Modeling 3D genomes:

Hormone induced changes in the structure of genomic Topologically Associating Domains (TADs)

Marc A. Marti-Renom
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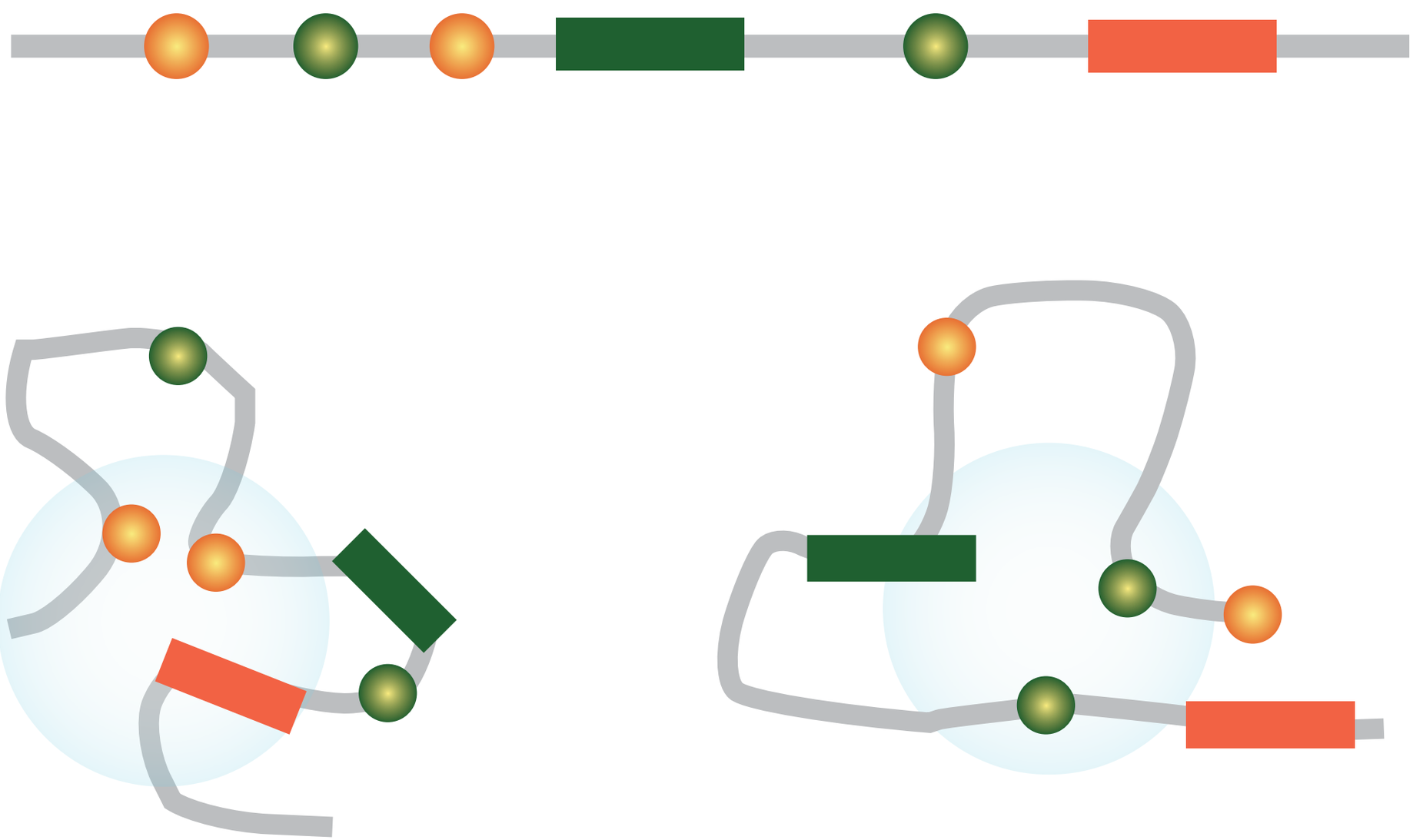
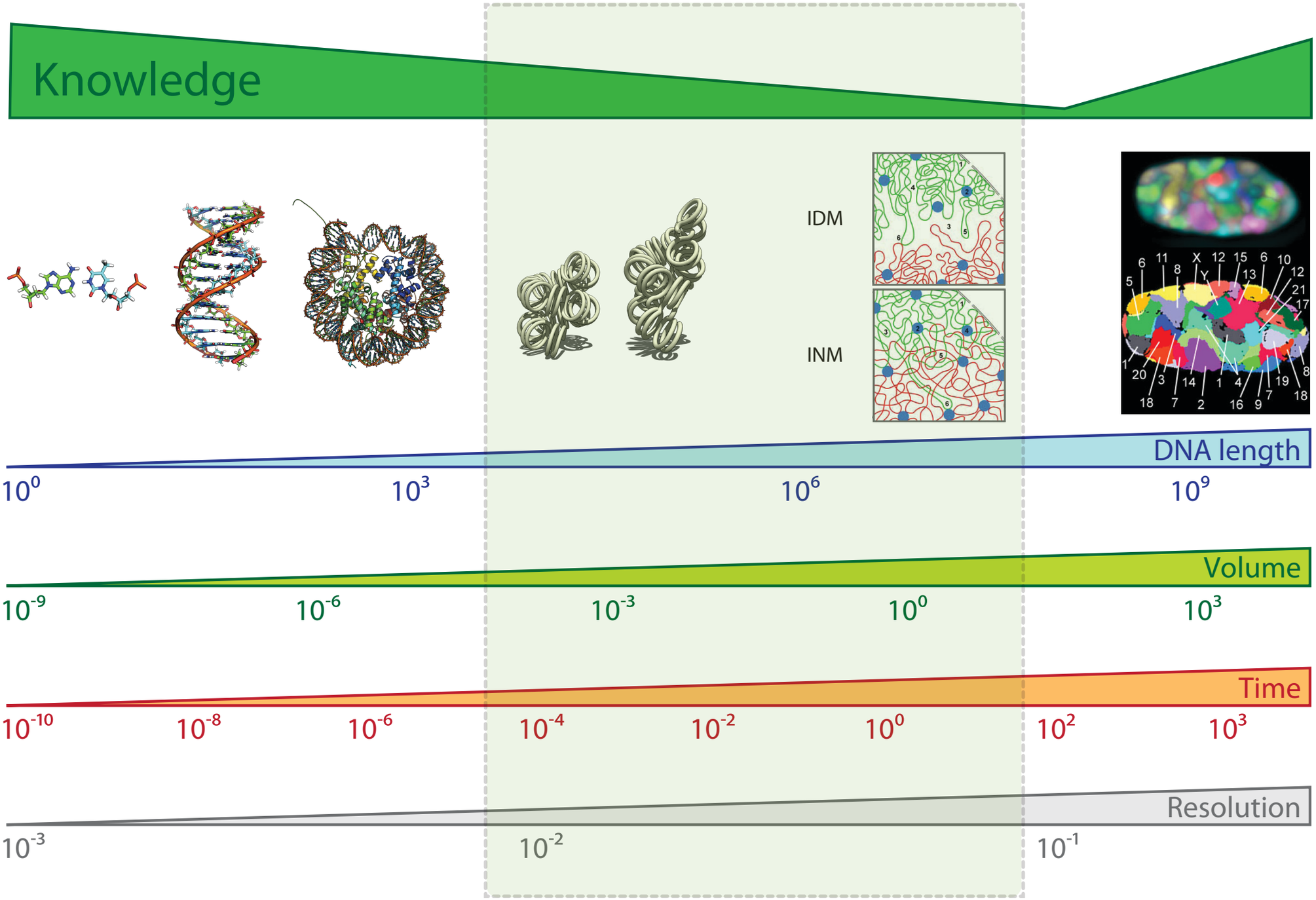
Complex genome organization



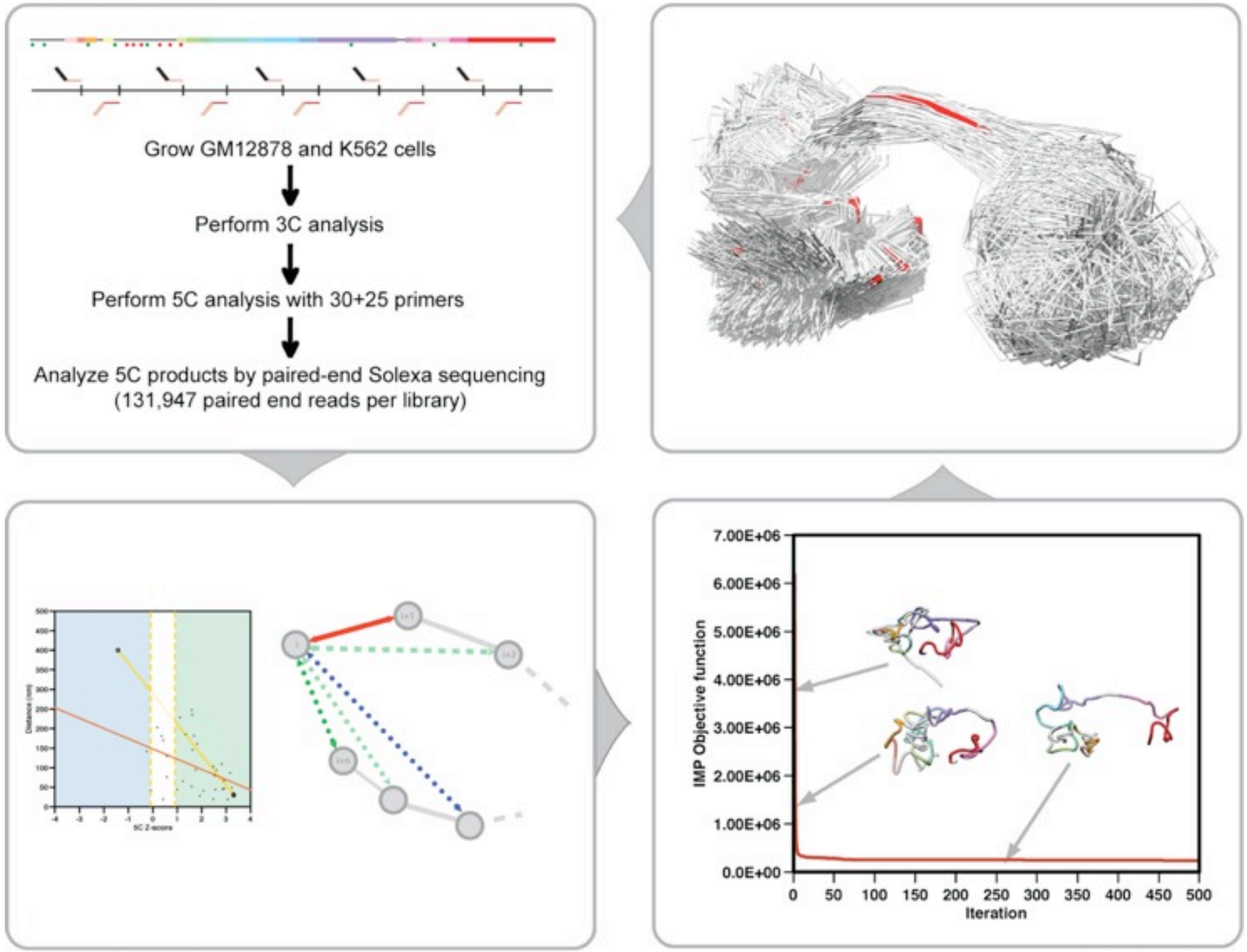
Resolution Gap

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



cnag CRG

Experiments

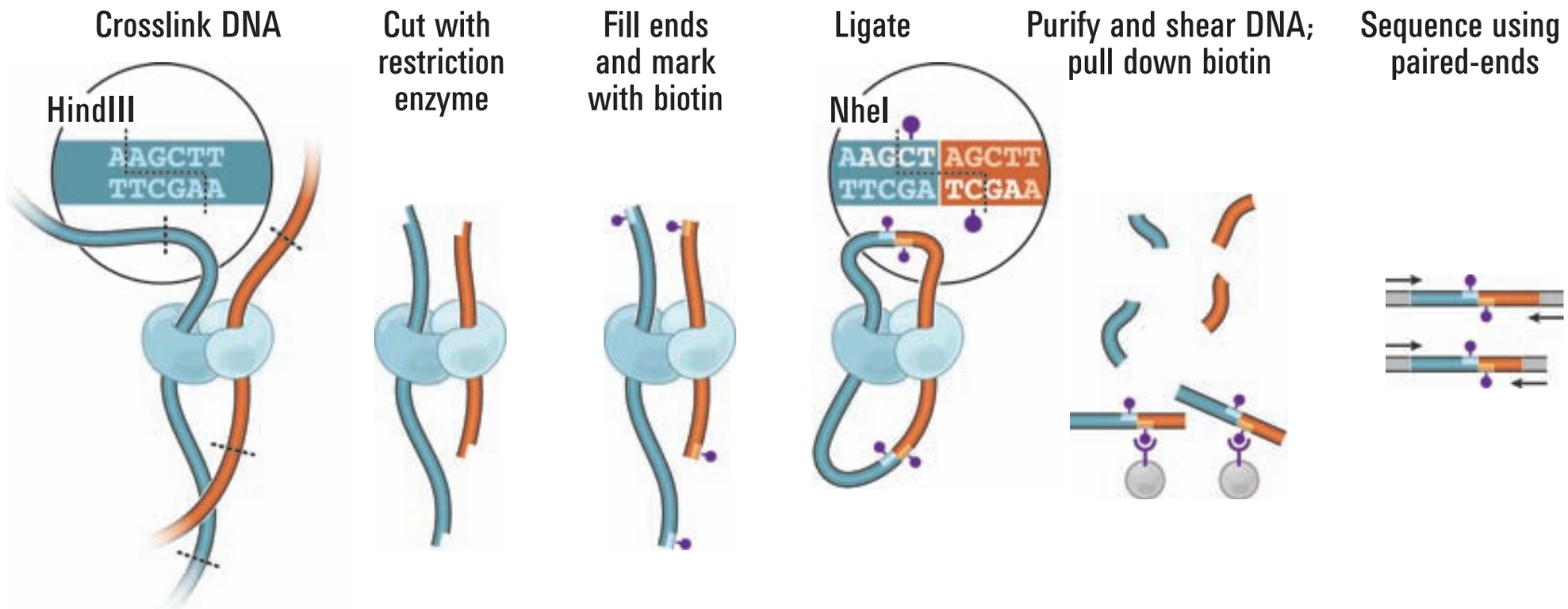


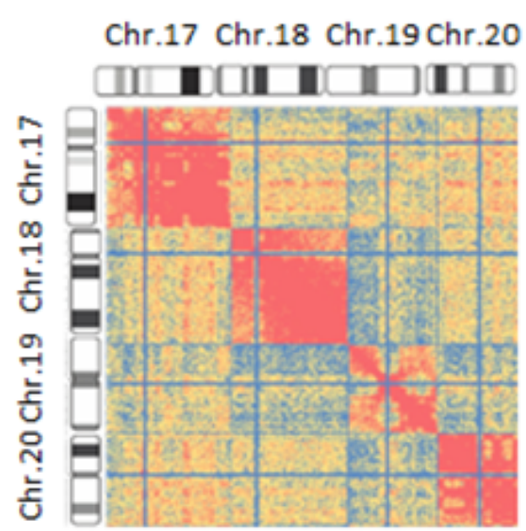
Computation

Hi technology

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

http://3dg.umassmed.edu





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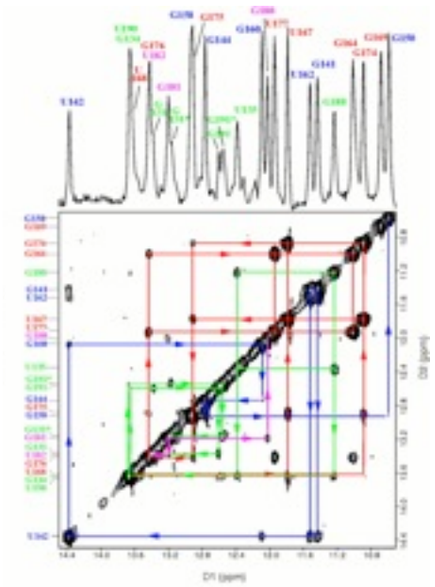
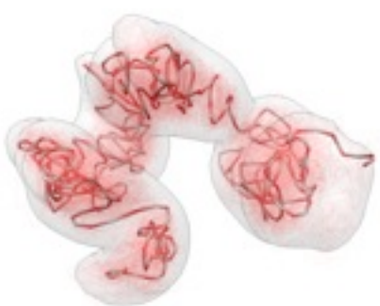
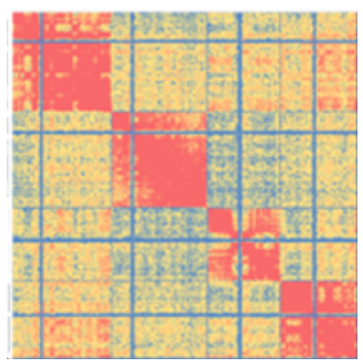


Biomolecular structure determination

2D-NOESY data



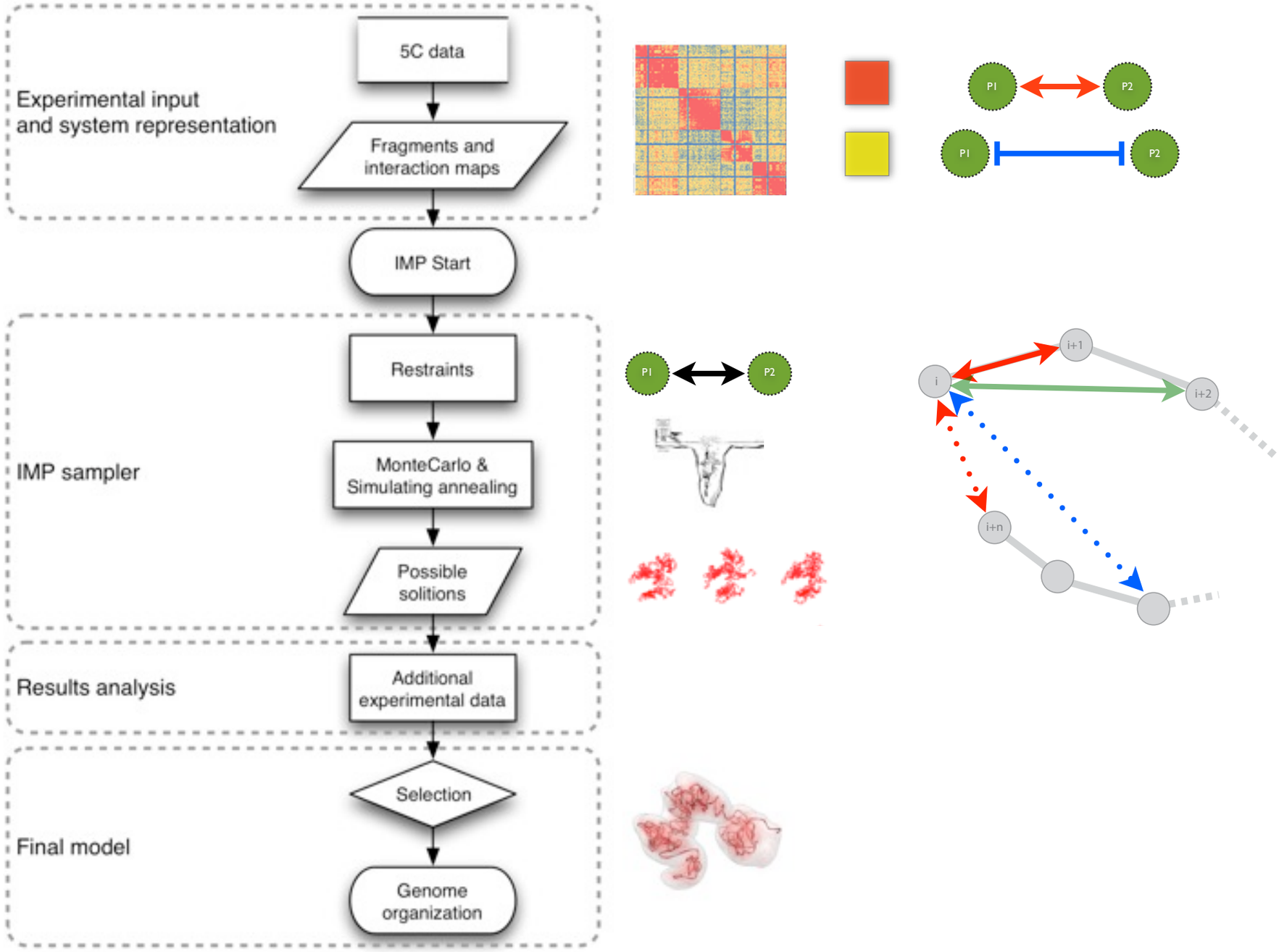


Chromosome structure determination

3C-based data

Integrative Modeling

http://www.integrativemodeling.org



Previous applications...

Baù, D. et al. Nat Struct Mol Biol (2011).
Umbarger, M. A. et al. Mol Cell (2011).

TECHNICAL REPORTS

nature structural & molecular biology

The three-dimensional folding of the α-globin gene domain reveals formation of chromatin globules

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We developed a general approach that combines chromosome conformation capture carbon copy (3C) with the Integrated Modeling Platform (IMP) to generate high-resolution three-dimensional models of chromatin at the megabase scale. We applied this approach to the ENm008 domain on human chromosome 16, containing the α-globin locus, which is expressed in K562 cells and silenced in lymphoblastoid cells (GM12878). The models accurately reproduce the known looping interactions between the α-globin genes and their regulatory elements in K562 cells. We also used our approach that the domain folds into a single globular conformation in GM12878 cells, whereas two globules are formed in K562 cells. The central cores of these globules are enriched for transcribed genes, whereas nontranscribed chromatin is more peripheral. We propose that globule formation represents a higher-order folding state related to clustering of transcribed genes around shared transcription machineries, as previously observed by microscopy.

Currently, efforts are directed at producing high-resolution genome sequences and at mapping the positions of functional elements or specific chromatin states are mapped onto the linear genomic sequence[1]. However, these linear representations do not indicate functional or structural relationships between distant elements. For instance, recent insights suggest that widely spaced functional elements cooperate to regulate gene expression by engaging in long-range chromatin looping interactions. The three-dimensional (3D) organization of chromosomes is thought to facilitate compartmentalization[2,3], chromatin organization[4] and spatial sequestration of genes and their regulatory elements[5–7], which may contribute to the regulation of the functional state of the genome. A general approach for determining the spatial organization of chromatin can aid in the identification of long-range relationships between genes and distant regulatory elements as well as in the identification of higher-order folding principles of chromatin in general.

Chromosome conformation capture (3C)-based assays are formaldehyde cross-linking followed by restriction digestion and intramolecular ligation to study chromatin looping interactions[8–11]. 3C-based assays have been used to show that specific elements such as promoters, enhancers and insulators are involved in the formation of chromatin loops[12,13,14–16]. The frequencies at which loci interact reflect chromatin folding[17–21], and thus comprehensive chromatin interaction data sets can help researchers build spatial models of chromatin.

Previously, chromatin conformation has been modeled using polymer models[6,22] and molecular dynamics simulations[23], which have proven valuable for understanding general features of chromatin folding and dynamics. Nevertheless, these approaches have generally not yet partially leverage the current wealth of experimental data on chromatin folding. Recently, experimentally driven approaches, in combination with computational modeling, have resulted in low-resolution models for the topological conformation of the immunoglobulin heavy chain[24], the *Hox*[25] loci and the yeast genome[26]. However, these methods were limited by the resolution and completeness of the input experimental data[26], by insufficient model representation, scoring and optimization[27], or by limited analysis of the 3D models[25].

To overcome such limitations, we developed a new approach that couples high-throughput 3C experiments[9] with the IMP[27]. We applied our approach to the human ENm008 region, a gene-dense domain of a 500-kilobase (kb) gene-dense domain located near the left telomere of human chromosome 16 (**Fig. 1a**). Embedded in this cluster of ubiquitously expressed housekeeping genes is the tissue-specific α-globin locus that is expressed only in erythroid cells. This 500-kb domain corresponds to the ENm008 region extensively studied by the ENCODE pilot project (**Fig. 1b**)[1].

The α-globin locus has been used widely as a model to study the mechanism of long-range and tissue-specific gene regulation[28–30]. Several cis-acting elements have been identified, these elements characterized by the presence of DNase I-hypersensitive sites (HSs) located 10 to 40 kb upstream of the ζ gene. One of these elements, HS40, is considered to be of particular importance[31–33]. This element can act as an enhancer in reporter constructs and its deletion greatly affects activation of the α-globin genes[31]. HS40 is bound by several erythroid transcription factors including GATA factors and NF-E2 (ref. 34). Notably, previous 3C studies have demonstrated direct long-range

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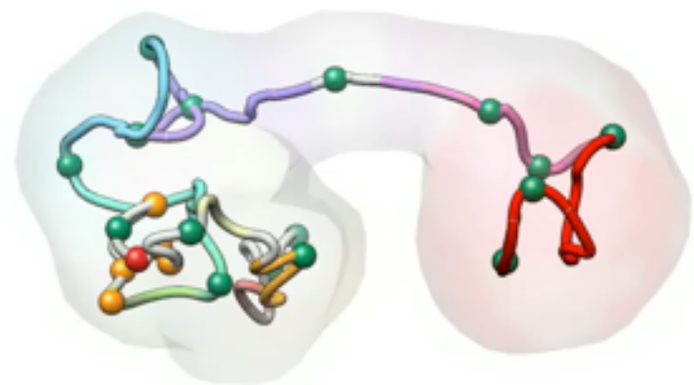

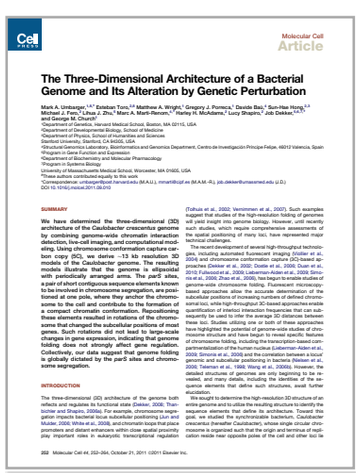

Cell PRESS

Molecular Cell
Article

The Three-Dimensional Architecture of a Bacterial Genome and Its Alteration by Genetic Perturbation

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DOI 10.1016/j.molcel.2011.08.010

SUMMARY

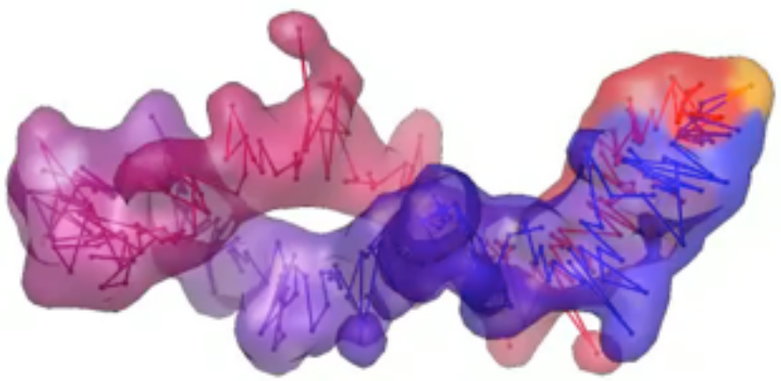
We have determined the three-dimensional (3D) architecture of the *Caulobacter crescentus* genome by combining genome-wide chromatin interaction detection, live-cell imaging, and computational modeling. Using chromosome conformation capture carbon copy (5C), we derive ~13 kb resolution 3D models of the *Caulobacter* genome. The resulting models illustrate that the genome is ellipsoidal with periodically arranged arms. The *parS* sites, a pair of short contiguous sequence elements known to be involved in chromosome segregation, are positioned at one pole, where they anchor the chromosome to the cell and contribute to the formation of a compact chromatin conformation. Repositioning these elements resulted in rotations of the chromosome that changed the subcellular positions of most genes. Such rotations did not lead to large-scale changes in gene expression, indicating that genome folding does not strongly affect gene regulation. Collectively, our data suggest that genome folding is globally dictated by the *parS* sites and chromosome segregation.

INTRODUCTION

The three-dimensional (3D) architecture of the genome both reflects and regulates its functional state (Dekker, 2008; Tharingselvan and Shapiro, 2009a). For example, chromosome segregation impacts bacterial locus subcellular positioning (Jun and Mulder, 2006; White et al., 2008), and chromatin loops that place promoters and distant enhancers within close spatial proximity play important roles in eukaryotic transcriptional regulation (Tolhuis et al., 2002; Vernimmen et al., 2007). Such examples suggest that studies of the high-resolution folding of genomes will yield insight into genome biology. However, until recently such studies, which require comprehensive assessments of the spatial positioning of many loci, have represented major technical challenges.

The recent development of several high-throughput technologies, including chromosome conformation capture (3C) (Dekker et al., 2002; Dostie et al., 2006; Duan et al., 2010; Fullwood et al., 2009; Lieberman-Aiden et al., 2009; Simonis et al., 2006; Zhao et al., 2006), has begun to enable studies of genome-wide chromosome folding. Fluorescent microscopy-based approaches allow the accurate determination of the subcellular positions of increasing numbers of defined chromosomal loci, while high-throughput 3C-based approaches enable quantification of interloci interaction frequencies that can subsequently be used to infer the average 3D distances between loci (Dekker, 2008; Duan et al., 2010). Recent studies have highlighted the potential of genome-wide studies of chromosome structure and have begun to reveal specific features of chromosome folding, including the transcription-based compartmentalization of the human nucleus (Lieberman-Aiden et al., 2009; Simonis et al., 2009) and the correlation between a locus' genomic and subcellular positioning in bacteria (Nielsen et al., 2006; Teleman et al., 1999; Wang et al., 2006b). However, the detailed structures of genomes are only beginning to be revealed, and many details, including the identities of the sequence elements that define such structures, await further investigation.

We sought to determine the high-resolution 3D structure of an entire genome and to utilize the resulting structure to identify the sequence elements that define its architecture. Toward this goal, we studied the synchronizable bacterium, *Caulobacter crescentus* (hereafter Caulobacter), whose single circular chromosome is organized such that the origin and terminus of replication reside near opposite poles of the cell and other loci be



On TADs and hormones

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 François le Dily

Miguel Beato

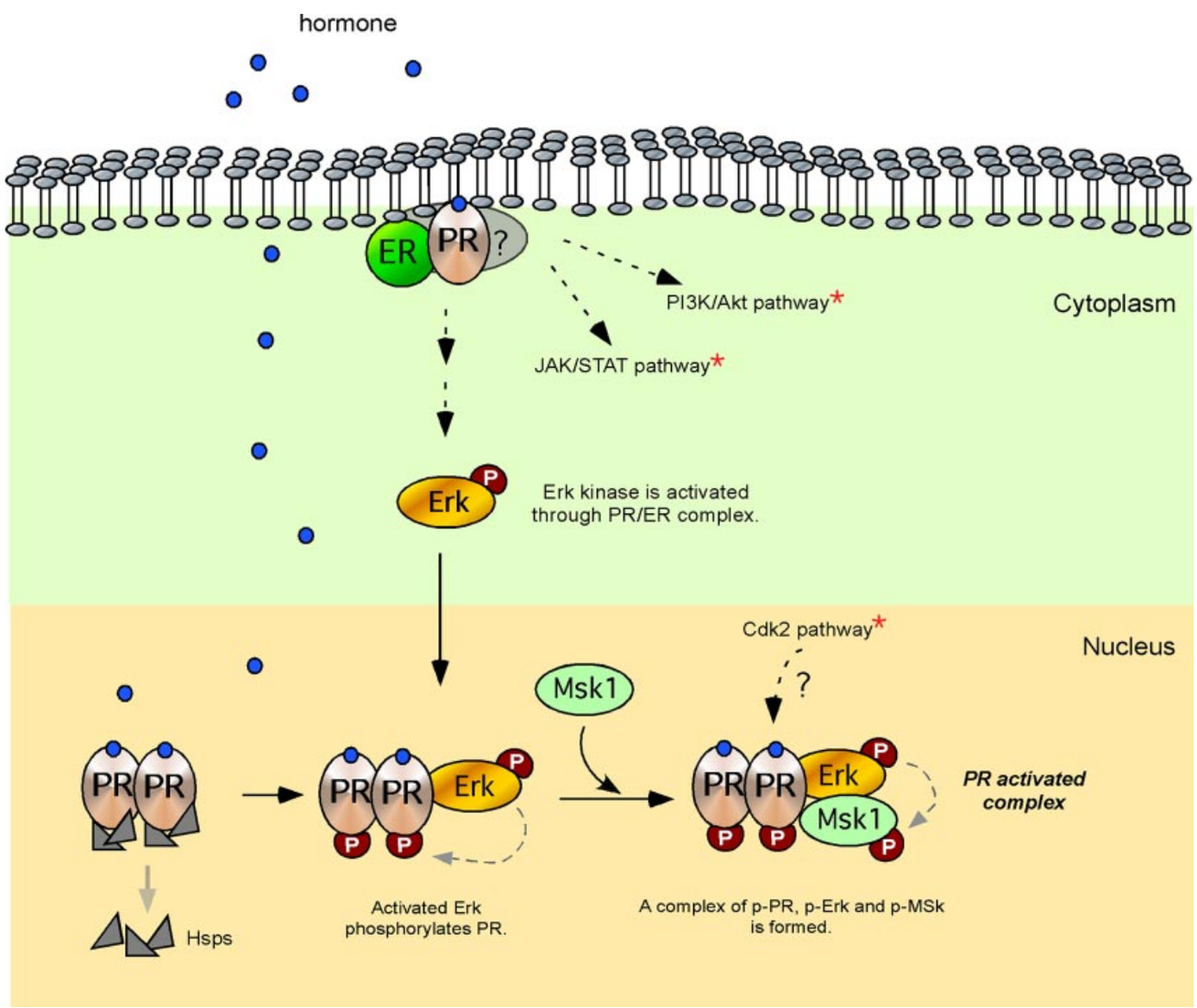
Chromatin and Gene Expression Group (CRG)

Guillaume Filion

Genome Architecture Group (CRG)

cnag CRG

Progesterone-regulated transcription in breast cancer



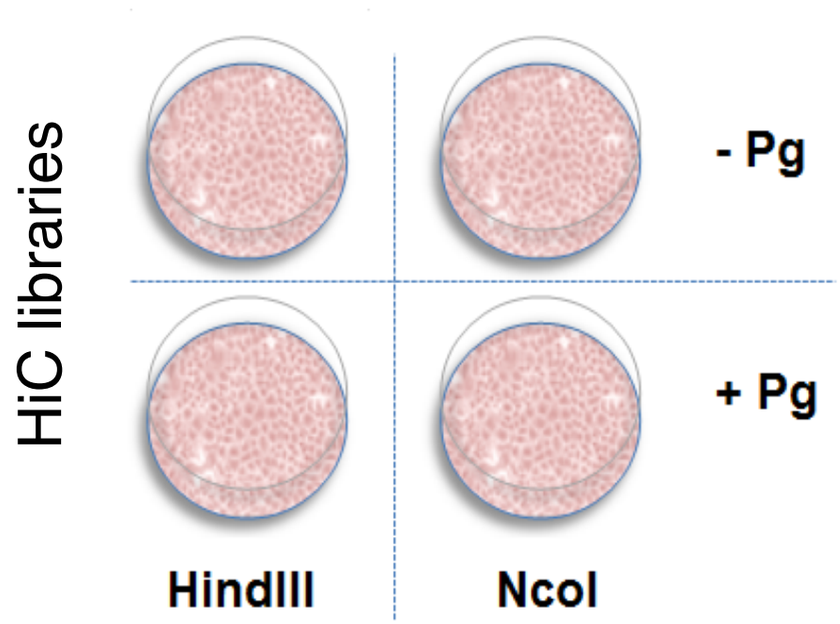
Vicent *et al* 2011, Wright *et al* 2012, Ballare *et al* 2012

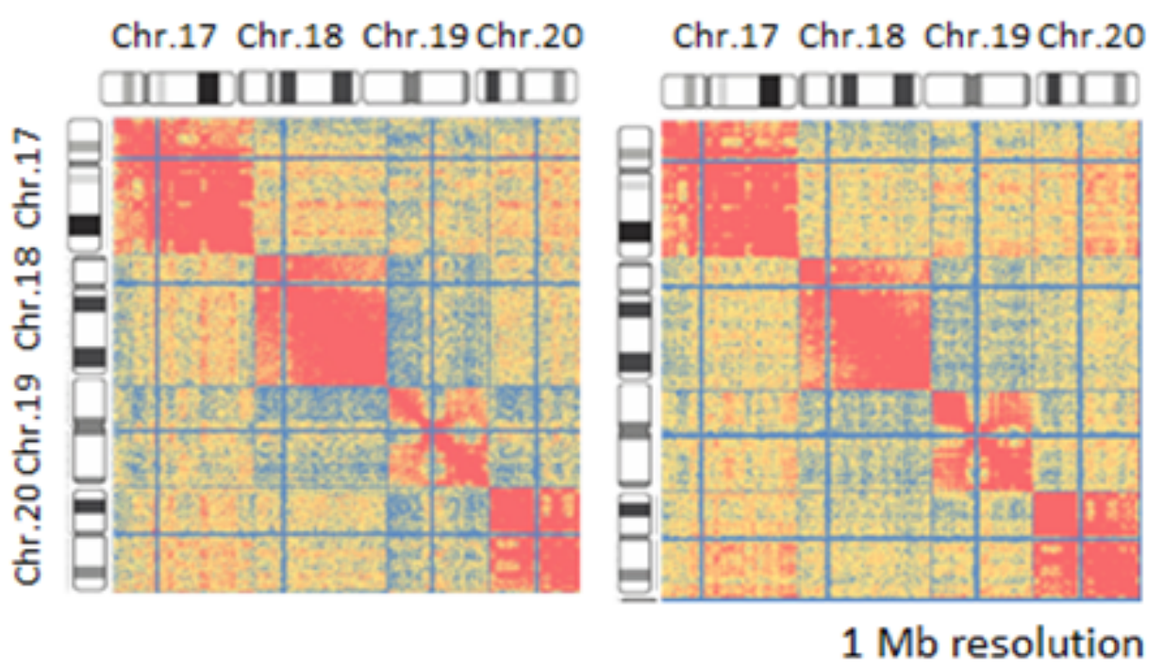
> 2,000 genes **Up**-regulated

> 2,000 genes **Down**-regulated

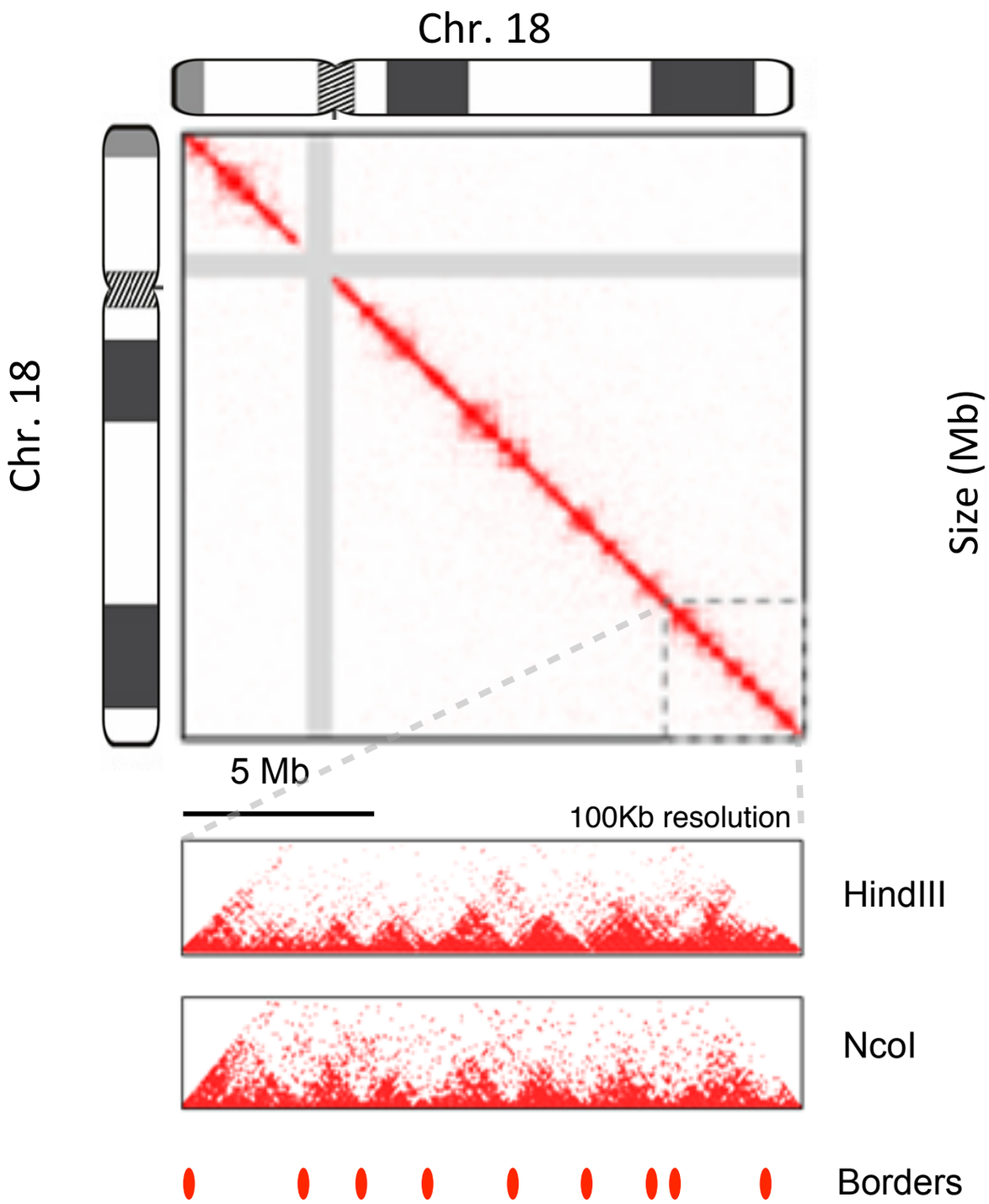
Regulation in 3D?

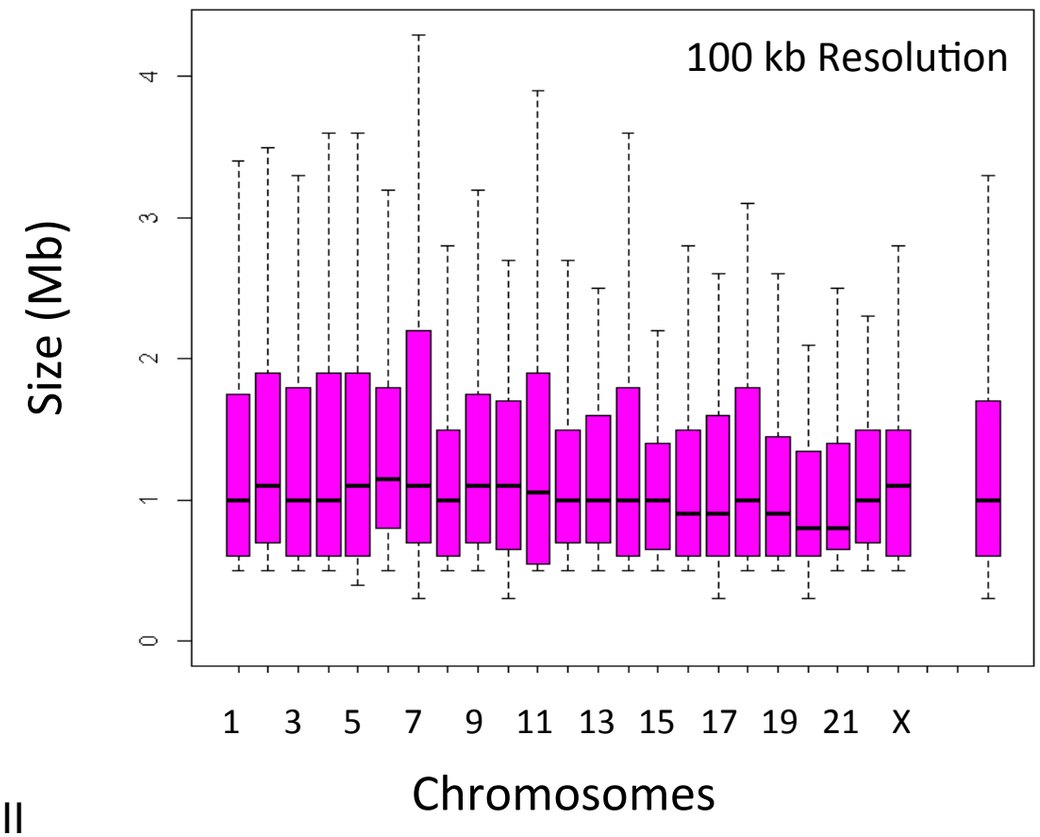
Experimental design



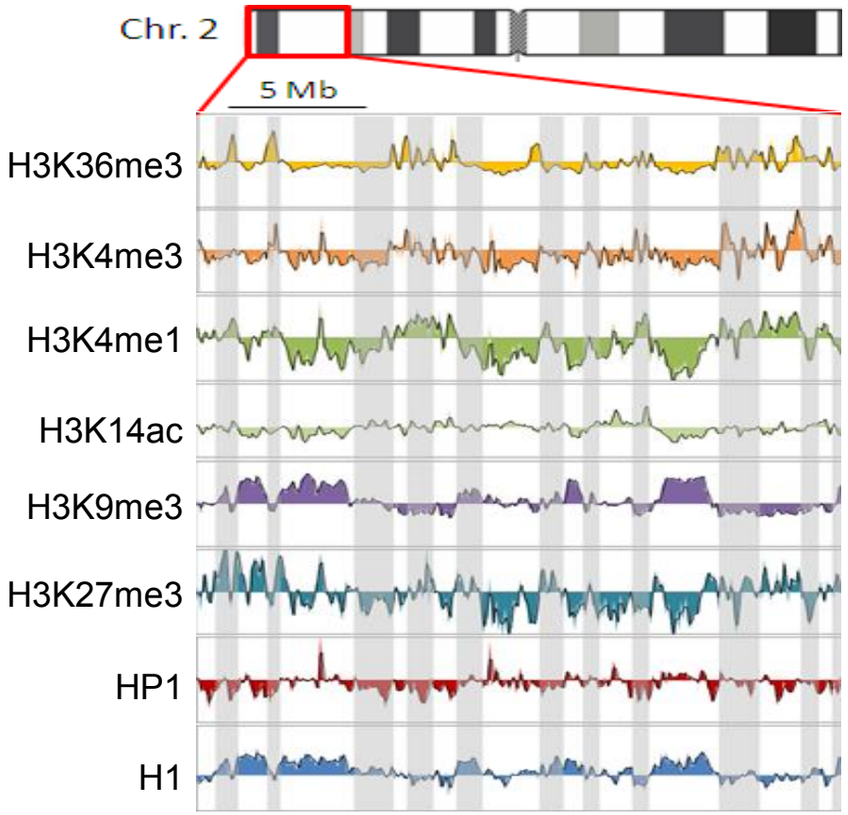


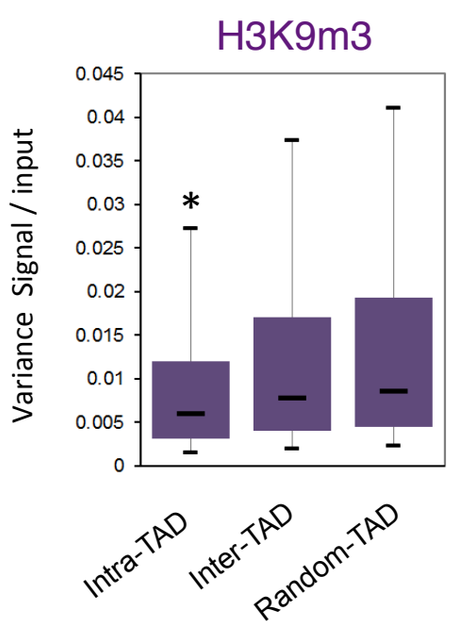
Chromosomes are organized into Topologically Associated Domains (TADs)

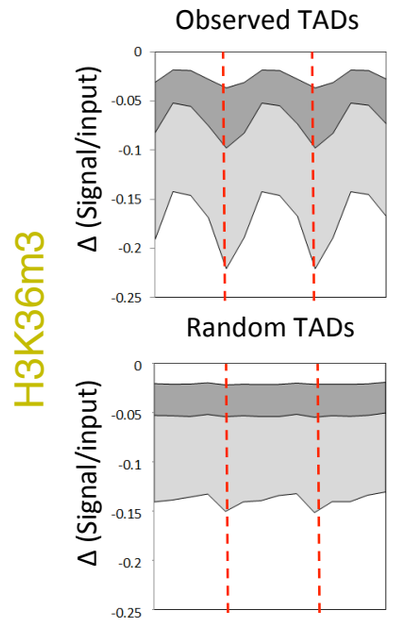


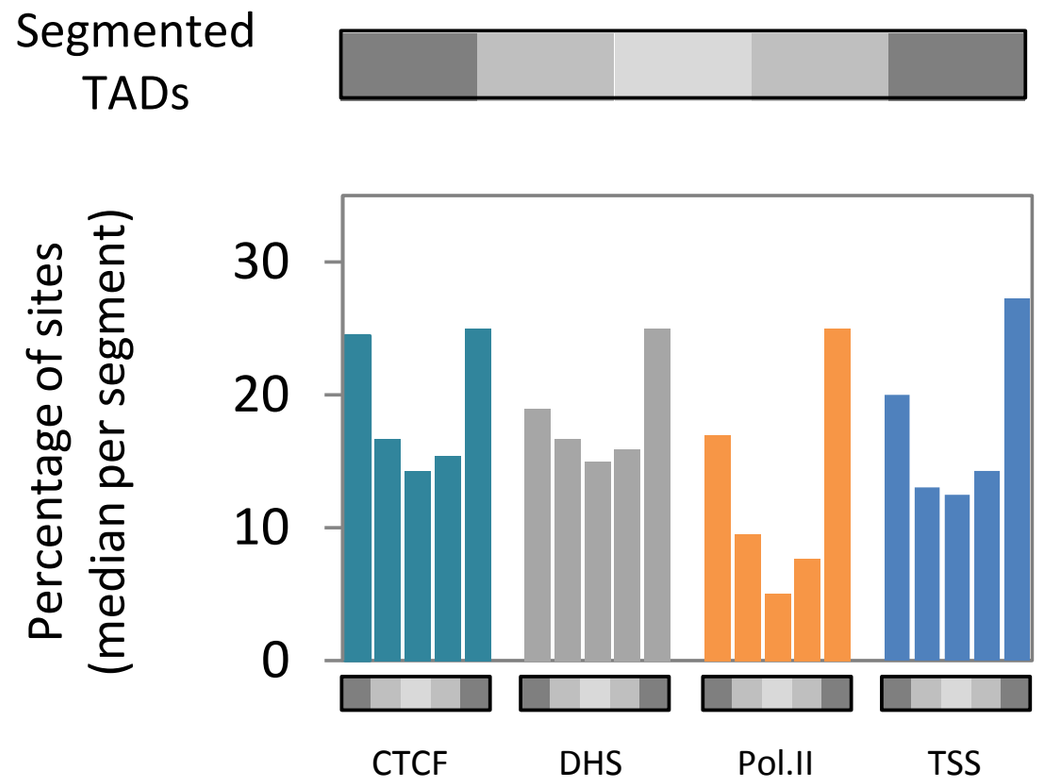


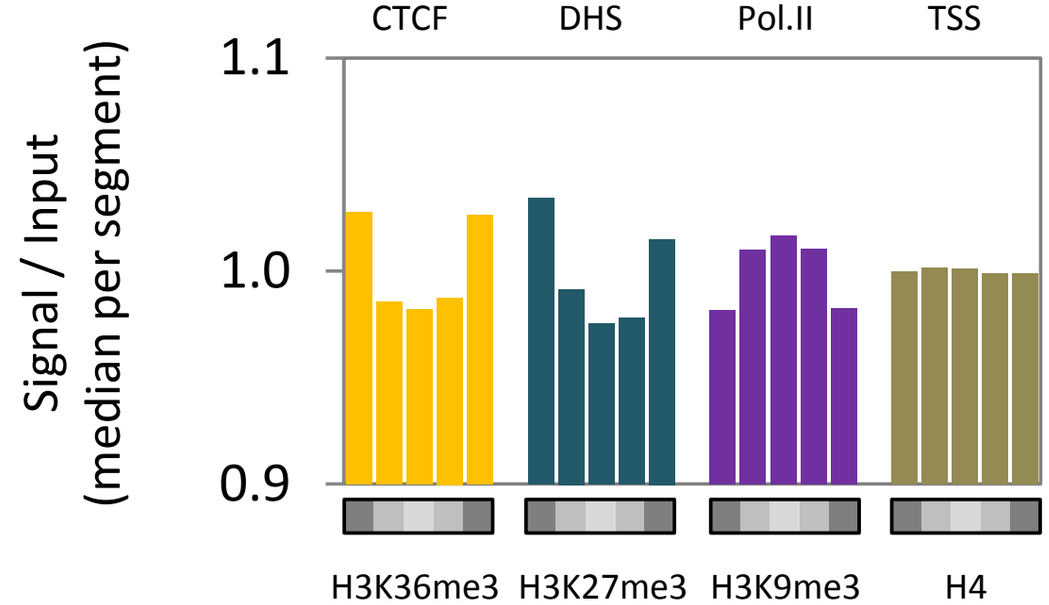
Are TADs homogeneous?



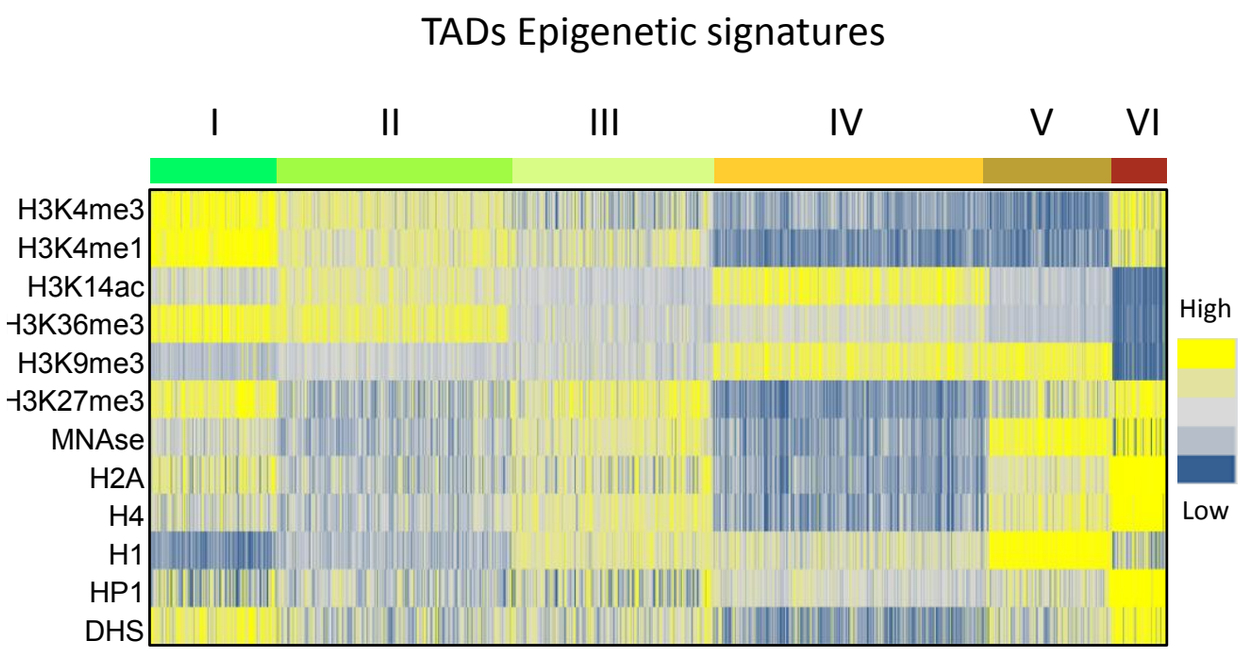


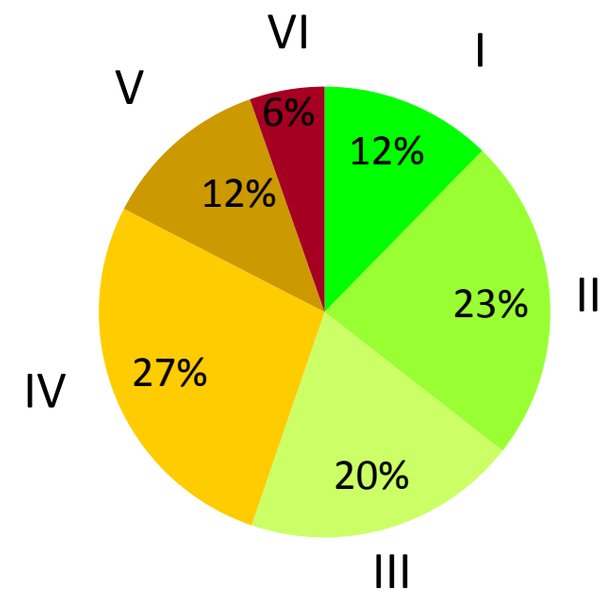


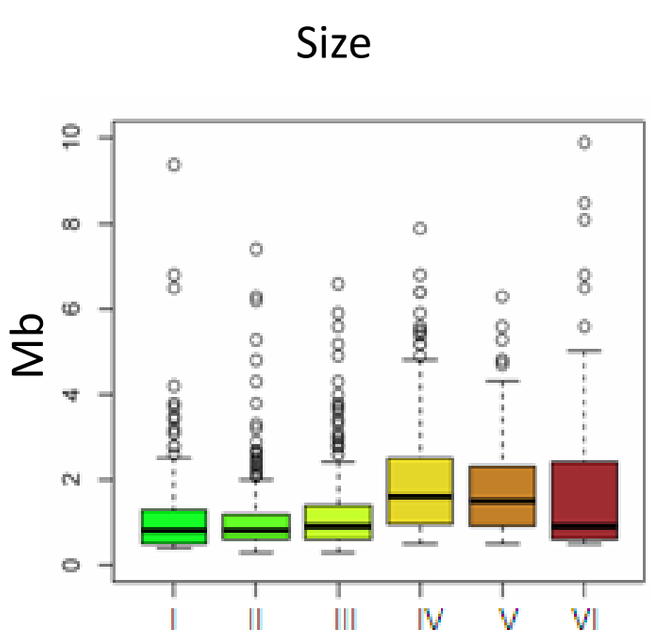


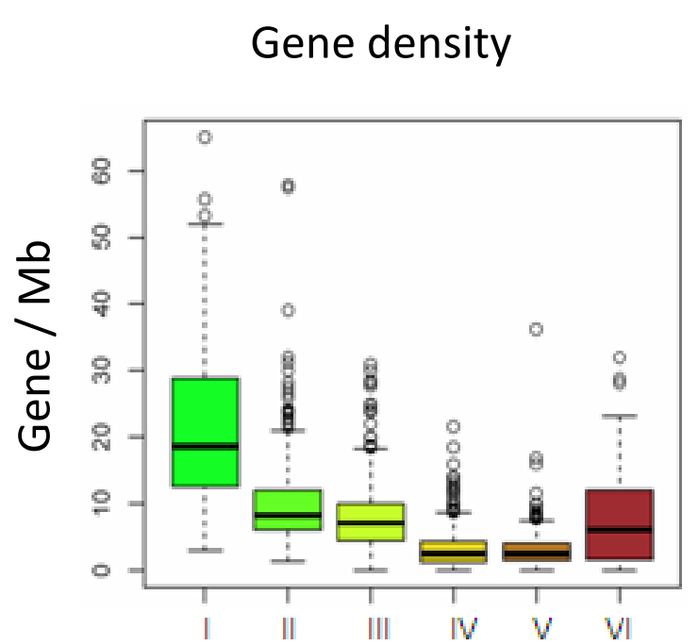


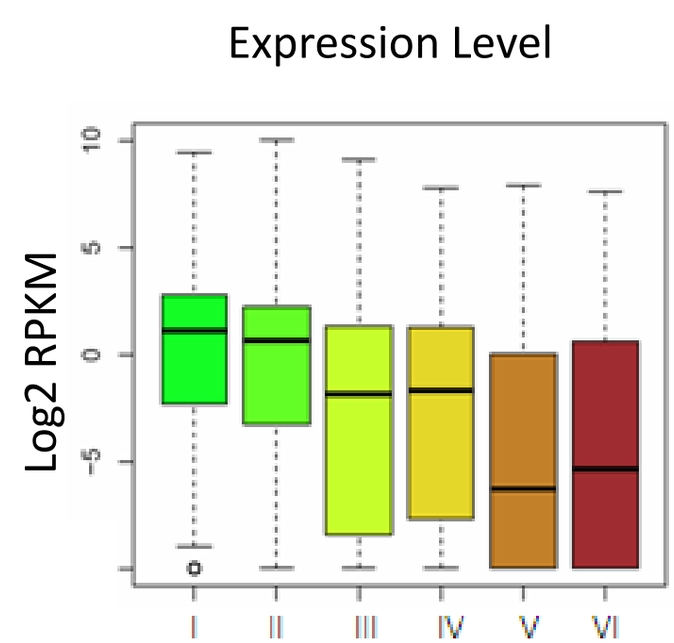
Functional Chromatin signatures of TADs



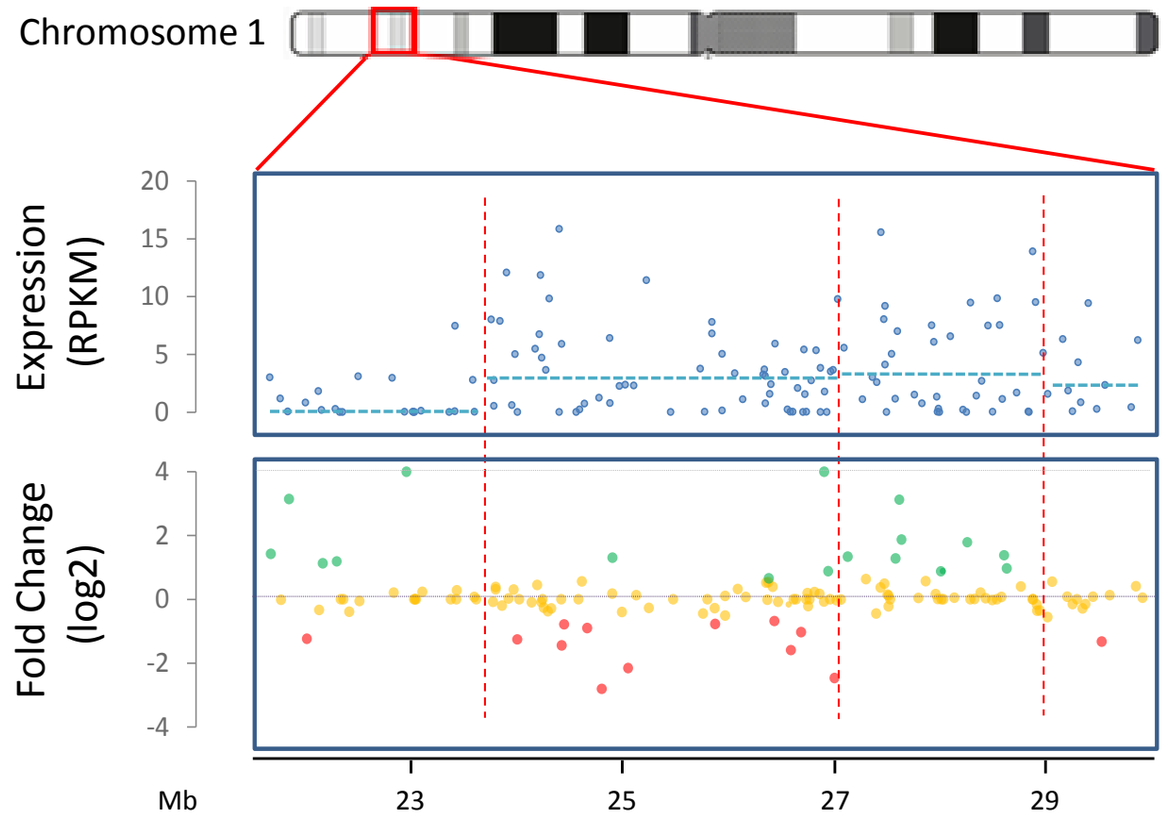


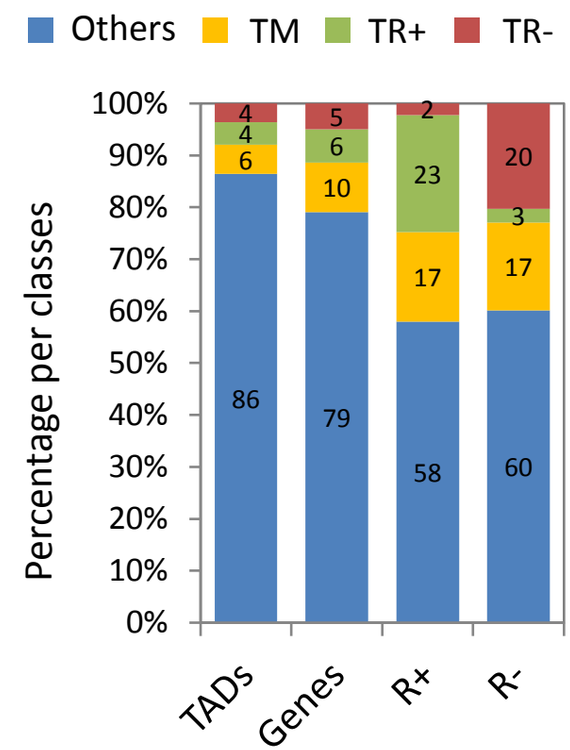




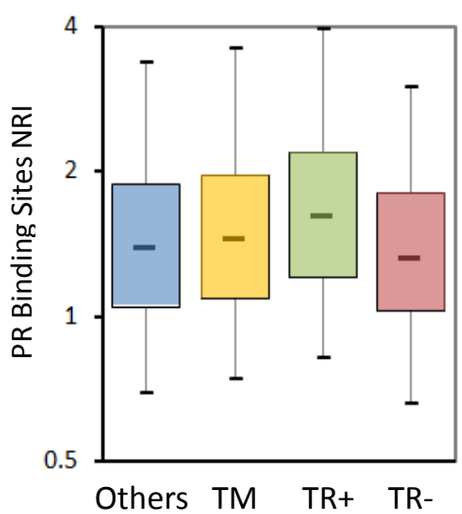


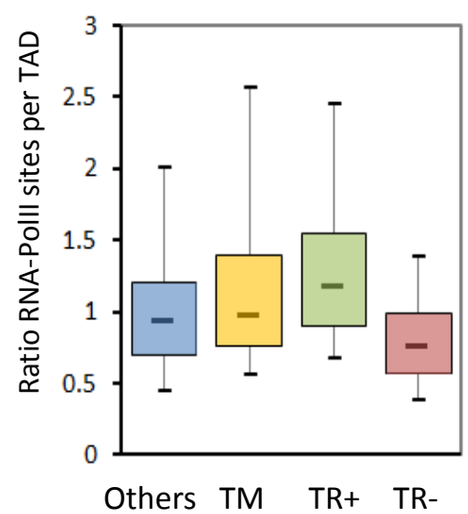
A role for TADs in Pg induced transcription changes

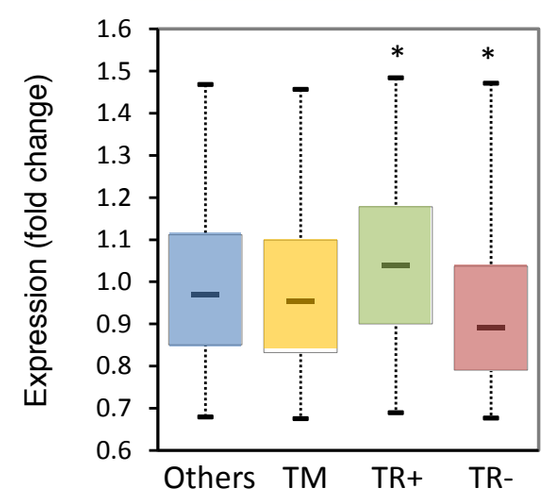




Non-regulated genes

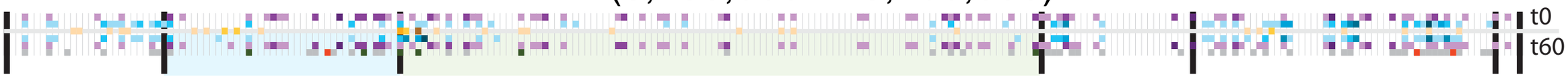






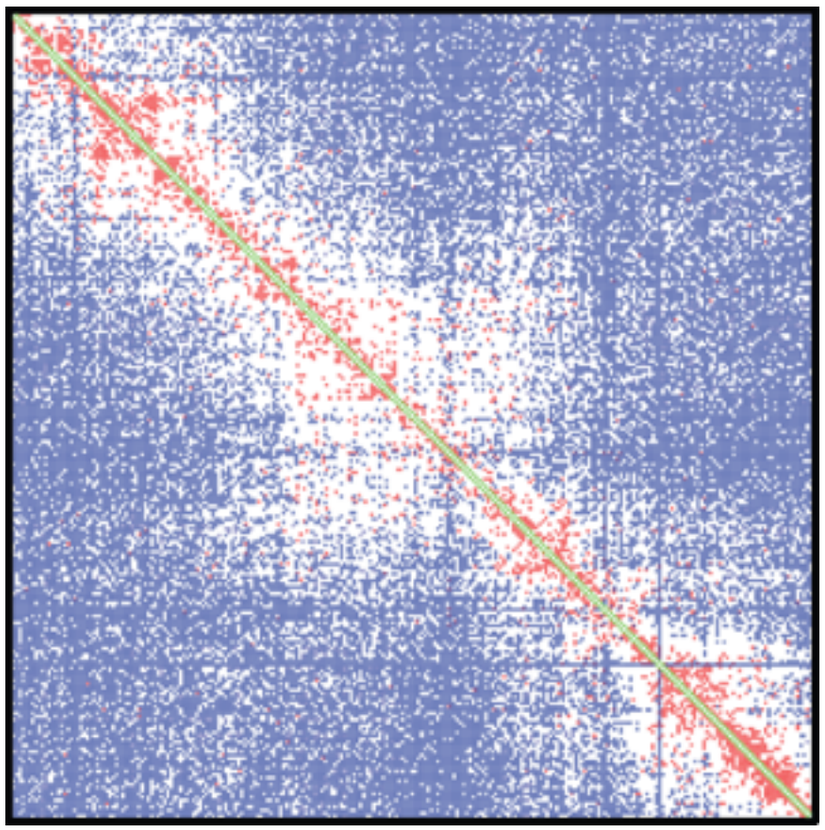
Modeling 3D TADs

R1 (8,480,000 - 13,460,000)

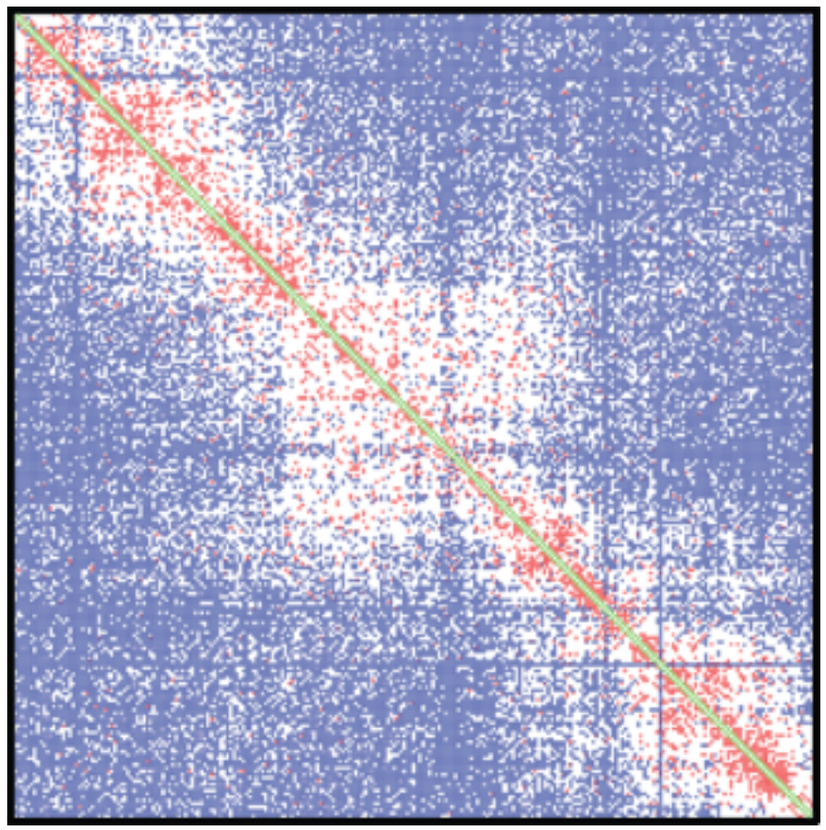


Harmonic · Harmonic Lower Bound · Harmonic Upper Bound

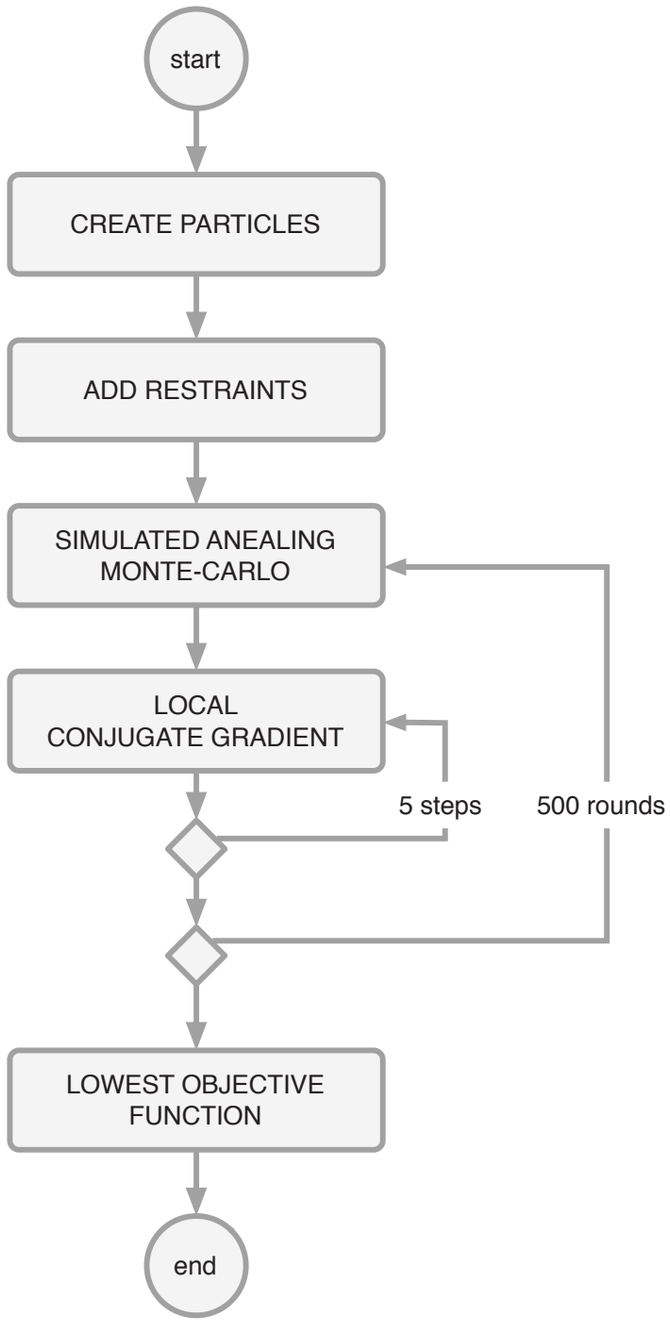
T0

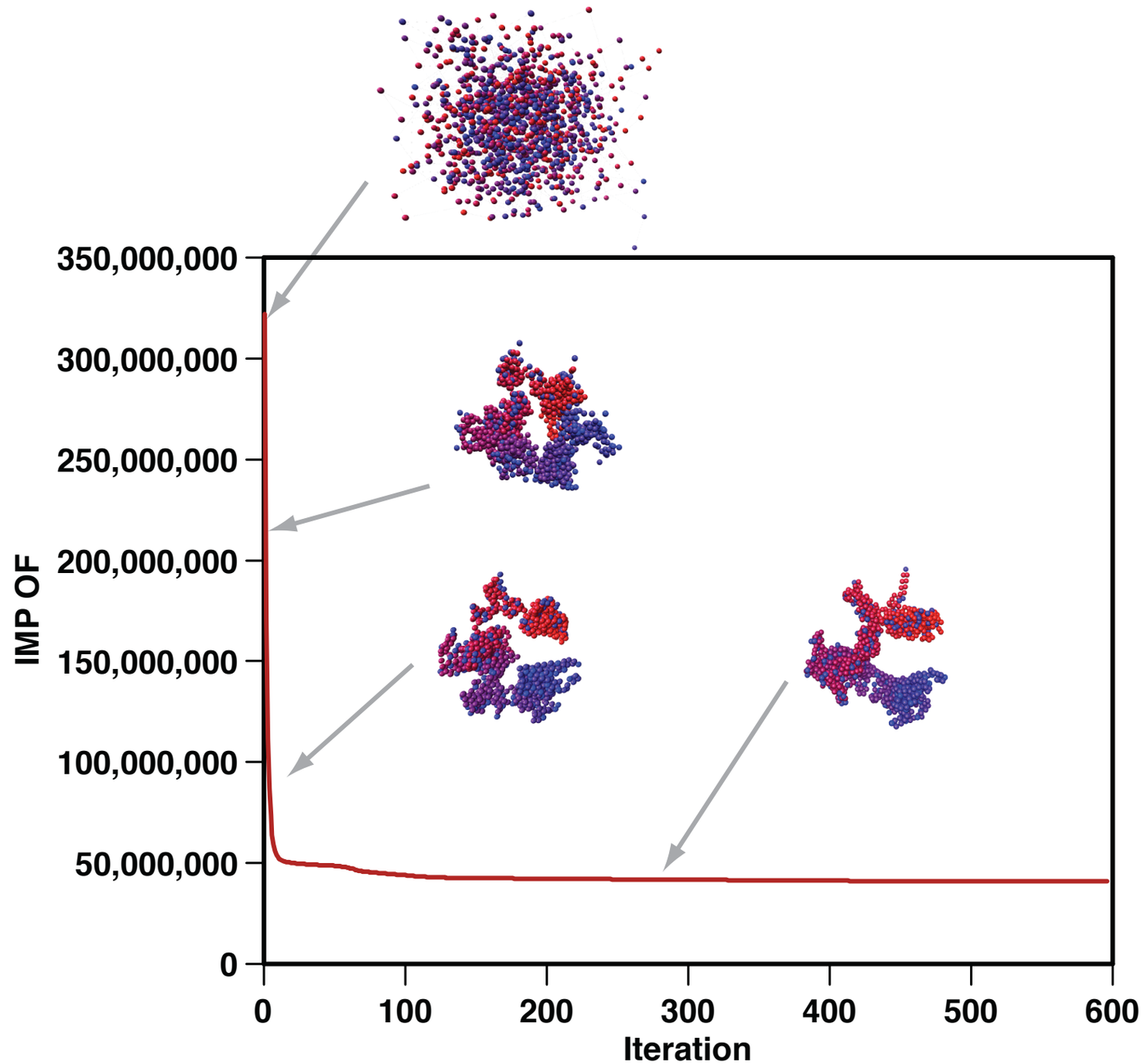


T60

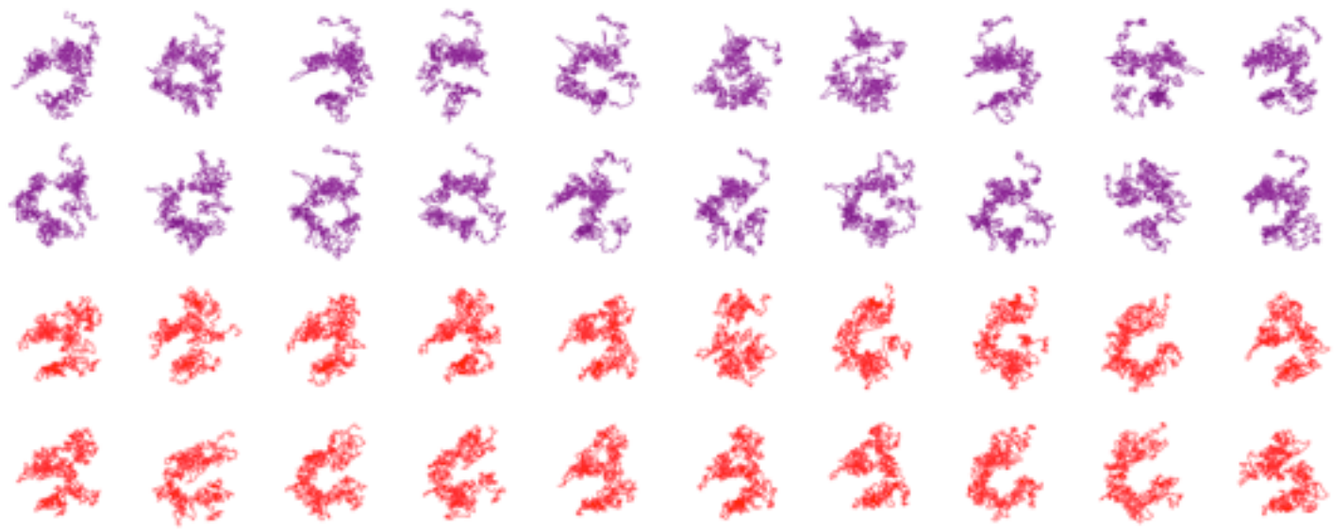


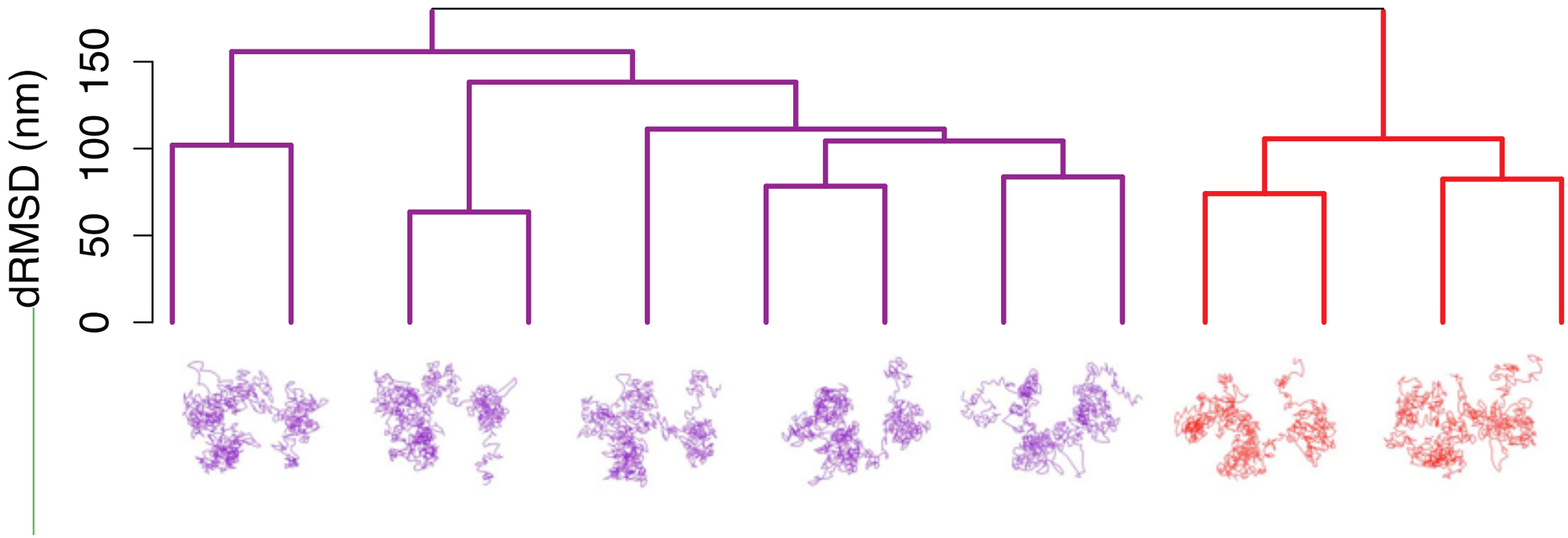
Optimization of the scoring function





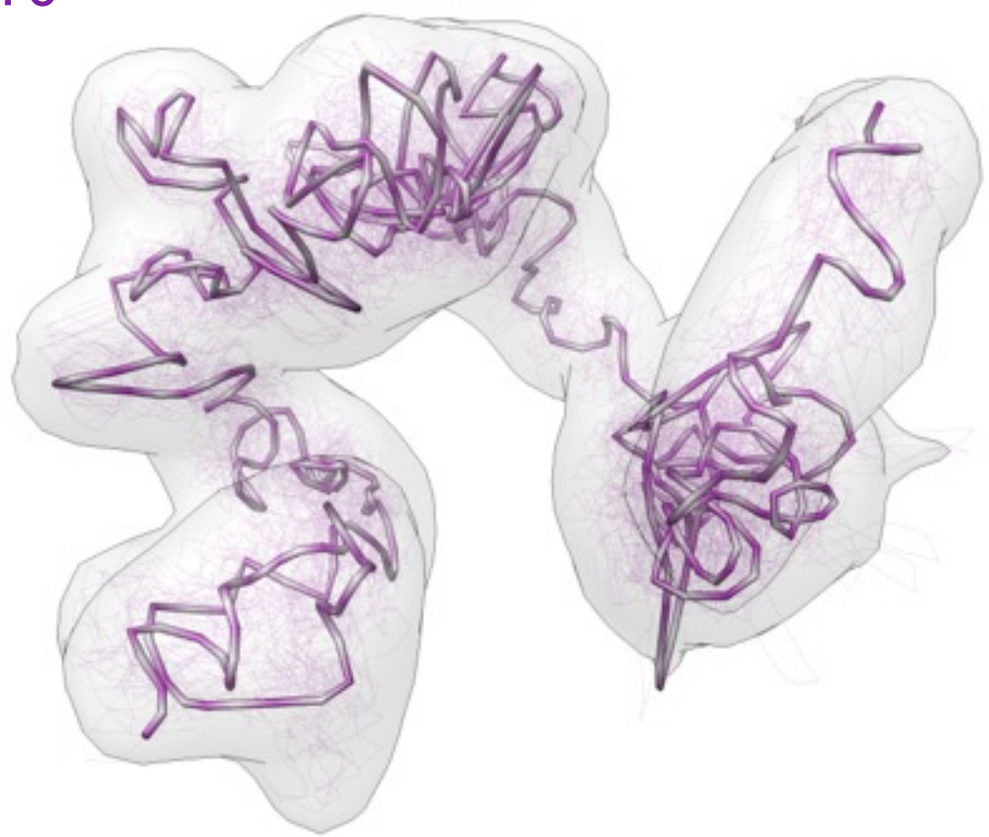
Clustering





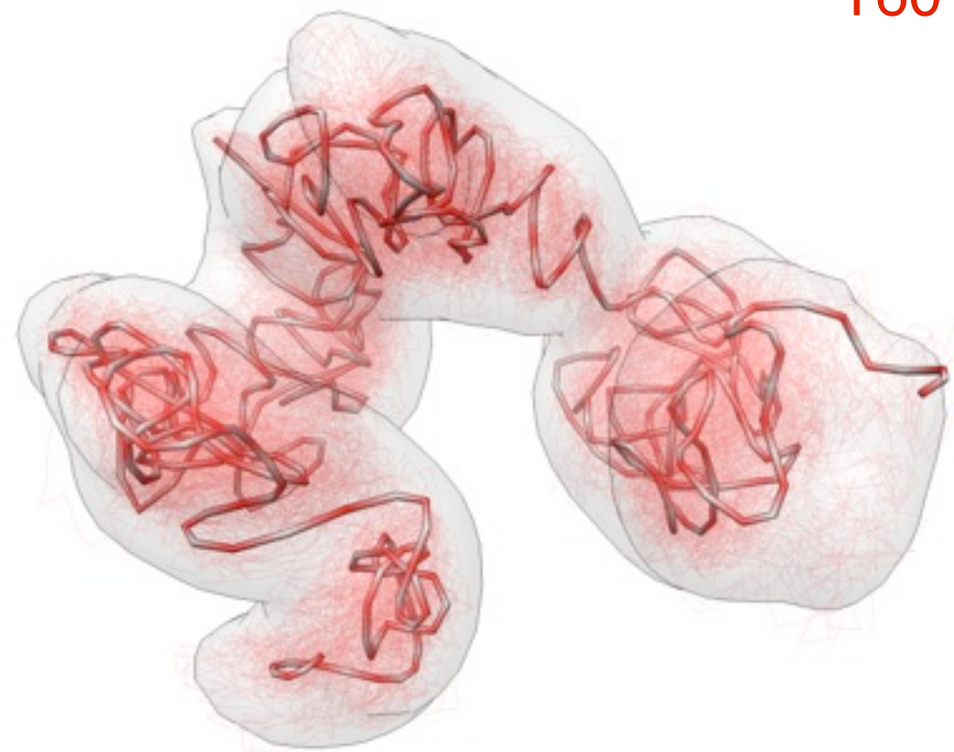
Clustering

T0



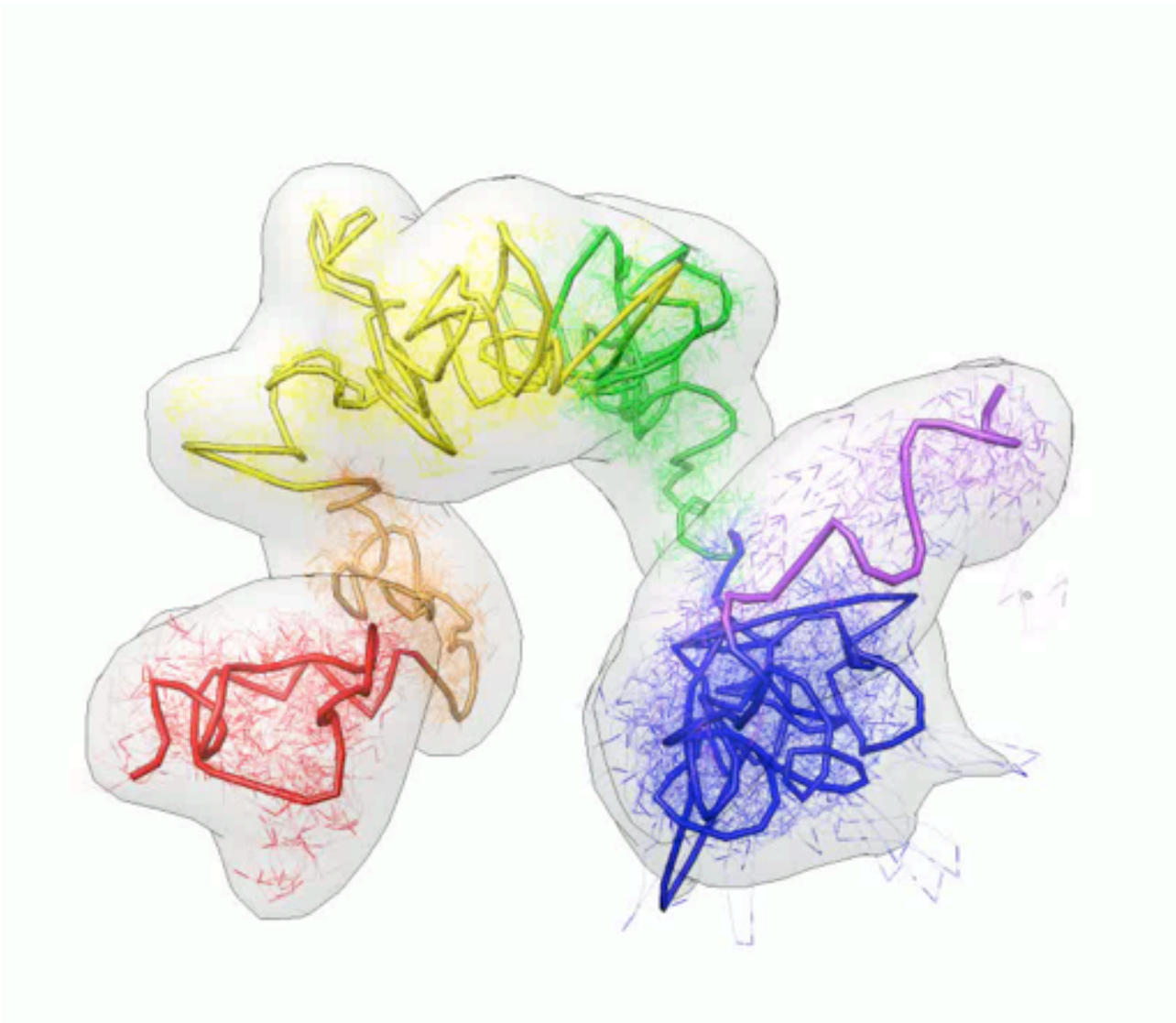
Cluster #3
13 models
MaxDist: ~1400nm

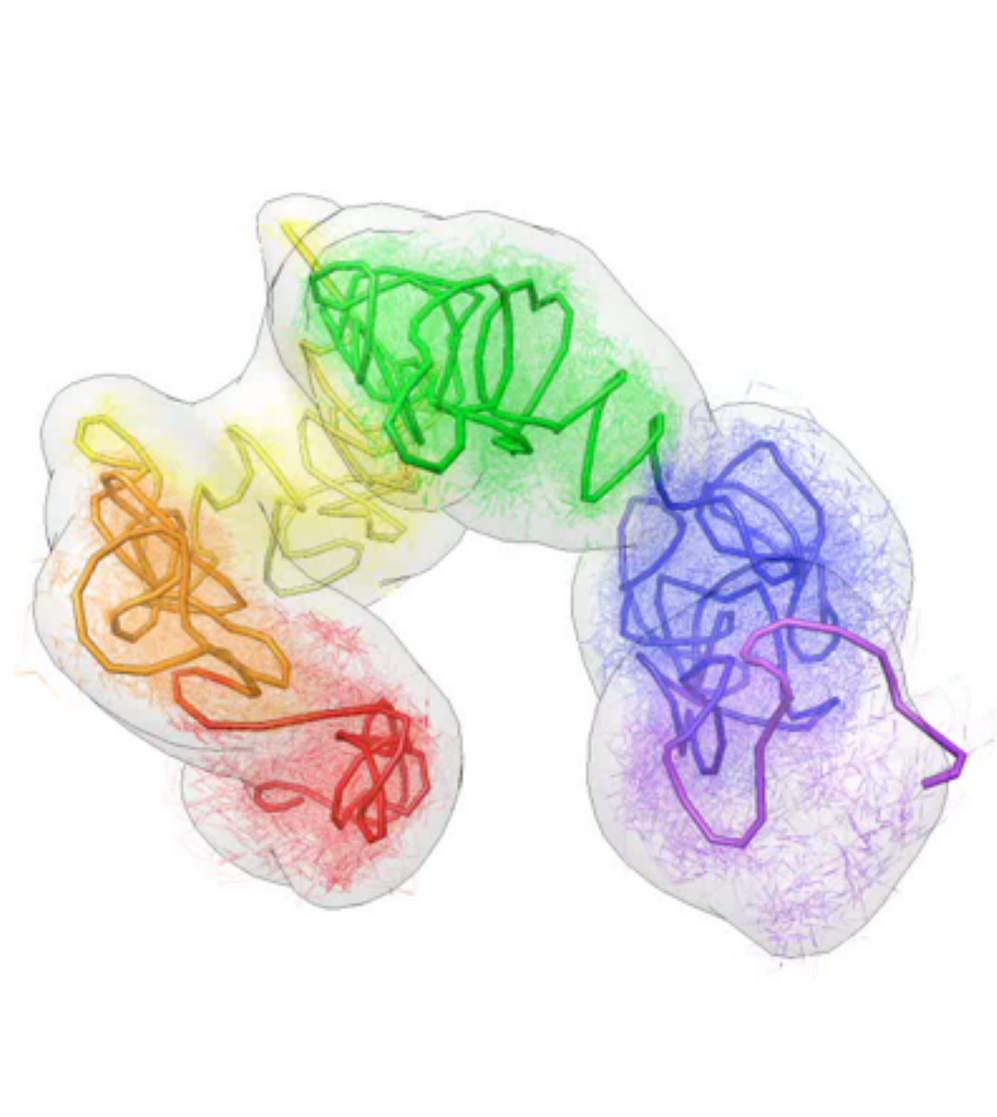
T60



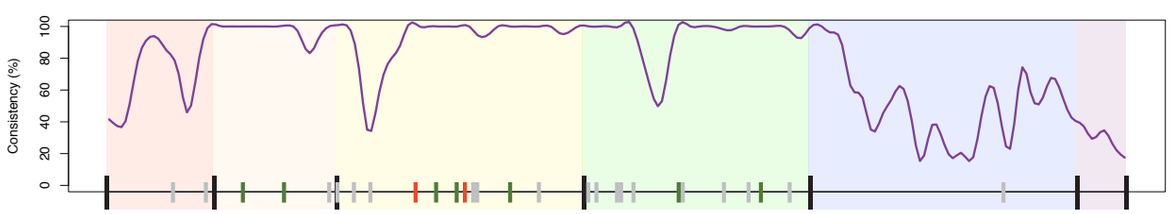
Cluster #1
42 models
MaxDist: ~1400nm

Structural TADs

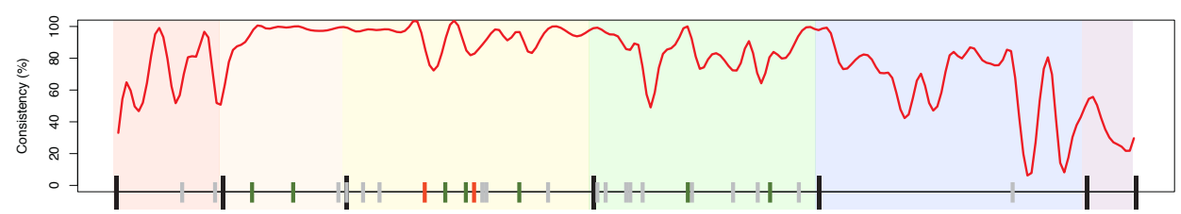




T0

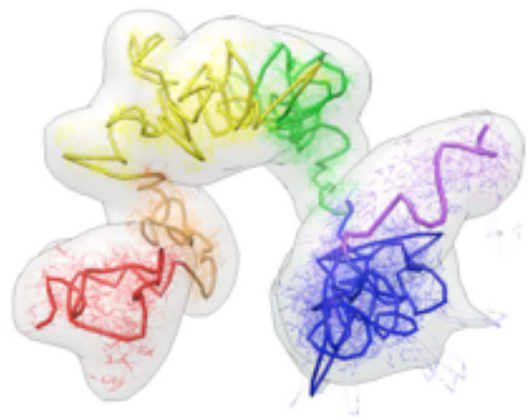


T60



Structural expansion of TADs

T0



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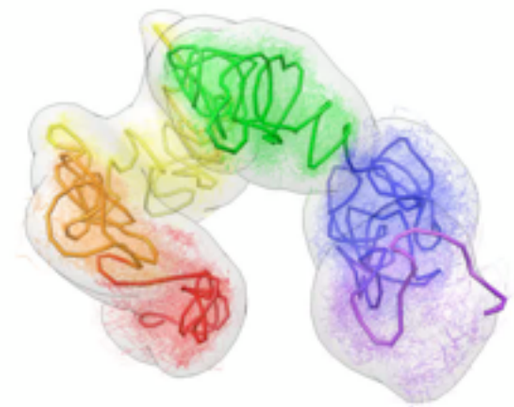
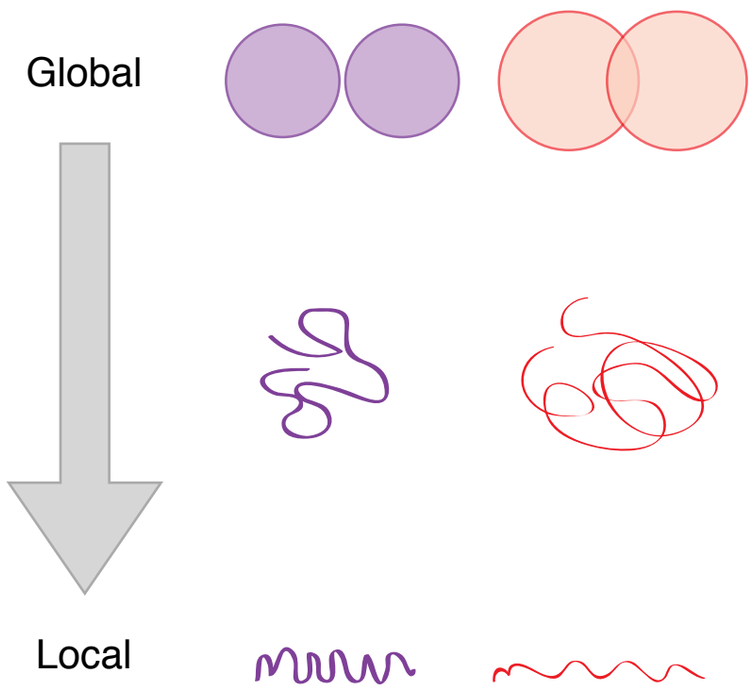
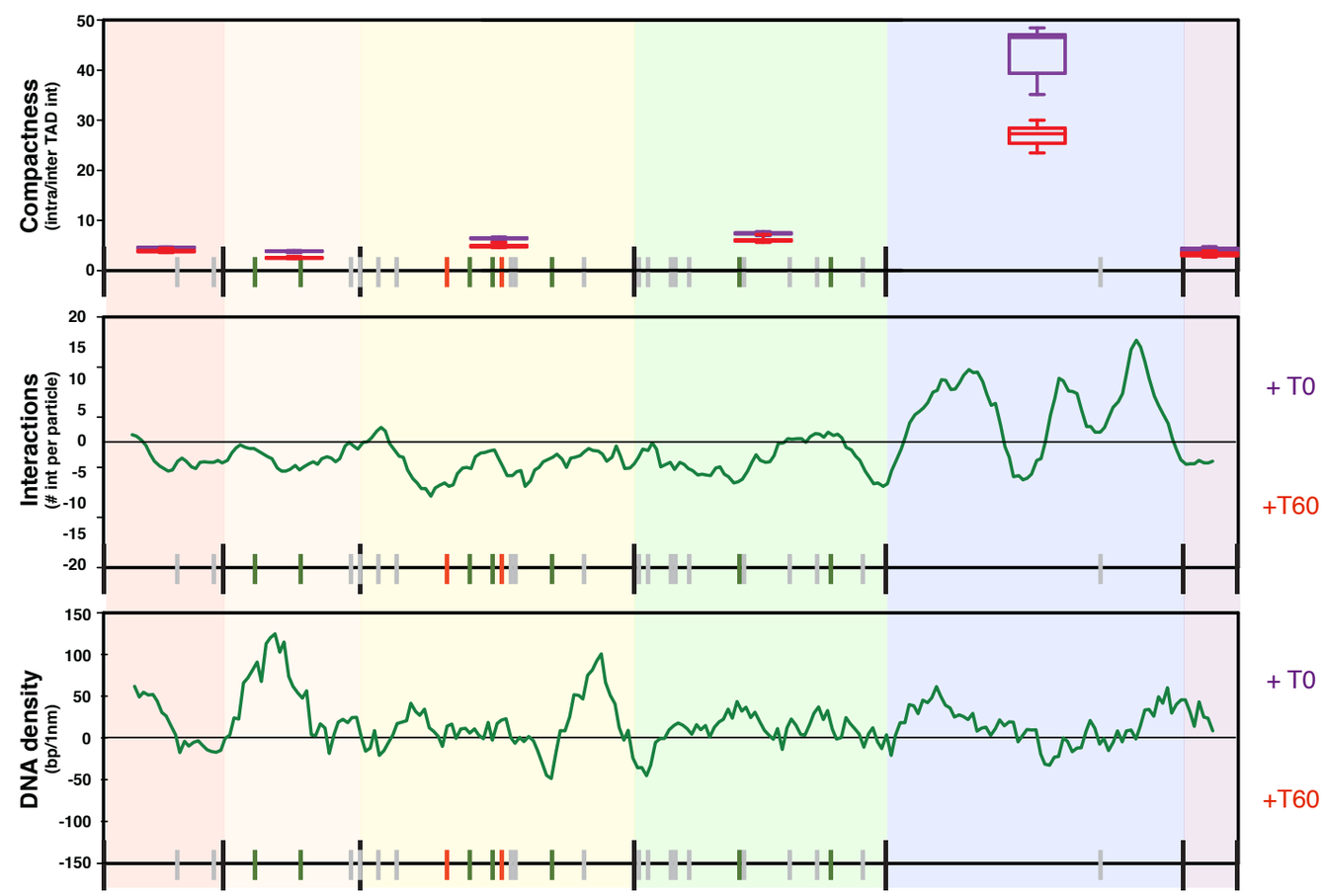
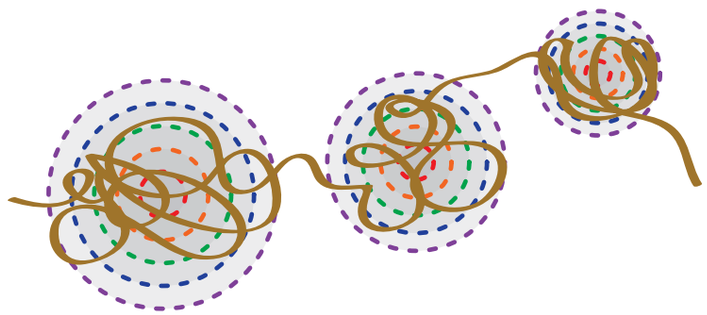
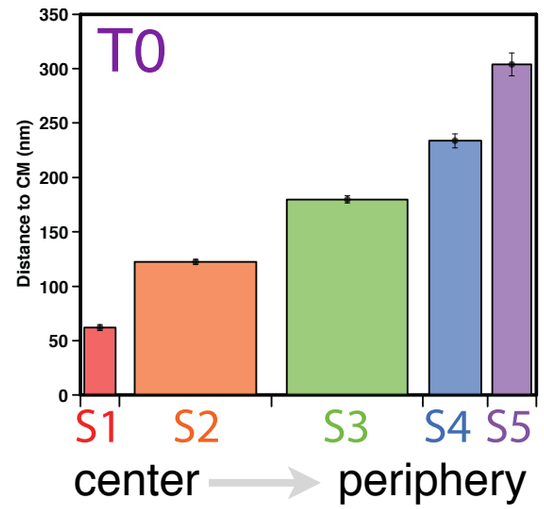


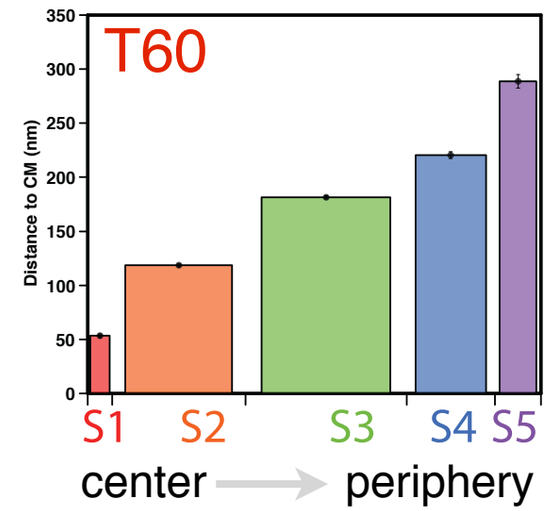


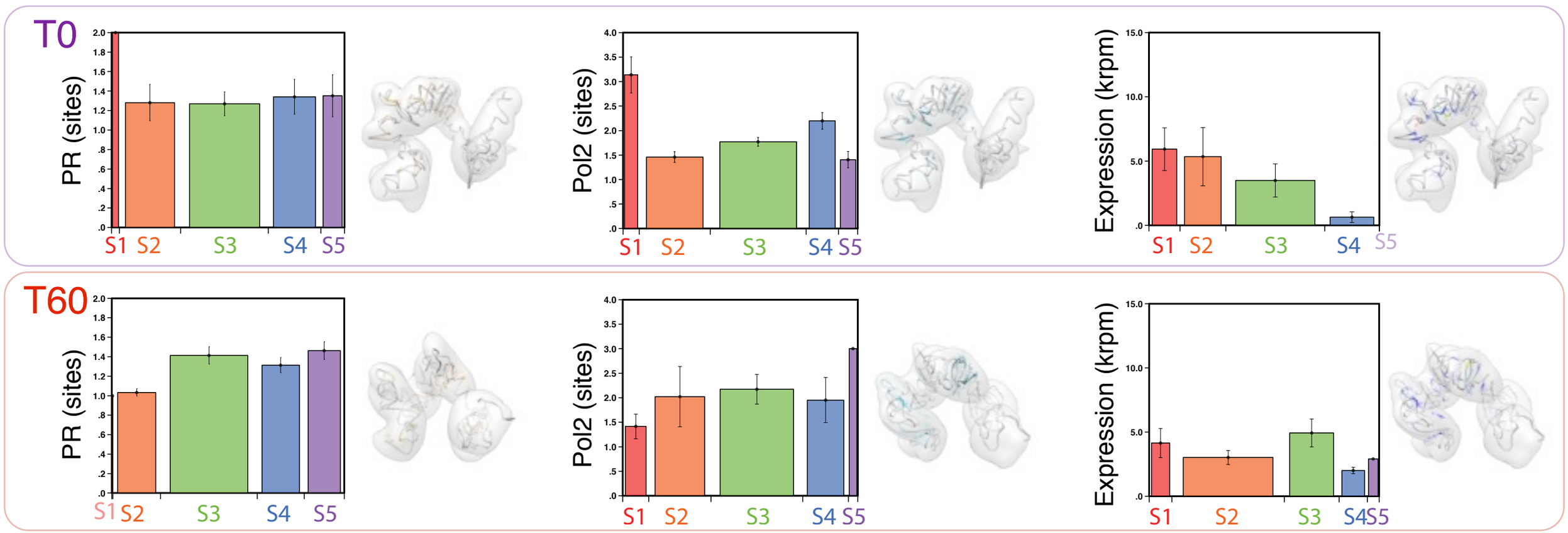


Functional TADs



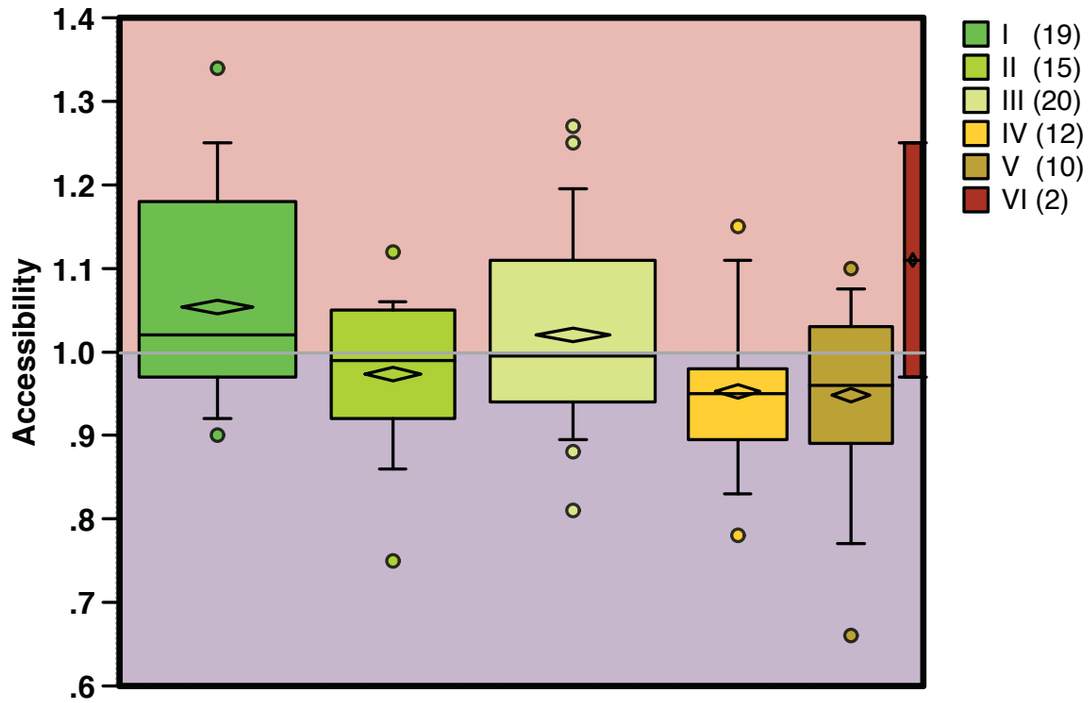


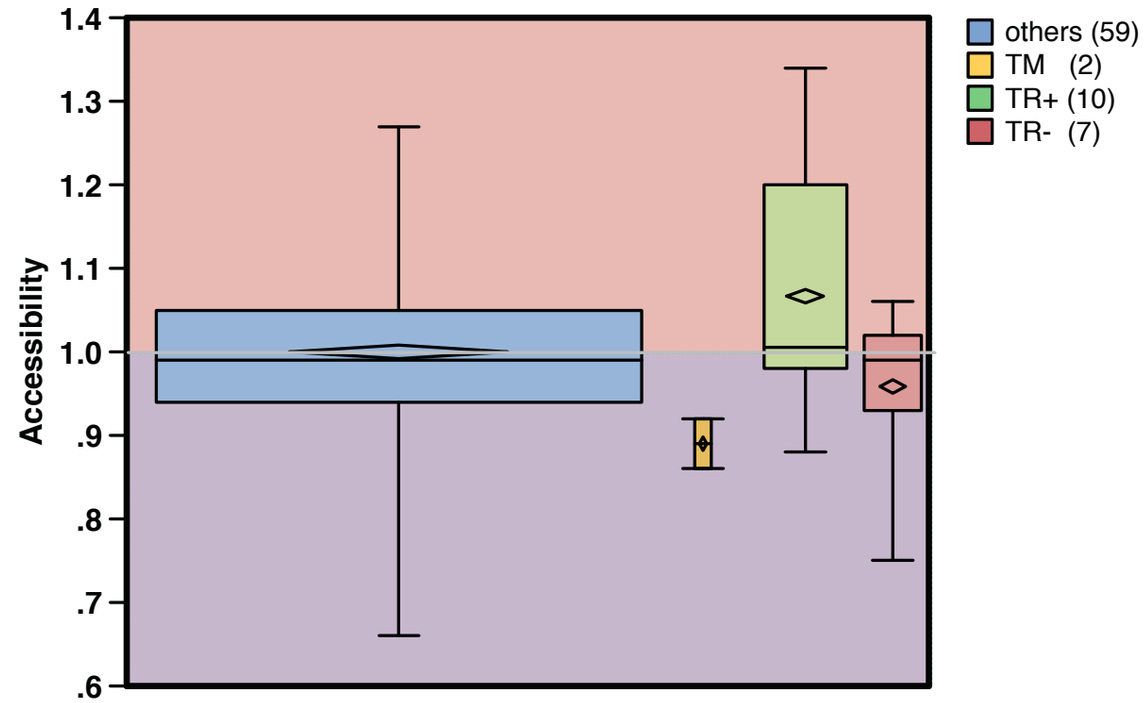


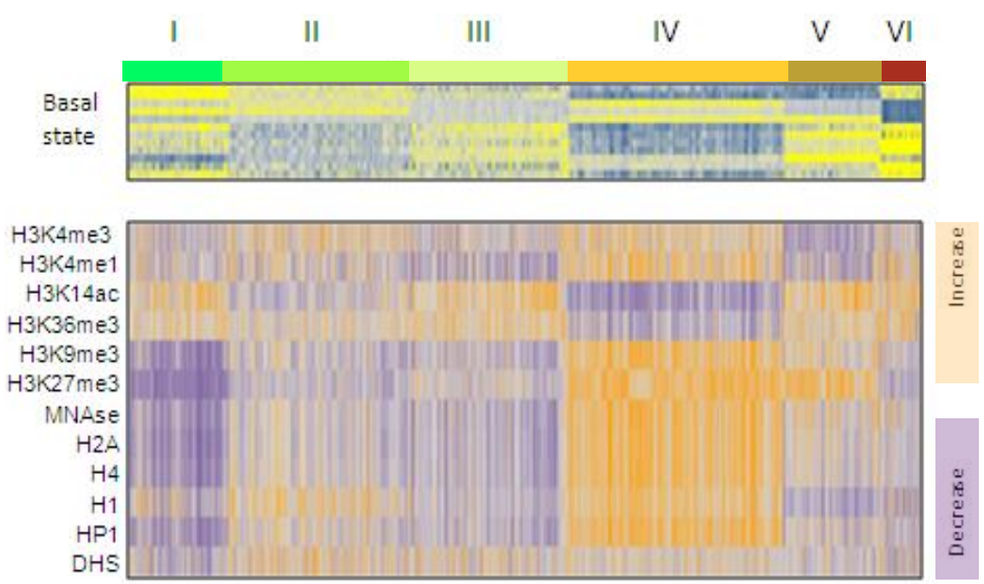


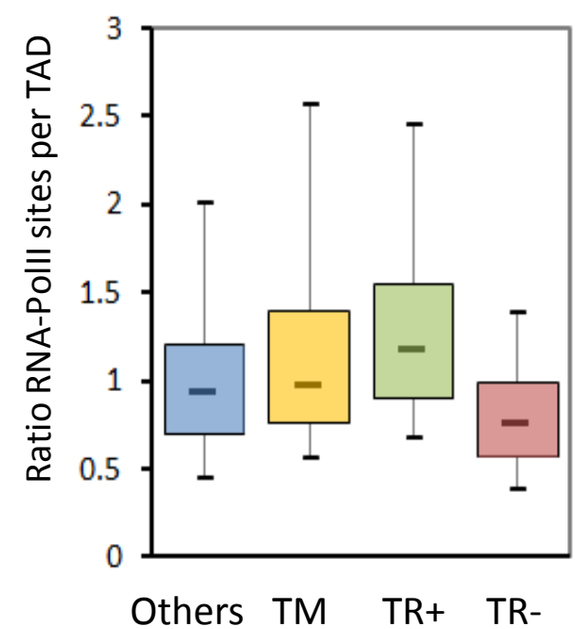
cnag CRG

TADs respond differently

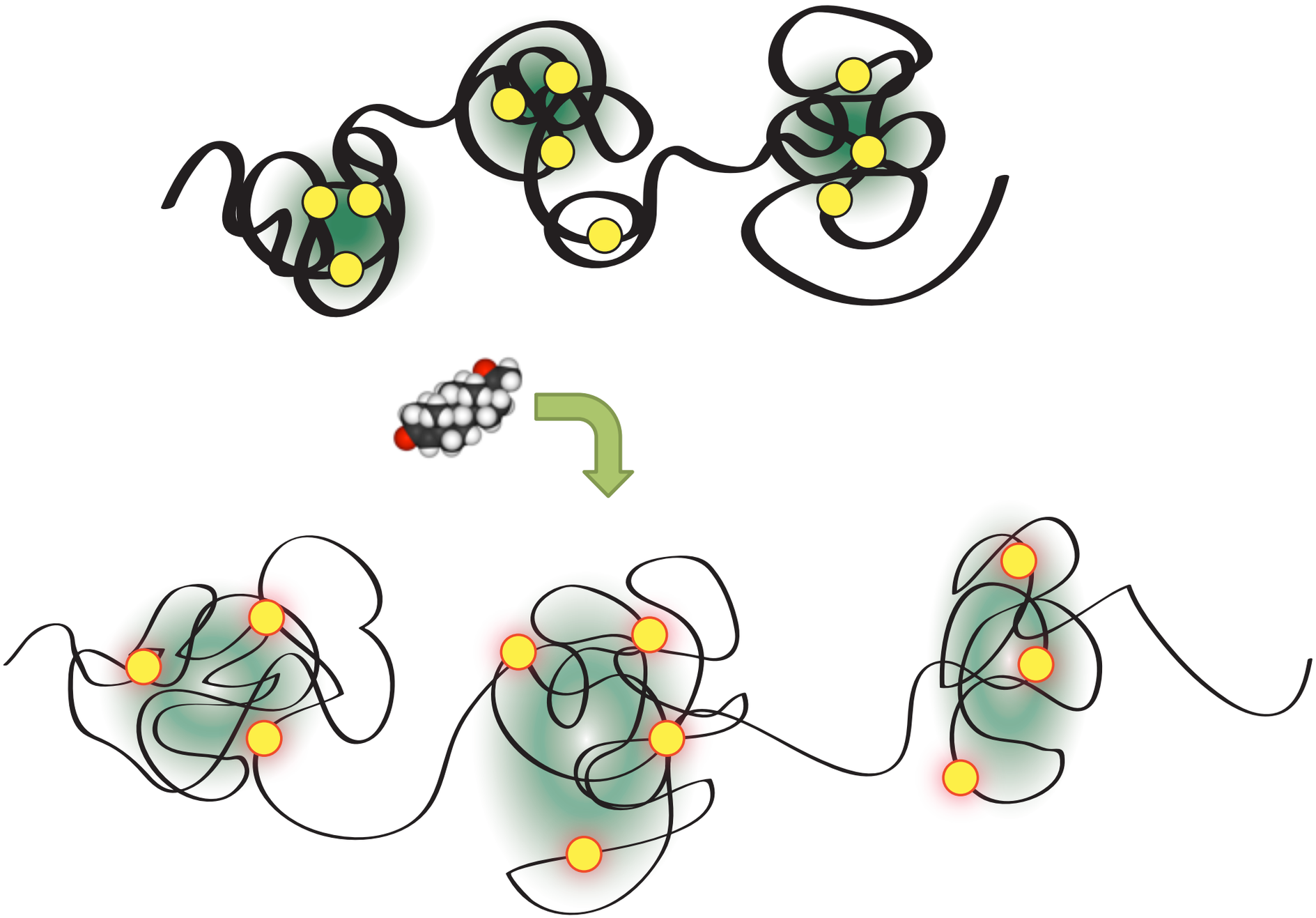








Model for TAD regulation



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