Skepthical review: *Linking Residue-Level Network Dynamics to Peptide Aggregate Stability: A Hierarchical Spectral Graph Analysis of KYFIL Self-Assembly*

Summary

The paper proposes a hierarchical, time-resolved graph-theoretic framework to analyze peptide self-assembly from MD. From a **500** ns trajectory of **30** KYFIL pentapeptides, the authors construct per-frame peptide-level contact graphs to identify aggregates (connected components) and track aggregate "lineages" across time via Jaccard overlap, then define "growth" and "dissolution" events (Sec. 2.1–2.2.2). For aggregates persisting at least **250** frames (**5** ns), the authors build residue-level heavy-atom contact graphs and compute spectral and standard graph metrics (algebraic connectivity/Fiedler value λ_2, density, clustering coefficient, centrality) as time series and around event-aligned windows (Sec. 2.3–2.4, Sec. 3.2–3.3).

The headline result is that while peptide-level aggregates are often large and connected, residue-level algebraic connectivity is reported as identically zero with (near-)zero variance across $>$ **62k** aggregate-frame graphs, whereas residue-level density and clustering vary and differ between event and stable windows (Sec. 3.2–3.3, Table 2). The overall direction—connecting macro aggregation dynamics to micro contact-network organization—is promising and timely. However, the current manuscript leaves key methodological steps under-specified (tracking/events/statistics/contact definition), contains at least one mathematical misinterpretation of λ_2, and lacks basic diagnostics to validate the striking $\lambda_{2,\text{res}} \equiv 0$ finding. The statistical comparisons risk overstating significance due to non-independence and size confounding, and the mechanistic interpretation is not yet anchored in structural/chemical observables. Strengthening reproducibility, validation, robustness, and positioning in biomolecular network literature would substantially improve the paper (Sec. 1–4).

Strengths

- Clear conceptual separation between peptide-level aggregation dynamics and residue-level contact organization, making the hierarchical pipeline easy to follow (Sec. 2.2–2.3).
- Systematic, time-resolved analysis of multiple graph metrics (λ_2, density, clustering, centrality) and explicit alignment of metric behavior to growth/dissolution events (Sec. 2.4, Sec. 3.1–3.3).
- Large-scale residue-level evaluation across $>$ **62,!000** aggregate-frame graphs, which in principle can provide robust descriptive statistics (Sec. 3.2).
- Aggregate lineage idea is useful for turning a fluctuating MD ensemble into analyzable "objects" with histories (Sec. 2.2.2, Sec. 3.1.1).

- Figures/tables (notably Fig. 1 and Table 1/2) generally support the narrative and help connect definitions to observed time-series behavior.

Major issues

1. **Algebraic connectivity (λ_2) is misinterpreted, undermining the central residue-level claim.** The manuscript states that $\lambda_2 = 0$ can occur for a connected graph with articulation points; for the standard (combinatorial or normalized) Laplacian, $\lambda_2 = 0$ if and only if the graph is disconnected. This directly affects the interpretation of $\lambda_{2,\text{res}} \equiv 0$ across all residue graphs (Sec. 3.2, Sec. 3.4; definitions in Sec. 2.3.2).

   Recommendation: Revise all discussion of $\lambda_{2,\text{res}} = 0$ to state explicitly that it implies ≥ 2 connected components (disconnection). If the goal is to capture "bottlenecks" in connected graphs, add (or substitute) appropriate measures (e.g., vertex/edge connectivity, articulation-point counts, bridge edges, k-core structure, or using λ_2 magnitude when $\lambda_2 > 0$). Also specify which Laplacian variant is used (combinatorial vs normalized) and keep the interpretation consistent throughout (Sec. 2.3.2, Sec. 3.2–3.4).

2. **Residue-level graph construction and spectral computation are under-specified and not validated, making the striking $\lambda_{2,\text{res}} \equiv 0$ (with ∼zero SD) ambiguous and potentially an artifact of contact definition, intra- vs inter-peptide edges, periodic boundary handling, or numerical thresholding (Sec. 2.3.1–2.3.2, Sec. 3.2, Table 2).**

   Recommendation: In Sec. 2.3.1, specify precisely: (i) periodic boundary condition handling (minimum-image convention); (ii) whether solvent/ions are excluded; (iii) whether intra-peptide residue contacts are included and any sequence-separation exclusion; (iv) whether edges are unweighted vs weighted (distance/frequency), and whether any temporal persistence filtering is applied. In Sec. 2.3.2, report eigen-solver details (dense vs sparse), numeric precision, and the tolerance used to treat eigenvalues as zero. In Sec. 3.2, add diagnostics that must accompany $\lambda_{2,\text{res}}$ claims: distributions of number of connected components, size/fraction of nodes in the largest component, and (optionally) articulation points/bridge edges. Strongly consider reporting λ_2 computed on the largest connected component (or a component-aware cohesion proxy) so λ_2 can vary meaningfully even when the full graph is disconnected. Finally, separate and report results for an inter-peptide-only residue graph (edges only between residues on different peptides), since intra-peptide edges can create many small cliques that trivially yield global disconnection.

3. **Aggregate tracking, lineage assignment, and event definitions are under-specified, limiting reproducibility and interpretability of the reported 1742 lineages, 28 persistent aggregates, and 22 dissolution / 27 growth events**

(Sec. 2.2.2, Sec. 2.4.2–2.4.3, Sec. 3.3). Handling of merges/splits, tie-breaking among multiple Jaccard matches, and operational definitions of "growth/dissolution/stable" windows remain ambiguous.

Recommendation: In Sec. 2.2.2, provide a fully specified algorithm (ideally pseudocode/flowchart): exact Jaccard threshold(s) used in results; whether matching is one-to-one or many-to-one; tie-breaking rules; explicit treatment of merges and splits and how lineage IDs propagate; and any temporal smoothing (e.g., ignoring one-frame bridges). In Sec. 2.4.2–2.4.3, give formal event definitions (thresholds on size change, minimum duration, and how lineage termination is defined), precise window indexing (e.g., $[t-49, t]$ vs $[t, t+49]$), rules for overlapping events/windows, and how "stable windows" are selected/matched. In Sec. 3.3, report the total number of frames/windows per category and clarify whether multiple events from the same lineage are treated as independent.

4. **Statistical testing is insufficiently specified and likely overstates significance due to non-independence (autocorrelation within lineages; repeated measures across frames) and multiple comparisons. Reporting "$p = 0.0$" is not acceptable, and the current analysis is largely retrospective rather than demonstrating predictive utility (Sec. 2.4.3, Sec. 3.3).**

   Recommendation: In Sec. 2.4.3, specify the exact tests, units of analysis, and assumptions (frame-level vs event-averaged vs lineage-averaged). Use methods that respect dependence (e.g., per-lineage aggregation with lineages as replicates; block bootstrap; or mixed-effects models with lineage random effects and time correlation). Apply and report multiple-comparison correction across metrics/event types (e.g., FDR). In Sec. 3.3, replace "$p = 0.0$" with p-values or bounds (e.g., $p < 1 \times 10^{-6}$), and add effect sizes with confidence intervals. To connect to the paper's implied stability-indicator narrative (Sec. 3.4), add a simple predictive evaluation (e.g., logistic regression/ROC-AUC) distinguishing event vs stable windows using density/clustering (and possibly derivatives), with cross-validation performed at the lineage level.

5. **Event-associated differences in residue-level density/clustering may be confounded by aggregate size (number of residues/nodes) and by how "persistent" aggregates are selected; this risks attributing trivial size effects to topology changes (Sec. 3.3.1–3.3.2; Figures 2–4).**

   Recommendation: In Sec. 3.3, control explicitly for size: regress metrics on event label with N as covariate (or use mixed models), stratify comparisons by narrow N ranges, and/or analyze size-invariant alternatives (e.g., mean degree, transitivity, or inter-peptide edge density only). Report whether the event vs stable differences persist after controlling for size. If not, temper claims in Sec. 3.4 accordingly.

6. **Key parameter choices and MD context are incomplete or inconsistent, limiting reproducibility and raising basic sanity-check concerns (Sec. 2.1–2.2, Sec. 3.2–3.3). In particular: (i) the equilibrium-frame count appears**

inconsistent with the stated duration/sampling/equilibrium start; and (ii) heavy-atom totals are arithmetically inconsistent (48 heavy atoms/peptide $\times 30 = 1440$ vs stated 1225).

Recommendation: In Sec. 2.1, provide essential MD details (force field, water model, thermostat/barostat, timestep, electrostatics, box/PBC, saving stride) or cite prior work and summarize them. Reconcile the frame-count/time inconsistency by stating exact first/last analyzed frame indices and times. Correct and explain heavy-atom counting (what is included/excluded). Add a single table listing all analysis parameters (cutoffs, Jaccard threshold, persistence threshold, window size, equilibrium start) with brief justification.

7. **Generality and robustness are not established: conclusions about λ_2 being uninformative and local metrics being better are currently drawn from one peptide sequence, one trajectory, and one set of thresholds (Sec. 3.4, Sec. 4).** Given $\lambda_{2,\mathrm{res}}$ sensitivity to contact definitions and intra/inter edges, broader claims are premature.

   Recommendation: In Sec. 3.4 and Sec. 4, qualify conclusions to "this KYFIL system under these analysis choices." Add robustness checks at minimum: vary residue contact cutoff (e.g., **3.5/4.0/4.5** Å) and peptide cutoff (e.g., $\pm$**0.5** Å), and show whether (a) $\lambda_{2,\mathrm{res}}$ remains identically zero and (b) event-associated density/clustering differences persist (with size control). If feasible, include an additional trajectory (different initial condition) or a second peptide sequence; otherwise present this explicitly as future work and avoid general statements.

8. **Physical/chemical interpretation is underdeveloped: the manuscript interprets metric changes (density/clustering around growth/dissolution) in terms of compaction or local motifs without independent structural/energetic observables, leaving the mechanism speculative (Sec. 3.3–3.4).**

   Recommendation: Augment Sec. 3.3–3.4 with concrete structure-linked analyses: radius of gyration/asphericity of aggregates; core–periphery partition (distance to aggregate COM) with metrics computed separately; contact decomposition by residue types (K/Y/F/I/L) and by backbone–backbone vs sidechain–sidechain; and/or edge persistence (contact lifetimes) around events. Include at least one illustrative snapshot showing how a peptide-level connected aggregate can yield a disconnected residue graph under the chosen definition. If these analyses are not added, explicitly label mechanistic claims as hypotheses and temper language in Sec. 3.4/Conclusion.

9. **Related work and positioning are currently skewed toward astrophysics/network references and do not adequately engage biomolecular network analysis and peptide aggregation literature, making novelty and relevance harder to assess (Sec. 1, Sec. 2, References).**

Recommendation: Revise the Introduction/Sec. 2 to cite and discuss relevant work on residue interaction networks, protein/peptide contact graphs from MD, dynamic network analysis in biomolecules, and prior graph descriptors used for aggregation/self-assembly. Retain non-biomolecular network references only when methodologically essential and explicitly justify their relevance. Update the reference list accordingly.

Minor issues

1. Methods–Results inconsistency on peptide-level metrics: Methods suggest metrics are computed on the full peptide graph each frame, while Results/Table 1 imply metrics are computed on the largest aggregate/component (Sec. 2.2.3 vs Sec. 3.1.2, Table 1).

   Recommendation: Explicitly define which graph each peptide-level metric is computed on (full **30**-node graph vs induced subgraph of the largest connected component). Update notation accordingly (e.g., $G_{\text{peptide,t}}$ vs $G_{\text{largest,t}}$) and ensure Table 1/Fig. 1 captions match.

2. Contact cutoff mismatch across scales (**4.5** Å peptide-level vs **4.0** Å residue-level) complicates interpretation of "connected peptide aggregate but disconnected residue network," which may be a thresholding artifact rather than a meaningful multiscale distinction (Sec. 2.2.1, Sec. 2.3.1, Sec. 3.2).

   Recommendation: Add a short clarification/diagnostic: quantify how often peptide edges correspond to at least one inter-peptide residue edge under the residue cutoff, and show sensitivity to harmonized cutoffs. If the mismatch is intentional, justify the physical rationale (and cite prior practice).

3. Fiedler vector analyses are introduced but not substantively used in Results, creating a Methods–Results gap (Sec. 2.2.3, Sec. 2.3.2 vs Sec. 3.2–3.3).

   Recommendation: Either add concrete Fiedler-vector results (e.g., peptide-level partitioning; localization of weak links) with a figure/example, or reduce emphasis in Methods and state explicitly that Fiedler vectors did not add interpretive value in this dataset.

4. Figure 1 and event-related figures need accessibility and interpretability improvements: small text, unclear subpanel mapping, insufficient marking of equilibrium phase and event windows, and potential confusion due to size-dependence of λ_2 (Fig. 1; Sec. 3.1–3.3; Figures 2–6).

   Recommendation: Increase font/line sizes and use a colorblind-safe palette; add (a–d) subpanel labels referenced in captions; shade/mark equilibrium bounds and event windows consistently; clarify whether plotted values are raw per-frame or smoothed (and how). Consider adding size-normalized comparisons or annotate expected λ_2 behavior vs N.

5. Captions/statistical reporting in Figures 2–6 are missing key information (sample sizes, unit of analysis, tests used, what each point represents), which makes the results hard to audit (Sec. 3.3).

   Recommendation: For each figure in Sec. 3.3, state: number of events/lineages/windows; whether values are frame-level or event-averaged; the statistical test/model; effect size; and p-values reported appropriately (not "0.0").

6. Reproducibility and usability: the work is algorithmic and large-scale (tens of thousands of graphs) but the manuscript does not report code availability, runtime, or scaling considerations (Sec. 2–3).

   Recommendation: Provide a code/data availability statement (repository link, versioning, key dependencies). Add a brief note on compute cost (hardware, runtime for graph construction and eigen-computation) and discuss scaling with number of residues/frames; mention practical optimizations (sparse matrices, restricting spectral analysis to selected frames, etc.).

7. Equilibrium onset at 100 ns is asserted but not demonstrated with standard MD observables (Sec. 2.1, Sec. 3.1).

   Recommendation: Add a brief supporting diagnostic (e.g., potential energy, RMSD, aggregate size plateau, or similar) or cite a source that directly supports this specific trajectory's equilibration behavior.

8. Metric-definition clarity: clustering definition (average of local clustering vs transitivity) and treatment of isolated nodes should be stated; also clarify whether graphs are simple (no self-loops/multiedges), since several identities depend on this (Sec. 2.4.1, Sec. 3.2, Table 2).

   Recommendation: In Sec. 2.4.1 (or a notation/definitions table), specify the exact NetworkX (or other) functions used, how isolates are handled, and confirm graphs are simple undirected graphs. Where you use identities (e.g., mean degree centrality equals density under standard normalization), state assumptions explicitly.

Very minor issues

1. Typographical and formatting problems (line breaks splitting words, inconsistent unit formatting for Å and time, inconsistent inline-math formatting, inconsistent section heading styles) reduce polish (Sec. 1–4).

   Recommendation: Proofread and standardize units (Å, ns/ps), math formatting for λ_2, citation style, and section numbering/heading capitalization across the manuscript.

2. Keywords are generic and do not reflect peptide self-assembly and graph/network focus (Abstract).

Recommendation: Replace with domain-specific keywords (e.g., peptide self-assembly, molecular dynamics, contact networks, dynamic graphs, spectral graph theory, algebraic connectivity).

3. Reference list formatting appears inconsistent, and several entries seem incomplete or not obviously relevant (References).

   Recommendation: Standardize bibliography entries (journal, volume, pages/DOI) and ensure in-text citations match. Remove clearly irrelevant citations or justify their methodological relevance in the text.

4. Some long, multi-clause sentences—especially in the Introduction and interpretive discussion—impair readability (Sec. 1, Sec. 3.4).

   Recommendation: Edit for shorter sentences with single main ideas, particularly where definitions and claims are made.

5. Notation inconsistencies (λ_2 vs $\lambda_{2,\mathrm{res}}$; $\mathrm{Fvec}_{\mathrm{res}}$ referenced but not clearly defined; graph indices) create avoidable friction (Sec. 2–3).

   Recommendation: Add a compact notation table defining all graph objects ($G_{\mathrm{peptide,t}}$; $G_{\mathrm{residue,agg,f}}$), indices ($t, f, \mathrm{agg}$), and all metrics (including Laplacian variant), and ensure consistent use in text/figures/tables.

Key statements and references

- • **Molecular dynamics simulations provide atomic-resolution insights into the dynamic processes of peptide self-assembly, capturing the transient nature of inter-peptide and intra-aggregate interactions over time, but traditional analyses that focus on average properties or specific interaction types can fail to capture the emergent collective network behavior underlying aggregate integrity and dynamics (Horowitz & Hughto, 2008).**
- *Reference(s):* Horowitz, C. J., & Hughto, J. 2008
- • **Spectral graph properties such as the algebraic connectivity (Fiedler value λ_2) and its corresponding Fiedler vector are established tools for probing global connectivity and structural bottlenecks in complex networks, and have been applied in recent graph-based analyses of physical systems (Yang and Yu, 2023; Strey et al., 2024).**
- *Reference(s):* Yang, D., & Yu, H.-B. 2023, Strey, S.-G., Castronovo, A., & Elumalai, K. 2024
- • **Network density and average clustering coefficient are standard graph-theoretical measures used to quantify overall connectivity and local cohesiveness in interaction networks, and have been employed in recent work to analyze the structure of astrophysical and dark-matter-related graphs (Pavlou et al., 2023; Yang and Yu, 2023).**

- *Reference(s):* Pavlou, O., Michos, I., Lesta, V. P., et al. 2023, Yang, D., & Yu, H.-B. 2023

- • **The time-evolving peptide-level graphs in this study were constructed following an approach in which, for each simulation frame, nodes represent peptides and undirected edges are added when the minimum heavy-atom distance between two peptides is below a fixed threshold, a methodology consistent with recent graph-based treatments of molecular or astrophysical systems (Zheng et al., 2023).**

- *Reference(s):* Zheng, S., Li, J., Wang, J., et al. 2023

- • **The identification and tracking of peptide aggregates over time used a connected-components representation of aggregates in each frame and a Jaccard-index-based lineage matching between consecutive frames, an approach aligned with recent work on tracking evolving structures or objects in time-resolved datasets (Remijan et al., 2021; Oliver and Buck, 2024; Zhuang et al., 2025).**

- *Reference(s):* Remijan, A., Xue, C., Margulès, L., et al. 2021, Oliver, W. H., & Buck, T. 2024, Zhuang, G., Song, W., Huang, J., et al. 2025

Mathematical consistency audit

This section audits **symbolic/analytic** mathematical consistency (algebra, derivations, dimensional/unit checks, definition consistency).

Maths relevance: light

The paper is primarily methodological and descriptive, using standard graph-theoretic definitions (contact graphs, connected components, density, clustering coefficient, Laplacian spectrum/algebraic connectivity) with minimal formal derivations. The main analytic vulnerability is an incorrect statement about what $\lambda_2 = 0$ implies, which affects the interpretation of a headline result ($\lambda_{2,\mathrm{res}}$ reported as identically zero).

Checked items

1. ✔ **Definition of algebraic connectivity** (Sec. 2.2.3, p.3 (and Sec. 2.3.2, p.4))
    - **Claim:** Algebraic connectivity λ_2 is the second smallest eigenvalue of the graph Laplacian matrix; for disconnected graphs $\lambda_2 = 0$.
    - **Checks:** definition consistency, notation consistency
    - **Verdict:** PASS; confidence: high; impact: moderate
    - **Assumptions/inputs:** Standard (combinatorial) graph Laplacian $L = D - A$ for a simple undirected graph, Eigenvalues ordered nondecreasing
    - **Notes:** The definition given matches the standard usage: λ_2 is the second smallest Laplacian eigenvalue; $\lambda_2 = 0$ for disconnected graphs.

2. ✖ **Incorrect implication: $\lambda_2 = 0$ for connected graphs with articulation points** (Sec. 3.2, p.6–7 (paragraph explaining Table 2))
    - **Claim:** "An algebraic connectivity of zero indicates that the graph is either disconnected into multiple components or is connected but contains articulation points (cut vertices) whose removal would disconnect it."
    - **Checks:** logical implication, graph spectral property consistency
    - **Verdict:** FAIL; confidence: high; impact: critical
    - **Assumptions/inputs:** Same λ_2 definition as Sec. 2.2.3/2.3.2 (second-smallest Laplacian eigenvalue)
    - **Notes:** Under the stated definition, $\lambda_2 = 0$ indicates the graph is disconnected. A connected graph can have articulation points while still having $\lambda_2 > 0$. The first incorrect step is the disjunction that includes "connected but contains articulation points" as a condition for $\lambda_2 = 0$.
3. ✔ **Density maximum-edges formula** (Sec. 3.1.2, p.6)
    - **Claim:** For an aggregate of size N, the maximum number of edges is $N(N-1)/2$.
    - **Checks:** combinatorial identity
    - **Verdict:** PASS; confidence: high; impact: minor
    - **Assumptions/inputs:** Simple undirected graph with no self-loops and no multi-edges
    - **Notes:** Correct for simple undirected graphs.
4. ✔ **Converting density to average degree (peptide-level)** (Sec. 3.1.2, p.6)
    - **Claim:** A density of 0.1485 for $N \approx 29$ implies each peptide is on average in contact with approximately $0.1485 \times (29-1) \approx 4.16$ other peptides.
    - **Checks:** algebraic identity
    - **Verdict:** PASS; confidence: high; impact: minor
    - **Assumptions/inputs:** Density defined as $2m/(N(N-1))$, Average degree equals $2m/N$
    - **Notes:** Identity holds: avg degree = density $\times(N-1)$. The factor-of-2 is handled correctly via the relationship between m and density.
5. ✔ **Converting density to average degree (residue-level)** (Sec. 3.2, p.7)
    - **Claim:** For ≈ 144 nodes and density 0.0272, each residue is in contact with approximately $0.0272 \times (144-1) \approx 3.89$ other residues on average.
    - **Checks:** algebraic identity
    - **Verdict:** PASS; confidence: high; impact: minor
    - **Assumptions/inputs:** Same density and average-degree identities as above
    - **Notes:** Same identity; algebra is consistent.

6. ✔ **Average degree centrality vs density** (Sec. 2.3.2, p.4 and Sec. 3.2/Table 2, p.6–7)
    - **Claim:** Average degree centrality is directly proportional to density; Table 2 shows identical mean values for density and average degree centrality.
    - **Checks:** algebraic identity, definition consistency
    - **Verdict:** PASS; confidence: high; impact: minor
    - **Assumptions/inputs:** Degree centrality for node v is $\deg(v)/(n-1)$, Graph is simple undirected
    - **Notes:** For simple undirected graphs, the average degree centrality equals density exactly: $\text{mean}(\deg/(n-1)) = (2m/n)/(n-1) = 2m/(n(n-1)) =$ density. Text says "proportional," but the implied computation is consistent.
7. ✔ **Residue count consistency check (symbolic)** (Sec. 3.2, p.6–7)
    - **Claim:** Average number of residues per residue-graph is consistent with average peptides per aggregate times 5 residues/peptide.
    - **Checks:** definition consistency, dimensional counting
    - **Verdict:** PASS; confidence: medium; impact: minor
    - **Assumptions/inputs:** Each peptide has exactly 5 residues, Residue-graph includes all residues from peptides in the aggregate
    - **Notes:** The stated relationship is symbolically correct: $N_{\text{res}} \approx 5 \times N_{\text{peptides}}$. Whether the averages numerically match is outside this audit.
8. ✔ **Persistence threshold unit conversion** (Sec. 2.1, p.3)
    - **Claim:** Persistence threshold 250 frames is equivalent to 5 ns given 20.0 ps per frame.
    - **Checks:** unit consistency
    - **Verdict:** PASS; confidence: high; impact: minor
    - **Assumptions/inputs:** Constant frame spacing of 20.0 ps
    - **Notes:** 250×20 ps $= 5000$ ps $= 5$ ns; conversion is consistent.
9. ⚠ **Metric-computation target graph (full graph vs largest component)** (Sec. 2.2.3, p.3 vs Sec. 3.1.2, p.5–6)
    - **Claim:** Methods: metrics computed for entire peptide-level graph each frame; Results: metrics computed for the largest aggregate/component each frame.
    - **Checks:** definition consistency, notation consistency
    - **Verdict:** UNCERTAIN; confidence: high; impact: moderate
    - **Assumptions/inputs:** $G_{\text{peptide},t}$ includes all peptides as nodes, Largest aggregate is a connected component of $G_{\text{peptide},t}$

- **Notes:** This is a definitional mismatch rather than a derivation error. Both approaches are valid but yield different λ_2/density/clustering series. The paper should explicitly state which graph is used for each reported statistic and figure.

10. ⚠ **Residue-level connectivity inference from $\lambda_{2,\text{res}} = 0$** (Sec. 3.2–3.4, pp.6–10 (interpretation of Table 2 and Figures 6–8))

    - **Claim:** $\lambda_{2,\text{res}} \equiv 0$ implies the residue network is fragmented/disconnected or minimally connected globally, motivating the conclusion that global connectivity metrics are not predictive here.
    - **Checks:** logical implication, dependency on definitions
    - **Verdict:** UNCERTAIN; confidence: medium; impact: critical
    - **Assumptions/inputs:** $\lambda_{2,\text{res}}$ computed on the residue-level graph as defined in Sec. 2.3.1, Standard λ_2 interpretation
    - **Notes:** If $\lambda_{2,\text{res}}$ is truly exactly 0, the correct inference is "disconnected" (not "minimally connected connected graph"). However, because the paper also misstates the $\lambda_2 = 0$ condition and does not specify the Laplacian variant, graph preprocessing (e.g., isolated nodes handling), or whether $\lambda_{2,\text{res}}$ is computed on the full residue set vs largest component, the analytic meaning of the reported identically-zero series cannot be fully verified from the PDF.

Limitations

- The PDF provides definitions but not explicit formulas for the Laplacian used (combinatorial vs normalized), eigen-solver conventions, or preprocessing (e.g., removal of isolated nodes), limiting verification of spectral claims beyond basic properties.
- Several potentially relevant quantities (e.g., Jaccard index formula, clustering coefficient definition variant) are referenced but not explicitly defined, so only standard interpretations can be assumed.
- This audit does not validate numerical values in tables/figures; it only checks algebraic/unit relationships and logical implications stated in the text.

Numerical results audit

This section audits **numerical/empirical** consistency: reported metrics, experimental design, baseline comparisons, statistical evidence, leakage risks, and reproducibility.

Seven numerical consistency checks were run: five passed and two failed. The failures are (i) a large mismatch between reported equilibrium-frame count and the stated duration/sampling (implying ≈ 1435.42 ns rather than ≈ 500 ns), and (ii) an incorrect multiplication for total heavy atoms (expected 1440 vs reported 1225). Unit conversion for the persistence threshold and several graph-density-derived interpretations were internally consistent within stated tolerances.

Checked items

1. ✖ **C01_frames_from_time_step** (p.2 §2.1 (Methods) + p.5 §3.1 (Results))
   - **Claim:** Simulation duration ≈ 500 ns with frames saved every 20.0 ps; analysis focuses on 100.0 ns onwards and reports 66,!771 frames in the equilibrium segment (100 ns onwards).
   - **Checks:** time_to_frame_count_consistency
   - **Verdict:** FAIL
   - **Notes:** Expected equilibrium frames: (500 − 100) ns/0.02 ns = 20,!000. Reported 66,!771. Implied total duration from reported frames: 100 ns + 66,!771 × 0.02 ns = 1435.42 ns.
2. ✔ **C02_persistence_threshold_frames_to_ns** (p.3 §2.1 (Methods) and p.5 §3.1.1)
   - **Claim:** Persistence threshold for aggregates: 250 frames, equivalent to 5 ns, given 20.0 ps per frame.
   - **Checks:** unit_conversion
   - **Verdict:** PASS
   - **Notes:** 250 frames ×20.0 ps/frame = 5000 ps = 5 ns.
3. ✖ **C03_total_heavy_atoms_sum** (p.5 §3.1)
   - **Claim:** Each KYFIL peptide contains 48 heavy atoms, summing to 1225 heavy atoms for the entire system of 30 peptides.
   - **Checks:** parts_to_total
   - **Verdict:** FAIL
   - **Notes:** 30 × 48 = 1440 heavy atoms, not 1225.
4. ✔ **C04_residues_per_graph_from_peptide_size** (p.6 §3.2)
   - **Claim:** Average number of residues per residue-level graph (144.24) is consistent with average parent aggregate size (≈ 28.89 peptides ×5 residues/peptide ≈ 144.45 residues).
   - **Checks:** derived_quantity_consistency
   - **Verdict:** PASS
   - **Notes:** Computed 28.89 × 5 = 144.45. Difference vs 144.24 is 0.21 residues (within tolerance).
5. ✔ **C05_peptide_density_implied_avg_degree** (p.6 §3.1.2 (text immediately after Table 1))
   - **Claim:** With mean peptide-level density 0.1485 for aggregate size ≈ 29, each peptide is on average in contact with approximately 0.1485 × (29 − 1) ≈ 4.16 other peptides.
   - **Checks:** graph_density_to_avg_degree

- **Verdict:** PASS
- **Notes:** $0.1485 \times (29 - 1) = 4.158$, consistent with ≈ 4.16.

6. ✔ **C06_residue_density_implied_avg_degree** (p.7 §3.2 (density interpretation) + Table 2)
   - **Claim:** Residue-level density ρ_{res} averaged 0.0272; for ≈ 144 nodes, each residue is in contact with approximately $0.0272 \times (144 - 1) \approx 3.89$ other residues.
   - **Checks:** graph_density_to_avg_degree
   - **Verdict:** PASS
   - **Notes:** $0.0272 \times (144 - 1) = 3.8896$, consistent with ≈ 3.89.
7. ✔ **C07_degree_centrality_equals_density_claim** (p.6 Table 2)
   - **Claim:** Table 2 reports Graph Density (ρ_{res}) mean= 0.0272 std= 0.0103 and Avg. Degree Centrality (res) mean= 0.0272 std= 0.0103 (identical).
   - **Checks:** internal_table_value_equality
   - **Verdict:** PASS
   - **Notes:** Printed values match exactly (means and stds).

Limitations

- Only parsed PDF text was available; no access to the underlying trajectory/topology files or computed time series/graphs, so most numerical results that depend on simulation data cannot be recomputed.
- Figures are not used for extracting numeric values (no plot-pixel reading); only numbers explicitly stated in text/tables are considered.
- Several statements use approximations (e.g., "≈ 500 ns", "≈ 29 peptides"); checks involving these require tolerances and may not conclusively indicate an error without exact endpoints/frame indexing details.