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

cnag CRG ICREA

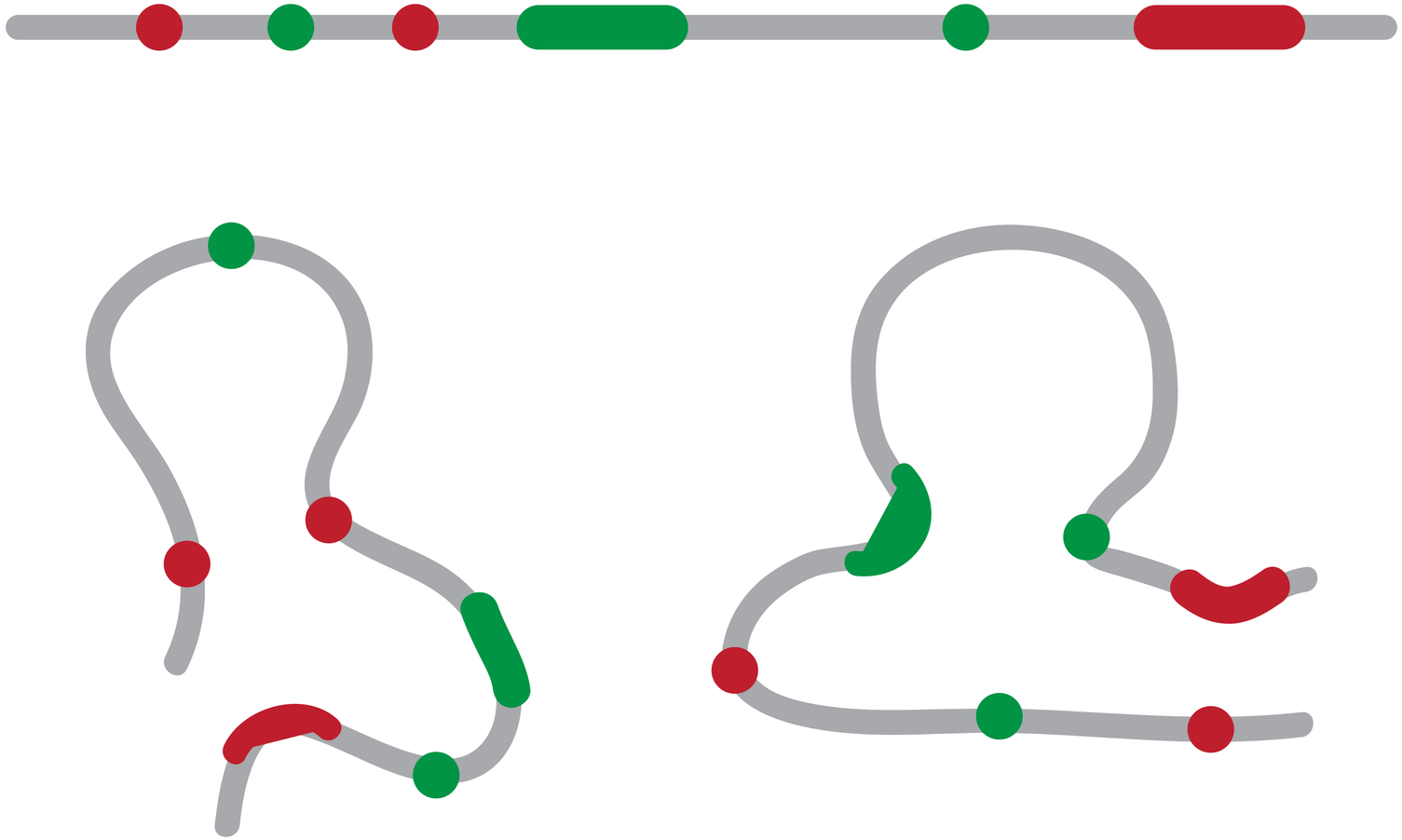
All you will see in the screen is here:

http://marciuslab.org/www/presentations/

I encourage you to:

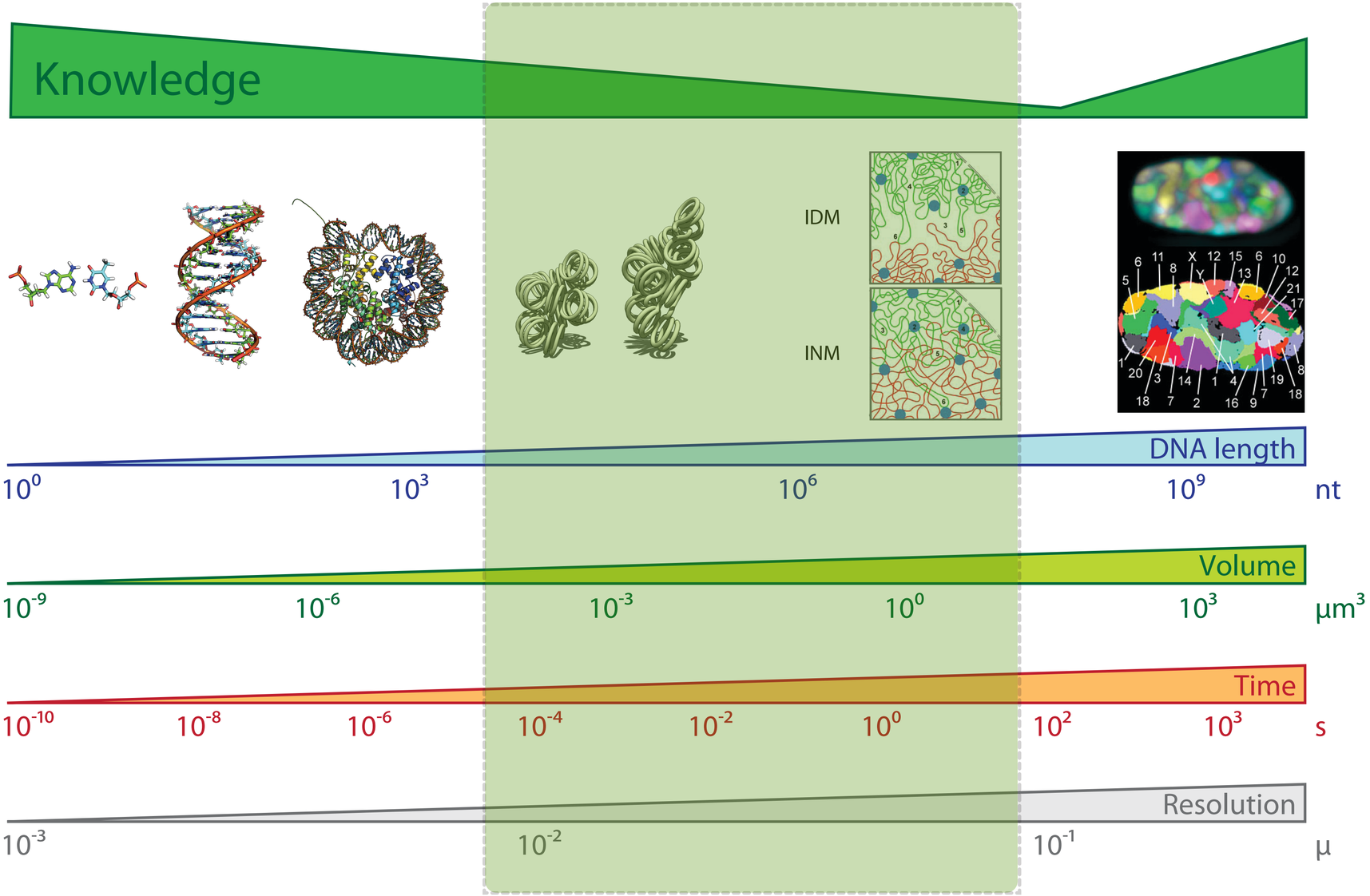
listen AND speak

not necessarily in this order… 😄



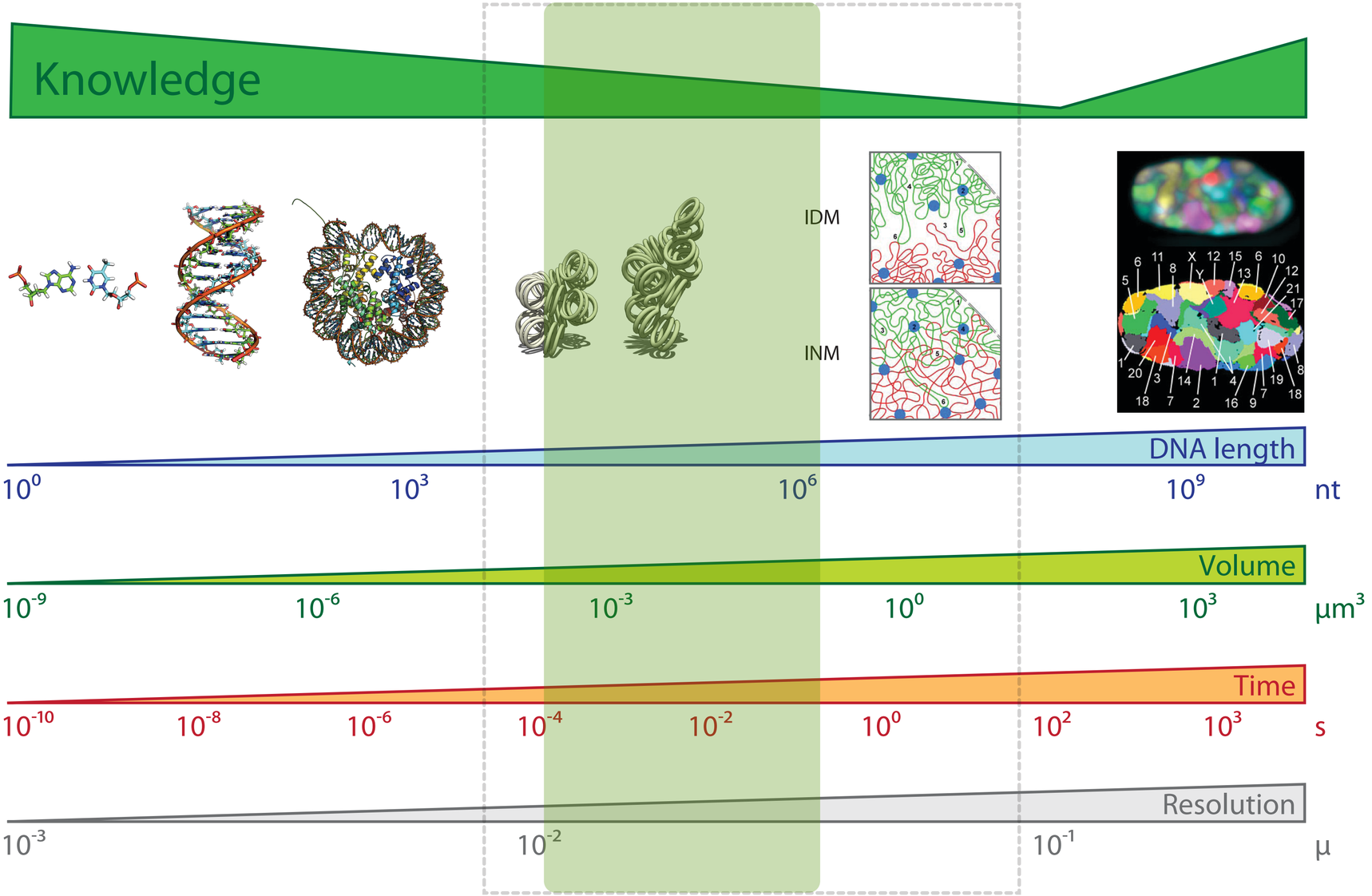
Resolution Gap

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



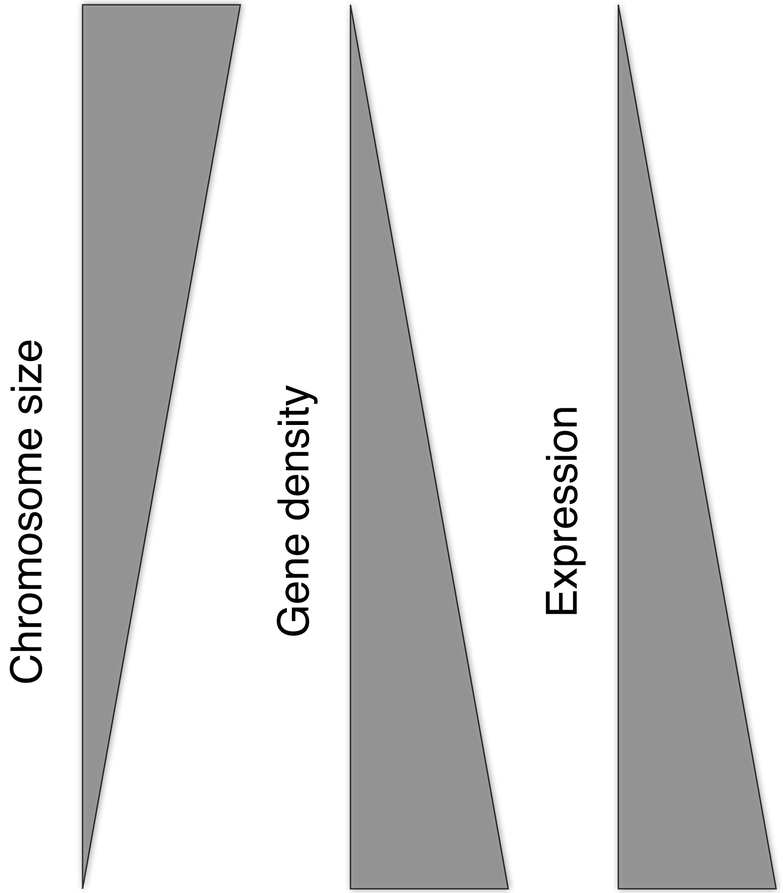
Resolution Gap

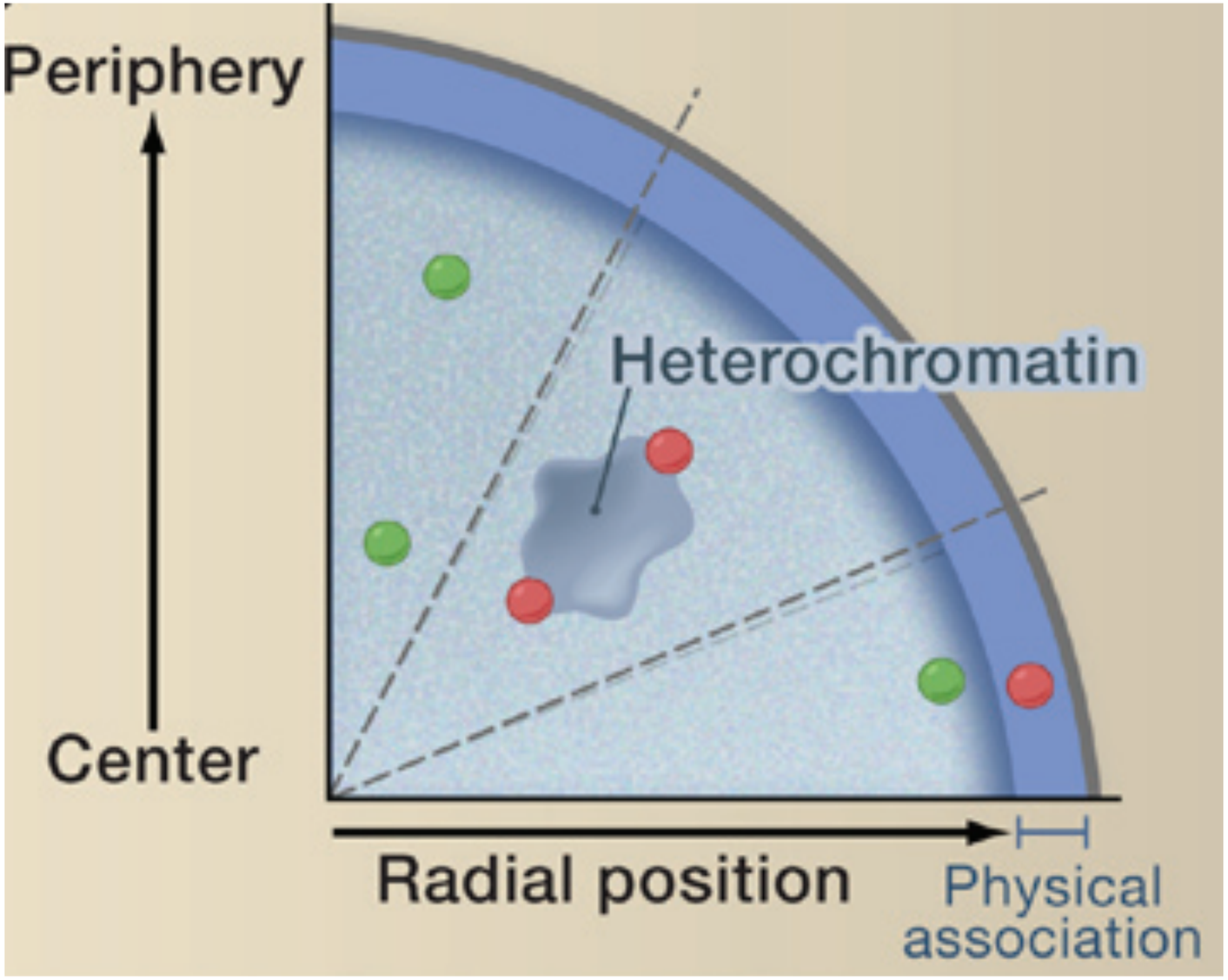
Marti-Renom, M. A. & Mirny, L. A. PLoS Comput Biol *7*, e1002125 (2011)



Level I: Radial genome organization

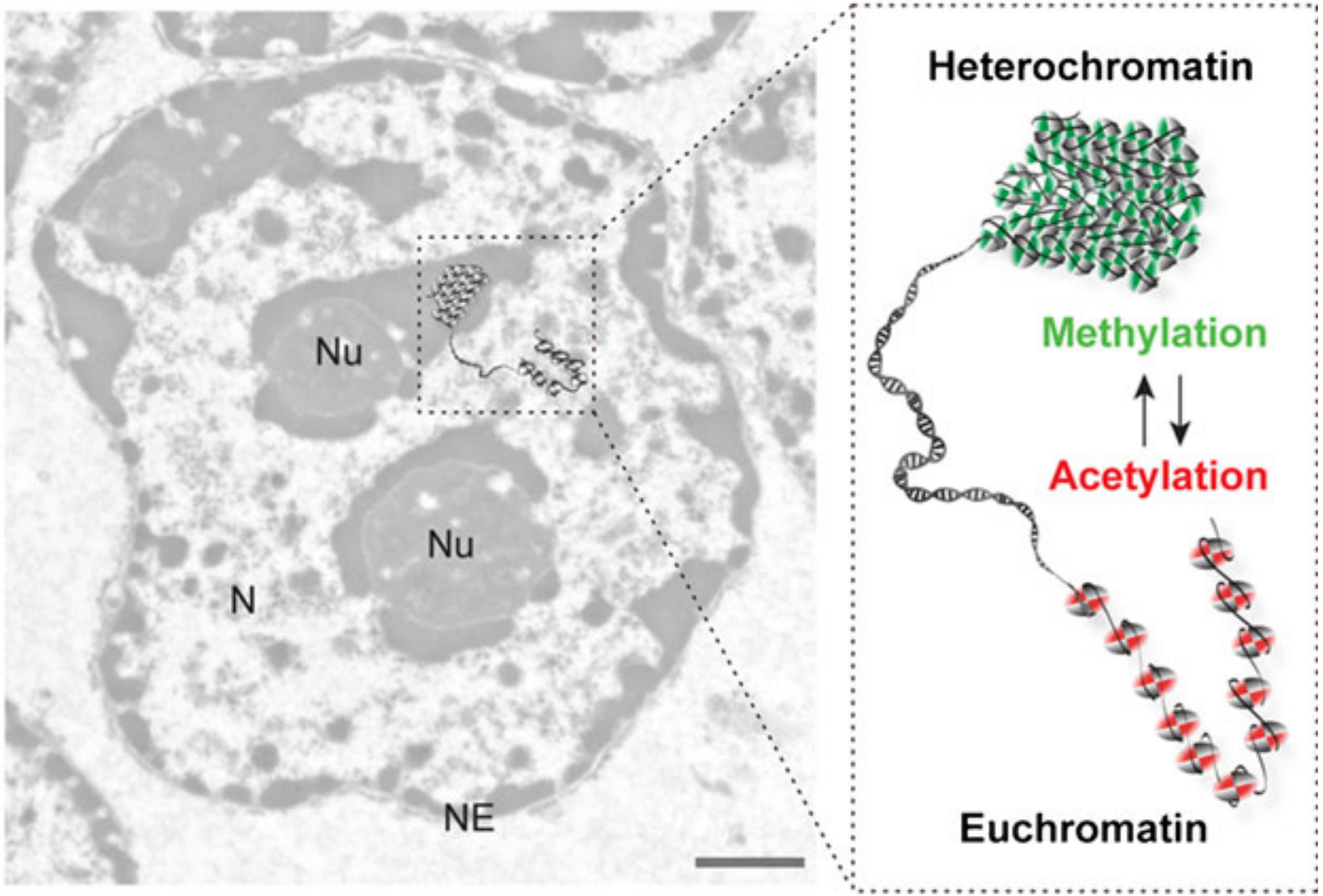
Takizawa, T., Meaburn, K. J. & Misteli, T. The meaning of gene positioning. Cell 135, 9–13 (2008).



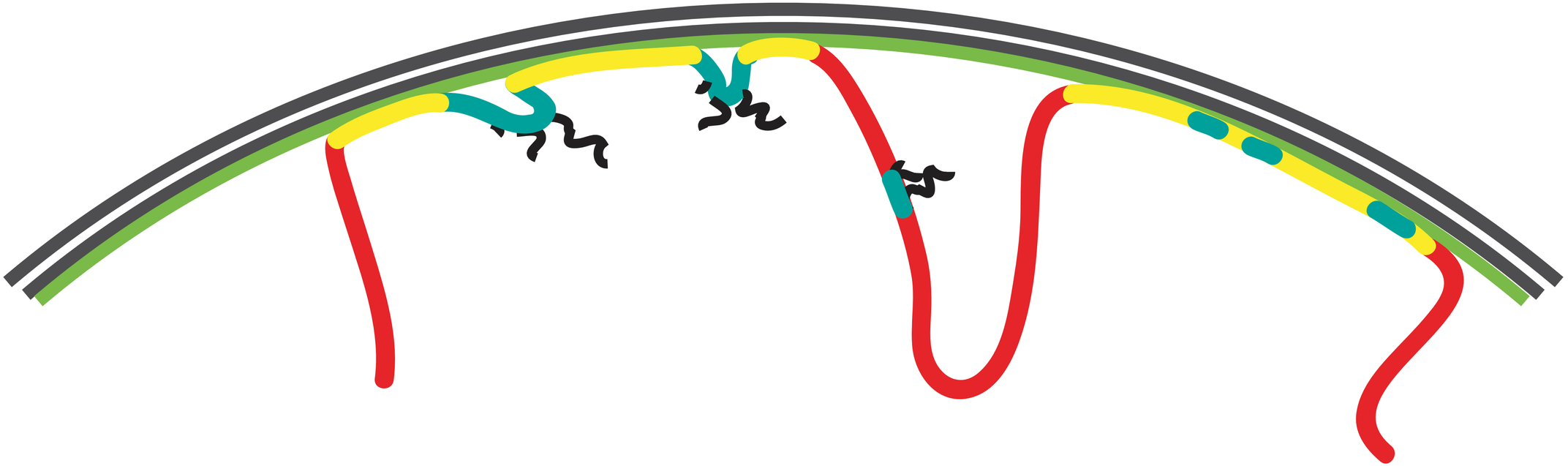


Level II: Euchromatin vs heterochromatin

Electron microscopy



Level III: Lamina-genome interactions



nuclear membrane
nuclear lamina

internal chromatin (mostly active)
lamina-associated domains (repressed)

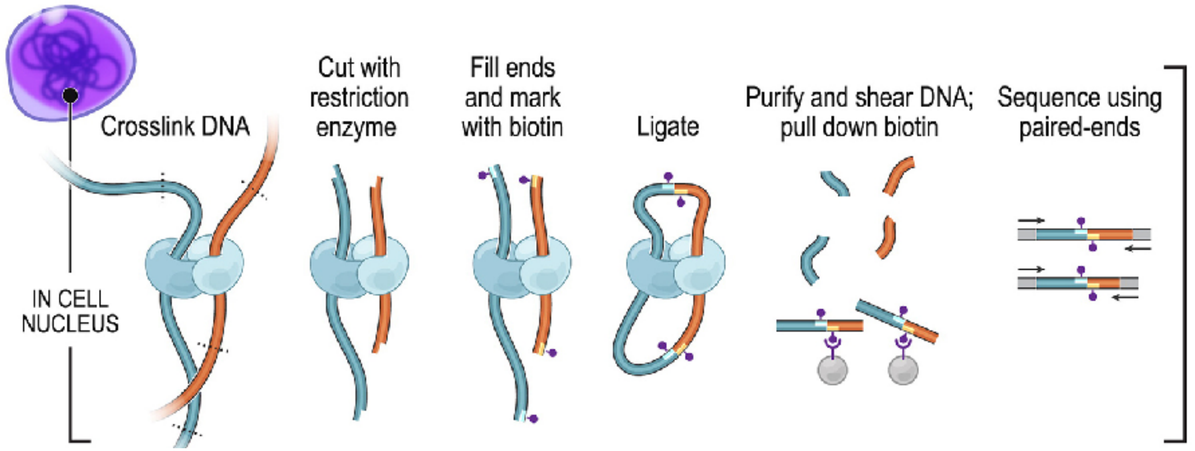
Genes

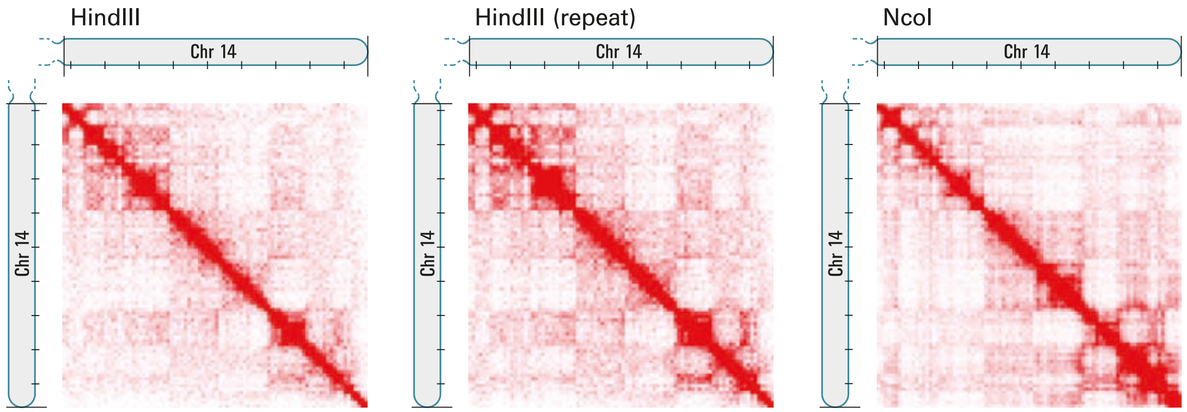
mRNA

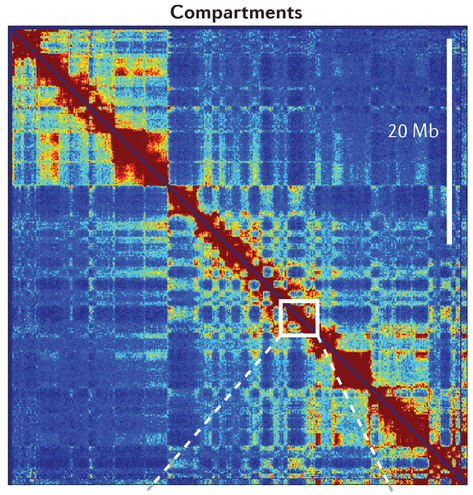
Adapted from Molecular Cell 38, 603-613, 2010

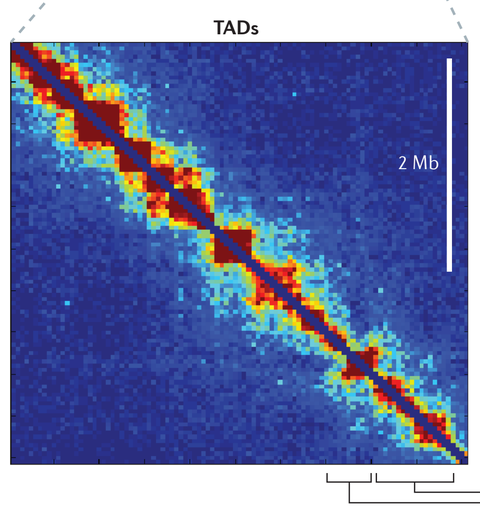
Level IV: Higher-order organization

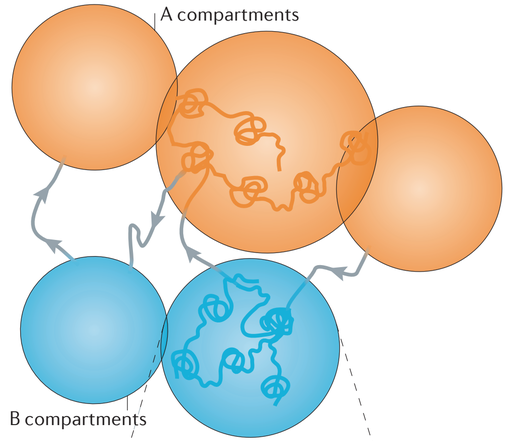
Dekker, J., Marti-Renom, M. A. & Mirny, L. A. Nat Rev Genet 14, 390–403 (2013).

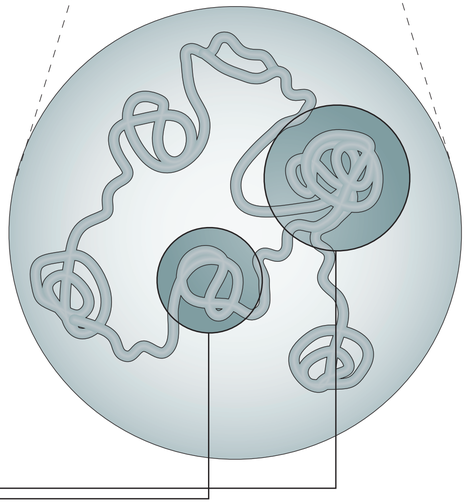




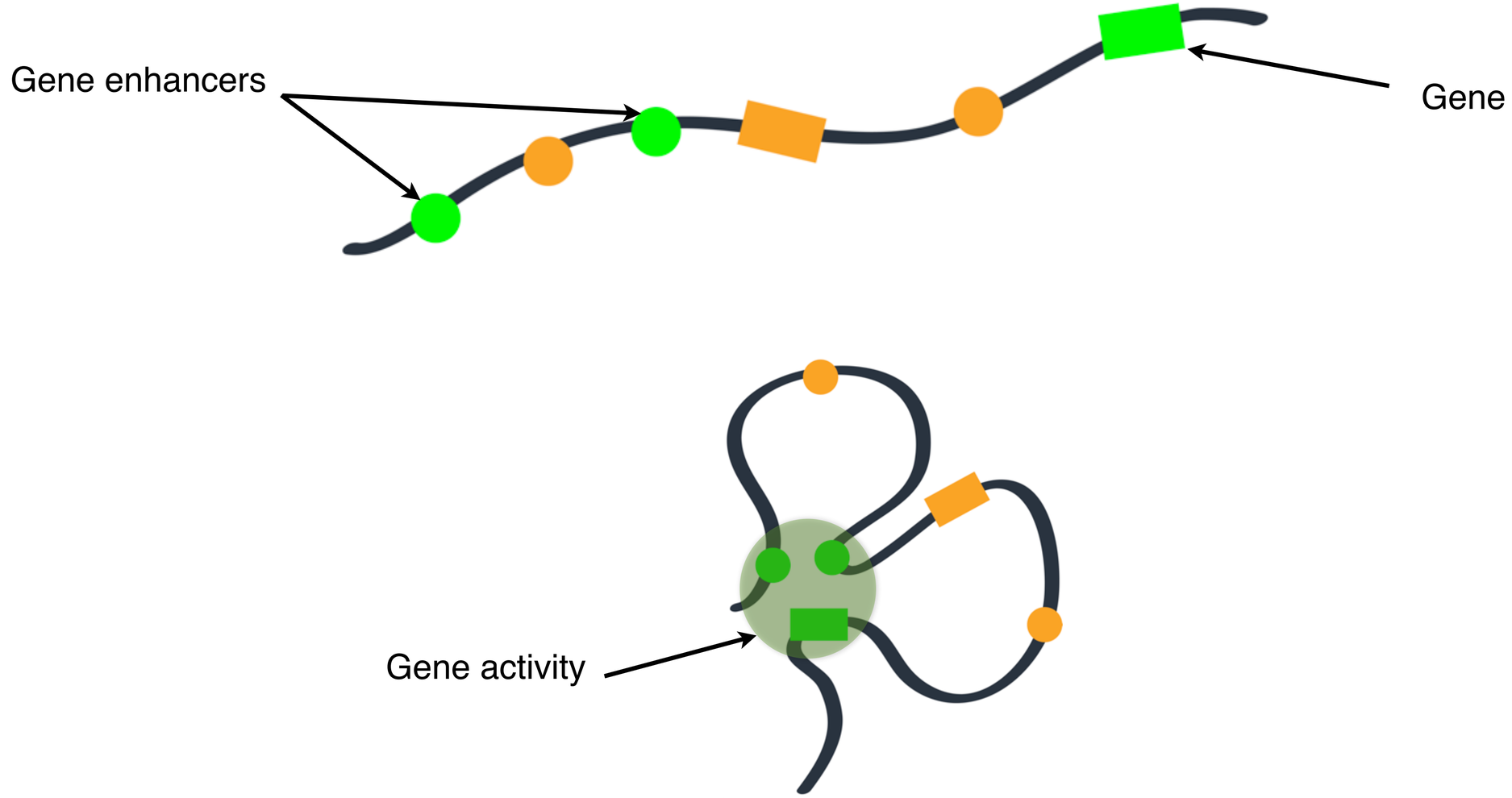






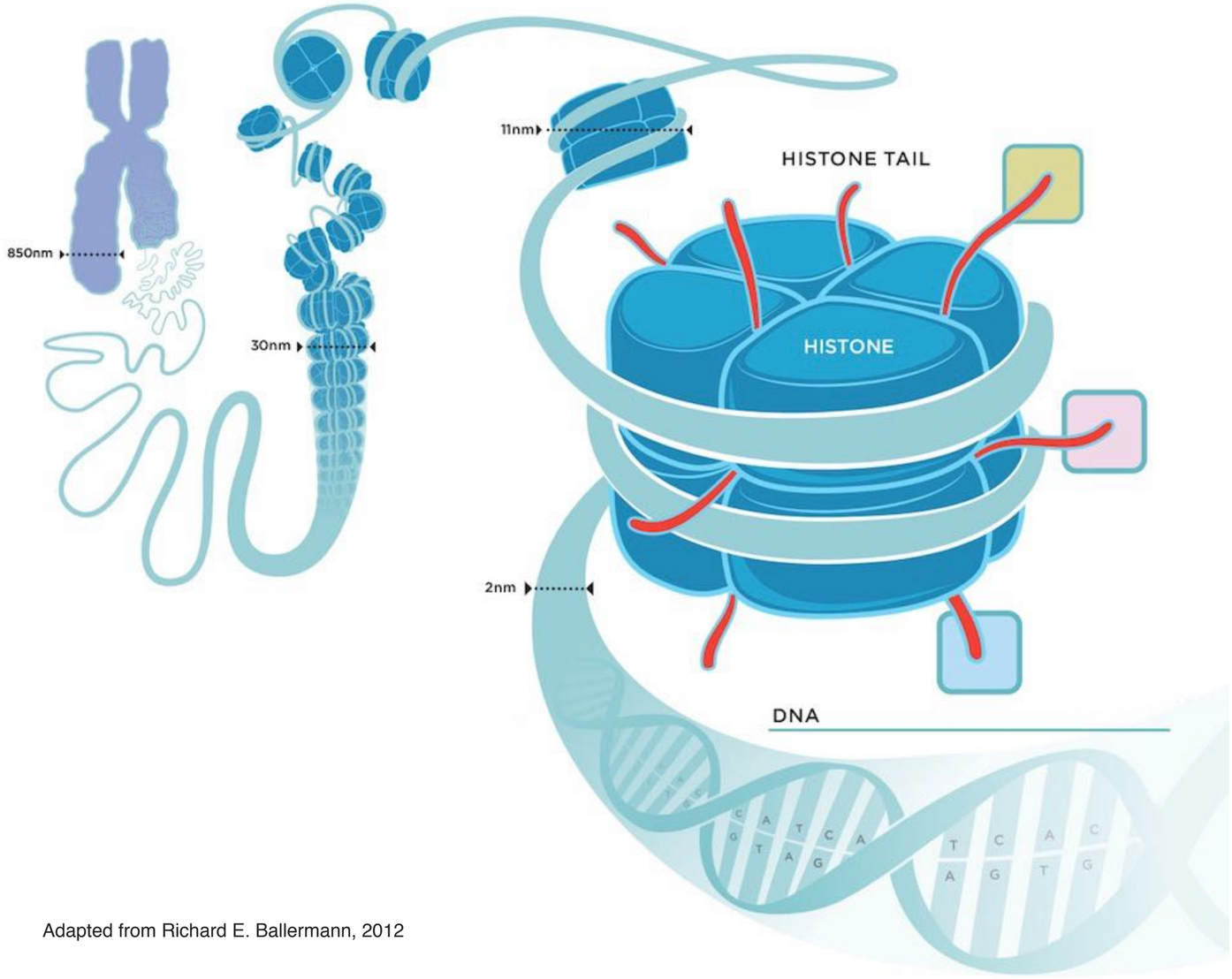


Level V: Chromatin loops



Level VI: Nucleosome

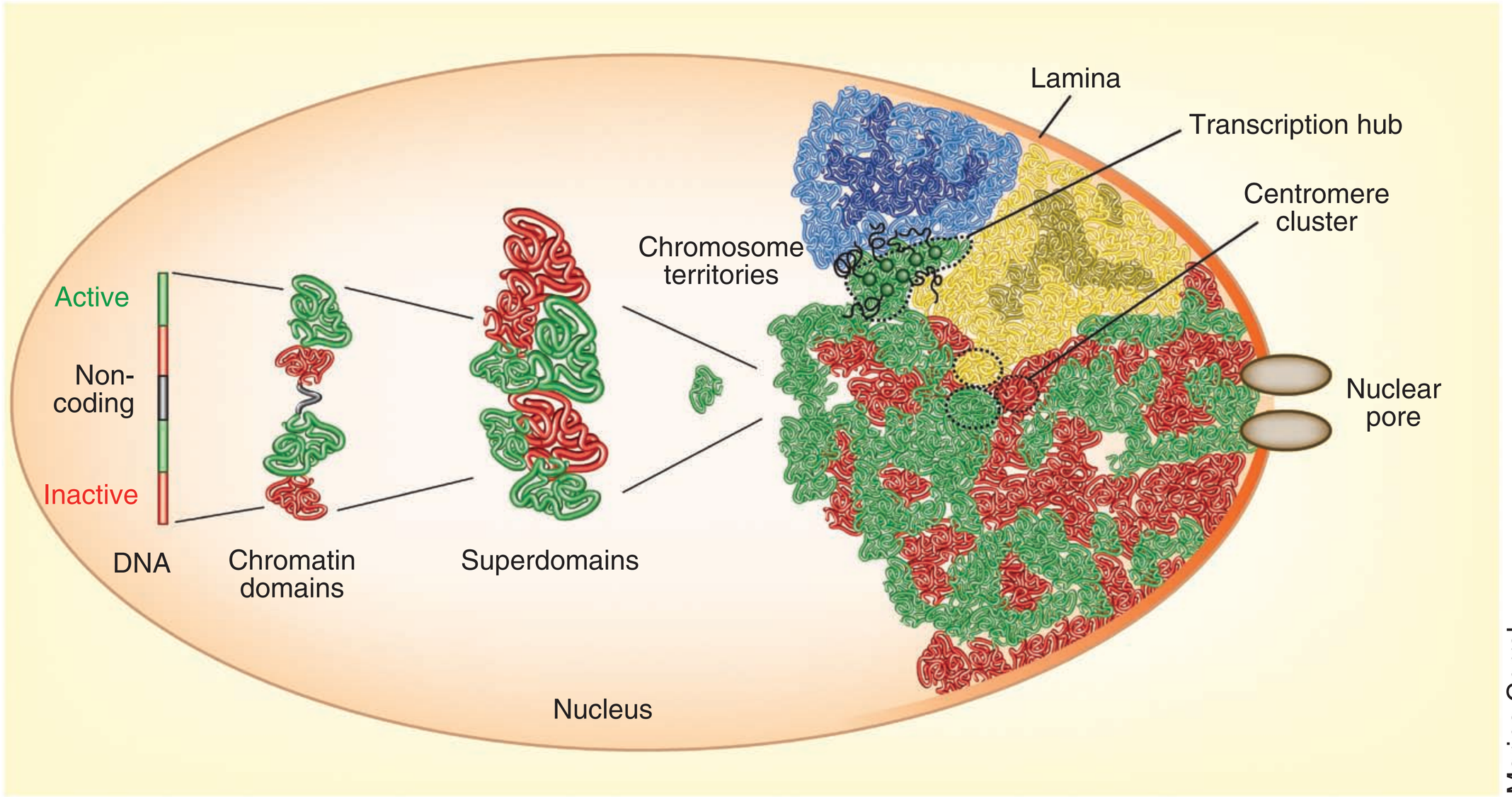
Chromosome **Chromatin fibre** **Nucleosome**



Adapted from Richard E. Ballermann, 2012

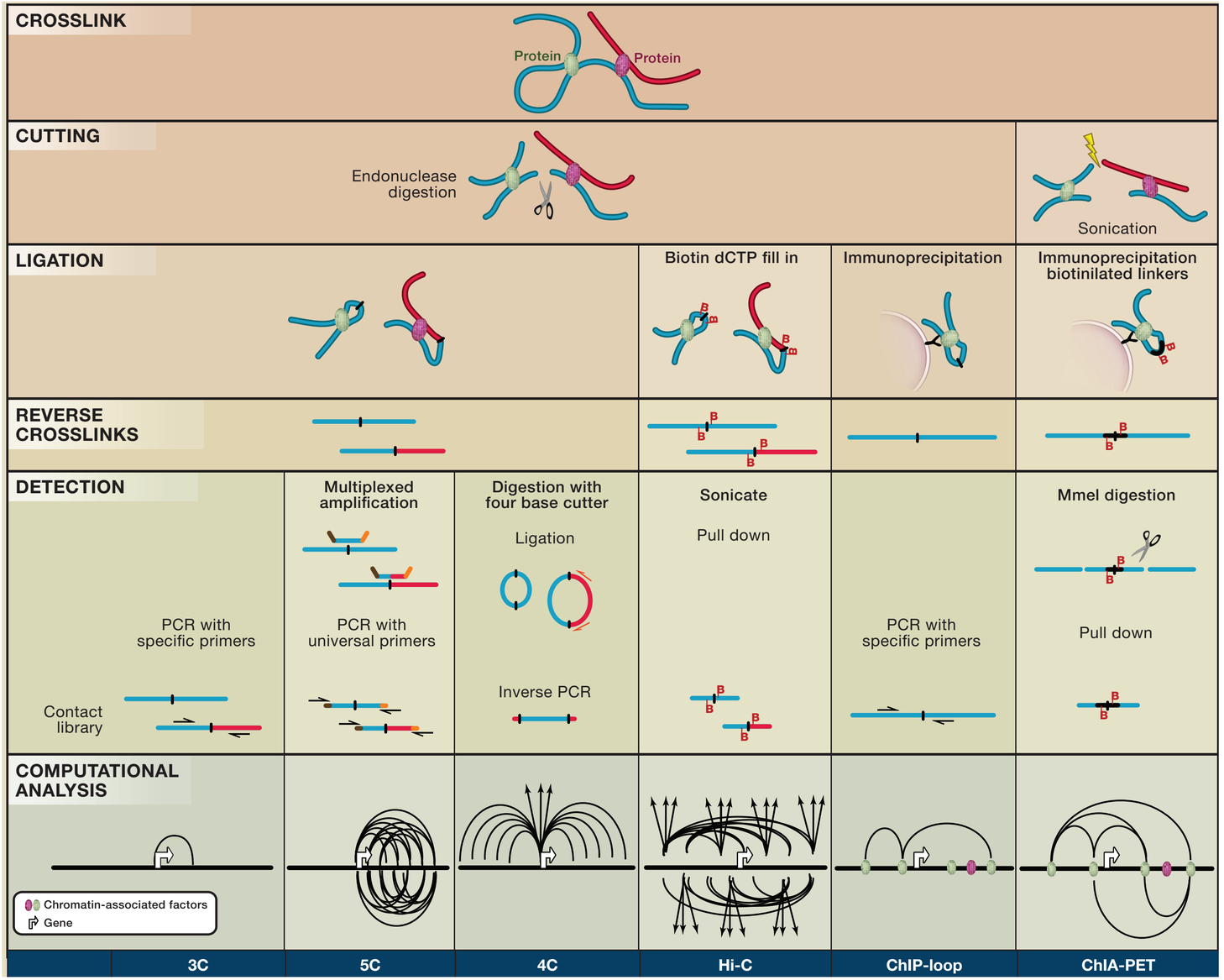
Complex genome organization

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



Marina Corral

Chromosome Conformation Capture



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

ARTICLE

doi:10.1038/nature12593

Single-cell Hi-C reveals cell-to-cell variability in chromosome structure

Takashi Nagano[1]*, Yaniv Lubling[2]*, Tim J. Stevens[3]*, Stefan Schoenfelder[1], Eitan Yaffe[2], Wendy Dean[4], Ernest D. Laue[3], Amos Tanay[2] & Peter Fraser[1]

LETTER

doi:10.1038/nature20158

Capturing pairwise and multi-way chromosomal conformations using chromosomal walks

Pedro Olivares-Chauvet[1], Zohar Mukamel[1], Aviezer Lifshitz[1], Omer Schwartzman[1], Noa Oded Elkayam[1], Yaniv Lubling[1], Gintaras Deikus[2], Robert P. Sebra[2] & Amos Tanay[1]

nature genetics ARTICLES

https://doi.org/10.1038/s41588-018-0161-5

Enhancer hubs and loop collisions identified from single-allele topologies

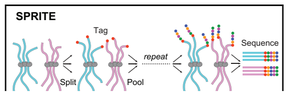
Amin Allahyar[1,2,7], Carlo Vermeulen[3,7], Britta A. M. Bouwman[3], Peter H. L. Krijger[3], Marjon J. A. M. Verstegen[3], Geert Geeven[3], Melissa van Kranenburg[3], Mark Pieterse[3], Roy Straver[1], Judith H. I. Haarhuis[4], Kees Jalink[5], Hans Teunissen[6], Ivo J. Renkens[1], Wigard P. Kloosterman[1], Benjamin D. Rowland[4], Elzo de Wit[4], Jeroen de Ridder[1]* and Wouter de Laat[3]*

Resource

Cell

Higher-Order Inter-chromosomal Hubs Shape 3D Genome Organization in the Nucleus

Graphical Abstract



Authors

Sofia A. Quinodoz, Noah Ollikainen, Barbara Tabak, ..., Patrick McDonel, Manuel Garber, Mitchell Guttman

Correspondence

mguttman@caltech.edu

nature COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-06961-0 OPEN

Chromatin conformation analysis of primary patient tissue using a low input Hi-C method

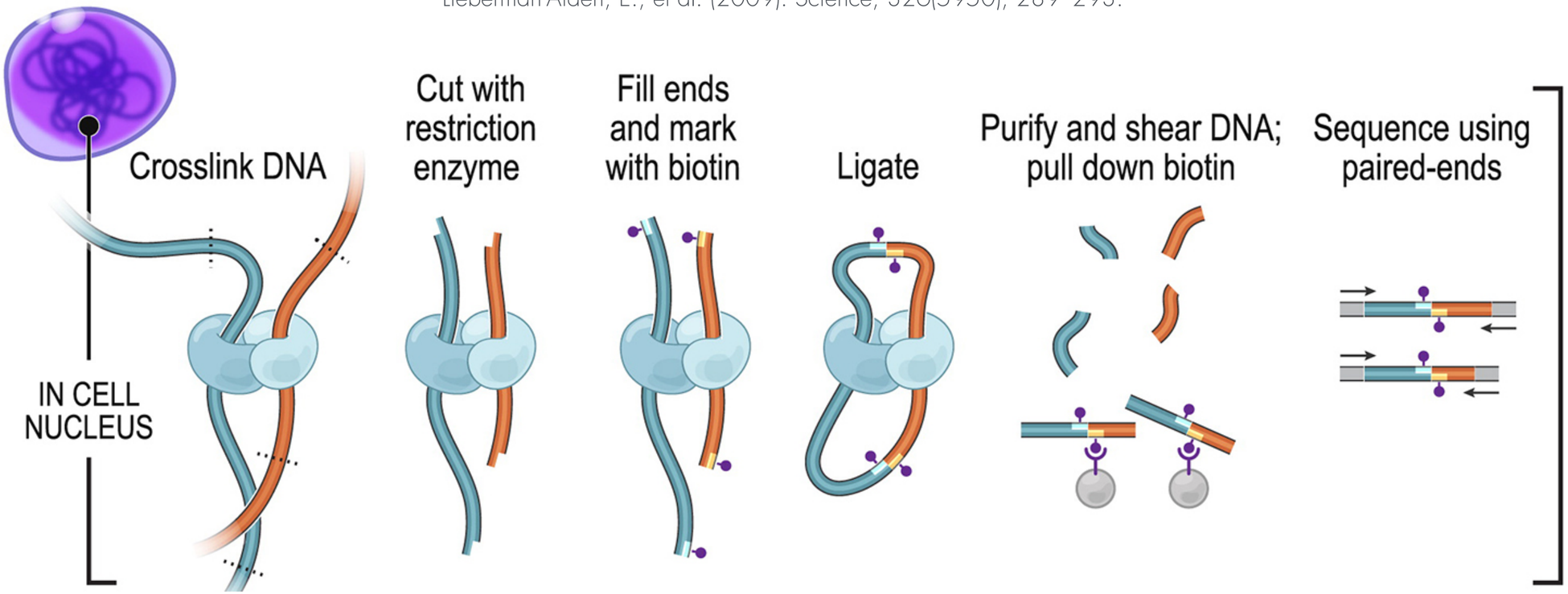
Noelia Díaz[1], Kai Kruse[1], Tabea Erdmann[2], Annette M. Staiger[3,4,5], German Ott[3], Georg Lenz[2] & Juan M. Vaquerizas[1]

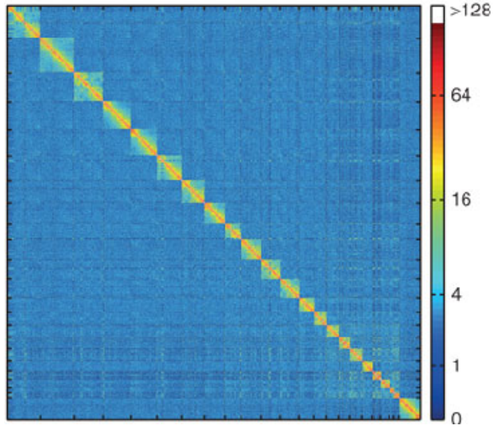
Compartment-dependent chromatin interaction dynamics revealed by liquid chromatin Hi-C

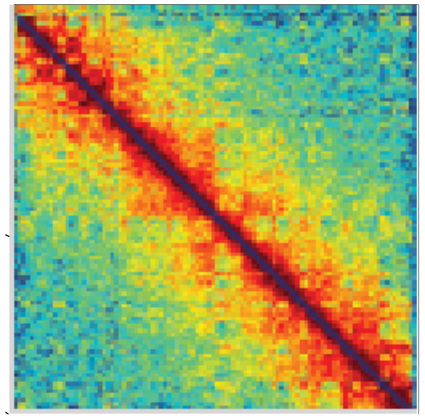
Houda Belaghzal[1*], Tyler Borrman[2*], Andrew D. Stephens[3], Denis L. Lafontaine[1], Sergey V. Venev[1], Zhiping Weng[2], John F. Marko[3,4], Job Dekker[1, 5, 6 #]

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.

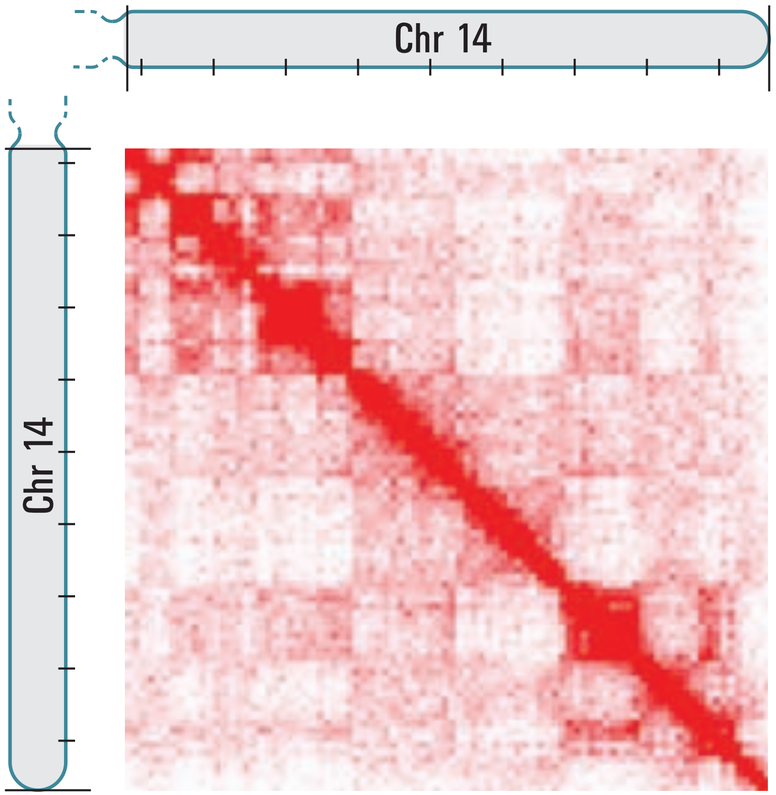


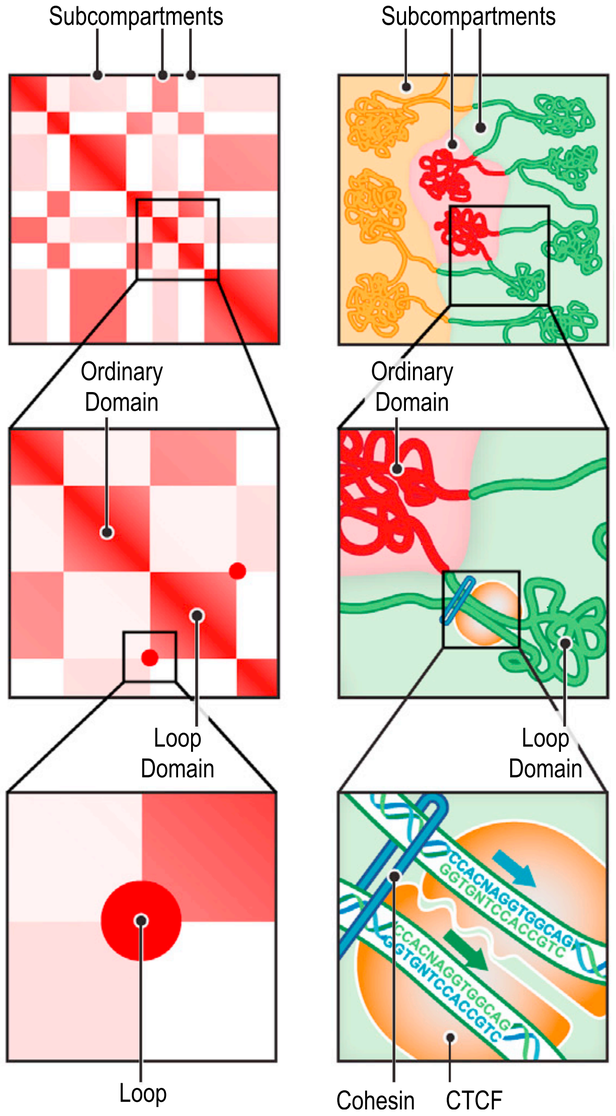




Hierarchical genome organisation

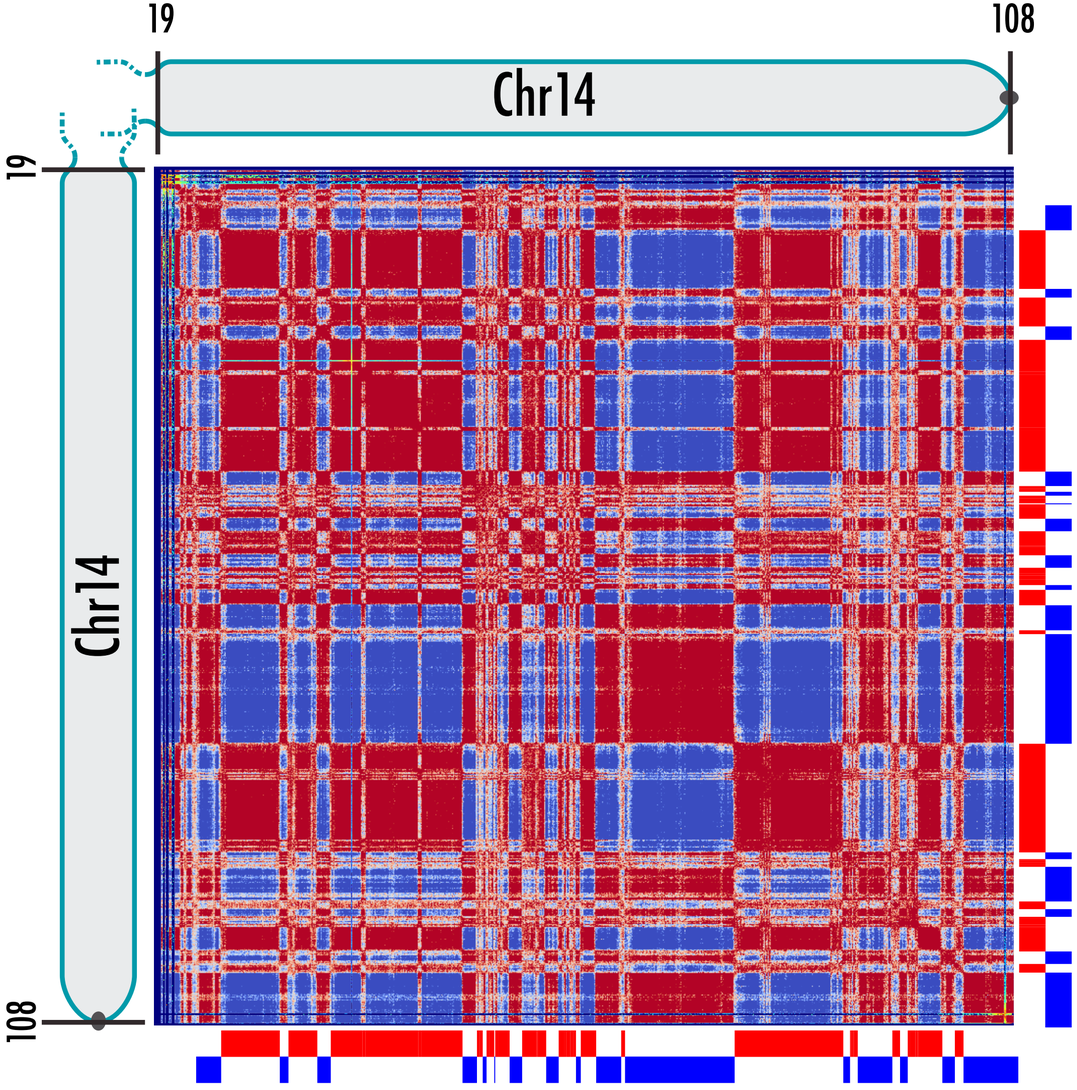
Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.
Rao, S. S. P., et al. (2014). Cell, 1–29.

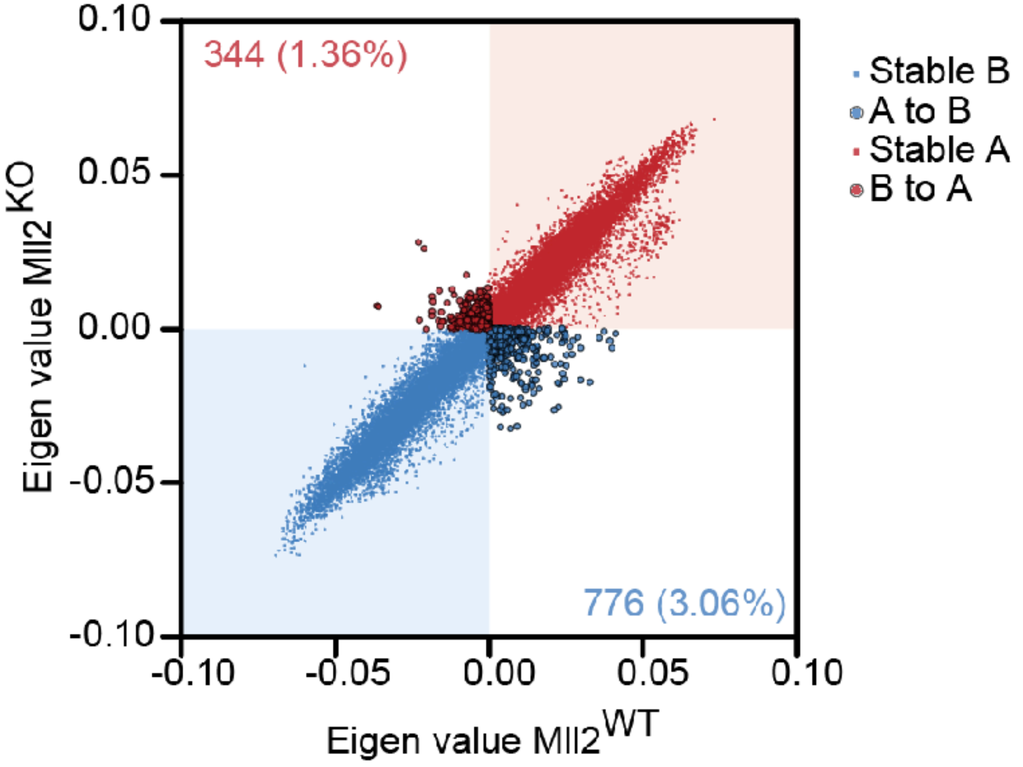




A/B Compartment

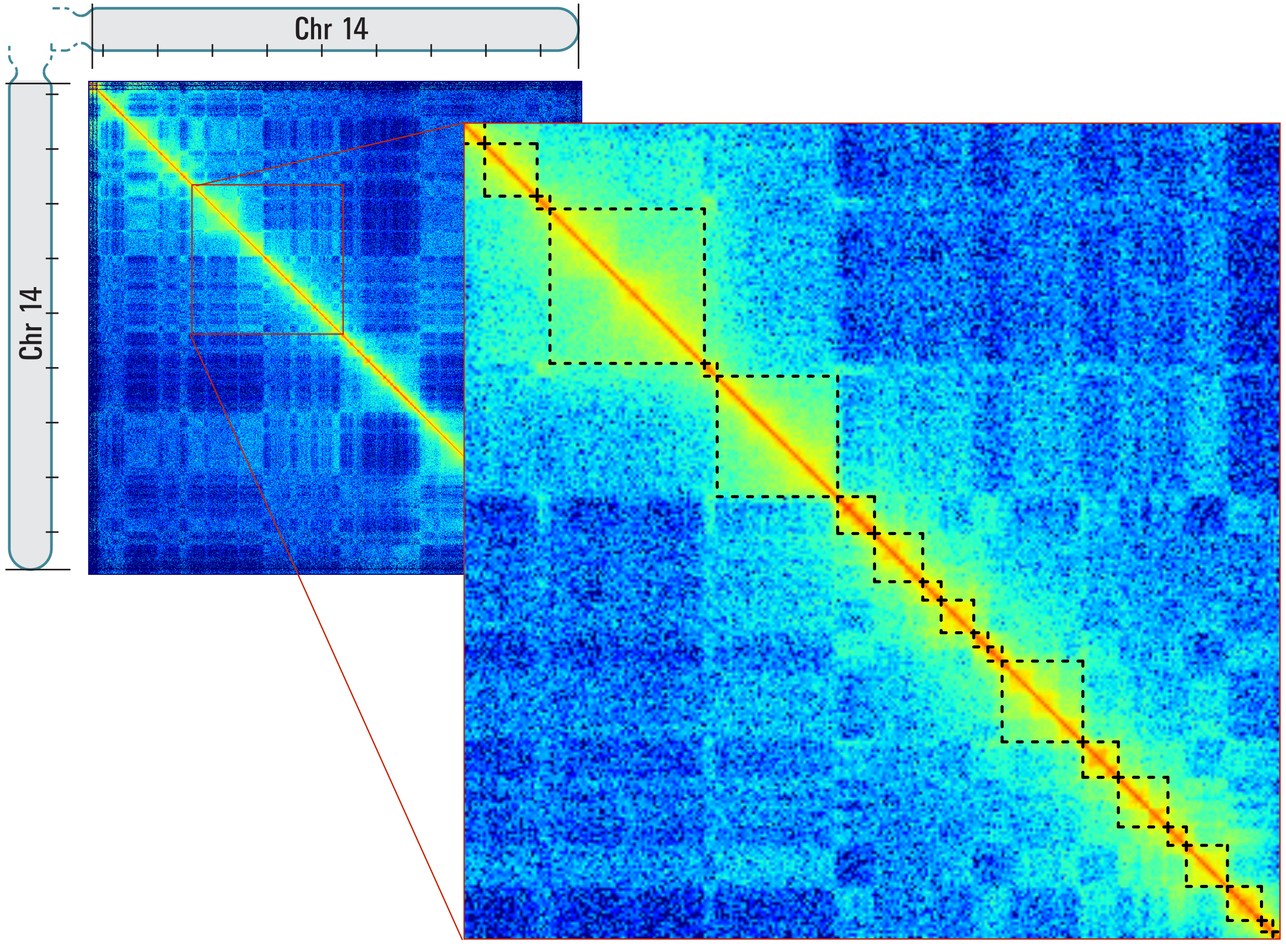
Chromosome 14

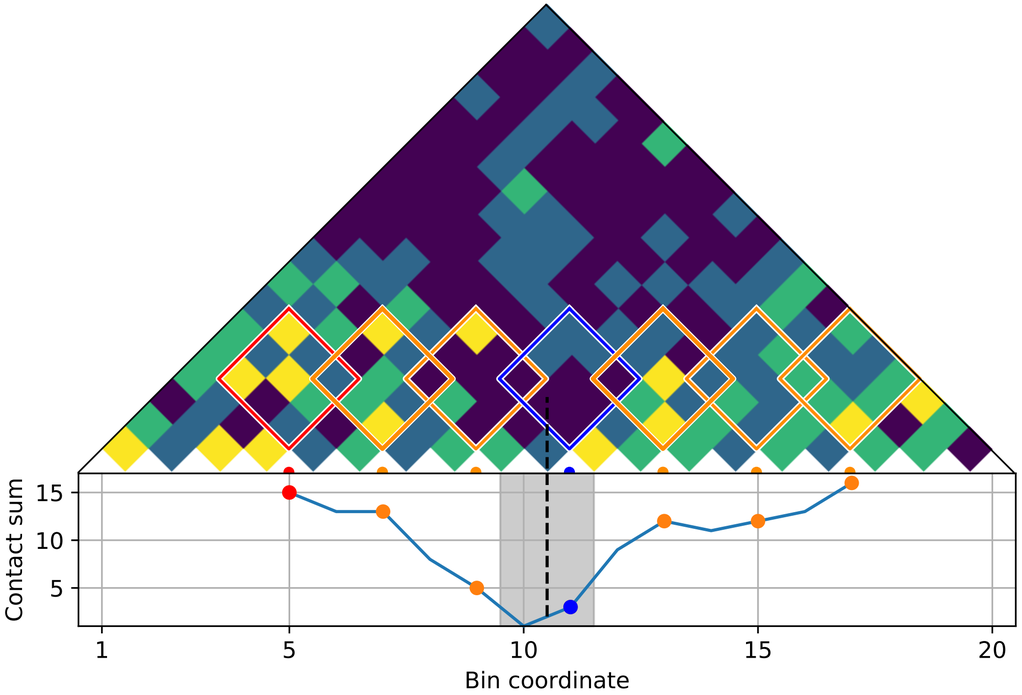




TADs

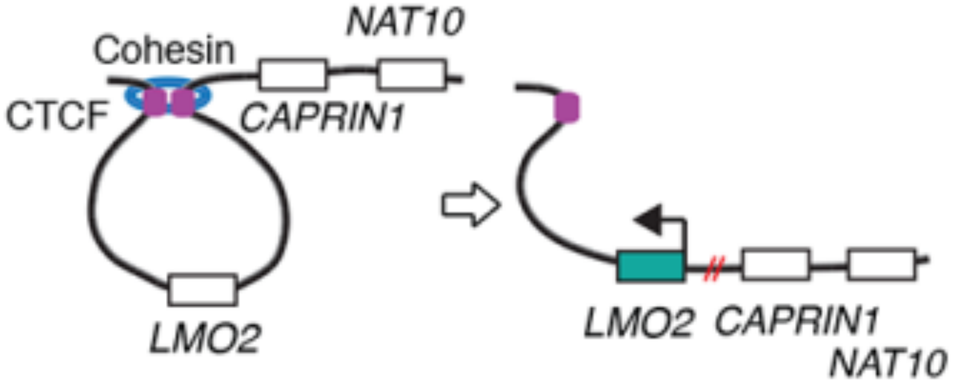
Chromosome 14

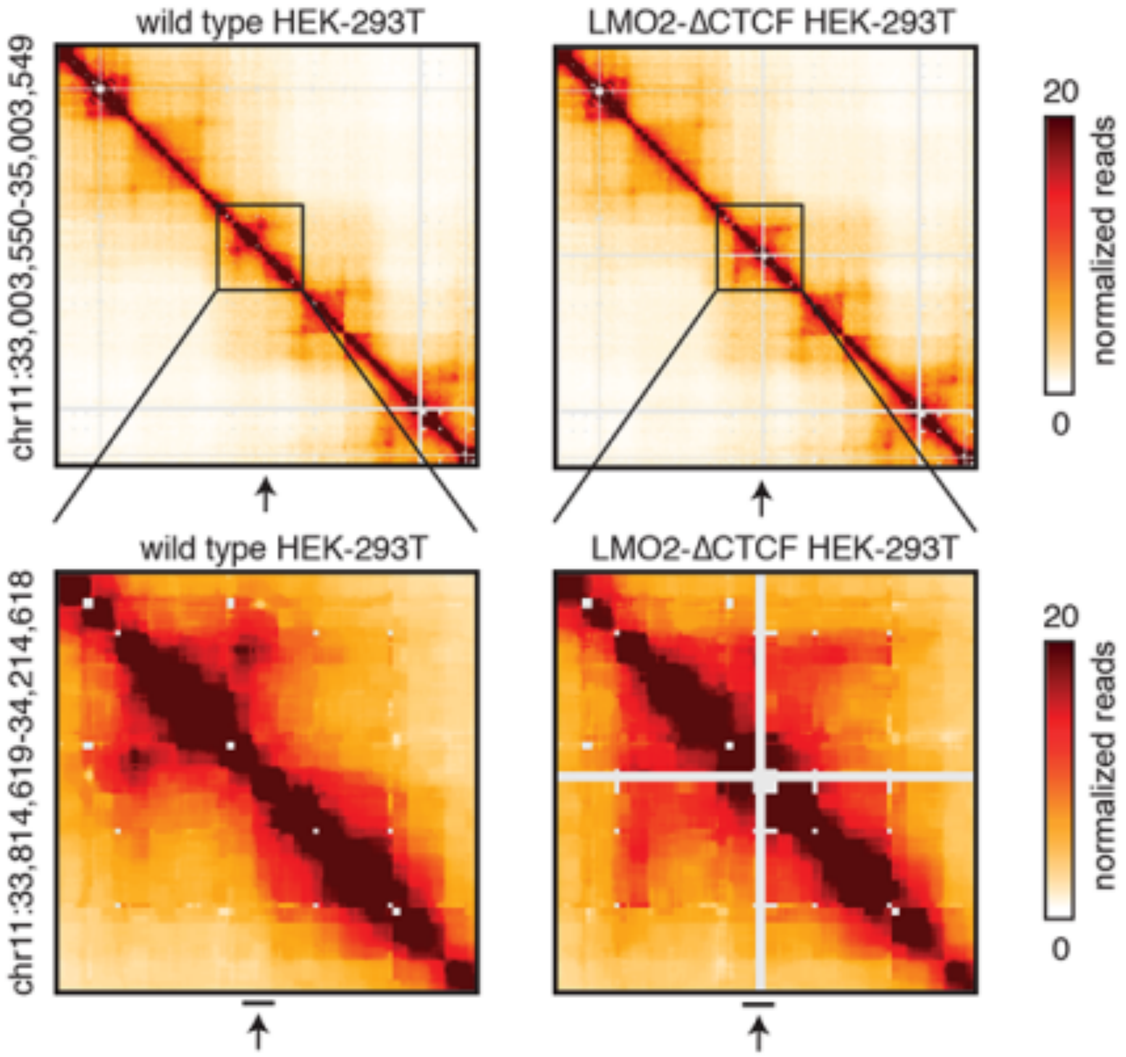




TADs are functional units

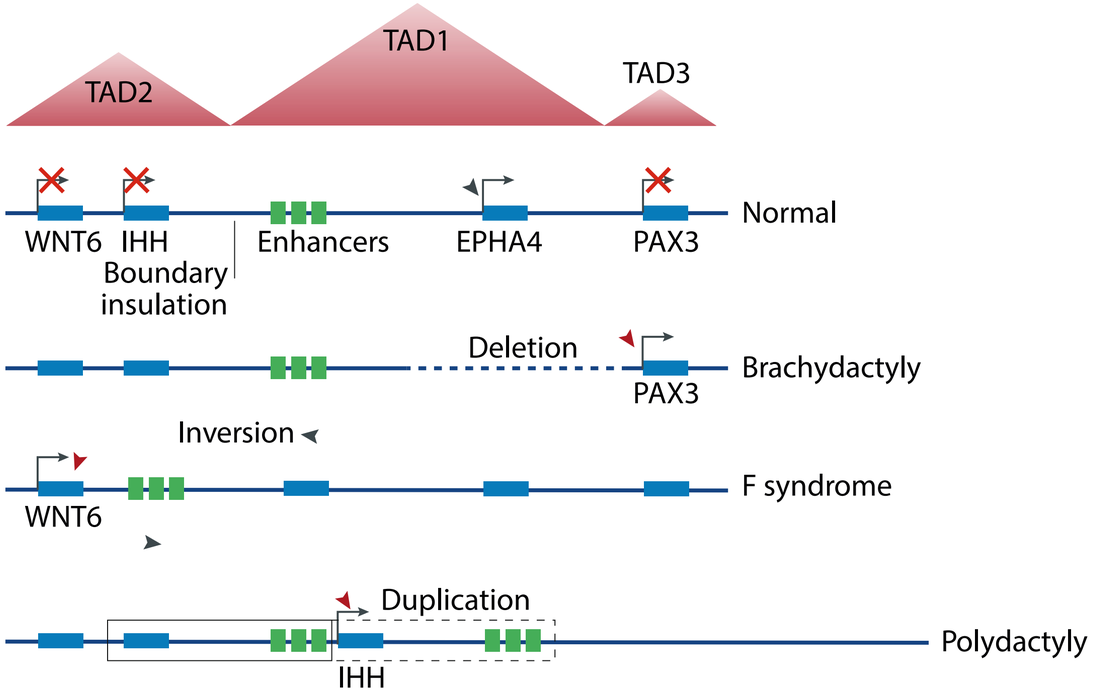
Hnisz, D., et al. (2016). Science



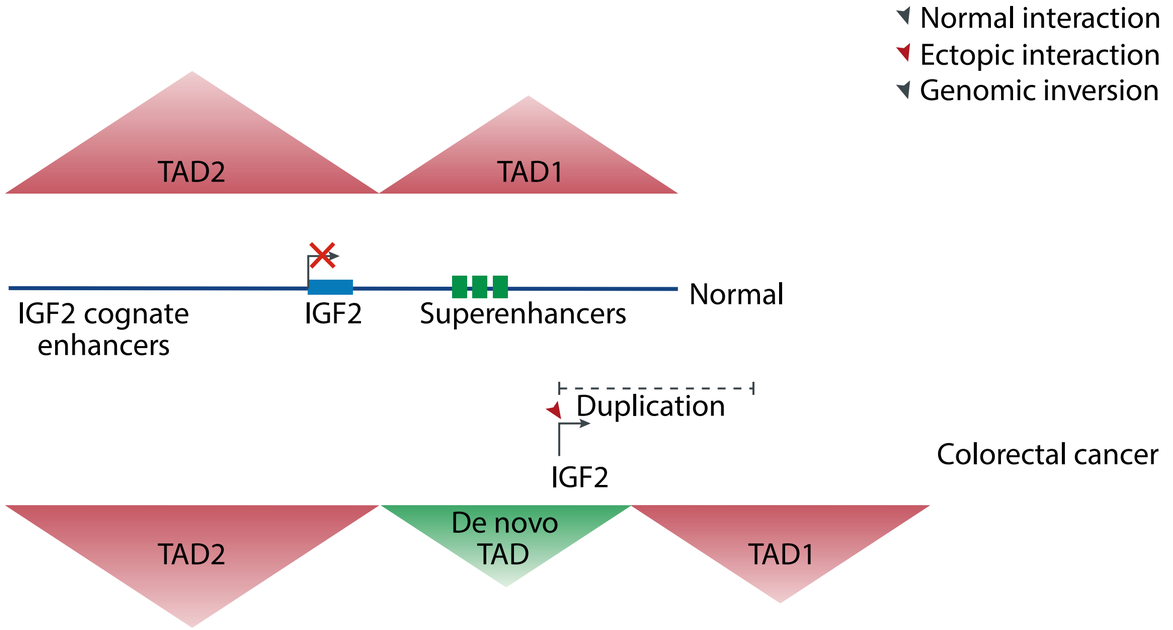


TADs are functional units

Figure adapted from Hui Zheng and Wei Xie. Nature Reviews Molecular Cell Biology (2019)



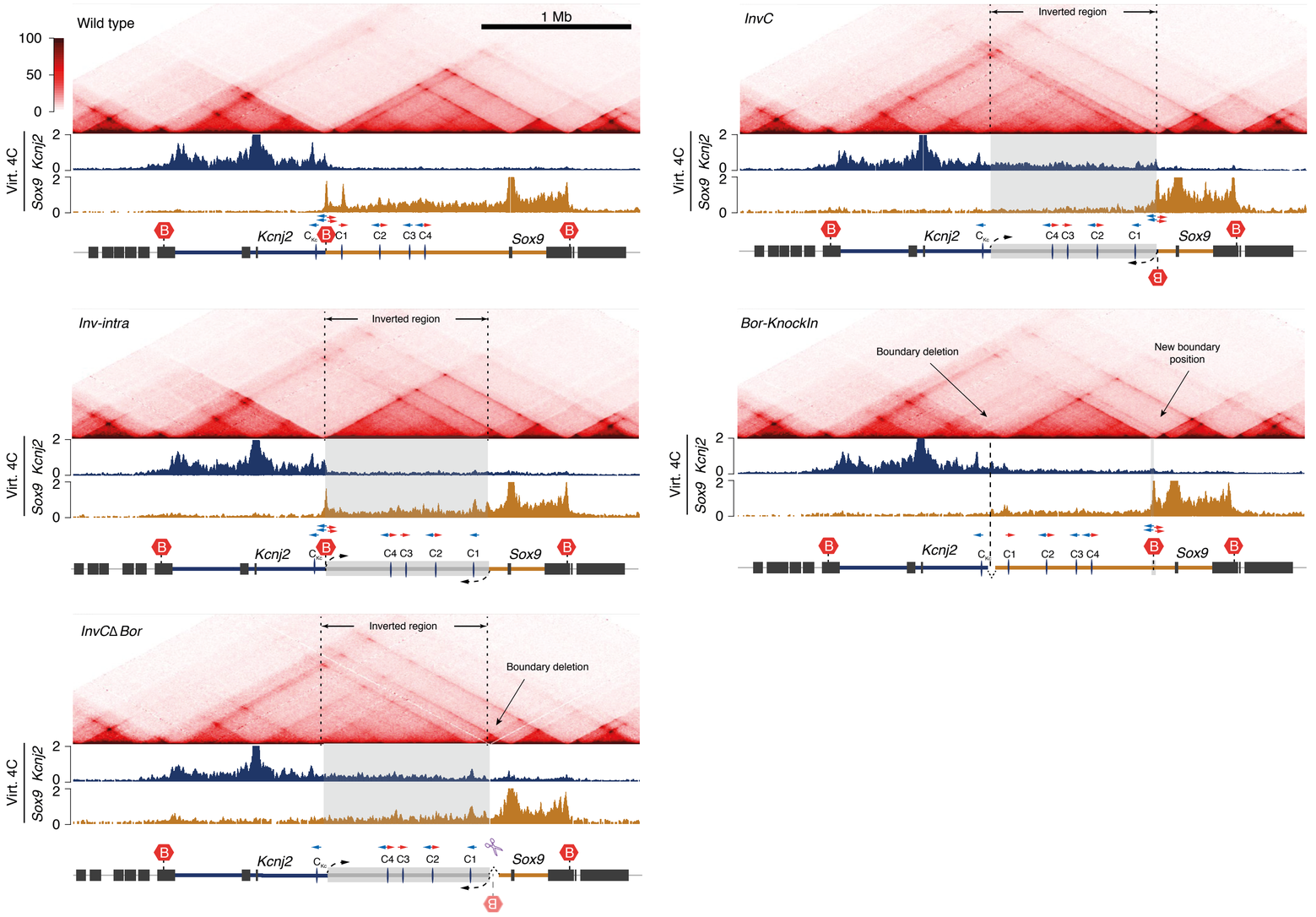
Lupianez, D. G. et al. Cell 161, 1012–1025 (2015)



Flavahan, W. A. et al. Nature 529, 110–114 (2016).

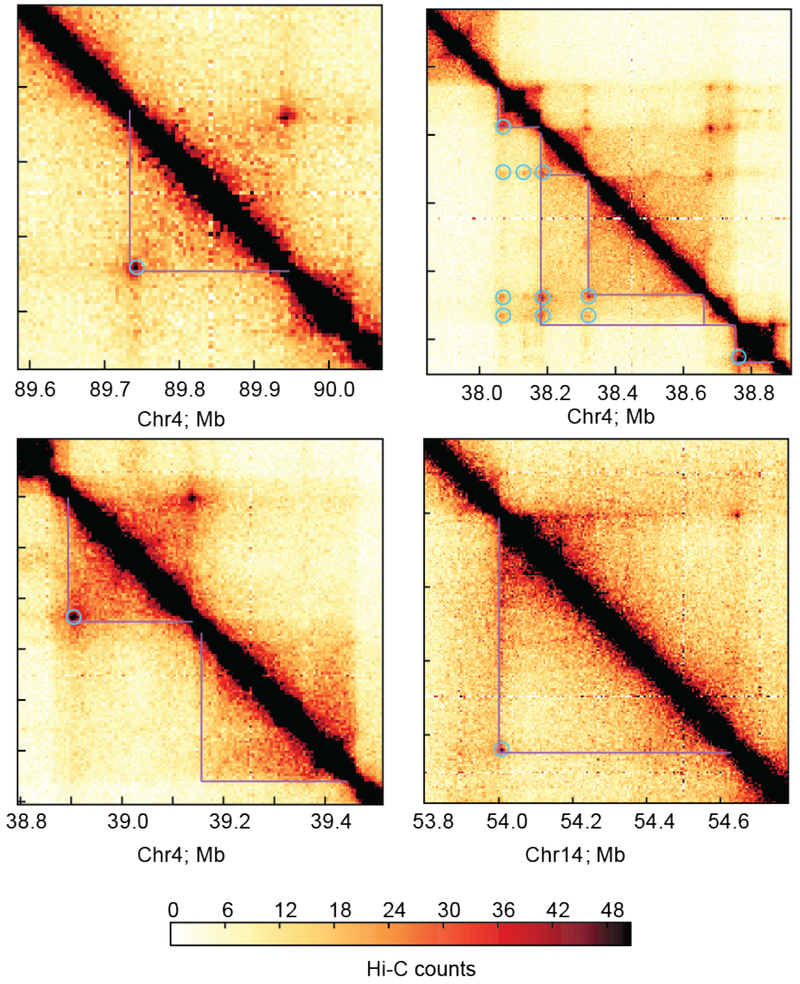
TADs are functional units

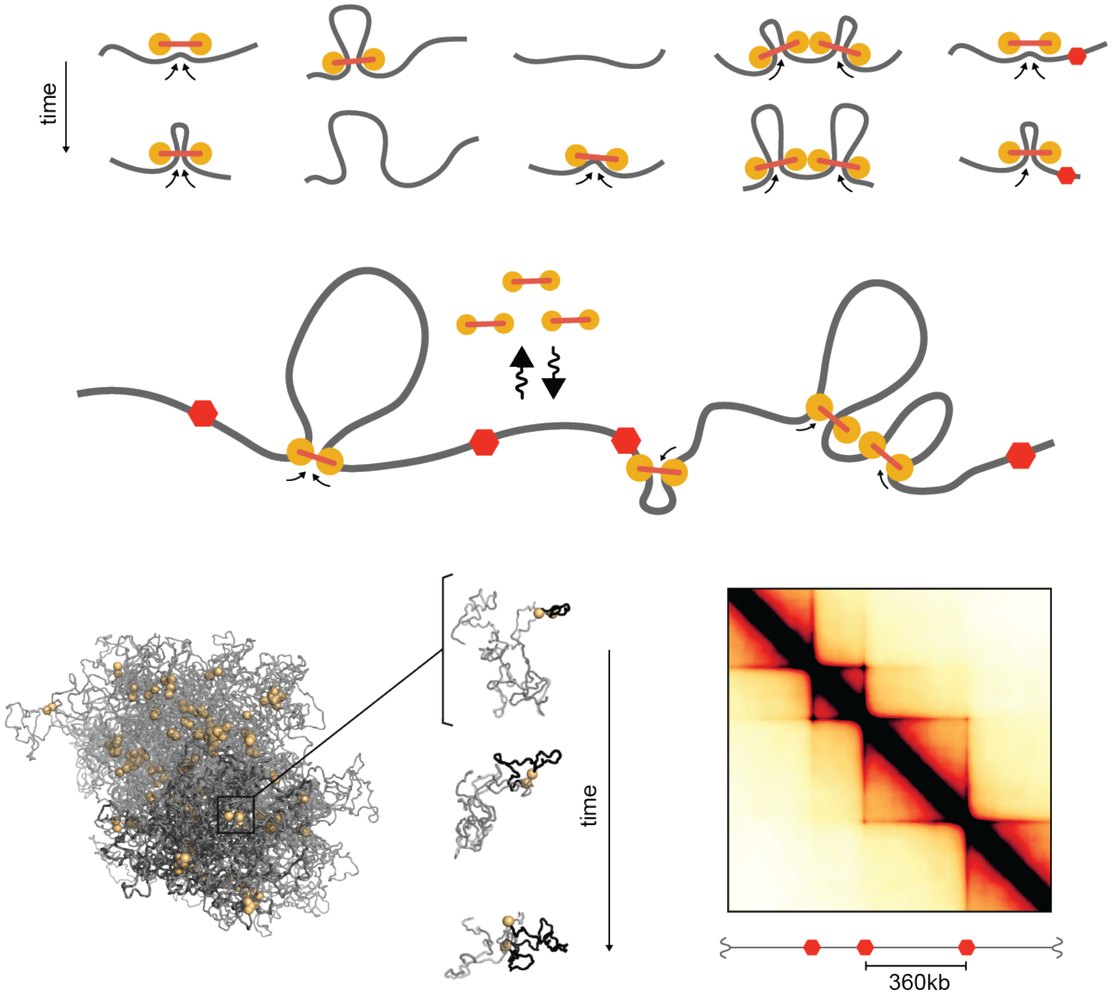
Despang, et al. (2019). Nature Genetics 51,1263–1271 (2019)



Loop-extrusion as a TAD forming mechanism

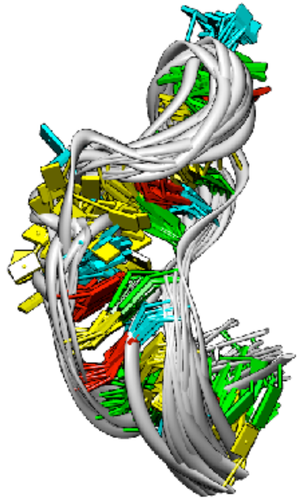
Fudenberg, G., Imakaev, M., Lu, C., Goloborodko, A., Abdennur, N., & Mirny, L. A. (2018).
Cold Spring Harb Symp Quant Biol 2017. 82: 45-55

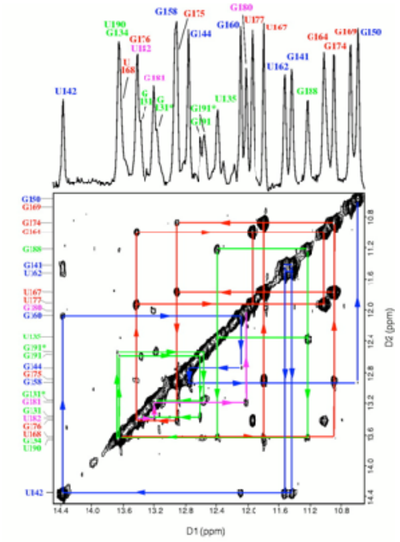




Restraint-based Modeling

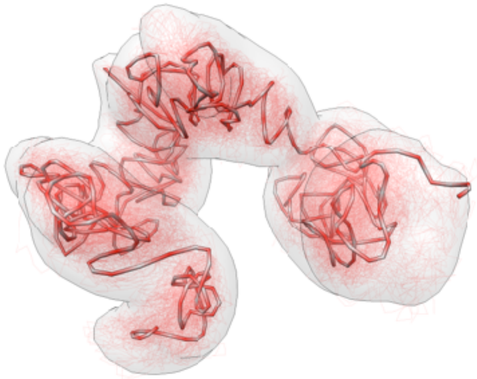
Baù, D. & Marti-Renom, M. A. Methods 58, 300–306 (2012).

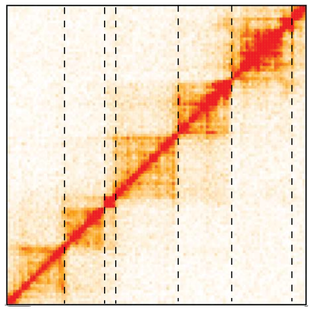




Biomolecular structure determination

2D-NOESY data





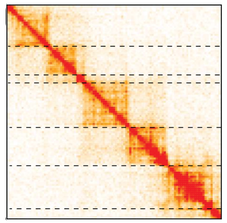
Chromosome structure determination

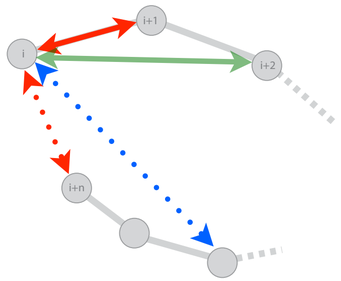
3C-based data

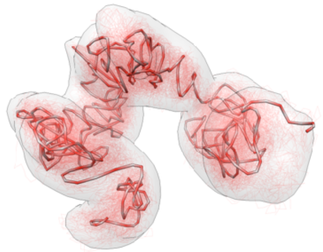
TADbit

http://3DGenomes.org

Serra, F., Baù, D. et al. PLOS CB (2017)





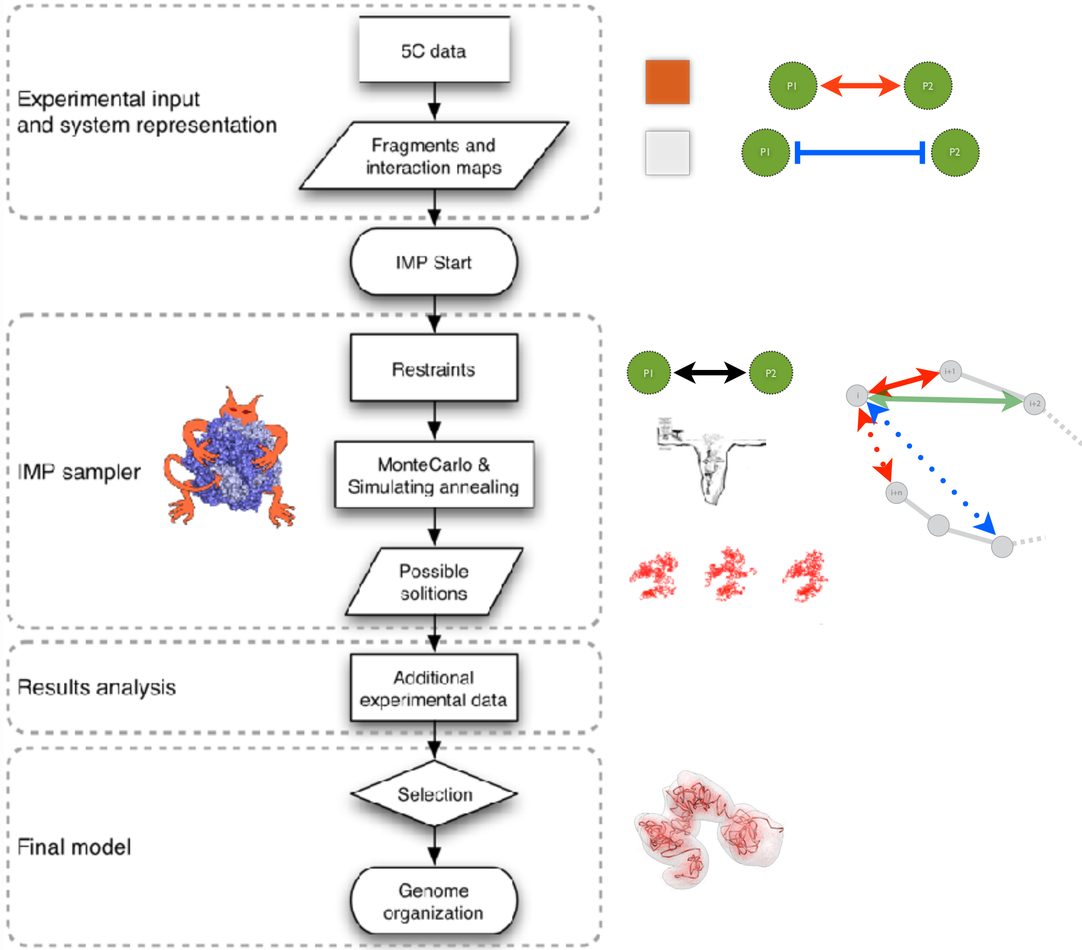


FastQ files to Maps

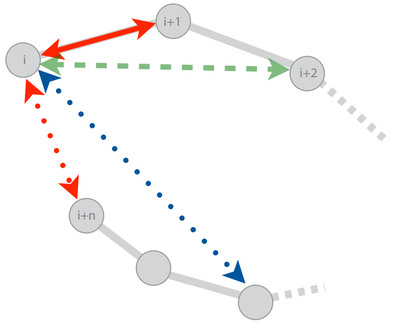
Map analysis

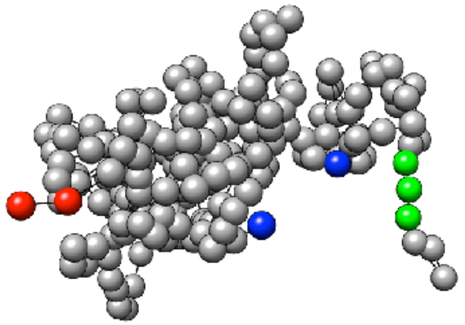
Model building

Model analysis



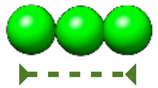
Model representation and scoring





d = d_0

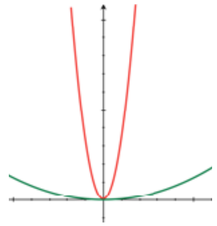
d < d_0



d > d_0

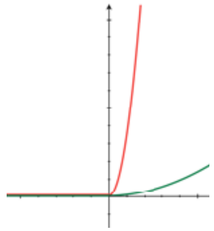
Harmonic

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



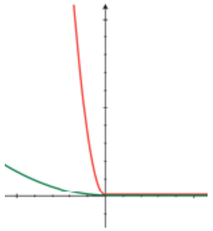
Harmonic Upper Bound

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

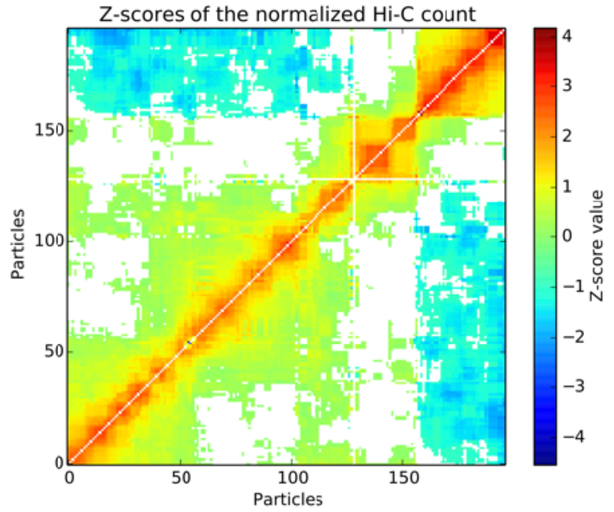


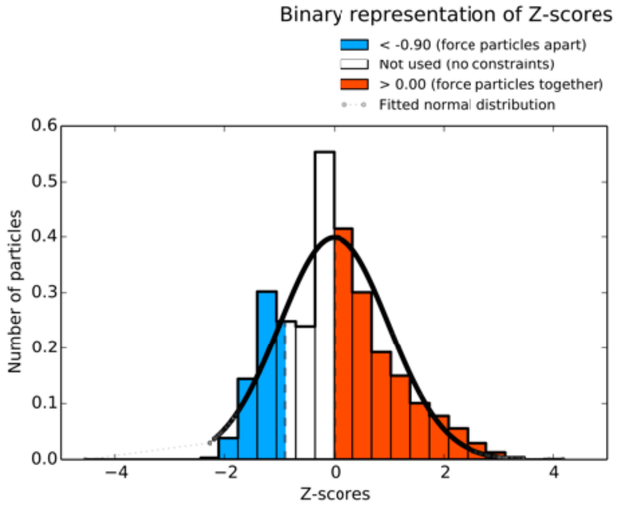
Harmonic Lower Bound

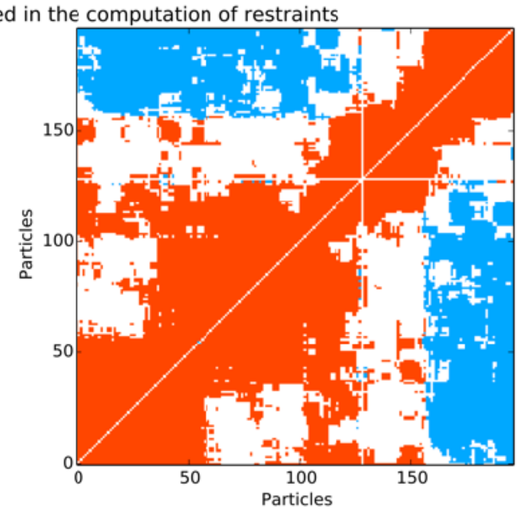
$$\begin{cases} if\ \ d_{i,j} \leq d^0_{i,j}; & lbH_{i,j} = k\left(d_{i,j} - d^0_{i,j}\right)^2 \\ if\ \ d_{i,j} > d^0_{i,j}; & lbH_{i,j} = 0 \end{cases}$$

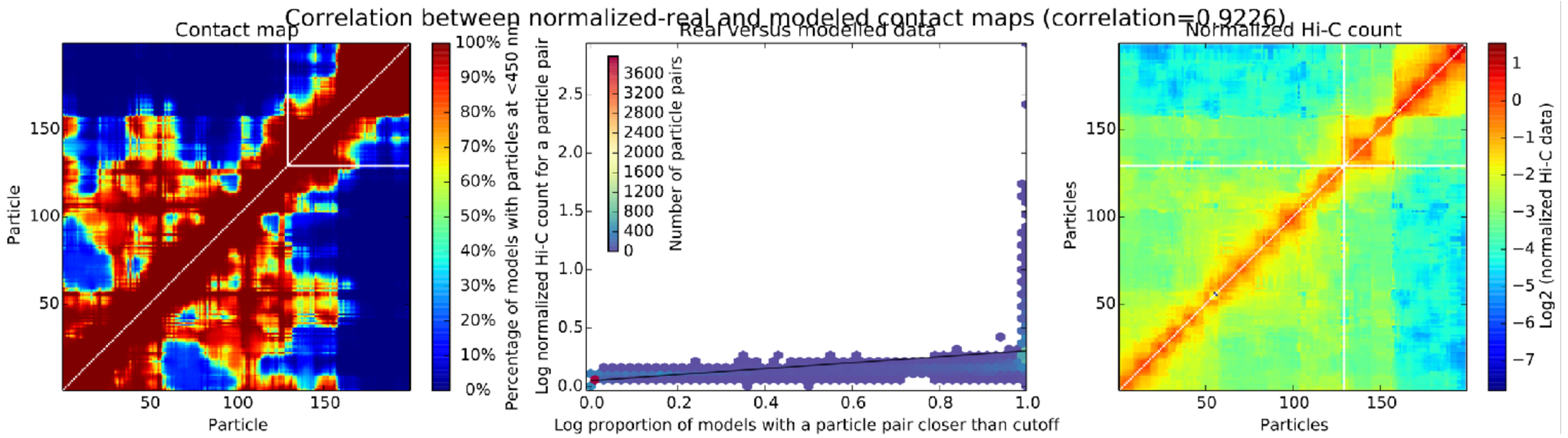


Parameter optimization

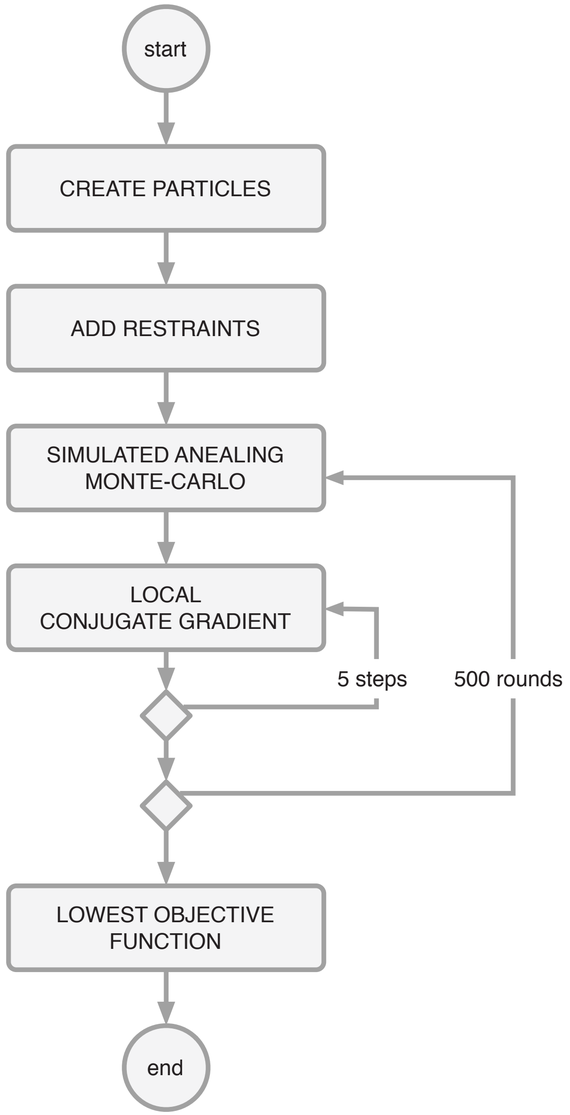


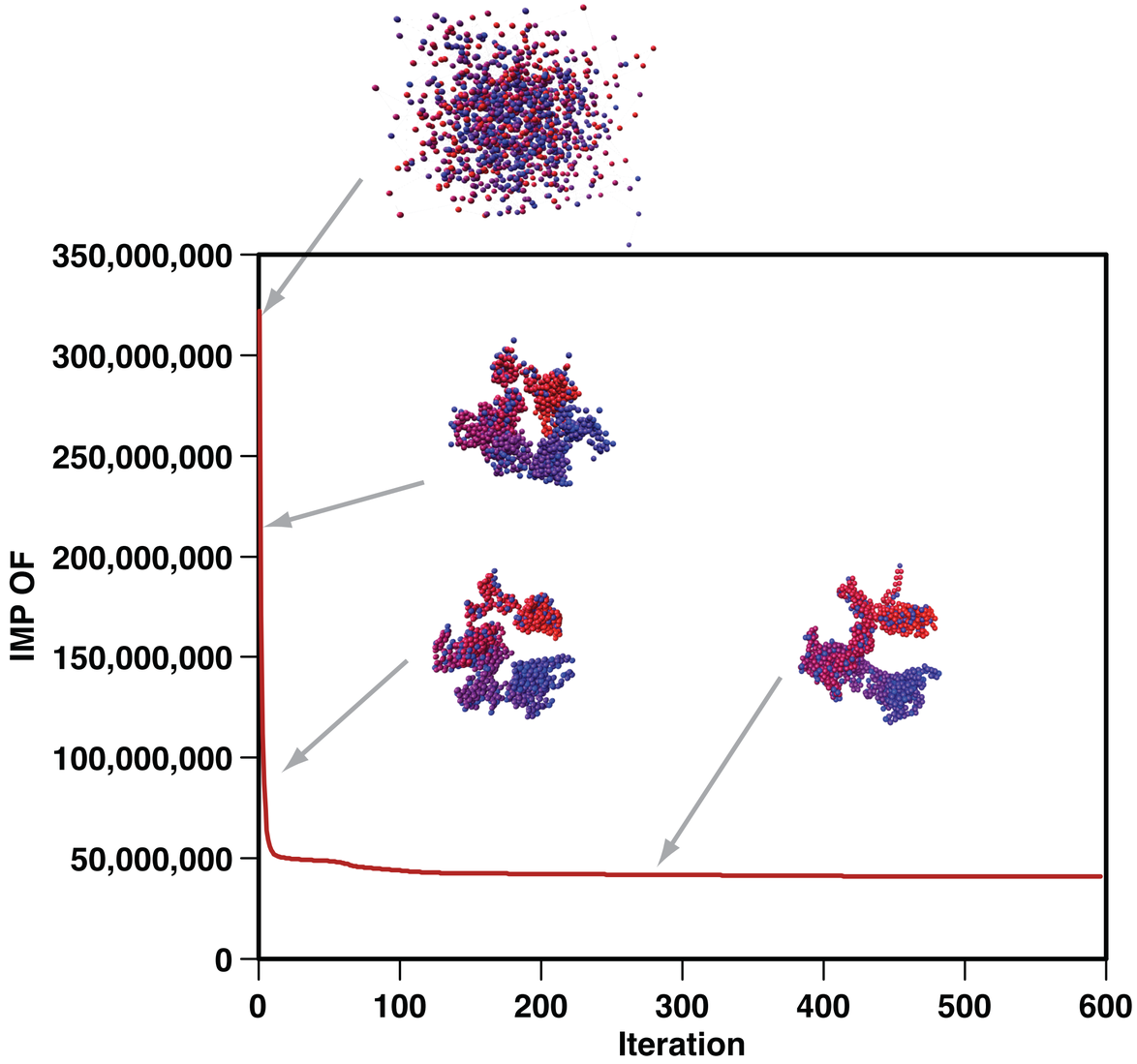




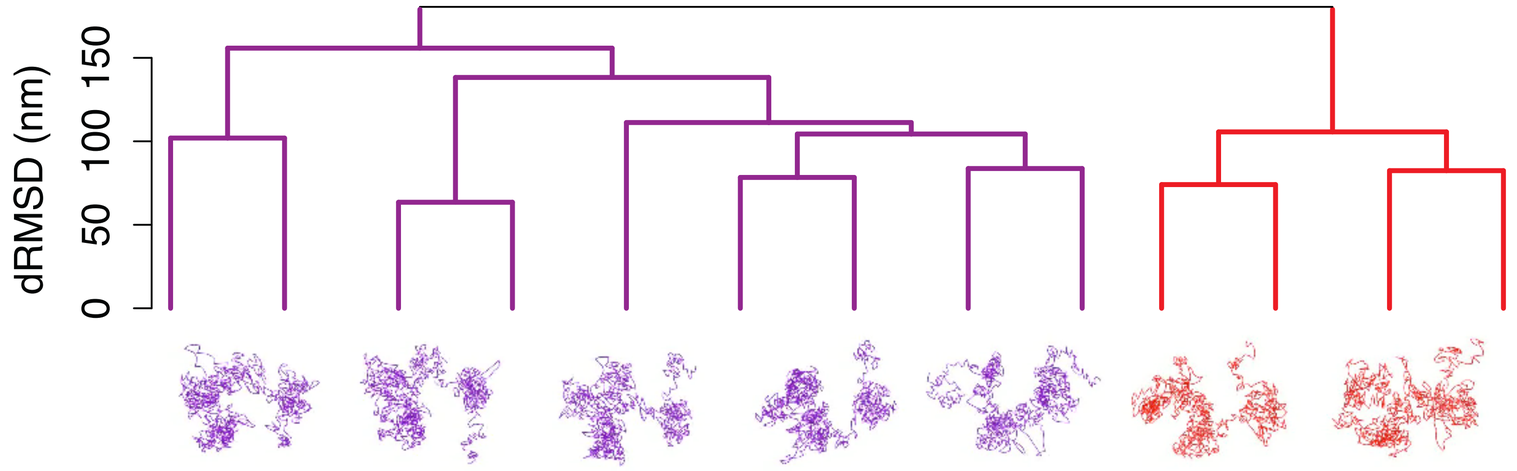


Optimization of the scoring function

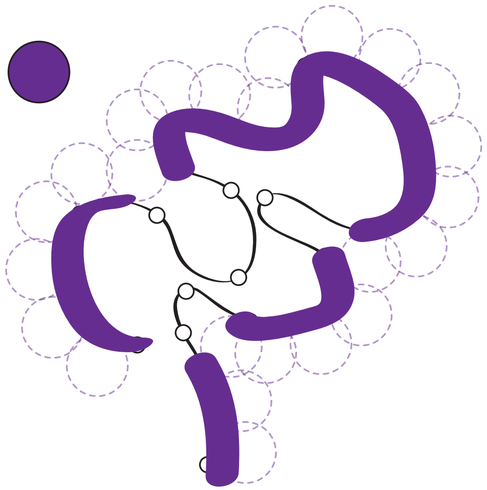




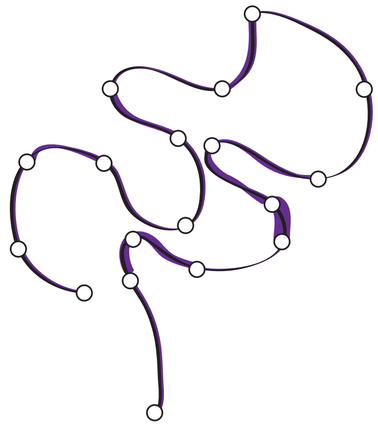
Model analysis: clustering and structural features



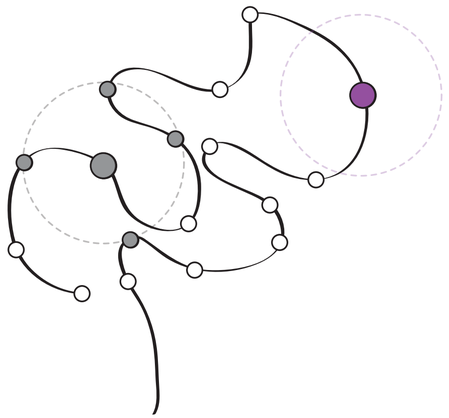
Accessibility (%)



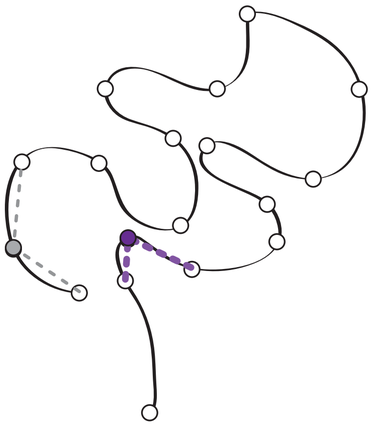
Density (bp/nm)



Interactions

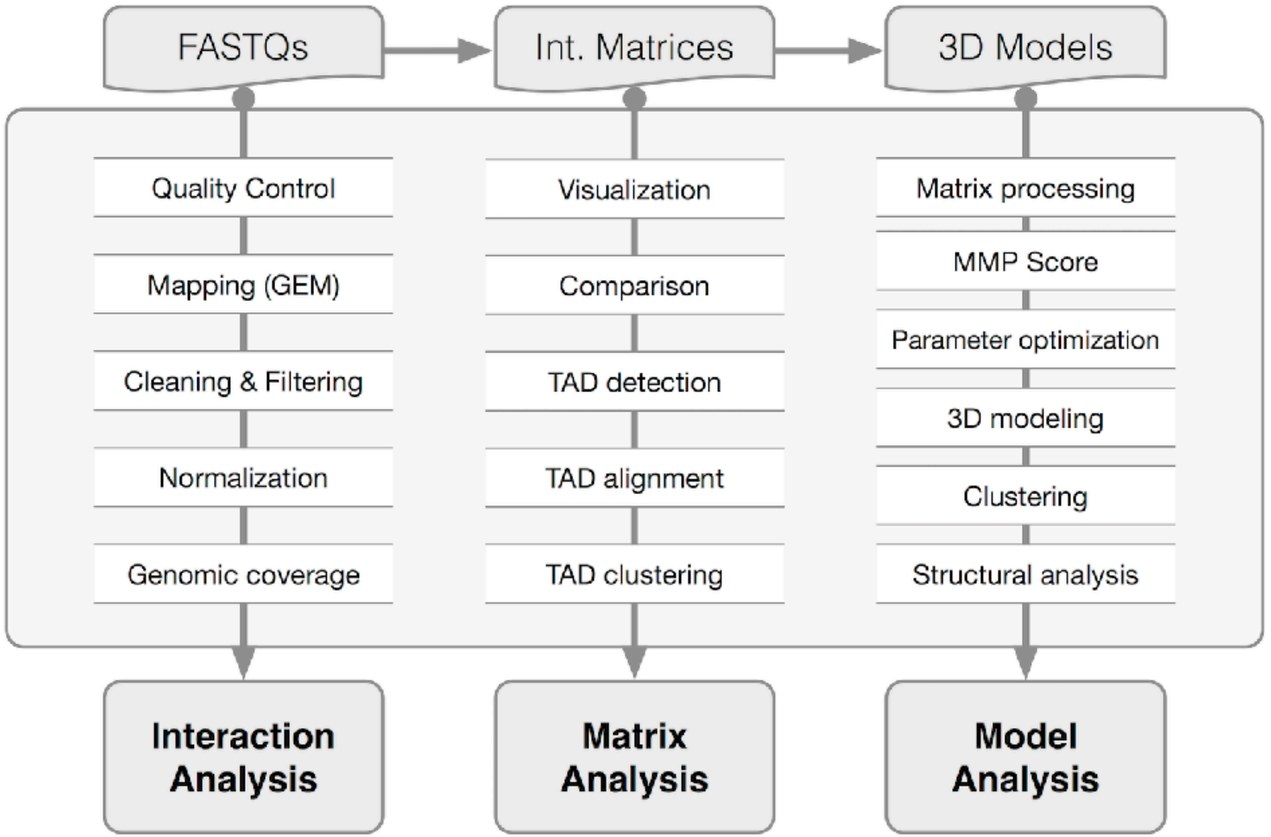


Angle



TADbit

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



- Baù, D. et al. Nat Struct Mol Biol (2011)
- Umbarger, M. A. et al. Mol Cell (2011)
- Le Dily, F. et al. Genes & Dev (2014)
- Belton, J.M. et al. Cell Reports (2015)
- Trussart M. et al. Nature Communication (2017)
- Cattoni, D. et al. Nature Communication (2017)
- Stadhouders R. et al. Nature Genetics (2018)
- Kojic, A., Cuadrado, A. et al. Nat Struct Mol Biol (2018)
- Beekman R. et al. Nature Medicine (2018)
- Mas, G. et al. Nature Genetics (2018)
- Pascual-Reguant, L. et al. Nature Communication (2018)
- Nir, Farabella, Perez-Estrada, et al. PLOS Genetics (2018)
- Cuadrado, Giménez-Llorente et al. 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
BMC Biology 17(1), 55, 2019

https://github.com/3DGenomes/tadbit
https://github.com/3DGenomes/MethodsMolBiol

DISCLAIMER — Many alternatives

Tool	Short-read aligner(s)	Mapping improvement	Read filtering	Read-pair filtering	Normalization	Visualization	Confidence estimation	Implementation language(s)
HiCUP [46]	Bowtie/Bowtie2	Pre-truncation	✓	✓	–	–	–	Perl, R
Hiclib [47]	Bowtie2	Iterative	✓[a]	✓	Matrix balancing	✓	–	Python
HiC-inspector [131]	Bowtie	–	✓	✓	–	✓	–	Perl, R
HIPPIE [132]	STAR	✓[b]	✓	✓	–	–	–	Python, Perl, R
HiC-Box [133]	Bowtie2	–	✓	✓	Matrix balancing	✓	–	Python
HiCdat [122]	Subread	–[c]	✓	✓	Three options[d]	✓	–	C++, R
HiC-Pro [134]	Bowtie2	Trimming	✓	✓	Matrix balancing	–	–	Python, R
TADbit [120]	GEM	Iterative	✓	✓	Matrix balancing	✓	–	Python
HOMER [62]	–	–	✓	✓	Two options[e]	✓	✓	Perl, R, Java
Hicpipe [54]	–	–	–	–	Explicit-factor	–	–	Perl, R, C++
HiBrowse [69]	–	–	–	–	–	✓	✓	Web-based
Hi-Corrector [57]	–	–	–	–	Matrix balancing	–	–	ANSI C
GOTHiC [135]	–	–	✓	✓	–	–	✓	R
HiTC [121]	–	–	–	–	Two options[f]	✓	✓	R
chromoR [59]	–	–	–	–	Variance stabilization	–	–	R
HiFive [136]	–	–	✓	✓	Three options[g]	✓	–	Python
Fit-Hi-C [20]	–	–	–	–	–	✓	✓	Python

Analysis methods for studying the 3D architecture of the genome
Ay, F. & Noble, W. S. Genome Biol. 16, 183 (2015).

DISCLAIMER — Many alternatives

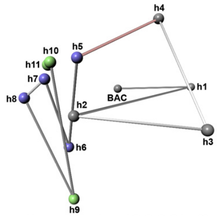
Method *available online	Representation	Scoring				Sampling	Models
		U_{3C}		U_{Biol}	U_{Phys}		
		$F_{ij} \rightarrow D_{ij}$ conversion	Functional form				
ChromSDE* [37]	Points	$D_{ij} = \begin{cases} (\frac{1}{F_{ij}})^{\alpha} \text{ if} F_{ij} > 0 \\ \infty \text{ if } F_{ij} = 0 \end{cases}$ α is optimized	$\sum_{(i,j)D_{ij}<\infty)} \frac{(r_{ij}^2 - D_{ij}^2)}{D_{ij}} - \lambda \sum_{(i,j)} r_{ij}^2$ where λ is set to 0.01	N/A	N/A	Deterministic semidefinite programming to find the coordinates	Consensus
ShRec3D* [38]	Points	$D_{ij} = \begin{cases} \left(\frac{1}{F'_{ij}}\right)^{\alpha} & \text{if } F'_{ij} > 0 \\ \frac{N^2}{\sum_{(i,j)} F_{ij}} & \text{if } F'_{ij} = 0 \end{cases}$ F'_{ij} is the original F_{ij} corrected to satisfy all triangular inequalities with the shortest path reconstruction	N/A	N/A	N/A	Deterministic transformations of D_{ij} into coordinates	Consensus
TADbit* [43]	Spheres	$D_{ij} \propto \begin{cases} \alpha F_{ij} + \beta & \text{if } F_{ij} < \gamma' \text{ or } F_{ij} > \gamma \\ \frac{s_i + s_j}{2} & \text{if } \lvert i-j \rvert = 1 \end{cases}$ α and β are estimated from the max and the min F_{ij}, from the optimized max distance and from the resolution. $\gamma' < \gamma$ are optimized too. s_i is the radius of particle i	$\sum_{(i,j)} k_{ij}(r_{ij} - D_{ij})^2$ where $k_{ij} = 5$ if $\lvert i-j \rvert = 1$ or proportional to F_{ij} otherwise	Yes	U_{excl} and U_{bond} have harmonic forms	Monte Carlo (MC) sampling with Simulated annealing and Metropolis scheme	Resampling
BACH* [45]	Points	$D_{ij} \propto \frac{B_i B_j}{F_{ij}^{\alpha}}$. The biases B_i and B_j and α are optimized	$b_{ij} D_{ij}^{1/\alpha} + c_{ij} \log(D_{ij})$ where b_{ij} and c_{ij} are optimized parameters	No	No	Sequential importance and Gibbs sampling with hybrid MC and adaptive rejection	Population
Giorgetti et al. [40]	Spheres	Particles interact with pair-wise well potentials of depths B_{ij} and contact radius a, which is larger than a hard-core radius and smaller than a maximum contact radius. The parameters are optimized over all the population of models		No	N/A	MC sampling with metropolis scheme	Population
Duan et al. [41]	Spheres	$\overline{F_{\lvert i-j \rvert}} = \frac{\sum_{k=0}^{N-1-\lvert i-j \rvert} F_{(k,k+\lvert i-j \rvert)}}{N-\lvert i-j \rvert}$ is the average of F_{ij} at genomic distance $\lvert i-j \rvert$ expressed in kb. $D_{ij} = \overline{F_{\lvert i-j \rvert}} \times 7.7 \times \lvert i-j \rvert$ assuming that α 1 kb maps onto 7.7 nm	$\sum_{(i,j)} (r_{ij} - D_{ij})^2$	Yes	U_{excl} and U_{bond} have harmonic forms	Interior-point gradient-based method	Resampling
MCMC5C* [49]	Points	$D_{ij} \propto \frac{1}{F_{ij}^{\alpha}}$ where is optimized	$\sum_{(i,j)} (F_{ij} - r_{ij}^{-1/\alpha})^2$	N/A	N/A	MC sampling with Markov chain based algorithm	Resampling
PASTIS* [47]	Points	$D_{ij} \propto \frac{1}{F_{ij}^{\alpha}}$ where α is optimized	$b_{ij} D_{ij}^{1/\alpha} + c_{ij} \log(D_{ij})$ where b_{ij} and c_{ij} are optimized parameters	No	No	Interior point and isotonic regression algorithms	Resampling
Meluzzi and Arya [48]	Spheres	$\sum_{(i,j)} k_{ij} r_{ij}^2$ where k_{ij} are adjusted such that the contact probabilities computed on the models match the F_{ij}		No	U_{excl} is a pure repulsive LJ potential. U_{bond} and U_{bend} have harmonic forms	Brownian dynamics	Resampling
AutoChrom3D* [44]	Points	$D_{ij} \propto \begin{cases} \alpha F_{ij} + \beta & \text{if } F_{min} < F_{ij} < F_{\gamma} \\ \alpha' F_{ij} + \beta' & \text{if } F_{\gamma} < F_{ij} < F_{max} \end{cases}$ where F_{min} (F_{max}) are the min(max) of F_{ij}. The parameters (α, β), (α', β') and F_{γ} are found using the nuclear size, the resolution and the decay of F_{ij} with $\lvert i-j \rvert$	$\sum_{(i,j)} \frac{(r_{ij} - D_{ij})^2}{D_{ij}^2}$	Yes	N/A	Non-linear constrained	Consensus
Kalhor et al. [14]	Spheres	$D_{ij} = R_{contact}$ to enforce the pair contact, if the normalized contact frequency F_{ij} is higher than 0.25. Otherwise the contact is not enforced	$\sum_{models} \sum_{(i,j)} k_{ij}(r_{ij} - D_{ij})^2$ where k_{ij} is different for pairs of particles, on different chromosomes, on the same chromosome, or connected	Yes	U_{excl} and U_{bond} have harmonic forms	Conjugate gradients sampling with Simulated annealing scheme	Population

* These methods are publicly available.

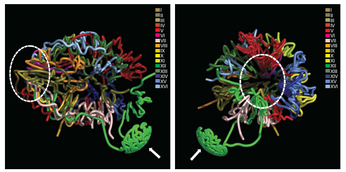
Restraint-based three-dimensional modeling of genomes and genomic domains.
Serra F, Di Stefano M, Spill YG, Cuartero Y, Goodstadt M, Baù D, Marti-Renom MA. FEBS Lett 589: 2987–2995 (2015)

Are the models correct?

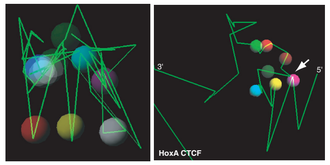
Trussart et al. NAR (2015)



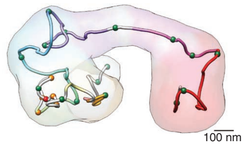
Jhunjhunwala (2008) Cell



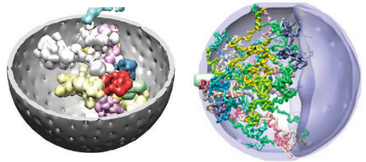
Duan (2010) Nature



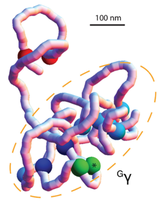
Fraser (2009) Genome Biology
Ferraiuolo (2010) Nucleic Acids Research



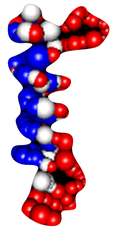
Baù (2011) Nature Structural & Molecular Biology



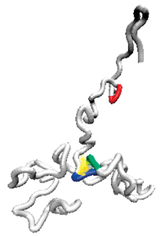
Kalhor (2011) Nature Biotechnology
Tjong (2012) Genome Research



Junier (2012) Nucleic Acids Research



Hu (2013) PLoS Computational Biology



Giorgetti, (2014) Cell

Nucleic Acids Research Advance Access published March 23, 2015

Nucleic Acids Research, 2015 **1**
doi: 10.1093/nar/gkv221

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

Marie Trussart[1,2], François Serra[3,4], Davide Baù[3,4], Ivan Junier[2,3], Luís Serrano[1,2,5] and Marc A. Marti-Renom[3,4,5,*]

[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

Received January 16, 2015; Revised February 16, 2015; Accepted February 22, 2015

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).

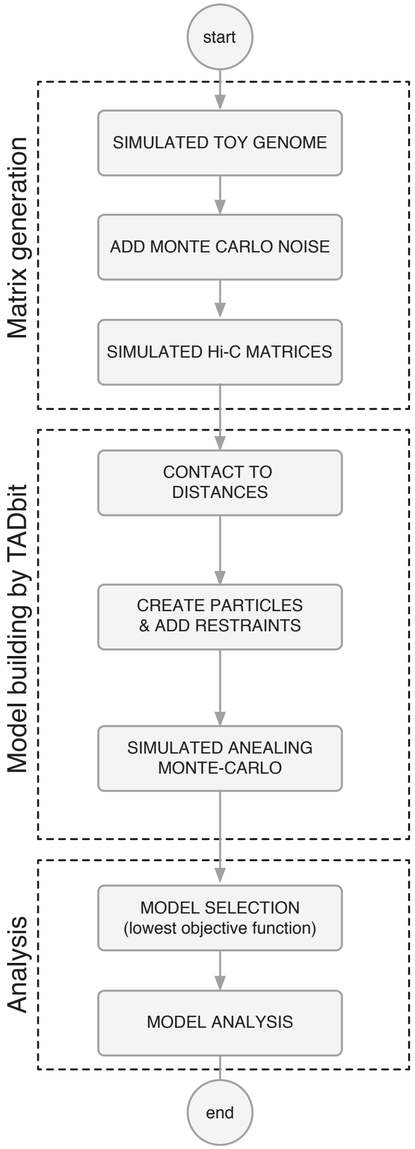
We recently developed a mean-field method for modeling 3D structures of genomes and genomic domains based on 3C interaction data (9). Our approach, called TADbit, was developed around the Integrative Modeling Platform (IMP, http://integrativemodeing.org), a general framework for restraint-based modeling 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

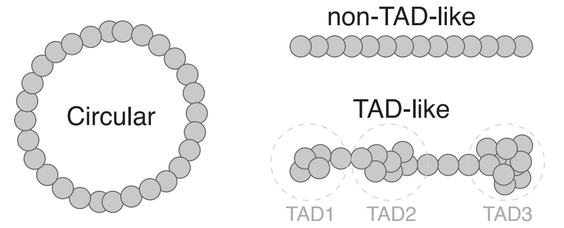
*To whom correspondence should be addressed. Tel: +34 934 020 542; Fax: +34 934 037 279; Email: mmarti@pcb.ub.cat

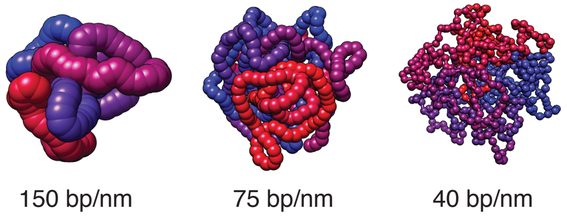
© The Author(s) 2015. Published by Oxford University Press on behalf of Nucleic Acids Research.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

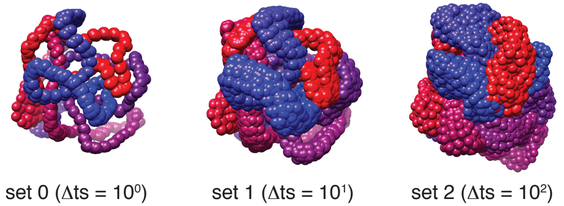
Downloaded from http://nar.oxfordjournals.org/ by guest on March 24, 2015

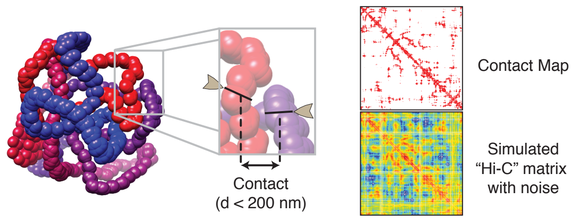
Toy models





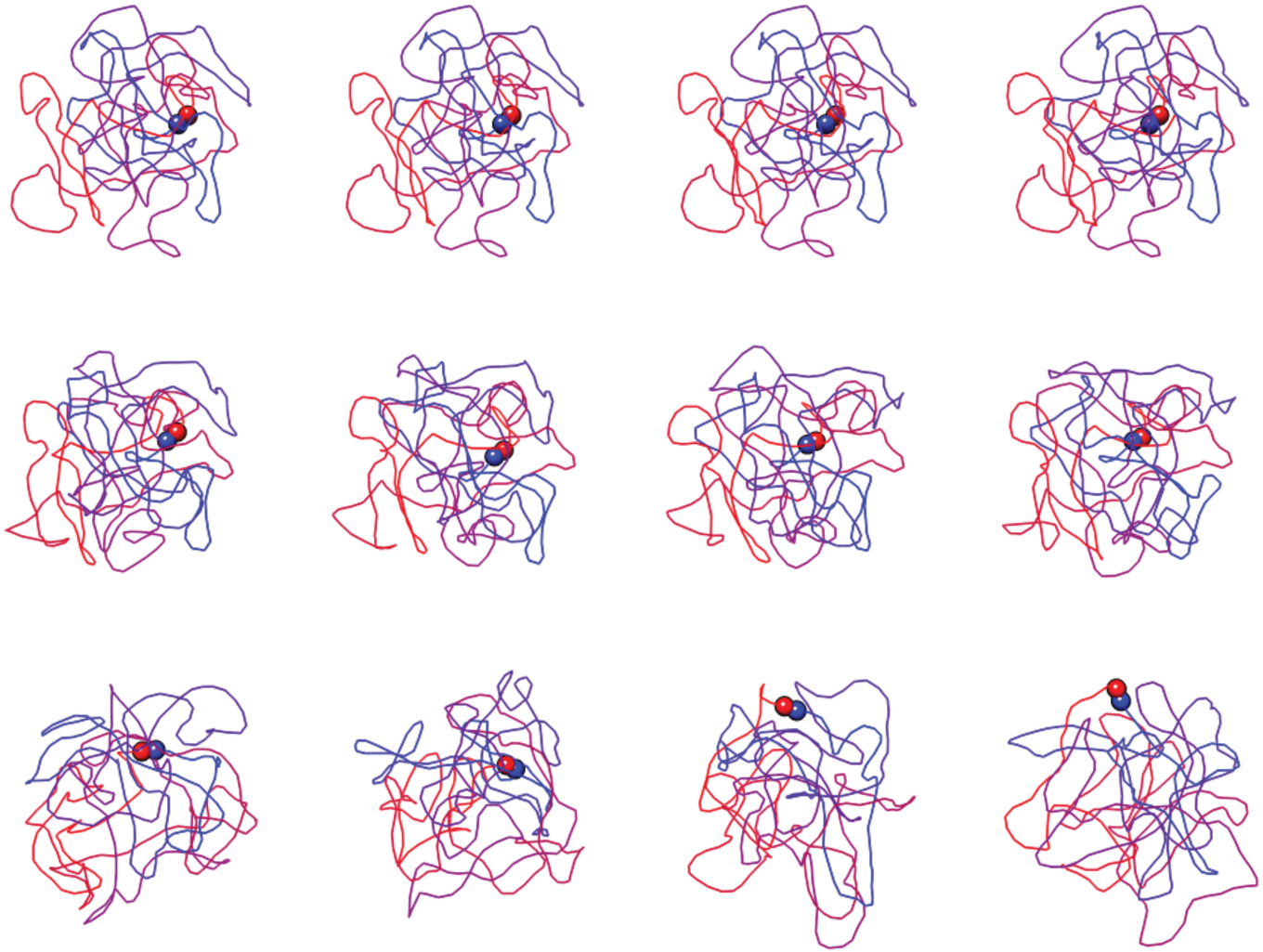




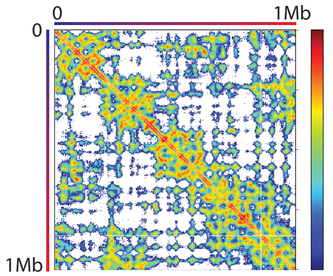


by Ivan Junier

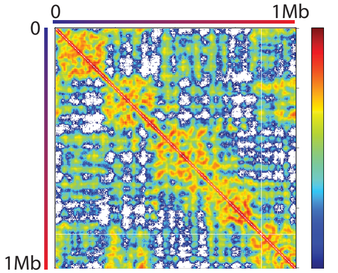
Toy interaction matrices



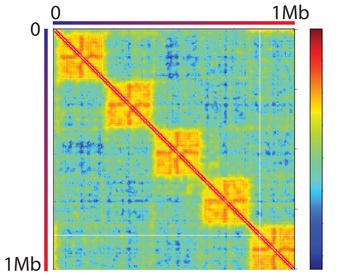
set 0 ($\Delta ts=10^0$)



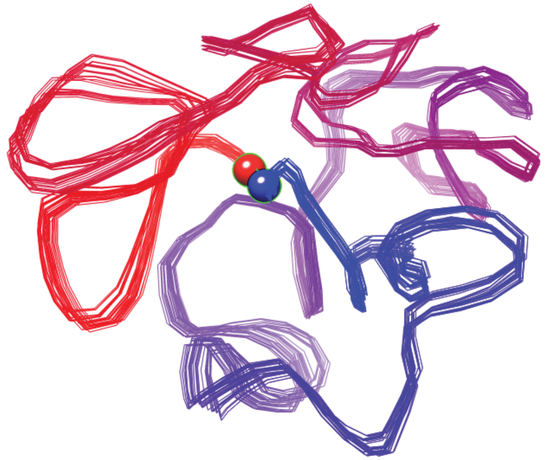
set 4 ($\Delta ts=10^4$)

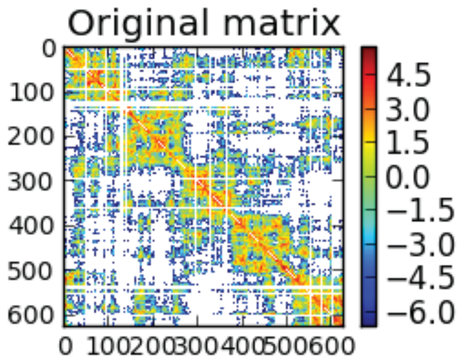


set 6 ($\Delta ts=10^6$)



Reconstructing toy models





chr40_TAD

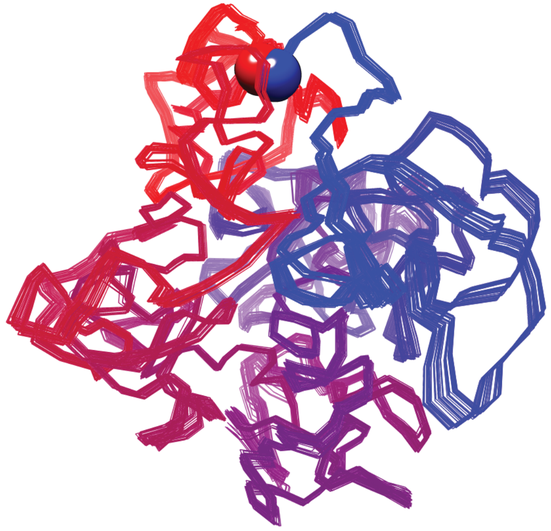
α=100

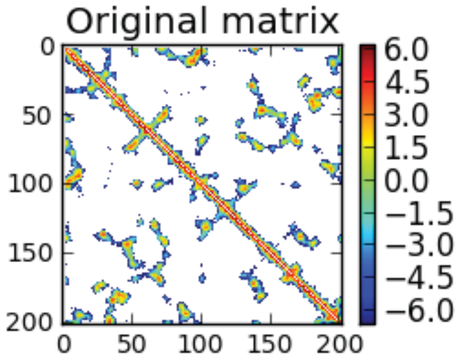
Δts=10

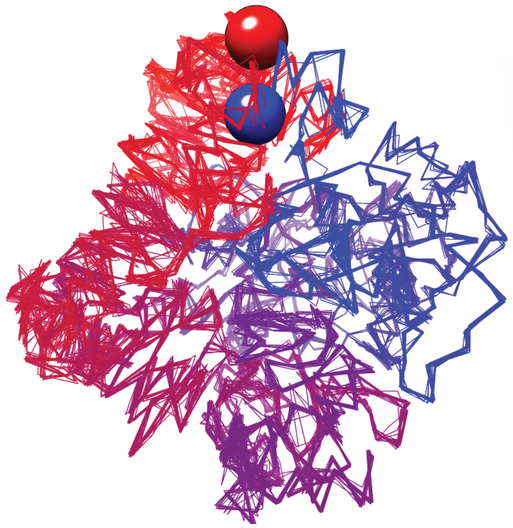
TADbit-SCC: 0.91

<dRMSD>: 32.7 nm

<dSCC>: 0.94







chr150_TAD

α=50

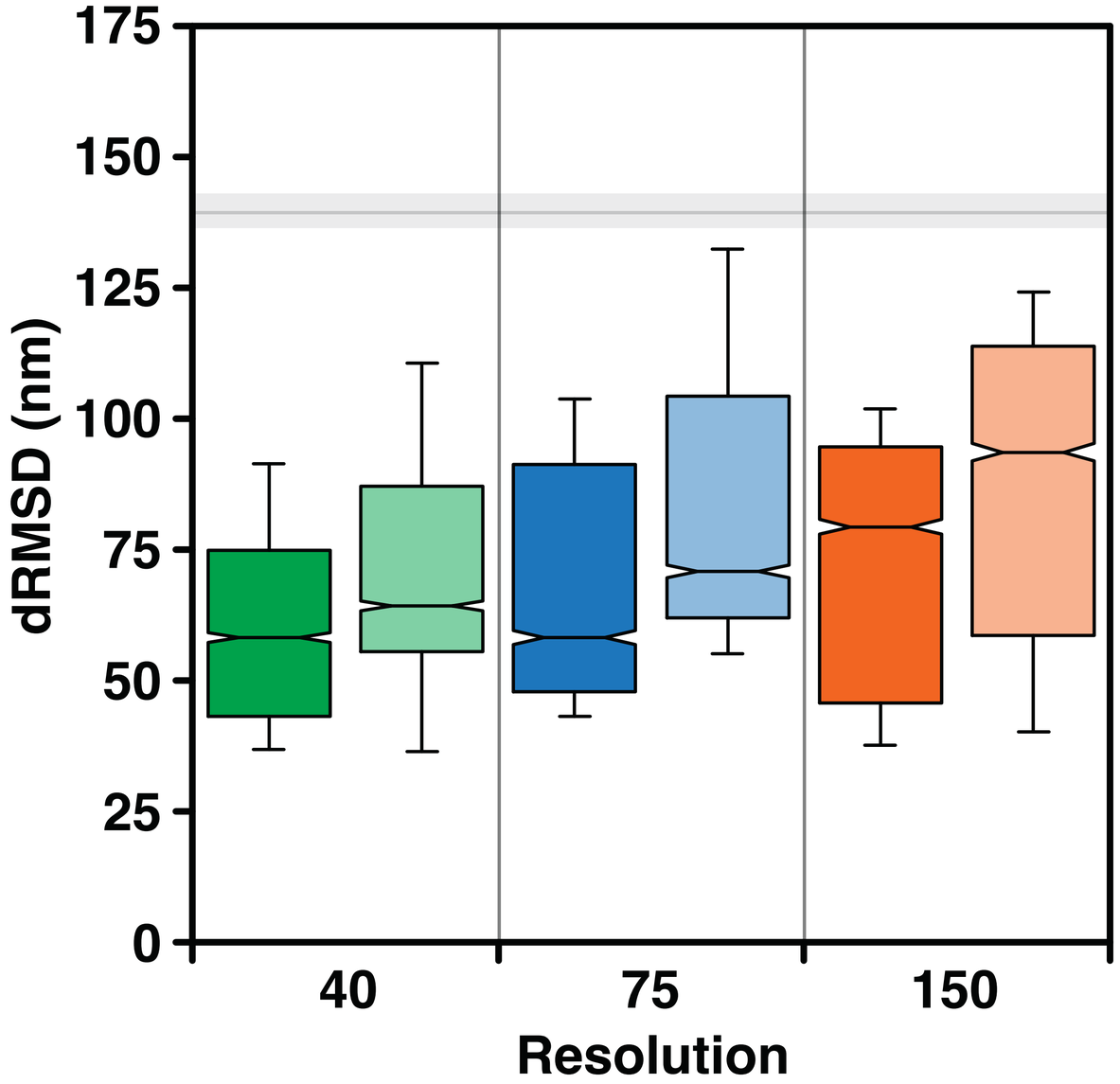
Δts=1

TADbit-SCC: 0.82

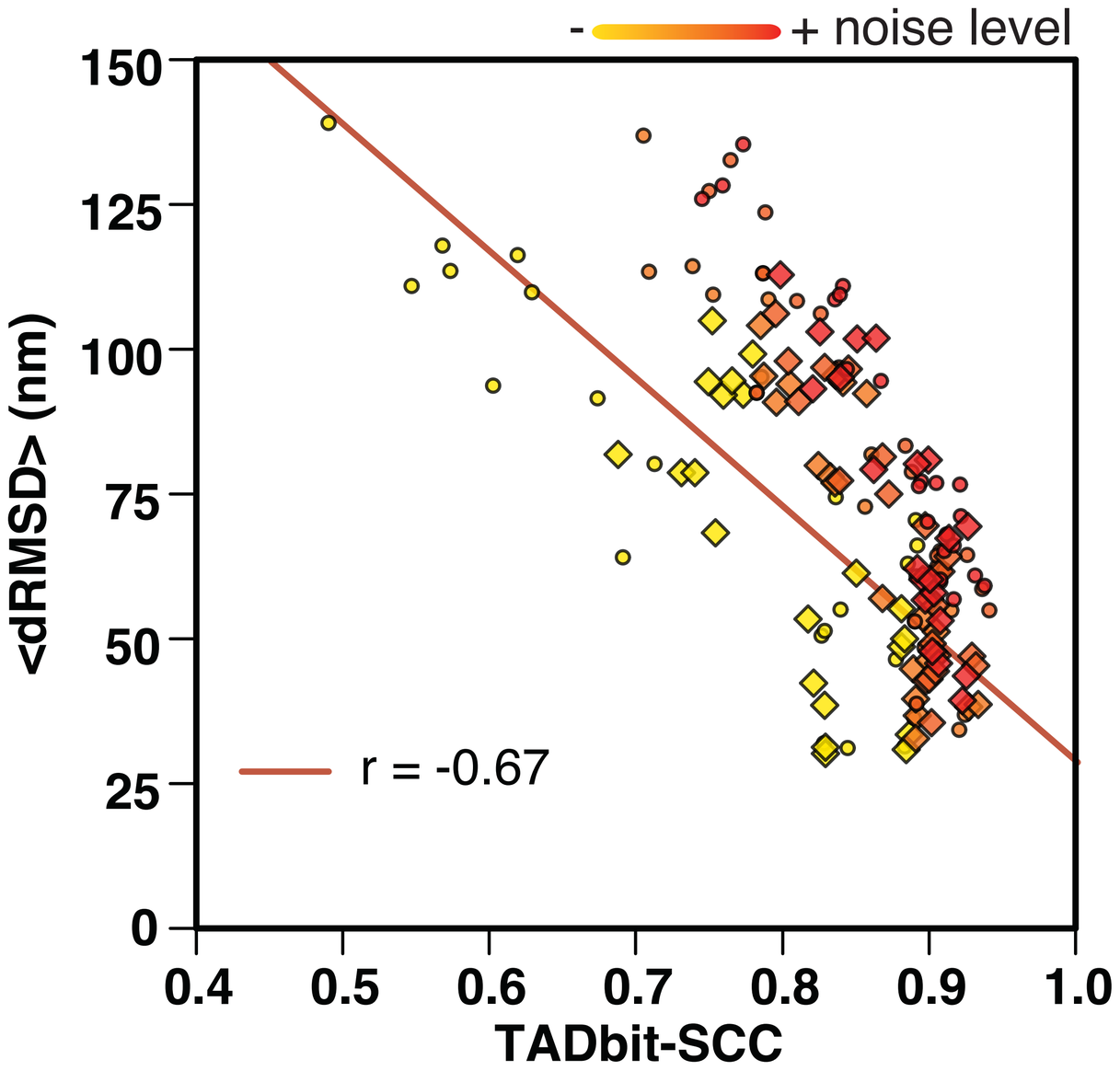
<dRMSD>: 45.4 nm

<dSCC>: 0.86

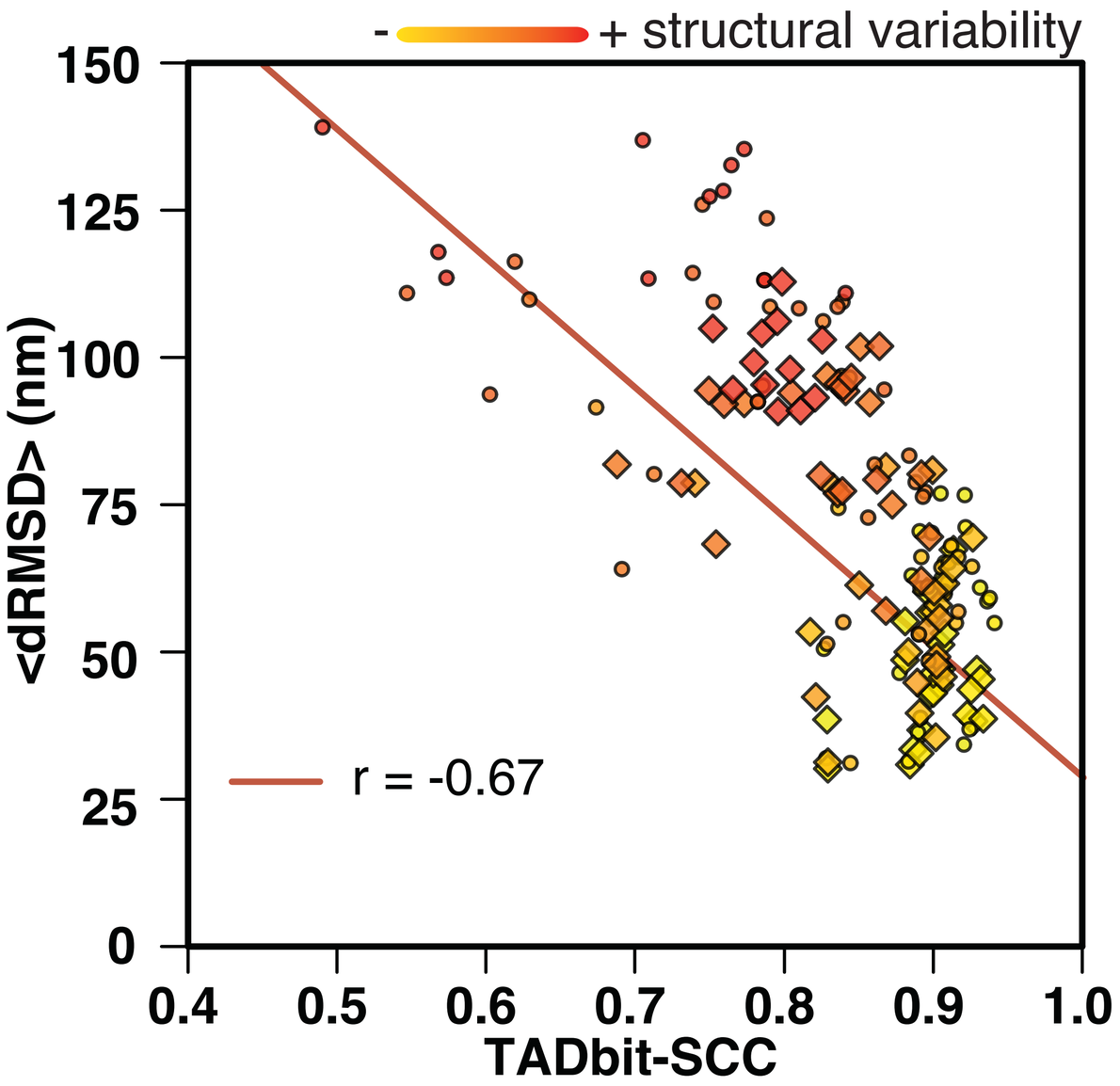
TADs & higher-res are "good"



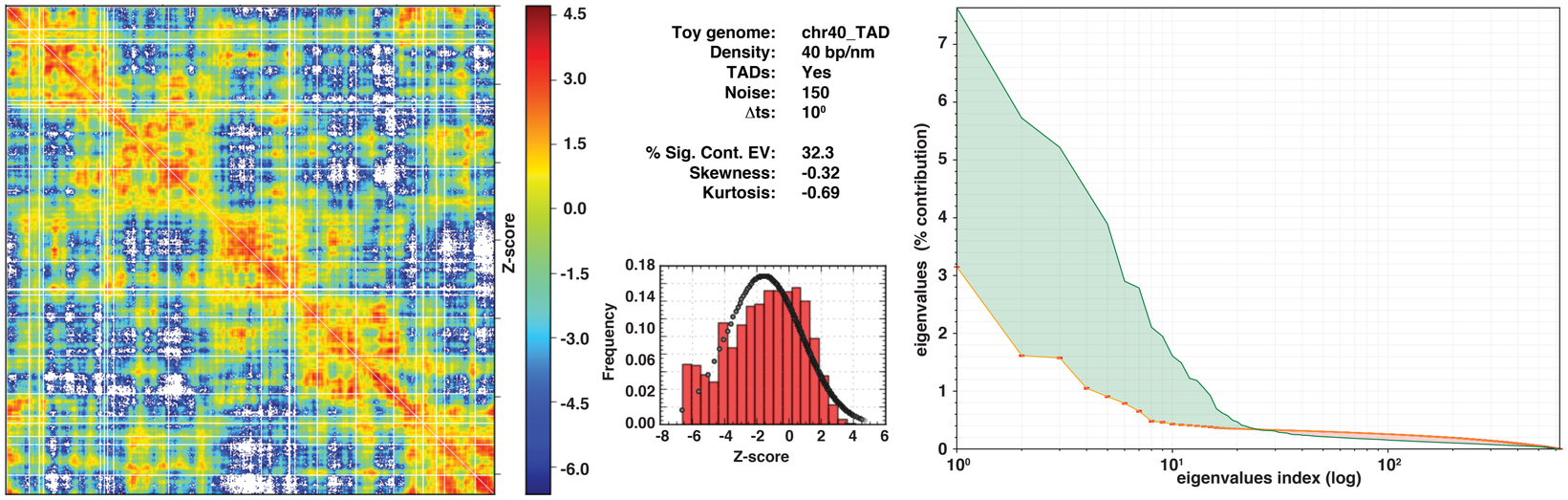
Noise is "OK"

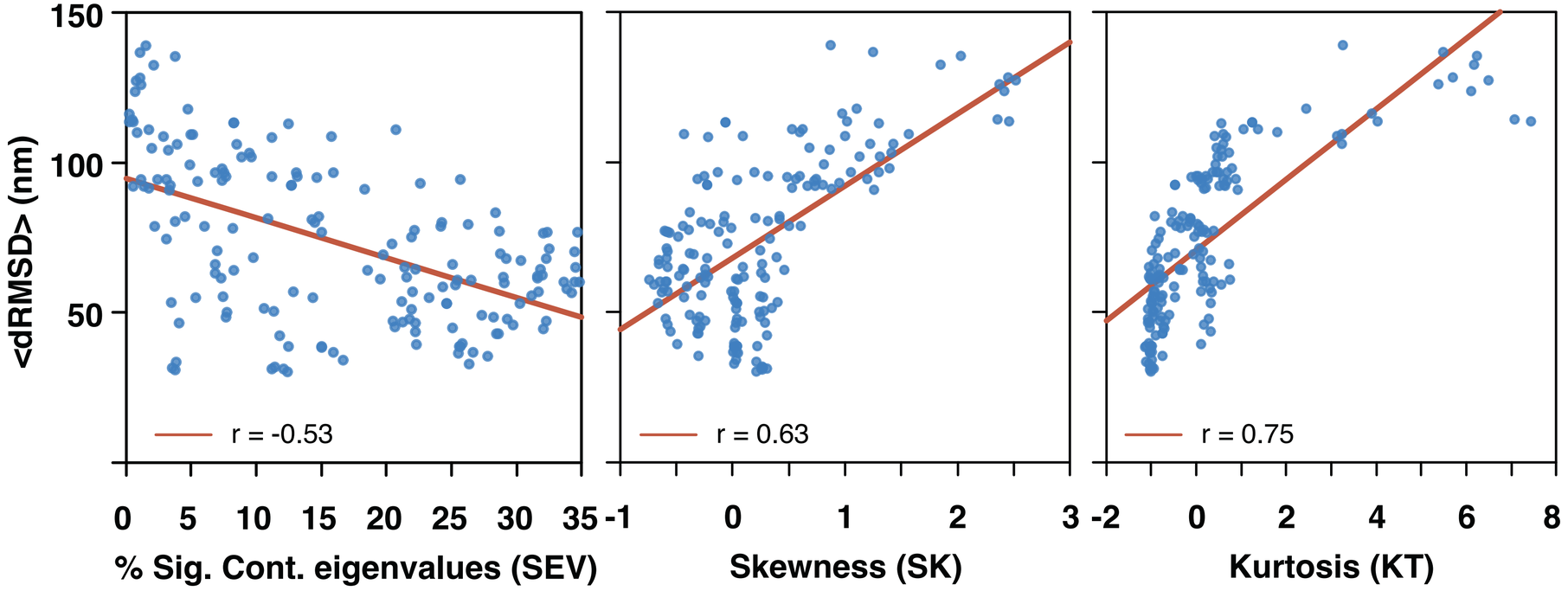

- + noise level
150
125
100
75
50
25
0
<dRMSD> (nm)
r = -0.67
0.4
0.5
0.6
0.7
0.8
0.9
1.0
TADbit-SCC

Structural variability is "NOT OK"

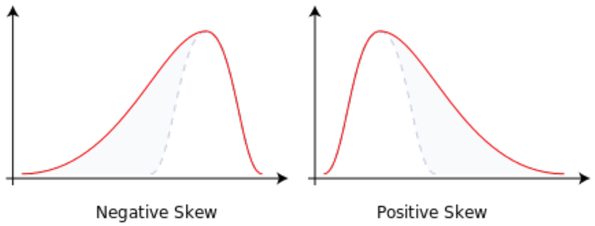


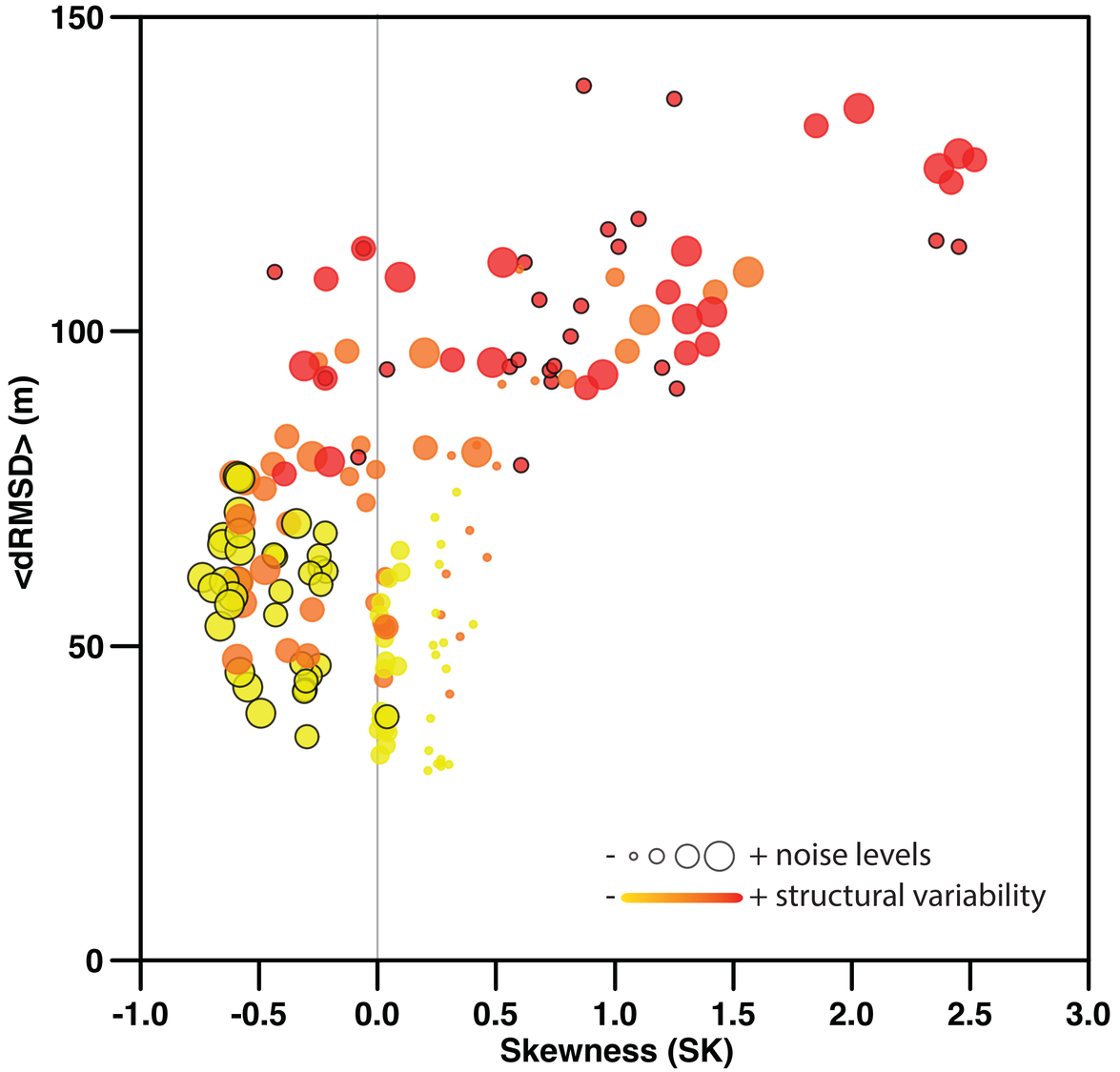
Can we predict the accuracy of the models?





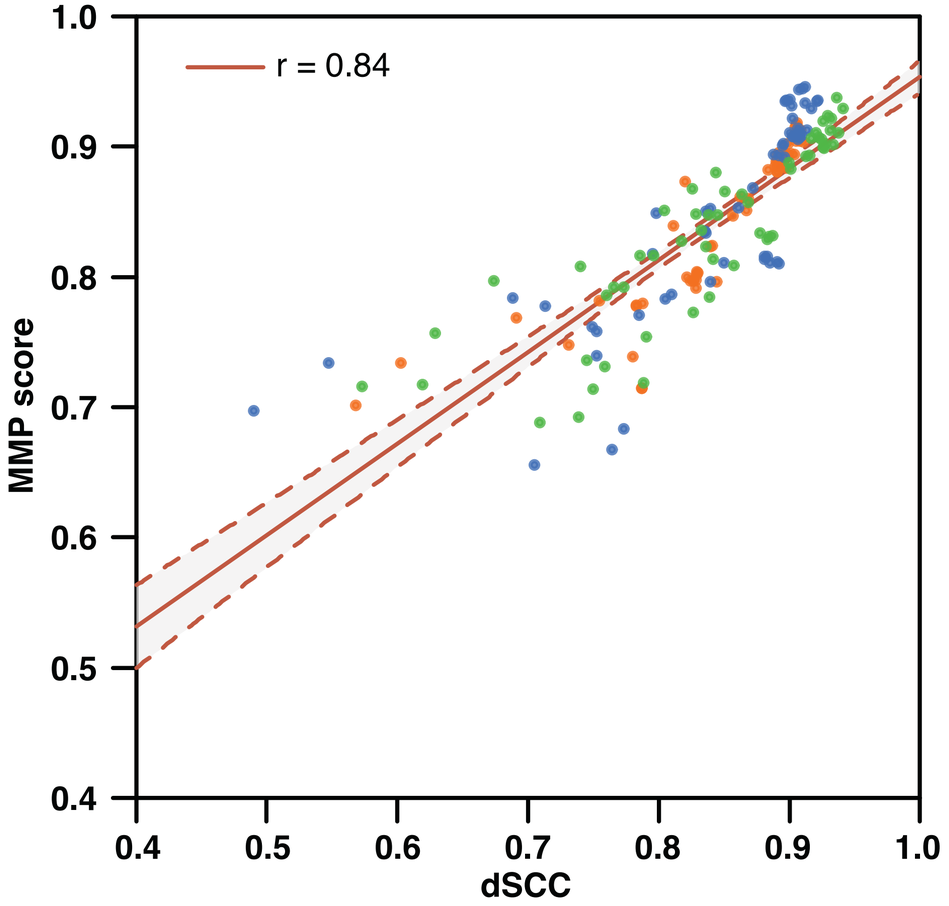
Skewness "side effect"





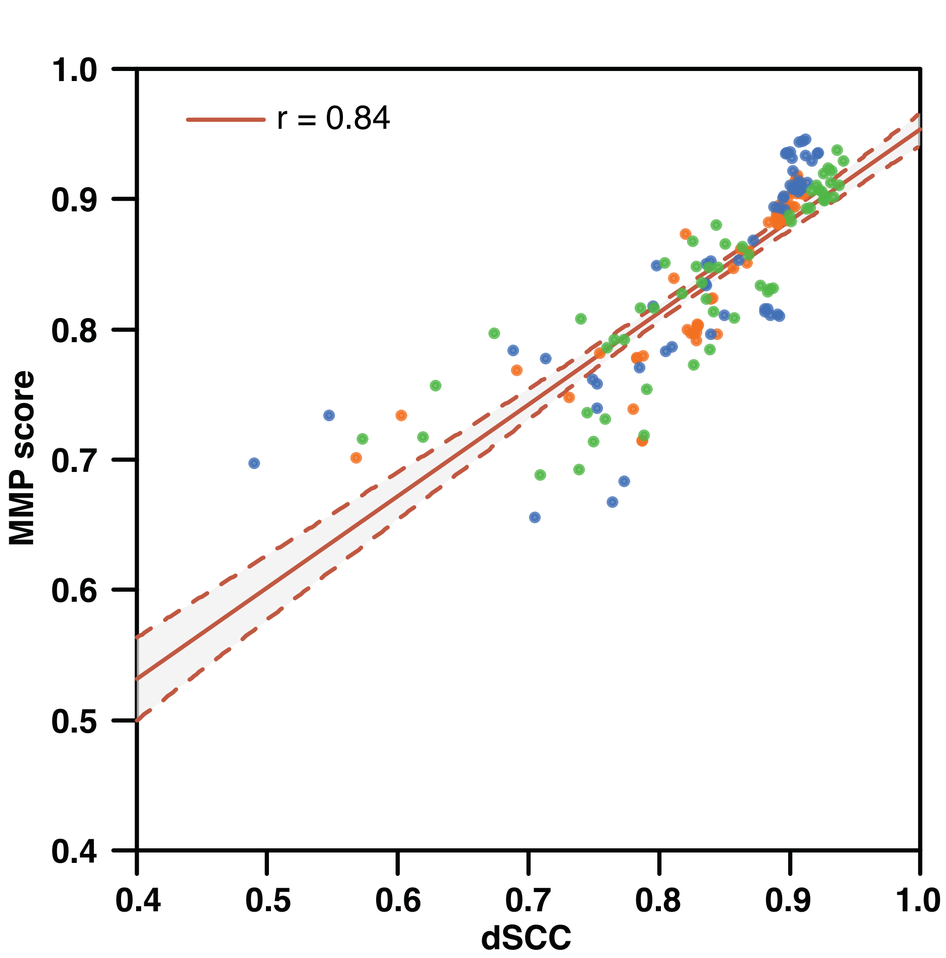
Can we predict the accuracy of the models?

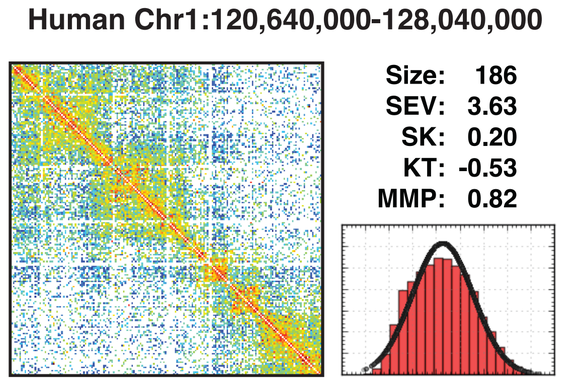
$$\mathrm{MMP} = -0.0002 * \mathrm{Size} + 0.0335 * \mathrm{SK} - 0.0229 * \mathrm{KU} + 0.0069 * \mathrm{SEV} + 0.8126$$

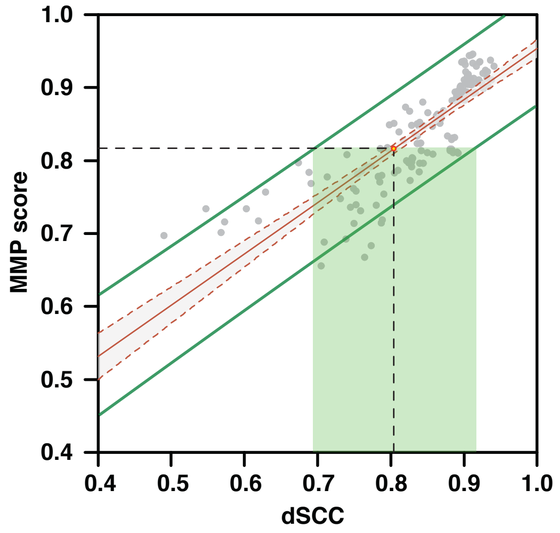


Can we predict the accuracy of the models?

$$MMP = -0.0002 * Size + 0.0335 * SK - 0.0229 * KU + 0.0069 * SEV + 0.8126$$







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

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

@marciuslab
@mamartirenom

David Castillo
Yasmina Cuartero
Marco Di Stefano
Irene Farabella
Silvia Galan
Mike Goodstadt
Rodrigo Jara
Maria Marti-Marimon
Francesca Mugianesi
Julen Mendieta
Juan Rodriguez
Paula Soler
Aleksandra Sparavier

.: Our current sponsors :.