Massachusetts Institute of Technology

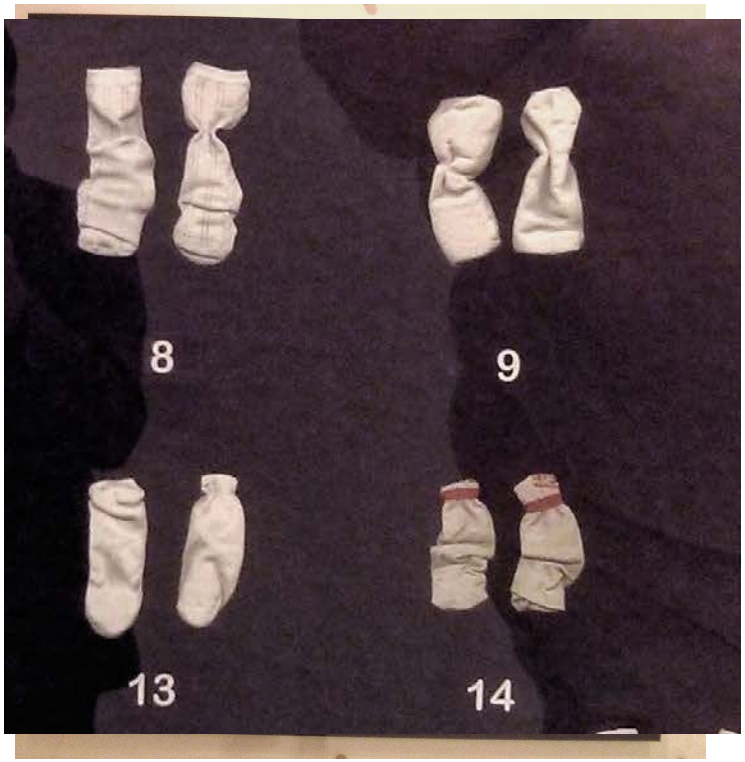
MIT DEPARTMENT OF PHYSICS

INSTITUTE FOR MEDICAL ENGINEERING & SCIENCE

Genome in 3D

Leonid Mirny

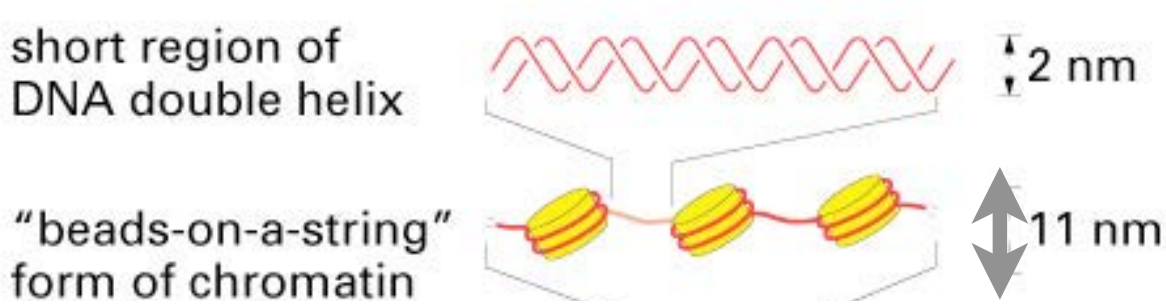
leonid@mit.edu



Andrea Duncan (2002)
Wellcome Collection, London

What we know

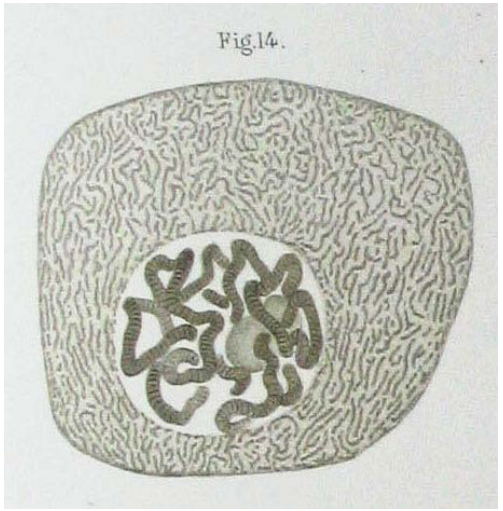
x5 compression



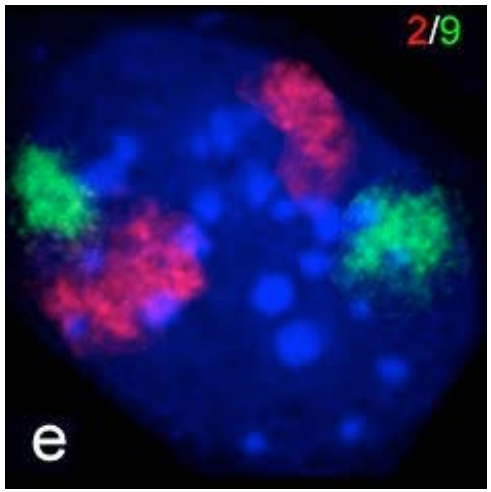
CHROMATIN FIBER

?

CHROMOSOMAL TERRITORIES



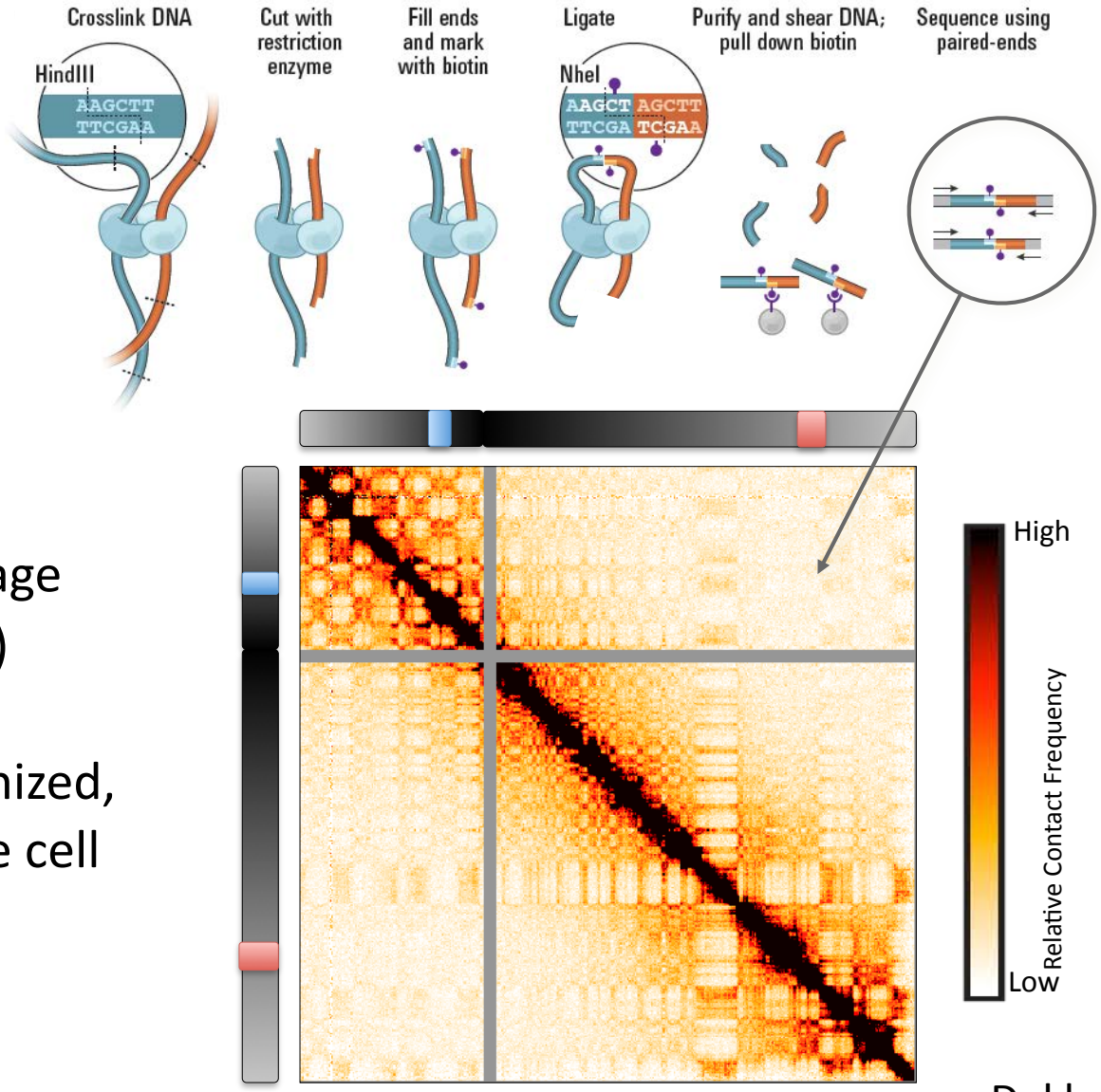
Walther Flemming in 1882



Thomas Cremer et al in 2005

5000 nm

Chromosome Conformation Capture (Hi-C)



- ensemble average (over 10^7 cells)

- unless synchronized, averages over the cell cycle as well!

Job Dekker

Dekker *et al.* Science 2002

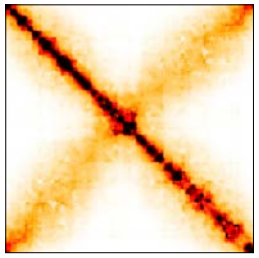
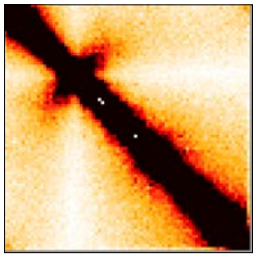
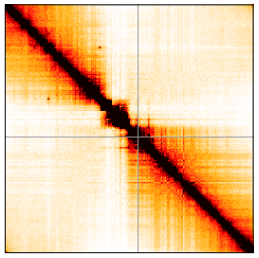
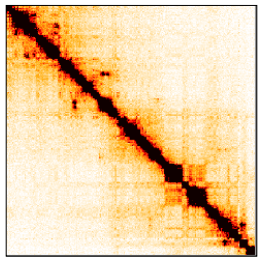
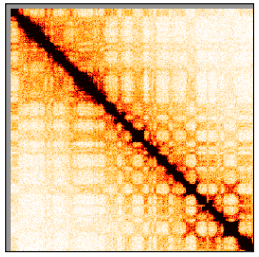
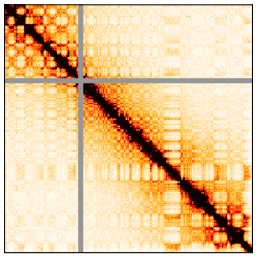
Lieberman-Aiden & van Berkum *et al.* Science 2009

Genome Folding across Kingdoms






C. crescentus *S. cerevisiae* *A. thaliana* *D. melanogaster* *M. musculus* *H. sapiens*

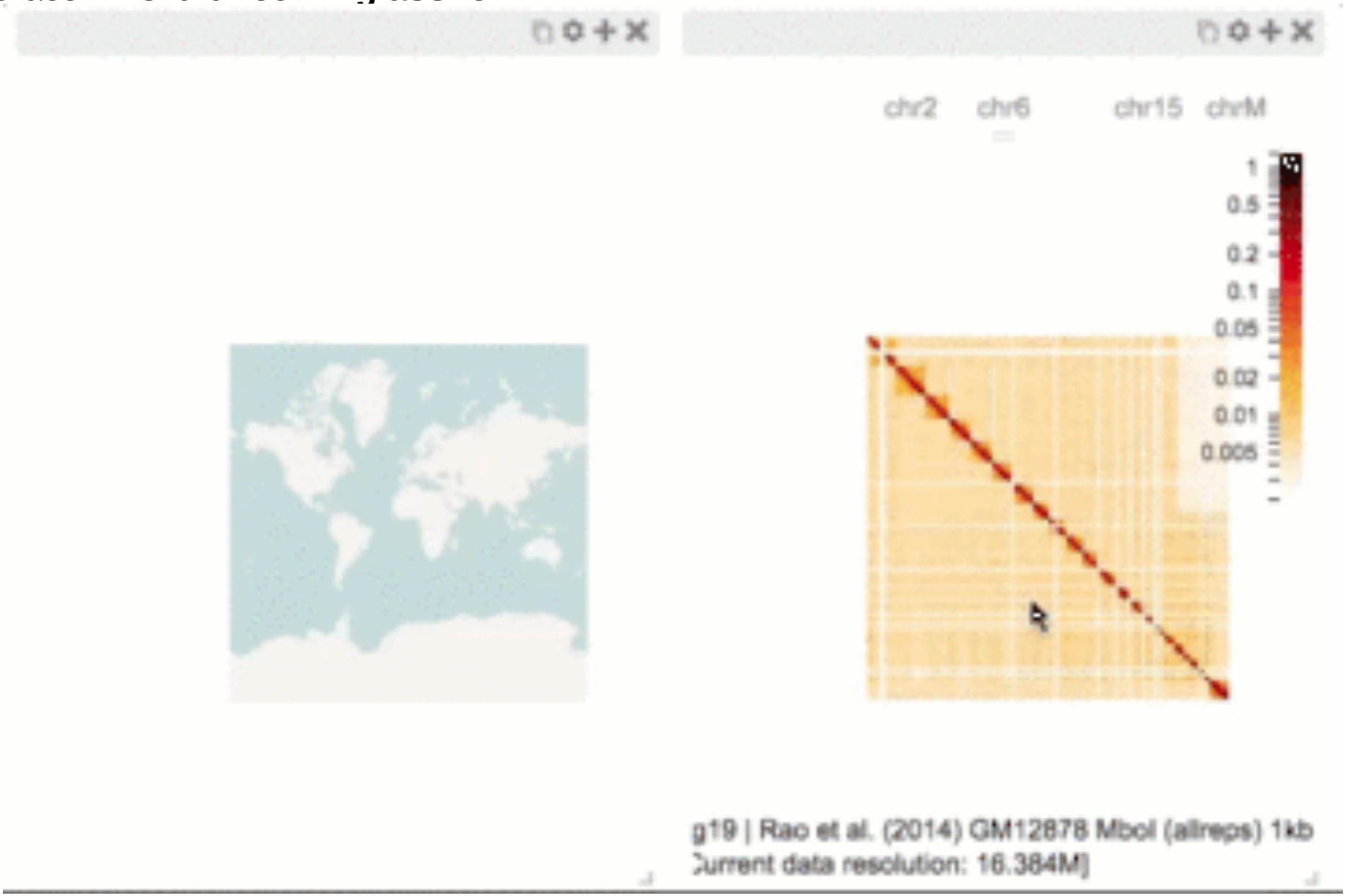







4 Mb 12 Mb 157 Mb 180 Mb 2.7 Gb 3 Gb

Bacteria Fungi Plants Insects Mammals

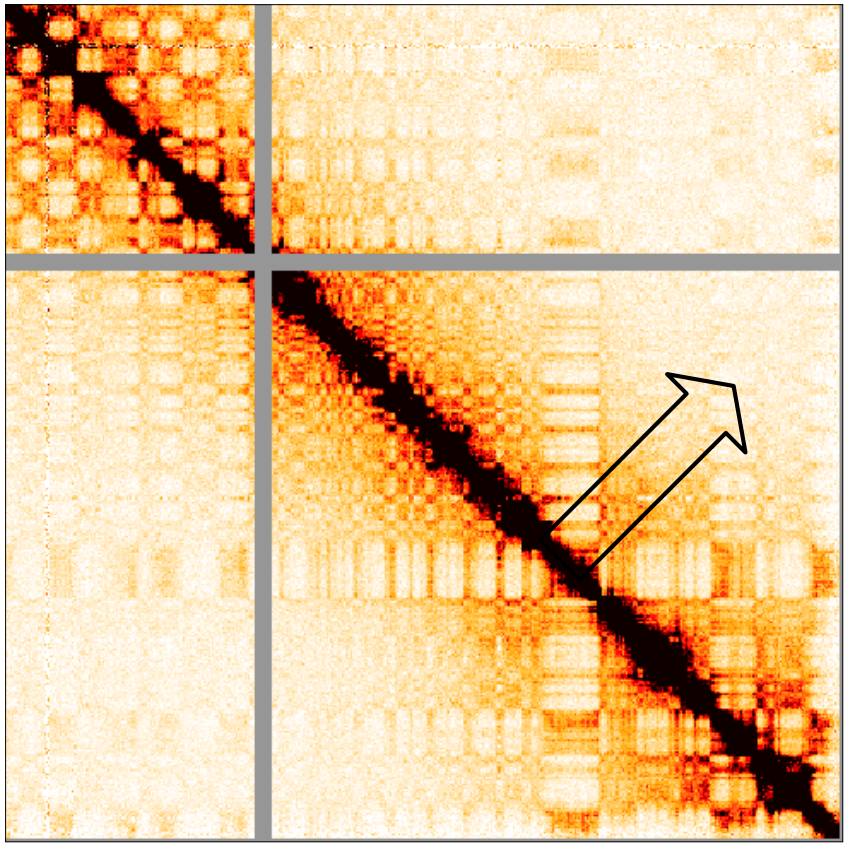
Chromosome Conformation Capture (Hi-C)

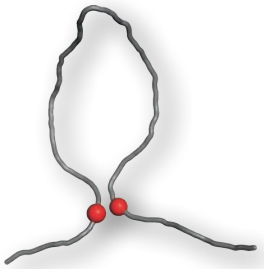
HiGlass Hi-C browser: **higlass.io**



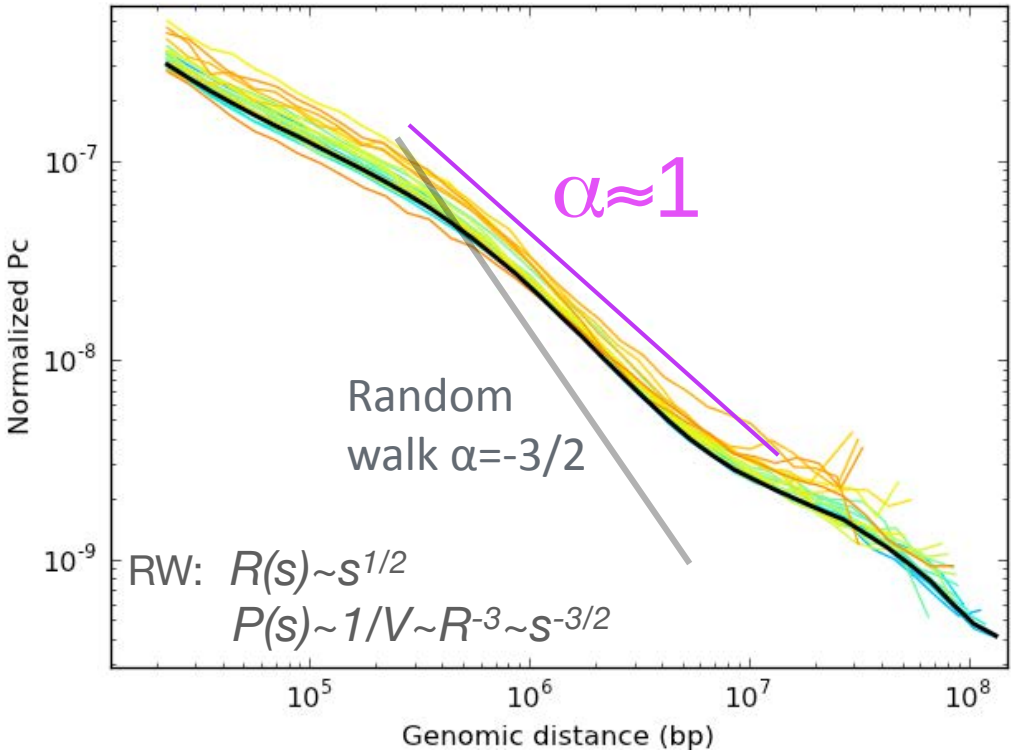
by Nils Gehlenborg and Peter Karpedjiev
bioRxiv (2017) Harvard University

Chromosomes: non-equilibrium polymer system





$$P(s) \sim s^{-\alpha}$$

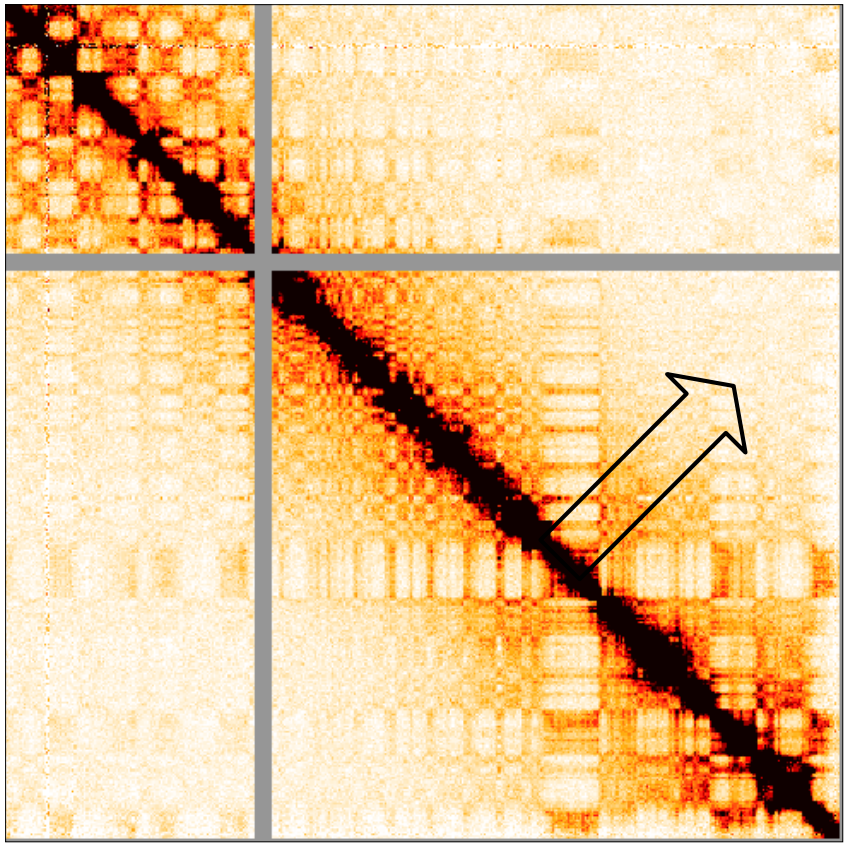


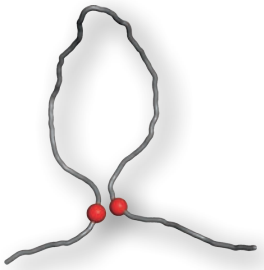
From a melt of rings to chromosome territories: the role of topological constraints in genome folding

Jonathan D Halverson[1], Jan Smrek[2], Kurt Kremer[3] and Alexander Y Grosberg[2,4]

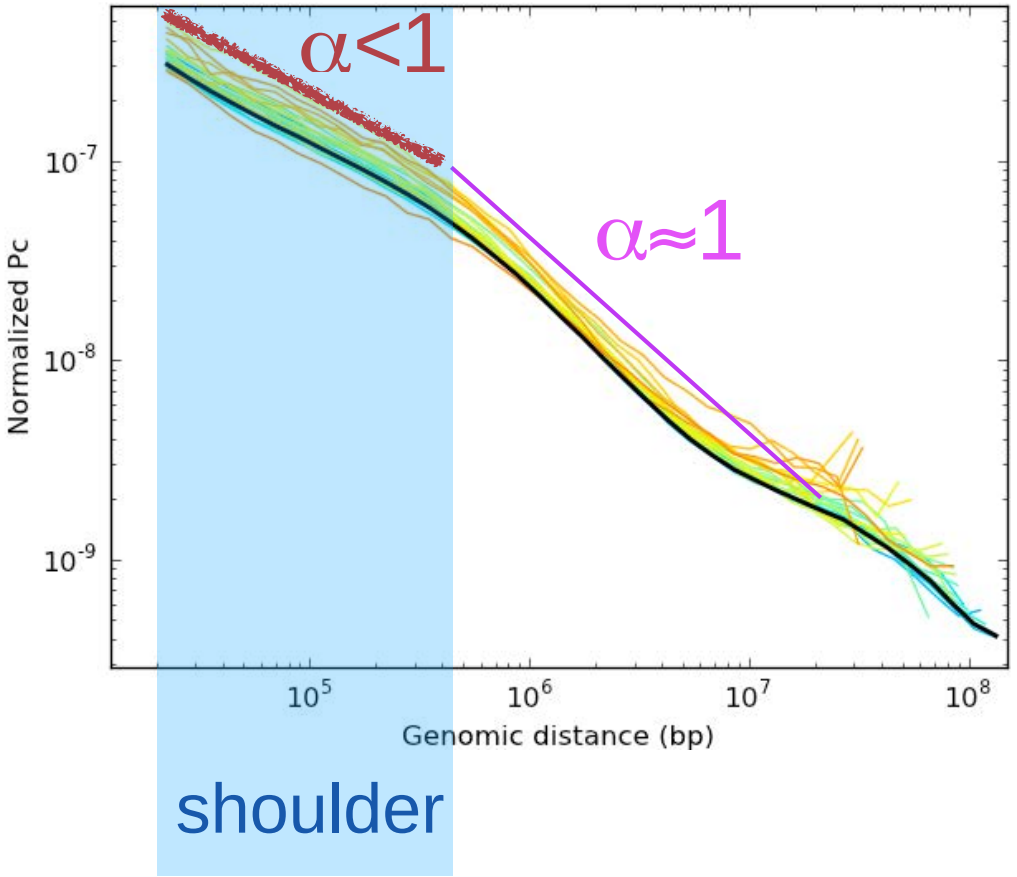
α≈1.15

Chromosomes: non-equilibrium polymer system



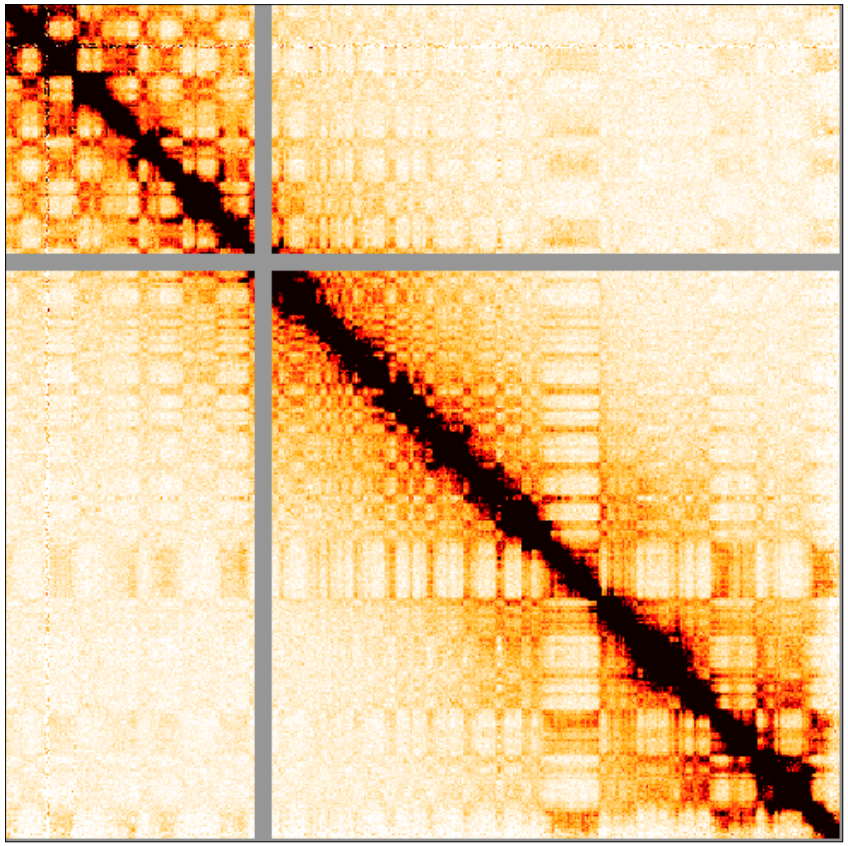


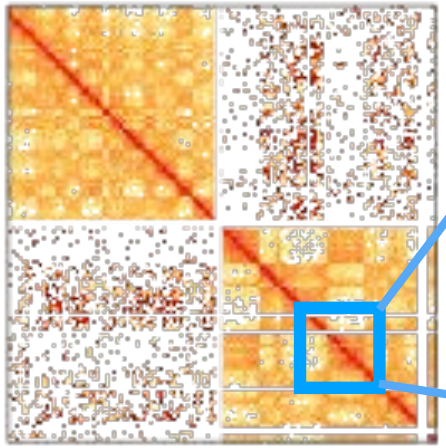
$$P(s) \sim s^{-\alpha}$$



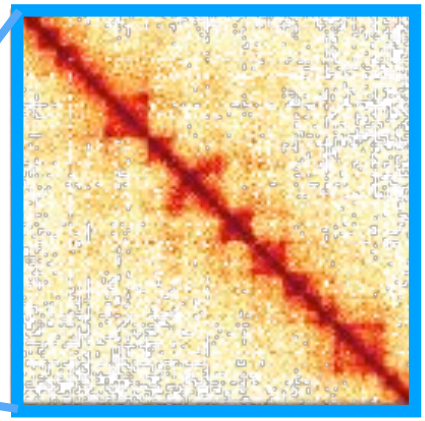
Multiple levels of organization

compartments and **domains**

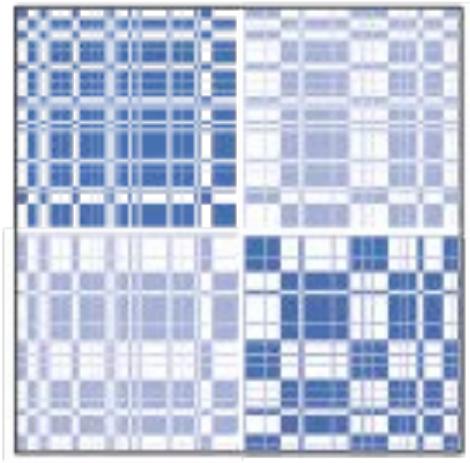




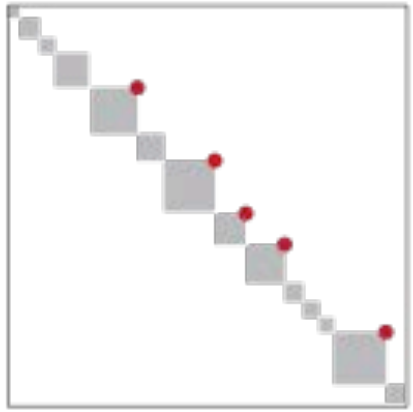
cis+trans Hi-C maps



short-distance (<few Mb)
cis Hi-C maps

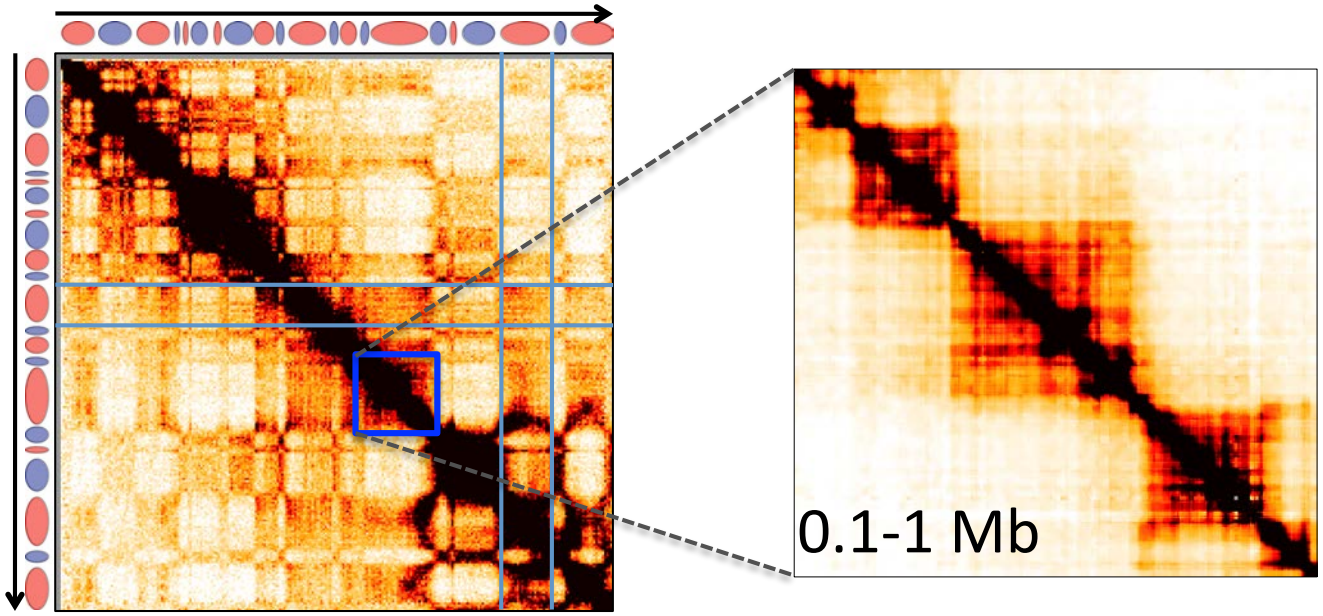


checkered pattern in cis & trans



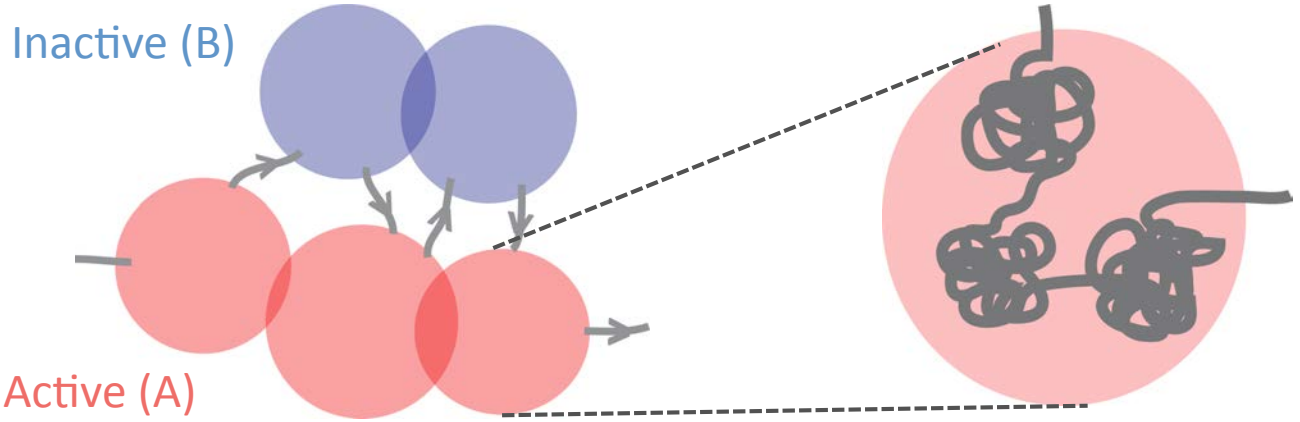
domains of local
contact enrichment

Mechanisms:
compartments and **domains**



A/B Compartments

Domains

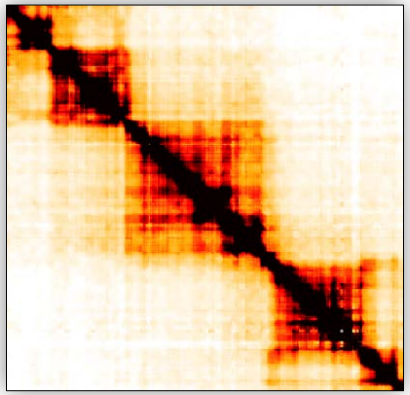


diblock copolymer

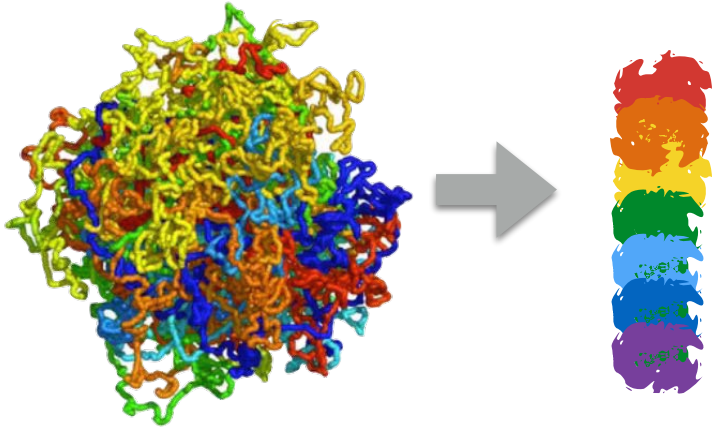
??????

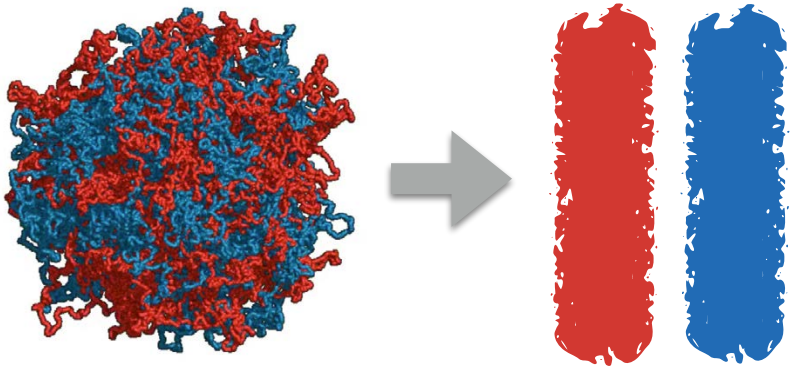
Problems

- Formation of TAD (domains) in in



- Compaction and segregation of chromosomes in mitosis

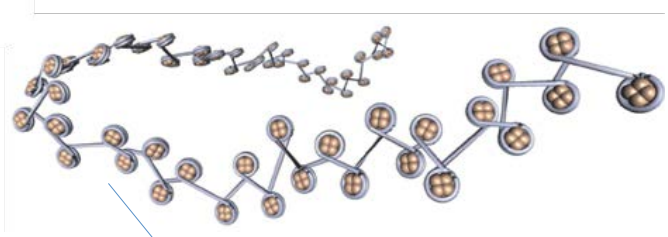




New process: **loop extrusion**

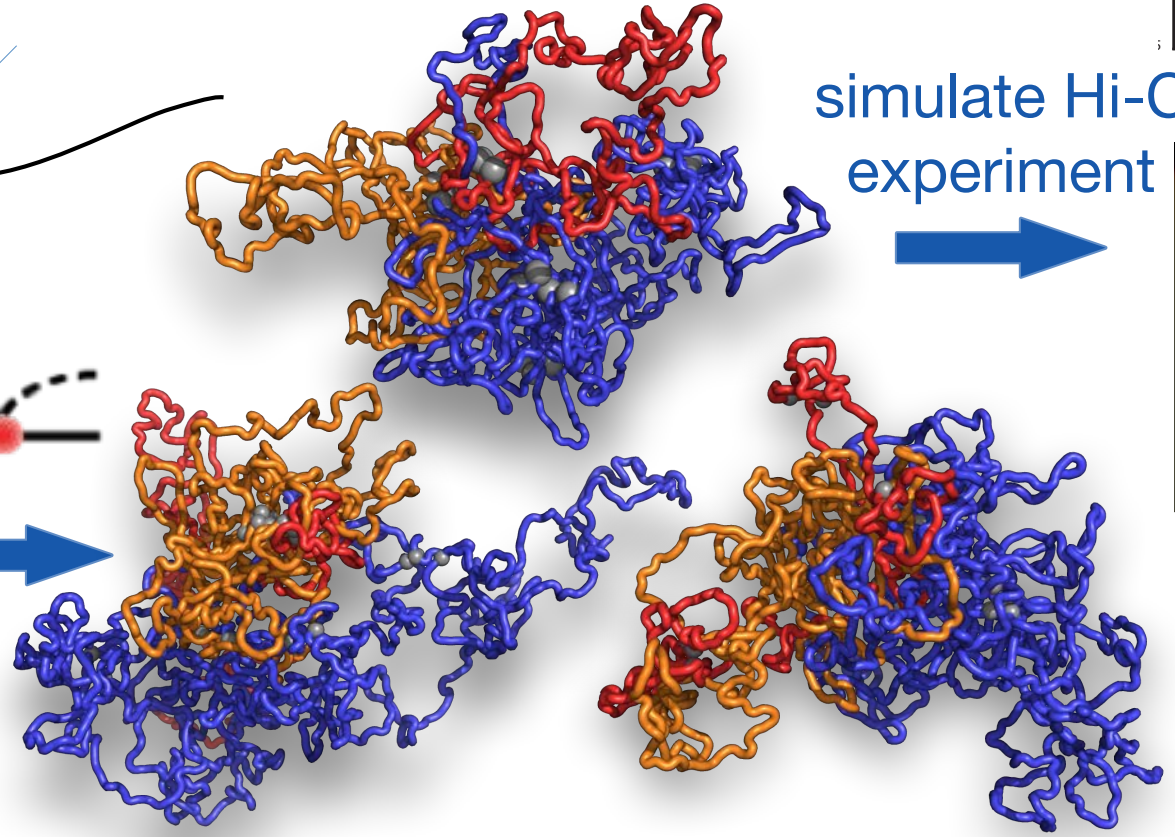
Occam's razor approach

USE PRIOR BIOLOGICAL KNOWLEDGE



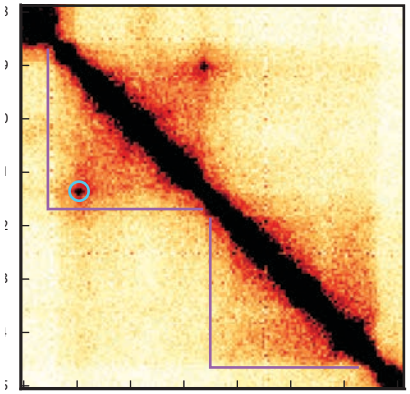
Model-specific constraints

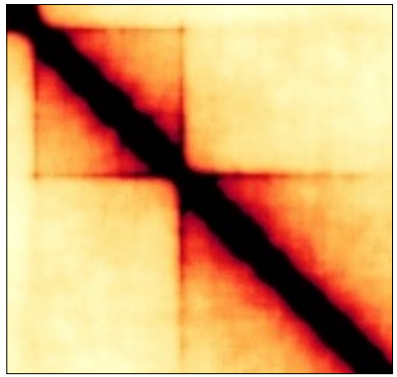
ensemble of conformations



simulate Hi-C experiment

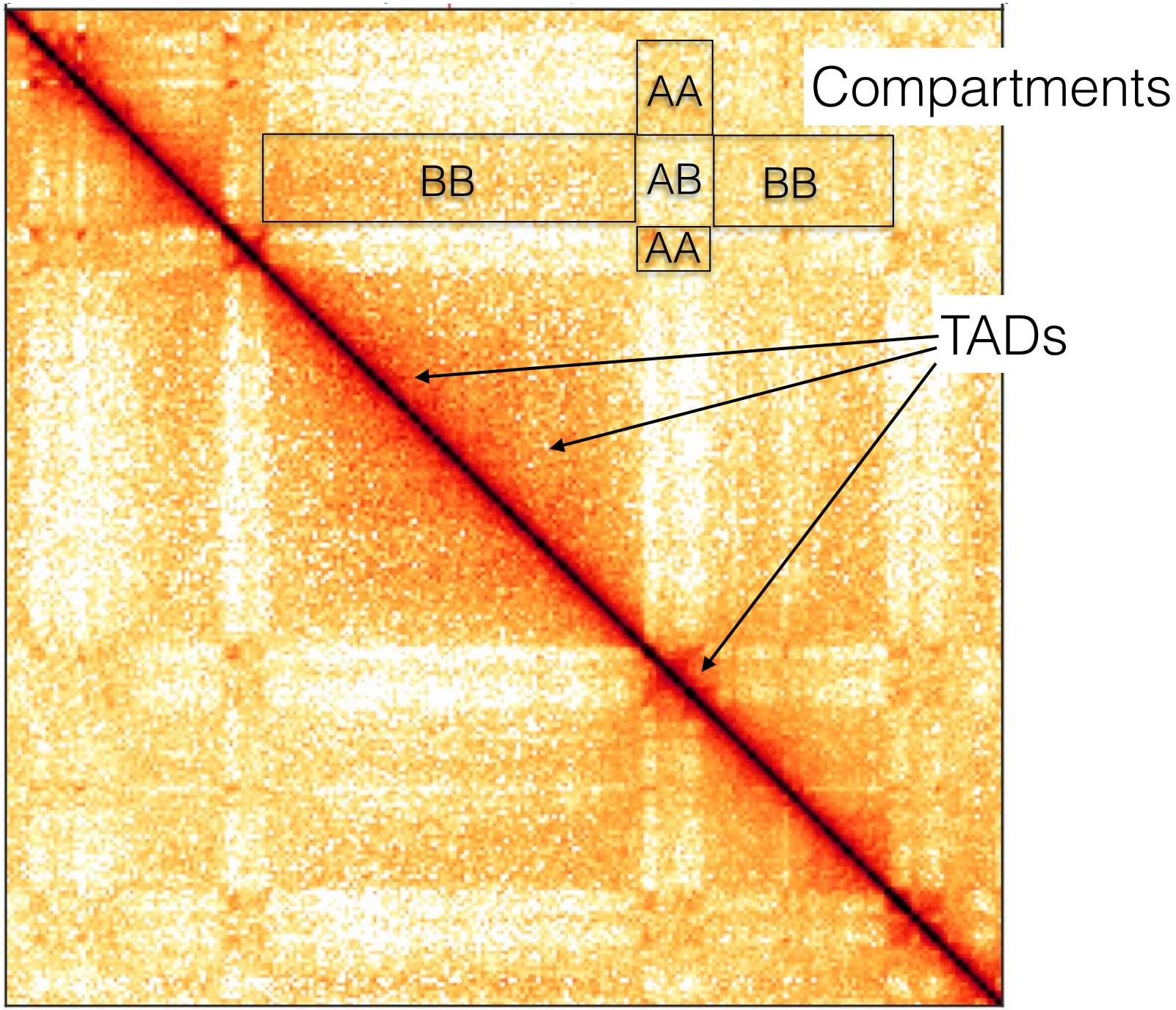
Hi-C data





Compartments

http://HiGlass.io

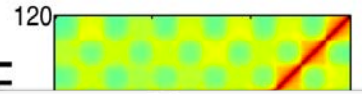


Mechanism of compartmentalization
microphone separation in polymers

Attractions (direct or mediated)

A-A

B-B



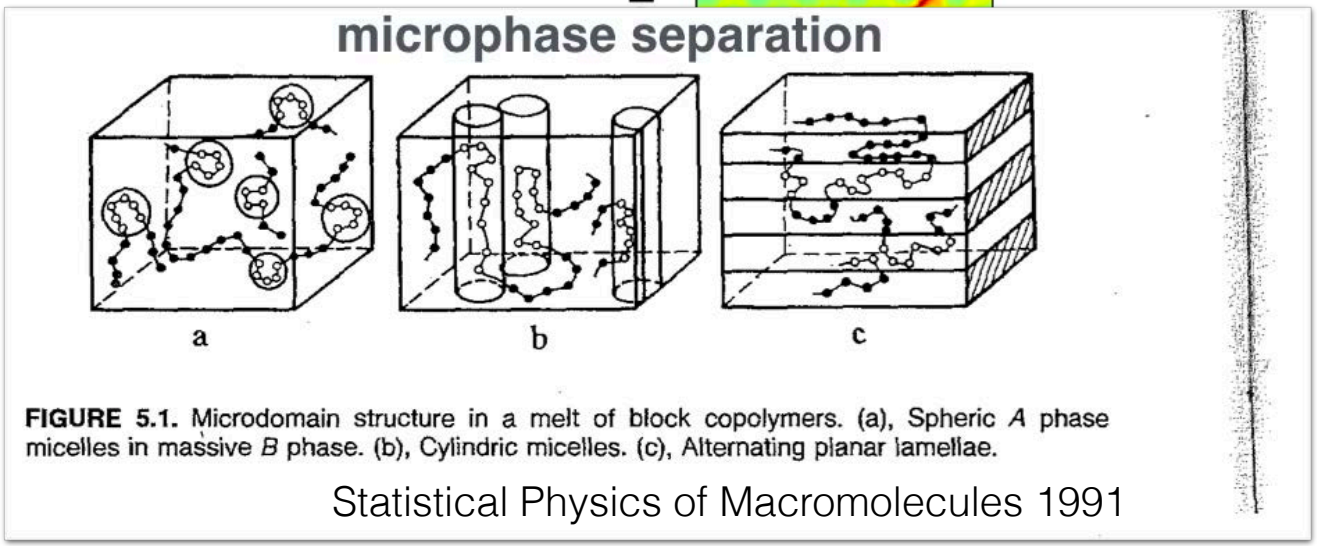
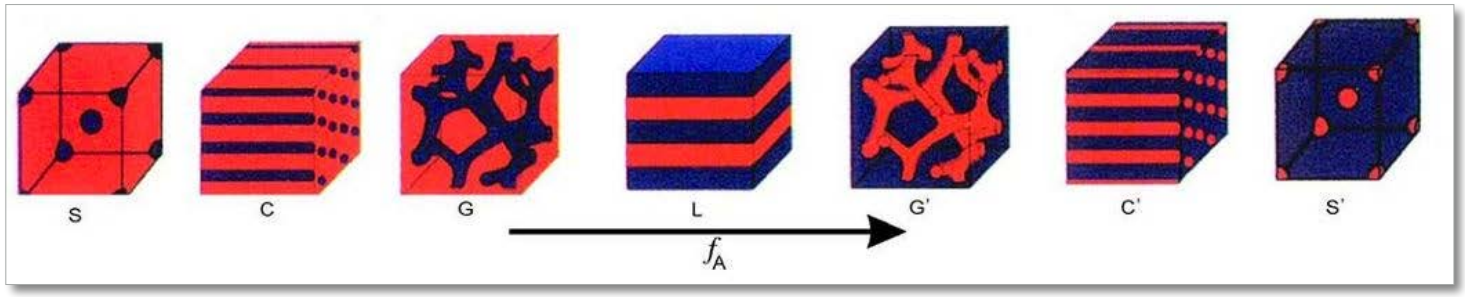


FIGURE 5.1. Microdomain structure in a melt of block copolymers. (a), Spheric *A* phase micelles in massive *B* phase. (b), Cylindric micelles. (c), Alternating planar lamellae.

Statistical Physics of Macromolecules 1991



Martin Falk
MIT

Yana
Feodorova,
Plovidv U

Irina
Solovei,
LMU

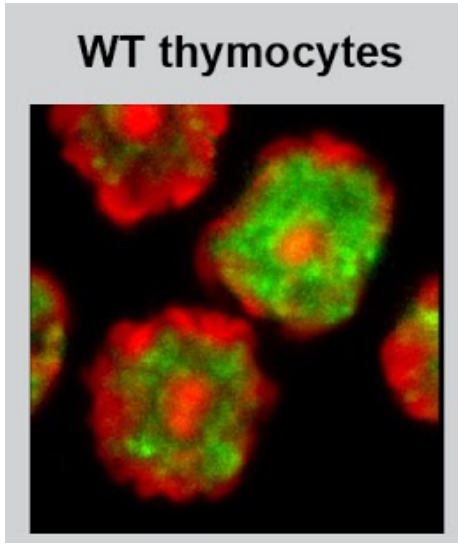
Job Dekker
UMass Medical

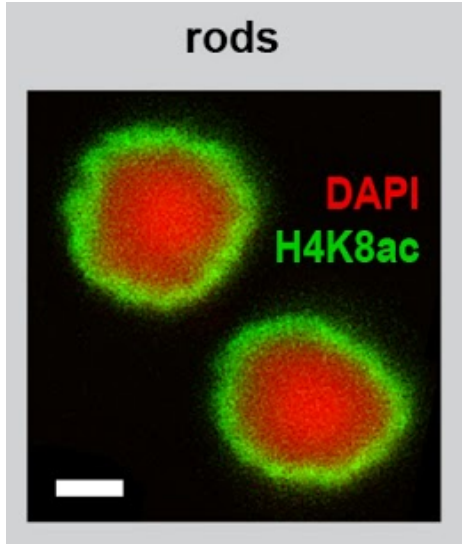
Mechanism of compartmentalization

Attractions (direct or mediated)

A-A
B-B
B-Lamina

Which are more important for compartmentalization?





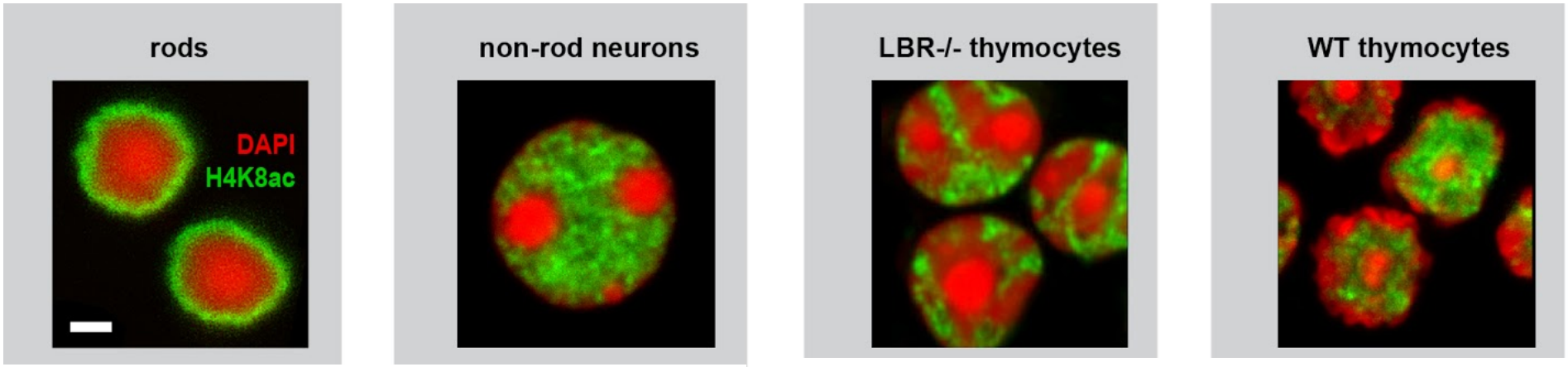
Nature 2019

Heterochromatin drives compartmentalization of inverted and conventional nuclei

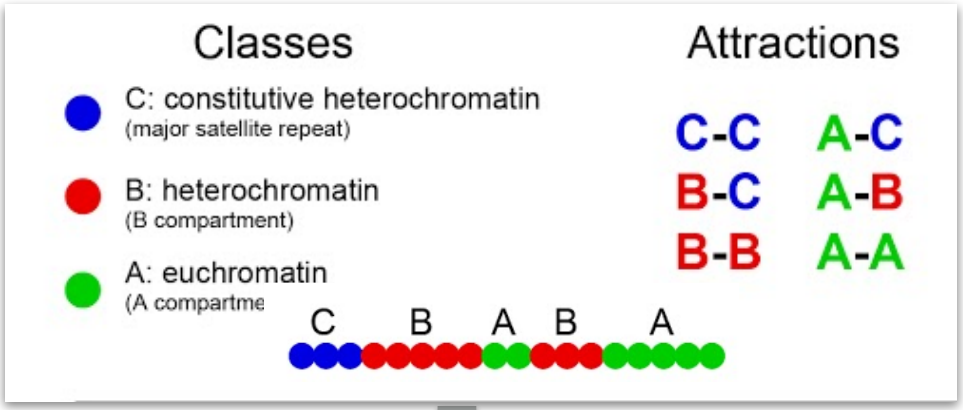
Martin Falk[1,8], Yana Feodorova[2,3,8], Natalia Naumova[4,5,8], Maxim Imakaev[1], Bryan R. Lajoie[4,6], Heinrich Leonhardt[3], Boris Joffe[3], Job Dekker[4], Geoffrey Fudenberg[1,7]*, Irina Solovei[3]* & Leonid A. Mirny[1]*

Mechanism of compartmentalization

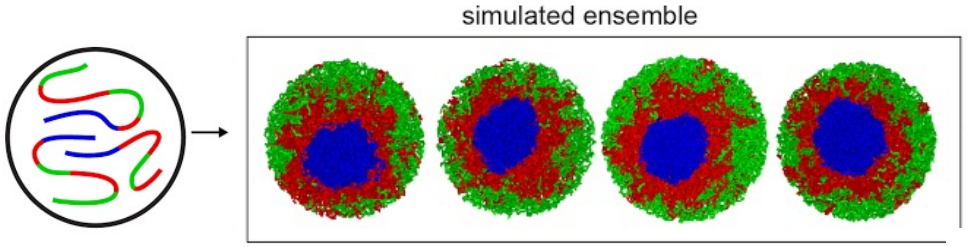
inverted **conventional** **inverted** **conventional**



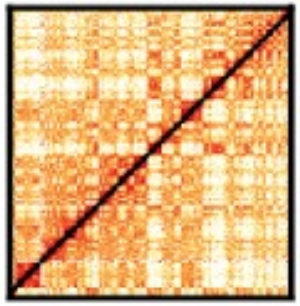
Mechanism of compartmentalization



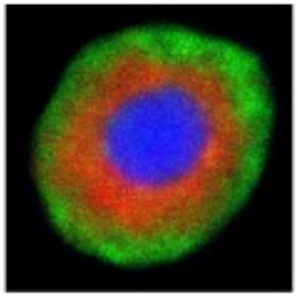
(micro)phase separation
in a block copolymer



Hi-C



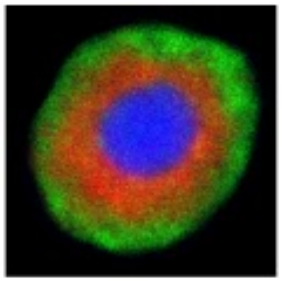
imaging

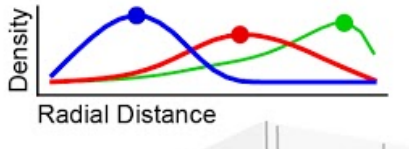


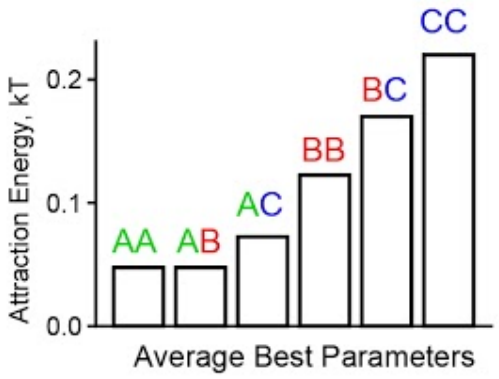
Inverted nucleus

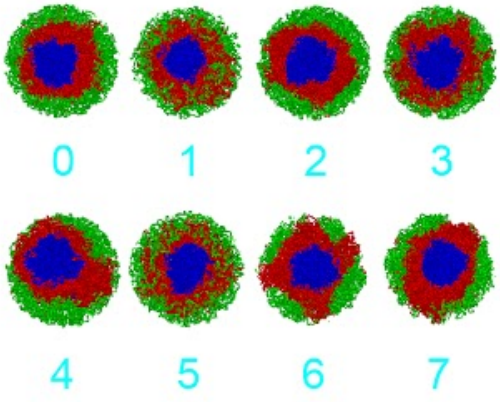
CC<BC<BB<AB<AA
BC<CC<BB<AB<AA
AA<AB<BB<BC<CC

….720 classes of models….



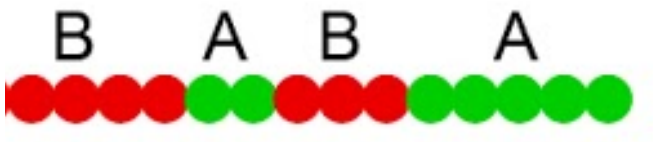






AA < AB < BB < BC < CC

Summary: Mechanism of compartmentalization

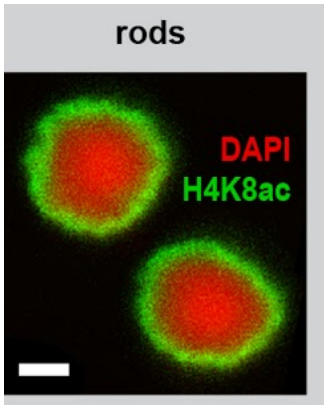


Attractions:

~~AA~~

BB <— *for compartmentalization*

B-Lamina <— *for positioning in the nucleus*



inverted *is the default state of the nucleus!*

inverted is new conventional

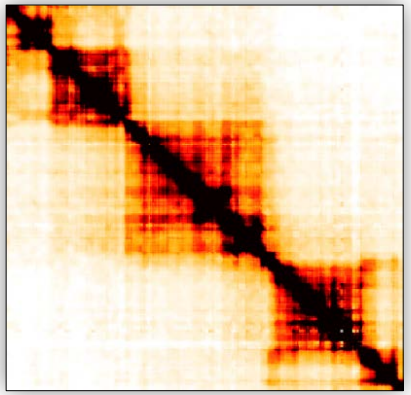
Nature 2019

Heterochromatin drives compartmentalization of inverted and conventional nuclei

Martin Falk[1,8], Yana Feodorova[2,3,8], Natalia Naumova[4,5,8], Maxim Imakaev[1], Bryan R. Lajoie[4,6], Heinrich Leonhardt[3], Boris Joffe[3], Job Dekker[4], Geoffrey Fudenberg[1,7]*, Irina Solovei[3]* & Leonid A. Mirny[1]*

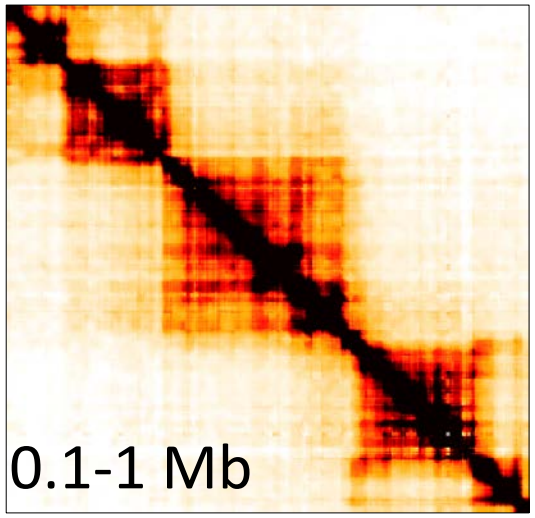
Problem

- Formation of TAD (domains) in

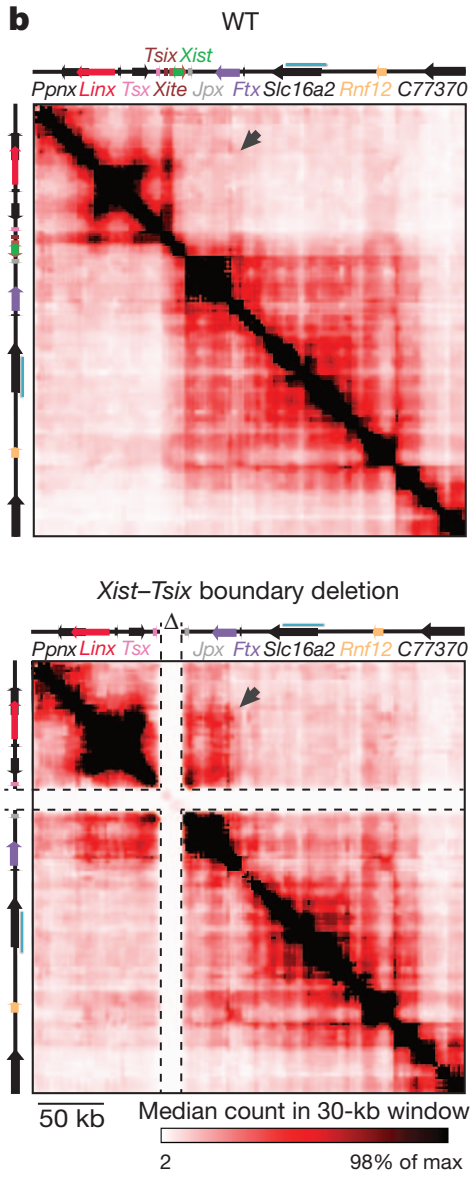


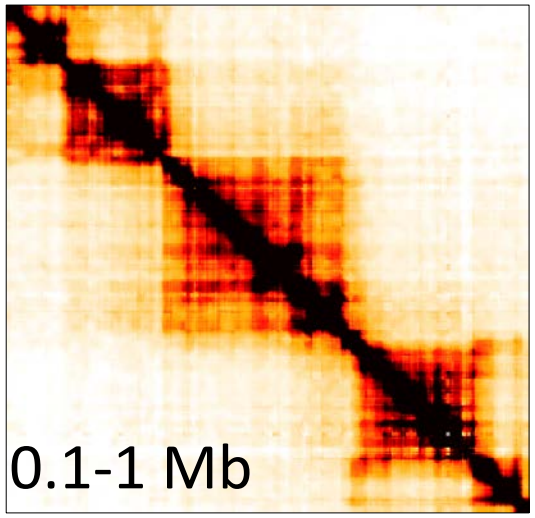
Problem

What's the mechanism of domain formation?



Domains boundaries are essential for domain formation





Teunissen, H., Splinter, E., Wijchers, P.J., Krijger, P.H., and de Laat, W. (2015). CTCF Binding Polarity Determines Chromatin Looping. Mol Cell *60*, 676-684.

Narendra V, et al. (2015) CTCF establishes discrete functional chromatin domains at the Hox clusters during differentiation. *Science (New York, NY)* 347(6225):1017–1021.

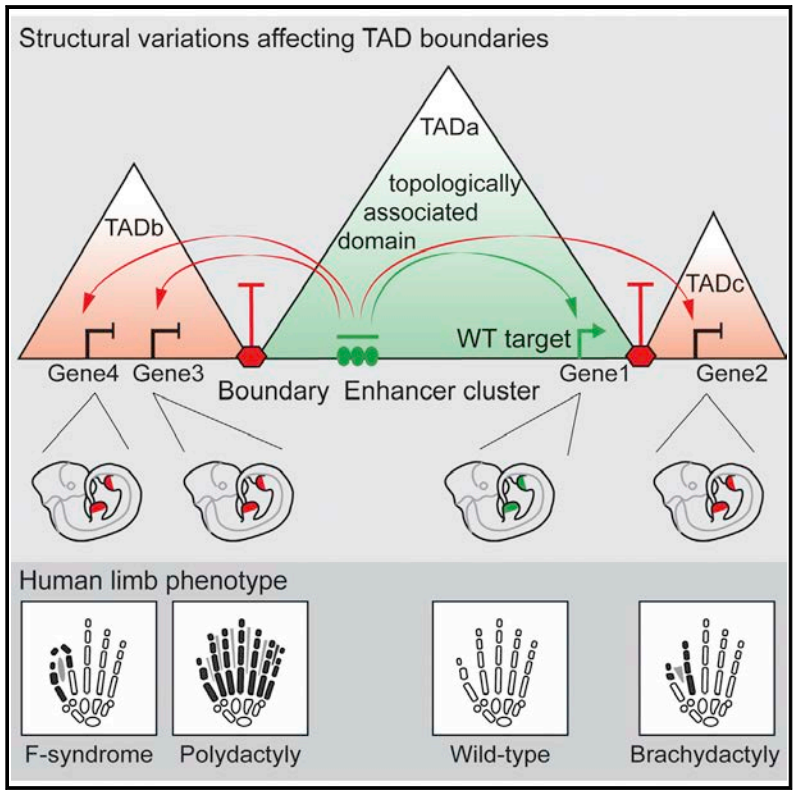
Guo Y, et al. (2015) CRISPR Inversion of CTCF Sites Alters Genome Topology and Enhancer/Promoter Function. *Cell* 162(4):900–910.

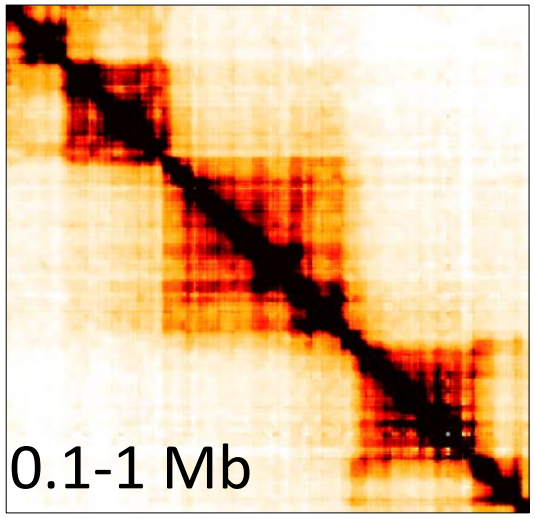
Sanborn AL, et al. (2015) Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. *Proceedings of the National Academy of Sciences* 112(47):E6456–65.

17 MAY 2012 | VOL 485 | NATURE |

Elphège P. Nora[1,2,3], Bryan R. Lajoie[4]*, Edda G. Schulz[1,2,3]*, Luca Giorgetti[1,2,3]*, Ikuhiro Okamoto[1,2,3], Nicolas Servant[1,5,6], Tristan Piolot[1,2,3], Nynke L. van Berkum[4], Johannes Meisig[7], John Sedat[8], Joost Gribnau[9], Emmanuel Barillot[1,5,6], Nils Blüthgen[7], Job Dekker[4] & Edith Heard[1,2,3]

Domains boundaries controls functional interactions





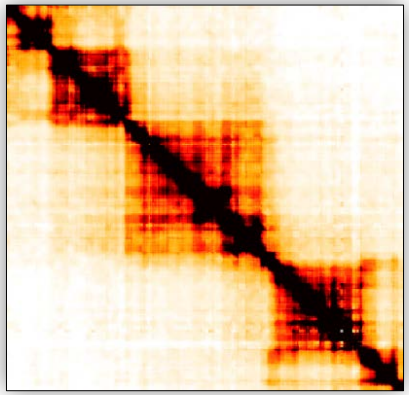
Disruptions of Topological Chromatin Domains Cause Pathogenic Rewiring of Gene-Enhancer Interactions

Darío G. Lupiáñez,[1,2] Katerina Kraft,[1,2] Verena Heinrich,[2] Peter Krawitz,[1,2] Francesco Brancati,[3] Eva Klopocki,[4] Denise Horn,[2] Hülya Kayserili,[5] John M. Opitz,[6] Renata Laxova,[6] Fernando Santos-Simarro,[7,8] Brigitte Gilbert-Dussardier,[9] Lars Wittler,[10] Marina Borschiwer,[1] Stefan A. Haas,[11] Marco Osterwalder,[12] Martin Franke,[1,2] Bernd Timmermann,[13] Jochen Hecht,[1,14] Malte Spielmann,[1,2,14] Axel Visel,[12,15,16] and Stefan Mundlos[1,2,14,*]

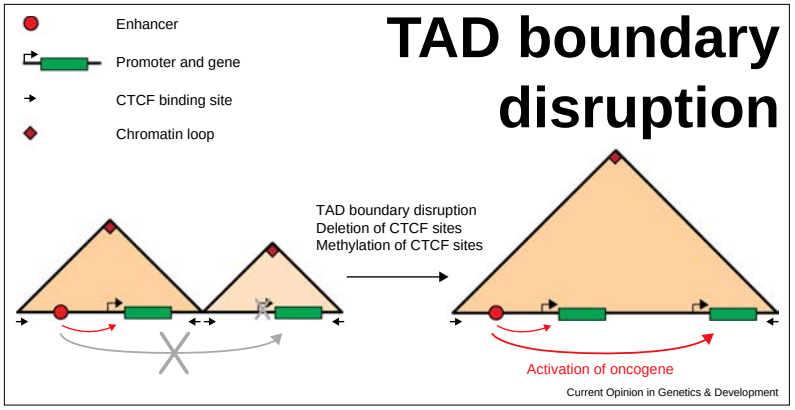
Lupiáñez et al., 2015, Cell *161*, 1–14
May 21, 2015
http://dx.doi.org/10.1016/j.cell.2015.04.004

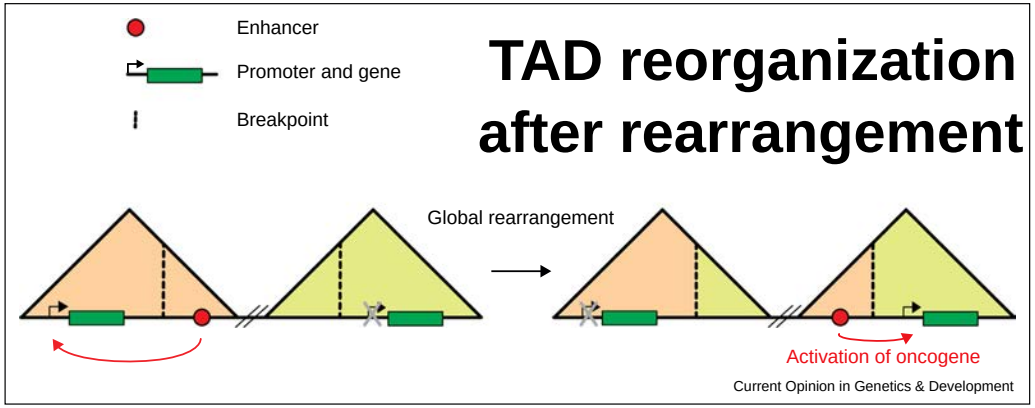
In cancer…

- Formation of TAD (domains) in in



Enhancer hijacking

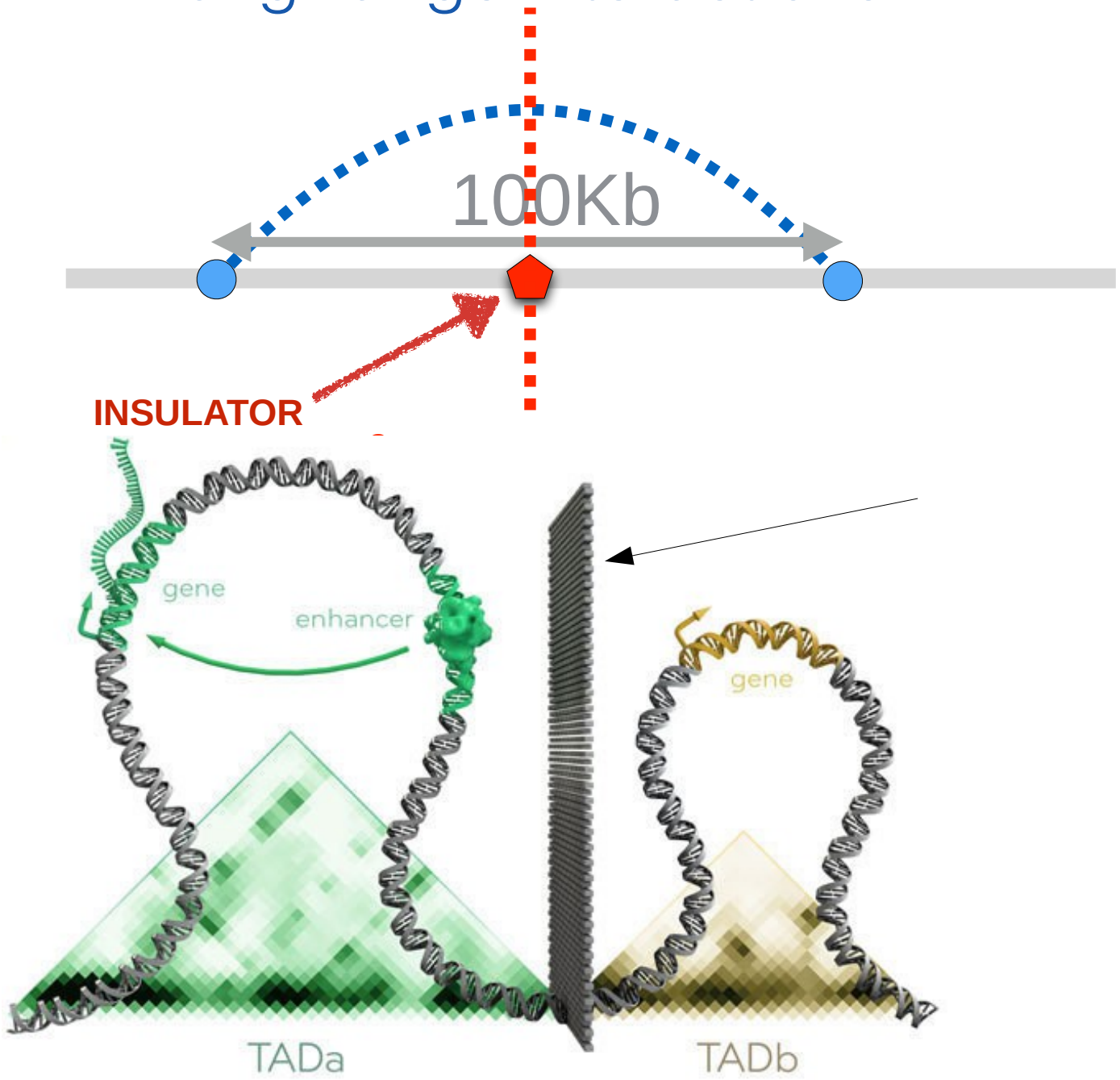




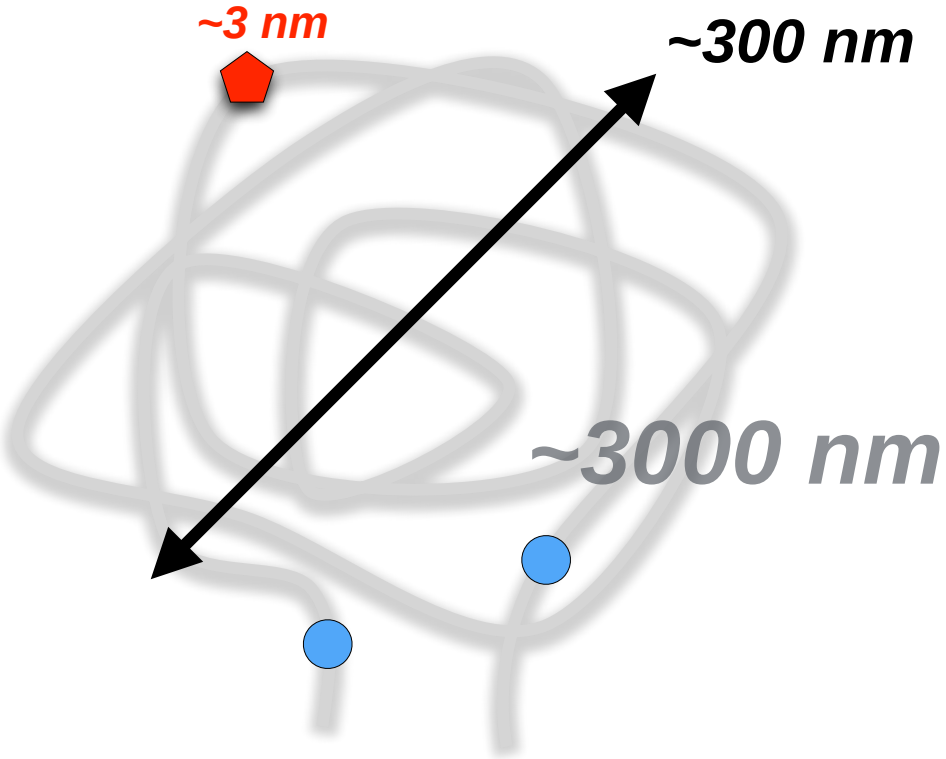
Current Opinion in Genetics & Development

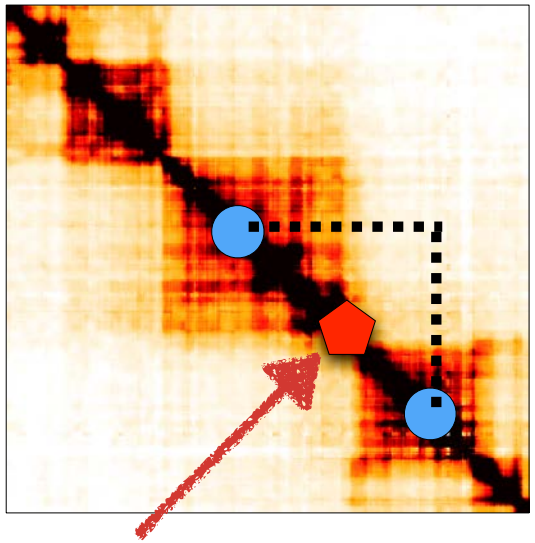
TAD disruption as oncogenic driver
Anne-Laure Valton and Job Dekker

PROBLEM 1: how can small proteins control long-range interactions?

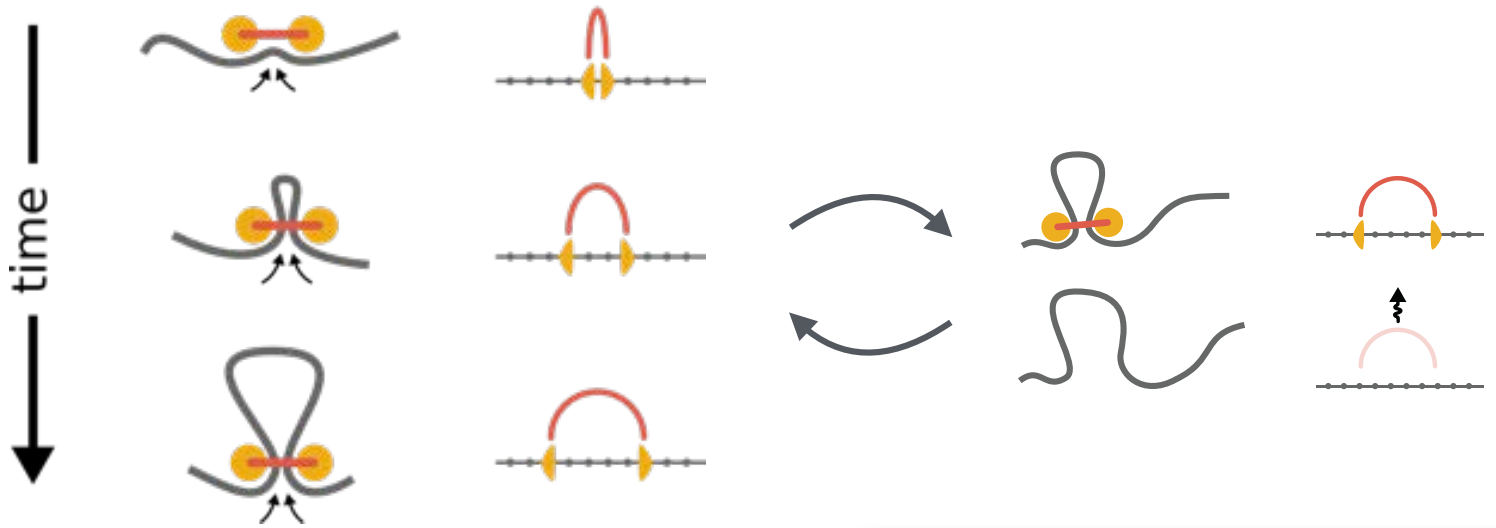


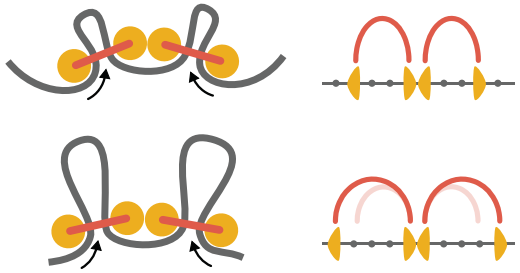
PROBLEM 1: Problem of scales

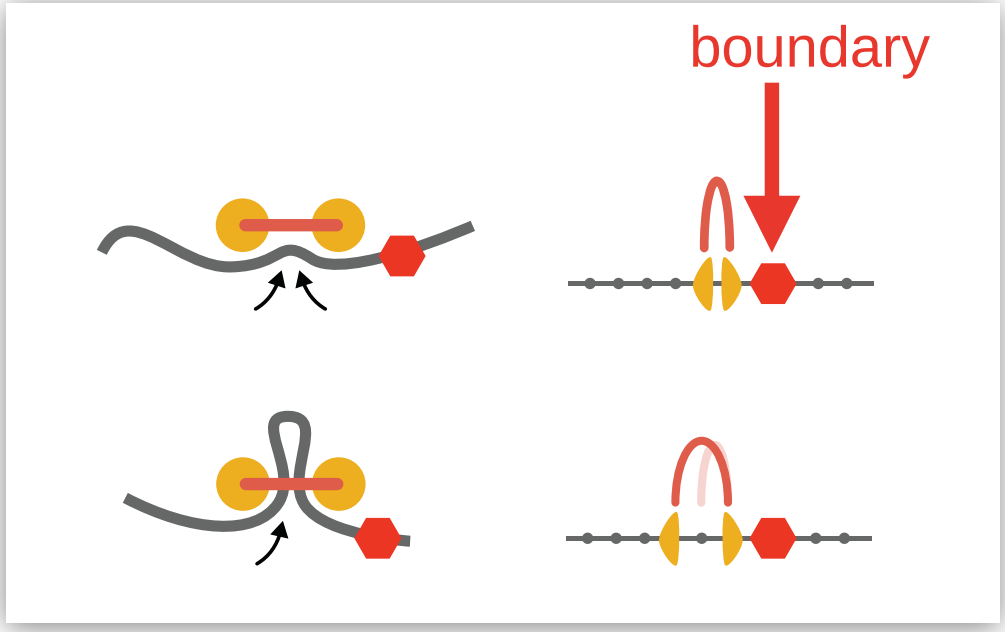




Loop extrusion with boundaries => TADs







Formation of Chromosomal Domains by Loop Extrusion
bioRxiv Aug 14 (2015)
Fudenberg, Imakaev et al.
DOI: 10.1101/024620

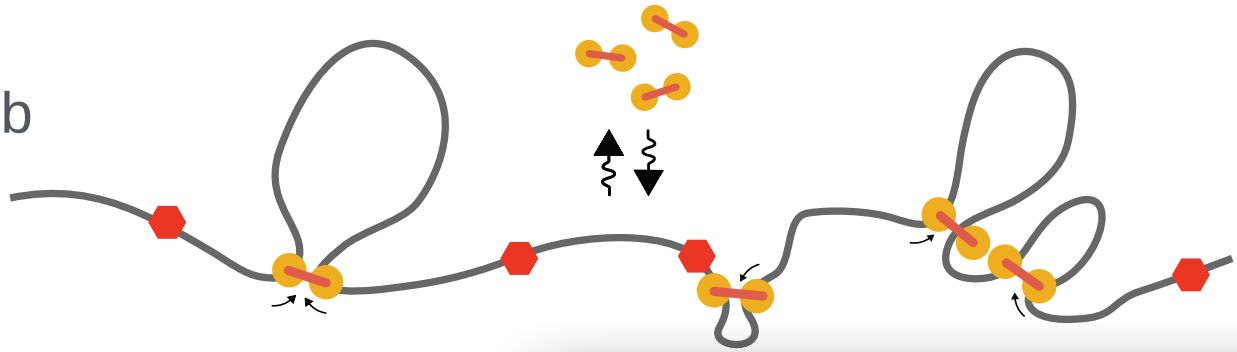
bioRxiv beta THE PREPRINT SERVER FOR BIOLOGY

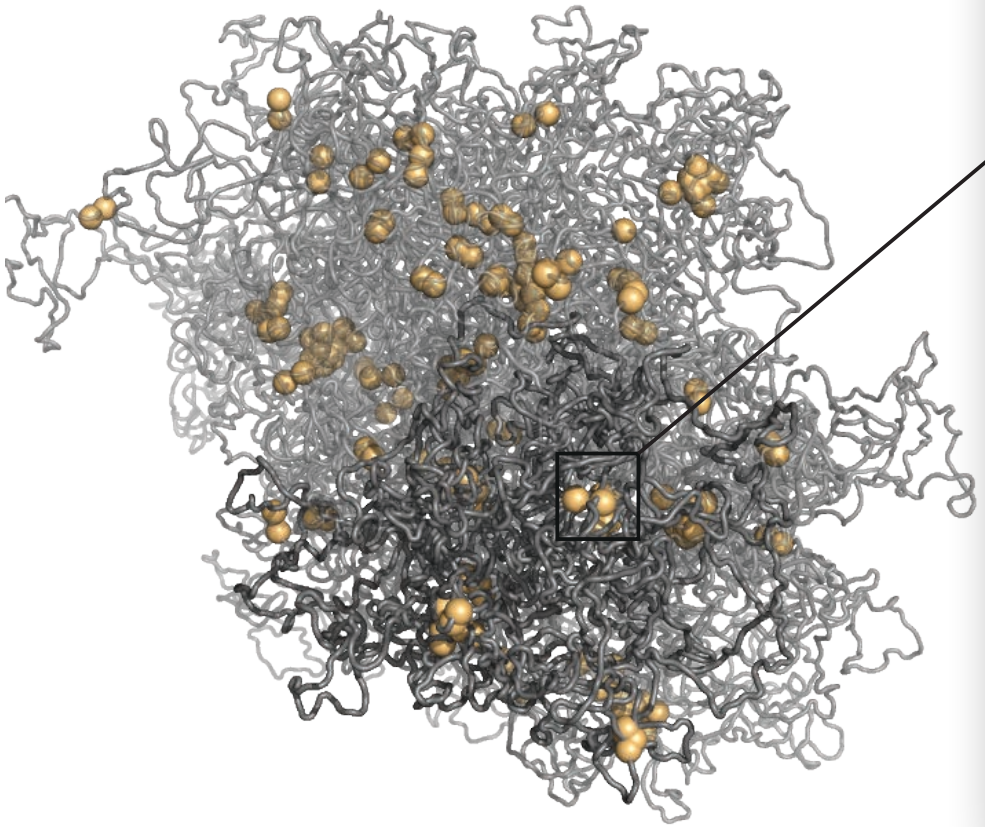
Cell Reports

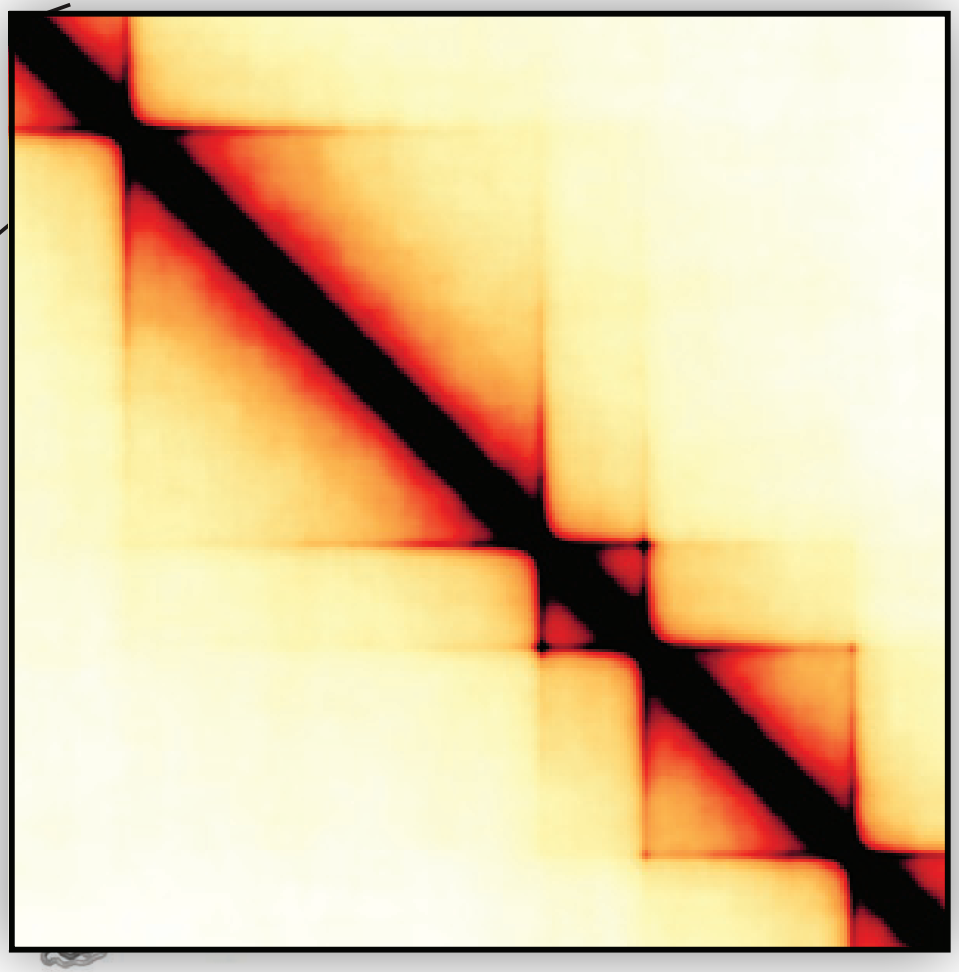
Loop extrusion with boundaries => TADs

Model

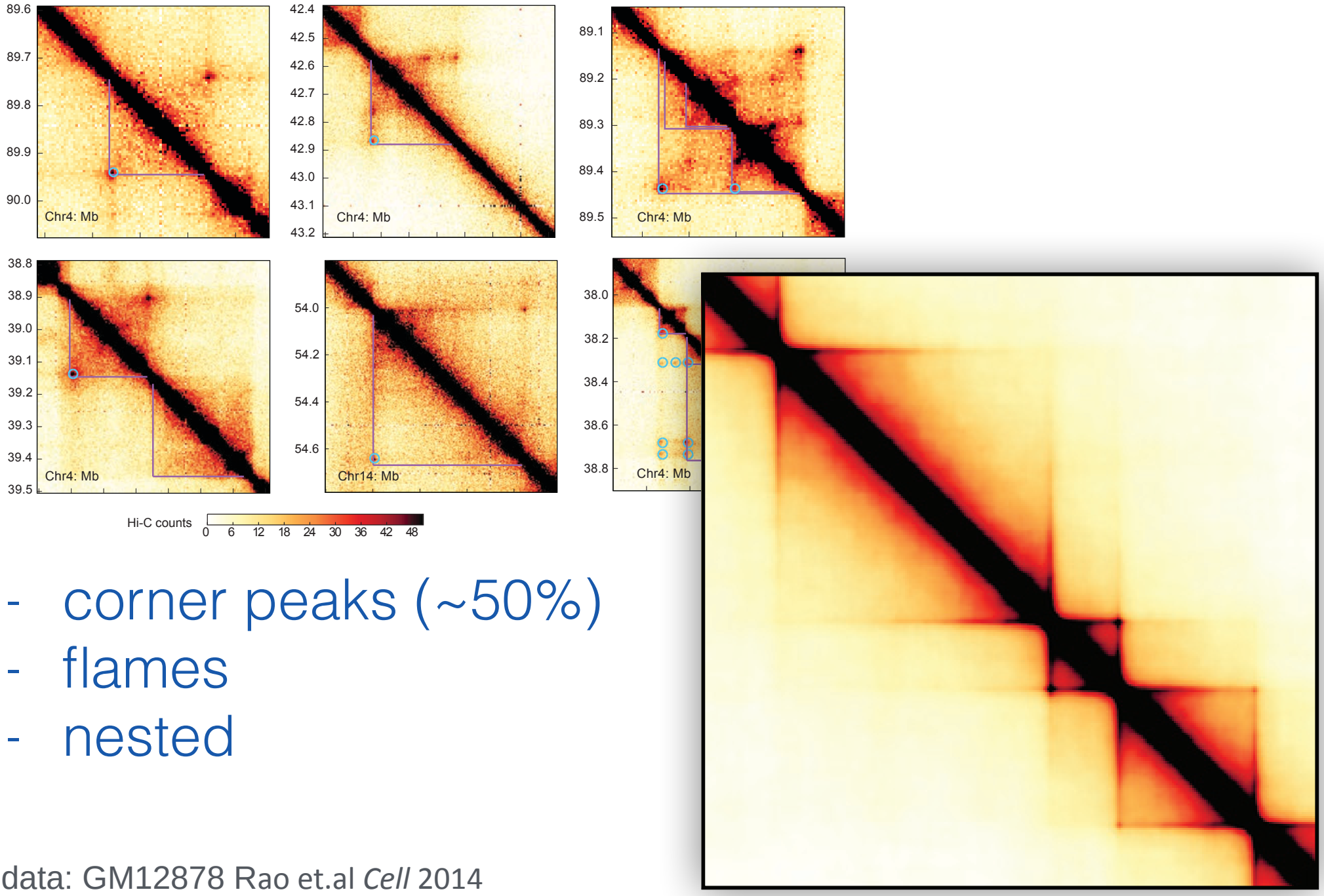
36 domains=10Mb







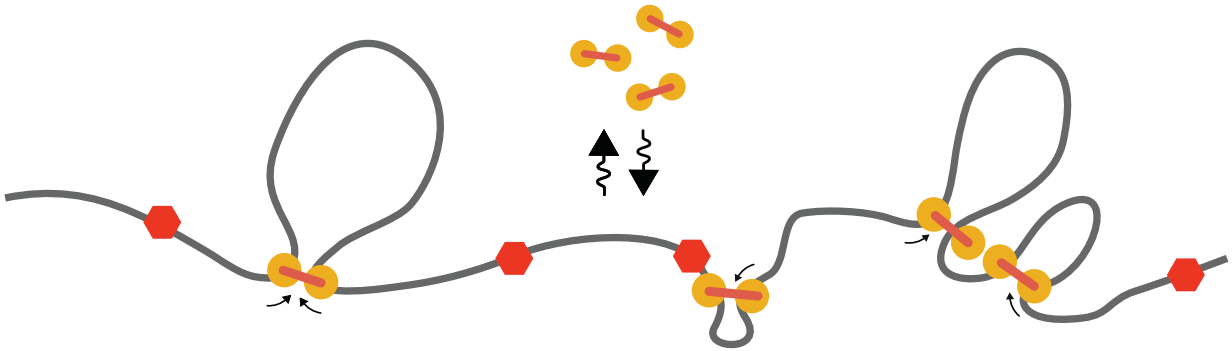
Complex architecture of TADs

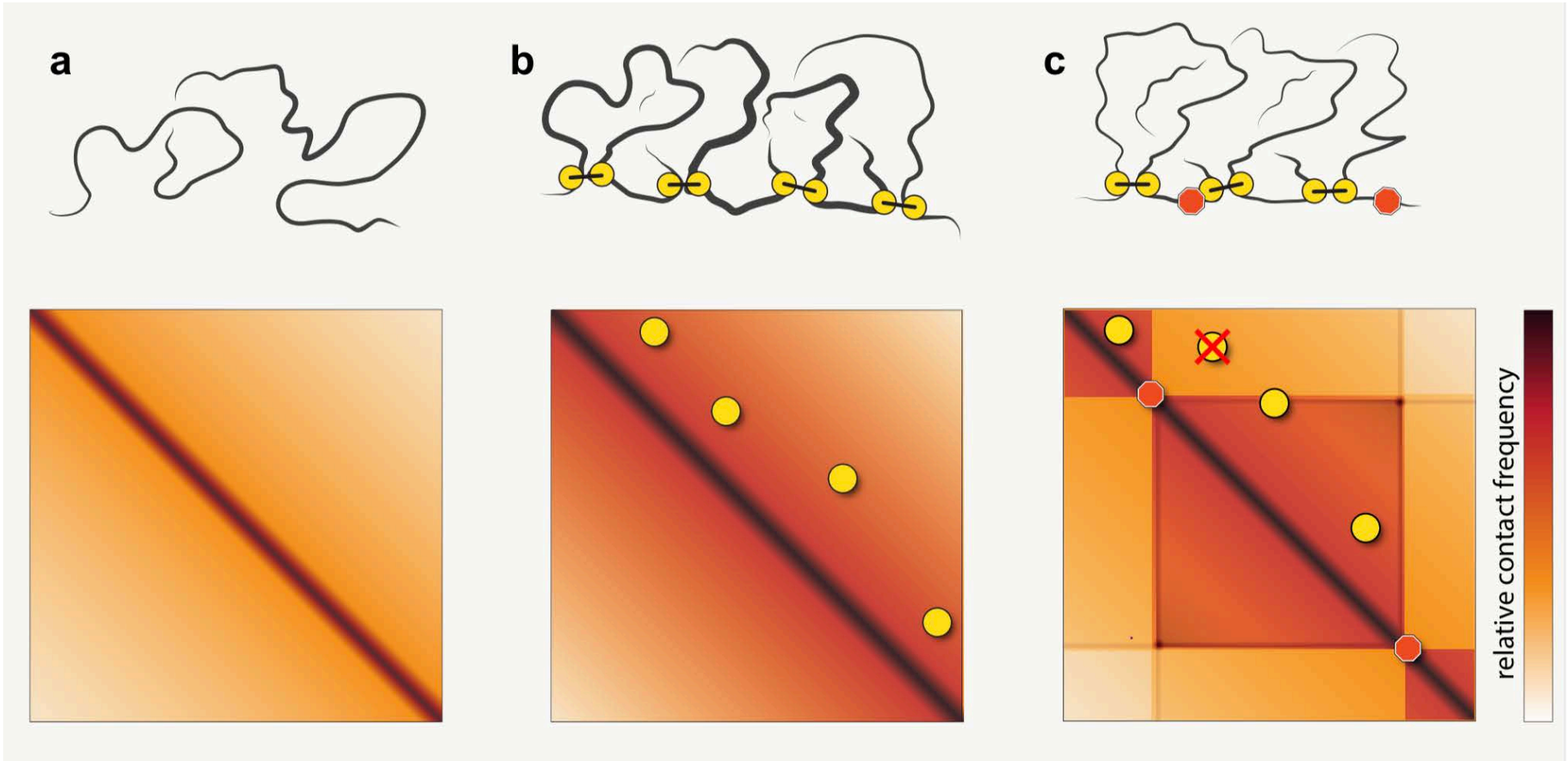


- corner peaks (~50%)
- flames
- nested

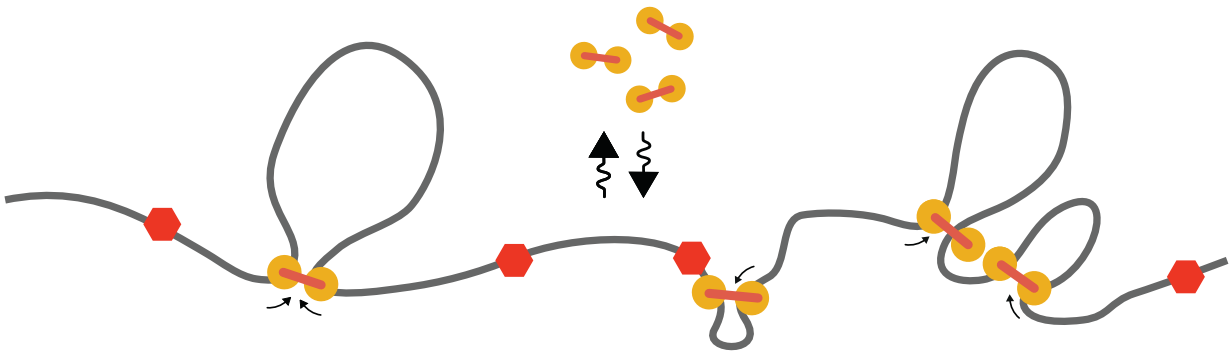
data: GM12878 Rao et.al *Cell* 2014

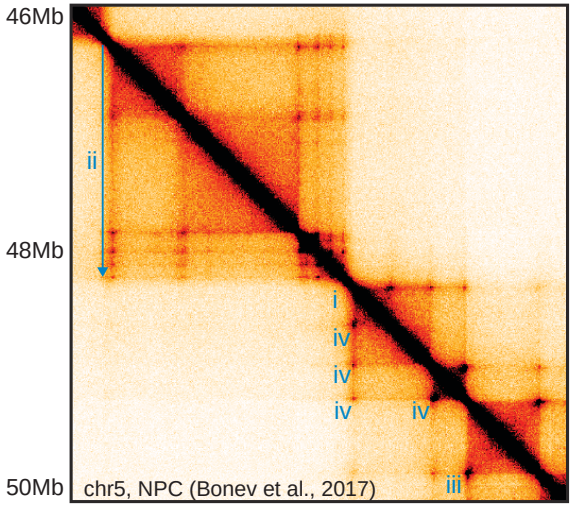
Loop extrusion => TADs, flames, dots, grids etc





PROBLEM 1: Formation of domains by loop extrusion



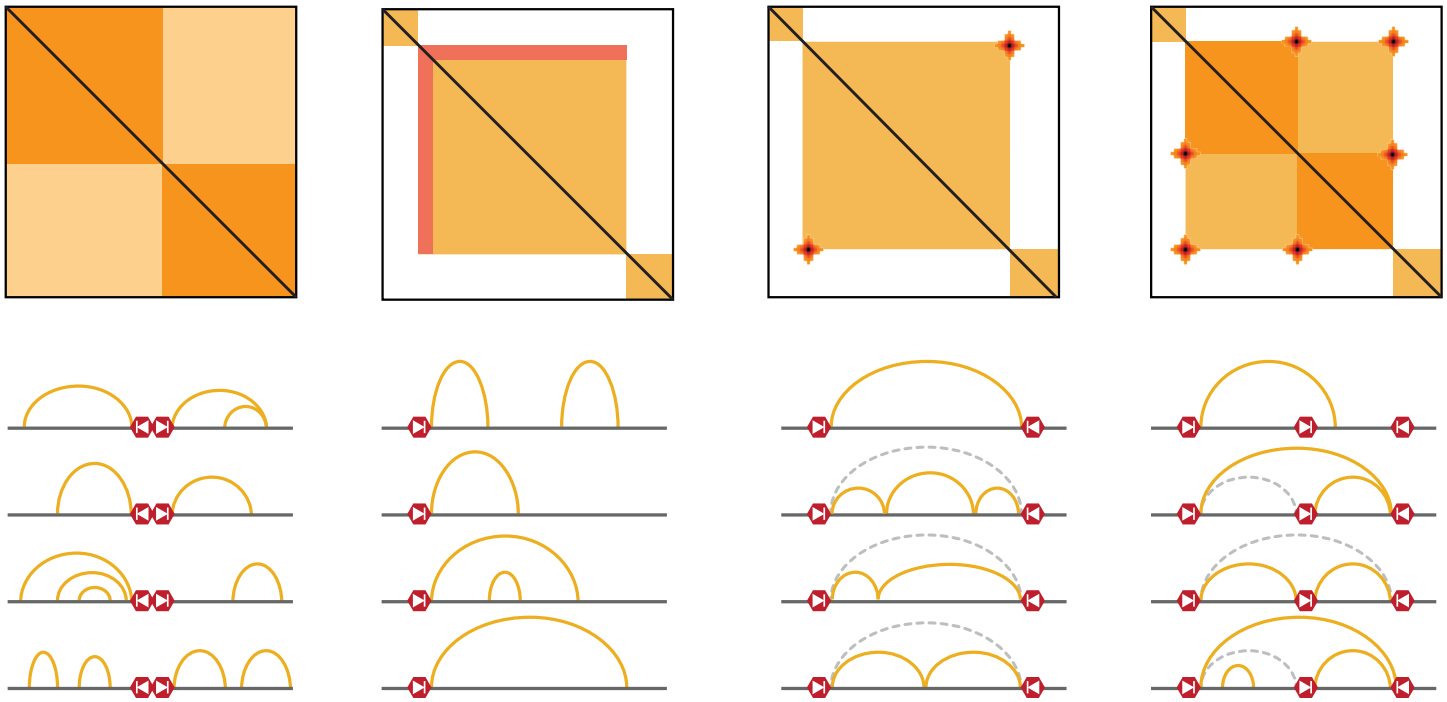


i insulation

ii flame (track)

iii corner peak

iv peak grid

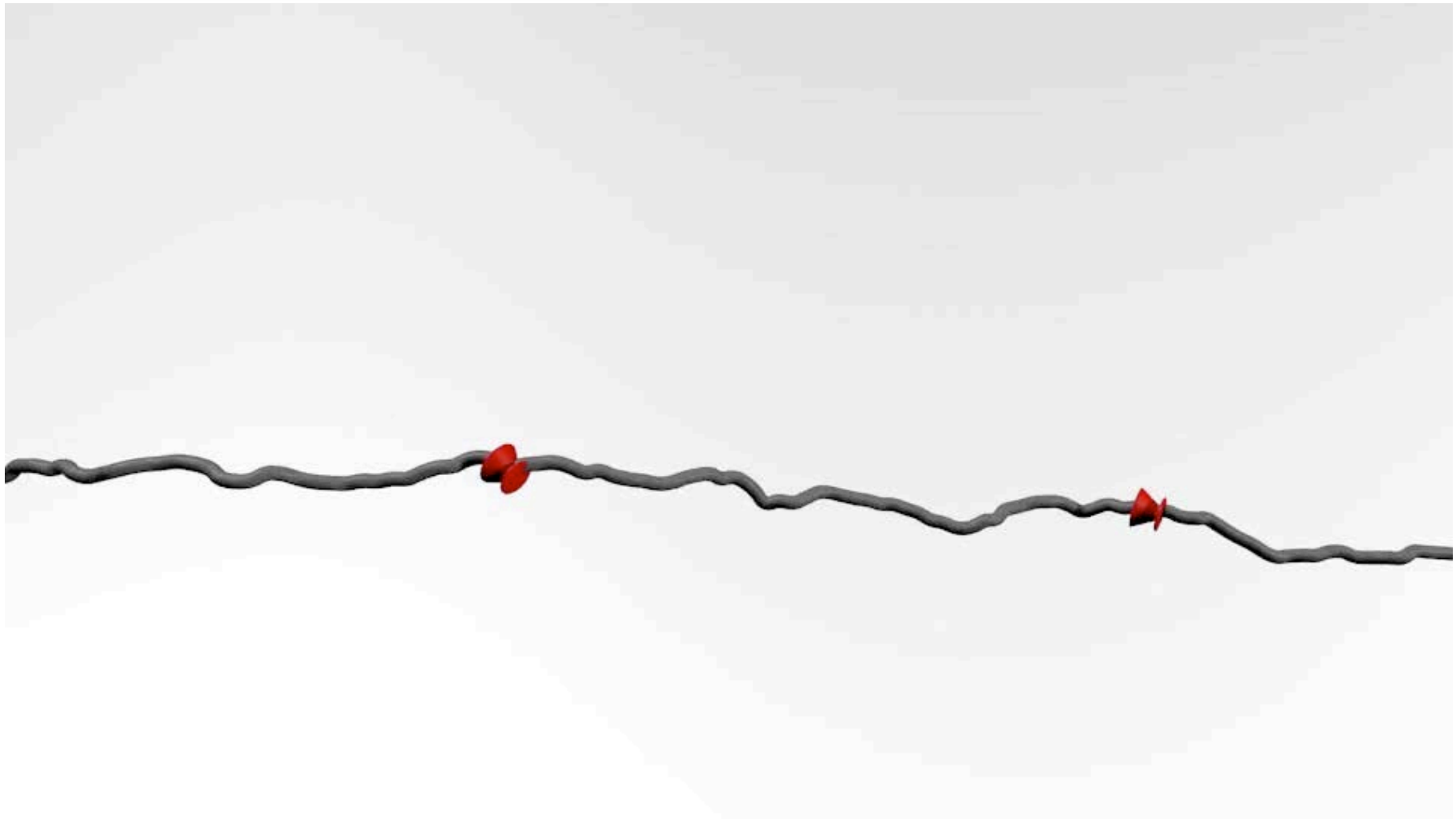


Scanning! ***<10%*** ***<10%***

Fudenberg et al Emerging Evidence of Chromosome Folding by Loop Extrusion
CSH Symposia in Qunat. Bio (2018)

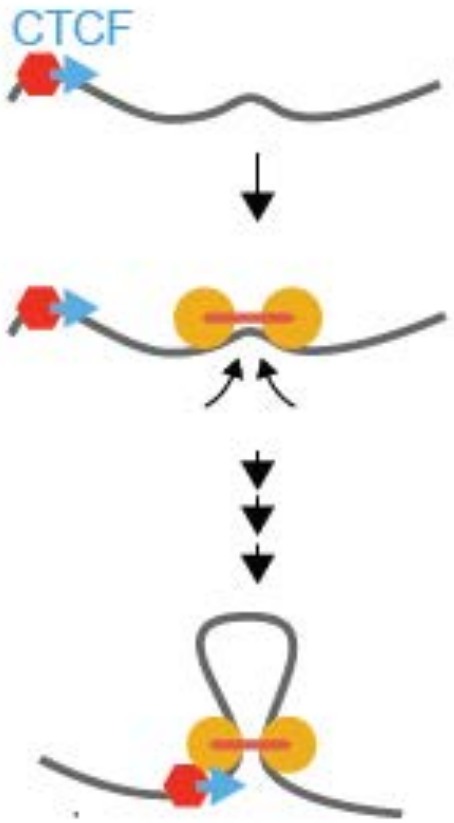
Domain — systems of actively extruded loops

YouTube Search youtube mirnylab

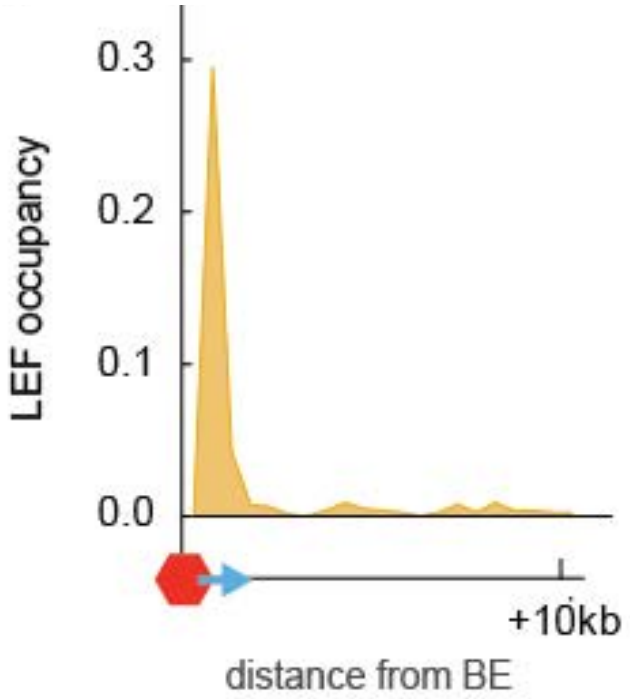


https://www.youtube.com/watch?v=8FW6gOx5lPI

Cohesin **—** accumulates near boundaries

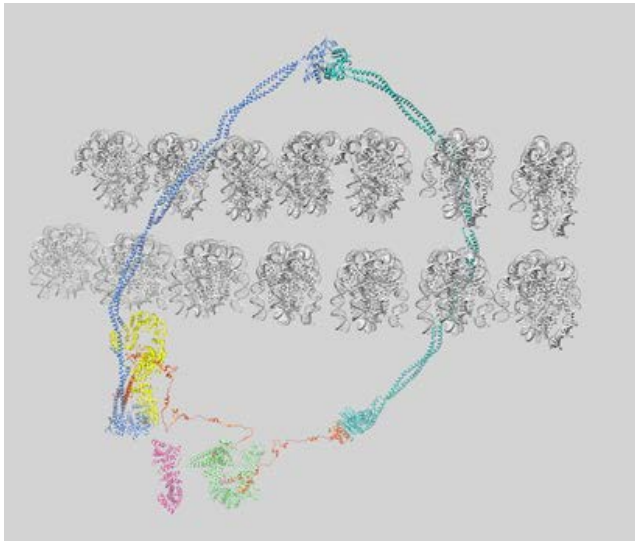


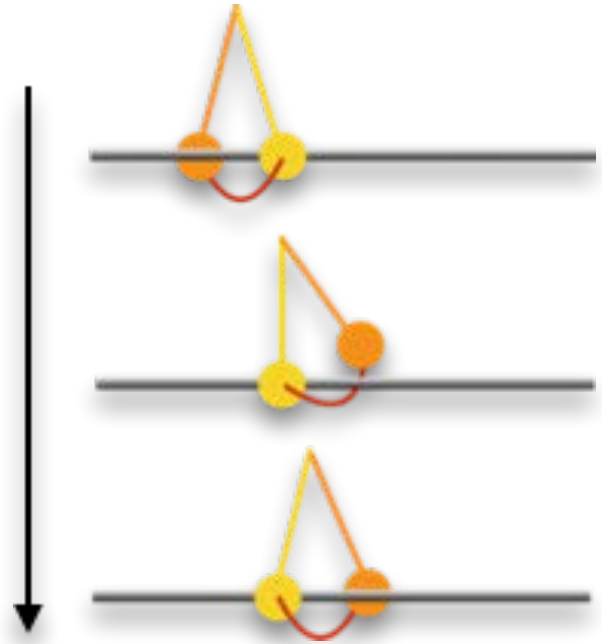
Model



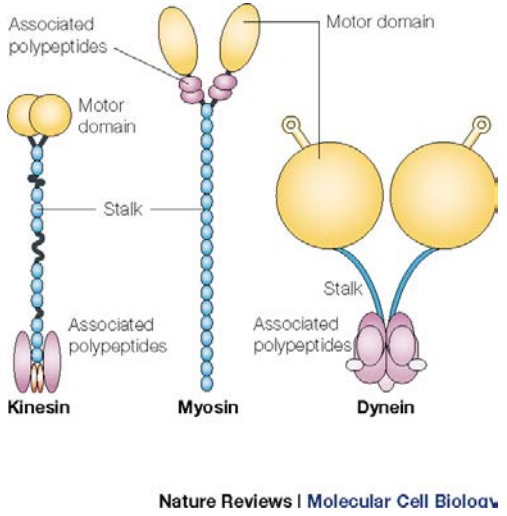
Hypothesis: **cohesin** (and other SMCs) — loop extruding motor

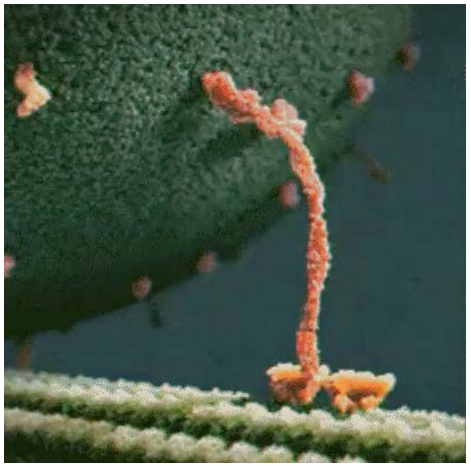
cohesin





Known molecular motors

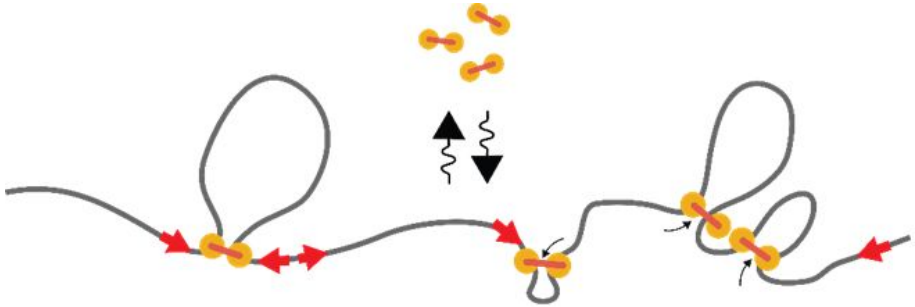




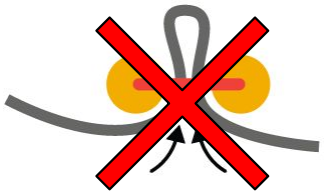
REVIEW: Fundenberg et al bioRxiv 2018

Testing the theory

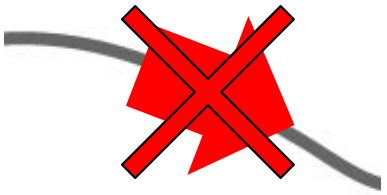
depleting
1. loop extruding enzyme (cohesin)
2. boundary element (CTCFs)



ΔNipbl = no extruders



CTCF-AID = no boundaries



Two independent modes of chromatin organization revealed by cohesin removal

Wibke Schwarzer[1]*, Nezar Abdennur[2]*, Anton Goloborodko[3]*, Aleksandra Pekowska[4], Geoffrey Fudenberg[5], Yann Loe-Mie[6,7], Nuno A Fonseca[8], Wolfgang Huber[4], Christian H. Haering[9], Leonid Mirny[3,5] & Francois Spitz[1,4,6,7]

2 NOVEMBER 2017 | VOL 551 | NATURE | 51

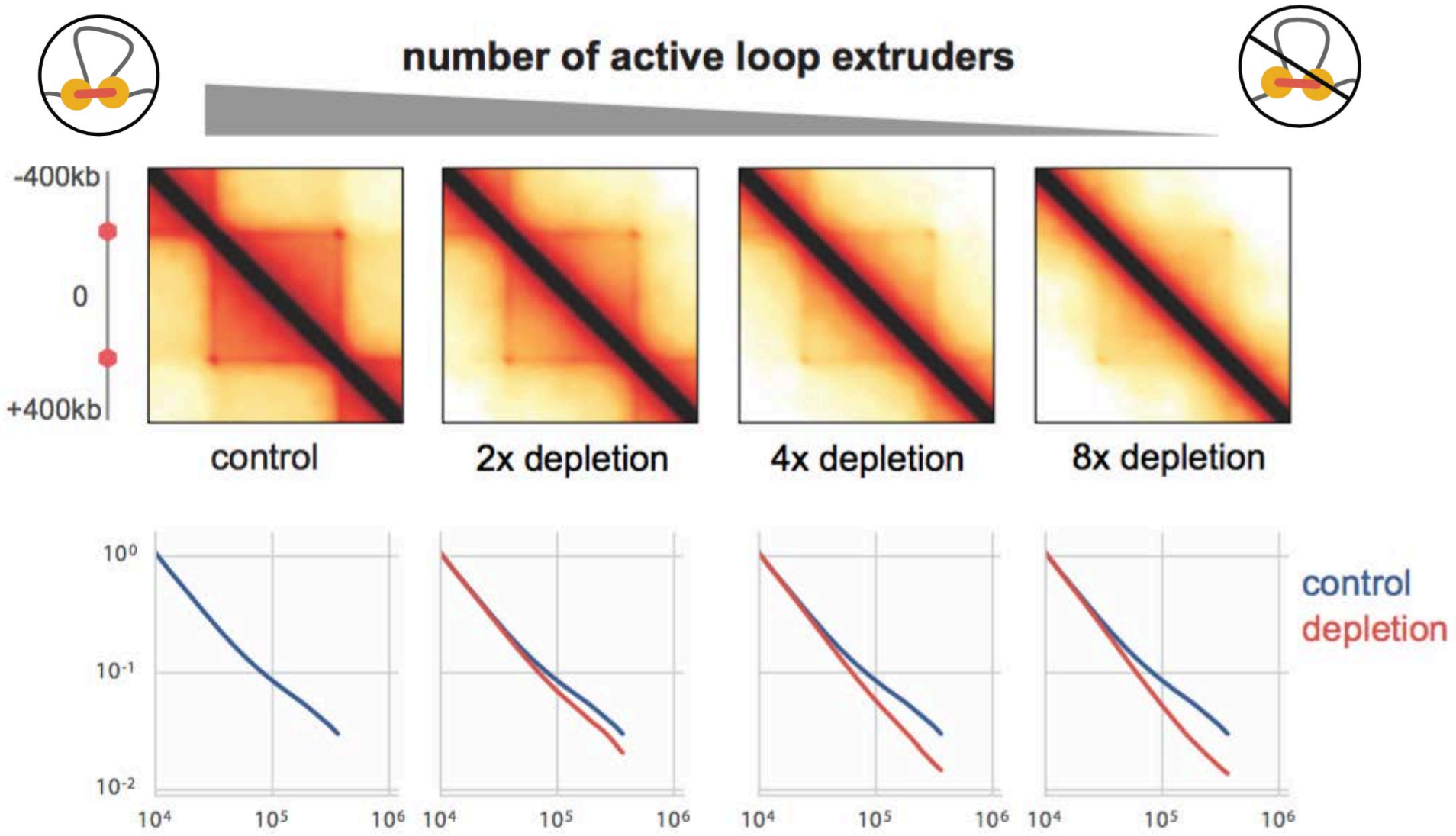
Targeted Degradation of CTCF Decouples Local Insulation of Chromosome Domains from Genomic Compartmentalization

Elphège P. Nora,[1,2,*] Anton Goloborodko,[3] Anne-Laure Valton,[4] Johan H. Gibcus,[4] Alec Uebersohn,[1,2,7] Nezar Abdennur,[3] Job Dekker,[4] Leonid A. Mirny,[3] and Benoit G. Bruneau[1,2,5,6,8,*]

Cell *169*, 930–944, May 18, 2017

Predictions

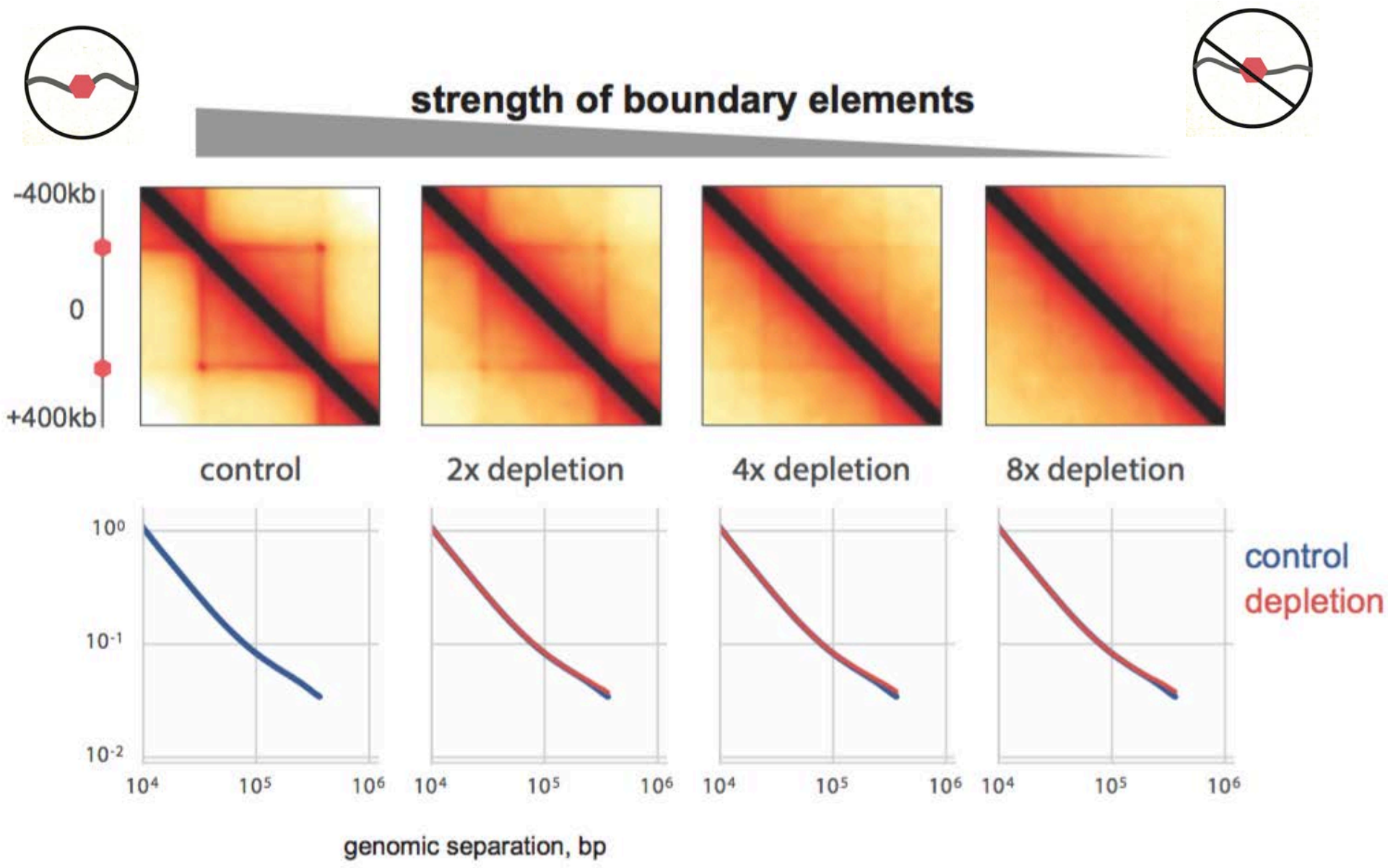
1. deplete loop extruding enzyme (cohesin)



Geoff Fudenberg (UCSF)

Predictions

2. deplete boundary element (CTCFs)

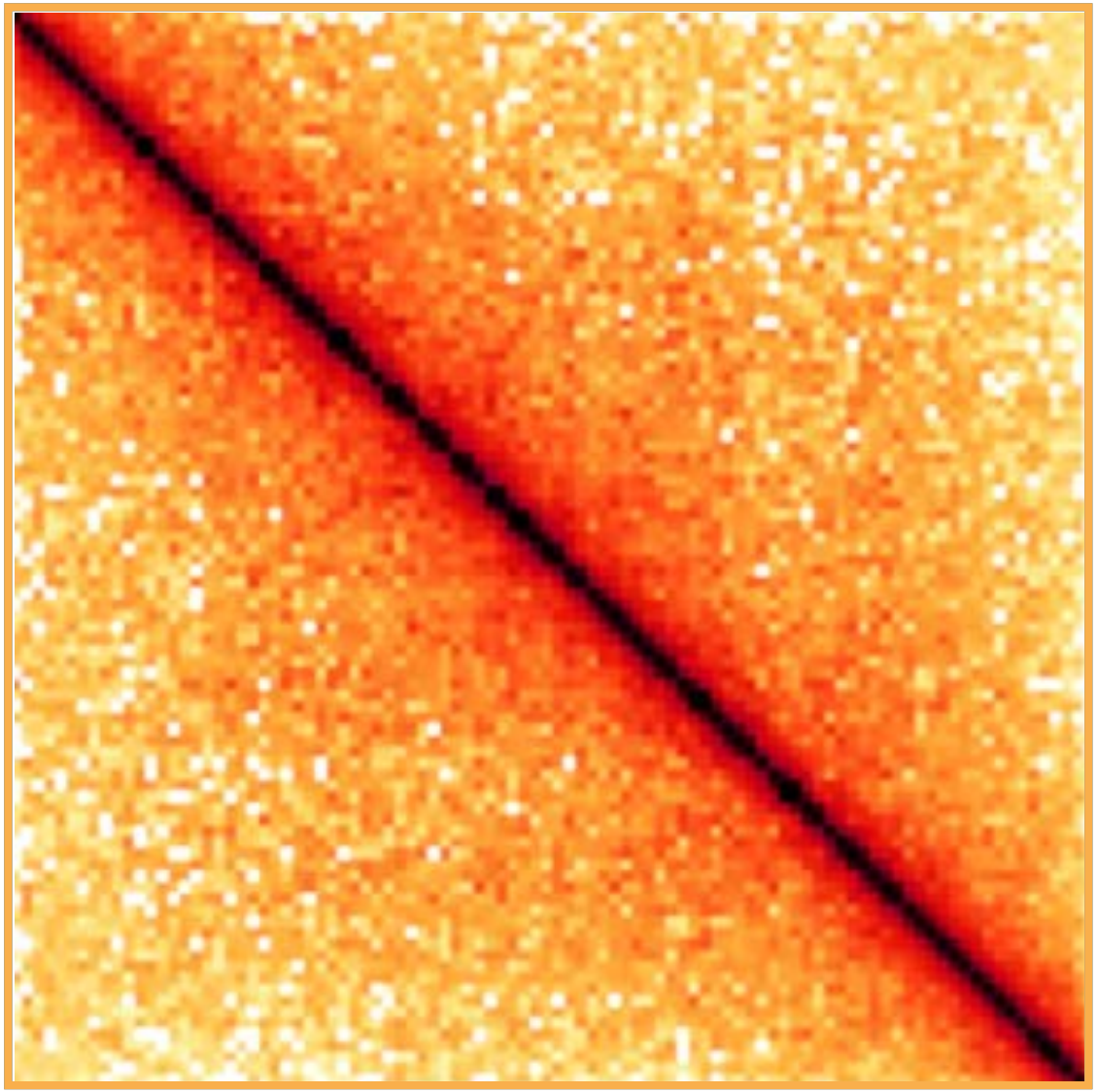


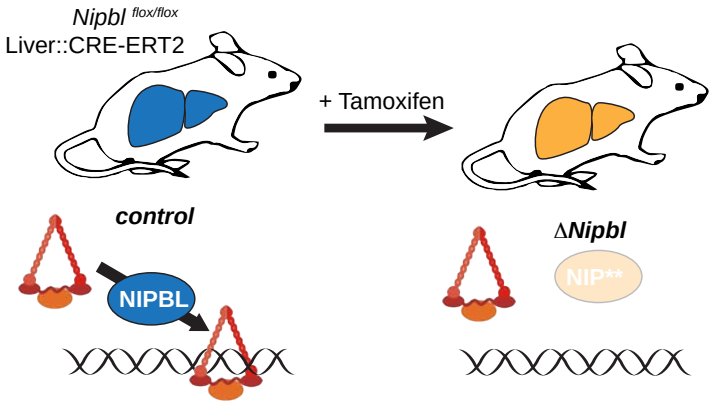
Geoff Fudenberg (UCSF)

Experiment

Domains disappear when ***cohesin*** is removed

Francois Spitz
Wibke Schwarzer

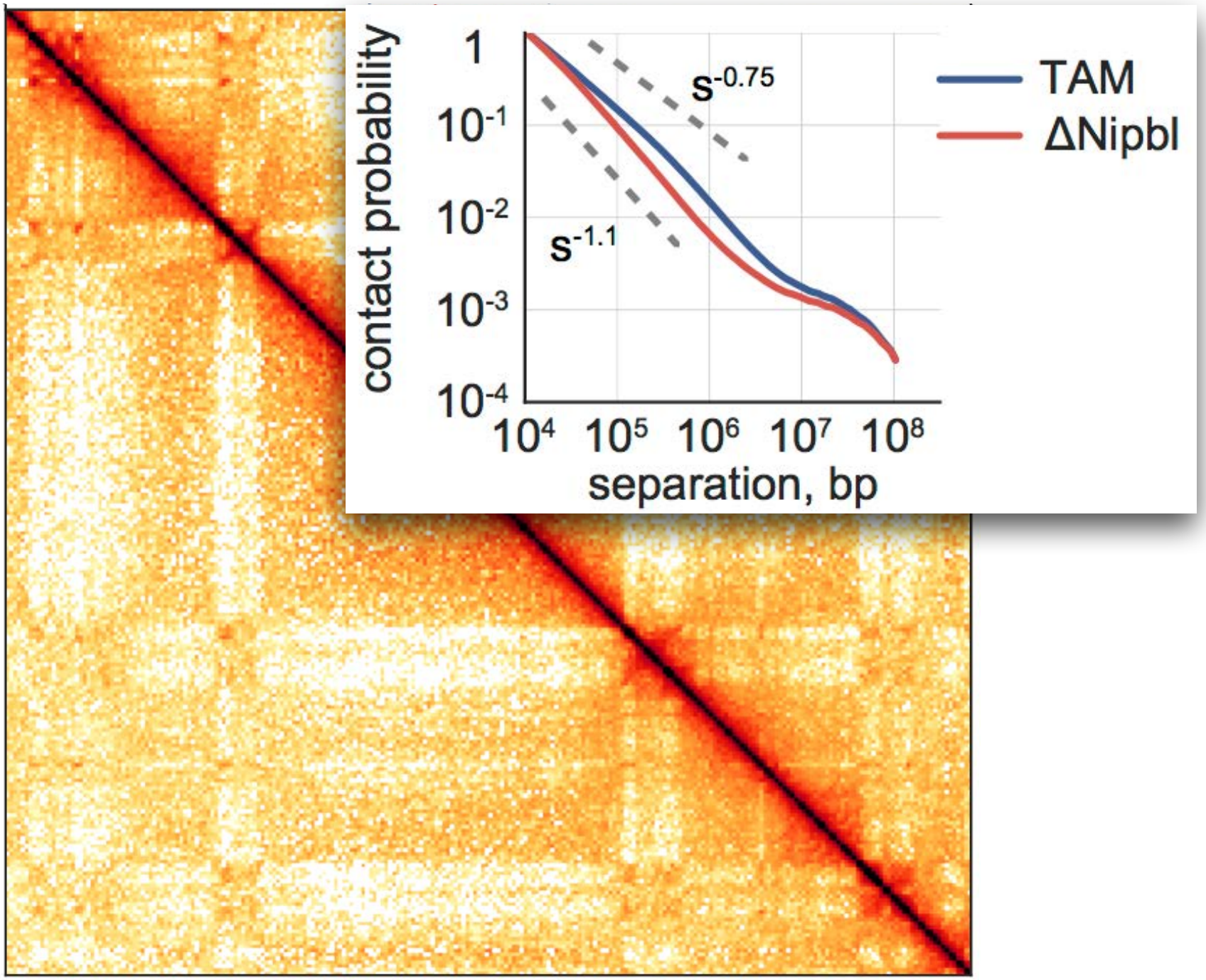




Nezar Abdennur
MIT Comp/Sys Biology

Anton Goloborodko
MIT Physics

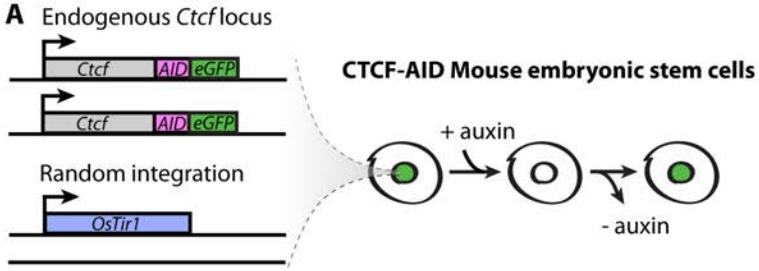
TADs (but not compartments) are cohesin-dependent

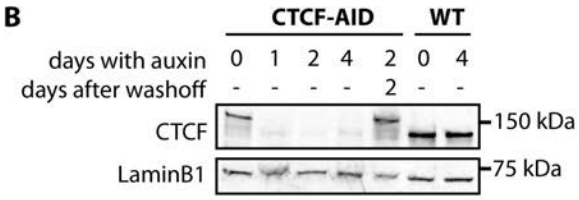


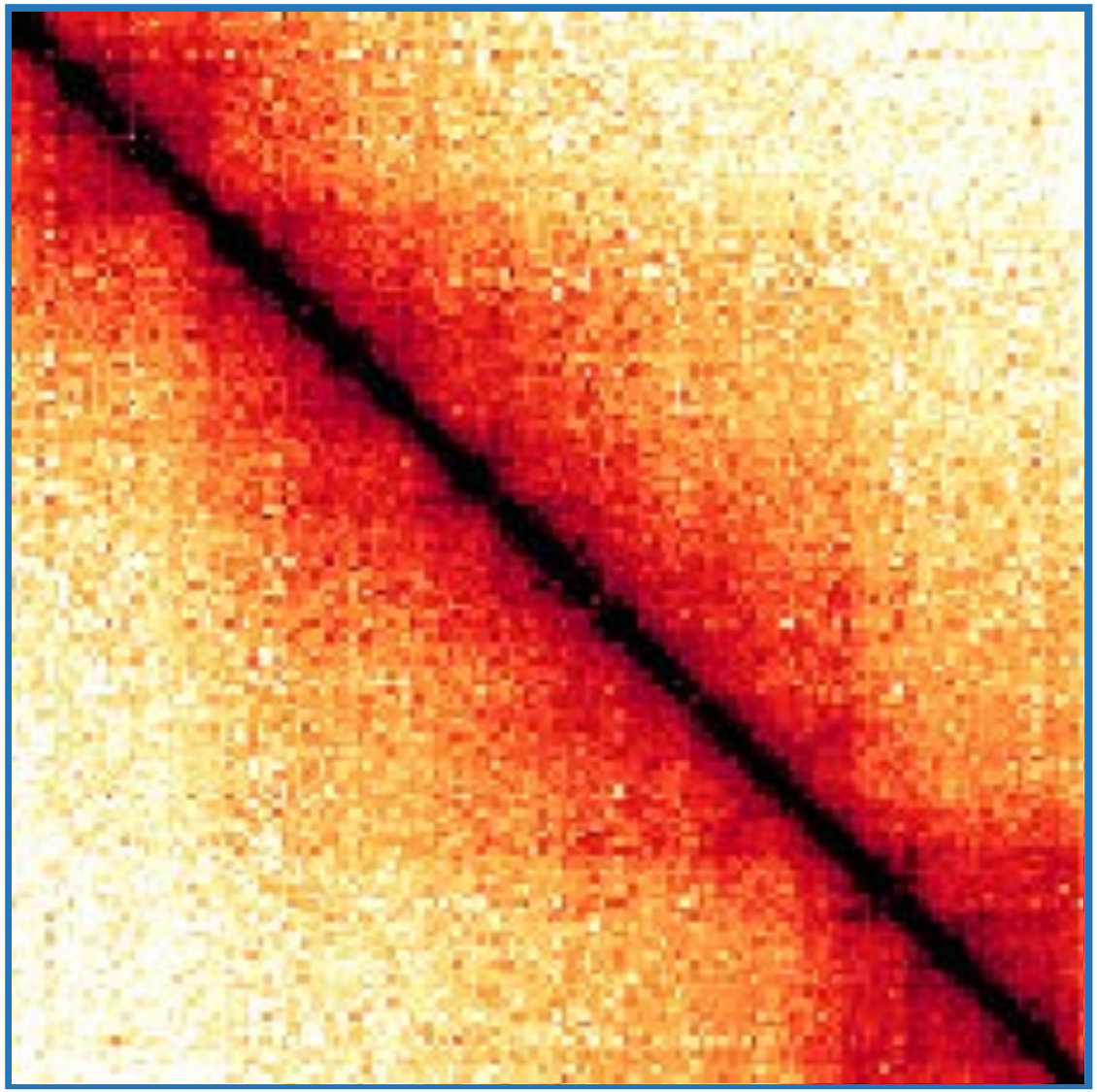
Experiment

Domains merge when ***CTCF*** is removed

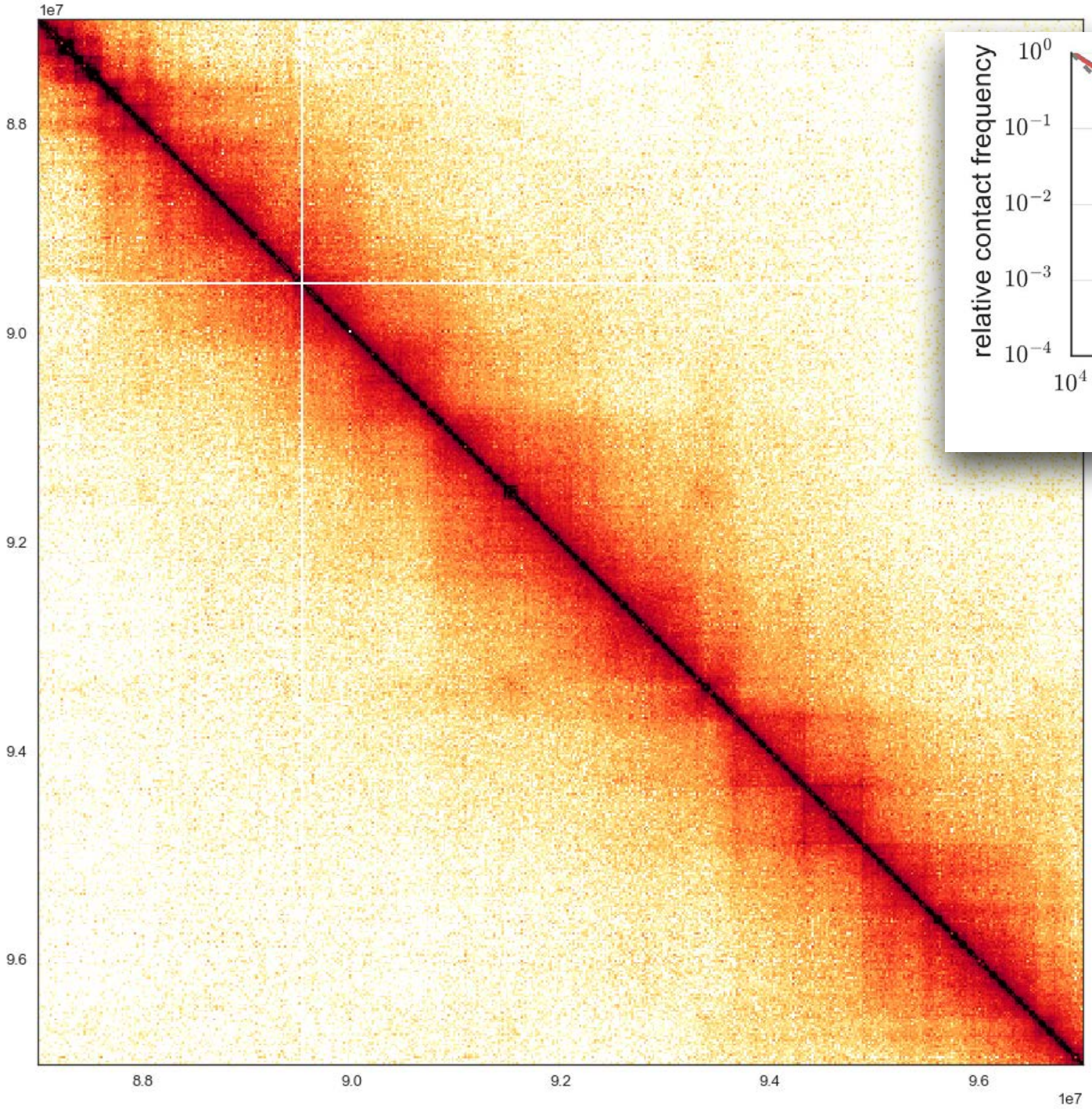
Elphege Nora
Anton Goloborodko
Benoit G. Bruneau
Job Dekker et al

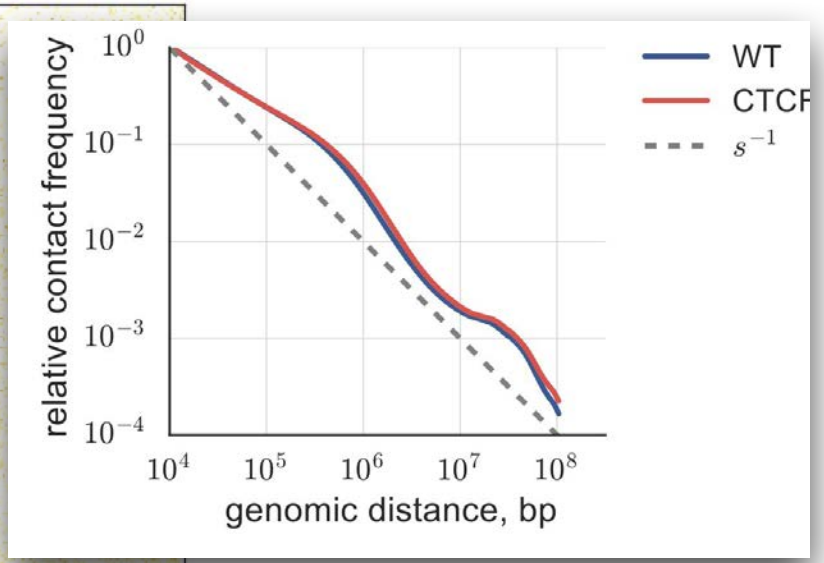




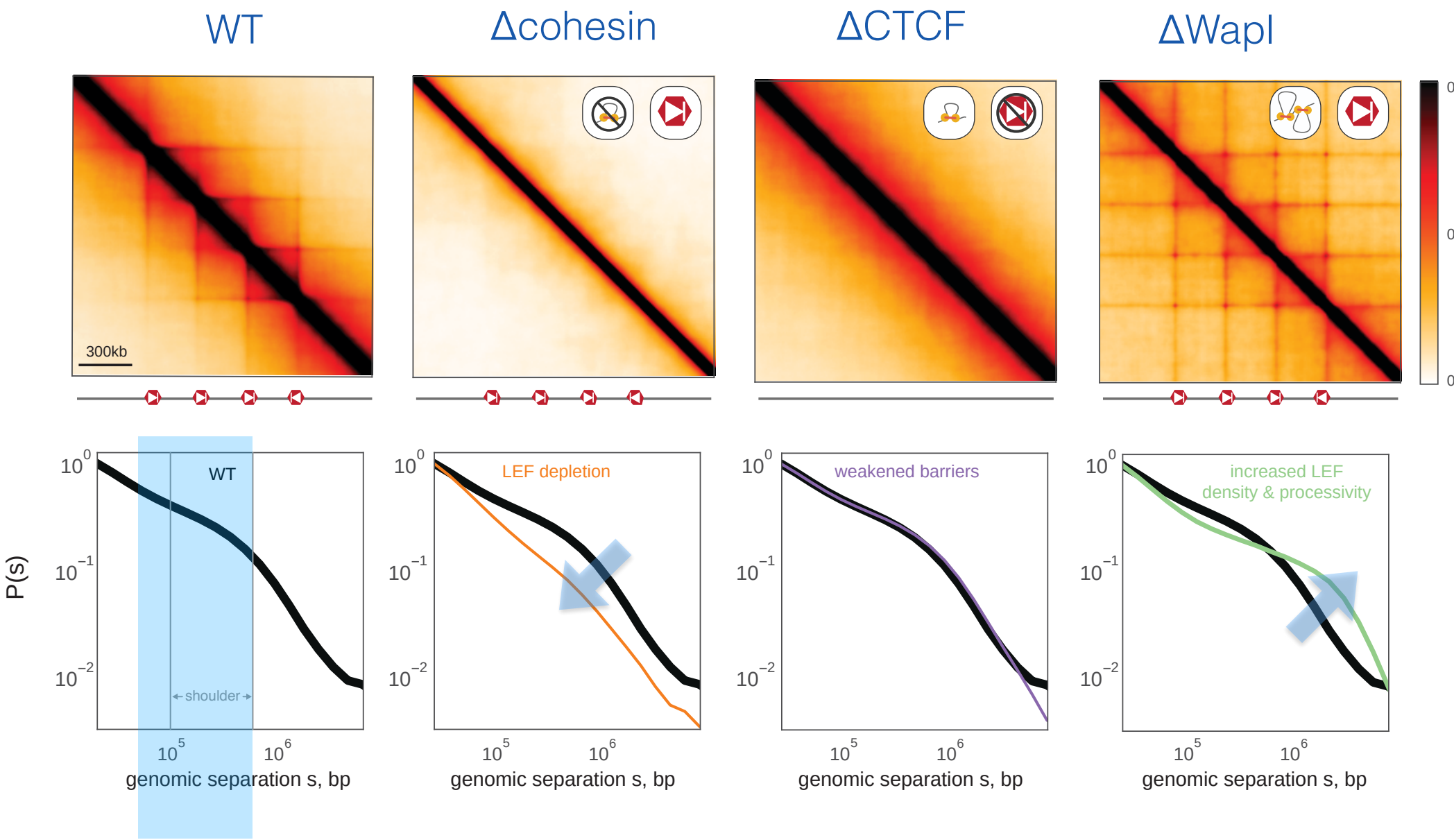


TADs merge with each other



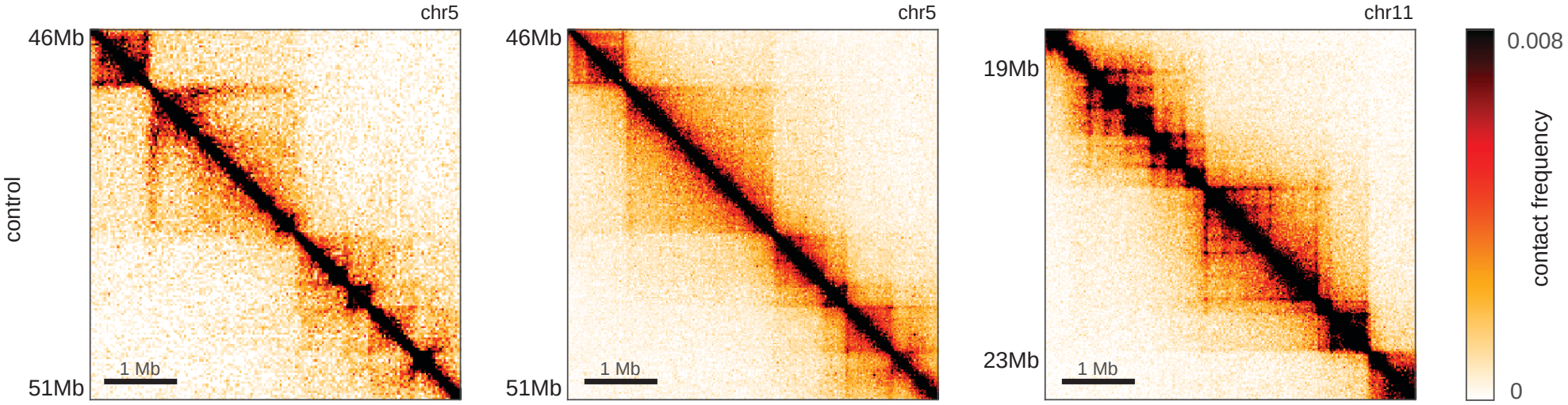


Testing loop extrusion
Predictions



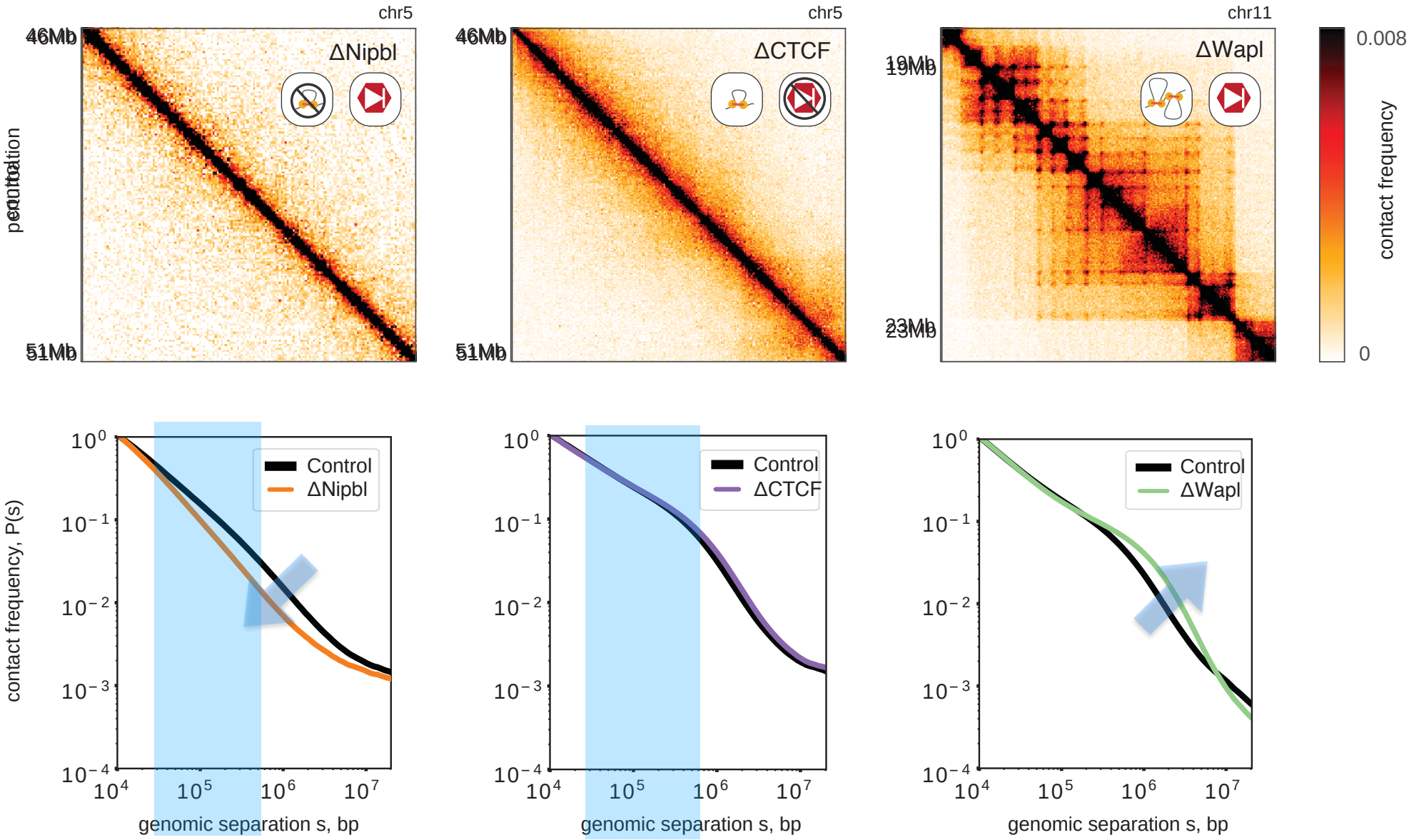
Testing loop extrusion

Experiments



Testing loop extrusion

Experiments



Cees Dekker
UT Delft

Single-molecule experiments

Real-time imaging of DNA loop extrusion by condensin

Science

Mahipal Ganji,[1] Indra A. Shaltiel,[2*] Shveta Bisht,[2*] Eugene Kim,[1] Ana Kalichava,[1] Christian H. Haering,[2†] Cees Dekker[1†]

REPORTS

Cite as: M. Ganji *et al.*, *Science* 10.1126/science.aar7831 (2018).

This video shows how a condensin protein gradually causes DNA to extrude a DNA loop over time.

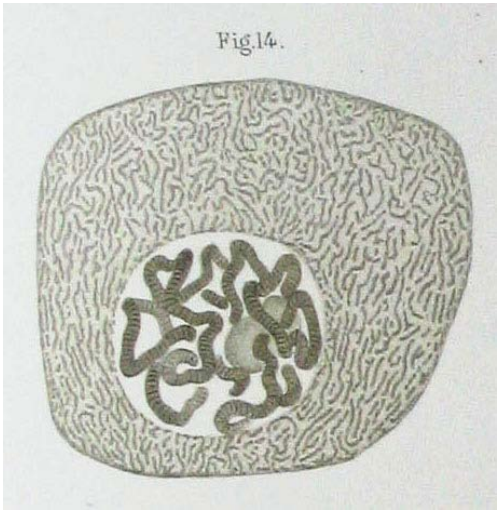
Cees Dekker lab, Kavli Institute of Nanoscience
Delft University of Technology
www.ceesdekkerlab.nl

This result is reported in Science (online, Febr.15, 2018)
Paper title: Real-time imaging of DNA loop extrusion by condensin
Authors: Mahipal Ganji (1), Indra A. Shaltiel (2), Shveta Bisht (2), Eugene Kim (1), Ana Kalichava (1), Christian H. Haering (2), Cees Dekker (1)
Affiliations: 1 Department of Bionanoscience, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, Netherlands.
2 Cell Biology and Biophysics Unit, Structural and Computational Biology Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

Take home message

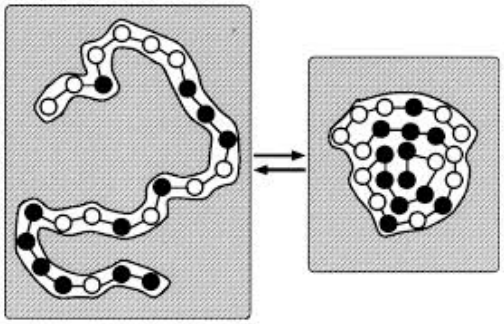
protein folding

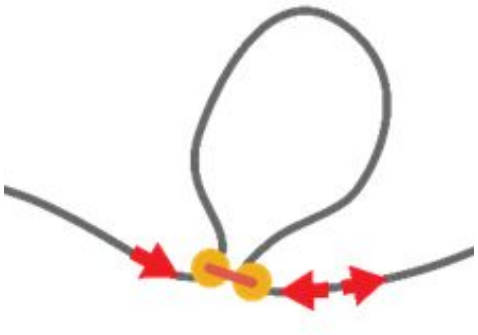
interactions
(energy)



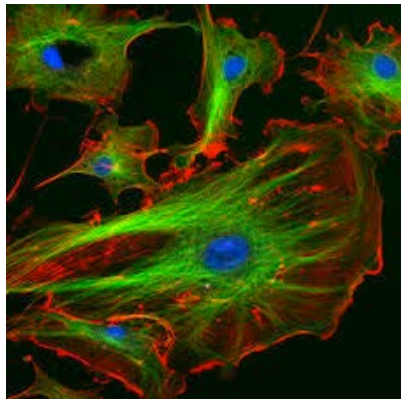
Walther Flemming in 1882

interactions
and
active processes

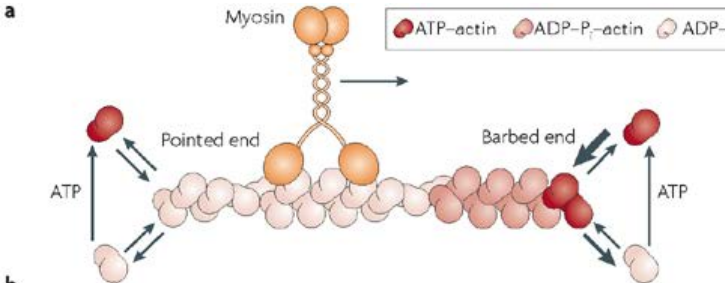




cytoskeleton

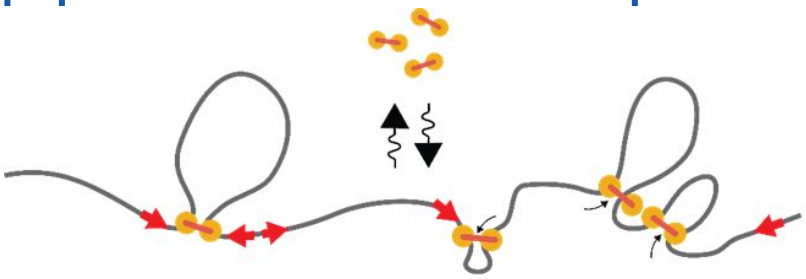


active processes

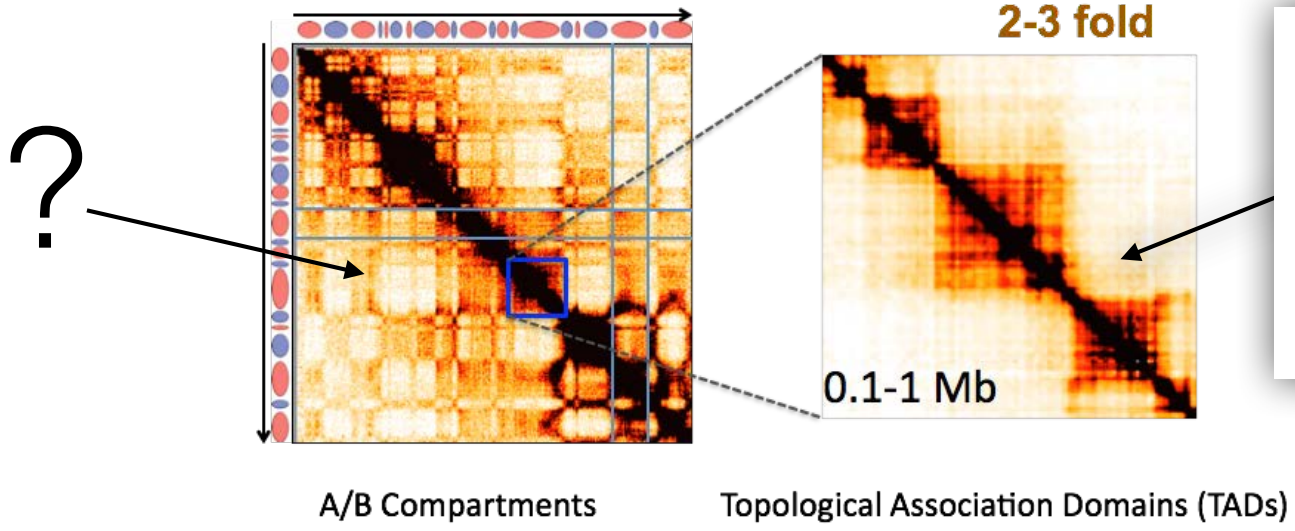


Conclusions

1. Strong experimental support of the loop extrusion hypothesis



2. **Domains** and **compartments** are formed by different mechanisms



Loop extrusion by cohesin

CTCF to stop it

G2 -> M

Biophysical Journal
Article

Biophysical Society

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RESEARCH ARTICLE

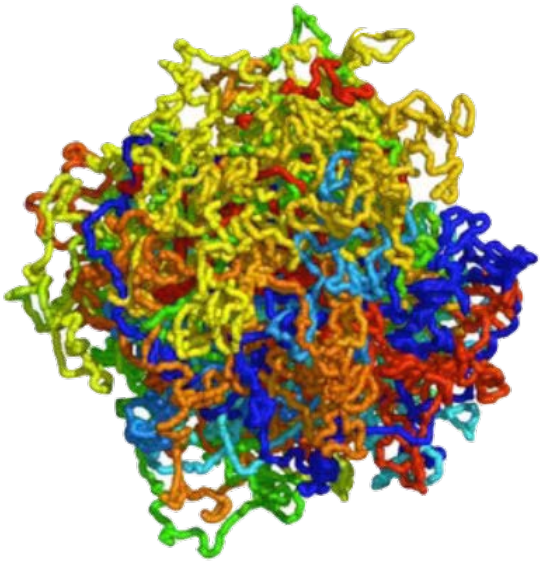
Chromosome Compaction by Active Loop Extrusion

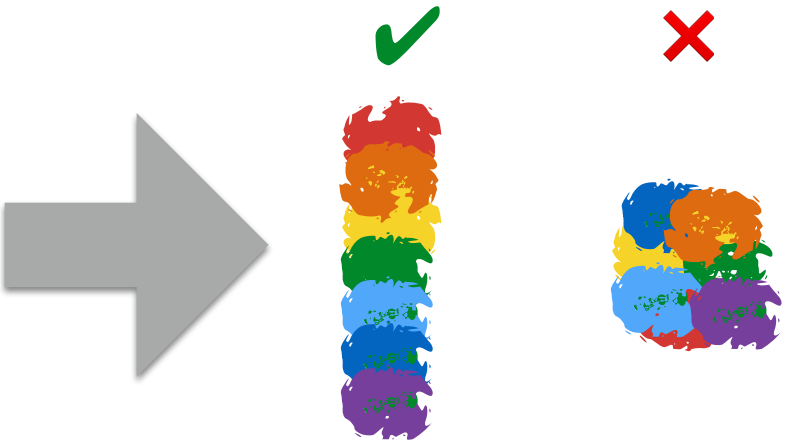
Anton Goloborodko,[1] John F. Marko,[2] and Leonid A. Mirny[1,3,*]

Compaction and segregation of sister chromatids via active loop extrusion

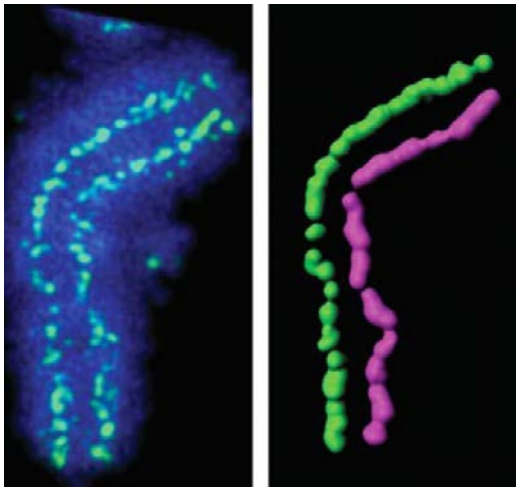
Anton Goloborodko[1], Maxim V Imakaev[1], John F Marko[2,4], Leonid Mirny[1,3]*

PROBLEM 2: how can chromosome condense while acquiring elongated morphology and linear order?



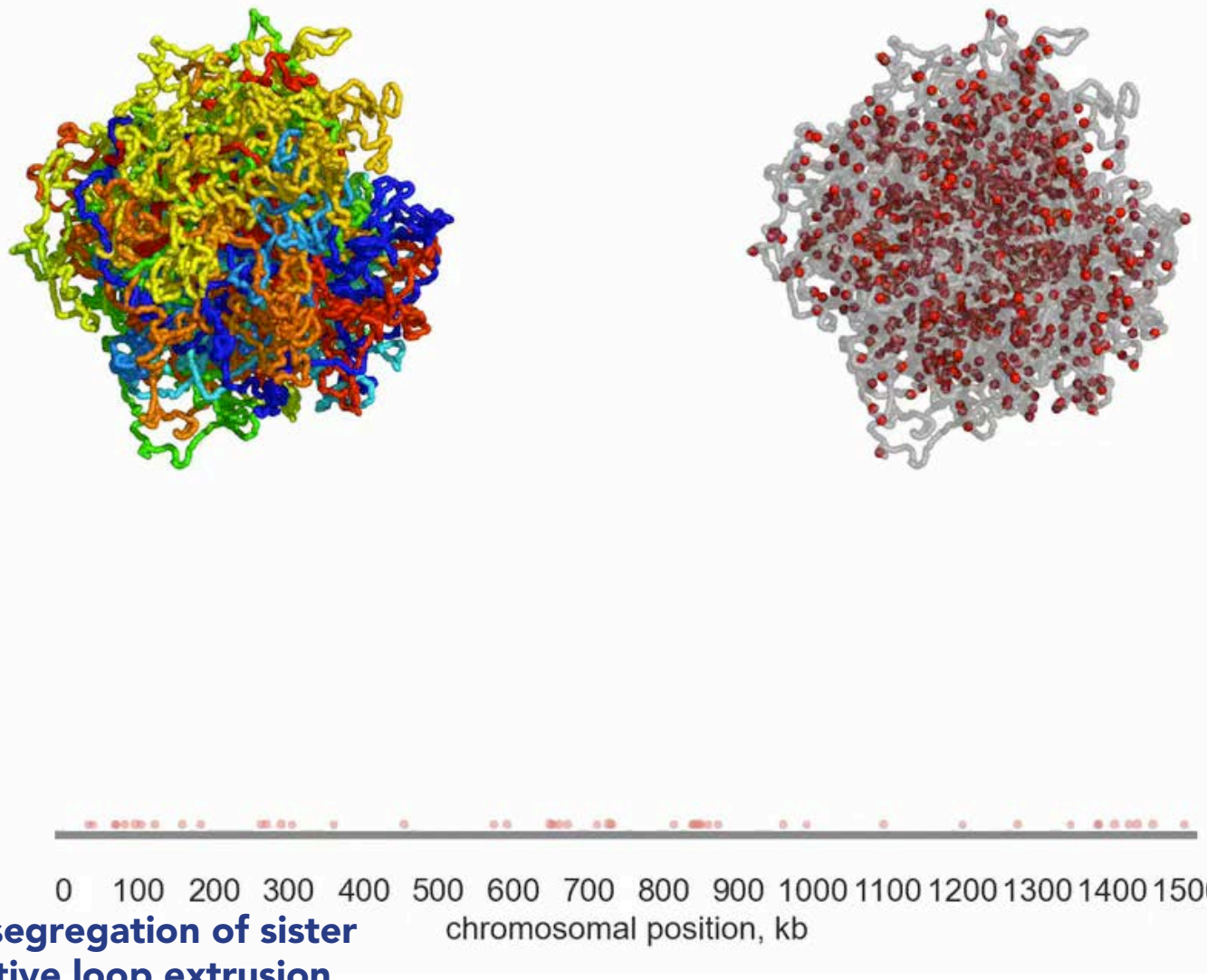


chromosomal axis



Loop extrusion is sufficient for chromosome condensation

https://www.youtube.com/watch?v=_Vc7__xfnfc



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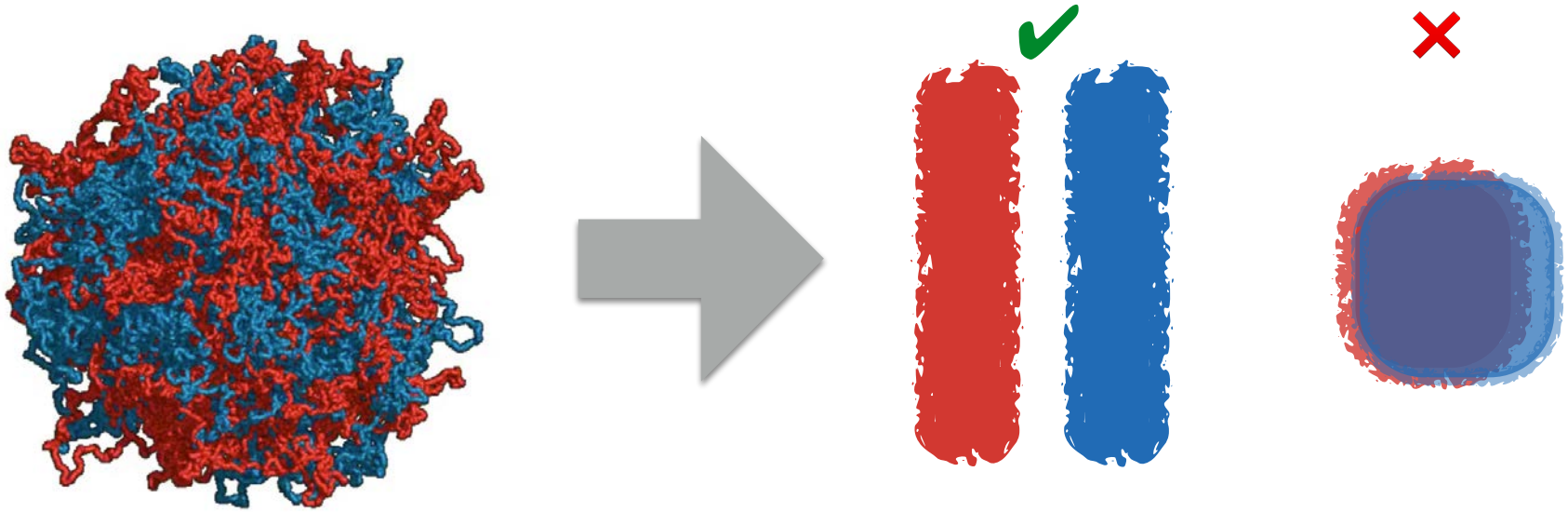
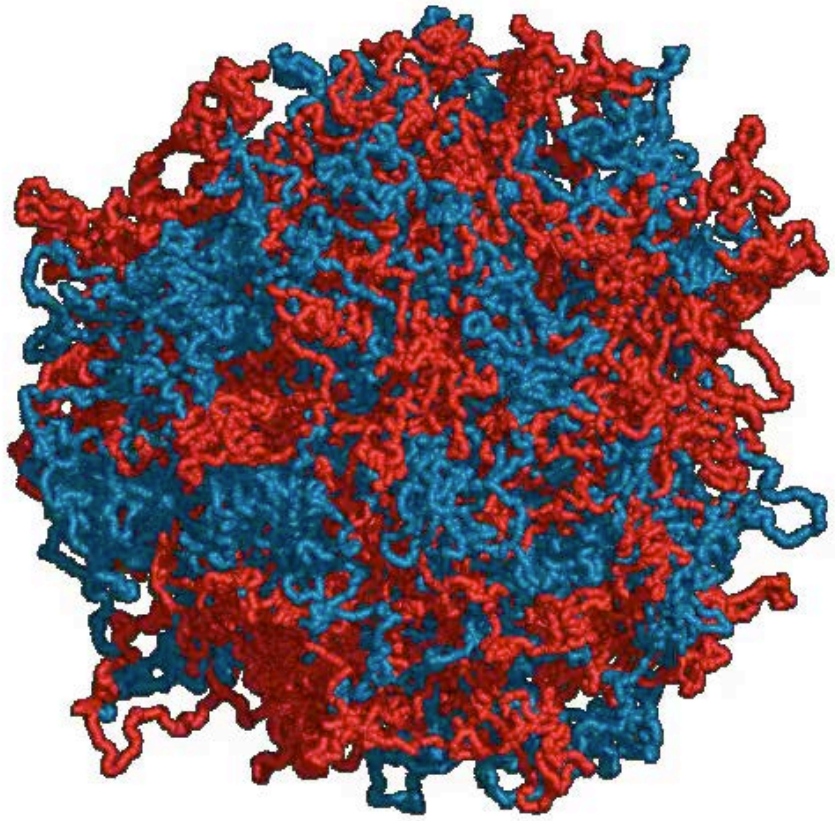
Compaction and segregation of sister chromatids via active loop extrusion

Anton Goloborodko[1], Maxim V Imakaev[1], John F Marko[2,4], Leonid Mirny[1,3]*

PROBLEM 3: how can two sister chromatids condense separately, i.e. segregate and disentangle



Loop extrusion is sufficient for sister segregation



https://www.youtube.com/watch?v=stZR5s9n32s

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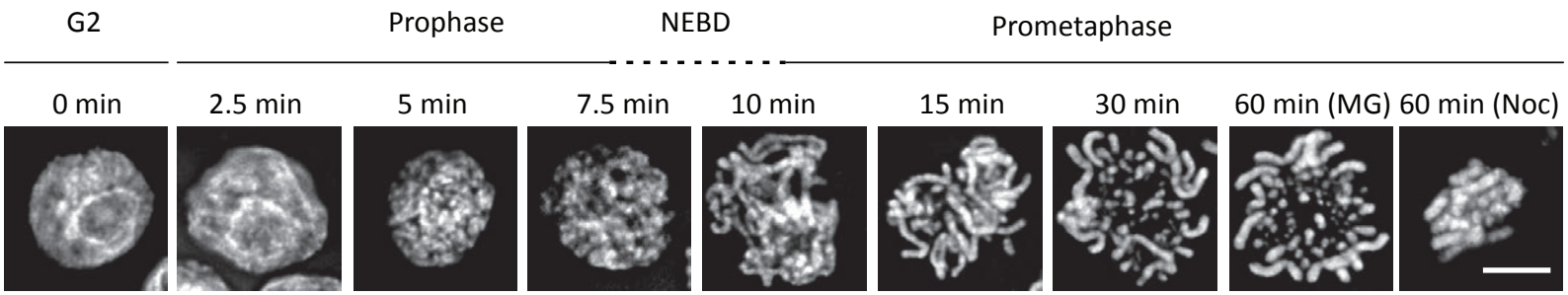
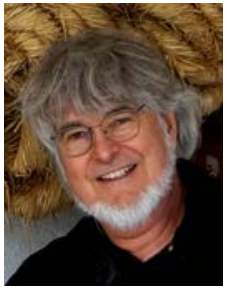
Compaction and segregation of sister chromatids via active loop extrusion

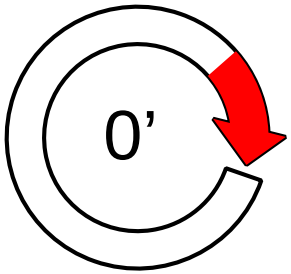
Anton Goloborodko[1], Maxim V Imakaev[1], John F Marko[2,4], Leonid Mirny[1,3]*

Hi-C of mitotic condensation

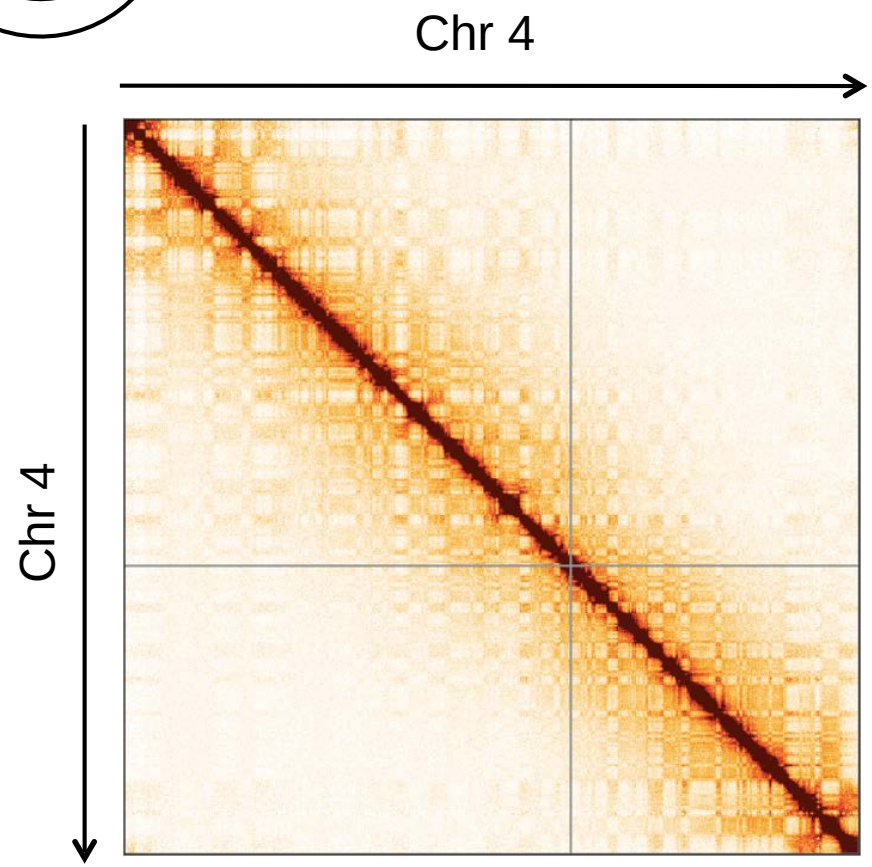


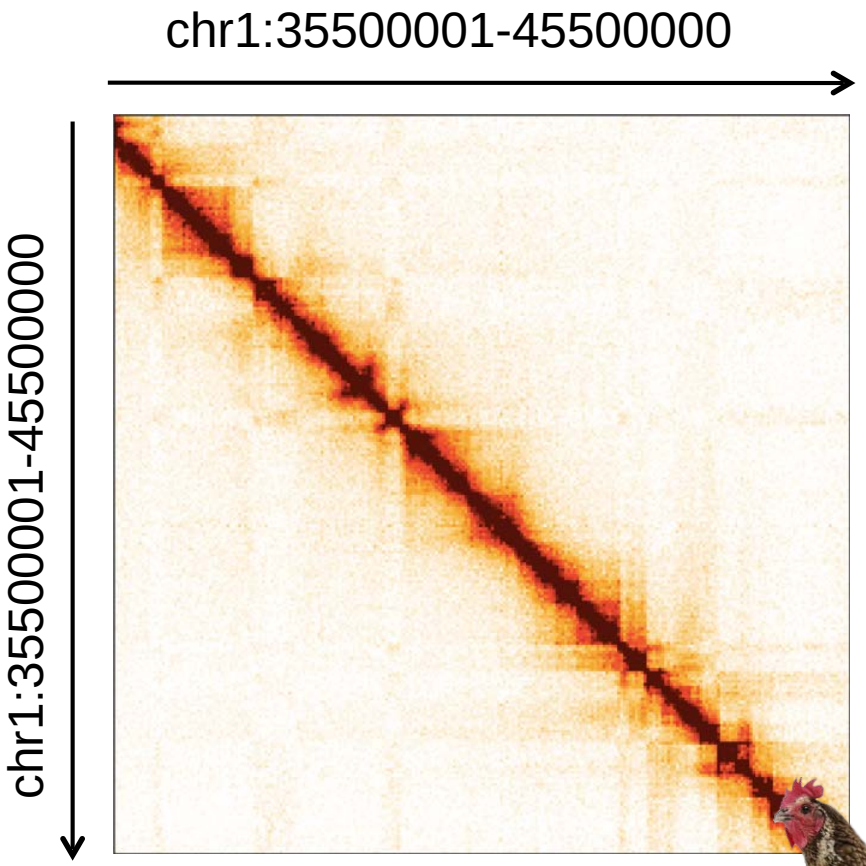


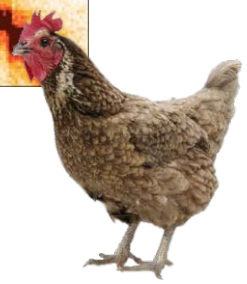
Bill Earnshaw **Job Dekker**
University of Edinburgh

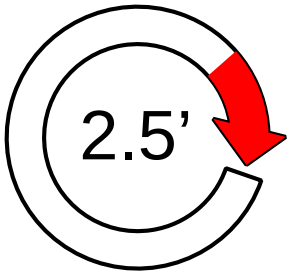


Hi-C on Synchronized DT-40









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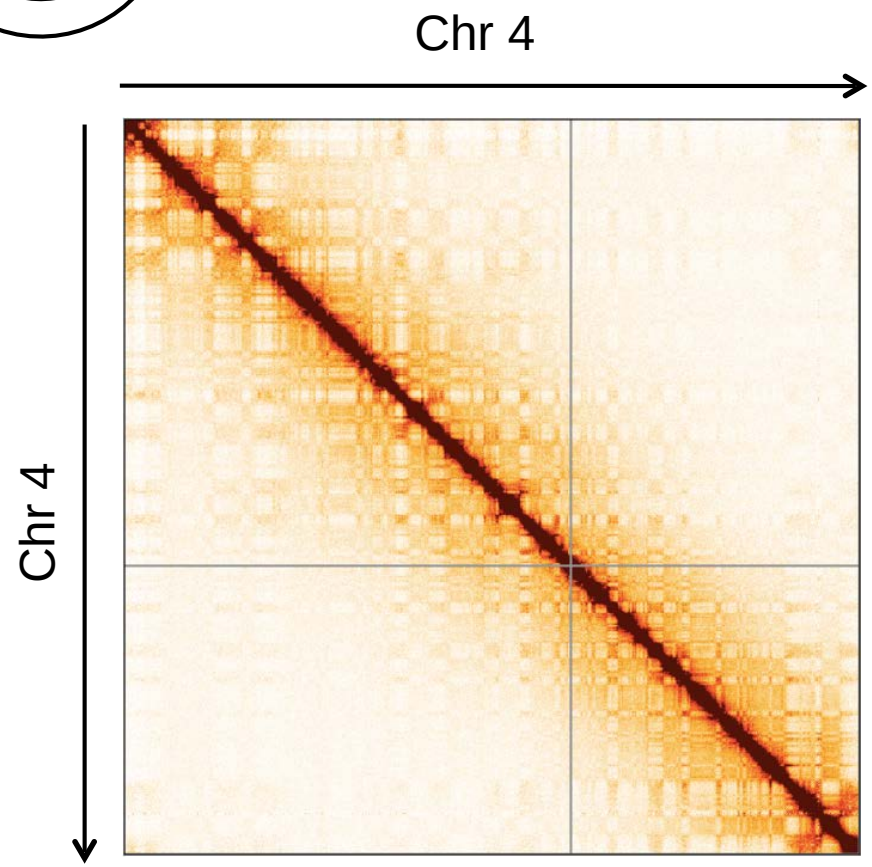

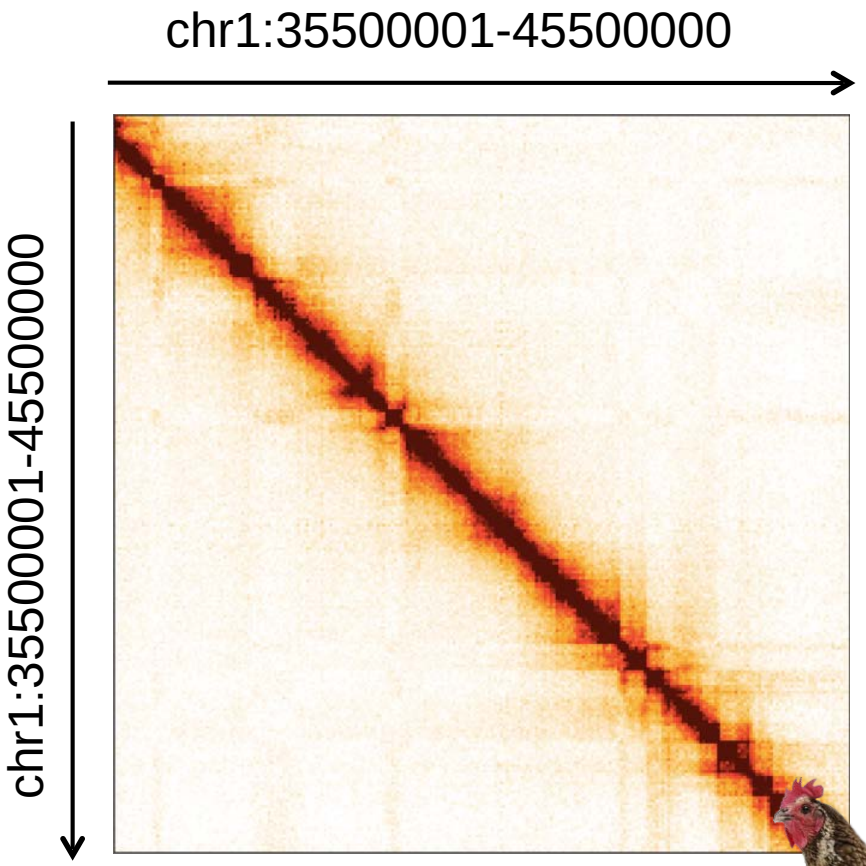

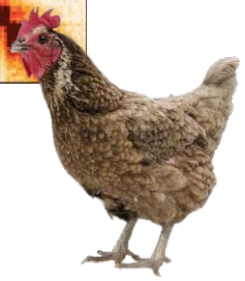

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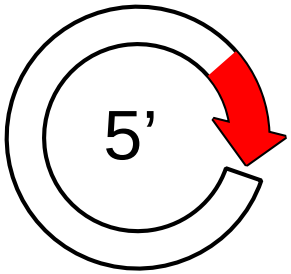

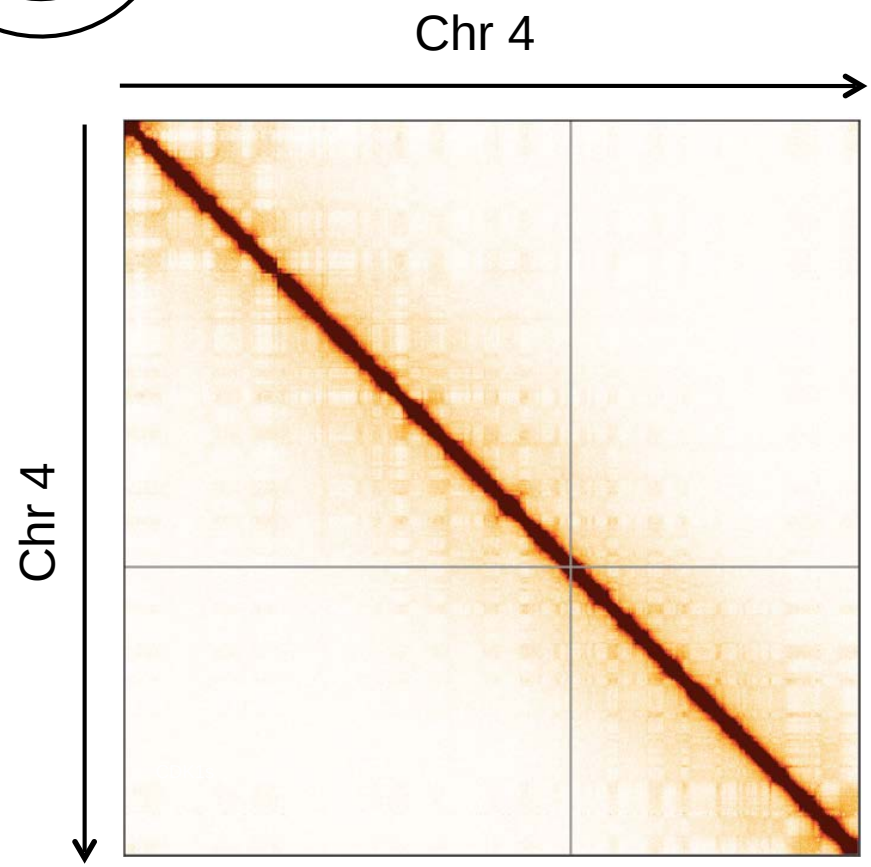

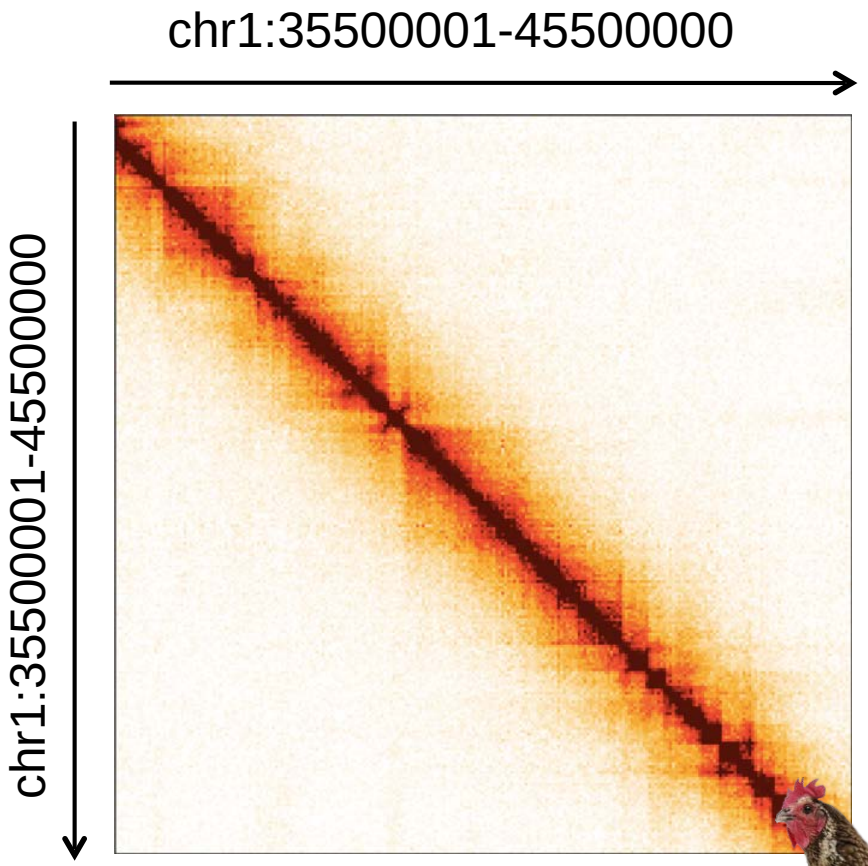

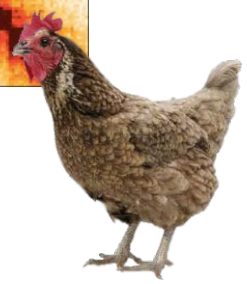

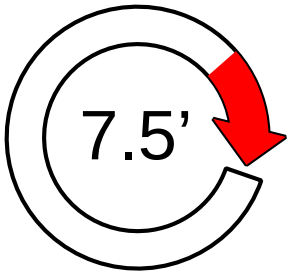

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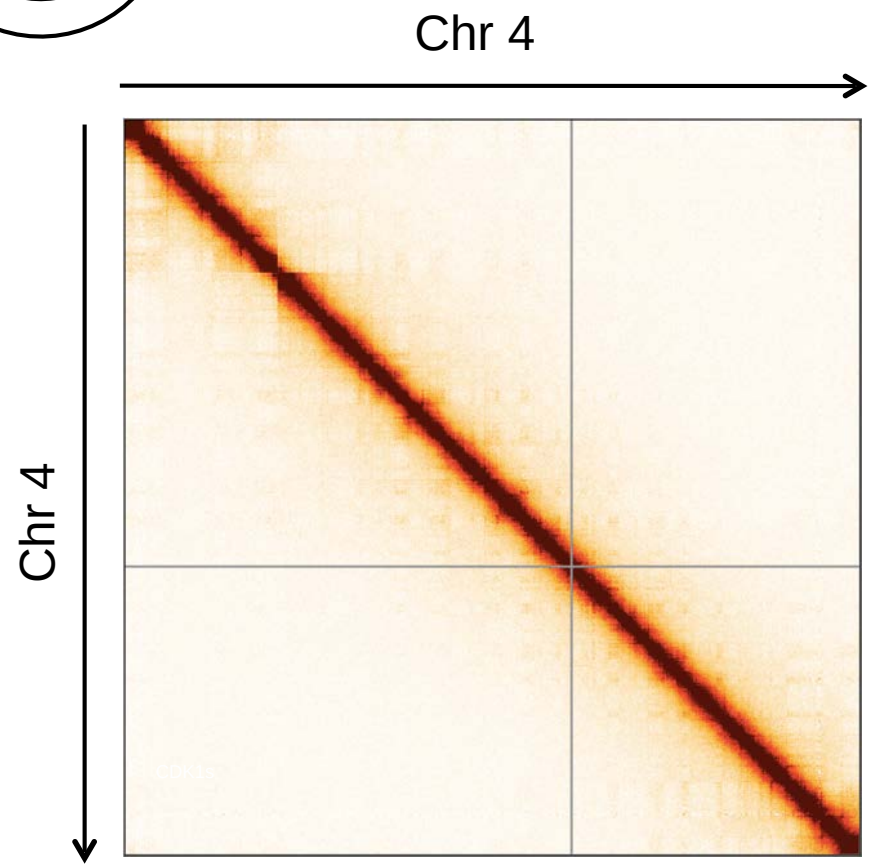

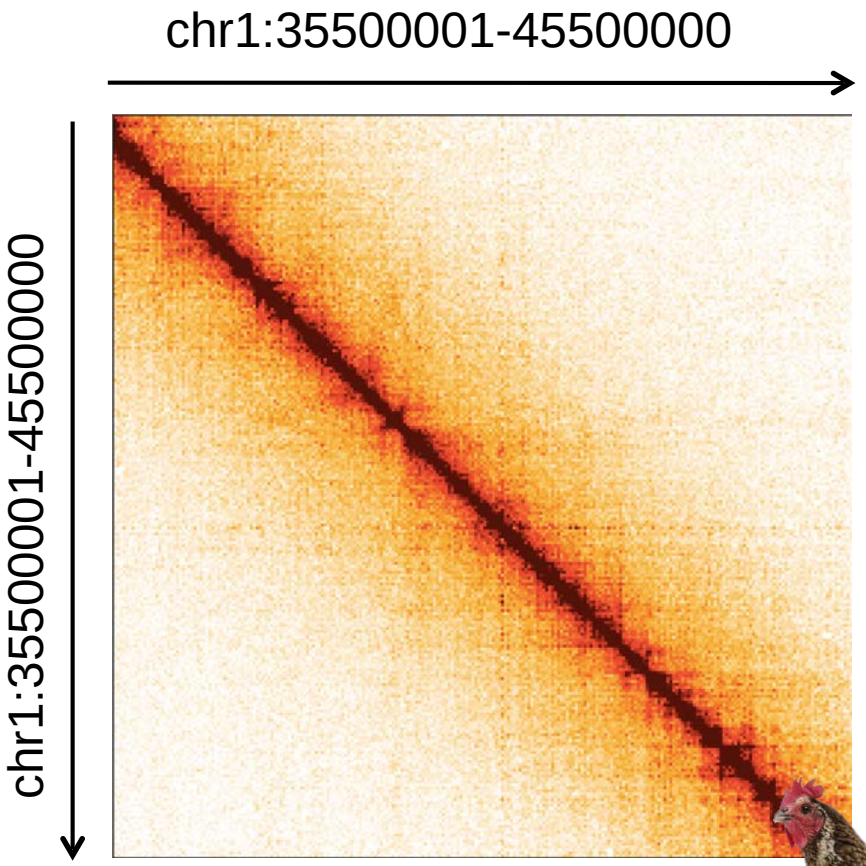

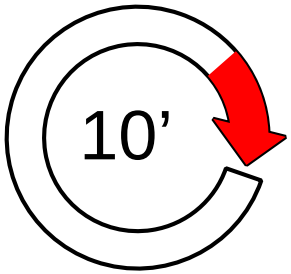

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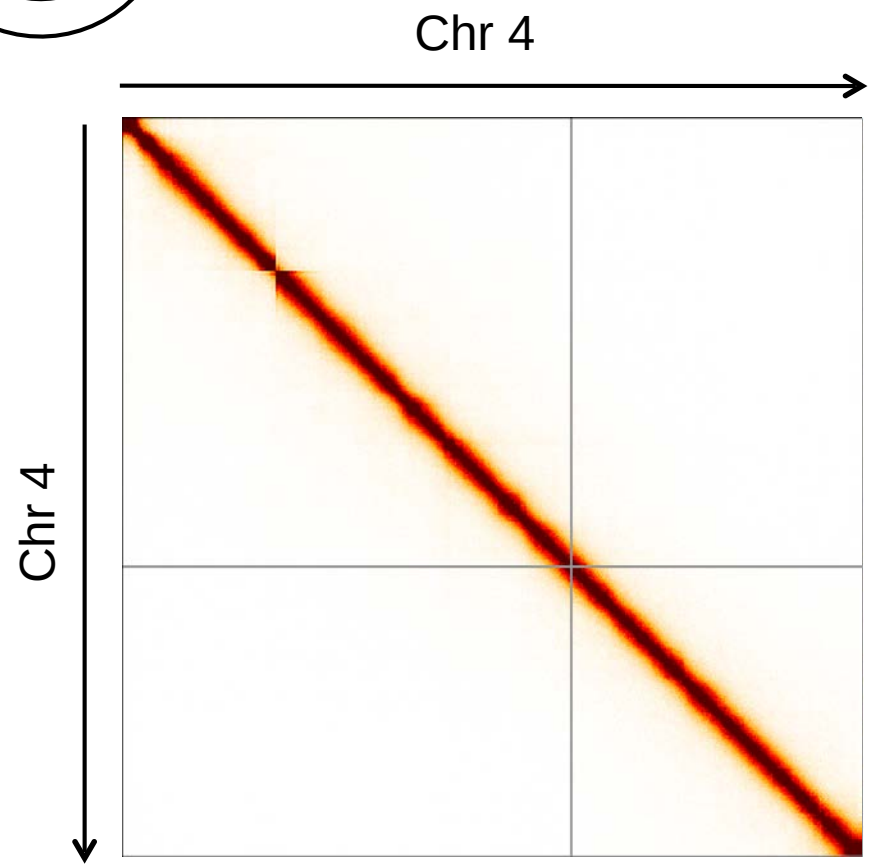

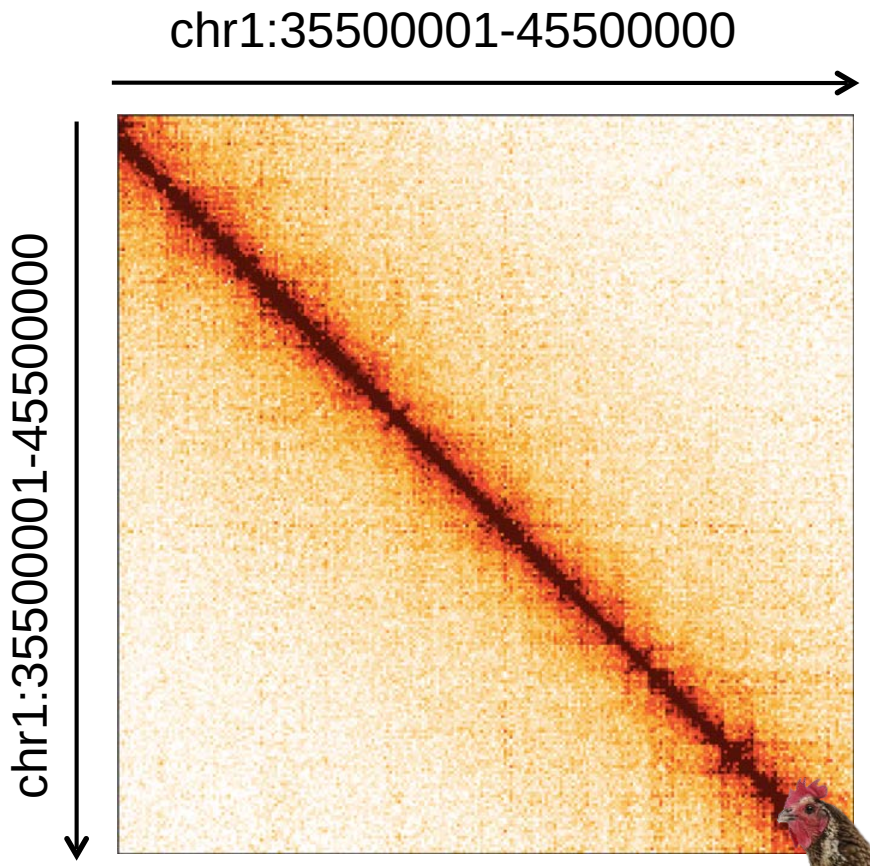

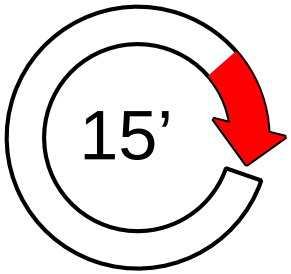

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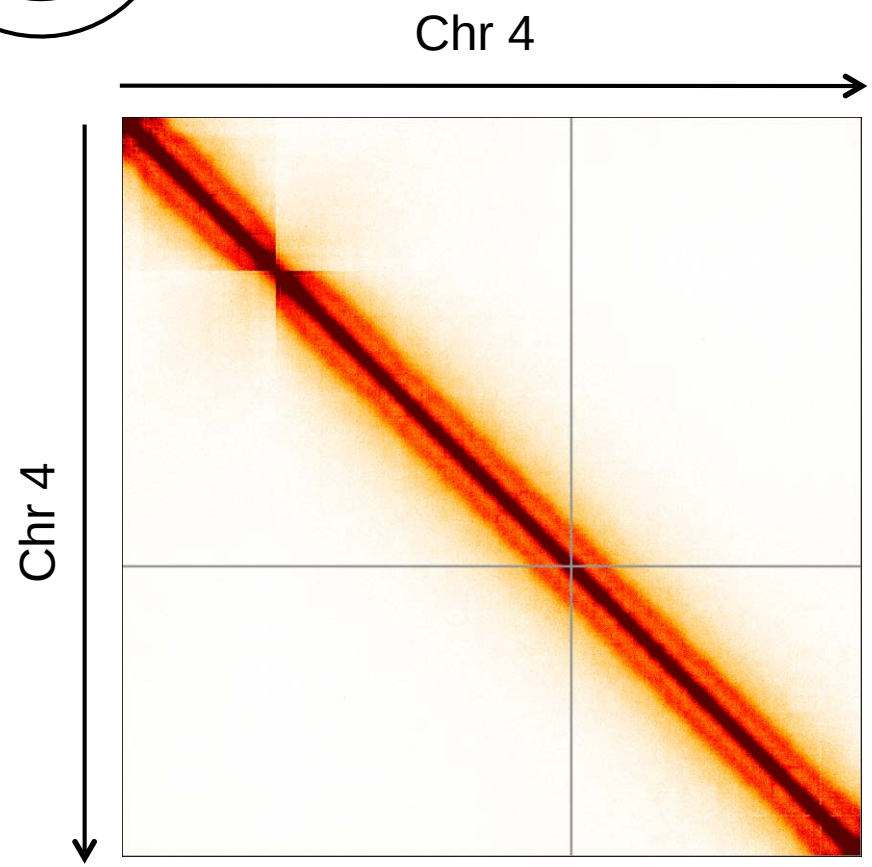

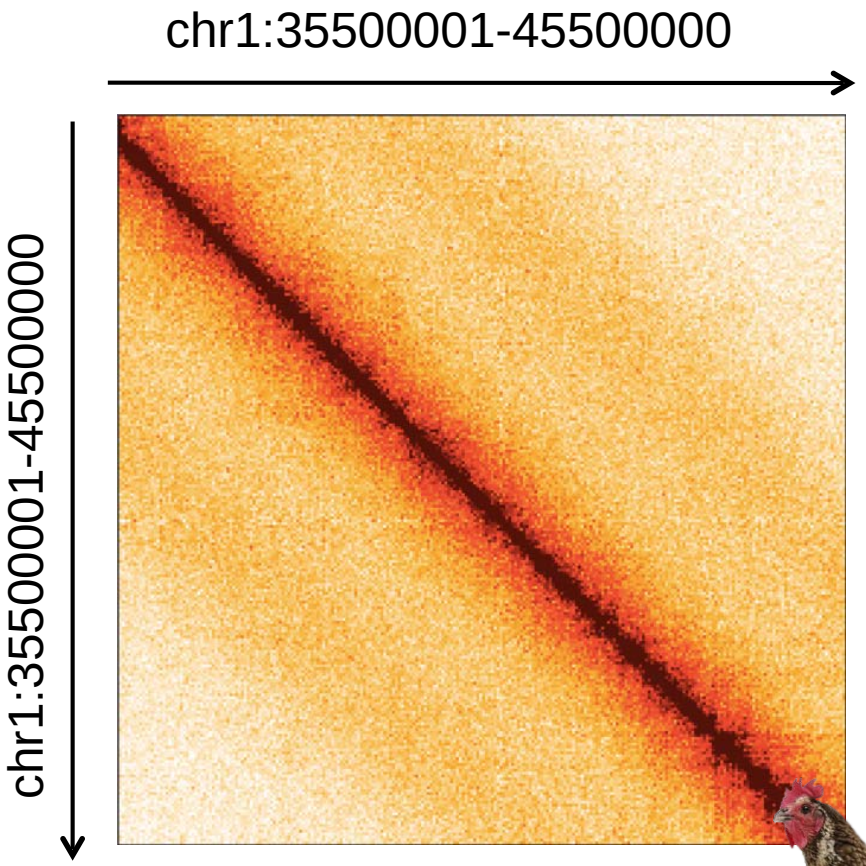

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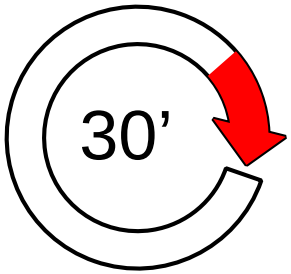
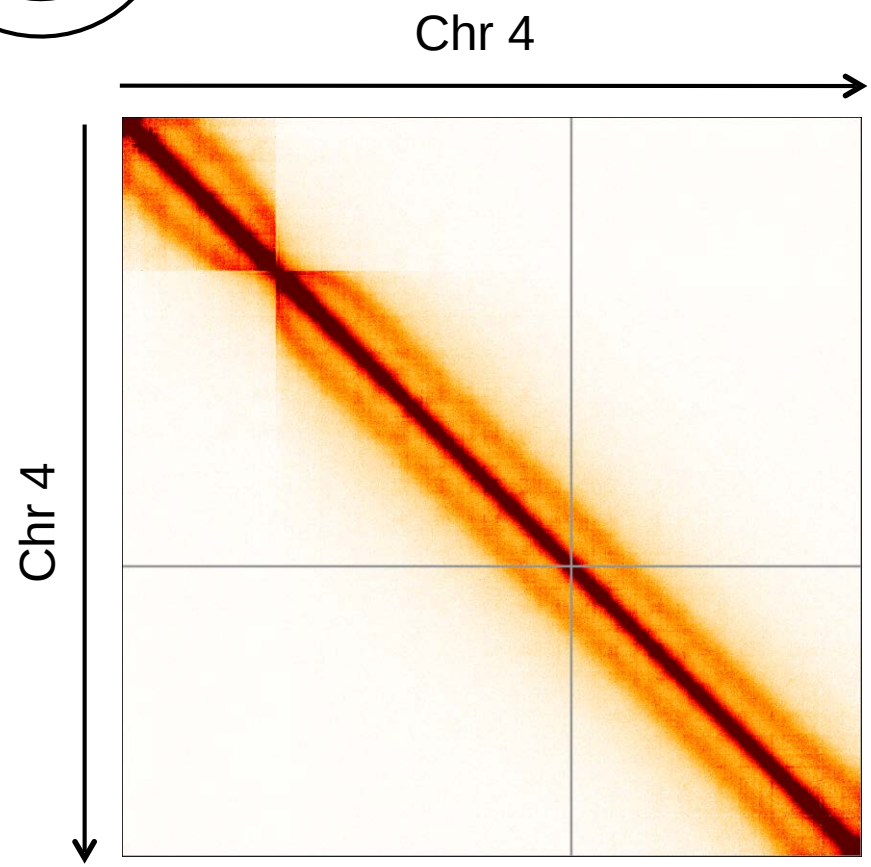
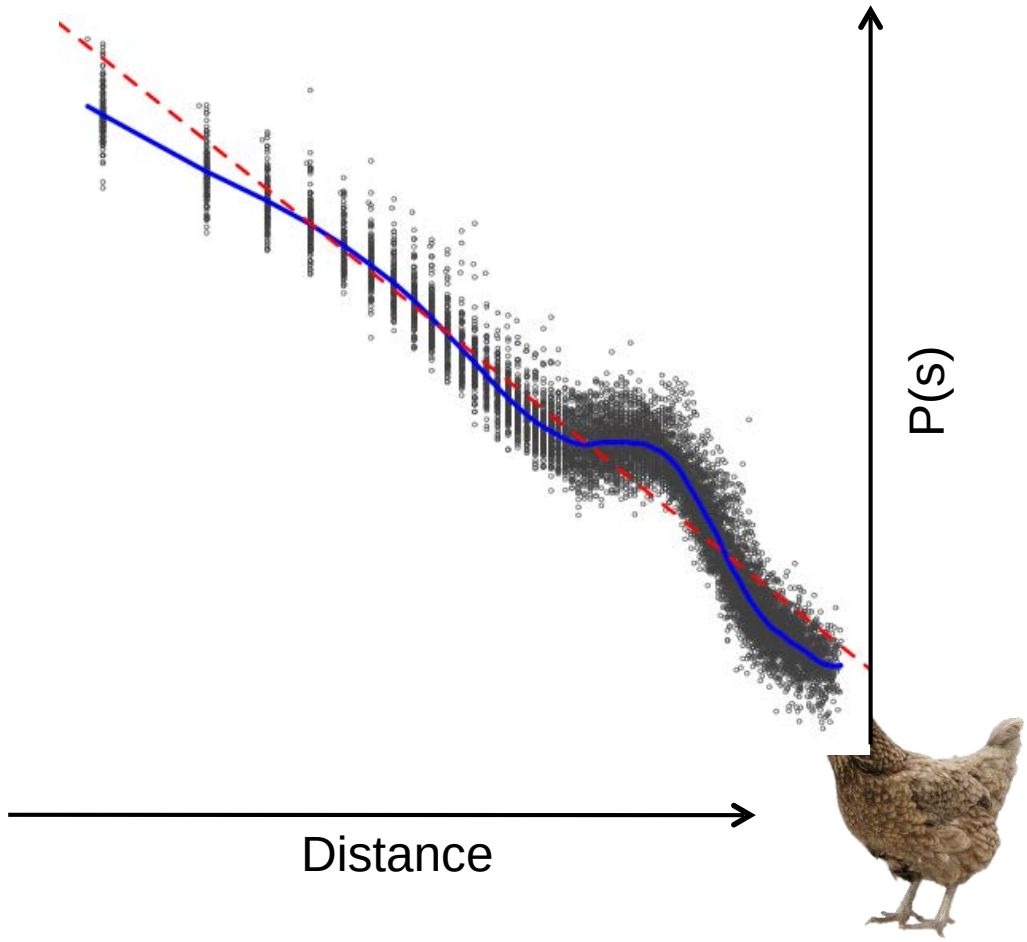
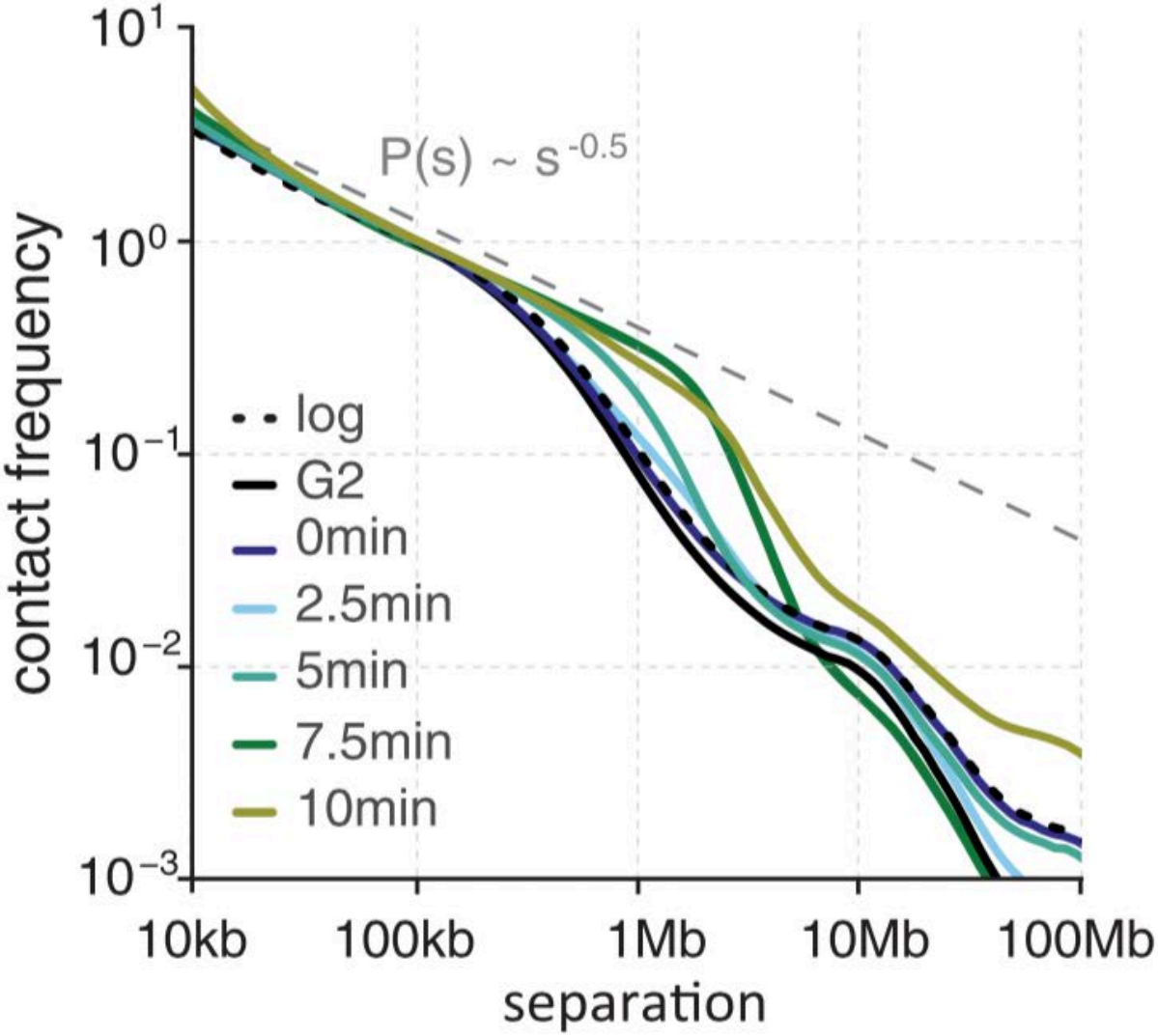
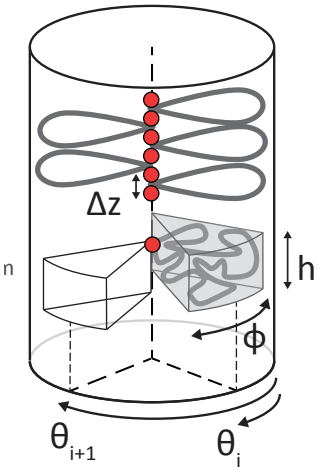


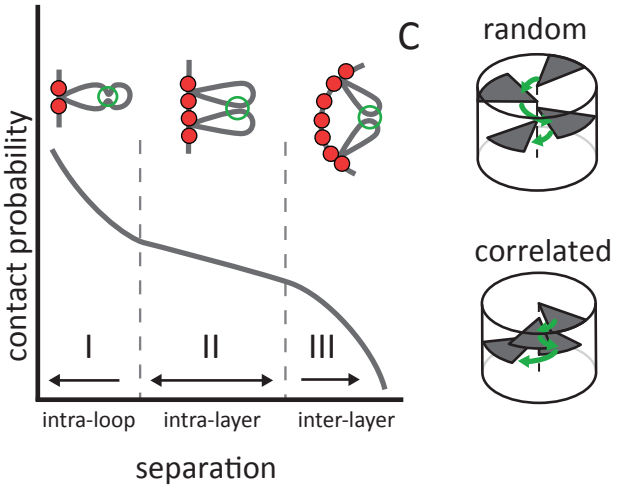


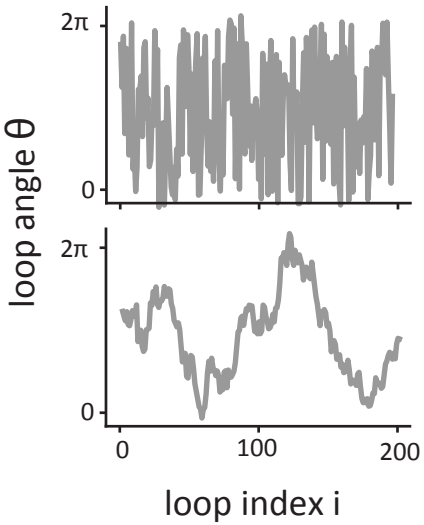


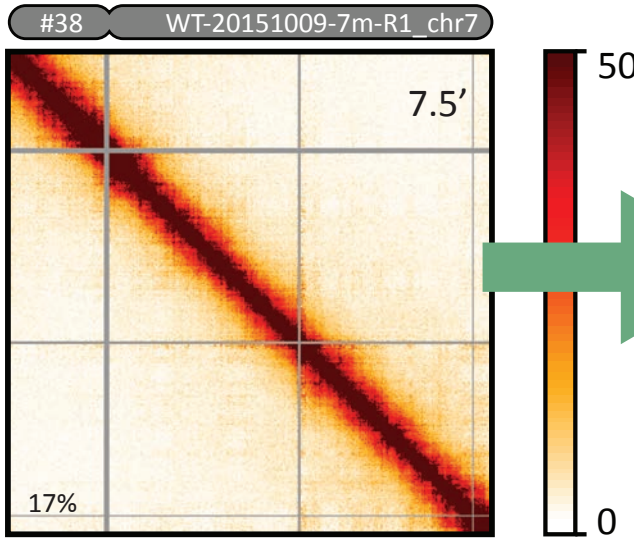

10^1
10^0
10^{-1}
10^{-2}
10^{-3}
contact frequency
P(s) ~ s$^{-0.5}$
log
G2
0min
2.5min
5min
7.5min
10min
10kb
100kb
1Mb
10Mb
100Mb
separation

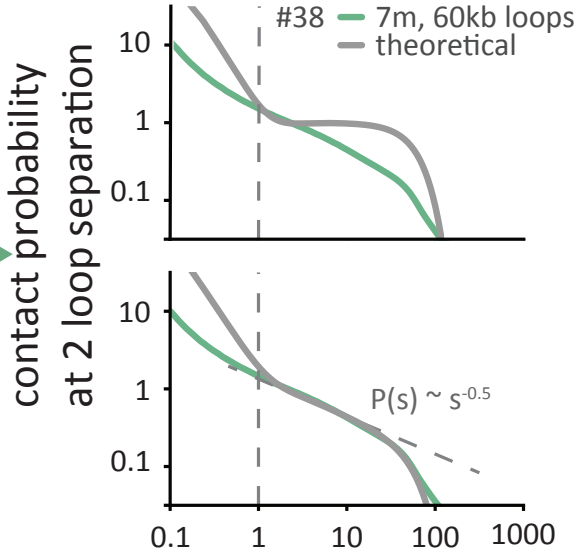
Model of prophase chromosomes



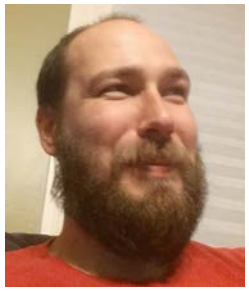








Anton Goloborodko
MIT Physics

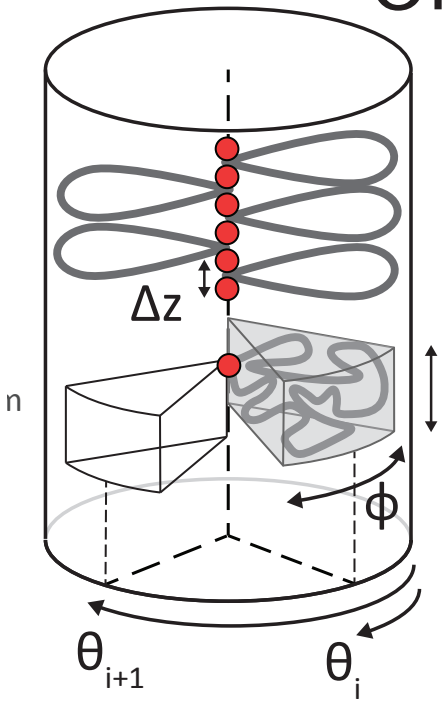


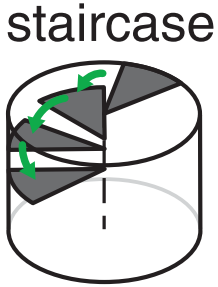
Anton Goloborodko
MIT Physics

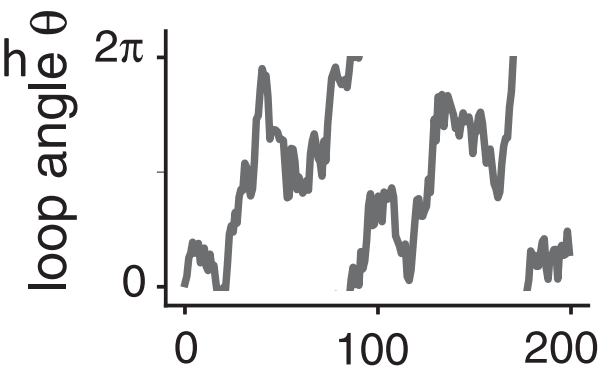
Model: metaphase with spiral scaffold

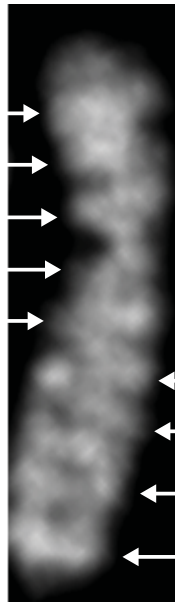
Ornstein–Uhlenbeck RW

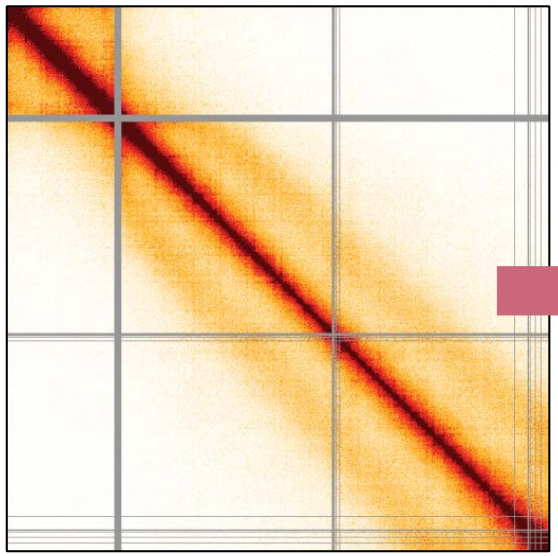
staircase

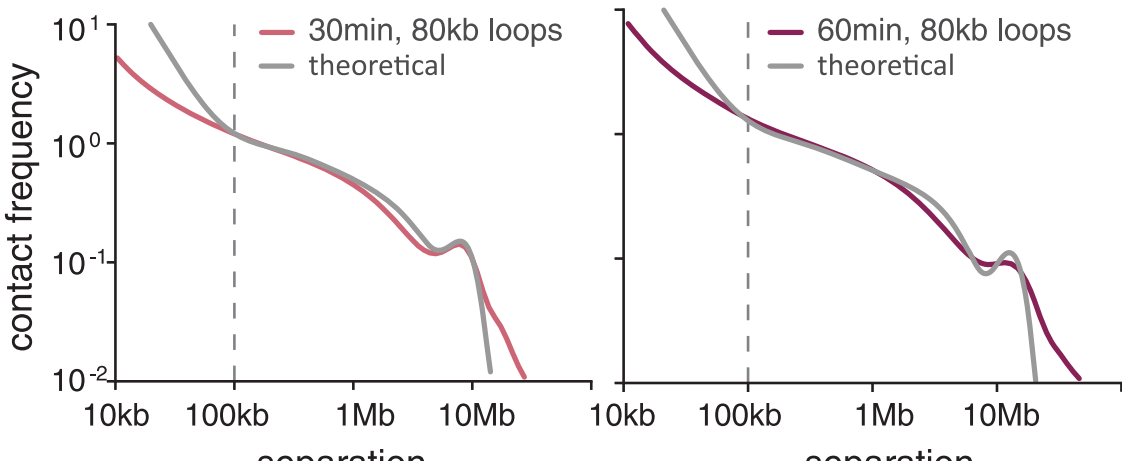






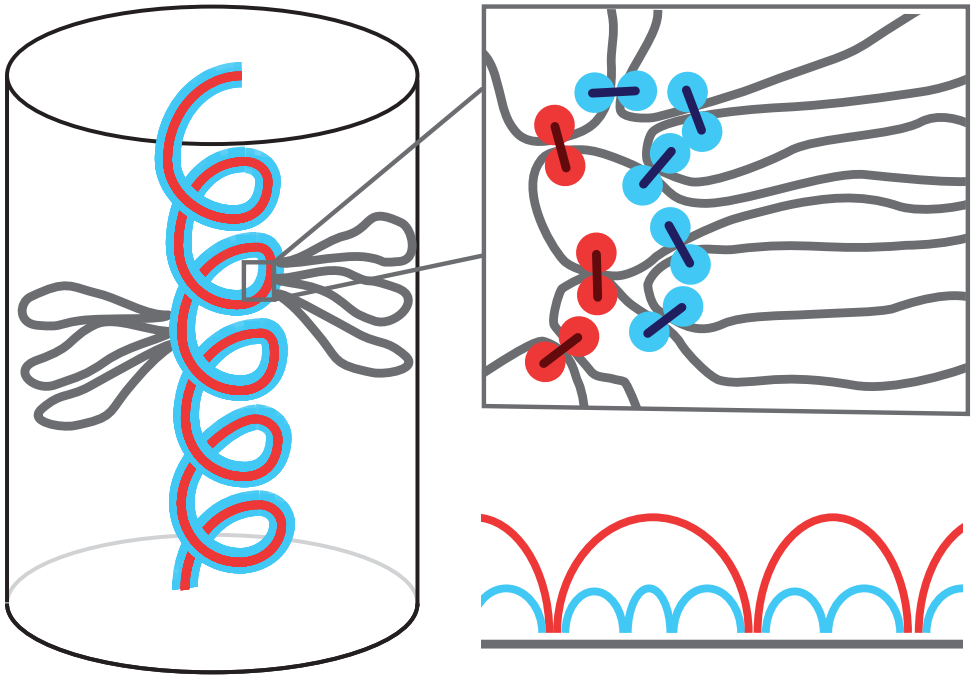


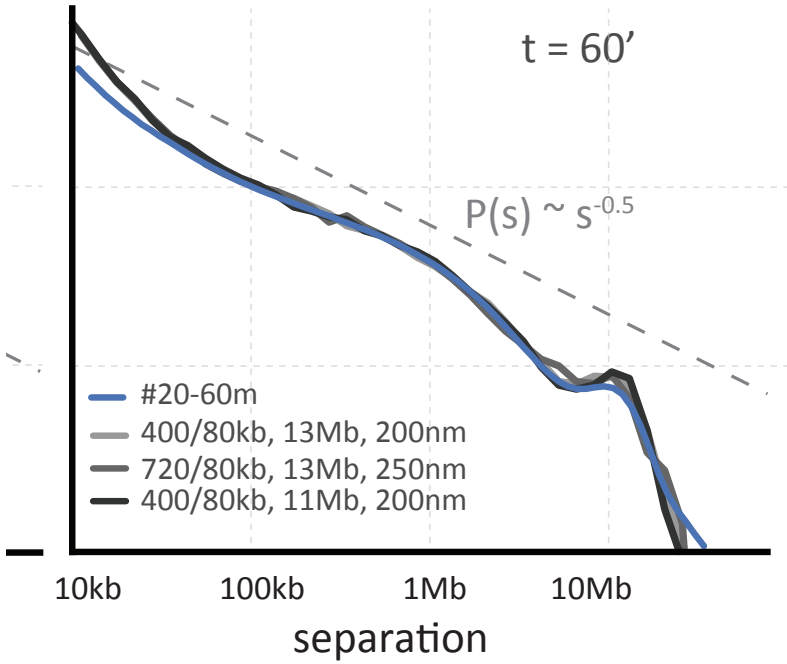




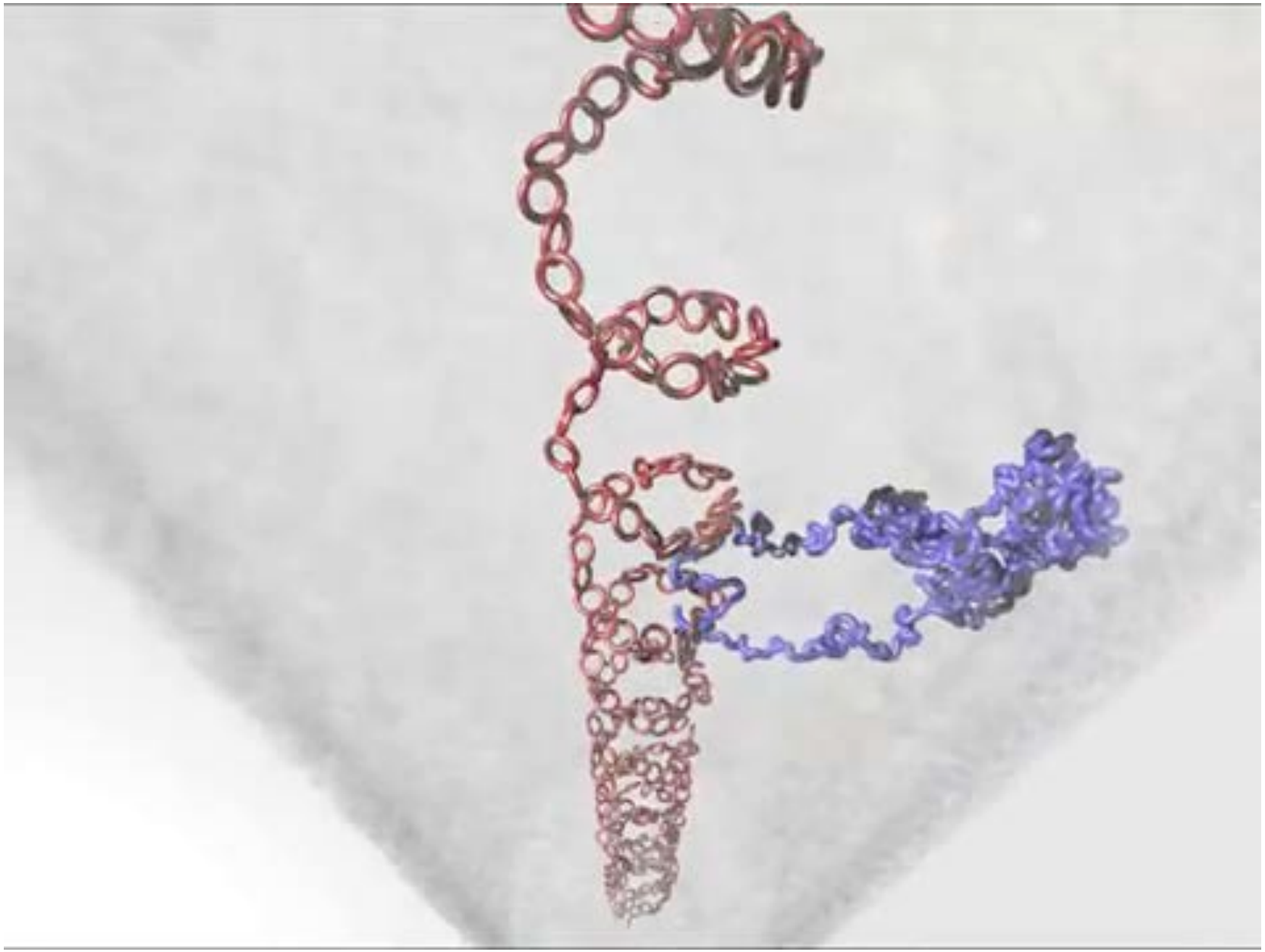
Model: metaphase

Nested loops: 400Kb and 80Kb loops on spiral scaffold



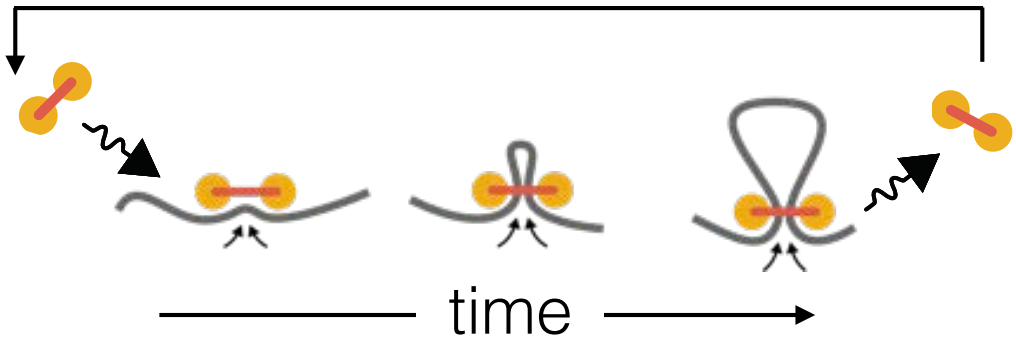


Model of mitotic chromosome



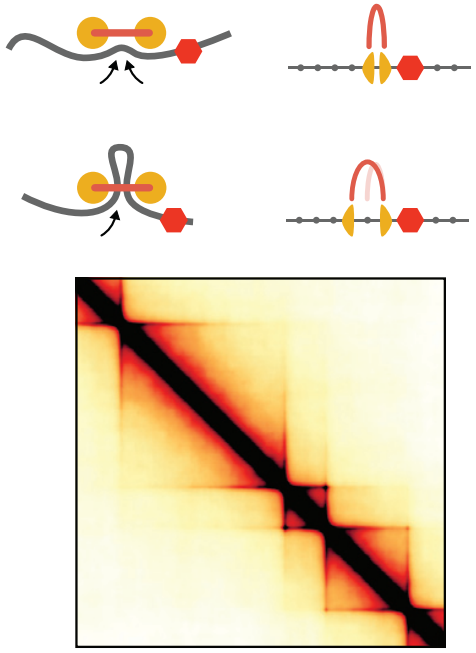
https://www.youtube.com/watch?v=cJSpWClqb7k

Summary: **chromosomes need a motor**

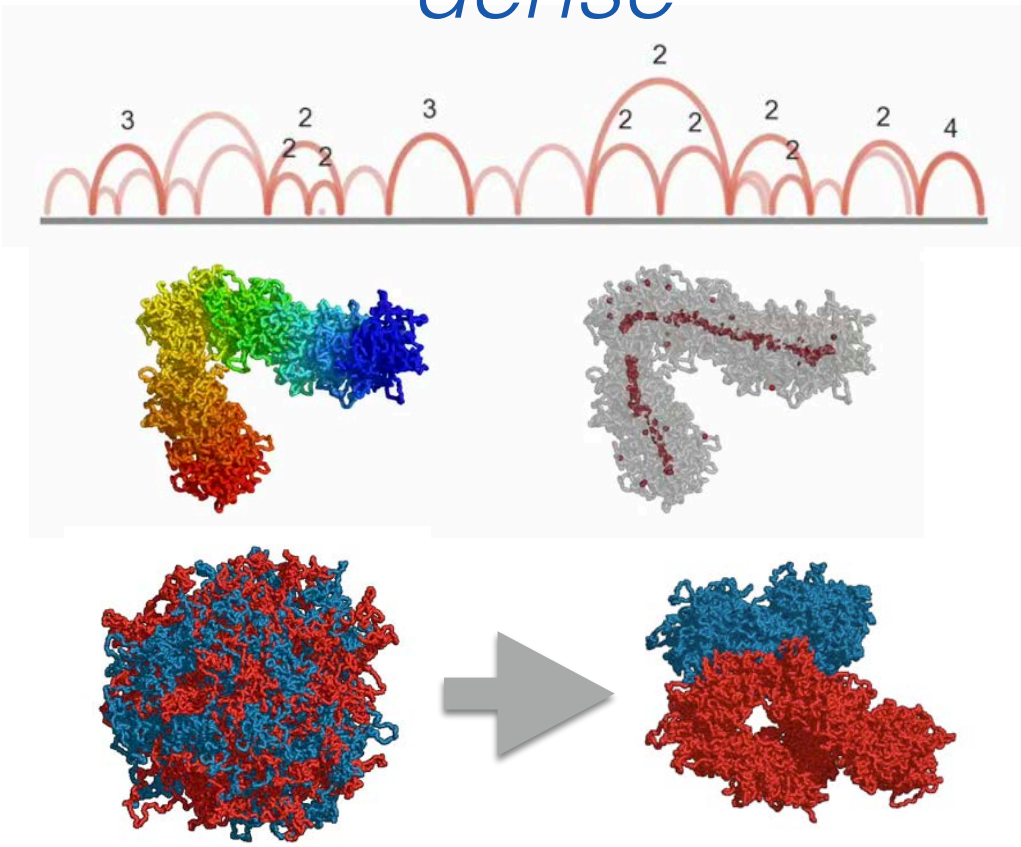


sparse

with borders



dense

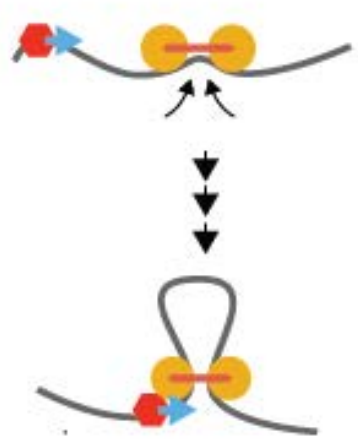


Fudenberg, Imakaev et al.
Formation of Chromosomal Domains by Loop Extrusion
bioRxiv Aug 14 (2015), Cell Reports (2016)

Goloborodko A et. al, *eLife* (2016)
Goloborodko A, Marko JF, Mirny LA *Biophysical J* (2016)

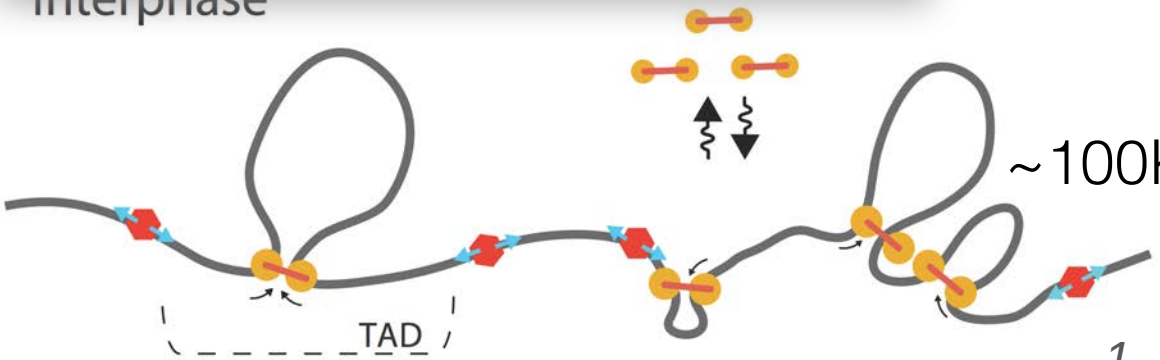
Summary

Active loop extrusion
by **cohesin**



blocked by **CTCF**

Interphase



~100Kb loops

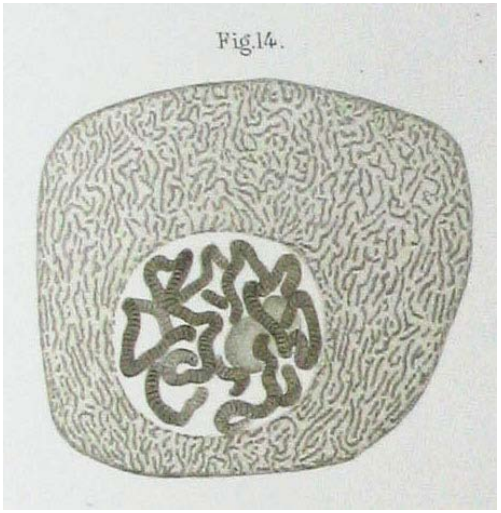
1. Remove boundaries
2. More (x5-10) loop extruding factors
cohesin** is replaced by **condensins

60 -> 600Kb

Take home message

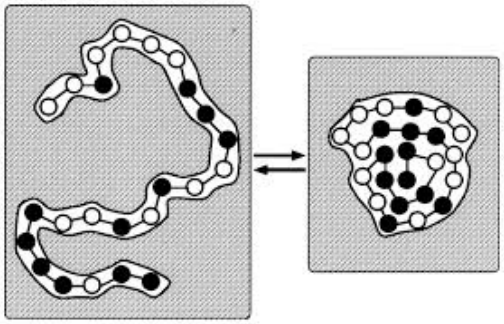
protein folding

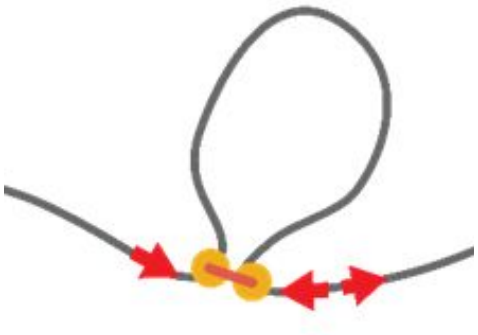
interactions
(energy)



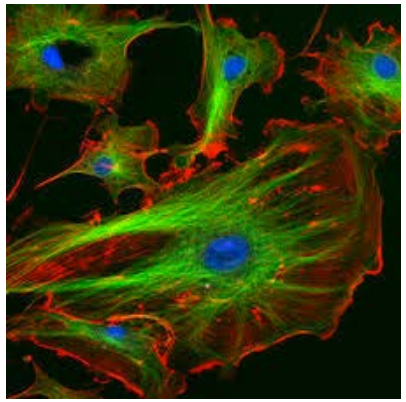
Walther Flemming in 1882

interactions
and
active processes

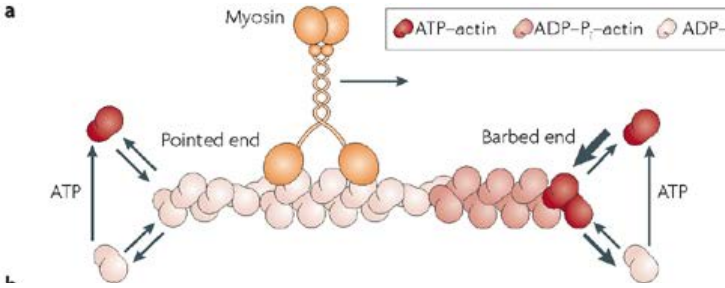




cytoskeleton



active processes



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MIT Physics

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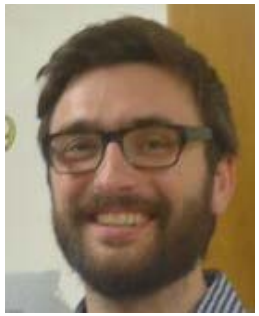
Hugo Brandao
Harvard Biophysics

Martin Falk
MIT



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Maxim Imakaev
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Simon Grosse-Holz
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Carolyn Lu
MIT senior

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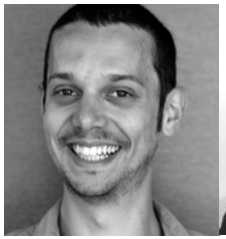

Elphege Nora
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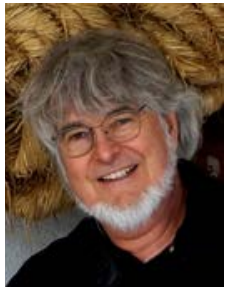


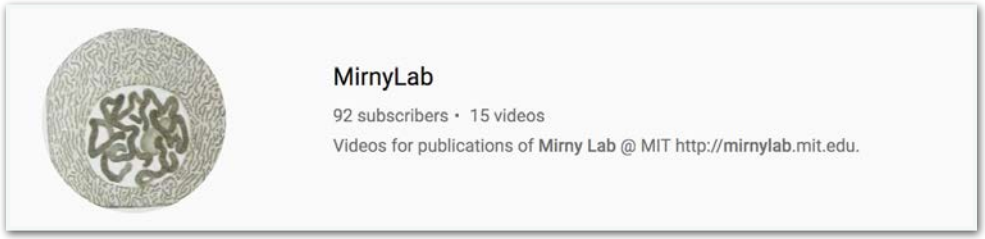
Bill Earnshaw
U of Edinburgh

http://mirnylab.mit.edu/resources/

Falk et al.,

Heterochromatin drives compartmentalization of inverted and conventional nuclei

Nature (2019)



Fudenberg et al.,

Emerging Evidence of Chromosome Folding by Loop Extrusion

CSH Symposia in Quantitative Biology (2018)

Schwarzer W, Abdennur N et al

Two independent modes of chromatin organization revealed by cohesin removal.

Nature (2017)

Nature April 19, 2017

nature International weekly journal of science

DNA's secret weapon against knots and tangles

A simple process seems to explain how massive genomes stay organized. But no one can agree on what powers it.

Elie Dolgin

19 April 2017