Structure determination of genomes and genomic domains by satisfaction of spatial restraints

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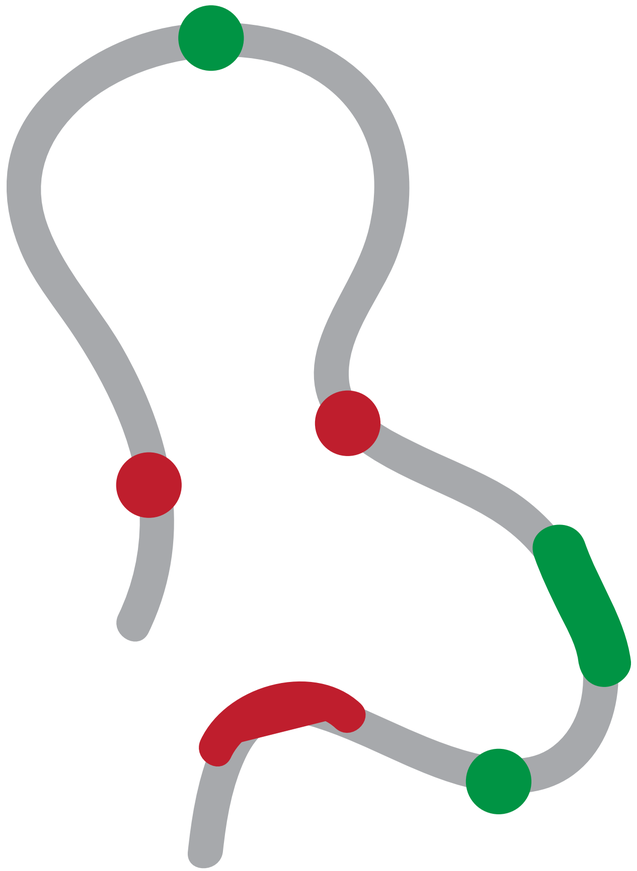
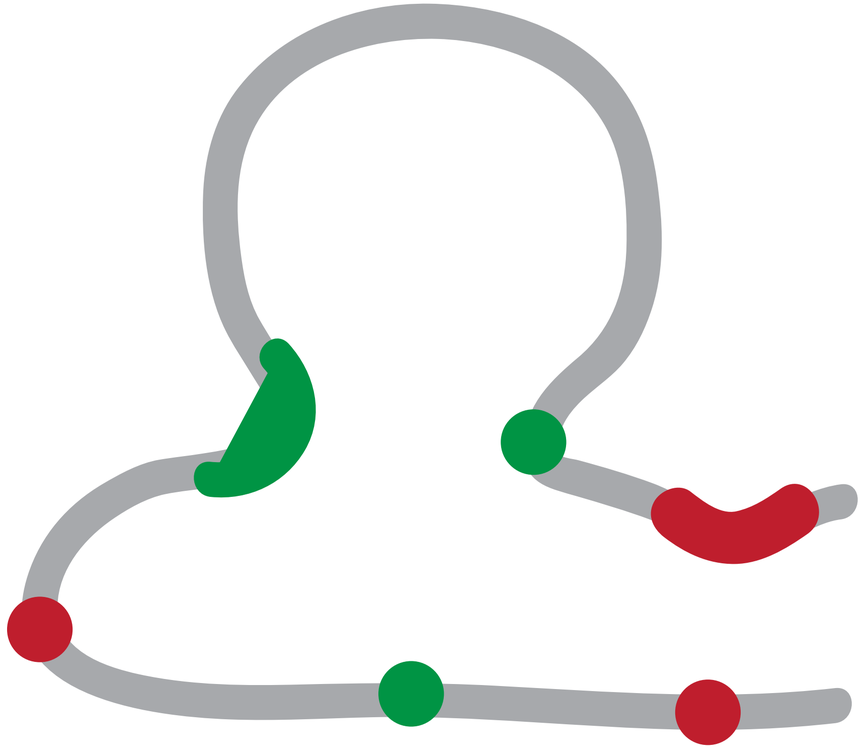
http://marciuslab.org
http://3DGenomes.org
http://cnag.crg.eu

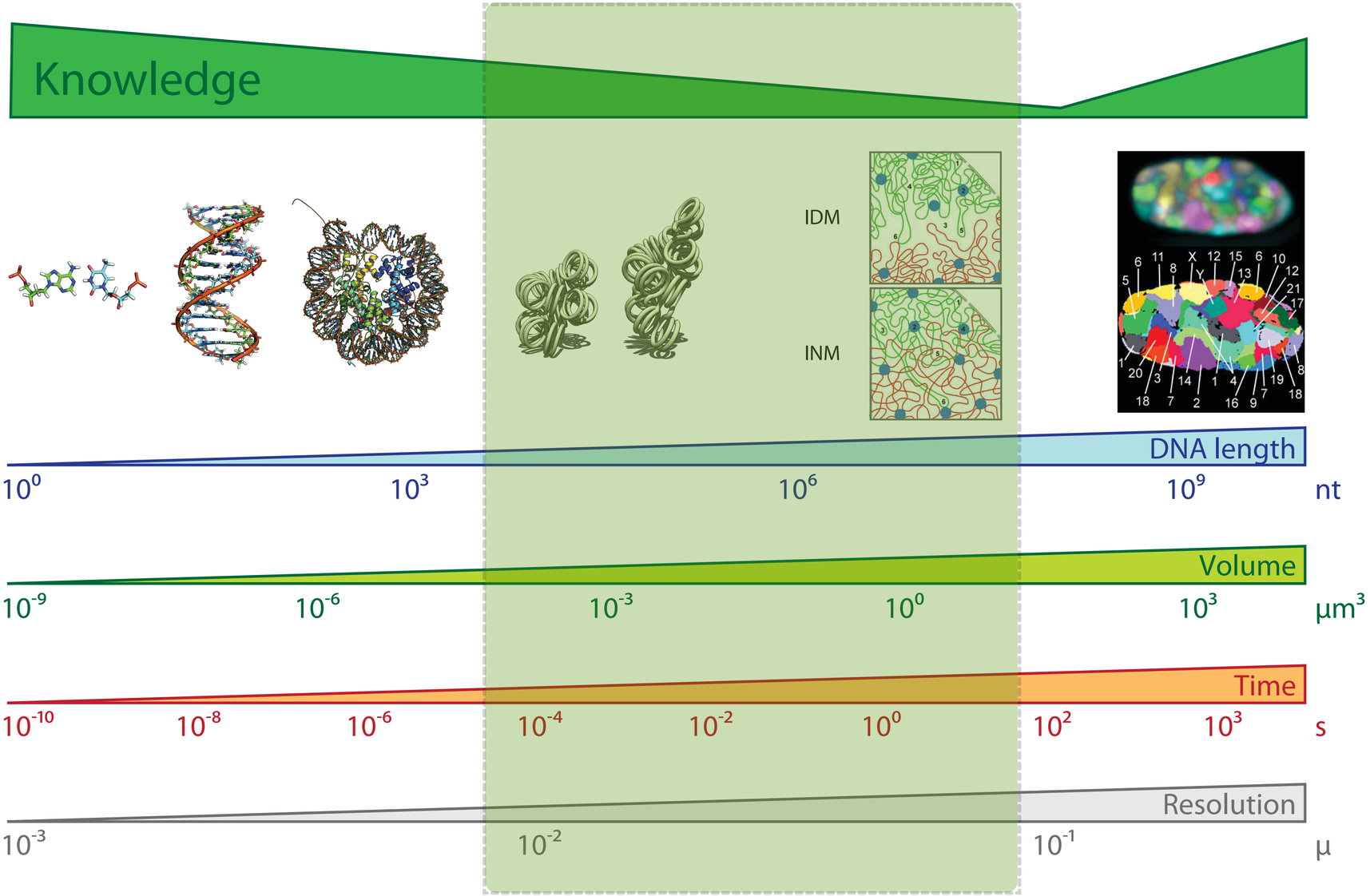
cnag CRG ICREA





Resolution Gap

Marti-Renom, M. A. & Mirny, L. A. PLoS Comput Biol *7*, e1002125 (2011)



Resolution Gap

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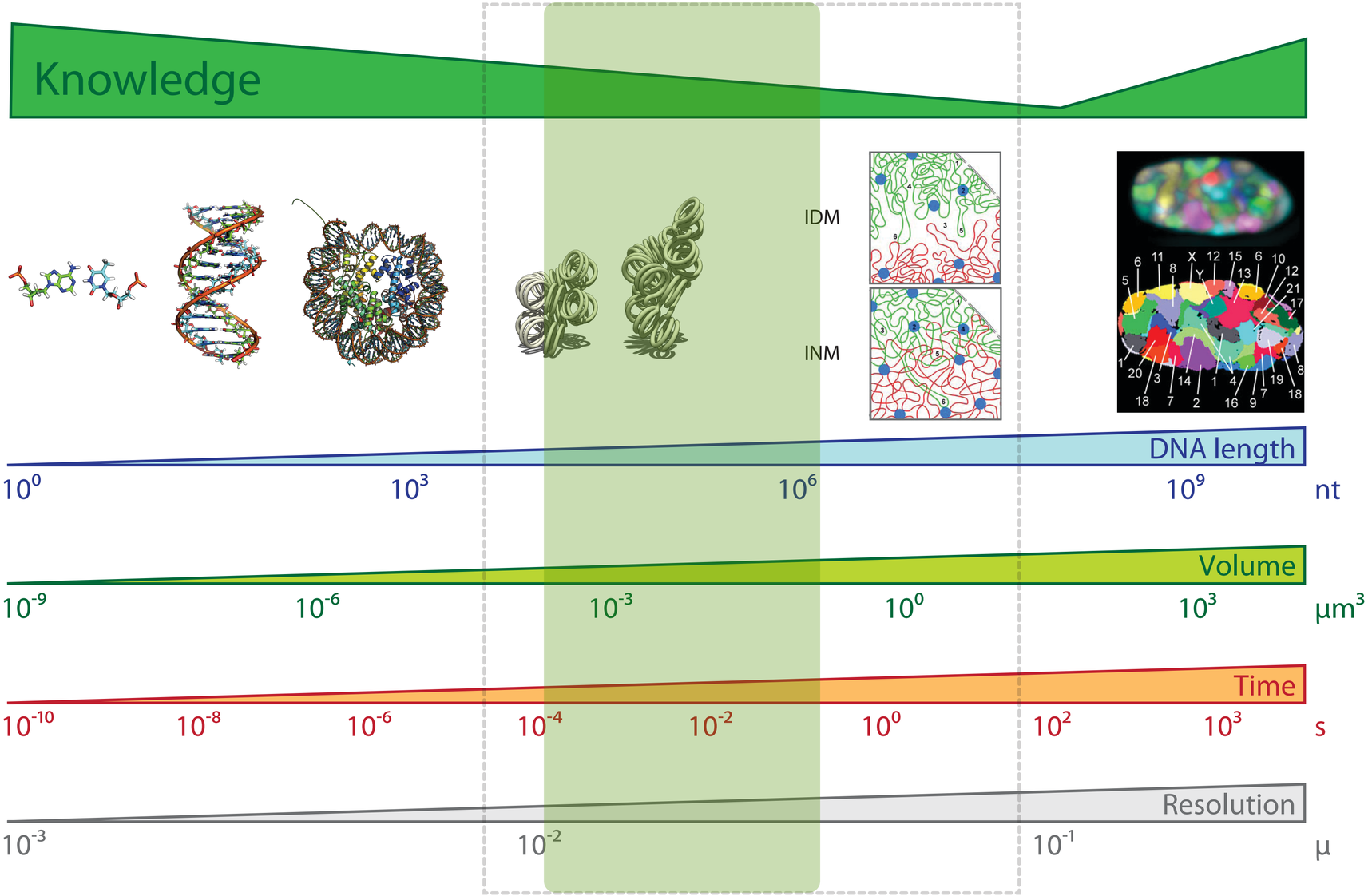
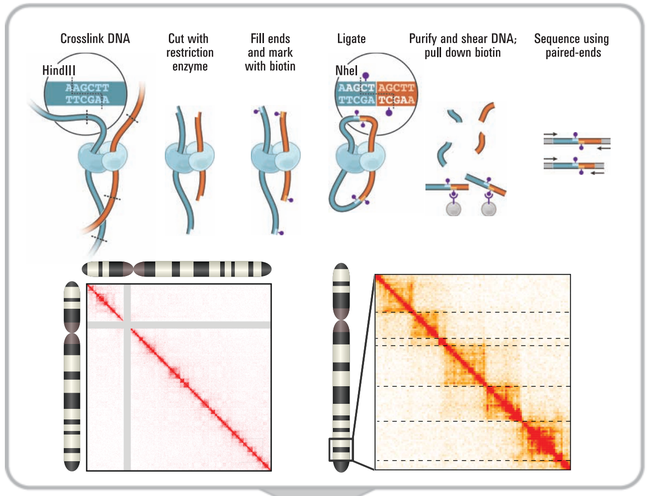
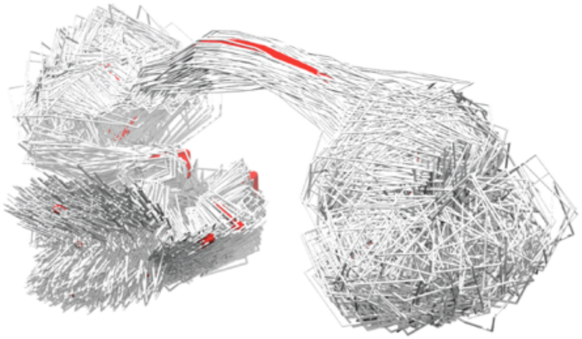


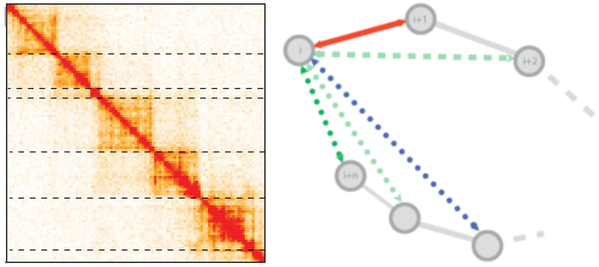
Hybrid Method

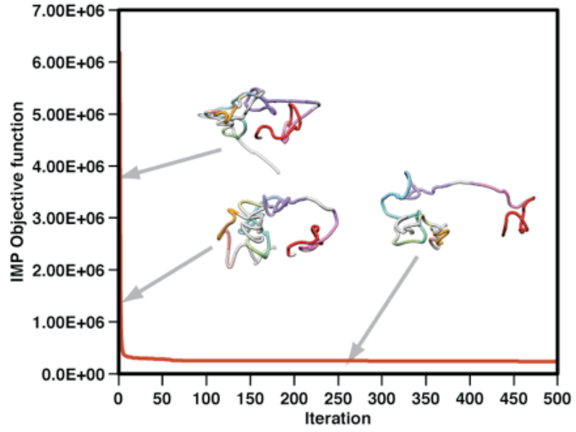
Baù, D. & Marti-Renom, M. A. Methods 58, 300–306 (2012).

Experiments





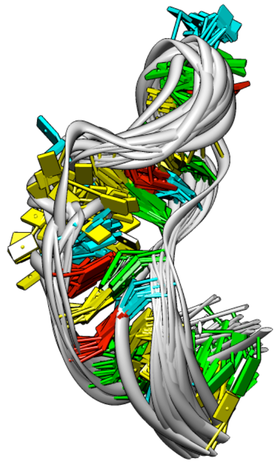


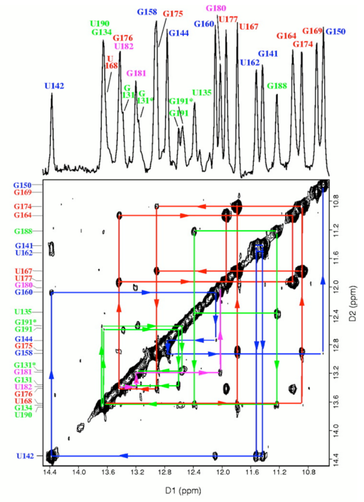


Computation

Restraint-based Modeling

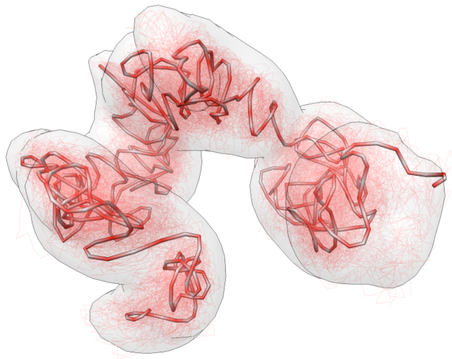
Baù, D. & Marti-Renom, M. A. Methods 58, 300–306 (2012).

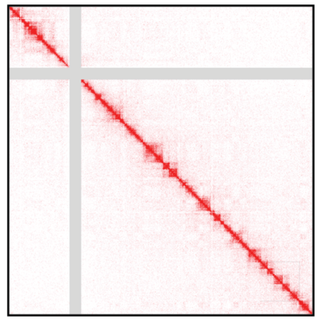




Biomolecular structure determination

2D-NOESY data





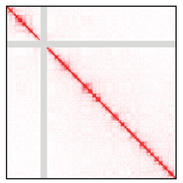
Chromosome structure determination

3C-based data

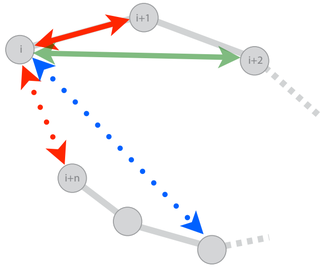
TADbit

http://3DGenomes.org

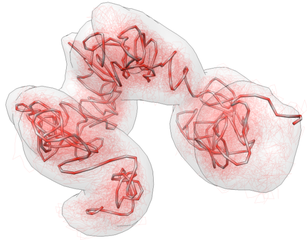
FastQ files to Maps



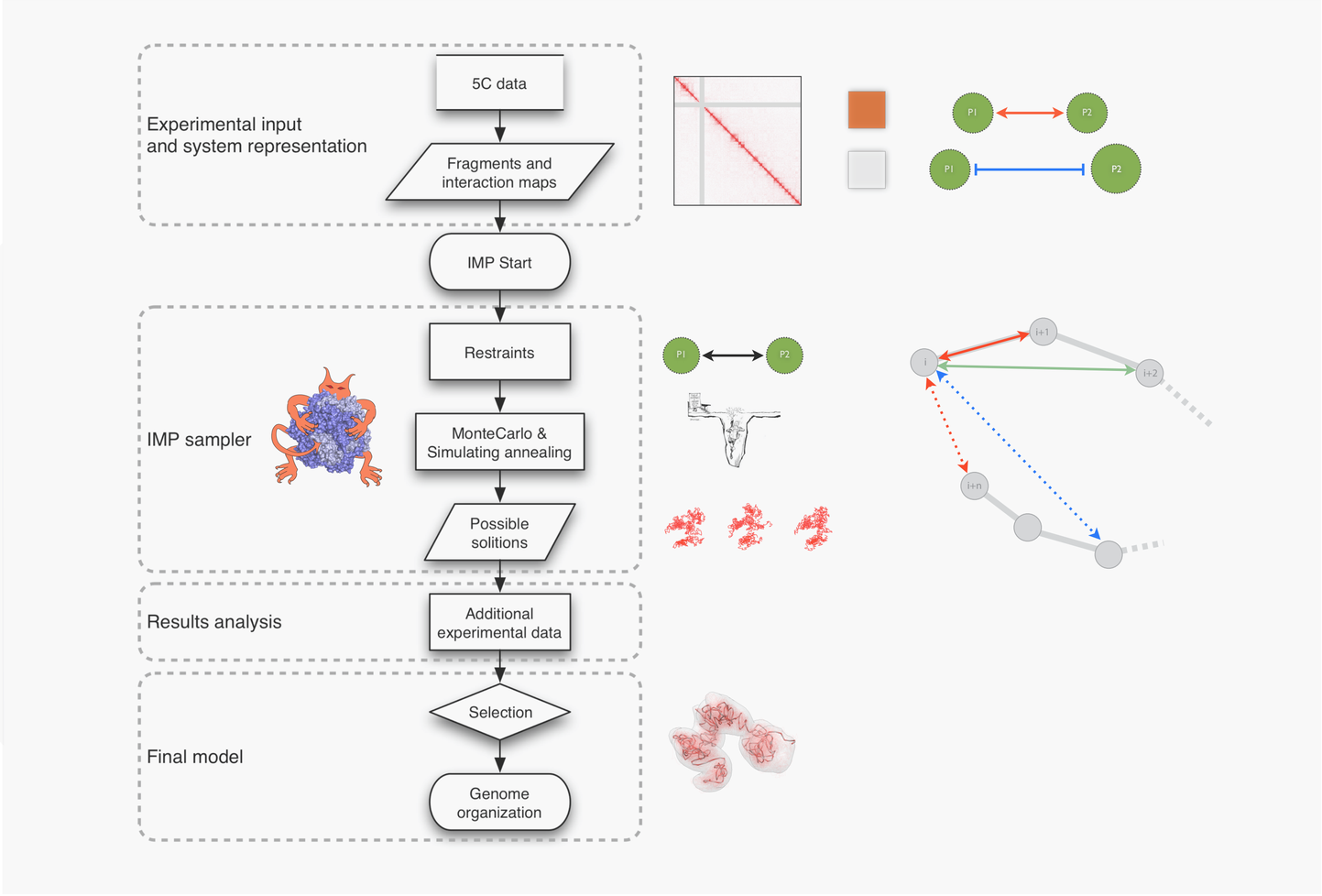
Map analysis



Model building

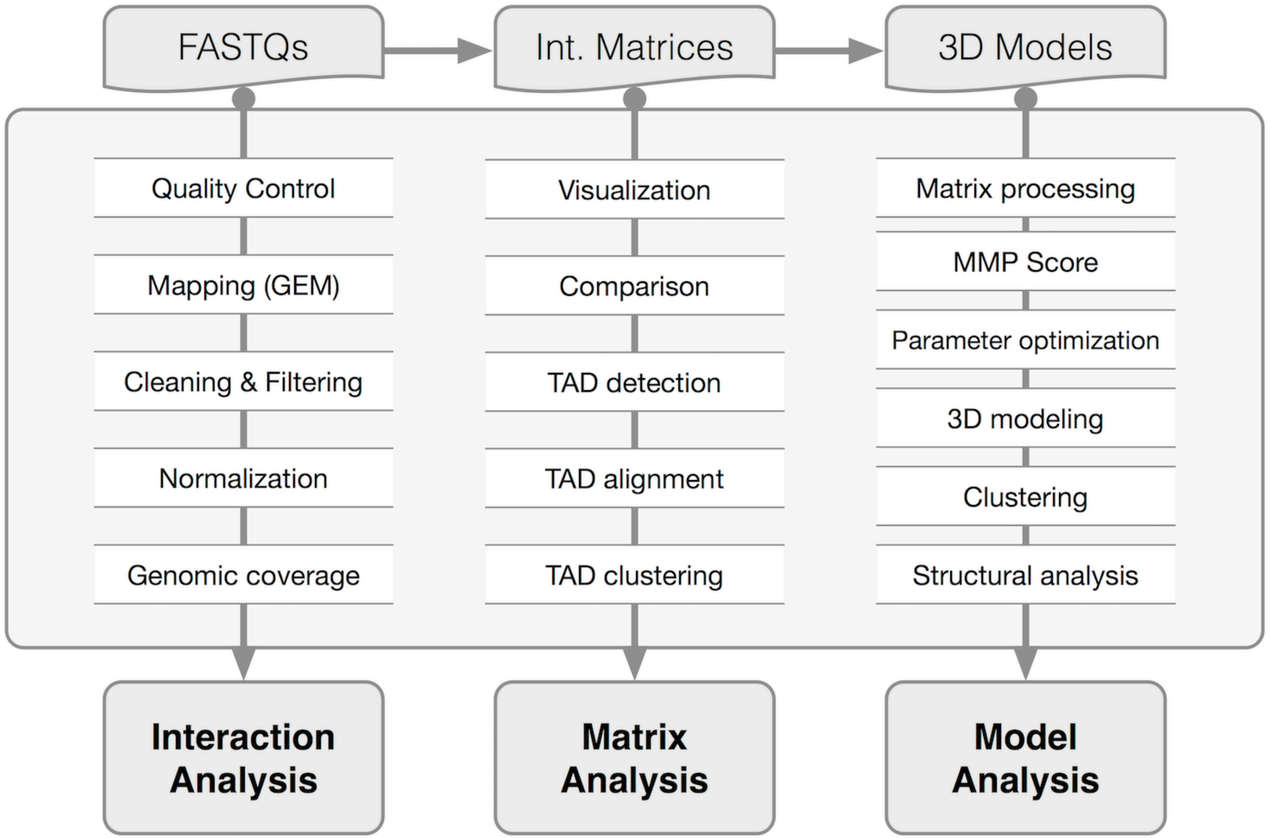


Model analysis



TADbit

Serra, Baù, et al. (2017). PLOS CompBio



- Baù, D. et al. Nat Struct Mol Biol (2011)
- Umbarger, M. A. et al. Mol Cell (2011)
- Le Dily, F. et al. Genes & Dev (2014)
- Belton, J.M. et al. Cell Reports (2015)
- Trussart M. et al. Nature Communication (2017)
- Cattoni, D. et al. Nature Communication (2017)
- Stadhouders R. et al. Nature Genetics (2018)
- Kojic, A., Cuadrado, A. et al. Nat Struct Mol Biol (2018)
- Beekman R. et al. Nature Medicine (2018)
- Mas, G. et al. Nature Genetics (2018)
- Pascual-Reguant, L. et al. Nature Communication (2018)
- Nir, Farabella, Perez-Estrada, et al. PLOS Genetics (2018)
- Cuadrado, Giménez-Llorente et al. (2019)
- Miguel-Escalada et al. (2019)
- Morf et al. (2019)

Nature Structural & Molecular Biology, 25(9), 766-777, 2018
Cell, 173(7), 1796-1809.e17, 2018
Structure, 26(6), 894-904.e2, 2018
Genome Research, 29(1), 29-39, 2019
Genome Research, 29(1), gr.238527.118, 2019

Can we walk the chromatin path in the nucleus?

by

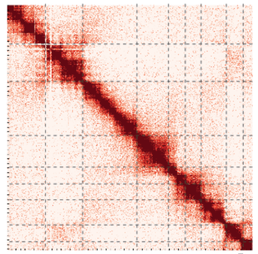
Integrating imaging and Hi-C maps with modeling.

by developing a method for

Oligopaint-based modeling of genomes

High-resolution imaging

Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



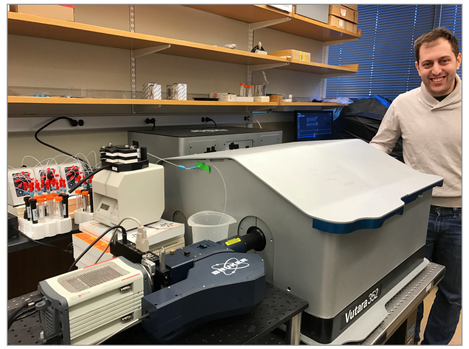
chr19:7,335,095-15,449,189
~8Mb

homologous 32-42bp

Beliveau et al. Nat. Comm. 2015

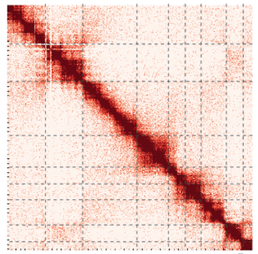
High-resolution imaging

Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



Guy Nir Harvard Med School

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



chr19:7,335,095-15,449,189
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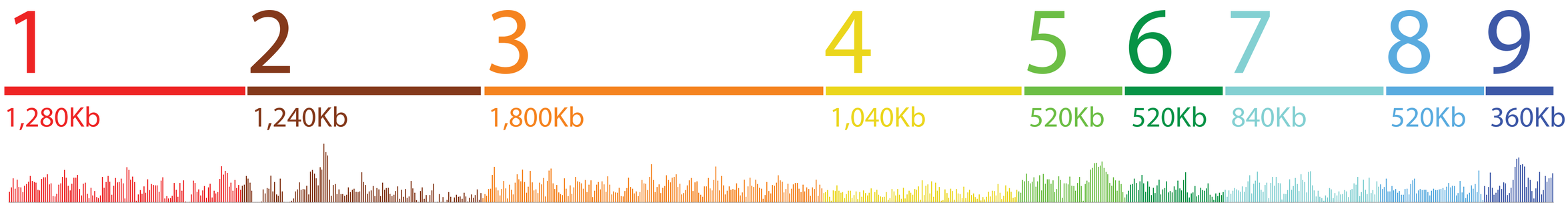
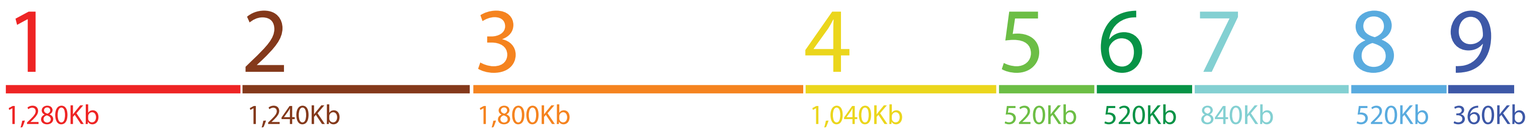


High-resolution imaging

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

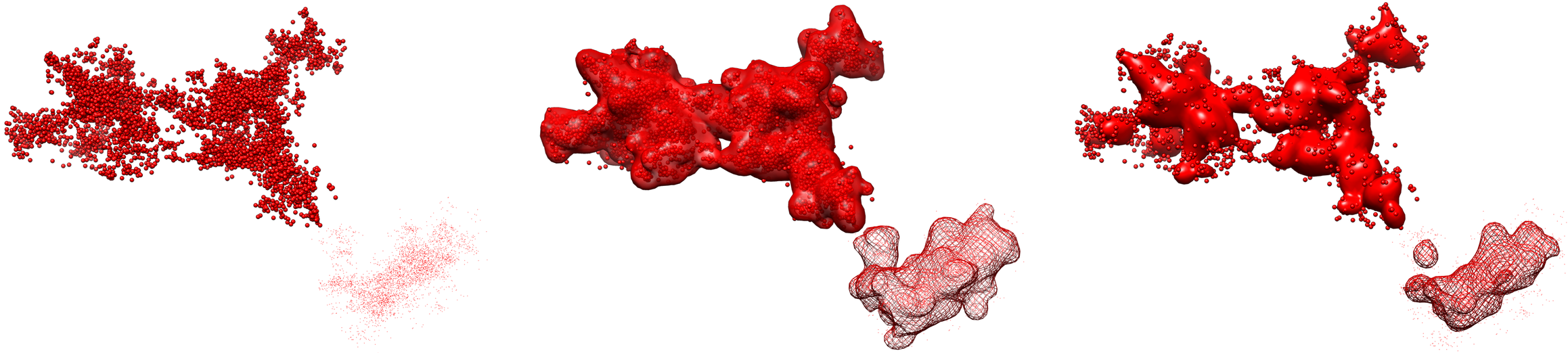


Cell-02

High-resolution imaging

XYZ points convolution into a density map

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

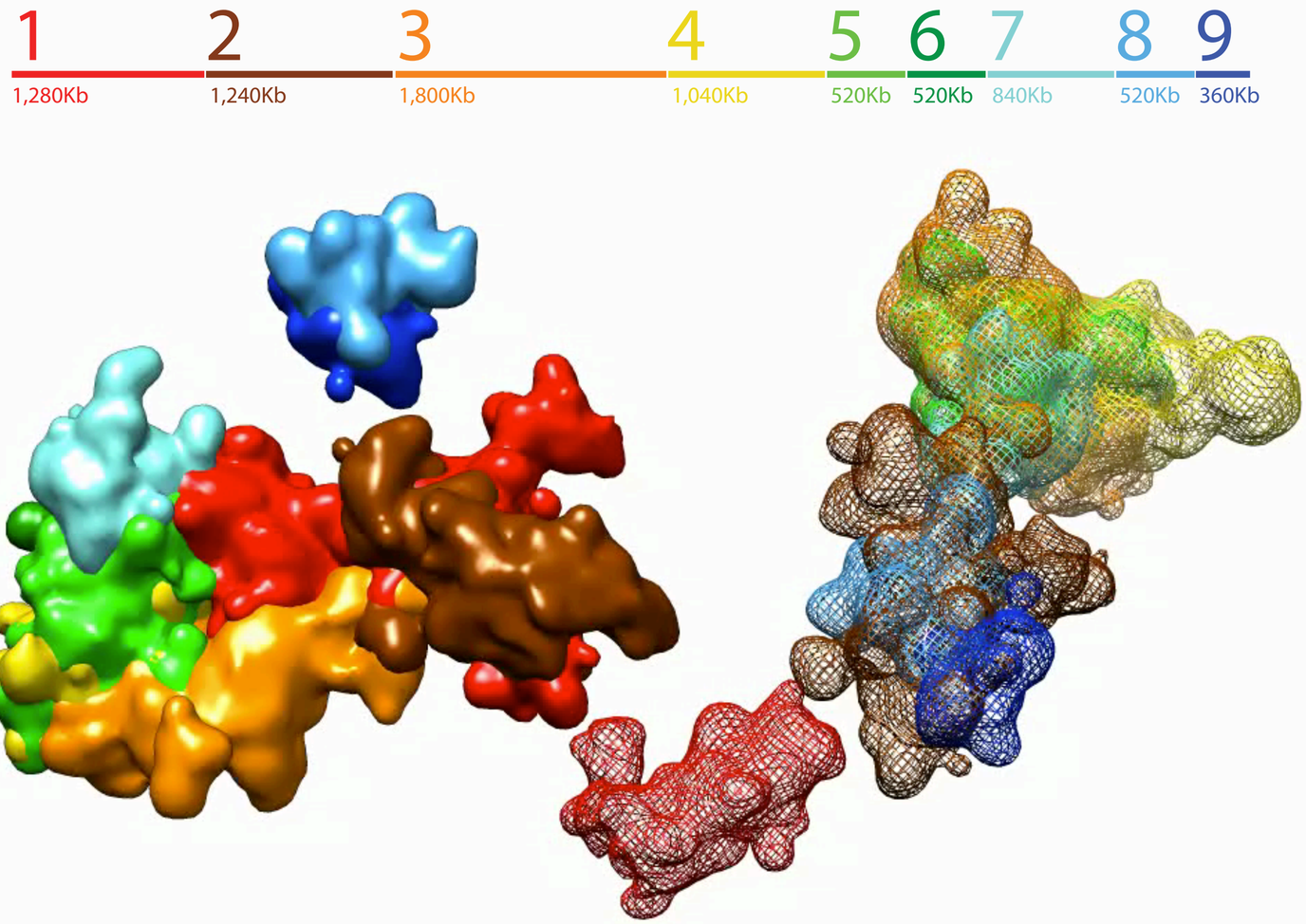


Cell-02 · Segment 1

Farabella et al, J Appl Crystallogr. 2015

Density maps

Cell-02 · Density map @ 50nm

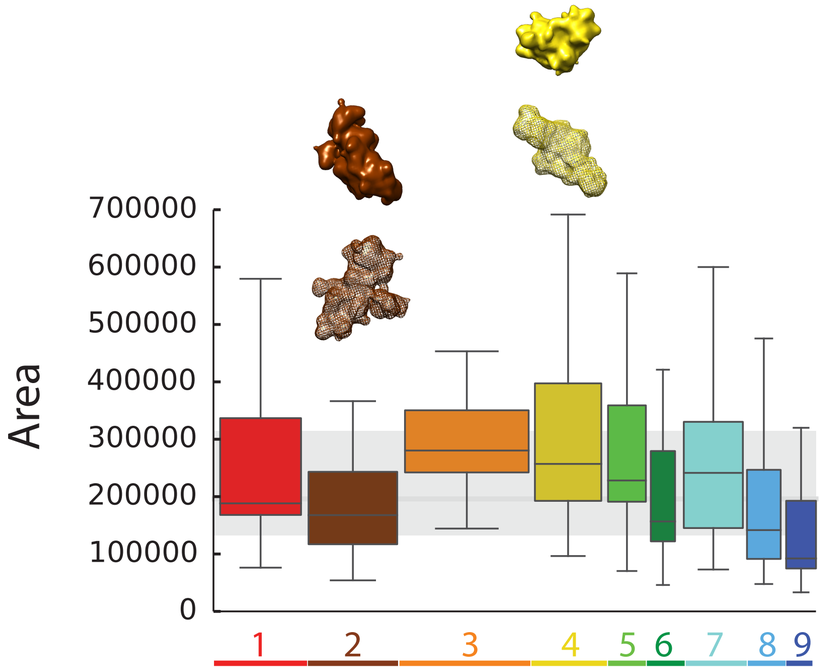


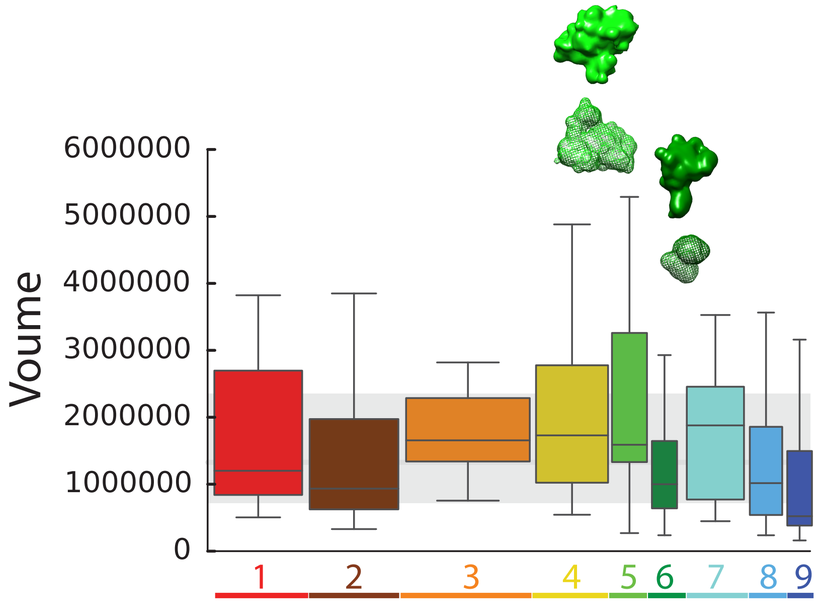
Area (nm^2)
Volume (nm^3)
Sphericity
Overlap (%)
Distance (nm)

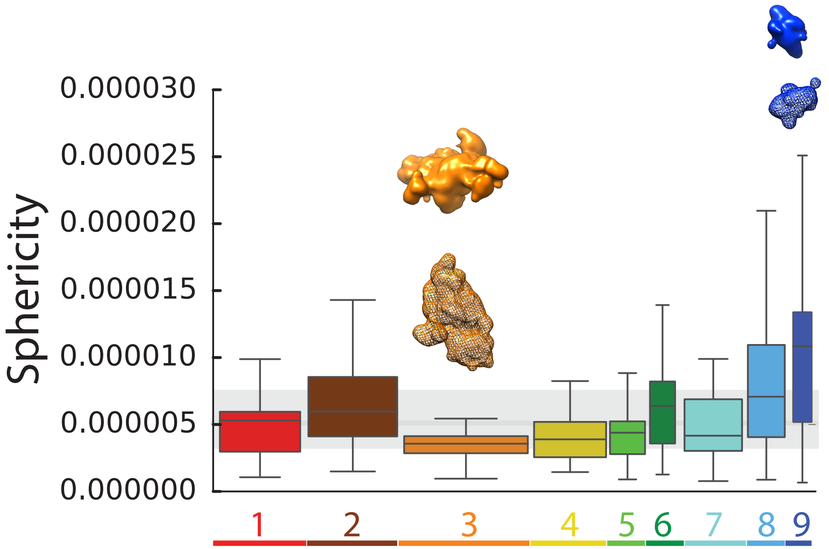
Farabella et al, J Appl Crystallogr. 2015

Structural features

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



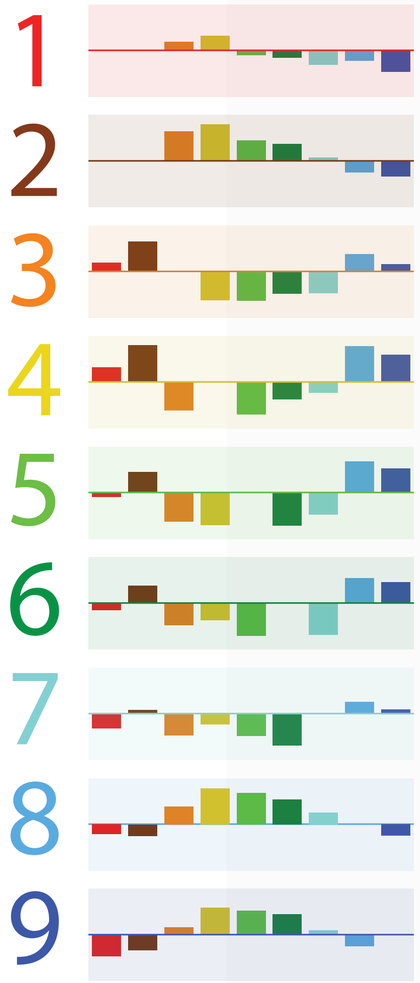


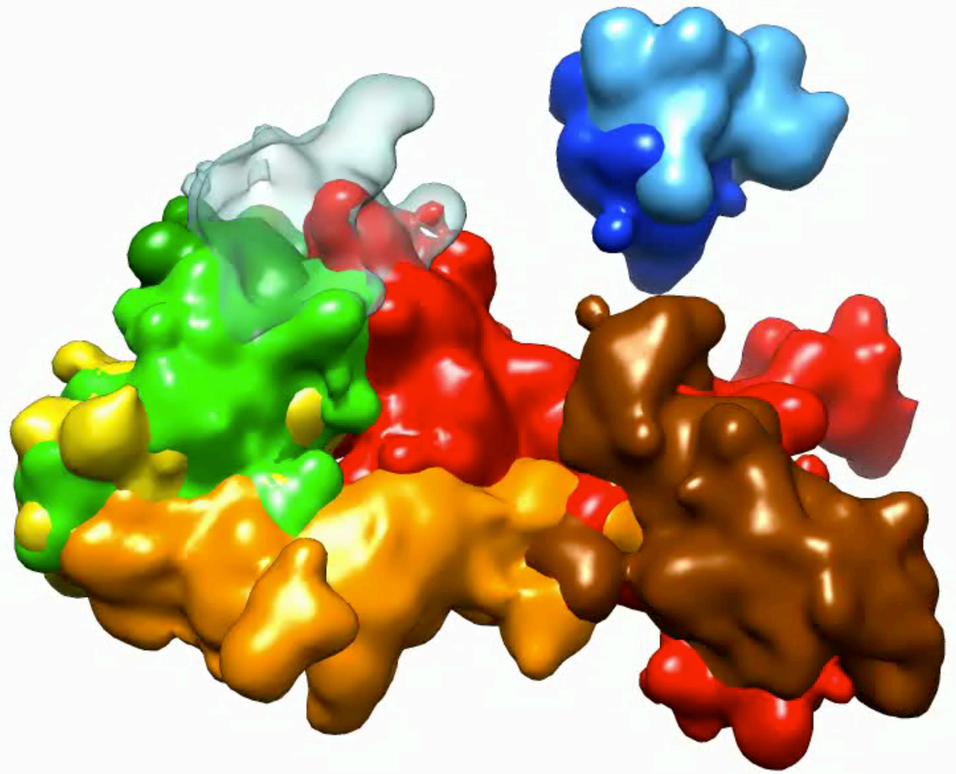


Spatial arrangement

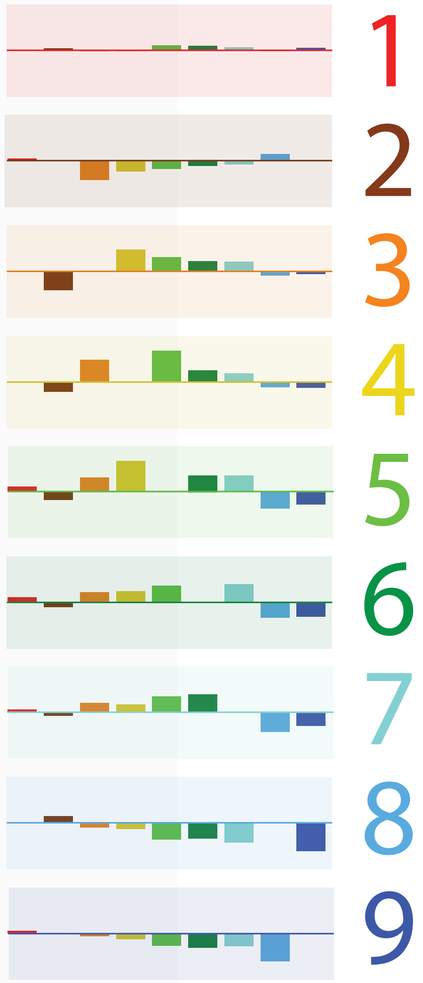
Distance and overlap of 19 cells each with 2 homologous resolved

Diff. distance



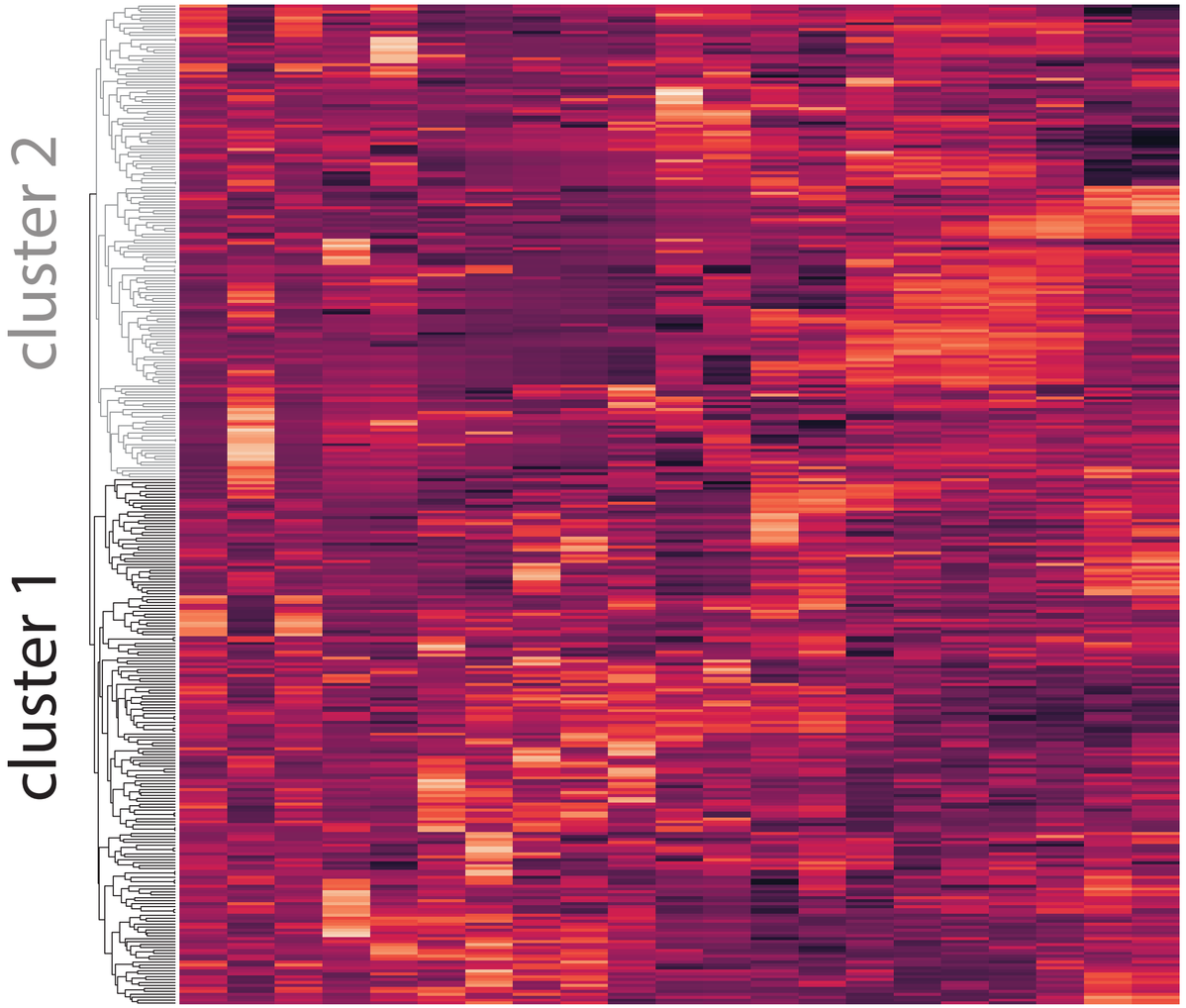


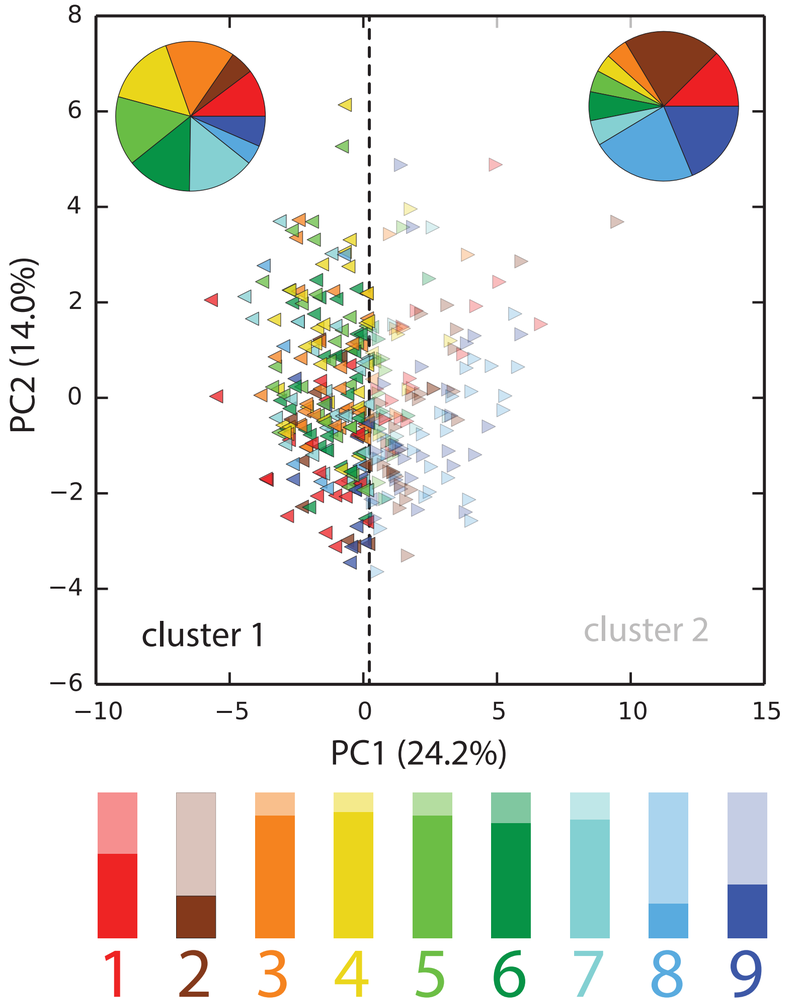
Diff. overlap



Structural clustering

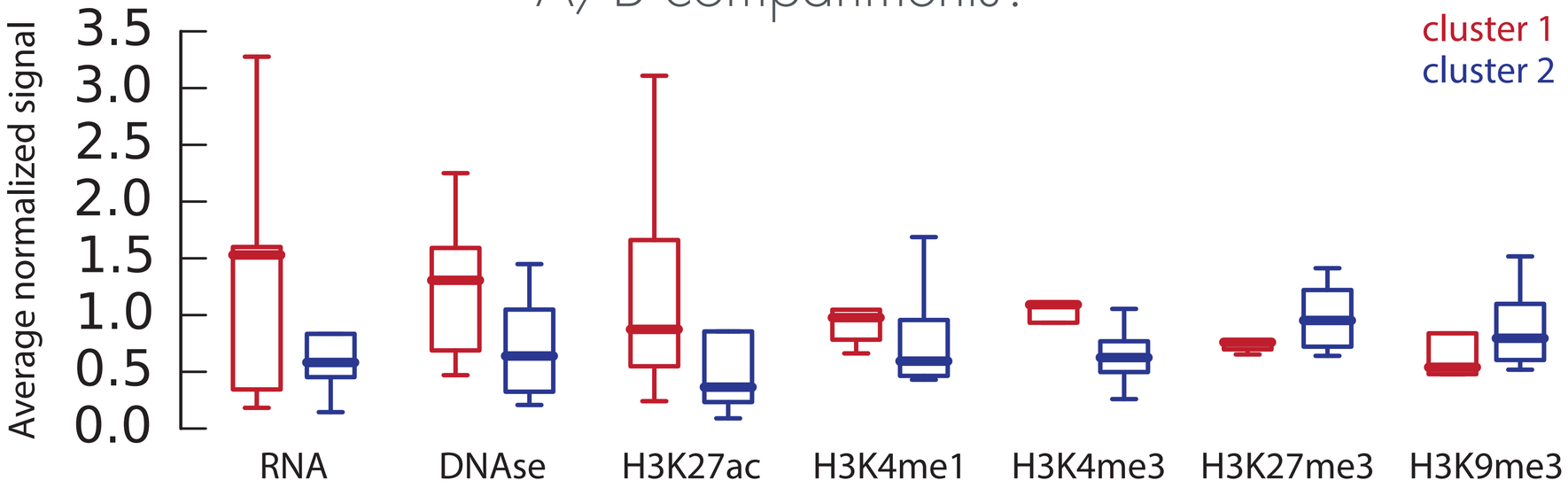
19 cells each with 2 homologous and 9 segments each (342)

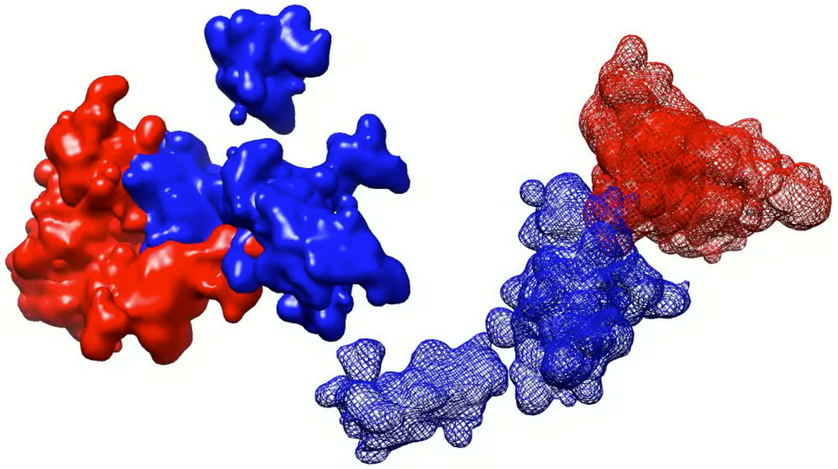


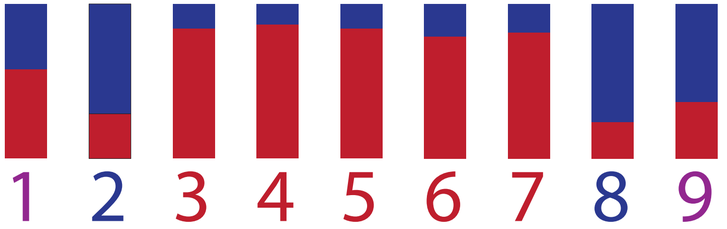


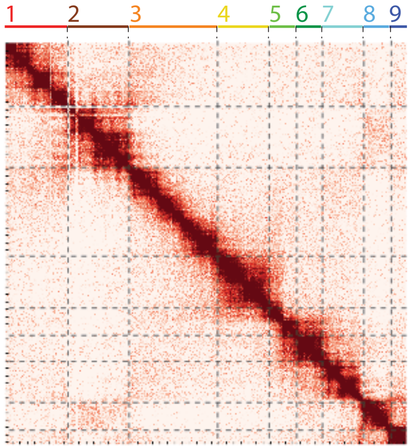
Cluster properties

A/B compartments?





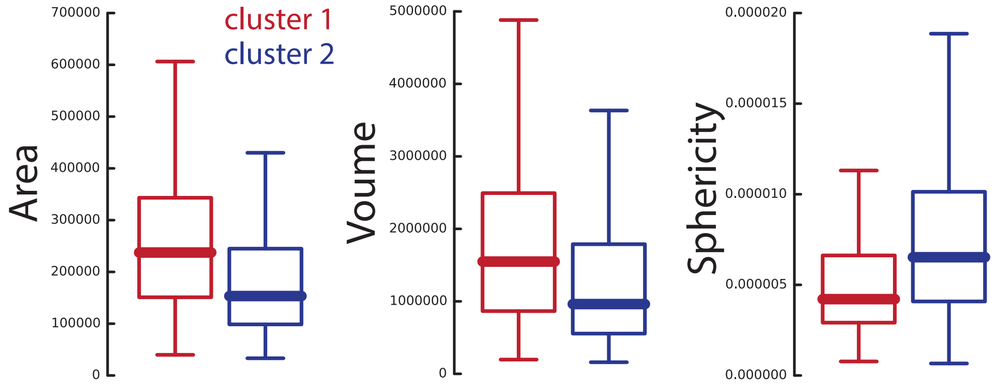


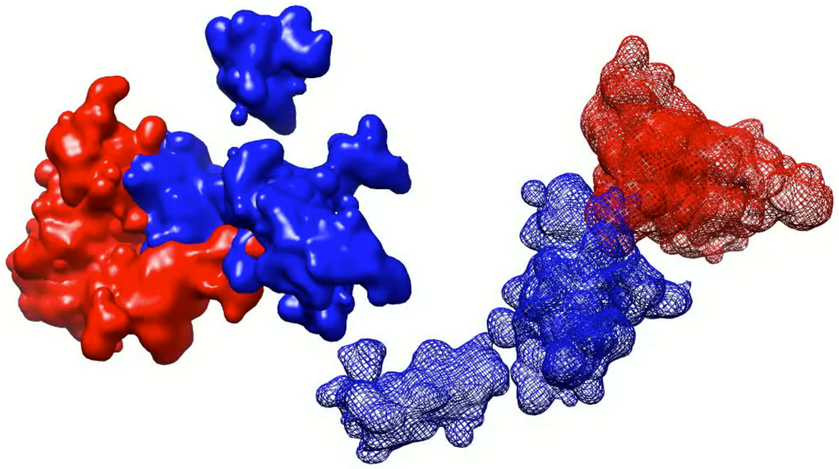


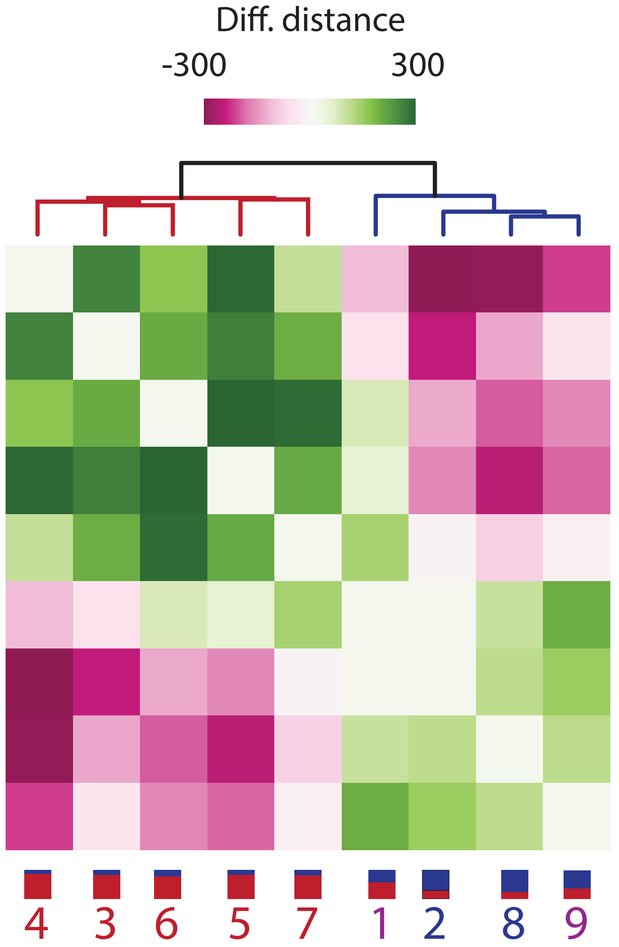
PGP1 ChIP-seq and Hi-C data from ENCODE and Lieberman-Aiden Lab, respectively

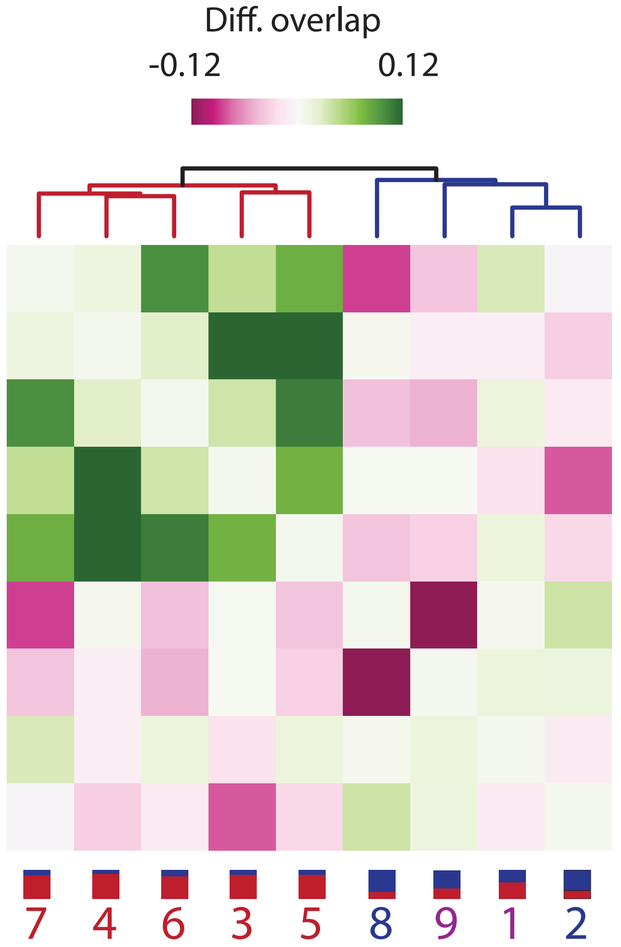
Cluster properties

A/B compartment properties









Can we walk the chromatin path in the nucleus?

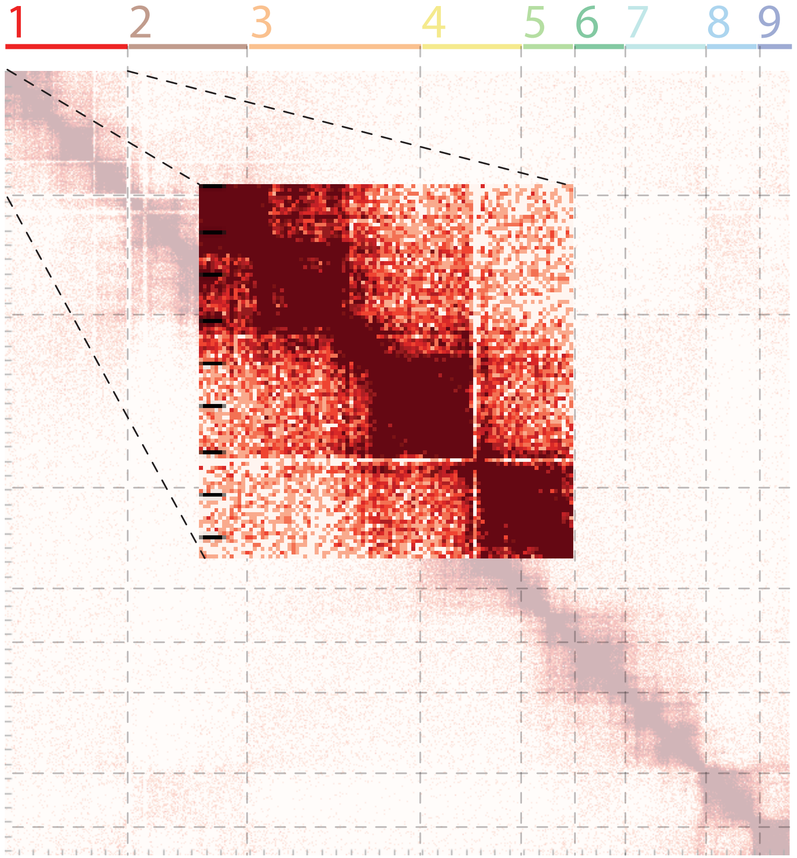
YES!

Can we increase the resolution of our data?

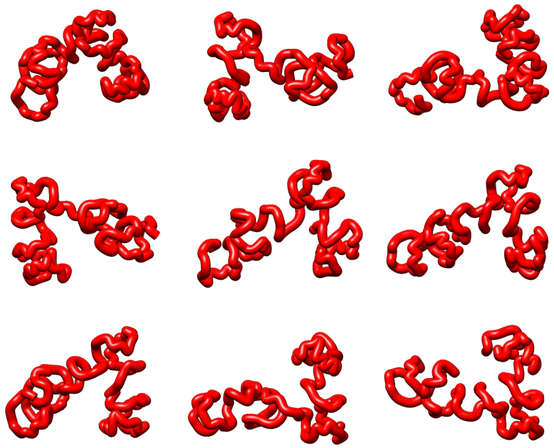
by fitting 3D models based on Hi-C interaction maps

Increasing resolution

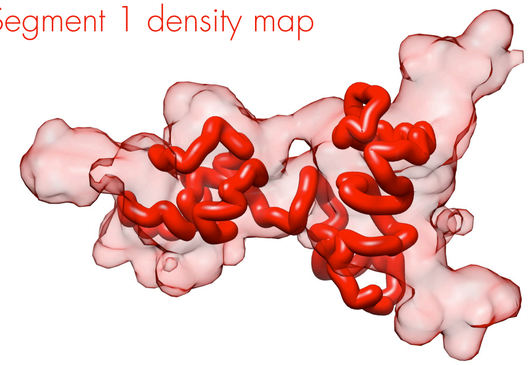
Rigid body fitting 3D structures based on Hi-C data



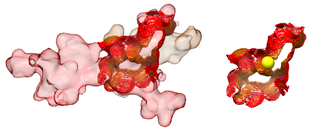
Segment 1 3D models



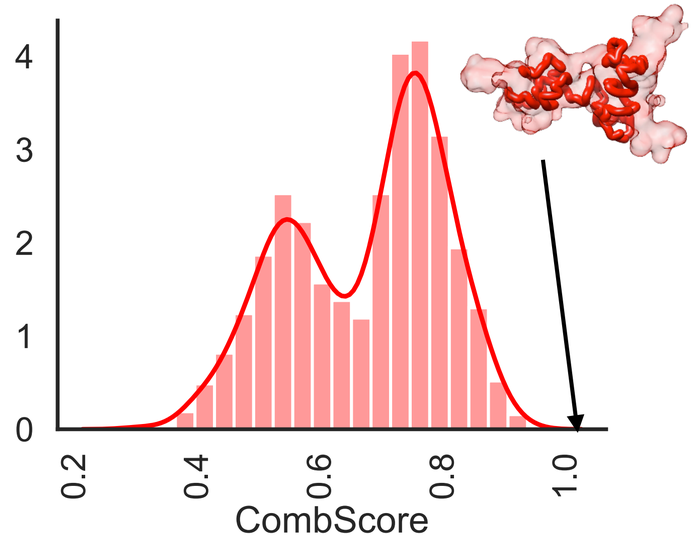
Segment 1 density map



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



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



TADbit

Serra, Baù, et al. PLOS CB 2017

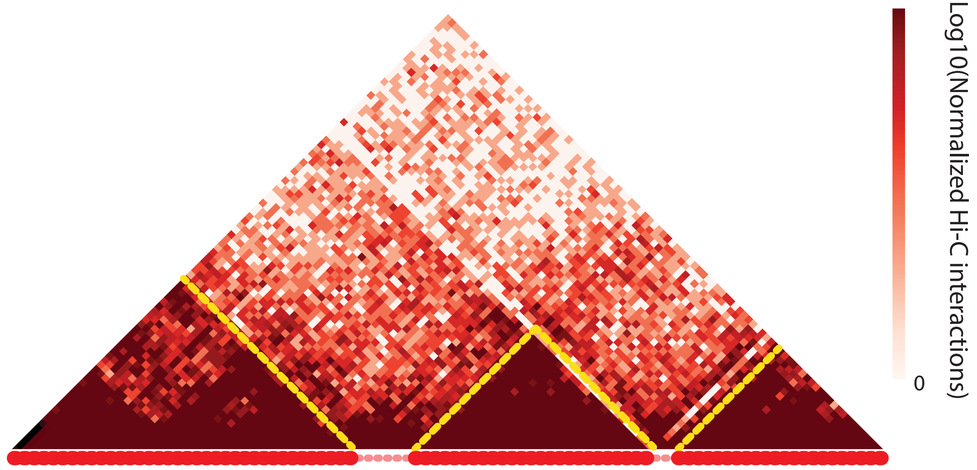
http://www.3DGenomes.org

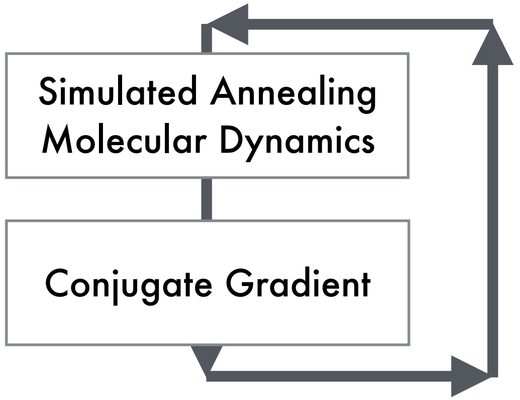
Farabella et al, J Appl Crystallogr. 2015

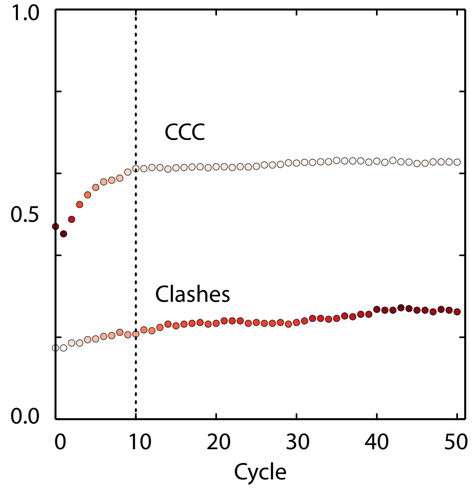
Roseman, 2000; Wriggers & Chacon, Structure 2001

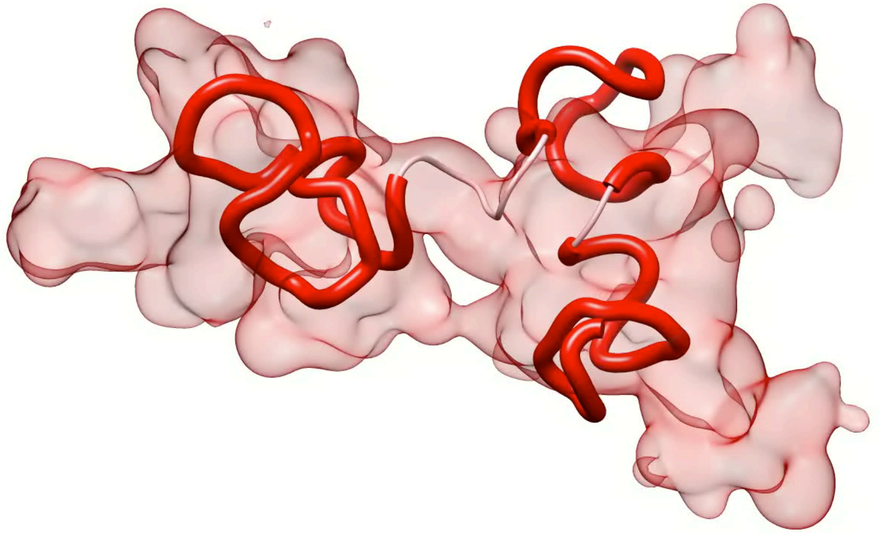
Increasing resolution

Flexible fitting 3D structures based on Hi-C data



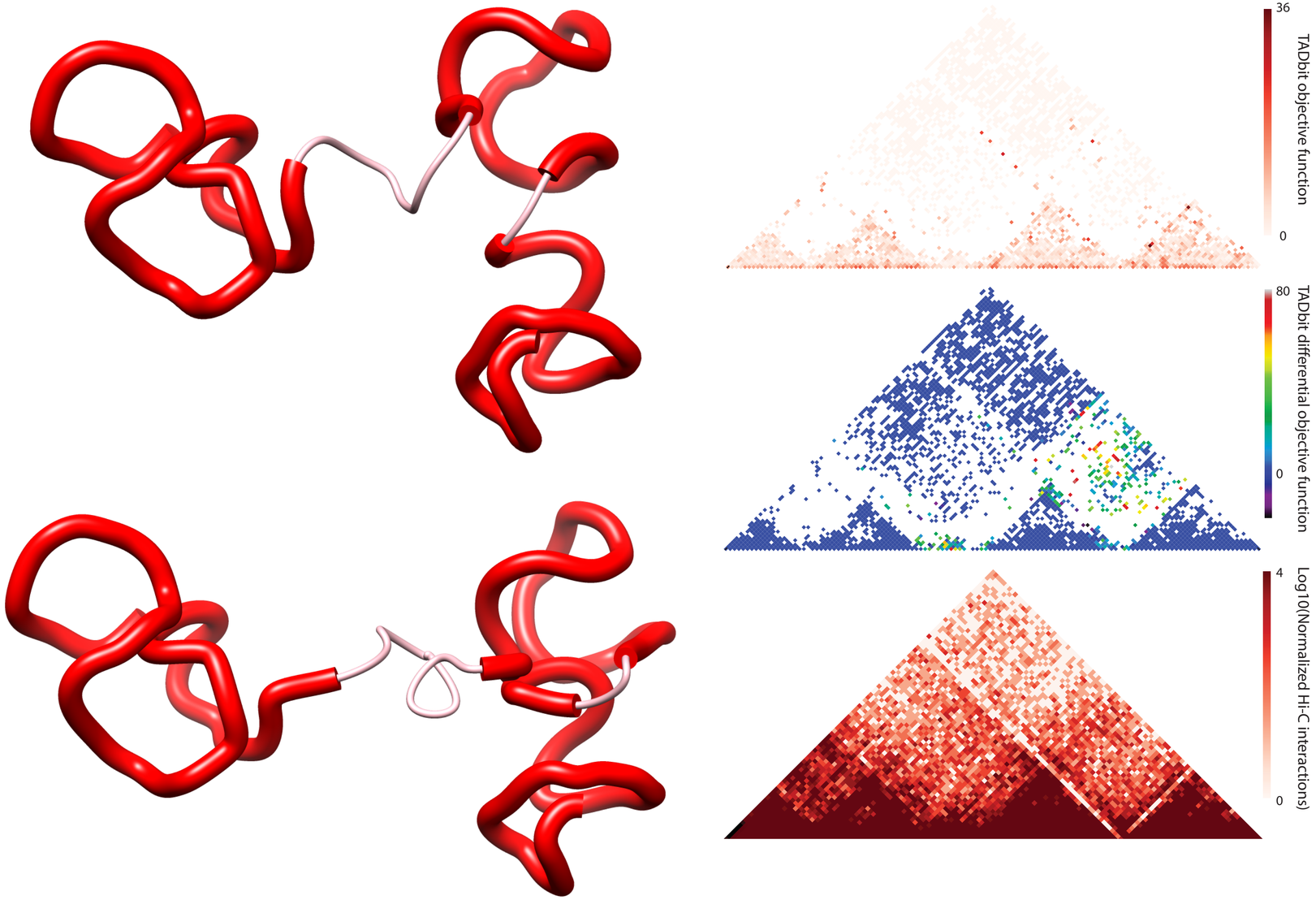




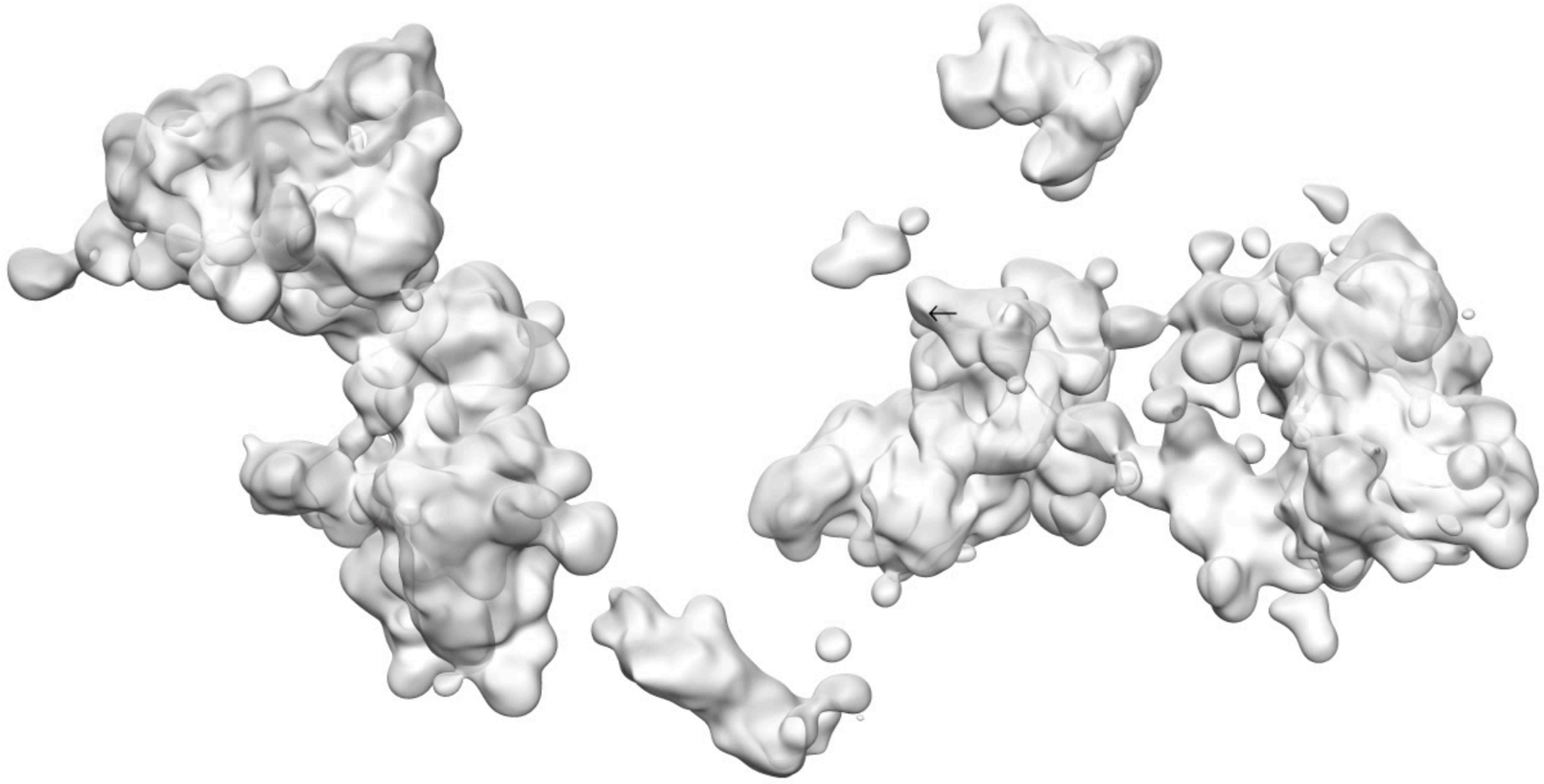


Increasing resolution

Flexible fitting 3D structures based on Hi-C data



Chromosome walking path @10Kb resolution



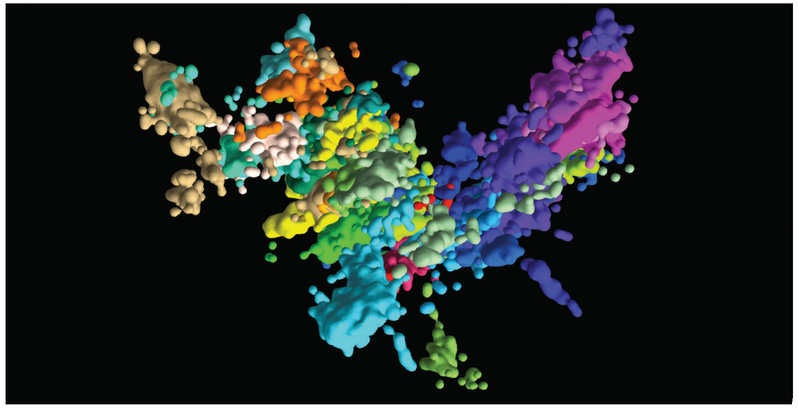
Jeffrey M. Perkel *Nature* **569**, 293-294 (2019)

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 ▶

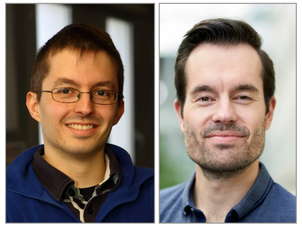
What next?

Is there a dynamic coupling between structure and gene activity?

data published in Nat Genetics January 2018 and method preprinted (BioRxived)

The End…

3D structural dynamics of the SOX2 locus activation

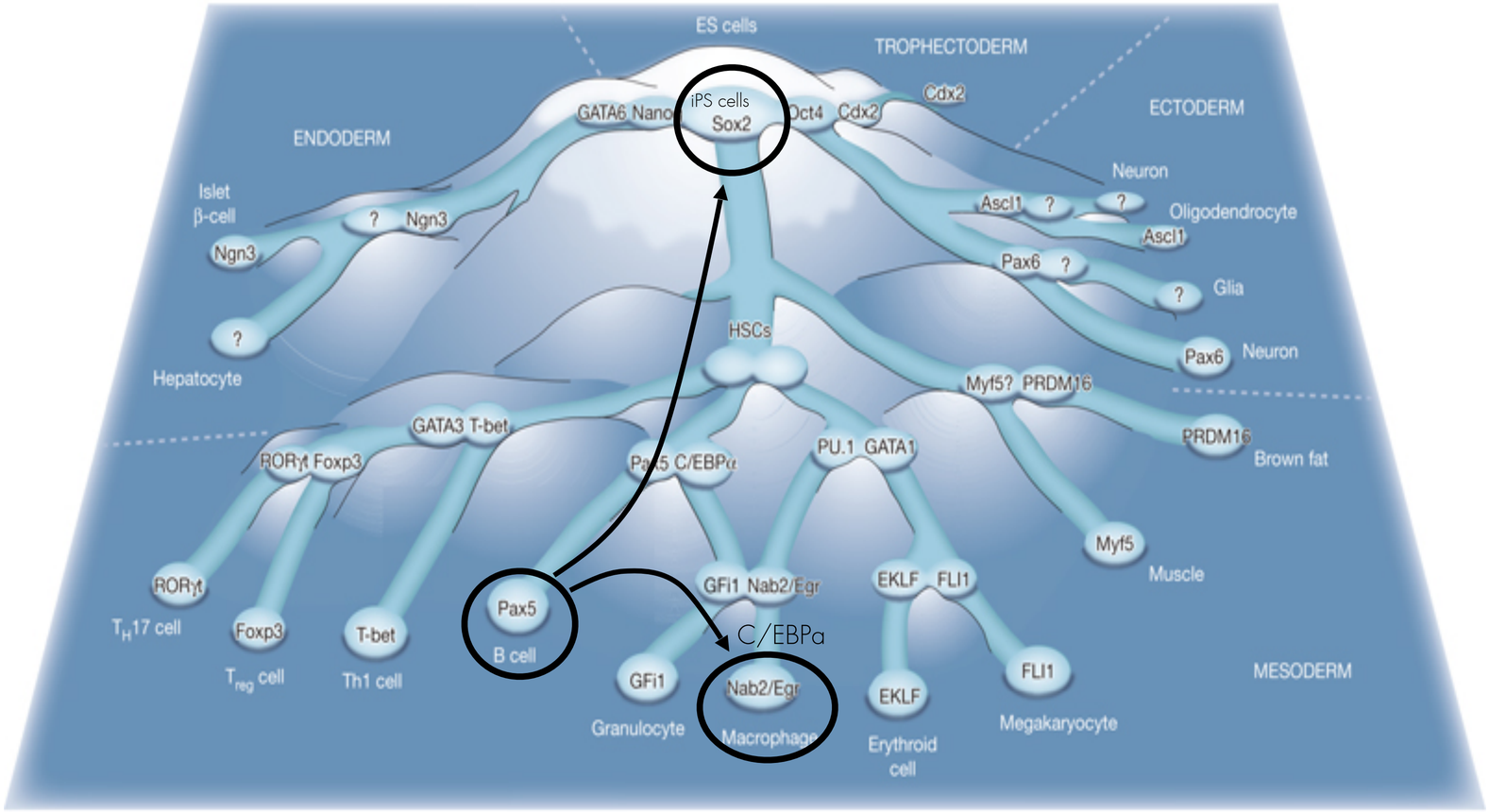


Marco di Stefano
Ralph Stadhouders
with Graf Lab (CRG, Barcelona)

Nature Genetics (2018) 50 238–249 & BioRxived

Transcription factors dictate cell fate

Graf & Enver (2009) Nature



Transcription factors (TFs) determine cell identity through gene regulation

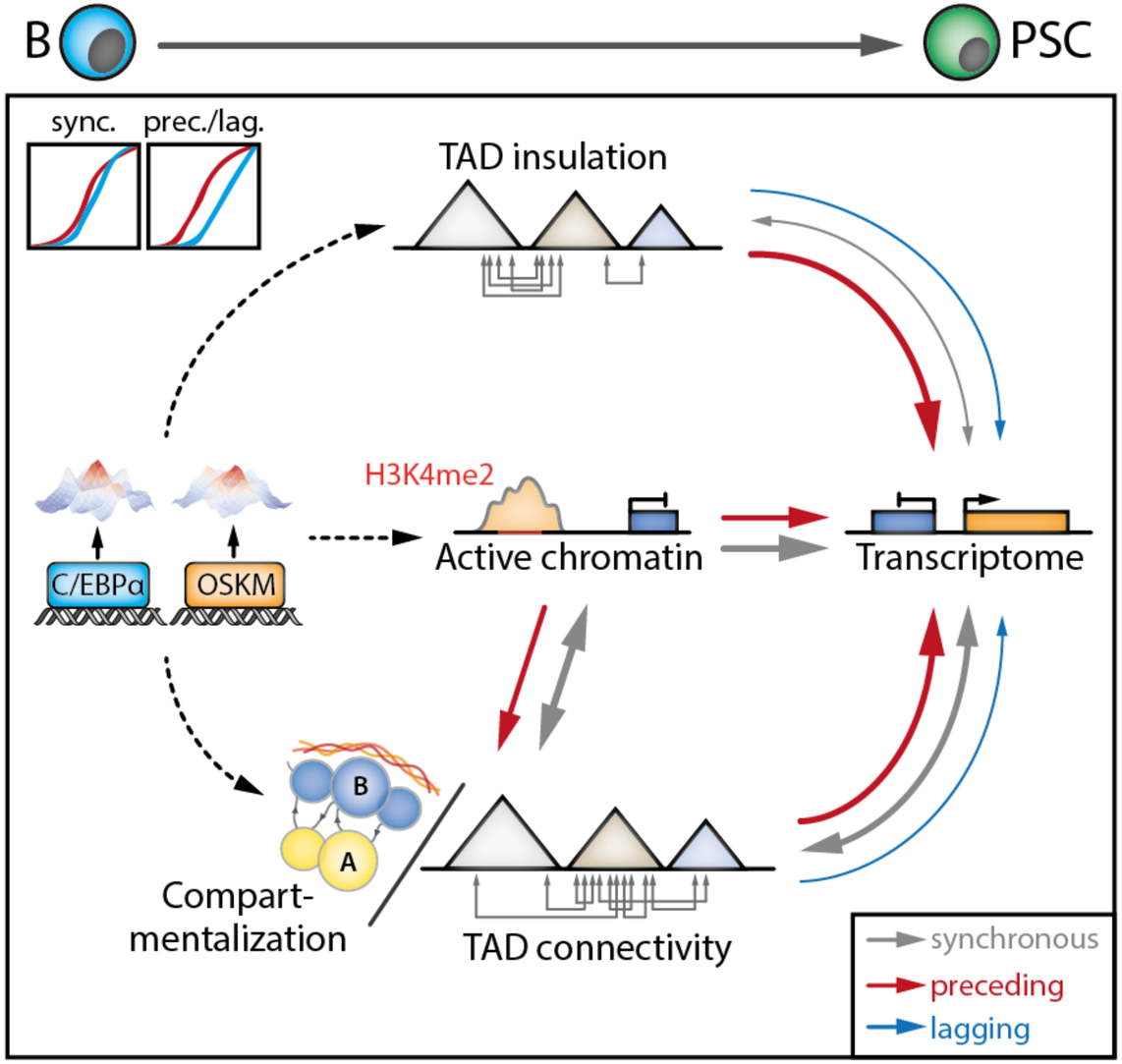
Normal 'forward' differentiation

Cell fates can be converted by enforced TF expression

Transdifferentiation or reprogramming

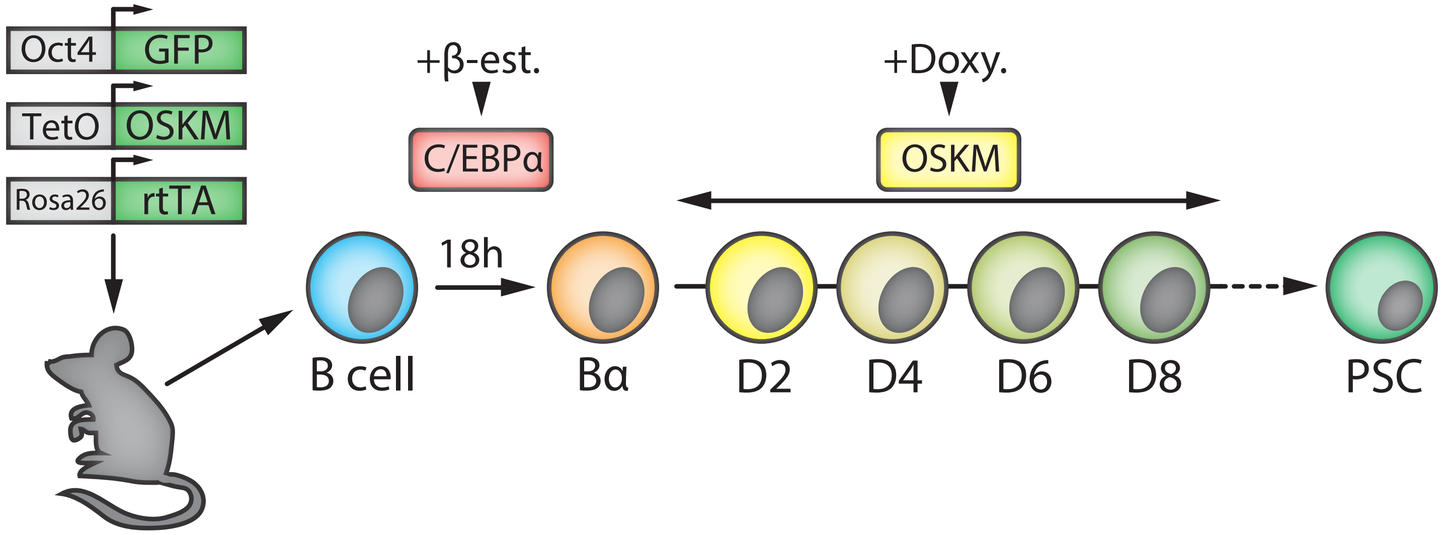
Interplay: topology, gene expression & chromatin

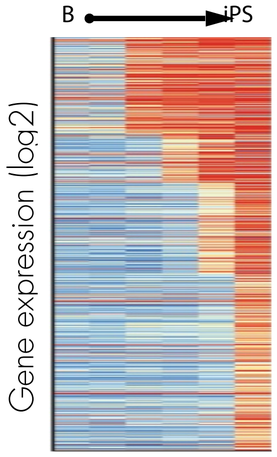
Stadhouders, R., Vidal, E. et al. (2018) Nature Genetics

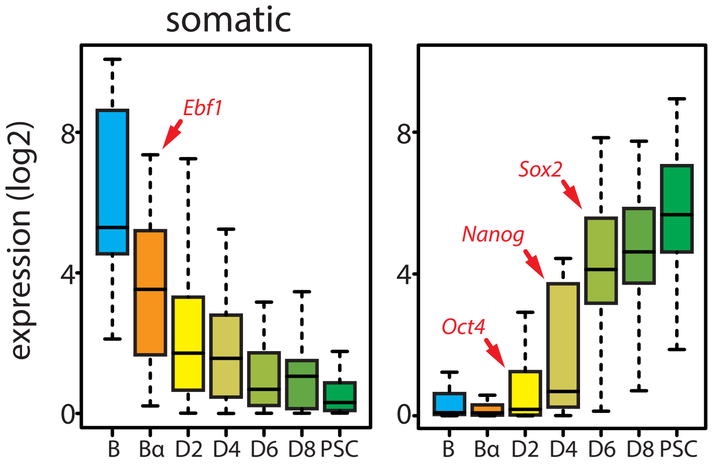


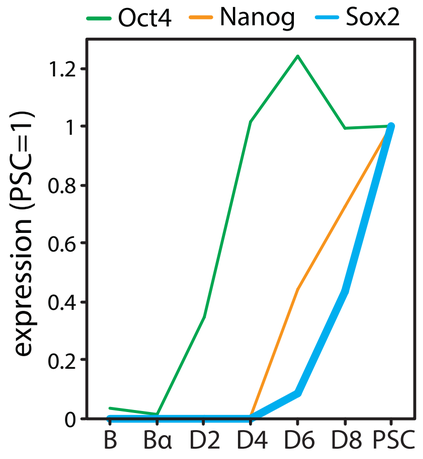
Reprogramming from B to PSC

Stadhouders, R., Vidal, E. et al. (2018) Nature Genetics



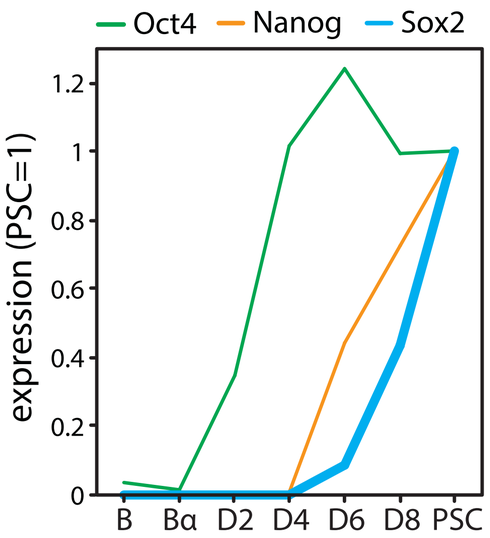


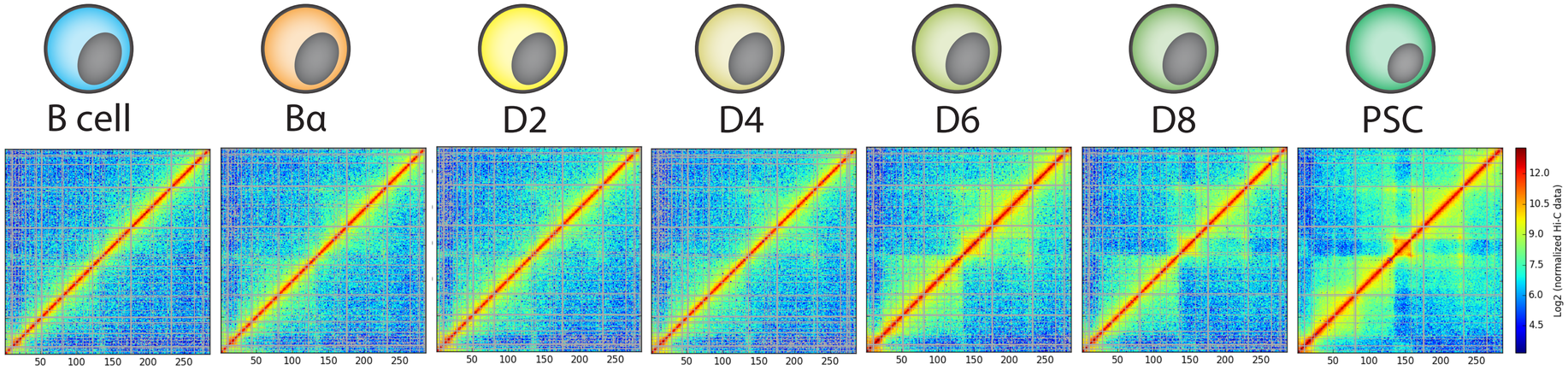




Hi-C maps of reprogramming from B to PSC

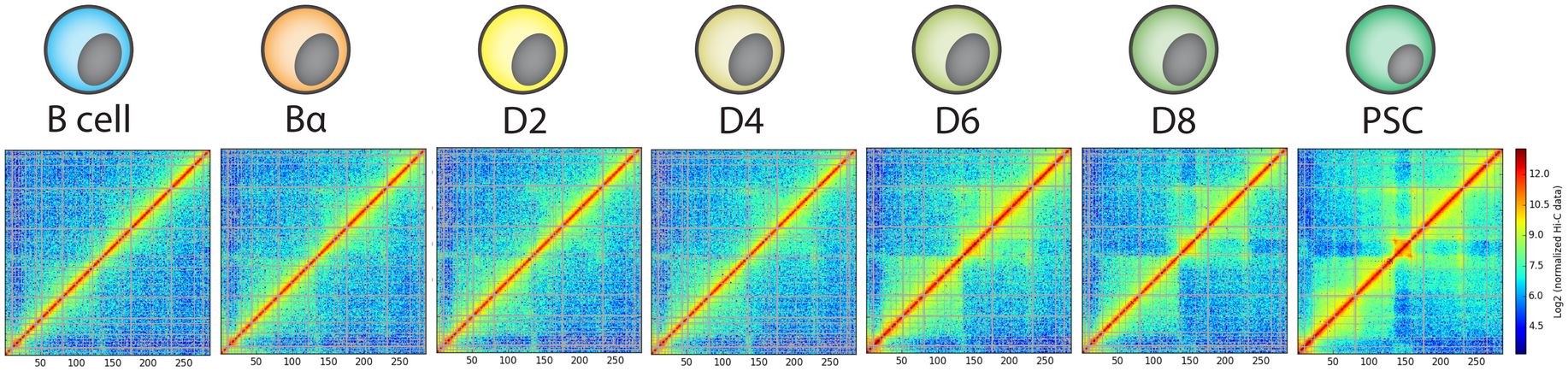
The SOX2 locus





Hi-C maps of reprogramming from B to PSC

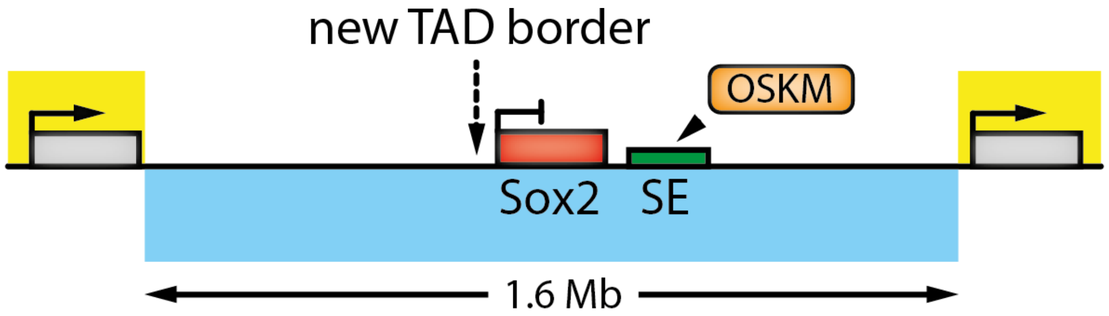
The SOX2 locus

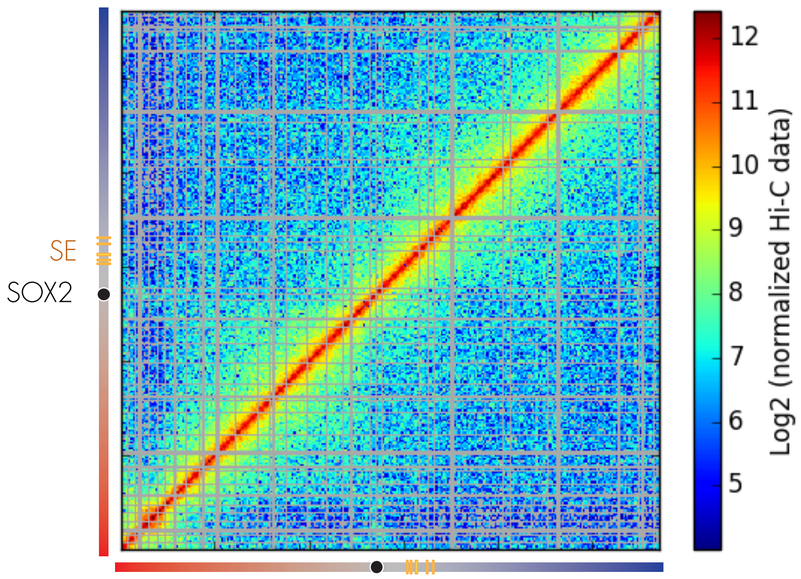


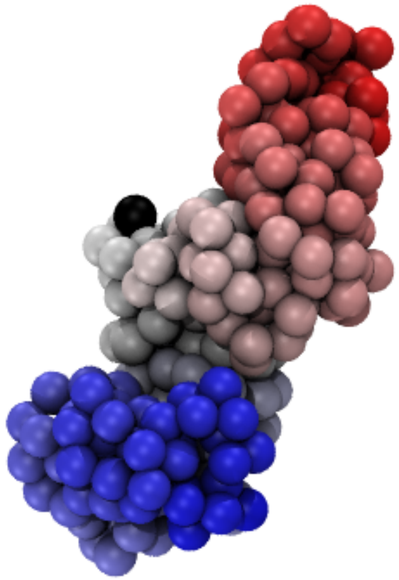
How does these structural rearrangements interplay with the transcription activity?

What are the main drivers of structural transitions?

TADbit modeling of SOX2 from B cells Hi-C





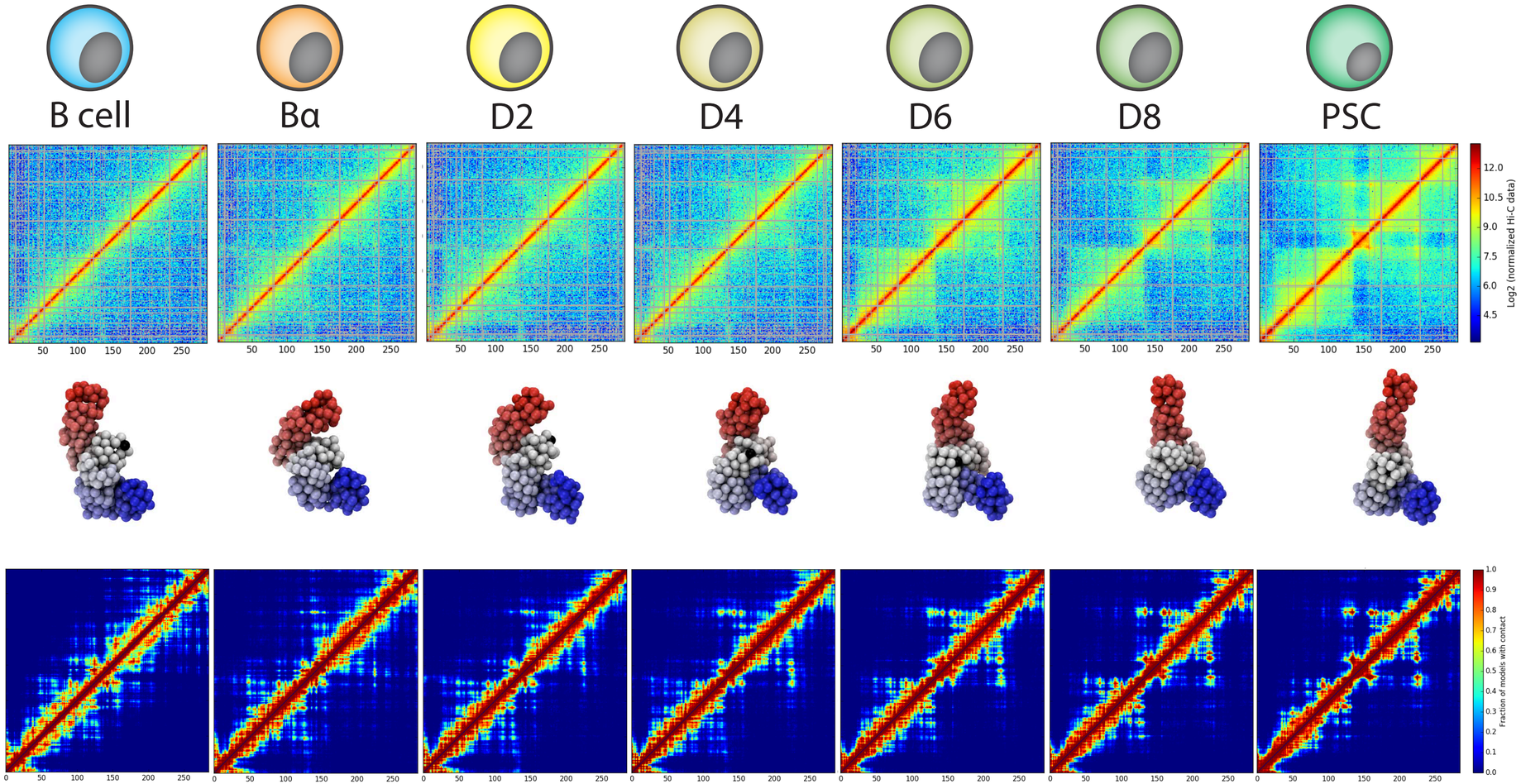


Optimal IMP parameters

lowfreq=0 , upfreq=1 , maxdist=200nm, dcutoff=125nm, particle size=50nm (5kb)

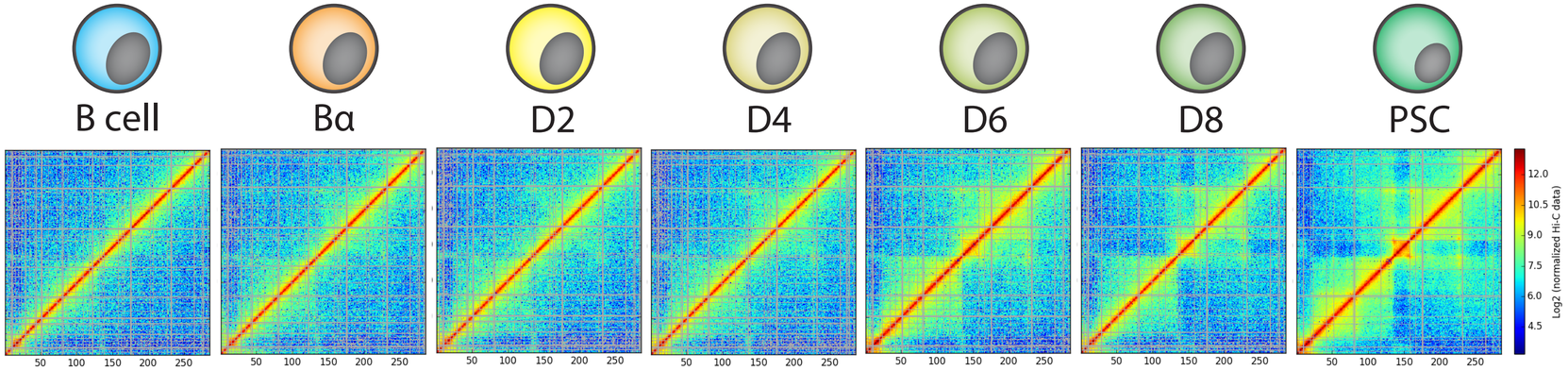
Models of reprogramming from B to PSC

The SOX2 locus



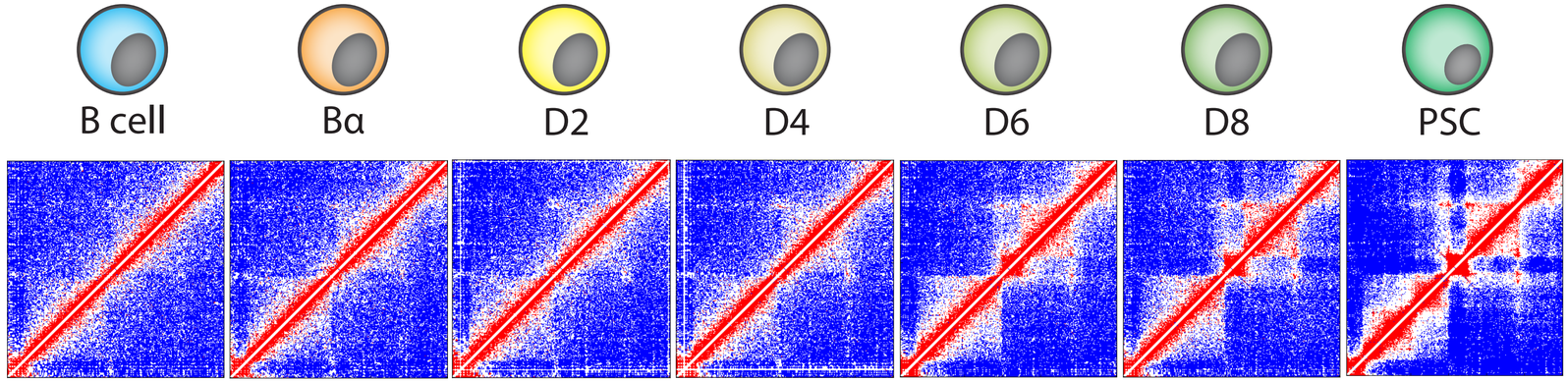
TADdyn: from time-series Hi-C maps to dynamic restraints

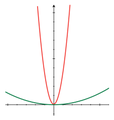
The SOX2 locus



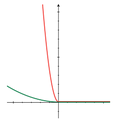
TADdyn: from time-series Hi-C maps to dynamic restraints

The SOX2 locus





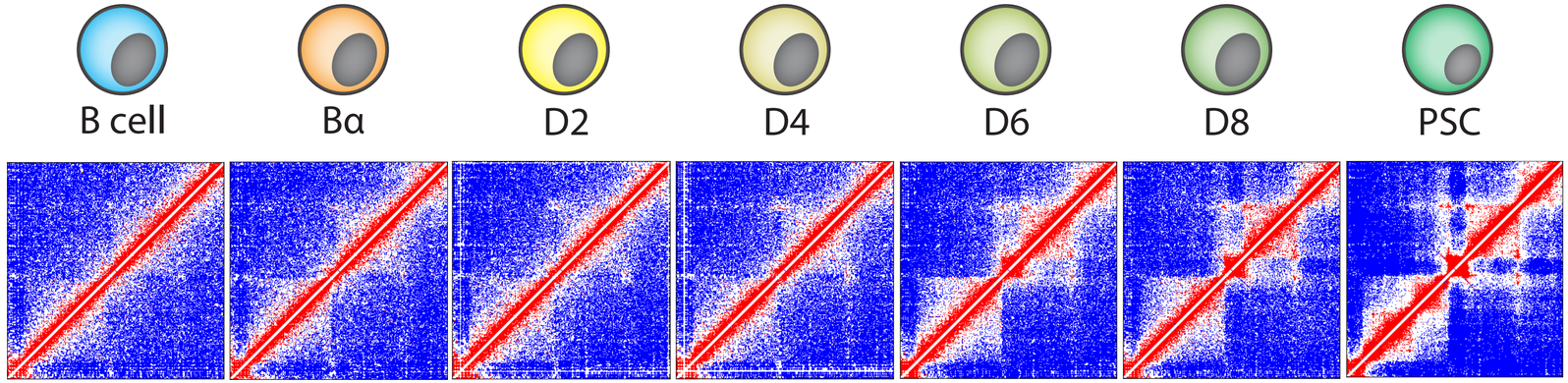
Harmonic

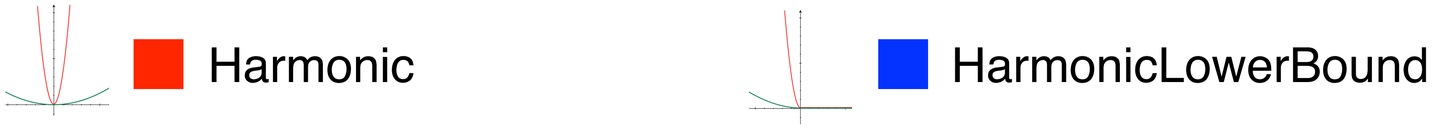


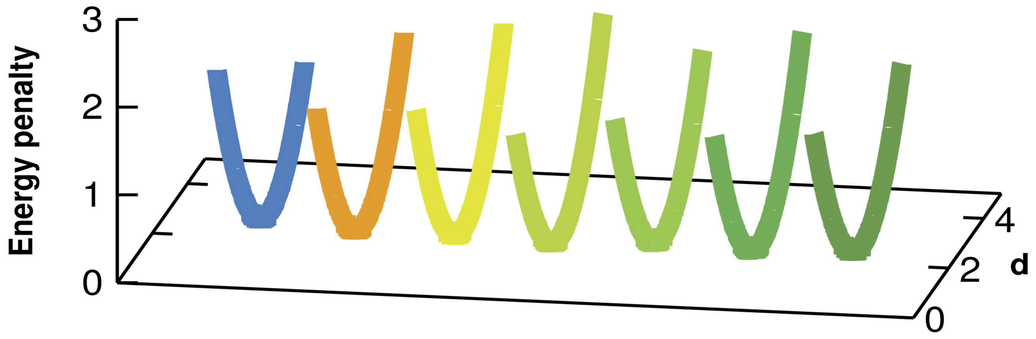
HarmonicLowerBound

TADdyn: from time-series Hi-C maps to dynamic restraints

The SOX2 locus



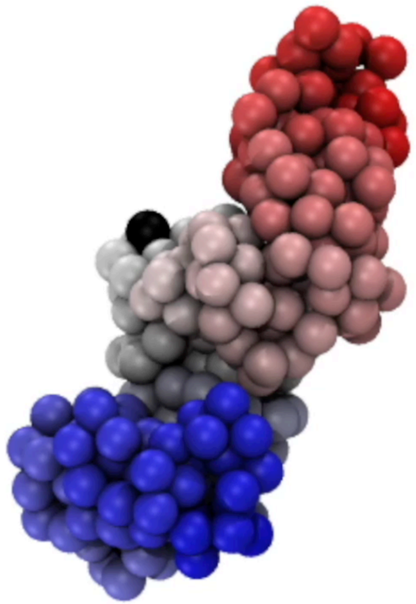


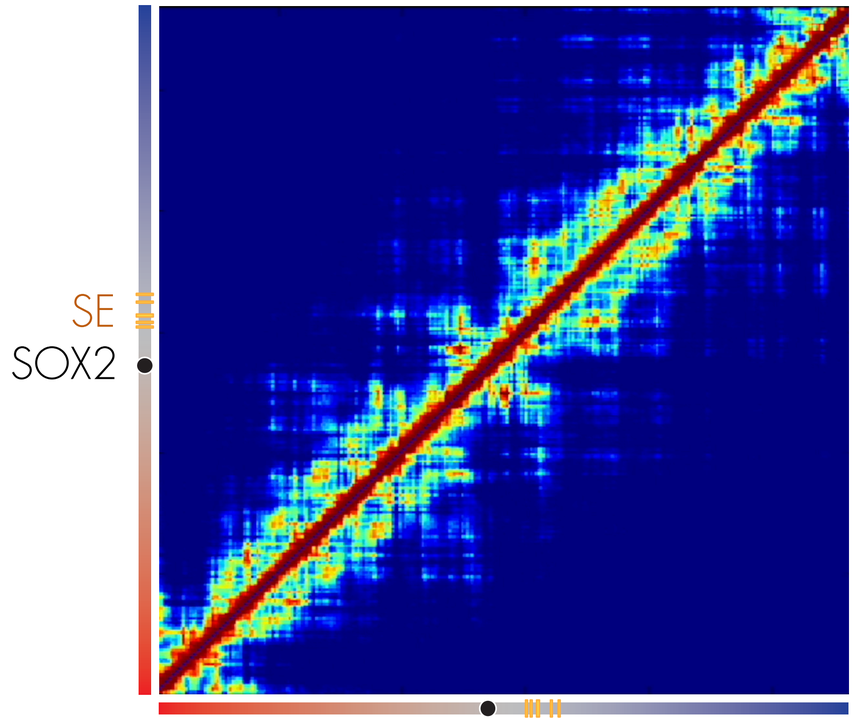


Transition	Stable	Vanishing	Raising
B -> Bα	18,612	6,984	7,290
Bα -> D2	18,512	7,390	6,687
D2 -> D4	18,369	6,830	6,893
D4 -> D6	18,971	6,291	7,289
D6 -> D8	20,167	6,093	6,250
D8 -> ES	20,679	5,738	6,173

SOX2 locus structural changes from B to PSC

Contacts





SOX2 locus structural changes from B to PSC

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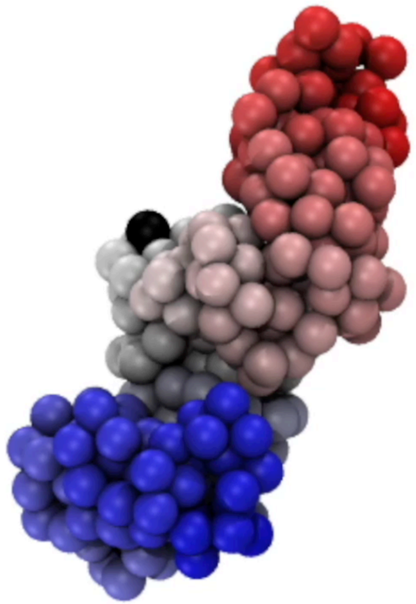
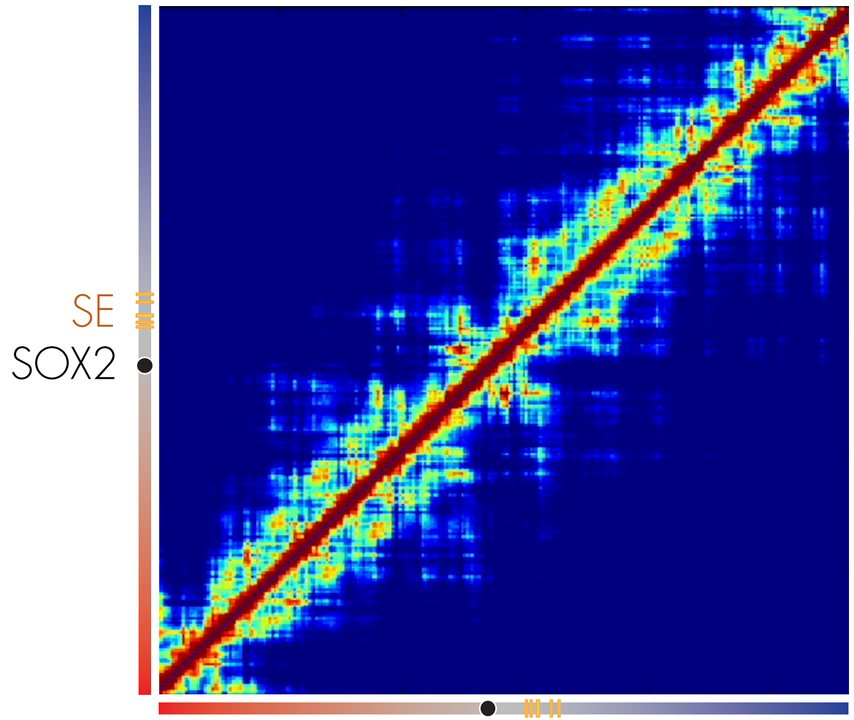
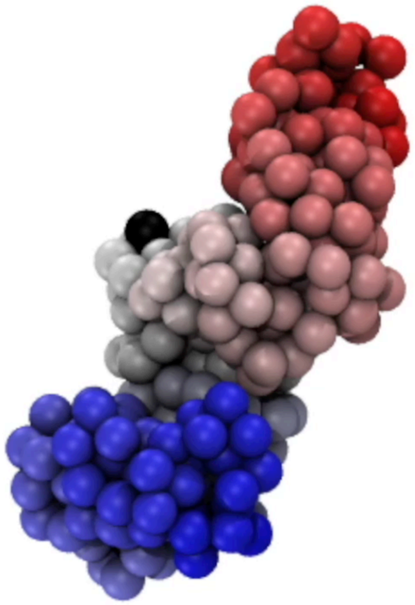
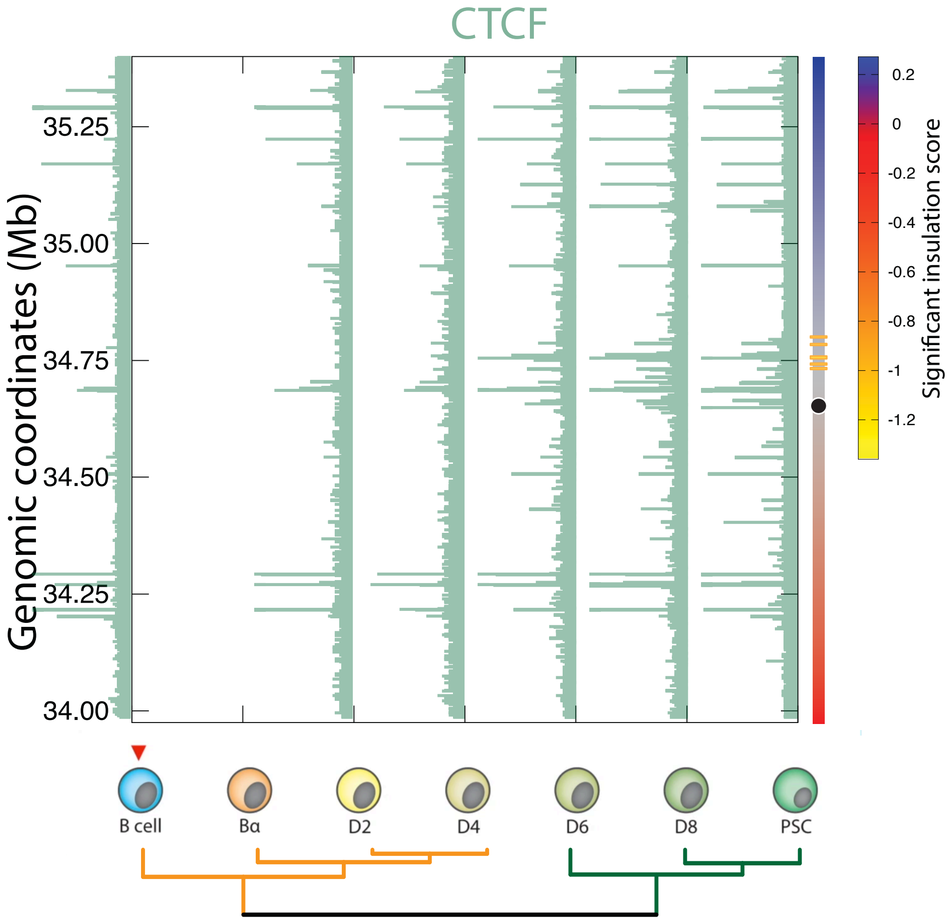




SOX2 locus structural changes from B to PSC

TAD borders





SOX2 locus structural changes from B to PSC

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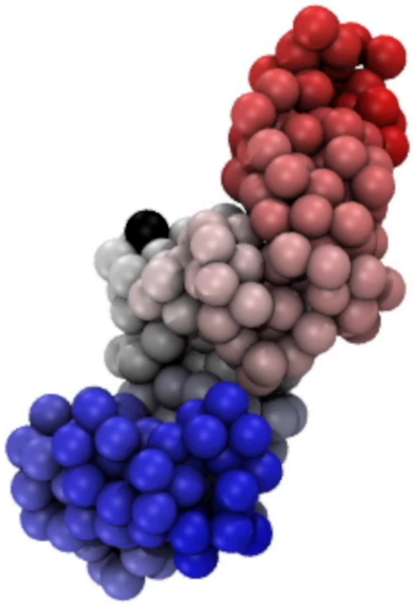
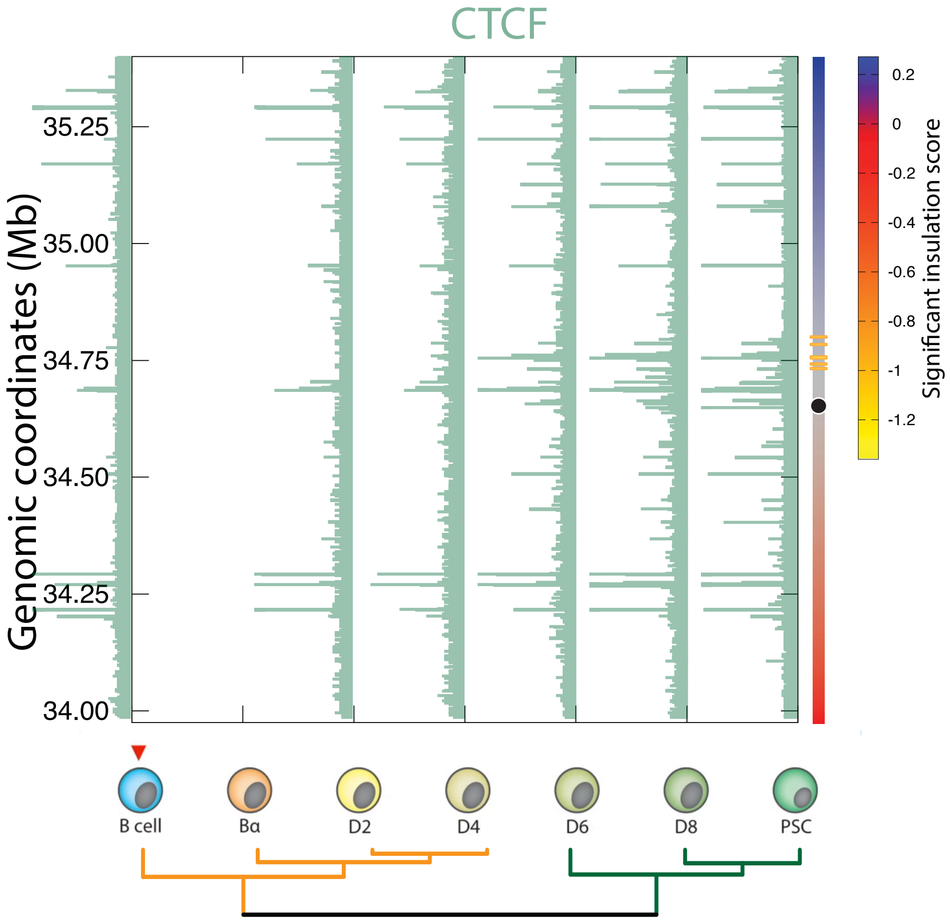
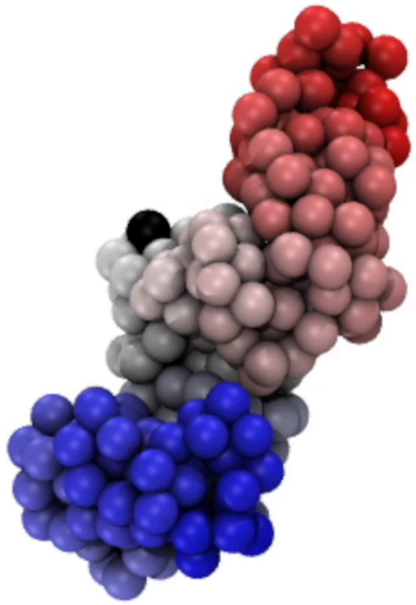
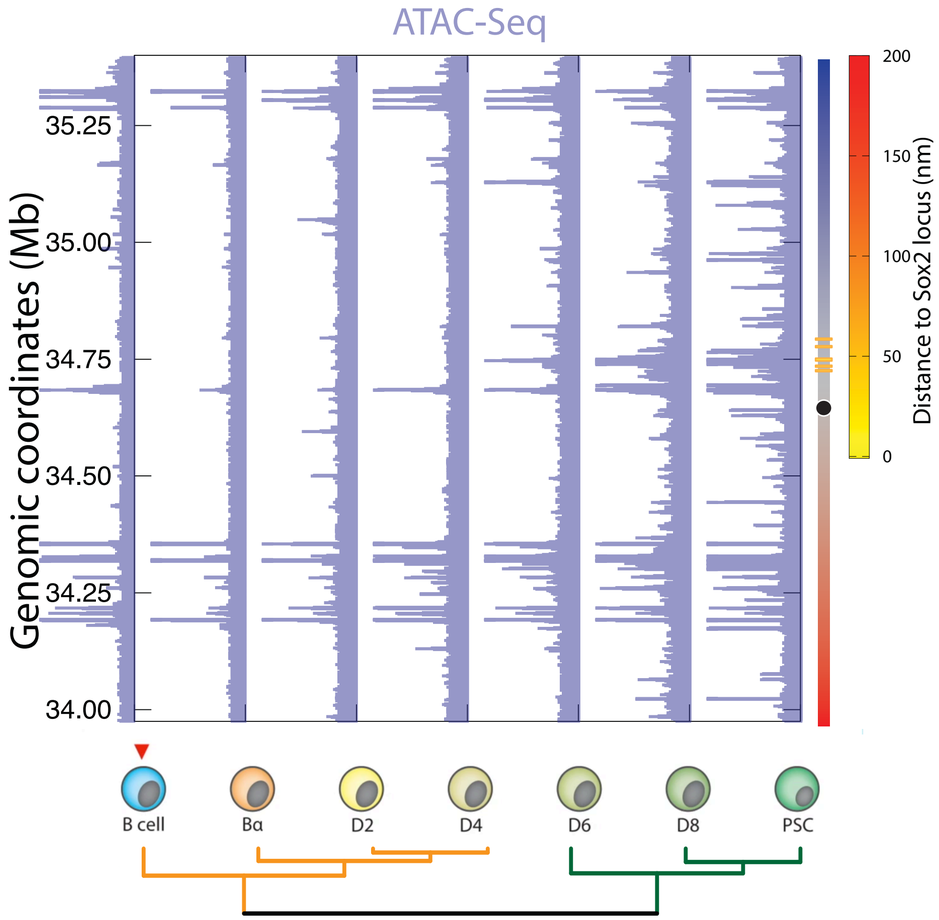




SOX2 locus structural changes from B to PSC

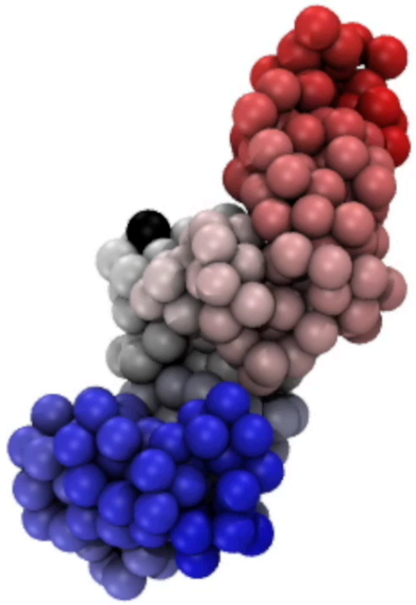
Distance to regulatory elements

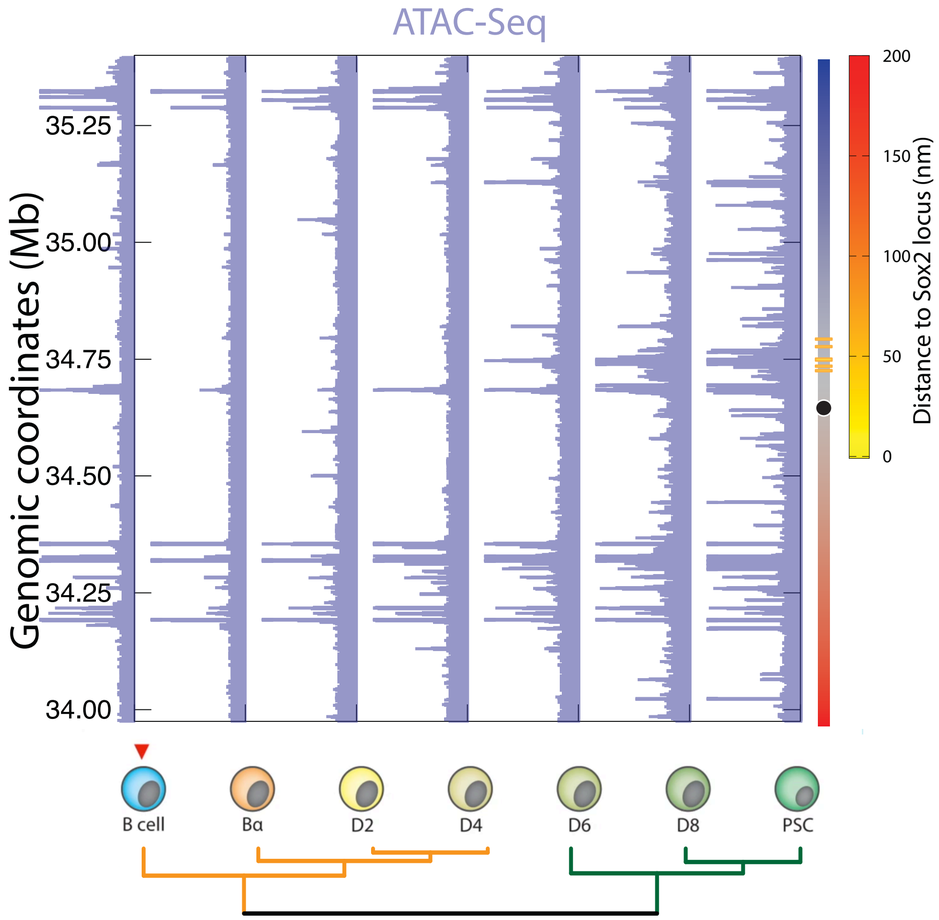




SOX2 locus structural changes from B to PSC

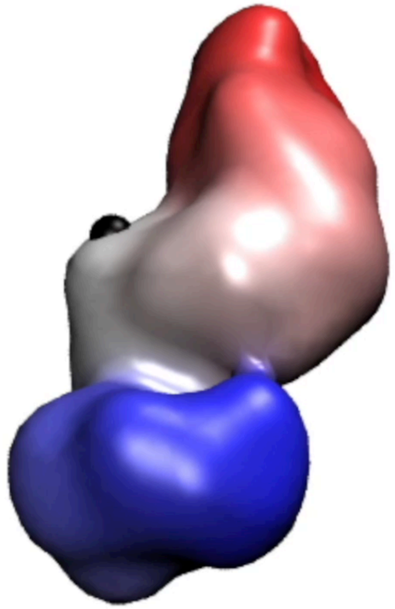
Distance to regulatory elements

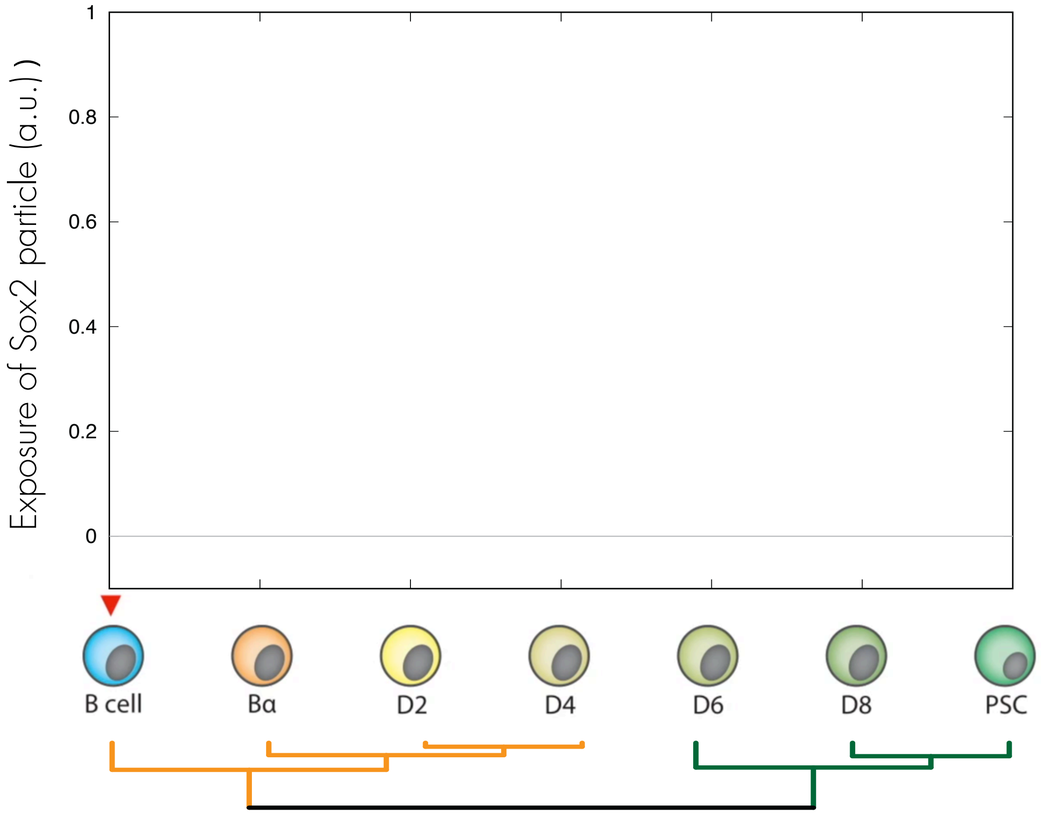




SOX2 locus structural changes from B to PSC

Structural exposure





SOX2 locus structural changes from B to PSC

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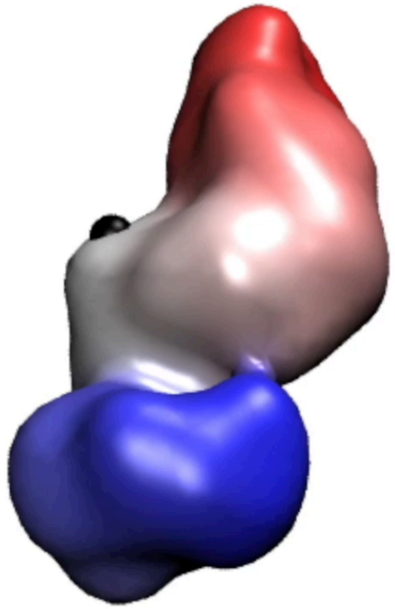
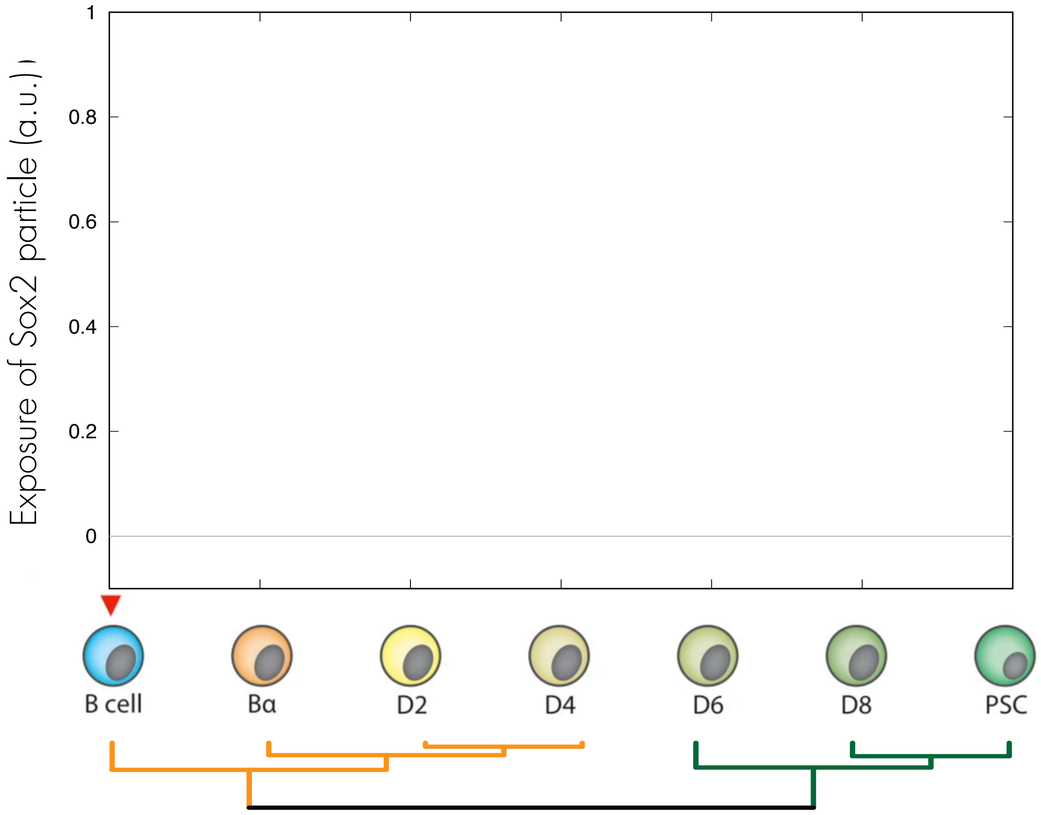
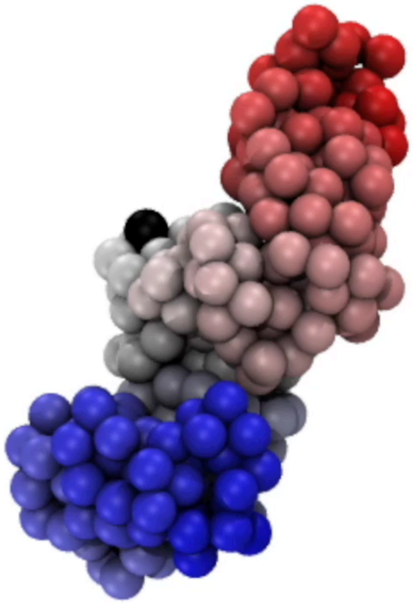
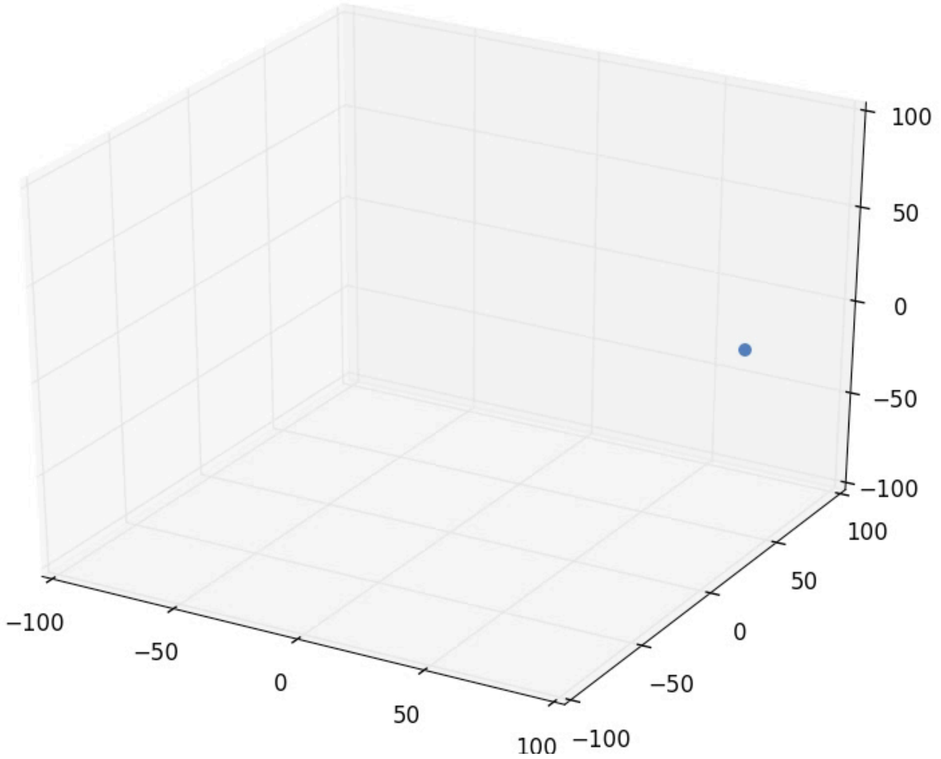


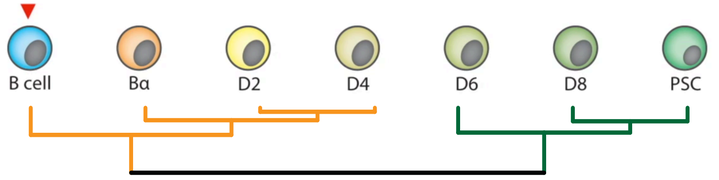


SOX2 locus dynamics changes from B to PSC

SOX2 displacement

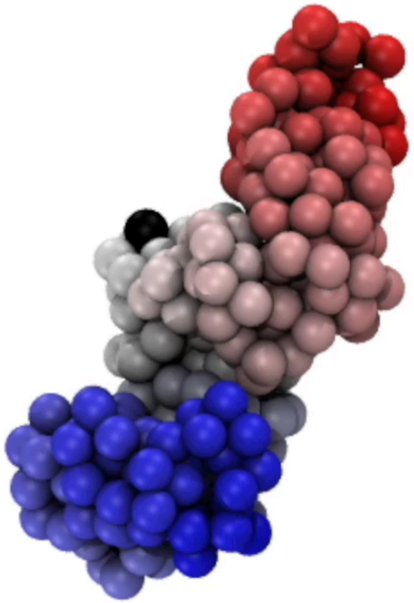


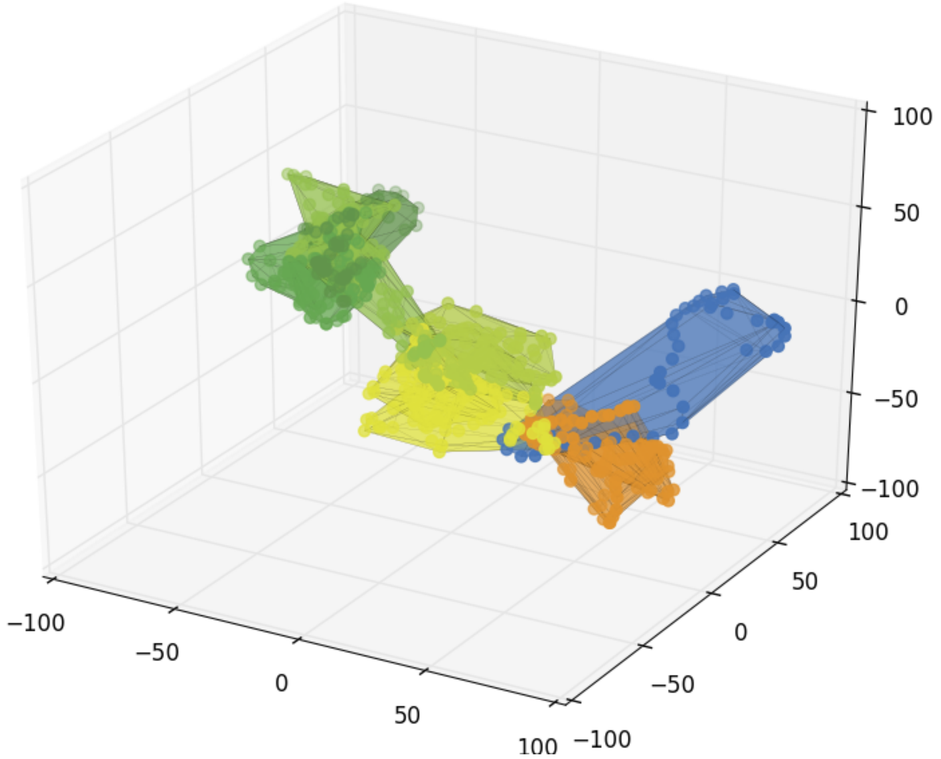


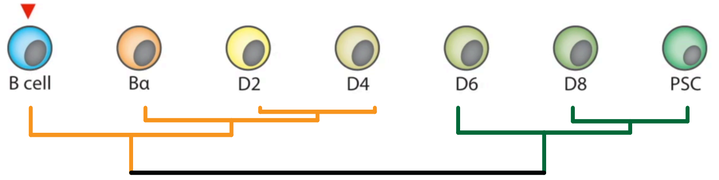


SOX2 locus dynamics changes from B to PSC

SOX2 displacement

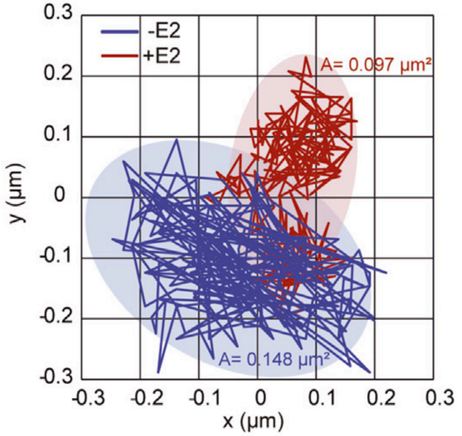






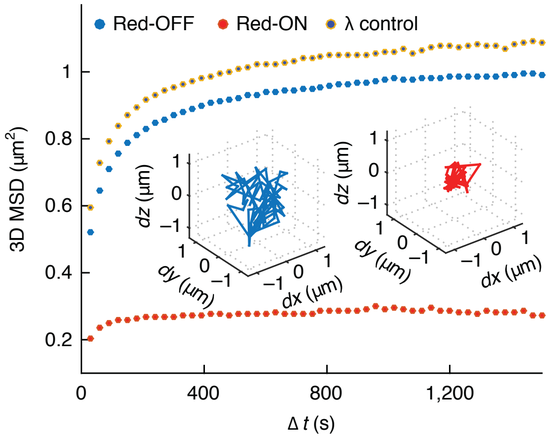
SOX2 locus dynamics changes from B to PSC

SOX2 displacement



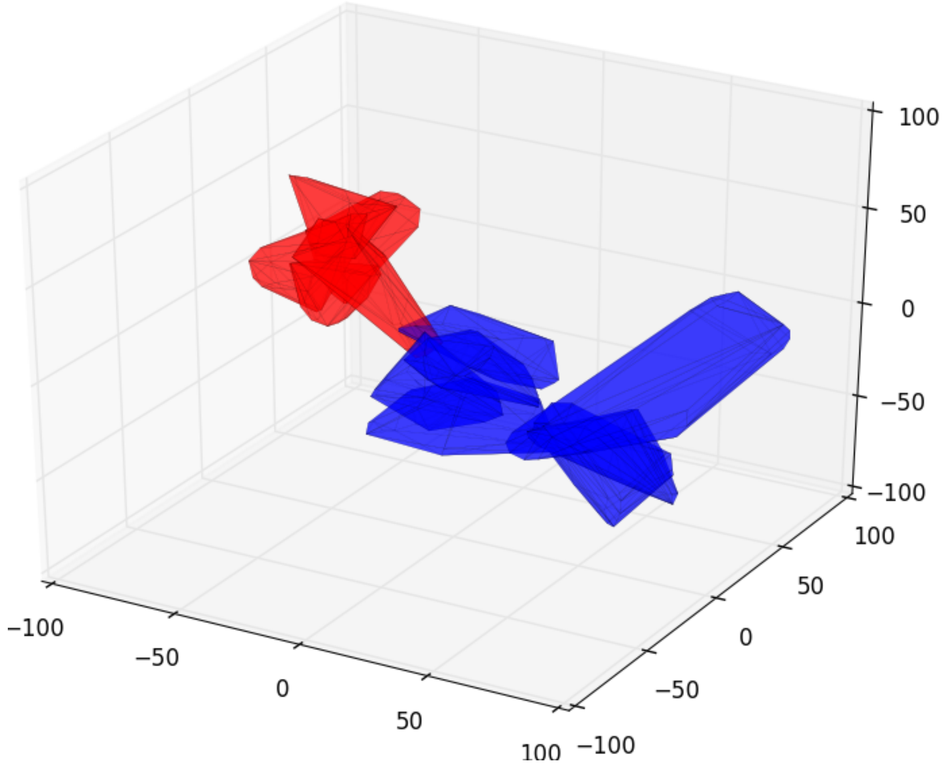
Two dimensional trajectories and area explored over 50s of the CCND1 locus recored before -E2 and after +E2 activation.

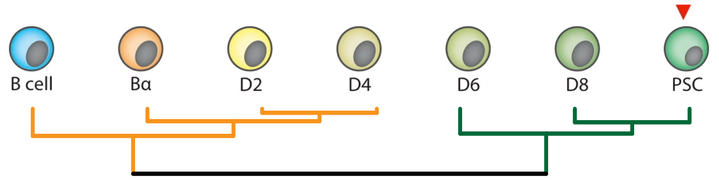
Germier ,T., et al, (2017) Biophys J.



Transcription affects the 3D topology of the enhancer-promoted enhancing its temporal stability and is associated with further spatial compaction.

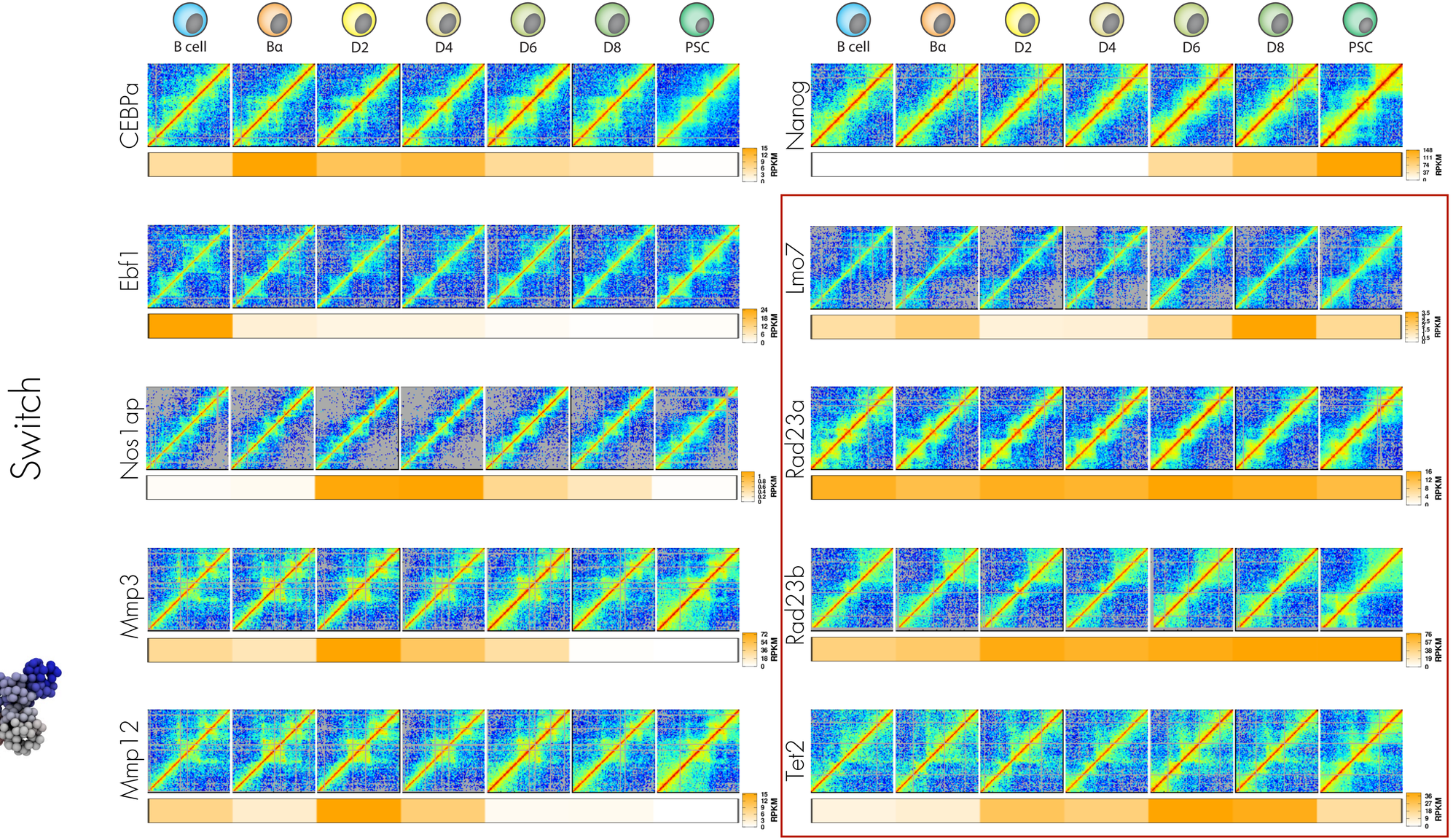
Chen ,T., et al, (2018) Nat. Genetics





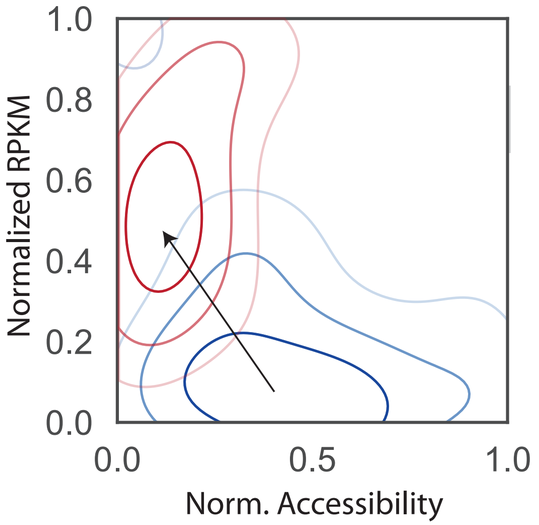
Structural changes from B to PSC

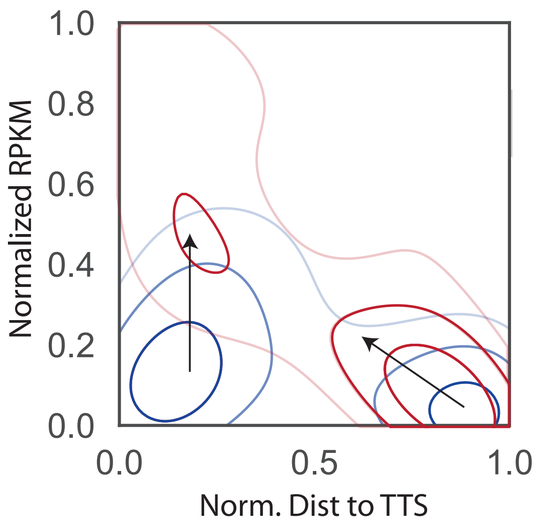
Other 10 loci



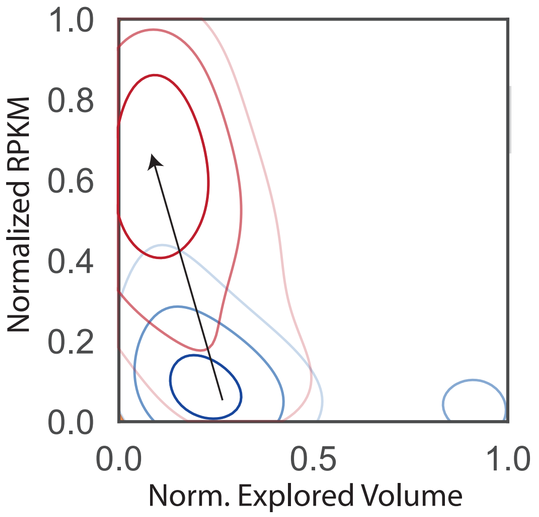
Dynamics of gene activation

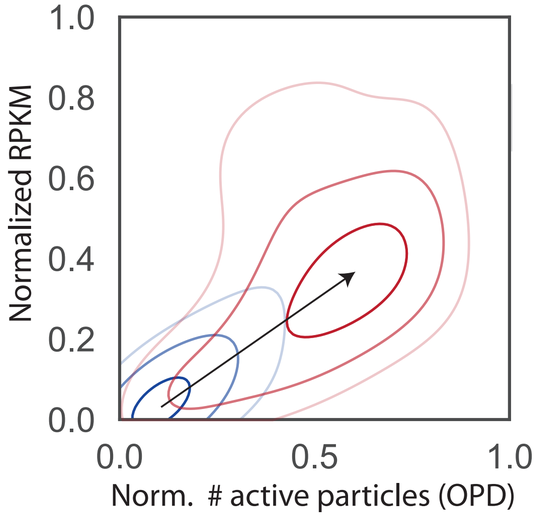
Trends in all 11 loci



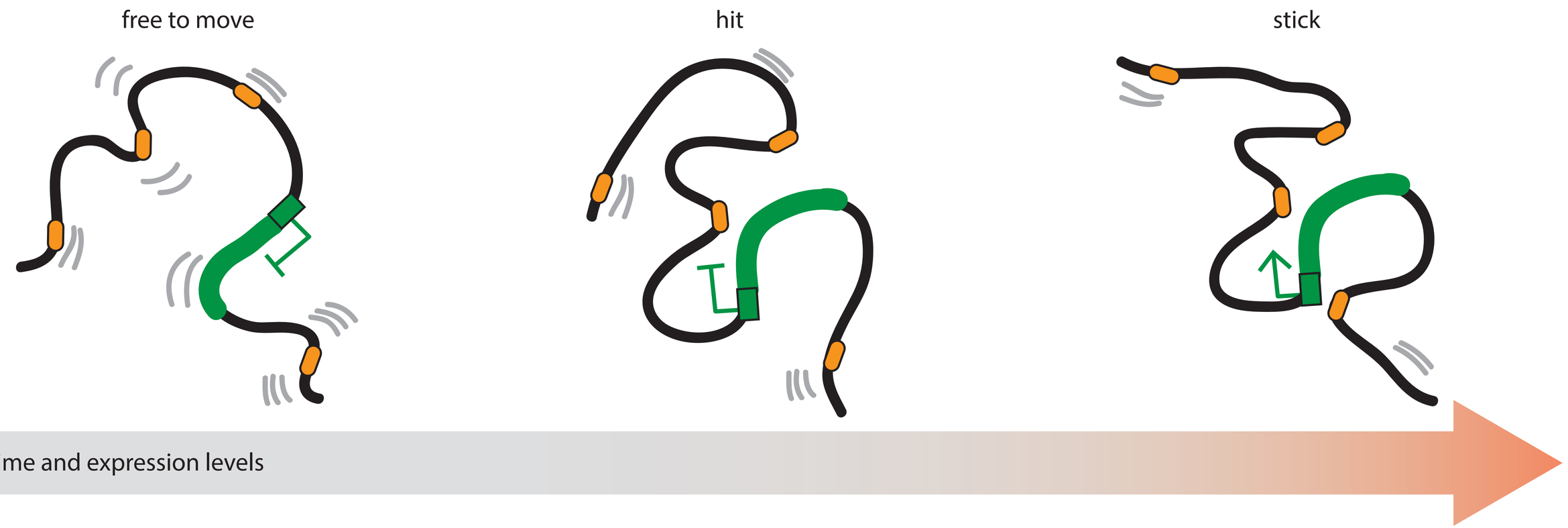


Active loci
Switching loci





A “hit-and-stick” model for transcriptional activation



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