Structure determination of genomes and genomic domains by satisfaction of spatial restraints
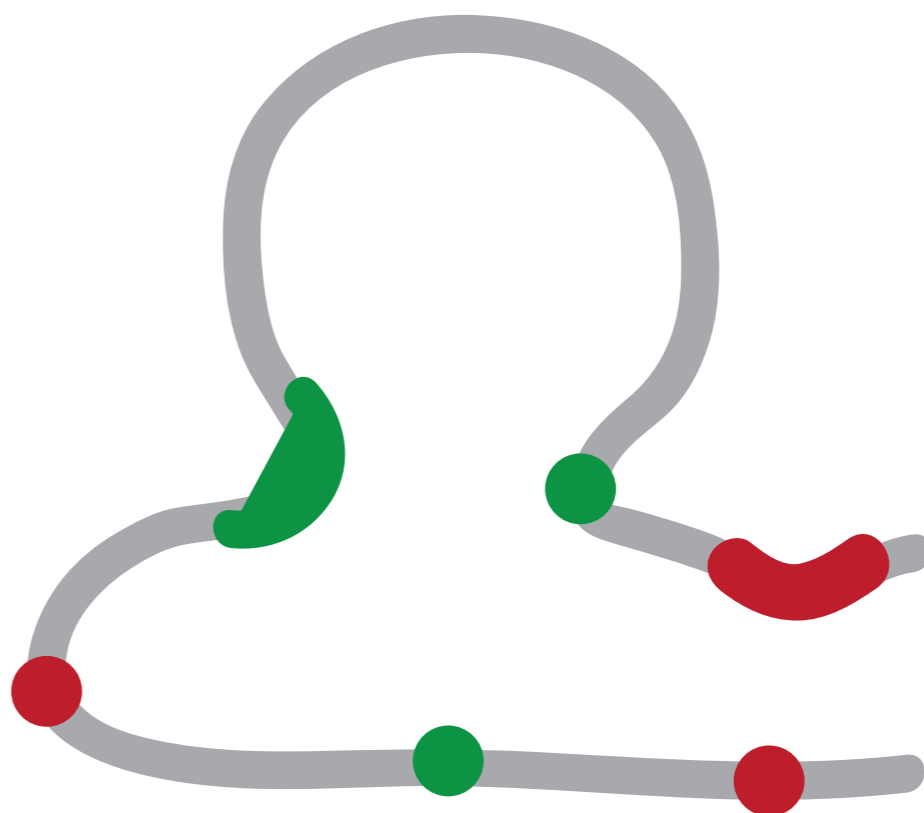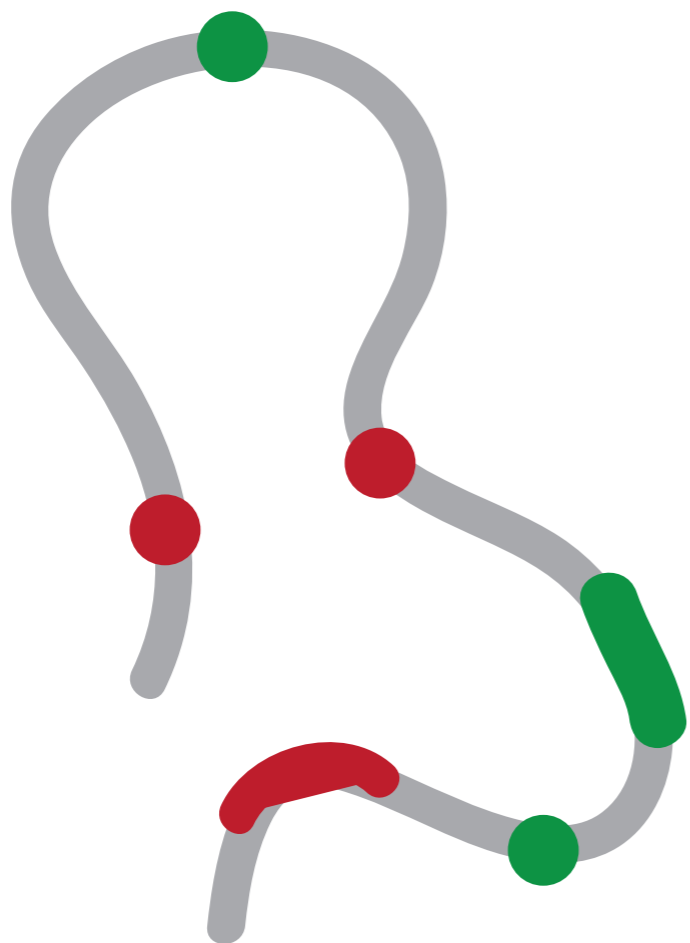
Marc A. Marti-Renom
Structural Genomics Group (ICREA, CNAG-CRG)
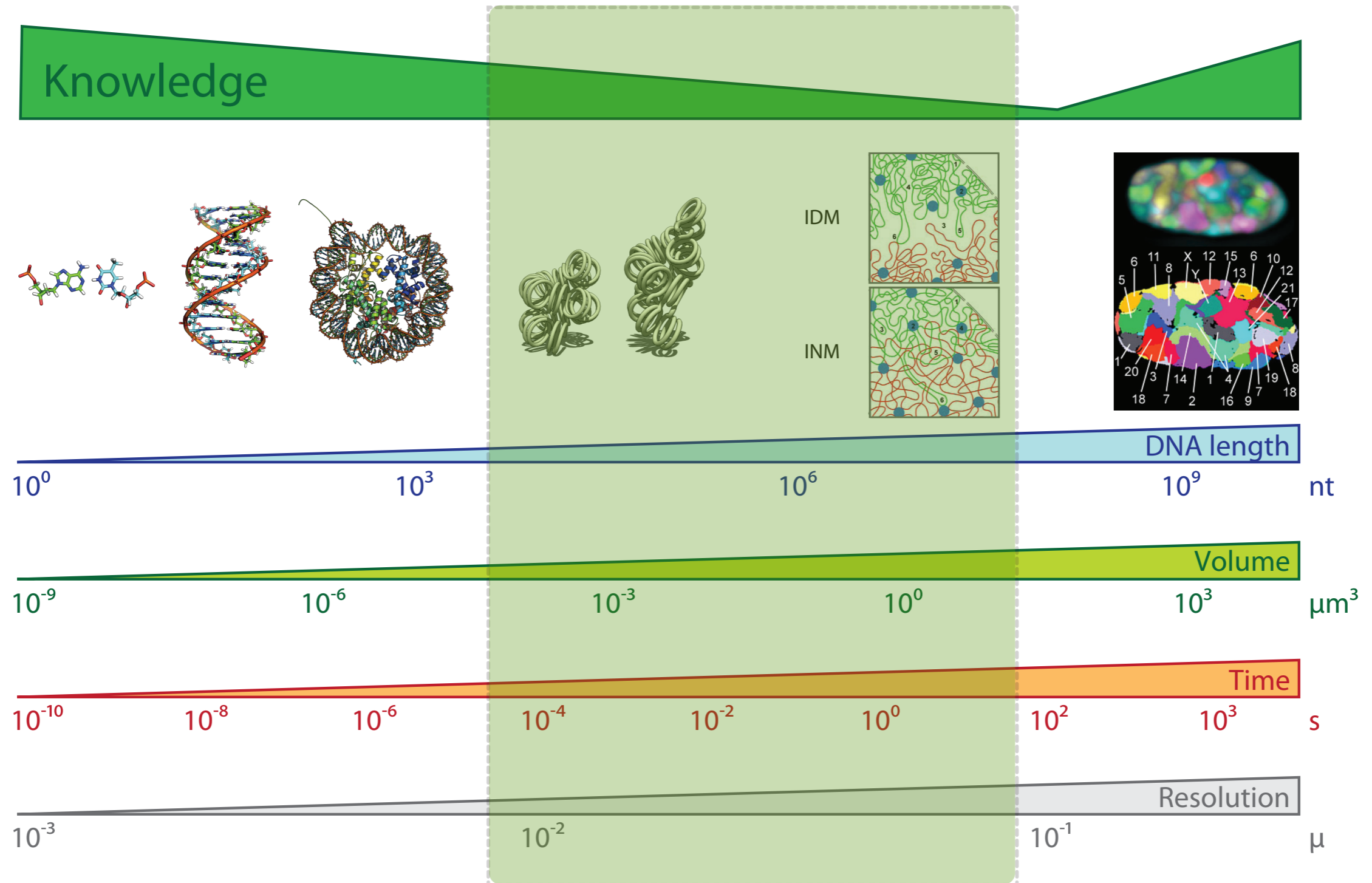
http://marciuslab.org
http://3DGenomes.org
http://cnag.crg.eu

cnag CRG ICREA

Resolution Gap

Level I: Radial genome organization

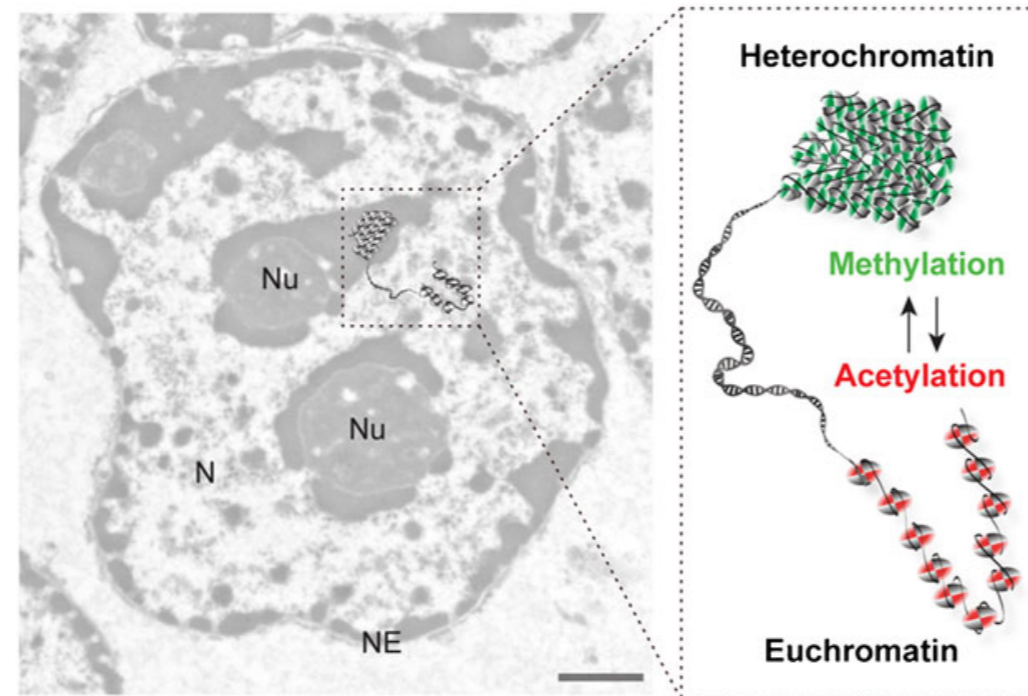Takizawa, T., Meaburn, K. J. & Misteli, T. The meaning of gene positioning. Cell 135, 9–13 (2008).
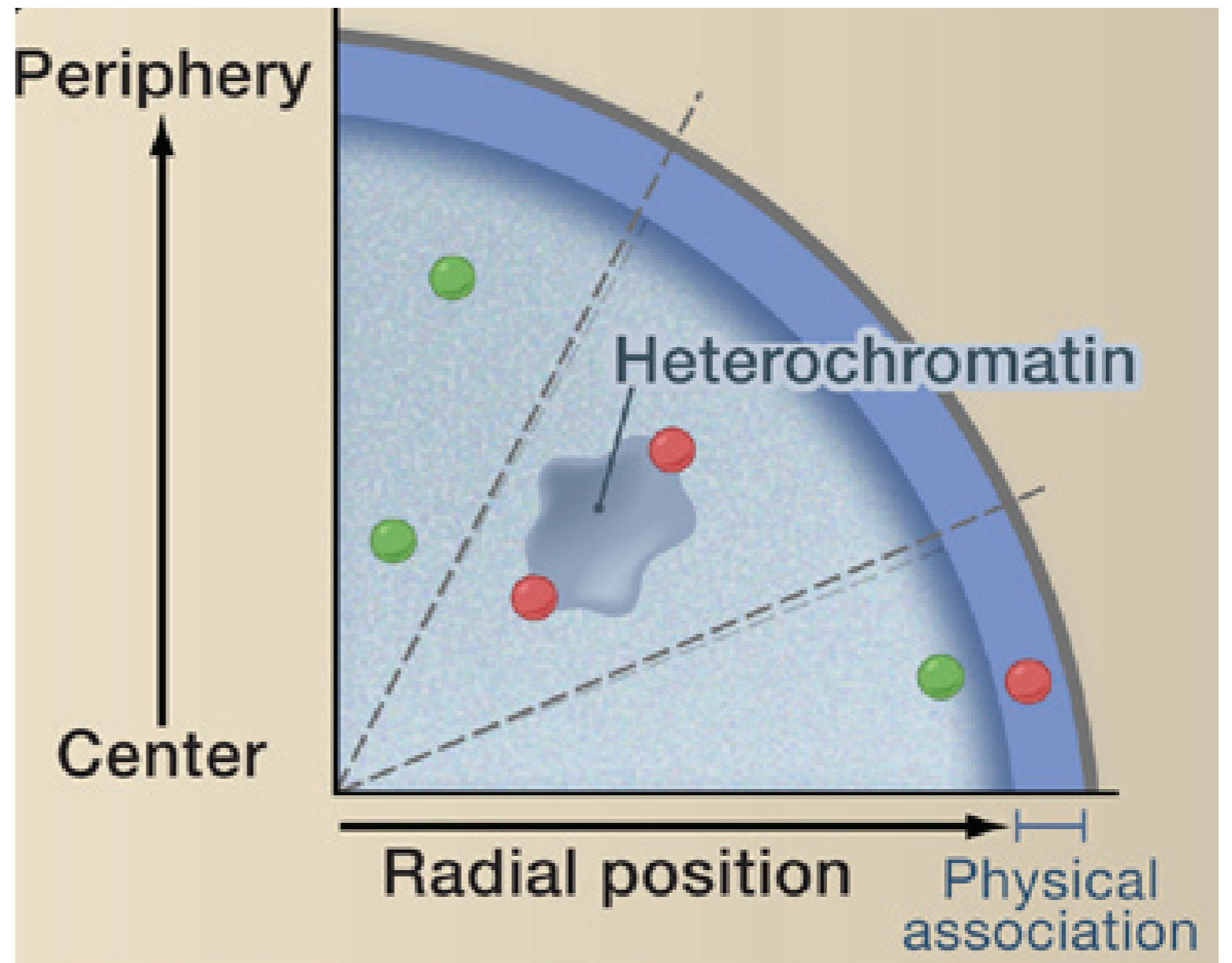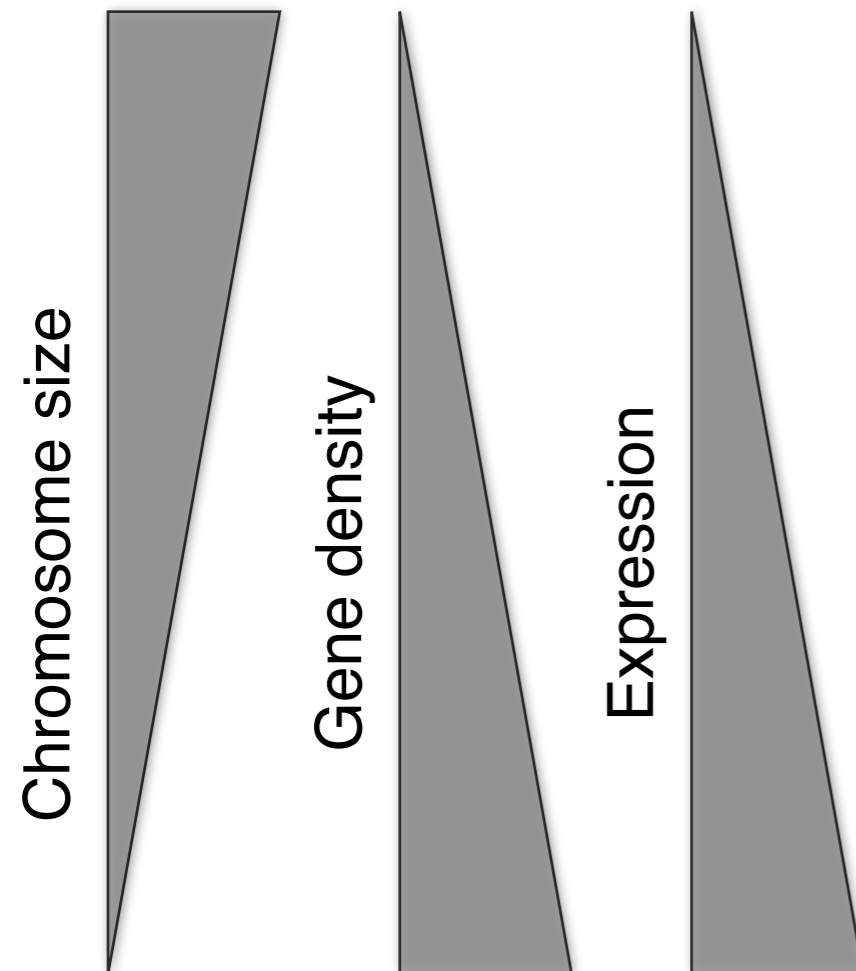
Level II: Euchromatin vs heterochromatin
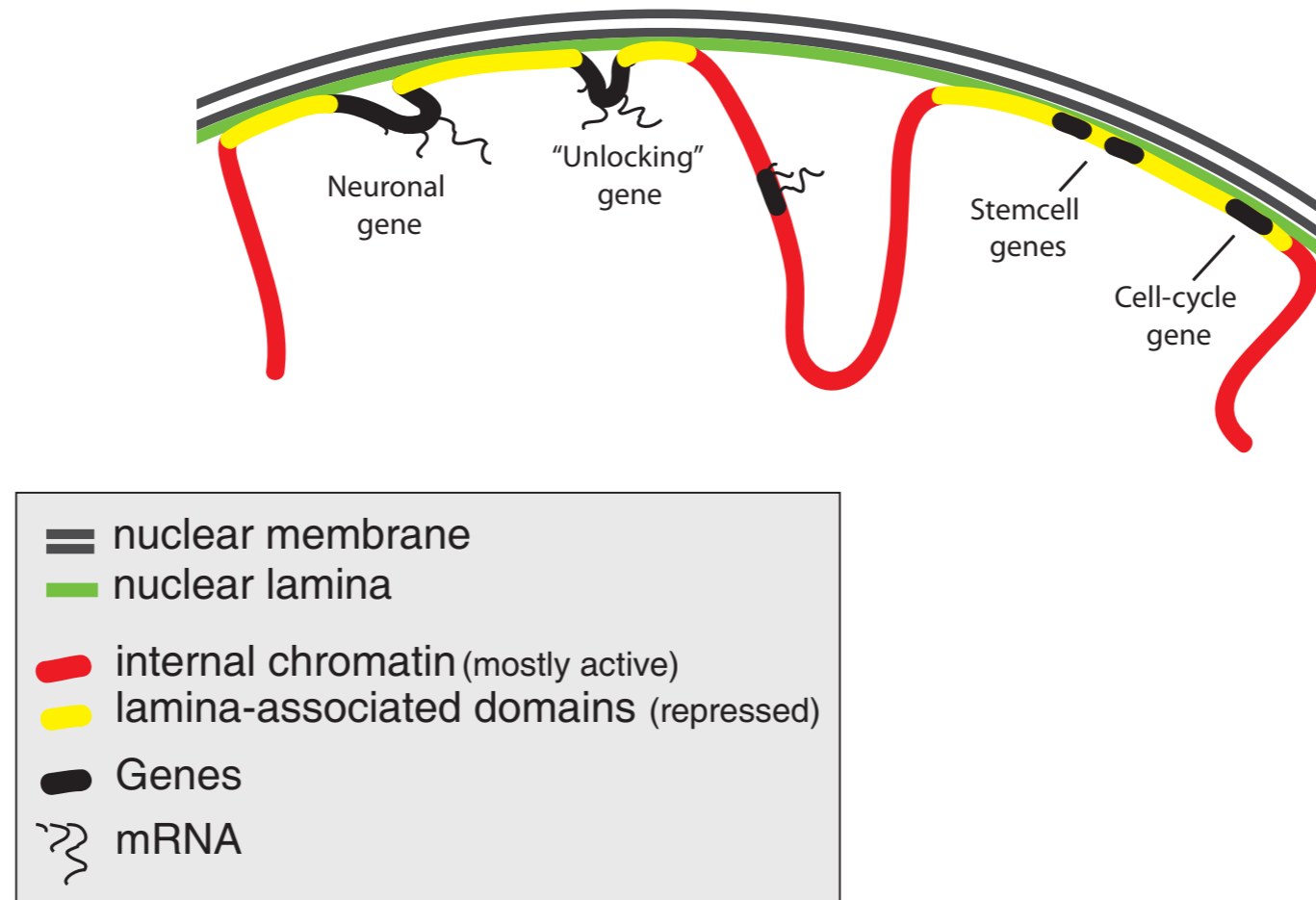
Electron microscopy

Euchromatin:
chromatin that is located away from the nuclear lamina, is generally less densely packed, and contains actively transcribed genes

Heterochromatin:
chromatin that is near the nuclear lamina, tightly condensed, and transcriptionally silent
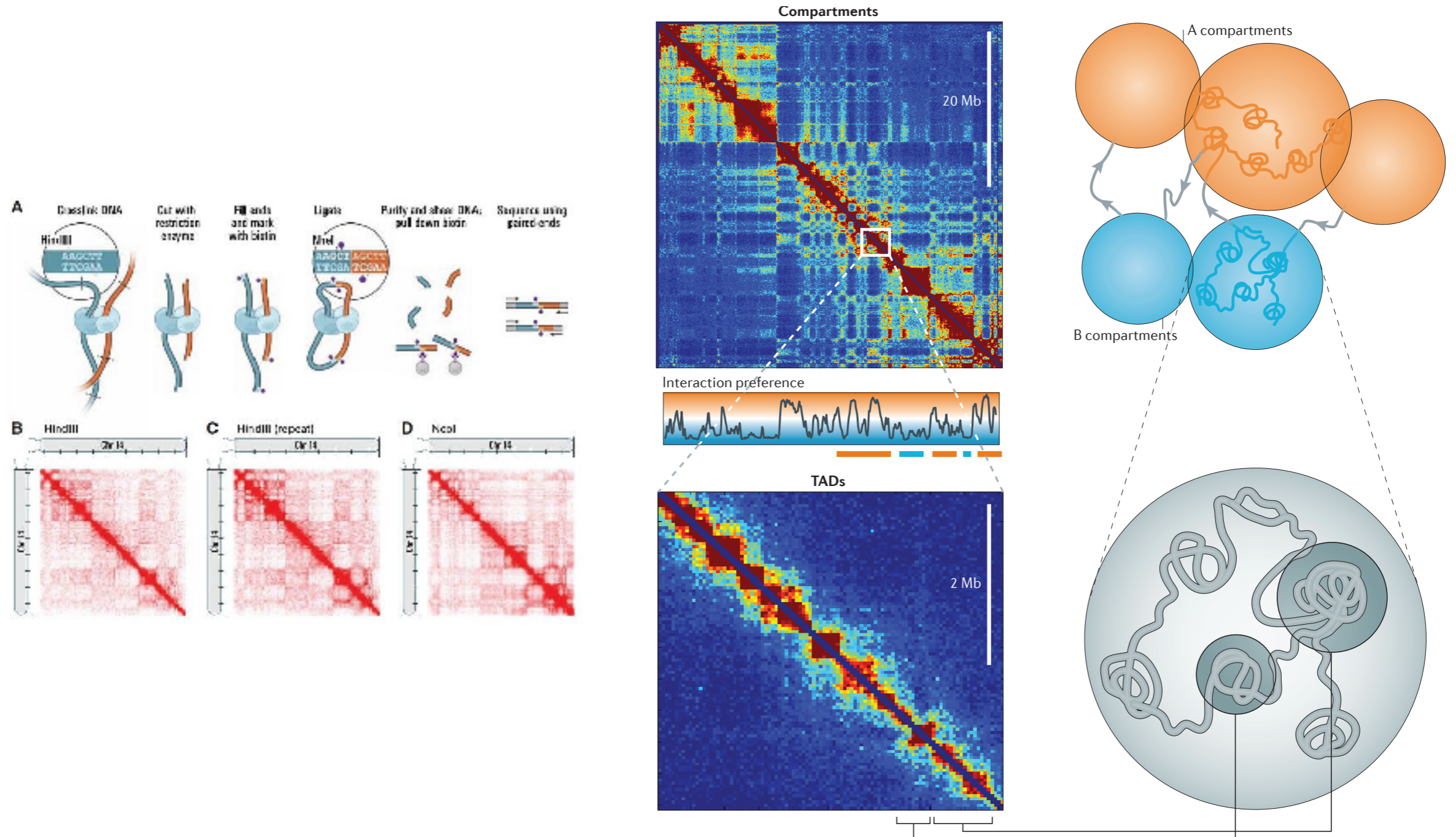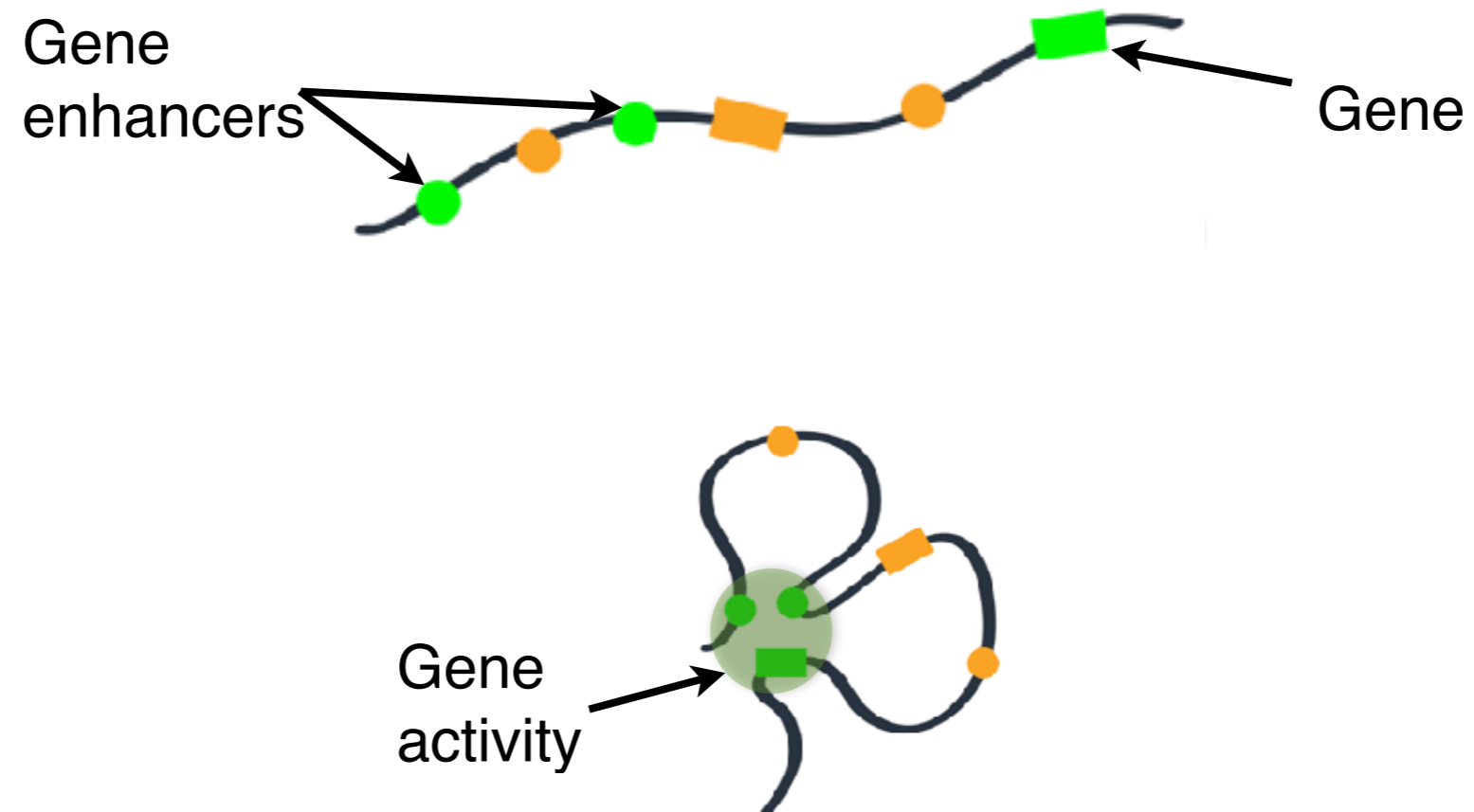
Level III: Lamina-genome interactions

Most genes in Lamina Associated Domains are transcriptionally silent, suggesting that **lamina-genome interactions** are widely involved in the control of **gene expression**

Level IV: Higher-order organization

# Level V: Chromatin loops


Gene enhancers

Gene

Gene activity

Loops bring distal genomic regions in close proximity to one another

This in turn can have profound effects on gene transcription

Enhancers can be thousands of kilobases away from their target genes in any direction (or even on a separate chromosome)
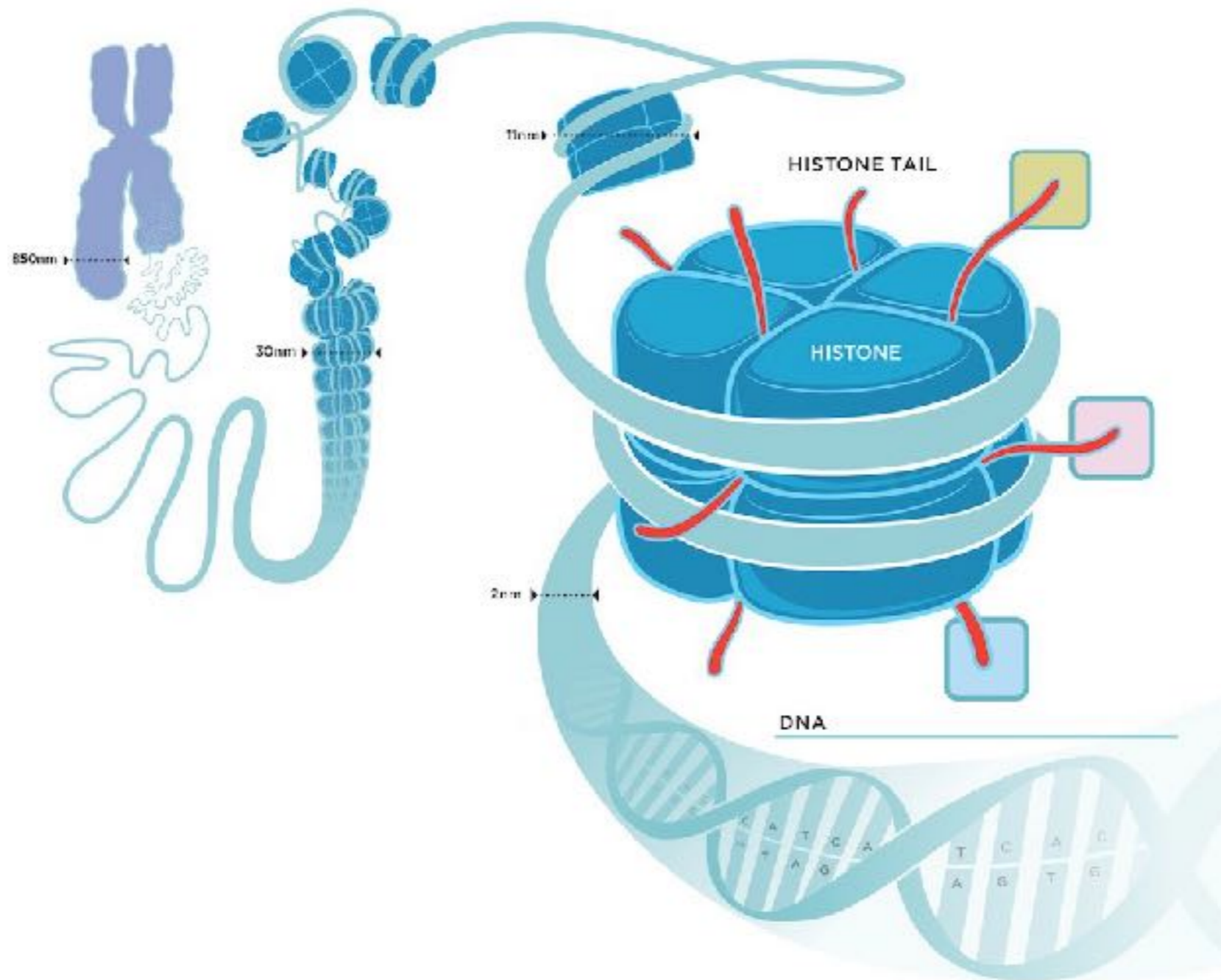
Level V: Loop-extrusion as a driving force

Fudenberg, G., Imakaev, M., Lu, C., Goloborodko, A., Abdennur, N., & Mirny, L. A. (2015).
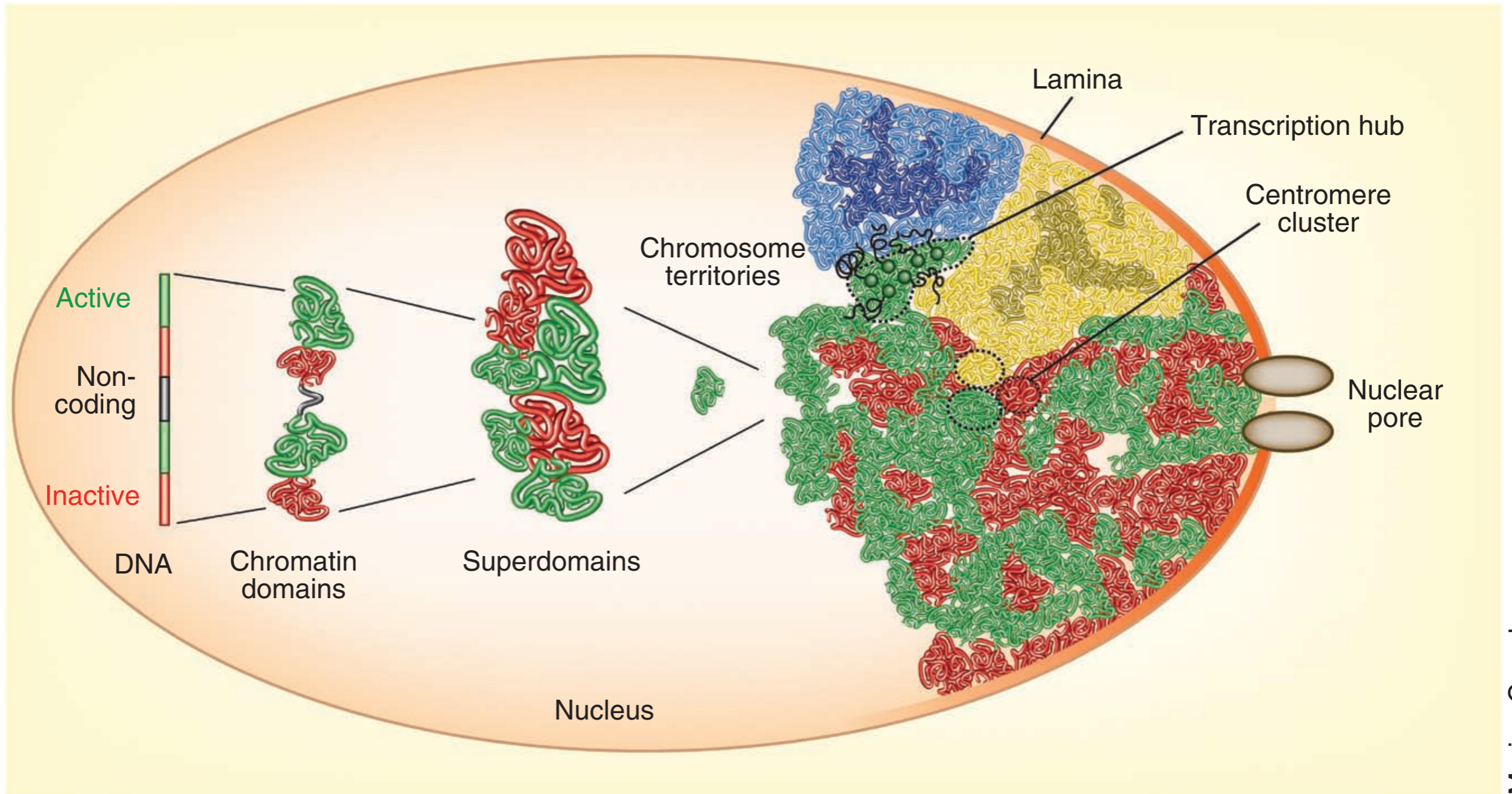Formation of Chromosomal Domains by Loop Extrusion. bioRxiv.
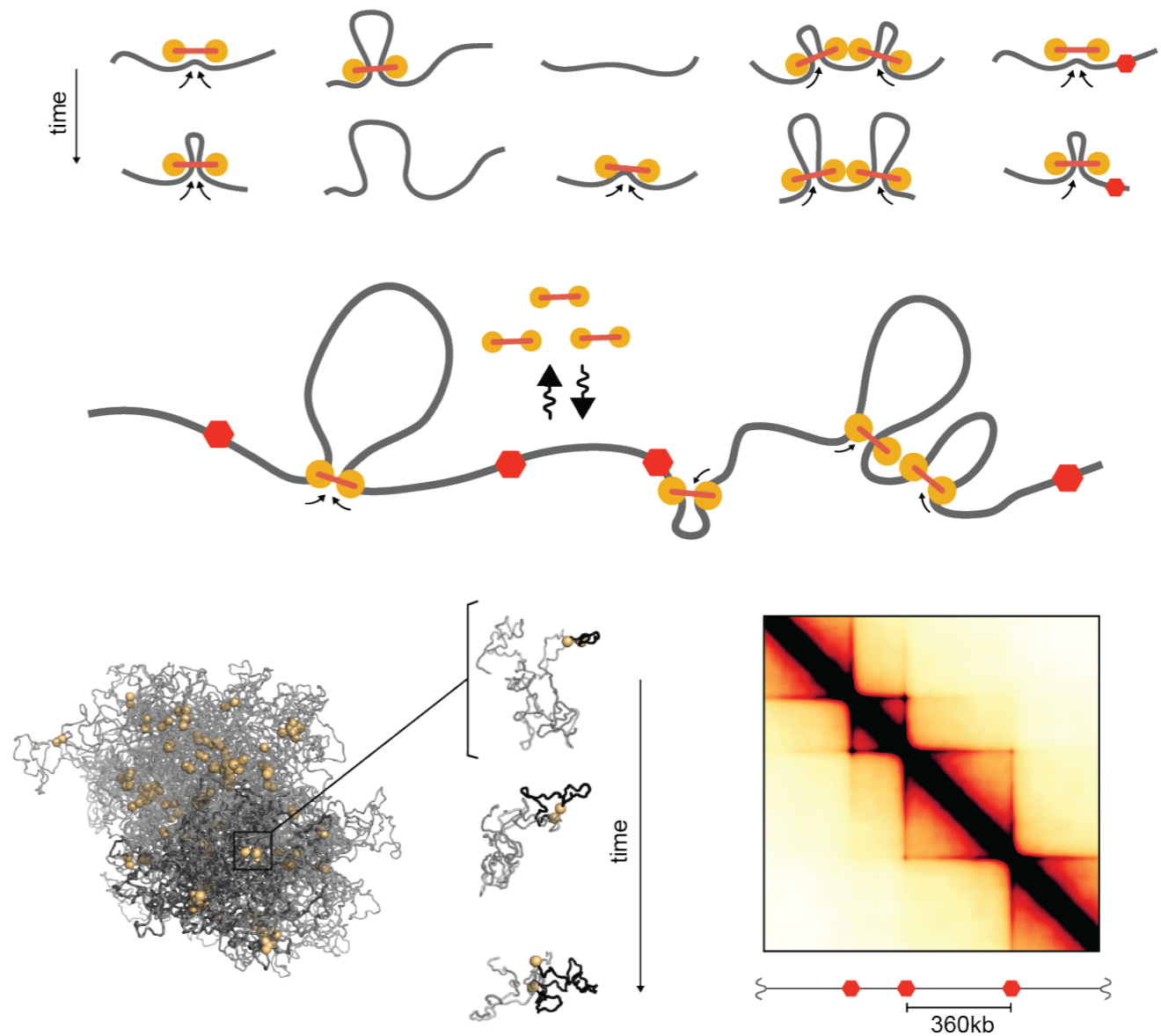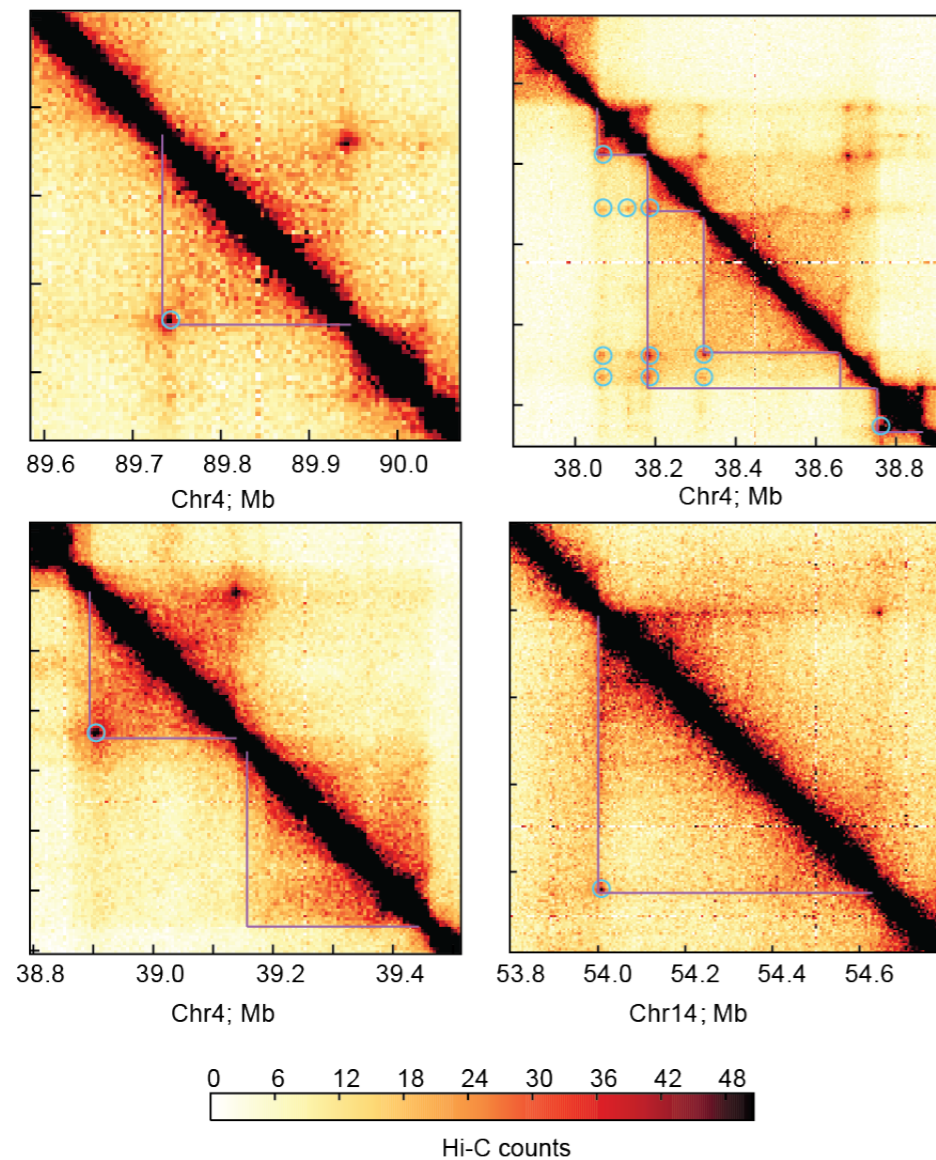
Level VI: Nucleosome

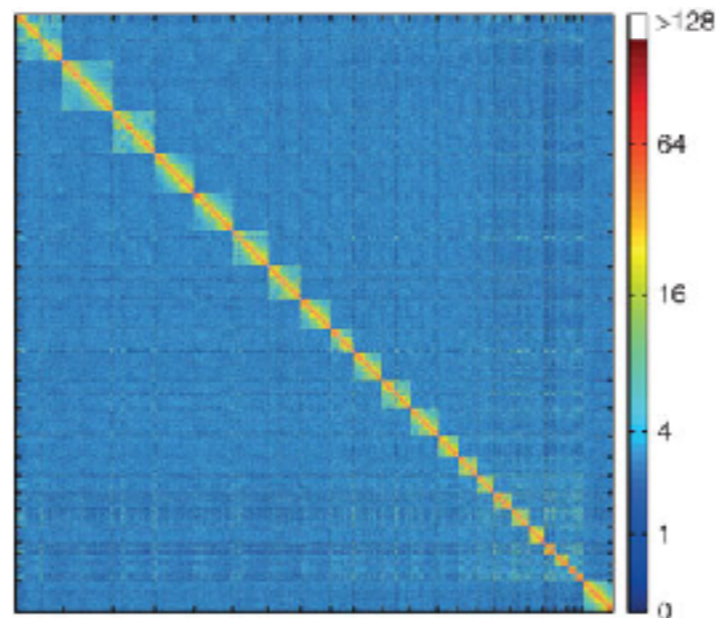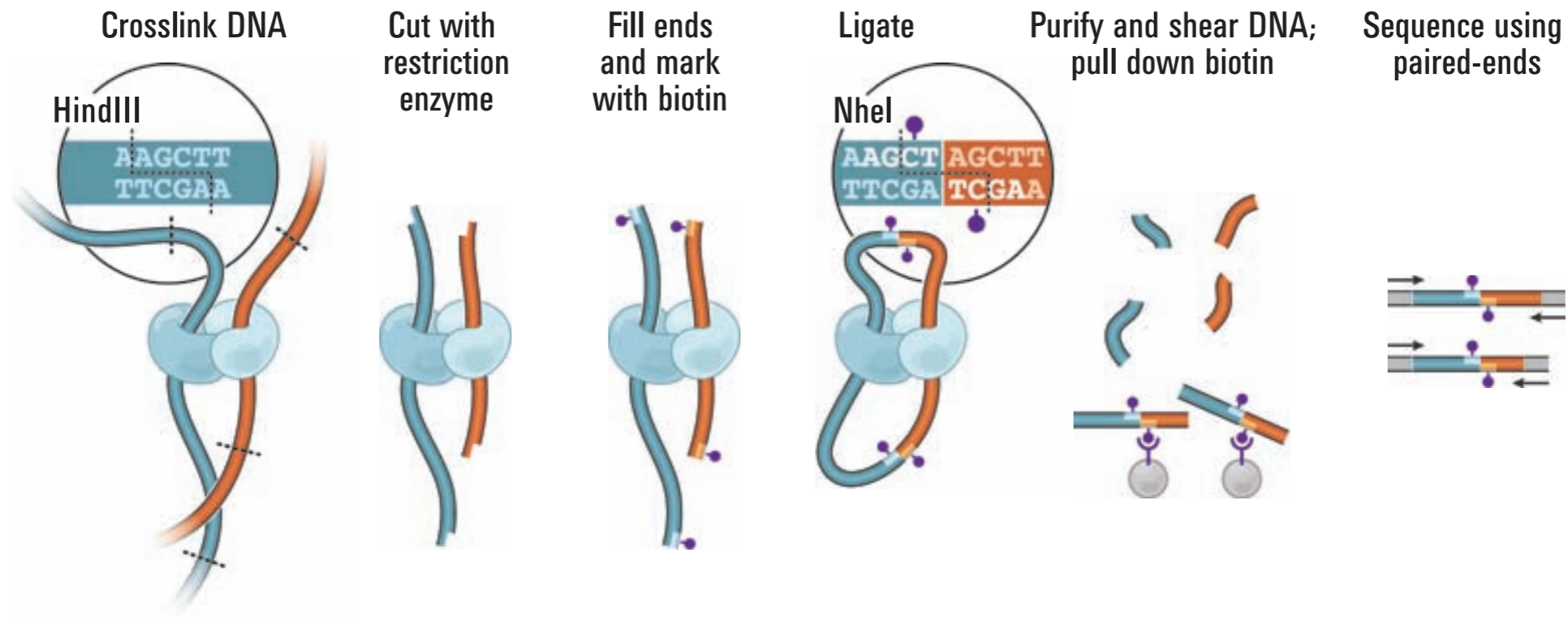**Chromosome**   **Chromatin fibre**   **Nucleosome**

Complex genome organization

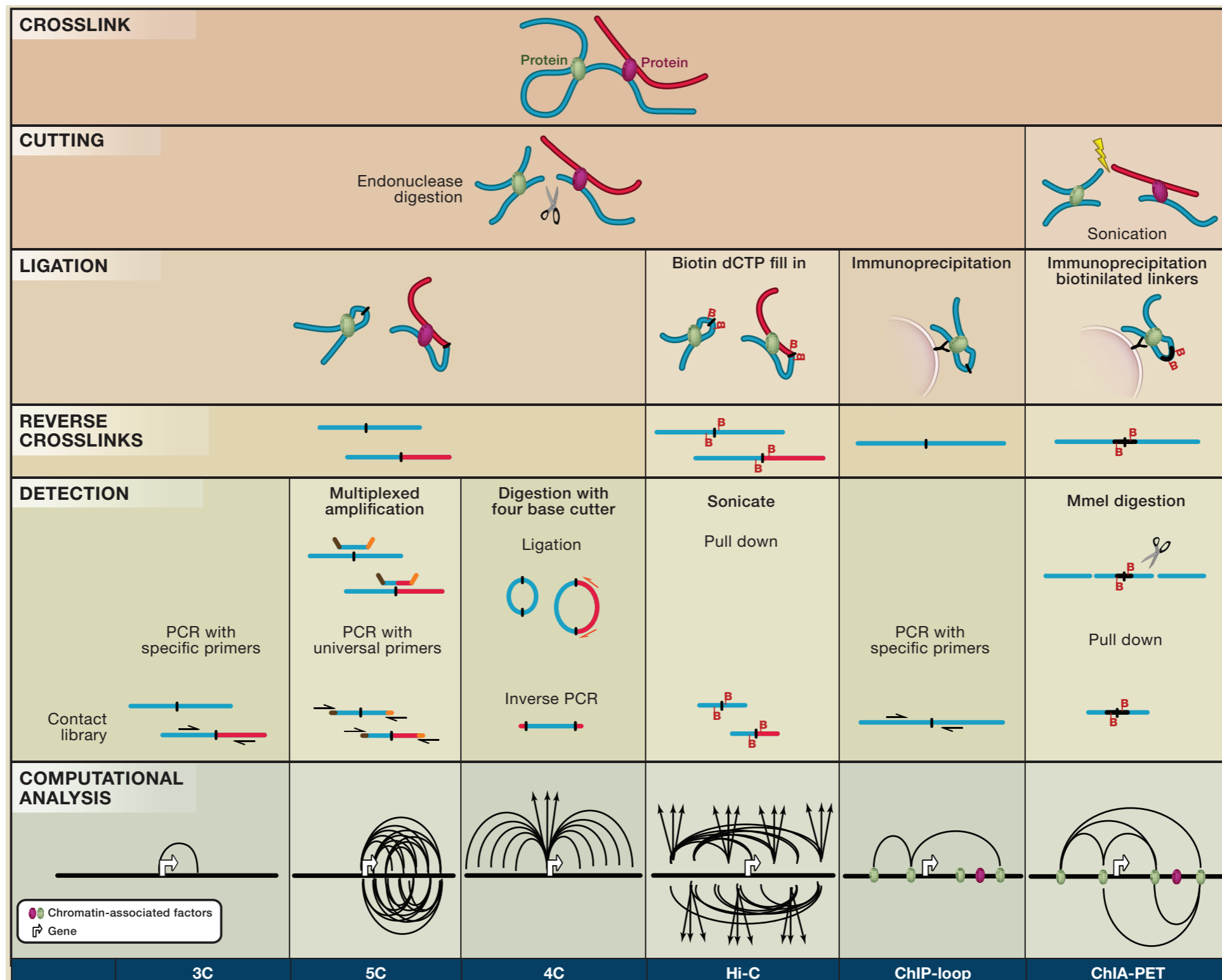Cavalli, G. & Misteli, T. Functional implications of genome topology. Nat Struct Mol Biol 20, 290–299 (2013).

Chromosome Conformation Capture

Dekker, J., Rippe, K., Dekker, M., & Kleckner, N. (2002). Science, 295(5558), 1306–1311.
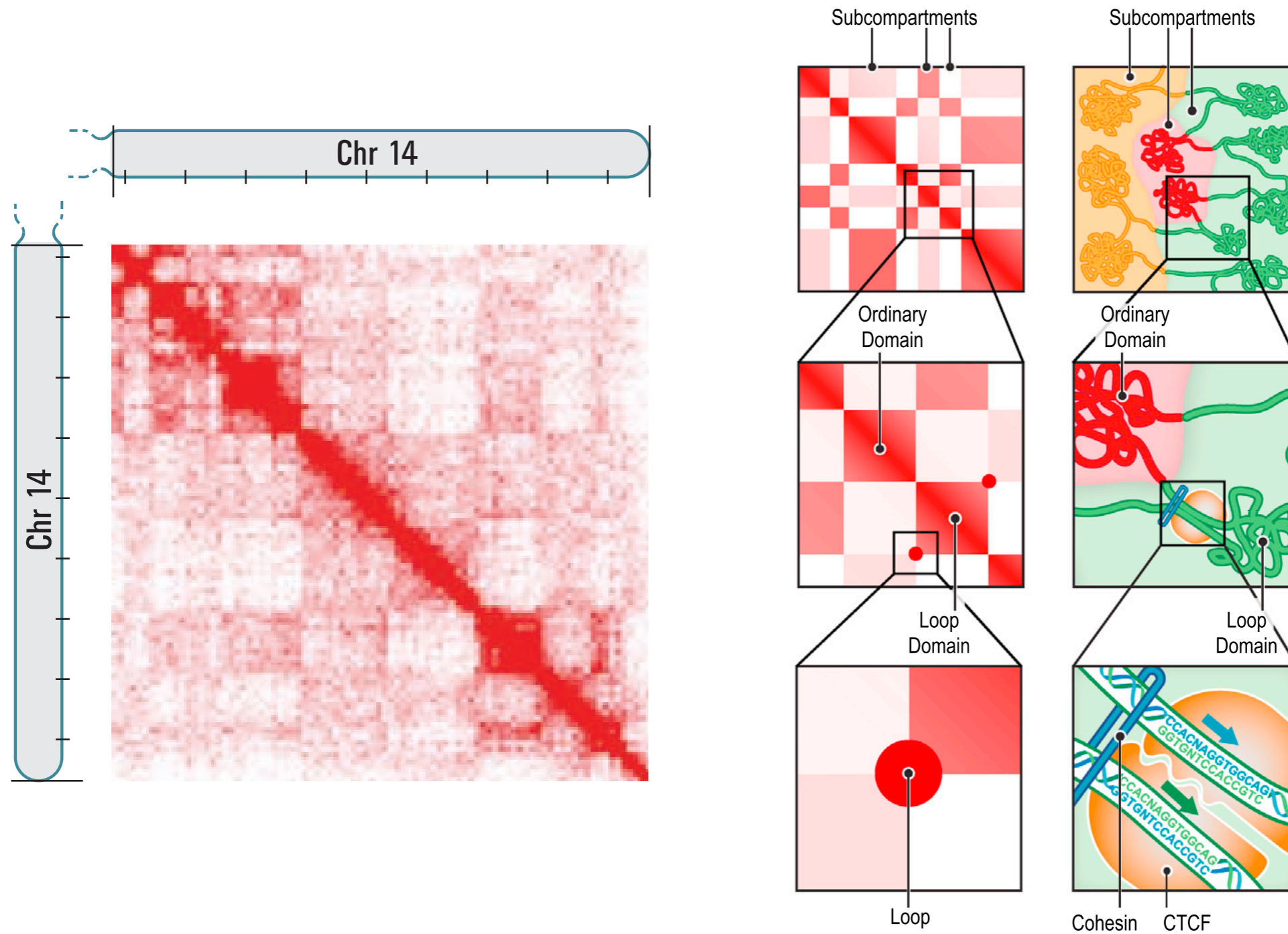Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.
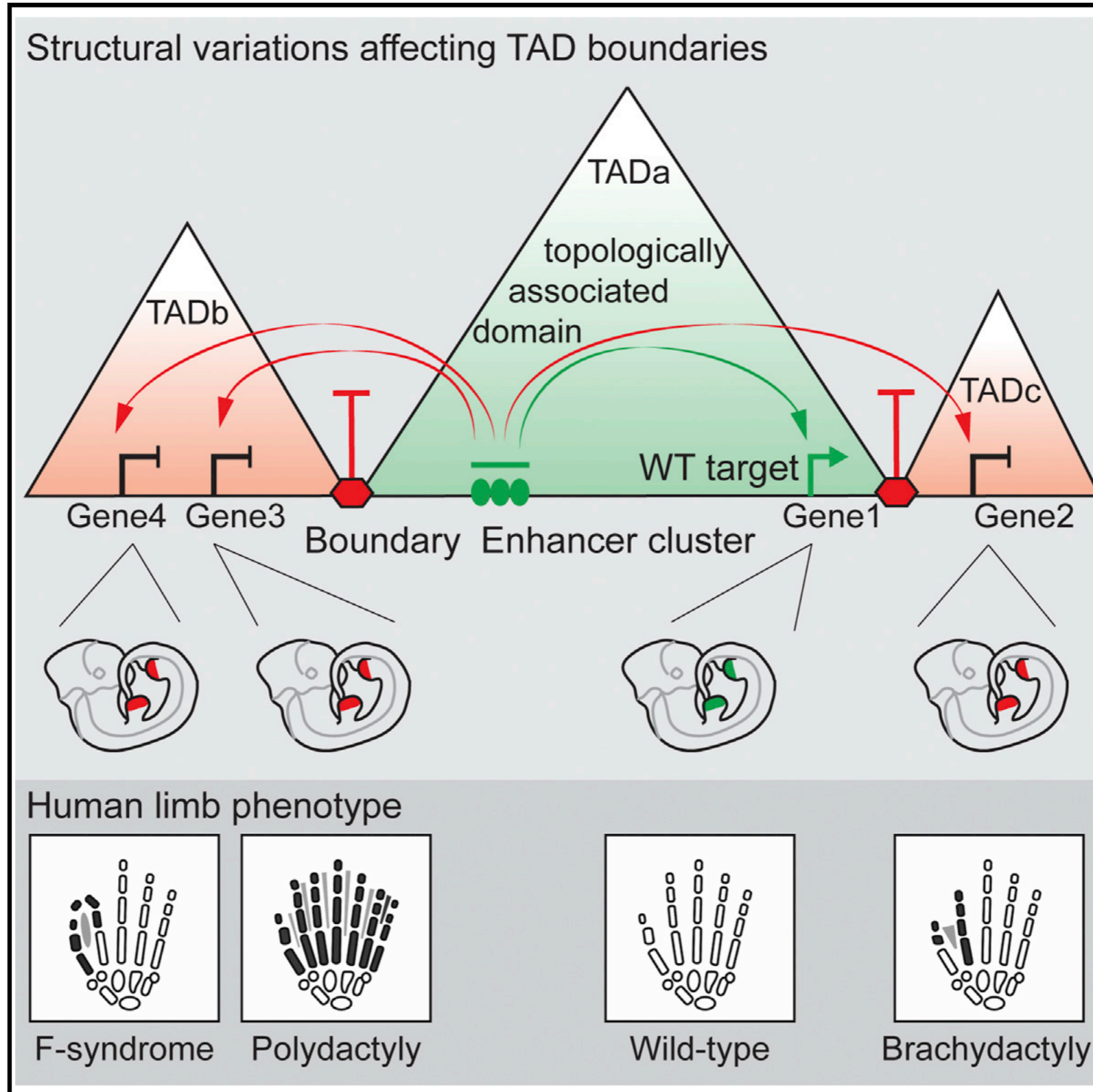
Chromosome Conformation Capture

Hakim, O., & Misteli, T. (2012). SnapShot: Chromosome Confirmation Capture. Cell, 148(5), 1068–1068.e2.
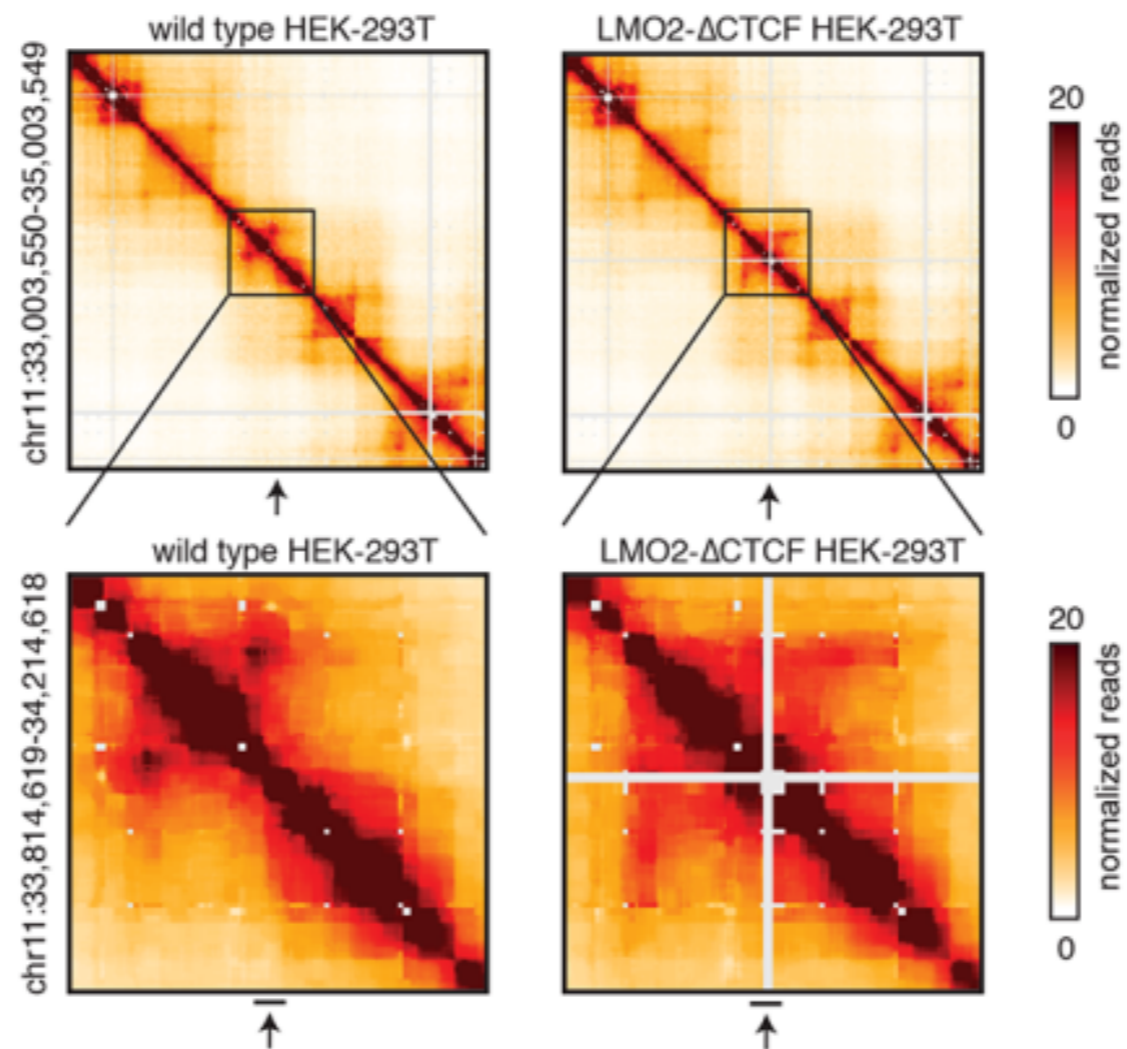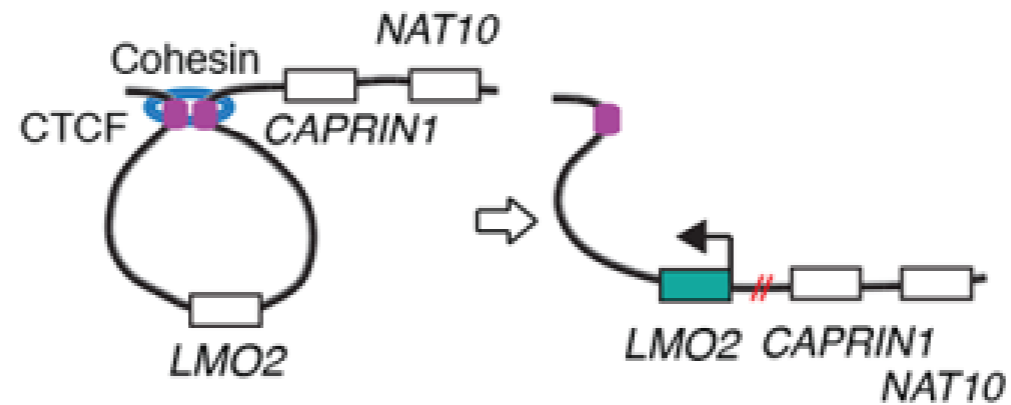
Hierarchical genome organisation

Chr 14

Chr 14

Subcompartments

Subcompartments

Loop

Cohesin   CTCF

Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.
Rao, S. S. P., et al. (2014). Cell, 1–29.
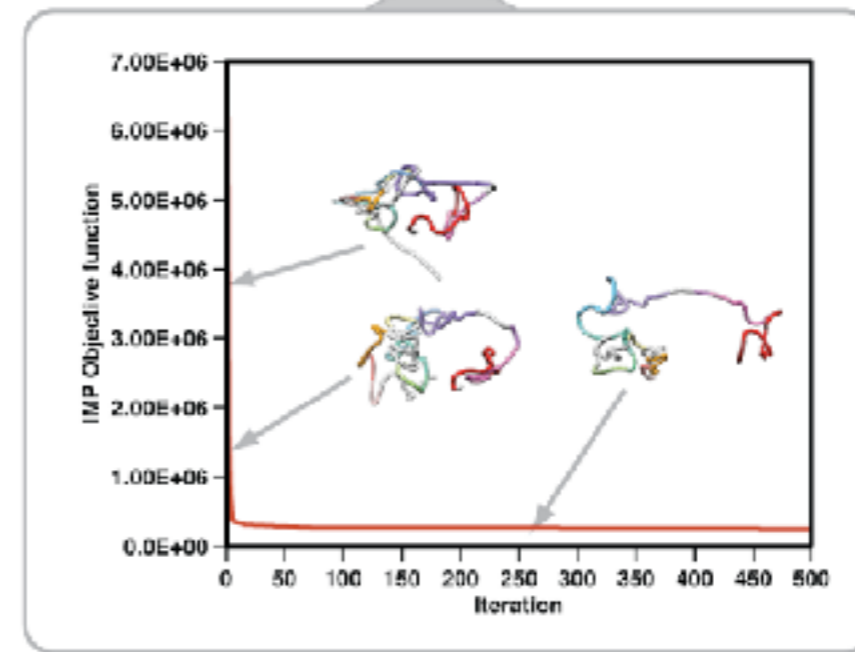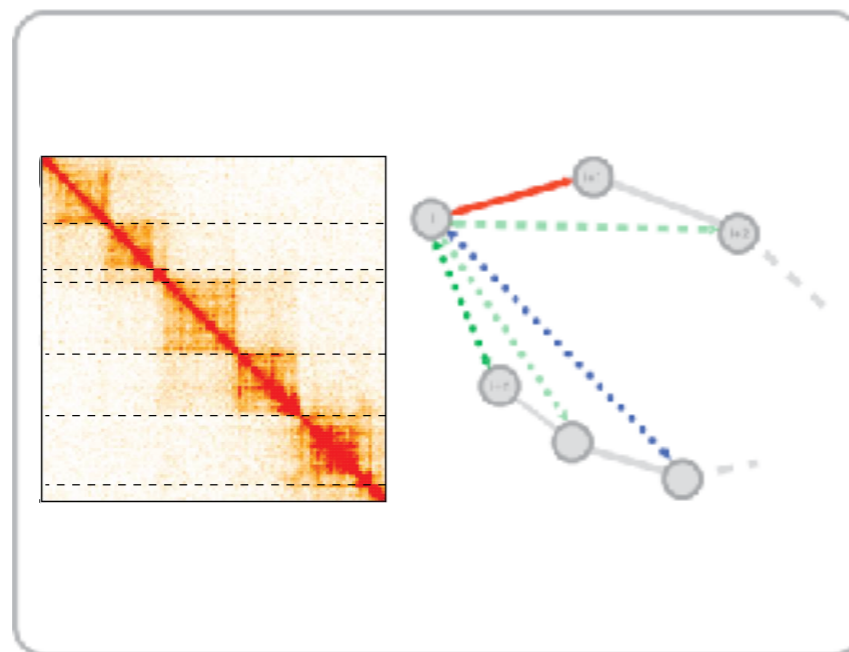
TADs are functional units
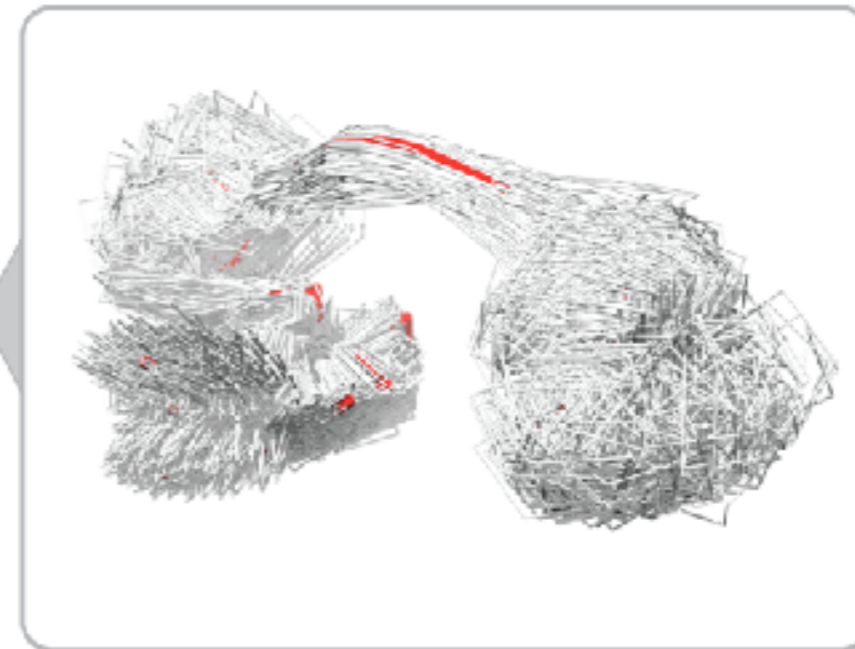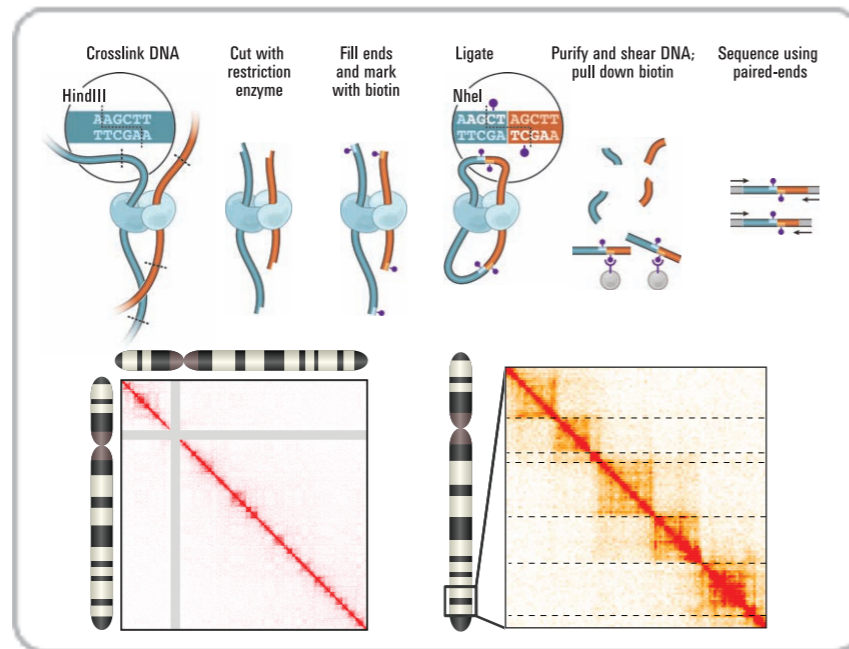
TADs are functional units

Hnisz, D., et al. (2016). Science, on line

Hybrid Method

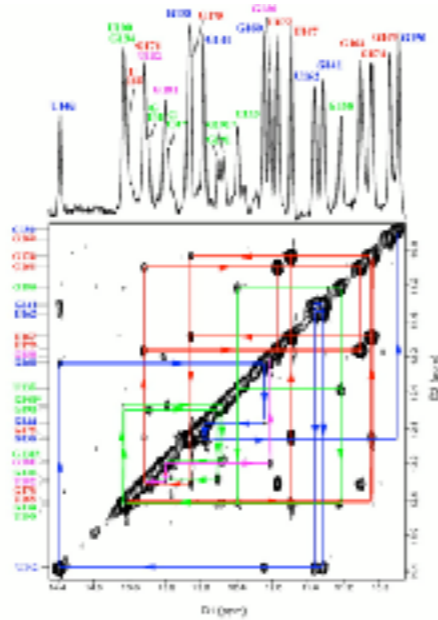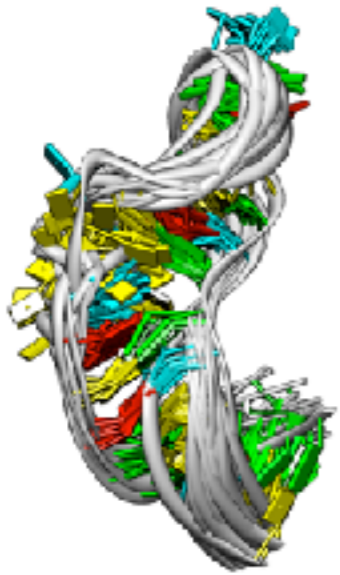Baù, D. & Marti-Renom, M. A. Methods 58, 300–306 (2012).
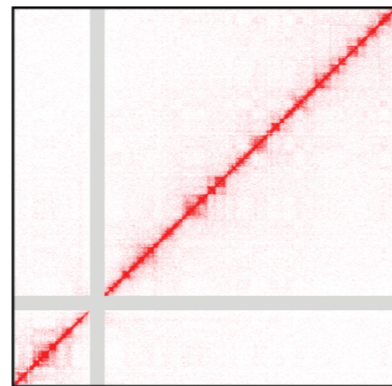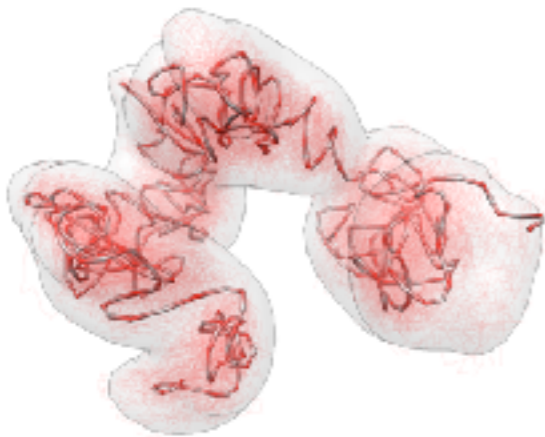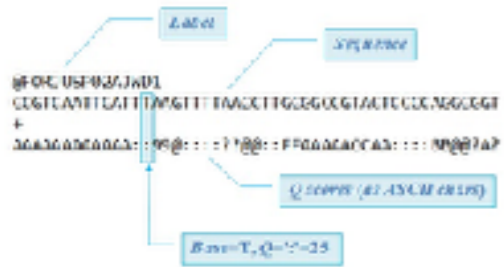
Experiments

Computation

Structure determination using Hi-C data
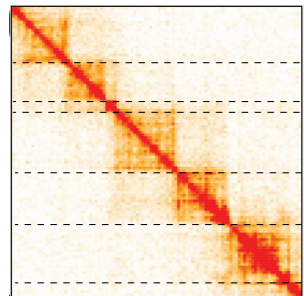

Biomolecular structure determination
2D-NOESY data

Chromosome structure determination
3C-based data
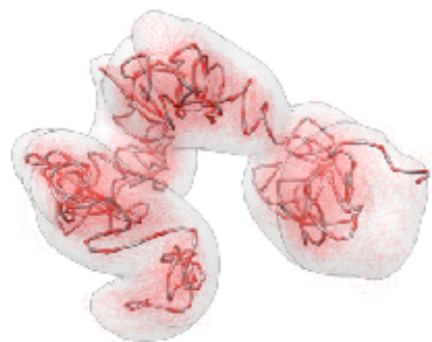
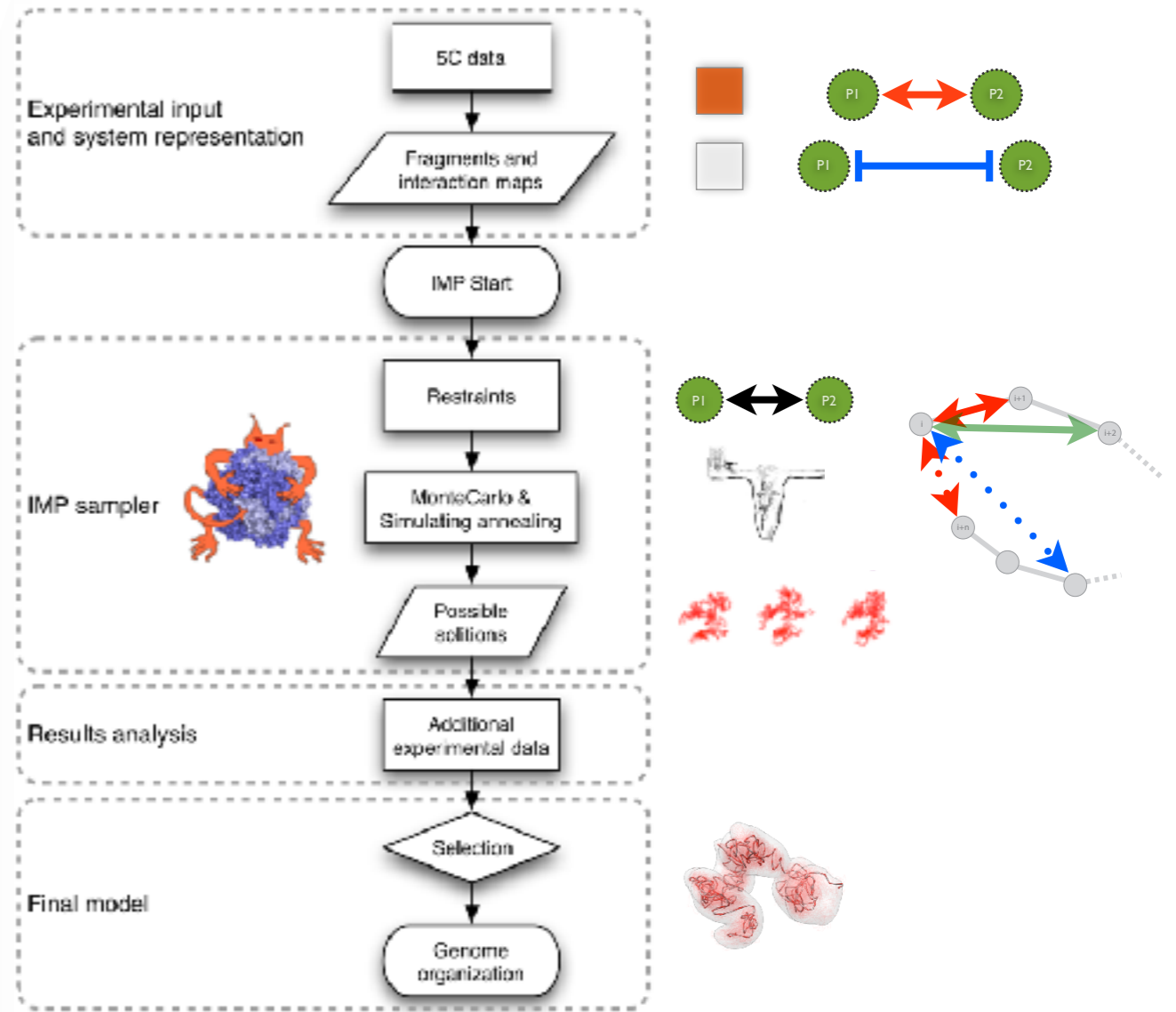TADbit

http://3DGenomes.org
http://www.integrativemodeling.org

FastQ files to Maps

Map analysis
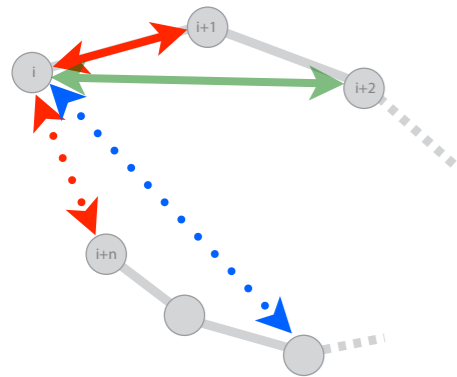
Model building

Model analysis

TADbit

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

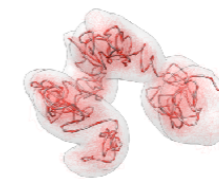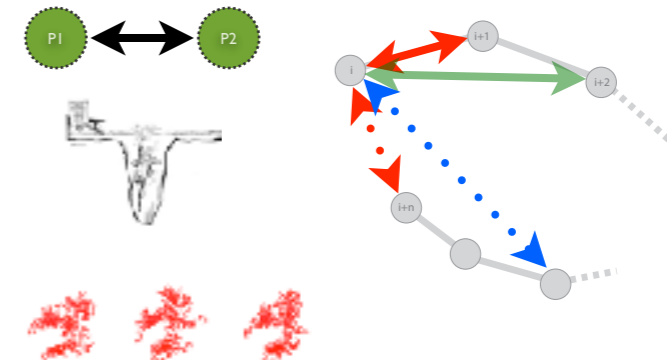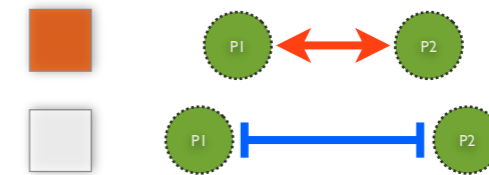| FASTQs	Int. Matrices	3D Models
Quality Control	Visualization	Matrix processing
Mapping (GEM)	Comparison	MMP Score
Cleaning & Filtering	TAD detection	Parameter optimization
Normalization	TAD alignment	3D modeling
Genomic coverage	TAD clustering	Clustering
		Structural analysis

Interaction Analysis | **Matrix Analysis** | **Model Analysis**

TADbit

http://3DGenomes.org
http://www.integrativemodeling.org

Model representation and scoring

d = d₀
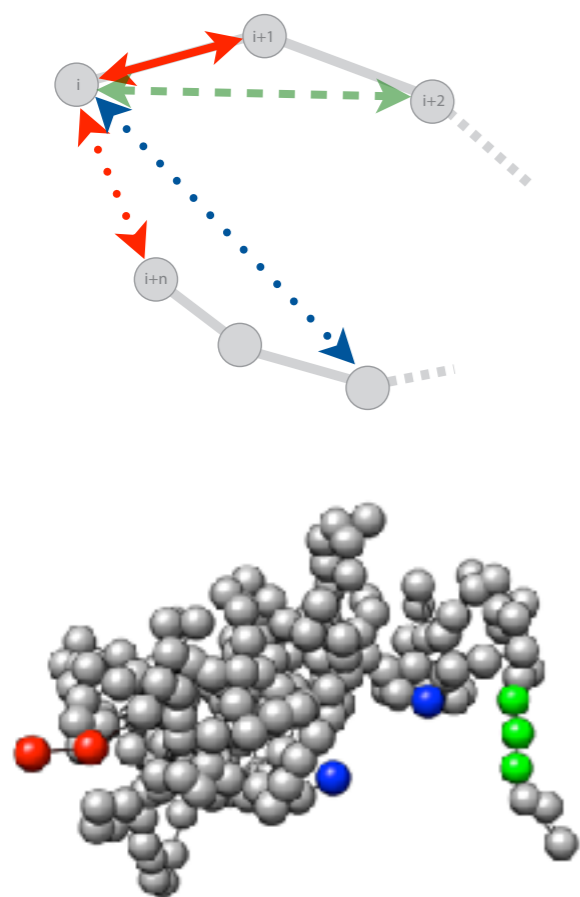
$$H_{i,j} = k\left(d_{i,j} - d_{i,j}^0\right)^2$$
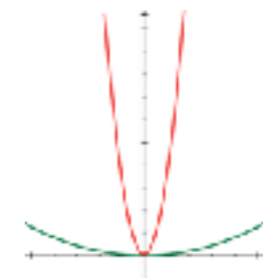
Harmonic

d < d₀

Harmonic Upper Bound

$$\begin{cases} if \ d_{i,j} \geq d_{i,j}^0; & ubH_{i,j} = k\left(d_{i,j} - d_{i,j}^0\right)^2 \\ if \ d_{i,j} < d_{i,j}^0; & ubH_{i,j} = 0 \end{cases}$$
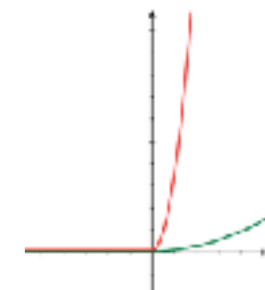
d > d₀

Harmonic Lower Bound

$$\begin{cases} if \ d_{i,j} \leq d_{i,j}^0; & lbH_{i,j} = k\left(d_{i,j} - d_{i,j}^0\right)^2 \\ if \ d_{i,j} > d_{i,j}^0; & lbH_{i,j} = 0 \end{cases}$$
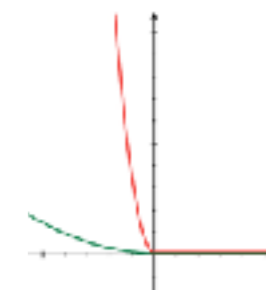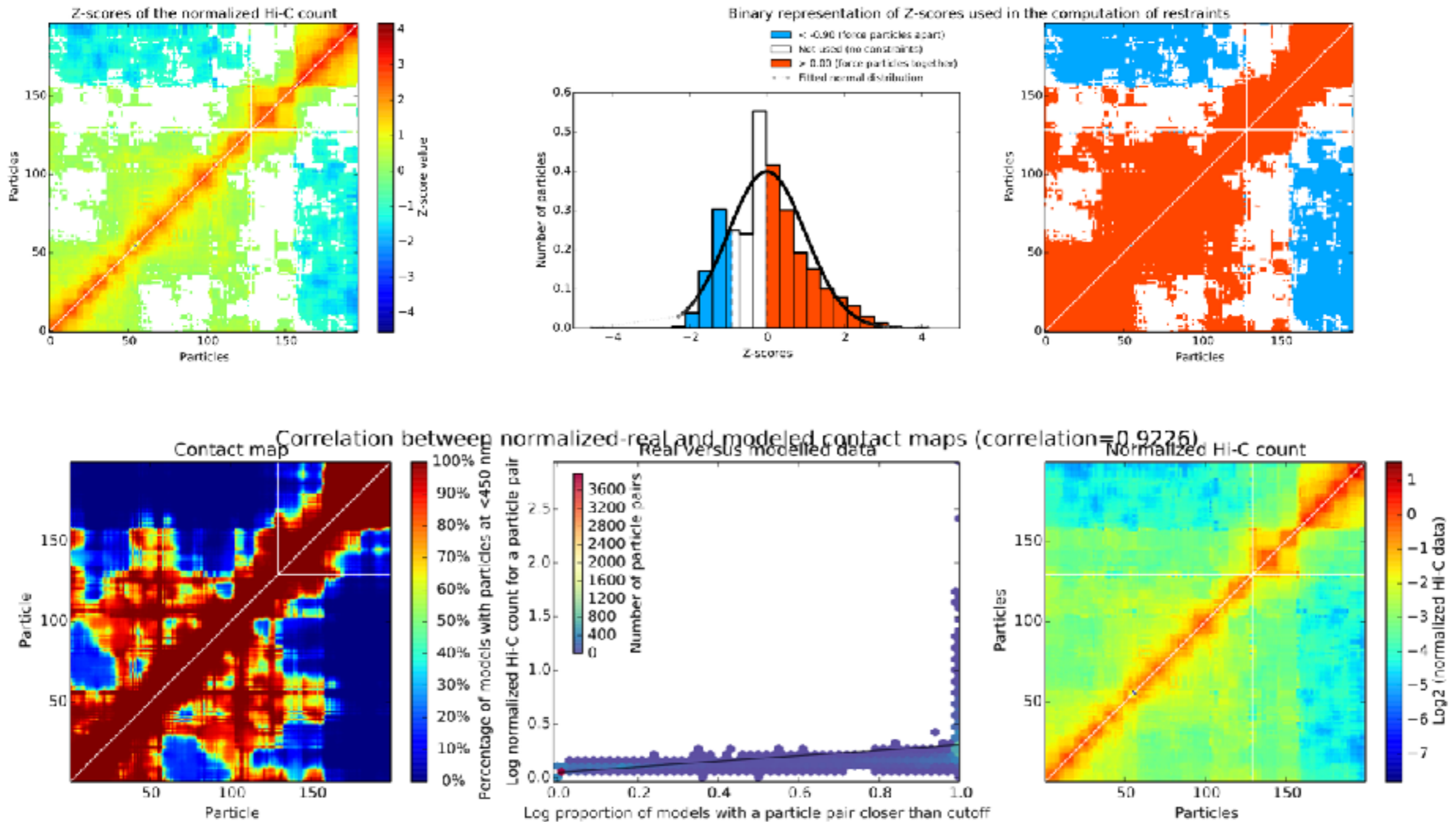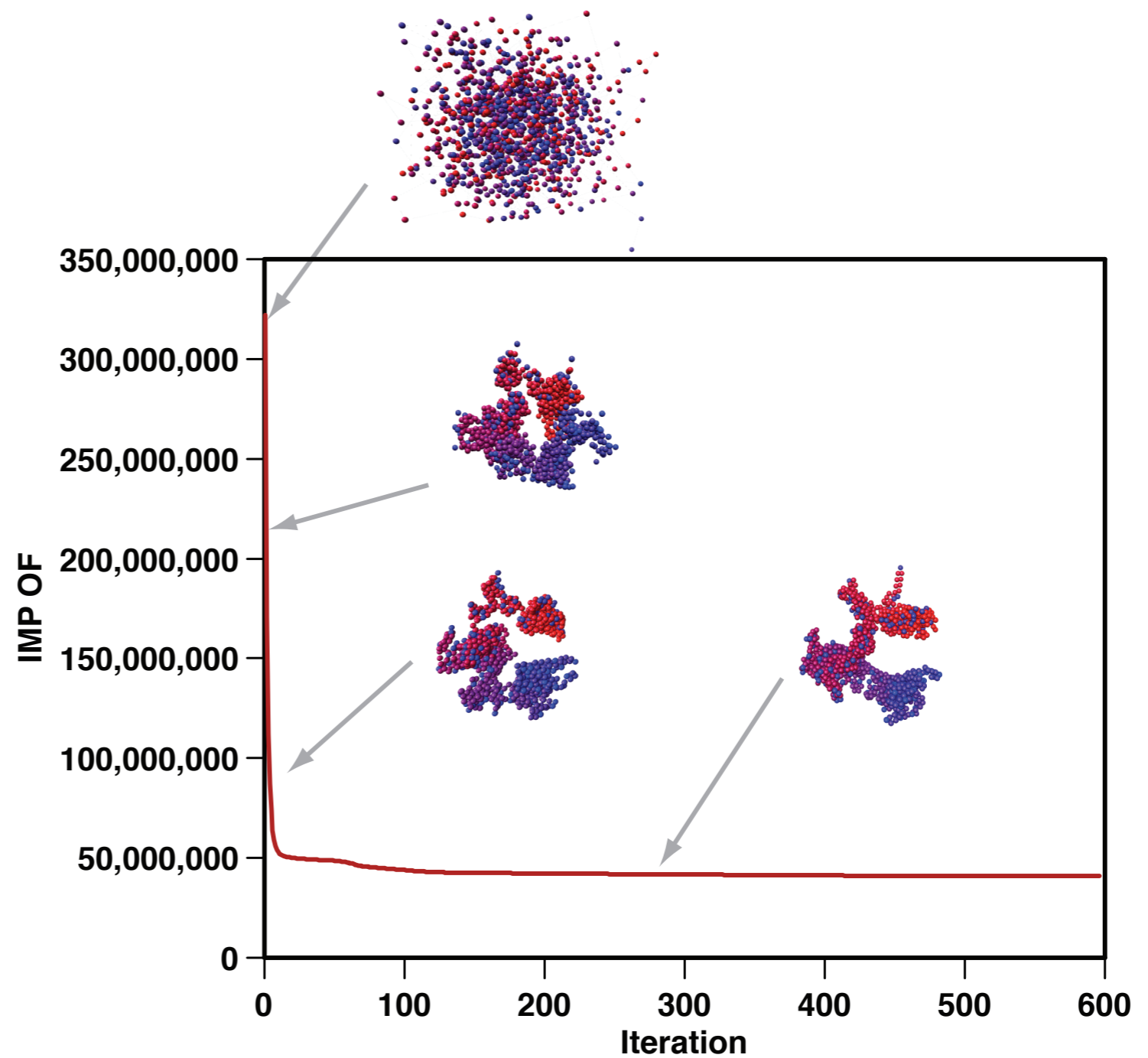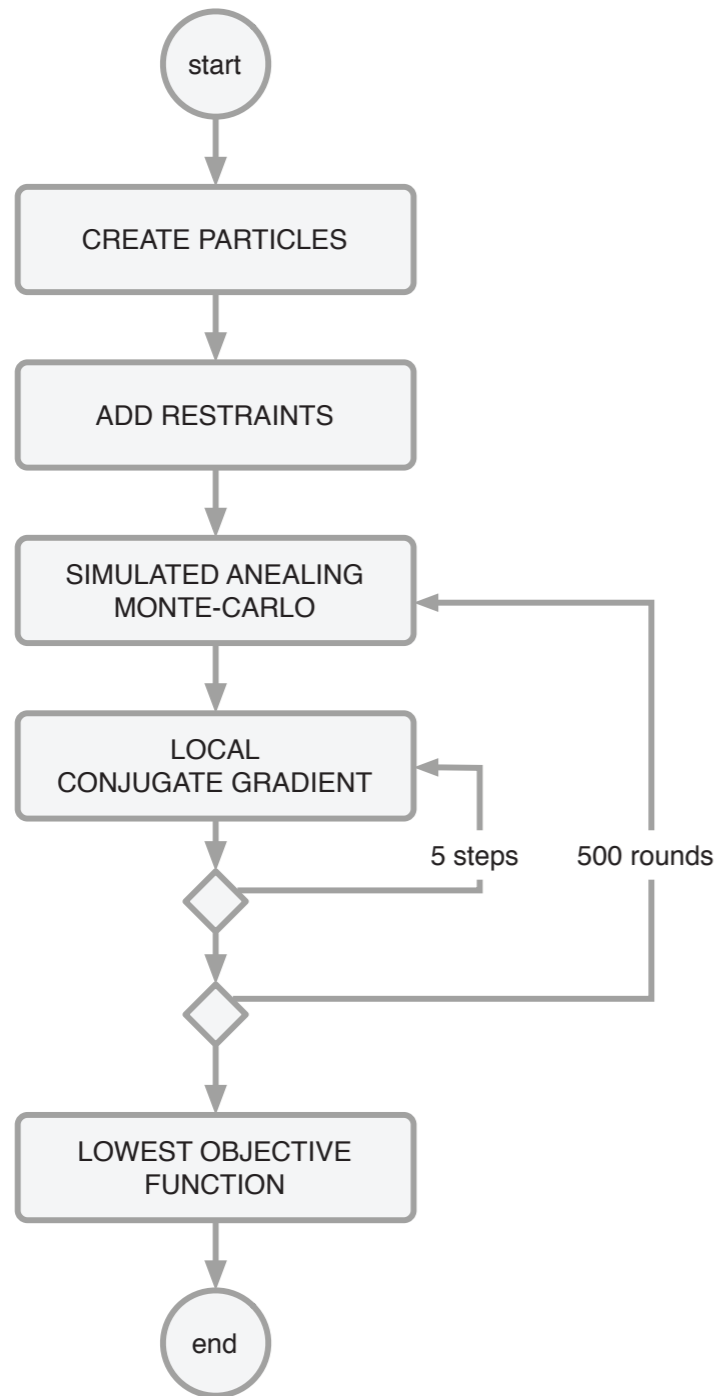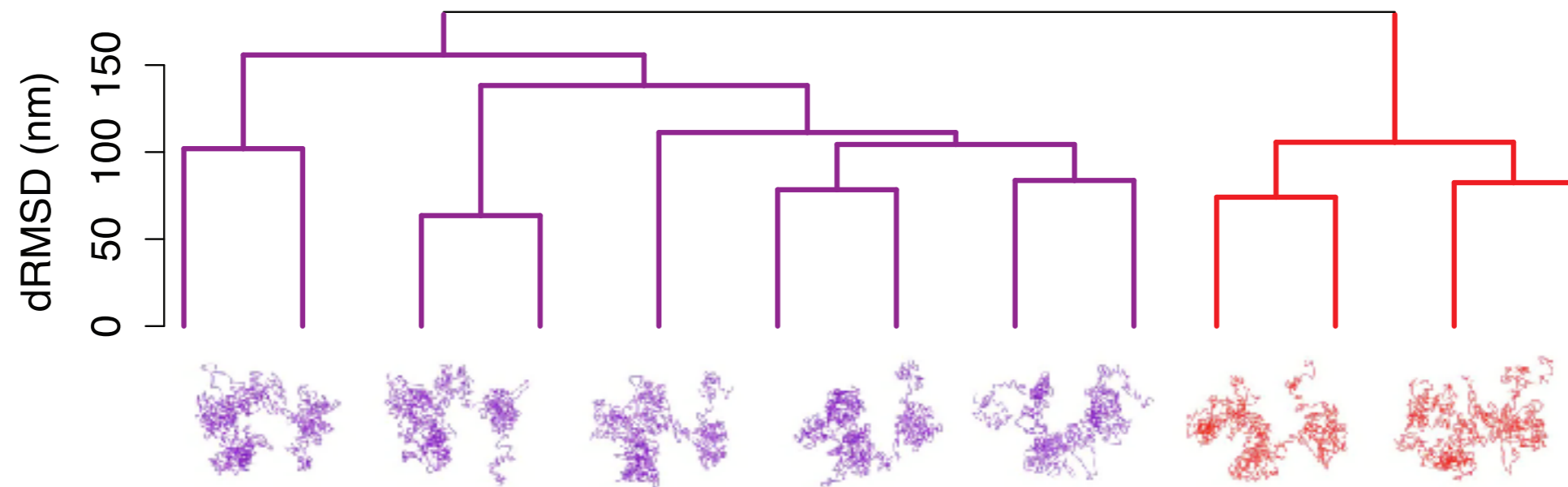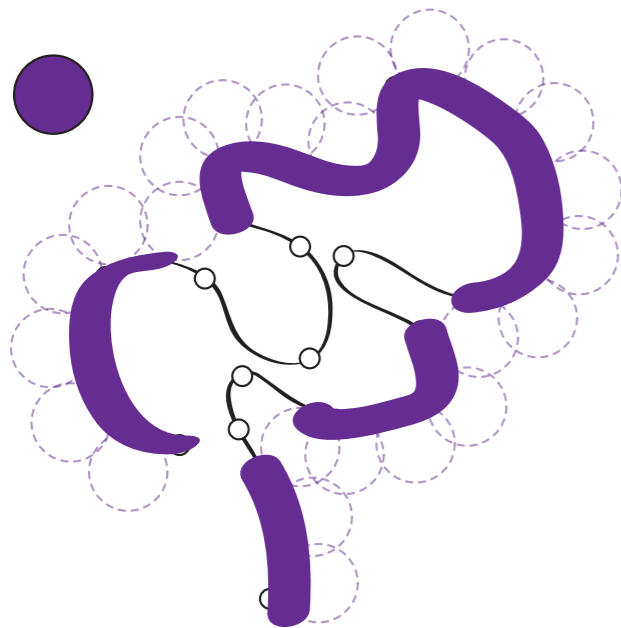
Parameter optimization

Optimization of the scoring function

Model analysis: clustering and structural features

Structuring the **COLORs** of chromatin

Fly Chromatin COLORs

Filion et al. (2010). Cell, 143(2), 212–224.

drawing by Guillaume Filion

Principal component analysis

Hidden Markov model

GREEN
HP1

YELLOW
Active

RED
Active

BLUE
Polycomb

BLACK
Repressive
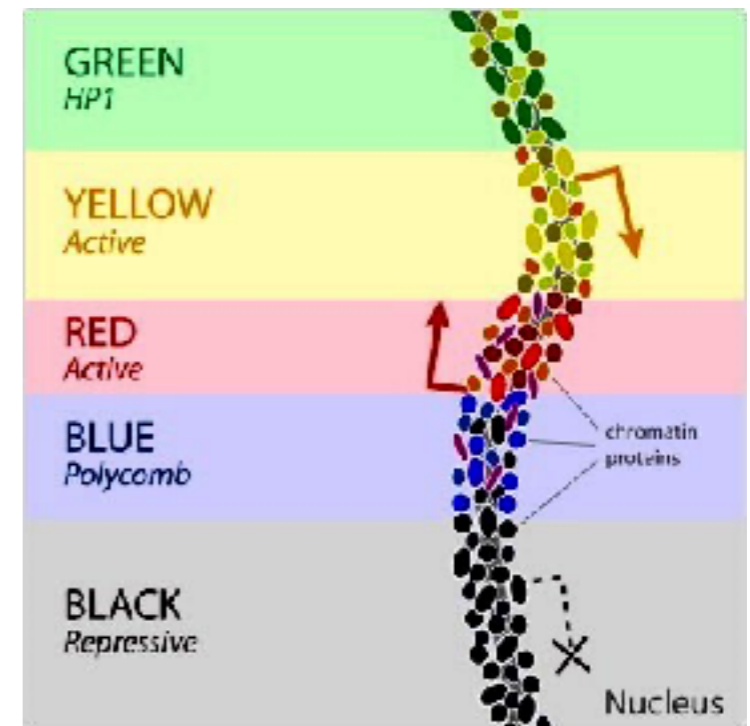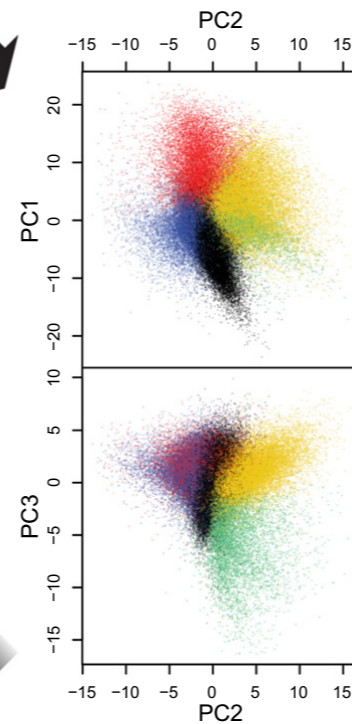
Fly Chromatin COLORs

Hou et al. (2012). Molecular Cell, 48(3), 471–484.
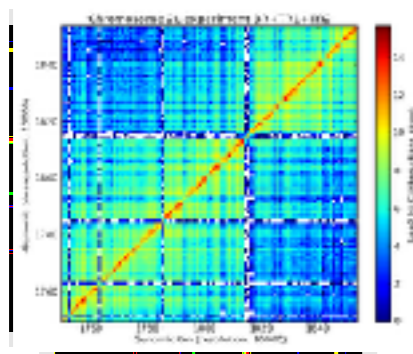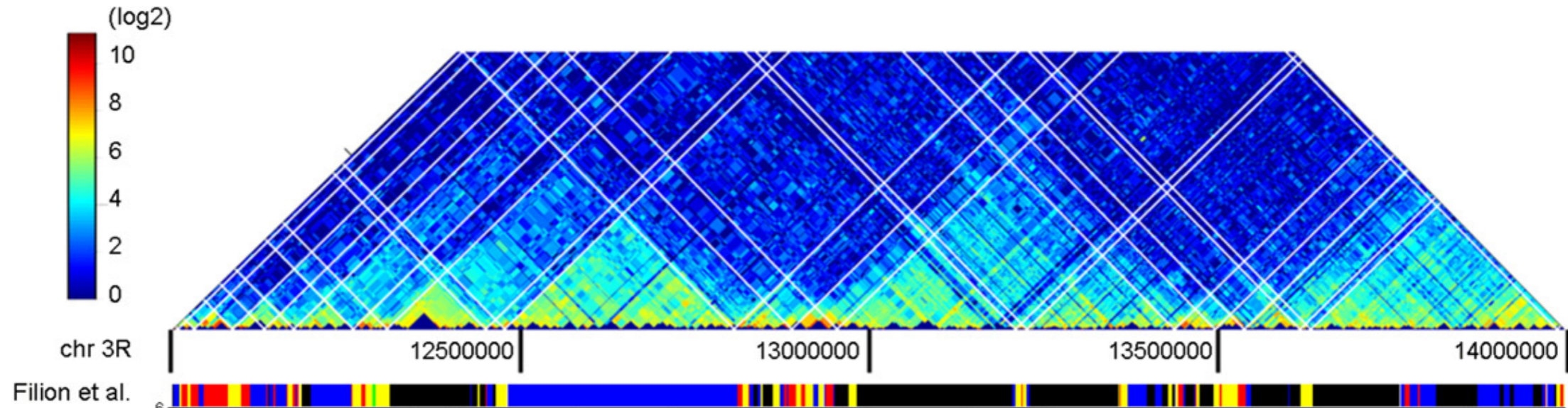
~200 regions of ~5Mb each
2Kb resolution

Mapping · Filtering · Normalizing
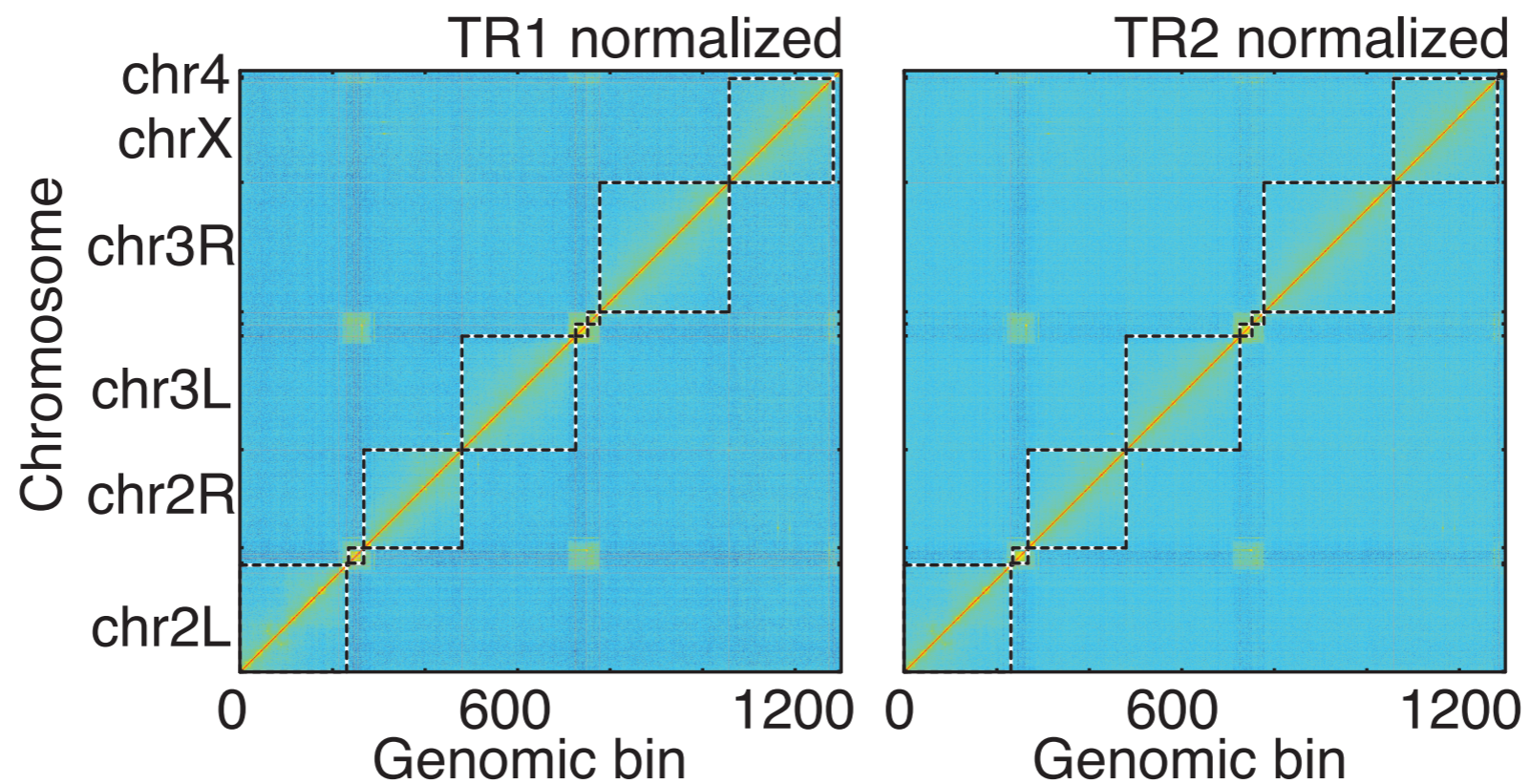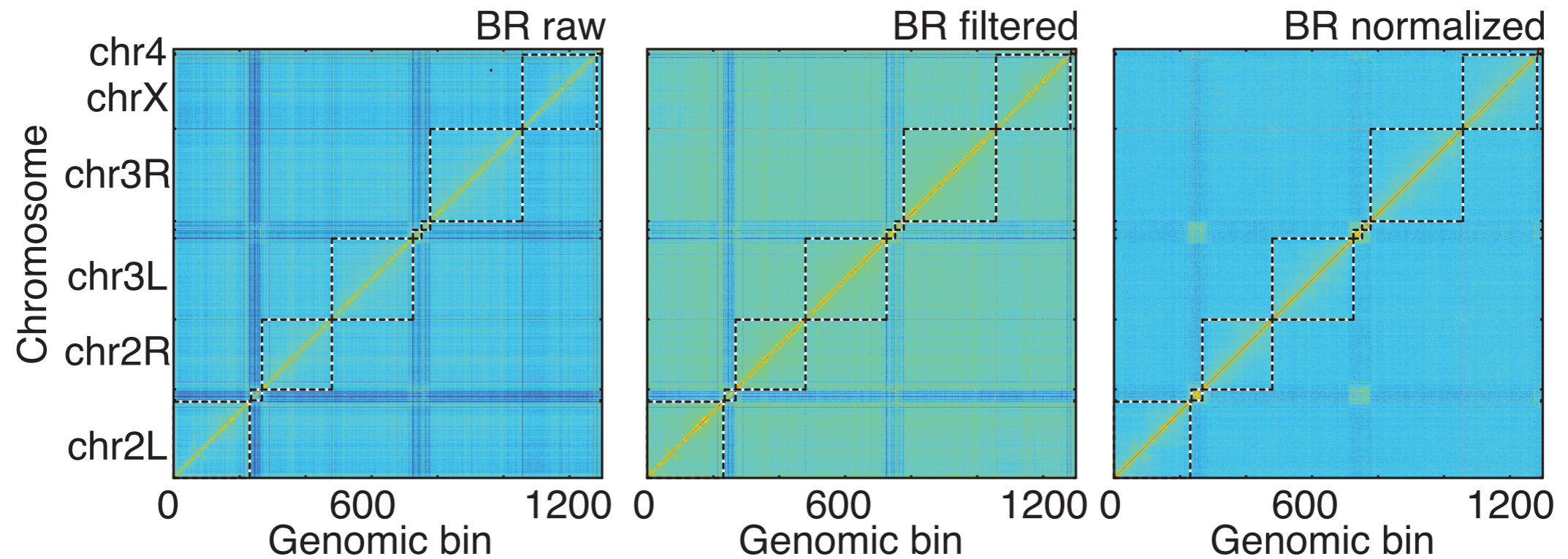
TAD detection · comparison
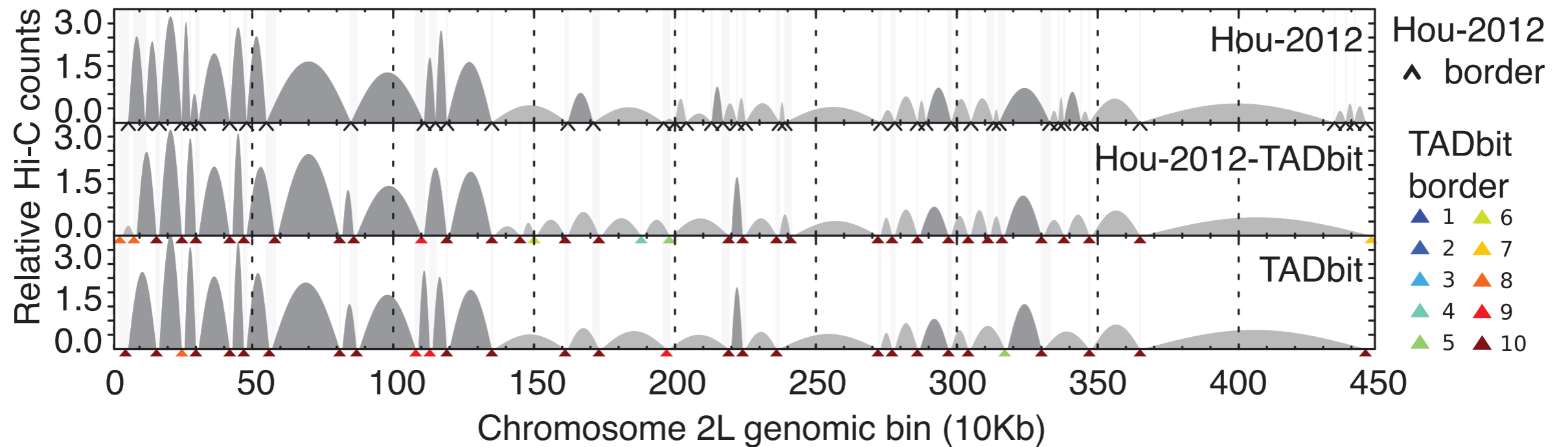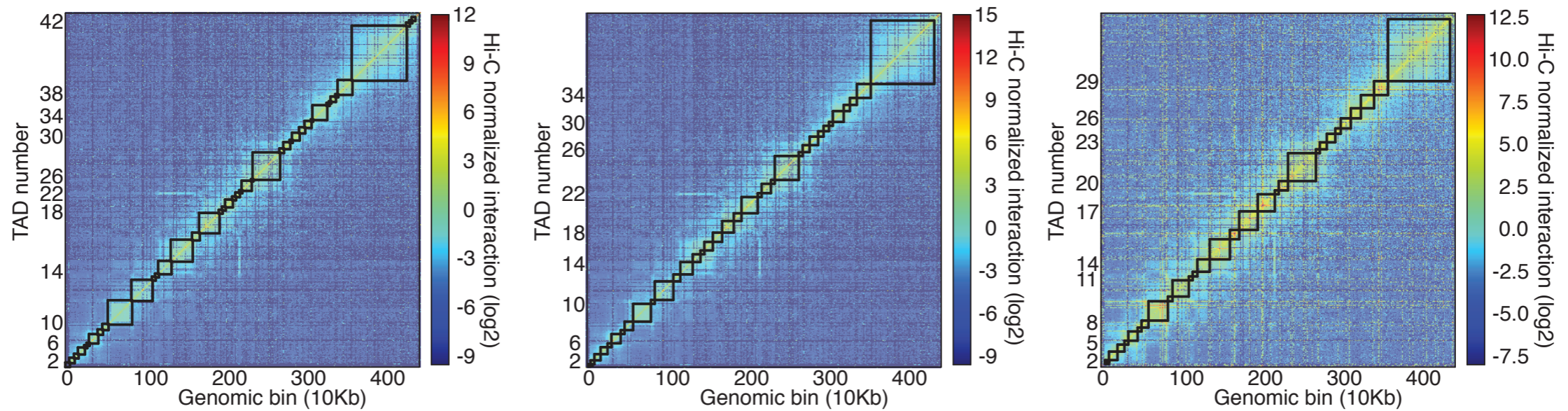
Structural properties

50 1Mb regions. 10 enriched for each color.

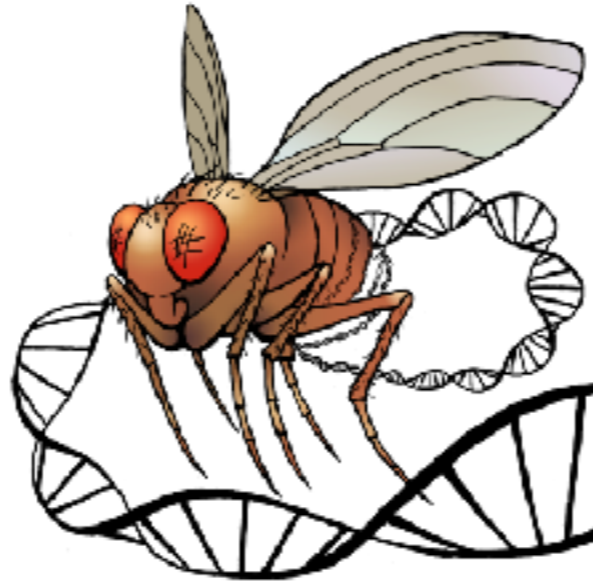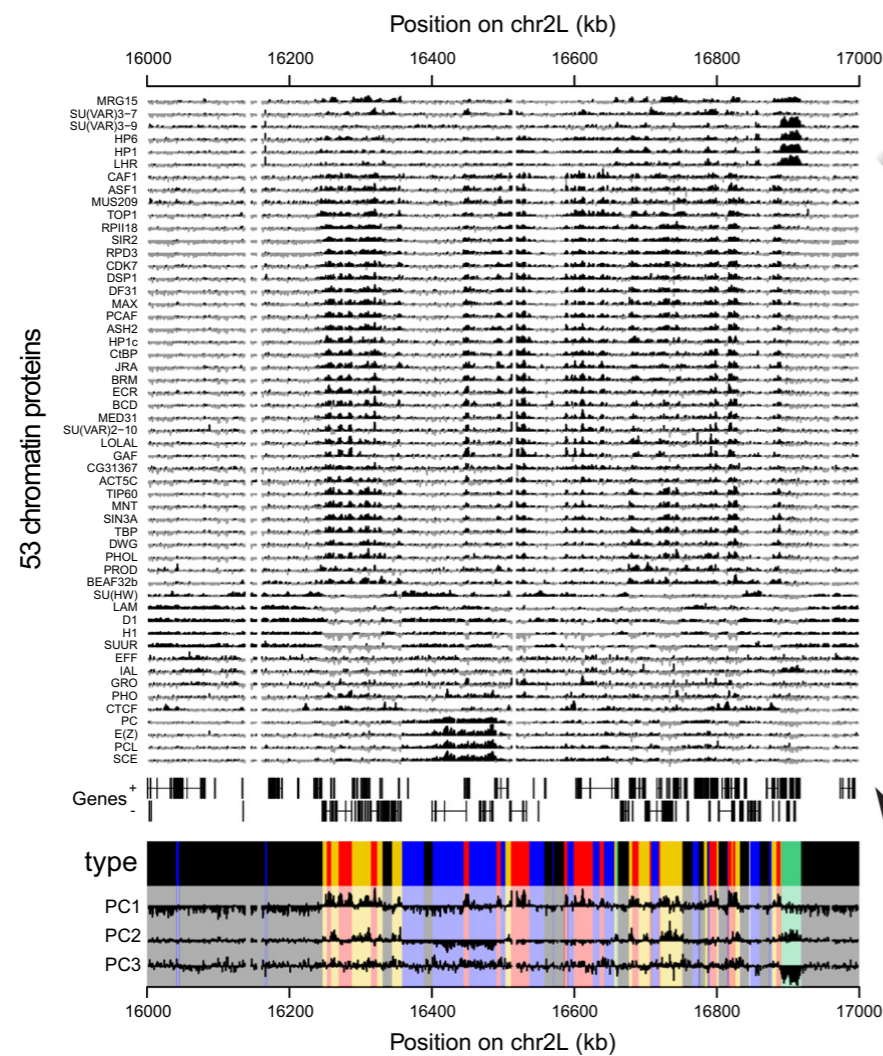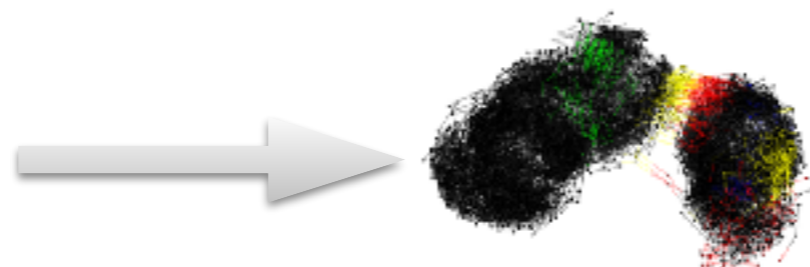| RED dense region	YELLOW dense region	GREEN dense region	BLUE dense region	BLACK dense region
3R:18920000-19920000	X:15590000-16600000	2R:510000-1530000	3L:210000-1230000	2L:17500000-18530000
22% 17% 0% 11% 45% 6%	0% 48% 4% 20% 26% 3%	0% 0% 100% 0% 0% 0%	11% 17% 0% 52% 13% 0%	1% 0% 0% 0% 98% 1%
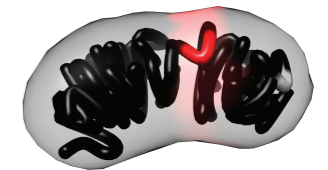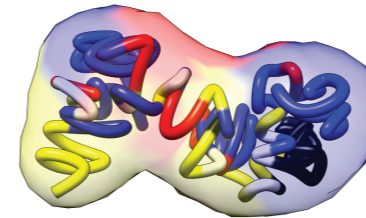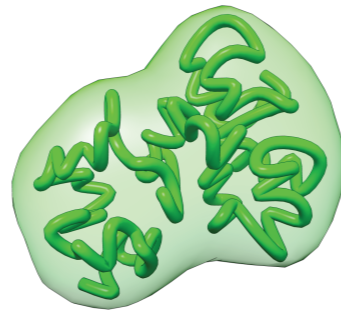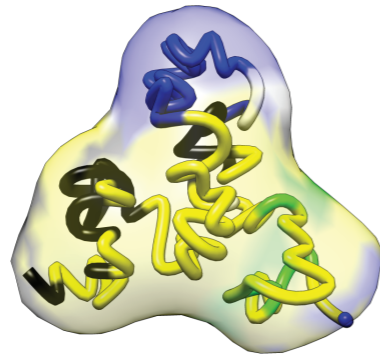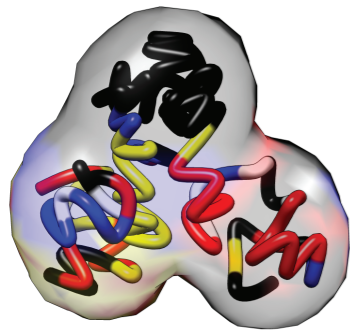
Accessibility (%) Density (bp/nm) Interactions Angle
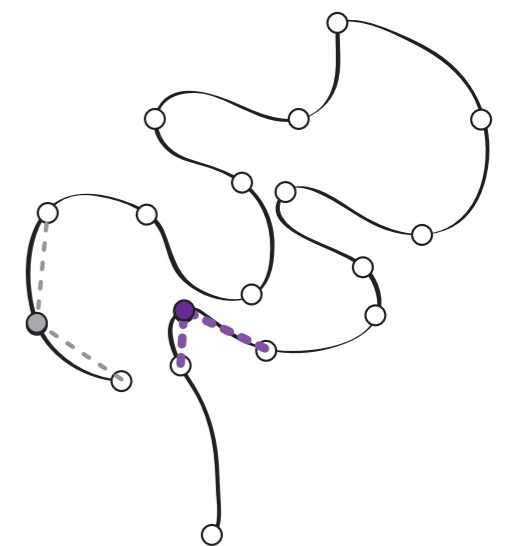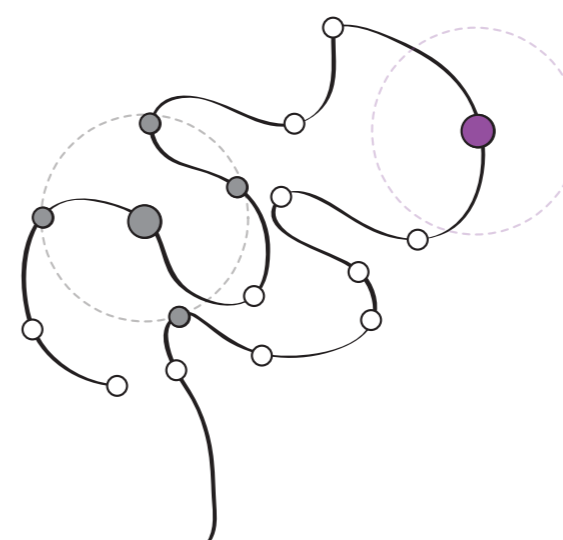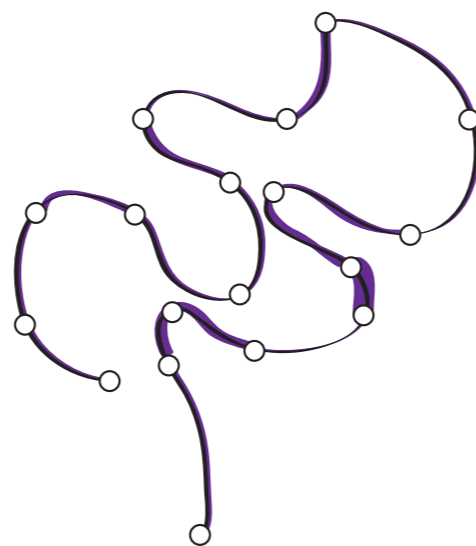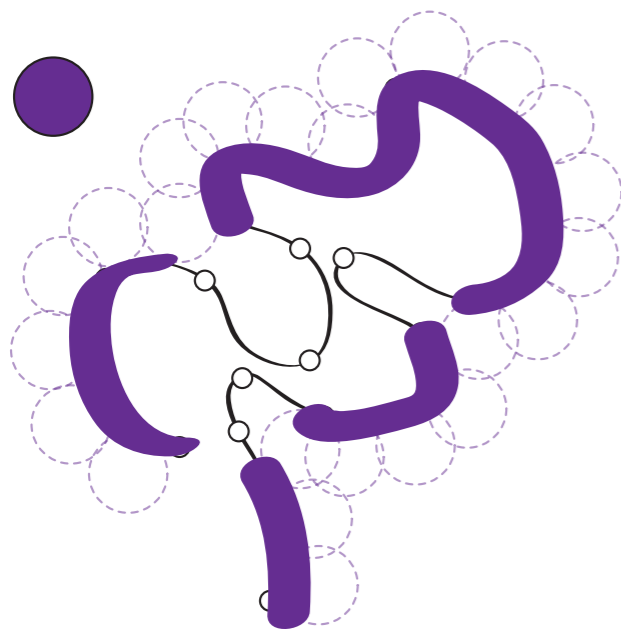
Structural COLORs
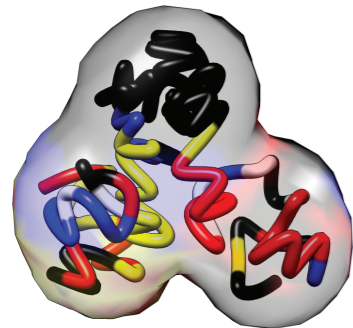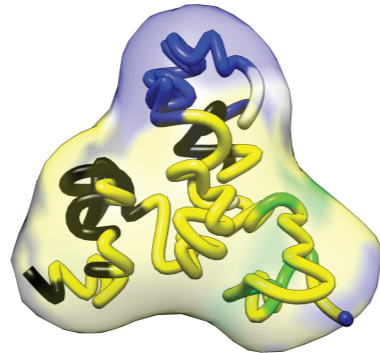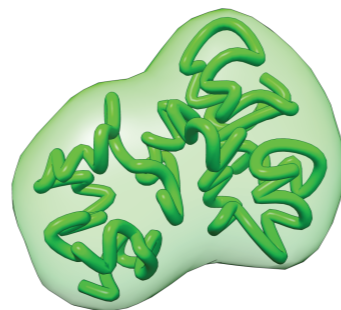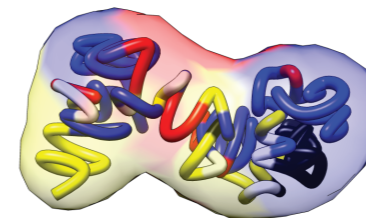
RED dense region	YELLOW dense region	GREEN dense region	BLUE dense region	BLACK dense region
3R:18920000-19920000	X:15590000-16600000	2R:510000-1530000	3L:210000-1230000	2L:17500000-18530000
22% 17% 0% 11% 45% 6%	0% 48% 4% 20% 26% 3%	0% 0% 100% 0% 0% 0%	11% 17% 0% 52% 13% 0%	1% 0% 0% 0% 98% 1%
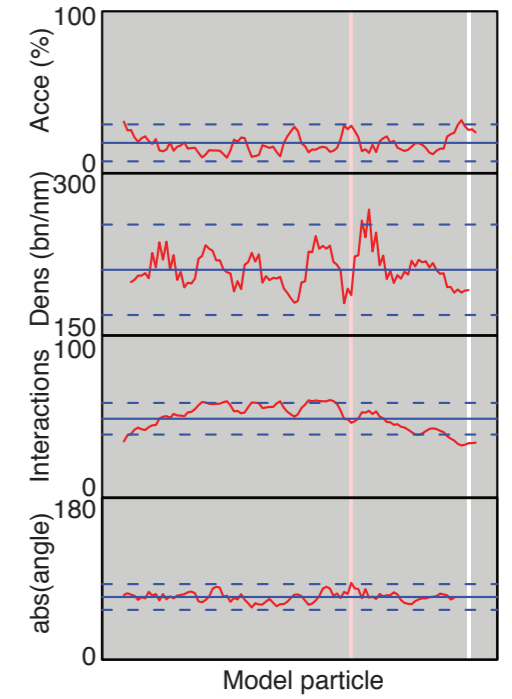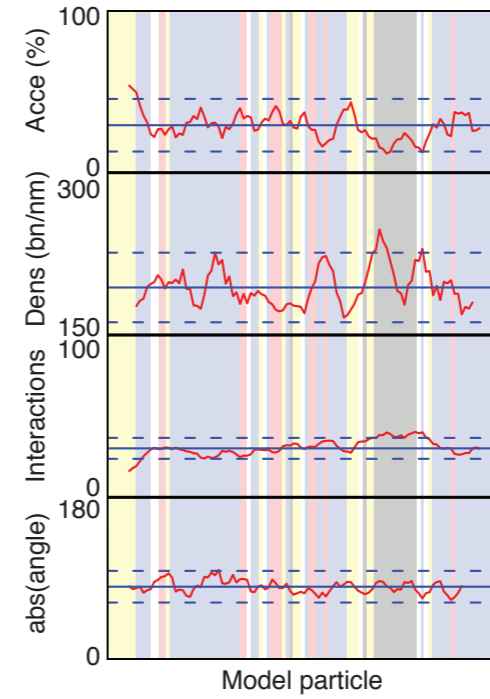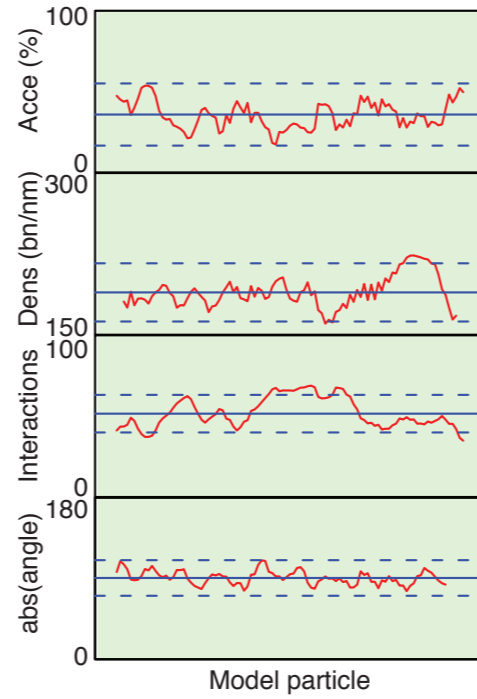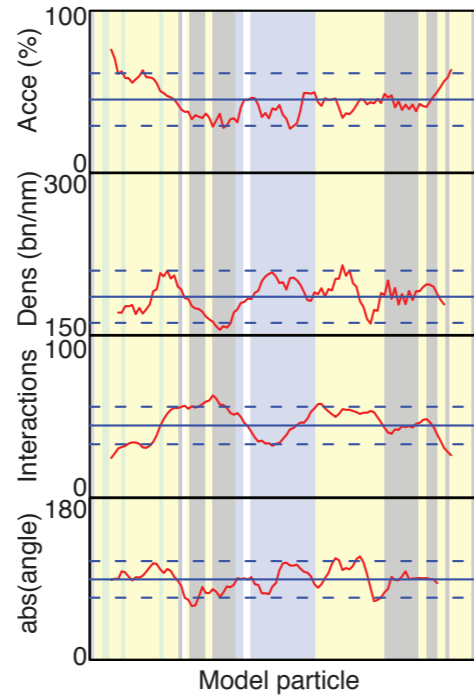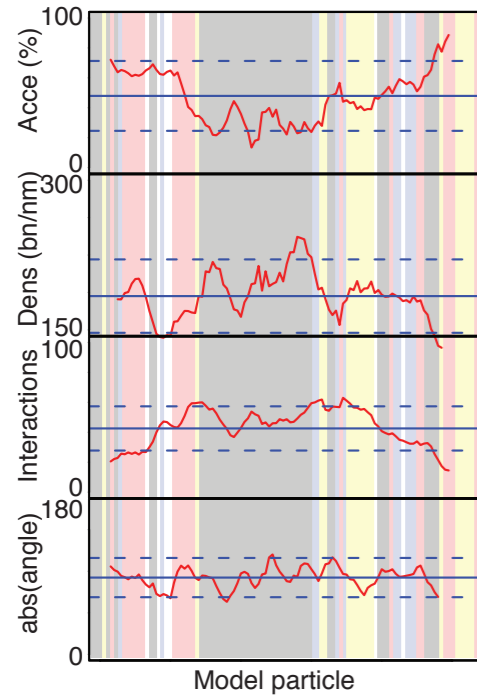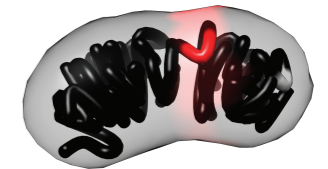
Structural COLORs
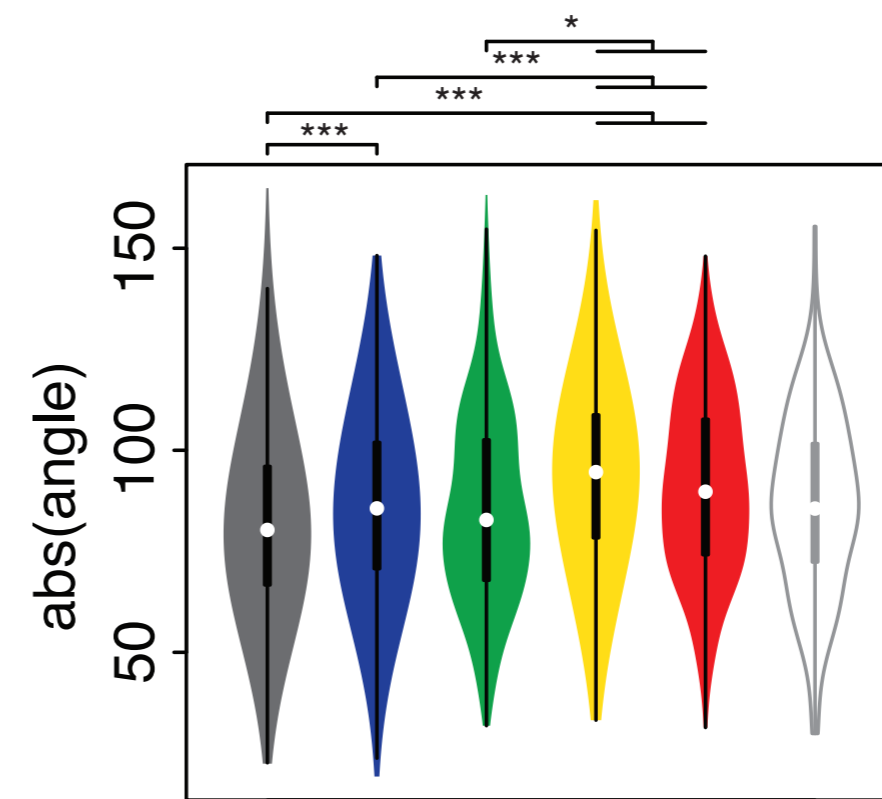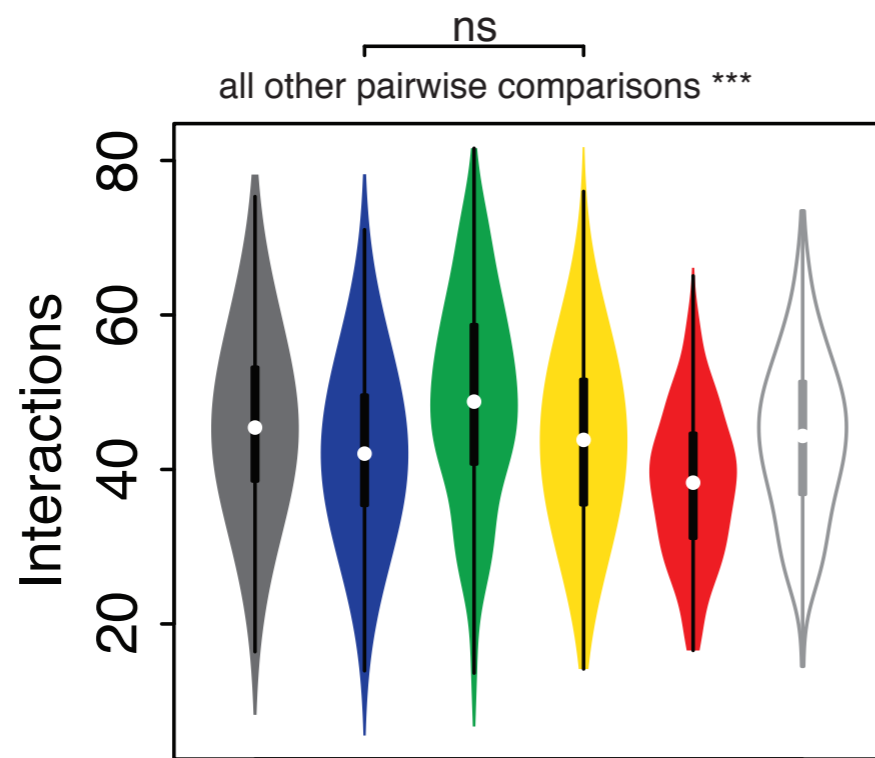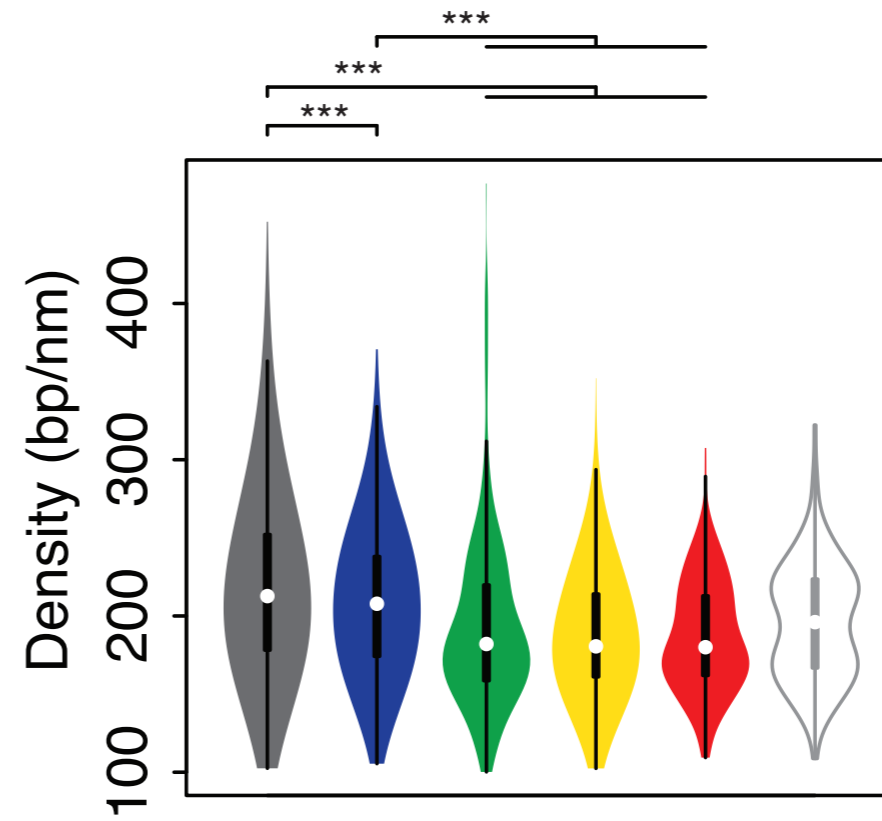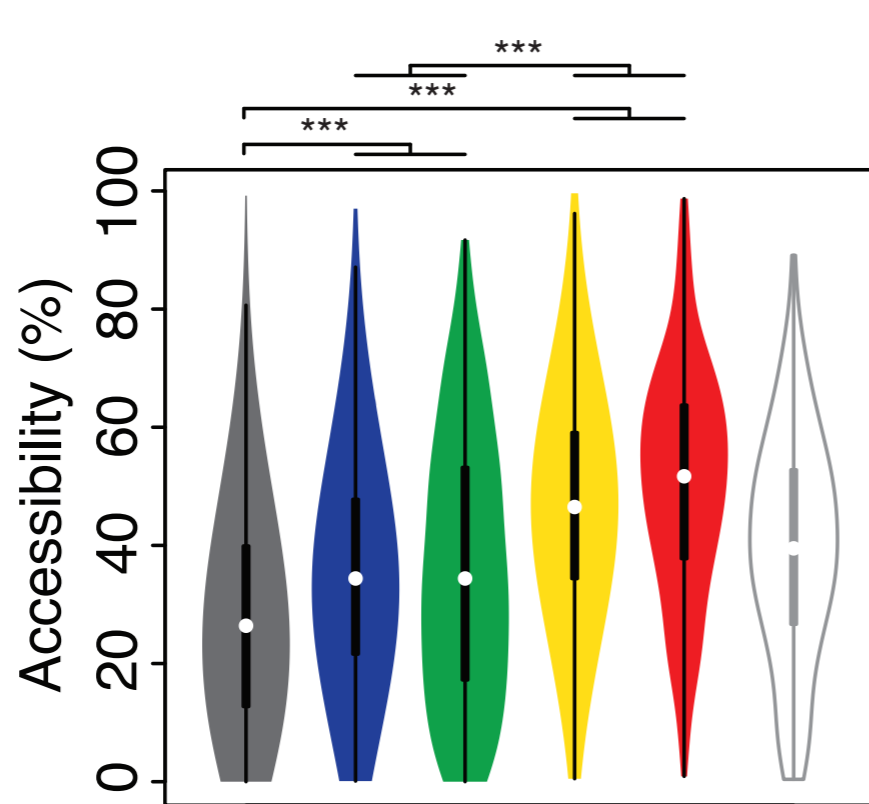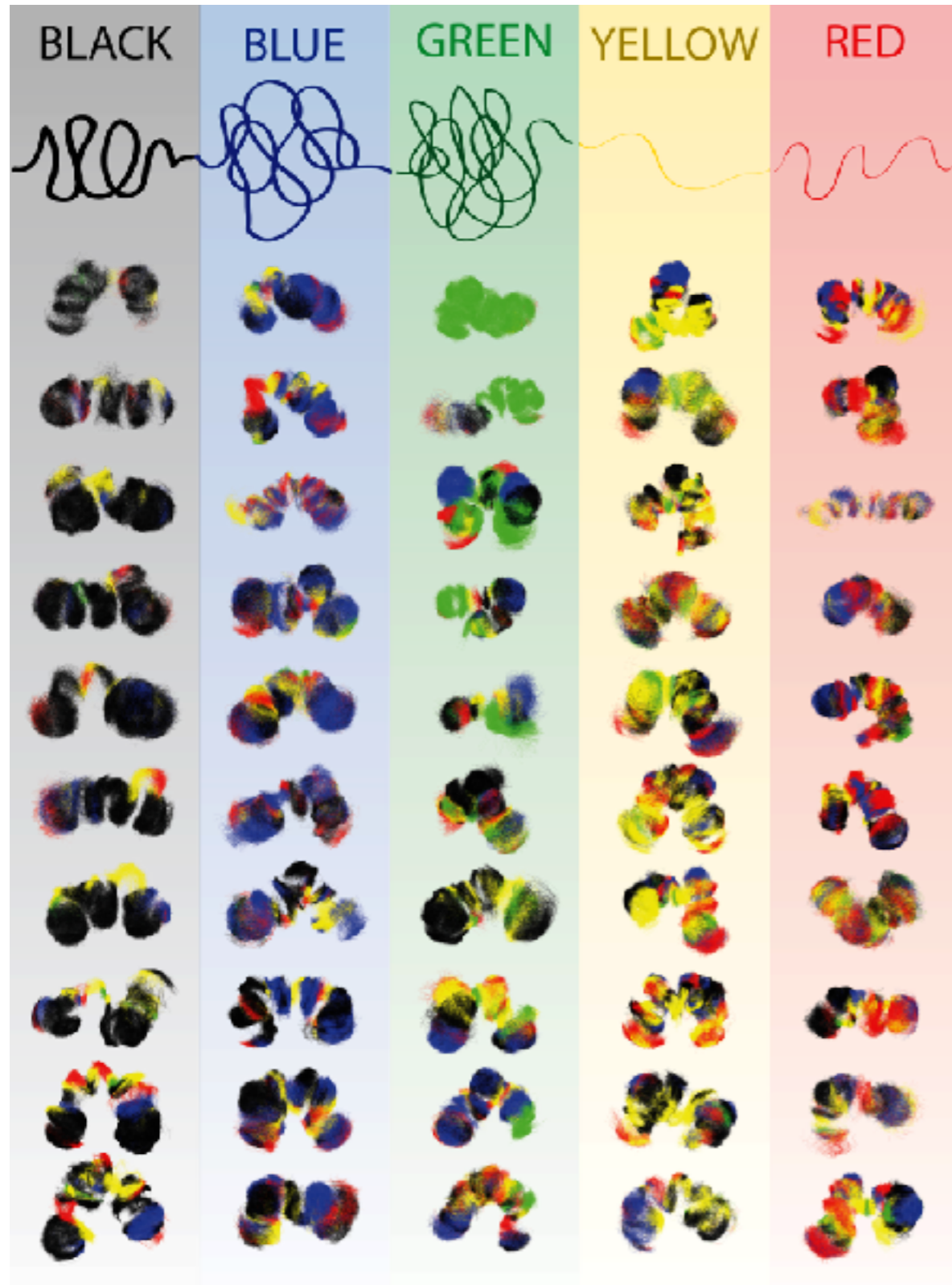
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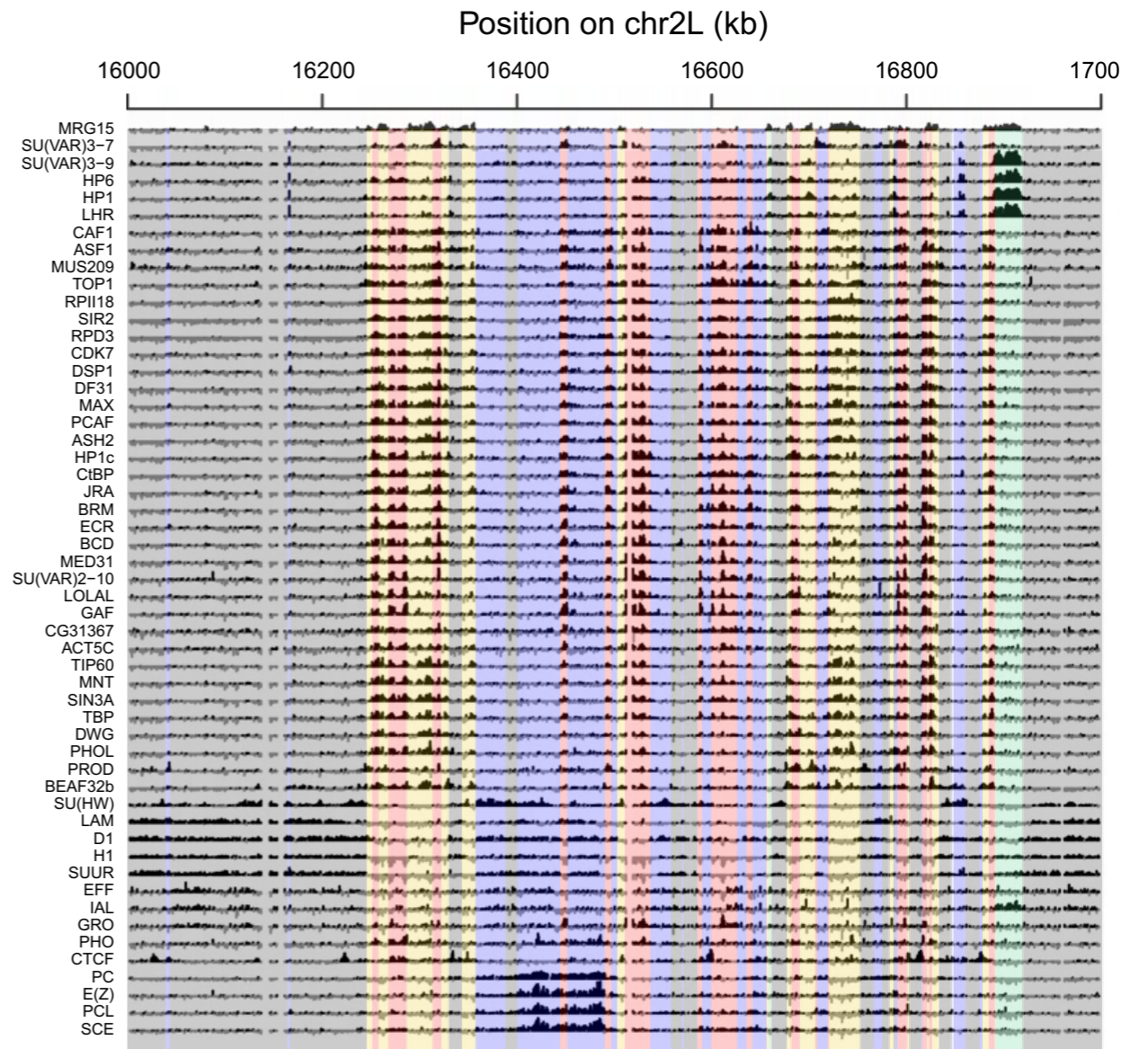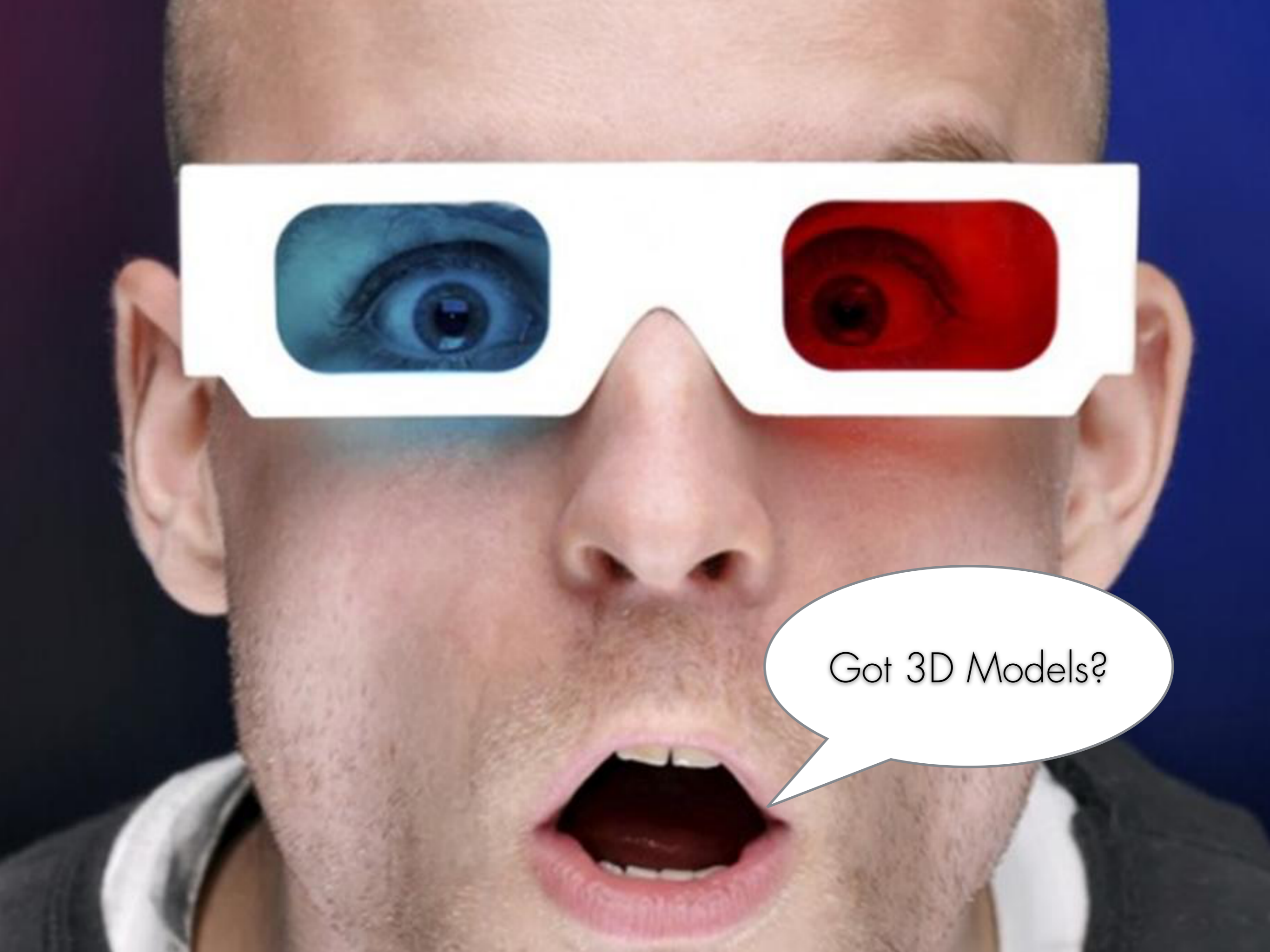
Got 3D Models?

Are the models correct?
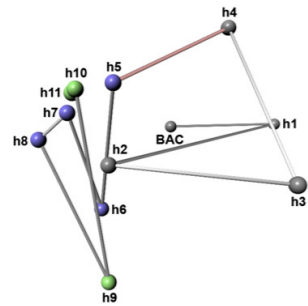
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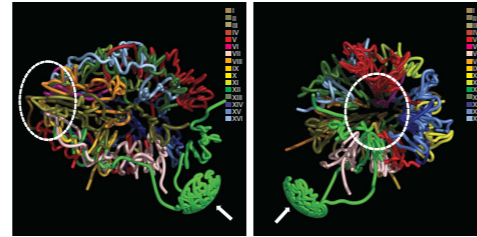
Duan (2010) Nature
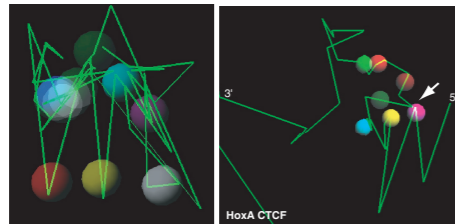
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Ferraiuolo (2010) Nucleic Acids Research
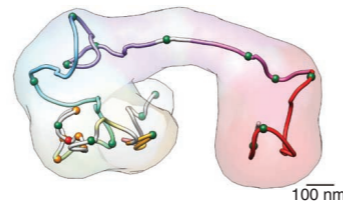
Baù (2011) Nature Structural & Molecular Biology

HoxA CTCF
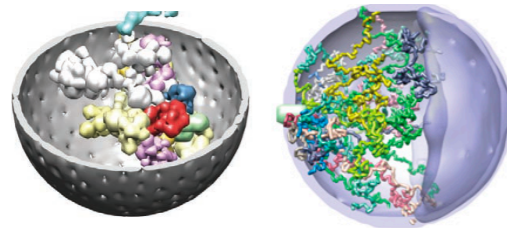
Kalhor (2011) Nature Biotechnology
Tjong (2012) Genome Research
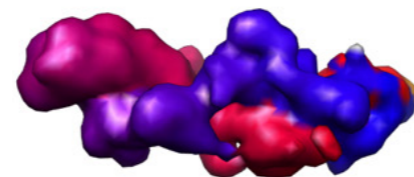
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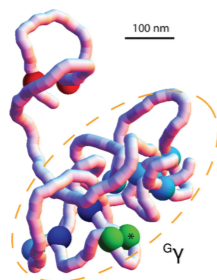
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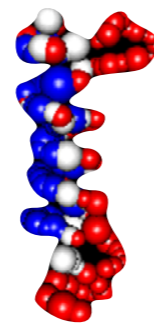
Hu (2013) PLoS Computational Biology

Assessing the limits of restraint-based 3D modeling of genomes and genomic domains

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[1]EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), Barcelona, Spain, [2]Universitat Pompeu Fabra (UPF), Barcelona, Spain, [3]Gene Regulation, Stem Cells and Cancer Program, Centre for Genomic Regulation (CRG), Barcelona, Spain, [4]Genome Biology Group, Centre Nacional d'Anàlisi Genòmica (CNAG), Barcelona, Spain and [5]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

ABSTRACT

Restraint-based modeling of genomes has been recently explored with the advent of Chromosome Conformation Capture (3C-based) experiments. We previously developed a reconstruction method to resolve the 3D architecture of both prokaryotic and eukaryotic genomes using 3C-based data. These models were congruent with fluorescent imaging validation. However, the limits of such methods have not systematically been assessed. Here we propose the first evaluation of a mean-field restraint-based reconstruction of genomes by considering diverse chromosome architectures and different levels of data noise and structural variability. The results show that: first, current scoring functions for 3D reconstruction correlate with the accuracy of the models; second, reconstructed models are robust to noise but sensitive to structural variability; third, the local structure organization of genomes, such as Topologically Associating Domains, results in more accurate models; fourth, to a certain extent, the models capture the intrinsic structural variability in the input matrices and fifth, the accuracy of the models can be *a priori* predicted by analyzing the properties of the interaction matrices. In summary, our work provides a systematic analysis of the limitations of a mean-field restrain-based method, which could be taken into consideration in further development of methods as well as their applications.

INTRODUCTION

Recent studies of the three-dimensional (3D) conformation of genomes are revealing insights into the organization and the regulation of biological processes, such as gene expression regulation and replication (1–6). The advent of the so-called Chromosome Conformation Capture (3C) assays (7), which allowed identifying chromatin-looping interactions between pairs of loci, helped deciphering some of the key elements organizing the genomes. High-throughput derivations of genome-wide 3C-based assays were established with Hi-C technologies (8) for an unbiased identification of chromatin interactions. The resulting genome interaction matrices from Hi-C experiments have been extensively used for computationally analyzing the organization of genomes and genomic domains (5). In particular, a significant number of new approaches for modeling the 3D organization of genomes have recently flourished (9–14). The main goal of such approaches is to provide an accurate 3D representation of the bi-dimensional interaction matrices, which can then be more easily explored to extract biological insights. One type of methods for building 3D models from interaction matrices relies on the existence of a limited number of conformational states in the cell. Such methods are regarded as mean-field approaches and are able to capture, to a certain degree, the structural variability around these mean structures (15).

We recently developed a mean-field method for modeling 3D structures of genomes and genomic domains based on 3C interaction data (9). Our approach, called TADbit, was developed around the Integrative Modeling Platform (IMP, http://integrativemodeing.org), a general framework for restraint-based modeling of 3D bio-molecular structures (16). Briefly, our method uses chromatin interaction frequencies derived from experiments as a proxy of spatial proximity between the ligation products of the 3C libraries. Two fragments of DNA that interact with high frequency are dynamically placed close in space in our models while two fragments that do not interact as often will be kept apart. Our method has been successfully applied to model the structures of genomes and genomic domains in eukaryote and prokaryote organisms (17–19). In all of our studies, the final models were partially validated by assessing their

Trussart, et al. (2015). Nucleic Acids Research.

Toy models

by Ivan Junier
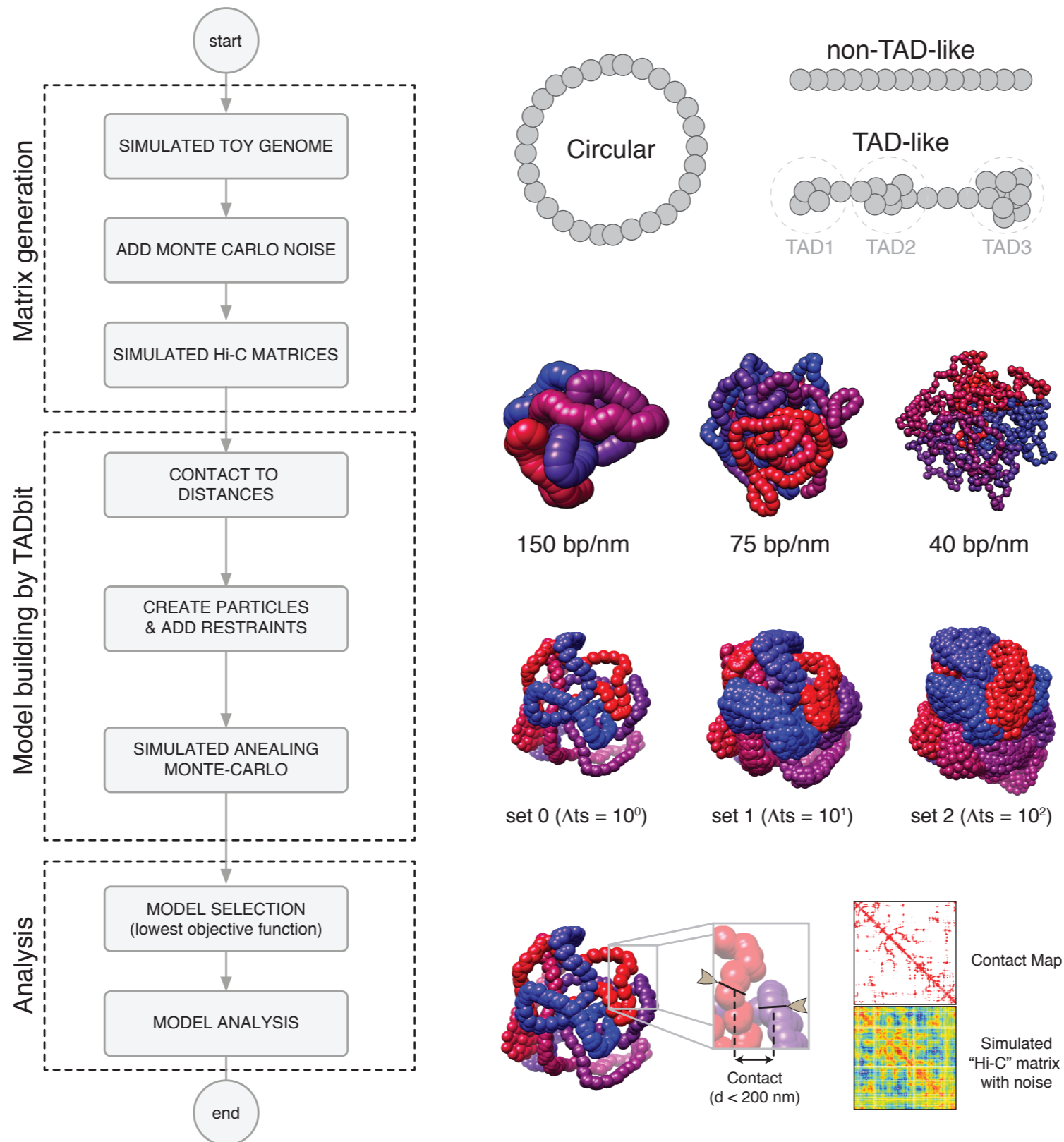
Toy interaction matrices

set 0 (△ts=10⁰)
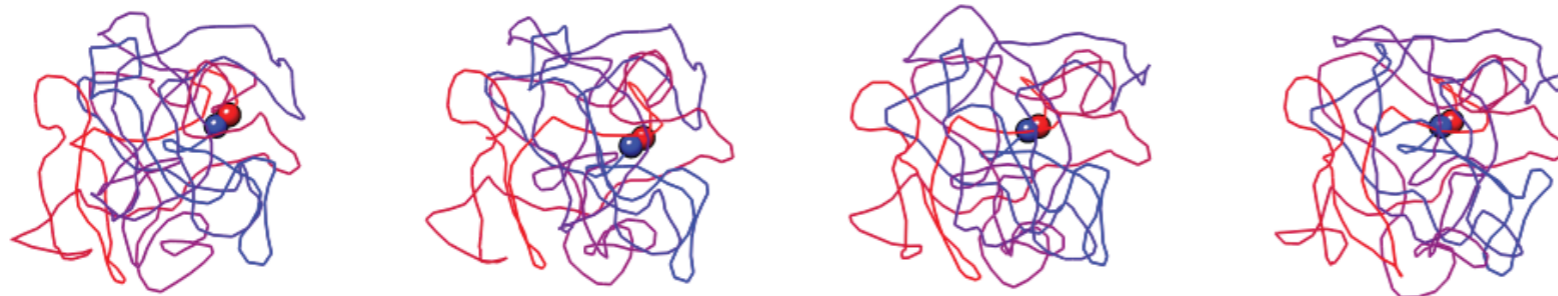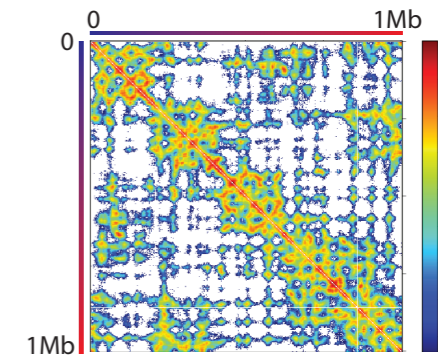
set 4 (△ts=10⁴)
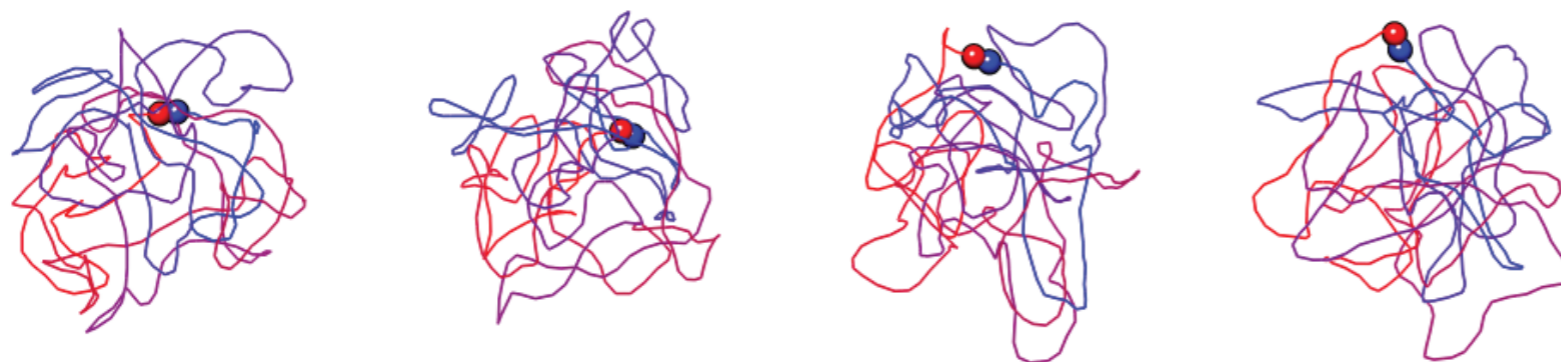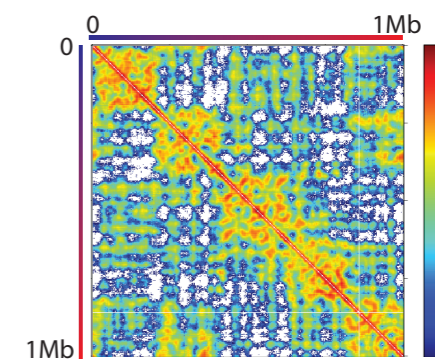
set 6 (△ts=10⁶)
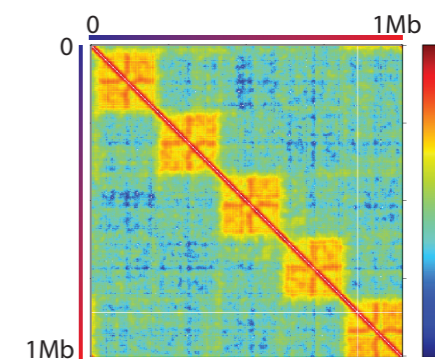
Reconstructing toy models
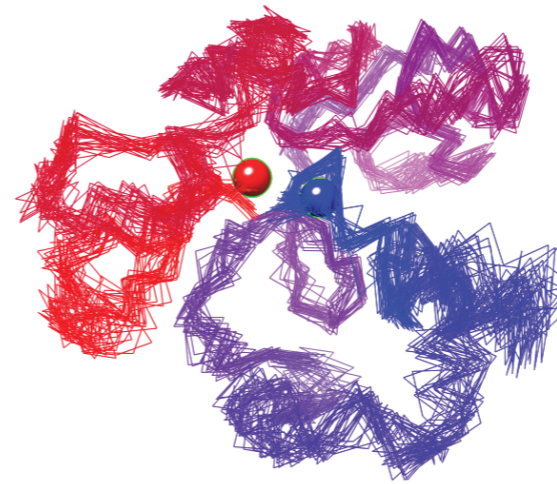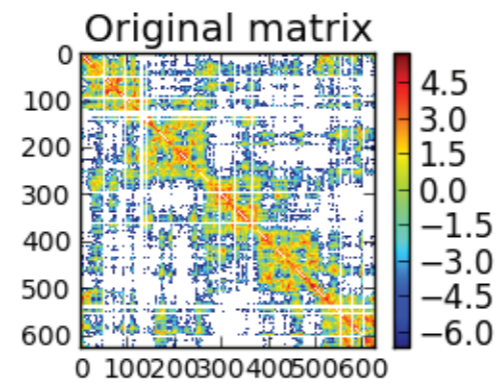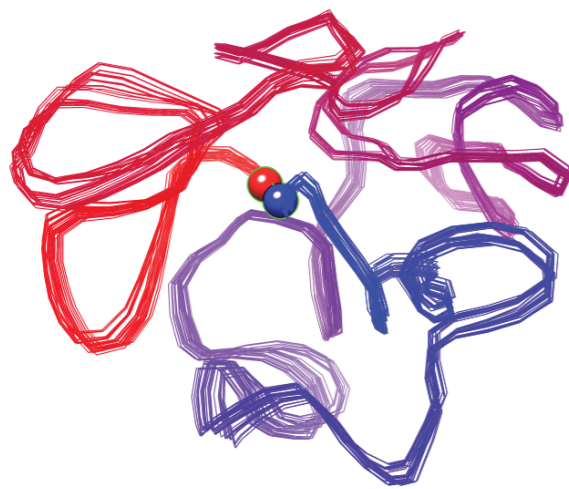
chr40_TAD
α=100
Δts=10
TADbit-SCC: 0.91
<dRMSD>: 32.7 nm
<dSCC>: 0.94
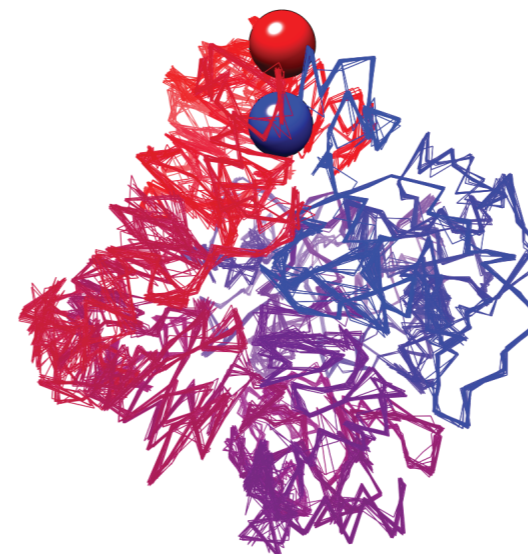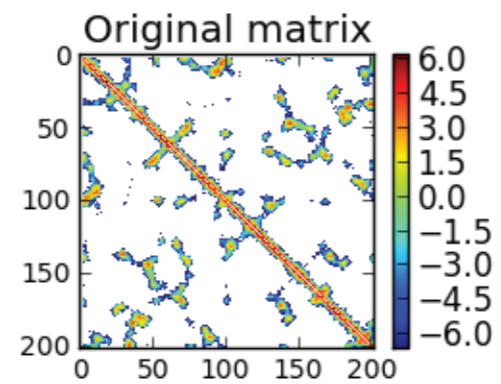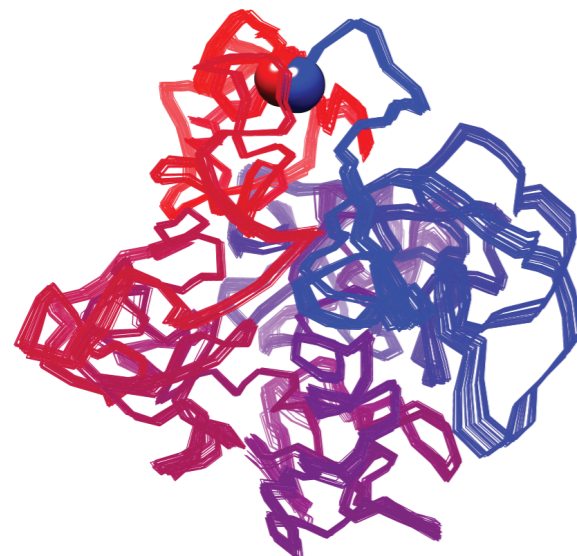
chr150_TAD
α=50
Δts=1
TADbit-SCC: 0.82
<dRMSD>: 45.4 nm
<dSCC>: 0.86

Noise is "OK"

- noise level +

<dRMSD> (nm)

TADbit-SCC

r = -0.67
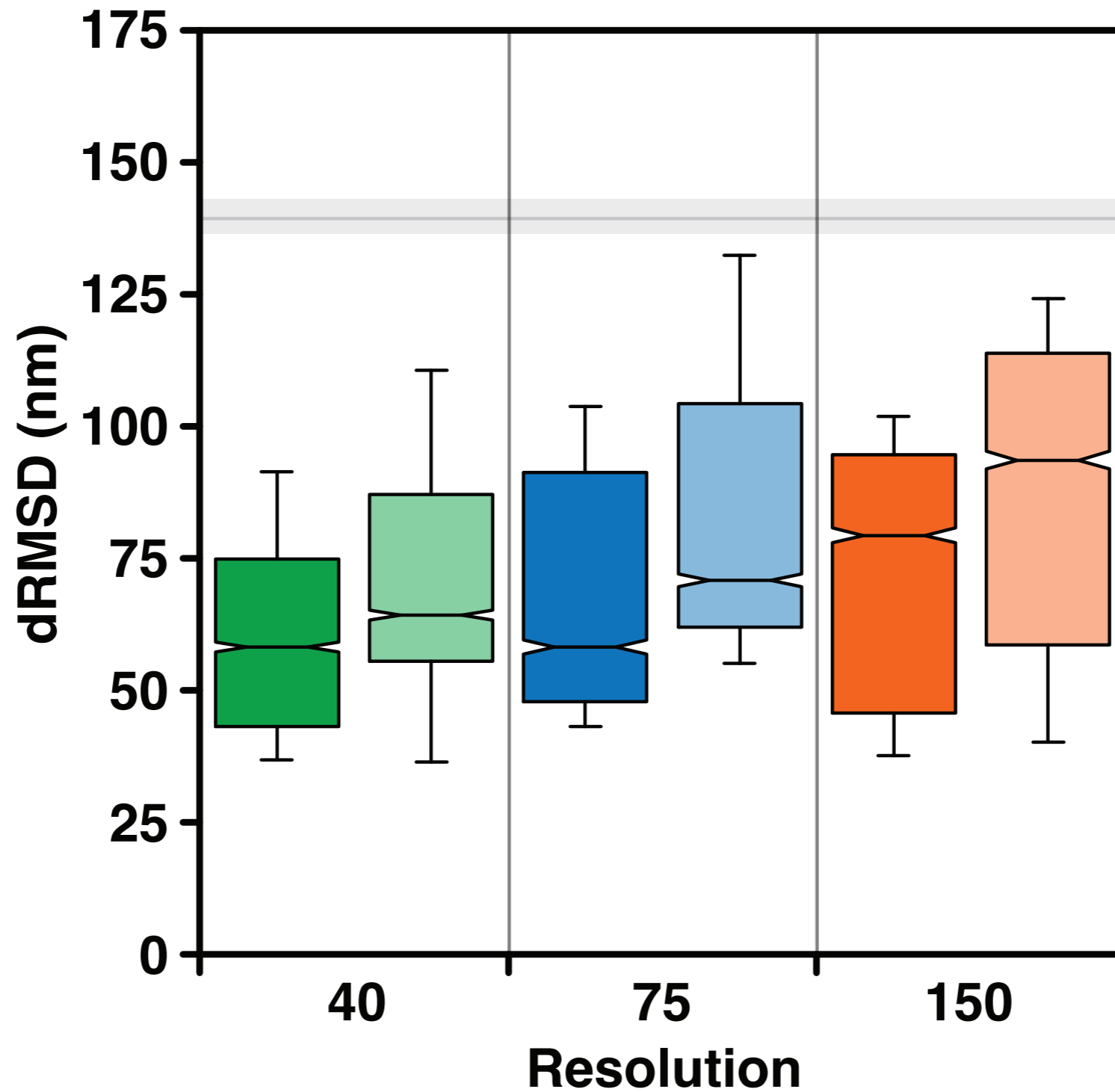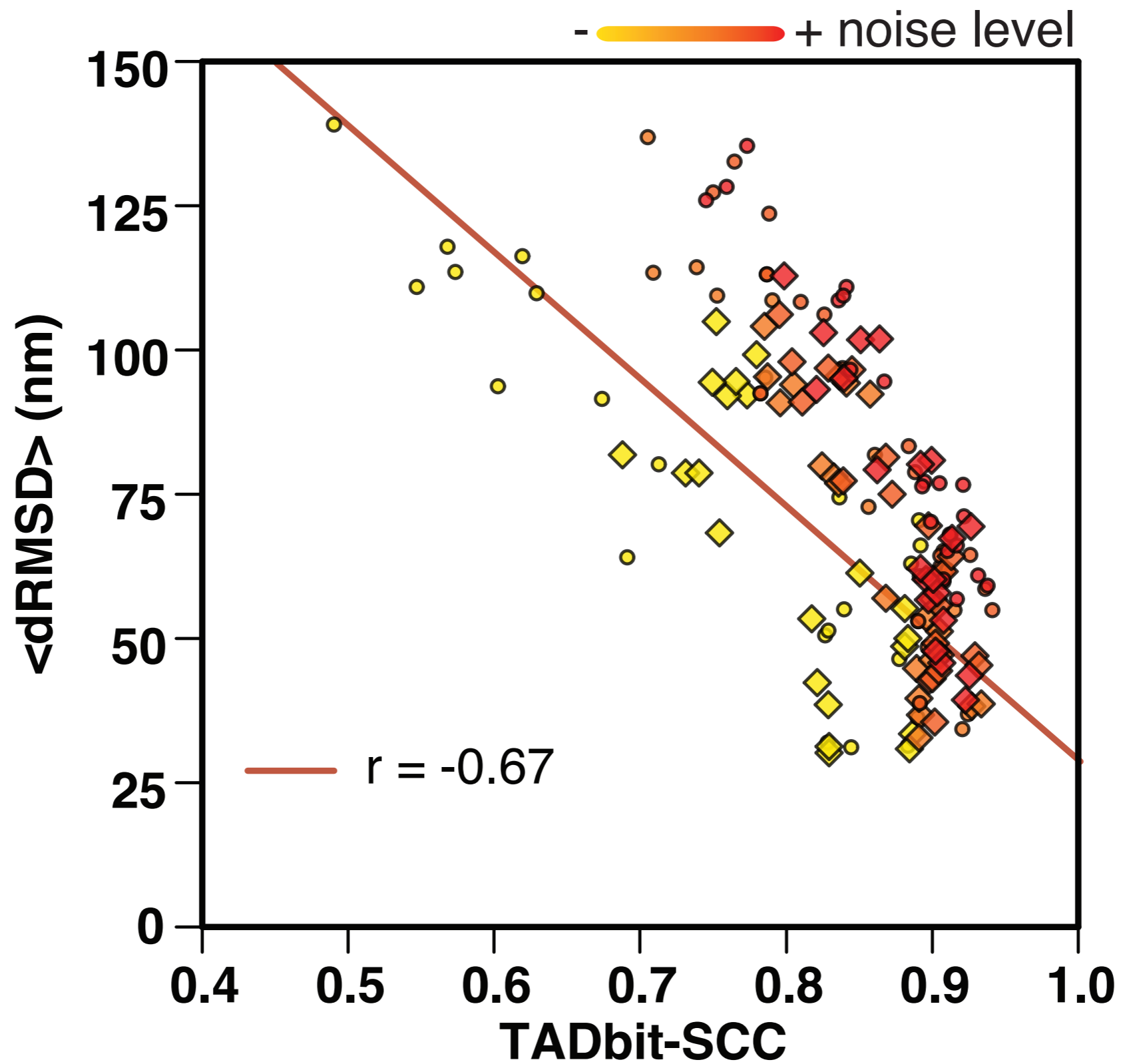
Structural variability is "NOT OK"

Can we predict the accuracy of the models?

Toy genome: chr40_TAD
Density: 40 bp/nm
TADs: Yes
Noise: 150
Δts: 10^0

% Sig. Cont. EV: 32.3
Skewness: -0.32
Kurtosis: -0.69
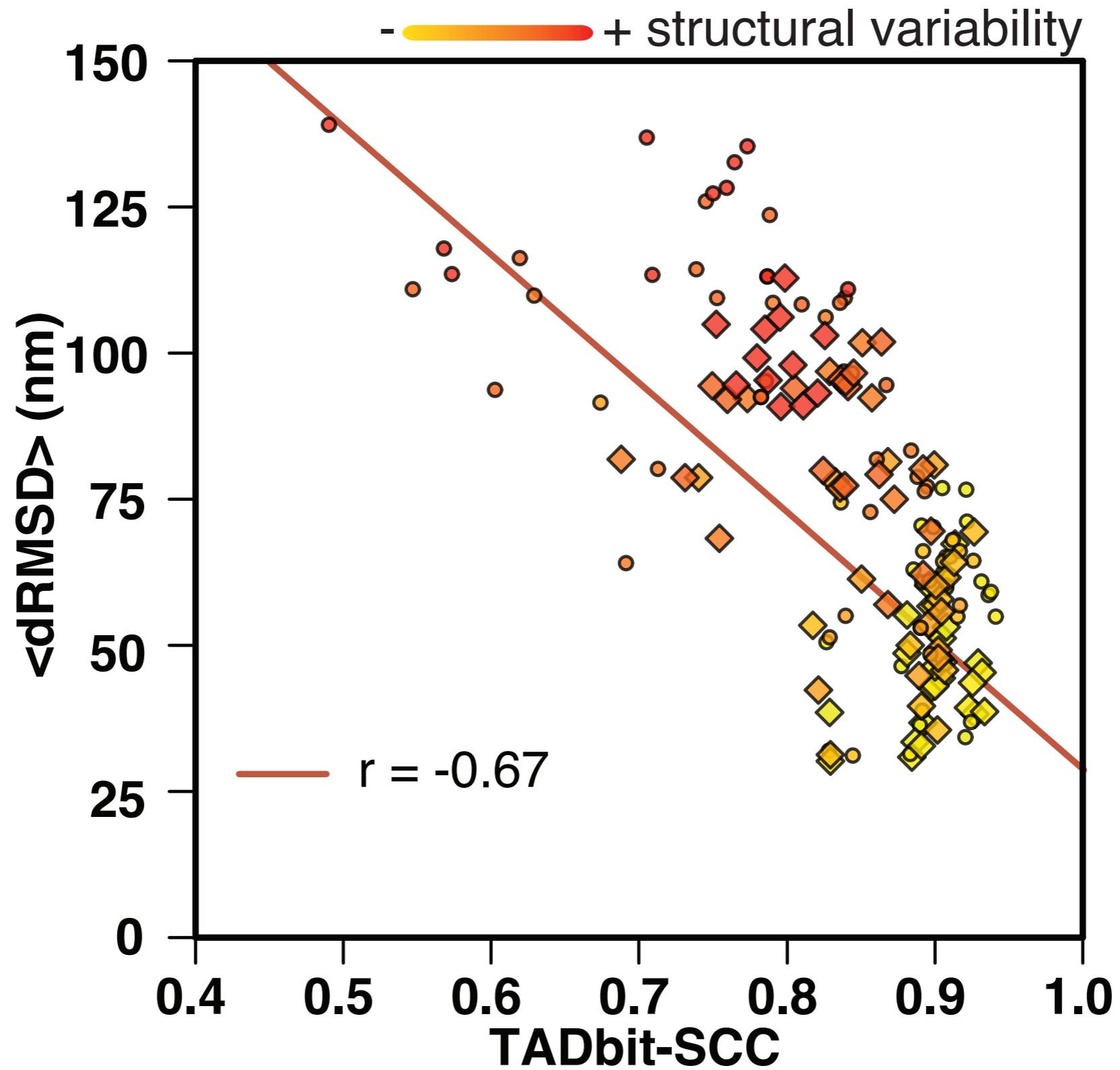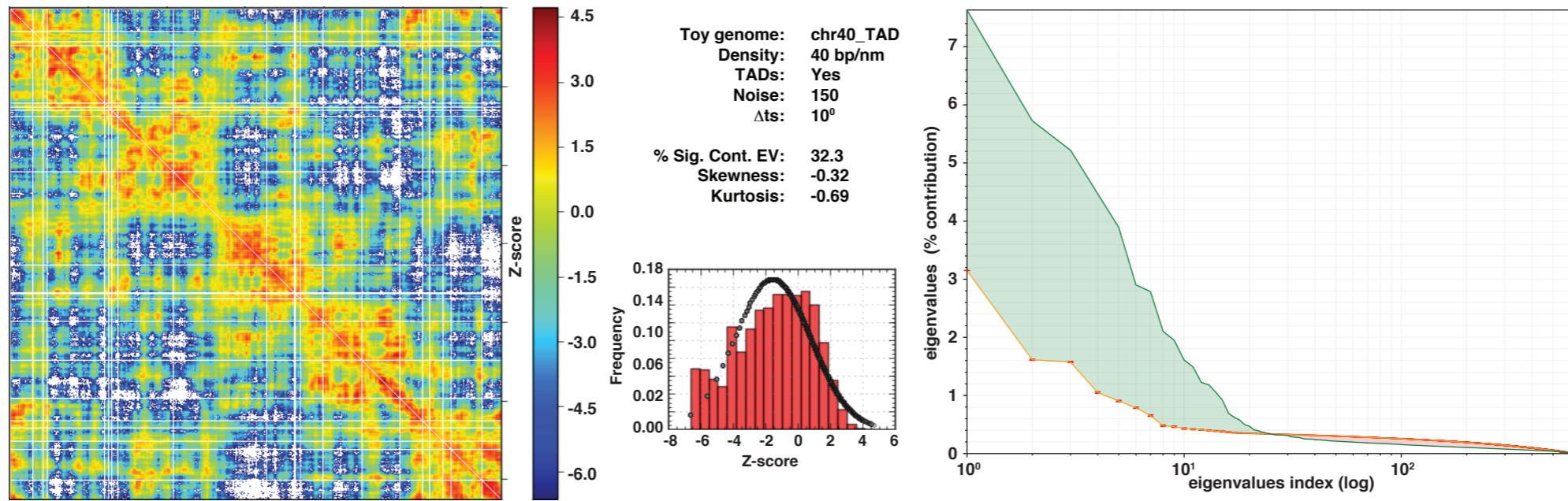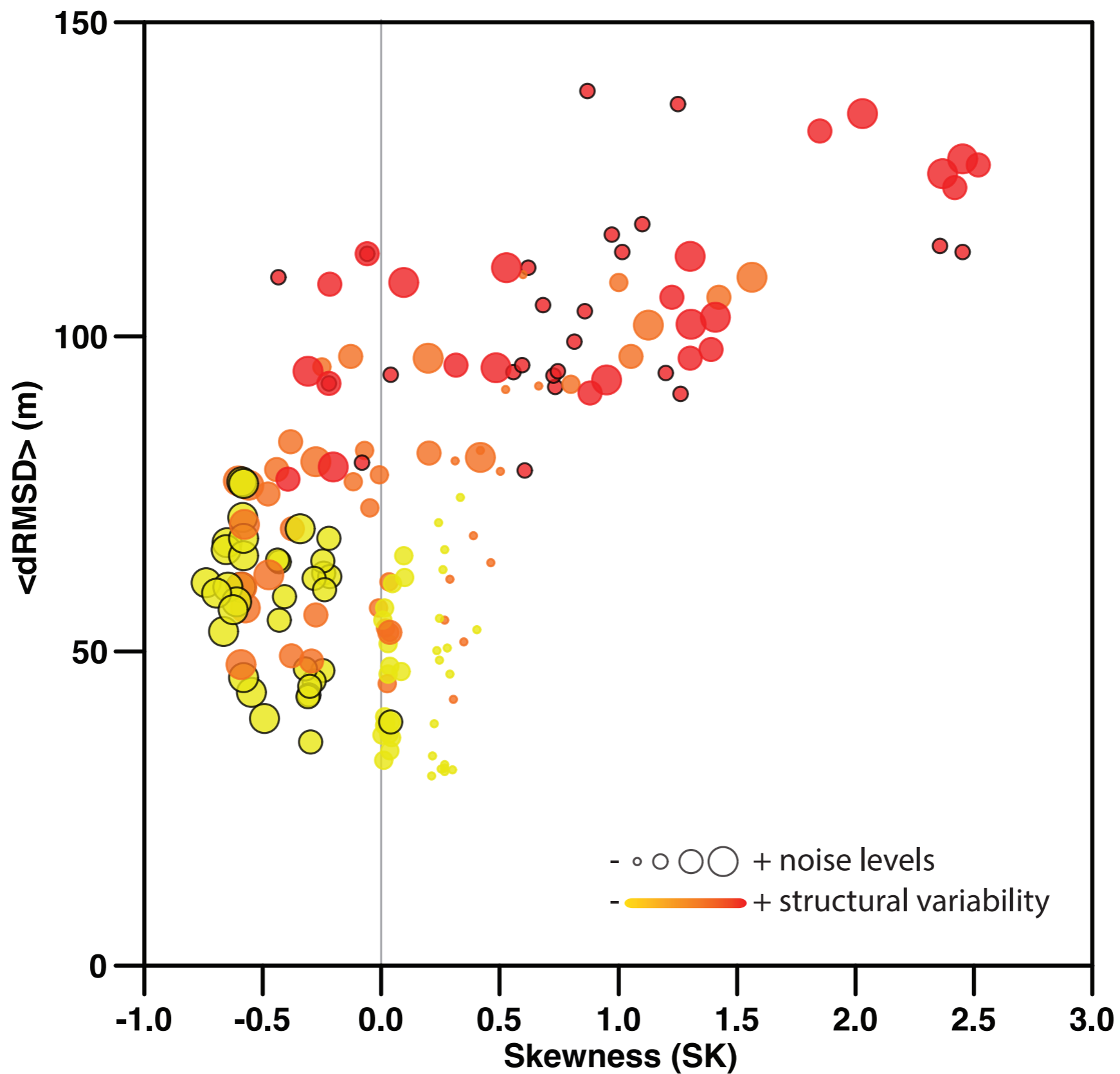
Can we predict the accuracy of the models?
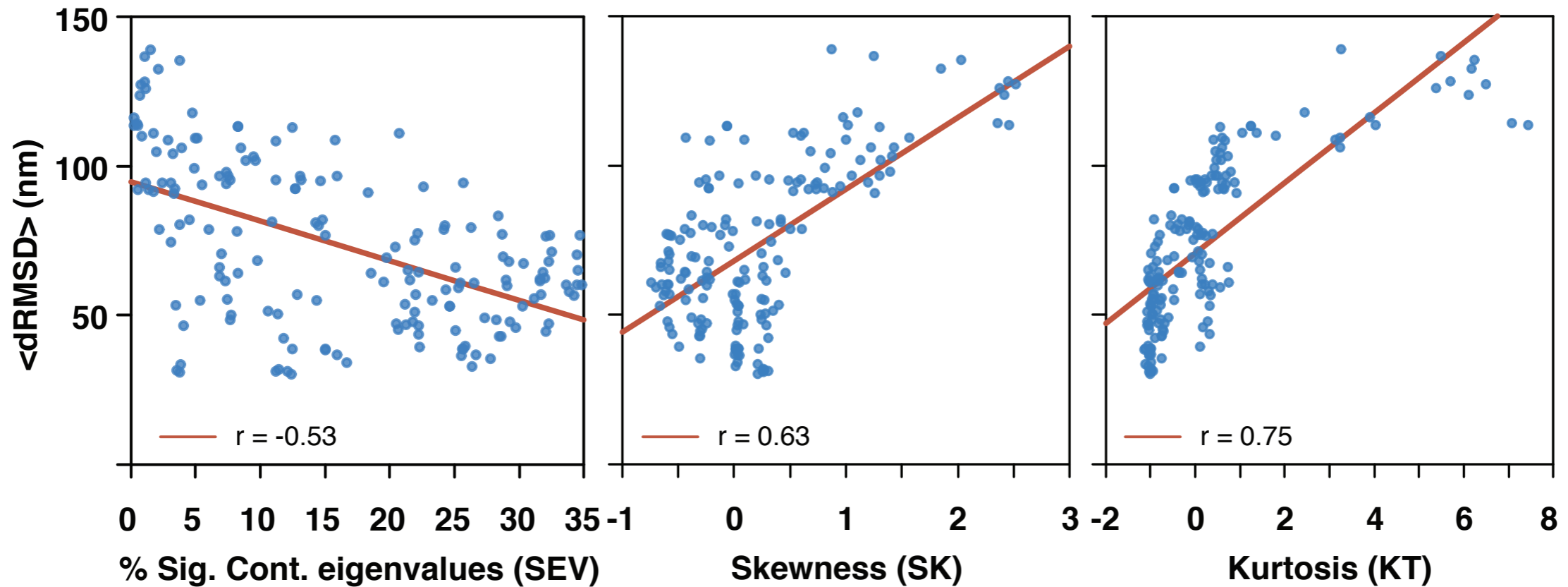
$$MMP = -0.0002 * \text{Size} + 0.0335 * \text{SK} - 0.0229 * KU + 0.0069 * \text{SEV} + 0.8126$$

Human Chr1:120,640,000-128,040,000

Size: 186
SEV: 3.63
SK: 0.20
KT: -0.53
MMP: 0.82

Higher-res is "good"

put your $$ in sequencing

Noise is "OK"

no need to worry much

Structural variability is "NOT OK"

homogenize your cell population!

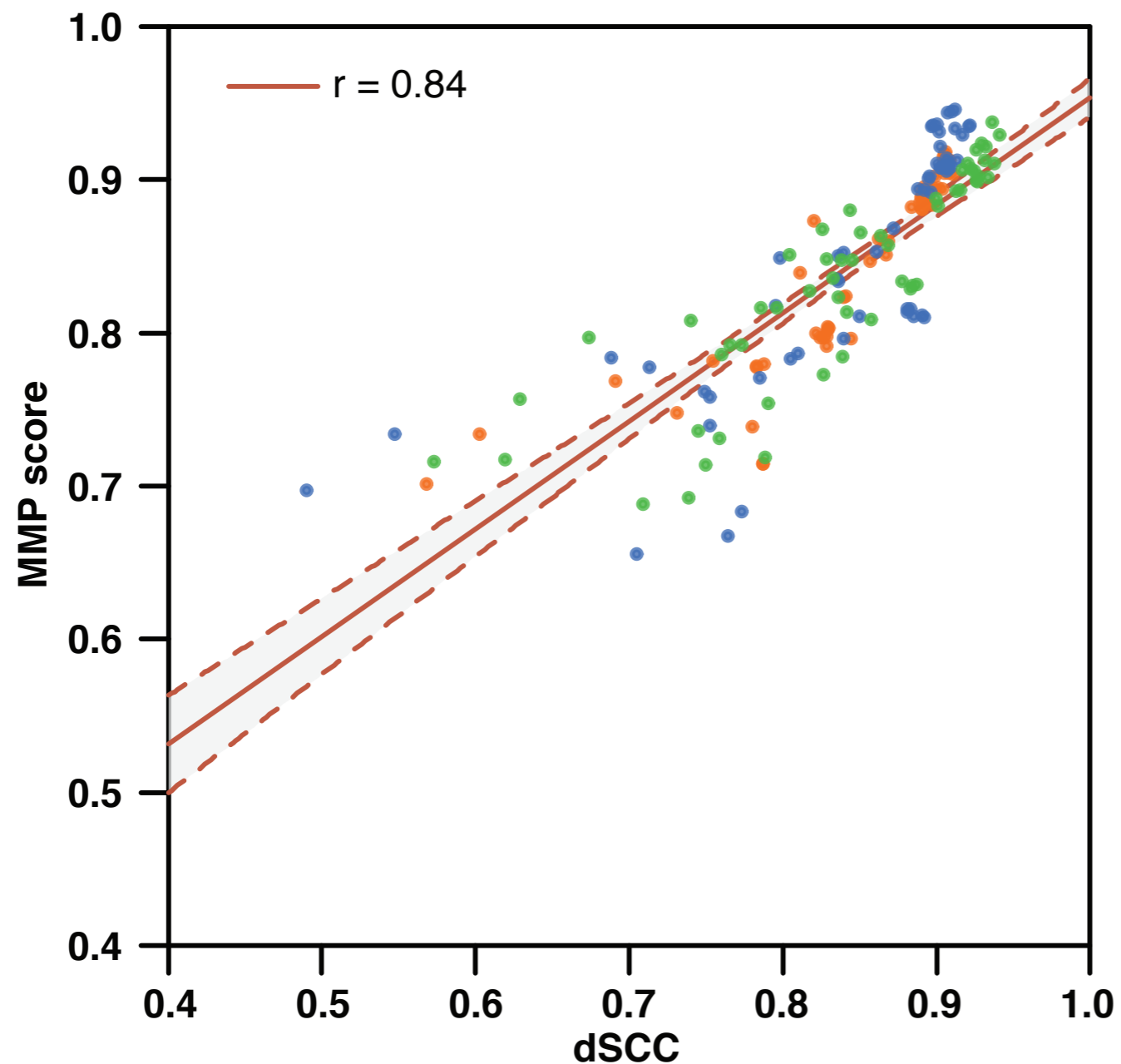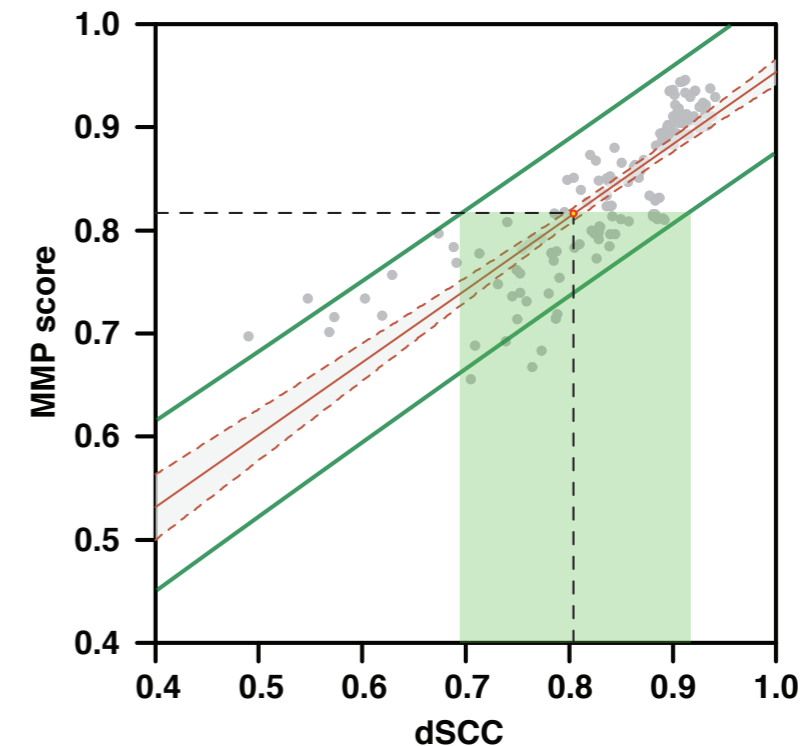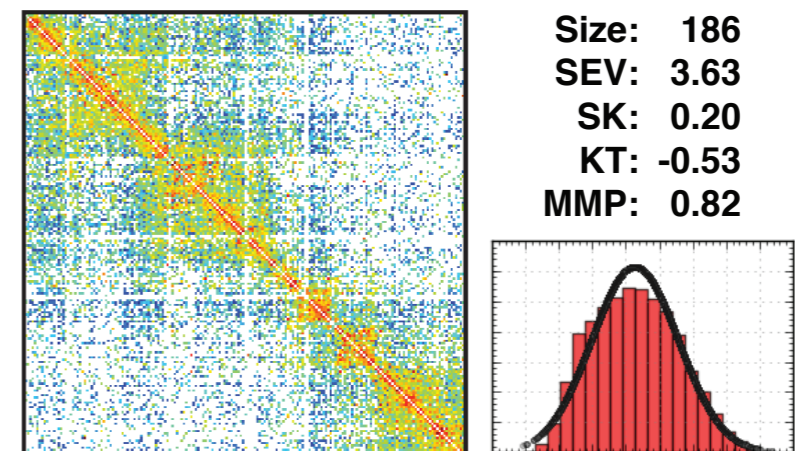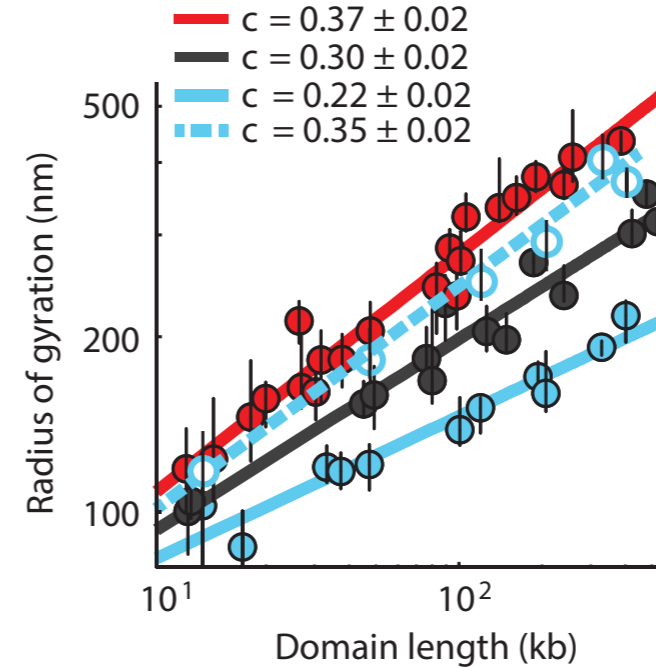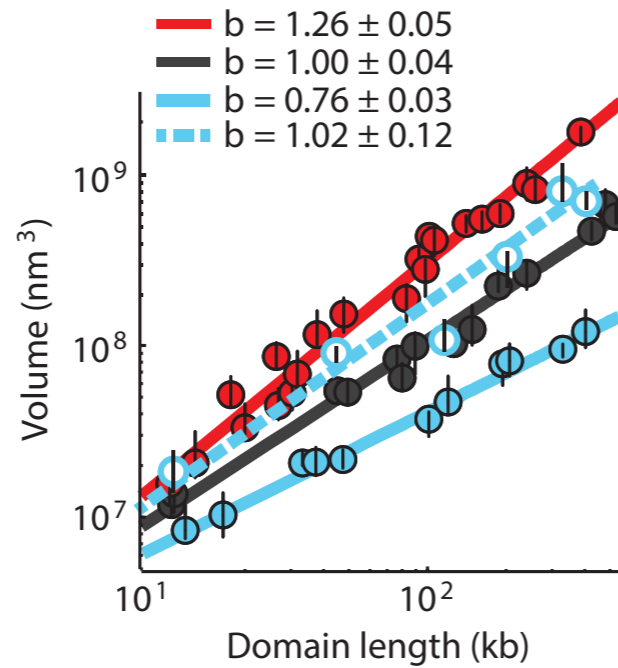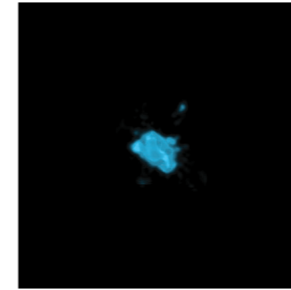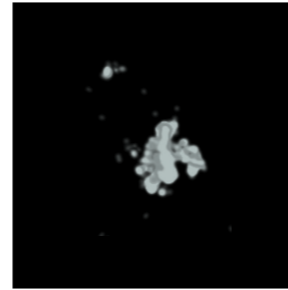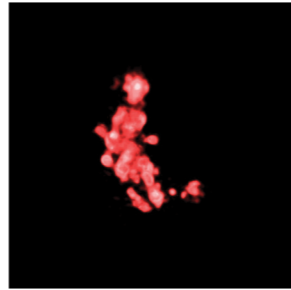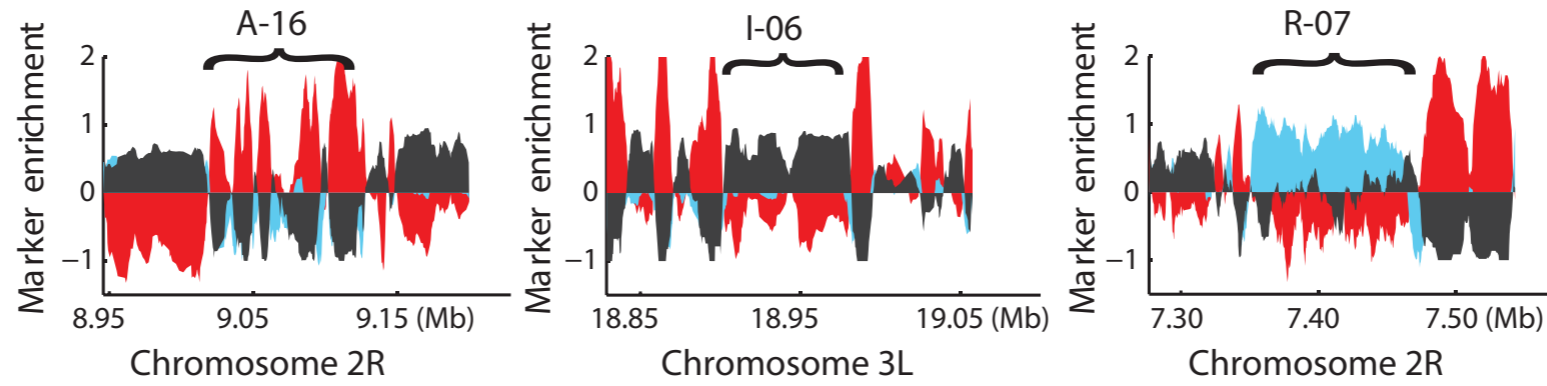…but we can differentiate between noise and structural variability

and we can a priori predict the accuracy of the models

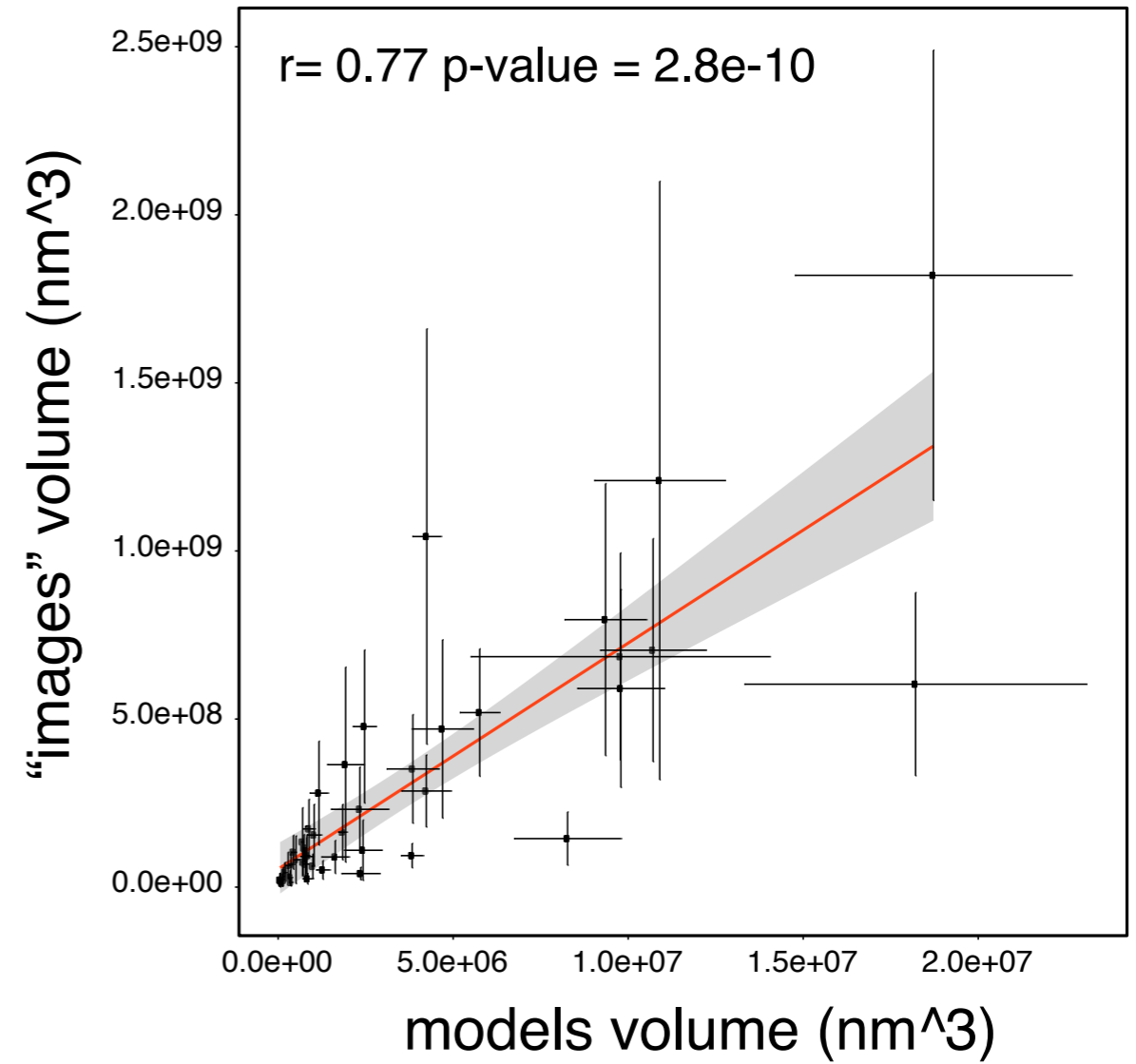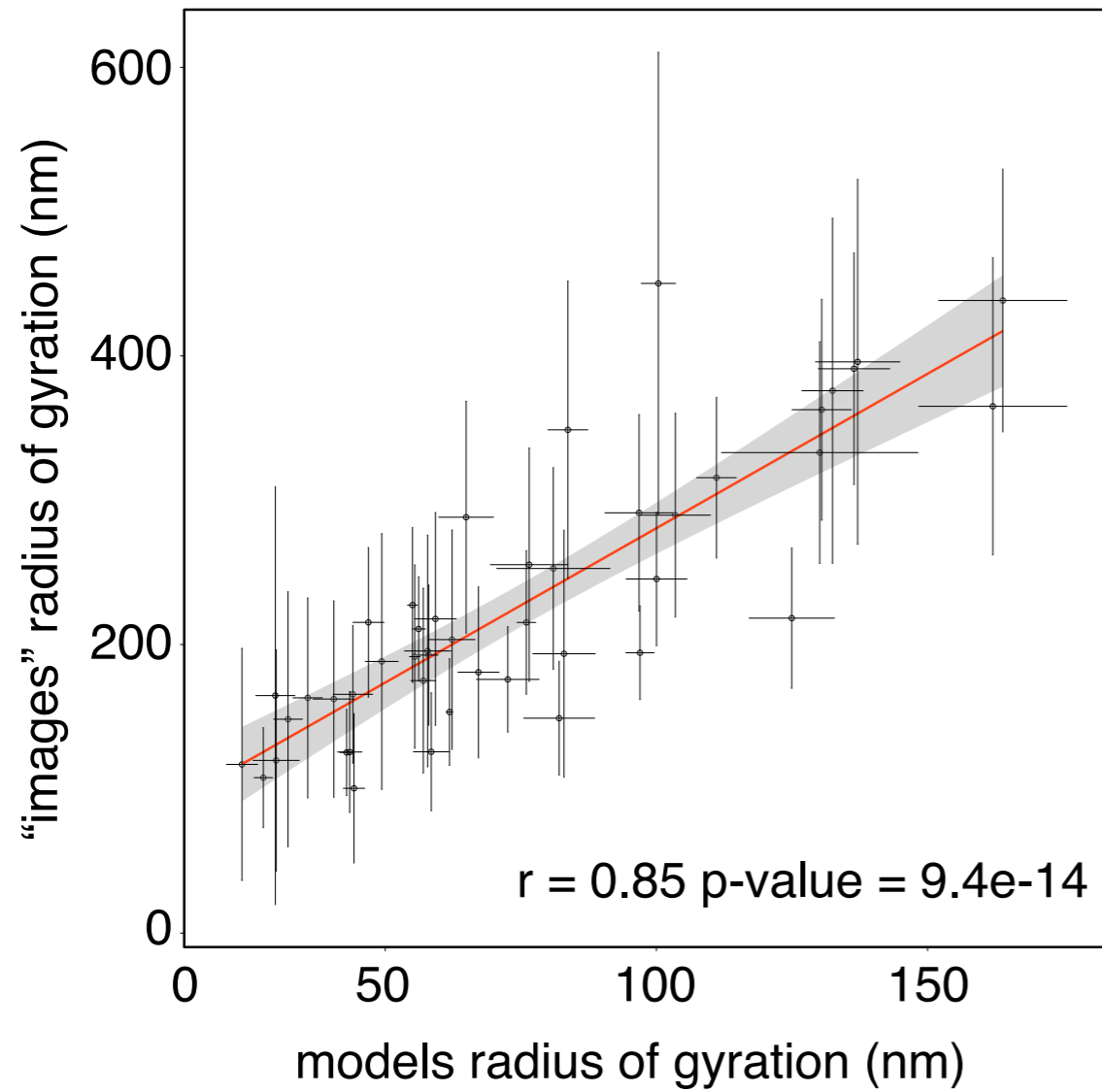But… what about direct validation of models?

Model accuracy

Boettiger, A. N., et al. (2016). Nature, 529, 418–422.

A-16
Marker enrichment
8.95   9.05   9.15 (Mb)
Chromosome 2R

I-06
Marker enrichment
18.85   18.95   19.05 (Mb)
Chromosome 3L

R-07
Marker enrichment
7.30   7.40   7.50 (Mb)
Chromosome 2R

b = 1.26 ± 0.05
b = 1.00 ± 0.04
b = 0.76 ± 0.03
b = 1.02 ± 0.12

Volume (nm³)
10^9
10^8
10^7
10^1 10^2
Domain length (kb)

c = 0.37 ± 0.02
c = 0.30 ± 0.02
c = 0.22 ± 0.02
c = 0.35 ± 0.02

Radius of gyration (nm)
500
200
100
10^1 10^2
Domain length (kb)

● Active   ● Inactive   ● Repressed   ○ Repressed (Ph KD)

Model accuracy (fly@2Kb)

Boettiger, A. N., et al. (2016). Nature, 529, 418–422.

r = 0.85 p-value = 9.4e-14

r= 0.77 p-value = 2.8e-10

Model accuracy (Human Chr21@40Kb)

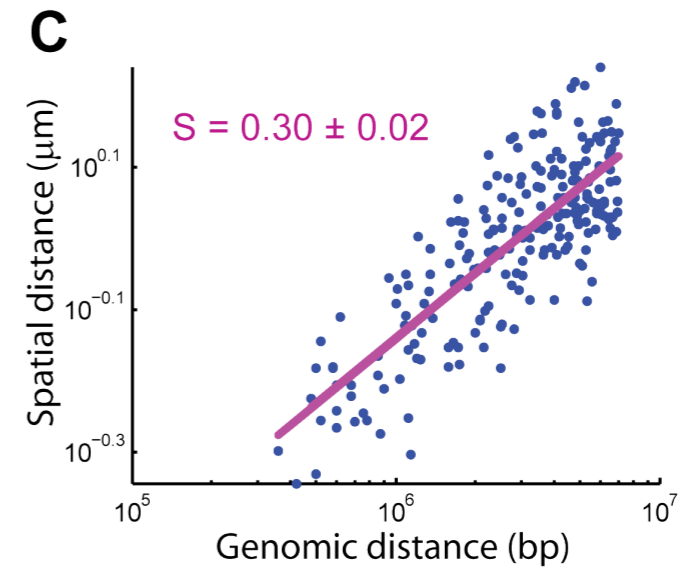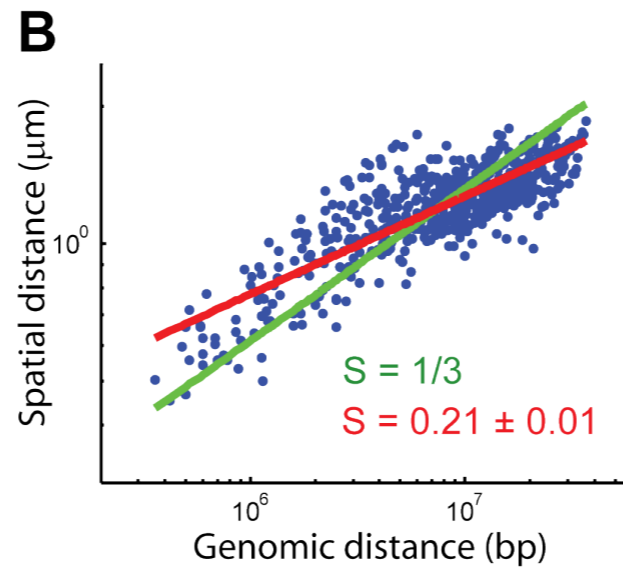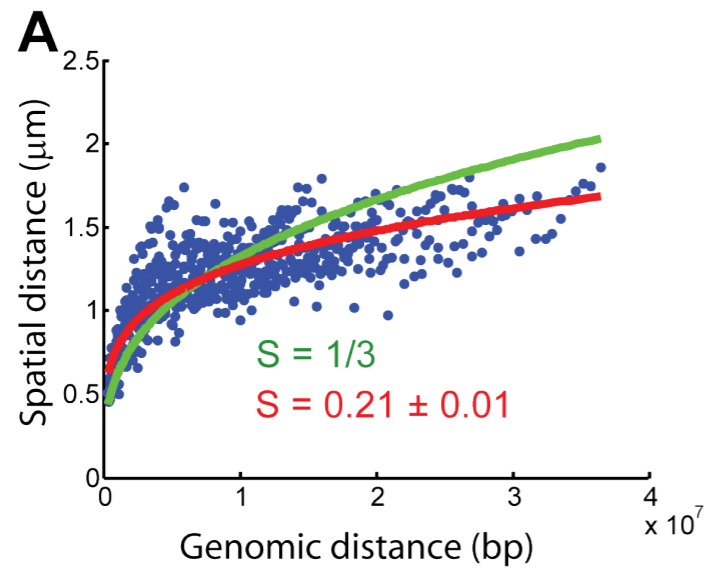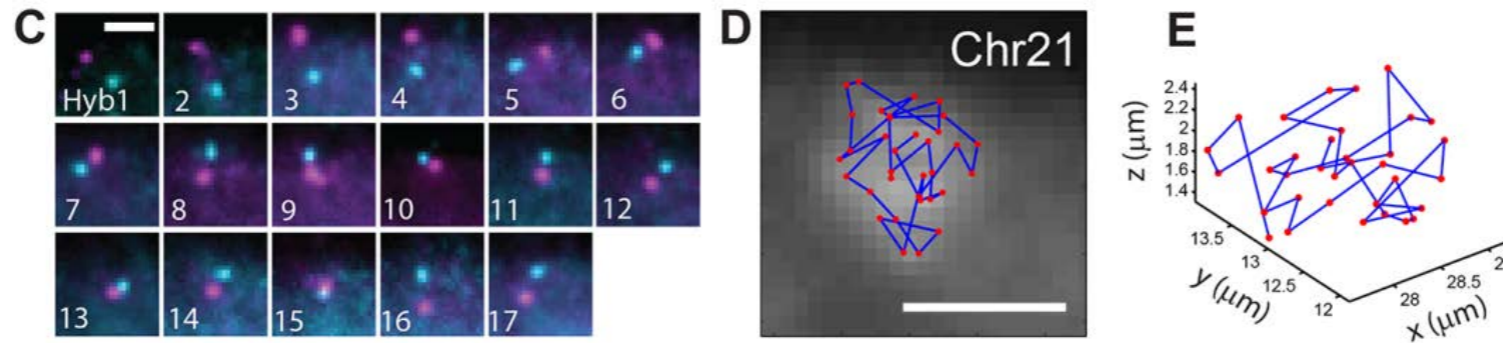Fig. 1. Mapping the spatial organization of the central 100-kb regions of all 34 TADs in chromosome 21 (Chr21) of IMR90 cells. A
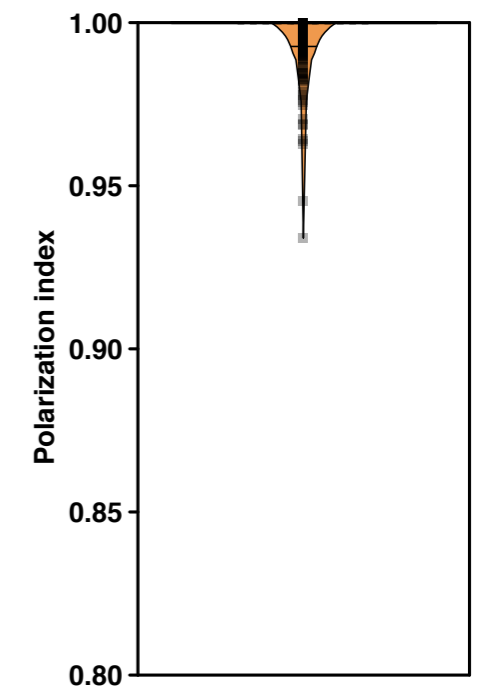
Model accuracy (Human Chr21@40Kb)

Wang, S., et al. (2016). Science 353(6299), 598–602.

Fig. 2. Spatial organization of compartments in individual chromosomes of Chr21.

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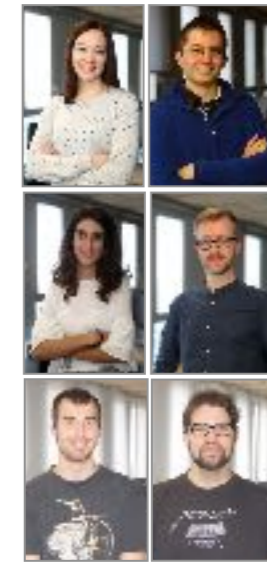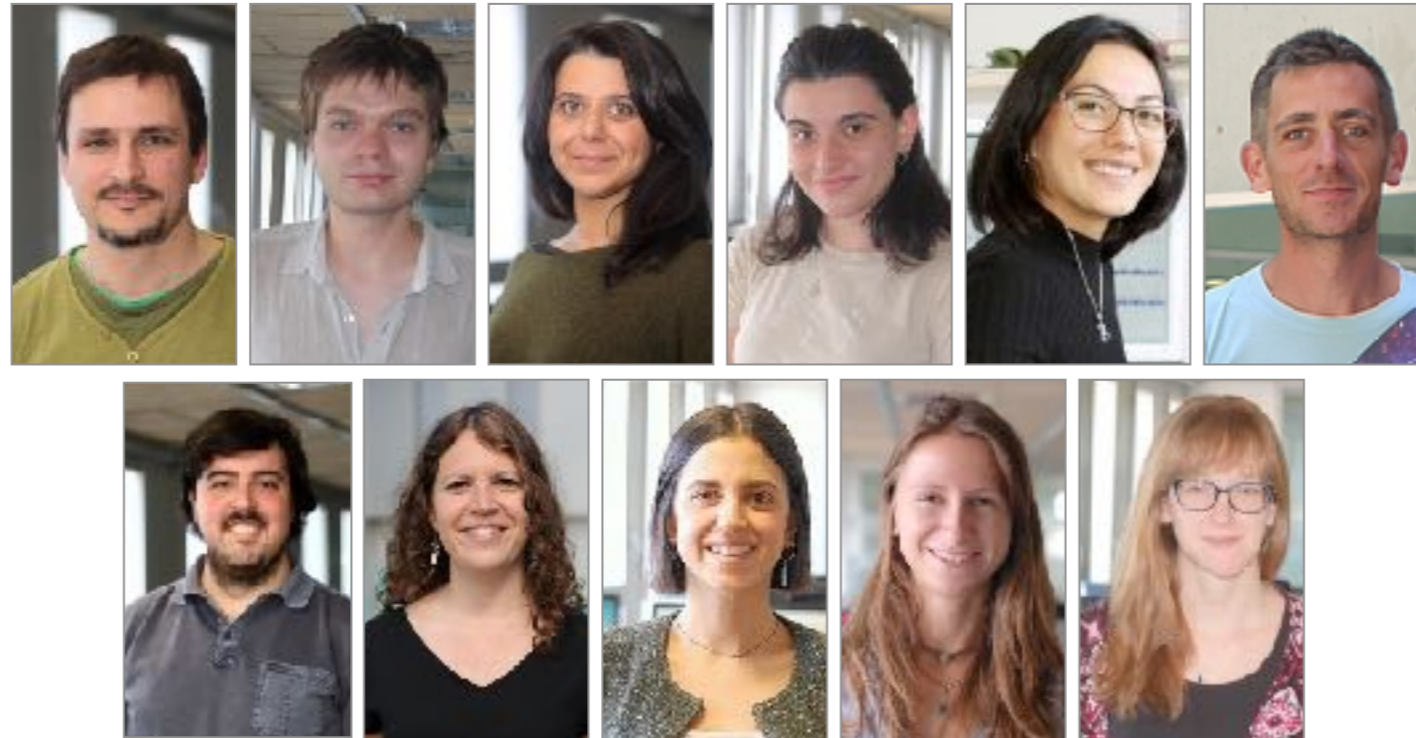http://3DGenomes.org
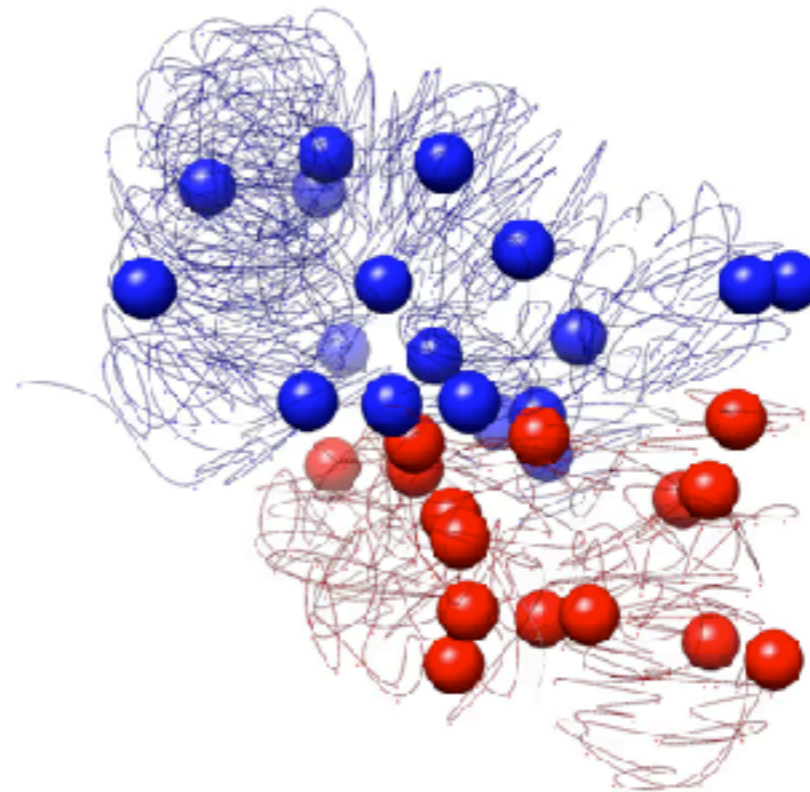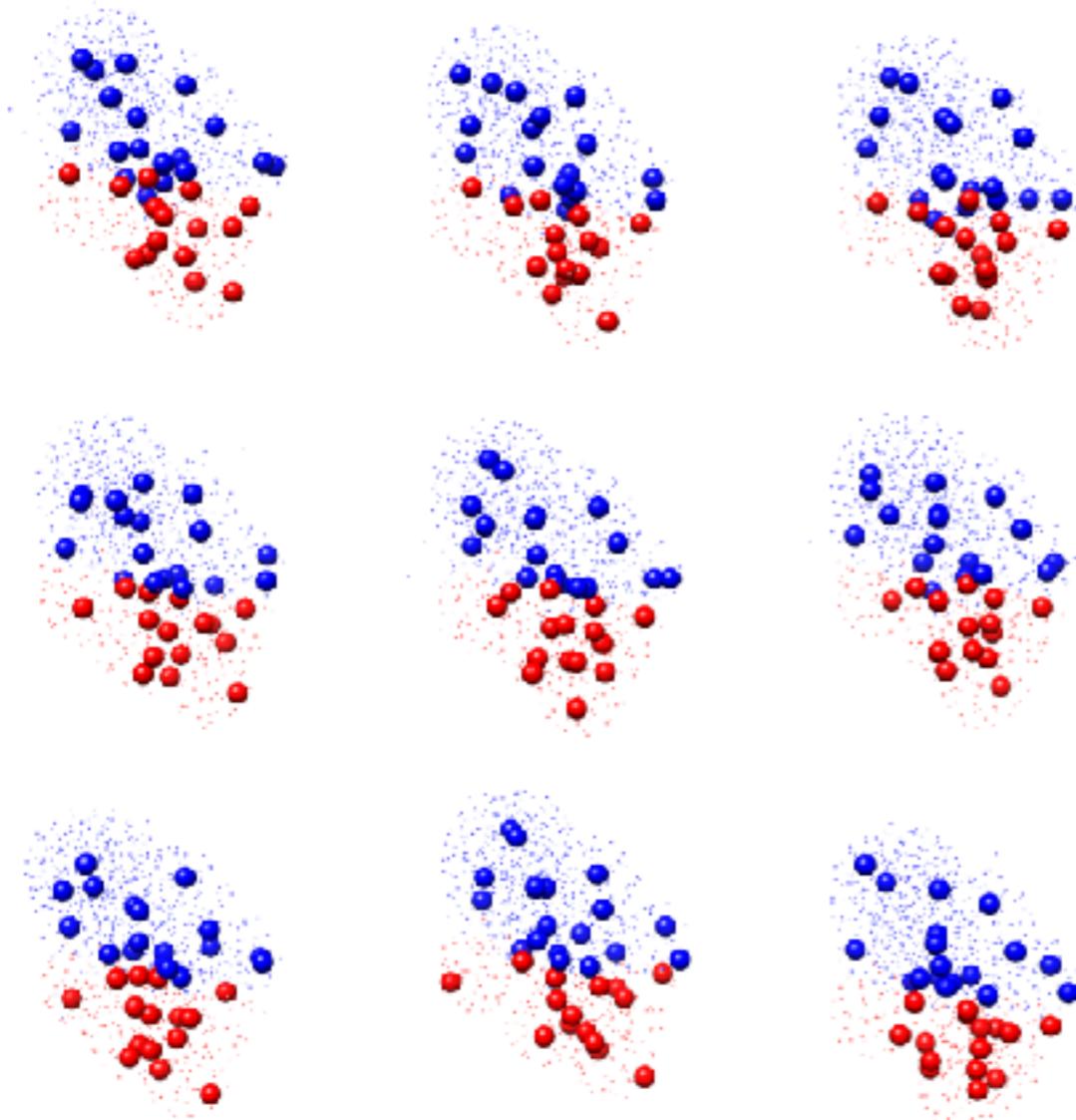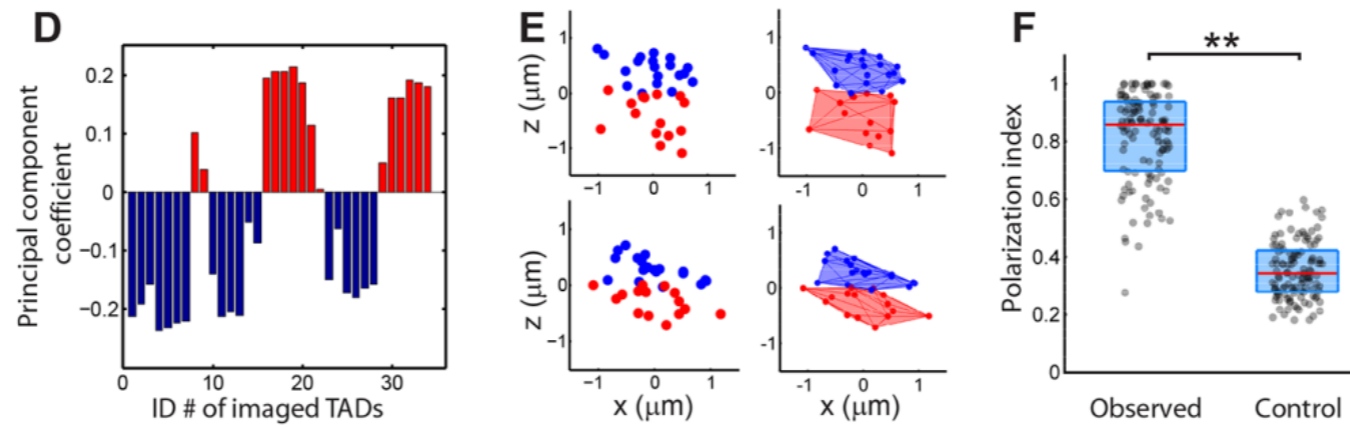
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@mamartirenom

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