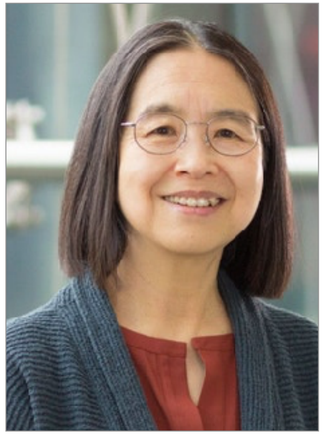
Chromosome walking with super-resolution imaging and modeling

Marc A. Marti-Renom
CNAG-CRG · ICREA

PLOS Genetics (2018) 14(12) e1007872



Guy Nir
Irene Farabella
Cynthia Perez-Estrada
with Wu Lab (HMS, Boston) & Aiden Lab (UT, Texas)

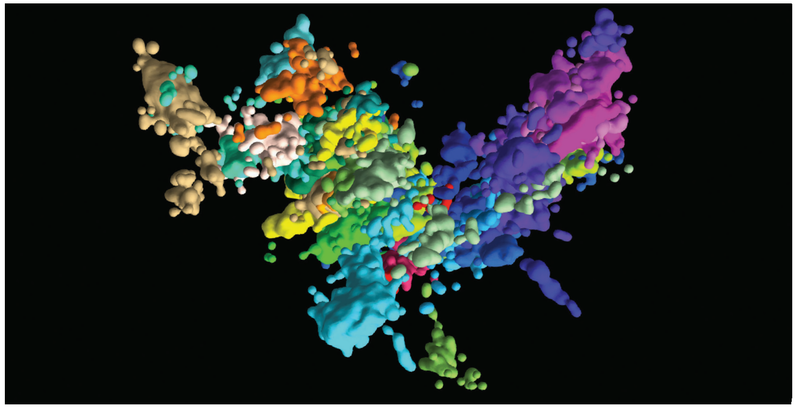
Jeffrey M. Perkel *Nature* **569**, 293-294 (2019)

TECHNOLOGY FEATURE

CHROMOSOMAL DNA COMES INTO FOCUS

Imaging techniques to probe the shape of chromatin are revealing the dynamism of the DNA–protein complex.

BOGDAN BINTU/THE XIAOWEI ZHUANG LABORATORY/ THE ALISTAIR BOETTIGER LABORATORY



This multicoloured image of chromatin was created using multiplexed fluorescence *in situ* hybridization and super-resolution microscopy.

BY JEFFREY M. PERKEL

Molecular models suggest that chromosomes assemble in an ordered, hierarchical way: DNA wraps around proteins called histones to form nucleosomes, which fold into 30-nanometre fibres, then 120-nanometre 'chromonema', and further into larger chromatin structures until they reach their most tightly coiled form — the characteristic X-shaped bodies.

Under the high-resolution microscopes of biophysicist Xiaowei Zhuang, these chromosomes resemble something from the mind of surrealist painter Salvador Dalí. Zhuang, who is at Harvard University in Cambridge, Massachusetts, is one of a growing number of researchers charting the topology of the genome to decode the relationship between chromatin structure and function. Using a highly multiplexed form of fluorescence *in situ* hybridization (FISH) in combination with super-resolution microscopy, Zhuang's team mapped several million bases of human chromosome 21 at 30 kilobase resolution, tracing their shape like a dot-to-dot puzzle[1]. The resulting multicoloured image resembles one of the melting clocks in Dalí's 1931 *The Persistence of Memory*.

But that was in just one cell. In each cell that Zhuang's team looked at, the chromosome assumed a different shape — each one a different solution to some ineffable cellular calculation. "There is very strong cell-to-cell heterogeneity," Zhuang says.

Ting Wu, a geneticist at Harvard Medical School in Boston, Massachusetts, who combined a similar super-resolution FISH approach with sequencing analysis to map a chunk of human chromosome 19 to 10 kilobase resolution in late 2018, observed similar heterogeneity[2]. The chromosomes in that study look more like space-filling protein models, and when the team overlaid markers of inactive and active chromatin, they observed distinct patterns. "We have never seen a structure of that 8.6-megabase region twice," says Wu. "The variability, which people had thought was there, and there are hints of, is truly astounding." Brian Beliveau, a genomic scientist at the University of Washington, Seattle, and a co-author of the paper, says bluntly: "Chromosomes are almost certainly like snowflakes."

A DEEPER LOOK

In biology, function derives from form. It is shape, as a result of amino-acid sequence, that determines whether a given protein acts as a structural scaffold, signalling molecule or enzyme. The same is probably true of the genome. But until recently, there was no easy way for researchers to determine that structure.

Using a sequencing-based method called Hi-C, which calculates the frequencies at which different chromosomal segments ▶

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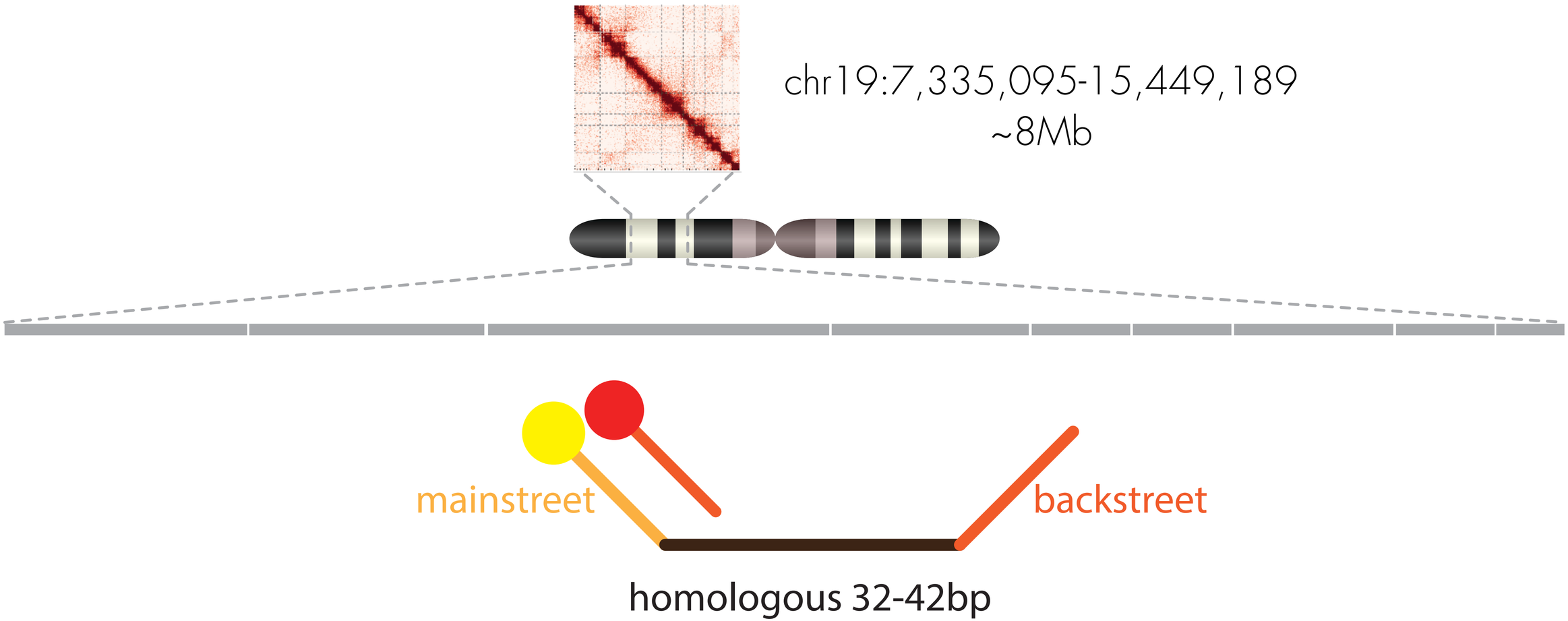
1. **Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells.** Bintu B, Mateo LJ, Su JH, Sinnott-Armstrong NA, Parker M, Kinrot S, Yamaya K, Boettiger AN, Zhuang X. Science. 2018 10 26; 362(6413) https://doi.org/10.1126/science.aau1783 PMID: 30361340
2. **Walking along chromosomes with super-resolution imaging, contact maps, and integrative modeling.** Nir G, Farabella I, Pérez Estrada C, Ebeling CG, Beliveau BJ, Sasaki HM, Lee SD, Nguyen SC, McCole RB, Chattoraj S, Erceg J, AlHaj Abed J, Martins NMC, Nguyen HQ, Hannan MA, Russell S, Durand NC, Rao SSP, Kishi JY, Soler-Vila P, Di Pierro M, Onuchic JN, Callahan SP, Schreiner JM, Stuckey JA, Yin P, Aiden EL, Marti-Renom MA, Wu CT. PLoS Genet. 2018 12; 14(12):e1007872 https://doi.org/10.1371/journal.pgen.1007872 PMID: 30586358
3. **Microscopy-Based Chromosome Conformation Capture Enables Simultaneous Visualization of Genome Organization and Transcription in Intact Organisms.** Cardozo Gizzi AM, Cattoni DI, Fiche JB, Espinola SM, Gurgo J, Messina O, Houbron C, Ogiyama Y, Papadopoulos GL, Cavalli G, Lagha M, Nollmann M. Mol Cell. 2019 Feb 12; https://doi.org/10.1016/j.molcel.2019.01.011 PMID: 30795893
4. **Visualizing DNA folding and RNA in embryos at single-cell resolution.** Mateo LJ, Murphy SE, Hafner A, Cinquini IS, Walker CA, Boettiger AN. Nature. 2019 Mar 18; https://doi.org/10.1038/s41586-019-1035-4 PMID: 30886393

High-resolution imaging

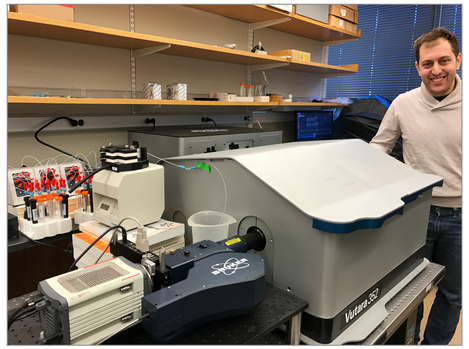
Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



Beliveau et al. Nat. Comm. 2015

High-resolution imaging

Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



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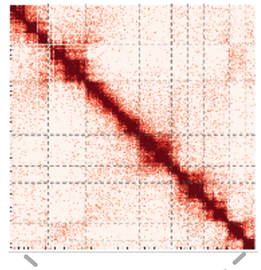
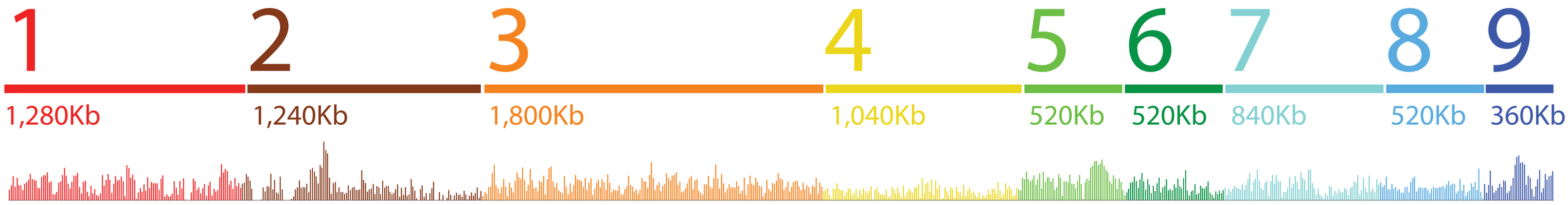
Bodgan Bintu Harvard
Carl Ebeling Bruker
Jeff Stuckey Bruker
John Schreiner Zero Epsilon
Steve Callahan Zero Epsilon

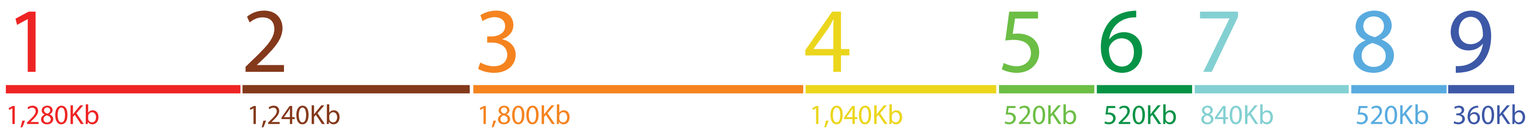


chr19:7,335,095-15,449,189
~8Mb



High-resolution imaging

Tracing chr19:7,335,095-15,449,189 ~8Mb

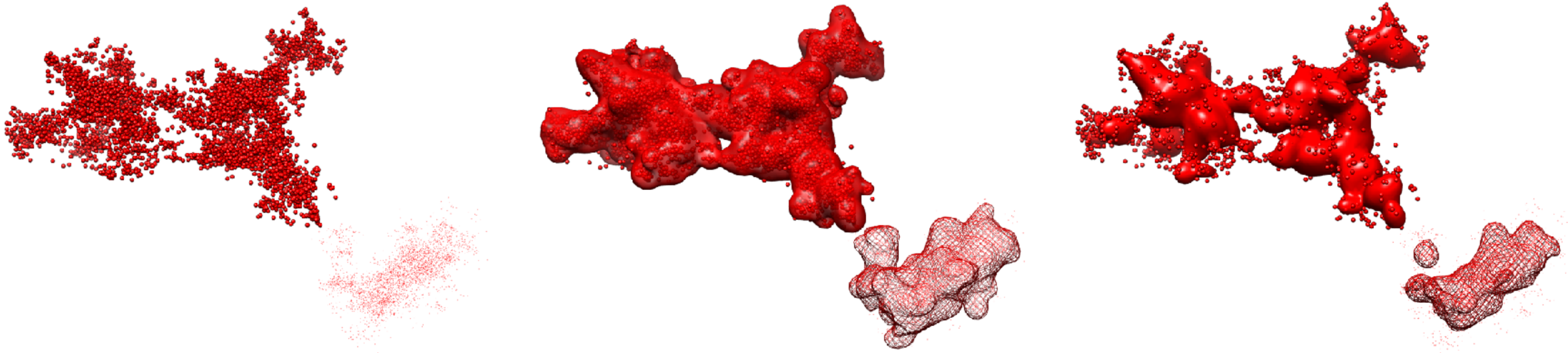


Cell-02

High-resolution imaging

XYZ points convolution into a density map

$$\rho(x,y,z) = \sum_N \frac{Z_N}{\left(\sigma\sqrt{2\pi}\right)^3} e^{-\frac{(x-x_n)^2+(y-y_n)^2+(z-z_n)^2}{2\sigma^2}}$$

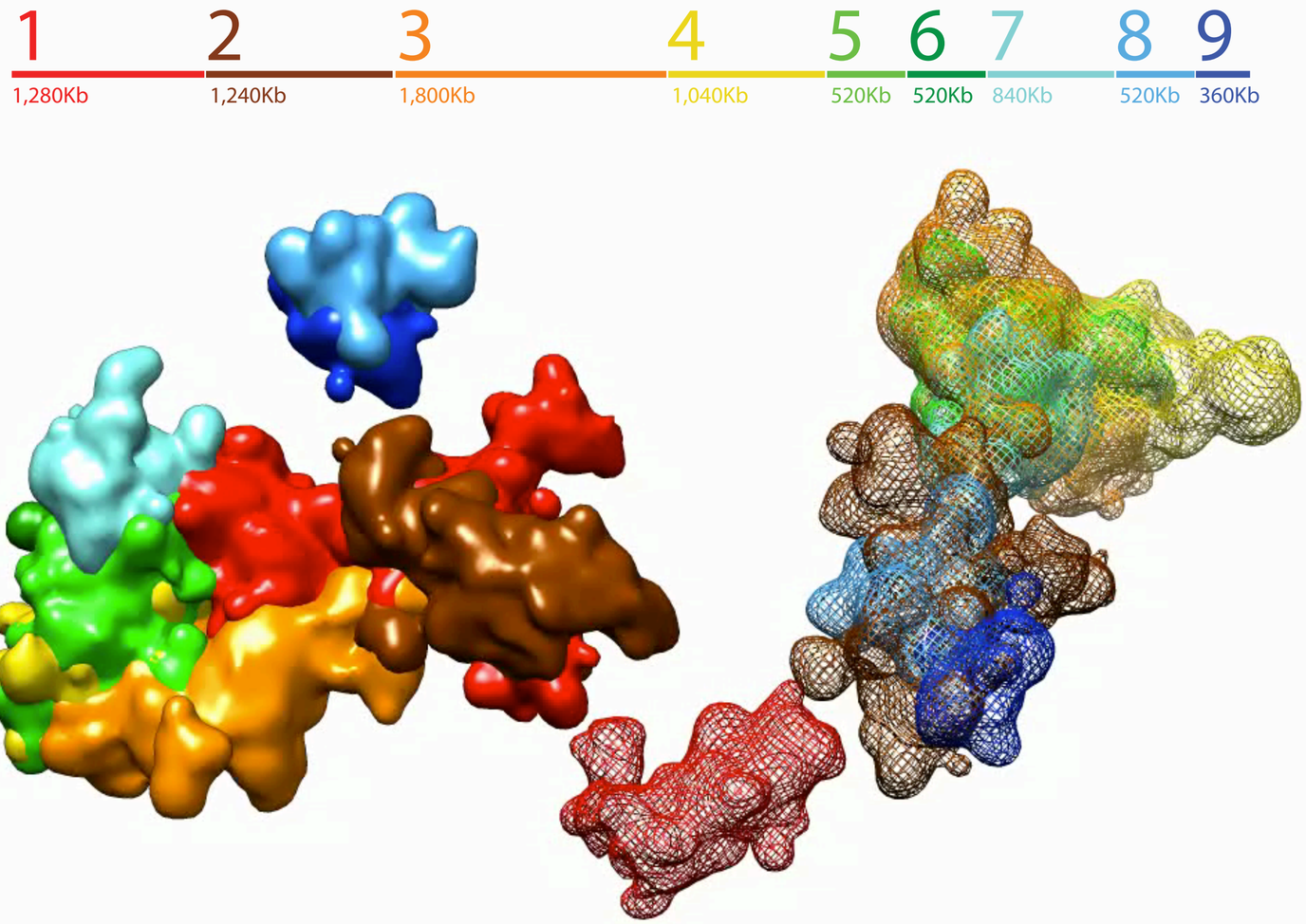


Cell-02 · Segment 1

Farabella et al, J Appl Crystallogr. 2015

Density maps

Cell-02 · Density map @ 50nm

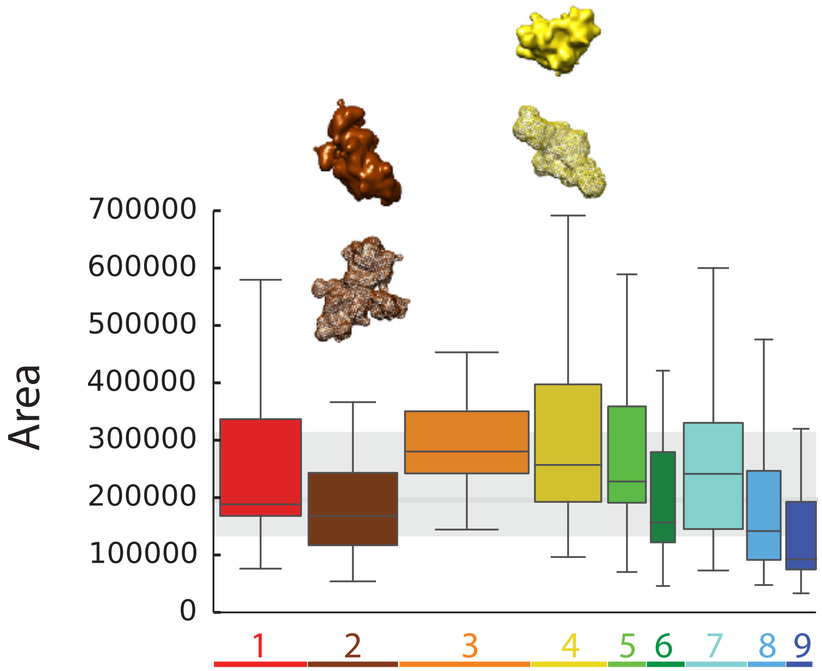


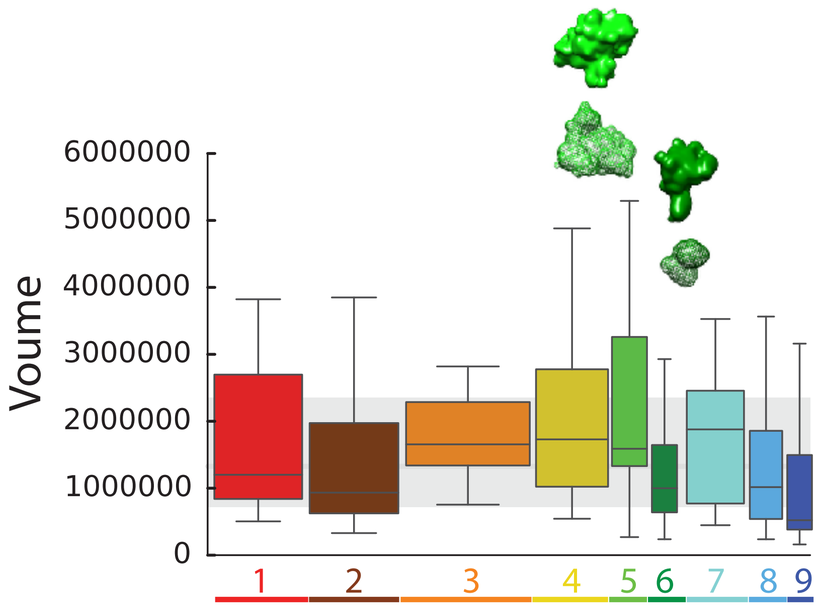
Area (nm^2)
Volume (nm^3)
Sphericity
Overlap (%)
Distance (nm)

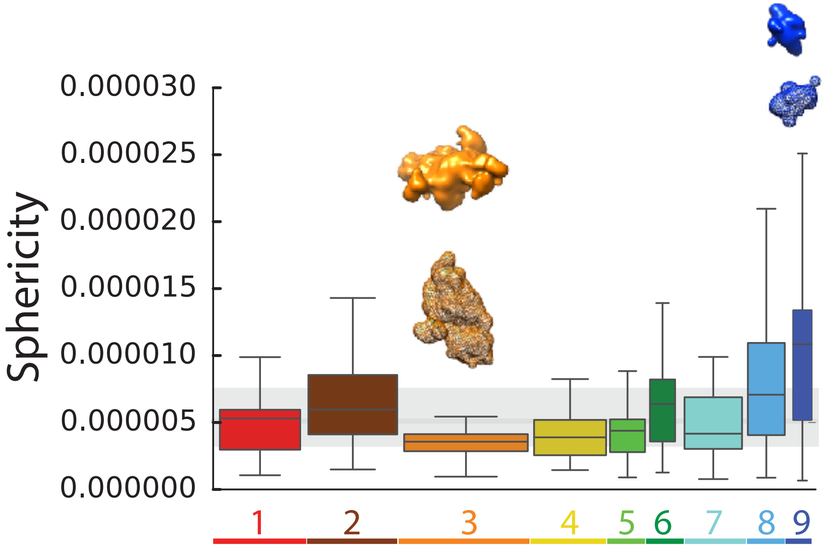
Farabella et al, J Appl Crystallogr. 2015

Structural features

Area, Volume and Sphericity of 19 cells each with 2 homologous resolved



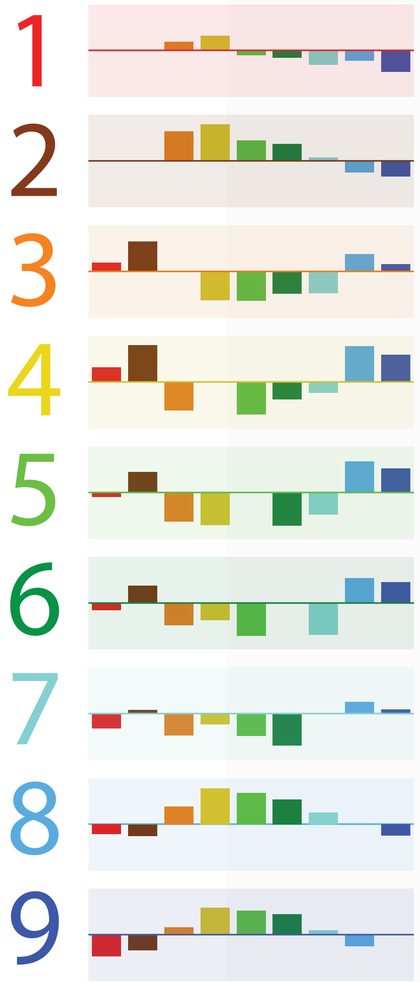


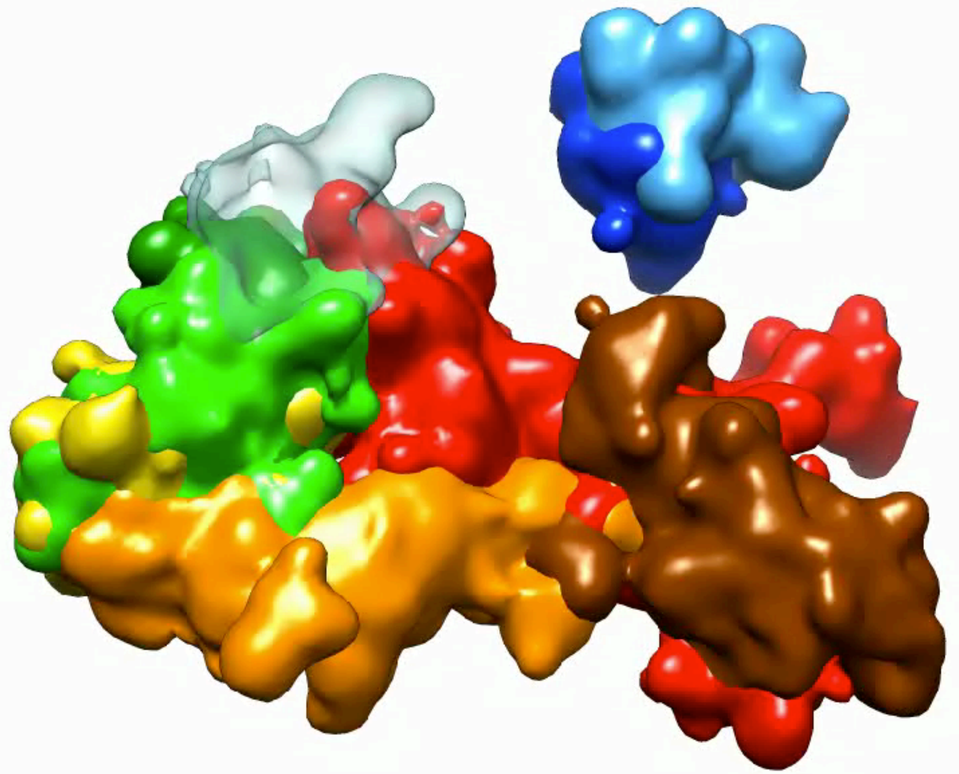


Spatial arrangement

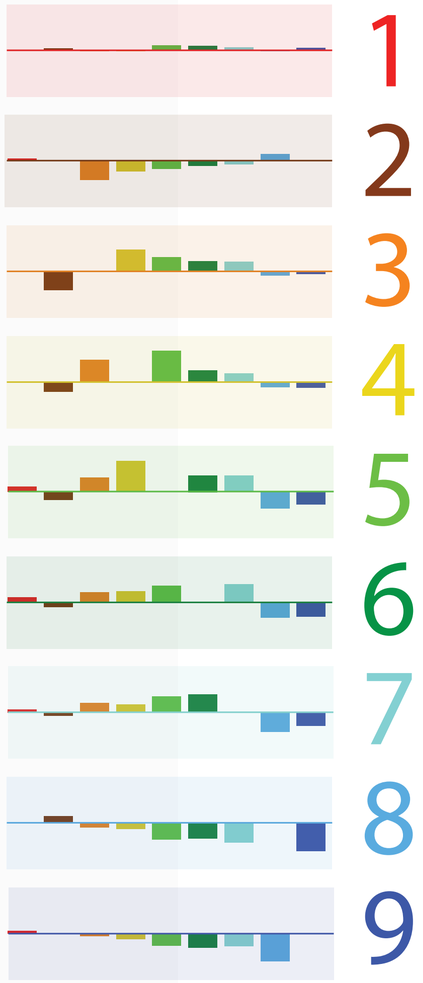
Distance and overlap of 19 cells each with 2 homologous resolved

Diff. distance



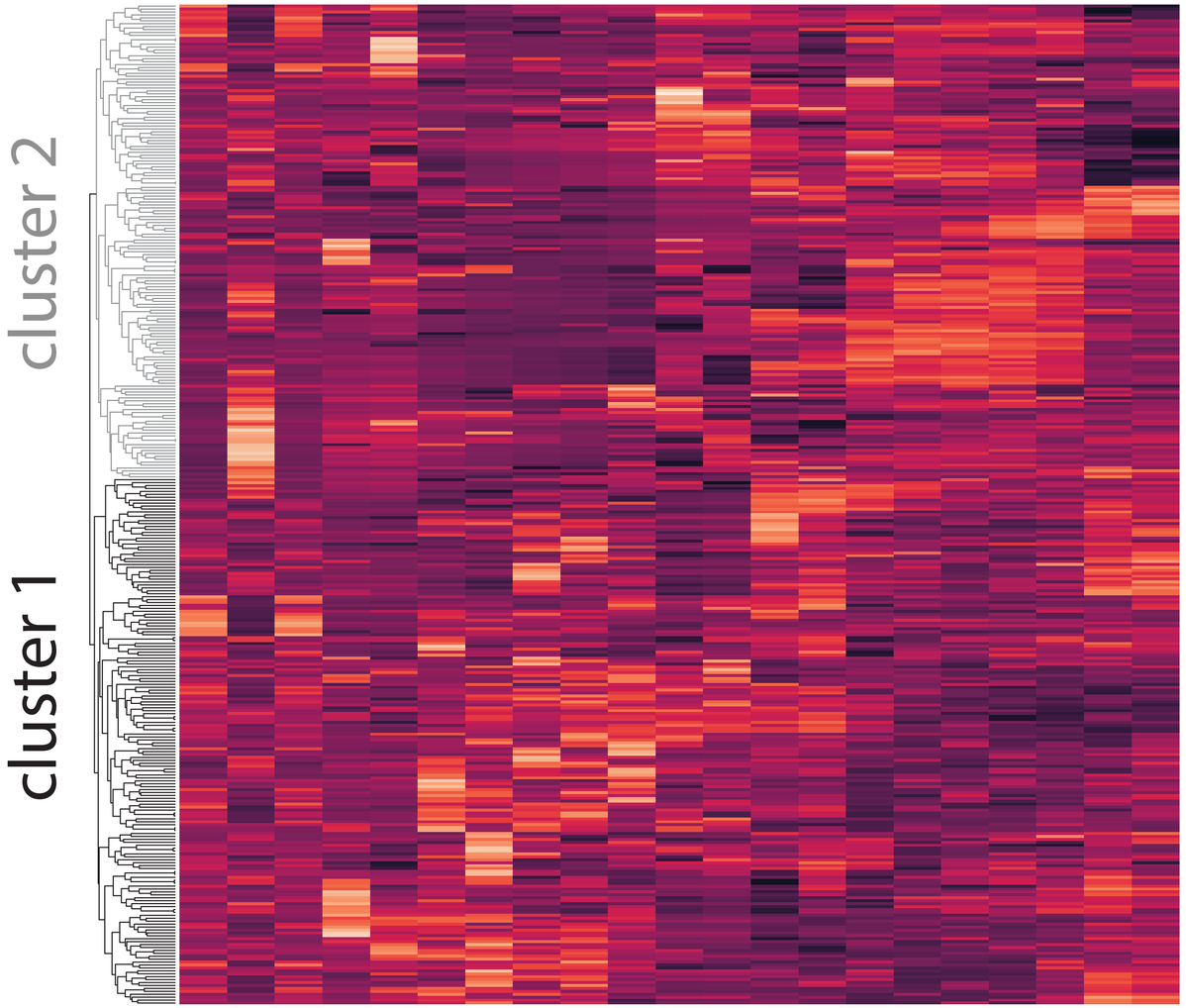


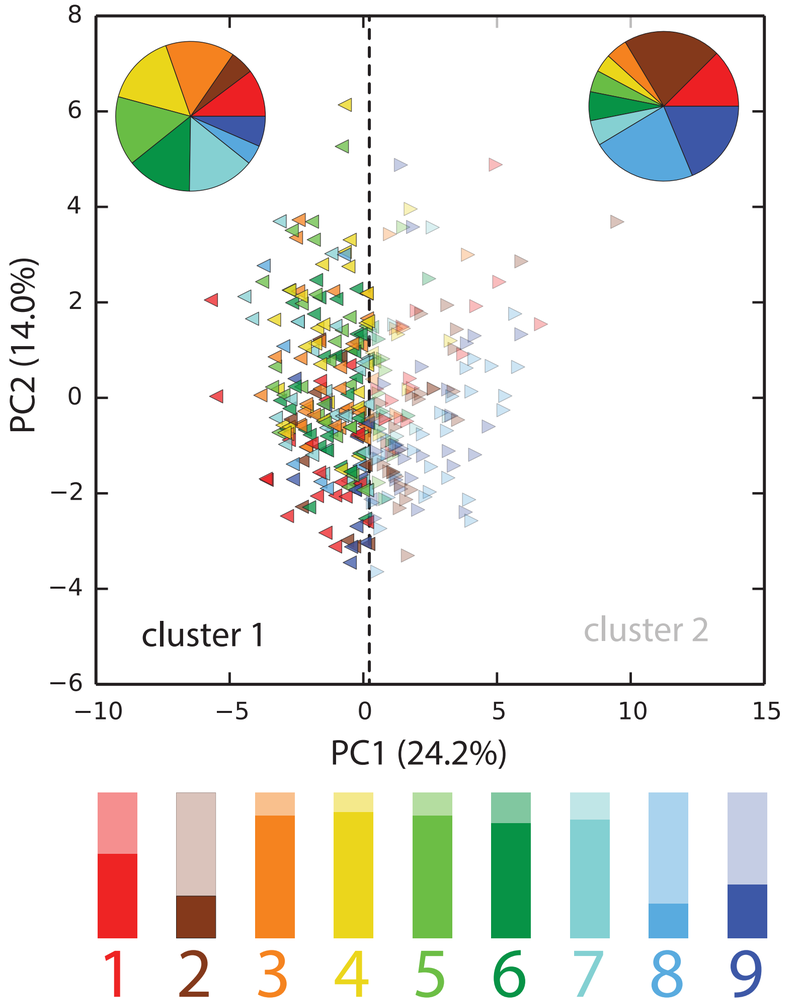
Diff. overlap



Structural clustering

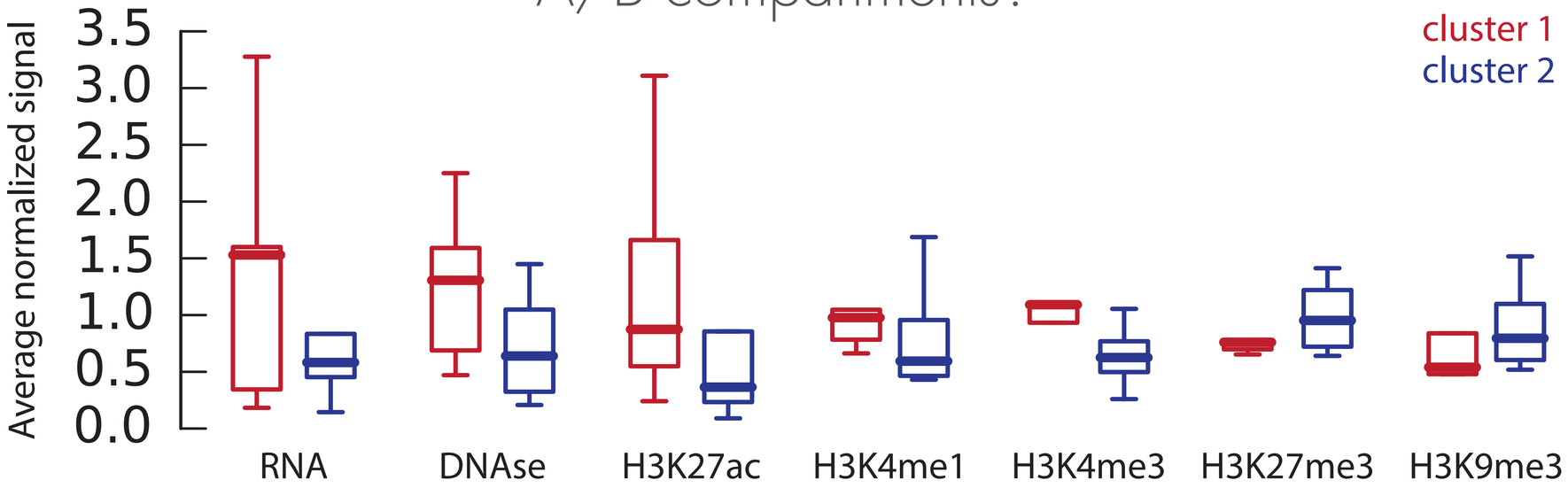
19 cells each with 2 homologous and 9 segments each (342)

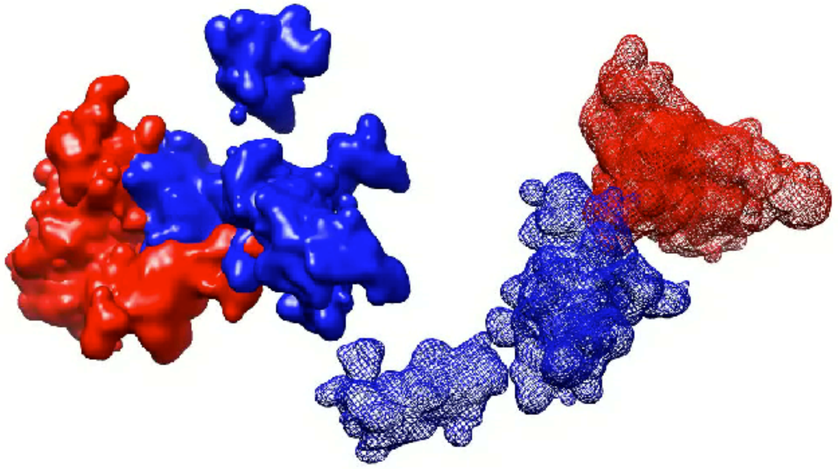


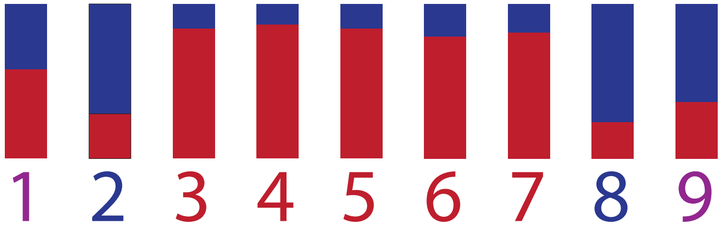


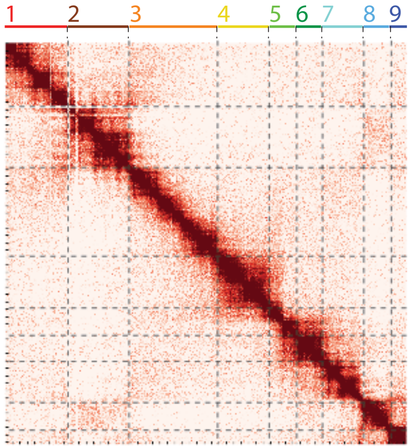
Cluster properties

A/B compartments?





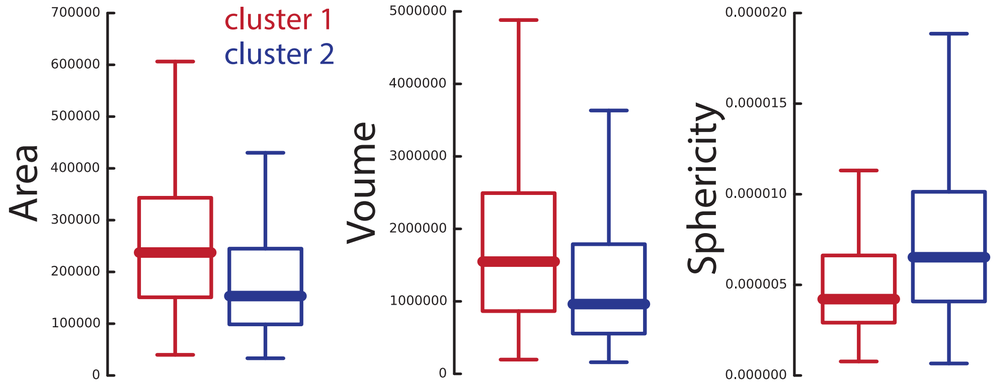


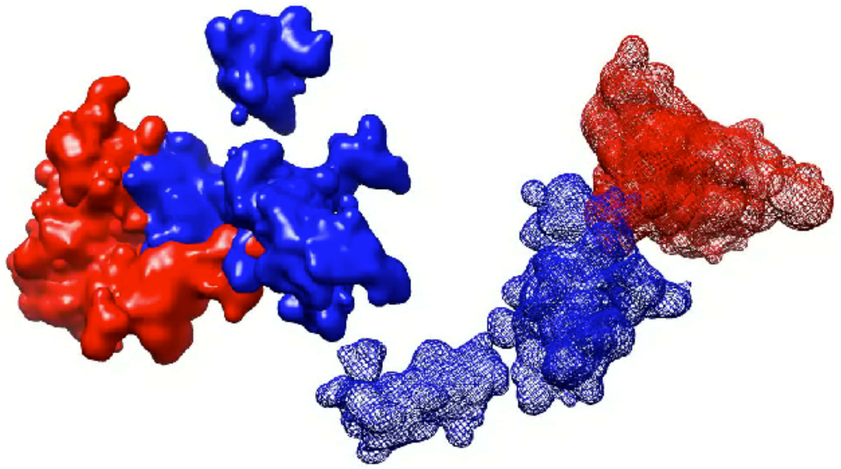


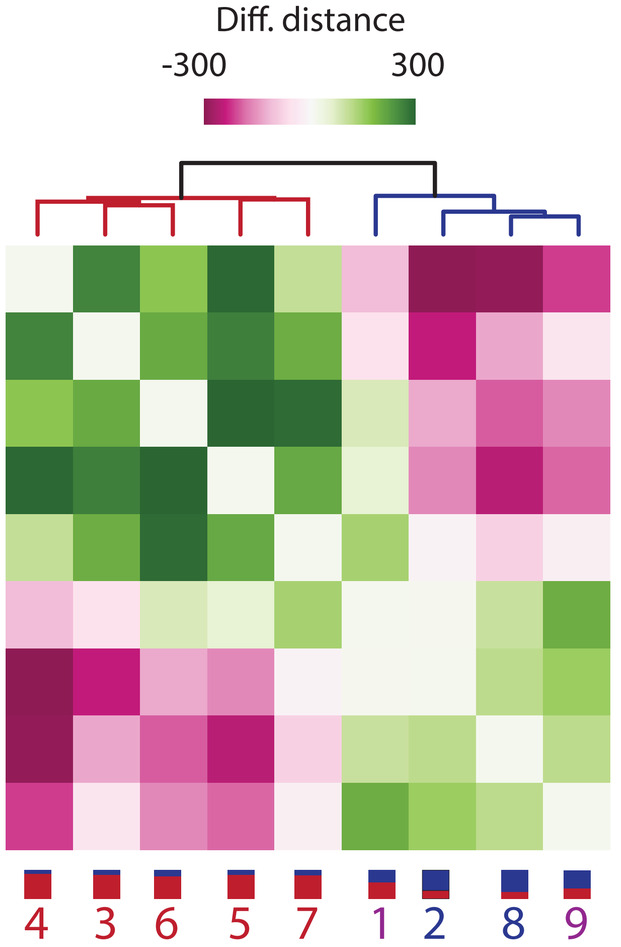
PGP1 ChIP-seq and Hi-C data from ENCODE and Lieberman-Aiden Lab, respectively

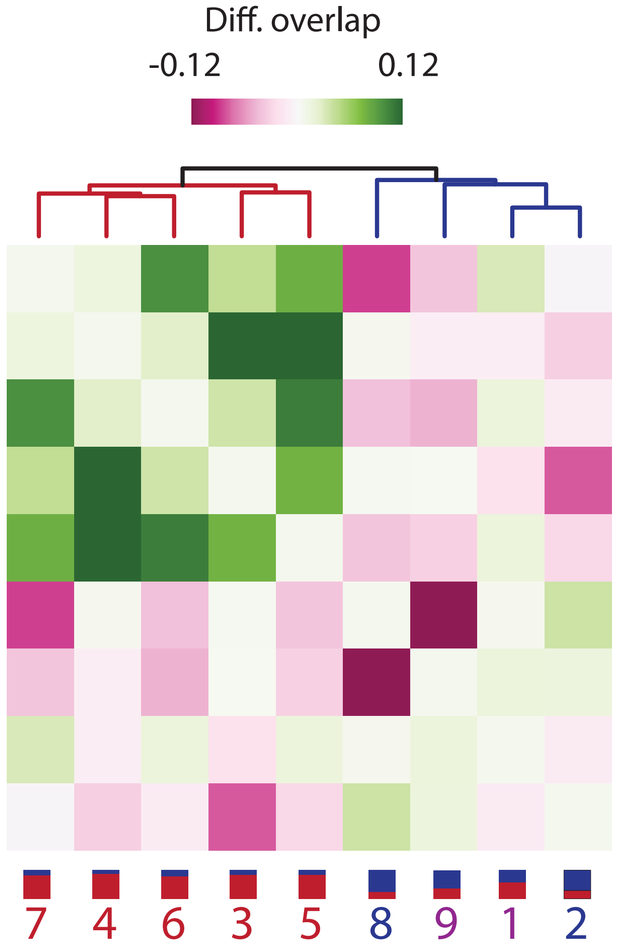
Cluster properties

A/B compartment properties



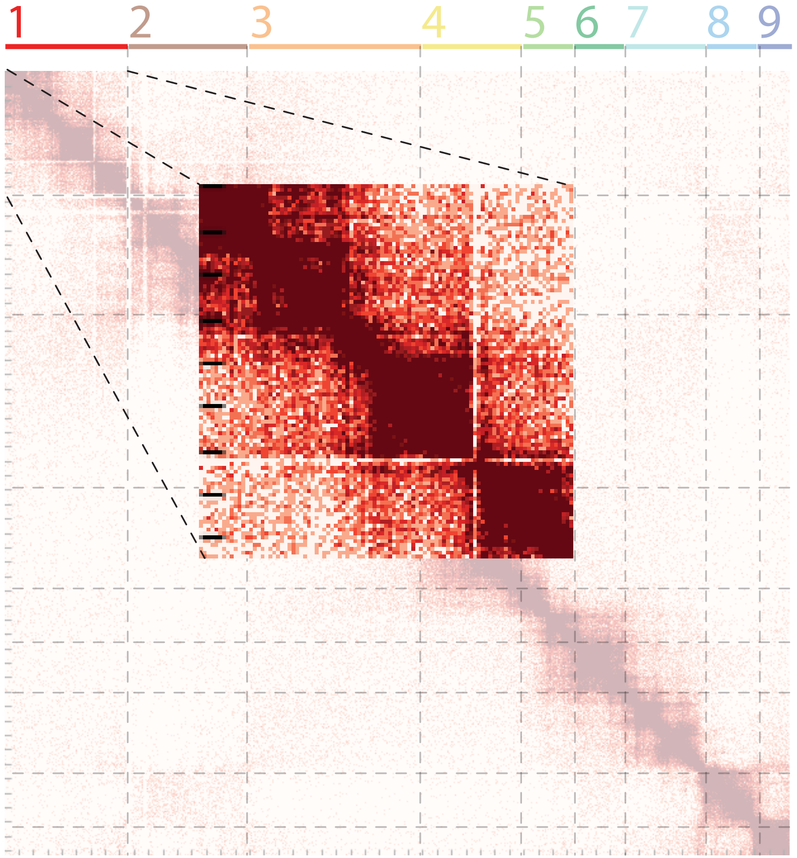




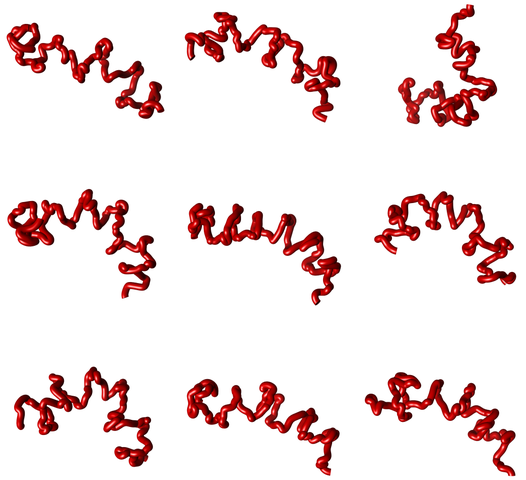


Increasing resolution

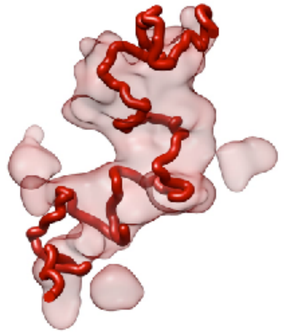
Rigid body fitting 3D structures based on Hi-C data



Segment 3 3D models

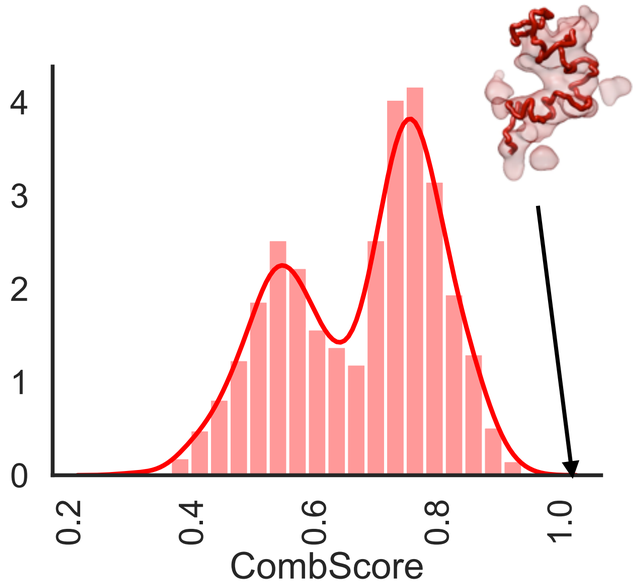


Segment 3 density map



$$\mathrm{ConS} = 1 - \frac{d_{P,COM}}{\max\left(d_{P,COM}\right)}$$

$$\mathrm{CCC} = \frac{\sum_{i=1}^{M}\left[\rho_i^{EM} - \bar{\rho}^{EM}\right]\left[\rho_i^{P} - \bar{\rho}^{P}\right]}{\sqrt{\sum_{i=1}^{M}\left[\rho_i^{EM} - \bar{\rho}^{EM}\right]^2 \sum_{i=1}^{M}\left[\rho_i^{P} - \bar{\rho}^{P}\right]^2}}$$



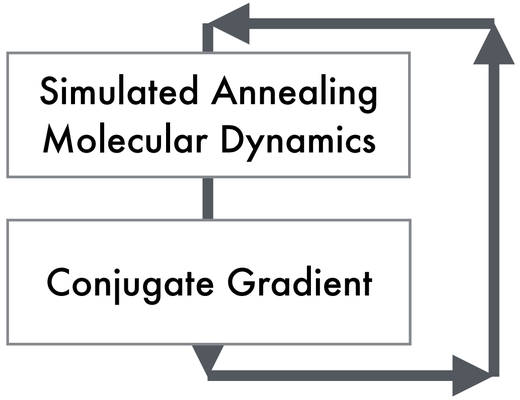
TADbit

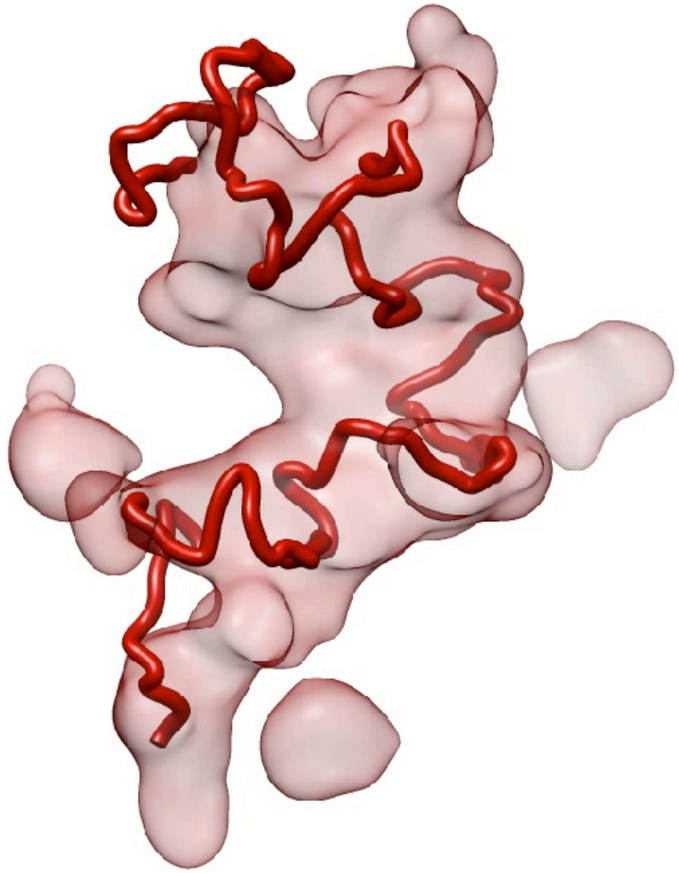
Serra, Baù, et al. PLOS CB 2017
http://www.3DGenomes.org

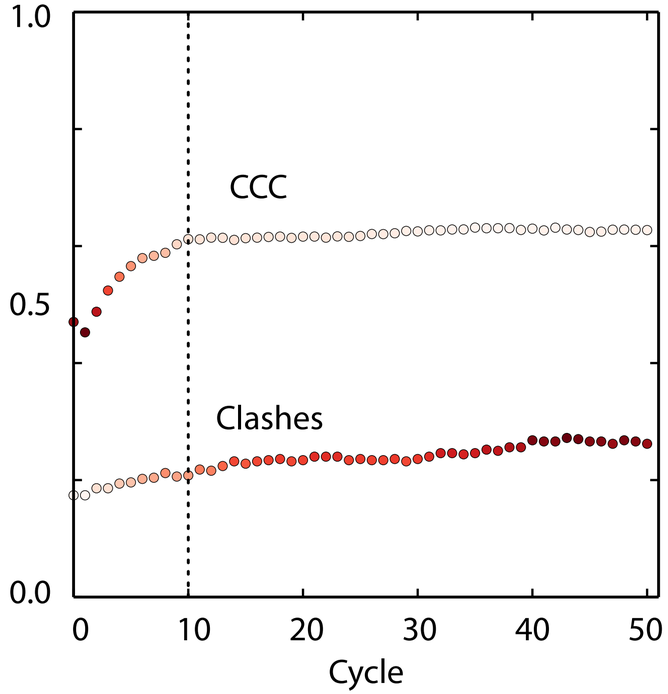
Farabella et al, J Appl Crystallogr. 2015
Roseman, 2000; Wriggers & Chacon, Structure 2001

Increasing resolution

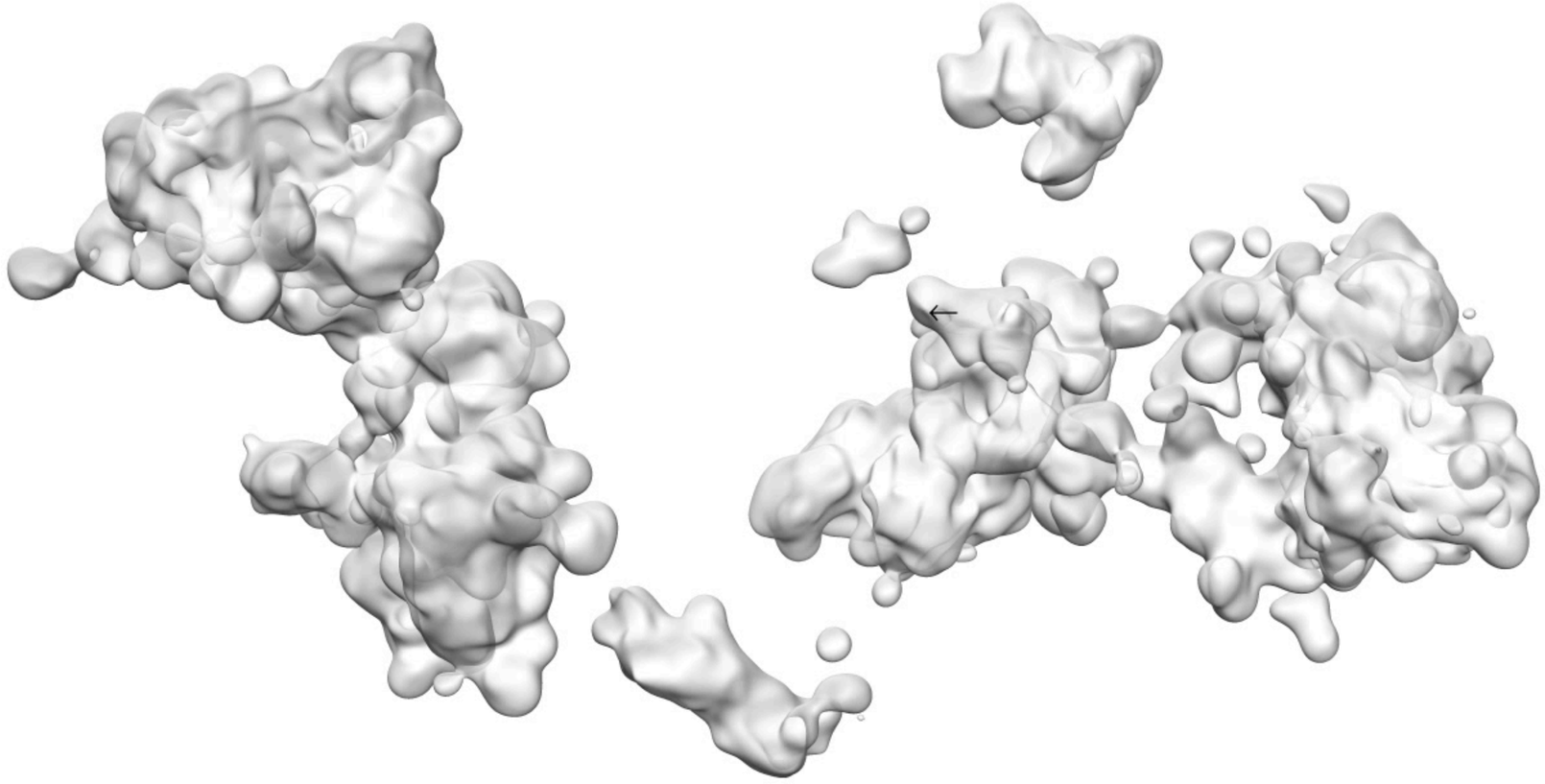
Flexible fitting 3D structures based on Hi-C data





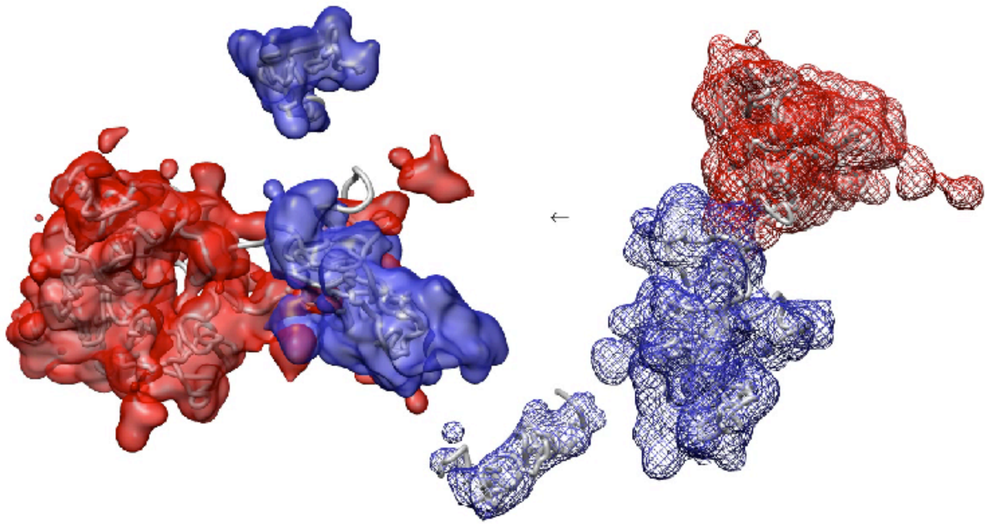


Chromosome walking path @10Kb resolution

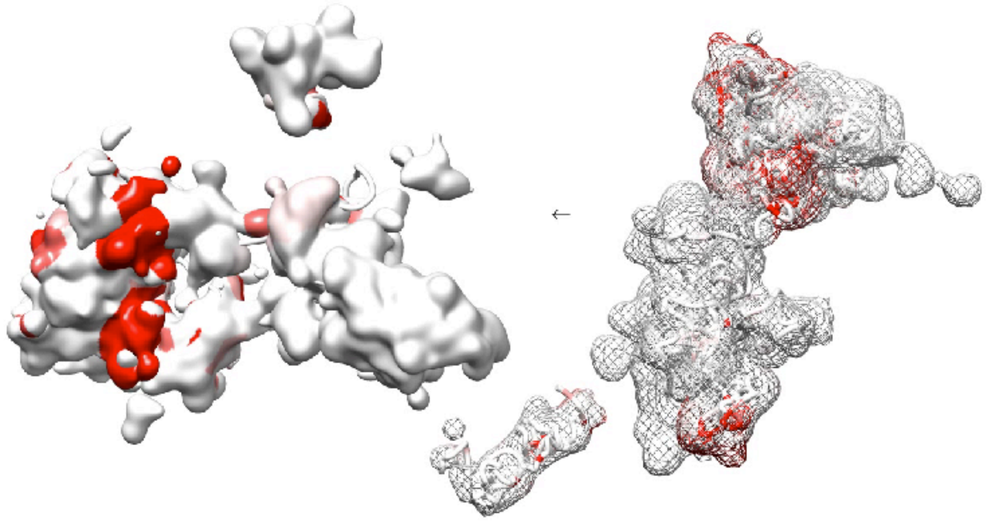


Mapping “omics”

3D organization of local structures



Both in “active” cluster
One in each cluster
Both in “inactive” cluster



μ mean value per 10kb μ+2σ

H3K4me1

http://marciuslab.org
http://3DGenomes.org

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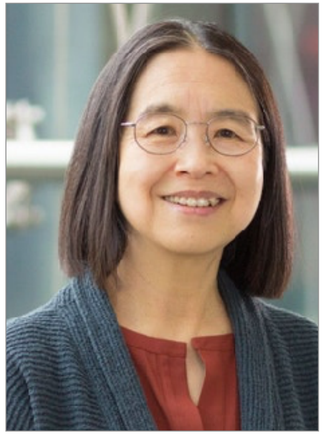
cnag CRG Centre for Genomic Regulation ICREA



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