Imaging nuclear organisation at the nanoscale

Is spatial chromosome organization random or heterogeneous?

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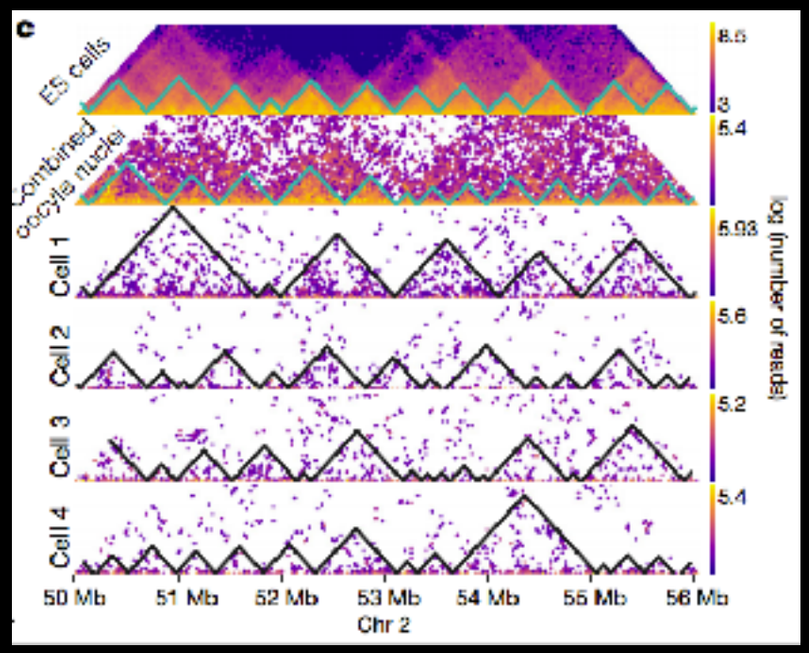
Organization of chromatin into TADs

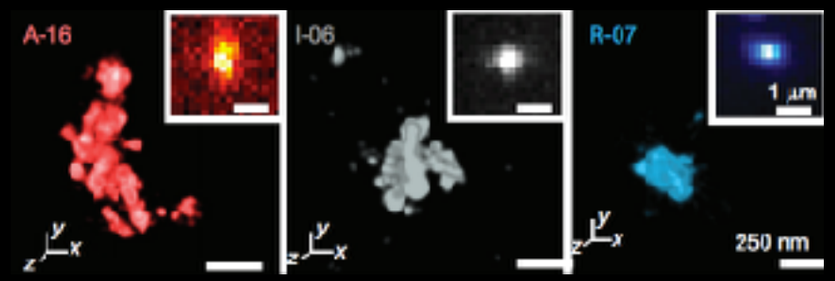
Single-cell Hi-C



Nagano, *Nature*, 2013
Flyamer, *Nature*, 2017
Stevens, *Nature*, 2017

TADs arise only after ensemble averaging?

Direct imaging of TADs



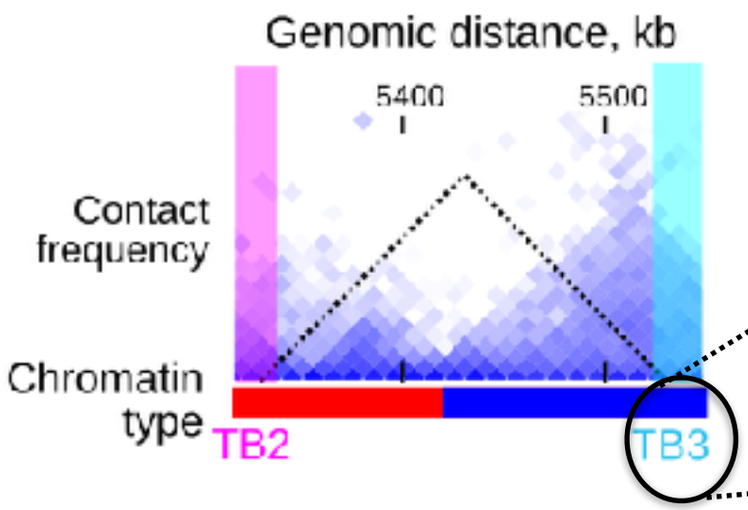
$$V^{TAD} \sim g^{b}$$

Boettiger, *Nature*, 2016
Fabre, *PNAS*, 2015
Szabo, Science Adv, 2018

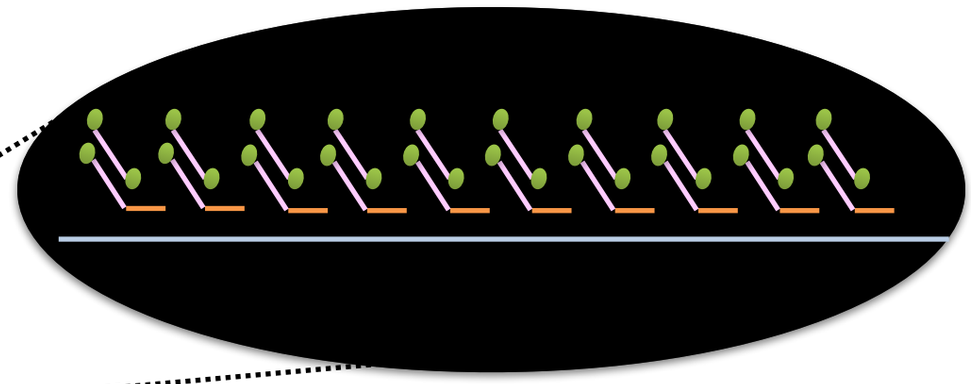
suggests that TADs exist in each cell

Develop methods to directly measure the absolute levels of heterogeneity to discern between these models

Organization of TAD borders one cell at a time



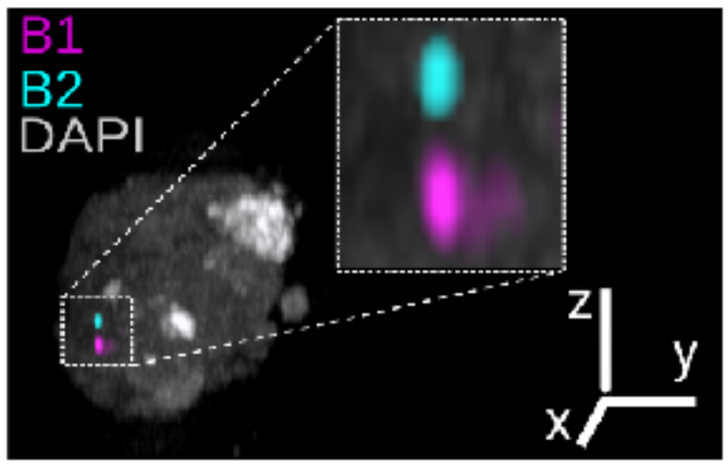
oligopaints



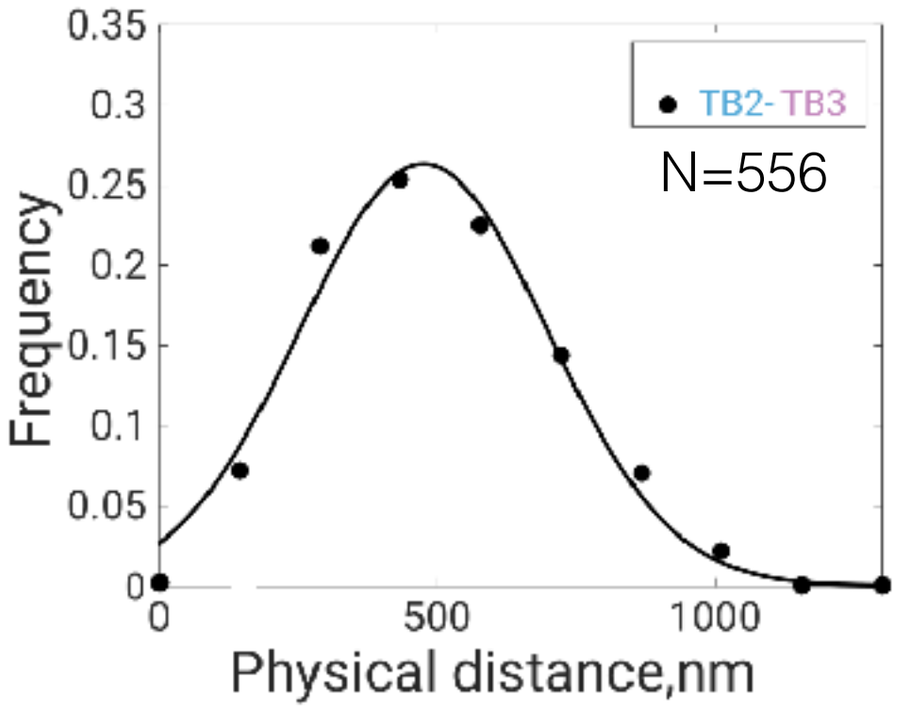
Beliveau et al., 2015

200-500 oligos/probe
~20 kb / probe

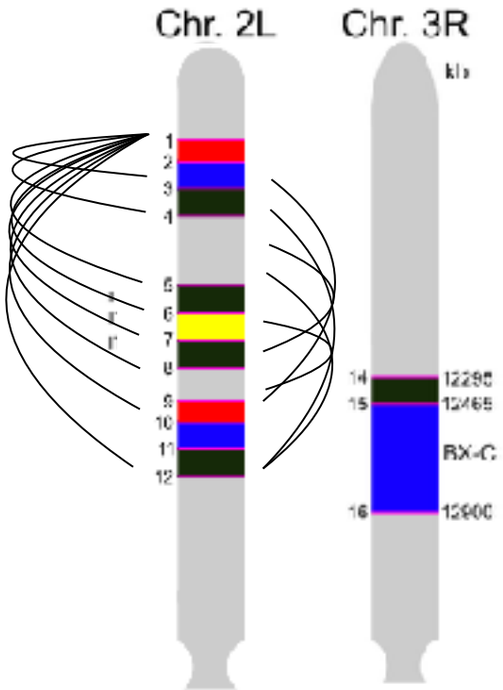
3D-SIM



Distribution of 3D physical distances



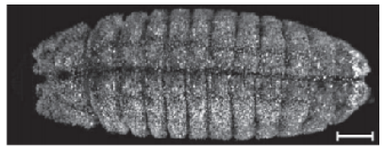
Measure distance distributions



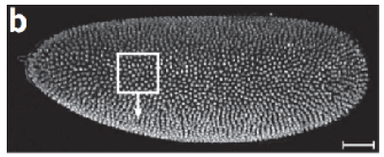
~30 loci combinations

2 chromosomes

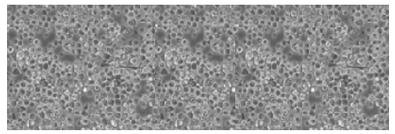
3 cell types



Late embryo

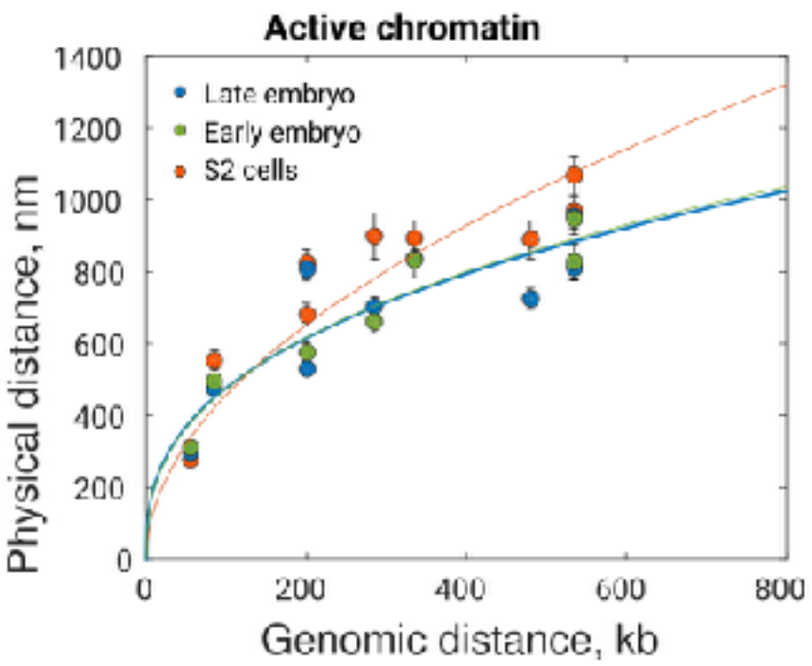


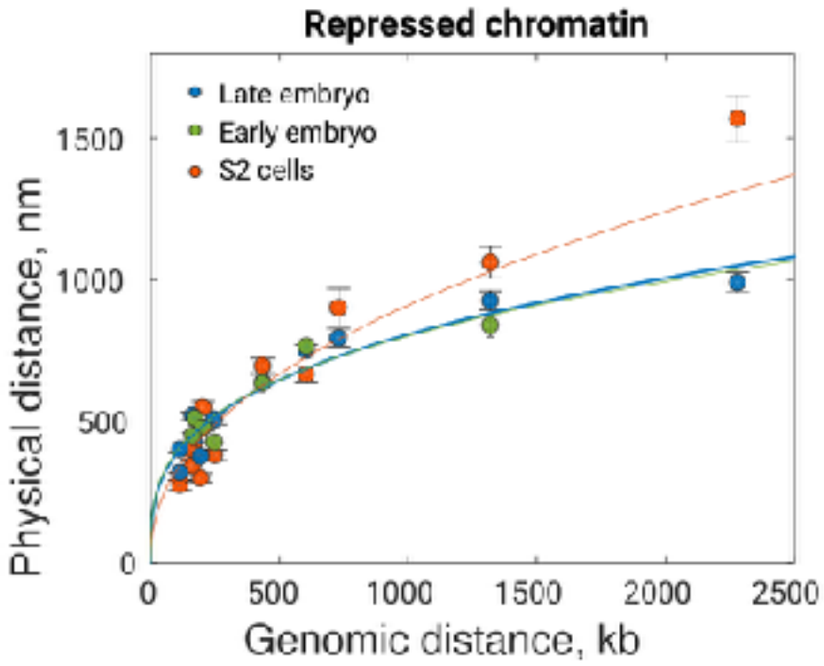
Early embryo



S2 cells

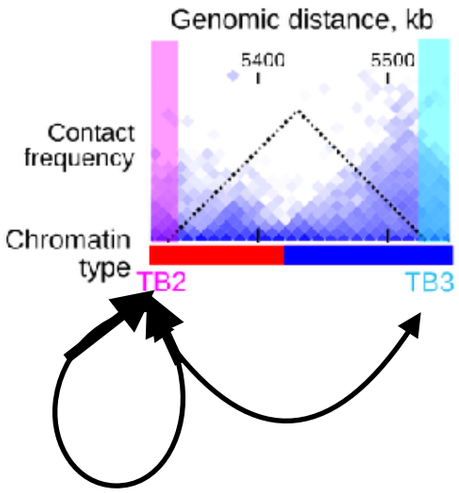
>100 distributions

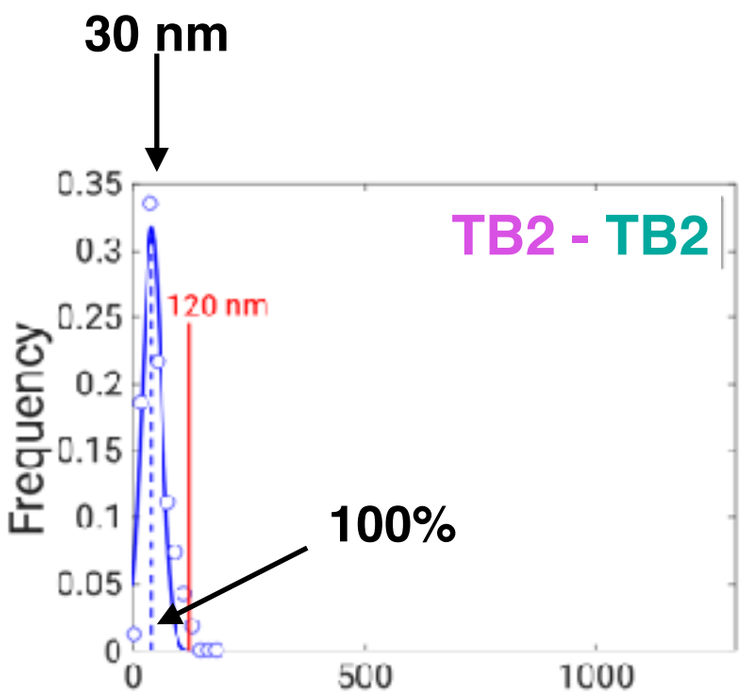


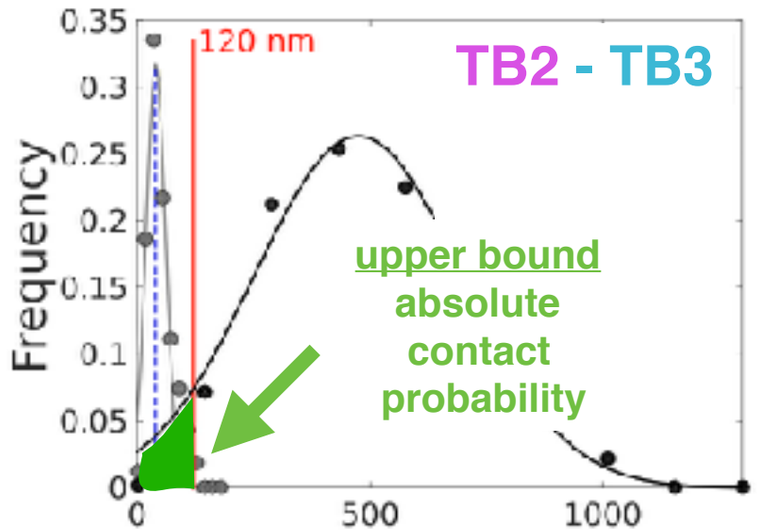


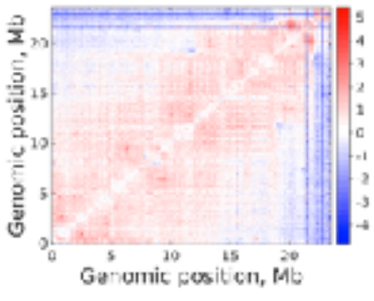
Boettiger, Nature, 2016

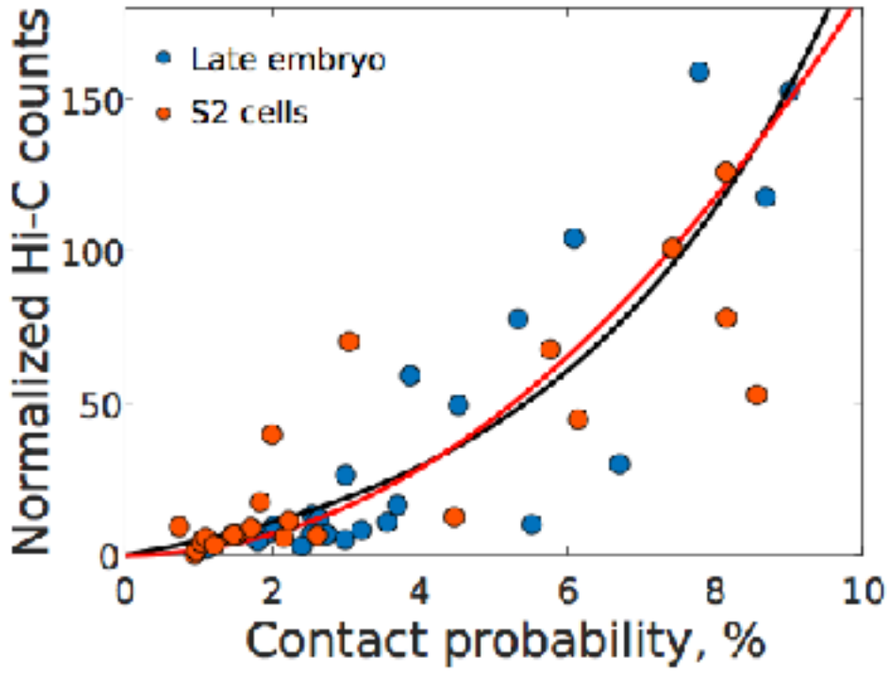
Measuring absolute contact frequencies in single cells









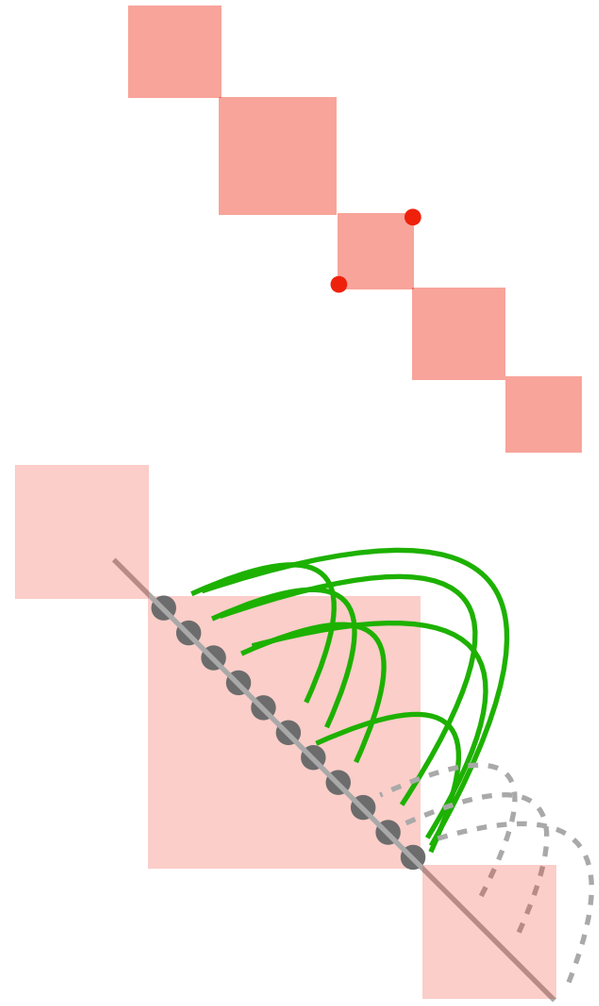


Normalised Hi-C counts
scale <u>non-linearly</u>
with
absolute contact
probabilities

Quantitative measurements of absolute contact probabilities

Do TAD borders loop in *Drosophila*?

Organization of TAD borders one cell at a time

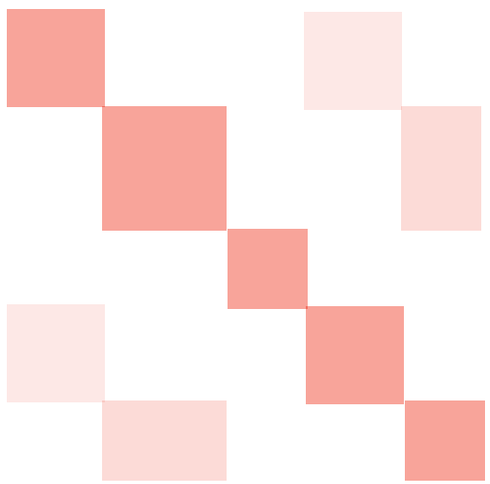


What makes a TAD?

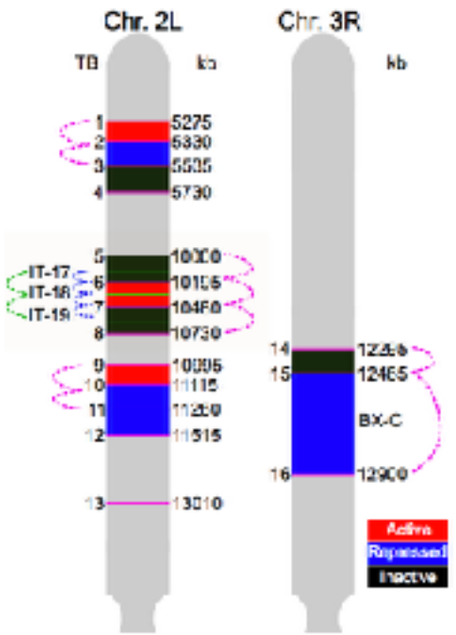
Measuring stochasticity within single TADs

How do TADs interact to form compartments?

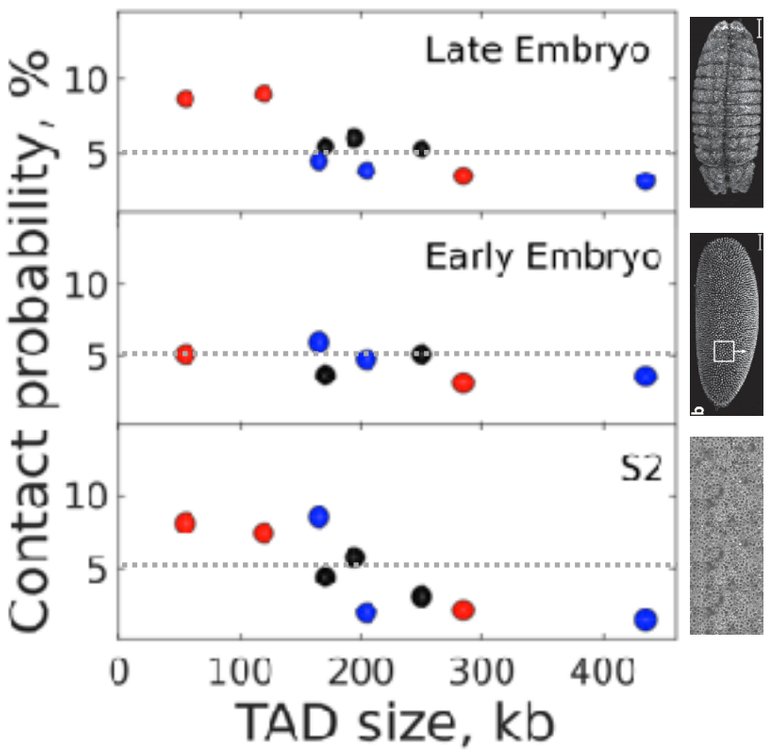
Epigenetic compartments super-resolved



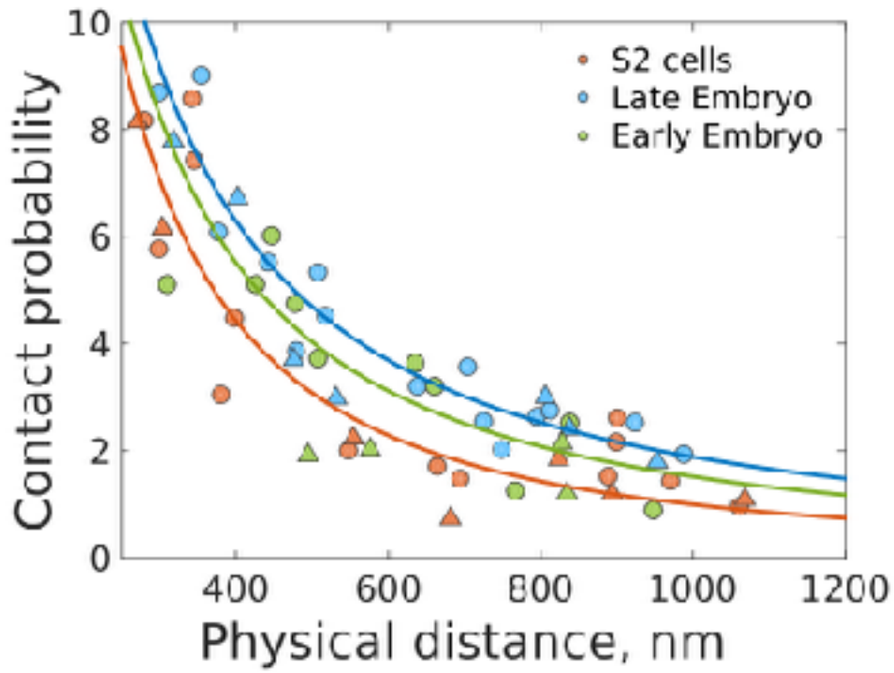
Probability of interaction between TAD borders



Consecutive borders



All borders

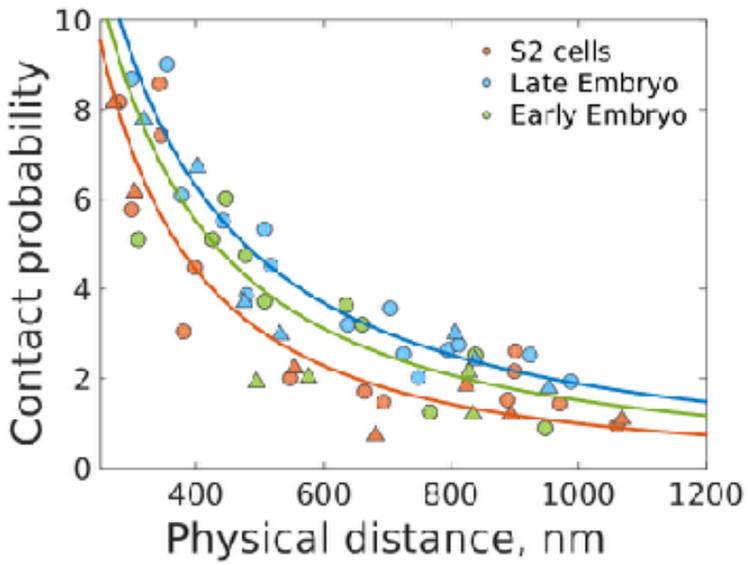


Absolute contact probabilities between consecutive TAD borders are small ~ 5%

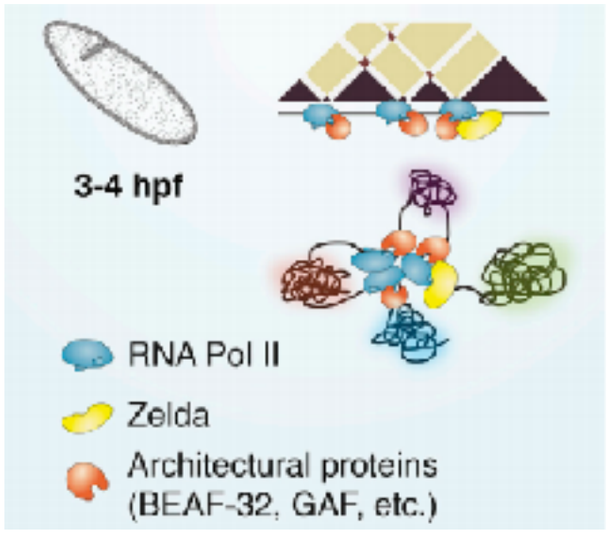
Contact probabilities are affected by genomic distance (power-law dependence)

Interactions between TAD borders in *Drosophila*

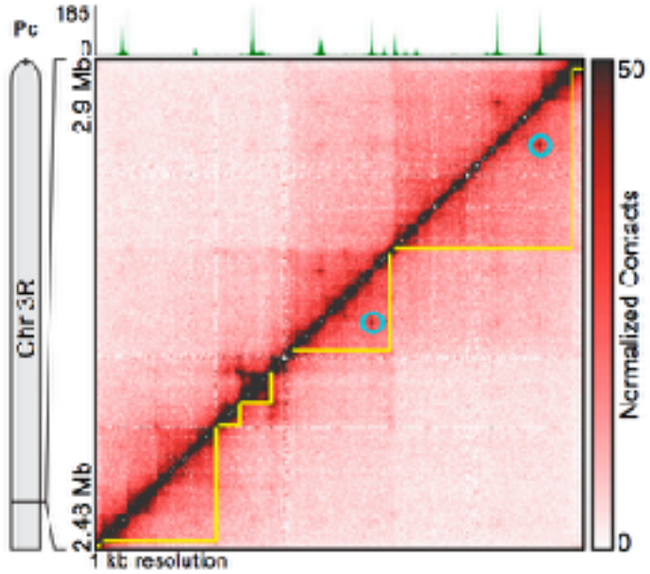
Single-cell



Hi-C



Hug, Cell, 2017



Eagen, PNAS, 2017

Our data shows very rare looping between TAD borders in *Drosophila* and suggests TAD borders act as barriers, not the bases of stable loops

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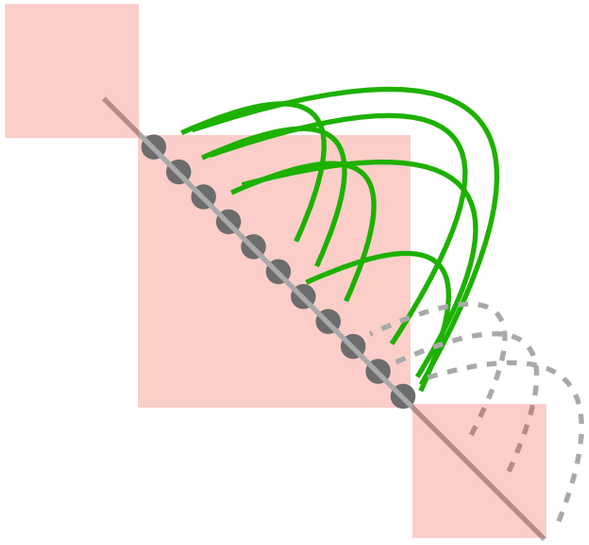
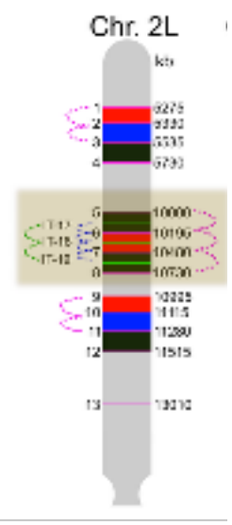
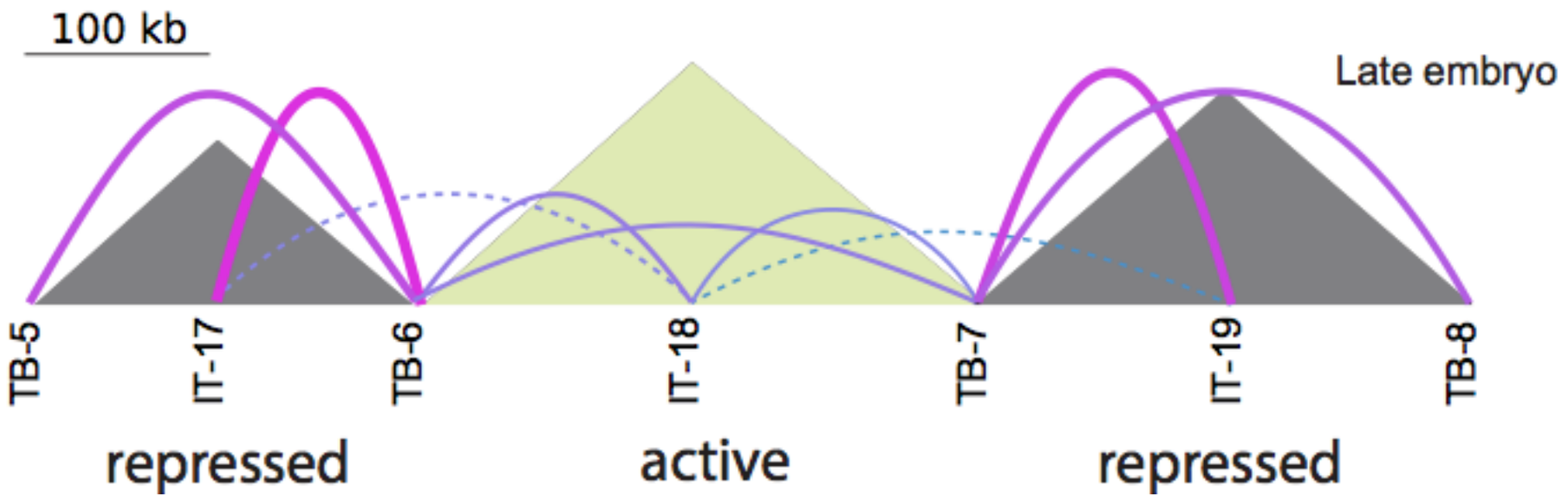


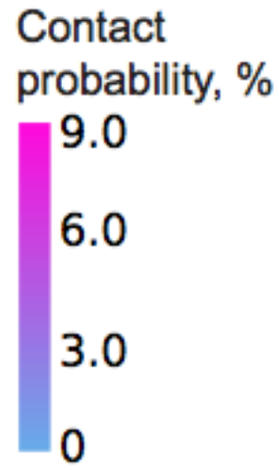
How do TADs interact to form compartments?

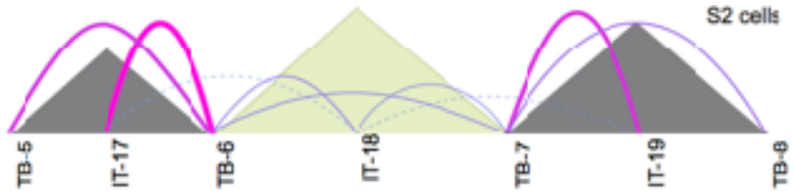
Epigenetic compartments super-resolved

Absolute contact frequencies within TADs









S2 cells/ early embryos

Contact probabilities remain small <10%

Contact probabilities are larger within TADs than between TADs

Null/repressed TADs show higher interaction frequencies than active TADs

Despite high heterogeneity, multiple, TAD-specifici contacts may be enough to account for TADs

Quantitative measurements of absolute contact probabilities

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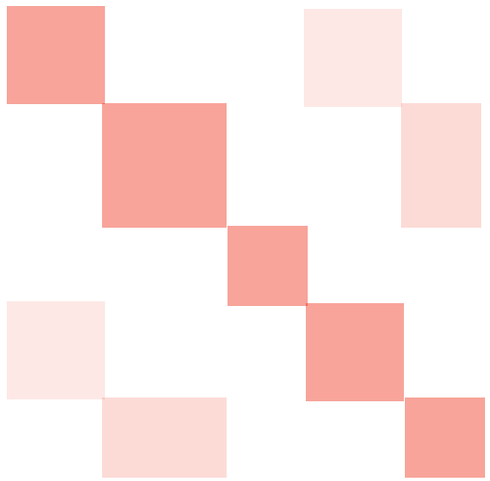
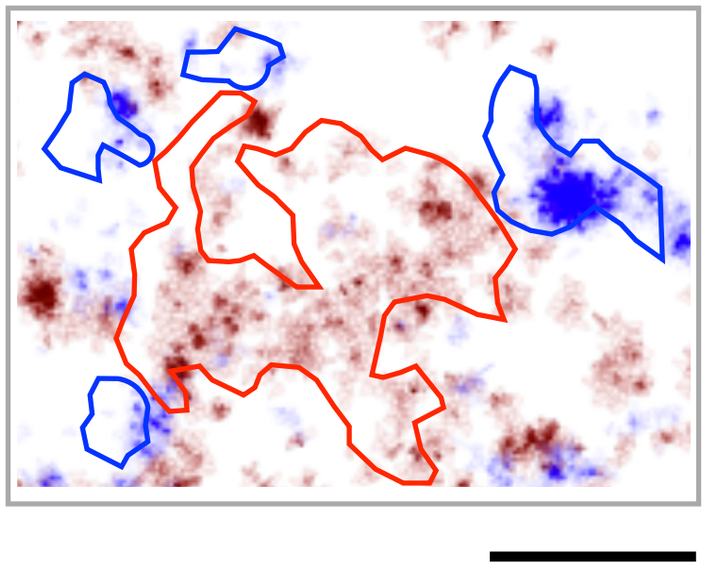


Active / repressed compartments visualized at super-resolution

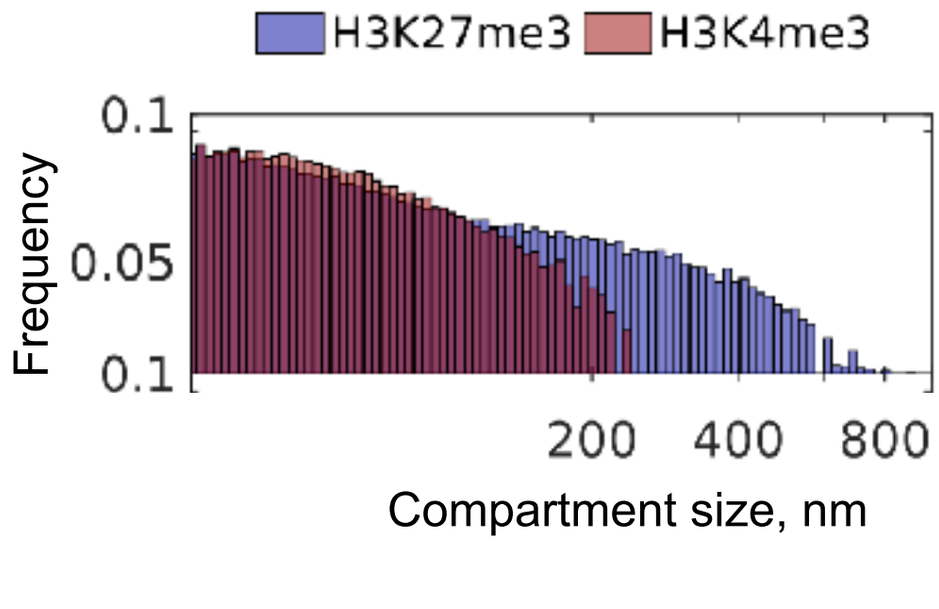
two colour dSTORM

Nanoscale segregation

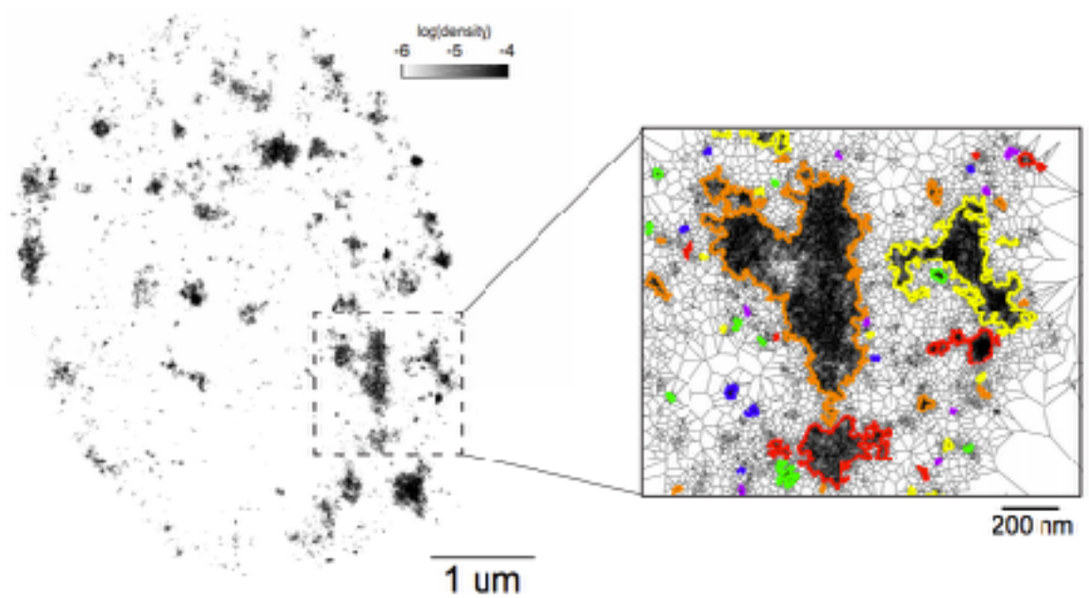
H3K27me3 H3K4me3



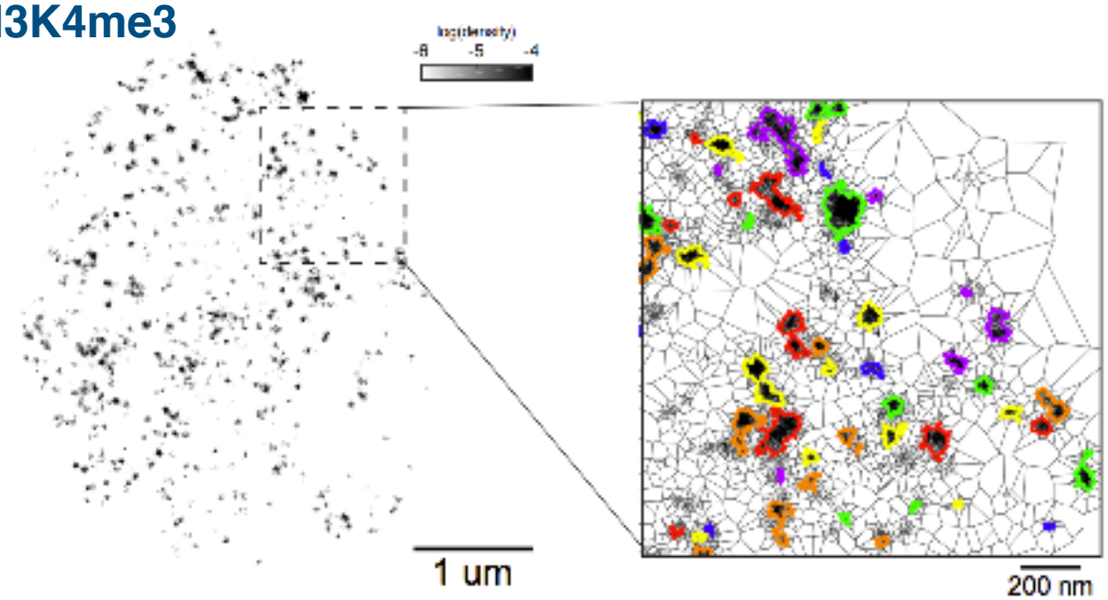
Domain sizes



H3K27me3

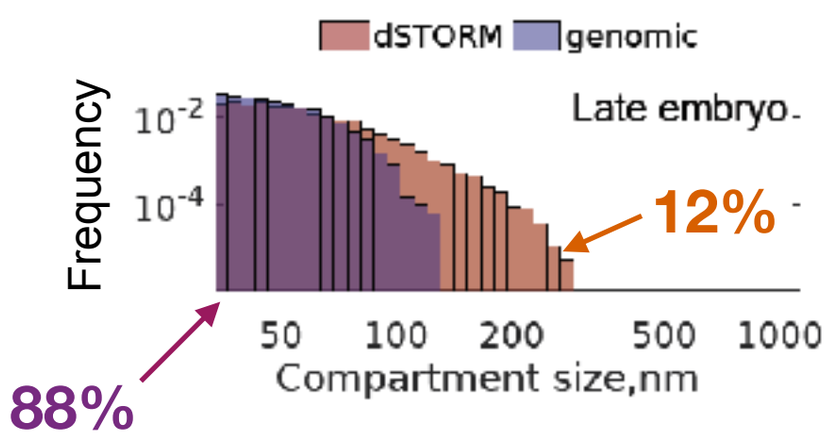


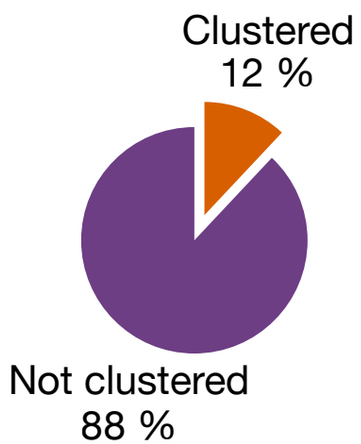
H3K4me3

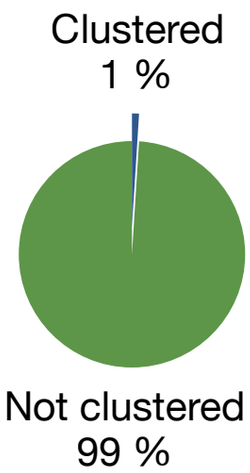


Probability of TAD clustering ~ 10%

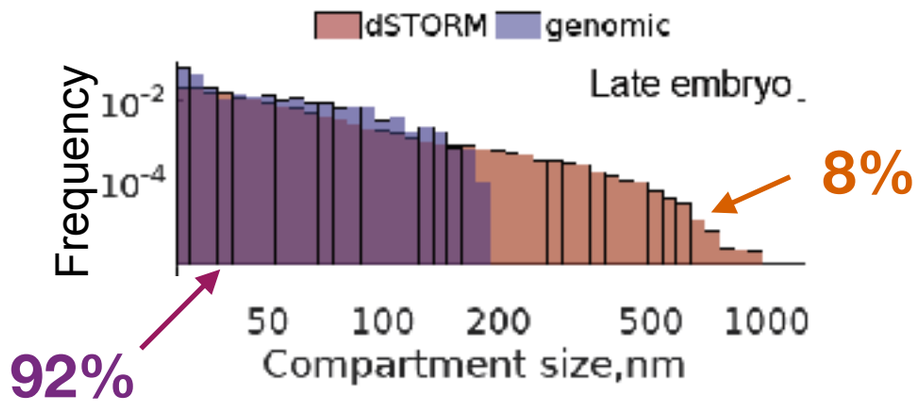
Active chromatin

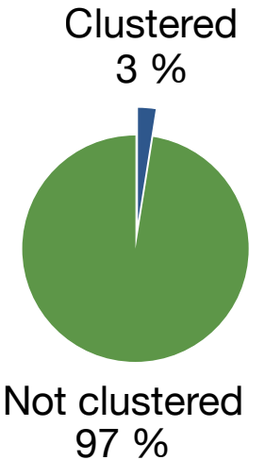






Repressed chromatin



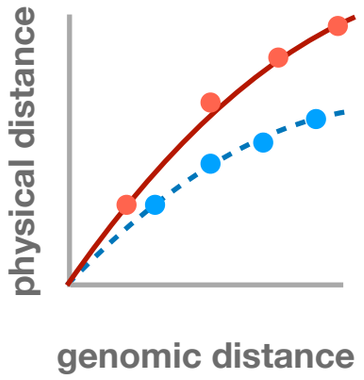


epigenetic domains exist at the single-cell level

clustering between domains of the same type exists but is rare and depends on cell type.

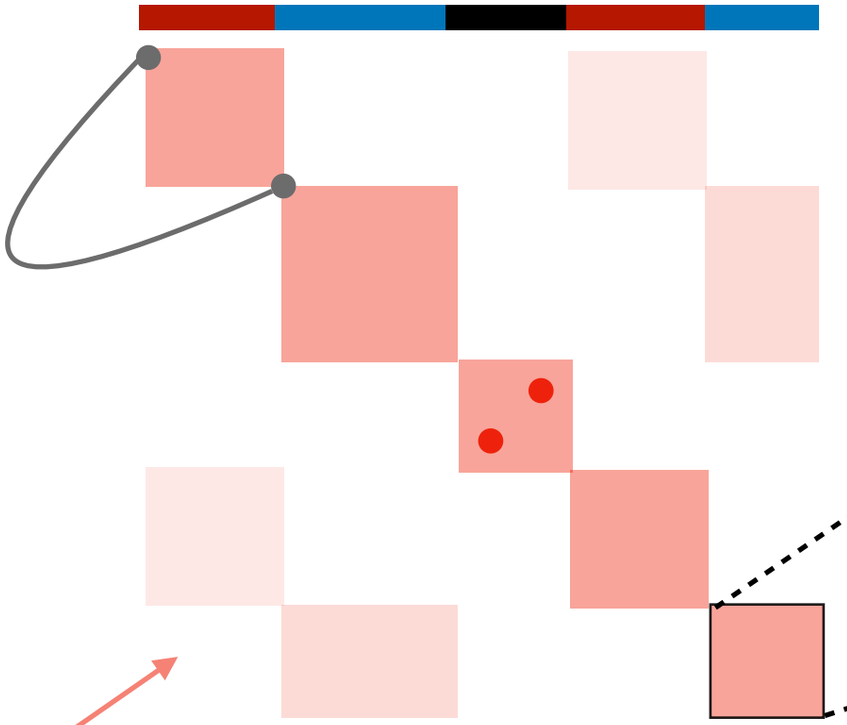
Randomness versus heterogeneity

Multiple, low-frequency yet specific interactions may be sufficient to organise chromatin at different scales

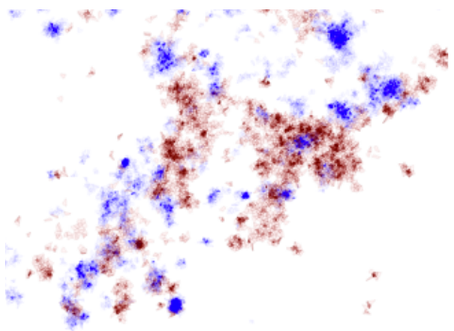


physical properties chromatin fiber

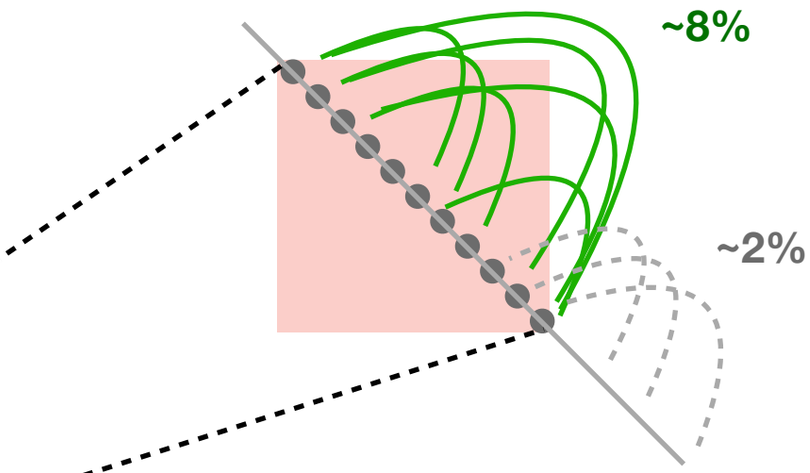
General principles of chromosome organization?



Direct visualisation of epigenetic domains,
[clustering is rare]



Internal TAD contacts are specific



Cattoni*, Cardozo-Gizzi*, Georgieva*, **Nat. Comm. (2017)**

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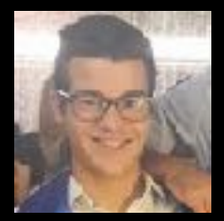

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