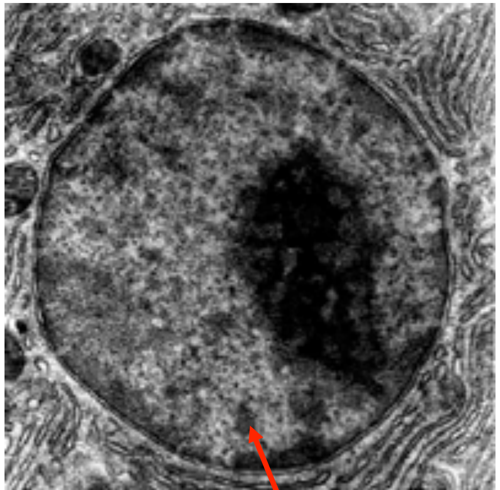
Super-resolution imaging reveals principles of physical chromatin folding in *eukaryotes*

Frédéric Bantignies

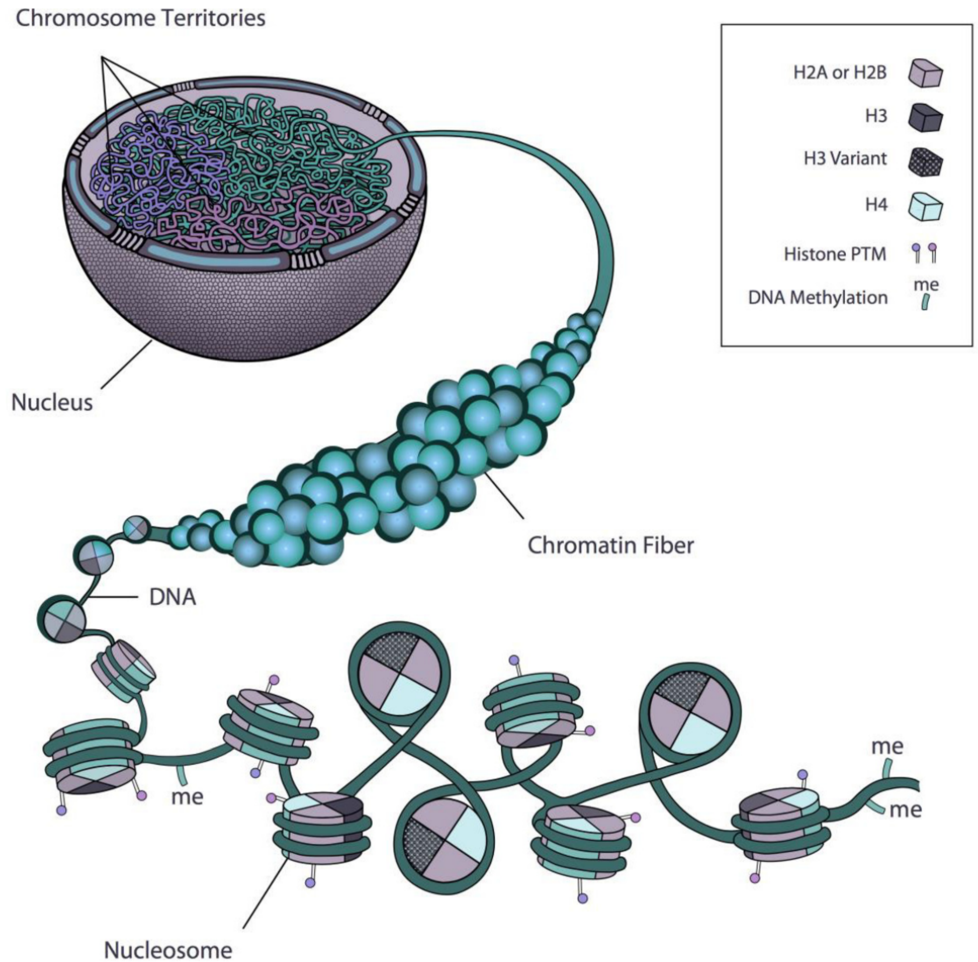
Chromosome Conformation Symposium - Toulouse 04/12/2019

Inside cell nucleus, the genome is highly compacted and folded as a chromatin fiber



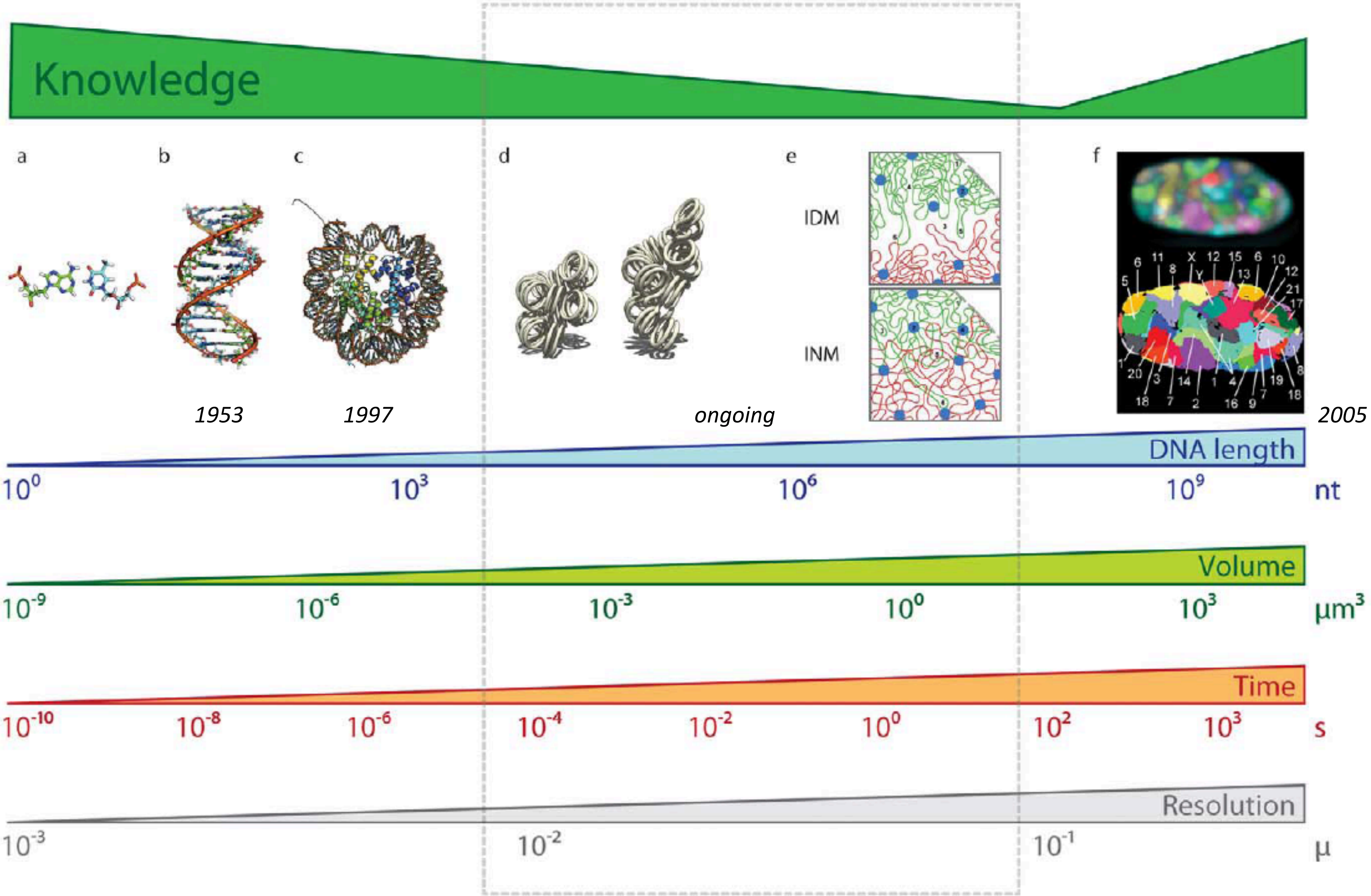
~10 µm

~2 meters of DNA



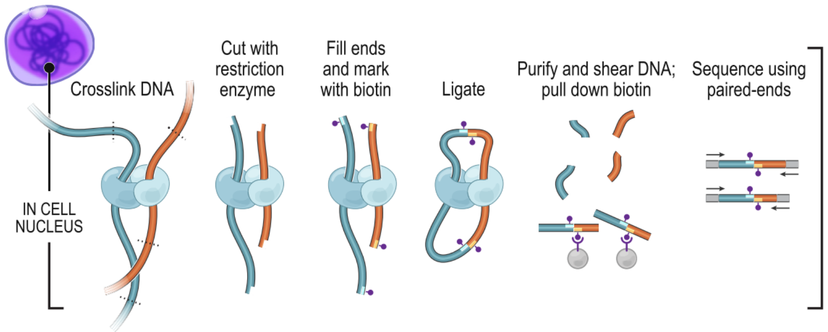
Rosa & Shaw, 2013

The different level of genome organization



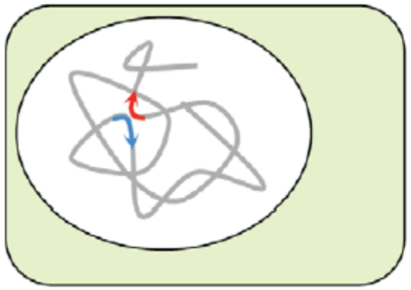
Marti-Renom and Mirny, *PLoSComputational Biology 2011*

Chromosome Conformation Capture (Hi-C)

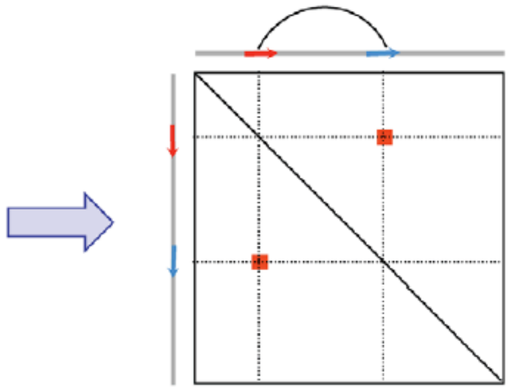


Lieberman *et al*, 2009 (Hi-C)
Rao *et al*, 2014 (in situ Hi-C)

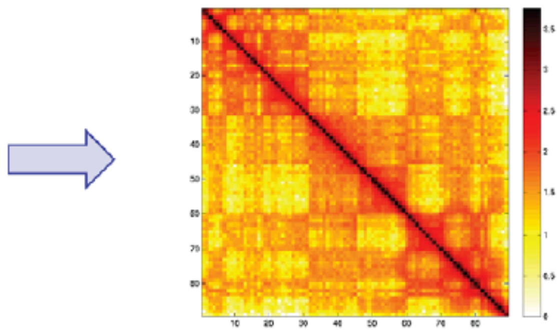
Chromatin crosslink inside nucleus



Generation of contact maps between all interacting fragments

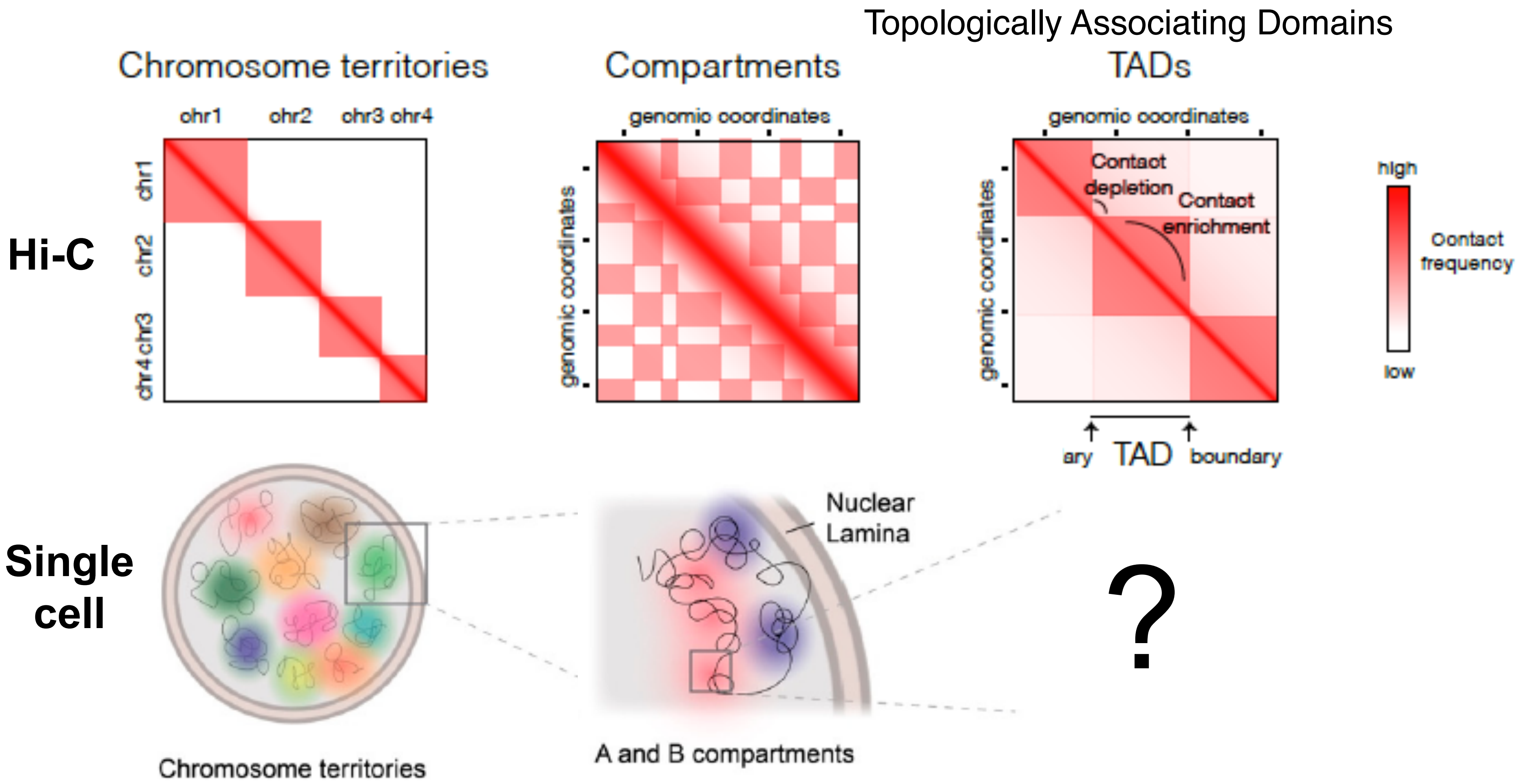


Contact density map



Marti-Renom and Mirny, *PLoSComputational Biology 2011*

Hi-C maps represent three main levels of genome folding



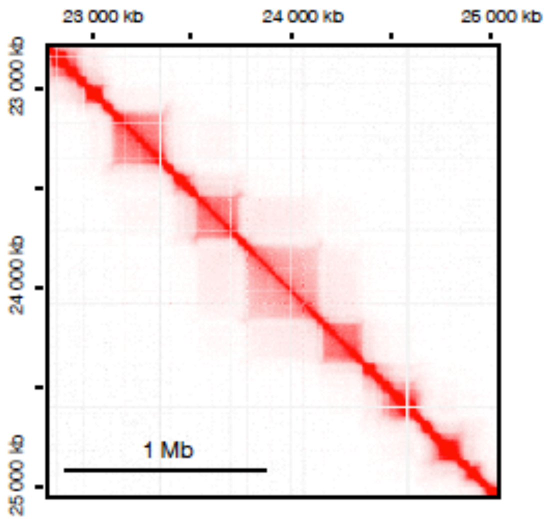
➢ **TADs represent genomic region of highly interacting chromatin with few interactions spanning their borders**

Adapted from Szabo, Bantignies, Cavalli, *Science Advances 2019*
and Mota-Gomez, Lupianez, *Genes 2019*

TADs are a conserved genomic feature with species specificities

Fly

Drosophila melanogaster

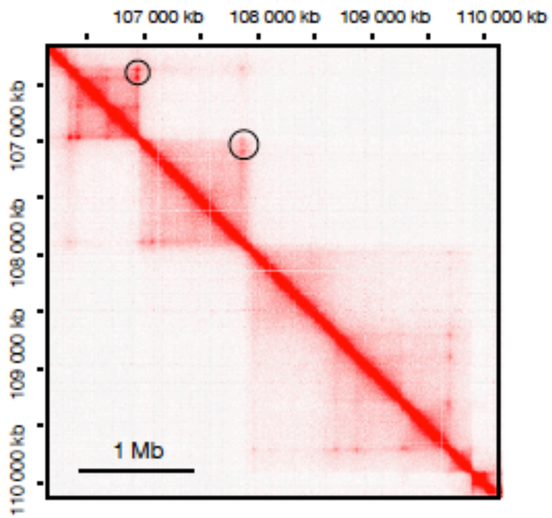


- Median size: ~ 100 kb
- Coincide well with the alternation of repressed and active chromatin marks

vs.

Mammals

Mus musculus



- Median size: ~ 900 kb
- Presence of corner peaks (structural architectural loops)
- Presence of Enhancer-Promoter loop (functional loops)

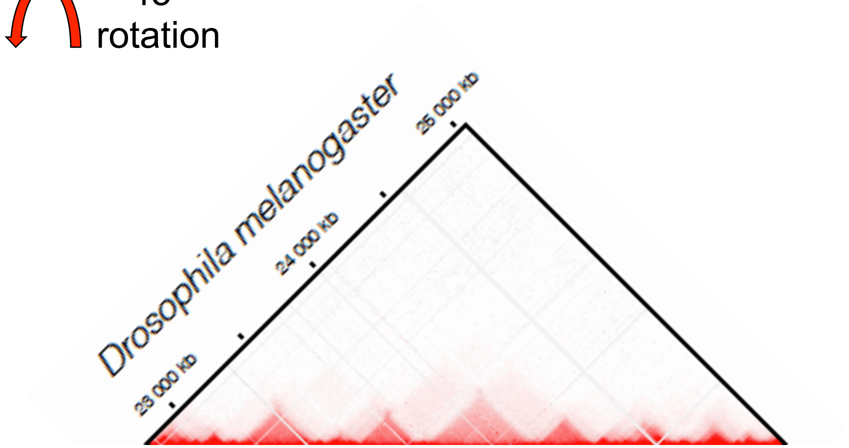
Sexton *et al.*, Cell 2012
Nora *et al.*, Nature 2012
Dixon *et al.*, Nature 2012
Hou *et al.*, Molecular Cell 2012

Adapted from Szabo, Bantignies, Cavalli, *Science Advances 2019*

TADs are considered as functional genomic units

45° rotation

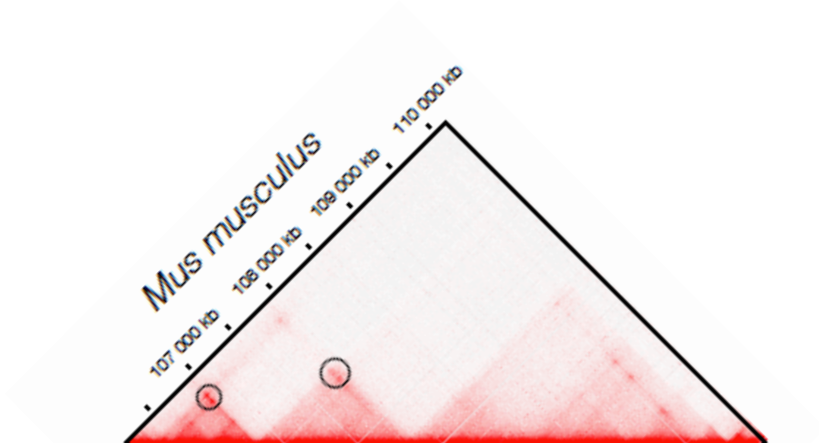
Fly



- Median size: ~ 100 kb

- Coincide well with the alternation of repressed and active chromatin marks (Sexton et al, 2012)

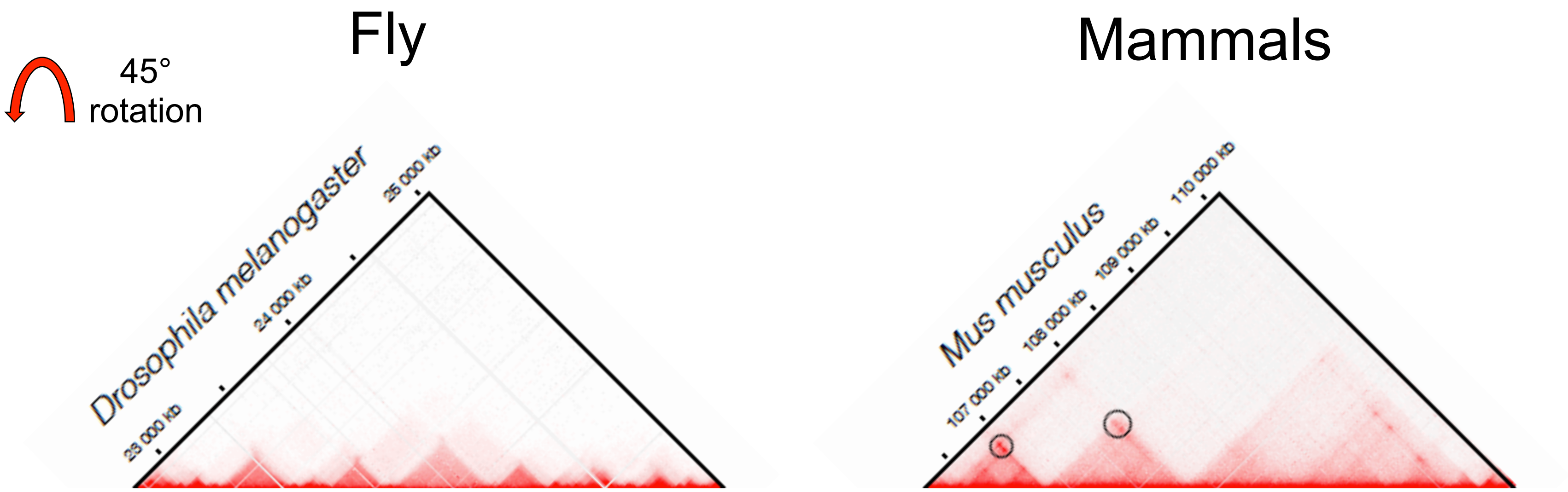
Mammals



- Median size: ~ 900 kb
- Presence of corner peaks (structural architectural loops)
- Presence of Enhancer-Promoter loop (functional loops)

- Genes within TADs are co-regulated (Nora *et al*, 2012; Zhan *et al*, 2017)
- Enhancer/promoter contacts are restricted within TADs (Symmons *et al*, 2014; Bonev *et al*, 2017)
- Disruption of boundary leads to ectopic gene expression (Lupianez *et al*, 2015; Hniz *et al*, 2016; Rodriguez-Carballo *et al*, 2017)

TADs are considered as functional genomic units



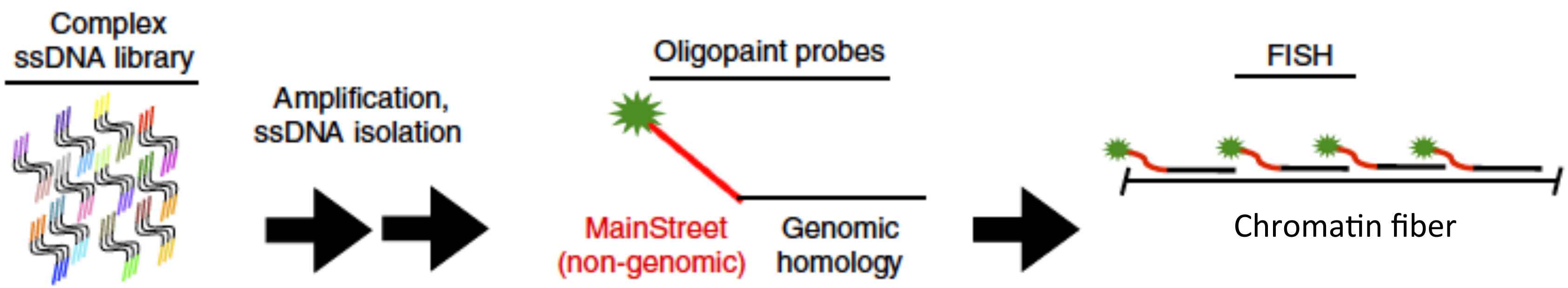
Whether TADs structure is compatible with their functional role ?

Indeed, they can represent the manifestation of average interactions from large cell populations and therefore we need to understand their structure before to claim that they represent functional domains

We undertook a structural approach combining
Hi-C / Oligopaint technology / super-resolution microscopy
in Drosophila

The Oligopaint 3D-FISH technology

- Represents a new generation of FISH probes entirely derived from synthetic DNA oligonucleotides
- Production of *ssDNA oligo pools* able to recognize any portion of the genome in various organisms, from 10 kb to several Mb, avoiding repetitive sequences



Beliveau *et al.,* Nature communications 2015*;* Beliveau *et al.*, PNAS 2018

https://oligopaints.hms.harvard.edu

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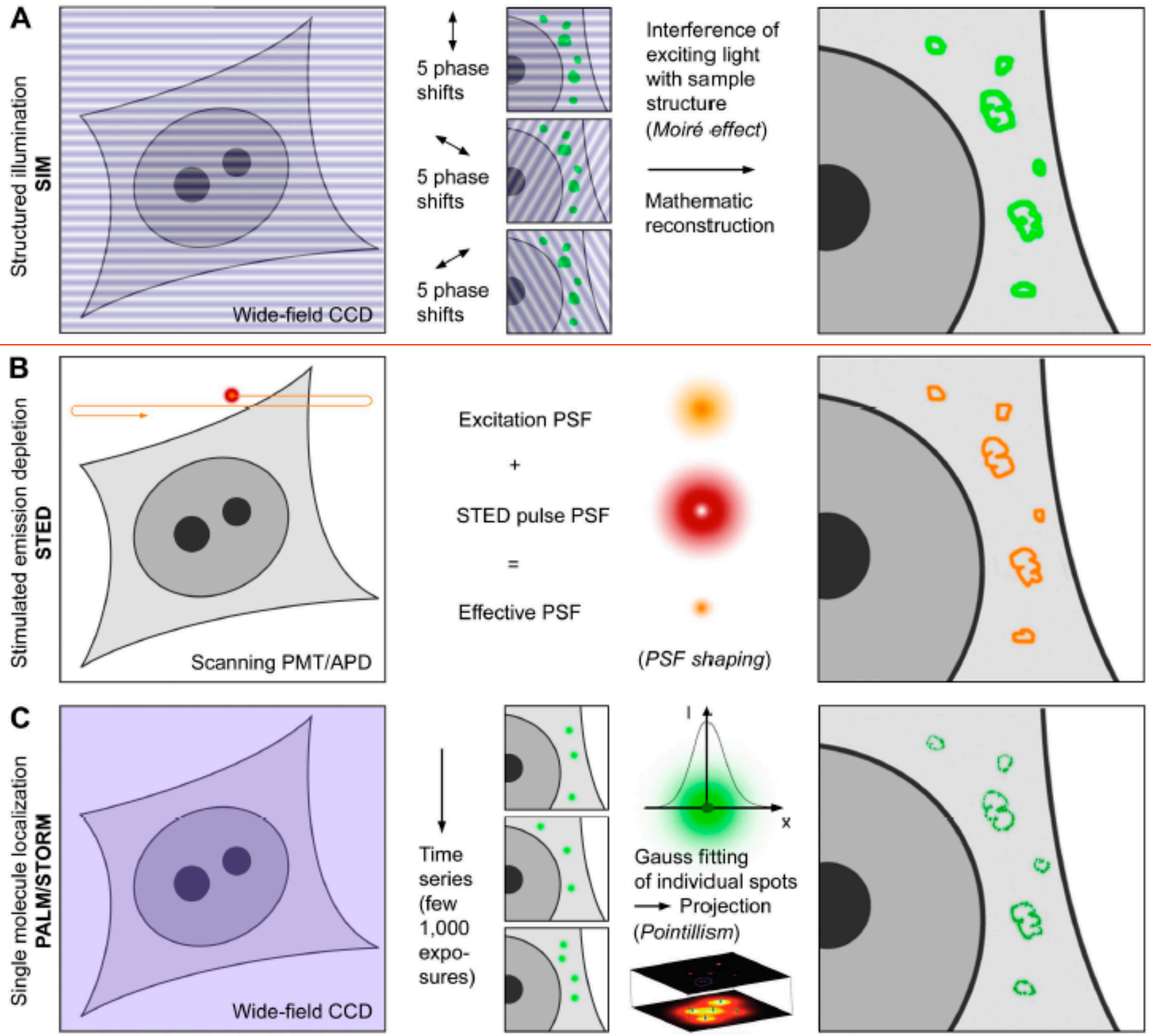
Super-Resolution Microscopy (SRM)



Axial resolution (estimated)

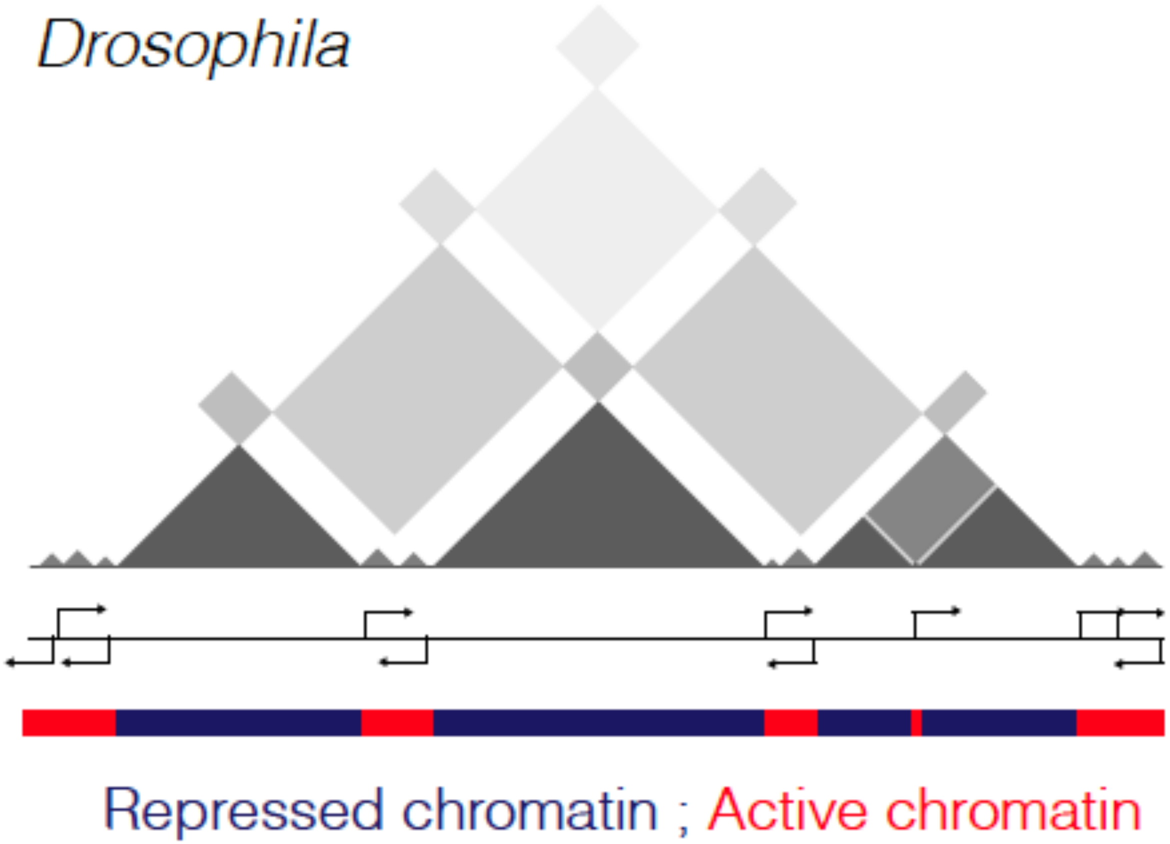
100 nm

50-80 nm

30 nm

Schermelleh, Heintzmann and Leonhardt, J.cell.Biol. 2010

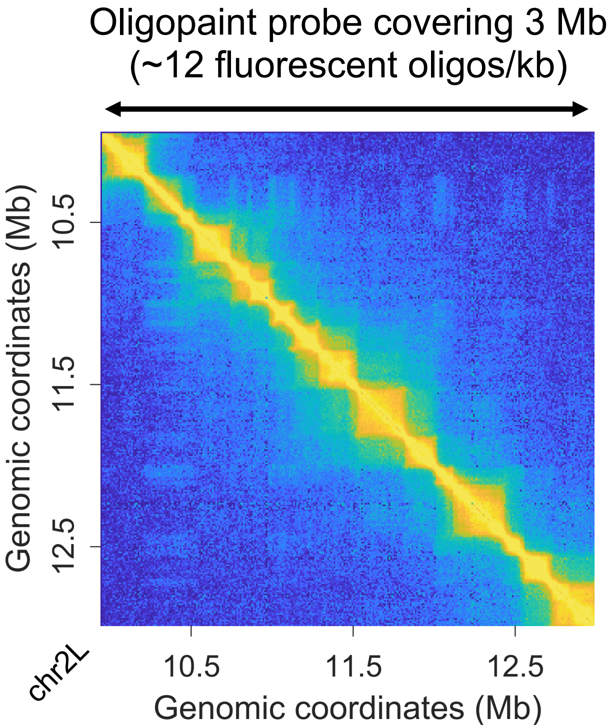
In *Drosophila*, TADs corresponds to the alternation of chromatin states



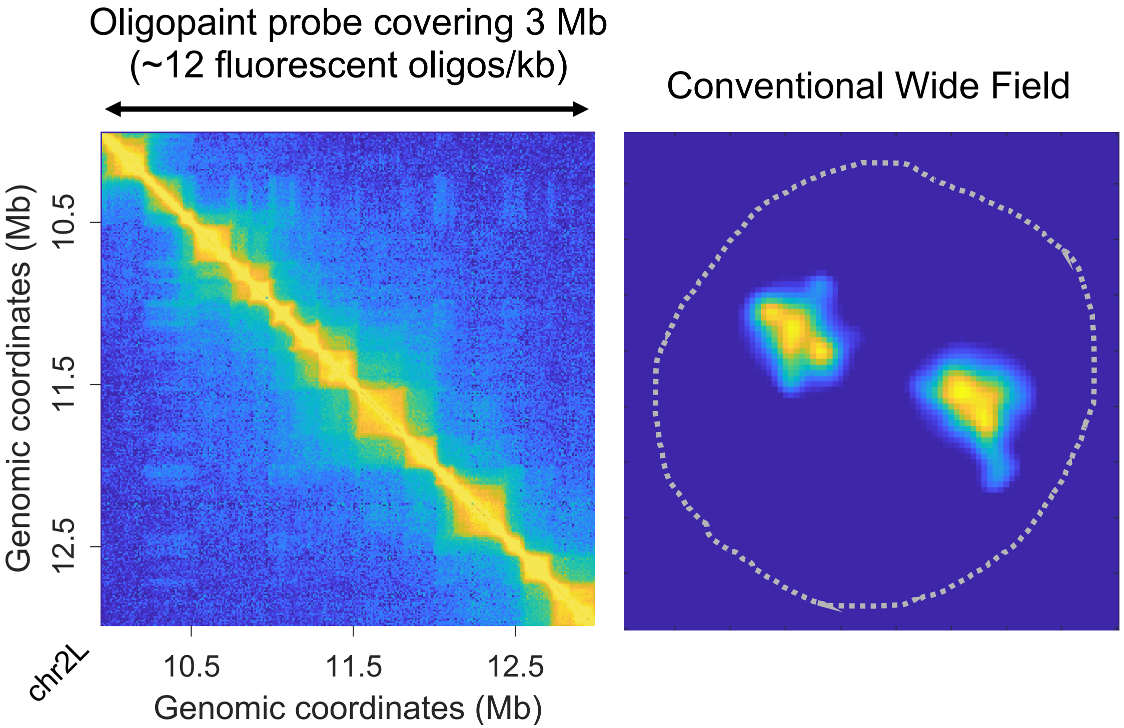
- Active chromatin: H3K4me3/H3K36me3/H3K27ac/gene dense/ubiquitously active
- Repressed chromatin: H3K27me3/Polycomb proteins or Void chromatin/gene poor/specific activation during developmental programs

Adapted from Szabo, Bantignies, Cavalli, Science Advances 2019

3D-SIM super-resolution imaging reveals chromatin nano-structures or nanocompartments



3D-SIM super-resolution imaging reveals chromatin nano-structures or nanocompartments



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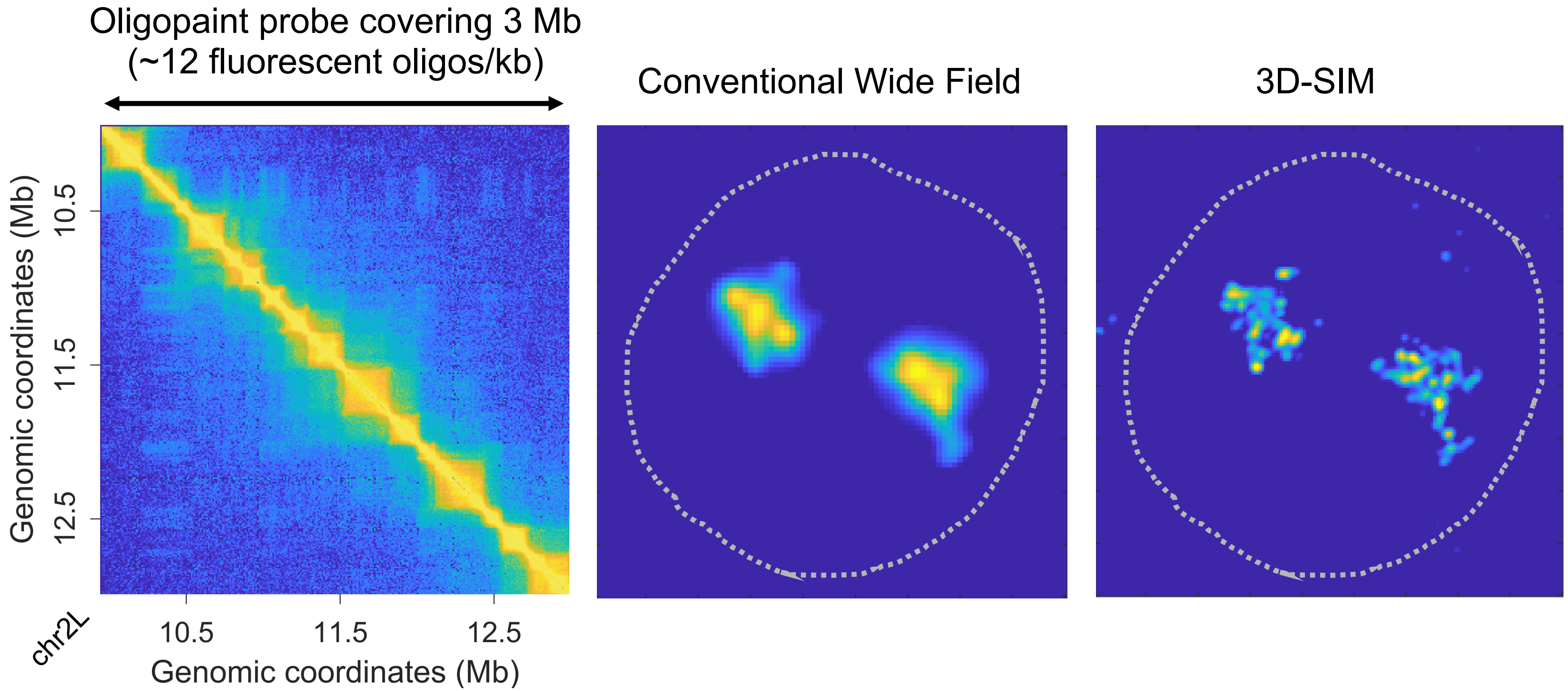

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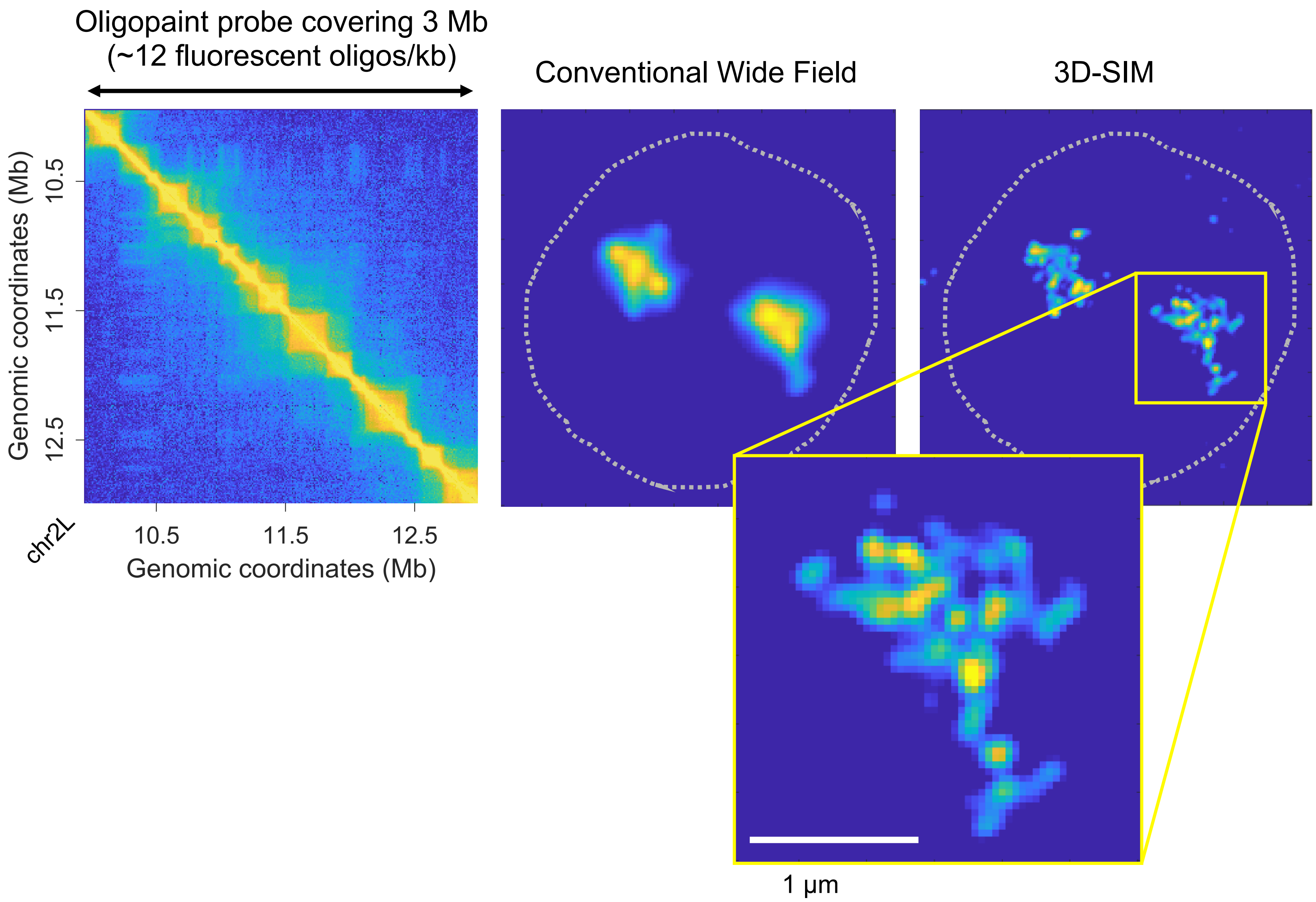
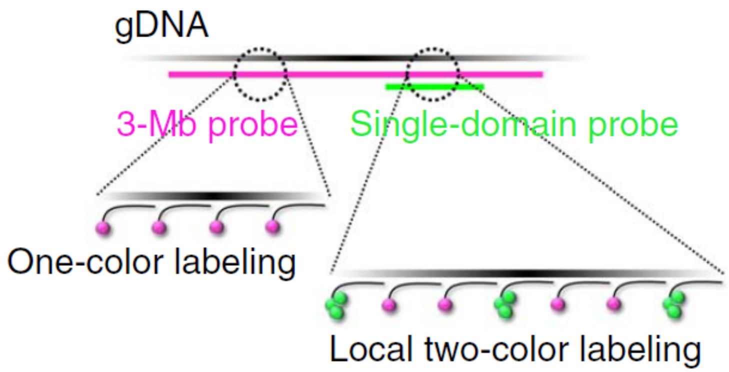
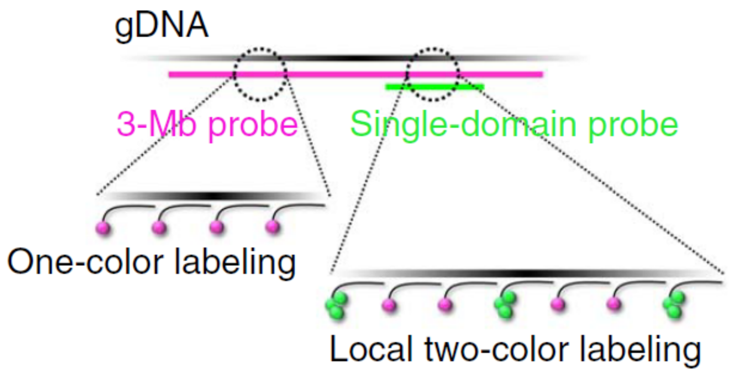
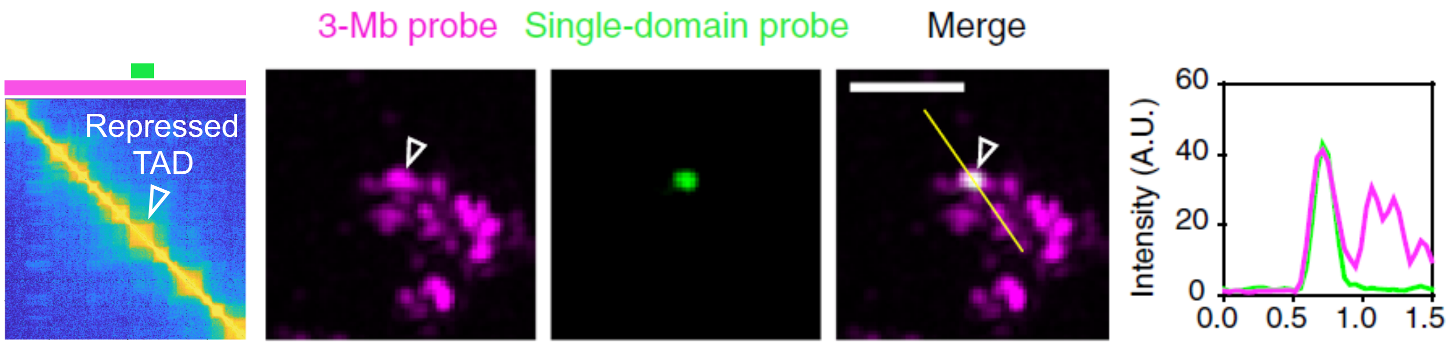


Dual labeling of the chromatin fiber



Local chromatin compaction reflects the chromatin state





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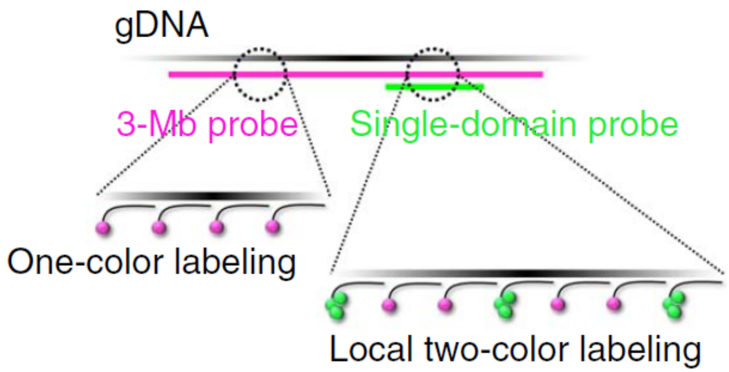
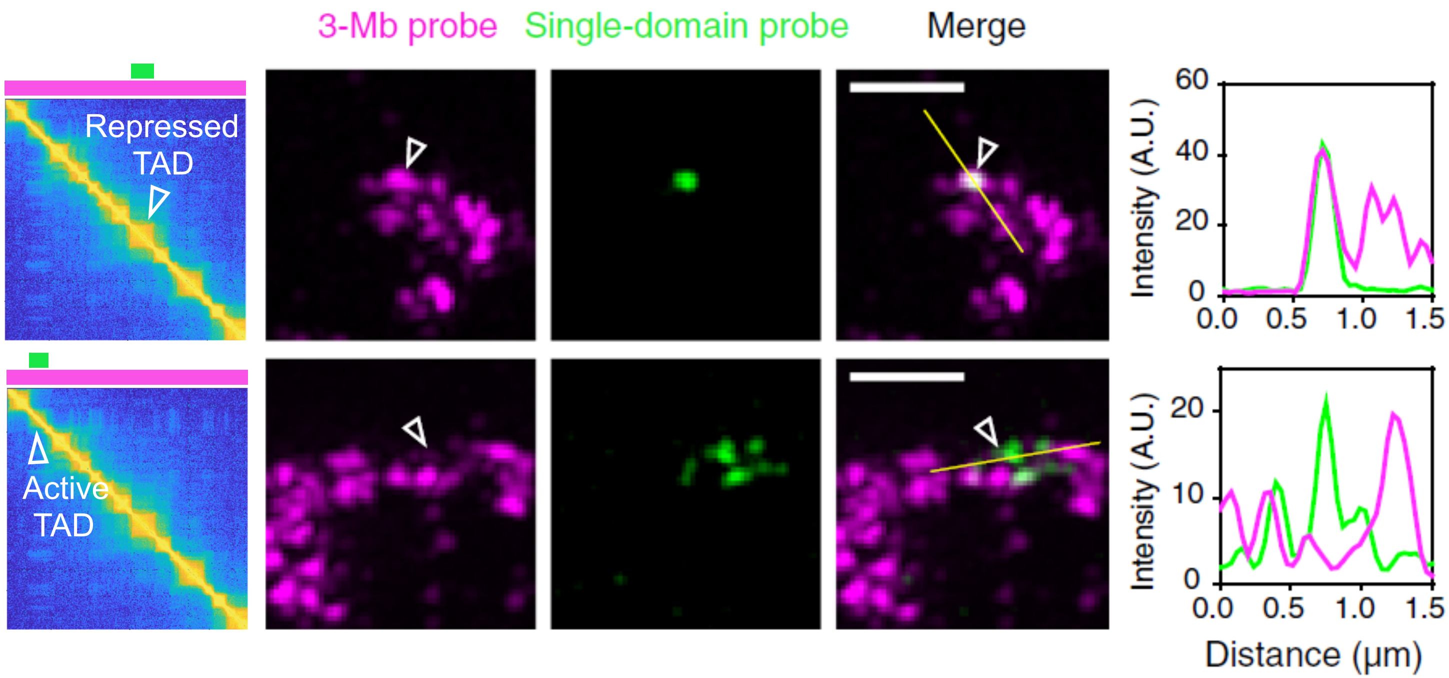
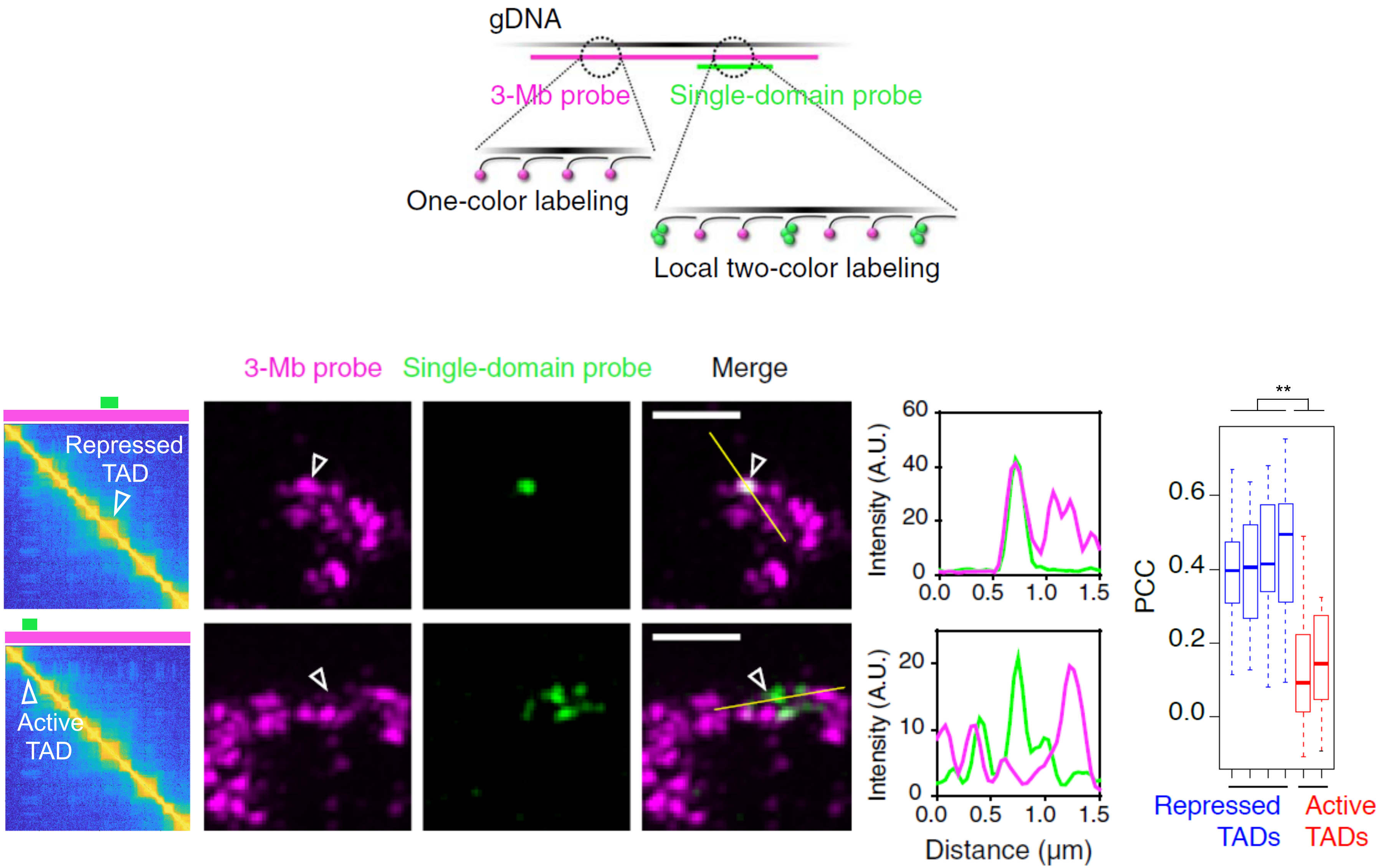
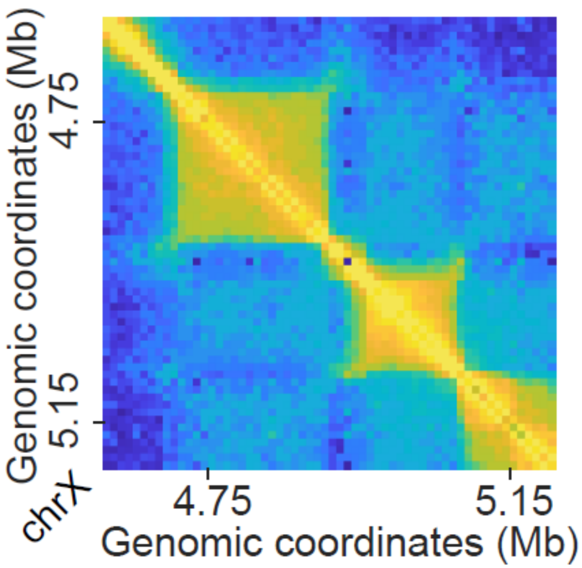




Local chromatin compaction reflects the chromatin state



Investigating TAD structures *in vivo*



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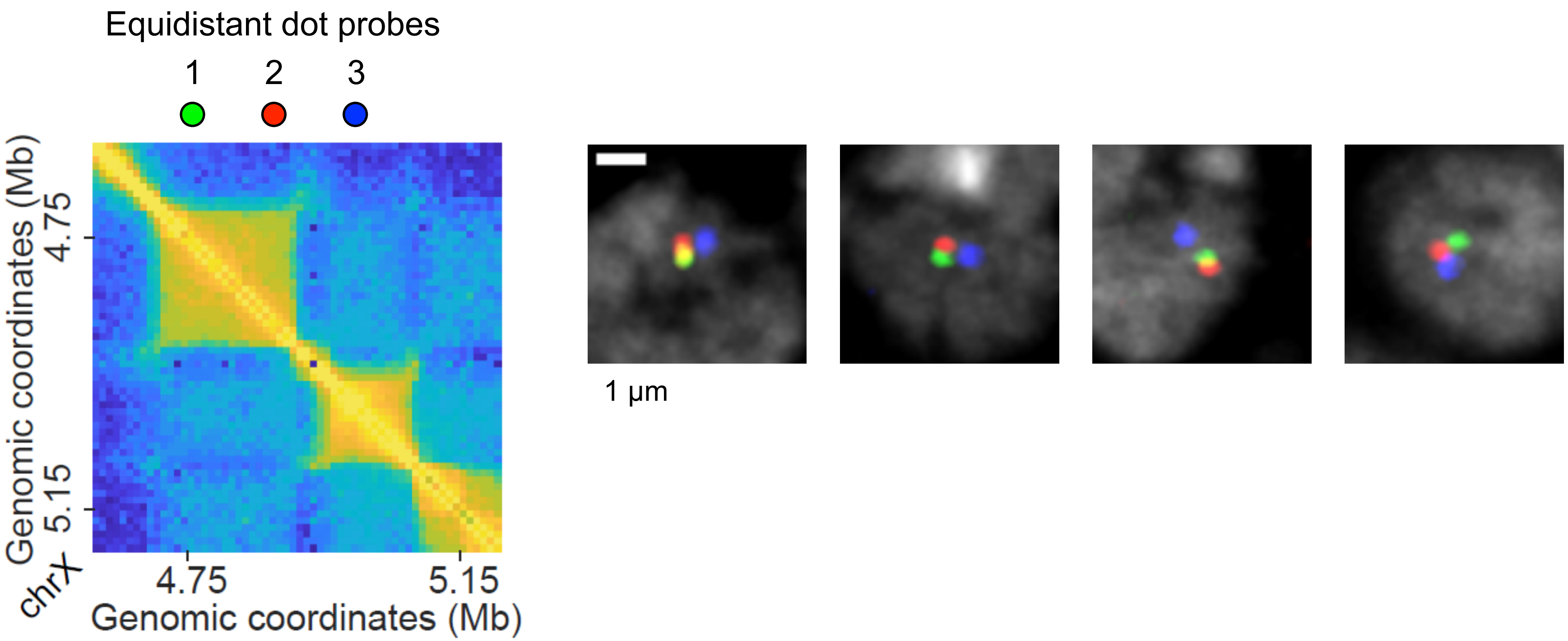
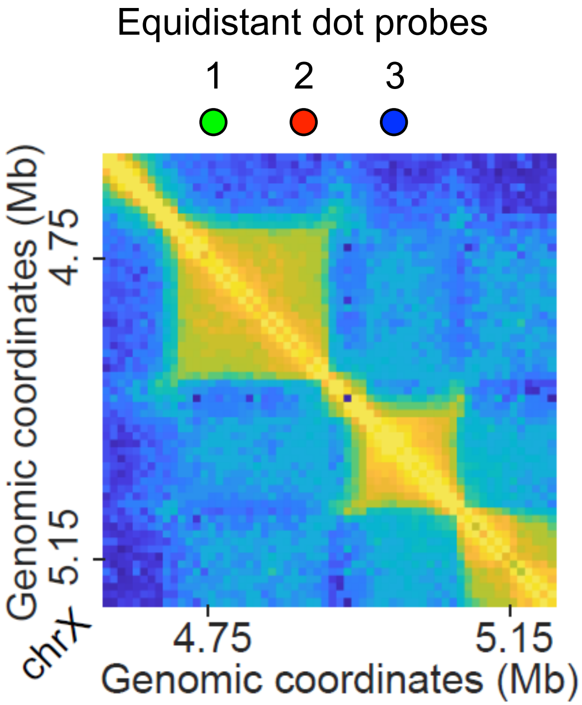
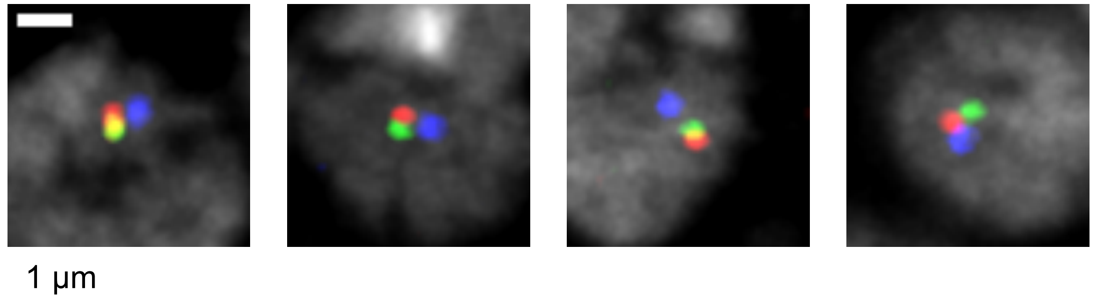
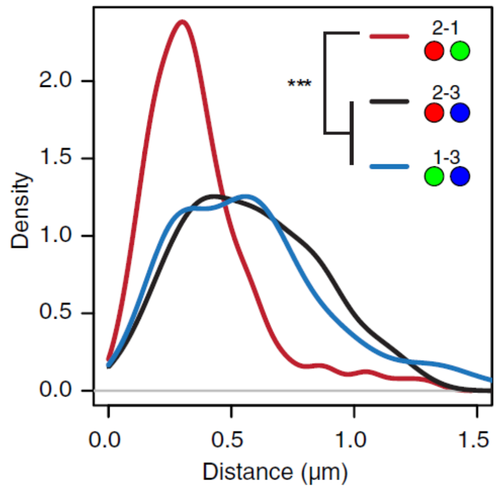


Repressed TADs spatially confine the chromatin fiber







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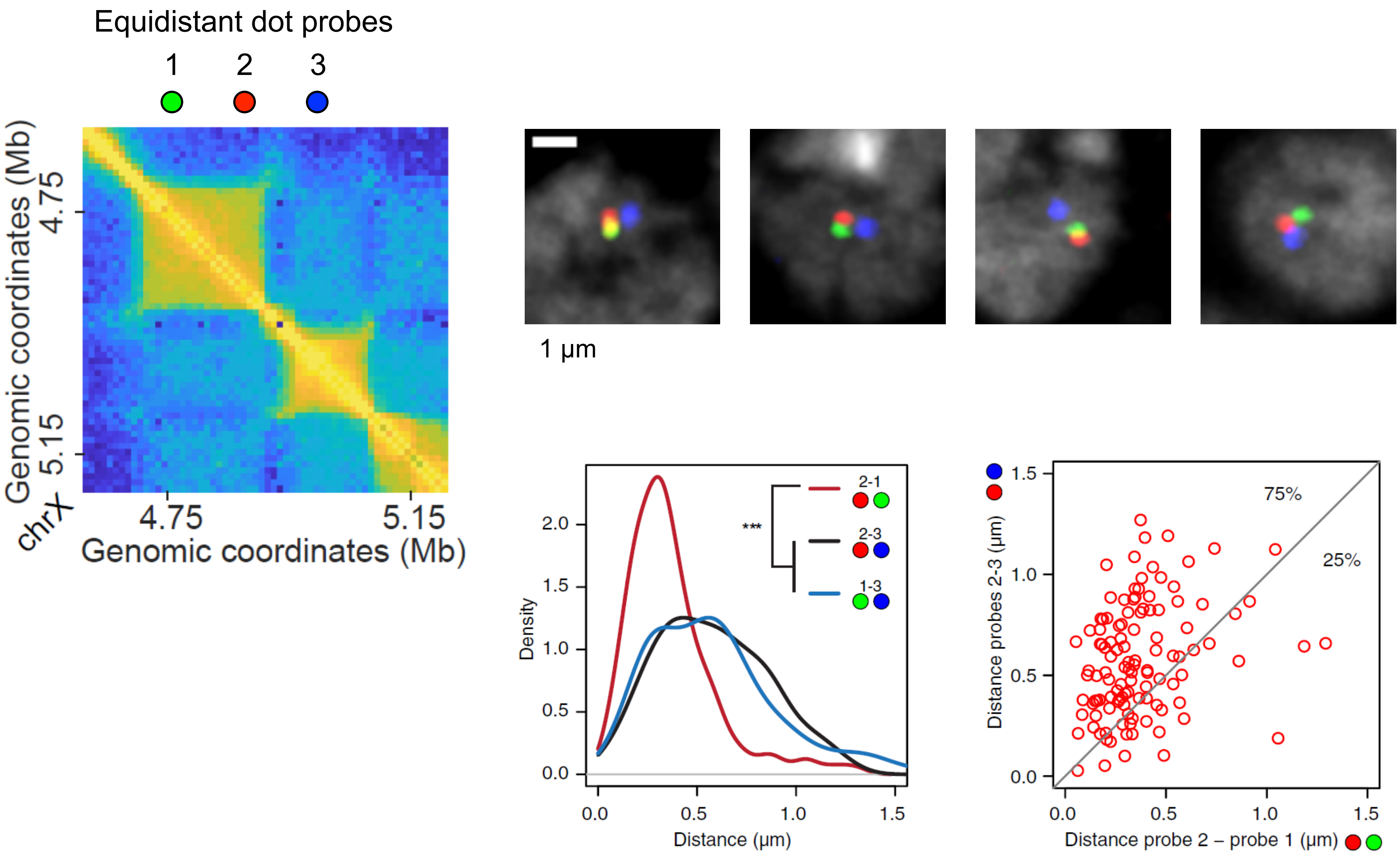
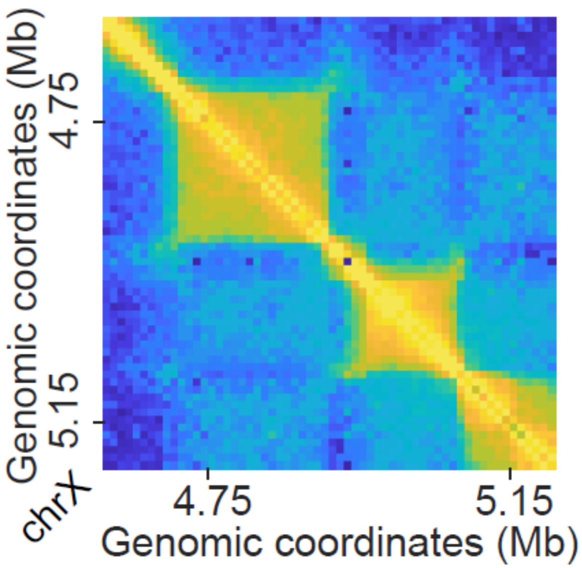


Repressed TADs form discrete 3D chromosomal units or nanocompartments



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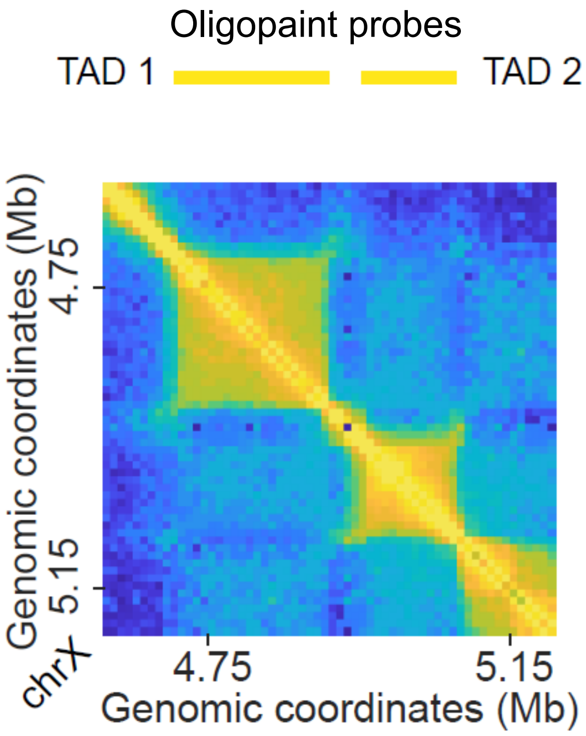

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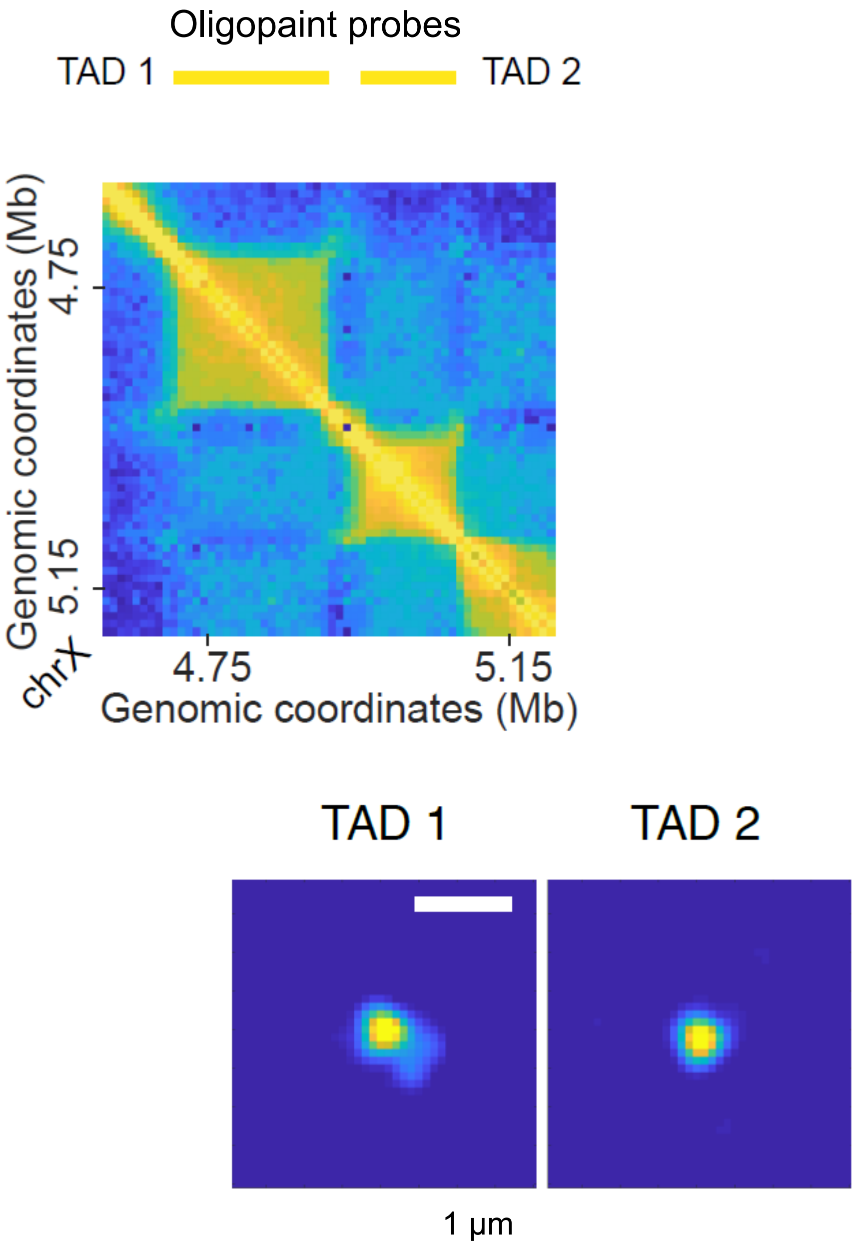

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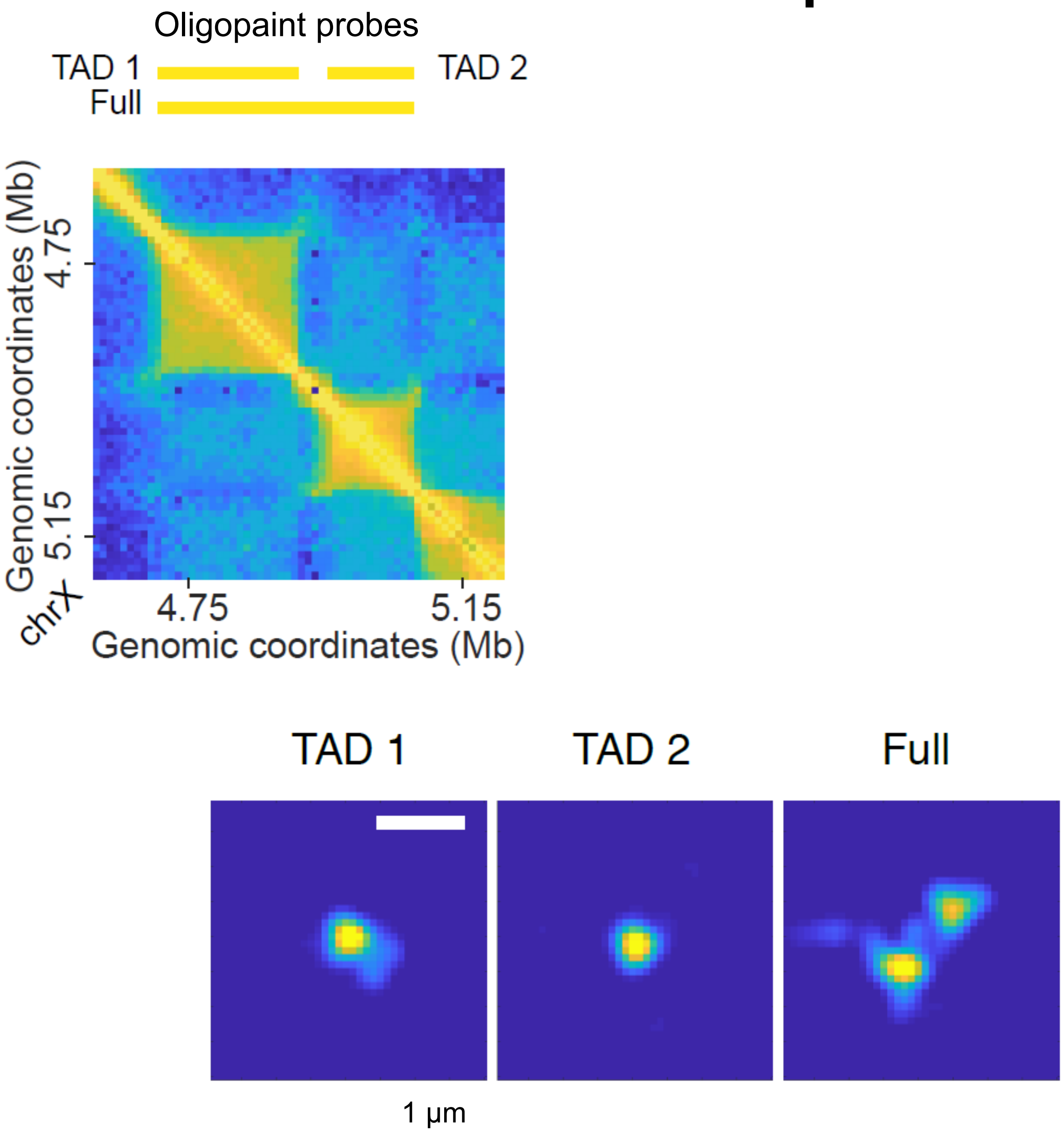

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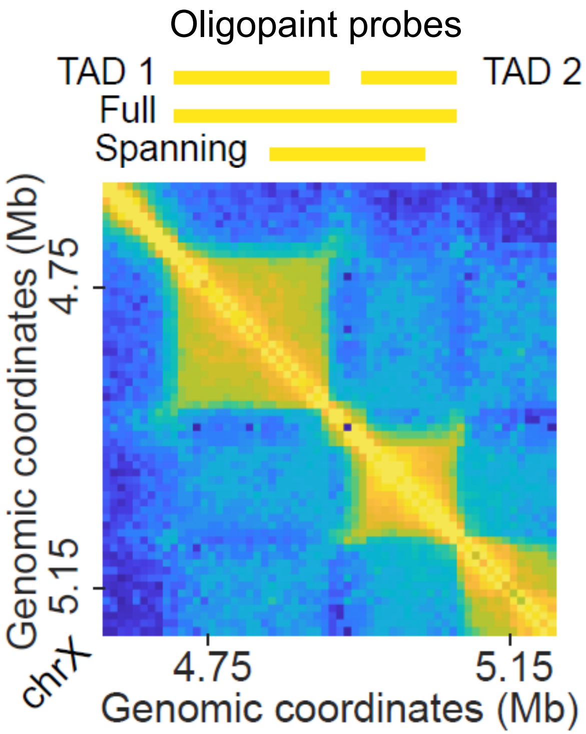

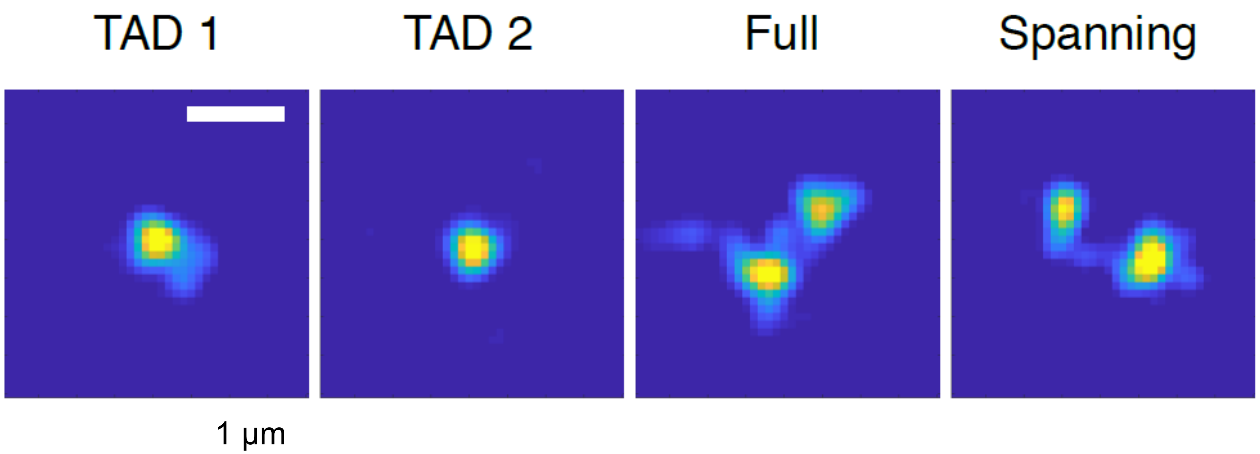

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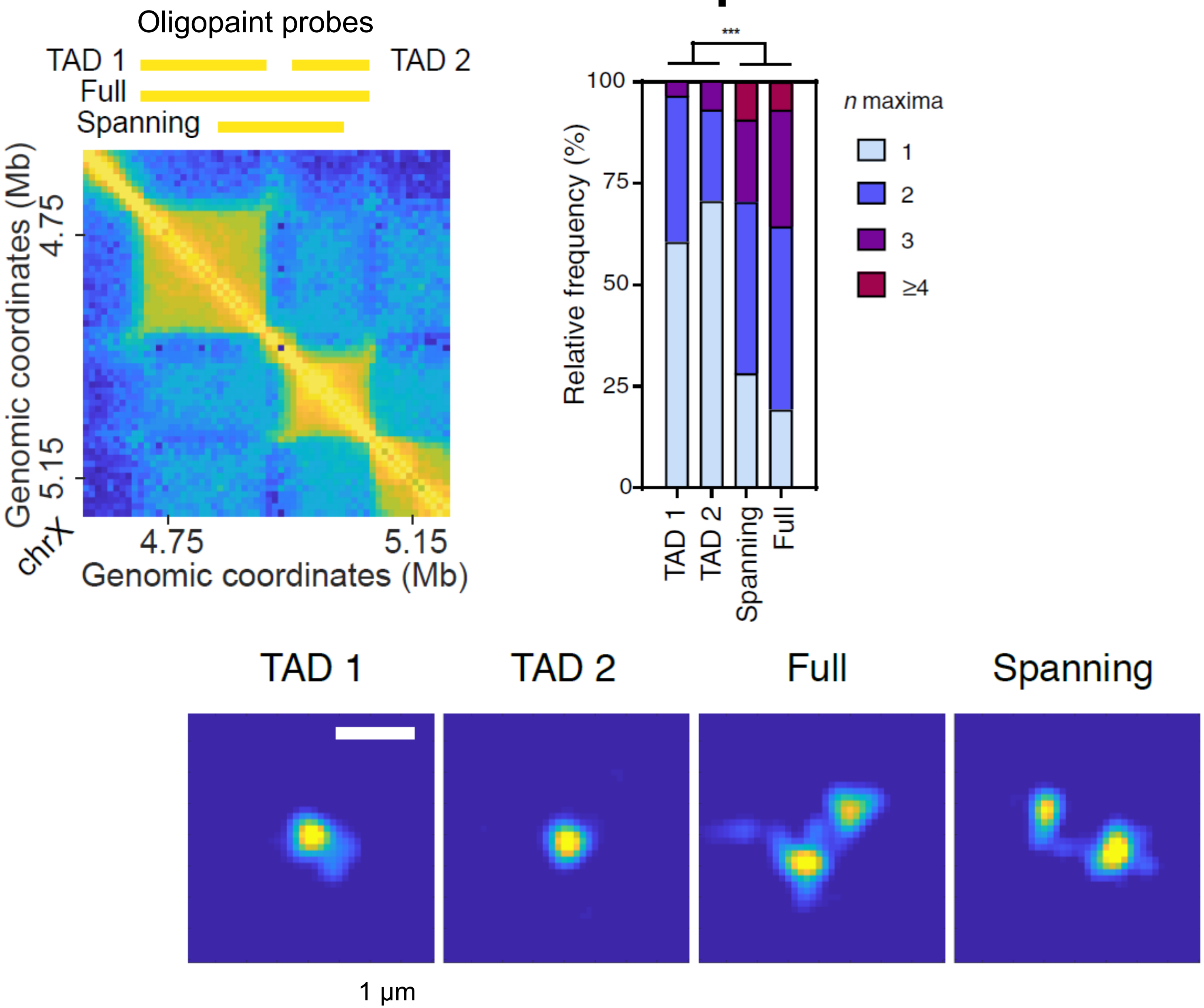

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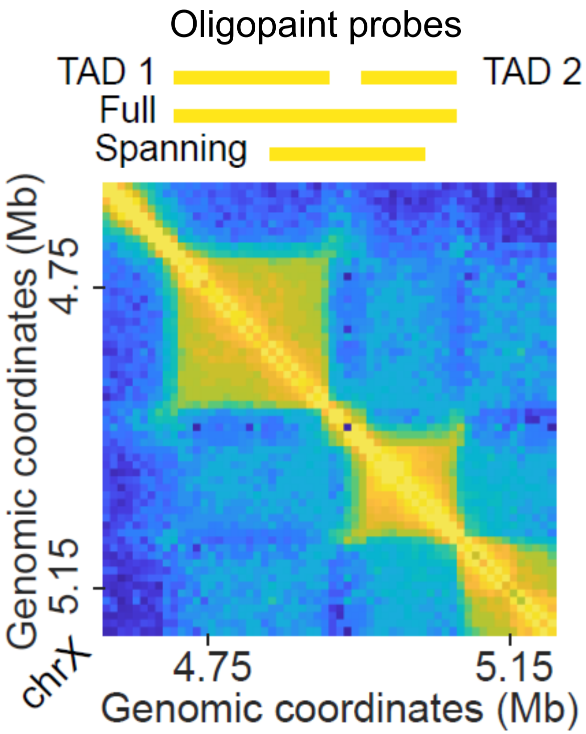
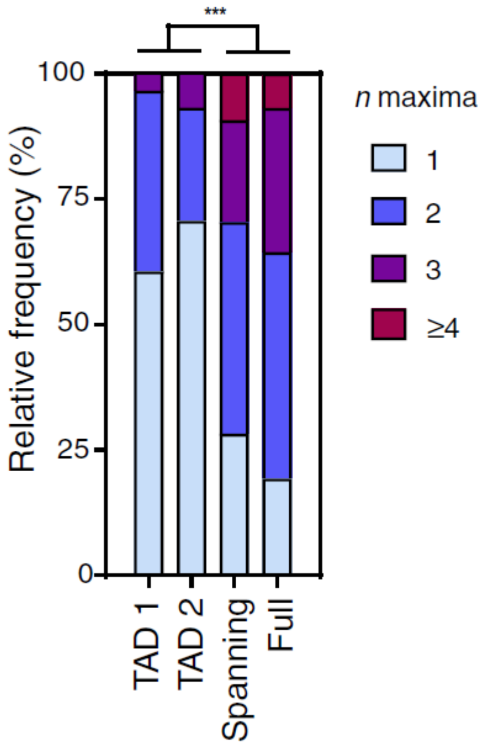
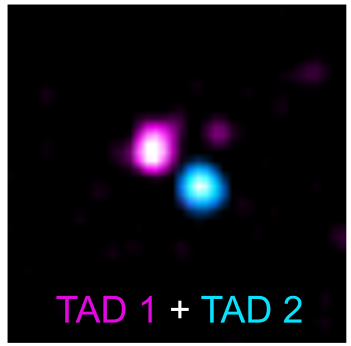
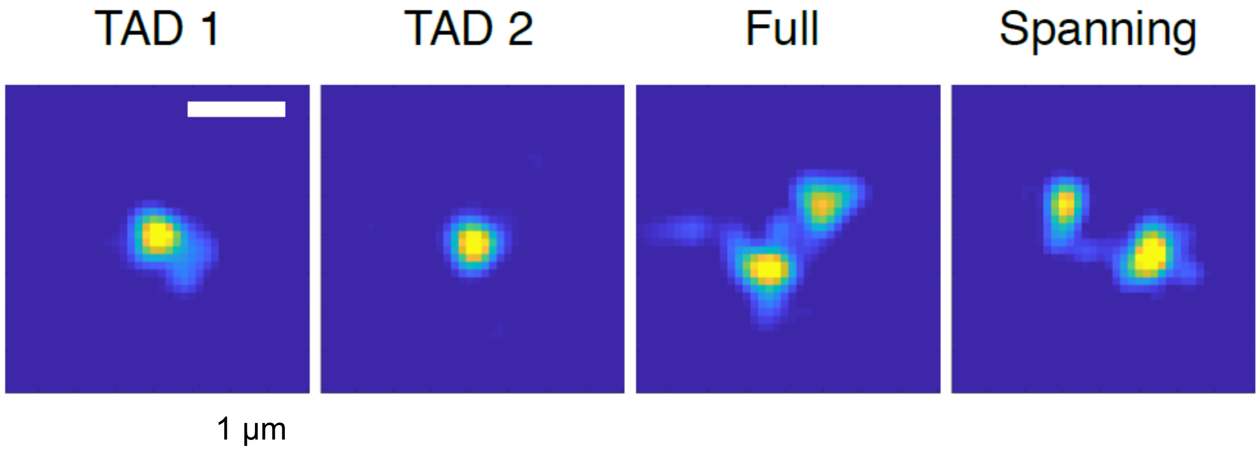






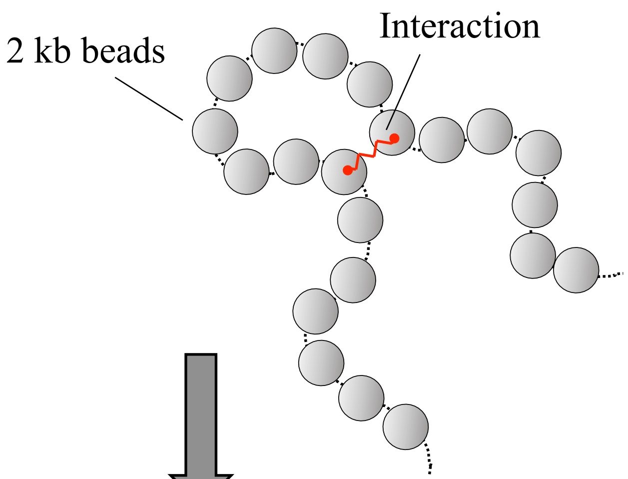
- No contacts in ~70% of the cells

- Overlap fraction < 0.1 in ~85% of the cells



Polymer modeling of the chromatin fiber

Self-avoiding and self-interacting polymer model of the region of interest



Optimize
interaction potentials

Simulate ensemble of
different configurations

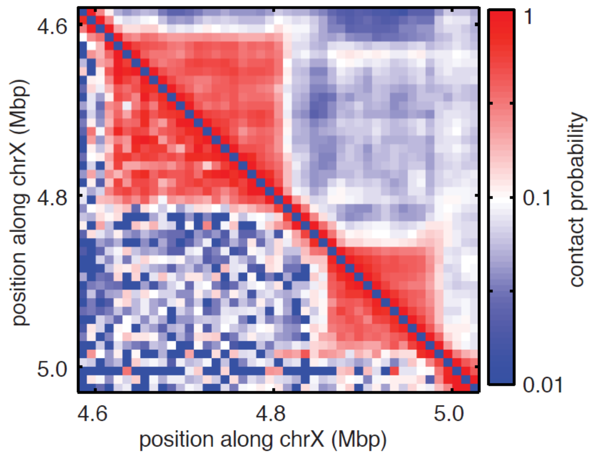
Simulated
contact probability
map

Compare with
experimental
Hi-C map

Adapted from Giorgetti *et al*, Cell 2014

Polymer modeling is consistent with the physical TAD-based chromatin compartmentalization

Simulated



Experimental

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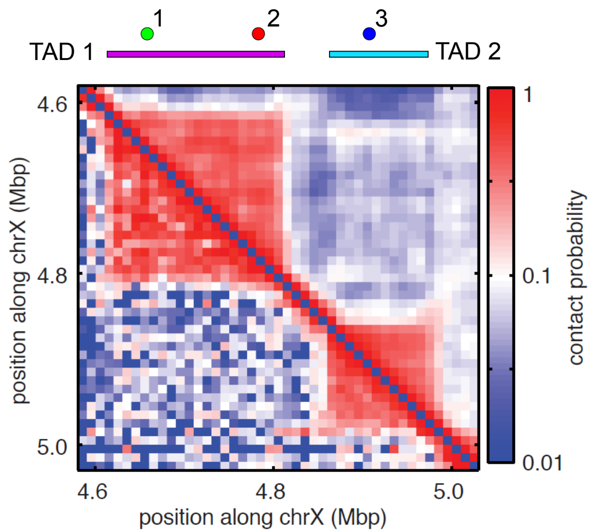
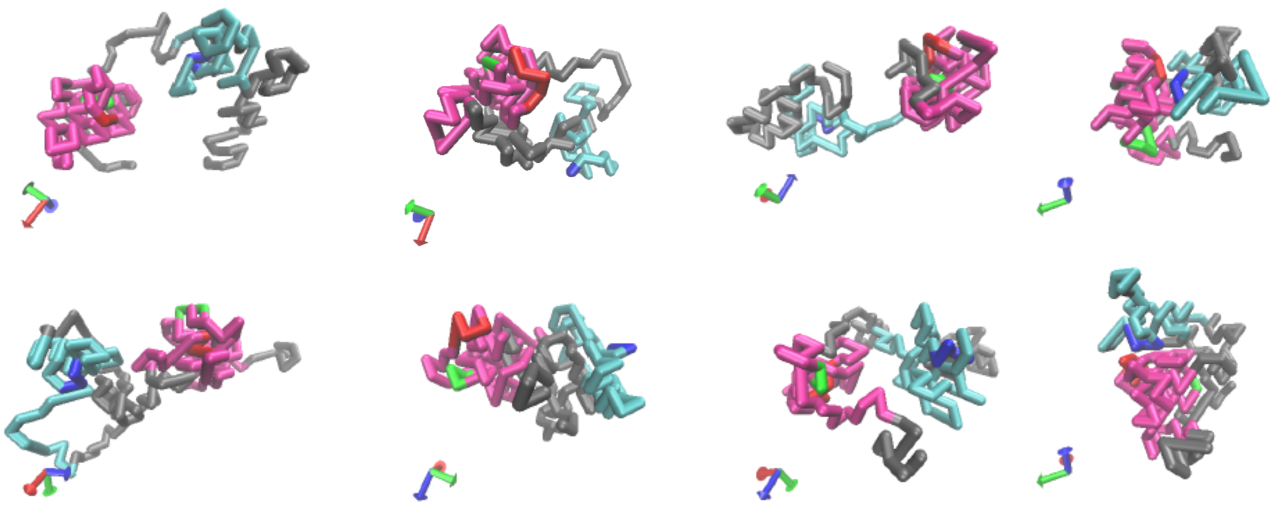
Polymer modeling is consistent with the physical TAD-based chromatin compartmentalization





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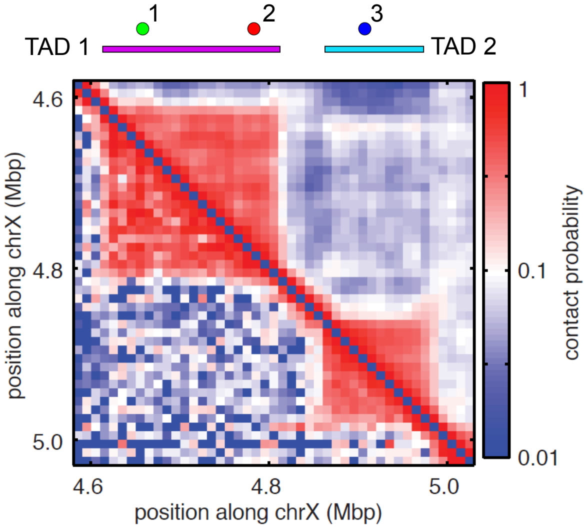

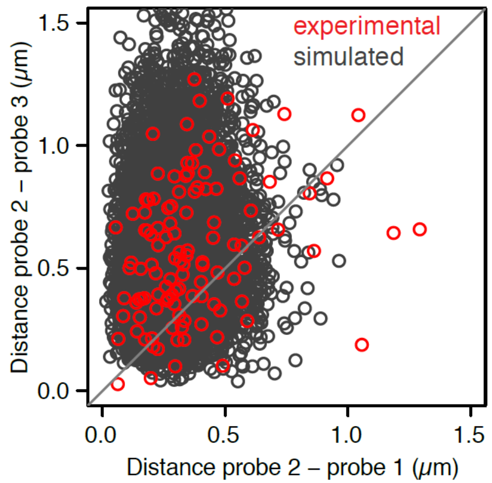

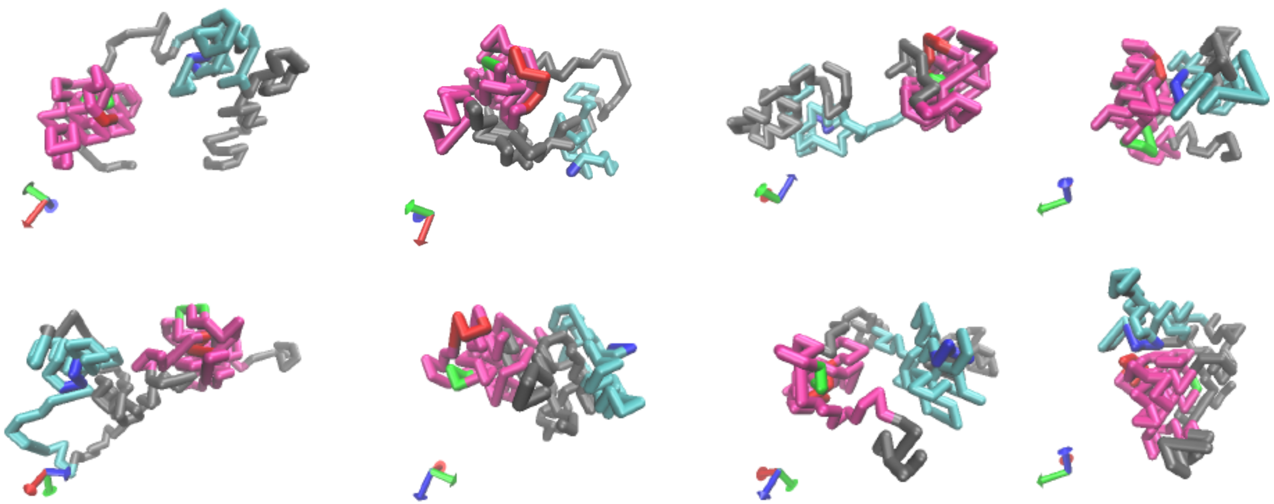

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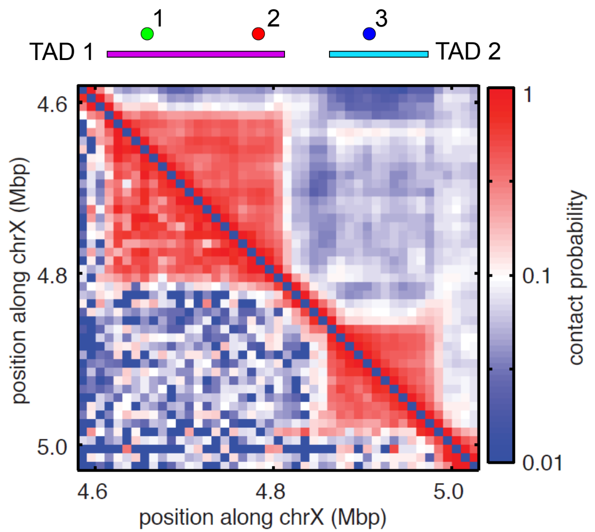
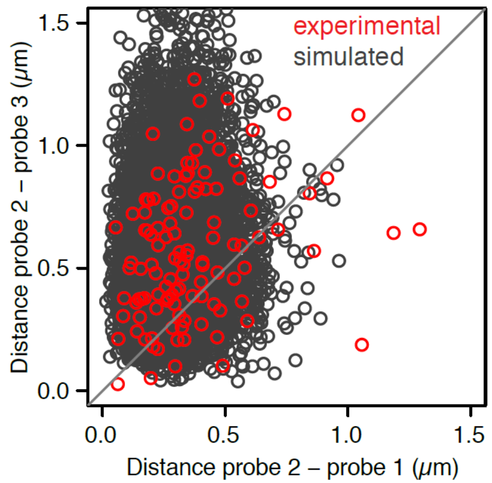
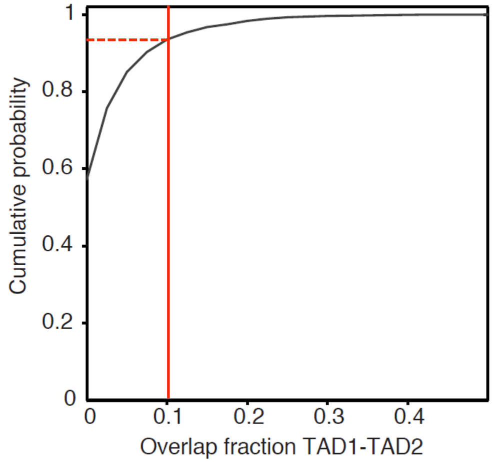
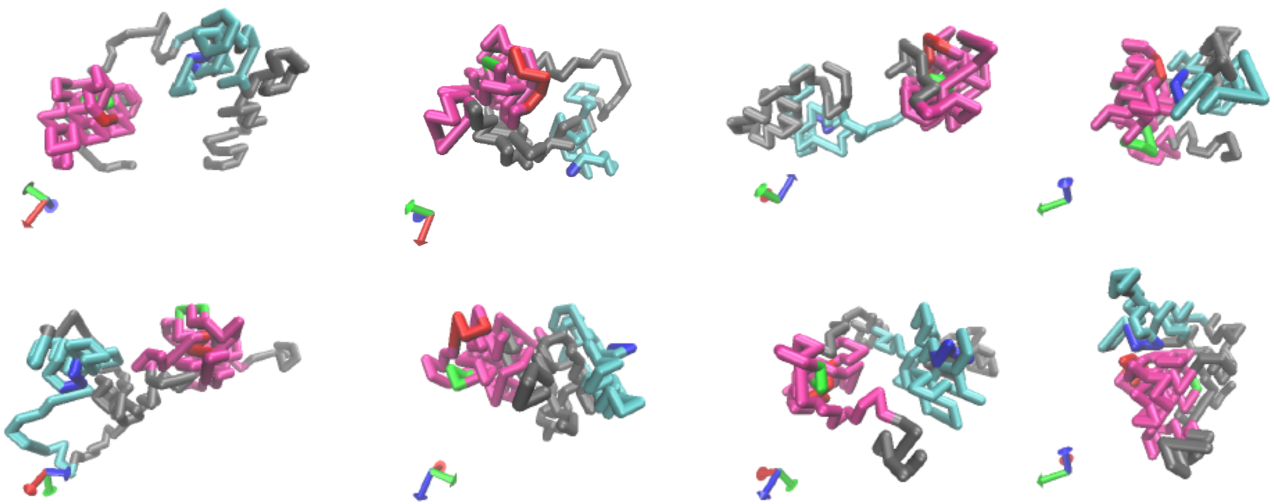
Polymer modeling is consistent with the physical TAD-based chromatin compartmentalization









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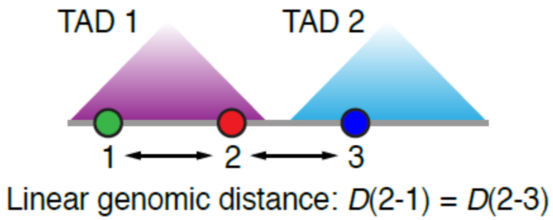
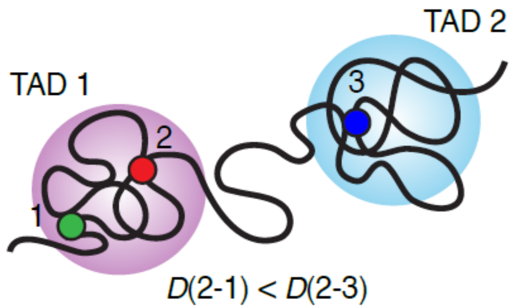
What about shorter *inter* versus *intra*-TAD distances?



75 %
of the cells



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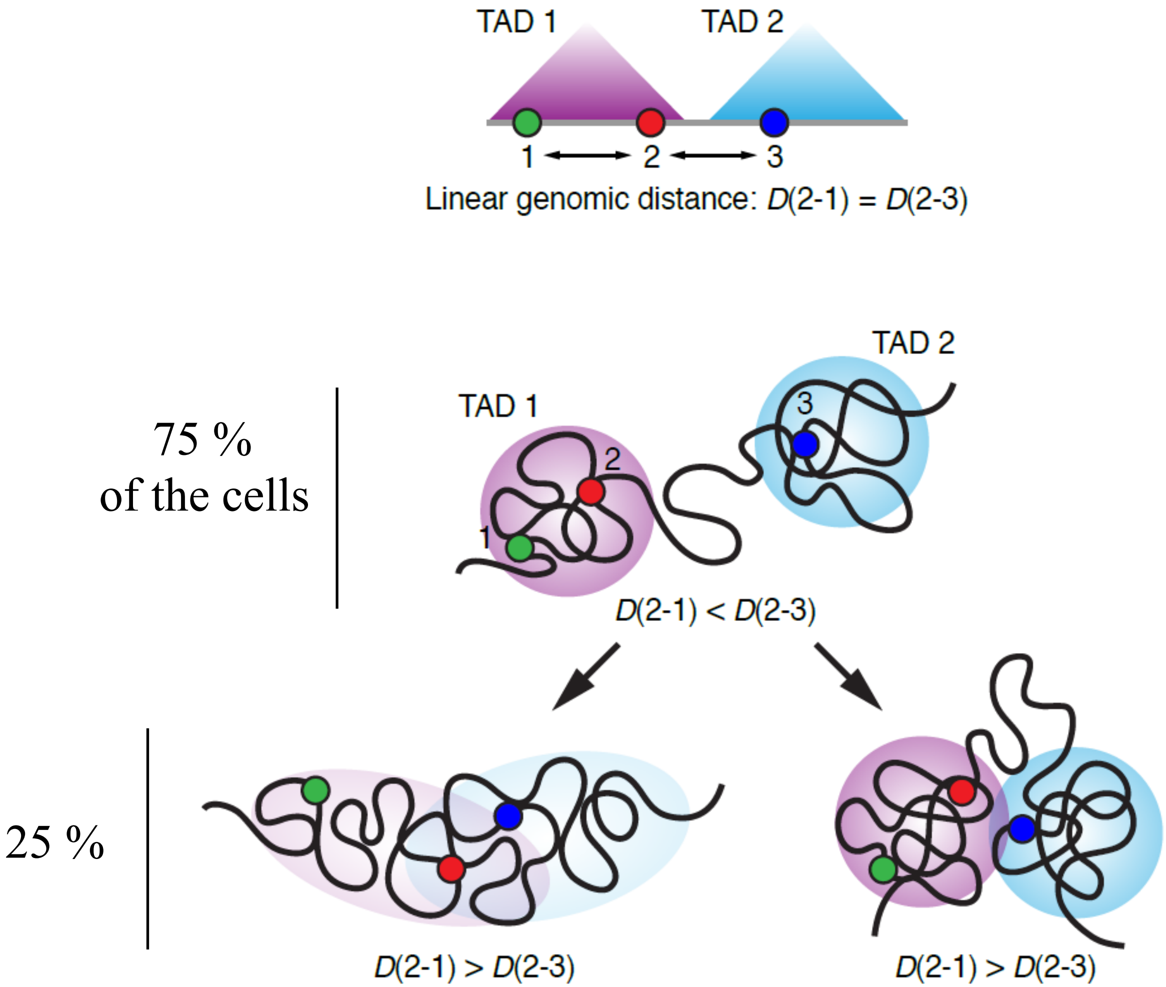
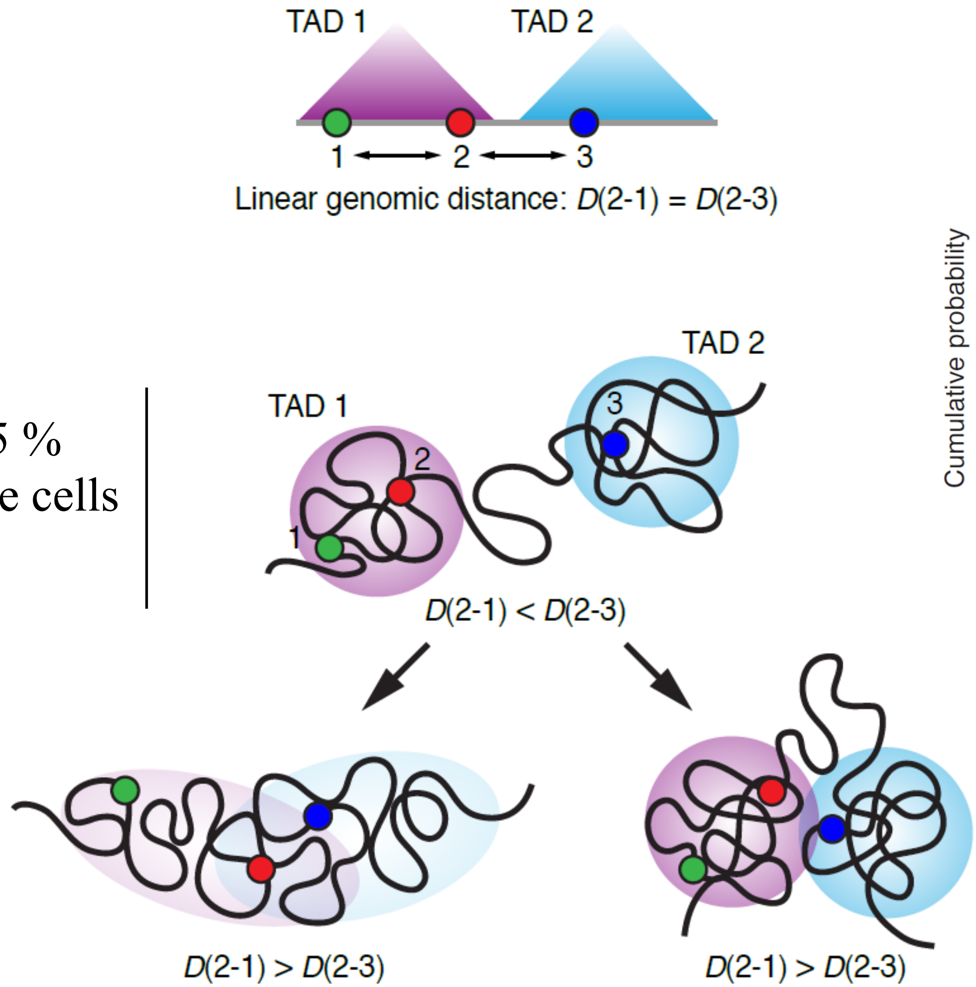
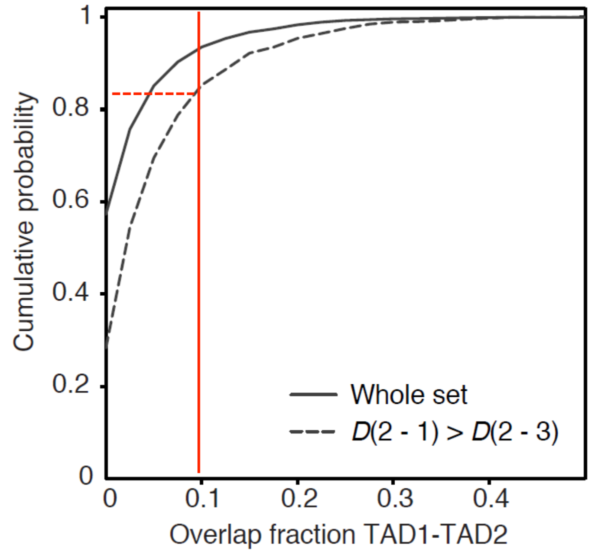


The relative TAD positioning can explain shorter *inter* versus *intra*-TAD distances

75 %
of the cells

25 %





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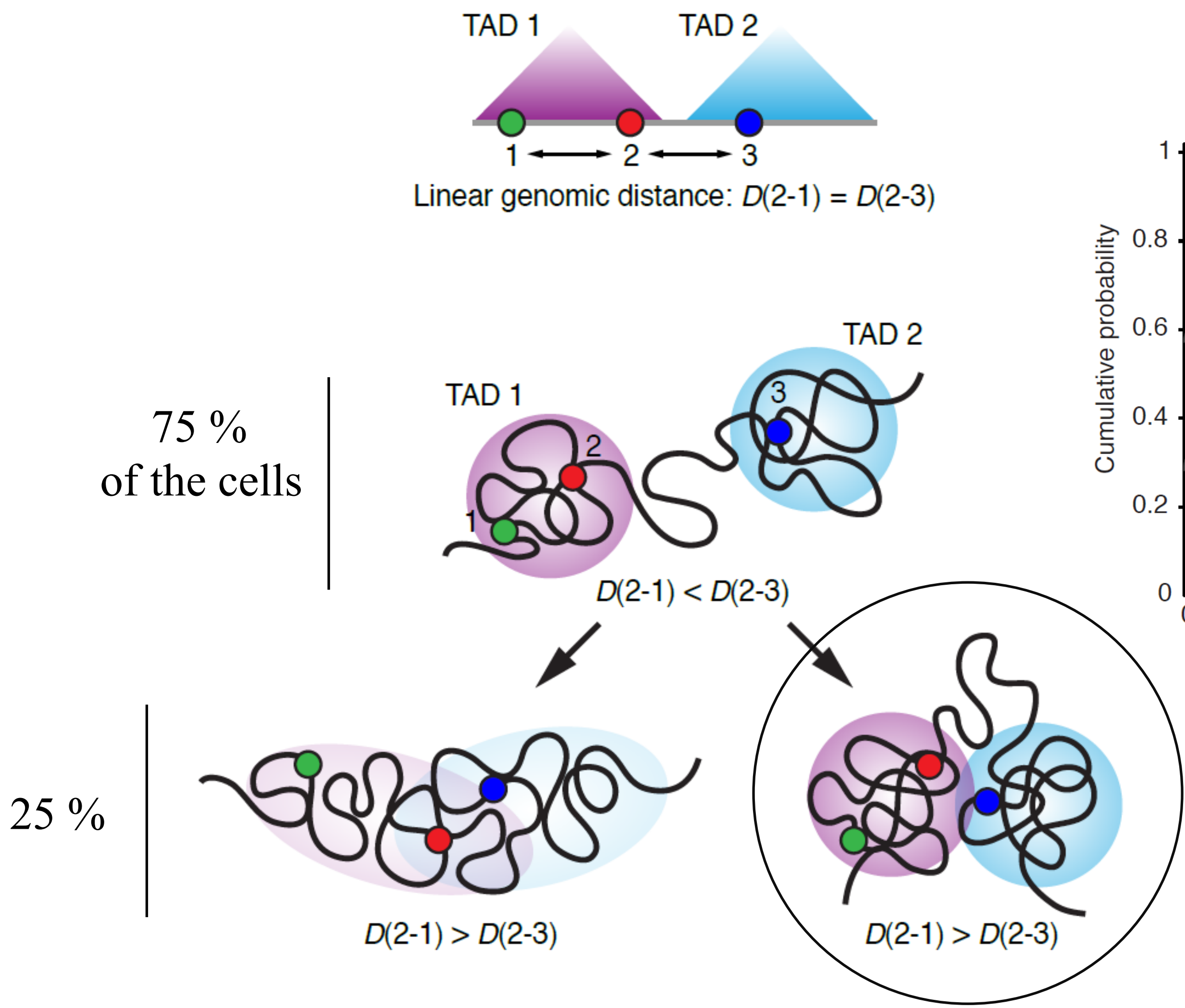
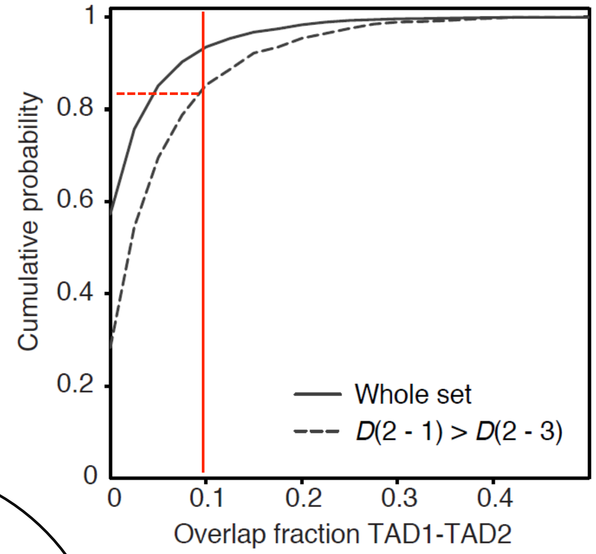
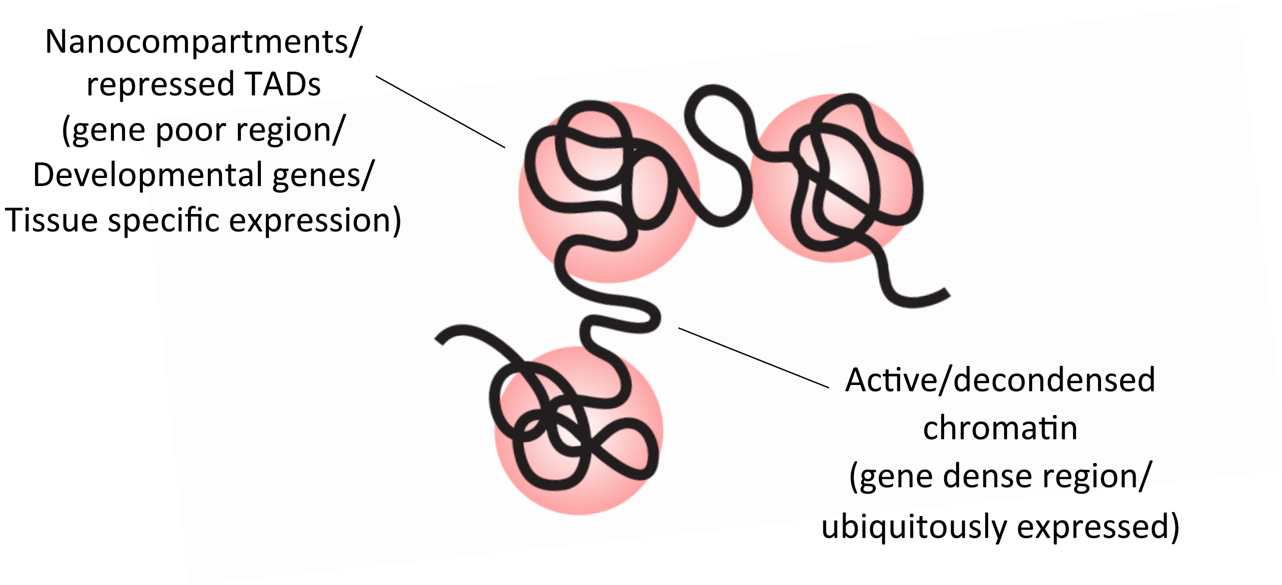




Organization of the chromatin fiber in *Drosophila* interphase nuclei



Szabo *et al.*, Science Advances 2018

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