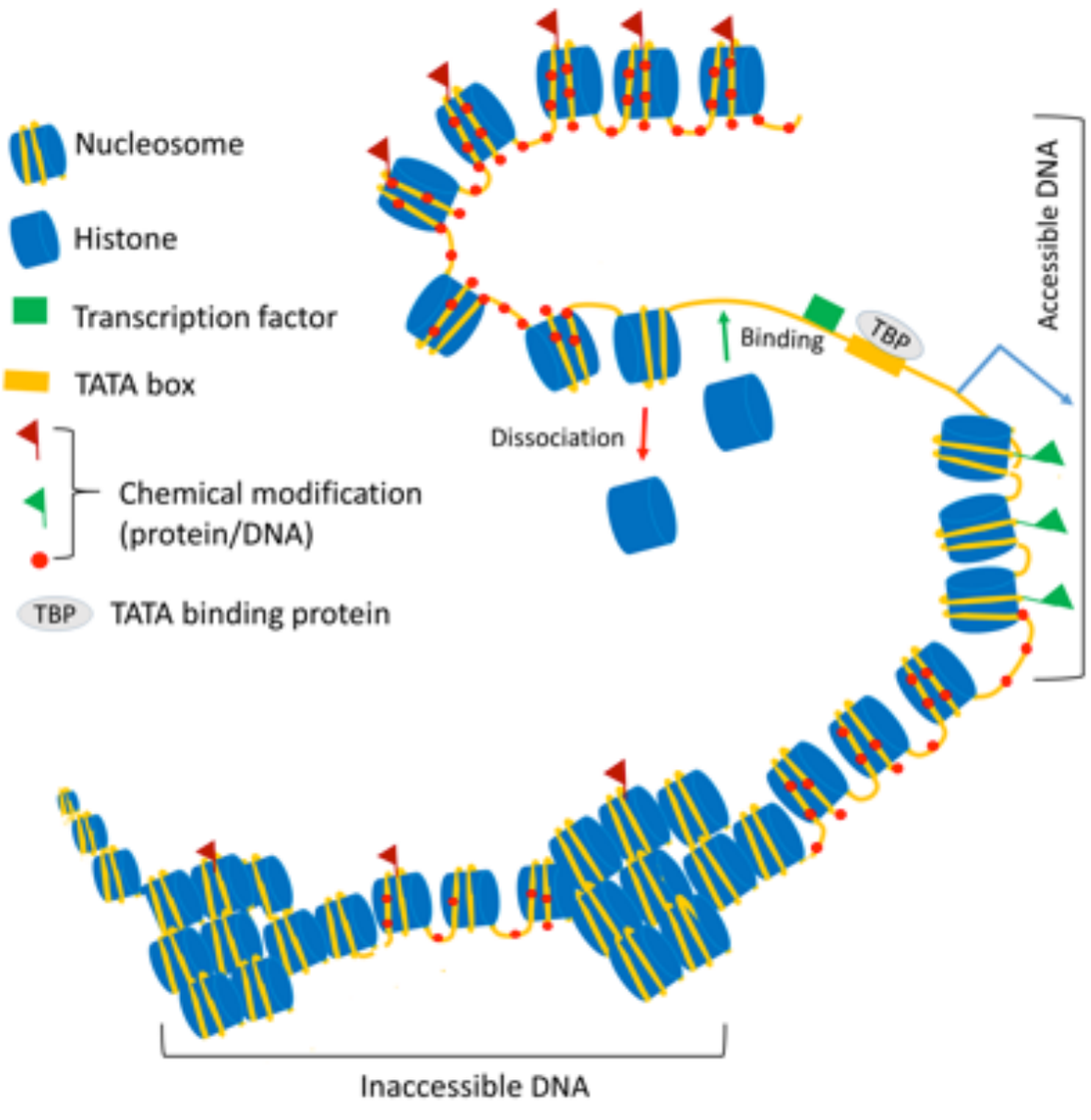
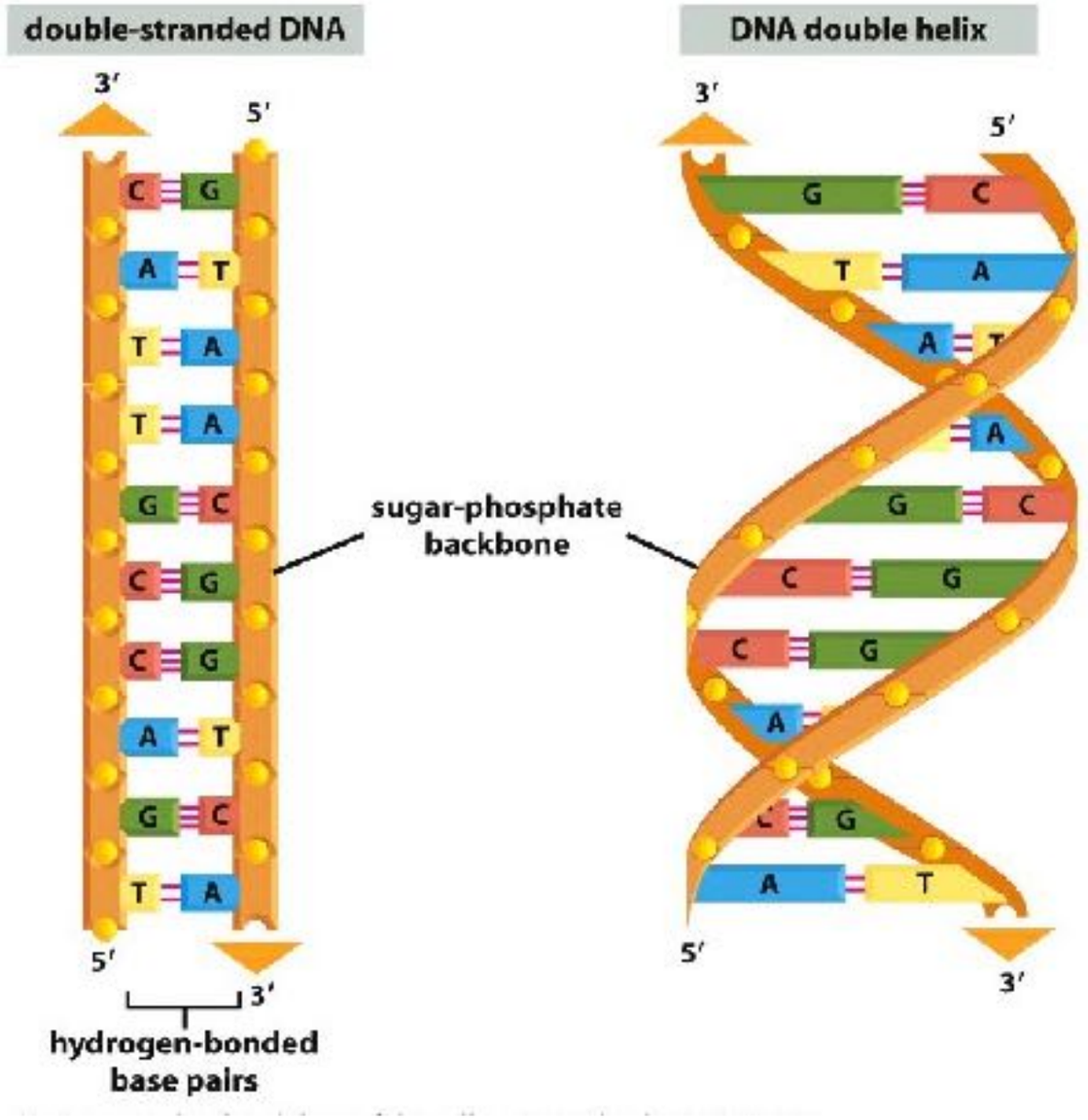
Stochasticity in nucleosome positioning: chromatin states and gene regulation



Ranjith Padinhateeri
Biosciences & Bioengineering
IIT Bombay

DNA : molecule that contains code for cellular processes



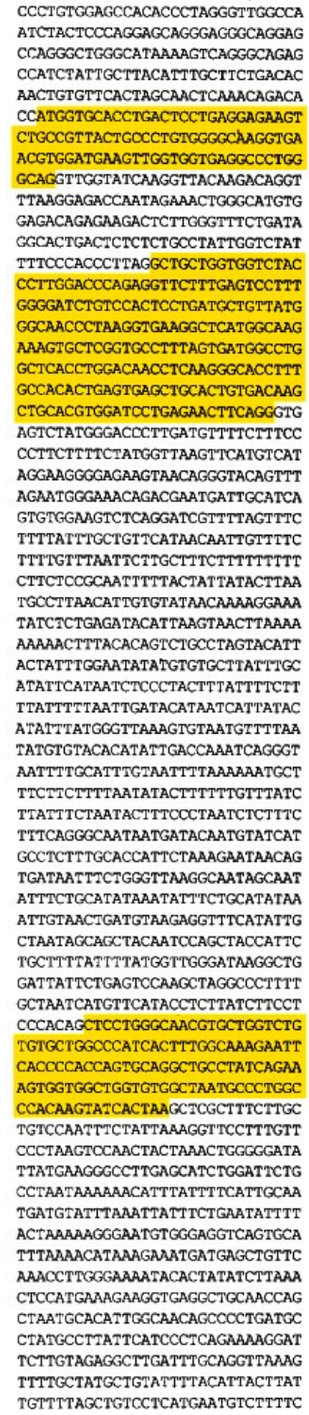
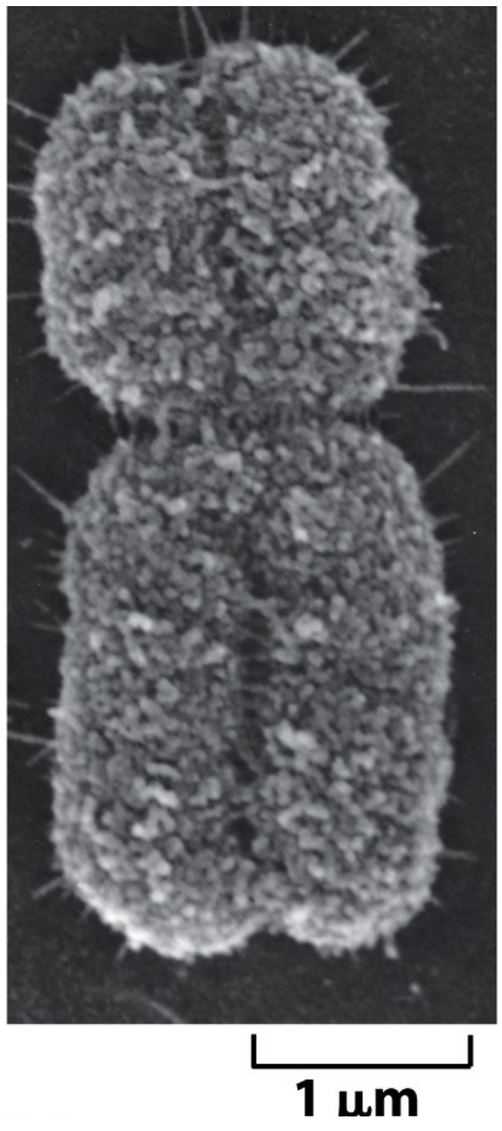


Figure credit: *Molecular Biology of the Cell* (© Garland Science 2008)

DNA needs to be read;
but cells need to prevent
“unwanted” reading!

DNA in cells is not bare; covered by large number of proteins => Chromatin



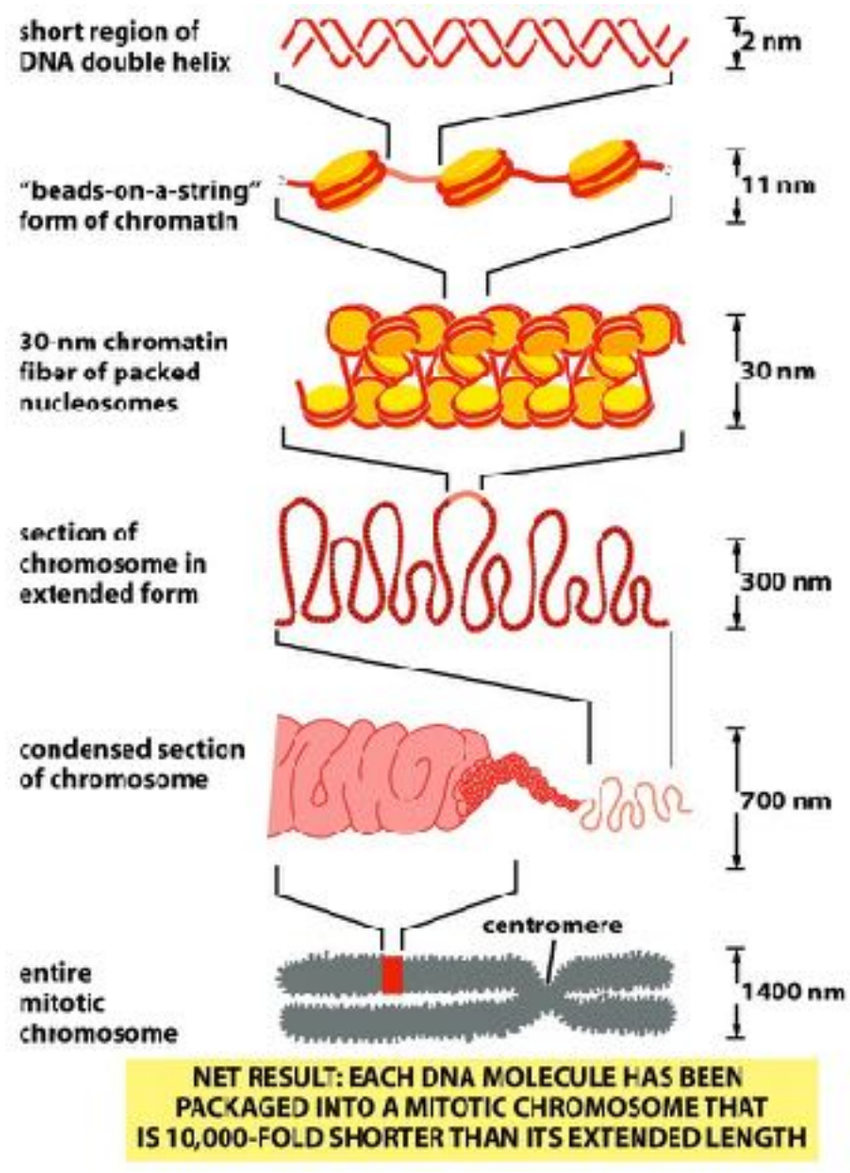
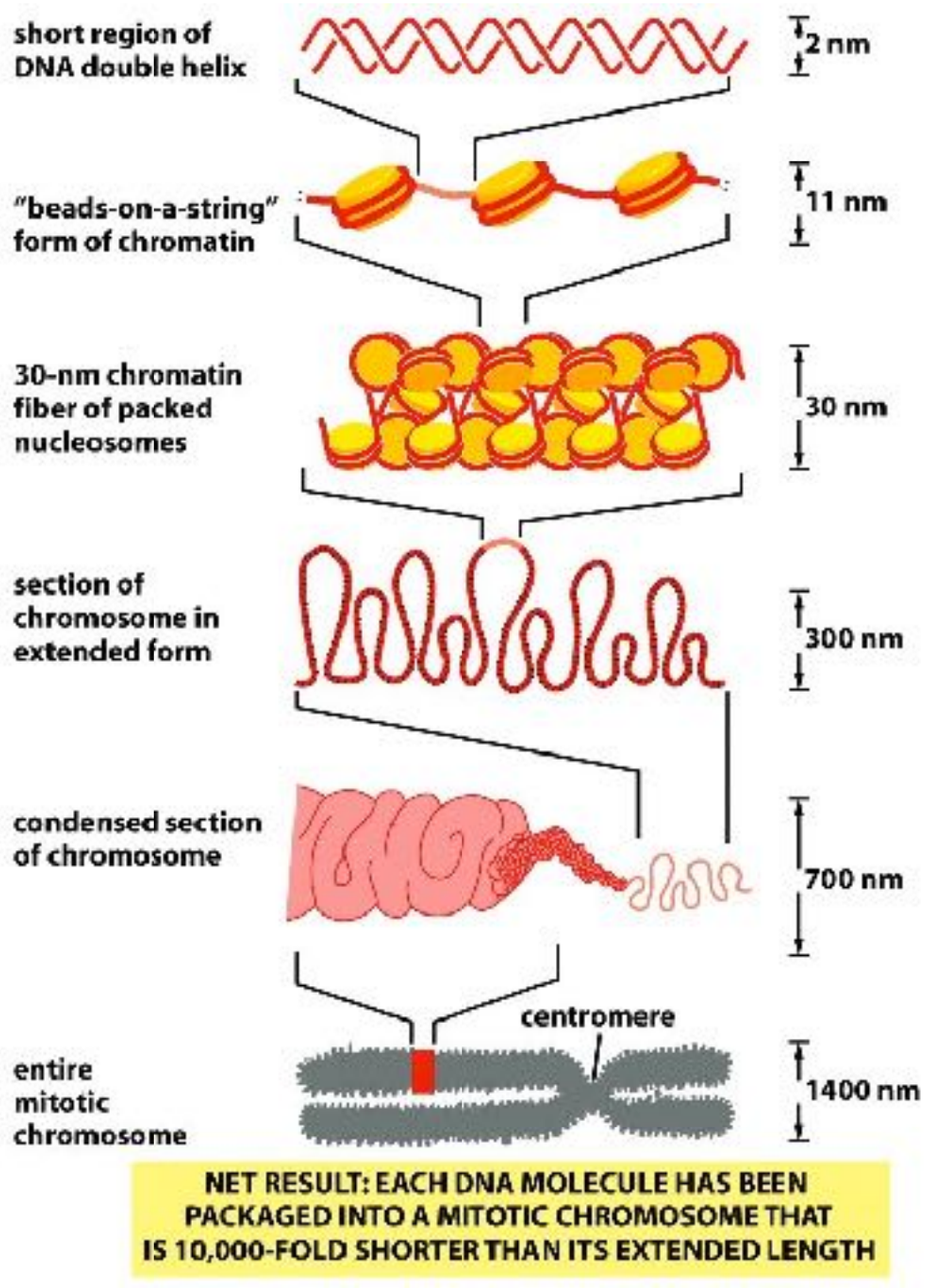


Figure credit: *Molecular Biology of the Cell* (© Garland Science 2008)

In cells, DNA is “actively” organized in 3D



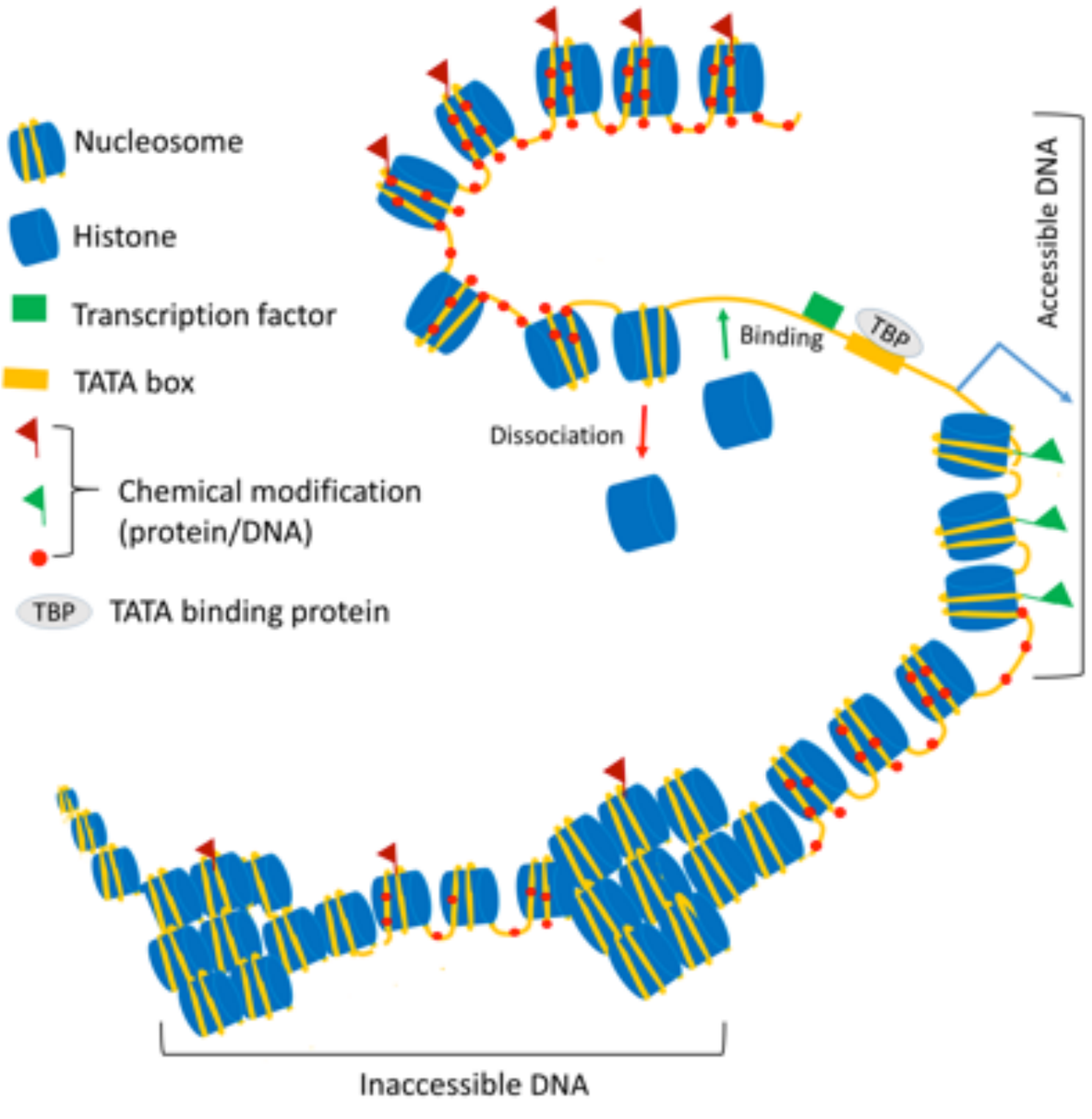
DNA

Array of nucleosomes

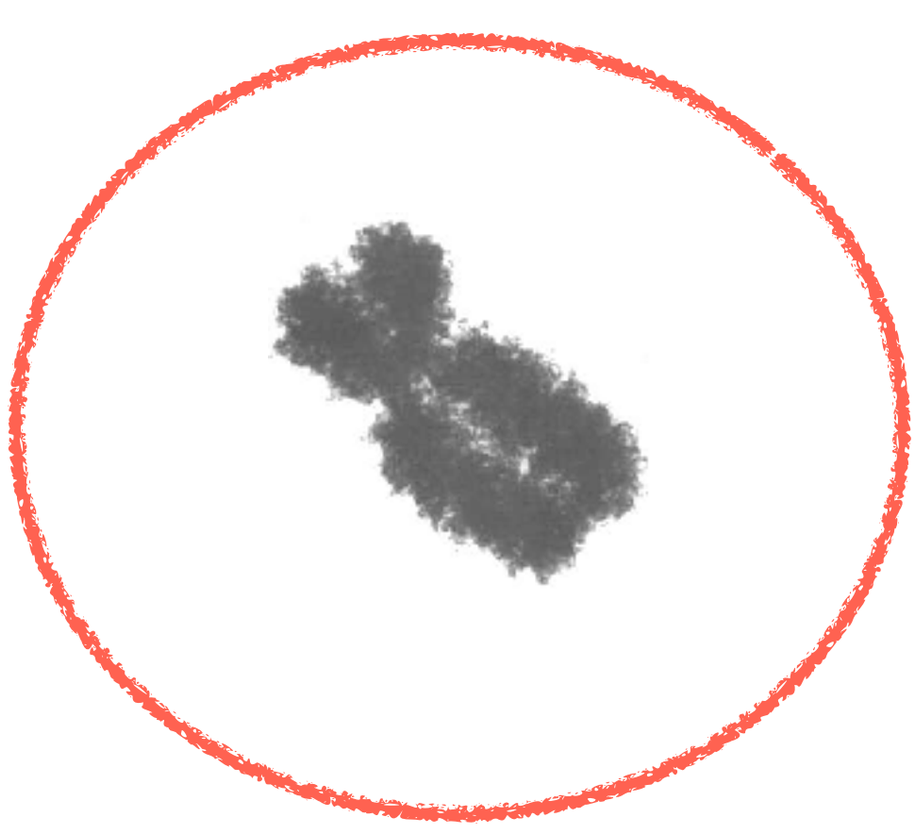
Different cells; different organization

Figure : *Molecular Biology of the Cell* (© Garland Science 2008)

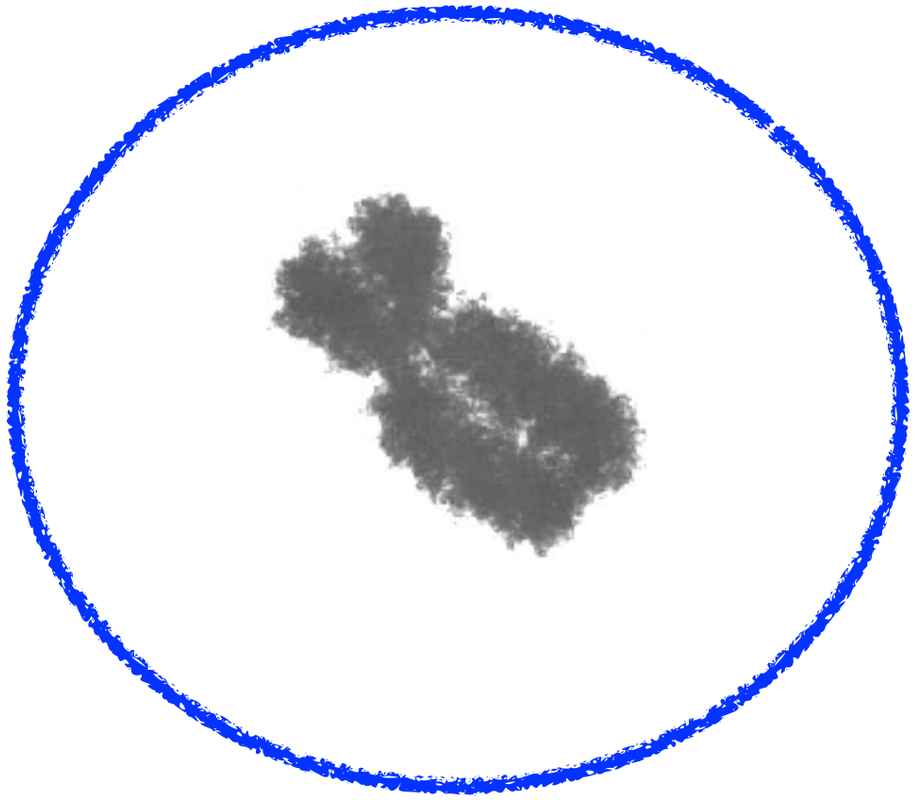
What is the organization of genome in living cells?



Different cells; but same DNA



Cells in our skin

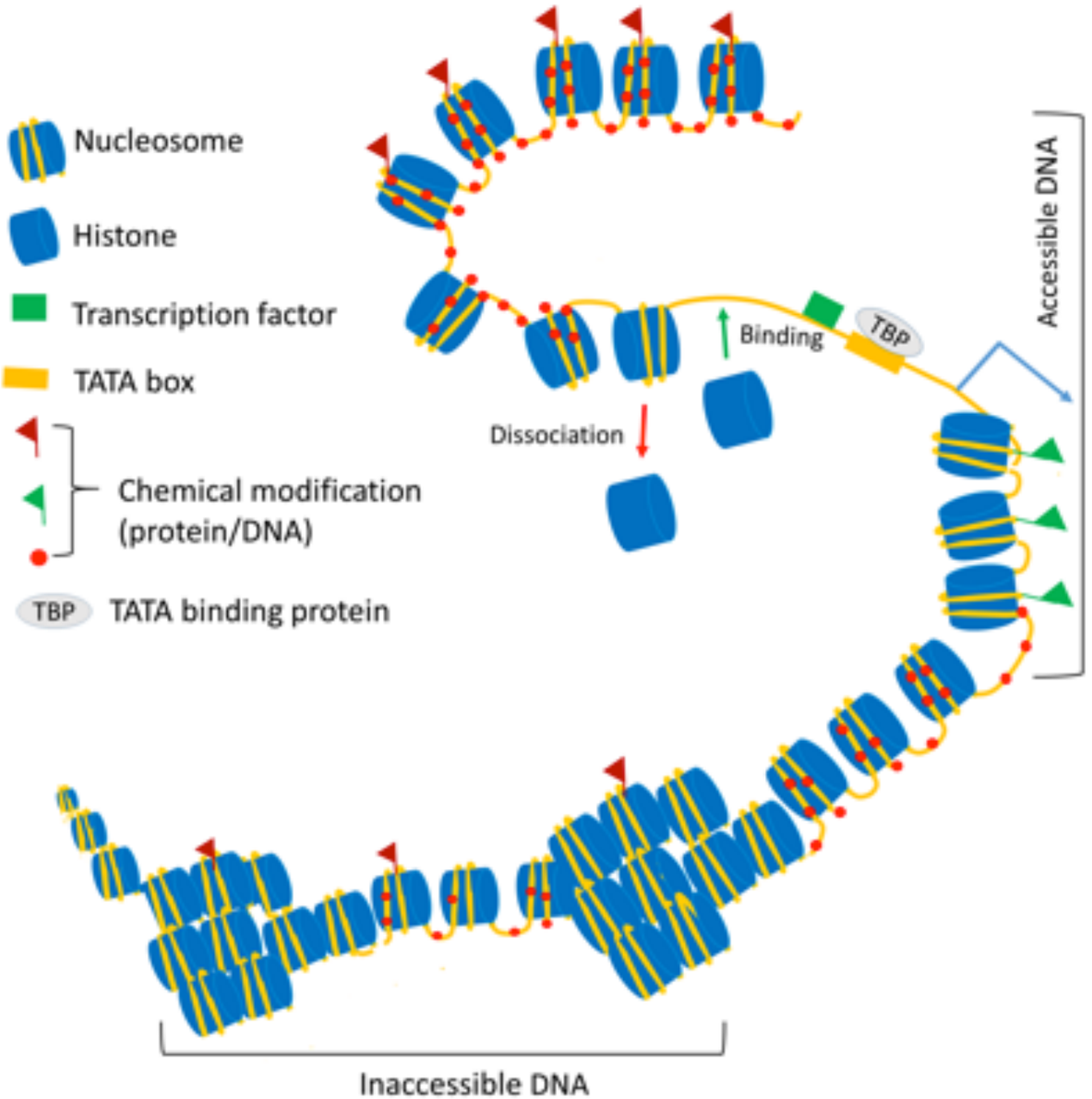


Cells in our eye

How do they show different behavior ?

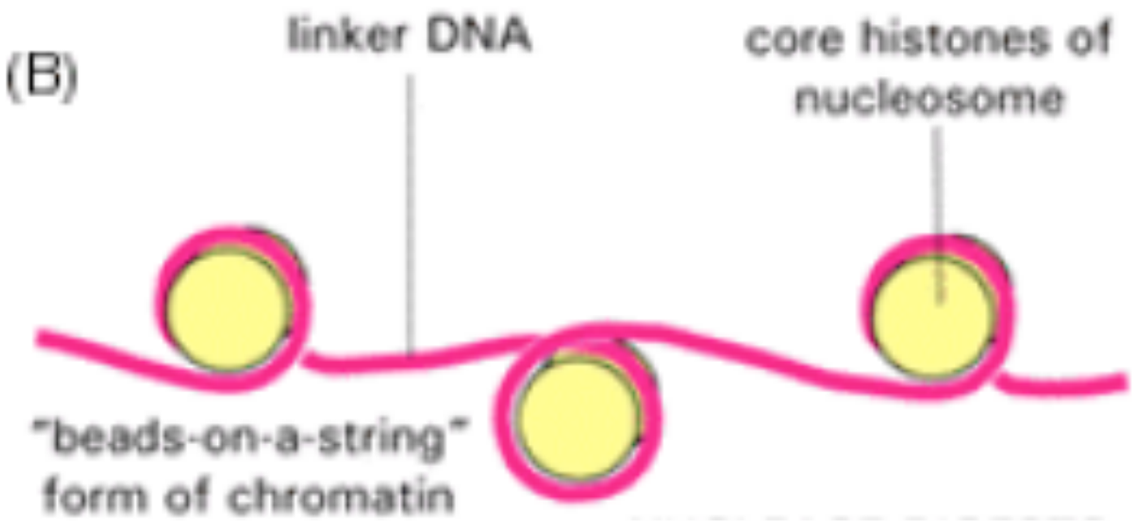
Fate/function of a cell is not decided by the DNA sequence alone; but also by different “states” of chromatin

Different cell types; different chromatin organization



“state” of a chromatin is actively maintained (ATP-dependent chromatin remodeling is crucial)

First level of packaging: nucleosome particles on a 1D track



Is there a pattern in the organization of these “ball”-like proteins —nucleosomes?

Nucleosome: Binding energy

$$\Delta G \approx -40 k_{\mathrm{B}} T$$

Forming a nucleosome (DNA wrapping around histone octamer) is energetically favorable

Nucleosome state is a highly stable state

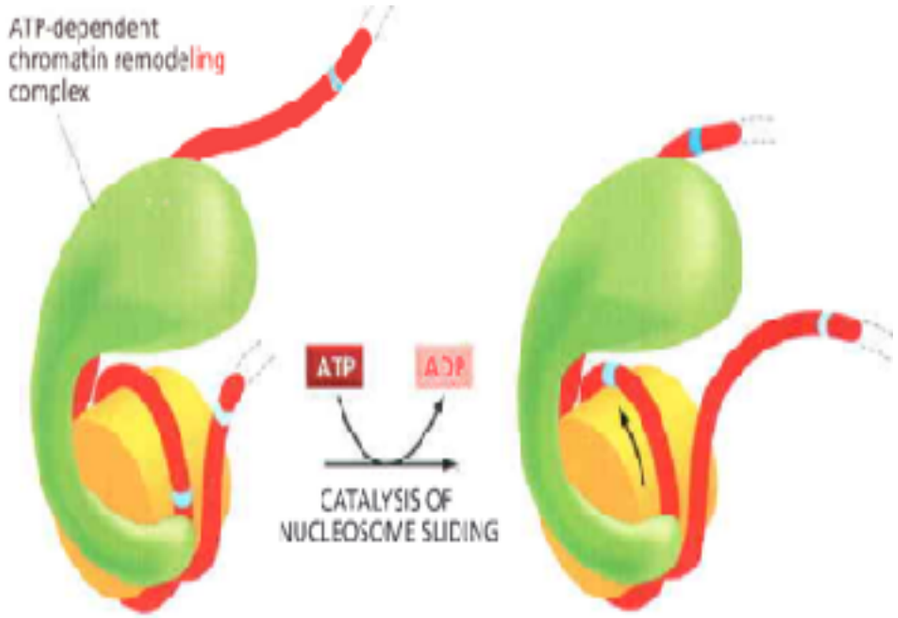
Yan et al, Mol. Bio. Cell (2007)

$$\Delta G \approx -40 k_{\mathrm{B}} T$$

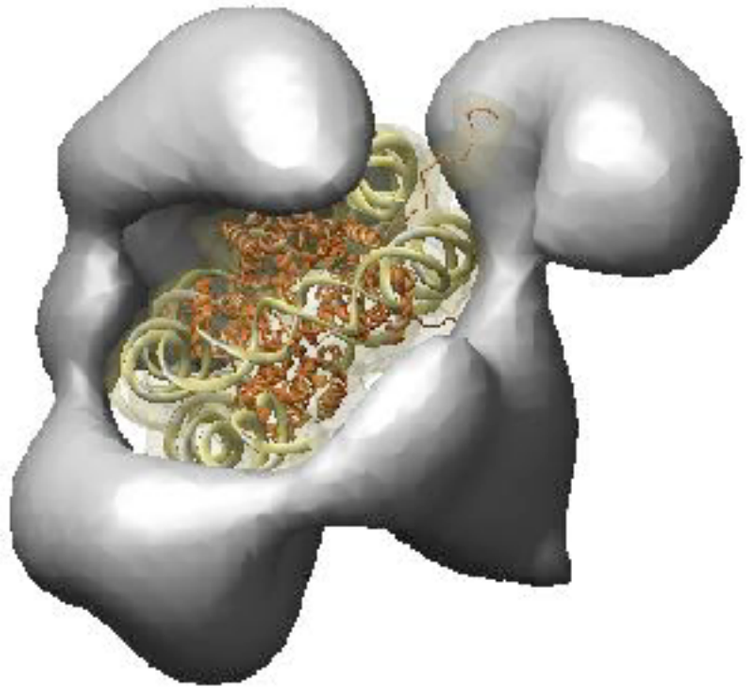
What does this suggest ?

- Any thermal dissociation will be nearly impossible
- Need extra machinery to slide/disassemble

Molecular machines use ATP, and rearrange nucleosome positions



ATP-dependent sliding (candidates: yeast ISWI, ACF etc — ISWI family)



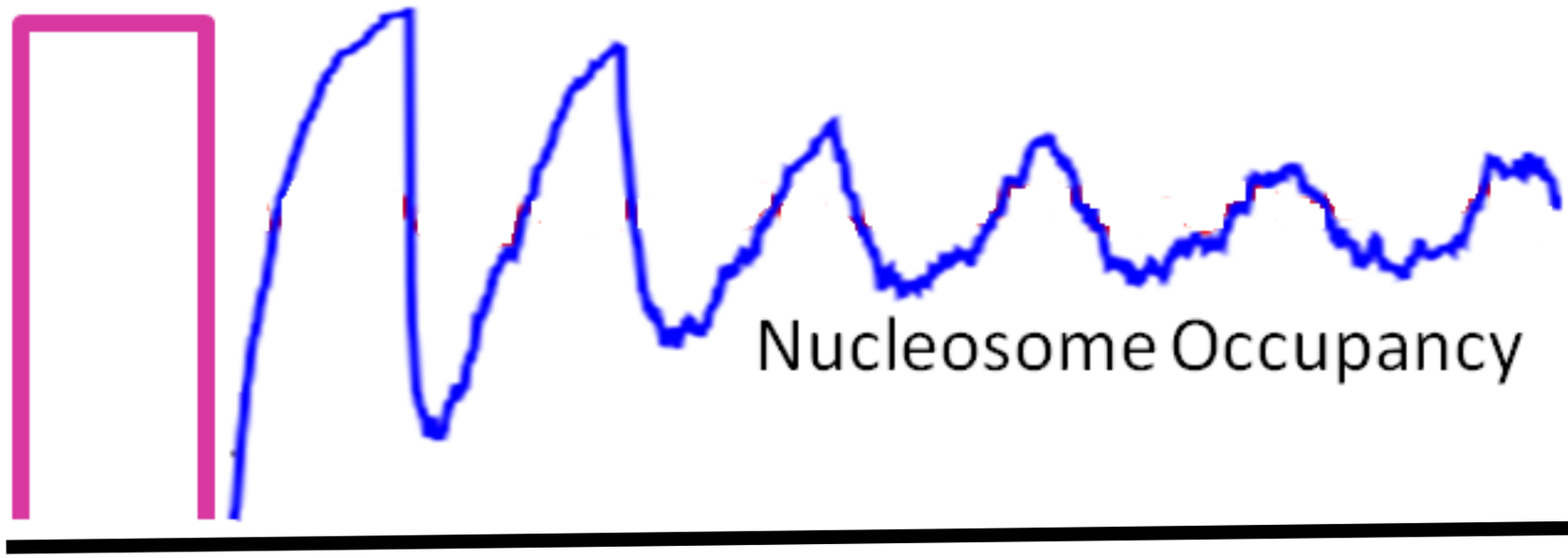
ATP-dependent disassembly (candidate: RSC, SWI/SNF family)

Figure: *Molecular Biology of the Cell* (© Garland Science 2008)

How are these nucleosomes—sterically interacting particles—organized inside the cells?

- Are they randomly organized?
- Is there a pattern?

Experiments: “non-random” organization of nucleosomes

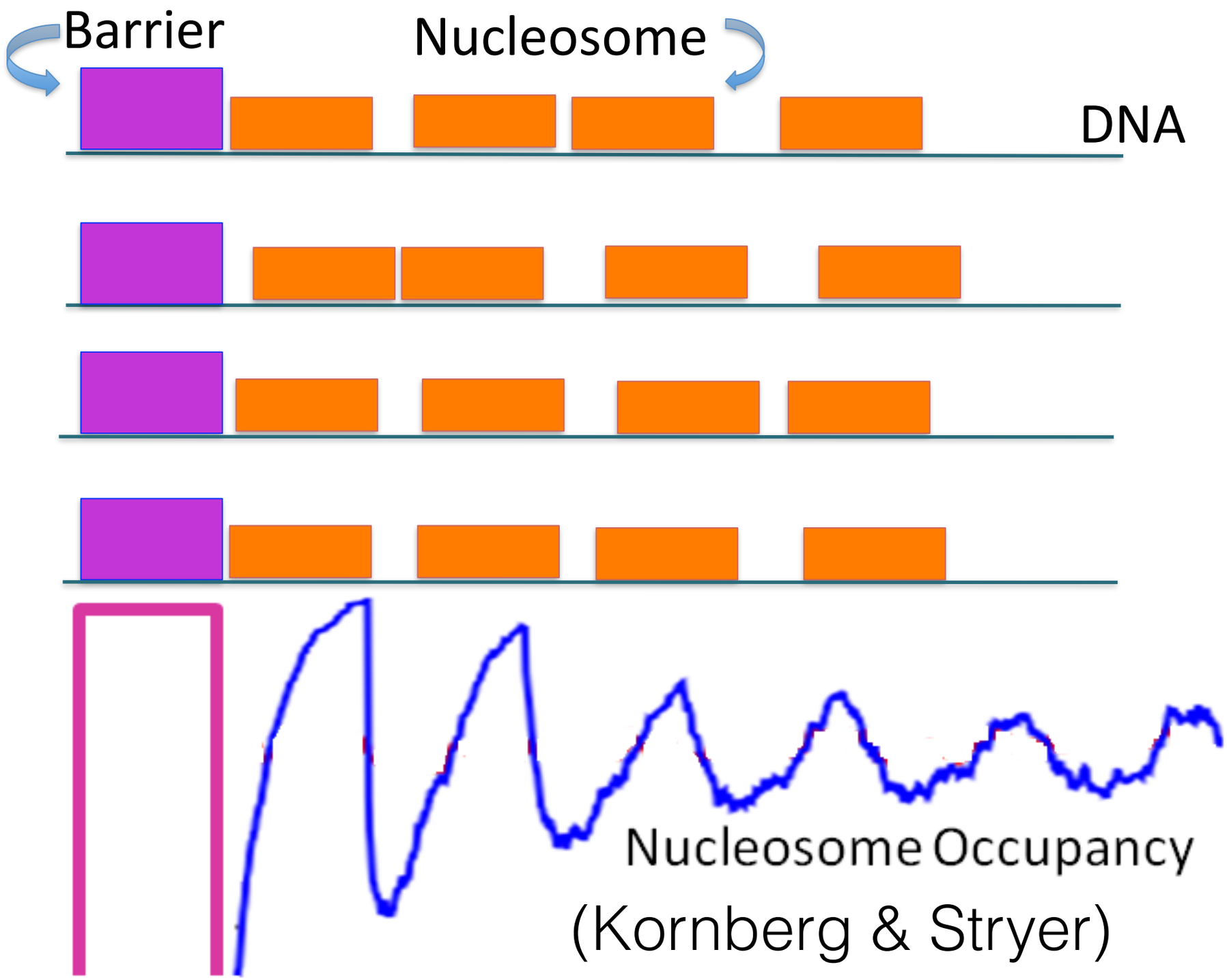


Nucleosome occupancy: Probability that a DNA site j is covered by a nucleosome

Volume 16 Number 14 1988 Nucleic Acids Research

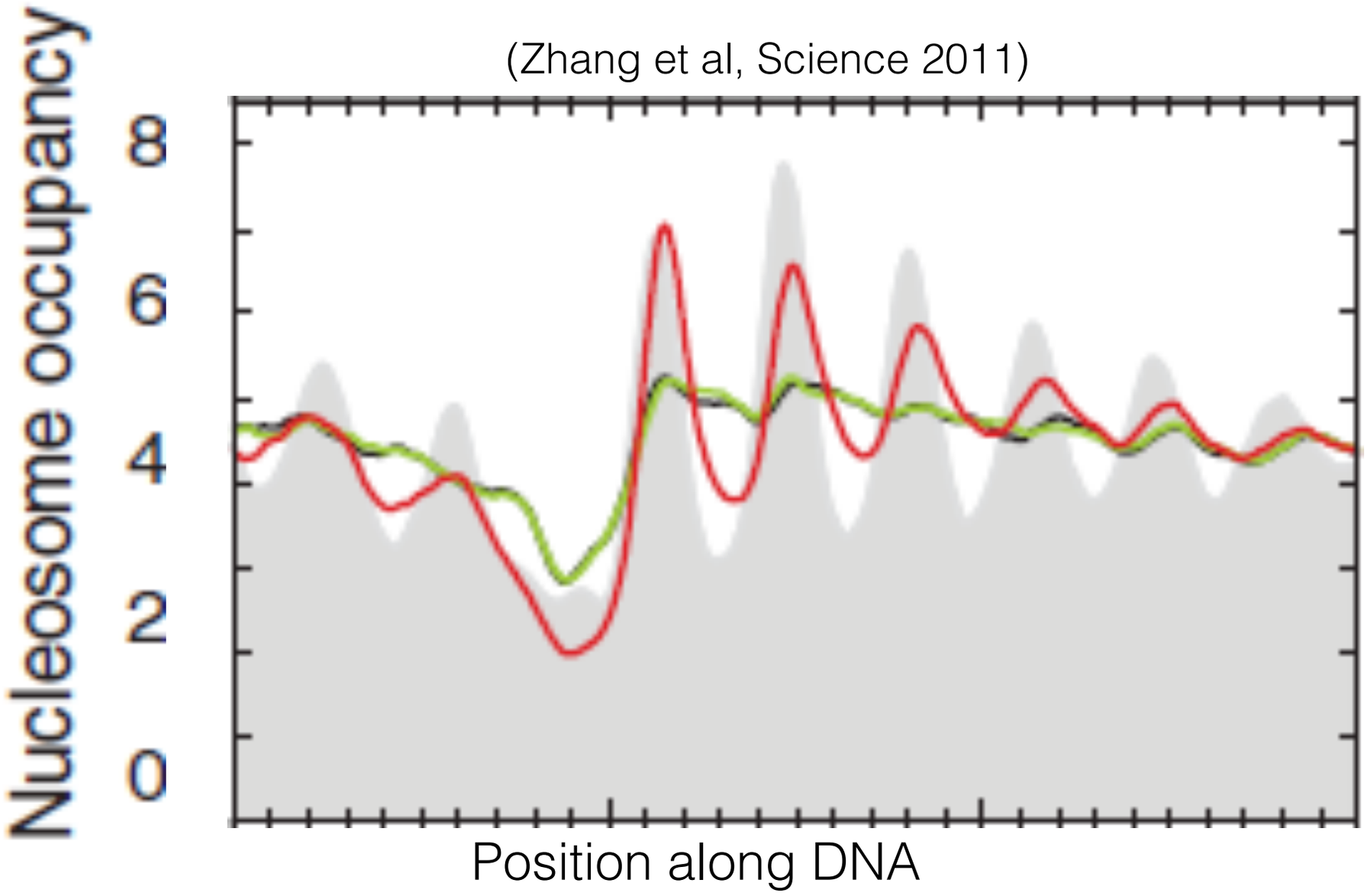
Statistical distributions of nucleosomes: nonrandom locations by a stochastic mechanism

Roger D.Kornberg and Lubert Stryer



ATP-dependent activity is necessary for maintaining nucleosome positioning

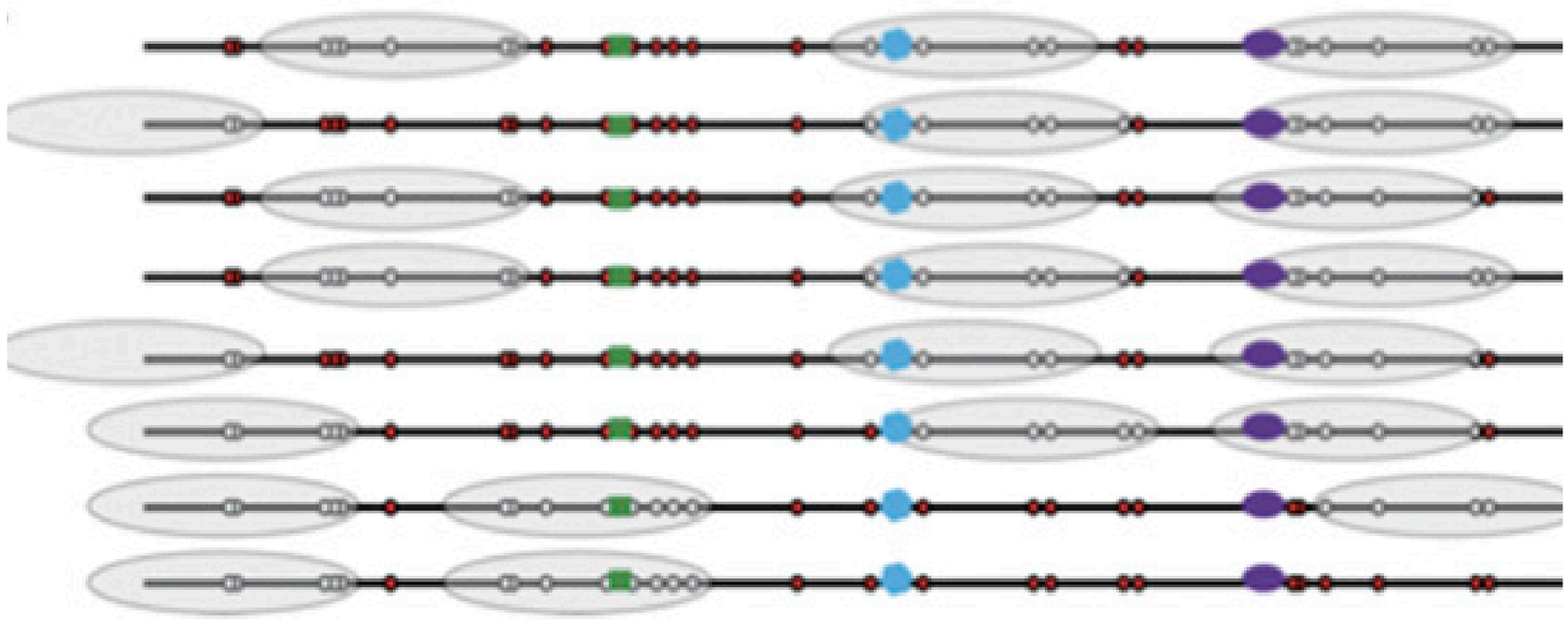
(Zhang et al, Science 2011)



Red: Cell extract with ATP

Green: Cell extract with no ATP

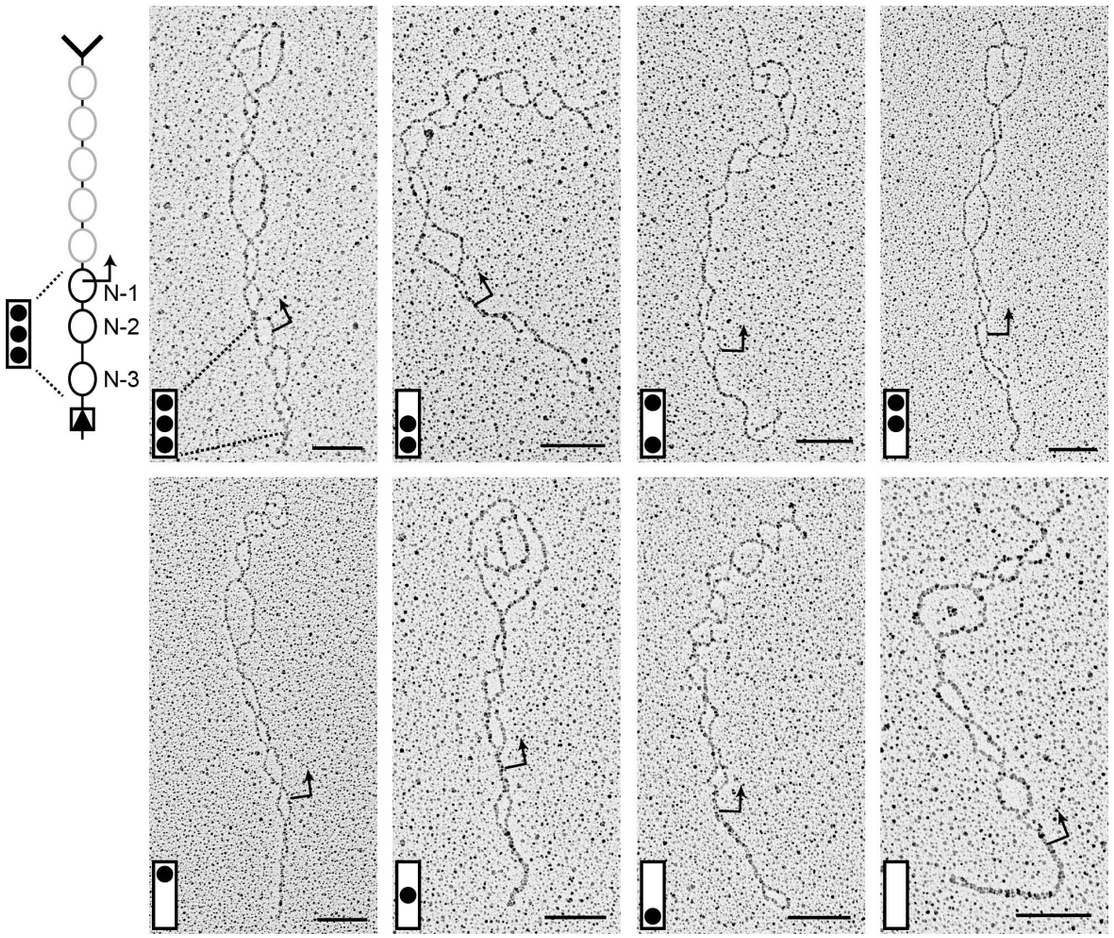
Stochasticity in nucleosome organization in promoters



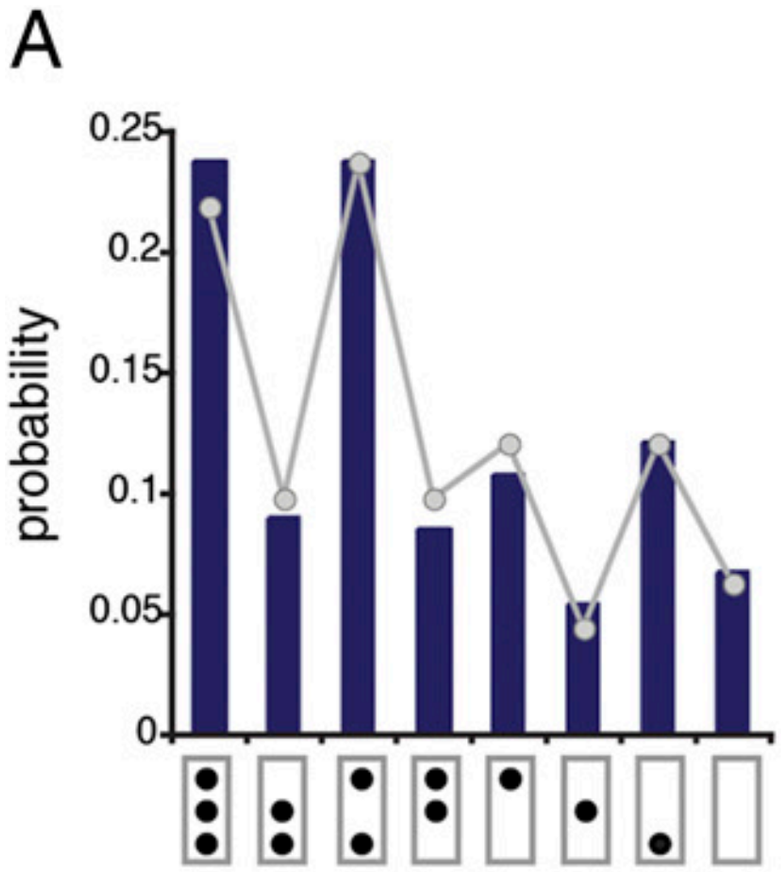
Different cells (under exactly the same condition) have different nucleosome organization

(Small et al, PNAS, 2014, Brown et al PLOS Biology, 2011)

Stochasticity in nucleosome organization near promoter regions—eight “states”



Brown et al, PLoS Biology, 2013
(Boeger Lab)



Brown & Beoger PNAS (2014)

Some questions we investigate…

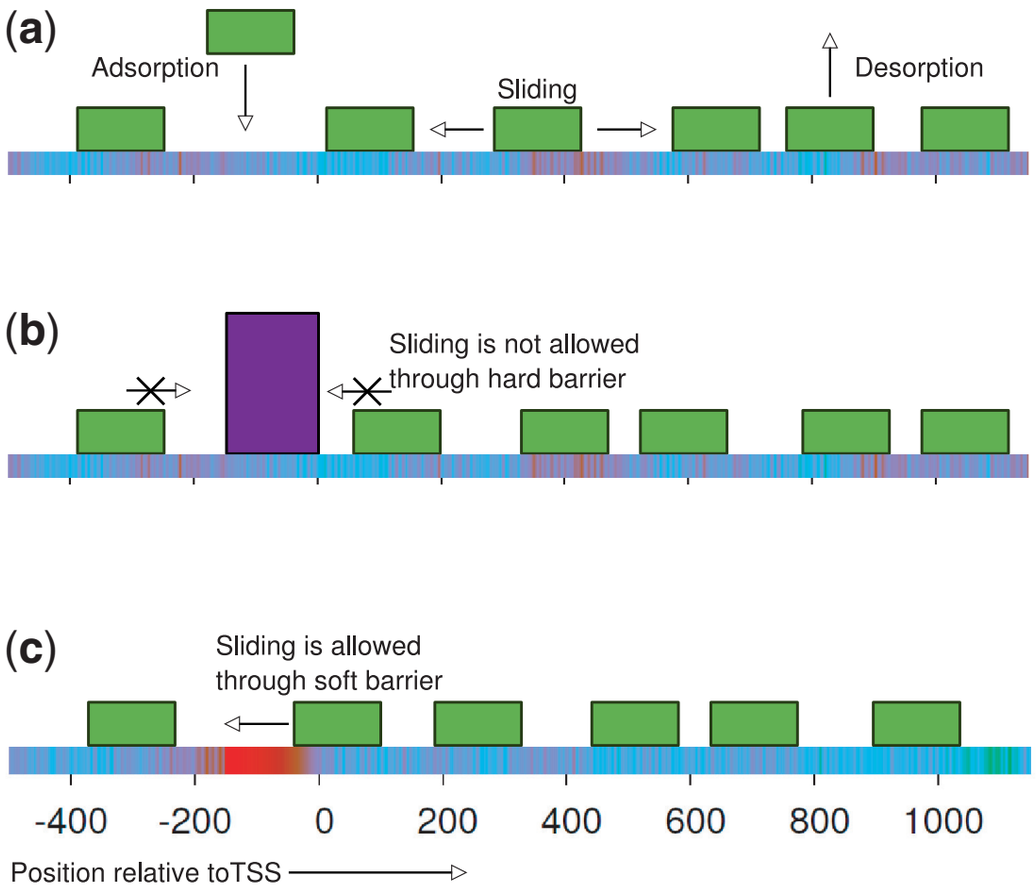
- Given that nucleosomes are dynamic (they can bind/ dissociate and slide), what must be the underlying dynamical rules to obtain the experimentally known pattern?

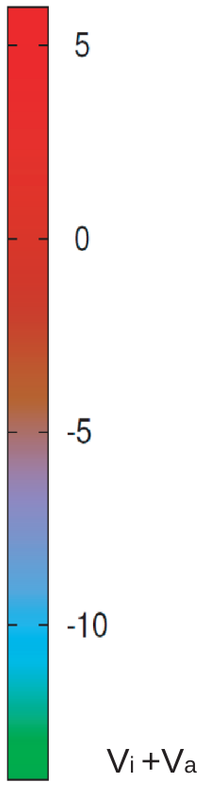
- What is the role of ATP-dependent chromatin remodeling in maintaining experimentally observed chromatin organization?

- How nucleosome organization would influence 3D looping of chromatin?

- How certain non-nucleosomal proteins might affect chromatin organization?

Part-1: 1D-Model with nucleosome binding, dissociation and sliding





With sequence effects

With steric interaction

$$r_{\text{on}} = r_0 \times f(\{l\})$$

$$r_{\text{off}} = (r_0 + r_a e^{V_a}) e^{V_i}$$

$$r_{\text{slide}} = 0 \text{ (without ATP)}$$

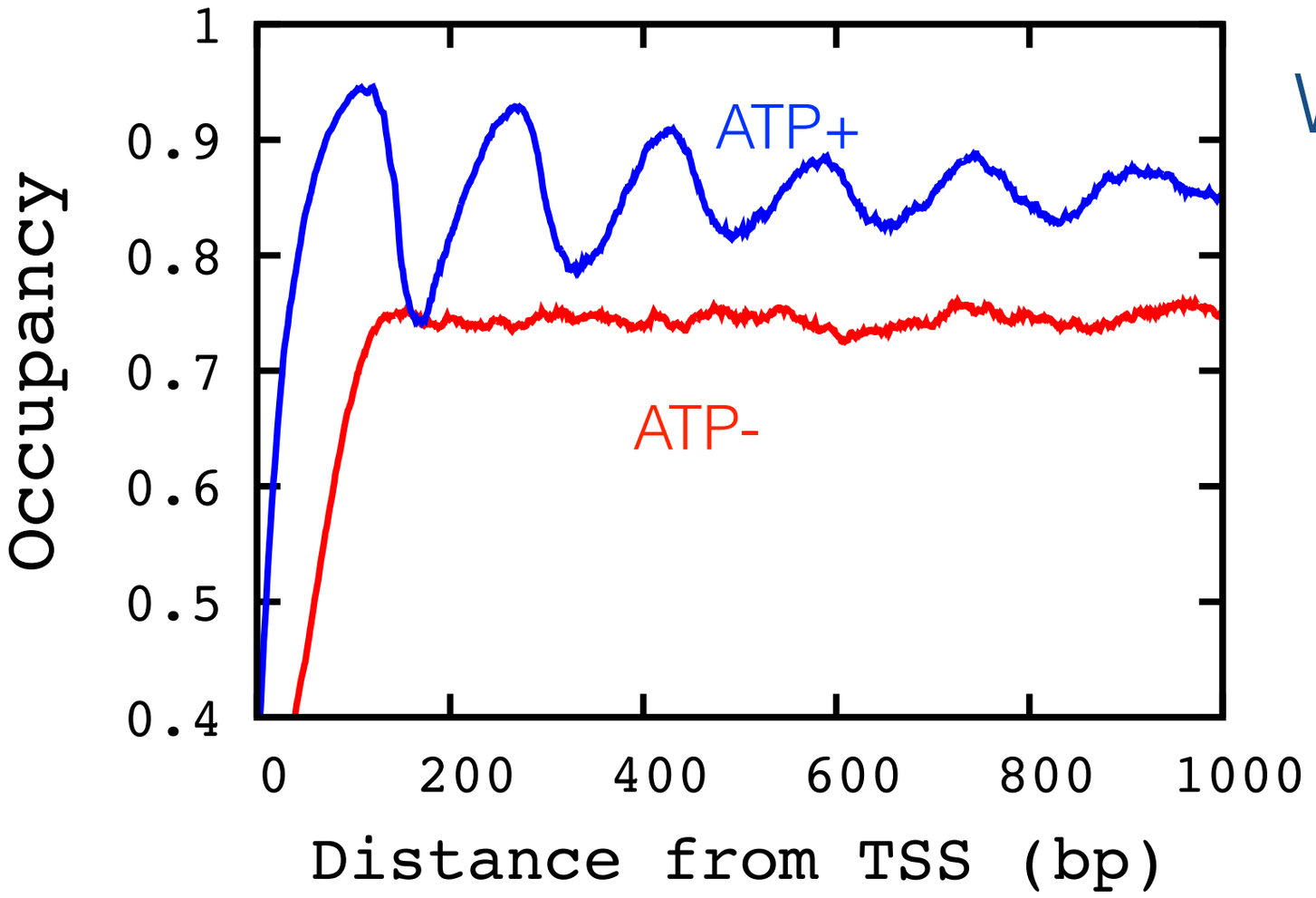
$$r_{\text{slide}} = r_s \text{ (with ATP)}$$

(with some sliding rule)

We build the model bottom up: start with minimal; add details
We do kinetic Monte Carlo simulations

Jyotsana Parmar et al NAR (2014), and NAR (2016)

How ATP-dependent moves (sliding/ disassembly) would affect occupancy?



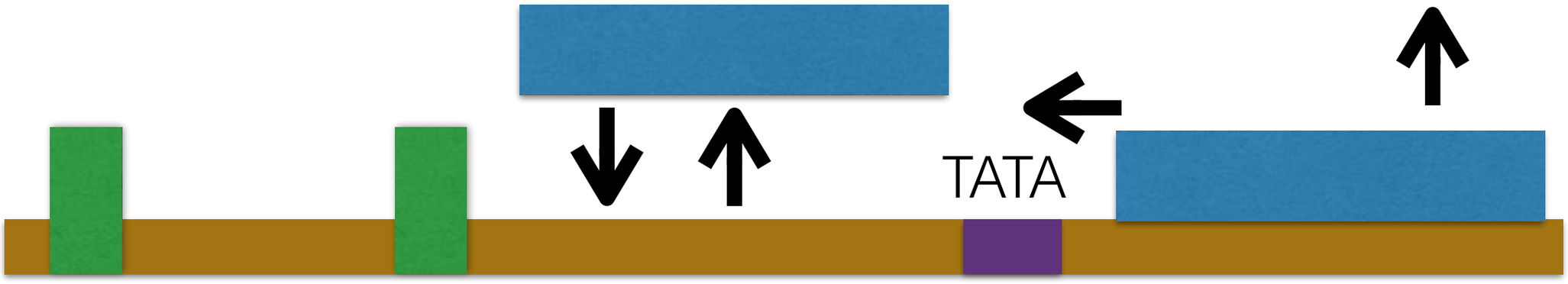
With ATP-dependent moves

No ATP-dependent moves

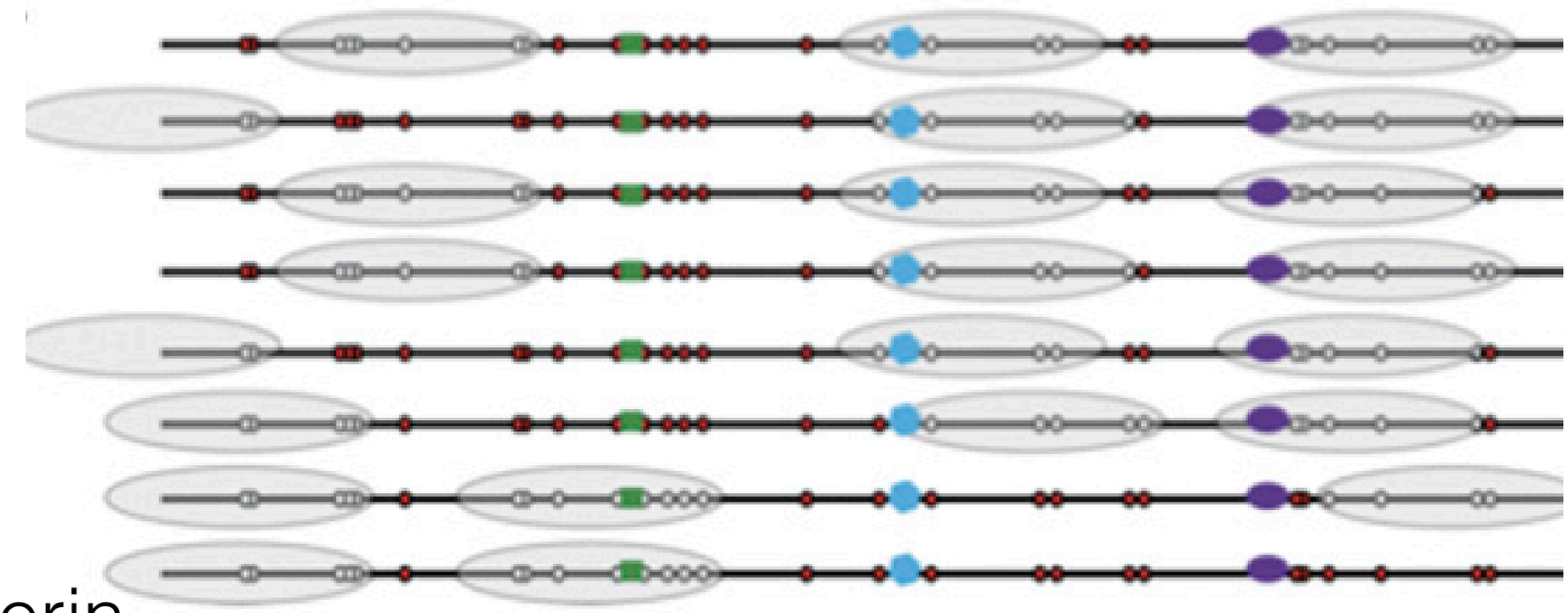
Our theory explains the reasons behind disappearance of oscillatory positioning (need activity to “feel” the barrier)

Jyotsana Parmar, JF Marko and RP, Nucleic Acids Research (2014)

What could be the underlying kinetics that will lead to the experimentally seen nucleosome organization?



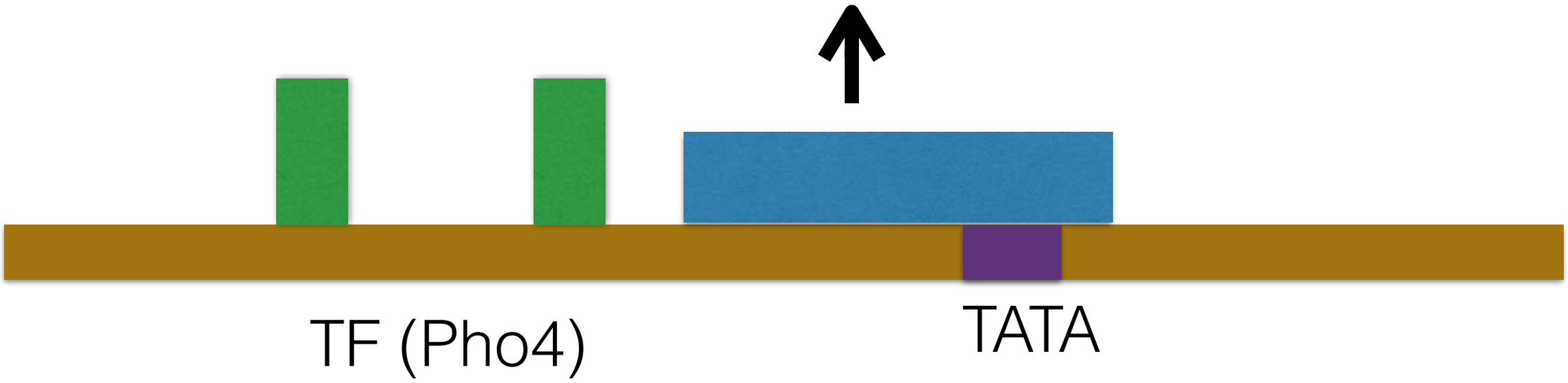
TFs



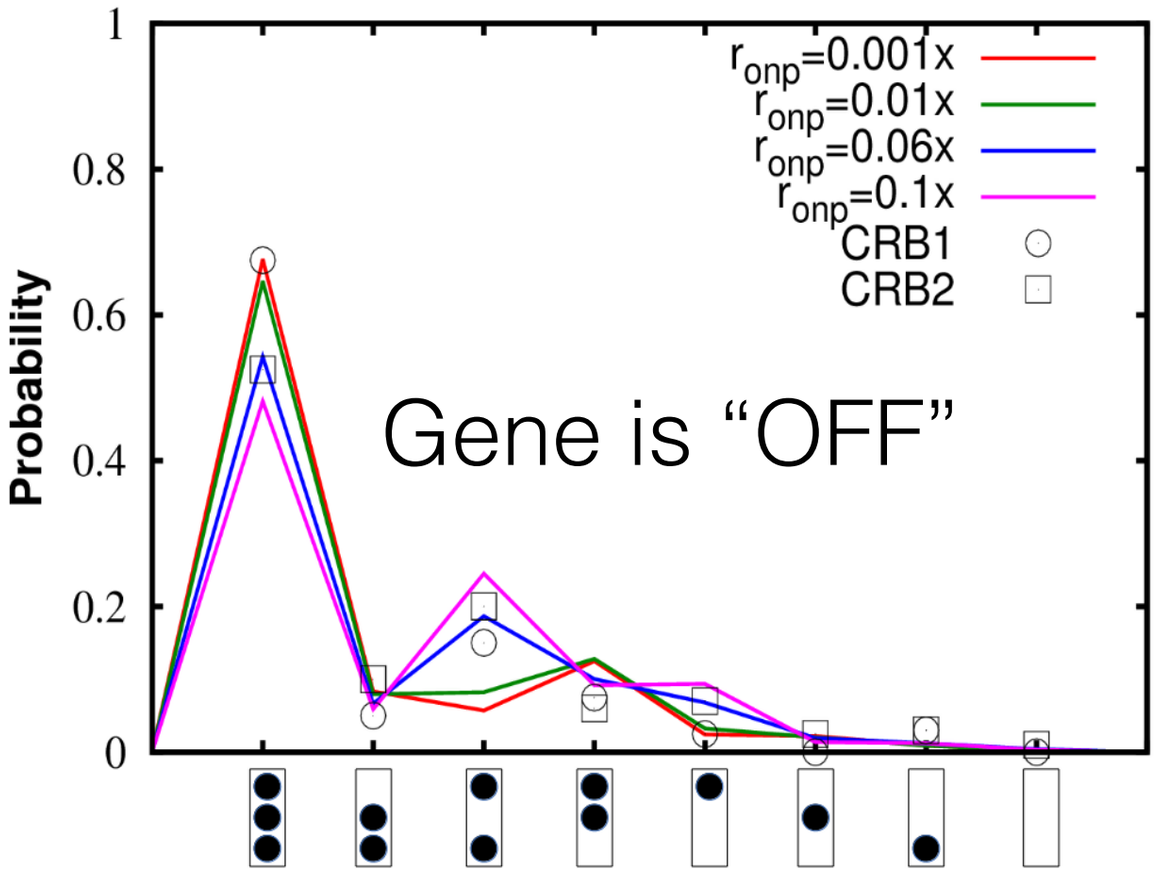
Hungyo Kharerin

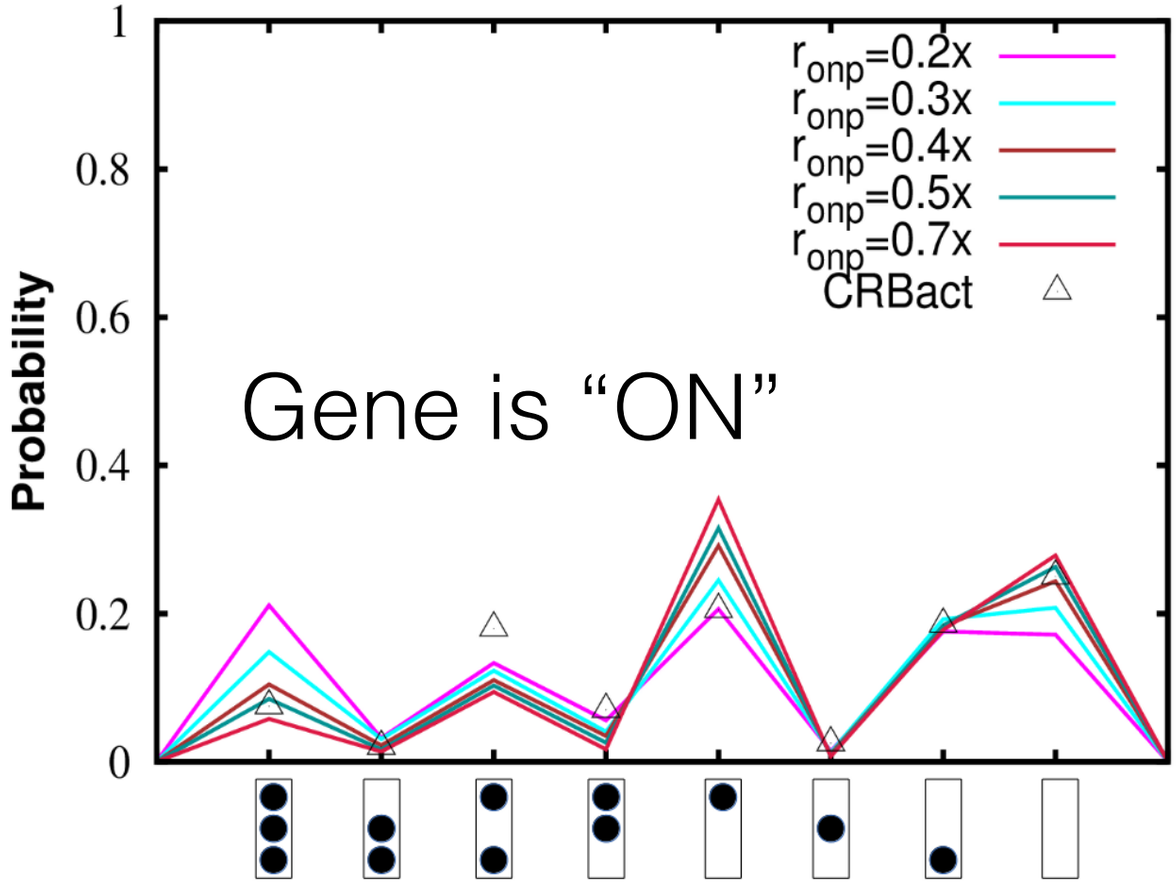
Beyond known nucleosome kinetics two more events needed:

- Binding of certain extra proteins (transcription factors).
- Nucleosome removal coupled with pho4 binding(local remodeling)



ATP-dependent disassembly is absolutely necessary to maintain these different states in each case



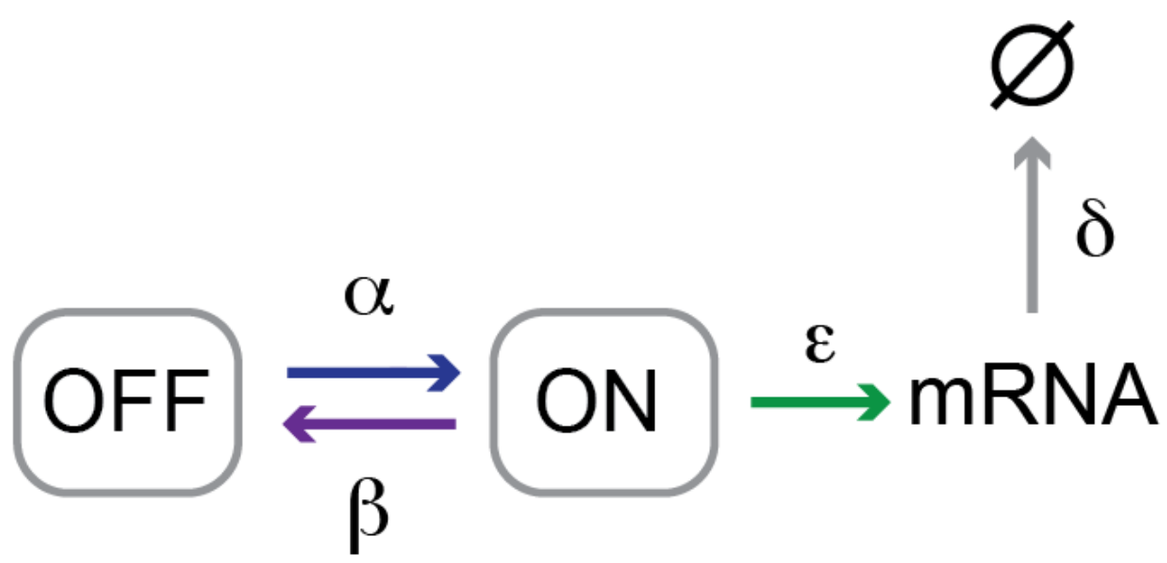


Off state: Nucleosome kinetics + TF binding

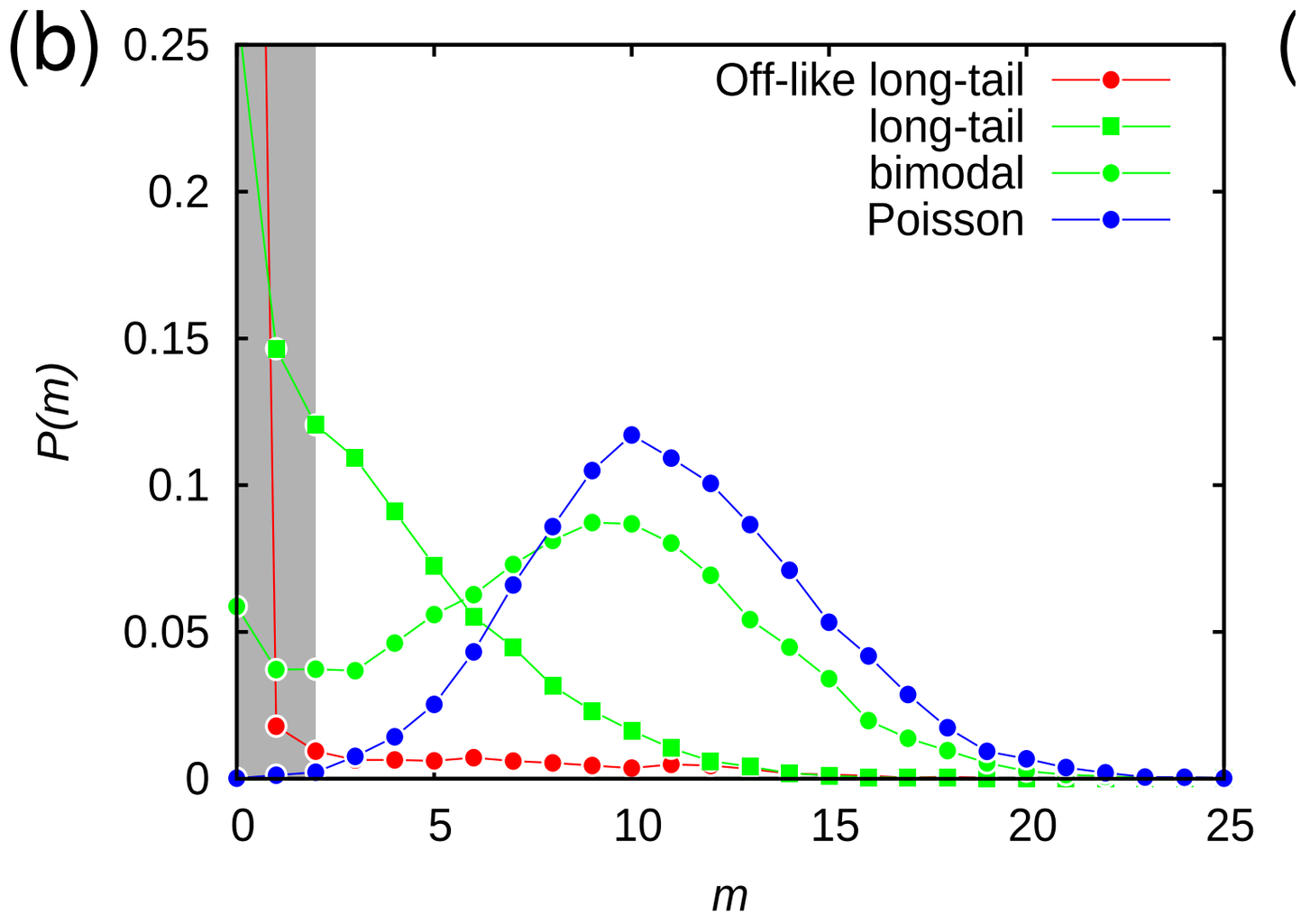
On state: Nucleosome kinetics + TF binding + local nucleosome disassembly coupled to protein binding

Hungyo Kharerin, PJ Bhat, JF Marko and RP Scientific Reports (2016)

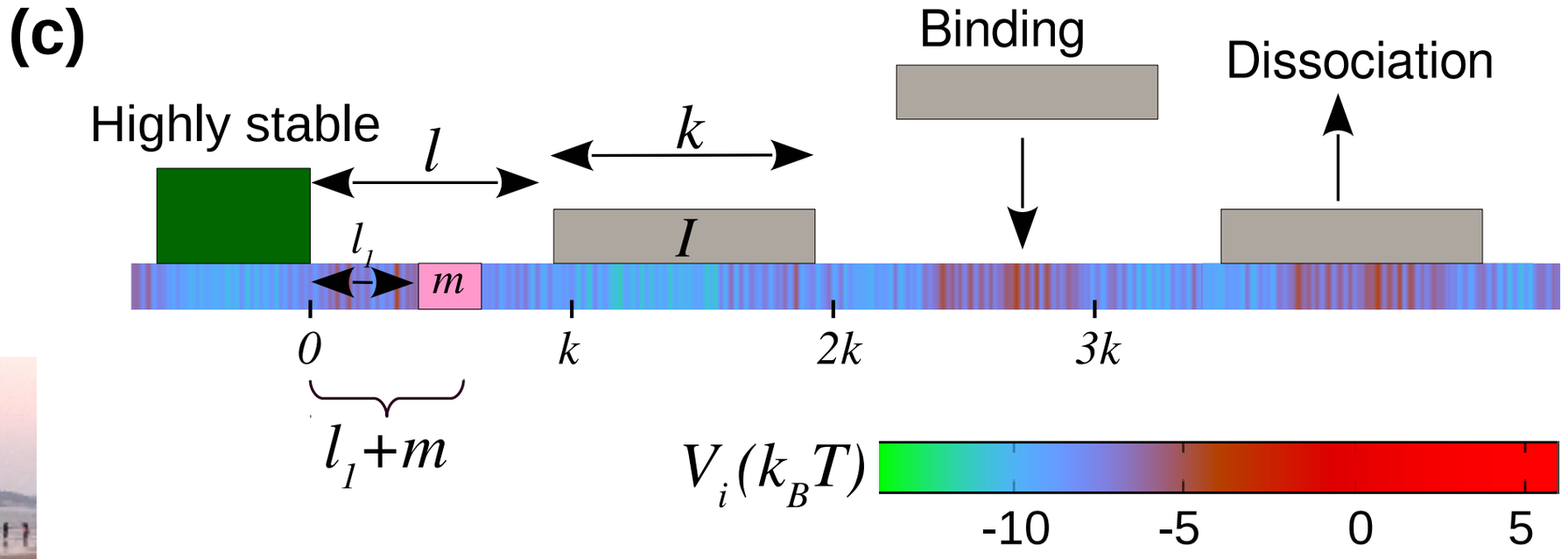
When the gene is “on”, we introduce mRNA production



mRNA distribution

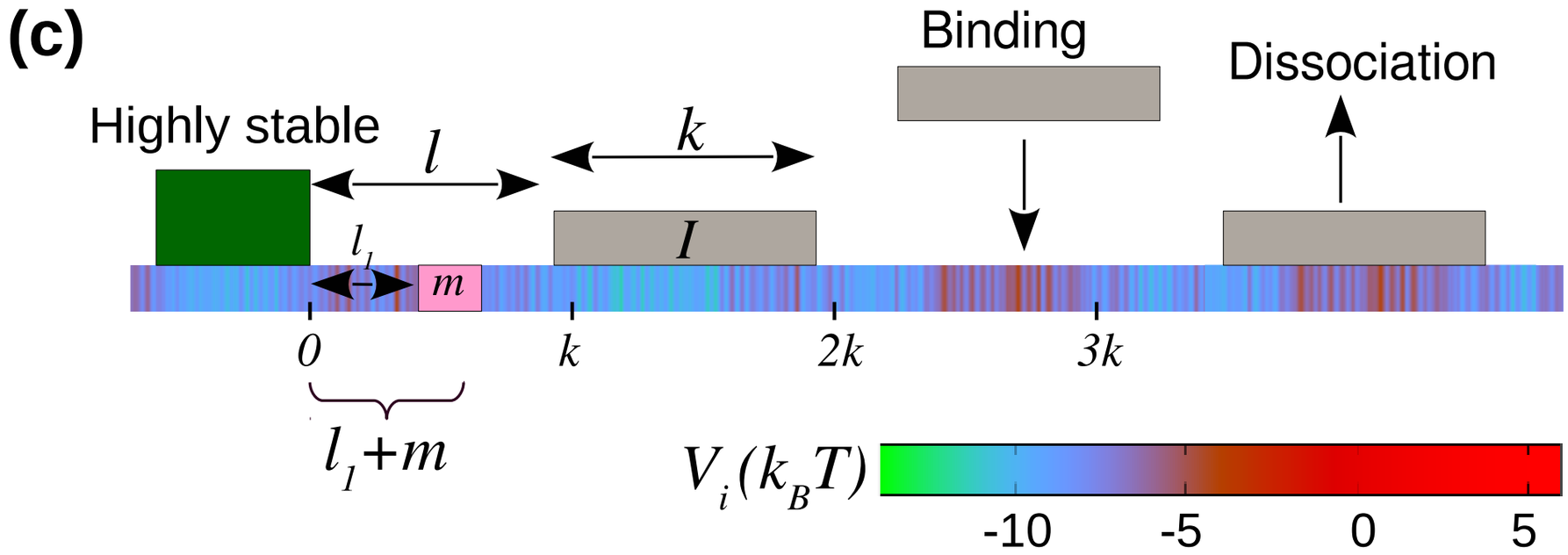


Given nucleosome dynamics, we can compute, how long a particular region of interest will remain “open” (exposed) before it getting covered by another nucleosome



(Jyotsana Parmar, Dibyendu Das)

We compute “first passage time” of covering the patch of size “m”

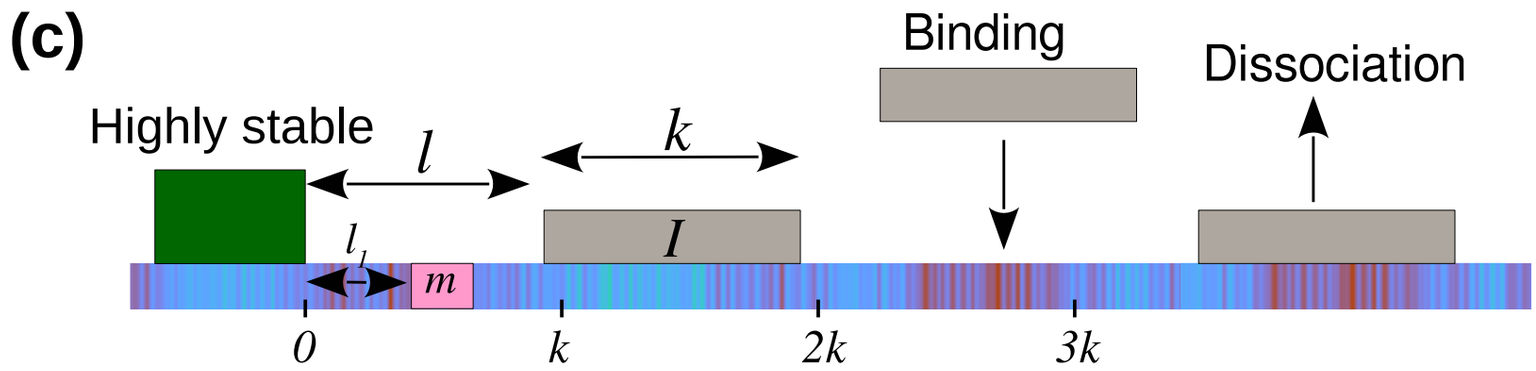


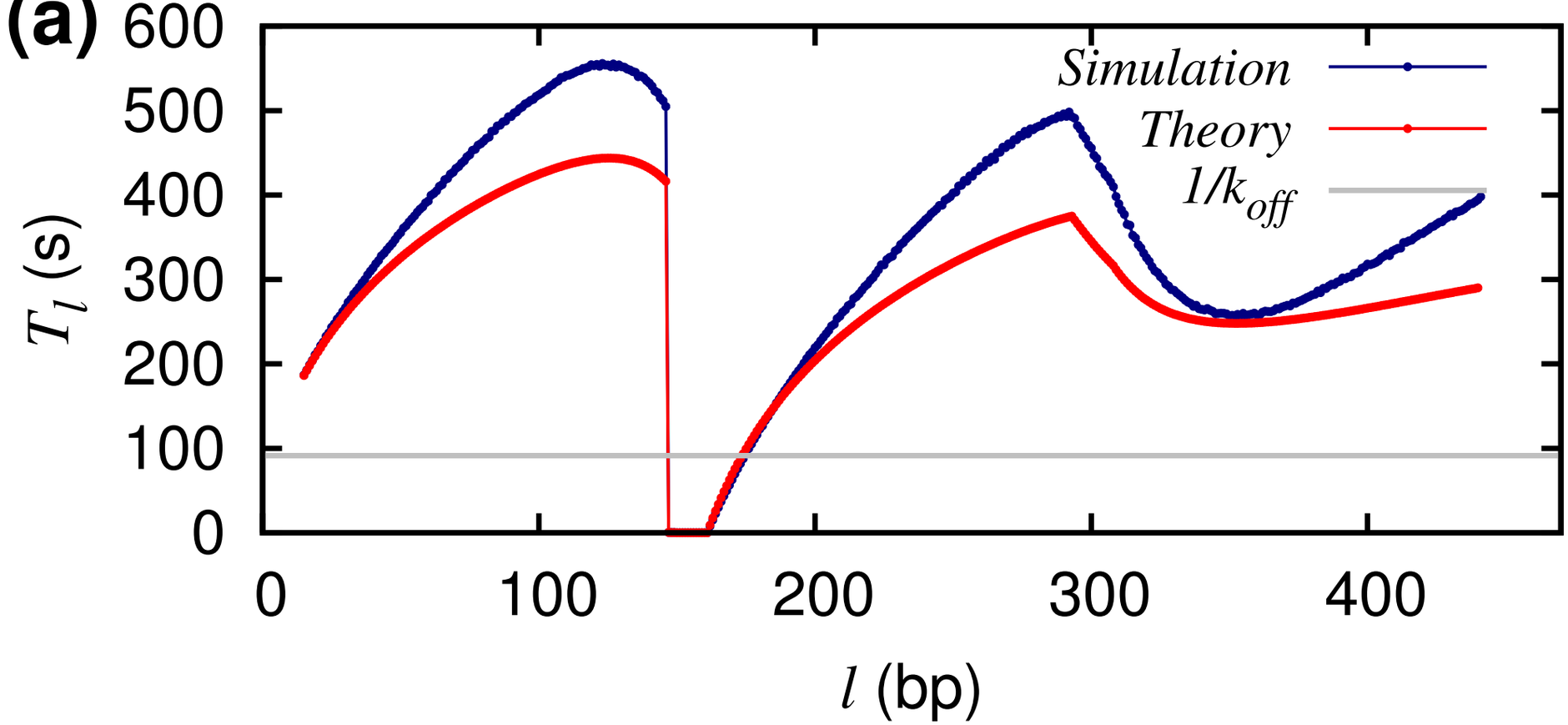
$$T_l - \sum_{\tilde{l}=l+k}^{3k-1} P_{\rm ss}(\tilde{l}-l-k)\; T_{\tilde{l}} = \frac{1}{k_{\rm off}}.$$

When binding-dissociation dominates over sliding, we can compute it analytically

Jyotsana Parmar, Dibyendu Das & RP, Nucleic Acids Res. (2016)

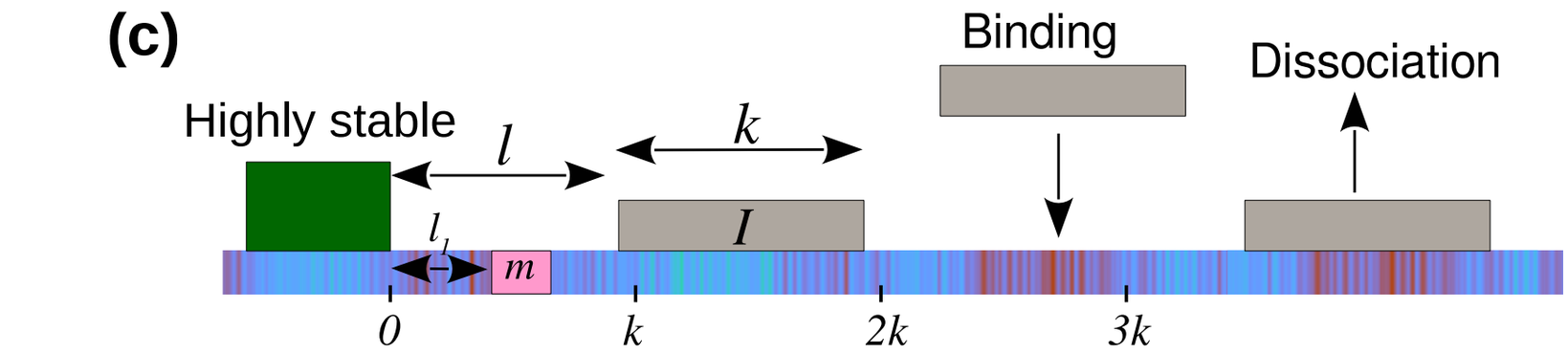
Mean exposure time is very different from any of the known timescales in the problem.

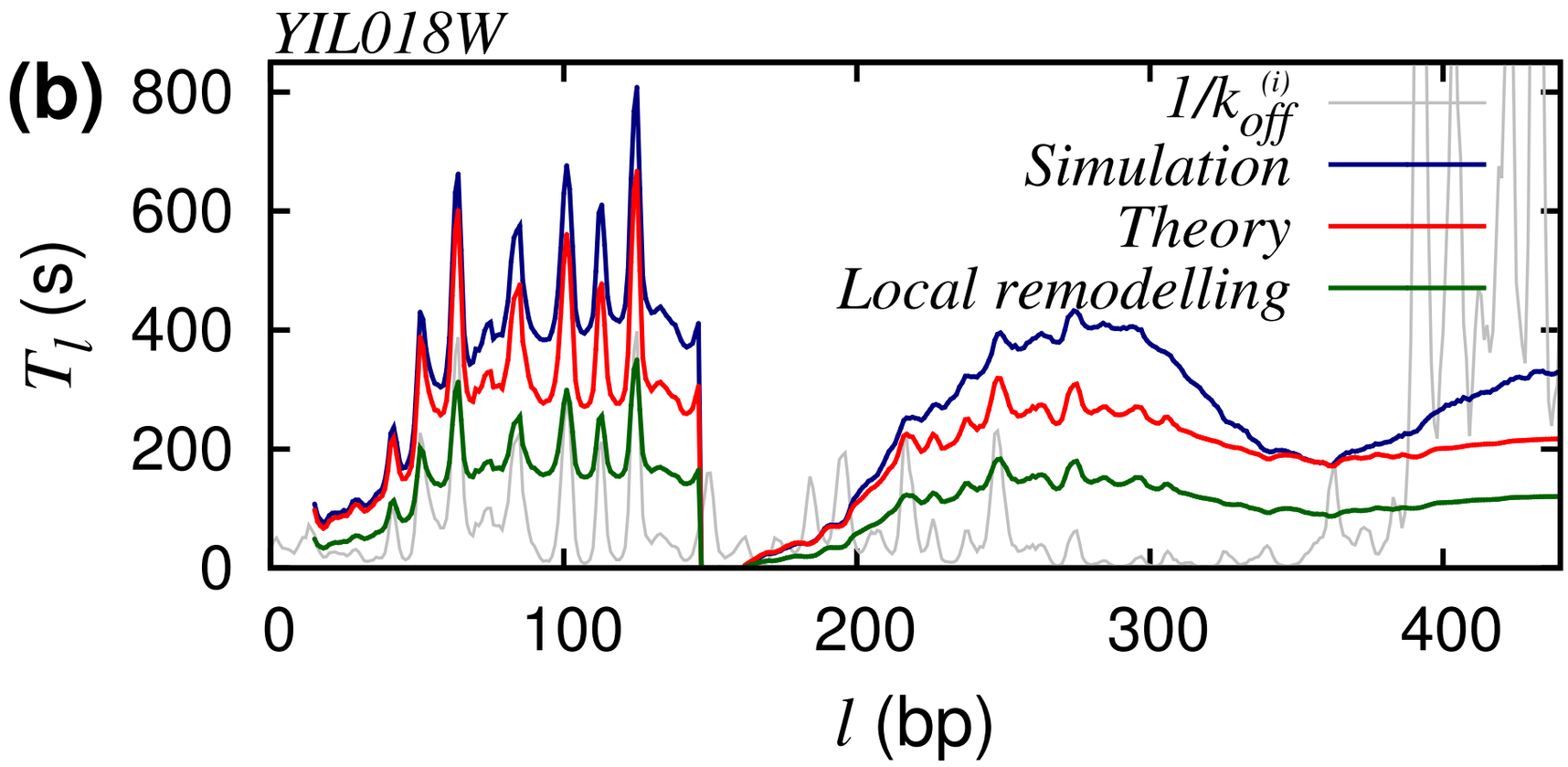




Jyotsana Parmar, Dibyendu Das & RP, Nucleic Acids Res (2015)

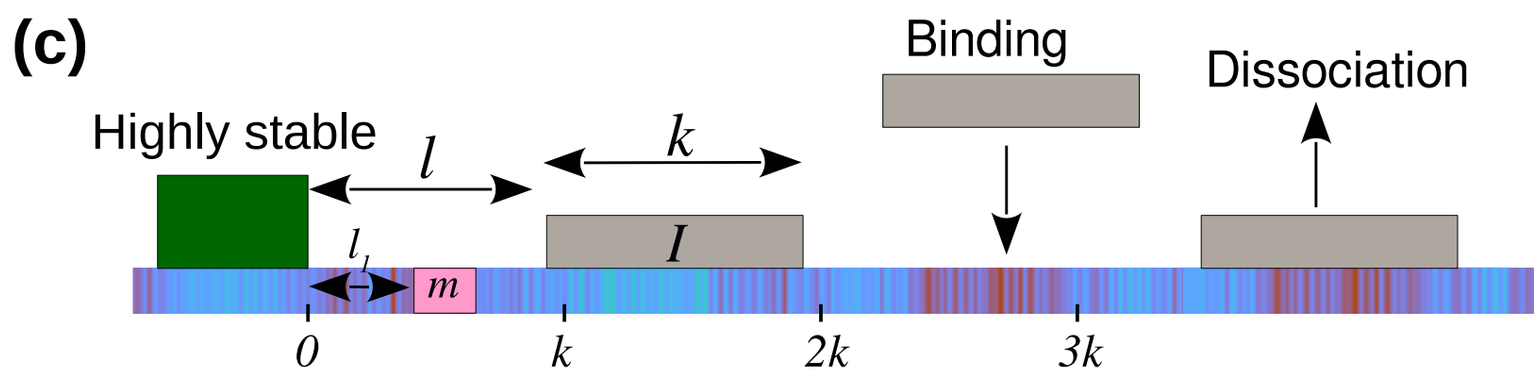
We can compute the same with sequence-dependent rates

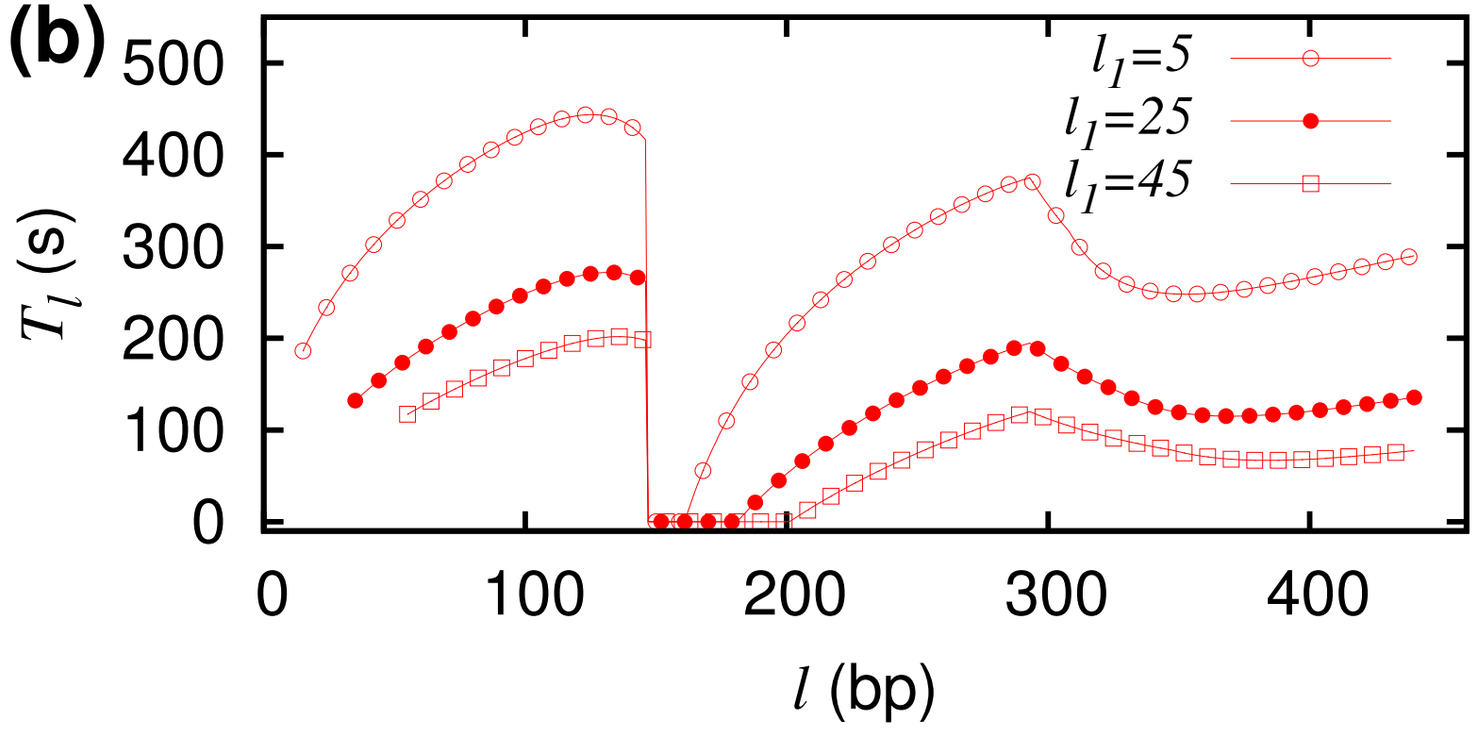




Jyotsana Parmar, Dibyendu Das & RP, Nucleic Acids Res (2015)

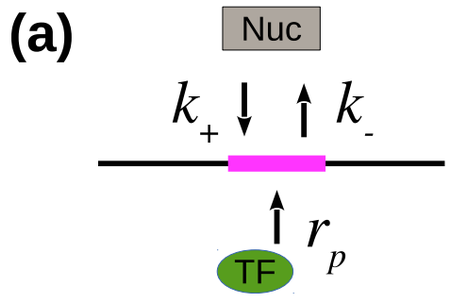
Promoter architecture matters

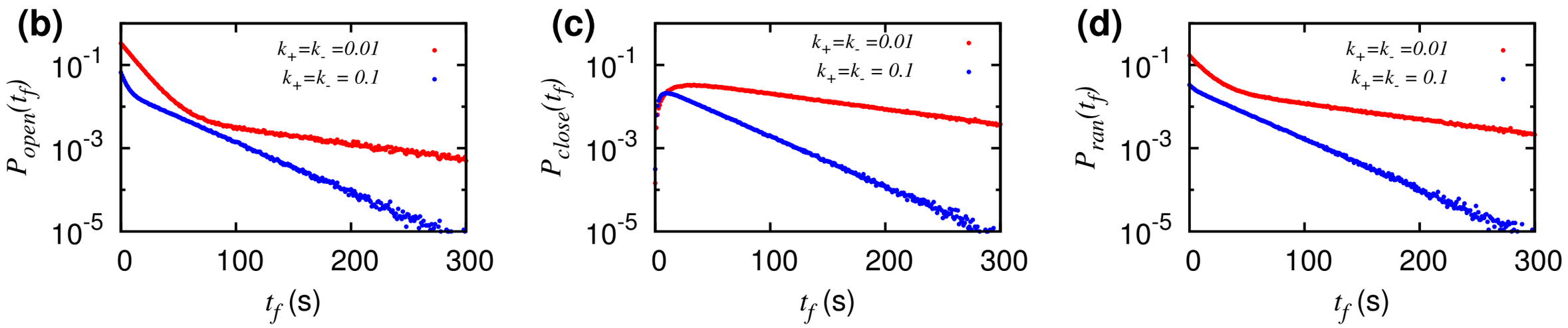




Jyotsana Parmar, Dibyendu Das & RP, Nucleic Acids Res (2015)

Distribution of TF binding times



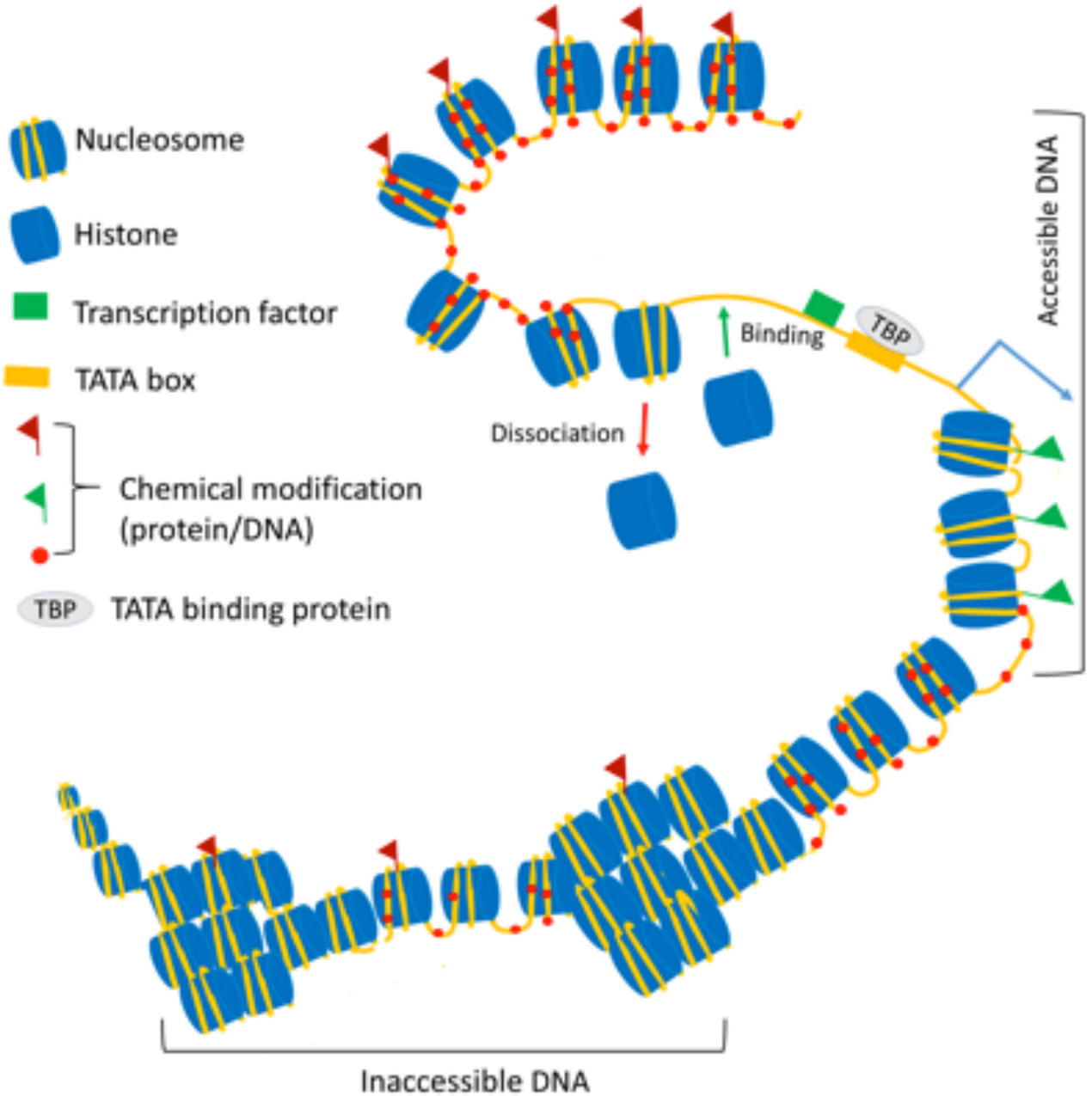


r_p=1/15s

t_f is the time it takes for a the TF to bind

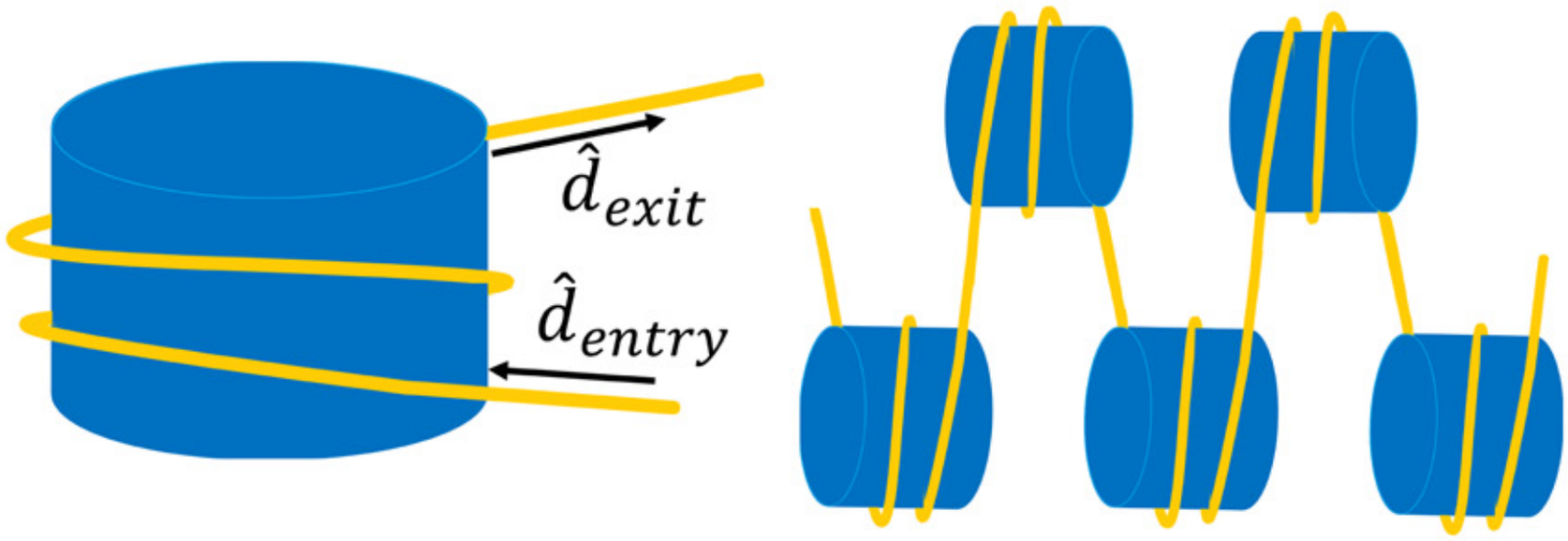
Same nucleosome occupancy; but different TF binding times

Part-2: How do nucleosomes influence 3D organization of the chromatin?



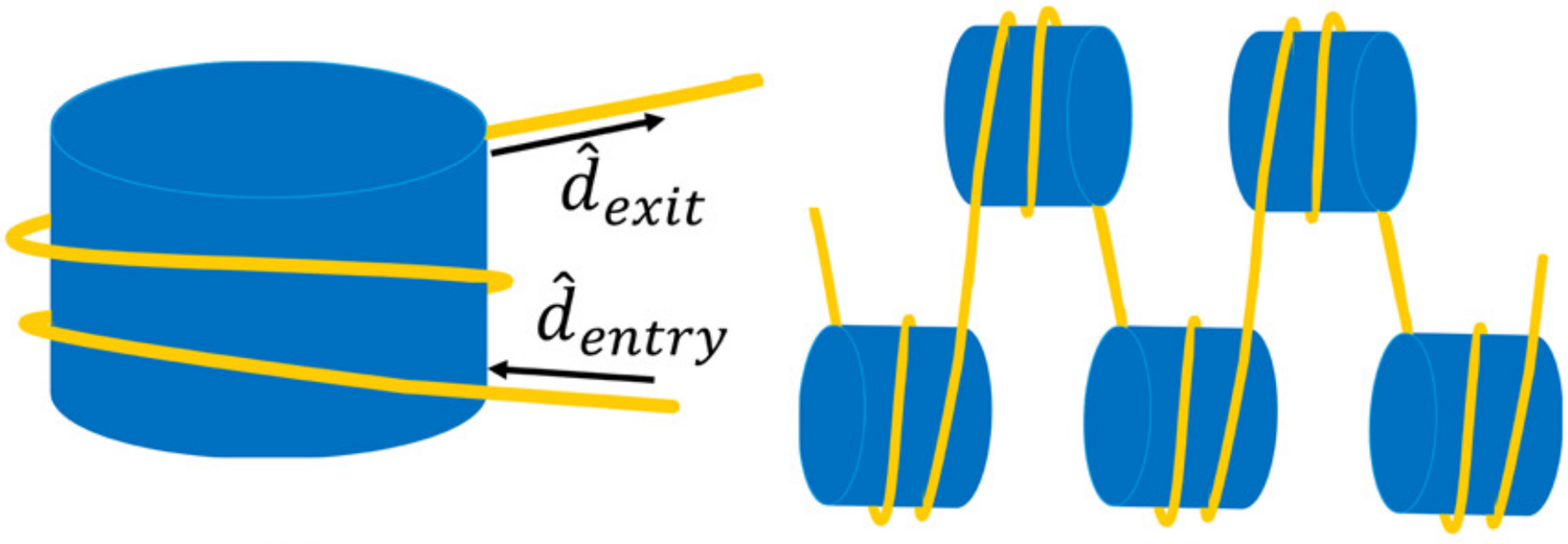
Gaurav Bajpai

Prevalent theory: zig-zag

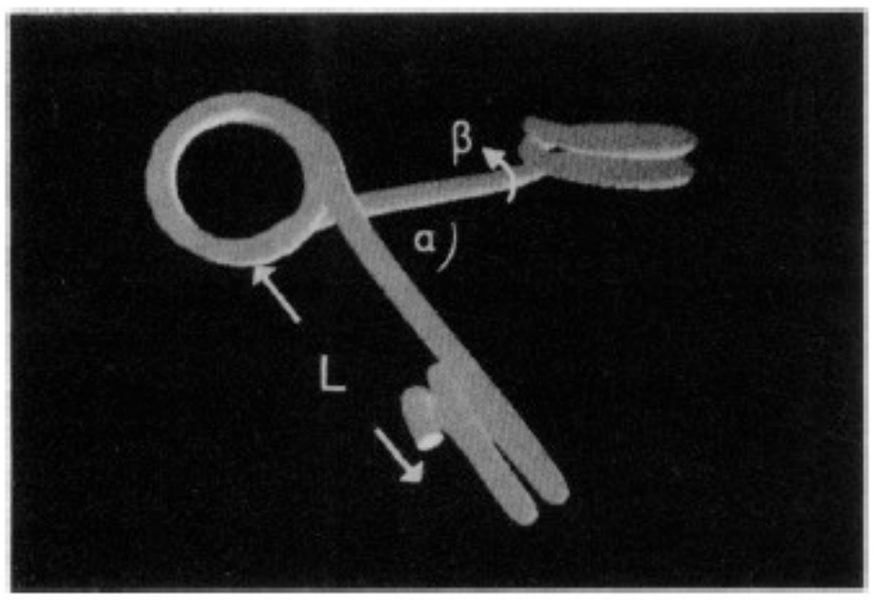


(a) (b)

Physical basis of zig-zag



(a) (b)



* Entry/exit angle of DNA

* Stiffness of linker region

Woodcock et al PNAS (1993)

In situ/In vivo experiments: No regular 30nm structure!

PNAS

Analysis of cryo-electron microscopy images does not support the existence of 30-nm chromatin fibers in mitotic chromosomes in situ

Mikhail Eltsov[a,b,1,2], Kirsty M. MacLellan[a,c,1], Kazuhiro Maeshima[d,1], Achilleas S. Frangakis[b,e], and Jacques Dubochet[a,f]

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Human mitotic chromosomes consist predominantly of irregularly folded nucleosome fibres without a 30-nm chromatin structure

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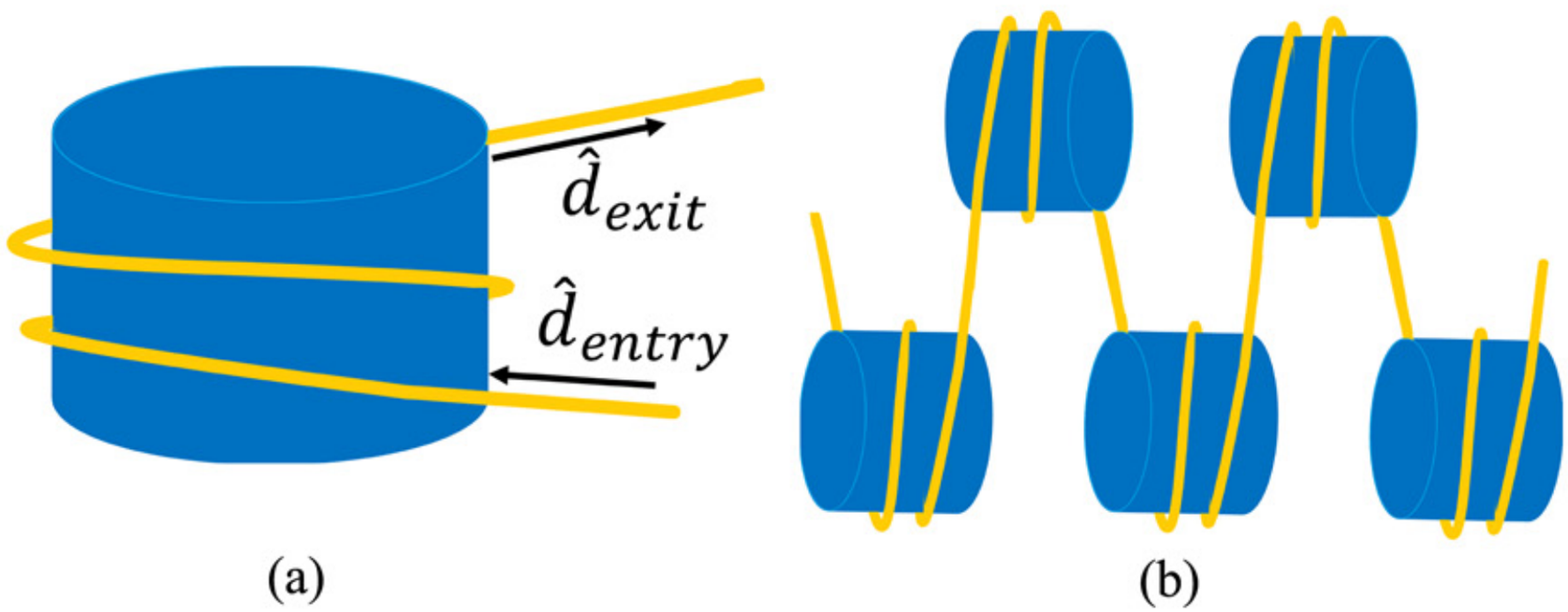
A long strand of DNA is wrapped around core histones to form a nucleosome structure like 'beads on a string' (Kornberg and Lorch, 1999). It has long been assumed that this nucleosome fibre is folded into 30-nm chromatin fibres (Alberts *et al*, 2007) and that condensins are involved in further regular chromatin folding (Swedlow and Hirano,

Elstov et al PNAS 2007, Maeshima Lab, EMBO J (2012),
Decker Lab, Oliver Rando Lab, Cell (2015)

Models so far: DNA+histones

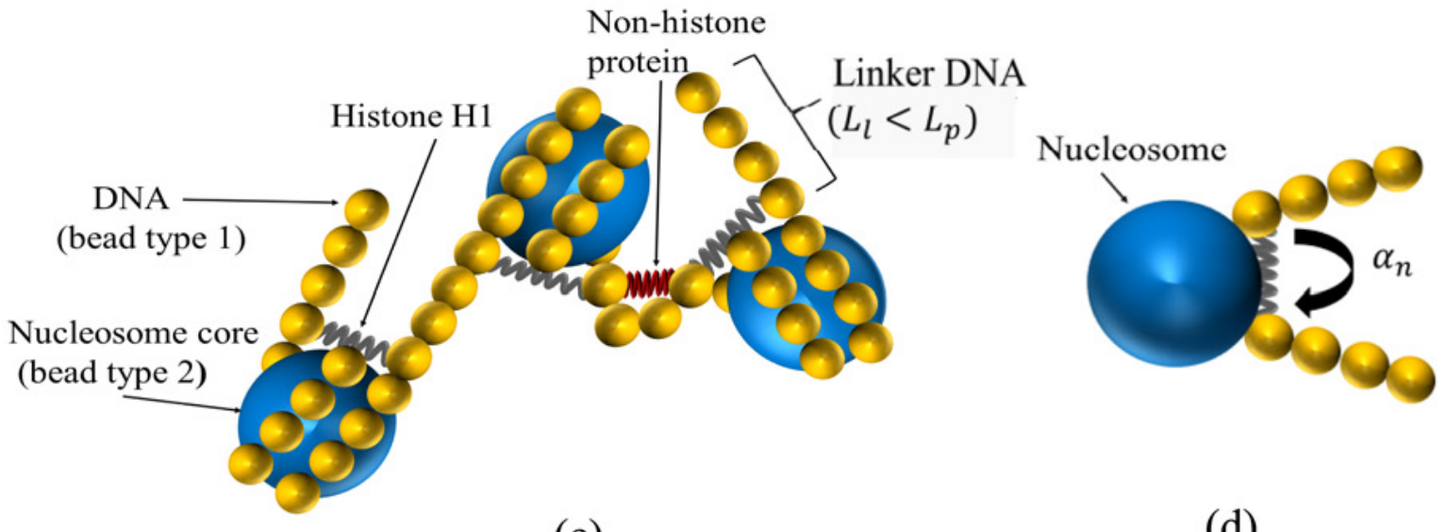


Models so far only consider histones (core histones+H1)
That too mostly regular organization of histones!

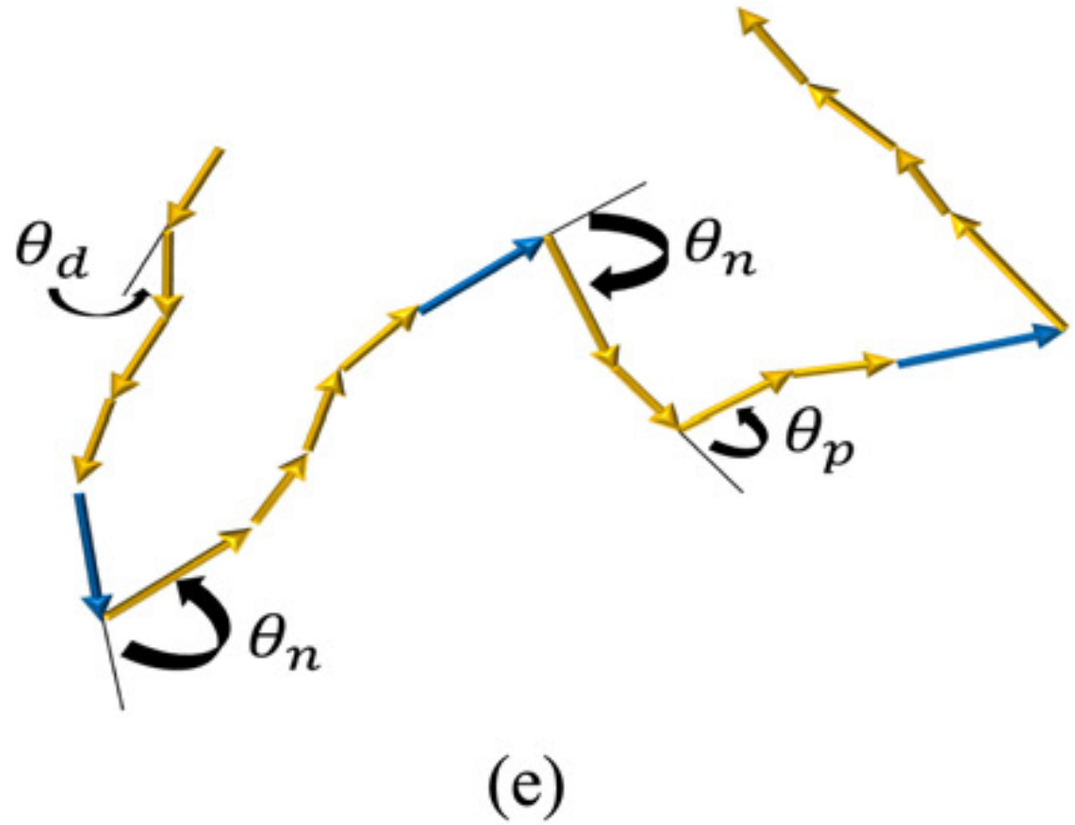
Woodcock et al,
Tamar Schlick lab, Langowski lab

- No model so far has accounted for the role of DNA-bending non-histone proteins (eg. HMG/nhp6)
- What would be the chromatin organization in the length-scale of a gene (a few genes) given a concentration of DNA-bending proteins and a given nucleosome organization?

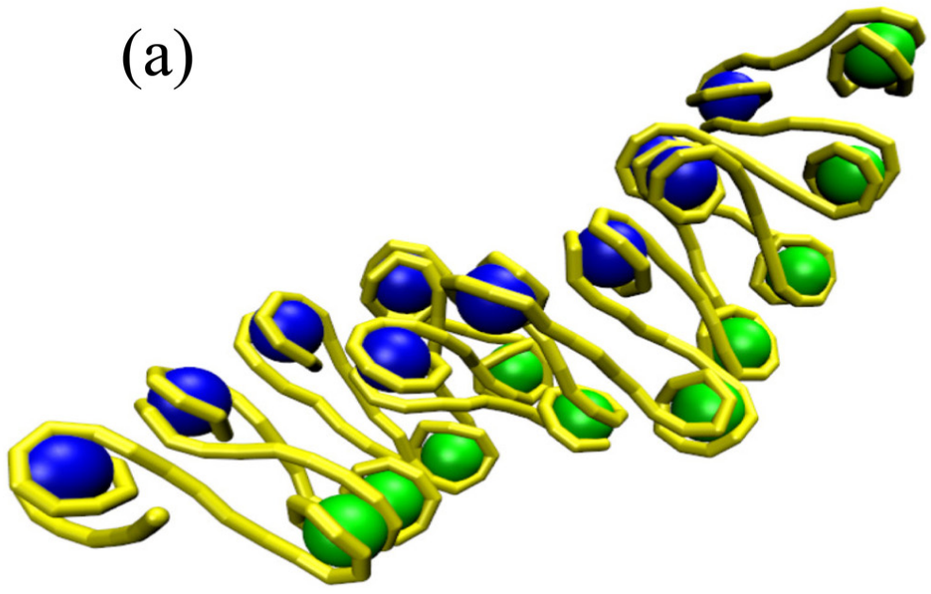
We do polymer simulations accounting for nucleosomes and DNA-bending proteins

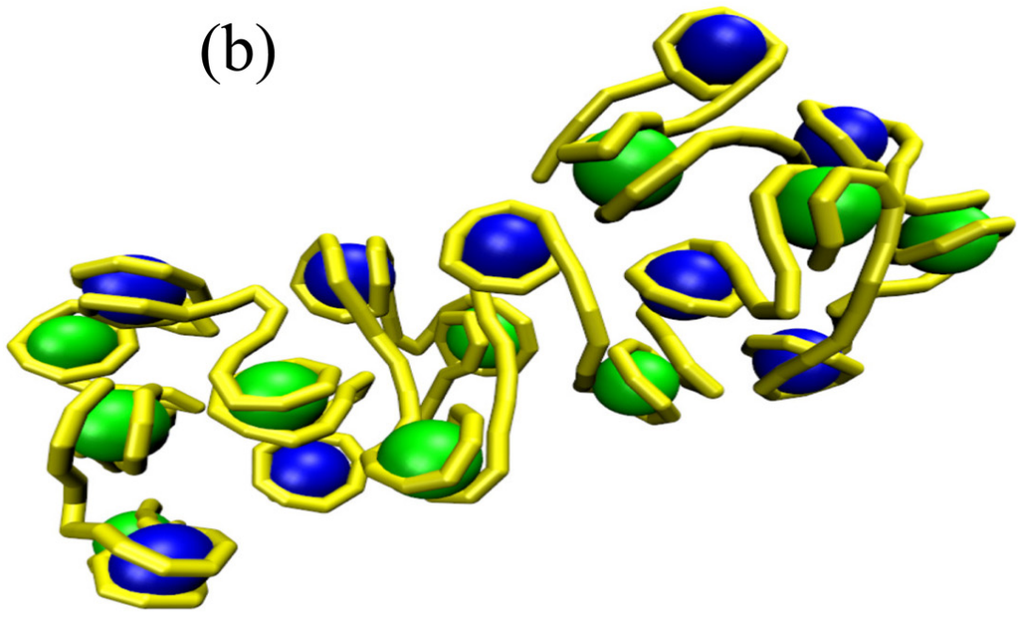


Brownian dynamics simulation



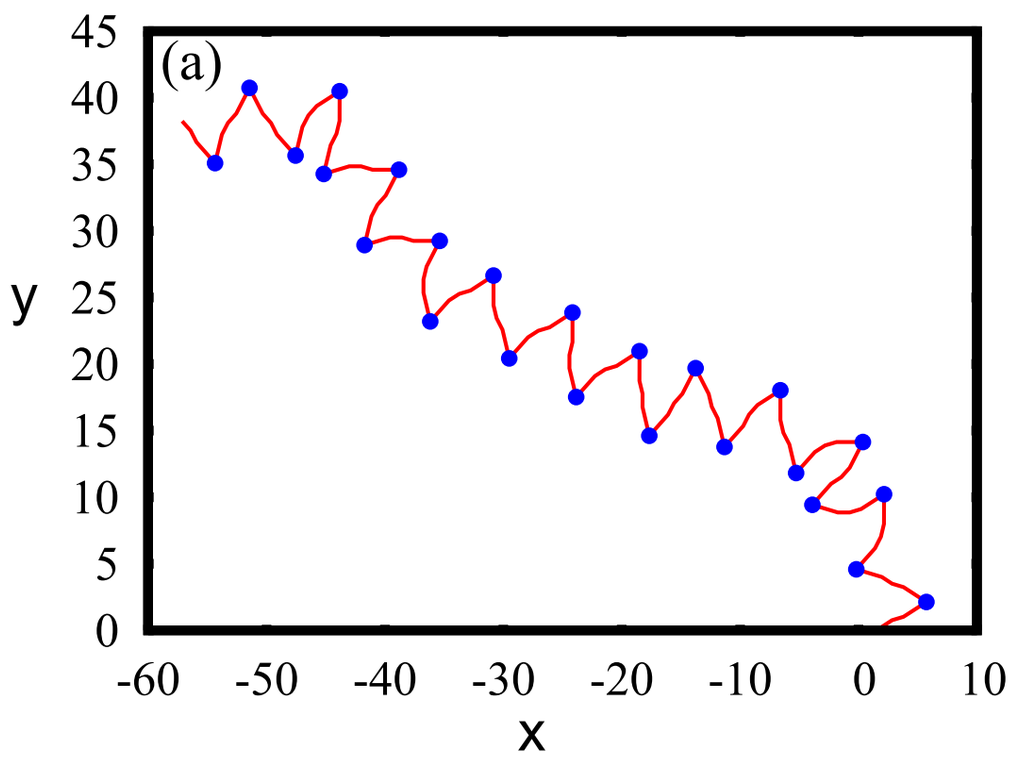
Freely rotating Chain model for polymers

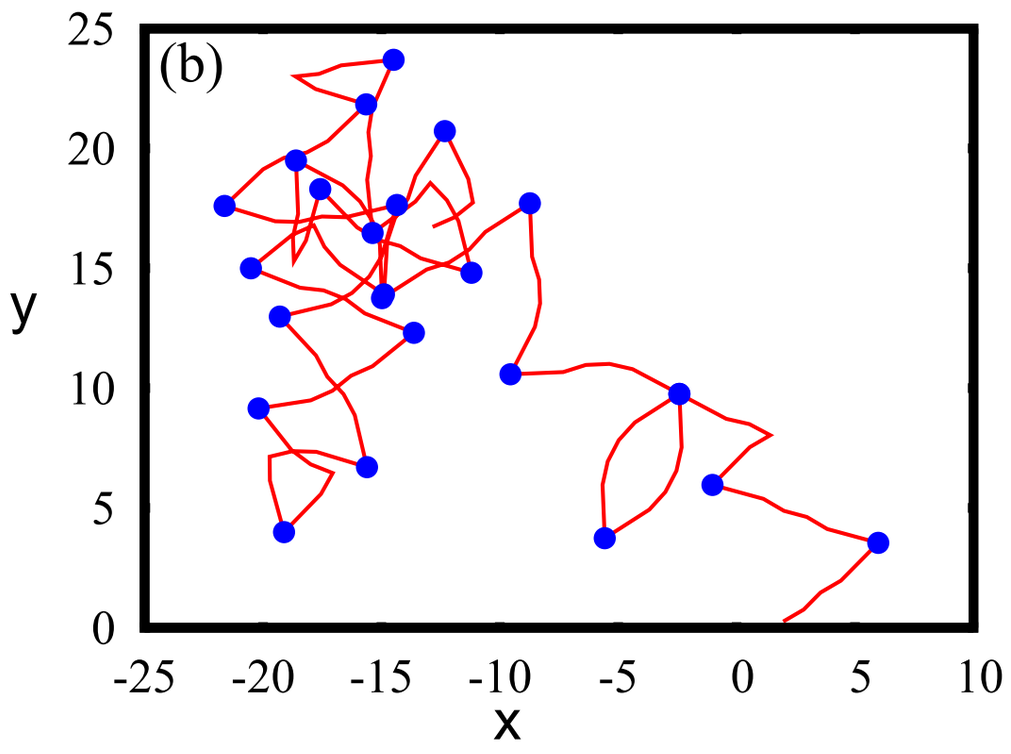




No non-histone protein

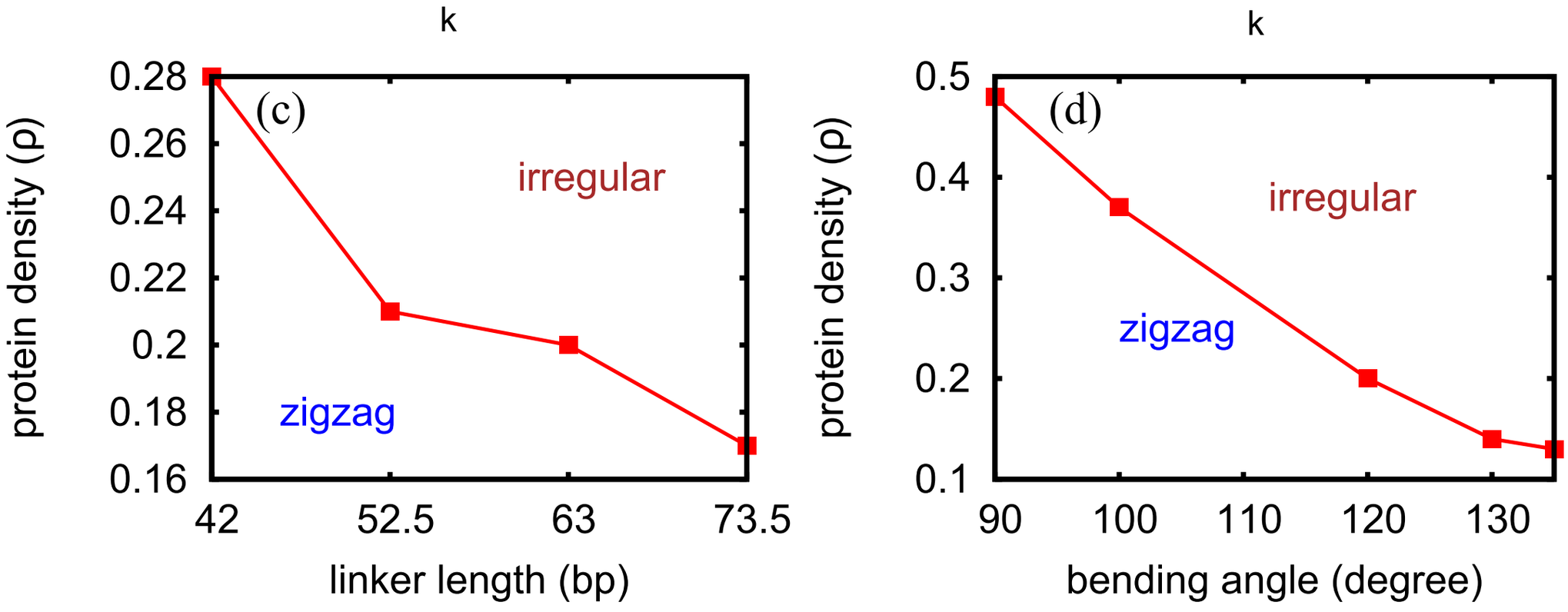
With non-histone protein





Gaurv Bajpai et al (2017) PLOS Comp. Bio.

Under what conditions will one observe “irregular” chromatin?

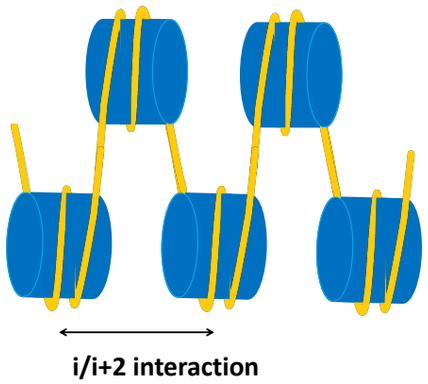


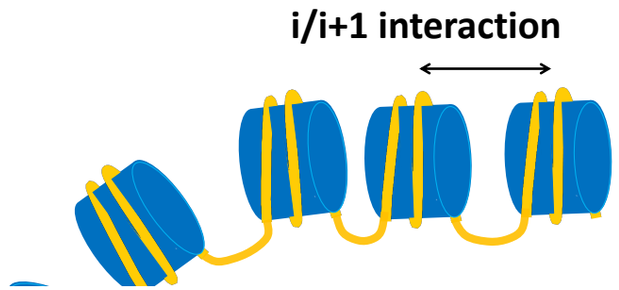
Bajpai et al (2017) PLOS Comp. Bio.

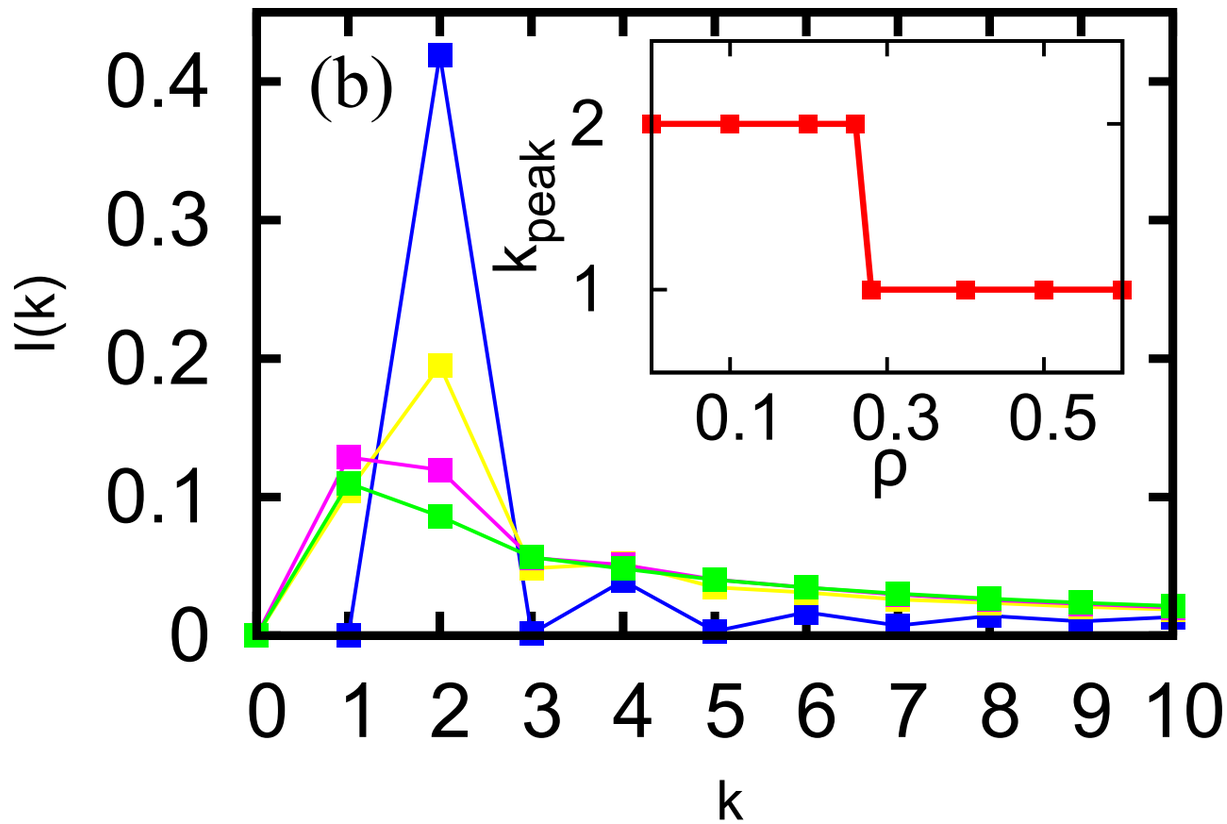
What is the density of non-histone proteins beyond which the zig-zag would disappear?

Is that density biologically relevant?

Probability of neighboring nucleosomes to interact



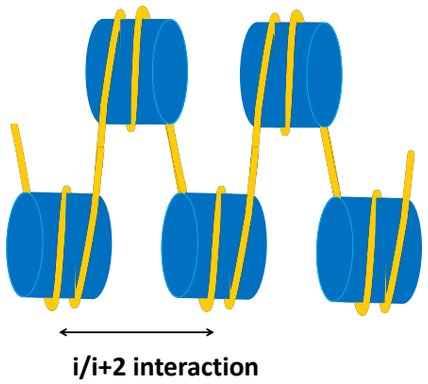


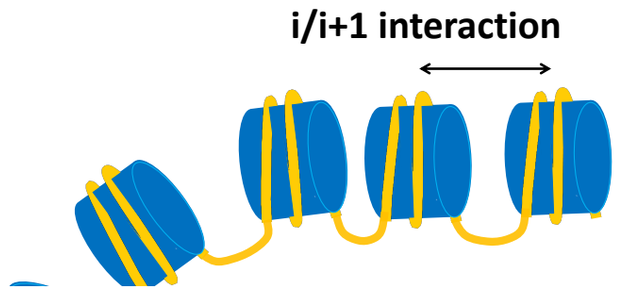


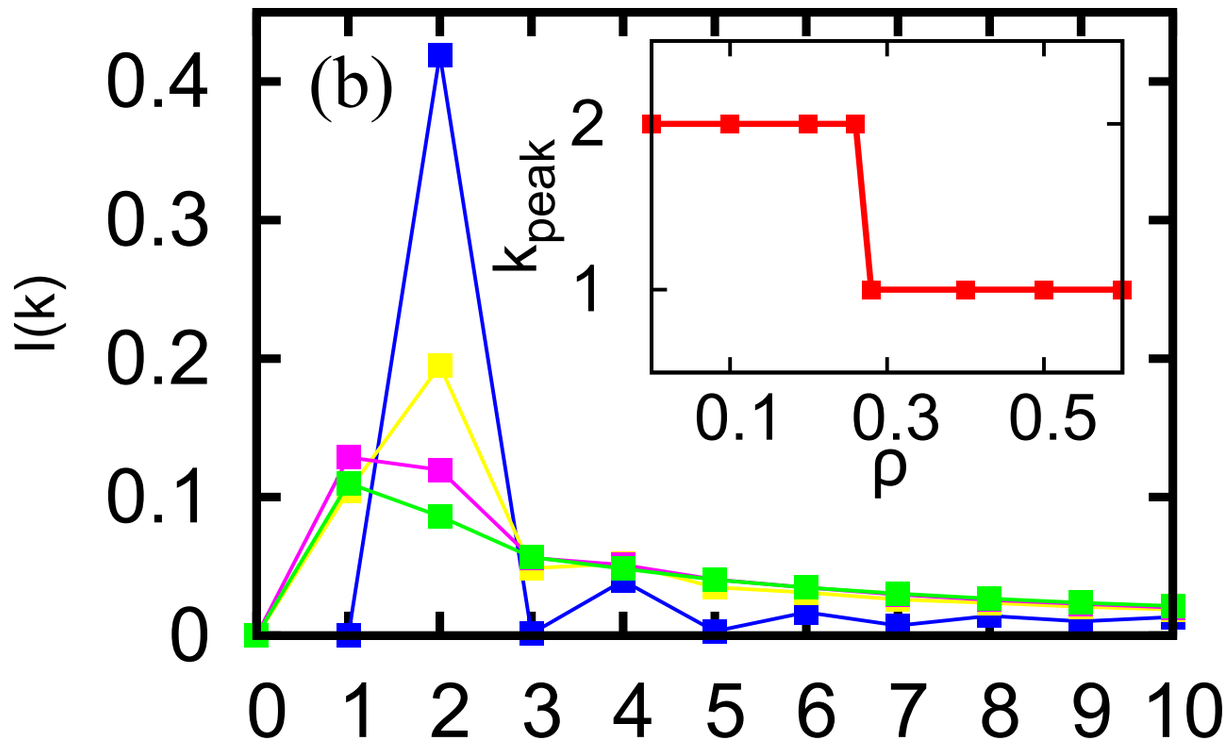
When NHP density~30%, I(1)=I(2)

Bajpai et al (2017) PLOS Comp. Bio.

Probability of neighboring nucleosomes to interact





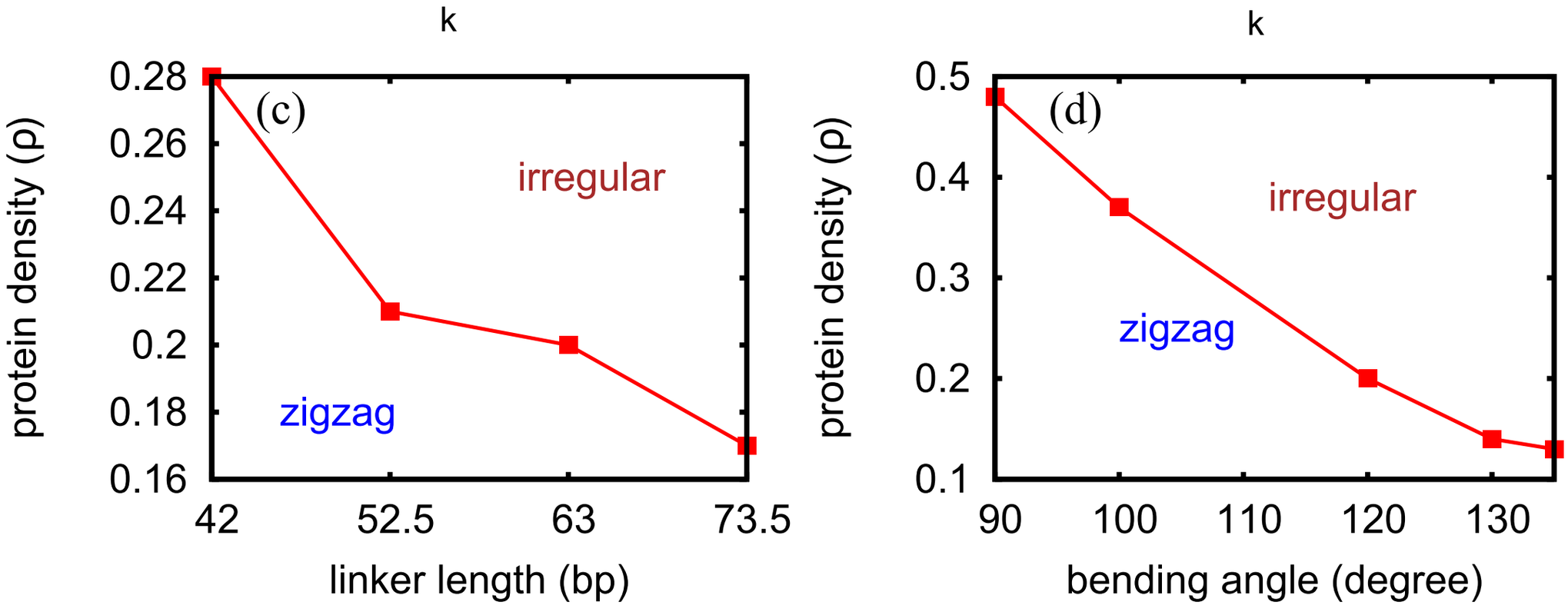


When NHP density~30%, $I(1) \overset{k}{=} I(2)$

Neighbor and next-neighbor nucleosomes interact.
Comparable with recent Micro-C (Hi-C) experiments

Bajpai et al (2017) PLOS Comp. Bio.

Under what conditions will one observe “irregular” chromatin?



Bajpai et al (2017) PLOS Comp. Bio.

Summary

- Physical models for nucleosome positioning
- Dynamics of nucleosomes
- How nucleosome positioning affect 3D organization of chromatin
- DNA-bending non-histone protein will influence 3D organization of chromatin

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http://www.bio.iitb.ac.in/~ranjith/