



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

**Marc A. Marti-Renom**

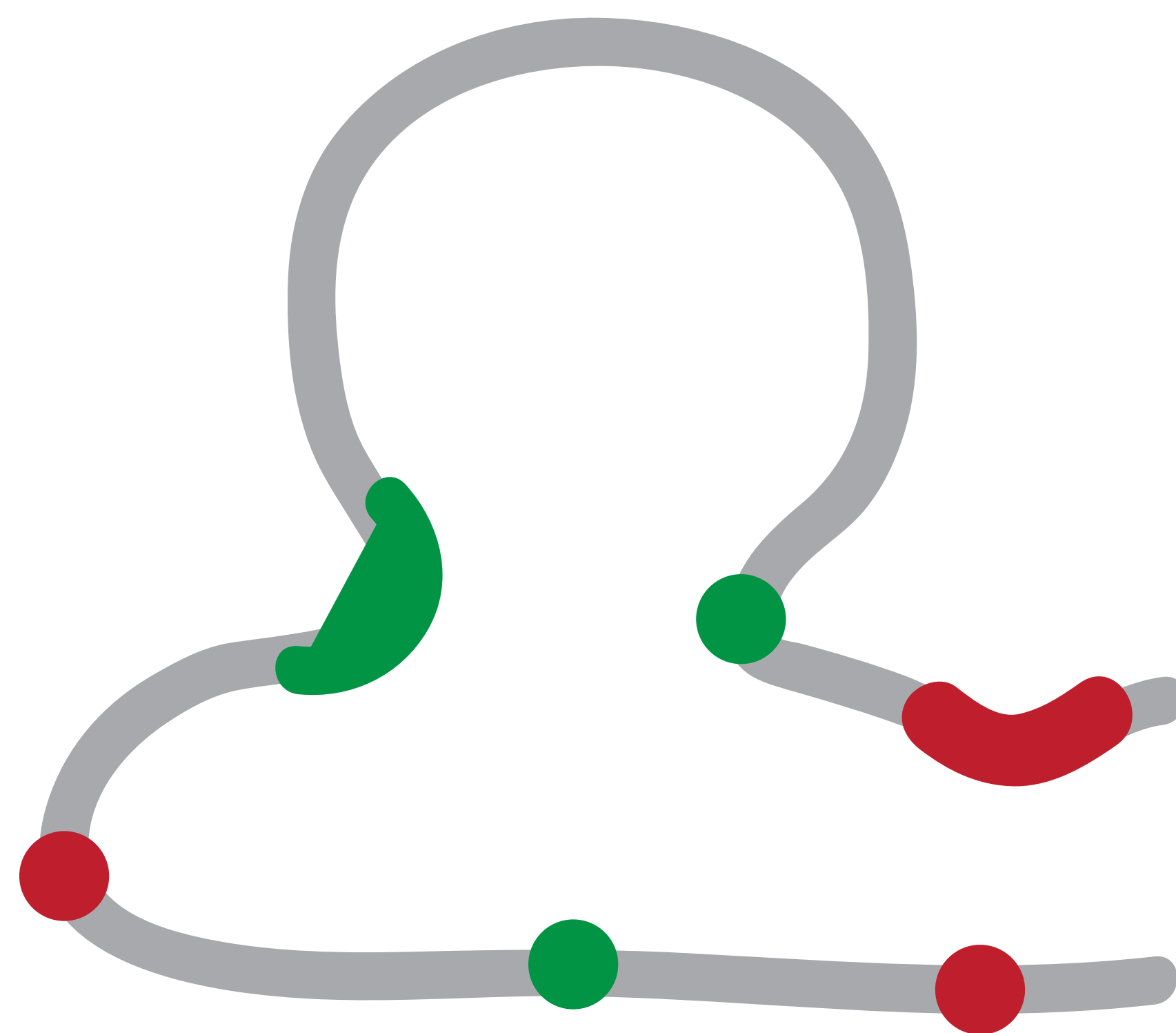
CNAG-CRG · ICREA

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

HUMAN “The Movie” by Yann Arthus-Bertrand

**cnag** CRG<sup>®</sup> ICREA

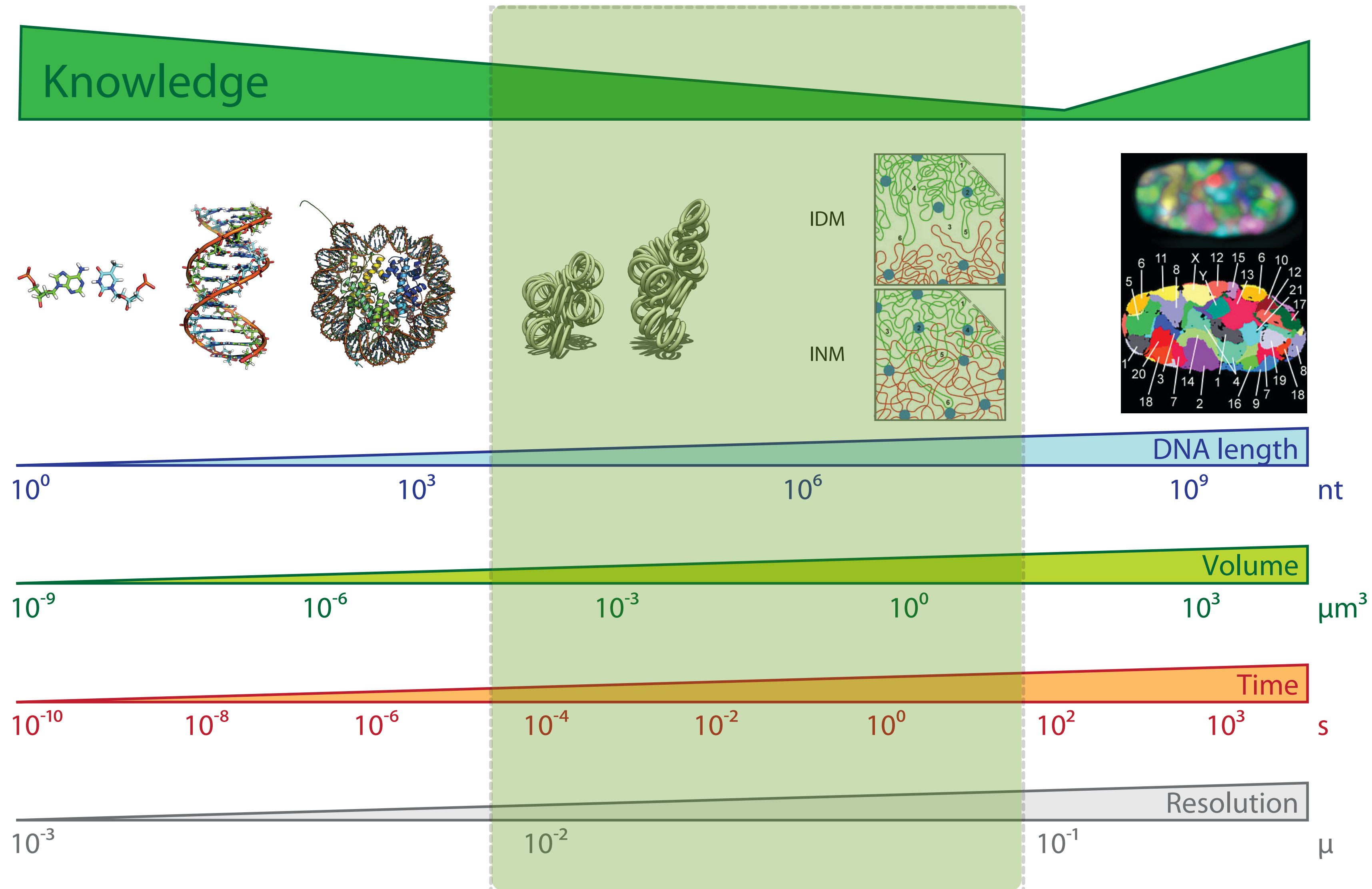






# Resolution Gap

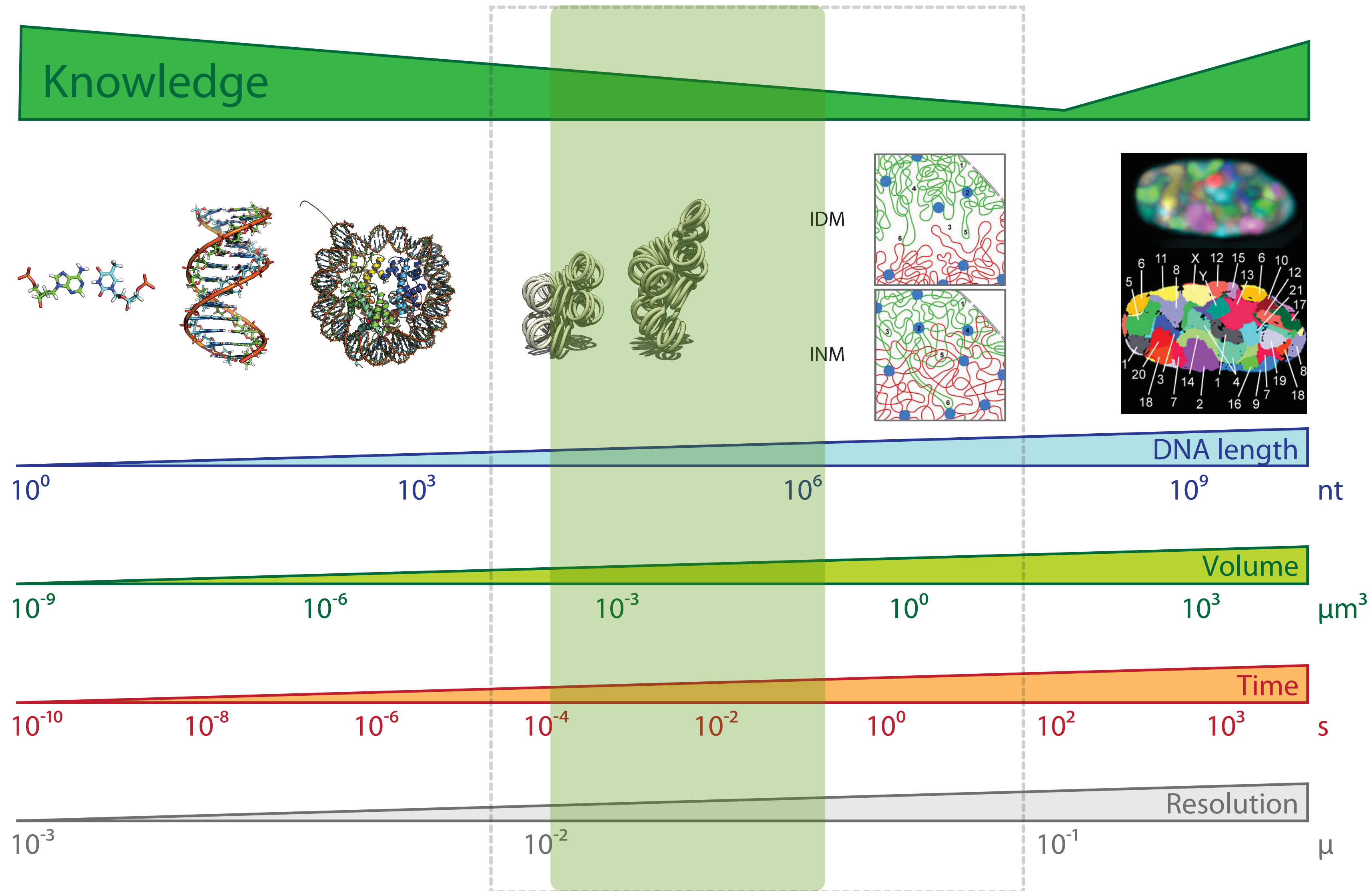
Marti-Renom, M. A. & Mirny, L. A. PLoS Comput Biol 7, e1002125 (2011)





# Resolution Gap

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

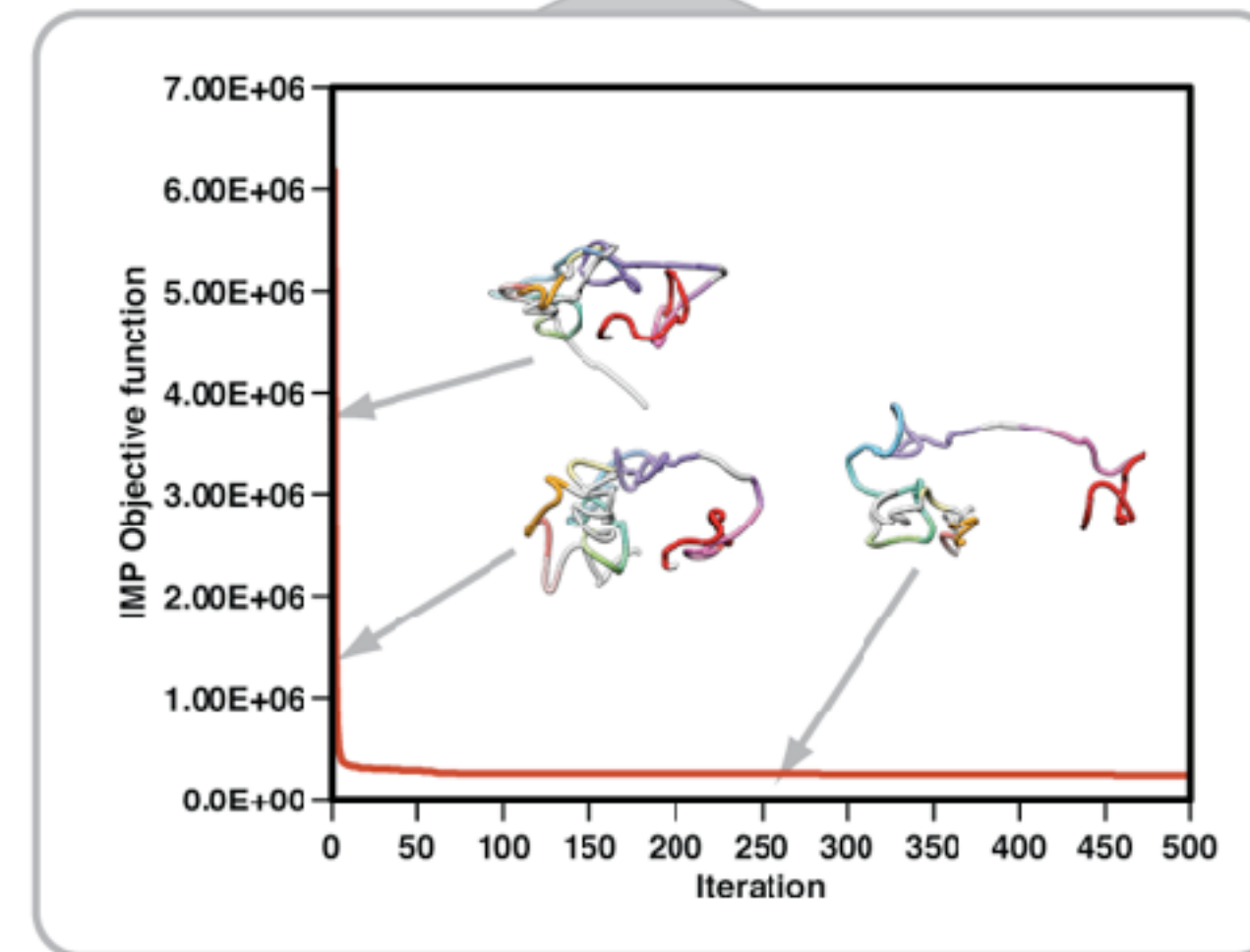
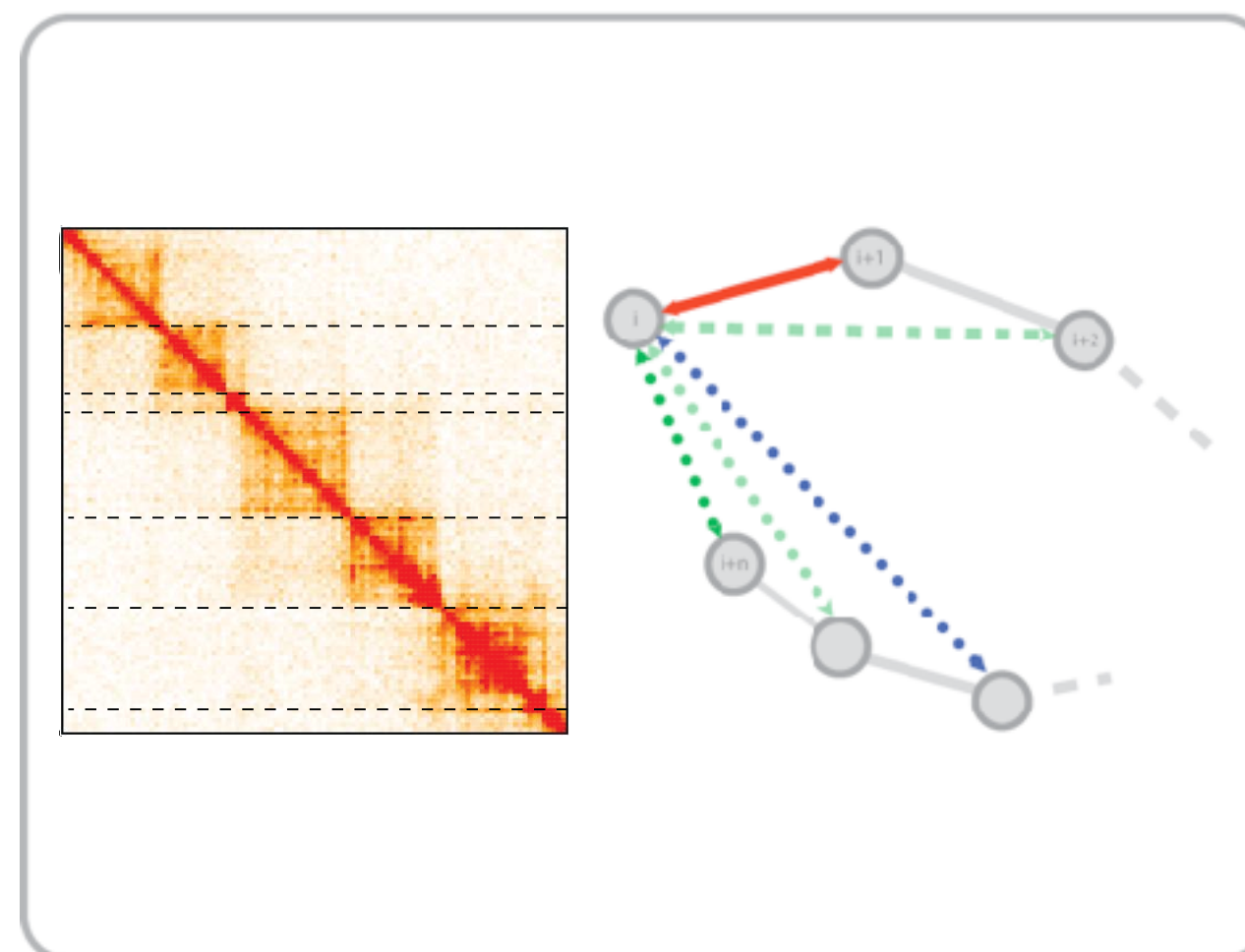
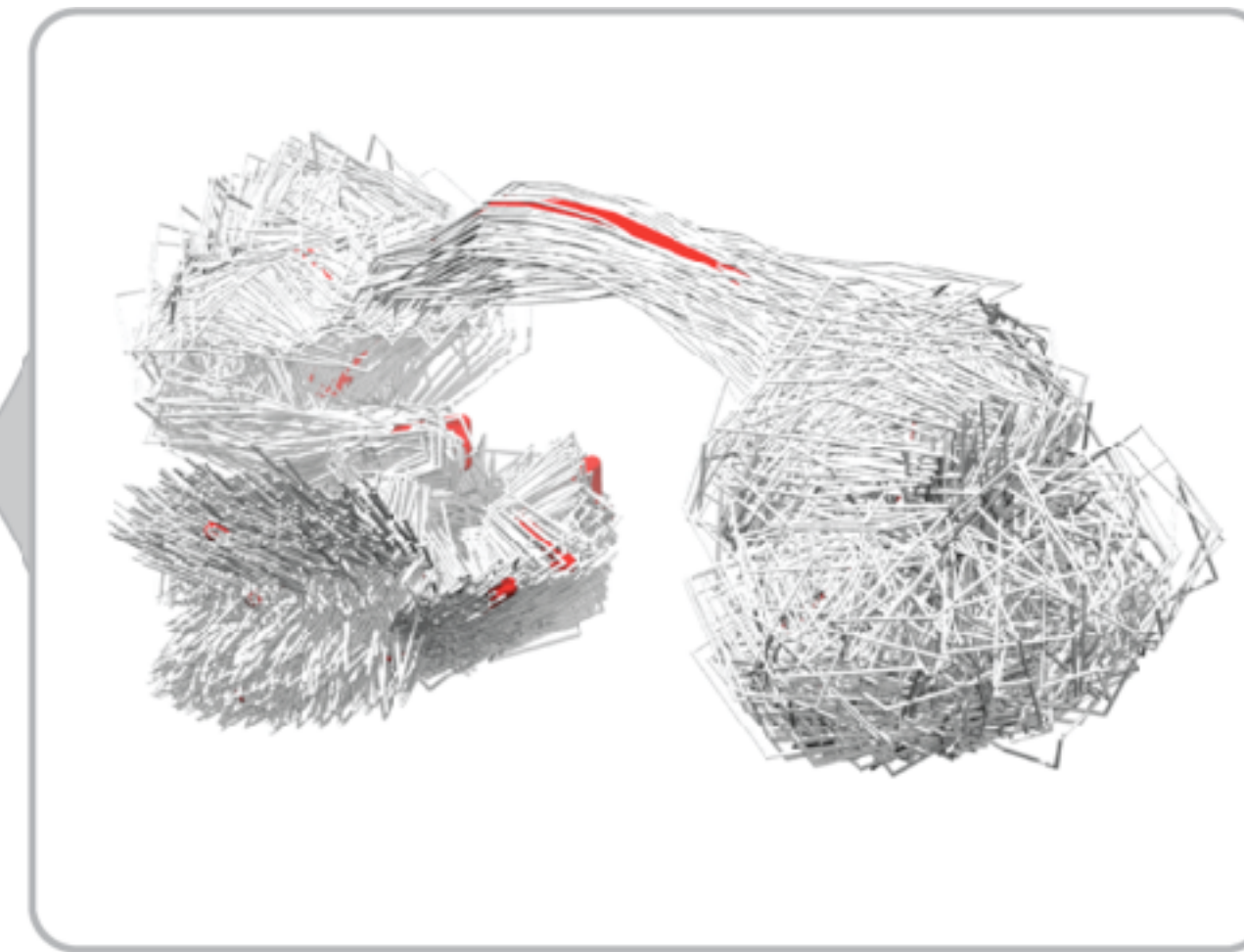
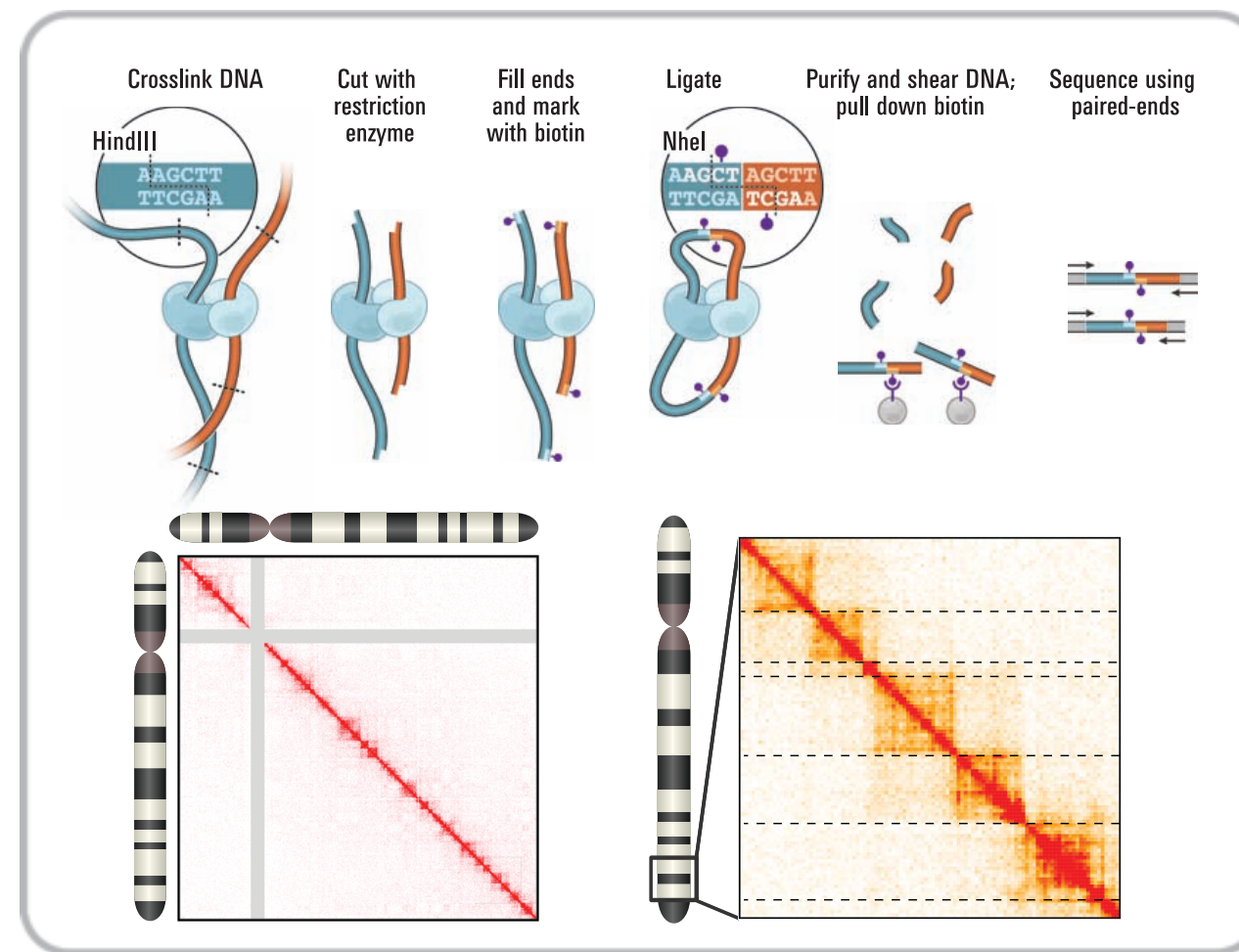




# Hybrid Method

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

## Experiments



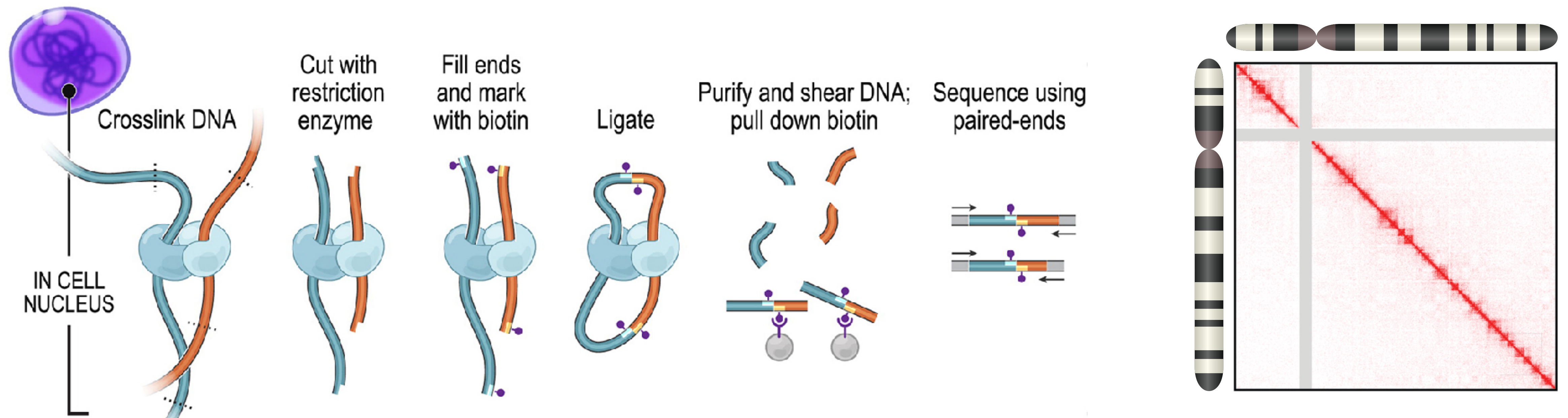
## Computation



# Chromosome Conformation Capture

Dekker, J., Rippe, K., Dekker, M., & Kleckner, N. (2002). *Science*, 295(5558), 1306–1311.

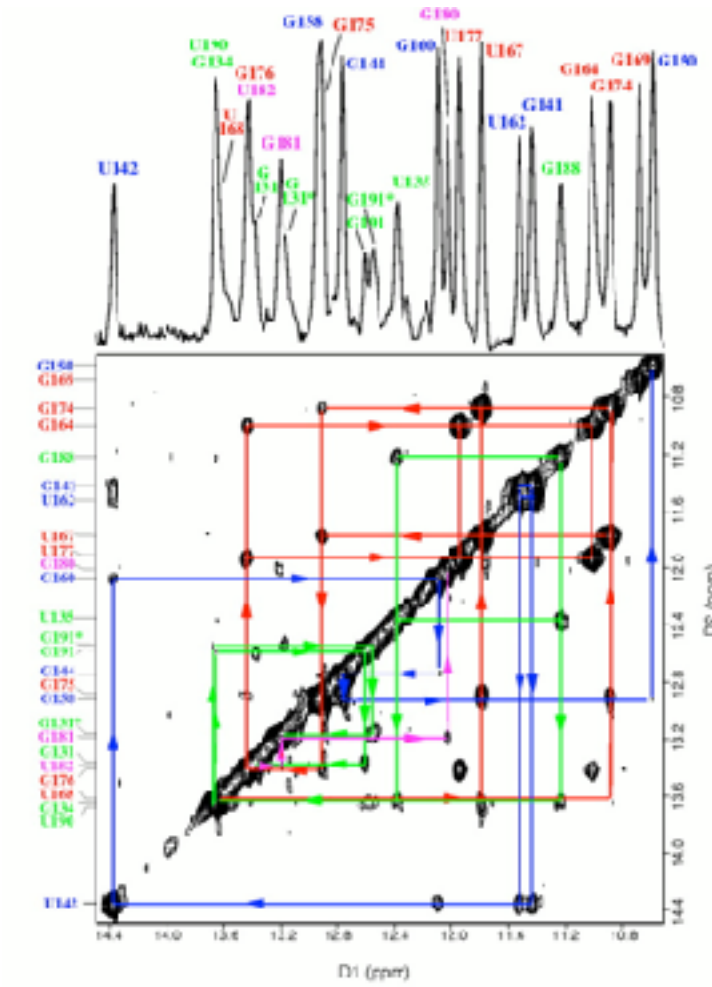
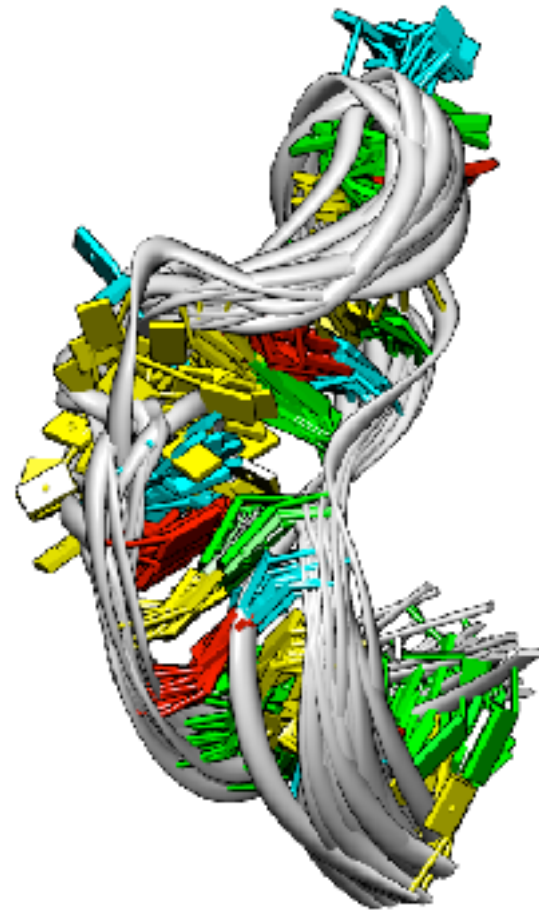
Lieberman-Aiden, E., et al. (2009). *Science*, 326(5950), 289–293.



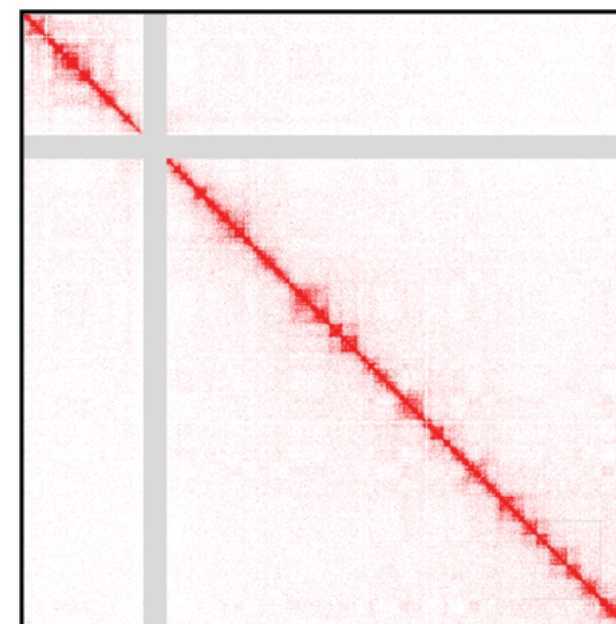
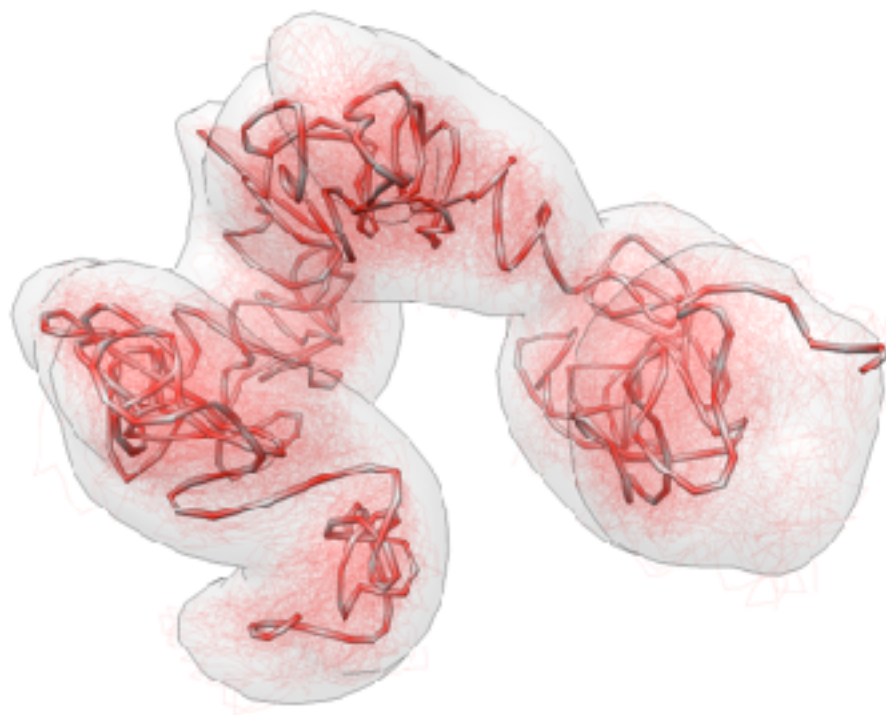


# Restraint-based Modeling

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



Biomolecular structure determination  
2D-NOESY data



# Chromosome structure determination

## 3C-based data





<http://3DGenomes.org>

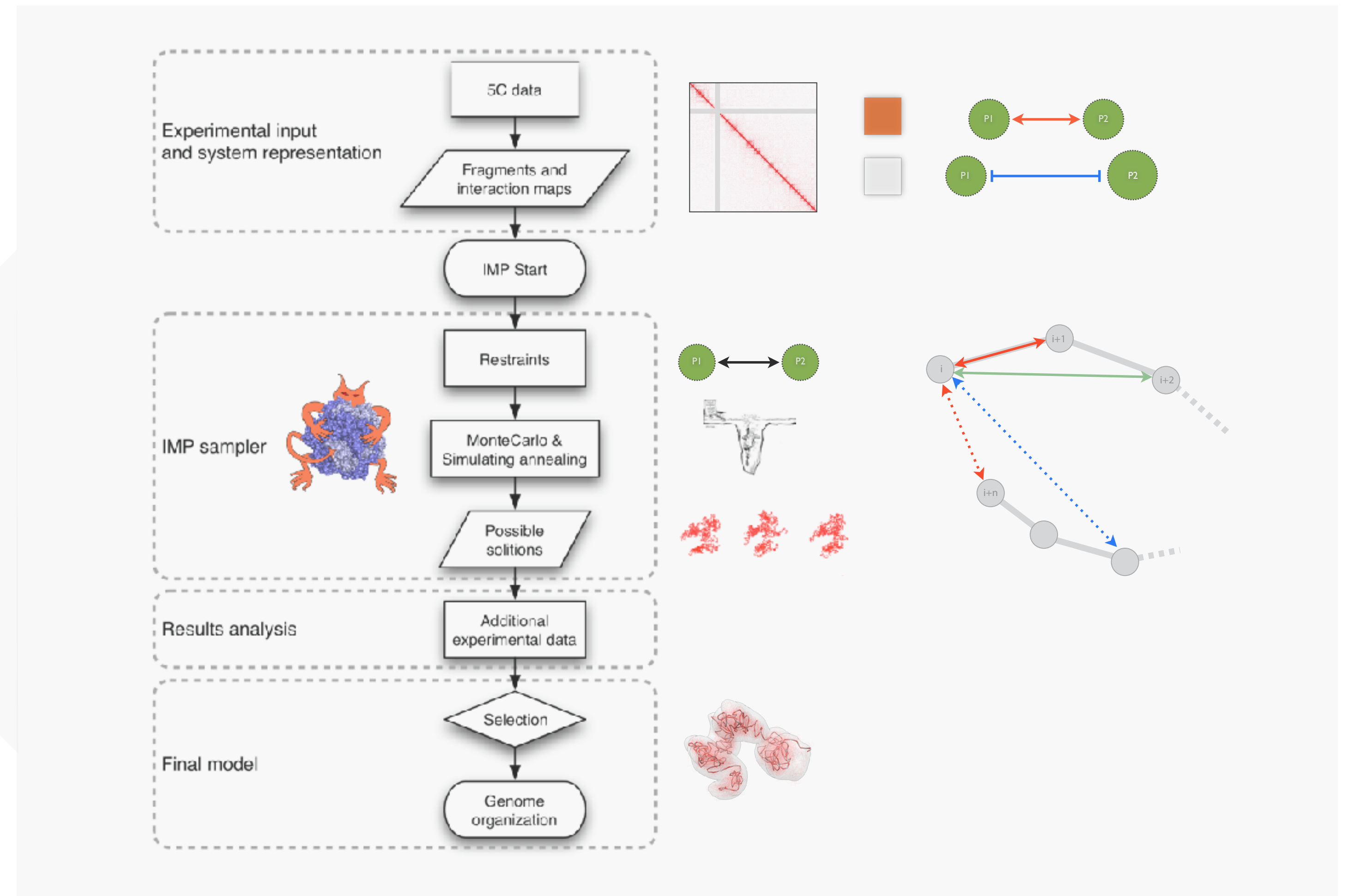
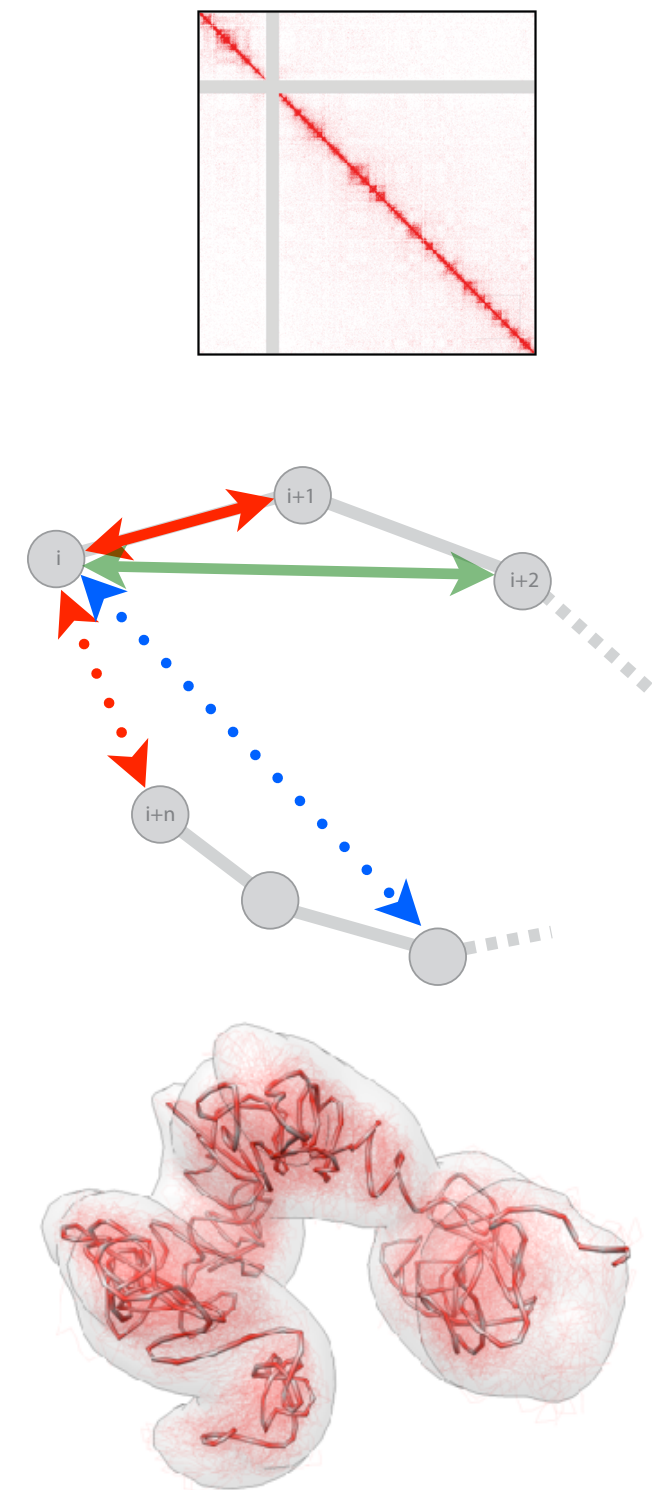


FastQ files to Maps

Map analysis

Model building

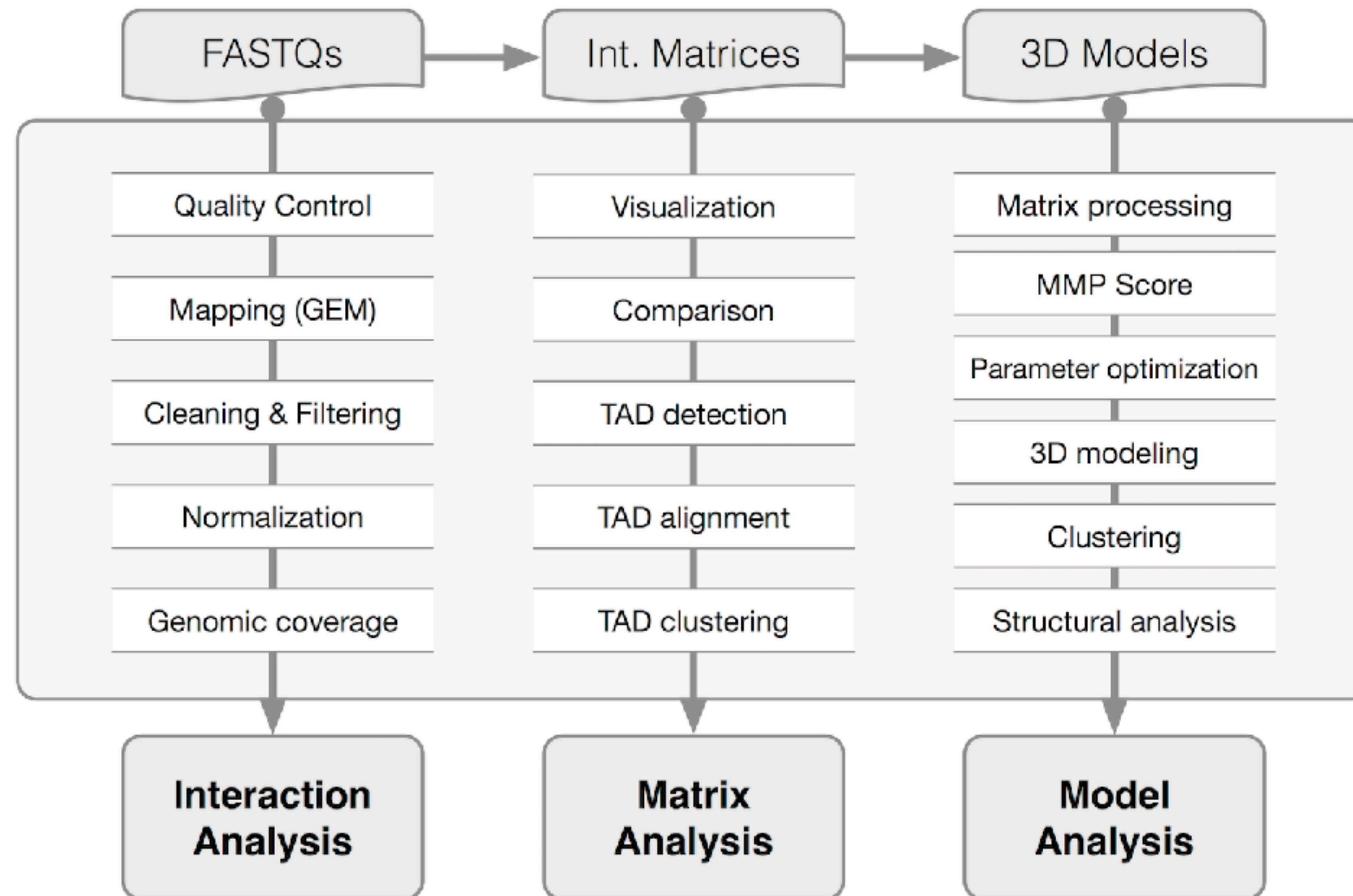
Model analysis







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. Cell Reports (2019)
- Vara et al. Cell Reports (2019)
- Miguel-Escalada et al. Nature Genetics (2019)
- Morf et al. Nature Biotechnology (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  
 Cell Systems 9, 1–13.e1–e6, 2019





Photo by David Oliete - [www.davidoliete.com](http://www.davidoliete.com)

# Chromosome walking with super-resolution imaging and modeling



**Guy Nir**

**Irene Farabella**

**Cynthia Perez-Estrada**

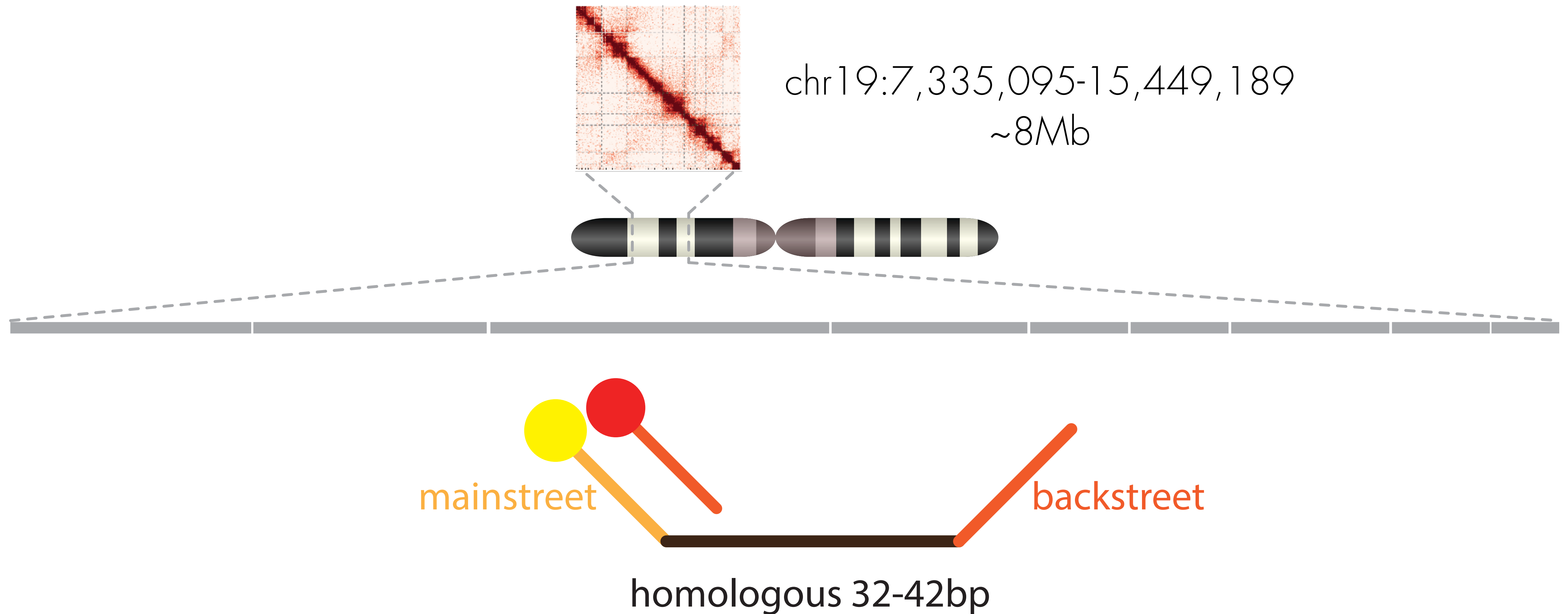
with Wu Lab (HMS, Boston) & Aiden Lab (UT, Texas)

PLOS Genetics (2018) 14(12) e1007872



# High-resolution imaging

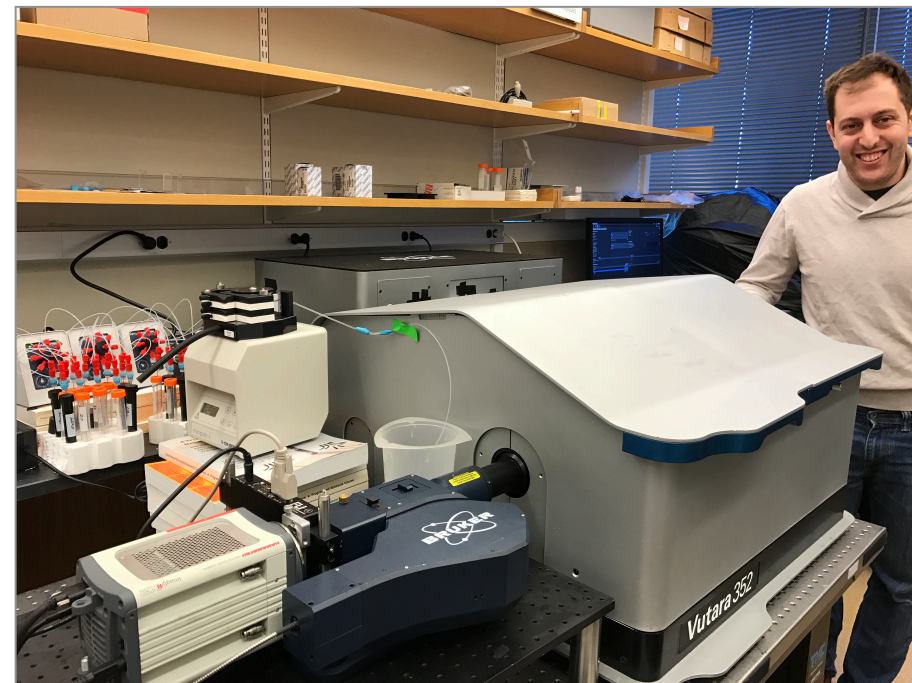
Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells





# High-resolution imaging

Tracing chromosomes with OligoSTORM & fluidics cycles in PGP1 cells



**Guy Nir** Harvard Med School

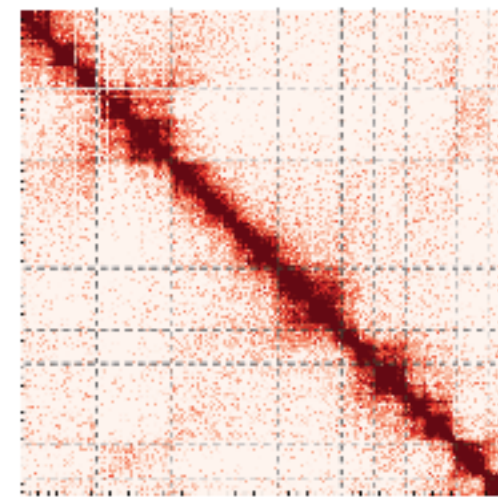
**Bodgan Bintu** Harvard

**Carl Ebeling** Bruker

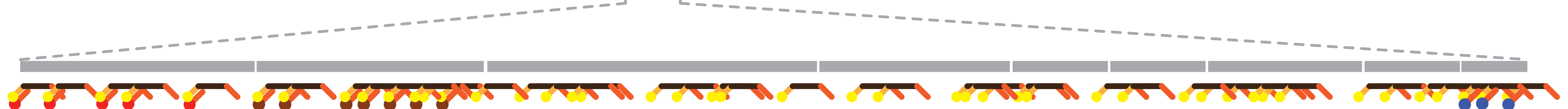
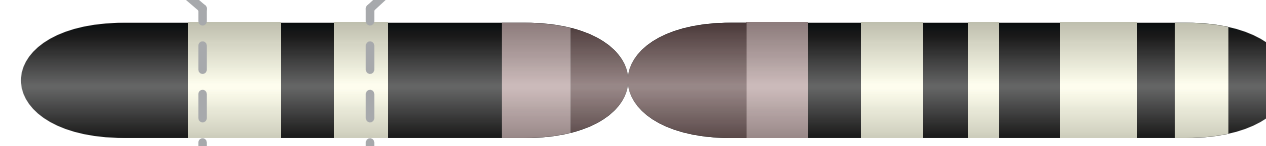
**Jeff Stuckey** Bruker

**John Schreiner** Zero Epsilon

**Steve Callahan** Zero Epsilon



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



1

1,280Kb

2

1,240Kb

3

1,800Kb

4

1,040Kb

5

520Kb

6

520Kb

7

840Kb

8

520Kb

9

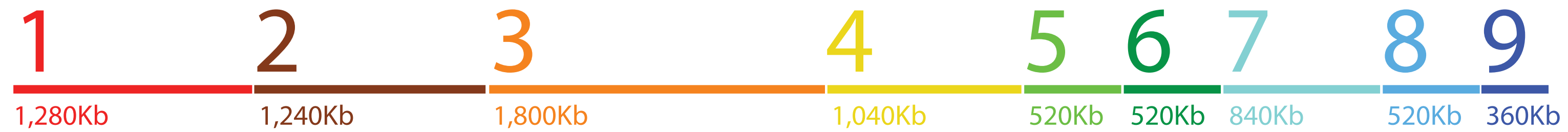
360Kb





# High-resolution imaging

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

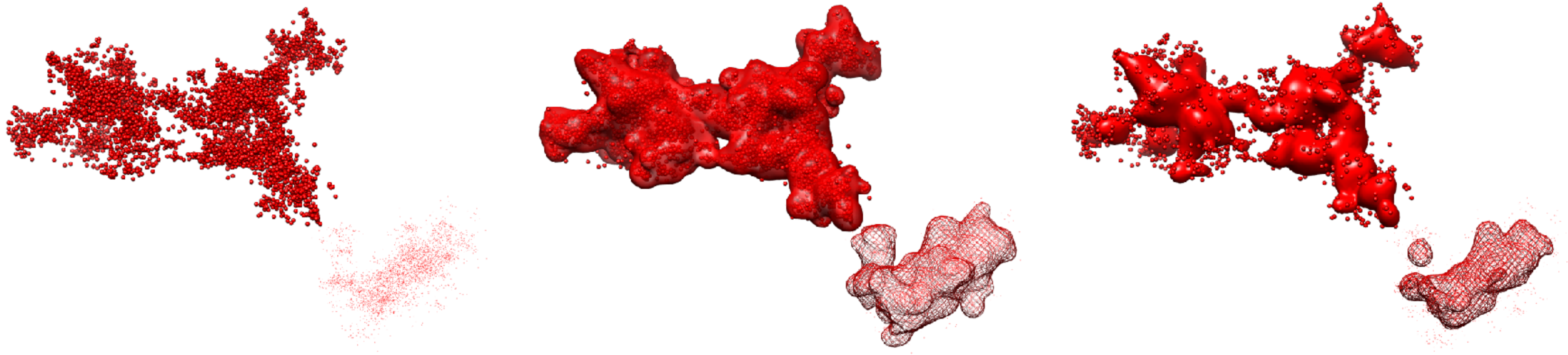




# High-resolution imaging

XYZ points convolution into a density map

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

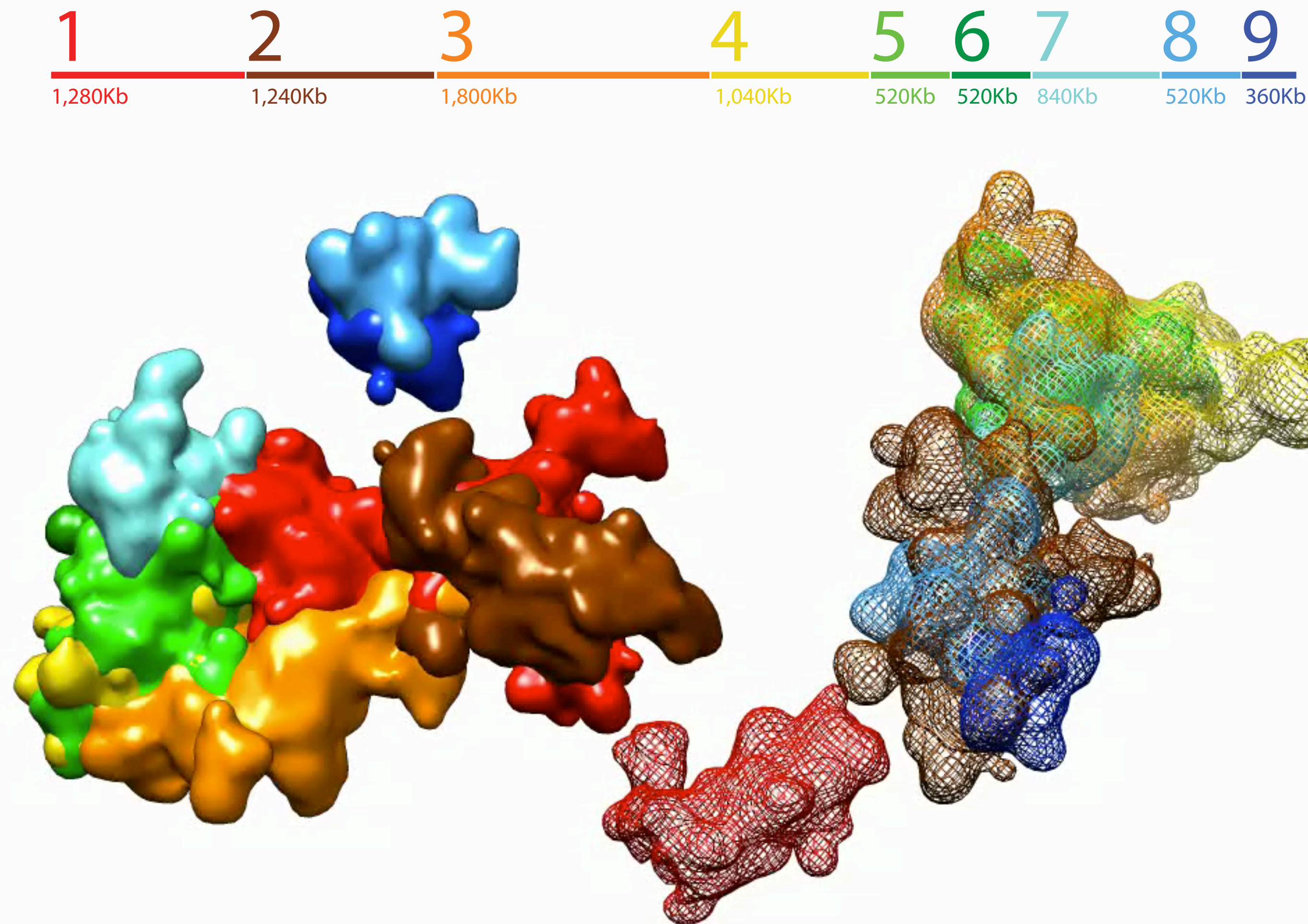


Cell-02 · Segment 1



# Density maps

Cell-02 · Density map @ 50nm



Area (nm<sup>2</sup>)

Volume (nm<sup>3</sup>)

Sphericity

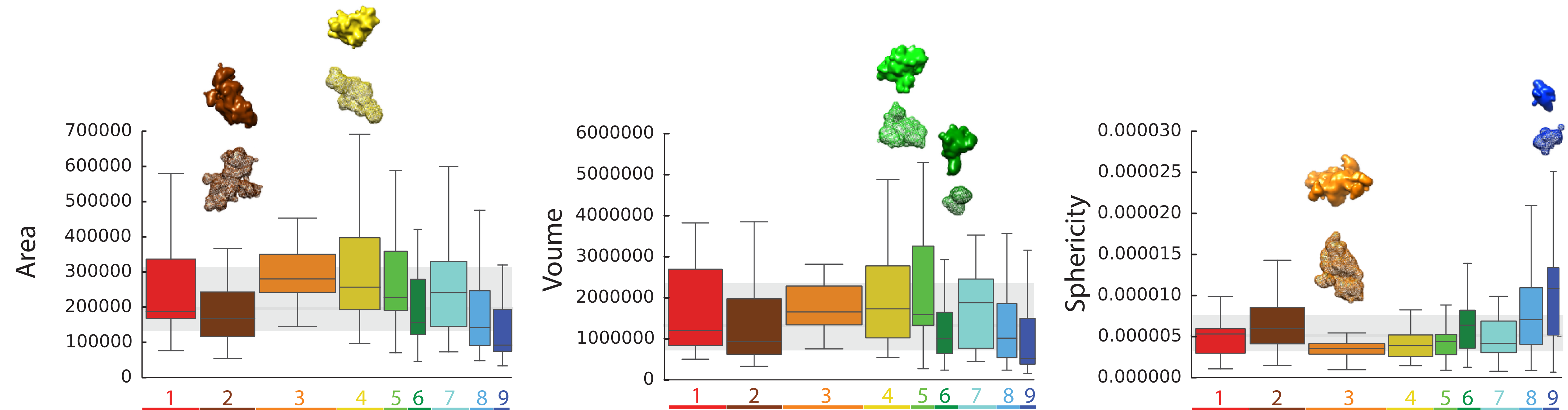
Overlap (%)

Distance (nm)



# Structural features

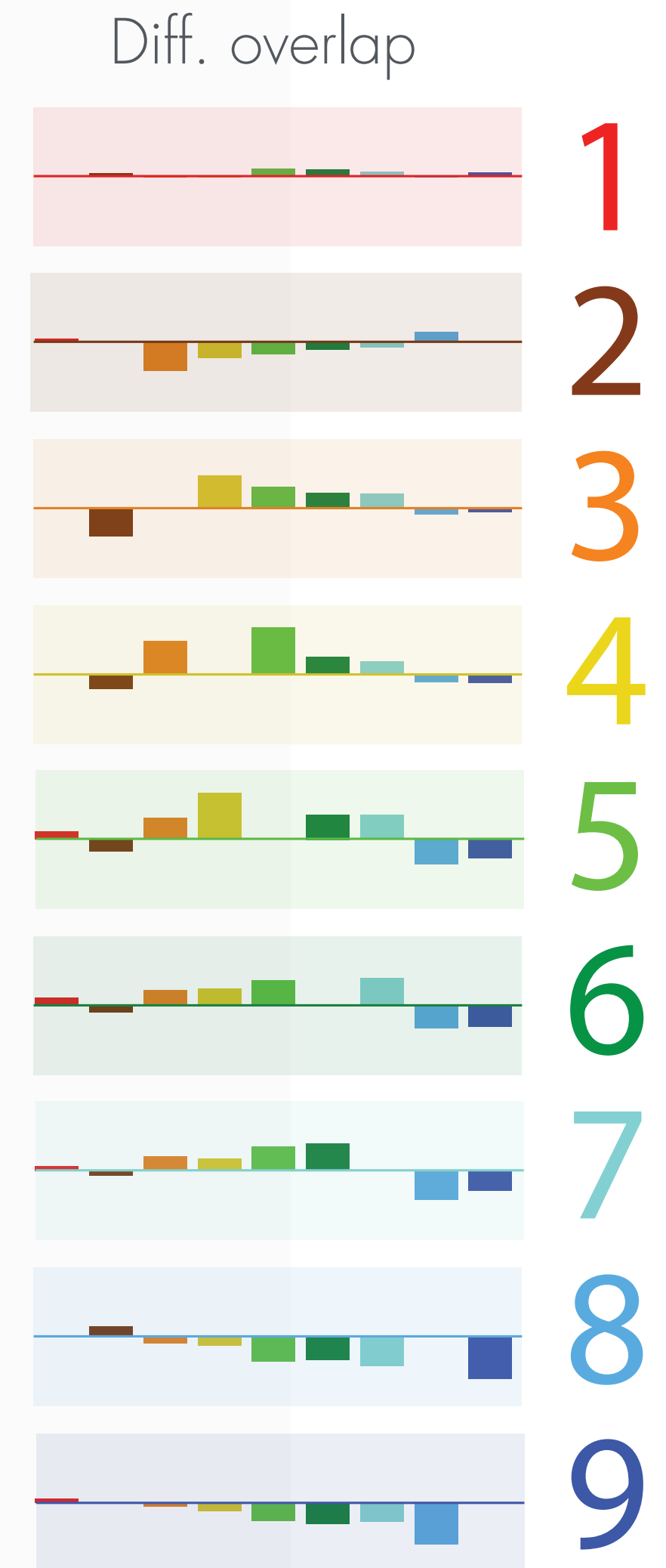
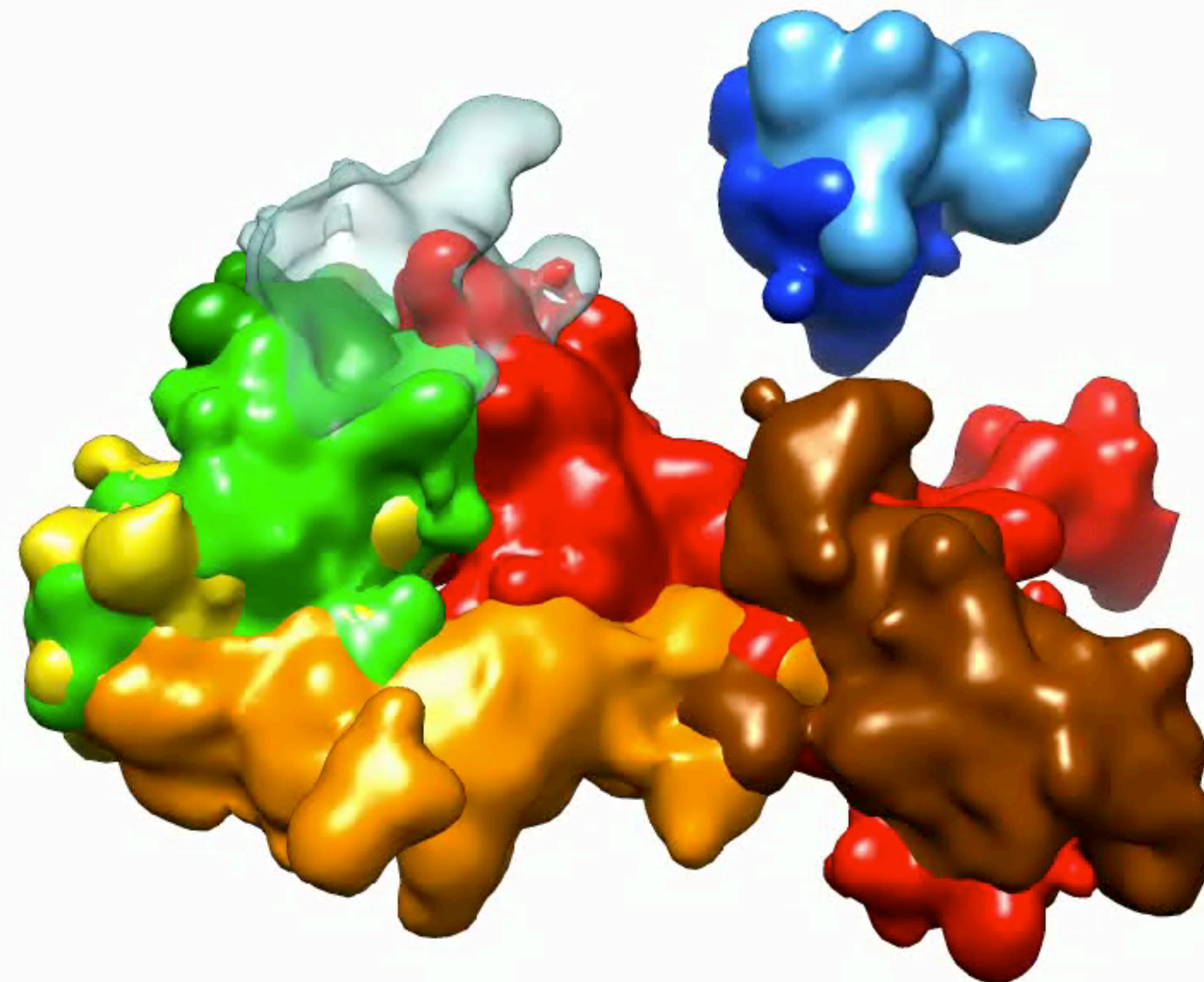
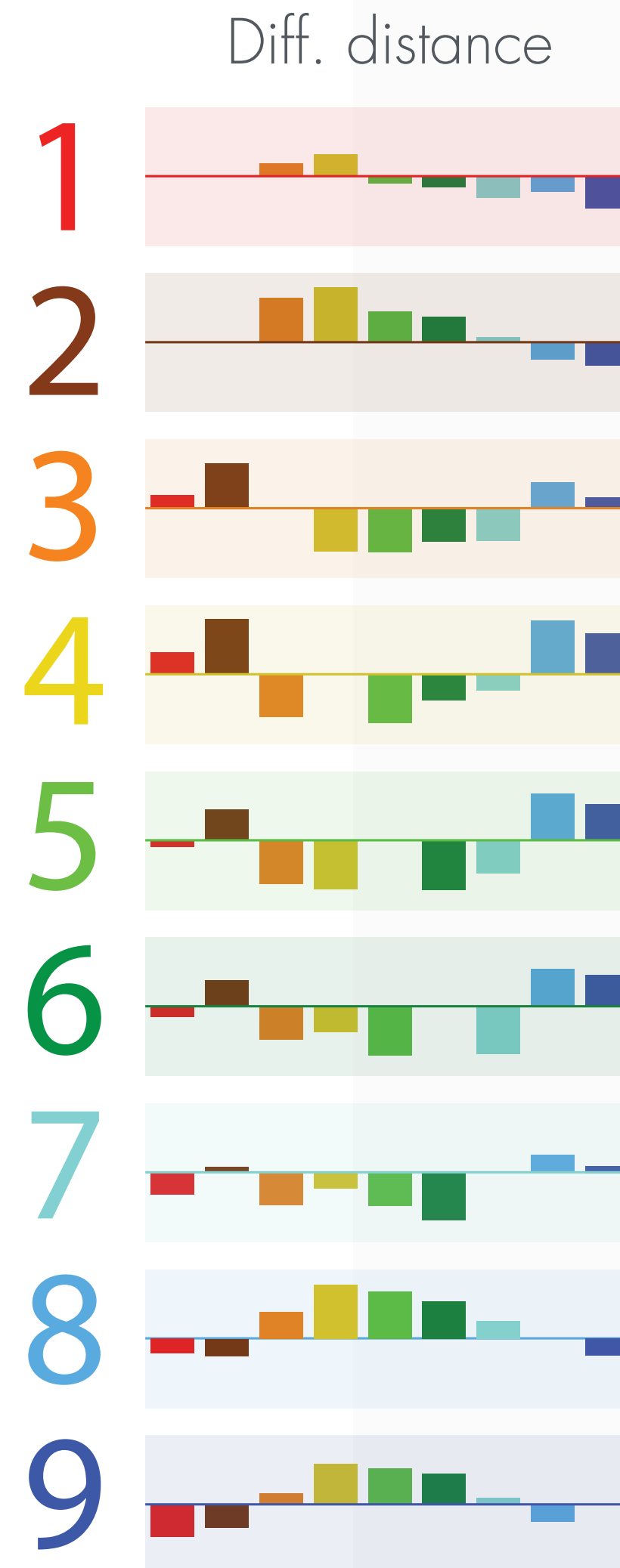
Area, Volume and Sphericity of 19 cells each with 2 homologous resolved





# Spatial arrangement

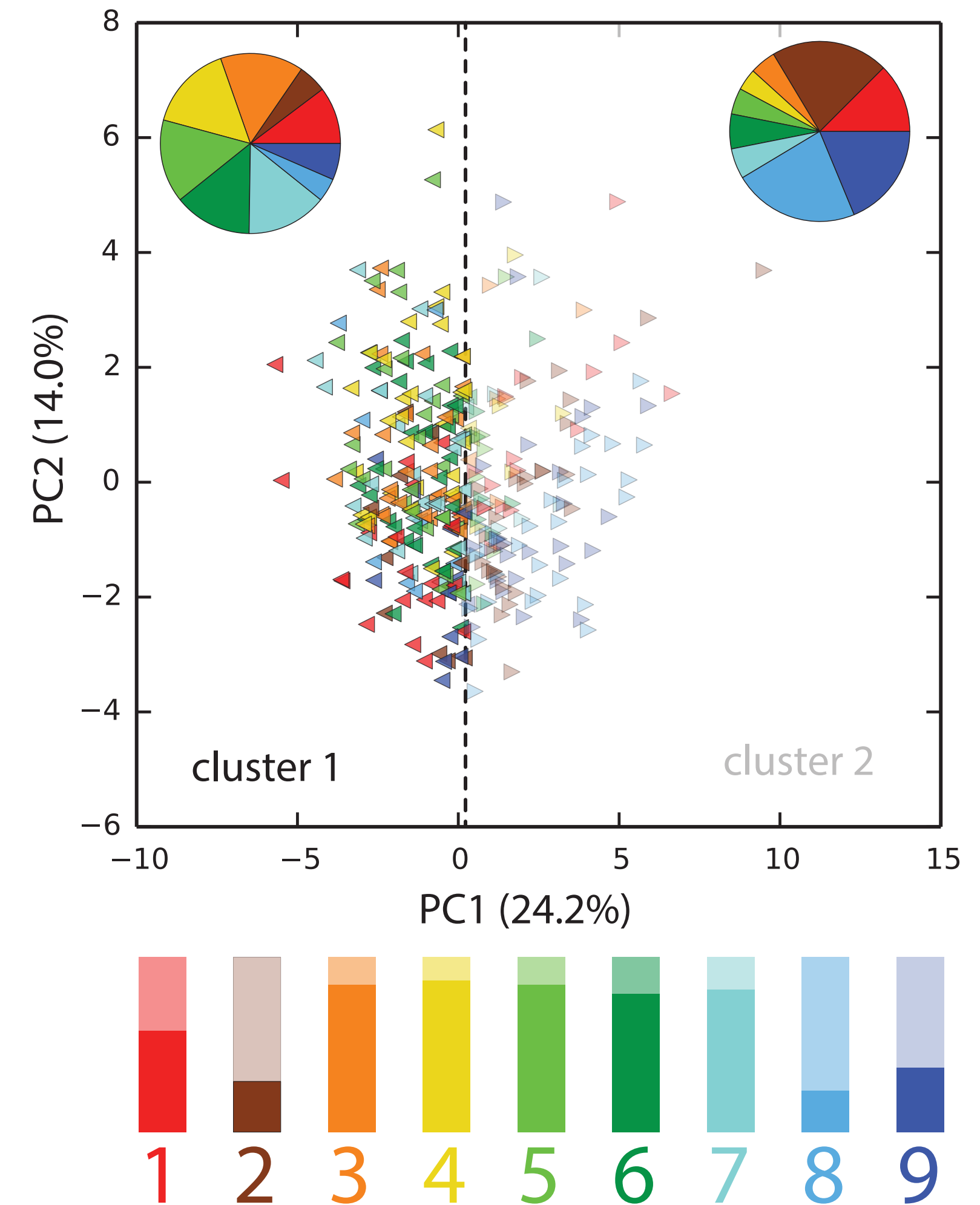
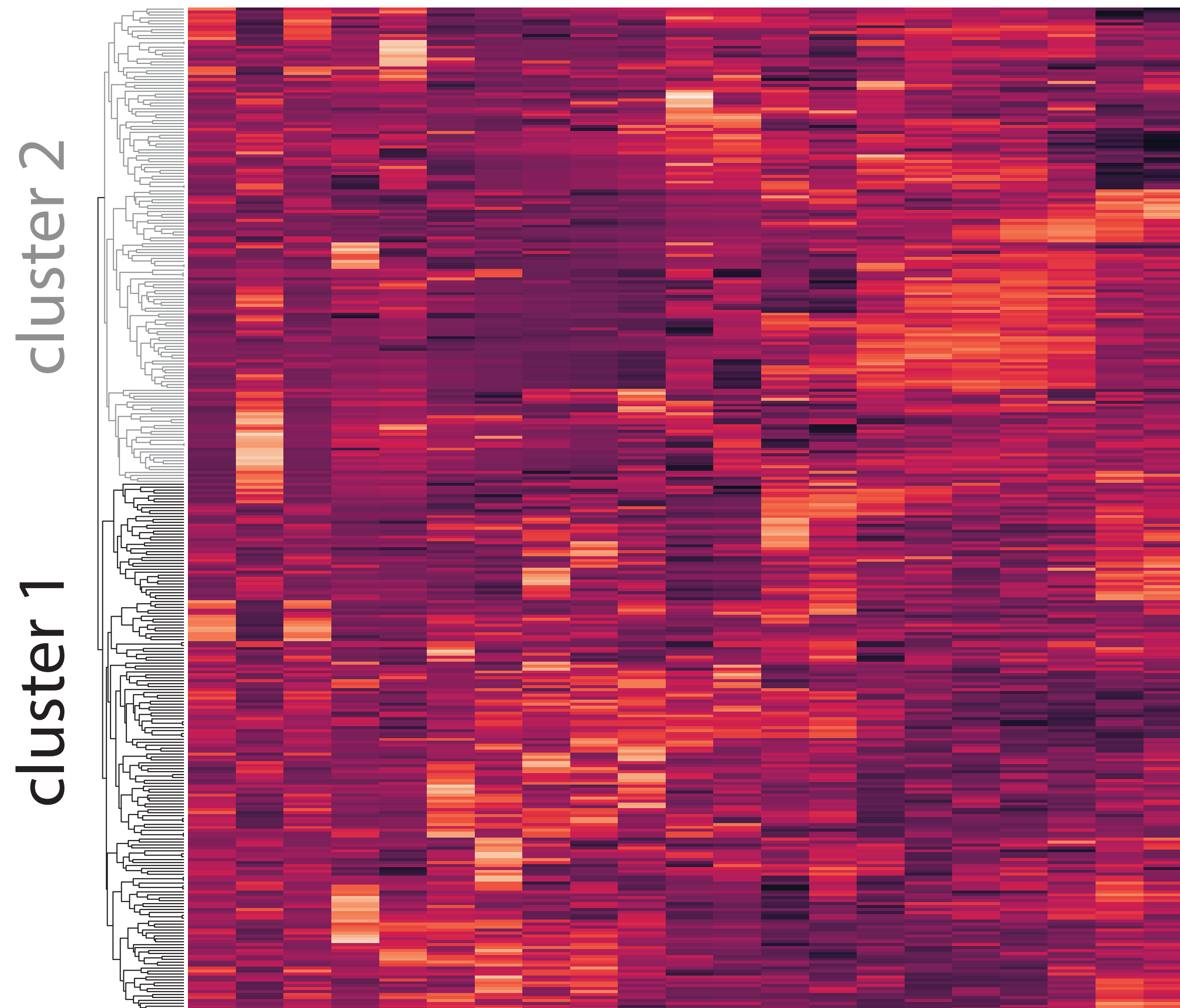
Distance and overlap of 19 cells each with 2 homologous resolved





# Structural clustering

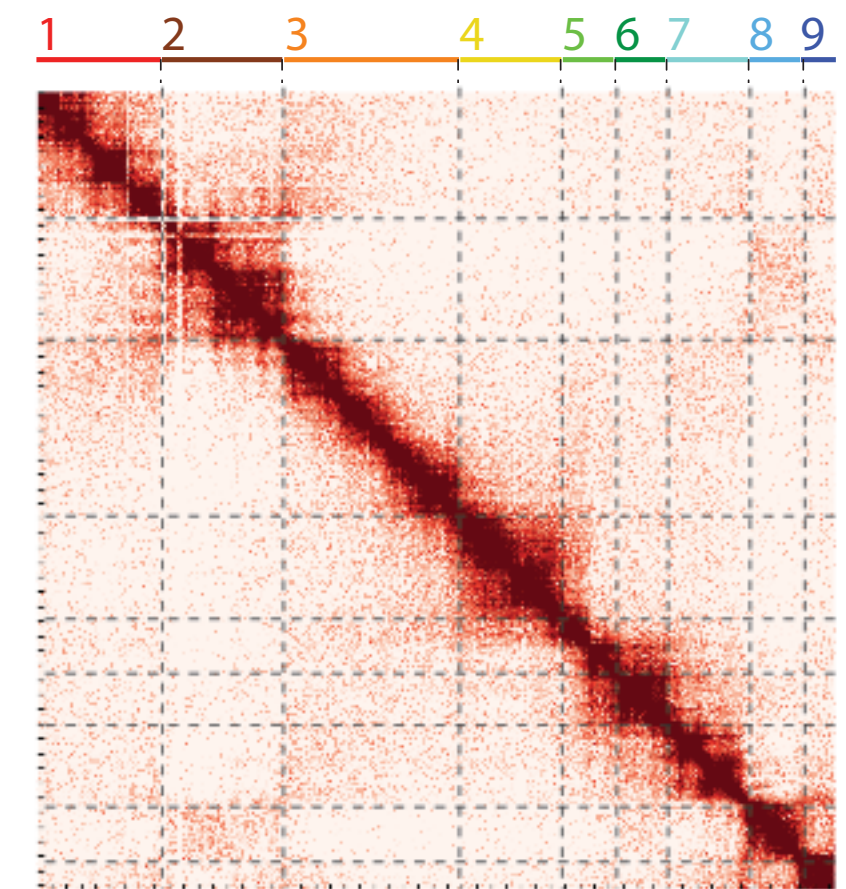
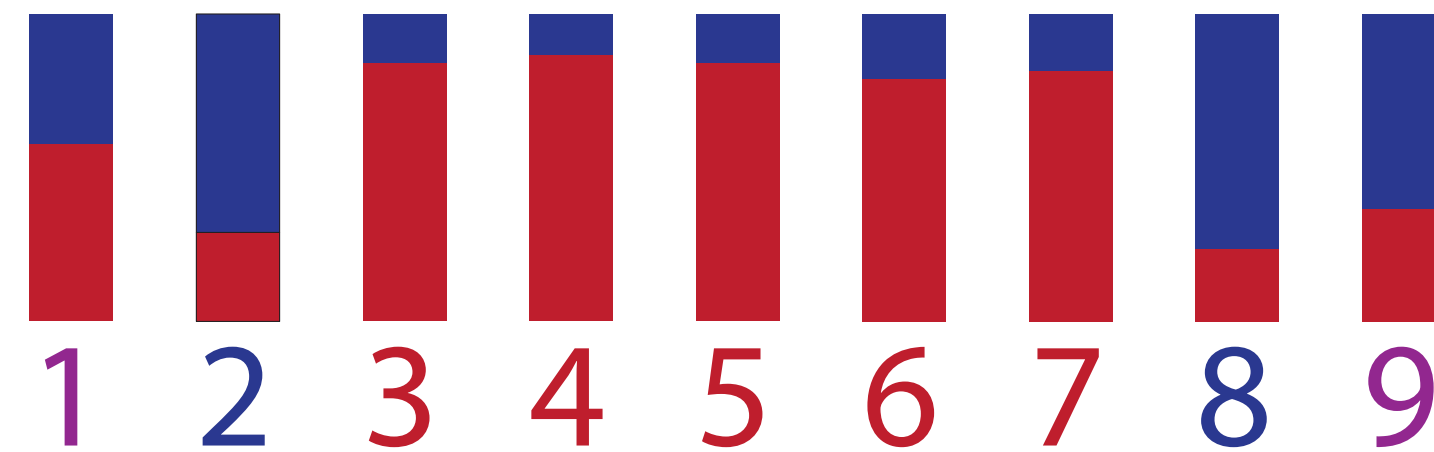
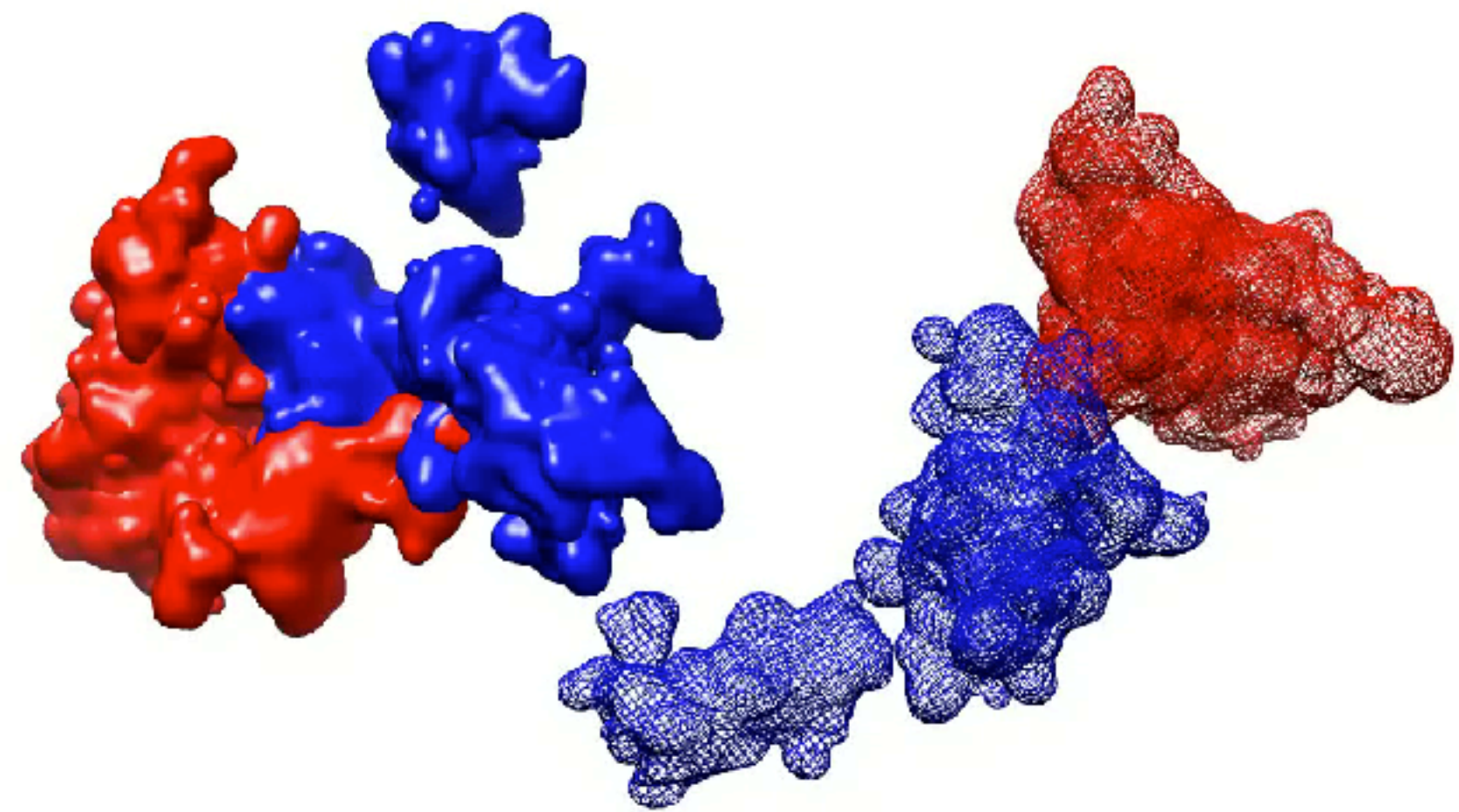
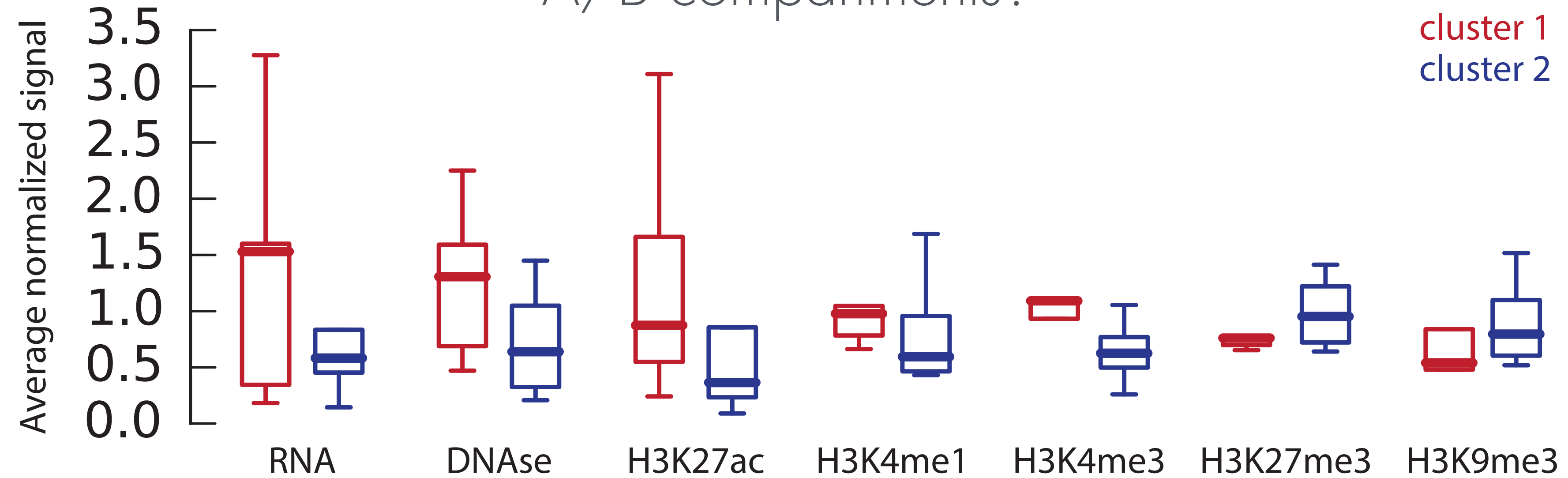
19 cells each with 2 homologous and 9 segments each (342)





# Cluster properties

A/B compartments?

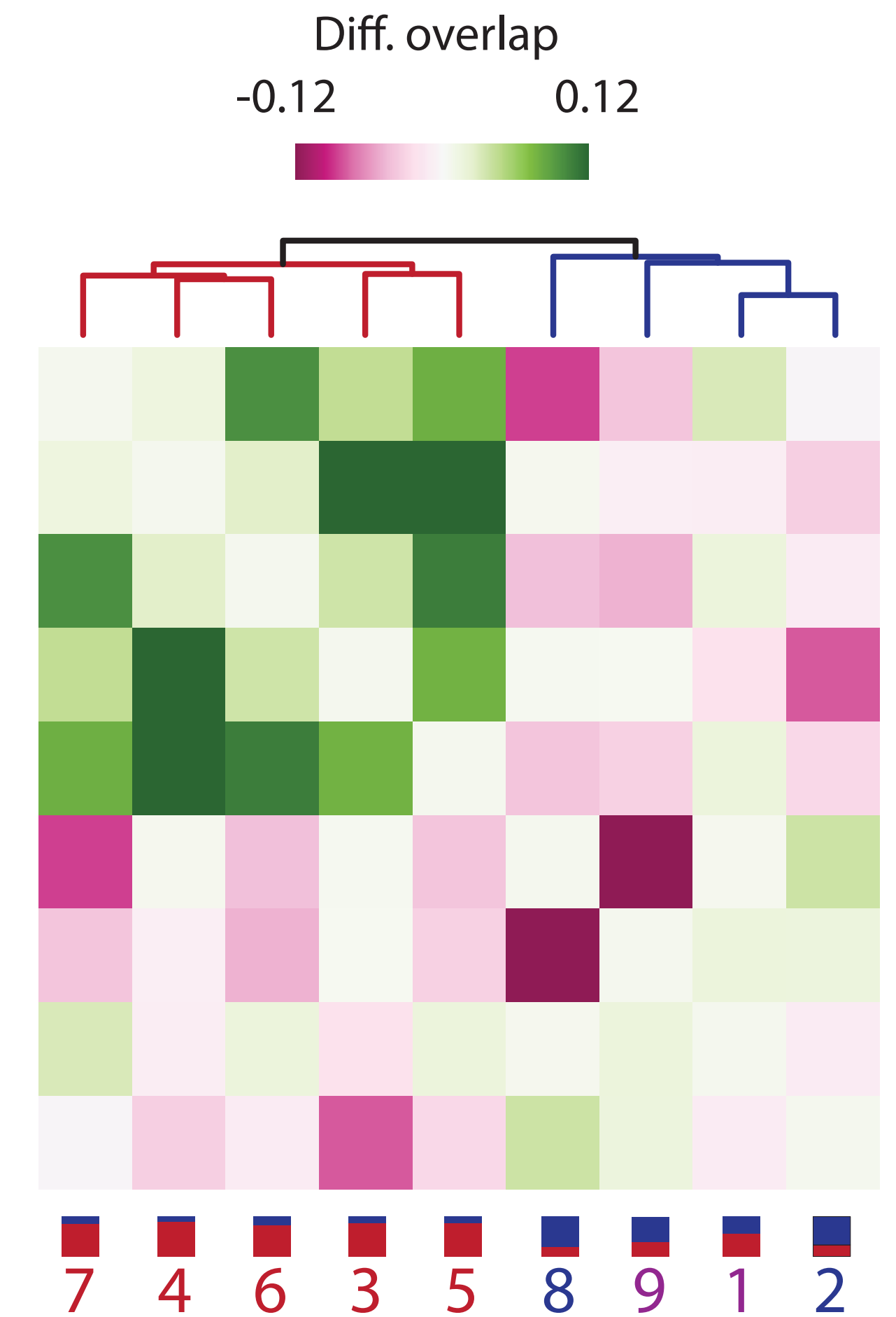
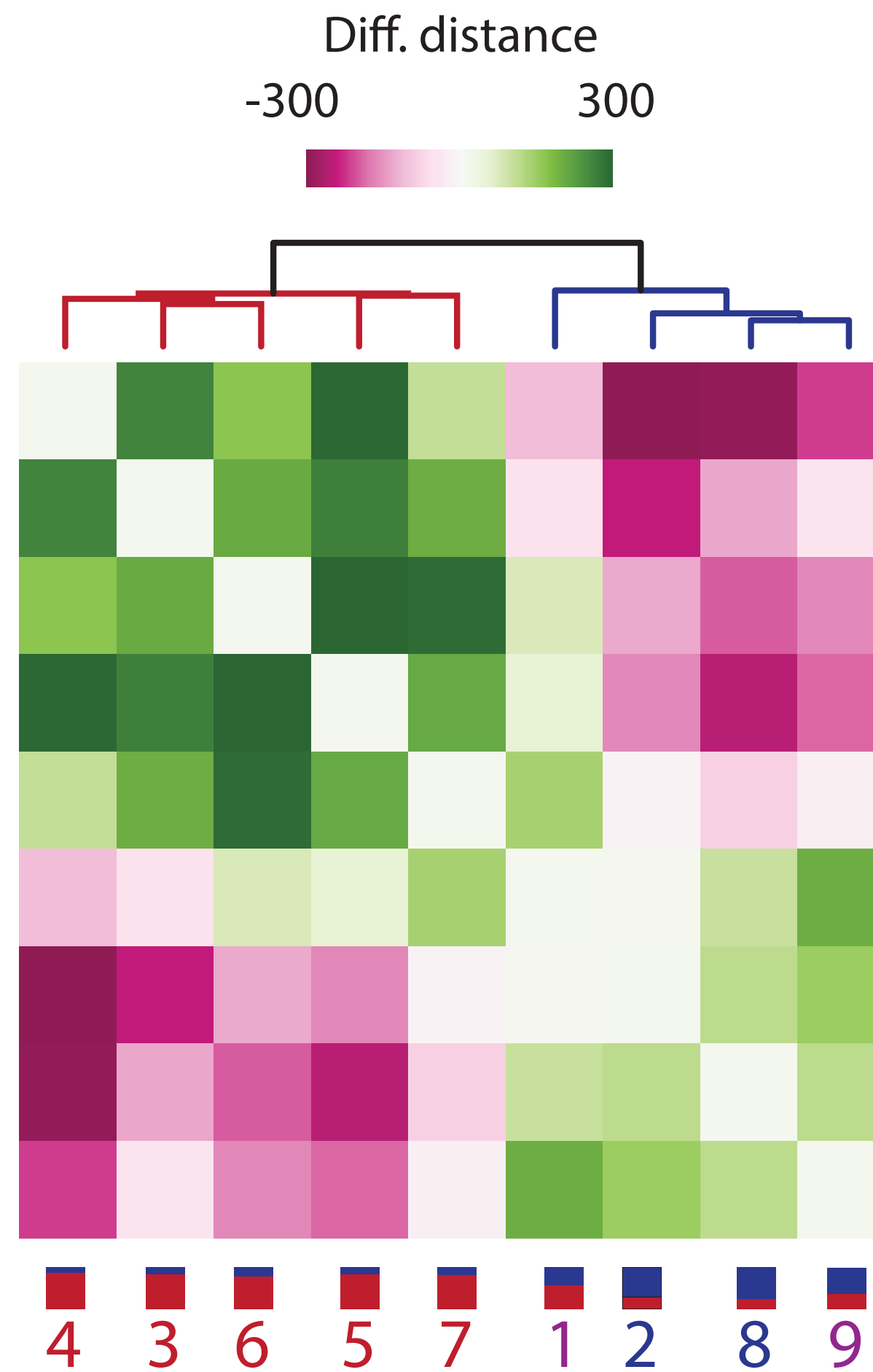
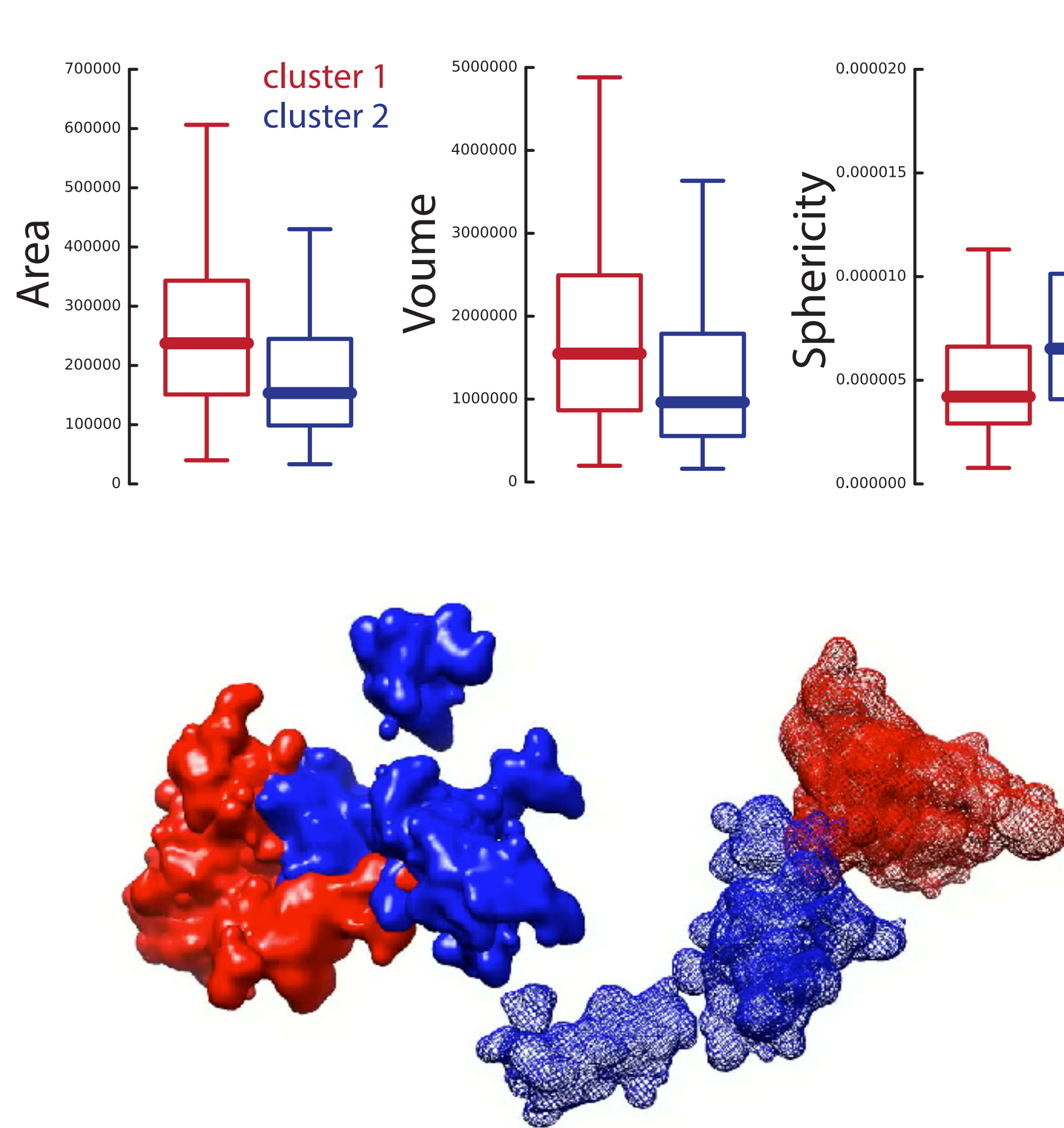


PGP1 ChIP-seq and Hi-C data from ENCODE and Lieberman-Aiden Lab, respectively



# Cluster properties

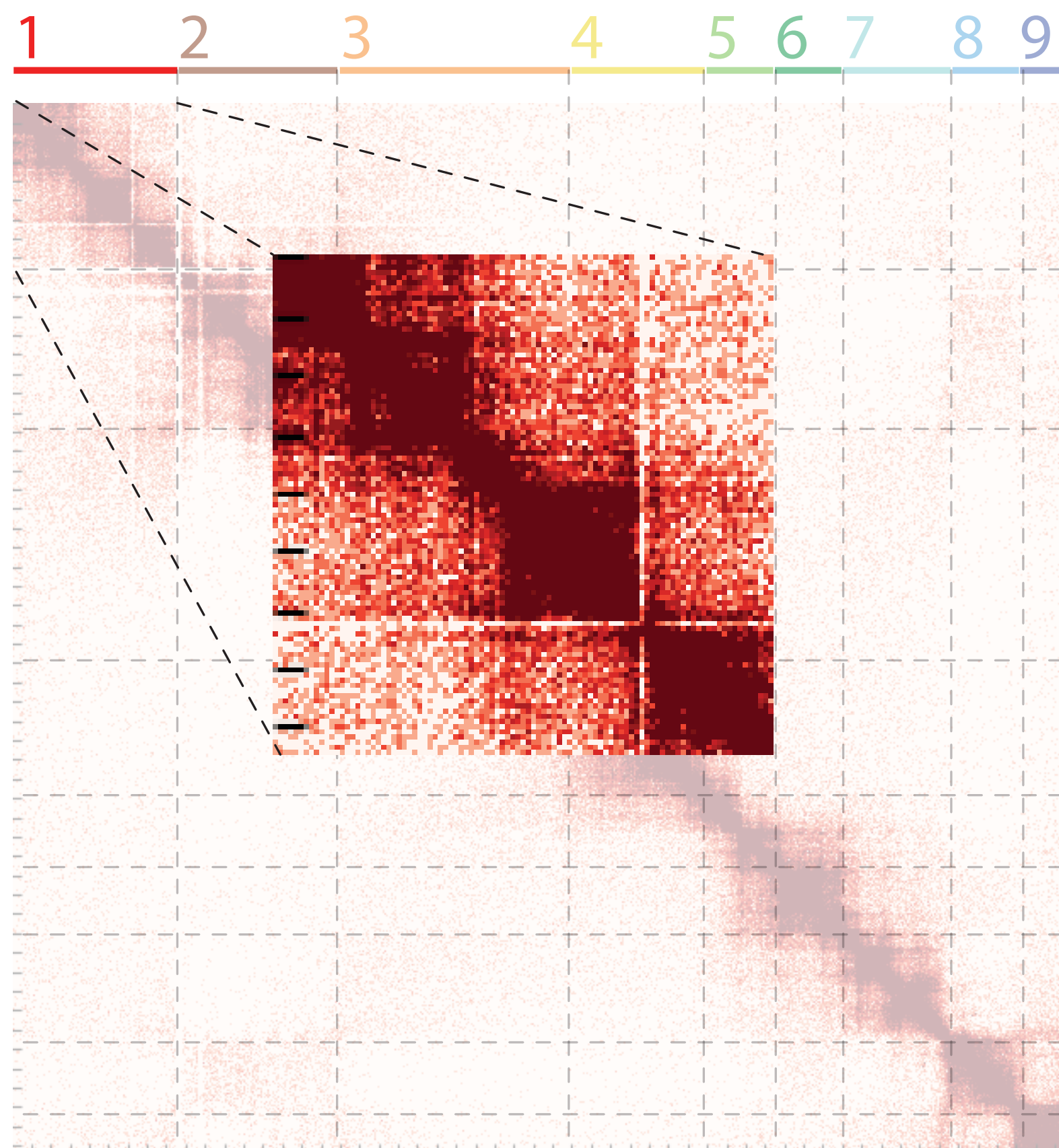
## A/B compartment properties



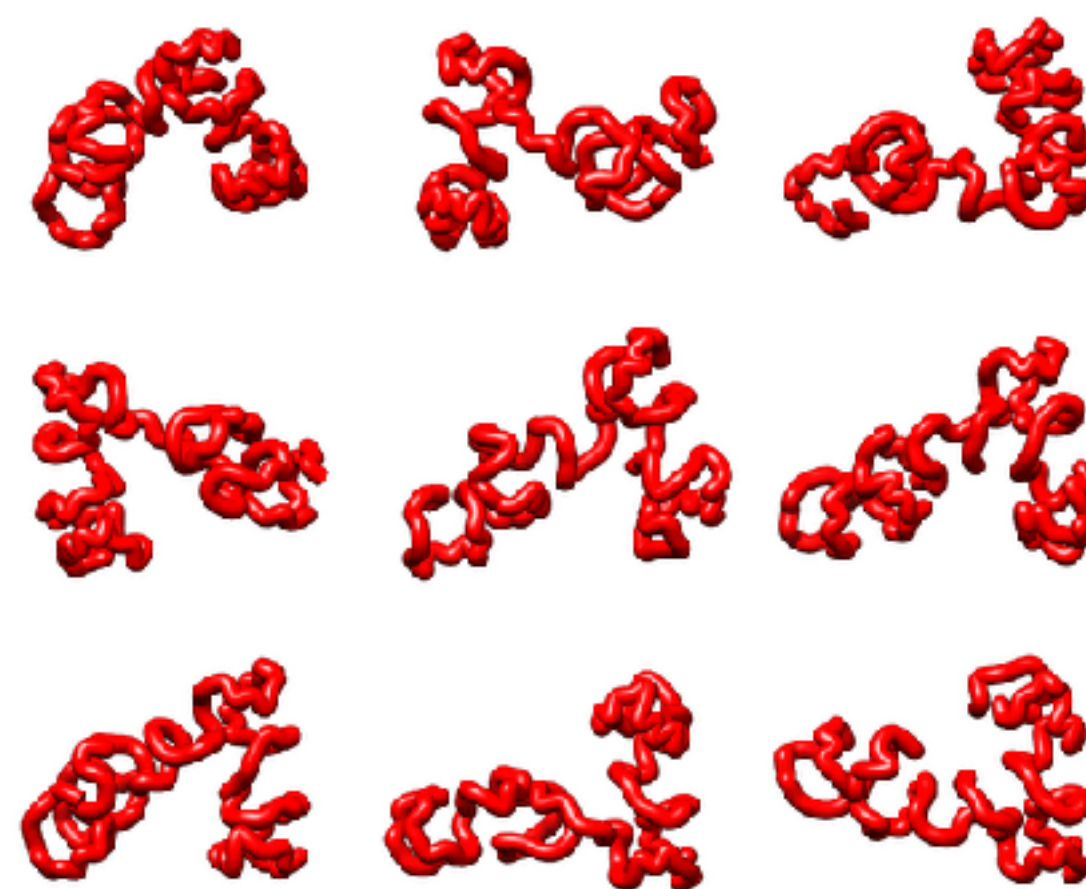


# Increasing resolution

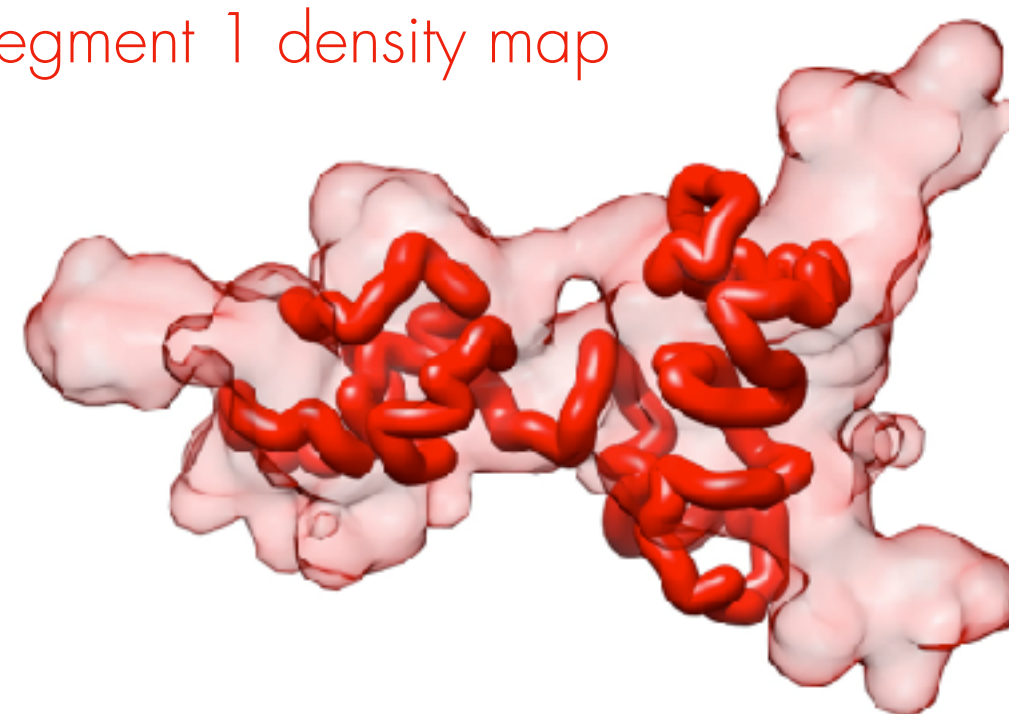
Rigid body fitting 3D structures based on Hi-C data



Segment 1 3D models

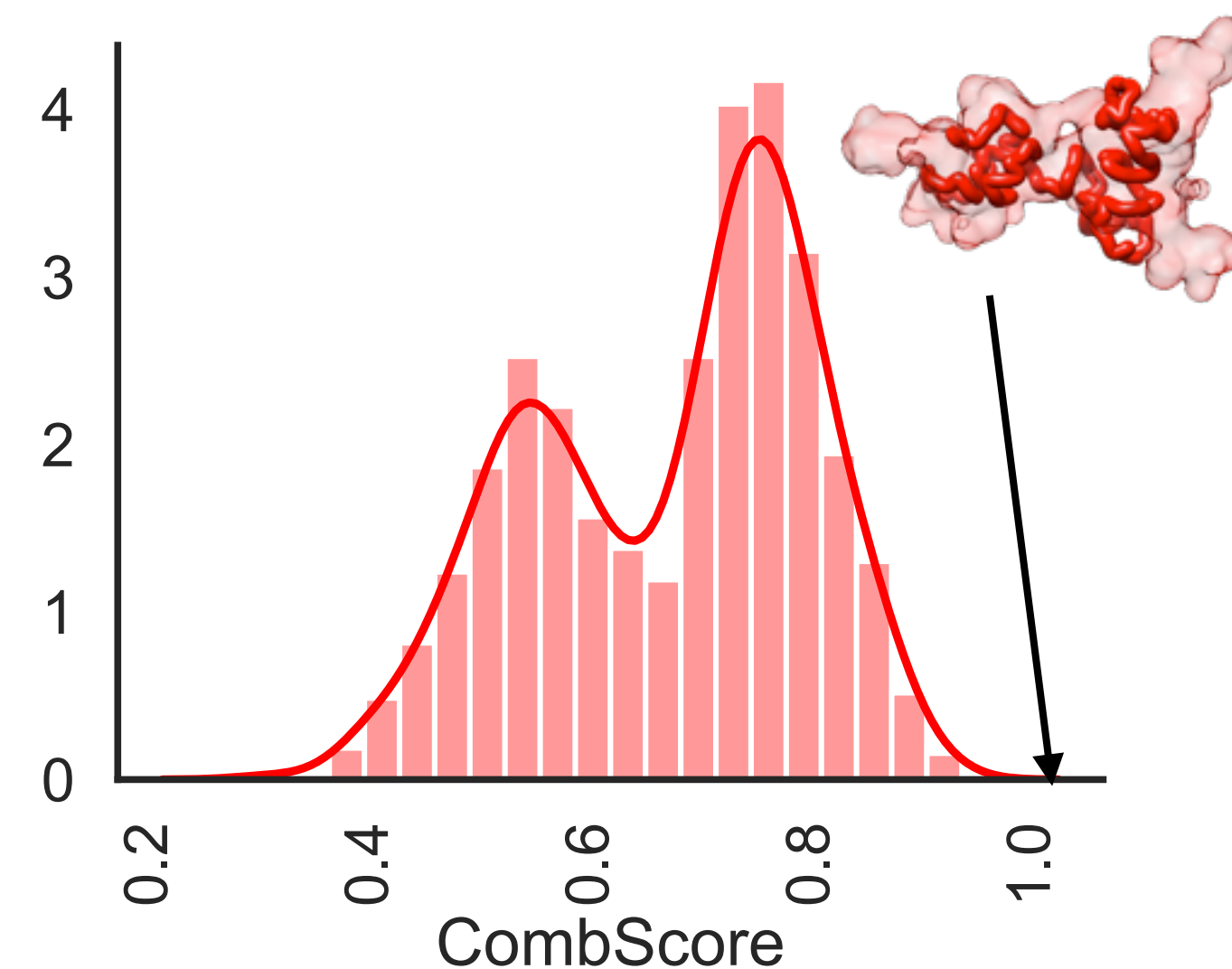


Segment 1 density map



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

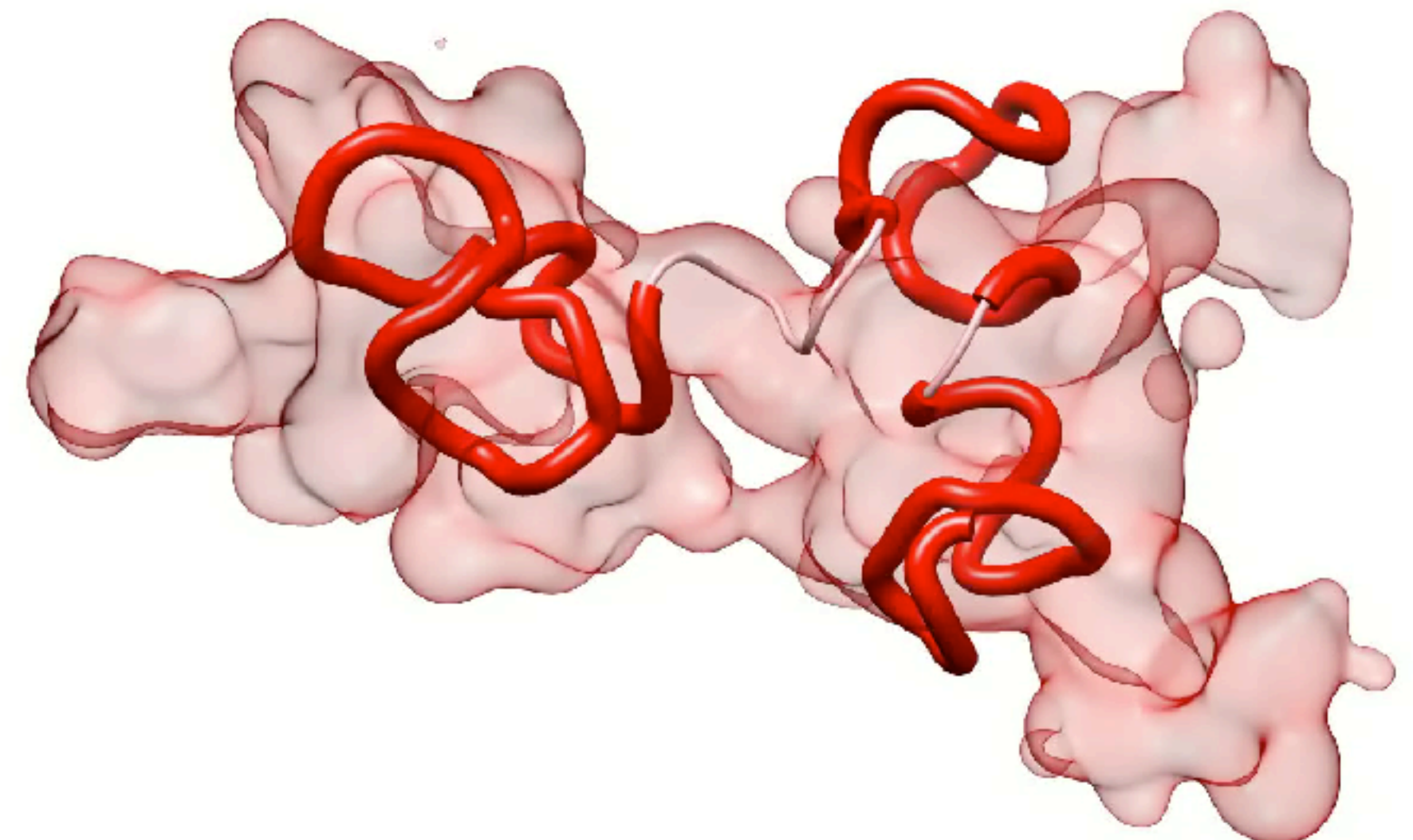
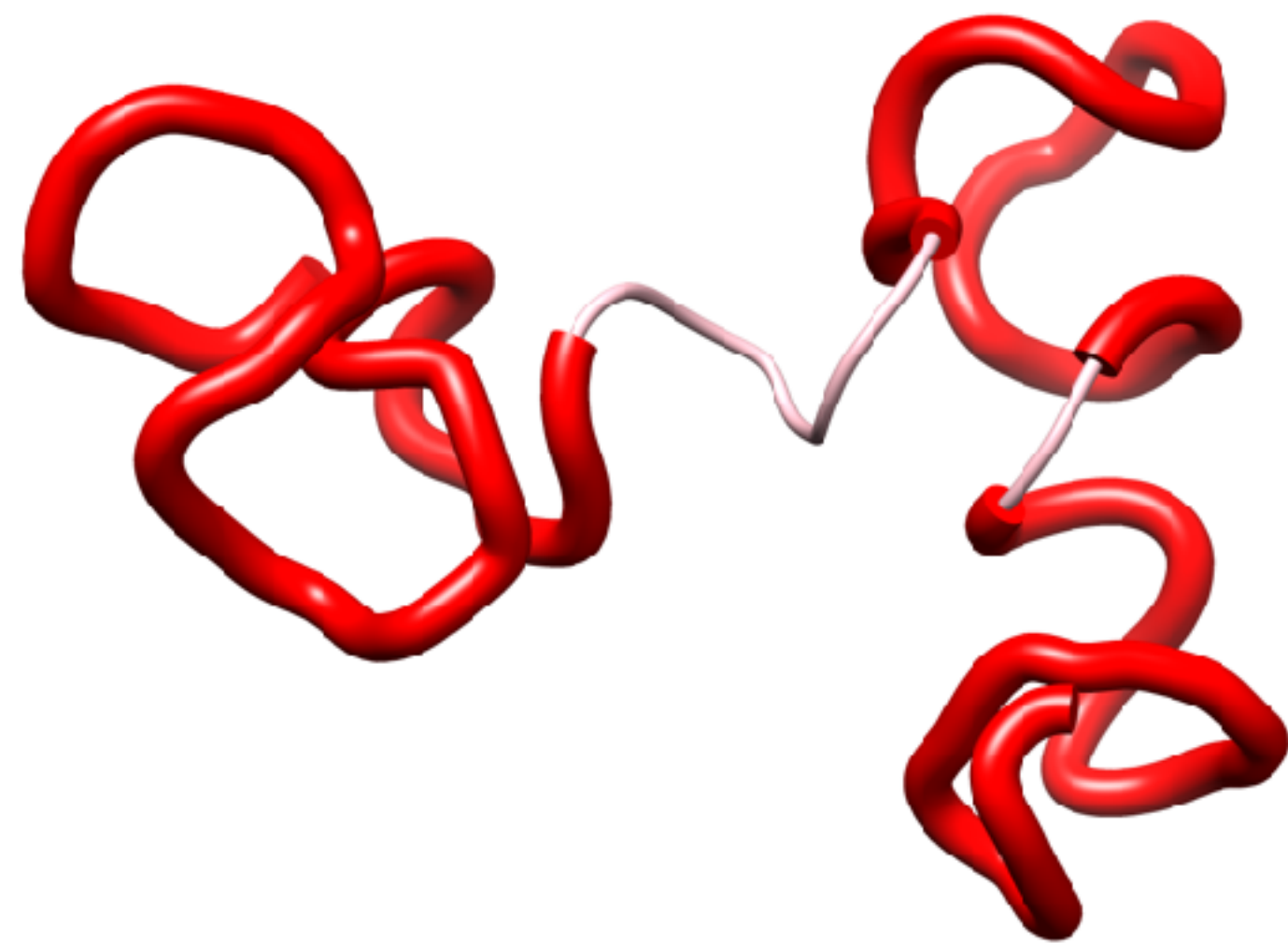
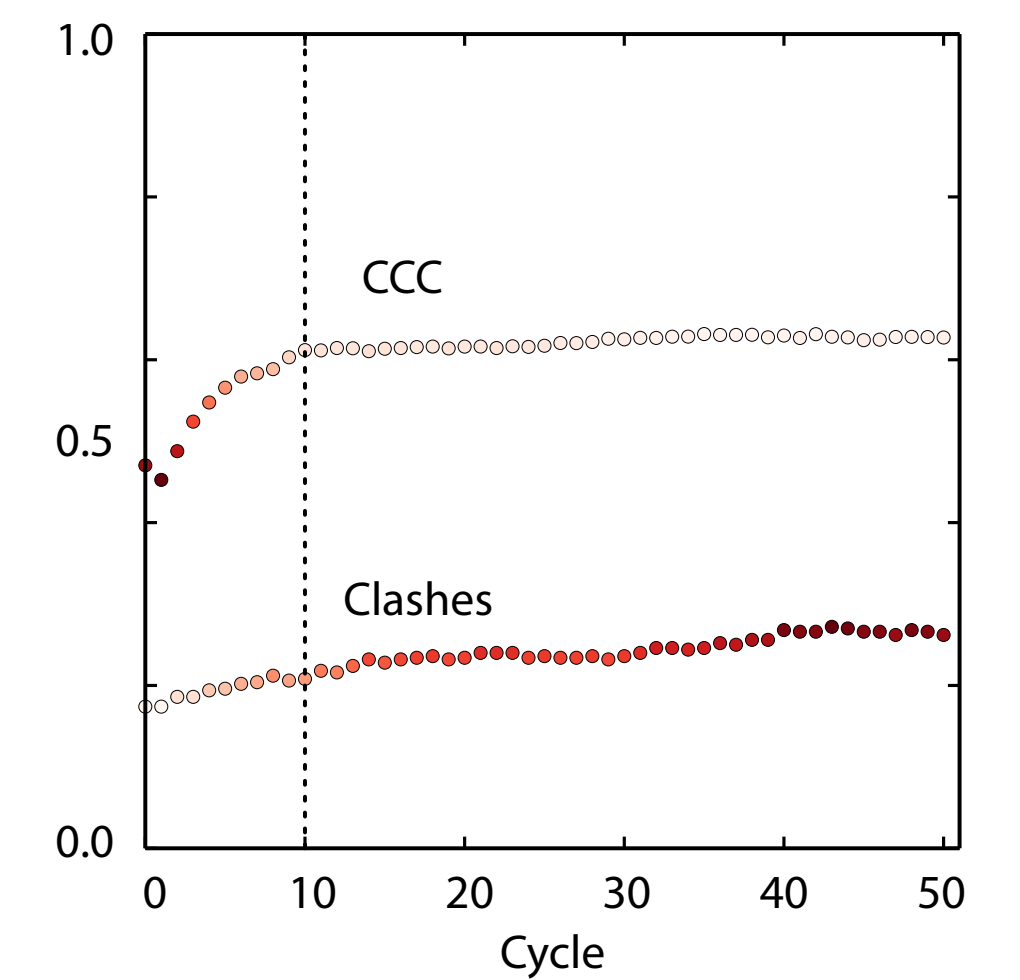
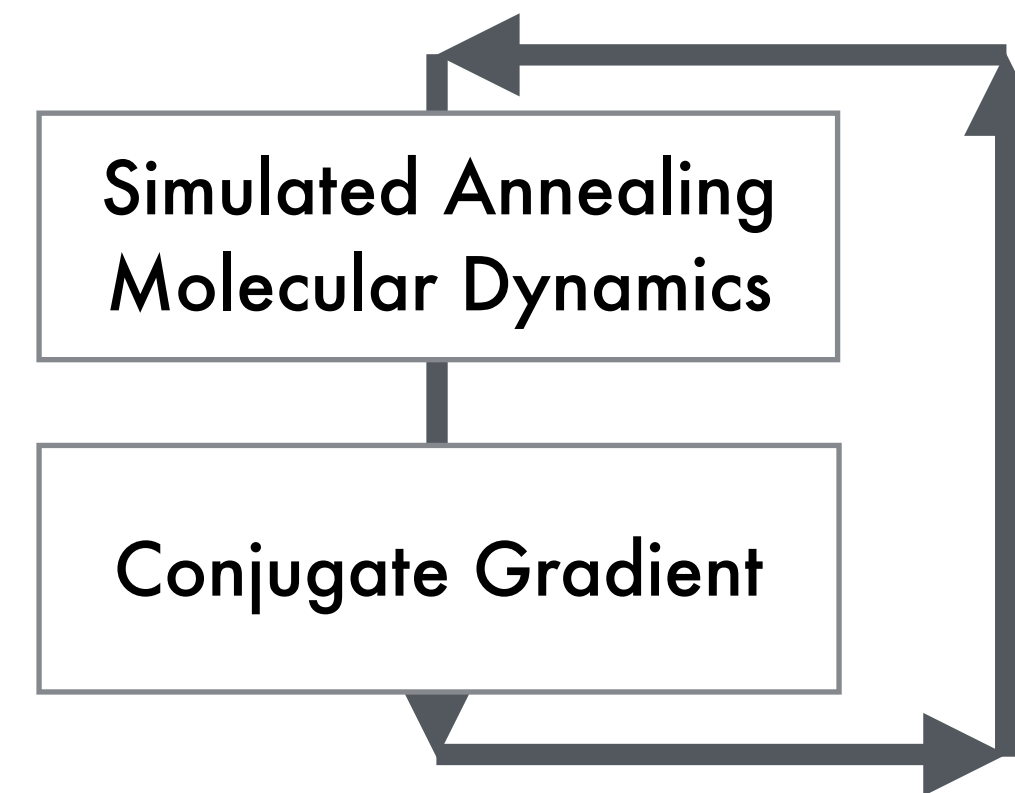
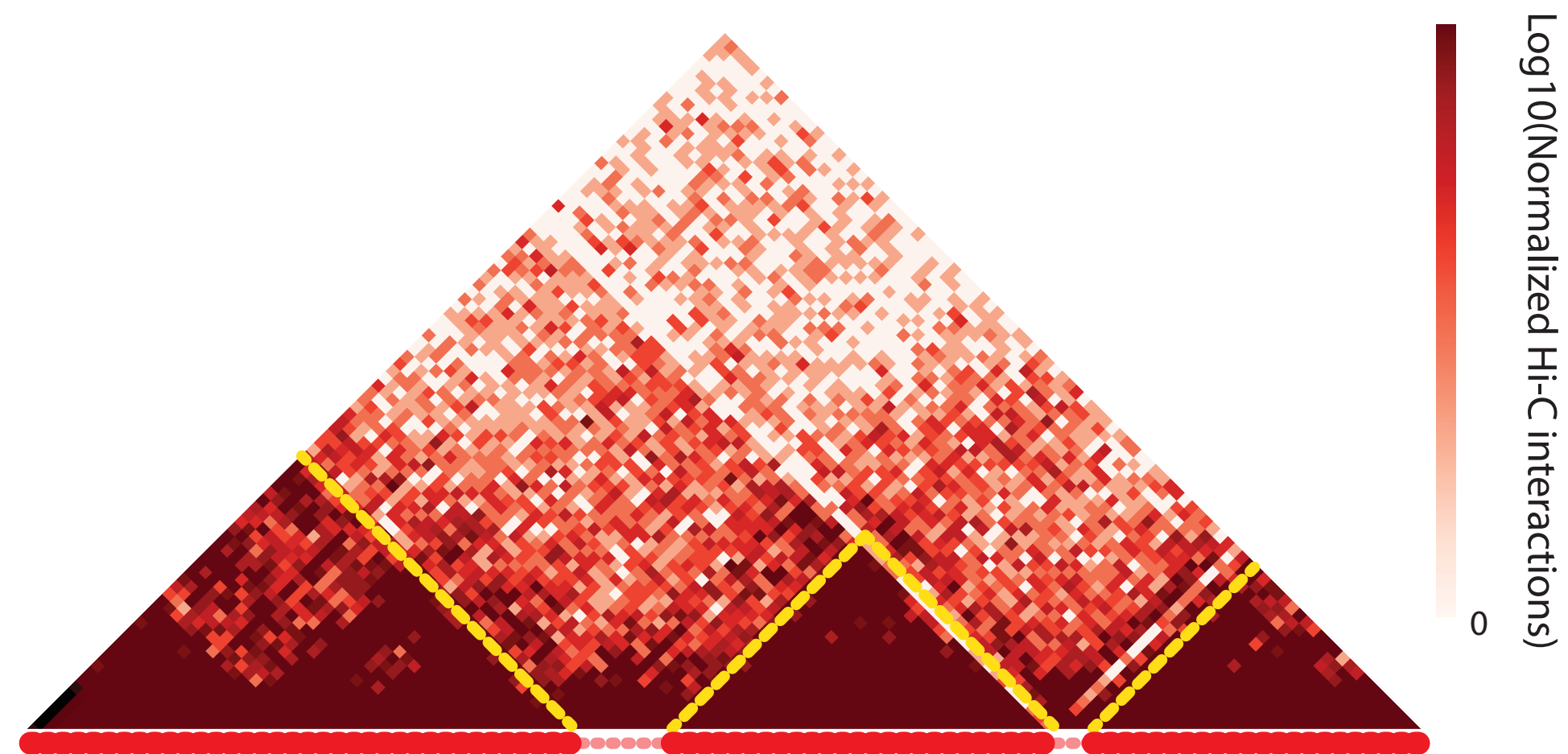
$$\text{CCC} = \frac{\sum_{i=1}^M [\rho_i^{EM} - \bar{\rho}^{EM}] [\rho_i^P - \bar{\rho}^P]}{\sqrt{\sum_{i=1}^M [\rho_i^{EM} - \bar{\rho}^{EM}]^2 \sum_{i=1}^M [\rho_i^P - \bar{\rho}^P]^2}}$$





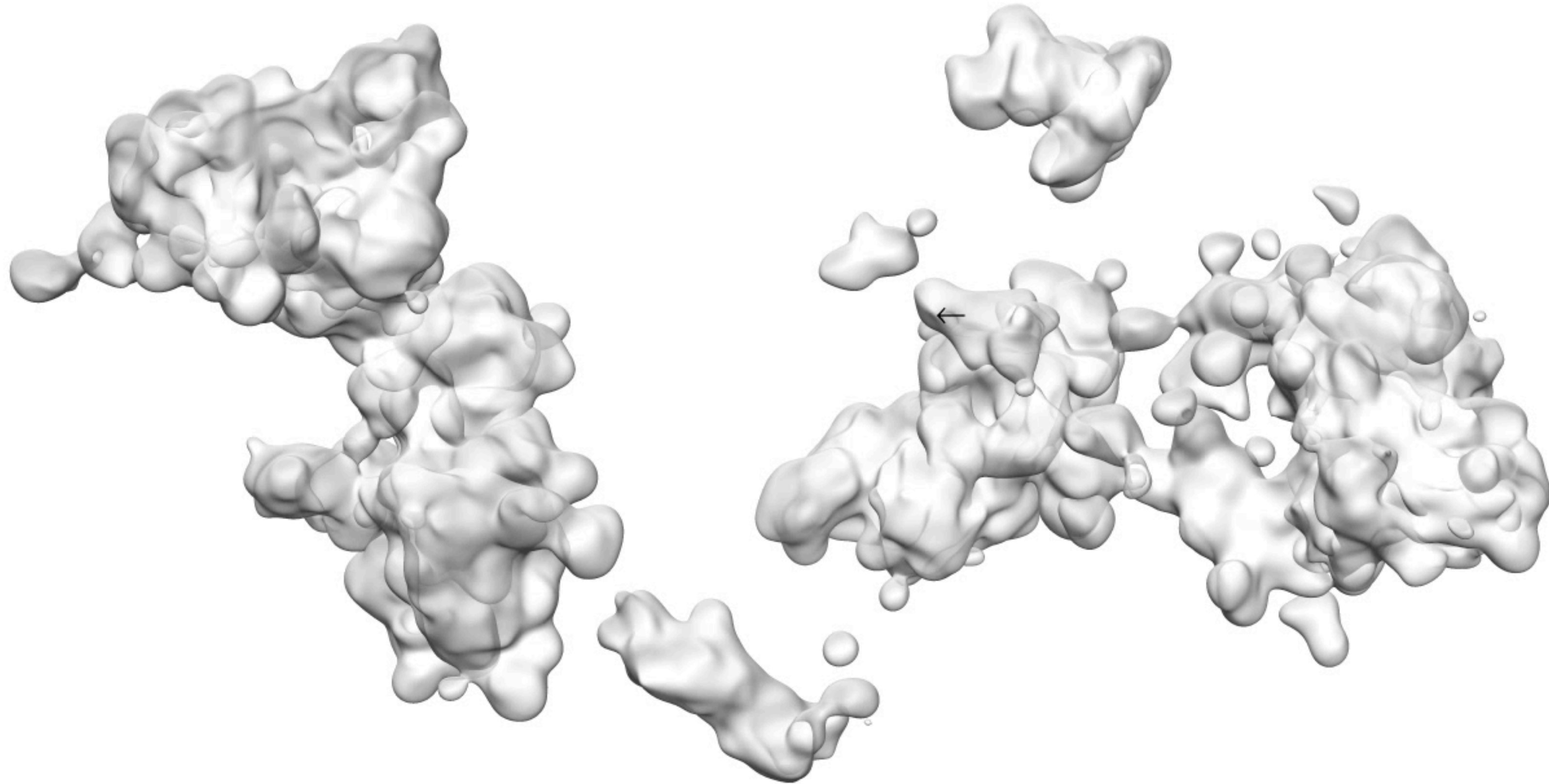
# Increasing resolution

Flexible fitting 3D structures based on Hi-C data





# Chromosome walking path @10Kb resolution







# Dynamics of gene activation



**Marco Di Stefano**  
**Ralph Stadhouders**  
**Enrique Vidal**

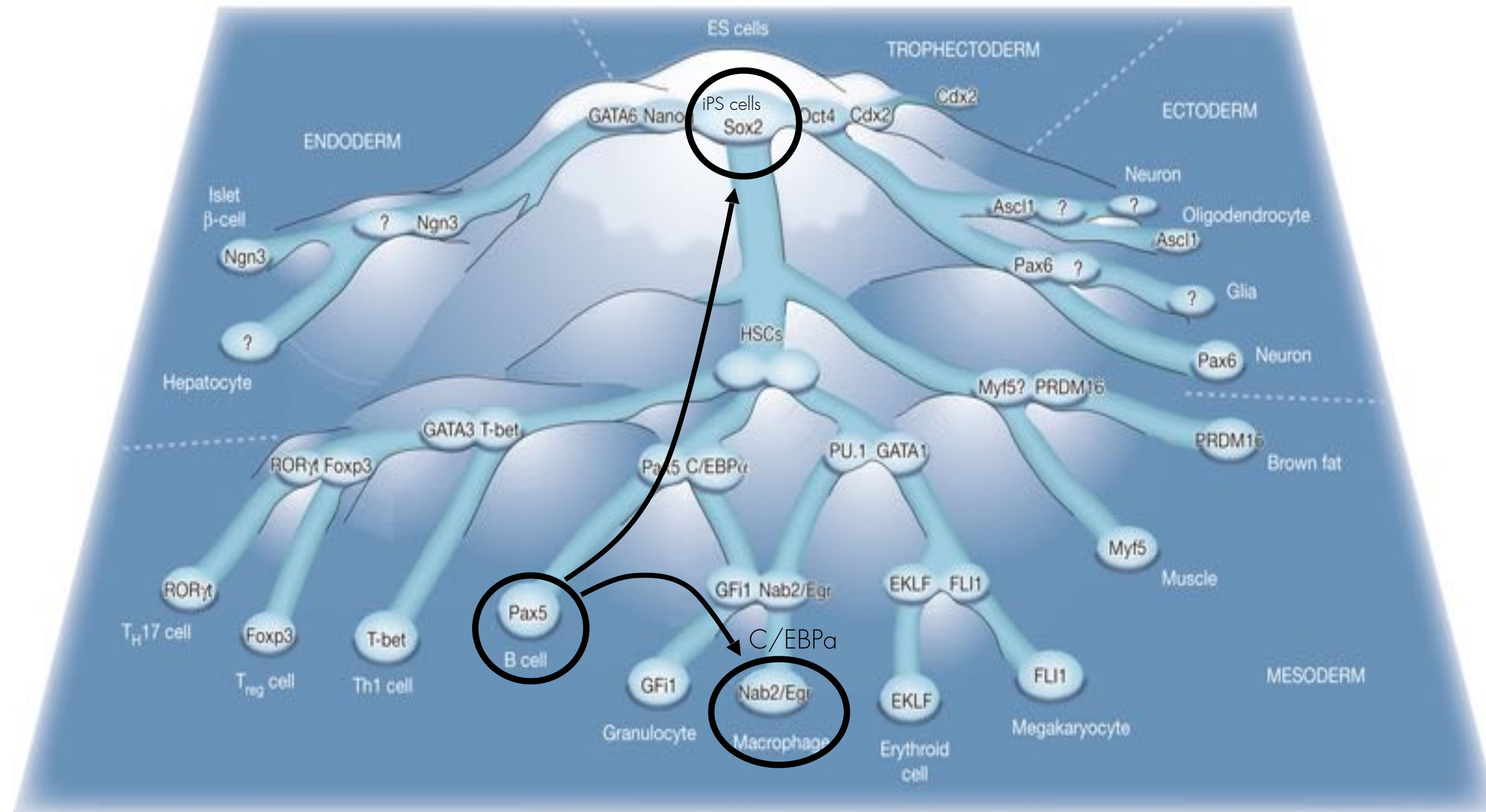
with Graf Lab (CRG, Barcelona)

Nature Genetics (2018) 50 238–249 & BioRxiv/under review



# 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**

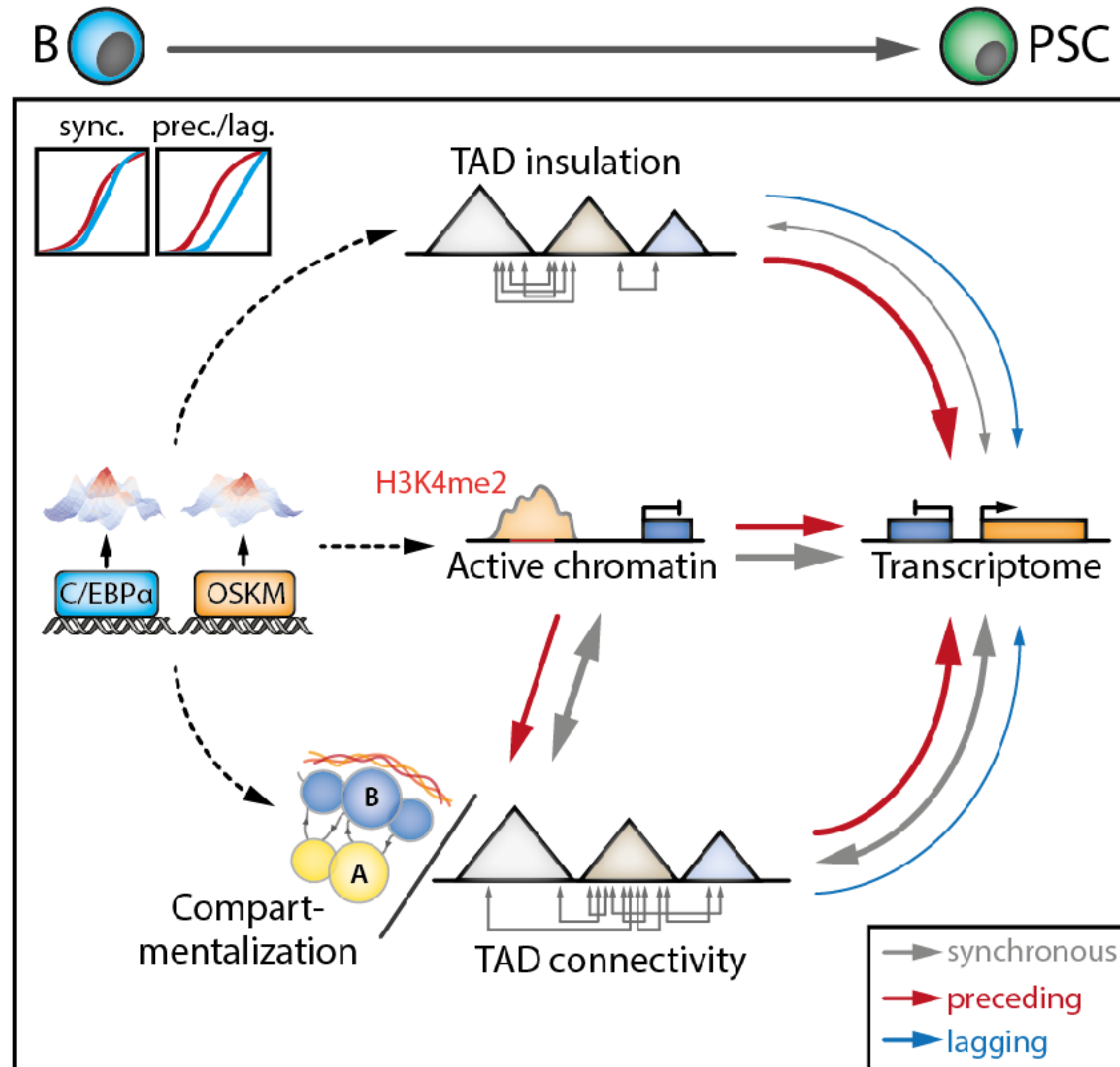
Transdifferentiation or reprogramming



# Interplay: topology, gene expression & chromatin

Graf & Enver (2009) Nature

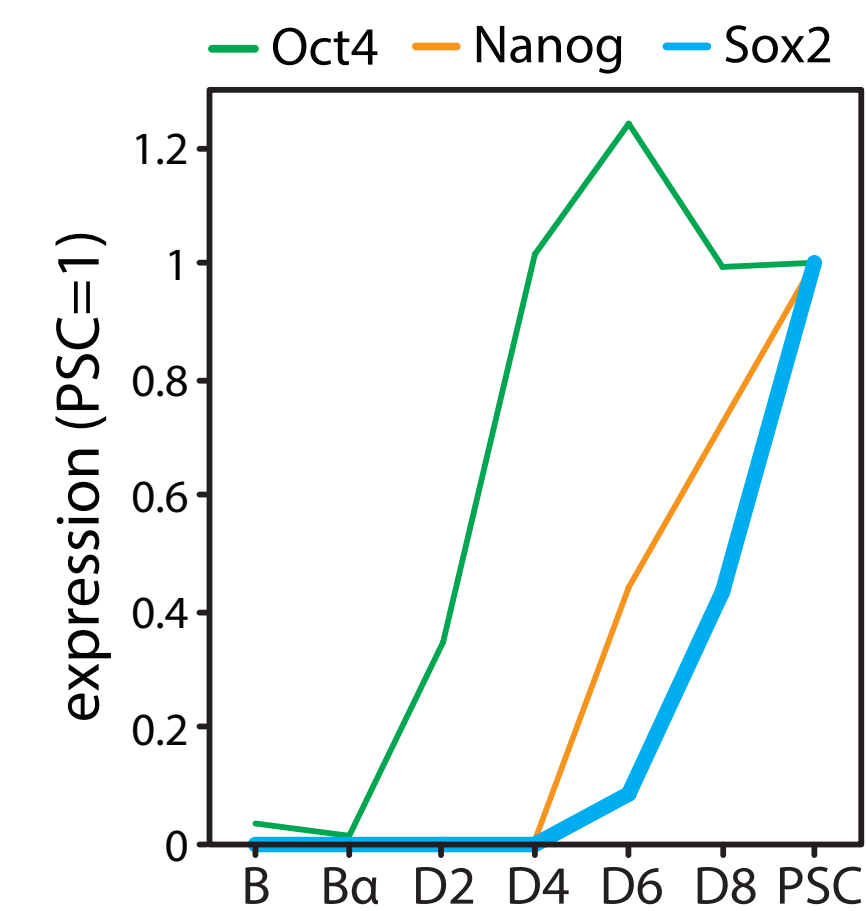
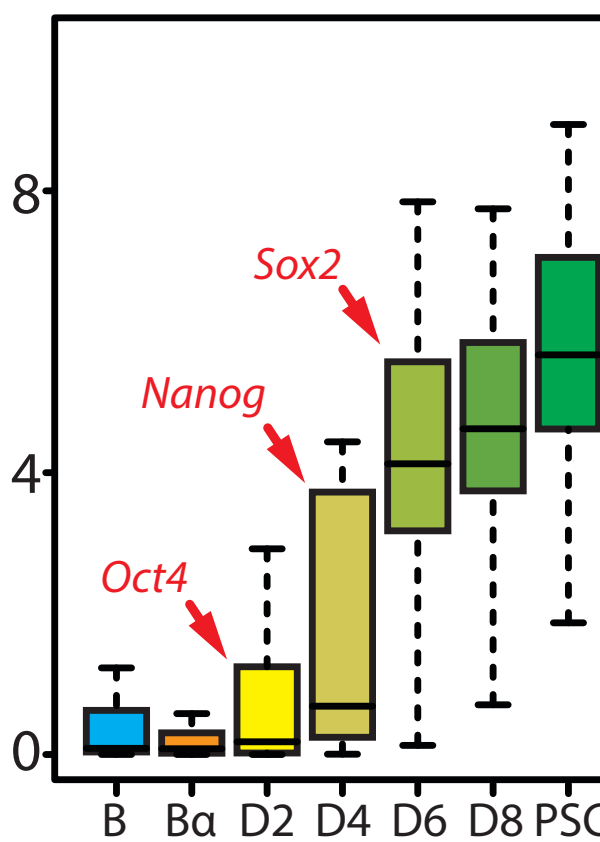
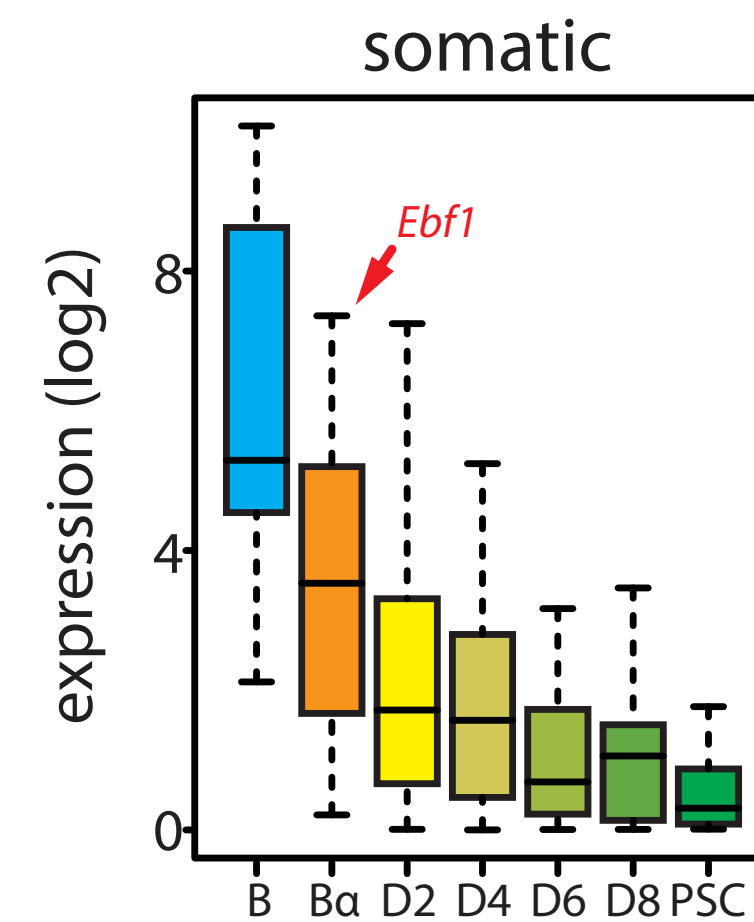
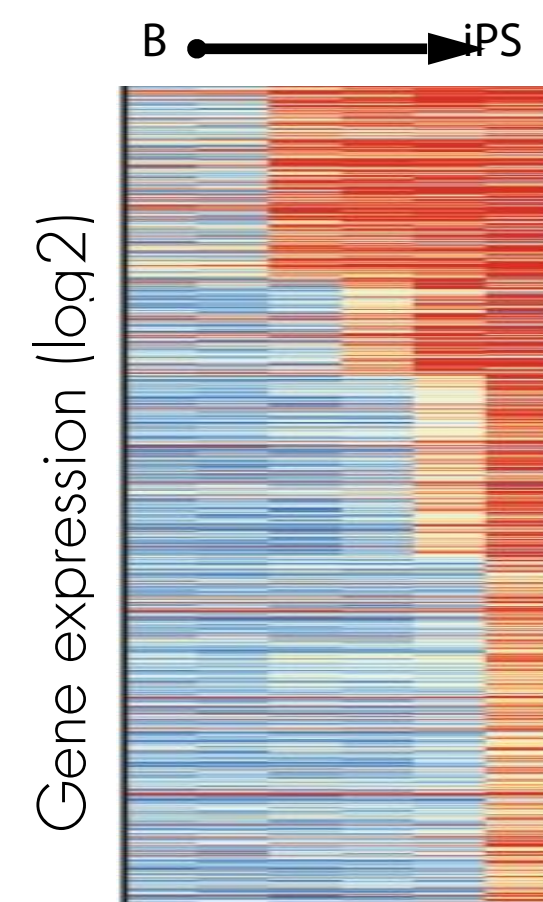
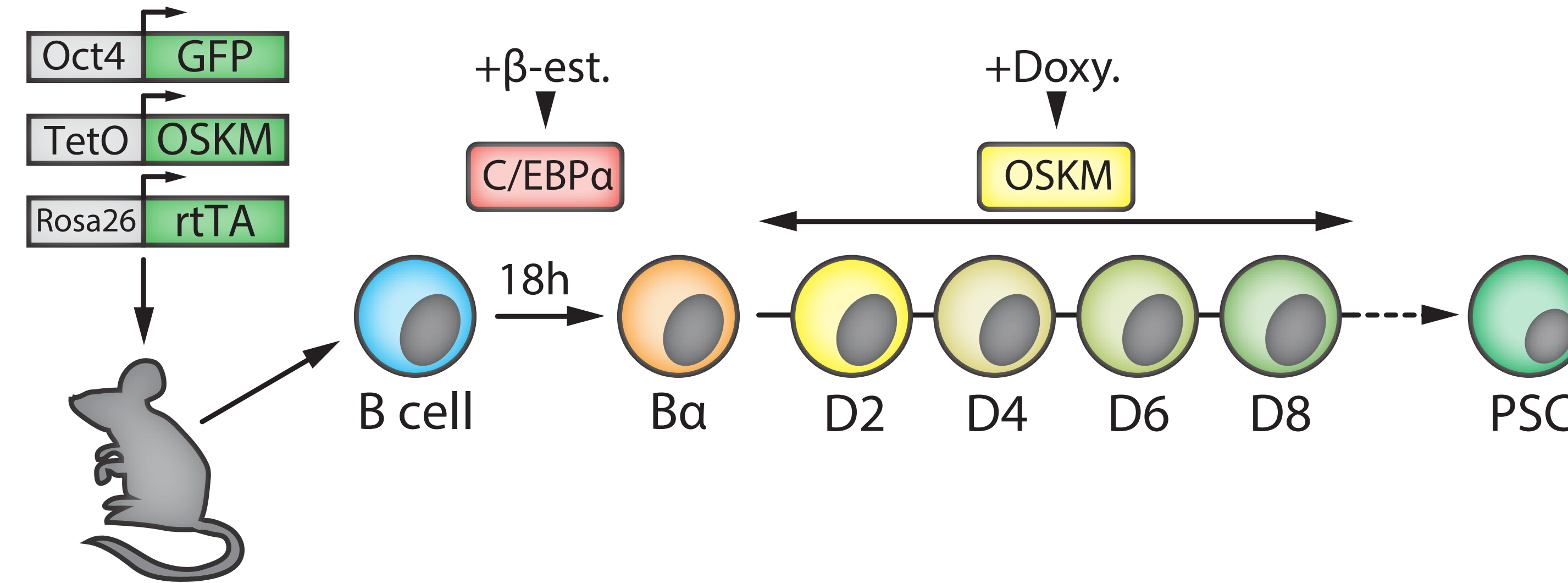
Stadhouders, R., Vidal, E. et al. (2018) Nature Genetics





# Reprogramming from B to PSC

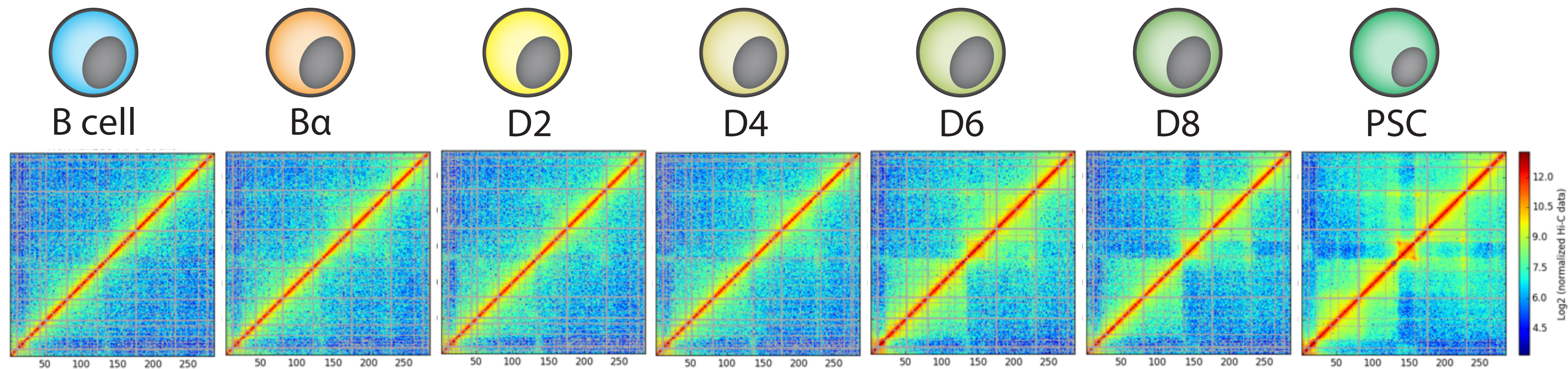
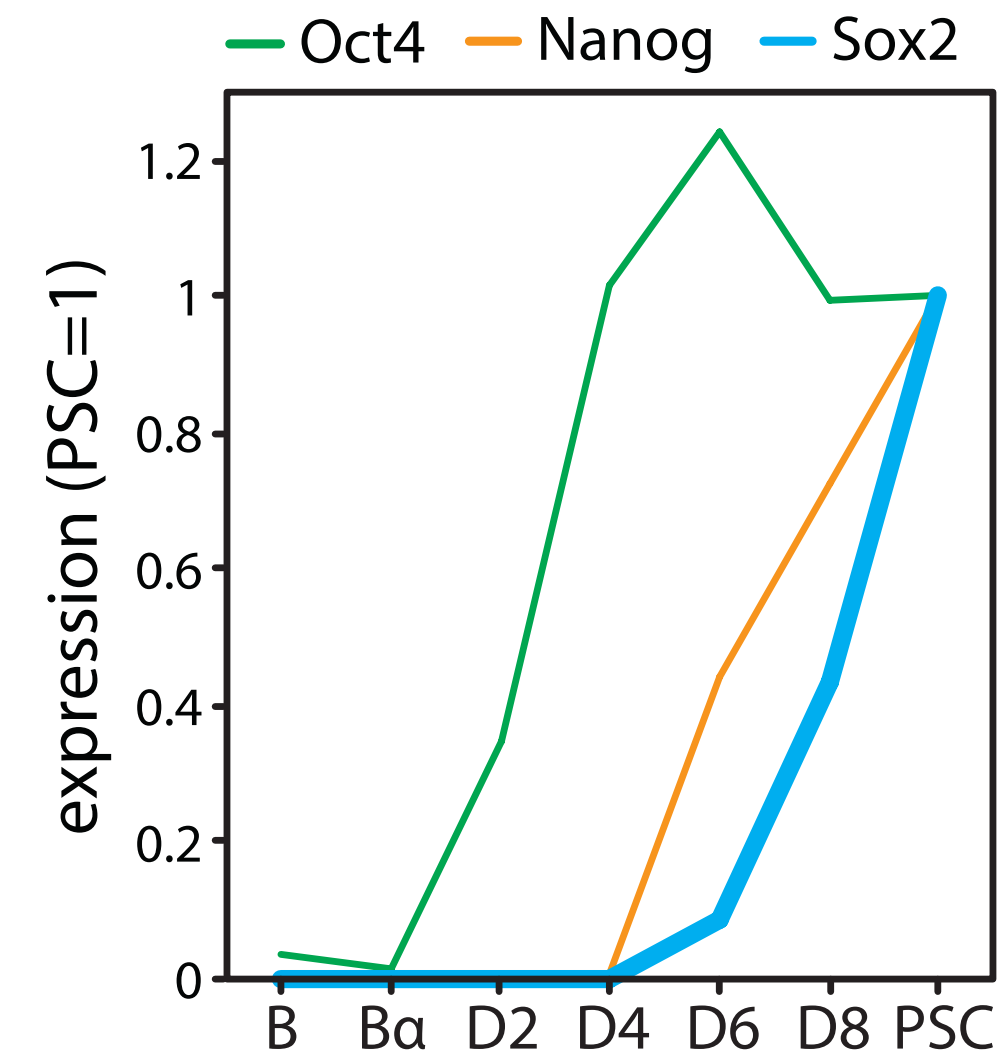
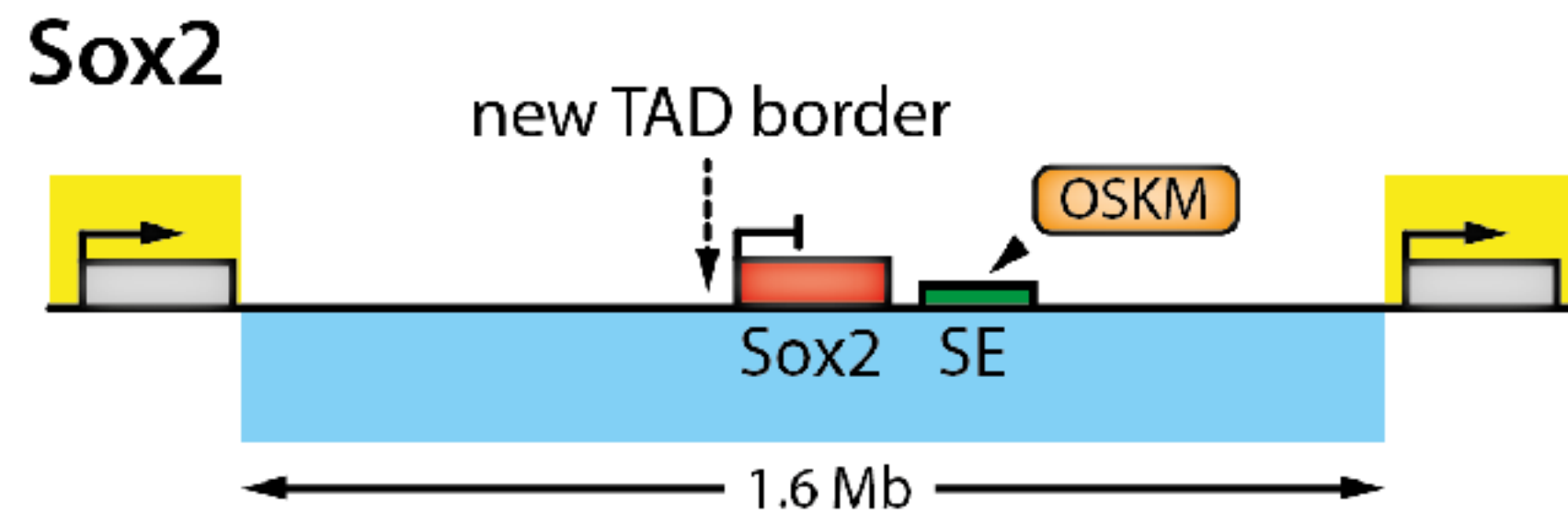
Stadhouders, R., Vidal, E. et al. (2018) Nature Genetics





# Hi-C maps of reprogramming from B to PSC

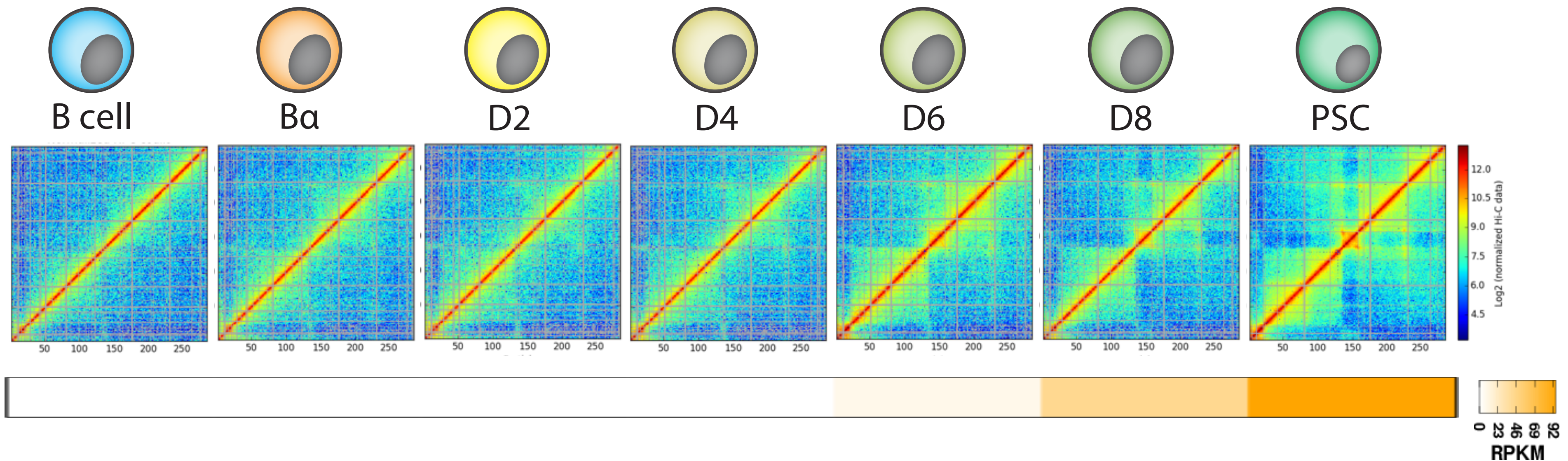
## The SOX2 locus





# Hi-C maps of reprogramming from B to PSC

The SOX2 locus

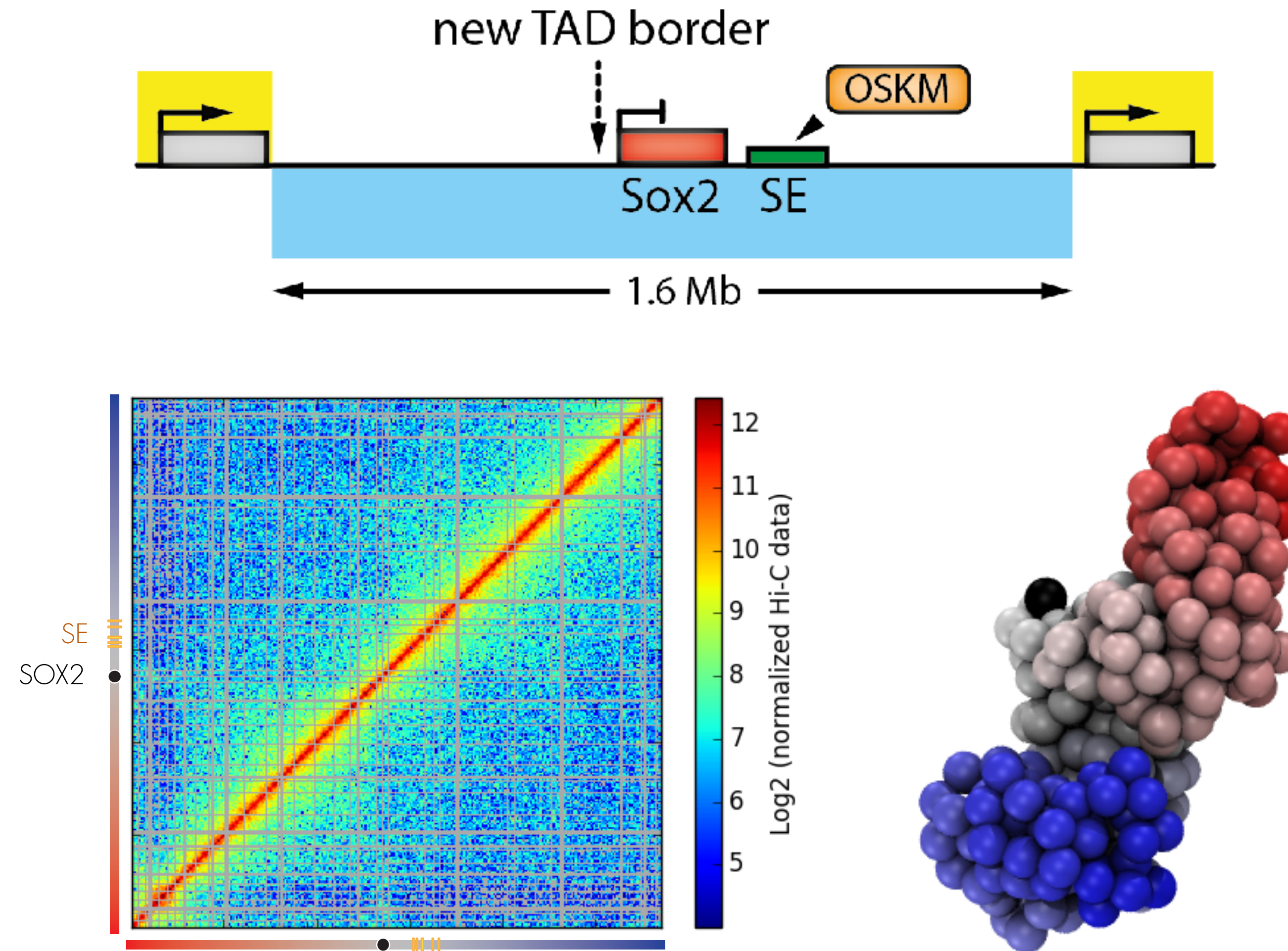


How do these structural rearrangements interplay with the transcription activity?

What are the main drivers of structural transitions?



# TADbit modeling of SOX2 from B cells Hi-C

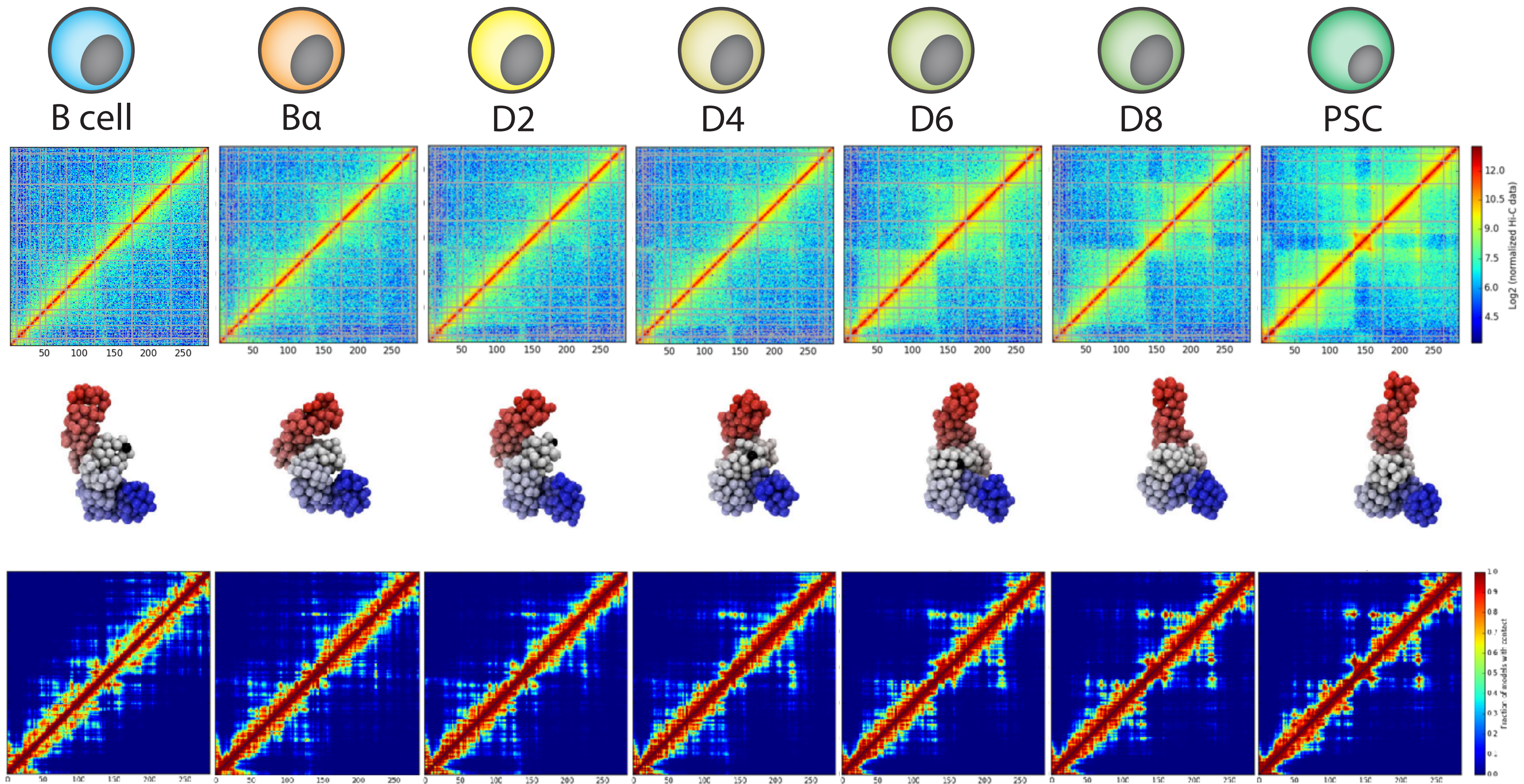


Optimal IMP parameters  
lowfreq=0 , upfreq=1 , maxdist=200nm, dcutoff=125nm, particle size=50nm (5kb)



# Models of reprogramming from B to PSC

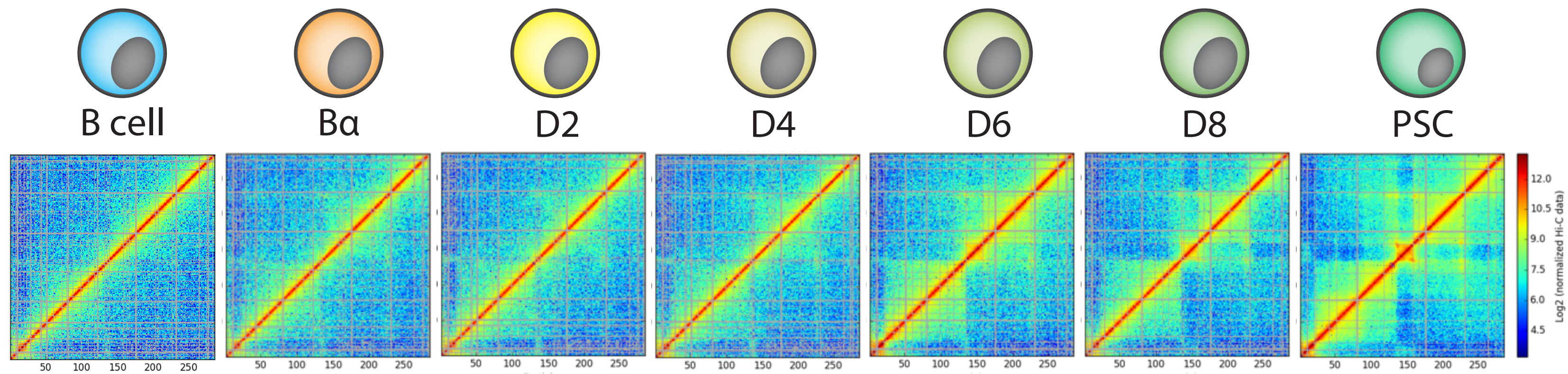
## The SOX2 locus





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

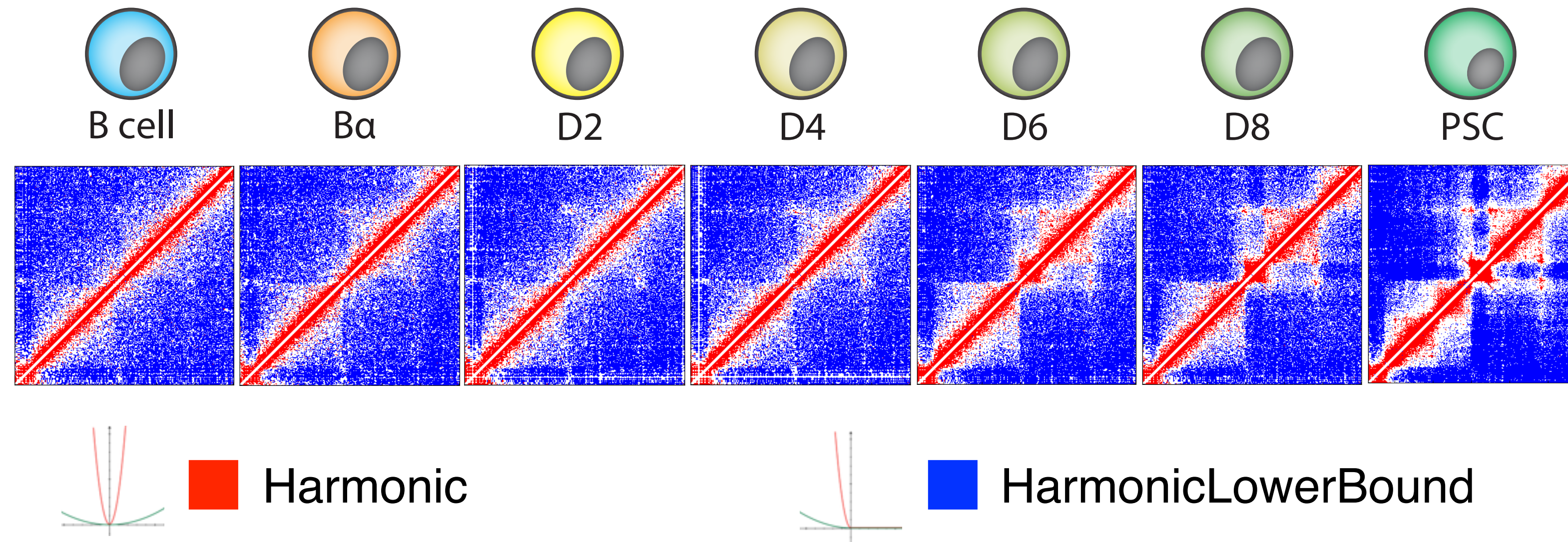
The SOX2 locus





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

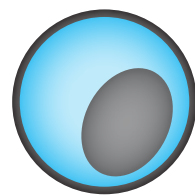
The SOX2 locus



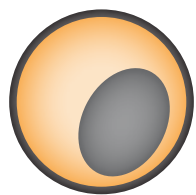


# TADdyn: from time-series Hi-C maps to dynamic restraints

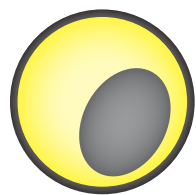
The SOX2 locus



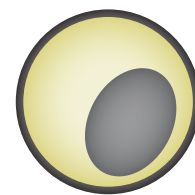
B cell



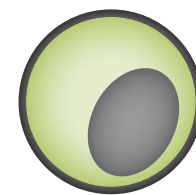
B $\alpha$



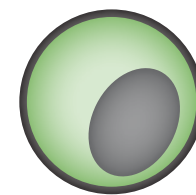
D2



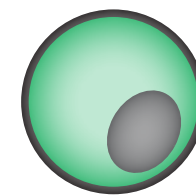
D4



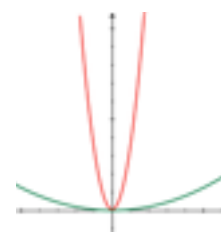
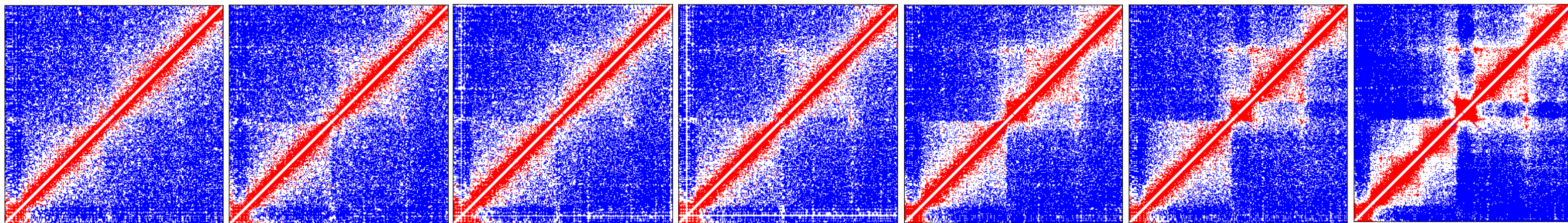
D6



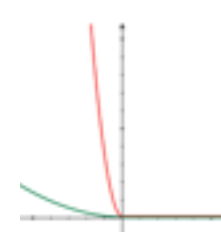
D8



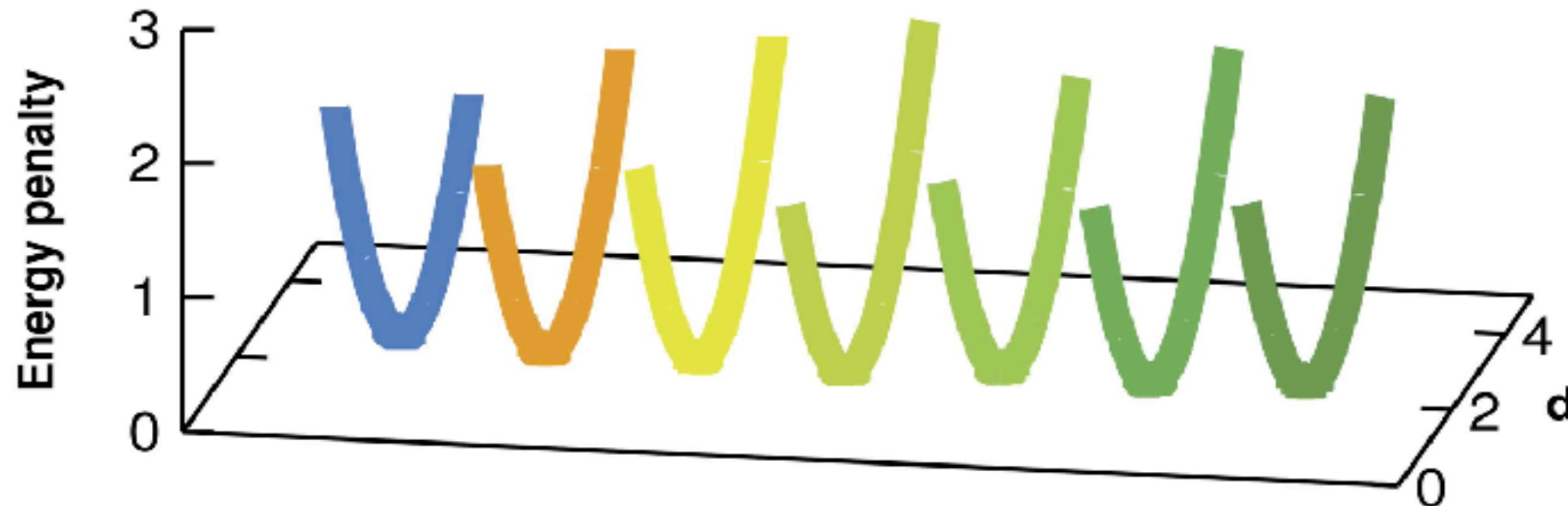
PSC



Harmonic



HarmonicLowerBound

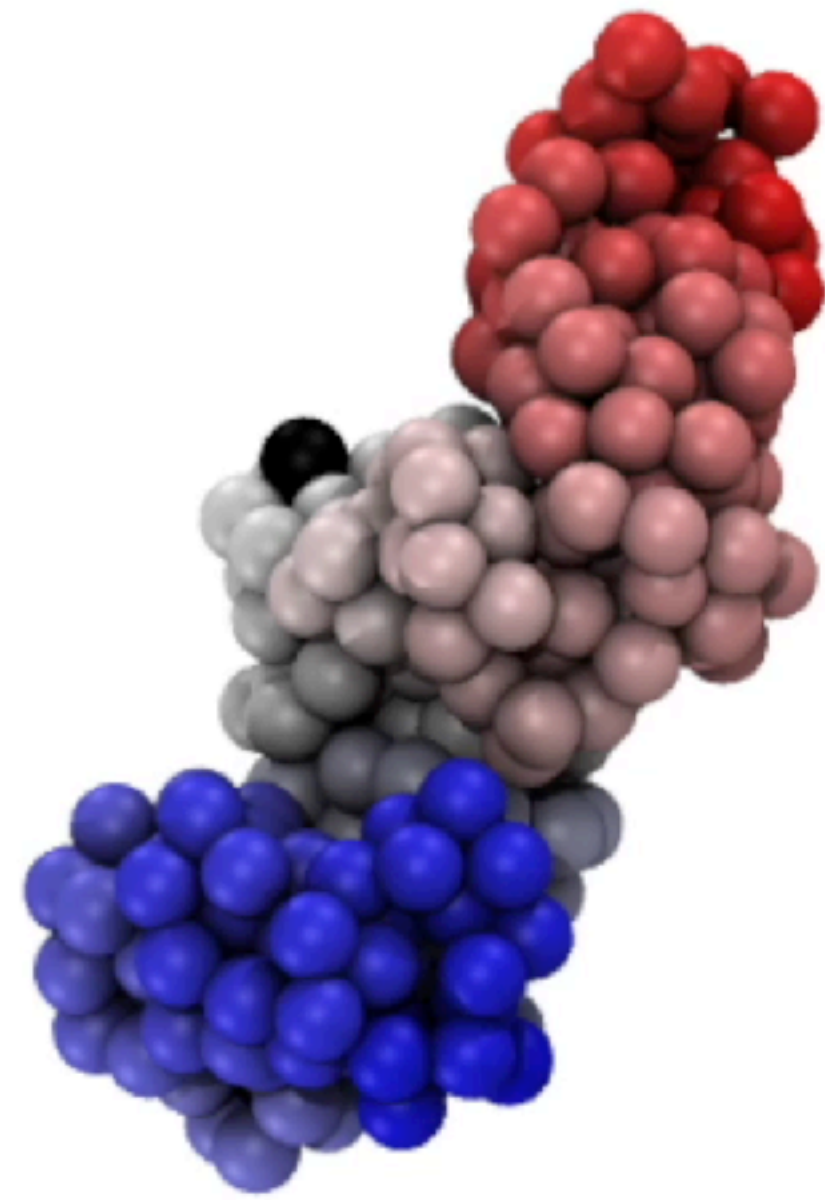


Transition	Stable	Vanishing	Raising
B -> B $\alpha$	18,612	6,984	7,290
B $\alpha$ -> D2	18,512	7,390	6,687
D2 -> D4	18,369	6,830	6,893
D4 -> D6	18,971	6,291	7,289
D6 -> D8	20,167	6,093	6,250
D8 -> ES	20,679	5,738	6,173

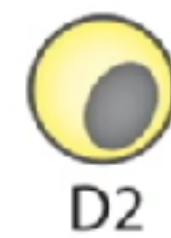
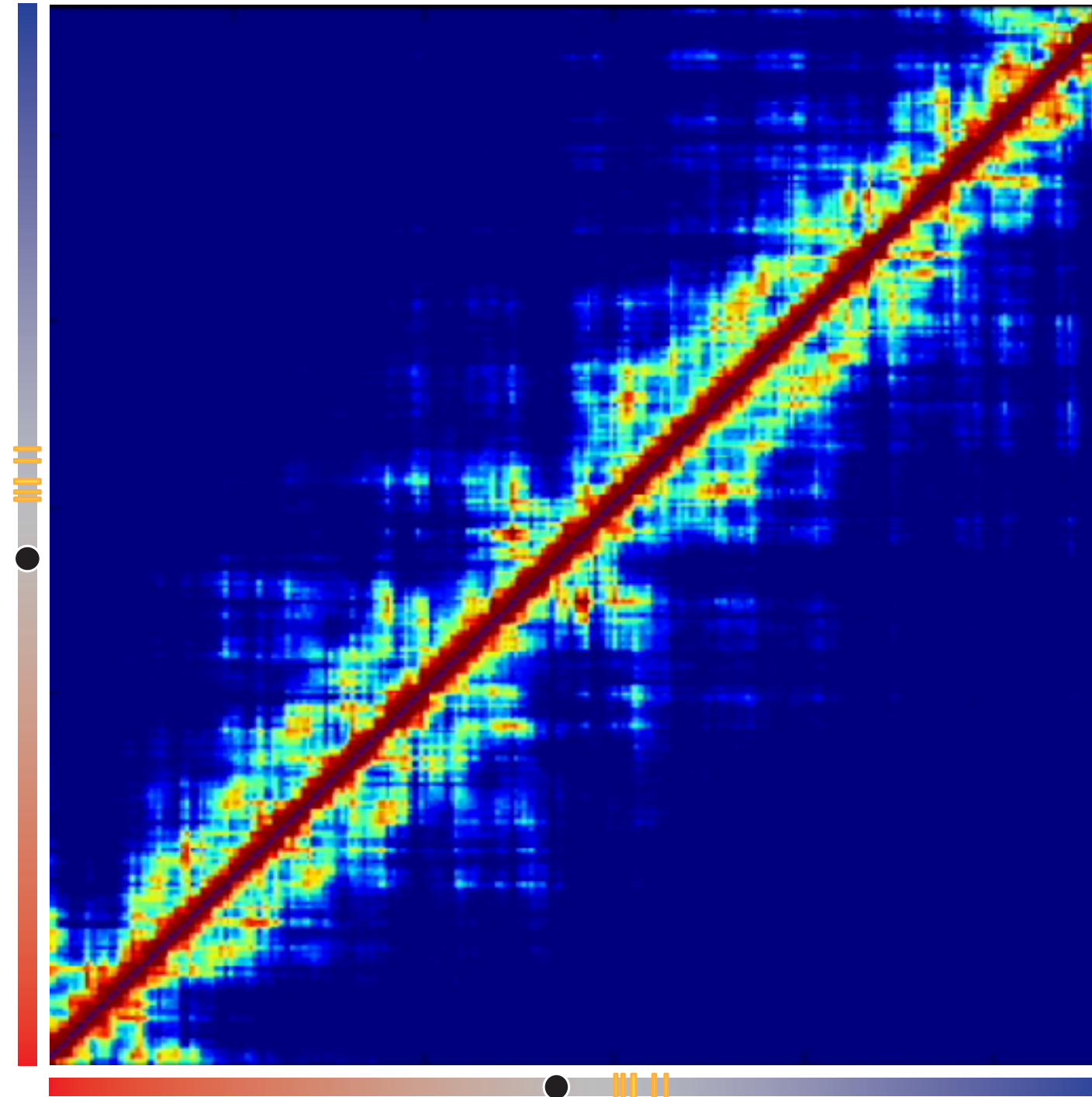


# SOX2 locus structural changes from B to PSC

Contacts



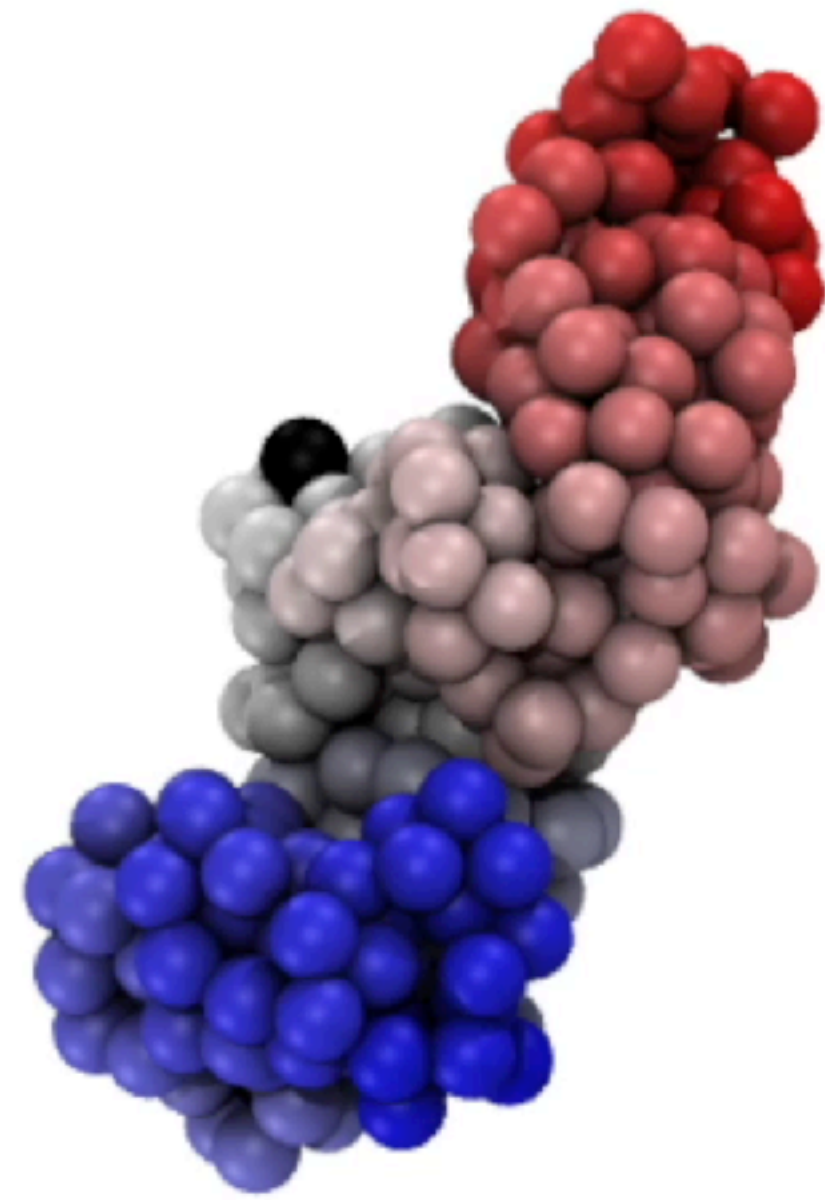
SE  
SOX2



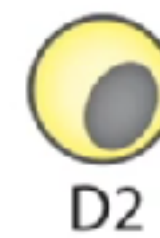
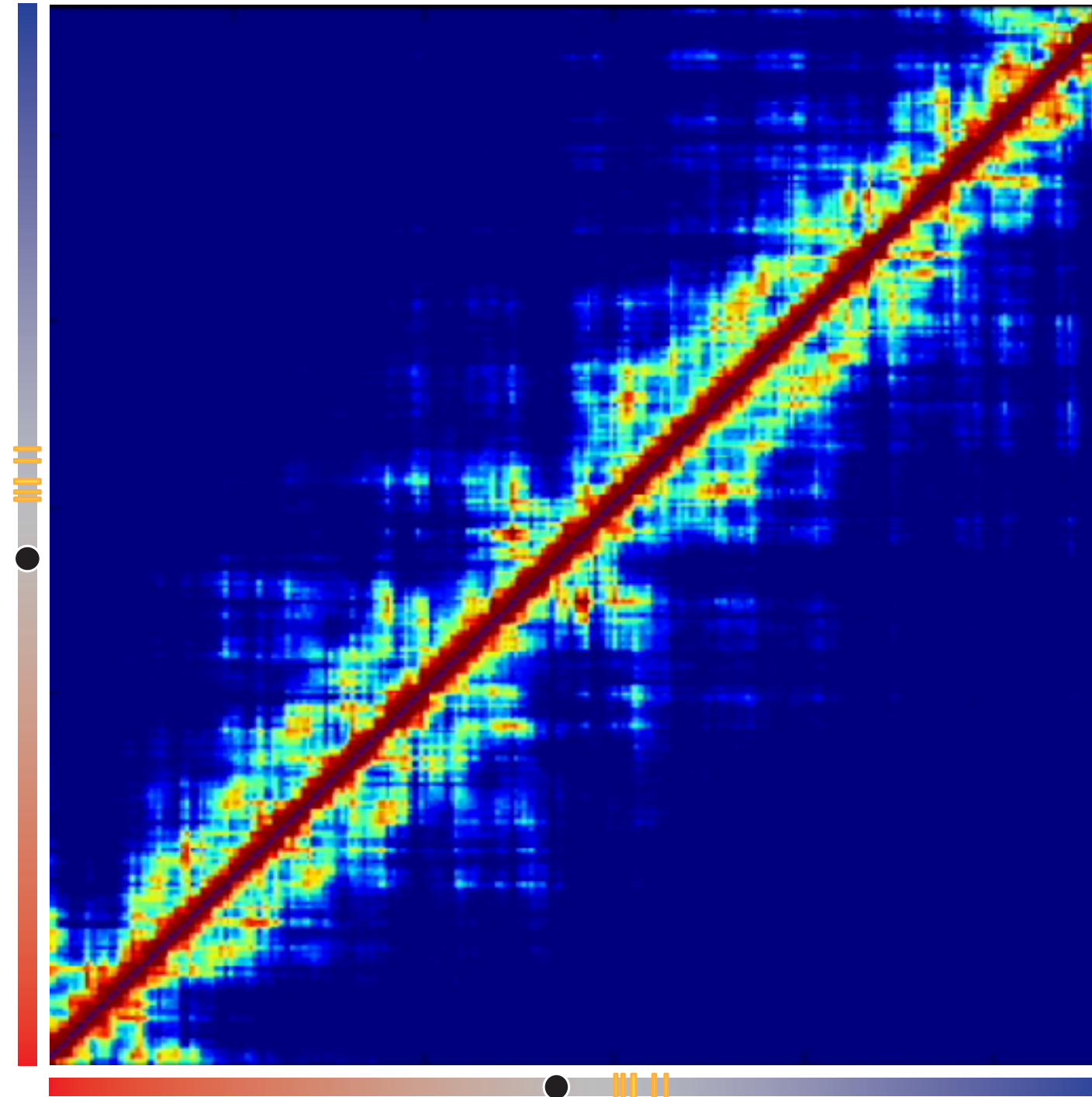


# SOX2 locus structural changes from B to PSC

Contacts



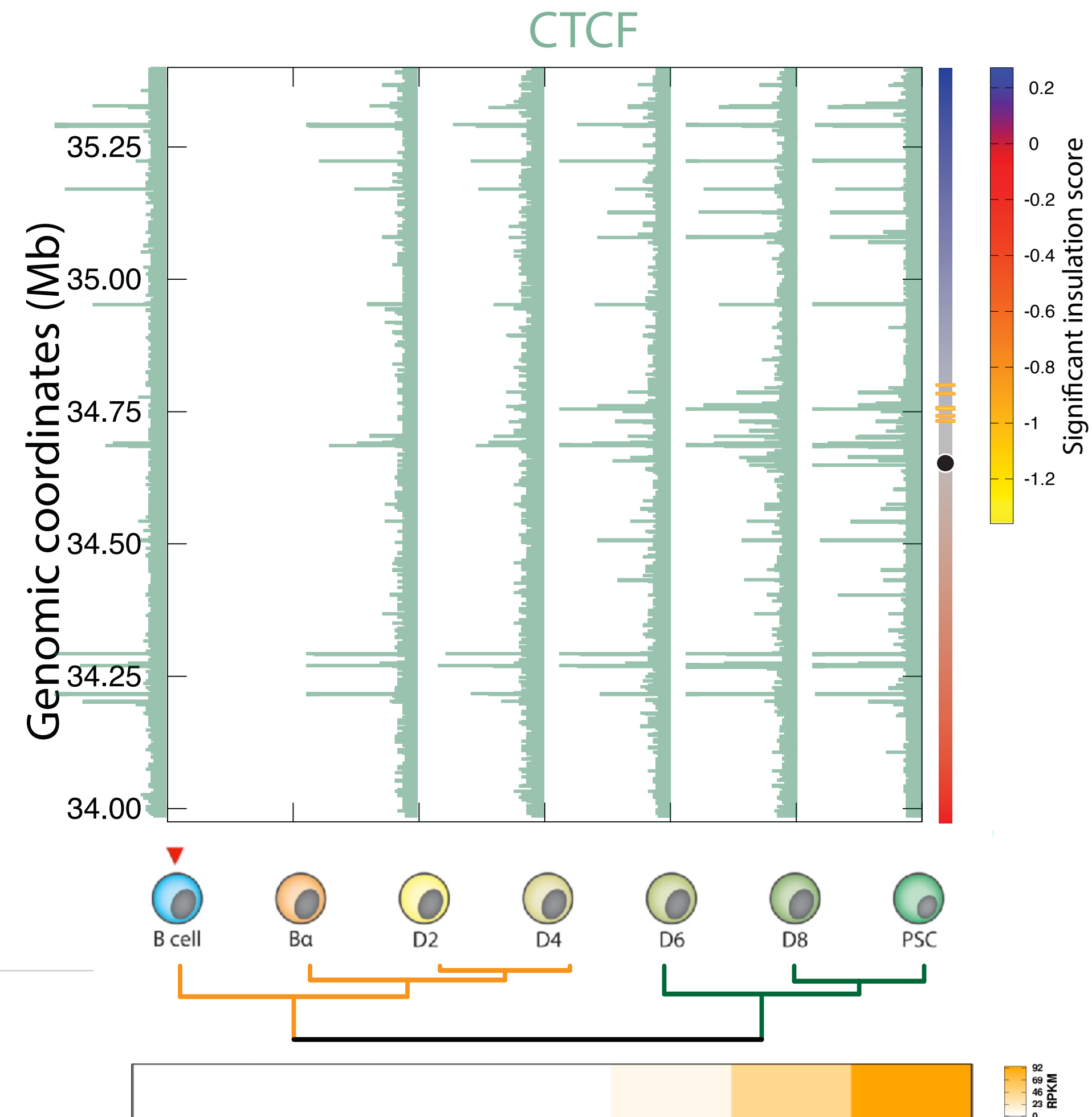
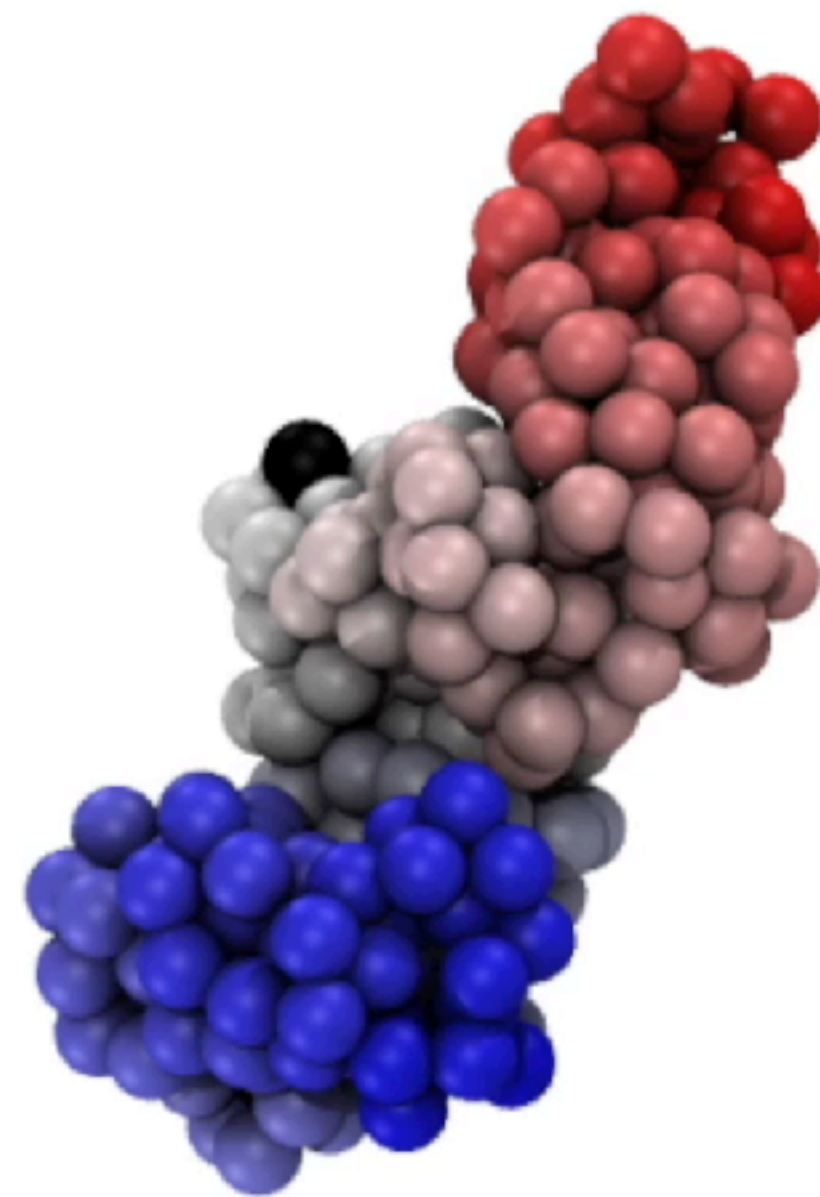
SE  
SOX2





# SOX2 locus structural changes from B to PSC

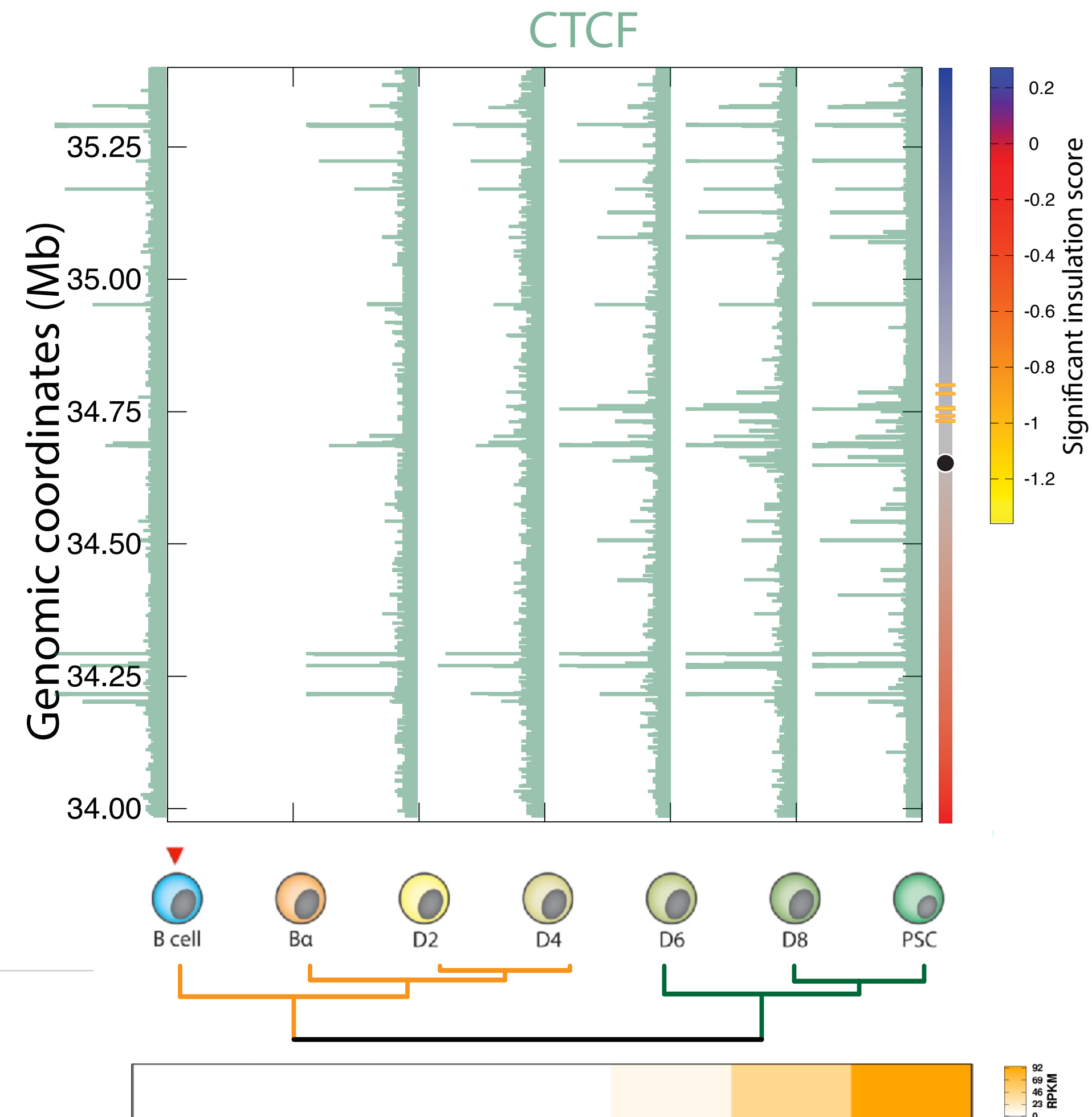
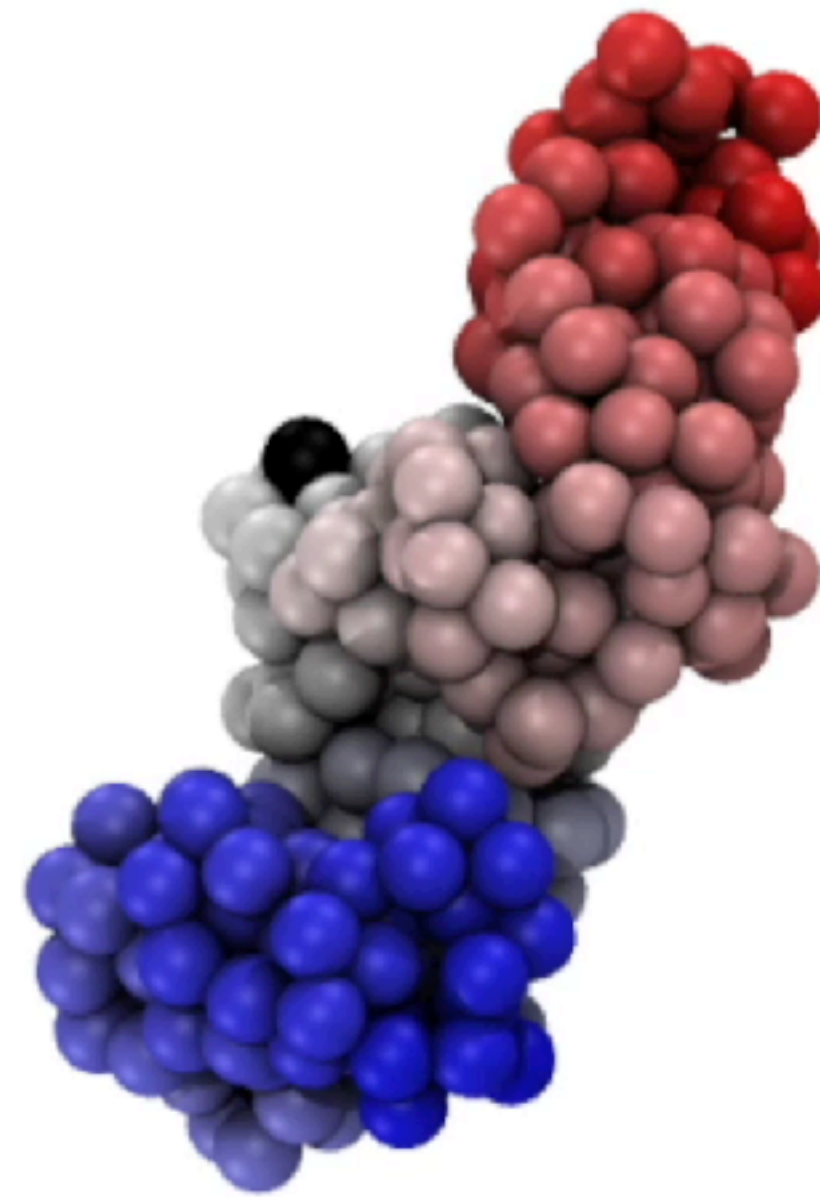
TAD borders





# SOX2 locus structural changes from B to PSC

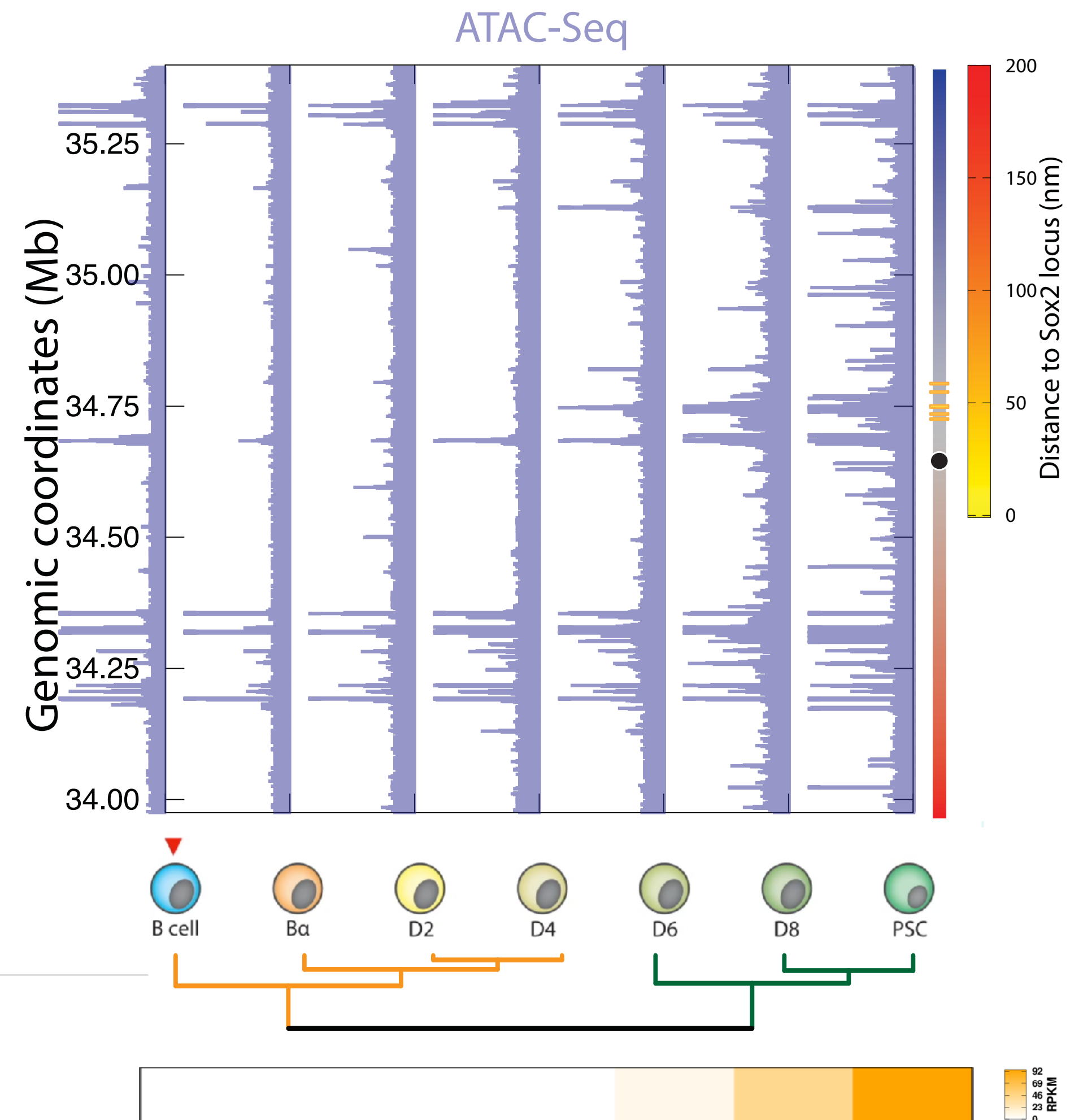
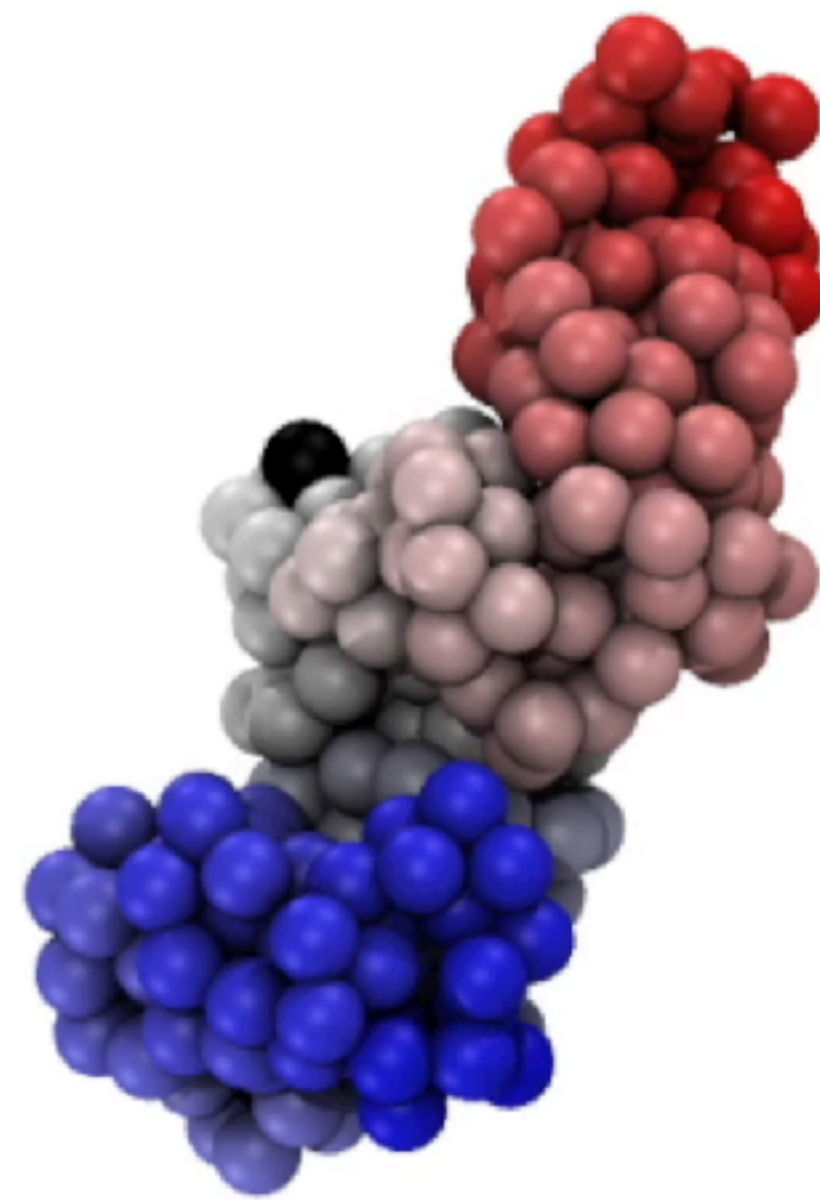
TAD borders





# SOX2 locus structural changes from B to PSC

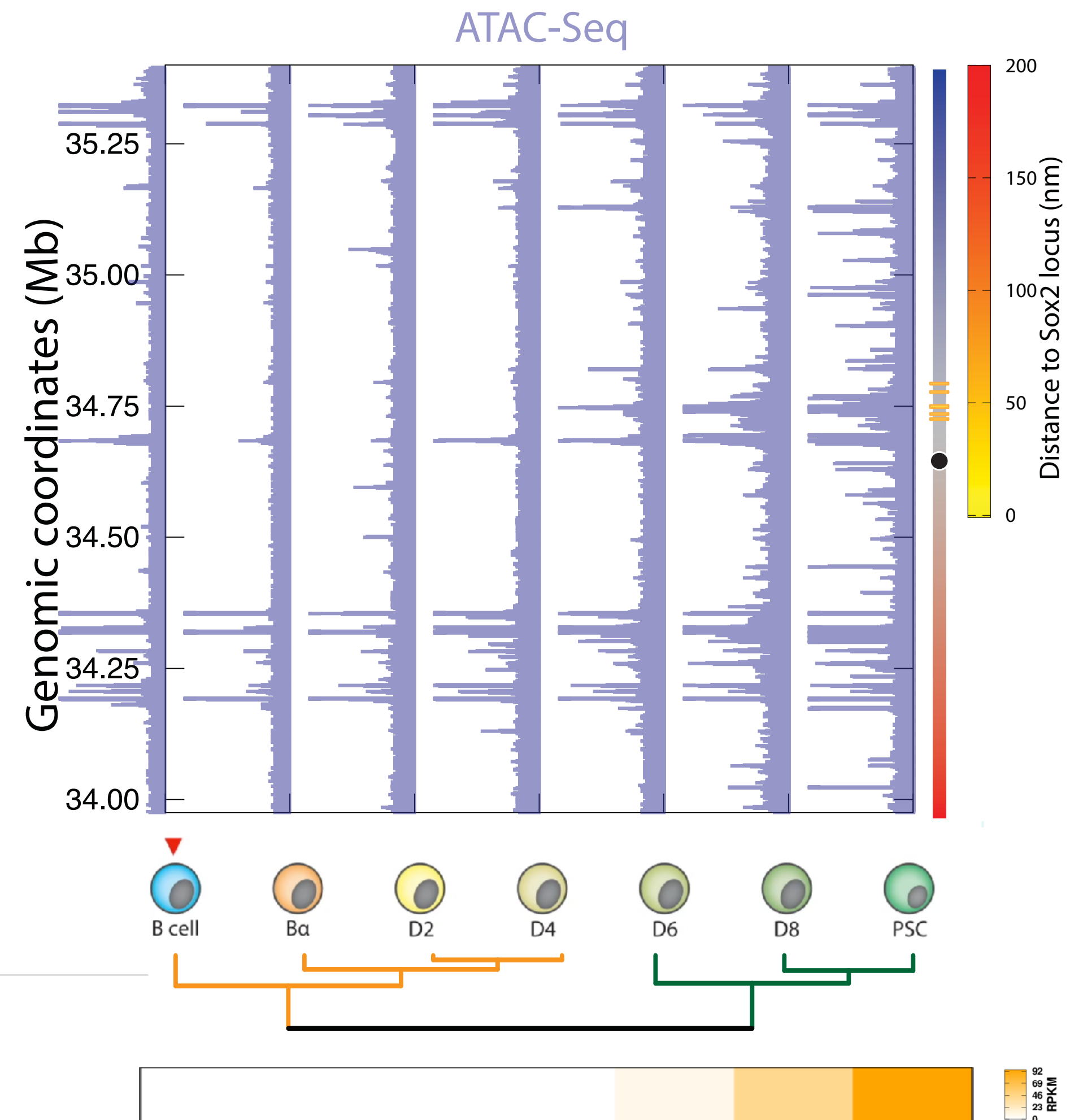
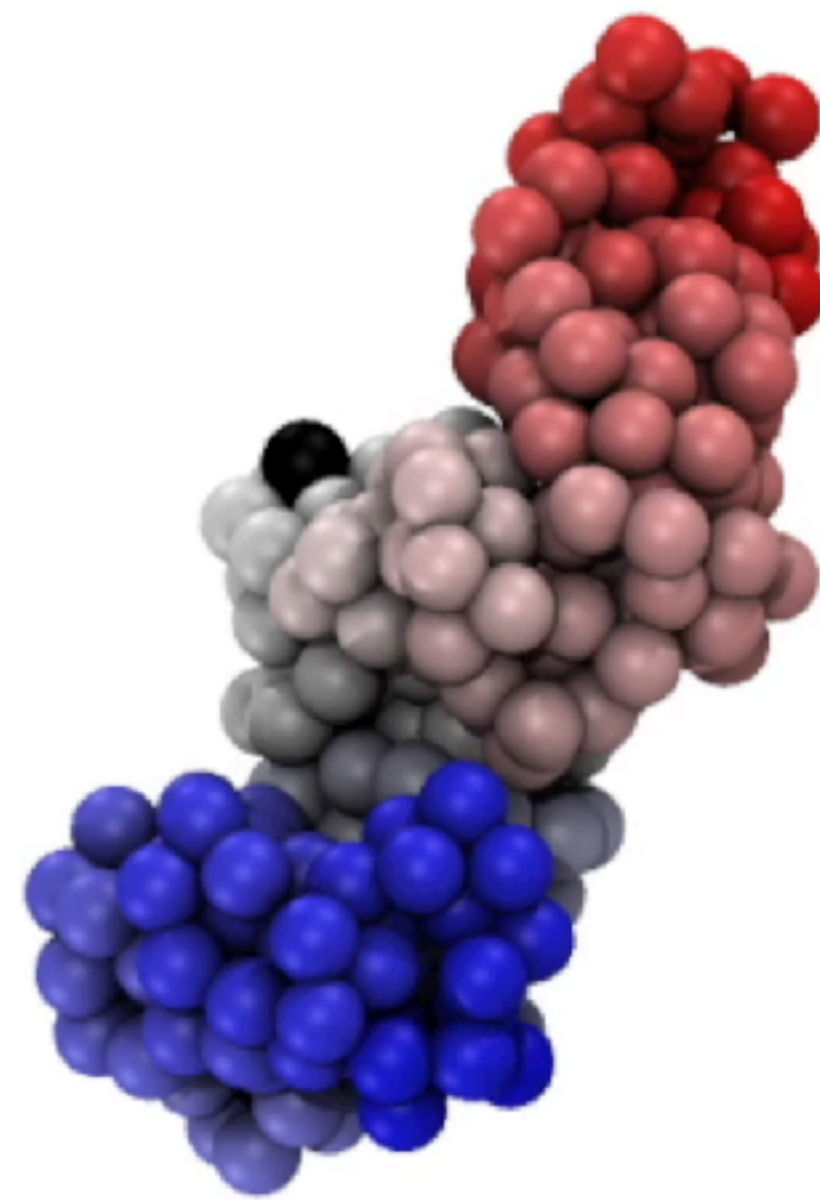
Distance to regulatory elements





# SOX2 locus structural changes from B to PSC

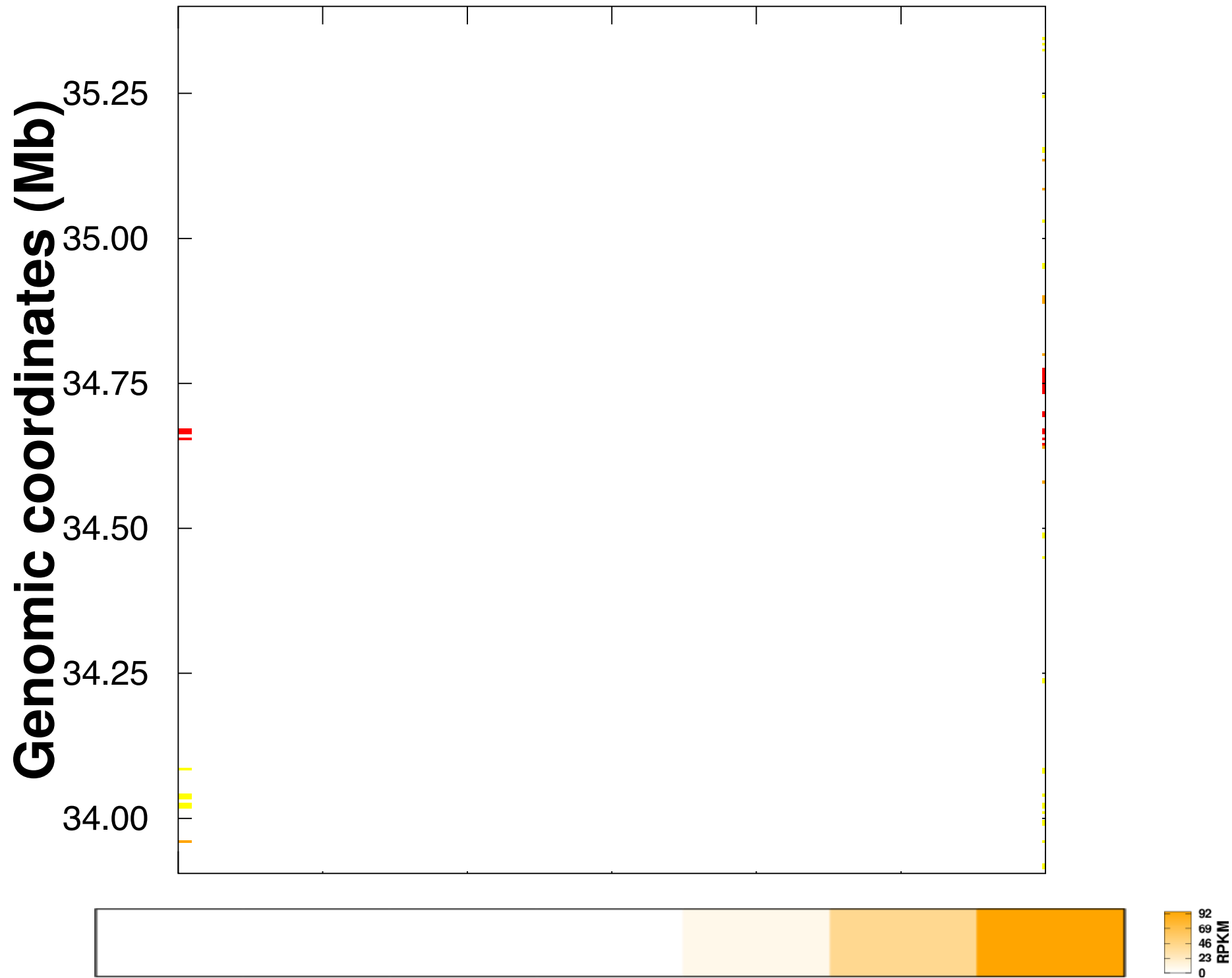
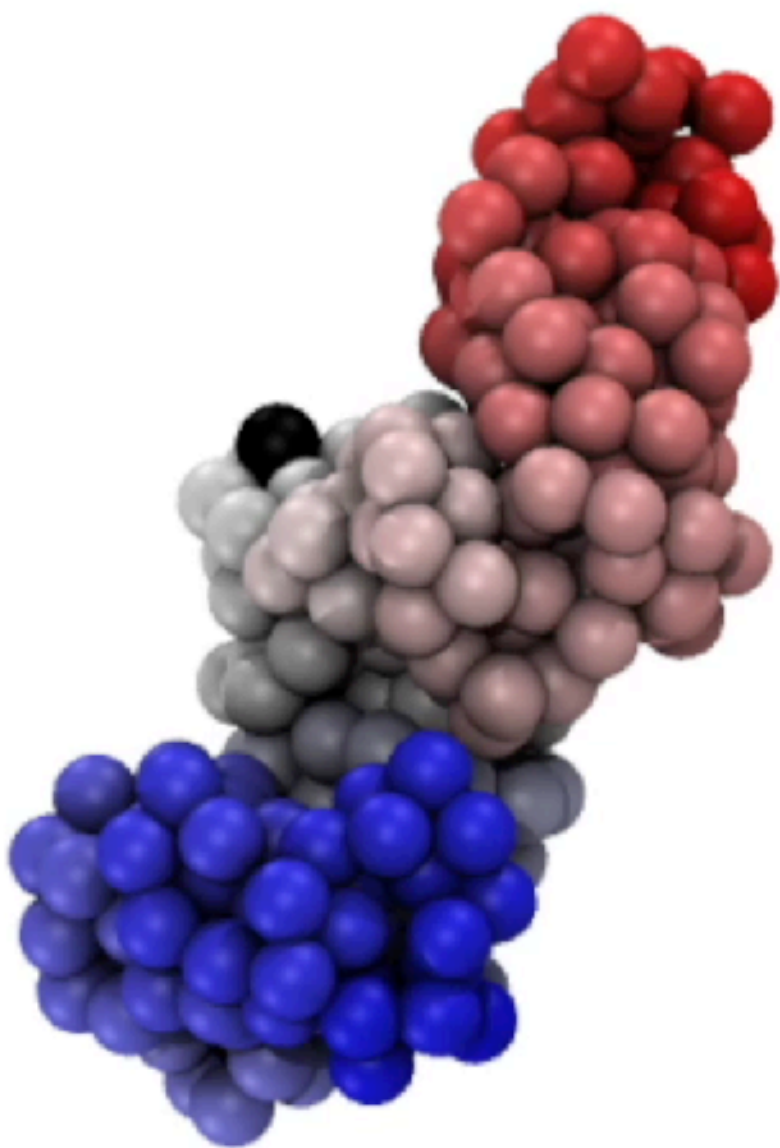
Distance to regulatory elements





# SOX2 locus structural changes from B to PSC

Chromatin Activity



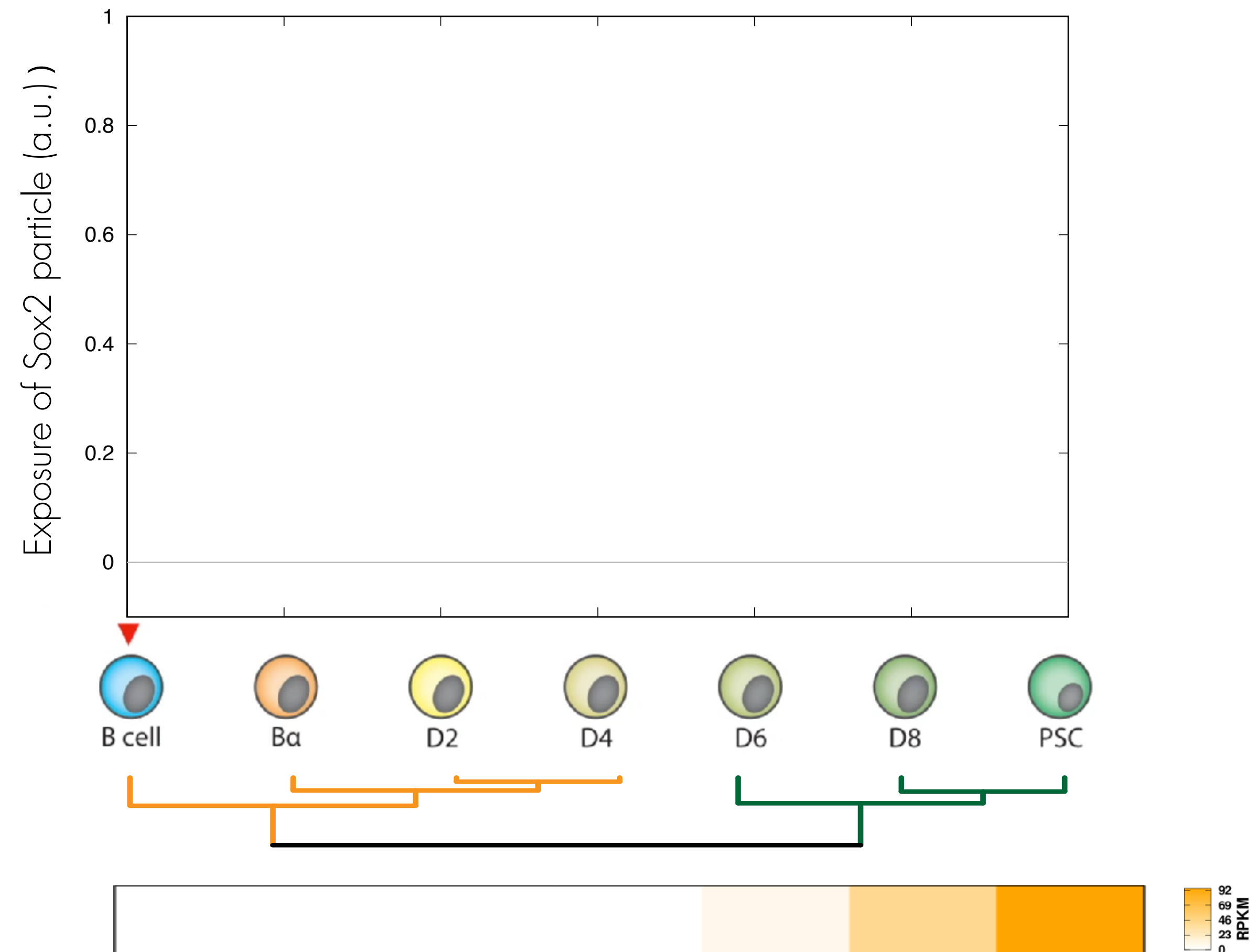
	B	Ba	D2	D4	D6	D8	PSC
A	9	6	7	13	13	22	48
AP	4	1	4	4	4	13	23
APD	3	1	1	1	4	10	15





# SOX2 locus structural changes from B to PSC

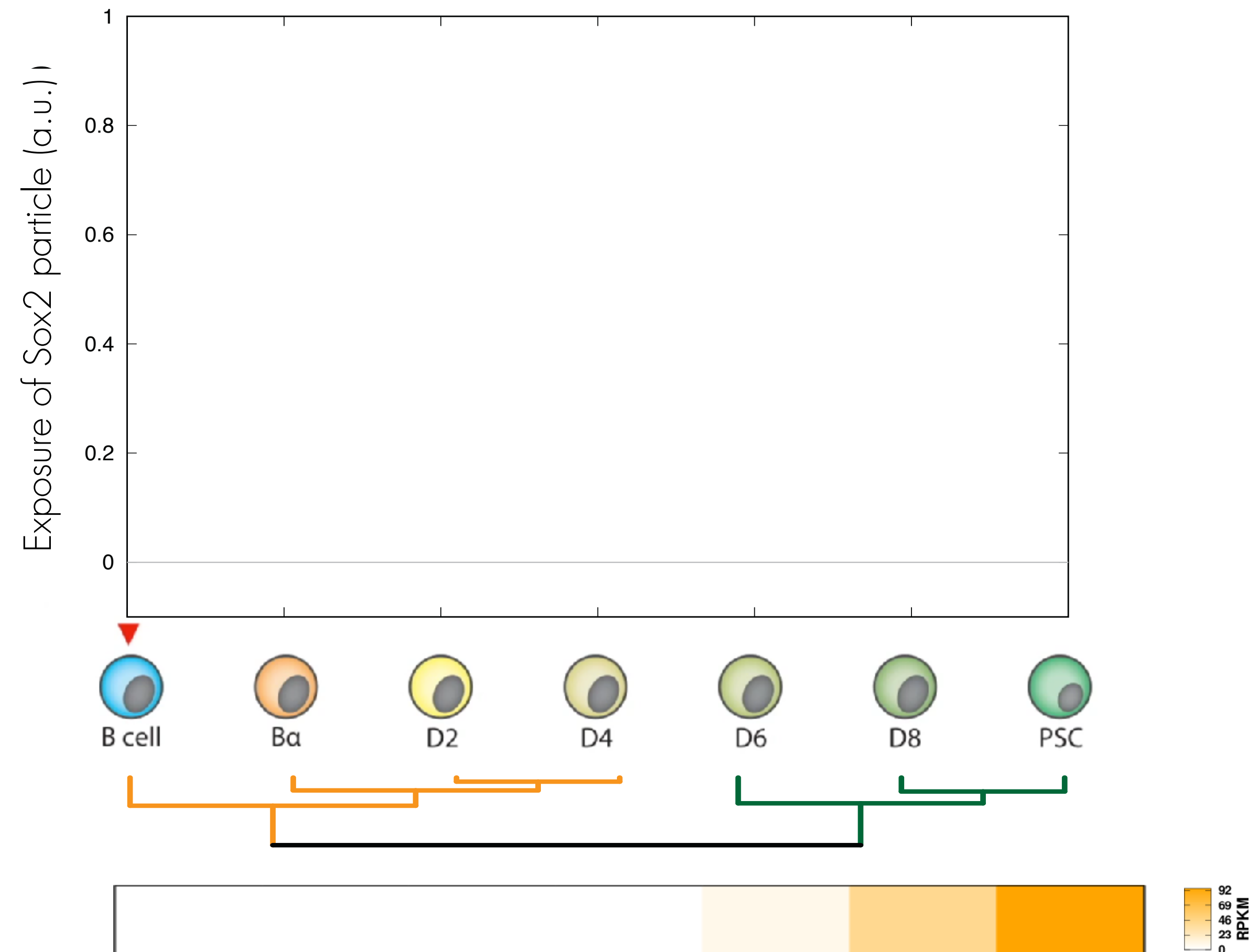
Structural exposure





# SOX2 locus structural changes from B to PSC

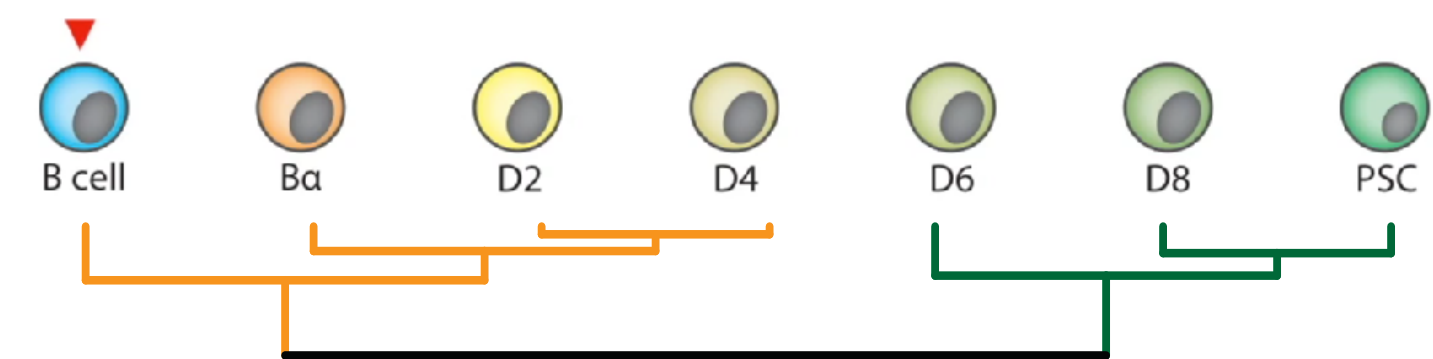
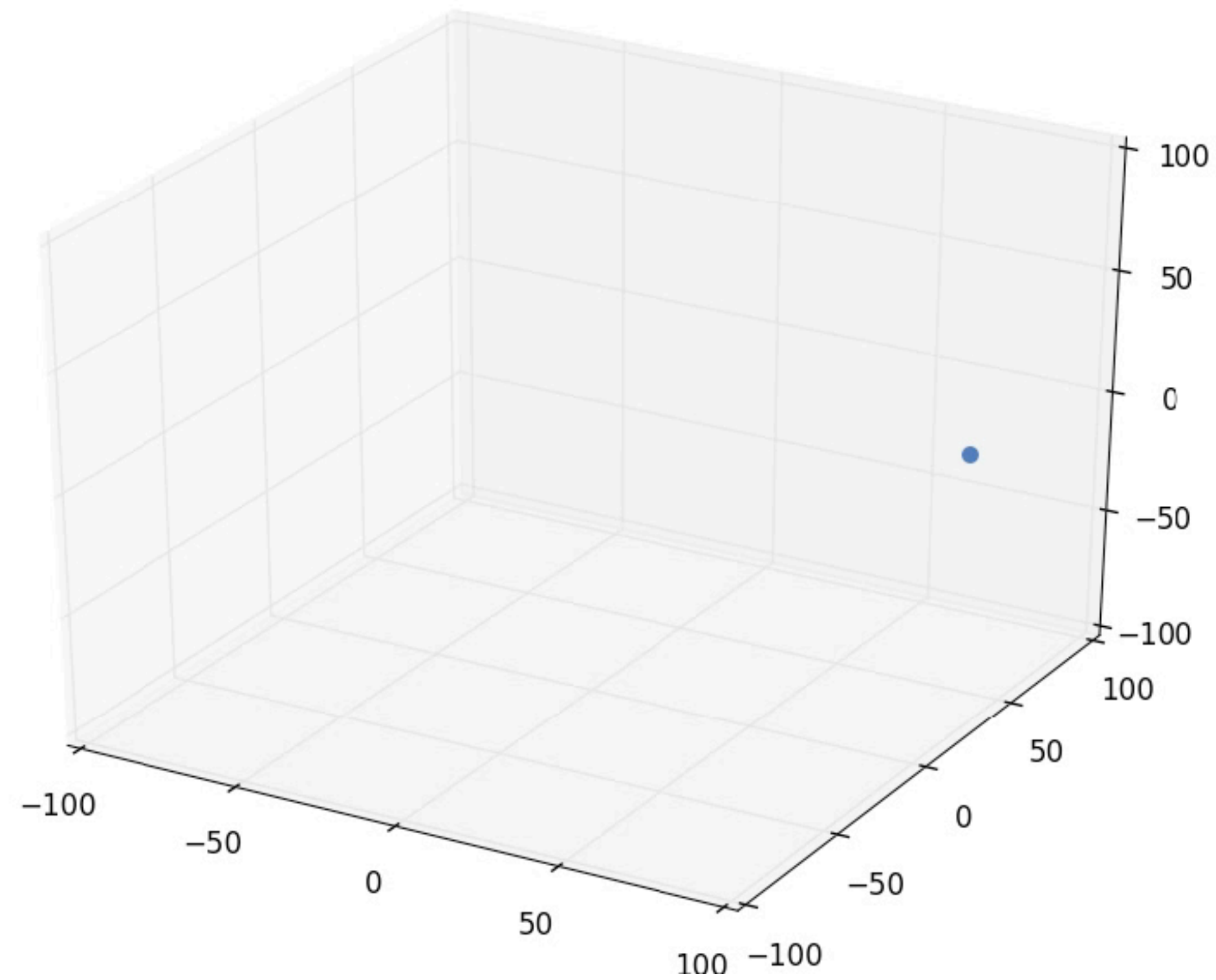
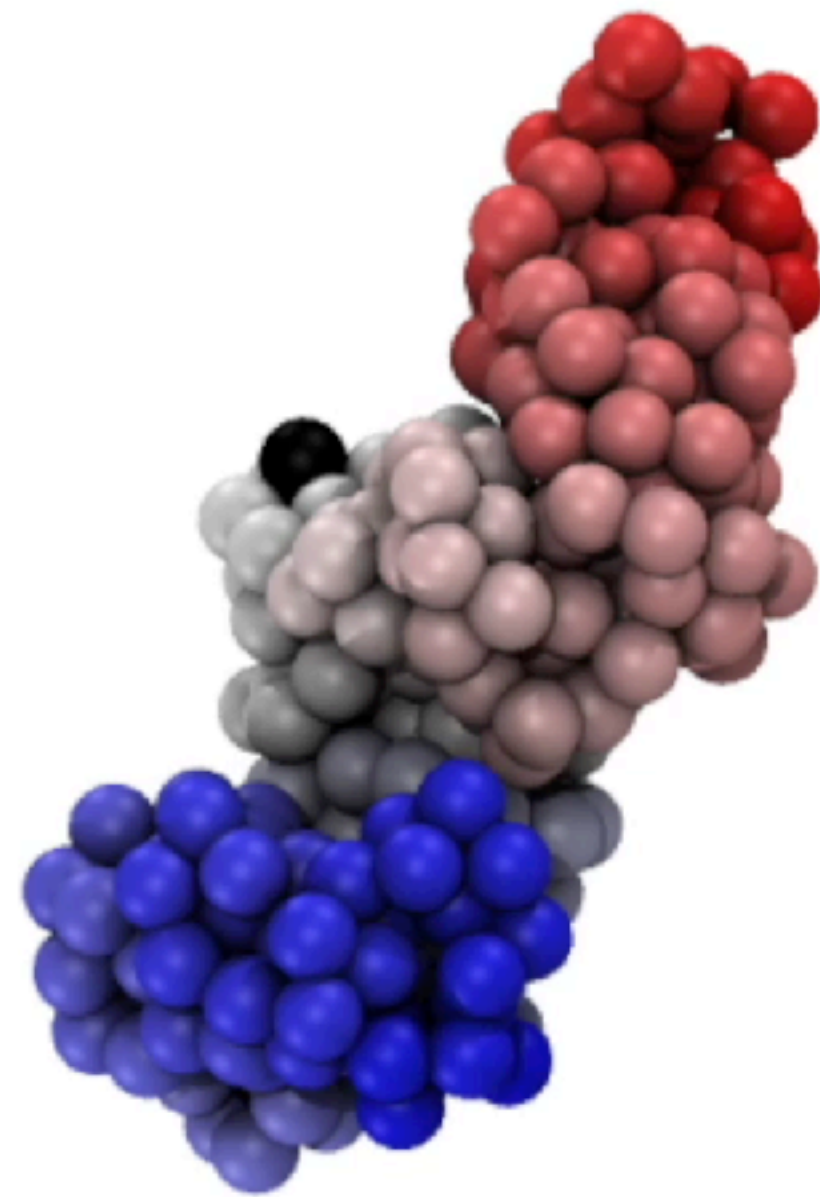
Structural exposure





# SOX2 locus dynamics changes from B to PSC

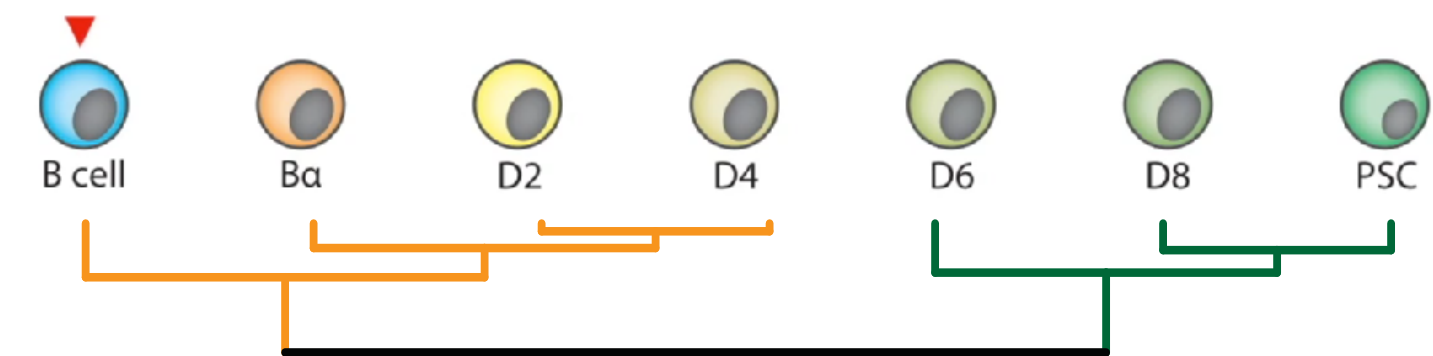
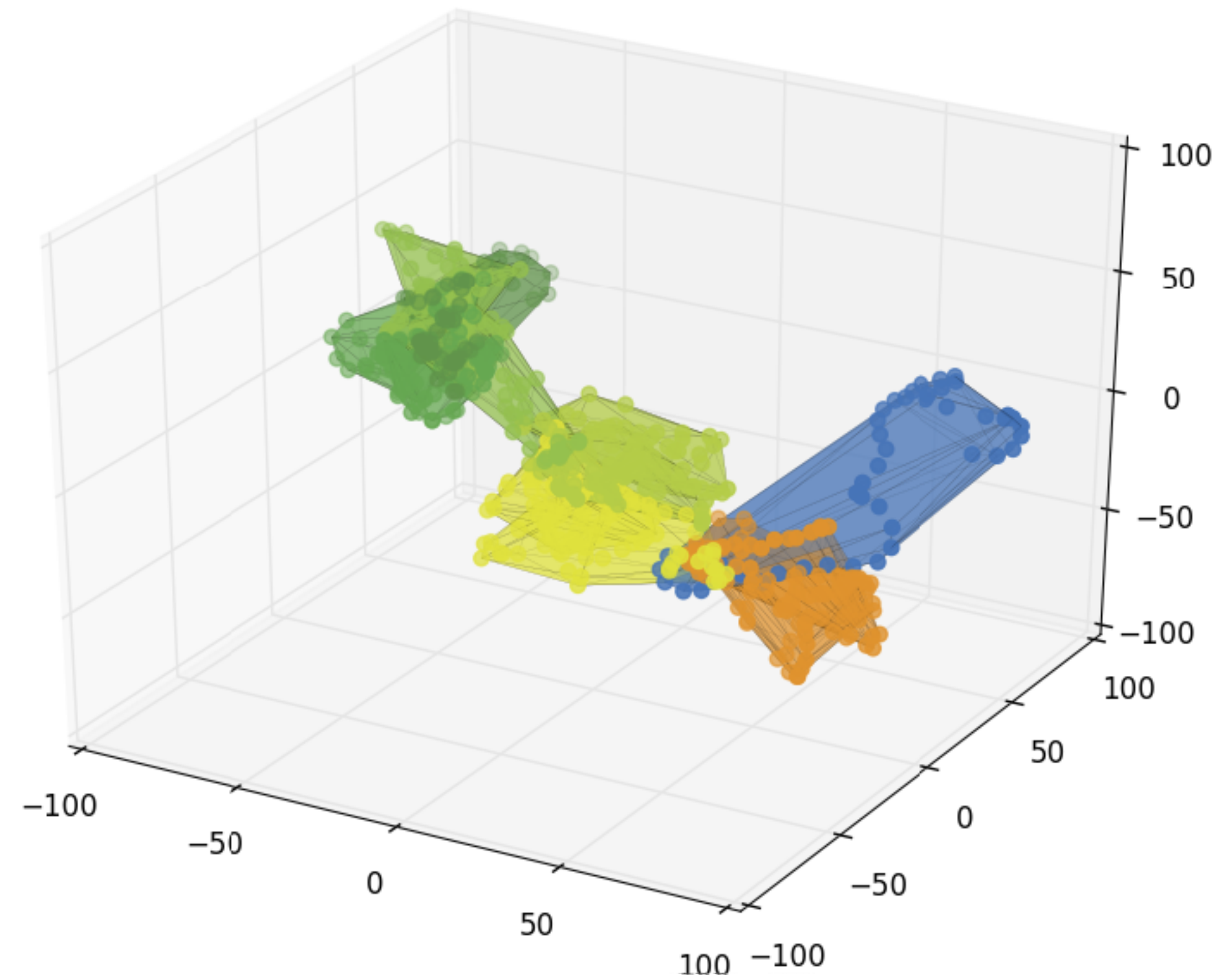
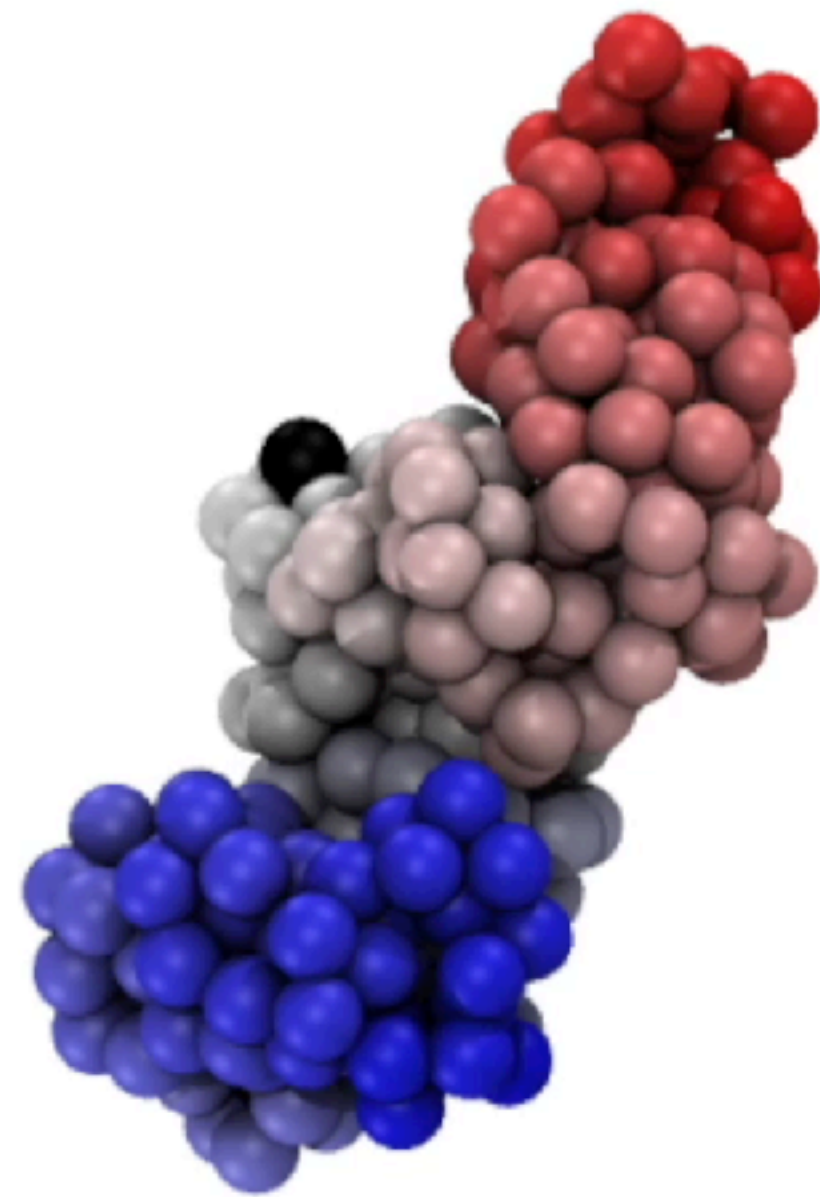
## SOX2 displacement





# SOX2 locus dynamics changes from B to PSC

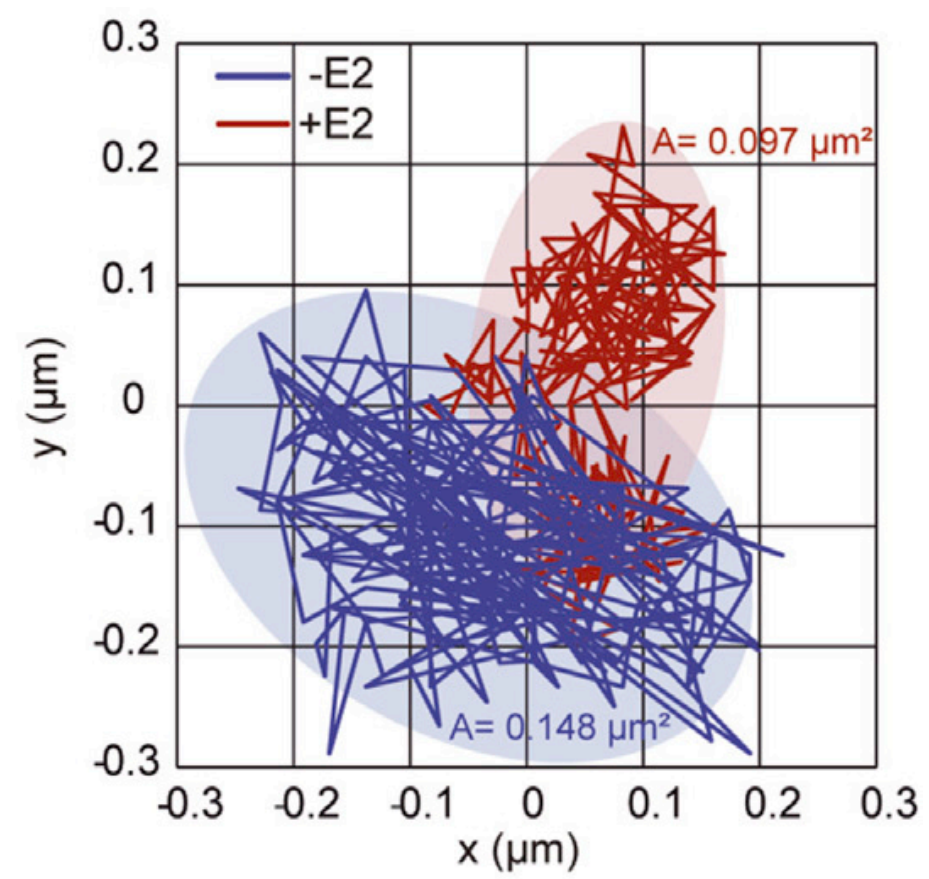
SOX2 displacement





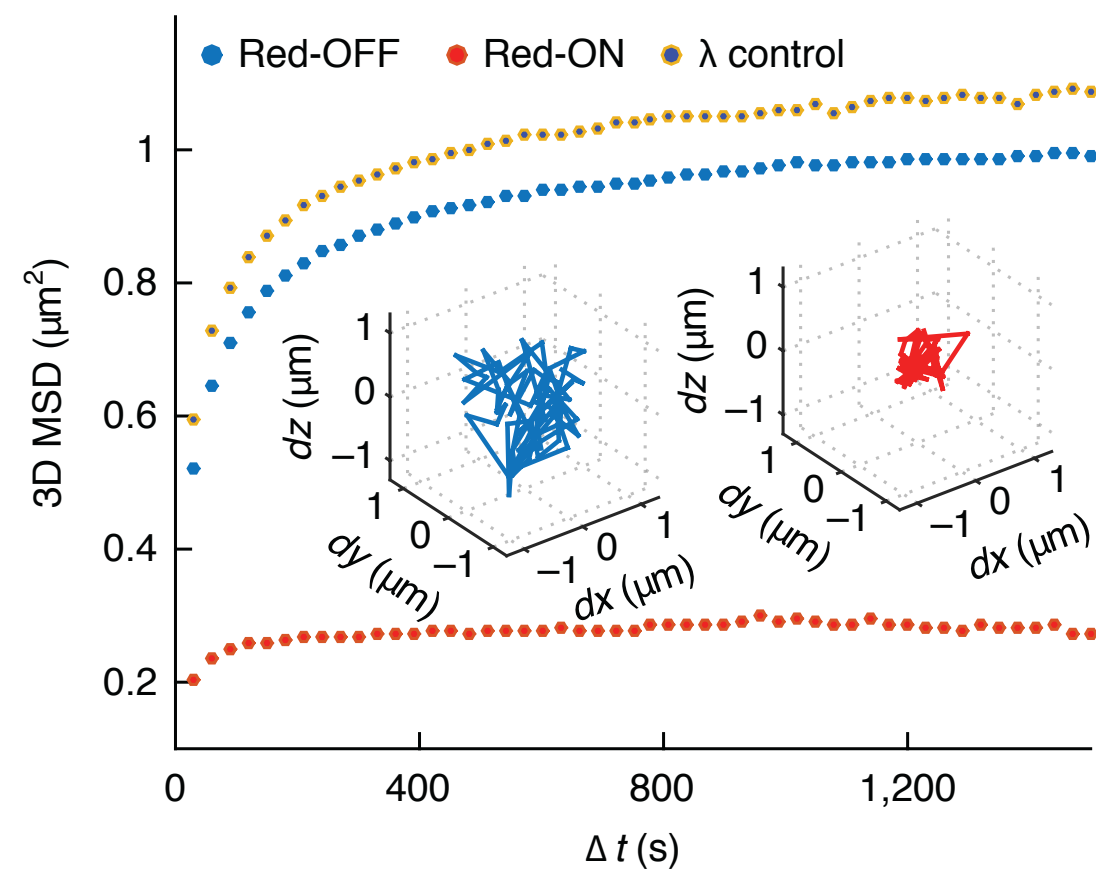
# SOX2 locus dynamics changes from B to PSC

## SOX2 displacement



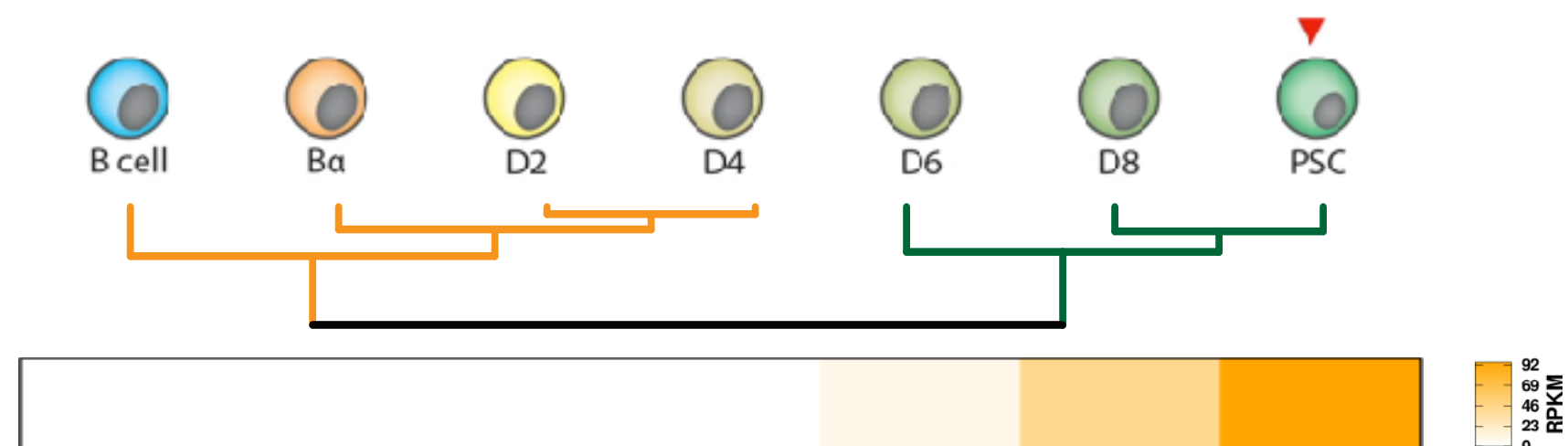
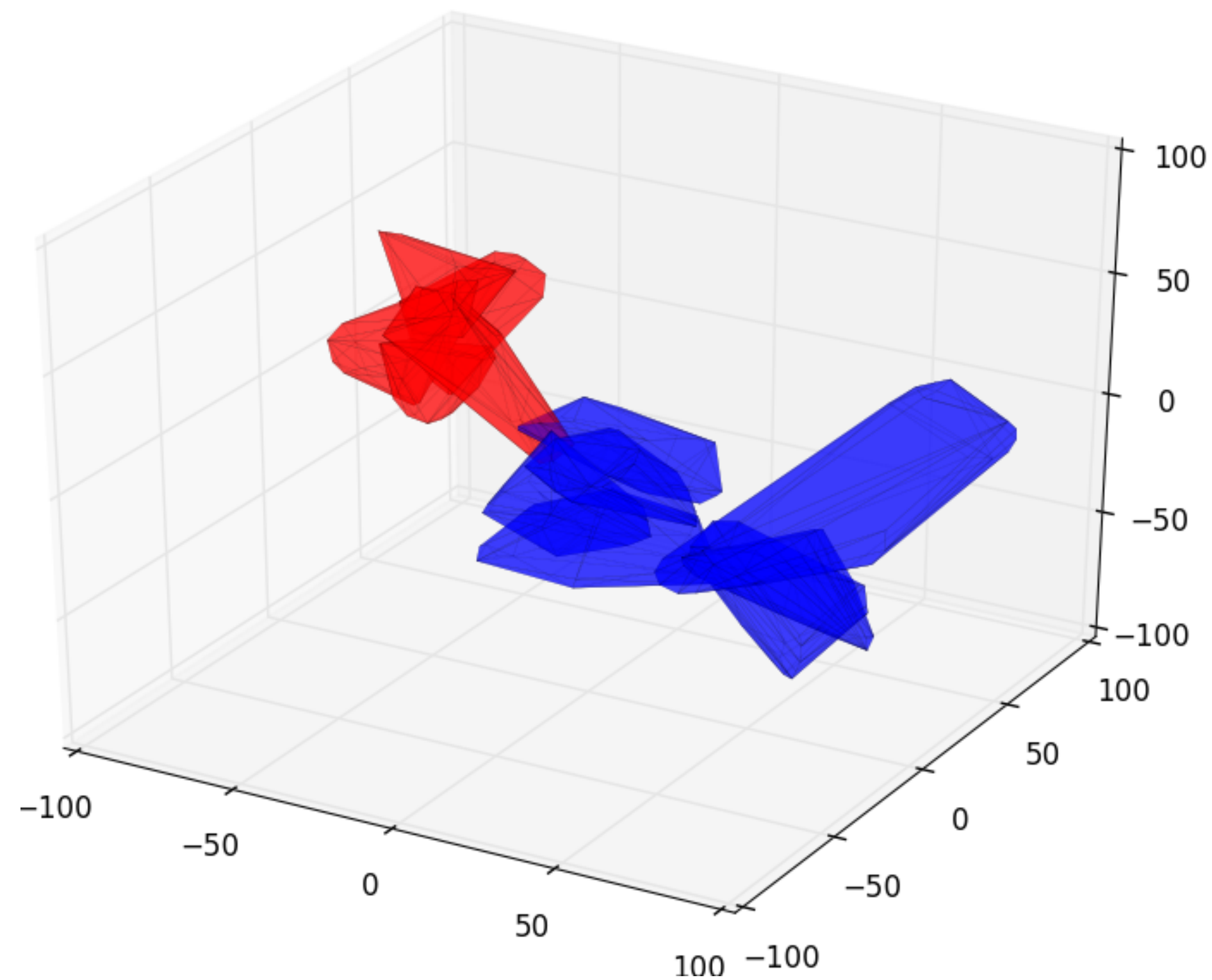
Two dimensional trajectories and area explored over 50s of the CCND1 locus recored before -E2 and after +E2 activation.

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



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

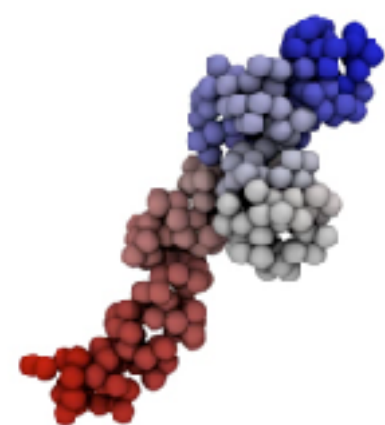
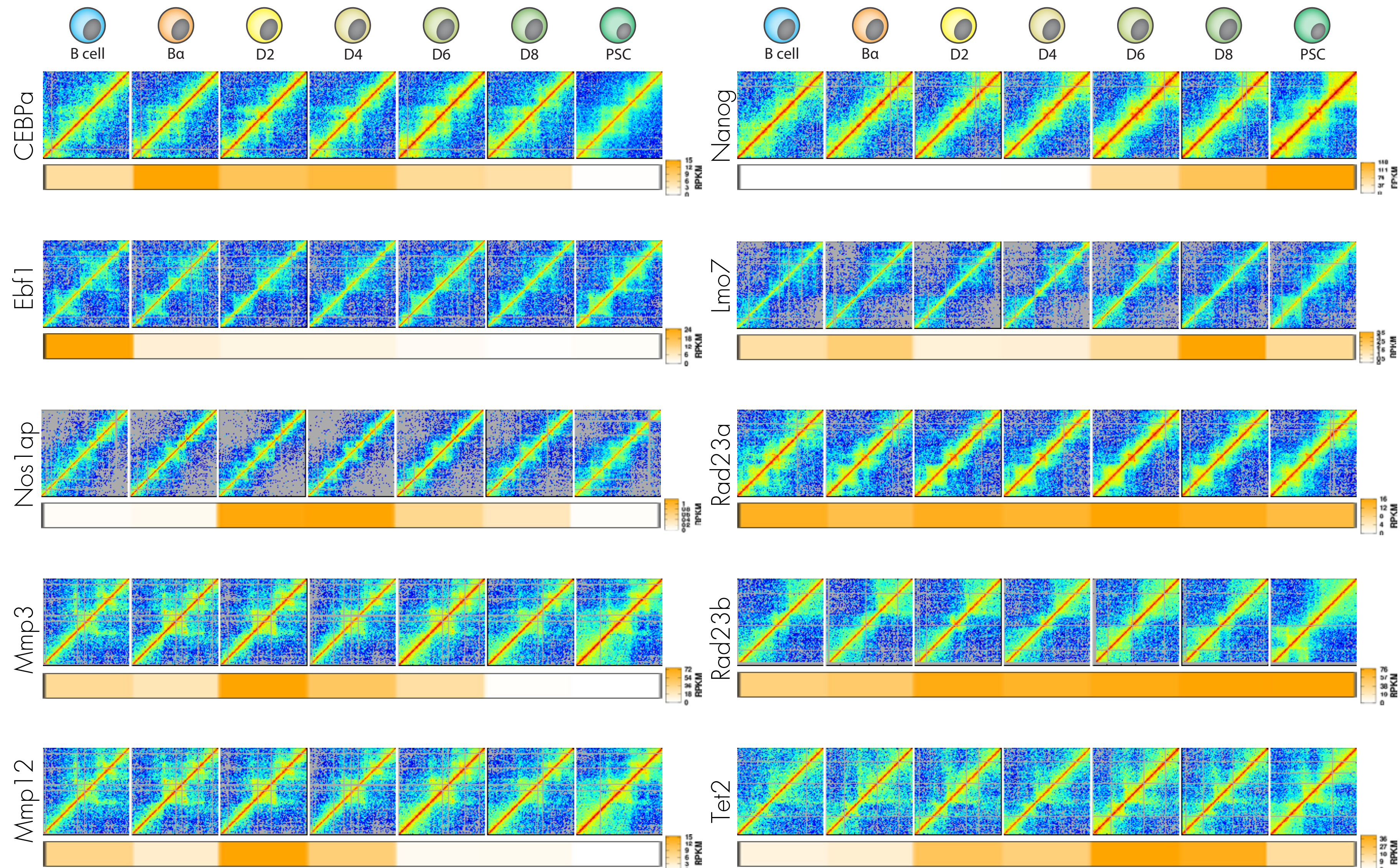
Chen ,T., et al, (2018) Nat. Genetics





# Structural changes from B to PSC

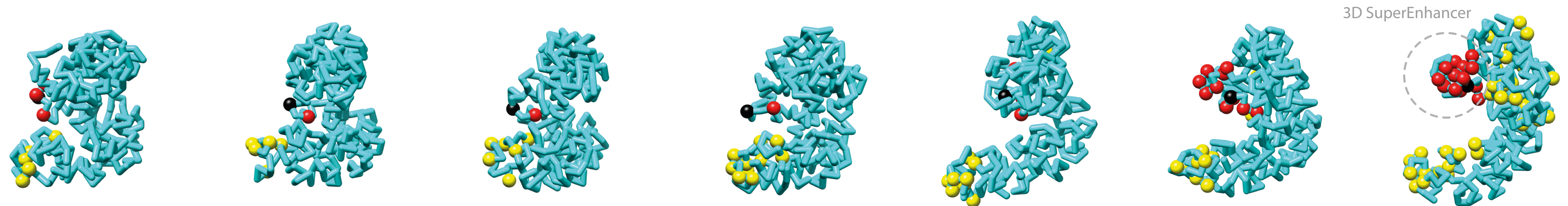
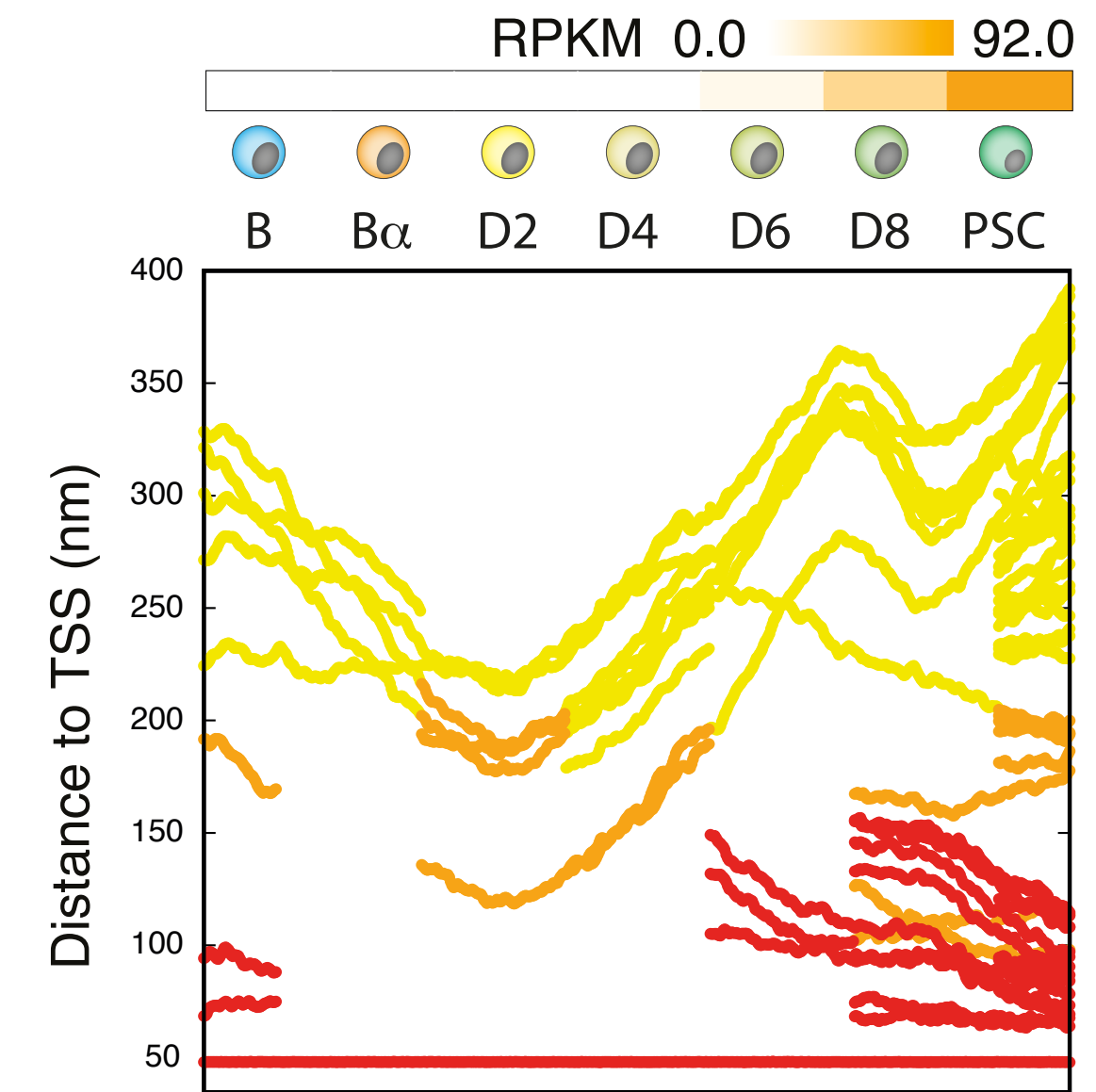
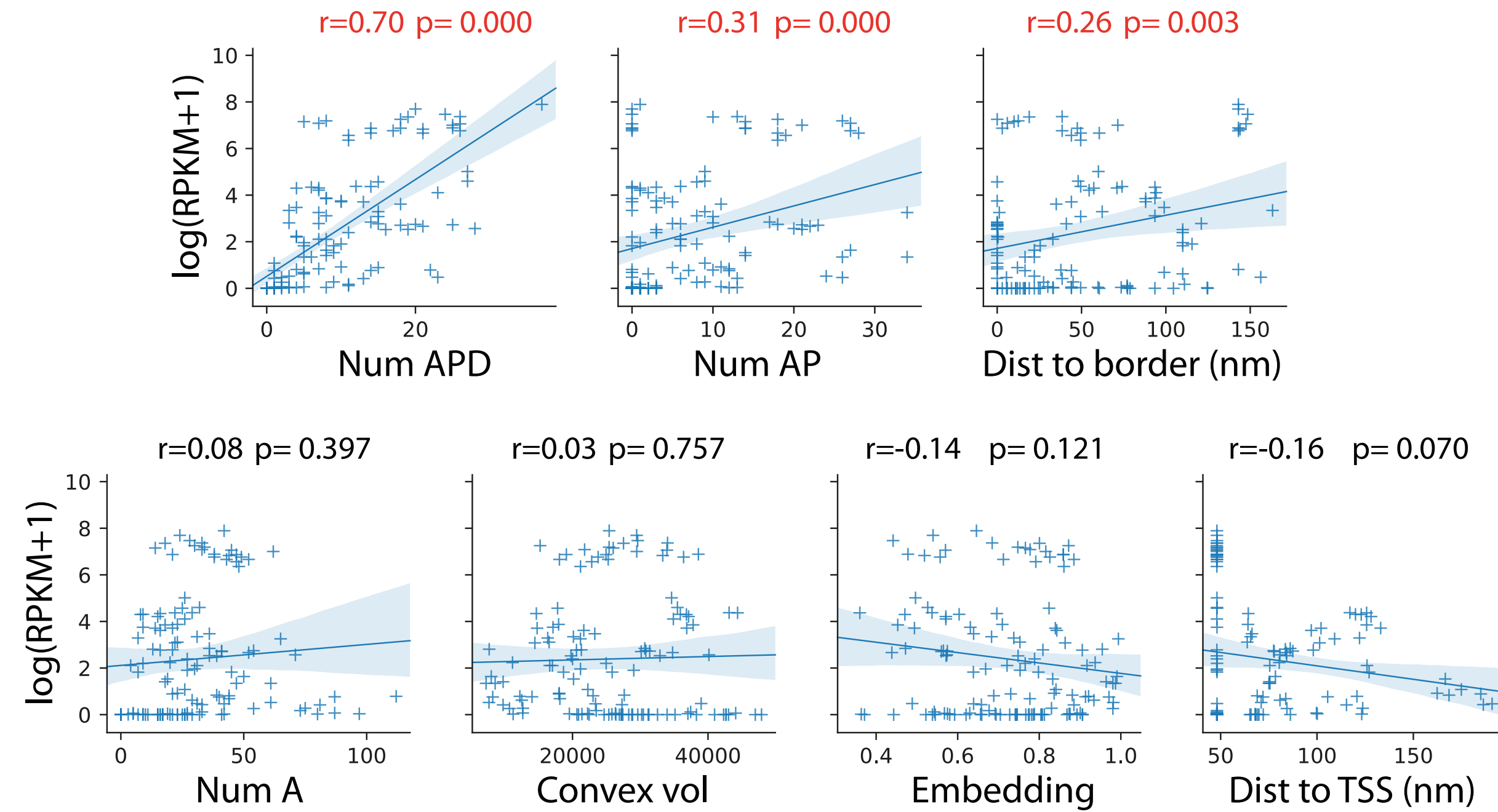
Other 21 loci





# Dynamics of gene activation

Trends in 21 loci



Time and expression levels



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

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