Visualizing chromosome organization and transcription in single cells

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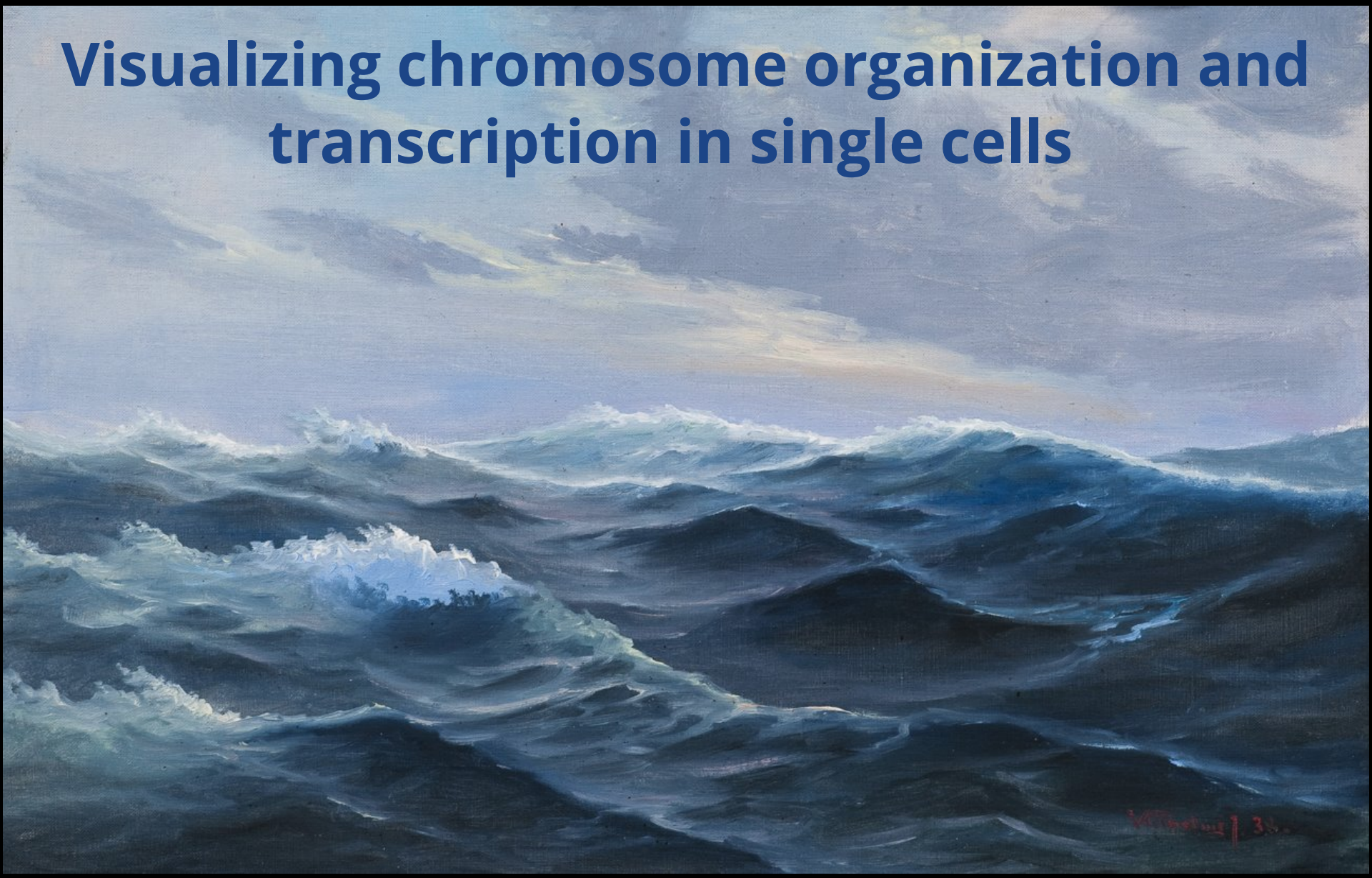
Visualizing chromosome organization and transcription in single cells



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for talk feedback!

Scan
to discover !

https://tinyurl.com/y4ydcc8v

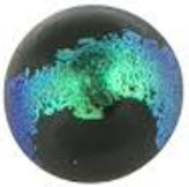
Chromosomes and DNA

DNA

~1 m crystallographic length

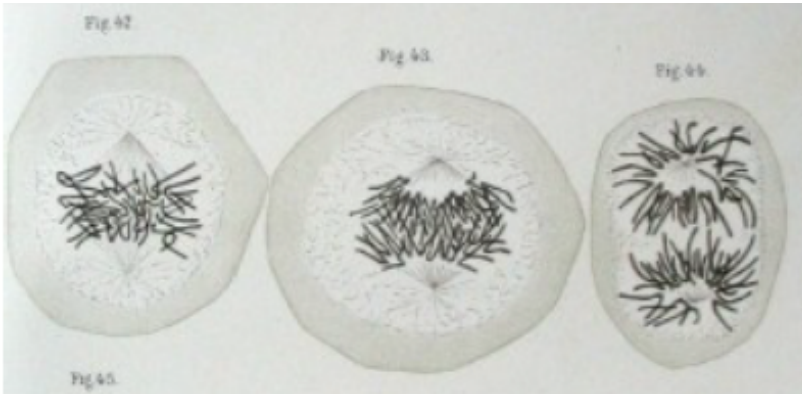
2 km

5 um nucleus



1 cm

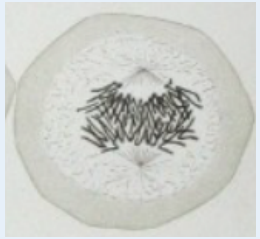
Walther Flemming



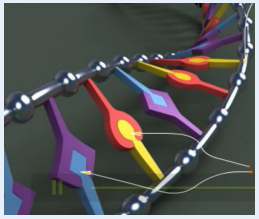
Chromosomes in mitotic cells, c.a. 1885

Multiscale chromosome organization

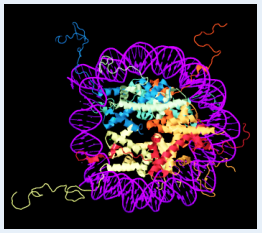
Mitotic chromosomes
Flemming, 1885



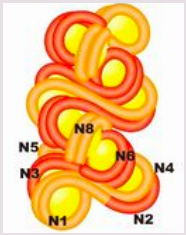
B-DNA
Watson & Crick, 1953
Franklin & Gosling, 1953



Nucleosomes
Luger, 1997



Chromatin fibers, loops, etc



Chromosome Territories
Bolzer, 2005

size

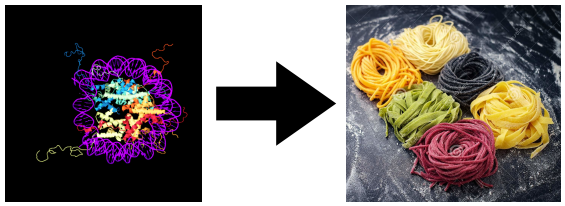
2nm

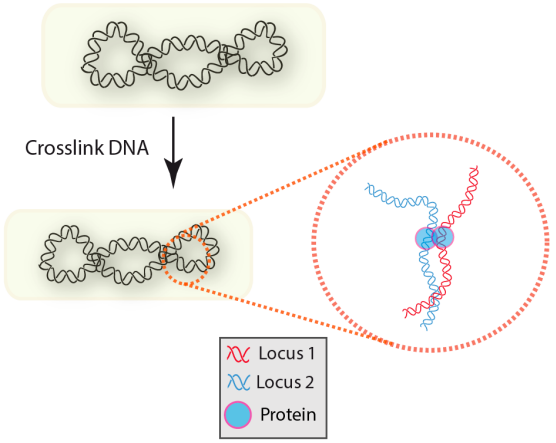
~10nm, 180bp

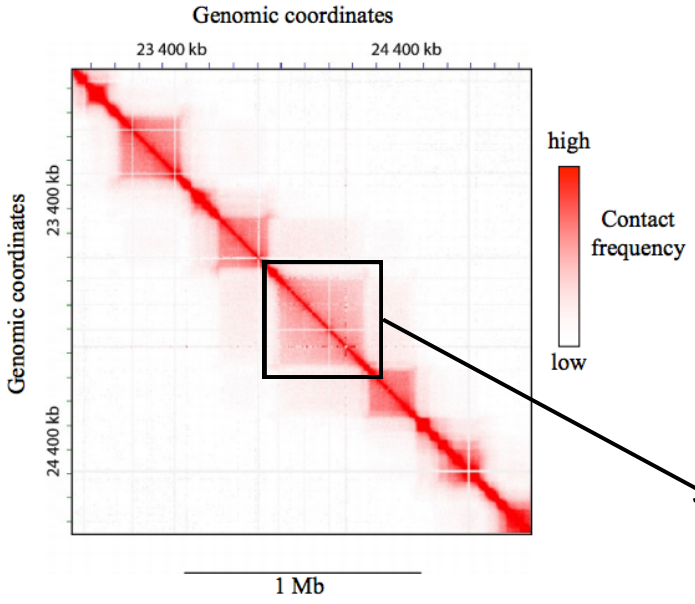
~200nm, >100kb

~1um, >10Mbp

Sequencing-based technologies







High-throughput sequencing

GAAGTGCGGTTCAACACCCTCCG

chromosome conformation capture

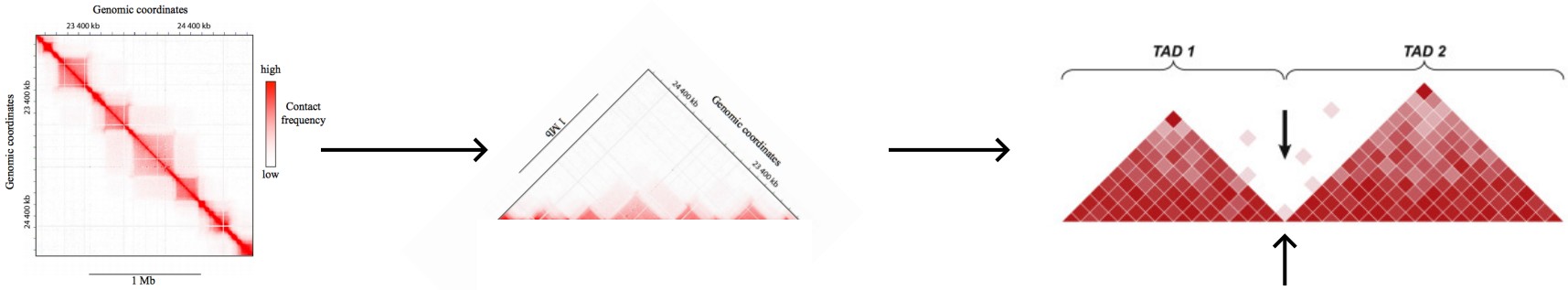
(3C-seq, **Hi-C**, etc)

TAD: Topological Associating Domains

(Sexton, 2012, Dixon, 2012; Nora, 2012)

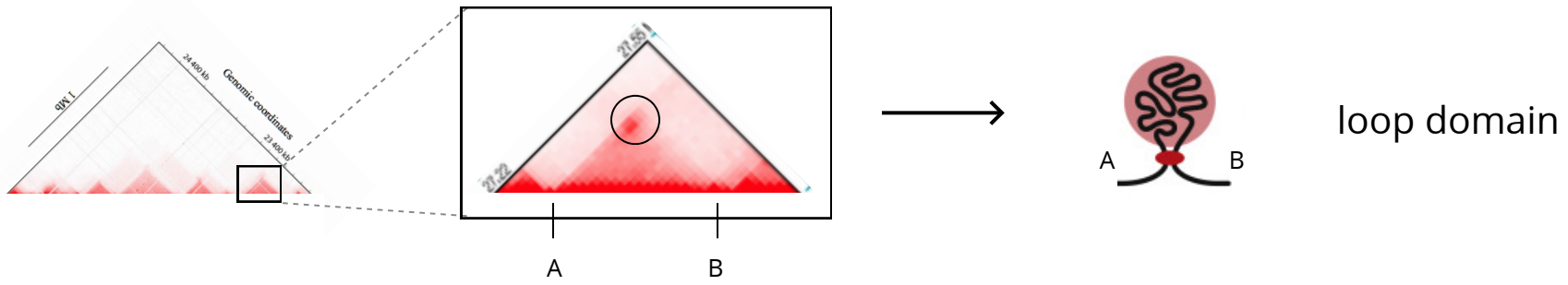
TADs: structural features

Domain borders



housekeeping genes
RNApol2
H3K4me1
chromatin insulators (e.g. CTCF)

Border Loop



loop domain

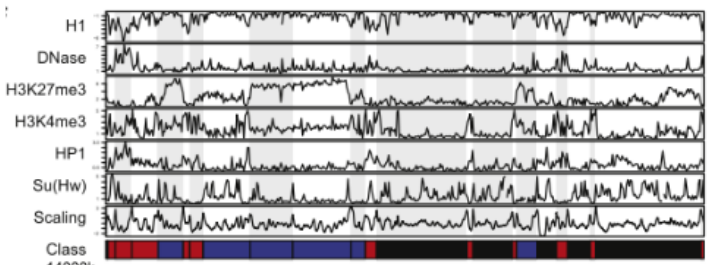
Rao (2015)

epigenetic domains and TADs

NGS: chromatin immuno-precipitation (ChIP)

epigenetic domains

Filion (2011)



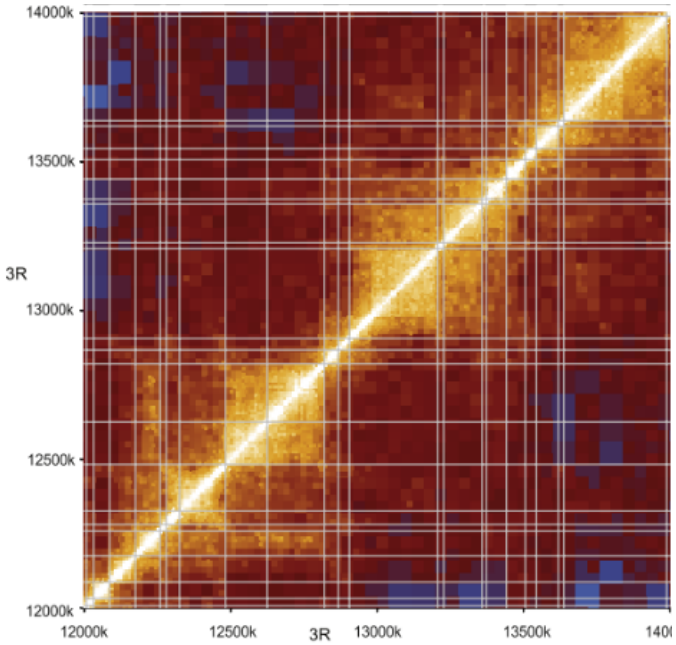
Active: RNA polymerase

Polycomb: H3K27me3

Active **Polycomb** **Void**

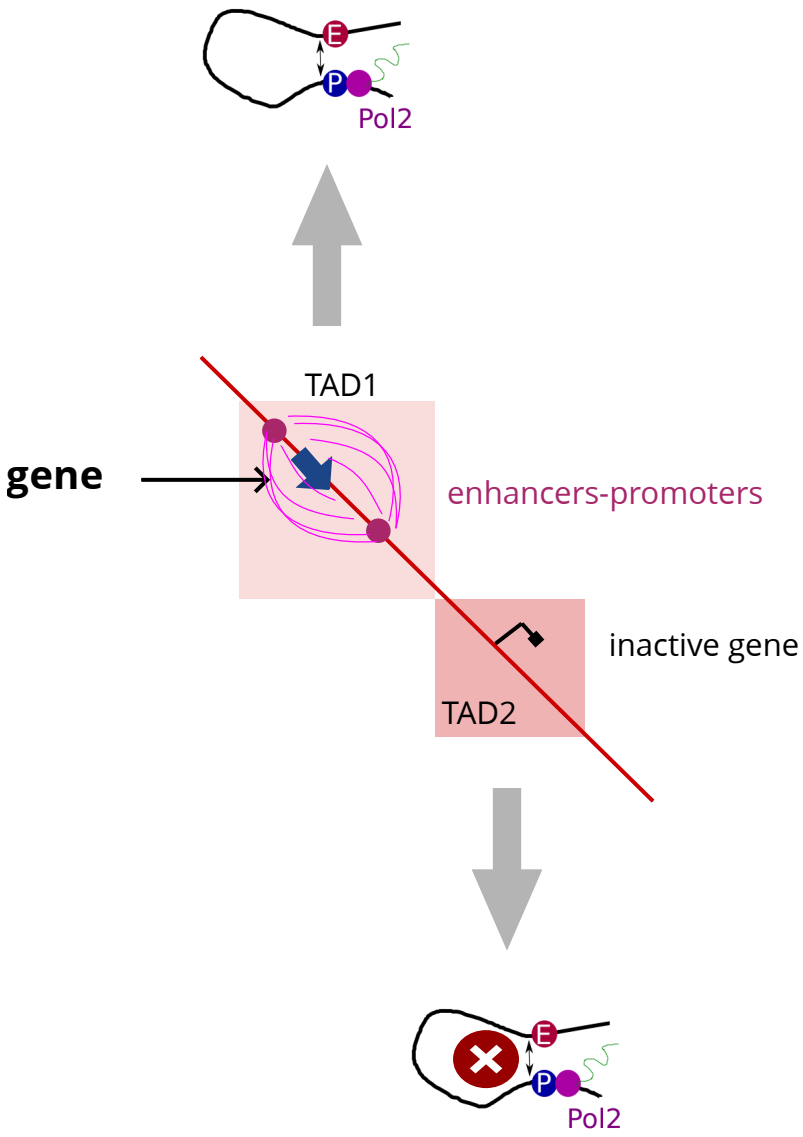
NGS: Hi-C

TADs



(**Sexton, 2012**; Dixon, 2012; Nora, 2012)

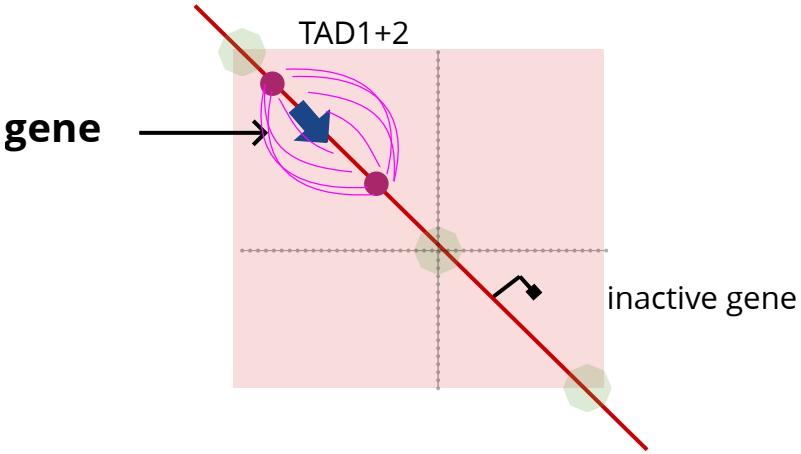
Do TADs have a functional role?



- TADs are conserved among species (Dixon, 2012)
- Internal structure of TADs changes between cell types (Phillips-Cremins, 2013)
- removal of border leads to ectopic expression and developmental defects and to cancers (Lupiañez, 2015; Norhcott, 2014)

TADs are functional units of chromosome organization

Genetic manipulation: are TADs correlated to function **?**



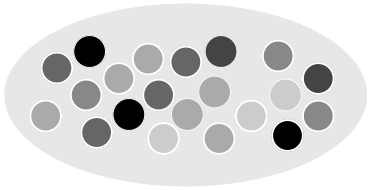
- Loss of TAD insulation lead to small effect in global transcription (Nora, 2017; Schwarzer, 2017; Rao, 2017; Despang, 2017).
- Fusion of TADs may not change gene expression (Rodriguez-Carballo, 2019; Williamson, 2019).
- Large TAD rearrangements lead to small changes in gene expression (Ghavi-Helm, 2019).
- Enhancers can communicate across TAD boundaries (Galupa, 2020)

Do TADs play major roles in transcriptional regulation ?

Does TAD structure change upon transcriptional activation?

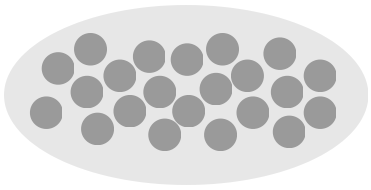
Ensemble sequencing technologies: Hi-C, Chip-seq, RNAseq...

single-cell

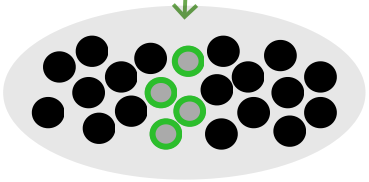


cannot dissect heterogeneity

ensemble

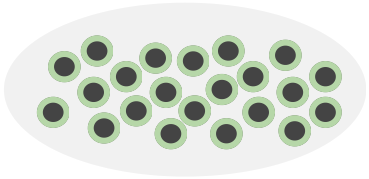


expressing



do not maintain spatial information

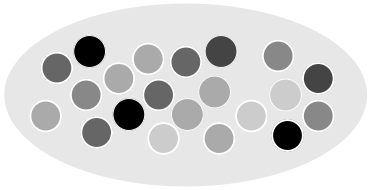
wild-type



It is hard to correlate 3D structure and transcriptional state !

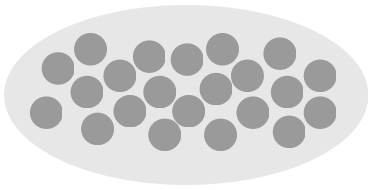
Ensemble sequencing technologies: Hi-C, Chip-seq, RNAseq...

single-cell



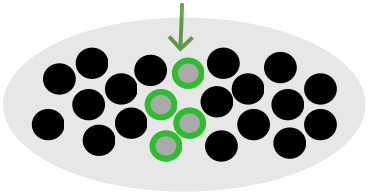
cannot dissect heterogeneity

ensemble



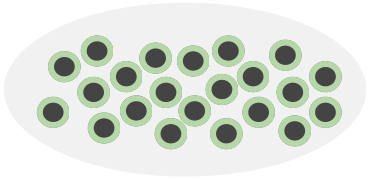
expressing

wild-type

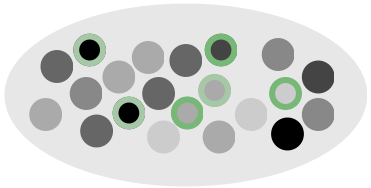


do not maintain spatial information

wild-type

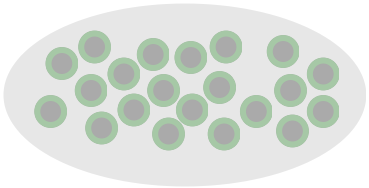


mutant



cannot correlate 3D structure and transcriptional state !

mutant



visualize TADs and transcription simultaneously in single cells?

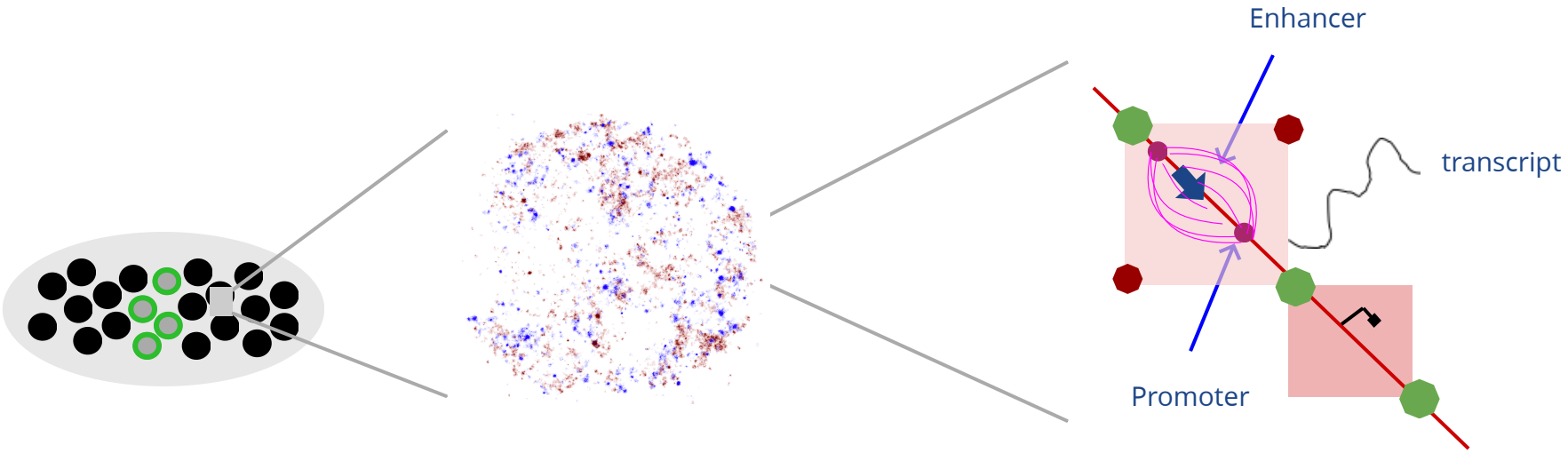


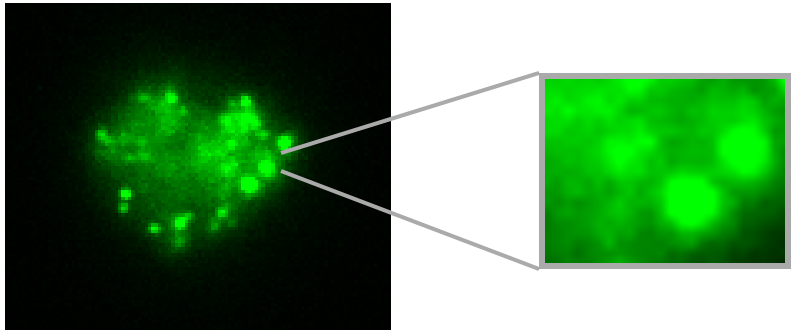
image **single-cells** with spatial resolution

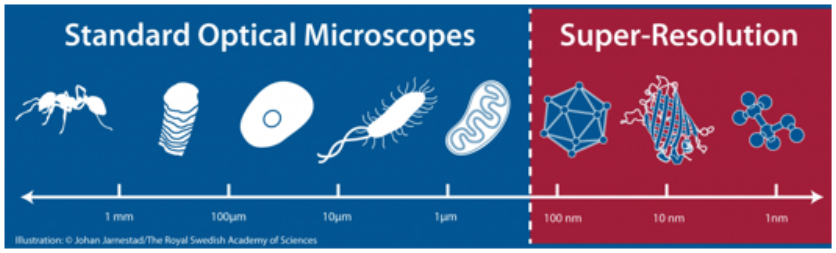
resolve **chromosome structure**

detect **transcription!**

Seeing is believing
The truth about spatial resolution

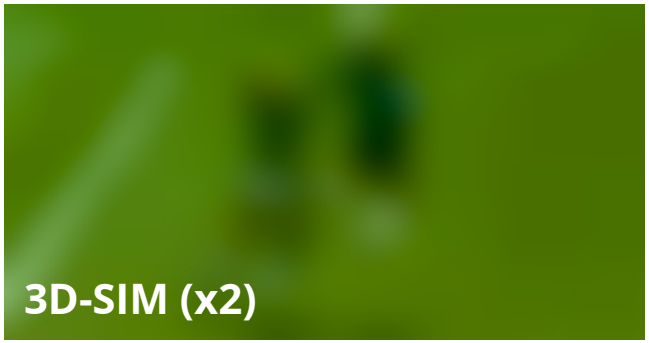
Repressive domains (H3K27me3)





Betzig, Zhuang, Gustafson Labs

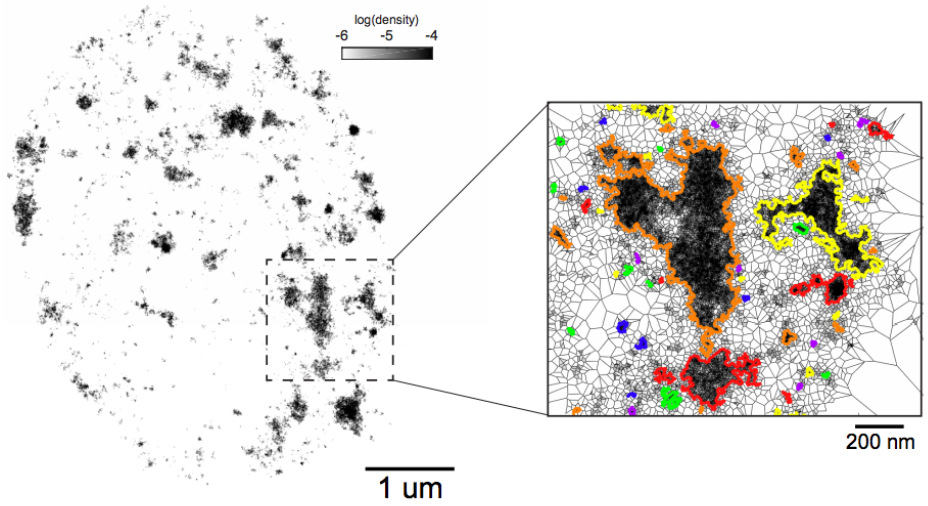
3D-SIM --> 2x resolution
STORM/PALM --> 10x resolution



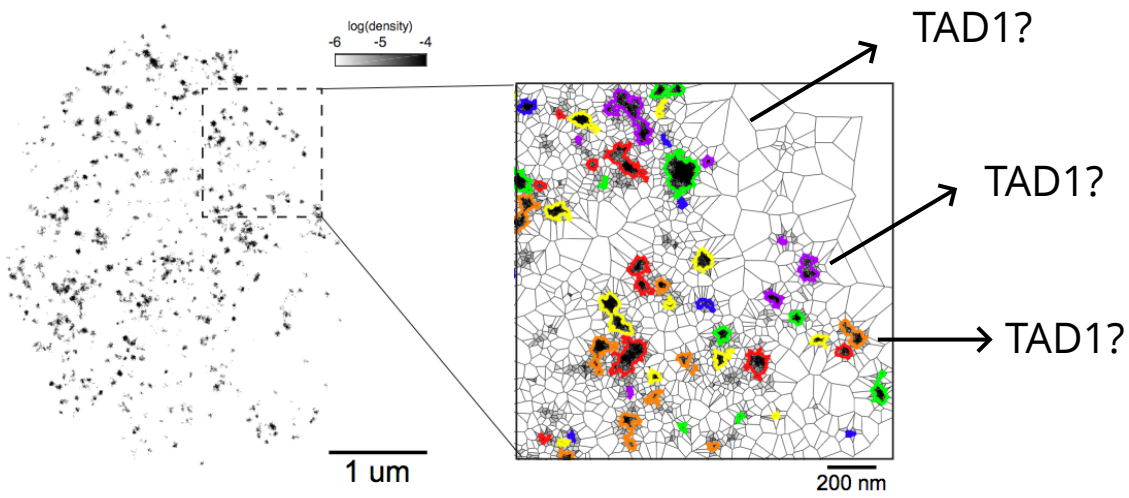
"hand of god"

Visualizing epigenetic domains

H3K27me3



H3K4me3

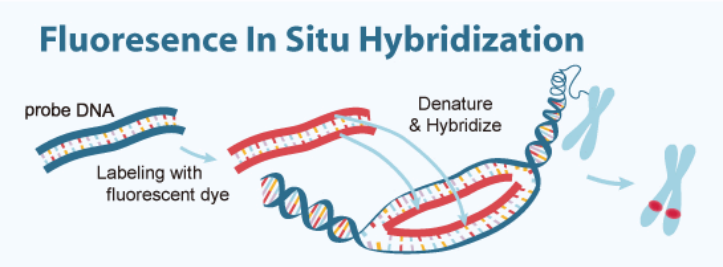


Cattoni, **Nat. Comm** (2017)

spatial resolution

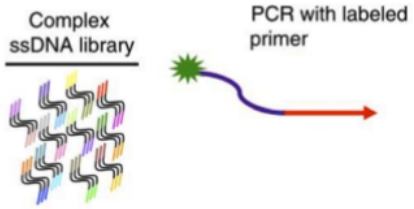
We don't know what we are looking at !

DNA labeling strategies



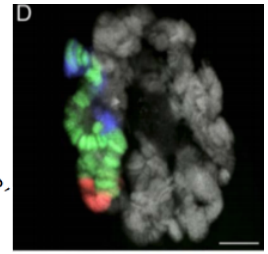
labeling short DNA regions (<10kb)

Oligopaints - Next Generation **Synthesis**



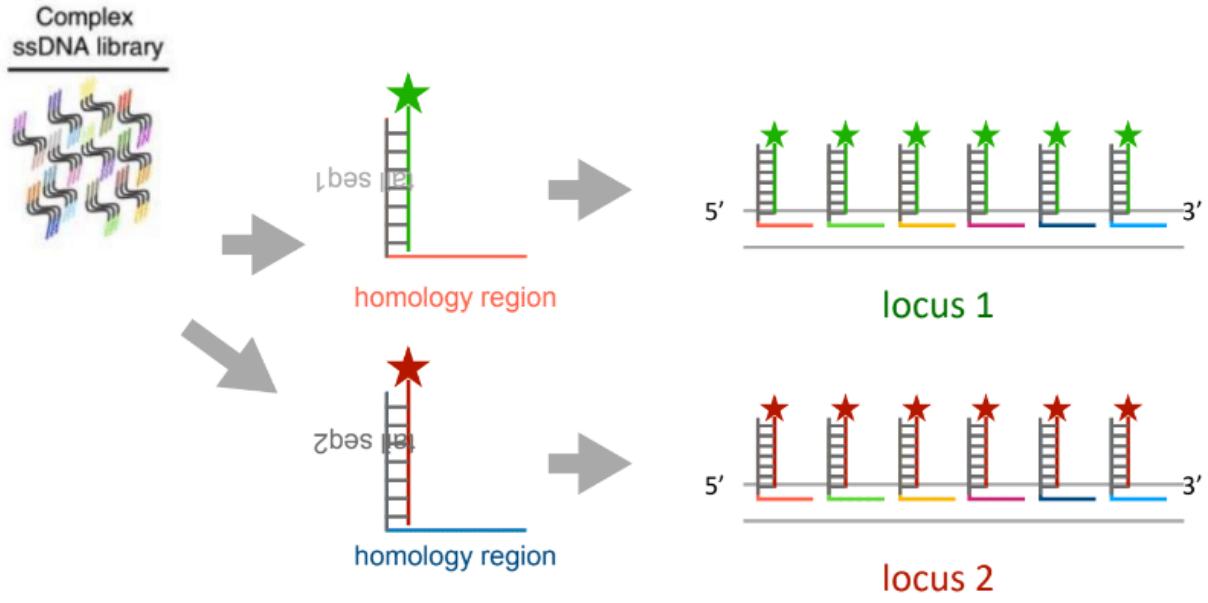
> 10 000 short oligonucleotides

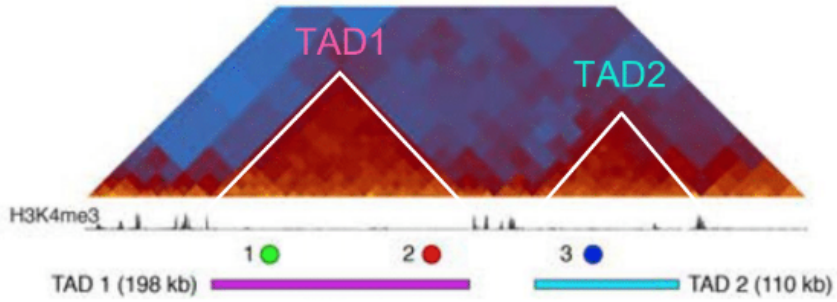
1 kb-2Mb



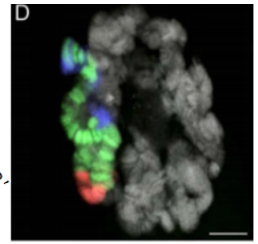
Beliveau, *et al*, 2012
Beliveau, *et al*, 2015

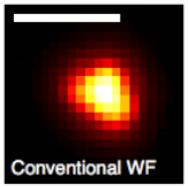
multi-color oligoPaints

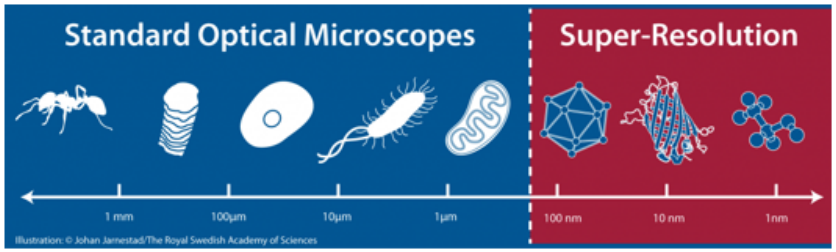




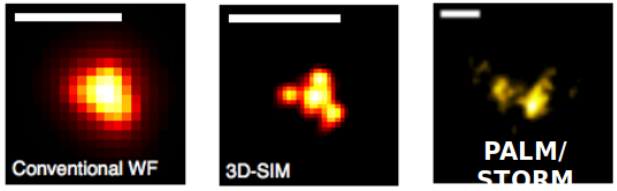
oligoPaints + super-resolution





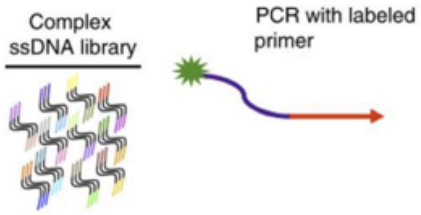


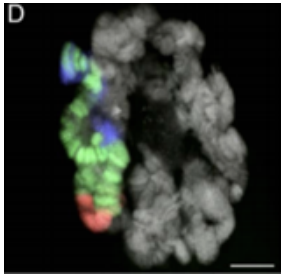
Betzig, Zhuang, Gustafson Labs



x2 x10

oligopaint FISH

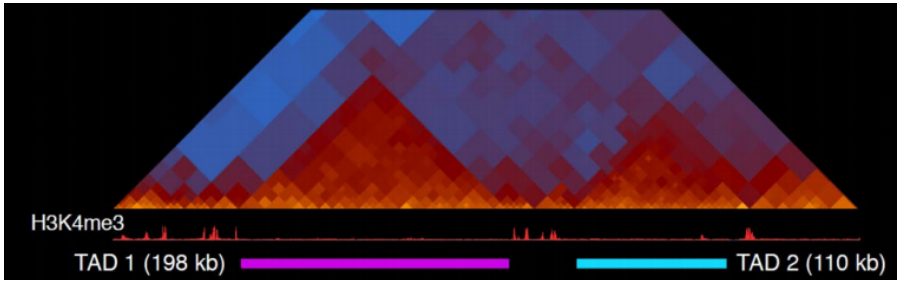




Beliveau (2012)

oligopaint + 3D-SIM/ STORM

Q.Szabo, D. Jost, D. Cattoni, F. Bantignies, G. Cavalli

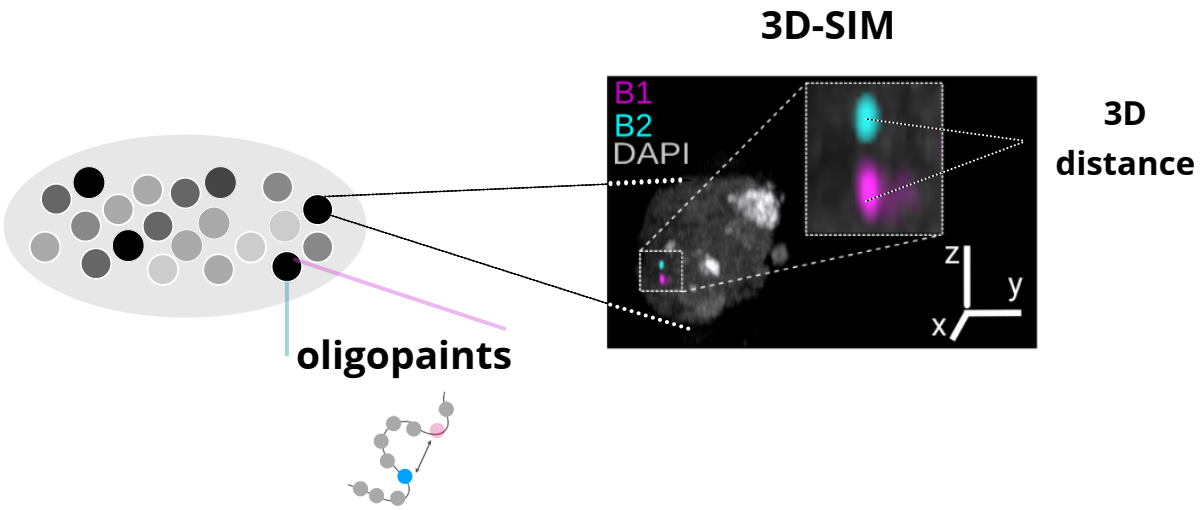


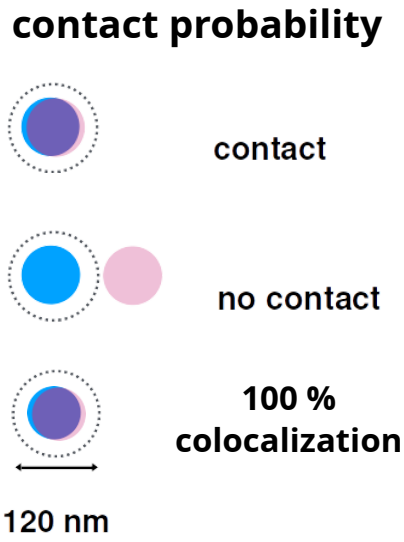
Szabo, **Science Adv.** (2018)

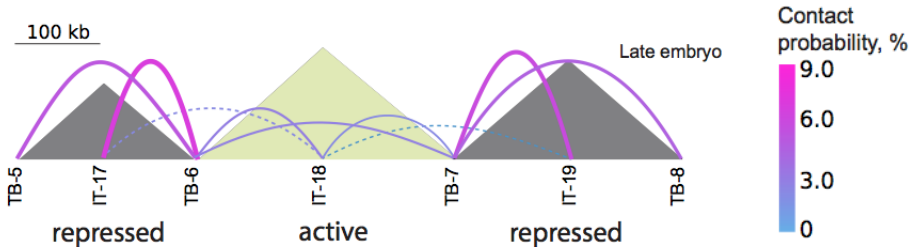
no contact
~68%

Visualization of TADs with spatial resolution but limited labeling specificity

measuring contact frequencies by microscopy





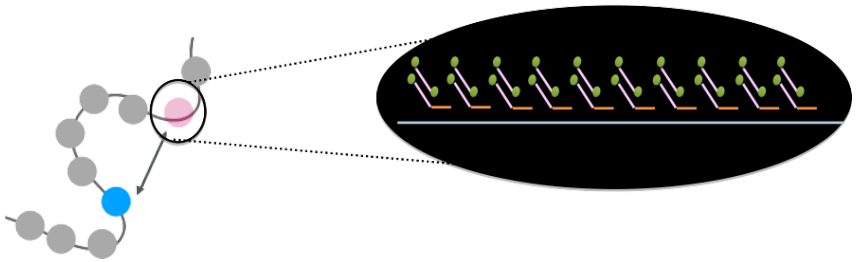


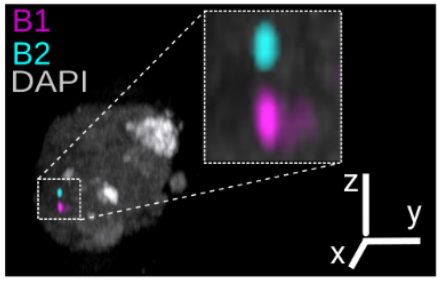
Can only see two DNA regions at once

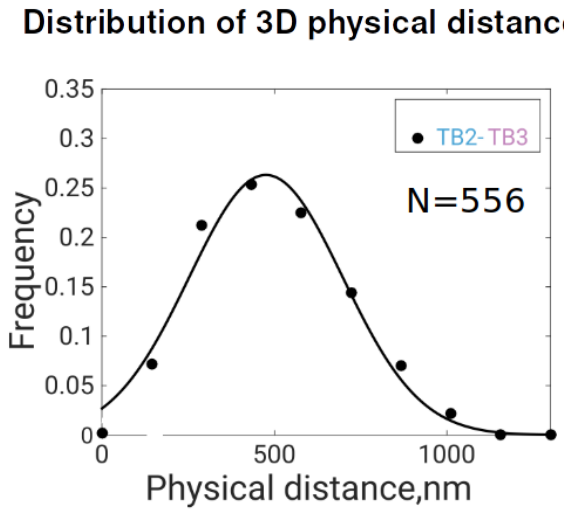
Cattoni, **Nat. Comm** (2017)

Finn, **Cell** (2018)

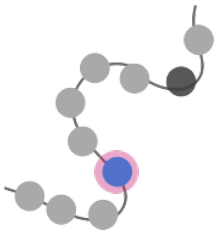
measuring contact frequencies by microscopy

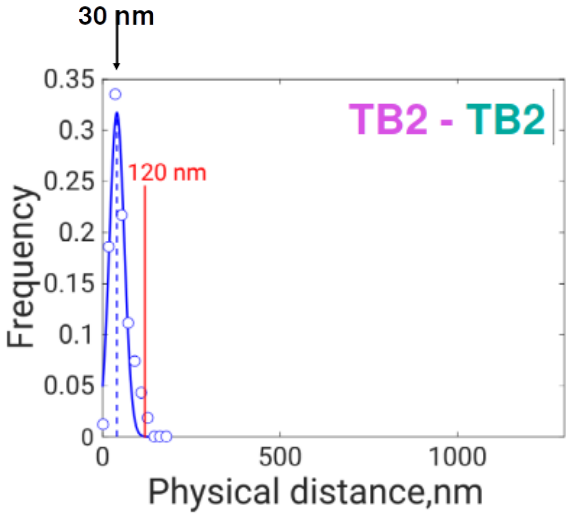


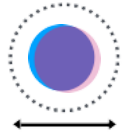




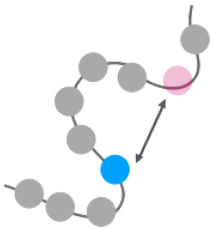
measuring contact frequencies by microscopy

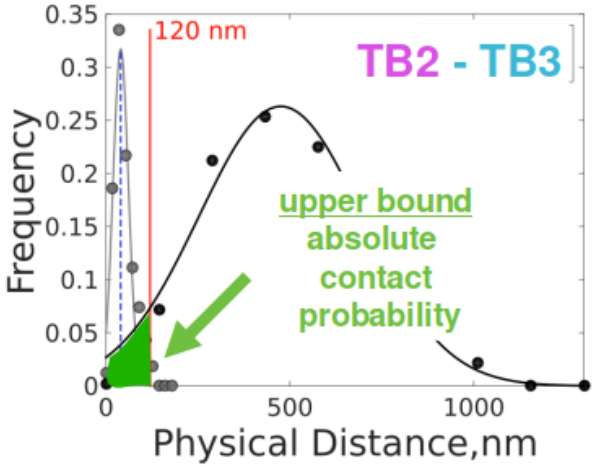






120 nm

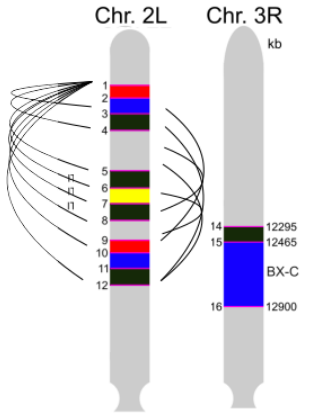


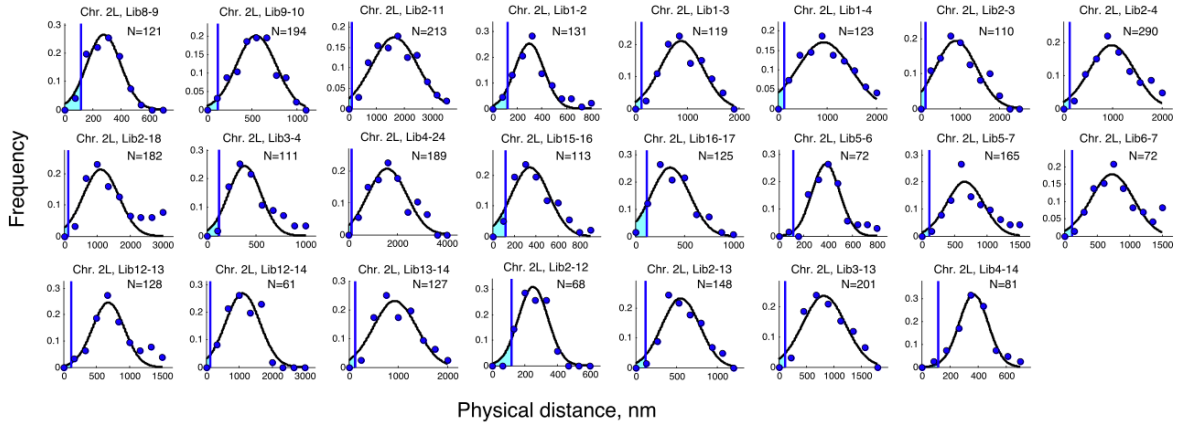


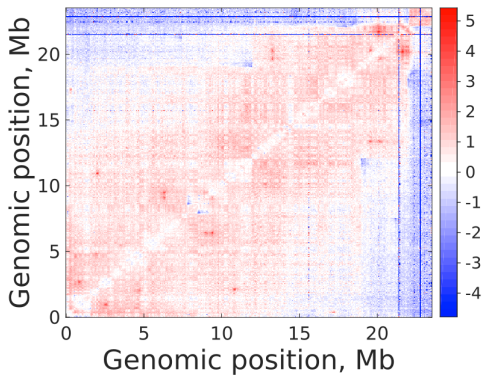
contact

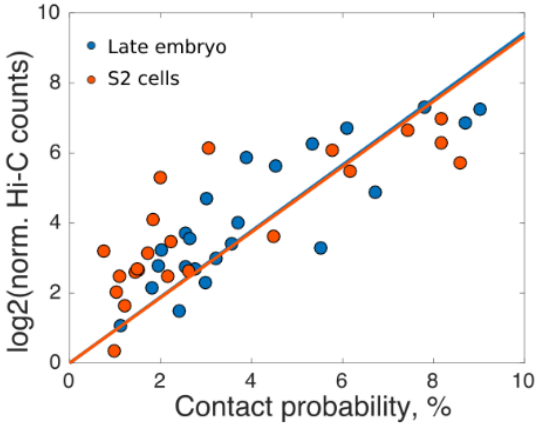
no contact

comparing Hi-C and microscopy contacts

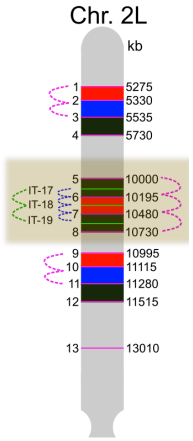


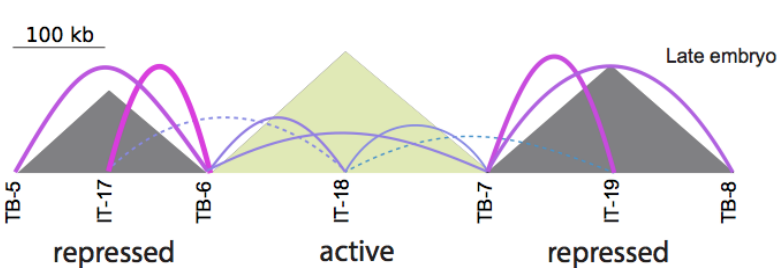


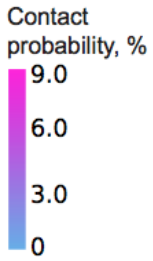




microscopy contacts in a TAD



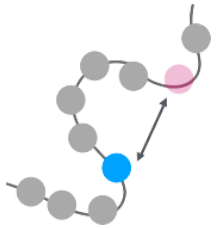




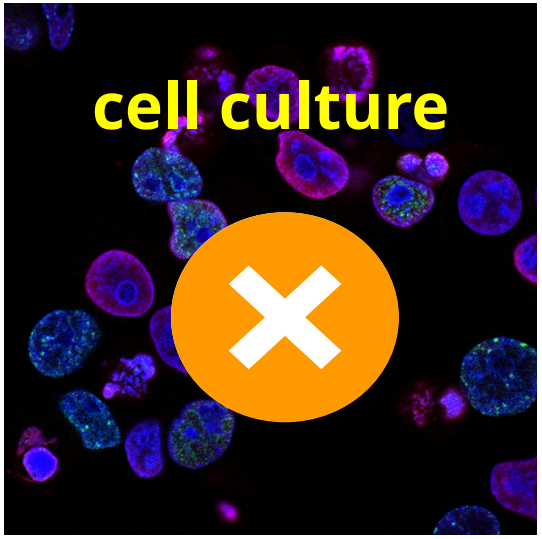
TADs exist in every cell!

How can TADs exist in every cell and have such low contact frequencies?

PROBLEM 1: Can only see 2 loci at any given time !



PROBLEM 2: Model system



different cell cycle stages

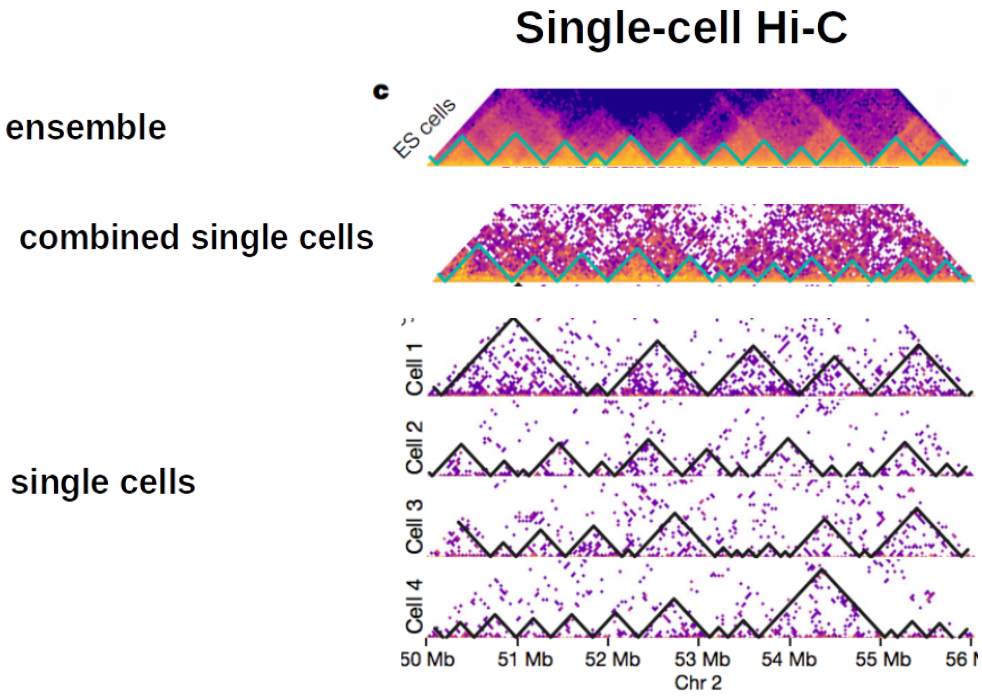
different transcriptional programs

different morphologies

different DNA structures

intrinsic SC heterogeneity

Looking at the 3D genome with sc-HiC

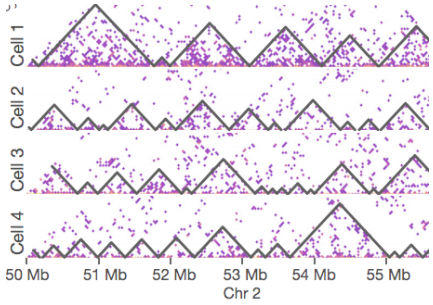


Flyamer, *Nature*, 2017
Stevens, *Nature*, 2017

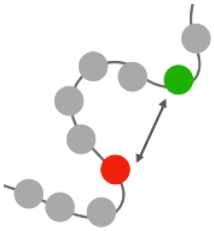
Do TADs arise only after ensemble averaging?

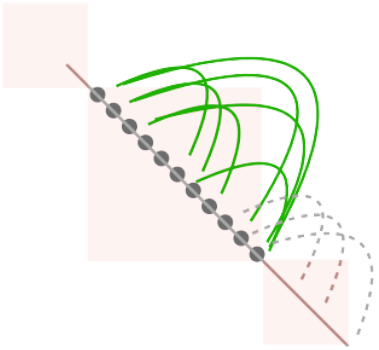
TADs exist in every cell!

TADs as ensemble averages



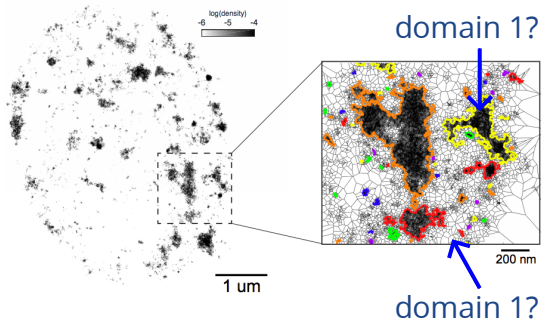
quantify contact probabilities at the single cell level?



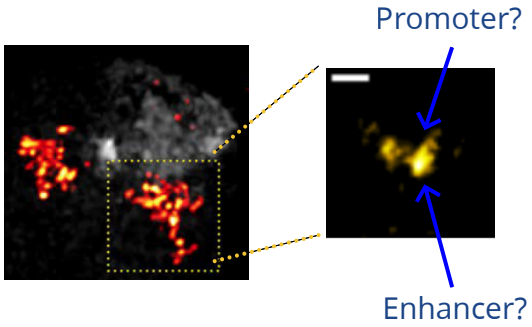


Spatial Genomics v0.1

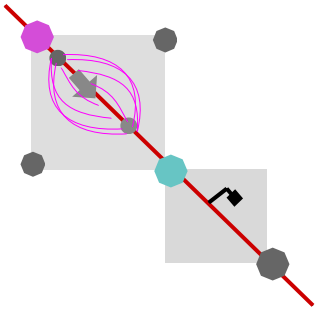
2-color STORM
of epigenetic domains



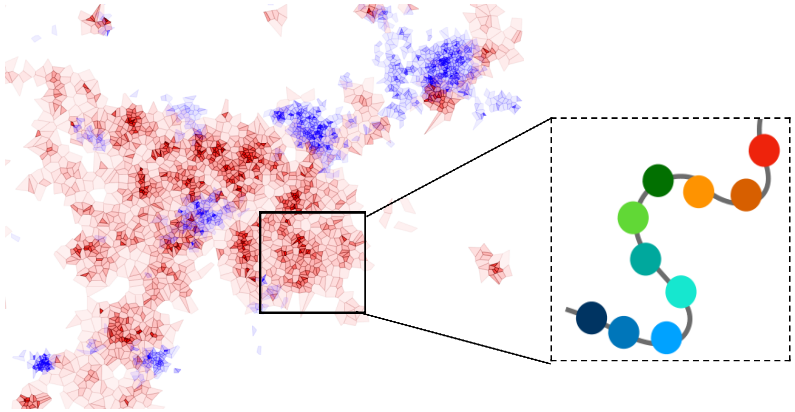
Super-resolution imaging
+ Oligopaints



Contact probabilities by imaging



holy grail?



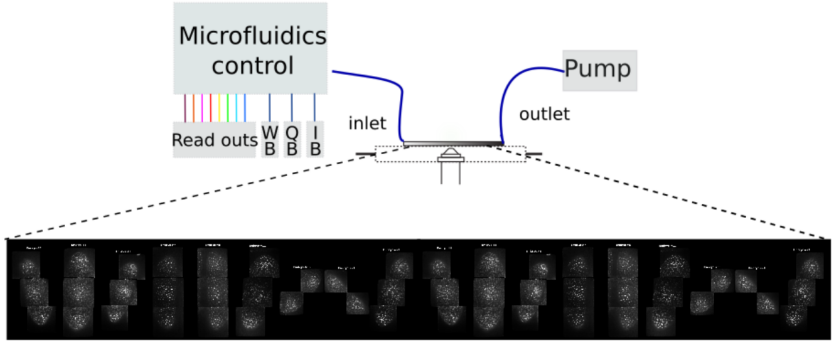
spatial resolution

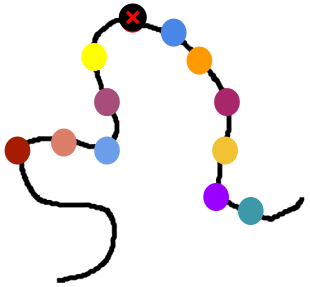
labeling specificity

multiplexing

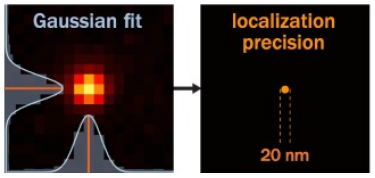
Hi-M: Spatial Genomics v1.0

Sequential imaging



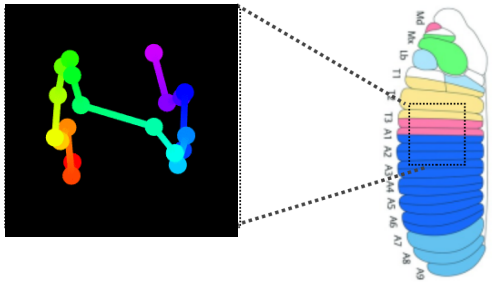


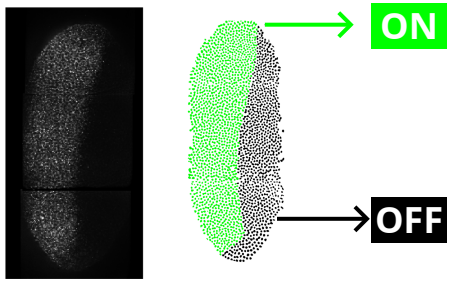
3D position



3D coordinates of each locus with genomic specificity & nanometer precision!

Hi-M: reconstructing 3D path of DNA + transcription

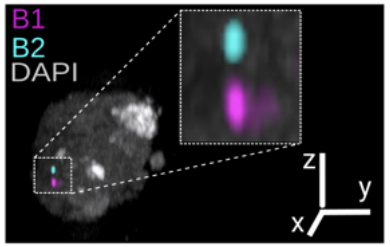




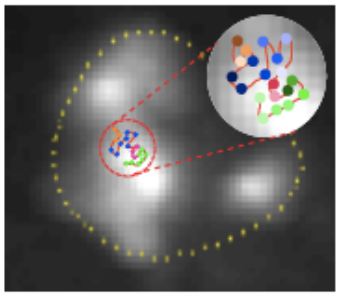
Cardozo, **Mol Cell** (2019); Cardozo, **Nature Protocols** (2020)
Bintu, **Science** (2018); Mateo, **Nature** (2019)

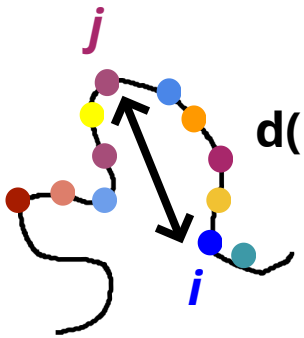
Hi-M benchmarks: mean distances

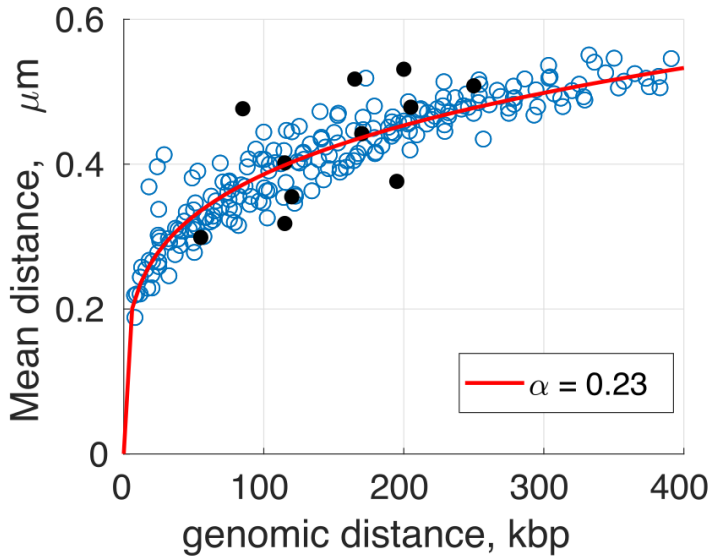
3D-SIM



Hi-M

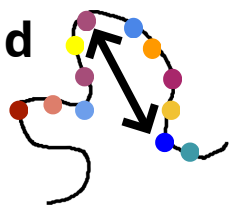


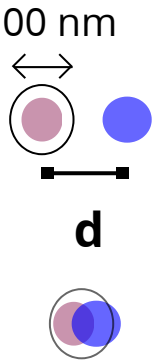




Cardozo, **Mol Cell** (2019), **Nature Protocols** (2020)

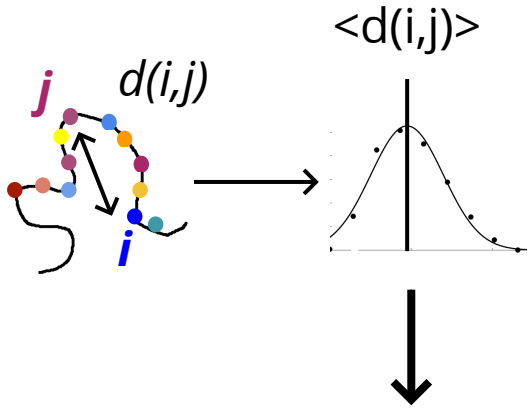
Hi-M benchmarks: contact frequencies





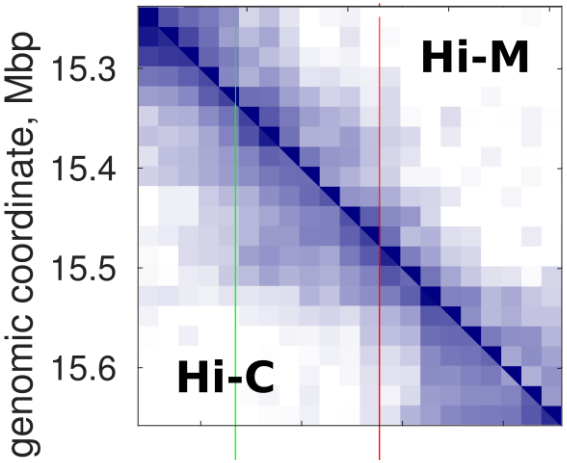
no contact

contact

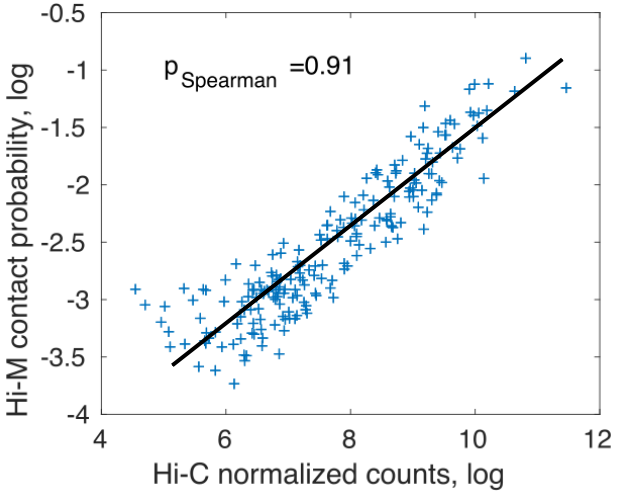


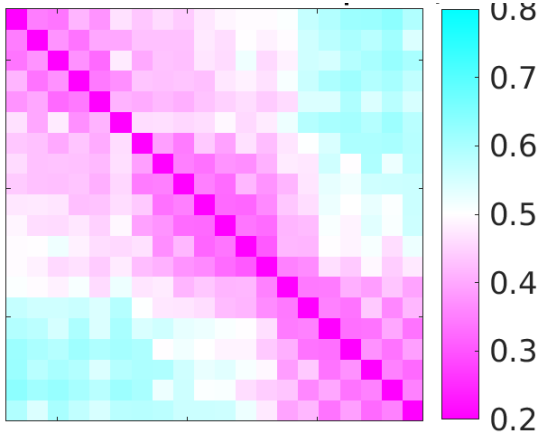
contact probability map

pairwise distance map



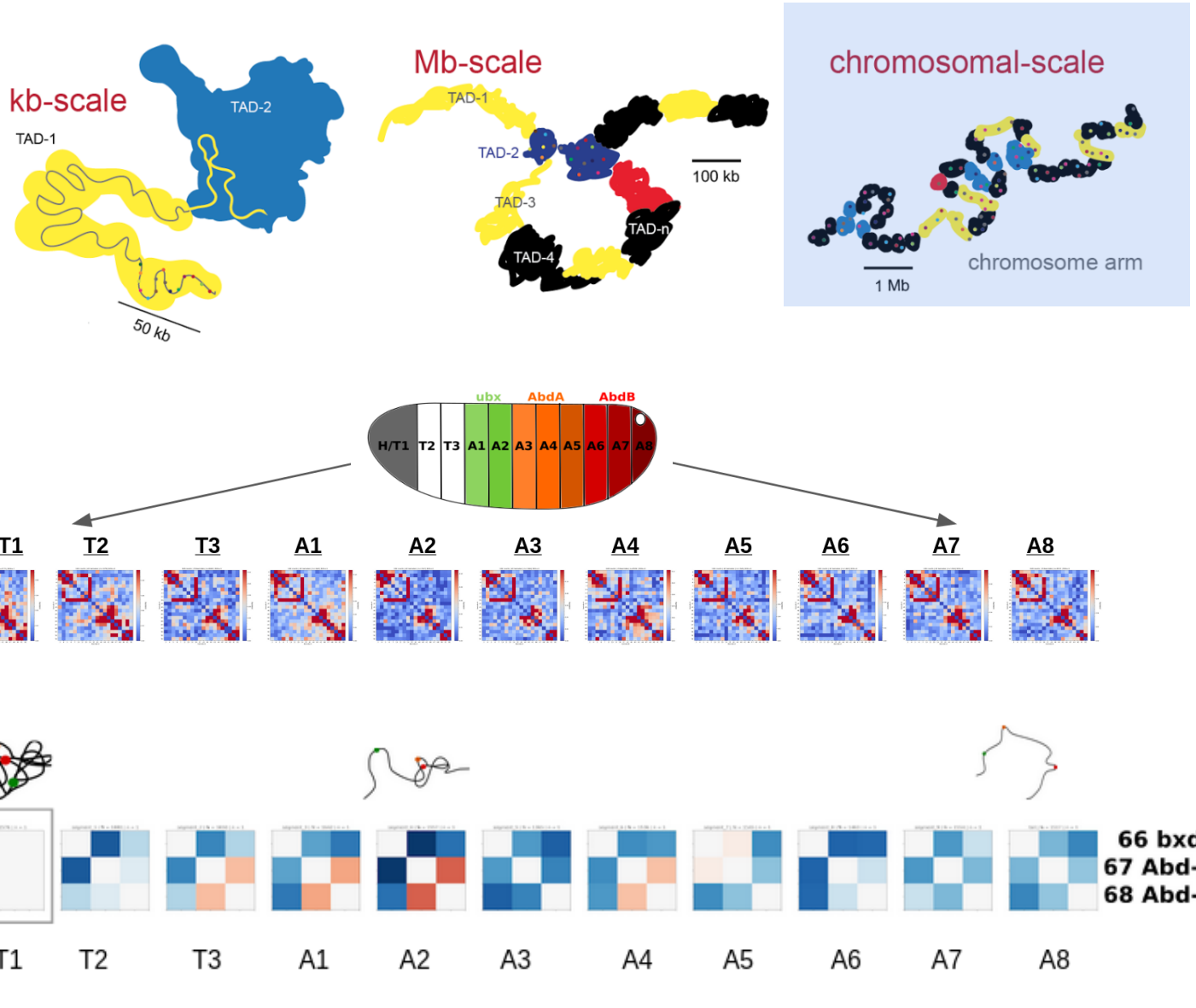
sna locus,
chr2L





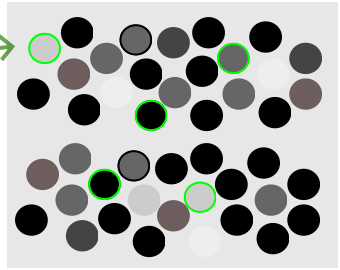
Cardozo, **Mol Cell** (2019)

Hi-M can address multiple scales



cell-cultures

expressing

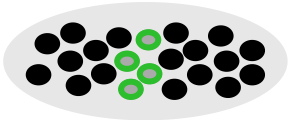


variable morphologies

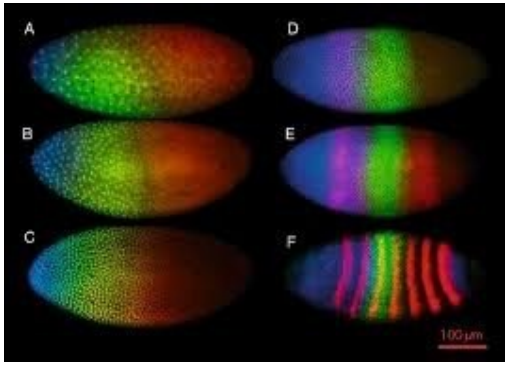
different cell cycle stages

different transcriptional levels

fly embryo



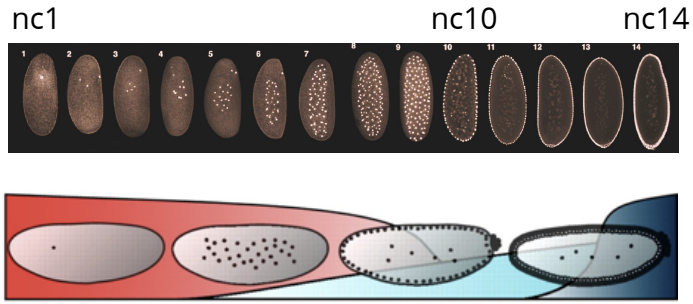
RNA expression



Transcription is encoded in space

A primer on Drosophila development II

Transcription during development



degradation
maternal RNA

zygotic
transcription

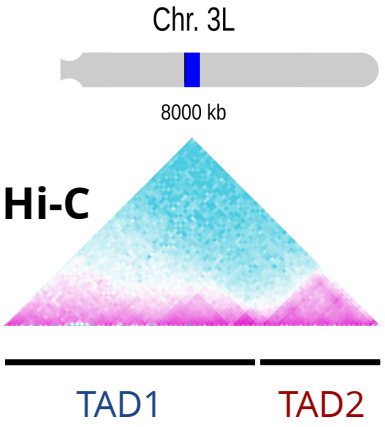
Cell division during development

M. Lagha's Lab

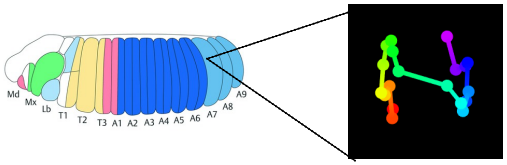
transcription
cell cycle

Encoded in time and space

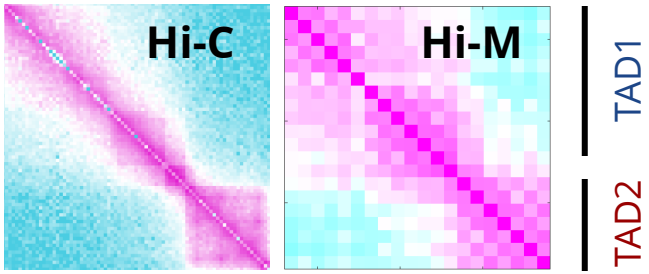
fly embryo



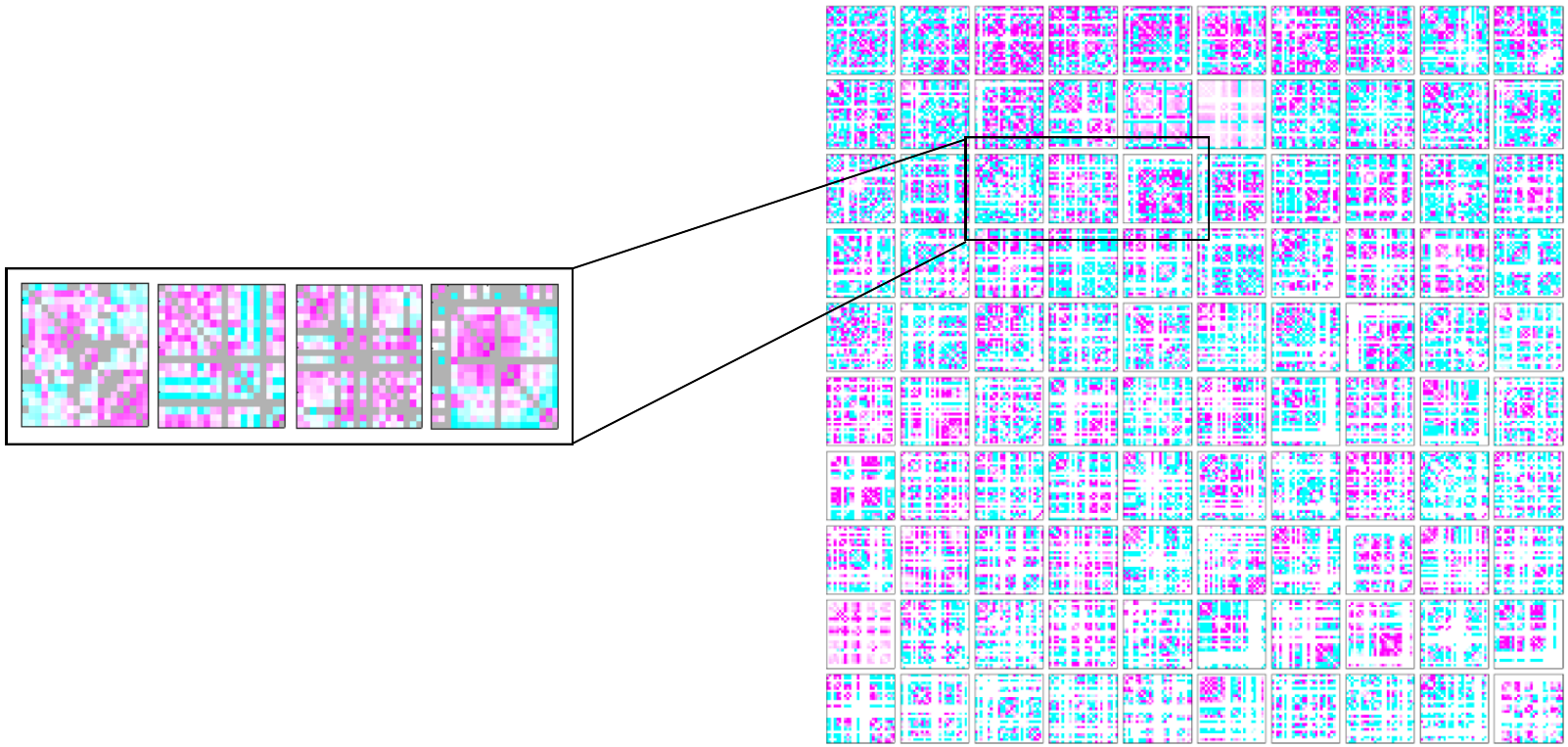
Hi-M

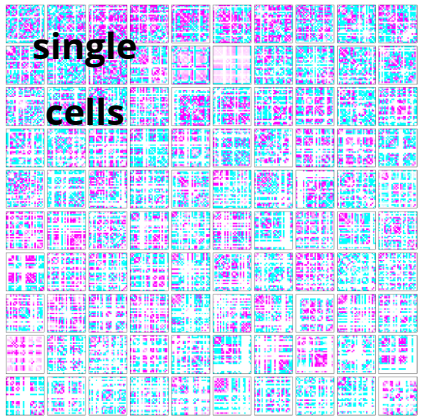


ensemble



single cells





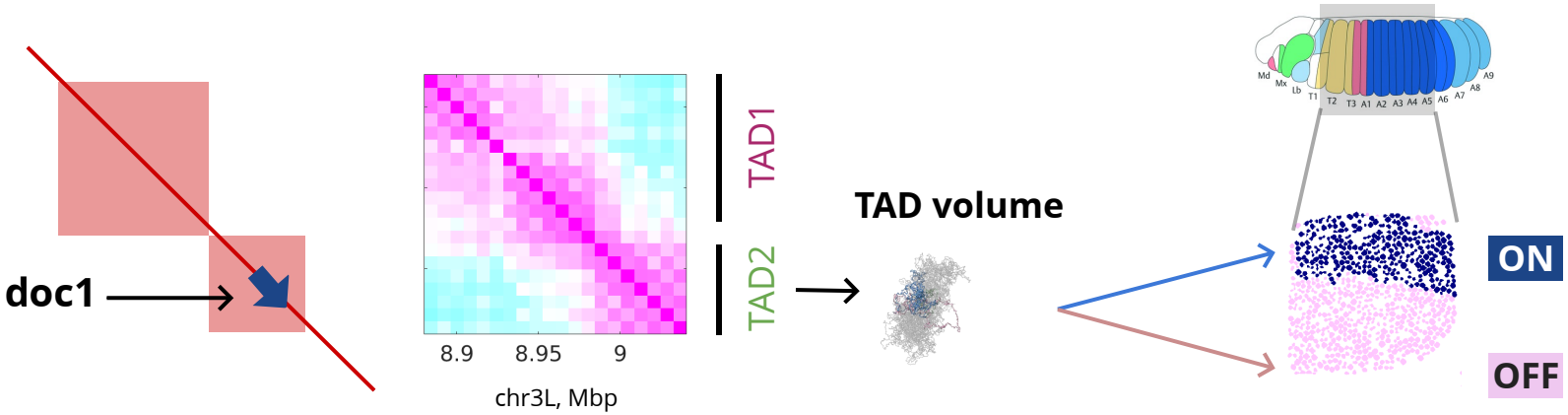
? ? ? ?

What is the source of this heterogeneity?

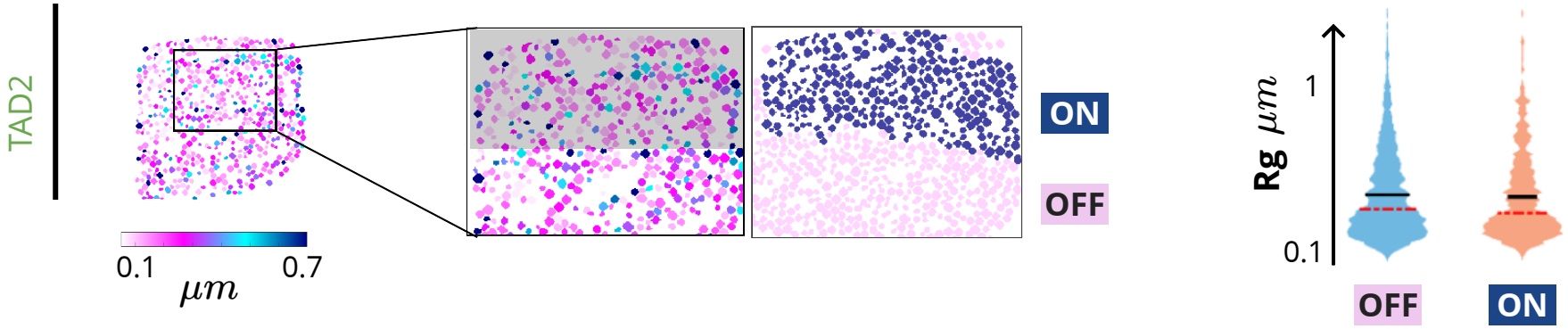
? ? ? ?

M. Lagha's Lab

do TADs expand/contract upon transcriptional activation?

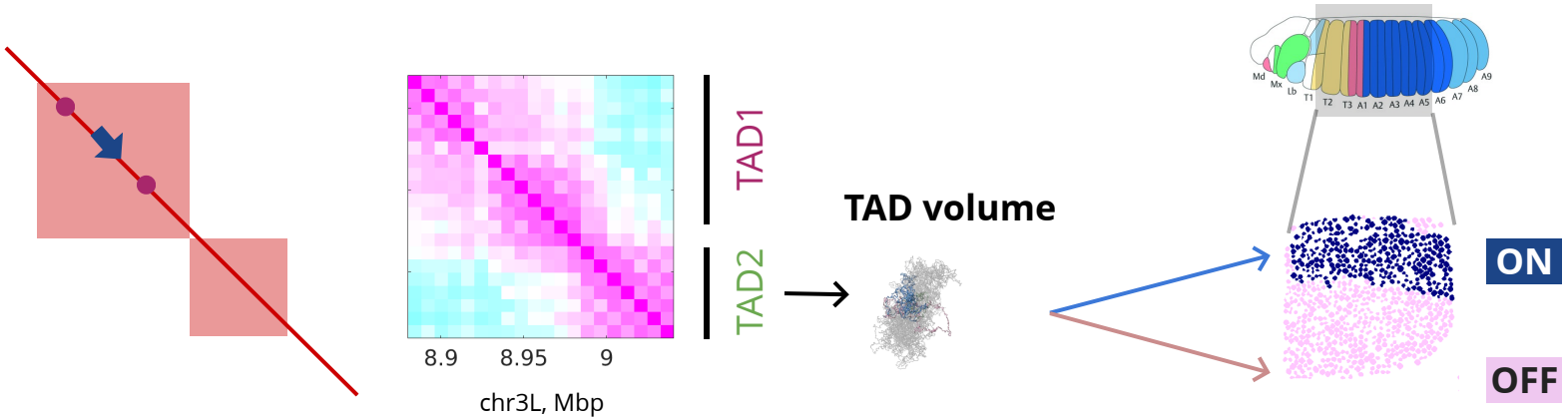


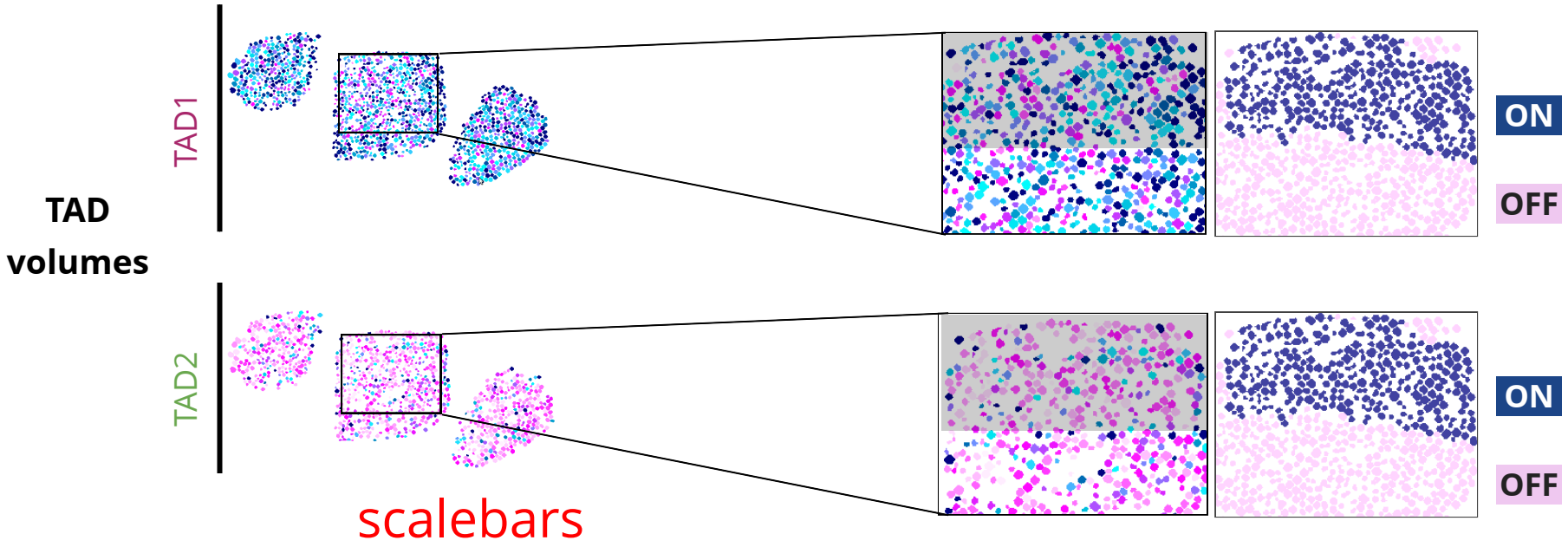
TAD volumes



TAD shape alone does not seem to determine function

do TADs expand/contract upon transcriptional activation?

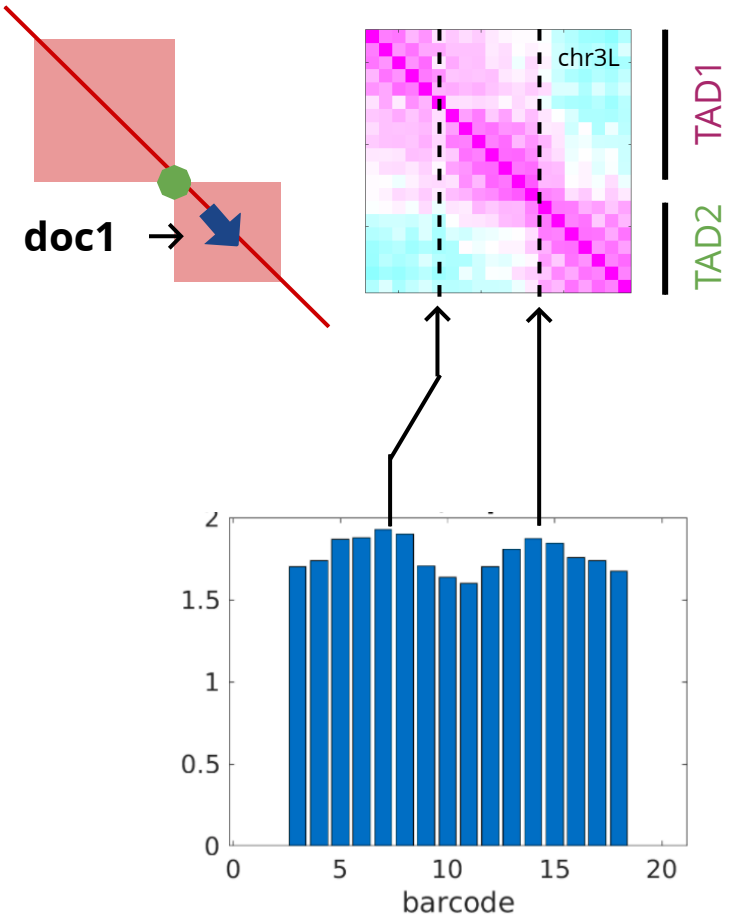




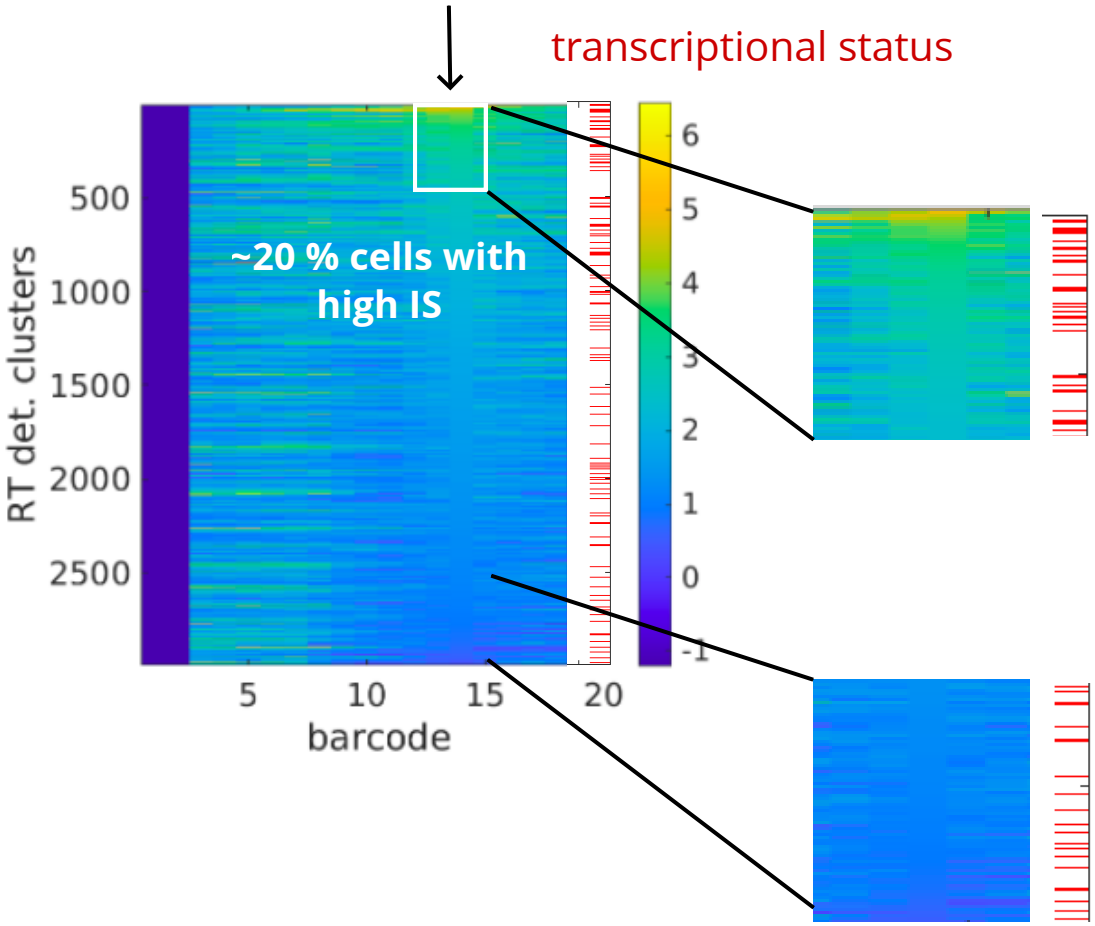
TAD shape alone does not seem to determine function

Are borders correlated to transcription ?

mean insulation score

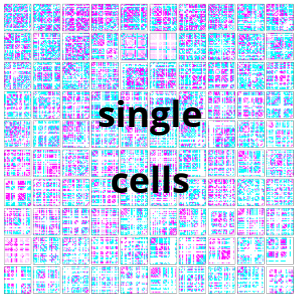


single-cell insulation score



Borders not strongly correlated to transcriptional status

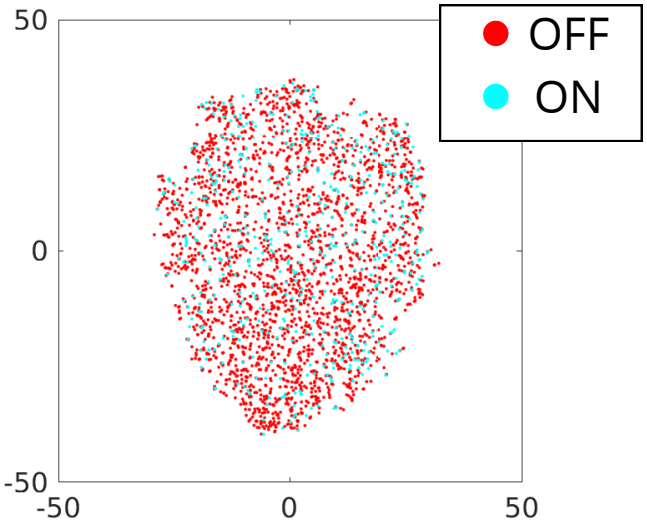
Does 3D organization change between ON/OFF cells?



sc **Architectures**

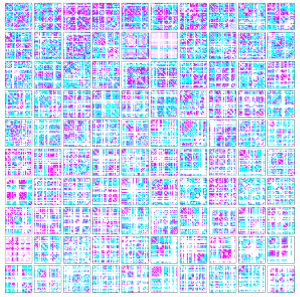
Markus Goetz

UMAP clustering

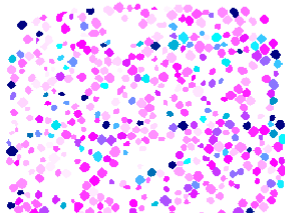


Impossible to distinguish pairwise maps of **ON cells** from **OFF cells**

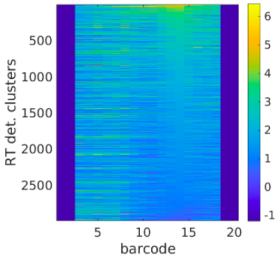
single cells



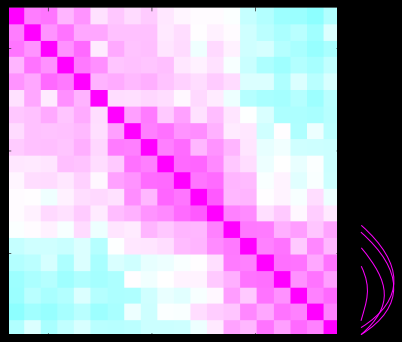
sc Volumes



sc Borders



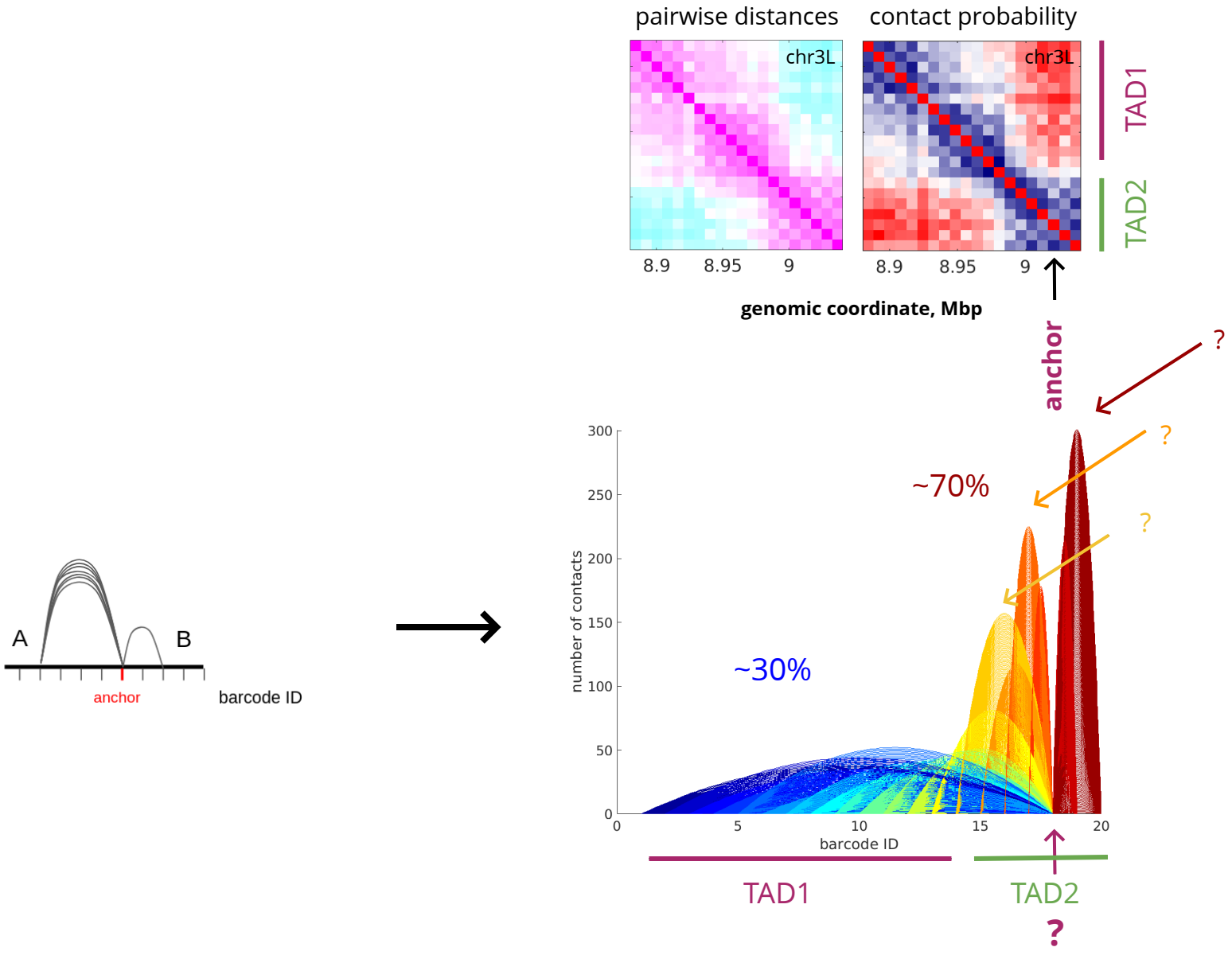
not correlated to function ?



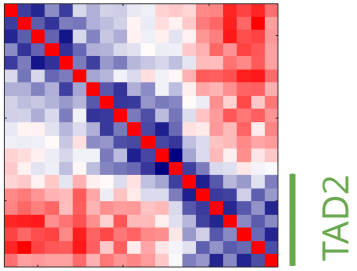
Intra-TAD interactions?

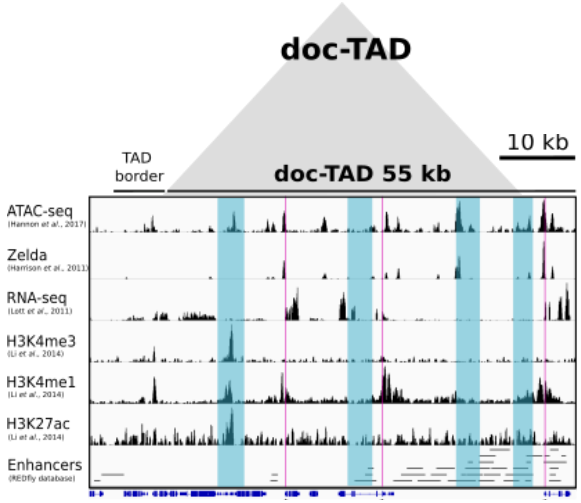
↓

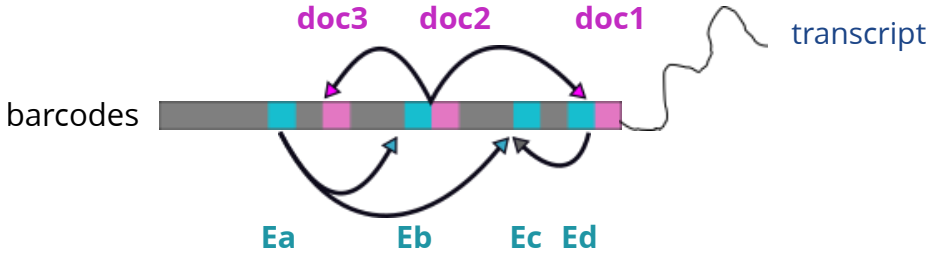
function?



Loci within TADs prefer to interact with loci in the same TAD

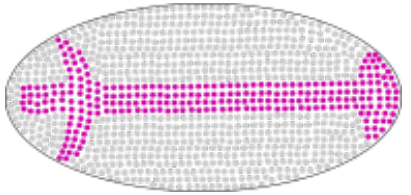




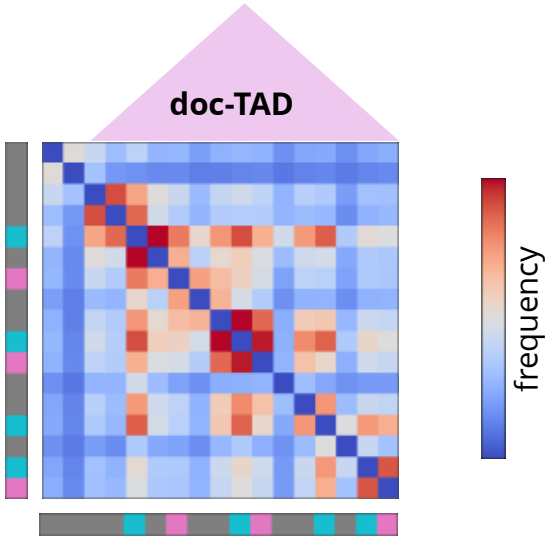


Hi-M

doc
ON cells

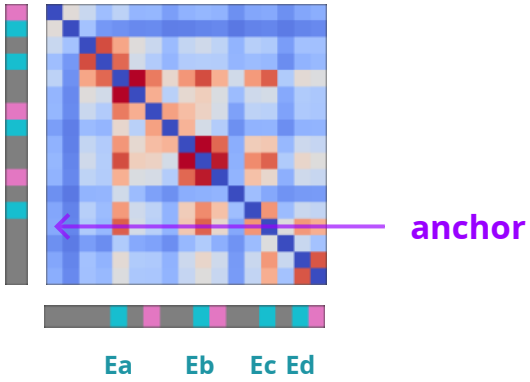


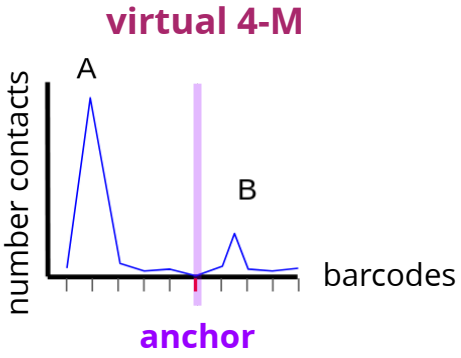
3x resolution
increase

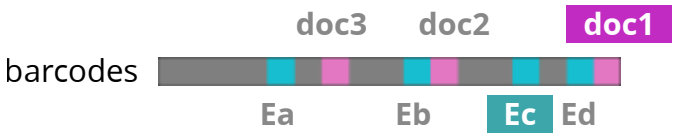


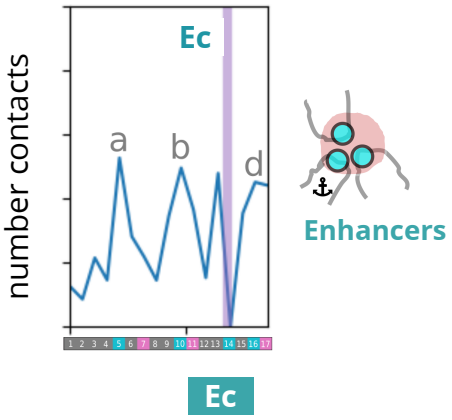
Hi-M detects **specific looping** interactions

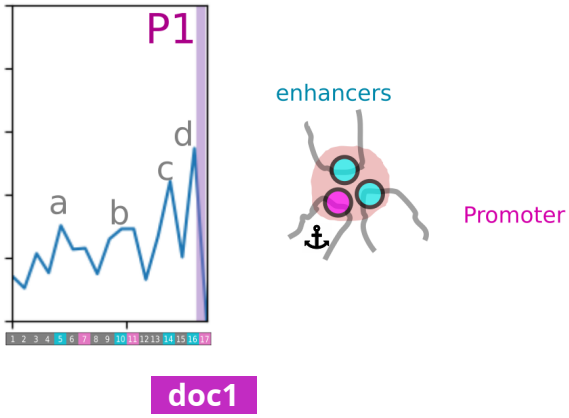
decoding preferential 3D networks





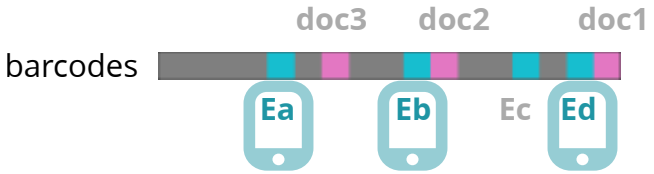




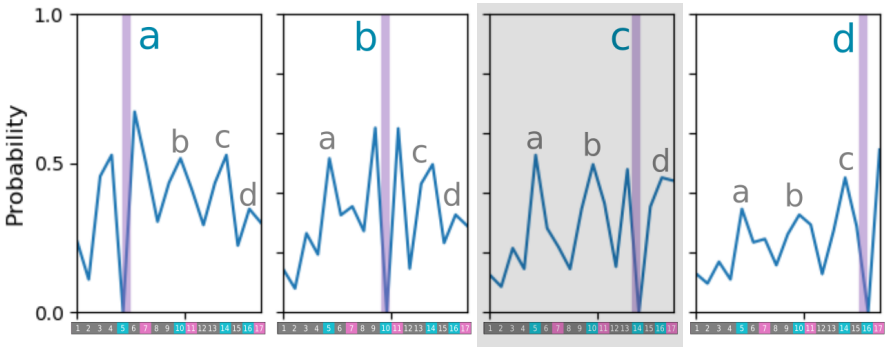


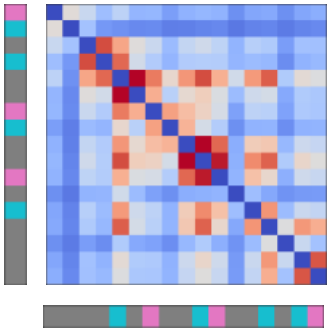
We can detect E-E and E-P contacts in ON cells !

are cis-RE interactions widespread?

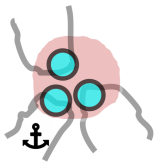


Enhancers

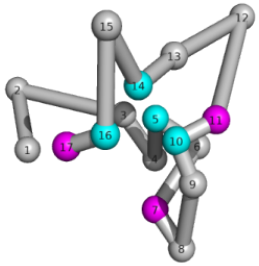




topological representation (ShRec3D) Lesne (2015)

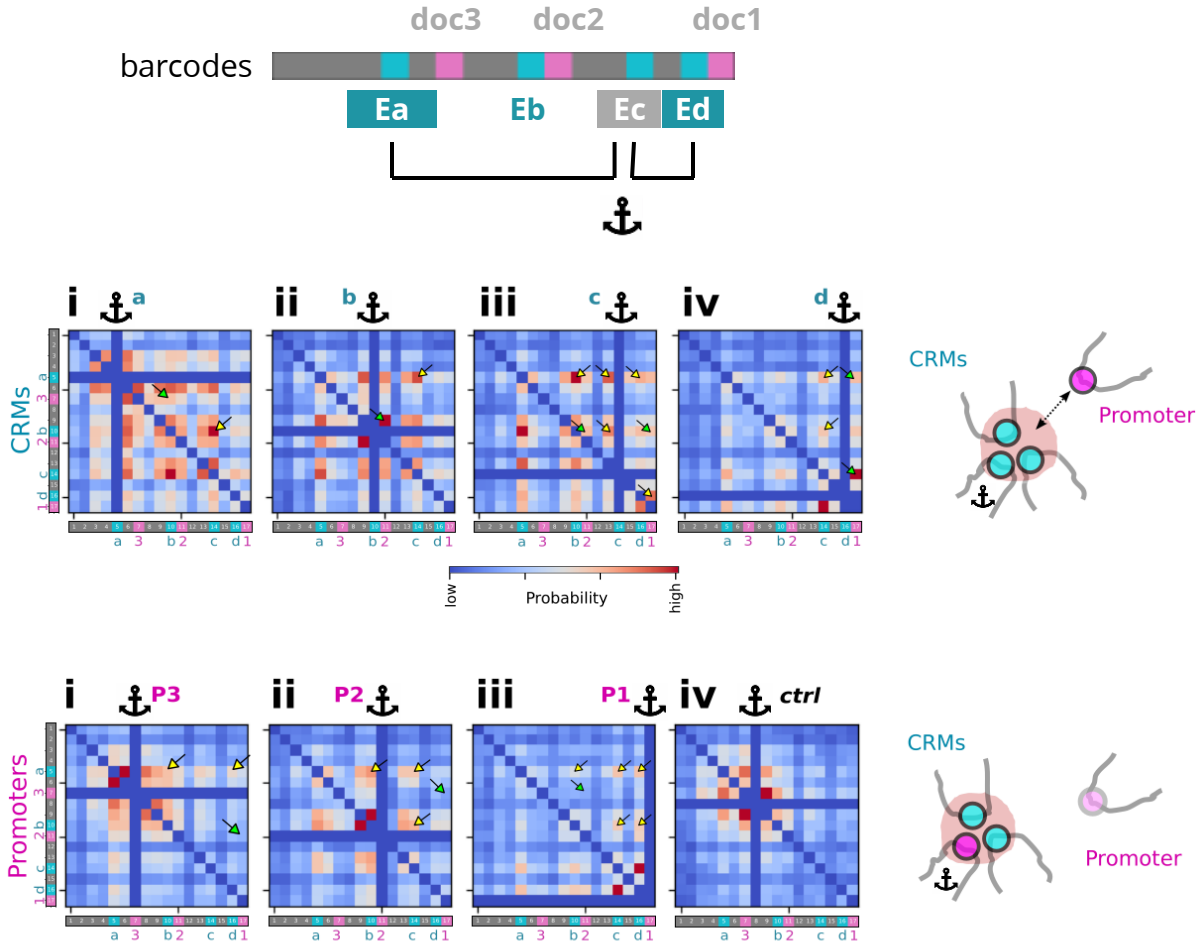


Enhancers



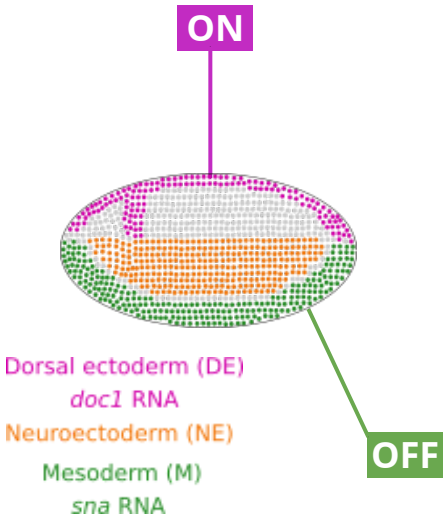
do **enhancer** form clusters in single cells?

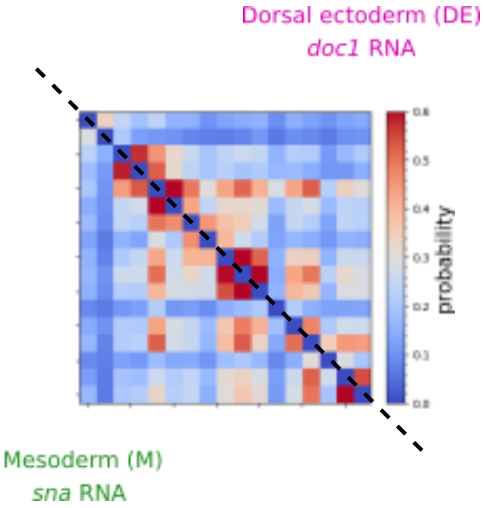
3-way interactions

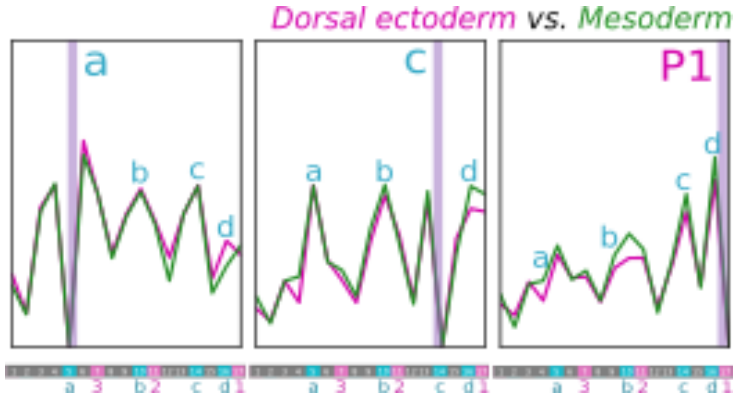


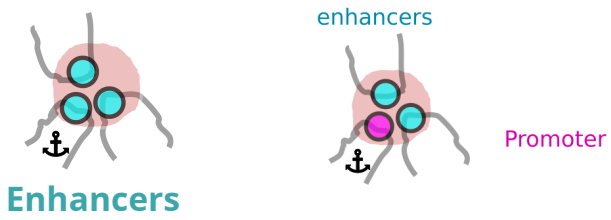
Promoters contact enhancer clusters in single cells

Surely these cis-RE networks are specific to ON cells!

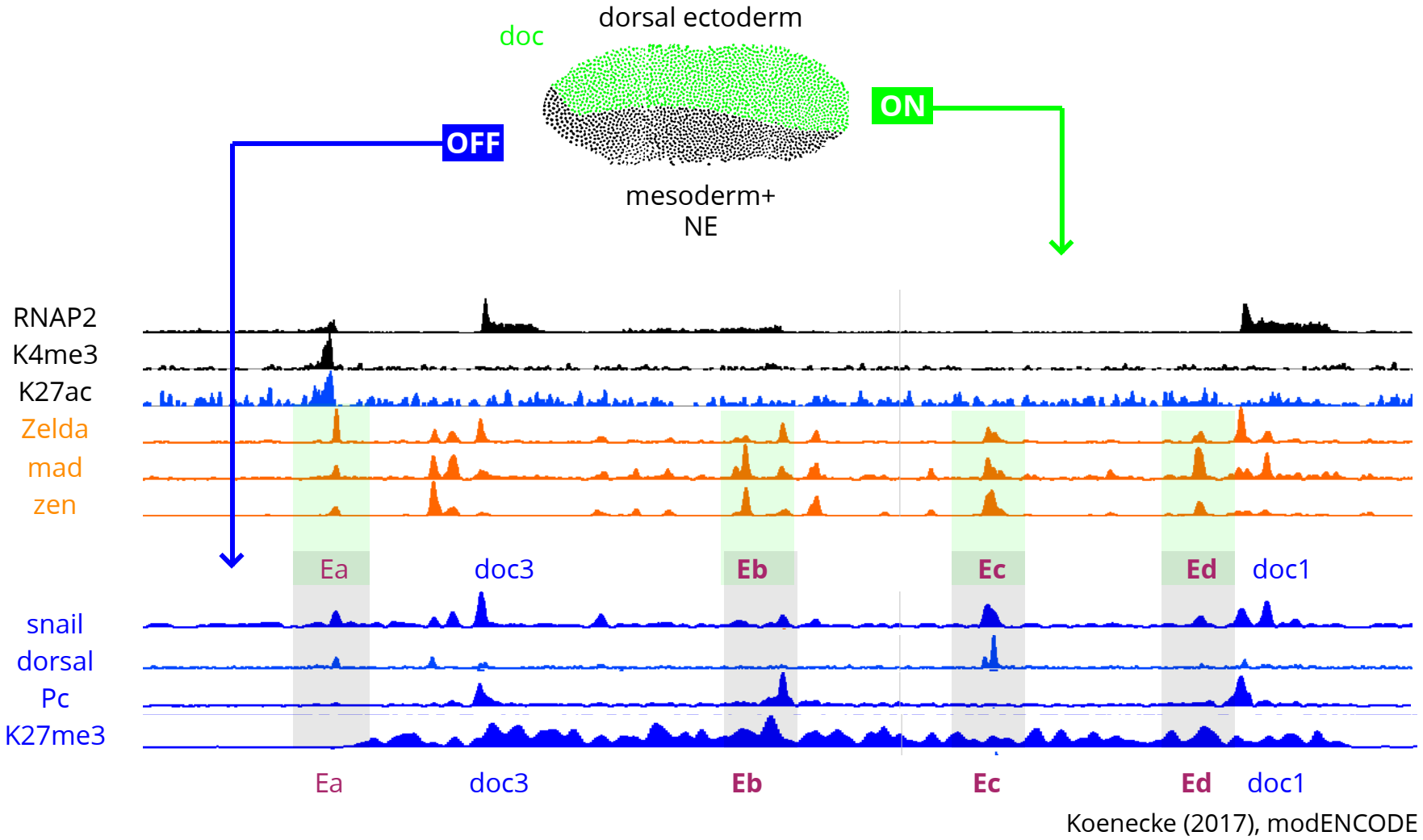








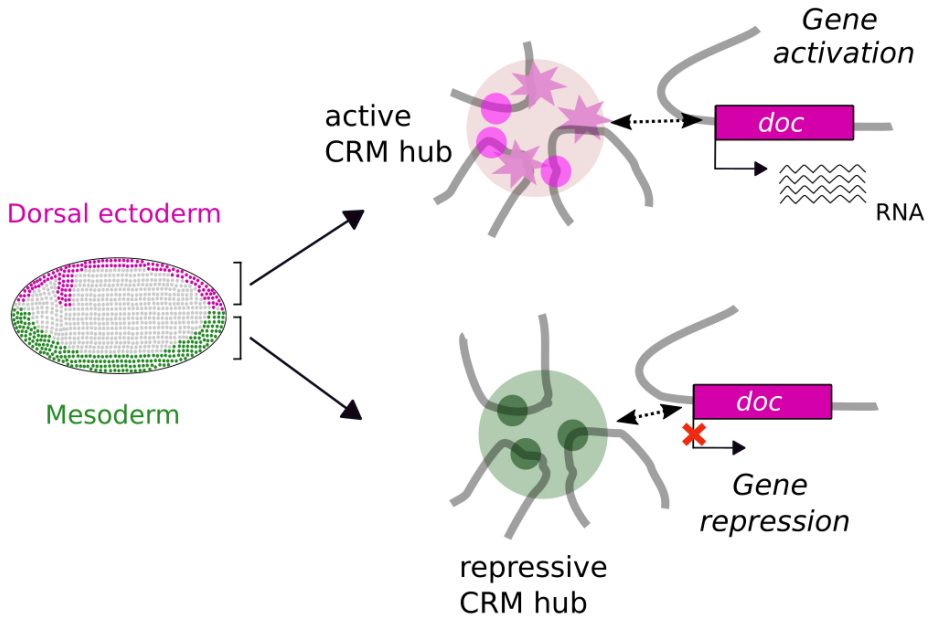
Promoters are still contacting enhancer clusters in OFF cells ! Why?



Koenecke (2017), modENCODE

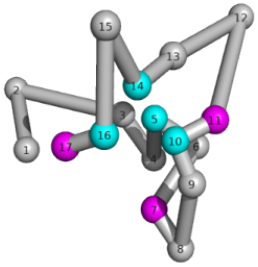
Enhancers are occupied by transcriptional activators AND repressors

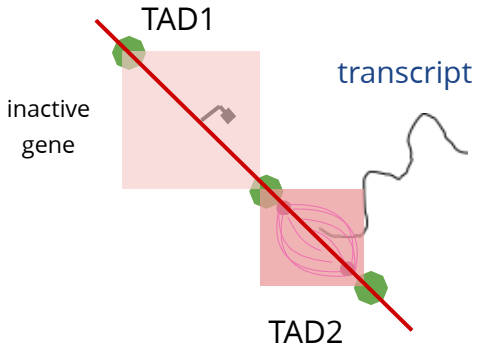
Chromatin architecture plays a double role in transcriptional regulation



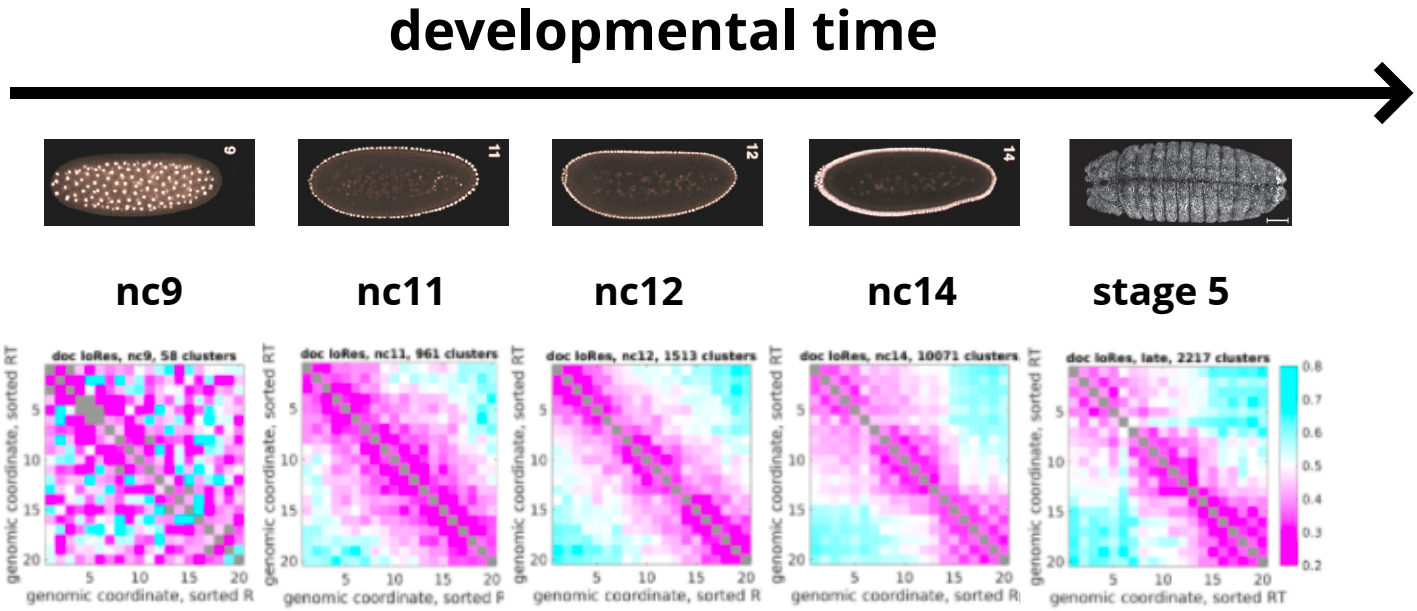
Do TADs / transcription shape the 3D network of cis-RE?

Are cis-RE interactions established before or after TADs and onset of transcription during development?

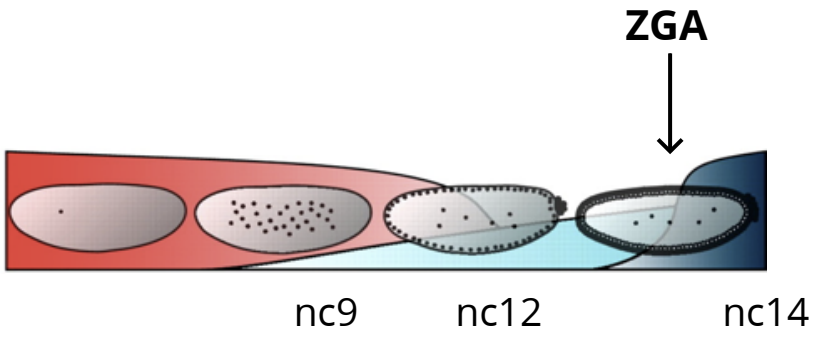




3D TAD folding during development

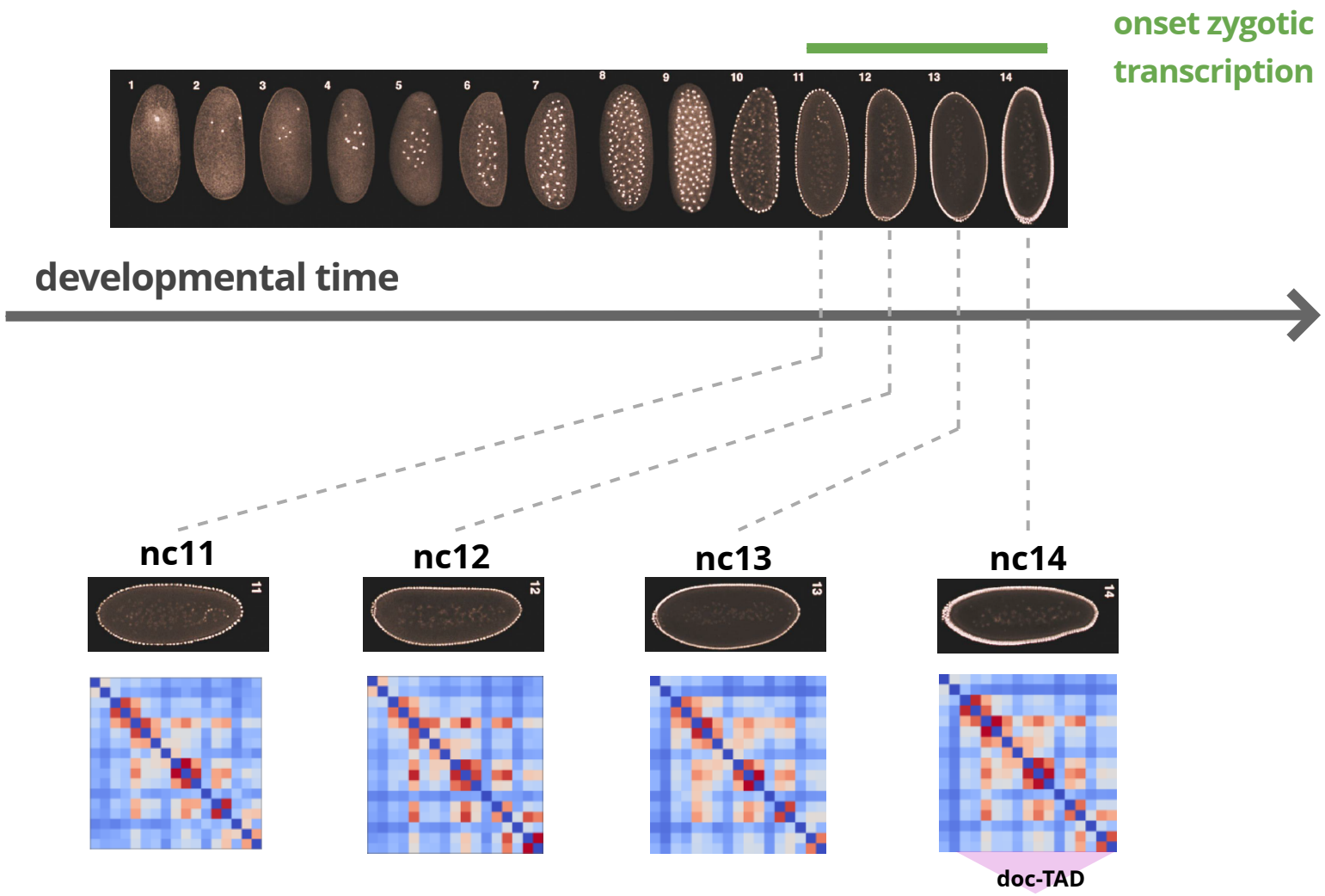


TADs emerge at nc14



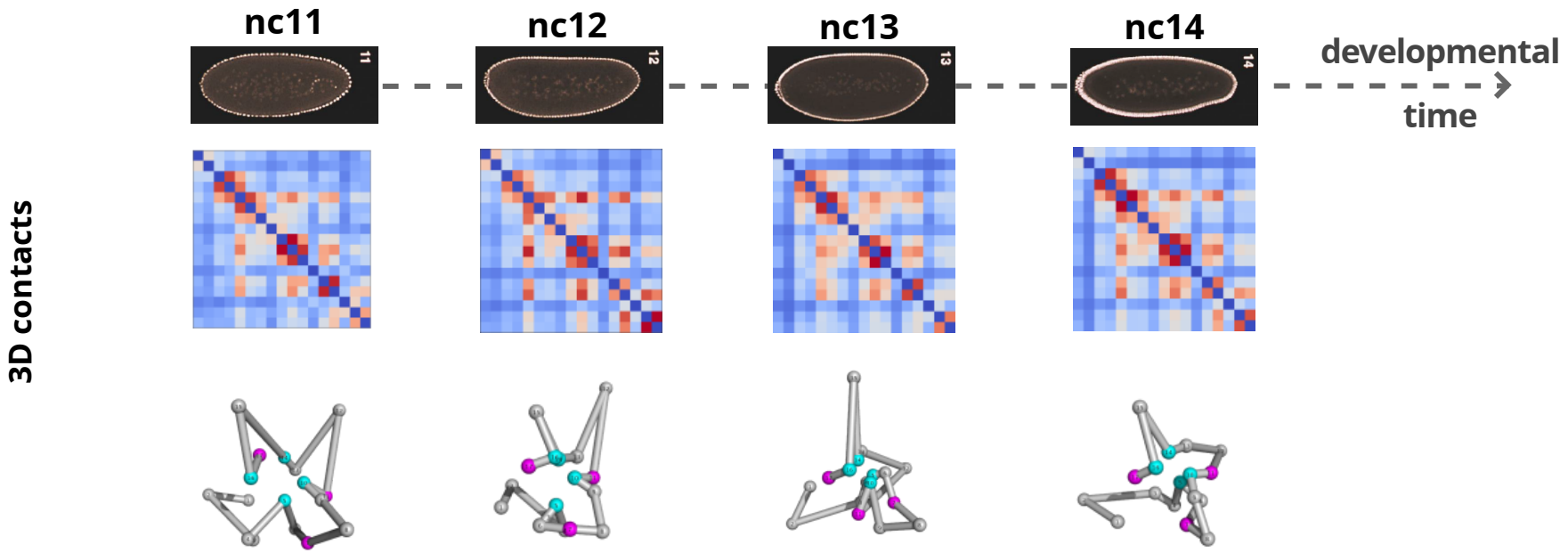
Hug, **Cell** (2017); Oiyama, **Mol.Cell** (2018); Cardozo, **Mol Cell** (2019)

When are cis-RE interactions established?

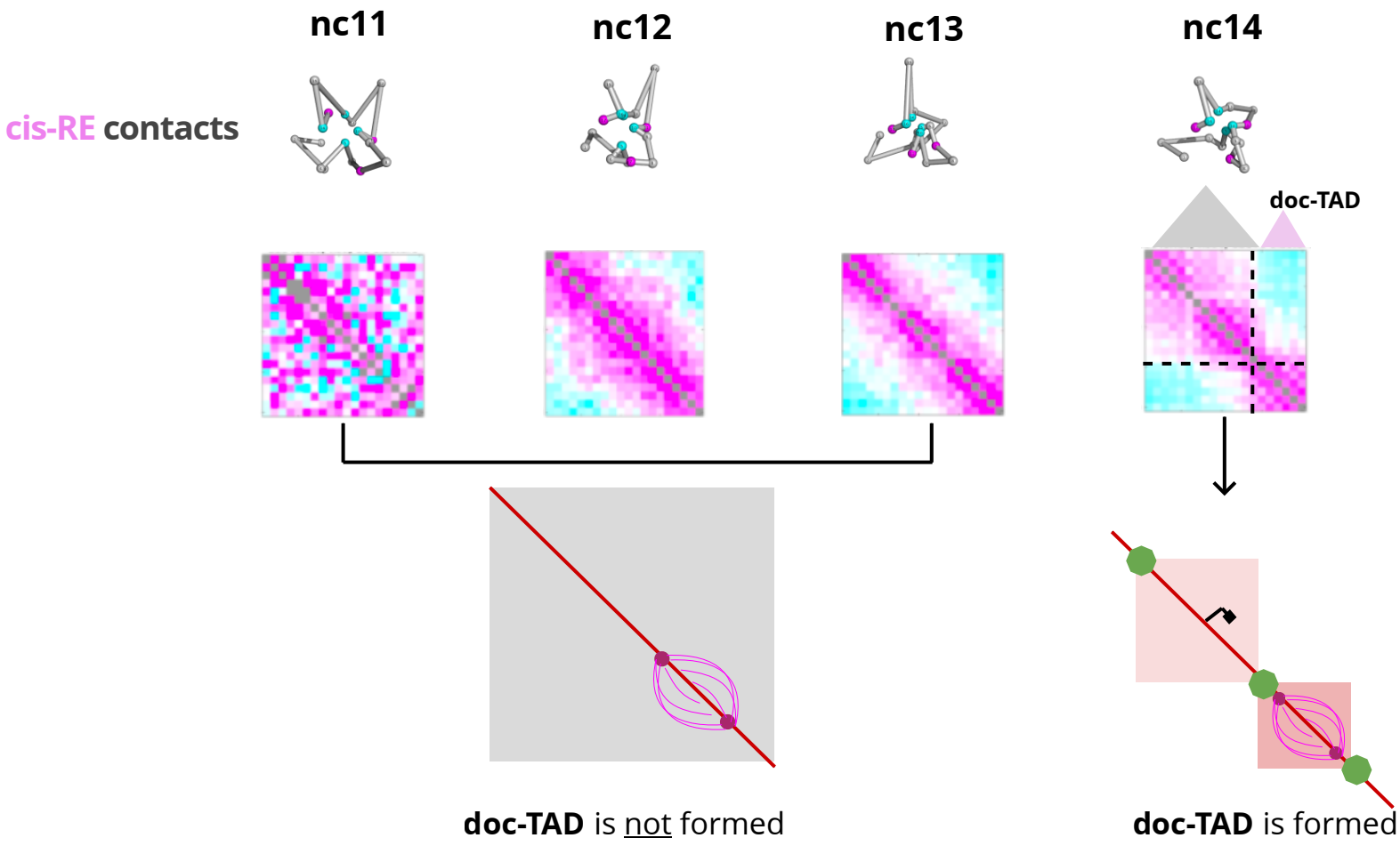


Is the docTAD established as early?

3D networks of cis-RE elements during development



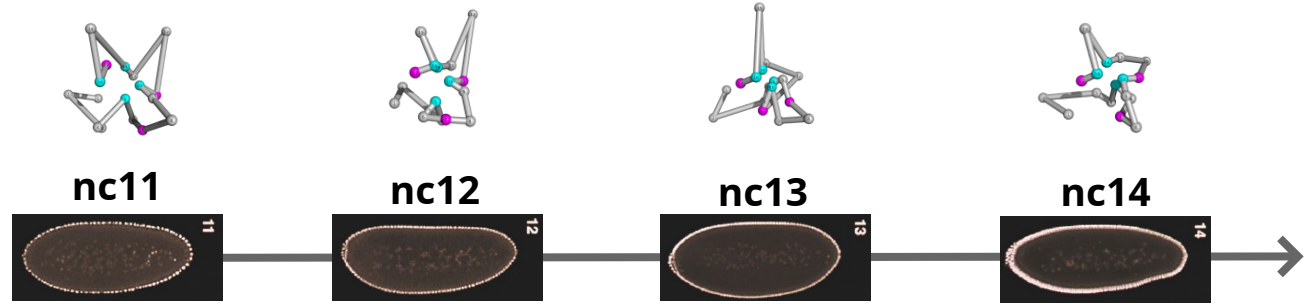
Is the doc-TAD established as early?



Hug, **Cell** (2017); Ogiyama, **Mol Cell** (2018)

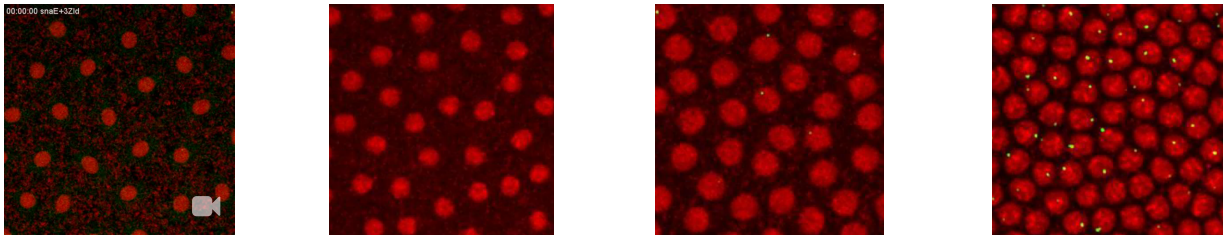
cis-RE networks precede the establishement of doc-TADs at nc14

Do cis-RE networks appear before transcriptional activation of doc genes?

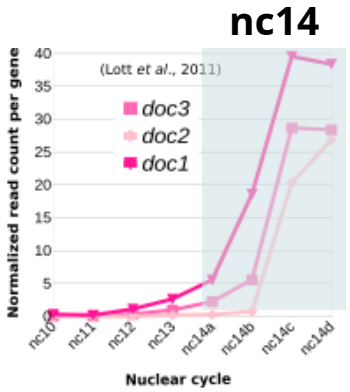


cis-RE contacts

developmental time

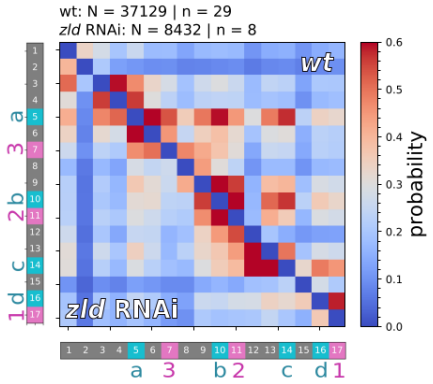


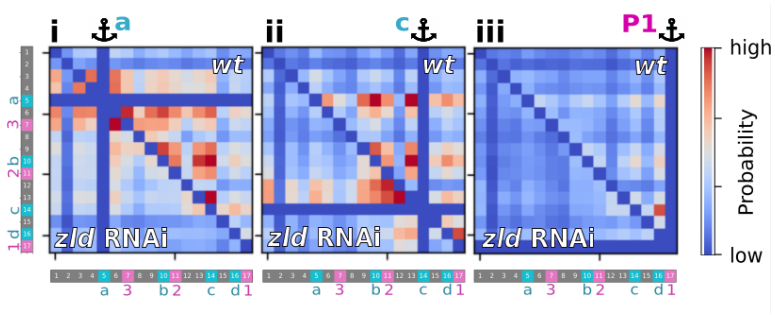
RNAseq

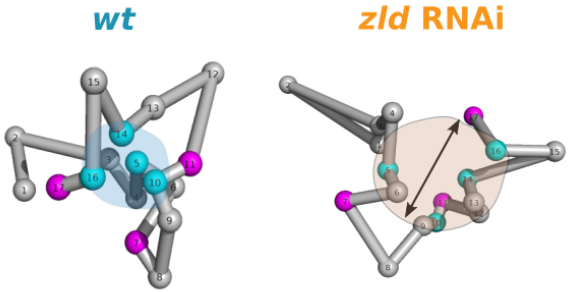


cis-RE networks are established prior to transcriptional activation

What factors are required to form cis-RE networks ?



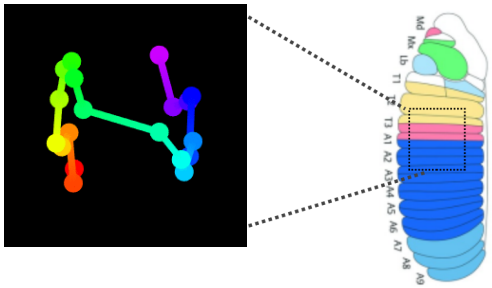




zelda depletion leads to loss of cis-regulatory interactions

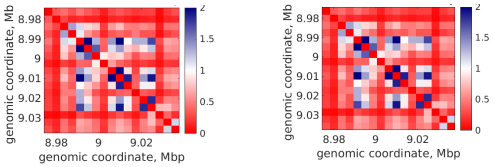
Take home messages

Hi-M



detect **cis-regulatory** logic
&
transcriptional state

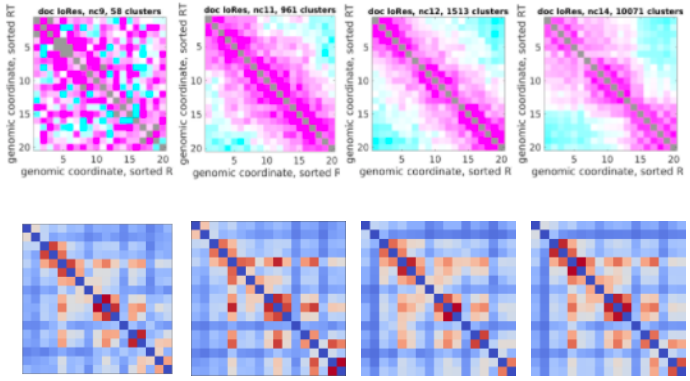
OFF ON



<u>cis-RE hubs</u>
activators AND repressors

TADs

c-RE



3D network of **cis-RE**
<u>precedes</u>
TADs &
transcriptional activation

Espinola, Gotz, etal. BioRxiv (2020)

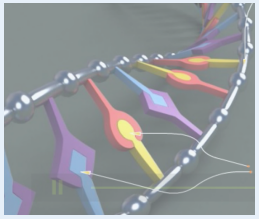
Ing-Simmon, etal. BioRxiv (2020)

Multiscale chromosome organization

Mitotic chromosomes
Flemming, 1885

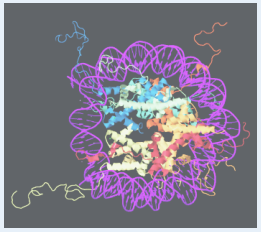
single-cell imaging
structurally heterogeneous

B-DNA
Watson & Crick, 1953
Franklin & Gosling, 1953



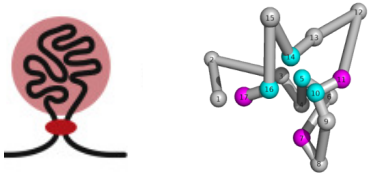
unclear how 3D structure encodes function

Nucleosomes
Luger, 1997



Use of interdisciplinary methods will be key to get full understanding

TADs, hubs, compartments, etc



~200nm, 50kb - 1 Mb

Chromosome Territories
Bolzer, 2005

3D genomics 2020 !

Acknowledgments

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Postdoc
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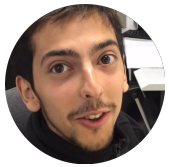
IR INSERM
Physics

Christophe Houbron
IE INSERM
Biology



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Mounia Lagha
IGMM, CNRS

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for talk feedback!

Scan
to discover !

did you understand?

where you lost? where?

were you confused by a reference?