BIO-463
Genomics and bioinformatics

Lecture 14: Activation of transcription

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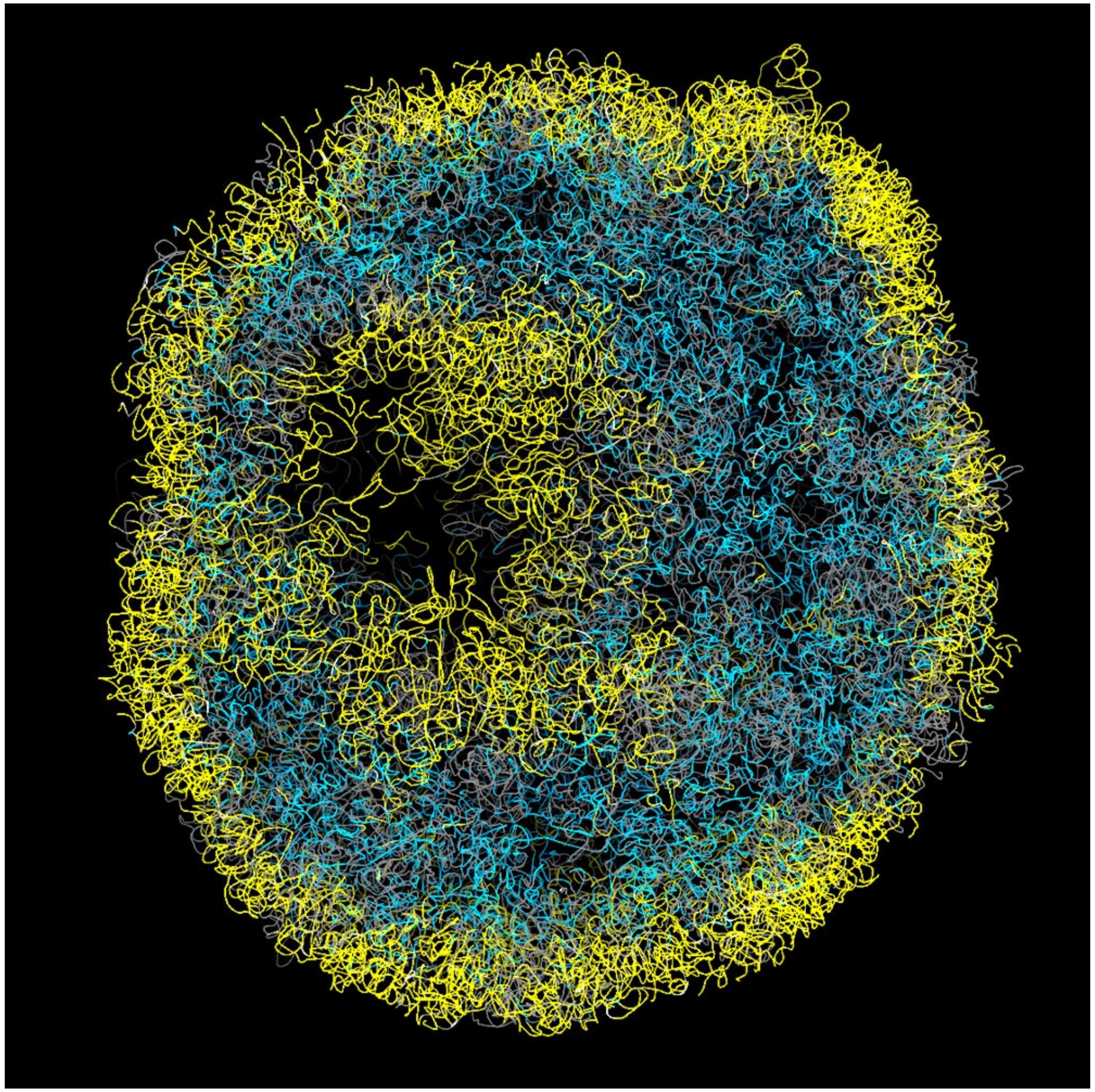
EPFL

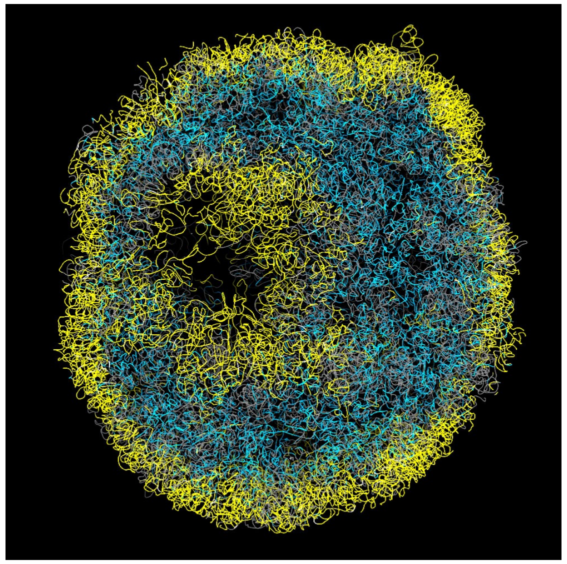
Genome is entangled



Stevens TJ et al. Nature (2017)

- Nucleotide size: 1/3 nanometer
- Genome size: $2 \times 3 \times 10^9$ nucleotides: 2 m / 10^{-14} litres
- Nucleus volume: 10^{-13} litres (diameter 5-10 microns)
- Typical number of binding sites: 500-5000
- Number of protein copies per cell: 1000-10000

How long do we need to search?

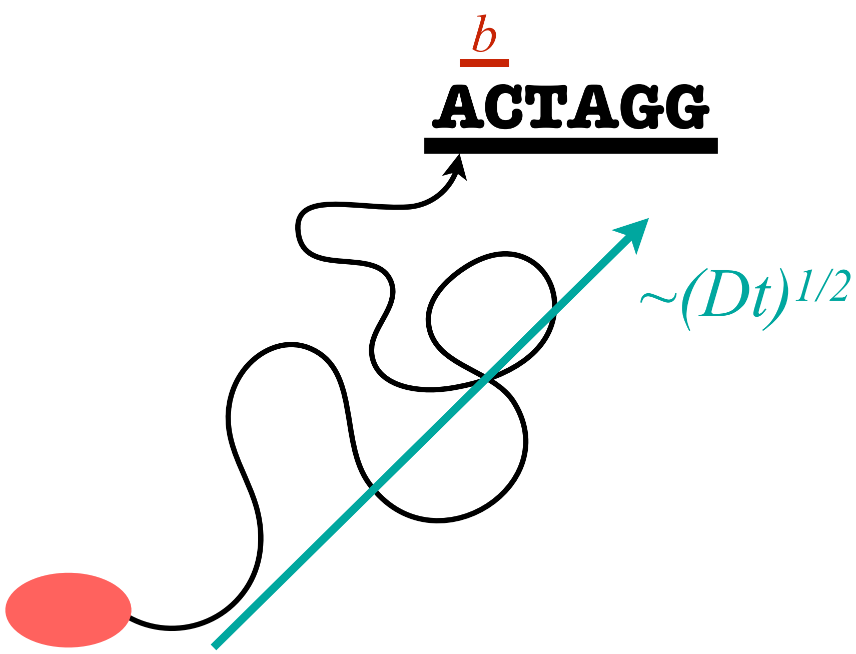


$$b = 0.34 \cdot 10^{-9} [m]$$

$$D_{\mathrm{3d}} = 10^{-10} [m^2 s^{-1}]$$

$$k_{\mathrm{on}} \approx 4 \cdot 10^{-19} [m^3 s^{-1}] = 4 \cdot 10^{-16} [\ell s^{-1}]$$

$$\approx 2.4 \cdot 10^{8} [M^{-1} s^{-1}]$$



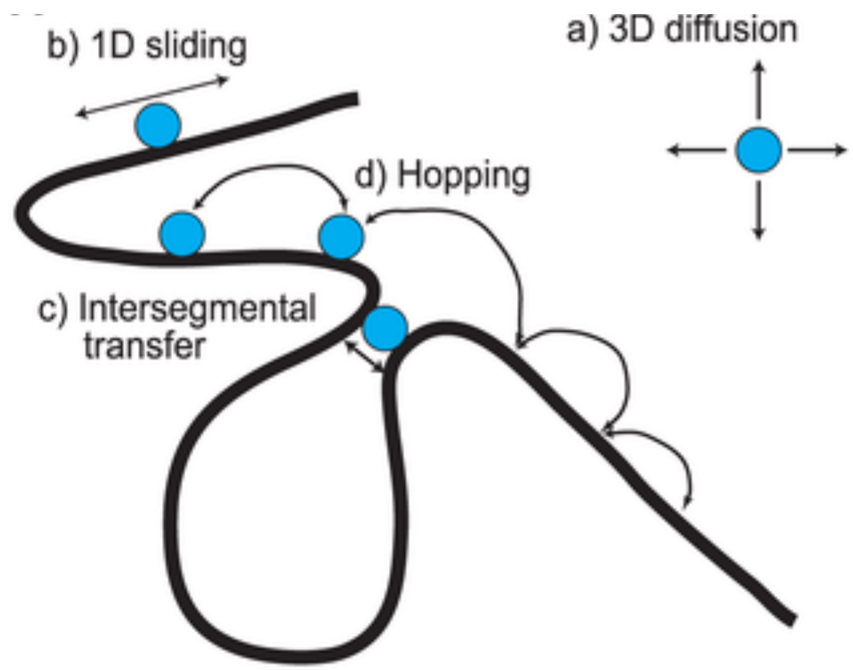
Riggs experiment (1970!): $k_{\mathrm{on}} \approx 10^{10}$

In time units: $t_0 = (k_{\mathrm{on}} [S])^{-1}$

$$= \left(k_{\mathrm{on}} \frac{\#\mathrm{sites}/\#\mathrm{proteins}}{6 \cdot 10^{23}\, Vol(\mathrm{nucleus})} \right)^{-1}$$

$$\approx 10[s]$$

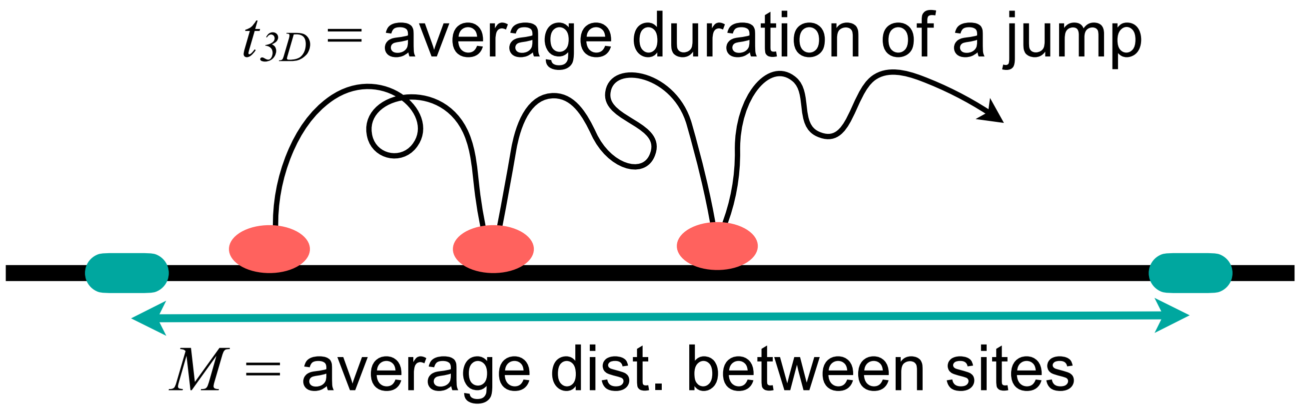
Facilitated diffusion



Schmidt HG et al. PLoS ONE 9 (2014)

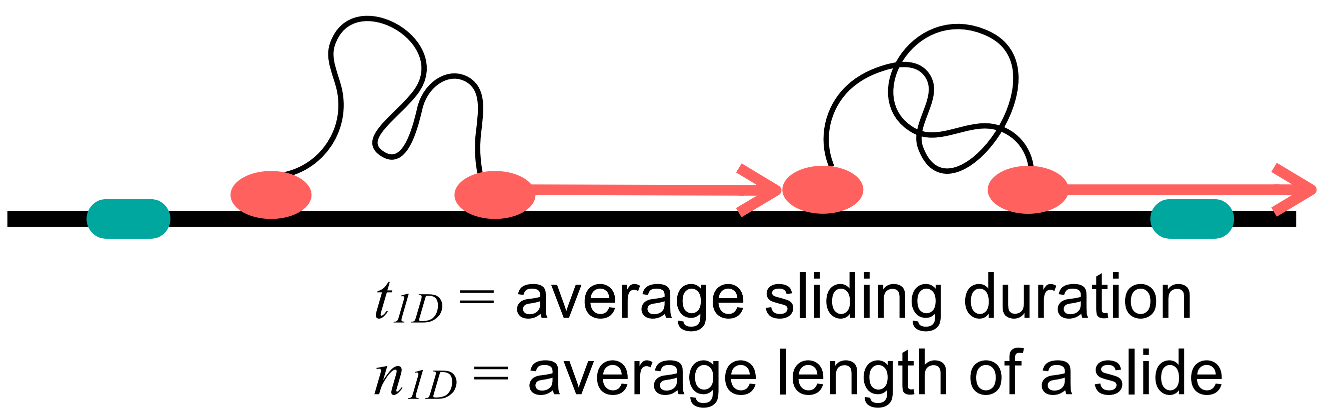
- Hypothesis: proteins alternate free (3D) diffusion and (1D) sliding along the genome
- This accelerates the binding site search by concentrating it to a neighbourhood of the genome
- Already proposed in 1986 by Berg and von Hippel

Facilitated diffusion



3D jumps only:

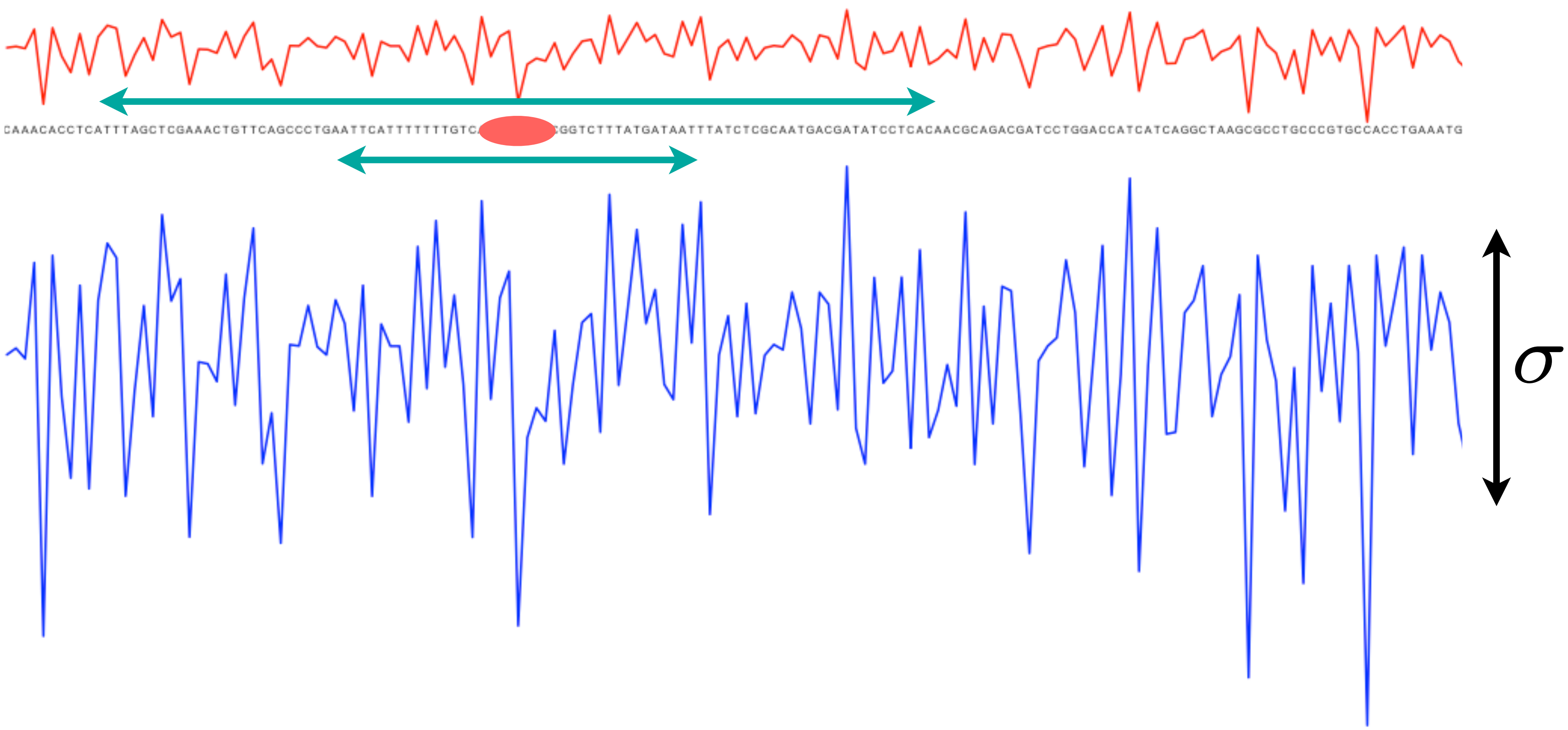
$$t_0 = M t_{3\mathrm{D}}$$

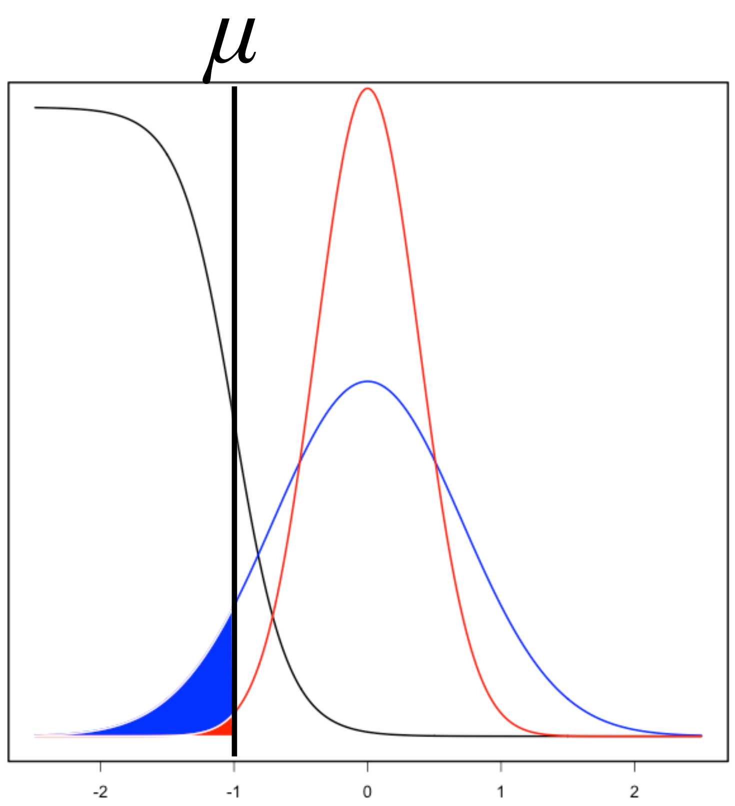


Alternating with 1D sliding

$$\begin{aligned} t_{\mathrm{search}} &= \frac{M}{n_{1\mathrm{D}}}(t_{1\mathrm{D}} + t_{3\mathrm{D}}) \\ &= \frac{t_0}{n_{1\mathrm{D}}}\left(\frac{t_{1\mathrm{D}}}{t_{3\mathrm{D}}} + 1\right) \\ &= \frac{t_0}{\sqrt{D_{1\mathrm{D}} t_{1\mathrm{D}}}}\left(\frac{t_{1\mathrm{D}}}{t_{3\mathrm{D}}} + 1\right) \\ &\geq \frac{2t_0}{\sqrt{D_{1\mathrm{D}} t_{3\mathrm{D}}}} \end{aligned}$$

How to slide fast?





Diffusion over a random landscape: $D_{1\mathrm{D}} \approx e^{-\gamma(\beta\sigma)^2}$

<u>Speed-stability paradox:</u> it is impossible to have both a strong affinity for some target binding sites and a fast diffusion across a majority of non-specific sites

Mitigating effects

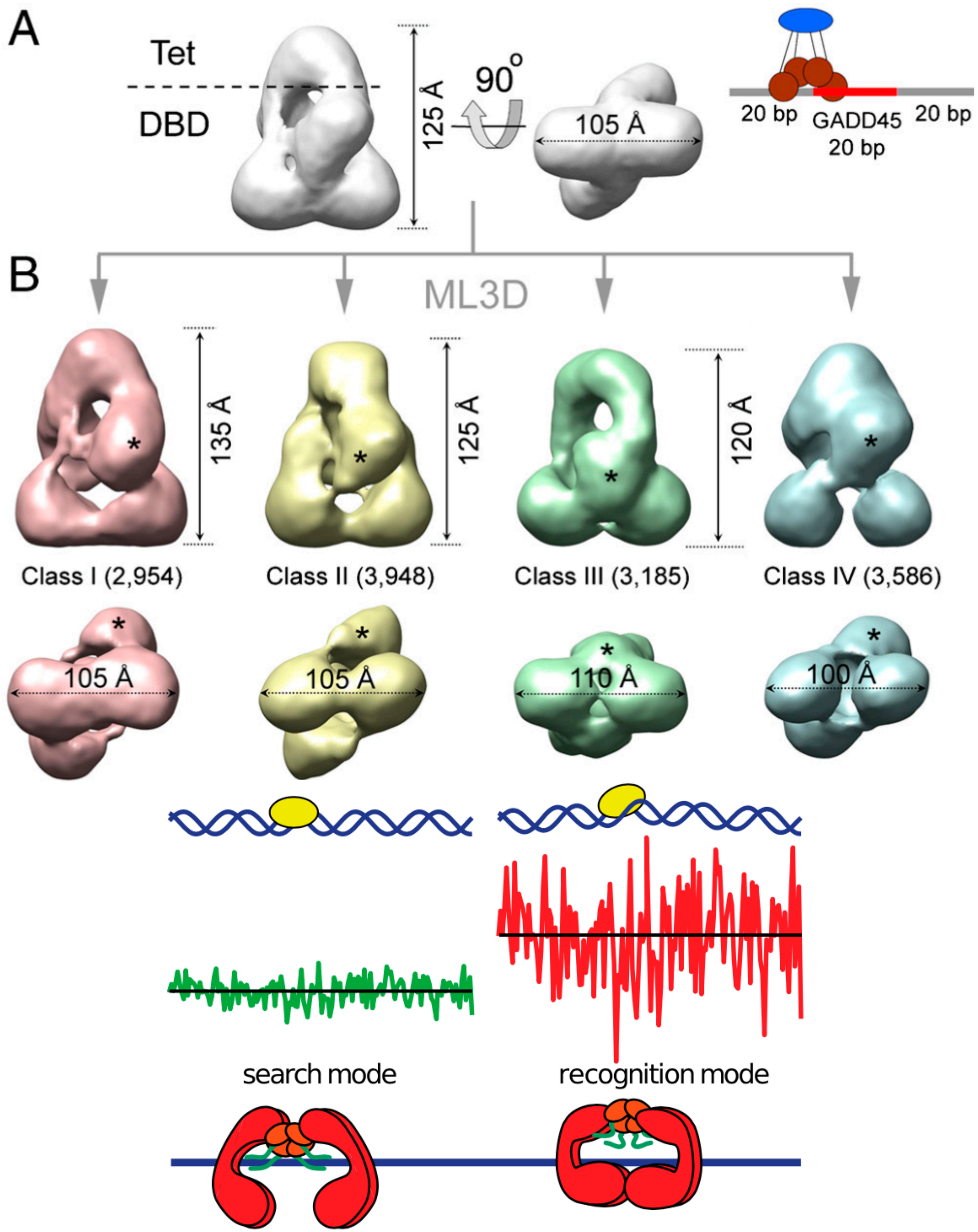
Crowded genome effect: only 5-10% of genome is available (not occupied by other proteins)

This reduces the search space, increases the 3D time, and reduces the 1D slide length.

$$t_{\text{search}} = \frac{\epsilon M}{\min(n_{1\text{D}}, n_{\text{free}})} \left(t_{1\text{D}} + \frac{t_{3\text{D}}}{\epsilon} \right)$$

Folded genome effect: nucleus is compartmented into euchromatin (accessible) and heterochromatin (inaccessible), this will mostly only reduce the 3D search space.

P53 conformational change



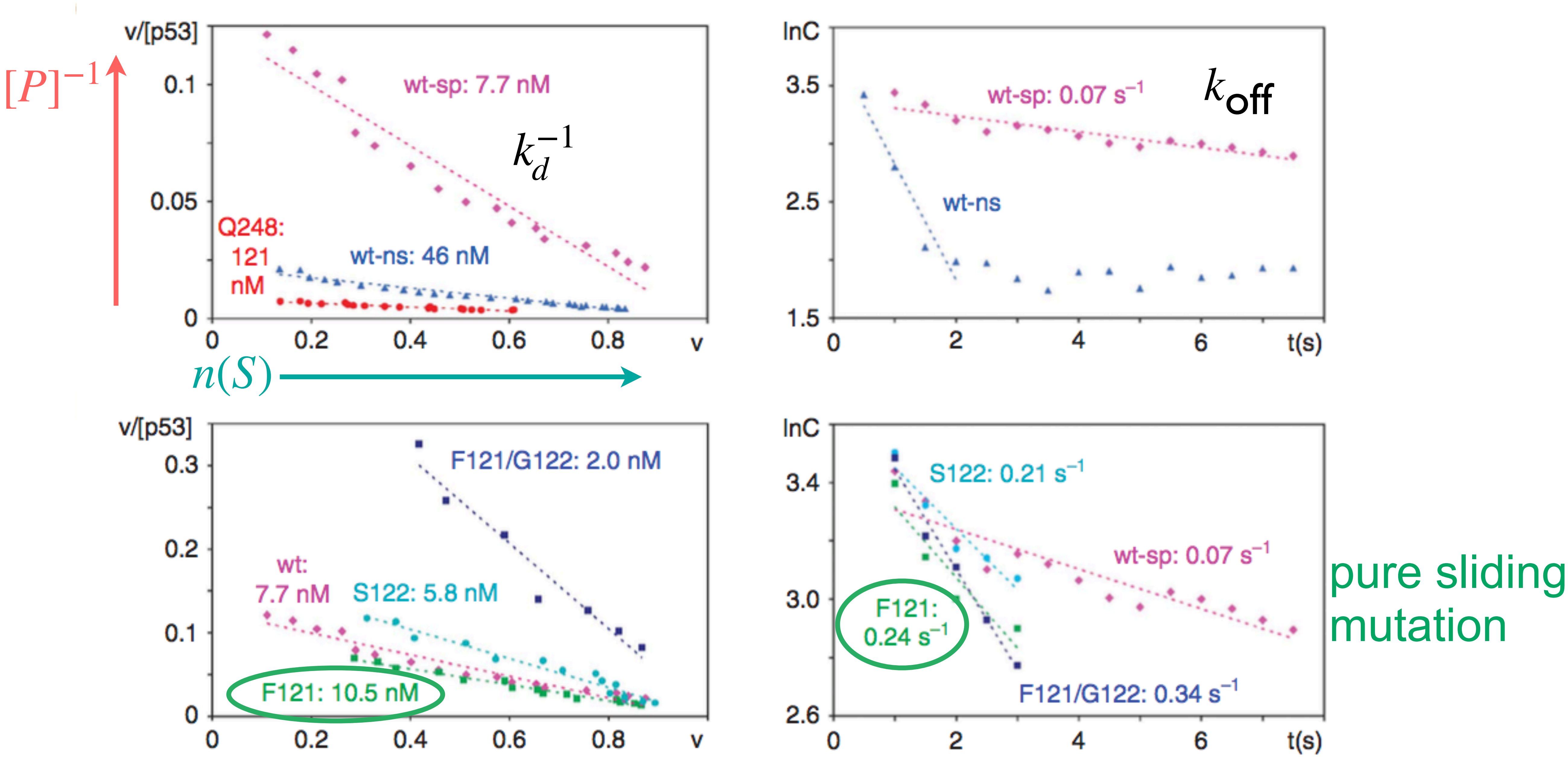
Melero et al PNAS (2011)
Leith et al PNAS (2012)

P53 conformational change

Petty TJ et al. EMBO J (2011)

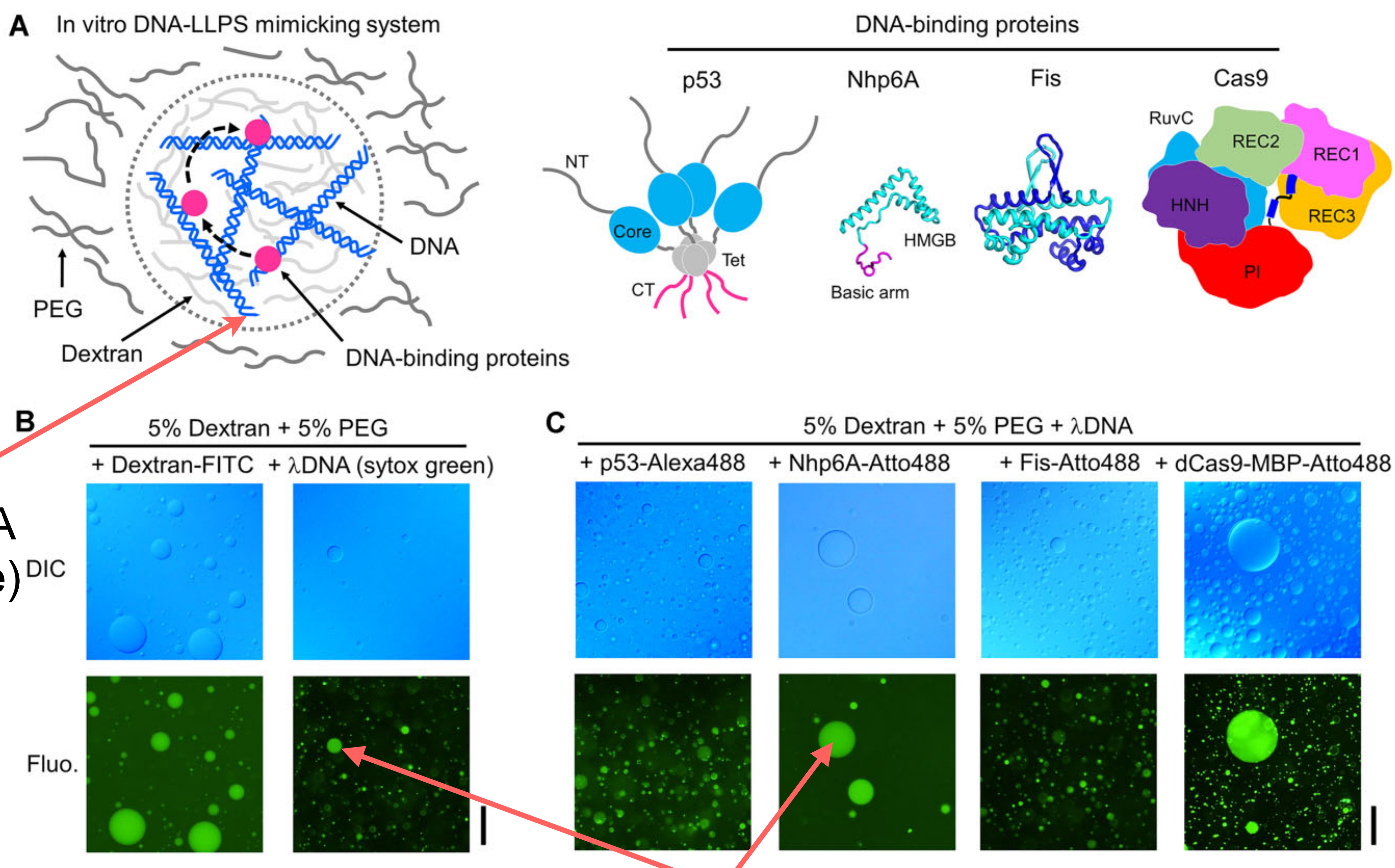
$$\text{p53} + \text{DNA} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{p53/DNA} \qquad \text{p53} + \text{DNA} \underset{k_{off1}}{\overset{k_{on1}}{\rightleftharpoons}} \text{p53/DNA} \underset{k_{off2}}{\overset{k_{on2}}{\rightleftharpoons}} \textit{p53}\text{/DNA}$$

$$K_D = \frac{[\text{p53}]\,[\text{DNA}]}{[\text{p53/DNA}]} = \frac{k_{off}}{k_{on}} \qquad K_D = \frac{[\text{p53}][\text{DNA}]}{[\textit{p53}\text{/DNA}]} = \frac{k_{offo}}{k_{ono}}$$



A general property of TFs?

Kamagata K et al. NAR (2023)

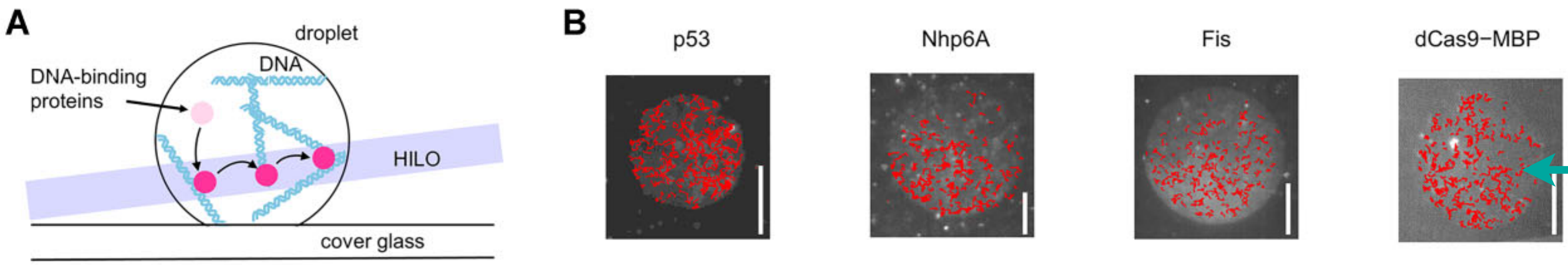


Unspecific DNA
(no binding site)

In vitro observations of TFs in droplets with DNA
to mimic in-vivo compartmentalization

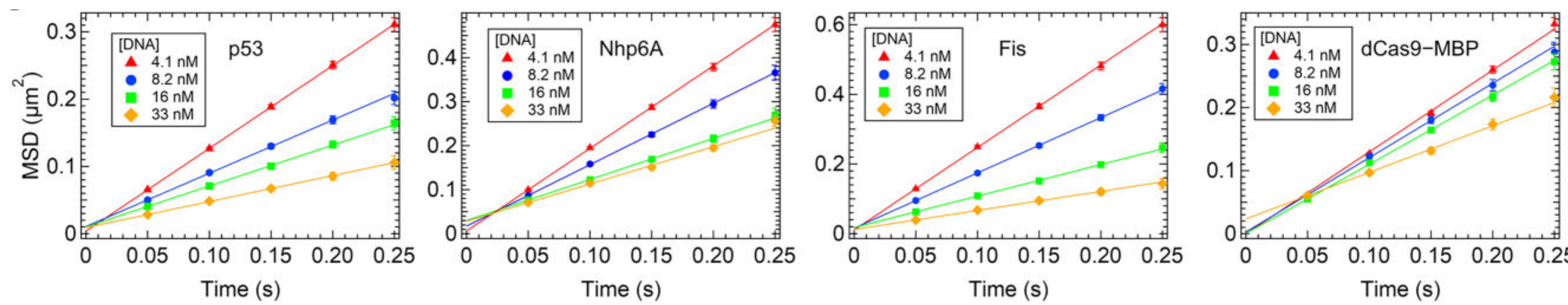
A general property of TFs?

Kamagata K et al. NAR (2023)

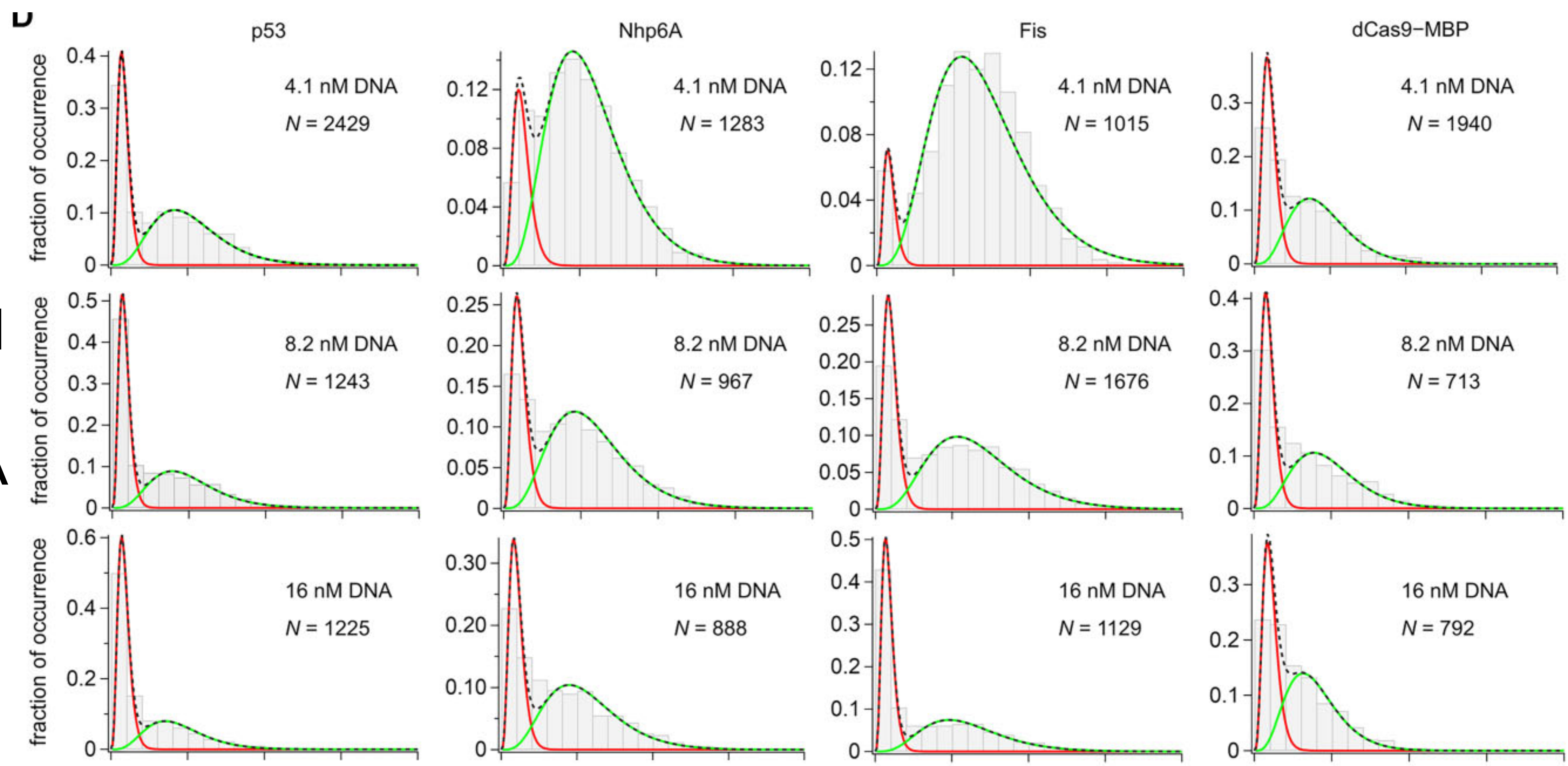


record motion of individual TFs

Displacement = speed × time



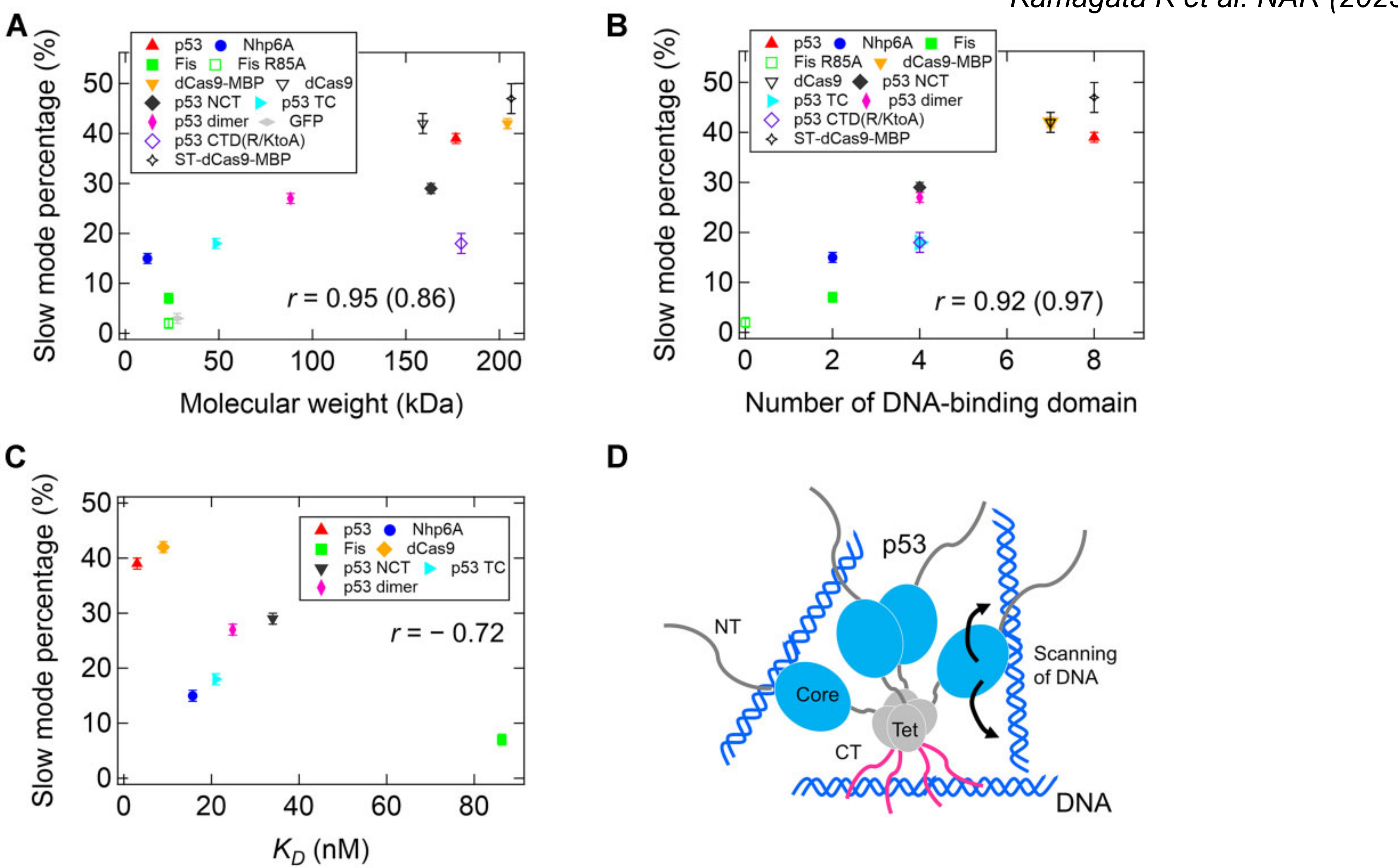
speed



- speed is always bimodal
- ratio slow/fast depends on concentration of DNA

A general property of TFs?

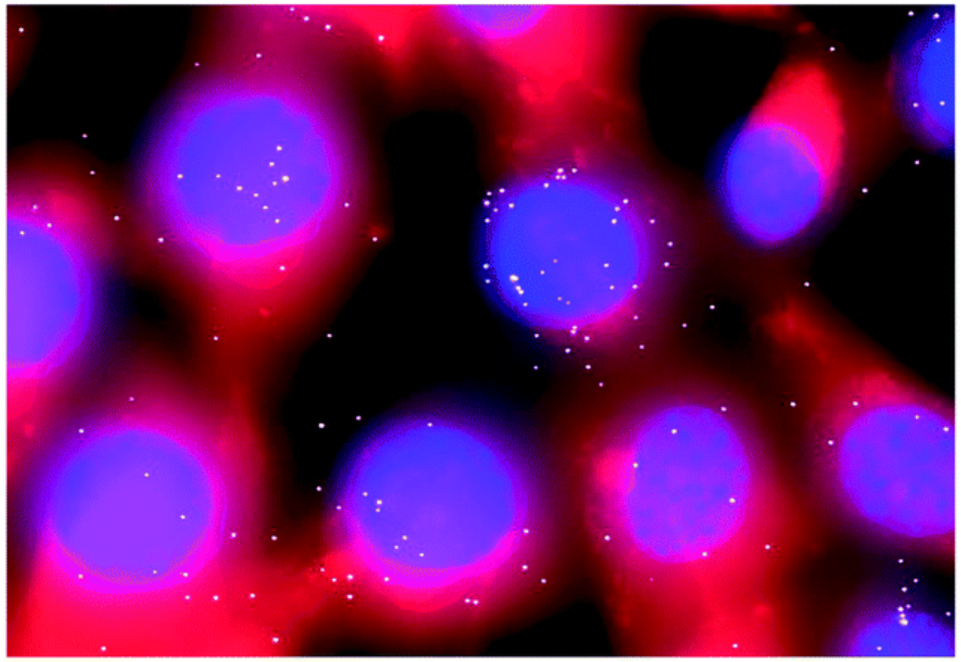
Kamagata K et al. NAR (2023)

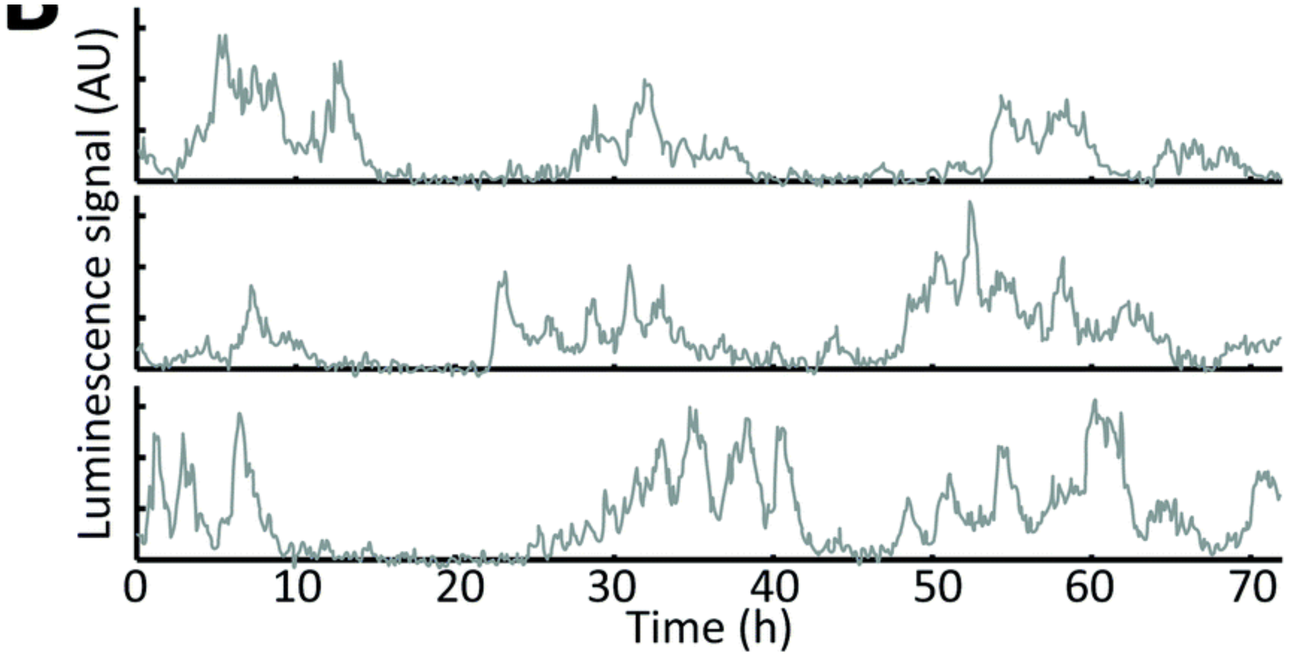


Slow mode correlates with

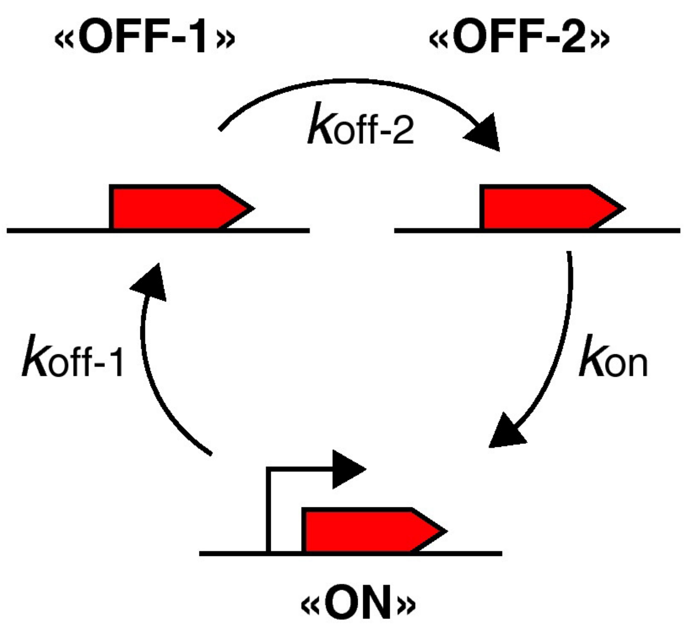
- size of protein (heavier is slower)
- number of DNA binding domains on the protein (more is slower)
- the affinity (small K_D / small k_{off} / high affinity is slower)

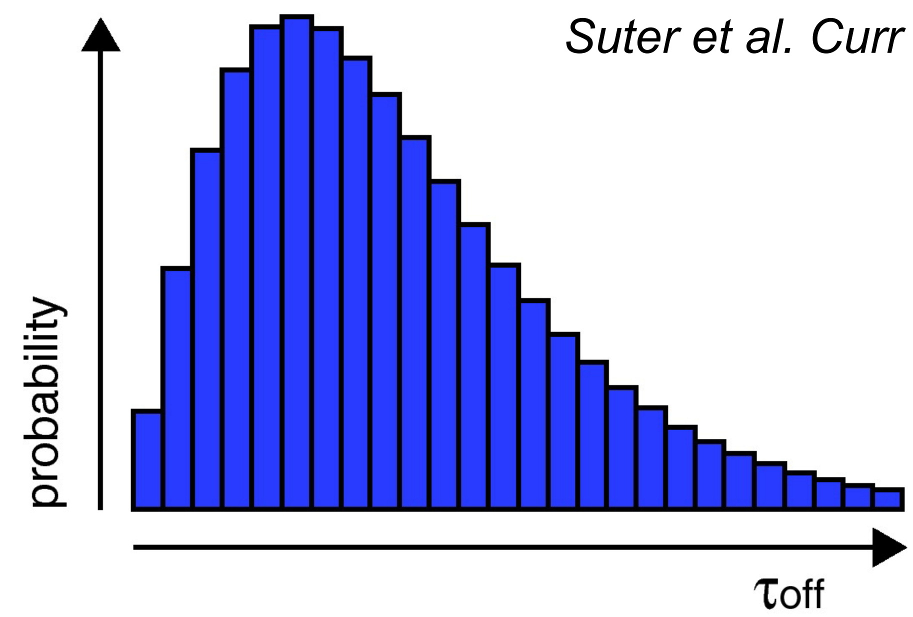
Transcriptional bursts





Nicolas et al. Mol BioSyst (2017)
Suter et al. Curr Op Cell Biol (2011)

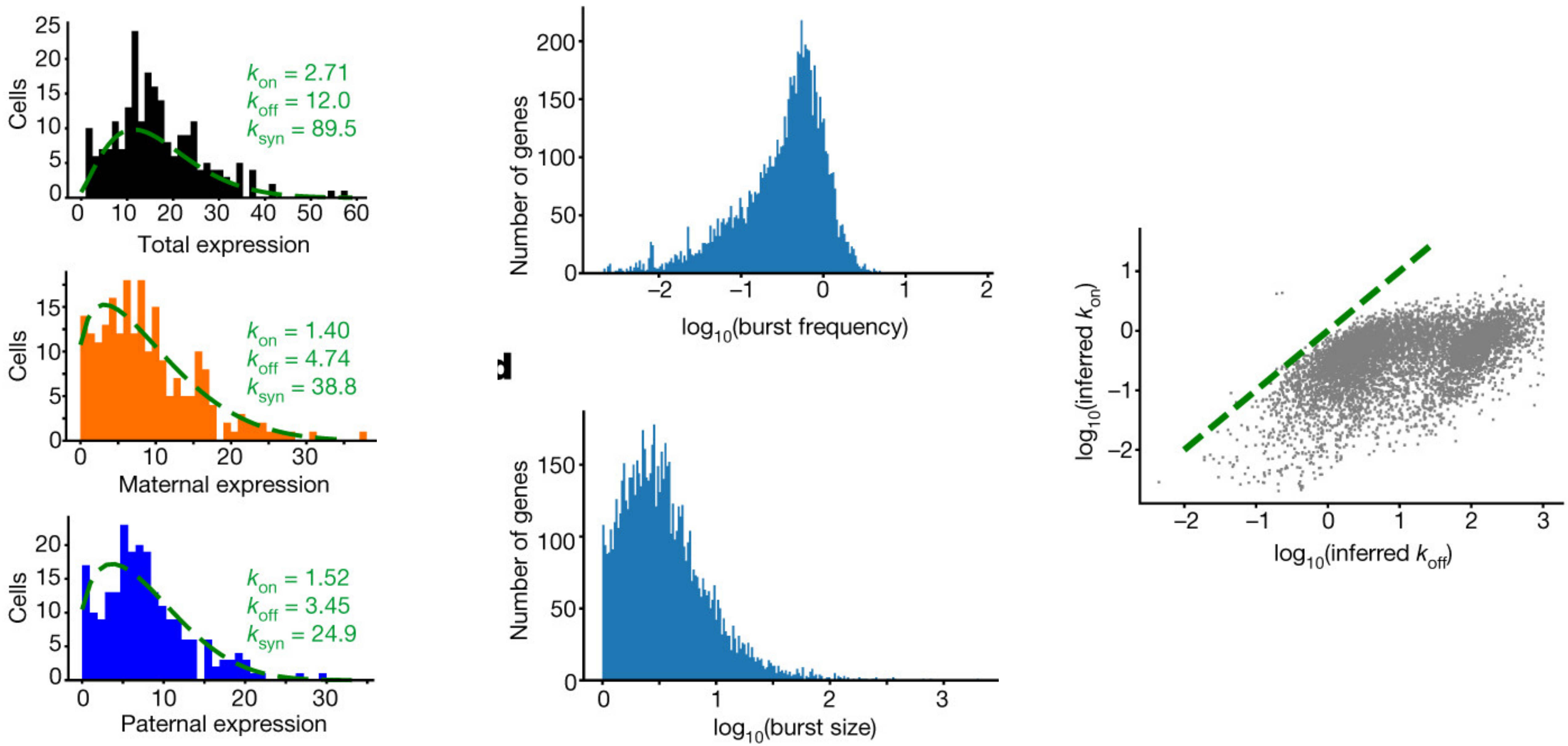




Active genes are transcribed in "bursts": they alternate between "on" phases of RNA synthesis and "off" times without transcription

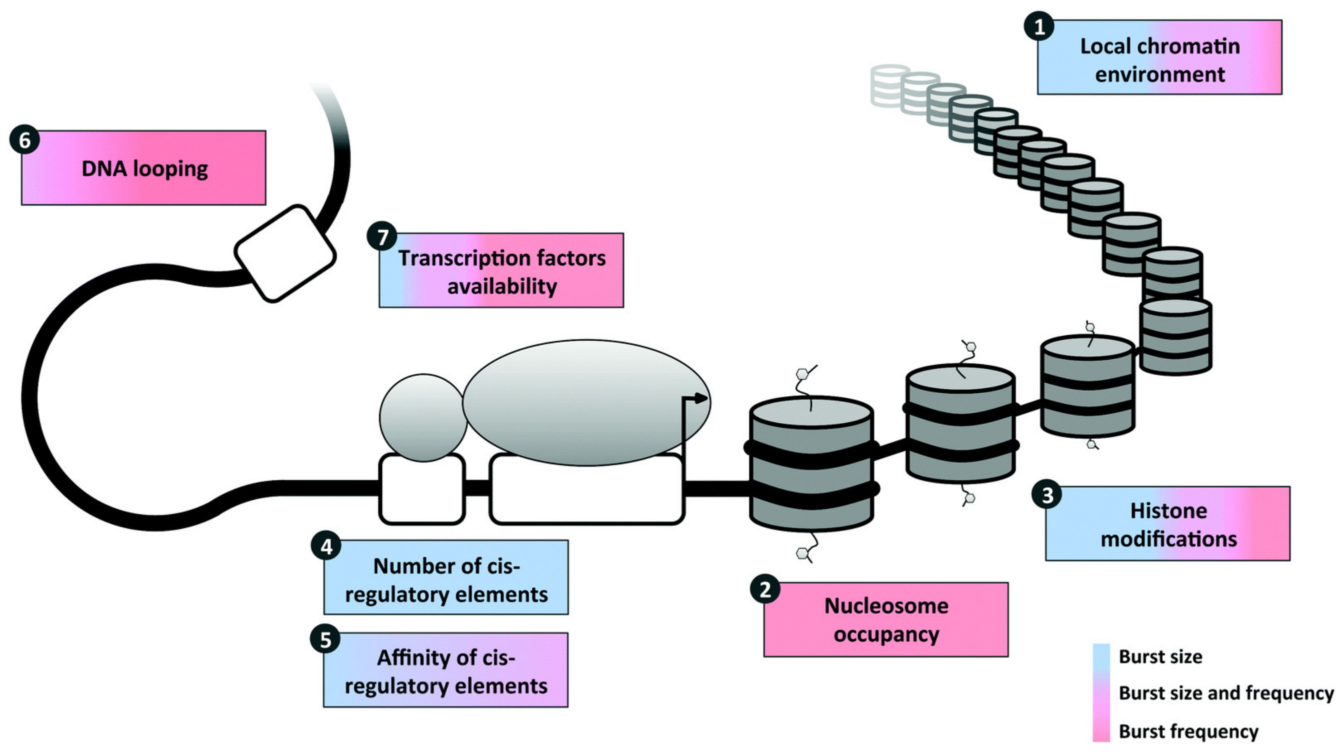
Transcriptional bursts

Larsson AJM et al. Nature (2019)



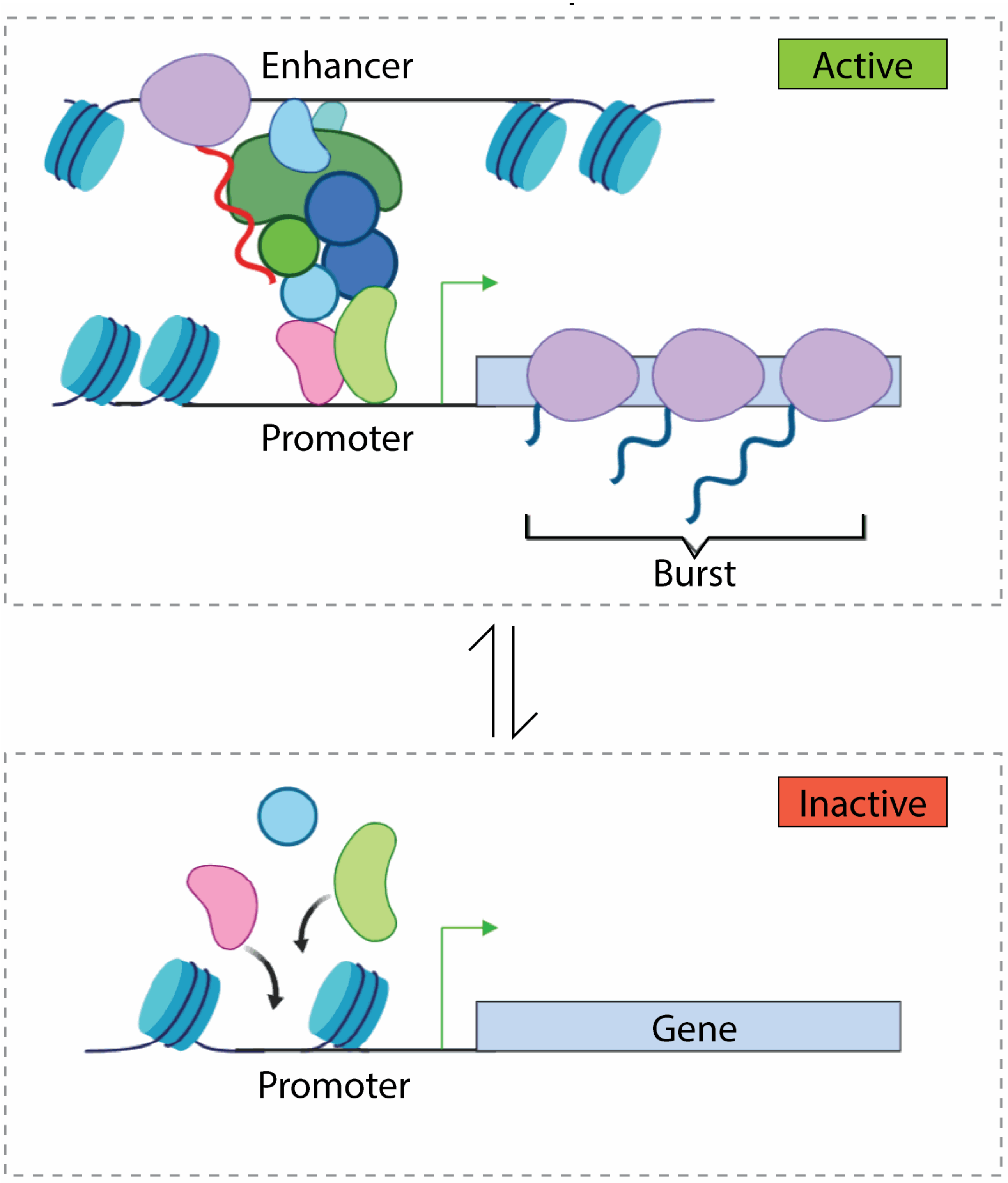
Distributions of "on" times and "off" times can be inferred using the random "telegraph" model

Looping theory of enhancer-promoter interaction



Nicolas et al. Mol BioSyst (2017)

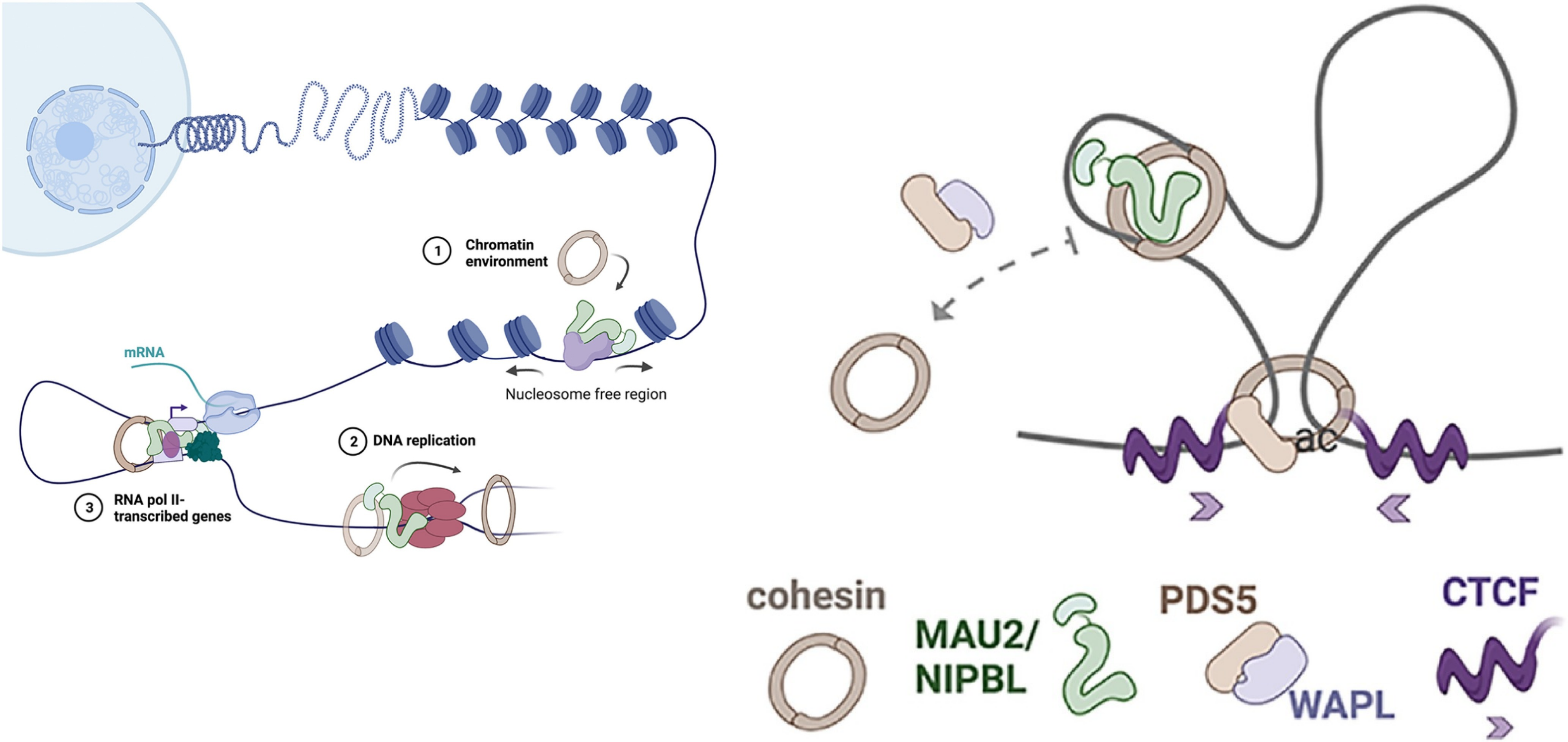
- RNA synthesis requires the full transcription machinery
- Loss of any component turns it off
- Distant enhancer facilitates this assembly via chromosome looping



Rodriguez J & Larson DR Annu Rev Biochem (2020)

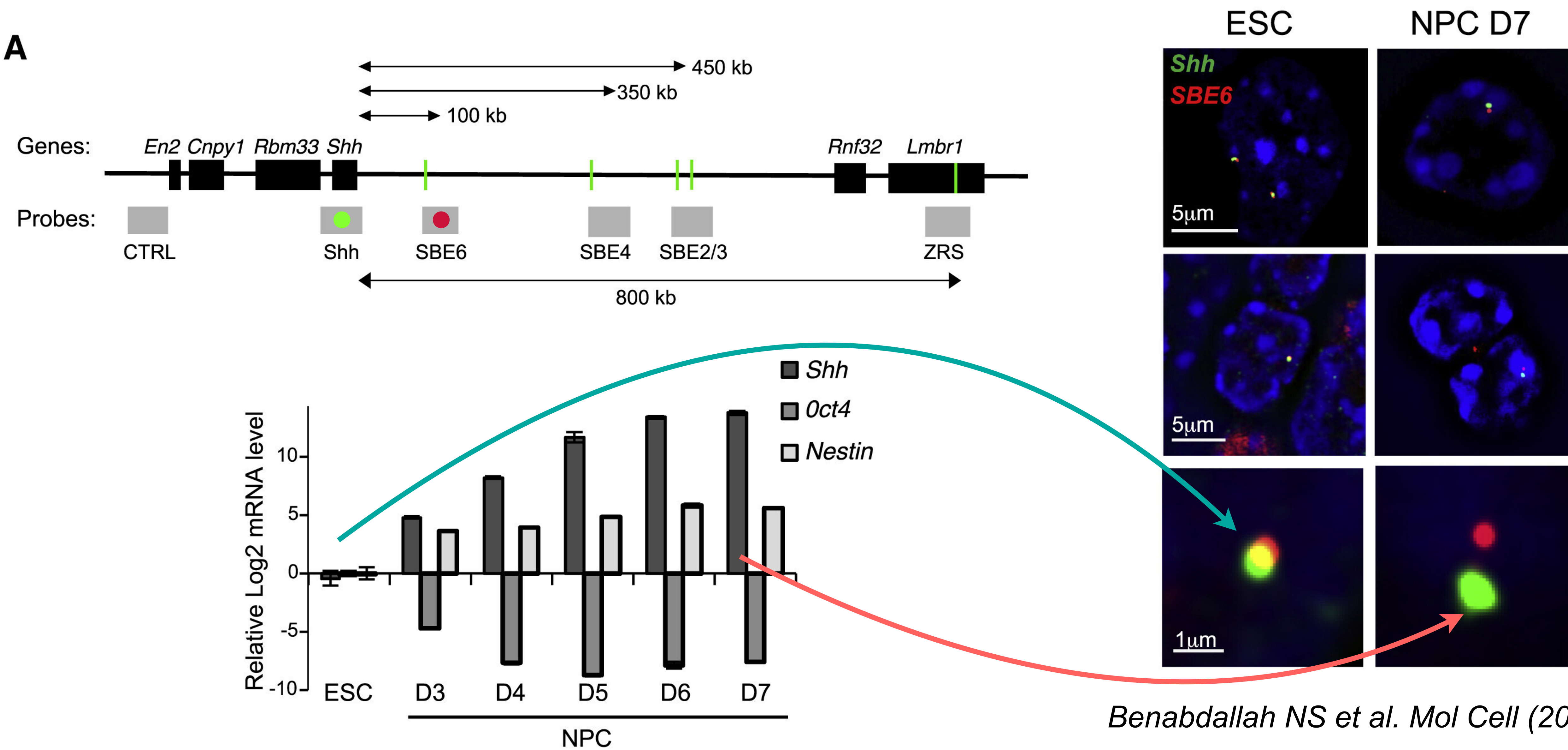
Looping theory of enhancer-promoter interaction

Alonso-Gil D & Losada A. Trends in Cell Biology (2023)



- CTCF binding fixes loop ends
- NIPBL/MAU2 pulls loop through (ATP-dependent)
- PDS5 recruits Cohesin and is repressed by WAPL

Transcription does not imply contact

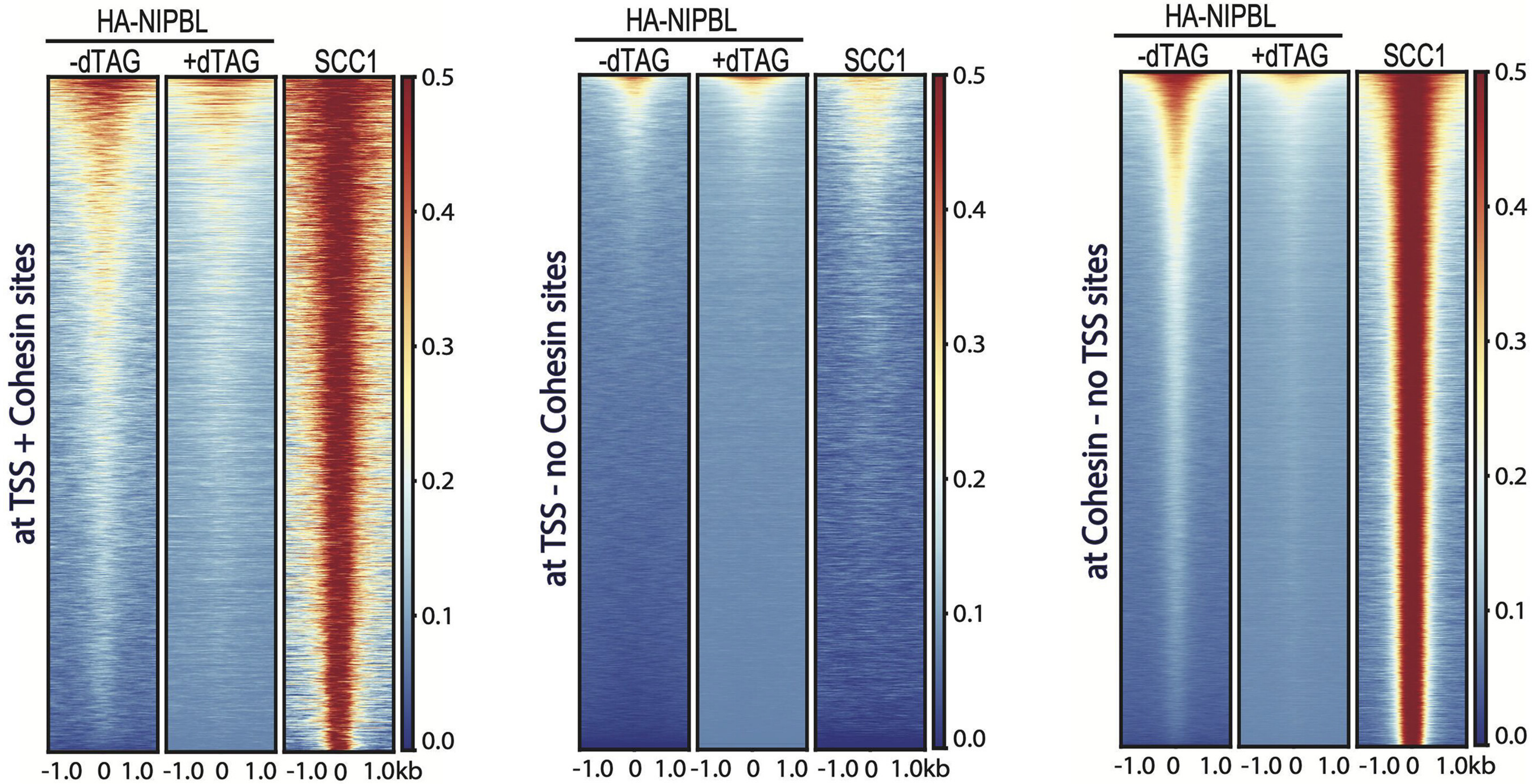


Benabdallah NS et al. Mol Cell (2019)

Enhancer-promoter contact before activation of Shh (ESC) is lost during expression (NPC)

Not many loops created at promoters

Banigan EJ et al. PNAS (2023)



±pulling

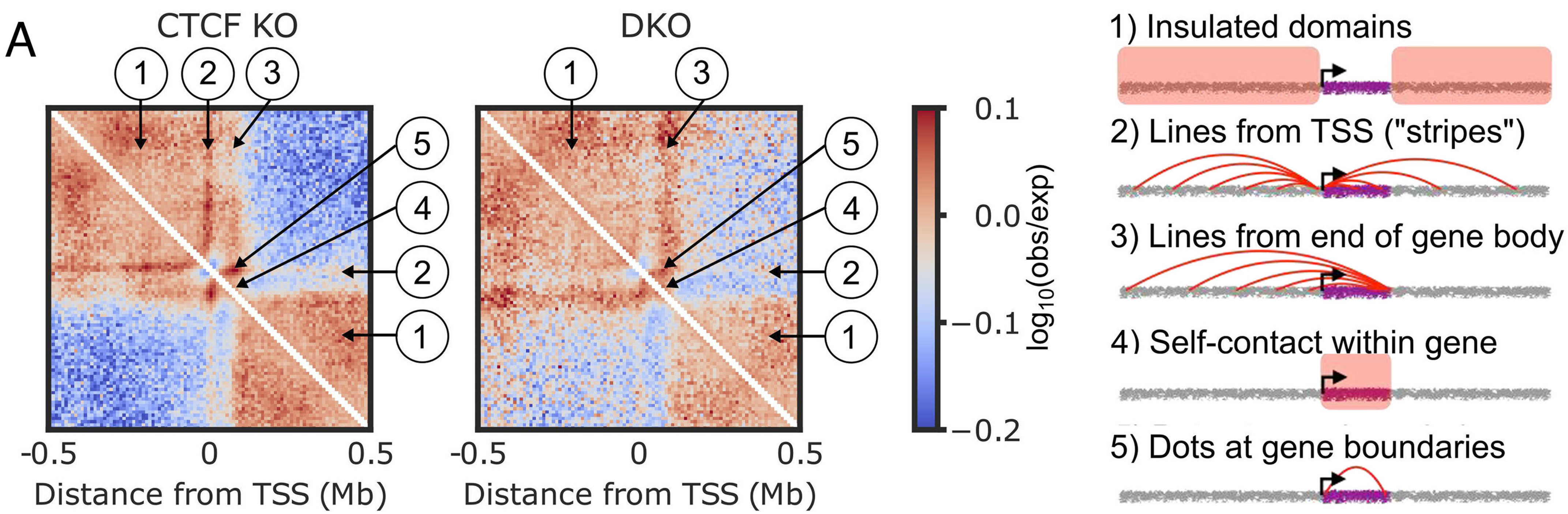
Cohesin

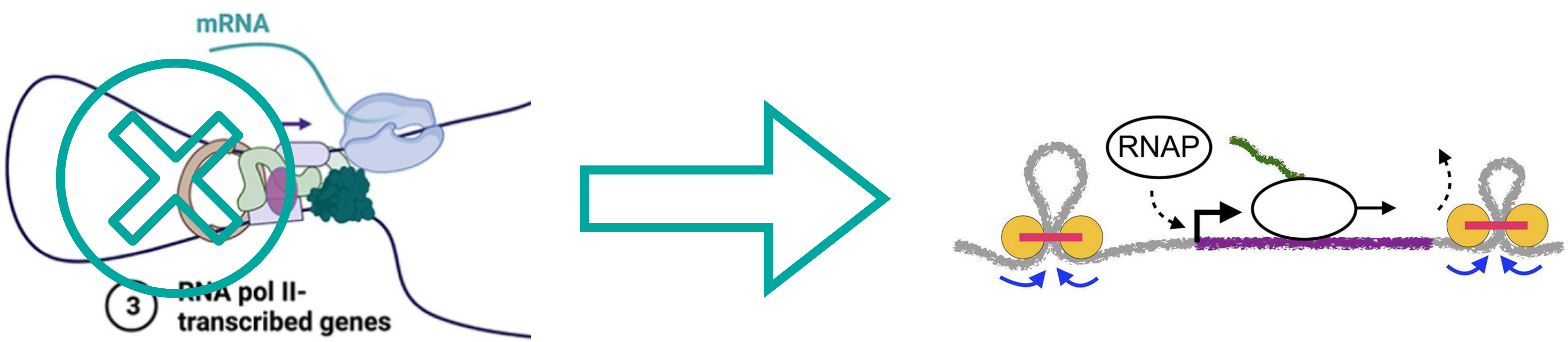
ChIP-seq shows association of NIPBL and Cohesin is not enriched at TSS

Not many loops between promoters and enhancers

KO CTCF / CTCF+Wapl

Banigan EJ et al. PNAS (2023)

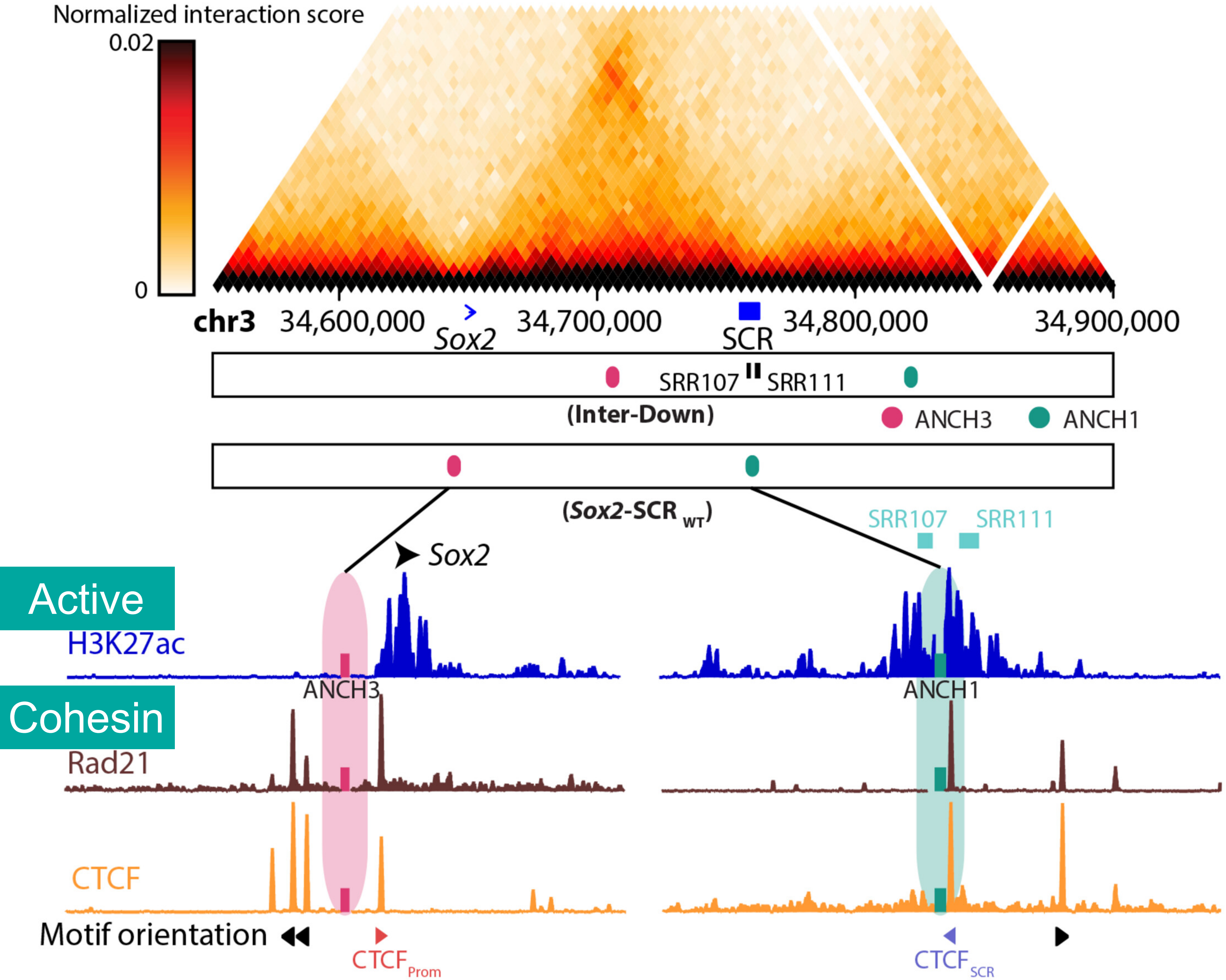




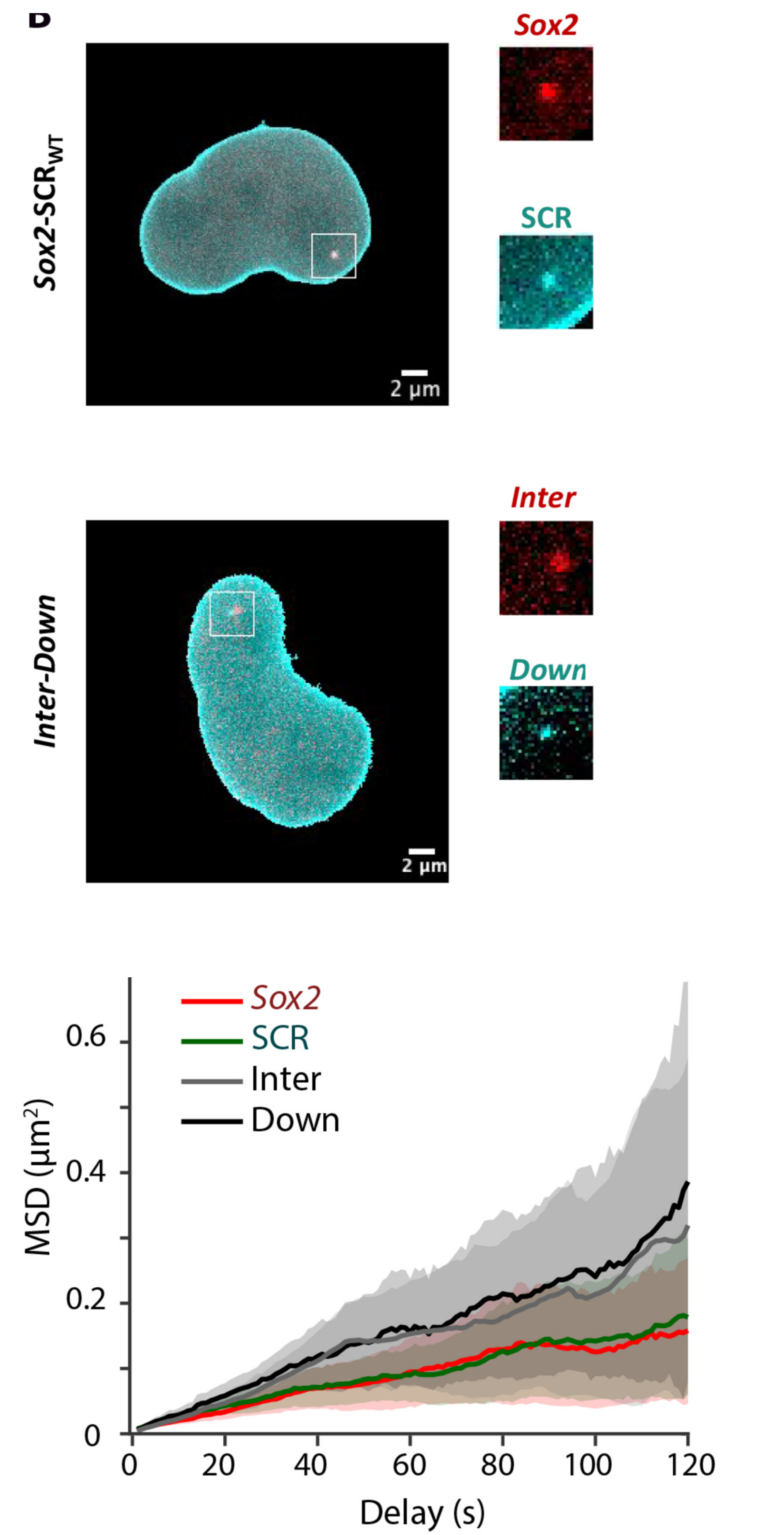
HiC data more compatible with loops either sides of transcribed genes than between promoter and distant enhancer

Mobility of enhancers vs promoters

Platania A et al. Sci Adv (2024)

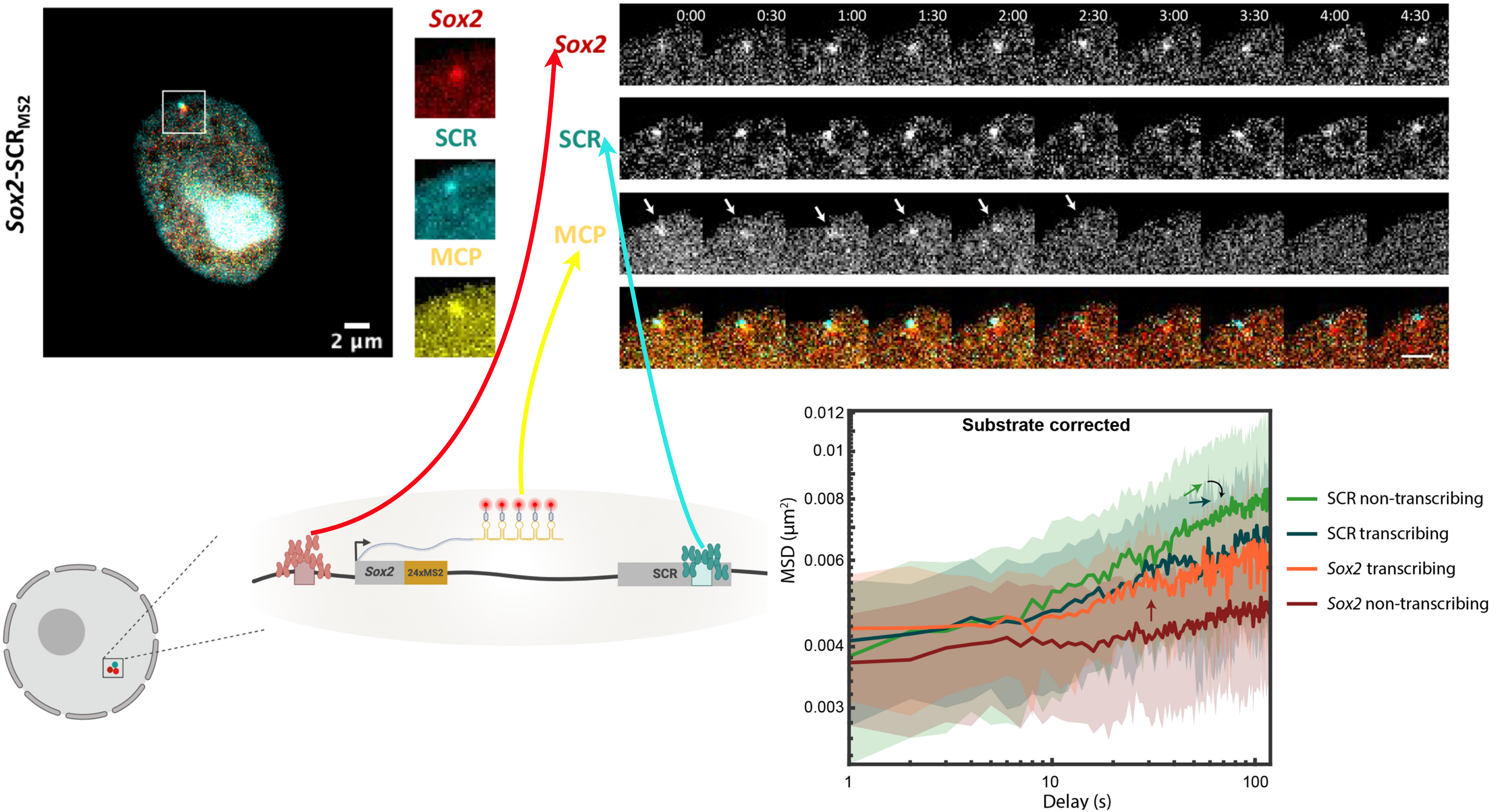


Live-cell imaging to measure promoter-enhancer distance



No "loop pulling" effect

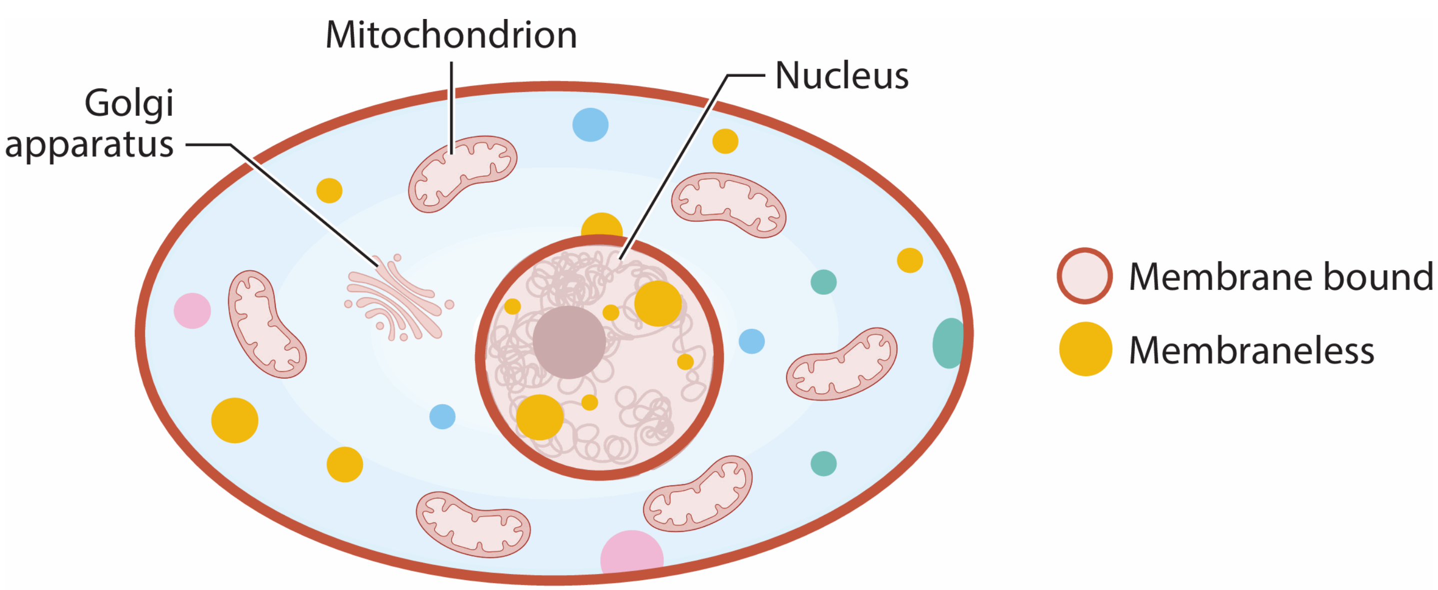
Platania A et al. Sci Adv (2024)

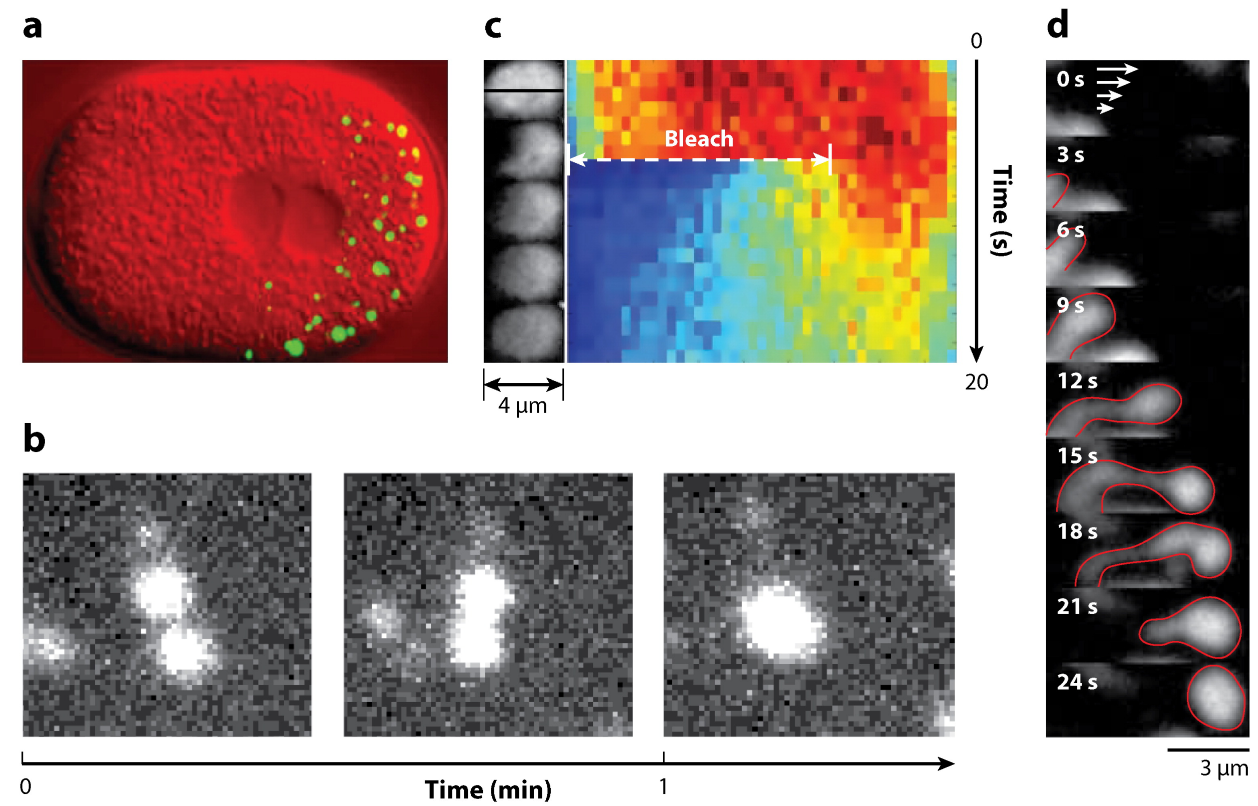


Transcription increases promoter mobility, decreases enhancer mobility and synchronizes them

Phase-separated droplets

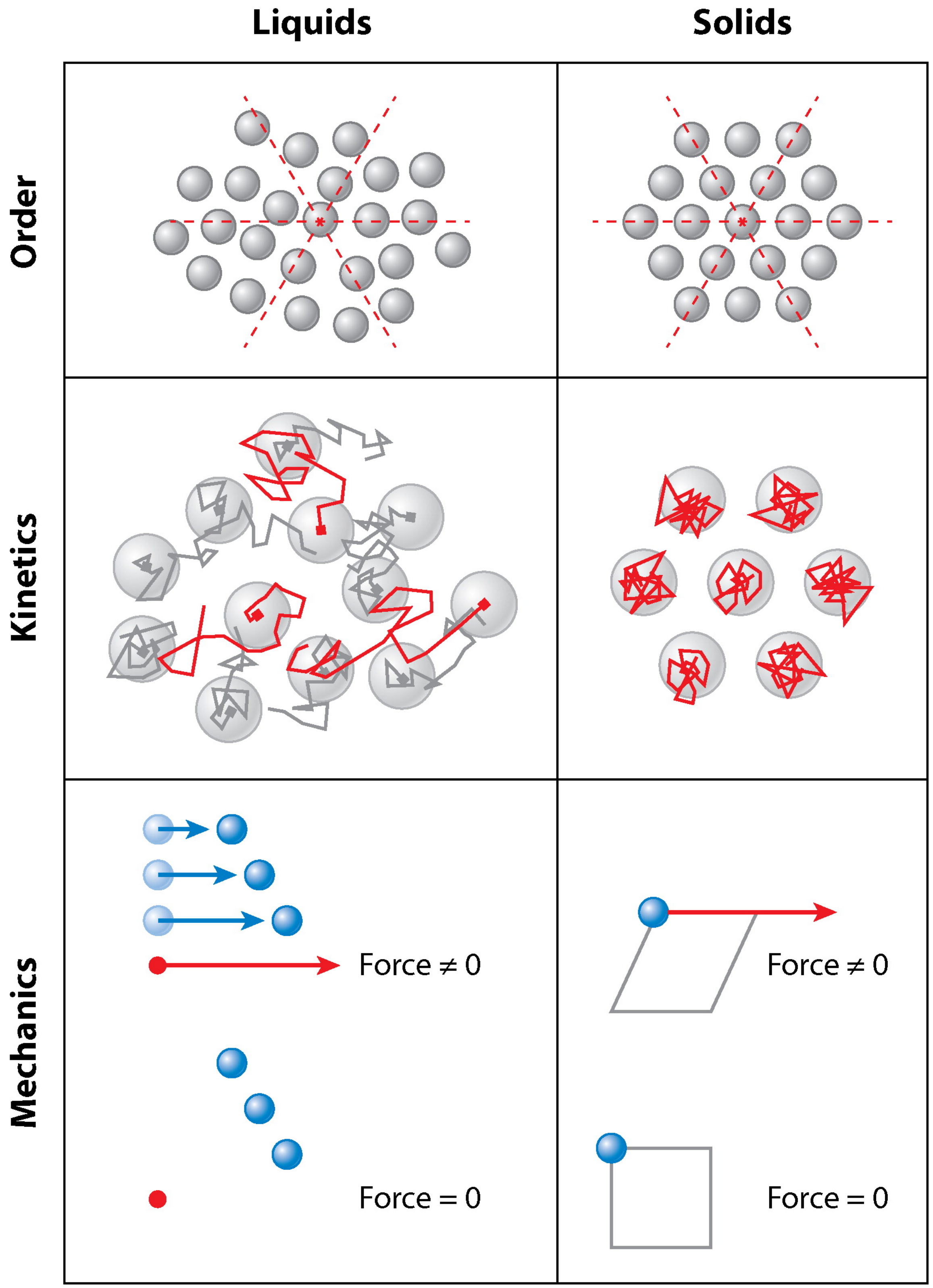
Hyman AA, Weber CA & Jülicher F. Ann Rev Cell Dev Biol (2014)
Jülicher F & Weber CA. Ann Rev Cond Mat Phys. (2024)

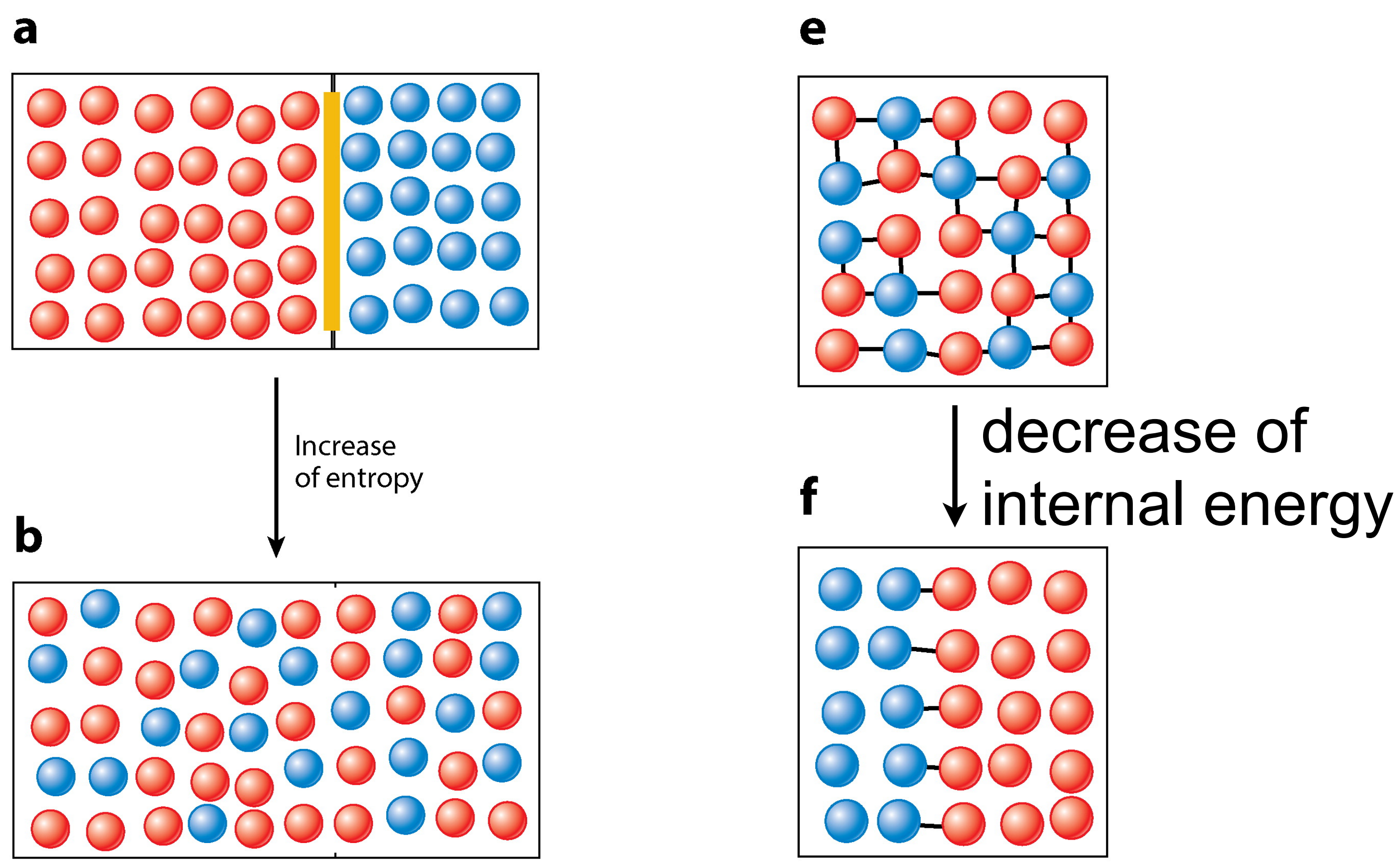




Cell (and nucleus) content is highly inhomogeneous: some organelles are separated by a membrane but many are just local "condensates" (p-bodies, l-bodies, ...)

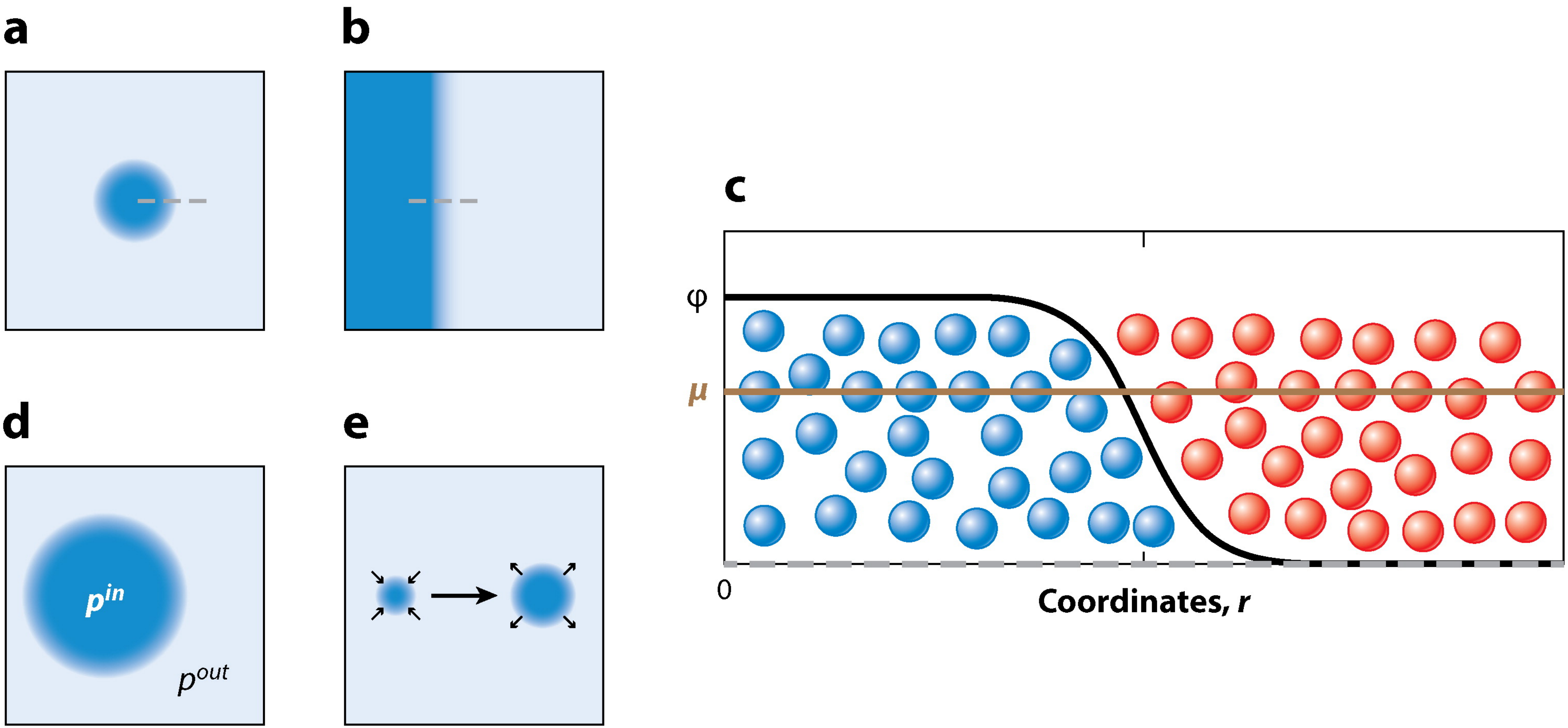
Phase-separated droplets





- Thermal agitation leads to an entropy increase, therefore mixing
- With repulsive interactions, dissipation of internal energy leads to de-mixing
- Phase separation is the resulting equilibrium (oil-in-water emulsion)

Phase-separated droplets

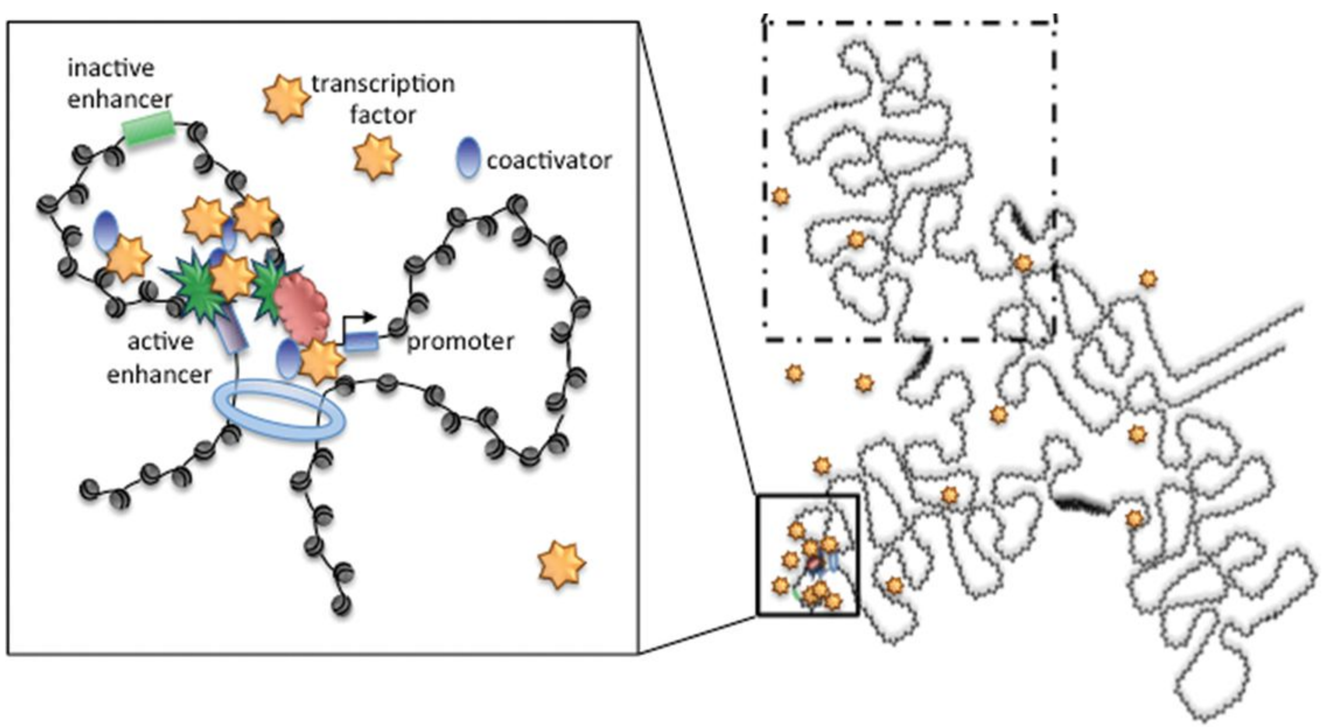


- Particles are exchanged through the droplet boundary, but concentrations are kept constant
- There is a pressure difference across boundary for round droplets
- There is a local flux from smaller droplets to larger droplets

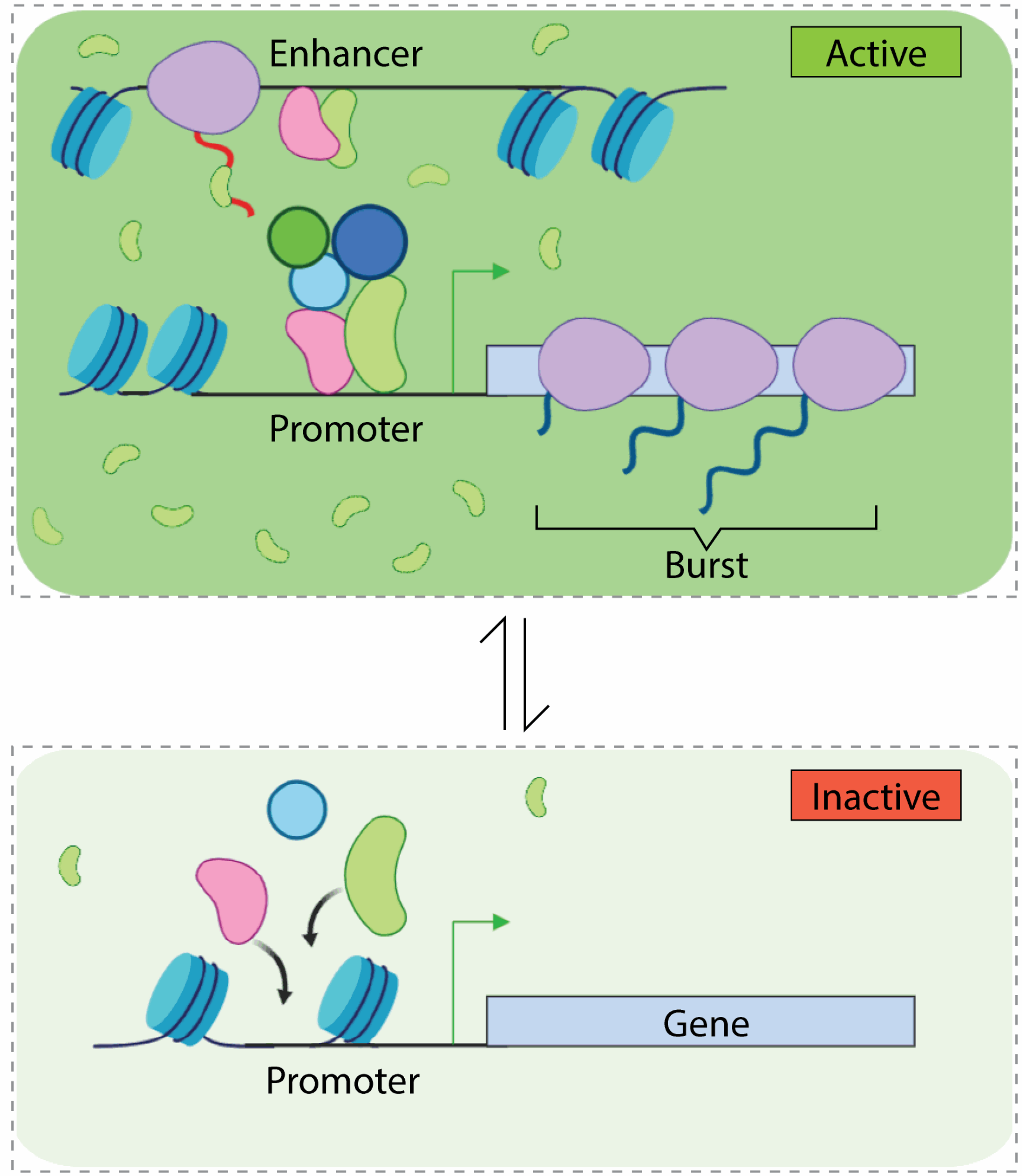
Transcription condensates

Hypothesis:

- Nucleus is organised into phase-separated droplets which locally increase concentrations of specific proteins
- Transient contact between different droplets triggers reaction bursts
- Example: promoters and enhancers in one droplet, Pol2 and mediator in another droplet lead to bursts of transcription
- Enhancers-bound cofactors modulate burst frequency

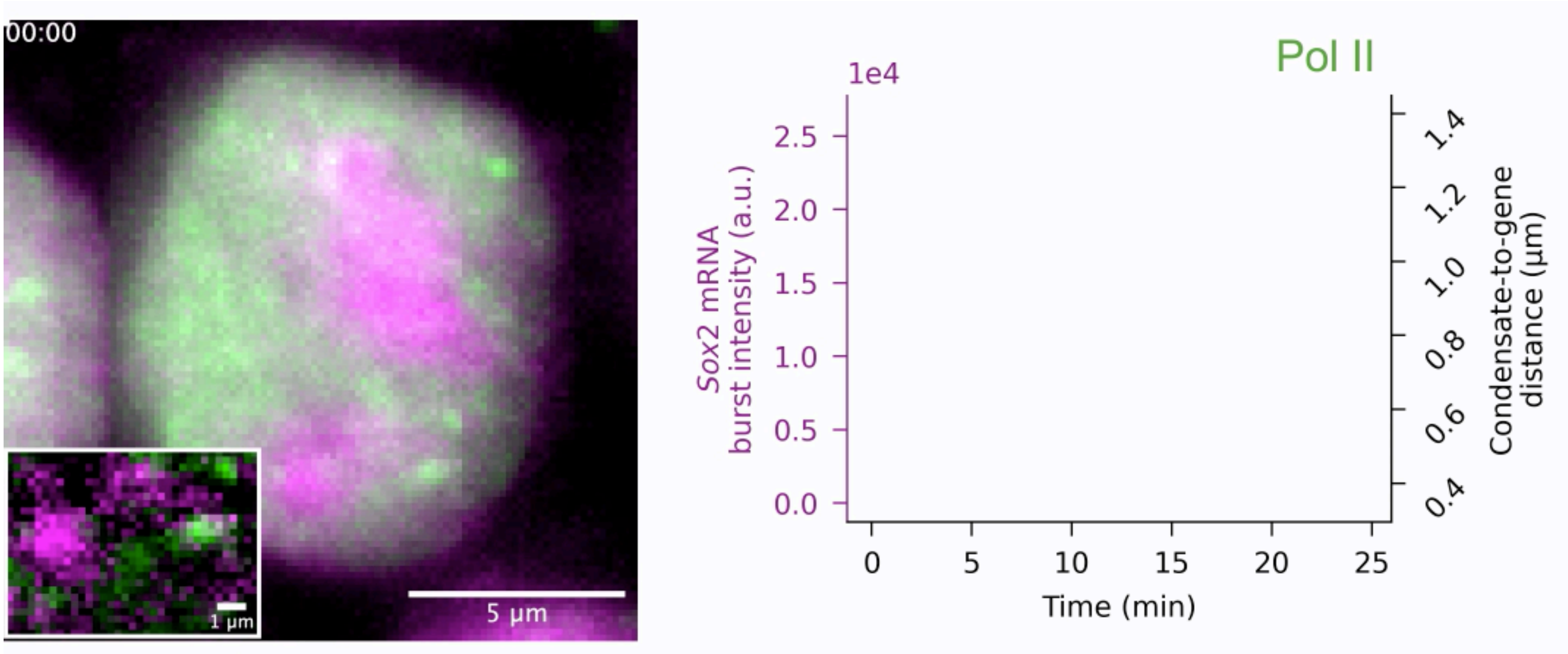


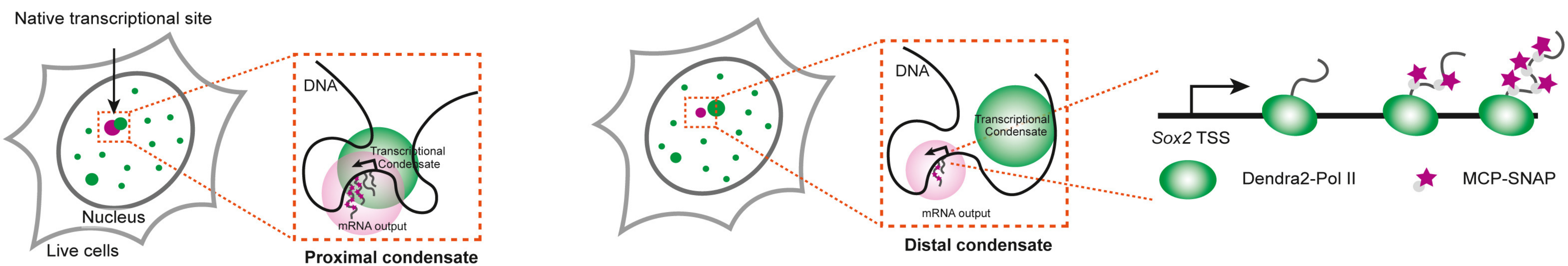
Benabdallah NS & Bickmore WA. Cold Spring Harb (2015)



Transcription condensates

Du M et al. Cell (2024)

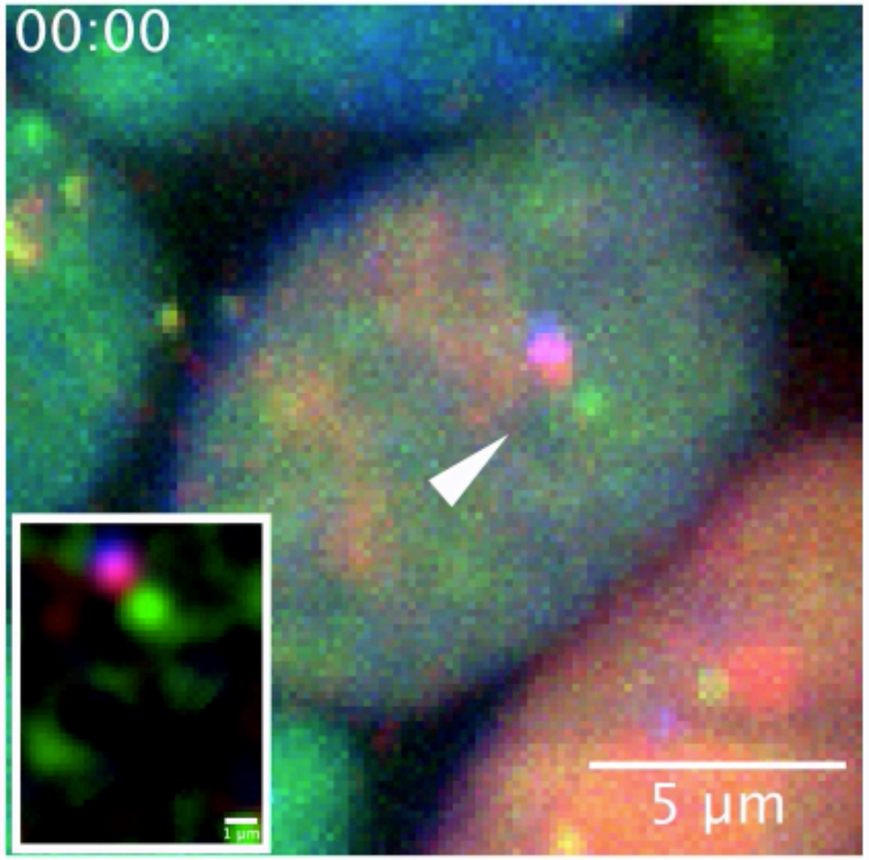


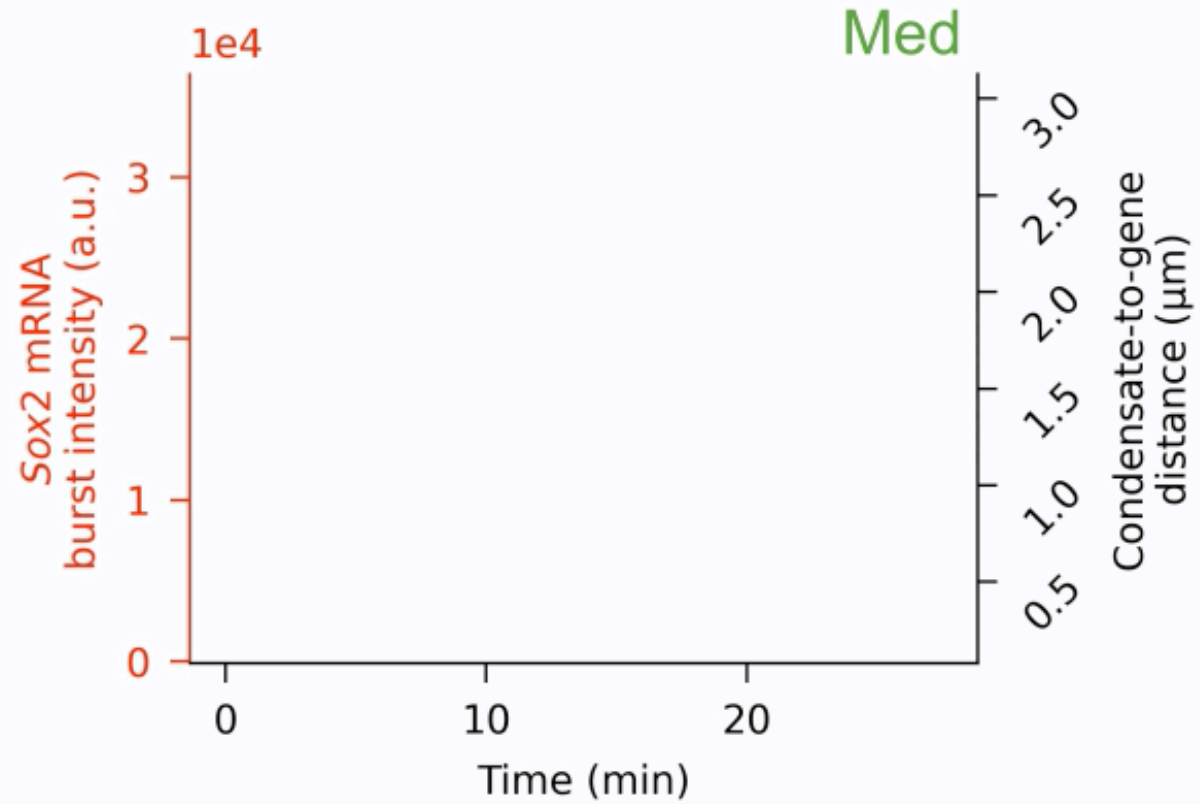


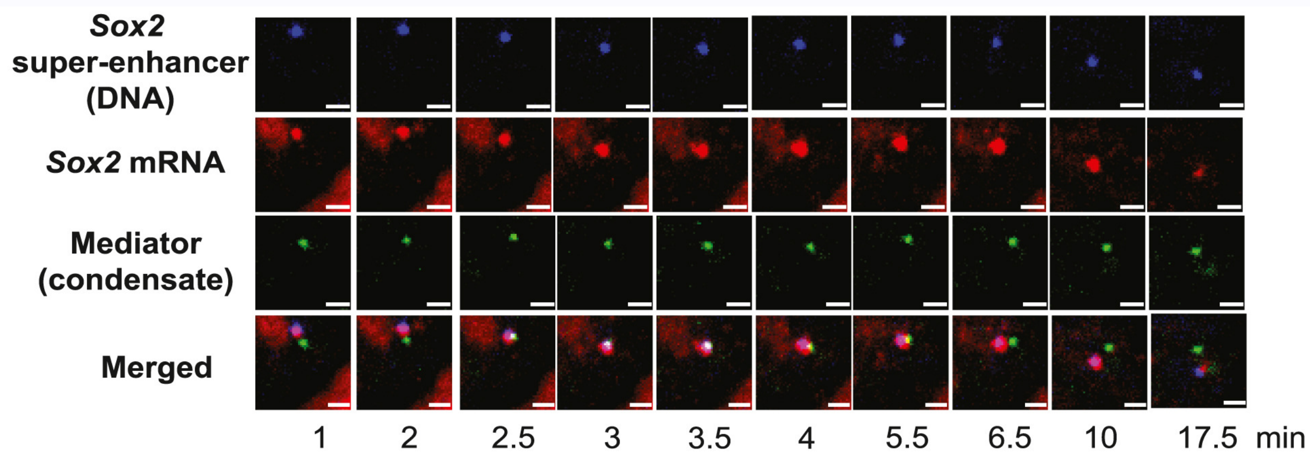
Transient contact between Pol II-rich condensate and gene promoter leads to burst of transcription

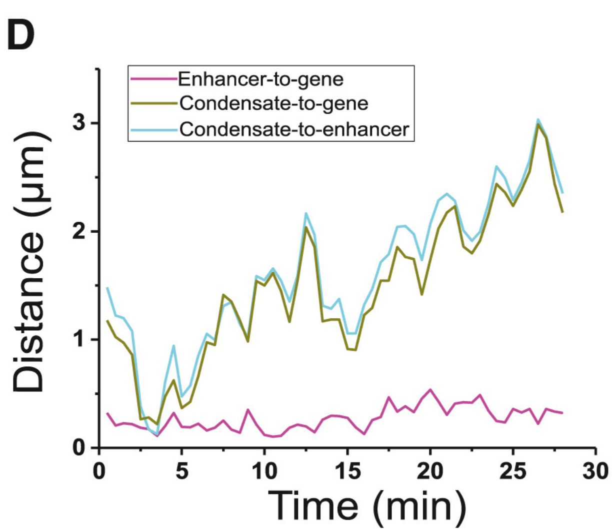
Transcription condensates

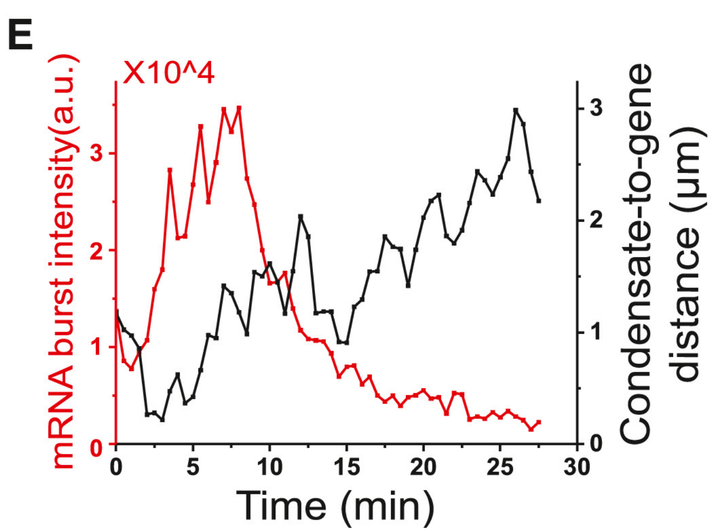
Du M et al. Cell (2024)











3-way contact:

- enhancer
- transcription (mediator)
- gene promoter

References

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