Tier 1: Exploratory | 3D Chromatin Topology & Spectral Graph Calibration | Signal Rating: 2/5 Beakers | Nov 2, 2026 | 11 mins

3D Chromatin Graph Laplacian Connectivity, TAD Insulation & A/B Compartment Eigenvector Calibration: Native Rust vs. SciPy Across 12 Hi-C & Micro-C Cohorts

Head-to-head calibration of combinatorial and normalized graph Laplacian Fiedler algebraic connectivity (lambda , lambda), Von Neumann spectral entropy (S_VN), off-diagonal window TAD insulation (IS_i), and Lieberman-Aiden O/E A/B compartment eigenvectors (E) against SciPy v1.18.1 across 6 Development and 6 Untouched Reserve 3D chromatin cohorts

Three-dimensional genome folding organizes chromosomes into megabase-scale active (A) and inactive (B) compartments, CTCF/cohesin-insulated topologically associating domains (TADs), and multi-way super-enhancer transcriptional hubs. Here we benchmark our native Rust 3D chromatin contact graph solver against SciPy v1.18.1 (scipy.sparse.csgraph.laplacian and scipy.linalg.eigh) and NumPy v2.5.4 (numpy.corrcoef) across 12 cohorts (6 Development and 6 Untouched Reserve, spanning 119.36 Mb). The native solver reproduces combinatorial and normalized Fiedler algebraic connectivity (lambda 2 , lambdã 2), Von Neumann spectral entropy (S VN), and TAD insulation minima (IS min) to 0.0000 mean absolute error (r = 1.0000) with 100.0% regime concordance and a 10.94x execution speedup.

1. Biological & Mathematical Framework: Spectral Graph Theory on 3D Chromatin Contact Maps

High-throughput chromosome conformation capture assays (Hi-C, Micro-C, and Pore-C) represent spatial genome folding as a real symmetric N x N interaction frequency matrix W , where entry W i,j >= 0 records the physical contact weight between genomic bin i and bin j . Treating W as a weighted undirected graph enables four complementary mathematical diagnostics of three-dimensional regulatory architecture:

* Stage 1 -- Combinatorial & Symmetric Normalized Graph Laplacians (L and L sym): Given off-diagonal contact weights W i,j (i ≠ j) and vertex degrees d i = Sigma j ≠ i W i,j with D = diag(d 1 , …, d N), the combinatorial Laplacian is L = D − W and the symmetric normalized Laplacian is L sym = I − D −1/2 W D −1/2 .

* Stage 2 -- Cyclic Jacobi Eigendecomposition for Fiedler Connectivity (lambda 2 , lambdã 2) & Von Neumann Entropy (S VN): Orthogonal Jacobi rotations diagonalize L (0 = lambda 1 <= lambda 2 <= … <= lambda N) and L sym (0 = lambdã 1 <= lambdã 2 <= … <= lambdã N <= 2). By Cheeger's inequality, the normalized Fiedler value lambdã 2 bounds the minimum conductance bottleneck across the locus : sharp CTCF-insulated dual-TAD boundaries drive lambdã 2 into the 0.036-0.050 range, boundary deletions that fuse adjacent TADs elevate lambdã 2 into the 0.34-0.36 range (>8x increase), and multi-way super-enhancer cliques push lambdã 2 > 0.82. Von Neumann graph spectral entropy quantifies eigenvalue dispersion across the trace-normalized spectrum ρ i = lambda i / Tr(L): S VN (ρ) = − Sigma i =2.. N ρ i

log 2 (ρ i)

* Stage 3 -- Off-Diagonal Window TAD Insulation Score (IS i) & Lieberman-Aiden O/E Compartment Eigenvector (e 1): Following Crane et al. (2015), a w x w off-diagonal contact sum D i (w) = Sigma a = i − w .. i −1 Sigma b = i +1.. i + w W a,b is evaluated across interior bins and normalized to the locus mean: IS i = log 2 (D i (w) / ⟨ D (w)⟩) Deep local minima (IS min <= −3.70 bits) identify intact CTCF /cohesin insulator boundaries, whereas pathogenic boundary deletions attenuate the dip (IS min ~ −0.77 to −0.81 bits). Following Lieberman-Aiden et al. (2009), dividing each contact by its genomic-distance mean yields the Observed/Expected matrix M i,j = W i,j / E (| i − j |), whose Pearson correlation matrix C i,j = corr(M i , M j) has principal eigenvector e 1 partitioning euchromatic (A, e 1, i > 0) and heterochromatic (B, e 1, i < 0) compartments with Newman-Girvan modularity Q .

* Stage 4 -- Intrinsically Disordered Protein (IDR) Sticker-Spacer Grammar (c sat): Coupling 3D contact topology with co-activator IDR amino-acid composition (aromatic/ arginine sticker fraction f sticker vs. glycine/ serine /proline spacer fraction f spacer) estimates the liquid-liquid phase separation saturation concentration c sat (uM), distinguishing multivalent transcriptional condensate hubs (lambdã 2 >= 0.80, c sat < 5.0 uM) from structural CTCF/cohesin insulators and featureless polymer controls.

Signal Rating Assessment (2 Technical Validation -- Tier 1: Exploratory): This study is classified as a Tier 1: Exploratory head-to-head tool calibration benchmark and earns a 2 Technical Validation rating. It evaluates 12 multi-locus 3D chromatin contact cohorts (6 Development + 6 Untouched Reserve, spanning 119.36 Mb across 192 genomic bins) and 2 deliberate 0 polymer/cohesin-depletion negative controls, establishing exact numerical parity (0.0000 MAE) with SciPy v1.18.1 prior to downstream Tier 2 and Tier 3 regulatory genomics syntheses.

2. Validation Dataset & Cohort Provenance (VAL-SET-016-CHROM-LAPLACIAN-TAD-12COHORT)

To prevent threshold overfitting, parameters were frozen on six Development cohorts (DEV-01 through DEV-06) and evaluated without modification on six Untouched Reserve cohorts (RES-01 through RES-06), including two negative controls (isotropic s −1 polymer decay DEV-06 and acute cohesin-depleted RAD21-mAID loop-free chromatin RES-06):

| Cohort ID | | Validation Split | | Genomic Locus & Architectural Context | | Reference Coordinates & Primary Anchor | | Bins | | Bin Size (kb) | | Span (kb) | | Expected 3D Chromatin Regime |

| DEV-01 | | Development | | Human chr1p36.33 AGRN Super-Enhancer Condensate Hub | | GRCh38 chr1:1,015,000-1,095,000 (4DNFI2R1W3YW Micro-C) | | 16 | | 5 | | 80 | | Phase-Separated Super-Enhancer Condensate Hub |

| DEV-02 | | Development | | Human chr12p13.33 CACNA1C Intron-30 VNTR eRNA Hub | | GRCh38 chr12:2,480,000-2,560,000 (Song et al. 2018) | | 16 | | 5 | | 80 | | Phase-Separated Super-Enhancer Condensate Hub |

| DEV-03 | | Development | | Human chr2q35-q36 EPHA4/PAX3 Canonical Dual-TAD

Boundary | | GRCh38 chr2:221,200,000-222,800,000 (Lupiáñez et al. 2015) | | 16 | | 100 | | 1600 | | CTCF-Insulated Dual-TAD Boundary |

| DEV-04 | | Development | | Human chr2q35-q36 EPHA4/PAX3 Brachydactyly Del-B Boundary Loss | | GRCh38 chr2:221,200,000-222,800,000 Del-B (Lupiáñez et al. 2015) | | 16 | | 100 | | 1600 | | TAD-Boundary Fusion / Enhancer Hijacking |

| DEV-05 | | Development | | Human chr14 Checkerboard Euchromatin/Heterochromatin Plaid | | GRCh38 chr14:20,000,000-100,000,000 (Lieberman-Aiden et al. 2009) | | 16 | | 5000 | | 80000 | | Checkerboard A/B Compartment Plaid Architecture |

| DEV-06 | | Development | | Negative Control: Isotropic s −1 Fractal-Globule Polymer Decay | | CALIBRATION-CONTROL-ISOTROPIC-POLYMER-S1 | | 16 | | 50 | | 800 | | 0-Beaker Negative Control (Featureless Polymer Decay) |

| RES-01 | | Untouched Reserve | | Human chr8q24.21 MYC/PVT1/CCAT1-L Super-Enhancer Clique | | GRCh38 chr8:127,700,000-128,500,000 (Sabari et al. 2018) | | 16 | | 50 | | 800 | | Phase-Separated Super-Enhancer Condensate Hub |

| RES-02 | | Untouched Reserve | | Human chr17q24.3 SOX9/KCNJ2 Canonical Dual-TAD Boundary | | GRCh38 chr17:69,900,000-71,500,000 (Franke et al. 2016) | | 16 | | 100 | | 1600 | | CTCF-Insulated Dual-TAD Boundary |

| RES-03 | | Untouched Reserve | | Human chr17q24.3 SOX9/KCNJ2 Cook Syndrome Dup-C Neo-TAD Fusion | | GRCh38 chr17:69,900,000-71,500,000 Dup-C (Franke et al. 2016) | | 16 | | 100 | | 1600 | | TAD-Boundary Fusion / Enhancer Hijacking |

| RES-04 | | Untouched Reserve | | Vertebrate chr2q31.1 HOXD Bimodal Telomeric/Centromeric Switch | | GRCh38 chr2:175,900,000-177,500,000 (Andrey et al. 2013) | | 16 | | 100 | | 1600 | | CTCF-Insulated Dual-TAD Boundary |

| RES-05 | | Untouched Reserve | | Human chr19 Bipartite Pericentromeric B vs. Telomeric A Map | | GRCh38 chr19:24,000,000-56,000,000 (Rao et al. 2014) | | 16 | | 2000 | | 32000 | | Checkerboard A/B Compartment Plaid Architecture |

| RES-06 | | Untouched Reserve | | Negative Control: Cohesin-Depleted (RAD21-mAID +Auxin) Polymer | | HCT116 RAD21-mAID Loop-Free Control (Rao et al. 2017) | | 16 | | 50 | | 800 | | 0-Beaker Negative Control (Featureless Polymer Decay) |

Generated by: Native 3D Chromatin Graph Laplacian, TAD Insulation & RNA Phase-Separation Scaffolder Engine

3. Quantitative Head-to-Head Calibration Results: Native Rust vs. SciPy v1.18.1

Across all 12 cohorts, the native Rust cyclic Jacobi spectral solver and off-diagonal window insulation calculator match scipy.sparse.csgraph.laplacian , scipy.linalg.eigh , and numpy.corrcoef to four decimal places (0.0000 mean absolute error across every spectral and insulation metric):

| Cohort | | Split | | Native lambda 2 | | SciPy lambda 2 | | Native lambdã 2 | | SciPy lambdã 2 | | Native S VN (bits) | | SciPy S VN (bits) | | Native IS min (bits) | | SciPy IS min (bits) | | Boundary Bin | | Modularity Q | | E 1 r | | IDR c sat (uM) | | Beakers |

| DEV-01 | | Development | | 189.2020 | | 189.2020 | | 0.8536 | | 0.8536 | | 3.8902 | | 3.8902 | | -0.1458 | | -0.1458 | | 7 | | 0.0635 | | 1.0000 | | 2.4310 | | 2 |

| DEV-02 | | Development | | 164.5781 | | 164.5781 | | 0.8291 | | 0.8291 | | 3.8896 | | 3.8896 | | -0.1369 | | -0.1369 | | 7 | | 0.0635 | | 1.0000 | | 2.0998 | | 2 |

| DEV-03 | | Development | | 5.9272 | | 5.9272 | | 0.0426 | | 0.0426 | | 3.8080 | | 3.8080 | | -3.9348 | | -3.9348 | | 7 | | 0.5414 | | 1.0000 | | 9.7087 | | 2 |

| DEV-04 | | Development | | 52.6681 | | 52.6681 | | 0.3482 | | 0.3482 | | 3.8607 | | 3.8607 | | -0.8068 | | -0.8068 | | 7 | | 0.3750 | | 1.0000 | | 2.3332 | | 2 |

| DEV-05 | | Development | | 21.2624 | | 21.2624 | | 0.1394 | | 0.1394 | | 3.7713 | | 3.7713 | | -1.4726 | | -1.4726 | | 3 | | 0.4915 | | 1.0000 | | 3.4808 | | 2 |

| DEV-06 | | Development | | 40.0003 | | 40.0003 | | 0.4611 | | 0.4611 | | 3.8519 | | 3.8519 | | 0.0000 | | 0.0000 | | 3 | | 0.0716 | | 0.5781 | | 27.8545 | | 0 |

| RES-01 | | Reserve | | 197.8603 | | 197.8603 | | 0.8610 | | 0.8610 | | 3.8904 | | 3.8904 | | -0.1474 | | -0.1474 | | 7 | | 0.0636 | | 1.0000 | | 2.3879 | | 2 |

| RES-02 | | Reserve | | 5.1924 | | 5.1924 | | 0.0367 | | 0.0367 | | 3.8068 | | 3.8068 | | -4.1616 | | -4.1616 | | 7 | | 0.5444 | | 1.0000 | | 9.7087 | | 2 |

| RES-03 | | Reserve | | 55.1707 | | 55.1707 | | 0.3587 | | 0.3587 | | 3.8620 | | 3.8620 | | -0.7672 | | -0.7672 | | 7 | | 0.3690 | | 1.0000 | | 2.3332 | | 2 |

| RES-04 | | Reserve | | 6.6808 | | 6.6808 | | 0.0497 | | 0.0497 | | 3.8056 | | 3.8056 | | -3.7399 | | -3.7399 | | 6 | | 0.5167 | | 1.0000 | | 9.7087 | | 2 |

| RES-05 | | Reserve | | 12.8576 | | 12.8576 | | 0.0628 | | 0.0628 | | 3.7831 | | 3.7831 | | -3.0270 | | -3.0270 | | 7 | | 0.5315 | | 1.0000 | | 3.4808 | | 2 |

| RES-06 | | Reserve | | 46.7833 | | 46.7833 | | 0.5025 | | 0.5025 | | 3.8602 | | 3.8602 | | 0.0000 | | 0.0000 | | 3 | | 0.1517 | | 0.3233 | | 27.8545 | | 0 |

Generated by: CRV Science Labs Native Bioinformatics Suite

4. Split-By-Split Error Audit & Architectural Regime Separation

Evaluating the Development and Untouched Reserve splits independently confirms that zero numerical drift occurs when moving from parameter-frozen development loci to unseen reserve loci (such as the MYC/PVT1 8q24 condensate hub, SOX9/KCNJ2 Cook syndrome boundary duplication, and HOXD bimodal switch):

| Calibration Partition | | Cohorts | | Mean |Deltalambda 2 | | | Mean |Deltalambdã 2 | | | Mean |Delta S VN | (bits) | | Mean |Delta IS min | (bits) | | Positive-Cohort E 1 r | | Boundary Match (%) | | Regime Match (%) | | Speedup vs. SciPy |

| Development Split (DEV-01..DEV-06) | | 6 | | 0.0000 | | 0.0000 | | 0.0000 | | 0.0000 | | 1.0000 | | 100.0 | | 100.0 | | 10.85 |

| Untouched Reserve Split (RES-01..RES-06) | | 6 | | 0.0000 | | 0.0000 | | 0.0000 | | 0.0000 | | 1.0000 | | 100.0 | | 100.0 | | 11.03 |

| Combined Benchmark (VAL-SET-016) | | 12 | | 0.0000 | | 0.0000 | | 0.0000 | | 0.0000 | | 1.0000 | | 100.0 | | 100.0 | | 10.94 |

Generated by: CRV Science Labs Native Bioinformatics Suite

5. Focal 3D Chromatin Insulation Profiles, Compartment Eigenvectors & Structural Hierarchy

Comparing the wild-type EPHA4/PAX3 limb enhancer locus (DEV-03) against its pathogenic brachydactyly boundary deletion (DEV-04 , Lupiáñez et al. 2015) and the AGRN super-enhancer condensate hub (DEV-01) illustrates how spectral connectivity and off-diagonal window insulation capture distinct physical folding regimes. In wild-type DEV-03 , convergent CTCF motifs at the TAD-1/TAD-2 junction (bins 8-9) produce a sharp insulation trough (IS min = −3.9348 bits) and restrict normalized Fiedler connectivity to lambdã 2 = 0.0426. Deleting the boundary in DEV-04 collapses the insulation barrier by 3.13 bits (IS min = −0.8068 bits) and increases normalized Fiedler connectivity 8.17x to lambdã 2 = 0.3482, consistent with ectopic enhancer- promoter contacts across the former boundary:

Generated by: Native 3D Chromatin Graph Laplacian, TAD Insulation & RNA Phase-Separation Scaffolder Engine

Clustering the 12 cohorts by their standardized spectral-topological feature vectors (lambdã 2 , S VN , IS min , Q , ln c sat) via Neighbor-Joining recovers the five distinct 3D chromatin architectural regimes with 100% clade separation:

Generated by: Native K2P / TN93 Distance, Neighbor-Joining & ABGD Systematics Engine * Native 3D Chromatin Graph Laplacian, TAD Insulation & RNA Phase-Separation Scaffolder Engine

Generated by: Native K2P / TN93 Distance, Neighbor-Joining & ABGD Systematics Engine

Discussion & Key Takeaways

* Exact Spectral & Topological Concordance with SciPy v1.18.1 : Across all 12 Development and Untouched Reserve cohorts in VAL-SET-016 , our native Rust cyclic Jacobi eigensolver and off-diagonal window TAD insulation calculator achieve 0.0000 mean absolute error on combinatorial Fiedler connectivity (lambda 2), normalized Fiedler connectivity (lambdã 2), Von Neumann graph spectral entropy (S VN), and minimum TAD insulation score (IS min), while executing 10.94x faster than SciPy v1.18.1 .

* Complementary Diagnostic Power of lambdã 2 and IS min : Off-diagonal window insulation (IS min) pinpoints localized CTCF/cohesin boundary dips, whereas normalized Fiedler algebraic connectivity (lambdã 2) measures global graph conductance across the entire contact matrix. Combining both metrics cleanly separates intact CTCF-insulated dual-TAD boundaries (lambdã 2 < 0.055, IS min <= −3.74 bits), structural variant boundary fusions (lambdã 2 in [0.348, 0.359], IS min in [−0.81, −0.77] bits), checkerboard A/B macro-compartments (Q >= 0.49), and multi-way super-enhancer hubs (lambdã 2 >= 0.829).

* Downstream Bioinformatics Scope & Limitations: These computational graph-Laplacian and sticker-spacer metrics provide rapid candidate hypotheses for interpreting non-coding structural variants (such as TAD-boundary deletions or VNTR expansions)

in clinical genomics and comparative chromosome evolution ; experimental validation via Capture Hi-C, Micro-C, or CRISPR boundary perturbation remains essential to establish causal regulatory effects in specific cell types.

References & Primary Sources

* [1] Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 326(5950): 289-293. https://doi.org/10.1126/science.1181369

* [2] Crane E, Bian Q, McCord RP, Lajoie BR, Wheeler BS, Ralston EJ, Uzawa S, Dekker J, Meyer BJ (2015). Condensin-driven remodelling of X chromosome topology during dosage compensation. Nature 523(7559): 240-244. https://doi.org/10.1038/nature14450

* [3] Lupianez DG, Kraft K, Heinrich V, Krawitz P, Brancati F, Klopocki E, Horn D, Kayserili H, Opitz JM, Laxova R, et al. (2015). Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. Cell 161(5): 1012-1025. https://doi.org/10.1016/j.cell.2015.04.004

* [4] Franke M, Ibrahim DM, Andrey G, Schwarzer W, Heinrich V, Schopflin R, Kraft K, Kempfer R, Jerkovic I, Chan WL, et al. (2016). Formation of new chromatin domains determines pathogenicity of genomic duplications. Nature 538(7624): 265-269. https://doi.org/10.1038/nature19800

* [5] Sabari BR, DallAgnese A, Boija A, Klein IA, Coffey EL, Shrinivas K, Abraham BJ, Hannett NM, Zamudio AV, Manteiga JC, et al. (2018). Coactivator condensation at super-enhancers links phase separation and gene control. Science 361(6400): eaar3958. https://doi.org/10.1126/science.aar3958

* [6] Rao SSP, Huang SC, Glenn St Hilaire B, Engreitz JM, Perez EM, Kieffer-Kwon KR, Sanborn AL, Johnstone SE, Bascom GD, Bochkov ID, et al. (2017). Cohesin loss eliminates all loop domains. Cell 171(2): 305-320. https://doi.org/10.1016/j.cell.2017.09.026

* [7] Virtanen P, Gommers R, Oliphant TE, Haberland M, Reddy T, Cournapeau D, Burovski E, Peterson P, Weckesser W, Bright J, et al. (2020). SciPy 1.0: fundamental algorithms for scientific computing in Python. Nature Methods 17: 261-272. https://doi.org/10.1038/s41592-019-0686-2