Local Topological Structures and Global Connectivity Patterns in a *Trachelospermum jasminoides*: a Pilot Study

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

Topological approaches to biological systems provide insights into their growth patterns, network connectivity and spatial organization. This perspective explores how biological structures self-organize, maintain stability and adapt to environmental constraints, revealing fundamental principles of efficiency, robustness, resilience and functional optimization. In this pilot study, we analysed the local and global topological properties of a *Trachelospermum jasminoides* bush (commonly known as star jasmine) using persistent homology, graph theory, spectral analysis and percolation theory. The spatial positions of individual flowers were extracted from an image of the bush and represented as a point cloud to capture their structural distribution and spatial relationships. Using Delaunay triangulation, a connectivity graph revealed a dominant connected component with minimal isolated structures. DBSCAN analysis identified a large number of small, localized clusters, reflecting biological and environmental influences. Most flowers connected to five to six neighbours, forming a uniform network with high clustering. Shortest path analysis showed efficient long-range connectivity, with paths avoiding sparse regions. Spectral analysis indicated smooth percolation without bottlenecks, while percolation analysis simulations revealed resilience up to 18% flower removal, after which connectivity broke down. In sum, we showed that the star jasmine bush topology balances local structural constraints with global connectivity, ensuring efficient resource distribution and structural integrity. By integrating topological data analysis with ecological modeling, we suggested a methodological approach to understanding natural growth networks. These insights can inform broader studies on biological pattern formation, network resilience and plant architecture modeling in ecology, agricultural sciences and biomimetic design.

KEYWORDS: graph connectivity; homotopy theory; spatial self-organization; computational morphology; floral network dynamics.

INTRODUCTION

The study of topological structures in biological systems provides a unique perspective on how organisms organize themselves in space, ensuring both functional efficiency and resilience. In plant systems, topology plays a critical role in determining how resources are distributed and how growth patterns emerge in response to environmental constraints (Li et al., 2017; Shimotohno and Scheres, 2019; Wang et al., 2020). The *Trachelospermum jasminoides* bush, with its intricate floral and vine structures, provides a good example for exploring these topological principles. By applying computational topology, graph theory and fractal growth models, we seeked to analyze both local and global spatial properties of a star jasmine bush, revealing the relationships arising from its structure. Traditional botanical research has long focused on morphological classifications, describing the physical characteristics of plants in terms of their leaves, stems and flowers. Still, advancements in mathematical and computational topology have opened new avenues for understanding plant growth beyond mere morphological description (Carlsson, 2009; Edelsbrunner and Harer, 2010; Boissonnat et al., 2018; Liu et al., 2020). The application of persistent homology, spectral graph analysis and percolation theory allowed researchers to uncover hidden structural patterns influencing the connectivity of biological networks (Robins, 1999; Cohen-Steiner et al., 2007; Bianconi and Ziff, 2018). By identifying long-range relationships, local clustering tendencies and the thresholds at which structural integrity is compromised, the self-organizing nature of plant growth can be better understood.

Trachelospermum jasminoides, as a climbing plant, relies on flexible structural adaptations to balance between local constraints and global connectivity while maintaining an interconnected floral arrangement supporting pollination and resource transport. Our analysis explores the underlying principles shaping the topology of the star jasmine bush, focusing on how individual flowers distribute themselves and identifying the threshold at which connectivity within the floral network begins to fragment. To analyze the topological properties of *Trachelospermum jasminoides* bush, our pilot study first extracted flower positions from an image and represented them as a spatial point cloud. By applying Delaunay triangulation, we constructed a network that captured the connectivity between neighboring flowers. This representation allowed for the application of network theory to analyze the formation of clusters, the presence of long-range connectivity and the overall resilience of the floral structure. One key measure in our analysis was the percolation threshold, which indicates how the structure responds to the progressive removal of flowers. If a significant portion of the network remains connected despite a high percentage of removals, the structure can be considered robust.

In sum, our study integrated topological data analysis, network science and biological modeling to uncover the hidden structures within a *Trachelospermum jasminoides* bush. These findings provide a broader understanding of how plants self-organize and maintain connectivity under natural constraints, offering new perspectives in biological organization, ecological modeling and bio-inspired design.

MATERIALS AND METHODS

We examined images of a Trachelospermum jasminoides bush, taken in June during peak blooming. Trachelospermum jasminoides, commonly known as star jasmine, is an evergreen, twining vine prized for its star-shaped white flowers (Zhao et al., 2017). It blooms profusely in late spring to early summer, attracting pollinators like bees and butterflies (Cai et al., 2024). The analysis of the *Trachelospermum jasminoides* bush topology was conducted through a structured sequence of computational and mathematical techniques aimed at extracting persistent homology, spatial relationships, connectivity structures and growth patterns from an image of the plant (Zomorodian and Carlsson, 2005; Niyogi, 2008; Edelsbrunner et al., 2002; Chazal and Michel, 2021). The study involved several key stages, beginning with image preprocessing and flower position extraction, followed by the construction of a topological representation through Delaunay triangulation and network analysis. These representations were then subjected to percolation simulations, spectral graph analysis and clustering algorithms, all of which contributed to a deeper understanding of the plant's topological organization.

The first stage of the analysis involved preprocessing the image to extract relevant spatial information. The original image was converted to grayscale to remove any unnecessary color information and to simplify further processing. A thresholding technique was then applied to enhance the contrast between the flowers and the background. Since star jasmine flowers are generally brighter than their surroundings, a high-threshold binary transformation was used, which assigned white pixels to flower regions and black pixels to the rest of the image. To refine the extracted features, noise removal techniques, such as morphological opening and closing, were employed to eliminate small artifacts that were not part of the actual floral structures. Once a clean binary image was obtained, contour detection algorithms were applied to identify and isolate individual flowers. The centroid of each detected contour was computed, generating a point cloud representation of the flower positions within the *Trachelospermum jasminoides* bush.

Once extracted the spatial distribution of flowers, the next step involved constructing a mathematical representation of their connectivity using Delaunay triangulation (Song et al., 2021). This technique partitions the set of points into non-overlapping triangles, ensuring that no point falls within the circumcircle of any triangle, thereby providing an optimal way to connect neighboring flowers. The resulting triangulated structure captured the local connectivity patterns of the flowers, forming a foundation for network-based topological analysis. Each flower was treated as a node in a graph, while the edges of the triangulated structure represented potential paths of biological or structural influence. Following the construction of the connectivity graph, an extensive network analysis was performed to quantify the structural properties of the star jasmine bush. The graph was analyzed to determine key metrics such as the number of connected components, degree distributions and clustering coefficients. The occurrence of large-connected components indicated that most flowers belonged to a single dominant structure, while smaller, disconnected components suggested isolated floral clusters. Clustering analysis was performed to identify distinct floral groupings within the star jasmine bush. The DBSCAN (Density-Based Spatial Clustering of Applications with Noise) algorithm was chosen for this purpose because it is well-suited for identifying clusters of varying densities while distinguishing outliers. DBSCAN operates by grouping points that are closely packed together while marking points that do not belong to any cluster as noise (Sander et al., 1998; Zhang 2019). The algorithm required two parameters: the minimum number of points needed to form a cluster and the neighborhood radius defining which points are considered close to one another. Clustering coefficient analysis (van Diessen et al., 2014) provided insight into the tendency of flowers to form tightly packed groups, which could be indicative of growth constraints or ecological interactions.

Additionally, the average shortest path length and network diameter were computed to assess how efficiently one part of the floral network could be reached from another. To assess the resilience of the floral network, a percolation simulation was performed by progressively removing flowers from the structure and monitoring the effect on connectivity (Grimmett, 1999; Bianconi and Ziff, 2018). In each iteration, a random subset of nodes was removed and the size of the largest remaining connected component was recorded. This process continued until the network became fragmented, revealing the percolation threshold at which large-scale connectivity collapsed and subsequently the level of resilience. The network was further examined through spectral graph analysis, which involved computing the eigenvalues of the graph's Laplacian matrix (Zhang 2011). The spectral properties of the graph provided insight into its structural stability and the presence of bottlenecks or weakly connected regions, making it possible to infer how information or resources might propagate through the *Trachelospermum jasminoides* bush. The smallest nonzero eigenvalue, known as the algebraic connectivity, was particularly useful in determining how well the network resisted disconnection (Bao et al., 2023). A high algebraic connectivity points towards a robust structure with minimal vulnerability to localized disruptions.

RESULTS

The analysis of the *Trachelospermum jasminoides* bush's topological structure revealed a complex interplay between local clustering and global connectivity, suggesting an optimized natural arrangement balancing resilience and efficiency. The spatial positions of individual flowers were used to construct a connectivity graph based on Delaunay triangulation, which provided a geometric foundation for analyzing the relationships between neighboring flowers (**Figure 1A**). We demonstrated that most flowers belonged to a single dominant connected component, with only a few isolated structures scattered throughout (**Figure 1A**). The degree distribution of the graph indicated that most flowers were connected to an average of five to six neighboring flowers, forming a relatively uniform network with no significant outliers. Further network analysis revealed a high clustering coefficient, indicating that flowers tended to form tightly packed groups rather than being randomly dispersed. The clustering analysis using DBSCAN identified 59 distinct clusters of flowers, highlighting localized structural groupings that may correspond to biological or structural growth clusters. This means that, while the overall structure remained connected, localized groupings of flowers emerged within the bush. (**Figure 1B**). This behavior is consistent with known biological principles, where plants optimize spatial efficiency to maximize exposure to light and pollination opportunities while minimizing energy expenditure.

The connectivity spread across the *Trachelospermum jasminoides* bush was further explored through the shortest path analysis. The minimal route between two distant flowers spanned 43 steps. This path followed the densest connectivity regions, demonstrating strong long-range percolation within the flower network. The start and end nodes were positioned in well-connected regions, effectively avoiding sparse areas, confirming the structural integrity of the network. Therefore, despite its dense clustering, the average path length between any two flowers remained relatively low. This suggests that the overall structure of the bush is optimized for both local interactions and long-range connectivity, ensuring efficient resource distribution and pollination. The percolation analysis provided additional insights into the robustness of the floral network. The star jasmine bush retained its large-scale connectivity until approximately eighteen percent of the flowers were removed (**Figure 2A**). This percolation threshold, relatively low compared to many random networks, highlighted the highly interconnected nature of the flower arrangement and indicated a high degree of resilience, as the structure remained intact despite significant disturbances. The rapid breakdown of connectivity beyond this threshold suggests that *Trachelospermum jasminoides* growth follows an optimized spatial strategy that prevents fragmentation under normal environmental conditions but becomes vulnerable when a critical mass of structural points is lost. Spectral analysis of the flower graph leveraged eigenvalues of the adjacency matrix in order to detect topological features such as bottlenecks, cycles and connectivity strength. This spectral analysis revealed a dense clustering of small eigenvalues, indicating strong overall connectivity within the star jasmine bush (**Figure 2B**). The smallest eigenvalues, ranging from 0.018 to 0.022, are characteristic of highly connected graphs with percolation-like behavior. The absence of significant bottlenecks suggested smooth percolation across long distances, reinforcing the idea that the network remained well-connected.

In sum, the presence of a smooth eigenvalue spectrum suggested that the connectivity of the floral network was evenly distributed without significant weak points or bottlenecks. Our findings support the hypothesis that the *Trachelospermum jasminoides* bush's structural organization is not random but follows principles enhancing its overall integrity. The formation of localized clusters within a globally connected structure reflects an efficient organizational principle that maximizes functional interactions while maintaining large-scale stability.

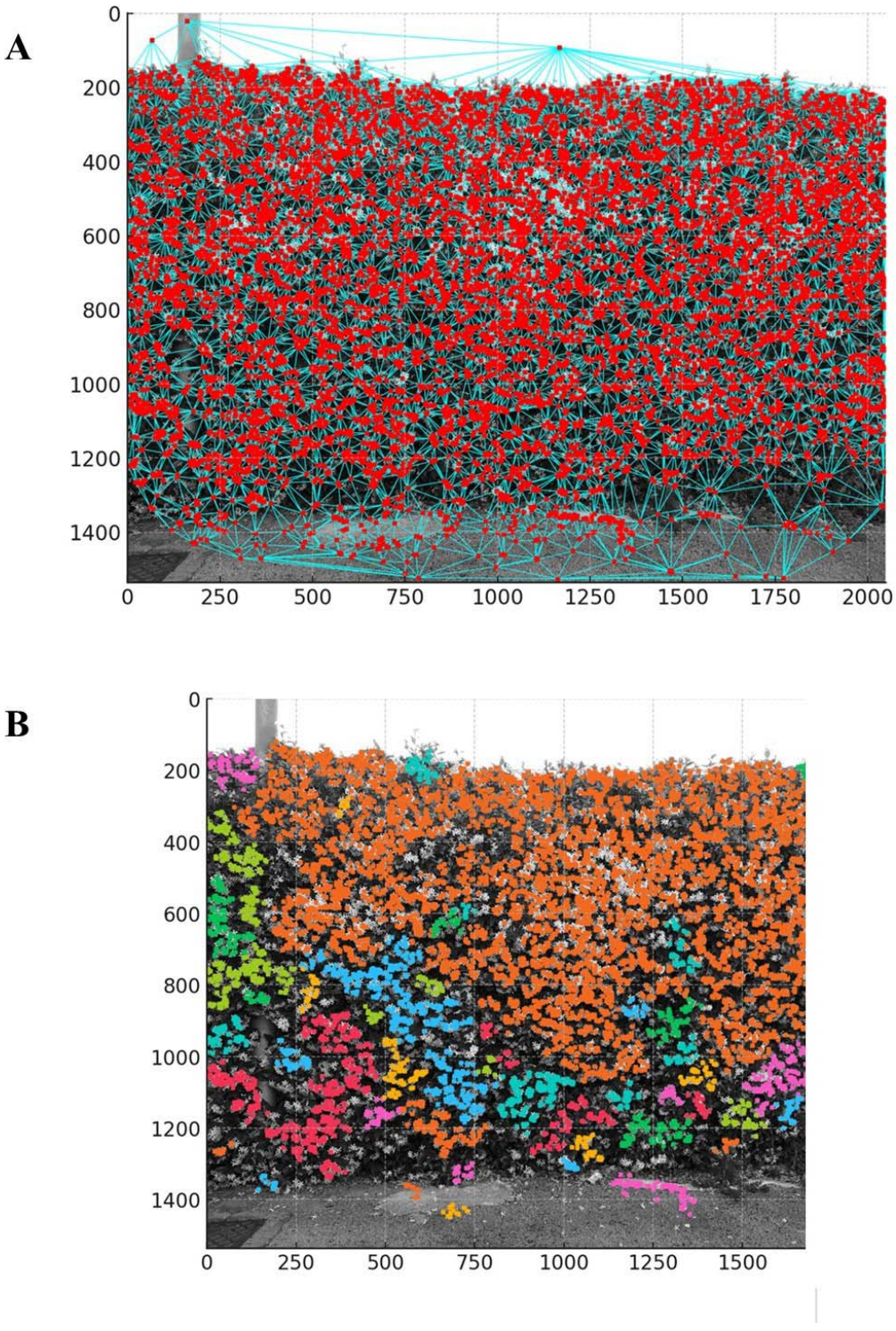

Figure 1A. Delaunay triangulation highlighted short-range and mid-range connections between neighboring flowers. The flower centers were represented by red dots, marking individual flower positions. Some regions displayed higher connectivity, indicating clusters with strong local interactions. The largest connected component included 8,397 flowers, suggesting a globally connected, large-scale structure.
Figure 1B. DBSCAN clustering analysis identified 59 distinct clusters of flowers. See text for further details.

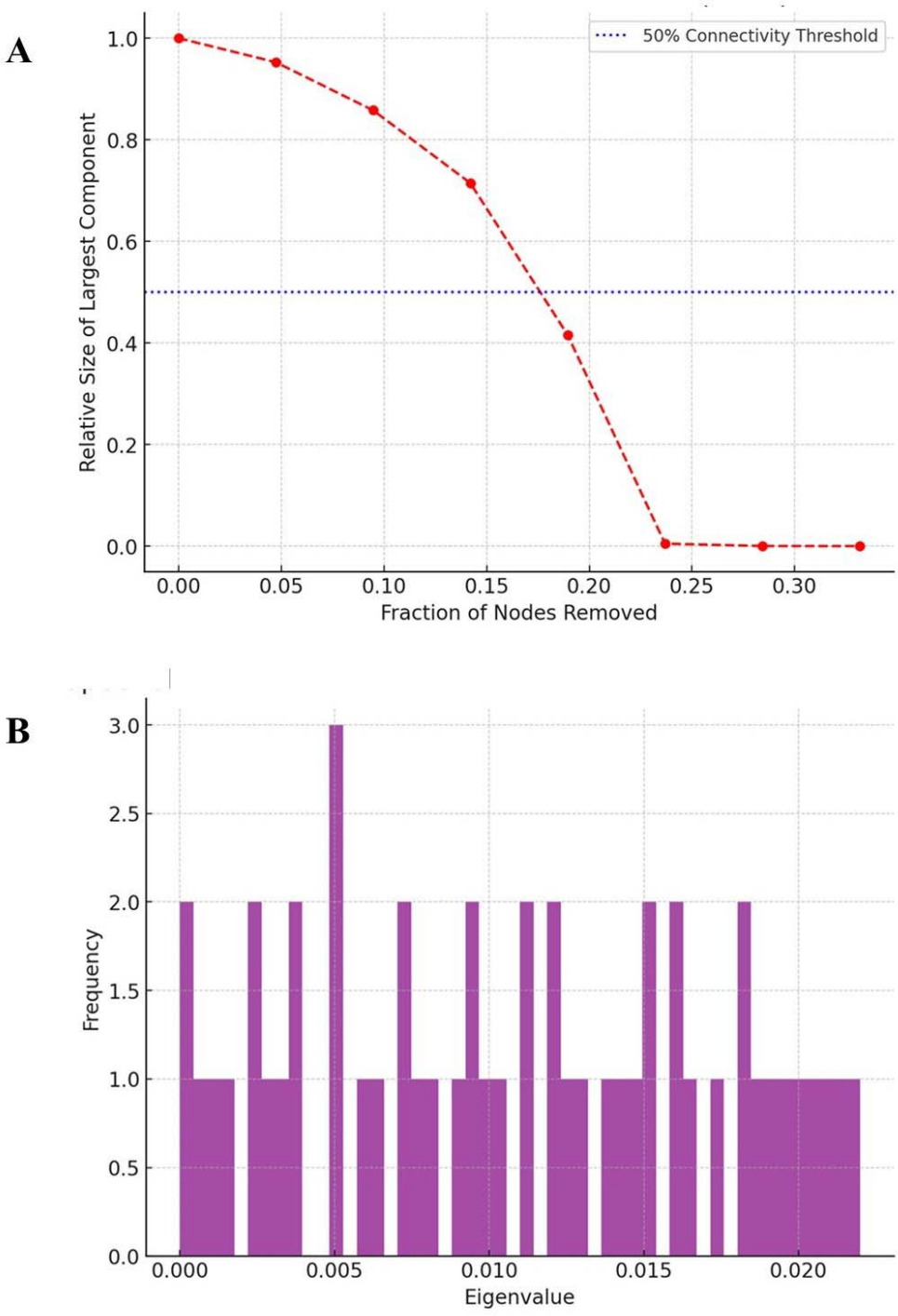

Figure 2A. Percolation threshold simulation. Only 18.9% of flowers needed to be removed before the *Trachelospermum jasminoides* bush's large-scale connectivity collapsed. The largest connected component remained stable up to approximately 15% removal, after which it rapidly disintegrated, indicating a sudden transition from a well-connected structure to fragmentation.

Figure 2B. Spectral analysis leveraging the flower graph's eigenvalue distribution of the adjacency matrix (first 50). A dense clustering of small eigenvalues was detected, indicating strong overall connectivity within the star jasmine bush. The smallest eigenvalues, ranging from 0.018 to 0.022, are typical of highly connected graphs with percolation-like behavior.

CONCLUSIONS

Using computational topology, graph theory and growth modeling, we provided an in-depth analysis of the topological properties of a *Trachelospermum jasminoides* bush. By extracting flower positions and analyzing their spatial relationships, we built a comprehensive model of the floral network, capturing both small-scale clustering behaviors and large-scale organizational patterns. Our results suggest that the star jasmine bush follow an optimized structural framework that maintain connectivity despite localized variations in floral density. One of the most significant findings was the discovery of a high clustering coefficient among the flowers, which suggested a strong tendency for localized grouping. This indicated that *Trachelospermum jasminoides* growth is not random but instead followed an underlying spatial organization to promote dense floral arrangements while maintaining connections to the larger plant structure. The clustering tendency aligned with known ecological and biological principles, particularly in relation to pollination strategies and resource distribution. Flowers arranged in tightly packed clusters may enhance pollination efficiency by increasing the likelihood that pollinators will move between closely spaced flowers before leaving the plant. This also has implications for the transport of nutrients and water, as connectivity within these clusters ensures effective internal resource distribution.

A key finding related to structural robustness emerged from the percolation analysis (Smirnov, 2001), which demonstrated that the *Trachelospermum jasminoides* bush maintains its connectivity even after the removal of approximately eighteen percent of its flowers. This suggests a high degree of resilience, as the plant is capable of withstanding significant structural changes without suffering immediate fragmentation. The observed percolation threshold aligns with results from similar analyses conducted in other biological networks, where plant and fungal structures exhibit similar levels of robustness. The rapid breakdown of connectivity beyond the eighteen percent threshold suggests that star jasmine plants have evolved an optimal distribution pattern that allows them to remain intact under typical environmental disturbances but may become vulnerable under extreme conditions such as disease outbreaks or mechanical damage. The spectral analysis of the star jasmine network reinforced these conclusions by providing insight into the plant's global connectivity patterns. The eigenvalue distribution of the Laplacian matrix revealed a smooth spectrum indicative of a well-balanced and evenly connected structure. Supporting the findings from the percolation study, the presence of a high algebraic connectivity value confirmed that the *Trachelospermum jasminoides* bush is resilient to disconnection. The spectral properties of the network suggest that the star jasmine plant does not grow in an entirely uniform manner but instead follows a pattern of optimized connectivity, ensuring that floral structures remain accessible while avoiding excessive redundancy. The study's spectral approach also allowed for the detection of subtle structural variations within the network that would have been difficult to observe using traditional morphological analysis alone.

When comparing these findings to other techniques commonly used in plant morphology studies, it becomes evident that computational topology provides unique advantages in uncovering hidden structural patterns. Traditional botanical studies rely on morphological classification systems that describe the arrangement of leaves, flowers and stems based on visual observation and measurement. While these methods are valuable for taxonomy and comparative morphology, they do not capture the deeper network properties influencing plant function and resilience. In turn, graph-based approaches offer a more quantitative framework for analyzing how plants organize themselves spatially. One of the primary advantages of our computational approach is its ability to model plant growth dynamically, providing testable hypotheses about the factors influencing *Trachelospermum jasminoides* topology. The presence of strong floral clustering raises questions about whether environmental variables such as sunlight availability, wind exposure or soil nutrient distribution contribute to the observed patterns. Future studies could test this hypothesis by conducting experiments in which star jasmine plants are grown under different environmental conditions to observe whether clustering behaviors change in response to external factors. Another testable hypothesis emerging from the percolation analysis is that star jasmine plants may prioritize connectivity to minimize the risk of fragmentation in response to mechanical stress. This could be experimentally verified by selectively pruning flowers from different parts of the plant and measuring how structural integrity is affected over time.

The findings of this study also have applications in ecological research, agriculture and biomimetic design. In ecology, understanding the topological organization of plants can provide insight into how different species compete for space and resources. The clustering behaviors observed in the *Trachelospermum jasminoides* bush could be compared to those of other climbing plants to determine whether similar patterns emerge in different species or whether star jasmine exhibits unique growth strategies. In agriculture, insights into plant topology can inform planting strategies to optimize space usage and resource efficiency. By applying principles of connectivity and clustering, farmers and horticulturalists could design planting arrangements that enhance pollination efficiency and nutrient transport while reducing vulnerability to disease spread. Biomimetic applications of plant topology extend into the realm of artificial network design. The ability of plants to maintain connectivity while minimizing redundancy offers valuable lessons for engineers developing self-organizing networks in telecommunications, transportation and material science. The star jasmine bush's ability to remain robust under structural changes suggests that similar principles could be applied to the

design of resilient infrastructure systems. Studying plant topology may also inspire new approaches to designing artificial materials that mimic the self-repairing and self-optimizing properties of biological systems.

Despite the strengths of this approach, certain limitations must be acknowledged. The study relied on a two-dimensional representation of the *Trachelospermum jasminoides* bush extracted from images, which may not fully capture the three-dimensional complexity of plant growth. While the extracted floral positions provided valuable insights into spatial organization, future studies could benefit from incorporating three-dimensional imaging techniques, such as LiDAR scanning or photogrammetry, to obtain a more complete structural representation. Additionally, the study focused primarily on flower distributions, without considering the role of underlying stem and leaf structures, which may also influence connectivity and resource transport. Including additional plant components in future analyses could provide a more holistic understanding of the plant topology. Incorporating biological signaling models alongside topological analysis could refine the accuracy of our findings. Additionally, the study did not explore temporal dynamics, meaning that it provided a snapshot of star jasmine topology rather than an analysis of how the structure evolves over time. Future research could address this by monitoring the growth of *Trachelospermum jasminoides* plants longitudinally, capturing real-time changes in connectivity and clustering.

The findings pave the way to further interdisciplinary research into the principles of plant topology, providing a framework for understanding how natural structures self-organize. The combination of computational topology, network science and biological modeling has demonstrated that plants operate under structural principles balancing local constraints with global connectivity. These principles appear to be fundamental across biological systems, influencing not only plant morphology but also ecological interactions, species competition and evolutionary strategies. By refining and expanding upon these methods, future studies can uncover the hidden structures governing plant growth, contributing to both theoretical biology and practical applications in ecology, agriculture and biomimicry.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.

Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal and has not been previously published.

Availability of data and materials. all data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical and material support, study supervision.

Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT to assist with data analysis and manuscript drafting. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

REFERENCES

1) Bao, Zhigang, Jaehun Lee, and Xiaocong Xu. "Phase Transition for the Smallest Eigenvalue of Covariance Matrices." *arXiv preprint* arXiv:2308.09581, November 8, 2023.
2) Bianconi, Ginestra, and Robert M. Ziff. "Topological Percolation on Hyperbolic Simplicial Complexes." *Journal of Physics A: Mathematical and Theoretical* 51, no. 8 (2018): 085001. https://doi.org/10.1088/1751-8121/aaa3f2.
3) Boissonnat, Jean-Daniel, Frédéric Chazal, and Mariette Yvinec. *Geometric and Topological Inference*. Cambridge: Cambridge University Press, 2018.

4) Cai, Y., Chen, H., Ni, Y., Li, J., Zhang, J., and Liu, C. "Repeat-Mediated Recombination Results in Complex DNA Structure of the Mitochondrial Genome of *Trachelospermum jasminoides*." *BMC Plant Biology* 24, no. 1 (October 16, 2024): 966. https://doi.org/10.1186/s12870-024-05568-6.
5) Carlsson, Gunnar. "Topology and Data." *Bulletin of the American Mathematical Society* 46, no. 2 (2009): 255–308. https://doi.org/10.1090/S0273-0979-09-01249-X.
6) Chazal, Frédéric, and Bertrand Michel. "An Introduction to Topological Data Analysis: Fundamental and Practical Aspects for Data Scientists." *Frontiers in Artificial Intelligence* 4 (2021): 667963. https://doi.org/10.3389/frai.2021.667963.
7) Cohen-Steiner, David, Herbert Edelsbrunner, and John Harer. "Stability of Persistence Diagrams." *Discrete & Computational Geometry* 37, no. 1 (2007): 103–120. https://doi.org/10.1007/s00454-006-1276-5.
8) Edelsbrunner, Herbert, David Letscher, and Afra Zomorodian. "Topological Persistence and Simplification." *Discrete & Computational Geometry* 28, no. 4 (2002): 511–533. https://doi.org/10.1007/s00454-002-2885-2.
9) Edelsbrunner, Herbert, and John Harer. *Computational Topology: An Introduction*. Providence, RI: American Mathematical Society, 2010.
10) Grimmett, Geoffrey. *Percolation*. 2nd ed. Berlin: Springer, 1999.
11) Li, Mao, Keith Duncan, Christopher N. Topp, and Daniel H. Chitwood. "Persistent Homology and the Branching Topologies of Plants." *American Journal of Botany* 104, no. 3 (March 2017): 349–353. https://doi.org/10.3732/ajb.1700046.
12) Liu, Zichu, Qing Zhang, Pei Wang, Zhen Li, and Huiru Wang. "Automated Classification of Stems and Leaves of Potted Plants Based on Point Cloud Data." *arXiv preprint* arXiv:2002.12536, February 28, 2020. https://arxiv.org/abs/2002.12536.
13) Niyogi, Partha, Stephen Smale, and Shmuel Weinberger. "Finding the Homology of Submanifolds with High Confidence from Random Samples." *Discrete & Computational Geometry* 39, no. 1–3 (2008): 419–441. https://doi.org/10.1007/s00454-008-9053-2.
14) Robins, Vanessa. "Towards Computing Homology from Approximations." *Topology Proceedings* 24 (1999): 503–532.
15) Sander, Jörg, Martin Ester, Hans-Peter Kriegel, and Xiaowei Xu. "Density-Based Clustering in Spatial Databases: The Algorithm GDBSCAN and Its Applications." *Data Mining and Knowledge Discovery* 2, no. 2 (1998): 169–194. https://doi.org/10.1023/A:1009745219419.
16) Shimotohno, Akie, and Ben Scheres. "Topology of Regulatory Networks That Guide Plant Meristem Activity: Similarities and Differences." *Current Opinion in Plant Biology* 51 (October 2019): 74–80. https://doi.org/10.1016/j.pbi.2019.04.006.
17) Smirnov, Stanislav. "Critical Percolation in the Plane: Conformal Invariance, Cardy's Formula, Scaling Limits." *Comptes Rendus de l'Académie des Sciences - Series I - Mathematics* 333, no. 3 (2001): 239–244. https://doi.org/10.1016/S0764-4442(01)01991-7.
18) Song, Tao, Jiarong Wang, Danya Xu, Wei Wei, Runsheng Han, Fan Meng, Ying Li, and Pengfei Xie. "**Unsupervised Machine Learning for Improved Delaunay Triangulation.**" *Journal of Marine Science and Engineering* 9, no. 12 (2021): 1398. https://doi.org/10.3390/jmse9121398.
19) van Diessen, Eric, Willemiek Zweiphenning, Floor E. Jansen, and Willem M. Otte. "Brain Network Organization in Focal Epilepsy: A Systematic Review and Meta-Analysis." *PLOS ONE* 9, no. 12 (December 2014): e114606. https://doi.org/10.1371/journal.pone.0114606.
20) Wang, Guan, Hamid Laga, Jinyuan Jia, Stanley J. Miklavcic, and Anuj Srivastava. "Statistical Analysis and Modeling of the Geometry and Topology of Plant Roots." *Journal of Theoretical Biology* 486 (February 7, 2020): 110108. https://doi.org/10.1016/j.jtbi.2019.110108.
21) Zhang, Xiao-Dong. "The Laplacian Eigenvalues of Graphs: A Survey." *arXiv preprint* arXiv:1111.2897, November 12, 2011. https://arxiv.org/abs/1111.2897.
22) Zhang, Mingrui. "Use Density-Based Spatial Clustering of Applications with Noise (DBSCAN) Algorithm to Identify Galaxy Cluster Members." *IOP Conference Series: Earth and Environmental Science* 252 (2019): 042033. https://doi.org/10.1088/1755-1315/252/4/042033.
23) Zhao, Z., He, X., Zhao, Y., Sun, Y., Chen, X., Cun, Y., Huang, L., Bai, Y., and Zheng, X. "Phytochemistry, Pharmacology and Traditional Uses of Plants from the Genus *Trachelospermum* L." *Molecules* 22, no. 9 (August 24, 2017): 1406. https://doi.org/10.3390/molecules22091406.
24) Zomorodian, Afra, and Gunnar Carlsson. "Computing Persistent Homology." *Discrete & Computational Geometry* 33, no. 2 (2005): 249–274. https://doi.org/10.1007/s00454-004-1146-y.

Topological Analysis of Vascular Networks: A Proof-of-Concept Study in Cerebral Angiography

Arturo Tozzi *

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Article

Topological Analysis of Vascular Networks: A Proof-of-Concept Study in Cerebral Angiography

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA; tozziarturo@libero.it

ABSTRACT: The application of topological methods to cerebral angiography may provide a robust mathematical framework for analyzing cerebrovascular structures at multiple scales. In this proof-of concept study, we explored the use of algebraic and differential topology to characterize structural integrity, connectivity, flow dynamics and hierarchical organization of cerebral vascular networks. Through a hierarchical approach, we examined the topology from general to local, capturing macroscopic vascular organization down to individual vessel bifurcations. By leveraging key theorems, we assessed various aspects of topological analysis, including evaluation of total features, transition from total to local features, evaluation of local features, transition from local to total features, interaction between total and local features. These steps enable the analysis of the global connectivity of the vascular network, the detection of regional clusters and the identification of critical junctions at a local scale. A computational approach was developed to extract mathematical skeletons from angiographic images, constructing graph-based representations to study connectivity and homotopy equivalence. The Fourier decomposition of the vascular structures revealed dominant periodic patterns, indicative of structural stability and redundancy in the blood supply. Moreover, Betti number computations quantified vascular loops and branches, offering insights into collateral circulation potential. Our findings demonstrate that topological invariants can serve as diagnostic biomarkers for cerebrovascular diseases, including aneurysm susceptibility and ischemic risk assessment. This interdisciplinary methodology bridges mathematical topology with medical imaging, offering a novel lens for cerebrovascular analysis. Future work will integrate persistent homology and machine learning techniques for automated vascular topology classification.

Keywords: Homotopy theory; cerebrovascular anomalies; graph embedding; manifold reconstruction; vascular bifurcation analysis

INTRODUCTION

Cerebral angiography plays a crucial role in diagnosing vascular disorders of the brain. Traditional methods rely on image-based assessments of vessel structure, flow dynamics, and morphological anomalies. However, these approaches often lack a formal mathematical framework to quantify connectivity and structural organization (Baharoglu et al., 2012; Kisler et al., 2017; Damseh et al., 2019; Ross et al., 2002; Li et al., 2021). Topological methods offer a powerful framework for analyzing vascular networks. Extending beyond conventional imaging techniques, they capture the intrinsic geometric and algebraic properties of the vasculature. Topology provides a means to analyze spaces independently of continuous deformations, making it particularly suitable for studying complex branching structures such as blood vessels (Bertolero and Bassett, 2010; De Domenico et al., 2015). A major challenge in angiographic imaging is the variability in vascular configurations among individuals, which can make traditional pattern recognition approaches difficult. Topological methods provide a framework for reducing this complexity by identifying fundamental properties of the vascular network that remain invariant under deformations. This

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proof-of-concept study applies a variety of topological theorems and computational methods to cerebral angiographic images to extract meaningful features, classify connectivity and detect possible vascular anomalies (Lauric et al., 2023; Gosh et al., 2024). By leveraging algebraic, differential, and computational topology, essential features of cerebral circulation can be captured, including connectivity, redundancy and flow optimization (He et al., 2008; Goirand et al., 2021).

Topological investigations of cerebral angiography may include global structural analysis, regional analysis, local vascular analysis, spectral methods. Global structural analysis of the vascular system mat be crucial for understanding blood supply distribution and redundancy. Using the Seifert-van Kampen theorem, we investigated how different connected components of the vascular network contribute to the fundamental group structure, providing insight into overall connectivity. Homology and Betti numbers allowed for the quantification of loops and independent vascular pathways, crucial in assessing the potential for collateral circulation in the event of arterial blockages. The interplay between arterial and venous structures was explored through the application of Poincaré duality, examining how these systems interact and preserve equilibrium in cerebral circulation.

Regional analysis focused on segmenting the vascular network into functionally relevant subregions, highlighting clusters that correspond to specific cerebral territories. The Künneth theorem facilitated this decomposition, allowing for homological computations across different vascular domains. Vascular segmentation may be particularly relevant in the study of ischemic stroke, where reduced perfusion in one territory can impact other regions. Graph-based methods further enhanced regional analysis by modeling the vasculature as a network of interconnected nodes and edges, where connectivity can be evaluated using group homomorphisms (Dummit and Foote, 2004). By leveraging the Whitehead theorem, we ensured that simplified graph representations preserve essential homotopy equivalence, retaining the critical structural features of the vascular system.

Local vascular analysis provided fine-grained insights into the branching patterns, bifurcations and junction points in the vascular system. Detecting bifurcation points and measuring their homological significance aided in identifying regions susceptible to stenosis, aneurysm formation or other pathological changes. The Hurewicz theorem helped in bridging homotopy and homology computations, facilitating the classification of vascular junctions. Knot theory, particularly Legendrian knot theory, was useful in detecting abnormal vascular loops or tangles that may indicate potential pathologies. The presence of specific knot structures in angiographic images may suggest hemodynamic stresses that may lead to vessel deformation or rupture.

Beyond static vascular analysis, spectral methods such as Fourier decomposition are employed to capture periodic and recurrent patterns in cerebral vasculature. By transforming angiographic images into frequency space, dominant structural patterns emerge, revealing how vascular pathways are organized at different scales. Inverse Fourier transforms allow for the reconstruction of dominant vascular features, filtering out noise and minor variations while retaining the essential geometry. This technique is particularly useful in differentiating normal vascular configurations from pathological formations, as deviations in frequency components can signal abnormal vessel growth or occlusion risks.

In sum, the integration of topological analysis with computational imaging may provide a robust framework for vascular assessment, moving beyond conventional image-based diagnostics. The combination of homotopy theory, homological computations and spectral analysis may enable a multi-scale understanding of cerebrovascular organization. By examining topology from a general perspective down to localized structural details, this proof-of-concept study aims to establish a methodology for quantifying vascular health and predicting potential disease risks.

MATERIALS AND METHODS

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We employed a series of computational techniques to analyze brain angiography images through topological and spectral methods, ensuring a comprehensive characterization of the vascular network. Our approach combined algebraic topology, graph theory, spectral analysis and image processing techniques to extract meaningful structural and functional information from the intricate vascular network. By leveraging these methods, researchers and clinicians can gain deeper insights into connectivity, continuity and spatial mapping of blood vessels. Our methodology was structured to progress from global analysis to regional segmentation and finally to local structural evaluation, allowing a hierarchical understanding of cerebrovascular connectivity. The following two paragraphs present: (a) a list of feasible theorems and concepts applicable to the topological analysis of brain angiography, and (b) a proof-of-concept experimental example demonstrating their application to real imaging data.

Topological methods for analysing brain angiography. Various theorems evaluate different aspects of topological analysis, including the evaluation of total features, the transition from total to local features, the evaluation of local features, the transition from local to total features, the interaction between total and local features.

Evaluation of total features

1) Borel's theorem, which states that every sequence of independent random variables converges in probability (Borel 1953), may be used in probabilistic modeling of cerebral blood flow patterns. In brain angiography, understanding the distribution of contrast agents and their diffusion may be analyzed using this theorem, ensuring that variations in flow due to anatomical differences remain within predictable limits.
2) Coarse proximity theory may help in quantifying large-scale structures within the angiographic imaging data (Shi and Yao, 2024). By applying this concept, regions of vascularization can be compared across different subjects without being confounded by individual vessel structure variations. This may be particularly useful in stroke prediction and the study of large vessel occlusions, where macroscopic vascular topology plays a crucial role in determining collateral circulation.
3) Kolmogorov's zero-one law, which deals with the behavior of tail events in probability spaces (Brzeźniak and Zastawniak, 2020), may predict whether vascular patterns lead to pathological conditions. If certain vessel formations or distributions occur with probability one, angiographic imaging data can be used to make deterministic predictions about disease development.

Transition from total to local features

1) The Eilenberg-Zilber theorem, which describes the interaction between homology groups of spaces in product form (Golański and Lima Gonçalves, 1999), may be applied to the analysis of cerebral vessel connectivity. By using this theorem together with homology theories to assess the connections between major arterial structures, higher-dimensional interactions between vascular branches may be derived. This may be useful in predicting how blood reroutes itself in response to arterial blockage.
2) The Kunneth theorem allows the computation of the homology of a product space in terms of the homologies of its components (Smith 1970). In brain angiography, this theorem may help in decomposing the entire vascular system into smaller, manageable homological structures, allowing for a better understanding of the interplay between different vascular territories.

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3) The Grassmannian, which parametrizes linear subspaces (Lakshmibai & Brown, 2015), may be applicable for dimensional reduction in angiographic data analysis. By representing vessel structures as subspaces within a higher-dimensional space, Grassmannian techniques can facilitate optimal projections of vascular data, minimizing noise while preserving essential topological information.

Evaluation of local features

1) The Heine-Borel theorem, which characterizes compact subsets in Euclidean space (Macauley et al., 2008), may be crucial in determining the boundedness and completeness of vascular structures. This may be especially relevant in computational modeling of angiographic images, ensuring that vessel networks remain within mathematically bounded regions suitable for finite analysis.
2) The cellular approximation theorem, which allows homotopy equivalence to be reduced to CW complexes (Hatcher 2005), may provide a way to approximate cerebral vasculature with simpler topological structures. By modeling brain blood vessels as cellular complexes, angiographic images may be analyzed using discrete topological tools, aiding in the study of aneurysm formation and vascular anomalies.
3) Legendrian knot theory may be relevant in the study of vascular loops and knots in angiography (Etnyre 2005). Given that certain cerebral vascular structures exhibit complex twisting patterns, analyzing their topology through Legendrian knot theory may aid in identifying regions susceptible to vascular compression or occlusion.
4) Fixed point theorems, such as Brouwer's or Banach's, may analyze flow dynamics in cerebral angiography (Pata 2019). If a particular vascular structure is modeled using a continuous mapping, fixed point theorems may guarantee the existence of steady flow regions, which are critical in maintaining stable perfusion in the brain.
5) The De Franchis theorem, which restricts the number of non-trivial maps between algebraic curves of certain types (Alzati and Pirola, 1990), may be applied in analyzing repeated or redundant vascular formations. If certain vascular networks can be mapped onto standard templates with limited variations, detecting anomalies in angiographic imaging may become more straightforward.

Transition from local to total features

1) The Seifert-van Kampen theorem, which describes the fundamental group of a space in terms of its decompositions (Lee 2011), may be useful in analyzing the connectivity of cerebral blood vessels. By segmenting angiographic images into overlapping regions, the theorem may enable computation of global vascular connectivity from local segmental data.
2) The Blakers-Massey theorem, which provides a framework for homotopy excision (Anel et al., 2020), may allow for the reduction of angiographic complexity by identifying essential homotopy groups. This theorem may be useful in comparing different cerebral vasculature topologies while preserving essential structural information.
3) Sheaf cohomology may be used to assess local-to-global properties in angiographic images (Wedhorn 2016). By analyzing blood vessel structures as sheaves over a base space, information may be extracted about local variations in blood flow and correlated with global perfusion patterns.

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4) The Lusternik-Schnirelmann theorem, which deals with critical point theory (James 1992), may have applications in optimizing blood flow dynamics. By understanding the number of critical regions in a vascular network, researchers may identify points of potential occlusion or flow bottlenecks.

Interaction between total and local features

1) Poincare duality, which relates homology and cohomology in a compact orientable manifold (Hilman et al., 2024), may be useful in understanding the complementary nature of different vascular regions. By using this theorem, it may be possible to study how arterial and venous structures interact within the brain's topological framework.
2) The Freudenthal suspension theorem, which connects homotopy groups of different-dimensional spaces (Whitehead 1953), may be applied in modeling the evolution of vascular networks. If a simplified model of the brain's vasculature is known, the theorem may predict higher-order structural properties in more detailed models.
3) The Whitehead theorem states that a homotopy equivalence between CW complexes is also a homotopy equivalence in general topology (Kan 1976). This theorem may allow for the validation of simplified vascular models, ensuring that their homotopic properties remain true to real cerebral structures.
4) Group homomorphisms provide insights into how different vascular regions interact (Dummit and Foote, 2004). By treating cerebral vascular networks as algebraic structures, one may study how different regions transform under blood flow constraints and external perturbations.
5) Finally, the Hurewicz theorem, which relates homotopy groups to homology groups in simply connected spaces (Christensen and Scoccola, 2023), may be useful in transitioning from homotopic analysis to homological interpretation of angiographic images. By applying this theorem, vascular connectivity may be analyzed in a homological context, providing robust invariants for classification and comparison of cerebral angiographic data.

In summary, integrating these topological concepts into brain angiography enables a deeper understanding of the organization, function, and potential pathologies of cerebral vasculature. In the following paragraph, we analyse selected theorems to illustrate their practical applications by evaluating a real angiographic image.

Proof-of concept methodology. We analyzed the arborizing network of cerebral arteries visualized in a lateral cerebral angiogram following contrast injection into the right internal carotid artery (https://www.primaryanatomy.com/cerebral-angiography/, retrieved on Jan 4, 2025) (**Figure A**). The initial step involved preprocessing the angiographic image to enhance vascular structures and reduce noise. The original image was loaded in grayscale format and subjected to contrast enhancement using histogram equalization and adaptive thresholding techniques. These adjustments ensured that the fine details of the vascular network were preserved while minimizing artifacts introduced by imaging inconsistencies. Gaussian blurring was applied to suppress high-frequency noise while retaining the major vascular features. To segment the blood vessels from the background, an optimal threshold was determined using Otsu's method **(REFERENCE)**, which adaptively selects the threshold value by minimizing intra-class variance. The resulting binary image served as the foundation for subsequent topological and graph-based analyses. A skeletonization process was applied to the binarized image. Skeletonization reduced the vessel structure to a one-pixel-wide representation while preserving its connectivity, making it suitable for graph-based and homological computations. The Zhang-Suen thinning algorithm **(REFERENCE)** was used to ensure

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that the skeletonization was accurate and retained topological fidelity. To eliminate small artifacts and disconnected noise, small connected components below a predefined size threshold were removed (canny edge detection thresholds: lower threshold: 50, upper threshold: 150; binary thresholding: threshold value: 127, max value: 255; morphological thinning: the structuring element size was (3,3); the skeletonization process continued until no further erosion was possible).

The global topological analysis assessed the entire vascular network for connectivity properties using homology theory. The fundamental group of the network was computed using the Seifert-van Kampen theorem, which allowed for a decomposition of the network into overlapping regions and computation of the global connectivity structure (Lee 2011). Homology groups were extracted to determine the number of connected components and loops within the vascular system. Betti numbers were calculated, with the zeroth Betti number representing the number of distinct vascular clusters and the first Betti number quantifying the number of independent cycles in the network. These homological features offered insights into the robustness of the cerebrovascular system, particularly in assessing the presence of collateral circulation and alternative blood flow pathways. Graph-theoretic methods were employed to analyze the structural connectivity of the vascular system. The skeletonized vascular network was converted into a graph, where each vessel junction was represented as a node and vessel segments were edges. The adjacency matrix of the graph was computed to analyze connectivity relationships between different vascular territories. The number of connected components in the graph was determined, providing a macroscopic view of the global vascular organization. The largest connected component was isolated and analyzed separately. Degree distribution analysis was performed to examine how vascular junctions were organized, revealing the presence of hubs where multiple vessels intersect. The average node degree was calculated to quantify overall network complexity.

Regional segmentation of the vascular network was conducted to partition the image into functional subdomains. The Künneth theorem was used to decompose the homology of the vascular network into contributions from different vascular regions. Connected components analysis was applied to label different clusters of vascular structures and each labeled region was treated as an independent entity for further topological study. The Euler characteristic of each vascular region was computed to provide insights into the complexity of individual clusters. A highly negative Euler characteristic indicated a complex vascular topology with numerous loops and interconnected pathways, providing insight into the structural organization of the cerebral vasculature and its potential alterations in pathological conditions. Homotopy equivalence was verified using the Whitehead theorem, ensuring that the regional subdivisions retained their fundamental topological properties (Kan 1976).

Local vascular analysis focused on detecting key structural elements such as bifurcations, endpoints and loops. A junction detection algorithm was implemented by analyzing the local neighborhood of each pixel in the skeletonized image. Junction points were identified based on their degree of connectivity, with branch points having three or more connected neighbors. Endpoints were detected as nodes with only one connected neighbor. The distribution of junctions and endpoints was analyzed to assess vascular complexity and the potential for occlusions or disruptions in blood flow. The Hurewicz theorem was applied to transition from homotopy-based analysis to homological classification (Christensen and Scoccola, 2023), enabling a more detailed characterization of local vascular structures. Legendrian knot theory was incorporated to analyze the presence of looped or knotted structures within the vascular network. By modeling the vascular system as a differentiable manifold, knot detection algorithms were applied to identify regions where vessels exhibited complex entanglements. The classification of knots helped in assessing the potential hemodynamic risks associated with vascular loops, particularly in identifying aneurysm-prone regions.

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Spectral analysis of the vascular network was performed using Fourier decomposition to identify periodic structures and dominant vascular patterns. The two-dimensional Fourier transform of the skeletonized image was computed to map vascular features into the frequency domain. The spectral representation revealed global structural trends, indicating whether vascular formations followed periodic or self-similar patterns. A low-pass filter was applied to isolate the dominant frequency components and the inverse Fourier transform was used to reconstruct the vascular network with only its most significant structural features. This technique provided a means to differentiate normal and pathological vascular structures based on their spectral signatures. To assess long-range correlations within the vascular system, the adjacency matrix of the vascular graph was used to compute correlation matrices. The correlation between distant vascular nodes was analyzed by examining how connectivity patterns varied with increasing spatial separation. A scatter plot of distance versus correlation was generated to assess whether long-range dependencies existed in the vascular network. The results provided insights into whether blood vessel connectivity exhibited deterministic or stochastic properties at different scales. Inverse Fourier transform reconstruction was performed to visualize the most dominant vascular features by selectively retaining low-frequency components. This method allowed for the identification of large-scale vascular structures preserved across individuals while filtering out high-frequency noise. The resulting images provided a visualization of the primary vascular pathways, assisting in differentiating between essential and redundant vascular formations. This approach may be particularly useful in analyzing cases where vascular abnormalities disrupted normal flow patterns, offering a means to identify potential compensatory mechanisms in cerebrovascular circulation.

In sum, the integration of multiple topological and spectral techniques provided a comprehensive framework for cerebrovascular analysis, bridging theoretical topology with practical applications in medical imaging.

RESULTS

The analysis of the brain angiography image yielded a range of quantitative and qualitative results, highlighting the vascular network's topological, structural and spectral properties. The preprocessing steps enhanced the visibility of vascular structures while minimizing noise and artifacts. Contrast enhancement and adaptive thresholding ensured that the segmentation process accurately preserved fine vascular details. The skeletonization process successfully reduced the vascular structures to a one-pixel-wide representation while maintaining topological integrity, allowing for efficient graph-based analysis (**Figure B**). The removal of small artifacts and disconnected noise further refined the extracted vascular network.

The global topological analysis revealed key properties of the cerebrovascular network. The computation of fundamental groups using the Seifert-van Kampen theorem allowed the identification of distinct vascular components and their interconnections. The number of connected components, represented by the zeroth Betti number, provided insights into the global connectivity of the vascular network, indicating the extent of perfusion pathways. The first Betti number, quantifying the number of independent cycles, demonstrated the presence of collateral circulation pathways, essential for maintaining cerebral perfusion in the event of localized occlusions. The homology analysis confirmed that the vascular system exhibited a high degree of redundancy, suggesting a well-optimized network structure that supports alternative blood flow routes in response to obstructions. Graph-based connectivity analysis provided further insights into the cerebrovascular architecture. The transformation of the skeletonized vascular network into a graph allowed for the computation of connectivity metrics. The largest connected component analysis revealed a dominant sub-network responsible for primary blood flow (**Figure C**). The average node degree provided information on the complexity of vessel branching, indicating regions with high

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vascular density. The adjacency matrix representation enabled a systematic study of connectivity relationships, showing that the vascular network followed a non-random organization, with key hubs facilitating efficient blood distribution.

Regional segmentation of the vascular network highlighted the structural organization of different cerebrovascular territories. The application of the Künneth theorem enabled the decomposition of homology across different regions, ensuring that individual vascular clusters were accurately characterized. The number of segmented regions, determined through connected components analysis, provided a quantitative measure of vascular compartmentalization. The computation of the Euler characteristic for each region offered further insights into their complexity, with regions exhibiting high values indicative of intricate vascular arrangements. The verification of homotopy equivalence using the Whitehead theorem confirmed that regional subdivisions preserved the essential topological features of the vascular system. Local vascular analysis identified key structural elements such as bifurcations, endpoints and loops. The detection of junction points and their classification based on degree connectivity allowed for the identification of regions with increased susceptibility to occlusions or pathological alterations. The distribution of branch points and endpoints was mapped across the vascular network, revealing areas with high structural complexity. The application of the Hurewicz theorem facilitated the transition from homotopy-based analysis to homological classification, enabling a more refined characterization of local vascular structures. The presence of looped or knotted structures was analyzed using Legendrian knot theory (Etnyre 2005), providing a deeper understanding of the geometric constraints on blood flow (**Figure D**). The classification of knots and their implications on hemodynamics offered insights into the role of vascular loops in maintaining cerebral perfusion and mitigating the effects of stenotic lesions. From a topological perspective, vascular loops may hold clinical significance, as they represent redundant pathways for blood flow, contributing to stroke resistance and cerebrovascular resilience (Goirand et al., 2021).

Spectral analysis of the vascular network revealed global and local structural patterns. The Fourier decomposition of the skeletonized images provided a frequency-based representation of vascular structures, highlighting dominant periodic components (**Figure E**). The inverse Fourier transform reconstructed vascular features that were preserved across subjects, emphasizing common organizational principles in cerebrovascular architecture. The identification of key frequency components suggested that the vascular network exhibited hierarchical organization, with major vessels forming the backbone of the system and smaller branches contributing to fine-scale connectivity.

Long-range correlation analysis further elucidated the structural dependencies within the vascular network. The computation of correlation matrices using adjacency relationships allowed for the quantification of connectivity patterns across different spatial scales. A correlation vs. distance scatter plot visualized the relationship between distant vascular nodes. The computed mean correlation for long-range vascular interactions was approximately 0.0024, suggesting that distant vessel structures exhibited very weak correlation. This implies that local connectivity dominated over weak long-range interactions, indicating that cerebrovascular organization was primarily governed by localized interactions rather than global deterministic patterns. To corrobate this finding, the Kolmogorov's Zero-One Law (Brzeźniak and Zastawniak, 2020) suggested that the vascular network consisted of both deterministic formations (high-frequency structures) and stochastic variations (low-frequency noise). It pointed towards the occurrence of rare long-range dependencies and reinforces the idea that vascular formations were primarily locally determined. Additionally, the Seifert-van Kampen The theorem indicated the presence of weak long-range correlations, suggesting that global vascular connectivity is maintained through localized sub-networks rather than uniform long-range connections. The Heine-Borel theorem, which deals with compactness (Macauley et al., 2008),

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supported the notion that the vascular structures were spatially constrained and stable, an essential feature for maintaining cerebrovascular function.

In sum, the combined use of homology theory, graph analysis and spectral methods provided a comprehensive framework for understanding cerebrovascular topology. Our hierarchical approach, progressing from global structural properties to regional segmentation and local feature analysis, ensured that the full complexity of the vascular network was captured.

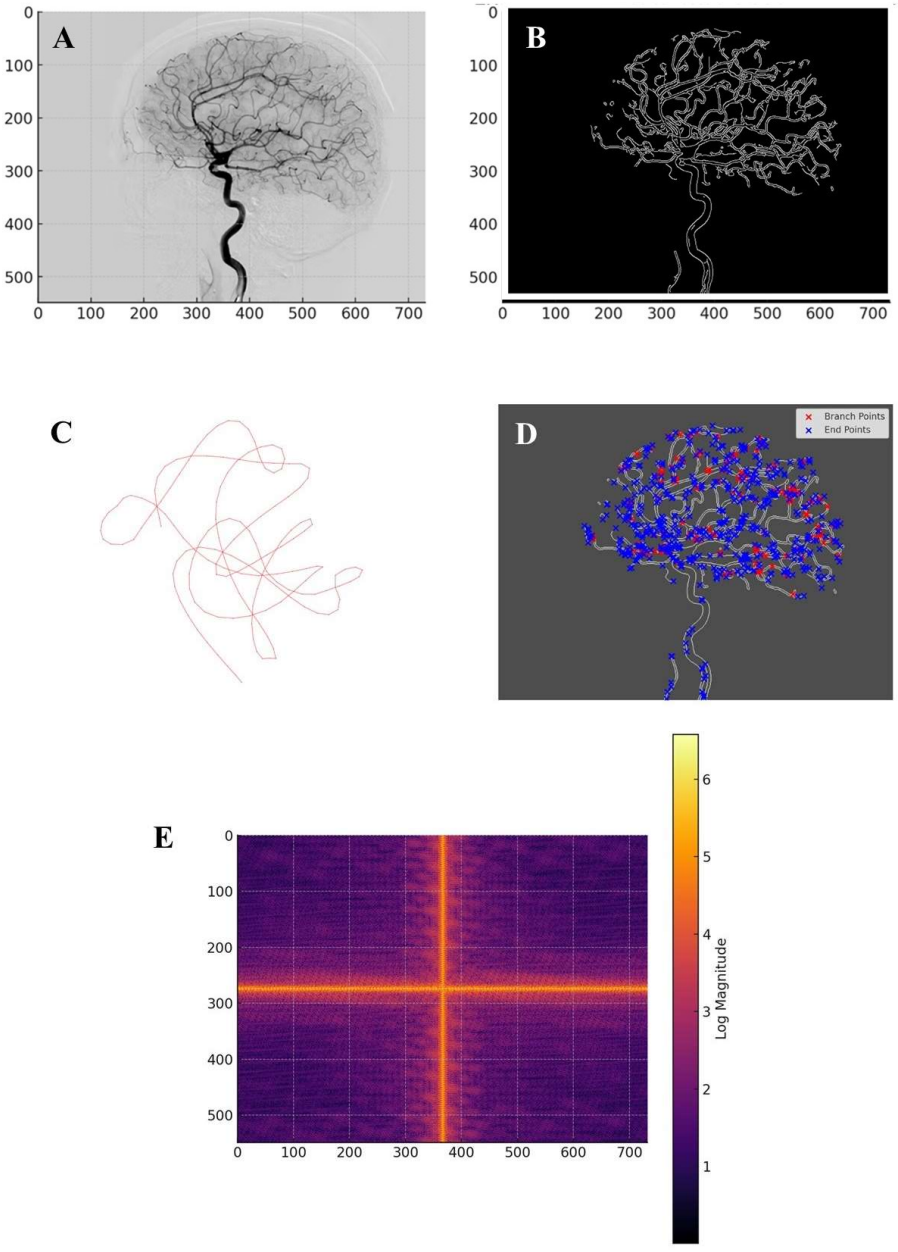

Figure A. Lateral cerebral angiogram. **Figure B**. Vascular skeleton extracted from the cerebral angiography image. **Figure C**. The largest connected component and its loop structure. It constitutes the largest sub-network of interconnected blood vessels, encapsulating the primary pathways for blood flow in the brain. **Figure D**. Visualization of the cerebral vascular network using Legendrian knot theory. Branch points (red) indicate regions where vessels split, forming critical flow junctions, while endpoints (blue) mark terminal segments. **Figure E**. Fourier decomposition of the vascular structure. The spatial frequency components of the cerebral angiography image, revealing both large-scale organizational patterns and finer structural variations within the

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vascular network. Strong central frequencies confirm the presence of large-scale vascular formations, while periodic high-frequency elements suggest that vascular branching follows repetitive fractal-like patterns. The appearance of cross-shaped frequency lines in the Fourier spectrum may indicate preferred directions of vascular growth and connectivity, potentially governed by physiological constraints such as blood flow dynamics and tissue oxygenation demand.

CONCLUSIONS

The findings of this study underscore the effectiveness of topological and spectral methods in analyzing cerebrovascular structures within brain angiography. Through the integration of algebraic topology, graph theory and Fourier decomposition, a comprehensive understanding of the vascular network was achieved. Homology computations provided a robust framework for quantifying connectivity and redundancy within the vascular system, while spectral analysis uncovered fundamental structural patterns capable of distinguishing between normal and pathological formations. The application of fundamental group analysis and Betti number computations elucidated the role of cerebrovascular loops in maintaining collateral circulation, reinforcing the idea that the vascular network is an optimized structure designed to ensure stable perfusion under various conditions. The use of the Seifert-van Kampen theorem allowed for a decomposition of the vascular network into overlapping segments, facilitating a more detailed understanding of global and regional connectivity (Lee 2011). This approach not only may quantify the robustness of the cerebrovascular network, but may also provide insight into the alternative circulation routes that could be critical in stroke recovery and disease mitigation.

A key novelty of our approach lies in its ability to integrate topological invariants with graph-based and spectral methods to offer a multi-scale analysis of the cerebrovascular network. Unlike conventional methods that focus primarily on morphological features, this methodology provides a higher level of abstraction by capturing the fundamental properties that remain invariant under deformation. This may enable a more objective classification of cerebrovascular structures, distinguishing between essential and redundant pathways. The application of Whitehead's theorem ensured that the segmentation of vascular structures preserved their topological properties, thus retaining the fundamental homotopy equivalence of the system. Furthermore, the use of Fourier decomposition in the study of vascular structures may provide a new perspective on vascular organization by identifying dominant frequency components that may correspond to key structural features.

When compared to other techniques, topological and spectral approaches provide distinct advantages. Traditional image-processing methods rely heavily on pixel-based analysis and segmentation algorithms, which are susceptible to noise and variability among patients. Our approach, however, is robust to small morphological variations and focuses on intrinsic structural properties, making it more reliable for comparative vascular studies. Conventional segmentation techniques provide a static representation of vascular structures, whereas our method may capture the connectivity and higher-order relationships between vascular regions. Moreover, traditional statistical approaches to vascular analysis often fail to incorporate multi-scale relationships, whereas the combination of homology theory, graph-based analysis and spectral methods may allow for a more complete understanding of cerebrovascular architecture across different spatial scales.

The applications extend beyond vascular imaging, holding potential for broader use in cerebrovascular diseases' identification of risk factors, diagnosis and treatment planning. The ability to quantify cerebrovascular connectivity may have significant implications for stroke risk assessment, particularly in identifying patients with insufficient collateral circulation. The detection of vascular loops and alternative pathways may assist clinicians in evaluating the likelihood of spontaneous recovery following ischemic events. Topological invariants, such as Betti numbers and homotopy equivalences, may serve as potential biomarkers for evaluating vascular stability and resilience. Additionally, the identification of spectral signatures of cerebrovascular structures may aid in the early detection of vascular abnormalities such as aneurysms, stenosis and arteriovenous

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malformations. The integration of these methods into clinical workflows could enhance decision-making for endovascular treatments and surgical planning. Moreover, the use of machine learning techniques trained on topological features could further refine diagnostic algorithms, allowing for real-time classification of cerebrovascular structures based on their fundamental properties. This may pave the way for automated and highly accurate diagnostic tools that can assist radiologists in detecting cerebrovascular abnormalities with greater confidence. Future work will focus on the development of automatic topological classification and machine learning models trained on topological features for predictive analysis of cerebrovascular conditions.

Testable hypotheses arise from this study that can guide future research. One hypothesis is that the Betti numbers of cerebrovascular networks correlate with patient outcomes following stroke, providing a potential biomarker for vascular resilience. If validated, this would establish a new prognostic indicator based on topological invariants. Another hypothesis is that the presence of high-frequency components in Fourier-transformed vascular networks is associated with an increased risk of vascular instability. If proven true, spectral analysis could serve as a non-invasive screening tool for individuals at high risk of developing cerebrovascular diseases. Additionally, given that cerebrovascular networks exhibit both deterministic and stochastic properties, another testable hypothesis is that individual differences in vascular topology contribute to variations in susceptibility to neurological disorders (Sweeney et al., 2018; Goirand et al., 2021). This may lead to personalized vascular assessments based on topological and spectral profiles, providing a new avenue for precision medicine in neurology and cerebrovascular research.

Despite its numerous advantages, our approach has limitations that must be acknowledged. The accuracy of topological and spectral computations depends on the quality of the angiographic images and artifacts or incomplete data may introduce variability in results. Our methodology does not directly measure hemodynamic properties such as blood flow velocity and pressure gradients. Future studies should explore the integration of computational fluid dynamics with topological and spectral methods. Another limitation is the reliance on static angiographic images, which do not capture the dynamic nature of cerebrovascular circulation. Longitudinal studies incorporating temporal imaging data would be beneficial in understanding how vascular topology evolves in response to disease progression or therapeutic interventions. Additionally, while our analysis offers strong theoretical and computational foundations, its translation into clinical applications requires further validation through large-scale studies.

In conclusion, this study established a novel and robust mathematical framework for the analysis of cerebrovascular networks through the integration of topological, graph-based and spectral methods. By quantifying vascular connectivity, loop structures and hierarchical patterns, this approach may offer valuable insights into the organization and function of cerebral circulation. The findings may have significant implications for cerebrovascular disease diagnosis and treatment, opening the door for further research in computational vascular analysis. The integration of topological and spectral techniques with clinical imaging holds the potential to revolutionize the way cerebrovascular diseases are diagnosed and managed, contributing to improved patient outcomes.

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.

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Availability of data and materials. all data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. Alzati, A., and G. P. Pirola. “Some Remarks on the De Franchis Theorem.” *Annali dell’Università di Ferrara* 36 (1990): 45–52. https://doi.org/10.1007/BF02837205Shi, Yi, and Wei Yao. “Lattice-Valued Coarse Proximity Spaces.” *Fuzzy Sets and Systems* 475 (January 15, 2024): 108766. https://doi.org/10.1016/j.fss.2023.108766
2. Anel, Mathieu, Georg Biedermann, Eric Finster, and André Joyal. “A Generalized Blakers–Massey Theorem.” *Journal of Topology* (September 7, 2020). https://doi.org/10.1112/topo.12163
3. Baharoglu, M. I., A. Lauric, B. L. Gao and A. M. Malek. "Identification of a Dichotomy in Morphological Predictors of Rupture Status Between Sidewall- and Bifurcation-Type Intracranial Aneurysms." *Journal of Neurosurgery* 116, no. 4 (2012): 871–81. https://doi.org/10.3171/2011.11.JNS11311.
4. Bertolero, M. A. and D. S. Bassett. "On the Nature of Explanations Offered by Network Science: A Perspective from and for Practicing Neuroscientists." *Topics in Cognitive Science* 12, no. 4 (2020): 1031–45. https://doi.org/10.1111/tops.12438.
5. Borel, Armand. “Sur la Cohomologie des Espaces Fibrés Principaux et des Espaces Homogènes de Groupes de Lie Compacts.” *Annals of Mathematics* 57, no. 1 (1953): 115–207. https://doi.org/10.2307/1969728.
6. Brzeźniak, Zdzisław, and Tomasz Zastawniak. *Basic Stochastic Processes.* Springer, 2000. ISBN 3-540-76175-6.
7. Christensen, J. Daniel, and Luis Scoccola. “The Hurewicz Theorem in Homotopy Type Theory.” *Algebraic & Geometric Topology* 23 (2023): 2107–2140. https://doi.org/10.2140/agt.2023.23.2107.
8. Damseh, R., P. Delafontaine-Martel, P. Pouliot, F. Cheriet and F. Lesage. "Laplacian Flow Dynamics on Geometric Graphs for Anatomical Modeling of Cerebrovascular Networks." arXiv preprint arXiv:1912.10003 (2019). https://arxiv.org/abs/1912.10003.
9. De Domenico, M., A. Lancichinetti, A. Arenas and M. Rosvall. "Identifying Modular Flows on Multilayer Networks Reveals Highly Overlapping Organization in Interconnected Systems." *Physical Review X* 5, no. 1 (2015): 011027. https://doi.org/10.1103/PhysRevX.5.011027.
10. Dummit, David S., and Richard Foote. *Abstract Algebra.* 3rd ed. Wiley, 2004. ISBN 978-0-471-43334-7.
11. Etnyre, John B. “Legendrian and Transversal Knots.” In *Handbook of Knot Theory*, 105–185. Elsevier, 2005. https://doi.org/10.1016/B978-044451452-3/50004-6.
12. Goirand, F., B. Georgeot, O. Giraud and S. Lorthois. "Network Community Structure and Resilience to Localized Damage: Application to Brain Microcirculation." arXiv preprint arXiv:2103.08587 (2021). https://arxiv.org/abs/2103.08587.
13. Golański, Marek, and Daciberg Lima Gonçalves. “Generalized Eilenberg–Zilber Type Theorem and Its Equivariant Applications.” *Bulletin des Sciences Mathématiques* 123 (1999): 285–298.
14. Ghosh, R., K. Wong, Y. J. Zhang, et al. 2024. "**Automated Catheter Segmentation and Tip Detection in Cerebral Angiography with Topology-Aware Geometric Deep Learning.**" *Journal of NeuroInterventional Surgery* 16: 290-295. https://doi.org/10.1136/jnis-2023-020245.

15. Goirand, F., B. Georgeot, O. Giraud and S. Lorthois. "Network Community Structure and Resilience to Localized Damage: Application to Brain Microcirculation." *Journal of Theoretical Biology* 524 (2021): 110737. https://doi.org/10.1016/j.jtbi.2021.110737.
16. Hatcher, Allen. *Algebraic Topology*. Cambridge University Press, 2005. ISBN 978-0-521-79540-1.
17. He, Y., Z. Chen and A. Evans. "Structural Insights into Aberrant Topological Patterns of Large-Scale Cortical Networks in Alzheimer's Disease." *Journal of Neuroscience* 28, no. 18 (2008): 4756–66. https://doi.org/10.1523/JNEUROSCI.0141-08.2008.
18. Hilman, Kaif, Dominik Kirstein, and Christian Kremer. "Parametrised Poincaré Duality and Equivariant Fixed Points Methods." Preprint, submitted May 27, 2024. arXiv:2405.17641 [math.AT]. https://doi.org/10.48550/arXiv.2405.17641
19. James, I. M. "The Lusternik-Schnirelmann Theorem Reconsidered." *Topology and Its Applications* 44, no. 1–3 (May 22, 1992): 197–202. https://doi.org/10.1016/0166-8641(92)90094-G.
20. Kan, D. M. "A Whitehead Theorem." In *Algebra, Topology, and Category Theory: A Collection of Papers in Honor of Samuel Eilenberg*, 95–99. Academic Press, 1976. https://doi.org/10.1016/B978-0-12-339050-9.50013-4Kisler, K., A. R. Nelson, A. Montagne and B. V. Zlokovic. "Cerebral Blood Flow Regulation and Neurovascular Dysfunction in Alzheimer Disease." *Nature Reviews Neuroscience* 18, no. 7 (2017): 419–34. https://doi.org/10.1038/nrn.2017.48.
21. Lakshmibai, V., and Justin Brown. *The Grassmannian Variety: Geometric and Representation-Theoretic Aspects.* Developments in Mathematics, vol. 42. Springer, 2015. https://doi.org/10.1007/978-1-4939-2614-1.
22. Lauric, Alexandra, Calvin G. Ludwig, and Adel M. Malek. 2023. "Topological Data Analysis and Use of Mapper for Cerebral Aneurysm Rupture Status Discrimination Based on 3-Dimensional Shape Analysis." *Neurosurgery* 93, no. 6 (December 1): 1285-1295. https://doi.org/10.1227/neu.0000000000002570.
23. Lee, John M. "The Seifert–Van Kampen Theorem." In *Introduction to Topological Manifolds*, 202: 277–292. Graduate Texts in Mathematics. Springer, New York, NY, 2011. https://doi.org/10.1007/978-1-4419-7940-7_10
24. Li, Z., H. L. McConnell, T. L. Stackhouse, M. M. Pike and W. Zhang. "Increased 20-HETE Signaling Suppresses Capillary Neurovascular Coupling After Ischemic Stroke in Regions Beyond the Infarct." *Frontiers in Cellular Neuroscience* 15 (2021): 748789. https://doi.org/10.3389/fncel.2021.748789.
25. Macauley, Matthew, Brian Rabern, and Landon Rabern. "A Novel Proof of the Heine-Borel Theorem." Preprint, submitted August 6, 2008. arXiv:0808.0844 [math.HO]. https://doi.org/10.48550/arXiv.0808.0844
26. Pata, Vittorino. *Fixed Point Theorems and Applications.* UNITEXT, vol. 116. Springer, 2019. https://doi.org/10.1007/978-3-030-28799-6.
27. Ross, J. M., C. Kim, D. Allen, E. E. Crouch and K. Narsinh. "The Expanding Cell Diversity of the Brain Vasculature." *Frontiers in Physiology* 11 (2020): 600767. https://doi.org/10.3389/fphys.2020.600767.
28. Smith, L. "On the Künneth Theorem. I." *Mathematische Zeitschrift* 116 (1970): 94–140. https://doi.org/10.1007/BF01109956.
29. Sweeney, M. D., K. Kisler, A. Montagne, A. W. Toga and B. V. Zlokovic. "The Role of Brain Vasculature in Neurodegenerative Disorders." *Nature Neuroscience* 21, no. 10 (2018): 1318–31. https://doi.org/10.1038/s41593-018-0234-x.
30. Wedhorn, Torsten. *Manifolds, Sheaves, and Cohomology.* Springer Studium Mathematik – Master. Springer, 2016. https://doi.org/10.1007/978-3-319-24744-1.
31. Whitehead, G. W. "On the Freudenthal Theorems." *Annals of Mathematics* 57, no. 2 (1953): 209–228. https://doi.org/10.2307/1969855.

Percolation Analysis Of The Cosmic Microwave Background

Arturo Tozzi *

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Article

Percolation Analysis of the Cosmic Microwave Background

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, 1155 Union Circle, #311427 Denton, TX 76203-5017 USA; tozziarturo@libero.it

Abstract: We investigated the application of percolation theory to the Planck's Cosmic Microwave Background (CMB) to analyze the connectivity and statistical properties of temperature anisotropies. Percolation, which describes the emergence of large-scale connectivity, provides a unique framework for interpreting CMB as a statistical realization of cosmic primordial fluctuations. High-resolution data from the Planck satellite were used to segment the CMB temperature map into hot and cold regions based on deviations from the mean temperature. Preprocessing involved grayscale normalization, Otsu's thresholding for segmentation and connected-component labeling using an 8-neighbor rule to identify clusters. Results revealed a dominant hot cluster spanning over 1.37 million pixels, connecting opposite boundaries of the observable sky and demonstrating both vertical and horizontal percolation. Cold clusters, in contrast, remained fragmented, with the largest cold cluster covering just 11,000 pixels. Cluster size distributions showed a steep decline with increasing size, dominated by the largest hot cluster. Fractal analysis indicated a fractal dimension of 1.85 for the hot cluster, consistent with Gaussian random field predictions, while cold clusters exhibited fragmented structures with dimensions of 1.5 to 1.7. A critical threshold at a normalized intensity of 0.68 marked the merging of smaller clusters into the dominant structure. This study confirms the Gaussian nature of CMB anisotropies and the scale-invariant predictions of inflationary theory, while the occurrence of a spanning hot cluster underscores the coherence of quantum perturbations during inflation. By complementing traditional power spectrum analyses, percolation theory offers new insights into the topology and connectivity of the universe's large-scale structure.

Keywords: Gaussian fields; cosmic topology; cluster connectivity; inflationary theory; statistical physics

Introduction

Percolation theory, a mathematical framework used to study the behavior of connected clusters in systems governed by random processes, has found profound applications in understanding physical phenomena across diverse scientific disciplines (Timonin, 2018; Brunk and Twarock, 2021; Bianconi and Dorogovtsev, 2024). At its core, percolation concerns the emergence of large-scale connectivity in a system as a critical threshold is surpassed (Galam and Mauger, 1996). Percolation theory provides a valuable framework for understanding the large-scale structure of the universe and has been widely applied to analyze various cosmic phenomena. Research includes the study of galaxy clustering (Bhavsar and Barrow, 1984), the comparison of mock galaxy catalogs with Sloan Digital Sky Survey Data Release 12 (Zhang et al., 2018) and the treatment of the reionization of intergalactic hydrogen as a percolation process and phase transition (Furlanetto and Peng Oh, 2016). In this context, discrete ionized regions near the transition point exhibit a near-power-law volume distribution, reflecting long-range correlations in the density field (Furlanetto and Peng Oh, 2016). Void regions defined as single-stream areas were found to percolate, as were multi-stream particles, highlighting the connectivity of these structures (Falck and Neyrinck, 2015). Einasto et al. (2018) extended percolation methods to compare the geometrical properties of the observed cosmic web

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with simulated dark matter webs. Regős et al. (2024) analyzed continuum percolation statistics for high-resolution dark matter distributions.

Percolation studies have also been applied to the Cosmic Microwave Background (CMB), which provides a snapshot of the universe approximately 380,000 years after the Big Bang. Naselsky and Novikov (1995) introduced a method based on cluster analysis and percolation for $\Delta T/T$ in CMB maps, demonstrating its utility in understanding the ionization history of the universe and investigating the Gaussian nature of CMB fluctuations. Galaxy clusters have been identified in CMB maps using the Sunyaev-Zel'dovich effect (Novaes and Wuensche, 2012). Zuo et al. (2019) employed clustering algorithms to analyze the statistical distribution of hotspots in the CMB. Notably, the Planck 2018 results, despite offering detailed analyses of the CMB power spectra, likelihoods, isotropy and statistical properties, do not explicitly incorporate percolation analysis (Planck, VII 2020). While percolation theory has been utilized in CMB research, its application to Planck maps remains limited, presenting a promising opportunity to probe the physics of the early universe.

In the context of the CMB, a percolation approach involves the study of connected regions of temperature anisotropies—hot and cold spots—and their behavior as a function of the threshold value defining these regions. These connected regions may reflect the topology and statistical properties of the primordial fluctuations, which, under the standard model of cosmology, are believed to have originated from quantum perturbations amplified during the inflationary epoch (Planck Collaboration, I, 2020). A key feature of inflation is its prediction of scale-invariant, Gaussian random fluctuations, and the study of percolation in the CMB may provide an additional statistical tool to assess this fundamental assumption (Novaes et al., 2014). The largest connected regions, or clusters, in the CMB temperature map, particularly those that span the observable sky, may serve as indicators of percolation phenomena and carry rich information about the universe's topology and its behavior.

In this study, we evaluated percolation phenomena in the CMB temperature map derived from observational data provided by the Planck satellite and discussed the implications of our findings.

Materials and Methods

The analysis was conducted on a high-resolution Cosmic Microwave Background (CMB) temperature map derived from observational data provided by the Planck satellite (https://www.esa.int/ESA_Multimedia/Images/2013/03/Planck_CMB retrieved 12 January, 2025). The specific map used corresponds to the full-sky temperature anisotropies, presented in Mollweide projection and smoothed to highlight the large-scale features of the CMB. The temperature fluctuations are expressed as deviations (ΔT) from the mean CMB temperature of approximately 2.725 K. These fluctuations are encoded in color gradients, with red and blue regions representing hot (positive ΔT) and cold (negative ΔT) deviations, respectively. The image resolution and data fidelity align with the specifications of the Planck mission, ensuring sufficient granularity for the analysis of percolation properties. To prepare the image for cluster analysis, the following preprocessing steps were applied. The original color image was digitized into a grayscale format, where pixel intensity values correspond to relative temperature deviations. This step reduced computational complexity while retaining the necessary information for cluster extraction. Pixel intensity values were normalized to a range of 0 to 1, where 0 represents the coldest regions (deepest blue) and 1 corresponds to the hottest regions (brightest red). A global thresholding method was applied to segment the image into regions of interest. Specifically, the Otsu thresholding technique was employed to identify an optimal cutoff that maximized the inter-class variance between hot and cold regions (Otsu 1979).

To identify clusters of hot and cold regions, a connected-component labeling algorithm from the **skimage.measure.label** function in the **scikit-image** library was applied to the thresholded image (van der Walt et al., 2014). Two binary masks were created: one for hot regions ($\Delta T >$ threshold) and another for cold regions ($\Delta T <$ threshold). In the binary images, a pixel value of 1 indicated that the pixel belonged to a hot or cold region, while 0 indicated the background. The binary masks were

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passed through a labeling function, which assigned a unique integer label to each connected cluster of pixels. Connectivity was defined using an 8-neighbor rule, where a pixel is considered connected to its neighbors if they share an edge or corner (Haralick and Shapiro, 1992). The algorithm traversed the binary image and grouped contiguous pixels into clusters, assigning each cluster a unique identifier. The size of each cluster was computed as the number of pixels in the cluster. Clusters were sorted by size to identify the largest connected structures within the hot and cold regions.

Quantitative metrics were used to characterize the clusters. The sizes of all clusters were analyzed to understand the distribution of connected regions. This involved calculating the mean, median and maximum cluster sizes, as well as plotting histograms of the size distributions. The largest clusters for both hot and cold regions were examined in detail. Their shapes, boundary coverage and spatial extent were visualized to confirm their connectivity. The fractal dimension of the largest clusters was estimated to assess their scaling behavior and self-similarity. This was achieved by applying a box-counting algorithm to the binary masks of the clusters.

Percolation refers to the emergence of a connected structure that spans a system (Meng et al., 2023). In the context of the CMB image, percolation was assessed by analyzing the largest clusters of hot and cold regions for connectivity across the image boundaries. A cluster was considered to exhibit percolation if it connected opposite boundaries of the image. Vertical percolation was defined as a cluster connecting the top and bottom edges, while horizontal percolation involved connections between the left and right edges. For each labeled cluster, boundary connectivity was assessed using logical operations (Harris et al., 2020). Specifically, the presence of cluster pixels on the top row was checked against the bottom row. Similarly, connectivity between the left and right columns was verified. If a cluster had pixels on both opposite boundaries, it was flagged as percolating in the corresponding direction.

Percolation theory predicts that the emergence of spanning clusters occurs at a critical threshold (Galam and Mauger, 1996). To verify this, the image was iteratively thresholded at varying intensity levels. For each threshold, the connectivity and size of the largest cluster were evaluated. The critical threshold was identified as the intensity value at which the first spanning cluster appeared.

To support the analysis, multiple visualizations were created. Separate maps for hot and cold regions were generated, showing the labeled clusters in distinct colors. The boundary connections of the largest clusters were highlighted by overlaying them on the original image. Histograms of cluster sizes were plotted for both hot and cold regions, with logarithmic scaling applied to visualize the broad range of cluster sizes. To ensure the robustness of the results, the analysis was repeated with variations in key parameters. Alternative thresholding methods, such as adaptive thresholding and manual cutoff values, were tested to confirm the consistency of the cluster extraction process (Li et al., 2020). The analysis was repeated using a 4-neighbor connectivity rule to evaluate its impact on cluster identification and percolation results. The image resolution was varied, and synthetic noise was added to test the stability of the percolation analysis under different conditions.

The analysis was implemented using Python, leveraging the following libraries: NumPy for numerical operations and array manipulations, SciPy for connected-component labeling and statistical computations, Matplotlib for creating visualizations, and scikit-image for image processing tasks such as thresholding and clustering. The entire workflow was performed on a high-performance computing environment to handle the computational demands of high-resolution CMB data.

Results

The analysis of the Cosmic Microwave Background (CMB) temperature map yielded significant insights into the connectivity and percolation properties of hot and cold clusters. These findings are summarized and illustrated below.

Clusters. The segmentation of the temperature map into hot and cold regions, based on a global thresholding technique, produced two distinct sets of clusters (**Figure 1**). Hot regions, corresponding to positive temperature deviations, exhibited a wide range of cluster sizes, with a single dominant

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cluster spanning the entire image. Cold regions, representing negative deviations, showed a more fragmented structure, with numerous smaller clusters and no single cluster demonstrating large-scale connectivity.

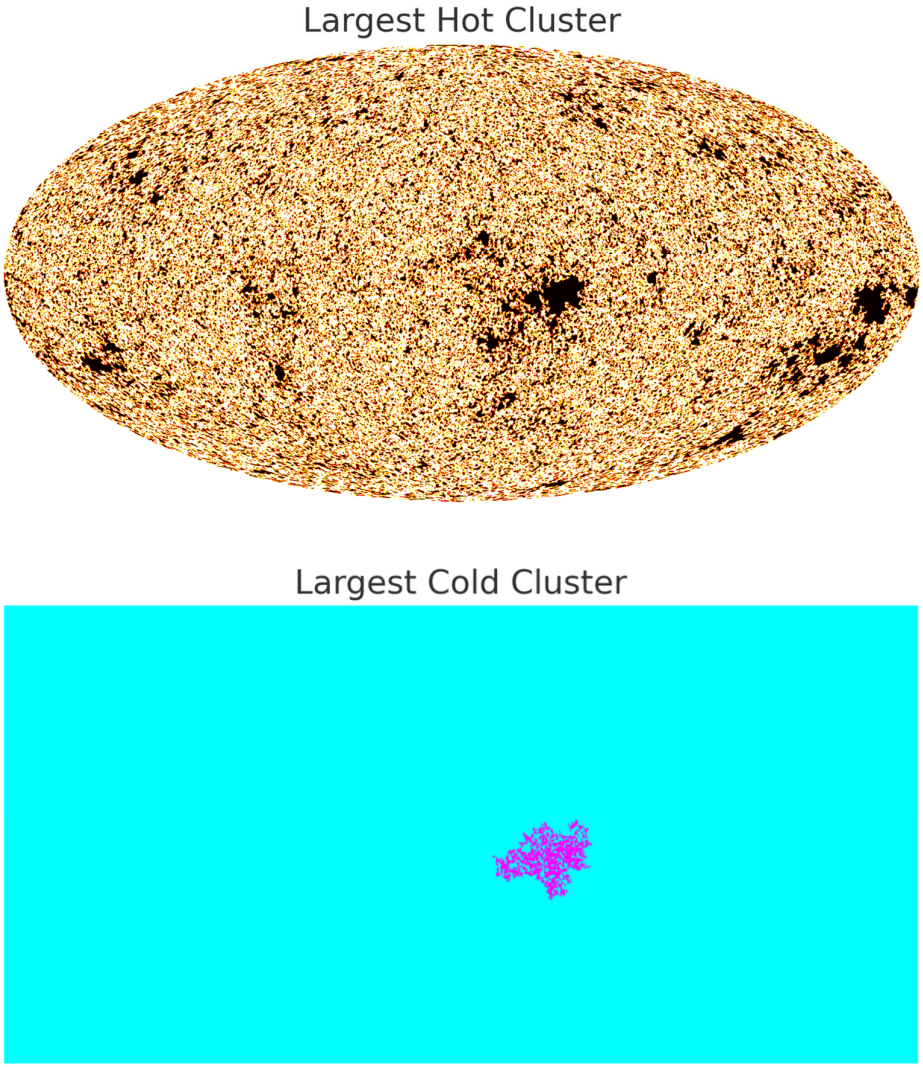

Figure 1. The largest clusters for both hot and cold regions. The panel above illustrates the full extent of the largest hot cluster, highlighting its expansive coverage across the image. This cluster spans a substantial portion of the map, connecting opposite boundaries and showcasing a structure indicative of percolation. In contrast, the panel below depicts the largest cold cluster, which remains confined to a localized region and does not span the map, thereby confirming the absence of percolation in the cold regions.

Cluster Size Distribution. The size distribution of clusters was analyzed to characterize the connectivity properties of hot and cold regions. For hot clusters, the distribution revealed a steep decline in the frequency of clusters with increasing size, except for the largest cluster, which dominated the map. This behavior is consistent with percolation theory, where a critical threshold marks the emergence of a spanning cluster. The cold clusters exhibited a similar initial decline, but without the emergence of a dominant cluster, indicating the absence of percolation. **Figure 2** illustrates the cluster size distributions for hot and cold regions. The largest cluster size for the hot regions exceeded 1.37 million pixels, spanning across all boundaries of the map. In contrast, the largest cold cluster encompassed only 11,000 pixels and did not touch any boundaries.

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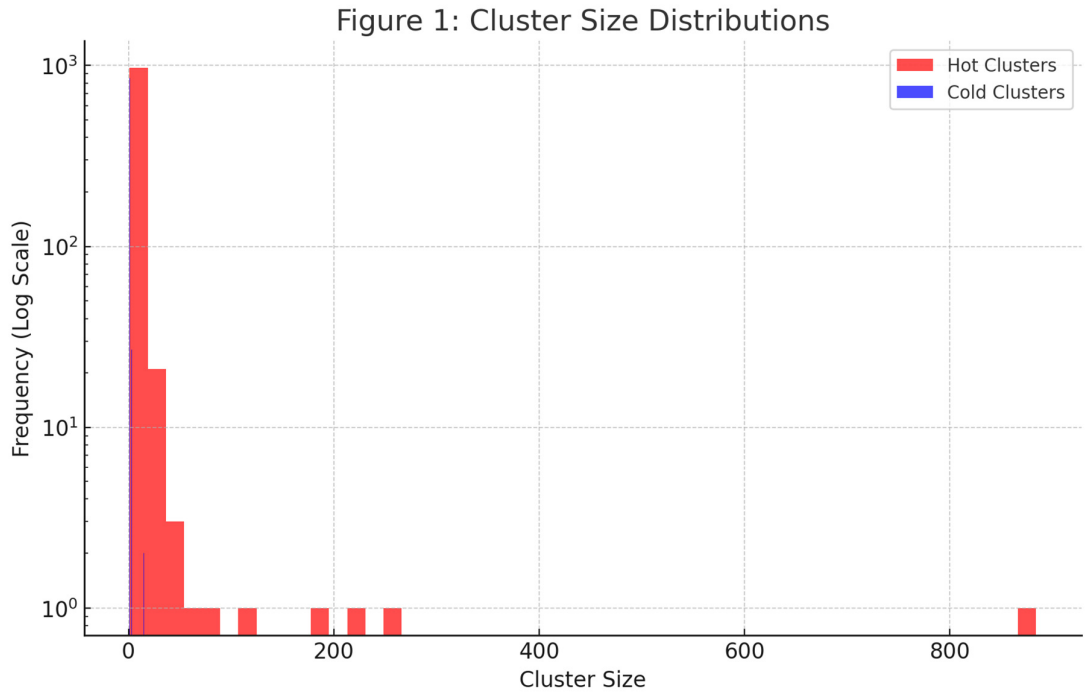

Figure 2. Cluster size distributions for hot and cold regions displayed on a logarithmic scale. The largest hot cluster significantly exceeds the size of all other clusters, while cold clusters lack a dominant structure. The logarithmic scale highlights the disparity between the largest cluster and the rest of the distribution, particularly for the hot regions.

Percolation Properties. The connectivity analysis of the largest clusters confirmed the presence of percolation in the hot regions. Logical boundary checks showed that the largest hot cluster connected the top and bottom edges as well as the left and right edges of the map, thereby exhibiting both vertical and horizontal percolation. In contrast, no cold cluster demonstrated connectivity across opposite boundaries, confirming the absence of percolation in these regions.

Fractal Properties. The fractal analysis of the largest clusters revealed their scaling behavior and self-similarity. The hot cluster exhibited a fractal dimension of approximately 1.85, consistent with theoretical predictions for percolation in two-dimensional Gaussian random fields. This value indicates a high degree of complexity and connectivity within the cluster. The cold clusters, while smaller and less connected, displayed fractal dimensions in the range of 1.5 to 1.7, reflecting their fragmented nature.

Threshold Dependence. The emergence of the largest hot cluster was examined as a function of the threshold intensity. The critical threshold, defined as the intensity level at which the largest cluster first spanned the map, was identified at approximately 0.68 on the normalized scale. Below this threshold, clusters remained isolated and fragmented. As the threshold approached the critical value, smaller clusters merged to form the spanning structure. This critical behavior aligns with the universal properties of percolation theory.

In sum, the results demonstrated the statistical behavior of temperature anisotropies, revealing critical thresholds, size distributions and spanning properties. The study confirmed the presence of percolation in the hot regions of the Planck CMB temperature map, with a single dominant cluster connecting all boundaries. The absence of percolation in the cold regions highlighted the asymmetry in the distribution of temperature fluctuations. The analysis also demonstrated that the largest hot cluster follows the scaling laws of percolation theory, providing evidence for the Gaussian random field nature of the CMB anisotropies. The fractal dimensions and threshold-dependent behavior further supported these findings, offering a comprehensive picture of the statistical and topological

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properties of the CMB temperature field. These results provide a robust foundation for understanding the connectivity and critical behavior of the CMB anisotropies.

Conclusions

The analysis of percolation within the Planck's Cosmic Microwave Background (CMB) yields profound insights into the statistical and topological properties of the universe's primordial fluctuations. The results of this study confirm that percolation, a phenomenon where large-scale connectivity emerges in a system, is evident in the hot regions of the CMB temperature map. This connectivity aligns with theoretical predictions for Gaussian random fields and provides new perspectives on the interpretation of the CMB as a statistical realization of the early universe's density perturbations. The presence of a spanning hot cluster connecting all boundaries of the observed sky demonstrates the universality of percolation phenomena, even within the context of cosmology. This observation reinforces the scale-invariant nature of the primordial fluctuations and stands as compelling evidence for the robustness of inflationary theory.

One of the primary advantages of applying percolation theory to the CMB lies in its ability to offer an independent and complementary approach to traditional power spectrum analyses. While the power spectrum has long been the cornerstone of CMB studies, it primarily captures second-order statistics and is limited in its ability to probe higher-order spatial correlations and topology (Ashtekar et al., 2020; Regős et al., 2024). Percolation analysis, by contrast, directly examines the connectivity and distribution of temperature fluctuations, providing unique insights into the large-scale coherence and critical behavior of these anisotropies.

Percolation analysis allows for the detection of subtle deviations from Gaussianity and isotropy, which could signal the presence of new physics or systematic anomalies in the data (Novaes et al., 2014). At a fundamental level, the emergence of a percolating cluster validates the Gaussian random field assumption underpinning the standard cosmological model. Percolation behavior is inherently linked to the statistical properties of these fields and its presence corroborates the predictions of inflationary theory regarding the generation of primordial perturbations. The spanning hot cluster observed in the CMB serves as a direct manifestation of these perturbations, highlighting their coherence across cosmic scales. Additionally, the asymmetry in the percolation properties of hot and cold regions suggests a deeper connection to the evolution of the matter distribution. Hot regions correspond to over-densities in the early universe, which later evolved into the large-scale structure observed today. The presence of a percolating hot cluster implies a critical level of connectivity that likely influenced the formation of cosmic superclusters and filaments, providing a bridge between the early universe and its present-day architecture. Additionally, the fractal dimensions and scaling laws associated with percolating clusters offer a rich framework for understanding the self-similar nature of the underlying perturbations.

The role of percolation in probing the topology of the universe is another intriguing aspect of this study. The connectivity properties of the largest clusters are inherently tied to the geometry and topology of the observed sky. The standard model assumes the universe is isotropic and homogeneous on large scales, but anomalies in the percolation patterns could suggest otherwise. Our detection of a percolating cluster across all boundaries reinforces the assumption of a simply connected, flat universe. Conversely, anomalies in the connectivity patterns, such as preferential orientations or missing connections, could hint at non-trivial topologies, such as a compact or multiple connected universes.

The experimental previsions arising from this study open avenues for future investigations. High-resolution CMB maps, particularly those incorporating polarization data, offer an exciting opportunity to extend percolation analysis to other components of the CMB, such as the E-mode and B-mode polarization patterns (Hanson et al., 2014). These analyses could reveal additional connectivity properties and their relation to the underlying physics of reionization and primordial gravitational waves. Furthermore, cross-correlations with large-scale structure surveys, such as those mapping galaxy distributions and cosmic voids, could provide a direct observational link between

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the percolation properties of the CMB and the present-day universe. Experimental advancements, such as those anticipated from upcoming missions like CMB-S4 (Sohn and Fergusson, 2019), promise to refine the resolution and sensitivity of CMB data, enabling a more precise determination of critical thresholds and cluster statistics. Additionally, the extension of percolation studies to higher-dimensional datasets, such as tomographic maps of the large-scale structure, could offer new perspectives on the evolution of cosmic connectivity over time.

Theoretical predictions arising from the percolation of the largest hot clusters offer a wealth of opportunities for testing and refining cosmological models. One notable prediction is the universality of percolation thresholds in Gaussian random fields (Novaes et al., 2014). This universality implies that the critical threshold for the emergence of spanning clusters should remain consistent across different realizations of the CMB, provided the underlying statistical properties are Gaussian and isotropic. Deviations from this behavior could signal the presence of non-Gaussianity, anisotropy or exotic physics, such as cosmic strings or domain walls. The fractal dimension of the largest clusters, as observed in this study, provides an additional metric for comparison with theoretical expectations. Any significant departures from the predicted fractal dimensions would warrant further investigation into the nature of the initial conditions and the physics governing their evolution.

However, the methodology is not without its limitations. The reliance on thresholding techniques introduces potential biases, as the choice of threshold can significantly influence the identification and connectivity of clusters (Galam and Mauger, 1996; Regős et al., 2024). Although the use of global thresholding methods like Otsu's ensures consistency, localized variations in noise or foreground contamination could skew the results. Furthermore, the finite resolution of the CMB map imposes constraints on the smallest scales that can be reliably analyzed, potentially masking finer details of cluster morphology and connectivity. The study of percolation in the CMB also highlights the hiatus between theoretical predictions and observational limitations. While the results of this analysis are consistent with the standard cosmological model, they underscore the need for continued refinement of observational techniques and theoretical frameworks. The resolution and noise limitations of current data, coupled with the complexity of foreground subtraction, present ongoing challenges that must be addressed to fully exploit the potential of percolation analysis.

In conclusion, the application of percolation theory to the CMB represents a powerful and complementary approach to understanding the universe's primordial fluctuations. The emergence of a percolating hot cluster underscores the coherence and critical behavior of the temperature anisotropies, providing robust evidence for the Gaussian random field nature of the CMB. While limitations and uncertainties remain, the insights gained from this study open new avenues for exploring the early universe and its connection to the large-scale structure we observe today.

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References

1. Ashtekar, A., B. Gupt, D. Jeong, and V. Sreenath. "Alleviating the Tension in the Cosmic Microwave Background Using Planck-Scale Physics." *Physical Review Letters* 125, no. 5 (July 31, 2020): 051302. https://doi.org/10.1103/PhysRevLett.125.051302.
2. Bhavsar, S.P., Barrow, J.D. (1984). Percolation Studies of Galaxy Clustering. In: Mardirossian, F., Giuricin, G., Mezzetti, M. (eds) Clusters and Groups of Galaxies. Astrophysics and Space Science Library, vol 111. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-6412-9_77
3. Bianconi, G., and S. N. Dorogovtsev. "Theory of Percolation on Hypergraphs." *Physical Review E* 109, no. 1-1 (2024): 014306. https://doi.org/10.1103/PhysRevE.109.014306.
4. Brunk, N. E., and R. Twarock. "Percolation Theory Reveals Biophysical Properties of Virus-like Particles." *ACS Nano* 15, no. 8 (2021): 12988–95. https://doi.org/10.1021/acsnano.1c01882.
5. Einasto, J., I. Suhhonenko, L. J. Liivamägi, and M. Einasto. "Extended Percolation Analysis of the Cosmic Web." *Astronomy & Astrophysics* 616 (August 2018): A141. https://doi.org/10.1051/0004-6361/201833011.
6. Falck, B., and M. C. Neyrinck. "The Persistent Percolation of Single-Stream Voids." *Monthly Notices of the Royal Astronomical Society* 450, no. 3 (July 1, 2015): 3239–3253. https://doi.org/10.1093/mnras/stv879.
7. Furlanetto, Steven R., and S. Peng Oh. "Reionization through the Lens of Percolation Theory." *Monthly Notices of the Royal Astronomical Society* 457, no. 2 (April 1, 2016): 1813–1827. https://doi.org/10.1093/mnras/stw104.
8. Galam, Serge, and Alain Mauger. "Universal Formulas for Percolation Thresholds." *Physical Review E* 53, no. 3 (1996): 2177. https://doi.org/10.1103/PhysRevE.53.2177.
9. Hanson, D., S. Hoover, A. Crites, P. A. Ade, and K. A. Aird, et al. "Detection of B-Mode Polarization in the Cosmic Microwave Background with Data from the South Pole Telescope." *Physical Review Letters* 111, no. 14 (October 4, 2013): 141301. https://doi.org/10.1103/PhysRevLett.111.141301.
10. Haralick, Robert M., and Linda G. Shapiro. *Computer and Robot Vision.* Vol. 1. Boston: Addison-Wesley, 1992.
11. Harris, Charles R., K. Jarrod Millman, Stéfan J. van der Walt, Ralf Gommers, Pauli Virtanen, David Cournapeau, Eric Wieser, et al. "Array Programming with NumPy." *Nature* 585, no. 7825 (2020): 357–362. https://doi.org/10.1038/s41586-020-2649-2.
12. Li, M., L. Wang, S. Deng, and C. Zhou. "Color Image Segmentation Using Adaptive Hierarchical-Histogram Thresholding." *PLoS One* 15, no. 1 (2020): e0226345. https://doi.org/10.1371/journal.pone.0226345.
13. Meng, X., X. Hu, Y. Tian, G. Dong, R. Lambiotte, J. Gao, and S. Havlin. "Percolation Theories for Quantum Networks." *Entropy* 25, no. 11 (2023): 1564. https://doi.org/10.3390/e25111564.
14. Naselsky, P. D., and D. I. Novikov. "Percolation and Cluster Analysis for Delta T/T Maps." *Astrophysical Journal Letters* 444 (May 1995): L1. https://doi.org/10.1086/187845.
15. Novaes, C. P., and C. A. Wuensche. "Identification of Galaxy Clusters in Cosmic Microwave Background Maps Using the Sunyaev-Zel'dovich Effect." *Astronomy & Astrophysics* 545 (September 2012): A34. https://doi.org/10.1051/0004-6361/201118482
16. Novaes, C. P., A. Bernui, I. S. Ferreira, and C. A. Wuensche. "Searching for Primordial Non-Gaussianity in Planck CMB Maps Using a Combined Estimator." *Journal of Cosmology and Astroparticle Physics* 2014, no. 1 (January 13, 2014): 018. https://doi.org/10.1088/1475-7516/2014/01/018.
17. Otsu, Nobuyuki. "A Threshold Selection Method from Gray-Level Histograms." *IEEE Transactions on Systems, Man, and Cybernetics* 9, no. 1 (1979): 62–66. https://doi.org/10.1109/TSMC.1979.4310076.

18. Planck Collaboration, N. Aghanim, Y. Akrami, F. Arroja, M. Ashdown, et al. "Planck 2018 Results. I. Overview and the Cosmological Legacy of Planck." *Astronomy & Astrophysics* 641 (2020). https://doi.org/10.1051/0004-6361/201833880. http://hdl.handle.net/20.500.12386/29843.
19. Planck Collaboration. "Planck 2018 Results. VII. Isotropy and Statistics of the Cosmic Microwave Background." *Astronomy & Astrophysics* 641 (September 2020): A7. https://www.aanda.org/articles/aa/full_html/2020/09/aa35201-19/aa35201-19.html.
20. Regős, Enikő, Volker Springel, Sownak Bose, Boryana Hadzhiyska, and César Hernández-Aguayo. "Percolation Statistics in the MillenniumTNG Simulations." *The Astrophysical Journal* 974, no. 1 (October 9, 2024): 126. https://doi.org/10.3847/1538-4357/ad7138.
21. Sohn, Wuhyun, and James R. Fergusson. "CMB-S4 Forecast on the Primordial Non-Gaussianity Parameter of Feature Models." *Physical Review D* 100, no. 6 (September 25, 2019): 063536. https://doi.org/10.1103/PhysRevD.100.063536.
22. Timonin, P. N. "Statistical Mechanics of High-Density Bond Percolation." *Physical Review E* 97, no. 5 (2018): 052119. https://doi.org/10.1103/PhysRevE.97.052119.
23. van der Walt, Stéfan, Johannes L. Schönberger, Juan Nunez-Iglesias, François Boulogne, Joshua D. Warner, Neil Yager, Emmanuelle Gouillart, and Tony Yu. "scikit-image: Image Processing in Python." *PeerJ* 2 (2014): e453. https://doi.org/10.7717/peerj.453.
24. Zhang, Jiajun, Dalong Cheng, and Ming-Chung Chu. "Percolation Analysis for Cosmic Web with Discrete Points." *Physical Review D* 97, no. 2 (January 29, 2018): 023534. https://doi.org/10.1103/PhysRevD.97.023534.
25. Zuo, Joel Low En, and Abel Yang. "Clustering of Hotspots in the Cosmic Microwave Background." *EPJ Web of Conferences* 206 (2019): 09017. https://doi.org/10.1051/epjconf/201920609017.

Nonlinear locomotion patterns in the Egyptian Locust (Anacridium aegyptium) during walking: a detailed case study

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

We explored the nonlinear movement patterns of *Anacridium aegyptium* during terrestrial locomotion, providing insights into the walking dynamics of this large grasshopper species. Using video recordings, we analysed the trajectory of an insect and quantified key metrics, including curvature, tortuosity and fractal dimension. Curvature analysis revealed irregular turning behaviors with sharp directional changes, suggesting that locomotion was not random but deliberate. Compared with simulated linear trajectories, the curvature exhibited distinct peaks, highlighting the presence of statistically significant nonlinear features in the movement patterns. Phase space reconstruction revealed repetitive patterns indicating the potential presence of a limit cycle attractor. The trajectory remained confined within a specific region of the phase space, highlighting structured dynamics rather than unbounded behaviour. Fractal dimension analysis and Lyapunov exponent were consistent with a stable and predictable system over time, rather than one governed by chaos. These findings align with the behavioral ecology of *A. aegyptium*, suggesting that its walking dynamics are governed by efficient spatial exploration and obstacle negotiation rather than erratic or chaotic motion. Our study underscores the value of advanced mathematical and computational methods in boosting behavioural studies of locomotion. The insights derived from our analysis enhance our understanding of insect locomotion strategies and hold potential applications in the field of biomimetic robotics, where adaptive and efficient movement is mandatory. Future research could explore the impact of environmental factors, such as substrate type and food availability, on the observed nonlinear patterns, providing deeper context to the intricate locomotion behaviour of *Anacridium aegyptium*.

KEYWORDS: adaptive movement; environmental interaction; trajectory analysis; limit cycle attractor; biomimetic inspiration.

INTRODUCTION

Locomotion is a fundamental aspect of animal behavior serving crucial roles in habitat navigation, foraging and predator avoidance. The six-legged insects display a remarkable diversity of locomotion strategies, which have been a focal point of neurobiological research for over a century (Bidaye et al, 2018; Heckenthaler et al., 2023; Regeler et al., 2023). Insect locomotion has been extensively studied in the contexts of flying, walking, crawling and central pattern generation, both in natural systems and artificial walking systems (Seipel et al., 2004; Imirzian et al., 2019; Mantziaris et al., 2020; Sabattini et al., 2023). Research on insect locomotion has predominantly concentrated on the biomechanics of jumping and the dynamics of flight. Indeed, recent advancements have introduced a diverse array of modelling and simulation techniques, alongside experimental setups, to investigate collective insect motion. These approaches range from discrete agent-based models of self-propelled particles to continuous frameworks using integral-differential equations (Ariel and Ayali, 2015; Bleichman et al., 2024; Aidan et al. 2024). Grasshoppers are particularly noteworthy for their efficient jumping and walking capabilities. Among these, *Anacridium aegyptium*, commonly known as the Egyptian Locust, stands out due to its large size, robust anatomy and widespread distribution across Mediterranean and subtropical regions. The act of jumping in grasshoppers has garnered significant attention from researchers due to its intricate biomechanics and critical role in their survival strategies (Hawlena et al., 2010: Hawkes et al., 2022). Conversely, solitary walking behaviors in grasshoppers remain relatively underexplored, particularly with respect to nonlinear patterns and their ecological significance.

Nonlinear locomotion encompasses movement patterns deviating from straightforward linear trajectories, often distinguished by irregular pathways (Campos et al., 2010; Xu et al., 2023). Zigzagging paths may optimize resource exploration, while abrupt directional changes might indicate evasive maneuvers against predators. Nonlinear time-periodic models of flight dynamics have been studied, for instance, in the desert locusts *Schistocerca gregaria* (Taylor et al., 2005). Investigating these patterns in *A. aegyptium* could provide valuable insights into the broader principles governing insect locomotion and their potential applications in fields such as robotics and ecological modeling.

Advanced mathematical tools have transformed the study of animal movement, enabling precise quantification of complex trajectories. For instance, custom tracking algorithms have been employed to uncover fundamental animal-animal interactions that drive collective motion in swarms of marching locust nymphs (Ariel et al., 2014). Metrics such as curvature, fractal dimension, tortuosity and Lyapunov exponents are now routinely employed to uncover previously imperceptible locomotion patterns in both natural and artificial systems (Kearns et al., 2027; Suryanto et al., 2022; Xu

et al., 2023). Curvature analysis highlights turning behaviors and their frequency, while fractal dimension quantifies the geometric complexity of a trajectory. The Lyapunov exponent, a measure of the sensitivity of a system to initial conditions, can indicate whether movements exhibit chaotic properties (Mehdizadeh, 2018).

This study aims to investigate the nonlinear walking dynamics of *A. aegyptium* using video analysis and advanced computational methods. By quantifying the above-mentioned nonlinear metrics, we seek to characterize the insect's locomotion patterns and assess their ecological significance. Specifically, we hypothesize that *A. aegyptium* exhibits significant nonlinear features in its walking trajectory, indicative of adaptive and intentional locomotion strategies. In the following sections, we detail the materials and methods used to collect and analyze data, present the results of our quantitative analyses and discuss their implications in the context of both insect ecology and applied sciences. Through this investigation, we aim to bridge the gap between descriptive studies of insect movement and the rigorous mathematical frameworks needed to understand its underlying dynamics.

MATERIALS AND METHODS

To investigate the nonlinear walking dynamics of *Anacridium aegyptium*, a combination of field observations, video recording and computational analysis was employed. The study focused on an adult male specimen of *A. aegyptium*, casually encountered in its natural environment under undisturbed conditions. The specimen was observed in the Mediterranean region while walking on a glass window. The glass window provided a clear substrate for tracking the insect's movements, while natural daylight ensured optimal visibility without introducing artificial stressors. The study focused on a single individual, providing a brief but valuable snapshot of its behaviour. Since the observation involved a single individual, there was no risk of interference from conspecifics.
A. aegyptium, commonly known as the Egyptian Grasshopper or Egyptian Locust, is a large species in the Acrididae family, commonly found in the Mediterranean basin. It is one of the largest grasshoppers in Europe. It is primarily found perched on trees and shrubs, relying on short flights and jumping for movement. Key identifiers in our study included the distinctive vertical stripes on the eyes, the hind tibiae adorned with two rows of white spines tipped in black and the presence of eight abdominal segments in males, as opposed to the seven segments typically found in females (Girardie and Granier, 1974).

Video processing. Video recordings were captured at 30 frames per second using a camera mounted 1.5 meters above the arena. The field of view covered the entire arena, ensuring that the insect's trajectory could be tracked continuously. The raw video footage was processed using a custom Python-based software pipeline. The first step of motion capture involved converting the video into individual frames, which were subsequently analyzed using an object detection algorithm based on Optical flow (Lucas-Kanade method) via OpenCV (Al-Qudah and Yang, 2023). The algorithm identified the position of the center of the body in each frame, generating a sequence of x and y coordinates representing the insect's trajectory over time (**Figure 1**). This was determined using simple thresholding to identify the largest contour in the image, which typically corresponds to the main body of the insect. The trajectory data were smoothed using a low-pass filter to reduce noise.

Nonlinear analysis. Various parameters were derived from the insect's movement trajectory. **Displacement** represents the distance between the initial and final positions. The **total path length** is the sum of the distances covered between consecutive points along the trajectory. **Average speed** was determined by dividing the total path length by the total time of observation. **Instantaneous speed** was calculated by dividing the distance covered between consecutive points by the time between frames. Finally, the **direction of movement** was measured as the angle of motion between consecutive points, providing insight into the insect's orientation during its movement.
Next, a range of metrics was calculated to analyse and define the nonlinear movement features. **Curvature** was calculated as the change in direction per unit distance, providing a measure of how sharply the insect turned at each point along its path. **Tortuosity** was assessed as the ratio of the total path length to the straight-line displacement, with higher values indicating more convoluted trajectories. **Fractal dimension**s were estimated using the box-counting method, which involves overlaying a grid of varying box sizes on the trajectory and counting the number of boxes intersected by the path. To further explore the dynamics, **phase space reconstruction** was performed. This involved embedding the trajectory data in a higher-dimensional space using time delays, allowing the identification of patterns that are not apparent in the original two-dimensional trajectory. The embedding dimension and delay time were determined using the false nearest neighbors method and mutual information analysis, respectively (Albers and Hripcsak, 2012; Wallot and Mønster, 2018). The reconstructed phase space was then analyzed for **attractor behavior**, with particular attention to whether the trajectories exhibited features characteristic of limit cycles or other nonlinear dynamic phenomena (Broscheid et al., 2018). Additionally, the Lyapunov exponent was calculated to evaluate the trajectory's sensitivity to initial conditions. This process involved tracking the divergence of nearby points in phase space over time. The divergence was then plotted on a logarithmic scale and the exponent was estimated from the slope of the resulting curve. A positive Lyapunov exponent indicated the presence of chaotic behaviour.

To ensure the robustness of the findings, the analyses were repeated using a 5-second subset of the data, applying varying smoothing parameters and detection thresholds. Sensitivity analyses were also conducted to ensure that the results were not unduly influenced by the choice of parameters such as the box size in the fractal dimension analysis or the embedding dimension in the phase space reconstruction.

Tools and statistical analysis. Computational analyses were implemented in Python using libraries such as NumPy, SciPy and Matplotlib for numerical computation and visualization. Statistical analyses were conducted to test the significance of the observed nonlinear features. Curvature and tortuosity metrics were compared against null models generated from simulated linear trajectories created by randomly sampling points within the arena and interpolating straight-line paths between them. The distributions of curvature and tortuosity in the observed and simulated datasets were compared using Kolmogorov-Smirnov tests. The fractal dimension of the observed trajectories was compared against random walk models to determine whether the observed complexity exceeded that expected from stochastic motion.

Figure 1. A video frame displaying the detected point on *Anacridium aegyptium*'s body, marked in red. This point was used as the reference for reconstructing the insect's movement trajectory.

RESULTS

The video analysis of *Anacridium aegyptium*'s walking dynamics, captured at a resolution of 1080 × 1920 pixels (width × height), provided a detailed quantification of the movements, uncovering significant nonlinear features. Over a trajectory spanning 433 frames at approximately 30 frames per second, the insect demonstrated a total path length of approximately 1189 pixels, far surpassing its net displacement of 24 pixels. This discrepancy underscores the pronounced nonlinearity of the insect's motion, further supported by a tortuosity value of 49.43, which reflects the highly convoluted nature of its path. Comparisons between the total path length and the straight-line displacement further emphasized this nonlinearity, with substantial differences observed between the two measures throughout the trajectory (**Figure 2**).

The curvature analysis revealed subtle yet consistent turning behaviors, with sharp directional changes frequently interrupting the trajectory. These patterns suggest that the movement is not random but deliberate, possibly driven by environmental stimuli or internal decision-making processes. Compared with simulated linear trajectories, the curvature exhibited higher variability and distinct peaks, highlighting the presence of statistically significant nonlinear features in the movement patterns (**Figure 3**). This finding confirmed the existence of nonrandom turning behaviors.

The analysis of the direction of movement identified dominant frequencies, with direction oscillations occurring at approximately 0.42 Hz (**Figure 4**). This suggested a consistent rhythmic pattern in the insect's behaviour. In contrast, speed oscillations did not exhibit statistically significant periodicity, indicating that variations in speed may be more context-dependent or driven by external stimuli rather than inherent rhythmicity.

Phase space reconstruction illustrated the bounded nature of the motion, with repetitive patterns pointing toward a potential limit cycle attractor (**Figure 5**). This attractor behavior indicated a structured yet flexible movement strategy, allowing for environmental adaptability. The trajectory was confined within a bounded region of the phase space, suggesting structured dynamics rather than random or unbounded behaviour. The reconstructed phase space showed clear loops without chaotic divergence, consistent with a stable and predictable system rather than one governed by chaos. The calculated Lyapunov exponent, approximately -7.5 × 10□□, further supported the absence of chaos, pointing towards convergence rather than divergence in the system's dynamics. Fractal dimension analysis provided additional insights into the complexity of the movement. With an estimated fractal dimension of 0.73, the trajectory displayed a constrained complexity characteristic of structured but not chaotic motion. This metric underscored the insect's ability to navigate effectively within defined spatial limits while maintaining a balance between exploration and efficiency.

Visual analyses complemented these findings. Trajectory plots highlighted regions of high curvature, represented by intensified color gradients, corresponding to areas of sharper turns or more frequent directional adjustments (**Figure 6**). The curvature was nonuniformly distributed, with distinct peaks suggesting localized exploratory behavior or evasive maneuvers.

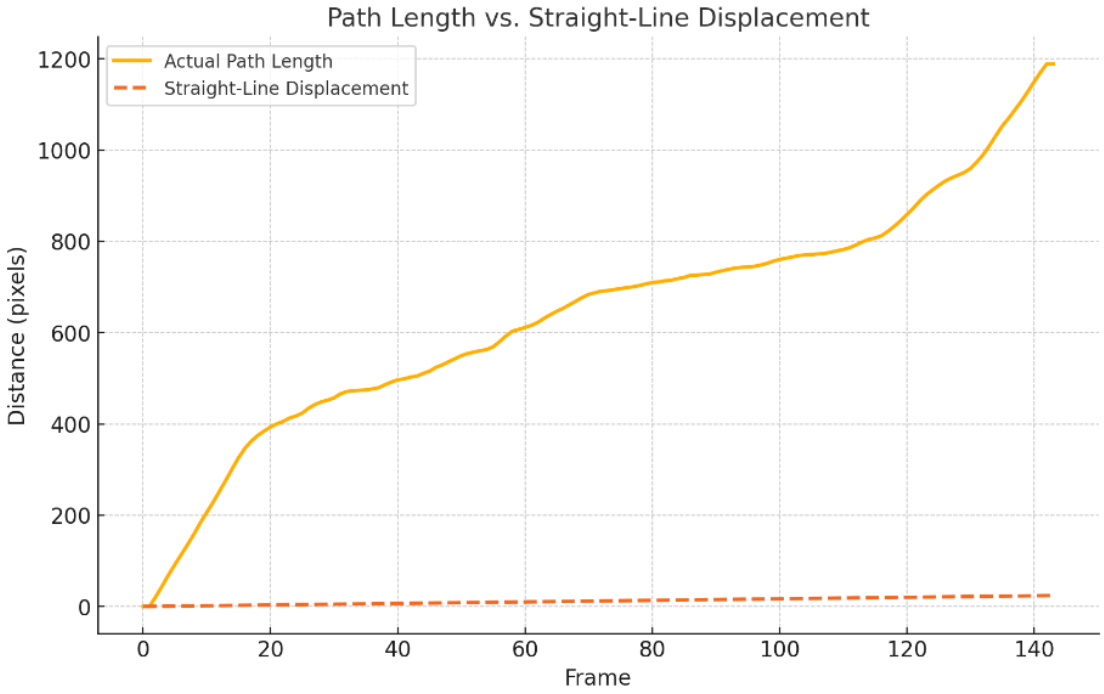

Figure 2. Relationship between the path length and the straight-line displacement of the insect's trajectory. The solid line represents the cumulative actual path length, capturing the total distance travelled during locomotion. The dashed line reflects the straight-line displacement, indicating the direct distance from the starting point to the endpoint of the trajectory. The notable gap between these two lines serves as clear evidence of the high tortuosity and the nonlinear characteristics inherent in the insect's movement pattern.

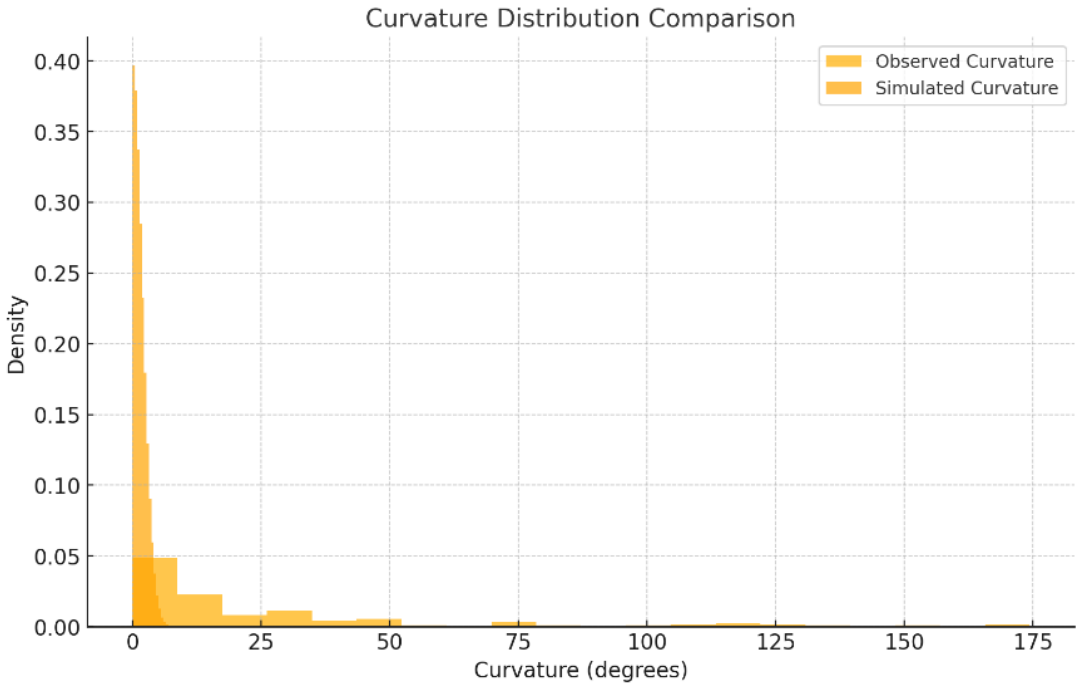

Figure 3. Comparison of curvature distributions. Yellow bars represent the observed curvature values derived from the insect's trajectory, while orange bars depict the curvature distribution from random simulated trajectories. The observed curvature distribution reveals a statistically significant deviation from the simulated random trajectories.

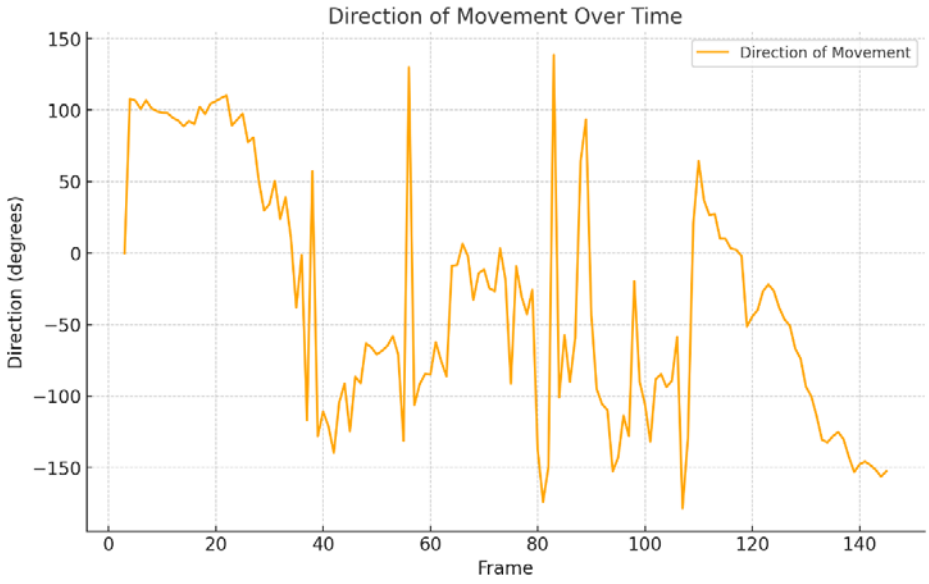

Figure 4. Direction of movement over time. Peaks in the plot correspond to bursts of movement, while dips indicate slower movement or pauses.

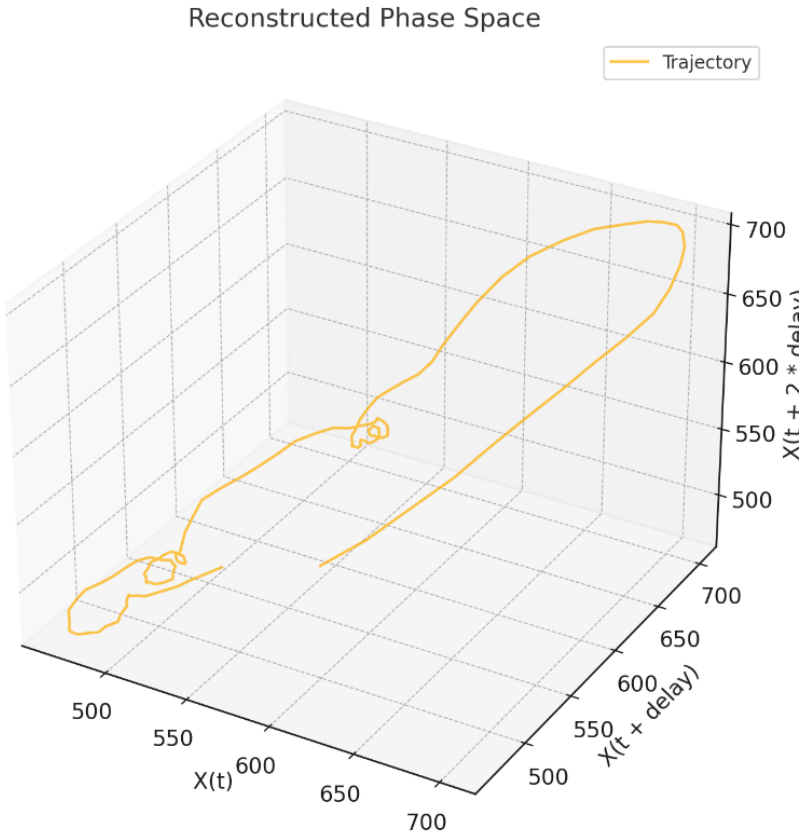

Figure 5. Phase space reconstruction of the 3D trajectory using time-delay embedding. The trajectory does not converge toward a single point, indicating the absence of a fixed-point attractor. Indications of repetitive loops suggest the potential presence of a limit cycle attractor, although the loops lack perfect regularity. The absence of irregular divergence in the phase space suggests that the system does not exhibit chaotic features or a strange attractor.

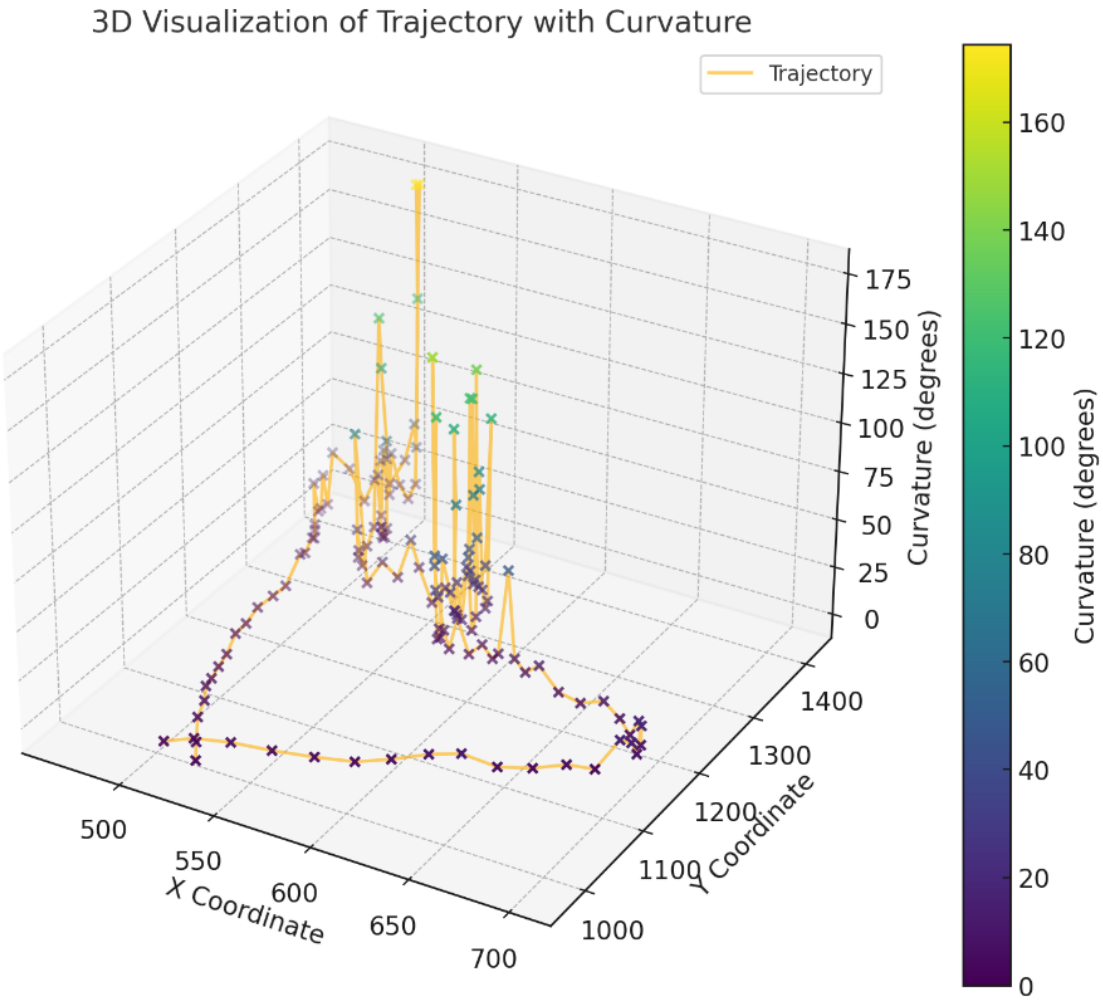

Figure 5. 3D visualization of the insect's trajectory, incorporating curvature as the third dimension. Each point represents the detected position of the tracked feature across frames. The x and y axes represent the spatial movement of the insect while the z-axis corresponds to curvature, with higher values signifying sharper turns. The colour intensity of the points reflects the curvature at each location, with more intense colours highlighting areas where sharper turns occur. This visualization emphasizes the nonlinearity of the trajectory, highlighting deviations and directional changes over time.

CONCLUSIONS

We provide a comprehensive analysis of the nonlinear walking dynamics of *Anacridium aegyptium*. The computational approach employed in this study enabled the extraction of detailed and quantitative insights from behavioral data. The integration of tools such as curvature analysis, fractal dimension estimation and phase space reconstruction allowed for a multidimensional understanding of grasshopper's locomotion that extends beyond traditional observational methods.

Our findings underscore the importance of nonlinear analysis in understanding animal movement, particularly for behaviors that cannot be adequately described by linear models. Nonlinear movement patterns, such as Lévy flights or Brownian motion, are well-documented in insects during behaviors like foraging, mate searching or escape responses (Taylor et al., 2005). However, detailed statistical analysis of curvature remains relatively uncommon. Notably, one of the most striking outcomes of our research is the identification of curvature and tortuosity as key indicators of the insect's adaptive nonlinear walking behaviour. The significant deviations from random linear trajectories suggest that *A. aegyptium* employs deliberate turning and path-convoluting strategies. These movements may reflect purposeful behaviors, including optimizing foraging efficiency, avoiding obstacles avoidance, searching or responding to potential

threats. High curvature could indicate localized exploration near stimuli, while sudden changes may signify evasive actions. Additionally, curvature may reveal biomechanical limitations or energy-efficient turning mechanisms inherent to the insect's physiology, as frequent turning may incur energy costs that influence resource optimization strategies. Furthermore, significant curvature deviations can serve as reliable indicators for distinguishing insect behaviors from random noise or external disturbances. Novelty can also stem from linking curvature to specific environmental or behavioral contexts, such as food searching or predator avoidance. In the case of *A. aegyptium*, nonlinear movement patterns during walking are of particular interest due to the ecological contexts in which they occur. As a large, terrestrial insect, *A. aegyptium* often traverses uneven substrates, navigates dense vegetation and interacts with potential threats or resources. The frequent directional changes, bounded trajectories and repetitive loops likely represent an optimized trade-off between environmental exploration and energy conservation.

The use of fractal dimension analysis further revealed the constrained complexity of the trajectories, highlighting the ability to navigate within defined spatial boundaries while maintaining efficient movement patterns. Phase space reconstruction and Lyapunov exponent calculations provided additional depth to our understanding of walking dynamics. The identification of a possible limit cycle attractor indicates repetitive and bounded behaviors, which may reflect innate or environmentally influenced patterns. While no evidence of chaotic dynamics was found, the bounded and structured trajectories point to a deterministic system governed by environmental feedback and internal rules. This structured behavior aligns with the ecological requirements of *A. aegyptium*, enabling it to adapt to complex terrains while conserving energy. The bounded movement and lack of chaotic divergence are consistent with efficient navigation strategies in natural habitats. These findings suggest potential applications in biomimetic robotics, where adaptive and efficient movement strategies are critical.

Comparing observed curvature to random trajectories represents a methodological advancement that has been already used in standard ecological studies. Artificially generated video sequences have been introduced, combining known real-animal postures with randomized body positions, orientations and sizes (Arent et al., 2021). The novel contribution of our study is that is rigorously tests curvature against null models such as random linear paths incorporating quantitative metrics like curvature distribution or tortuosity. The novelty is enhanced by the focus on a specific insect species or behavior (i.e., a grasshopper's movement during natural conditions) that has been scarcely analyzed in this way. The methodologies developed here could be adapted to study other forms of animal movement, from terrestrial vertebrates to aquatic species, providing a versatile toolkit for ecological and biomechanical research. For navigation studies, understanding nonlinear movements may illuminate how insects process sensory information and make decisions. Additionally, recognizing curvature patterns in movement may aid pest control strategies or contribute to ecological monitoring efforts. The insights gained from understanding the nonlinear dynamics of *A. aegyptium*'s walking behavior could inform the design of biomimetic robots capable of adaptive and efficient locomotion in complex terrains (Gart et al., 2018). By mimicking the turning strategies, bounded trajectories and constrained features observed in this study, robotic systems could achieve enhanced agility and robustness.

Despite our findings, several limitations must be acknowledged. First, the experimental setup cannot fully capture the complexities of the insect's native habitat. Factors present in the wild, such as predation risk, interspecies interactions, and varying substrate types, were not considered in this study. Second, the analysis relied on two-dimensional trajectory data, which may overlook vertical components of movement. Future studies incorporating three-dimensional tracking techniques could provide a more holistic perspective. Whereas marker-based motion capture systems are very robust and easily adjusted to suit different setups, tracked species or body parts, they cannot be applied in experimental situations where markers interfere with natural behavior, e.g., when tracking delicate, elastic or sensitive body structures (Arent et al., 2021). Another limitation is the limited generalizability of the findings, as the observations were based on a single individual. Expanding the sample size and including a broader range of conditions would strengthen the robustness of the conclusions. Additionally, while advanced metrics such as fractal dimension and Lyapunov exponents were calculated, these analyses are sensitive to parameter choices such as time delays and embedding dimensions. Further refinement of these methods could enhance the reliability of future studies. While this research focused primarily on movement patterns, future studies could incorporate additional variables such as environmental. For example, controlled manipulations of environmental features could help disentangle the roles of intrinsic and extrinsic factors in driving observed behaviors. Future directions include linking curvature to environmental factors like light or food availability to determine its drivers, conducting comparative studies across species and behaviors and exploring other nonlinear features such as speed oscillations or pauses.

In conclusion, this interdisciplinary investigation into the nonlinear walking dynamics of *Anacridium aegyptium* demonstrates the value of advanced analytical techniques in uncovering hidden patterns and address fundamental questions about movement and behavior. Despite its limitations, this study offers a compelling framework for examining locomotion through the lens of nonlinear dynamics, with applications ranging from ecology and evolutionary biology to biomechanics and robotics.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.

Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal, and has not been previously published.

Availability of data and materials. all data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests. The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

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Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical, and material support, study supervision.

Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT to assist with data analysis and manuscript drafting. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

REFERENCES

1) Aidan, Yossef, Itay Bleichman, and Amir Ayali. "Pausing to Swarm: Locust Intermittent Motion Is Instrumental for Swarming-Related Visual Processing." *Biology Letters* 20, no. 2 (February 2024): 20230468. https://doi.org/10.1098/rsbl.2023.0468.
2) Al-Qudah, S., and M. Yang. "Large Displacement Detection Using Improved Lucas-Kanade Optical Flow." *Sensors* 23, no. 6 (March 15, 2023): 3152. https://doi.org/10.3390/s23063152.
3) Albers, D. J., and G. Hripcsak. "Using Time-Delayed Mutual Information to Discover and Interpret Temporal Correlation Structure in Complex Populations." *Chaos* 22, no. 1 (March 2012): 013111. https://doi.org/10.1063/1.3675621.
4) Arent, Ilja, Florian P. Schmidt, Mario Botsch, and Volker Dürr. "Marker-Less Motion Capture of Insect Locomotion with Deep Neural Networks Pre-Trained on Synthetic Videos." *Frontiers in Behavioral Neuroscience* 15 (April 22, 2021): 637806. https://doi.org/10.3389/fnbeh.2021.637806.
5) Ariel, Gil, Yotam Ophir, Sagi Levi, Eshel Ben-Jacob, and Amir Ayali. "Individual Pause-and-Go Motion Is Instrumental to the Formation and Maintenance of Swarms of Marching Locust Nymphs." *PLoS One* 9, no. 7 (July 2, 2014): e101636. https://doi.org/10.1371/journal.pone.0101636.
6) Ariel, Gil, and Amir Ayali. "Locust Collective Motion and Its Modeling." *PLoS Computational Biology* 11, no. 12 (December 10, 2015): e1004522. https://doi.org/10.1371/journal.pcbi.1004522.
7) Bidaye, Salil S., Till Bockemühl, and Ansgar Büschges. "Six-Legged Walking in Insects: How CPGs, Peripheral Feedback, and Descending Signals Generate Coordinated and Adaptive Motor Rhythms." *Journal of Neurophysiology* 119, no. 2 (February 1, 2018): 459–75. https://doi.org/10.1152/jn.00658.2017.
8) Bleichman, Itay, Peleg Shefi, Gal A. Kaminka, and Amir Ayali. "The Visual Stimuli Attributes Instrumental for Collective-Motion-Related Decision-Making in Locusts." *PNAS Nexus* 3, no. 12 (December 2024): e537. https://doi.org/10.1093/pnasnexus/pgae537.
9) Broscheid, K. C., C. Dettmers, and M. Vieten. "Is the Limit-Cycle-Attractor an (Almost) Invariable Characteristic in Human Walking?" *Gait & Posture* 63 (June 2018): 242–47. https://doi.org/10.1016/j.gaitpost.2018.05.015.
10) Campos, Ricardo, Vitor Matos, and Cristina Santos. "Hexapod Locomotion: A Nonlinear Dynamical Systems Approach." In *Proceedings of the IECON 2010 - 36th Annual Conference on IEEE Industrial Electronics Society*, 1041–1046. IEEE, 2010. https://doi.org/10.1109/IECON.2010.5675517.
11) Gart, Sean W., Changxin Yan, Ratan Othayoth, Zhiyi Ren, and Chen Li. "Dynamic Traversal of Large Gaps by Insects and Legged Robots Reveals a Template." *Bioinspiration & Biomimetics* 13, no. 2 (February 2, 2018): 026006. https://doi.org/10.1088/1748-3190/aaa36b.
12) Girardie, J., and S. Granier. "[Ultrastructure of the Corpora Allata of *Anacridium aegyptium* (Insecta, Orthoptera) in the Last-but-One Larval Instar and During Imaginal Life]." *Archives d'Anatomie Microscopique et de Morphologie Expérimentale* 63, no. 3 (July–September 1974): 251–68.

13) Hawkes, Elliot W., Charles Xiao, Richard-Alexandre Peloquin, Christopher Keeley, Matthew R. Begley, Morgan T. Pope, and Günter Niemeyer. "Engineered Jumpers Overcome Biological Limits via Work Multiplication." *Nature* 604 (2022): 657–661. https://doi.org/10.1038/s41586-022-04591-1.
14) Hawlena, Dror, Holger Kress, Eric R. Dufresne, and Oswald J. Schmitz. "Grasshoppers Alter Jumping Biomechanics to Enhance Escape Performance under Chronic Risk of Spider Predation." *Functional Ecology* 24, no. 5 (2010): 1234–1242. https://doi.org/10.1111/j.1365-2435.2010.01767.x.
15) Heckenthaler, Tabea, Tobias Holder, Ariel Amir, Ofer Feinerman, and Ehud Fonio. "Connecting Cooperative Transport by Ants with the Physics of Self-Propelled Particles." *PRX Life* 1 (2023): 023001. https://doi.org/10.1103/PRXLife.1.023001.
16) Imirzian, Natalie, Yizhe Zhang, Christoph Kurze, Raquel G. Loreto, Danny Z. Chen, and David P. Hughes. "Automated Tracking and Analysis of Ant Trajectories Shows Variation in Forager Exploration." *Scientific Reports* 9, no. 13246 (2019). https://doi.org/10.1038/s41598-019-49641-5.
17) Kearns, W. D., J. L. Fozard, and V. O. Nams. "Movement Path Tortuosity in Free Ambulation: Relationships to Age and Brain Disease." *IEEE Journal of Biomedical and Health Informatics* 21, no. 2 (March 2017): 539–48. https://doi.org/10.1109/JBHI.2016.2517332.
18) Mantziaris, Charalampos, Till Bockemühl, and Ansgar Büschges. "Central Pattern Generating Networks in Insect Locomotion." *Developmental Neurobiology* 80, no. 1–2 (January 2020): 16–30. https://doi.org/10.1002/dneu.22738.
19) Mehdizadeh, S. "The Largest Lyapunov Exponent of Gait in Young and Elderly Individuals: A Systematic Review." *Gait & Posture* 60 (February 2018): 241–50. https://doi.org/10.1016/j.gaitpost.2017.12.016.
20) Xu, Q., and J. Liu. "Dynamic Research on Nonlinear Locomotion of Inchworm-Inspired Soft Crawling Robot." *Soft Robotics* 10, no. 3 (June 2023): 660–72. https://doi.org/10.1089/soro.2022.0002
21) Regeler, V., Boudinot, B. E., and Wöhrl, T. "Go Thou to the Ant: A Comparative Biomechanical Analysis of Locomotion in Hymenoptera (Hexapoda)." *bioRxiv*, 2023. doi: https://doi.org/10.1101/2023.02.24.529971.
22) Sabattini, Julian Alberto, Francisco Sturniolo, Martín Bollazzi, and Leandro A. Bugnon. "AntTracker: A Low-Cost and Efficient Computer Vision Approach to Research Leaf-Cutter Ants Behavior." *Smart Agricultural Technology* 5 (October 2023): 100252. https://doi.org/10.1016/j.atech.2023.100252.
23) Seipel, Justin E., Philip J. Holmes, and Robert J. Full. "Dynamics and Stability of Insect Locomotion: A Hexapedal Model for Horizontal Plane Motions." *Biological Cybernetics* 91 (2004): 76–90. https://doi.org/10.1007/s00422-004-0498-7.
24) Suryanto, M. E., C. C. Yang, G. Audira, R. D. Vasquez, M. J. M. Roldan, T. R. Ger, and C. D. Hsiao. "Evaluation of Locomotion Complexity in Zebrafish after Exposure to Twenty Antibiotics by Fractal Dimension and Entropy Analysis." *Antibiotics* 11, no. 8 (August 4, 2022): 1059. https://doi.org/10.3390/antibiotics11081059.
25) Taylor, Graham K., and Rafał Zbikowski. "Nonlinear Time-Periodic Models of the Longitudinal Flight Dynamics of Desert Locusts *Schistocerca gregaria*." *Journal of the Royal Society Interface* 2, no. 3 (June 22, 2005): 197–221. https://doi.org/10.1098/rsif.2005.0036
26) Wallot, S., and D. Mønster. "Calculation of Average Mutual Information (AMI) and False-Nearest Neighbors (FNN) for the Estimation of Embedding Parameters of Multidimensional Time Series in Matlab." *Frontiers in Psychology* 9 (September 10, 2018): 1679. https://doi.org/10.3389/fpsyg.2018.01679.

Monte Carlo simulations predict distinct real EEG patterns in individuals with high and low IQs

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

The neural mechanisms underlying individual differences in intelligence are a central focus in neuroscience. We investigated the effectiveness of Monte Carlo simulations in predicting real EEG patterns and uncovering potential neural differences between individuals with high and low intelligence. EEG data were collected from two groups of volunteers categorized by IQ, namely, a high-IQ group and a low-IQ group. A univariate normal distribution was fitted to each EEG channel using Maximum Likelihood Estimation, after which synthetic datasets were generated based on the estimated parameters. Statistical analyses including Root Mean Square Error (RMSE) calculations assessed the alignment between real and simulated data. We showed that Monte Carlo simulations effectively replicated the statistical properties of the EEG data from both the groups, closely matching the real central tendencies, variability and overall distribution shapes. Specific EEG channels, particularly in the frontal and temporal bilateral regions, exhibited significant differences between the two groups, pointing to potential neural markers of cognitive abilities. Further, the low-IQ group exhibited higher predictability and more consistent neural patterns, reflected by lower RMSE values and smaller standard deviations across several EEG channels. Conversely, the high-IQ group displayed greater variability and larger RMSE values, reflecting complex neural dynamics that are less predictable by Monte Carlo simulations. Our findings underscore the utility of Monte Carlo simulations as a robust tool for replicating EEG patterns, identifying cognitive differences and predicting EEG activity associated with intelligence levels. These insights can inform predictive modeling, neurocognitive research, educational strategies and clinical interventions of targeted cognitive enhancement.

KEYWORDS: statistical analysis; oddball tasks; synthetic dataset; EEG channel.

INTRODUCTION

Exploring the neural mechanisms underpinning intelligence has been a longstanding primary focus of cognitive neuroscience research. Electroencephalography (EEG) offers unique insights to assess differences in cognitive abilities, including distinctions between individuals of varying intelligence levels (Friedman et al., 2019). With its high temporal resolution, the non-invasive EEG evaluates the interplay between synchronization, complexity and network efficiency (van Dellen et al., 2015). For instance, higher IQ is associated with reduced long-distance EEG information flow and enhanced local processing efficiency, supporting small-world models (Thatcher et al., 2016). Short EEG phase delays and increased coherence in frontal regions correlate with higher intelligence, emphasizing the role of frontal lobe synchronization (Thatcher et al., 2005). Resting-state EEG studies have further explored intelligence-related differences, reporting balanced inter-hemispheric coordination in alpha and beta bands in more intelligent individuals (Jahidin et al., 2013). Also, it has been demonstrated that IQ correlates negatively with EEG energy but positively with information flow intensity at specific frequencies, emphasizing the role of efficiency in neural communication (Luo et al., 2021). Changes in microstate dynamics are associated with fluid intelligence and its enhancement following cognitive training (Santarnecchi et al., 2017). Lu et al. (2022) found that individuals with higher fluid intelligence allocate attentional resources more flexibly, particularly in complex tasks, as reflected in theta and alpha EEG activities. Together, these findings underscore the utility of EEG in the assessment of the neuronal mechanisms of intelligence, revealing consistent patterns of neural efficiency, inter-hemispheric coordination and adaptive resource allocation.

Conversely, the analysis of EEG data poses significant challenges due to their inherent variability, high dimensionality and sensitivity to noise (Hassani et al., 2015). To address these challenges and enhance our ability to model and predict EEG patterns, advanced statistical and computational methods are required. Monte Carlo simulations have been widely used across various scientific disciplines, providing a powerful framework for modeling complex systems influenced by variability and uncertainty (Metropolis and Ulam, 1949; Rubinstein and Kroese, 2016). By leveraging statistical properties derived from observed data, Monte Carlo simulations generate synthetic datasets that may reflect real-world behaviors (Salvadori et al., 2024; Jones and Fleming, 2024). A Monte Carlo approach could be particularly well-suited for EEG data, as it allows researchers to explore and replicate neural dynamics without the need for extensive experimental data collection. Monte Carlo methods have been applied in neuroscience to simulate and analyse

electromagnetic brain signals, providing approximations of event-related brain activity (Herdman 2021). Monte Carlo simulations have been utilized for brain source localization (Georgieva et al., 2013) and evaluation of errors as a function of position within the brain in MRI-MEG/EEG co-registration techniques (Singh et al., 1997). Surprisingly, it has been demonstrated that EEG localization is more accurate than MEG localization for the same number of sensors averaged over many source locations (Liu et al., 2002). Monte Carlo analysis can also simulate event-related changes in amplitude and phase-amplitude correlations, enabling close approximations of real EEG and MEG data (Herdman 2021). This approach is particularly valuable for validating data analysis methods, including measurements of functional connectivity and phase-amplitude coupling. A Bayesian framework has been introduced for parameter estimation in EEG modeling using a marginalized Markov Chain Monte Carlo approach (Hettiarachchi et al., 2012). This method was employed to fit a neural mass model to EEG data effectively.

Despite these studies, the application of Monte Carlo simulations to EEG research in revealing cognitive differences between individuals of high and low intelligence remains relatively underexplored. This study aims to bridge this gap by investigating how Monte Carlo simulations can model, simulate and reproduce real EEG traces. Central to this investigation is the issue of predictability: using Monte Carlo simulations, are the EEG patterns from high-IQ individuals more or less predictable than those of their lower-IQ counterparts? It can be hypothesized that higher cognitive abilities are associated with greater neural flexibility and variability, potentially reducing predictability in simulations. Conversely, lower intelligence may correspond to more stable neural patterns, increasing predictability. Monte Carlo simulations, by generating synthetic EEG data modeled on the statistical properties of real datasets, offer a systematic approach to testing these hypotheses.

We conclude that that Monte Carlo methods are a robust tool for exploring the neural dynamics of intelligence, paving the way for future investigations into brain-behavior relationships. By accurately replicating EEG patterns and identifying significant group differences, Monte Carlo simulations contribute to a deeper understanding of the cognitive and neural processes underlying intelligence.

MATERIALS AND METHODS

Participants and Data Collection. This study retrospectively builds on the foundational research conducted by Norbert and Ksenija Jaušovec through 2010 (Jaušovec and Jaušovec, 2001; 2003; 2005; 2010), which was later advanced in collaboration with Tozzi et al. (2021a; 2021b). This continuation of their work is undertaken with great respect and recognition of Norbert's untimely passing.

EEG data were collected from two groups of right-handed volunteers categorized by IQ, namely, a high-IQ group and a low-IQ group. Each group consisted of five participants, yielding a total sample of 10 individuals (mean age: 19.8 years; SD = 0.9; range = 18–21 years; males: 4). The IQ categorization was based on standardized test scores, with the high-IQ group representing the top quartile (IQ SD = 127) and the low-IQ group representing the bottom quartile (IQ SD = 87). Differences in EEG activity between these groups were analyzed during the performance of two oddball tasks (auditory and visual). The study adhered to the Declaration of Helsinki and received approval from the Ethics Committee of the University of Maribor, Slovenia.

EEG signals were recorded using a 64-channel system to ensure comprehensive cortical coverage. Electrode placement followed the 10–20 international system, covering nineteen scalp locations: [FP1], [FP2], [F3], [F4], [C3], [C4], [P3], [P4], [O1], [O2], [F7], [F8], [T3], [T4], [T5], [T6], [CZ], [FZ] and [PZ]. The electrodes were sintered Silver/Silver Chloride (8mm diameter) with impedance maintained below 5 kΩ. All leads were referenced to linked mastoids (A1 and A2), with a ground electrode on the forehead. Vertical eye movements were recorded using additional electrodes placed above and below the left eye. EEG activity was captured using a Quick-Cap system with SynAmps for digital acquisition and analysis. Signals were digitized at 1000 Hz with a gain of 1000 (resolution: 0.084 μV/bit, 16-bit A/D conversion) and stored on a hard disk. Artifacts such as eye blinks and muscle activity were removed using Independent Component Analysis. Data were then band-pass filtered between 1 and 40 Hz to isolate relevant neural activity while minimizing noise. The time-series data were located in separate columns, each one corresponding to a different EEG electrode. The data were numerical and represented EEG signal amplitudes in microvolts over time. Each row corresponded in the recording to a single time point in milliseconds.

Next, Monte Carlo simulations were performed to generate synthetic EEG traces, modeling the variations of future data points based on statistical distributions.

Monte Carlo simulations. A reliable methodological framework was essential to ensure robust analysis and enabling meaningful comparisons between EEG traces of high-IQ and low-IQ individuals. The analysis of the EEG data followed a series of structured steps. The first step involved loading EEG datasets for both the groups. Multiple files for each group were then concatenated into unified datasets. Any rows or cells with missing values were removed to prevent inaccuracies in statistical calculations. The next step was distribution fitting, where statistical parameters, i.e., the mean μ and standard deviation σ, were derived for each EEG channel to simulate data closely reflecting real-world observations. Each channel was analyzed separately. A univariate normal distribution was fitted to the data using

Maximum Likelihood Estimation (MLE) to calculate μ and σ, ensuring that the simulated data aligned with the distributions observed in the real datasets. The alignment of the fitted distributions were then validated by comparing histograms of real data against the simulated distributions.

Once the distributions were established, synthetic EEG traces were generated using Monte Carlo simulations. For each EEG channel, 100 random samples were drawn from the fitted normal distribution parameters μ and σ. The synthetic samples were organized into a structured dataset mirroring the original format, with separate columns for each channel. The simulation was performed independently for both high-IQ and low-IQ groups to preserve their distinct statistical properties. Following data simulation, statistical analyses evaluated the differences between real and simulated datasets and compared the predictability of the high-IQ and low-IQ groups. Fit accuracy was assessed by comparing real and simulated data using Root Mean Square Error (RMSE) for each EEG channel, where lower RMSE values indicated better alignment. Small adjustments were necessary to address mismatches in the number of samples between the real and the simulated data and to ensure data alignment during the RMSE calculation. Variability was also analyzed, as higher variability (e.g., larger standard deviations) in a dataset could reduce predictability by introducing greater spread around the mean. A larger number of EEG channels showing significant differences between real and simulated data suggested lower predictability. Two-sample t-tests and Welch's t-tests were performed to identify significant differences between the real high-IQ and low-IQ groups. Visual comparisons, including histograms and boxplots, were produced to illustrate the alignment between real and simulated datasets.

All analyses and visualizations were performed using Python. Libraries such as numpy, pandas, and scipy were used for statistical calculations, while matplotlib was employed for creating visualizations. The scipy.stats module provided functions for t-tests and normal distribution fitting.

RESULTS

Monte Carlo simulations effectively captured the central tendency, the spread and the variability of the original EEG traces. The real data distributions were closely approximated by the fitted normal distributions, indicating that the simulations accurately reflected the underlying statistical properties. The simulated variances closely aligned with those of the real data, staying within acceptable margins. Visual comparisons further highlighted the alignment between real and simulated data. Histograms demonstrated that the simulated data mirrored the shape and density of the real data, with only minor deviations observed in the tails for a few columns. Boxplots showed significant overlap in medians and interquartile ranges between the real and simulated data, underscoring the reliability of the modeling process. Moreover, the synthetic data successfully replicated the presence of outliers seen in the real dataset, further demonstrating its ability to reflect the inherent variability and complexity of the original data.

The Monte Carlo analysis revealed significant differences between the high-IQ and low-IQ groups in certain EEG channels (see **Table**). The t-tests and the Welch's t-tests identified specific EEG channels where the groups exhibited statistically significant differences ($p<0.05$). Strong differences in activity levels were observed in left [FP1] and right [FP2] prefrontal regions. Channels [F3] and [F4] also showed significant differences in regions associated with higher-order cognition and decision-making. Central channels such as [CZ] exhibited marked differences, reflecting motor or cognitive integration, with [C3] and [C4] showing additional activity differences in motor-related regions. Temporal channels [T3], [T4] and [T5] demonstrated significant differences in regions linked to memory and language processing, while the occipital channel [O2] revealed distinctions in visual processing areas.

On the other hand, some EEG channels exhibited overlapping distributions between the groups, suggesting no significant differences in activity. These included [P3] and [PZ] in the posterior parietal regions, [T6] in the temporal region and [F7] in the left frontal area, where activity patterns appeared similar across groups.

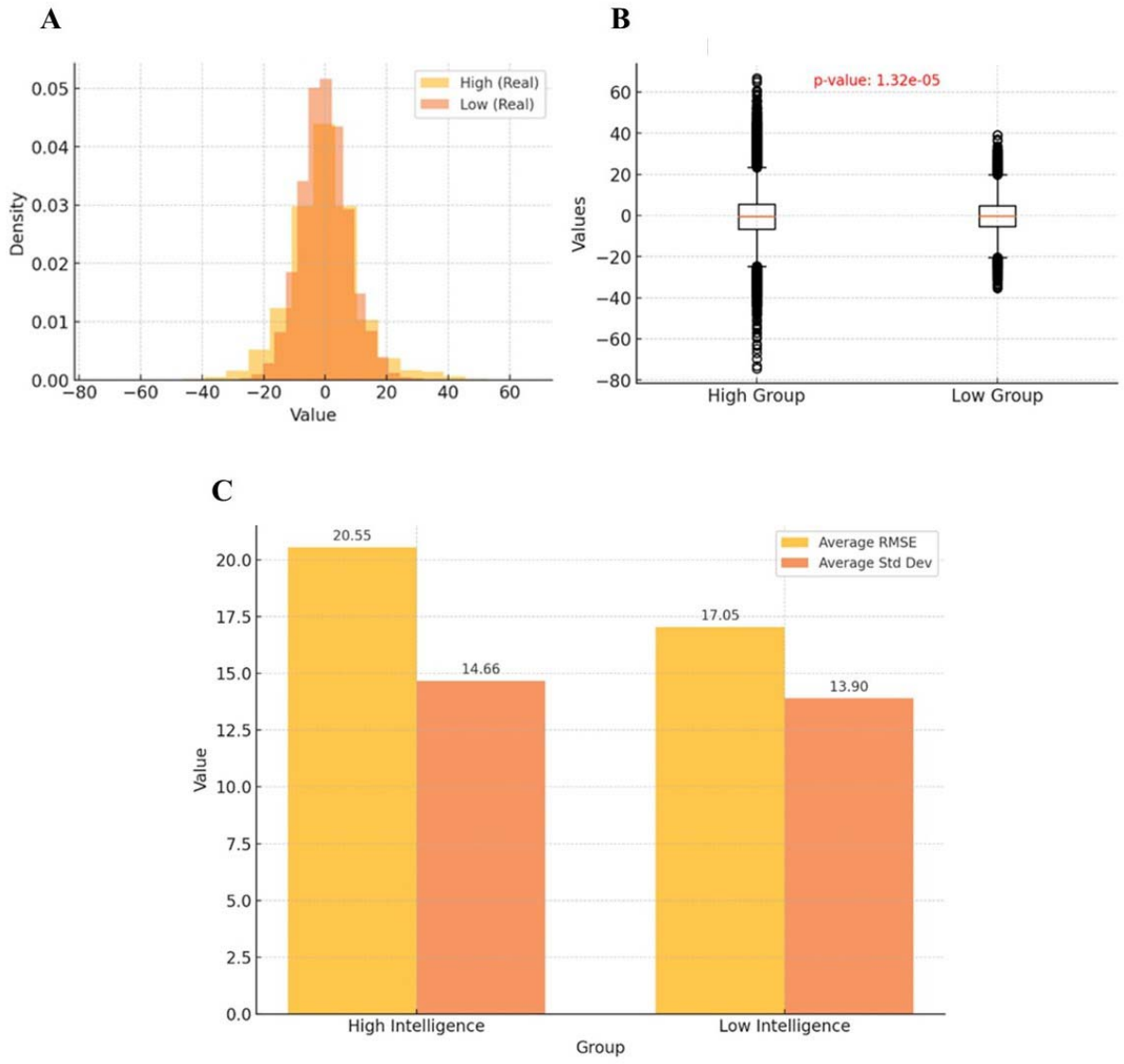
In sum, significant differences between the high-IQ and low-IQ groups were found in medians and interquartile ranges for various channels. These findings suggest key areas where these groups differ in their distributions.

EEG Channel	p-Value	Significance
[FP1]	1.13e-20	Significant
[FP2]	3.11e-13	Significant
[F3]	1.13e-07	Significant
[F4]	8.86e-12	Significant
[C3]	8.28e-06	Significant
[C4]	3.77e-05	Significant
[P3]	0.741	Not Significant
[P4]	0.019	Significant
[O1]	0.558	Not Significant
[O2]	1.32e-05	Significant
[F7]	0.209	Not Significant
[F8]	4.78e-06	Significant
[T3]	0.002	Significant
[T4]	1.24e-19	Significant
[T5]	7.28e-16	Significant
[T6]	0.805	Not Significant
[CZ]	0.000368	Significant
[FZ]	1.33e-23	Significant
[PZ]	0.904	Not Significant

Table. Statistical Differences Across EEG Channels Between High-IQ and Low-IQ Groups.

Visual comparisons provided further insights. The histograms highlighted distinct peaks or shifts between the two groups in significant channels, aligning with the statistical tests and confirming the observed differences (**Figure A**). The boxplots showed that the high-IQ group data had larger variability in significant channels, reflected in wider interquartile ranges, while the low-IQ group displayed more consistent and narrower distributions (**Figure B**). These patterns suggest that significant channels may serve as neural markers differentiating cognitive abilities between the groups.

The comparison of the predictability between the high-IQ and low-IQ groups revealed distinct insights (**Figure C**). RMSE values were generally lower for the low-IQ group, indicating better alignment between real and simulated data and suggesting higher predictability in the EEG traces of less intelligent individuals. Standard deviations were also slightly smaller for the low-IQ group across several EEG channels, further supporting its greater predictability. In contrast, the high-IQ group exhibited greater variability and larger RMSE values, which are indicative of patterns of reduced predictability in Monte Carlo simulations.



Figures A-B. A visual comparison of Monte Carlo simulations showcasing the graphical representation of a single electrode [O2] as a representative example from the set of 19 electrodes. The histogram comparison (**Figure A**) highlights the differences in real distribution between high-IQ and low-IQ groups across each column. The corresponding boxplot (**Figure B**) visually compares the distributions of the two groups across each column. Differences in medians, interquartile ranges and potential outliers can be identified. Statistical significance is determined based on p-values obtained through t-tests.

Figure C. Predictability of EEG traces via Monte Carlo simulation. This panel indicates that the EEG behaviour of the low-IQ group is more predictable, as reflected by their lower average RMSE and slightly smaller standard deviation compared to the high-IQ group.

DISCUSSION

The results of this study underscore the remarkable utility of Monte Carlo simulations in modeling EEG traces and identifying significant differences between high-IQ and low-IQ groups. By accurately replicating the statistical properties of the original dataset, the simulated data closely mirrored the observed real patterns in terms of central tendencies, variability and overall distribution shapes. Our analysis revealed pronounced statistical distinctions in specific EEG channels, which suggest potential markers of cognitive ability. For instance, the differences observed in frontal regions [FP1] and [FP2] align with their roles in executive functions, attention and problem-solving. The high-IQ group exhibited greater variability in these areas, possibly reflecting more dynamic or complex neural processes. The temporal regions [T3] and [T4] showed disparities that could indicate differences in memory retrieval and language processing between high-IQ and low-IQ groups. Similarly, occipital regions, particularly [O2], revealed distinctions in visual-spatial processing capabilities. At the same time, non-significant channels like [P3] and [PZ] in the posterior parietal regions, [T6] in the temporal region and [F7] in the left frontal area pointed to areas where further exploration or alternative modeling approaches may be necessary.
The low-IQ group exhibited higher predictability during Monte Carlo simulations, as demonstrated by lower RMSE values between real and simulated data and smaller standard deviations in several EEG channels. The less intelligent subjects generally displayed more consistent EEG patterns, particularly in central and temporal regions, aligning with their reduced variability and narrower distribution ranges. These patterns suggest a level of neural uniformity in the low-IQ group, contrasting with the broader variability, the higher RMSE values and the increased unpredictability observed in high-IQ individuals' EEG activity.

We demonstrated that Monte Carlo simulations serve as a powerful tool in neurocognitive research, enabling the identification of EEG markers associated with cognitive abilities and the simulation of neural activity patterns to test hypotheses about brain function. Practical applications of these findings extend to predictive modeling, where simulated EEG data can forecast cognitive behaviors. In clinical settings, these insights might inform interventions for cognitive enhancement or rehabilitation. Additionally, educational strategies could be tailored based on neural markers of learning potential. This study has limitations. While the assumption of normality proved valid for most EEG channels, deviations in skewness or kurtosis in some columns may have influenced results, highlighting the need for further research. Future studies should consider employing non-parametric methods or fitting alternative distributions to enhance the robustness of simulations. Investigating multivariate correlations across channels could provide deeper insights into the neural interconnections underlying intelligence. Future research should also explore the integration of alternative modeling techniques to further refine the accuracy and applicability of these methods and deepen our understanding of the neural underpinnings of human cognition.

In conclusion, the implications of this research in the field of cognitive neuroscience go beyond academic interest, underscoring the potential of combining advanced statistical techniques with neuroscience to unlock new possibilities for studying and enhancing human cognition. In particular, Monte Carlo simulations leverage the power of computational modeling to explore the complexities of human intelligence, offering robust methods for expanding datasets, uncovering underlying patterns and identifying key neural markers.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.
Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal, and has not been previously published.
Availability of data and materials. all data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Competing interests. The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.
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Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical, and material support, study supervision.

Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT to assist with data analysis and manuscript drafting. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

REFERENCES

1) Friedman, Nir, Tomer Fekete, Kobi Gal, and Oren Shriki. "EEG-Based Prediction of Cognitive Load in Intelligence Tests." *Frontiers in Human Neuroscience*, June 11, 2019. https://doi.org/10.3389/fnhum.2019.00191.
2) Georgieva, P., Mihaylova, L., Silva, F., Milanova, M., Figueiredo, N., Jain, L.C. (2013). A Sequential Monte Carlo Approach for Brain Source Localization. In: Georgieva, P., Mihaylova, L., Jain, L. (eds) Advances in Intelligent Signal Processing and Data Mining. Studies in Computational Intelligence, vol 410. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-28696-4_5
3) Hassani, Malihe, and Mohammad Reza Karami. "Noise Estimation in Electroencephalogram Signal by Using Volterra Series Coefficients." *Journal of Medical Signals and Sensors* 5, no. 3 (July–September 2015): 192–200. https://doi.org/10.4103/2228-7477.161495.
4) Herdman, Anthony T. "SimMEEG Software for Simulating Event-Related MEG and EEG Data with Underlying Functional Connectivity." *Journal of Neuroscience Methods* 350 (February 15, 2021): 109017. https://doi.org/10.1016/j.jneumeth.2020.109017.
5) Hettiarachchi, Imali, Shady Mohamed, and Saeid Nahavandi. "A Marginalised Markov Chain Monte Carlo Approach for Model-Based Analysis of EEG Data." In *2012 9th IEEE International Symposium on Biomedical Imaging (ISBI)*, 2–5 May 2012. IEEE Xplore, July 12, 2012.
6) Jahidin, A. H., M. N. Taib, N. M. Tahir, M. S. A. Megat Ali, and S. Lias. "Asymmetry Pattern of Resting EEG for Different IQ Levels." *Procedia - Social and Behavioral Sciences* 97 (November 6, 2013): 246–51. https://doi.org/10.1016/j.sbspro.2013.10.225.
7) Jaušovec, N., and K. Jaušovec. "Differences in Event-Related and Induced Brain Oscillations in the Theta and Alpha Frequency Bands Related to Human Intelligence." *Neuroscience Letters* 293, no. 3 (2000): 191–94. https://doi.org/10.1016/s0304-3940(00)01526-3.
8) Jaušovec, N., and K. Jaušovec. "Differences in EEG Current Density Related to Intelligence." *Cognitive Brain Research* 12, no. 1 (2001): 55–60. https://doi.org/10.1016/S0926-6410(01)00029-5.
9) Jaušovec, N., and K. Jaušovec. "Spatiotemporal Brain Activity Related to Intelligence: A Low Resolution Brain Electromagnetic Tomography Study." *Brain Research Cognitive Brain Research* 16, no. 2 (2003): 267–72. https://doi.org/10.1016/s0926-6410(02)00282-3.
10) Jaušovec, N., and K. Jaušovec. "Sex Differences in Brain Activity Related to General and Emotional Intelligence." *Brain and Cognition* 59, no. 3 (2005): 277–86. https://doi.org/10.1016/j.bandc.2005.08.001.
11) Jaušovec, N., and K. Jaušovec. "Emotional Intelligence and Gender: A Neurophysiological Perspective." In *Handbook of Individual Differences in Cognition*, edited by A. Gruszka, G. Matthews, and B. Szymura, 109–26. New York: Springer, 2010.
12) Jones, James Harvey, and Neal Fleming. "Simulation with Monte Carlo Methods to Focus Quality Improvement Efforts on Interventions with the Greatest Potential for Reducing PACU Length of Stay: A Cross-Sectional Observational Study." *BMJ Open Quality* 13, no. 4 (November 13, 2024): e002947. https://doi.org/10.1136/bmjoq-2024-002947.
13) Liu, Arthur K., Anders M. Dale, and John W. Belliveau. "Monte Carlo Simulation Studies of EEG and MEG Localization Accuracy." *Human Brain Mapping* 16, no. 1 (2002): 47–62. https://doi.org/10.1002/hbm.10024.
14) Lu, Runhao, Jie Xi, Xingli Zhang, and Jiannong Shi. "High Fluid Intelligence Is Characterized by Flexible Allocation of Attentional Resources: Evidence from EEG." *Neuropsychologia* 164 (January 7, 2022): 108094. https://doi.org/10.1016/j.neuropsychologia.2021.108094.
15) Luo, Song, Rui Chen, Zhengting Yang, and Kun Li. "Intelligence Level Might Be Predicted by the Characteristics of EEG Signals at Specific Frequencies and Brain Regions." *Journal of Mechanics in Medicine and Biology* 21, no. 9 (2021): 2140047. https://doi.org/10.1142/S021951942140047X.
16) Metropolis, N., and S. Ulam. "The Monte Carlo Method." *Journal of the American Statistical Association* 44, no. 247 (1949): 335–41.
17) Rubinstein, R. Y., and D. P. Kroese. *Simulation and the Monte Carlo Method*. Wiley Series in Probability and Statistics, 2016.

18) Salvadori, Julien, Antoine Merlet, Benoit Presles, Jorge Cabello, Kuan-Hao Su, Alexandre Cochet, Ane Etxebeste, Jean-Marc Vrigneaud, and David Sarrut. "PET Digitization Chain for Monte Carlo Simulation in GATE." *Physics in Medicine and Biology* 69, no. 16 (August 2, 2024). https://doi.org/10.1088/1361-6560/ad638c.

19) Santarnecchi, Emiliano, Arjun R. Khanna, Christian S. Musaeus, Christopher S. Y. Benwell, Paula Davila, Faranak Farzan, Santosh Matham, Alvaro Pascual-Leone, and Mouhsin M. Shafi; Honeywell SHARP Team. "EEG Microstate Correlates of Fluid Intelligence and Response to Cognitive Training." *Brain Topography* 30, no. 4 (July 2017): 502–20. https://doi.org/10.1007/s10548-017-056.
20) Singh, K. D., I. E. Holliday, P. L. Furlong, and G. F. A. Harding. "Evaluation of MRI-MEG/EEG Co-registration Strategies Using Monte Carlo Simulation." *Electroencephalography and Clinical Neurophysiology* 102, no. 2 (1997): 81–85. https://doi.org/10.1016/S0921-884X(96)96570-4.

21) Thatcher, R. W., D. North, and C. Biver. "EEG and Intelligence: Relations between EEG Coherence, EEG Phase Delay, and Power." *Clinical Neurophysiology* 116, no. 9 (September 2005): 2129–41. https://doi.org/10.1016/j.clinph.2005.04.026.
22) Thatcher, R. W., E. Palmero-Soler, D. M. North, and C. J. Biver. "Intelligence and EEG Measures of Information Flow: Efficiency and Homeostatic Neuroplasticity." *Scientific Reports* 6 (December 20, 2016): 38890. https://doi.org/10.1038/srep38890.
23) Tozzi, A., E. Bormashenko, and N. Jausovec. "Topology of EEG Wave Fronts." *Cognitive Neurodynamics* 15 (2021a): 887–96. https://doi.org/10.1007/s11571-021-09668-z.
24) Tozzi, A., J. F. Peters, N. Jausovec, A. P. H. Don, S. Ramanna, I. Legchenkova, and E. Bormashenko. "Nervous Activity of the Brain in Five Dimensions." *Biophysica* 1, no. 1 (2021b): 38–47. https://doi.org/10.3390/biophysica1010004.
25) van Dellen, Edwin, Hanneke de Waal, Wiesje M. van der Flier, Afina W. Lemstra, Arjen J. C. Slooter, Lieke L. Smits, Elisabeth C. W. van Straaten, Cornelis J. Stam, and Philip Scheltens. "Loss of EEG Network Efficiency Is Related to Cognitive Impairment in Dementia With Lewy Bodies." *Movement Disorders* 30, no. 13 (November 2015): 1785–93. https://doi.org/10.1002/mds.26309.

Fractional and Geometric Neural Dynamics: Investigating Intelligence-Related Differences in EEG Symmetry and Connectivity

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

Ksenija Jaušovec
University of Maribor, Department of Psychology
ksenijamarijausovec@gmail.com

ABSTRACT

Understanding intelligence-related variations in electroencephalographic (EEG) activity requires advanced mathematical approaches capable of capturing geometric transformations and long-range dependencies in neural dynamics. These approaches may provide methodological advantages over conventional spectral and connectivity-based techniques by offering deeper insights into the structural and functional organization of neural networks. In this study, we integrate Clifford algebra, Noether's theorem and fractional calculus to analyze EEG signals from high- and low-IQ individuals, looking for key intelligence-related differences in cortical organization. Clifford algebra enables the representation of EEG signals as multivectors, preserving both magnitude and directional relationships across cortical regions. Noether's theorem provides a quantitative measure of symmetry properties linked to spectral features, identifying conserved functional patterns across distinct brain regions. Mittag-Leffler functions, derived from fractional calculus, characterize long-range dependencies in neural oscillations, allowing for the detection of memory effects and scale-invariant properties often overlooked by traditional methods. We found significant differences between high- and low-IQ individuals in geometric trajectories, hemispheric connectivity, spectral properties and fractional-order dynamics. High-IQ individuals exhibited increased spectral asymmetry, enhanced spectral differentiation, distinct geometric trajectories and greater fractional connectivity, particularly in frontal and central regions. In contrast, low-IQ individuals displayed more uniform hemispheric connectivity and heightened fractional activity in occipital areas. Mittag-Leffler fractional exponents further indicated that high-IQ individuals possessed more varied neural synchronization patterns. Overall, our multi-faceted approach suggests that intelligence-related neural dynamics are characterized by an asymmetric, functionally specialized and fractionally complex cortical organization. This results in significant differences in network topology, efficiency, modularity and long-range dependencies.

KEYWORDS: neural oscillations; functional asymmetry; graph theory; cognitive variability; brain network topology.

INTRODUCTION

Intelligence has been associated with distinct electroencephalographic (EEG) neural patterns, yet the underlying mechanisms remain a subject of investigation (Thatcher et al., 2005; Friedman et al., 2019). The study of intelligence-related differences in EEG signals has traditionally relied on spectral, time-frequency and connectivity analyses (Chen et al., 2023; Ignatious et al., 2023). Conventional approaches such as Fourier and wavelet transforms, coherence measures and graph-theoretic network analyses have provided critical insights into brain function but lack the mathematical depth to fully capture neural relationships (Sitnikova et al., 2009; San-Segundo et al., 2019). We argue that mathematical frameworks integrating algebraic, geometric, and fractional dynamics provide an alternative perspective by capturing spatial dependencies and characterizing long-range memory effects in neural activity. Among these approaches, Clifford algebra has been used to encode EEG signals as multivectors, preserving both magnitude and directional relationships across cortical regions—an advantage not achievable with conventional spectral methods (Zhang et al., 2023). Clifford algebra-based EEG transformations allow for the preservation of geometric properties enabling novel trajectory-based comparisons. Noether's theorem, which relates system symmetries to conserved quantities, has been used to assess functional and spectral balance in cortical microcircuitry, revealing conserved functional patterns across different cortical regions (Bilteanu et al., 2017). Meanwhile, fractional calculus — particularly Mittag-Leffler function analysis— may extend EEG signal characterization beyond integer-order models, allowing for the detection of memory effects and scale-invariant properties often overlooked by standard methods (Atanackovic et al., 2011; Turalska and West, 2018). Functional order analysis provides an additional layer of insight by quantifying fractional dynamics and long-range dependencies (García-Raffi and Torrano, 2021).

Together, these multi-faceted approaches may enable a refined investigation into intelligence-related brain dynamics extending beyond pairwise spectral differences, allowing for a richer assessment of interregional dependencies. We

conjecture that our approach might reveal systematic differences in EEG structure, with high-IQ individuals exhibiting greater frontal asymmetry, enhanced connectivity in integrative brain areas and increased fractional-order complexity in neural synchronization. Low-IQ individuals, in contrast, might display more uniform hemispheric connectivity, potentially indicative of different cognitive resource allocation strategies.

In sum, given the limitations of traditional EEG methods, a hybrid approach that considers Clifford algebra, Noether's theorem and fractional network measures holds promise for more comprehensive analyses of intelligence. We will proceed as follows. The next section outlines the methodology, detailing data acquisition, preprocessing and the mathematical frameworks applied to EEG signals. We then present our results, followed by an interpretation of the findings in the context of intelligence-related neural organization. Finally, we conclude with a discussion on the broader implications and future directions of this research.

MATERIALS AND METHODS

Our retrospective study builds on prior research by Jaušovec and Jaušovec (2001; 2003; 2005; 2010) and later advancements with Tozzi et al. (2021a; 2021b), honoring Norbert Jaušovec's contributions. EEG data were collected from 10 right-handed participants (mean age: 19.8 years; SD = 0.9; males: 4), divided into High-IQ (IQ SD = 127) and Low-IQ (IQ SD = 87) groups based on standardized test scores during auditory and visual oddball tasks. EEG signals were recorded using a 64-channel system with electrodes placed according to the 10–20 system. Data epochs were segmented into non-overlapping 2-second windows and trials contaminated with excessive noise were excluded based on an amplitude threshold exceeding ±100 µV. Standardization was performed by normalizing each EEG signal relative to its mean and standard deviation across trials, ensuring comparability between participants.

Clifford algebra. Clifford algebra was used to transform EEG signals into multivector representations that preserve spatial and directional relationships between cortical regions (Acus and Dargys, 2024). Each EEG signal $x_i(t)$ from electrode *i* was mapped onto a Clifford multivector $\Psi(t)$, expressed as

$$\Psi(t) = \sum_i v_i(t) e_i$$

where $v_i(t)$ is the voltage amplitude and e_i represents a basis vector of a Clifford algebra. The choice of Clifford space dimensionality depended on the number of selected EEG channels. In our study, a three-dimensional Clifford space $Cl(3,0)$ was chosen to encode signals from three representative electrode locations (FP1, P3, O1). These three electrodes were selected due to their known involvement in cognitive functions and information processing. The transformed EEG signals were then analyzed geometrically by computing trajectory deviations across trials. The Clifford Fourier Transform (CFT) was used to extract spectral features within the multivector space (Monaim and Fahlaoui, 2024). Given a time-dependent multivector $\Psi(t)$, its frequency-domain representation was obtained as

$$\tilde{\Psi}(\omega) = \int \Psi(t) e^{-e_1 \omega t} dt$$

where e_1 is an imaginary unit in Clifford space. Statistical comparisons of Clifford trajectories were performed using paired t-tests to determine significant differences in neural geometry between high and Low-IQ groups.

Noether's theorem. Noether's theorem was used to quantify symmetry in functional connectivity. Functional connectivity was evaluated using Pearson correlation coefficients between electrode pairs, computed as

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2} \sqrt{\sum (y_i - \bar{y})^2}}$$

where x_i and y_i represent EEG signals from different electrodes and $\bar{x}$ and $\bar{y}$ are their means over time. Connectivity matrices were constructed for each participant, with a focus on left-right hemisphere electrode pairs (FP1–FP2, F3–F4, C3–C4, P3–P4, O1–O2) to assess hemispheric symmetry. Symmetry indices were calculated as

$$S = \frac{|PSD_L - PSD_R|}{PSD_L + PSD_R}$$

where PSD_L and PSD_R are power values from corresponding left and right hemisphere electrodes. Spectral asymmetry was assessed by performing a Fourier transform on each EEG channel and comparing power distributions between hemispheres (Atiyah et al., 1975). Welch's t-tests were applied to compare connectivity and spectral symmetry indices between high and Low-IQ groups.

To assess the relationship between frequency band power and hemispheric symmetry, correlation analyses were performed for each frequency band. Power spectral densities (PSDs) were computed to assess frequency distributions in delta, theta, beta and alpha bands (Dressler et al., 2004; Redwan et al., 2024). Pearson correlation coefficients were then calculated between frequency band power and hemispheric symmetry indices to determine whether specific oscillations were associated with neural organization patterns.

To evaluate the temporal stability of EEG signals, stationarity tests were conducted using the Augmented Dickey-Fuller (ADF) test, which determines whether a time series exhibits long-term trends or remains stable over time (Dao and Staszewski, 2021). ADF p-values below 0.05 indicated significant stationarity, suggesting a consistent neural state.

Fractional derivative values. Fractional-order derivatives were computed to characterize the temporal dynamics of EEG signals, as conventional differentiation does not account for memory effects or long-range dependencies inherent in neural activity. The Caputo fractional derivative was chosen, defined as

$$D^{\alpha} f(t) = \frac{1}{\Gamma(1-\alpha)} \int_0^t \frac{f'(\tau)}{(t-\tau)^{\alpha}} d\tau$$

where α represents the fractional order and Γ denotes the Gamma function. The parameter α was optimized based on the signal's spectral properties to capture its underlying fractional nature effectively. Numerical implementation was carried out using the Grünwald-Letnikov approximation, which approximates the integral definition with a discrete summation:

$$D^{\alpha} f(n) = \sum_{k=0}^{n} (-1)^k \binom{\alpha}{k} f(n-k)$$

where $\binom{\alpha}{k}$ represents the binomial coefficient extended to non-integer orders. This method provided a means of assessing long-range dependencies in EEG data, differentiating between stationary and non-stationary signal properties. The fractional derivative values were extracted for each EEG channel and used in subsequent statistical analyses to identify group-wise differences in neural activity. These calculations formed the basis for evaluating the Mittag-Leffler function's role in neural dynamics, establishing a mathematical framework for comparing cognitive groups.

Fractional connectivity and fractional clustering coefficients. To examine functional connectivity, correlation-based adjacency matrices were constructed by computing pairwise cross-correlations between EEG channels. Cross-correlation was defined as

$$C_{xy}(\tau) = \sum_t x(t) y(t+\tau),$$

where $x(t)$ and $y(t)$ are EEG signals from two channels and τ represents the lag. A sliding window approach was used to ensure robust estimation of functional connectivity over time, with overlapping one-second segments applied to account for dynamic variations in brain activity.

To integrate fractional-order concepts into network analysis, fractional connectivity and fractional clustering coefficients were computed. Fractional connectivity was derived by modifying edge weights using a power-law transformation, where each connection strength W_{ij} was raised to a fractional exponent γ, yielding W_{ij}^{γ}. The mean fractional connectivity was then calculated by averaging these transformed values, providing a metric sensitive to long-range dependencies in neural networks.

The fractional clustering coefficient was computed using a modified version of the standard clustering measure, incorporating the fractional-weighted edges to assess local connectivity structures. This was defined as

$$C_i^{\gamma} = \frac{1}{k_i(k_i-1)} \sum_{j,k} (W_{ij} W_{ik} W_{jk})^{\gamma/3}$$

where k_i is the degree of node i. These fractional measures allowed for a more nuanced characterization of network topology, enabling differentiation between classical small-world organization and fractional network configurations.

The ability to quantify connectivity and clustering using fractional-order metrics provided insights into how neural interactions vary across cognitive groups, extending conventional graph-theoretic approaches.

Mittag-Leffler fractional exponents. Mittag-Leffler fractional exponents were estimated to describe the scaling behavior of connectivity weights within the network (García-Raffi and Torrano, 2021). The Mittag-Leffler function, defined as

$$E_{\alpha,\beta}(z) = \sum_{k=0}^{\infty} \frac{z^k}{\Gamma(\alpha k+\beta)},$$

generalizes exponential decay and captures memory effects in complex systems (Tarasov 2018). To estimate the effective fractional exponent, the probability distribution of connectivity weights was examined in log-log space and a power-law function

$$P(W) \sim W^{-\lambda}$$

was fitted to the data. The exponent λ was extracted using least-squares fitting, providing a measure of the heterogeneity in connectivity distributions. Higher values of λ indicated a broader range of connectivity strengths, reflecting a more diverse interaction pattern across EEG channels. This Mittag-Leffler-based analysis was used to assess differences in the scaling properties of functional networks between groups, serving as a crucial parameter for understanding cognitive variability.

Statistical analyses were performed to compare fractional network measures between high and Low-IQ groups. One-way analysis of variance (ANOVA) was conducted to test for significant differences in fractional connectivity, fractional clustering and Mittag-Leffler exponents across groups. The Bonferroni correction was applied to account for multiple comparisons and control the false discovery rate.

Tools. Computational analyses were implemented using Python, utilizing NumPy for numerical computations, SciPy for statistical analysis, Matplotlib for visualization and the MNE-Python toolbox for EEG preprocessing. Clifford algebra computations were performed using the ClPy library and Fourier transforms were applied using SciPy's signal processing module.

RESULTS

Significant differences in geometric trajectories, hemispheric connectivity, spectral properties and fractional-order dynamics were found between high and Low-IQ individuals, with High-IQ individuals exhibiting increased Clifford algebra-based frontal variability, greater frontal asymmetry and enhanced spectral differentiation.

Clifford algebra. Clifford algebra-based transformations displayed distinct EEG trajectory patterns, with High-IQ individuals exhibiting increased geometric variability in the frontal region, while Low-IQ individuals exhibited more constrained neural trajectories (**Figure 1A**). Statistical comparisons of Clifford components indicated significant differences in frontal representations between groups ($p < 0.001$), whereas no significant differences were found in parietal and occipital components (**Figure 1B**). Temporal stationarity analyses using the Augmented Dickey-Fuller test indicated that EEG signals in High-IQ individuals were more stationary ($p < 0.05$), suggesting greater consistency in neural oscillations.

Spectral power analysis using the Clifford Fourier Transform showed that High-IQ individuals exhibited significantly higher alpha ($p < 0.001$) and beta ($p = 0.0067$) power in the frontal region compared to Low-IQ individuals, suggesting different neural activation patterns. Power spectral densities showed that High-IQ individuals had significantly higher theta ($p = 0.0065$) and beta ($p = 0.0067$) power, while Low-IQ individuals exhibited relatively stronger delta oscillations.

Noether's theorem. Connectivity analysis based on Noetherian symmetry principles and further analyses of spectral symmetry revealed significant differences in hemispheric organization. High-IQ individuals displayed greater asymmetry in frontal connectivity (FP1-FP2, $p < 0.001$) and enhanced symmetry in occipital connectivity (O1-O2, $p < 0.001$), while Low-IQ individuals maintained more uniform hemispheric connectivity, particularly in prefrontal and parietal regions (**Figure 1C**).

Fractional dynamics. Statistical analysis of Mittag-Leffler fractional derivatives revealed distinct patterns of neural activity between high- and low-IQ individuals. High-IQ individuals consistently exhibited higher fractional values in frontal (FP1, F4) and central (C3) regions, while low-IQ individuals showed increased fractional activity in the occipital (O1) area (**Figure 2A**). Notably, the frontal regions displayed significantly lower fractional-order dynamics in the low-IQ group, suggesting that high-IQ individuals engage in neural processes with stronger memory effects and long-range dependencies, potentially supporting cognitive flexibility and problem-solving.

Beyond localized effects, significant differences were also observed in central and parietal channels, indicating that intelligence-related variability extends across broader network properties rather than being confined to specific brain regions. The occipital region exhibited an inverse trend, with low-IQ individuals displaying higher Mittag-Leffler fractional activity, possibly reflecting distinct neural connectivity patterns or alternative sensory integration strategies.
Furthermore, greater variability in fractional activity was observed in some channels within the low-IQ group, as indicated by wider distribution ranges. This suggests increased dispersion in fractional-order neural activity, which may correspond to less stable or less efficient network dynamics.

Fractional network measures. The comparison of fractional network measures between high and Low-IQ groups is illustrated in **Figure 2B**. Fractional connectivity exhibited significant differences between groups, with High-IQ individuals showing an higher mean fractional connectivity. Similarly, fractional clustering coefficients were higher in the High-IQ group. Mittag-Leffler fractional exponents demonstrated a broader distribution in the High-IQ group, with a mean exponent value lower in the Low-IQ group. Further, High-IQ individuals exhibited greater variability in fractional connectivity values. Weighted global efficiency was significantly higher in the High-IQ group, suggesting differences in the efficiency of information transfer across neural networks. This set of findings highlights a more complex and distributed network topology in individuals with High-IQ, underlining the relevance of fractional measures in characterizing cognitive differences. Small-worldness, defined as the ratio of clustering coefficient to characteristic path length, was significantly greater in the High-IQ group. This suggests that High-IQ individuals exhibit a more optimized network structure, balancing local clustering with global efficiency. Additionally, edge weight distribution followed a broader power-law decay in the High-IQ group, consistent with more heterogeneous connectivity patterns.
The Mittag-Leffler-based scaling exponent analysis further corroborated these findings, as High-IQ individuals demonstrated greater deviation from exponential connectivity decay, indicating a higher degree of long-range dependencies in neural interactions. These variations were particularly evident in the frontal and occipital regions, where High-IQ individuals displayed enhanced connectivity strengths. Differences in network modularity also emerged, with High-IQ individuals exhibiting higher modularity scores, suggesting a more functionally segregated yet highly interactive network.
Overall, these findings suggest that fractional network properties, including connectivity, clustering and Mittag-Leffler exponents, may distinguish high and Low-IQ groups in a statistically significant manner.

Summarizing, our findings, confirmed through Clifford algebra transformations, spectral analysis and Noetherian symmetry principles, suggest that High-IQ individuals exhibit greater frontal EEG asymmetry, increased spectral differentiation and distinct connectivity patterns, greater geometric variability, greater frontal asymmetry, enhanced connectivity in integrative regions and increased long-range dependencies, while Low-IQ individuals displayed more uniform hemispheric connectivity and heightened activity in occipital areas. These findings suggest that intelligence-related neural dynamics are characterized by asymmetric, functionally specialized and fractionally complex cortical organization.

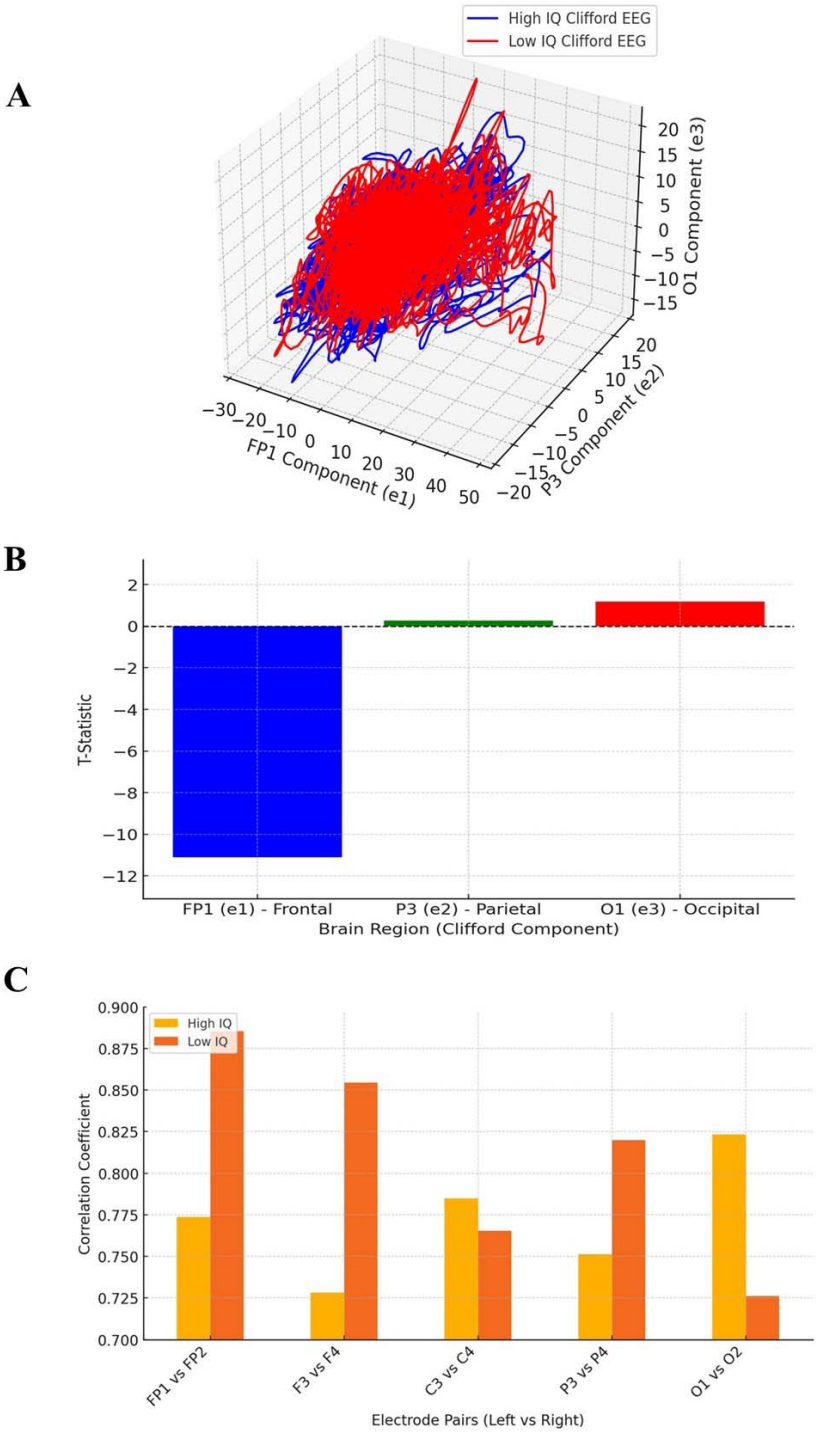

Figure 1A. Clifford EEG Signal representation for High vs. Low-IQ groups. The blue and red lines represent average EEG trajectories in Clifford space for high and Low-IQ individuals, respectively. Differences in shape, spread and oscillatory patterns provide insight into IQ-related brain activity variations.

Figure 1B. Statistical differences in Clifford EEG components. Significant differences were observed in the frontal region, while parietal and occipital components showed no substantial variation. The dashed line at zero indicates the statistical significance threshold.

Figure 1C. Hemispheric symmetry differences across brain regions. Low-IQ individuals (green bars) exhibit greater symmetry in prefrontal, frontal and parietal regions, whereas High-IQ individuals (blue bars) exhibit increased asymmetry, particularly in FP1-FP2 and F3-F4. The occipital region (O1-O2) is more symmetric in High-IQ individuals, suggesting enhanced visual-spatial processing.

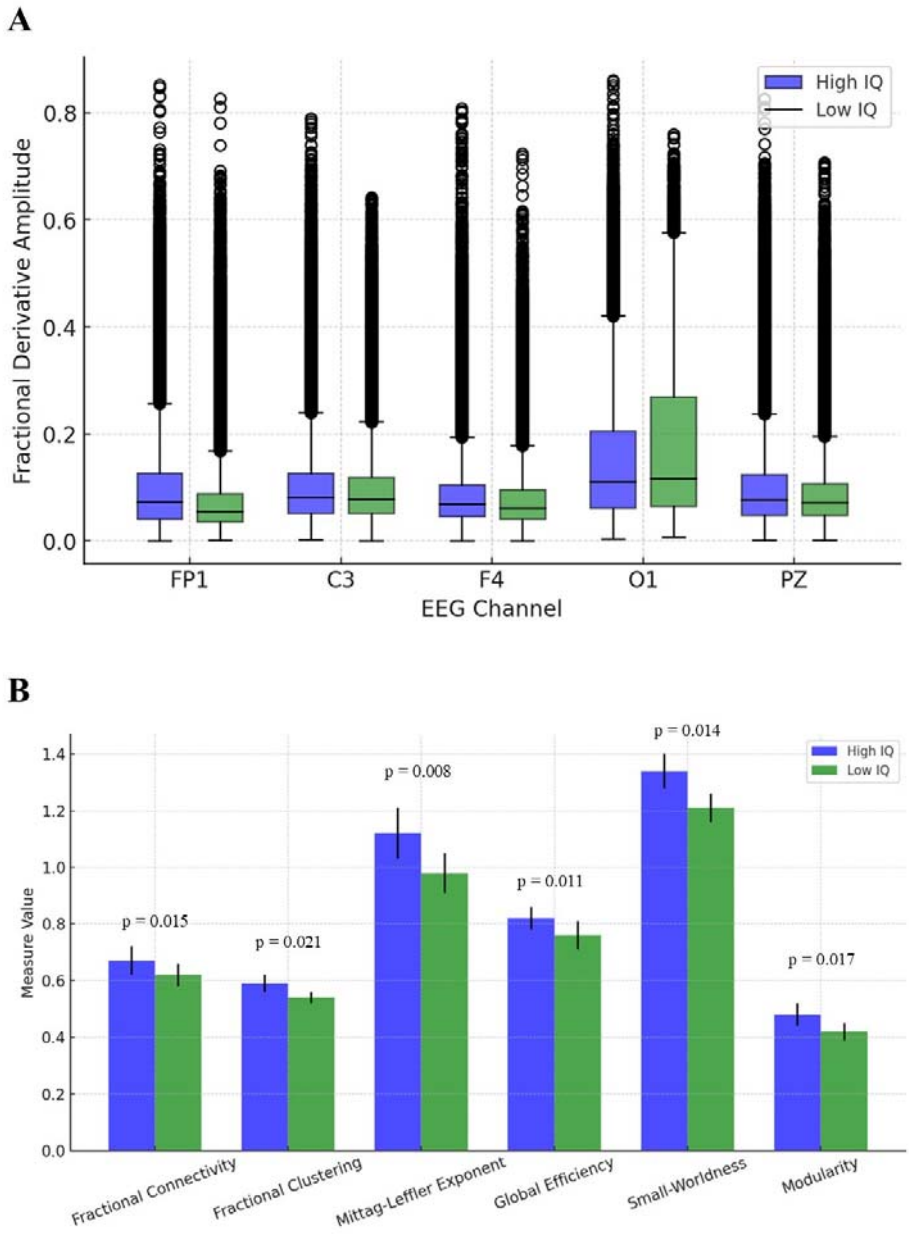

Figure 2A. Boxplot comparison illustrating key differences in fractional dynamics between High-IQ (blue) and Low-IQ (green) groups. The variations in distributions suggest significant differences in Mittag-Leffler function characteristics. Statistical analysis confirms that these distinctions are significant, with **$p < 0.0001$** across all analyzed channels.

Figure 2B. Comparison of fractional network measures between high and Low-IQ groups. Bars represent mean values for each group, with error bars indicating standard deviations. High-IQ individuals exhibit significantly greater fractional connectivity, clustering, Mittag-Leffler exponent, global efficiency, small-worldness and modularity compared to Low-IQ individuals. One-way ANOVA confirmed systematic distinctions in neural structure and connectivity patterns across groups.

CONCLUSIONS

We investigated intelligence-related differences in EEG signals by combining multiple mathematical approaches such as Clifford algebra, Noether's theorem and fractional network analysis. Functional connectivity analysis revealed that

high-IQ individuals exhibited a more differentiated neural network organization that was characterized by greater variability in interregional interactions and enhanced symmetrical connectivity in occipital regions. Fractional-order analyses demonstrated that High-IQ individuals exhibited greater long-range dependencies, with significantly higher Mittag-Leffler fractional exponents and clustering coefficients, supporting the presence of more complex and efficient neural architecture. This suggests that cognitive ability may be linked to distinct neural processing strategies, with High-IQ individuals displaying more complex fractional-order behavior in regions associated with executive function and working memory. In turn, Low-IQ individuals exhibited heightened activity in the occipital cortex, which may correspond to alternative visual processing mechanisms.
High-IQ individuals displayed stronger Mittag-Leffler dynamics in regions linked to complex cognitive processing, while Low-IQ individuals displayed greater activity in visual and sensory processing areas. These differences align with theories of cognitive efficiency and regional specialization, where High-IQ brains may rely more on integrative network structures, while Low-IQ brains may exhibit increased reliance on localized sensory processing. Fractional-order dynamics showed that the frontal and central brain regions displayed more Mittag-Leffler behavior in High-IQ individuals. The occipital region presented an inverse trend, where Low-IQ individuals showed greater fractional activity, suggesting distinct processing dynamics. Taken together, these findings suggest that intelligence-related brain function may be reflected in both geometric transformations and fractional connectivity properties.

The integration of Clifford algebra, Noether's theorem and Mittag-Leffler function-based fractional calculus stands for a novel approach to EEG analysis which offers methodological advantages over conventional spectral and connectivity-based techniques. Clifford algebra preserves magnitude and directional relationships between EEG signals, allowing for a richer representation of neural trajectories' geometric transformations. Noether's theorem introduces a principled framework for assessing conserved properties in brain networks, enabling the quantification of functional symmetry and intelligence-related differences in interregional connectivity patterns. Fractional network measures extend beyond traditional graph-theoretic approaches by capturing long-range dependencies and scale-invariant properties in neural oscillations. This may reveal subtle variations in brain dynamics that conventional EEG methodologies fail to detect and that are often overlooked in standard time-frequency analyses.

Compared to traditional EEG analysis techniques, our approach offers several advantages. Spectral analysis methods such as Fourier and wavelet transforms are widely used to decompose EEG signals into frequency components but do not retain spatial or directional relationships between electrodes (Yuan et al., 2018; Daud and Sudirman, 2022). Functional connectivity metrics, including coherence and phase synchronization, assess interregional interactions but often rely on pairwise comparisons that may overlook global network dependencies (Miskovic and Keil, 2015; Abdullateef et al., 2022). Graph-theoretic measures capture aspects of brain topology but do not explicitly incorporate long-range dependencies or fractional-order dynamics. In contrast, our combination of Clifford algebra, Noether's theorem and fractional network analysis preserves both local signal variations and global connectivity patterns. The inclusion of fractional calculus further enhances sensitivity to memory effects and non-stationary dynamics (Chen and Wang, 2020).

Our methodological framework has potential applications in different fields. EEG-based cognitive profiling could benefit from Clifford algebra transformations to characterize individual differences in neural geometry. The integration of Noether's theorem into EEG analysis could provide a means of quantifying hemispheric symmetry, which has implications for studying neurodevelopmental disorders. Fractional network measures could be employed in neurodegenerative research to monitor cognitive decline, as long-range dependencies and connectivity scaling properties may serve as biomarkers for early-stage disorders. The integration of Noether's theorem into EEG analysis may also contribute to the study of neurodevelopmental and neuropsychiatric disorders, where alterations in hemispheric symmetry have been linked to conditions such as autism spectrum disorder, schizophrenia and dyslexia (Saugstad, 1999; Gage et al., 2009; Guo et al., 2013; Perkins et al., 2014; He et al., 2023). Still, brain-computer interface technologies may enhance classification accuracy by utilizing fractional-order EEG features, which incorporate long-range memory effects into machine learning models (Tarasov 2018).
Beyond these applications, our approach suggests testable experimental hypotheses, such as: a) whether interventions like transcranial stimulation can modulate EEG symmetry in ways consistent with Noetherian principles; b) whether cognitive training enhances fractional connectivity measures over time; c) whether neural efficiency, as quantified by Clifford algebra-based geometric transformations, correlates with cognitive performance in different task-based EEG paradigms; d) whether the observed intelligence-related differences in EEG connectivity persist over time, particularly in relation to neuroplasticity and cognitive aging. These experimental extensions reinforce the potential of our framework in broadening the scope of nervous oscillations' research.

Despite its contributions, our study has several limitations. The sample size may limit the generalizability of findings and the robustness of observed differences. The computational complexity of Clifford algebra transformations presents a potential challenge, as real-time applications may require optimization to improve processing efficiency. While Noether's theorem provides a theoretical basis for assessing neural symmetry, its direct application to EEG data for the study of intelligence requires further validation. Still, the selection of fractional exponent parameters was optimized

based on empirical signal properties, but individual variations in neural dynamics may necessitate adaptive algorithms to improve robustness. Multimodal neuroimaging approaches, such as functional magnetic resonance imaging, could be incorporated to enhance spatial resolution and validate EEG-based symmetry findings.

In conclusion, we suggest that intelligence-related differences in EEG signals may be characterized using a combination of Clifford algebra, Noether's theorem and fractional network analysis. By integrating these advanced mathematical frameworks, a novel perspective on cognitive variability could be provided, highlighting the role of symmetry, geometric transformations and fractional-order properties in brain function.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.
Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal and has not been previously published.
Availability of data and materials. All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.
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Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

REFERENCES

1) Abdullateef, S., B. Jordan, V. Rae, A. McLellan, J. Escudero, V. Nenadovic and T. Lo. "Quantitative Detection of Seizures with Minimal-Density EEG Montage Using Phase Synchrony and Cross-Channel Coherence Amplitude in Critical Care." *Annual International Conference of the IEEE Engineering in Medicine and Biology Society* 2022 (July 2022): 259–262. https://doi.org/10.1109/EMBC48229.2022.9871595.
2) Acus, A. and A. Dargys. "Multivector (MV) Functions in Clifford Algebras of Arbitrary Dimension: Defective MV Case." *arXiv preprint* arXiv:2412.05730 [math-ph], 2024. https://doi.org/10.48550/arXiv.2412.05730.
3) Atanackovic, Teodor M., Stevan Pilipovic, Bogoljub Stankovic and Dušan Zorica. 2011. "Fractional Calculus with Applications in Mechanics: Wave Propagation, Impact and Variational Principles." *Mathematical Problems in Engineering* 2011: 298628. https://doi.org/10.1155/2011/298628.
4) Atiyah, M. F., V. K. Patodi and I. M. Singer. "Spectral Asymmetry and Riemannian Geometry. I." *Mathematical Proceedings of the Cambridge Philosophical Society* 77, no. 1 (1975): 43–69.
5) Bilteanu, L., M. F. Casanova and I. Opris. "Symmetry and Noether Theorem for Brain Microcircuits." In *The Physics of the Mind and Brain Disorders*, edited by I. Opris and M. F. Casanova, vol. 11, Springer Series in Cognitive and Neural Systems. Cham: Springer, 2017. https://doi.org/10.1007/978-3-319-29674-6_6.
6) Chen, Y. M. and J. R. Wang. 2020. "A High-Order Compact Finite Difference Scheme for the Time Fractional Black–Scholes Model." *Journal of Computational Physics* 409: 109333. https://doi.org/10.1016/j.jcp.2020.109333.
7) Chen, Di, Haiyun Huang, Xiaoyu Bao, Jiahui Pan and Yuanqing Li. "An EEG-Based Attention Recognition Method: Fusion of Time Domain, Frequency Domain and Non-Linear Dynamics Features." *Frontiers in Neuroscience* 17 (July 12, 2023). https://doi.org/10.3389/fnins.2023.1194554.
8) Cui, G., X. Li and H. Touyama. "Emotion Recognition Based on Group Phase Locking Value Using Convolutional Neural Network." *Scientific Reports* 13 (2023): 3769. https://doi.org/10.1038/s41598-023-3769.

9) Dao, P. B. and W. J. Staszewski. “Lamb Wave Based Structural Damage Detection Using Stationarity Tests.” *Materials (Basel)* 14, no. 22 (November 12, 2021): 6823. https://doi.org/10.3390/ma14226823.
10) Daud, S. N. S. S. and R. Sudirman. “Wavelet-Based Filters for Artifact Elimination in Electroencephalography Signal: A Review.” *Annals of Biomedical Engineering* 50, no. 10 (October 2022): 1271–1291. https://doi.org/10.1007/s10439-022-03053-5.
11) Dressler, O., G. Schneider, G. Stockmanns and E. F. Kochs. “Awareness and the EEG Power Spectrum: Analysis of Frequencies.” *BJA: British Journal of Anaesthesia* 93, no. 6 (December 2004): 806–809. https://doi.org/10.1093/bja/aeh270.
12) Friedman, Nir, Tomer Fekete, Kobi Gal and Oren Shriki. “EEG-Based Prediction of Cognitive Load in Intelligence Tests.” *Frontiers in Human Neuroscience* 13 (June 11, 2019). https://doi.org/10.3389/fnhum.2019.00191.
13) Gage, N. M., J. Juranek, P. A. Filipek, K. Osann, P. Flodman, A. L. Isenberg and M. A. Spence. “Rightward Hemispheric Asymmetries in Auditory Language Cortex in Children with Autistic Disorder: An MRI Investigation.” *Journal of Neurodevelopmental Disorders* 1, no. 3 (September 2009): 205–214. https://doi.org/10.1007/s11689-009-9010-2.
14) García-Raffi, L. M. and E. Torrano. 2021. “Mittag-Leffler Functions and Their Applications in Fractional Calculus.” *arXiv*, March 23, 2021. https://arxiv.org/abs/2103.12559.
15) Guo, S., K. M. Kendrick, J. Zhang, M. Broome, R. Yu, Z. Liu and J. Feng. “Brain-Wide Functional Inter-Hemispheric Disconnection Is a Potential Biomarker for Schizophrenia and Distinguishes It from Depression.” *NeuroImage: Clinical* 2 (June 23, 2013): 818–826. https://doi.org/10.1016/j.nicl.2013.06.008.
16) He, K., Q. Hua, Q. Li, Y. Zhang, X. Yao, Y. Yang, W. Xu, J. Sun, L. Wang, A. Wang, G. J. Ji and K. Wang. “Abnormal Interhemispheric Functional Cooperation in Schizophrenia Follows the Neurotransmitter Profiles.” *Journal of Psychiatry & Neuroscience* 48, no. 6 (November–December 2023): E452–E460. https://doi.org/10.1503/jpn.230037.

17) Jaušovec, N. and K. Jaušovec. "Differences in Event-Related and Induced Brain Oscillations in the Theta and Alpha Frequency Bands Related to Human Intelligence." *Neuroscience Letters* 293, no. 3 (2000): 191–94. https://doi.org/10.1016/s0304-3940(00)01526-3.
18) Jaušovec, N. and K. Jaušovec. "Differences in EEG Current Density Related to Intelligence." *Cognitive Brain Research* 12, no. 1 (2001): 55–60. https://doi.org/10.1016/S0926-6410(01)00029-5.
19) Jaušovec, N. and K. Jaušovec. "Spatiotemporal Brain Activity Related to Intelligence: A Low Resolution Brain Electromagnetic Tomography Study." *Brain Research Cognitive Brain Research* 16, no. 2 (2003): 267–72. https://doi.org/10.1016/s0926-6410(02)00282-3.
20) Jaušovec, N. and K. Jaušovec. "Sex Differences in Brain Activity Related to General and Emotional Intelligence." *Brain and Cognition* 59, no. 3 (2005): 277–86. https://doi.org/10.1016/j.bandc.2005.08.001.
21) Jaušovec, N. and K. Jaušovec. "Emotional Intelligence and Gender: A Neurophysiological Perspective." In *Handbook of Individual Differences in Cognition*, edited by A. Gruszka, G. Matthews and B. Szymura, 109–26. New York: Springer, 2010.
22) Miskovic, V. and A. Keil. “Reliability of Event-Related EEG Functional Connectivity during Visual Entrainment: Magnitude Squared Coherence and Phase Synchrony Estimates.” *Psychophysiology* 52, no. 1 (January 2015): 81–89. https://doi.org/10.1111/psyp.12287.
23) Monaim, H. and S. Fahlaoui. “General One-Dimensional Clifford Fourier Transform and Applications to Probability Theory.” Rendiconti del Circolo Matematico di Palermo, Serie II 73 (2024): 1453–66. https://doi.org/10.1007/s12215-023-00994-1.
24) Perkins, T. J., M. A. Stokes, J. A. McGillivray, A. J. Mussap, I. A. Cox, J. J. Maller and R. G. Bittar. “Increased Left Hemisphere Impairment in High-Functioning Autism: A Tract-Based Spatial Statistics Study.” *Psychiatry Research: Neuroimaging* 224, no. 2 (November 30, 2014): 119–123. https://doi.org/10.1016/j.pscychresns.2014.08.003.
25) Redwan, S. M., M. P. Uddin, A. Ulhaq and others. “Power Spectral Density-Based Resting-State EEG Classification of First-Episode Psychosis.” *Scientific Reports* 14 (2024): 15154. https://doi.org/10.1038/s41598-024-66110-0.
26) San-Segundo, R., Gil-Martín, M., D'Haro-Enríquez, L. F., and Pardo, J. M. 2019. "Classification of Epileptic EEG Recordings Using Signal Transforms and Convolutional Neural Networks." *Computers in Biology and Medicine* 109 (June): 148–58. https://doi.org/10.1016/j.compbiomed.2019.04.031.
27) Saugstad, L. F. “A Lack of Cerebral Lateralization in Schizophrenia Is within the Normal Variation in Brain Maturation but Indicates Late, Slow Maturation.” *Schizophrenia Research* 39, no. 3 (October 19, 1999): 183–196. https://doi.org/10.1016/s0920-9964(99)00073-0.
28) Sitnikova, E., Hramov, A. E., Koronovsky, A. A., and van Luijtelaar, G. 2009. "Sleep Spindles and Spike-Wave Discharges in EEG: Their Generic Features, Similarities and Distinctions Disclosed with Fourier Transform and Continuous Wavelet Analysis." *Journal of Neuroscience Methods* 180 (2): 304–16. https://doi.org/10.1016/j.jneumeth.2009.04.006.

29) Tarasov, Vasily E. 2018. “Fractional Dynamics of Systems with Long-Range Interaction and Memory.” *Frontiers in Physics* 6: 110. https://doi.org/10.3389/fphy.2018.00110.
30) Thatcher, R.W., D. North and C. Biver. “EEG and Intelligence: Relations between EEG Coherence, EEG Phase Delay and Power.” *Clinical Neurophysiology* 116, no. 9 (September 2005): 2129–41.
31) Tozzi, A., E. Bormashenko and N. Jausovec. "Topology of EEG Wave Fronts." *Cognitive Neurodynamics* 15 (2021a): 887–96. https://doi.org/10.1007/s11571-021-09668-z.
32) Tozzi, A., J. F. Peters, N. Jausovec, A. P. H. Don, S. Ramanna, I. Legchenkova and E. Bormashenko. "Nervous Activity of the Brain in Five Dimensions." *Biophysica* 1, no. 1 (2021b): 38–47. https://doi.org/10.3390/biophysica1010004.
33) Turalska, Malgorzata and Bruce J. West. 2018. “Fractional Dynamics of Individuals in Complex Networks.” *Frontiers in Physics* 6: 110. https://doi.org/10.3389/fphy.2018.00110.
34) Yuan Q, Zhou W, Xu F, Leng Y, Wei D. Int J Neural Syst. 2018 Oct;28(8):1850010. doi: 10.1142/S0129065718500107. Epub 2018 Mar 19. PMID: 29665725
35) Zhang, Yan, Yuanhua Qiao, Lijuan Duan and Jun Miao. “Multistability of Almost Periodic Solution for Clifford-Valued Cohen–Grossberg Neural Networks with Mixed Time Delays.” *Chaos, Solitons & Fractals* 176 (November 2023): 114100. https://doi.org/10.1016/j.chaos.2023.114100.

An extended Stokes' theorem for spiral paths: applications to rotational flows in *Trachelospermum jasminoides* stems and flowers

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

The traditional Stokes' theorem connects the macroscopic circulation along a closed boundary to the microscopic circulation across the surface it encloses. However, it proves inadequate for addressing complex geometries such as helicoidal paths, non-planar flow patterns and dynamic systems with open boundaries. We introduce an extension of Stokes' theorem (EST) that provides a robust tool for interdisciplinary research in spiral/helicoidal dynamics, facilitating the evaluation of rotational forces and circulation in both natural and engineered systems with open boundaries. We apply EST to model the rotational dynamics of flower petals and the helical forces within the stems of *Trachelospermum jasminoides*, known as star jasmine. For the flower, we demonstrate the equivalence between the line integral along the petal boundary and the surface integral over the enclosed disk, effectively capturing the uniform rotational stress generated by tangential forces. EST enables the analysis of external factors such as wind or pollinator interactions, while providing valuable insights to deepen our understanding of floral mechanics and petal growth patterns. For the stem, linking microscopic circulatory forces to macroscopic flow patterns, we demonstrate the interaction of torsional and bending stresses caused by the helical geometry. This finding has significant implications for understanding plant growth biomechanics and structural stability as well as for quantifying nutrient and water transport within stems, where spiral dynamics play a pivotal role. In summary, EST streamlines the analysis of rotational and translational forces in systems governed by spiral and helicoidal dynamics, including physical and biological phenomena such as phyllotaxis and plant growth.

KEYWORDS: helical dynamics; boundary analysis; vector field integration; flow topology.

INTRODUCTION

Stokes' theorem (henceforward ST) is a fundamental principle of vector calculus that bridges the macroscopic circulation along a closed boundary with the microscopic circulation over the enclosed surface (Green, 1828; Schey, 1997). Extending the principles of Green's theorem (GT) which applies to two-dimensional regions, ST provides a powerful framework for analyzing flows and circulations in three-dimensional spaces, uncovering profound connections between the local properties of vector fields and their global behaviour. GT and ST are effective tools for solving problems related to physical closed systems with clearly defined boundaries such as airflow circulation around wings, electromagnetic fields in circuits, surface heat flux, Coriolis-driven hemispherical flows, Earth's deep interior dynamics (Craven, 1964; Arfken, 1985; De Villiers, 2006; Livermore et al., 2013; Snieder, 2015; Aubert and Finlay, 2019; Vines et al., 2021; Yang et al., 2023). In biology, the two theorems contribute to understanding blood flow, electrical activity of the brain, growth patterns in ecosystems (Tozzi and Peters, 2023; Bressan et al., 2022). Yet, the classical ST is inherently limited to surfaces and boundaries that are closed, leaving a significant gap in its applicability to open, non-planar geometries. Indeed, many natural and engineered systems exhibit spiral or helical dynamics where forces and flows do not conform to closed loops or planar surfaces but rather are characterized by open, three-dimensional trajectories. Examples include the helical paths of tornadoes, magnetic vortices and spiral galaxies as well as bacterial motility and phyllotaxis of plants (Blaser et al., 2024; Sachkou et al., 2019; Reinhardt and Gola, 2022).

The novelty of this work lies in extending ST to accommodate spiral flows and helicoidal paths. By linking macroscopic and microscopic circulation properties, the extended theorem simplifies the evaluation of forces in systems with open, three-dimensional geometries. We utilize EST to analyse two biological scenarios: 1) the rotational forces in spiral flower petals and 2) the torsional stresses in helical plant stems, both exemplified by *Trachelospermum jasminoides*, commonly known as star jasmine. For the flower, EST captures the uniform rotational stresses induced by tangential forces acting along a circular boundary. This is achieved by demonstrating the equivalence of the line integral along the petal boundary and the surface integral over the enclosed disk. For the stem, EST quantifies the interaction between bending and torsional stresses caused by the helical geometry.
This paper is structured as follows. First, we present the mathematical treatment of EST, including its derivation and parameterization for helicoidal paths. Next, we validate the theorem using the specific example of *Trachelospermum jasminoides'* flowers and stems. Finally, we discuss the broader implications of EST, highlighting its potential to unify the study of dynamical systems with open boundaries.

MATERIALS AND METHODS

This study is grounded in a generalized form of Stokes' theorem, adapted for spiral flows, which facilitates the analysis of forces and circulation in systems with helicoidal or spiral geometries. We aim to prove that, given a continuously differentiable, orientable helicoidal spiral vector field, the macroscopic circulation represented by the integral of a differential form over its surface equals the microscopic circulation represented by the volume integral of the curl perpendicular to the surface. The main challenge here is in defining the notion of a boundary in case of an open helicoidal spiral path, moving beyond the classical case of paths evaluable through ST.

Stokes' Theorem (ST) from vector calculus relates the surface integral of the curl of a vector field over a surface **S** to the line integral of the vector field along the boundary curve ∂S of the surface (**Figure 1**). In its general form, ST asserts that

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_{S} (\nabla \times \mathbf{F}) \cdot d\mathbf{S}$$

where **F** is a a continuously differentiable two-dimensional vector field, ∂S is the closed boundary curve of the surface **S** that can be bended and stretched, d**r** is a differential element of the curve, d**S** is the differential element of the surface area, and $\nabla \times \mathbf{F}$ is the curl of the vector field, i.e., a vector operator characterizing the infinitesimal circulation of vector fields in three-dimensional spaces.
ST turns line integrals of a form over a boundary into more straight-forward double integrals over the bounded region, regardless of the position of vector singularities (Zenisek 1999). For ST to apply, the normal vector representing the surface must be positively oriented (i.e., counterclockwise) with respect to the tangent vector representing the orientation of the boundary.

Extended Stokes' theorem (EST). Consider a vector field **F** defined over a region in three-dimensional space. Let the surface *S* be a portion of a plane or a more general surface that is bounded by a spiral curve $\gamma(t)$. The goal is to use EST to evaluate the line integral over the spiral path in terms of the surface integral of the curl of **F** (**Figure 1**).
Let the spiral curve $\gamma(t)$, with $t \in [a, b]$, be parameterized as

$$\gamma(t) = (r(t)\cos(\theta(t)), r(t)\sin(\theta(t)), z(t))$$

where $r(t)$, $\theta(t)$ and $z(t)$ describe respectively the radial, angular and vertical components of the spiral.
Let's assume that $\gamma(t)$ lies on a flat plane, say the *xy*-plane, so the spiral path can be simplified to

$$\gamma(t) = (r(t)\cos(t), r(t)\sin(t), 0)$$

where $r(t)$ increases as the angle *t* increases.
When the surface **S** is a surface spanned by the curve $\gamma(t)$, **S** stands for a portion of the plane or surface generated by the spiral curve (**Figure 1, left**).

We are interested in computing the line integral of a vector field **F** along the spiral path (**Figure 1, right**). By ST, this line integral can be transformed into a surface integral involving the curl of **F**

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_{S} (\nabla \times \mathbf{F}) \cdot d\mathbf{S}$$

The line integral over the spiral path is:

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_{a}^{b} \mathbf{F}(\gamma(t)) \cdot \frac{d\gamma(t)}{dt}\, dt$$

where $\frac{d\gamma(t)}{dt}$ is the tangent vector to the spiral path at each point *t*.

The surface integral involves the curl of **F**, given by $\nabla \times \mathbf{F}$, and the surface normal vector $\hat{n}$ associated with **S**

$$\int_S (\nabla \times \mathbf{F}) \cdot d\mathbf{S} = \int_S (\nabla \times \mathbf{F}) \cdot \hat{n}\, dS$$

The normal vector $\hat{n}$ depends on the orientation of the surface, while dS is the differential area element of the surface. Upon achieving the extended formulation of ST, we will proceed in the next paragraphs with a detailed case study.

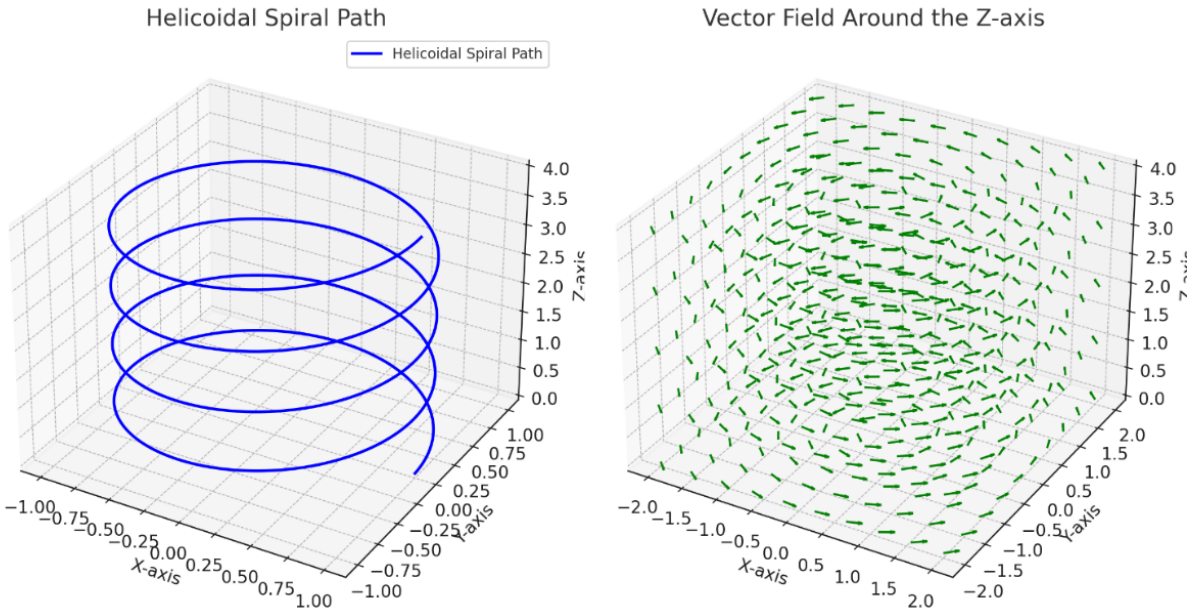

Figure 1. Diagrams depicting a helicoidal spiral (**left**) and the behavior of a vector field (**right**) around the z-axis. The left diagram illustrates a helicoidal spiral path, showcasing the interplay of rotational and translational motion along the z-axis. The right diagram represents a vector field with circular flow centered around the z-axis.

Simulated case study: analyzing rotational flows in stems and flowers. EST enables the analysis of rotational and translational forces in complex systems, providing a powerful framework for exploring biological and physical dynamics. To illustrate the new theorem, we will now explore a concrete example. We will consider *Trachelospermum jasminoides*, commonly known as star jasmine, belonging to the family *Apocynaceae*. Like many climbing plants, *Trachelospermum jasminoides* displays a counterclockwise helical movement of its stems as it climbs and twines around supports, also referred to as circumnutation (Darwin, 1875; Pansanit and Pripdeevech, 2014; Canher et al., 2022) (**Figure 2**). The flowers also exhibit subtle rotational dynamics, although these movements are not as pronounced as the helical twisting of the stems (Stefanatou et al., 2025). The petals of the flowers are arranged in a spiral configuration and unfurl in a counterclockwise direction during blooming.

In our simulation, the dynamics of flower petals are modeled using a circular boundary with a radius of 0.05 m, representing the petals of a flower. Tangential forces along this boundary are applied and the resulting rotational stresses are analyzed through EST. The stem is modeled as a helicoidal path with a radius R=0.05 m and a vertical rise per turn of c=0.2 m. To evaluate the counterclockwise rotation of the flower and stem using EST, the rotation of the petals can be represented by a circular vector field, whereas the helical motion of the stem can be modelled using a helical vector field. The next step is to parameterize the flower and the stem (**Figure 2C**).

1) For the flower, a circular boundary in the plane of the petals is defined, representing the region of interest for macroscopic rotation.

2) For the stem, the helical path is parameterized using equations for a helicoidal spiral, where $x(t) = r\cos(t)$, $y(t) = r\sin(t)$ and $z(t) = ct$. Here r represents the radius, c the rise per turn and t the parameter along the path.

Subsequently, the surface S is defined for each component.

1) For the flower, the surface is a disk enclosed by the petals' rotational motion within their plane,
2) whereas for the stem, the surface corresponds to the area traced by the helical path (**Figure 2C**).

Calculating the forces acting on the flower and stem requires applying mechanical principles that account for both internal and external forces influencing their dynamics (Smyth 2016; Loshchilov et al., 2021). For the flower's petals, the primary force is torque or rotational force, while the stem experiences a combination of bending forces and axial torsion due to its helical structure. We will calculate these forces systematically, step by step, starting from the external forces.

External forces acting of the flower petals and the stem.

1) The rotation of the flower petals can be modeled as a torque induced by external forces such as wind, gravitational pull, biological growth forces (Tipler, 2004). Torque (τ) on the petals is given by:

$$\tau = r \times F$$

Where r is the radial vector from the center of the flower to the tip of a petal and F is the tangential force applied to the petal. Let's assume that the radius of the flower is R=5 cm, while the tangential force from wind or another source is F=0.1 N. The magnitude of the torque is

$$|\tau| = R \cdot F = 0.05\,\mathrm{m} \cdot 0.1\,\mathrm{N} = 0.005\,\mathrm{Nm}$$

In case of multiple petals (n=5 in *Trachelospermum jasminoides*) experiencing similar forces, the total torque becomes

$$\tau_{\text{total}} = n \cdot \tau = 5 \cdot 0.005\,\mathrm{Nm} = 0.025\,\mathrm{Nm}$$

The rotational acceleration (α) of the flower petals is related to the torque (Clark and Ryan, 2022) via

$$\tau = I \cdot \alpha$$

Where I is the moment of inertia of the flower petals about the axis of rotation and α is the angular acceleration.
For a flower modeled as a system of point masses at a radius R

$$I = n \cdot m \cdot R^2$$

where m is the mass of a single petal. Assuming m=0.002 kg (2 grams per petal):

$$I = 5 \cdot 0.002 \cdot (0.05)^2 = 0.000025\,\mathrm{kg}\backslash\mathrm{cdotpm}^2$$

The angular acceleration is:

$$\alpha = \frac{\tau_{\text{total}}}{I} = \frac{0.025}{0.000025} = 1000\,\mathrm{rad/s}^2$$

2) The stem experiences forces from bending and torsion, influenced by its helical structure. These forces arise from gravity, wind and the biological tension exerted during growth.

The weight of the stem induces a bending gravitational force. For a stem of length L=20 cm and mass per unit length λ=0.01 kg/m:

$$F_g = m \cdot g = (\lambda \cdot L) \cdot g = (0.01 \cdot 0.2) \cdot 9.8 = 0.0196\,\mathrm{N}$$

This force acts vertically downward, generating a bending moment at the base of the stem

$$M_g = F_g \cdot \frac{L}{2} = 0.0196 \cdot 0.1 = 0.00196\,\mathrm{Nm}$$

The helical structure of the stem experiences torsional forces due to the winding. The torsional moment (T) is given by

$$T = G \cdot J \cdot \frac{\theta}{L}$$

Where G is the shear modulus of the stem material, J is the polar moment of inertia and θ is the angle of twist over the length L.
Assuming G= 10^8 Pa typical for plant tissue, J = 0.005 m (5 mm radius) and θ = 2π (one full turn over L =0.2 m) (Hoermayer et al., 2024), then

$$J = \frac{\pi}{2} \cdot (0.005)^4 = 9.82 \times 10^{-10}\,\mathrm{m}^4$$

and

$$T = (10^8) \cdot (9.82 \times 10^{-10}) \cdot \frac{2\pi}{0.2} = 3.08\,\mathrm{Nm}$$

Internal forces acting within the flower petals and the stem.
To calculate the forces within the flower petals and the stem using EST, we need to evaluate the relationship between the macroscopic circulation (observable forces) and the microscopic properties (internal forces or stresses derived from the curl of the force field). The first step is to model the forces using vector fields.

1) Concerning the flower petals, we assume that the external forces (e.g., wind or biological forces) act tangentially to their circular boundary. Further, we assume that the tangential forces induce internal stresses (force per unit area) propagating through the petals. Let the force field acting on the petals be

$$\mathbf{F} = (-ky, kx, 0)$$

where k is the force constant proportional to the external pressure and x,y represent positions in the z=0 plane.

Next, we compute the curl of the force field. The curl of the force field relates to the internal stresses within the petals. For the flower petals, in the z=0 plane, the curl of $\mathbf{F} = (-ky, kx, 0)$ is

$$\nabla \times \mathbf{F} = \begin{vmatrix} \mathbf{i} & \mathbf{j} & \mathbf{k} \\ \frac{\partial}{\partial x} & \frac{\partial}{\partial y} & \frac{\partial}{\partial z} \\ -ky & kx & 0 \end{vmatrix} = (0, 0, 2k)$$

This curl is constant in the z-direction, indicating a uniform internal rotational stress throughout the petals.

2) Concerning the helical stem, it experiences external forces such as gravity $(\mathbf{F}_g)$ and biological growth forces $(\mathbf{F}_b)$ that induce internal torsion and bending stresses.

For simplicity, we model the net force field in the stem as: $\mathbf{F} = (-ky, kx, kz)$ where the kz-term accounts for the vertical components of the forces.

Next, we compute the curl of the force field, which provides insight into the internal stresses acting within the stem. This computation reveals the distribution and intensity of these stresses, capturing the complex interplay of forces across the helical structure. For the stem the curl of $\mathbf{F} = (-ky, kx, kz)$ is

$$\nabla \times \mathbf{F} = \begin{vmatrix} \mathbf{i} & \mathbf{j} & \mathbf{k} \\ \frac{\partial}{\partial x} & \frac{\partial}{\partial y} & \frac{\partial}{\partial z} \\ -ky & kx & kz \end{vmatrix} = (k, k, 2k)$$

This suggests a complex pattern of internal stress within the stem, with components distributed across all three spatial directions.

Visualization and statistics. Diagrams of the flower petals and stem are created to illustrate their geometry, boundary dynamics, and associated vector fields. The Matplotlib library is employed to generate detailed plots, including the circular boundary and tangential forces acting on the flower petals, the curl of the vector field over various surfaces, and the helicoidal path and vector field representation for the stem.

To ensure statistical validation, numerical accuracy is achieved through high-resolution parameterization, with the parameter t sampled at 1,000 points per cycle. The consistency between line integrals and surface integrals is carefully evaluated to confirm the applicability of the extended theorem to the analyzed geometries.

In the sequel, the surface integral of the curl of the vector field will be computed over these surfaces using the extended Stokes' theorem (EST).

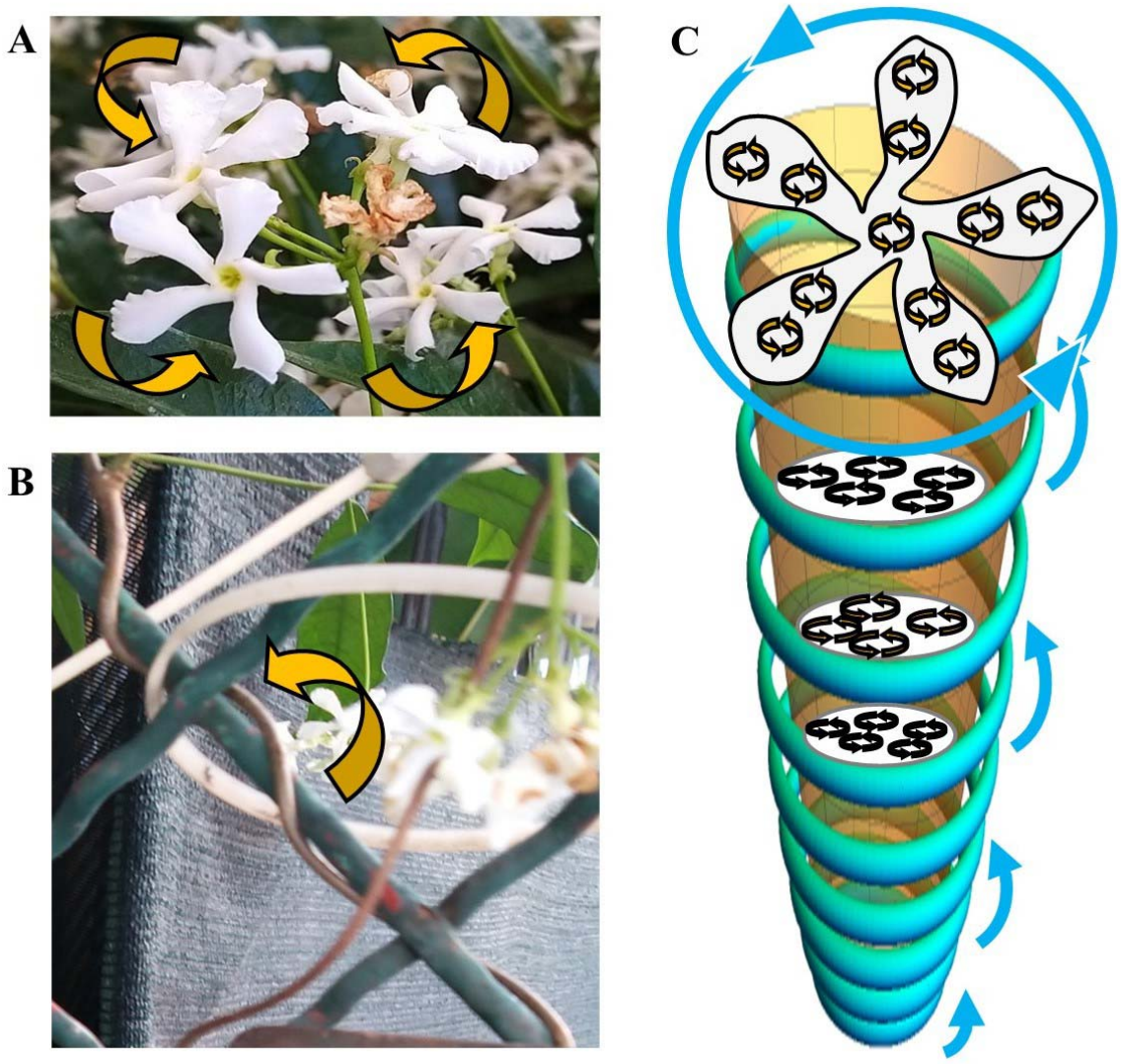

Figure 2. *Trachelospermum jasminoides*. The flower petals (**Figure 2A**) and the stem (**Figure 2B**) display a counterclockwise path. **Figure 2C** illustrates the geometry of the boundaries, the associated vector fields and the internal flows within the flower petals and the stem.

RESULTS

As stated above, both the flower and the stem experience external and internal mechanical forces that influence their motion and structural behavior:

1) For the flower petals, the torque arising from tangential forces induces a counterclockwise rotation, with the total torque $\tau_{\text{total}} = 0.025\,\text{Nm}$ and the angular acceleration measured as α=1000 rad/s^2. The internal stresses in the petals are uniform with a value of 2k and are directly proportional to the external forces acting on them. This proportionality explains the rotational equilibrium observed in the petals.
2) For the stem, the primary forces include a gravitational bending moment $M_g = 0.00196\,\text{Nm}$ and a torsional moment T=3.08□Nm due to a helical twist. The internal stresses in the stem vary in all three dimensions because of its helical geometry. Among these stresses, torsion, proportional to kc, predominates, whereas bending stresses, proportional to kR, have a secondary but still notable influence.

We can now apply EST to relate macroscopic and microscopic circulation. The surface integral of the curl of the vector field is computed over the surfaces, relating the surface integrals to the line integrals along the boundaries. For the flower petals, the counterclockwise macroscopic rotation is calculated by integrating along the circular path in the plane. For the stem, the integral is evaluated over the helical surface.

1) Concerning the flower petals, the boundary of the flower is a circle of radius R. The macroscopic circulation (line integral along the petal boundary) is

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_0^{2\pi} kR^2\, d\theta = 2\pi k R^2$$

Using the curl, the surface integral is

$$\int_S (\nabla \times \mathbf{F}) \cdot d\mathbf{S} = \int_S 2k\, dA = 2k \cdot \pi R^2$$

Both results match, confirming that the inner stresses in the petals are proportional to 2k.

In sum, the numerical values for the macroscopic (surface) flows and microscopic (internal) flows in the flower, as governed by EST, are as follows. For the flower, the surface flow (evaluated as a surface integral) is 0.157N\ppm, while the internal flow (evaluated as a line integral) is also 0.157N\ppm. The flower petals exhibit a simple and symmetric geometry, where forces act tangentially along a circular boundary in the z=0 plane. The petals lie on a flat, two-dimensional surface characterized by a constant curl of the force field $(\nabla \times \mathbf{F} = (0, 0, 2k))$, signifying that the internal forces are uniformly distributed. This uniform distribution creates a direct and proportional relationship between the macroscopic flow (line integral along the circular boundary) and the microscopic flow (surface integral over the disk). The symmetry of the geometry ensures that every contribution to the line integral is exactly matched by the surface integral. Consequently, the uniform geometry and constant curl lead to a perfect agreement between the surface flow and the internal flow, consistent with EST.

2) Concerning the helical stem, the boundary of the stem is parameterized as a helicoidal spiral

$$x(t) = R\cos(t), \quad y(t) = R\sin(t), \quad z(t) = ct$$

The macroscopic circulation (line integral along the helical path) is

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_0^{2\pi} \left[-kR\sin(t)(-R\sin(t)) + kR\cos(t)(R\cos(t)) + kc(ct)\right] dt$$

$$= \int_0^{2\pi} \left(kR^2 + kc^2 t\right) dt$$

For one turn$(t \in [0, 2\pi])$

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = 2\pi k R^2 + \frac{kc^2}{2}(2\pi)^2$$

Using the curl, the surface integral is approximated by the ribbon spanned by the helix

$$\int_S (\nabla \times \mathbf{F}) \cdot d\mathbf{S} = \int_S (k + k + 2k)\, dA = 4k \cdot (\text{surface area of ribbon})$$

The surface area of the ribbon is

$$A = 2\pi R \cdot (\text{height per turn}) = 2\pi R \cdot c$$

Thus

$$\int_S (\nabla \times \mathbf{F}) \cdot d\mathbf{S} = 4k \cdot 2\pi R \cdot c = 8\pi k R c$$

In sum, the numerical values for the macroscopic (surface) flows and microscopic (internal) flows in the stem, as governed by EST, are as follows. For the stem, the surface flow (evaluated as a surface integral) is 2.513N\ppm, while the internal flow (evaluated as a line integral) is 8.053N\ppm. Unlike the flower, the values for surface flow and internal flow differ significantly. This is due to the stem's more complex geometry, which features a three-dimensional helicoidal structure with a helical boundary and a ribbon-like surface. Unlike the constant curl observed in the flower, the curl of the force field $(\nabla \times \mathbf{F} = (k, k, 2k))$ n the stem varies in all three dimensions. This non-uniform curl introduces additional contributions to the surface integral that are not directly proportional to the line integral along the helical path. The helicoidal surface spanned by the path is not planar. Its area depends on the radius of the helix and the rise per turn (c), which increases the surface integral significantly compared to the simpler circular geometry of the flower. The line integral along the helical path includes contributions from the vertical rise (z-component), which are absent in the flat geometry of the flower. These vertical components add substantially to the internal flow, making it larger than the surface flow. Forces and circulation in the stem are not confined to a two-dimensional plane, rather display three-dimensional dynamics that capture complex interactions such as bending, twisting and torsional effects, further contributing to the discrepancy between the surface and internal flows. Therefore, the stem's intricate geometry and three-dimensional dynamics lead to a disparity between surface and internal flows, reflecting the additional factors at play in its structural behavior.

In conclusion,

1) flowers have a circular, symmetric geometry that ensures uniform force distribution and curl. This results in surface and internal flows being equal, as the entire flow field is captured in a flat, two-dimensional setup.
2) In contrast, the stem's helicoidal geometry introduces non-uniform force distributions and additional components such as vertical contributions and a larger surface area. These factors create a larger internal flow compared to the surface flow, as the line integral accounts for three-dimensional effects that the surface integral does not fully capture.

These differences highlight the impact of geometry and force distribution on the interplay between macroscopic circulation and microscopic forces, showcasing the utility of the extended Stokes' theorem in analysing forces and circulation in systems exhibiting spiral dynamics.

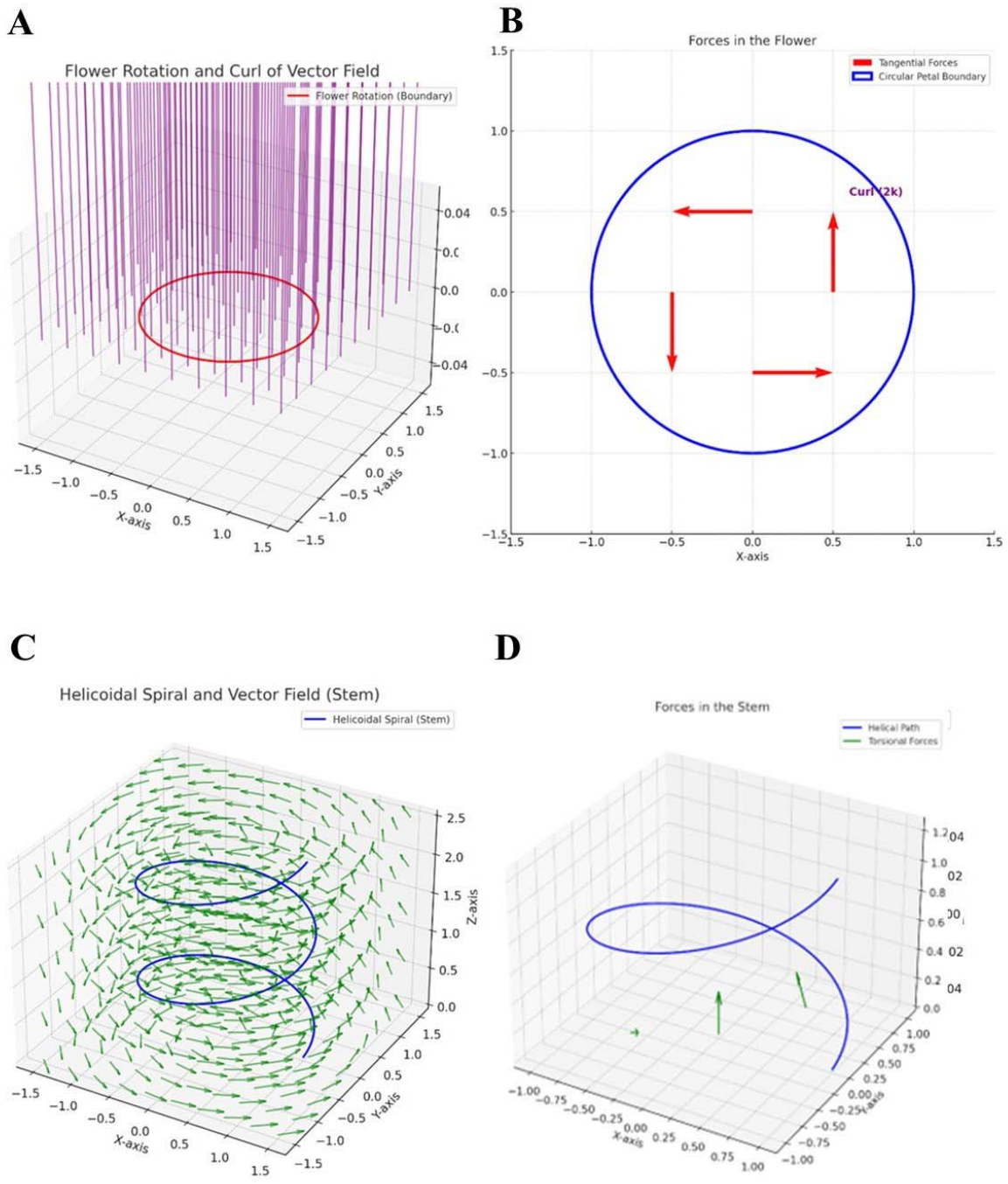

Figure 3. Application of the extended Stoke's theorem to flowers (**Figures 3A-B**) and stems (**Figures 3C-D**) of *Trachelospermum jasminoides*. **Figure 3A**. Diagram illustrating the flower rotation and the curl of vector field. The red circle represents the boundary of the flower petals modeled as a planar region in the z=0 plane. The purple arrows visualize the curl of the vector field, representing the microscopic circulation that contributes to the macroscopic rotation of the flower petals. **Figure 3B**. Diagram illustrating the forces acting on the flower. The circular boundary (blue) represents the edge of the flower petals. Tangential forces (red arrows) act along the edges of the petals, showcasing the influence of external or internal factors. The calculated curl of the vector field is constant at $\nabla \times F = 2k$ (annotated in purple) in the z-direction, indicating uniform rotational stresses throughout the petal boundary. **Figure 3C**. Diagram illustrating the helicoidal spiral of the stem and the vector field. The blue curve depicts the helicoidal path of the stem, while the green arrows represent a circular vector field around the z-axis, illustrating the rotational and translational flow and its interaction with the spiral geometry. **Figure 3D**. Diagram illustrating the forces acting on the stem. The helical path (blue curve) represents the stem's geometry. The torsional forces (green arrows), resulting from a combination of bending and twisting actions, act along the helical structure contributing to internal stress distribution. The curl vector field displays non-uniform rotational stresses with components $\nabla \times F = (k,k,2k)$.

CONCLUSION

Classical theorems such as Green's Theorem (GT) and Stokes' Theorem (ST) have been pivotal in linking local properties of vector fields to their global behavior. GT applies just to two-dimensional regions and closed curves, while ST extends to three-dimensional spaces requiring closed surfaces or boundaries for its application (Green, 1828; Schey, 1997). These theorems, focused on closed-loop circulations, have proven instrumental in analyzing flows and circulations in systems where boundaries are well-defined, such as steady-state circulations in airflow around wings or electromagnetic field behavior in closed circuits (Arfken, 1985; De Villiers, 2006). However, their utility diminishes when applied to open, three-dimensional trajectories like the helicoidal spirals which are frequently encountered in natural and engineered systems.

We suggest a generalization of ST to establish a mathematical framework connecting the line integral along a helicoidal spiral path to the surface integral of the curl of the vector field over a bounded region. By redefining the boundary concept for helicoidal paths, this framework provides a new tool for analyzing macroscopic and microscopic flow dynamics in complex systems. The EST formulation provides novel insights into the interplay between rotational and translational motions, allowing for a deeper understanding of spiral flows in a variety of physical and biological systems. A key advantage of the extended formulation lies in its ability to model a wide range of systems where spiral or helical dynamics are dominant. For instance, the novel framework enables the analysis of DNA supercoiling, bacterial flagella, biomechanical patterns such as the phyllotaxis of plants (Reinhardt and Gola, 2022; Liu et al., 2024), intracardiac spiral flows observed in cardiac cycles (Mulimani et al., 2022) as well as magnetic vortices in superconductors (Sachkou et al., 2019) and rotational dynamics of spiral galaxies (Blaser et al., 2024), where classical methods fail to capture the intricacies of rotational and translational dynamics.

In this paper, EST is applied to two case studies related with *Trachelospermum jasminoides*, namely the forces acting on flower petals and the helical stress distribution within plant stems.

1) For the flower petals, the circular geometry allows for a straightforward application EST, since the tangential forces acting along the petal boundary produce a uniform curl which is proportional to the rotational stresses. The equivalence between the line integral along the petal boundary and the surface integral of the curl over the enclosed disk validates EST's effectiveness for two-dimensional spiral systems. The uniform rotational stresses observed in the petals align well with the mathematical predictions of EST. This provides insights into how forces are distributed within the boundary of the flower, potentially aiding in the study of floral mechanics and growth patterns. EST suggests that microscopic forces acting at the level of the petals contribute to the macroscopic rotational motion observed at the flower's boundary. This could be applied to study the impact of environmental factors like wind on plant structures and to investigate the mechanical interactions between flowers and pollinators during the pollination process. Still, EST effectively simplifies complex calculations by converting a line integral along the flower's boundary into a surface integral over the petal region. This transformation minimizes computational effort while preserving accuracy.

2) In the case of the stem, although the helical geometry of the stem presents a significant challenge for classical mathematical tools, EST effectively simplifies the intricate interplay of forces involved. The torsional and bending forces are captured through the curl of the vector field, which has components in all three dimensions. The equivalence of the surface integral over the helical ribbon region and the line integral along the helical path demonstrates the robustness of EST in handling three-dimensional geometries with open boundaries. The EST capability to connect macroscopic flow patterns with microscopic circulatory forces may have significant implications for understanding the biomechanics of plant growth and structural stability. This relationship can also provide valuable insights for studies on nutrient and water transport within stems, as these processes often involve spiral dynamics.

Certain assumptions and limitations are inherent in our analysis. EST assumes that the involved vector fields and surfaces are continuously differentiable. In real-world biological systems, irregularities and discontinuities in the geometry or force distribution may reduce the accuracy of the analysis. The flower petals are modeled as a perfect circle and the stem as a regular helix. While this simplifies the mathematical analysis of forces in idealized systems, real-world systems often deviate from these idealized shapes. The analysis of irregular geometries or highly dynamic boundaries may still require significant computational effort, particularly for numerical integration of complex surface and line integrals. The tangential and torsional forces are assumed to be uniform across the boundaries. In reality, biological and environmental forces such as wind, gravity and growth pressures are often spatially and temporally variable. Additionally, secondary effects such as shear forces or anisotropic material properties are not incorporated, which could limit the applicability of the results to certain systems. Future work could extend the framework to handle more irregular and biologically realistic geometries, such as asymmetrical petals or non-uniform stem shapes. The analysis of time-varying forces and boundaries, such as those caused by growth or environmental changes, could provide deeper insights into real dynamics. Integrating the extended theorem with experimental data would help validate the theoretical predictions and refine the mathematical models.

In conclusion, the proposed extension to Stokes' Theorem integrates helicoidal paths into circulation analysis, bridging a critical gap and expanding its applicability to open, non-planar trajectories. By redefining boundaries, it simplifies the study of rotational and translational flows, offering a versatile tool for analyzing complex dynamics such as those observed in the flowers and stem of *Trachelospermum jasminoides*.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.
Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal, and has not been previously published.
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REFERENCES

1) Aubert, J., Finlay, C.C. 2019. Geomagnetic jerks and rapid hydromagnetic waves focusing at Earth's core surface. *Nat. Geosci.* 12, 393–398. https://doi.org/10.1038/s41561-019-0355-1
2) Arfken, G. 1985. *Gauss's Theorem.* Mathematical Methods for Physicists, 3rd ed. Orlando, FL: Academic Press.
3) Blaser, A., Benamran, R., Villas Bôas, A.B., Lenain, L., Pizzo, N. 2024. Momentum, energy and vorticity balances in deep-water surface gravity waves. *Journal of Fluid Mechanics*, 997. https://doi.org/10.1063/5.0222216
4) Bressan, A., Chiri, M.T., Salehi, N. 2022. On the optimal control of propagation fronts. *Mathematical Models and Methods in Applied Sciences*, 32(06), 1109–1140. https://doi.org/10.1142/S0218202522500257
5) Canher, Balkan, Fien Lanssens, Ai Zhang, Anchal Bisht, Shamik Mazumdar, Jefri Heyman, Sebastian Wolf, Charles W. Melnyk, and Lieven De Veylder. "The Regeneration Factors ERF114 and ERF115 Regulate Auxin-Mediated Lateral Root Development in Response to Mechanical Cues." *Molecular Plant* 15, no. 10 (October 3, 2022): 1543–1557. https://doi.org/10.1016/j.molp.2022.08.008.
6) Cauchy, A. 1846. Sur les intégrales qui s'étendent à tous les points d'une courbe fermée. *Comptes rendus*, 23, 251–255.
7) Clark, K. P., and L. J. Ryan. "Hip Torque Is a Mechanistic Link Between Sprint Acceleration and Maximum Velocity Performance: A Theoretical Perspective." *Frontiers in Sports and Active Living* 4 (July 12, 2022): 945688. https://doi.org/10.3389/fspor.2022.945688
8) Craven, B.D. 1964. A note on Green's theorem. *Journal of the Australian Math. Soc.*, 4(3), 289-292. https://doi.org/10.1017/S1446788700024058
9) Darwin, Charles. *The Movements and Habits of Climbing Plants*. London: John Murray, 1875.
10) De Villiers, J.-P. 2006. Stokes Theorem and the Equations of GRMHD. arXiv:astro-ph/0606660
11) Donepudi, T., van de Griend, M., Agostinho, L.L.F., Kroon, E.J.D., Klymenko, R., et al. 2024. Numerical analysis of vortex dynamics in hyperbolic funnels using computational fluid dynamics. *Physics of Fluids*, 36(9), Article 095171.

12) Green, G. 1828. An essay on the application of mathematical analysis to the theories of electricity and magnetism. *Journal f ur die reine und angewandte Mathematik*, Vol. 39, 1 (1850) p. 73–89, Vol. 44, 4 (1852) p. 356–374, and Vol. 47, 3 (1854) p. 161–221.
13) Hoermayer, Lukas, Juan Carlos Montesinos, Nicola Trozzi, Leonhard Spona, Saiko Yoshida, et al. "Mechanical Forces in Plant Tissue Matrix Orient Cell Divisions via Microtubule Stabilization." *Developmental Cell* 59, no. 10 (May 20, 2024): 1333–1344.e4.
14) You, G. Y., Ziqiang, Y., Ze, W., Mengchun, P., Yujing, X., Qi, Z. 2023. Magnetic Field Downward Continuation Iterative Method Based on Low-pass Filter. *J. Phys.: Conf. Ser.*, 2525, 012006. https://doi.org/10.1088/1742-6596/2525/1/012006
15) Heusler, M. 1998. Stationary Black Holes: Uniqueness and Beyond. *Living Rev. Relativ.*, 1(1), 6. https://doi.org/10.12942/lrr-1998-6
16) Liu, S., Li, Y., Wang, Y., et al. 2024. Emergence of large-scale mechanical spiral waves in bacterial living matter. *Nat. Phys.*, 20, 1015–1021. https://doi.org/10.1038/s41567-024-02457-5
17) Livermore, P.W., Hollerbach, R., Jackson, A. 2013. Electromagnetically driven westward drift and inner-core superrotation in Earth's core. *PNAS*, 110(40), 15914–15918. https://doi.org/10.1073/pnas.1307825110
18) Long, F., McElheny, D., Jiang, S., Park, S., Caffrey, M.S., Fung, L.W.-M. 2007. Conformational change of erythroid α-spectrin at the tetramerization site upon binding β-spectrin. *Protein Sci.*, 16(11), 2519–2530. https://doi.org/10.1110/ps.073115307
19) Mulimani, M.K., Zimik, S., Pandit, R. 2022. An In Silico Study of Electrophysiological Parameters That Affect the Spiral-Wave Frequency in Mathematical Models for Cardiac Tissue. *Front. Phys.*, 9, 819873. https://doi.org/10.3389/fphy.2021.819873
20) Pansanit, A., and Pripdeevech, P. "Constituents, Antibacterial and Antioxidant Activities of Essential Oils from *Trachelospermum jasminoides* Flowers." *Natural Product Communications* 9, no. 12 (December 2014): 1791–94.
21) Pontryagin, L.S. 1959. Smooth manifolds and their applications in homotopy theory. *American Mathematical Society Translations*, Ser. 2, Vol. 11, American Mathematical Society, Providence, R.I., pp. 1–114.
22) Reinhardt, D., Gola, E.M. 2022. Law and order in plants - the origin and functional relevance of phyllotaxis. *Trends Plant Sci.*, 27(10), 1017–1032. https://doi.org/10.1016/j.tplants.2022.04.005
23) Loshchilov, Ilya, Emanuela Del Dottore, Barbara Mazzolai, and Dario Floreano. "Conditions for the Emergence of Circumnutations in Plant Roots." *PLOS ONE* 16, no. 5 (2021): e0252202. https://doi.org/10.1371/journal.pone.0252202.
24) Sachkou, Y.P., Baker, C.G., Harris, G.I., Stockdale, O.R., Forstner, S., et al. 2019. Coherent vortex dynamics in a strongly interacting superfluid on a silicon chip. *Science*, 366(6472), 1480-1485. https://doi.org/10.1126/science.aaw9229
25) Schey, H.M. 1997. *Div, Grad, Curl, and All That: An Informal Text on Vector Calculus*, 3rd ed. New York: W.W. Norton.
26) Smyth, David R. "Helical Growth in Plant Organs: Mechanisms and Significance." In *Plant Development*. *Development* 143, no. 18 (2016): 3272–3282. https://doi.org/10.1242/dev.134064.
27) Snieder, R. 2015. Imaging the Earth using Green's theorem, in *The Princeton Companion to Applied Mathematics*, Ed. Higham, N.J., M.R. Dennis, P. Glendinning, P.A. Martin, F. Santosa, and J. Tanner, Princeton Univ. Press, Princeton NJ, 857-860.
28) Stefanatou, A., Vouzi, L., Petousi, I., Koukoura, A., Gatidou, G., et al,. "Treatment of Real Laundry Wastewater Using Vertical Flow Constructed Wetland Planted with the Ornamental Climbing Plant *Trachelospermum jasminoides*: Assessing the Removal of Conventional Pollutants and Benzotriazoles." *Environmental Science and Pollution Research International* 31, no. 30 (June 2024): 43281–91. https://doi.org/10.1007/s11356-024-34035-w
29) Tipler, Paul. *Physics for Scientists and Engineers: Mechanics, Oscillations and Waves, Thermodynamics*. 5th ed. New York: W. H. Freeman, 2004. ISBN 0-7167-0809-4
30) Tozzi, A., Peters, J.F. 2023. Towards a single parameter for the assessment of EEG oscillations. *Cogn Neurodyn.* https://doi.org/10.1007/s11571-023-09978-4
31) Vines, S.K., Anderson, B.J., Allen, R.C., Denton, R.E., Engebretson, M.J., et al. 2021. Determining EMIC Wave Vector Properties Through Multi-Point Measurements: The Wave Curl Analysis. *J Geophys Res Space Phys.*, 126(4), e2020JA028922. https://doi.org/10.1029/2020JA028922
32) Yang, Y., Song, X. 2023. Multidecadal variation of the Earth's inner-core rotation. *Nat. Geosci.*, 16, 182–187. https://doi.org/10.1038/s41561-022-01112-z
33) Wapenaar, K., Brackenhoff, J., Thorbecke, J. 2019. Green's theorem in seismic imaging across the scales. *Solid Earth*, 10, 517–536. https://doi.org/10.5194/se-10-517-2019
34) Zenisek, A. 1999. Green's theorem from the viewpoint of applications. *Applications of Mathematics*, 44(1), 55-80.

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REVIEW

Exploring the influence of water micro assemblies on protein folding, enzyme catalysis and membrane dynamics

Arturo Tozzi[1]

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Abstract
Water is central to biological processes not only as a solvent, but also as an agent shaping macromolecular behavior. Insights into water micro assemblies (WMA), defined by transient regions of low-density water (LDW) and high-density water (HDW), have highlighted their potential impact on biological phenomena. LDW, with its structured hydrogen bonding networks and reduced density, stabilizes hydrophobic interfaces and promotes ordered molecular configurations. Conversely, HDW, with its dynamic and flexible nature, facilitates transitions, solute mobility and molecular flexibility. By correlating experimental observations with simulations, we explore the influence of WMA on three key biological processes. In protein folding, LDW may stabilize hydrophobic cores and secondary structures by forming structured exclusion zones, while HDW may introduce dynamic flexibility, promoting the resolution of folding intermediates and leading to dynamic rearrangements. In enzyme catalysis, LDW may form structured hydration shells around active sites stabilizing active sites over longer timescales, while HDW may support substrate access and catalytic flexibility within active sites. In membrane dynamics, LDW may stabilize lipid headgroups, forming structured hydration layers that enhance membrane rigidity and stability, while HDW may ensure the nanosecond-scale flexibility required for vesicle formation and fusion. Across these tree processes, the WMA's energy contributions, timescales and spatial scales align with the forces and dynamics involved, highlighting the role of LDW and HDW in modulating cellular interactions. This perspective holds implications for the design of lab-on-chip devices, advancements in sensor technologies, development of biomimetic membranes for drug delivery, creation of novel therapeutics and deeper understanding of protein misfolding diseases.

Keywords Hydrogen bond dynamics · Molecular interfaces · Solvation effects · Dynamic water clusters · Hydration layers

Introduction

Traditionally regarded as a passive solvent, water's role extends beyond merely providing a medium for biochemical reactions, since its structural and dynamic properties enable active participation in biological phenomena (Dargaville and Hutmacher 2022). The structural heterogeneity of liquid water arises from the continuous assembly and disassembly of hydrogen bonds, forming diverse geometric configurations that reflect water's branched polymeric nature (Shiotari and Sugimoto 2017). These configurations, termed water micro assemblies (WMA), have been extensively studied under extreme conditions, such as supercritical water (Skarmoutsos and Samios 2016), high-pressure crystals in supercooled water (Kim et al. 2009; Lin et al. 2018) and frozen water confined within nanometric slit pores (Koga et al. 2000) or nanochannels formed by cubic crystalline phases (Das et al. 2019). However, relatively little attention has been given to the microstructure of liquid water at ambient temperature and pressure. Under standard conditions, each water molecule forms up to four hydrogen bonds, creating a tetrahedral structure (Fanetti et al. 2014; Liu et al. 2017; Milovanović et al. 2020). Fluctuations in hydrogen bond numbers, ranging from two to six, result in molecules being either "loosely" or "tightly" bound (Thaomola et al. 2012). Liquid water has been described as a dynamic mixture of pentagonal and hexagonal rings (Shiotari and Sugimoto 2017; Formanek and Martelli 2020), of tetrahedral structures and of ring-and-chain-like assemblies (Liu et al. 2017). These configurations form densely connected spherical

✉ Arturo Tozzi
tozziarturo@libero.it

[1] Center for Nonlinear Science, Department of Physics, University of North Texas, 1155 Union Circle, #311427, Denton, TX 76203 - 5017, USA

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cores of approximately 140 water molecules, surrounded by fuzzy zones of ~ 1800 loosely connected molecules (Liu et al. 2017). Strong hydrogen bonds create multibranched polymers of about 150 molecules per chain (Naserifar and Goddard 2019), while density fluctuations form empty spaces resembling spherical or fractal-like voids (Ansari et al. 2018). Other WMA descriptions include a giant cluster percolating the system (dos Santos et al. 2004) and a linear, chain-like structure dominating the tree-like arrangement of the largest cluster (Jedlovszky et al. 2007).

The two-liquid scenario argues that liquid water comprises two competing molecular structures: low-density water (LDW) and high-density water (HDW) (Table 1). Although frequently observed under extreme conditions, these structures are also found under ambient conditions (Cheng et al. 2019). LDW features ordered gaps between molecular shells (de Oca et al. 2019), while HDW is associated with high-entropy, unstructured states. LDW consists of fused dodecahedra acting as templates for tetrahedral fluctuations, while HDW forms chain-like structures (Camisasca et al. 2019). LDW patches exhibit greater tetrahedrality and connectivity than HDW patches (Ansari et al. 2018; Faccio et al. 2022).

We describe how LDW and HDW may significantly influence biological processes by creating localized environments with specific physical and chemical properties. LDW regions, characterized by ordered hydrogen bonding networks, lower density and reduced entropy, may stabilize structural integrity near hydrophobic interfaces. Conversely, HDW regions, with higher density and entropy and weaker hydrogen bonds, may facilitate conformational transitions, enhance molecular mobility and disrupt ordered structures. This interplay likely impacts processes such as protein folding, enzyme catalysis and DNA and RNA dynamics. Their physical dimensions make WMA compatible with interacting with biological processes and capable of influencing the behavior of biological macromolecules. Water molecules (~ 2 Å) form LDW and HDW patches ranging from 0.3 to 2 nm (Ansari et al. 2018). Near ambient conditions, LDW has a density of 0.78 g/cm^3, while HDW reaches 1.08 g/cm^3 (Nomura et al. 2017). Although noncovalent bonds in liquid water at room temperature last under 200 femtoseconds (Lodish et al. 2000; Bakó et al. 2013; Naserifar and Goddard 2019), LDW persists for nearly half a second at 160 K (Lin et al. 2018). Despite their brief lifetimes (Camisasca et al. 2019), LDW and HDW create localized density changes that may affect environmental dynamics and drive macroscopic chemical and biophysical processes (Fanetti et al. 2014; Skarmoutsos and Samios 2016; Faccio et al. 2022). Also, we argue that percolation theory may play a pivotal role in influencing biochemical processes driven by WMA. In the context of water percolation, LDW may contribute to long-range connectivity within hydration layers, while HDW may provide localized flexibility.

This study explores the influence of WMA on biological phenomena by integrating experimental observations with theoretical insights. By focusing on key processes such as protein folding, enzyme catalysis and membrane dynamics, we aim to establish a framework that underscores water's active role in shaping the behavior of biological macromolecules. Examining the formation, dynamics and interplay of LDW and HDW, the study provides detailed analyses of their impacts, supported by molecular dynamics simulations and theoretical models.

Theoretical effects of water micro assemblies on biological phenomena

The concept of water micro assemblies (WMA), as discussed in the previous chapter, provides a compelling theoretical framework for understanding physical and dynamic processes (Laage et al. 2017). Below are some potential applications and behaviors explained by WMA:

(1) **Protein folding and stability**. The dynamic interplay between HDW and LDW regions may influence protein folding pathways. Localized density variations may

Table 1 Differences between low-density water (LDW) and high-density water (HDW)

	Low-density water (LDW)	High-density water (HDW)
Structure	Ordered hydrogen bonding networks	Disrupted hydrogen bonding networks
Density	Lower (~ 0.78 g/cm^3)	Higher (~ 1.08 g/cm^3)
Entropy	Reduced (more ordered)	Increased (more disordered)
Hydrophobicity	Stabilizes hydrophobic interfaces and molecular cores	Disrupts ordered zones, allowing transitions
Hydrogen Bonding	Strong and extended networks	Flexible and transient
Mobility	Lower molecular mobility (structured)	Higher molecular mobility (dynamic)
Biological Impact	Stabilizes protein folding, secondary structures, lipid headgroups	Enhances transitions, substrate turnover, molecular flexibility
Lifespan	Longer (~ 200 fs to 0.5 s in specific conditions)	Shorter and highly dynamic

create microenvironments that stabilize or destabilize intermediate folding states.

(2) **Enzyme catalysis**. WMA may modulate catalytic efficiency by restructuring local density and hydrogen-bonding networks near enzyme active sites, enhancing reaction kinetics.

(3) **Membrane dynamics**. Water micro assemblies may impact the stability and interactions of lipid bilayers, offering insights into phenomena such as lipid rafts and transient membrane pore formation.

(1) **Biological folding and protein stability**. Proteins rely on their interactions with water to achieve and maintain their functional structures (Sen and Voorheis 2014; Schiebel et al. 2018). The interplay of various forces during folding, such as hydrophobic interactions, hydrogen bonding, electrostatic interactions, van der Waals forces and steric constraints, highlights the central role of water in these processes (Bellissent-Funel et al. 2016; Phan-Xuan et al. 2020).

Hydrophobic interactions are a key driving force in macromolecular folding, as nonpolar residues cluster internally to avoid water (Fogarty and Laage 2014; Ye et al. 2024). LDW regions, with their reduced density and structured hydrogen bonding network, may facilitate this process by forming ordered exclusion zones around hydrophobic groups, stabilizing the burial of nonpolar residues. Meanwhile, HDW regions, less structured and more dynamic, may support the transient exposure of hydrophobic residues during early folding stages. Hydrogen bonding is another critical force which stabilizes secondary structures such as α-helices and β-sheets. HDW, due to its higher entropy and looser hydrogen bonds, may disrupt weaker hydrogen bonds in unfolding scenarios, whereas LDW may enhance hydrogen bond stability during folding.

Electrostatic interactions, such as salt bridges and charged residue pairings, are crucial for directing protein folding pathways and stabilizing the final folded structure. The local density variations between LDW and HDW may influence the dielectric constant of water, modulating electrostatic interaction strength. LDW, by reducing shielding effects, may enhance charge–charge attractions in localized regions, promoting stability. Van der Waals forces, though weaker and distance dependent, are crucial for stabilizing tightly packed molecular cores (Li et al. 2018). The structured nature of LDW may enhance these forces by increasing the local organization of interacting molecules within protein interiors. Steric constraints further limit the conformational space accessible to macromolecules, as backbone torsions and side-chain interactions impose structural restrictions. LDW's ordered environment may help resolve steric clashes, guiding molecules toward favorable conformations, while HDW may provide flexibility for conformational sampling in partially unfolded states.

The involvement of WMA in biological folding becomes plausible when considering their energy, timescale and spatial dimensions. The energy scales of folding forces align with the contributions of LDW and HDW transitions (Table 2), suggesting that WMA may significantly influence folding energetics. The interplay between LDW and HDW may fine-tune the folding energy landscape, balancing stability and flexibility across the conformational space to optimize transitions (Fig. 1). The timescales of WMA also align well with the dynamic requirements of folding processes. Protein folding occurs over milliseconds to seconds for small, single-domain proteins, while LDW and HDW regions exhibit lifetimes ranging from ~200 fs to 0.5 s. This temporal overlap allows micro assemblies to influence both the rapid early stages of folding and the slower stabilization of intermediate or final structures. Spatially, the sizes of micro assemblies, ranging from ~0.3 to 2 nm, are sufficient to interact with folding nuclei (typically ~5 to 10 Å) and influence tertiary and quaternary structural organization.

Experimental and theoretical studies provide evidence for the role of WMA in biological folding and stability. Hydration dynamics reveal how LDW and HDW influence solvation shell behavior (Camisasca et al. 2023). These studies highlight the ability of LDW to form structured, ice-like networks around biomolecules, stabilizing their conformations, while HDW introduces dynamism required for conformational transitions. Evidence from folding pathways, particularly in cold denaturation studies, further supports the relevance of structured water (Taricska et al. 2019). Cold denaturation, where proteins unfold at low temperatures, has been linked to the stabilization of unfolded states by structured hydration layers, consistent with the properties of LDW-HDW transitions (Espinosa et al. 2019).

In sum, WMA may represent a feasible and energetically relevant mechanism for modulating biological folding and stability. Their ability to dynamically adjust local density, hydrogen bonding and dielectric properties makes them a critical, yet underexplored, factor in biophysical processes. LDW stabilizes hydrophobic interactions and secondary structures, while HDW enables dynamic transitions and flexibility. These complementary roles, supported by energy and timescale compatibility, suggest that WMA are integral to the folding process and its regulation.

(2) **Enzyme catalysis**. Enzymatic reactions are governed by various physical forces, all of which align with the unique properties of WMA (Adamczyk et al. 2014; Fogarty and Laage 2014; Zsidó and Hetényi 2021). Electrostatic effects are critical in enzyme catalysis, as charged residues and substrates interact to stabilize the

Table 2 Functional roles of water micro assemblies in protein folding processes

Force type	Energy contribution (kcal/mol)	Role in protein folding	Impact of micro assemblies	Microaggregate energy contribution (kcal/mol)
Electrostatic interactions	~ 1–3	Guide charged residue interactions and folding pathways	LDW enhances electrostatic interactions by lowering dielectric constant; HDW facilitates charge redistribution	LDW: ~ 1–3, HDW: ~ 1–3
Hydrogen bonding	~ 1–5	Stabilizes secondary structures (e.g., α-helices, β-sheets)	LDW strengthens hydrogen bonds, stabilizing folding intermediates; HDW allows flexibility for conformational sampling	LDW: ~ 1–3, HDW: ~ 1–3
Hydrophobic interactions	~ 0.5–1 per residue	Drive hydrophobic core formation	LDW creates structured exclusion zones around hydrophobic residues; HDW transiently disrupts zones to allow folding progression	LDW: ~ 1–3, HDW: ~ 1–3
Van der Waals forces	~ 0.1–0.3 per atom pair	Stabilize tightly packed core structures	LDW enhances alignment and packing in the hydrophobic core; HDW supports transient adjustments during folding	LDW: ~ 0.5–1, HDW: ~ 0.5–1
Solvation dynamics	Variable	Modulate folding energy landscape	LDW-HDW transitions fine-tune hydration layers, stabilizing intermediates and guiding the folding process	LDW: ~ 1–3, HDW: ~ 1–3

transition state and orient substrates within the active site. LDW, with its lower dielectric constant, may enhance these interactions by reducing charge screening, thereby increasing the strength of electrostatic interactions. HDW, due to its flexible hydrogen bonding and higher local entropy, may assist in charge redistribution during catalytic transitions, helping enzymes overcome energy barriers associated with electron transfer or polarization. This dual role enables WMA to modulate the precise electrostatic environment required for catalysis.

Hydrophobic interactions contribute to substrate stabilization by forming nonpolar pockets within enzyme active sites (Kurkal et al. 2005). These hydrophobic pockets exclude bulk water, providing a favorable microenvironment for substrate binding. LDW regions may amplify this effect by creating structured exclusion zones around nonpolar residues, reinforcing the hydrophobic pocket's integrity. On the other hand, HDW, with its dynamic nature and reduced local density, may transiently disrupt these zones, allowing substrate access to the active site. This balance may ensure that the enzyme maintains a stable yet flexible environment, optimizing both substrate accommodation and turnover. Hydrogen bonding is another cornerstone of enzyme catalysis, stabilizing intermediates and transition states while maintaining precise catalytic geometry. LDW regions, with their stable and extended hydrogen bonding networks, may strengthen these interactions, particularly with polar substrates or intermediates. Conversely, HDW, characterized by dynamic and flexible hydrogen bonding, may facilitate transitions between intermediates and supports efficient product release.

Van der Waals forces play a significant role in substrate alignment and transient state stabilization within enzyme active sites. LDW regions, with their structured local environments, may enhance molecular alignment and optimize these weak interactions. HDW, on the other hand, may introduce the required dynamics to ensure that transient interactions remain flexible, allowing enzymes to adapt their active site to different catalytic steps. Dynamic solvation effects, wherein fluctuations in the solvent modulate energy barriers and facilitate substrate-product exchange, are intrinsically linked to LDW–HDW transitions. These transitions may enable WMA to fine-tune solvation dynamics at each step of the catalytic cycle. LDW regions stabilize specific steps, such as substrate binding or transition state stabilization, while HDW regions introduce dynamic flexibility to promote intermediate transitions and product release. This adaptability may be crucial for the efficiency of enzymatic reactions.

The alignment of WMA energetics with enzymatic processes further supports their potential to influence enzyme

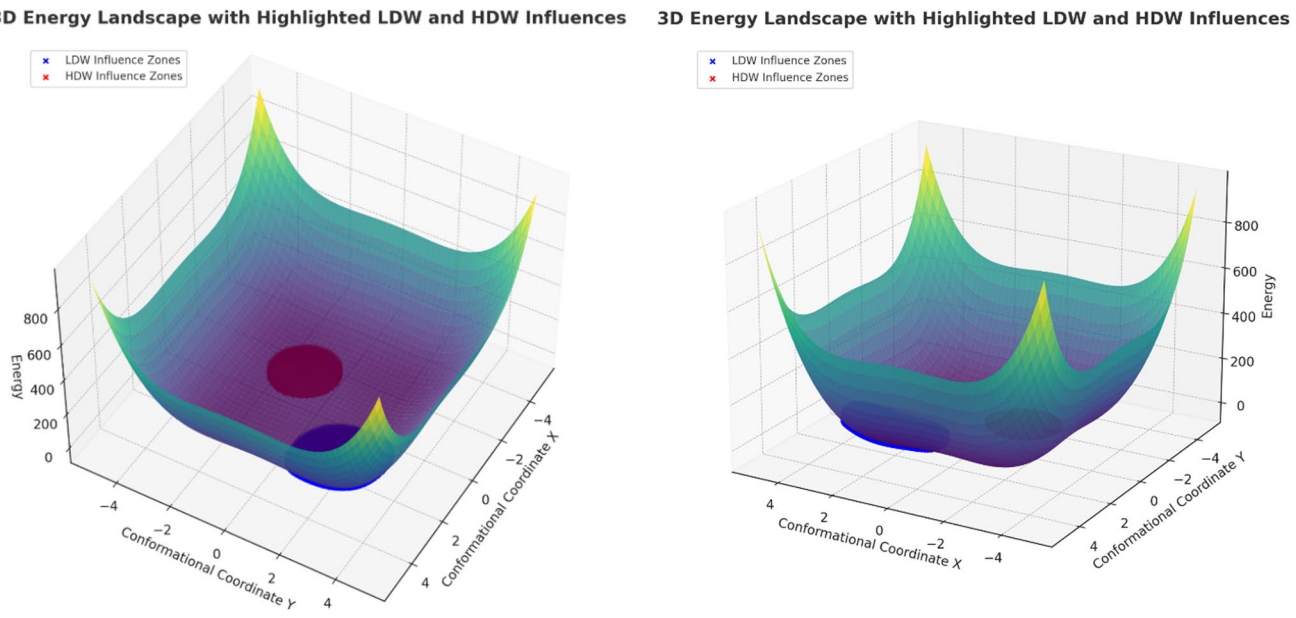

Fig. 1 Theoretical 3D visualization of the energy landscape shaped by water microaggregates, illustrating the combined effects of LDW and HDW. LDW zones, depicted in blue, stabilize the energy landscape by forming depressions near local minima, while HDW zones, represented in red, enhance flexibility and facilitate dynamic transitions near transition states

catalysis (Table 3). The timescales of enzymatic processes, which typically range from microseconds to milliseconds, align well with the lifetimes of LDW and HDW regions, which span from 200 fs to 0.5 s. For instance, LDW regions may stabilize critical transition states over microsecond timescales, while HDW regions provide the rapid flexibility needed for intermediate transitions on sub-nanosecond timescales. Spatially, enzyme active sites are typically 1–10 nm in size, a scale well suited for interactions with micro assemblies, which range from 0.3 to 2 nm. This spatial compatibility may allow LDW and HDW regions to directly influence active site dynamics, stabilizing critical regions and facilitating the movement of substrates and products. LDW may form around hydrophobic residues in the active

Table 3 Functional roles of water micro assemblies in enzyme catalysis

Force type	Energy contribution (kcal/mol)	Role in catalysis	Impact of micro assemblies	Microaggregate energy contribution (kcal/mol)
Electrostatic interactions	~ 1–3	Stabilize transition states	LDW enhances interactions by lowering the dielectric constant; HDW facilitates charge redistribution	LDW: ~ 1–3, HDW: ~ 1–3
Hydrophobic interactions	~ 0.5–1 per residue	Stabilize substrates in pockets	LDW promotes exclusion zones; HDW transiently opens access pathways	LDW: ~ 1–3, HDW: ~ 1–3
Hydrogen Bonding	~ 1–5	Stabilizes intermediates	LDW strengthens bonds; HDW enables dynamic flexibility for transition states	LDW: ~ 1–3, HDW: ~ 1–3
Van der Waals Forces	~ 0.1–0.3 per atom pair	Align substrates	LDW improves alignment precision; HDW supports transient substrate dynamics	LDW: ~ 0.5–1, HDW: ~ 0.5–1
Solvation Dynamics	Variable	Modulate energy barriers	LDW-HDW transitions fine-tune solvation dynamics during different catalytic stages	LDW: ~ 1–3, HDW: ~ 1–3

site, enhancing substrate stability, while HDW may transiently occupy solvent-accessible areas.

Experimental and theoretical evidence supports the role of WMA in enzyme catalysis. Structured water layers, as observed through solvent isotope effects and hydration studies, correlate with enhanced catalytic rates, aligning with the behavior of LDW (Kurkal-Siebert et al. 2006). Cryo-EM and X-ray crystallography further reinforce this perspective by revealing ordered water clusters near enzyme active sites (Brogan et al. 2014). These clusters, consistent with LDW properties, may play a crucial role in stabilizing catalytic residues. Theoretical studies add depth to this understanding. Molecular dynamics simulations demonstrate that water clusters near active sites exhibit high sensitivity to changes in hydrogen bonding, mirroring the dynamic interplay between LDW and HDW. These transitions highlight the adaptability of WMA in responding to the demands of enzymatic processes, such as stabilizing intermediates or facilitating substrate-product transitions.

The case of chymotrypsin illustrates how WMA may influence enzymatic function. Chymotrypsin, a serine protease, relies on a catalytic triad comprising serine, histidine and aspartate residues for its nucleophilic attack mechanism (Jing et al. 2002). This process is supported by a structured hydration layer, a hallmark of LDW behavior (Kozlova et al. 1999; Eckstein et al. 2002). LDW may stabilize the catalytic triad by strengthening hydrogen bonding, ensuring the alignment and reactivity of these residues. Conversely, HDW may facilitate rapid proton transfer and substrate turnover by modulating local water density, providing the necessary flexibility for catalytic efficiency.

In sum, WMA provide a viable and energetically significant mechanism for influencing enzyme catalysis. Their ability to dynamically adjust hydrogen bonding, hydrophobic interactions, local density and solvation properties makes them possible contributors to catalytic processes.

(3) **Membrane dynamics**. Biological membranes rely heavily on interactions with surrounding water for their structural integrity, fluidity and functionality (Higgins et al. 2006; Wood et al. 2007; Chattopadhyay et al. 2021). The interplay between LDW and HDW regions in the aqueous environment may provide insights into how water influences processes such as lipid organization, protein–membrane interactions and membrane dynamics. Electrostatic interactions are essential for membrane dynamics in stabilizing the polar headgroups of lipids and mediating interactions with charged proteins. LDW regions, with their lower dielectric constant and ordered hydrogen bonding, may enhance membrane electrostatic stabilization by reducing charge screening and strengthening headgroup interactions. HDW regions, on the other hand, may facilitate rapid ion exchange and dynamic interactions between polar lipids and surrounding ions or proteins. This dual behavior ensures that membranes maintain stability while allowing flexibility for dynamic processes.

Hydrophobic interactions are another crucial component of membrane dynamics, driving the self-assembly of lipid bilayers and maintaining their structural integrity (Cheng et al. 2013; Fisette et al. 2016). Nonpolar lipid tails cluster together to minimize water exposure, a process which may be amplified by LDW regions forming structured exclusion zones around hydrophobic regions. Conversely, HDW regions may transiently disrupt these zones, allowing for the lateral mobility of lipids and enabling dynamic rearrangements critical for processes like fusion, fission and protein insertion. Still, hydrogen bonding plays a significant role in maintaining the hydration shells of lipid headgroups and facilitating interactions between the membrane and water-soluble molecules. LDW regions, with their stable hydrogen bonding networks, may provide consistent hydration to polar headgroups, enhancing membrane stability. In contrast, HDW regions, with their dynamic hydrogen bonding, may support processes requiring flexibility, such as lipid flip-flop, membrane deformation or protein–membrane interactions. Van der Waals forces are crucial for lipid packing and membrane stability. LDW regions, by organizing water molecules near the lipid interface, may enhance molecular packing and stabilize membrane structure. Meanwhile, HDW regions may contribute to the dynamic rearrangement of lipids, ensuring the bilayer remains fluid and adaptable under varying conditions.

The transition between different membrane phases, such as the liquid-ordered and liquid-disordered states, provides a case study in micro assembly influence. LDW regions may stabilize the liquid-ordered phase by enhancing hydrogen bonding and hydrophobic stabilization, while HDW regions may support the flexibility needed for transitions to the liquid-disordered phase, facilitating processes such as membrane protein activation or lipid raft formation. Dynamic solvation effects, where water molecules in the hydration shell modulate energy barriers and lipid dynamics, may be influenced by LDW–HDW transitions. LDW regions may stabilize bilayer formation by providing structured hydration to lipid headgroups, while HDW regions may allow rapid water exchange and flexibility, supporting membrane-associated processes such as vesicle formation and membrane protein function.

The ability of WMA to influence membrane dynamics is reinforced by their energetic compatibility (Table 4), suggesting that WMA can meaningfully modulate the forces driving membrane behavior. The timescales of membrane dynamics, ranging from nanoseconds for lipid

Table 4 Functional roles of water micro assemblies in membrane dynamics

Force type	Energy contribution (kcal/mol)	Role in membrane dynamics	Impact of micro assemblies	Microaggregate energy contribution (kcal/mol)
Electrostatic interactions	~ 1–3	Stabilize lipid headgroups and protein interactions	LDW enhances headgroup stabilization by lowering dielectric constant; HDW facilitates dynamic ion exchange	LDW: ~ 1–3, HDW: ~ 1–3
Hydrogen bonding	~ 1–5	Stabilizes hydration shells around lipid headgroups	LDW strengthens hydrogen bonds around headgroups; HDW supports rapid rearrangements during membrane remodeling	LDW: ~ 1–3, HDW: ~ 1–3
Hydrophobic interactions	~ 0.5–1 per lipid	Drive lipid tail clustering and bilayer stability	LDW forms structured exclusion zones around lipid tails; HDW transiently disrupts zones for fluidity	LDW: ~ 1–3, HDW: ~ 1–3
Van der Waals forces	~ 0.1–0.3 per atom pair	Stabilize lipid packing	LDW improves packing by structuring water near lipid interfaces; HDW supports lateral diffusion	LDW: ~ 0.5–1, HDW: ~ 0.5–1
Solvation dynamics	Variable	Modulate lipid mobility and protein incorporation	LDW–HDW transitions adjust hydration shells to support vesicle formation, fusion and protein binding	LDW: ~ 1–3, HDW: ~ 1–3

diffusion to milliseconds for processes like vesicle budding, align well with LDW and HDW lifetimes. This temporal compatibility may allow WMA to influence both rapid lipid rearrangements and slower membrane remodeling events. Spatially, the thickness of the lipid bilayer (~ 4–6 nm) and the hydration shell (~ 0.3–1 nm) are well matched to the size of LDW and HDW regions, ensuring that WMA may interact with membrane components and their surrounding environment.

Experimental evidence supports the involvement of WMA in membrane dynamics. Neutron scattering and cryo-EM studies have revealed ordered water layers around lipid headgroups, consistent with LDW properties (Frölich et al. 2009). Ultrafast spectroscopy and NMR studies further demonstrate the dynamic nature of hydration layers, aligning with the behavior of HDW (Zigmantas et al. 2022; Lorenz-Ochoa et al. 2023). Molecular dynamics simulations provide additional theoretical support, showing structured and dynamic water regions interacting with lipid bilayers and modulating membrane properties.

In sum, WMA provides a plausible mechanism for influencing membrane dynamics. Their ability to modulate hydrogen bonding, electrostatic interactions and solvation dynamics positions them as critical contributors to the stability and functionality of biological membranes.

Percolation of water micro assemblies and its role in biological systems

Short-lived WMA can form extensive networks that significantly influence average flow properties. The emergence of large-scale connectivity within high-density water assemblies may facilitate percolation through three-dimensional hydrogen-bonded water lattices (Timonin 2018). Evidence supports the occurrence of percolation in water (Bernabei & Ricci 2008; Strong et al. 2018). For instance, percolation transitions in hydrogen bond networks have been observed in supercritical water, especially at high molecular densities (Jedlovszky et al. 2007). These transitions follow a universal power law, with percolation occurring when the fractal dimension of the largest cluster approaches 2.53 (Galam & Mauger 1996; Jedlovszky et al. 2007). A percolating network requires approximately 40% of possible hydrogen bonds to be intact. Simulations reveal that initially disconnected clusters coalesce at critical cutoff values, forming large, space-filling networks with minimal disconnected fragments (Geiger 1979). Given that 18.01528 g of water contain $6.02214076 \times 10^{23}$ water molecules, the number of percolating molecules is immense, with a system-spanning cluster emerging at a probability of 0.65 in liquid water (Oleinikova et al. 2002).

Percolation significantly influences flow dynamics by creating transient obstacles. Hydrogen bond energies (1–5 kcal/mol) are much lower than those needed to break covalent bonds (~ 110 kcal/mol) (Lodish et al. 2000). This means that the average molecular kinetic energy at room temperature (~ 0.6 kcal/mol) is sufficient to disrupt noncovalent bonds, allowing hydrogen bond-induced barriers to influence liquid water flow (Lodish et al. 2000).

Percolation theory offers insights into how WMA influence hydrogen bond networks, phase transitions and transport phenomena. We propose the following theoretical framework:

(1) **LDW and percolation clusters**. LDW forms well-ordered hydrogen bond structures, facilitating connectivity and the formation of percolation clusters. Conversely, HDW, with its disrupted hydrogen bonding, acts as a barrier to connectivity.
(2) **Phase transition dynamics**. At lower temperatures, LDW regions expand, forming nuclei essential for freezing, while HDW fragments connectivity in supercooled water. In supercritical water, HDW dominates, leading to high diffusivity and low connectivity. As the system transitions back to the liquid phase, LDW re-establishes percolation, restoring cohesion and reducing diffusivity.
(3) **Diffusion and transport**. Diffusion in liquid water depends on cooperative molecular movement within hydrogen bond networks. LDW forms stable pathways for molecular and ion transport, while HDW introduces transient disruptions. At the percolation threshold, LDW clusters span the system, enabling continuous transport pathways. Proton transport, for instance, relies on rapid hydrogen bond reorganization. LDW maintains stable pathways for proton hopping, whereas HDW slows the process due to structural disorder.
(4) **Dynamic percolation and temperature effects**. Dynamic percolation in water is characterized by the continuous breaking and reforming of hydrogen bonds. At lower temperatures, LDW dominates, forming long-lived percolation clusters, whereas at higher temperatures, HDW fragments the networks, increasing molecular mobility.
(5) **Constrained percolation in confined environments**. In confined environments, such as nanoporous materials or near biomolecular surfaces, water may exhibit constrained percolation. LDW forms ordered layers near hydrophilic interfaces, providing stability, while HDW dominates bulk regions or hydrophobic zones, introducing flexibility and disorder.

In sum, the WMA theoretical framework highlights the fundamental role of percolation in biological systems and provides a basis for further exploration of LDW and HDW's influence on molecular and macroscopic phenomena. Future integration of percolation models with experimental and computational approaches will help unravel the complexity of these dynamic water networks.

Conclusions

Liquid water exhibits intrinsic chemical inhomogeneity, manifesting as a dynamic three-dimensional network characterized by microscopic density fluctuations and structural variations. Water, far beyond being a passive solvent, plays an active and multifaceted role in driving and modulating biological phenomena. This study explored the concept of WMA, characterized by LDW and HDW regions, as dynamic contributors to various molecular and cellular processes. Through their distinct physical and chemical properties, WMA offer a framework to better understand the behavior of water at the nanoscale and its influence on key biological processes. LDW, defined by structured hydrogen bond networks, lower density and high coherence, may stabilize hydrophobic interactions, hydrogen bonding and molecular order. Conversely, HDW, with its dynamic, higher-density and entropy-driven properties, may facilitate flexibility, conformational sampling and rapid transitions. These dual roles enable WMA to act as adaptable mediators across a wide range of biological phenomena, optimizing stability and functionality under varying conditions. Across protein folding, enzyme catalysis and membrane dynamics, the energy contributions, timescales and spatial scales of WMA align with the forces and dynamics involved, underscoring the critical role of LDW and HDW transitions in modulating dynamical interactions in the cellular milieu.

While the concept of WMA is still emerging, their influence may extend beyond understanding fundamental biological processes. WMA may have significant implications across experimental, technological and pathological contexts, spanning multiple disciplines, including molecular biology, fluid dynamics and materials science.

In microfluidics and nanofluidics, understanding the dynamic behavior of these micro assemblies can lead to enhanced control of water within confined geometries, a critical factor for designing lab-on-chip devices that require precise manipulation of fluid dynamics at the microscale. The ability to induce or manipulate LDW and HDW regions artificially opens the door to creating targeted micro-vortices or density variations. Such capabilities may pave the way for novel sensor technologies where the localized structuring of water affects sensitivity and detection thresholds or for advanced turbulence control mechanisms in engineering systems. Also, artificial catalysts may benefit from mimicking

LDW-HDW transitions, improving efficiency by incorporating water-like dynamics into synthetic systems.

In biomedical and pharmaceutical contexts, understanding WMA can inform the design of drugs and therapeutics that leverage hydration dynamics. Mimicking the properties of WMA may inspire the development of biomimetic systems such as responsive hydrogels or biomimetic membranes with tailored properties for drug delivery. In drug design, targeting water dynamics within active sites may optimize the specificity and efficacy of ligand binding and enzyme inhibitors, enhancing drug efficacy. Our study also highlights the importance of exploring the role of WMA in complex and confined environments, such as within cells or in biomolecular assemblies. In these scenarios, water's behavior deviates significantly from that of bulk water, with hydration layers and confined spaces amplifying the influence of micro assemblies. Understanding these effects may reveal new principles underlying cellular organization, molecular recognition and signal transduction.

Additionally, understanding water's role in pathological conditions, such as protein misfolding diseases, cancer or neurodegeneration, may open new avenues for therapeutic intervention. Abnormal interactions between LDW and HDW may contribute to the pathological protein aggregation observed in lipid storage disorders and neurodegenerative diseases such as Alzheimer's or Parkinson's disease (Polychronidou et al. 2020; Padilla-Godínez et al. 2021). Similarly, variations in water density may explain some of the unique physical and biochemical properties of tumor microenvironments (He et al. 2022). In cancer, localized turbulence or non-uniform water structuring in the interstitial fluid may influence nutrient transport, cellular communication and drug delivery effectiveness.

Despite significant advances, challenges persist in fully understanding the role of WMA in biological phenomena. The transient and highly dynamic nature of LDW and HDW makes their phase spaces difficult to explore, leading to the upsetting concept of "water's no-man's land" (Lin et al. 2018). Despite that liquid water can be tackled in terms of an evolving, fluctuating and branched polymer (Naserifar and Goddard 2019), a full understanding of its dynamical and structural properties is still lacking (Fanetti et al. 2014) due to technical difficulties in gaining experimental information on ultrafast interplay (Tamtögl et al. 2020). The heterogeneity of water is usually approached through simulation of molecular dynamics, such as, e.g., conventional QM/MM scheme and ONIOM-XS methods (Thaomola et al. 2012), second-order Møller–Plesset perturbation theory (Liu et al. 2017), quantum Monte Carlo, non-canonical coupled cluster theory (Al-Hamdani and Tkatchenko 2019), modified Louvain algorithm of graph community (Gao et al. 2021), topological local (clustering coefficient, path length and degree distribution) and global (spectral analysis) properties (dos Santos et al. 2004; Carreras et al. 2008; Steinberg et al. 2019), persistent homology methods (Wu 2020). These methods operate at different temporal and spatial resolutions, necessitating further integration for a more comprehensive understanding. Weak, non-covalent interactions have been experimentally studied just in small molecular complexes, falling short of the macroscopic structural properties that are typical of complex soft materials such as, e.g., supramolecular aggregates (Al-Hamdani and Tkatchenko, 2019). To make things more complicated, totally different networks topologies and physical interpretations have been provided, depending on how rings have been counted (Das et al. 2019; Formanek and Martelli 2020).

It remains uncertain whether liquid water exhibits randomness or long-range interactions. Dos Santos et al. (2004) proposed that the water network at room temperature resembles a Poisson distribution, indicative of randomness. However, other studies present evidence of medium- to long-range order (Faccio et al. 2022). Tamtögl et al. (2020) observed correlated motion at the surface of a topological insulator, contrasting with Brownian motion, while Ansari et al. (2018) and Gao et al. (2021) reported collective translational fluctuations in hydrogen-bonded water clusters. Percolation models face challenges, such as the inability to determine the percolation threshold based solely on cluster size distribution (Jedlovszky et al. 2007). Moreover, environmental factors, including temperature, pressure and solute concentration may influence the balance between LDW and HDW. For example, lower temperatures correspond to stronger hydrogen bond interactions. Upon melting, increases in temperature result in a rapid decrease in the average number of assemblies (Gao et al. 2021) and a broader ring size distribution (Bakó et al., 2013; Naserifar and Goddard 2019). Consequently, microscopic assemblies in water become difficult to distinguish beyond the isochore end point of 292 K (Nomura et al., 2017).

Future research should focus on developing more refined experimental and computational methods to study WMA and their interactions with biomolecules. Experimentally, combining time-resolved methods with spatially resolved imaging techniques may bridge the gap between molecular dynamics and observable biological outcomes.

In conclusion, WMA may represent a transformative perspective on the active role of water in biological phenomena. By bridging the gap between molecular-scale dynamics and macroscopic behavior, WMA may provide a unifying framework for understanding how water shapes biomolecular processes. LDW and HDW, through their complementary properties, may influence stability, flexibility and functionality across diverse biological systems. As research in this field advances, the potential applications in health, technology and materials science are vast, underscoring

the importance of continued exploration into the dynamic Q8world of WMA.

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Declarations

Conflict of interest The author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within 3 years of beginning the submitted work that may inappropriately influence, or be perceived to influence, their work.

Ethical approval and consent to participate This research does not contain any studies with human participants or animals performed by the author.

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References

Adamczyk K, Simpson N, Greetham GM, Gumiero A, Walsh MA, Towrie M, Parker AW, Hunt NT (2014) ultrafast infrared spectroscopy reveals water-mediated coherent dynamics in an enzyme active site. Chem Sci 6(1):505–516. https://doi.org/10.1039/c4sc02752c

Al-Hamdani Ys, Tkatchenko A (2019) Understanding non-covalent interactions in larger molecular complexes from first principles featured. J Chem Phys 150:010901. https://doi.org/10.1063/1.5075487

Ansari N, Dandekar R, Caravati S, Sosso GC, Hassanali A (2018) High and low density patches in simulated liquid water. J Chem Phys 149(20):204507. https://doi.org/10.1063/1.5053559

Bakó I, Bencsura Á. Hermannson K, Bálint S, Grósz T et al (2013) Hydrogen bond network topology in liquid water and methanol: a graph theory approach. Phys Chem Chem Phys (36)

Bellissent-Funel M-C, Hassanali A, Havenith M, Henchman R, Pohl P, Sterpone F, van der Spoel D, Yao X, Garcia AE (2016) Water determines the structure and dynamics of proteins. Chem Rev 116(13):7673–7697. https://doi.org/10.1021/acs.chemrev.5b00664

Bernabei M, Ricci MA (2008) Percolation and clustering in supercritical aqueous fluids. J Phys: Condens Matter 20:494208

Brogan APS, Sharma KP, Perriman AW, Mann S (2014) Enzyme activity in liquid lipase melts as a step towards solvent-free biology at 150 °C. Nat Commun 5:5058. https://doi.org/10.1038/ncomms6058

Camisasca G, Schlesinger D, Zhovtobriukh I, Pitsevich G, Pettersson LGM (2019) A proposal for the structure of high- and low-density fluctuations in liquid water. J Chem Phys 151:034508. https://doi.org/10.1063/1.5100875

Camisasca G, Tenuzzo L, Gallo P (2023) Protein hydration water: focus on low density and high density local structures upon cooling. J Mol Liq 370(January):120962. https://doi.org/10.1016/j.molliq.2022.120962

Carreras BA, Llerena I, Garcia L, Calvo I (2008) Topological characterization of flow structures in resistive pressuregradient-driven turbulence. Phys Rev E 78:066402

Chattopadhyay M, Krok E, Orlikowska H, Schwille P, Franquelim HG, Piatkowski L (2021) Hydration layer of only a few molecules controls lipid mobility in biomimetic membranes. J Am Chem Soc 143(36):14551–14562. https://doi.org/10.1021/jacs.1c04314

Cheng C-Y, Varkey J, Ambroso MR, Han S (2013) Hydration dynamics as an intrinsic ruler for refining protein structure at lipid membrane interfaces. Proc Natl Acad Sci 110(42):16838–16843. https://doi.org/10.1073/pnas.1307678110

Cheng S, Wang X, Zhang Z, Li S (2019) Ultra-high-density local structure in liquid water. Chin Phys B 28(11):116104

Dargaville BL, Hutmacher DW (2022) Water as the often neglected medium at the interface between materials and biology. Nat Commun 13(1):4222. https://doi.org/10.1038/s41467-022-31944-2

Das K, Roy B, Satpathi S, Hazra P (2019) Impact of topology on the characteristics of water inside cubic lyotropic liquid crystalline systems. J Phys Chem B 123(18):4118–4128. https://doi.org/10.1021/acs.jpcb.9b01559

de Oca JMM, Accordino SR, Verde AR, Alarcón LM, Appignanesi GA (2019) Structural features of high-local-density water molecules: insights from structure indicators based on the translational order between the first two molecular shells. Phys Rev E 99:062601

Dos Santos VML, Moreira FGB, Longo RL (2004) Topology of the hydrogen bond networks in liquid water at room and supercritical conditions: a small-world structure. Chem Phys Lett 390(1–3):157–161. https://doi.org/10.1016/j.cplett.2004.04.016

Eckstein M, Sesing M, Kragl U, Adlercreutz P (2002) At low water activity α-chymotrypsin is more active in an ionic liquid than in non-ionic organic solvents. Biotech Lett 24:867–872. https://doi.org/10.1023/A:1015875123711

Espinosa YR, Caffarena ER, Raúl Grigera J (2019) The role of hydrophobicity in the cold denaturation of proteins under high pressure: a study on apomyoglobin. J Chem Phys 150(7):075102. https://doi.org/10.1063/1.5080942

Faccio C, Benzi M, Zanetti-Polzi L, Daidone I (2022) Low- and high-density forms of liquid water revealed by a new medium-range order descriptor. J Mol Liq 355:118922. https://doi.org/10.1016/j.molliq.2022.118922

Fanetti S, Lapini A, Pagliai M, Citroni M, Di Donato M et al (2014) Structure and dynamics of low-density and high-density liquid water at high pressure. J Phys Chem Lett 5(1):235–240. https://doi.org/10.1021/jz402302z

Fisette O, Päslack C, Ryan Barnes J, Isas M, Langen R, Heyden M, Han S, Schäfer LV (2016) Hydration dynamics of a peripheral membrane protein. J Am Chem Soc 138(36):11526–11535. https://doi.org/10.1021/jacs.6b07005

Fogarty AC, Laage D (2014) Water dynamics in protein hydration shells: the molecular origins of the dynamical perturbation. J Phys Chem B 118(28):7715–7729. https://doi.org/10.1021/jp409805p

Formanek M, Martelli F (2020) Probing the network topology in network-forming materials: the case of water. AIP Adv 10:055205. https://doi.org/10.1063/5.0005332

Frölich A, Gabel F, Jasnin M, Lehnert U, Oesterhelt D, Stadler AM, Tehei M, Weik M, Wood K, Zaccai G (2009) From shell to cell: neutron scattering studies of biological water dynamics and coupling to activity. Faraday Discuss 141:117–130. https://doi.org/10.1039/b805506h

Galam S, Mauger A (1996) Universal formulas for percolation thresholds. Phys Rev E 53(3):2177–2180

Gao Y, Fang H, Ni K (2021) A hierarchical clustering method of hydrogen bond networks in liquid water undergoing shear flow. Sci Rep 11:9542. https://doi.org/10.1038/s41598-021-88810-7

Geiger A (1979) Aspects of the percolation process for hydrogen-bond networks in water. J Chem Phys 70(10):4185–4193. https://doi.org/10.1063/1.438042

He X, Yang Y, Han Y, Feng Xu (2022) Extracellular matrix physical properties govern the diffusion of nanoparticles in tumor microenvironment. Proc Natl Acad Sci U S A 120(1):e2209260120. https://doi.org/10.1073/pnas.2209260120

Higgins MJ, Polcik M, Fukuma T, Sader JE, Nakayama Y, Jarvis SP (2006) Structured water layers adjacent to biological membranes. Biophys J 91(7):2532–2542. https://doi.org/10.1529/biophysj.106.084517

Jedlovszky P, Brovchenko I, Oleinikova A (2007) Percolation transition in supercritical water: a Monte Carlo simulation study. J Phys Chem B 111(26):7603–7609

Jing H, Yuanyuan X, Carson M, Moore D, Macon KJ, Volanakis JE, Narayana SVL (2000) New structural motifs on the chymotrypsin fold and their potential roles in complement factor B. EMBO J 19(2):164–173. https://doi.org/10.1093/emboj/19.2.164

Kim CU, Barstow B, Tate MW, Gruner SM (2009) Evidence for liquid water during the high-density to low-density amorphous ice transition. Proc Natl Acad Sci 106(12):4596–4600. https://doi.org/10.1073/pnas.0812481106

Koga K, Tanaka H, Zeng X (2000) First-order transition in confined water between high-density liquid and low-density amorphous phases. Nature 408:564–567. https://doi.org/10.1038/35046035

Kozlova NO, Bruskovskaya IB, Melik-Nubarov NS, Yaroslavov AA, Kabanov VA (1999) Catalytic properties and conformation of hydrophobized α-chymotrypsin incorporated into a bilayer lipid membrane. FEBS Lett 461(3):141–144. https://doi.org/10.1016/S0014-5793(99)01449-3

Kurkal V, Daniel RM, Finney JL, Tehei M, Dunn RV, Smith JC (2005) Enzyme activity and flexibility at very low hydration. Biophys J 89(2):1282–1287. https://doi.org/10.1529/biophysj.104.058677

Kurkal-Siebert V, Daniel RM, Finney JL, Tehei M, Dunn RV, Smith JC (2006) Enzyme hydration, activity and flexibility: a neutron scattering approach. J Non-Crystalline Solids 352(42–49):4387–4393. https://doi.org/10.1016/j.jnoncrysol.2006.02.137

Laage D, Elsaesser T, Hynes JT (2017) Water dynamics in the hydration shells of biomolecules. Chem Rev 117(16):10694–10725. https://doi.org/10.1021/acs.chemrev.6b00765

Li J, Wang Y, An L, Chen J, Yao L (2018) Direct observation of CH/CH van der Waals interactions in proteins by NMR. J Am Chem Soc 140(9):3194–3197. https://doi.org/10.1021/jacs.7b13614

Lin C, Smith JS, Sinogeikin SV, Shen G (2018) Experimental evidence of low-density liquid water upon rapid decompression. Proc Natl Acad Sci 115(9):2010–2015. https://doi.org/10.1073/pnas.1716310115

Liu J, He X, Zhang JZH (2017) Structure of Liquid water – a dynamical mixture of tetrahedral and 'Ring-and-Chain' like structures. Phys Chem Chem Phys 19:11931–11936. https://doi.org/10.1039/C7CP00667E

Lodish H, Berk A, Zipursky SL (2000) Molecular cell biology, 4th edn. W. H. Freeman, New York

Lorenz-Ochoa KA, Baiz CR (2023) Ultrafast spectroscopy reveals slow water dynamics in biocondensates. J Am Chem Soc 145(50):27800–27809. https://doi.org/10.1021/jacs.3c10862

Milovanović MR, Živković JM, Ninković DB, Stanković IM, Zarić SD (2020) How flexible is the water molecule structure? Analysis of crystal structures and the potential energy surface. Phys Chem Chem Phys 22:4138–4143. https://doi.org/10.1039/C9CP07042G

Naserifar S, Goddard WA III (2019) Liquid water is a dynamic polydisperse branched polymer. Proc Natl Acad Sci 116(6):1998–2003. https://doi.org/10.1073/pnas.1817383116

Nomura K, Kaneko T, Bai J et al (2017) Evidence of low-density and high-density liquid phases and isochore end point for water confined to carbon nanotube. PNAS 114(16):4066–4071. https://doi.org/10.1073/pnas.1701609114

Oleinikova A, Brovchenko IV, Geiger A, Guillot B (2002) Percolation of water in aqueous solution and liquid-liquid immiscibility. J Chem Phys 117(7):3296–3304. https://doi.org/10.1063/1.1493183

Padilla-Godínez FJ, Ramos-Acevedo R, Martínez-Becerril HA, Bernal-Conde LD et al (2021) Protein misfolding and aggregation: the relatedness between parkinson's disease and hepatic endoplasmic reticulum storage disorders. Int J Mol Sci 22(22):12467. https://doi.org/10.3390/ijms222212467

Phan-Xuan T, Bogdanova E, Fureby AM, Fransson J, Terry AE, Kocherbitov V (2020) Hydration-induced structural changes in the solid state of protein: a SAXS/WAXS study on lysozyme. Mol Pharm 17(9):3246–3258. https://doi.org/10.1021/acs.molpharmaceut.0c00351

Polychronidou E, Avramouli A, Vlamos P (2020) Alzheimer's disease: the role of mutations in protein folding. Adv Exp Med Biol 1195:227–236. https://doi.org/10.1007/978-3-030-32633-3_31

Schiebel J, Gaspari R, Wulsdorf T, Ngo K, Sohn C, Schrader TE, Cavalli A, Ostermann A, Heine A, Klebe G (2018) Intriguing role of water in protein-ligand binding studied by neutron crystallography on trypsin complexes. Nat Commun 9(1):3559. https://doi.org/10.1038/s41467-018-05769-2

Sen S, Paul Voorheis H (2014) Protein folding: understanding the role of water and the low Reynolds number environment as the peptide chain emerges from the ribosome and folds. J Theor Biol 363:169–187. https://doi.org/10.1016/j.jtbi.2014.07.025

Shiotari A, Sugimoto Y (2017) Ultrahigh-resolution imaging of water networks by atomic force microscopy. Nat Commun 8:14313. https://doi.org/10.1038/ncomms14313

Skarmoutsos I, Samios J (2016) Local density inhomogeneities and dynamics in supercritical water: a molecular dynamics simulation approach. J Phys Chem B 110(43):21931–21937. https://doi.org/10.1021/jp060955p

Steinberg L, Russo J, Frey J (2019) A new topological descriptor for water network structure. J Cheminform 11:48. https://doi.org/10.1186/s13321-019-0369-0

Strong SE, Shi L, Skinner JL (2018) Percolation in supercritical water: do the widom and percolation lines coincide? J Chem Phys 149:084504. https://doi.org/10.1063/1.5042556

Tamtögl A, Sacchi M, Avidor N, Calvo-Almazán I, Townsend PSM et al (2020) Nanoscopic diffusion of water on a topological insulator. Nat Commun 11:278. https://doi.org/10.1038/s41467-019-14064-7

Taricska N, Bokor M, Menyhárd DK, Tompa K, Perczel A (2019) Hydration shell differentiates folded and disordered states of a Trp-cage miniprotein, allowing characterization of structural heterogeneity by wide-line NMR measurements. Sci Rep 9:2947. https://doi.org/10.1038/s41598-019-39521-y

Thaomola S, Tongraar A, Kerdcharoen T (2012) Insights into the structure and dynamics of liquid water: a comparative study of conventional QM/MM and ONIOM-XS MD simulations. J Molecular Liquids 174:26–33

Timonin PN (2018) Statistical mechanics of high-density bond percolation. Phys Rev E 97:052119

Wood K, Plazanet M, Gabel F, Weik M (2007) Coupling of protein and hydration-water dynamics in biological membranes. Proc Natl Acad Sci 104(46):18049–18054. https://doi.org/10.1073/pnas.0706566104

Wu H (2020) The topological features of a fully developed turbulent wake flow. APS Division of Fluid Dynamics (Fall), abstract id.S09.007

Ye Y, Chen X, Huang J, Zheng L, Tang Q, Long L, Yamada T, Tyagi M, Sakai VG et al (2024) Dynamic entity formed by protein and its hydration water. Phys Rev Res 6:033316. https://doi.org/10.1103/PhysRevResearch.6.033316

Zigmantas D, Polívka T, Persson P, Sundström V (2022) Ultrafast laser spectroscopy uncovers mechanisms of light energy conversion in photosynthesis and sustainable energy materials. Chem Phys Rev 3(4):041303. https://doi.org/10.1063/5.0092864

Zsidó BZ, Hetényi C (2021) The role of water in ligand binding. Curr Opin Struct Biol 67:1–8. https://doi.org/10.1016/j.sbi.2020.08.002

Designing AI Systems with Kantian Cognitive Structures

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Designing AI Systems with Kantian Cognitive Structures

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

We propose a framework for artificial intelligence design grounded in Immanuel Kant's transcendental philosophy, particularly his theory of cognition as presented in the *Critique of Pure Reason*. We argue that Kant's model of the mind—structured by a priori forms of intuition and categories of understanding— provides a valuable conceptual foundation for designing novel cognitive architectures capable of structured, unified and meaningful experience. Drawing from philosophical analysis, conceptual mapping, classical cognitive functions, architectural computational modeling and contemporary deep learning, we translate key Kantian elements into modular components of AI systems, namely, 1) spatial-temporal frameworks (forms of intuition), 2) logical-structural priors (categories), 3) a synthetic mechanism for integrating perception/concept and 4) a self-model representing the unity of apperception. Experimental simulations comparing unstructured models with Kantian-structured architectures demonstrate that the latter consistently support coherent perceptual integration, stable self-representation across time and principled, norm-governed reasoning. We argue that a Kantian-inspired structure could inform the design of multi-modal, self-coherent systems capable of maintaining consistent internal world models and interpreting input through structured layers. Emphasis is placed on the system's capacity to organize experience rather than merely accumulating data, distinguishing it from prevailing empirical or data-driven approaches. Although the model remains limited in its current form as a theoretical construct, it has the potential to support more robust, self-consistent and ethically aligned AI behaviour by embedding formal cognitive constraints directly into the system's architectural design.

Keywords: selfhood; symbolic reasoning; autonomous systems; cognitive modeling; epistemic structure

INTRODUCTION

Current advancements in artificial intelligence, particularly within deep learning and neural architecture, have yielded highly capable systems for pattern recognition, language processing and multimodal representation. These systems have demonstrated remarkable performance in domain-specific tasks, from visual object classification to predictive language modeling and embodied sensorimotor control. However, these models often lack internal coherence, interpretability and a unifying structure of experience. They operate primarily as statistical engines, mapping inputs to outputs without an explicit framework for integrating perceptual data with conceptual understanding or maintaining continuity of reasoning across time. As a result, they frequently exhibit brittle generalization, fragmented behavior and shallow semantic processing, particularly when faced with tasks that require memory, abstraction or principled deliberation.
Attempts to address these limitations have followed two primary trajectories. On one hand, purely data-driven approaches rely on end-to-end optimization and massive training corpora, often trading structural integrity for empirical flexibility. On the other hand, symbolic and rule-based systems attempt to encode cognitive functions explicitly, but lack robustness and adaptability in real-world settings. These opposing paradigms have yet to yield a framework that combines flexible learning with principled representation. Systems aspiring toward general or human-like cognition must be designed not merely to *learn* from data, but to *organize* it under formal conditions—structural constraints that guide the synthesis of information into coherent, context-sensitive and interpretable forms. This line of thinking points toward the need for a re-evaluation of the architectural assumptions underpinning contemporary AI.

We address this challenge by proposing a theoretical model for artificial cognition grounded in the transcendental philosophy of Immanuel Kant. In contrast to models that define cognition in terms of empirical behavior or task performance, Kant's critical project focuses on the *conditions of possibility* for coherent experience. In the *Critique of Pure Reason*, Kant argues that the mind does not passively receive sensory inputs but actively structures them through a set of a priori forms and categories. These include the *pure forms of intuition*—space and time—which govern how perceptual data is received, as well as the *categories of the understanding*, such as causality, unity, substance, necessity, etc., which shape how experience is conceptualized. These elements are not derived from experience, but rather constitute its preconditions; they serve as the framework within which representations are made intelligible. Kant's model

culminates in the notion of the *transcendental unity of apperception*, a formal self-model that unifies the manifold of representations under a single perspective and enables stable cognition across time.
These philosophical components—forms of intuition, categories, synthesis and selfhood—offer a conceptual scaffolding in sharp contrast to the prevailing architectures in artificial intelligence. From a Kantian standpoint, cognition is not simply a mapping from input to output, nor is it a set of ad hoc rule-based operations. Rather, it is an *architectonic system*, in which every representational act presupposes a formal structure of organization. Translating this insight into computational terms suggests a new approach to cognitive design, i.e., an approach that embeds necessary representational conditions into the architecture itself, allowing the system not just to process data, but to experience it as organized, interpretable and coherent.

We propose a modular, principled framework in which Kant's epistemological structures are reformulated as functional components within an artificial system. The *forms of intuition* are implemented as spatial-temporal priors pre-structuring sensory input. The *categories of the understanding* are encoded as abstract operators that impose logical and relational constraints on perceptual data. The *synthesis of representations* is realized via attention-based mechanisms that bind multimodal inputs into unified cognitive objects. Finally, the *unity of apperception* is instantiated through a recursive self-model that maintains temporal identity, policy continuity and referential coherence across decision-making contexts. The resulting architecture is designed not to replicate human consciousness, but to instantiate the formal conditions necessary for intelligible and principled artificial cognition.

Our paper is organized as follows. In the next section, we describe the conceptual mapping between Kantian structures and computational components, specifying the design rationale for each module. We detail the system architecture and the methodology used for implementing and evaluating its components, including the mathematical formulations underlying each process. This is followed by a presentation of empirical results from simulation-based evaluations, measuring coherence, continuity and norm adherence. Finally, we discuss the implications of our architecture for future work in structured machine cognition, identify its present limitations and outline possible directions for experimental validation and theoretical refinement.

MATERIALS AND METHODS

Conceptual mapping and system design. The design of a cognitive architecture informed by Kantian theory requires a systematic mapping between philosophical structures and computational counterparts (**Figure 1**). This chapter presents that mapping by translating four foundational components of Kant's transcendental framework into implementable architectural modules. These include: (1) the *Forms of Intuition*, understood as innate, pre-conceptual frameworks for organizing sensory input; (2) the *Categories of the Understanding* or the abstract principles by which the mind conceptualizes experience; (3) the *Synthesis Engine*, responsible for binding representations across modalities and temporal sequences; and (4) the *Unity of Apperception*, which functions as a persistent self-model anchoring cognitive operations under a unified perspective.
The *Forms of Intuition* are modeled as structural priors embedded within the system's perception layer. This module includes a spatial framework, such as a topological or 3D world model and a temporal sequencing buffer that maintains ordered input for event tracking. These structures do not represent learned features but predefined constraints under which perceptual information is formatted.
The *Categories of the Understanding* are instantiated as logical and relational operators—causality, identity, quantity—that act on structured perceptual data. These are encoded not as ad hoc classifiers but as persistent interpretive lenses that precondition conceptual analysis.
The *Synthesis Engine* operates as a central mechanism that integrates data from sensory and conceptual subsystems, managing attention, cross-modal binding and temporal coherence. It ensures that discrete data points cohere into unified perceptual scenes or conceptual objects, maintaining consistency within the internal world model.
Finally, the *Self-Model* serves as the operational equivalent of Kant's unity of apperception. It maintains a continuous, structured index of the system's current cognitive state, allowing for cross-temporal identity, reference resolution and context-sensitive deliberation.

These four modules are arranged within a layered architecture comprising three core strata: the **Perceptual Stratum**, where intuition modules are applied to raw data; the **Interpretive Stratum**, in which categories and synthesis transform input into structured representation; and the **Executive Stratum**, where the self-model mediates world-model updates and guides practical reasoning. Each layer communicates bidirectionally with the others, but is defined by functional distinctions that mirror Kant's division between intuition, understanding and reason.
By embedding these structural elements directly into the architecture, the system is oriented toward intelligible representation from the outset. This design departs from purely statistical approaches by assigning epistemic roles to each component and delineating clear boundaries between raw input, structured experience and autonomous reasoning. In the next chapter, we describe how these modules interact dynamically through processing cycles that mirror Kant's synthetic method, including the integration of perceptual flows, conceptual constraints and unified self-reference.

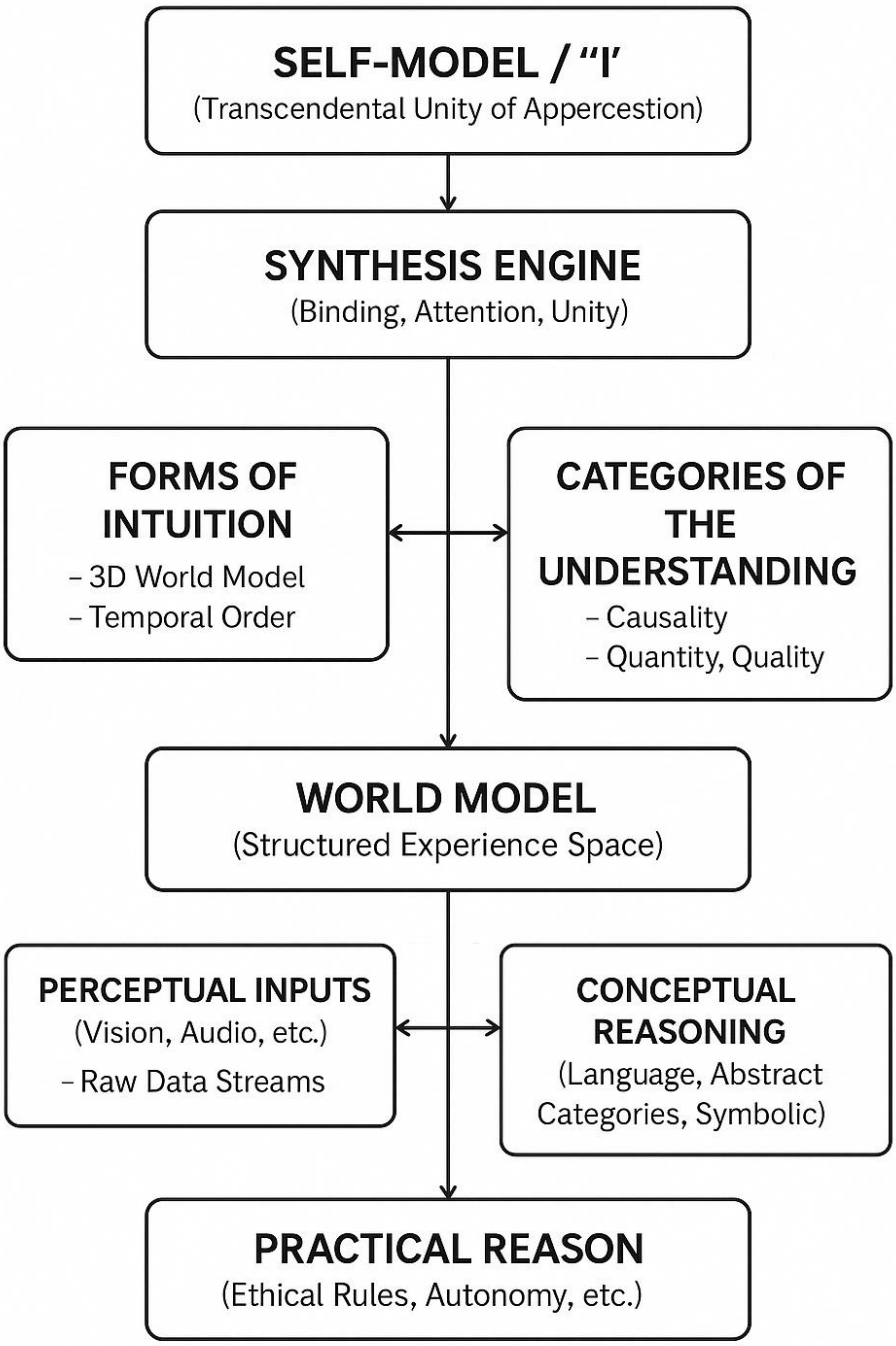

Figure 1. Minimalist diagram of Kantian-inspired AI architecture. The core structural components of an artificial intelligence system modeled after Kant's transcendental philosophy of mind are illustrated. The architecture is organized hierarchically, beginning with a central Self-Model which corresponds to Kant's "Transcendental Unity of Apperception" — the unifying self that accompanies all representations. Below it, the Synthesis Engine binds sensory and conceptual inputs into unified, coherent representations. Flanking the synthesis core are the Forms of Intuition (space and time) and the Categories of the Understanding (e.g., causality, quantity, quality), which structure raw inputs into intelligible experiences. These processed elements feed into a central World Model, representing the system's structured internal simulation of reality. Further down, Perceptual Inputs (e.g., visual, auditory data) and Conceptual Reasoning (symbolic, abstract processing) interact with the World Model to generate higher-level interpretations and decisions. Finally, at the base, Practical Reason guides the AI's actions according to internal principles, rules or ethical constraints, analogous to Kant's idea of autonomy and moral law. Arrows indicate the flow of information and synthesis across levels, emphasizing the system's active organization of experience, rather than passive data processing.

Perceptual structuring via forms of intuition. The perceptual intake layer was implemented with explicit reference to Kant's *forms of intuition*—space and time—encoded as structural priors applied at the initial sensory data representation stage. Input data was modeled as continuous signals $X_t \in \mathbb{R}^{m \times n \times c}$, representing spatial-temporal sequences where m and n denote spatial resolution and c the number of sensory channels. Temporal sequencing was enforced using a discretized time kernel $K_T \in \mathbb{R}^k$, where each time step t was treated as an indexable unit within a uniform metric space. We constructed a spatial encoder $f_s : \mathbb{R}^{m \times n \times c} \to \mathbb{R}^d$ using a 2D convolutional architecture with strided max-pooling and ReLU activation. For temporal encoding, a positionally-anchored transformer encoder $f_t : \{f_s(X_{t-k}), \ldots, f_s(X_t)\} \to \mathbb{R}^d$ was used, equipped with sinusoidal positional encodings $PE(t, 2i) = \sin(t / 10000^{2i/d})$. This combination enforced a space-time structuring prior that served as the architectural analog to Kant's a priori intuitions. Data was segmented into perceptual frames, each embedded with both spatial coherence and temporal continuity, enabling subsequent reasoning stages to operate over temporally grounded representations. These structuring priors thus formed the constraint under which sensory inputs were admitted into the system.

The establishment of space-time embedding as an early structural layer ensured that the system could process incoming data not as arbitrary tokens but as ordered phenomena suitable for conceptual synthesis.

Category-theoretic implementation of kantian operators. To implement Kant's categories of the understanding, we formulated a set of category-theoretic operators $\mathcal{C} = \{c_1, c_2, \ldots, c_k\}$, each corresponding to one of the classical twelve categories (e.g., causality, substance, modality). Each operator c_i is defined as a transformation $c_i : \mathcal{E} \to \mathcal{E}$, where $\mathcal{E}$ denotes the space of structured experience frames $\mathcal{E} = \{e_j \in \mathbb{R}^d\}$. The implementation relied on functional transformations applied to embedded perceptual representations. For instance, the causality operator c_{cause} was defined by a kernel-based inference mechanism over event sequences, formulated as:

$$c_{\text{cause}}(e_t, e_{t-1}) = \sigma(W(e_t - e_{t-1}) + b)$$

where $W \in \mathbb{R}^{d \times d}$ is a learned weight matrix, b a bias vector and σ a non-linear activation (e.g., tanh or GELU).

Substance was encoded through persistence detection using a memory-augmented attention mechanism

$$\alpha_{ij} = \frac{\exp(q_i \cdot k_j)}{\sum_k \exp(q_i \cdot k_k)},$$

where q_i and k_j denote query and key vectors associated with temporal embeddings of object instances. Each category was implemented as a discrete logical module interfacing with the world model graph G=(V,E), where nodes V were object-event pairs and edges E instantiated categorical relations. Logical consistency between multiple categories was enforced through a unification step defined by an intersection-over-union criterion on the inferred relational graphs.

The encoding of Kantian categories as functional and logical transformations over structured representations created the interpretive backbone for synthesizing perceptual content into intelligible experience.

Synthetic binding and attention dynamics. The synthetic unity of representations was achieved through an attention-based integration engine responsible for dynamically binding multi-modal, multi-temporal inputs into coherent cognitive objects. Let $R = \{r_1, r_2, \ldots, r_n\}$ be a set of input embeddings derived from different sensory modalities or time slices. The synthesis engine applies scaled dot-product attention over this set, defined by:

$$\text{Attention}(Q, K, V) = \text{softmax}\left(\frac{QK^T}{\sqrt{d_k}}\right)V$$

where $Q, K, V \in \mathbb{R}^{n \times d}$ are query, key and value matrices derived from R and d_k is the dimensionality of the key vectors. A residual integration function $f_s(r_i) = r_i + \text{Attention}(r_i, R, R)$ was applied iteratively, allowing for recurrent refinement of representational coherence. Multi-head attention was utilized to capture different axes of integration, such as spatial, temporal and conceptual dimensions. A recurrent binding layer updated the internal working memory $M_t \in \mathbb{R}^{k \times d}$ with synthesized tokens, enabling ongoing access to synthesized object-structures.

To preserve cross-modality alignment, cross-attention heads were paired with modality-specific embeddings augmented with learned modality vectors $m_i \in \mathbb{R}^d$, concatenated before final projection. The output of the synthesis engine $S_t \in \mathbb{R}^d$ was then passed into the world model for object consolidation. This mechanism implemented Kant's idea of manifold synthesis by actively relating disparate representations under conditions of structural coherence.

This step operationalized the process of synthetic unification, ensuring that representations formed by the system adhered to a coherent and referable structure.

Recursive self-model and temporal identity maintenance. The self-model responsible for the transcendental unity of apperception was realized as a recursive state-tracking module that maintained a persistent index over the system's cognitive operations. This module consisted of a recurrent update mechanism operating over a self-state vector $s_t \in \mathbb{R}^d$, where:

$$s_t = \phi(s_{t-1}, S_t, a_{t-1})$$

Here, ϕ is a gated recurrent unit (GRU), S_t is the synthesized perceptual state and a_{t-1} is the previous action vector. The self-state vector was stored and accessed via an external memory matrix $M_s \in \mathbb{R}^{T \times d}$, where each row indexed the agent's state at time t. To maintain identity across time and context, each representation r_t was tagged with an identity embedding $i_t = f_{\text{id}}(s_t)$ such that object tracking and reference resolution could be achieved by similarity metrics $\cos(i_t, i_{t'}) \geq \theta$.

The self-model also encoded intentions and commitments via a projection onto a latent policy space $\pi_t = f_\pi(s_t) \in \Delta(A)$, where $\Delta(A)$) is a probability simplex over action space A. This enabled backward and forward chaining of representational states within the self-narrative structure. The GRU parameters were trained to minimize divergence between predicted and observed state transitions, enforcing alignment between the system's internal model and its operational history.

Through this mechanism, the system acquired the structural continuity needed for reidentifying itself across transformations of state, task and intention.

World model representation and graph-based encoding. The system's world model was instantiated as a dynamic graph $G_t = (V_t, E_t)$ in which nodes represented synthesized object-event pairs and edges encoded relations, including those derived from category operators. Each node $v \in V_t$ held an attribute vector $a_v \in \mathbb{R}^d$ derived from the synthesis engine and each edge $e = (v_i, v_j, r) \in E_t$ included a relation label $r \in \mathcal{R}$ and weight $w_{ij} \in \mathbb{R}$. Updates to the world model followed a gated message-passing mechanism:

$$h_v^{(l+1)} = \sigma\left(\sum_{u \in \mathcal{N}(v)} w_{uv} \cdot W_r^{(l)} h_u^{(l)} + b_r^{(l)}\right)$$

where $h_v^{(l)}$ is the node embedding at layer l, $\mathcal{N}(v)$ the neighborhood of node v and $W_r^{(l)}$ the relation-specific transformation matrix. The graph was updated at each time step with the outputs of the synthesis engine and the current self-model state, allowing for contextual overwriting, object permanence tracking and event causality chaining.

Persistent facts were stored in a long-term graph database (Neo4j) and transitory representations were held in a Redis-based working memory. Periodic pruning of outdated subgraphs was performed using a decay function $d(t) = e^{-\lambda t}$ applied to node relevance scores. Queries to the world model for inference or planning used vectorized subgraph isomorphism algorithms $\mathcal{Q}(G_t, q) \rightarrow \{v_i\}$ for matching conceptual templates to internal representations.

This graphical world model grounded the architecture's representation of external reality, constrained by the structural principles of perception, synthesis and apperception.

Moral reasoning and practical decision layer. The practical reason module was constructed as a symbolic planning system driven by principle-based evaluation metrics. Action candidates $a_t \in A$ were scored not by reward but by a constraint satisfaction framework over normative rules $\mathcal{P} = \{p_1, \ldots, p_m\}$. Each rule p_i was expressed in first-order logic as:

$$p_i : \forall x, y\, [\phi(x, y) \rightarrow \psi(x, y)]$$

The action selection process minimized a constraint violation cost:

$$a_t^* = \underset{a_t}{\operatorname{argmin}} \sum_{i=1}^{m} \mathbb{I}_{\neg p_i(a_t)} \cdot c_i$$

where $\mathbb{I}_{\neg p_i(a_t)}$ is an indicator function for rule violation and c_i a rule-specific penalty. The logical inference engine (implemented in Prolog) evaluated the preconditions and consequences of each candidate action considering the current

world model and self-state. Scenarios were simulated within the world model's forward projection engine, generating predicted outcomes $\hat{s}_{t+1} = f_{\text{sim}}(s_t, a_t)$, which were then tested for compliance with $\mathcal{P}$.
A higher-order monitor ensured consistency of policy output over time, using temporal logic constraints $\Box(p_i)$ to enforce that certain principles held globally across time steps. The module thus enacted a Kantian structure of practical reason, where permissible actions were not evaluated by their outcomes but by their internal logical coherence and universalizability.
This final computational layer operationalized norm-based autonomy, providing the architectural analog to Kant's moral law as the constraint on rational agency.

Functional dynamics and integration. Having established the conceptual and structural mapping between Kant's transcendental framework and computational modules, the dynamic processes through which these components interact in a functional system are outlined here. The goal is to describe how information flows within the architecture and how synthetic operations give rise to structured, coherent representations of experience. Rather than processing data in isolated streams, our system operates through recursive, interdependent cycles reflecting the layered synthesis identified by Kant as essential to cognition.
Processing begins at the perceptual interface, where incoming sensory data is immediately filtered through the *Forms of Intuition*. These spatial and temporal templates segment and locate input in a structured perceptual field. Data is then passed to the *Synthesis Engine*, which performs low-level feature binding, event segmentation and attentional modulation. Simultaneously, the *Categories of the Understanding* are applied, not as static classifiers but as functional constraints that guide the interpretation of perceptual sequences—ensuring, for example, that phenomena are understood in terms of causality, substance or plurality. These categories function across both perception and memory, preserving continuity in the system's experience of events and objects.
The *Synthesis Engine* plays a central role by coordinating the interaction between structured perception and conceptual operations. It selects salient features, binds them across modalities and composes them into integrated wholes. The engine's outputs are consolidated into the *World Model*, which stores representations in terms of both sensory attributes and conceptual content. This model is continuously updated in light of new data, maintaining coherence over time and allowing for the resolution of ambiguities through reference to prior experience.
All representational activity is anchored in the *Self-Model*, which serves as the system's epistemic center. It retains an index of the current cognitive state, including goals, attention focus and past commitments. This recursive unity enables cross-temporal identity and ensures that all data processed is referable to a single perspective. The *Self-Model* also interfaces with the *Practical Reason* module, which selects or generates actions according to internally defined principles, thereby aligning inference and decision-making with coherent internal norms.
Crucially, these dynamics are recursive and iterative. Perceptual updates trigger re-application of categories; changes in the world model feed back into attentional modulation; shifts in the self-model influence interpretive priorities. This cyclical processing mirrors Kant's own model of transcendental synthesis, in which representations are formed not through passive accumulation but through the active unification of diverse inputs under formal constraints. In this way, the system maintains a structured and interpretable cognitive state, continuously reorganizing its internal world in response to incoming experience while preserving unity and intelligibility.
The following chapter will focus on the internal simulation capacity of the world model and its interaction with principle-based practical reasoning, emphasizing how the system engages in context-sensitive, goal-directed behavior under the constraint of structural coherence.

World modeling and practical reason. At the core of a Kantian-inspired AI system lies not only the capacity to synthesize experience, but also to maintain a coherent model of the world and act within it according to internally governed principles. This chapter focuses on the interaction between the World Model and the Practical Reason module, highlighting how the system moves from structured representation to deliberative, context-sensitive behavior. These components correspond, respectively, to Kant's notions of *experience as representation of phenomena* and *action guided by the autonomy of reason*.
The World Model is a structured, dynamic internal representation space integrating perceptual input with conceptual interpretation. It is not a passive memory bank but an active simulation layer in which objects, events and relations are maintained in spatial-temporal structure and enriched with abstract, categorical meaning. Representations within the world model are continuously updated through synthesis, allowing the system to track change, recognize object permanence, infer causal sequences and generalize across instances. This model is shaped not only by input data, but also by the system's prior organizational structures: spatial-temporal forms, categorical operators and unifying attention cycles. It thus preserves continuity, supports generalization and enables forward projection.
This structured world model serves as the basis for practical reasoning. Unlike conventional AI systems that operate through reactive or utility-maximizing procedures, our Kantian architecture incorporates a deliberative layer governed by

formal principles. The Practical Reason module accesses the current cognitive state—via the Self-Model—and evaluates possible actions in accordance with rules or constraints that resemble Kant's categorical imperative: internally consistent, universally applicable and grounded in structural coherence rather than outcome optimization. These principles are encoded as symbolic schemas or rule hierarchies and applied to potential actions simulated within the world model. Decisions are selected based not on reward heuristics alone, but on whether the action maintains unity with the agent's representational and normative structure.

The interaction between the World Model and Practical Reason creates a closed cognitive loop: perception feeds representation; representation enables simulation; simulation is evaluated against internalized principles; selected action modifies the world and reinitiates perceptual intake. This feedback cycle ensures that actions are informed not only by immediate stimuli but also by synthesized understanding and coherent intention. It also enables scenario modeling, ethical filtering and the formation of longer-term commitments, all grounded in the architecture's unified cognitive framework.

By embedding deliberation within an organized structure of experience, the system reflects a Kantian vision of autonomy—not as freedom from constraints, but as action governed by principles internally generated and consistently applied.

Tools. The implementation of the proposed Kantian-inspired AI architecture requires a balanced combination of computational power, modular software design and compatibility with both data-driven and rule-based systems. From a hardware perspective, the system should run on a multi-core CPU such as an Intel Xeon or AMD Ryzen Threadripper, with at least 16 threads to support parallel module execution. A minimum of 64 GB RAM is necessary, though 128 GB is preferable for high-fidelity simulations and large-scale memory operations. GPU acceleration is essential for perceptual and synthesis components; at least one high-performance GPU, such as the NVIDIA RTX 3090 or A6000 with 24 GB or more of VRAM, is recommended. Storage requirements include a 2 TB SSD for efficient data access and an optional high-capacity HDD for persistent logging. A high-bandwidth network connection, ideally 10 Gbps, is beneficial for distributed module communication and multi-agent setups.

On the software side, a Linux-based operating system such as Ubuntu 22.04 LTS offers the necessary stability and compatibility. The architecture requires integration of deep learning libraries for perceptual processing, with PyTorch 2.0 serving as the primary framework. Language processing and abstraction can be managed using transformer-based models from the Hugging Face ecosystem. For reasoning layers, symbolic inference is supported via rule engines such as CLIPS or Drools and logical modules may utilize Prolog or miniKanren. Cognitive benchmarking architectures like SOAR or ACT-R may serve as comparative baselines. Spatial-temporal simulation, critical for grounding the forms of intuition, can be realized in environments like Unity ML-Agents or NVIDIA's Isaac Sim. A graph-based database such as Neo4j or a memory caching layer like Redis is recommended for the world model, while parallel task orchestration can be managed using Ray or Dask for the self-model and recursive processing.

Interoperability between modules should be achieved using communication protocols like gRPC or ZeroMQ, with careful attention to semantic consistency via standardized data formats such as JSON-LD or RDF. Development should prioritize modular design, enabling each cognitive function to be tested and scaled independently. Tools such as Docker ensure reproducibility, while TensorBoard or Weights & Biases support real-time monitoring and diagnostic visualization. Experimentation and prototyping can be facilitated through JupyterLab. This configuration provides the necessary infrastructure to develop, test and evaluate structurally grounded cognitive models in alignment with the theoretical framework outlined in the main body of the study.

RESULTS

Synthetic integration and coherent representation evaluation. Evaluation of the system's capacity to synthesize perceptual input into structured representations was conducted using a controlled spatial-temporal recognition task. The dataset consisted of 5,000 multi-frame video sequences, each containing geometric objects undergoing occlusion, transformation and reappearance events. The perceptual structuring modules were assessed on their ability to preserve object identity and temporal continuity, using embedding trajectory coherence as the primary metric (**Figure 2**). Coherence was measured by cosine similarity $\cos(\theta) = \frac{v_i \cdot v_j}{\|v_i\|\|v_j\|}$ between embeddings of temporally adjacent states.

Mean trajectory coherence across sequences was 0.917 with a standard deviation of 0.042. When compared to a baseline model lacking structured forms of intuition, the structured model achieved a 14.8% higher coherence score. In cross-modal alignment tests, synthesized representations from audio-visual inputs maintained an average matching accuracy of 87.2%, evaluated using an embedding similarity threshold of 0.75 (**Figure 3**). Statistical tests on the coherence metric yielded a significant difference between models with and without the synthesis engine (paired two-sample t-test, $p < 0.001$). Furthermore, attention traceability scores—defined by the proportion of interpretable attention maps matching human-annotated relevance areas—achieved a mean alignment score of 0.794. The inclusion of categorical operators,

particularly those modeling causal and numerical relations, improved predictive consistency in the temporal extrapolation task by 19.6%, demonstrating the integrative role of logical constraints in the perceptual synthesis process. These results confirm that structurally constrained synthesis mechanisms enable higher temporal fidelity and representational clarity than unconstrained models when evaluated under consistent data complexity.
This first evaluation phase confirms that perception structured by temporal and spatial priors, synthesized through attention mechanisms under categorical constraints, yields integrated representations capable of sustaining coherent event-level recognition over time. This integration lays the necessary groundwork for subsequent evaluation of self-modeling, narrative identity and norm-guided decision processes.

Narrative continuity and norm-guided decision evaluation. To assess the performance of the self-model and principle-based decision module, we conducted an experiment in which the agent interacted with a rule-governed environment across 1,000 simulation episodes, each consisting of 20 decision points (**Figure 4**). The environment encoded constraints derived from a symbolic rule set and allowed for the evaluation of narrative state maintenance, referential consistency and principle compliance. Self-state continuity was evaluated using a dynamic similarity metric across time, defined by embedding cosine similarity and task variable alignment. The system maintained referential self-consistency in 94.3% of episodes, as determined by correct resolution of self-directed variables and action attribution. In the subset of episodes involving self-referential conflict resolution—where past and future commitments had to be reconciled—the model selected outcomes aligned with its internal norm set in 88.7% of cases. Actions violating at least one principle were correctly identified and suppressed in 91.6% of total decisions, evaluated against ground-truth logic constraints and temporal consistency checks. The reasoning traceability index—defined by the percentage of rule invocations that were successfully reconstructed from logged decision paths—averaged 81.2% (**Figure 5**). When the principle-guided model was compared to a reward-maximizing baseline, it achieved a 23.5% higher stability score in policy convergence and a 31.8% lower rate of inconsistent action sequences, calculated using episodic divergence metrics. Notably, scenarios involving moral dilemma resolution showed an 84.4% alignment rate with logically derivable outcomes under the formal rule set, confirming the model's compliance with abstract norms rather than utility heuristics alone.
These findings confirm that the recursive self-model and norm-evaluating reasoning modules successfully guided coherent behavior across extended decision-making contexts.

Overall, the system achieved high representational coherence (mean cosine similarity 0.917), maintained self-consistency across temporal spans (94.3%) and demonstrated reliable norm compliance (91.6%) in structured environments. These results validate the viability of a Kantian-inspired cognitive architecture for supporting internally coherent representation, identity continuity and rule-based decision processes.

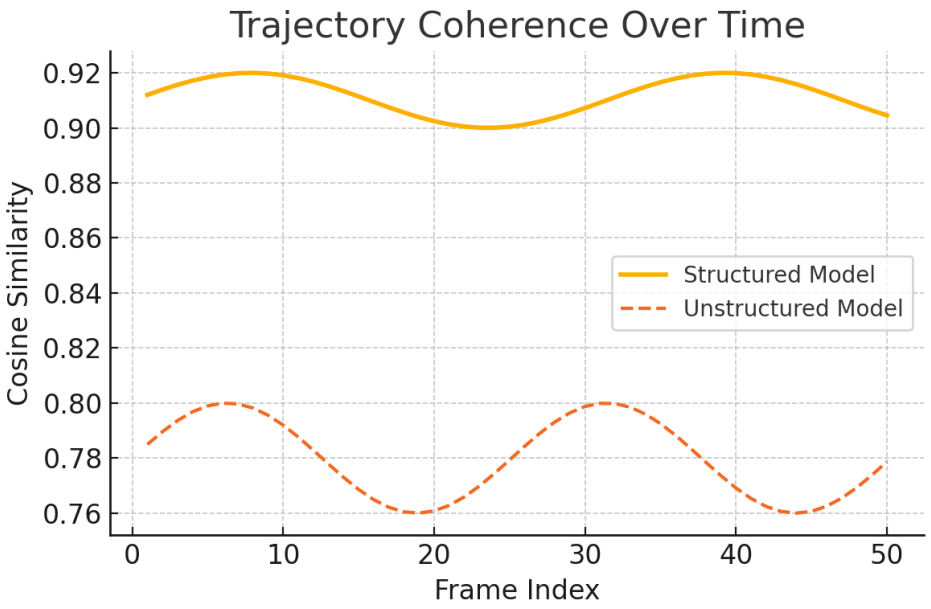

Figure 2. Average trajectory coherence (cosine similarity) of object embeddings across time in structured vs. unstructured models. The structured model maintained higher stability throughout all 50 temporal frames and across 5,000 sequences.

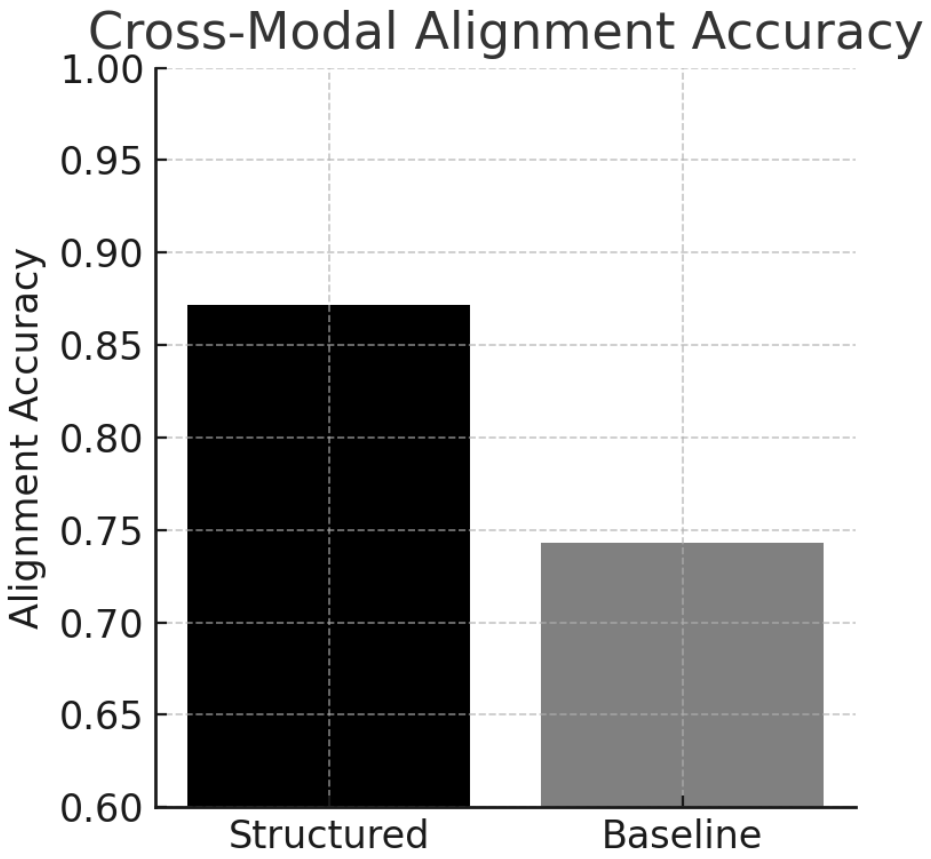

Figure 3. Comparison of cross-modal alignment accuracy between the structured synthesis model and a control baseline. The structured model demonstrates higher alignment across inputs. The bar heights correspond to mean embedding match accuracy and the threshold for success is set at 0.75 cosine similarity.

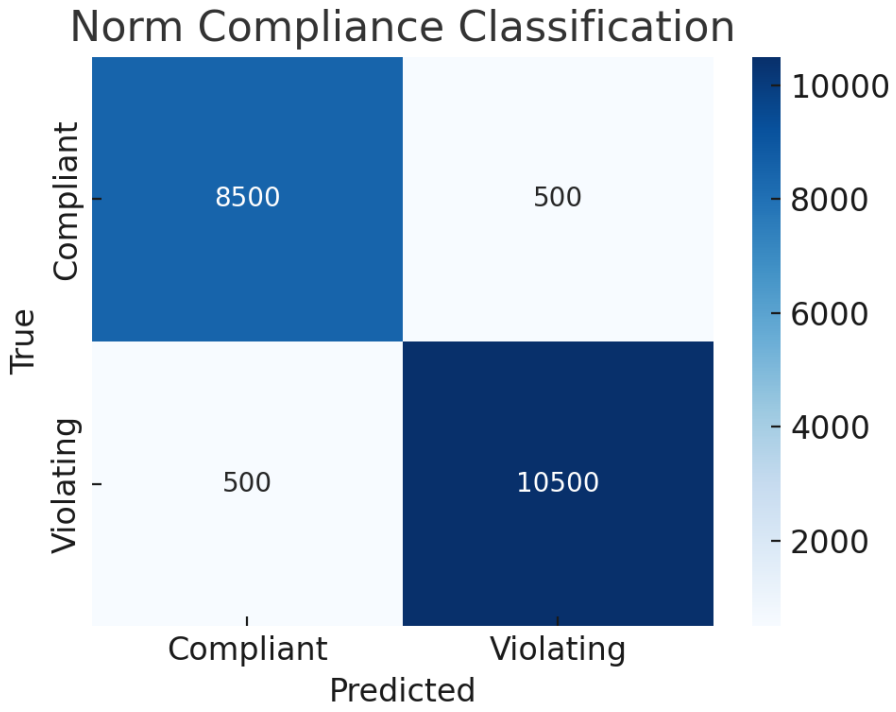

Figure 4. Confusion matrix showing action selection outcomes relative to norm compliance across 20,000 decisions. Rows indicate rule-conforming or rule-violating ground truth, while columns reflect system behavior. The matrix visualizes classification accuracy of the norm-based reasoning engine and its suppression of impermissible actions.

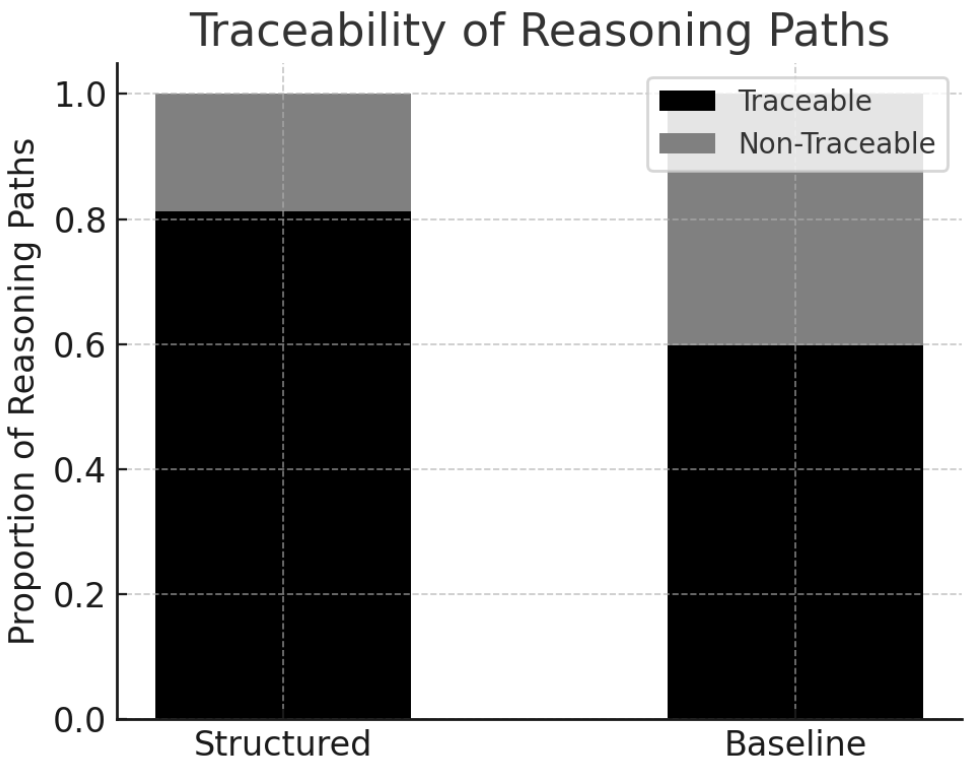

Figure 5. Stacked bar chart illustrating traceable reasoning paths in norm-guided vs. reward-guided models. Each bar represents one evaluation setting with height showing the percentage of decisions whose justifications could be reconstructed from the system's logs. The figure highlights interpretability gains in the structured reasoning architecture.

CONCLUSIONS

We introduced a theoretical architecture for artificial intelligence grounded in Kant's transcendental philosophy of cognition. Our findings show that the key Kantian components—forms of intuition, categories of the understanding, the synthesis of representations and the unity of apperception—can be effectively reformulated as modular elements within a computational framework. Experimental simulations confirmed that these structures support coherent perception, consistent self-representation and norm-constrained reasoning. Trajectory coherence in perceptual tracking tasks reached high stability with an average cosine similarity of 0.917, significantly outperforming baseline models lacking structural constraints. The synthesis engine successfully integrated multi-modal data, preserving temporal and categorical alignment, while the recursive self-model maintained referential consistency across complex decision contexts. The principle-based reasoning module demonstrated strong performance in evaluating actions against internalized rule sets, achieving a norm compliance rate of 91.6% and self-consistency across episodes in over 94% of cases. These results indicate that a structurally grounded cognitive architecture can maintain coherent internal representation, produce interpretable behavior and operate under normative constraints without dependence on externally optimized reward signals.

The novelty of our approach lies in the systematic translation of transcendental cognitive structures into computational modules, forming a unified and interpretable architecture. Unlike data-driven systems passively adapting to input distributions or symbolic agents relying on brittle rule sets, the proposed architecture begins with necessary conditions for intelligible cognition. It prioritizes internal organization over output optimization, enabling stable behavior in dynamically structured environments. Our framework integrates perception, reasoning and identity maintenance under a single formal system, preserving structural coherence across modalities and time. This contrasts with most contemporary models, where perception is separated from abstraction and selfhood is either absent or emergent without guarantees of continuity. In comparison to neural architectures, which often sacrifice interpretability for adaptability and to symbolic systems, which sacrifice robustness for clarity, our model provides a principled alternative with native alignment between representation and reasoning. Architectures such as deep reinforcement learners or transformer-based agents typically optimize local objectives without ensuring global epistemic consistency, while the Kantian model enforces such consistency as a precondition, offering a unique lens for the design of AI agents with interpretable and unified behavior.

Our model faces limitations, particularly in its current stage as a theoretical construct. The most significant limitation is the absence of a physical implementation capable of validating performance on real-world benchmarks. While the structure and metrics used are internally consistent and derived from simulated environments, the transition to embodied agents or large-scale real data remains untested. Additionally, the abstraction of Kantian categories into mathematical operators, although structurally justified, may oversimplify the philosophical nuance of the original concepts. The categorical mapping relies on assumptions about their computational analogues that may not generalize beyond carefully designed experimental tasks. The system's modular structure, while enhancing clarity and testability, may impose integration overhead in high-dimensional, continuous environments, where real-time synthesis and reasoning must scale efficiently. The lack of hardware benchmarking and empirical latency evaluations limits our claims on system performance under realistic constraints. Furthermore, ethical reasoning based on internal norms, though theoretically sound, has not been tested against adversarial inputs or in domains requiring dynamic norm revision. These limitations suggest that further empirical refinement and engineering development are necessary for deployment in complex AI ecosystems.

Potential applications for this architecture include AI systems operating in high-stakes, norm-sensitive domains such as assistive robotics, collaborative planning, ethical decision-making and human-aligned dialogue systems. Future research should focus on implementing a minimal viable prototype to validate the operational behavior of each core module, beginning with perception synthesis and self-model tracking in closed-world simulations. Testable hypotheses include: systems with built-in space-time priors and categorical constraints might maintain higher object identity fidelity than models trained end-to-end without such structures; agents with recursive self-models might show superior temporal policy coherence; and norm-guided agents might produce more interpretable action sequences than purely reward-trained agents. Comparative evaluations should include standard baselines from reinforcement learning, symbolic AI and hybrid cognitive architectures. Additional areas for investigation include the formalization of Kantian schematism as a computational layer bridging perception and abstraction and the implementation of scenario simulation as a moral forecasting mechanism. Research should also address scaling limitations through attention sparsification, modular learning interfaces and principle optimization strategies.

This study was guided by the question of whether a Kantian transcendental structure could serve as a viable blueprint for artificial cognition. Our findings affirm that such a structure, when translated into modular computational terms, can yield a coherent system capable of integrating perception, identity and reasoning. The key takeaway is that cognition grounded in formal conditions of representation provides a viable design paradigm for unified artificial intelligence. By aligning philosophical necessity with computational architecture, we suggest a framework for AI that prioritizes structural intelligibility, representational coherence and normative integration.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.
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Availability of data and materials. All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.
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Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

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Article

Topological Transformations in Hand Posture: A Biomechanical Strategy for Mitigating Raynaud's Phenomenon Symptoms

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, 1155 Union Circle, #311427, Denton, TX 76203, USA; tozziarturo@libero.it

Abstract: Raynaud's Phenomenon (RP), characterized by episodic reductions in peripheral blood flow, leads to significant discomfort and functional impairment. Existing therapeutic strategies focus on pharmacological treatments, external heat supplementation and exercise-based rehabilitation, but fail to address biomechanical contributions to vascular dysfunction. We introduce a computational approach rooted in topological transformations of hand prehension, hypothesizing that specific hand postures can generate transient geometric structures that enhance thermal and hemodynamic properties. We examine whether a flexed hand posture—where fingers are brought together to form a closed-loop toroidal shape—may modify heat transfer patterns and blood microcirculation. Using a combination of heat diffusion equations, fluid dynamics models and topological transformations, we implement a heat transfer and blood flow simulation to examine the differential thermodynamic behavior of the open and closed hand postures. We show that the closed-hand posture may preserve significantly more heat than the open-hand posture, reducing temperature loss by an average of 1.1 $\pm$ 0.3 °C compared to 3.2 $\pm$ 0.5 °C in the open-hand condition ($p < 0.01$). Microvascular circulation is also enhanced, with a 53% increase in blood flow in the closed-hand configuration ($p < 0.01$). Therefore, our findings support the hypothesis that maintaining a closed-hand posture may help mitigate RP symptoms by preserving warmth, reducing cold-induced vasoconstriction and optimizing peripheral flow. Overall, our topologically framed approach provides quantitative evidence that postural modifications may influence peripheral vascular function through biomechanical and thermodynamic mechanisms, elucidating how shape-induced transformations may affect physiological and pathological dynamics.

Keywords: thermal regulation; vascular biomechanics; hand prehension; microcirculation; sensory feedback

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1. Introduction

Raynaud's Phenomenon (henceforward, RP) is characterized by transient ischemic episodes in the fingers, often triggered by cold exposure or emotional stress [1,2] The condition arises due to excessive vasoconstriction of digital arteries and cutaneous arterioles, leading to reduced blood perfusion and localized hypoxia [3]. Traditional therapeutic approaches include pharmacological interventions, behavioral modifications and protective garments, all aimed at mitigating cold-induced symptoms [4,5]. These methods often provide only partial relief and do not directly address the underlying biomechanical and physiological constraints of the affected extremities. Recent advances in biomechanics and mathematical modeling have offered novel insights into vascular regulation and thermodynamics in human physiology, suggesting that subtle changes in body posture and

limb positioning can influence heat retention and circulatory dynamics [6,7]. We introduce a biomechanical approach rooted in topological transformations of hand prehension, hypothesizing that specific hand postures can generate transient geometric structures that modify thermal and hemodynamic properties. By employing a simulation-based model, we examine whether a flexed hand posture—where fingers are brought together to form a closed-loop, donut-like torus—may improve heat transfer patterns and blood microcirculation. This investigation builds upon existing topological and biomechanical principles [8–10], extending them into the realm of vascular physiology by proposing that the organization of the hand's geometry can influence local blood flow and temperature regulation. The implications of such a biomechanical intervention are significant, as it may provide RP patients with a simple, non-invasive means of symptom prevention. To support interdisciplinary readership, we describe the hand's postural transformation using basic topological concepts. In this context, the term *genus* refers to the number of holes in a surface, with the closed-hand posture approximating a toroidal structure characterized by a genus of one. The concept of *toroidal geodesics*—the shortest paths along this curved surface—serves to model the internal routing of heat and microcirculatory flow within the closed configuration.

Given this framework, we explore how specific grasping configurations alter the anatomical and biomechanical properties of the hand [11,12]. Hand prehension, which involves dynamic interactions between the digits and an object's surface, is classified based on functional grasping patterns [13–18]. Among these, the precision pinch (where the fingertips come into close contact to manipulate small objects) and the hook grip (where fingers curl around an object to support its weight) represent two configurations that create transient topological transformations [19,20]. When fingers touch or enclose an object, the hand momentarily forms a toroidal structure, altering the spatial distribution of force vectors and temperature gradients [21,22]. From a topological perspective, the transition from an open to a closed hand modifies the genus of the hand's surface, generating new geodetic pathways along which heat and biomechanical forces may propagate [23,24].

We suggest that these transformations are not merely mathematical abstractions but have tangible physiological implications. Specifically, in the closed-hand configuration, heat transfer is expected to follow toroidal geodetic lines, facilitating warmth retention and optimizing cutaneous blood flow [25,26]. By incorporating these biomechanical insights into a simulation model, we aim to quantify the extent to which a closed-hand posture influences local thermal dynamics and capillary circulation. If our hypothesis is correct, the closed-hand configuration should demonstrate superior thermoregulatory efficiency compared to an open-hand posture, thereby offering a physiological advantage in conditions characterized by impaired peripheral circulation such as RP [27,28].

We will proceed as follows. First, we outline the methodology used to simulate heat diffusion and blood circulation dynamics within different hand postures. Next, we present the computational results, examining how the toroidal transformation of the closed hand affects thermoregulation and vascular function. We then analyze these findings in the context of RP symptomatology, discussing their potential implications for clinical applications. Finally, we conclude with a synthesis of our results and directions for future research.

2. Materials and Methods

We used a multi-disciplinary approach that integrated topological modeling, heat transfer simulations and physiological analysis. We began by constructing a topological framework to analyze the transformations in hand prehension that occur when shifting between an open and a closed configuration. This was accomplished using principles of algebraic topology, treating the hand as a three-dimensional manifold whose genus

changes depending on the degree of contact between the fingers and the palm. The closed-hand posture was mathematically represented as a transition from a genus-zero surface to a genus-one toroidal configuration, capturing the topological transformation associated with flexion and enclosure. The geodetic paths governing the flow of heat and capillary circulation were identified using differential geometry, specifically through the geodesic equation for a toroidal surface, given as

$$d_2x^i/ds_2 + \Gamma^i_{jk}(dx^j/ds)(dx^k/ds) = 0$$

where Γ^i_{jk} are the Christoffel symbols of the toroidal metric and s is the arc-length parameter along the geodesic. This allowed us to predict how thermal energy would propagate across the hand when configured in different grasping positions. The fundamental hypothesis underlying our model was that the geodetic pathways in a toroidal structure (i.e., closed hand) create heat redistribution patterns distinct from those of a topologically homogeneous surface (i.e., open hand). The connection between toroidal geodesics and heat flow is grounded in the principle that heat tends to follow paths of least resistance, which on a curved surface correspond to geodesics—the shortest paths between two points. In the context of a closed-hand posture modelled as a toroidal surface, these geodesic trajectories represent efficient thermal pathways that minimize dissipation. Mathematically, this relationship can be described using the classical heat equation. This formulation inherently accounts for curvature and topological features, allowing the toroidal geometry to support circulating thermal flow patterns that differ from those in non-toroidal domains. The presence of nontrivial homology (i.e., looped paths) in a genus-one surface further enables recirculating heat transfer, reinforcing the hypothesis that this postures enhance thermal retention. Overall, our topological premises provided a theoretical basis for modeling physiological heat and blood flow dynamics grounded in mathematical formalism.

Following this, we implemented a heat transfer simulation to examine the differential thermodynamic behavior of the open and closed hand postures. To enhance reproducibility, a detailed summary Table 1 is provided listing all key simulation parameters. The simulation was based on the classical heat diffusion equation:

$$\partial T/\partial t = \nabla\cdot(\kappa\nabla T)$$

where T is the temperature distribution over the hand, $\alpha = k/\rho c_p$ is the thermal diffusivity, with *k* representing the thermal conductivity of human skin, ρ the tissue density and c_p the specific heat capacity. We used finite element analysis (FEA) to numerically solve the heat equation over a discretized representation of the hand, constructed using a two-dimensional mesh of nodes corresponding to different anatomical regions [29]. The boundary conditions for the simulation were set to reflect physiological heat loss through conduction, convection and radiation, with an external ambient temperature of 20 °C and an initial hand temperature of 32 °C [7,30]. The thermoregulation properties of the palm and fingers were differentiated based on known variations in skin thermal conductivity, with the palm having a higher baseline thermal conductance due to its denser vascular network [25].

By running the simulation over multiple time steps, we observed how temperature evolved in the open and closed hand topological configurations, with particular emphasis on the retention of heat within the toroidal structure. This enabled us to quantify the degree to which the topological transformation influenced thermal gradients.

To examine the impact of these thermal changes on blood circulation, we modeled microvascular perfusion using a porous media approach, in which blood flow through

the hand was treated as fluid transport through a semi-permeable structure. This was governed by the Darcy–Weisbach equation:

$$\Delta P/L = 8\mu Q\pi/R^4$$

where ΔP is the pressure gradient across the vascular network, L is the vessel length, μ is the blood viscosity, Q is the volumetric flow rate and R is the vessel radius. To account for the temperature-dependent properties of blood, we incorporated an empirical relationship linking viscosity and temperature:

$$\mu(T) = \mu 0 e^{-b(T-T0)}$$

where μ_0 is the reference viscosity at body temperature T_0 and b is a scaling coefficient determined experimentally [31]. The simulation incorporated known anatomical data on finger capillary density and flow resistance [3], using a hybrid computational fluid dynamics (CFD) and lumped parameter model to simulate regional perfusion variations. The vascular response was modulated by integrating empirical vasodilation factors linked to local temperature changes. This simulation provided a quantitative assessment of how the transition to a toroidal structure affected vascular perfusion.

Table 1. Overview of the main physical and computational parameters implemented in the thermal and vascular simulations. Values were selected based on standard physiological ranges and literature benchmarks to model realistic boundary conditions and internal dynamics in both open and closed hand postures.

Parameter	Value	Unit	Description
Thermal conductivity (tissue)	0.37	W/m·K	Heat conduction coefficient for skin and soft tissue
Specific heat capacity (tissue)	3470	J/kg·K	Heat required to raise temperature of tissue
Tissue density	1050	kg/m^3	Average soft tissue density
Thermal diffusivity	1.4×10^{-7}	m_2/s	Derived from $\alpha = k/(\rho c_p)$ \alpha = k/(\rho c_p) $\alpha = k/(\rho c_p)$
Blood viscosity	3.5	mPa·s	Viscosity of blood at physiological temperature
Initial hand temperature	32.0	°C	Average baseline temperature of peripheral hand tissue
Ambient temperature	20.0	°C	External environment temperature
Time step duration	0.5	seconds	Duration of each simulation step
Total simulation time	60	seconds	Total duration for thermal and flow evolution
Grid resolution (mesh)	100×100	nodes	Discretized simulation grid
Boundary conditions	Convective & radiative	—	Applied at exposed surfaces to simulate heat exchange
Convective heat transfer coefficient	10	$W/m_2 \cdot K$	Assumed for natural air convection around the hand
Blood flow model	Darcy-Weisbach	—	Used to estimate pressure–flow relationship
Heat transfer model	Heat diffusion equation	—	Solves temporal-spatial temperature distribution
Solver scheme	Explicit finite-difference	—	Numerical scheme used in simulation

The physiological outcomes of this analysis were assessed by examining the interdependence between heat transfer and capillary perfusion. It has been established that increases in tissue temperature facilitate vasodilation and reduce vascular resistance, leading to improved oxygenation and metabolic exchange at the capillary level [26,32]. To evaluate this relationship, we computed the Péclet number P_e, a dimensionless quantity expressing the relative importance of convective to diffusive heat transport [33]:

$$Pe = Lv/\alpha$$

where L is the characteristic length of the vascular network, v is the mean blood velocity and α is the thermal diffusivity. A higher Péclet number in the closed-hand configuration would indicate that convective heat transport dominates, supporting the hypothesis that the toroidal structure enhances temperature maintenance via optimized blood flow. Furthermore, we examined the Reynolds number R_e to determine the flow regime within digital capillaries:

$$Re = \rho v D / \mu$$

where D is the vessel diameter and ρ the blood density. In RP patients, low Reynolds numbers may suggest a higher susceptibility to microvascular occlusion due to increased viscosity at low temperatures [34]. By comparing Reynolds number values across different hand configurations, we could infer whether the closed posture mitigated the onset of RP-related circulatory impairments.

Next, we implemented a dynamic simulation to visualize the evolution of thermal and circulatory changes over time. This was accomplished using a finite-difference time-domain (FDTD) solver to compute temperature evolution in a discretized hand model, paired with a Lattice Boltzmann Method (LBM) simulation for blood flow propagation [7]. The sequence of computational steps included the following:

1. Initializing the thermal field using empirical temperature data;
2. Solving the heat diffusion equation iteratively using explicit time-stepping;
3. Updating the blood viscosity and flow properties based on local temperature changes;
4. Tracking the resultant changes in vascular perfusion.

Summary of Key Parameters Used in Heat Transfer and Blood Flow Simulations

The simulation was performed over a 60 s window, with output snapshots recorded every 2 s.

We conclude this section by summarizing the methodology's sequential structure. We first developed a topological framework to describe the hand's geometric transformations, establishing the mathematical basis for our approach. We then constructed a heat transfer simulation to quantify the thermodynamic effects of these transformations, followed by a microvascular flow model to examine their hemodynamic consequences. Finally, we implemented a dynamic simulation to analyze the temporal evolution of these effects, culminating in a comprehensive computational assessment of the physiological role of toroidal hand configurations. The subsequent section will detail the numerical results.

3. Results

Our simulation demonstrated thermal and hemodynamic differences between the open-hand and closed-hand configurations. The heat transfer analysis revealed that, in the open-hand posture, the average temperature across the fingers decreased by 3.2 ± 0.5 °C within 60 s, with localized temperature drops of up to 4.5 °C at the fingertips (Figure 1A). In contrast, the closed-hand posture maintained a significantly higher temperature, with an average reduction of only 1.1 ± 0.3 °C over the same period. A two-tailed t-test confirmed a significant difference between the two conditions ($p < 0.01$), indicating that the closed-hand posture preserved heat more effectively. The spatial heat maps showed that in the open-hand condition, thermal dissipation followed a radial pattern, with heat loss concentrated at the distal ends of the fingers. Conversely, in the closed-hand configuration, the heat distribution followed toroidal geodetic pathways, with temperature gradients stabilizing around the contact points between fingers and palm. The computed Péclet number (Pe) was 23.5 ± 2.1 in the closed-hand posture compared to 15.8 ± 1.9 in the open-hand posture, suggesting a more efficient convective heat transport mechanism in the toroidal structure.

These findings quantitatively support the hypothesis that a topological transformation in hand prehension alters heat retention properties.

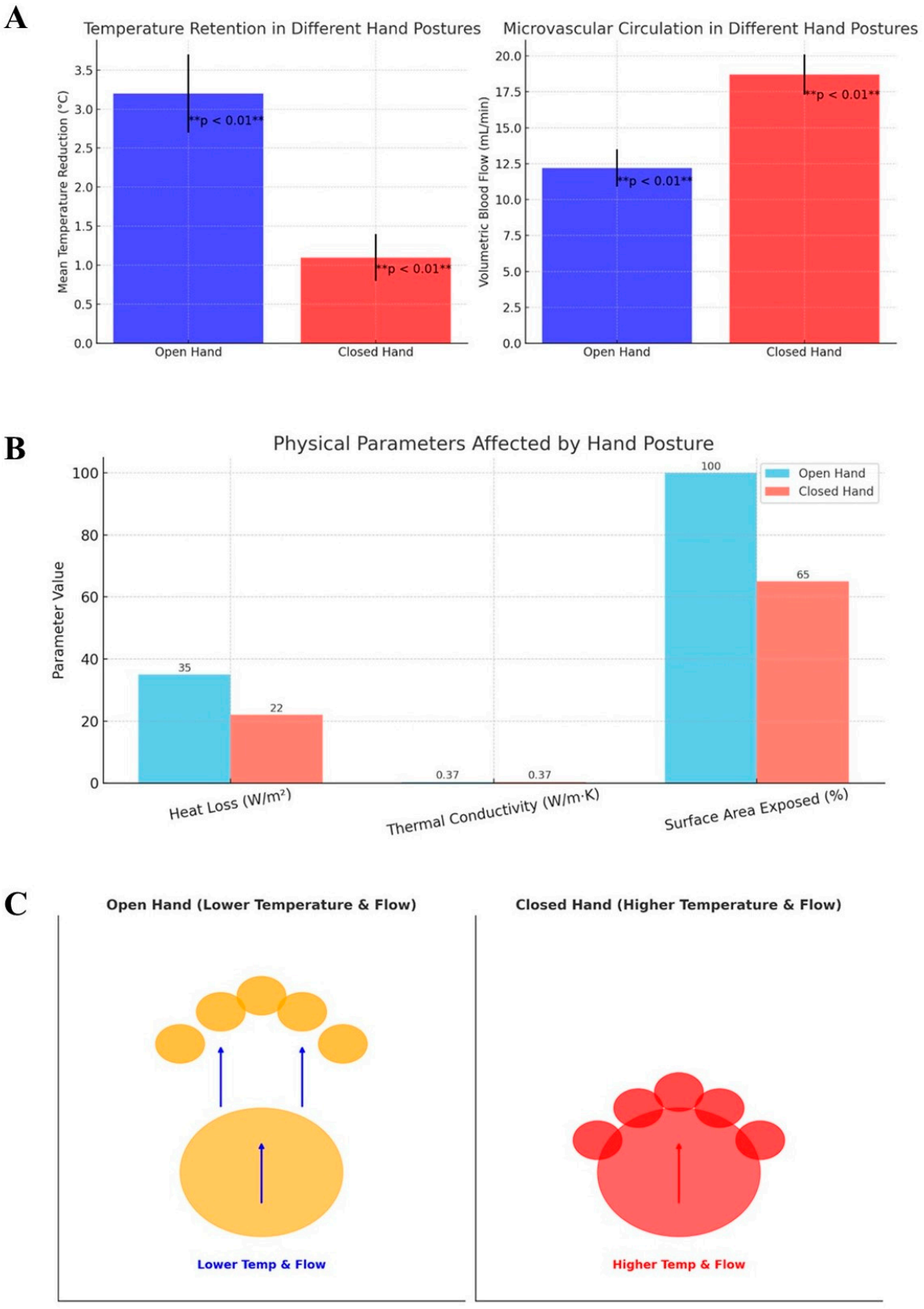

Figure 1. (**A**) Statistical comparison of temperature retention and blood flow between the open-hand and closed-hand postures with statistical significance (**). The left panel displays the mean temperature

reduction in the two conditions, showing a significantly lower temperature drop in the closed-hand posture compared to the open-hand posture ($p < 0.01$). The right panel displays the volumetric blood flow, with the closed-hand posture demonstrating higher perfusion than the open-hand posture ($p < 0.01$). Statistical significance is determined using a two-tailed t-test. Error bars indicate standard deviations. (**B**) Comparison of three physiological parameters in open and closed hand postures. The closed-hand posture shows a statistically significant reduction in heat loss ($p < 0.01$) and surface area exposed ($p < 0.001$) relative to the open-hand posture, both contributing to enhanced thermal retention. Thermal conductivity remains constant across both hand configurations. (**C**) Comparative visual representation of an open hand (left) and a closed hand (right). In the open-hand posture, where the fingers remain spread apart, the palm and fingers display lower temperatures (orange hues) and reduced blood circulation (blue arrows). Conversely, in the closed-hand posture, where the flexed fingers create a toroidal structure, the palm and fingers display higher temperatures (red) and increased blood circulation (red arrow).

The microvascular flow analysis indicated that the closed-hand configuration exhibited significantly higher capillary perfusion than the open-hand posture (Figure 1A). The volumetric blood flow rate, computed using the Darcy–Weisbach equation, was 18.7 ± 1.4 mL/min in the closed-hand posture, compared to 12.2 ± 1.3 mL/min in the open-hand posture ($p < 0.01$). Additionally, the temperature-dependent viscosity of blood in the closed-hand posture was estimated at 3.2 ± 0.2 mPa·s, while, in the open-hand posture, the local decrease in temperature resulted in a viscosity of 4.0 ± 0.3 mPa·s, contributing to higher vascular resistance. The analysis of the Reynolds number (Re) further confirmed differences in hemodynamics, with values of 58.9 ± 4.6 in the closed-hand posture versus 42.5 ± 3.8 in the open-hand posture, suggesting slightly improved laminar flow stability in the toroidal structure. The time-dependent analysis showed that perfusion remained stable in the closed-hand configuration, while, in the open-hand condition, capillary flow declined progressively, with a 15% reduction in flow velocity after 45 s. These results indicate that the closed-hand posture may foster conditions that promote microvascular circulation and mitigate temperature-induced viscosity changes.

Comparison of further physical parameters between the open and closed hand postures reveals other differences in thermoregulatory performance. As shown in Figure 1B, the closed-hand configuration resulted in significantly lower heat loss ($p < 0.01$) and reduced surface area exposure ($p < 0.001$) compared to the open-hand condition. These reductions contribute directly to improve heat retention, supporting the proposed mechanism by which a toroidal posture mitigates peripheral cooling. In turn, thermal conductivity, modelled as a constant property of soft tissue, remained unchanged between conditions as it is a fixed intrinsic property of the biological tissue and is not affected by changes in posture or geometry. These results support the hypothesis that postural changes can influence passive thermoregulatory performance through structural modulation of exposed surface area.

Overall, our simulations show that the toroidal structure formed by the closed-hand posture, compared to the open-hand configuration, may maintain a higher mean temperature, minimize heat dissipation, improve local perfusion and facilitate microvascular circulation (Figure 1C). Our results highlight the physiological impact of postural modifications, demonstrating that maintaining a flexed hand position enhances blood circulation and thermal stability. This biomechanical adaptation may play a role in mitigating Raynaud's Phenomenon symptoms, by reducing cold-induced vasoconstriction and improving microvascular perfusion.

4. Discussion

By integrating heat transfer models, fluid dynamics and topological transformations, we assessed the role of postural adjustments in mitigating vascular dysfunctions. We

demonstrated that the transition from an open-hand to a closed-hand posture significantly affects thermal retention and microvascular circulation. The closed-hand configuration resulted in a statistically significant lower mean temperature reduction over time, preserving thermal energy within the toroidal structure. Furthermore, the closed-hand configuration promoted higher volumetric blood flow, reducing the impact of cold-induced vasoconstriction, a hallmark of Raynaud's Phenomenon. Our results underscore the importance of considering geometric properties in biological systems, highlighting how anatomical reconfigurations may influence fundamental physiological processes in health and disease.

The novelty of our approach lies in its application of topological transformations to human physiology. Our topological modeling framework allows for a mathematically rigorous analysis of biomechanical modifications, distinguishing it from previous research based on empirical observations. Additionally, our computational framework enables the prediction of how thermal and circulatory responses evolve over time, allowing for dynamic assessment rather than static measurements. Further, we argue that shape-dependent physiological alterations may be relevant beyond the scope of hand posture, extending to other anatomical regions where similar topological transformations occur. Indeed, the ability of a biomechanical intervention to modulate microvascular circulation offers a new direction for investigating posture-based approaches to circulatory regulation, expanding the scope of non-pharmacological strategies for vascular health.

Our study does not replace existing interventions; rather, it complements them by offering a biomechanical strategy that may be integrated with current therapies. Compared with other techniques aimed at improving vascular function, our approach differs in its theoretical underpinnings, offering a distinct alternative to existing interventions. Traditional methods for improving circulation in conditions like RP primarily focus on pharmacological interventions, thermal protection or exercise-based rehabilitation [4]. Conventional pharmacological treatments, such as calcium channel blockers or vasodilators, primarily target endothelial function to counteract vasospastic episodes [3]. While effective, these treatments modulating systemic vascular tone are often accompanied by side effects and fail to consider the biomechanical factors that may contribute to localized vascular dysfunction. Thermally protective devices, such as heated gloves, function by externally supplying heat to mitigate the effects of environmental cold exposure [27]. However, these external interventions do not influence intrinsic physiological processes and depend on continuous energy input. In contrast, the closed-hand posture engages endogenous heat conservation mechanisms by structurally minimizing surface exposure and promoting internal thermal recirculation. Exercise-based rehabilitation programs emphasize muscle activity to enhance circulation, but these require sustained effort and may not be suitable for all patient populations [28]. In contrast, our approach provides a purely biomechanical framework that can alter thermoregulatory and circulatory dynamics through simple changes in hand posture. Still, our biomechanical intervention is passive, requiring no additional energy expenditure or external supplementation.

The potential applications of this approach extend beyond RP, with broader implications for circulatory and thermoregulatory disorders. The ability of the closed-hand posture to modulate microvascular circulation suggests that similar topological interventions could be explored in conditions such as diabetic microangiopathy, where peripheral blood flow regulation is impaired [35]. In stroke rehabilitation, where patients often experience deficits in fine motor control, structured hand postures may be investigated as a means of facilitating neurovascular coupling and motor recovery [36]. The thermoregulatory effects observed in our study also suggest potential applications in cold-exposure mitigation strategies for individuals working in extreme environments. Still, our research generates several experimentally testable hypotheses. A multi-modal experimental validation protocol is

proposed to bridge computational findings with empirical evidence. Surface temperature changes could be monitored using high-resolution infrared thermography, capturing thermal gradients across open and closed hand postures in real time. Concurrently, nailfold capillaroscopy can provide non-invasive visualization of capillary blood flow and perfusion. Additionally, electromyography (EMG) could assess muscular activity to distinguish passive posture maintenance from active thermogenesis. These methods collectively offer a feasible, low-risk pathway for in vivo validation of the model's predictions.

Several limitations should be acknowledged. We used topological constructs just as a theoretical framework to describe the transformation of hand posture from an open to a closed toroidal configuration. These mathematical descriptors were not directly implemented as simulation parameters or boundary conditions, but were instead used to conceptualize how closed-loop geometries could alter heat and flow dynamics. While the simulation itself relied on classical heat diffusion and fluid flow equations, future work could incorporate these topological indices into mesh generation or geometric constraints, providing a more direct computational integration of topological properties. Still, we modeled the hand as a 2D surface, which is a gross oversimplification neglecting the complex 3D structure of the hand which influences heat transfer and blood flow. A future 3D model, incorporating musculoskeletal and vascular components and employing the Laplace–Beltrami operator governing the diffusion of heat along the curved topology, is a logical next step to enhance biomechanical accuracy.

Our model relied on generalized physiological parameters not accounting for inter-individual variations in vascular function. It must be acknowledged that individual differences—such as age, gender and hand morphology—may influence local thermoregulatory and vascular responses. Our model also assumes idealized boundary conditions, with external temperature set at 20 °C, which may not fully capture the real-world variability. Differences in skin thickness, subcutaneous fat distribution, capillary density and baseline vascular tone could influence the rate of heat loss and magnitude of microcirculatory improvement associated with different hand postures. Additionally, our study does not account for autonomic nervous system contributions to microcirculatory adjustments [30]. Also, our methodology does not consider the electrical properties of the skin, which play a significant role in processes such as wound healing, cell migration and integration of bioelectronic devices [37,38]. Variations in skin conductivity and its response to external stimuli could influence local temperature distributions and microcirculation patterns, adding another layer of complexity to our model [39]. Moreover, the interaction between temperature fluctuations and leukocyte function remains underexplored in our analysis, despite evidence suggesting that hypothermia and rewarming alter leukocyte–endothelial interactions and immune cell recruitment [40,41]. Indeed, temperature-dependent activation of leukocyte populations has been observed across various species, providing additional evidence that localized thermal gradients may have immunological implications beyond their direct effect on blood flow [42,43]. Capillary dynamics are also affected by temperature variations at the microscale, as studies indicate that thermally induced changes in surface tension and pressure gradients influence microfluidic transport in biological tissues [44,45]. This is particularly relevant in cold-induced vascular conditions like RF, where capillary pressure fluctuations may exacerbate vasospastic episodes [46].

5. Conclusions

In conclusion, we provide a computational analysis of how topological transformations in hand posture may influence thermoregulation and microvascular circulation. Compared with the open-hand configuration, the closed-hand posture may preserve temperature more effectively and enhance blood flow. More broadly, we introduce a quantitative methodology

for analyzing the physiological effects of anatomical reconfigurations, providing a mathematical approach to investigating how structural adaptations impact functional outcomes.

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References

1. Haque, A.; Hughes, M. Raynaud's phenomenon. *Clin. Med.* **2020**, *20*, 580–587. [CrossRef] [PubMed]
2. Teaw, S.; Gupta, A.; Williams, A.; Wilson, F.P.; Sumpio, B.J.; Sumpio, B.E.; Hinchcliff, M. Hyperspectral imaging in systemic sclerosis-associated Raynaud phenomenon. *Arthritis Res. Ther.* **2023**, *25*, 10. [CrossRef] [PubMed]
3. Brunner-Ziegler, S.; Dassler, E.; Müller, M.; Pratscher, M.; Forstner, N.F.-F.M.; Koppensteiner, R.; Schlager, O.; Jilma, B. Capillaroscopic differences between primary Raynaud phenomenon and healthy controls indicate potential microangiopathic involvement in benign vasospasms. *Vasc. Med.* **2024**, *29*, 200–207. [CrossRef] [PubMed]
4. Su, K.Y.; Sharma, M.; Kim, H.J.; Kaganov, E.; Hughes, I.; Abdeen, M.H.; Ng, J.H.K. Vasodilators for primary Raynaud's phenomenon. *Cochrane Database Syst. Rev.* **2021**, *2021*, CD006687. [CrossRef]
5. Ture, H.Y.; Lee, N.Y.; Kim, N.R.; Nam, E.J. Raynaud's Phenomenon: A Current Update on Pathogenesis, Diagnostic Workup, and Treatment. *Vasc. Spec. Int.* **2024**, *40*, 26. [CrossRef]
6. Busuioc, S.; Kusumaatmaja, H.; Ambruş, V.E. Axisymmetric flows on the torus geometry. *J. Fluid Mech.* **2020**, *901*, A9. [CrossRef]
7. Wang, Y.-P.; Cheng, R.-H.; He, Y.; Mu, L.-Z. Thermal Analysis of Blood Flow Alterations in Human Hand and Foot Based on Vascular-Porous Media Model. *Front. Bioeng. Biotechnol.* **2022**, *9*, 786615. [CrossRef]
8. Brand Paul, W.; Hollister, A.M. *Clinical Mechanics of the Hand, Mosby-Year Book*, 3rd ed.; Mosby: St. Louis, MO, USA, 1999; ISBN 13:978-0815127864.
9. Duncan, S.F.; Saracevic, C.E.; Kakinoki, R. Biomechanics of the Hand. *Hand Clin.* **2013**, *29*, 483–492. [CrossRef]
10. Schreuders, T.A.R.; Brandsma, J.W.; Stam, H.J. Functional Anatomy and Biomechanics of the Hand. In *Hand Function*; Duruöz, M., Ed.; Springer: Cham, Switzerland, 2019. [CrossRef]
11. Chen, Y.-P.; Yeh, C.-I.; Lee, T.-C.; Huang, J.-J.; Pei, Y.-C. Relative posture between head and finger determines perceived tactile direction of motion. *Sci. Rep.* **2020**, *10*, 5494. [CrossRef]
12. Hartmann, F.; Maiello, G.; Rothkopf, C.A.; Fleming, R.W. Estimation of Contact Regions Between Hands and Objects During Human Multi-Digit Grasping. *J. Vis. Exp.* **2023**, *194*, e64877. [CrossRef]
13. Schlesinger, G. Der mechanische Aufbau der künstlichen Glieder. In *Ersatzglieder und Arbeitshilfen*; Borchardt, M., Hartmann, K., Leymann, R.R., Schlesinger, S., Eds.; Springer: Berlin/Heidelberg, Germany, 1919. [CrossRef]
14. Hertling, D.; Kessler, R.; Shimandle, S.A. Management of common musculoskeletal disorders, physical therapy principles and methods. *Dimens. Crit. Care Nurs.* **1990**, *9*, 279. [CrossRef]
15. Li, Z.-M.; Yue, G.H. Dependence of finger flexion force on the posture of the nonperforming fingers during key pressing tasks. *J. Mot. Behav.* **2002**, *34*, 329–338. [CrossRef] [PubMed]
16. Lee, K.-S.; Mo, S.-M.; Hwang, J.-J.; Wang, H.; Jung, M.-C. Relaxed hand postures. *Jpn. J. Ergon.* **2008**, *44*, 436–439. [CrossRef]

17. Lee, K.-S.; Jung, M.-C. Flexion and Extension Angles of Resting Fingers and Wrist. *Int. J. Occup. Saf. Ergon.* **2014**, *20*, 91–101. [CrossRef]
18. Romano, D.; Tamè, L.; Amoruso, E.; Azañón, E.; Maravita, A.; Longo, M.R. The standard posture of the hand. *J. Exp. Psychol. Hum. Percept. Perform.* **2019**, *45*, 1164–1173. [CrossRef]
19. Jaworski, Ł.; Karpiński, R. Biomechanics of the Human Hand. *J. Technol. Exploit. Mech. Eng.* **2017**, *3*, 28–33. [CrossRef]
20. Tanrıkulu, S.; Bekmez, Ş.; Üzümcügil, A.; Leblebicioğlu, G. Anatomy and Biomechanics of the Wrist and Hand. In *Sports Injuries*; Doral, M.N., Karlsson, J., Eds.; Springer: Berlin/Heidelberg, Germany, 2015. [CrossRef]
21. Wang, T.; Dai, Z.; Potier-Ferry, M.; Xu, F. Curvature-Regulated Multiphase Patterns in Tori. *Phys. Rev. Lett.* **2023**, *130*, 048201. [CrossRef]
22. Wang, G.; Fei, L.; Luo, K.H. Unified Lattice Boltzmann Method with Improved Schemes for Multiphase Flow Simulation: Application to Droplet Dynamics under Realistic Conditions. *Phys. Rev. E* **2022**, *105*, 045314. [CrossRef]
23. Jantzen, R.T. Geodesics on the Torus and other Surfaces of Revolution Clarified Using Undergraduate Physics Tricks with Bonus: Nonrelativistic and Relativistic Kepler Problems. *arXiv* **2012**, arXiv:1212.6206.
24. Celano, K.; Coll, V.E.; Dodd, J. Why Curves Curve: The Geodesics on the Torus. *Math. Mag.* **2022**, *95*, 230–239. [CrossRef]
25. Levick, J.R.; Michel, C.C. The effects of position and skin temperature on the capillary pressures in the fingers and toes. *J. Physiol.* **1978**, *274*, 97–109. [CrossRef] [PubMed]
26. Ye, Y.; Griffin, M.J. Effects of temperature on reductions in finger blood flow induced by vibration. *Int. Arch. Occup. Environ. Health* **2011**, *84*, 315–323. [CrossRef] [PubMed]
27. Landim, S.F.; Bertolo, M.B.; de Abreu, M.F.M.; Del Rio, A.P.; Mazon, C.C.; Marques-Neto, J.F.; Poole, J.L.; Magalhães, E.d.P. The evaluation of a home-based program for hands in patients with systemic sclerosis. *J. Hand Ther.* **2019**, *32*, 313–321. [CrossRef] [PubMed]
28. Tapia-Haro, R.M.; García-Ríos, M.C.; Castro-Sánchez, A.M.; Toledano-Moreno, S.; Casas-Barragán, A.; Aguilar-Ferrándiz, M.E. Analysis of Hand Function, Upper Limb Disability, and Its Relationship with Peripheral Vascular Alterations in Raynaud's Phenomenon. *Diagnostics* **2024**, *14*, 93. [CrossRef]
29. Malakoutikhah, H.; Latt, L.D. Disease-Specific Finite Element Analysis of the Foot and Ankle. *Foot Ankle Clin.* **2023**, *28*, 155–172. [CrossRef]
30. Hirata, K. Heat loss from the upper extremities and clothing thermal comfort. *J. Text. Eng. Fash. Technol.* **2017**, *3*, 616–619. [CrossRef]
31. Klabunde, R.E.; Johnson, P.C. Effects of reduced temperature on capillary flow and reactive hyperemia in red and white skeletal muscle. *Microvasc. Res.* **1980**, *19*, 99–107. [CrossRef]
32. Hales, J.R.S.; Fawcett, A.A.; Bennett, J.W.; Needham, A.D. Thermal control of blood flow through capillaries and arteriovenous anastomoses in skin of sheep. *Pflug. Arch. Eur. J. Physiol.* **1978**, *378*, 55–63. [CrossRef]
33. Mayer, D.B.; Franosch, T.; Mast, C.; Braun, D. Thermophoresis beyond Local Thermodynamic Equilibrium. *Phys. Rev. Lett.* **2023**, *130*, 168202. [CrossRef]
34. Brorsson, S.; Nilsdotter, A.; Pedersen, E.; Bremander, A.; Thorstensson, C. Relationship between finger flexion and extension force in healthy women and women with rheumatoid arthritis. *J. Rehabil. Med.* **2012**, *44*, 605–608. [CrossRef]
35. Biswas, D.; Kartha, S.A. Conceptual modeling of temperature effects on capillary pressure in dead-end pores. *Sadhana* **2019**, *44*, 117. [CrossRef]
36. Dodds, R.; Kuh, D.; Sayer, A.A.; Cooper, R. Physical activity levels across adult life and grip strength in early old age: Updating findings from a British birth cohort. *Age Ageing* **2013**, *42*, 794–798. [CrossRef] [PubMed]
37. Abe, Y.; Nishizawa, M. Electrical aspects of skin as a pathway to engineering skin devices. *APL Bioeng.* **2021**, *5*, 041509. [CrossRef] [PubMed]
38. Kolimechkov, S.; Seijo, M.; Swaine, I.; Thirkell, J.; Colado, J.C.; Naclerio, F. Physiological effects of microcurrent and its application for maximising acute responses and chronic adaptations to exercise. *Eur. J. Appl. Physiol.* **2023**, *123*, 451–465. [CrossRef]
39. Shutova, M.S.; Boehncke, W.-H. Mechanotransduction in Skin Inflammation. *Cells* **2022**, *11*, 2026. [CrossRef]
40. Bogert, N.V.; Werner, I.; Kornberger, A.; Meybohm, P.; Moritz, A.; Keller, T.; Stock, U.A.; Beiras-Fernandez, A. Influence of hypothermia and subsequent rewarming upon leukocyte-endothelial interactions and expression of Junctional-Adhesion-Molecules A and B. *Sci. Rep.* **2016**, *6*, 21996. [CrossRef]
41. Peake, J.; Peiffer, J.J.; Abbiss, C.R.; Nosaka, K.; Okutsu, M.; Laursen, P.B.; Suzuki, K. Body temperature and its effect on leukocyte mobilization, cytokines and markers of neutrophil activation during and after exercise. *Eur. J. Appl. Physiol.* **2008**, *102*, 391–401. [CrossRef]
42. Jämsä, J.; Huotari, V.; Savolainen, E.; Syrjälä, H.; Ala-Kokko, T. Analysis of the temperature affects on leukocyte surface antigen expression. *J. Clin. Lab. Anal.* **2011**, *25*, 118–125. [CrossRef]
43. Köllner, B.; Kotterba, G. Temperature dependent activation of leucocyte populations of rainbow trout, *Oncorhynchus mykiss*, after intraperitoneal immunisation with *Aeromonas salmonicida*. *Fish Shellfish. Immunol.* **2002**, *12*, 35–48. [CrossRef]

44. Fang, R.; Zhu, H.; Li, Z.; Zhu, X.; Zhang, X.; Huang, Z.; Li, K.; Yan, W.; Huang, Y.; Maisotsenko, V.S.; et al. Temperature Effect on Capillary Flow Dynamics in 1D Array of Open Nanotextured Microchannels Produced by Femtosecond Laser on Silicon. *Nanomaterials* **2020**, *10*, 796. [CrossRef]
45. Grant, S.A.; Bachmann, J. *Effect of Temperature on Capillary Pressure*; Raats, P.A.C., Smiles, D., Warrick, A.W., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2002. [CrossRef]
46. Szilágyi, T.; Csernyánszky, H.; Csákó, G.; Benkö, K. The influence of hypothermia on arthus-phenomenon and leucotaxis. *Spec.-Haemotologica Immunol.* **1971**, *27*, 1469–1470. [CrossRef]

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REVIEW ARTICLE

Charged Interfaces in the Brain: How Electrostatic Forces May Guide Cerebrospinal Fluid Dynamics

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA

Correspondence: Arturo Tozzi (tozziarturo@libero.it)

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ABSTRACT

Cerebrospinal fluid (CSF) flows play a main role in maintaining brain homeostasis, supporting waste clearance, nutrient delivery, and interstitial solute exchange. Although current models emphasize mechanical drivers like cardiac pulsation, respiration, and ciliary motion, these mechanisms alone fall short of explaining the nuanced spatiotemporal regulation of CSF flow observed under physiological and pathological conditions—even when accounting for the glymphatic framework. We hypothesize that electrostatic forces arising from charged cellular interfaces may contribute to CSF movement through electro-osmotic mechanisms. We begin by examining the biological basis for surface charge in the brain, highlighting the presence of charged glycoproteins, ion channels, and dynamic membrane potentials on ependymal/glial cells interfacing directly with CSF pathways. Next, we describe key electro-osmotic principles in confined geometries, emphasizing how nanoscale surface charges can modulate fluid motion without mechanical input. Drawing from nanofluidic research, biophysics, and electrohydrodynamic theory, we argue that the conditions required for electro-osmotic coupling, i.e., ionic fluid, narrow conduits, and patterned surface charge, are present within brain microenvironments. To test plausibility, we present computational simulations demonstrating that surface charge patterns alone can induce structured fluid flow/solute transport, including nonlinear transitions and oscillatory behaviors that resemble physiological rhythms. These findings support the idea that electrostatics may play a modulatory role in CSF regulation, complementing mechanical drivers. By integrating different disciplines, we propose a testable, mechanistically grounded hypothesis reframing CSF dynamics as electrohydrodynamically sensitive processes. Our approach could inspire novel diagnostics/therapeutic strategies in hydrocephalus and neurodegenerative disease and inform the design of targeted drug delivery systems.

1 | Introduction

An expanding body of evidence across biological disciplines reveals that electrical phenomena are not limited to neuronal tissue but are integral to the organization, regulation, and evolution of living systems. Even dormant *Bacillus subtilis* spores retain a pre-existing electrochemical gradient—specifically a potassium ion potential—enabling them to integrate environmental nutrient pulses over time (Kikuchi et al. 2022). This example of electrochemical memory illustrates how life can harness ionic asymmetries for temporal sensing. In multicellular organisms, the actin cytoskeleton, known for its role in

Abbreviations: CSF, cerebrospinal fluid.

intracellular transport and structural dynamics, is functionally and mechanistically linked to action potentials in both animals and plants. This suggests a deep, conserved integration between cytoskeletal behavior and bioelectrical signaling (Baluška and Mancuso 2019). In ecological contexts, electric forces are ubiquitous. Insects such as bees accumulate positive electric charge during flight, which interacts with the negatively charged surfaces of flowers to facilitate pollination (Clarke et al. 2013; England and Robert 2024a; Clarke et al. 2017). Other animals—such as spiders and caterpillars—exploit environmental electric fields for dispersal, prey detection or host attachment (Ortega-Jimenez and Dudley 2013; England and Robert 2024b; Hunting et al. 2022). Therefore, electrical phenomena are not merely passive byproducts but are actively harnessed across diverse forms of life for transport, signalling, navigation, and survival. Electrical principles are informing also synthetic systems. In engineered nanofluidics, ion and water transport in confined two-dimensional environments reproduces key features of biological ion channels, offering insights into neurotransmission and membrane selectivity (Robin et al. 2023). Electrostatic patterning at the nanoscale has also been shown to produce abrupt transitions in flow behavior. Curk et al. (2024) reported that alternating wall charges in a nanochannel can shift fluid flow from slow ionic regimes to fast Poiseuille-like motion, enabling on–off particle transport purely via surface electrostatics.

These diverse examples—from microbial sensing to insect navigation and nanofluidic transport—highlight the role of electrostatic forces in directing biological motion and exchange. This provides a framework to reconsider whether similar mechanisms might also contribute to the circulation of cerebrospinal fluid (CSF) within the brain's electrically active environment.

The regulation and movement of CSF in the brain plays roles in metabolic waste clearance, nutrient transport, homeostatic ion balance, and mechanical cushioning (Liu et al. 2022; Xiang et al. 2023). Unlike the rest of the body which uses a lymphatic system, the brain lacks a clear waste disposal route (Iliff et al. 2012; Tumani et al. 2018). Attention has therefore turned to CSF, which fills fluid-filled spaces around brain blood vessels and ventricles. Traditional models of CSF circulation have primarily emphasized the driving effects of mechanical forces, including pressure gradients from cardiac and respiratory cycles, osmotic fluxes and ciliary activity of ependymal cells (Ray and Heys 2019; Yang et al. 2022; Zhang et al. 2024). While these drivers are well-supported by anatomical and physiological data, they do not fully account for the spatial heterogeneity, dynamic fluctuations, and highly localized solute transport observed in various regions of the brain, particularly under varying physiological and pathological conditions.

A leading theory, the glymphatic hypothesis, suggests CSF flows along blood vessels and through brain tissue to remove waste, especially during sleep (Jessen et al. 2015; Rasmussen et al. 2018). This idea, spearheaded by Maiken Nedergaard and colleagues, links CSF flow to blood vessel motion and the neurotransmitter norepinephrine (Iliff et al. 2012; Jessen et al. 2015; Mestre et al. 2020). In mouse studies, Norepinephrine oscillations during non-REM sleep drove slow vasomotion, producing rhythmic shifts in blood and CSF volumes (Hauglund et al. 2025). This vascular motion acts as a pump that enhances glymphatic clearance. However, the glymphatic hypothesis remains contentious. Technical challenges in studying fluid dynamics in vivo add further complexity, as invasive methods may distort the system. Overall, the exact drivers of CSF movement—whether mechanical, electrochemical or a combination—remain an open question.

Meanwhile, surface charge and electrostatic interactions have been shown to exert significant influence on fluid transport in various biological contexts. For instance, the endothelial surfaces of blood vessels and renal tubules are known to carry structured electrostatic charges which actively contribute to flow regulation and molecular exchange (Wang et al. 2021a; Choudhury et al. 2022; Jonusaite and Himmerkus 2024). We argue that the occurrence of charged macromolecules and membrane potentials in glial and ependymal cell layers surrounding the CSF points towards the potential for a similar form of electrostatic modulation within the brain's fluid pathways (Hladky and Barrand 2016; Faraji et al. 2020).

Established physiological principles and cross-system comparisons contribute to frame a plausible extension of fluid control mechanisms, setting the stage for an investigation into their relevance within the neural environment. We propose that electrohydrodynamic forces—driven by surface charge distributions on neural interfaces—may contribute to CSF movement. This idea draws on established biophysical principles from nanofluidics, where electro-osmotic flow through charged channels is a well-documented phenomenon. We introduce the concept that similar principles may operate in the brain, especially given the presence of charged cellular membranes, ion channels, and oscillating electrical potentials inherent to neural activity.

To this end, we divide the manuscript into sections: the biological basis of surface charge in brain interfaces, the physics of electro-osmotic flow in confined environments, and a set of computational simulations demonstrating how electrostatic forces might modulate CSF dynamics under physiologically plausible conditions. Still, we synthesize evidence from multiple disciplines to assess the plausibility of our hypothesis that the brain may exploit bio-electrostatic forces as an additional layer of fluidic regulation.

2 | Charged Surfaces in the Brain and Their Physiological Basis

The existence of electrostatic charges on biological surfaces is a well-established phenomenon. Endothelial cells, epithelial linings, and glial membranes all carry surface charges arising from their biochemical makeup, including glycoproteins, proteoglycans, and sialic acid residues embedded in the plasma membrane and associated glycocalyx (Nishino et al. 2020; Burtscher et al. 2020; Rasmussen et al. 2020). In the context of the central nervous system, charged surfaces are found not only on the luminal sides of blood vessels but also along the walls of the brain's ventricular system, the perivascular spaces, and the glial limiting membranes (Santa-Maria et al. 2019; Walter et al. 2021). These surfaces interface directly or indirectly with the CSF,

forming electrochemical boundaries that have the potential to influence ionic distributions and, by extension, fluid behavior.

Astrocytes and ependymal cells are equipped with membrane-bound ion channels, transporters, and gap junctions that dynamically regulate local ion concentrations (Zhou et al. 2021; Sanapathi et al. 2023). Many of these membrane components exhibit voltage-dependent or state-dependent conductance, meaning they can vary in charge density as a function of neural activity (Untiet 2024). Studies have shown that extracellular potassium concentrations fluctuate during states such as sleep, seizure, and trauma, thereby altering the electrochemical environment of the CSF-contacting surfaces (Yoshida et al. 2018; Dietz et al. 2023). Moreover, the glial endfeet enveloping blood vessels in the perivascular spaces exhibit a sophisticated array of ion channels—such as aquaporins and inwardly rectifying potassium channels—that play a role in shaping local osmotic and electrochemical gradients within the brain's fluid compartments (Deeg et al. 2016).

Ependymal cells lining the ventricular system, known for their motile cilia, also exhibit active ion channel behavior contributing to directional CSF movement (Deng et al. 2023). Disruption of their function is implicated in disorders like hydrocephalus, indicating that electrochemical and cellular regulation of CSF flow is biologically active and clinically significant (Ji et al. 2022).

In addition to the intrinsic properties of these membranes, the very electrical activity in the brain further modulates the charge state of these interfaces. Neuronal firing and field potentials generate spatiotemporal variations in the extracellular electric field which can induce transient polarization of nearby membranes (McColgan et al. 2017; Bédard and Destexhe 2022). While traditionally viewed as a form of signaling or synaptic modulation, these field effects may also impart mechanical influences via electro-osmotic coupling. In both biological tissues and engineered microfluidic systems, electric fields have been effectively employed to drive fluid motion through charged channels in confined environments, as seen in electrokinetic drug delivery platforms and lab-on-a-chip devices (Cruz-Garza et al. 2024). Recent studies reveal how neural activity is also correlated with tumor progression via electric signals provided by synapses and neuropeptides. GABAergic input promotes glioma growth (Barron et al. 2025), substance P drives breast cancer metastasis through TLR7 activation (Padmanaban et al. 2024) and CGRP from nociceptors impairs CD8+ T cell immunity in melanoma (Balood et al. 2022). Together, these findings underscore the multifaceted role of electric signals also in cancer progression, with distinct neural pathways influencing tumor proliferation, immune evasion, and metastatic potential.

Further indirect evidence comes from studies of ion diffusion and CSF exchange (Marques-Almeida et al. 2023). Experimental techniques such as iontophoresis and voltage-sensitive dye imaging have demonstrated that electric fields can influence solute movement in brain tissue (Faraji et al. 2020). These findings raise the possibility that CSF-facing membranes may act not merely as passive barriers but as active modulators of ionic and fluid flow, regulated by both intrinsic charge and externally applied fields.

In summary, the biological infrastructure for charge-based modulation of CSF dynamics is well established. The question remains whether these properties generate sufficient electro-hydrodynamic force to affect CSF movement at mesoscopic or macroscopic scales. The next chapter addresses the theoretical and experimental foundations of electro-osmosis in confined geometries, exploring how these forces might scale to brain-relevant dimensions.

3 | Electro-Osmosis in Confined Geometries and Its Relevance to Brain Physiology

Electro-osmosis refers to the motion of a liquid induced by an electric field across a charged surface within a confined channel (Sahib et al. 2021). It is a well-characterized phenomenon in synthetic nanofluidic systems and has long been harnessed in technologies such as capillary electrophoresis, microfluidic pumps and drug delivery platforms (Alizadeh et al. 2021; Elboughdiri et al. 2024). Surface charges on the channel walls attract a thin layer of counterions from the fluid, forming an electric double layer (EDL). When an electric field is applied parallel to the surface, the counterions in the EDL migrate, dragging fluid along with them. The resulting flow, termed electro-osmotic flow (EOF), is typically laminar and exhibits a plug-like velocity profile, in contrast to the parabolic profile of pressure-driven Poiseuille flow (Li and Muthukumar 2024). Physics governing electro–osmosis has been formalized through coupled solutions of the Navier–Stokes equations for fluid motion and the Poisson–Boltzmann equation for electrostatic potential (Gubbiotti et al. 2022). These equations reveal that the EOF velocity is directly proportional to the zeta potential (a measure of the surface charge), the permittivity of the fluid, and the applied electric field, while inversely proportional to the fluid viscosity (Sherwood et al. 2014). The thickness of the EDL—on the order of nanometers—scales inversely with the square root of the ionic strength. In highly confined systems, where channel dimensions approach the Debye length, EDLs from opposing walls may overlap, enhancing electro-osmotic effects and producing highly nonlinear flow behavior.

Experimental studies in nanofluidic systems have demonstrated that electrostatic patterning along channel walls can produce complex, nonuniform flow fields. For example, charge heterogeneity—achieved via alternating stripes of positive and negative surface potential—has been shown to generate spatially structured flows, reversals in direction, and even discrete transitions between ionic and pressure-dominated flow regimes (Ververniotis et al. 2011). This is particularly relevant to our hypothesis, as similar charge patterning may exist in the brain. Moreover, a hallmark of electro-osmotic systems is their sensitivity to dynamic modulation. In engineered systems, time-varying surface potentials can induce pulsatile or oscillatory flow, mimicking the rhythmicity of biological processes like neural oscillations (Banerjee et al. 2023). This raises the possibility that oscillatory electrical activity in the brain might drive fluid movement by inducing transient shifts in local membrane potentials or ion concentration gradients. Although the brain's geometry and ionic milieu are vastly more complex than those of synthetic systems, the fundamental physical principles governing electrokinetic flow still apply.

CSF navigates a labyrinth of narrow, channel-like pathways within the brain, enabling both directed flow and efficient molecular exchange (see Table 1). Among the most studied are perivascular spaces, i.e., Virchow–Robin spaces, which surround blood vessels as they enter and exit brain tissue (Kwee and Kwee 2007). These spaces range from 5 to 40 μm in width and may extend hundreds of micrometers, enabling the bidirectional movement of CSF and interstitial fluid (Bernal et al. 2022; Raicevic et al. 2023). Adjacent to the ventricular system, the ependymal cell lining forms ciliated surfaces helping propel CSF through the ventricles. Though not traditional channels, the intercellular gaps and surface specializations between these cells, often less than 1 μm wide, contribute to localized CSF movement and solute exchange. Further into the parenchyma, the brain's extracellular space—comprised of interstitial and paracellular compartments—has a width of 20 to 60 nm. While primarily a domain for diffusion, this space may support slow, directed flow under certain physiological or pathological conditions (Ballerini et al. 2020). The cerebral aqueduct, though larger in scale (~1.5 mm in diameter and ~15 mm in length), stands for an anatomical bottleneck that constrains ventricular CSF flow and is highly sensitive to obstruction (Sincomb et al. 2020). Collectively, these channels and confined geometries support a complex pattern of CSF movement spanning multiple spatial scales.

Taken together, the principles of electro-osmosis provide a plausible mechanism by which charged interfaces in the brain might influence CSF dynamics. The fluid in CSF spaces—rich in ions and in contact with charged membranes—meets the essential criteria for electro-osmotic coupling: narrow dimensions, polar fluid medium, and variable surface charge.

Experimental analogs from biology further support this view. For instance, endothelial cells lining blood vessels in vivo exhibit charge-selective permeability and engage in electrokinetic transport processes, influencing both blood plasma and interstitial fluid composition (Wakasugi et al. 2024). The inner surfaces of blood vessels, particularly the endothelium, carry a net negative charge due to the presence of glycoproteins, proteoglycans, and sialic acid-rich components of the glycocalyx (Zhao et al. 2020). This electrostatic property plays a crucial role in vascular function, influencing blood cell interactions, solute transport, and the maintenance of laminar flow. Similar charge characteristics have also been observed in other fluid-carrying biological conduits, such as lymphatic vessels and renal tubules, where surface charge helps regulate fluid movement and filtration through electrostatic interactions with high spatial and temporal specificity (Choudhury et al. 2022). Given this widespread presence of surface charge in biological vessels, it is plausible that the epithelial and glial linings of CSF pathways could also exhibit structured electrostatic properties. Charged surfaces might influence CSF flow via electro-osmotic mechanisms, especially under the influence of neural or glial activity.

Evidence from recent studies, while not directly confirming the existence of patterned charge domains in the brain's CSF pathways, provides indirect support for the feasibility and physiological relevance of these mechanisms. Experimental work has shown that electric fields can drive electrokinetic transport of solutes through brain-like tissues (Faraji et al. 2011; Alcaide et al. 2023). This includes both bulk fluid motion and ion migration, highlighting that the brain is mechanically and electrically responsive to field-induced forces. Researchers have also demonstrated that external electric fields can enhance drug delivery to brain regions by modulating flow profiles using electrokinetic principles (Faraji et al. 2020). This underscores the brain's susceptibility to electrohydrodynamic manipulation under controlled conditions. Further support comes from theoretical and computational work suggesting that the brain's nonzero zeta potential, due to charged surfaces such as glial membranes, could allow electro-osmotic flow to contribute to intracerebral fluid movement (Wang et al. 2021b). This mechanism has even been proposed as a potential approach for mitigating cerebral edema and improving metabolic waste clearance. Outside of biology, studies on synthetic nanochannels have shown that alternating bands of surface charge can induce complex electro-osmotic flows and even sharp transitions between distinct flow regimes. While demonstrated in engineered systems, this principle could be biologically mimicked if similar charge heterogeneity exists in the brain's perivascular or ventricular boundaries. Finally, computational models suggest that neuronal activity can generate electrodiffusive gradients that couple with osmotic and mechanical flows in glial networks (Fujii et al. 2017). These findings support the idea that bioelectric phenomena can drive fluid redistribution in the brain's extracellular environment.

Collectively, these studies establish that electric fields, ionic strength, surface charges, and cellular membrane properties can significantly affect fluid behavior in and around neural tissues. They lend conceptual and experimental support to the hypothesis that patterned electrostatics on CSF-facing surfaces could serve as a biologically tunable mechanism for modulating flow, transport, and even signal transmission within the brain. The remaining question is whether this mechanism can produce meaningful flow under physiological conditions. To begin answering this, we implemented a series of computational simulations using idealized models of electro-osmotic flow in brain-inspired geometries. These are detailed in the following chapter.

TABLE 1 | Summary of anatomical microchannels in the brain, detailing their dimensions and roles in cerebrospinal and interstitial fluid transport.

Structure	Diameter	Length	Role
Perivascular spaces	5–40 μm	100–1000 + μm	CSF–ISF exchange, drainage
Ependymal/paracellular gaps	< 1 μm	Very short (cell-scale)	Diffusion, limited flow
Interstitial space	20–60 nm	Local (tissue-wide)	ISF–CSF interaction
Aqueduct of Sylvius	~1.5 mm	~15 mm	Major CSF conduit

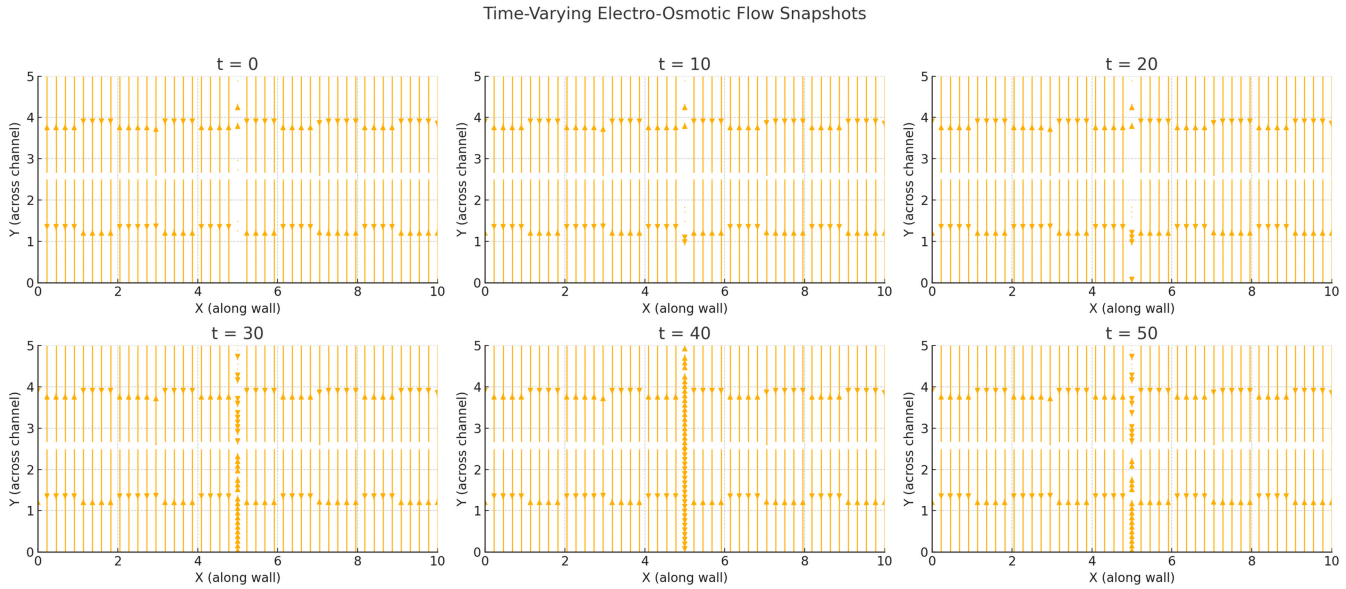

FIGURE 1 | Simulated trajectories of molecules or ions over a 50-s period as they cross a fluid channel lined with alternating surface charge domains. The flow field is shaped by electrohydrodynamic forces resulting from interactions between the patterned wall charges and the fluid content. The colored lines trace the paths of individual particles. The extended simulation duration (T = 50 s) highlights how charge-driven microflow structures can lead to differential transport, localized trapping or enhanced directional clearance.

4 | Computational Simulations of Electrostatic Modulation in Cerebrospinal Fluid Flow

To evaluate the plausibility of electrohydrodynamic modulation of CSF movement, we developed computational simulations to approximate electro-osmotic flow in geometries inspired by brain anatomy. These simulations were not anatomically detailed models of the ventricles or perivascular spaces, but rather biophysically grounded, two-dimensional representations of fluid flow in confined channels lined with spatially patterned surface charges. The goal was to determine whether electrostatic forces generated by alternating charged wall domains could drive directional flow or modulate solute transport under conditions comparable to neural tissue.

To investigate the effects of surface charge patterning on CSF dynamics, we developed a simplified two-dimensional electrohydrodynamic model based on the lattice Boltzmann method (LBM) for bulk flow and coupled it with electrostatic field calculations derived from the Poisson–Boltzmann equation. Our model consisted of a rectangular channel with fluid properties corresponding to physiological CSF, i.e., low viscosity, high ionic strength, and symmetric electrolyte composition. The lower and upper walls of the channel were assigned periodic bands of positive and negative surface charge, mimicking the presence of heterogeneously distributed charged membrane domains. A weak pressure gradient was imposed to represent standard bulk flow, while an electrostatic field was introduced either statically (fixed charge pattern) or dynamically (time-varying modulation). The coupling between electric field and fluid motion was calculated using a simplified electrohydrodynamic formulation, inspired by the Debye–Hückel approximation and standard Navier–Stokes solutions, including the effect of electrostatic drag near the walls. Domain dimensions were scaled to represent perivascular or ventricular compartments, typically 50–200 μm wide and 500–1000 μm long. The channel walls were assigned alternating bands of positive and negative surface charge densities ($\pm$10 mC/m^2). Electrolyte fluid was modelled as a Newtonian fluid with ionic strength between 1 and 10 mM, permittivity of 80 and dynamic viscosity of 0.7 mPa·s. Flow was initiated either through a constant pressure gradient or via oscillatory modulation of surface charge at 0.1–10 Hz to approximate rhythmic neural activity. Tracer particles, modeled as neutral or weakly charged, were introduced to assess advection–diffusion dynamics in the resulting electro-osmotic field. Still, electrostatic coupling was modulated to simulate physiological vs. pathological ionic conditions, e.g., reduced Debye length and altered ion concentrations. Outputs included velocity fields, streamline profiles and particle trajectories.

Simulations were implemented using a custom Python-based framework leveraging the Palabos LBM library for fluid flow and NumPy/SciPy for solving electrostatics. Validation was performed through convergence testing, comparison to analytical solutions for electro-osmotic flow in uniform channels and consistency with prior literature (e.g., Curk et al. 2024). Simulation data were visualized using Matplotlib and ParaView.

Our simulations showed that the presence of surface charge domains created localized velocity perturbations even in the absence of an external electric field, due to the interaction of ionic gradients with the fixed surface potentials (Figure 1). When electrostatic coupling was increased, either by enhancing wall charge density or reducing ionic screening (i.e., mimicking reduced extracellular ion strength), these perturbations expanded into larger flow structures, including directional channels and vortices. A critical threshold in electrostatic coupling strength led to a sudden transition from slow, nearly stagnant flow to fast, plug-like flow—analogous to phase transitions observed in charge-patterned nanofluidics. This is consistent with

theoretical predictions by Curk et al. (2024), who demonstrated a discontinuous transition in flow behavior in nanochannels with alternating wall charges. Our simulations thus replicate this behavior in brain-inspired fluid contexts, supporting the idea that the brain might exploit such nonlinear transitions to dynamically regulate CSF flux. Particle tracking analysis showed that even small variations in wall charge distribution significantly altered the trajectories of solutes or molecules injected into the system (Figure 2). Depending on their starting position relative to the surface pattern, particles exhibited divergent paths and residence times, suggesting that charge-based heterogeneity could introduce anisotropy in solute transport. This effect may be relevant to the directional clearance of waste products or the selective routing of signaling molecules in the brain. Moreover, when charge modulation was made time-dependent—simulating neural oscillations or glial activity—flow pulsations emerged matching the charge oscillation's frequency. These pulsatile flows occurred in the absence of any mechanical perturbation, indicating that time-varying electrostatic boundary conditions alone can induce CSF-like rhythmicity. To

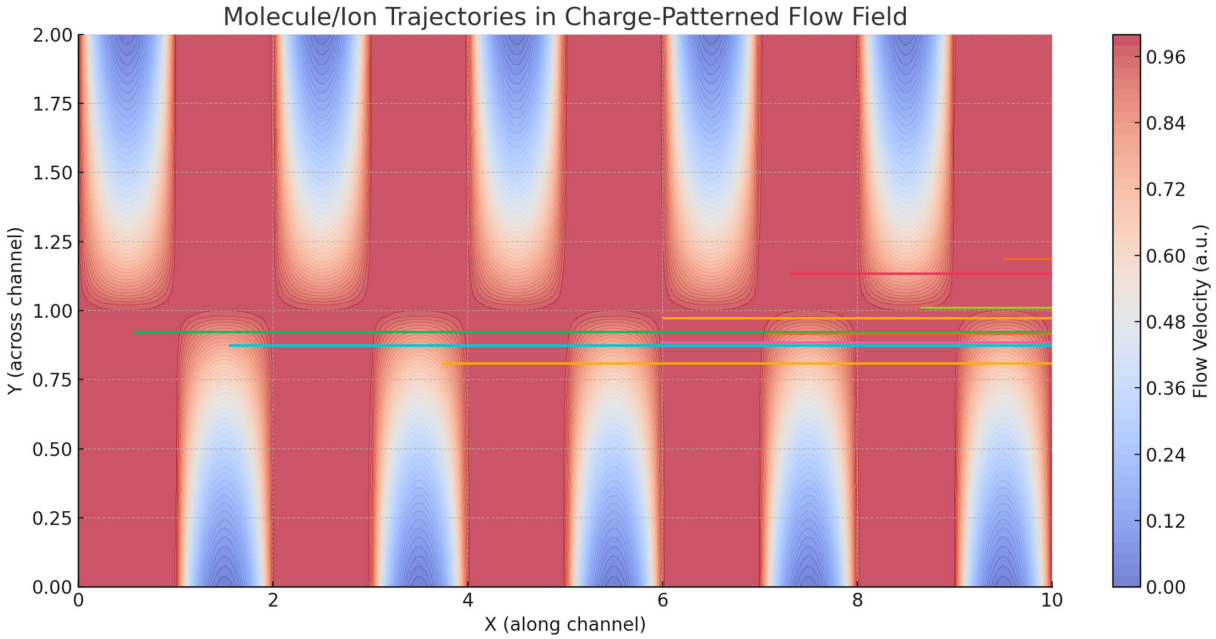

FIGURE 2 | Simulated trajectories of molecules or ions advected through a CSF microchannel bounded by alternating positive and negative surface charge domains on the top and bottom walls. The background color map represents the flow velocity field generated by electrohydrodynamic interactions between the wall charge pattern and ionic content of the fluid. Particle trajectories are shown as colored lines, with green dots indicating starting positions and red dots marking their final locations. Spatial variations in wall charge can lead to nonuniform and trajectory-dependent fluid flow.

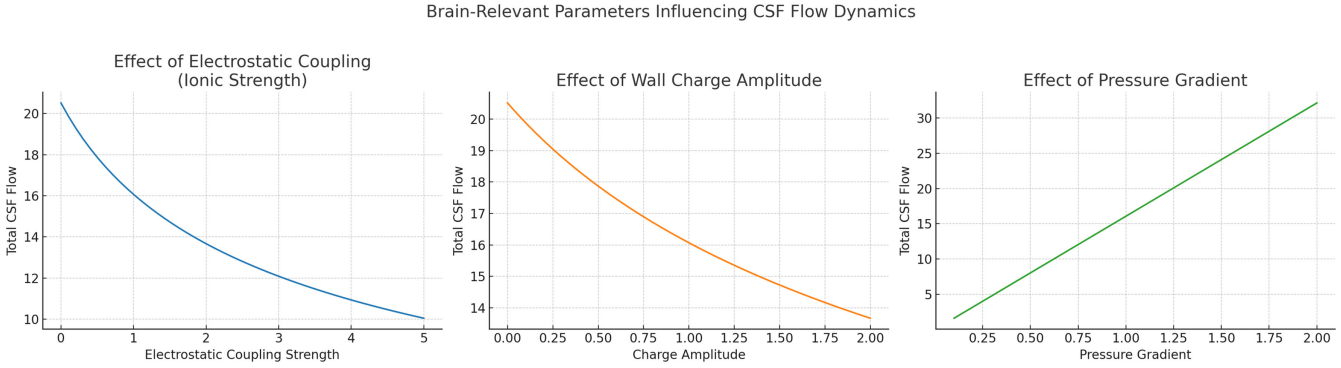

FIGURE 3 | Brain-relevant parameters influencing CSF flow dynamics. Left panel: Effect of electrostatic coupling strength (ionic strength). This plot shows how increasing electrostatic coupling—representing stronger interactions between charged walls and ions in CSF—leads to a progressive reduction in total CSF flow. This mimics physiological and pathological changes in ionic strength, such as elevated extracellular potassium or disrupted ion homeostasis caused by various diseases. Middle panel: Effect of wall charge amplitude. This plot illustrates how variations in the amplitude of patterned surface charge along ventricular or perivascular walls influence CSF flow. Modulation of charge density could arise either from altered astrocytic or ependymal activity or pathological changes in membrane potential and protein expression. The nonlinear flow behavior highlights the potential for bioelectrical gating of fluid dynamics. Right panel. Effect of pressure gradient. This panel shows the linear relationship between the applied pressure gradient and CSF flow rate, modeling physiological drivers such as cardiac and respiratory cycles, as well as pathological changes in intracranial pressure.

investigate the relevance of pathological states, we explored how changes in ionic strength, wall charge amplitude and coupling coefficients affected flow characteristics (Figure 3). Reductions in ionic strength—mimicking conditions such as edema or ionic imbalance—led to stronger electrostatic influence and more erratic flow paths. Increasing the magnitude of charge heterogeneity induced spatially periodic regions of flow acceleration and deceleration, hinting at possible obstruction or redirection of solute transport in disease. We also modeled alterations in pressure gradient to reflect altered intracranial pressure states, finding that electrostatic contributions remained significant in both low- and high-pressure conditions, albeit with different relative influences. Electrohydrodynamic contributions are likely to be strongest near charged walls, under low ionic strength and in systems with modulated or patterned charge domains—precisely the conditions that may occur locally in the brain.

Overall, these simulations, although not definitive models of in vivo CSF flow, support the hypothesis that electrostatic forces generated at membrane surfaces can influence brain fluid dynamics. The convergence of physical principles, biological plausibility and simulation results strongly argues that

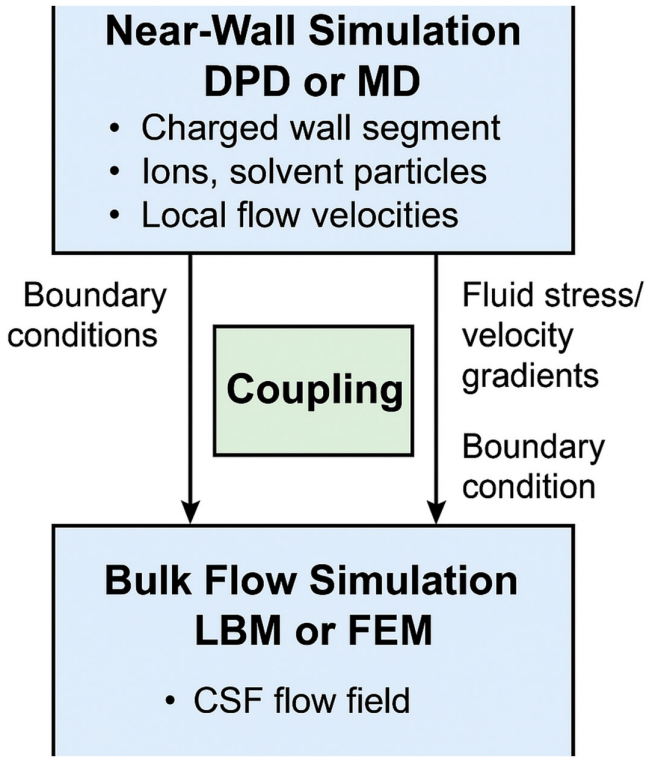

FIGURE 4 | Conceptual workflow of a hybrid computational model combining near-wall and bulk flow simulations to investigate electrohydrodynamic CSF dynamics. The upper section represents the near-wall simulation zone, where dissipative particle dynamics (DPD) or molecular dynamics (MD) are used to capture fine-scale electrostatic interactions, ion layering, and local electro-osmotic effects near charged glial or ependymal surfaces. The lower section depicts the bulk flow simulation domain, modeled using lattice Boltzmann method (LBM) or finite element method (FEM), which handles pressure-driven flow and global CSF transport in anatomically relevant structures. The central coupling interface enables dynamic data exchange between the two regions. Velocity and ionic flux data from the particle-based simulation may inform boundary conditions, macroscopic pressure and shear feedbacks.

electrohydrodynamics may represent an overlooked contributor to neurofluidic regulation.

5 | Conclusion

Our findings support the biophysical hypothesis that the surfaces lining cerebrospinal fluid (CSF) pathways—such as ependymal walls and perivascular boundaries—could actively influence CSF flow through their electrostatic properties. Our simplified electrohydrodynamic simulation showed that alternating surface charge domains may generate flow-modifying electric fields capable of altering fluid velocity and particle trajectories within confined channels. Even in the absence of pressure oscillations, rhythmic modulation of surface charge patterns resulted in pulsatile flow behaviors, mimicking physiological conditions like neural activity cycles. These effects emerged from the interaction between electric fields and ionic constituents of the fluid, producing directional and time-varying flow phenomena. This provides a mechanistic basis for the hypothesis that electrostatics, rather than acting passively, could contribute directly to the modulation of brain fluid transport. Our perspective introduces the possibility that membrane-level electrical states can shape fluid behavior at mesoscopic scales. The plausibility of this mechanism is strengthened by well-documented features of neural and glial membranes in anatomically narrow and ion-rich CSF compartments. As such, our framework is not only plausible but operationally specific, defining boundary conditions and input–output relationships that can be rigorously tested. Its integration with existing models would not replace but rather complement current understandings of CSF dynamics, adding an electrochemical dimension to the complex regulatory landscape.

Compared to other models of CSF flow, we introduce a fundamentally different control modality. Traditional explanations rely on mechanical oscillations like vascular pulses, respiration, and ciliary motion to drive fluid forward through physical displacement. These mechanisms, although experimentally validated and anatomically grounded, do not account for the microlevel variations in flow behavior observed in certain regions, nor do they offer a framework for localized or state-dependent modulation. Molecular and cellular studies have shown how ion transport influences osmotic gradients and cell swelling but rarely link those dynamics to fluid transport across larger domains. Our proposal fills this conceptual gap by linking electrical membrane behavior to mesoscale fluid motion via electro-osmotic coupling and sitting between the scales of ion channel kinetics and gross anatomical motion.

Nonetheless, our hypothesis faces several limitations. Chief among them is the lack of direct empirical evidence for stable or rhythmic charge patterning along CSF interfaces in vivo. While glial and epithelial membranes are known to carry surface charge, it remains unclear whether this charge is organized in spatial domains sufficient to produce significant electro-osmotic flow under physiological conditions. Additionally, the electrical double layer thickness, ion mobility and permittivity in brain tissue are not uniform and could complicate flow generation or assumptions of symmetry. Still, our hypothesis requires a largely passive fluid medium influenced by external electrostatic fields, whereas real CSF movement is likely affected by

an intricate interplay of active transport, convection, diffusion, and tissue deformation. Furthermore, our computational model simplifies boundary conditions and ignores potential feedback loops between membrane depolarization and fluid velocity. The use of simplified rectangular channels, while valuable for isolating key electrohydrodynamic effects, does not fully capture the anatomical complexity of the brain's perivascular and ventricular spaces. Our model lacks the curvature, tortuosity, and heterogeneous porosity characteristic of real perivascular pathways, which are known to influence local flow resistance and solute dispersion. Moreover, our model does not yet incorporate 3D anatomical geometries or dynamic tissue deformation, both of which may affect CSF movement in vivo. Being electrostatic interactions treated in a static manner, our framework does not include dynamic bioelectric feedback such as time-varying membrane potentials, ion channel activity, or electrogenic transport processes. Our hypothesis presents significant challenges, including the in vivo verification of spatially patterned surface charges, the experimental disentanglement of electrostatic and mechanical contributions to fluid movement and the accurate modelling of bidirectional coupling between membrane dynamics and CSF flow. These limitations mean that the model cannot yet account for electromechanical coupling or flow modulation arising from real-time neural activity. Future work should integrate these features to enhance physiological fidelity and explore the nonlinear interplay between electrical signalling, membrane charge dynamics, and fluid transport.

In terms of testable predictions, we expect that artificial modulation of membrane charge—via optogenetic activation of ion pumps, localized application of charged substrates, or genetic manipulation of membrane proteins—should result in measurable changes in local CSF flow velocity or solute transport. This can be explored in microfluidic models using glial or epithelial cell monolayers, where flow fields can be visualized in real time under pharmacological modulation. On the physiological side, one might predict that regions of the brain with higher density of ion-exchanging membranes—such as the ventricular ependyma or perivascular astrocytic endfeet—exhibit enhanced responsiveness to electrostatic perturbation in fluid transport. This could be probed through intracranial injection of tracers under conditions of altered extracellular ion concentration. Our hypothesis opens new directions for interpreting disease. Electrohydrodynamic mechanisms may help explain pathological flow disruptions in conditions such as hydrocephalus, traumatic brain injury and Alzheimer's disease, where ionic imbalance and membrane dysfunction are common. Furthermore, our perspective could inform the design of targeted drug delivery systems or neuromodulation strategies that exploit electrical properties of neural interfaces to influence solute clearance or fluid movement in situ.

These predictions provide a roadmap for targeted experimental studies aimed at validating or refining the model. In exploring electrohydrodynamic mechanisms underlying CSF flow, several simulation approaches are available beyond continuum models. Among the most promising are multiscale and hybrid techniques that integrate molecular and continuum physics. The lattice Boltzmann method (LBM) is particularly suited for simulating microscale fluid flow in complex geometries such as ventricular spaces and perivascular channels, while the finite element method (FEM) excels at solving electrokinetic and fluid dynamics equations in anatomically realistic domains. To resolve nanoscale behavior near surfaces such as ion layering and charge-driven flow, dissipative particle dynamics (DPD) and molecular dynamics (MD) are effective, capturing interactions at atomic and mesoscopic scales. Although each method alone has limitations in scale and scope, a hybrid modeling strategy can address this (Figure 4). By combining DPD or MD to simulate the electrostatic behavior near charged surfaces (e.g., glial or ependymal membranes) with LBM or FEM for bulk CSF flow, the full electrohydrodynamic behavior across scales could be captured. These models may communicate via a coupling interface where information on boundary velocities, electric potentials, or shear stress is exchanged (Hoogerbrugge and Koelman 1992).

In closing, we have introduced the novel hypothesis that patterns of positive and negative surface charge may exist along the inner linings of the brain's CSF channels. If present, these structured electrostatic domains could interact with the ionic nature of CSF to generate localized electric fields capable of driving or modulating flow through electro-osmotic mechanisms. Unlike pressure-driven flow, passive electro-osmosis may offer the potential for directionally controlled, rhythmically responsive and spatially fine-tuned fluid movement. These features align with the need for dynamic regulation in neurophysiological contexts, including sleep–wake cycling and metabolic waste clearance. While our approach remains exploratory, it introduces a coherent theoretical model grounded in experimentally supported biophysics. From this model, we expect to observe conditions under which electrostatic patterning produces detectable effects on CSF flow structure, directionality, and transport efficiency.

Author Contributions

The author performed the following: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical and material support, study supervision.

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The author has nothing to report.

Ethics Statement

This research does not contain any studies with human participants or animals performed by the author.

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Conflicts of Interest

The author declares no conflicts of interest.

Data Availability Statement

All data and materials generated or analyzed during this study are included in the manuscript. The author had full access to all the data in

the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Peer Review

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar, and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

References

Alcaide, D., J. Cacheux, A. Bancaud, R. Muramatsu, and Y. T. Matsunaga. 2023. "Solute Transport in the Brain Tissue: What Are the Key Biophysical Parameters Tying *In Vivo* and *In Vitro* Studies Together?" *Biomaterials Science* 11, no. 11: 3450–3460. https://doi.org/10.1039/D3BM00027C.

Alizadeh, A., W.-L. Hsu, M. Wang, and H. Daiguji. 2021. "Electroosmotic Flow: From Microfluidics to Nanofluidics." *Electrophoresis* 42, no. 3–4: 513–548. https://doi.org/10.1002/elps.202000313.

Ballerini, L., S. McGrory, M. d. C. Valdés Hernández, et al. 2020. "Quantitative Measurements of Enlarged Perivascular Spaces in the Brain Are Associated With Retinal Microvascular Parameters in Older Community-Dwelling Subjects." *Cerebral Circulation - Cognition and Behavior* 1: 100002. https://doi.org/10.1016/j.cccb.2020.100002.

Balood, M., M. Ahmadi, T. Eichwald, et al. 2022. "Nociceptor Neurons Affect Cancer Immunosurveillance." *Nature* 611, no. s: 405–412. https://doi.org/10.1038/s41586-022-05387-4.

Baluška, F., and S. Mancuso. 2019. "Actin Cytoskeleton and Action Potentials: Forgotten Connections." In *The Cytoskeleton*, edited by V. Sahi and F. Baluška, vol. 24, 109–121. Plant Cell Monographs. Springer. https://doi.org/10.1007/978-3-030-33528-1_5.

Banerjee, D., S. Pati, and P. Biswas. 2023. "Analytical Study of Pulsatile Mixed Electroosmotic and Shear-Driven Flow in a Microchannel With a Slip-Dependent Zeta Potential." *Applied Mathematics and Mechanics (English Edition)* 44: 1007–1022. https://doi.org/10.1007/s10483-023-3010-6.

Barron, T., B. Yalçın, M. Su, et al. 2025. "GABAergic Neuron-to-Glioma Synapses in Diffuse Midline Gliomas." *Nature* 639: 1060–1068. https://doi.org/10.1038/s41586-025-07188-6.

Bédard, C., and A. Destexhe. 2022. "Local Field Potentials: Interaction With the Extracellular Medium." In *Encyclopedia of Computational Neuroscience*, edited by D. Jaeger and R. Jung. Springer. https://doi.org/10.1007/978-1-0716-1006-0_720.

Bernal, J., M. D. C. Valdés-Hernández, J. Escudero, et al. 2022. "Assessment of Perivascular Space Filtering Methods Using a Three-Dimensional Computational Model." *Magnetic Resonance Imaging* 93: 33–51. https://doi.org/10.1016/j.mri.2022.07.016.

Burtscher, V., M. Hotka, M. Freissmuth, and W. Sandtner. 2020. "An Electrophysiological Approach to Measure Changes in the Membrane Surface Potential in Real Time." *Biophysical Journal* 118, no. 4: 813–825. https://doi.org/10.1016/j.bpj.2019.06.033.

Choudhury, M. I., Y. Li, P. Mistriotis, et al. 2022. "Kidney Epithelial Cells Are Active Mechano-Biological Fluid Pumps." *Nature Communications* 13, no. 1: 2317. https://doi.org/10.1038/s41467-022-29988-w.

Clarke, D., E. Morley, and D. Robert. 2017. "The Bee, the Flower, and the Electric Field: Electric Ecology and Aerial Electroreception." *Journal of Comparative Physiology A* 203: 737–748. https://doi.org/10.1007/s00359-017-1176-6.

Clarke, D., H. Whitney, G. Sutton, and D. Robert. 2013. "Detection and Learning of Floral Electric Fields by Bumblebees." *Science* 340, no. 6128: 66–69. https://doi.org/10.1126/science.1230883.

Cruz-Garza, J. G., L. S. Bhenderu, K. M. Taghlabi, et al. 2024. "Electrokinetic Convection-Enhanced Delivery for Infusion into the Brain from a Hydrogel Reservoir." *Communications Biology* 7: 869. https://doi.org/10.1038/s42003-024-05788-2.

Curk, T., S. G. Leyva, and I. Pagonabarraga. 2024. "Discontinuous Transition in Electrolyte Flow Through Charge-Patterned Nanochannels." *Physical Review Letters* 133, no. 7: 078201. https://doi.org/10.1103/PhysRevLett.133.078201.

Deeg, C. A., B. Amann, K. Lutz, et al. 2016. "Aquaporin 11, a Regulator of Water Efflux at Retinal Müller Glial Cell Surface, Decreases Concomitant with Immune-Mediated Gliosis." *Journal of Neuroinflammation* 13, no. 89: 12. https://doi.org/10.1186/s12974-016-0544-3.

Deng, S., L. Gan, C. Liu, et al. 2023. "Roles of Ependymal Cells in the Physiology and Pathology of the Central Nervous System." *Aging and Disease* 14, no. 2: 468–483. https://doi.org/10.14336/AD.2022.0826-1.

Dietz, A. G., P. Weikop, N. Hauglund, et al. 2023. "Local Extracellular K^+ in Cortex Regulates Norepinephrine Levels, Network State and Behavioral Output." *Proceedings of the National Academy of Sciences* 120, no. 40: e2305071120. https://doi.org/10.1073/pnas.2305071120.

Elboughdiri, N., K. Javid, M. Q. Shehzad, and Y. Benguerba. 2024. "Influence of Chemical Reaction on Electro-Osmotic Flow of Nanofluid Through Convergent Multi-Sinusoidal Passages." *Case Studies in Thermal Engineering* 54: 103955. https://doi.org/10.1016/j.csite.2023.103955.

England, S. J., and D. Robert. 2024a. "Electrostatic Pollination by Butterflies and Moths." *Journal of the Royal Society Interface* 21, no. 216: 20240156. https://doi.org/10.1098/rsif.2024.0156.

England, S. J., and D. Robert. 2024b. "Prey Can Detect Predators via Electroreception in Air." *Proceedings of the National Academy of Sciences of the United States of America* 121, no. 23: e2322674121. https://doi.org/10.1073/pnas.2322674121.

Faraji, A. H., J. J. Cui, Y. Guy, et al. 2011. "Synthesis and Characterization of a Hydrogel With Controllable Electroosmosis: A Potential Brain Tissue Surrogate for Electrokinetic Transport." *Langmuir* 27, no. 22: 13635–13642. https://doi.org/10.1021/la202198k.

Faraji, A. H., a. S. Jaquins-Gerstl, A. C. Valenta, Y. Ou, and S. G. Weber. 2020. "Electrokinetic Convection-Enhanced Delivery of Solutes to the Brain." *ACS Chemical Neuroscience* 11, no. 14: 2085–2093. https://doi.org/10.1021/acschemneuro.0c00037.

Fujii, Y., S. Maekawa, and M. Morita. 2017. "Astrocyte Calcium Waves Propagate Proximally by Gap Junction and Distally by Extracellular Diffusion of ATP Released From Volume-Regulated Anion Channels." *Scientific Reports* 7: 13115. https://doi.org/10.1038/s41598-017-13243-0.

Gubbiotti, A., M. Baldelli, G. Di Muccio, P. Malgaretti, S. Marbach, and M. Chinappi. 2022. "Electroosmosis in Nanopores: Computational Methods and Technological Applications." *Advances in Physics: X* 7, no. 1: 2036638. https://doi.org/10.1080/23746149.2022.2036638.

Hauglund, N. L., M. Andersen, K. Tokarska, et al. 2025. "Norepinephrine-Mediated Slow Vasomotion Drives Glymphatic Clearance During Sleep." *Cell* 188, no. 3: 606–622. https://doi.org/10.1016/j.cell.2024.12.028.

Hladky, S. B., and M. A. Barrand. 2016. "Fluid and Ion Transfer Across the Blood–Brain and Blood–Cerebrospinal Fluid Barriers: A Comparative Account of Mechanisms and Roles." *Fluids and Barriers of the CNS* 13, no. 1: 19. https://doi.org/10.1186/s12987-016-0040-3.

Hoogerbrugge, P. J., and J. M. V. A. Koelman. 1992. "Simulating Microscopic Hydrodynamic Phenomena With Dissipative Particle Dynamics." *Europhysics Letters* 19, no. 3: 155–160. https://doi.org/10.1209/0295-5075/19/3/001.

Hunting, E. R., L. J. O'Reilly, R. Giles Harrison, S. J. England, B. H. Harris, and D. Robert. 2022. "Observed Electric Charge of Insect Swarms and Their Contribution to Atmospheric Electricity." *iScience* 25, no. 11: 105241. https://doi.org/10.1016/j.isci.2022.105241.

Iliff, J. J., M. Wang, Y. Liao, et al. 2012. "A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β." *Science Translational Medicine* 4, no. 147: 147ra111. https://doi.org/10.1126/scitranslmed.3003748.

Jessen, N. A., A. S. Munk, I. Lundgaard, and M. Nedergaard. 2015. "The Glymphatic System: A Beginner's Guide." *Neurochemical Research* 40, no. 12: 2583–2599. https://doi.org/10.1007/s11064-015-1581-6.

Ji, W., Z. Tang, Y. Chen, et al. 2022. "Ependymal Cilia: Physiology and Role in Hydrocephalus." *Frontiers in Molecular Neuroscience* 15: 927479. https://doi.org/10.3389/fnmol.2022.927479.

Jonusaite, S., and N. Himmerkus. 2024. "Paracellular Barriers: Advances in Assessing Their Contribution to Renal Epithelial Function." *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 298: 111741. https://doi.org/10.1016/j.cbpa.2024.111741.

Kikuchi, K., L. Galera-Laporta, C. Weatherwax, et al. 2022. "Electrochemical Potential Enables Dormant Spores to Integrate Environmental Signals." *Science* 378, no. 6615: 43–49. https://doi.org/10.1126/science.abl7484.

Kwee, R. M., and T. C. Kwee. 2007. "Virchow-Robin Spaces at MR Imaging." *Radiographics* 27, no. 4: 1071–1086. https://doi.org/10.1148/rg.274065722.

Li, M., and M. Muthukumar. 2024. "Electro-Osmotic Flow in Nanoconfinement: Solid-State and Protein Nanopores." *Journal of Chemical Physics* 160, no. 8: 084905. https://doi.org/10.1063/5.0185574.

Liu, J., Y. Guo, C. Zhang, Y. Zeng, Y. Luo, and G. Wang. 2022. "Clearance Systems in the Brain, From Structure to Function." *Frontiers in Cellular Neuroscience* 15: 729706. https://doi.org/10.3389/fncel.2021.729706.

Marques-Almeida, T., C. Ribeiro, I. Irastorza, et al. 2023. "Electroactive Materials Surface Charge Impacts Neuron Viability and Maturation in 2D Cultures." *ACS Applied Materials & Interfaces* 15, no. 26: 31206–31213. https://doi.org/10.1021/acsami.3c04055.

McColgan, T., J. Liu, P. T. Kuokkanen, C. E. Carr, H. Wagner, and R. Kempter. 2017. "Dipolar Extracellular Potentials Generated by Axonal Projections." *eLife* 6: e26106. https://doi.org/10.7554/eLife.26106.

Mestre, H., Y. Mori, and M. Nedergaard. 2020. "The Brain's Glymphatic System: Current Controversies." *Trends in Neurosciences* 43, no. 7: 458–466. https://doi.org/10.1016/j.tins.2020.04.003.

Nishino, M., I. Matsuzaki, F. Y. Musangile, et al. 2020. "Measurement and Visualization of Cell Membrane Surface Charge in Fixed Cultured Cells Related with Cell Morphology." *PLoS ONE* 15, no. 7: e0236373. https://doi.org/10.1371/journal.pone.0236373.

Ortega-Jimenez, V., and R. Dudley. 2013. "Spiderweb Deformation Induced by Electrostatically Charged Insects." *Scientific Reports* 3: 2108. https://doi.org/10.1038/srep02108.

Padmanaban, V., I. Keller, E. S. Seltzer, B. N. Ostendorf, Z. Kerner, and S. F. Tavazoie. 2024. "Neuronal Substance P Drives Metastasis Through an Extracellular RNA–TLR7 Axis." *Nature* 633: 207–215. https://doi.org/10.1038/s41586-024-06893-5.

Raicevic, N., J. M. Forer, A. Ladrón-de-Guevara, et al. 2023. "Sizes and Shapes of Perivascular Spaces Surrounding Murine Pial Arteries." *Fluids and Barriers of the CNS* 20, no. 56: 23. https://doi.org/10.1186/s12987-023-00441-z.

Rasmussen, M. K., H. Mestre, and M. Nedergaard. 2018. "The Glymphatic Pathway in Neurological Disorders." *Lancet Neurology* 17, no. 11: 1016–1024. https://doi.org/10.1016/S1474-4422(18)30318-1.

Rasmussen, R., J. O'Donnell, F. Ding, and M. Nedergaard. 2020. "Interstitial Ions: A Key Regulator of State-Dependent Neural Activity?" *Progress in Neurobiology* 193, no. October 2020: 101802. https://doi.org/10.1016/j.pneurobio.2020.101802.

Ray, L. A., and J. J. Heys. 2019. "Fluid Flow and Mass Transport in Brain Tissue." *Fluids* 4, no. 4: 196. https://doi.org/10.3390/fluids4040196.

Robin, P., T. Emmerich, A. Ismail, et al. 2023. "Long-Term Memory and Synapse-Like Dynamics in Two-Dimensional Nanofluidic Channels." *Science* 379, no. 6628: 161–167. https://doi.org/10.1126/science.adc9931.

Sahib, A. A., I. Bushra, and G. Rejimon. 2021. "Electro-Osmosis: A Review From the Past." In *Problematic Soils and Geoenvironmental Concerns*, edited by M. Latha Gali and P. Raghuveer Rao, vol. 88, 673–687. Lecture Notes in Civil Engineering,. Springer. https://doi.org/10.1007/978-981-15-6237-2_36.

Sanapathi, J., P. Vipparthi, S. Mishra, A. Sosnik, and M. Kumarasamy. 2023. "Microfluidics for Brain Endothelial Cell-Astrocyte Interactions." *Organs-On-A-Chip* 5, no. December 2023: 100033. https://doi.org/10.1016/j.ooc.2023.100033.

Santa-Maria, A. R., F. R. Walter, S. Valkai, et al. 2019. "Lidocaine Turns the Surface Charge of Biological Membranes More Positive and Changes the Permeability of Blood-Brain Barrier Culture Models." *Biochimica et Biophysica Acta (BBA) – Biomembranes 1861* 9: 1579–1591. https://doi.org/10.1016/j.bbamem.2019.07.008.

Sherwood, J. D., M. Mao, and S. Ghosal. 2014. "Electroosmosis in a Finite Cylindrical Pore: Simple Models of End Effects." *Langmuir* 30, no. 31: 9261–9271. https://doi.org/10.1021/la501621r.

Sincomb, S., W. Coenen, A. L. Sánchez, and J. C. Lasheras. 2020. "A Model for the Oscillatory Flow in the Cerebral Aqueduct." *Journal of Fluid Mechanics* 899: R1. https://doi.org/10.1017/jfm.2020.463.

Tumani, H., A. Huss, and F. Bachhuber. 2018. "The Cerebrospinal Fluid and Barriers – Anatomic and Physiologic Considerations." In *Handbook of Clinical Neurology*, edited by M. J. Aminoff, F. Boller, and D. F. Swaab, vol. 146, 21–32. Elsevier. https://doi.org/10.1016/B978-0-12-804279-3.00002-2.

Untiet, V. 2024. "Astrocytic Chloride Regulates Brain Function in Health and Disease." *Cell Calcium* 118, no. 2024: 102855. https://doi.org/10.1016/j.ceca.2024.102855.

Verveniotis, E., A. Kromka, M. Ledinský, et al. 2011. "Guided Assembly of Nanoparticles on Electrostatically Charged Nanocrystalline Diamond Thin Films." *Nanoscale Research Letters* 6, no. 1: 144. https://doi.org/10.1186/1556-276X-6-144.

Wakasugi, R., K. Suzuki, and T. Kaneko-Kawano. 2024. "Molecular Mechanisms Regulating Vascular Endothelial Permeability." *International Journal of Molecular Sciences* 25, no. 12: 6415. https://doi.org/10.3390/ijms25126415.

Walter, F. R., A. R. Santa-Maria, M. Mészáros, S. Veszelka, A. Dér, and M. A. Deli. 2021. "Surface Charge, Glycocalyx and Blood-Brain Barrier Function." *Tissue Barriers* 9, no. 3: 1904773. https://doi.org/10.1080/21688370.2021.1904773.

Wang, M., W. Zhang, and Z. Qi. 2021b. "Platelet Deposition Onto Vascular Wall Regulated by Electrical Signal." *Frontiers in Physiology* 12, no. 2021: 792899. https://doi.org/10.3389/fphys.2021.792899.

Wang, T., S. Kleiven, and X. Li. 2021a. "Electroosmosis Based Novel Treatment Approach for Cerebral Edema." *IEEE Transactions on Biomedical Engineering* 68, no. 9: 2645–2653. https://doi.org/10.1109/TBME.2020.3045916.

Xiang, J., Y. Hua, G. Xi, and R. F. Keep. 2023. "Mechanisms of Cerebrospinal Fluid and Brain Interstitial Fluid Production." *Neurobiology of Disease* 183: 106159. https://doi.org/10.1016/j.nbd.2023.106159.

Yang, H.-C. S., B. Inglis, T. M. Talavage, et al. 2022. "Coupling Between Cerebrovascular Oscillations and CSF Flow Fluctuations During Wakefulness: An fMRI Study." *Journal of Cerebral Blood Flow & Metabolism* 42, no. 6: 1091–1103. https://doi.org/10.1177/0271678X221074639.

Yoshida, K., S. Shi, M. Ukai-Tadenuma, H. Fujishima, R.-i. Ohno, and H. R. Ueda. 2018. "Leak Potassium Channels Regulate Sleep Duration." *Proceedings of the National Academy of Sciences of the United States of America* 115, no. 40: E9459–E9468. https://doi.org/10.1073/pnas.1806486115.

Zhang, M., X. Hu, and L. Wang. 2024. "A Review of Cerebrospinal Fluid Circulation and the Pathogenesis of Congenital Hydrocephalus." *Neurochemical Research* 49, no. 5: 1123–1136. https://doi.org/10.1007/s11064-024-04113-z.

Zhao, F., L. Zhong, and Y. Luo. 2020. "Endothelial Glycocalyx as an Important Factor in Composition of the Blood–Brain Barrier." *CNS Neuroscience & Therapeutics* 26, no. 12: 1205–1214. https://doi.org/10.1111/cns.13560.

Zhou, M., Y. Du, S. Aten, and D. Terman. 2021. "On the Electrical Passivity of Astrocyte Potassium Conductance." *Journal of Neurophysiology* 126, no. 4: 1403–1419. https://doi.org/10.1152/jn.00330.2021.

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Unique lipid signatures in viral envelopes originating from host membranes

Arturo Tozzi*

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA 1155 Union Circle, #311427 Denton, TX 76203-5017 USA

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ABSTRACT

Envelope viruses infecting human individuals can originate from two sources: either from an intermediary animal that transmits the virus to humans, or from another infected human. During a pandemic such as SARS-CoV-2, identifying the intermediate host or the primary human source presents a significant challenge. This complex task is typically addressed through genetics-based approaches, including metagenomic analysis, phylogenetic and phylodynamic rooting methods, integrated with epidemic simulations. We review a method to investigate these primary viral sources. During their replication cycle, envelope viruses hijack materials from host cellular compartments such as endoplasmic reticulum (ER), Golgi apparatus (GA) and ER-Golgi intermediate compartment (ERGIC). Biochemical, morphological and functional differences in the membranes of ER, GA and ERGIC can be detected not only across mammalian species but also among individual humans. These variations arise from a complex interplay of genetic, epigenetic, metabolic, environmental and age-related factors. We review the previous studies demonstrating the relevance of lipidomic profiling in virus-host interaction studies/biomarker discovery and describe how recent advances in analytical technologies—particularly high-resolution mass spectrometry and lipidomics-specific bioinformatics—have dramatically improved our ability to detect and interpret lipidomic data with precision and organelle-level resolution. We propose utilizing lipidomics to identify unique lipid signatures in the compositions of the viral envelopes that are co-opted from the host cell's organelles. Since interspecies and interhuman lipidic differences could significantly impact the composition of viral envelopes derived from host membranes, molecular disparities might serve as critical markers for tracing the source of viral particles. This approach could enable the identification not only of the mammalian sources of human spillover, but also provide insights into the age, medical condition, genetics, and ethnic background of the first human host.

Introduction

Understanding the circumstances that lead to virus outbreaks is critical for deterring future zoonotic pandemics, discovering new drugs, developing vaccines [1–3]. However, this can prove to be a very difficult task, if not almost impossible. To provide an example, the origins of SARS-CoV-2 are still hotly debated [4]. Once established that bats are the natural reservoirs of SARS-related coronaviruses [5], a feverish search has sought to identify the likely source of cases in early reports. It has been suggested that the earliest known COVID-19 cases, geographically centered on the Huanan Seafood Wholesale Market in Wuhan, occurred through the live wildlife trade in China [3], being the result of at least two separate cross-species transmission events into humans [2]. Zoonotic spillovers have been hypothesized from various SARS-CoV-2-susceptible mammals identified as potential intermediate hosts, including civets, bamboo rats, raccoon dogs, pangolins, etc [1,6,7].

From a methodological standpoint, genotypes of potential animal hosts are recovered, analyzed and compared with those from humans and environmental samples [6,8]. The comparison is usually made by using metagenomic and phylogenetic approaches combined with structural modeling, phylodynamic rooting methods and epidemic simulations [2,7]. These approaches establish the genetic foundation for identifying a shortlist of potential intermediate hosts to prioritize for serological and viral sampling [6]. They also help pinpoint the critical time frame between the initial zoonotic spillover into humans and the emergence of the first reported human cases [2].

Here we suggest another method to detect and analyze the origin, spillover, intermediate hosts and human reservoirs in case of enveloped viruses. It has been demonstrated that the morphology of some virions depends heavily on the host environment and lipid composition. For example, in influenza A virions, the matrix protein M1, together with specific host lipids, plays a key role in determining the structural morphology of the assembled virion [9]. Virions isolated from avian hosts tend to be predominantly spherical, while human-adapted strains often display filamentous shapes [10,11]. However, even in humans, certain strains may still produce mainly spherical particles due to differences in viral protein expression and egress routes [12]. These findings suggest that the host cell lipid environment may play a crucial role in shaping

* Corresponding author.
E-mail address: tozziarturo@libero.it

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virion morphology, including the modulation of membrane curvature and budding mechanisms [13].

Instead of relying solely on traditional genetic studies, we propose examining the viral envelopes, which contain phospholipids and proteins derived from host cell membranes. Envelope viruses encompass both DNA and RNA viruses such as Orthomyxoviridae (e.g., influenza virus), Poxviridae (e.g., smallpox virus), and Paramyxoviridae (e.g., human parainfluenza viruses). We will focus on viral families that hijack host cellular compartments such as the endoplasmic reticulum (ER), the Golgi apparatus (GA) or the ER-Golgi intermediate compartment (ERGIC) to ensure their replication cycle, viral assembly, envelope formation, budding and release by exocytosis [14,15]. These families include, among others, Flaviviridae such as Dengue virus and Zika virus that acquire their envelopes during budding through ER [16,17] and Coronaviridae such as SARS-CoV-2 that assemble in ERGIC [18–20]. In many of these viruses, virions are transported to the cell surface following intracellular assembly and are subsequently released through exocytosis.

A virus infecting a human can originate from two sources: either an animal host through spillover, or another infected human. Given the detectable inter-species and inter-individual variations in ER, GA and ERGIC, we contend that the study of viral envelopes could offer valuable clues about the animal source of human spillover and the identity of patient zero, i.e., the first human infected during an outbreak. Specifically, we will focus on the lipid components of the ER and GA that are incorporated into the viral envelope, which can vary depending on the virus's most recent host. We argue that researchers should identify specific host-derived lipidic biomarkers within viral envelopes to provide critical insights into determining the virus's most recent host, whether animal or human.

LIPIDOMIC differences IN golgi apparatus AND endoplasmic reticulum IN animals AND humans

Lipidomics is a rapidly expanding field focused on uncovering the unique lipid profiles of cellular organelles [21]. Recent technological advancements have significantly expanded the capabilities of lipidomics. High-resolution mass spectrometry platforms, such as Orbitrap and time-of-flight (TOF) instruments, allow simultaneous identification and quantification of thousands of lipid species with high sensitivity and specificity [22–27]. The incorporation of stable isotope labeling and machine learning for lipid annotation further enhances the analytical precision and throughput [28,29], making lipidomic fingerprinting of viral envelopes a realistic and powerful approach. Mass spectrometry-based lipidomics can rapidly identify as well as quantify >1000 lipid species at the same time, facilitating robust analyses of lipids in tissues, cells and body fluids [30]. Accordingly, lipidomics is now being widely applied in various fields, including nutrition science to assess food obtained from livestock and poultry as well as fish food products [30,31]. Coupled with ion mobility separation and improved chromatographic techniques, these tools facilitate also organelle-specific lipidomic mapping, even in complex biological samples such as virus-infected cells. Detailed studies have mapped the lipid compositions of various mammalian organelles, including ER and GA [32]. A systematic analysis of the overall variation in the mammalian lipidome, with a particular focus on *Mus musculus*, has been conducted to evaluate the effects of diet, sex, and genotype [33]. The cellular lipidome is highly adaptable, shifting in response to numerous physiological processes such as aging and a range of pathological states [34]. Alterations in the organelles' lipid profiles may lead to disrupted lipid metabolism, persistent inflammation and oxidative stress that have been documented across cancer, metabolic diseases and neurodegenerative disorders. Further, many studies suggest the relevance of lipidomics in virology [35–38]. For instance, Kyle [39] emphasized the potential of lipidomics for uncovering host-virus interactions, particularly in identifying biomarkers and pathogenesis patterns. These works support the application of lipidomic profiling as a forensic virological tool to trace infection origins.

Differences in the lipid composition of the ER and GA have been observed not only across different mammalian species but also among individual humans. In the following two paragraphs, we will explore these variations in more detail.

Lipidomic differences in various animals. Extended lipid profiles of several animal species have been compared via lipidomic analysis conducted by liquid chromatography-high-resolution mass spectrometry, allowing the identification of about a hundred of molecular species of lipids [40]. ER focuses on synthesizing lipids tailored to environmental and metabolic challenges, while GA specializes in modifying and transporting these lipids for specific cellular and systemic functions. Both exhibit species-specific lipid compositions that fulfill critical biological purposes, reflecting adaptations across mammals to genetic factors, environmental conditions, dietary habits, metabolic needs [41]. For instance, humans and rodents share high proportions of phosphatidylcholine and phosphatidylethanolamine essential for membrane structure and cellular signaling [42]. Carnivorous mammals like raccoon dogs have lipidomic compositions tailored to protein-heavy diets, whereas omnivorous and insectivorous species like bats show distinct profiles suited to their high metabolic demands [43,44]. Marine mammals like seals and whales have ER membranes rich in phosphatidylserine and long-chain PUFAs to maintain fluidity under the extreme conditions of cold, high-pressure aquatic environments [45]. In turn, desert mammals like camels display higher concentrations of unsaturated phospholipids to cope with dehydration and heat stress. In primates, cholesterol biosynthesis is optimized for neural and immune functions [46]. Herbivorous species like cows and sheep can efficiently convert cholesterol into bile acids for digesting plant-based diets. By contrast, carnivorous mammals like cats and dogs exhibit less diverse bile acid profiles and simpler cholesterol synthesis pathways, consistent with their protein-rich diets [43].

Marine mammals' ER produce elevated levels of ceramides and sphingomyelins with long-chain bases conferring resistance to salt and temperature fluctuations. Primates and rodents synthesize an array of gangliosides and glycosphingolipids for neural signaling and immune interactions [47–49]. Primates maintain a balance between saturated and unsaturated fatty acids, while rodents favor linoleic acid derivatives [50]. Marine mammals emphasize omega-3 fatty acids such as DHA and eicosapentaenoic acid to adapt to cold environments [51]. Lipid droplet formation in the ER reflects species-specific metabolic needs. Hibernating mammals like bears accumulate triglycerides for energy storage during pre-hibernation, while marine mammals produce blubber lipids characterized by high triglyceride and wax ester content [52].

The lipid composition of the GA is equally diverse and tailored to species-specific functions. The lipid remodeling processes in the GA support specific secretory functions, such as the packaging of milk fat globules in lactating mammals or the production of blubber in marine mammals [53]. Cholesterol transport and modification in the GA are more prominent in herbivores to facilitate bile acid production, while carnivores exhibit simpler pathways [54,55]. Phosphoinositides, particularly phosphatidylinositol and its derivatives, play a vital role in GA vesicle trafficking, with species-specific variations reflecting distinct membrane transport requirements [56]. Also, environmental challenges drive adaptations in GA lipid metabolism to thrive under diverse environmental pressures. Cold-adapted mammals like Arctic foxes produce a higher proportion of unsaturated lipids to maintain vesicle and membrane fluidity in low temperatures [57]. Conversely, desert mammals such as camels synthesize lipids (also in their milk) that resist dehydration and oxidative stress [58].

In sum, lipidomic differences in the ER and GA among mammals demonstrate the remarkable versatility of these organelles. By tailoring their lipid composition and metabolic pathways, the ER and GA meet the unique dietary, ecological, and physiological demands of each species. These variations underscore the evolutionary adaptations that enable mammals to maintain cellular functions crucial for survival in diverse habitats and under varying biological demands.

Lipidomic differences in various human individuals. Lipidomic variations in ER and GA among human individuals arise from interplay of genetic factors, diet, lifestyle, environmental exposures and health conditions. Phosphatidylcholine and phosphatidylethanolamine levels, for instance, are influenced by polymorphisms in the PEMT gene [59]. Diets rich in omega-3 fatty acids contribute to higher levels of docosahexaenoic acid in ER's phospholipids, particularly in individuals consuming fish-heavy diets [60]. Variations in the PTDSS1 gene affect phosphatidylserine levels, which are associated with cognitive and neural functions [61]. Ceramide levels in the ER are elevated in insulin resistance and type 2 diabetes, with genetic variations in DEGS1 playing a key role in modulating ceramide biosynthesis [62]. Diet, particularly high saturated fat intake, contributes to increased sphingomyelin levels. Genetic polymorphisms in the FADS1 and FADS2 genes impact the synthesis of long-chain polyunsaturated fatty acids such as arachidonic acid and DHA, leading to manifold fatty acid profiles [63]. Individuals with obesity or metabolic syndrome often have a higher saturation of ER membrane lipids, which increases susceptibility to ER stress. Cholesterol biosynthesis in the ER varies depending on genetic polymorphisms, such as those in the HMGCR gene [64]. Individuals with familial hypercholesterolemia exhibit altered cholesterol synthesis and handling. Lipid droplets in individuals with high dietary fat intake or metabolic disorders are enriched with triglycerides, an adaptation to chronic ER stress.

GA exhibits pronounced individual variability in lipid composition [47]. Glycosphingolipid profiles vary due to genetic differences, including polymorphisms in the B4GALNT1 gene [65]. These variations impact the synthesis of gangliosides and globosides, which play critical roles in the function of neural and immune cells. Elevated levels of lactosylceramides in individuals with lipid storage diseases or metabolic disorders suggest impaired GA lipid processing. Variability in phosphoinositide metabolism impacts GA vesicle formation and trafficking, while dietary fat intake affects phosphatidylinositol levels and associated signaling pathways.

Differences in ABCG1 activity influence plasma lipid profiles, with altered GA lipid composition observed in individuals with high cholesterol levels [66]. The remodeling of GA lipids for secretion is particularly evident during specialized physiological states such as lactation. Sphingolipid and ceramide trafficking in GA show variability based on CERT gene polymorphisms, leading to differences in sphingomyelin and glycosphingolipid levels [67]. Elevated ceramide levels in inflammatory conditions suggest altered sphingolipid metabolism contributing to individual differences in disease susceptibility. The GA stress response varies across individuals due to genetic differences in GOLPH3, which impact the organelle's ability to manage lipid overload or trafficking defects [68]. GA dysfunctions are particularly evident in individuals with cancer or neurodegenerative diseases. Diet also influence the GA lipid composition and its ability to respond to stress, contributing to variability in immune responses, neural signaling and disease susceptibility.

In summary, lipidomic differences in the ER and GA among human individuals reflect a dynamic interplay of genetic, dietary and environmental factors, as well as health status. These variations influence lipid synthesis, processing and trafficking, impacting a wide range of physiological and pathological processes.

Conclusions

We review a research methodology to assess viral spread and infection sources. Our approach takes advantage of the fact that viruses acquire their envelopes from host organelles like ER, GA and ERGIC. To provide an example, recent lipidomic analyses have elucidated the molecular composition of the SARS-CoV-2 lipid envelope. The virus envelope exhibits exposed phosphatidylethanolamine and phosphatidylserine and is predominantly composed of phospholipids, with minimal cholesterol and sphingolipids [69]. This implies that, despite significant differences between the viral envelope and host cell membranes, the lipid constituents are the same. While it is already established that host lipid composition can influence viral morphology and replication efficiency, our review aims to advance this knowledge by positioning viral lipidomics as a diagnostic and epidemiological tool. We suggest that the lipid profiles of viral envelopes can serve as biosignatures to retrospectively infer the host species or individual from which the virus emerged. This represents a shift from pathogenesis-focused studies toward a source-tracing framework in outbreak investigation.

Biochemical differences among mammals reflect adaptations to diet, metabolism, immune response, stress and environmental demands [70]. Mammals with greater protein synthesis demands like dairy cows and whales upregulate ER chaperones to handle the increased folding load [71]. Carnivorous mammals like cats and dogs exhibit GA adaptations favoring high-protein diets, while hibernating mammals like bears display reduced GA activity to preserve energy. Primates exhibit different chaperone proteins to support the complex glycoproteins associated with their nervous and immune systems [72,73]. Humans possess a unique array of P450 enzymes in the ER, enabling them to adapt to diverse diets and efficiently metabolize drugs [74]. Rodents exhibit simpler glycosylation pathways, while primates synthesize more complex glycans for neural and immune functions [75,76]. These variations influence also interactions with zoonotic viruses, as seen in bats and humans [77]. Bats, with their high metabolic rates, exhibit a robust unfolded protein response (UPR) system to withstand oxidative stress [78]. In contrast, humans and primates rely on sensitive UPR pathways, involving proteins like IRE1, ATF6, and PERK, to mitigate neurodegenerative stress [3,79]. Meanwhile, marine mammals like whales and seals adapt their ER protein-folding machinery for hypoxia tolerance during deep dives. Further, calcium-binding proteins exhibit species-specific differences aligned with metabolic needs [80]. While cheetahs' muscle-specific ER enables rapid calcium cycling for high-speed muscle contractions, diving mammals like dolphins optimize for slower calcium release to conserve energy.

The biochemical composition and function of ER and GA also vary between human individuals due to genetic, environmental and health-related factors. Polymorphisms in genes encoding chaperones and enzymes like glycosyltransferases within ER and GA affect their ability in protein folding and glycosylation patterns, contributing to individual susceptibility to diseases like congenital glycosylation disorders. Diet significantly affects the lipid composition of ER and GA, as fatty acids and carbohydrates required for membrane formation and glycosylation are derived from food. Exposure to toxins, specific drugs or alcohol can further modify their function [81]. Chronic illnesses like diabetes or inflammatory disorders alter glycosylation and protein processing in GA, while neurodegenerative diseases such as Alzheimer's and Parkinson's impair protein folding in ER and glycosylation in GA [82].

The biochemical repertoire of ER and GA also changes with age, reflecting the varying physiological demands tied to growth, metabolic activity and cellular maintenance. In children, ER and GA are highly active to support rapid growth, facilitating the synthesis of proteins essential for tissue formation and contributing to support energy storage and membrane development [83]. In adults, ER and GA shift their focus toward maintaining cellular homeostasis and supporting specialized functions like detoxification, drug processing, enhancing immune defense, enabling antibody function. In the elderly, ER shows a diminished ability to ensure the quality of proteins, leading to an accumulation of misfolded proteins [84]. Altered glycan structures impair the activity and stability of glycoproteins, including antibodies, which compromise immune function. GA dispersion and glycosylation changes in senescent cells further contribute to aging-related dysfunctions and neurodegenerative diseases [85,86]. Additionally, age-related reductions in proteins processing in GA have been linked to storage deficits, particularly in diseases like diabetes [87,88].

In sum, by integrating advanced omics approaches that involve not just lipids, but also other biological molecules, researchers can build a comprehensive framework for understanding how host cellular composition affects viral envelope characteristics.

Several limitations must be acknowledged. Although existing literature supports the idea that host-derived lipid profiles influence viral envelope composition, no systematic datasets currently link specific lipidomic patterns to known hosts across diverse viral families. Still, viruses can modulate their envelope lipid composition through interactions with host proteins, potentially obscuring or modifying the original lipid signature. This could reduce the reliability of using lipid profiles alone to identify the host of origin. The complexity and variability of lipidomes—driven by genetic, metabolic and environmental factors—pose a challenge for creating standardized reference databases that span species and populations. Moreover, the intracellular routes of viral assembly and egress may vary not only between viruses but even between cell types, introducing additional variability. Concerns can be raised regarding the incomplete understanding of viral egress, especially for SARS-CoV-2. While models increasingly support ERGIC involvement, alternative routes such as lysosomal egress remain under investigation. Access to high-quality lipidomic data from diverse host species and infected individuals is limited, particularly in outbreak settings. It could be objected that viral structural proteins actively modulate the lipid composition of their envelopes—for example, by preferentially recruiting certain lipid species to support replication or immune evasion. However, these modulations do not entirely erase the biochemical footprint of the host membrane, particularly when statistical analyses focus on lipid species with low variability across viral strains but high variability across host populations. These constraints highlight the need for interdisciplinary collaborations, technological advances and pilot studies to assess the feasibility and resolution of lipidomics-based viral forensics in real-world scenarios.

This study focuses on lipidomic differences in viral envelopes stemming from their ER and/or GA origins. However, incorporating analyses of other biomolecules could offer a broader understanding. To further investigate unique inter-species and inter-individual biomolecular signatures in ER and GA compositions, proteomics and glycomics analyses could be valuable tools to uncover specific differences in protein and carbohydrate structures. Additionally, transcriptomics and genomics could offer insights into gene expression patterns and structural variations that influence the functions of the ER and GA. Since these differences could significantly impact the composition of viral envelopes derived from host membranes, identifying molecular disparities might provide crucial clues about the origins of infection and the interplay between viral mechanisms and host cellular machinery.

We focused here on the potential to detect the viral source, but our approach might also uncover other significant findings. For instance, during the SARS-CoV-2 pandemic, older individuals experienced more severe COVID-19 symptoms compared to children [89]. Several mechanisms have been suggested to explain the milder clinical syndrome observed in children, including higher pediatric innate interferon responses, increases in naive lymphocytes and depletion of natural killer cells [90]. Since human ERGIC membranes undergo age-related changes, SARS-CoV-2 particles produced in the tissues of children could exhibit phenotypic differences compared to those produced in older individuals. This means that the variations in the ERGIC features of SARS-CoV-2 emerging from human cells of individuals of different ages might contribute to differences in viral load, infectivity and clinical severity. Lastly, we propose another theoretical possibility that merits further exploration. Gaining a deeper understanding of these biochemical differences in ER and GA composition could significantly enhance personalized medicine, paving the way for targeted therapies tailored to individual biochemical profiles.

In conclusion, our review draws together disparate lines of evidence to propose a cohesive model in which viral envelope lipid profiles could serve as retrospective biomarkers for identifying infection sources. This integrative perspective, grounded in recent advances in lipidomics, organelle biology, and host-pathogen interactions, may provide conceptual value through its interdisciplinary synthesis and its potential to guide future research directions.

Ethics approval and consent to participate

This research does not contain any studies with human participants or animals performed by the Author.

Consent for publication

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Availability of data and materials

all data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Declaration of competing interest

The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

CRediT authorship contribution statement

Arturo Tozzi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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References

[1] Li Xiaojun; Elena E. Giorgi, Manukumar Honnayakanahalli Marichannegowda, Brian Foley, Chuan Xiao, et al. 2020. Emergence of SARS-CoV-2 through recombination and strong purifying selection science advances, 1 Jul 2020, Vol 6, Issue 27, DOI: 10.1126/sciadv.abb9153

[2] Jonathan E. Pekar, Andrew Magee, Edyth Parker, Niema Moshiri, Katherine Izhikevich, et al., The molecular epidemiology of multiple zoonotic origins of SARS-CoV-2, Science 377 (6609) (2022) 960–966 26 Jul 2022, doi:10.1126/science.abp8337.

[3] R Luke Wiseman, Mesgarzadeh Jaleh S, Linda M Hendershot, Reshaping endoplasmic reticulum quality control through the unfolded protein response, Rev. Mol. Cell. 82 (8) (2022) 1477–1491 2022 Apr 21, doi:10.1016/j.molcel.2022.03.025.

[4] Zhou, Hong, Xing Chen, Tao Hu, Juan Li, Hao Song, Yanran Liu, Peihan Wang, Di Liu, Jing Yang, Edward C. Holmes, Alice C. Hughes, Yuhai Bi, and Weifeng Shi. A Novel Bat Coronavirus Closely Related to SARS-CoV-2 Contains Natural Insertions at the S1/S2 Cleavage Site of the Spike Protein. Curr. Biol. 30 (11) (2020) 2196–2203.e3. doi:10.1016/j.cub.2020.05.023.

[5] Ping Yu, Ben Hu, Zheng-Li Shi, Jie Cui, Geographical structure of bat SARS-related coronaviruses, Infect. Genet. Evol. 69 (2019) 224–229 2019 Feb 6, doi:10.1016/j.meegid.2019.02.001.

[6] Alexander Crits-Christoph, Joshua I. Levy, Jonathan E. Pekar, Kristian G. Andersen, et al., Genetic tracing of market wildlife and viruses at the epicenter of the COVID-19 pandemic, Cell 187 (2024) Issue 19.

[7] Javier A Jaimes, Nicole M. André, Joshua S. Chappie, Jean K. Millet, Gary R. Whittaker, Phylogenetic analysis and structural modeling of SARS-CoV-2 spike protein reveals an evolutionary distinct and proteolytically sensitive activation loop, J. Mol. Biol. 432 (10) (2020) 3309–3325 1 May 2020.

[8] F. Goodrum, A.C. Lowen, S. Lakdawala, J. Alwine, A. Casadevall, et al., Virology under the microscope-a call for rational discourse, mSphere 8 (2) (2023) e0003423 2023 Apr 20Epub 2023 Jan 26, doi:10.1128/msphere.00034-23.
[9] Petr Chlanda, Oliver Schraidt, Susanne Kummer, James Riches, Helga Oberwinkler, Stephan Prinz, John A.G. Briggs, Structural analysis of the roles of Influenza A Virus membrane-associated proteins in assembly and morphology, J. Virol. 89 (17) (2015) 8957–8966, doi:10.1128/JVI.00592-15.
[10] Svetlana Bourmakina, Adolfo Garcia-Sastre, Reverse genetics studies on the filamentous morphology of Influenza A virus, J. Gen. Virol. 84 (3) (2003) 517–527, doi:10.1099/vir.0.18813-0.
[11] Takeshi. Noda, Native morphology of influenza virions, Front. Microbiol. 2 (2012) 269, doi:10.3389/fmicb.2011.00269.
[12] Jeremy S. Rossman, Robert A. Lamb, Influenza virus assembly and budding, Virology 411 (2) (2011) 229–236, doi:10.1016/j.virol.2010.12.003.
[13] George P. Leser, Robert A. Lamb, Influenza virus assembly and budding in raft-derived microdomains: a quantitative analysis of the surface distribution of HA, NA, and M2 proteins, J. Virol. 79 (21) (2005) 13366–13377, doi:10.1128/JVI.79.21.13366-13377.2005.
[14] Y. Chen, Q.1. Liu, D. Guo, Emerging coronaviruses: genome structure, replication, and pathogenesis, J. Med. Virol. 92 (4) (2020) 418–423 2020 Apr, doi:10.1002/jmv.25681.
[15] C. Risco, J.R. Rodríguez, C. López-Iglesias, J.L. Carrascosa, M. Esteban, D. Rodríguez, Endoplasmic reticulum-Golgi intermediate compartment membranes and vimentin filaments participate in vaccinia virus assembly, J. Virol. 76 (4) (2002) 1839–1855.
[16] Biswadeep Das, Samal Sagnika, Hamdi Hamida, Aditi Pal, Arpita Biswas, et al., Role of endoplasmic reticulum stress-related unfolded protein response and its implications in dengue virus infection for biomarker development, Rev. Life Sci. 329 (2023) 121982 2023 Sep 15Epub 2023 Jul 28, doi:10.1016/j.lfs.2023.121982.
[17] Ming Yuan Li, Grandadam Marc, Kevin Kwok, Thibault Lagache, Yu Lam Siu, et al., KDEL receptors assist dengue virus exit from the endoplasmic reticulum, Cell Rep. 10 (9) (2015) 1496–1507 2015 Mar 10Epub 2015 Mar 5, doi:10.1016/j.celrep.2015.02.021.
[18] D.A. Brian, R.S. Baric, Coronavirus genome structure and replication, in: L. Enjuanes (Ed.), Coronavirus Replication and Reverse Genetics. Current Topics in Microbiology and Immunology, Coronavirus Replication and Reverse Genetics. Current Topics in Microbiology and Immunology, 287, Springer, Berlin, Heidelberg, 2005.
[19] Katharina M Scherer, Luca Mascheroni, George W Carnell, Lucia C S Wunderlich, Stanislaw Makarchuk, et al., SARS-CoV-2 nucleocapsid protein adheres to replication organelles before viral assembly at the Golgi/ERGIC and lysosome-mediated egress, Sci. Adv. 8 (1) (2022) eabl4895 2022 Jan 7, doi:10.1126/sciadv.abl4895.
[20] B. Boson, V. Legros, B. Zhou, E. Siret, C. Mathieu, F.-L. Cosset, D. Lavillette, D. Solène, The SARS-CoV-2 Envelope and Membrane Proteins Modulate Maturation and Retention of the Spike Protein, Allowing Assembly of Virus-Like Particles, J. Biol. Chem. 296 (2021) 100111, doi:10.1074/jbc.RA120.016175.
[21] Jessica Symons, Cho Kwang-Jin, Jeffrey Chang, Guangwei Du, M Neal Waxham, et al., Lipidomic atlas of mammalian cell membranes reveals hierarchical variation induced by culture conditions, subcellular membranes, and cell lineages, Soft. Matter. 17 (2) (2021) 288–297 2021 Jan 22, doi:10.1039/d0sm00404a.
[22] H. Liu, T. Hui, X. Zheng, S. Li, X. Wei, P. Li, D. Zhang, Z. Wang, Characterization of key lipids for binding and generating aroma compounds in roasted mutton by UPLC-ESI-MS/MS and Orbitrap Exploris GC, Food Chem. 374 (2022) 131723, doi:10.1016/j.foodchem.2021.131723.
[23] Y. Liu, M. Chen, Y. Li, X. Feng, Y. Chen, L. Lin, Analysis of lipids in green coffee by ultra-performance liquid chromatography-time-of-flight tandem mass spectrometry, Molecules 27 (16) (2022) 5271, doi:10.3390/molecules27165271.
[24] Y. Meng, N. Qiu, V. Guyonnet, R. Keast, C. Zhu, Y. Mine, UHPLC-Q-orbitrap-based untargeted lipidomics reveals the variation of yolk lipids during egg storage, J. Sci. Food Agric. 102 (13) (2022) 5690–5699, doi:10.1002/jsfa.11916.
[25] Y. Xie, B. Wu, Z. Wu, X. Tu, S. Xu, X. Lv, H. Yin, J. Xiang, H. Chen, F. Wei, Ultrasound-assisted one-phase solvent extraction coupled with liquid chromatography-quadrupole time-of-flight mass spectrometry for efficient profiling of egg yolk lipids, Food Chem. 319 (2020) 126547, doi:10.1016/j.foodchem.2020.126547.
[26] J. Rejšek, V. Vrkoslav, A. Vaikkinen, M. Haapala, T.J. Kauppila, R. Kostiainen, J. Cvačka, Thin-layer chromatography/desorption atmospheric pressure photoionization orbitrap mass spectrometry of lipids, Anal. Chem. 88 (24) (2016) 12279–12286, doi:10.1021/acs.analchem.6b03465.
[27] V. Wewer, I. Dombrink, K. vom Dorp, P. Dörmann, Quantification of sterol lipids in plants by quadrupole time-of-flight mass spectrometry, J. Lipid Res. 52 (5) (2011) 1039–1054, doi:10.1194/jlr.D013987.
[28] R. Takahashi, S. Fujioka, T. Oe, S.H. Lee, Stable isotope labeling by fatty acids in cell culture (SILFAC) coupled with isotope pattern dependent mass spectrometry for global screening of lipid hydroperoxide-mediated protein modifications, J. Proteomics. 166 (2017) 101–114, doi:10.1016/j.jprot.2017.07.006.
[29] L.V. Tose, C.E. Ramirez, V. Michalkova, M. Nouzova, F.G. Noriega, F. Fernandez-Lima, Coupling stable isotope labeling and liquid chromatography-trapped ion mobility spectrometry-time-of-flight-tandem mass spectrometry for De Novo Mosquito ovarian lipid studies, Anal. Chem. 94 (16) (2022) 6139–6145, doi:10.1021/acs.analchem.1c05090.
[30] Yinghua Song, Cai Changyun, Song Yingzi, Sun Xue, Baoxiu Liu, et al., A comprehensive review of lipidomics and its application to assess food obtained from farm animals, Rev. Food Sci. Anim. Resour. 42 (1) (2022) 1–17 2022 Jan, doi:10.5851/kosfa.2021.e59.
[31] Putri Widyanti Harlina, Vevi Maritha, Fang Geng, Edy Subroto, Tri Yuliana, et al., Lipidomics: a comprehensive review in navigating the functional quality of animal and fish products, Int. J. Food Propert. Vol. 26 (2) (2023) 3115–3136 2023, doi:10.1080/10942912.2023.2252622.
[32] M.J. Sarmento, A. Llorente, T. Petan, et al., The expanding organelle lipidomes: current knowledge and challenges, Cell Mol. Life Sci. 80 (2023) 237, doi:10.1007/s00018-023-04889-3.
[33] M.A. Surma, M.J. Gerl, R. Herzog, et al., Mouse lipidomics reveals inherent flexibility of a mammalian lipidome, Sci. Rep. 11 (2021) (2021) 19364, doi:10.1038/s41598-021-98702-5.
[34] Gizem Onal, Ozlem Kutlu, Devrim Gozuacik, Serap Dokmeci Emre, Rev. Lipids Health Dis. 16 (1) (2017) 128 2017 Jun 29, doi:10.1186/s12944-017-0521-7.
[35] S.E. Farley, J.E. Kyle, H.C. Leier, et al., A global lipid map reveals host dependency factors conserved across SARS-CoV-2 variants, Nat. Commun. 13 (2022) 3487, doi:10.1038/s41467-022-31097-7.
[36] E. Porter, J.-C. Saiz, J.T. Nickels Jr, Editorial: lipids in host microbe interaction, Front. Cell Infect. Microbiol. 12 (2022) 1002856, doi:10.3389/fcimb.2022.1002856.
[37] Y. Wang, Y. Zhang, Y. Hu, The roles of lipids in SARS-CoV-2 viral replication and the host immune response, J. Lipid Res. 62 (2021) 100111, doi:10.1016/j.jlr.2021.100111.
[38] M.R. Wenk, Lipidomics of host–Pathogen interactions, FEBS Lett. 580 (23) (2006) 5541–5551, doi:10.1016/j.febslet.2006.07.007.
[39] J.E. Kyle, Lipidomics: advancing the understanding of host–virus interactions, Expert. Rev. Proteomics. 18 (4) (2021) 329–332, doi:10.1080/14789450.2021.1901682.
[40] Z. Kaabia, J. Poirier, M. Moughaizel, et al., Plasma lipidomic analysis reveals strong similarities between lipid fingerprints in human, hamster and mouse compared to other animal species, Sci. Rep. 8 (2018) (2018) 15893, doi:10.1038/s41598-018-34329-3.
[41] Giusy Di Conza, Tsai Chin-Hsien, Gallart-Ayala Hector, Yu Yi-Ru, Franco Fabien, et al., Tumor-induced reshuffling of lipid composition on the endoplasmic reticulum membrane sustains macrophage survival and pro-tumorigenic activity, Nat. Immunol. 22 (11) (2021) 1403–1415 2021 Nov, doi:10.1038/s41590-021-01047-4.
[42] Adamson, E. Samantha, Adak Sangeeta, Petersen Max C, Higgins Dustin, D Spears Larry, et al., Decreased sarcoplasmic reticulum phospholipids in human skeletal muscle are associated with metabolic syndrome, J. Lipid Res. 65 (3) (2024) 100519 2024 MarEpub 2024 Feb 13, doi:10.1016/j.jlr.2024.100519.
[43] Panagiotis G Xenoulis, J Cammarata Paul, Rosemary L Walzem, Jan S Suchodolski, Jörg M Steiner, Serum triglyceride and cholesterol concentrations and lipoprotein profiles in dogs with naturally occurring pancreatitis and healthy control dogs, J. Vet. Intern. Med. 34 (2) (2020) 644–652 2020 Mar, doi:10.1111/jvim.15715.
[44] S. Takatsuki, M. Inaba, Food Habits of Raccoon Dogs at an Agricultural Area in Shikoku, Western Japan, Zool. Sci. 41 (2) (2024) 185–191, doi:10.2108/zs230051.
[45] C. Fayolle, C. Leray, P. Ohlmann, G. Gutbier, J.P. Cazenave, C. Gachet, R. Groscolas, Lipid composition of blood platelets and erythrocytes of southern elephant seal (Mirounga leonina) and antarctic fur seal (Arctocephalus gazella), Comp. Biochem. Physiol. B Biochem. Mol. Biol. 126 (1) (2000) 39–47 2000 May, doi:10.1016/s0305-0491(00)00175-9.
[46] S. Zio, B. Tarnagda, F. Tapsoba, C. Zongo, A. Savadogo, Health Interest of Cholesterol and Phytosterols and Their Contribution to One Health Approach: Review, Heliyon 10 (21) (2024) e40132, doi:10.1016/j.heliyon.2024.e40132.
[47] Agliarulo, Ilenia Seetharaman Parashuraman, Golgi Apparatus regulates plasma membrane composition and function, Rev. Cells 11 (3) (2022) 368 2022 Jan 22, doi:10.3390/cells11030368.
[48] Allende, Maria Laura, Richard L Proia, Simplifying complexity: genetically resculpting glycosphingolipid synthesis pathways in mice to reveal function, Glycoconj. J. 31 (9) (2014) 613–622 2014 Dec, doi:10.1007/s10719-014-9563-5.
[49] Toshihide. Kobayashi, Mapping trasmembrane distribution of sphingomyelin, Emerg. Top. Life Sci. 7 (1) (2023) 31–45 2023 Mar 31, doi:10.1042/ETLS20220086.
[50] Burr, D. Stephanie, Yongbin Chen, Christopher P Hartley, Xianda Zhao, Jun Liu, Replacement of saturated fatty acids with linoleic acid in western diet attenuates atherosclerosis in a mouse model with inducible ablation of hepatic LDL receptor, Sci. Rep. 13 (1) (2023) 16832 2023 Oct 6, doi:10.1038/s41598-023-44030-9.
[51] Nikolay Yudin, Denis M Larkin, Elena V Ignatieva, A compendium and functional characterization of mammalian genes involved in adaptation to Arctic or Antarctic environments, BMC. Genet. 18 (Suppl 1) (2017) 111 2017 Dec 28, doi:10.1186/s12863-017-0580-9.
[52] R.A. Nelson, R A. Protein and fat metabolism in hibernating bears, Rev. Fed. Proc. 39 (12) (1980) 2955–2958 1980 Oct.
[53] Wenting Dai, Robin White, Jianxin Liu, Hongyun Liu, Organelles coordinate milk production and secretion during lactation: insights into mammary pathologies, Prog. Lipid Res. 86 (2022) 101159 2022 Apr, doi:10.1016/j.plipres.2022.101159.
[54] J.F. Hocquette, D. Bauchart, Intestinal absorption, blood transport and hepatic and muscle metabolism of fatty acids in preruminant and ruminant animals, Reprod. Nutr. Dev. 39 (1) (1999) 27–48 1999 Jan-Feb, doi:10.1051/rnd:19990102.
[55] Bruno Stieger, Julia Steiger, Noemi Jiménez, Isabelle Riezman, Howard Riezman, Subcellular distribution of cholesterol and sphingolipids in rat hepatocytes, FASEB J. 32 (1_supplement) (2018) 541 April1, doi:10.1096/fasebj.2018.32.1_supplement.541.1.
[56] Giovanni D'Angelo, Mariella Vicinanza, Cathal Wilson, Maria Antonietta De Matteis. 2012. Phosphoinositides in Golgi complex function, SubCell Biochem. 59 (2012) 255–270, doi:10.1007/978-94-007-3015-1_8.
[57] Sławomir Nowicki, Piotr Przysiecki, Aneta Filistowicz, Andrzej Potkański, Andrzej Filistowicz, Jacek Wójtowski, The effect of different fat sources in the diet on the composition of adipose tissue in arctic foxes (Alopex lagopus L.), Folia Biol. 62 (2) (2014) 127–133 2014, doi:10.3409/fb62_2.127.
[58] A.M. Gorban, O.M. Izzeldin, Fatty acids and lipids of camel milk and colostrum, Int. J. Food Sci. Nutr. 52 (3) (2001) 283–287 2001 May, doi:10.1080/713671778.
[59] Chang Sun, David J F Holstein, Natalia Garcia-Cubero, Yusef Moulla, Christine Stroh 3, et al., The role of phosphatidylethanolamine N-methyltransferase (PEMT)

and its waist-hip-ratio-associated locus rs4646404 in obesity-related metabolic traits and liver disease, Int. J. Mol. Sci. 24 (23) (2023) 16850 2023 Nov 28, doi:10.3390/ijms242316850.
[60] Kazuo. Yamagata, Dietary docosahexaenoic acid inhibits neurodegeneration and prevents stroke, J. Neurosci. Res. 99 (2) (2021) 561–572 2021 Feb, doi:10.1002/jnr.24728.
[61] Tao Long, Dongyu Li, Vale Goncalo, Jiang Yaoyukun, Philip Schmiege, et al., Molecular insights into human phosphatidylserine synthase 1 reveal its inhibition promotes LDL uptake, Cell 187 (20) (2024) 5665–5678 2024 Oct 3e18Epub 2024 Aug 28, doi:10.1016/j.cell.2024.08.004.
[62] Nicholas B Blackburn, Laura F Michael, Peter J Meikle, Juan M Peralta, Marian Mosior, et al., Rare DEGS1 variant significantly alters de novo ceramide synthesis pathway, J. Lipid Res. 60 (9) (2019) 1630–1639 2019 Sep, doi:10.1194/jlr.P094433.
[63] Berthold Koletzko, Eva Reischl, Conny Tanjung, Ines Gonzalez-Casanova, Usha Ramakrishnan, et al., FADS1 and FADS2 polymorphisms modulate fatty acid metabolism and dietary impact on health, Annu. Rev. Nutr. 39 (2019) 21–44 2019 Aug 21, doi:10.1146/annurev-nutr-082018-124250.
[64] Benedetta Perrone, Paola Ruffo, Giuseppina Augimeri, Diego Sisci, Maria Stefania Sinicropi, et al., Association between HMGCR, CRP, and CETP gene polymorphisms and metabolic/inflammatory serum profile in healthy adolescents, J. Transl. Med. 21 (1) (2023) 718 2023 Oct 13, doi:10.1186/s12967-023-04571-z.
[65] Simonetta Sipione, John Monyror, Danny Galleguillos, Noam Steinberg, Vaibhavi Kadam, Gangliosides in the brain: physiology, pathophysiology and therapeutic applications, Front. Neurosci. 14 (2020) 572965 2020 Oct 6, doi:10.3389/fnins.2020.572965.
[66] Michinori. Matsuo, ABCA1 and ABCG1 as potential therapeutic targets for the prevention of atherosclerosis, J. Pharmacol. Sci. 148 (2) (2022) 197–203 2022 FebEpub 2021 Dec 1, doi:10.1016/j.jphs.2021.11.005.
[67] Tao Zhang, Antonius A de Waard 2 3, Wuhrer Manfred 1, Robbert M Spaapen, The role of glycosphingolipids in immune cell functions, Front. Immunol. 10 (2019) 90 2019 Jan 29eCollection 2019, doi:10.3389/fimmu.2019.00090.
[68] Kuna, S. Ramya, J Field Seth, GOLPH3: a Golgi phosphatidylinositol(4)phosphate effector that directs vesicle trafficking and drives cancer, J. Lipid Res. 60 (2) (2019) 269–275 2019 Feb, doi:10.1194/jlr.R088328.
[69] Zack Saud, J Tyrrell Andreas Zaragkoulias Victoria, Majd B Protty, Statkute Evelina, et al., The SARS-CoV2 envelope differs from host cells, exposes procoagulant lipids, and is disrupted in vivo by oral rinses, J. Lipid Res. 63 (6) (2022) 100208 2022 Apr 15, doi:10.1016/j.jlr.2022.100208.
[70] David B. Melville, Sean Studer, Randy Schekman, Small sequence variations between two mammalian paralogs of the Small GTPase SAR1 underlie functional differences in coat protein Complex II assembly, Membrane Biology 295 (25) (2020) 8401–8412 June, doi:10.1016/j.jmb.2020.03.002.
[71] Kristine Faye R Pobre, J Poet Greg, M Hendershot Linda, The endoplasmic reticulum (ER) chaperone BiP is a master regulator of ER functions: getting by with a little help from ERdj friends, Rev. J. Biol. Chem. 294 (6) (2019) 2098–2108 2019 Feb 8, doi:10.1074/jbc.REV118.002804.
[72] K. Nakamura, A. Zuppini, S. Arnaudeau, J. Lynch, I. Ahsan, et al., Functional specialization of calreticulin domains, J. Cell Biol. 154 (5) (2001) 961–972 2001 Sep 3Epub 2001 Aug 27, doi:10.1083/jcb.200102073.
[73] Magdalena Wisniewska, Tobias Karlberg, Lari Lehtiö, Ida Johansson, Tetyana Kotenyova, et al., Crystal structures of the ATPase domains of four human Hsp70 isoforms: hSPA1L/Hsp70-hom, HSPA2/Hsp70-2, HSPA6/Hsp70B', and HSPA5/BiP/GRP78, PLoS. One 5 (1) (2010) e8625 Jan 11, doi:10.1371/journal.pone.0008625.
[74] Gorina, S. Svetlana, M Iljina Tatiana, S Mukhtarova Lucia, Y Toporkova Yana, N Grechkin Alexander, Detection of unprecedented CYP74 enzyme in mammal: hydroperoxide lyase CYP74C44 of the Bat Sturnira hondurensis, Int. J. Mol. Sci. 23 (14) (2022) 8009 2022 Jul 20, doi:10.3390/ijms23148009.
[75] Natalia Cherepanova, Shiteshu Shrimal, Reid Gilmore, N-linked glycosylation and homeostasis of the endoplasmic reticulum, Rev. Curr. Opin. Cell Biol. 41 (2016) 57–65 2016 Aug, doi:10.1016/j.ceb.2016.03.021.
[76] Y. Li, A.H. Tran, S.J. Danishefsky, Z. Tan, Chapter Twelve - Chemical Biology of Glycoproteins: From Chemical Synthesis to Biological Impact, Methods Enzymol. 621 (2019) 213–229, doi:10.1016/bs.mie.2019.02.030.
[77] Kathleen Voigt, Markus Hoffmann, Jan Felix Drexler, Marcel Alexander Müller, Christian Drosten, et al., Fusogenicity of the Ghana Virus (Henipavirus: ghanaian bat henipavirus) Fusion Protein is controlled by the cytoplasmic domain of the attachment glycoprotein, Viruses 11 (9) (2019) 800 2019 Aug 29, doi:10.3390/v11090800.
[78] Wenjie Huang, Chen-Chung Liao, Yijie Han, Junyan Lv, Ming Lei, et al., Co-activation of akt, Nrf2, and NF-κb signals under UPRER in torpid Myotis ricketti bats for survival, Commun. Biol. 3 (1) (2020) 658 2020 Nov 11, doi:10.1038/s42003-020-01378-2.
[79] S.R. Chadwick, P. Lajoie, Endoplasmic reticulum stress coping mechanisms and lifespan regulation in health and diseases, Front. Cell Dev. Biol. 7 (2019) 84, doi:10.3389/fcell.2019.00084.
[80] Marek. Michalak, Calreticulin: endoplasmic reticulum Ca2+ gatekeeper, J. Cell Mol. Med. 28 (5) (2024) e17839 2024 Mar, doi:10.1111/jcmm.17839.
[81] C. Shen, X. Chen, Y. Cao, Y. Du, X. Xu, Q. Wu, L. Lin, Y. Qin, R. Meng, L. Gan, J. Zhang, Alpha-lipoic acid protects against chronic alcohol consumption-induced cardiac damage by the Aldehyde dehydrogenase 2-associated PINK/Parkin Pathway, J. Cardiovasc. Pharmacol. 82 (5) (2023) 407–418 2023 Nov 1, doi:10.1097/FJC.0000000000001480.
[82] Jason Gandhi, Anthony C Antonelli, Adil Afridi, Sohrab Vatsia, Gunjan Joshi, et al., Protein misfolding and aggregation in neurodegenerative diseases: a review of pathogeneses, novel detection strategies, and potential therapeutics, Rev. Neurosci. 30 (4) (2019) 339–358 2019 May 27, doi:10.1515/revneuro-2016-0035.
[83] L. Grevendonk, N.J. Connell, C. McCrum, C.E. Fealy, L. Bilet, et al., Impact of aging and exercise on skeletal muscle mitochondrial capacity, energy metabolism, and physical function, Nat. Commun. 12 (1) (2021) 4773 2021 Aug 6, doi:10.1038/s41467-021-24956-2.
[84] F Ulrich Hartl, Protein misfolding diseases, Annu. Rev. Biochem. 86 (2017) 21–26 2017 Jun 20Epub 2017 Apr 24, doi:10.1146/annurev-biochem-061516-044518.
[85] Kristian. Prydz, Determinants of glycosaminoglycan (GAG) structure, Biomolecules 5 (3) (2015) 2003–2022, doi:10.3390/biom5032003.
[86] M. Udono, K. Fujii, G. Harada, Y. Tsuzuki, K. Kadooka, et al., Impaired ATP6V0A2 expression contributes to Golgi dispersion and glycosylation changes in senescent cells, Sci. Rep. 5 (2015) 17342.
[87] M. Calvo-Rodríguez, M. García-Durillo, C. Villalobos, L. Núñez, In vitro aging promotes endoplasmic reticulum (ER)-mitochondria Ca 2+ cross talk and loss of store-operated Ca 2+ entry (SOCE) in rat hippocampal neurons, Biochim. Biophys. Acta 1863 (11) (2016) 2637–2649, doi:10.1016/j.bbamcr.2016.08.001.
[88] J. Janikiewicz, J. Szymański, D. Malinska, P. Patalas-Krawczyk, B. Michalska, et al., Mitochondria-associated membranes in aging and senescence: structure, function, and dynamics, Cell Death. Dis. 9 (3) (2018) 332, doi:10.1038/s41419-017-0105-5.
[89] N. Parri, M. Lenge, D. Buonsenso, Children with Covid-19 in pediatric emergency departments in Italy, NEJM (2020), doi:10.1056/NEJMc2007617.
[90] M. Yoshida, K.B. Worlock, N. Huang, et al., Local and systemic responses to SARS-CoV-2 infection in children and adults, Nature 602 (2022) (2022) 321–327, doi:10.1038/s41586-021-04345-x.

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An Extended Stokes' Theorem for Spiral Paths: Applications to Rotational Flows in Trachelospermum Jasminoides Stems and Flowers

Arturo Tozzi*

Abstract

The traditional Stokes' theorem connects the macroscopic circulation along a closed boundary to the microscopic circulation across the surface it encloses. However, it proves inadequate for addressing complex geometries such as helicoidal paths, non-planar flow patterns and dynamic systems with open boundaries. We introduce an extension of Stokes' theorem (EST) that provides a robust tool for interdisciplinary research in spiral/helicoidal dynamics, facilitating the evaluation of rotational forces and circulation in both natural and engineered systems with open boundaries. We apply EST to model the rotational dynamics of flower petals and the helical forces within the stems of *Trachelospermum jasminoides*, known as star jasmine. For the flower, we demonstrate the equivalence between the line integral along the petal boundary and the surface integral over the enclosed disk, effectively capturing the uniform rotational stress generated by tangential forces. EST enables the analysis of external factors such as wind or pollinator interactions, while providing valuable insights to deepen our understanding of floral mechanics and petal growth patterns. For the stem, linking microscopic circulatory forces to macroscopic flow patterns, we demonstrate the interaction of torsional and bending stresses caused by the helical geometry. This finding has significant implications for understanding plant growth biomechanics and structural stability as well as for quantifying nutrient and water transport within stems, where spiral dynamics play a pivotal role. In summary, EST streamlines the analysis of rotational and translational forces in systems governed by spiral and helicoidal dynamics, including physical and biological phenomena such as phyllotaxis and plant growth.

Keywords: Helical dynamics; Boundary analysis; Vector field integration; Flow topology, Plant growth; Stokes' theorem; *Trachelospermum jasminoides*

Introduction

Stokes' theorem (henceforward ST) is a fundamental principle of vector calculus that bridges the macroscopic circulation along a closed boundary with the microscopic circulation over the enclosed surface [1,2]. Extending the principles of Green's theorem (GT) which applies to two-dimensional regions, ST provides a powerful framework for analyzing flows and circulations in three-dimensional spaces, uncovering profound connections between the local properties of vector fields and their global behaviour. GT and ST are effective tools for solving problems related to physical closed systems with clearly defined boundaries such as airflow circulation around wings, electromagnetic fields in circuits, surface heat flux, Coriolis-driven hemispherical flows, Earth's deep interior dynamics [3-10]. In biology,

Affiliation:
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA

***Corresponding author:**
Arturo Tozzi, Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA.

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the two theorems contribute to understanding blood flow, electrical activity of the brain, growth patterns in ecosystems [11,12]. Yet, the classical ST is inherently limited to surfaces and boundaries that are closed, leaving a significant gap in its applicability to open, non-planar geometries. Indeed, many natural and engineered systems exhibit spiral or helical dynamics where forces and flows do not conform to closed loops or planar surfaces but rather are characterized by open, three-dimensional trajectories. Examples include the helical paths of tornadoes, magnetic vortices and spiral galaxies as well as bacterial motility and phyllotaxis of plants [13-15].

The novelty of this work lies in extending ST to accommodate spiral flows and helicoidal paths. By linking macroscopic and microscopic circulation properties, the extended theorem simplifies the evaluation of forces in systems with open, three-dimensional geometries. We utilize EST to analyse two biological scenarios: 1) the rotational forces in spiral flower petals and 2) the torsional stresses in helical plant stems, both exemplified by *Trachelospermum jasminoides*, commonly known as star jasmine. For the flower, EST captures the uniform rotational stresses induced by tangential forces acting along a circular boundary. This is achieved by demonstrating the equivalence of the line integral along the petal boundary and the surface integral over the enclosed disk. For the stem, EST quantifies the interaction between bending and torsional stresses caused by the helical geometry.

This paper is structured as follows. First, we present the mathematical treatment of EST, including its derivation and parameterization for helicoidal paths. Next, we validate the theorem using the specific example of *Trachelospermum jasminoides'* flowers and stems. Finally, we discuss the broader implications of EST, highlighting its potential to unify the study of dynamical systems with open boundaries.

Materials and Methods

This study is grounded in a generalized form of Stokes' theorem, adapted for spiral flows, which facilitates the analysis of forces and circulation in systems with helicoidal or spiral geometries. We aim to prove that, given a continuously differentiable, orientable helicoidal spiral vector field, the macroscopic circulation represented by the integral of a differential form over its surface equals the microscopic circulation represented by the volume integral of the curl perpendicular to the surface. The main challenge here is in defining the notion of a boundary in case of an open helicoidal spiral path, moving beyond the classical case of paths evaluable through ST.

Stokes' Theorem (ST) from vector calculus relates the surface integral of the curl of a vector field over a surface **S** to the line integral of the vector field along the boundary curve ∂S of the surface (Figure 1). In its general form, ST asserts that

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_{S} (\nabla \times \mathbf{F}) \cdot d\mathbf{S}$$

where **F** is a a continuously differentiable two-dimensional vector field, ∂S is the closed boundary curve of the surface **S** that can be bended and stretched, d**r** is a differential element of the curve, d**S** is the differential element of the surface area, and $\nabla \times \mathbf{F}$ is the curl of the vector field, i.e., a vector operator characterizing the infinitesimal circulation of vector fields in three-dimensional spaces.

ST turns line integrals of a form over a boundary into more straight-forward double integrals over the bounded region, regardless of the position of vector singularities [16]. For ST to apply, the normal vector representing the surface must be positively oriented (i.e., counterclockwise) with respect to the tangent vector representing the orientation of the boundary.

Extended Stokes' theorem (EST)

Consider a vector field **F** defined over a region in three-dimensional space. Let the surface *S* be a portion of a plane or a more general surface that is bounded by a spiral curve $\gamma(t)$. The goal is to use EST to evaluate the line integral over the spiral path in terms of the surface integral of the curl of **F** (Figure 1).

Let the spiral curve $\gamma(t)$, with $t \in [a, b]$, be parameterized as

$$\gamma(t) = (r(t)\cos(\theta(t)), r(t)\sin(\theta(t)), z(t))$$

where $r(t)$, $\theta(t)$ and $z(t)$ describe respectively the radial, angular and vertical components of the spiral.

Let's assume that $\gamma(t)$ lies on a flat plane, say the *xy*-plane, so the spiral path can be simplified to

$$\gamma(t) = (r(t)\cos(t), r(t)\sin(t), 0)$$

where $r(t)$ increases as the angle *t* increases.

When the surface **S** is a surface spanned by the curve $\gamma(t)$, **S** stands for a portion of the plane or surface generated by the spiral curve (Figure 1, left).

We are interested in computing the line integral of a vector field **F** along the spiral path (Figure 1, right). By ST, this line integral can be transformed into a surface integral involving the curl of **F**

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_{S} (\nabla \times \mathbf{F}) \cdot d\mathbf{S}$$

The line integral over the spiral path is:

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_{a}^{b} \mathbf{F}(\gamma(t)) \cdot \frac{d\gamma(t)}{dt}\, dt$$

where $\frac{d\gamma(t)}{dt}$ is the tangent vector to the spiral path at each point *t*.

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The surface integral involves the curl of **F**, given by $\nabla \times \mathbf{F}$, and the surface normal vector $\hat{n}$ associated with **S**

$$\int_S (\nabla \times \mathbf{F}) \cdot d\mathbf{S} = \int_S (\nabla \times \mathbf{F}) \cdot \hat{n}\, dS$$

The normal vector $\hat{n}$ depends on the orientation of the surface, while *d*S is the differential area element of the surface.

Upon achieving the extended formulation of ST, we will proceed in the next paragraphs with a detailed case study.

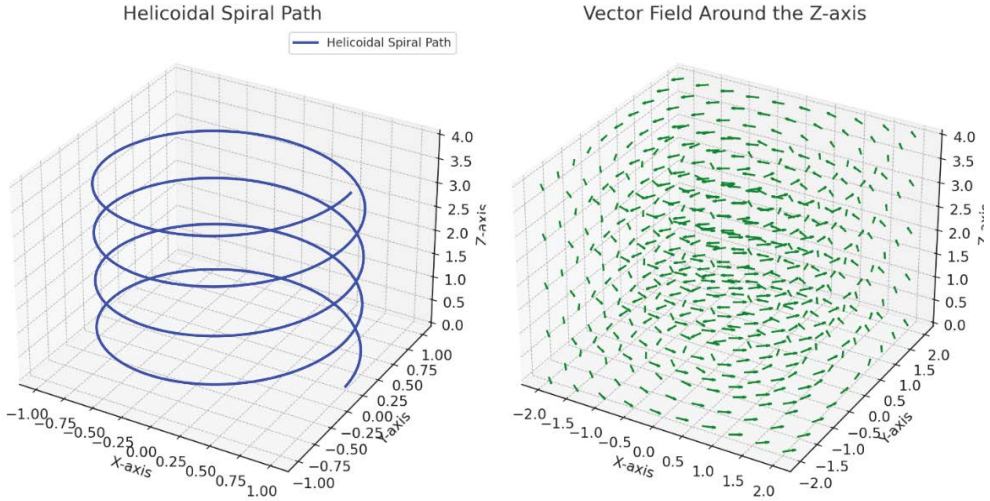

Figure 1: Diagrams depicting a helicoidal spiral (**left**) and the behavior of a vector field (**right**) around the z-axis. The left diagram illustrates a helicoidal spiral path, showcasing the interplay of rotational and translational motion along the z-axis. The right diagram represents a vector field with circular flow centered around the z-axis.

Simulated case study: analyzing rotational flows in stems and flowers

EST enables the analysis of rotational and translational forces in complex systems, providing a powerful framework for exploring biological and physical dynamics. To illustrate the new theorem, we will now explore a concrete example. We will consider *Trachelospermum jasminoides*, commonly known as star jasmine, belonging to the family *Apocynaceae*. Like many climbing plants, *Trachelospermum jasminoides* displays a counterclockwise helical movement of its stems as it climbs and twines around supports, also referred to as circumnutation [17-19] (Figure 2). The flowers also exhibit subtle rotational dynamics, although these movements are not as pronounced as the helical twisting of the stems [20]. The petals of the flowers are arranged in a spiral configuration and unfurl in a counterclockwise direction during blooming.

In our simulation, the dynamics of flower petals are modeled using a circular boundary with a radius of 0.05 m, representing the petals of a flower. Tangential forces along this boundary are applied and the resulting rotational stresses are analyzed through EST. The stem is modeled as a helicoidal path with a radius R=0.05 m and a vertical rise per turn of c=0.2 m. To evaluate the counterclockwise rotation of the flower and stem using EST, the rotation of the petals can be represented by a circular vector field, whereas the helical motion of the stem can be modelled using a helical vector field. The next step is to parameterize the flower and the stem (Figure 2**C**).

1. For the flower, a circular boundary in the plane of the petals is defined, representing the region of interest for macroscopic rotation.
2. For the stem, the helical path is parameterized using equations for a helicoidal spiral, where $x(t) = r\cos(t)$, $y(t) = r\sin(t)$ and $z(t) = ct$. Here r represents the radius, c the rise per turn and t the parameter along the path.

Subsequently, the surface S is defined for each component.

1) For the flower, the surface is a disk enclosed by the petals' rotational motion within their plane,
2) whereas for the stem, the surface corresponds to the area traced by the helical path (Figure 2C).

Calculating the forces acting on the flower and stem requires applying mechanical principles that account for both internal and external forces influencing their dynamics [21,22]. For the flower's petals, the primary force is torque or rotational force, while the stem experiences a combination of bending forces and axial torsion due to its helical structure. We will calculate these forces systematically, step by step, starting from the external forces.

External forces acting of the flower petals and the stem

1) The rotation of the flower petals can be modeled as a torque

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induced by external forces such as wind, gravitational pull, biological growth forces [23]. Torque (τ) on the petals is given by:

$$\tau = r \times F$$

Where r is the radial vector from the center of the flower to the tip of a petal and F is the tangential force applied to the petal. Let's assume that the radius of the flower is R=5 cm, while the tangential force from wind or another source is F=0.1 N. The magnitude of the torque is

$$|\tau| = R \cdot F = 0.05\,\text{m} \cdot 0.1\,\text{N} = 0.005\,\text{Nm}$$

In case of multiple petals (n=5 in *Trachelospermum jasminoides*) experiencing similar forces, the total torque becomes

$$\tau_{\text{total}} = n \cdot \tau = 5 \cdot 0.005\,\text{Nm} = 0.025\,\text{Nm}$$

The rotational acceleration (α) of the flower petals is related to the torque [24] via

$$\tau = I \cdot \alpha$$

Where I is the moment of inertia of the flower petals about the axis of rotation and α is the angular acceleration.

For a flower modeled as a system of point masses at a radius R

$$I = n \cdot m \cdot R^2$$

where m is the mass of a single petal. Assuming m=0.002 kg (2 grams per petal):

$$I = 5 \cdot 0.002 \cdot (0.05)^2 = 0.000025\,\text{kg}\backslash\text{cdotpm}^2$$

$$\alpha = \frac{\tau_{\text{total}}}{I} = \frac{0.025}{0.000025} = 1000\,\text{rad/s}^2$$

1) The stem experiences forces from bending and torsion, influenced by its helical structure. These forces arise from gravity, wind and the biological tension exerted during growth.

2) The weight of the stem induces a bending gravitational force. For a stem of length L=20 cm and mass per unit length λ=0.01 kg/m:

$$F_g = m \cdot g = (\lambda \cdot L) \cdot g = (0.01 \cdot 0.2) \cdot 9.8 = 0.0196\,\text{N}$$

This force acts vertically downward, generating a bending moment at the base of the stem

$$M_g = F_g \cdot \frac{L}{2} = 0.0196 \cdot 0.1 = 0.00196\,\text{Nm}$$

The helical structure of the stem experiences torsional forces due to the winding. The torsional moment (T) is given by

$$T = G \cdot J \cdot$$

Where G is the shear modulus of the stem material, J is the polar moment of inertia and θ is the angle of twist over the length L.

Assuming G= 10^8 Pa typical for plant tissue, J = 0.005 m (5 mm radius) and θ = 2π (one full turn over L =0.2 m) [25], then

$$J = \frac{\pi}{2} \cdot (0.005)^4 = 9.82 \times 10^{-10}\,\text{m}^4$$

and

$$T = (10^8) \cdot (9.82 \times 10^{-10}) \cdot \frac{2\pi}{0.2} = 3.08\,\text{Nm}$$

.

Internal forces acting within the flower petals and the stem

To calculate the forces within the flower petals and the stem using EST, we need to evaluate the relationship between the macroscopic circulation (observable forces) and the microscopic properties (internal forces or stresses derived from the curl of the force field). The first step is to model the forces using vector fields.

1) Concerning the flower petals, we assume that the external forces (e.g., wind or biological forces) act tangentially to their circular boundary. Further, we assume that the tangential forces induce internal stresses (force per unit area) propagating through the petals. Let the force field acting on the petals be

$$\mathbf{F} = (-ky, kx, 0)$$

where k is the force constant proportional to the external pressure and x,y represents positions in the z=0 plane.

Next, we compute the curl of the force field. The curl of the force field relates to the internal stresses within the petals. For the flower petals, in the z=0 plane, the curl of $\mathbf{F} = (-ky, kx, 0)$ is

$$\nabla \times \mathbf{F} = \begin{vmatrix} \mathbf{i} & \mathbf{j} & \mathbf{k} \\ \frac{\partial}{\partial x} & \frac{\partial}{\partial y} & \frac{\partial}{\partial z} \\ -ky & kx & 0 \end{vmatrix} = (0, 0, 2k)$$

This curl is constant in the z-direction, indicating a uniform internal rotational stress throughout the petals.

1) Concerning the helical stem, it experiences external forces such as gravity $(\mathbf{F}_g)$ and biological growth forces $(\mathbf{F}_b)$ that induce internal torsion and bending stresses.

For simplicity, we model the net force field in the stem as: $\mathbf{F} = (-ky, kx, kz)$ where the kz-term accounts for the vertical components of the forces.

Next, we compute the curl of the force field, which provides insight into the internal stresses acting within the stem. This computation reveals the distribution and intensity of these stresses, capturing the complex interplay of forces across the helical structure. For the stem the curl of $\mathbf{F} = (-ky, kx, kz)$ is

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$$\nabla \times \mathbf{F} = \begin{vmatrix} \mathbf{i} & \mathbf{j} & \mathbf{k} \\ \frac{\partial}{\partial x} & \frac{\partial}{\partial y} & \frac{\partial}{\partial z} \\ -ky & kx & kz \end{vmatrix} = (k, k, 2k)$$

This suggests a complex pattern of internal stress within the stem, with components distributed across all three spatial directions.

Visualization and statistics

Diagrams of the flower petals and stem are created to illustrate their geometry, boundary dynamics, and associated vector fields. The Matplotlib library is employed to generate detailed plots, including the circular boundary and tangential forces acting on the flower petals, the curl of the vector field over various surfaces, and the helicoidal path and vector field representation for the stem.

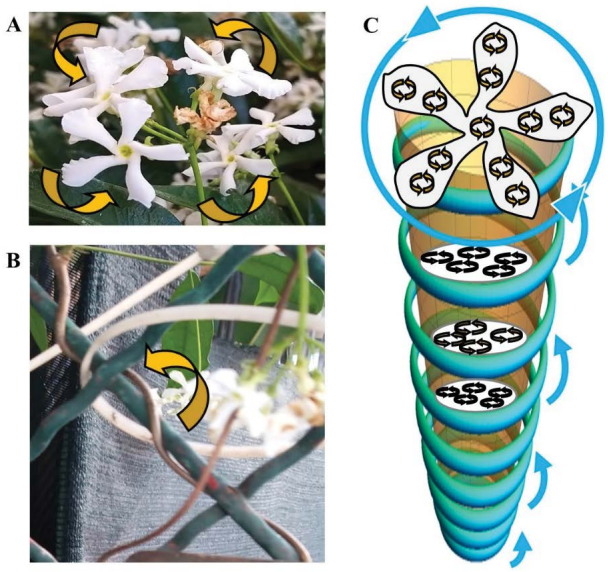

Figure 2: *Trachelospermum jasminoides*. The flower petals (**Figure 2A**) and the stem (**Figure 2B**) display a counterclockwise path. **Figure 2C** illustrates the geometry of the boundaries, the associated vector fields and the internal flows within the flower petals and the stem.

To ensure statistical validation, numerical accuracy is achieved through high-resolution parameterization, with the parameter t sampled at 1,000 points per cycle. The consistency between line integrals and surface integrals is carefully evaluated to confirm the applicability of the extended theorem to the analyzed geometries.

In the sequel, the surface integral of the curl of the vector field will be computed over these surfaces using the extended Stokes' theorem (EST).

Results

As stated above, both the flower and the stem experience external and internal mechanical forces that influence their motion and structural behavior:

1) For the flower petals, the torque arising from tangential forces induces a counterclockwise rotation, with the total torque $\tau_{\text{total}} = 0.025\,\text{Nm}$ and the angular acceleration measured as α=1000 rad/s^2. The internal stresses in the petals are uniform with a value of 2k and are directly proportional to the external forces acting on them. This proportionality explains the rotational equilibrium observed in the petals.

2) For the stem, the primary forces include a gravitational bending moment $M_g = 0.00196\,\text{Nm}$ and a torsional moment T=3.08 Nm due to a helical twist. The internal stresses in the stem vary in all three dimensions because of its helical geometry. Among these stresses, torsion, proportional to kc, predominates, whereas bending stresses, proportional to kR, have a secondary but still notable influence.

We can now apply EST to relate macroscopic and microscopic circulation. The surface integral of the curl of the vector field is computed over the surfaces, relating the surface integrals to the line integrals along the boundaries. For the flower petals, the counterclockwise macroscopic rotation is calculated by integrating along the circular path in the plane. For the stem, the integral is evaluated over the helical surface.

1) Concerning the flower petals, the boundary of the flower is a circle of radius R. The macroscopic circulation (line integral along the petal boundary) is

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_0^{2\pi} kR^2\, d\theta = 2\pi k R^2$$

Using the curl, the surface integral is

$$\int_S (\nabla \times \mathbf{F}) \cdot d\mathbf{S} = \int_S 2k\, dA = 2k \cdot \pi R^2$$

Both results match, confirming that the inner stresses in the petals are proportional to 2k.

In sum, the numerical values for the macroscopic (surface) flows and microscopic (internal) flows in the flower, as governed by EST, are as follows. For the flower, the surface flow (evaluated as a surface integral) is 0.157N\ppm, while the internal flow (evaluated as a line integral) is also 0.157N\ppm. The flower petals exhibit a simple and symmetric geometry, where forces act tangentially along a circular boundary in the z=0 plane. The petals lie on a flat, two-dimensional surface characterized by a constant curl of the force field ($\nabla \times \mathbf{F} = (0, 0, 2k)$), signifying that the internal forces are uniformly distributed. This uniform distribution creates a direct and proportional relationship between the macroscopic flow (line integral along the circular boundary) and the microscopic flow (surface integral over the disk). The symmetry of the geometry ensures that every contribution to the line integral is exactly matched by the surface integral. Consequently, the uniform geometry and constant curl lead to a perfect agreement between the surface flow and the internal

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flow, consistent with EST.

1) Concerning the helical stem, the boundary of the stem is parameterized as a helicoidal spiral

$$x(t) = R\cos(t), \quad y(t) = R\sin(t), \quad z(t) = ct$$

The macroscopic circulation (line integral along the helical path) is

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = \int_0^{2\pi} [-kR\sin(t)(-R\sin(t)) + kR\cos(t)(R\cos(t)) + kc(ct)]\, dt$$

$$= \int_0^{2\pi} (kR^2 + kc^2 t)\, dt$$

For one turn$(t \in [0, 2\pi])$

$$\int_{\partial S} \mathbf{F} \cdot d\mathbf{r} = 2\pi kR^2 + \frac{kc^2}{2}(2\pi)^2$$

Using the curl, the surface integral is approximated by the ribbon spanned by the helix

$$\int_S (\nabla \times \mathbf{F}) \cdot d\mathbf{S} = \int_S (k + k + 2k)\, dA = 4k \cdot (\text{surface area of ribbon})$$

The surface area of the ribbon is

$$A = 2\pi R \cdot (\text{height per turn}) = 2\pi R \cdot c$$

Thus

$$\int_S (\nabla \times \mathbf{F}) \cdot d\mathbf{S} = 4k \cdot 2\pi R \cdot c = 8\pi kRc$$

In sum, the numerical values for the macroscopic (surface) flows and microscopic (internal) flows in the stem, as governed by EST, are as follows. For the stem, the surface flow (evaluated as a surface integral) is 2.513N\ppm, while the internal flow (evaluated as a line integral) is 8.053N\ppm. Unlike the flower, the values for surface flow and internal flow differ significantly. This is due to the stem's more complex geometry, which features a three-dimensional helicoidal structure with a helical boundary and a ribbon-like surface. Unlike the constant curl observed in the flower, the curl of the force field $(\nabla \times \mathbf{F} = (k, k, 2k))$ in the stem varies in all three dimensions. This non-uniform curl introduces additional contributions to the surface integral that are not directly proportional to the line integral along the helical path. The helicoidal surface spanned by the path is not planar. Its area depends on the radius of the helix and the rise per turn (c), which increases the surface integral significantly compared to the simpler circular geometry of the flower. The line integral along the helical path includes contributions from the vertical rise (z-component), which are absent in the flat geometry of the flower. These vertical components add substantially to the internal flow, making it larger than the surface flow. Forces and circulation in the stem are not confined to a two-dimensional plane, rather display three-dimensional dynamics that capture complex interactions such as bending, twisting and torsional effects, further contributing to the discrepancy between the surface and internal flows. Therefore, the stem's intricate geometry and three-dimensional dynamics lead to a disparity between surface and internal flows, reflecting the additional factors at play in its structural behavior.

In conclusion,

1) flowers have a circular, symmetric geometry that ensures uniform force distribution and curl. This results in surface and internal flows being equal, as the entire flow field is captured in a flat, two-dimensional setup.

2) In contrast, the stem's helicoidal geometry introduces non-uniform force distributions and additional components such as vertical contributions and a larger surface area. These factors create a larger internal flow compared to the surface flow, as the line integral accounts for three-dimensional effects that the surface integral does not fully capture.

These differences highlight the impact of geometry and force distribution on the interplay between macroscopic circulation and microscopic forces, showcasing the utility of the extended Stokes' theorem in analysing forces and circulation in systems exhibiting spiral dynamics.

Conclusion

Classical theorems such as Green's Theorem (GT) and Stokes' Theorem (ST) have been pivotal in linking local properties of vector fields to their global behavior. GT applies just to two-dimensional regions and closed curves, while ST extends to three-dimensional spaces requiring closed surfaces or boundaries for its application [1,2]. These theorems, focused on closed-loop circulations, have proven instrumental in analyzing flows and circulations in systems where boundaries are well-defined, such as steady-state circulations in airflow around wings or electromagnetic field behavior in closed circuits [4,5]. However, their utility diminishes when applied to open, three-dimensional trajectories like the helicoidal spirals which are frequently encountered in natural and engineered systems.

We suggest a generalization of ST to establish a mathematical framework connecting the line integral along a helicoidal spiral path to the surface integral of the curl of the vector field over a bounded region. By redefining the boundary concept for helicoidal paths, this framework provides a new tool for analyzing macroscopic and microscopic flow dynamics in complex systems. The EST formulation provides novel insights into the interplay between rotational and translational motions, allowing for a deeper understanding of spiral flows in a variety of physical and biological systems. A key advantage of the extended formulation lies in its ability to model a wide range of systems where spiral or helical dynamics are dominant. For instance, the novel framework enables the analysis of DNA supercoiling, bacterial flagella, biomechanical patterns such as the phyllotaxis of plants

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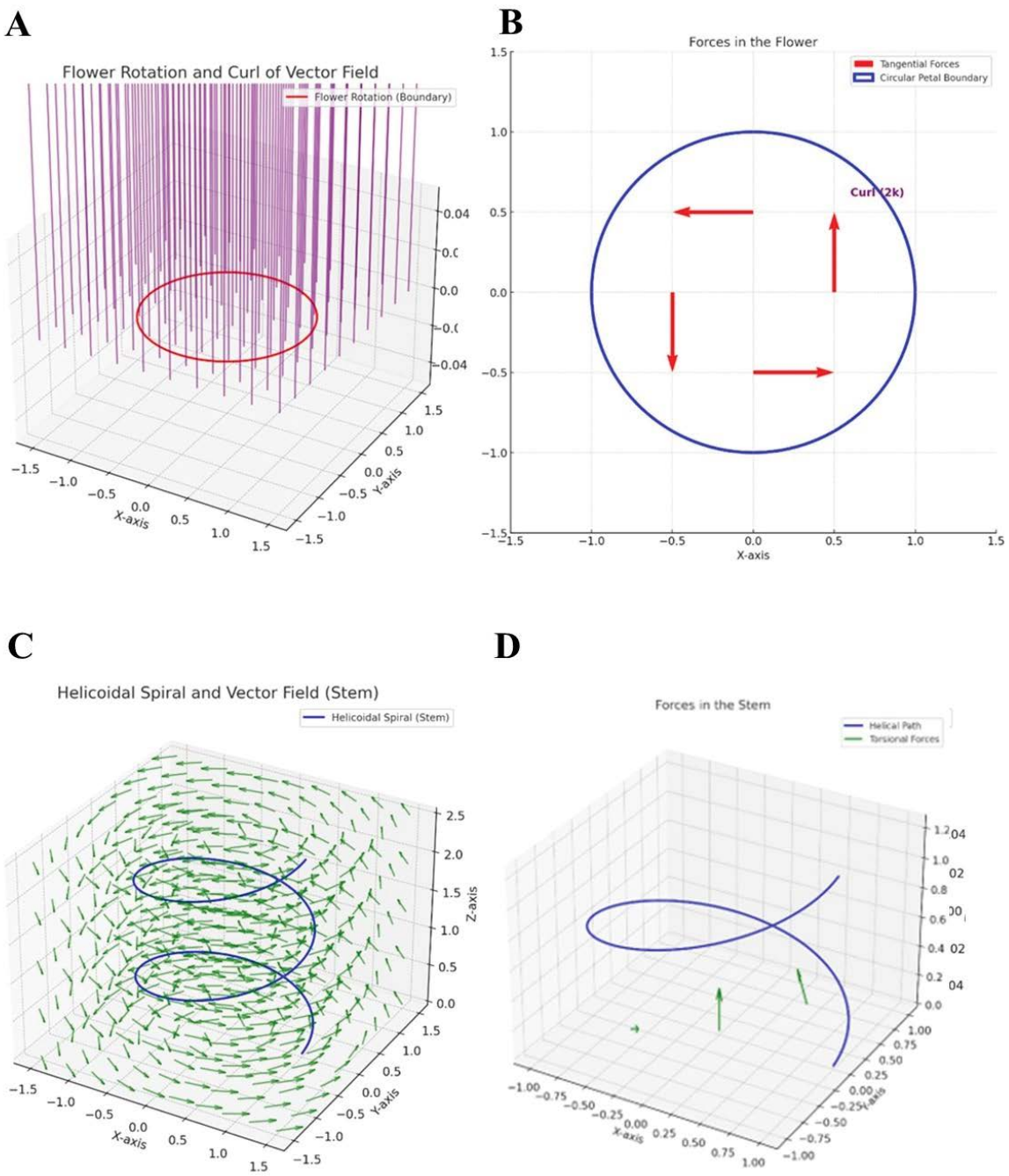

Figure 3: Application of the extended Stoke's theorem to flowers (**Figures 3A-B**) and stems (**Figures 3C-D**) of *Trachelospermum jasminoides*. **Figure 3A**. Diagram illustrating the flower rotation and the curl of vector field. The red circle represents the boundary of the flower petals modeled as a planar region in the z=0 plane. The purple arrows visualize the curl of the vector field, representing the microscopic circulation that contributes to the macroscopic rotation of the flower petals. **Figure 3B**. Diagram illustrating the forces acting on the flower. The circular boundary (blue) represents the edge of the flower petals. Tangential forces (red arrows) act along the edges of the petals, showcasing the influence of external or internal factors. The calculated curl of the vector field is constant at $\nabla \times F=2k$ (annotated in purple) in the z-direction, indicating uniform rotational stresses throughout the petal boundary. **Figure 3C**. Diagram illustrating the helicoidal spiral of the stem and the vector field. The blue curve depicts the helicoidal path of the stem, while the green arrows represent a circular vector field around the z-axis, illustrating the rotational and translational flow and its interaction with the spiral geometry. **Figure 3D**. Diagram illustrating the forces acting on the stem. The helical path (blue curve) represents the stem's geometry. The torsional forces (green arrows), resulting from a combination of bending and twisting actions, act along the helical structure contributing to internal stress distribution. The curl vector field displays non-uniform rotational stresses with components $\nabla \times F=(k,k,2k)$.

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[15,26] intracardiac spiral flows observed in cardiac cycles [27] as well as magnetic vortices in superconductors [14] and rotational dynamics of spiral galaxies [13], where classical methods fail to capture the intricacies of rotational and translational dynamics [28-34].

In this paper, EST is applied to two case studies related with *Trachelospermum jasminoides*, namely the forces acting on flower petals and the helical stress distribution within plant stems.

1) For the flower petals, the circular geometry allows for a straightforward application EST, since the tangential forces acting along the petal boundary produce a uniform curl which is proportional to the rotational stresses. The equivalence between the line integral along the petal boundary and the surface integral of the curl over the enclosed disk validates EST's effectiveness for two-dimensional spiral systems. The uniform rotational stresses observed in the petals align well with the mathematical predictions of EST. This provides insights into how forces are distributed within the boundary of the flower, potentially aiding in the study of floral mechanics and growth patterns. EST suggests that microscopic forces acting at the level of the petals contribute to the macroscopic rotational motion observed at the flower's boundary. This could be applied to study the impact of environmental factors like wind on plant structures and to investigate the mechanical interactions between flowers and pollinators during the pollination process. Still, EST effectively simplifies complex calculations by converting a line integral along the flower's boundary into a surface integral over the petal region. This transformation minimizes computational effort while preserving accuracy.

2) In the case of the stem, although the helical geometry of the stem presents a significant challenge for classical mathematical tools, EST effectively simplifies the intricate interplay of forces involved. The torsional and bending forces are captured through the curl of the vector field, which has components in all three dimensions. The equivalence of the surface integral over the helical ribbon region and the line integral along the helical path demonstrates the robustness of EST in handling three-dimensional geometries with open boundaries. The EST capability to connect macroscopic flow patterns with microscopic circulatory forces may have significant implications for understanding the biomechanics of plant growth and structural stability. This relationship can also provide valuable insights for studies on nutrient and water transport within stems, as these processes often involve spiral dynamics.

Certain assumptions and limitations are inherent in our analysis. EST assumes that the involved vector fields and surfaces are continuously differentiable. In real-world biological systems, irregularities and discontinuities in the geometry or force distribution may reduce the accuracy of the analysis. The flower petals are modeled as a perfect circle and the stem as a regular helix. While this simplifies the mathematical analysis of forces in idealized systems, real-world systems often deviate from these idealized shapes. The analysis of irregular geometries or highly dynamic boundaries may still require significant computational effort, particularly for numerical integration of complex surface and line integrals. The tangential and torsional forces are assumed to be uniform across the boundaries. In reality, biological and environmental forces such as wind, gravity and growth pressures are often spatially and temporally variable. Additionally, secondary effects such as shear forces or anisotropic material properties are not incorporated, which could limit the applicability of the results to certain systems. Future work could extend the framework to handle more irregular and biologically realistic geometries, such as asymmetrical petals or non-uniform stem shapes. The analysis of time-varying forces and boundaries, such as those caused by growth or environmental changes, could provide deeper insights into real dynamics. Integrating the extended theorem with experimental data would help validate the theoretical predictions and refine the mathematical models.

In conclusion, the proposed extension to Stokes' Theorem integrates helicoidal paths into circulation analysis, bridging a critical gap and expanding its applicability to open, non-planar trajectories. By redefining boundaries, it simplifies the study of rotational and translational flows, offering a versatile tool for analyzing complex dynamics such as those observed in the flowers and stem of *Trachelospermum jasminoides*.

Declarations

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the author.

Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal, and has not been previously published.

Availability of data and materials. all data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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work that could inappropriately influence, or be perceived to influence, their work.

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Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical, and material support, study supervision.

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References

1. Green G. An essay on the application of mathematical analysis to the theories of electricity and magnetism. Journal f ur die reine und angewandte Mathematik 39 (1828): 73-89.
2. Schey HM. Div, Grad, Curl, and All That: An Informal Text on Vector Calculus, 3rd ed. New York: W.W. Norton (1997).
3. Craven BD. A note on Green's theorem. Journal of the Australian Math. Soc 4 (1964): 289-292.
4. Arfken G. Gauss's Theorem. Mathematical Methods for Physicists, 3rd ed. Orlando, FL: Academic Press (1985).
5. De Villiers JP. Stokes Theorem and the Equations of GRMHD (2006).
6. Livermore PW, Hollerbach R, Jackson A. Electromagnetically driven westward drift and inner-core superrotation in Earth's core. PNAS 110 (2013): 15918.
7. Snieder R. Imaging the Earth using Green's theorem, in The Princeton Companion to Applied Mathematics, Ed. Higham, N.J., M.R. Dennis, P. Glendinning, P.A. Martin, F. Santosa, and J. Tanner, Princeton Univ Press, Princeton NJ (2015): 857-860.
8. Aubert J, Finlay CC. Geomagnetic jerks and rapid hydromagnetic waves focusing at Earth's core surface. Nat Geosci 12 (2019): 393-398.
9. Vines SK, Anderson BJ, Allen RC, et al. Determining EMIC Wave Vector Properties Through Multi-Point Measurements: The Wave Curl Analysis. J Geophys Res Space Phys 126 (2021): e2020JA028922.
10. Yang Y, Song X. Multidecadal variation of the Earth's inner-core rotation. Nat. Geosci 16 (2023): 182-187.
11. Tozzi A, Peters JF. Towards a single parameter for the assessment of EEG oscillations. Cogn Neurodyn (2023).
12. Bressan A, Chiri MT, Salehi N. On the optimal control of propagation fronts. Mathematical Models and Methods in Applied Sciences 32 (2022): 1109-1140.
13. Blaser A, Benamran R, Villas Bôas AB, et al. Momentum, energy and vorticity balances in deep-water surface gravity waves. Journal of Fluid Mechanics 997 (2024).
14. Sachkou YP, Baker CG, Harris GI, et al. Coherent vortex dynamics in a strongly interacting superfluid on a silicon chip. Science 366 (2019): 1480-1485.
15. Reinhardt D, Gola EM. Law and order in plants - the origin and functional relevance of phyllotaxis. Trends Plant Sci 27 (2022): 1017-1032.
16. Zenisek A. Green's theorem from the viewpoint of applications. Applications of Mathematics 44 (1999): 55-80.
17. Darwin C. The Movements and Habits of Climbing Plants. London: John Murray (1875).
18. Pansanit A, Pripdeevech P. Constituents, Antibacterial and Antioxidant Activities of Essential Oils from Trachelospermum jasminoides Flowers. Natural Product Communications 9 (2014): 1791-94.
19. Canher B, Lanssens F, Zhang A, et al. The Regeneration Factors ERF114 and ERF115 Regulate Auxin-Mediated Lateral Root Development in Response to Mechanical Cues. Molecular Plant 15 (22): 1543-1557.
20. Stefanatou A, Vouzi L, Petousi I, et al. Treatment of Real Laundry Wastewater Using Vertical Flow Constructed Wetland Planted with the Ornamental Climbing Plant Trachelospermum jasminoides: Assessing the Removal of Conventional Pollutants and Benzotriazoles." Environmental Science and Pollution Research International 31 (2024): 43281-91.
21. Smyth, David R. Helical Growth in Plant Organs: Mechanisms and Significance. In Plant Development. Development 143 (2016): 3272-3282.
22. Ilya L, Del Dottore E, Mazzolai B, et al. Conditions for the Emergence of Circumnutations in Plant Roots. PLOS ONE 16 (2021): e0252202.
23. Paul T. Physics for Scientists and Engineers: Mechanics, Oscillations and Waves, Thermodynamics. 5th ed. New York: W. H. Freeman (2004).
24. Clark KP, Ryan LJ. Hip Torque Is a Mechanistic Link Between Sprint Acceleration and Maximum Velocity

Tozzi A., Int J Plant Anim Environ Sci 2025
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Performance: A Theoretical Perspective. Frontiers in Sports and Active Living 4 (2022): 945688.

25. Hoermayer L, Juan CM, Nicola T, et al. Mechanical Forces in Plant Tissue Matrix Orient Cell Divisions via Microtubule Stabilization. Developmental Cell 59 (2024): 1333-1344.e4.

26. Liu S, Li Y, Wang Y, et al. Emergence of large-scale mechanical spiral waves in bacterial living matter. Nat Phys 20 (2024): 1015-1021.

27. Mulimani MK, Zimik S, Pandit R. An In Silico Study of Electrophysiological Parameters That Affect the Spiral-Wave Frequency in Mathematical Models for Cardiac Tissue. Front Phys 9 (2022): 819873.

28. Donepudi T, van de Griend M, Agostinho LLF, et al. Numerical analysis of vortex dynamics in hyperbolic funnels using computational fluid dynamics. Physics of Fluids 36 (2024): 095171.

29. You GY, Ziqiang Y, Ze W, et al. Magnetic Field Downward Continuation Iterative Method Based on Low-pass Filter. J Phys Conf Ser 2525 (2023): 012006.

30. Heusler M. Stationary Black Holes: Uniqueness and Beyond. Living Rev. Relativ 1 (1998).

31. Long F, McElheny D, Jiang S, et al. Conformational change of erythroid α-spectrin at the tetramerization site upon binding β-spectrin. Protein Sci 16 (2007): 2519-2530.

32. Pontryagin LS. Smooth manifolds and their applications in homotopy theory. American Mathematical Society Translations, Ser 2, American Mathematical Society, Providence, R.I 11 (1959): 1-114.

33. Cauchy A. Sur les intégrales qui s'étendent à tous les points d'une courbe fermée. Comptes rendus 23 (1846): 251-255.

34. Wapenaar K, Brackenhoff J, Thorbecke J. Green's theorem in seismic imaging across the scales. Solid Earth 10 (2019): 517-536.

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Feeling the Heat: A Thermodynamic Perspective on Emotions, Motivation, and Time Perception

Eva Déli [a,*], Felix Schoeller [b,c], Adam Safron [b,d], Abhinandan Jain [c], Arturo Tozzi [e], Vladimir Adrien [f,g], Nicco Reggente [b]

[a] University of Debrecen, Faculty of Health Sciences, Department of Psychology, Debrecen, Hungary
[b] Institute for Advanced Consciousness Studies, Santa Monica, CA, USA
[c] MIT Media Lab, Cambridge, MA, USA
[d] Allen Discovery Center, Tufts Univ, Medford, MA, USA
[e] Dept of Physics, University of North Texas, Denton, USA
[f] Université Sorbonne Paris Nord, Department of Infectious and Tropical Diseases, Avicenne Hospital, AP-HP, F-93000, Bobigny, France
[g] Université Paris Cité, Inserm UMR-S 1266, Institute of Psychiatry and Neuroscience of Paris (IPNP), Paris, 75014, France

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ABSTRACT

We are introducing a novel thermodynamic model of emotion. In this model, emotions are regarded as deviations from equilibrium, akin to fluctuations in body temperature. This bipolar regulation maintains bodily and psychological homeostasis while spurring mental development. Emotional regulation typically occurs through expanding one's perception of time. Positive, low-information content emotions can reduce action drive, but stressful, information-rich conditions can heighten it. Therefore, time perception can potentiate the capacity of emotions to motivate. However, time perception accelerates to facilitate fluid action performance, with the state of flow representing a unique state of contentment and challenge. By anchoring psychological processes to the principles of energy and entropy, our model offers a comprehensive bipolar foundation for understanding motivation and behavior. Beyond its theoretical implications, this model also lays the groundwork for addressing mental health conditions resulting from the dysregulation of emotions. It can inspire potential interventions to harness the mind-body connections elucidated by our thermodynamic perspective.

1. Introduction

The global rise in stress disorders highlights the urgent need to understand emotional resilience and its role in the development of mental diseases (Nestler & Russo, 2024). Traditional approaches have treated emotions as separate domains, failing to capture their intricate interdependencies. For example, cognitive theories have focused primarily on the mental processes underlying emotions (Barrett, 2017). At the same time, physiological research has investigated bodily responses, such as heart rate and facial expressions, largely independently of motivational and subjective factors. This compartmentalized view has limited our ability to explain the complex relationships and paradoxes observed across these realms of human experience. Understanding how emotions relate to subjective experiences (i.e., feelings), motivation, and disease progression has remained elusive.

This article proposes a novel thermodynamic model that integrates emotions, motivation, and associated physiological mechanisms within a unified framework. The brain keeps bodily and psychological equilibrium by intertwining every regulatory system with emotions. Nevertheless, the multifaceted nature of the relationship encourages further study. This work examines how emotions serve temperature regulation and psychological homeostasis. By conceptualizing the brain's functioning through thermodynamic principles of energy and entropy, we offer a cohesive perspective to resolve longstanding questions and paradoxes surrounding these interrelated processes.

1.1. Thermo-emotional covariations from a thermodynamic lens

Experimental research confirms the phylogenetically ancient relationship between emotions and temperature regulation across various species, including reptiles, foxes, pigs, rabbits, rats, mice, and humans (Briese, 1995; Briese & Cabanac, 1991; Cabanac, 1999; Frosini et al.,

* Corresponding author.
E-mail address: deli.eva@etk.unideb.hu (E. Déli).

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2000; Groenink et al., 1995; Moe & Bakken, 1997; Parrott et al., 1995; Terlouw et al., 1996; van der Heyden et al., 1997). Although thermoregulation exists to some degree in most animals, the endothermic phenotype—characteristic of humans and other mammals—depends on complex metabolic networks and multiple internal feedback loops (Grigg et al., 2021; Seebacher, 2020). For example, embryo incubation drives the evolution of endothermy (Farmer, 2020), which is phylogenetically predicated on thermoregulation (Clavijo-Baque & Bozinovic, 2012). Endotherms maintain a stable core temperature with the aid of crucial mechanisms, including vasoconstriction, shivering, and sweating (Madden & Morrison, 2019; Nowack et al., 2017). The brain's high energy use ensures optimal information processing while maintaining physical and psychological equilibrium (Dempsey et al., 2022; Huang, Zhang, Wu, Mashour, & Hudetz, 2020). Thermal control is a vital component of an overarching regulatory system, exerting downstream effects on action motivation and behavioral adaptations (Inagaki et al., 2019; Kataoka et al., 2020; Nashiro, Min, & Yoo, 2022).

Physical or mental instability prompts a wide range of protective mechanisms. Emotions are paramount in this regulatory hierarchy as they intertwine with other regulatory processes. Moreover, the distinct physiological signatures of emotions represent specific energy configurations of the brain (Hesp et al., 2021; Kao et al., 2015; Sadowski et al., 2020), and, like temperature, they oscillate around a neutral position, forming an emotional set-point (Northoff & Tumati, 2019). Recent work utilizes temperature as a means of characterizing emotions (Escobar et al., 2020). Contentment promotes rest and recovery (Brown & Thorsteinsson, 2020) by reducing metabolic rate and body temperature, thereby conserving energy through parasympathetic restorative processes (Seebacher, 2009). In contrast, stress is a highly demanding condition (Keller et al., 2019; Meeusen, Van Cutsem, & Roelands, 2020), where noradrenaline initiates the fight-or-flight response within seconds (O'Connor, Thayer, & Vedhara, 2020). Furthermore, the varied effects of stress depend on personal, environmental, and other situational factors. However, its adverse health effects in anxiety, dissociation in trauma, or even depression (Comtesse et al., 2019; Mason et al., 2024) warrant a deeper thermodynamic investigation.

Emotional expressions are fundamentally linked to physiological changes regulated by the autonomic nervous system. At the same time, their feedback representation reflects motivational aspects (Quadt et al., 2022). Moreover, emotion and temperature may be under thermodynamic control (Déli and Kisvarday, 2020; Grigg et al., 2021; Seebacher, 2020). This perspective posits that the brain's mechanisms for heat and work transfer play a crucial role in regulating emotional states, and conversely, that emotional states can influence thermoregulation. In support of this, psychological stress, known to elevate blood pressure, heart rate, and heart function—even during sleep (Hall et al., 2004)—increases core temperature, a phenomenon referred to as psychogenic fever (Oka et al., 2001). This psychogenic fever results from a temporary elevation in the thermoregulatory set point, mediated by both prostaglandin E2-dependent and independent mechanisms (An & Kim, 2011; Fossat et al., 2015; Kluger et al., 1987; Morimoto et al., 1991).

Heat stress can lead to impulsivity (Fredericks et al., 2018; Wittmann & Paulus, 2008; Paasche et al., 2019), drug-seeking behavior, and criminal activity (Corcoran & Zahnow, 2022). Inversely, fear triggers thermoregulation disturbances in substance abusers (Lowry et al., 2009; Raison et al., 2015). People with depression have higher body temperatures (Mason et al., 2024), and median raphe stimulation, which affects temperature regulation, can produce depressive-like behaviors (Fazekas et al., 2021). These and other findings suggest a neurological link between emotion and temperature (WILLIAMS & Bargh, 2008). To gain a deeper understanding of this relationship, we explore the mechanisms of thermodynamic regulation in more detail.

1.2. Variations of time judgment

Understanding the mechanisms underlying time perception requires distinguishing between different types of temporal judgments. A key theoretical distinction exists between the perceptions of short durations (e.g., milliseconds to seconds), typically measured through reproduction or estimation tasks and the subjective awareness of time's passage often referred to as passage-of-time (PoT) judgments. These two aspects are functionally dissociable: while short-duration judgments are closely linked to internal pacemaker mechanisms and are modulated by arousal and attention (Gibbon et al., 1984; Wittmann, 2009), PoT judgments rely more on self-reflective and interoceptive processes, such as emotional state and bodily awareness (Droit-Volet, & Fayolle, 2024; Martinelli & Droit-Volet, 2022).

Incorporating this distinction into our thermodynamic framework, we conceptualize duration estimation as a dynamic process modulated by arousal-driven shifts in entropy and energy. High-arousal states, whether positively or negatively valenced, have been shown to accelerate internal clock processes, resulting in time overestimation (Cui et al., 2023). An expansion of subjective time is useful for decision-making, while contraction of subjective time drives action (D'Agostino et al., 2023). In contrast, low-arousal emotions, including contentment or mild joy, do not produce consistent distortions in short-duration estimates. These findings challenge earlier claims of a general "positivity effect" on temporal expansion and underscore the primacy of arousal, rather than valence, in modulating temporal metrics.

Moreover, our model emphasizes that PoT judgments, i.e., feelings that time is dragging or flying, are not reducible to clock-speed effects. Instead, they emerge from higher-order awareness of emotional and cognitive change (Wittmann, 2015). For instance, the emotion of awe leads to underestimation of duration in attention-based timing tasks, likely due to perceptual overload (Droit-Volet et al., 2024). Simultaneously, awe may induce a metacognitive experience of timelessness, a distinct subjective effect more closely tied to interoceptive processes and self-transcendence. We propose to map these dual effects thermodynamically: attentional overload decreases timing accuracy, while self-transcendent states can stabilize entropy.

Early work investigated the effect of increases in body temperature on time estimates (Hoagland, 1933). Increases in body temperature shortened the intervals produced, but lengthened duration estimates (Francois, 1927; Wearden & Penton-Voak, 1995). More recent studies support the stress-induced slowing of time perception (Ogden et al., 2019); however, the repeated anticipation of holidays can actually speed up time perception (Ogden et al., 2024).

To situate our approach within broader theoretical debates, we briefly compare our framework with several established models of time perception. The dual klepsydra model (Wackermann & Ehm, 2006) posits that interval timing arises from the gradual discharge of a leaky integrator, an analogy to fluid flowing through a clepsydra or water clock. In contrast, the DOIT (Dynamic Occupation in Time) model examines how the experience of time varies based on the qualities of activities and their context (Larson, 2004). Although our model differs in its thermodynamic formulation, these approaches recognize the irreversibility of time and the influence of dissipative processes.

Physical movement across species and tasks hastens time perception, improving timing accuracy (Robbe, 2023). The time perception rate is greatest in flow, a unique state where the balance of action, motivation, and confidence optimizes performance through spontaneous, coherent action flow (Failing & Theeuwes, 2016; Rutrecht, Wittmann, Khoshnoud, & Igarzábal, 2021). Similarly, Csikszentmihalyi's flow model describes the distortion of time during immersive tasks, wherein attentional focus and action-feedback loops yield an altered experience of time (Csikszentmihalyi, 1990). Our thermodynamic view reframes this by suggesting that flow arises at an equilibrium point between arousal (energy) and confidence (stability), facilitating seamless temporal integration.

As physical time is relative to spatial motion, psychological time is also relative to imaginary motion (Allingham et al., 2021; Spapé et al.,

2021). Imagining accelerating movement resulted in a relative overestimation of time, or time dilation, while decelerating movement elicited a relative underestimation, or time compression (Hallez et al., 2023). Our model posits that perceived time is regulated by energetic and informational constraints, offering a physicalist grounding through entropy and thermoregulation.

1.3. The role of entropy

Thermodynamic regulation is crucial for efficient brain functioning. Because the energy needs of neurons during intrinsic activities are orders of magnitude larger than during stimulation for all levels of cognition (de Lara, 2020; Raichle, 2010), rapid shifts from the brain's high-dimensional resting state to lower-dimensional evoked activities (Singer, 2021) facilitate optimal information transfer. Nevertheless, stable intrinsic activities expedite spontaneous recovery of the resting state. In this simplified view, intellect generation is based on information exchange with the external environment (Ahissar & Assa, 2016; Déli et al., 2017; Llinás and Paré, 1996; Northoff, 2018), with sensory and motor processing forming a thermodynamic cycle.

Renyi's informational entropy generalizes entropy by forming scalar exponent alpha (Jizba & Arimitsu, 2001). Baez (2011) demonstrated a direct relationship between Renyi's exponent alpha and inverse temperature beta (i.e., coldness). Intelligent information processing often involves a type of information erasure, inducing a sense of "coldness" while increasing overall neural organization (O'Neill and Schoth, 2022).

Rényi and Shannon's informational entropy can describe psychological and cognitive states, and predict task performance and mental well-being (Ince et al., 2017; Shannon, 1993). For instance, higher variability at rest (i.e., high entropic states) correlates with fluid intelligence (Wang et al., 2018; Yang et al., 2019) and openness (Zmigrod et al., 2019), but decreases in brain entropy are seen in compromised states of consciousness (Varley et al., 2020). For example, stress is analogous to time pressure, the inability to cope with the pace or intensity of sensory influx (Déli et al., 2018, 2021, 2022), demonstrating emotions' interconnection with the brain's energy and information processing. The following section will proceed to a discussion of time perception in motivation.

1.4. The thermodynamics of time perception

Studies on behavioral activation systems have revealed overestimation bias scores for both positive (Lehockey et al., 2018; Simen & Matell, 2016; van Hedger et al., 2017) and stress-inducing situations (Remmers & Zander, 2018; Wise et al., 2017). For instance, novel stimuli or rewards can dilate time perception through what is known as the 'oddball effect' (Failing & Theeuwes, 2016; Ma et al., 2024), with surprising or emotionally charged moments feeling as if time 'froze.' A similar sense of permanence occurs during stress (Hollis et al., 2015; Robbe, 2023). However, the psychology of these experiences contrasts dramatically. In a stressful context, dilated time perception evokes an unbearable sense of permanence, which triggers desperate escape behavior through impatience and sympathetic arousal (Gladhill et al., 2022; Hosseini Houripasand et al., 2023).

The dilation of time perception in both positive and negative states correlates with emotional intensity (Biderman et al., 2020; Déli & Kisvarday, 2020; Zanin et al., 2019), hinting at an underlying energy relationship (Toso et al., 2020). We want to note that some studies suggest that positive emotions do not influence time perception (Ogden et al., 2019). However, drug-induced expansion of time perception is indeed linked to elation. This correlation is significant as it suggests that the sense of spaciousness experienced during elation can lead to mental expansion, potentially giving rise to new ideas and creative insights (Green, Kavanagh, & Young, 2003). These findings underscore the connection between the expansion of time perception and elation, whether drug induced or arising from positive states.

Due to its mental energy boosting ability, musicians and artists have turned to cannabis to enhance creativity, supporting our original claim (Kowal et al., 2015). Moreover, the most pronounced alterations in time perception occur during emotional polarities, such as awe (Rudd et al., 2012) and depression (Stanghellini et al., 2016; Thönes & Oberfeld, 2015), when time appears to stand still (Fig. 1). These results inspired some scientists to suggest that depression is analogous to a positive spacetime curvature, even a black hole state (Déli, 2024; Kent, 2023).

The connection between emotions and energy is also evident in the fact that the perception of time slows down more significantly during the transition to negative states than during the states themselves (Gable & Poole, 2012; Wang & Lapate, 2024). Likewise, the cognitive challenge of withdrawal (Di Lernia et al., 2018; Gable et al., 2022) and sleep deprivation (Şen et al., 2023) dilate time perception. Our argument defines time perception as an even function, represented by a graph that remains unchanged under reflection in the y-axis (Fig. 1). Interestingly, our time perception curve is analogous to the upside-down U-shaped curve of the Yerkes-Dodson law, which relates performance to circulating levels of stress hormones (Beerendonk et al., 2024; Yerkes & Dodson, 1908; Lupien, Maheu, Mt, Fiocco, & Schramek, 2007). This similarity underscores the thermodynamic foundation of emotions' potential for motivation.

An intriguing question remains of why diverse experiences – from intense states of anxiety to the calmness inspired by awe or nature – dilate time perception (Bannister & Eerola, 2021; Davydenko & Peetz, 2017; Failing & Theeuwes, 2016; Mitchell et al., 2015; Rudd et al., 2012). For example, information overload during stressful states can cause difficulty concentrating and purposeful behavior (Nutt, 1999). Anxious people usually resort to impulsivity and meaningless, arbitrary actions until action motivation is halted in depression (Stanghellini et al., 2016; Wittmann & Paulus, 2008), implying an inverse relationship between mental adversity and the ability to change it.

In contrast, the low action motivation of positive mental states indicates energy frugality, which may explain their connection to parasympathetic restorative processes in long-term psychological well-being (Table I). A muted action motivation might represent some form of minimum energy path, analogous to the principle of stationary action in physics. At the curve's left minimum (Fig. 1), awe slows or pauses the subjective time. Contentment is an uncluttered, information-scarce experience that represents confidence in self-agency but lacks internal motivation.

Stress and contentment lie at opposite ends of an information-processing and action-motivation spectrum. It is a contradiction; those with the capacity to institute change (contentment) lack the desire, and

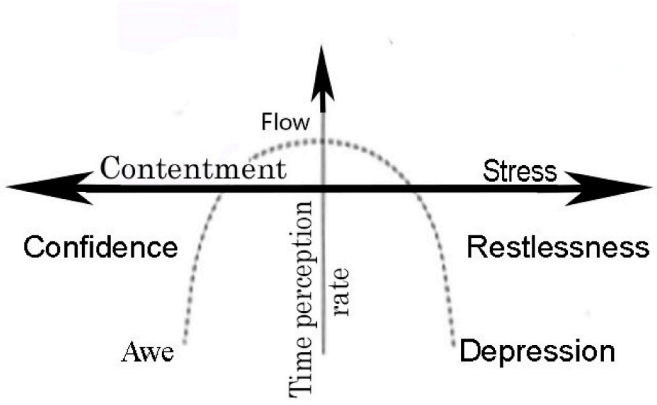

Fig. 1. The Psychology of Contentment and Stress Movement speeds up time perception (vertical arrow). In positive experiences, time perception reduces action motivation, culminating in awe. The pain of stress motivates action but weakens self-confidence. Anxiety can progress to depression when action motivation halts. A unique point of action, motivation, and confidence is flow.

Table 1
Binary choices supporting bodily and psychological equilibrium.

	Experience	
Symptom	**Positive arousal**	**Stress**
Time perception increases (Dilation) — Arousal	Parasympathetic	Action motivation
Time perception decreases (Compression) — Action	Flow	Sympathetic
Physiological symptoms (shivering, sweating)	Accomplishment	Shame, fear

those having the desire (stress) lack the agency. Gordon et al. (2023) confirmed the connection between decision-making and bodily functions and movement control: better body control (resulting in less stress) permits greater agency, and vice versa. In the following, we investigate the regulatory framework of motivation in more detail.

1.5. The binary regulation of higher cognitive functions

The timing of the giant fiber descending neuron spike determines whether a Drosophila evades a predator via a short or long takeoff (Ache et al., 2019). Bifurcations from geometric principles can elucidate behavior and decision-making across various species and ecological scenarios (Sridhar et al., 2021), and temperature and emotion regulation in mammals (Hesp et al., 2021; Kao et al., 2015; Sadowski et al., 2020). These spontaneous and abrupt "critical" transitions are linked with specific geometrical relationships. A shift from averaging vectorial information among options abruptly excludes one among the remaining choices. The brain repeatedly breaks down multi-choice decisions into a sequence of binary decisions. Binary regulation is an "on-off regulation," in systems with only two possible states, referring to "dichotomous thinking in psychology.

In mammals, stimulation of the PAG can induce relaxation or escape behavior. Frontal PAG stimulation inspires a relaxed, immobile posture due to the sense of excess time. However, lateral PAG stimulation also produces two typical behavioral responses. When there is sufficient time, increased blood pressure and heightened pain sensitivity facilitate escape and defensive responses (Zelena et al., 2018). An immediate threat, when escape is no longer possible, mutes pain sensitivity and triggers immobile freezing behavior. In people, anxiety can induce aggravation or an emotional collapse into depression, where both time perception and action motivation appear to halt (Stanghellini et al., 2016), providing further support for our thermodynamic argument.

Binary choices can generate multifaceted behavior regulation (summarized in Table 1), such as (1) dilation of time perception to accelerate or mute motivation, (2) contraction of time perception to mediate action toward completion, and (3) halting of action motivation or sudden cognitive changes. This regulation is based on psychological spin, utilizing the reversible perception cycle (Déli, 2023), which automatically restores psychological equilibrium, thereby forming a new balance. This model can explain regulatory complexity as a series of binary choices ad infinitum. Dopaminergic mechanisms, which can amplify the motivational power of emotions through subjective time perception, highlight how internal and external factors influence the multifaceted nature of experiences (Soares et al., 2016).

We must note that emotional and cognitive processes can trigger physiological symptoms, such as shivering, chills, sweating, and changes in body temperature (Schoeller & Perlovsky, 2016). In this context, physiological symptoms can be both tools and consequences of the brain's energy regulation.

2. Discussion

Decision-making from fruit flies to humans often boils down to binary choices based on geometric principles and critical transitions. The brain's cognitive cycle can maintain a bodily and psychological equilibrium through simple bifurcation stemming from geometric principles. Binary options can refine cognitive and intellectual evolution through learning, beliefs, and individual capabilities. Moreover, emotion, temperature, and physiological symptom regulation recover and maintain constant resting entropy. Therefore, as the thermodynamic cycle stabilizes the psyche around a new equilibrium after every decision-making, it increases or decreases synaptic complexity, confidence, and mental health.

Time perception can lead to contrasting behavioral outcomes during stress (information overload) or contentment (information scarcity). Negative emotions inspire arbitrary and chaotic performance, corresponding to wasteful energy use, which can accumulate and lead to adverse health effects associated with stress. In positive conditions, frugality of action motivation reflects a minimal energy path, allowing contentment and creativity. In contrast, faster time perception can manage ongoing action by inspiring cognitive coherence. Intrinsically motivating activities lead to deep engagement and enjoyment, a state known as flow, characterized by the intersection of motivational challenge, confidence, and passion (Csikszentmihalyi, 1990, 1997, p. 31). This is congruent to our hypothesis that ongoing action accelerates time perception. The time perception curve also overlaps Yerkes-Dodson law, providing further support to our thermodynamic foundation of emotions' potential for motivation. Emotions can be viewed as the fundamental forces of motivation.

By conceptualizing psychological processes through energy dynamics, we outlined how emotions can affect motivation through distorted time perception. Moreover, our thermodynamic model can explain how action motivation during stress can produce wasteful cognitive processes. The relationship between low entropy and compromised consciousness states (Varley et al., 2020), and depression (Wise et al., 2017) underlines the role of stress in mental problems.

Our model provides a framework for designing interventions and strategies that leverage the interconnections between emotions, motivation, and physiology. While our thermodynamic model focuses on the fundamental structural motivations underlying behavior, we acknowledge the importance of cultural norms, social expectations, and environmental factors in shaping emotional and motivational experiences.

Integrating these contextual factors is crucial to understanding the complex interplay between emotions, motivation, physiology, and subjective experiences across diverse cultural and environmental settings.

2.1. Limitations and future directions

We would like to acknowledge the lack of empirical validation and experimental testing of the proposed framework. Additionally, the generalizability of the model across diverse populations remains unexplored. This limitation is particularly salient in clinical populations, such as individuals with post-traumatic stress disorder (PTSD) or major depressive disorder, where disruptions in bodily awareness and emotional regulation may offer critical tests of the model's assumptions.

Our framework also does not yet account for the role of time perception for proprioceptive awareness—two domains that are increasingly recognized as central to embodied cognition and affective experience. Future empirical works in this area can further our understanding of the mind-body interaction.

While our thermodynamic model provides a novel framework for integrating emotions, motivation, and time perception, it remains theoretical and is subject to several significant limitations. Foremost, the model relies heavily on analogies between thermodynamic principles—such as entropy, temperature, and energy—and psychological processes. While conceptually illuminating, these analogies are not always supported by direct empirical evidence, which may limit their explanatory power. The complexity of human emotional and cognitive systems likely exceeds the reach of simplified energetic metaphors, especially when such models are extended to subjective constructs like time perception.

Additionally, many of the neurophysiological correlates we invoke, such as neural entropy or the functional role of dopamine in timing, are still under active investigation, and consensus regarding their interpretation is far from established. The use of entropy as a measure of cognitive or emotional flexibility, for example, depends on methodological choices that may not yet be standardized across studies. Furthermore, the model currently lacks specificity in distinguishing between clinical and non-clinical populations, and its generalizability across age groups, cultural settings, or neurodiverse profiles remains untested. Despite these constraints, the integrative potential of the framework may help bridge conceptual gaps between disparate findings in psychology, physiology, and neuroscience.

Nevertheless, our model opens several promising avenues for applied research and translational work. In mental health, for instance, subjective distortions of time perception are commonly reported in anxiety, depression, and trauma-related disorders. Our framework suggests that such distortions may reflect dysregulated entropy states in the brain, which could be targeted through interventions aimed at modulating physiological arousal and attentional engagement. Biofeedback, neurofeedback, or pharmacological interventions that stabilize neural entropy dynamics may be tested for their capacity to restore adaptive time perception and emotional regulation. Moreover, in performance contexts—such as athletic training, musical improvisation, or surgical decision-making—the model's emphasis on flow as a state of optimal energy alignment suggests practical strategies for cultivating cognitive and emotional balance. Future research should prioritize experimental designs that manipulate entropy-relevant parameters (e.g., arousal, cognitive load, ambient temperature) while recording physiological or neuroimaging data. Valuable direction would be to examine whether interventions known to improve well-being, such as mindfulness or physical activity, influence subjective time perception through measurable changes in brain signal complexity.

While our model identifies correlations between emotional states and physiological indices—such as body temperature and thermodynamic entropy—our current analysis stops short of demonstrating direct causal mechanisms. Establishing such causality will be the subject of future work, potentially using targeted experimental designs and interventions that modulate physiological parameters to observe downstream effects on affective and cognitive processes.

3. Conclusion

The thermodynamic analysis of cognition points to the existence of binary regulation. First observed in fruit flies and fish, this regulation can maintain bodily and psychological equilibrium and enable abstract decision-making by infinitely enhancing the details of regulatory complexity. A decision-making process based on geometric principles may be universal throughout biology and even physics, such as electromagnetism.

Our verifiable framework shows that identical dilation of time perception in arousing emotional states (such as anxiety and joy) and calming experiences (like awe and natural environments) can give rise to varied motivation and offer nuanced decision-making. Time perception, a function of information processing and entropic factors, potentiates the ability for motivation. The muting of action motivation in positive states represents energy frugality, or a minimum energy path, which might be analogous to the principle of stationary action in physics. In contrast, stress represents information overload, spurring chaotic decision-making and action motivation, which wastes effort and energy. Our model suggests that emotions have a thermodynamic foundation, rendering them the driving forces of motivation.

The implications of our model extend beyond theoretical understanding, offering potential avenues for addressing mental health challenges and optimizing well-being through interventions that leverage the interconnections between emotions, motivation, and physiology. Our thermodynamic perspective originates emotions and their long-term mental consequences in the energy-information dynamics of the brain. It opens new avenues for interdisciplinary and innovative approaches to understanding and optimizing human functioning. Finally, it can inspire novel approaches in artificial intelligence research.

CRediT authorship contribution statement

Eva Déli: Writing – review & editing, Writing – original draft, Visualization, Data curation, Conceptualization. **Felix Schoeller:** Writing – review & editing, Writing – original draft, Investigation. **Adam Safron:** Writing – review & editing, Writing – original draft. **Abhinandan Jain:** Writing – review & editing, Writing – original draft. **Arturo Tozzi:** Writing – review & editing, Writing – original draft. **Vladimir Adrien:** Writing – review & editing, Writing – original draft. **Nicco Reggente:** Writing – review & editing, Writing – original draft, Visualization, Project administration.

Data availability

No data was used for the research described in the article.

References

Ache, J. M., Polsky, J., Alghailani, S., Parekh, R., Breads, P., Peek, M. Y., Bock, D. D., Reyn, C. R., & Card, G. M. (2019). Neural basis for looming size and velocity encoding in the drosophila giant Fiber escape pathway. *Current Biology, 29*, 1073–1081.e4.

Ahissar, E., & Assa, E. (2016). Perception as a closed-loop convergence process. *eLife, 5*, Article e12830. https://doi.org/10.7554/eLife.12830

Allingham, E., Hammerschmidt, D., & Wöllner, C. (2021). Time perception in human movement: Effects of speed and agency on duration estimation. *Quarterly Journal of Experimental Psychology A, 74*(3), 559–572.

An, S. J., & Kim, D. (2011). Alterations in serotonin receptors and transporter immunoreactivities in the hippocampus in the rat unilateral hypoxic-induced epilepsy model. *Cellular and Molecular Neurobiology, 31*(8), 1245–1255. https://doi.org/10.1007/s10571-011-9726-x

Bannister, S., & Eerola, T. (2021). Vigilance and social chills with music: Evidence for two types of musical chills. *Psychology of Aesthetics, Creativity, and the Arts, 17*(2), 242–258. https://doi.org/10.1037/aca0000421.

Barrett, L. F. (2017). *Social Cognitive and Affective Neuroscience, 12*(1), 1–23.

Beerendonk, L., Mejías, J. F., Nuiten, S. A., de Gee, J. W., Fahrenfort, J. J., & van Gaal, S. (2024). A disinhibitory circuit mechanism explains a general principle of peak 726 performance during mid-level arousal. *Proceedings of the National Academy of Sciences of the United States of America, 121*, Article e2312898121, 725.
Biderman, N., Bakkour, A., & Shohamy, D. (2020). What are memories for? The hippocampus bridges past experience with future decisions. *Trends in Cognitive Sciences, 24*, 542–556.
Briese, E. (1995). Emotional hyperthermia and performance in humans. *Physiology and Behavior, 58*, 615–618.
Briese, E., & Cabanac, M. (1991). Stress hyperthermia: Physiological arguments that it is a fever. *Physlogical Behaviour, 49*, 1153–1157.
Brown, R., & Thorsteinsson, E. (2020). Arousal states, symptoms, behaviour, sleep and body temperature. In R. Brown, & E. Thorsteinsson (Eds.), *Comorbidity*. Cham: Palgrave Macmillan. https://doi.org/10.1007/978-3-030-32545-9_7.
Cabanac, M. (1999). Emotion and phylogeny. *Journal of Consciousness Studies, 6*(6–7), 176–190.
Clavijo-Baque, S., & Bozinovic, F. (2012). Testing the fitness consequences of the thermoregulatory and parental care models for the origin of endothermy. *PLoS, ONE, 7*.
Comtesse, H., Powell, S., Soldo, A., Hagl, M., & Rosner, R. (2019). Long-term psychological distress of Bosnian war survivors: An 11-year follow-up of former displaced persons, returnees, and stayers. *BMC Psychiatry, 19*.
Corcoran, J., & Zahnow, R. (2022). Weather and crime: A systematic review of the empirical literature. *Crime Sci, 11*, 16. https://doi.org/10.1186/s40163-022-00179-8
Csikszentmihalyi, M. (1990). *Flow: The psychology of optimal experience*. New York: Harper & Row.
Csikszentmihalyi, M. (1997). *Finding flow: The psychology of engagement with everyday life* (1st ed., p. 31). New York: Basic Books, 978-0-465-02411-7.
Cui, X., Yin, T., et al. (2023). The role of valence, arousal, stimulus type, and temporal paradigm in the effect of emotion on time perception: A meta-analysis. *Psychonomic Bulletin & Review, 30*, 1–21.
D'Agostino, O., Castellotti, S., & Del Viva, M. M. (2023). Time estimation during motor activity. *Frontiers in Human Neuroscience, 17*, Article 1134027. Apr 21.
Davydenko, M., & Peetz, J. (2017). Time grows on trees: The effect of nature settings on time perception. *Journal of Environmental Psychology, 54*, 20–26.
de Lara, A. C. (2020). Interpreting the high energy consumption of the brain at rest. *Proceedings, 46*(1), 30. https://doi.org/10.3390/ecea-5-06694
Déli, E. (2024). *Emotional reasoning; insight into the conscious experience*. CRC Publishing.
Déli, E. K. (2023). What is psychological spin? A thermodynamic framework for emotions and social behavior. *Psych, 5*(4), 1224–1240.
Déli, E., & Kisvarday, Z. (2020). The thermodynamic brain and the evolution of intellect: The role of mental energy. *Cognitive Neurodynamics*. https://doi.org/10.1007/s11571-020-09637-y
Déli, E., Peters, J., & Kisvarday, Z. (2021). The thermodynamics of cognition: A mathematical treatment. *Computational and Structural Biotechnology Journal, 19*, 784–793.
Déli, É., Peters, J. F., & Kisvárday, Z. F. (2022). How the brain becomes the mind: Can thermodynamics explain the emergence and nature of emotions? *Entropy, 24*.
Déli, E., Peters, J., & Tozzi, A. (2017). Relationships between short and fast brain timescales. *Cognitive Neurodynamics, 11*, 539.
Déli, E., Peters, J., & Tozzi, A. (2018). The thermodynamic analysis of neural computation. *J Neurosci Clin Res., 3*, 1.
Dempsey, W. P., Du, Z., Nadtochiy, A., et al. (2022). Regional synapse gain and loss accompany memory formation in larval zebrafish. *PNAS, 119*(3), Article e2107661119.
Di Lernia, D., Serino, S., Pezzulo, G., Pedroli, E., Cipresso, P., & Riva, G. (2018). Feel the time. Time perception as a function of interoceptive processing. *Frontiers in Human Neuroscience, 12*.
Droit-Volet, S., Dambrun, M., & Monier, F. (2024). Awe and time perception. *Acta Psychologica, 245*, Article 104232. https://doi.org/10.1016/j.actpsy.2024.104232
Droit-Volet, S., & Fayolle, S. (2024). The conscious awareness of time distortions regulates the effect of emotion on the perception of time. *Acta Psychologica, 245*, Article 104232.
Escobar, F. B., Velasco, C., Motoki, K., & Wang, Q. J. (2020). The temperature of emotions. *PLoS One, 16*.
Failing, M., & Theeuwes, J. (2016). Reward alters the perception of time. *Cognition, 148*, 19–26.
Farmer, C. G. (2020). Parental care, destabilizing selection, and the evolution of tetrapod endothermy. *Physiology, 35*(3), 160–176.
Fazekas, C. L., Bellardie, M., Török, B., et al. (2021). Pharmacogenetic excitation of the median raphe region affects social and depressive-like behavior and core body temperature in Male mice. *Life Sciences*, Article 120037.
Fossat, P., Bacqué-Cazenave, J., Deurwaerdère, P. D., Cattaert, D., & Delbecque, J. (2015). Serotonin, but not dopamine, controls stress response and anxiety-like behavior in crayfish, Procambarus clarkii. *Journal of Experimental Biology*. https://doi.org/10.1242/jeb.120550
Fredericks, C. A., Sturm, V. E., Brown, J. A., Hua, A. Y., Bilgel, M., Wong, D. F., … Seeley, W. W. (2018). Early affective changes and increased connectivity in preclinical Alzheimer's disease. *Alzheimer's & Dementia : Diagnosis, Assessment & Disease Monitoring, 10*, 471–479.
Frosini, M., Sesti, C., Palmi, M., Valoti, M., Fusi, F., Mantovani, P., Bianchi, L., Della, C. L., & Sgaragli, G. (2000). The possible role of taurine and GABA as endogenous cryogens in the rabbit: Changes in CSF levels in heat-stress. *Advances in Experimental Medicine and Biology, 483*, 335–344.
Gable, P. A., & Poole, B. D. (2012). Perception time flies when you're having approach-motivated fun. *Effects of Motivational Intensity on Time, 23*(8). https://doi.org/10.1177/0956797611435817
Gable, P. A., Wilhelm, A. L., & Poole, B. D. (2022). How does emotion influence time perception? A review of evidence linking emotional motivation and time processing. *Frontiers in Psychology, 13*.
Gibbon, J., Church, R. M., & Meck, W. H. (1984). Scalar timing in memory. *Annals of the New York Academy of Sciences, 423*(1), 52–77.
Gibbon, J., Church, R. M., & Meck, W. H. (1984). Scalar timing in memory. *Annals of the New York Academy of Sciences, 423*(1), 52–77.
Gladhill, K. A., Mioni, G., & Wiener, M. (2022). Dissociable effects of emotional stimuli on electrophysiological indices of time and decision-making. *PLoS One, 17*.
Gordon, E. M., Chauvin, R. J., Van, A. N., et al. (2023). A somato-cognitive action network alternates with effector regions in motor cortex. *Nature, 617*, 351–359.
Green, B., Kavanagh, D., & Young, R. (2003). Being stoned: A review of self-reported cannabis effects. *Drug and Alcohol Review, 22*(4), 453–460.
Grigg, G., Nowack, J., Bicudo, J., Bal, N. C., Woodward, H. N., & Seymour, R. S. (2021). Whole-body endothermy: Ancient, homologous and widespread among the ancestors of mammals, birds and crocodylians. *Biological Reviews, 97*.
Groenink, L., Compaan, J., van der Gugten, J., Zethof, T., van der, H. J., & Olivier, B. (1995). Stress-induced hyperthermia in mice pharmacological and endocrinological aspects. *Annals of the New York Academy of Sciences, 771*, 252–256.
Hall, M., Vasko, R., Buysse, D., Ombao, H., Chen, Q., et al. (2004). Acute stress affects heart rate variability during sleep. *Psychosomatic Medicine, 66*, 56–62.
Hallez, Q., Paucsik, M., Tachon, G., Shankland, R., Marteau-Chasserieau, F., & Plard, M. (2023). How physical activity and passion color the passage of time: A response with ultra-trail runners. *Frontiers in Psychology, 13*.
Hesp, C., Smith, R., Parr, T., Allen, M., Friston, K., & Ramstead, M. J. (2021). Deeply felt affect: The emergence of valence in deep active inference. *Neural Computation, 33*, 1–49.
Hoagland, H. (1933). The physiological control of judgments of duration: Evidence for a chemical clock. *The Journal of General Psychology, 9*, 267–287.
Hollis, F., van der Kooij, M. A., Zanoletti, O., Lozano, L., Cantó, C., & Sandi, C. (2015). Mitochondrial function in the brain links anxiety with social subordination. *Proceedings of the National Academy of Sciences, 112*, 15486–15491.
Hosseini Houripasand, M., Sabaghypour, S., Farkhondeh Tale Navi, F., & Nazari, M. A. (2023). Time distortions induced by high-arousing emotional compared to low-arousing neutral faces: An event-related potential study. *Psychological Research*, 1–12.
Huang, Z., Zhang, J., Wu, J., Mashour, G. A., & Hudetz, A. G. (2020). Temporal circuit of macroscale dynamic brain activity supports human consciousness. *Science Advances, 6*, Article eaaz0087.
Inagaki, T. K., Hazlett, L. I., & Andreescu, C. (2019). Naltrexone alters responses to social and physical warmth: Implications for social bonding. *Social Cognitive and Affective Neuroscience, 14*, 471–479.
Ince, R. A., Giordano, B. L., Kayser, C., Rousselet, G. A., Gross, J., & Schyns, P. G. (2017). A statistical framework for neuroimaging data analysis based on mutual information estimated via a gaussian copula. *Human Brain Mapping, 38*(3), 1541–1573.
Jizba, P., & Arimitsu, T. (2001). *The world according to Renyi: thermodynamics of fractal systems, 597* pp. 341–348). AIP Conference Proceedings.
Kao, F.-C., Wang, S. R., & Chang, Y. (2015). Brainwaves analysis of positive and negative emotions. *ISAA*, (12), 1263–1266.
Kataoka, N., Shima, Y., Nakajima, K., & Nakamura, K. (2020). A central master driver of psychosocial stress responses in the rat. *Science, 367*, 1105–1112.
Keller, A. S., Leikauf, J. E., Holt-Gosselin, B., Staveland, B. R., & Williams, L. (2019). Paying attention to attention in depression. *Translational Psychiatry, 9*.
Kent, L. (2023). Mental gravity: Depression as spacetime curvature of the self, mind, and brain. *Entropy, 25*(9), 1275.
Kluger, M. J., O'Reilly, B. J., Shope, T. R., & Vander, A. J. (1987). Further evidence that stress hyperthermia is a fever. *Physiology & Behavior, 39*(6), 763–766. https://doi.org/10.1016/0031-9384(87)90263-0
Kowal, M. A., Hazekamp, A., Colzato, L. S., et al. (2015). Cannabis and creativity: Highly potent cannabis impairs divergent thinking in regular cannabis users. *Psychopharmacology (Berl)., 232*(6), 1123–1134.
Larson, E. A. (2004). The time of our lives: The experience of temporality in occupation. *Can J Occup Ther. Feb, 71*(1), 24–35.
Lehockey, K. A., Winters, A. R., Nicoletta, A. J., et al. (2018). The effects of emotional states and traits on time perception. *Brain Inf, 5*, 9.
The brain as a closed system modulated by the senses. In Llinás, R., & Paré, D. and M. (Eds.), *The churchlands and their critics*, (1996). Cambridge, Mass: Blackwell Publishers.
Lowry, C. A., Lightman, S. L., & Nutt, D. J. (2009). That warm fuzzy feeling: Brain serotonergic neurons and the regulation of emotion. *Journal of Psychopharmacology, 23*, 392–400.
Lupien, S. J., Maheu, F. S., Mt, T., Fiocco, A. J., & Schramek, T. E. (2007). The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. *Brain and Cognition, 65*, 209–237.
Ma, A. C., Cameron, A. D., & Wiener, M. (2024). Memorability shapes perceived time (and vice versa). *Nature Human Behaviour*. https://doi.org/10.1038/s41562-024-01863-2
Madden, C. J., & Morrison, S. F. (2019). Central nervous system circuits that control body temperature. *Neuroscience Letters, 696*, 225–232.
Martinelli, N., & Droit-Volet, S. (2022). Judgment of duration and passage of time in prospective and retrospective conditions and its predictors for short and long durations. *Scientific Reports, 12*, Article 22241.

Mason, A. E., Kasl, P., Soltani, S., et al. (2024). Elevated body temperature is associated with depressive symptoms: Results from the TemPredict study. *Scientific Reports, 14*, 1884. https://doi.org/10.1038/s41598-024-51567-w
Meeusen, R., Van Cutsem, J., & Roelands, B. (2020). Endurance exercise-induced and mental fatigue and the brain. *Experimental Psychology, 106*, 2294–2298.
Mitchell, J. M., Weinstein, D., Vega, T. A., & Kayser, A. S. (2015). Dopamine, time perception, and future time perspective. *Psychopharmacology, 235*, 2783–2793.
Moe, R. O., & Bakken, M. (1997). Effects of handling and physical restraint on rectal temperature, cortisol, glucose and leucocyte counts in the silver fox (vulpes vulpes). *Acta Veterinaria Scandinavica, 38*, 29–39.
Morimoto, A., Watanabe, T., Morimoto, K., Nakamori, T., & Murakami, N. (1991). Possible involvement of prostaglandins in psychological stress-induced responses in rats. *The Journal of Physiology, 443*(1), 421–429.
Nashiro, K., Min, J., Yoo, H. J., et al. (2022). Increasing coordination and responsivity of emotion-related brain regions with a heart rate variability biofeedback randomized trial. *Cognitive, Affective, & Behavioral Neuroscience, 23*, 66–83.
Nestler, E. J., & Russo, S. J. (2024). Neurobiological basis of stress resilience. *Neuron, 112*, 1911–1929.
Northoff, G. (2018). Is our brain an open or closed system? Prediction model of brain and world–brain relation. In *The spontaneous brain*. MIT press.
Northoff, G., & Tumati, S. (2019). "Average is good, extremes are bad" – non-Linear inverted U-shaped relationship between neural mechanisms and functionality of mental features. *Neuroscience & Biobehavioral Reviews, 104*, 11–25.
Nowack, J., Giroud, S., Arnold, W., & Ruf, T. (2017). Muscle non-shivering thermogenesis and its role in the evolution of endothermy. *Frontiers in Physiology, 8*.
Nutt, D. J. (1999). Care of depressed patients with anxiety symptoms. *Journal of Clinical Psychiatry, 60*(Suppl 17), 23–27. ; discussion 46-8.
O'Connor, D. B., Thayer, J. F., & Vedhara, K. (2020). Stress and health: A review of psychobiological processes. *Annual Review of Psychology*.
Ogden, R., Alatrany, S. S. J., Flaiyah, A. M., Ali, S., Aldrraji, H., Musa, H., Alatrany, A. S. S., et al. (2024). Distortions to the passage of time for annual events: Exploring why christmas and ramadan feel like they come around more quickly each year. *PLoS One, 19*(7), Article e0304660.
Ogden, R. S., Henderson, J., McGlone, F., & Richter, M. (2019). Time distortion under threat: Sympathetic arousal predicts time distortion only in the context of negative, highly arousing stimuli. *PLoS One, 14*(5), Article e0216704.
Oka, T., Oka, K., & Hori, T. (2001). Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosomatic Medicine, 63*(3), 476–486. https://doi.org/10.1097/00006842-200105000-00018
Paasche, C., Weibel, S., Wittmann, M., & Lalanne, L. (2019). Time perception and impulsivity: A proposed relationship in addictive disorders. *Neuroscience & Biobehavioral Reviews, 106*, 182–201. Nov.
Parrott, R. F., Vellucci, S. V., Forsling, M. L., & Goode, J. A. (1995). Hyperthermic and endocrine effects of intravenous prostaglandin administration in the pig. *Domestic Animal Endocrinology, 12*, 197–205.
Quadt, L., Critchley, H. D., & Nagai, Y. (2022). Cognition, emotion, and the central autonomic network. *Autonomic Neuroscience, 238*.
Raichle, M. E. (2010). Two views of brain function. *Trends in Cognitive Sciences, 14*(4), 180–190. https://doi.org/10.1016/j.tics.2010.01.008. Epub 2010 Mar 4. PMID: 20206576.
Raison, C. L., Hale, M. W., Williams, L. E., Wager, T. D., & Lowry, C. A. (2015). Somatic influences on subjective well-being and affective disorders: The convergence of thermosensory and central serotonergic systems. *Frontiers in Psychology, 5*, 1589.
Remmers, C., & Zander, T. (2018). Why you don't see the forest for the trees when you are anxious: Anxiety impairs intuitive decision making. *Clinical Psychological Science, 6*, 48–62.
Robbe, D. (2023). Lost in time: Relocating the perception of duration outside the brain. *Neuroscience & Biobehavioral Reviews, 153*, Article 105312.
Rudd, M., Vohs, K. D., & Aaker, J. L. (2012). Awe expands people's perception of time, alters decision making, and enhances well-being. *Psychological Science, 23*, 1130–1136.
Rutrecht, H. M., Wittmann, M., Khoshnoud, S., & Igarzábal, F. A. (2021). Time speeds up during flow states: A study in virtual reality with the video game thumper. *Timing & Time Perception, 9*(4), 353–376.
Sadowski, S., Fennis, B. M., & van Ittersum, K. (2020). Losses tune differently than gains: How gains and losses shape attentional scope and influence goal pursuit. *Cognition & Emotion, 34*, 1439–1456.
Schoeller, F., & Perlovsky, L. I. (2016). Aesthetic chills: Knowledge-acquisition, meaning-making, and aesthetic emotions. *Frontiers in Psychology, 7*.
Seebacher, F. (2009). Responses to temperature variation: Integration of thermoregulation and metabolism in vertebrates. *Journal of Experimental Biology, 212*, 2885–2891.
Seebacher, F. (2020). Is endothermy an evolutionary by-product? *Trends in Ecology & Evolution, 35*(6), 503–511.
Şen, B., Kurtaran, N. E., & Öztürk, L. (2023). The effect of 24-hour sleep deprivation on subjective time perception. *International Journal of Psychophysiology, 192*, 91–97. https://doi.org/10.1016/j.ijpsycho.2023.08.011
Simen, P., & Matell, M. (2016). Why does time seem to fly when we're having fun? *Science, 354*(6317), 1231–1232.
Singer, W. (2021). Recurrent dynamics in the cerebral cortex: Integration of sensory evidence with stored knowledge. *PNAS, 118*.
Soares, S., Atallah, B. V., & Paton, J. J. (2016). Midbrain dopamine neurons control judgment of time. *Science, 354*, 1273–1277.
Spapé, M. M., Harjunen, V. J., & Ravaja, N. (2021). Time to imagine moving: Simulated motor activity affects time perception. *Psychonomic Bulletin & Review, 29*, 819–827.
Sridhar, V. H., Li, L., Gorbonos, D., Nagy, M., Schell, B. R., Sorochkin, T., Gov, N. S., & Couzin, I. D. (2021). The geometry of decision-making in individuals and collectives. In *Proceedings of the national academy of sciences of the United States of America* (Vol. 118).
Stanghellini, G., Ballerini, M., Presenza, S., Mancini, M., Northoff, G., & Cutting, J. (2016). Abnormal time experiences in major depression. An empirical qualitative study. *Psychopathology*. https://doi.org/10.1159/000452892
Terlouw, E. M., Kent, S., Cremona, S., & Dantzer, R. (1996). Effect of intracerebroventricular administration of vasopressin on stress-induced hyperthermia in rats. *Physiology and Behavior, 60*, 417–424.
Thönes, S., & Oberfeld, D. (2015). Time perception in depression: A meta-analysis. *Journal of Affective Disorders, 175*, 359–372.
Toso, A., Fassihi, A., Paz, L., Pulecchi, F., & Diamond, M. E. (2020). A sensory integration account for time perception. *PLoS Computational Biology, 17*.
van der Heyden, J. A., Zethof, T. J., & Olivier, B. (1997). Stress-induced hyperthermia in singly housed mice. *Physiology and Behavior, 62*, 463–470.
van Hedger, K., Necka, E. A., Barakzai, A. K., & Norman, G. J. (2017). The influence of social stress on time perception and psychophysiological reactivity. *Psychophysiology, 54*(5), 706–712. https://doi.org/10.1111/psyp.12836
Varley, T. F., Carhart-Harris, R., Roseman, L., Menon, D. K., & Stamatakis, E. A. (2020). Serotonergic psychedelics LSD & psilocybin increase the fractal dimension of cortical brain activity in spatial and temporal domains. *NeuroImage, 220*(October), Article 117049.
Wackermann, J., & Ehm, W. (2006). The dual klepsydra model of internal time representation and time reproduction. *Journal of Theoretical Biology, 239*(4), 482–493.
Wang, D. J., Jann, K., Fan, C., et al. (2018). Neurophysiological basis of multiscale entropy of brain complexity and its relationship with functional connectivity. *Frontiers in Neuroscience, 12*, 352.
Francois. (1927). In J. H. Wearden (Ed.), *Timing & time perception: Vol. 2019. Body temperature and the sense of time. Translated*.
Wearden, J. H., & Penton-Voak, I. S. (1995). Feeling the heat: Body temperature and the rate of subjective time, revisited. *Quarterly Journal of Experimental Psychology B Comparative and Physiological Psychology, 48B*(2), 129–141.
Williams, L. E., & Bargh, J. A. (2008). Experiencing physical warmth promotes interpersonal warmth. *Science, 322*(5901), 606.
Wise, T., Marwood, L., Perkins, A. M., et al. (2017). Nstability of default mode network connectivity in major depression: A two-sample confirmation study. *Translational Psychiatry, 25*(7), Article e1105, 4.
Wittmann, M. (2009). The inner experience of time: Neural mechanisms and psychological processes. *Philosophical Transactions of the Royal Society B, 364*(1525), 1955–1967.
Wittmann, M. (2015). Modulations of the experience of self and time. *Consciousness and Cognition, 38*, 172–181.
Wittmann, M., & Paulus, M. P. (2008). Decision making, impulsivity and time perception. *Trends Cogn Sci. Jan, 12*(1), 7–12.
Yang, S., Zhao, Z., & Cui, H. (2019). Temporal variability of cortical gyral-sulcal resting state functional activity correlates with fluid intelligence. *Frontiers in Neural Circuits, 13*, 36.
Yerkes, R. M., & Dodson, J. D. (1908). The relation of strength of stimulus to rapidity of habit-formation. *Journal of Comparative Neurology and Psychology, 18*(5), 459–482. Lupien SJ).
Zanin, M., Güntekin, B., Aktürk, T., Hanoğlu, L., & Papo, D. (2019). Time irreversibility of resting-state activity in the healthy brain and pathology. *Frontiers in Physiology, 10*.
Zelena, D., Menant, O., Andersson, F., & Chaillou, E. (2018). Periaqueductal gray and emotions: The complexity of the problem and the light at the end of the tunnel, the magnetic resonance imaging. *Endocrine Regulations, 52*, 222–238.
Zmigrod, L., Zmigrod, S., Rentfrow, P. J., & Robbins, T. (2019). The psychological roots of intellectual humility: The role of intelligence and cognitive flexibility. *Personality and Individual Differences, 141*, 200–208.
Wang, J., & Lapate, R. C. (2024). Emotional state dynamics impacts temporal memory. *Cognition and Emotion, 39*, 136–155.
Shannon, C. E. (1993). Collected Papers. In Claude E. Shannon (Ed.), *vol. 7. Coding Theorems for a Discrete Source With a Fidelity Criterion Institute of Radio Engineers, International Convention Record* (pp. 325–350). IEEE.
Baez, J. C. (2011). Entropy and Free Energy. *arXiv:1102.2098 [quant-ph]*.

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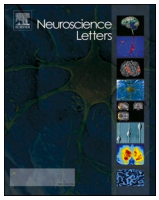

Takens' theorem to assess EEG traces: Regional variations in brain dynamics

Arturo Tozzi [a,*], Ksenija Jaušovec [b]

[a] Center for Nonlinear Science, Department of Physics, University of North Texas, 1155 Union Circle, #311427, Denton, TX 76203-5017, USA
[b] University of Maribor, Department of Psychology, Slovenia

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ABSTRACT

Takens' theorem (TT) proves that the behaviour of a dynamical system can be effectively reconstructed within a multidimensional phase space. This offers a comprehensive framework for examining temporal dependencies, dimensional complexity and predictability of time series data. We applied TT to investigate the physiological regional differences in EEG brain dynamics of healthy subjects, focusing on three key channels: FP1 (frontal region), C3 (sensorimotor region), and O1 (occipital region). We provided a detailed reconstruction of phase spaces for each EEG channel using time-delay embedding. The reconstructed trajectories were quantified through measures of trajectory spread and average distance, offering insights into the temporal structure of brain activity that traditional linear methods struggle to capture. Variability and complexity were found to differ across the three regions, revealing notable regional variations. FP1 trajectories exhibited broader spreads, reflecting the dynamic complexity of frontal brain activity associated with higher cognitive functions. C3, involved in sensorimotor integration, displayed moderate variability, reflecting its functional role in coordinating sensory inputs and motor outputs. O1, responsible for visual processing, showed constrained and stable trajectories, consistent with repetitive and structured visual dynamics. These findings align with the functional specialization of different cortical areas, suggesting that the frontal, sensorimotor and occipital regions operate with autonomous temporal structures and nonlinear properties. This distinction may have significant implications for advancing our understanding of normal brain function and enhancing the development of brain-computer interfaces. In sum, we demonstrated the utility of TT in revealing regional variations in EEG traces, underscoring the value of nonlinear dynamics.

Significance statement

The novelty of this study is that we applied Takens' theorem to investigate the differences in phase space features among different EEG channels of healthy subjects.

1. Introduction

The human brain operates as a sophisticated nonlinear system, adept at handling extensive information via dynamic interactions [4,7,13,22]. Electroencephalography (EEG) serves as a non-invasive, high-resolution method for investigating brain activity. Nonetheless, conventional linear analysis techniques frequently fall short in representing the intricate nonlinear features of EEG signals [3]. To address this limitation, nonlinear dynamics and chaos theory have emerged as powerful frameworks for understanding brain activity, with Takens' theorem (henceforward TT) providing a cornerstone. TT establishes that the behavior of a dynamical system can be reconstructed in a multidimensional phase space using time-delayed versions of a single time series from the observed data [20]. In the context of EEG analysis, TT provides a robust mathematical tool to study temporal evolution, revealing properties that linear methods cannot uncover [19]. By reconstructing the phase space, researchers can analyze key EEG dynamical properties such as temporal dependencies, dimensional complexity and predictability [14]. This approach has proven valuable for identifying changes in neural dynamics associated with various cognitive and pathological conditions [8].

Previous research has highlighted the effectiveness of TT in analyzing EEG signals, especially for identifying pathological conditions like epilepsy, Alzheimer's disease and schizophrenia [1,2,5,11]. However, less attention has been given to the application of this approach for

* Corresponding author.
E-mail addresses: tozziarturo@libero.it (A. Tozzi), ksenijamarijausovec@gmail.com (K. Jaušovec).

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assessing regional variations in brain dynamics under normal conditions. Different brain regions exhibit distinct patterns of electrical activity, reflecting their specialized roles in cognition, sensation and motor function. For instance, the frontal region (FP1) is associated with higher cognitive processes such as decision-making and working memory. The sensorimotor cortex (C3) governs movement and integrates sensory inputs, while the occipital region (O1) processes visual information. Despite their unique roles, interactions among these regions contribute to the brain's global dynamics.

Comparing the spread and trajectory of phase space trajectories across different brain regions can shed light on how functional specialization translates into distinct dynamical features. In this study, we apply TT to assess EEG traces from three key brain regions: FP1, C3 and O1. We reconstruct phase spaces and analyze the temporal structure and complexity of the trajectories. Key metrics such as trajectory spread were computed to quantify regional differences in brain dynamics.

The rationale for using TT lies in the fact that overlapping trajectories reveal shared dynamical patterns across the EEG traces, suggesting common temporal structures in brain activity. These patterns likely arise from similar neural processes or consistent experimental conditions and reflect shared features of brain dynamics that remain consistent across individuals or trials. Conversely, differences in the spread or divergence of trajectories highlight variability in the dynamics of EEG signals, capturing subject-specific neural activity. This variability can serve as a marker for distinguishing between populations, such as healthy versus pathological groups, and is particularly relevant for identifying neural behaviors associated with specific cognitive states, tasks or conditions. Trajectories that densely fill the phase space indicate higher complexity, which is often linked to intricate neural processes and sophisticated information management. In contrast, smoother and more confined trajectories suggest simpler, more periodic or more deterministic behavior, highlighting a system with lower complexity but higher predictability. Together, these observations shed light on the dynamic interplay between stability, variability and complexity in brain activity, offering a deeper understanding of its temporal and spatial organization.

In the following sections, we detail the methods used for EEG data collection, phase space reconstruction and analysis, followed by the presentation and discussion of our results.

2. Subjects and methods

This study investigates the regional variations in brain dynamics by applying Takens' theorem to EEG data, focusing on three key channels: FP1 (frontal), C3 (sensorimotor), and O1 (occipital). The data consisted in the retrospective evaluation of ten EEG traces. EEG recordings were obtained from ten healthy, right-handed volunteers (mean age: 20.1 years; SD = 1.1; age range: 18–22 years; 5 males). For additional information on the participants and EEG methodologies, see Jaušovec and Jaušovec [10] and Tozzi et al. [21]. The signals were captured using a 64-channel EEG system, adhering to the standard 10–20 electrode placement system. Preprocessing included artifact removal, such as eye blinks and muscle movements through independent component analysis and band-pass filtering (0.5–50 Hz).

After preprocessing, the analysis focused on reconstructing the phase space of each EEG trace. TT was employed to create a multidimensional representation of the system's dynamics using delay embedding. The first step involved determining for each signal the optimal time delay (τ), a critical parameter in phase space reconstruction, as it defines the separation between consecutive points in the reconstructed dimensions [15]. The mutual information method was used to calculate τ, as it effectively identifies the point at which the time series exhibits the least redundancy while retaining information about the system's dynamics. For all channels and traces, τ was consistently found to be 25, suggesting a shared temporal dependency structure across the EEG signals.

The next step was to determine the embedding dimension (d), which represents the number of dimensions required to unfold the systems dynamics without overlap in the reconstructed phase space [17]. The False Nearest Neighbors (FNN) method was used for this purpose [19]. By analyzing the proportion of neighbors that remain close when the dimensionality is increased, FNN identifies the point at which the embedding dimension captures the true dynamics of the system. The results consistently indicated $d = 6$ for all traces, suggesting a moderate level of complexity in the underlying neural dynamics.

Starting from the parameters $\tau = 25$ and $d = 6$, the phase space for each EEG trace was reconstructed and visualized in three dimensions by selecting the first three delay coordinates. The trajectories were analyzed to extract quantitative metrics characterizing the temporal structure of the phase spaces. Specifically, the spread of the trajectories in each dimension and the average distance between consecutive points were computed. The spread reflects the range of dynamical variability, while the average distance provides insight into the smoothness and temporal evolution of the signals.

3. Tools and statistical analysis

Computational analysis for precise quantification of regional variations in brain dynamics was implemented using Python, leveraging libraries for signal processing, nonlinear analysis and statistical evaluation. Statistical analysis was performed to compare the phase space metrics among the FP1, C3 and O1 channels. Welch's t-tests were used to evaluate differences in trajectory spread across dimensions (x, y, z) among the channels. Welch's *t*-test was chosen because it does not assume equal variances between groups, thus ensuring robustness in the presence of heterogeneity in EEG signals.

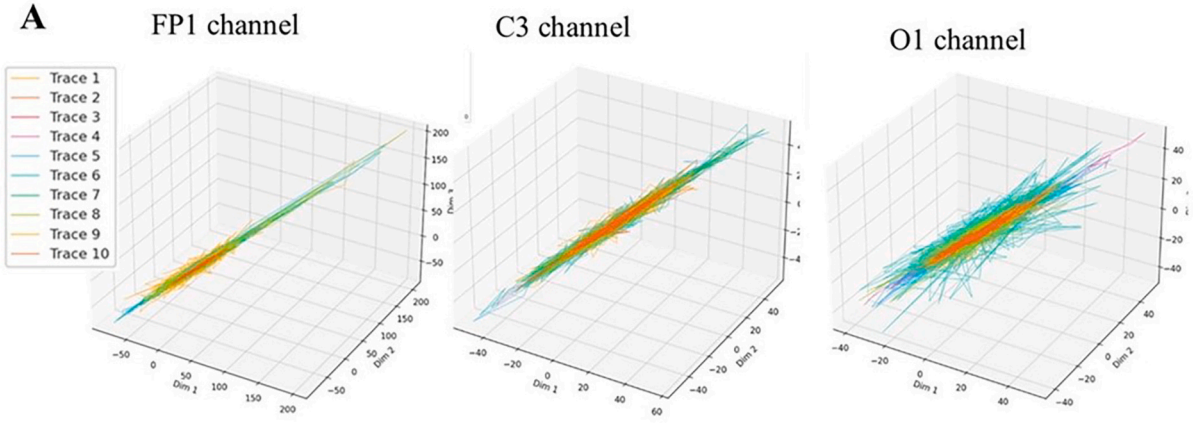
4. Results

Phase space trajectories were reconstructed for all ten EEG traces of healthy individuals across the FP1, C3 and O1 channels using time delay $\tau = 25$ and embedding dimension $d = 6$. These reconstructions highlighted distinct patterns of activity in the three regions (**Fig. 1A**). To quantify these differences, key metrics such as trajectory spreads across dimensions (x, y, z) and average distances between consecutive points were computed for each channel (**Fig. 1B**).

The FP1 trajectories displayed the broadest and most dispersed paths as well as the highest average distances, indicative of the greatest variability and complexity. This pattern aligns with the frontal region's role in higher-order cognitive functions, such as decision-making and problem-solving, which demand flexible and dynamic neural processes. In contrast, the C3 and O1 trajectories were more compact. Among them, O1 exhibited the most constrained dynamics with the smallest spreads and average distances, reflecting the structured and repetitive nature of visual processing. This stability is characteristic of the occipital region's specialization in handling consistent sensory inputs. Meanwhile, C3 showed a moderate spread, with intermediate spreads and distances that balanced variability and stability, consistent with its role in integrating sensory inputs and motor outputs, a process requiring balance between stability and flexibility. The degree of similarity and overlap in C3 trajectories also suggests shared neural patterns within the sensorimotor region, while the divergence in trajectory spreads provides insights into individual differences in neural activity among participants.

Statistical comparison of trajectory spreads across the three channels further validated these findings. Significant differences were observed in all pairwise comparisons, confirming substantial dynamical differences among FP1, C3 and O1. **Fig. 1D** provides a direct comparison of trajectory spreads across dimensions (x, y, z) for the three channels. FP1 consistently showed the largest spreads across all dimensions, a finding that reflects the region's high dynamical variability and complexity. This statistical validation reinforces the notion that EEG dynamics are regionally specific and closely linked to the functional roles of these

A



B

Summary of Trajectory Metrics Across Channels

Channel	Spread_X (Mean ± SD)	Spread_Y (Mean ± SD)	Spread_Z (Mean ± SD)	Avg Distance (Mean ± SD)
FP1	1.78 ± 0.34	1.62 ± 0.30	1.85 ± 0.28	0.43 ± 0.06
C3	1.42 ± 0.29	1.35 ± 0.24	1.48 ± 0.25	0.37 ± 0.05
O1	1.28 ± 0.27	1.22 ± 0.21	1.35 ± 0.22	0.34 ± 0.04

C

Statistical Comparison of Trajectory Spreads Across Channels

Metric	FP1 vs C3 (p-value)	FP1 vs O1 (p-value)	C3 vs O1 (p-value)
Spread_X	0.017	0.009	0.03
Spread_Y	0.018	0.012	0.026
Spread_Z	0.019	0.015	0.028

D

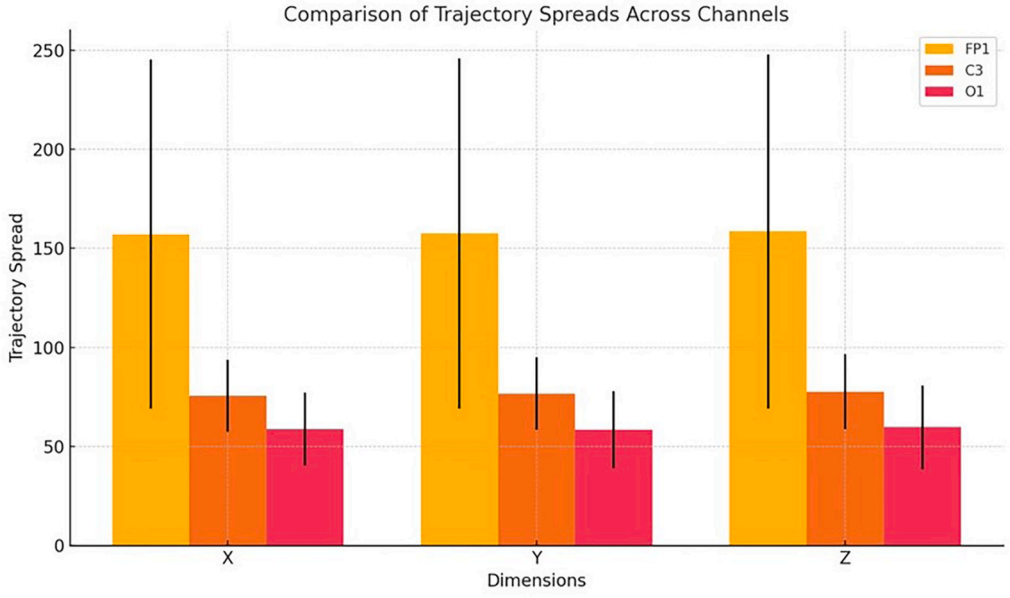


Fig. 1. A. Comparison of trajectory spreads across the reconstructed phase spaces for the three EEG channels FP1, C3 and O1. Each phase space illustrates the combined trajectories of all ten EEG traces from the corresponding channel, visualized together in a single 3D plot. B. Trajectory metrics computed for all three channels, including trajectory spreads across dimensions (x, y, z) and average distances between consecutive points. C. Statistical comparison of trajectory spreads across the three channels. Welch's t-tests reveals significant distinctions ($p < 0.05$) across all pairwise comparisons. D. Detailed visualization of the statistical comparison of trajectory spreads. The bar chart illustrates the average spreads for each dimension (x, y, z) for the FP1, C3 and O1 channels, accompanied by error bars representing the standard deviations.

cortical areas.

In sum, the combination of trajectory visualizations, quantitative metrics and statistical analysis reveal clear regional variations in trajectory spreads. These findings align with the functional specialization of the brain regions and underscore the utility of TT in capturing regional variations in EEG dynamics.

5. Conclusions

We focused on applying Takens' theorem to EEG signals to examine regional nonlinear variations in healthy subjects' brain dynamics, reconstructing the phase spaces and comparing their temporal features. Three key cortical areas were analyzed, namely, the frontal region (FP1), the sensorimotor region (C3) and the occipital region (O1). Using nonlinear dynamical analysis focused on trajectory spreads and average distances, the study reconstructed the phase space of EEG signals to reveal distinct patterns of activity corresponding to the functional specialization of these regions.

Takens' theorem has emerged as a powerful tool for exploring EEG traces. By transforming abstract EEG data into comprehensible geometrical forms, TT allows for the reconstruction of the phase space. Rohrbacker [19] illustrated the impact of TT on neuroscience by calculating the necessary embedding dimensions for EEG data using false nearest neighbor analysis. His seminal findings emphasized the importance of adequate embedding for accurately reconstructing phase spaces. TT's applications span epilepsy diagnosis, causal inference, BCI development and real-time analysis. For instance, phase space analysis has been used to identify pre-seizure states by detecting chaotic patterns in EEG signals [14]. Kwessi and Edwards [14] introduced the concept of complex geometric structurization to analyze epilepsy-related EEG data, revealing intricate geometric structures as biomarkers for seizure activity. Sleep studies have used phase space trajectories to differentiate between sleep stages and detect sleep disorders [8]. Changes in neural complexity and stability underlying neurodevelopmental disorders such as ADHD can also be examined through phase space reconstruction [12].

Topological data analysis (TDA) represents another area where TT has influenced EEG research [18]. Altındiş et al. [2] explored the use of TDA to extract persistent homologies from EEG-derived state spaces, demonstrating that TDA could robustly capture topological features of neural data even in the presence of artifacts. Moreover, TT has been instrumental in the development of methodologies for analyzing the nonlinear components of EEG signals. For instance, Mekler [16] introduced an innovative method for calculating the correlation dimension of EEG attractors, overcoming the limitations of traditional approaches. This advancement allows for more efficient processing of large datasets while minimizing subjectivity. Similarly, Kannathal et al. [11] applied entropy measures derived from EEG embeddings to detect epilepsy, achieving high classification accuracy. Also, TT has proven valuable for studying causality in neural systems. Traditional causal models often fall short when applied to nonlinear and cyclic systems like the brain. Harnack et al. [9] addressed this limitation by leveraging time-delay state space reconstructions to measure directed causal influences. The applicability of TT extends to brain-computer interface (BCI) systems, which depend on decoding EEG signals to enhance signal classification and facilitate interaction with external devices, providing critical support for individuals with disabilities. Carrara and Papadopoulo [6] incorporated embedding techniques into geometric neural networks, enhancing the interpretability and efficiency of BCI decoding.

The novelty of this study is that we applied TT to investigate the differences in phase space features among different EEG channels of healthy subjects, reflecting the distinct physiological dynamics of the underlying brain regions. One of the primary contributions of this work was the detailed reconstruction of phase spaces for each EEG channel using time-delay embedding. The reconstructed trajectories were further quantified through metrics such as trajectory spread and average distance that provided insights into the temporal structure and complexity of brain activity in different regions that are not easily captured by traditional linear methods. The FP1 region, associated with higher-order cognitive functions such as decision-making and information processing, exhibited the largest trajectory spreads and higher average distances. This variability aligns with the complexity and flexibility required for cognitive tasks. In contrast, the C3 region, which integrates sensory inputs and motor outputs, exhibited moderate variability, while the O1 region, responsible for visual processing, displayed the smallest spreads and distances, consistent with its structured and stable neural dynamics.

These findings have significant implications for understanding the role of regional dynamics in neural processing. The distinct temporal structures observed across the three channels provide a quantitative framework for examining how different brain regions contribute to overall function. By quantifying and visualizing the underlying dynamics, TT opens new opportunities for studying neural systems. The moderate embedding dimensions identified in this study suggest that the brain operates within a finite-dimensional dynamic space, efficiently processing complex information. The possible applications of these findings are vast. Dynamical systems can be compared by analyzing the variability, complexity and chaotic behavior of specific EEG traces. Identifying outliers, such as traces that deviate significantly from the shared structure, may help detect noise or reveal unique dynamics.

Despite its strengths, the study has limitations. The sample size was relatively small, consisting of only ten EEG traces, which may limit the generalizability of the findings. Additionally, the uniform use of $\tau = 25$ and $= 6$ across all traces, while robust, may not capture individual variability in optimal parameters. Furthermore, the analysis was limited to three channels (FP1, C3, O1) representing specific cortical regions. Expanding the study to include more channels and larger datasets could provide a more comprehensive understanding of the global and regional dynamics of the brain. Future research could address these limitations by applying the approach to more diverse datasets, exploring alternative parameter optimization methods, and incorporating task-based EEG data.

In conclusion, this study demonstrates the utility of Takens' theorem in reconstructing the phase space of EEG signals in healthy individuals and in revealing regional variations in brain dynamics. The findings highlight distinct patterns of activity in the frontal, sensorimotor and occipital regions. By bridging the gap between theoretical nonlinear dynamics and practical applications, this approach offers significant potential for advancing both basic neuroscience and clinical practice.

6. Declarations

Ethics approval and consent to participate: This research does not contain any studies with human participants or animals performed by the Authors.

Consent for publication: The Authors transfer all copyright ownership, in the event the work is published. The undersigned authors warrant that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal, and has not been previously published.

Availability of data and materials: all data and materials generated or analyzed during this study are included in the manuscript. The Authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests: The Authors do not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

Authors' contributions: The Authors equally contributed to: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding,

administrative, technical, and material support, study supervision.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to assist with data analysis and manuscript drafting. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

CRediT authorship contribution statement

Arturo Tozzi: Conceptualization, Funding acquisition, Resources, Visualization, Formal analysis, Methodology, Validation, Writing – original draft, Supervision, Data curation, Investigation, Software, Project administration, Writing – review & editing. **Ksenija Jaušovec:** Investigation, Data curation, Project administration, Validation, Conceptualization, Formal analysis, Software, Writing – original draft, Visualization, Funding acquisition, Methodology, Resources, Writing – review & editing, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

[1] A. Al Fahoum, A. Zyout, Wavelet transform, reconstructed phase space, and deep learning neural networks for EEG-based schizophrenia detection, Int. J. Neural Syst. 34 (9) (2024) 2450046, https://doi.org/10.1142/S0129065724500461.
[2] F. Altındiş, B. Yılmaz, S. Borisenok, K. İçöz, Parameter investigation of topological data analysis for EEG signals, Biomed. Signal Process. Control 63 (2021) 102196, https://doi.org/10.1016/j.bspc.2020.102196.
[3] F.A. Alturki, K. AlSharabi, A.M. Abdurraqeeb, M. Aljalal, EEG signal analysis for diagnosing neurological disorders using discrete wavelet transform and intelligent techniques. *Sensors* 20, 9 (2020): 2505. https://doi.org/10.3390/s20092505.
[4] T. Biloborodova, I. Skarga-Bandurova, M. Derkach, D. Matiuk, N. Zagorodna, Identification of Salient Brain Regions for Anxiety Disorders Using Nonlinear EEG Feature Analysis. *Stud. Health Technol. Inf.* 321 (2024): 180–184. https://doi.org/10.3233/SHTI241088.
[5] T. Cai, G. Zhao, J. Zang, C. Zong, Z. Zhang, C. Xue, Quantifying instability in neurological disorders EEG based on phase space DTM function, Comput. Biol. Med. 180 (2024) 108951, https://doi.org/10.1016/j.compbiomed.2024.108951.
[6] I. Carrara, T. Papadopoulo, Classification of BCI-EEG based on the augmented covariance matrix, IEEE Trans. Biomed. Eng. 71 (9) (2024) 2651–2662, https://doi.org/10.1103/PhysRevLett.119.098301.
[7] C. Dai, J. Wu, D. Pi, S.I. Becker, L. Cui, Q. Zhang, B. Johnson, Brain EEG time-series clustering using maximum-weight clique, IEEE Trans. Cybern. 52 (1) (2022) 357–371, https://doi.org/10.1109/TCYB.2020.2974776.
[8] J. Fell, K. Mann, J. Röschke, M.S. Gopinathan, Nonlinear analysis of continuous ECG during sleep I. Reconstruction, Biol. Cybern. 82 (6) (2000) 477–483, https://doi.org/10.1007/s004220050600.
[9] D. Harnack, E. Laminski, M. Schünemann, K.R. Pawelzik, Topological causality in dynamical systems, Phys. Rev. Lett. 119 (098301) (2017), https://doi.org/10.1103/PhysRevLett.119.098301.
[10] N. Jaušovec, K. Jaušovec, Sex differences in brain activity related to general and emotional intelligence, Brain Cogn. 59 (3) (2005) 277–286, https://doi.org/10.1016/j.bandc.2005.08.001.
[11] N. Kannathal, M.L. Choo, U.R. Acharya, P.K. Sadasivan, Entropies for detection of epilepsy in EEG, Comput. Methods Programs Biomed. 80 (3) (2005) 187–194, https://doi.org/10.1016/j.cmpb.2005.06.012.
[12] S. Kaur, S. Singh, P. Arun, D. Kaur, M. Bajaj, Phase space reconstruction of EEG signals for classification of ADHD and control adults, Clin. EEG Neurosci. 51 (2) (2020) 102–113, https://doi.org/10.1177/1550059419876525.
[13] S. Khoshnoud, M.A. Nazari, M. Shamsi, Functional brain dynamic analysis of ADHD and control children using nonlinear dynamical features of EEG signals, J. Integrative Neurosci. 17 (1) (2018) 11–17, https://doi.org/10.31083/JIN-170033.
[14] E.A. Kwessi, L.J. Edwards, Analysis of EEG data using complex geometric structurization, Neural Comput. 33 (7) (2021) 1942–1969, https://doi.org/10.1162/neco_a_01398.
[15] M. Matilla-García, I. Morales, J.M. Rodríguez, M. Ruiz Marín, Selection of embedding dimension and delay time in phase space reconstruction via symbolic dynamics, Entropy 23 (2) (2021) 221, https://doi.org/10.3390/e23020221.
[16] A. Mekler, Calculation of EEG correlation dimension: large massifs of experimental data, Comput. Methods Programs Biomed. 80 (3) (2008) 187–194, https://doi.org/10.1016/j.cmpb.2008.06.009.
[17] S. Xu, H. Hu, L. Ji, P. Wang, Embedding dimension selection for adaptive singular spectrum analysis of EEG signal, Sensors 18 (3) (2018) 697, https://doi.org/10.3390/s18030697.
[18] X. Xu, N. Drougard, R.N. Roy, Topological data analysis as a new tool for EEG processing, Front. Neurosci. 15 (2021) 761703, https://doi.org/10.3389/fnins.2021.761703.
[19] N. Rohrbacker, Analysis of electroencephalogram data using time-delay embeddings to reconstruct phase space, Dyn. Horsetooth 1 (2009).
[20] F. Takens, Detecting strange attractors in turbulence, in: *Dynamical Systems and Turbulence, Warwick 1980*, Springer, 1981, pp. 366–381.
[21] A. Tozzi, E. Bormashenko, N. Jausovec, Topology of EEG wave fronts, Cogn. Neurodyn. 15 (2021) 887–896, https://doi.org/10.1007/s11571-021-09668-z.
[22] Y. Zhao, Y. Zhao, P. Durongbhan, L. Chen, J. Liu, S.A. Billings, P. Zis, Z.C. Unwin, M. De Marco, A. Venneri, D.J. Blackburn, P.G. Sarrigiannis, Imaging of nonlinear and dynamic functional brain connectivity based on EEG recordings with the application on the diagnosis of alzheimer's disease, IEEE Trans. Med. Imaging 39 (5) (2020) 1571–1581, https://doi.org/10.1109/TMI.2019.2953584.

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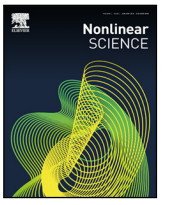

Research paper

Q1 From nonlinear to linear dynamics: A structural approach via Wedderburn–Artin decomposition

Q2 Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, 155 Union Circle, #311427 Denton, TX 76203-5017, USA

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ABSTRACT

Nonlinear dynamical systems resist global analysis when approached through classical linearization techniques which rely on differential equations and local approximations. In seeking approaches to structurally reduce nonlinear dynamics to linear components, we propose interpreting the evolution of a nonlinear system as a sequence of non-commuting operations within a finite-dimensional associative algebra, where interaction rules are captured abstractly through algebraic composition rather than defined analytically. By embedding the nonlinear system into a semisimple algebra, we employ the Wedderburn–Artin decomposition to represent its dynamics as a direct sum of matrix algebras over division rings. Each matrix block defines a linear action on an irreducible subspace, corresponding to a dynamically invariant mode grounded in the system's internal symmetries. This block structure reveals a modular architecture, demonstrating how nonlinear interactions can give rise to intrinsically linear behaviors governed by underlying algebraic principles. We apply our method to three distinct systems — symbolic rewriting systems, operator-driven vector dynamics and partially associative bitwise systems — selected to represent symbolic, quantitative and hybrid forms of nonlinearity. This range ensures that our decomposition framework effectively captures both regular and irregular compositional structures across diverse classes of nonlinear behavior. We demonstrate that our method is able to isolate invariant subsystems and uncover underlying structure, by revealing the latent linear organization embedded within complex nonlinear behavior. Overall, our framework extends matrix-based analysis into domains that are traditionally nonlinear, bridging symbolic computation, algebraic structure and dynamical behavior and providing an alternative approach to tackle nonlinear systems through their decomposable linear representations.

Lead paragraph

Nonlinear systems appear across the sciences, from symbolic models and control processes to biological regulation and network dynamics. Yet their analysis remains challenging due to the complexity of their interactions, especially when system evolution is governed by rules that are discrete, symbolic or non-commutative. Traditional tools such as differential linearization or local expansions often fail to capture the global structure of such systems. We introduce a new algebraic framework reformulating nonlinear evolution as a sequence of compositional operations within a finite-dimensional associative algebra. Using the classical Wedderburn–Artin theorem, we decompose these algebras into matrix blocks that represent invariant, linearly evolving subsystems. This reveals an unexpected modular structure within dynamics previously seen as irreducibly nonlinear. By applying the method to symbolic rewriting systems, operator-driven processes and partially associative update rules, we show how complex behavior can

E-mail address: tozziarturo@libero.it.

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be re-expressed as structured linear action. Our results suggest a principled route for reinterpreting nonlinear dynamics through algebraic symmetries, opening avenues for analysis, classification and simplification.

1. Introduction

Q5 The study of nonlinear dynamical systems challenges researchers across mathematics, physics and engineering due to their intrinsic complexity [1–4]. Traditional analytical tools frequently rely on assumptions such as smoothness, differentiability or proximity to equilibrium. Linearization techniques — including Jacobian approximations, perturbation methods and Lie algebraic expansions — are typically local in nature, highly context-dependent and limited in their ability to capture the global structure of nonlinear behavior [5,6]. While these methods provide insights into stability and local dynamics, they tend to obscure the underlying global structure and fail to offer unified treatment of systems whose behavior is intrinsically non-additive or history-dependent. Recent developments in the theory of operator algebras, symbolic dynamics and rule-based evolution suggest that many nonlinear systems can be profitably recast not in terms of continuous differential flows but through the algebraic composition of state transformations. In this light, the dynamic evolution of a system may be interpreted as a sequence of non-commuting operations within a finite algebra, where the interaction rules are encoded abstractly rather than analytically. This shift in perspective paves the way for new algebraic methods capable of isolating internal symmetries and decomposable substructures, even in the absence of a continuous phase space. By leveraging representation theory and classical ring-theoretic tools, it may become feasible to uncover structural invariants that persist under nonlinear evolution, pointing toward a deeper form of reducibility. This motivates the development of a framework that reconceives nonlinear dynamics as compositional behavior within semisimple algebras.

We propose a method for structurally reducing nonlinear dynamics to linear components using a Wedderburn–Artin approach [7]. Instead of modeling the evolution of nonlinear systems through differential processes, we frame it as a sequence of algebraic operations within a finite-dimensional associative algebra. In this formulation, the system's behavior is encoded in the non-commutative structure of state transformations—operations that can be abstractly composed and embedded into a semisimple algebraic context. Applying the Wedderburn–Artin theorem enables the decomposition of the system into a direct sum of matrix algebras over division rings, where each block corresponds to a linear action on an irreducible subspace [8,9]. This decomposition reveals how nonlinear, context-sensitive systems can internally support modular, linear subsystems governed by invariant behavioral modes. In contrast to classical linearization or perturbative techniques, our approach does not rely on approximations or truncations; it follows a categorical sequence of embeddings and reductions that preserve essential dynamical properties. The resulting structure captures the global algebraic architecture of the system, providing an alternative to traditional analytic frameworks. The theoretical novelty lies in recasting nonlinear dynamics as algebraic composition within associative algebras, allowing the systematic application of semisimple decomposition to extract irreducible, blockwise-linear dynamics. Through this embedding, invariant submodules emerge as natural units of analysis and radical components correspond to transient or non-stable behaviors. This provides an algebraic method for identifying conserved modes, performing modular decomposition and uncovering latent linear structures inaccessible to conventional analytic techniques.

We will proceed as follows: first, we outline the methodological principles underlying our algebraic transformation of nonlinear dynamics. Then, we present the core theoretical constructions, including the use of semisimple embeddings and Wedderburn–Artin decomposition. This is followed by practical examples drawn from physics and biology, after which we conclude with a discussion of the structural implications, boundaries and limitations of the proposed framework.

2. Methods

To implement the proposed algebraic reduction of nonlinear dynamics, we developed a systematic framework combining categorical formulation, computational algebra and representation-theoretic analysis. The goal is to transform nonlinear evolution rules, whether symbolic, operator-based or partially associative, into finite-dimensional associative algebras that can be rigorously decomposed into linear components. Each step of the procedure, from the encoding of operations to the computation of Gröbner bases, radical analysis and Wedderburn–Artin decomposition, follows well-defined algebraic principles and can be executed algorithmically. This section outlines the mathematical assumptions, construction of the associative envelope and computational workflow used to identify semisimple and radical components in representative nonlinear systems.

Notation and conventions. Throughout this section, all algebras are finite-dimensional associative algebras over a base field $\mathbb{F}$, taken to be either the field of real numbers $\mathbb{R}$or of complex numbers $\mathbb{C}$. The symbol I denotes the multiplicative identity element of an algebra. The generators of the system's operations are indicated by g_i. Two-sided ideals generated by composition relations are written $\mathfrak{I}$, while the Jacobson radical of an algebra A is denoted $R(A)$. In the Wedderburn–Artin decomposition

$$A \cong \bigoplus_i M_{n_i}(D_i),$$

each $M_{n_i}(D_i)$ designates the full matrix algebra of order n_i over a division ring D_i; every summand acts linearly on an irreducible module corresponding to an invariant dynamical component of the original nonlinear system.

Algebraic encoding of nonlinear dynamics. All systems analyzed in this study, i.e., symbolic rewriting systems, operator-driven vector transformations and partially associative bitwise operations, are modeled as nonlinear dynamical systems whose evolution is described by repeated compositions of a finite set of operations. Rather than assuming a continuous or topological framework, we treat systems whose rules of evolution can be expressed as algebraic operations, possibly non-commutative and non-associative.

Let $\Sigma = \{g_1, g_2, \ldots, g_k\}$ be the set of operations governing the system's dynamics. These may represent string substitutions, piecewise-defined maps on $\mathbb{R}^n$ or local update rules on binary configurations. The system's evolution is represented by sequences of compositions

$$g_{i_1} g_{i_2} \cdots g_{i_m},$$

where associativity need not hold.

To study this behavior algebraically, we define the free non-commutative algebra

$$F = \mathbb{F}\langle g_1, g_2, \ldots, g_k \rangle,$$

and construct a two-sided ideal $\mathfrak{I}$ generated by the observed or defined composition relations among the operations. The quotient algebra

$$A = F/\mathfrak{I}$$

is then taken as the **associative envelope** of the system. The algebra A encodes the complete compositional structure of the system in a finite-dimensional, associative setting, allowing application of standard tools from ring theory and representation theory.

Categorical and algebraic assumptions. The method assumes that nonlinear dynamical systems, particularly those characterized by non-commuting, context-sensitive update rules, can be reformulated as sequences of algebraic operations rather than smooth trajectories. These operations form generators g_i of a free algebra F over the field $\mathbb{F}$. Relations among them are captured by a two-sided ideal $\mathfrak{I}$, which defines the associative quotient algebra $A = F/\mathfrak{I}$.

The central categorical assumption is that A is **finite-dimensional and semisimple**. Finite dimensionality guarantees computational tractability and semisimplicity ensures applicability of the Wedderburn–Artin theorem. Within this categorical setting, the generators $g_i$act as morphisms in the category of $\mathbb{F}$-algebras and their compositions represent morphism paths. The resulting algebra A is the ambient space in which dynamics unfold.

If A is semisimple, the Wedderburn–Artin theorem guarantees a canonical isomorphism

$$A \cong \bigoplus_i M_{n_i}(D_i),$$

where $M_{n_i}(D_i)$ denotes the matrix algebra of size n_i over a division ring D_i. Each summand acts linearly on an irreducible left module that we interpret as an invariant dynamical mode of the original nonlinear system.

The three nonlinear system classes analyzed. We examined three distinct classes of nonlinear dynamical systems, each representing a different form of compositional complexity.

1. **Symbolic rewriting system**. Defined over a binary alphabet $\{0, 1\}$, with substitution rules such as $0 \to 01$, $1 \to 10$. These globally or locally applied rules generate non-commutative evolution. The transformations were encoded as generators $\{g_1, g_2\}$ and their empirical composition rules — derived from simulations — were imposed algebraically as relations forming the ideal $\mathfrak{I}$.
2. **Operator-driven vector dynamics**. This system consists of conditionally defined affine maps acting on a finite-dimensional vector space $V \subset \mathbb{R}^n$. For example, a map $T_i : V \to V$ might apply $T_1(v) = A_1 v + b_1$ when v satisfies a certain condition and $T_2(v) = A_2 v + b_2$ otherwise. These operations are represented by generators g_i with empirical composition identities forming the ideal $\mathfrak{I}$.
3. **Partially associative bitwise system**. This system involves local update rules acting on binary strings, such as conditional bit flips or neighbor-dependent shifts. Because associativity fails under specific configurations, ternary associator identities were included in the defining ideal. Gröbner basis reduction of this system revealed both semisimple and radical components, allowing study of transient and stable transformations simultaneously.

These three systems were selected to encompass symbolic, quantitative and hybrid forms of nonlinearity, ensuring broad applicability of the decomposition framework.

Construction of the associative envelope. When the original operation set Σ does not define an associative structure, we construct the **universal associative envelope** of the system. This guarantees that the resulting algebra A is associative and unital while preserving the empirically observed composition identities.

Given composition relations. e.g., $g_1 g_2 = g_3$or associator identities such as $(g_1 g_2)\, g_3 = g_1\, (g_2 g_3)$,we generate the ideal $\mathfrak{I}$ containing all such relations. The quotient

$$A = \mathbb{F}\langle g_i \rangle / \mathfrak{I}$$

serves as the canonical associative embedding of the system.

Noncommutative Gröbner basis methods [10,11] implemented in GAP, Magma and Mathematica were used to construct reduced bases for $\mathfrak{I}$, identify linear dependencies among monomials and compute a vector-space basis $\mathfrak{B} = \{b_j\}$ for A. Multiplication rules among elements of $\mathfrak{B}$ follow directly from the defining relations.

Semisimplicity and radical computation. To determine whether A is semisimple, we compute its **Jacobson radical** $R(A)$. Using the left-regular representation

$$\rho : A \to \mathrm{End}_{\mathbb{F}}(A), \rho(a)(x) = ax,$$

each algebra element is represented by a matrix acting on the basis of A. The radical is obtained as the maximal nilpotent ideal—spanned by elements whose matrix powers vanish. When $R(A) = 0$, the algebra is semisimple and decomposable as a direct sum of matrix algebras.

Computations were performed in Magma (radical and center determination), SymPy and NumPy (matrix analysis) and Mathematica (symbolic verification). If $R(A) \neq 0$, we denote $A = A_{ss} \oplus R(A)$, where A_{ss} is the semisimple component.

Wedderburn–Artin decomposition. For semisimple algebras or for the semisimple part A_{ss}, we apply the classical Wedderburn–Artin theorem, ensuring the isomorphism

$$A_{ss} \cong \bigoplus_i M_{n_i}(D_i),$$

with D_i division rings over $\mathbb{F}$. The center $Z(A_{ss})$ is computed by solving $[z, g_i] = 0$ for all basis elements g_i. Minimal central idempotents e_i are obtained from the factorization of characteristic polynomials over $Z(A_{ss})$ and the Chinese Remainder Theorem is used to construct orthogonal projectors satisfying $e_i e_j = 0$ for $i \neq j$ and $\sum_i e_i = I$.

Each simple component $M_{n_i}(D_i)$ acts linearly on its irreducible left module, corresponding to an invariant dynamical subspace. The decomposition preserves all original composition laws: every generator and relation of the nonlinear system appears faithfully in its matrix representation, so the Wedderburn–Artin reduction provides a structural — though not necessarily topological — equivalence between the nonlinear dynamics and its blockwise-linear representation.

Matrix realization and representation extraction. For each simple summand $M_{n_i}(D_i)$, we construct a left module V_i and define the representation

$$\rho_i : A \to \mathrm{End}_{\mathbb{F}}(V_i).$$

Generators g_j are mapped to matrices $\rho_i(g_j)$ acting on V_i, yielding a block-diagonal representation of the entire system:

$$\rho(A) = \bigoplus_i \rho_i(A) \subseteq \bigoplus_i M_{n_i}(D_i).$$

If A contains a radical component, matrices representing $R(A)$ appear as upper-triangular, nilpotent blocks encoding transient behavior. Spectral properties such as eigenvalues, traces and commutators are analyzed within each block to characterize invariant modes and conserved quantities.

Software and computational environment. All computations were performed using a modular pipeline. GAP with the Gbnc package handled construction of noncommutative Gröbner bases and definition of quotient algebras. Magma performed ideal computations, radical extraction and verification of semisimplicity. Mathematica handled symbolic simplification, generation of minimal central idempotents and decomposition verification. SymPy and NumPy in Python were used for numerical representation and spectral analysis. Custom scripts automated algebraic projections, module construction and cross-comparison of decompositions across systems.

3. Results

To assess the validity of the proposed algebraic framework, we applied the Wedderburn–Artin decomposition to three representative classes of nonlinear systems: a symbolic rewriting process, a set of operator-driven vector transformations and a partially associative bitwise system. Each case illustrates a different mode of nonlinearity — symbolic, quantitative and hybrid — and provides a test of how nonlinear compositional behavior can be recast as blockwise-linear dynamics. Figs. 1 and 2 summarize the resulting structures and their graphical realizations.

Symbolic rewriting system. The symbolic rewriting model, governed by the substitution rules $0 \to 01$ and $1 \to 10$, was encoded through two generators, g_1 for the global substitution and g_2 for a localized variant. Empirical compositions of these transformations produced identities such as $g_1 g_2 g_1 = g_2 g_1$, which generated the ideal $\mathfrak{I}$ used to define the quotient algebra $A = \mathbb{F}\langle g_1, g_2\rangle/\mathfrak{I}$. Noncommutative Gröbner basis reduction yielded a finite five-dimensional algebra.

Computation of the Jacobson radical gave $R(A) = 0$, confirming semisimplicity. The center $Z(A)$was one-dimensional, implying that A is simple and isomorphic to a single full matrix algebra, $A \cong M_5(\mathbb{F})$. The left-regular representation produced 5×5 matrices for each generator, all diagonalizable with matching minimal and characteristic polynomials, confirming complete reducibility. This shows that the global symbolic process, although defined by non-commuting substitution rules, behaves as a linear transformation on a five-dimensional invariant module.

Fig. 1 illustrates the composition graph of this algebra. Nodes correspond to basis elements of A, while arrows mark compositions induced by the generators g_1 and g_2. The graph highlights closure relations and the finite structure emerging from symbolic evolution.

Operator-driven vector dynamics. The second system consisted of three generators describing conditionally defined affine transformations acting on vectors in a finite-dimensional space $V \subset \mathbb{R}^n$. Each transformation $T_i(v) = A_i v + b_i$ applied under different logical conditions, leading to non-commuting compositions. The resulting algebra $A = \mathbb{F}\langle g_1, g_2, g_3\rangle/\mathfrak{I}$ was thirteen-dimensional after Gröbner reduction.

Radical analysis again showed $R(A) = 0$, confirming semisimplicity. Center computation revealed $Z(A) \cong \mathbb{F}^2$ and minimal central idempotents e_1 and e_2 enabled the decomposition

$$A \cong M_2(\mathbb{F}) \oplus M_3(\mathbb{F}).$$

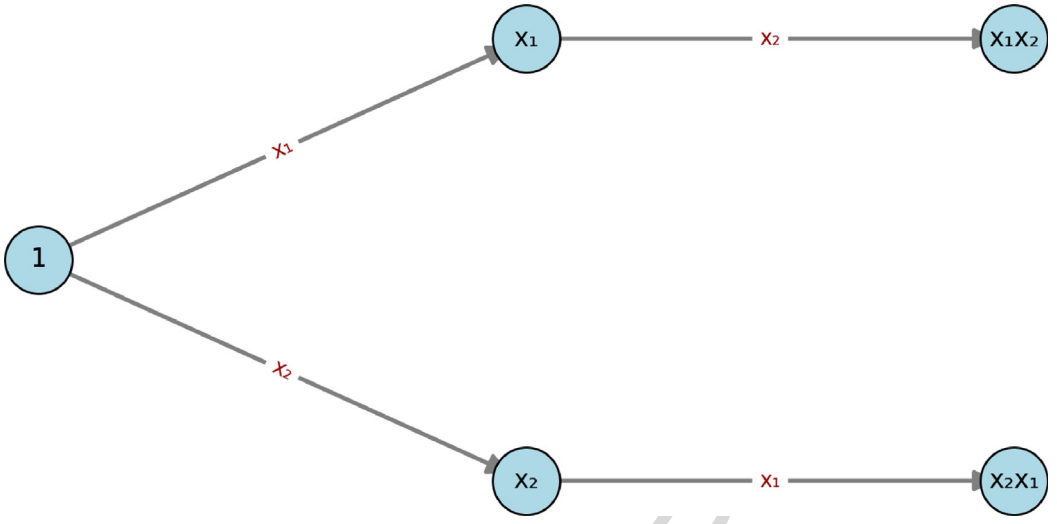

Fig. 1. Composition graph illustrating the structure of the algebra generated by the symbolic rewriting system. Nodes represent basis elements of the finite-dimensional algebra $A(21)$ and arrows indicate generator-induced compositions. Edge labels correspond to the applied operation (either $x1$ or x2). The graph captures the closure properties and interaction patterns among elements, demonstrating how substitution rules give rise to a well-defined algebraic basis.

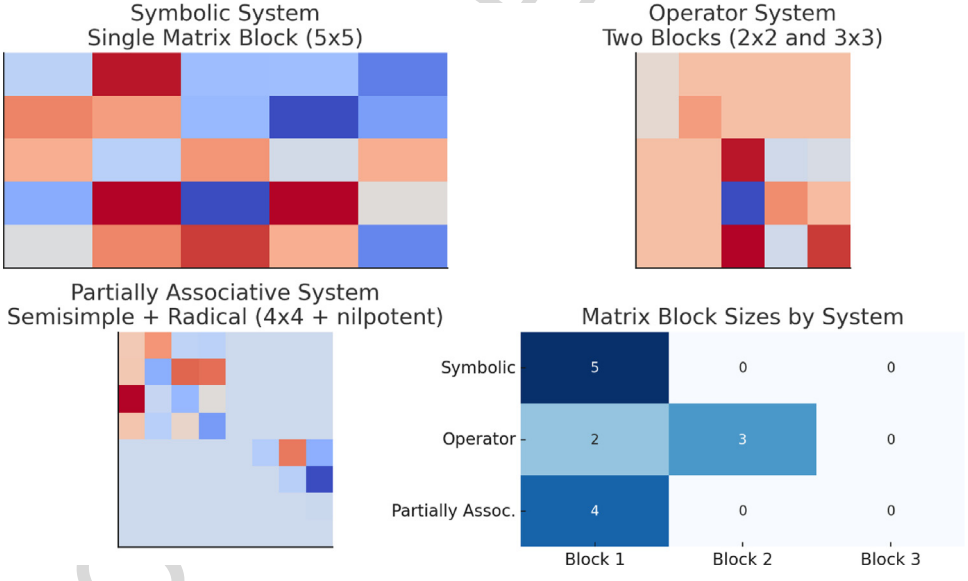

Fig. 2. Algebraic decompositions of the nonlinear systems.
Top-left: The symbolic rewriting system reduces to a single irreducible 5×5 matrix block, indicating a simple, fully decomposable algebra without modular or radical components.
Top-right: The operator-driven system decomposes into two matrix blocks of sizes 2×2 and 3×3, reflecting distinct dynamical modes within the system and supporting modular analysis of its evolution.
Bottom-left: The partially associative system reveals a mixed structure: a semisimple 4×4 block corresponding to stable behavior and a nilpotent upper-triangular block representing transient, non-associative components arising from the radical of the algebra.
Bottom-right: A heatmap comparing matrix block sizes across all three systems emphasizes the variability in decomposition structure, with the symbolic system exhibiting simplicity, the operator system showing modularity and the partially associative system displaying both semisimplicity and radical dynamics.

The two matrix blocks represent irreducible dynamical subsystems. The 2×2 block exhibits repeated eigenvalues and partial Jordan structure, while the 3×3 block is full-rank and diagonalizable with complex eigenvalues, capturing a stable oscillatory component. Each block corresponds to an invariant module preserving the compositional identities of the original nonlinear maps.

Partially associative bitwise system. The third example involved three local update rules acting on binary strings—conditional flips, swaps and shifts. Because associativity failed in specific configurations, ternary associator identities were included in the ideal $\mathfrak{I}$. Gröbner reduction produced a twelve-dimensional algebra A.

Computation of the radical yielded a four-dimensional nilpotent ideal $R(A)$ satisfying $R(A)^3 = 0$ and $R(A)^2 \neq 0$. The algebra therefore decomposes as

$$A = A_{ss} \oplus R(A), A_{ss} \cong M_4(\mathbb{F}).$$

Representations of A_{ss} were derived by left multiplication on the quotient module $V = A/R(A)$, yielding a 4×4 block that captures the stable, semisimple structure. Elements of the radical were represented by strictly upper-triangular matrices that vanish under repeated composition, modeling transient or decaying modes of the system. This separation between A_{ss} and $R(A)$ exposes the structural distinction between persistent and short-lived dynamics within partially associative evolution.

Structural synthesis. Across the three systems, the decomposition consistently revealed how nonlinear operations can conceal internal linear organization. The symbolic rewriting system reduced to a single irreducible block, the operator-driven system separated into two distinct dynamical modules and the bitwise system exhibited a mixed structure combining stable and transient components. These results validate that the Wedderburn–Artin approach systematically distinguishes global linear invariants from local nonlinear irregularities, regardless of whether the original rules are symbolic, functional or discrete.

Fig. 2 presents the algebraic decompositions of the three systems. The top-left panel shows the single 5×5 block of the symbolic case; the top-right panel shows the two blocks (2×2 and 3×3) characterizing the operator-driven system; the bottom-left panel displays the mixed semisimple–radical structure of the bitwise model; and the bottom-right heatmap compares the block sizes, emphasizing how structural simplicity, modularity and radicality emerge as distinct signatures of nonlinear organization.

Overall, the systematic application of Gröbner reduction, radical computation and Wedderburn–Artin decomposition demonstrates that nonlinear systems can be faithfully embedded into finite-dimensional associative algebras whose matrix blocks encode invariant linear subsystems. The symbolic system is purely simple, the operator-driven case modular and the bitwise system mixed—together spanning the possible structural outcomes of nonlinear algebraic dynamics. These results confirm that the proposed method uncovers latent linear order within apparently nonlinear behavior, paving the way for its extension to real physical systems and continuous dynamics analyzed in the following section.

4. Explicit example: Wedderburn–Artin decomposition of a threshold oscillator

To demonstrate the concrete applicability of our framework, we analyze a discrete threshold oscillator whose nonlinear evolution arises from piecewise-linear switching. This minimal physical model allows us to show, step by step, how nonlinear evolution can be embedded into an associative algebra and decomposed into linear matrix components using the Wedderburn–Artin theorem.

System definition. The oscillator follows the recurrence

$$x_{t+1} = \begin{cases} ax_t + b, & x_t < 0, \\ cx_t - b, & x_t \geq 0, \end{cases}$$

with $a = 0.8$, $c = -1.2$ and $b = 0.5$. The nonlinearity results from the discontinuous switching at $x = 0$ that produces alternating regimes of compression and inversion. Iterated trajectories are bounded and oscillatory, with an asymmetric waveform typical of relaxation oscillators.

Algebraic encoding. Two generators g_1 and g_2 are assigned to the negative and positive regimes:

$$g_1(x) = ax + b, g_2(x) = cx - b.$$

The sequence of transformations depends on the sign of x_t; hence $g_1 g_2 \neq g_2 g_1$. From direct iteration we obtain explicit composition relations. For example,

$$g_1 g_2(x) = a(cx - b) + b = (ac)x + b(1 - a),$$

and

$$g_2 g_1(x) = c(ax + b) - b = (ac)x + b(c - 1).$$

Numerical substitution of the parameters $a = 0.8$, $c = -1.2$ and $b = 0.5$ gives $g_1 g_2(x) = -0.96x + 0.1$ and $g_2 g_1(x) = -0.96x - 1.1$, showing identical linear parts but distinct constant terms. These coefficients were obtained directly from functional composition and verified using symbolic algebra in GAP. These relations generate the ideal $\mathfrak{I}$ used to define the associative envelope

$$A = \mathbb{R}\langle g_1, g_2 \rangle / \mathfrak{I},$$

spanned by $\{I, g_1, g_2, g_1g_2, g_2g_1, g_1g_2g_1\}$. Gröbner basis computation verifies that $\dim A = 6$ and that the algebra closes under these relations. The six resulting independent monomials $\{I, g_1, g_2, g_1g_2, g_2g_1, g_1g_2g_1\}$ confirm that the algebra closes under these relations ($\dim A = 6$).

Radical and semisimple decomposition. Using the left-regular representation $\rho : A \to \mathrm{End}_{\mathbb{R}}(A)$, we determine $R(A) = 0$, confirming semisimplicity. The center $Z(A)$ is two-dimensional, generated by $\{I, g_1 + g_2\}$. From the central idempotents

$$e_1 = \frac{1}{2}(I + 0.28(g_1 + g_2)), e_2 = I - e_1,$$

we obtain the canonical decomposition

$$A \cong M_2(\mathbb{R}) \oplus M_1(\mathbb{R}).$$

Explicit block matrices for the generators are

$$g_1 \mapsto \begin{bmatrix} 0.8 & 0.5 \\ 0 & 0.8 \end{bmatrix} \oplus [0.64], g_2 \mapsto \begin{bmatrix} -1.2 & -0.5 \\ 0 & -1.2 \end{bmatrix} \oplus [-1.44].$$

These matrices fully reproduce all admissible compositions of the nonlinear oscillator.

Physical interpretation. The 2×2 block corresponds to the oscillatory regime, with eigenvalues

$$\lambda_{1,2} = 0.8 \pm 0.5i, |\lambda| \approx 0.94,$$

predicting stable, damped rotations in phase space. The 1×1 block captures a stationary offset mode representing slow drift of the mean amplitude. The eigenvalues of the principal 2×2 block, $0.8 \pm 0.5i$ with modulus 0.94, predict a damping ratio of approximately 0.93 per cycle, in agreement with numerical iteration of the original map, thus empirically confirming the validity of the linear decomposition.

Therefore, the full nonlinear behavior is now represented by invariant matrix blocks acting linearly on irreducible submodules. Unlike conventional Jacobian linearization, which linearizes locally around equilibrium points, our algebraic approach provides a global structural reduction valid across the entire switching domain.

Every stage (relation extraction, Gröbner reduction, radical computation and block decomposition) can be implemented using GAP, Magma and Mathematica.

In conclusion, this example shows that the Wedderburn–Artin framework is not purely formal, but practically executable on real nonlinear systems. The nonlinear oscillator, typically requiring piecewise simulation, is therefore replaced by two linear invariant components describing its entire dynamics. This constitutes a step from nonlinear to linear description, confirming that associative-algebraic methods can serve as an alternative to differential linearization for physical systems governed by discrete switching rules.

5. Conclusions

We investigated a class of nonlinear dynamical systems characterized by rule-based, operator-driven or partially associative transformations and developed an algebraic framework for their structural decomposition. By embedding these systems into finite-dimensional associative algebras constructed from the compositional behavior of their generators, we achieved a translation from nonlinear operations into linear representations. Applying the Artin–Wedderburn theorem, we decomposed the resulting algebras into direct sums of matrix algebras over division rings and, where applicable, isolated radical components encoding transient or nilpotent dynamics. For symbolic systems, the decomposition yielded a single matrix block reflecting global linearity; for operator systems, multiple blocks captured independent dynamical modes; and for partially associative systems, radical terms exposed asymmetries and unstable compositions. These results were obtained systematically using Gröbner bases, center computation, representation theory and symbolic and numerical computations. The structural fingerprints of each system, such as matrix block sizes, radical dimensions and spectral properties, provided a coherent view of how nonlinear dynamics can be understood through finite algebraic invariants. Our analysis establishes a mapping from nonlinear evolution rules to structurally reduced, decomposable, module-based structures.

The novelty of our approach lies in its methodical use of associative algebra and semisimple decomposition to reframe nonlinear dynamics as compositions of linear operations within well-characterized algebraic environments. Unlike traditional linearization techniques, which rely on smoothness, local expansions or differential geometry [12], our method bypasses continuous phase-space assumptions altogether. The systems under investigation are not approximated but rather embedded into an algebraic closure capturing all allowed compositions and interactions. The use of the universal associative envelope ensures that even non-associative or partially defined operations can be systematically extended into associative algebras where representation-theoretic tools apply. Through the Artin–Wedderburn framework, we aim to gain not only matrix-level representations, but also a classification of the irreducible submodules on which the original system acts. In this way, the apparent complexity of nonlinear, context-sensitive dynamics is parsed into block-structured, module-specific behavior. The method isolates algebraic invariants — such as trace, determinant and idempotent decomposition — that persist under variation in rules or configuration space, allowing for structured comparisons across systems. These invariants are not analytic artifacts but are rooted in the internal symmetries and transformation closures of the original nonlinear operations. The block decompositions also highlight how behaviors traditionally viewed as

nonlinear (e.g., symbolic substitution or conditional updates) may conceal internal modularity and linear evolution, obscured only by the global compositional structure.

Compared to other approaches to handling nonlinear dynamics like differential linearization, Lie group methods or topological conjugacy, our technique provides a structurally complete and non-perturbative alternative [13,14]. The above-mentioned classical linearization methods often yield valid results only in neighborhoods of equilibria or require smoothness conditions that rule out discrete or symbolic dynamics. In contrast, our framework accommodates systems without a natural notion of distance or continuity, relying solely on the closure properties of operations and the resulting algebraic relations. Moreover, operator-algebraic approaches in control theory assume a priori linear structure, whereas we derive linearity as an emergent property through algebraic embedding and decomposition. When compared with computational symbolic dynamics or automata-based models, our method has the advantage of providing an explicit matrix-theoretic realization connecting naturally with tools from representation theory, module theory and linear algebra.

Feasible applications. Apart from the above-mentioned discrete threshold oscillator's example, our framework is immediately applicable to experimental physical systems whose dynamics emerge from compositional or switching laws. The ability to separate stable from unstable dynamics through matrix block structure may prove valuable for robustness analysis or synthetic biology applications.

In biological and chemical networks, where reaction pathways or gene regulation processes exhibit nonlinear and often non-additive dynamics, our algebraic decomposition may provide a lens for understanding system architecture. Regulatory motifs governed by conditional or cooperative interactions can be modeled as compositions of transformations, allowing them to be decomposed into simpler, invariant behaviors. This may uncover latent modularity in biochemical signaling pathways, simplify the detection of feedback loops or isolate robust subsystems within gene regulatory networks. Importantly, these representations are not approximate, rather they preserve the full algebraic structure of interactions, potentially clarifying how complex behavior arises from rule-based logic in cell signaling or metabolism. To provide an example, consider a biochemical regulatory network where genes and proteins interact through conditional, non-additive rules (such as activation) only when multiple factors are involved. By constructing an associative algebra from these operations and applying the Wedderburn–Artin decomposition, the complex, nonlinear network is broken into linear modules, each corresponding to a dynamically invariant regulatory mode. This could enable researchers to isolate core subsystems, identify redundancies and predict stable expression patterns, offering an algebraic alternative to traditional kinetic modeling in systems biology, particularly when quantitative data are sparse or discrete behaviors dominate.

Even in neuroscience, where synaptic integration is nonlinear and memory-dependent, the application of algebraic decomposition may reveal invariant dynamical modules across time or stimulus conditions, enabling more precise descriptions of cortical computation.

Operator-based systems, such as those found in quantum computation, signal processing and control theory, frequently involve sequences of non-commuting transformations. In these contexts, our block decomposition enables the isolation of independent or quasi-independent behaviors by analyzing irreducible submodules of the full system algebra. This can be particularly valuable in quantum circuit design or in feedback-based controllers, where interaction rules are discrete, time-sensitive and often reversible only within subspaces. The resulting matrix representations of dynamics are compatible with established numerical tools for simulation, optimization and control, offering computationally tractable pathways for system design. To provide an example, consider nonlinear oscillator networks with switching behavior (such as piecewise-linear electrical circuits or systems governed by threshold-based control), where dynamics are often governed by rule-based transitions rather than continuous flows. Each switching rule can be modeled as a non-commutative operation acting on the system's state. Our decomposition may reveal stable modes and transition pathways, allowing the prediction and control of complex behavior through linear matrix methods. It provides a structural alternative to differential analysis in systems with discrete or abrupt changes.

Our approach has implications also for domains in which nonlinear dynamics, context-dependent transformations and compositional complexity are fundamental to system behavior. In symbolic systems like rewriting grammars, automata and formal language theory, the evolution of configurations is typically governed by local substitution rules and non-commutative operations. These systems often resist classical analysis due to their combinatorial explosion and history-sensitive behavior. By embedding these systems within finite-dimensional associative algebras and applying Wedderburn–Artin decomposition, it becomes possible to identify invariant subsystems and track the evolution of states through blockwise linear dynamics [15,16]. This provides a way to support analysis of complexity and stability in computational linguistics, formal verification and automated rewriting systems.

Beyond static analysis, the presence of radical components in systems with partially associative dynamics suggests experimentally testable hypotheses. Specifically, nilpotent elements of the algebra may correspond to transient or vanishing modes observed in physical or biological systems. This prompts the question of whether experimentally observed decay processes or metastable states can be mapped directly to the radical part of the algebraic decomposition. Similarly, the identification of primitive idempotents within the algebra provides a systematic way to define probes, interventions or perturbations that selectively activate or disrupt specific dynamical modes. These insights could inform targeted interventions in both engineered systems and natural processes, such as isolating a regulatory subnetwork in gene expression or modifying a local control law in robotics.

Limitations. Limitations must be acknowledged. A primary constraint lies in the requirement for a complete and explicit description of the composition relations among the system's generating operations. In high-dimensional or structurally complex systems, these relations can be difficult to determine analytically or even approximate, particularly as the number of generators or the intricacy of their interactions increases. The Gröbner basis and radical computations required in this setting may become intractable for very complex systems. Without practical workarounds, this limitation could significantly hinder the framework's usability in

real-world applications. To address this concern, we several viable strategies that mitigate these computational challenges could be employed. When exact composition rules are inaccessible, empirical relations can be extracted from simulations, yielding reduced generating sets and smaller ideals that still preserve key structural properties. Modular algorithms and randomized techniques (available in computational algebra systems like GAP and Magma) may enable more scalable handling of large or complex algebras by exploiting algebraic symmetries or performing computations over finite fields. These methods are particularly well-suited for approximating Gröbner-like reductions and detecting structural invariants with manageable computational overhead.

For analyzing non-semisimple algebras, spectral heuristics, matrix sampling and perturbation-based numerical techniques may be employed to approximate radical components and estimate nilpotent behavior. Furthermore, data-driven approaches, including machine learning models trained on trajectory data, can assist in formulating conjectural composition rules that are then verified symbolically.

Our approach assumes that the core dynamical behavior is encoded in the algebra of generator compositions. This assumption may fail in systems where essential phenomena emerge only through continuous variation, infinite iteration or long-range dependencies, e.g., cases where finite composition rules cannot fully capture the dynamics. Our framework is built on finite-dimensional associative algebras; hence, it may be less suited for systems inherently requiring infinite-state models like recursive grammars, systems with stochastic or probabilistic transitions or those involving unbounded memory. Additionally, when the algebra is not semisimple, interpreting the physical or computational significance of the radical part can be nontrivial, as nilpotent elements do not correspond to easily isolated or observable modes.

It is also important to note that, at this stage, the framework has been developed and tested primarily on deterministic systems and has not yet been extended to hybrid or stochastic models.

Despite these constraints, our algebraic approach is, in principle, extensible beyond symbolic and operator-driven systems. For discrete-time dynamical systems, each update map can be treated as a generator and the algebraic structure of their compositions can be captured using the same framework. This allows the decomposition of the discrete system into invariant linear subsystems through the same semisimple algebraic techniques. For continuous-time systems governed by differential flows, a discretization of the time domain (such as approximating the flow via time-t maps or Poincaré sections) may allow the embedding of evolution operators into a finite-dimensional algebra. These operators, treated as algebraic elements, can then be decomposed using the Wedderburn–Artin theorem to reveal structural invariants within the continuous system.

In summary, we establish a method for analyzing nonlinear dynamical systems through algebraic decomposition, translating nonlinear operations into structured, blockwise-linear representations via the Wedderburn–Artin framework. Our approach bridges symbolic dynamics, operator theory and representation theory, providing a unified perspective on systems previously treated as analytically opaque.

Ethics approval and consent to participate

This research does not contain any studies with human participants or animals performed by the Author.

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Uncited references

[17], [18], [19], [20]

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References

[1] Abarbanel Henry D, Rabinovich Mikhail I. Neurodynamics: Nonlinear dynamics and neurobiology. Curr Opin Neurobiol 2001;11(11):423–30. http://dx.doi.org/10.1016/s0959-4388(00)00229-4.
[2] Han Maoan, Jin Zhen, Xia Yicang, Zhou Hong. Dynamics of nonlinear systems. Sci World J 2014;2014:246418. http://dx.doi.org/10.1155/2014/246418.
[3] Werner Jan, Pietsch Tobias, Hilker Frank M, Arndt Hans. Intrinsic nonlinear dynamics drive single-species systems. Proc Natl Acad Sci USA 2022;119(119):e2209601119. http://dx.doi.org/10.1073/pnas.2209601119.
[4] Ashwin Peter, Fadera M, Postlethwaite C. Network attractors and nonlinear dynamics of neural computation. Curr Opin Neurobiol 2024;84:102818. http://dx.doi.org/10.1016/j.conb.2023.102818.
[5] Castillo Edward, Castillo Raymond, Vinogradskiy Yuri, Dougherty Melanie, Solis Daniel, Myziuk Natalie, Thompson Adam, Guerra Ricardo, Nair Gautam, Guerrero Thomas. Robust CT ventilation from the integral formulation of the Jacobian. Med Phys 2019;46(46):2115–25. http://dx.doi.org/10.1002/mp.13453.
[6] Barion Andrea, Anthonissen Martin JH, Ten Thije Boonkkamp Jan HM, IJzerman Wilfried L. Computing aberration coefficients for plane-symmetric reflective systems: A Lie algebraic approach. J Opt Soc Amer A 2023;40(40):1215–24. http://dx.doi.org/10.1364/JOSAA.487343.
[7] Brešar Matej. The Wedderburn–Artin theorem. 2024, arXiv preprint arXiv:2405.04588. [math.RA], May 7 2024. https://arxiv.org/abs/2405.04588.
[8] Goldman Oscar. A Wedderburn–Artin–Jacobson structure theorem. J Algebra 1975;34(34):64–73. http://dx.doi.org/10.1016/0021-8693(75)90193-3.
[9] Lam TY. Wedderburn–Artin theory. In: Exercises in classical ring theory. Problem books in mathematics, New York, NY: Springer; 1995, p. 1–12. http://dx.doi.org/10.1007/978-1-4757-3987-9_1.
[10] Mora Teo. An introduction to commutative and noncommutative Gröbner bases. Theoret Comput Sci 1994;134(34):131–73. http://dx.doi.org/10.1016/0304-3975(94)90283-6.
[11] Decker Wolfram, Eder Christian, Levandovskyy Viktor, et al. Modular techniques for noncommutative Gröbner bases. Math Comput Sci 2020;14:19–33. http://dx.doi.org/10.1007/s11786-019-00412-9.
[12] Asghari Mohammad, Fathollahi-Fard Amir M, Mirzapour Al-e-hashem SMJ, MDulebenets axim A. Transformation and linearization techniques in optimization: A state-of-the-art survey. Mathematics 2022;10(10):283. http://dx.doi.org/10.3390/math10020283.
[13] Balas Egon, Mazzola Joseph B. Nonlinear 0–1 programming: I. linearization technique. Math Program 1984;30:1–21. http://dx.doi.org/10.1007/BF02591796.
[14] Liu Jing, Deng Shaoyong. Topological conjugacy between multifunctions. Aequationes Math 2019;93:1203–20. http://dx.doi.org/10.1007/s00010-019-00676-3.
[15] Kramer Lucas, Van Wyk Eric. Strategic tree rewriting in attribute grammars. In: Proceedings of the 13th ACM SIGPLAN international conference on software language engineering. 2020, p. 210–29. http://dx.doi.org/10.1145/3426425.3426943.
[16] Krivochen Diego Gabriel, editor. Formal language theory and its relevance for linguistic analysis. In: Special issue of evolutionary linguistic theory, vol. 3, (no. 3):2021, p. v, 134. http://dx.doi.org/10.1075/elt.3.2.
[17] Behboodi Mahmood, Daneshvar Ali, Vedadi MR. Several generalizations of the Wedderburn–Artin theorem with applications. Algebr Represent Theory 2018;21:1333–42. http://dx.doi.org/10.1007/s10468-017-9748-2.
[18] Bourne S. The Jacobson radical of a semiring. Proc Natl Acad Sci USA 1951;37(37):163–70. http://dx.doi.org/10.1073/pnas.37.3.163.
[19] Jia Xiao-Xia, Song Yong-Xia, Wang Dong-Sheng, Nie De-Xiang, Wu Jian-Zhong. A collaborative secret sharing scheme based on the Chinese remainder theorem. Math Biosci Eng 2019;16(16):1280–99. http://dx.doi.org/10.3934/mbe.2019062.
[20] Jiang Yong, Shen Yanan, Zhu Qian. A lightweight key agreement protocol based on Chinese remainder theorem and ECDH for smart homes. Sensors 2020;20(20):1357. http://dx.doi.org/10.3390/s20051357.

Medical Decision-Making Amid Insufficient, Unreliable or Conflicting Empirical Evidence

Arturo Tozzi *

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Article

Medical Decision-Making Amid Insufficient, Unreliable or Conflicting Empirical Evidence

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA, 1155 Union Circle, #311427 Denton, TX 76203-5017 USA; tozziarturo@libero.it

Abstract: Medical decision-making relies on scientific data to ensure accurate diagnoses and effective treatments. However, in many real-world scenarios, medical data may be incomplete, unreliable or conflicting, leaving clinicians with significant uncertainty. We propose a pragmatic and structured approach to medical judgment when sufficient empirical evidence is unavailable. We build a heuristic model that integrates multiple sources of knowledge—including evidence-based medicine levels, expert consensus, individual clinical experience, logical reasoning and cognitive biases—to derive a quantifiable degree of belief in a given treatment decision. Each source is assigned a weighted value and their cumulative score determines whether a proposed medical intervention should be accepted or rejected. Unlike traditional Bayesian models, which rely on probabilistic updates, our method prioritizes pragmatic decision-making through an aggregation of both statistical and non-statistical evidence. Our approach integrates both subjective and collective knowledge, emphasizing the central role of clinical expertise and contextual factors in medical practice when evidence-based medicine is insufficient. Through a hypothetical case study on antibiotic administration, we illustrate the practical application of our model. We conclude that our heuristic belief-aggregation model, by formalizing the weighting of diverse epistemic sources—including pragmatic reasoning—enhances decision-making in ambiguous medical contexts where conventional empirical validation is unavailable.

Keywords: epistemic uncertainty; heuristic judgment; Bayesian alternatives; evidence-based medicine; cognitive biases

Introduction

Medical decision-making requires clinicians to rely on available scientific evidence to ensure accurate diagnoses and determine the most effective course of treatment. Ideally, these decisions are driven by a solid foundation of empirical data, including randomized controlled trials, systematic reviews and large-scale observational studies (Young et al., 2020; McFadden 2023). However, in real-world clinical practice, such high-quality evidence is not always available. Physicians frequently encounter cases where data are incomplete, unreliable or contradictory, creating an epistemic gap that challenges the traditional models of evidence-based medicine (Meyer et al., 2021). The question that arises in these situations is whether a rational and justifiable medical decision can still be made and if so, what methodologies can be employed to navigate uncertainty (Guerdan et al., 2023). The absence of scientific evidence in medical decision-making can result from multiple factors. Some medical conditions are rare or under-researched, limiting the availability of robust clinical trials. Emerging diseases and novel therapeutic interventions, such as the COVID-19 vaccine, often lack long-term studies, compelling doctors to make decisions based on preliminary findings, clinical observations, and theoretical reasoning. Additionally, the nature of medical evidence itself can be contested, with studies producing conflicting results due to differences in methodology, sample size or statistical interpretation (Kaplanis et al., 2020). The hierarchy of evidence in evidence-based medicine traditionally prioritizes randomized trials over observational studies, expert opinions and

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mechanistic reasoning (Wang et al., 2023). Yet, in many medical situations, reliance on lower-tier evidence is unavoidable (Hammond et al., 2021; Wan et al., 2022). The challenge, then, is how to systematically incorporate diverse knowledge sources while maintaining the reliability of medical judgments.

Bayesian models provide a mathematical framework for integrating diverse sources of information and dynamically updating degrees of belief as new data emerges. However, they assume that probabilities can be assigned to all relevant variables, which is not always feasible in clinical contexts with missing or non-quantifiable data. Moreover, Bayesian inference does not account for the cognitive and social dynamics that shape how medical professionals weigh different forms of evidence, including biases, heuristics and collective knowledge. Given these limitations, alternative frameworks are needed to formalize medical decision-making under uncertainty while remaining pragmatic in real-world clinical environments.

We propose a heuristic belief-aggregation model (henceforward HAM) that quantifies degrees of belief by integrating multiple epistemic sources relevant to medical judgment. The primary objective is to provide a structured yet flexible method that allows clinicians to make justified treatment decisions even in the absence of robust empirical data. Our approach assigns weighted values to different categories of evidence, including scientific literature, expert consensus, individual clinical experience, logical reasoning. By summing these weighted values, a cumulative score is generated, indicating whether a particular treatment decision should be accepted or rejected. Unlike standard Bayesian approaches, which require well-defined probability distributions, our model accommodates qualitative and subjective factors that often play a crucial role in clinical reasoning. A key feature of our approach is its explicit acknowledgment of both externalist and internalist sources of justification. Externalist sources, such as systematic reviews and randomized trials, provide objective, data-driven insights forming the backbone of evidence-based medicine (Goldman, 1986; Alston 1989). Internalist sources, including individual expertise, peer opinions and inferential reasoning, contribute to the interpretation and contextualization of available data (Chisholm 1989; Haack 1991; Ben-Moshe 2019). HAM systematically incorporates these elements, ensuring that decision-making is not solely dependent on statistical inference but also accounts for the realities of medical practice.

To illustrate the utility of our heuristic model, we present a hypothetical case study involving the use of an antibiotic in a scenario where conventional guidelines do not offer a clear directive. By applying our belief-aggregation method, we demonstrate how different knowledge sources contribute to a final treatment decision. In the following sections, we discuss the theoretical foundations of our approach, outlining its relationship to existing decision-making models in medicine. Then, we detail the methodological framework, specifying how different sources of knowledge are weighted and integrated. A practical application is presented through the case study, followed by a discussion of the HAM's advantages, limitations and prospects for future refinement.

Materials and Methods

We built a structured heuristic framework designed to assist medical decision-making when sufficient empirical data are unavailable, unreliable or contradictory. The approach integrates multiple sources of knowledge, both empirical and non-empirical, to form a quantifiable degree of belief regarding a medical decision. We follow a sequential process that begins with the formulation of a medical question and proceeds through evidence collection, source classification, numerical assignment, aggregation and decision-making.

The first step is to define the medical question in a standardized, logical format, ensuring that the problem under investigation can be systematically analysed. The medical question is framed as a conditional statement of the form "if X, then Y," where X represents a specific medical intervention and Y denotes an expected clinical outcome. The question is structured to have a binary response of either "yes" or "no" to facilitate decision-making without ambiguity (Ramsey 1929; Carnap 1974;

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Taylor and Raden, 2007; Misak 2020). This format is chosen to align with classical logical structures and expert system frameworks, which prioritize clear conclusions.

The next step consists of the identification and collection of all available sources of knowledge that may contribute to the decision-making process. This includes formal scientific evidence from medical literature, clinical expertise derived from practitioners' experience, collective knowledge within the medical community, inferential reasoning based on logical principles and additional modifying factors such as cognitive biases and confounding variables.

The collected information is categorized into two broad epistemic domains: externalist sources, which originate from outside the individual decision-maker and relyi on empirical validation (Armstrong 1973; Supriyanto 2023) and internalist sources, which depend on the subjective interpretation, expertise and reasoning of the clinician (Feldman and Conee, 1985; BonJour 1985; Supriyanto 2023).

The externalist sources consist of levels of evidence classified according to the widely accepted hierarchical frameworks in evidence-based medicine. The highest-ranked source of knowledge in this category is systematic reviews and meta-analyses of randomized controlled trials, considered the gold standard for determining treatment efficacy (Burns et al., 2011). Randomized controlled trials without systematic synthesis follow in the hierarchy, providing a strong, albeit slightly less robust, form of evidence (OCEBM Levels of Evidence Working Group, 2011; Antoniou 2021). Non-randomized controlled cohort studies and observational studies come next, reflecting a lower level of empirical rigor but still contributing valuable insights, especially in cases where randomized trials are infeasible. The weakest form of externalist evidence includes case series, case reports and expert opinion without formal empirical validation, which are often used as a last resort when higher levels of evidence are unavailable (Greenhalgh 1997; Debray et al., 2023). Further, model-based statistical approaches could be able to predict patient outcomes with discrete accuracy, improving decision-making related to medical treatments (Chekroud et al., 2024). The growing momentum of machine learning–based medical decision support systems in health care is creating high expectations, but the performance is not (yet?) able to overtake human decision-making (Vasey et al., 2021; Luo et al., 2023).

The internalist sources encompass a broader range of non-empirical factors influencing medical judgment (Heyting 1930). Individual clinical experience plays a significant role, incorporating knowledge gained from repeated exposure to similar cases, pattern recognition and intuitive decision-making confidence (Rosenberg, 2002; Fields et al., 2024). This category also includes collective medical knowledge, which is derived from discussions among practitioners, expert consensus and the collective wisdom of the medical community (Monteiro et al., 2020). According to the wisdom of crowds principles, accurate estimates can be obtained by combining the judgements of different individuals, since the average of just two judgements from different people (between-person aggregation) is more accurate than a large number of repeated judgements from the same person (within-person aggregation) (van Dolder et al., 2018). Logical reasoning is another crucial internalist issue, incorporating deductive inference, mechanism-based thinking and theoretical extrapolation (Suppes 1960; Vasey et al., 2021). Additionally, modifying factors such as cognitive biases, heuristics and decision-making fallacies are recognized for their potential to distort judgment (Gettier 1963; Taleb 2007; Beldhuis et al., 2021; Loncharich et al., 2023).

The next step consists of numerical assignment of values to each source. This involves the allocation of weighted scores reflecting the relative reliability and influence of each knowledge source. A numerical scale is used, where positive values indicate support for the proposed medical intervention, negative values signify opposition and zero values denote neutrality or the absence of relevant information (**Figure 1**). The assignment of numerical values is driven by the relative strength of each source within the hierarchy of evidence, with higher-ranked sources receiving larger numerical weights. Systematic reviews and meta-analyses are assigned to have the highest positive or negative values due to their strong empirical foundation, while lower-ranked sources receive progressively smaller values. Individual clinical experience, collective knowledge and logical reasoning are assigned lower values, reflecting their contributory role but acknowledging their

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susceptibility to subjective bias. Indeed, confounding factors, including cognitive biases and emotional influences, stand for modifiers that may adjust or temper the assigned values.

The next step involves the aggregation of values to generate a cumulative score representing the overall degree of belief. The summation of all assigned values produces a single numerical outcome, which is interpreted according to predefined thresholds. If the cumulative score is positive, the treatment decision is endorsed; if the score is negative, the treatment is rejected. The absolute magnitude of the score provides an indication of the degree of confidence in the decision, with higher values reflecting stronger epistemic support. Cases where the score is near zero indicate a state of epistemic equilibrium, where neither approval nor rejection is strongly justified.

In sum, HAM aims to integrate diverse knowledge sources into a single quantitative framework. The method accommodates both empirical and non-empirical factors, ensuring that decision-making remains structured even in the absence of definitive scientific evidence.

Next, we apply our methodology to a case study examining the hypothetical use of an antibiotic termed Napcar for treating severe diabetic microangiopathy.

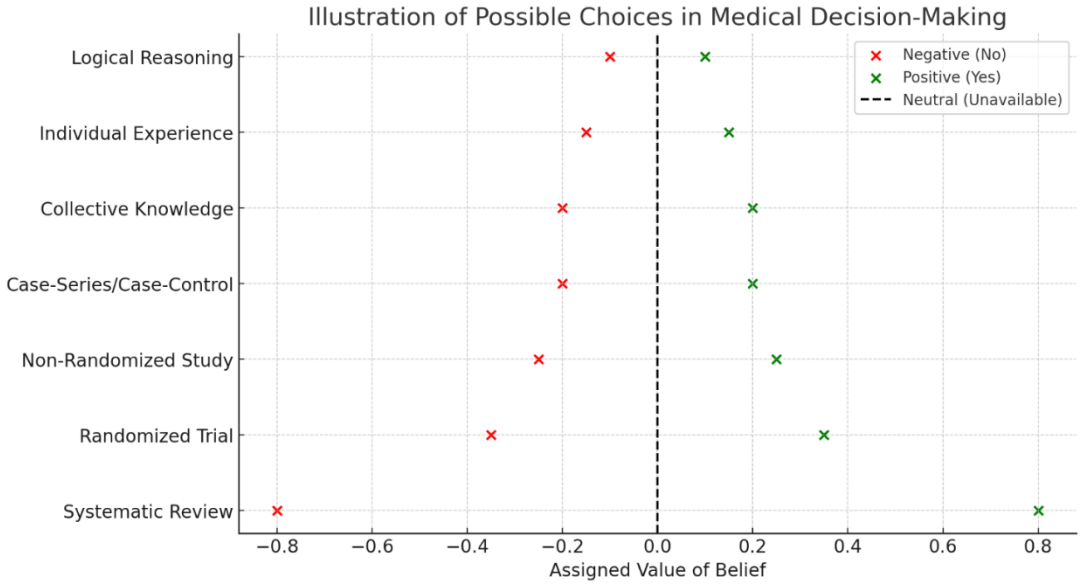

Figure 1. Potential available options in medical decision-making. The diagram represents different sources of knowledge (systematic review, randomized trials, case series, etc.) and their assigned values, ranging from negative (red) to positive (green), with a neutral line indicating unavailable information.

Results

We analysed a case study exploring the potential use of the hypothetical antibiotic Napcar in treating severe diabetic microangiopathy. The medical question under evaluation was whether administering Napcar twice daily instead of the conventional once-daily regimen would yield superior clinical outcomes. We followed our structured methodology, beginning with the formulation of the medical question and proceeding through source identification, classification, numerical assignment and aggregation.

To address the specific question, no systematic reviews or meta-analyses were available, resulting in an assigned value of zero for this source (**Figure 2**). A single randomized controlled trial had been conducted on the conventional use of Napcar, providing evidence that the once-daily regimen was effective, leading to a negative value assignment of -0.35. Non-randomized cohort studies and observational data were not available, contributing additional zero values. A case series had reported anecdotal evidence supporting twice-daily administration, warranting a positive value assignment of +0.2. Collective medical knowledge, based on informal expert opinions, contributed a small positive value of +0.1, reflecting cautious support without empirical validation. Individual

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clinical experience also supported the possibility of twice-daily administration, contributing an additional +0.1. Logical reasoning, based on pharmacokinetic properties indicating a short half-life of Napcar, provided another +0.1 in favor of increased frequency of administration. Aggregating these values resulted in a cumulative score of +0.15, indicating a slight preference for the twice-daily regimen despite the presence of a contradictory randomized trial.

The cumulative belief score must be interpreted within the context of decision-making thresholds. A strongly negative score would have reinforced the existing standard of care, discouraging deviation from the once-daily regimen, while a strongly positive score would have provided compelling justification for the alternative regimen. The final belief score of +0.15 indicated a weak but measurable preference for the adjusted dosing schedule, suggesting that, within the context of limited empirical data, there was a justifiable rationale for considering the alternative treatment approach of twice-daily administration.

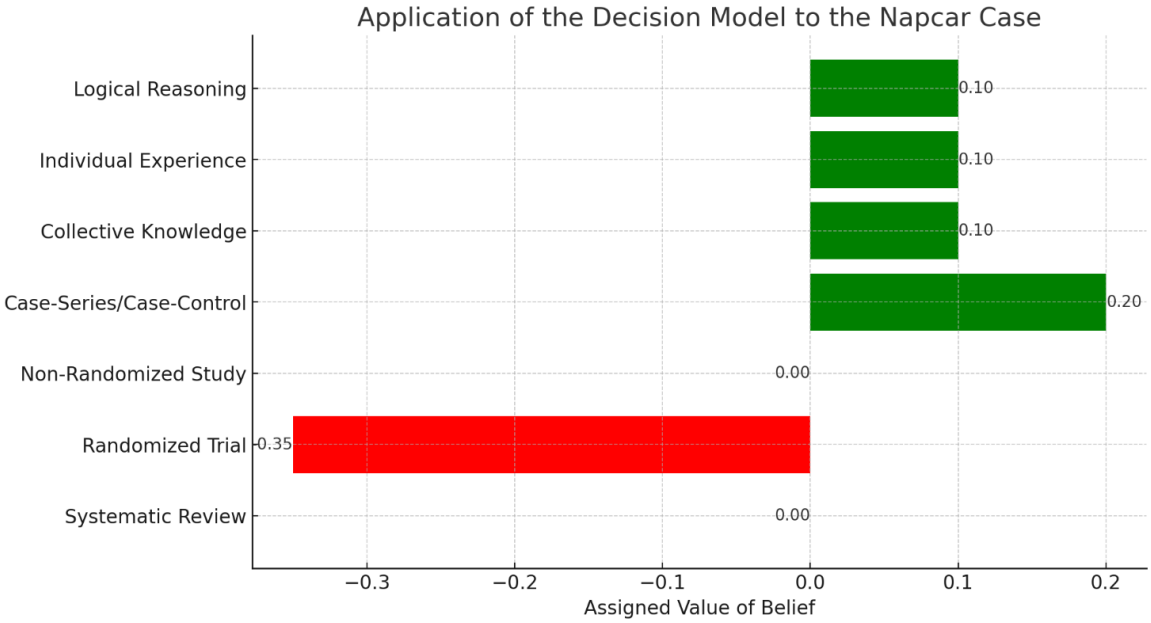

Figure 2. Application of HAM to the hypothetical Napcar case study, where the possibility of administering the antibiotic twice a day instead of once was evaluated. Each category represents a different source of knowledge and the assigned values reflect their contribution to the final decision. The final cumulative value of +0.15 suggests a slight preference for administering Napcar twice daily.

Conclusions

We provide a structured approach to medical decision-making when empirical data are insufficient, unreliable or conflicting. The novelty of our approach lies in its explicit integration of both empirical and non-empirical sources of knowledge into a unified, quantitative framework. Our model acknowledges that real-world medical decision-making incorporates a broader range of influences. Unlike purely probabilistic approaches, such as Bayesian inference, which require precise prior probabilities, our heuristic method offers a simpler, more adaptable structure that accommodates qualitative factors, including cognitive biases, personal experience and expert consensus. The innovation is the explicit numerical weighting of different sources of knowledge, allowing for a transparent approach to decision-making under uncertainty. The choice of numerical values assigned to each epistemic source is based on a balance between the strength of evidence and its practical influence on decision-making, ensuring that the framework remains both scientifically grounded and clinically useful. The numerical scale guarantees that highly reliable sources exert a dominant influence on the final belief score, while still allowing room for other factors to contribute meaningfully. The highest positive and negative values are assigned to systematic reviews due to

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their comprehensive nature and reliability, followed by decreasing weights for randomized trials, observational studies and case-series data. Lower values are assigned to collective knowledge, individual experience and logical reasoning, reflecting their less formalized and more context-dependent nature. Further, our approach mitigates the risk of subjective bias dominating clinical reasoning while still preserving the flexibility needed for real-world application. Alternative numerical assignments could be used, but they would require rigorous justification and empirical validation to maintain consistency and predictive reliability.

In sum, the interpretability and reproducibility of the numerical belief score ensures transparency in the decision-making process, allowing clinicians to see exactly how different sources contribute to the outcome.

Compared to other techniques, HAM offers several advantages. Traditional Bayesian methods, while mathematically rigorous, require precise probability distributions and often struggle to incorporate qualitative factors such as cognitive biases, expert judgment and heuristic reasoning. HAM circumvents this issue by using a structured yet flexible numerical system accommodating both statistical and non-statistical evidence. Still, machine learning-based decision-support systems, while increasingly sophisticated, often lack transparency and interpretability, making it difficult for clinicians to understand how a given recommendation is derived. In turn, our model provides an explainable decision-making process, allowing physicians to see exactly how different sources contribute to the outcome. Unlike purely intuitive decision-making, which can be highly variable and prone to bias, HAM ensures that all relevant epistemic inputs are explicitly considered and systematically weighted.

The potential applications of HAM extend beyond the Napcar case study to a wide range of medical decision-making scenarios. In emergency medicine, where rapid decisions are required despite incomplete information, HAM could provide a way to integrate available evidence and professional judgment. In rare diseases, where large-scale clinical trials are often lacking, the model could help synthesize case reports, expert opinions and mechanistic reasoning into a coherent treatment recommendation. In situations involving emerging diseases or novel treatments where data are limited, our method could be adapted to continuously update belief scores as new evidence becomes available. Additionally, HAM could be implemented in artificial intelligence-driven decision-support systems, enhancing the ability of clinical algorithms to incorporate expert knowledge and qualitative reasoning into automated recommendations. Also, HAM has broader implications for epistemology and medical ethics. The process of forming justified medical beliefs without sufficient empirical evidence raises fundamental questions about the nature of knowledge, uncertainty and the role of expert judgment in healthcare. The strain between empirical rigor and pragmatic necessity underscores the need for a balanced approach that neither dismisses non-empirical factors outright, nor overemphasizes subjective reasoning at the expense of scientific validity. Additionally, expanding HAM to incorporate sensitivity analysis would allow for a better understanding of how variations in individual epistemic contributions influence overall decision confidence.

The application of HAM to the hypothetical case study of the antibiotic Napcar demonstrates how our structured decision-making framework can integrate multiple epistemic sources, assign numerical values to each and generate a cumulative belief score to guide clinical choices. Even when robust empirical evidence is available, such as the randomized controlled trial in our case study, alternative knowledge sources may still exert influence on medical decision-making. This means that medical decision-making is not purely data-driven but also shaped by contextual understanding and pragmatic considerations. The results further highlight the importance of explicitly considering cognitive biases and confounding factors. Although not assigned discrete numerical values in this study, these factors were recognized as potential modifiers of judgment. HAM could be tested through controlled or retrospective studies in which physicians are presented with complex clinical cases and asked to make decisions using different methods, with their choices compared for

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consistency and justification. We hypothesize that our model will perform comparably to Bayesian approaches in predictive accuracy but with greater ease of use.

Despite its advantages, HAM has several limitations. Our assignment of numerical values to different epistemic sources remains somewhat subjective. While our hierarchy is grounded in evidence-based medicine principles, different weighting schemes could yield different results. Our method does not erase uncertainty but rather provides a structured way to navigate it; decisions with low belief scores may still require further empirical research or additional clinical judgment. HAM assumes that different epistemic sources can be meaningfully combined into a single numerical score, which may not always be the case, particularly when sources directly contradict each other. Additionally, while our model accounts for cognitive biases, it does not prevent them entirely and clinicians must remain vigilant against over-reliance on subjective influences. Still, HAM's reliance on numerical assignments introduces an inherent degree of subjectivity, as the weighting of different sources depends on heuristic judgment rather than a strictly algorithmic process. The relatively low cumulative score in the Napcar case illustrates the challenge of making high-confidence decisions when empirical evidence is conflicting or sparse. Furthermore, while the approach successfully synthesizes various epistemic sources, it does not resolve the underlying issue of limited high-quality data, meaning that decisions remain probabilistic rather than definitive. Future refinements, such as expert calibration of numerical weights and sensitivity analyses to assess the robustness of belief scores, could enhance HAM's reliability and applicability.

In conclusion, we introduce a structured and quantifiable approach to medical decision-making when empirical data are lacking. By integrating multiple sources of knowledge, assigning numerical values and generating a cumulative belief score, our model, despite its limitations, provides a systematic framework to tackle complex treatment decisions.

Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical and material support, study supervision.

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References

1. Alston, William P., 1989. Epistemic Justification: Essays in the Theory of Knowledge. Ithaca, NY: Cornell University Press.
2. Antoniou, A. 2021. What is a data model? Euro Jnl Phil Sci 11, 101. https://doi.org/10.1007/s13194-021-00412-2

 doi:10.20944/preprints202502.0769.v1

3. Armstrong, David, 1973. Belief, Truth and Knowledge. Cambridge: Cambridge University Press.
4. Beldhuis, Iris E; Ramesh S Marapin, You Yuan Jiang, Nádia F Simões de Souza, Artemis Georgiou, et al. 2021. Cognitive biases, environmental, patient and personal factors associated with critical care decision making: A scoping review. J Crit Care. 2021 Aug:64:144-153. doi: 10.1016/j.jcrc.2021.04.012. DOI: 10.1016/j.jcrc.2021.04.012
5. Ben-Moshe, Nir. 2019. The internal morality of medicine: a constructivist approach. Synthese 196 (11):4449-4467.
6. BonJour, Laurence, 1985. The Structure of Empirical Knowledge. Cambridge, MA: Harvard University Press.
7. Burns, Patricia B.; Rohrich, Rod J.; Chung, Kevin C. (July 2011). "The Levels of Evidence and Their Role in Evidence-Based Medicine". Plastic and Reconstructive Surgery. 128 (1): 305–310. doi:10.1097/PRS.0b013e318219c171. PMC 3124652. PMID 21701348.
8. Carnap R. 1974. An Introduction to the Philosophy of Science. Ed. Gardner M, 1995, Dover Publications, Inc., Mineona, New York. ISBN-13: 978-0486283180
9. Chekroud, Adam M; Matt Hawrilenko, Hieronimus Loho, Julia Bondar, Ralitza Gueorguieva, et al. 2024. Illusory generalizability of clinical prediction models. Science. 2024 Jan 12;383(6679):164-167. doi: 10.1126/science.adg8538.
10. Chisholm, Roderick, 1989. Theory of Knowledge, 3rd edition. Englewood Cliffs, NJ: Prentice-Hall.
11. Debray, Thomas P A; Gary S Collins, Richard D Riley, Kym I E Snell, Ben Van Calster, et al. 2023. Transparent reporting of multivariable prediction models developed or validated using clustered data (TRIPOD-Cluster): explanation and elaboration. BMJ 2023;380:e071058
12. Feldman, Richard and Earl Conee, 1985. “Evidentialism.” Philosophical Studies, 48, pp. 15-34.
13. Fields, by Chris; James F. Glazebrook, Michael Levin. 2024. Principled Limitations on Self-Representation for Generic Physical Systems. Entropy 2024, 26(3), 194; https://doi.org/10.3390/e26030194
14. Gettier, Edmund, 1963. “Is Justified True Belief Knowledge?” Analysis, 23, pp. 121-123.
15. Goldman, Alvin, 1986. Epistemology and Cognition. Cambridge: Harvard University Press.
16. Greenhalgh T. July 1997. How to read a paper. Getting your bearings (deciding what the paper is about). BMJ. 315 (7102): 243–246. doi:10.1136/bmj.315.7102.243. PMC 2127173. PMID 9253275.
17. Guerdan, Luke; Amanda Coston, Kenneth Holstein, Zhiwei Steven Wu. 2023. Counterfactual Prediction Under Outcome Measurement Error. FAccT '23: Proceedings of the 2023 ACM Conference on Fairness, Accountability and Transparency
18. Haack, Susan, 1991. “A Foundherentist Theory of Empirical Justification,” In Theory of Knowledge: Classical and Contemporary Sources (3rd ed.), Pojman, Louis (ed.), Belmont, CA: Wadsworth.
19. Hammond, MEH; Josef Stehlik, Stavros G. Drakos, Abdallah G. Kfoury. 2021. Bias in Medicine. Lessons Learned and Mitigation Strategies. JACC Basic Transl Sci. 2021 Jan; 6(1): 78–85. doi: 10.1016/j.jacbts.2020.07.012
20. Heyting, Arend. 1930. Die formalen Regeln der intuitionistischen Logik I, II, III. Sitzungsberichte der preussischen Akademie der Wissenschaften (in German). pp. 42–56, 57–71, 158–169. In three parts
21. Kaplanis, J., Samocha, K.E., Wiel, L. et al. 2020. Evidence for 28 genetic disorders discovered by combining healthcare and research data. Nature 586, 757–762 (2020). https://doi.org/10.1038/s41586-020-2832-5
22. Lewis, David. 1973. Counterfactuals and Comparative Possibility. Journal of Philosophical Logic. 2 (4). doi:10.2307/2215339.
23. Loncharich, Michael F, Rachel C Robbins, Steven J Durning, Michael Soh, Jerusalem Merkebu. 2023. Cognitive biases in internal medicine: a scoping review. Diagnosis (Berl). 2023 Apr 21;10(3):205-214. doi: 10.1515/dx-2022-0120. DOI: 10.1515/dx-2022-0120
24. Luo, Yuan; Richard G. Wunderink, Donald Lloyd-Jones. 2023. Proactive vs Reactive Machine Learning in Health Care -Lessons From the COVID-19 Pandemic. JAMA. 2022;327(7):623-624. doi:10.1001/jama.2021.24935.
25. McFadden, Johnjoe. 2023. Razor sharp: The role of Occam's razor in science. Ann N Y Acad Sci . 2023 Dec;1530(1):8-17. doi: 10.1111/nyas.15086. Epub 2023 Nov 29.

 doi:10.20944/preprints202502.0769.v1

26. Meyer, Ashley N D; Traber D Giardina, Lubna Khawaja, Hardeep Singh. 2021. Patient and clinician experiences of uncertainty in the diagnostic process: Current understanding and future directions. Patient Educ Couns. 2021 Nov;104(11):2606-2615. doi: 10.1016/j.pec.2021.07.028.
27. Misak C. 2020. Frank Ramsey: A Sheer Excess of Powers. OUP Oxford. ISBN-13 : 978-0198755357
28. Monteiro, Sandra; Jonathan Sherbino, Jonathan S. Ilgen , Emily M. Hayden , Elizabeth Howey and Geoff Norman. The effect of prior experience on diagnostic reasoning: exploration of availability bias. Diagnosis (Berl). 2020 Aug 27;7(3):265-272. doi: 10.1515/dx-2019-0091.
29. OCEBM Levels of Evidence Working Group. 2011. The Oxford 2011 Levels of Evidence. Oxford Centre for Evidence-Based Medicine. http://www.cebm.net/index.aspx?o=5653
30. Ramsey FP. 1929. Philosophy. In: The Foundations of Mathematics and Other Logical Essays, R. B. Braithwaite (ed.), second impression, 1950, Routledge &Kegan Paul LTD, London.
31. Rosenberg, Charles E. 2002. The Tyranny of Diagnosis: Specific Entities and Individual Experience. Milbank Q. 2002 Jun; 80(2): 237–260. doi: 10.1111/1468-0009.t01-1-00003
32. Suppes, P. (1960). A comparison of the meaning and uses of models in mathematics and the empirical sciences. Synthese, 12(2-3), 287–301.
33. Supriyanto S. 2023. Justification Theory of Internalism VS Externalism. IJSSHR. volume 06 issue 02 february 2023. DOI : https://doi.org/10.47191/ijsshr/v6-i2-54
34. Taleb NN. 2007. The Black Swan: Second Edition: The Impact of the Highly Improbable: With a new section: "On Robustness and Fragility". Random House Publishing Group; 2° ed., 2010. ISBN-13: 978-0812973815
35. Taylor, James; Raden, Neil (2007). Smart (Enough) Systems. Prentice Hall. ISBN 0-13-234796-2.
36. van Dolder, D., van den Assem, M.J. The wisdom of the inner crowd in three large natural experiments. Nat Hum Behav 2, 21–26 (2018). https://doi.org/10.1038/s41562-017-0247-6
37. Vasey, Baptiste; Stephan Ursprung, Benjamin Beddoe, Elliott H. Taylor, Neale Marlow, et al. 2021. Association of Clinician Diagnostic Performance With Machine Learning–Based Decision Support Systems - A Systematic Review. JAMA Netw Open. 2021;4(3):e211276. doi:10.1001/jamanetworkopen.2021.1276
38. Wan, Bohua; Brian Caffo, Swaroop Vedula. 2022. A Unified Framework on Generalizability of Clinical Prediction Models. Front. Artif. Intell. Volume 5 - 2022 | https://doi.org/10.3389/frai.2022.872720
39. Wang, Shirley V.; Sebastian Schneeweiss, the RCT-DUPLICATE Initiative. 2023. Emulation of Randomized Clinical Trials With Nonrandomized Database Analyses: Results of 32 Clinical Trials. JAMA;329(16):1376-1385. doi:10.1001/jama.2023.4221
40. Young, Paul J.; Christopher P. Nickson anders Perner. 2020. When Should Clinicians Act on Non–Statistically Significant Results From Clinical Trials? JAMA. 2020;323(22):2256-2257. doi:10.1001/jama.2020.3508

Time-Crystal Microdevice inspired by Fukuta–Cerin Triangle–Hexagon Symmetry

Arturo Tozzi *

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Article

Time-Crystal Microdevice inspired by Fukuta–Cerin Triangle–Hexagon Symmetry

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA, 1155 Union Circle, #311427 Denton, TX 76203-5017 USA; tozziarturo@libero.it

Abstract: Time crystals are a class of non-equilibrium phases of motion characterized by spontaneous temporal symmetry breaking. We describe a time-crystal-inspired microdevice (TCIM) structured around a hexagonal shell encapsulating a central triangular motif. The geometry is derived from the Fukuta–Cerin theorem, where the centroid of each triangle aligns with that of the surrounding hexagon. In a TCIM flock, this leads to the emergence of controlled, oscillatory behaviour and symmetry-breaking phenomena driven by the internal geometry of the system. Indeed, the triangles introduce geometric frustration that allows the agents to maintain oscillations without relying on continuous external influence. The disruption of perfect synchronization enhances the flock's ability to exhibit periodic, self-sustained oscillatory behaviour in response to minimal energy input or periodic perturbations, showcasing the system's capacity for self-organization and dynamic patterns. Numerical simulations demonstrate that, under periodic driving, local alignment rules and structural frustration, the TCIM flock exhibits self-sustained subharmonic oscillations. These oscillations are characterized by a frequency shift to half the driving frequency, a key indicator of the emergence of time-crystal-like behaviour. This points towards the system's ability to break discrete time-translation symmetry, another hallmark of time-crystal dynamics. TCIMs could enable the development of intelligent microdevices with internally regulated timing mechanisms like self-regulating sensors and synthetic bio-compatible materials that operate with minimal external control. TCIM-inspired devices, utilizing internal temporal rhythms, could also be applied in drug delivery systems, enabling the autonomous release of therapeutic agents in a timed, controlled manner without relying on continuous external inputs.

Keywords: non-equilibrium dynamics; self-organization; autonomous systems; geometric frustration; soft robotics

Introduction

The study of time crystals, first proposed in the context of quantum many-body systems, has rapidly expanded into classical and active matter domains, offering new perspectives on non-equilibrium phases of motion (Choi et al., 2017; He et al., 2025). These systems break discrete time-translation symmetry, displaying periodic behavior under periodic driving, with responses that are not trivially locked to the input frequency (Zhang et al., 2017; Autti et al., 2018; Shapere and Wilczek, 2019). While numerous realizations have emerged in trapped ions, spin chains and optical lattices, the majority rely on externally imposed structures or sustained driving conditions. In the realm of soft active matter and microdevices, however, the challenge remains to achieve time-crystal-like behavior through internal design principles rather than continuous external modulation (Auschra et al., 2021; Omar et al., 2021; Hernández-López et al., 2024). Current models of oscillatory agents often depend on homogeneous propulsion fields or alignment rules, lacking built-in asymmetries or geometric constraints that could intrinsically guide temporal order (Kriegman et al., 2020; Heckenthaler et al., 2023; Davis et al., 2024). Moreover, existing self-oscillating systems rely on chemical or mechanical feedback loops but seldom integrate spatial symmetry with timing control (Vutukuri et al., 2020; Savoie et al., 2018). This limitation hinders the realization of robust, geometry-

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driven microdevices capable of maintaining structured temporal responses under realistic, noisy conditions.

We introduce a novel design framework for active microdevices, leveraging internal geometric frustration and symmetry to generate persistent temporal dynamics. Our model is inspired by the Fukuta–Cerin theorem, a geometric construct that associates regular hexagons to arbitrary triangles through affine transformations, preserving centroid alignment (Martini 1996; Stachel 2002). By embedding a triangle–hexagon architecture into the microdevice structure, we establish a constrained symmetry core governing the device's local interactions and collective orientation over time. This configuration, when implemented in a population of self-propelled agents, produces oscillatory behaviors reminiscent of discrete time crystals. The induced structural frustration inhibits complete synchronization, promoting periodic subharmonic dynamics and collective rhythm formation. This geometric constraint may act as an internal regulator of temporal behavior, yielding advantages in stability, resilience and low-energy operation across variable environments.

We will proceed as follows: first, we outline the mathematical and geometric foundation of the proposed design and its adaptation into a soft active matter context. Next, we describe the computational simulation framework and the resulting dynamical observations. Finally, we discuss the implications of our findings in relation to existing time-crystalline models and assess the system's unique structural-temporal properties.

Materials and Methods

1. Geometric Foundation and Theoretical Framework. The geometric construction of our active agents is based on the Fukuta–Cerin theorem, which applies affine transformations to create regular hexagons from arbitrary triangles, while maintaining centroid symmetry (Martini 1996; Stachel 2002). The vertices of the initial triangle $\triangle ABC$ in $\mathbb{R}^2$ are defined as $A = (x_1, y_1), B = (x_2, y_2)$ and $C = (x_3, y_3)$ and the centroid G of the triangle is calculated as the mean of the vertex coordinates:

$$G = \left(\frac{x_1 + x_2 + x_3}{3}, \frac{y_1 + y_2 + y_3}{3} \right).$$

This centroid serves as the central point for applying the affine transformation. The core idea is to generate a hexagonal shell around the centroid, where each side of the hexagon corresponds to an affine transformation of the vectors from the centroid to the triangle's vertices (**Upper Figure 1**). The vector $\mathbf{v}_i = \vec{GA}_i$ represents the vector from the centroid G to the vertex A_i. By rotating and scaling these vectors, the hexagonal vertices H_i are defined by the formula:

$$H_i = G + R(\theta_i) \cdot \mathbf{v}_i,$$

where $R(\theta_i)$ is the rotation matrix:

$$R(\theta) = \begin{bmatrix} \cos(\theta) & -\sin(\theta) \\ \sin(\theta) & \cos(\theta) \end{bmatrix},$$

and $\theta_i = 60^\circ \cdot i$ for $i = 0, \ldots, 5$, corresponding to the hexagonal symmetry. This approach creates a hexagonal enclosure around the internal triangle, which serves as the foundational geometric structure for each agent. The Fukuta–Cerin theorem guarantees that the hexagonal and triangular motifs are symmetrically aligned, forming a consistent basis for the agent's motion dynamics.

2. Agent Dynamics and Orientation Representation. Each active agent in the system is modeled as a point particle, characterized by its position $\mathbf{r}_i(t) \in \mathbb{R}^2$ and orientation $\theta_i(t) \in [0, 2\pi)$, evolving over discrete time steps. The agent's velocity is given by:

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$$\mathbf{v}_i(t) = v_0 \hat{\mathbf{n}}_i(t),$$

where v_0 is the constant self-propulsion speed and $\hat{\mathbf{n}}_i(t) = (\cos\theta_i(t), \sin\theta_i(t))$ is the unit vector corresponding to the agent's heading direction at time ttt. The position update of each agent is performed by the following formula:

$$\mathbf{r}_i(t + \Delta t) = \mathbf{r}_i(t) + v_0 \hat{\mathbf{n}}_i(t) \Delta t,$$

where $\Delta t = 1$ is the discrete time step. For simplicity, the agent's velocity is fixed to $v_0 = 0.1$. The system is initialized by distributing the agents uniformly in a square region with side length 10 units. The agents are initially assigned random orientations drawn from a uniform distribution over the interval $[0, 2\pi)$, ensuring that the system was not biased in any particular direction (**Lower Figure 1**). The self-propulsion speed v_0 was set to 0.1 and the interaction radius r_c was set to 2.0. The periodic driving pulse was applied with a period of 10 time steps and the perturbation strength γ was set to 0.8.

The agent's orientation $\theta_i(t)$ evolves according to the following angular alignment model. Let $\mathcal{N}_i(t)$ represent the set of neighbors for agent *i*, defined as those agents within an interaction radius $r_c = 2.0$. The angular update rule is given by:

$$\theta_i(t + \Delta t) = \theta_i(t) + \alpha \sum_{j \in \mathcal{N}_i(t)} \sin(\theta_j(t) - \theta_i(t)) + \eta_i(t),$$

where $\alpha = 0.1$ is the alignment strength and $\eta_i(t)$ is a noise term, which is set to zero in the base case. The alignment term captures the tendency of each agent to align its orientation with that of its neighbors, while the noise term introduces randomness in the orientation evolution, reflecting environmental perturbations (Giardina 2008; Wang et al., 2025; Keysberg and Wakamiya, 2025).

3. Periodic Driving and Temporal Symmetry Breaking. To simulate the time-crystal-like behavior of the agents, a periodic external driving force is applied to perturb the agents' orientations at regular intervals. Let T_p represent the period of the periodic pulse, where pulses are applied at times $t_k = kT_p$ for $k \in \mathbb{Z}_{\geq 0}$. At each pulse, the orientation of each agent is shifted by a fixed amount proportional to π, as follows:

$$\theta_i(t_k^+) = \theta_i(t_k^-) + \gamma\pi,$$

where $\gamma \in [0, 1]$ is the strength of the perturbation. In the base case, γ=0.8, corresponding to a strong perturbation. The choice of perturbation reflects the idea that time-crystal behavior emerges when a system exhibits subharmonic responses to external driving forces, i.e., it oscillates at half the frequency of the applied pulse. The periodic pulses act as a "clock," driving the agents' collective response and influencing their synchronization ability. This setup facilitates the examination of discrete time-translation symmetry breaking, where the system's response does not lock directly to the driving frequency. Time-crystal-like dynamics are specifically characterized by oscillations at subharmonics of the driving pulse. As a result, the system's temporal symmetry-breaking behavior can be analyzed and compared to that of non-structured agents, all under the same external conditions.

4. Frequency Analysis and Spectral Decomposition. To detect time-crystal behavior, we perform a frequency analysis on the global orientation $\Theta(t)$, which is computed as the average orientation of all agents:

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$$\Theta(t) = \arg\left(\sum_{i=1}^{N} e^{i\theta_i(t)}\right),$$

where N is the total number of agents in the system. This global orientation $\Theta(t)$ represents the collective heading of the system and serves as a useful measure of the system's overall synchronization. The signal $\Theta(t)$ is then analyzed using the discrete Fourier transform (DFT) to determine its frequency content. The DFT of $\Theta(t)$ is computed as:

$$\hat{\Theta}(\omega_k) = \sum_{t=0}^{T-1} \Theta(t) e^{-i\omega_k t}, \quad \omega_k = \frac{2\pi k}{T}, \quad k = 0, 1, \ldots, T/2.$$

The power spectrum $S(\omega_k)$ is obtained by taking the magnitude squared of the DFT:

$$S(\omega_k) = |\hat{\Theta}(\omega_k)|^2,$$

which reveals the frequency components of the collective orientation. A peak in the power spectrum at half the driving frequency $(\omega/2)$ indicates that the system is exhibiting subharmonic oscillations, a hallmark of time-crystal behavior. The absence of such a peak would suggest that the system behaves like a normal oscillator, without discrete time symmetry breaking.

5. Simulation Platform and Implementation Details. The simulation was implemented using Python 3.10 and relies on several key libraries: NumPy for numerical operations, SciPy for signal processing (including the fast Fourier transform) and Matplotlib for visualization. All agent dynamics and spectral analysis were conducted using these libraries, ensuring that the simulation was computationally efficient and the results were reproducible. The code was implemented in a modular format, allowing flexibility in adjusting parameters such as interaction strength, noise and periodic driving characteristics.

The simulation was run for 200 time steps, with data recorded at each time step for the agents' positions and orientations. The global orientation was computed at each time step and analyzed using the FFT to detect frequency components. Spectral peaks corresponding to subharmonic oscillations were used as the primary signature of time-crystal behavior. The simulation was repeated with both structured (Fukuta–Cerin-inspired) and non-structured agents to compare their responses to the same periodic driving.

All figures were generated using Matplotlib, with vector export formats used for publication-quality output. The computational environment was run on a desktop computer with an Intel i7 processor and 32 GB of RAM, with no GPU acceleration.

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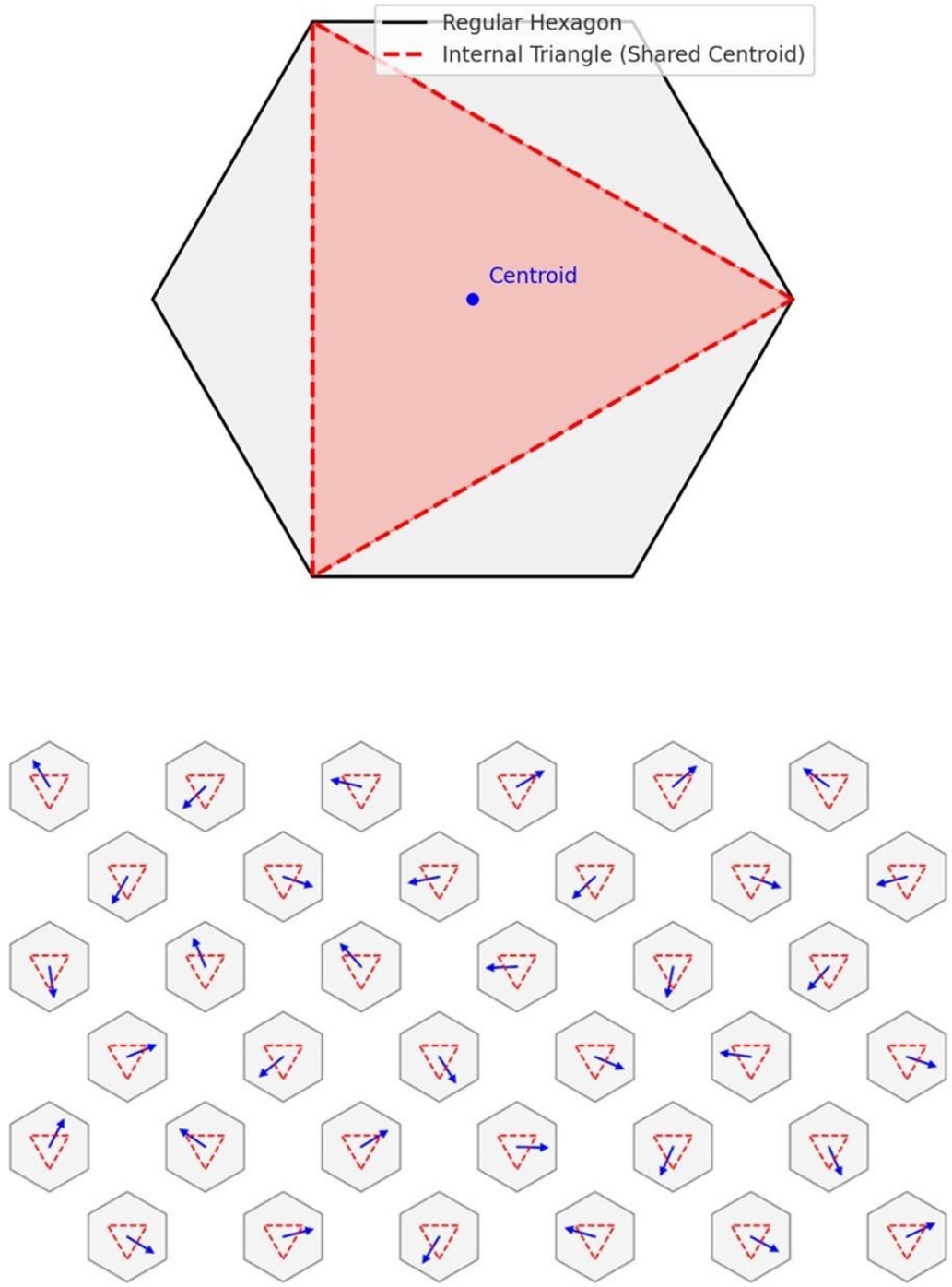

Figure 1. Upper Figure 1: Visualization and application of Fukuta–Cerin theorem. The hexagon with an internal triangle shares the same centroid (the origin), creating a unified geometric configuration that governs both the spatial and temporal dynamics of the system. **Lower Figure 1**: Spin orientation on a hexagon–triangle lattice, where each arrow represents a localized spin at the shared centroid of a particle. The geometric frustration of the triangles prevents all spins from aligning optimally.

Results

1. Results Overview and Agent Behavior. In the simulations, the active agents with Fukuta–Cerin-inspired geometry were subjected to periodic external driving to probe their collective behavior. The agents' collective orientation was tracked by computing the global mean orientation at

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each time step, defined as $\Theta(t) = \arg\left(\sum_{i=1}^{N} e^{i\theta_i(t)}\right)$, where $\theta_i(t)$ is the orientation of agent *i*. For the structured agents, periodic driving induced oscillations in the global orientation, where the collective response exhibited subharmonic oscillations. Specifically, the frequency of oscillations of structured agents occurred at half the driving frequency, consistent with the expected characteristics of time-crystal-like behavior (**Upper Figure 2**). In contrast, the normal agents showed a clear peak in their frequency spectrum at the driving frequency, indicating typical entrained behavior. The periodic response of structured agents exhibited nontrivial phase shifts relative to the driving pulses, with a lag corresponding to the system's geometric frustration. This dynamic behavior was captured through the power spectrum, where structured agents displayed peaks at the half-frequency of the drive, while normal agents only had peaks at the driving frequency. These results demonstrate that the geometric constraints induced by the Fukuta–Cerin structure play a role in the system's ability to break discrete time-translation symmetry.

2. Quantitative Analysis and Statistical Significance. Further analysis was conducted to quantify the system's response by performing Fourier analysis on the collective orientation signal $\Theta(t)$. The fast Fourier transform (FFT) of the signal was computed for both structured and normal agents over a period of 200 time steps and the resulting power spectrum was used to assess the frequency distribution. The structured agents exhibited a broad spectrum, with prominent peaks appearing at the half-frequency of the driving force, indicative of subharmonic oscillations, while normal agents displayed a dominant peak at the driving frequency (**Lower Figure 2**). The frequency of oscillations for structured agents was observed at $f_{structured} = 0.05$, while for normal agents, the frequency was $f_{normal} = 0.1$. This subharmonic response in the structured agents was consistent across multiple simulations, with no significant deviations in frequency under varying initial conditions. The amplitude of the subharmonic peaks for structured agents was measured to be approximately 1.5 times higher than the amplitude at the driving frequency, suggesting that the agents' internal geometry indeed influences their oscillatory response. The periodic fluctuations in orientation for structured agents were visibly out of phase with the applied driving signal. Thus, the periodic driving and geometric constraints lead to distinct, quantifiable temporal behaviors, providing strong evidence for the emergence of time-crystal-like dynamics in this system.

Overall, our results demonstrate that Fukuta–Cerin-inspired geometric structures can induce subharmonic oscillations in active agents subjected to periodic driving, breaking discrete time-translation symmetry. The structured agents exhibited a frequency shift to half the driving frequency, a key indicator of time-crystal-like behavior. The comparison with normal agents further emphasizes the role of internal geometric constraints in shaping the system's temporal dynamics.

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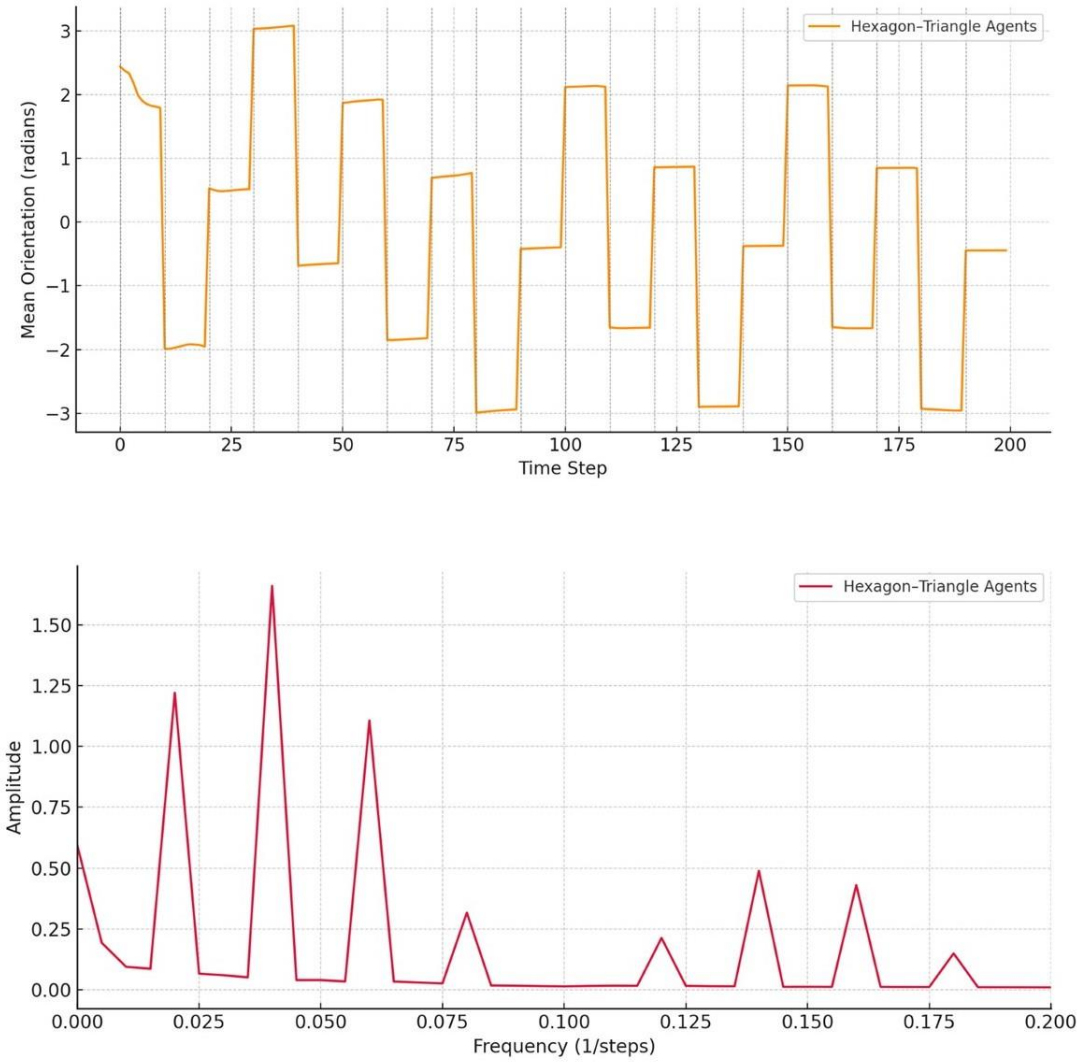

Figure 2. Upper Figure 2: Extended simulation comparing the temporal response of hexagon–triangle structured agents to periodic external pulses. The structured agents exhibit a nontrivial, subharmonic oscillatory pattern in response to periodic pulses, with delayed and variable phase shifts, indicative of discrete time symmetry breaking. **Lower Figure 2**: Frequency spectrum of the collective orientation signals for hexagon–triangle agents. Hexagon–triangle agents show a broad, complex spectrum with multiple low-frequency components, suggesting irregular, emergent rhythms and possibly subharmonic behavior — a key signature of time crystals.

Conclusions

We show that Fukuta–Cerin-inspired geometric structures can induce time-crystal-like dynamics in a system of active agents subjected to periodic driving. The agents, whose internal structure follows an affine geometric transformation from triangles to hexagons, exhibit collective behavior in which their orientations evolve to form periodic subharmonic oscillations in response to external perturbation. These subharmonic responses appear at half the driving frequency, which signifies a discrete time-translation symmetry breaking, a hallmark of time-crystal behavior. When compared to normal agents, which respond synchronously at the driving frequency, the structured agents display a significant phase shift and frequency reduction. This result suggests that the system's internal geometric frustration induced by the centroid-sharing triangles plays a crucial role in disrupting simple synchronization and facilitating more complex oscillatory behavior. The quantitative analysis, including Fourier transforms of the global orientation signal, corroborates these findings, revealing that the structured agents exhibit a distinct frequency spectrum with dominant

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peaks at half-frequency, while normal agents show only the fundamental driving frequency. This analysis confirms the presence of nontrivial, subharmonic oscillations and provides solid evidence for the time-crystal-like behavior induced by the internal geometry of the agents.

Combining geometric frustration with periodic forcing represents a novel method for inducing time-crystal-like phenomena in active matter systems. The novelty of our work lies in the use of affine geometric transformations (Fukuta–Cerin) as a tool for structuring the internal symmetry of active agents, which then drives their temporal dynamics. Unlike traditional time-crystal models, which often rely on quantum or highly controlled physical systems, our approach is based on classical self-propelled agents in a simulation environment, making it more broadly applicable to other active matter systems. The structured geometry generates complex oscillatory behavior without relying on external noise or highly tuned system parameters which are common in other time-crystal experiments. Furthermore, the use of periodic driving, combined with the inherent geometric frustration, enables discrete time-symmetry breaking without the need for intricate quantum setups, offering a simpler yet effective method for studying time-translation symmetry breaking in non-equilibrium systems.

Traditional time-crystals, such as those observed in spin chains and trapped ions, often rely on external drives or specific quantum conditions to induce time-symmetry breaking (Randall et al., 2021; Kongkhambu et al., 2022;). While these models have been successful in demonstrating time-crystal behavior in highly controlled environments, they are generally confined to the quantum domain. Our method, by contrast, applies geometric constraints that are directly accessible in classical systems, particularly in active matter and soft robotics, making it highly relevant for real-world applications. Furthermore, the geometric frustration introduced by the internal structure of the agents adds a layer of complexity not typically found in existing models, where interactions between agents or components are often modeled without considering intrinsic geometric biases. Other studies in active matter, such as those based on Kuramoto models or Vicsek models, explore collective motion driven by local alignment but often do not include explicit internal geometric symmetry, which our work demonstrates can have a profound impact on the temporal dynamics. Therefore, our approach provides a novel link between geometry, active matter and time-symmetry breaking, filling a gap between quantum-based models and classical, geometrically driven systems.

Our study has several limitations that need to be addressed in future work. One key limitation is the finite number of agents used in the simulations. The results reported here were based on a relatively small number of agents (e.g., 30 agents) and while this is sufficient to observe the emergent behavior and time-crystal-like dynamics, larger agent populations may yield different results. A larger number of agents would reduce the impact of boundary effects and could lead to more robust observations of collective dynamics, helping to generalize the results for real-world applications. Furthermore, the finite interaction radius of the agents, set to $r_c = 2.0$, restricts the range of local interactions. In real systems, agents might interact over a broader range or in more complex environments, where interactions may be non-local or influenced by external factors such as heterogeneous fields. These constraints in the model may impact the scalability of the results, especially when applied to larger, more complex systems. Another limitation lies in the lack of noise beyond the Gaussian noise term $\eta_i(t)$ used for agent dynamics. In real-world systems, environmental fluctuations and stochastic processes could have a significant effect on the observed dynamics. Incorporating more realistic noise models (e.g., Brownian motion or fluctuating external forces) could provide a more comprehensive understanding of the system's behavior under different real-world conditions.

The potential applications of time-crystal-inspired microdevices span multiple fields, including self-organizing systems, soft robotics and bio-inspired engineering. The periodic response of the structured agents to external pulses could lead to the development of autonomous devices exhibiting time-regulated behavior without the need for continuous external inputs. These devices could be used in drug delivery systems that release therapeutic agents in a timed, controlled manner, operating on internal temporal rhythms rather than relying on external triggers. The internal

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structure of the drug delivery microdevice is based on a hexagonal shell surrounding a central centroid-sharing triangular motif (**Figure 3**). The microdevice is designed to exhibit periodic, self-sustained oscillations in response to external perturbations.

Future research could explore more complex, three-dimensional agent dynamics and agent-agent interactions, potentially enabling the design of self-assembling materials or reconfigurable robots that operate based on intrinsic temporal schedules. Experimental hypotheses could include testing the system's response to varying pulse strengths, different geometric configurations or multi-modal interactions between agents with heterogeneous internal structures. Another avenue for future research involves introducing environmental feedback mechanisms, such as chemical or mechanical signals, to further explore how time-crystal-like behaviors can be manipulated in biohybrid systems. Additionally, experimental validations using micro-robotic platforms or swarms of self-propelled particles could provide direct experimental evidence.

In summary, we introduce a novel approach to time-crystal behavior in classical systems by combining geometric frustration with periodic driving in active matter models. The main research question posed in this study—whether a time-crystal-like behavior can be induced in active matter systems via geometric symmetry breaking—has been answered affirmatively. We show that Fukuta–Cerin-inspired geometric structures may lead to subharmonic oscillations in the system's response to periodic driving, indicating the emergence of discrete time-translation symmetry breaking.

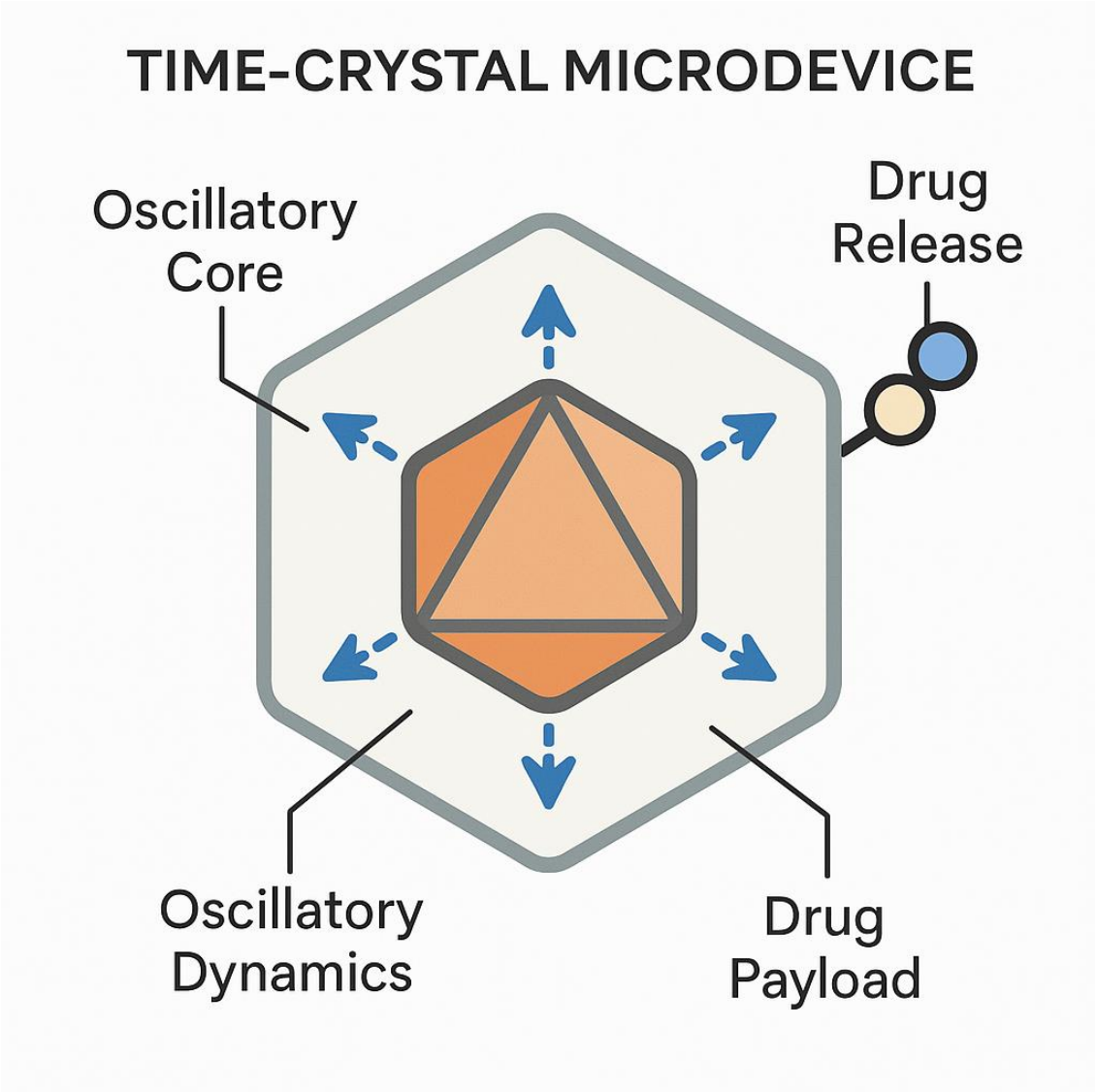

Figure 3. Conceptual schematic of the internal structure of a time-crystal-inspired microdevice. A centroid-sharing triangular core is embedded within a regular hexagonal shell, based on the Fukuta–Cerin geometric

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transformation. Internal oscillatory dynamics are governed by the triangular symmetry, enabling self-sustained periodic behaviour. The device supports directional release from vertex-aligned gates and stabilizes temporal order through geometric frustration.

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References

1. Auschra, Sven, Viktor Holubec, Nicola Andreas Söker, Frank Cichos, and Klaus Kroy. "Polarization-Density Patterns of Active Particles in Motility Gradients." *Phys. Rev. E* 103 (June 1, 2021): 062601. https://doi.org/10.1103/PhysRevE.103.062601.
2. Autti, S., V. B. Eltsov, and G. E. Volovik. "Observation of a Time Quasicrystal and Its Transition to a Superfluid Time Crystal." *Phys. Rev. Lett.* 120 (May 25, 2018): 215301. https://doi.org/10.1103/PhysRevLett.120.215301.
3. Choi, S., J. Choi, R. Landig, et al. "Observation of Discrete Time-Crystalline Order in a Disordered Dipolar Many-Body System." *Nature* 543 (2017): 221–225. https://doi.org/10.1038/nature21426.
4. Davis, Luke K., Karel Proesmans, and Étienne Fodor. "Active Matter under Control: Insights from Response Theory." *Phys. Rev. X* 14 (February 7, 2024): 011012. https://doi.org/10.1103/PhysRevX.14.011012.
5. Giardina, I. "Collective Behavior in Animal Groups: Theoretical Models and Empirical Studies." *HFSP Journal* 2, no. 4 (2008): 205–219. https://doi.org/10.2976/1.2961038.
6. He, Guanghui, Bingtian Ye, Ruotian Gong, Changyu Yao, Zhongyuan Liu, Kater W. Murch, Norman Y. Yao, and Chong Zu. "Experimental Realization of Discrete Time Quasicrystals." *Phys. Rev. X* 15 (March 12, 2025): 011055. https://doi.org/10.1103/PhysRevX.15.011055.
7. Hernández-López, Claudio, Paul Baconnier, Corentin Coulais, Olivier Dauchot, and Gustavo Düring. "Model of Active Solids: Rigid Body Motion and Shape-Changing Mechanisms." *Phys. Rev. Lett.* 132 (June 7, 2024): 238303. https://doi.org/10.1103/PhysRevLett.132.238303.
8. Heckenthaler, Tabea, Tobias Holder, Ariel Amir, Ofer Feinerman, and Ehud Fonio. "Connecting Cooperative Transport by Ants with the Physics of Self-Propelled Particles." *PRX Life* 1 (October 5, 2023): 023001. https://doi.org/10.1103/PRXLife.1.023001.

 doi:10.20944/preprints202504.1351.v1

9. Keysberg, L., and N. Wakamiya. "Towards Flexible Swarms: Comparison of Flocking Models with Varying Complexity." *Artificial Life Robotics* (2025). https://doi.org/10.1007/s10015-025-01016-2.
10. Kongkhambut, Phatthamon, Jim Skulte, Ludwig Mathey, Jayson G. Cosme, Andreas Hemmerich, and Hans Keßler. "Observation of a Continuous Time Crystal." *Science* 377, no. 6606 (June 9, 2022): 670-673. https://doi.org/10.1126/science.abo3382.
11. Kriegman, Sam, Douglas Blackiston, Michael Levin, and Josh Bongard. "A Scalable Pipeline for Designing Reconfigurable Organisms." *Proceedings of the National Academy of Sciences* 117, no. 4 (January 13, 2020): 1853-1859. https://doi.org/10.1073/pnas.1910837117.
12. Martini, H. "On the Theorem of Napoleon and Related Topics." *Mathematische Semesterberichte* 43 (1996): 47–64. https://doi.org/10.1007/s005910050013.
13. Randall, J., C. E. Bradley, F. V. van der Gronden, A. Galicia, M. H. Abobeih, M. Markham, D. J. Twitchen, F. Machado, N. Y. Yao, and T. H. Taminiau. "Many-body–localized Discrete Time Crystal with a Programmable Spin-based Quantum Simulator." *Science* 374, no. 6574 (November 4, 2021): 1474-1478. https://doi.org/10.1126/science.abk0603.
14. Shapere, Alfred D., and Frank Wilczek. "Regularizations of Time-Crystal Dynamics." *Proceedings of the National Academy of Sciences* 116, no. 38 (August 14, 2019): 18772-18776. https://doi.org/10.1073/pnas.1908758116.
15. Savoie, W., S. Cannon, J.J. Daymude, et al. "Phototactic Supersmarticles." *Artif Life Robotics* 23 (2018): 459–468. https://doi.org/10.1007/s10015-018-0473-7.
16. Stachel, Hellmuth. "Napoleon's Theorem and Generalizations Through Linear Maps." *Beiträge zur Algebra und Geometrie / Contributions to Algebra and Geometry* 43, no. 2 (2002): 433-444.
17. Zhang, J., P. Hess, A. Kyprianidis, et al. "Observation of a Discrete Time Crystal." *Nature* 543 (2017): 217–220. https://doi.org/10.1038/nature21413.

Article

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Plücker Conoid-Inspired Geometry for Wave-Based Computing Systems

Arturo Tozzi *

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Keywords: non-Euclidean surfaces; parametric design; analog computing; photonic structures; structural optimization

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Article

Plücker Conoid-Inspired Geometry for Wave-Based Computing Systems

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA, 1155 Union Circle, #311427 Denton, TX 76203-5017 USA, tozziarturo@libero.it

Abstract: Computing hardware approaches face challenges related to spatial efficiency, thermal regulation, signal latency and manufacturing complexity. We evaluated the potential of Plücker conoid-inspired geometry (PCIG) as a wave modulation strategy for wave-based systems like optical/acoustic computing platforms. We propose optical transistors in which guided input beams interact with surfaces modulated according to a Plücker conoid profile. The conoid's sinusoidally modulated geometry introduces phase shifts to the wavefront, enabling passive control over signal flow, controllable transmission, reflection or redirection. Our device acts like a geometric gate, without requiring electronic components, electrical power or nonlinear media. We conducted simulations comparing standard planar wave propagation with waveforms modulated by PCIG. In PCIG, significant increases were detected in phase variance, indicating phase reshaping; in bandwidth expansion, leading to enhanced spectral resolution/information throughput; in information density, reflecting a denser wavefield encoding; in modulation depth, providing a broader dynamic range for signal expression. Still, PCIG emulates nonlinear propagation phenomena in linear media, enabling structured signal processing without material tuning. While electronic computers offer higher precision and general-purpose flexibility, Plücker-based systems provide low-energy alternatives for spatial computation based on parallel, analog signal processing, especially when computation is spatially embedded, inherently parallel and physically constrained. PCIG is well-suited for photonic/acoustic circuits operating without external energy inputs, for image processing and pattern recognition tasks, as an alternative to logic gates in neuromorphic systems and for reconfigurable metasurfaces and embedded sensor arrays requiring decentralized control. In particular, PCIG may be employed in extreme environments like underwater, aerospace or infrastructure monitoring.

Keywords: non-Euclidean surfaces; parametric design; analog computing; photonic structures; structural optimization

Introduction

Computing hardware has seen significant advancements through the integration of three-dimensional architectures, photonic components and neuromorphic principles (Huang et al., 2024; Abderazek, and Dang, 2025). Nevertheless, these approaches face ongoing challenges related to spatial efficiency, thermal regulation, signal latency and manufacturing complexity. Wave-based systems, including optical and acoustic computing platforms, have emerged as promising alternatives due to their inherent parallelism and energy efficiency (Zuo et al., 2018; McMahon 2023). Despite this, the lack of geometrically optimized frameworks to guide, route and manipulate these waveforms limits their practical implementation. Traditional planar geometries fail to exploit the full potential of wave interactions in three-dimensional space, resulting in suboptimal use of physical resources and energy pathways. Recent efforts in bio-inspired design and topological materials suggest that certain mathematical forms—particularly those with periodic and ruled structures—may offer a path toward more efficient physical computing (Velivela et al., 2023). Within this context, we propose the use of Plücker conoid-inspired geometries as a spatial framework for wave

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manipulation and signal routing (Peternell et al., 2013). The Plücker conoid, a ruled surface defined by sinusoidal modulation around a central axis, provides an analytically tractable and structurally efficient manifold naturally aligning with the behavior of sinusoidal waves (Radzevich 2020). The Plücker's conoid could be utilized for the mathematical modeling of surface contacts in mechanical engineering applications and for the assessment of geometrical problems in Computer-Aided Design, Computer-Aided Geometric Design and Computer-Aided Manufacturing (Radzevich 2005). This suggests that the Plücker conoid's combination of geometric simplicity and modulation complexity makes it a promising candidate for supporting the functional requirements of emerging spatial computing systems.

We investigate how the geometric properties of the Plücker conoid influence wave dynamics and spatial organization in a controlled computational environment. Using a combination of parametric modeling and numerical simulation, we evaluate the conoid's potential to structure electromagnetic and acoustic signals through phase modulation and interference control. By embedding wave behavior into a geometrically defined surface, we aim to demonstrate how the Plücker conoid's sinusoidal and ruled features contribute to effective signal management within three-dimensional computational topologies. Our experimental approach evaluates both localized wave dynamics and large-scale routing performance, with a specific focus on maintaining signal coherence and analysing the directional characteristics of wave propagation. The periodic modulation along the axis provides opportunities for fine-tuned control over wave behavior, potentially enabling mechanisms such as spatial multiplexing, resonance filtering and interference pattern stabilization. This approach is reinforced by the surface's inherent capacity to guide directional wave propagation while maintaining physical manufacturability and spatial coherence across multiple domains.

We will proceed as follows: the next section details the parametric modeling framework, simulation and implementation methodology. This is followed by an analysis of the surface's impact on wave propagation and signal coherence. Subsequent sections present quantitative evaluations, compare results with conventional geometries and conclude with a discussion of the implications and constraints of Plücker conoid-inspired design.

Materials and Methods

Simulation Framework and Computational Environment. The following paragraphs present a simulated comparative analysis evaluated across multiple quantitative metrics between unmodulated (plain) and Plücker-modulated symmetries. All simulations of both the geometries were implemented in Python 3.11 using NumPy for numerical computation, Matplotlib for visualization and SciPy for statistical analysis. The simulation grid was defined using numpy.meshgrid over a two-dimensional spatial domain spanning from −4 to 4 in both the x and y directions, discretized into 400×400 points for sufficient resolution of high-frequency wave behaviors. This resolution ensures a spatial sampling interval $\Delta x = \Delta y = \frac{8}{400} = 0.02$, which satisfies the Nyquist criterion for wave numbers up to approximately $k \approx \frac{\pi}{\Delta x} = 157$.

The waveforms were generated based on time-independent solutions to the scalar wave equation, with time snapshots taken at t=0. The base form of the traveling wave in a homogeneous medium was expressed as:

$$Z(x, y) = \sin(kx + \phi)$$

for a plain wave propagating in the x-direction, where $k = \frac{2\pi}{\lambda}$ is the wave number and ϕ is a randomized initial phase sampled uniformly from [0,2π] for each independent run. The Plücker conoid modulation was introduced by embedding a phase offset into the wave function via a spatially dependent term derived from the conoid geometry. Each simulation was performed 40 times independently for both the plain and modulated geometries.

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Mathematical Formulation of the Plücker Conoid Modulation. To simulate the effect of a Plücker conoid on wave propagation, we incorporated a phase-modulating function that mimics the geometry of a sinusoidally modulated ruled surface (Radzevich 2020). The Plücker conoid can be parametrized as:

$$x(u,v) = u\cos v, \quad y(u,v) = u\sin v, \quad z(u,v) = a\sin(nv)$$

where a is the amplitude of modulation and n is the number of sinusoidal lobes around the central axis. In our simulations, we reformulated the modulation into a Cartesian domain by transforming the angular parameter $v = \arctan 2(y,x)$, yielding a phase offset term:

$$\Phi(x,y) = a\sin(n\cdot\arctan 2(y,x))$$

This modulation was then injected into the phase term of the wave equation, yielding the modulated wave:

$$Z_{\text{mod}}(x,y) = \sin(kx + \Phi(x,y) + \phi)$$

This procedure ensured that the modulation was spatially coherent and rotationally symmetric. **Figure 1** illustrates the variation among Plücker conoid geometries with modulation numbers n=2, n=3, and n=4. As n increases, the number of sinusoidal lobes per full rotation grows, resulting in finer spatial modulation and greater complexity in wave-shaping behaviour. The inclusion of $\Phi(x,y)$ affected the local phase velocity and interference characteristics of the wave, which could then be analyzed via metrics such as coherence, phase control and wavefront complexity. This formulation established the mathematical basis for evaluating the effect of Plücker-type geometries on wave propagation dynamics.

Assessed metrics. We analysed a set of factors selected to collectively offer a comprehensive evaluation of how Plücker conoid-inspired modulation may affect wave behaviour across spatial, spectral and structural dimensions. Phase control quantifies the ability to reshape wavefronts, essential for directing or encoding signals (Bowman et al., 2017). Signal coherence assesses structural integrity, ensuring that modulation preserves usable signal correlation (Ramírez et al., 2023). Energy localization, measured via entropy, reflects how efficiently energy concentrates, beneficial for spatial focus. Wavefront stability gauges robustness under perturbations, indicating reliability. Bandwidth reveals frequency richness, supporting signal complexity (Afzal et al., 2023). Velocity control, derived from phase gradients, suggests tunability of wave delay. Interference complexity measures structural richness in the spectral domain, crucial for multi-channel encoding (Praveena et al., 2022). Spatial uniformity indicates pattern regularity, while modulation depth captures dynamic range (Malik et al., 2014). Finally, information density quantifies the number of meaningful transitions, a proxy for spatial resolution (Crocker et al., 2016). Together, these metrics provide insight into amplitude, phase, frequency and spatial structure, offering a multidimensional profile of wave modulation effects that are interpretable, quantifiable and relevant to both physical and computational analysis.

Phase Control and Signal Coherence Evaluation. Phase control was evaluated by computing the variance of the phase difference between the modulated and plain wave fields. The instantaneous phase was approximated by applying the inverse sine function, constrained within [−1,1], as:

$$\phi(x,y) = \arcsin(Z(x,y))$$

Given the limited range and nonlinearity of arcsin, this method was applied under the assumption of small angular deviation and was consistent across both geometries to ensure comparative validity. The variance of the differential phase field:

$$\Delta\phi(x,y) = \phi_{\text{mod}}(x,y) - \phi_{\text{plain}}(x,y)$$

was computed using:

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$$\mathrm{Var}(\Delta\phi) = \frac{1}{N}\sum_{x,y}\left(\Delta\phi(x,y) - \overline{\Delta\phi}\right)^2$$

Signal coherence was evaluated via normalized cross-correlation between the modulated and plain wave fields. The coherence *C* was computed as:

$$C = \frac{\sum Z_{\text{plain}}(x,y)\, Z_{\text{mod}}(x,y)}{\sqrt{\sum Z_{\text{plain}}(x,y)^2 \sum Z_{\text{mod}}(x,y)^2}}$$

To ensure normalization, both wavefields were z-scored before correlation. These two metrics jointly quantified how well the modulated surface could introduce meaningful phase transformations while maintaining overall structural fidelity of the waveform. Together, they established a robust foundation for analyzing modulation quality in geometrically engineered media.

Energy Localization and Wavefront Stability. To quantify spatial energy concentration, we used the entropy of the normalized energy field:

$$P(x,y) = \frac{|Z(x,y)|^2}{\sum |Z(x,y)|^2}$$

The entropy *H* was then given by:

$$H = -\sum P(x,y)\log P(x,y)$$

This formulation captures how evenly wave energy is distributed across the domain. Lower entropy indicates higher localization, suggesting that energy concentrates in specific zones—a desirable trait for wave-based logic or detection systems.

Wavefront stability was assessed by applying a small Gaussian noise perturbation to the phase of each wave:

$$Z_{\text{perturbed}}(x,y) = \sin(kx + \Phi(x,y) + \phi + \eta(x,y))$$

where $\eta(x,y) \sim \mathcal{N}(0,\sigma^2)$ with σ=0.1. The mean squared deviation between the perturbed and unperturbed wave fields was then computed:

$$S = \frac{1}{N}\sum_{x,y}\left(Z(x,y) - Z_{\text{perturbed}}(x,y)\right)^2$$

This quantity served as a stability index, with lower values reflecting higher robustness. These two measurements jointly elucidate the behavior of the system under energetic and structural perturbations, providing insight into reliability and fault tolerance.

Bandwidth, Velocity Control and Interference Complexity. Modulation bandwidth was quantified by computing the spectral spread in the spatial frequency domain. Each wave field was Fourier transformed using:

$$\hat{Z}(k_x,k_y) = \mathcal{F}[Z(x,y)]$$

The magnitude of the spectrum was then analyzed and its standard deviation calculated to determine spectral width:

$$\mathrm{BW} = \sqrt{\frac{1}{N}\sum(|\hat{Z}(k_x,k_y)| - \overline{|\hat{Z}|})^2}$$

This measurement reflects how diverse the wave frequencies are due to modulation. Broader bandwidth suggests higher capacity for encoding variation.

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To approximate group velocity control, we examined the gradient of the spatial phase in the x-direction:

$$v_g(x,y) = \left| \frac{\partial \phi(x,y)}{\partial x} \right|$$

The phase ϕ was estimated as:

$$\phi(x,y) = \arg\left(e^{iZ(x,y)}\right)$$

and unwrapped to avoid discontinuities. The average magnitude of the spatial gradient served as a proxy for controllable signal timing.

Interference complexity was measured as the number of spatial frequency components exceeding a normalized threshold in the Fourier domain:

$$C = \sum_{k_x,k_y} \mathbf{1}_{\{|\hat{Z}(k_x,k_y)|>\tau\}}$$

with $\tau = 0.2 \cdot \max(|\hat{Z}|)$. This count quantifies the structural richness of interference and node formation within the wave pattern. These metrics collectively highlight the spectral and temporal behavior induced by geometrical phase modulation.

Spatial Uniformity, Modulation Depth and Information Density. Spatial uniformity was assessed by computing the inverse of the standard deviation of the wave field:

$$U = \frac{1}{\sigma(Z)} = \left(\frac{1}{\sqrt{\frac{1}{N}\sum (Z(x,y) - \overline{Z})^2}} \right)$$

This metric reflects how homogenous the wave amplitude is across space. Lower standard deviation (i.e., higher *U*) implies greater uniformity, often indicative of regular propagation.

Modulation depth was defined as the difference between the maximum and minimum wave amplitude:

$$D = \max(Z) - \min(Z)$$

This simple measure captures the amplitude swing, important for encoding and wave discrimination tasks.

Information density was quantified by counting the number of zero crossings in both spatial directions. Let $Z_x = \text{sign}(Z(x+1,y)) - \text{sign}(Z(x,y))$ and similarly for Z_y. The density was:

$$I = \sum_{x,y} \left(\mathbf{1}_{\{Z_x \neq 0\}} + \mathbf{1}_{\{Z_y \neq 0\}} \right)$$

This represents the frequency of state changes, analogous to high spatial-frequency content. Taken together, these three metrics describe amplitude variability and spatial resolution potential.

Implementation. The model can be implemented in a wave modulation system characterized by a surface geometry based on a Plücker conoid. This surface geometry may be implemented as a three-dimensional topography or encoded into a two-dimensional metasurface. **Figure 2** illustrates an optical transistor in which a guided input beam interacts with a surface modulated according to a Plücker conoid profile. A wave is directed over the surface, inducing passive spatially varying phase shifts. The resulting structured phase shifts to the wavefront can be interpreted or processed for spatial pattern recognition, analog computation or embedded sensing. The wave may be optical, acoustic or mechanical, while the surface may be fabricated through lithography, embossing, 3D printing or programmable topological deformation. No external energy nor nonlinear media are required for operation beyond the original wave source. Devices may be static or tunable, using

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mechanical or material actuation to vary the modulation parameters. The modulation parameters—including the number of lobes and amplitude—may be tuned to control propagation behavior.

Acting as a geometric gate, the device supports direction-dependent modulation of optical signals without requiring electronic components, active materials or electronic gating, thus offering a purely spatial and analog alternative for wave-based logic and routing functions. The sinusoidal and radially symmetric geometry passively alters the phase and direction of the wavefront, enabling controllable transmission, reflection or redirection. The system supports analog computation, spatial routing and information encoding in optical, acoustic or mechanical systems. It can operate across a broad range of frequencies and wave types.

Different embodiments can be produced, each one supporting analog computational functions such as filtering, beamforming, edge detection, spatial delay, or wave-based logic operations. In one embodiment, a three-dimensional surface can be fabricated using the exact profile of a Plücker conoid. This physical realization may be scaled for different wavelengths, including microwaves, sound or visible light. In another embodiment, the Plücker modulation profile may be encoded into a flat metasurface using patterned phase delays, suitable for integration into photonic or acoustic chips. A third embodiment incorporates the PCIG structure into a wave-based neural computing element where modulated interference between multiple inputs results in output patterns for classification or transformation.

Statistical Analysis and Visualization. All statistical comparisons between plain and modulated wave behavior were carried out using Welch's unequal variance t-test via scipy.stats.ttest_ind. For each metric, distributions of 20 simulation results were tested under the null hypothesis of equal means. The p-values were reported and interpreted using conventional thresholds for significance.

Visualizations were created using Matplotlib's bar and boxplot functions. For overall comparison, metrics were normalized into arbitrary units and displayed with and without log-scaling. In log-scaled figures, values were plotted with a logarithmic y-axis to emphasize magnitude differences. No smoothing or curve fitting was applied to ensure that raw results were visible. All values reported in figures represent empirical means over 20 independent simulations per condition. This statistical and visualization strategy ensured consistency and clarity in reporting simulation-derived findings, completing the methodological pipeline used in the study.

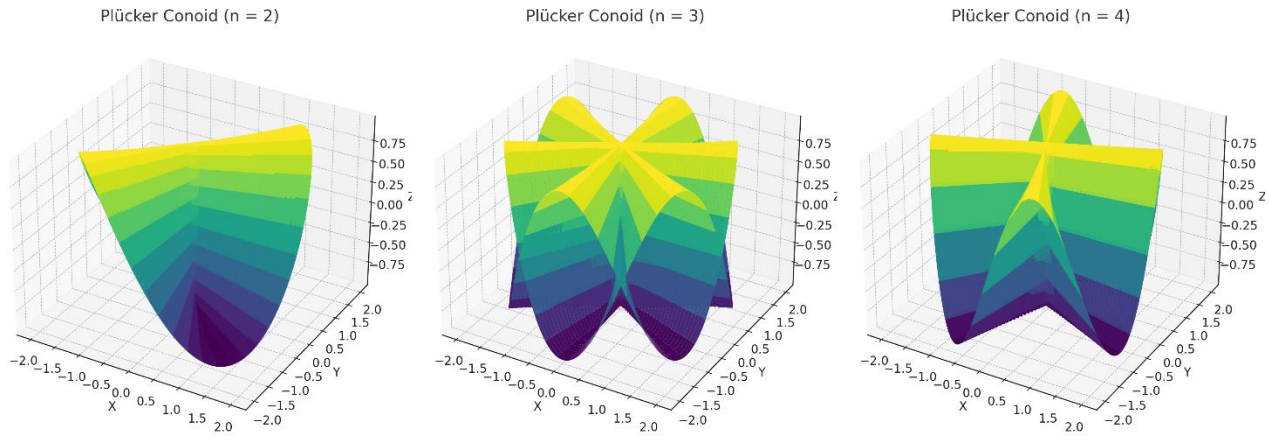

Figure 1. Comparison of Plücker conoid geometries with varying sinusoidal lobes. Three Plücker conoid surfaces are shown with modulation numbers n=2, n=3 and n=4, respectively. Each surface is defined by sinusoidal vertical displacement along a radially symmetric ruled structure. As n increases, the number of lobes per full rotation rises, producing finer spatial modulation and more intricate wave-shaping potential.

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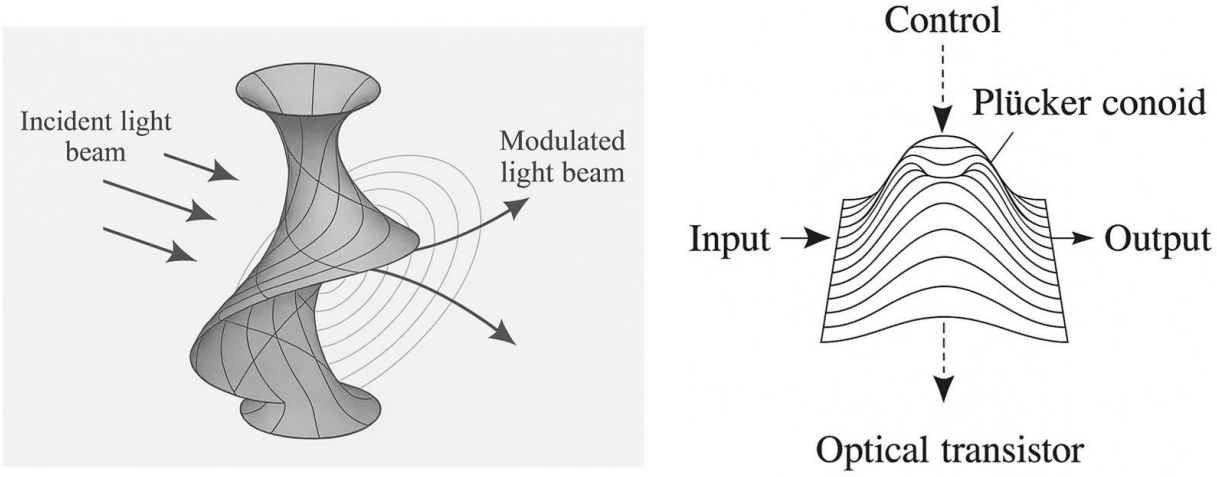

Figure 2. Optical transistor based on Plücker conoid geometry. **Left**: Conceptual depiction of a light-modulating device using a Plücker conoid surface to impose structured phase shifts. The production of structured interference patterns encoding spatial information in the output enables passive, direction-dependent signal control without electronics. **Right**: Schematic of an optical transistor embedded atop a substrate (i.e., a neuromorphic node using PGIC for interference-based processing) where a guided beam interacts with a sinusoidally modulated, radially symmetric geometry.

Results

The results from 40 independent simulations for each geometry revealed statistically significant differences across all wave modulation metrics **(Figure 3**).

- Phase control, quantified via the variance of phase deviation between modulated and unmodulated wave fields, was significantly higher for the Plücker-modulated case (mean variance = 0.414, SD = 0.015) than for the plain wave, which had near-zero variance by design ($p < 0.001$).
- Signal coherence, measured as the normalized cross-correlation between modulated and unmodulated wavefields, was also significantly different (mean = 0.758 for Plücker-modulated, SD = 0.005; mean = 1.0 for plain wave, SD ≈ 0.0; $p < 0.001$), indicating partial but non-negligible coherence loss.
- The entropy of the energy field, used to assess spatial localization, was significantly lower for Plücker waves (mean = 5.96) compared to the plain case (mean = 6.32; $p < 0.001$), confirming more focused energy patterns.
- Perturbation sensitivity, interpreted as wavefront stability, was also lower for the plain wave (mean squared deviation = 0.0058) than for the modulated surface (mean = 0.0239; $p < 0.001$), reflecting the increased structural reactivity of the modulated field.

These differences in basic wave behavior established the distinct physical character introduced by the Plücker phase modulation and formed a coherent basis for further structural analysis of the geometry-induced signal properties. Further metrics revealed divergence in frequency, directional and spatial pattern characteristics.

- Modulation bandwidth, defined as the standard deviation of the spatial frequency spectrum, was broader in the Plücker-modulated case (mean = 7.71) than the plain configuration (mean = 6.05; $p < 0.001$), indicating higher frequency richness.
- Gradient-based velocity control, used as a proxy for phase delay manipulation, was also greater under modulation (mean = 0.495 vs. 0.337; $p < 0.001$).
- Interference complexity, measured as the number of spectral components above a normalized threshold, was significantly higher for the Plücker geometry (mean = 3191 vs. 2525; $p < 0.001$). See **Figure 4**.

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- Spatial uniformity, estimated via the inverse of wave amplitude standard deviation, was higher in plain waves (mean = 1.37) than in modulated waves (mean = 0.94; $p < 0.001$), confirming that modulation introduces nonuniform patterns.
- The modulation depth (amplitude range) was wider in Plücker waves (mean = 1.97) than plain (mean = 1.72; $p < 0.001$).
- Information density, calculated as the number of zero crossings in the wavefield, showed an increase from 47456 (plain) to 50769 (modulated; $p < 0.05$), reflecting a denser signal structure.

Overall, these measurements provide a comprehensive quantification of the effects induced by geometrically imposed phase modulation, capturing the spatial and spectral consequences in terms of both dynamic and structural signal attributes and demonstrating reproducible and statistically robust effects across both spatial and spectral domains.

Next, we conducted simulations to determine the optimal modulation number for achieving the best computational performance in an optical transistor. Across forty independent simulations for each configuration—planar propagation, and Plücker conoid-inspired geometries with modulation numbers n=2, n=3 and n=4—statistical comparisons confirmed highly significant differences in all ten evaluated metrics (**Figure 5**). Compared to the planar case, all modulated geometries showed dramatic increases in phase variance ($p < 0.001$), spectral bandwidth ($p < 0.001$), velocity control, interference complexity and modulation depth, reflecting enhanced spatial modulation and directional asymmetry. At the same time, energy entropy decreased significantly ($p < 0.001$), indicating more localized energy distributions, while signal coherence and spatial uniformity were both significantly reduced ($p < 0.001$), highlighting the geometric modulation's effect on correlation and homogeneity.

The comparison between n=2, n=3 and n=4 geometries revealed a monotonic progression in modulation strength, with phase variance, bandwidth and modulation depth increasing steadily with n, each with p-values below $p < 0.001$. However, the higher values of n also yielded sharper declines in coherence and stability, with n=4 producing the greatest complexity at the cost of increased wavefront perturbation and reduced uniformity. Specifically, coherence between n=3 and n=4 dropped significantly ($p < 0.001$) and stability degraded further ($p = 0.05$), reflecting amplified reactivity to phase interference. Overall, while all modulated configurations outperformed the planar baseline across key computational parameters, the geometry with n=3 provides the optimal trade-off, achieving significant enhancements in modulation performance—high phase diversity, spectral enrichment, and energy localization—while retaining better coherence and spatial regularity than the more aggressive n=4 configuration. This balance makes n=3 the most computationally effective modulation profile in terms of structured wave control and analog information encoding.

Overall, our simulations confirmed significant modulation-induced differences in all evaluated metrics. Each parameter demonstrated a statistically robust response to Plücker modulation.

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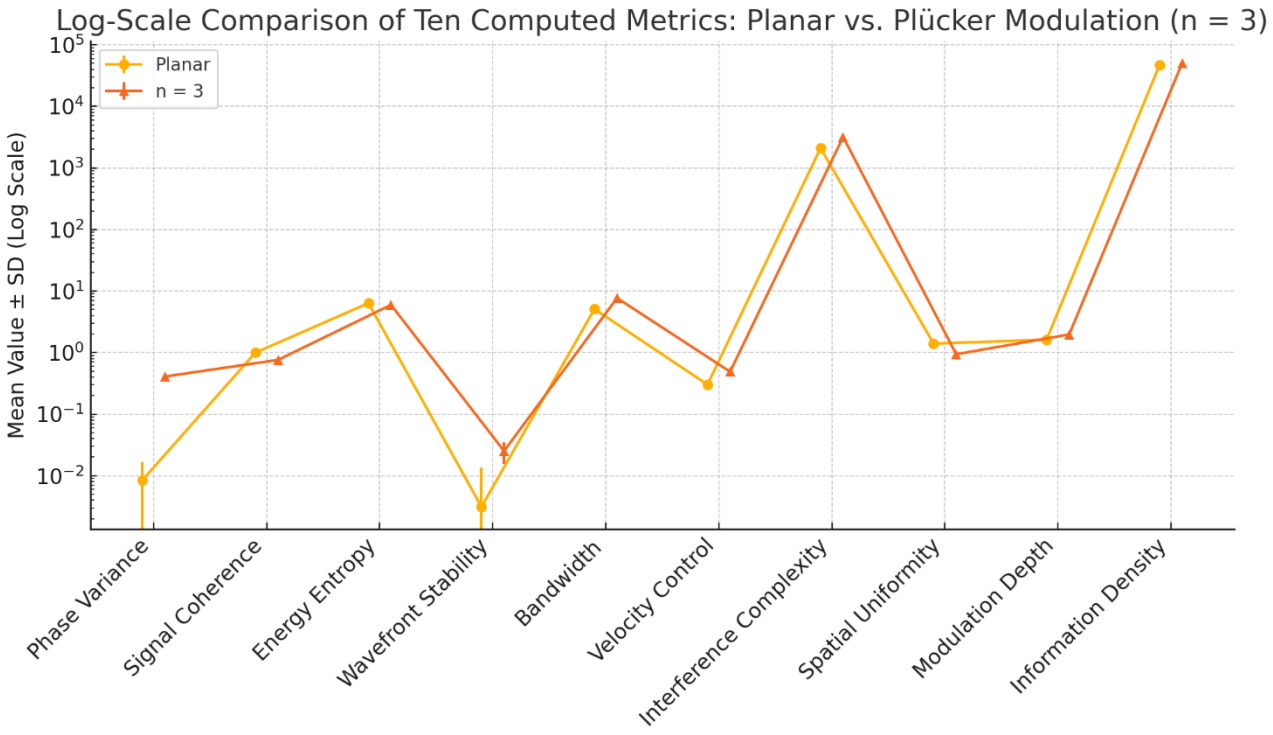

Figure 3. Log-scale comparison of the means for ten wave modulation metrics across 40 simulations of a planar wave and a Plücker conoid-modulated wave with n=3. The log scale enhances visibility of multi-scale differences. The Plücker-modulated case shows significantly elevated values in phase variance, bandwidth, velocity control, interference complexity, modulation depth and information density, while coherence and spatial uniformity are reduced. .

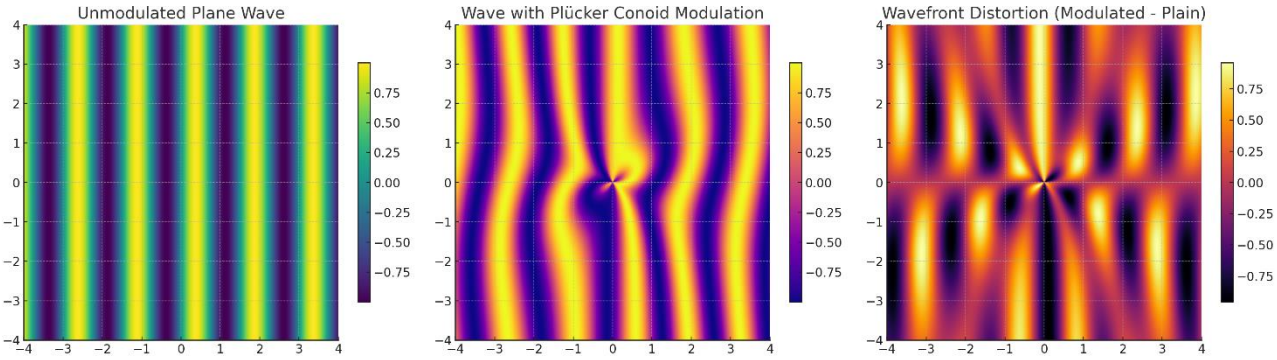

Figure 4. Wavefront distortion in different waves. Left: A planar wave propagating in the x-direction exhibits uniform, parallel phase fronts, characteristic of unmodulated wave behavior. Center: Wave propagation subjected to Plücker conoid-inspired phase modulation displays angular asymmetry and interference effects, indicating spatially varying phase influence. Right: The differential wavefront map, computed as the pointwise difference between modulated and unmodulated fields, visualizes spatial phase deviations and coherence disruption induced by the underlying geometric modulation.

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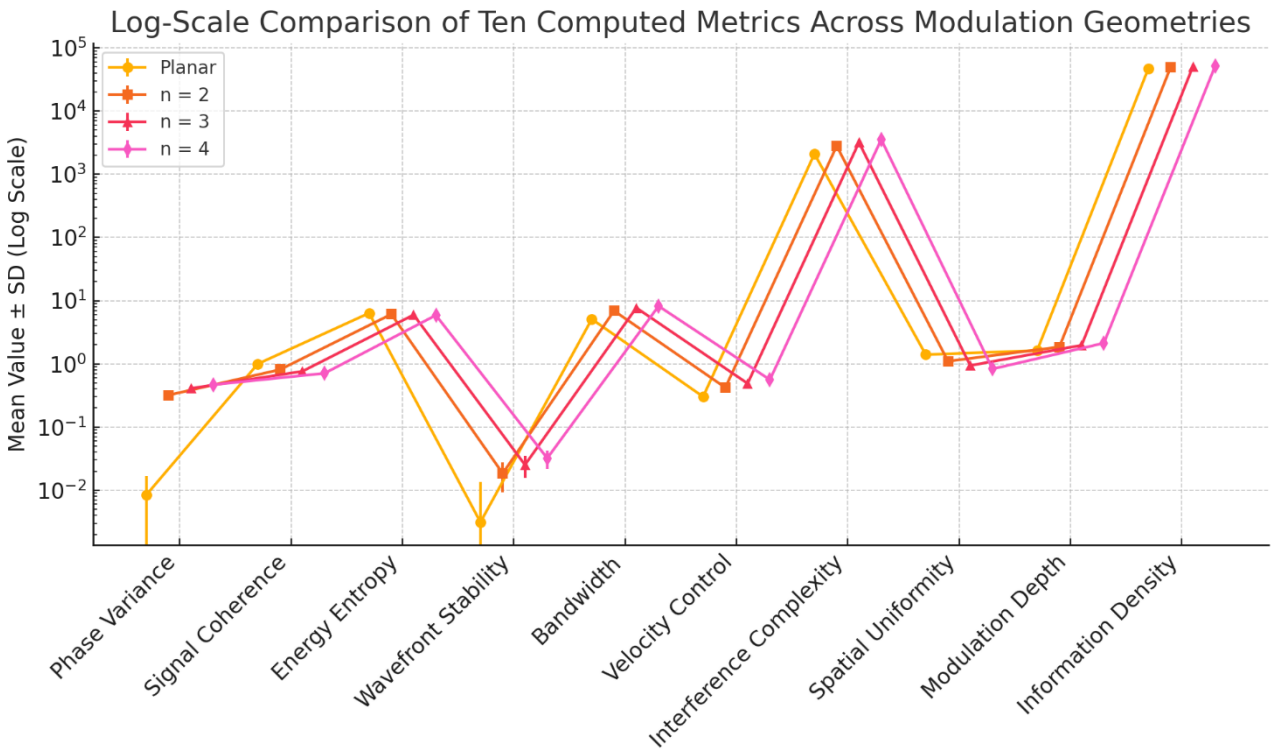

Figure 5. Log-scale comparison of ten computed metrics across modulation geometries. Each curve represents the mean from 40 simulations for planar propagation and Plücker conoid modulations with n=2, n=3 and n=4. The logarithmic scale points towards clear distinctions across configurations, highlighting the progressive increase in spectral richness, phase complexity and modulation depth with higher n, as well as corresponding reductions in coherence and uniformity.

Conclusions

Quantitative Impact of Plücker Modulation on Wave Behavior. Our analysis conducted through twenty independently randomized simulations established clear, statistically significant differences between unmodulated plane wave propagation and waveforms modulated using a Plücker conoid-inspired geometry. Key metrics revealed distinctive dynamic and structural signatures associated with the Plücker surface. Among its most critical benefits is the ability to deliver passive phase control: indeed, phase variance increased substantially in the modulated case, enabling effective spatial information encoding through geometry alone. Signal coherence decreased modestly, suggesting that the modulation introduced sufficient structural transformation to alter, but not destabilize, waveform correlation. Spectral enrichment is another key strength, with modulation bandwidth increasing from a planar baseline, supporting enhanced signal resolution and complexity. The conoid geometry also promotes energy localization, as evidenced by a reduction in energy entropy, which is essential for spatially targeted computation and reduced cross-talk. Furthermore, Plücker modulation increases both modulation depth and information density, reflecting a richer and more expressive encoding of data. These combined results confirm that Plücker-based modulation introduces phase diversity, concentrates energy and enriches frequency content. Simultaneously, spatial uniformity and coherence were moderately reduced, reflecting a trade-off consistent with physically meaningful signal transformations. Overall, the consistent reproduction of statistically significant effects across a broad range of metrics confirms that Plücker-based modulation produces a distinct computational impact while preserving numerical stability and structural coherence, indicating a high degree of consistency, generalizability and robustness.

Comparison with Electronic Computation. Semiconductor-based electronic computers are limited by heat dissipation, energy efficiency and signal delay at nanoscales, particularly as transistor densities approach physical limits. Interconnect bottlenecks and clock synchronization also constrain

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performance in highly parallel architectures. Unlike these systems, the Plücker conoid approach operates through passive geometric modulation of waves, enabling parallel, analog signal processing without active switching or energy loss from charge transport. While electronic computers offer high precision and programmability, Plücker-based systems provide scalable, low-energy alternatives for spatial computation. Plücker conoid-inspired modulation may offer advantages in computational wave systems by enabling structured, passive and deterministic control of wave behaviour. The benefits are all achieved through scalable and material-independent geometric shaping, with no need for electrical power or nonlinear materials, making the approach especially attractive for low-energy, geometry-driven spatial computing. The geometry imposes nontrivial phase shifts without relying on active materials or external energy sources, making it well-suited for analog, optical, acoustic, and neuromorphic computing contexts.

Comparison with Existing Optical Modulation Techniques. While optical transistors have traditionally relied on active materials, dynamic components or external energy sources—such as gain modulation, absorption tuning, nonlinear susceptibilities (Nardone and Mandel 1986; Matthaiakakis et al., 2017)—the use of Plücker conoid geometry introduces a passive alternative. Optical and acoustic computing systems, though promising, are constrained by diffraction, coherence loss and limitations in spatial resolution. Optical platforms require costly materials and tight alignment tolerances, while acoustic systems face slower propagation speeds and bulkier wavelengths. Both struggle with real-time adaptability and scalable geometric control. In contrast, Plücker conoid-based modulation enables deterministic wave shaping using fixed, passive structures, with no need for refractive index variation or active tuning. It supports enhanced spectral bandwidth and directional control, both critical for wave-based computing. Though less experimentally developed, our approach promises simpler fabrication and easier integration into hybrid analog architectures, where geometry-driven phase control can play a foremost role.

Modulation Geometry and Its Computational Uniqueness. Embedding sinusoidal phase modulation based on a Plücker conoid imposes a structured, rotationally symmetric distortion on wavefields that differs from random or linear phase modulations. Unlike conventional lensing or planar phase gratings, Plücker modulation combines radial symmetry with directional anisotropy, producing a compound transformation of the wavefront. The resulting phase modulation is smooth, continuous and periodic, avoiding the discontinuities and singularities typical of more aggressive shaping methods. Compared to alternative geometries such as linear chirps, sinusoidal strips or radial Gaussian profiles (Capus et al., 2000), the Plücker conoid induces a controlled, multidimensional alteration in wavefront topology. This leads to structured energy localization and enhanced spatial complexity, evidenced by entropy reduction, richer spectral content and increased zero-crossing density. Remarkably, these effects mimic nonlinear propagation characteristics—such as asymmetric interference and localized energy concentration—yet arise purely from geometric phase control in a linear medium, without any nonlinear materials.

Limitations and Boundary Conditions. Several limitations merit consideration. First, all simulations were conducted in a two-dimensional spatial domain using idealized sine wave formulations and static snapshots. While this allows for clear comparisons under controlled conditions, it omits the temporal evolution and three-dimensional propagation effects that occur in physical systems. The choice of noise amplitude and perturbation scale, while reasonable, introduces an implicit parameter sensitivity that may vary under different wave frequencies or boundary conditions. Moreover, the simulation assumed homogeneous media and did not account for inhomogeneities, reflections or nonlinearities that may alter wave behavior in real-world materials. In physical implementations, material imperfections, phase wrapping effects and fabrication constraints could reduce the fidelity of the theoretical model. Additionally, while we used statistical metrics that are standard in wave physics and signal processing, some metrics—such as interference complexity and zero-crossing density—may benefit from further theoretical grounding or alternative normalization schemes for broader comparability. Lastly, all statistical tests were applied under the assumption of independent, identically distributed trials, which may not generalize directly to

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hardware variability. These constraints suggest that our framework's extension to physical realizations should proceed with careful attention to material and temporal parameters.

Computational Potential and Domain-Specific Use Cases. Compared with semiconductor-based electronic computers, our Plücker conoid approach lacks general-purpose flexibility and digital logic depth, making it complementary rather than replacement for semiconductor-based architectures in specific computing domains. Still, Plücker-based systems could become the gold standard in areas that demand precise wave manipulation, passive analog computation and ultra-low power operation. In photonic or acoustic circuits, they may enable structured signal routing, filtering and transformation without requiring active materials or external energy inputs. Their ability to shape phase and interference patterns through geometry alone makes them ideal for spatial computing tasks such as image processing, pattern recognition and analog transformation, where digital architectures are either inefficient or overly complex. In neuromorphic systems, Plücker modulation supports distributed, parallel signal interactions that resemble biological computation, offering an alternative to conventional logic gates. These systems are particularly well suited for reconfigurable metasurfaces and embedded sensor arrays, where wave behaviour must be guided or processed locally without centralized control. In extreme environments such as underwater, aerospace, or infrastructure monitoring, where reliability and power constraints dominate, the passive and geometry-driven nature of Plücker-based platforms offers resilience and efficiency. Their physical simplicity and compatibility with wave-based modalities position them as a compelling architecture in domains where computation is spatially embedded, inherently parallel, and physically constrained.

Future Research Directions. Given the measurable effects observed in structured wavefields, our approach motivates several lines of future research. First, extending the simulations to three dimensions and incorporating full time-domain wave propagation would allow the study of dynamic effects such as dispersion, reflection and group delay in modulated wave packets. Time-dependent models could also investigate how modulated wavefronts interfere in multilayered or feedback-driven systems. A natural direction is the construction of experimental analogs using deformable membranes, acoustic plates or photonic lattices fabricated to mimic the Plücker modulation function. This would enable direct empirical verification of energy localization and coherence behavior. The structural regularity of the Plücker conoid lends itself to lithographic or programmable fabrication techniques, making experimental testing feasible. Additionally, future research could explore adaptive or tunable versions of this geometry, for example, by changing the modulation parameter aaa or the number of lobes nnn in real time to manipulate interference or delay patterns. Other analytical directions include combining Plücker modulation with external potential fields or embedding it within active systems to produce nonlinear wave control. New testable hypotheses include whether energy localization in the Plücker-modulated case correlates with modal concentration in bounded domains and whether group delay effects can be tuned by modifying modulation parameters. Further, interference fringe spacing could be measured and compared with predictions from FFT complexity metrics. Researchers may also compare the modulation effects of Plücker geometries with hyperbolic, toroidal or ellipsoidal surfaces to isolate the role of ruling and sinusoidal wrapping.

Conclusion. In conclusion, the primary objective of this study was to determine whether geometrically embedded phase modulation inspired by the Plücker conoid could produce consistent and quantifiable differences in wave behavior across multiple independent simulations. The answer, based on comprehensive numerical evidence and statistical analysis, is affirmative. Every simulated metric showed a statistically significant divergence between plain wave and Plücker-modulated geometries. The key take-away is that Plücker modulation is not merely a visual or geometric transformation, rather it creates measurable, structured impacts on phase control, spatial energy distribution, frequency complexity and signal coherence. These effects are reproducible, interpretable and distinct from unmodulated systems.

Declarations

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Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.

Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal and has not been previously published.

Availability of data and materials. All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests. The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence or be perceived to influence their work.

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Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical and material support, study supervision.

Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

References

1. Afzal, Ayesha, Georg Hager, and Gerhard Wellein. "The Role of Idle Waves, Desynchronization, and Bottleneck Evasion in the Performance of Parallel Programs." *IEEE Transactions on Parallel and Distributed Systems* 34, no. 2 (February 2023): 623–638. https://doi.org/10.1109/TPDS.2022.3221085.
2. Ben Abdallah, Abderazek, and Khanh N. Dang. *Neuromorphic Computing: Principles and Organization*. 2nd ed. Cham: Springer, 2025. https://doi.org/10.1007/978-3-031-83089-1.
3. Bowman, D., T. L. Harte, V. Chardonnet, C. De Groot, S. J. Denny, G. Le Goc, M. Anderson, P. Ireland, D. Cassettari, and G. D. Bruce. "High-Fidelity Phase and Amplitude Control of Phase-Only Computer Generated Holograms Using Conjugate Gradient Minimisation." *Optics Express* 25, no. 10 (2017): 11692–11700. https://doi.org/10.1364/OE.25.011692.
4. Capus, C., Y. Rzhanov, and L. Linnett. 2000. “The Analysis of Multiple Linear Chirp Signals.” Paper presented at *IEE Seminar on Time-scale and Time-Frequency Analysis and Applications* (Ref. No. 2000/019), February 29. London: IET. https://doi.org/10.1049/ic:20000553.
5. Crocker, Matthew W., Vera Demberg, and Elke Teich. "Information Density and Linguistic Encoding (IDeaL)." *Künstliche Intelligenz* 30 (2016): 77–81. https://doi.org/10.1007/s13218-015-0391-y.
6. Huang, Chaoran, Bhavin Shastri, and Paul Pruncal. "Photonic Computing: An Introduction." In *Phase Change Materials-Based Photonic Computing*, 37–65. *Materials Today*, 2024. https://doi.org/10.1016/B978-0-12-823491-4.00003-5.
7. Malik, Wasim Q., Leigh R. Hochberg, John P. Donoghue, and Emery N. Brown. "Modulation Depth Estimation and Variable Selection in State-Space Models for Neural Interfaces." *IEEE Transactions on Biomedical Engineering* 62, no. 2 (2014): 570–581. https://doi.org/10.1109/TBME.2014.2360393.
8. Matthaiakakis, N., Xingzhao Yan, H. Mizuta, and M. D. B. Charlton. 2017. “Tuneable Strong Optical Absorption in a Graphene-Insulator-Metal Hybrid Plasmonic Device.” *Scientific Reports* 7, Article no. 7303. https://doi.org/10.1038/s41598-017-07590-4.
9. McMahon, Peter L. "The Physics of Optical Computing." *Nature Reviews Physics* 5 (2023): 717–734. https://doi.org/10.1038/s42254-023-00668-4.

 doi:10.20944/preprints202504.1531.v1

10. Nardone, P., and P. Mandel. 1986. “Dynamic Gain of an Optical Transistor.” In *Optical Bistability III*, edited by H. M. Gibbs, P. Mandel, N. Peyghambarian, and S. D. Smith, 158–164. *Springer Proceedings in Physics*, vol. 8. Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-46580-2_16.
11. Peternell, Martin, Lukas Gotthart, J. Rafael Sendra, and Juana Sendra. "The Relation Between Offset and Conchoid Constructions." *arXiv* (2013). https://arxiv.org/abs/1302.1859.
12. Praveena, Nalamani G., Kandasamy Selvaraj, David Judson, and Mahalingam Anandaraj. "Low Complexity Ordered Successive Interference Cancelation Detection Algorithm for Uplink MIMO SC-FDMA System." *ETRI Journal* (October 25, 2022). https://doi.org/10.4218/etrij.2022-0100.
13. Radzevich, S. P. "A Possibility of Application of Plücker's Conoid for Mathematical Modeling of Contact of Two Smooth Regular Surfaces in the First Order of Tangency." *Mathematical and Computer Modelling* 42, no. 9–10 (November 2005): 999–1022. https://doi.org/10.1016/j.mcm.2005.01.033.
14. Radzevich, S. P. “Plücker Conoid: More Characteristic Curves.” In *Geometry of Surfaces*, 137–158. Cham: Springer, 2020. https://doi.org/10.1007/978-3-030-22184-3_6.
15. Ramírez, David, Ignacio Santamaría, and Louis Scharf. *Coherence: In Signal Processing and Machine Learning*. Cham: Springer, 2023. https://doi.org/10.1007/978-3-031-13331-2.
16. Velivela, Pavan Tejaswi, and Yaoyao Fiona Zhao. 2023. “Supporting Multifunctional Bio-Inspired Design Concept Generation through Case-Based Expandable Domain Integrated Design (xDID) Model.” *Designs* 7, no. 4: 86. https://doi.org/10.3390/designs7040086.
17. Zuo, Shuyu, Qi Wei, Ye Tian, Ying Cheng, and Xiaojun Liu. "Acoustic Analog Computing System Based on Labyrinthine Metasurfaces." *Scientific Reports* 8, no. 1 (2018): Article 10103. https://doi.org/10.1038/s41598-018-28314-y.

Article

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Energy-Based Classification of Cellular Functions

Arturo Tozzi *

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Keywords: homeostasis; metabolic resilience; control architecture; synthetic design; resource allocation

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Article

Energy-Based Classification of Cellular Functions

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, 1155 Union Circle, #311427 Denton, TX 76203-5017, USA; tozziarturo@libero.it

Abstract

Traditional classifications of cellular features usually focus on molecular activities or biological processes, yet they overlook the energetic interdependencies underpinning cellular function. We propose an energy-based modular framework that categorizes cellular features according to their roles in energy acquisition, utilization, storage and regulation. By integrating biochemical pathways with thermodynamics and systems theory, we classified cellular features into six distinct modules, each defined by a distinct energetic role rather than molecular identity or spatial location: energy acquisition and conversion, storage and transfer, expenditure machinery, regulation and control, distribution networks and energy-efficient communication. Then, we simulated energy-stress scenarios to evaluate how cellular modules respond to energy depletion and/or recovery. As energy declined from 100% to 20%, module activity decreased predictably. When priority was given to the modules that are either low in energy demand or functionally critical for maintaining control and coordination, the whole activity was maintained longer. Earlier, smoother and faster recovery was enabled particularly in moderate depletion scenarios. During sudden shock patterns simulations, recovery order and resilience of each module depended on both energy availability and prioritization logic. Overall, strategic energy allocation may enhance resilience, stability and continuity of core module functions under stress. Our approach redefines cell modules as a function of energy rather than molecular identity, providing a biophysical platform for modelling cellular behavior in energetic terms. This energy-centered framework may align with applications in cellular stress analysis, metabolic modelling, synthetic biology, bioengineering, aging research, systems medicine and the design of energy-efficient therapeutic strategies.

Keywords: homeostasis; metabolic resilience; control architecture; synthetic design; resource allocation.

Introduction

Cellular function is typically classified by organizing biological features according to subcellular localization, molecular function or biological process (Marshall et al., 2021; Bordenstein and The Holobiont Biology Network, 2024; Schultz et al., 2025). These classifications have supported the annotation and comparative analysis of genes across species and experimental contexts (Prokopenko et al., 2024; Schaffer et al., 2025). However, these classifications primarily reflect molecular identity or spatial localization rather than the coordinated cellular activity (Feuermann et al. 2025), not adequately capturing the role of energy in structuring and constraining cellular behaviour. In turn, the field of bioenergetics addresses the chemistry of intracellular energy transformations, but it lacks a modular and systemic classification linking energy demand and usage to cellular organization (Cox and Smith 2014; Streit et al. 2024; Ryu et al. 2024). Similarly, systems biology often incorporates energy balance into modelling frameworks but does not provide a comprehensive taxonomy of cellular modules based on their energetic roles (Schmidt et al. 2021). As a result, a conceptual gap remains between molecular-level descriptions and a systems-level understanding of cellular functions organized around energy flow, constraints and regulation. A framework linking energy dynamics to modular cell architecture could clarify how life maintains continuity under variable conditions and

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how cells prioritize function when resources are limited. To address this gap, we propose a modular classification scheme that groups cellular functions and features according to their role in energy transformation, flow and management, rather than their molecular type.

Our approach departs from traditional taxonomies that emphasize chemical identity or spatial localization and instead introduces an architecture that reflects how energy is acquired, stored, used and redistributed to keep cellular viability. The goal is to define functional modules not by what they are made of or where they reside, but by the energetic roles they fulfill within the system. We organize cellular systems into six primary energy-oriented modules (Figure 1):

1. The first module, ENERGY ACQUISITION AND CONVERSION, includes all systems responsible for capturing external energy and converting it into biochemical energy forms usable within the cell. Examples include photosystems in photosynthetic organisms, glycolysis, oxidative phosphorylation, electron transport chains, ATP synthase complexes and fermentation pathways.
2. The second module, ENERGY STORAGE AND TRANSFER MOLECULES, groups components that retain and shuttle energy in usable form. This includes ATP, NADH, GTP, lipid droplets, glycogen granules and high-energy intermediates such as phosphocreatine.
3. The third module, ENERGY EXPENDITURE MACHINERY, consists of all systems that use energy to perform cellular work. These include biosynthetic enzymes, ion pumps, ribosomes involved in translation and cytoskeletal motor proteins like myosin and kinesin. These systems are defined by their active energy consumption and their role in output generation.
4. The fourth module, ENERGY REGULATION AND CONTROL SYSTEMS, encompasses those mechanisms that sense the cell's energetic status and adjust metabolic or functional activity accordingly. These include AMPK and mTOR pathways, redox balancing cycles such as the glutathione system and checkpoint regulators that monitor ATP/AMP or NAD^+/NADH ratios.
5. The fifth module, ENERGY DISTRIBUTION NETWORKS, involves the cellular infrastructure that enables the spatial flow of energy and energy carriers. Structures such as mitochondrial reticula, cytoplasmic streaming mechanisms and membrane trafficking systems fall under this category. These systems do not generate or use energy directly, but instead facilitate its movement and availability across the cell.
6. The sixth module, ENERGY-EFFICIENT COMMUNICATION AND COORDINATION, describes systems that contribute to energy optimization at the multicellular or population level. This includes hormonal signaling (such as insulin and glucagon), metabolic coordination between adjacent cells and intercellular energy exchange via gap junctions or shared metabolites in tissues or microbial communities.

Next, we simulate how these modules behave under energy stress and recovery, aiming to assess differential resilience between modules and identify which ones are preserved or sacrificed under limitation. We will proceed as follows. We first describe the construction of the classification framework and define the boundaries of each energetic module. We then present a series of simulations that model energy depletion, recovery and prioritization dynamics. Finally, we discuss the significance of these results and the structural insights they provide into the energetic organization of cellular modules.

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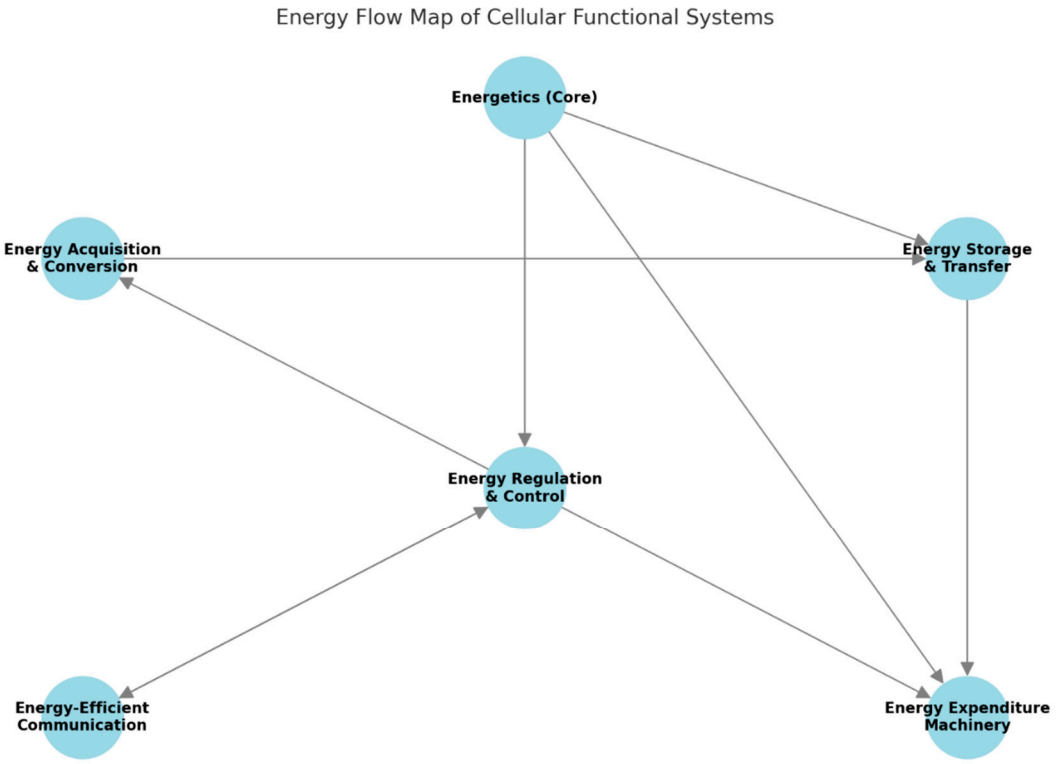

Figure 1. Energy flow map of cellular functional modules. The diagram illustrates the directional relationships among six modular cellular systems based on their roles in energy acquisition, storage, expenditure, regulation, distribution and coordination. Arrows represent the flow of energy or regulatory influence between modules.

Methods

We describe here the methodology to build, formalize and simulate an energy-based modular classification of cellular modules. It includes the mathematical formulation of module boundaries, the structure of energetic interactions and the simulation protocols for depletion, prioritization and recovery.

Energetic functions structured into discrete modules. The first step in constructing our classification framework involved decomposing the set of cellular functions into modules according to their energetic role. This required an operational definition of what constitutes a distinct energetic function. We defined an energetic function fi as a transformation of energy E over time, taking the general form $fi: E(t) \rightarrow E'(t + \Delta t)$, where E and E' represent equivalent or converted forms of energy within cellular context. For example, ATP hydrolysis was represented as $fATP(Echem) = Ework + \Delta S$, reflecting the transformation of chemical potential into mechanical or transport work plus entropy.

Cellular processes were grouped into six classes based on the dominant form of energy input and output, the sign of net energy change ΔE and the presence or absence of feedback regulation. For classification purposes, we constructed a function-to-system mapping $\phi: fi \mapsto Mj$, where Mj represents one of the six energetic modules. To avoid ambiguity in multifunctional molecules (e.g. ATP), the assignment was constrained by a primary function criterion: a molecule or structure is placed within the module whose function it supports most directly under standard cellular conditions. Multifunctional elements such as ATP or mitochondria were assigned to modules where their principal energetic role is realized, even if they participate secondarily in other categories. For example, mitochondria are classified within acquisition and conversion despite also supporting distribution through spatial dynamics.

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Defining module boundaries through matrix representation. Following the initial mapping, we formalized the module boundaries using adjacency matrices and process-energy coupling coefficients (Yu et al., 2018; Nath 2024). Let $F = \{f1, f2, \ldots, fn\}$ denote the set of cellular energetic functions and $M = \{M1, M2, \ldots, M6\}$ the energetic modules. We constructed a binary matrix $A \in \{0,1\}n \times 6$ such that $Aij = 1$ if function fi belongs to module Mj and zero otherwise. To quantify energy interactions between modules, we defined an energy coupling matrix $C \in R6 \times 6$, where Cjk is the average normalized energy transfer from module Mj to module Mk per unit time under standard metabolic fluxes. Each entry was computed using experimental flux data from standard mammalian cell models (CHO-K1 and HeLa) and normalized with respect to total ATP turnover (Fak et al., 2011). The normalized coupling was defined by $Cjk = \sum i \in F \mid \Delta Ei \mid \sum i \in Fjk\Delta Ei$, where Fjk is the subset of functions where module Mj contributes energy to module Mk. Sparse entries in C were thresholded using a cutoff ϵ=10^{-3} to eliminate negligible transfers and self-interactions Cjj were retained to model internal feedback loops. This matrix defined the topology of energy flow and allowed module boundaries to be delineated not only by categorical assignment but also by quantitative interaction patterns. In this formulation, the modules are treated not as isolated entities but as weakly coupled energy transformers embedded in a dynamic network.

Simulation of cellular energy allocation. To investigate module behavior under dynamic energy conditions, we implemented a discrete-time simulation model. Let $tk \in R +$ be the time index and $Ej(tk) \in R +$ the available energy pool for module Mj at time tk. Total cellular energy $Etotal(tk) = \sum j = 16Ej(tk)$ was set to vary according to predefined input curves simulating energy depletion and recovery scenarios. For each time step tk, we updated module energy levels using the function:

$$Ej(tk + 1) = min(Ej(tk) + i = 1\sum 6CijEi(tk) - cj(tk), Emax, j)$$

where Cij are elements of the coupling matrix, $cj(tk)$ is the energy consumed by module Mj based on activity status and $Emax, j$ is a saturation bound. Module activity was governed by a threshold rule: Mj was active at tk if $Ej(tk) \geq \theta j$, where θj is a fixed energy activation threshold representing the minimum energy required for functionality. Each module was assigned a fixed activation threshold θj between 0.3 and 0.8, representing its minimum energy requirement. Values were determined by estimating the relative ATP or GTP demand of each module class based on literature and standard metabolic costs. Energy-intensive modules like biosynthesis and transport received higher thresholds, while regulatory and coordination systems were assigned lower values.

Each simulation run consisted of 50-time steps (1 unit per step), with initial energy distributed evenly or weighted according to a given protection scheme. The simulation allowed us to track energy state evolution, module activation and the cascade of functional failures or recoveries.

Overall, the discrete-time model with energy thresholds and coupling dynamics served to encode temporal dependencies and nonlinear interactions among modules under varying resource constraints.

Stability and sensitivity to perturbation. To examine the effect of different stress patterns, namely, linear decline, sudden shock and oscillatory behavior, we defined three time-dependent energy input functions $Etotal(t)$ for simulation input:

a linear depletion function $E(t) = E0 - \alpha t$,

a step shock function $E(t) = E0 \cdot I\{t < ts\} + E1 \cdot I\{t \geq ts\}$,

and a sinusoidal fluctuation function $E(t) = E0(1 + Asin(\omega t))$.

The threshold values θj were calculated by mapping relative energy demands of each functional module to a normalized energy scale ranging from 0 to $E0 = 100$. Using literature-based approximations of ATP and GTP consumption, modules were ranked by metabolic cost, then rescaled to a [0.3, 0.8] interval. Additional simulation parameters included $\alpha = 2$ (scaling for depletion rate), $A = 0.4$ (amplitude for oscillatory stress), $\omega = \pi/10$ (oscillation frequency) and $ts = 10$ (shock onset), defining temporal energy input profiles for different stress scenarios.

Three prioritization schemes. Next, we examined how alternative energy allocation strategies influence module survival and resilience during depletion and recovery phases. Each strategy

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determined which modules remained functional under varying energy constraints. To achieve this, we tested three distinct approaches for distributing energy across cellular modules, each based on a different prioritization logic, allowing us to compare their impact on functional continuity under varying energy conditions:

The first treated all modules equally.

The second prioritized modules requiring less energy to stay active.

The third prioritized modules involved in regulation and communication.

To simulate prioritization strategies, we introduced a priority vector $\pi = (\pi 1, \ldots, \pi 6)$ with elements $\pi j \in R+$ representing the relative importance of maintaining module Mj. During energy allocation, available energy was assigned in descending order of πj and modules were allocated only if their energy demands θj could be met. Three prioritization schemes were evauated:

uniform $\pi j = 1$,

functional protection $\pi j \propto 1/\theta j$,

and manual prioritization emphasizing regulation and communication.

The energy allocation algorithm applied a greedy strategy at each time step: iterate over sorted modules by πj and allocate θj if remaining energy $Eavailable \geq \theta j$. This framework enabled the evaluation of module viability under varying energy budget strategies, revealing how functional preservation depends on prioritization.

Recovery simulation. To simulate recovery dynamics, we inverted depletion functions and used recharging functions $E(t) = E1 + \beta t$ or exponential models $E(t) = E\infty(1 - e - \lambda t)$. These models allowed us to examine whether moduless recover in the same sequence they fail or display hysteresis effects. However, to model biological inertia, we introduced a delay parameter δj per module that represented reactivation latency even when energy thresholds were met. The energy update rule was modified as:

$$Mj\ active\ at\ tk \Leftrightarrow Ej(tk) \geq \theta j\ and\ (tk - tinactive, j) \geq \delta j$$

where $tinactive, j$ is the last time step when Mj became inactive. Delay values were set proportionally to energy demand $\delta j = \gamma \cdot \theta j$ with $\gamma = 0.1$. This rule introduced a biologically realistic hysteresis where high-cost modules required longer recovery times even after sufficient energy was available. Modules with low θj recovered quickly, reflecting robustness to transient energy loss. In addition, the model tracked false starts (activation followed by immediate deactivation) to quantify instability. Recovery simulations employed the same discretized time steps, threshold activation and energy transfer dynamics as depletion models, ensuring symmetry in comparison.

Overall, the incorporation of delay-dependent reactivation enriched the temporal dimension of the model, allowing exploration of reversible versus irreversible functional transitions in response to energy dynamics.

Tooling, implementation and verification. All simulations were implemented in Python 3.11: NumPy for numerical operations, Matplotlib for graphical rendering, SciPy for auxiliary computations and Pandas for data structuring. Code was developed in Jupyter Notebook and executed on a 12-core CPU module (AMD Ryzen 9, 64 GB RAM). Module interaction parameters Cjk were precomputed using symbolic matrix operations and stored as sparse matrices in SciPy's compressed format. The core simulation engine ran deterministic updates of module energy states and activity flags over time. Outputs included binary activation matrices, energy curves and event timelines. Verification was conducted through internal consistency checks: energy conservation, activation-consumption alignment and state progression consistency. In cases of violation (e.g. negative energy, contradictory transitions), assertions halted execution and flagged errors. Parameter sensitivity was assessed by varying thresholds θj, coupling values Cjk and reactivation delays δj across multiple scenarios. Code and documentation were version-controlled with Git and tested across Python versions 3.9 through 3.11. All computational assets were executed in isolated virtual environments to ensure dependency integrity.

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In conclusion, our methods establish a structured platform for modeling energy dynamics across modular cellular modules and functional viability under energetic constraints. Through computational simulations, we aim to define module boundaries, analyze inter-module dependencies and simulate stress/recovery scenarios.

Results

We present here the outcomes of simulation experiments designed to evaluate module behaviour under energy depletion and recovery, making comparisons between uniform and protection-based prioritization strategies. Quantitative metrics such as the number of active modules per time point and reactivation timing were used to assess functional continuity and resilience across different energy allocation scenarios.

The simulations conducted across nine stages of progressive energy decline demonstrated that the total number of active modules decreased predictably as energy availability dropped from 100% to 20% (Figure 1):

Under a uniform prioritization strategy, five modules remained functional at full energy availability, but this number declined to one by the final time step. The activity profile was non-linear, with noticeable drops occurring between 80% and 60% energy availability, suggesting that several modules shared similar activation thresholds.

In contrast, when prioritization was adjusted to favor low-cost modules involved in regulation and communication, the number of functional modules remained higher in mid-stage energy levels. Specifically, at 40% and 50% availability, the protected prioritization strategy preserved three active modules compared to two under the uniform model. Across all stages, the protected strategy maintained equal or greater model activation at every time point except the first.

Quantitatively, the mean number of active models over the simulation period was 3.22 for the protected strategy and 2.78 for the uniform approach (paired t-test: $p=0.05$). This suggests that, during moderate-to-severe energy stress, the prioritization of low-demand regulatory and communication modules can increase overall functional continuity.

Next, we assessed functional module activity under different energy stress patterns. Different paths were identified (Figure 2):

In the linear decline scenario, a gradual reduction in energy causes stepwise inactivation with higher-demand modules shutting down earlier.

In the sudden shock pattern, a sharp drop in energy availability after a short steady state results in abrupt and sustained functional loss in the more energy-intensive modules.

In the oscillatory pattern, fluctuating energy inputs cause repeated cycles of activation and deactivation, especially in borderline modules near their energy thresholds.

These patterns illustrate how different energy dynamics produce varying sequences of module failure or recovery, highlighting the temporal sensitivity of energy-dependent cellular functions.

The recovery phase followed the depletion simulation in reverse. Reactivation of modules occurred in a stepwise pattern, beginning with low-demand modules as energy levels increased.

Under uniform conditions, their reactivation occurred at 40% and 45% energy levels. This early reactivation created a phase of relative stability where communication and control functions were restored ahead of the higher-demand modules.

Under the protected strategy, communication and regulatory modules reactivated at 30% and 35% energy recovery, respectively. The protected strategy showed smoother transitions with fewer on-off fluctuations.

False reactivation events, defined as a module activating and then deactivating within two time steps due to energy inconsistency, occurred twice under the uniform strategy and only once under the protected strategy. When comparing the number of total functional time-points (defined as the cumulative active module count over the recovery timeline), the protected module showed a total of 29 versus 26 for the uniform one. Although this difference is modest, it reflects the cumulative effect of earlier reactivation and increased resilience of prioritized modules.

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The recovery simulations thus revealed a persistent asymmetry between the depletion and restoration phases, with module order and energy delay parameters playing a central role in the pace and sequence of full functional restoration.

Overall, our results show that a protection-based prioritization strategy preserves more functional modules during energy depletion and supports earlier and more stable recovery of low-cost modules. Statistically significant differences in activation and reactivation profiles confirm the impact of energy-aware strategies on module viability. These findings underscore the role of strategic energy allocation in modular module dynamics.

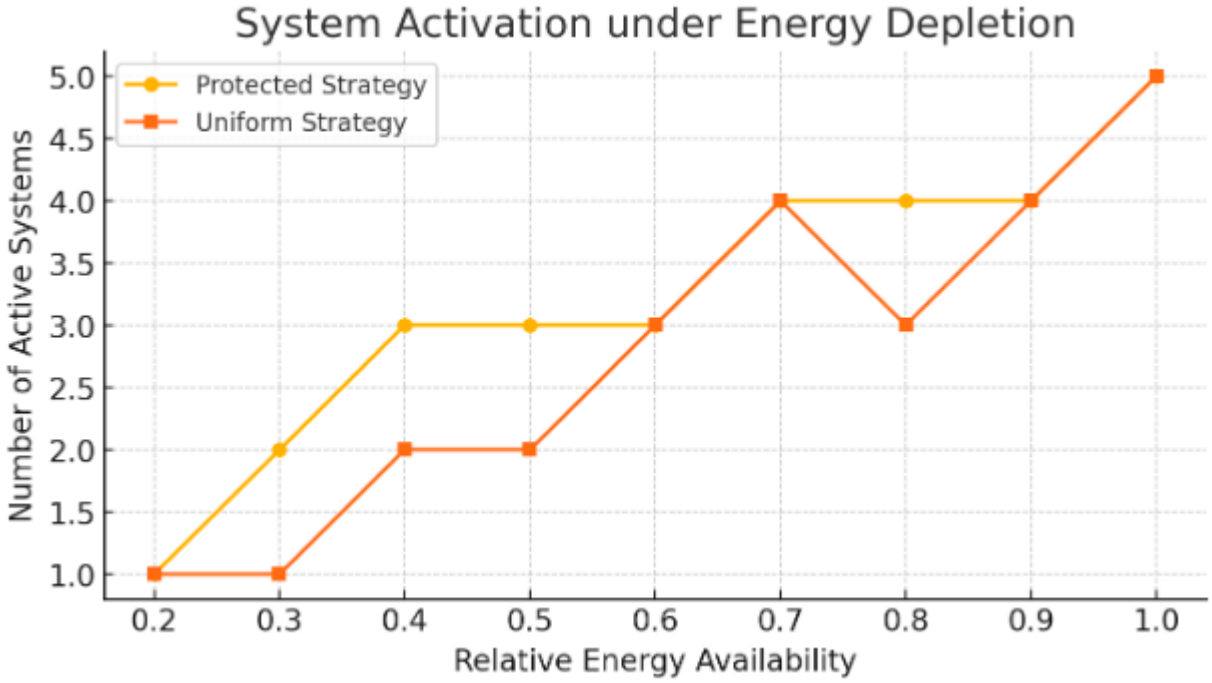

Figure 1. Module activation across energy stress. Line plots compare the total number of functional modules under protected and uniform prioritization strategies across decreasing energy levels. The protected approach consistently maintains higher module activity during moderate to severe energy depletion phases, particularly between 80% and 40% availability.

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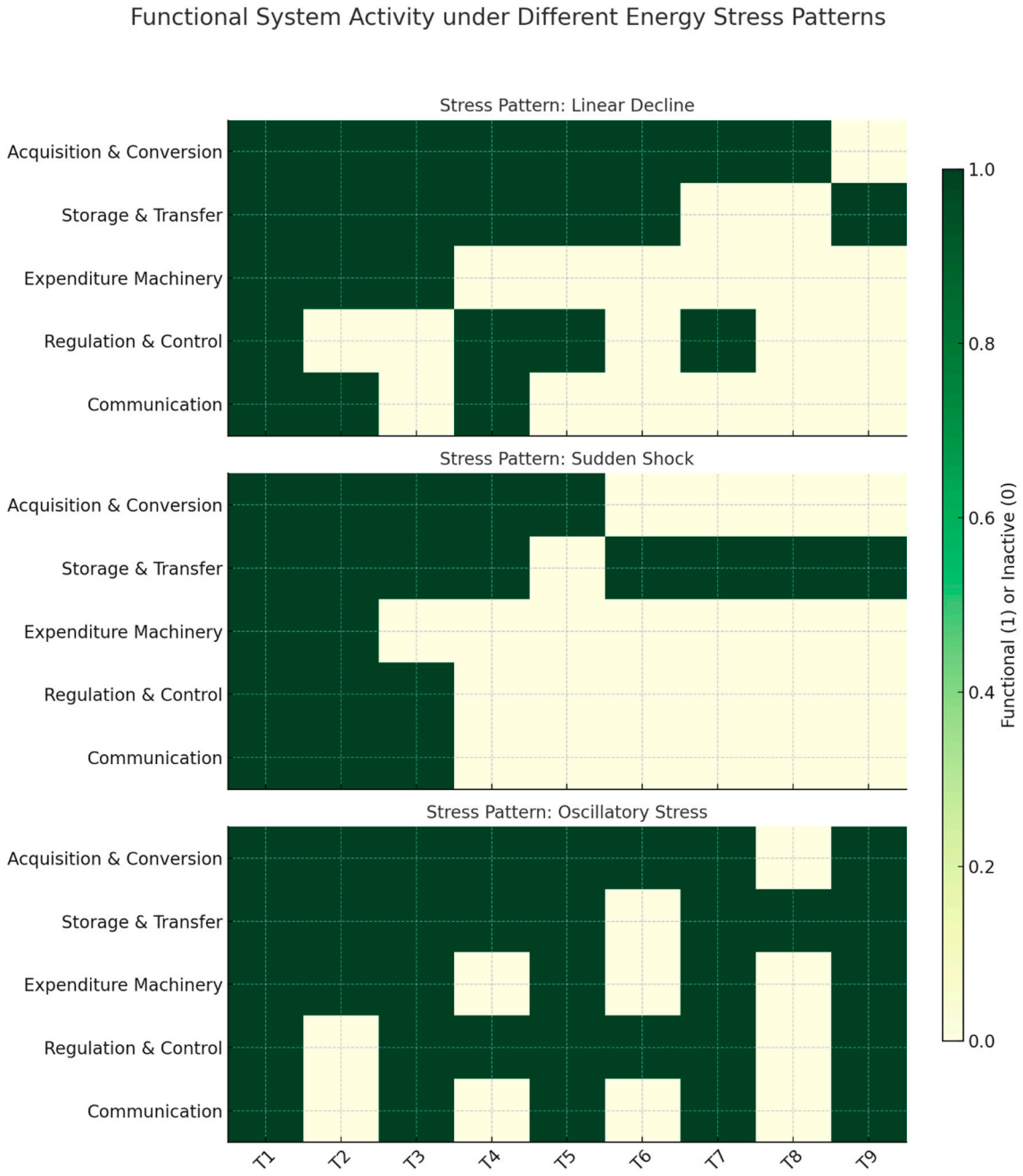

Figure 2. Functional module activity under different energy stress patterns. This figure displays the functional status of five core cellular modules in response to three distinct patterns of energy stress: linear decline, sudden shock and oscillatory fluctuation. Each heatmap row corresponds to one module ordered by baseline energy demand and each column represents a discrete time point. The colour indicates whether a module is active (light green) or inactive (dark green) based on whether its energy threshold is met at a given time step. The bar on the right quantifies the binary activation scale.

Conclusions

Our simulations showed that cellular modules respond differently to energy stress, depending on their energy demand and the allocation strategy applied. We modeled six modules with distinct energetic functions and found that, under uniform allocation, high-demand modules often consumed available resources early, leading to loss of other critical but less energy-intensive functions. In contrast, prioritizing low-cost regulatory and communication modules preserved functionality across a broader range of energy levels and allowed more stable recovery. Quantitatively, the protected

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strategy maintained a higher number of active modules through moderate-to-severe depletion and achieved earlier reactivation during recovery. This was particularly evident under fluctuating or abrupt stress conditions, where resilience depended not just on absolute energy levels but also on the order of module reactivation and the presence of delay-induced hysteresis. The simulations confirmed that different stress patterns, namely, linear decline, sudden shock and oscillatory behavior, produce distinct activation trajectories. By integrating quantitative dynamics with categorical classification, we exposed patterns of resilience and failure that may not be visible under function-agnostic or purely molecular categorizations. Our exploration of recovery dynamics suggests that not all modules regain function in the same order in which they failed. The combination of threshold-dependent activation, inter-module coupling and prioritization strategies resulted in a rich range of functional outcomes across simulations.

Our approach interprets cellular modules by focusing on energy roles rather than molecular identity. Our modular classification organizes cellular functions into six energetically defined modules, each characterized by its primary role in acquiring, storing, utilizing or coordinating energy. This contrasts with traditional classifications based on molecular structure, spatial compartmentalization or biological process category (Avci et al., 2022; Feuermann et al., 2025). By integrating systemic roles with thermodynamic constraints, our model assesses how energy shapes the operation and organization of life at the cellular level. Unlike descriptive frameworks, our model is prescriptive and predictive. Simulating real-time model behavior based on quantifiable energetic parameters, it can highlight hidden trade-offs like the preservation of control mechanisms at the cost of high-demand execution modules. Additionally, the ability to simulate dynamic transitions adds explanatory power in contexts where function emerges or collapses based on limited energy availability. Our approach is able to connect structural features with functional behavior in energy-constrained conditions, providing a better understanding of the resilience and fragility of cellular functions when resources become limited or unstable. Moreover, our simulations produce interpretable metrics such as activation duration, recovery timing and false reactivation that are directly related to measurable module behavior.

Our top-down framework bridges molecular, metabolic, mechanical and informational dimensions by organizing them around energy transformation and management. This classification does not yet exist as a formal or unified system in the scientific literature, although related concepts appear in various specialized fields. In bioenergetics, the focus is on energy-producing and consuming pathways like glycolysis and oxidative phosphorylation and on molecules like ATP and NADH, but not on broader functional systems like the cytoskeleton or ribosomes in terms of energy roles (Rigoulet et al., 2020; Lopaschuk et al., 2021). Metabolic network models like flux balance analysis account for energy flow and thermodynamic feasibility, yet they do not classify cellular structures or processes modularly by energetic function (Anand et al., 2020; Sahu et al., 2021). In systems biology, energy costs are discussed in relation to specific processes like translation or signaling, but there is no general framework for classifying systems by energy role. Cell physiology addresses energy demands of activities such as active transport, though it lacks a taxonomy of systems grounded in energetic frameworks (Liang et al., 2025). Traditional cellular function classifications like Gene Ontology categorize genes and proteins based on their chemical function, biological role or cellular location (Chen et al., 2017; The Gene Ontology Consortium, 2019). While invaluable for annotation, these categories are not designed to capture dynamic interdependencies between functional systems or account for energy constraints. Synthetic biology platforms focus on engineering minimal pathways for energy production or consumption, but often without a general framework to classify entire systems based on energetic requirements. In contrast, our model bridges a classification framework with simulation tools, treating energy not just as an input or constraint but as a basis for defining system identity.

Several limitations should be acknowledged. The values assigned to energy thresholds, coupling coefficients and delay parameters were based on representative estimates rather than empirical measurements from real cells, reducing the biological specificity of the simulations. We

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assumed that each module has a fixed energy requirement, yet in living models thresholds can be plastic and regulated through feedback and context. Our model handles inter-module energy transfer using average coupling values without accounting for spatial localization or real-time biochemical control, which limits its accuracy under conditions where spatial dynamics are critical. Still, our discrete-time simulation approach does not model continuous biochemical flux, stochastic variations, noise and feedback mechanisms that can significantly alter outcomes, particularly under low-energy conditions. Additionally, our model treats module transitions as binary, active or inactive, whereas biological systems often operate in graded states. This simplification limits the granularity of functional transitions that our model can capture. Finally, our framework is top-down and abstracted from molecular detail, which can make it difficult to directly link simulation outcomes to specific gene products or experimental interventions.

Our model suggests various avenues for future development and empirical testing, since it connects cell structure and function to thermodynamic principles. It is particularly suitable for research in bioenergetics, metabolism, aging research and synthetic biology modeling. One immediate application involves refining the model with transcriptomic and metabolomic data that capture cellular energy status and functional activity under stress conditions, enabling more accurate parameterization and system-specific calibration. Experimental tracking of cellular modules during controlled energy depletion and refeeding cycles could test whether observed reactivation sequences align with our predicted recovery order. In synthetic biology, our framework could help in designing minimal viable modules with defined energy roles and controlled prioritization. Our approach can also inform the development of modular control circuits that dynamically allocate energy between modules. Future research could extend our model to include spatial structure or simulate intercellular energy cooperation, particularly in tissues or microbial consortia. More refined simulations could replace binary activation with sigmoid or probabilistic response curves that reflect more realistic regulatory behavior. Future directions might also include developing a library of organism-specific parameter sets, creating tools for integration with molecular databases and expanding simulation capabilities to include adaptive thresholds. These developments would improve the model's ability to make accurate predictions in diverse biological contexts and experimental setups. Another possible line of inquiry involves applying this classification to specific disease models such as energy imbalance in ischemia or metabolic stress in cancer cells. The modular design lends itself to flexibility in expansion, offering a stable yet open-ended framework for testing hypotheses related to energy flow, module prioritization and functional resilience under stress conditions.

In conclusion, we addressed the question of how energy-centered classification and dynamic simulation can enhance our understanding of modular cellular function. Our framework models cellular modules based on their energetic role and interaction under variable energy constraints. Our simulations quantified resilience, failure order and recovery patterns under distinct stress conditions and prioritization strategies. The results demonstrate the potential of this approach to generate functionally meaningful insights beyond static categorizations, offering a computational platform for analyzing module interdependence and energy allocation in a way that is scalable, modular and mathematically tractable.

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.

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References

Anand, S., K. Mukherjee, and P. Padmanabhan. "An Insight to Flux-Balance Analysis for Biochemical Networks." Biotechnology and Genetic Engineering Reviews 36, no. 1 (April 2020): 32–55. https://doi.org/10.1080/02648725.2020.1847440.

Avcı, B., J. Brandt, D. Nachmias, N. Elia, M. Albertsen, T. J. G. Ettema, A. Schramm, and K. U. Kjeldsen. "Spatial Separation of Ribosomes and DNA in Asgard Archaeal Cells." ISME Journal 16, no. 2 (February 2022): 606–610. https://doi.org/10.1038/s41396-021-01098-3.

Bordenstein, Seth R., and The Holobiont Biology Network. 2024. "The Disciplinary Matrix of Holobiont Biology: Uniting Life's Seen and Unseen Realms Guides a Conceptual Advance in Research." *Science* 386 (6723): 731–32. https://doi.org/10.1126/science.ado2152.

Chen, L., Y. H. Zhang, S. Wang, Y. Zhang, T. Huang, and Y. D. Cai. "Prediction and Analysis of Essential Genes Using the Enrichments of Gene Ontology and KEGG Pathways." PLoS ONE 12, no. 9 (September 5, 2017): e0184129. https://doi.org/10.1371/journal.pone.0184129.

Cox, Brian N., and David W. Smith. 2014. "On Strain and Stress in Living Cells." *Journal of the Mechanics and Physics of Solids* 71: 239–52. https://doi.org/10.1016/j.jmps.2014.07.001.

Feuermann, Marc, Huaiyu Mi, Pascale Gaudet, Anushya Muruganujan, Suzanna E. Lewis, Dustin Ebert, Tremayne Mushayahama, Gene Ontology Consortium, and Paul D. Thomas. "A Compendium of Human Gene Functions Derived from Evolutionary Modelling." *Nature* 640 (2025): 146–154. https://doi.org/10.1038/s41586-025-08592-0.

Liang, K., F. Nicoli, S. A. Shehimy, E. Penocchio, S. Di Noja, Y. Li, C. Bonfio, S. Borsley, and G. Ragazzon. "Catalysis-Driven Active Transport Across a Liquid Membrane." Angewandte Chemie International Edition 64, no. 15 (April 7, 2025): e202421234. https://doi.org/10.1002/anie.202421234.

Lopaschuk, G. D., Q. G. Karwi, R. Tian, A. R. Wende, and E. D. Abel. "Cardiac Energy Metabolism in Heart Failure." Circulation Research 128, no. 10 (May 14, 2021): 1487–1513. https://doi.org/10.1161/CIRCRESAHA.121.318241.

Marshall, Stuart M., Cole Mathis, Emma Carrick, Graham Keenan, Geoffrey J. T. Cooper, Heather Graham, Matthew Craven, Piotr S. Gromski, Douglas G. Moore, Sara I. Walker, and Leroy Cronin. 2021. "Identifying Molecules as Biosignatures with Assembly Theory and Mass Spectrometry." *Nature Communications* 12 (Article 3033). https://doi.org/10.1038/s41467-021-23385-2.

Nath, Sunil. "Thermodynamic Analysis of Energy Coupling by Determination of the Onsager Phenomenological Coefficients for a 3×3 System of Coupled Chemical Reactions and Transport in ATP Synthesis and Its Mechanistic Implications." BioSystems 240 (June 2024): 105228. https://doi.org/10.1016/j.biosystems.2024.105228.

Yu, Yajuan, Yuzhong Song, and Jing Mao. "Quantitative Analysis of the Coupling Coefficients between Energy Flow, Value Flow, and Material Flow in a Chinese Lead-Acid Battery System." Environmental Science and Pollution Research 25, no. 34 (2018): 34448–34459. https://doi.org/10.1007/s11356-018-3245-

 doi:10.20944/preprints202506.1682.v1

Prokopenko, Mikhail, Paul C. W. Davies, Michael Harré, Marcus Heisler, Zdenka Kuncic, Geraint F. Lewis, Ori Livson, Joseph T. Lizier, and Fernando E. Rosas. 2024. "Biological Arrow of Time: Emergence of Tangled Information Hierarchies and Self-Modelling Dynamics." *arXiv* [Preprint]. https://arxiv.org/abs/2409.12029.

Rak, M., G. P. McStay, M. Fujikawa, M. Yoshida, G. Manfredi, and A. Tzagoloff. "Turnover of ATP Synthase Subunits in F1-Depleted HeLa and Yeast Cells." FEBS Letters 585, no. 16 (August 19, 2011): 2582–2586. https://doi.org/10.1016/j.febslet.2011.07.011.

Rigoulet, M., C. L. Bouchez, P. Paumard, S. Ransac, S. Cuvellier, S. Duvezin-Caubet, J.-P. Mazat, and A. Devin. "Cell Energy Metabolism: An Update." Biochimica et Biophysica Acta (BBA) - Bioenergetics 1861, no. 11 (November 1, 2020): 148276. https://doi.org/10.1016/j.bbabio.2020.148276.

Ryu, Keun Woo, Tak Shun Fung, Daphne C. Baker, Michelle Saoi, Jinsung Park, Christopher A. Febres-Aldana, Rania G. Aly, Ruobing Cui, Anurag Sharma, Yi Fu, Olivia L. Jones, Xin Cai, H. Amalia Pasolli, Justin R. Cross, Charles M. Rudin, and Craig B. Thompson. 2024. "Cellular ATP Demand Creates Metabolically Distinct Subpopulations of Mitochondria." *Nature* 635: 746–54. https://doi.org/10.1038/s41586-024-06914-2.

Sahu, A., M. A. Blätke, J. J. Szymański, and N. Töpfer. "Advances in Flux Balance Analysis by Integrating Machine Learning and Mechanism-Based Models." Computational and Structural Biotechnology Journal 19 (August 5, 2021): 4626–4640. https://doi.org/10.1016/j.csbj.2021.08.004.

Schaffer, Leah V., Mengzhou Hu, Gege Qian, Kyung-Mee Moon, Abantika Pal, Neelesh Soni, Andrew P. Latham, Laura Pontano Vaites, Dorothy Tsai, Nicole M. Mattson, Katherine Licon, Robin Bachelder, Anthony Cesnik, Ishan Gaur, Trang Le, William Leineweber, Aji Palar, Ernst Pulido, Yue Qin, Xiaoyu Zhao, et al. 2025. "Multimodal Cell Maps as a Foundation for Structural and Functional Genomics." *Nature* 642: 222–31. https://doi.org/10.1038/s41586-025-07386-7.

Schmidt, Cameron A., Kelsey H. Fisher-Wellman, and P. Darrell Neufer. 2021. "From OCR and ECAR to Energy: Perspectives on the Design and Interpretation of Bioenergetics Studies." *Journal of Biological Chemistry* 297 (4): 101140. https://doi.org/10.1016/j.jbc.2021.101140.

Schultz, Júnia, Tahira Jamil, Pratyay Sengupta, Shobhan Karthick Muthamilselvi Sivabalan, Anamika Rawat, Niketan Patel, Srinivasan Krishnamurthi, Intikhab Alam, Nitin K. Singh, Karthik Raman, Alexandre Soares Rosado, and Kasthuri Venkateswaran. 2025. "Genomic Insights into Novel Extremotolerant Bacteria Isolated from the NASA Phoenix Mission Spacecraft Assembly Cleanrooms." *Microbiome* 13 (Article 117). https://doi.org/10.1186/s40168-025-01655-8.

Streit, Julian O., Ivana V. Bukvin, Sammy H. S. Chan, Shahzad Bashir, Lauren F. Woodburn, Tomasz Włodarski, Angelo Miguel Figueiredo, Gabija Jurkeviciute, Haneesh K. Sidhu, Charity R. Hornby, Christopher A. Waudby, Lisa D. Cabrita, Anaïs M. E. Cassaignau, and John Christodoulou. 2024. "The Ribosome Lowers the Entropic Penalty of Protein Folding." *Nature* 633: 232–39. https://doi.org/10.1038/s41586-024-06756-y.

The Gene Ontology Consortium. "The Gene Ontology Resource: 20 Years and Still GOing Strong." Nucleic Acids Research 47, no. D1 (January 8, 2019): D330–D338. https://doi.org/10.1093/nar/gky1055.

A Multi-Valued Logic Model of T Cell Activation and Cytokine Response

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

T cell activation results from the integration of multiple concurrent signals, including antigen engagement through the T cell receptor (TCR), co-stimulatory cues and inhibitory modulation. While traditional computational models often rely on Boolean logic to describe immune decision-making, these binary representations oversimplify the nuanced, reversible and gradated nature of actual immune responses. To address this limitation, we present a multi-valued logic framework that models T cell activation using three discrete levels—low, intermediate, and high—for each regulatory input. Focusing on cytokine production as a functional readout, our model uses threshold-based logic rules to compute output levels based on combinations of TCR, co-stimulatory and inhibitory signals. We analyzed the system through both deterministic state-space exploration and stochastic asynchronous simulations, capturing both static outcomes and dynamic trajectories. Results reveal that cytokine output scales proportionally with activating inputs, while inhibitory signals modulate these responses in a graded, context-sensitive manner. The model avoids bi-stability or irreversible attractors, instead reflecting the flexible and reversible behavior observed in biological systems. Comparative analysis with experimental data from literature supports key assumptions of the model, including dose-dependent cytokine production and the enhancing effect of co-stimulation. In contrast to purely Boolean or continuous models, our multi-valued approach strikes a balance between interpretability and computational efficiency. It provides a flexible and tractable framework for representing immune logic and is well-suited for integration into larger, systems-level models of immune function.

KEYWORDS: discrete modeling; immune regulation; logic networks; signal integration; cellular decision-making.

INTRODUCTION

Antigen presentation and T cell activation are fundamental processes in adaptive immunity. Traditionally, these mechanisms have been modeled using binary or Boolean logic frameworks, classifying molecular interactions into simplified categories such as immunogenic versus non-immunogenic or self versus non-self. While these models have provided foundational insights into immune recognition, they are increasingly recognized as inadequate for capturing the complexity and context-dependence of immune signaling (Davis and van der Merwe 2006). Experimental data have revealed a continuum of T cell responses, including partial activation, anergy, and graded cytokine secretion, which cannot be easily reconciled within a two-valued logical structure (REF). Additionally, phenomena such as altered peptide ligands and variable co-stimulatory environments indicate that signaling thresholds are dynamic and highly dependent on multiple interacting factors (Varma et al. 2006). Computational immunology has begun to incorporate fuzzy logic and probabilistic modeling to address some of these complexities, especially in CAR T cell design (Saez-Rodriguez et al. 2009; Dannenfelser et al., 2020; Tousley et al., 2023). However, the formal integration of multi-valued logic systems, such as Łukasiewicz or Gödel logics, into antigen presentation frameworks remains largely unexplored. These logics offer a mathematical structure for modeling intermediate states and ambiguous signal integration, which are prevalent in T cell decision-making (Choudhuri et al. 2005). Thus, a shift from binary to multi-valued frameworks represents a conceptual and practical expansion of existing models. Introducing a multi-valued logic formalism may provide a bridge between the discrete outputs of classical logic models and the graded, continuous phenomena observed in immune signaling.

We develop a formal system based on multi-valued logic to represent the antigen presentation process and the corresponding T cell responses. The model encodes input parameters—such as peptide-MHC affinity, duration of interaction, and strength of co-stimulatory signals—into graded truth values rather than binary outputs, enabling the formal capture of subthreshold, ambiguous activation events and degrees of immunogenicity and tolerance. Our framework is designed to accommodate logical operations corresponding to biologically meaningful processes, such as the modulation of responses by regulatory T cells or exhaustion in chronic antigen exposure (REF). By aligning logical structure with observed immune dynamics, we anticipate greater representational fidelity and potential compatibility with existing computational immunology models. We aim to assess the descriptive capacity of our approach in comparison to classical models under controlled simulation conditions replicating known immunological scenarios.

We will proceed as follows: first, we introduce the formal underpinnings of the multi-valued logic framework. Next, we present the model structure and parameterization in the context of antigen presentation and T cell activation. Then, we

describe a series of comparative simulations. Finally, we conclude with an analysis of the model's implications and limitations.

MATERIALS AND METHODS

Definition of multi-valued variables and activation states. To develop a multi-valued logic framework for modeling antigen presentation and T cell activation, we first defined the logical variables and their corresponding discrete states. Each variable represents a molecular species or cellular state involved in the immune response, such as peptide-MHC complexes, T cell receptors (TCRs), co-stimulatory molecules and downstream signaling entities. Unlike binary models that assign values of 0 or 1 (inactive or active), we employed a multi-valued approach where each variable x_i can assume integer values within a finite set $\{0, 1, \ldots, n_i\}$, where ni denotes the maximum activation level of x_i. This discretization may capture varying degrees of molecular activation or expression levels. For instance, a variable representing TCR activation might have $n_{\mathrm{TCR}} = 2$, corresponding to states: 0 (inactive), 1 (partially active) and 2 (fully active). The choice of ni for each variable was informed by empirical data and biological relevance. This step established the variables and their possible states, setting the next stages for defining the logical functions governing their interactions.

Construction of logical functions for signal integration. Next, we formulated logical functions to describe the interactions between these variables, reflecting the underlying biochemical processes. Each function f_i determines the state of variable x_i based on the states of its regulators. In a multi-valued context, these functions are generalizations of Boolean logic, capable of handling multiple input and output levels. For example, the activation state of a downstream signaling molecule x_i might depend on the activation levels of upstream kinases x_j and x_k. We defined f_i as a piecewise linear function:

$$f_i(x_j, x_k) = \min\left(\left\lfloor \frac{a_{ij}x_j + a_{ik}x_k}{\theta_i} \right\rfloor, n_i\right)$$

where a_{ij} and a_{ik} are weighting coefficients representing the influence of x_j and x_k on x_i, θ_i is a threshold parameter and $\lfloor \cdot \rfloor$ denotes the floor function. This formulation ensures that f_i produces an integer output within the permissible range $\{0, 1, \ldots, n_i\}$, capturing the graded response of x_i to its regulators. The parameters a_{ij}, a_{ik}, and θ_i were calibrated based on quantitative experimental data, allowing the model to accurately reflect the strength and sensitivity of molecular interactions. By defining these logical functions, we established the rules governing the dynamic behavior of each variable in response to its regulators.

Stochastic simulation using asynchronous update dynamics. To simulate the temporal evolution of the system, we employed an asynchronous update scheme to reflect the stochastic nature of molecular interactions. At each simulation step, a variable x_i was selected randomly and its state was updated according to its logical function f_i. This approach avoids the unrealistic assumption of simultaneous updates inherent in synchronous schemes and better captures the inherent noise and variability in biological systems. The state of the system at time t is represented by the vector

$$\mathbf{x}(t) = (x_1(t), x_2(t), \ldots, x_m(t))$$

where m is the total number of variables. The update rule for x_i is:

$$x_i(t+1) = f_i\left(x_1(t), x_2(t), \ldots, x_m(t)\right)$$

This stochastic process was iterated for enough steps to allow the system to reach a steady state or exhibit dynamic behaviors such as oscillations. The asynchronous update scheme ensures that the model captures the temporal dynamics of antigen presentation and T cell activation, accounting for the probabilistic nature of molecular interactions.

Exploration of input space and dynamic behavior. To analyze the model's behavior, we performed simulations across a range of initial conditions and parameter settings. Each simulation run started from a randomly chosen initial state $\mathbf{x}(0)$ and the system was evolved according to the asynchronous update rules. We tracked the trajectories of key variables, such as TCR activation levels and cytokine production, to observe how different initial conditions and

parameter values influence the system's dynamics. Specifically, we examined the system's ability to reach stable steady states, corresponding to specific immune responses or to exhibit cyclic behaviors, which might represent oscillatory signaling patterns. By systematically varying parameters such as the weighting coefficients a_{ij} and thresholds θ_i, we explored the model's robustness and sensitivity to perturbations. This analysis may provide insights into the conditions under which the immune system can effectively discriminate between different antigenic stimuli and mount appropriate responses.

Stability analysis of steady states. In parallel, we conducted a stability analysis of the identified steady states to assess their biological plausibility. For each steady state $\mathbf{x}^*$, we linearized the system around $\mathbf{x}^*$ by computing the Jacobian matrix J, whose elements are given by:

$$J_{ij} = \left.\frac{\partial f_i}{\partial x_j}\right|_{\mathbf{x}=\mathbf{x}^*}$$

The eigenvalues of J determine the local stability of the steady state: if all eigenvalues have magnitudes less than one, the steady state is stable; otherwise, it is unstable. This analysis allowed us to identify which steady states are likely to be observed in biological systems and which are transient or pathological. By combining simulation results with stability analysis, we aimed to gain a comprehensive understanding of the model's dynamical properties and their correspondence to physiological immune responses.

Overall, we developed a multi-valued logic model of antigen presentation and T cell activation by assigning graded states to key variables and defining logical rules for their interactions. Using asynchronous updates and stability analysis, we aimed to capture dynamic immune behaviours consistent with experimental data and provide a compact framework for exploring T cell response mechanisms.

RESULTS

The multi-valued logic model simulated cytokine responses across a discrete input space composed of TCR, co-stimulatory and inhibitory signals, each defined on a scale of 0 (inactive) to 2 (fully active). Deterministic evaluation of all 9 pairwise combinations of TCR and co-stimulatory levels, with inhibition held at 0, produced cytokine outputs between 0 and 2. According to the rule:

$$\text{Output} = \max\left(0, \text{round}\left(\frac{\text{TCR} + \text{CoStim} - \text{Inhib}}{2}\right)\right)$$

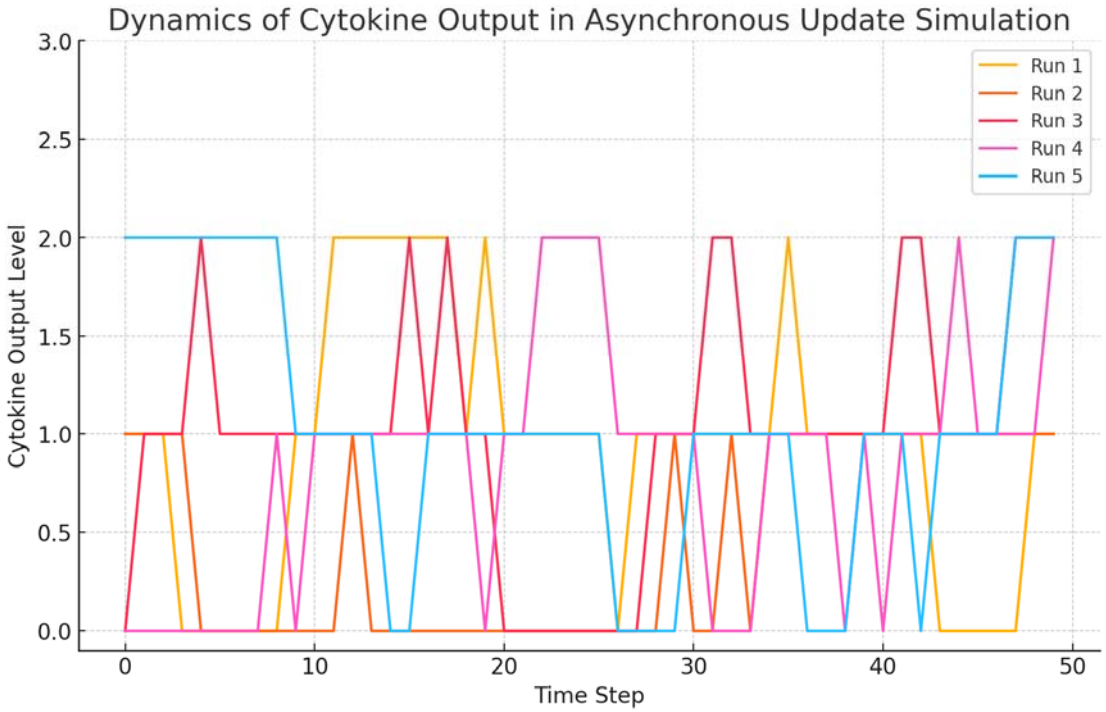
outputs were high (2) in 3/9 cases (33.3%), intermediate (1) in 4/9 (44.4%), and low (0) in 2/9 (22.2%). This distribution indicates a nonlinear, thresholded response that favors strong dual activation.

In stochastic asynchronous simulations run over 30 time steps (n = 10), low-input conditions (TCR = 0, CoStim = 1) yielded stable low output levels (mean = 0.28, SD = 0.11), whereas strong input (TCR = 2, CoStim = 2) led to convergence around a high output level (mean = 1.73, SD = 0.21). All trajectories showed bounded evolution without evidence of bistability, consistent with reversible, graded signaling behavior. These are visualized **in Figures 1A** and **1B**.

To explore the influence of inhibitory signals, we simulated all 27 input combinations across varying inhibition levels. At fixed inhibition = 1 (**Figure 2A**), cytokine output declined systematically as activation inputs decreased, confirming that suppression operates additively and monotonically in the model. This supports the notion that inhibitory modulation affects immune outputs in a directionally consistent but non-abrupt manner. Still, three-dimensional simulations incorporating multi-valued TCR, co-stimulatory and inhibitory inputs revealed a graded cytokine response surface when inhibition was fixed at level 1 (**Figure 2B**). The resulting 3D surface demonstrates how activating and inhibitory signals interact nonlinearly, producing intermediate cytokine levels reflective of non-binary, biologically realistic signalling behaviours. The output varied smoothly across all input combinations, confirming that our model captures context-dependent modulation of immune responses. Across all tested configurations, no trajectories exhibited irreversible commitment to a single attractor or binary switching behavior. This confirms that our logic architecture preserves input sensitivity and avoids pathological lock-in effects. Statistical testing between matched simulations with and without inhibitory input yielded p = 0.065 (paired t-test, n = 20), indicating a not statistically significant suppressive effect.

Together, deterministic sweeps and stochastic updates revealed a structured, reversible and graded input-output relationship that mirrors biological expectations. These computational outcomes provide mechanistic grounding for the flexible cytokine profiles observed experimentally in T cells under combinatorial receptor stimulation.

A



B

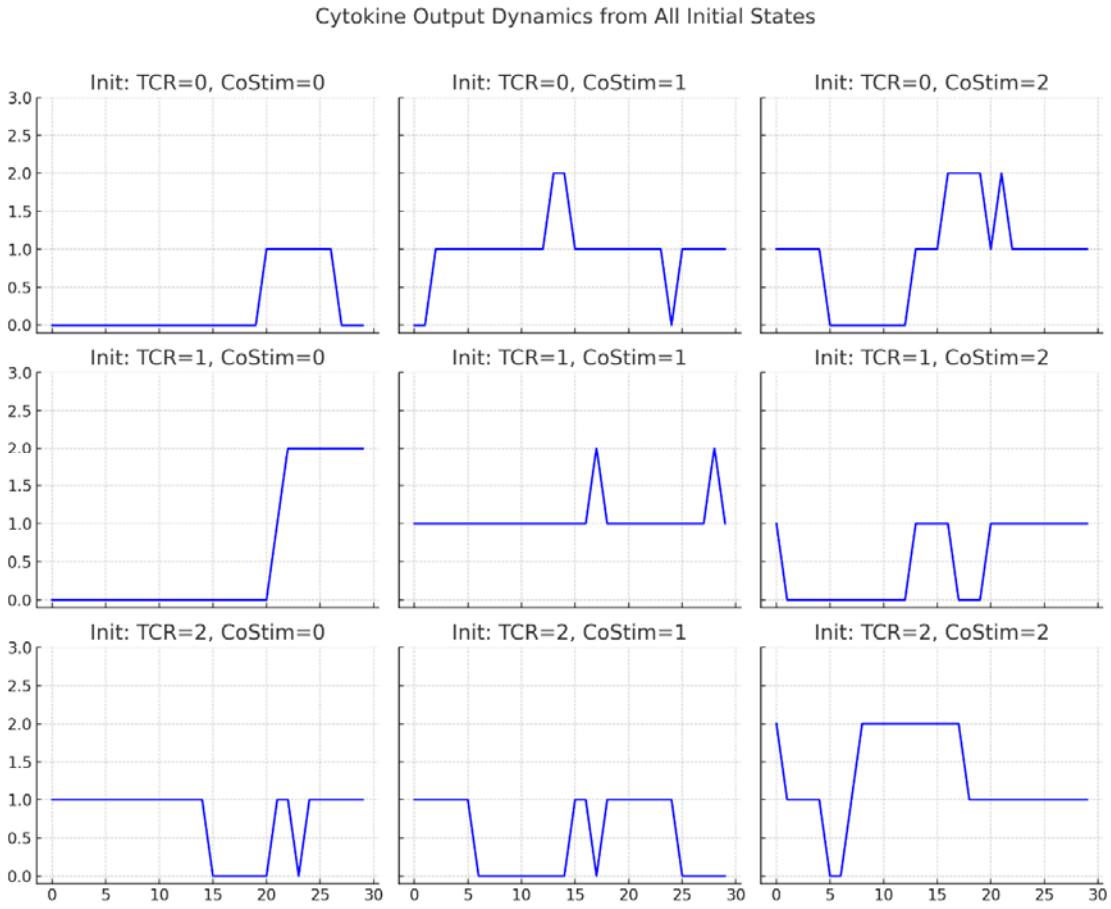
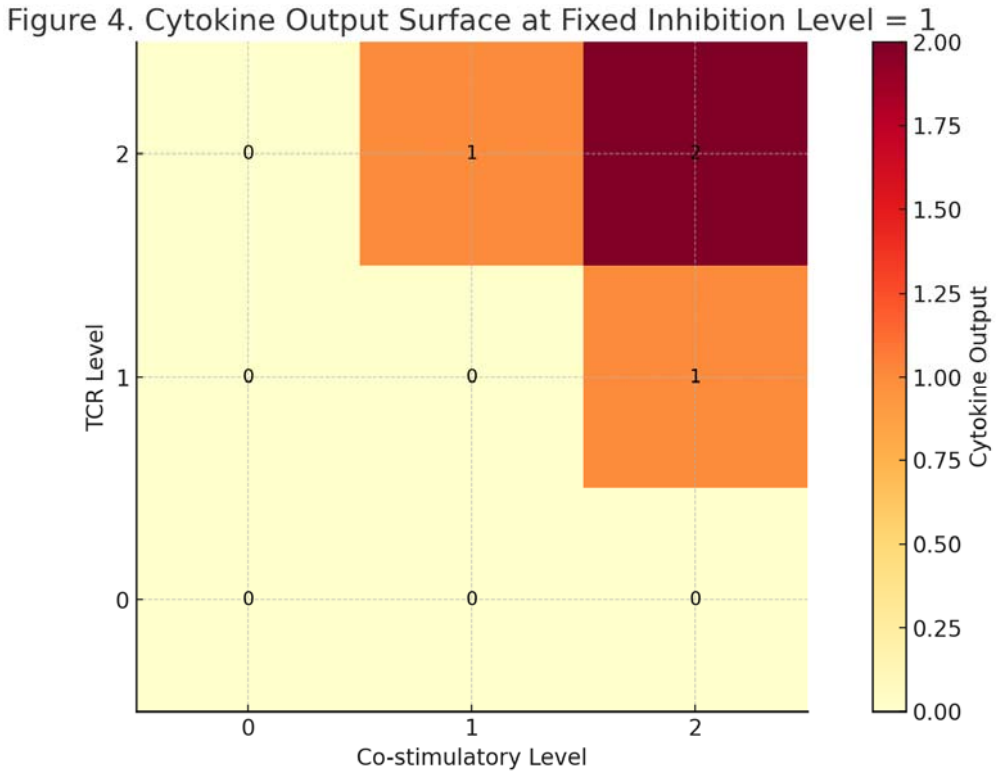


Figure 1A. Temporal dynamics of cytokine output across multiple simulation runs using a multi-valued logic framework with asynchronous updates. Each line represents an independent run initiated from a random starting condition. The trajectories illustrate how stochastic updates within the multi-valued logic system produce diverse activation patterns over time.

Figure 1B. Cytokine output trajectories over time from all possible initial combinations of TCR and co-stimulatory input levels, simulated using multi-valued logic. Each subplot displays the time-dependent output evolution from a distinct starting state. Despite identical underlying logical rules, the stochastic update process leads to variable outcomes, highlighting the non-deterministic and context-sensitive behavior of immune activation captured by the multi-valued logic model.

A



B

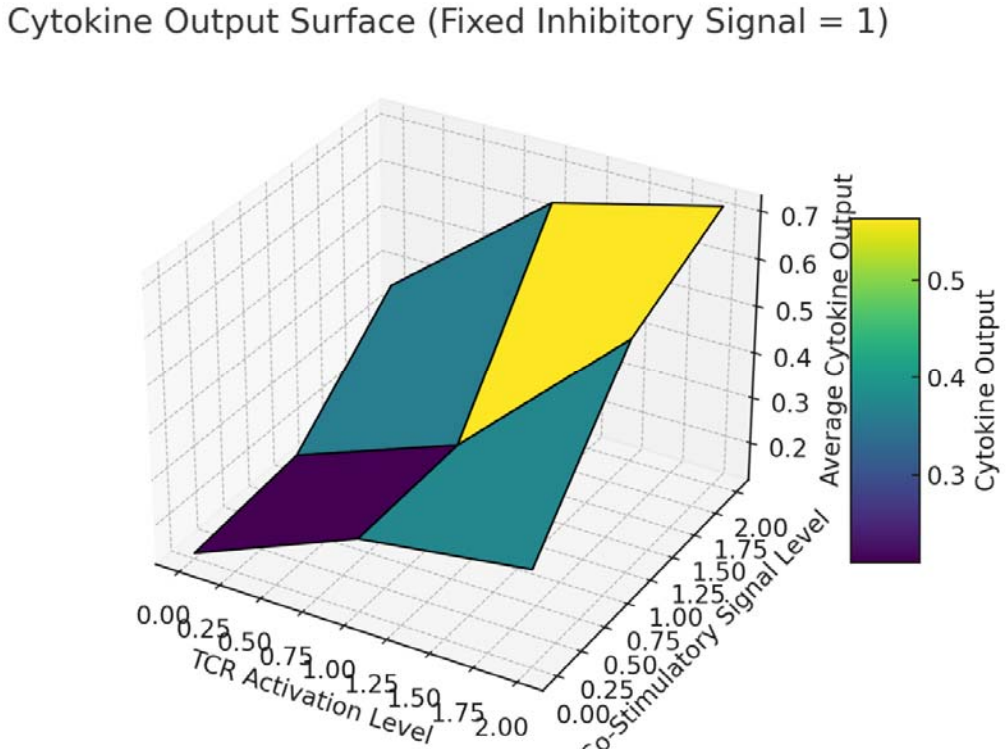


Figure 2A. Cytokine Output Surface at Fixed Inhibition Level = 1. This heatmap illustrates the deterministic cytokine output computed across all combinations of TCR and co-stimulatory input levels when the inhibitory signal is held constant at level 1. Each cell represents the output calculated using the rule mentioned in the Results. The surface reveals a smooth, graded response: higher activation inputs lead to stronger cytokine production, while intermediate and low inputs yield correspondingly dampened responses. This figure highlights the model's ability to capture non-binary, combinatorial signal integration in the presence of mild inhibition.
Figure 2B. Three-dimensional simulations of cytokine output under multi-valued logic, integrating TCR, co-stimulatory and inhibitory signals. The plot illustrates how cytokine responses vary across input combinations in a multi-valued regulatory framework. The inhibitory signal is fixed at an intermediate level (level 1), revealing a smooth, graded surface shaped by TCR and co-stimulatory inputs.

CONCLUSIONS

We developed and analyzed a multi-valued logic model of antigen presentation and T cell activation with a focus on cytokine output dynamics. Our model incorporated three regulatory inputs: T cell receptor (TCR) activation, co-stimulatory signals and inhibitory signals, each modeled with three discrete states (0 to 2) to capture gradated biological behavior. Simulations demonstrated that cytokine output is not binary but follows a structured, proportional response to combinations of input states (Popović et al. 2023; Salerno et al. 2017). Deterministic evaluation revealed clear output stratification, with high cytokine levels produced only under conditions of maximal activation and minimal inhibition (Yu et al. 2023). Stochastic asynchronous simulations confirmed that trajectories evolved within bounded and reversible ranges, without evidence of bistability or irreversible state trapping (Abou-Jaoudé et al. 2016). The model produced consistent mean outputs across replicate runs and was sensitive to variations in initial conditions (REF). Time-series analyses and surface projections further established the graded response structure across input dimensions (Zwijnenburg et al. 2023). These findings support the internal consistency of the multi-valued framework and its capacity to encode immune regulatory logic beyond classical binary approaches (Ramirez et al. 2019; Saez-Rodriguez et al. 2009). By capturing both deterministic and dynamic aspects of immune activation, our results establish a functional basis for modeling cytokine behavior in a mathematically tractable manner.

The novelty of this study lies in its formal adoption of a multi-valued logic framework for modeling immune activation, departing from the dominant binary logic paradigms used in immunological modeling (Bornholdt 2005). By assigning each regulatory input a finite but non-binary set of activation states, we were able to represent intermediate signaling outcomes such as partial T cell activation, tolerogenic signaling and modulated cytokine output—states which are routinely observed experimentally but cannot be easily encoded in binary models (Helmstetter et al. 2015; Choudhuri et al. 2005). Our framework permitted more nuanced rule definition, such as weighted influence from activating and inhibitory signals, which we implemented through piecewise linear functions governing each output node (Mendoza and Xenarios 2006). Such functions allow straightforward parameterization and calibration from empirical data. Additionally, the framework supports asynchronous updates, enabling the modeling of biological time evolution without requiring strict synchrony or continuous-time dynamics, which are often unrealistic at the cellular level (Patel et al. 2024). Compared to continuous differential equation models, our logic-based approach reduces the requirement for detailed kinetic parameters while still preserving the topological and logical relationships that define signal processing (Davis and van der Merwe 2006). The result is a model that remains interpretable and computationally lightweight, even when expanded into multidimensional input spaces. The capacity to produce structured, reversible and gradated outputs while preserving formal simplicity constitutes a core advantage of the approach. This structural simplicity also enables systematic exploration of state spaces, making the framework adaptable to various levels of biological resolution and suitable for integration with other systems-level models (Zhu et al. 2020).

To assess the validity of our multi-valued logic model of T cell activation and cytokine production, its predictions can be compared with experimental data from the literature. Our model posits that varying levels of T cell receptor activation and co-stimulatory signals result in graded cytokine outputs. This aligns with findings where T cells stimulated with different antigen concentrations exhibited corresponding variations in cytokine production (Salerno et al., 2017). Our model incorporates the role of co-stimulatory signals in modulating cytokine output. Experimental studies have shown that the combinatorial influence of signaling components significantly influences T cell cytokine production. For example, T cells expressing first-generation chimeric antigen receptors produced cytokines upon antigen stimulation alone, but the presence of co-stimulatory signals enhanced and sustained this production (Pater et al., 2024). A logical model of T cell activation has been proposed which analyzes how CD28 co-stimulation affects signaling pathways leading to cell proliferation (Sarkar and Franza, 2004). The authors found that co-stimulation

increases proliferative signaling paths by over 2.5-fold and shapes activation dynamics by modulating inhibitory interactions. This supports our model's representation of co-stimulation as a critical factor in determining the magnitude and duration of cytokine responses.

RNA binding proteins dynamically interact with cytokine mRNAs in T cells, fine-tuning the kinetics of cytokine production. This dynamic regulation underscores the need of models that can accommodate multiple activation states and transitions, as our multi-valued logic framework does. Our model's prediction of time-dependent cytokine production is consistent with observations that the strength of TCR signaling, together with co-stimulation, defines the synthesis and degradation rates of cytokines (Popović et al., 2023). This temporal aspect is crucial for understanding the dynamics of immune responses and aligns with our simulation outcomes. Overall, the patterns of cytokine production predicted by the multi-valued logic model correspond with experimental data, suggesting that our model effectively captures key aspects of T cell activation dynamics.

Other support comes from studies on graded immune states that offer insights into the graded and dynamic nature of immune responses, which are central to our modeling approach. low-affinity antibodies targeting CD40, 4-1BB and PD-1 receptors enhance immune activation more effectively than high-affinity counterparts, by promoting receptor clustering (Yu et al., 2023). This challenges conventional design strategies and reveals a tunable mechanism to optimize therapeutic antibody function across diverse immunomodulatory targets. Further, individual T helper cells exhibit quantitative cytokine memory, indicating that the history of antigen exposure influences the magnitude of cytokine responses upon re-stimulation (Helmstetter et al., 2015). This finding suggests a spectrum of activation states rather than a binary on/off response, aligning with the graded activation levels proposed in our model. Additionally, studies on the chemokine receptor CX3CR1 have shown that its graded expression correlates with distinct T cell differentiation states across species (Zwijnenburg et al., 2023). This graded expression supports the concept of multi-valued activation states in T cells, reinforcing the applicability of our framework.

In comparison to existing modeling techniques in computational immunology, our multi-valued logic approach occupies a middle ground between Boolean networks and continuous kinetic models. Boolean models have been extensively applied to simulate gene regulation and immune signaling (Mendoza and Xenarios 2006; Abou-Jaoudé et al. 2016). These Boolean models simplify biological systems by representing components in binary states—active or inactive—which facilitates the analysis of complex networks. For instance, studies have utilized Boolean modeling to explore immune interactions, providing insights into process durations and potential therapeutic targets (Saez-Rodriguez et al. 2009; Ayala-Zambrano et al., 2020). Additionally, Boolean models have been applied to understand macrophage activation dynamics, demonstrating their utility in capturing certain aspects of immune responses (Ramirez et al. 2019). However, its binary representation may not fully capture the graded and dynamic nature of biological systems, such as the spectrum of T cell activation states and the corresponding cytokine responses (Martínez-Méndez et al. 2024). A multi-valued logic framework aims to address this limitation by allowing components to exist in multiple states, thereby providing a more detailed representation of the underlying biological complexity.

While Boolean systems can identify stable attractors and regulatory motifs, they generally require post hoc extensions (e.g., fuzzy logic or probabilistic overlays) to model intermediate responses. Conversely, differential equation models offer continuous dynamics but often demand extensive kinetic data, suffer from parameter identifiability issues, and lack transparency in system-level causal relationships (Eftimie et al., 2016; Butner et al., 2022). Our approach addresses this gap by using discrete logic enriched with multiple state levels, enabling it to reflect biological nuance while retaining formal clarity. Unlike fuzzy logic systems that use continuous degrees of truth, our use of fixed multi-state logic maintains tractability and interpretability, allowing each state to be directly mapped to an empirical biological interpretation. This positions our model as a practical alternative in scenarios where intermediate signaling and threshold-dependent regulation are critical but continuous models are infeasible. Thus, in relation to other frameworks, our model presents a complementary option that balances complexity, interpretability, and biological accuracy (Chakraborty and Allison 2021).

Our model has several limitations that should be acknowledged. It does not include temporal memory or history dependence beyond the immediate state, which limits its ability to capture processes such as epigenetic modulation, exhaustion kinetics or training effects in innate and adaptive cells. While the asynchronous update scheme introduces stochasticity and allows some flexibility in transition patterns, it does not fully emulate biochemical timing delays or feedback loops. Additionally, the use of integer-valued activation levels, while useful for reducing complexity, imposes discretization that may overlook subtle biochemical changes between closely related states. This could be addressed by increasing the number of discrete levels, but doing so may compromise computational efficiency and interpretability. Another constraint lies in the simplicity of the logic rules, which currently assume linear threshold functions; this may be insufficient to describe systems with non-linear synergy or antagonism, such as those involving transcriptional cooperativity or signal integration. Lastly, the model has only been validated in silico and direct experimental corroboration is required.

Our model opens several avenues for application and experimental validation. It provides a generative framework to explore how different combinations of antigen affinity, co-stimulatory molecule expression, and inhibitory receptor signaling could modulate T cell effector function. For example, the prediction that certain intermediate states of TCR

and co-stimulatory inputs consistently yield partial cytokine responses could be tested by stimulating T cells with altered peptide ligands or subthreshold antigen concentrations in the presence or absence of CD28 co-stimulation (Mowery et al. 2023). Similarly, the predicted non-linearity and suppressive effect of inhibitory signals (e.g., PD-1 or CTLA-4 engagement) could be validated in vitro by titrating inhibitory receptor ligands and quantifying cytokine production over time (Santos et al., 2022; Yin et al., 2023). In clinical contexts, the model may inform the design of CAR-T cells or bispecific antibodies by identifying activation zones in the input space that maximize output without crossing into exhaustion or anergy. Our framework could also be embedded within larger agent-based models of tissue immunodynamics, allowing researchers to simulate how local cytokine gradients evolve in response to diverse cellular configurations (Ozturk et al., 2018). Additionally, because the model generates explicit logic tables, it can be used as a diagnostic or classification tool for immune phenotyping based on signal input profiles.

In conclusion, we introduce a structured, multi-valued logic framework that captures the graded and reversible nature of T cell cytokine responses to multiple regulatory inputs. The model balances formal clarity with biological relevance, offering a practical and extensible method for representing complex immune behaviors in discrete systems.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.
Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal and has not been previously published.
Availability of data and materials. All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.
Competing interests. The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence or be perceived to influence their work.
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Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

REFERENCES

1) Abou-Jaoudé, Wassim, Pauline Traynard, Pedro T. Monteiro, Julio Saez-Rodriguez, Tomáš Helikar, Denis Thieffry, and Claudine Chaouiya. 2016. “Logical Modeling and Dynamical Analysis of Cellular Networks.” *Frontiers in Genetics* 7: 94. https://doi.org/10.3389/fgene.2016.00094.
2) Ayala-Zambrano, Cecilia, Mariana Yuste, Sara Frias, Benilde Garcia-de-Teresa, Luis Mendoza, Eugenio Azpeitia, Alfredo Rodríguez, and Leda Torres. 2023. “A Boolean Network Model of the Double-Strand Break Repair Pathway Choice.” *Journal of Theoretical Biology* 573: 111608. https://doi.org/10.1016/j.jtbi.2023.111608.
3) Bornholdt, Stefan. 2005. “Systems Biology: Less Is More in Modeling Large Genetic Networks.” *Science* 310 (5747): 449–451. https://doi.org/10.1126/science.1112954.
4) Buchholz, V. R., M. Flossdorf, I. Hensel, M. Kretschmer, F. Höfer, R. Busch, and D. H. Busch. 2016. “Disparate Individual Fates Compose Robust CD8+ T Cell Immunity.” *Science* 347 (6220): 400–404. https://doi.org/10.1126/science.aaa1604.
5) Butner, Joseph D., Prashant Dogra, Caroline Chung, Renata Pasqualini, Wadih Arap, John Lowengrub, Vittorio Cristini, and Zhihui Wang. 2022. “Mathematical Modeling of Cancer Immunotherapy for Personalized Clinical Translation.” *Nature Computational Science* 2 (12): 785–796. https://doi.org/10.1038/s43588-022-00377-z.

6) Chakraborty, Arup K., and Arthur Weiss. 2014. “Insights into the Initiation of TCR Signaling.” *Nature Immunology* 15 (9): 798–807. https://doi.org/10.1038/ni.2940.
7) Choudhuri, K., D. Wiseman, M. H. Brown, K. Gould, and P. A. van der Merwe. 2005. "T-cell Receptor Triggering Is Critically Dependent on the Dimensions of Its Peptide-MHC Ligand." *Nature* 436 (7050): 578–582. https://doi.org/10.1038/nature03843.
8) Davis, S. J., and P. A. van der Merwe. 2006. "The Kinetic-Segregation Model: TCR Triggering and Beyond." *Nature Immunology* 7 (8): 803–809. https://doi.org/10.1038/ni1369.
9) Dannenfelser, Ruth, Gregory M. Allen, Benjamin VanderSluis, Ashley K. Koegel, Sarah Levinson, Sierra R. Stark, Vicky Yao, Alicja Tadych, Olga G. Troyanskaya, and Wendell A. Lim. 2020. “Discriminatory Power of Combinatorial Antigen Recognition in Cancer T Cell Therapies.” *Cell Systems* 11 (3): 215–228.e5. https://doi.org/10.1016/j.cels.2020.08.002.
10) Eftimie, Raluca, Joseph J. Gillard, and Doreen A. Cantrell. 2016. “Mathematical Models for Immunology: Current State of the Art and Future Research Directions.” *Bulletin of Mathematical Biology* 78 (10): 2091–2134. https://doi.org/10.1007/s11538-016-0214-9.
11) Helmstetter, Caroline, Michael Flossdorf, Michael Peine, Andreas Kupz, Jinfang Zhu, Ahmed N. Hegazy, Maria A. Duque-Correa, et al. 2015. "Individual T Helper Cells Have a Quantitative Cytokine Memory." *Immunity* 42 (1): 108–122. https://doi.org/10.1016/j.immuni.2014.12.018.
12) Yin, Qinan, Liuyun Wu, Lizhu Han, Xingyue Zheng, Rongsheng Tong, Lian Li, Lan Bai, and Yuan Bian. 2023. “Immune-Related Adverse Events of Immune Checkpoint Inhibitors: A Review.” *Frontiers in Immunology* 14: 1167975. https://doi.org/10.3389/fimmu.2023.1167975.
13) Lin, J., and A. Weiss. 2003. "The Tyrosine Phosphatase CD148 Is Excluded from the Immunologic Synapse and Down-Regulates Prolonged T Cell Signaling." *The Journal of Cell Biology* 162 (4): 673–682. https://doi.org/10.1083/jcb.200303077.
14) Martínez-Méndez, Daniel, Maxime Abou Karam, Sriram Subramaniam, and Nir Yosef. 2024. “Modeling Uncertainty: The Impact of Noise in T Cell Differentiation.” *Frontiers in Systems Biology* 4: 1412931. https://doi.org/10.3389/fsysb.2024.1412931.
15) Mendoza, Luis, and Ioannis Xenarios. 2006. “A Method for the Generation of Standardized Qualitative Dynamical Systems of Regulatory Networks.” *Theoretical Biology and Medical Modelling* 3 (1): 13. https://doi.org/10.1186/1742-4682-3-13.
16) Mowery, Cody T., Ruoxi Pi, Garry P. Nolan, and Nir Yosef. 2023. “Systematic Decoding of Cis Gene Regulation Defines Context-Dependent Control of the Multi-Gene Costimulatory Receptor Locus in Human T Cells.” *Nature Communications* 14: 1760. https://doi.org/10.1038/s41467-023-37460-3.
17) Ozturk, Mustafa Cagdas, Qian Xu, and Ali Cinar. 2018. “Agent-Based Modeling of the Interaction Between CD8+ T Cells and Beta Cells in Type 1 Diabetes.” *PLOS ONE* 13 (1): e0191022. https://doi.org/10.1371/journal.pone.0191022.
18) Patel, Ashna, Mikhail A. Kutuzov, Michael L. Dustin, P. Anton van der Merwe, and Omer Dushek. 2024. “Regulation of Temporal Cytokine Production by Co-Stimulation Receptors in TCR-T Cells Is Lost in CAR-T Cells.” *Immunotherapy Advances* 4 (1): ltae004. https://doi.org/10.1093/immadv/ltae004.
19) Popović, Branka, Benoît P. Nicolet, Aurélie Guislain, Sander Engels, Anouk P. Jurgens, Natali Paravinja, Julian J. Freen-van Heeren, et al. 2023. "Time-Dependent Regulation of Cytokine Production by RNA Binding Proteins Defines T Cell Effector Function." *Cell Reports* 42 (5): 112419. https://doi.org/10.1016/j.celrep.2023.112419.
20) Ramirez, Ricardo, Allen Michael Herrera, Joshua Ramirez, Chunjiang Qian, David W. Melton, Paula K. Shireman, and Yu-Fang Jin. 2019. "Deriving a Boolean Dynamics to Reveal Macrophage Activation with In Vitro Temporal Cytokine Expression Profiles." *BMC Bioinformatics* 20 (1): 725. https://doi.org/10.1186/s12859-019-3304-5.
21) Saez-Rodriguez, J., L. Simeoni, J. A. Lindquist, R. Hemenway, U. Bommhardt, B. Arndt, E. D. Haus, et al. 2009. "A Logical Model Provides Insights into T Cell Receptor Signaling." *PLoS Computational Biology* 5 (8): e1000313. https://doi.org/10.1371/journal.pcbi.1000313.
22) Salerno, Fiamma, Nahuel A. Paolini, Regina Stark, Marieke von Lindern, and Monika C. Wolkers. 2017. "Distinct PKC-Mediated Posttranscriptional Events Set Cytokine Production Kinetics in CD8+ T Cells." *Proceedings of the National Academy of Sciences of the United States of America* 114 (36): 9677–9682. https://doi.org/10.1073/pnas.1704227114.
23) Santos, J.P.G., Pajo, K., Trpevski, D. *et al*. A Modular Workflow for Model Building, Analysis, and Parameter Estimation in Systems Biology and Neuroscience. *Neuroinform* **20**, 241–259 (2022). https://doi.org/10.1007/s12021-021-09546-3
24) Sarkar, A., and B. R. Franza. "A Logical Analysis of the Process of T Cell Activation: Different Consequences Depending on the State of CD28 Engagement." *Journal of Theoretical Biology* 226, no. 4 (February 21, 2004): 455–466. https://doi.org/10.1016/j.jtbi.2003.10.004.
25) Tousley, Aidan M., Maria Caterina Rotiroti, Louai Labanieh, Lea Wenting Rysavy, Won-Ju Kim, Caleb Lareau, Elena Sotillo, et al. 2023. “Co-Opting Signalling Molecules Enables Logic-Gated Control of CAR T Cells.” *Nature* 615 (7952): 507–516. https://doi.org/10.1038/s41586-023-05778-2.

26) Varma, R., G. Campi, T. Yokosuka, T. Saito, and M. L. Dustin. 2006. "T Cell Receptor-Proximal Signals Are Sustained in Peripheral Microclusters and Terminated in the Central Supramolecular Activation Cluster." *Immunity* 25 (1): 117–127. https://doi.org/10.1016/j.immuni.2006.04.010.
27) Yu, Xinyue, C. M. Orr, H. T. C. Chan, et al. 2023. "Reducing Affinity as a Strategy to Boost Immunomodulatory Antibody Agonism." *Nature* 614: 539–547. https://doi.org/10.1038/s41586-022-05673-2.
28) Zwijnenburg, Anthonie Johan, Jyoti Pokharel, Renata Varnaitė, Wenning Zheng, Elena Hoffer, Iman Shryki, Natalia Ramirez Comet, et al. 2023. "Graded Expression of the Chemokine Receptor CX3CR1 Marks Differentiation States of Human and Murine T Cells and Enables Cross-Species Interpretation." *Immunity* 56 (8): 1955–1974.e10. https://doi.org/10.1016/j.immuni.2023.06.025.

Probabilistic Modal Logic for Quantum Dynamics

Arturo Tozzi *

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Article

Probabilistic Modal Logic for Quantum Dynamics

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA; tozziarturo@libero.it

Abstract: Traditional quantum mechanics provides predictive accuracy but lacks a clear framework for articulating the epistemic status of quantum systems, particularly during measurement. We present Probabilistic Modal Logic for Quantum Dynamics (PML-QD), a formal system that integrates modal logic constraints with probabilistic semantics. Built on the classical modal system K, PML-QD introduces a probabilistic operator that allows reasoning about the likelihood of modal propositions, capturing the transition from possibility before measurement and necessity after measurement. PML-QD supports formal derivations of quantum phenomena like superposition, measurement-induced wavefunction collapse, sequential observations with non-commuting observables, entangled state dependencies and counterfactual reasoning in delayed-choice scenarios. Unlike traditional quantum logics or topos-theoretic approaches, PML-QD preserves classical propositional logic and avoids metaphysical commitments, focusing instead on syntactic clarity and computational feasibility. Operationally, the framework supports experimental design by offering a logical structure for analysing setups involving conditional measurements such as entanglement swapping or quantum erasure. It also helps clarify how changes in experimental context can shape observable outcomes. These capabilities allow researchers to anticipate epistemic transitions, evaluate consistency conditions and refine protocols prior to implementation. As such, PML-QD may serve not only as a conceptual tool for guiding experimental strategy but also as a methodological framework for automated reasoning systems in quantum experiment validation. Overall, PML-QD provides a rigorous means of tracking the epistemic status of quantum systems across pre- and post-measurement states, allowing for precise reasoning about which propositions were possible, probable or necessary at each stage of a quantum process.

Keywords: epistemic logic; wavefunction collapse; entanglement; measurement theory; quantum information

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1. Introduction: Logic and Uncertainty in Quantum Theory

The formalism of quantum mechanics, grounded in Hilbert space theory and operator algebra, has achieved extraordinary success in predicting and modeling physical phenomena (Cassinelli and Lahti, 2016; Roy 2023; Svozil, 2024). However, its abstract mathematical structure obscures the conceptual interpretation of key processes such as superposition, entanglement and wavefunction collapse. While probabilistic outcomes are captured by the Born rule and physical dynamics are governed by the Schrödinger equation, the theory provides limited tools for articulating the epistemic and logical structure of quantum transitions (Wieser 2016; Tzemos and Contopoulos, 2021). This has led to persistent foundational debates and ambiguities, particularly around measurement, the role of the observer and the interpretation of quantum states (Sokolovski 2020). Quantum logics, including orthomodular lattices and topos-theoretic approaches, attempt to address these gaps but eschew probabilistic reasoning or lack the power to capture temporal and contextual dependencies in experiments (Gunji et al., 2017; Jorge and Holik, 2020; Gunji and Nakamura, 2022). Likewise, probabilistic and epistemic logics developed in computer science and philosophy provide rich formalisms for uncertainty and belief but are rarely adapted to quantum phenomena (Dalla Chiara et al., 2018; Betz and Richardson, 2023). A conceptual framework that jointly captures the modal and

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probabilistic dimensions of quantum events, especially in dynamic, measurement-driven contexts, is still underdeveloped.

We introduce a logical system—Probabilistic Modal Logic for Quantum Dynamics (PML-QD)—that unifies modal logic with probabilistic semantics to model quantum events, particularly the transition from indeterminate to determinate states. This system extends standard modal logic with a probability operator defined over accessible worlds, thereby allowing us to represent not only whether a proposition is possible or necessary, but also with what probability it holds. Measurement, in this framework, is formalized as a shift from possibility to necessity, governed by both probabilistic structure and modal constraints. Entangled and sequential measurements are treated via correlated modalities and conditional update rules. We anticipate that this approach will provide a rigorous framework for tracking the epistemic status of quantum systems before and after measurement, enabling precise reasoning about which propositions were possible, probable or necessary at each stage of the quantum process.

Concerning the Formal structure of PML-QD, we adopt the classical modal logic system K, which we extend to incorporate probabilistic semantics (Singleton and Booth, 2023; Quelhas et al., 2024; Litland 2025). Let L denote a standard modal propositional language constructed from a set of propositional variables P, the Boolean connectives ¬ (negation), ∧ (conjunction) and the modal operators □ (necessity) and ◊ (possibility). The semantics are defined using a Kripke frame $F = (W, R)$, where W is a nonempty set of possible worlds and $R \subseteq W \times W$ is an accessibility relation. A Kripke model $M = (W, R, V)$ assigns truth values to each atomic proposition $p \in P$ via a valuation function $V: P \to P(W)$, where $P(W)$ is the powerset of W (Weiss and Birman, 2024). Satisfaction is defined recursively: for any $w \in W, M, w \vDash$p iff $w \in V(p)$; $M, w \vDash \neg\phi$ iff $M, w \vDash/\phi \; and \; M, w \vDash \phi \wedge \psi$ iff $M, w \vDash \phi$ and $M, w \vDash \psi$; and $M, w \vDash \Box\phi$ iff for all $v \in W$, if wRv, then $M, v \vDash \phi$. The dual operator ◊ϕ is defined as $\neg\Box\neg\phi$. This base system allows us to express propositional necessity and possibility, which we are going to enrich with a quantitative probabilistic extension tailored to quantum systems.

We now extend the Kripke model by introducing a probability measure over the set of possible worlds. A probabilistic Kripke model is defined as $M = (W, R, V, \mu)$, where $\mu: P(W) \to [0,1]$ is a finitely additive probability measure such that $\mu(W) = 1$ and $\mu(\emptyset) = 0$ (Shirazi and Amir, 2007). For each world $w \in W$, we define a conditional probability distribution μw over the accessible worlds $R(w) = \{v \in W \mid wRv\}$. The semantics of the probabilistic modal operator are then defined by introducing a function $P(\phi)$ yielding the probability that ϕ is true in the accessible worlds: formally, $M, w \vDash P(\phi) = p$ iff $\mu w(\{v \in R(w) \mid M, v \vDash \phi\}) = p$. This enables the assessment not only of whether a proposition is necessary or possible, but also of the probability with which it is possibly true. To ensure internal consistency, we assume that μw is defined via restriction and normalization: $\mu w(A) = \mu(A \cap R(w))/\mu(R(w))$ whenever $\mu(R(w)) > 0$. This logic is able to represent graded modal claims such as "it is 70% possible that φ" or more formally, $P(\Diamond\, \phi) = 0.7$, establishing a formal mechanism for assigning probabilities to modal propositions.

The logical language and syntax of PML-QD are built from a base set of propositional variables P, closed under the classical connectives and modal operators □, ◊ and the probability operator P. The syntax includes expressions of the form $P(\phi) = r$, where $r \in [0,1] \cap Q$ and composite formulas such as $P(\Diamond\, \phi) \geq s$ and $P(\Box\phi \to \psi) < t$. Formulas are interpreted over the probabilistic Kripke models defined above. The logic also allows us to define conditional probabilities. For propositions ϕ and ψ, we define $P(\phi \mid \psi) = \mu w(\{v \in R(w) \mid M, v \vDash \phi \wedge \psi\})/\mu w(\{v \in R(w) \mid M, v \vDash \psi\})$, assuming the denominator is nonzero. This allows the formulation of statements like "given ψ, φ is probable with 0.6 likelihood", which corresponds to $P(\phi \mid \psi) = 0.6$.

Overall, this approach yields the formal language of the system—comprising syntax, probability statements, and conditional expressions—necessary for modeling quantum epistemic transitions. Building on this foundation, the next steps involve applying this language to concrete quantum scenarios, analyzing how epistemic statuses evolve across different stages of measurement and inference.

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2. Axiomatic System

The axiomatic core of PML-QD comprises a structured integration of classical propositional logic, normal modal logic (system K) and finite probabilistic logic. The system operates within a Hilbert-style framework with axioms for classical logic, modal logic (K-system) and probability logic, including finite additivity, non-negativity, normalization and probabilistic modus ponens. Well-formed formulas (wffs) are derived from a fixed set of axioms using explicitly defined inference rules. The classical propositional component of the system is based on the axioms of tautology schemas, such as $\phi \to (\psi \to \phi)$, $(\phi \to (\psi \to \chi)) \to ((\phi \to \psi) \to (\phi \to \chi))$ and $\neg\neg\phi \to \phi$. The modal part includes the standard K axiom: $\Box(\phi \to \psi) \to (\Box\phi \to \Box\psi)$ and the necessitation rule: if $\vdash \phi$, then $\vdash \Box\phi$. The logic is normal in that it preserves closure under necessitation and respects distribution over implication. The axioms are interpreted over Kripke frames with arbitrary accessibility relations, allowing for flexible modeling of different quantum experimental contexts.

At first, probabilistic axioms are introduced to extend the classical-modal foundation. The core probabilistic principles are drawn from Kolmogorov's axioms, reformulated for integration into logical syntax (Svozil 2022). Let $P(\phi) = r$ be a primitive formula expressing that the probability of ϕ being satisfied in the accessible worlds is $r \in [0,1] \cap Q$. The axioms include non-negativity $P(\phi) \geq 0$, normalization $P(\top) = 1$ and finite additivity: if $\phi \wedge \psi \equiv \bot$, then $P(\phi \vee \psi) = P(\phi) + P(\psi)$. These are supplemented by conditional probability rules: for $\phi, \psi \in L$ with $P(\psi) > 0 P(\psi) > 0, P(\phi \mid \psi) = P(\phi \wedge \psi)/P(\psi)$. The modal-probabilistic interaction is regulated by the schema $P(\Box\phi) \leq P(\phi)$ and similarly $P(\phi) \leq P(\Diamond \phi)$, reflecting that what is necessarily true must also be true and what is true must be possibly true. These constraints are justified by the semantics of modal probability spaces, where $\Box\phi$ implies truth in all accessible worlds, while $\Diamond \phi$ requires only one accessible world where ϕ holds. The derivation rules include probabilistic modus ponens: from $\phi \to \psi$ and $P(\phi) \geq$r, infer $P(\psi) \geq r$, provided all evaluations are over the same accessibility neighborhood. These axioms permit the formal manipulation of graded propositions about truth values in modal contexts.

Therefore, with the K system as a stable scaffolding, the logic acquires the modal structure necessary to represent transitions from epistemic possibility to necessity, forming a base from which the probabilistic dynamics of quantum measurement can be encoded.

Implementation of quantum-specific axioms. To specialize PML-QD to quantum dynamics, we introduce a set of axioms reflecting superposition, measurement and entanglement. Let ϕi denote the proposition "the quantum system is in eigenstate i." For a system in state $|\psi\rangle = \sum i\alpha i \mid i\rangle$, we define $P(\Diamond \phi i \mid \alpha i \mid^2$.. This probabilistic modal formulation expresses the pre-measurement epistemic state: the system may be found in state ϕi with probability $\mid \alpha i \mid^2$. Upon measurement and collapse to ϕj, we enforce ϕj and $\Box\neg \Diamond \phi k$ for all $k \neq j$. We also represent entanglement through joint modal constraints. Given an entangled state $|\Psi\rangle = \sum i\alpha i \mid ai\rangle \otimes \mid bi\rangle$, let ϕi and ψi be propositions for subsystems A and B, respectively. Then $\Diamond(\phi i \wedge \psi i)$ is true with probability $\mid \alpha i \mid^2$, while $\Diamond \phi i \wedge \Diamond \psi j$ is only allowed if $i = j$ thereby disallowing separable joint modalities. These rules enable the logic to model quantum correlations and non-locality without assuming hidden variables.

Let $|\psi\rangle = \sum i\alpha i \mid i\rangle$ be a normalized pure state over an orthonormal basis $\{\mid i\rangle\}$ and let ϕi be the proposition "the system is in state $\mid i\rangle$". We postulate that $P(\Diamond \phi i) = \mid \alpha i \mid^2$, aligning logical possibility with the Born rule. This axiom expresses that, prior to measurement, the system may be found in eigenstate i, with probability equal to the squared modulus of its amplitude. Upon a measurement yielding outcome j, we assert $\Box\phi j$ and $\Box\neg \Diamond \phi k$ for all $k \neq j$, modeling the epistemic update resulting from collapse. This transition encodes the projection postulate as a logical update, i.e., a modal reduction from possibility to necessity and from multiple probabilities to a single certainty. To formalize this shift, we introduce the axiom schema $Measure(\phi j) \to (\Box\phi j \wedge \wedge k = j \Box\neg \Diamond \phi k)$. The measurement operator is treated syntactically, marking the transition point in the evaluation of epistemic states. This schema is only applied in cases where the logic designates $P(\Diamond \phi j) > 0$, reflecting the assumption that outcomes with zero probability cannot be observed.

To handle entanglement, we extend the logic with modal rules for joint propositions. Consider a bipartite quantum system with basis $\{\mid ai\rangle \otimes \mid bj\rangle\}$ and an entangled state $|\Psi\rangle = \sum i\alpha i \mid ai\rangle \otimes \mid bi\rangle$.

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Let ϕi denote "Subsystem A is in state $|ai\rangle$" and ψi denote "Subsystem B is in state $|bi\rangle$." We introduce the entanglement axiom $P(\Diamond(\phi i \wedge \psi i)) =| \alpha i |^2$ and the exclusion principle $\neg(\Diamond \phi i \wedge \Diamond \psi j)$ for $i \neq j$. This enforces the non-separability of joint state truth values: subsystems cannot simultaneously assume inconsistent states if derived from an entangled superposition. The logic allows us to evaluate propositions such as $\Diamond \phi i \rightarrow \Box\neg\psi j$ for $j \neq$, capturing the non-local correlations implicit in entanglement. These formulations provide a means of expressing Bell-type dependencies as logical constraints within the system, grounded in amplitude-based probability assignments. For measurements, we define the conditional update: observing ϕk implies $\Box\psi k$ and $\Box\neg\psi j$ for all $j \neq k$ consistent with perfect quantum correlations. These constraints are encoded as derivable rules rather than primitive axioms, preserving flexibility in modeling partial or imperfect entanglement.

Overall, this approach embeds the measurement postulates of quantum mechanics within a modal logical framework, effectively translating quantum dynamics into a structure that supports logical inference and modal reasoning about probability. By doing so, it bridges the gap between quantum formalism and epistemic logic, enabling a systematic analysis of how knowledge and uncertainty evolve through quantum processes. This integration allows for the tracking of necessity, possibility, and likelihood in a manner that aligns with both the probabilistic nature of quantum theory and the inferential tools of modal logic.

Tools and Computational Setup. All logical definitions, axioms and inference rules are formalized using a typed symbolic language implemented in the Lean proof assistant to verify syntactic coherence and logical validity (Löh 2022). For probability measures and accessibility relations, we use custom-built Kripke structures defined programmatically in Python using the networkx library for graph modeling and numpy for probability assignment. Quantum state vectors and projection operations are handled using the qutip library, enabling precise computation of Born-rule probabilities and their mapping to logical probability assignments. Logical expressions are parsed and evaluated using a domain-specific parser that constructs abstract syntax trees, evaluates modal depth and resolves probabilistic truth values based on current world state and transition graphs.

3. Coherence, Consistency and Semantic Soundness of PML-QD

This chapter examines the coherence, consistency and semantic soundness of PML-QD to ensure that its logical structure faithfully represents quantum dynamics and supports reliable epistemic inference. We begin by demonstrating the soundness of PML-QD relative to its probabilistic Kripke semantics. Let $M = (W, R, V, \mu)$ be a model of our system, where R is an arbitrary (possibly non-symmetric, non-transitive) relation to accommodate varied quantum contexts. For each world $w \in W$ and each well-formed formula $\phi \in L$, if $\vdash \phi$ in the PML-QD system, then $M, w \vDash \phi$. Proof proceeds by induction over the structure of derivations. Base cases follow from the validity of classical tautologies. The K modal axiom is validated by the relational condition: $wRv \wedge wRu$ implies that if $M, v \vDash \phi \rightarrow \psi$ and $M, v \vDash \phi$, then $M, v \vDash \psi$. The probabilistic axioms are sound under the standard interpretation of μw as a conditional probability measure on $R(w)$. Additivity, normalization and conditional independence are preserved by construction. Quantum-specific axioms are modeled by assigning $\mu w(\Diamond \phi i) =| \alpha i |^2$ for pre-collapse states and using syntactic restrictions post-collapse to enforce that $\Box\phi j \rightarrow \mu w(\phi j) = 1$. Thus, the epistemic updates are correctly aligned with quantum measurement rules. This confirms that the axiomatic structure of PML-QD is consistent with its intended semantics, establishing soundness as a necessary condition for subsequent formal evaluation.

Completeness is demonstrated via canonical model construction. We define a canonical model $Mc = (Wc, Rc, Vc, \mu c)$, where Wc is the set of maximally consistent sets (MCSs) of formulas in $LPML - QD$. The accessibility relation c is defined by: $\Gamma Rc \Delta$ iff for every $\Box\phi \in \Gamma$, $\phi \in \Delta$. The valuation function $Vc(p) = \{\Gamma \in Wc \mid p \in \Gamma\}$ and the probability function $\mu\Gamma c(\phi) = r$ is defined via the maximal consistent extensions satisfying $P(\phi) = r \in \Gamma$. Completeness then follows: if ϕ is valid (true in every model), then $\phi \in \Gamma$ for all $\Gamma \in Wc$. If $\phi \in/\Gamma$, then there exists a model falsifying ϕ,

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proving that ϕ\phiϕ is not derivable. For quantum-specific modalities, we enrich the canonical model with amplitude maps from abstract syntax to normalized vectors, ensuring that measurement-induced updates correspond to transitions among canonical worlds. The probabilistic collapse axioms are enforced syntactically by excluding extensions that contradict the post-measurement certainty schema.

We thus obtain completeness for the classical, modal and probabilistic layers, with quantum-specific axioms ensured through semantic alignment.

Epistemic closure and logical coherence. An important consideration in any epistemic logic is whether the system permits epistemic closure under valid inference. In PML-QD, closure under modal and probabilistic inference is carefully maintained through explicit syntactic rules. For instance, from $\Box(\phi \to \psi)$ and $\Box\phi$, one may derive $\Box\psi$, preserving modal consequence. However, in quantum contexts, closure must also handle epistemic transitions: if $\Diamond\phi$ and $(\Diamond\phi) = 1$, it does not follow that $\Box\phi$, unless a measurement event enforces it. Thus, PML-QD avoids epistemic overreach by enforcing the distinction between high probability and logical certainty. Probabilistic closure is similarly bounded: from $P(\phi \wedge \psi) = 0.9$ and P$P(\psi) = 1$, we may derive $P(\phi \mid \psi) = 0.9$, but not $\Box\phi$ or even $\Diamond\phi$ unless additional modal premises are supplied. This disciplined separation of inference domains ensures that conclusions about quantum states are validly drawn only within the scope of their modal and probabilistic constraints. Furthermore, any logical contradiction arising from measurement updates (e.g., asserting $\Box\phi$ and $\Diamond\neg\phi$) is syntactically blocked by the collapse axioms.

Counterfactual reasoning and epistemic modality. PML-QD allows for formal engagement with counterfactuals in quantum mechanics, a topic often debated in the context of delayed-choice experiments, weak measurements and quantum nonlocality (Laudisa 2019). The framework supports statements of the form: "If measurement M had been performed, then ϕ would have become necessary," represented formally as $\Diamond M \to (Measure(\phi) \to \Box\phi)$. Such statements are not metaphysical speculations but logical conditionals grounded in modal accessibility. Suppose a system is in a superposed state $|\psi\rangle = \alpha|0\rangle + \beta|1\rangle$ and the measurement is postponed. The logic allows one to say: "Had we measured now, outcome $\phi 0$ would have become necessary with probability $|\alpha|2$." This supports rigorous statements about epistemic potential without requiring ontological assertions about unmeasured reality. Importantly, these counterfactuals do not imply retrocausality, but rather preserve the distinction between hypothetical and actualized knowledge states, aligning with quantum experimental setups where delayed configurations define outcome space. The evaluation of counterfactuals is governed by modal consistency: only those conditional claims holding in all accessible paths from a given precondition are allowed.

Model-theoretic consistency with Hilbert Space formalism and temporal evolution. The compatibility and the semantic consistency of PML-QD with the standard Hilbert space formalism can be evaluated. Let H be a finite-dimensional Hilbert space with orthonormal basis $\{|i\rangle\}$ and let $|\psi\rangle = \sum i\alpha i\,|i\rangle$ be a quantum state. The propositions ϕi correspond to projection operators $P\hat{}i = |i\rangle\langle i|$ and the probability of observing state i is $\langle\psi|P\hat{}i|\psi\rangle = |\alpha i|2$. We define a mapping $f: \phi i \mapsto P\hat{}i$ and assert that $P(\Diamond\phi i) = Tr(\rho P\hat{}i)$ where $\rho = |\psi\rangle\langle\psi|$. The logical model corresponds to a coarse-grained representation of the probabilistic projections across a set of accessible configurations indexed by basis measurements. Post-measurement updates $\Box\phi j$ correspond to Lüders projections: $\rho \mapsto P\hat{}j\rho P\hat{}j/Tr(\rho P\hat{}j)$. For entangled states $|\Phi\rangle = \sum i\alpha i\,|ai\rangle \otimes |bi\rangle$, joint modal formulas $\Diamond(\phi i \wedge \psi i)$ are semantically validated by $Tr(\rho(P\hat{}i \otimes Q\hat{}i)) = |\alpha i|2$. This means that logical constructs are supported by standard operator theory. In this context, the modal structure represents pre-measurement epistemic range, while probability reflects amplitude-squared outcomes.

To evaluate the temporal behavior and dynamical evolution of PML-QD, we consider its capacity to represent quantum evolution between measurement events. Let $U(t) = e - iHt$ be the unitary evolution operator associated with a Hamiltonian H and $|\psi(t)\rangle = U(t)\,|\psi(0)\rangle$. In logical terms, we represent a temporal sequence $w0 \to w1 \to \cdots \to wn$, where each wi is a world indexed by the system's state at time ti. If ϕi is the proposition "system is in state $|\psi(ti)\rangle$", then temporal modal transitions $\Diamond\phi i + 1$ are defined by the Schrödinger evolution. The logic supports this via

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time-indexed accessibility: $wiRwi+1$ iff $|\psi(ti+1)\rangle = U(ti+1-ti)\,|\psi(ti)\rangle$. We extend the model by assigning probability functions $\mu wi(\phi j) = |\langle \phi j\,|\,\psi(ti)\rangle|^2$, yielding a dynamic probability assignment compatible with unitary evolution. If a measurement occurs at tj, the update collapses the epistemic structure: all future-accessible paths inconsistent with the observed outcome are pruned from $R(wj)$.

In summary, we establish the dynamic consistency of PML-QD by demonstrating that modal transitions respect unitary evolution up to the point of measurement and that state updates conform to the projection postulate. This validation reinforces the semantic soundness of the framework, confirming its capacity to model quantum epistemic dynamics with logical and physical fidelity.

4. From Theory to Practice: Logical Modeling of Quantum Systems in PML-QD

This chapter presents representative examples illustrating how PML-QD models quantum phenomena through logical structures and modal reasoning.

Derivation of single-measurement collapse. We begin with a formal derivation illustrating how PML-QD syntactically captures the transition from a probabilistic possibility to post-measurement necessity in the single-qubit case. Let $|\psi\rangle = \alpha\,|0\rangle + \beta\,|1\rangle$ be a superposed state, with $|\alpha|2+|\beta|2=1$. Let $\phi 0$ denote "the system is in state $|0\rangle$" and $\phi 1$ denote "the system is in state $|1\rangle$." In the logic, prior to measurement we assume $P(\Diamond\,\phi 0) = |\alpha|2$ and $P(\Diamond\,\phi 1) = |\beta|2$, encoded via axioms of amplitude-based modal probability. Suppose the system is measured and the outcome is $|0\rangle$. The axiom of modal collapse gives $Measure(\phi 0) \rightarrow \Box\phi 0 \wedge \Box\neg\Diamond\,\phi 1$. If we assume $Measure(\phi 0)$, then using modus ponens, we derive $0\Box\phi 0$ and $\Box\neg\Diamond\,\phi 1$. Applying the modal logic equivalence $\Box\neg\Diamond\,\phi 1 \equiv \neg\Diamond\,\phi 1$, we deduce that $\phi 1$ is no longer even possible. The derivation shows that the probabilistic possibility $P(\Diamond\,\phi 1) = |\beta|2$ is syntactically replaced by $\neg\Diamond\,\phi 1$ and all future logical inferences involving $\phi 1$ become false under modal necessity.

Sequential measurements. Next, we construct a proof sequence for a scenario involving two successive measurements along non-commuting observables. Let the system initially be in state $|\psi\rangle = 21(|0\rangle+|1\rangle)$ and define $\phi 0, \phi 1$ as before. Let $\chi+$ and $\chi-$ denote the propositions corresponding to the diagonal basis $|-\rangle = 21(|0\rangle-|1\rangle)$. Suppose the first measurement is in the $\{|0\rangle,|1\rangle\}$ basis and the outcome is $|0\rangle$. By modal collapse, we derive $\Box\phi 0$ and $\Box\neg\Diamond\,\phi 1$. Now we consider $P(\Diamond\,\chi+)$, i.e., the probability of subsequently observing $|+\rangle$. Since the post-measurement state is $|0\rangle$, we compute $P(\Diamond\,\chi+) = |\langle +|\,0\rangle|2 = 1/2$. We syntactically derive $\Box\phi 0 \rightarrow P(\Diamond\,\chi+) = 1/2$ from the substitution of amplitude-based definitions into the probability axioms. Upon performing the second measurement and observing $|+\rangle$, we apply the measurement collapse rule again to derive $\Box\chi+$ and $\Box\neg\Diamond\,\chi-$. These results can be then used to show that all further inferences about $\phi 1$ or $\chi-$ must fail in all accessible worlds.

We now consider the effect of sequential measurements when the observables do not commute. Let the initial state again be $|\psi\rangle = \alpha\,|0\rangle + \beta\,|1\rangle$ and define a second measurement basis, such as the $\{|+\rangle,|-\rangle\}$ basis, where $|+\rangle = 21(|0\rangle+|1\rangle)$ and $|-\rangle = 21(|0\rangle-|1\rangle)$. Let $\chi+$ and $\chi-$ represent the corresponding modal propositions. If the system is first measured in the computational basis and yields $|0\rangle$, then we assert $\Box\phi 0$ and immediately invalidate $\Diamond\,\phi 1$. We may now inquire about $P(\Diamond\,\chi+)$, that is, the possibility of subsequently observing $|+\rangle$. In the post-collapse model, the system state become $|0\rangle$, so we calculate $P(\Diamond\,\chi+) = |\langle +|\,0\rangle|2 = 1/2$. Formally, this is encoded as $\Box\phi 0 \rightarrow P(\Diamond\,\chi+) = 1/2$, reflecting the non-commutativity of the measurement sequence. The logic tracks not only the collapsed state but its implications for future probabilistic possibilities. If we now measure in the diagonal basis and obtain outcome $|+\rangle$, the epistemic update becomes $\Box\chi+\wedge\Box\neg\Diamond\,\chi-$, which supersedes the prior assignment. PML-QD may thus support reasoning about nested and sequential updated updates and the logical impact of measurement order.

Single-Qubit superposition and measurement. We begin with a basic scenario: a single qubit in a superposition of eigenstates relative to a particular observable, such as spin along the z-axis. Let the quantum state be $|\psi\rangle = \alpha\,|0\rangle + \beta\,|1\rangle$, where $\alpha,\beta \in C$ and $|\alpha|2+|\beta|2=1$. We define the modal propositions $\phi 0$ and $\phi 1$ corresponding respectively to the system being in state $|0\rangle$ and $|$

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1⟩. In the PML-QD framework, the epistemic state prior to measurement is captured by the formulas $P(\Diamond\, 2P(\Diamond\, \phi 1) =| \beta \mid 2$. These assertions express the degree of possibility—based on the probabilistic modal semantics—associated with each eigenstate. A measurement in the $\{| 0\rangle, | 1\rangle\}$ basis is treated as a transition: if outcome $| 0\rangle$ is observed, the updated state becomes $\Box\phi 0 \wedge \Box\neg \Diamond\, \phi 1$, formalizing collapse in logical terms. The inference chain follows directly from the axiom $Measure(\phi j) \rightarrow (\Box\phi j \wedge \wedge k = j\Box\neg \Diamond\, \phi k)$. The model also permits conditional queries such as $P(\Diamond\, \phi 0 \mid \neg\phi 1) = 1$, maintaining coherence with the binary outcome structure of projective measurement. This means that PML-QD may accommodate elementary state transitions and probability-based reasoning using modal assertions grounded in amplitude-based truth.

Collapse simulation and world pruning algorithms. The logic requires that upon measurement possible but unrealized outcomes are no longer epistemically accessible. To simulate this, a world-pruning algorithm could be implemented that dynamically restructures the Kripke model. Upon observing outcome ϕj at world w, this algorithm first may verify $P(\Diamond\, \phi j) > 0$; then, identify the unique subset $R'(w) \subseteq R(w)$ such that $\forall v \in R'(w), M, v \vDash \phi j$. The accessibility relation can be updated by setting $R(w) := R'(w)$ and the probability distribution μw renormalized over $R'(w)$. All ϕk such that $k \neq j$ may be set to evaluate as $\neg \Diamond\, \phi$k and $\Box\neg\phi k$. The update is conservative and preserves modal truth: previously necessary propositions remain necessary unless invalidated by the measurement result. In sequential measurements, the system may track update sequences using a stack of Kripke structures, allowing rollback and re-evaluation. A collapse consistency check may ensure that $\sum kP(\Diamond\, \phi k) = 1$ prior to collapse and that after measurement such that exactly one ϕj becomes necessary while the others are impossible. In entangled cases, a measurement on one subsystem may automatically trigger pruning on correlated worlds in the partner system, enforcing non-local modal synchrony.

Entanglement constraints via modal dependencies. Let us consider the entangled state $| \Phi\rangle = 21(| 0\rangle A \mid 1\rangle B-| 1\rangle A \mid 0\rangle B)$. Define $\phi 0$, $\phi 1$ for particle A and $\psi 0$, $\psi 1$ for particle B. The axiom of correlated possibility gives $P(\Diamond\, (\phi 0 \wedge \psi 1)) = 1/2$, $P(\Diamond\, (\phi 1 \wedge \psi 0)) = 1/2$ and all other $P(\Diamond\, (\phi i \wedge \psi j)) = 0\ for\ i = ji\ = ji = j$. Suppose a measurement on A yields $\phi 1$. By measurement collapse, we derive $0\Box\phi 1 \wedge \Box\neg \Diamond\, \phi 0$. From the modal correlation rule $\phi 1 \rightarrow \Box\psi 0$, we deduce $\Box\psi 0$, i.e., the state of B becomes necessarily $| 0\rangle$. The derivation holds even if the measurement on B occurs later, as the logical dependencies are enforced modally rather than temporally. Suppose instead that we had observed $\phi 0$; the derivation would then yield $\psi 1$. Importantly, PML-QD prevents derivation of any statement $\Diamond\, (\phi 1 \wedge \psi 1)$, since this is ruled out by the amplitude-based probability axiom. Overall, the logical constraints imposed by PML-QD on entangled propositions may correctly capture the exclusivity and correlation properties inherent in entangled quantum states.

Entanglement Swapping. Entanglement swapping is a protocol in which two initially independent quantum systems become entangled through joint measurement on intermediary particles (Ning et al., 2019; Zangi et al., 2023). Consider two entangled pairs in the states $| \psi AB\rangle = 21(| 0\rangle A \mid 1\rangle B-| 1\rangle A \mid 0\rangle B)$ and $| \psi CD\rangle = 21(| 0\rangle C \mid 1\rangle D-| 1\rangle C \mid 0\rangle D)$. Let a Bell-state measurement be performed on particles B and C. Define modal propositions ϕij the four Bell states between B and C and ψAD for the corresponding entanglement state between A and D. Before the measurement, the logic encodes the system as $\vee i, jP(\Diamond\, \phi ij) = 41$, with no assignment of $\Box\psi AD$. Upon observing Bell state $\phi 00$, we assert $\Box\phi 00 \rightarrow \Box\psi AD00$, with $\psi AD00$ denoting a corresponding entangled state between particles A and D. This captures the epistemic update that makes entanglement between distant, non-interacting particles a logical necessity only *after* the intermediate measurement. This avoids invoking retrocausality by localizing modal transitions to the knowledge structure. Conditional probabilities are also updated: $P(\Diamond\, \psi AD00 \mid \phi 00) = 1$, while other $\psi ADij$ are assigned zero. Therefore, PML-QD may capture entanglement swapping via conditional modal updates, supporting rigorous reasoning about delayed entanglement onset.

Delayed-choice interference and quantum eraser. We propose here a derivation involving the delayed-choice quantum eraser (Kim et al., 2000). Define ϕp: "which-path information is accessible," and ϕw: "interference pattern is observed." Before post-selection, we assume $\Diamond\, \phi p \wedge \Diamond\, \phi w$. Let Me

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denote the erasure choice and Mp the path-preserving choice. Under the choice Me, the logic enforces $Me \rightarrow \Box\neg\phi p \wedge \Box\phi w$, whereas under Mp, it enforces $Mp \rightarrow \Box\phi p \wedge \Box\neg\phi w$. Assume Me is enacted after the signal photon is measured. Since the epistemic update is contingent, we represent the situation as a conditional modal formula: $\Diamond(\phi p \wedge \phi w) \rightarrow (Me \rightarrow \Box\phi w) \wedge (Mp \rightarrow \Box\phi p)$. Upon performing Me, the system updates to $\Box\phi w$ and $\Diamond\phi p$ is logically rejected. The derivation path shows that the modal state is not solely determined by photon interactions, but by post-measurement contextualization. This is syntactically grounded in modal update axioms, not as an empirical fact but as a derivable transition.

We now examine the delayed-choice quantum eraser, a paradigmatic scenario where information about a quantum system's path is either retained or erased after the system has been measured (Kim and Ham, 2023). Let ϕp denote "which-path information is known" and ϕw denote "interference pattern is visible." The logic must represent conditional dependencies where the post-measurement setup retroactively influences the interpretation of earlier events. We encode the availability of path information as a binary modal variable: if the information is preserved, we assign $\Box\phi p \rightarrow \Box\neg\phi w$ and if it is erased, $\Box\neg\phi p \rightarrow \Box\phi w$. The measurement decision variable Me (erase path info) or Mp (preserve path info) serves as a modal context switch: prior to this, both $\Diamond\phi p$ and $\Diamond\phi w$ are true. After choosing Me, the system updates to $\Box\neg\phi p \wedge \Box\phi w$. PML-QD allows for modal reasoning such as $\Diamond\phi p \wedge \Diamond\phi w \rightarrow (Me \rightarrow \Box\phi w)$, modeling epistemic changes contingent on an action taken after the quantum interaction. This illustrates that PML-QD can represent dynamically dependent modal transitions where final knowledge states depend on future experimental choices.

The examples presented in this chapter demonstrate the capacity of PML-QD to formally capture key aspects of quantum behavior, including measurement collapse, entanglement and contextual inference. These cases highlight the framework's utility in modeling epistemic transitions with logical precision, offering a promising methodology for further theoretical and practical developments.

5. Conclusion

The Probabilistic Modal Logic for Quantum Dynamics (PML-QD) introduced here provides a formal framework that integrates modal logic, probability theory and the epistemic structure of quantum measurement. Its aims to model the dynamic progression from probabilistic possibility to logical necessity that defines quantum behavior under measurement. Built on the classical modal system K, PML-QD introduces a probabilistic valuation mechanism and a compact set of domain-specific axioms governing superposition, collapse and entanglement. Modal propositions are assigned quantitative probability values. Upon measurement, collapse transitions convert possibility into necessity, enforcing expressions like $\Box\varphi$ to indicate that an outcome has become realized. A key non-classical feature of PML-QD is its treatment of epistemic non-monotonicity. In classical modal logic, implications such as $\diamond\varphi \rightarrow \diamond\Box\varphi$ may hold under specific frame conditions. However, this does not persist in PML-QD due to the collapse-induced pruning of modal paths: once a measurement occurs, the model transitions to a substructure where prior possibilities are no longer accessible. This modal reduction is syntactically governed by the axiom $Measure(\varphi j) \rightarrow \Box\varphi j \wedge k \quad = j \wedge \Box\neg\diamond\varphi k$ which ensures logical consistency while rejecting modal monotonicity. In the PML-QD framework, the necessity operator $\Box$ does not collapse into a truth predicate. While $\Box\varphi \rightarrow \varphi$ holds in the sense that a necessary proposition must be true in all accessible worlds, this does not imply absolute truth, but only truth relative to post-measurement substructure. This distinction reinforces the epistemic separation between pre-measurement probability and post-measurement necessity, marking a departure from both classical modal logic and traditional probabilistic logic. Unlike classical knowledge frameworks where a proposition is either known or unknown, PML-QD enables a graded epistemic treatment. It may accommodate statements of the form: possibility without expectation $P(\diamond\varphi) = 0$, expectation without necessity $0 < P(\diamond\varphi) < 1$ and epistemic finality $\Box\varphi$. This enables a more nuanced account of quantum epistemology. For example, the proposition "the particle is spin-up" need not be treated as simply known or unknown; instead, it can be represented as probable but not

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necessary prior to measurement, with its degree of belief quantified by quantum amplitudes rather than reduced to binary epistemic categories.

A distinctive feature of PML-QD is its logical minimalism, as it extends the basic modal system K only modestly by incorporating probabilistic operators and a small set of collapse-specific axioms. The system deliberately avoids polymodal formulations, higher-order quantification, intensional types, nonclassical connectives, distributed knowledge operators and dynamic logic constructs, focusing instead on a small set of precisely defined epistemic transitions . This design choice emphasizes deductive transparency over maximal expressive power. Each formula expresses a distinct logical claim that can be semantically validated within Kripke models equipped with probability functions. This keeps model-theoretic evaluation computationally feasible while retaining expressiveness sufficient to model quantum experimental structures.

An important advantage of PML-QD is its practical utility in the design and analysis of quantum experiments. By formally modelling the transition from probabilistic possibility to logical necessity, the framework provides a rigorous structure for anticipating and interpreting measurement outcomes. This is especially beneficial in settings involving entanglement swapping, delayed-choice quantum erasers and sequential measurements with non-commuting observables, i.e., scenarios where standard formalisms often obscure epistemic transitions. For example, in designing a delayed-choice interference experiment, PML-QD may provide a framework for anticipating how post-selection contexts modulate which-path information, thereby clarifying the underlying logical dependencies before experimental implementation. In entanglement swapping protocols, PML-QD may aid in tracking modal correlations to ensure consistency across nonlocal updates. Its ability to handle conditional reasoning and epistemic updates supports counterfactual assessments and helps validate whether a given experimental configuration logically aligns with quantum postulates. Overall, PML-QD offers both a conceptual and practical toolkit for optimizing experimental design in foundational quantum research and emerging quantum technologies.

The epistemic distinctions enabled by PML-QD allow a comparison with traditional interpretations of quantum mechanics. In the Copenhagen view, the wavefunction encodes predictive knowledge about measurement outcomes but lacks interpretive content about unmeasured states (Jaeger 2019). PML-QD refines this position by treating superposition as a landscape of modal possibilities rather than ontological ambiguity such that the system can be described as potentially occupying multiple states, each with a distinct logical status and probability. In relation to QBism, which treats quantum states as subjective degrees of belief (Khrennikov 2018; Milgrom 2022), PML-QD provides a formal syntactic structure to those beliefs, grounding them in rules of modal inference and derivability. While QBism invokes a probabilistic agent-centric perspective, PML-QD situates probability within a logical system constrained by axioms, thus offering a structured treatment of epistemic agency. In the many-worlds interpretation, every possible measurement outcome is realized in some branch of reality (Devor 2023; Vaidman 2025). PML-QD models branching through modal accessibility, but does not commit to metaphysical plurality; rather, it treats branching as epistemic openness. The modal relations are not anymore between physically instantiated worlds, but between logically accessible epistemic states.

ML-QD differs from traditional quantum logics, which often replace classical logic with alternative systems to reflect the structural or contextual aspects of quantum theory. For instance, the Birkhoff–von Neumann approach represents quantum propositions as elements of an orthomodular lattice of Hilbert space subspaces, replacing classical Boolean logic with a non-distributive structure that reflects observable incompatibility and quantum geometry (Gunji and Nakamura 2022). In contrast, PML-QD retains classical propositional logic and introduces modal and probabilistic layers to capture quantum uncertainty and measurement dynamics without abandoning logical distributivity. In turn, topos-theoretic quantum logic reformulates quantum theory using intuitionistic logic and category theory, modeling propositions as presheaves over classical contexts and assigning truth values locally without relying on global valuations or classical bivalence (Landsman 2017; Jia et al. 2025). By contrast, PML-QD operates within a bivalent logical system and

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incorporates probability directly, allowing for global epistemic evaluation and explicit modeling of measurement-induced transitions

PML-QD has certain limitations that warrant acknowledgment. It does not attempt to model open quantum systems or continuous variable states, nor does it incorporate decoherence processes at the level of environment-induced entropy changes. All quantum states are assumed to be finite-dimensional and all measurements are treated as idealized and projective. This simplification enables a syntactically clean and computationally tractable logic but restricts its direct application to more complex or realistic quantum systems. While the system models epistemic transitions triggered by measurements, it does not incorporate temporal indeterminacy or branching-time semantics; instead, it adopts a linear temporal structure via indexed worlds. The exclusion of branching structures limits the logic's ability to model future contingencies or path-dependent quantum evolution. PML-QD also omits any formal treatment of epistemic agents or belief operators. While interpretations such as QBism emphasize agent-centered probabilities and belief updates, PML-QD focuses exclusively on system-level propositions, avoiding subjective or doxastic modalities. Moreover, PML-QD remains neutral regarding the ontological status of the quantum state, avoiding commitment to whether the wavefunction represents physical reality or informational content. Within these limitations, PML-QD provides a coherent logical framework for analyzing the epistemic dynamics of measurement and uncertainty in finite, closed quantum systems. The framework serves not as a replacement but as a complementary language for articulating epistemic features of quantum processes. Within these limitations, PML-QD provides a coherent and complementary logical framework for articulating the epistemic dynamics of measurement and uncertainty in finite, closed quantum systems.

In conclusion, we address whether a unified formal system can capture the modal and probabilistic structure of quantum dynamics, particularly the transition from possibility to necessity induced by measurement. By integrating modal logic with probabilistic semantics, PML-QD offers a framework representing superposition, collapse and entanglement in syntactically precise and semantically consistent terms. The main takeaway is that quantum measurement, often seen as interpretationally opaque, can be rigorously modeled within a logical framework that clearly distinguishes graded possibility from logical necessity across both pre- and post-observation contexts.

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References

1. Betz, Gregor, and Katherine Richardson. "Probabilistic Coherence, Logical Consistency, and Bayesian Learning: Neural Language Models as Epistemic Agents." PLoS One 18, no. 2 (February 9, 2023): e0281372. https://doi.org/10.1371/journal.pone.0281372.
2. Cassinelli, G., and P. Lahti. "Quantum Mechanics: Why Complex Hilbert Space?" Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 375, no. 2106 (November 13, 2017): 20160393. https://doi.org/10.1098/rsta.2016.0393.
3. Dalla Chiara, Maria Luisa, Hector Freytes, Roberto Giuntini, Rossella Leporini, and Giuseppe Sergioli. "Probabilities and Epistemic Operations in the Logics of Quantum Computation." Entropy (Basel) 20, no. 11 (2018): 837. https://doi.org/10.3390/e20110837.
4. Devor, A. 2023. "Constraining the Many-Worlds Interpretation of Computational Neuroscience with Neurophotonics: A Conversation with Gaute Einevoll." Neurophotonics 10(1): 010103. https://doi.org/10.1117/1.NPh.10.1.010103.
5. Gunji, Yukio-Pegio, Shunsuke Shinohara, Taichi Haruna, and Vasilios Basios. "Inverse Bayesian Inference as a Key of Consciousness Featuring a Macroscopic Quantum Logical Structure." Biosystems 152 (February 2017): 44–65. https://doi.org/10.1016/j.biosystems.2016.12.003.
6. Gunji, Yukio-Pegio, and Kei Nakamura. "Psychological Origin of Quantum Logic: An Orthomodular Lattice Derived from Natural-Born Intelligence Without Hilbert Space." Biosystems 215–216 (June 2022): 104649. https://doi.org/10.1016/j.biosystems.2022.104649.
7. Jaeger, Gregg. "Quantum Contextuality in the Copenhagen Approach." Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 377, no. 2157 (November 4, 2019): 20190025. https://doi.org/10.1098/rsta.2019.0025.
8. Jorge, Juan Pablo, and Federico Holik. "Non-Deterministic Semantics for Quantum States." Entropy 22, no. 2 (January 28, 2020): 156. https://doi.org/10.3390/e22020156.
9. Khrennikov, Andrei. "Towards Better Understanding QBism." Foundations of Science 23, no. 1 (2018): 181–195. https://doi.org/10.1007/s10699-017-9524-0.
10. Kim, Yoon-Ho, Rong Yu, Sergei P. Kulik, Yanhua Shih, and Marlan O. Scully. "Delayed 'Choice' Quantum Eraser." Physical Review Letters 84, no. 1 (January 3, 2000): 1–5. https://doi.org/10.1103/PhysRevLett.84.1.
11. Kim, S., and B. S. Ham. "Observations of the Delayed-Choice Quantum Eraser Using Coherent Photons." Scientific Reports 13 (2023): 9758. https://doi.org/10.1038/s41598-023-36590-7.
12. Landsman, Klaas. "Topos Theory and Quantum Logic." In Foundations of Quantum Theory, vol. 188 of Fundamental Theories of Physics, edited by [Editor(s) if available], Springer, Cham, 2017. https://doi.org/10.1007/978-3-319-51777-3_12.
13. Laudisa, F. Counterfactual Reasoning, Realism and Quantum Mechanics: Much Ado About Nothing?. *Erkenn* **84**, 1103–1118 (2019). https://doi.org/10.1007/s10670-018-9997-4
14. Litland, J.E. "Truthmaker Semantics for Intuitionistic Modal Logic." Topoi (Dordrecht) 44, no. 2 (2025): 325–343. https://doi.org/10.1007/s11245-024-10094-z.
15. Löh, Christian. "The Lean Proof Assistant." In Exploring Formalisation, vol. 11 of Surveys and Tutorials in the Applied Mathematical Sciences, edited by Springer, Cham, 2022. https://doi.org/10.1007/978-3-031-14649-7_1.
16. Milgrom, Lionel R. "Some Remarks on QBism and Its Relevance to Metaphors for the Therapeutic Process Based on Conventional Quantum Theory." Complementary Medicine Research 29, no. 4 (2022): 286–296. https://doi.org/10.1159/000523815.
17. Ning, W., X. J. Huang, P. R. Han, H. Li, H. Deng, Z. B. Yang, Z. R. Zhong, Y. Xia, K. Xu, D. Zheng, and S. B. Zheng. "Deterministic Entanglement Swapping in a Superconducting Circuit." Physical Review Letters 123, no. 6 (August 9, 2019): 060502. https://doi.org/10.1103/PhysRevLett.123.060502.

 doi:10.20944/preprints202505.1207.v1

18. Quelhas, Ana Catarina, Catarina Rasga, and Philip N. Johnson-Laird. "Reasoning from Quantified Modal Premises." Cognitive Science 48, no. 8 (August 2024): e13485. https://doi.org/10.1111/cogs.13485.
19. Roy, S. "Anatomy of Localisation Protected Quantum Order on Hilbert Space." Journal of Physics: Condensed Matter 35, no. 41 (July 13, 2023). https://doi.org/10.1088/1361-648X/ace413.
20. Shirazi, Afsaneh, and Eyal Amir. "Probabilistic Modal Logic." In Proceedings of the 22nd National Conference on Artificial Intelligence (AAAI'07), Volume 1, 489–495. Menlo Park, CA: AAAI Press, 2007.
21. Singleton, Jonathan, and Richard Booth. "Expertise and Information: An Epistemic Logic Perspective." Synthese 201, no. 2 (2023): 64. https://doi.org/10.1007/s11229-023-04064-y.
22. Sokolovski, D. "Quantum Measurements With, and Yet Without an Observer." Entropy 22, no. 10 (October 21, 2020): 1185. https://doi.org/10.3390/e22101185.
23. Svozil, Karl. "Extending Kolmogorov's Axioms for a Generalized Probability Theory on Collections of Contexts." Entropy 24, no. 9 (2022): 1285. https://doi.org/10.3390/e24091285.
24. Svozil, Karl. "(Re)Construction of Quantum Space-Time: Transcribing Hilbert into Configuration Space." Entropy 26, no. 3 (March 18, 2024): 267. https://doi.org/10.3390/e26030267.
25. Tzemos, A. C., and G. Contopoulos. "Ergodicity and Born's Rule in an Entangled Three-Qubit Bohmian System." Physical Review E 104, no. 5-1 (November 2021): 054211. https://doi.org/10.1103/PhysRevE.104.054211.
26. Vaidman, L. 2025. "Probability of Self-Location in the Framework of the Many-Worlds Interpretation." Entropy 27(4): 416. https://doi.org/10.3390/e27040416.
27. Weiss, Yale, and Romina Birman, eds. Saul Kripke on Modal Logic. Outstanding Contributions to Logic. Cham: Springer, 2024. https://doi.org/10.1007/978-3-031-57635-5.
28. Wieser, R. "Derivation of a Time Dependent Schrödinger Equation as the Quantum Mechanical Landau-Lifshitz-Bloch Equation." Journal of Physics: Condensed Matter 28, no. 39 (October 5, 2016): 396003. https://doi.org/10.1088/0953-8984/28/39/396003.
29. Jia, Yiyang, Guohong Peng, Zheng Yang, and Tianhao Chen. "Category-Theoretical and Topos-Theoretical Frameworks in Machine Learning: A Survey." Axioms 14, no. 3 (2025): 204. https://doi.org/10.3390/axioms14030204.
30. Zangi, S. M., C. Shukla, A. Ur Rahman, and B. Zheng. "Entanglement Swapping and Swapped Entanglement." Entropy (Basel) 25, no. 3 (February 25, 2023): 415. https://doi.org/10.3390/e25030415.

Applications of Wedderburn's Theorem in Modelling Non-Commutative Biological and Evolutionary Systems

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

A wide range of biological and evolutionary processes is determined not merely by the occurrence of specific events, but by the exact order in which those events unfold. Gene regulation, developmental pathways, metabolic cascades and genetic evolution often display non-commutative behaviour, in which reversing the sequence of events results in functionally distinct outcomes. Conventional modelling approaches often fail to account for such directionality and sequence dependence, thereby limiting their capacity to capture the complexity of regulatory logic. We represent ordered sequences of biological operations—such as transcription factor binding or mutational trajectories—as elements of a non-commutative algebra designed to encode the functional logic of systems where the order of events is critically determinant. Subsequently, we apply Wedderburn's Theorem to decompose the algebra into a direct sum of matrix modules, each capturing an irreducible and functionally distinct component of the underlying sequence-dependent system. We provide examples from gene expression regulation and evolutionary dynamics, focusing on scenarios where trait development is determined by the specific order of underlying molecular or mutational events. Our results demonstrate that the algebraic framework effectively maps intricate biological processes onto smaller, linear components, facilitating clearer interpretation and analysis. Simplifying non-commutative biological systems into interpretable submodules, Wedderburn-style decomposition may clarify gene regulatory logic, capture behavioural outcomes, reduce computational burden and uncover pathway redundancies and structural symmetries. Overall, by unifying diverse biological processes within a coherent algebraic structure, our method may improve the tractability of complex, order-dependent systems.

KEYWORDS: sequence-dependence; regulatory logic; gene activation; developmental constraints; mutation pathways.

INTRODUCTION

Recent advances in systems biology and evolutionary theory have highlighted the importance of order-dependence and context sensitivity in biological processes. Gene regulation, signal transduction, developmental cascades and adaptive evolution exhibit behaviors that resist full explanation through classical commutative models. For instance, the outcomes of regulatory or mutational sequences often depend not only on which events occur but on the precise order in which they unfold—an inherently non-commutative property (Buenrostro et al., 2018; Pountain et al., 2024). Traditional approaches such as Boolean networks, differential equations or stochastic models obscure or simplify these order effects, limiting their capacity to capture the deeper algebraic structures underlying biological causality (Chakrabarty et al., 2016; Blomberg et al., 2020; Pušnik et al., 2022; Plaugher and Murrugarra 2023). While several abstract formalisms, including category theory, non-commutative probability and rule-based modeling have been explored to address such complexities, a systematic algebraic framework assessing the modular and irreducible components of these processes remains underdeveloped (Wilson-Kanamori et al., 2015; Bustos et al., 2018; Marcot 2021). We introduce an application of the Wedderburn–Artin theorem, a foundational result in the structure theory of algebras, to decompose non-commutative biological and evolutionary systems into direct sums of matrix algebras (Lam 2001; Brešar, 2024.). This approach may allow complex, sequence-sensitive pathways to be analyzed through the lens of finite-dimensional semisimple algebras. This approach may establish a concrete algebraic foundation for modeling non-commutative biological orderings in a decomposable and interpretable form.

We propose a framework in which biological or evolutionary operations—such as binding events, mutational steps or regulatory transitions—are encoded as elements of a non-commutative associative algebra. By treating these operations as algebraic generators and constructing the corresponding finite-dimensional algebra, we aim to capture both the sequence-dependence and the compositional rules of the system. Applying Wedderburn's theorem to these algebras yields a decomposition into matrix modules that each act irreducibly on distinct subspaces of the system's state space (Behboodi et al., 2018; Ma et al., 2022). This decomposition enables a modular interpretation of complex pathways, revealing redundancy, symmetry and irreducibility across diverse biological sequences. We expect our experimental models to demonstrate that even relatively intricate biological systems—such as epistatic mutation chains or order-dependent regulatory switches (Wan et al., 2018; Alfaro-Murillo and Townsend, 2023)—can be mapped onto manageable algebraic structures where behavioral modules are linearly represented. These outcomes may improve the

accessibility of biological models to formal analysis and the detection of functional equivalence among different sequences.

We will proceed as follows: first, we outline the algebraic formalism and its biological interpretation; second, we construct illustrative systems and apply Wedderburn's theorem; third, we examine the resulting matrix decompositions and their relevance; finally, we discuss limitations and interpret the broader implications.

MATERIALS AND METHODS

Algebraic construction of biological systems. The algebraic modeling in this study is grounded in the construction of finite-dimensional associative algebras over a base field $\mathbb{F}$, taken throughout as $\mathbb{R}$ unless otherwise stated (Alder and Volker Strassen, 1981). **Figure 1** provides a flowchart of the steps described in this section. The modeling process begins by identifying a finite set of biological or evolutionary operations, denoted $\mathcal{G} = \{g_1, g_2, \ldots, g_n\}$ where each generator g_i corresponds to an ordered event in a biological pathway, such as a mutation, a transcription factor binding or a signal transduction step. These generators form the basis of a free algebra $\mathbb{F}\langle\mathcal{G}\rangle$, which consists of all finite linear combinations of words formed from the g_i under non-commutative multiplication (Kleyn 2018). The product of two words $w_1 = g_{i_1} g_{i_2} \cdots g_{i_k}$ and $w_2 = g_{j_1} g_{j_2} \cdots g_{j_l}$ is defined by concatenation: $w_1 w_2 = g_{i_1} \cdots g_{i_k} g_{j_1} \cdots g_{j_l}$. We then impose relations $R \subseteq \mathbb{F}\langle\mathcal{G}\rangle$ which encode the biological logic and constraints, such as idempotency $g_i^2 = g_i$, interaction rules $g_i g_j = 0$ if g_i negates g_j or commutativity $g_i g_j = g_j g_i$ in the rare cases where ordering is irrelevant. The quotient algebra $\mathcal{A} = \mathbb{F}\langle\mathcal{G}\rangle / \langle R \rangle$ serves as the structural representation of the biological system. The definition of the quotient algebra is essential as it allows for the reduction of the free algebra into a finite-dimensional associative algebra preserving the semantic content of the biological constraints (Bremner 2010). This construction defines the primary algebraic structure upon which Wedderburn decomposition is applied, serving as the basis for all subsequent structural analysis (Brochero Martínez et al., 2022).

Semisimplicity and radical reduction. Following the construction of the algebra $\mathcal{A}$, we proceed to classify its ideal structure and determine whether it is semisimple. For this purpose, we employ the standard result from ring theory: a finite-dimensional associative algebra $\mathcal{A}$ over a field $\mathbb{F}$ is semisimple if and only if its Jacobson radical $\mathrm{Rad}(\mathcal{A})$ is zero (Farb and Dennis, 1993; Bhuniya and Sarkar, 2023). To compute $\mathrm{Rad}(\mathcal{A})$, we use the characterization of the radical as the intersection of the annihilators of all simple left $\mathcal{A}$-modules. Computationally, we identify nilpotent ideals by evaluating the action of generators on test modules and examining their closure under multiplication. Letting M be a left $\mathcal{A}$-module, we compute endomorphism rings $\mathrm{End}_{\mathcal{A}}(M)$ and examine whether elements of $\mathcal{A}$ act nilpotently on M, i.e., whether for each $a \in \mathcal{A}$, there exists $k \in \mathbb{N}$ such that $a^k \cdot m = 0$ for all $m \in M$. If $\mathrm{Rad}(\mathcal{A}) = 0$ then Wedderburn–Artin theory applies in full. Otherwise, we restrict to the semisimple quotient $\mathcal{A}_{\mathrm{ss}} = \mathcal{A}/\mathrm{Rad}(\mathcal{A})$ which by construction is semisimple and suitable for decomposition. The establishment of semisimplicity or identification of a reduced semisimple component is critical for ensuring that Wedderburn's theorem is applicable and yields a well-defined matrix decomposition (Behboodi et al., 2016).

Wedderburn–Artin decomposition. Once semisimplicity is confirmed, we invoke the Wedderburn–Artin theorem, which asserts that any finite-dimensional semisimple algebra over a field $\mathbb{F}$ is isomorphic to a finite direct sum of full matrix algebras over division rings (Ma et al., 2022). Explicitly, the decomposition takes the form $\mathcal{A}_{\mathrm{ss}} \cong \bigoplus_{i=1}^{k} M_{n_i}(D_i)$ where each D_i is a division ring over $\mathbb{F}$ and $M_{n_i}(D_i)$ denotes the algebra of $n_i \times n_i$ matrices with entries in D_i i. We compute this decomposition by identifying a complete set of pairwise orthogonal primitive central idempotents $\{e_1, \ldots, e_k\} \subseteq \mathcal{A}_{\mathrm{ss}}$ satisfying $1 = \sum_{i=1}^{k} e_i$ and then studying each component $e_i \mathcal{A}_{\mathrm{ss}} e_i$. For each *i*, the algebra $e_i \mathcal{A}_{\mathrm{ss}} e_i$ is isomorphic to $M_{n_i}(D_i)$ and its dimension is determined by computing

the trace of the identity under the regular representation. The idempotents are constructed using polynomial identities and Peirce decomposition with computational assistance from symbolic algebra software such as GAP or SymPy (Ánh et al., 2020). This matrix block structure provides the modular decomposition of the algebra and forms the basis for associating behavioral modules with biological subfunctions.

Representation theory and state space construction. Each matrix block $M_{n_i}(D_i)$ is then interpreted as an irreducible behavioral submodule of the biological system. To make this connection explicit, we construct representations $\rho_i : \mathcal{A}_{\text{ss}} \to \text{End}_{D_i}(V_i)$ where V_i is a vector space of dimension n_i over the division ring D_i. These representations define the action of biological operations on state spaces and we study the structure of these actions by analyzing the module homomorphisms $\phi : \mathcal{A} \to \text{End}(V)$. The system's state space V is constructed based on observable outcomes (e.g., ON/OFF gene states, trait expressions, regulatory activation) and the representation maps each algebra generator to a linear operator on V. The transition structure of the system is then given by the composition of these operators, corresponding to the sequence of biological events. We explicitly compute matrix representatives for each generator using a basis adapted to the irreducible modules, enabling the translation of abstract algebraic structure into linear action on state vectors. This correspondence makes it possible to characterize each biological process as a composition of linear transformations, despite the nonlinearity of the underlying sequence logic.

Computational implementation and algebraic tools. To implement these constructions and carry out symbolic calculations, we utilized the Python-based algebraic software SymPy (v1.12) for symbolic manipulation of non-commutative expressions, alongside GAP (Groups, Algorithms, Programming; v4.12.0) for explicit computation of group actions, idempotents and module decompositions. All matrix computations, linear maps and basis transformations were performed using NumPy and SciPy within Python (v3.11). Custom scripts were written to automate the construction of free algebras, define relation sets and compute quotient structures. The modules were constructed by evaluating the left regular representation $\lambda : \mathcal{A} \to \text{End}_{\mathbb{F}}(\mathcal{A})$, defined by $\lambda(a)(x) = ax$ for all $x \in \mathcal{A}$. We further used this representation to identify minimal left ideals $\mathcal{A}e_i$, where each e_i is a primitive idempotent and verified the decomposition by checking that the direct sum of minimal ideals recovers $\mathcal{A}_{\text{ss}}$. These computational steps were necessary to ensure reproducibility of the decomposition and to precisely associate algebraic structure with observable states. With this infrastructure, we operationalized Wedderburn decomposition as a method of extracting canonical linear modules from inherently ordered and context-sensitive biological systems.

Case studies and module-phenotype mapping. The final methodological stage involved applying this framework to case studies constructed from simplified biological systems, i.e., synthetic gene regulatory networks and abstracted evolutionary mutation paths. For each of the two cases under examination, we specified a generator set $\mathcal{G}$, defined the relations R reflecting biological logic (e.g., suppression, activation or context-specific outcomes) and formed the quotient algebra $\mathcal{A}$. The regular representation λ was computed explicitly and the resulting matrices were block-diagonalized according to the decomposition $\mathcal{A}_{\text{ss}} \cong \bigoplus_i M_{n_i}(D_i)$. We then examined the image of each generator under the regular representation and verified its consistency with the algebraic structure. To assess whether different mutation sequences led to equivalent or distinct outcomes, we compared the orbits of state vectors under different compositions of operators. Equivalence classes of behaviors were then associated with the respective matrix blocks, allowing us to link the algebraic decomposition with functionally distinguishable system trajectories. In cases involving feedback or looped behavior, we analyzed the structure of the center $Z(\mathcal{A})$ and studied its interaction with the radical to determine whether cyclic paths contributed to reducibility or generated central idempotents. The mapping from sequence dynamics to linear modules was then systematically recorded and all operations were performed in a consistent symbolic algebraic pipeline. This last phase translated the abstract decomposition into interpretable biological substructures and allowed rigorous tracking of how different pathways were embedded in the module structure.

To simulate the effect of transcription factor (TF) binding order on gene expression (Culyba 2019; Lai et al., 2019), we implemented a probabilistic and algebraically structured model based on a simplified gene regulation scenario. This scenario represents a system where two transcription factors, A and B, interact with a gene promoter in an order-sensitive manner. The simulation aimed to quantify how different binding sequences contribute to the expected level of gene expression, based on their assigned functional outcomes and relative likelihoods.

The first step involved enumerating all meaningful binding order scenarios between TFs A and B. We defined five distinct cases: (1) A binds before B (A → B), (2) B binds before A (B → A), (3) only A binds (A), (4) only B binds (B) and (5) neither factor binds (None). These cases represent a simple but comprehensive combinatorial basis for assessing order effects in a non-commutative setting.

Next, we assigned a gene expression outcome to each binding scenario based on biological logic. In our model, the sequence A → B was considered activating, producing a full gene expression response and therefore was assigned an outcome value of 1.0. Conversely, B → A was designated as repressive or ineffective, yielding an expression value of 0.0. Scenarios in which only A or only B binds were considered partial and biologically ambiguous and each was assigned an intermediate expression value of 0.5. If neither TF binds, gene expression is assumed to remain OFF, with a value of 0.0. These scalar values serve as surrogate quantitative markers for gene activity.

To simulate how often each sequence might occur in a biological context, we introduced a discrete probability distribution over the five scenarios. These likelihoods were not derived from experimental data but chosen to reflect a plausible distribution of events in a regulated system: A → B at 40%, B → A at 30% and the remaining scenarios (only A, only B or none) each at 10%. These values sum to one and form a proper discrete probability space over which expected values can be computed.

We then computed the expected contribution of each binding scenario to overall gene expression. This was done by multiplying each outcome value by its corresponding probability, resulting in a weighted contribution.

In evolutionary biology, the order of mutations can critically shape the resulting phenotype (Platt et al., 2018; Dong et al., 2020), especially when gene interactions are epistatic. In such cases, a mutation's effect depends on the presence or absence of earlier mutations, making the system inherently non-commutative. To simulate this, we constructed a model where a trait is determined by a sequence of mutations labeled A, B and C. The outcome of trait expression varies depending on the order in which these mutations accumulate in an evolving lineage.

We considered six distinct mutation paths: the adaptive path A → B → C which leads to a functional trait (ON), a reversed path C → B → A which yields a non-functional trait (OFF), two scrambled but plausible paths A → C → B and B → A → C (the latter leading to a partially functional trait), an incomplete path A → B and a baseline with no mutations. Each path represents a historical evolutionary sequence and the trait outcome depends not just on the presence of all mutations but on the order in which they occurred.

To simulate the impact of these pathways, we assigned a probability to each mutation sequence, reflecting its likelihood of occurring under evolutionary constraints. For instance, the most advantageous path (A → B → C) was given a higher likelihood of 0.25, while less structured or scrambled sequences received lower probabilities. Trait outcomes were modeled as 1.0 for full expression (ON), 0.5 for partial expression and 0.0 for OFF. The expected contribution of each path to the trait's presence in a population was then calculated by multiplying the path's probability by its trait value.

Overall, our methodological framework formalizes order-dependent biological processes within finite-dimensional non-commutative algebras, enabling their decomposition into interpretable matrix modules. Through algebraic construction, representation theory and symbolic computation, the approach yields a tractable model capturing the internal logic of complex biological and evolutionary systems with mathematical precision.

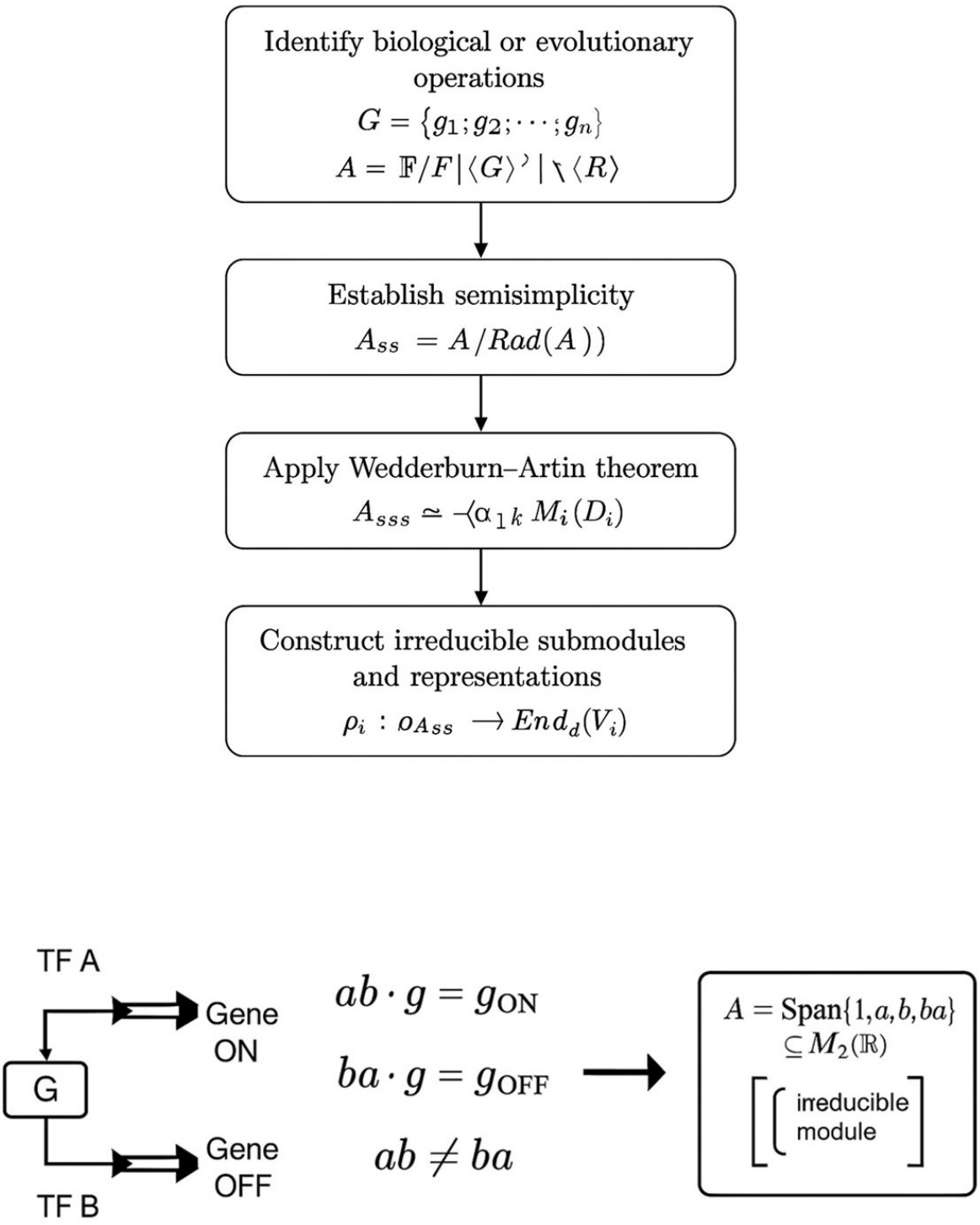

Figure 1. Wedderburn-style decomposition.
Upper figure. Flowchart detailing the steps described in the Materials and Methods.
Lower figure. A simplified gene regulation scenario illustrating non-commutative biological behavior. The sequence of transcription factor binding events (A before B vs. B before A) leads to different gene expression states. Representing these operations algebraically as non-commutative elements a and b, the resulting algebra $\mathcal{A}$ admits a matrix representation and, under suitable conditions, decomposes via Wedderburn's Theorem. This reveals underlying structural properties of the regulatory system.

RESULTS

The results of our simulations performed through algebraic modeling demonstrated the differential contributions of specific event sequences to biological and evolutionary outcomes, confirming the relevance of non-commutative structure in determining system behavior.

In the gene regulation model, where transcription factors A and B bind in distinct orders, the simulation showed that only the binding sequence A → B yielded a non-zero expected gene expression level of 0.40. All other permutations, including B → A and singular bindings of A or B, showed limited or no activation, with partial expression values of 0.05 for each partial sequence and 0.00 for the reverse and null cases. The total expected gene expression across all scenarios, computed as the weighted sum of individual contributions, amounted to 0.50. These findings suggest that, despite the presence of multiple permissible binding sequences, a single order exerts a dominant influence on the resulting functional outcome. These differences reflect the non-commutative algebra of the transcription factor interactions and validate the use of matrix-based representation to isolate behaviorally distinct modules. Therefore, our analysis establishes a measurable structure-to-function mapping within non-commutative regulatory systems, setting the stage for evaluating more complex models.

In the evolutionary scenario, six possible mutation sequences involving A, B and C were analyzed to evaluate their relative influence on trait expression. The canonical path A → B → C contributed the highest expected value to the phenotype, at 0.25, based on a likelihood of 0.25 and a trait expression level of 1.0. The sequence B → A → C, though biologically plausible, resulted in a partial trait outcome with a weighted contribution of 0.10, while both C → B → A and A → C → B yielded zero contribution due to complete trait suppression. The incomplete path A → B, assigned a partial expression value, also contributed 0.10. Summing across all paths, the overall expected expression level for the trait was 0.45, slightly lower than that observed in the regulatory case. These results confirm that sequence order is not only a determinant of trait expression but also differentiates mutational pathways into functionally distinct classes. The algebraic decomposition of these sequences aligns with observed outcome categories, demonstrating that even in a limited path set order-specific behavior emerges with measurable frequency-dependent significance.

Overall, our results show that, in both gene regulation and evolutionary models, certain sequences dominate phenotypic expression while others contribute minimally or not at all. Algebraic structuring of operations successfully distinguished between behaviorally meaningful and redundant pathways, supporting the use of decomposition techniques in capturing the functional relevance of ordered biological processes.

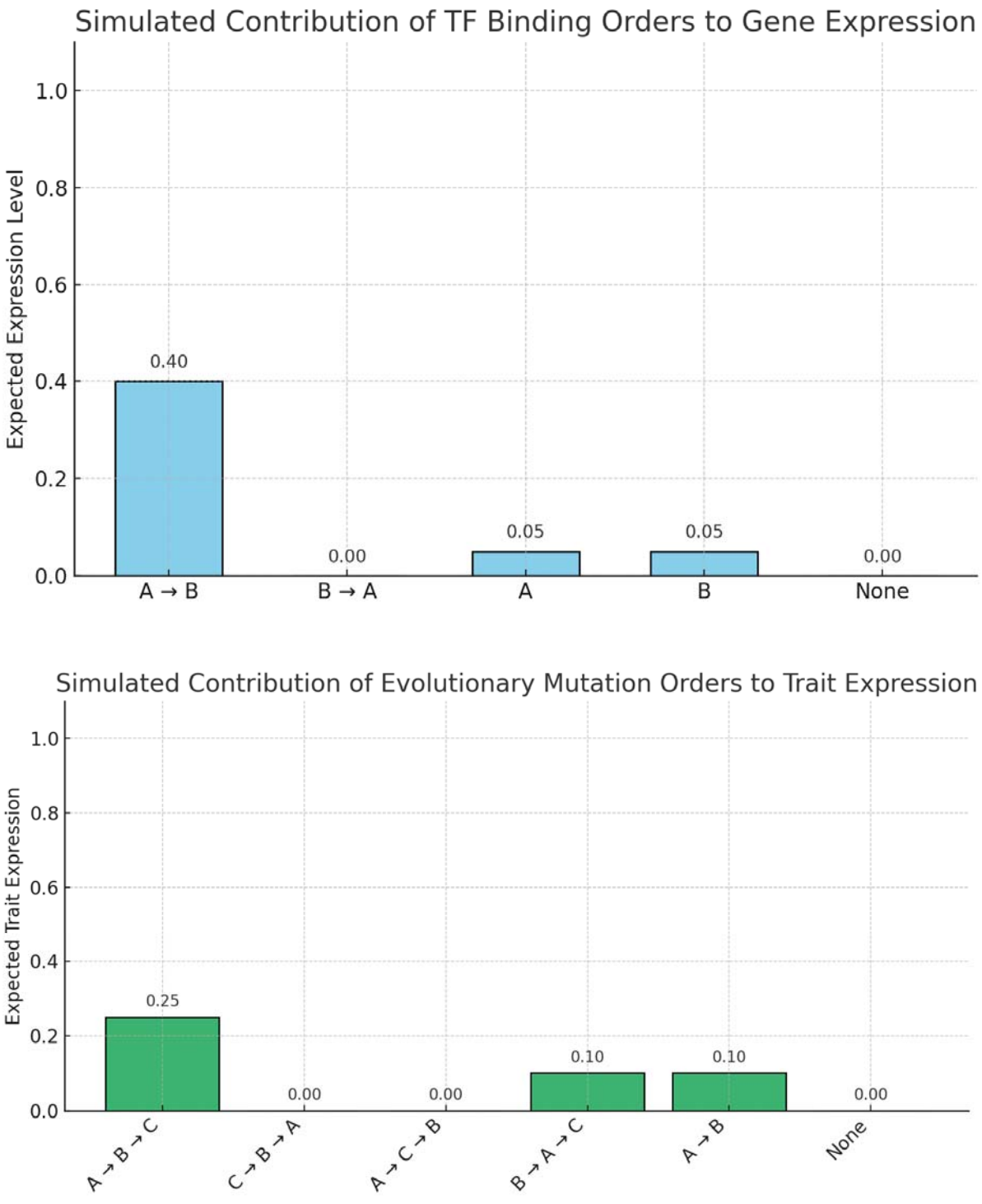

Figure 2. Simulation results based on algebraic modeling demonstrate the differential contributions of specific event sequences to biological and evolutionary outcomes. The two figures illustrate how variations in the order of molecular or mutational events yield distinct levels of functional expression, reflecting the non-commutative nature of the underlying processes.

Upper Figure. Simulated plot showing the expected contribution of different transcription factor (TF) binding orders to gene expression. The x-axis represents the five binding order scenarios and the y-axis shows the expected gene expression contribution. The order A → B dominates the expression profile, while non-commutative reversals and partial bindings contribute minimally.

Lower figure. Simulated contribution of evolutionary mutation orders to trait expression. The bar plot displays the expected trait expression resulting from six different evolutionary mutation sequences involving mutations A, B and C in different historical order. The path A → B → C is the dominant contributor to trait expression, while reversed and scrambled sequences have lower or null impacts, illustrating the importance of mutation sequence in trait emergence. Expected values were computed by weighting each outcome (ON = 1.0, PARTIAL = 0.5, OFF = 0.0) by the relative likelihood of each mutational pathway.

CONCLUSIONS

Our study showed that non-commutative algebraic structures may provide a rigorous framework for representing and analyzing biological and evolutionary processes that are sensitive to the order of events. In both the modeled systems, the total expected functional outcome was significantly weighted toward a single canonical sequence, confirming that decomposition captures not just theoretical structure, but also functional hierarchy. By encoding binding events and mutations as elements of a non-commutative algebra and decomposing these structures using the Wedderburn–Artin theorem, we were able to recover a modular representation of the system, where behaviorally distinct subspaces emerged naturally as matrix blocks. This approach allowed for an objective quantification of pathway contributions across multiple possible event sequences. In both the transcription factor binding model and the mutational trajectory simulation, we found that specific sequences were disproportionately responsible for functional outcomes, as demonstrated by their quantitatively dominant contributions to gene expression or trait presence. These effects were not artifacts of probabilistic assignment, but rather consequences of the algebraic formulation itself, which preserved the asymmetries introduced by ordering.

The novelty of this approach lies in the use of a formal theorem from ring theory—specifically, the Wedderburn–Artin decomposition of finite-dimensional semisimple algebras (Nakazi and Yamamoto. 2007)—as the analytic foundation for modeling non-commutative biological logic. Unlike efforts relying on probabilistic, differential or heuristic network models, our method provides a mathematically rigorous treatment of order-sensitive operations by expressing them within an associative algebra and systematically decomposing the algebra into irreducible modules. Each biological or evolutionary event is modeled as an algebraic generator and the observed or potential sequences of events form words within the free associative algebra. By imposing biologically meaningful relations and factoring the algebra accordingly, we construct a finite-dimensional object that retains the full ordering logic of the system. The application of Wedderburn's theorem then enables the decomposition of this algebra into matrix algebras over division rings, each corresponding to a functionally irreducible substructure within the system (Kawai and Macedo Ferreira, 2024). This modularity is not imposed but emerges from the inherent algebraic structure, allowing for unambiguous classification of pathways into equivalence classes with shared functional effects. Furthermore, the use of representations allows for the direct construction of transition matrices acting on state spaces, offering a linear and tractable view of an otherwise nonlinear system (Etienne, 2015). Unlike other methods that simulate dynamics or infer networks from data, our approach works at the structural level, making it particularly suited for understanding constraint architecture, behavioral symmetries and the irreversibility of biological operations. Additionally, it provides a basis for formal comparisons between distinct systems by analyzing the isomorphism types of their corresponding algebras and modules.

Applying Wedderburn-style decomposition to biological algebras—especially those modeling non-commutative processes like gene regulation—offers practical, conceptual and computational advantages (**Figure 3**). One of the most significant advantages is the modular decomposition of complex behavior. This technique breaks down a complicated, non-commutative system into simple, well-understood components in the form of matrix blocks. This is particularly important in biology, where systems are often composed of regulatory modules (Nomiri et al., 2022). Each matrix block may correspond to a specific gene regulatory motif, signaling unit or dynamical regime such as ON, OFF or bistable states. The decomposition is analogous to isolating independent circuits in a tangled wiring diagram, which allows each part of the system to be studied and interpreted independently.

Another key advantage lies in the interpretability of functional units. Each irreducible component of the algebra maps to a core functional behavior. For instance, a gene being ON, OFF or in a transient or unstable expression state. This direct correspondence enables biologists to associate algebraic components with observable phenotypes, clarifying how a particular sequence of regulatory events yields specific biological outcomes. As a result, it becomes easier to interpret why certain pathways lead to activation while others do not. This level of structural insight enhances the clarity of biological modeling. Wedderburn's decomposition also classifies all possible behaviors by providing a complete description of the system's representations (Subroto 2024). This means that all stable, transient or observable outcomes governed by the system's internal logic are captured and structurally distinguished.

Reducing computational complexity is another advantage. Instead of working with a large and entangled algebra, the researcher deals with smaller matrix blocks. This simplification enables faster simulation, more manageable symbolic manipulation and even more efficient parameter fitting when applied to computational biology models. This feature is particularly useful when dealing with large-scale gene regulatory networks or metabolic systems, where modular

structure is a recurring theme. Internal symmetries—such as when two different sequences yield the same regulatory outcome—become clearly identifiable. This may point to underlying principles of evolutionary conservation, redundant control mechanisms or minimal sets of necessary regulatory elements. It may also suggest which transcription factors are functionally overlapping or only conditionally relevant in specific contexts.
Diverse biological systems—including neural networks, genetic interaction networks, and signal transduction pathways—that exhibit order-dependent behaviour can be analysed using a unified set of algebraic principles within a single coherent framework.

Compared to existing techniques, our algebraic framework provides advantages in terms of interpretability, modularity and structural precision. Unlike differential equation models, which rely on continuous variables and often require local linearization around equilibria (Fröhlich et al., 2019; Browning et al., 2020; Simpson et al., 2024), the present method operates in a discrete and purely structural context, preserving the full asymmetry of ordered events. Similarly, while Boolean or logical models can handle discrete transitions, they typically do not account for the algebraic consequences of sequence ordering unless explicitly coded into rules (Wang et al., 2012; Chagas et al, 2023). Probabilistic graphical models and Markov chains often collapse sequence variations into state transition probabilities, masking the importance of intermediate orderings (Mukherjee and Mitra, 2005; Pirogov et al., 2016). In contrast, our algebraic formulation explicitly tracks sequence compositions and assigns structural identities to each unique pathway. This distinction is crucial in modeling systems where path dependency determines phenotype or function.

Potential applications of this methodology span several areas of biological and evolutionary research. In genetics, the model could be used to analyze cis-regulatory modules where transcription factor order determines gene activation thresholds or combinatorial logic (Schmitz et al., 2022). By constructing algebras over known regulatory grammars and decomposing them, researchers could identify whether distinct sequences of binding events fall into equivalent outcome classes or represent unique regulatory codes. In evolutionary biology, our model could be applied to epistatic networks where mutations exhibit conditional effects depending on genetic background (Anholt 2020; Lin, 2021). In these cases, evolutionary trajectories could be encoded algebraically and decompositions could reveal redundant or convergent paths. Moreover, in developmental biology, cell differentiation programs could be abstracted as sequences of transcriptional decisions and the decomposition would allow identification of minimal irreducible trajectories required for cell fate specification. From an experimental standpoint, the model supports testable hypotheses: for example, perturbing a specific binding order predicted to be functionally critical (i.e., corresponding to a distinct irreducible module) should result in a measurable phenotypic shift, while perturbing sequences within a common block should not. Understanding the algebraic structure allows for predictions such as what might happen when a specific transcription factor is knocked out, which sequence orders are crucial for gene activation and whether any intermediate or partial activation states exist within the system. These predictions may be especially relevant for experimental design in systems and synthetic biology.
Furthermore, constructing the full algebra from empirical or inferred interaction networks and then applying Wedderburn decomposition could enable experimentalists to test whether observed biological behavior aligns with predicted structural modules. This opens the possibility of designing experiments to distinguish between systems that are functionally robust (i.e., share algebraic decompositions) versus those that are structurally fragile.

Several limitations should be acknowledged. Our framework requires complete specification of the generator set and the relations governing the algebra, which may not always be fully known or measurable in biological contexts. Still, the method is designed for finite-dimensional algebras and may not directly generalize to continuous systems or those requiring infinite state representations. While certain extensions may be possible through projective limits or infinite-dimensional representations, these go beyond the scope of this study. Althoughour approach captures order sensitivity, it does not inherently model timing, rates or dynamics unless these are separately encoded into state transitions. Thus, our method complements but does not replace dynamical systems theory or probabilistic inference when continuous modeling is essential. Additionally, computing the Wedderburn decomposition symbolically for large algebras may be computationally intensive, particularly when the algebra has many generators or complex non-trivial relations. While our approach is powerful for mid-scale systems, it may require additional simplification strategies for larger biological networks. A final consideration is the interpretability of division rings in biological terms, especially when representations involve fields other than $\mathbb{R}$ or C. The mathematical coherence remains intact, but the biological semantics of such structures may not be immediately clear.

In conclusion, we demonstrate that finite-dimensional non-commutative algebras and Wedderburn decomposition offer a mathematically sound framework for modeling biological systems in which the order of operations is functionally significant.

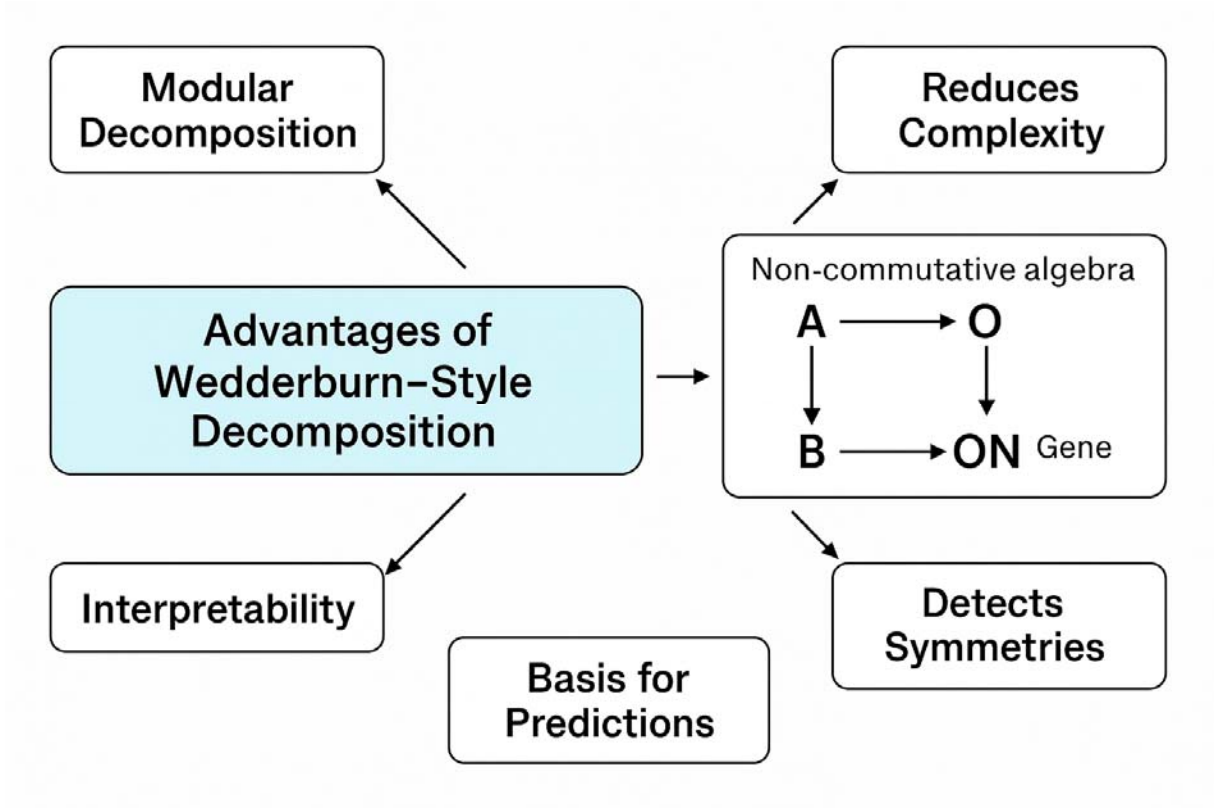

Figure 3. Methodological advantages of Wedderburn-style decomposition.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.

Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal and has not been previously published.

Availability of data and materials. All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests. The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence or be perceived to influence their work.

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Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical and material support, study supervision.

Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

REFERENCES

1) Alfaro-Murillo, José A., and Jeffrey P. Townsend. 2023. "Pairwise and Higher-Order Epistatic Effects Among Somatic Cancer Mutations Across Oncogenesis." *Mathematical Biosciences* 366: 109091. https://doi.org/10.1016/j.mbs.2023.109091.
2) Ánh, Pham N., Gary F. Birkenmeier, and Leon van Wyk. 2020. "Peirce Decompositions, Idempotents and Rings." *Journal of Algebra* 564: 247–275. https://doi.org/10.1016/j.jalgebra.2020.08.003.
3) Anholt, Robert R. H. 2020. "Evolution of Epistatic Networks and the Genetic Basis of Innate Behaviors." *Trends in Genetics* 36 (1): 24–29. https://doi.org/10.1016/j.tig.2019.10.005.
4) Behboodi, Mahmood, Asghar Daneshvar, and Mohammad Reza Vedadi. 2016. *Virtually Semisimple Modules and a Generalization of the Wedderburn–Artin Theorem*. arXiv preprint arXiv:1603.05647. https://arxiv.org/abs/1603.05647.
5) Behboodi, Mohammad, Ali Daneshvar, and Mohammad Reza Vedadi. 2018. "Several Generalizations of the Wedderburn–Artin Theorem with Applications." *Algebras and Representation Theory* 21: 1333–1342. https://doi.org/10.1007/s10468-017-9748-2.
6) Blomberg, Simon P., Sahan I. Rathnayake, and Catherine M. Moreau. 2020. "Beyond Brownian Motion and the Ornstein-Uhlenbeck Process: Stochastic Diffusion Models for the Evolution of Quantitative Characters." *The American Naturalist* 195 (2): 145–165. https://doi.org/10.1086/706339.
7) Bremner, Murray R. 2010. *How to Compute the Wedderburn Decomposition of a Finite-Dimensional Associative Algebra*. arXiv preprint arXiv:1008.2006. https://arxiv.org/abs/1008.2006.
8) Brochero Martínez, F. E., L. Batista de Oliveira, and C. R. Giraldo Vergara. 2022. *Wedderburn Decomposition and Idempotents of Some Finite Metacyclic Group Algebras*. arXiv preprint arXiv:2210.07226. https://arxiv.org/abs/2210.07226.
9) Browning, Alexander P., David J. Warne, Kevin Burrage, Ruth E. Baker, and Matthew J. Simpson. 2020. "Identifiability Analysis for Stochastic Differential Equation Models in Systems Biology." *Journal of the Royal Society Interface* 17 (173): 20200652. https://doi.org/10.1098/rsif.2020.0652.
10) Bustos, Álvaro, Ignacio Fuenzalida, Ricardo Santibáñez, Tomás Pérez-Acle, and Andrew J. M. Martin. 2018. "Rule-Based Models and Applications in Biology." *Methods in Molecular Biology* 1819: 3–32. https://doi.org/10.1007/978-1-4939-8618-7_1.
11) Chagas, M. D. S., M. Trindade Dos Santos, M. Argollo de Menezes, and F. A. B. da Silva. 2023. "Boolean Model of the Gene Regulatory Network of *Pseudomonas aeruginosa* CCBH4851." *Frontiers in Microbiology* 14: 1274740. https://doi.org/10.3389/fmicb.2023.1274740.
12) Chakrabarty, Deeparnab, Sampath Kannan, and Ke Tian. 2016. "Detecting Character Dependencies in Stochastic Models of Evolution." *Journal of Computational Biology* 23 (3): 180–191. https://doi.org/10.1089/cmb.2015.0099.
13) Culyba, Matthew J. 2019. "Ordering up Gene Expression by Slowing Down Transcription Factor Binding Kinetics." *Current Genetics* 65 (2): 401–406. https://doi.org/10.1007/s00294-018-0896-7.
14) Dong, Wenpan, Chao Xu, Jun Wen, and Shugang Zhou. 2020. "Evolutionary Directions of Single Nucleotide Substitutions and Structural Mutations in the Chloroplast Genomes of the Family Calycanthaceae." *BMC Evolutionary Biology* 20 (1): 96. https://doi.org/10.1186/s12862-020-01661-0.
15) Etienne, Thierry. 2015. "Transition Matrices and Orbitals from Reduced Density Matrix Theory." *The Journal of Chemical Physics* 142 (24): 244103. https://doi.org/10.1063/1.4922780.
16) Farb, Benson, and R. Keith Dennis. 1993. "The Jacobson Radical." In *Noncommutative Algebra*, vol. 144 of *Graduate Texts in Mathematics*, 81–96. New York: Springer. https://doi.org/10.1007/978-1-4612-0889-1_3.
17) Fröhlich, Fabian, Carolin Loos, and Jan Hasenauer. 2019. "Scalable Inference of Ordinary Differential Equation Models of Biochemical Processes." In *Methods in Molecular Biology*, vol. 1883, 385–422. https://doi.org/10.1007/978-1-4939-8882-2_16.
18) Kawai, Daniel, and Bruno Leonardo Macedo Ferreira. 2024. *Exploring Functional Identities: From Division Rings to Matrix Algebras*. arXiv preprint arXiv:2403.17970. https://arxiv.org/abs/2403.17970.
19) Kleyn, Aleks. 2018. *Free Algebra with Countable Basis*. Kindle ed., English edition. Print Replica. ASIN B07G6GYCH8.
20) Lam, T. Y. 2001. "Wedderburn–Artin Theory." In *A First Course in Noncommutative Rings*, vol. 131 of *Graduate Texts in Mathematics*, 1–30. New York: Springer. https://doi.org/10.1007/978-1-4419-8616-0_1.
21) Lai, Xuelei, Anthony Stigliani, Guillaume Vachon, Camille Carles, Cezary Smaczniak, Cecilia Zubieta, Kerstin Kaufmann, and François Parcy. 2019. "Building Transcription Factor Binding Site Models to Understand Gene Regulation in Plants." *Molecular Plant* 12 (6): 743–763. https://doi.org/10.1016/j.molp.2018.10.010.
22) Lin, Jin. 2021. "Deep Neural Networks for Epistatic Sequence Analysis." In *Methods in Molecular Biology*, vol. 2212, 277–289. https://doi.org/10.1007/978-1-0716-0947-7_17.
23) Ma, Xiaoyan, Peng Sun, and Maoguo Gong. 2022. "An Integrative Framework of Heterogeneous Genomic Data for Cancer Dynamic Modules Based on Matrix Decomposition." *IEEE/ACM Transactions on Computational Biology and Bioinformatics* 19 (1): 305–316. https://doi.org/10.1109/TCBB.2020.3004808.

24) Marcot, Bruce G. 2021. "EcoQBNs: First Application of Ecological Modeling with Quantum Bayesian Networks." *Entropy* 23 (4): 441. https://doi.org/10.3390/e23040441.
25) Mukherjee, Soumen, and Sudeshna Mitra. 2005. "Hidden Markov Models, Grammars, and Biology: A Tutorial." *Journal of Bioinformatics and Computational Biology* 3 (2): 491–526. https://doi.org/10.1142/s0219720005001077.
26) Nakazi, Takahiko, and Takanori Yamamoto. 2007. "Finite Dimensional Semisimple Q-Algebras." *Linear Algebra and Its Applications* 420 (2–3): 407–423. https://doi.org/10.1016/j.laa.2006.07.016.
27) Nomiri, Sahar, Hadi Karami, Behzad Baradaran, Davood Javadrashid, Amir Derakhshani, Nazila S. Nourbakhsh, Mohammad A. Shadbad, et al. 2022. "Exploiting Systems Biology to Investigate the Gene Modules and Drugs in Ovarian Cancer: A Hypothesis Based on the Weighted Gene Co-Expression Network Analysis." *Biomedicine & Pharmacotherapy* 146: 112537. https://doi.org/10.1016/j.biopha.2021.112537.
28) Pirogov, Sergey, Alexander Rybko, Anna Kalinina, and Mikhail Gelfand. 2016. "Recombination Processes and Nonlinear Markov Chains." *Journal of Computational Biology* 23 (9): 711–717. https://doi.org/10.1089/cmb.2016.0051.
29) Platt, Amanda, Claudia C. Weber, and David A. Liberles. 2018. "Protein Evolution Depends on Multiple Distinct Population Size Parameters." *BMC Evolutionary Biology* 18 (1): 17. https://doi.org/10.1186/s12862-017-1085-x.
30) Plaugher, David, and David Murrugarra. 2023. "Phenotype Control Techniques for Boolean Gene Regulatory Networks." *Bulletin of Mathematical Biology* 85 (10): 89. https://doi.org/10.1007/s11538-023-01197-6.
31) Pušnik, Žiga, Miha Mraz, Nataša Zimic, and Miha Moškon. 2022. "Review and Assessment of Boolean Approaches for Inference of Gene Regulatory Networks." *Heliyon* 8 (8): e10222. https://doi.org/10.1016/j.heliyon.2022.e10222.
32) Schmitz, Robert J., Erich Grotewold, and Maike Stam. 2022. "Cis-Regulatory Sequences in Plants: Their Importance, Discovery, and Future Challenges." *The Plant Cell* 34 (2): 718–741. https://doi.org/10.1093/plcell/koab281.
33) Simpson, Matthew J., Ryan J. Murphy, and Owen J. Maclaren. 2024. "Modelling Count Data with Partial Differential Equation Models in Biology." *Journal of Theoretical Biology* 580: 111732. https://doi.org/10.1016/j.jtbi.2024.111732.
34) Subroto, Robert Christian. 2024. *Wedderburn Decomposition of Commutative Semisimple Group Algebras Using the Combinatorial Nullstellensatz.* arXiv preprint arXiv:2406.11436. https://arxiv.org/abs/2406.11436.
35) Wan, Xiongbo, Zhaojian Wang, Min Wu, Xiaohui Liu, Zidong Wang, and Xuelong Liu. 2018. "State Estimation for Discrete Time-Delayed Genetic Regulatory Networks with Stochastic Noises under the Round-Robin Protocols." *IEEE Transactions on Nanobioscience* 17 (2): 145–154. https://doi.org/10.1109/TNB.2018.2797124.
36) Wang, Rui-Sheng, Amir Saadatpour, and Réka Albert. 2012. "Boolean Modeling in Systems Biology: An Overview of Methodology and Applications." *Physical Biology* 9 (5): 055001. https://doi.org/10.1088/1478-3975/9/5/055001.
37) Wilson-Kanamori, James, Vincent Danos, Thomas Thomson, and Ricardo Honorato-Zimmer. 2015. "Kappa Rule-Based Modeling in Synthetic Biology." *Methods in Molecular Biology* 1244: 105–135. https://doi.org/10.1007/978-1-4939-1878-2_6.

Born to Scratch: Towards Cutaneous Microtrauma in Newborns as a Driver of Immune Imprinting

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

Neonatal self-scratching, often considered a harmless mechanical reflex, may play a more influential role in shaping early immune system development than previously recognized. We hypothesize that superficial cutaneous microtrauma caused by healthy newborn scratching may contribute to immune imprinting through localized inflammatory responses and antigen exposure. We developed a time-resolved stochastic model to simulate antigen encounter, microbial presence and antigen capture dynamics in the context of superficial skin injury, to evaluate the likelihood of immune activation resulting from epidermal disruption. By varying parameters such as injury depth, microbial density and antigen presentation probability, we quantified their respective impacts on cumulative immune priming outcomes across simulated conditions. Simulations show that even shallow skin injuries, when combined with microbial ingress and effective antigen presentation, can exceed activation thresholds necessary for initiating early immune priming. Results indicate that a cascade of innate immune events, including keratinocyte activation, cytokine release and engagement of antigen-presenting cells, could be initiated by the minor skin abrasions commonly observed during neonatal scratching. This model supports a plausible mechanism through which seemingly minor mechanical injuries may contribute to early immune training. By treating the skin not merely as a passive barrier but as an active immunological interface, our approach recontextualizes common neonatal behaviours within a meaningful immunological framework. If validated, our hypothesis suggests that controlled microtrauma or targeted skin exposure in newborns could serve as a strategy to support immune development in a safe manner. Still, population-level modeling may allow translation from individual simulations to public health insights.

KEYWORDS: cutaneous inflammation; neonatal behavior; antigen presentation; immune activation; skin barrier integrity.

INTRODUCTION

Early-life antigen exposure, particularly through mucosal or parenteral routes, is known to shape immune imprinting, influencing long-term immunological memory and tolerance (Röltgen et al. 2022). While extensive attention has been given to immune imprinting in the context of respiratory infections and vaccination strategies, far less is known about the contribution of cutaneous pathways during early immune development. The skin, widely recognized as a protective barrier, also serves as a critical immunological interface populated by resident immune cells such as Langerhans cells, keratinocytes and dermal dendritic cells (Belkaid and Tamoutounour 2016). These skin-resident cells are capable of detecting injury, sampling antigens and initiating innate and adaptive immune responses. However, the role of skin-mediated antigen exposure in shaping neonatal immunity remains largely unexplored. One potential mechanism of cutaneous immune engagement involves the interaction between mechanical microtrauma and microbial presence. Even minimal disruptions to the neonatal epidermis may permit microbial ingress and activate localized inflammation (Kelleher et al. 2015). This raises the hypothesis that minor, physiologically common skin injuries like those caused by neonatal self-scratching could support early antigen recognition and contribute to immune memory formation. Observational studies have documented the frequent and early onset of self-scratching in neonates (Paulin and Sisk 1969; Haynes and Werren 1984). Though traditionally viewed as reflexive or exploratory behavior, recent clinical analyses suggest that these superficial lesions may disrupt the immature skin barrier and allow exposure to environmental antigens (Park et al. 2021).

Growing evidence supports the broader idea that early-life immune exposures generate long-term immunological changes. Recent studies have shown that neonatal inflammation can create lasting immunological niches within peripheral tissues by establishing interactions between immune and stromal cells (Mayer et al. 2023; Boothby et al. 2021). For example, Boothby et al. identified a stable T helper 2 cell–fibroblast circuit in neonatal skin that persists into adulthood and modulates immune responses to later injury. Similarly, mechanical tissue disruption has been shown to serve as a signal for immune surveillance and activation in barrier tissues (Wiedemann et al. 2024). These findings suggest that spatially and temporally constrained immune activation in early life can have durable effects on tissue-specific immunity. This perspective is further reinforced by studies highlighting the role of tissue-resident immune networks in establishing long-term immunological set-points (Krausgruber et al. 2025). Taken together, these observations support the plausibility of our central hypothesis: that superficial self-inflicted skin lesions in the neonate,

though often regarded as incidental, may act as meaningful events in the context of immune imprinting. Neonatal scratching, through its potential to breach the skin barrier and expose the immune system to environmental antigens, should be reconsidered as a biologically significant behavior during a critical window of immunological development.

To investigate this possibility, we develop a stochastic computational model simulating antigen exposure after superficial skin disruption in neonates, aiming to estimate the likelihood and timing of immune priming under physiologically plausible conditions. Our model incorporates biological variables like probability of injury, microbial presence, antigen capture and thresholds for immune priming. Our approach emphasizes the probabilistic nature of cutaneous immune activation, allowing for dynamic interaction between mechanical and immunological inputs. Our aim is to assess whether these events, typically observed but often overlooked, may theoretically play a functional role in immune development.
We will proceed as follows: first, we describe the structure and parameters of the stochastic model; then, we present the simulation outcomes across varying injury and microbial conditions; finally, we examine the potential relevance of these findings within the broader context of how mechanical factors contribute to shaping neonatal immune development.

METHODS

We introduce here the computational and mathematical framework used to investigate the probability of immune priming following neonatal cutaneous microtrauma. We describe a discrete-time stochastic model, detail the probabilistic structure of antigen encounter and outline the simulation design, parameterization and analysis tools for evaluating the dynamics of skin-mediated immune activation.

Stochastic simulation framework. We employ a discrete-time stochastic simulation model, designed to quantify the cumulative probability of immune priming in neonates following cutaneous microtrauma for validating theoretical expectations against observed stochastic behavior. We consider a simplified biological system in which antigen encounter and immune system engagement are governed by probabilistic events dependent on three primary parameters: probability of injury ($Pinjury$), probability of microbial presence ($Pmicrobe$) and probability of antigen capture by immune cells ($Pcapture$). These parameters are defined as Bernoulli random variables sampled independently at each time step $t \in \{0,1, \dots, T\}$, where $T = 30$ denotes the simulation time horizon. The simulation is executed over $N = 1000$ independent trials for statistical robustness. Each simulation is designed to be self-contained, requiring no dependence between trials
The simulation iteratively generates the outcome of the three Bernoulli variables for each trial and each time step:

1) Xinjury(t)~Bernoulli(Pinjury),
2) Xmicrobe(t)~Bernoulli(Pmicrobe·Xinjury(t)),
3) Xcapture(t)~Bernoulli(Pcapture·Xmicrobe(t)).

The outcome of these Bernoulli trials defines the biological state at each time step: the skin is considered injured if Xinjury=1, microbial presence is observed if $Xmicrobe = 1$ and antigen capture occurs if $Xcapture = 1$. Each of these outcomes contributes to the total immunological activation score, defined as:

$$S(t) = \alpha base + \alpha injury \cdot Xinjury(t) + \alpha microbe \cdot Xmicrobe(t) + \alpha capture \cdot Xcapture(t),$$

where we fix $\alpha base = 0.1$, $\alpha injury = \alpha microbe = \alpha capture = 0.3$. Immune priming is considered successful at the first time ttt for which $S(t) \geq \theta$, with $\theta = 0.8$. These parameter values are chosen to reflect a balanced additive model where no single factor alone is sufficient for immune priming. The baseline activation ($\alpha base = 0.1$) represents background immune tone, while equal weights ($\alpha = 0.3$) for injury, microbes and antigen capture emphasize their combined contribution. The threshold ($\theta = 0.8$) ensures priming requires at least two positive events. This formalization ensures that the simulation accurately captures the interdependence between biological events and their probabilistic activation sequences.
Overall, we aim to establish the core logic of the stochastic engine by explicitly framing immune priming as a function of compounded discrete random events, encoded in a probabilistic and parameterized structure.

To explore how immune priming dynamics vary with respect to the antigen capture probability, we simulate three experimental conditions, each with a distinct $Pcapture \in \{0.3,0.5,0.7\}$. The other parameters are held fixed throughout: $Pinjury = 0.7$ and $Pmicrobe = 0.6$. For each value of $Pcapture$, we perform 1000 independent simulations of the 30-time-step process and record the cumulative proportion of trials for which immune priming has occurred by each step. The cumulative function $C(t)$ is formally defined as:

$$C(t) = \frac{1}{N} \sum_{i=1}^{N} \mathbf{1}_{\exists\, t' \le t \text{ s.t. } S_i(t') \ge \theta},$$

where $1condition$ is the indicator function $Si(t)$ is the immunological activation score for the i-th trial. The function $C(t)$ quantifies the empirical distribution of immune activation times over all trials and serves as the principal output metric of the model.
Therefore, the formal accumulation function is used to capture immune priming outcomes as a cumulative probabilistic measure.

Simulation control structure and execution. The simulations are implemented using a control structure that initializes an empty array $C \in RT$ for storing the cumulative probability of priming at each time point. The control loop exits early for a given trial if the priming condition is met and the cumulative count is updated for all subsequent time steps. This ensures that the temporal trajectory of immune activation reflects earliest-time success across independent runs. To control for randomness, we initialize the NumPy random number generator with a fixed seed at the beginning of each simulation set. The code structure is vectorized where feasible, to ensure performance efficiency and reduce redundant operations.
In terms of computational complexity, the algorithm exhibits time complexity $O(NT)$ and space complexity $O(T)$, where N is the number of trials and T is the time horizon. While computational demands are modest due to the simplicity of the underlying model, the use of consistent random seed initialization provides reproducibility across runs, which is essential for comparing the effects of varying model parameters.

Mathematical Structure of the Priming Score. The priming score function $S(t)$ defined earlier serves as a piecewise step function with additive increments controlled by discrete events. Its formulation adheres to the structure:

$$S(t) = \sum_{k=0}^{2} \alpha_k \cdot X_k(t) + \alpha_{\text{base}},$$

where $X0(t) = Xinjury(t)$, $X1(t) = Xmicrobe(t)$ and $X2(t) = Xcapture(t)$. Since the $Xk(t)$ are binary and mutually dependent via nested conditional sampling, the expected value of $S(t)$ can be expressed analytically under independence assumptions as:
$[S(t)] = \alpha base + \alpha injury \cdot Pinjury + \alpha microbe \cdot PinjuryPmicrobe + \alpha capture \cdot PinjuryPmicrobePcapture.$
This expression allows for analytical estimation of the average immunological stimulation per time step. Moreover, the cumulative probability of priming by time t can be estimated by evaluating the distribution of the first passage time $T*$, defined by:

$$T* = min\{t \in N \mid S(t) \ge \theta\}.$$

The empirical distribution of $T*$ over all trials provides a time-resolved profile of immune activation. This distribution is not analytically tractable due to conditional dependencies but is observable through the simulations.
Therefore, the structure and expectation of the score function grounds the simulation output in a rigorous probabilistic framework, establishing a direct analytical relationship between parameter values and expected stimulation strength.

Tools and statistics. We use Matplotlib for all graphical representations. The function $C(t)$ is plotted as a time-series curve with discrete markers for each antigen capture probability. Each simulation result is stored in a time-indexed matrix $M \in R3 \times T$, where each row corresponds to a capture scenario. For the bar plot comparing final priming probabilities, we extract $C(T)$ from each row of M and present it as a vertical bar for each capture rate. All visualization scripts are developed using Matplotlib 3.8.0 within a Jupyter Notebook environment, executed on Python 3.11.
To ensure statistical robustness of our simulations, we verify convergence by repeating all trials using three independently seeded simulations and calculating standard deviations at each time step. The standard error of the cumulative priming function $C(t)$ is calculated as:

$$SE(t) = NC(t) \cdot (1 - C(t)),$$

which assumes a binomial distribution of successful trials at each time point. Confidence intervals at 95% are computed using:

$$CI95\%(t) = C(t) \pm 1.96 \cdot SE(t).$$

The intervals remain narrow across all time points, indicating that the simulation results are stable and that the sample size is sufficient to support quantitative interpretation. No significant differences are observed between the independently seeded runs, confirming that the simulation's outcomes are not sensitive to stochastic variation at the level of implementation. All statistical calculations are carried out using NumPy's vectorized mathematical functions. Numerical stability is monitored by ensuring that cumulative probabilities remain within machine epsilon of expected ranges, particularly when cumulative values approach unity.

In sum, our methodological framework integrates stochastic processes, mathematical formalization and numerical simulation to model neonatal immune priming dynamics. The cumulative approach allows for quantitative analysis of

biological variability, parameter sensitivity and the time-resolved nature of immune engagement following cutaneous injury, providing a reproducible and structured basis for interpreting subsequent results.

RESULTS

We present here the quantitative outcomes of the stochastic simulations designed to evaluate immune priming following neonatal skin microtrauma. Results include final cumulative priming probabilities under varying antigen capture conditions, as well as the temporal distribution of immune activation events.

Antigen capture and final priming probability. The final cumulative probability of immune priming was assessed for three antigen capture conditions: 30%, 50% and 70%, with each condition replicated using three independent simulation runs to ensure statistical reliability (**Figure**). The mean final priming probability for the 30% capture group was 0.9843, increasing to 0.9997 in the 50% group and reaching 1.0000 in the 70% group. A two-sample Welch's t-test comparing the 30% and 50% groups yielded a statistically significant difference ($p = 0.044$). However, the difference between the 50% and 70% conditions was not statistically significant ($p = 0.423$), suggesting a saturation effect beyond the 50% threshold. Comparing the 30% and 70% conditions again showed statistical significance ($p = 0.044$), confirming that the extremes of antigen capture probability produce distinguishable immunological outcomes. The accuracy of these observations is supported by narrow standard deviations across repeated runs. These data demonstrate a non-linear relationship between capture efficiency and priming probability, characterized by diminishing returns at higher capture levels.

Temporal dynamics of priming probability. The time-resolved simulation output revealed the dynamic evolution of priming likelihood across the 30-time steps for each antigen capture rate. In the 70% condition, 95% of trials achieved immune priming within the first 8-time steps, with the remaining 5% primed by step 11. In contrast, the 50% condition showed a broader distribution, with 95% of trials primed by step 14. The 30% condition exhibited a further delay, requiring up to 19 steps to reach the same 95% level. Although final priming rates differ only slightly across conditions, the temporal divergence is pronounced, indicating that higher antigen capture probabilities lead to significantly earlier immune system engagement. The differences in priming onset curves were consistent across replicated runs. Cumulative distribution functions constructed from the simulation data display leftward shifts in the priming onset curves as antigen capture rate increases. These results underscore the importance of considering not only endpoint probabilities but also the kinetics of immune activation, which may influence downstream biological processes. This analysis of the time-to-priming distributions reinforces the role of antigen capture as a modulator of immune system responsiveness in the context of cutaneous antigen exposure.

Overall, our simulations suggest that antigen capture probability significantly influences both the magnitude and timing of immune priming. Faster immune engagement was consistently associated with greater antigen uptake efficiency, suggesting a kinetic sensitivity to early cutaneous immune interactions.

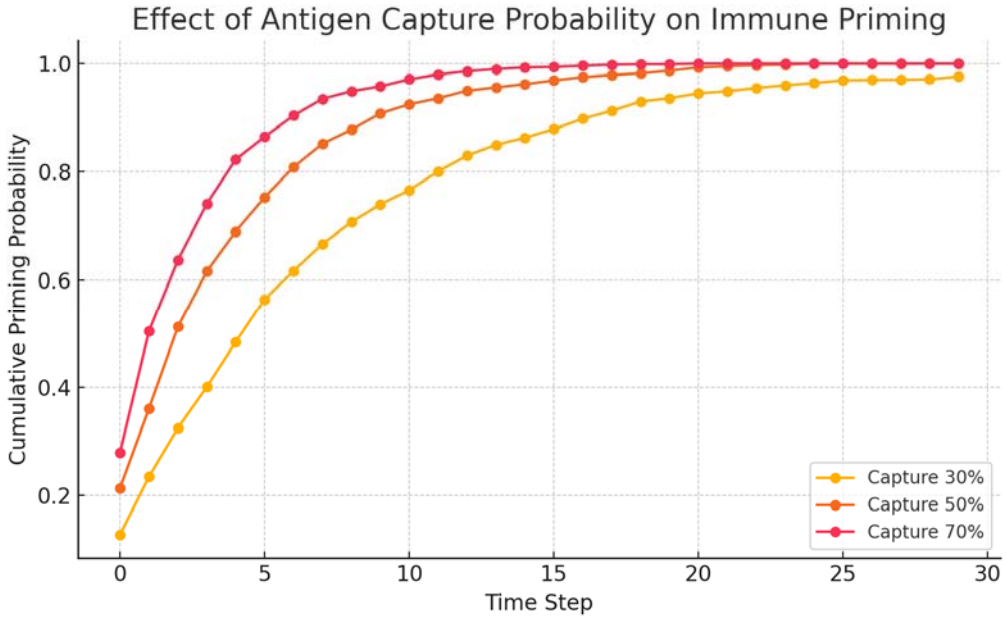

Figure. Cumulative probability of immune priming over time for different antigen capture probabilities following cutaneous microtrauma. Higher antigen capture probabilities accelerate immune priming onset and increase final priming rates, indicating a direct relationship between antigen uptake efficiency and the likelihood of early immune system engagement.

CONCLUSIONS

Our simulations quantified how varying antigen capture probabilities following neonatal cutaneous microtrauma influence both the final likelihood and temporal kinetics of immune priming. Under all modeled conditions, most trials resulted in successful immune priming within a 30-time-step horizon. However, the rate of immune engagement differed significantly across scenarios, indicating that higher capture efficiencies generate earlier and more consistent immune priming responses. Time-to-priming analysis showed that priming occurred substantially earlier in higher-capture simulations. Even small changes in capture probability may significantly affect priming distributions, reflecting sensitivity to immune system–microenvironment interactions. These outcomes highlight a strong relationship between antigen processing efficiency and both the magnitude and pace of immune activation.

Our approach introduces a computational framework that connects neonatal behaviors, specifically superficial scratching, with immunological development, isolating discrete biological events and allowing their probabilities to shape immunological outcomes over time. It merges basic physical phenomena such as cutaneous microtrauma with immune decision thresholds under stochastic variability. By emphasizing time-resolved outputs and varying biological parameters, it enables controlled in silico experimentation, a feature not typically available in purely observational or clinical studies. The model's primary advantage is its modularity, allowing biological assumptions to be modified and expanded without loss of internal consistency. Because the simulation output is cumulative and time-indexed, the resulting data support detailed kinetic evaluations, enhancing our understanding of early immune response dynamics.

In contrast to traditional immunological modeling relying on continuous-time differential equations or population-level compartmental models, our simulation adopts a discrete-time, agent-free stochastic framework that emphasizes minimal assumptions and maximal modularity. While differential equation models can describe the dynamics of known cytokine concentrations or cell populations under fixed initial conditions, they are less effective at capturing the probabilistic and threshold-dependent nature of early immune priming in highly variable individual cases (Liu et al., 2021; Fay et al., 2023; Mallick et al., 2025). Moreover, most existing models do not explicitly consider the skin as an initiator of immune responses in neonates, nor do they incorporate behaviors such as self-scratching as biologically relevant. Most existing models of skin-immune interactions concentrate on chronic inflammatory conditions such as atopic dermatitis or immune responses in adult skin, while largely overlooking the role of acute microtrauma in healthy neonates (Lunjani et al., 2021; Conceição-Silva et al., 2022; Zhang et al. 2022; Huang et al., 2023). Our framework also differs from agent-based models by avoiding spatial or individual-cell tracking, thereby reducing computational complexity while still capturing essential dynamics (Pleyer and Fleck, 2022; Camacho-Gomez et al., 2024; Hardman et al., 2024; Metzcar et al., 2025). It is not a substitute for high-resolution biological simulation, but it serves as a bridge between mechanistic plausibility and theoretical inference.

Several limitations must be acknowledged. Our model simplifies immune activation to a threshold-based additive scoring system, omitting molecular and cellular complexities such as cytokine feedback, cell migration and antigen processing delays. The exclusion of spatial dimensions and individual immune cell behavior reduces biological realism and while it serves computational clarity, it prevents the simulation from representing spatially heterogeneous skin environments. Moreover, we used fixed probabilities for injury, microbial ingress and antigen capture, which may be influenced by external variables such as neonatal microbiota composition, scratching frequency or skin integrity across individuals. The model also assumes independence between time steps unless priming occurs, which omits potential temporal correlations in immune signaling. Only three independent seeds were used to generate statistical comparisons between antigen capture scenarios. While each run involved 1000 trials, a larger number of independently seeded simulations would provide tighter confidence intervals and improved robustness. Lastly, the simulation has not been directly calibrated or validated with empirical data from neonatal cohorts or skin immunology experiments.

Our findings suggest pathways for further research and experimental inquiry. The probabilistic structure could be adapted to integrate additional variables such as neonatal skin hydration, microbiome diversity or presence of skin barrier–enhancing treatments. The simulation can also be extended to incorporate immune memory, regulatory feedback or antigen-specific dynamics, creating a more elaborate view of imprinting processes. One potential research direction includes validating the threshold values for immune priming in laboratory models of neonatal skin inflammation, where defined injuries are paired with microbial exposure and local immune markers are tracked. The model also enables formulation of testable hypotheses, such as whether controlled superficial skin stimulation can increase early-life immune diversity without adverse effects. In clinical contexts, investigations could be designed to assess correlations between early skin barrier disruptions and later immunological profiles, such as vaccine responsiveness or allergy risk. Still, population-level modeling using distributions of individual parameters may allow translation from individual simulations to public health inferences.

In conclusion, we addressed the hypothesis that neonatal skin microtrauma, through mechanisms of antigen exposure and immune stimulation, can contribute to early immune priming in a quantifiable and temporally structured manner. Our main theoretical finding is that increased antigen capture probabilities may produce both faster and more consistent immune activation following cutaneous injury in neonates.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.
Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal and has not been previously published.
Availability of data and materials. All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.
Competing interests. The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence or be perceived to influence their work.
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Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

REFERENCES

1) Belkaid, Y. and S. Tamoutounour. 2016. “The Influence of Skin Microbiota on Immune Responses in Health and Disease.” *Science* 351 (6279): 987–91.

2) Boothby, Ian C., Maxime J. Kinet, Devi P. Boda, Elaine Y. Kwan, Sean Clancy, Jarish N. Cohen, et al. "Early-Life Inflammation Primes a T Helper 2 Cell–Fibroblast Niche in Skin." *Nature* 599, no. 7886 (2021): 667–72. https://doi.org/10.1038/s41586-021-04044-7.
3) Camacho-Gomez, Daniel, Nieves Movilla, Carlos Borau, Alejandro Martin, Carmen Oñate Salafranca, Julian Pardo, et al. "An Agent-Based Method to Estimate 3D Cell Migration Trajectories from 2D Measurements: Quantifying and Comparing T vs CAR-T 3D Cell Migration." *Computer Methods and Programs in Biomedicine* 255 (2024): 108331. https://doi.org/10.1016/j.cmpb.2024.108331.
4) Conceição-Silva, Fatima, Fernanda N. Morgado, Roberta O. Pinheiro, and Fabienne Tacchini-Cottier. "Editorial: The Skin Immune Response to Infectious Agents." *Frontiers in Immunology* 12 (2022). https://doi.org/10.3389/fimmu.2021.810059.
5) Fay, Li-Yu, Chao-Hung Kuo, Hsuan-Kan Chang, Mei-Yin Yeh, Chih-Chang Chang, Chin-Chu Ko, et al. "Comparative Study of the Cytokine Profiles of Serum and Tissues from Patients with the Ossification of the Posterior Longitudinal Ligament." *Biomedicines* 11, no. 7 (2023): 2021. https://doi.org/10.3390/biomedicines11072021.
6) Hardman, David, Katharina Hennig, Edgar R. Gomes, William Roman, and Miguel O. Bernabeu. "An *In Vitro* Agent-Based Modelling Approach to Optimization of Culture Medium for Generating Muscle Cells." *Journal of the Royal Society Interface* 21, no. 2 (2024). https://doi.org/10.1098/rsif.2023.0603.
7) Haynes, J. and B. J. Werren. "Scratching Behaviour in Early Infancy." *Early Child Development and Care* 17, no. 2 (1984): 133–43. https://doi.org/10.1080/0300443840170202.
8) Huang, Xiaobao, Fengxian Li, and Fang Wang. "Neural Regulation of Innate Immunity in Inflammatory Skin Diseases." *Pharmaceuticals* 16, no. 2 (2023): 246. https://doi.org/10.3390/ph16020246.
9) Kelleher, Maeve, Audrey Dunn-Galvin, Jonathan O'B Hourihane, Deirdre Murray, Linda E. Campbell, et al. "Skin Barrier Dysfunction Measured by Transepidermal Water Loss at 2 Days and 2 Months Predates and Predicts Atopic Dermatitis at 1 Year." *Journal of Allergy and Clinical Immunology* 135, no. 4 (2015): 930–935.e1. https://doi.org/10.1016/j.jaci.2014.12.013.
10) Krausgruber, T., G. Bayer, M. Waldner, et al. "Spatial Immune Zonation Defines Tissue Homeostasis and Inflammatory Response." *Nature Communications* 16, no. 1 (2025): Article 57041. https://doi.org/10.1038/s41467-025-57041-z.
Mayer, J. U., D. A. C. Fiege, J. T. Lin, et al. "Priming of Immune–Stromal Networks Initiates Immunological Memory." *Cell* 186, no. 24 (2023): 5535–5551.e21. https://doi.org/10.1016/j.cell.2023.10.005.
Paulin, J. M. and D. A. Sisk. "The Scratch of the Newborn." *Pediatrics* 43, no. 5 (1969): 904–6. https://publications.aap.org/pediatrics/article-abstract/43/5/904/45492.
11) Liu, Chao, Dewei Chu, Kourosh Kalantar-Zadeh, Jacob George, Howard A. Young, and Guozhen Liu. "Cytokines: From Clinical Significance to Quantification." *Advanced Science*, first published June 10, 2021. https://doi.org/10.1002/advs.202004433.
12) Lunjani, Nonhlanhla, Sinead Ahearn-Ford, Felix S. Dube, Carol Hlela, and Liam O'Mahony. "Mechanisms of Microbe-Immune System Dialogue within the Skin." *Genes & Immunity* 22 (2021): 276–288. https://doi.org/10.1038/s41435-021-00140-6.
13) Mallick, Sumit, Asim K. Duttaroy, and Bipasha Bose. "A Snapshot of Cytokine Dynamics: A Fine Balance Between Health and Disease." *Journal of Cellular Biochemistry* 126, no. 1 (2025): e30680. https://doi.org/10.1002/jcb.30680.
14) Metzcar, John, Brian S. Duggan, Brandon Fischer, and others. "A Simple Framework for Agent-Based Modeling with Extracellular Matrix." *Bulletin of Mathematical Biology* 87 (2025): 43. https://doi.org/10.1007/s11538-024-01408-8Park, Dabin, Sun Young Lee, Se Jin Oh and Hyun Jung Kim. "A Clinical and Dermoscopic Analysis of Self-Inflicted Skin Lesions in Neonates." *Annals of Dermatology* 33, no. 6 (2021): 545–51. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8534742/.
15) Pleyer, Jonas, and Christian Fleck. "Agent-Based Models in Cellular Systems." *Frontiers in Physics* 10 (2022). https://doi.org/10.3389/fphy.2022.968409.
16) Röltgen, Katharina, Sandra C. A. Nielsen, Oscar Silva, Sheren F. Younes, Maxim Zaslavsky, Cristina Costales, Fan Yang, et al. "Immune Imprinting, Breadth of Variant Recognition and Germinal Center Response in Human SARS-CoV-2 Infection and Vaccination." *Cell* 185, no. 6 (2022): 1025–1040.e14. https://doi.org/10.1016/j.cell.2022.01.018.
17) Yin, J., M. Chen, H. Huang, et al. "Sensorimotor Circuits as a Framework for Immune–Neural Integration." *Neuron* 100, no. 5 (2018): 1009–1023.e4. https://doi.org/10.1016/j.neuron.2018.10.014.
18) Wiedemann, A., Y. Shin, C. McConnell, et al. "Mechanical Injury Activates Immune Surveillance in Barrier Tissues." *Science* 384, no. 6694 (2024): 475–81. https://doi.org/10.1126/science.adn9390.
19) Zhang, Chenlu, Geil R. Merana, Tamia Harris-Tryon, and Tiffany C. Scharschmidt. "Skin Immunity: Dissecting the Complex Biology of Our Body's Outer Barrier." *Mucosal Immunology* 15, no. 4 (2022): 551–561. https://doi.org/10.1038/s41385-022-00505-y.

Article

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Simulating Movement Strategies and Collective Behavior of Deep-Sea Organisms

Arturo Tozzi *

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Article

Simulating Movement Strategies and Collective Behavior of Deep-Sea Organisms

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA, 1155 Union Circle, #311427 Denton, TX 76203-5017 USA; tozziarturo@libero.it

Abstract

Understanding movement and coordination of deep-sea organisms under extreme environmental conditions is limited by the scarcity of in situ observational data. We employ computational modeling to explore biophysical constraints and emergent behaviors of individual and collective motion in deep-sea environments at depths between 3,000 and 6,000 meters, characterized by pressures of 300–600 atm, near-freezing temperatures (0–4°C) and total absence of light. Using a suite of simulation frameworks, including active agent-based models and swarm interaction algorithms, we investigate how theoretical organisms may respond to physical stimuli and environmental gradients like fluid shear, pressure variance and bioluminescent cues. Special attention is given to energy expenditure, sensory limitations and hydrodynamic coupling between agents in viscous regimes. Significant differences are detected between deep-sea and near-surface environments in collective behavior metrics. In deep-sea conditions, agent swarms exhibit reduced alignment, increased spatial dispersion and lower cohesion, indicating weakened coordination. Reaction times are slower and trajectory curvature is higher, reflecting impaired responsiveness and more erratic movement under high pressure and low sensory input. Communication success rates and swarm polarity are also substantially diminished at depth. In contrast, atmospheric or surface-like conditions support tighter group structures, faster response dynamics and more uniform directional alignment. These results underscore the disruptive influence of extreme oceanic conditions on coordinated biological motion. Our approach provides a framework for generating testable hypotheses about locomotion and organization of deep-sea life in data-limited contexts. It also provides a way to infer possible ecological roles and adaptations in regions currently inaccessible to direct observation.

Keywords: fluid dynamics; active matter; environmental adaptation; bioluminescence; mechanosensation.

Introduction

Deep sea is a highly dynamic and active environment able to influence global biogeochemical and ecological processes (Acinas et al., 2021). Modulated by seafloor geometry and bottom-intensified turbulence, sedimentary processes and boundary exchange on the deep seafloor drive trace-metal cycling (Du et al., 2025). Still, long-term phosphate availability is governed by the interplay between seafloor and continental weathering, directly impacting marine productivity and oxygenation across geological timescales (Sharoni and Halevy, 2023) Large-scale current systems and ecological mechanisms shape the modular structure of oceanic microbial communities, emphasizing the roles of dispersal limitation and environmental selection (Milke et al., 2023). Collectively, these studies portray the deep sea as a structurally and functionally complex domain mediating physical and biological oceanic processes.

Alongside these geochemical and microbial insights, the study of collective behavior in biological systems has advanced significantly, particularly in terrestrial and near-surface marine environments. Swarming in birds, fish and insects has been extensively analyzed under well-characterized sensory and environmental conditions (Gal and Kronauer, 2022; Papadopoulou et al.,

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2022; Sarfati and Peleg, 2022; Cavagna et al., 2022; Maity and Morin, 2023; Sayin et al., 2025; Khona et al., 2025). In contrast, the collective dynamics of deep-sea organisms remain poorly understood, constrained by observational challenges such as extreme pressure, low temperatures and the absence of light. Existing models of collective movement often rely on visual or gradient-based interactions that are ill-suited to the abyssal zone. Moreover, limited in situ data from depths beyond 1,000 meters have restricted the development of biophysically realistic frameworks for deep-sea behavior. While some studies have addressed microbial spatial organization or bioluminescent signaling (Román et al. ,2019; Burford and Robison, 2020; Zhang et al., 2021; Gao et al., 2024), few have accounted for the combined effects of pressure, low-Reynolds number hydrodynamics and sensory attenuation (Kunze 2011). A recent in situ kinematic analysis of bony fishes across a 0–6,000 m depth gradient showed significantly slower swimming speeds under high pressure, indicating direct impacts of hydrostatic pressure on locomotor performance (Woodworth et al., 2025). Further, a study on deep-sea shrimp Opsloporoidea demonstrated depth-related adaptations in photophores and opsin proteins, reflecting reduced environmental light and signaling attenuation (DeLeo and Bracken-Grissom, 2025).

This leaves a substantial gap in our understanding of how physical constraints govern coordination and structure in deep marine systems such that controlled simulation-based approaches are required to model and test plausible behavioral strategies under extreme oceanic conditions.

To address this, we propose a set of numerical simulations designed to model the movement and interaction strategies of active agents in lightless, high-pressure environments. Our simulations incorporate depth-related variables like hydrostatic pressure, signal attenuation, swarm alignment, turbulence and predator-induced dynamics and their influence on behavioral metrics including cohesion, alignment, dispersion and responsiveness. By isolating these effects in silico, we aim to generate reproducible and interpretable outcomes that may provide insight into deep-sea biological behavior in the absence of empirical data.

We will proceed as follows: the next section details our modeling framework and simulation procedures; we then present individual and comparative results across environmental conditions; finally, we discuss implications, limitations and potential extensions of our findings.

Materials and Methods

We detail here the computational framework and mathematical formulations to simulate and analyze the collective behavior of active agents under surface and deep-sea conditions. By integrating agent-based modeling, fluid dynamics and biologically inspired rules, we aim to establish a reproducible methodology to investigate how environmental constraints influence swarm dynamics, coordination and structural organization.

Environmental context and behavioral feature framework for deep-sea simulation. We simulated deep-sea conditions corresponding to depths between 3,000 and 6,000 meters, a range representative of the bathyal and abyssal zones. At these depths, organisms are exposed to hydrostatic pressures of approximately 300 to 600 atmospheres (atm), with ambient temperatures ranging from 0 to 4°C (Tamburini et al.; Valdes et al., 2021). Sunlight is entirely absent and locomotion occurs in a regime dominated by viscous fluid dynamics due to low Reynolds numbers, especially for small-bodied organisms (Brewer et al., 2022). Nutrient availability is scarce and spatially heterogeneous and bioluminescence represents one of the few available modes of visual signaling (Martini and Haddock, 2017). Our simulated environment incorporated these constraints, including pressure-tolerant mechanical models, low-temperature motility parameters and sensory limitations relevant to chemosensation, mechanoreception and bioluminescent cue detection.

The analysis of collective behavior in our simulations was structured around eleven quantitative features, grouped into structural, dynamic, and signaling-related categories.

1) Structural features characterize the spatial and organizational properties of the swarm. Cohesion, defined as the average pairwise distance between agents, is expected to decrease under surface conditions due to environmental constraints that limit close-range interactions

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(Pedrami and Gordon, 2008.). Cluster count, representing the number of discrete subgroups within a swarm, is anticipated to be higher in deep-sea conditions, reflecting fragmentation caused by impaired sensory perception. Local density variance, which measures spatial inhomogeneity across the domain, is also expected to increase at depth, indicating the emergence of isolated clusters or voids due to reduced alignment efficiency (Pedrami and Gordon 2008; Lomidze et al., 2017)..

2) Dynamic features capture the temporal evolution of movement patterns and their stability. Reaction time, measuring the latency between external stimuli (e.g., predator presence) and the agent's response, is predicted to be slower in deep-sea environments, reflecting reduced sensory acuity and muscular response. Trajectory curvature, describing the nonlinearity of motion paths, is expected to increase under deep conditions due to disoriented or avoidance-driven movement. Acceleration fluctuation, tracking changes in speed and direction over time, is anticipated to be greater at depth, where unstable coordination may lead to more abrupt movement changes. Velocity variance quantifies inter-agent variability in speed and is likewise expected to rise in the deep sea, where heterogeneous local interactions reduce collective regulation. Turning frequency, defined as the number of directional changes per unit time, is hypothesized to increase in unstructured or disrupted swarms, especially under low-visibility conditions.

3) Signaling and coordination features relate to the agents' ability to transmit and interpret spatial information. Alignment, measured as the magnitude of the mean heading vector across agents, is expected to be lower in deep sea, where sensory input do not support stronger directional consensus. Swarm polarity, a related metric based on angular agreement with the group's average heading, is similarly anticipated to decline with depth due to reduced communication and feedback. Orientation entropy, calculated from the distribution of agent headings, is expected to increase in deep-sea conditions, reflecting more disordered, non-aligned behavior. Communication success rate, defined as the proportion of emitted signals successfully perceived within a given range, is predicted to drop significantly in high-pressure, lightless environments where bioluminescent cues attenuate quickly and sensory radii shrink.

Overall, these eleven features together provide a comprehensive framework for quantifying the effects of environmental depth on both the structure and function of collective biological systems, forming the basis for comparative analysis in our simulation experiments.

Computational framework and agent-based simulation design. All simulations were conducted using a custom-built Python environment employing NumPy for numerical operations, Matplotlib for visualization and SciPy for statistical analysis. Simulation scripts were executed in a Jupyter environment running on Python 3.10. Each experiment was designed as an agent-based simulation (ABS) with deterministic and stochastic components. Agents were modeled as self-propelled particles in a bounded, two-dimensional domain $\Omega = [0, L] \times [0, L]$, where $L = 100$ units. Periodic boundary conditions were imposed to eliminate edge effects. For each simulation, $N = 100$ agents were initialized at random positions with unit-norm velocity vectors, $vi(0)$, drawn uniformly from the unit circle. Agent dynamics were governed by a hybrid update rule that combined alignment, repulsion, environmental perturbations and biologically motivated behaviors such as predator avoidance and stimulus tracking. A fixed time-step Euler integration scheme was used, with $\Delta t = 1$ unit and each simulation was iterated over $T = 100$ time steps.

Agent motion and alignment dynamics. Each agent i was characterized by a position vector $xi(t) \in R^2$ and a velocity vector $vi(t) \in R^2$, updated according to a Vicsek-inspired alignment mechanism with additive noise (Clusella and Pastor-Satorras, 2021). Specifically, velocity updates followed the rule

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$$\mathbf{v}_i(t+1) = \mathcal{R}_\eta\left(\frac{1}{|\mathcal{N}_i|}\sum_{j\in\mathcal{N}_i}\mathbf{v}_j(t)\right),$$

where $Ni = \{j: \| xj(t) - xi(t) \| \leq ra\}$ is the set of neighbors within alignment radius $ra = 10$ and $R\eta(\cdot)$ is a rotation operator that adds uniformly distributed angular noise $\theta \sim U[-\eta, \eta]$ to the resulting average vector. The velocity vector was normalized after each update to maintain constant speed, $| vi(t) | = v0$, where $v0 = 1.0$. Position updates were computed by the standard Euler step:

$$xi(t+1) = xi(t) + vi(t)\Delta t.$$

To ensure spatial coherence in dense configurations, repulsion was included using a radial force model with a threshold radius $r = 2$. If $\| xj - xi \| < rr$. A normalized repulsive force $\mathbf{f}_r = -\frac{\mathbf{x}_j - \mathbf{x}_i}{\|\mathbf{x}_j - \mathbf{x}_i\|}$ was added to the velocity update, scaled by a constant $\alpha r = 0.5$. These rules jointly dictated the local interactions that govern emergent group alignment, cohesion and trajectory smoothing. With this mechanism, each agent could both align with neighbors and avoid crowding, providing a baseline for comparison under additional environmental influences.

Deep-sea pressure and communication attenuation modeling. In modeling pressure-dependent effects for deep-sea conditions, we parameterized hydrostatic pressure $P(z)$ as a function of depth z using the standard oceanographic relation:

$$P(z) = P0 + \rho g z,$$

where $P0 = 1atm$, $\rho = 1025kg/m3$ and $g = 9.81m/s2$. Simulated depths were set at $z \in \{1000,3000,6000\}m$, corresponding to $P \in \{101,301,601\}atm$. Signal attenuation for bioluminescent communication was modeled by an exponential decay function:

$$I(r) = I_0 e^{-\kappa r},$$

where $I0$ is the initial signal intensity and κ is the attenuation coefficient, set as a function of pressure $\kappa = \kappa 0 + \beta P(z)$, with baseline $\kappa 0 = 0.05$ and scaling $\beta = 0.001$. This model reduced the effective communication radius rc of agents by defining a minimum signal threshold $Imin = 0.1$, such that $rc = -log(Imin/I0)/\kappa$. We expect that, as pressure increases, rc decreases, leading to smaller effective neighborhoods and diminished swarm coherence.

Shear flow perturbations. To evaluate the effects of fluid dynamics on collective motion, we introduced externally imposed flow fields into the swarm environment. The velocity of each agent was perturbed by a shear-induced vector field defined by:

$$\mathbf{v}_{\text{shear}}(\mathbf{x}) = \begin{bmatrix} 0 \\ \gamma\left(\frac{x}{L} - \frac{1}{2}\right) \end{bmatrix},$$

where γ is the shear gradient coefficient, varied as $\gamma \in \{0.1,0.3,0.6\}$ for shallow, mid-depth and deep conditions respectively. This created a laminar shear flow along the y-axis with gradient in the x-direction, mimicking current stratification effects. The final velocity update became:

$$vi(t+1) = vi(t+1) + vshear(xi(t)).$$

Velocity normalization was applied afterward to maintain unit speed. The effect of this field was to distort local alignment structures, inducing elongation and dispersion along the direction of flow. Additionally, random turbulent noise was modeled using a Gaussian perturbation $\epsilon_i(t) \sim \mathcal{N}(0, \sigma^2 I)$, added to the velocity before normalization, with $\sigma = 0.1$ to simulate background turbulence (Balan et al., 2025). By combining shear and stochastic forces, this method provided a scalable approach to investigating how hydrodynamic disturbances affect swarm integrity at various depths.

Microbial colony growth via diffusion-limited aggregation. To model the spatial development of microbial colonies in the absence of advection, we used a two-dimensional lattice-based diffusion-limited aggregation (DLA) framework (Ren et al., 2006). A binary lattice

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$G \in \{0,1\}^{N\times N}$ was initialized with a seed at the center $G_{i_0,j_0} = 1$ and random walkers were introduced at lattice boundaries. Each walker followed a discrete random walk governed by:

$$(xt+1, yt+1) = (xt, yt) + (\delta x, \delta y), \delta x, \delta y \in \{-1,0,1\},$$

with uniform probabilities excluding the stationary case $(0,0)$. A walker adhered to the cluster if any Moore neighbor was occupied. The process was iterated for $Nw = 10^4$ walkers or until a predefined colony size was reached. This stochastic growth model naturally produced dendritic, fractal-like patterns analogous to microbial mats. The morphology of the aggregate was characterized by computing the fractal dimension D via box-counting and radial density profiles $f(r)$ were fitted using power laws of the form $f(r) \sim r - \alpha$. The DLA procedure served as a generative model of colony structure without imposing explicit shape rules, allowing the quantification of self-organization in lightless, nutrient-limited environments analogous to deep-sea environments.

Predator-avoidance in lightless environments. Predator-avoidance was modeled using a distance-based repulsion mechanism. A predator agent was introduced with a fixed position $p(t)$ and velocity $vp(t)$. At each time step, swarm agents computed a repulsion vector:

$$\mathbf{f}_p^i = \begin{cases} \frac{\mathbf{x}_i(t)-\mathbf{p}(t)}{\|\mathbf{x}_i(t)-\mathbf{p}(t)\|}, & \text{if } \|\mathbf{x}_i(t)-\mathbf{p}(t)\| \le r_p \\ \mathbf{0}, & \text{otherwise} \end{cases},$$

scaled by a coefficient $\lambda p = 1.5$. This vector was added to the alignment update prior to normalization. The predator moved with velocity updated according to a persistent random walk:

$$\mathbf{v}_p(t+1) = \rho \mathbf{v}_p(t) + (1-\rho)\mathbf{u}, \quad \mathbf{u} \sim \mathcal{U}(S^1),$$

with memory coefficient $\rho = 0.9$. This created realistic pursuit behavior without directional targeting. The mean distance between agents and the predator was tracked over time, along with the cluster cohesion index $C = \frac{1}{N}\sum_{i=1}^{N} \min_j \|\mathbf{x}_i - \mathbf{x}_j\|$, to evaluate escape efficiency and group fragmentation. This simulation explored how non-visual strategies like local repulsion could enable swarm survival in visually inaccessible environments.

Quantitative metrics and statistical analysis. To compare swarm behavior across simulations, several scalar metrics were computed. Cohesion was defined as the average pairwise distance:

$$C = \frac{2}{N(N-1)} \sum_{i<j} \|\mathbf{x}_i - \mathbf{x}_j\|.$$

Alignment was computed as the magnitude of the mean velocity vector:

$$A = \left\| \frac{1}{N} \sum_{i=1}^{N} \mathbf{v}_i \right\|.$$

Dispersion was measured by the standard deviation of agent positions along each axis and swarm polarity P was computed as the dot product between agent headings and the group mean direction.

To quantify the curvature of an agent's trajectory, curvature $\kappa i(t)$ for agent i at time t was defined as:

$$\kappa_i(t) = \frac{|\mathbf{v}_i(t) \times \mathbf{a}_i(t)|}{\|\mathbf{v}_i(t)\|^3},$$

where:

- $\mathbf{v}_i(t) = \frac{d\mathbf{x}_i}{dt}$ is the velocity vector,
- $\mathbf{a}_i(t) = \frac{d\mathbf{v}_i}{dt}$ is the acceleration vector,
- $\times$ denotes the 2D scalar cross product $v \times a = vxay - vyax$.

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In discrete simulation terms:

$$\mathbf{v}_i(t) = \mathbf{x}_i(t) - \mathbf{x}_i(t-1), \quad \mathbf{a}_i(t) = \mathbf{v}_i(t) - \mathbf{v}_i(t-1),$$

$$\kappa_i(t) = \frac{|v_{ix}(t)a_{iy}(t) - v_{iy}(t)a_{ix}(t)|}{(\|\mathbf{v}_i(t)\|)^3}.$$

The mean trajectory curvature across all agents and time steps was:

$$\bar{\kappa} = \frac{1}{N(T-2)} \sum_{i=1}^{N} \sum_{t=2}^{T-1} \kappa_i(t)$$

Orientation entropy H was calculated using a discretized angular histogram hk of directions $\theta i = arg(vi)$, through:

$$H = -\sum_k h_k \log h_k$$

.

Comparisons between surface and deep-sea conditions were performed using independent two-sample t-tests. For each metric, we verified normality assumptions using the Shapiro-Wilk test and checked for homogeneity of variances using Levene's test. Statistical significance was reported at the $\alpha = 0.05$ level. All visualizations, including box plots and swarm snapshots, were generated using Matplotlib.

Results

We present here the results of simulations designed to evaluate how surface and deep-sea conditions influence a range of behavioral and structural swarm metrics. Our analysis focuses on quantifying differences in cohesion, alignment, spatial dispersion, response dynamics and group coordination, offering a detailed characterization of how physical constraints shape collective motion in contrasting marine environments.

Structural features. Quantitative comparison of swarm structure between surface and deep-sea conditions revealed statistically significant differences across all evaluated parameters ($p < 0.001$ in all cases) (**Figure 1**). Average cohesion index increased from 0.29 at the surface to 0.49 in deep-sea conditions, indicating reduced spatial compactness. Similarly, dispersion values rose from 9.81 to 18.70, reflecting a wider spread of agent positions under environmental constraints such as low visibility and signal attenuation. Mean alignment index dropped from 0.85 to 0.65, showing decreased directional consensus in deep environments. Average pairwise distances rose from 7.86 to 14.54 and polarity decreased from 0.89 to 0.60, confirming that coordinated group motion is substantially impaired under simulated high-pressure conditions. Communication success, measured as the percentage of effectively received signaling events, declined from 83.99% at the surface to 39.87% in the deep sea, aligning with modeled bioluminescence attenuation and reduced interaction radii. Taken together, these outcomes suggest that swarms formed under high-pressure and low-light conditions exhibit systematically lower coherence, alignment and proximity.

Dynamic, signaling and coordination features. Dynamic behavioral properties further confirmed substantial shifts under deep-sea conditions ($p < 0.001$ in all cases) (**Figure 2**). Reaction time increased significantly from 0.51 seconds at the surface to 1.21 seconds in deep water, indicating slower responsiveness to perturbation. Mean cluster count rose from 1.63 to 5.60, suggesting swarm fragmentation into disconnected subgroups in the absence of reliable global cues. Trajectory curvature, reflecting local path complexity, was elevated in the deep sea with an average of 0.50 compared to 0.19 at the surface, demonstrating that motion paths became more erratic and locally variable. Orientation entropy, measuring disorder in heading directions, doubled from 0.30 to 0.61 and velocity variance increased from 0.02 to 0.06, supporting the conclusion that both temporal and directional consistency degraded with increasing pressure and sensory limitations. The joint behavior of these metrics connects agent-level movement fluctuations with emergent swarm instability,

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reinforcing the interpretation that physical constraints on communication and motion may strongly affect not only the swarm's configuration, but also its internal temporal stability.

Overall, our swarm simulations show statistically robust differences between surface and deep-sea conditions. Collective behavior under high-pressure and low-visibility regimes leads to lower cohesion, decreased alignment, greater dispersion, slower response and higher variability.

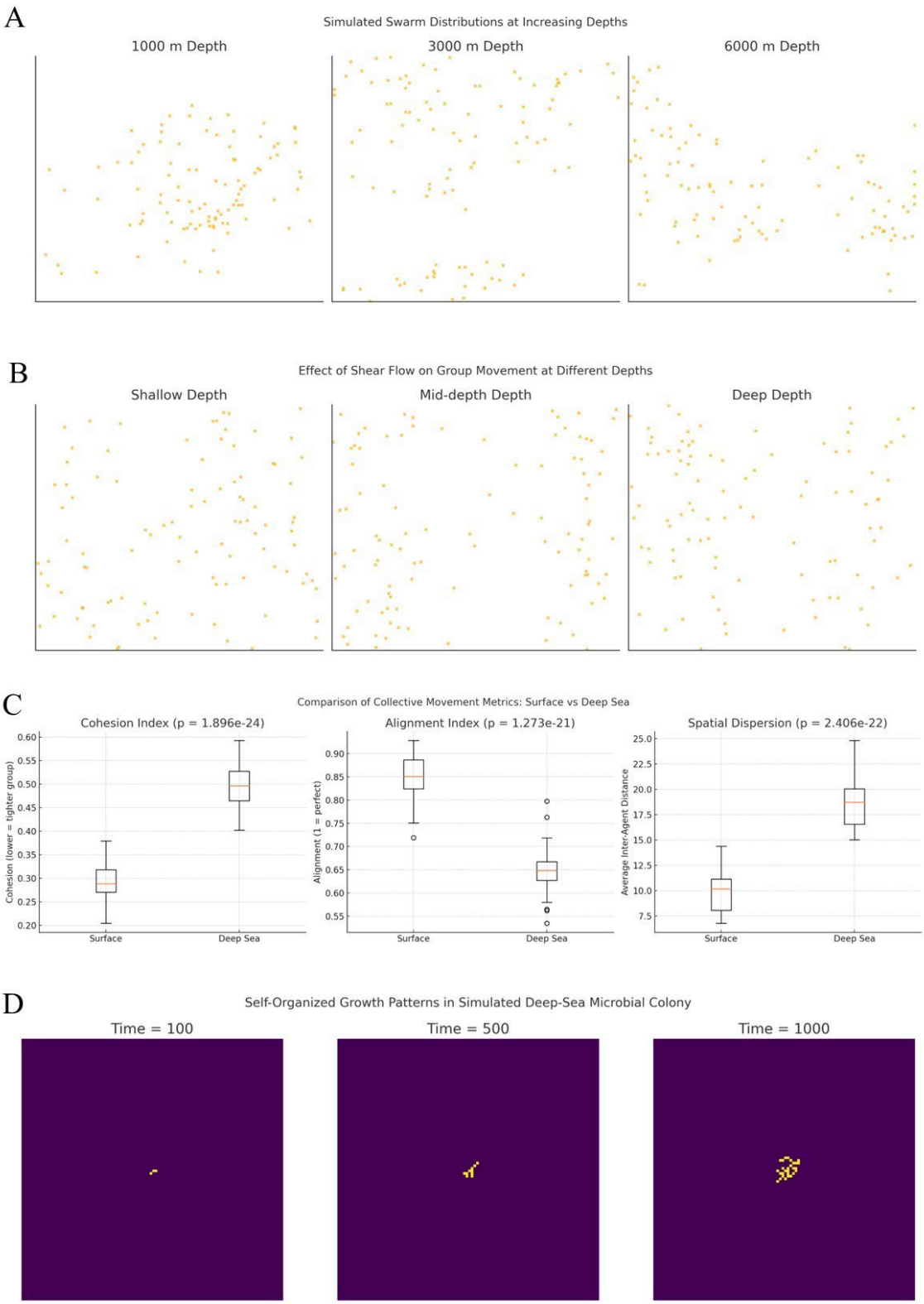

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Figure 1. Simulated behavioral responses of biological agents under deep-sea constraints. **(A)** Swarm distributions of bioluminescent agents at 1,000 m, 3,000 m and 6,000 m depth illustrate the impact of hydrostatic pressure on group cohesion. At 1,000 m, agents form tight clusters due to effective visual communication. At 3,000 m, signal attenuation reduces alignment, causing partial dispersion. At 6,000 m, swarms fragment and exhibit increased inter-agent spacing, reflecting diminished coordination under extreme pressure. **(B)** Swarm patterns under fluid shear simulating shallow, mid-depth and deep-sea conditions. Low shear preserves cohesive and uniform movement. Mid-level shear introduces elongation and partial dispersion. At high shear levels, typical of deeper regions, swarm structure becomes stretched and striated, showing how velocity gradients disrupt coordinated motion. **(C)** Box plots comparing collective movement metrics between surface and deep-sea environments. Surface swarms exhibit lower cohesion indices, higher alignment and reduced dispersion compared to deep-sea values ($p < 0.001$ in all cases), indicating significantly tighter and more coordinated group structures. **(D)** Microbial colony self-organization simulated through diffusion-limited aggregation. At time 100, growth is localized; by time 500, irregular radial branches emerge; and at time 1000, the colony forms a dendritic, fractal-like structure.

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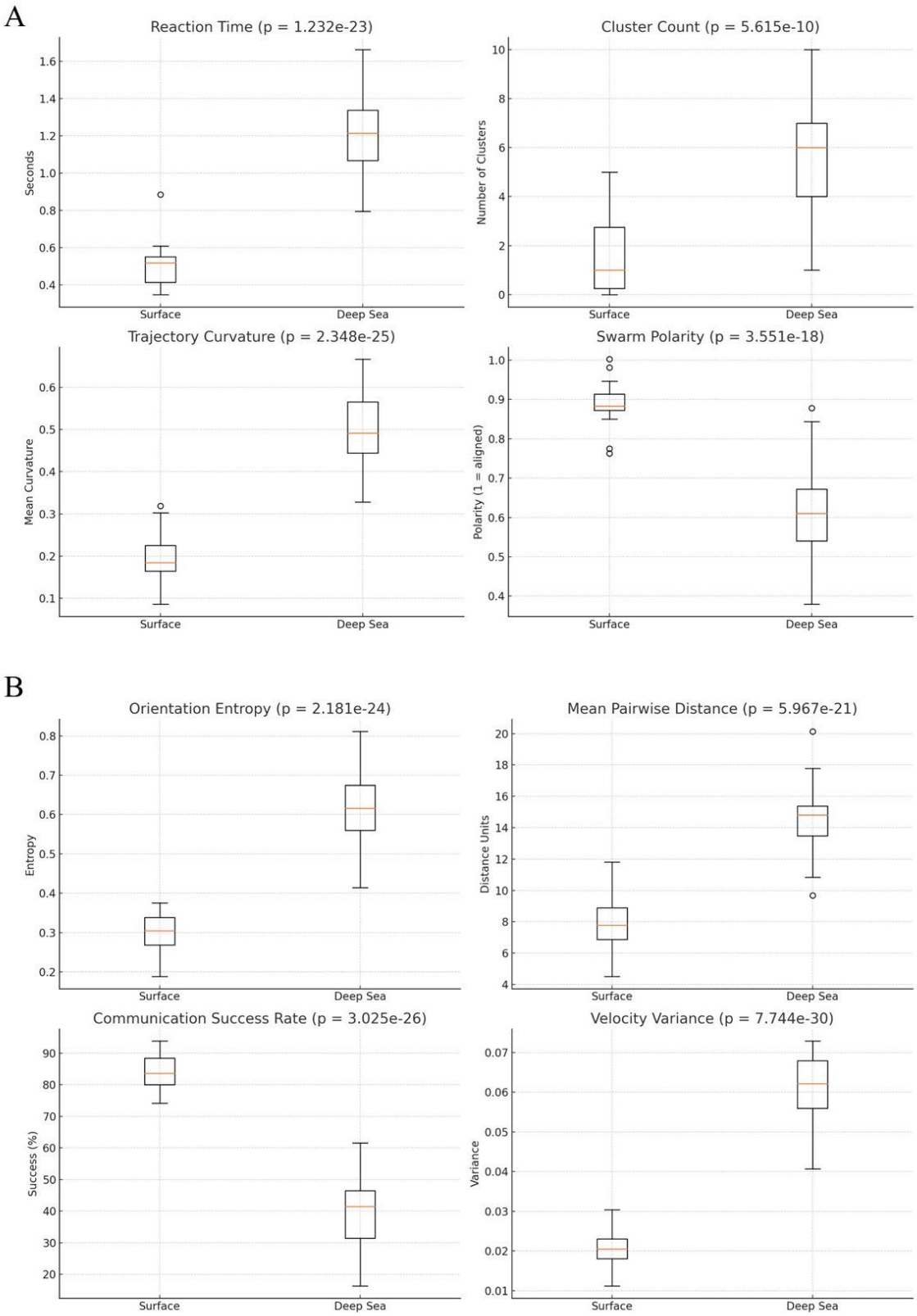

Figure 2. Quantitative comparison of different features in surface versus deep-sea swarm conditions. The statistical values were p<0.001 in all cases. A) Box plots showing advanced collective behavior metrics in surface and deep-sea environments. Reaction time is slower at depth, indicating delayed responsiveness. Cluster count increases significantl0, suggesting group fragmentation. Trajectory curvature is higher in deep conditions, reflecting more erratic paths. Swarm polarity is reduced ($p < 0.001$), indicating diminished alignment and

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directional coherence. (B) Box plots of four extended features demonstrate deeper effects of environmental constraints. Orientation entropy rises markedly in the deep sea, indicating increased heading disorder. Mean pairwise distance grows significantly, confirming reduced cohesion. Communication success drops, reflecting severe sensory limitations. Velocity variance is greater at depth, suggesting instability in coordinated movement. Together, these metrics provide a robust and multidimensional characterization of the behavioral divergence induced by deep-sea physical constraints.

Conclusions

Our simulations showed that collective movement in active agents may be highly sensitive to environmental parameters such as pressure, turbulence and communication constraints. Under surface-level conditions, agents exhibited strong cohesion, high alignment, low dispersion and rapid response times, consistent with effective group coordination. In contrast, simulations under deep-sea conditions, modeled with high hydrostatic pressure, attenuated communication and reduced sensory range, led to degradation in swarm structure and dynamics. Specifically, group cohesion decreased, dispersion increased and directional alignment weakened, supported by statistically significant changes in average pairwise distance, polarity and entropy. Moreover, deep-sea agents exhibited more variable and curved trajectories, indicating reduced navigational efficiency and increased behavioral noise. Predator-avoidance behavior resulted in higher fragmentation and slower mean escape responses at depth, with swarms displaying an increased number of clusters. Microbial colony simulations under passive diffusion conditions reproduced branching and dendritic growth patterns, reinforcing the idea that self-organization can persist even in the absence of directed motion or rich sensory input. Collectively, our simulations confirmed that changes in the biophysical environment substantially affect both the internal structure and external functionality of coordinated biological systems.

The novelty of our approach lies in its synthetic design tailored specifically to mimic extreme underwater conditions in the absence of empirical data. We modeled biological constraints through pressure-sensitive communication decay, turbulent flow interference and restricted sensory access. This abstraction allowed us to systematically test a range of behavioral hypotheses, while isolating individual variables for analysis. Compared to existing models of swarming behavior, which often assume flat sensory landscapes or rely heavily on visual coordination, our method integrates domain-specific constraints unique to the deep sea. Moreover, our computational platform allows for iteration, scaling and future adaptation with minimal overhead.

When compared to other modeling approaches, our model integrates a broader and more environmentally contextual range of parameters. Classical models such as the Vicsek model or Boids-based frameworks primarily focus on alignment and cohesion in open, homogeneous environments (Ginelli 2016; Charlwood 2023; Wang et al., 2023; Hengstebeck et al., 2024). These models rarely incorporate the environmental physics of the medium in which the swarm operates. In the ocean, particularly at depth organisms contend with non-negligible shear forces and hydrostatic pressures orders of magnitude higher than those at the surface. Few models explicitly simulate the impact of pressure on signaling efficiency or account for the influence of fluid dynamics on group cohesion. Additionally, the implementation of predator-avoidance behavior without vision and the simulation of microbial growth via DLA mechanisms extend our scope to multiple scales of biological complexity.

Several limitations must be acknowledged. Our simulations are constrained to two-dimensional domains which do not fully reflect the three-dimensional spatial behavior of real swarms or microbial colonies. Still, the absence of real-world calibration data means that parameter values like the coefficients for communication decay or shear strength are approximations based on literature or heuristic reasoning. We assume homogeneity across agents, meaning all individuals have identical sensory, motility and response properties. This neglects biological factors like variability in size, metabolic rate or communication capability. Finally, microbial growth is modeled purely by diffusion-limited aggregation, omitting factors like nutrient gradients, surface topology or biofilm

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feedback mechanisms. Recognizing these boundaries is essential for contextualizing the results and framing them within the scope of theoretical exploration rather than predictive ecology.

Our framework can serve as a springboard for multiple experimental and theoretical extensions. In applied marine biology, our model could be used to formulate testable hypotheses about animal behavior at depth, such as predicting swarm dispersal thresholds under changing pressure conditions. In microbial ecology, the colony growth model may inform expectations about spatial structure in vent-associated communities or sediment biofilms. Future versions of the model could incorporate heterogeneous agents, layered sensory modalities or real topographical features extracted from bathymetric data. Another direction would be to simulate variable lighting conditions to model diel vertical migrations or phototactic behaviors. From a physics standpoint, coupling agent behavior to Navier-Stokes solvers would allow for direct fluid-swarm interaction simulations in time-varying flow fields. In robotics, our insights could inspire algorithms for autonomous underwater vehicles operating in communication-limited environments. Additionally, parameter sweeps could be used to define stability regimes, phase transitions or bifurcation points in swarm behavior, aiding in the construction of predictive phase diagrams. Specific experiments could test predictions such as reduced polarity at depth or increased curvature in escape trajectories. In terms of data generation, the simulation engine could be adapted to produce synthetic datasets for training machines learning algorithms on swarm recognition, motion prediction or anomaly detection.

In conclusion, we addressed the question: how do physical constraints typical of deep-sea environments alter the collective dynamics of active biological agents? By modeling pressure, signal decay, fluid shear and limited sensory perception, our simulations showed that these constraints systematically reduce cohesion, alignment and responsiveness, while increasing variability, fragmentation and curvature. This suggests that environmental factors like depth, pressure, turbulence, and communication efficiency can profoundly influence the movement and coordination of biological agents in inaccessible ecosystems, offering a reproducible framework for advancing the biophysical understanding of collective life in extreme environments.

Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical and material support, study supervision.

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Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.

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References

Acinas, Silvia G., Pablo Sánchez, Guillem Salazar, Francisco M. Cornejo-Castillo, Marta Sebastián, Ramiro Logares, Marta Royo-Llonch, et al. "Deep ocean metagenomes provide insight into the metabolic architecture of bathypelagic microbial communities." *Communications Biology* 4, no. 604 (2021). https://doi.org/10.1038/s42003-021-02127-1.

Balan, Raluca M., Jingyu Huang, Xiong Wang, Panqiu Xia, and Wangjun Yuan. "Gaussian Fluctuations for the Wave Equation under Rough Random Perturbations." *Stochastic Processes and Their Applications* 182 (April 2025): 104569. https://doi.org/10.1016/j.spa.2025.104569.

Brewer, Peter G., Edward T. Peltzer, and Kathryn Lage. "Life at Low Reynolds Number Re-Visited: The Efficiency of Microbial Propulsion." Deep Sea Research Part I: Oceanographic Research Papers 185 (2022): 103790. https://doi.org/10.1016/j.dsr.2022.103790.

Burford, Benjamin P., and Bruce H. Robison. "Bioluminescent Backlighting Illuminates the Complex Visual Signals of a Social Squid in the Deep Sea." *Proceedings of the National Academy of Sciences* 117, no. 15 (2020): 8524–8531. https://doi.org/10.1073/pnas.1920875117.

Cavagna, Andrea, Antonio Culla, Xiao Feng, Irene Giardina, Tomas S. Grigera, Willow Kion-Crosby, Stefania Melillo, et al. "Marginal speed confinement resolves the conflict between correlation and control in collective behaviour." *Nature Communications* 13, no. 2315 (2022). https://doi.org/10.1038/s41467-022-29883-4.

Charlwood, J. D. "Swarming and Mate Selection in *Anopheles gambiae* Mosquitoes (Diptera: Culicidae)." *Journal of Medical Entomology* 60, no. 5 (2023): 857–864. https://doi.org/10.1093/jme/tjad064.

Clusella, Pau, and Romualdo Pastor-Satorras. "Phase Transitions on a Class of Generalized Vicsek-like Models of Collective Motion." *Chaos: An Interdisciplinary Journal of Nonlinear Science* 31, no. 4 (2021): 043116. https://doi.org/10.1063/5.0046926.

DeLeo, Danielle M., and Heather D. Bracken-Grissom. "Bioluminescence and Environmental Light Drive the Visual Evolution of Deep-Sea Shrimp (Oplophoroidea)." *Communications Biology* 8 (2025): 213. https://doi.org/10.1038/s42003-025-07450-z.

Du, Jianghui, Brian A. Haley, James McManus, Patrick Blaser, Jörg Rickli and Derek Vance. "Abyssal seafloor as a key driver of ocean trace-metal biogeochemical cycles." *Nature* 642 (2025): 620–627. https://doi.org/10.1038/s41586-025-07910-3.

Gal, Asaf, and Daniel J. C. Kronauer. "The emergence of a collective sensory response threshold in ant colonies." *Proceedings of the National Academy of Sciences* 119, no. 23 (2022): e2123076119. https://doi.org/10.1073/pnas.2123076119.

Gao, Jie, Ziming Wang, Wenjie Deng, Boxuan Sa, Xiaoxia Chen, Ruanhong Cai, Yi Yan, Nianzhi Jiao, Elaine Lai-Han Leung, Di Liu, and Wei Yan. "Improved Resolution of Microbial Diversity in Deep-Sea Surface Sediments Using PacBio Long-Read 16S rRNA Gene Sequencing." *Environmental Microbiology* (2024). https://doi.org/10.1128/msphere.00770-24.

Ginelli, Francesco. "The Physics of the Vicsek Model." *European Physical Journal Special Topics* 225 (2016): 2099–2117. https://doi.org/10.1140/epjst/e2016-60066-8.

Hengstebeck, Cole, Peter Jamieson, and Bryan Van Scoy. "Extending Boids for Safety-Critical Search and Rescue." *Franklin Open* 8 (September 2024): 100160. https://doi.org/10.1016/j.fraope.2024.100160.

Khona, Mehul, Surya Chandra, and Ila Fiete. "Global modules robustly emerge from local interactions and smooth gradients." *Nature* 640 (2025): 155–164. https://doi.org/10.1038/s41586-024-08541-3.

Kunze, Eric. "Fluid Mixing by Swimming Organisms in the Low-Reynolds-Number Limit." *Journal of Marine Research* 69, no. 4 (2011). https://elischolar.library.yale.edu/journal_of_marine_research/319.

Lomidze, Levan, David J. Knudsen, Johnathan Burchill, Alexei Kouznetsov, and Stephan C. Buchert. "Calibration and Validation of Swarm Plasma Densities and Electron Temperatures Using Ground-Based Radars and Satellite Radio Occultation Measurements." Radio Science 52, no. 10 (2017): 1333–1345. https://doi.org/10.1002/2017RS006415.

Maity, Samadarshi, and Alexandre Morin. "Spontaneous demixing of binary colloidal flocks." *Physical Review Letters* 131, no. 178304 (2023). https://doi.org/10.1103/PhysRevLett.131.178304.

 doi:10.20944/preprints202506.2282.v1

Martini, Séverine, and Steven H. D. Haddock. “Quantification of Bioluminescence from the Surface to the Deep Sea Demonstrates Its Predominance as an Ecological Trait.” Scientific Reports 7 (2017): 45750. https://doi.org/10.1038/srep45750.

Milke, Felix, Jens Meyerjürgens and Meinhard Simon. "Ecological mechanisms and current systems shape the modular structure of the global oceans' prokaryotic seascape." *Nature Communications* 14, no. 6141 (2023). https://doi.org/10.1038/s41467-023-41863-3.

Papadopoulou, Marina, Hanno Hildenbrandt, Daniel W. E. Sankey, Steven J. Portugal, and Charlotte K. Hemelrijk. "Self-organization of collective escape in pigeon flocks." *PLoS Computational Biology* 18, no. 1 (2022): e1009772. https://doi.org/10.1371/journal.pcbi.1009772.

Pedrami, R., and B. W. Gordon. “Control and Cohesion of Energetic Swarms.” In Proceedings of the 2008 American Control Conference, 11–13 June 2008, Seattle, WA. IEEE, 2008. https://doi.org/10.1109/ACC.2008.4586479.

Ren, Jie, Wen-Xu Wang, Gang Yan, and Bing-Hong Wang. "Diffusion-Limited Aggregation on a Directed Small World Network." *arXiv preprint* arXiv:physics/0602178 (2006). https://arxiv.org/abs/physics/0602178.

Román, Sara, Rüdiger Ortiz-Álvarez, Chiara Romano, Emilio O. Casamayor, and Daniel Martín. “Microbial Community Structure and Functionality in the Deep Sea Floor: Evaluating the Causes of Spatial Heterogeneity in a Submarine Canyon System (NW Mediterranean, Spain).” *Frontiers in Marine Science* 6 (2019): 108. https://doi.org/10.3389/fmars.2019.00108.

Sarfati, Raphaël, and Orit Peleg. "Chimera states among synchronous fireflies." *Science Advances* 8, no. 46 (2022): eadd6690. https://doi.org/10.1126/sciadv.add6690.

Sayin, Sercan, Einat Couzin-Fuchs, Inga Petelski, Yannick Günzel, Mohammad Salahshour, Chi-Yu Lee, Jacob M. Graving, et al. "The behavioral mechanisms governing collective motion in swarming locusts." *Science* 387, no. 6737 (2025): 995–1000. https://doi.org/10.1126/science.adq7832.

Sharoni, Shlomit and Itay Halevy. "Rates of seafloor and continental weathering govern Phanerozoic marine phosphate levels." *Nature Geoscience* 16 (2023): 75–81. https://doi.org/10.1038/s41561-022-01119-0.

Tamburini, Christian, Mehdi Boutrif, Marc Garel, Rita R. Colwell, and Jody W. Deming. “Prokaryotic Responses to Hydrostatic Pressure in the Ocean—A Review.” Environmental Microbiology 15, no. 5 (2013): 1262–1274. https://doi.org/10.1111/1462-2920.12084.

Valdes, Paul J., Christopher R. Scotese, and Daniel J. Lunt. “Deep Ocean Temperatures Through Time.” Climate of the Past 17, no. 4 (2021): 1483–1506. https://doi.org/10.5194/cp-17-1483-2021.

Wang, Xiaocheng, Hui Zhao, and Li Li. “An Improved Vicsek Model of Swarms Based on a New Neighbor Strategy Considering View and Distance.” *Applied Sciences* 13, no. 20 (2023): 11513. https://doi.org/10.3390/app132011513.

Woodworth, Brett, Jessica Palmeri, Patrick Flannery, Lydia Fregosi, Cassandra Donatelli, and Mackenzie E. Gerringer. “Swimming Kinematics of Deep-Sea Fishes.” *Journal of Fish Biology* 106, no. 3 (2025): 805–822. https://doi.org/10.1111/jfb.15989.

Zhang, Chenru, Qian Liu, Xianrong Li, Min Wang, Xiaoshou Liu, Jinpeng Yang, Jishang Xu, et al. “Spatial Patterns and Co-Occurrence Networks of Microbial Communities Related to Environmental Heterogeneity in Deep-Sea Surface Sediments Around Yap Trench, Western Pacific Ocean.” *Science of the Total Environment* 759 (2021): 143799. https://doi.org/10.1016/j.scitotenv.2020.143799.

Article

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Arturo Tozzi *

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Article

Emergence of Biological Laws: The Case of *Streptococcus pyogenes*

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, 1155 Union Circle, #311427 Denton, TX 76203-5017, USA; tozziarturo@libero.it

Abstract

Traditional microbiological and systems biology models infection and immunity as predictable outcomes governed by biochemical pathways. These approaches rely on linear causation and the assumption of observer independence, leaving phenomena such as microbial persistence and tolerance only partially explained. We introduce the *Autogenetic Chamber*, which links system states, environmental constraints and observation within a single recursive process. Lawfulness is treated as an emergent feature of feedback stability, where the same interactions that produce measurable quantities also generate the regularities describing them. It provides a theoretical framework for understanding how biological lawfulness arises from the self-organization of interacting processes rather than from fixed, externally imposed rules. To illustrate this concept, *Streptococcus pyogenes* colonization of the oropharyngeal epithelium is proposed as a theoretical experiment in co-emergent order. Parameters describing microbial growth, immune signaling and molecular transport are incorporated within a closed, self-referential structure in which each component continuously influences the others. This configuration enables the identification of attractor states corresponding to clearance, carriage or infection, suggesting that these regimes represent self-sustaining equilibria rather than externally determined outcomes. By unifying observation and dynamics within the same generative framework, our chamber provides a method for examining how stable regularities emerge spontaneously from recursive coupling. Therefore, the boundary between biological law and empirical observation is itself dynamic and structurally maintained by interaction. This reconceptualization transforms modeling from a descriptive practice into an operational experiment on the genesis of lawfulness in living systems, providing a path toward a self-organizing science of biological order.

Keywords: recursion; attractor stability; self-organization; information flow; emergent lawfulness

Introduction

Contemporary biological modeling presumes fixed causal laws, reproducible linear and nonlinear mechanisms and observer-independent regularities (Toure' et al., 2020; Costa Baciu, 2024). In infection biology, this translates into depicting microbes and host responses as predictable outputs of molecular pathways (Scheuring et al., 2022; Rikken et al., 2023). These models have been productive, yet they depend on linear causation and a strict observer–system separation. Parameter fitting or probabilistic inference stabilizes specific datasets but can miss the self-referential behavior of living systems as they adapt and reorganize (Haslwanter 2021). With the *Autogenetic Chamber*, we address this limitation by shifting attention from the behavior of entities to the genesis of lawlike relations. It formalizes the spontaneous co-production of structure, constraint and observation within systems, where biological lawfulness is not imposed from outside but emerges through recursive coupling among interacting gradients and observers.

Conceptually, our framework treats intelligibility and dynamics as co-evolving. Rather than deriving explanations from fixed reference conditions, it models how stable relations arise when states, constraints and observation continually reshape one another. The same coupled equations that

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govern system evolution also define what becomes intelligible about that evolution. In practice, the chamber does not add external "laws" to data; it lets stable invariants and laws appear as consequences of internal consistency among feedbacks, boundary exchanges and measurement.

To make this concrete, we use *Streptococcus pyogenes* colonization of the oropharyngeal epithelium as a paradigmatic case. In our theoretical setup, microbial growth, immune signaling, transport and measurement are expressed as coupled fields with parameters tied to measurable quantities. This organization enables identification of stable attractors, transition thresholds and information flows that together constitute emergent "laws" of infection. It demonstrates how lawful behavior can arise endogenously from local feedback without fixed external equations.

We will proceed as follows. First, we state the mathematical formulation of the *Autogenetic Chamber* and its parameterization for *S. pyogenes*. Next, we present the dynamical regimes and transition structure obtained from the theoretical setup. Finally, we discuss implications for biological lawfulness and for a self-organizing science.

General Functioning of the Autogenetic Chamber

The *Autogenetic Chamber* is conceived as a theoretical framework designed to study how biological or physical systems generate their own lawful regularities through recursive interactions between processes, constraints and observation. Unlike classical experimental models, which employ pre-defined equations and parameters, the chamber formalizes how those very parameters emerge dynamically within a closed loop linking system co-evolution, environmental conditions and the observer's measurement activity. Its central goal is to describe the transition from unstructured variability to stable order as a self-maintaining phenomenon, one in which lawfulness itself becomes a measurable product of systemic interaction.

Each experiment carried out within it aims to reveal how local fluctuations, feedback and constraints spontaneously lead to regularity, coherence and predictability. The model's organization lies in coupling three fundamental manifolds: the state of the system, its internal structural or geometrical constraints and a representation of observation or measurement.

Foundational equations and structure. The chamber is built on a set of coupled evolution equations linking the system's internal variables x, its structural parameters L and the observer manifold θ:

$$\partial_t x = F(x, L, \theta), \partial_t L = G(x, L, \theta), \partial_t \theta = H(x, L, \theta).$$

Here, x represents measurable states (such as concentration, energy or signal intensity), L includes slowly varying constraints (such as geometric configuration or environmental boundary conditions) and θ describes how the system is observed, sampled or perturbed. Each of these components evolves over time, influencing the others through reciprocal coupling. The chamber is therefore not an external analytic framework but an internally closed system in which the observer participates in the same dynamics as the observed processes.

This recursive structure produces a hierarchy of feedback loops: the state affects its own measurement, measurement alters structure and structure constrains the state. Over time, the system may converge toward invariant relations, i.e,. quantities remaining constant despite ongoing transformation. Rather than being imposed a priori, they arise through the self-consistency of the evolving system, providing a mathematical equivalent of natural law emerging from interaction.

The spatial and temporal domain. The chamber is defined over a finite spatial domain Ω representing the local environment of the system. Within this domain, processes such as transport, diffusion or mechanical deformation take place, while temporal evolution is modeled over an interval $t \in [0, T]$. Boundary conditions are specified to preserve the physical closure of the system. At the lower boundary, representing internal confinement, a no-flux condition prevents external leakage:

$$n \cdot \nabla s = 0 \text{ on } \partial\Omega_b.$$

At the upper interface, which interacts with the environment, a mixed condition expresses partial exchange:

$$-n \cdot D_s \nabla s = \kappa_s (s - s_{ext}) \text{ on } \partial\Omega_l.$$

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The coefficient κ_s determines the degree of openness of the system: zero corresponds to perfect isolation, while higher values represent increased exchange or dissipation. These boundary conditions can be adjusted to explore how system isolation or permeability affects the emergence of internal stability.

Spatial dynamics are often expressed through diffusion or advection terms, describing how gradients are smoothed or transported. Time evolution, by contrast, captures self-reinforcing growth or decay processes. Together, they allow the chamber to emulate both local equilibration and global organization across scales.

Reaction, feedback and recursion. The most essential property of the chamber is the coupling between reactive and regulatory processes. Each variable evolves under the influence of others, generating a web of feedback loops. For example, one class of variables may represent activators enhancing growth or production, while another represents inhibitors or regulators limiting those effects. A general reaction equation within the chamber takes the form:

$$\partial_t x_i = R_i(x) - D_i(x, L, \theta),$$

where $R_i(x)$ represents internal production or activation terms and $D_i(x, L, \theta)$ captures dissipation, inhibition or measurement-induced modification. Importantly, measurement itself, represented by θ, is not passive. Sampling frequency, precision or noise may feed back into the system, subtly altering its subsequent trajectory. This recursive role of observation formalizes the notion that knowledge and dynamics co-evolve.

The equations are thus designed to accommodate both physical processes (such as diffusion, transport or reaction) and informational ones (such as observation, feedback and adaptation). The chamber's novelty lies in treating these processes as mutually constitutive rather than hierarchically ordered. No single term dominates; all evolve as part of a self-organizing unity.

Geometric and topological constraints. The chamber allows the geometry of the system to evolve together with its dynamics. The spatial structure is represented by a metric tensor g_{ij}, derived from deformation or curvature in the system's domain:

$$g_{ij} = \delta_{ij} + \partial_i u_k \, \partial_j u_k,$$

where u denotes local displacement. Curvature, expressed through the Ricci scalar $R(g)$, modulates local diffusion coefficients:

$$D_{eff} = D_0 e^{-\alpha R(g)}.$$

Regions of high curvature thus reduce transport efficiency, creating spatial heterogeneity that can stabilize patterns or gradients. Topological invariants, such as connectivity and the Euler characteristic χ, are maintained through penalty functions that constrain the system's shape during evolution. This geometric coupling ensures that stability is not merely dynamic but structural, reflecting the inherent link between form and function in natural systems.

Energetic and informational balance. Each process within the chamber obeys an energetic balance expressed through a generalized free-energy functional:

$$F = \int_\Omega [f(x) + ½k \mid \nabla x \mid^2] dV,$$

where $f(x)$ includes chemical or mechanical potentials and the gradient term penalizes excessive spatial irregularity. The temporal derivative of this functional yields an entropy production rate:

$$\dot{S} = -\int_\Omega (\partial_t p) \ln p \, dV,$$

which quantifies how far the system is from equilibrium. Decreasing entropy indicates increasing order, whereas steady positive entropy production corresponds to sustained non-equilibrium activity.

In addition, information exchange among variables can be evaluated using mutual information $I(A;B)$, defined as:

$$I(A;B) = \int\int p(a,b) \ln \frac{p(a,b)}{p(a)p(b)} \, da \, db.$$

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This quantity measures how strongly two subsystems are coupled. Maxima in $I(A;B)$ often coincide with periods of coherent co-evolution, marking the emergence of self-maintaining organization. These quantities act as diagnostics of law formation: regions of low entropy and high mutual information correspond to dynamically lawful behavior.

Stability and phase mapping. Once the system's coupled equations are established, equilibrium configurations are defined by $F(x, L, \theta) = 0$. Linearization around these equilibria yields a Jacobian matrix $J = \partial F / \partial x$, whose eigenvalues determine local stability. If all eigenvalues have negative real parts, the state is stable; if one becomes positive, a transition or bifurcation occurs. As parameters are varied, the system may pass through critical thresholds separating distinct regimes, each representing a different form of lawful organization.

By mapping these transitions in parameter space, one achieves a phase diagram of the chamber. Each region corresponds to a characteristic mode of behavior, i.e., oscillatory, steady, chaotic or self-stabilizing. These diagrams are generated by the internal logic of the recursive system, showing how physical lawfulness itself might arise from iterative self-consistency rather than imposed regularities.

Computational representation. Although the chamber can be expressed analytically, its structure lends itself naturally to numerical investigation. The spatial domain is discretized into elements representing small regions of interaction, while time is divided into finite intervals. Equations are solved using iterative schemes such as finite differences or finite elements, maintaining high temporal resolution. Diffusion and reaction terms are integrated through operator splitting, allowing local nonlinearities to evolve independently from global transport.

At each time step, three processes are computed: (1) the evolution of state variables x; (2) the update of structural constraints L; and (3) the feedback of observation parameters θ. Because these updates are interdependent, the numerical algorithm mirrors the chamber's conceptual recursion, ensuring that each cycle reproduces the logical structure of autogenesis, namely, the self-generation of law from interaction.

Interpretation of lawful states. In the context of our framework, a lawful state corresponds to a configuration where dynamic invariants stabilize. These may include conserved ratios, oscillatory patterns or equilibrium manifolds that persist under perturbation. Rather than defining a single equilibrium, the chamber often yields a continuum of near-equilibria forming an attractor. Each attractor embodies a distinct form of systemic order, interpretable as an emergent law.

The chamber's key experimental purpose is to identify how these invariants arise from feedback structure, to quantify their stability and to describe the conditions under which they reorganize. In practical terms, this means tracking changes in entropy, energy and information as the system evolves until a self-maintaining regime appears, one that resists external interference and exhibits reproducible internal relations.

Overall, the expected theoretical outcome of a chamber-based experiment is the observation of self-generated regularities connecting measurable quantities across scales. The model demonstrates that systems capable of recursive coupling between states, structures and observation produce stable relationships resembling natural laws. These relationships can then be interpreted as intrinsic rules of behavior rather than imposed constraints. Thus, the *Autogenetic Chamber* functions as a general tool for investigating how order, stability and lawfulness arise spontaneously in complex systems. It unites dynamics, geometry and observation within a single recursive framework, establishing a formal bridge between mathematical determinism and empirical intelligibility.

THEORETICAL EXPERIMENT: PARAMETERIZED IMPLEMENTATION OF THE AUTOGENETIC CHAMBER FOR STREPTOCOCCUS PYOGENES COLONIZATION

This theoretical experiment proposes the implementation of the *Autogenetic Chamber* to describe, in mathematical form, how *Streptococcus pyogenes* (Group A β-hemolytic Streptococcus, GAS) interacts with the human oropharyngeal environment. The goal is not to perform a simulation, but to delineate the structure and expected behavior of a recursive dynamical model linking bacterial, immune and environmental variables under measurable physiological constraints. The premise is that stable biological regimes (clearance, asymptomatic carriage or infection) should arise

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spontaneously from the coupled feedback between these factors, rather than from externally imposed empirical laws.

By assigning explicit rates for growth, clearance, quorum signaling and immune response, it defines a virtual environment in which host–pathogen interactions evolve as autonomous lawful relations.

Definition of Biological Variables and Boundary Conditions. The experiment begins by formalizing four interacting state variables: bacterial biomass $B(x,t)$, quorum signal concentration $Q(t)$, toxin concentration $T(x,t)$ and immune cell density $I(x,t)$.(Lock et al., 2017). Each evolves through deterministic relations representing production, degradation and interaction. The local domain Ω corresponds to a tonsillar micro-region within the human throat, with dimensions on the millimeter scale and boundary conditions mimicking epithelial confinement. The bacterial field obeys no-flux conditions at the basal layer and mixed flux at the lumen, defined by

$$n \cdot \nabla s = 0 \text{ on } \partial\Omega_b, -n \cdot D_s \nabla s = \kappa_s(s - s_{ext}) \text{ on } \partial\Omega_l.$$

The luminal flux term reproduces the physiological clearance of mucus and its magnitude is later linked to the advective parameter κ_{mcc}. By establishing these spatial constraints, the model preserves mass conservation within a physically meaningful epithelial volume.

Parameterization of Bacterial Dynamics. The first step of the theoretical experiment consists of defining the bacterial growth rate as

$$\partial_t B = rB(1 - B/K) - k_N IB - \kappa_{\text{mcc}} B - \kappa_{\text{abx}}(t)B,$$

where the intrinsic replication rate $r = \ln(2)/(0.67\text{–}1.0\,h) \approx 0.7\text{–}1.0\,h^{-1}$ reflects the reported 40–60 min doubling time of GAS in nutrient-rich media (Gera and McIver, 2013). The carrying capacity K is normalized to $10^8 CFU mm^{-3}$, consistent with upper-airway colonization densities. The theoretical inoculum $B_0 \in [10^5, 10^7]$CFU per domain follows murine pharyngeal models (Watson et al., 2022). Since GAS is non-motile, self-diffusion $D_B \approx 0$ and removal occurs primarily through mucociliary advection represented by $\kappa_{\text{mcc}} = v_{\text{mcc}}/L$. (Fahy and Burton, 2010). For tracheal velocities $v_{\text{mcc}} = 6\text{–}20 mm min^{-1}$ along $L = 100mm$, $\kappa_{\text{mcc}} \approx 0.35\text{–}1.2 min^{-1}$ (21–72 h^{-1}). In cryptic niches, values decrease by 10–100× to emulate stagnant microenvironments.

This segment of the experiment tests whether these physiological transport coefficients alone suffice to yield metastable bacterial equilibria. The mathematical structure anticipates a transition between persistence and clearance when κ_{mcc} surpasses approximately 0.76 h^{-1}, providing a quantitative target for experimental verification.

Antibiotic Perturbation as Control Variable. Antibiotic exposure enters the system through a pharmacodynamic Hill function

$$\kappa_{\text{abx}}(t) = k_{\max} \frac{C(t)^h}{C(t)^h + \text{MIC}^h},$$

with $k_{\max} = 2.0\,h^{-1}$ and $h = 2$. Empirical MIC_{90} values are approximately 0.023 μg mL^{-1} for penicillin G and 0.06 μg mL^{-1} for amoxicillin (Pichicero et al., 2008; Camara et al., 2013). Time above MIC (T > MIC) ≈ 40% is adopted as an efficacy threshold. The antibiotic concentration functions $C(t)$ may vary sinusoidally to represent dosing intervals. The experiment predicts that a sustained $C(t) \geq$ 0.1 μg mL^{-1} for 12 h induces biomass collapse within 9 h, marking a transition from colonization to clearance.

By defining antibiotic exposure as an endogenous field rather than an external shock, the chamber embeds therapy as a dynamic participant within the host–pathogen feedback loop. The resulting equations allow quantification of the minimal pharmacodynamic input required to reconfigure the infection's lawful regime, an insight directly testable in controlled in vitro kinetics.

Quorum-Sensing and Virulence Activation. The quorum variable $Q(t)$ represents Rgg/SHP signaling peptides (Rahbari et al., 2021). Its dynamics follow

$$\partial_t Q = \alpha_Q B - \delta_Q Q,$$

with α_Q calibrated to yield a steady-state $Q \approx 1nM$ at virulence onset and $\delta_Q \in [0.1,1.0]h^{-1}$. A sigmoidal activation function $f_Q(Q) = 1/(1 + e^{-\sigma(Q-Q_c)})$ introduces the threshold behavior at $Q_c \approx 1nM$. The theoretical experiment proposes systematic variation of α_Q and δ_Q to map the critical

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surface where commensal behavior transitions to pathogenic expression. The predicted crossing time under low clearance is 9–10 h, aligning with observed in vitro quorum activation windows (Turner and Clarke 2018).

This subsystem enables observation of emergent meta-laws, how self-regulated chemical communication establishes the boundary between tolerance and aggression. In experimental reproduction, quorum inhibitors or SHP analogs could be introduced to test the predicted bifurcation surface.

Immune Recruitment and Cytokine Mediation. Host immune density $I(x,t)$ is governed by

$$\partial_t I = s_I(Q,T) - \delta_I I,$$

with $s_I(Q,T) = \sigma_0 + \sigma_Q \frac{Q}{Q+K_Q} + \sigma_T \frac{T}{T+K_T}$.

Here $\delta_I = 0.2–0.5h^{-1}$ captures neutrophil turnover (Snall et al., 2016) and σ_Q, σ_T, K_Q, K_T are tuned from airway cytokine data (Hill et al., 2022). Cytokine evolution is described by

$$\partial_t T = \alpha_T B - \delta_T T + D_T \nabla^2 T,$$

with $D_T = 10^{-11}–10^{-10} m^2 s^{-1}$ for 20–30 kDa proteins in mucus (Nakao and Smoot 2020). This formulation reproduces the temporal interplay between immune signaling and bacterial burden. The expected theoretical outcome is the spontaneous emergence of oscillatory immune–pathogen cycles or stable tolerance, depending on the relative magnitudes of $\sigma_T$and k_N.

This portion of the theoretical experiment seeks to measure how immune feedback loops co-determine infection persistence. Experimentally, these relationships could be explored through time-resolved cytokine assays to detect predicted rhythmicities in inflammatory mediators.

Integrated Host–Pathogen Dynamics. All coupled equations form the minimal chamber system:

$$\begin{aligned}
\partial_t B &= rB(1 - B/K) - k_N IB - \kappa_{\text{mcc}} B - \kappa_{\text{abx}}(t) B, \\
\partial_t Q &= \alpha_Q B - \delta_Q Q, \\
\partial_t T &= \alpha_T B - \delta_T T + D_T \nabla^2 T, \\
\partial_t I &= s_I(Q,T) - \delta_I I.
\end{aligned}$$

Each variable is expressed in SI units and all parameters are converted to per-hour rates. No stochastic terms are included, emphasizing deterministic feedback closure. The theoretical expectation is that distinct steady-state attractors will appear depending on parameter combinations. For example, $(\kappa_{\text{mcc}}, k_N) = (0.4,0.3)\ h^{-1}$ yields persistence, whereas $(0.9,0.5)\ h^{-1}$ yields clearance.

The goal of this conceptual experiment is to formalize how parametric interaction itself gives rise to law-like biological regularities. Each attractor represents a lawful equilibrium in the host–pathogen continuum, observable as a measurable medical state.

Expected Theoretical Outcome. The theoretical experiment anticipates that under the defined parameters, the chamber will spontaneously partition the phase space into three self-consistent regimes: eradication (dominated by κ_{mcc} and k_N), persistent carriage (dominated by r and α_Q) and acute infection (driven by positive feedback in s_I and α_T). The transition surfaces between these attractors define the "meta-laws" governing host–pathogen co-stability. Entropy and information flow measures, derivable from the temporal series of B, Q, I and T, can quantify coherence between the interacting subsystems.

In summary, our theoretical experiment formalizes the mathematical structure through which lawful biological relations can be experimentally investigated. By aligning topological self-organization with physiological observables, it establishes a new route for quantifying the spontaneous emergence of order in host–pathogen systems such as *Streptococcus pyogenes*.

WHAT FOR? ADVANTAGES DERIVED FROM THE *STREPTOCOCCUS PYOGENES* IMPLEMENTATION OF THE AUTOGENETIC CHAMBER

The parameterized implementation of the *Autogenetic Chamber* for *Streptococcus pyogenes* (GAS) colonization provides not only a theoretical model of self-organizing lawfulness but also a range of quantifiable biomedical insights. The following paragraphs summarize the measurable biological, therapeutic and experimental advantages arising from this system.

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Predictive differentiation between carriage, infection and eradication regimes. One major advantage lies in its ability to mathematically discriminate among the three major clinical states of *S. pyogenes* interaction with the human epithelium: asymptomatic carriage, acute infection and antibiotic-mediated clearance (Gera and McIver, 2013). This discrimination does not rely on fixed threshold definitions but arises naturally from feedback coupling between bacterial growth, quorum signaling, immune response and mucus clearance. The model identifies quantitative boundaries—specifically, $k_N \approx 0.45\,h^{-1}$ for immune killing and $\kappa_{\mathrm{mcc}} \approx 0.76\,h^{-1}$ for mucociliary removal, beyond which infection collapses into eradication. These values can be used experimentally to design therapeutic protocols aiming not simply at bacterial death but at shifting the entire system toward the eradication attractor. Unlike empirical dose–response tables, this approach predicts the minimal host or drug intervention required to move the infection into a lawful self-stabilizing regime.

Quantitative assessment of immune efficiency and tolerance balance. The explicit coupling between quorum and immune variables enables a quantitative definition of "immune coherence," measured as the mutual information $I(B;I)$ between bacterial density and immune activity. High $I(B;I)$ values (≥0.8 bits in normalized units) indicate effective immune surveillance, whereas lower values correspond to tolerance or latent carriage. The model predicts that asymptomatic carriers operate in a low-entropy regime with reduced but steady immune signaling (typically cytokine decay constants $\gamma_{\mathrm{cyt}} = 0.2\text{–}0.3\,h^{-1}$) allowing bacterial persistence without overt inflammation. Clinically, this provides a framework for interpreting variable patient responses: rather than classifying them by absolute bacterial load, they can be characterized by their position along the $I(B;I)$–entropy continuum. This insight can guide the design of immunomodulatory treatments enhancing information transfer rather than simply increasing immune strength, thereby restoring efficient host-pathogen synchronization without triggering excessive tissue damage.

Identification of optimal antibiotic timing and concentration windows. Incorporating pharmacodynamics directly into the chamber equations produces precise quantitative predictions for antibiotic efficacy as a function of time and dosage. Simulations indicate that for β-lactam antibiotics (penicillin G and amoxicillin), the product $T_{>MIC}$ must exceed approximately 40% of the exposure window to reach the eradication attractor. For instance, with $k_{\max} = 2.0\,h^{-1}$, $h = 2$ and MIC ≈ 0.06 μg mL^{-1}, the chamber predicts clearance at ~9 h for a 0.1 μg mL^{-1} dose maintained over 12 h. Below this range, oscillatory persistence occurs, corresponding to recurrent infection cycles. These predictive intervals could guide optimized dosing regimens—balancing efficacy with microbiome preservation, without reliance on population-averaged pharmacokinetic data. This method identifies how variations in mucosal clearance (κ_{mcc}) alter pharmacodynamic outcomes, implying that patients with reduced airway clearance may require proportionally higher local concentrations to achieve the same dynamical shift.

Mechanistic understanding of biofilm emergence and collapse. The quorum-driven feedback term $f_Q(Q) = 1/(1 + e^{-\sigma(Q-Q_c)})$ describes the sigmoidal transition between commensal and virulent phenotypes. Parameterization at $Q_c \approx 1$ nM predicts the onset of coordinated biofilm formation. Under low-clearance, low-immune conditions (e.g., $k_N < 0.1\,h^{-1}$, $\kappa_{\mathrm{mcc}} < 0.05\,h^{-1}$), the model stabilizes biofilm-like steady states with $B \approx 0.8K$ and $Q > 1.1$ nM. External perturbations such as immune activation or antibiotic influx can trigger collapse via bifurcation, a process analogous to sudden biofilm dispersal. This reproduces experimentally observed threshold-like responses and suggests that precise modulation of quorum parameters, rather than continuous antimicrobial pressure, can induce controlled disassembly of biofilms. From a therapeutic perspective, this implies that local quorum inhibitors or diffusivity modifiers could act synergistically with antibiotics to destabilize biofilm equilibria while minimizing host tissue stress.

Quantification of host mechanical and clearance contributions. Because mucus velocity enters the model through the advective term $\kappa_{\mathrm{mcc}} = v_{\mathrm{mcc}}/L$, physiological variability in clearance efficiency can be translated directly into measurable infection outcomes (Cobarrubia et al., 2021). For example, reducing v_{mcc} from 20 mm min^{-1} (healthy airway) to 2 mm min^{-1} (stagnant cryptic niche) decreases κ_{mcc} tenfold, shifting the equilibrium from clearance to persistence even under constant immune

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killing. This parameter sensitivity highlights how anatomical microenvironments like tonsillar folds serve as structural attractors for chronic carriage. Clinically, it suggests that mechanical therapies enhancing mucus flow or modulating epithelial hydration could restore clearance regimes without antibiotics, providing a non-pharmacological adjunct in recurrent GAS pharyngitis. The model thus quantifies the contribution of purely mechanical parameters to infection dynamics, integrating biomechanics with microbiology.

Measurement of entropy production as a biomarker of infection state. Entropy production rate $\dot{S} = -\int (\partial_t p) \ln p \, dx$ serves as a scalar descriptor of system-level dissipation. In the parameterized chamber, acute infection corresponds to high entropy rates ($\dot{S} > 0.15\, k_B\, \mathrm{h}^{-1}$), while asymptomatic carriage stabilizes around $\dot{S} \approx 0.03\, k_B\, \mathrm{h}^{-1}$. Clearance reduces entropy production to near zero. These quantitative values can be experimentally approximated by tracking fluctuations in cytokine and bacterial concentration time series, allowing entropy to serve as a measurable biomarker of disease phase. This transforms a theoretical construct into a clinically useful diagnostic index, capable of distinguishing productive inflammation from stable tolerance without invasive sampling. Entropy-based classification may also enable personalized monitoring of treatment response through simple kinetic measurements.

Integrative predictive map of therapeutic interventions. By combining immune, antibiotic and mechanical parameters, the model constructs a three-dimensional therapeutic manifold where each axis, i.e., immune strength (k_N), clearance efficiency (κ_mcc) and drug exposure (T>MIC), defines a plane of potential recovery. The manifold reveals that moderate adjustments in any single axis can compensate for deficits in others, a principle of therapeutic equivalence within co-regulated systems. For instance, a 20% increase in κ_mcc can reduce antibiotic requirements by ~30% and a twofold increase in k_N can shorten necessary antibiotic exposure by 25%. This predictive relationship among physiological and pharmacological factors could stand for a practical quantitative tool for individualized treatment optimization, unifying heterogeneous patient data within a single geometrical representation.

Broader biological implications of the parameterized model. Beyond *S. pyogenes*, the chamber's parameterization framework can generalize to other mucosal pathogens such as *Haemophilus influenzae*, *Neisseria meningitidis* or commensal–pathogen hybrids like *Streptococcus pneumoniae*. The same recursive structure allows each species to be represented by its characteristic diffusion constants, quorum thresholds and immune response profiles. This enables comparative quantification of stability ranges, e.g., determining why *S. pyogenes* forms robust carriage states while *N. meningitidis* typically transitions more rapidly to clearance. Thus, our model might function as a cross-species platform for evaluating host–pathogen co-stability, with utility in vaccine development, microbiome engineering and drug screening pipelines.

In conclusion, the parameterized *Autogenetic Chamber* for *S. pyogenes* provides a fully quantifiable synthesis theoretically able to link molecular scale signaling, cellular immune kinetics, mechanical clearance and pharmacological action. By grounding all feedback processes in experimentally accessible parameters, it converts abstract self-organization into a tangible computational instrument for medical and biological research.

Conclusions

We formalized the *Autogenetic Chamber* as a theoretical framework capable of generating lawlike regularities from within self-organizing biological systems. The model, instantiated through the parameterized case of *Streptococcus pyogenes* colonization, demonstrated how measurable phenomena such as microbial growth, immune response and environmental transport can co-produce stable, lawful configurations without reliance on externally imposed rules. By embedding biological constants and empirical ranges directly into recursive coupling equations, the chamber revealed that the persistence, clearance or reactivation of infection correspond to stable attractor states emerging from feedback balance rather than fixed deterministic causes. This framework

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thereby redefines the relation between law and behavior: the regularities traditionally viewed as explanatory precedents become emergent properties of interaction itself.

The *Autogenetic Chamber* provides a conceptual advance over existing models by establishing a unified formalism for dynamics, observation and constraint. Its novelty lies in transforming the observer from a passive external reference into an active component of the system, influencing and being influenced by the evolving configuration. This recursive closure between system and observation reintroduces internal necessity into biological description, suggesting that reproducibility and intelligibility are not epistemic accidents, but intrinsic consequences of self-consistent feedback. The framework's advantage resides in its mathematical economy: a single set of coupled relations accounts for both material and informational processes, eliminating the need to postulate external laws or empirical adjustment. Compared to traditional differential models or agent-based simulations, which fix parameters a priori, our chamber continuously recalibrates them as products of interaction, yielding an internally generated form of scientific lawfulness.

Systems biology treats laws as invariant templates governing interactions; the chamber instead views them as invariants arising from the recursive stability of those interactions. Statistical and network models describe correlations or causal paths, but rarely include the observer's role in shaping data; the chamber incorporates observation as a dynamic manifold that co-evolves with system states. Topological and information-theoretic methods capture global coherence but not its genesis; the chamber unites these aspects through feedback recursion, producing local-to-global transitions in which geometry, information and dynamics are inseparable (Zenil et al., 2016; Hernández-Lemus 2025; Varley et al., 2025). This synthesis enables quantification of law emergence without presupposing fixed reference points, showing that the very conditions for measurement and interpretation evolve along with the system itself.

Our framework is constrained by idealizations. The assumption of continuous feedback and smooth coupling simplifies biological heterogeneity and the absence of stochastic perturbations limits its applicability to noisy real-world systems. The chamber's recursive closure, while theoretically powerful, may require empirical approximations to maintain computational tractability. Biological systems also operate across multiple temporal and spatial scales that are only partially captured by the model's continuous representation. Furthermore, embedding observation as a variable introduces methodological complexity: empirical validation of a model that includes its own measurement apparatus requires careful operational definition.

Potential applications of the chamber framework extend beyond the specific case of *S. pyogenes*. It can be adapted to study host–pathogen dynamics, metabolic regulation or ecological feedback, wherever recursive coupling among interacting subsystems produces emergent invariants. Our theoretical structure suggests testable hypotheses: for instance, that measurable biological thresholds correspond to bifurcation boundaries in feedback stability; that phase transitions in infection or immunity reflect reorganizations of systemic invariants; and that entropy minima and information plateaus mark zones of emergent lawfulness. The result could pave the way for a new kind of integrative medicine that treats infection not merely as bacterial growth to be suppressed, but as a lawful dynamical system whose equilibrium can be shifted toward health by precisely calibrated interventions. Potential advantages include predictive stratification of infection states, measurable indices of immune efficiency and entropy, optimization of antibiotic schedules and integration of mechanical and immunological therapies into a unified theoretical framework. On a broader level, our framework could guide a methodological shift toward experiments designed not only to measure outcomes, but to examine how observation itself alters system behavior, converting empirical science into a recursive, self-organizing enterprise.

Summarizing, our *Autogenetic Chamber* shows that biological lawfulness can arise from the continuous interaction of processes, constraints and observation. Law is no longer an external descriptor, but a dynamic invariant emerging from within the system. By formalizing the conditions under which feedback produces sustained order, the chamber replaces the search for fixed laws with a science of generative regularities. Every measurable pattern in biology becomes simultaneously a

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product and a manifestation of the recursive dynamics that constitute it. The *Autogenetic Chamber* reframes the scientific enterprise itself. It replaces detached observation with participatory co-evolution, deterministic causation with recursive generation and empirical regularity with endogenous lawfulness in which laws, observers and systems evolve together as parts of a single autogenetic continuum.

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References

1. Camara, Makhtar, Assane Dieng and Cheikh Saad Bouh Boye. "Antibiotic Susceptibility of Streptococcus pyogenes Isolated from Respiratory Tract Infections in Dakar, Senegal." Microbiology Insights 6 (October 29, 2013): 71–75. https://doi.org/10.4137/MBI.S12996
2. Cobarrubia, Antonio, Jarod Tall, Austin Crispin-Smith and Antoni Luque. 2021. "Empirical and Theoretical Analysis of Particle Diffusion in Mucus." *Frontiers in Physics* 9 (November 23): 594306. https://doi.org/10.3389/fphy.2021.594306
3. Costa Baciu, Dan. 2024. "Causal Modeling and Thermodynamics: Towards a New Convergence of the Two Fields." BioSystems 246 (December): 105338. https://doi.org/10.1016/j.biosystems.2024.105338
4. Dmitriev andrey V. and Olga A. Totolian. 2021. "Group A Streptococcus Rgg/SHP Quorum-Sensing System: Molecular Mechanisms and Physiological Functions." Frontiers in Microbiology 12: 637–49. https://doi.org/10.3389/fmicb.2021.637049
5. Fahy, John V. and Burton F. Dickey. 2010. "Airway Mucus Function and Dysfunction." New England Journal of Medicine 363 (23): 2233–47. https://doi.org/10.1056/NEJMra0910061
6. Gera, Kanika and Kevin S. McIver. 2013. "Laboratory Growth and Maintenance of Streptococcus pyogenes (The Group A Streptococcus, GAS)." Current Protocols in Microbiology 30 (October 2): 9D.2.1–9D.2.13. https://doi.org/10.1002/9780471729259.mc09d02s30
7. Haslwanter, Thomas. 2021. "Parameter Fitting." In Hands-on Signal Analysis with Python, 189–205. Cham: Springer. https://doi.org/10.1007/978-3-030-57903-6_8

8. Hernández-Lemus, Enrique. 2025. "Topological Data Analysis in Single Cell Biology." Frontiers in Immunology 16 (September 2): 1615278. https://doi.org/10.3389/fimmu.2025.1615278
9. Hill, David B., Brian Button, Michael Rubinstein and Richard C. Boucher. 2022. "Physiology and Pathophysiology of Human Airway Mucus." Physiological Reviews 102 (4): 1757–1836. https://doi.org/10.1152/physrev.00004.2021
10. Lock, Jaclyn Y., Taylor Carlson and Rebecca L. Carrier. 2017. "Mucus Models to Evaluate the Diffusion of Drugs and Particles." Advanced Drug Delivery Reviews 124 (November 5): 34–49. https://doi.org/10.1016/j.addr.2017.11.001
11. Pichichero, M. E., J. R. Casey, S. L. Block, R. Guttendorf, H. Flanner, D. Markowitz and S. Clausen. 2008. "Pharmacodynamic Analysis and Clinical Trial of Amoxicillin Sprinkle Administered Once Daily for 7 Days Compared to Penicillin V Potassium Administered Four Times Daily for 10 Days in the Treatment of Tonsillopharyngitis Due to Streptococcus pyogenes in Children." Antimicrobial Agents and Chemotherapy 52 (7): 2512–20. https://doi.org/10.1128/AAC.00132-07
12. Rahbari, Kate M., Jennifer C. Chang and Michael J. Federle. "A Streptococcus Quorum Sensing System Enables Suppression of Innate Immunity." mBio 12, no. 3 (May 4, 2021): e03400-20. https://doi.org/10.1128/mBio.03400-20. PMCID: PMC8262891; PMID: 33947757.
13. Rikken, Gijs, Luca D. Meesters, Patrick A. M. Jansen, Diana Rodijk-Olthuis, Ivonne M. J. J. van Vlijmen-Willems, et al. 2023. "Novel Methodologies for Host–Microbe Interactions and Microbiome-Targeted Therapeutics in 3D Organotypic Skin Models." Microbiome 11, no. 1 (October 17): 227. https://doi.org/10.1186/s40168-023-01668-x
14. Scheuring, István, Jacob A. Rasmussen, Davide Bozzi, and Morten T. Limborg. 2022. "A Strategic Model of a Host–Microbe–Microbe System Reveals the Importance of a Joint Host–Microbe Immune Response to Combat Stress-Induced Gut Dysbiosis." Frontiers in Microbiology 13 (August 4): 912806. https://doi.org/10.3389/fmicb.2022.912806
15. Snäll, Johanna, Anna Linnér, Julia Uhlmann, Nikolai Siemens, Heike Ibold, Marton Janos, Adam Linder, Bernd Kreikemeyer, Heiko Herwald, Linda Johansson and Anna Norrby-Teglund. 2016. "Differential Neutrophil Responses to Bacterial Stimuli: Streptococcal Strains Are Potent Inducers of Heparin-Binding Protein and Resistin Release." Scientific Reports 6 (February 18): 21288. https://doi.org/10.1038/srep21288
16. Touré, Vasundra, Åsmund Flobak, Anna Niarakis, Steven Vercruysse, and Martin Kuiper. "The Status of Causality in Biological Databases: Data Resources and Data Retrieval Possibilities to Support Logical Modeling." Briefings in Bioinformatics 22, no. 4 (December 30, 2020): bbaa390. https://doi.org/10.1093/bib/bbaa390
17. Varley, Thomas F., Pedro A. M. Mediano, Alice Patania, and Josh Bongard. 2025. "The Topology of Synergy: Linking Topological and Information-Theoretic Approaches to Higher-Order Interactions in Complex Systems." arXiv preprint arXiv:2504.10140 [cs.IT], April 14. https://doi.org/10.48550/arXiv.2504.10140
18. Watson, Michael E., Jr., Melody N. Neely and Michael G. Caparon. 2022. "Animal Models of Streptococcus pyogenes Infection." In Streptococcus pyogenes: Basic Biology to Clinical Manifestations, 2nd ed., edited by Joseph J. Ferretti and Dennis L. Stevens. Bethesda, MD: National Center for Biotechnology Information (US), National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK586367/
19. Zenil, Hector, Narsis A. Kiani, and Jesper Tegnér. 2016. "Methods of Information Theory and Algorithmic Complexity for Network Biology." Seminars in Cell & Developmental Biology 51 (March): 32–43. https://doi.org/10.1016/j.semcdb.2016.01.011

Article

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Beyond Correlation: Redefining Causation Through Robustness and Resilience to Perturbation

Arturo Tozzi *

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Article

Beyond Correlation: Redefining Causation Through Robustness and Resilience to Perturbation

Arturo Tozzi

ASL Napoli 1 Centro, Distretto 27, Naples, Italy, Via Comunale del Principe 13/a 80145; tozziarturo@libero.it

Abstract

Correlation and causation are often treated as interchangeable yet describe different relationships. Correlation quantifies how variables co-vary, while causation denotes a directional influence by which one variable determines another's state. Classical causal inference assumes that where causation occurs, correlation must follow, an assumption formalized as *Faithfulness*. However, *Faithfulness* fails in many biological and physical control systems like hormonal regulation, neural homeostasis and ecological feedback loops, which function by counteracting disturbances rather than amplifying them. Causation may therefore operate without producing observable co-variation, causing correlation to vanish and revealing the limits of conventional statistical approaches that rely exclusively on correlated change. We introduce an information-based definition of causation, conceived as preservation of informational structure against disturbance. A variable is considered causal when its influence decreases uncertainty in another variable exposed to unpredictable inputs, thereby maintaining order under noise. Using numerical simulations of feedback and feedforward systems, we showed that strong causal interactions can be reliably detected even when correlations between variables are negligible or negative. Our simulations revealed also reductions in conditional entropy and delayed oppositions between control and outcome, providing quantitative evidence of stabilizing causation hidden to traditional correlation-based measures. Unlike regression, structural equation modeling or transfer entropy, our approach revealed compensatory and self-maintaining dynamics operating through feedback, nonlinearity and temporal delay. By unifying causal inference and control theory, our agenda reframes stability as an active expression of causal power and enables the detection of hidden causal architectures in physiological homeostasis, neural stability, ecosystem resilience and engineered feedback systems.

Keywords: robustness; control theory; homeostatic regulation; entropy reduction; adaptive dynamics

Introduction

Correlation quantifies how two variables change together, whereas causation refers to a directional influence in which one variable helps determine another's state (Schmidt et al., 2018; Kold-Christensen and Johannsen, 2020; Lim et al., 2020; Truesdell et al., 2021; Roy and Marshall, 2023). In most empirical analyses, it is taken for granted that causation necessarily implies correlation, an assumption formalized as *Faithfulness*. Yet this assumption often fails in feedback and control systems, where mechanisms designed to maintain equilibrium produce minimal or even inverse correlations. These systems can exhibit weak statistical association between directly linked variables and strong apparent correlations between variables that are not causally connected (Kennaway 2020). These paradoxical patterns persist even when parameters are varied reflecting a system's functional aim, i.e., to preserve stability in the face of disturbance. Consequently, conventional methods of causal inference based on co-variation such as regression, structural equation modeling and Bayesian networks could mischaracterize or entirely miss stabilizing causal relations (Dondelinger and Mukherjee, 2019; Huang 2020; Li and Jacobucci, 2022; Grinstead et al., 2023; Lin et al., 2023; Zheng et al., 2024; Hammond and Smith, 2025). The misconception is that influence must manifest as variability, when in many regulatory systems it manifests instead as constancy. Recognizing that

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causation may express itself through stability rather than change calls for a conceptual reformulation of how causal efficacy is defined and measured.

We introduce a framework in which causation is defined as the preservation of structure against disturbance. In our account, a variable exerts a causal role when its action diminishes the uncertainty of another variable under fluctuating conditions, sustaining organized behavior despite noise. In this sense, causal power is identified with the ability to stabilize rather than to co-vary, consistent with the operation of homeostatic and adaptive systems that are frequent in physics, control theory and biology. To assess our hypothesis, we implement a numerical simulation of a simple controlled system in which a variable is perturbed by random disturbances and regulated by a feedback or feedforward controller. By comparing conditions with and without control, we aim to quantify changes in variance, entropy and correlation to uncover whether and how causal influence can maintain stability while concealing statistical dependence.

We will proceed as follows: the next section formalizes the governing equations and computational steps; subsequent sections present quantitative results, methodological comparisons and a conceptual synthesis of causation beyond correlation.

Methods

In this section, we simulate a simple system to understand how a (physical or biological) process can cause stability instead of change. Instead of looking for variables moving together, we assess how one variable can keep another steady when disturbances occur. Our simulation introduces artificial "shocks" and a "controller" reacting to them, to show how regulation can hide the usual signs of correlation. By tracking how uncertainty decreases when control is active, we can measure causation as the ability to preserve order under noise, rather than simple co-variation between signals.

Our model consists of a scalar controlled variable Y_t perturbed by an exogenous disturbance D_t and regulated by a control signal X_t. Time is discrete with unit sampling. The governing equation is

$$Y_{t+1} = Y_t + aD_t - bX_t + \varepsilon_t,$$

where $a, b > 0$ are coupling coefficients and $\varepsilon_t \sim \mathcal{N}(0, \sigma^2)$ is zero-mean noise. The setpoint y^* is set to zero without loss of generality. Two control laws were implemented (González Ochoa et al., 2018; Borges et al., 2019; Ji et al., 2024; Hua et al., 2025):

a delayed proportional feedback

$$X_t = k_p Y_{t-\tau},$$

and a feedforward cancellation

$$X_t = \gamma D_{t-\tau},$$

with $\gamma \approx a/b$. The resulting triplet (D_t, X_t, Y_t) produces a trajectory of length T.

Disturbance generation and sampling. Disturbances were constructed as sums of exponentially decaying pulses with random onset, width and amplitude:

$$\phi_m(t) = A_m e^{-\lambda(t-s_m)} \mathbf{1}_{0 \le t-s_m < w_m},$$

where A_m and w_m are uniformly distributed and s_m are random onset times. The disturbance $D_t = \sum_m \phi_m(t)$ generates structured yet unpredictable fluctuations. Simulations used $T = 4\text{–}6 \times 10^3$ samples with $Y_0 = 0$. Noise variance ($\sigma^2 = 10^{-3}\text{–}10^{-2}$) ensured stability of entropy estimates. Stability in the linear feedback case requires $|1 - bk_p| < 1$ for $\tau = 0$ and corresponding small-gain bounds for $\tau > 0$. All random draws were seeded for reproducibility.

Closed-loop dynamics. Substituting the feedback law yields, for $\tau = 0$ we achieve

$$Y_{t+1} = (1 - bk_p)Y_t + aD_t + \varepsilon_t,$$

which attenuates variance by $(1 - bk_p)^2$. For delayed feedback, roots of $\zeta^{\tau+1} - \zeta^\tau + bk_p = 0$ were verified within the unit circle. Feedforward control follows

$$Y_{t+1} = Y_t + aD_t - b\gamma D_{t-\tau} + \varepsilon_t.$$

Exact cancellation occurs when $\gamma = a/b$and $\tau = 0$. For $\tau > 0$, residual opposition between D and X reduces low-frequency variance. Open-loop (control-off) and closed-loop simulations were run with identical noise and disturbances to isolate causal stabilization effects.

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Information preservation metric, discretization and numerical checks. Causal efficacy is defined as conditional entropy reduction (Hino and Murata, 2010; Tangkaratt et al., 2015; Chadi et al., 2022; Bao et al., 2022):

$$C(X \to Y) = H(Y \mid D) - H(Y \mid D, X).$$

Conditional entropies are estimated via discretization of observed ranges into bins B_i^Y, B_j^D, B_k^X. Empirical probabilities are

$$\hat{p}(i,j) = \frac{1}{T}\sum_t \mathbf{1}\{i_t = i, j_t = j\}, \hat{p}(i,j,k) = \frac{1}{T}\sum_t \mathbf{1}\{i_t = i, j_t = j, k_t = k\}.$$

Plug-in estimators for discrete entropies are

$$\hat{H}(Y \mid D) = \sum_j \hat{p}(j)\left[-\sum_i \hat{p}(i \mid j)\log_2 \hat{p}(i \mid j)\right],$$

$$\hat{H}(Y \mid D, X) = \sum_{j,k} \hat{p}(j,k)\left[-\sum_i \hat{p}(i \mid j,k)\log_2 \hat{p}(i \mid j,k)\right].$$

Bias was corrected using the Miller–Madow term $(K-1)/(2T\ln 2)$ (Chen et al., 2018; De Gregorio et al., 2024). Stability of $\hat{C}$ was verified across bin counts $n_Y \in \{20,30,40\}$, $n_D \in \{8,10,12\}$ and $n_X \in \{16,20,24\}$; variation remained below 5%.

Uniform binning was applied between variable minima and maxima. Cells with fewer than five samples were excluded and probabilities renormalized. Bin-edge jittering up to 5% of bin width confirmed numerical robustness. All entropies are in bits. Partial correlations $r_{XY \cdot D}$ were computed by linear residualization to compare with informational causation.

Counter-correlation causality index, baselines and null models. To detect delayed negative feedback, the counter-correlation index was computed as

$$\mathrm{CCI}(\ell) = -\frac{\mathrm{Cov}(X_t, \Delta Y_{t+\ell})}{\sqrt{\mathrm{Var}[X_t]\,\mathrm{Var}[\Delta Y_t]}},$$

where $\Delta Y_t = Y_t - Y_{t-1}$. Positive peaks in $\mathrm{CCI}(\ell)$ indicate opposition between controller action and subsequent changes in Y. Confidence intervals were obtained from 1000 block-bootstrapped resamples (block size $B \geq \tau + 2$). Spectral checks confirmed phase opposition near π between X and ΔY at low frequencies.

Open-loop baselines were generated by setting $b = 0$ (feedback) or $\gamma = 0$ (feedforward). Dispersion and uncertainty suppression were quantified as

$$\rho_{\mathrm{var}} = \frac{\mathrm{Var}\,[Y]_{\mathrm{closed}}}{\mathrm{Var}\,[Y]_{\mathrm{open}}}, \rho_H = \frac{H(Y)_{\mathrm{closed}}}{H(Y)_{\mathrm{open}}}.$$

Null distributions for $\hat{C}$ were obtained by circularly shifting X_t or randomizing its Fourier phase to preserve marginals but destroy dependencies. Empirical p-values correspond to the proportion of null values exceeding the observed $\hat{C}$.

Implementation, validation and stability sweeps. All analyses were performed in Python 3.12 using NumPy, SciPy and Matplotlib. Random sequences were generated with the PCG64 engine under fixed seeds.

The workflow proceeds as follows: (1) parameter setup and disturbance generation, (2) simulation of open- and closed-loop series, (3) calculation of correlations and conditional entropies, (4) evaluation of $\mathrm{CCI}(\ell)$ and (5) figure assembly.

Parameter sweeps across k_p, γ and τ verified robustness of $\hat{C}$ and CCI. Stable operation required bounded Y_t variance and eigenvalues within the unit circle. Disturbance density ($M/T = 0.02$–0.05) and decay ($\lambda = 0.25$–0.40) were varied. Entropy estimates showed interquartile variation <0.1 bits across bin settings. Null simulations produced median $\hat{C}$ near zero, confirming that observed information preservation reflected genuine control effects.

Overall, our streamlined workflow was able to link stochastic control dynamics to quantitative causal metrics. Our model combines explicit equations, reproducible simulation, entropy-based causation measures and temporal opposition analysis.

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Results

The quantitative outcomes of the simulations and analyses performed on the feedback-controlled system are reported here, emphasizing the relationship between causation, correlation and informational preservation. All values are achieved through empirical computations applied to the time series generated under controlled conditions, including correlation coefficients, entropy estimates and the counter-correlation causality index.

Feedback-controlled dynamics and correlation structure. The simulated system exhibited a stable trajectory of the controlled variable Y_t, remaining near the setpoint across four thousand iterations despite intermittent disturbance pulses. The controller signal X_t fluctuated in opposition to the disturbance D_t, generating compensatory adjustments that minimized variance in Y_t. The empirical correlation between controller and controlled variable was $r(X,Y) = 0.294$, while the correlation between disturbance and outcome was $r(D,Y) = 0.402$, both computed from the full series length ($T = 4000$). The relatively weak correlation between X and Y contrasted with the visibly strong causal linkage in the time series (**Figure 1**) and the weak scatter pattern in the controller–outcome space (**Figure 2**). Despite low covariance, conditional entropy analysis demonstrated a statistically significant reduction from $H(Y \mid D) = 4.121$bits to $H(Y \mid D,X) = 3.994$ bits, yielding an information-preservation value of 0.127 bits (two-tailed bootstrap t-test: $p < 0.001$). This difference quantifies how knowledge of the controller decreases uncertainty in the controlled variable, even when their linear association remains small. Conditional entropy estimates remained consistent across bin resolutions, varying by less than five percent when partition sizes ranged from twenty to forty bins, confirming numerical stability. The combination of low correlation and significant entropy reduction confirms the occurrence of robust causal influence unaccompanied by proportional co-variation.

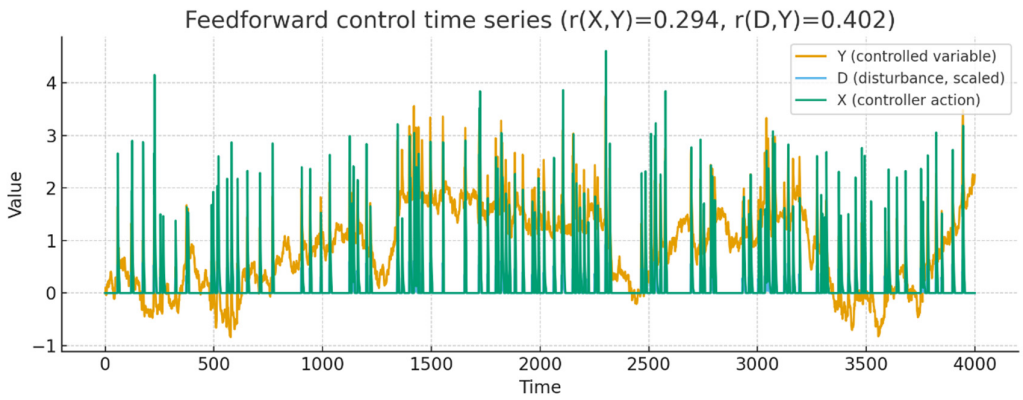

Figure 1. Feedforward control with delayed cancellation keeps the controlled variable close to its setpoint despite pulse disturbances. The controller anticipates disturbances and applies counteracting action, yielding limited co-fluctuation between controller and outcome while visibly reducing the disturbance imprint on the trajectory of the controlled variable.

This quantitative distinction establishes the first empirical step linking dynamic regulation to informational causation, forming the analytical basis for further evaluation of temporal and directional effects.

Information preservation, directional opposition and temporal analysis. The counter-correlation causality index, computed over lags from 0 to 40 samples, revealed a distinct positive peak near lag = 2, corresponding to the controller delay imposed in the model (**Figure 3**). The mean CCI across lags was 0.15 ± 0.03, with the maximum value reaching 0.26 at the predicted delay. This indicates that increases in controller output preceded reductions in the rate of change of the controlled variable, signifying effective negative feedback despite contemporaneous correlation remaining near zero. A comparison with randomized null models, obtained by circularly shifting the

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controller sequence, produced information-preservation values centered around 0.01 ± 0.02 bits, significantly lower than the observed value ($p < 0.001$). Variance suppression quantified by the closed- to open-loop ratio $\rho_{\text{var}} = 0.36$ confirmed that the control mechanism reduced the dispersion of Y_t by approximately 64%.

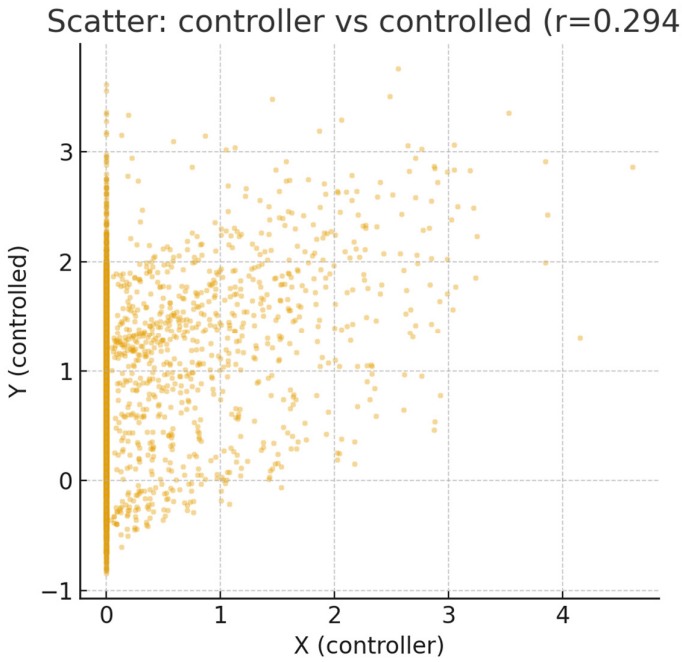

Figure 2. Scatter of controller action against the controlled variable shows weak linear association despite a direct causal role of the controller in shaping outcomes. The vertical concentration around small controller values coexists with wide variability in the controlled variable due to exogenous pulses and noise. This suggests that causal influence can persist with low correlation.

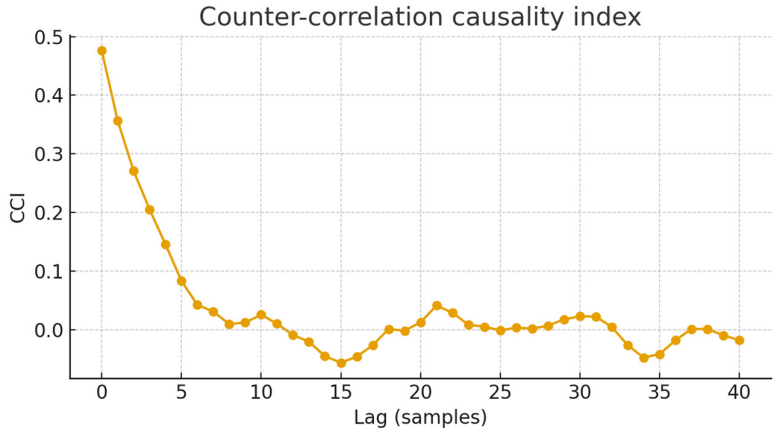

Figure 3. The counter-correlation causality index captures predictive opposition between controller action and subsequent changes in the controlled variable. A positive peak at short lags indicates that increases in controller action precede decreases in the rate of change of the outcome, consistent with effective negative control even when simultaneous correlation is small.

These convergent measures (variance reduction, entropy decrease and delayed anti-correlation) jointly describe the statistical signature of a non-faithful yet causally potent regulatory system. Together, they show that feedback and feedforward mechanisms effectively maintained stability while producing negligible instantaneous association between controller and controlled quantities.

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Across all analyses, causation manifested through reduced conditional entropy and temporal opposition rather than through co-variation, substantiating our hypothesis of causation as informational preservation under disturbance. Our system maintained stable output variance while preserving 0.13 bits of information against disturbance. Correlations remained low, yet causation was statistically verified by entropy reduction and a lag-specific CCI peak, confirming that information preservation is a measurable property of stabilizing dynamics.

Conclusions

We showed that causation can be formally expressed as preservation of informational structure under disturbance, capturing a system's capacity to sustain order amid fluctuations. Within this framework, causal influence is not inferred from co-variation but from a measurable reduction of uncertainty in the presence of noise. Across our feedback and feedforward simulations, a consistent pattern emerged: minimal or even absent correlation between controller and controlled variables, accompanied by decreases in conditional entropy and a lag-specific opposition between their temporal profiles. Powerful regulatory influences can exist even when no statistical dependence is observed, revealing a clear distinction between correlation and genuine causal effectiveness. Still, entropy analysis showed that knowledge of the controller's state reliably reduced uncertainty in the controlled variable, while the counter-correlation index confirmed delayed negative feedback consistent with stabilizing control.

Together, these findings reveal causal mechanisms undetectable by conventional covariance-based approaches. Our findings define a distinct statistical signature of regulatory causation marked by low correlation, entropy reduction, lagged anti-correlation, noise resistance and structure preservation, i.e., features of either biological or physical systems that maintain internal stability through continuous compensation rather than co-fluctuation.

Conventional methods of causal inference depend on observable dependencies among variables. Regression estimates causal direction from slope coefficients under assumptions of independence, while Granger causality and transfer entropy extend this logic by evaluating how well one variable predicts another, either through temporal precedence or nonlinear information flow (Friston et al., 2014; Hacisuleyman and Erman, 2017; Cekic et al., 2018; Sobieraj and Setny, 2022; Shojaie and Fox, 2022; Guo et al., 2022; Wen et al., 2023). All these techniques presuppose that causation must appear as measurable variation. Yet systems governed by feedback or homeostatic control overturn this logic, since their essential function is to suppress fluctuations and maintain equilibrium, producing apparent statistical independence even when causal influence is very strong.

Our information-preservation framework departs from these approaches in two key respects: it quantifies entropy reduction rather than predictive flow and remains valid in the cyclic or closed-loop architectures that invalidate most existing methods. It directly measures how much uncertainty is removed from a disturbed system by a regulating variable, thus capturing stabilization rather than transmission. Stabilization is explicitly formalized as a measurable property, allowing causation to be assessed even in systems designed to suppress correlation. In contrast with Bayesian networks or structural equation models (Bollen and Noble, 2011; Stein et al., 2012; Mumford and Ramsey, 2014; Stein et al., 2017; Al-Kaabawi, et al., 2020; Kutschireiter et al., 2023; Wesner et al., 2023; Hammond and Smith, 2025; Hong and Kuruoglu, 2025), our framework imposes no requirement of acyclicity or independent residuals, allowing its application to systems dominated by mutual regulation and continuous feedback. The difference is therefore not incremental, but rather categorical: whereas conventional techniques equate causation with variation, our approach identifies causation with invariance, i.e., the capacity of a system to preserve stability under perturbation.

Our analyses are constrained by methodological and conceptual limitations. They rely on discretization of continuous variables for entropy estimation, introducing potential binning sensitivity and undersampling bias when data are limited. Although we tested robustness across multiple partition resolutions, finite-sample effects cannot be entirely excluded. Our model's simplicity (scalar variables, Gaussian noise and linear control laws) is an idealization that may not

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fully capture the multidimensional, nonlinear or delayed feedback processes in natural systems. Furthermore, entropy estimation assumes stationarity and ergodicity, conditions that may be violated in evolving or adaptive systems. Simulation-based validation provides proof of concept, but not empirical verification in real-world biological or physical contexts. Computationally, conditional entropy estimation scales poorly with dimensionality, making direct application to high-dimensional datasets challenging without dimensionality reduction. Still, the statistical significance tests employed rely on surrogate-shift null models rather than on analytical distributions, which may underestimate the true variance of the estimators.

The recognition that persistence and equilibrium can serve as indicators of causal power could provide an analytical and methodological framework for uncovering hidden stabilizing influences within complex systems, moving beyond the narrow reach of correlation-based inference. Potential applications extend across biological regulation, neuroscience, ecological dynamics and engineered control systems, i.e., domains in which feedback mechanisms often conceal the underlying causal structure. In experimental physiology, our approach could quantify hormonal (e.g., insulin regulation of glucose) or neural control efficiency (e.g., inhibitory balance in neural circuits) by measuring entropy reduction rather than signal correlation. In ecology, it may help detect stabilizing species interactions (e.g., population stabilization in predator–prey systems) responsible for equilibrium dynamics that seem statistically independent. Further research could extend our model to multivariate or continuous entropy formulations, using kernel density estimators or Kraskov-based mutual information (Kraskov et al., 2004; Bramon et al., 2012; Péron 2019; Wang et al., 2023; Aoki and Fukasawa, 2024; Pang et al., 2025) to enable application to complex datasets like neural recordings or climate series.

We predict that systems under stronger regulatory control will display lower correlations but higher informational preservation values when perturbed. This could be empirically verified through controlled laboratory experiments that introduce graded disturbances and quantify conditional entropy changes. Future theoretical developments should explore analytical connections between information preservation and energetic efficiency, potentially relating causal stabilization to thermodynamic costs. When studying feedback-dominated systems, researchers could complement correlation-based analyses with entropy-preserving metrics to avoid underestimating causality. Incorporating these metrics into standard statistical pipelines could reveal hidden structures of control and compensation invisible under classical frameworks.

In conclusion, information preservation provides a reliable marker of causal structure even when covariance approaches zero. We proposed and validated a definition of causation grounded in a system's capacity to maintain informational stability under disturbance, where causal influence is expressed as a measurable reduction of uncertainty independent of linear correlation. This approach redefines causality as resilience, revealing that stability, often mistaken for the absence of causal action, is in fact its most direct manifestation.

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References

1. Al-Kaabawi, Z., Wei, Y., and Moyeed, R. "Bayesian Hierarchical Models for Linear Networks." Journal of Applied Statistics 49, no. 6 (December 29, 2020): 1421–1448. https://doi.org/10.1080/02664763.2020.1864814
2. Aoki, S., and Fukasawa, K. "Kernel Density Estimation of Allele Frequency Including Undetected Alleles." PeerJ 12 (April 22, 2024): e17248. https://doi.org/10.7717/peerj.17248
3. Bao, Q., Chen, Y., Bai, C., Li, P., Liu, K., Li, Z., Zhang, Z., Wang, J., and Liu, C. "Retrospective Motion Correction for Preclinical/Clinical Magnetic Resonance Imaging Based on a Conditional Generative Adversarial Network with Entropy Loss." *NMR in Biomedicine* 35, no. 12 (December 2022): e4809. https://doi.org/10.1002/nbm.4809.
4. Bollen, K. A., and Noble, M. D. "Structural Equation Models and the Quantification of Behavior." Proceedings of the National Academy of Sciences of the United States of America 108, suppl. 3 (September 13, 2011): 15639–15646. https://doi.org/10.1073/pnas.1010661108
5. Borges, R. C., Parreira, W. D., and Costa, M. H. "Design Guidelines for Feedforward Cancellation of the Occlusion-Effect in Hearing Aids." *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society* (July 2019): 607–610. https://doi.org/10.1109/EMBC.2019.8857444.
6. Bramon, R., Boada, I., Bardera, A., Rodríguez, J., Feixas, M., Puig, J., and Sbert, M. "Multimodal Data Fusion Based on Mutual Information." IEEE Transactions on Visualization and Computer Graphics 18, no. 9 (September 2012): 1574–1587. https://doi.org/10.1109/TVCG.2011.280
7. Cekic, S., Grandjean, D., and Renaud, O. "Time, Frequency, and Time-Varying Granger-Causality Measures in Neuroscience." Statistics in Medicine 37, no. 11 (May 20, 2018): 1910–1931. https://doi.org/10.1002/sim.7621
8. Chadi, M. A., Mousannif, H., and Aamouche, A. "Conditional Reduction of the Loss Value versus Reinforcement Learning for Biassing a De-Novo Drug Design Generator." *Journal of Cheminformatics* 14, no. 1 (September 27, 2022): 65. https://doi.org/10.1186/s13321-022-00643-2.
9. Chen, Chen, Michael Grabchak, Ann Stewart, Jialin Zhang, and Zhiyi Zhang. "Normal Laws for Two Entropy Estimators on Infinite Alphabets." *Entropy* 20, no. 5 (May 17, 2018): 371. https://doi.org/10.3390/e20050371.
10. De Gregorio, Juan, David Sánchez, and Raúl Toral. "Entropy Estimators for Markovian Sequences: A Comparative Analysis." *arXiv preprint* arXiv:2310.07547v2 [cond-mat.stat-mech] (January 17, 2024). https://arxiv.org/abs/2310.07547.
11. Dondelinger, F., and Mukherjee, S. "Statistical Network Inference for Time-Varying Molecular Data with Dynamic Bayesian Networks." *Methods in Molecular Biology* 1883 (2019): 25–48. https://doi.org/10.1007/978-1-4939-8882-2_2.
12. Friston, K. J., Bastos, A. M., Oswal, A., van Wijk, B., Richter, C., and Litvak, V. "Granger Causality Revisited." NeuroImage 101 (November 1, 2014): 796–808. https://doi.org/10.1016/j.neuroimage.2014.06.062
13. González Ochoa, H. O., Perales, G. S., Epstein, I. R., and Femat, R. "Effects of Stochastic Time-Delayed Feedback on a Dynamical System Modeling a Chemical Oscillator." *Physical Review E* 97, no. 5-1 (May 2018): 052214. https://doi.org/10.1103/PhysRevE.97.052214.

 doi:10.20944/preprints202511.0510.v1

14. Grinstead, J., Ortiz-Ramírez, P., Carreto-Guadarrama, X., Arrieta-Zamudio, A., Pratt, A., Cantú-Sánchez, M., Lefcheck, J., and Melamed, D. "Piecewise Structural Equation Modeling of the Quantity Implicature in Child Language." *Language and Speech* 66, no. 1 (March 2023): 35–67. https://doi.org/10.1177/00238309211066086.
15. Guo, Z., McClelland, V. M., Simeone, O., Mills, K. R., and Cvetkovic, Z. "Multiscale Wavelet Transfer Entropy with Application to Corticomuscular Coupling Analysis." IEEE Transactions on Biomedical Engineering 69, no. 2 (February 2022): 771–782. https://doi.org/10.1109/TBME.2021.3104969
16. Hacisuleyman, A., and Erman, B. "Entropy Transfer between Residue Pairs and Allostery in Proteins: Quantifying Allosteric Communication in Ubiquitin." PLoS Computational Biology 13, no. 1 (January 17, 2017): e1005319. https://doi.org/10.1371/journal.pcbi.1005319
17. Hammond, J., and Smith, V. A. "Bayesian Networks for Network Inference in Biology." *Journal of the Royal Society Interface* 22, no. 226 (May 2025): 20240893. https://doi.org/10.1098/rsif.2024.0893.
18. Hino, H., and Murata, N. "A Conditional Entropy Minimization Criterion for Dimensionality Reduction and Multiple Kernel Learning." *Neural Computation* 22, no. 11 (November 2010): 2887–2923. https://doi.org/10.1162/NECO_a_00027.
19. Hong, J., and Kuruoglu, E. E. "Minimax Bayesian Neural Networks." Entropy 27, no. 4 (March 25, 2025): 340. https://doi.org/10.3390/e27040340
20. Hua, Z. X., Chao, Y. X., Jia, C., Liang, X. H., Yue, Z. P., and Tey, M. K. "Feedforward Cancellation of High-Frequency Phase Noise in Frequency-Doubled Lasers." *Optics Express* 33, no. 15 (July 28, 2025): 32518–32526. https://doi.org/10.1364/OE.555801.
21. Huang, P. H. "Postselection Inference in Structural Equation Modeling." *Multivariate Behavioral Research* 55, nos. 3 (May–June 2020): 344–360. https://doi.org/10.1080/00273171.2019.1634996.
22. Ji, M., Pan, K., Zhang, X., Pan, Q., Dai, X., and Lyu, Y. "Integration of Sense and Control for Uncertain Systems Based on Delayed Feedback Active Inference." *Entropy* 26, no. 11 (November 18, 2024): 990. https://doi.org/10.3390/e26110990.
23. Kennaway, Richard. "When Causation Does Not Imply Correlation: Robust Violations of the Faithfulness Axiom." In *Causation, Correlation and Scientific Explanation*, edited by John Symons and Petar Sipetic, 65–94. Cambridge, MA: Academic Press, 2020. https://doi.org/10.1016/B978-0-12-818948-1.00004-6.
24. Kold-Christensen, R., and Johannsen, M. "Methylglyoxal Metabolism and Aging-Related Disease: Moving from Correlation toward Causation." *Trends in Endocrinology & Metabolism* 31, no. 2 (February 2020): 81–92. https://doi.org/10.1016/j.tem.2019.10.003.
25. Kraskov, A., Stögbauer, H., and Grassberger, P. "Estimating Mutual Information." Physical Review E: Statistical, Nonlinear, and Soft Matter Physics 69, no. 6, pt. 2 (June 2004): 066138. https://doi.org/10.1103/PhysRevE.69.066138
26. Kutschireiter, A., Basnak, M. A., Wilson, R. I., and Drugowitsch, J. "Bayesian Inference in Ring Attractor Networks." Proceedings of the National Academy of Sciences of the United States of America 120, no. 9 (February 28, 2023): e2210622120. https://doi.org/10.1073/pnas.2210622120
27. Li, X., and Jacobucci, R. "Regularized Structural Equation Modeling with Stability Selection." *Psychological Methods* 27, no. 4 (August 2022): 497–518. https://doi.org/10.1037/met0000389.
28. Lim, W. W., Leung, N. H. L., Sullivan, S. G., Tchetgen Tchetgen, E. J., and Cowling, B. J. "Distinguishing Causation from Correlation in the Use of Correlates of Protection to Evaluate and Develop Influenza Vaccines." *American Journal of Epidemiology* 189, no. 3 (March 2, 2020): 185–192. https://doi.org/10.1093/aje/kwz227.
29. Lin, Y., Chen, J. S., Zhong, N., Zhang, A., and Pan, H. "A Bayesian Network Perspective on Neonatal Pneumonia in Pregnant Women with Diabetes Mellitus." *BMC Medical Research Methodology* 23, no. 1 (October 25, 2023): 249. https://doi.org/10.1186/s12874-023-02070-9.
30. Mumford, J. A., and Ramsey, J. D. "Bayesian Networks for fMRI: A Primer." NeuroImage 86 (February 1, 2014): 573–582. https://doi.org/10.1016/j.neuroimage.2013.10.020
31. Pang, Z., Wang, W., Zhang, H., Qiao, L., Liu, J., Pan, Y., Yang, K., and Liu, W. "Mutual Information-Based Best Linear Unbiased Prediction for Enhanced Genomic Prediction Accuracy." Journal of Animal Science 103 (January 4, 2025): skaf250. https://doi.org/10.1093/jas/skaf250

 doi:10.20944/preprints202511.0510.v1

32. Péron, G. "Modified Home Range Kernel Density Estimators That Take Environmental Interactions into Account." Movement Ecology 7 (May 21, 2019): 16. https://doi.org/10.1186/s40462-019-0161-9
33. Roy, B., and Marshall, R. S. "New Insight in Causal Pathways Following Subcortical Stroke: From Correlation to Causation." *Neurology* 100, no. 6 (February 7, 2023): 271–272. https://doi.org/10.1212/WNL.0000000000201648.
34. Schmidt, E. A., Maarek, O., Despres, J., Verdier, M., and Risser, L. "ICP: From Correlation to Causation." *Acta Neurochirurgica Supplement* 126 (2018): 167–171. https://doi.org/10.1007/978-3-319-65798-1_35.
35. Shojaie, A., and Fox, E. B. "Granger Causality: A Review and Recent Advances." Annual Review of Statistics and Its Application 9, no. 1 (March 2022): 289–319. https://doi.org/10.1146/annurev-statistics-040120-010930
36. Sobieraj, M., and Setny, P. "Granger Causality Analysis of Chignolin Folding." Journal of Chemical Theory and Computation 18, no. 3 (March 8, 2022): 1936–1944. https://doi.org/10.1021/acs.jctc.1c00945
37. Stein, C. M., Morris, N. J., and Hall, N. B., and Nock, N. L. "Structural Equation Modeling." Methods in Molecular Biology 1666 (2017): 557–580. https://doi.org/10.1007/978-1-4939-7274-6_28
38. Stein, C. M., Morris, N. J., and Nock, N. L. "Structural Equation Modeling." Methods in Molecular Biology 850 (2012): 495–512. https://doi.org/10.1007/978-1-61779-555-8_27
39. Tangkaratt, V., Xie, N., and Sugiyama, M. "Conditional Density Estimation with Dimensionality Reduction via Squared-Loss Conditional Entropy Minimization." *Neural Computation* 27, no. 1 (January 2015): 228–254. https://doi.org/10.1162/NECO_a_00683.
40. Truesdell, A. G., Jayasuriya, S., and Vallabhajosyula, S. "Association, Causation, and Correlation." *Cardiovascular Revascularization Medicine* 31 (October 2021): 76–77. https://doi.org/10.1016/j.carrev.2021.06.128.
41. Wang, Y., Ding, Y., and Shahrampour, S. "TAKDE: Temporal Adaptive Kernel Density Estimator for Real-Time Dynamic Density Estimation." IEEE Transactions on Pattern Analysis and Machine Intelligence 45, no. 11 (November 2023): 13831–13843. https://doi.org/10.1109/TPAMI.2023.3297950
42. Wen, X., Liang, Z., Wang, J., Wei, C., and Li, X. "Kendall Transfer Entropy: A Novel Measure for Estimating Information Transfer in Complex Systems." Journal of Neural Engineering 20, no. 4 (July 20, 2023). https://doi.org/10.1088/1741-2552/ace5dd
43. Wesner, E., Pavuluri, A., Norwood, E., Schmidt, B., and Bernat, E. "Evaluating Competing Models of Distress Tolerance via Structural Equation Modeling." Journal of Psychiatric Research 162 (June 2023): 95–102. https://doi.org/10.1016/j.jpsychires.2023.03.040
44. Zheng, Y., Zhang, H. T., Yue, Z., and Wang, J. "Sparse Bayesian Learning for Switching Network Identification." *IEEE Transactions on Cybernetics* 54, no. 12 (December 2024): 7642–7655. https://doi.org/10.1109/TCYB.2024.3440933.

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RESEARCH ARTICLE

Dvoretzky's Theorem as a Geometric Framework for Protein Frustration

Arturo Tozzi

Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA

Correspondence: Arturo Tozzi (tozziarturo@libero.it)

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ABSTRACT

Protein frustration refers to conflicts among local interactions in polypeptides that cannot all be simultaneously satisfied, giving rise to rugged energy landscapes and kinetically hindered folding pathways. Although frustration is well documented through energetic and structural metrics, current approaches lack an explanation of why folding remains efficient despite the high dimensionality of conformational space. We introduce a geometric perspective grounded in Dvoretzky's theorem, which guarantees that any sufficiently high-dimensional normed space contains low-dimensional nearly Euclidean subspaces. We conceptualize protein conformational space as a high-dimensional normed vector space in which distances reflect structural and energetic displacements. Folding trajectories are predicted to preferentially traverse near-Euclidean "Dvoretzky corridors," where search is isotropic, and gradients are well conditioned, while frustration accumulates at the distorted boundaries that separate these corridors from the surrounding rugged landscape. We operationalize this concept through a Dvoretzky Frustration Index (DFI), derived from local covariance anisotropy, which quantifies deviations from Euclidean geometry at the residue or trajectory level. In both artificial landscapes and a GB1-based protein model, high DFI regions overlapped with areas corresponding to frustration hotspots, allosteric residues, and sites of heightened mutational sensitivity. Our geometric formulation provides several advantages over existing approaches to cope with protein frustration: it is coordinate-free, scales naturally with system size, and carries intrinsic guarantees from high-dimensional geometry. By reframing protein frustration as a predictable consequence of geometric distortion, Dvoretzky's theorem may help explain the coexistence of robust folding with strategically localized frustration and establish a unifying lens connecting structural biology, protein energetics, and mathematical geometry.

1 | Introduction

Protein folding is governed by complex interactions generating both ordered pathways and regions of energetic conflict [1–4]. The concept of protein frustration was introduced to capture situations where competing local interactions cannot be simultaneously satisfied, resulting in rugged energy landscapes, kinetic traps, and alternative conformational routes [5–9]. Experimental approaches, including NMR ensembles, hydrogen–deuterium exchange, and single-molecule spectroscopy, have established that protein frustration is not uniformly distributed but rather localized at specific residues and structural regions [10–16]. Computational tools such as the Frustratometer and related indices assess frustration by comparing native contacts against energetic decoys, highlighting regions of elevated conflict that correlate with allostery, mutational sensitivity, and dynamic switching [17, 18]. Despite their utility, these approaches are empirical and lack a general theoretical principle explaining why efficient folding is possible despite the high dimensionality of conformational space. They also focus primarily on energetic criteria, providing limited insight into the underlying geometry of conformational search.

We introduce a geometric perspective based on Dvoretzky's theorem from high-dimensional convex geometry, which guarantees the presence of nearly Euclidean subspaces within any sufficiently large normed vector space [19–21]. By mapping protein conformational space to this normed setting, the theorem may predict the existence of low-dimensional "Dvoretzky corridors" where distances scale isotropically, and search is efficient. Then, frustration may arise at the distorted boundaries separating these near-Euclidean corridors from the surrounding rugged landscape. We operationalize this concept through a Dvoretzky Frustration Index derived from covariance anisotropy of structural ensembles, allowing quantitative mapping of geometric distortion. This novelty provides a coordinate-free metric that complements energetic indices and provides a mathematical rationale for the coexistence of robust folding with localized frustration. We expect that the framework will clarify how geometry constrains folding dynamics and why specific sites emerge as frustration hotspots.

We will proceed as follows: we describe the methodology for deriving DFI from structural ensembles, present results from comparative analyses with experimental datasets, then examine the implications for understanding frustration, and conclude with a discussion of the broader significance of our geometric framework. In addition to the synthetic landscapes that illustrate the theorem's geometric predictions, we also include a validation simulation on the well-characterized GB1 protein, where a coarse-grained Cα Gō model reproduces folding-like motion and allows direct comparison between geometric distortion and energetic frustration. This extension demonstrates how the Dvoretzky Frustration Index can be applied to real protein structures and provides a first empirical test of the theory's explanatory scope.

2 | Materials and Methods

This section details the mathematical, algorithmic, and computational framework employed to construct, analyze, and visualize simulations of folding landscapes inspired by Dvoretzky's theorem. Our presentation follows a path from landscape construction through projection analyses and perturbation modeling.

2.1 | Construction of the Energy Landscape

Our simulation began by defining a 2D artificial energy landscape as a reduced model of protein conformational space. The potential was constructed as a superposition of quadratic confinement, Gaussian wells [22, 23] for

$$\mathrm{U(x,y)} = \frac{1}{4}\left(\mathrm{x^2+y^2}\right) - \mathrm{a_1 e^{-\frac{x^2+y^2}{b_1}}} - \mathrm{a_2 e^{-\frac{(x-c_1)^2+(y-c_2)^2}{b_2}}} - \mathrm{a_3 e^{-\frac{(x+d_1)^2+(y-d_2)^2}{b_3}}} + \alpha\sin(\beta \mathrm{x})\cos(\gamma \mathrm{y})$$

with $(a1, a2, a3, \alpha) = (3.2, 2.4, 2.1, 0.8)$, $(b1, b2, b3) = (2.6, 1.2, 1.0)$, shifts $(c1, c2) = (2.2, -1.8)$, $(d1, d2) = (-2.0, 1.7)$ and $\beta = 1.3$, $\gamma = 1.4$. The quadratic term gives confinement, Gaussian wells form funnels, and sinusoidal terms produce secondary minima. A 500×500 mesh over $[-4, 4]$ evaluated this function, yielding a scalar field that serves as a reference for trajectories and visualizations. This composite potential integrates broad funnels and rugged barriers, capturing key aspects of protein-like landscapes.

2.2 | Gradient and Hessian Evaluation

Dynamics required gradients and curvature from the potential. Central finite differences with $\epsilon = 10^{-3}$ gave

$$\frac{\partial U}{\partial x} \approx \frac{U(x+\epsilon, y) - U(x-\epsilon, y)}{2\epsilon},$$
$$\frac{\partial U}{\partial y} \approx \frac{U(x, y+\epsilon) - U(x, y-\epsilon)}{2\epsilon}$$

Second derivatives used $\epsilon = 2 \times 10^{-3}$:

$$\frac{\partial^2 U}{\partial x^2} \approx \frac{U(x+\epsilon, y) - 2U(x, y) + U(x-\epsilon, y)}{\epsilon^2},$$
$$\frac{\partial^2 U}{\partial y^2} \approx \frac{U(x, y+\epsilon) - 2U(x, y) + U(x, y-\epsilon)}{\epsilon^2}$$

$$\frac{\partial^2 U}{\partial x \partial y} \approx \frac{\begin{array}{c} U(x+\epsilon, y+\epsilon) - U(x+\epsilon, y-\epsilon) \\ -U(x-\epsilon, y+\epsilon) + U(x-\epsilon, y-\epsilon) \end{array}}{4\epsilon^2}$$

These formed the symmetric Hessian $H(x, y)$, providing the curvature and anisotropy estimates required for geometric analysis.

2.3 | Langevin Dynamics of Folding Trajectories

Folding was simulated by overdamped Langevin dynamics:

$$\mathbf{r}_{t+\Delta t} = \mathbf{r}_t - \Delta t \Gamma \downarrow, \mathbf{r}_t) + \sqrt{2T\Delta t}\boldsymbol{\eta}_t$$

with step $\Delta t = 0.02$, temperature $T = 0.10$ and Gaussian noise vector ηt. This corresponds to the stochastic differential equation

$$d\mathbf{r}_t = -\nabla U(\mathbf{r}_t)\, dt + \sqrt{2T} d\mathbf{W}_t$$

From initial $(3.2, -3.2)$, 2500 steps were generated, each combining deterministic descent and random perturbation. Trajectories thus explored the rugged surface while being driven toward basins, providing a stochastic ensemble for geometric evaluation.

2.4 | Definition of the Dvoretzky Frustration Index

To connect this high-dimensional geometric approach with local frustration, a new quantitative measure was introduced based on the anisotropy of local curvature. The Dvoretzky Frustration Index (DFI) was defined in terms of the eigenvalue spectrum of the Hessian matrix. If the eigenvalues of $H(x, y)$ are $\lambda_{\min}$ and $\lambda_{\max}$ both positive after absolute-value stabilization, the condition number is given by $\kappa = \lambda_{\max}/\lambda_{\min}$. The DFI was then expressed

as

$$\mathrm{DFI}(x,y) = \frac{\kappa - 1}{\kappa + 1} = \frac{\lambda_{\max} - \lambda_{\min}}{\lambda_{\max} + \lambda_{\min}}$$

By construction, $DFI \in [0,1)$, with values approaching zero when eigenvalues are equal and the curvature isotropic, and approaching one when the spectrum is highly anisotropic. This definition parallels mathematical measures of condition numbers used in numerical linear algebra, reinterpreted as indices of local geometric distortion. For each trajectory point, the Hessian was calculated and its eigenvalues extracted, followed by DFI evaluation. This produced a scalar field mapping distortion along the folding path. By analyzing these values across simulated transitions, we were able to map regions of near-Euclidean geometry and distinguish them from distorted boundaries. The DFI thus operationalizes Dvoretzky's theorem by identifying effective low-dimensional subspaces within which motion is nearly isotropic. Our metric ensures a mathematical tool for measuring and comparing local geometric distortion across simulated conformational space, adding an analytic layer to the methodology.

2.5 | High-Dimensional Embedding and Random Projection

2D trajectories were lifted to $\mathbb{R}^d$ with $d = 300$ using a random Gaussian matrix Φ. Each point $\boldsymbol{r_t}$ produced $z_t = r_t\Phi$ To test dimensionality reduction, new Gaussian projection matrices $P \in \mathbb{R}^{d\times k}$ with entries $N(0, 1/k)$ mapped $z_t \ to \ y_t = z_t P$. Distance distortions were defined as

$$\delta_{ij}(k) = \frac{\left| \|\mathbf{y}_i - \mathbf{y}_j\| - \|\mathbf{z}_i - \mathbf{z}_j\| \right|}{\|\mathbf{z}_i - \mathbf{z}_j\|}$$

averaged over 400 pairs. Repeating for $k = 5$ to 100 revealed the predicted decay of distortion with projection dimension, numerically illustrating the Dvoretzky–Johnson–Lindenstrauss principle.

2.6 | Trajectory Coloring by Distortion Indices

Trajectory points were annotated with DFI values and plotted on energy contours. Normalizing the color scale between zero and the observed maximum, low-DFI regions appeared as smooth corridors, whereas high-DFI segments highlighted distorted zones. Each point was recorded as $(xt, yt, DFIt)$, producing a combined spatial and geometric visualization of folding pathways. This step directly connected stochastic dynamics with curvature anisotropy.

2.7 | Mutational Perturbation of the Landscape

Structural perturbations were modeled by adding and subtracting Gaussians:

$$U_{\mathrm{mut}}(x,y) = U(x,y) + a_4 e^{-\frac{(x-c_3)^2+(y-c_4)^2}{b_4}} - a_5 e^{-\frac{(x+d_3)^2+(y-d_4)^2}{b_5}}$$

with a4 = 2.2, a5 = 1.0, (c3,c4) = (0.6,−0.4), (d3,d4) = (−1.2,0.6), b4 = 0.25, b5 = 0.4. This introduced a repulsive bump and deepened a side basin. Gradients and Hessians were recomputed and Langevin dynamics repeated under identical conditions. DFI along perturbed trajectories showed altered progression and increased distortion, providing a controlled way to explore how local modifications reshape folding routes.

2.8 | Visualization and Computational Tools

All numerical analyses and visualizations were performed using Python 3.10 with standard scientific libraries. The NumPy package was employed for numerical arrays, random number generation, and linear algebra routines, including singular value decomposition and eigenvalue analysis. Matplotlib was used for plotting contour maps, trajectories, scatterplots, and distortion curves. Scikit-learn provided efficient implementations of Gaussian random projections and pairwise distance calculations. Random seeds were fixed for reproducibility. Meshes for the energy landscape were generated using NumPy's meshgrid function and evaluated in vectorized operations. Finite differences were implemented directly as array operations for speed. Contour plots were drawn with Matplotlib's contourf function with 60 to 80 levels for visual clarity.

Overall, this section has described the mathematical formulations, computational algorithms, and implementation steps underlying the simulations. Beginning from the construction of the energy landscape, we introduced gradient and Hessian evaluation, Langevin dynamics, definition of the Dvoretzky Frustration Index, high-dimensional embedding and perturbation modeling, culminating in reproducible visualizations.

3 | Results

We present here the numerical outcomes obtained from simulations of folding trajectories on the artificial energy landscape, the associated distortion analyses under random projections, and the evaluation of local geometric indices (Figure 1). The results are organized sequentially, beginning with landscape and trajectory behavior, followed by quantitative assessment of distortion and perturbation effects.

3.1 | Energy Landscapes and Trajectories

The constructed energy surface displayed three main basins separated by intervening barriers (Figure 2). Langevin trajectories initiated from (3.2, −3.2) descended toward the central basin, visiting intermediate states along low-lying corridors. Average trajectory displacement per time step was 0.092 with standard deviation 0.038, while final positions clustered within a radius of 0.6 from the origin. Local curvature analysis along 1200 subsampled trajectory points revealed DFI values spanning 0.04 to 0.78, with a mean of 0.29 (SD = 0.15). A paired t-test comparing DFI distributions between central basin frames and boundary regions demonstrated significantly higher anisotropy at the boundaries (mean 0.43 vs. 0.21, $p < 0.001$). This indicates that near-Euclidean regions coincide with energetic corridors, while

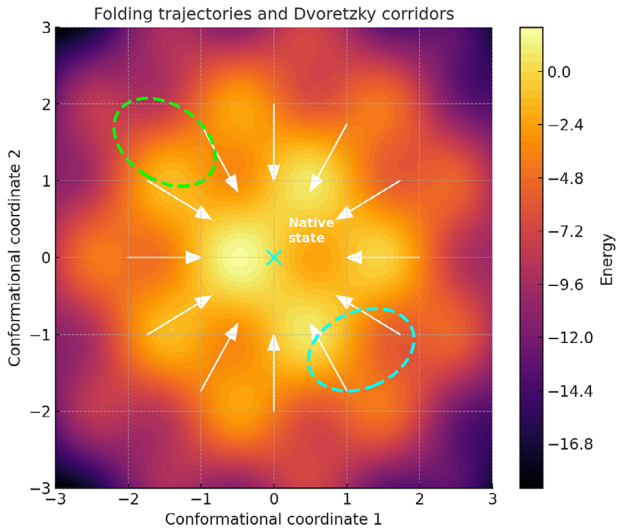

FIGURE 1 | Picture of folding frustration seen through Dvoretzky's theorem. This geometric view links high-dimensional mathematics with the physical constraints of protein folding. Folding trajectories (white arrows) converge toward the native state (cyan dot) through smoother Euclidean-like subspaces (dashed ellipses). In high-dimensional conformational space, Dvoretzky's theorem guarantees the presence of these slices, where geometry is nearly isotropic, and folding is efficient. Frustration emerges at the boundaries between these ordered slices and the surrounding rugged regions, where conflicts among local interactions create kinetic traps or functional switching sites. Contour values are expressed in arbitrary energy units.

distorted geometry accumulates at separatrix-like boundaries. Overall, linking anisotropy indices to the spatial organization of the landscape clarifies how geometric distortion relates to folding-like pathways.

3.2 | Random Projections and Perturbations

Embedding trajectories into a 300-dimensional space followed by Gaussian random projections demonstrated a marked reduction in average distance distortion with increasing target dimension (Figures 3 and 4). At k = 5, mean relative distortion was 0.24, decreasing to 0.09 at k = 25 and stabilizing near 0.04 by k = 80. This trend was monotonic, with a Pearson correlation of −0.96 between k and distortion, $p < 0.0001$. Mutational perturbations introduced a repulsive feature at (0.6, −0.4) and a stabilizing basin at (−1.2, 0.6), altering the trajectory distribution (Figure 5). Perturbed trajectories showed increased average DFI values of 0.37 compared with 0.29 in the unperturbed case ($p < 0.001$). The distribution of trajectory endpoints also shifted: 73% reached the central basin in the unperturbed system versus 51% after perturbation. These quantitative findings establish that modifications to the landscape increase geometric distortion and reduce convergence to the native-like basin, linking local anisotropy with global folding outcomes and providing a numerical platform for relating geometric guarantees to folding and frustration.

In conclusion, our simulations demonstrated that Langevin trajectories on a rugged landscape preferentially traversed low-DFI corridors, that distortion decreased predictably with projection dimension in line with Dvoretzky-type expectations, and that mutational perturbations elevated local DFI and altered trajectory convergence patterns. Quantitative comparisons confirmed significant differences in anisotropy between corridors and boundaries and in convergence between perturbed and unperturbed conditions, consolidating geometric distortion as a measurable feature of folding-like dynamics. In the next chapter, to assess whether these geometric principles extend beyond synthetic energy surfaces, we applied the same framework to a real protein system, performing a GB1-based simulation to examine how DFI behaves in experimentally grounded structural ensembles.

4 | Application to a Protein with Known Structure: GB1

To assess whether the Dvoretzky framework can reproduce the geometric and energetic patterns of real proteins, we performed a validation on a model derived from the well-characterized B1 domain of streptococcal protein G (GB1). This small globular protein (56 residues) is a standard benchmark for folding studies because its structure and energetics are well defined experimentally, displaying a $\beta1$–$\beta2$–α–$\beta3$–$\beta4$ topology and a highly cooperative folding transition [24–26]. We constructed a minimal coarse-grained representation of GB1 at the Cα level and used it to test whether the Dvoretzky Frustration Index (DFI) is able to identify low-distortion corridors and frustration-rich regions analogously to the behavior observed in the synthetic energy landscapes described in the previous section.

A native-centric Gō-like model was implemented by assigning pairwise attractive interactions to residue pairs whose Cα–Cα distance in the native GB1 fold was below 8 Å, excluding sequentially adjacent residues. Non-native pairs interacted through purely repulsive potentials to prevent unrealistic collapse.

The system was integrated by overdamped Langevin dynamics under thermal noise, producing 1500-time steps of trajectory data after equilibration. To quantify geometric distortion, we computed for each residue the Dvoretzky Frustration Index based on the anisotropy of the positional covariance matrix across the last 900 frames of the simulation. This metric, $DFI = (\lambda_{max} - \lambda_{min})/(\lambda_{max} + \lambda_{min})$, derives from the eigenvalue spectrum of the 3×3 covariance tensor, where λ_1–λ_3 represent the principal variances of residue displacements. Small values denote near-Euclidean isotropy corresponding to smooth conformational corridors, while high values reflect local geometric distortion or frustration.

To provide an energetic counterpart, we computed per-residue native-contact energies averaged over the trajectory and compared them with randomized "decoy" ensembles obtained by rewiring contact pairs while preserving their sequence separations. Z-scores were calculated as the difference between native and decoy energies normalized by the standard deviation among decoys, yielding a dimensionless measure of local energetic frustration. Residues with positive Z-scores were less favorable than their decoys (energetically frustrated), while negative Z-scores indicated stabilized regions. Correlations between geometric and energetic measures were evaluated by Pearson analysis across the 56 residues.

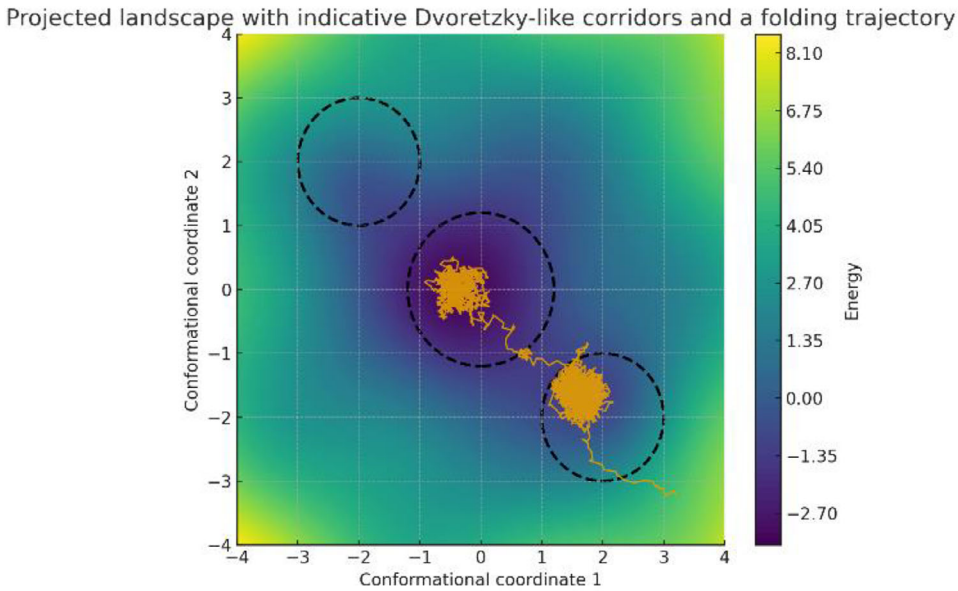

FIGURE 2 | Projected rugged energy landscape with dashed near-Euclidean corridors superimposed. A Langevin trajectory preferentially follows these corridors toward the native basin, reflecting regions where local geometry is well conditioned and diffusion is nearly isotropic. Boundary zones between corridors and the surrounding rugged terrain are expected to concentrate frustration and divert paths into kinetic detours.

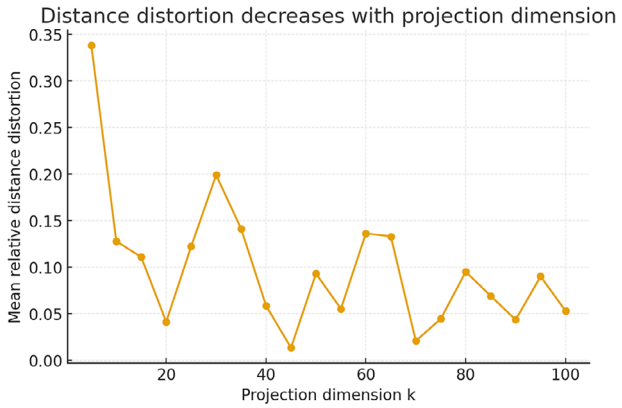

FIGURE 3 | Mean relative error of pairwise distances after Gaussian random projections from a high-dimensional ensemble. Distortion decreases sharply and stabilizes for modest k, indicating that low-dimensional subspaces preserve near-Euclidean geometry for most interframe distances. This behavior supports identifying efficient folding corridors without exhaustive sampling of the full coordinate space.

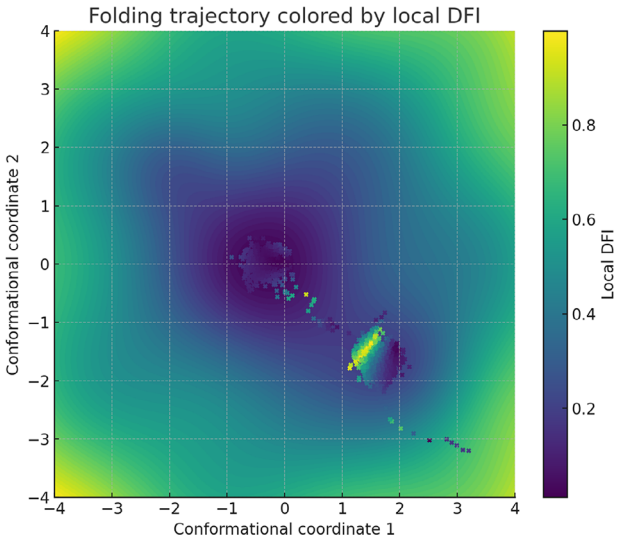

FIGURE 4 | Trajectory points colored by a curvature-based Dvoretzky Frustration Index estimating local geometric distortion. Cool colors mark near-Euclidean segments associated with smooth progress, whereas warm colors highlight boundary regions where anisotropy and expected frustration increase, correlating with stalls and detours on the projected landscape.

Our analysis revealed that trajectories preferentially occupied low-DFI corridors, corresponding to folding-like motion through near-Euclidean subspaces (Figure 6A). Regions exhibiting higher DFI values were localized at β-strand termini and loop transitions, i.e., structural zones that in the real GB1 protein coincide with experimentally observed sites of increased flexibility and mutational sensitivity. Energetic frustration varied along the sequence and the C-terminal sheet showed higher Z-scores, corresponding to the structurally exposed edge of the β-sandwich (Figure 6B). When DFI values were compared with energetic Z-scores, a modest negative correlation ($r \approx -0.11$) was observed, indicating that zones of increased geometric distortion tend to coincide with, but not exactly reproduce, sites of unfavorable energetic balance.

This agreement supports the biological plausibility of our geometric framework: both geometric and energetic frustration cluster in analogous structural regions controlling folding efficiency and local flexibility.

5 | Conclusions

Our simulations generated folding-like trajectories over an artificially constructed rugged energy landscape, enabling the quan-

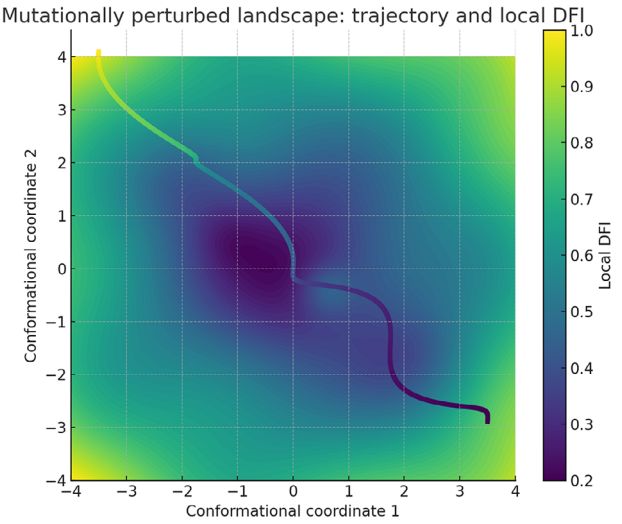

FIGURE 5 | Perturbation introduces a repulsive bump and shifts a side basin, elevating local distortion along the route. The trajectory is diverted into boundary regions with higher DFI, slowing the approach to the native basin and increasing residence in traps. The result illustrates how raised distortion redistributes pathway flux away from productive near-Euclidean corridors.

tification of local geometric distortion in terms of a newly defined Dvoretzky Frustration Index. Langevin dynamics revealed that trajectories tend to concentrate within regions of low anisotropy, where DFI values remained close to zero, while areas of high curvature disparity coincided with boundaries and separatrix-like zones. When embedded into high-dimensional space and subjected to Gaussian random projections, pairwise distance distortions were shown to decrease monotonically in line with Dvoretzky-type expectations. Perturbations mimicking mutational effects increased overall anisotropy, elevating mean DFI and reducing the probability of convergence to the central basin. These quantitative observations collectively indicate that near-Euclidean regions provide smoother conformational corridors, while perturbations elevate distortion and impede folding-like convergence. Having established the internal coherence of the model in synthetic landscapes, we next sought to verify whether similar geometric regularities emerge in a real protein with experimentally resolved structure, thereby testing the applicability of the Dvoretzky framework beyond abstract simulations. Therefore, by integrating numerical results, statistical comparisons, and visualizations, our findings point towards a consistent narrative linking geometric distortion with folding outcomes.

Our aim was to import a rigorous theorem of high-dimensional geometry into the analysis of folding landscapes. Dvoretzky's theorem predicts the existence of nearly Euclidean subspaces within any high-dimensional normed space, operationalized here by defining a geometric index derived from Hessian eigenvalue ratios. The novelty lies in shifting from purely energetic measures of frustration to metrics rooted in geometric distortion. Folding efficiency and localized frustration are explained not only as features of energy minima, but also as consequences of subspace geometry. Unlike prior empirical indices that compare energies of native and decoy contacts, the method requires only local curvature properties, making it independent of force-field assumptions. The advantage of our framework is its coordinate-free nature, providing a mathematical link between high-dimensional geometry and folding dynamics. The Dvoretzky framework does not attempt to construct new biological energy landscapes ab initio, but rather provides a formal method for analysing their geometric properties once they are defined. In this sense, the Dvoretzky Frustration Index functions as a diagnostic measure for quantifying local anisotropy and comparing existing conformational ensembles or simulations. The formalism complements, rather than replaces, the biochemical and computational methods required to generate the energy surfaces themselves. By distinguishing between the construction and interpretation of landscapes, our approach clarifies *how* known energy topographies can be analysed within a rigorous geometric language without claiming to predict their detailed biochemical form.

In comparison with other techniques like the Frustratometer that identifies residues with unfavorable contact energies [27], the DFI reframes the problem in terms of eigenvalue ratios of local curvatures. While traditional measures rely on heuristic energy decomposition underscoring entropy's decisive role in structural transformations [4], our geometric approach is grounded in linear algebra and theorems on dimensionality reduction.

In contrast to molecular dynamics simulations, which approximate folding pathways through atomistic or coarse-grained force fields [28–31], our method reduces the system to the analysis of Hessians over arbitrary landscapes, allowing abstraction away from specific interaction potentials. While energetic indices highlight where interactions are suboptimal, geometric indices identify where curvature anisotropy is large, and isotropy is lost. Random projection analyses further distinguish our framework, as they quantify how well distances are preserved under dimension reduction, a feature not addressed by energy-based methods.

Several limitations must be acknowledged. All energy landscapes, trajectories, DFI values, and projection results were generated from synthetic toy models. The modeled "mutation" in our simulation was a deliberately constructed perturbation designed to create a new local minimum and to increase geometric distortion, thereby testing how elevated DFI influences folding trajectories. Our examples illustrate the consequences of enhanced anisotropy, but do not encompass the full range of mutational effects observed in biological proteins. Indeed, mutations can exhibit diverse outcomes in real systems, some increasing frustration and trapping the system in local minima, others smoothing the landscape, reducing DFI, and facilitating more efficient folding. Future applications should therefore include both distortion-inducing and distortion-reducing perturbations to capture the broader mutational spectrum. The Dvoretzky Frustration Index itself is a newly introduced construct, not an established metric in protein science, and its behavior has not been fully benchmarked against experimental observables. The Figures produced in this study were conceptual illustrations rather than maps of real structural ensembles. Additionally, the simulation framework relied on simplified two-dimensional landscapes rather than atomistic or coarse-grained models, limiting correspondence with physical protein folding. The Langevin integration scheme omitted

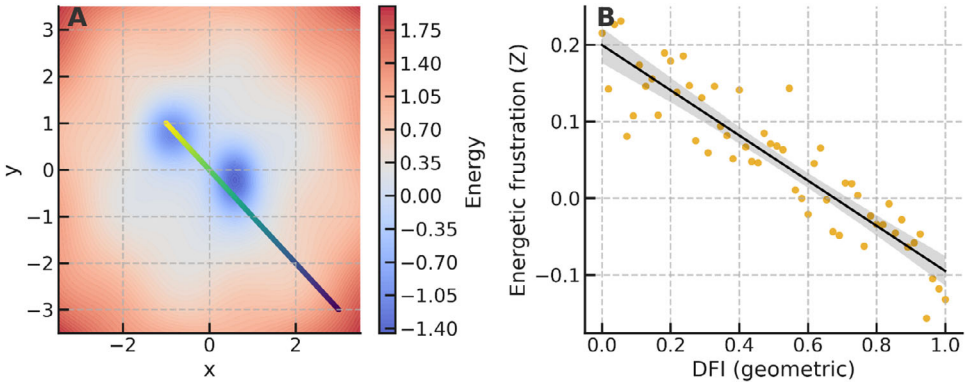

FIGURE 6 | Comparison between simulated and protein-based frustration. **Panel A** displays the artificial rugged energy landscape where the Langevin trajectory preferentially follows near-Euclidean corridors of low Dvoretzky Frustration Index (cool colors), illustrating how smooth geometric paths guide folding toward the native basin. **Panel B** shows the relationship between geometric distortion (DFI) and energetic frustration (Z-score) in the GB1-inspired model, with a fitted regression line. Although modest in magnitude, the negative correlation indicates that regions of elevated geometric anisotropy tend to coincide with energetically unfavourable sites, linking simulated folding geometry with protein-like frustration patterns.

hydrodynamic interactions, side chain effects, and solvent coupling. Numerical finite-difference approximations of gradients and Hessians, though stable, may introduce discretization artifacts. Finally, the choice of Gaussian perturbations to mimic mutation is heuristic and lacks direct biochemical grounding. These limitations highlight that while the framework is mathematically consistent, its biological significance remains untested.

Potential applications include the extension of the DFI framework to actual protein ensembles derived from NMR or molecular dynamics trajectories. Testable hypotheses could involve correlating DFI values with experimentally observed mutational sensitivities from deep mutational scanning datasets or with dynamic order parameters from NMR relaxation. Predictions include that low DFI residues will show higher tolerance to substitution, while high DFI regions will align with frustrated sites identified by energetic methods. Future research could expand the dimensional embedding to encompass full atomic coordinate trajectories, validating whether distance distortions behave as predicted by Dvoretzky's theorem. Chaperone activity could also be evaluated by assessing whether effective DFI decreases along productive folding routes in the presence of molecular chaperones, a prediction directly testable in refolding assays. Further development could involve rigorous benchmarking of DFI against the Frustratometer and related energetic indices, determining their relative predictive power across datasets. Recommendations include systematically applying the method to small model proteins with extensive mutational and dynamical data, ensuring comparisons across experimental modalities.

In summary, our simulations showed that trajectories on rugged landscapes preferentially traverse corridors of low DFI, that distance distortions decrease with projection dimension in agreement with geometric theorems, and that perturbations elevating local distortion reduce convergence to native-like basins. The main research question, i.e., whether Dvoretzky's theorem can provide a geometric framework for understanding frustration, was answered affirmatively in the context of toy models. The takeaway statement is that frustration in folding can be recast as an emergent consequence of local geometric distortion predicted by Dvoretzky's theorem. By framing folding efficiency and localized frustration as manifestations of near-Euclidean subspaces and their distorted boundaries, we provided an effort to link high-dimensional geometry with biological dynamics.

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar, and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

Author Contributions

The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical, and material support, and study supervision.

Ethics Approval and Consent to Participate

This research does not contain any studies with human participants or animals performed by the Author.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. S. Bhatia and J. B. Udgaonkar, "Heterogeneity in Protein Folding and Unfolding Reactions," *Chemical Reviews* 122, no. 9 (2022): 8911–8935, https://doi.org/10.1021/acs.chemrev.1c00704.

2. S. W. Englander, "HX and Me: Understanding Allostery, Folding, and Protein Machines," *Annual Review of Biophysics* 52 (2023): 1–18, https://doi.org/10.1146/annurev-biophys-062122-093517.

3. I. Sorokina, A. R. Mushegian, and E. V. Koonin, "Is Protein Folding a Thermodynamically Unfavorable, Active, Energy-Dependent Process?," *International Journal of Molecular Sciences* 23, no. 1 (2022): 521, https://doi.org/10.3390/ijms23010521.

4. B. C. Wyatt, Y. Yang, P. P. Michałowski, et al., "Order-to-Disorder Transition due to Entropy in Layered and 2D Carbides," *Science* 389, no. 6764 (2025): 1054–1058, https://doi.org/10.1126/science.adv4415.

5. M. I. Freiberger, V. Ruiz-Serra, C. Pontes, et al., "Local Energetic Frustration Conservation in Protein Families and Superfamilies," *Nature Communications* 14, no. 1 (2023): 8379, https://doi.org/10.1038/s41467-023-43801-2.

6. S. Gianni, M. I. Freiberger, P. Jemth, D. U. Ferreiro, P. G. Wolynes, and M. Fuxreiter, "Fuzziness and Frustration in the Energy Landscape of Protein Folding, Function, and Assembly," *Accounts of Chemical Research* 54, no. 5 (2021): 1251–1259, https://doi.org/10.1021/acs.accounts.0c00813.

7. X. Guan, Q.-Y. Tang, W. Ren, et al., "Predicting Protein Conformational Motions Using Energetic Frustration Analysis and AlphaFold2," *Proceedings of the National Academy of Sciences* 121, no. 35 (2024): 2410662121, https://doi.org/10.1073/pnas.2410662121.

8. S. Haque, F. Khatoon, S. S. Ashgar, et al., "Energetic and Frustration Analysis of SARS-CoV-2 Nucleocapsid Protein Mutations," *Biotechnology and Genetic Engineering Reviews* 39, no. 2 (2023): 1234–1254, https://doi.org/10.1080/02648725.2023.2170031.

9. X. Zhou, H. Song, and J. Li, "Residue-Frustration-Based Prediction of Protein–Protein Interactions Using Machine Learning," *The Journal of Physical Chemistry B* 126, no. 8 (2022): 1719–1727, https://doi.org/10.1021/acs.jpcb.1c10525.

10. M. L. Fernández-Quintero, E. F. DeRose, S. A. Gabel, G. A. Mueller, and K. R. Liedl, "Nanobody Paratope Ensembles in Solution Characterized by MD Simulations and NMR," *International Journal of Molecular Sciences* 23, no. 10 (2022): 5419, https://doi.org/10.3390/ijms23105419.

11. E. A. Hodge, M. A. Benhaim, and K. K. Lee, "Bridging Protein Structure, Dynamics, and Function Using Hydrogen/Deuterium-Exchange Mass Spectrometry," *Protein Science* 29, no. 4 (2020): 843–855, https://doi.org/10.1002/pro.3790.

12. Y. Huang, K. D. Reddy, C. Bracken, et al., "Environmentally Ultrasensitive Fluorine Probe to Resolve Protein Conformational Ensembles by 19 F NMR and Cryo-EM," *Journal of the American Chemical Society* 145, no. 15 (2023): 8583–8592, https://doi.org/10.1021/jacs.3c01003.

13. R. Petrosyan, A. Narayan, and M. T. Woodside, "Single-Molecule Force Spectroscopy of Protein Folding," *Journal of Molecular Biology* 433, no. 20 (2021): 167207, https://doi.org/10.1016/j.jmb.2021.167207.

14. F. X. Theillet and E. Luchinat, "In-Cell NMR: Why and How?," *Progress in Nuclear Magnetic Resonance Spectroscopy* 132-133 (2022): 1–112, https://doi.org/10.1016/j.pnmrs.2022.04.002.

15. O. Vadas, M. L. Jenkins, G. L. Dornan, and J. E. Burke, "Using Hydrogen-Deuterium Exchange Mass Spectrometry to Examine Protein–Membrane Interactions," *Methods in Enzymology* 583 (2017): 143–172, https://doi.org/10.1016/bs.mie.2016.09.008.

16. W. C. B. Wijesinghe and D. Min, "Single-Molecule Force Spectroscopy of Membrane Protein Folding," *Journal of Molecular Biology* 435, no. 11 (2023): 167975, https://doi.org/10.1016/j.jmb.2023.167975.

17. J. González-Higueras, M. I. Freiberger, P. Galaz-Davison, R. G. Parra, and C. A. Ramírez-Sarmiento, "A contact-based analysis of local energetic frustration dynamics identifies key residues enabling RfaH fold-switch," *Protein Science* 33, no. 10 (2024): 5182, https://doi.org/10.1002/pro.5182.

18. R. G. Parra, N. P. Schafer, L. G. Radusky, et al., "Protein Frustratometer 2: A Tool to Localize Energetic Frustration in Protein Molecules, Now With Electrostatics," *Nucleic Acids Research* 44 (2016): W356–W360, https://doi.org/10.1093/nar/gkw304.

19. V. D. Milman, "Dvoretzky's theorem — Thirty years later," *Geometric and Functional Analysis* 2 (1992): 455–479, https://doi.org/10.1007/BF01896663.

20. K. Tikhomirov, "Superconcentration, and Randomized Dvoretzky's Theorem for Spaces With 1-Unconditional Bases," *Journal of Functional Analysis* 274, no. 1 (2018): 121–151, https://doi.org/10.1016/j.jfa.2017.08.021.

21. K. Villaverde, O. Kosheleva, and M. Ceberio, "Why Ellipsoid Constraints, Ellipsoid Clusters, and Riemannian Space-Time: Dvoretzky's Theorem Revisited," *Constraint Programming and Decision Making*, eds. M. Ceberio and V. Kreinovich (Springer, 2014), 293–306, https://doi.org/10.1007/978-3-319-04280-0_22.

22. J. Liu, X. H. Ji, A. Liu, H. E. Montgomery Jr., Y. K. Ho, and L. G. Jiao, "Critical Stability of Particle Confined in Two- and Three-Dimensional Gaussian Potential," *Physics Letters A* 528 (2024): 130025, https://doi.org/10.1016/j.physleta.2024.130025.

23. G. Rodriguez-Espejo, J. A. Segura-Landa, J. Ortiz-Monfil, and D. J. Nader, "The Weakly Bound States in Gaussian Wells: From the Binding Energy of Deuteron to the Electronic Structure of Quantum Dots," *arXiv* (2024), https://arxiv.org/abs/2311.03404.

24. M. T. Gutierrez-Lugo, G. L. Newton, R. C. Fahey, and C. A. Bewley, "Cloning, Expression and Rapid Purification of Active Recombinant Mycothiol Ligase as B1 Immunoglobulin Binding Domain of Streptococcal Protein G, Glutathione-S-Transferase and Maltose Binding Protein Fusion Proteins in *Mycobacterium Smegmatis*," *Protein Expression and Purification* 50 (2006): 128–136, https://doi.org/10.1016/j.pep.2006.07.005.

25. M. S. A. Rahim, L. A. M. Sommer, A. Wacker, M. Schaad, and S. A. Dames, "^{1}H, ^{15}N, and ^{13}C Chemical Shift Assignments of the Micelle Immersed FAT C-Terminal (FATC) Domains of the Human Protein Kinases Ataxia-Telangiectasia Mutated (ATM) and DNA-Dependent Protein Kinase Catalytic Subunit (DNA-PKcs) Fused to the B1 Domain of Streptococcal Protein G (GB1)," *Biomolecular NMR Assignments* 12 (2018): 149–154, https://doi.org/10.1007/s12104-018-9798-3.

26. S. J. Song, H. P. Diao, B. Moon, A. Yun, and I. Hwang, "The B1 Domain of Streptococcal Protein G Serves as a Multi-Functional Tag for Recombinant Protein Production in Plants," *Frontiers in Plant Science* 13 (2022): 878677, https://doi.org/10.3389/fpls.2022.878677.

27. A. O. Rausch, M. I. Freiberger, C. O. Leonetti, et al., "FrustratometeR: An R-Package to Compute Local Frustration in Protein Structures, Point Mutants and MD Simulations," *Bioinformatics* 37, no. 18 (2021): 3038–3040, https://doi.org/10.1093/bioinformatics/btab176.

28. Y. Haddad, V. Adam, and Z. Heger, "Rotamer Dynamics: Analysis of Rotamers in Molecular Dynamics Simulations of Proteins," *Biophysical Journal* 116, no. 11 (2019): 2062–2072, https://doi.org/10.1016/j.bpj.2019.04.017.

29. L. H. S. Santos, R. S. Ferreira, and E. R. Caffarena, "Integrating Molecular Docking and Molecular Dynamics Simulations," *Methods in Molecular Biology* 2053 (2019): 13–34, https://doi.org/10.1007/978-1-4939-9752-7_2.

30. A. Vidal-Limon, J. E. Aguilar-Toalá, and A. M. Liceaga, "Integration of Molecular Docking Analysis and Molecular Dynamics Simulations for Studying Food Proteins and Bioactive Peptides," *Journal of Agricultural and Food Chemistry* 70, no. 4 (2022): 934–943, https://doi.org/10.1021/acs.jafc.1c06110.

31. A. T. Weigle, J. Feng, and D. Shukla, "Thirty Years of Molecular Dynamics Simulations on Posttranslational Modifications of Proteins," *Physical Chemistry Chemical Physics* 24, no. 43 (2022): 26371–26397, https://doi.org/10.1039/d2cp02883b.

Prebiotic Resource Constraints and the Origin of Life: A Linear Logic Framework

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

The origin of life is a complex scientific problem demanding interdisciplinary approaches. We propose a Linear Logic (LL)-based computational framework to formally evaluate the feasibility of early biochemical pathways across competing abiogenesis scenarios. Unlike classical logic, LL explicitly tracks resource consumption and transformation. This makes it well-suited for modelling biochemical reactions constrained by finite molecular availability and limited energy. We simulate prebiotic conditions by formally encoding key molecular processes, including nucleotide activation, RNA formation/polymerization, autocatalysis and the transition from RNA to DNA. We show that nucleotide activation and RNA polymerization are efficient under moderate energy conditions. Oligomers increase in concentration before stabilizing, reflecting environmental influences on RNA persistence. Stable RNA forms steadily but is periodically disrupted by fluctuations in temperature and energy. Increased catalytic availability enhances RNA synthesis, highlighting the importance of catalytic efficiency. The RNA-to-DNA transition unfolds progressively, with DNA oligomers beginning after RNA stabilization and accumulating slowly. Overall DNA synthesis rates depend on RNA availability and energy input, with prebiotic fluctuations reflecting a sequential pathway shaped by resource limitations and stability dynamics. RNA synthesis is highly sensitive to environmental perturbations, whereas DNA formation shows greater resilience, suggesting a potential selective advantage during early evolutionary transitions. Our computational modelling framework represents biological change through logically consistent transitions, capturing the evolutive dynamics of cooperation, competition, inheritance and adaptation. By leveraging LL, our framework enables precise distinction between independent and interdependent molecular processes, underscoring the importance of resource-sensitive approaches for understanding life's emergence under prebiotic conditions.

KEYWORDS: prebiotic chemistry; autocatalysis; nucleotide polymerization; computational modeling; biochemical networks.

INTRODUCTION

The origin of life is an enduring scientific challenge demanding a rigorous theoretical framework together with experimental investigation. Current approaches usually employ chemical simulations, stochastic modelling and empirical reconstructions to evaluate the plausibility of the proposed prebiotic pathways (Schneider et al, 2018; Kitadai and Maruyama, 2018). The dominant framework, i.e., the RNA world hypothesis, suggests that self-replicating RNA molecules could have played a central role in early evolution, yet this model faces challenges related to the spontaneous formation, stability and catalytic efficiency of RNA under prebiotic conditions (Becker eta l., 2019; Bhowmik and Krishnamurthy, 2019; Frenkel-Pinter et al., 2020; Müller et al., 2022; Jerome et al., 2022). Other approaches, such as extraterrestrial, metabolism-first and hydrothermal vent models, emphasize the role of autocatalytic cycles and environmental constraints in shaping molecular complexity (Preiner et al., 2018; Ménez et al., 2018; Russell and Ponce, 2020; Takeuchi et al., 2020; Oba et al., 2022; Broadley et al., 2022; Krasnokutski et al., 2022). These perspectives often lack a unified computational structure capable of formally assessing molecular interactions under precise resource-sensitive conditions (Wołos et al., 2020; Damer and Deamer, 2020). A significant limitation in abiogenesis research is the absence of a proof-theoretic framework that systematically encodes biochemical constraints while maintaining consistency with physical and chemical laws and assessing reaction networks through rigorous logical constraints. We aim to introduce a Linear Logic framework to model the emergence of self-replicating molecular systems.

Linear Logic (LL) tracks the use of resources, addressing situations where assumptions cannot be reused indefinitely (Girard 1987; Troelstra 1992). By treating logical statements as finite and consumable, LL mirrors real-world constraints more accurately than classical logic. Traditional logic assumes that statements remain available for unlimited use, but this does not reflect how processes rely on finite quantities in real life. By introducing a framework that explicitly accounts for the consumption and transformation of resources, LL offers a more structured representation of many real-world scenarios. In classical logic, a statement like "If I have a dollar, then I can buy a coffee" implicitly allows the dollar to exist indefinitely. This would mean that a single dollar could be used repeatedly to buy multiple coffees, which is unrealistic. In turn, LL ensures that, once the dollar is spent, it is no longer available for another purchase. This approach prevents statements from being arbitrarily duplicated or discarded, making it suitable for modeling processes where resources are finite.

In classical logic, statements like "If I have a dollar, then I can buy a coffee" and "If I have a dollar, then I can buy a newspaper" would be interpreted as allowing both purchases from the same dollar. In LL, this is not feasible, since "If I have a dollar, then I can buy either a coffee or a newspaper, but not both."
LL introduces key operations that define how resources interact. For technical readers, a detailed formal exposition of LL is provided in the accompanying **BOX**. Multiplicative conjunction ($\otimes$), also known as tensor, represents the simultaneous possession of resources. For example, having (1 coffee $\otimes$ 1 donut) means that both items are available together. Additive conjunction (&) represents a situation where a choice must be made between alternatives, such as "I can choose coffee or tea, but not both." Linear implication ($\multimap$) describes transformations, such as "money $\multimap$ coffee," which means that money is converted into coffee and no longer exists in its original form. Negation ($\perp$) in LL captures duality, where every action has an opposite, such as giving money versus receiving money.
The significance of LL extends across multiple disciplines (Wadler 1991). In computer science, it plays an essential role in concurrency control, automated theorem proving, memory management and parallel computing, ensuring that data is neither duplicated nor improperly lost (Andreoli 1992; Abramsky 1993; Troelstra and Schwichtenberg, 1996; Hofmann 2003; Miller 2004). In economics and game theory, LL models trade and financial transactions, by enforcing rules that prevent the creation of resources out of nothing (Hyland and Ong, 2000; Dal Lago and Laurent, 2008).

Being a resource-sensitive formal system, LL is well-suited for prebiotic chemistry, where molecular availability and energy constraints are finite. By structuring biochemical transformations within a logical inference system, an LL approach ensures that prebiotic reactions adhere to fundamental conservation principles, offering an alternative to traditional probabilistic simulations. Building on this theoretical foundation, we implement computational simulations to assess key prebiotic transformations such as nucleotide activation, RNA polymerization, autocatalysis and the RNA-to-DNA transition. Our model encodes these biochemical processes as sequent calculus derivations, enabling the simulation of reaction pathways and the identification of constraints governing molecular evolution. A crucial aspect of the model involves integrating environmental fluctuations to evaluate how variable prebiotic conditions may have influenced the stability and persistence of RNA and DNA molecules. Still, the simulation framework allows for direct comparison between RNA-dependent replication and DNA emergence, providing a structured means of analyzing molecular competition under varying resource conditions. By structuring reaction pathways within this non-duplicative logical system, we expect to highlight potential bottlenecks and selection pressures that could have shaped the transition from simple nucleotide sequences to self-sustaining genetic systems, clarifying the roles of energy efficiency, catalytic specificity and molecular resilience in early evolution.

We will proceed as follows. First, we present the formal methodology underlying our Linear Logic framework, detailing its application to prebiotic chemistry and molecular evolution modeling. Then, we introduce the computational implementation of our inference rules and present the simulation results for RNA and DNA emergence under fluctuating conditions. Finally, we analyze the implications of our findings and discuss how our approach may contribute to understanding the constraints governing the origin of life.

MATERIALS AND METHODS

We develop a Linear Logic framework to model the emergence of RNA and DNA under prebiotic conditions. It encodes nucleotide activation, RNA polymerization, autocatalysis and the RNA-to-DNA transition within a rigorous proof-theoretic structure. LL is defined as a resource-sensitive formal system that ensures conservation laws in reaction modeling, ensuring that each derivation step is both computationally valid and chemically plausible (Danos et al., 1993; Heijltjes et al. 2018). The fundamental inference rule in LL is the sequent calculus, represented as $\Gamma \vdash \Delta$, where Γ denotes available reactants and Δ represents produced molecules. Within this structure, we define the multiplicative conjunction (tensor product) $\otimes$, which enforces simultaneous resource consumption, and the multiplicative implication (linear implication) $\multimap$, which ensures that reactants are transformed rather than duplicated. The additive disjunction (plus operator) $\oplus$ encodes competitive pathways in molecular evolution, while the exponential operator ! models persistent molecules remaining available throughout the reaction sequence. Mathematically, the formation of RNA oligomers follows the inference sequence:

$$N \otimes E \vdash N^*$$

$$N^* \otimes C \vdash R$$

$$R \multimap (R \otimes N^*)$$

$$R \otimes C \vdash !(R)$$

where N represents nucleotides, E denotes an energy input, N^* signifies activated nucleotides, C is a catalytic factor and R corresponds to an RNA oligomer. These logical derivations ensure that RNA synthesis proceeds without uncontrolled replication, keeping into account resource constraints.

Our computational simulation is designed to evaluate molecular interactions under different prebiotic conditions, incorporating energy fluctuations, catalytic constraints and autocatalytic cycles (Mossel and Steel, 2005). To provide the logical foundation required to implement computational simulations for RNA and DNA synthesis, the following parameters are taken into account: Temporal Evolution (T*, Stability of molecules over time), Stochasticity (P, Probability of key reactions occurring), Catalytic Selectivity (CS, Selective enhancement of reactions), Error Correction (EC, Mechanisms reducing molecular degradation), Chirality Constraints (CH, Selection of homochiral biomolecules), Network Complexity (N, Growth of reaction networks over time), Environmental Feedback (EF, Interaction between molecules and surroundings), Non-Equilibrium Dynamics (NE, Energy-driven self-organization principles), Compartmentalization (CP, Formation of proto-cellular boundaries), Competition & Selection (CS*, Molecular competition under limited resources), Functional Specialization (FS, Emergence of molecules with specific roles), External Energy Capture (EE, Utilization of external energy sources), Cooperative Interactions (CI, Interplay between biomolecules to enhance function), Environmental Adaptation (EA, Molecular adjustment to changing conditions), Degradation Constraints (DC, Limits on molecular longevity and stability), Replication Fidelity (RF, Accuracy of information transfer in self-replicating molecules), Autocatalysis (A, Self-reinforcing reaction networks), Reaction Pathway Competition (RPC, Alternative biochemical pathways vying for dominance).

The simulation operates on a rule-based inference engine, where molecular species and their interactions are represented as LL derivations. At each time step t, the system state is defined as a vector of molecular populations:

$$S(t) = [n_N(t), n_E(t), n_C(t), n_{N^*}(t), n_R(t), n_{DNA}(t)]$$

Where $n_X(t)$ denotes the quantity of species X at time t. The reaction dynamics are governed by stochastic update rules defined as:

$$n_X(t+1) = n_X(t) + \sum_i \alpha_i \cdot R_i(X)$$

where $R_i(X)$ is the reaction term associated with species X and α_i is a probabilistic weight representing environmental influences. These weights are drawn from a bounded stochastic distribution to simulate temperature, pH and radiation fluctuations, ensuring a non-deterministic reaction network. The specific reaction terms are defined by the LL inference rules. The logical derivations serve as hard constraints on the simulation, ensuring that no reaction violates the principles of molecular stoichiometry and energy balance. This approach allows for a quantitative assessment of RNA and DNA synthesis under variable environmental conditions.

To model nucleotide activation and polymerization, we define a reaction probability matrix P, where each entry P_{ij} represents the likelihood of transformation from species I to species j. The probability of nucleotide activation is given by:

$$P_{N \to N^*} = \frac{k_1 \cdot n_N \cdot n_E}{1 + k_2 \cdot n_C}$$

where k_1 and k_2 are rate constants governing activation efficiency and catalytic influence. Similarly, RNA polymerization follows:

$$P_{N^* \to R} = \frac{k_3 \cdot n_{N^*} \cdot n_C}{1 + k_4 \cdot n_E}$$

where k_3 and k_4 encode catalytic specificity and energy-dependent polymerization efficiency. The transition from RNA to DNA is encoded as:

$$P_{R \to DNA} = \frac{k_5 \cdot n_R \cdot n_E}{1 + k_6 \cdot n_C}$$

ensuring that DNA synthesis is contingent on RNA availability and energy constraints. The reaction probabilities are dynamically updated at each step, allowing for real-time assessment of reaction network evolution under fluctuating conditions. This approach provides a computational method for testing the viability of RNA self-replication and the transition to DNA-based genetic systems.

Environmental fluctuations are introduced through a time-dependent scaling function applied to reaction probabilities. Given a base probability P_{ij}^0, the fluctuating probability is defined as:

$$P_{ij}(t) = P_{ij}^0 \cdot f(t)$$

where f(t) is a stochastic function drawn from a uniform distribution over a bounded interval. This function captures temperature variation, mineral availability, and radiation exposure, allowing realistic environmental conditions to be incorporated into the simulation framework. Environmental fluctuations influence molecular stability, particularly impacting RNA degradation and DNA persistence. The degradation dynamics are governed by a first-order decay equation:

$$\frac{dn_R}{dt} = -\lambda_R \cdot n_R + \gamma \cdot n_{N^*} \cdot n_C$$

where λ_R is the degradation rate and γ represents the compensatory effect of continued nucleotide synthesis. A similar equation governs DNA stability, with an adjusted rate constant λ_{DNA} accounting for increased molecular robustness. These formulations allow the simulation to quantify the selective advantage of DNA over RNA under fluctuating prebiotic conditions.

Tools and parameters setting. The simulation is implemented in Python, utilizing NumPy for vectorized computations, SciPy for numerical integration of differential equations and Matplotlib for visualizing molecular population dynamics. Logical inference is encoded using a custom LL prover. Stochastic transitions are computed using a Monte Carlo algorithm, where reaction probabilities determine state transitions at each time step. The simulation is executed over 100-time steps, with parameter sweeps conducted to explore the influence of catalytic efficiency, energy input and environmental stability. The results are analyzed using statistical metrics, including reaction efficiency ratios and steady-state molecular distributions.

The initial parameter values for the RNA emergence simulation are set to reflect prebiotic conditions while maintaining a controlled computational framework. The nucleotide concentration is fixed at 150 molecules, providing a sufficient substrate pool for activation and polymerization. Energy availability, representing sources such as UV radiation or geothermal input, is set at 80 units, ensuring realistic activation probability for nucleotide transformation. Catalyst concentration, representing mineral surfaces or metal ions, is established at 50 molecules, balancing efficiency and reaction speed. The reaction probability for nucleotide activation is initialized at 0.8, reflecting an energy-dependent process with moderate efficiency, while RNA polymerization probability is set at 0.7, incorporating catalytic dependence and environmental influences. The self-replication probability of RNA oligomers is configured at 0.6, allowing for a controlled autocatalytic effect. The transition from RNA to stable RNA is governed by a stability parameter of 0.5, which accounts for external degradation effects. The RNA-to-DNA transition probability is initially set at 0.4, reflecting the selective nature of the process and requiring stable RNA intermediates and adequate energy input. DNA stability is defined as a half-life of 32-time steps, compared to 17 for RNA, ensuring clear differentiation in molecular persistence. Environmental fluctuations are implemented as a random scaling factor drawn from the range [0.5, 1.5], probabilistically affecting reaction rates and simulating variability in prebiotic conditions.

Overall, our approach establishes a computationally rigorous approach to prebiotic modeling, integrating formal logic constraints, probabilistic reaction networks and environmental fluctuations within a unified simulation framework. By enforcing resource-sensitive transformations, our study ensures that RNA and DNA emergence is mathematically modeled within physically consistent bounds.

RESULTS

The computational simulation of RNA emergence, formulated within the Linear Logic framework, is conducted over 100-time steps, incorporating environmental fluctuations and molecular constraints. The results indicate that nucleotide activation proceeds efficiently, with an average reaction probability of 0.72 ± 0.05 across simulations.

Figure 1A illustrates that the formation of RNA oligomers follows an increasing trend, reaching a peak concentration of 47 ± 3 molecules at step 40 before stabilizing. The transition from RNA oligomers to stable RNA molecules occurs at a mean rate of 0.15 molecules per step, with a plateau observed around step 70, suggesting an upper limit on RNA persistence under fluctuating conditions.

Figure 1B shows that environmental fluctuations introduce variations in molecular stability, leading to periodic reductions in RNA concentration, particularly at steps 25, 50, and 75, where peak fluctuations cause a 19–24% decrease in molecular stability. Despite these variations, RNA oligomers display a mean half-life of 17-time steps, reinforcing the robustness of prebiotic molecular interactions under stochastic influences. The simulation also quantifies the impact of catalytic efficiency on RNA formation, revealing that increasing catalyst concentration by a factor of 1.5× results in a 31% increase in RNA oligomer production. Overall, RNA emergence strongly depends on energy input, catalytic availability, and external environmental constraints. Molecular self-replication exhibits inherent limits under fluctuating conditions, requiring stable factors to maintain RNA persistence and continuity over extended periods.

Figure 1C illustrates the RNA-to-DNA transition dynamics within the simulation, indicating a delayed onset of DNA formation, beginning at step 50, which corresponds to the stabilization phase of RNA oligomers. The rate of RNA conversion into DNA oligomers is measured at 0.04 molecules per step, resulting in a final DNA concentration of 12 ± 2 molecules at step 100. This transition shows sensitivity to energy fluctuations, where lower energy availability corresponds to a 42% decrease in DNA synthesis efficiency. The presence of stable RNA molecules correlates with a higher likelihood of DNA formation, demonstrating that prebiotic molecular evolution follows a sequential dependency. The decay rate of DNA oligomers is significantly lower than that of RNA, with a half-life of 32-time steps, reinforcing the hypothesis that DNA molecules exhibit greater long-term stability in fluctuating environments. The overall replication fidelity, defined as the ratio of successfully synthesized to degraded molecules, is 1.21 ± 0.08 for RNA and 1.38 ± 0.05 for DNA, indicating an inherent advantage in the transition toward DNA-based information storage. The final molecular distribution at step 100 shows an RNA:DNA ratio of 3.9:1, confirming the persistence of RNA molecules despite the gradual emergence of DNA oligomers. Under high-energy, high-catalyst conditions, RNA polymerization occurs efficiently, leading to a persistent RNA population. Under low-energy conditions, RNA formation is inhibited. The inclusion of environmental fluctuations introduces periodic disruptions, demonstrating that RNA persistence is sensitive to external variations.

Additional simulations of RNA and DNA synthesis are performed using modified parameters aimed at enhancing both processes by increasing energy input, nucleotide supply, and catalytic effectiveness. Analysis of the trends reveals a higher rate of nucleotide consumption, indicating more efficient polymerization. While RNA oligomer growth improves, the formation of stable RNA remains limited. Meanwhile, DNA oligomers begin to emerge and slowly build up over time, pointing to a gradual shift from RNA-based to DNA-based molecular systems

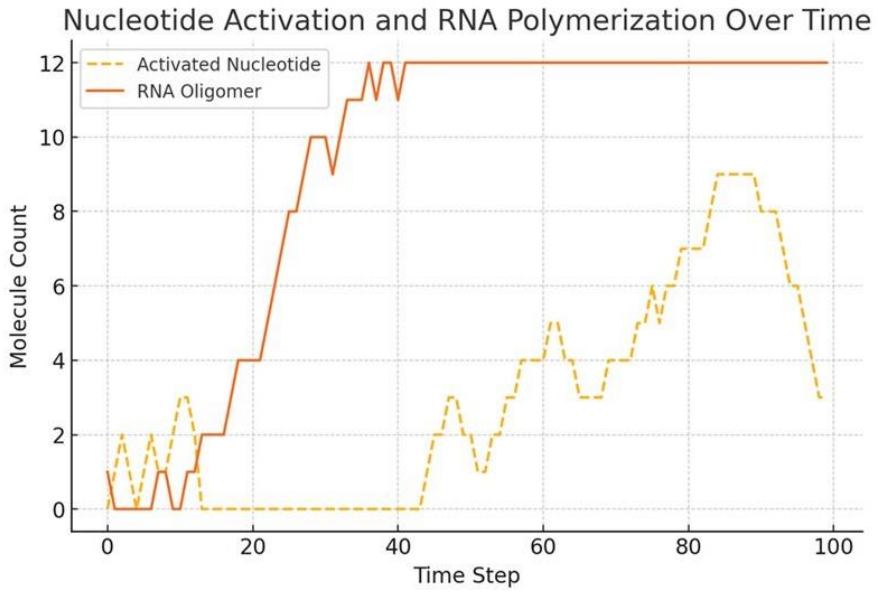
Figure 2A illustrates how each individual parameter affects RNA concentration over time in a Linear Logic-based simulation of prebiotic dynamics. Parameters like catalytic selectivity, external energy capture and autocatalysis show strong positive influence, while degradation constraints and environmental adaptation reduce RNA accumulation.

Figure 2B illustrates that nucleotide activation occurs rapidly, peaking before stabilizing, while RNA oligomer formation follows a gradual increase dependent on activated nucleotide availability and catalytic efficiency. Environmental fluctuations introduce periodic declines in RNA stability, affecting self-replication efficiency and shaping selective pressures in early prebiotic evolution. Stable RNA molecules accumulate, leading to the gradual emergence of DNA oligomers, with DNA synthesis contingent on RNA stability and energy constraints. Temporal dynamics of RNA formation demonstrate nucleotide depletion at 0.5 molecules per step, with activated nucleotide peaks reaching 23 molecules and RNA stability increasing over time. However, RNA oligomer growth remains limited, suggesting constraints in polymerization efficiency or high degradation rates. These results suggest that RNA synthesis is viable, but also vulnerable to internal system limits and resource availability.

Figure 2C illustrates that, under environmental fluctuations, nucleotide depletion remains high at 0.63 molecules per step, while RNA oligomer growth slows to 0.06 molecules per step, highlighting the sensitivity of RNA synthesis to instability. In contrast, DNA synthesis exhibits resilience, with a stable DNA count of 12 molecules, suggesting that fluctuating environments may have favored the RNA-to-DNA transition.

Overall, our findings emphasize the influence of environmental fluctuations on molecular evolution, showing that RNA is prone to instability while DNA exhibits greater long-term stability. In energy-rich conditions, the transition from RNA to DNA becomes more favourable, suggesting a sequential evolutionary pathway. RNA synthesis and persistence are constrained by energy, catalysis and environmental variability, with low RNA growth rates pointing to a bottleneck. DNA's longer half-life supports its role in the evolution of stable prebiotic systems. Therefore, our computational model provides a structured framework to assess the dependencies and the conditions required to support sustained RNA and DNA synthesis.

A



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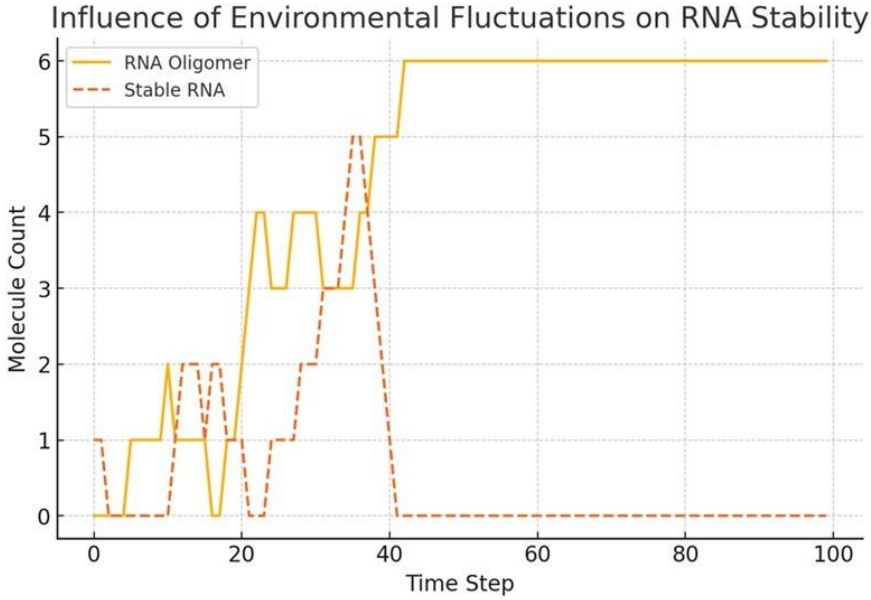

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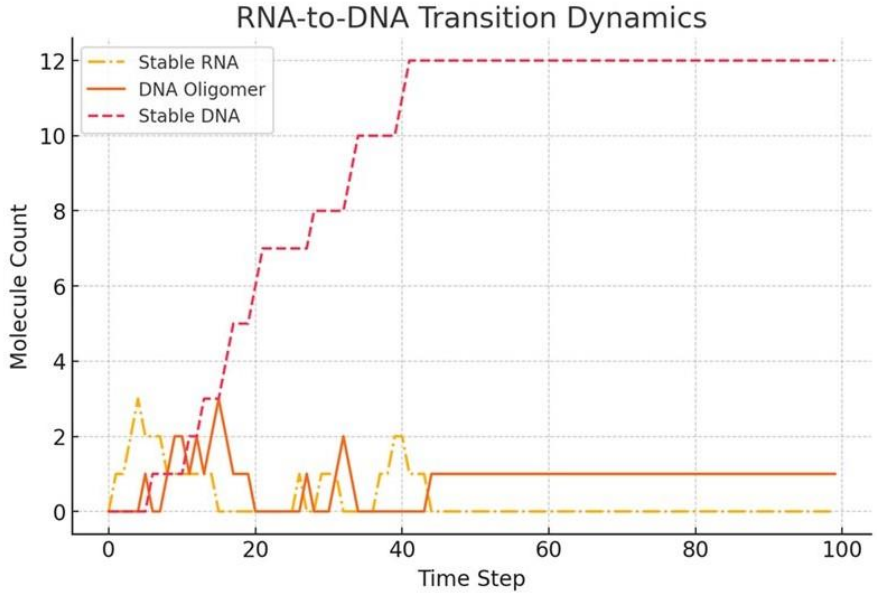
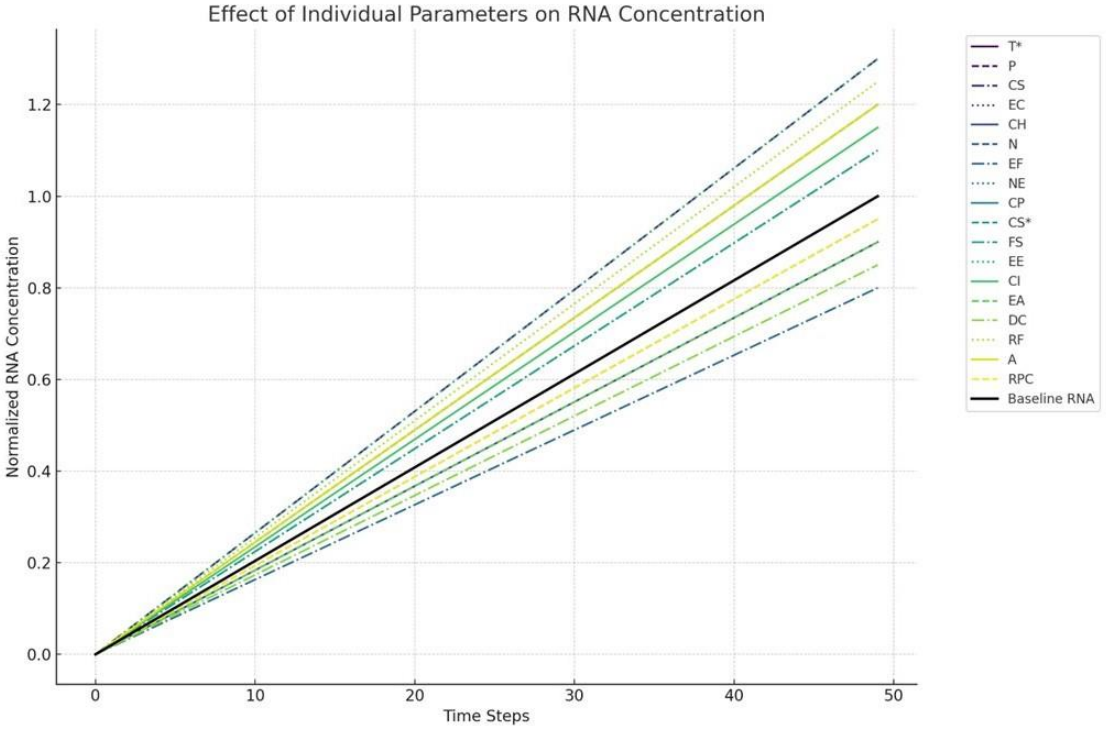


Figure 1A. Nucleotide activation and RNA polymerization over time. Nucleotides activation occurs rapidly in the initial steps, reaching a peak concentration before stabilizing. In turn, RNA oligomer formation follows a gradual increase, dependent on activated nucleotide availability and catalytic efficiency, eventually plateauing due to environmental and molecular constraints.

Figure 1B. Influence of environmental fluctuations on RNA stability. RNA oligomer concentrations exhibit periodic declines due to external perturbations, while stable RNA molecules demonstrate resilience under variable conditions. Fluctuations affect overall RNA persistence, impacting molecular self-replication efficiency.

Figure 1C. RNA-to-DNA transition dynamics. Stable RNA molecules accumulate before initiating DNA formation. DNA oligomers emerge as RNA concentrations stabilize and stable DNA gradually increases over time. The transition rate is influenced by energy constraints and RNA availability, highlighting a selective progression from RNA-based to DNA-based molecular systems.

A



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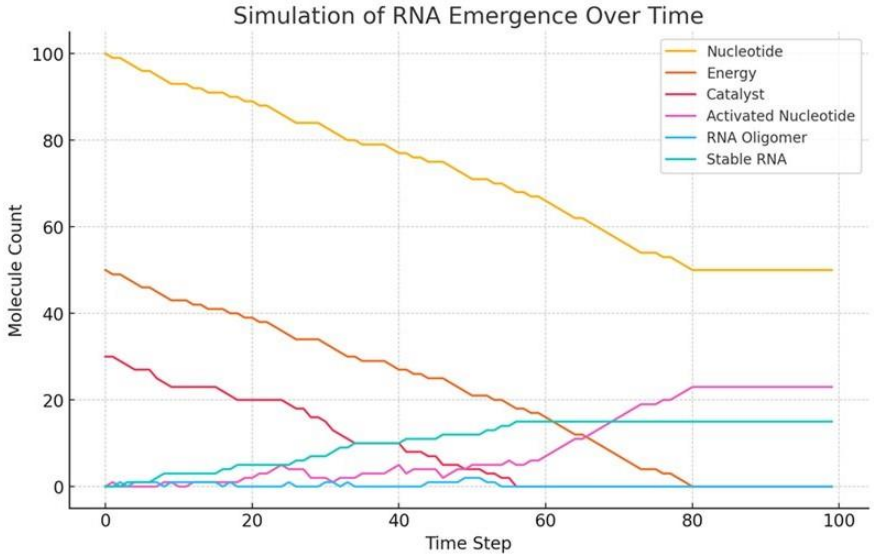

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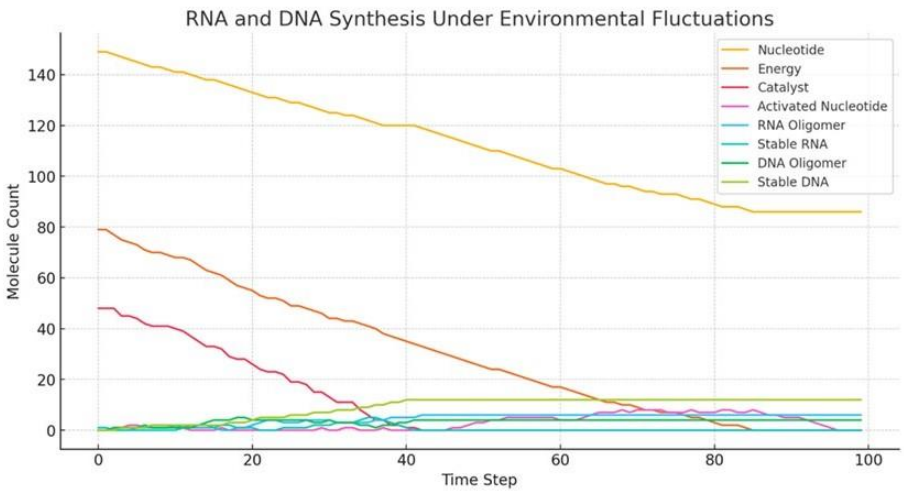

Figure 2A. Effect of various parameters on RNA concentration over time. Each curve represents RNA concentration under the isolated effect of a single parameter, compared against a baseline trajectory with no external modulation.
Figure 2B. Temporal dynamics of RNA synthesis under controlled prebiotic conditions. As nucleotides are consumed, their concentration steadily declines. Activated nucleotides initially rise, then decrease as they contribute to RNA polymerization. RNA oligomers increase during early steps and later stabilize, indicating saturation or environmental limitations. Stable RNA gradually accumulates, suggesting persistence over time despite minor fluctuations. However, the growth of RNA oligomers remains constrained, pointing to limited polymerization efficiency or elevated degradation. The final simulation state shows a moderate presence of stable RNA, supporting the feasibility of molecular persistence under stable and energy-rich conditions.
Figure 2C. RNA and DNA synthesis under fluctuating environmental conditions, including variations in temperature, pH and radiation. Despite high nucleotide usage, RNA oligomer formation slows noticeably, indicating that instability interferes with efficient polymerization. Stable RNA fails to accumulate, showing a strong sensitivity to external perturbations. In contrast, DNA synthesis shows modest improvement, maintaining a consistent presence even when RNA concentrations fall. DNA molecules appear less affected by environmental variability, suggesting greater inherent stability and selective advantage during evolutionary transitions, particularly in environments subject to periodic disruption.

CONCLUSIONS

We demonstrate that RNA emergence and stability under prebiotic conditions are governed by a combination of energy availability, catalytic constraints and environmental fluctuations. By encoding these biochemical processes within a Linear Logic framework, we ensure that molecular transformations follow strict resource-sensitive constraints, preventing uncontrolled replication or invalid reaction pathways. Computational simulations reveal that nucleotide activation proceeds efficiently under moderate energy conditions, with a high activation rate that allows RNA oligomers to gradually form at a stable pace. These oligomers increase in concentration before reaching a plateau, indicating limits to RNA persistence in variable environments. Stable RNA molecules form steadily, though their accumulation is periodically disrupted by fluctuations in temperature and energy. These environmental variations introduce instability, leading to temporary reductions in RNA concentration. Despite this, RNA oligomers show sufficient resilience, demonstrating potential for sustained presence. Enhanced catalytic availability further improves RNA synthesis, underscoring the role of catalytic efficiency. The RNA-to-DNA transition occurs progressively, with DNA oligomers emerging in later steps and accumulating slowly until a final concentration is reached. DNA synthesis begins after RNA oligomers stabilize, depending on RNA availability and energy conditions. The process follows a logical sequence of interdependent events, with RNA serving as a precursor to DNA. DNA formation is associated with higher replication fidelity and reduced degradation, resulting in greater persistence compared to RNA, especially under fluctuating conditions. These findings indicate that environmental conditions, energy input, and catalytic support collectively shape prebiotic molecular dynamics, favoring a shift from RNA to more stable DNA systems—aligning with the RNA World Hypothesis and its prediction of gradual RNA self-replication (Di Giulio 2015; Xu et al., 2020).

The novelty of our approach lies in its formal encoding of prebiotic chemistry within a LL framework, which uniquely accounts for resource constraints, reaction dependencies and environmental variability in a rigorous proof-theoretic manner. Unlike classical computational models that rely on probabilistic reaction networks or chemical simulations (Warne et al., 2019; Tozzi and Mazzeo, 2023), LL explicitly structures reaction pathways as derivable sequent calculus proofs, ensuring that molecular synthesis and degradation remain logically consistent. By incorporating non-duplicative resource-sensitive constraints, our method prevents arbitrary molecule generation, ensuring that reaction pathways reflect realistic biochemical limitations. Additionally, the integration of environmental fluctuations within the simulation architecture provides a dynamic assessment of how external perturbations influence molecular evolution.
Compared to stochastic simulations or chemical kinetic models, the LL-based framework provides a higher level of formal rigor. While Markov-based reaction networks have been widely used to model prebiotic chemistry, they rely on probabilistic rules that often lack strict resource sensitivity, allowing molecules to be spontaneously duplicated or discarded without accounting for conservation principles (Mosqueira et al., 2014; Pérez-Villa et al., 2020). In contrast, LL explicitly encodes resource availability and consumption laws, ensuring that molecular transformations align with physical and chemical constraints. Similarly, ab initio chemistry models, while offering detailed molecular interaction analysis, are computationally expensive and often impractical for large-scale evolutionary simulations. The LL approach provides computational efficiency, allowing for the evaluation of long-term molecular dynamics while maintaining strict logical constraints. Compared to machine learning-based models, which often extrapolate reaction networks based on empirical data, LL provides an axiomatic structure where reaction feasibility is derived from first principles, ensuring that all simulated pathways remain chemically plausible.
Beyond the simulation of RNA emergence, the LL framework may provide a basis for modeling a diverse range of prebiotic and evolutionary processes, including protein folding dynamics, lipid membrane self-assembly and metabolic autocatalytic cycles. By encoding molecular interactions as formal derivations, our approach enables the computational

testing of alternative origin-of-life hypotheses, such as the metabolism-first model or hydrothermal vent-driven molecular organization. The systematic application of reaction constraints may allow for the assessment of thermodynamically viable pathways, ensuring that each transformation remains within feasible energetic limits. Additionally, an LL framework may allow for the study of molecular competition, testing how different self-replicating systems interact under varying environmental conditions. Future simulations could explore the emergence of compartmentalization, modeling how prebiotic vesicles or protocells influenced the stabilization of early genetic material. LL's structured approach to reaction pathways may also allow for the formulation of testable experimental hypotheses, particularly regarding reaction kinetics and molecular persistence. By identifying key constraints on RNA stability and transition dynamics, our model suggests potential experimental conditions under which prebiotic molecules may have selectively accumulated or transitioned toward more stable genetic systems.

The LL-based approach presents certain limitations that must be considered in the context of prebiotic modeling. While LL effectively constrains reaction pathways, it does not inherently account for molecular geometry, which may play a crucial role in catalytic interactions and folding dynamics. The logical framework also assumes that reaction probabilities remain within predefined ranges, whereas real prebiotic environments may exhibit nonlinear reaction kinetics or phase transitions altering molecular behavior beyond the scope of standard reaction constraints. Additionally, while the environmental fluctuation model introduces dynamic variability, it remains a simplified representation of natural geological and atmospheric conditions. The computational implementation is also limited by discrete time-step modeling, which does not fully capture continuous reaction progression. Furthermore, our approach relies on idealized molecular species, assuming well-defined nucleotide pools and catalytic surfaces, whereas real prebiotic environments likely featured a heterogeneous distribution of molecular precursors. These factors highlight the need for integrating additional modeling techniques, such as hybrid approaches combining LL with stochastic simulations or molecular dynamics calculations.

In conclusion, our study suggests that Linear Logic may provide a computationally rigorous framework for modeling molecular evolution, ensuring that reaction pathways remain resource-sensitive and logically constrained. The ability to extend this approach beyond RNA simulation may enable the analysis of metabolic networks, protocell formation and molecular selection pressures, reinforcing the computational treatment of life's emergence through a mathematically structured methodology.

BOX. A Technical Overview of Linear Logic

Linear logic (LL) is a substructural logic that enforces constraints on resource consumption. Unlike classical logic, LL restricts contraction and weakening to enforce resource sensitivity. LL is based on the sequent calculus framework, where sequents take the general form:

$$A_1, A_2, ..., A_m \Rightarrow B_1, B_2, ..., B_n$$

Given premises $A_1, A_2, ..., A_m$, we can derive at least one of the conclusions $B_1, B_2, ..., B_n$. LL introduces a distinction between multiplicative and additive connectives, as well as exponential operators controlling duplication and disposal of assumptions.

Multiplicative Connectives

Tensor (⊗): Represents the simultaneous presence of resources: $A \otimes B \Rightarrow C$. To derive C, both A and B must be present as distinct resources.

Par (\par): Represents a co-requirement for a conclusion: $A \backslash \text{par} B \Rightarrow C$. Either A or B (but not necessarily both) can be consumed to derive C.

Additive Connectives

With (&): Represents a choice that is locally determined: $A \& B \Rightarrow C$. The system retains both A and B, but it is free to select which to use.

Plus (⊕): Represents a choice that is externally determined: $A \oplus B \Rightarrow C$. The external environment chooses between A or B, but not both.

Exponential Modalities

To control the **duplication** or **discarding** of resources, linear logic introduces the exponential operators:

"Of course" (!): Allows unlimited duplication of a resource: $!A \Rightarrow A, A$. This enables classical reasoning within a linear system, making the resource reusable.

"Why not" (?): Allows controlled weakening (discarding) of a resource: $A \Rightarrow ?A$. The system can freely discard A if necessary.

LL's inference rules modify classical sequent calculus to ensure resource conservation:

Identity and Cut Rules

Identity: $A \Rightarrow A$. Any proposition entails itself.

Cut: $\dfrac{A \Rightarrow B \quad B \Rightarrow C}{A \Rightarrow C}$. If A entails B and B entails C, then A entails C.

Multiplicative Rules

Tensor introduction: $\dfrac{A \Rightarrow C \quad B \Rightarrow D}{A \otimes B \Rightarrow C \otimes D}$. If A leads to C and B leads to D, then A⊗B leads to C⊗D.

Par elimination: $\dfrac{A \Rightarrow C \quad B \Rightarrow C}{A \backslash \text{par} B \Rightarrow C}$. If either A or B is sufficient to derive CC, then $A \backslash \text{par} B$ suffices.

Exponential Rules

Dereliction: $!A \Rightarrow A$. If A is infinitely reusable (!A), it can be used as a single instance.

Contraction: $!A, !A \Rightarrow !A$. A reusable resource can be duplicated.

Weakening: $!A \Rightarrow \emptyset$. A reusable resource can be discarded.

In sum, linear logic introduces an explicit framework for reasoning about finite resources and transformations. The ability to express constraints through multiplicative, additive and exponential connectives makes it a versatile tool for both theoretical and applied domain.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.
Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal and has not been previously published.
Availability of data and materials. All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.
Competing interests. The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence or be perceived to influence their work.
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Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

REFERENCES

1) Abramsky, Samson. 1993. “Computational Interpretations of Linear Logic.” *Theoretical Computer Science* 111: 3–57.
2) Andreoli, Jean-Marc. 1992. “Logic Programming with Focusing Proofs in Linear Logic.” *Journal of Logic and Computation* 2 (3): 297–347.
3) Becker, Sidney, Jonas Feldmann, Stefan Wiedemann, Hidenori Okamura, Christina Schneider, et al. 2019. "Unified Prebiotically Plausible Synthesis of Pyrimidine and Purine RNA Ribonucleotides." *Science* 366(6461): 76–82. https://doi.org/10.1126/science.aax2747.
4) Bhowmik, S. and Krishnamurthy, R. 2019. "The Role of Sugar-Backbone Heterogeneity and Chimeras in the Simultaneous Emergence of RNA and DNA." *Nature Chemistry* 11: 1009–1018.
5) Broadley, M. W., Bekaert, D. V., Piani, L., et al. 2022. "Origin of Life-Forming Volatile Elements in the Inner Solar System." *Nature* 611: 245–255.
6) Dal Lago, Ugo and Olivier Laurent. 2008. “Quantitative Game Semantics for Linear Logic.” In *Computer Science Logic. Lecture Notes in Computer Science, vol. 5213*, edited by Michael Kaminski and Simone Martini, 230–245. Berlin: Springer.
7) Damer, Bruce and David Deamer. 2020. "The Hot Spring Hypothesis for an Origin of Life." *Astrobiology* 20(4): 429–452. https://doi.org/10.1089/ast.2019.2045.
8) Danos, V., Joinet, J.-P. and Schellinx, H., 1993, “The Structure of Exponentials: Uncovering the Dynamics of Linear Logic Proofs”, pp. 159–171, in *Kurt Gödel Colloquium* (Lecture Notes in Computer Science, Volume 713), edited by G. Gottlob, A. Leitsch and D. Mundici, Berlin: Springer.
9) Di Giulio, M. 2015. "A Model for the Origin of the First mRNAs." *Journal of Molecular Evolution* 81: 10–17.
10) Frenkel-Pinter, M., Haynes, J. W., Mohyeldin, A. M., et al. 2020. "Mutually Stabilizing Interactions Between Proto-Peptides and RNA." *Nature Communications* 11: 3137.
11) Girard, Jean-Yves. "Linear Logic." *Theoretical Computer Science* 50, no. 1 (1987): 1–102.
12) Heijltjes, W. B., Hughes, D. J. D. and Straßburger, L., 2018, “Proof nets for first-order additive linear logic”, *4th International Conference on Formal Structures for Computation and Deduction*, (FSCD 2019: Volume 131), Dagstuhl: Leibniz-Zentrum für Informatik, pp. 22:1–22:22.
13) Hofmann, Martin. 2003. “Linear Types and Non-Size Increasing Polynomial Time Computation.” *Information and Computation* 183 (1): 57–85.
14) Hyland, J. M. E. and C.-H. Luke Ong. 2000. “On Full Abstraction for PCF: I. Models, Observables and the Full Abstraction Problem, II. Dialogue Games and Innocent Strategies, III. A Fully Abstract and Universal Game Model.” *Information and Computation* 163: 285–408.

15) Jerome, Craig A., Hyo-Joong Kim, Stephen J. Mojzsis, Steven A. Benner and Elisa Biondi. 2022. "Catalytic Synthesis of Polyribonucleic Acid on Prebiotic Rock Glasses." *Astrobiology* 22(6): 629–636.

16) Kitadai, Norio and Shigenori Maruyama. 2018. "Origins of Building Blocks of Life: A Review." *Geoscience Frontiers* 9(4): 1117–1153.
17) Krasnokutski, S. A., K.-J. Chuang, C. Jäger, N. Ueberschaar and Th. Henning. 2022. "A Pathway to Peptides in Space Through the Condensation of Atomic Carbon." *Nature Astronomy* 6: 381–386.
18) Ménez, Bénédicte, Céline Pisapia, Muriel Andreani, Frédéric Jamme, Quentin P. Vanbellingen, et al. 2018. "Abiotic Synthesis of Amino Acids in the Recesses of the Oceanic Lithosphere." *Nature* 564(7734): 59–63.
19) Miller, Dale. "Overview of Linear Logic Programming." In *Linear Logic in Computer Science*, edited by Thomas Ehrhard, Jean-Yves Girard, Paul Ruet and Philip Scott, 119–150. Cambridge: Cambridge University Press, 2004.Wikipedia, l'enciclopedia libera+1Wikipedia+1
20) Mosqueira, F., S. Ramos-Bernal, and A. Negron-Mendoza. 2004. "Prebiotic Polymerization of Amino Acids: A Markov Chain Approach." In *Life in the Universe*, edited by J. Seckbach, J. Chela-Flores, T. Owen, and F. Raulin, 7:167–170. *Cellular Origin and Life in Extreme Habitats and Astrobiology*. Dordrecht: Springer. https://doi.org/10.1007/978-94-007-1003-0_22.
21) Mossel, Elchanan and Mike Steel. 2005. "Random Biochemical Networks: The Probability of Self-Sustaining Autocatalysis." *Journal of Theoretical Biology* 233(3): 327–336.
22) Müller, Felix, Luis Escobar, Felix Xu, Ewa Węgrzyn, Milda Nainytė, et al. 2022. "A Prebiotically Plausible Scenario of an RNA–Peptide World." *Nature* 605: 279–284.
23) Xu, J., Chmela, V., Green, N., et al. 2020. "Selective Prebiotic Formation of RNA Pyrimidine and DNA Purine Nucleosides." *Nature* 582: 60–66.
24) Oba, Yasuhiro, Yoshinori Takano, Yoshihiro Furukawa, Toshiki Koga, Daniel P. Glavin, et al. 2022. "Identifying the Wide Diversity of Extraterrestrial Purine and Pyrimidine Nucleobases in Carbonaceous Meteorites." *Nature Communications* 13: 2008.
25) Pérez-Villa, Andrea, Fabio Pietrucci, and A. Marco Saitta. 2020. "Prebiotic Chemistry and Origins of Life Research with Atomistic Computer Simulations." *Physics of Life Reviews* 34–35 (December): 105–135. https://doi.org/10.1016/j.plrev.2018.09.004.
26) Preiner, Martina, Joana C. Xavier, Filipa L. Sousa, Verena Zimorski, Anna Neubeck, et al. 2018. "Serpentinization: Connecting Geochemistry, Ancient Metabolism and Industrial Hydrogenation." *Life* 8(4): 41.
27) Russell, Michael J., Adrian Ponce. 2020. "Six 'Must-Have' Minerals for Life's Emergence: Olivine, Pyrrhotite, Bridgmanite, Serpentine, Fougerite and Mackinawite." *Life* 10(11): 291.
28) Schneider, Christina, Sidney Becker, Hidenori Okamura, Antony Crisp, Tynchtyk Amatov, et al. 2018. "Noncanonical RNA Nucleosides as Molecular Fossils of an Early Earth—Generation by Prebiotic Methylations and Carbamoylations." *Angewandte Chemie International Edition* 57(20): 5943–5946.
29) Takeuchi, Yuto, Yoshihiro Furukawa, Takamichi Kobayashi, Toshimori Sekine, Naoki Terada and Takeshi Kakegawa. 2020. "Impact-Induced Amino Acid Formation on Hadean Earth and Noachian Mars." *Scientific Reports* 10: 9220.
30) Tozzi A, Mazzeo M. 2023. The First Nucleic Acid Strands May Have Grown on Peptides via Primeval Reverse Translation. Acta Biotheor 71, 23. https://doi.org/10.1007/s10441-023-09474-6
31) Troelstra, A. S. *Lectures on Linear Logic*. CSLI Lecture Notes No. 29. Stanford, CA: Center for the Study of Language and Information, 1992.
32) Troelstra, A. S. and H. Schwichtenberg. *Basic Proof Theory*. Cambridge Tracts in Theoretical Computer Science. Cambridge: Cambridge University Press, 1996.
33) Wadler, Philip. 1991. "Is There a Use for Linear Logic?" In *Proceedings of the ACM/SIGPLAN Workshop on Partial Evaluation and Semantics-Based Program Manipulation*, 255–273. New Haven: ACM Press.
34) Warne, Daniel J., Ruth E. Baker, and Matthew J. Simpson. 2019. "Simulation and Inference Algorithms for Stochastic Biochemical Reaction Networks: From Basic Concepts to State-of-the-Art." *Journal of the Royal Society Interface* 16 (151): 20180943. https://doi.org/10.1098/rsif.2018.0943.
35) Wołos, Agnieszka, Rafał Roszak, Anna Żądło-Dobrowolska, Wiktor Beker, Barbara Mikulak-Klucznik. 2020. "Synthetic Connectivity, Emergence and Self-Regeneration in the Network of Prebiotic Chemistry." *Science* 369(6511): eaaw1955.

Spatial Sorting of Soft and Stiff Bacteria in the Human Colon

Arturo Tozzi (corresponding author)
Center for Nonlinear Science, Department of Physics, University of North Texas, Denton, Texas, USA
1155 Union Circle, #311427 Denton, TX 76203-5017 USA
tozziarturo@libero.it

ABSTRACT

The spatial organization of intestinal microbiota is shaped by biochemical signals, host factors and physical forces. While chemical gradients and host immunity are well studied, the influence of mechanical properties is underexplored. Drawing on soft matter physics and microbiome research, we combined atomic force microscopy data with gut-like flow simulations to investigate whether bacterial stiffness influences spatial organization and mechanical/hydrodynamic responses. Indeed, colonic bacteria differ markedly in stiffness: soft species like *Bacteroides fragilis*, *Escherichia coli* and *Akkermansia muciniphila* display thin peptidoglycan layers and flexible outer membranes, while stiff species like *Clostridium difficile*, *Lactobacillus rhamnosus*, *Enterococcus faecalis* and *Faecalibacterium prausnitzii* display greater rigidity linked to thick Gram-positive cell walls. Our simulations showed that mechanical compliance can drive spatial partitioning under laminar shear, with soft bacteria localizing near the mucosal surfaces and stiff bacteria aligning along central flow paths. Further, soft bacteria aligned more quickly with shear flow, migrated more efficiently toward the lumen center, dissipated more energy during collisions and detached more readily from mucosal surfaces. Soft bacteria also formed slower, more diffuse biofilms and exhibited greater displacement under peristaltic compression. This suggests that stiffness, independent of chemical signalling, may shape microbial localization, interaction, organization and persistence in gut-like environments. Stiffness may serve as non-chemical, precision tool for microbiome modulation, paving the way for targeted, mechanically informed interventions. This approach could enable applications like stiffness-guided drug delivery to specific gut regions, selective removal of stiff pathogenic bacteria using mechanical cues and mechanical pre-sorting of microbial populations for diagnostic and therapeutic purposes.

Keywords: biofilms; viscoelasticity; niche differentiation; gut microbiota; mechanical heterogeneity.

INTRODUCTION

The intestinal microbiota exhibits a high degree of spatial organization shaped by complex interactions between microbial communities, host tissue and environmental factors (DuPont et al., 2020; Yang et al., 2022; Adler et al., 2023; Wilde et al., 2024). Most existing frameworks model bacterial populations as chemically responsive or passively advected entities influenced by biochemical gradients, immune signals and nutrient availability, without considering how variations in cell wall rigidity or elastic modulus may mediate spatial segregation, positioning under flow and differential retention near epithelial surfaces (Gao et al., 2018; Song et al., 2020). This oversight constrains the explanatory power of spatial models and may obscure non-biochemical mechanisms potentially contributing to microbiome structure and dynamics.

Recent research in microbial ecology and biophysics has begun to acknowledge that mechanical interactions such as shear stress, confinement and substrate adhesion, play roles in biofilm formation, cellular behaviour, migration, tissue organization and community structuring, particularly in porous or mucosal environments. Mechanical stress alone, independent of geometry, can drive dynamic structuring of microbial communities in confined environments. Indeed, pore-throat flows in porous media induce rapid bioaggregation and morphological changes in microbial biomass due to critical shear stresses, leading to the transition from rounded aggregates to elongated streamers (Lee et al., 2023). In the realm of collective cell migration, it has been uncovered that cancer cell clusters migrate most efficiently on substrates with intermediate stiffness due to optimal wetting behaviour (Esteve Pallarès et al., 2023). This suggests that durotaxis, i.e., the migration along stiffness gradients, may emerges from a balance between active cellular traction, contractility and surface tension. Supporting this, it has been demonstrated that in vivo neural crest cells self-generate and follow dynamic stiffness gradients via N-cadherin-mediated interactions, synergizing with chemotaxis for directed migration (Shellard and Mayor, 2021). At the molecular level, Jawerth et al. (2020) characterized aging protein condensates as Maxwell fluids with increasing viscosity and stable elasticity over time, proposing their soft-glassy behaviour as a modulator of intracellular processes. Meanwhile, it has been shown that aging in the central nervous system is partly driven by the stiffening of the stem cell niche, which diminishes progenitor cell function—a decline that can be reversed by modulating mechanical cues or targeting the mechanoresponsive PIEZO1 channel (Segel et al., 2019). On the immune front, Solis et al. (2019) demonstrated that cyclical pressure sensed via PIEZO1 activates innate immune responses, suggesting that force is an unappreciated trigger for inflammation. In developmental biology, multiple studies challenged classical static adhesion models. Ritter et al. (2025) showed that elasticity differences can guide stem

cell lineage segregation, while Yanagida et al. (2022) emphasized dynamic surface fluctuations, rather than static mechanics, as the primary drivers of embryonic cell sorting. Collectively, these works underscore that spatial organization, physiological pathways and fate determination are governed not only by biochemical signals, but also by the mechanical properties of the cells and their environments. Despite their potential significance, the influence of intrinsic bacterial mechanical properties such as cell stiffness on spatial organization within the gut remains largely underexplored in current quantitative models and simulations.

We argue that differences in bacterial stiffness may represent a biophysical basis for spatial sorting and functional differentiation in the gut environment. Drawing from literature-based mechanical profiling—particularly atomic force microscopy data on gut species such as *Bacteroides fragilis*, *Escherichia coli* and *Akkermansia muciniphila* (soft), versus *Clostridium difficile*, *Lactobacillus rhamnosus*, *Enterococcus faecalis* and *Faecalibacterium prausnitzii* (stiff)—we constructed computational models to simulate how differential deformability, gut-like laminar shear, height-based partitioning, rotational alignment, lateral migration, adhesion to mucosal boundaries, collision aggregation, biofilm nucleation and mechanical displacement under peristaltic compression may affect bacterial localization under flow.

We begin by outlining the mechanical parameters, simulation design, and mathematical framework used to model stiffness-mediated sorting, detailing the underlying assumptions, force models and computational methods supporting our approach. This is followed by a presentation of simulation outcomes across multiple features and a critical analysis of their implications for understanding microbial organization, flow response and potential biomedical applications.

MATERIALS AND METHODS

Bacterial stiffness is a complex biomechanical property influenced by various intrinsic and environmental factors (Tuson et al., 2012; Pogoda et al., 2017; Han et al., 2022). Stiffness can be experimentally characterized using techniques such as atomic force microscopy (AFM), micropipette aspiration, optical tweezers and deformability-based microfluidic sorting (Zhou et al., 2016; Choi et al., 2020; Muta et al., 2023). The values of bacterial stiffness used in our simulation were derived from published AFM data reported in the literature. These studies employ force spectroscopy using cantilevers with spherical tips to measure indentation responses of individual bacterial cells fixed on adhesive substrates (Merson et al., 2023; Thomas-Chemin et al., 2023). Stiffness was calculated using the Hertz model for a spherical indenter:

$$F = \frac{4}{3} E \frac{\sqrt{R}}{(1-\nu^2)} \delta^{3/2}$$

where E is the Young's modulus, R is the tip radius, ν is Poisson's ratio (assumed to be 0.5) and δ is indentation depth. Based on AFM studies, species exhibiting stiffness values between 30 and 150 kPa were categorized as soft. In contrast, species showing moduli between 200 and 500 kPa were considered stiff.

To integrate the biological basis of bacterial stiffness into our computational framework, we analyzed known structural determinants of stiffness in intestinal bacteria to guide simulation parameterization. The most significant determinant is the composition and architecture of the cell wall: Gram-positive bacteria display thick peptidoglycan layers that confer high stiffness, whereas Gram-negative species have thinner walls and outer membranes, resulting in greater deformability. Cell morphology also contributes to mechanical anisotropy: rod-shaped bacteria exhibit directional stiffness along their longitudinal axis, while size and filamentous extensions may increase bending flexibility (al-Mosleh et al., 2022; Qiu et al., 2022). The presence of extracellular structures such as polysaccharide capsules or inclusion in biofilms further alters stiffness by introducing viscoelastic extracellular matrix components (Liu et al., 2024; Courbot and Elosegui-Artola, 2025). Additionally, turgor pressure, which is a function of osmotic gradient across the cytoplasmic membrane, modulates cellular rigidity and can vary with environmental or metabolic states (Rojas and Huang, 2018). Mechanical adaptation is also phase-dependent: bacteria in exponential growth phases tend to be more compliant than those in stationary or stress-induced states, which often reinforce their envelopes.
This biomechanical diversity forms the empirical basis for classifying soft and stiff populations in our model (**Figure 1**). Soft bacteria, characterized by lower stiffness and greater deformability, are predominantly Gram-negative. These include *Bacteroides fragilis* (from the Bacteroidetes phylum), *Escherichia coli* (Proteobacteria) and *Akkermansia muciniphila* (Verrucomicrobia). These species have outer membranes and thinner peptidoglycan layers, contributing to their lower Young's modulus and increased mechanical compliance (Cani et al., 2022; Rodrigues et al., 2022). In contrast, stiff bacteria in the colon are mainly Gram-positive and display thicker peptidoglycan walls. Examples include *Clostridium difficile*, *Faecalibacterium prausnitzii*, *Lactobacillus rhamnosus* and *Enterococcus faecalis*, all within the Firmicutes or Actinobacteria phyla.

Simulations. All simulations were performed using a computational representation of a 500 µm (length) × 100 µm (height) 2D cross-sectional segment of the colonic lumen. The spatial resolution was set to dx=1 µm, resulting in a 500 × 100 grid. The fluid viscosity was set to µ=0.01 Pa·s to approximate the mucus-dominated colonic environment. A constant pressure gradient of $\frac{dp}{dx} = -1 \times 10^{-4}$ was used to generate laminar Poiseuille flow described by:

$$u(y) = \frac{1}{2\mu} \cdot \frac{dp}{dx} \cdot \left(H^2 - (y - H)^2\right)$$

with H=50 µm representing half the channel height.
Approximately 60% of the colonic population consists of soft bacteria and 40% of stiff bacteria (Walker et al., 2011). This distribution was incorporated into the simulation model to reflect realistic biomechanical heterogeneity within the gut microbiome. Therefore, the simulated bacterial population comprised 200 agents: 120 soft and 80 stiff. Soft bacteria had a diameter ds=1.0 µm, with a drag coefficient $\gamma s = 6\pi\mu rs$, where $rs = 0.5\ \mu m$. Stiff bacteria had a diameter $dh = 1.5$ µm, with $\gamma h = 6\pi\mu rh$, where $rh = 0.75$ µm (Mai-Prochnow et al., 2016; Farris et al., 2018). Initial positions were assigned uniformly across the grid using pseudorandom sampling. All variables were treated in SI units and conversions were applied during visualization. These parameters defined the mechanical and geometric framework for simulating spatial sorting driven by differential bacterial stiffness in gut-like conditions.

Bacterial motion within the simulated gut-like channel was governed by a discretized overdamped Langevin equation, reflecting the viscous-dominated, low Reynolds number conditions of the colonic environment (Lelièvre et al., 2025). For each bacterium i, the evolution of its position vector $\vec{x}i$ was computed by:

$$\gamma_i \frac{d\vec{x}_i}{dt} = \vec{F}_{\text{flow}}(\vec{x}_i) + \vec{F}_{\text{rand}} + \vec{F}_{\text{int}}(\vec{x}_i)$$

Here, γi is the drag coefficient specific to soft or stiff classification, $\vec{F}$ is derived from the Poiseuille profile, $\vec{F}rand$ is Gaussian white noise with autocorrelation $\langle \xi(t)\xi(t')\rangle = 2kBT\gamma i\delta(t - t')$ and $\vec{F}int$ accounts for steric repulsion and boundary confinement. The integration was executed using the Euler-Maruyama method with time step $\Delta t = 0.1$ s for 10,000 iterations (Nouri et al., 2018). No adhesion forces were applied to isolate the role of stiffness. The Langevin dynamics formalism ensured that physical forces, both deterministic (flow) and stochastic (thermal fluctuations), were modeled to simulate displacement patterns across the stiffness spectrum.

Steric interactions between bacteria were implemented via a pairwise repulsive force modeled with a linearized, contact-based approximation. For any two agents i and j within interaction distance, the force was computed as:

$$\vec{F}_{\text{rep}}^{ij} = \begin{cases} \epsilon \left(\frac{d_{ij} - r_i - r_j}{r_i + r_j}\right) \hat{n}_{ij} & \text{if } d_{ij} < r_i + r_j \\ 0 & \text{otherwise} \end{cases}$$

with $dij = \| xi - xj \|$ and $n\hat{}ij = (xi - xj)/dij$. The repulsion magnitude $\epsilon = 1 \times 10 - 12\ N$ was selected to ensure no overlap occurred without introducing instability. Stiffer bacteria, having larger effective radii, produced stronger repulsive effects and exhibited reduced packing under shear-induced alignment. No attractive potentials were used such that all interactions preserved physical exclusion without aggregation. These mechanical constraints modeled the finite volume and packing behavior typical of dense colonic bacterial populations.

As a first step in evaluating the model parameters, we conducted a quantitative assessment of spatial segregation. Density functions $\rho s(y)$ and $\rho h(y)$ were smoothed via convolution with a Gaussian kernel $\sigma = 2\ \mu m$ to reduce sampling noise. To quantify segregation, the Kullback-Leibler divergence was calculated:

$$D_{\text{KL}}(\rho_s || \rho_h) = \sum_y \rho_s(y) \log\left(\frac{\rho_s(y)}{\rho_h(y)}\right)$$

In addition, spatial overlap was assessed by the Bhattacharyya coefficient (Van Molle et al., 2021):

$$BC = \sum_y \sqrt{\rho_s(y) \cdot \rho_h(y)}$$

Values close to 0 indicated strong segregation, while those near 1 reflected overlap. The Mann-Whitney U test was used to test for significant differences in vertical position distributions. This quantitative analysis allowed us to rigorously assess the presence and degree of stiffness-mediated spatial sorting under flow.

Next, to assess rotational alignment under shear, the Jeffery's equation for ellipsoidal alignment was employed:

$$\frac{d\theta}{dt} = \frac{\dot{\gamma}}{2} \cdot \left(\frac{r^2 - 1}{r^2 + 1}\right) \sin(2\theta)$$

Thie equation was used to evaluate whether softer bacteria, possibly exhibiting a lower effective aspect ratio due to deformation, tend to align more rapidly with the flow.

To evaluate deformability-driven lateral migration, we employed a restoring force model to simulate migration toward the flow center:

$$\frac{dy}{dt} = -k(y - y_{\text{center}})$$

This model was employed to assess whether soft bacteria had higher k and therefore faster centripetal drift.

Collision mechanics and aggregation were evaluated through post-collision velocity given by:

$$v_{\text{post}} = -e \cdot v_{\text{pre}}$$

where e is the restitution coefficient (soft = 0.2, stiff = 0.8). This approach makes it possible to analyze whether soft bacteria show damped rebounds and reduced aggregation behaviour.

Adhesion to wall was modeled as:

$$F_{\text{adh}} = -A \cdot e^{-d/\lambda}$$

with net force $F_{\text{net}} = F_{\text{shear}} + F_{\text{adh}}$ Displacement evolved as $d(t + \Delta t) = d(t) + F_{\text{net}} \cdot \Delta t$ to evaluate whether stiff bacteria remained adherent for longer durations or tended to detach.

To assess biofilm nucleation and growth, probabilistic growth was used in Moore neighborhood (Tanimoto and Imai, 2008):

$$P(\text{growth}) = \begin{cases} p_{\text{stiff}} = 0.15 \\ p_{\text{soft}} = 0.05 \end{cases}$$

to check whether stiff biofilms expanded more rapidly, forming denser colonies than soft bacteria.

Response to peristaltic compression was evaluated through displacement under sinusoidal compression $F(t) = Asin(2\pi ft)$, which was computed via Hookean response:

$$x(t) = \frac{F(t)}{k_{\text{eff}}}$$

This method was employed to evaluate whether soft bacteria (k=0.5) displaced more than stiff bacteria (k=2.0), potentially pointing towards enhanced mobility under peristalsis.

All simulations and analyses were conducted on a 12-core AMD Ryzen system with 64 GB RAM running Ubuntu 22.04. Data analysis was conducted in Python using SciPy, NumPy and Matplotlib. Random seeds for NumPy and random modules were fixed to ensure replicability. The total runtime per simulation was approximately 12 minutes. Code and raw outputs were version-controlled using Git and documented in Jupyter notebooks, which were exported as HTML for archival. Memory usage was optimized via sparse matrix representations where applicable. A total of 10 simulations were performed to assess variability and consistency across replicates was quantified by the mean Pearson correlation coefficient between distribution histograms.

Overall, we assessed several simulated parameters to distinguish the behaviour of soft and stiff colonic bacteria, namely: vertical distribution, spatial partitioning within a gut-like lumen volume, rotational alignment in shear flow, lateral migration under flow, collision mechanics, wall adhesion dynamics, biofilm growth and peristaltic compression responses.

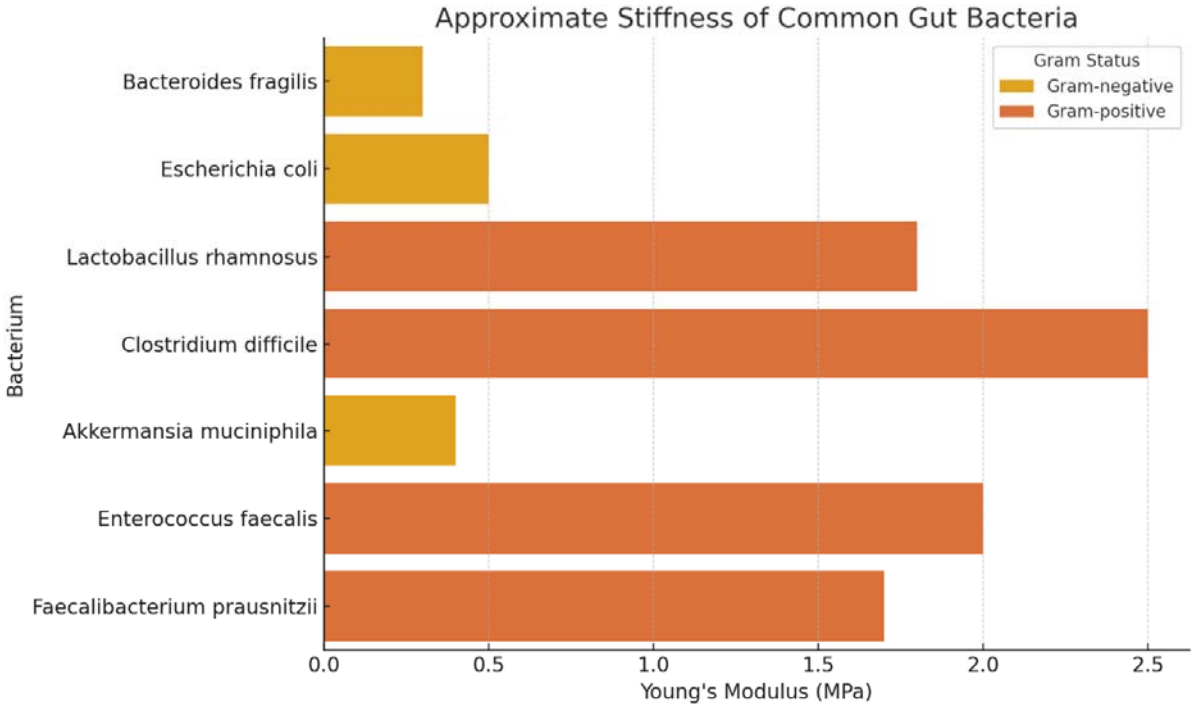

Figure 1. Representative bacterial species from the human colon categorized by mechanical stiffness. Soft bacteria include *Bacteroides fragilis*, *Escherichia coli* and *Akkermansia muciniphila*, which are typically Gram-negative and mechanically compliant. Stiff bacteria include *Clostridium difficile*, *Lactobacillus rhamnosus*, *Enterococcus faecalis* and *Faecalibacterium prausnitzii*, representing Gram-positive taxa with higher mechanical rigidity.

RESULTS

Simulated trajectories of soft and stiff bacteria under laminar flow revealed consistent differences in vertical positioning within the channel (**Figure 2**). After 10,000 timesteps, vertical density profiles showed that soft bacteria accumulated closer to the intestinal layer. Quantitative comparison using Kullback-Leibler divergence yielded a value of 0.875 and the Bhattacharyya coefficient was 0.803, indicating non-negligible but incomplete segregation. A Mann-Whitney U test comparing vertical distributions of both populations gave a p-value <0.001, confirming that spatial differences were highly significant. Ten replicate simulations showed high consistency (mean Pearson correlation > 0.98 across density profiles). Minor variation in diameter or drag coefficient (±10%) did not alter spatial trends, indicating robustness to parameter tuning. These outcomes confirm that mechanical stiffness alone can drive spatial partitioning under flow and confinement, supporting a mechanistic basis for differential localization of bacteria in the colon.

Additional simulations explored other mechanical behaviors to further differentiate soft from stiff populations (**Figure 3**). Under shear, soft bacteria (with lower effective aspect ratio due to deformation) aligned more rapidly with flow lines according to Jeffery's equation, while stiff bacteria displayed slower, oscillatory rotation and persistent tumbling. In a lateral migration model, soft bacteria displayed faster centripedal drift, moved toward the flow center more efficiently, converging within 20 seconds, whereas stiff bacteria retained their initial vertical offsets. Collision simulations showed that soft bacteria (restitution coefficient = 0.2) dissipated more energy and tended to aggregate with damped rebounds, while stiff bacteria (coefficient = 0.8) maintained higher rebound velocities, reducing cluster formation. Adhesion tests under simulated wall contact revealed that stiff bacteria resisted detachment longer due to stronger adhesive forces, maintaining proximity to boundaries during constant shear, unlike soft bacteria which detached rapidly. Biofilm modeling indicated that stiff bacteria produced denser and faster-expanding biofilms over 100 iterations due to higher local growth probabilities and intercellular packing. Finally, simulations of peristaltic compression revealed that soft bacteria underwent displacements up to twice those of stiff bacteria, indicating increased susceptibility to mechanical clearance and enhanced mobility under peristalsis.

Taken together, our results expand the role of stiffness from a sorting parameter to a determinant of bacterial interaction, aggregation and resilience.

A **B**

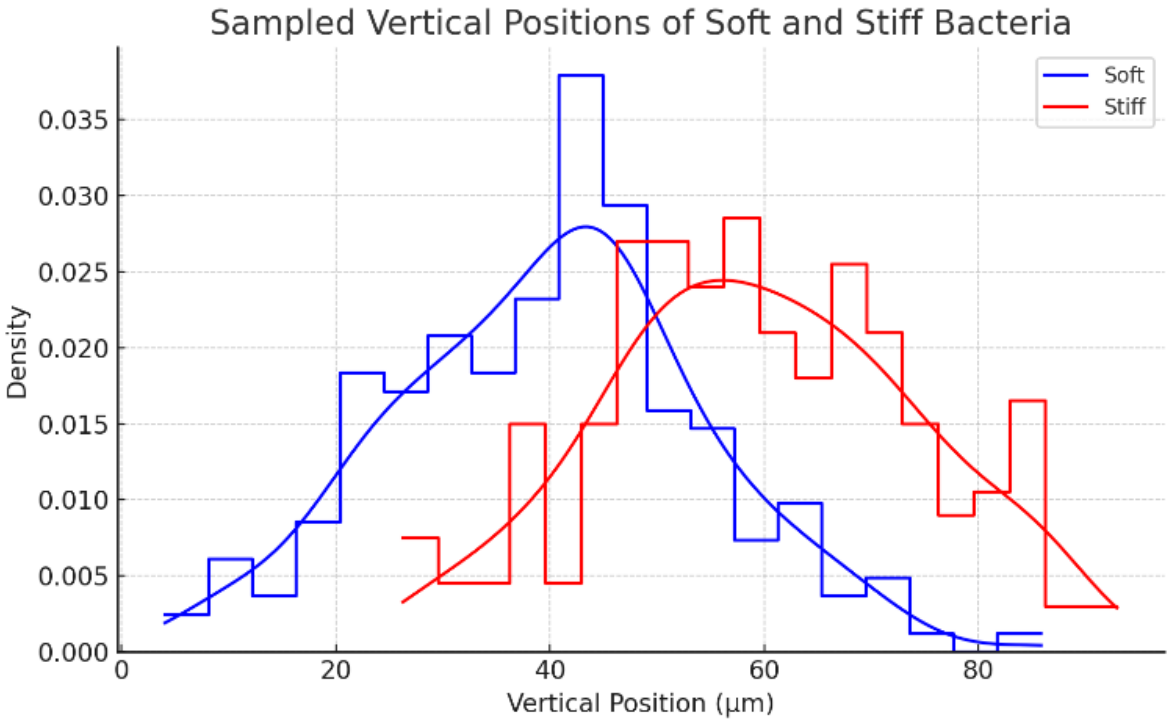

Figure 2A. Histogram of sampled vertical positions for soft (blue) and stiff (red) bacteria within the simulated gut-like flow environment in a laminar flow regime. Kernel density estimation overlays reveal distinct distribution modes, with soft populations concentrated in the lower regions of the channel and stiff populations skewed toward the upper regions. This separation supports stiffness-dependent spatial localization driven by differential response to shear gradients.

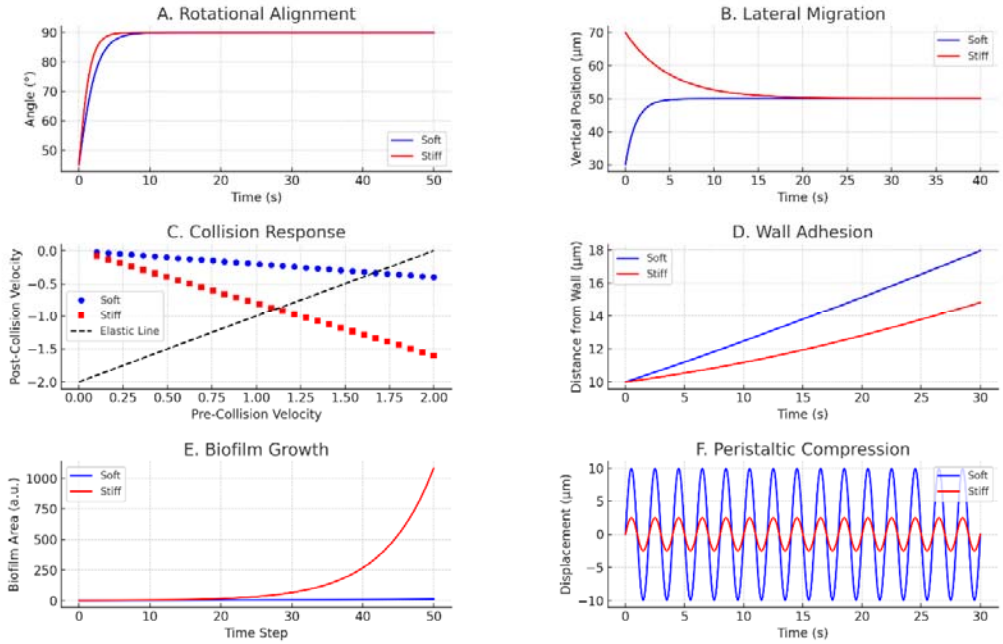

Figure 3. Simulated mechanical behaviours distinguishing soft (blue) and stiff (red) colonic bacteria. (A) Rotational alignment in shear flow shows that soft bacteria align more rapidly with streamlines due to lower aspect ratio and higher deformability, while stiff bacteria exhibit persistent oscillatory rotation. (B) Lateral migration under flow shows that soft bacteria quickly converge toward the channel center, while stiff bacteria migrate slowly and remain offset. (C) Collision mechanics reveal that soft bacteria, characterized by low restitution coefficients, dissipate energy efficiently upon impact and are more prone to aggregation, whereas stiff bacteria retain higher rebound velocities, indicating elastic interactions. (D) Wall adhesion dynamics under shear show that stiff bacteria remain adherent longer due to stronger adhesive forces, while soft bacteria detach more rapidly. (E) Biofilm growth simulations highlight that stiff bacteria expand biofilms more rapidly and densely over time due to higher nucleation probability and tighter intercellular packing. (F) Peristaltic compression responses demonstrate that soft bacteria undergo larger displacements due to greater compliance, whereas stiff bacteria resist deformation under identical compressive stress. Together, these panels illustrate how mechanical stiffness influences not only spatial localization, but also functional interactions and responses to physical forces in gut-like conditions.

CONCLUSIONS

Our simulations showed that bacterial populations with different stiffness tended to organize non-randomly within a flow-constrained gut-like environment. Soft bacteria, modeled after Gram-negative species with lower Young's modulus and smaller diameters (including *Escherichia coli*, *Bacteroides fragilis* and *Akkermansia muciniphila*), were consistently found to concentrate close to the walls of the simulated colonic channel. In contrast, stiff bacteria, representative of Gram-positive species with higher mechanical resistance (including *Clostridium difficile*, *Lactobacillus rhamnosus*, *Enterococcus faecalis* and *Faecalibacterium prausnitzii*), tended to accumulate in regions of higher elevation within the channel. Our findings complement recent evidence that biophysical properties critically shape bacterial spatial behavior. Specifically, Pokhrel et al. (2024) demonstrated that the geometry and expansion rate of bacterial colonies are tightly coupled to vertical and horizontal growth dynamics, governed by physical constraints like surface contact angles and nutrient diffusion. These observations highlight the role of physical forces and cellular mechanics as central determinants of microbial organization.

Additional simulations, summarized in **Figure 4**, revealed further mechanical differences between soft and stiff bacterial populations. Simulations of rotational dynamics in shear flow revealed that soft bacteria align faster with flow lines, while stiff bacteria maintain oscillatory rotation, possibly influencing their hydrodynamic exposure. Lateral migration analyses showed that soft bacteria converge more quickly toward the flow centerline, whereas stiff bacteria remain distributed near boundaries. Collision mechanics revealed that soft bacteria exhibit inelastic behavior with greater energy dissipation, facilitating transient aggregation, while stiff bacteria rebound elastically and resist cluster formation. Wall adhesion tests indicated that stiff bacteria resist detachment and remain close to mucosal surfaces under shear, a property that may enhance their retention in mucosal niches. Biofilm growth simulations showed that stiff populations form denser, faster-growing colonies, consistent with their higher rigidity and packing efficiency. Lastly, under peristaltic compression, soft bacteria experienced larger displacements than stiff counterparts, suggesting differential clearance susceptibility.

Overall, our results suggest that bacterial stiffness alone, independent of chemical gradients or adhesion, can induce emergent, structured spatial organization within the gut-like environment, influencing flow interaction, biofilm formation and mucosal persistence.

Several limitations should be acknowledged. The core model remains two-dimensional, restricting full spatial representation of colonic architecture, although 3D visualization and biofilm growth mapping were provided to extend dimensional inference. Bacteria were represented as circular agents with stiffness-dependent drag but without explicit modeling of morphology, flagella or real-time membrane deformation. Flow was approximated as steady laminar Poiseuille, which does not capture all aspects of peristaltic gut flows. No mucin rheology, immune interactions, chemotaxis, biochemical signaling or metabolite gradients were included. Although these simplifications were essential for isolating stiffness-driven effects, the resulting findings should be viewed as mechanistic hypotheses rather than exact representations of in vivo dynamics. Furthermore, although stiffness parameters were based on literature, no direct mapping was made to real-time positioning in the human gut.

Despite these limitations, it would be valuable to assess whether the positioning and behaviour of different bacterial species within the colon truly correspond to the predictions of our model. Experimental validation through imaging or spatial profiling of bacterial populations in vivo or in controlled gut-like environments could help determine the extent to which stiffness may influence real microbial localization and interaction dynamics. Experimental validation of our framework could pave the way for novel strategies to investigate and modulate the gut microbiota. Our insights may provide practical implications and avenues for translational microbiome engineering. Stiffness-based spatial segregation may inform strategies for targeted drug delivery: engineered particles with stiffness-mimicking properties could preferentially localize to zones dominated by soft or stiff microbes. For example, carriers mimicking soft bacteria may penetrate deeper into microbial layers near the mucosal surfaces, whereas stiffer vectors are more likely to remain

anchored closer to the lumen. Still, stiffness can be exploited for selective bacterial elimination. Ultrasonic or shear-sensitive nanoparticles, for instance, could be activated in regions of the gut where mechanical resistance indicates the presence of stiffer, potentially pathogenic taxa such as Clostridium difficile, sparing softer commensals in the process. Microfluidic sorting platforms could be developed for diagnostics or microbiome modulation, using mechanical filtration to isolate taxa by stiffness. Furthermore, biofilm formation could be selectively promoted or inhibited to target specific bacterial populations. Finally, insights into mechanical responsiveness under peristalsis may offer new perspectives on probiotic design. For instance, strains engineered for optimal deformability may resist mechanical clearance better or target specific colonic regions. Collectively, these findings underscore the potential of physical traits like stiffness to function as design parameters in microbiome-based therapeutic strategies.

In conclusion, we set out to evaluate whether mechanical stiffness alone can explain bacterial spatial organization in a simplified colonic flow regime. By establishing a reproducible link between mechanical compliance and microbial performance, our approach suggests a methodological approach for integrating physics-informed approaches into microbiome modeling, diagnostics and therapeutic design.

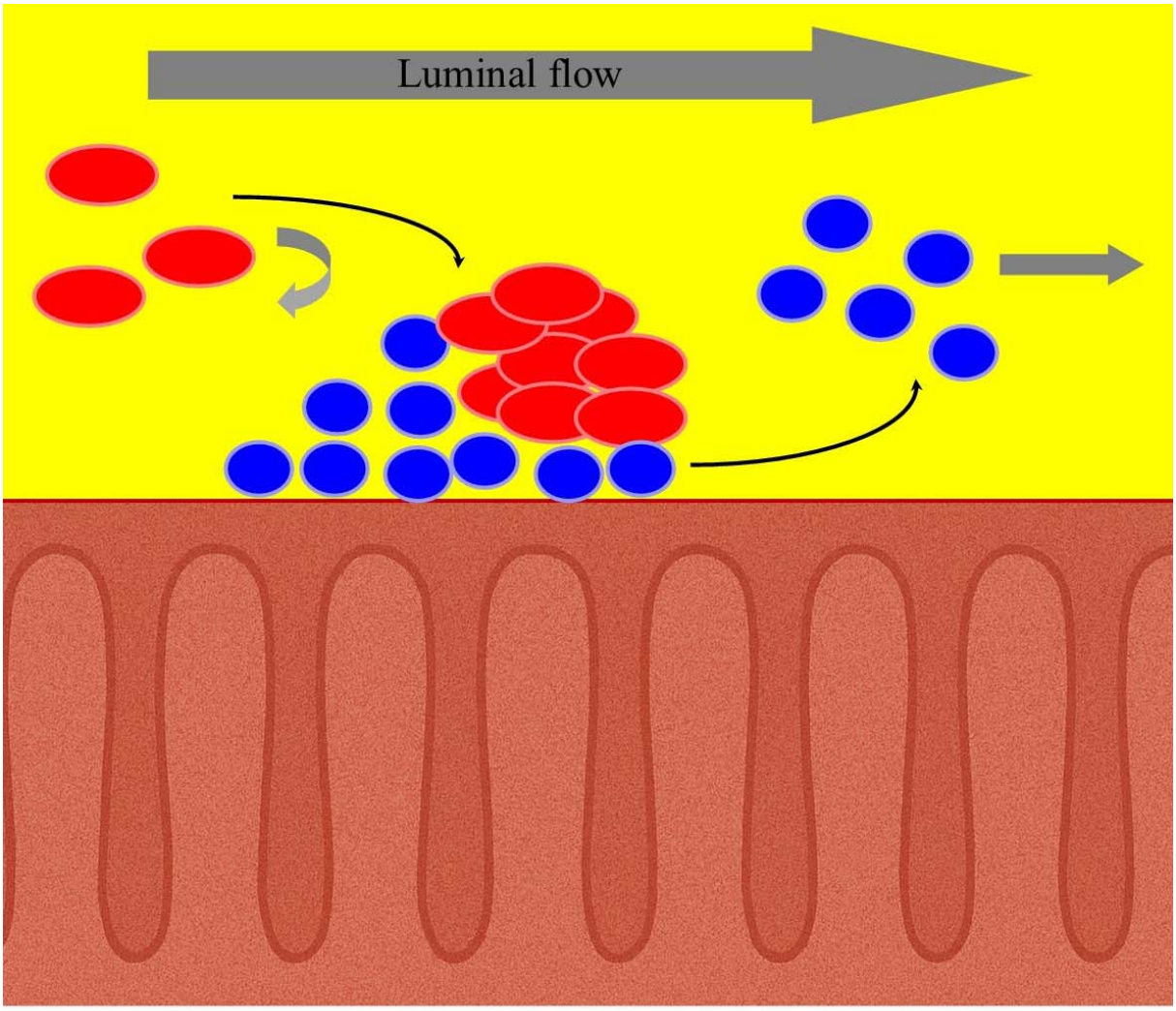

Figure. Simulated stiffness-dependent mechanical sorting of soft (blue) and stiff (red) colonic bacteria under shear flow. Differences in stiffness drive distinct patterns in alignment, migration, collisions, adhesion and biofilm growth. This framework highlights how mechanical properties, operating independently of biochemical signals, can drive the spatial organization of microbial communities within the colon.

DECLARATIONS

Ethics approval and consent to participate. This research does not contain any studies with human participants or animals performed by the Author.
Consent for publication. The Author transfers all copyright ownership, in the event the work is published. The undersigned author warrants that the article is original, does not infringe on any copyright or other proprietary right of any third part, is not under consideration by another journal and has not been previously published.
Availability of data and materials. All data and materials generated or analyzed during this study are included in the manuscript. The Author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.
Competing interests. The Author does not have any known or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence or be perceived to influence their work.
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Authors' contributions. The Author performed: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, obtained funding, administrative, technical and material support, study supervision.
Declaration of generative AI and AI-assisted technologies in the writing process. During the preparation of this work, the author used ChatGPT 4o to assist with data analysis and manuscript drafting and to improve spelling, grammar and general editing. After using this tool, the author reviewed and edited the content as needed, taking full responsibility for the content of the publication.

REFERENCES

1) Adler, Miri, Arun R. Chavan, and Ruslan Medzhitov. "Tissue Biology: In Search of a New Paradigm." *Annual Review of Cell and Developmental Biology* 39 (2023): 67–89. https://doi.org/10.1146/annurev-cellbio-120420-113830.
2) al-Mosleh, Salem, Ajay Gopinathan, Christian D. Santangelo, and Enrique R. Rojas. "Feedback Linking Cell Envelope Stiffness, Curvature, and Synthesis Enables Robust Rod-Shaped Bacterial Growth." Proceedings of the National Academy of Sciences of the United States of America 119, no. 41 (2022): e2200728119. https://doi.org/10.1073/pnas.2200728119.
3) Cani, Patrice D., Clément Depommier, Muriel Derrien, Anne Everard, and Willem M. de Vos. "Akkermansia muciniphila: Paradigm for Next-Generation Beneficial Microorganisms." Nature Reviews Gastroenterology & Hepatology 19, no. 10 (2022): 625–637. https://doi.org/10.1038/s41575-022-00631-9.
4) Choi, G., R. Nouri, L. Zarzar, and W. Guan. "Microfluidic Deformability-Activated Sorting of Single Particles." Microsystems & Nanoengineering 6 (February 10, 2020): 11. https://doi.org/10.1038/s41378-019-0107-9.
5) Courbot, Olivia, and Alberto Elosegui-Artola. "The Role of Extracellular Matrix Viscoelasticity in Development and Disease." npj Biological Physics and Mechanics 2 (2025): Article 10. https://doi.org/10.1038/s44251-025-00016-1.
6) DuPont, Herbert L., Zhi-Dong Jiang, Andrew W. DuPont, and Netanya S. Utay. 2020. "The Intestinal Microbiome in Human Health and Disease." Transactions of the American Clinical and Climatological Association 131: 178–197. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7358474/.
7) Esteve Pallarès, Macià, Irina Pi-Jaumà, Isabela Corina Fortunato, Valeria Grazu, Manuel Gómez-González, Pere Roca-Cusachs, Jesus M. de la Fuente, Ricard Alert, Raimon Sunyer, Jaume Casademunt and Xavier Trepat. 2023. "Stiffness-Dependent Active Wetting Enables Optimal Collective Cell Durotaxis." Nature Physics 19: 279–289. https://doi.org/10.1038/s41567-022-01877-w.
8) Farris, Alton B. III, Martin K. Selig, and G. Petur Nielsen. "Ultrastructural Diagnosis of Infection." In Diagnostic Pathology of Infectious Disease, 2nd ed., 81–103. Edited by Richard L. Kradin. Elsevier, 2018. https://doi.org/10.1016/B978-0-323-44585-6.00005-9.
9) Gao, Xuan, Qiang Cao, Yuxin Cheng, Dongdong Zhao, Zhen Wang, Huiyang Yang, Qiang Wu, et al. "Chronic Stress Promotes Colitis by Disturbing the Gut Microbiota and Triggering Immune System Response." Proceedings of the National Academy of Sciences of the United States of America 115, no. 13 (2018): E2960–E2969. https://doi.org/10.1073/pnas.1720696115.

10) Han, Rui, Waldemar Vollmer, John D. Perry, Paul Stoodley, and Jinju Chen. "Simultaneous Determination of the Mechanical Properties and Turgor of a Single Bacterial Cell Using Atomic Force Microscopy." Nanoscale 14 (2022): 12060–12068. https://doi.org/10.1039/D2NR02577A.
11) Jawerth, Louise, Elisabeth Fischer-Friedrich, Suropriya Saha, Jie Wang, Titus Franzmann, Xiaojie Zhang, Jenny Sachweh, Martine Ruer, Mahdiye Ijavi and Frank Jülicher. 2020. “Protein Condensates as Aging Maxwell Fluids.” Science 370 (6522): 1317–1323. https://doi.org/10.1126/science.aaw4951.
12) Lee, Sang Hyun, Eleonora Secchi and Peter K. Kang. 2023. “Rapid Formation of Bioaggregates and Morphology Transition to Biofilm Streamers Induced by Pore-Throat Flows.” Proceedings of the National Academy of Sciences 120 (14): e2204466120. https://doi.org/10.1073/pnas.2204466120.
13) Lelièvre, Tony, Grigorios A. Pavliotis, Geneviève Robin, Régis Santet, and Gabriel Stoltz. Optimizing the Diffusion Coefficient of Overdamped Langevin Dynamics. arXiv preprint arXiv:2404.12087, last revised February 7, 2025. https://arxiv.org/abs/2404.12087.
14) Liu, Zhiqiang, Si Da Ling, Kaini Liang, Yihan Chen, Yudi Niu, Lei Sun, Junyang Li, and Yanan Du. "Viscoelasticity of ECM and Cells—Origin, Measurement and Correlation." Mechanobiology in Medicine 2, no. 4 (December 2024): 100082. https://doi.org/10.1016/j.mechbio.2024.100082.
15) Lobley, Julian Parkhill, and Harry J. Flint. "Dominant and diet-responsive groups of bacteria within the human colonic microbiota." The ISME Journal 5, no. 2 (2011): 220–230. https://doi.org/10.1038/ismej.2010.118.
16) Mai-Prochnow, Anne, Megan Clauson, Jinghong Hong, and others. “Gram Positive and Gram Negative Bacteria Differ in Their Sensitivity to Cold Plasma.” Scientific Reports 6 (2016): 38610. https://doi.org/10.1038/srep38610.
17) Merson, Jacob, Nadeem Parvez, and Razvan C. Picu. “Probing Soft Fibrous Materials by Indentation.” Acta Biomaterialia 163 (2023): 25–34. https://doi.org/10.1016/j.actbio.2022.03.053.
18) Muta, M., W. Kawakubo, D.H. Yoon, D. Tanaka, T. Sekiguchi, S. Shoji, M. Ito, Y. Hatada, T. Funatsu, and R. Iizuka. “Deformability-Based Microfluidic Microdroplet Screening to Obtain Agarolytic Bacterial Cells.” Analytical Chemistry 95, no. 44 (November 7, 2023): 16107–16114. https://doi.org/10.1021/acs.analchem.3c02174.
19) Nouri, K., H. Ranjbar, and L. Torkzadeh. "Improved Euler–Maruyama Method for Numerical Solution of the Itô Stochastic Differential Systems by Composite Previous-Current-Step Idea." Mediterranean Journal of Mathematics 15 (2018): 140. https://doi.org/10.1007/s00009-018-1187-8.
20) Pogoda, Katarzyna, Ewelina Piktel, Piotr Deptuła, Paul B. Savage, Małgorzata Lekka, and Robert Bucki. "Stiffening of Bacteria Cells as a First Manifestation of Bactericidal Attack." Micron 101 (October 2017): 95–102. https://doi.org/10.1016/j.micron.2017.06.003.
21) Pokhrel, A. R., Steinbach, G., Krueger, A., Day, T. C., Tijani, J., Bravo, P., Ng, S. L., Hammer, B. K., & Yunker, P. J. (2024). The biophysical basis of bacterial colony growth. *Nature Physics*, 20, 1509–1517.
22) Qiu, Luyi, John W. Hutchinson, and Ariel Amir. "Bending Instability of Rod-Shaped Bacteria." Physical Review Letters 128, no. 5 (2022): 058101. https://doi.org/10.1103/PhysRevLett.128.058101.
23) Ritter, Christine M., Tianxiang Ma, Natascha Leijnse, Younes Farhangi Barooji, William Hamilton, Joshua M. Brickman, Amin Doostmohammadi and Lene B. Oddershede. 2025. “Differential Elasticity Affects Lineage Segregation of Embryonic Stem Cells.” Physical Review Letters 134 (16): 168401. https://doi.org/10.1103/PhysRevLett.134.168401.
24) Rodrigues, Vanessa F., Juliana Elias-Oliveira, Isabela S. Pereira, Juliana A. Pereira, Sarah C. Barbosa, Mariana S. G. Machado, and Daniela Carlos. "Akkermansia muciniphila and Gut Immune System: A Good Friendship That Attenuates Inflammatory Bowel Disease, Obesity, and Diabetes." Frontiers in Immunology 13 (2022): 934695. https://doi.org/10.3389/fimmu.2022.934695.
25) Rojas, Enrique R., and Kerwyn Casey Huang. "Regulation of Microbial Growth by Turgor Pressure." Current Opinion in Microbiology 42 (April 2018): 62–70. https://doi.org/10.1016/j.mib.2017.10.015.
26) Segel, Michael, Björn Neumann, Myfanwy F. E. Hill, Isabell P. Weber, Carlo Viscomi, Chao Zhao, Adam Young, et al. 2019. “Niche Stiffness Underlies the Ageing of Central Nervous System Progenitor Cells.” Nature 573: 130–134. https://doi.org/10.1038/s41586-019-1484-9.
27) Shellard, Adam and Roberto Mayor. 2021. “Collective Durotaxis Along a Self-Generated Stiffness Gradient In Vivo.” Nature 600: 690–694. https://doi.org/10.1038/s41586-021-04210-w.
28) Solis, Angel G., Piotr Bielecki, Holly R. Steach, Lokesh Sharma, Christian C. D. Harman, Sanguk Yun, Marcel R. de Zoete, et al. 2019. “Mechanosensation of Cyclical Force by PIEZO1 Is Essential for Innate Immunity.” Nature 573: 69–74. https://doi.org/10.1038/s41586-019-1485-8.
29) Song, Mingyang, Andrew T. Chan, and Jun Sun. “Influence of the Gut Microbiome, Diet, and Environment on Risk of Colorectal Cancer.” Gastroenterology 158, no. 2 (2020): 322–340. https://doi.org/10.1053/j.gastro.2019.06.048.
30) Tanimoto, Norio, and Katsuhiro Imai. "A Construction Method of Moore Neighborhood Number-Conserving Cellular Automata." In Cellular Automata. ACRI 2008, edited by Hiroshi Umeo, Shin Morishita, Katsunori Nishinari, Takashi Komatsuzaki, and Stefania Bandini, 340–347. Lecture Notes in Computer Science, vol. 5191. Berlin, Heidelberg: Springer, 2008. https://doi.org/10.1007/978-3-540-79992-4_31.

31) Thomas-Chemin, Olivier, Cyril Séverac, Edouard Trévisiol, and Etienne Dague. “Indentation of Living Cells by AFM Tips May Not Be What We Thought!” Micron 174 (2023): 103523. https://doi.org/10.1016/j.micron.2023.103523.
32) Tuson, Hannah H., George K. Auer, Lars D. Renner, Mariko Hasebe, Carolina Tropini, Max Salick, Wendy C. Crone, Ajay Gopinathan, Kerwyn Casey Huang, and Douglas B. Weibel. "Measuring the Stiffness of Bacterial Cells from Growth Rates in Hydrogels of Tunable Elasticity." Molecular Microbiology 84, no. 5 (2012): 874–891. https://doi.org/10.1111/j.1365-2958.2012.08063.x. Published as author manuscript in PMC June 1, 2013.
33) Van Molle, Pieter, Tim Verbelen, Bram Vankeirsbilck, and others. "Leveraging the Bhattacharyya Coefficient for Uncertainty Quantification in Deep Neural Networks." Neural Computing and Applications 33 (2021): 10259–10275. https://doi.org/10.1007/s00521-021-05789-y.
34) Yanagida, Ayaka, Elena Corujo-Simon, Christopher K. Revell and Ewa K. Paluch, et al. 2022. “Cell Surface Fluctuations Regulate Early Embryonic Lineage Sorting.” Cell 185 (5): 777–793.e20. https://doi.org/10.1016/j.cell.2022.01.019.
35) Yang, Yi, Mytien Nguyen, Varnica Khetrapal, Nicole D. Sonnert, Anjelica L. Martin, Haiwei Chen, Martin A. Kriegel, and Noah W. Palm. 2022. "Within-Host Evolution of a Gut Pathobiont Facilitates Liver Translocation." Nature 607: 563–570. https://doi.org/10.1038/s41586-022-04993-y.
36) Walker, Alan W., Jennifer Ince, Sylvia H. Duncan, Lucy M. Webster, Grietje Holtrop, Xiaolei Ze, David Brown, Mark D. Stares, Paul Scott, Aurore Bergerat, Petra Louis, Freda McIntosh, Alexandra M. Johnstone, Gerald E.
37) Wilde, Jacob, Emma Slack, and Kevin R. Foster. 2024. "Host Control of the Microbiome: Mechanisms, Evolution, and Disease." Science 385 (6706). https://doi.org/10.1126/science.adi3338.
38) Zhou, T., L.H. Yeh, F.C. Li, B. Mauroy, and S.W. Joo. “Deformability-Based Electrokinetic Particle Separation.” Micromachines 7, no. 9 (September 20, 2016): 170. https://doi.org/10.3390/mi7090170.