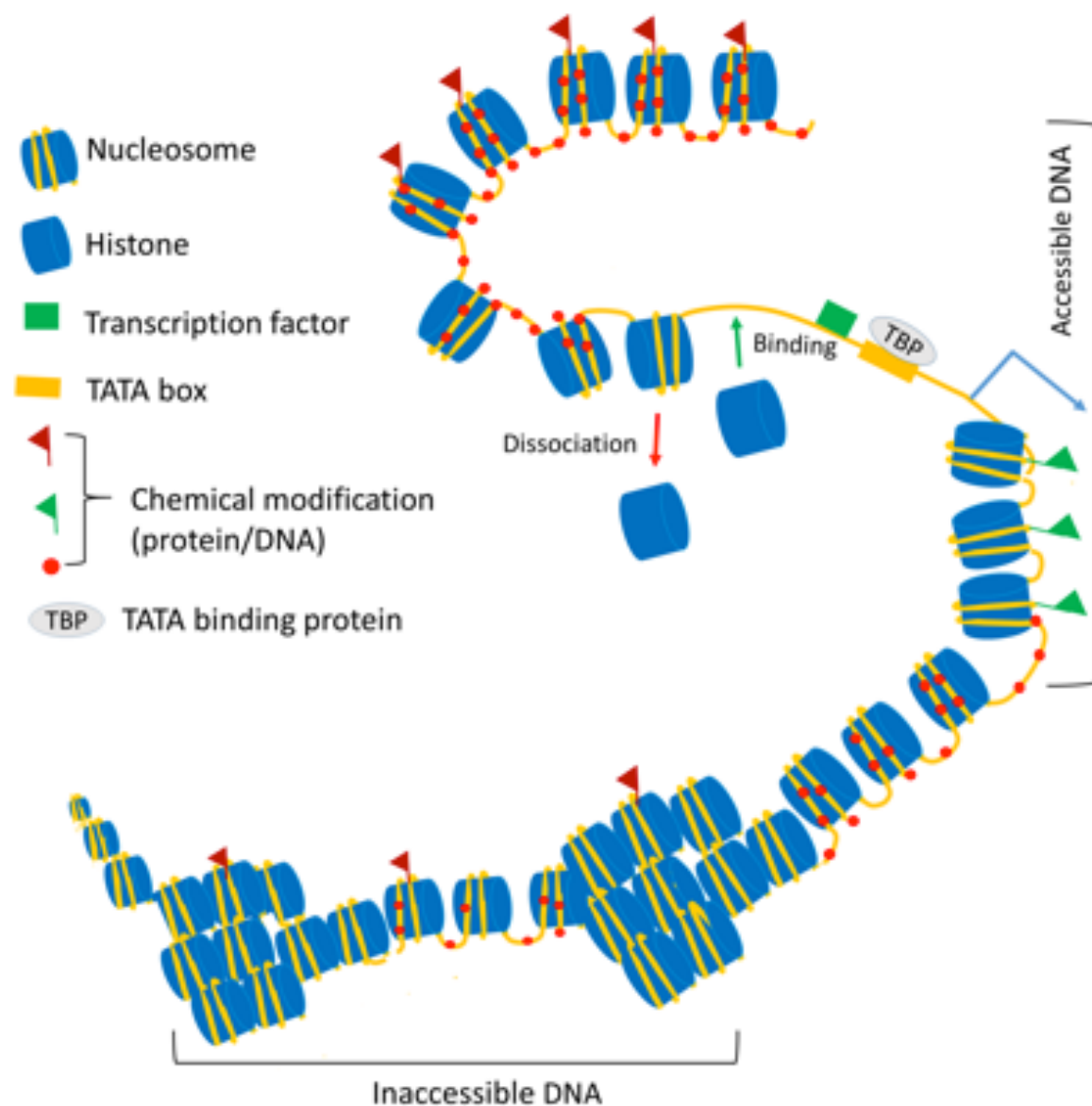
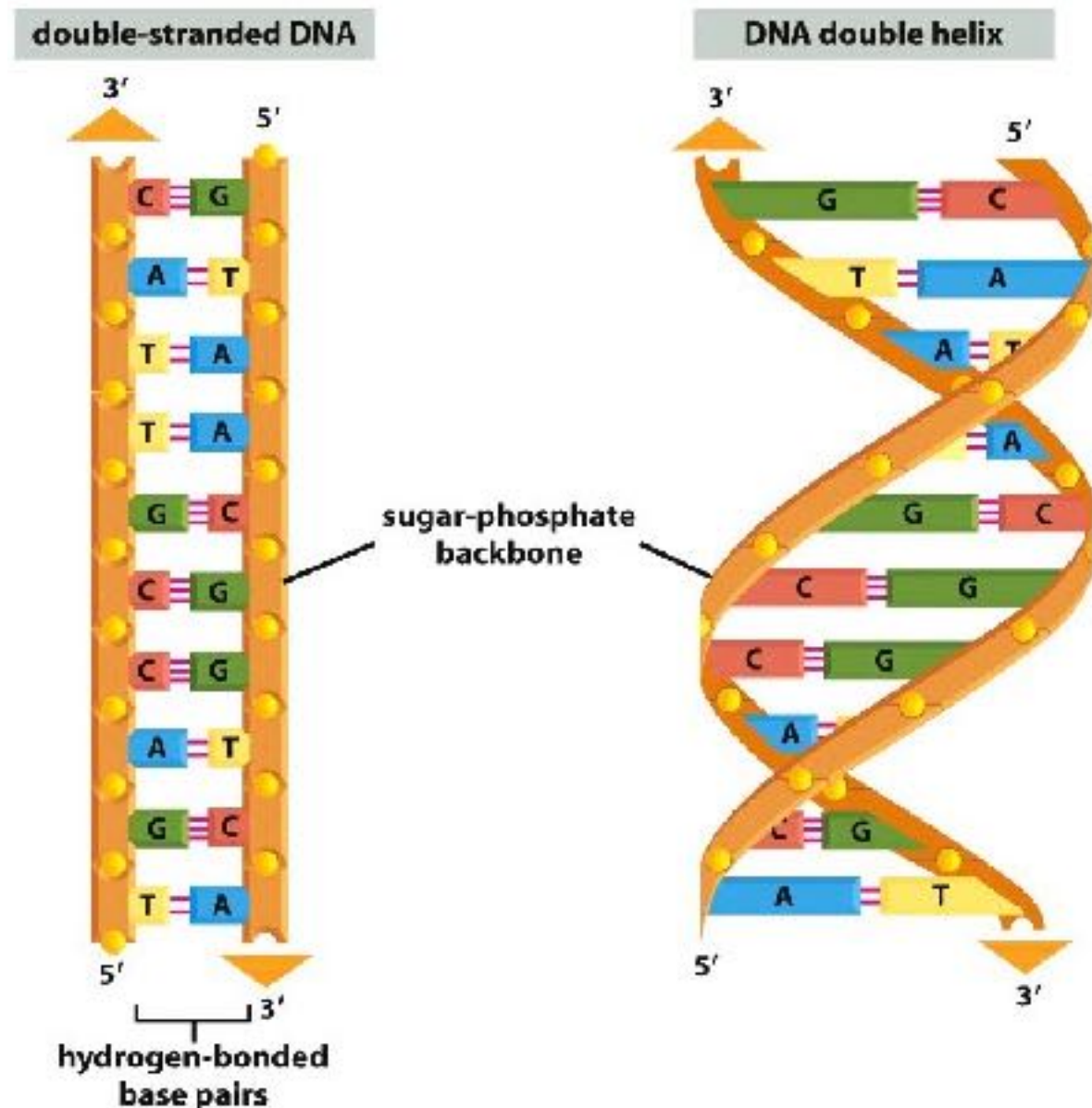


# Stochasticity in nucleosome positioning: chromatin states and gene regulation



Ranjith Padinhateeri  
Biosciences & Bioengineering  
IIT Bombay

# DNA : molecule that contains code for cellular processes



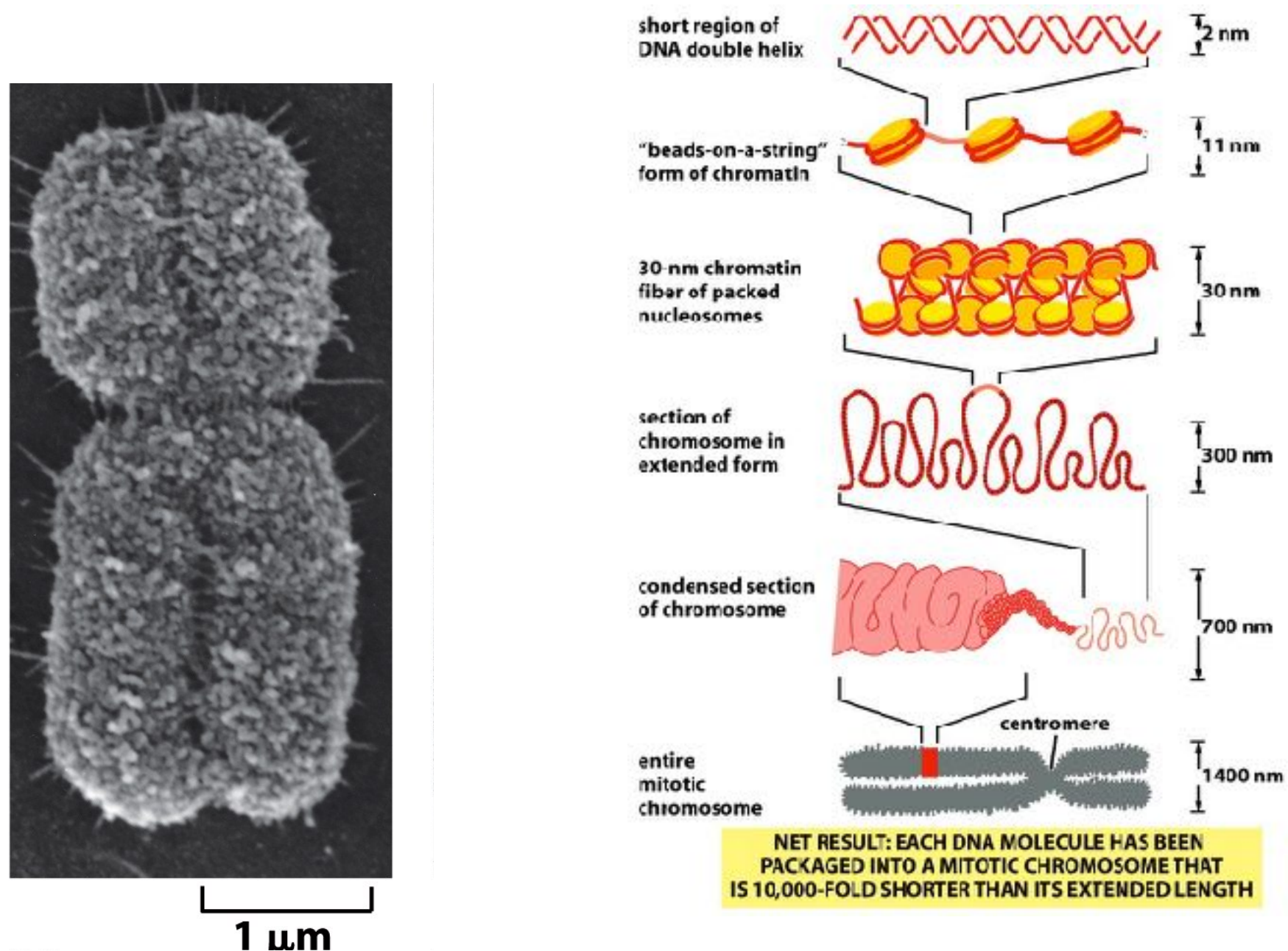
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Figure credit: *Molecular Biology of the Cell* (© Garland Science 2008)

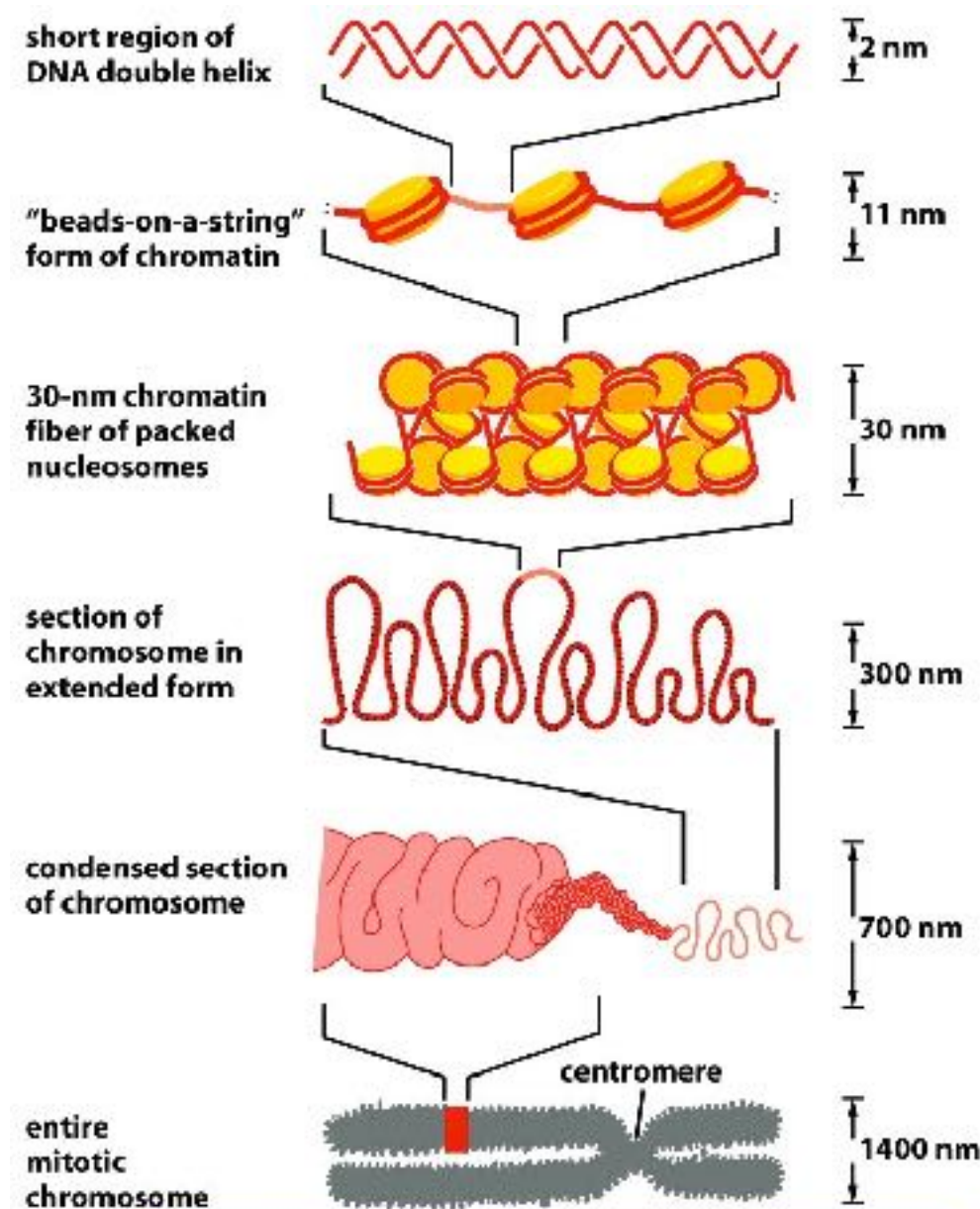
DNA needs to be read;  
but cells need to prevent  
“unwanted” reading!

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





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



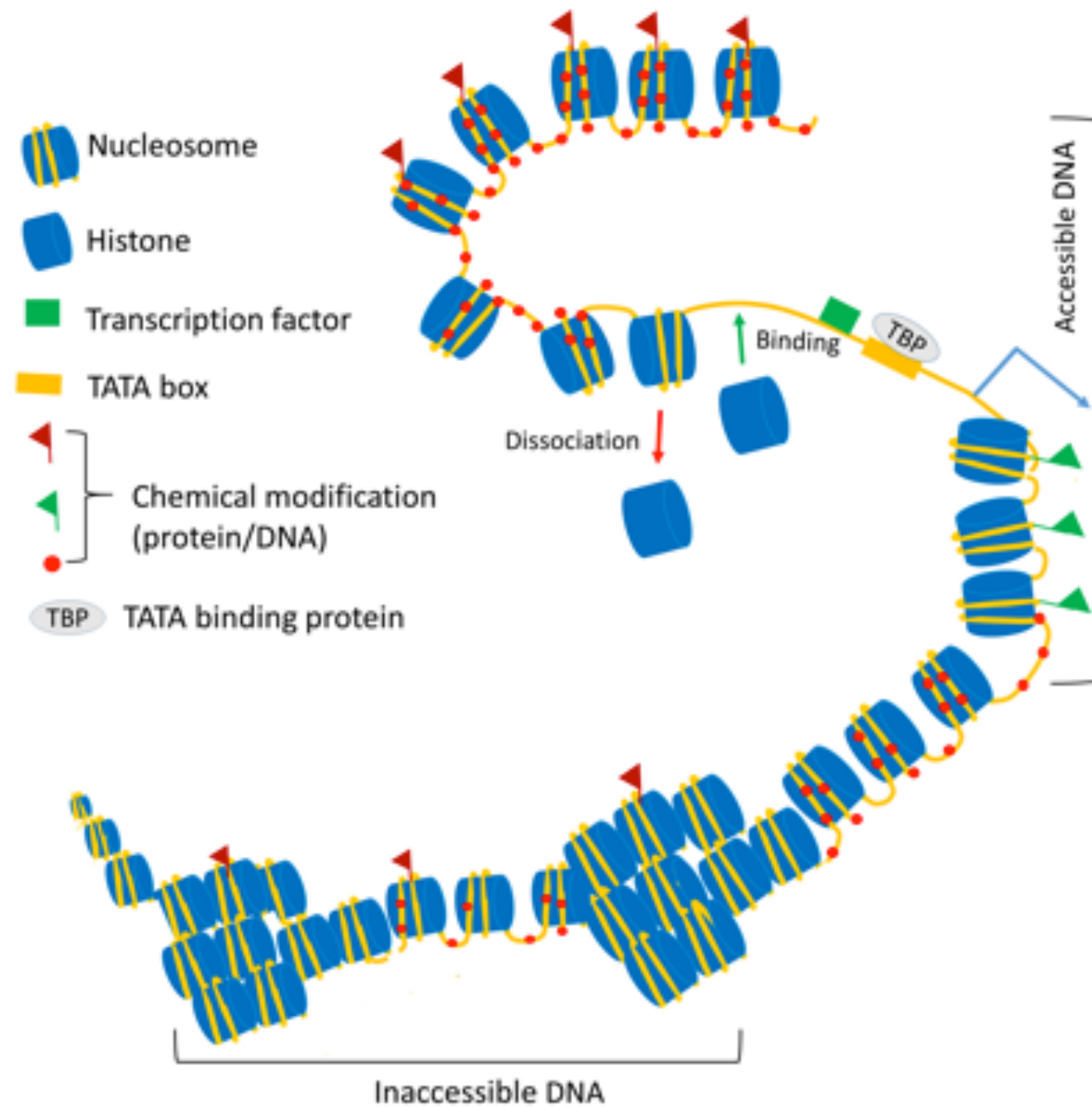
DNA

Array of nucleosomes

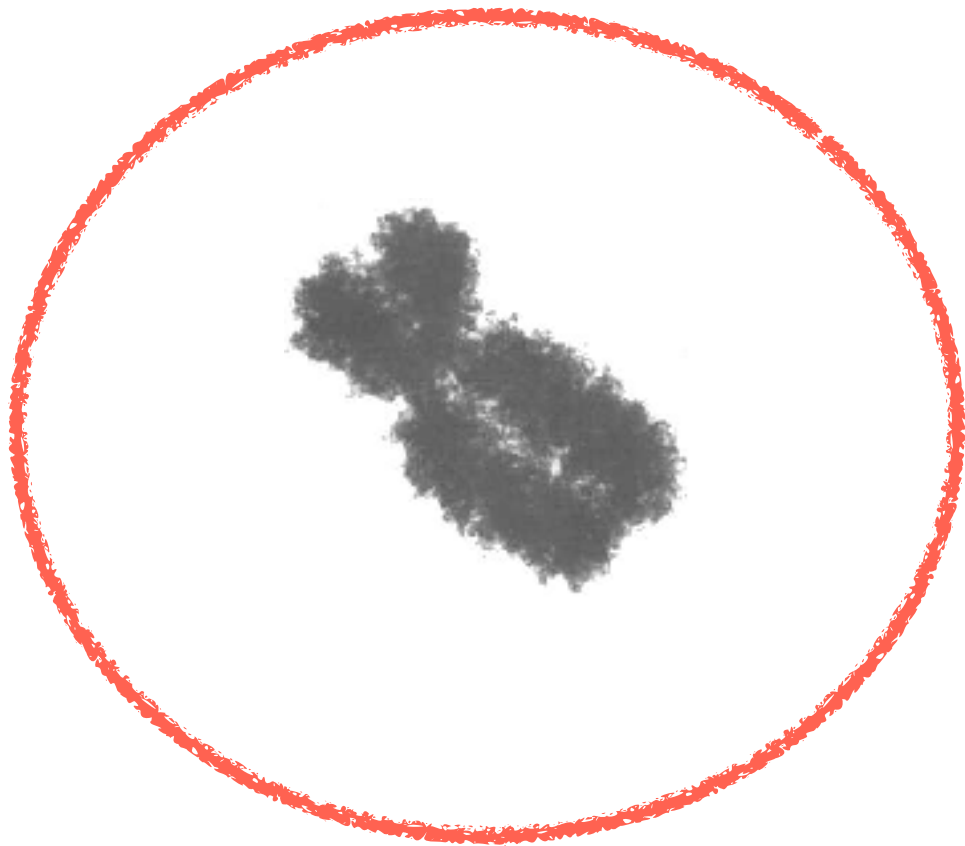
Different cells;  
different organization

**NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH**

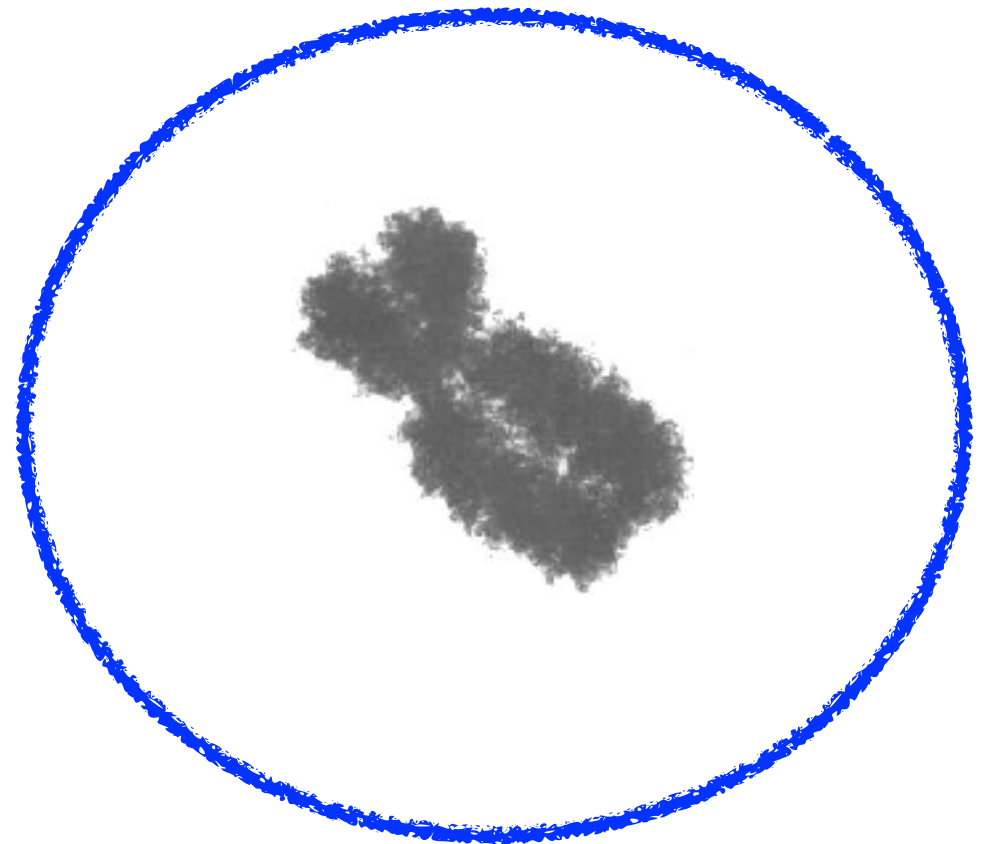
# What is the organization of genome in living cells?



# Different cells; but same DNA



Cells in our skin



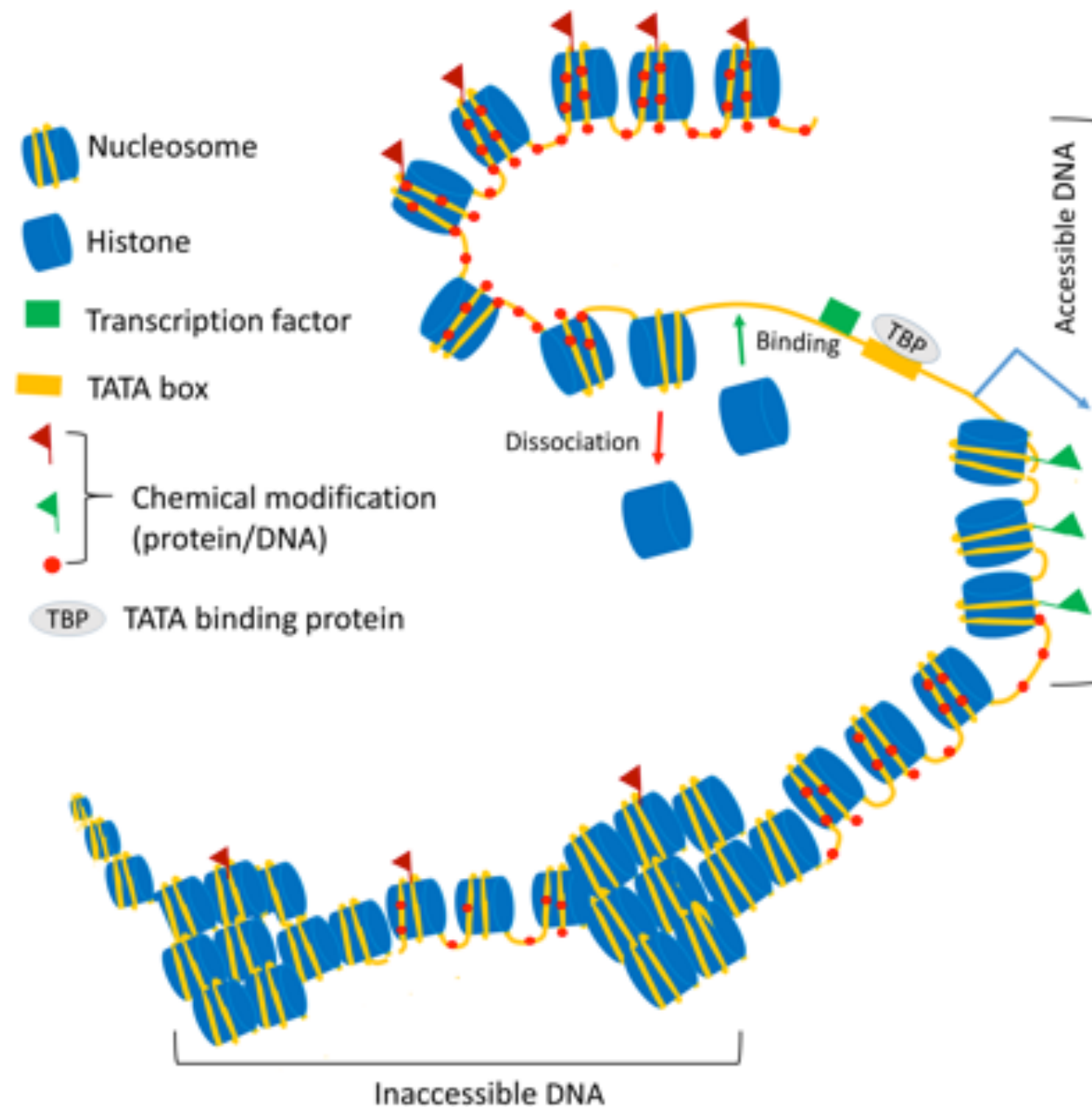
Cells in our eye

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

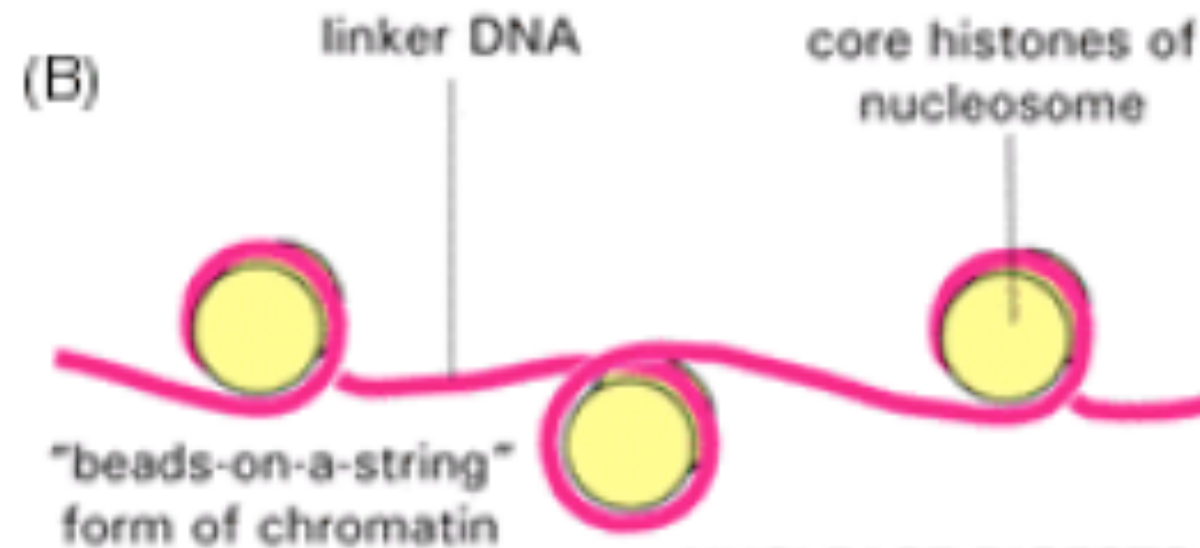


# Different cell types; different chromatin organization



“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



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

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)

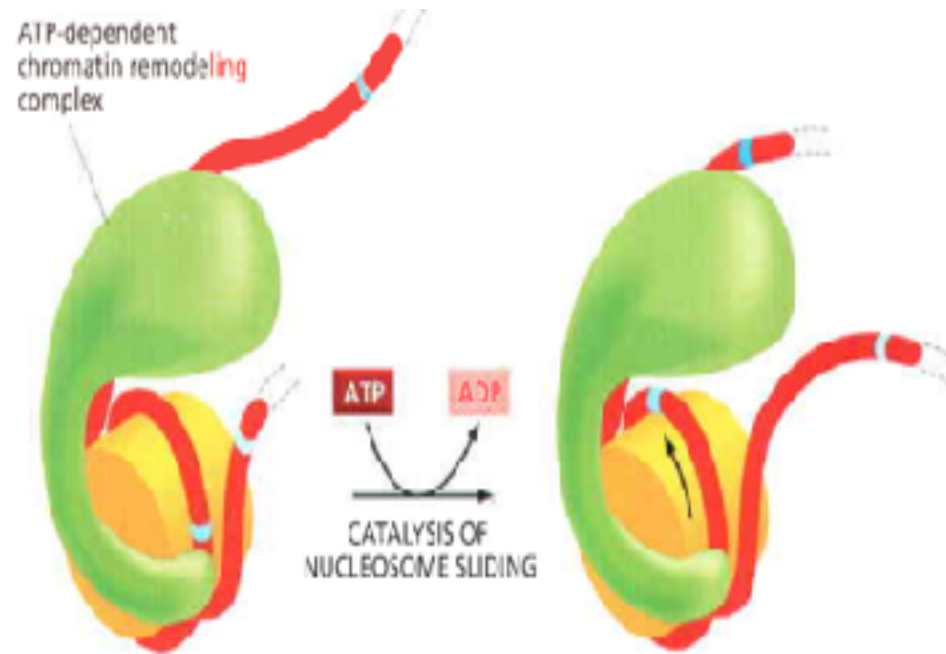
$$\Delta G \approx -40k_{\text{B}}T$$

What does this suggest ?

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

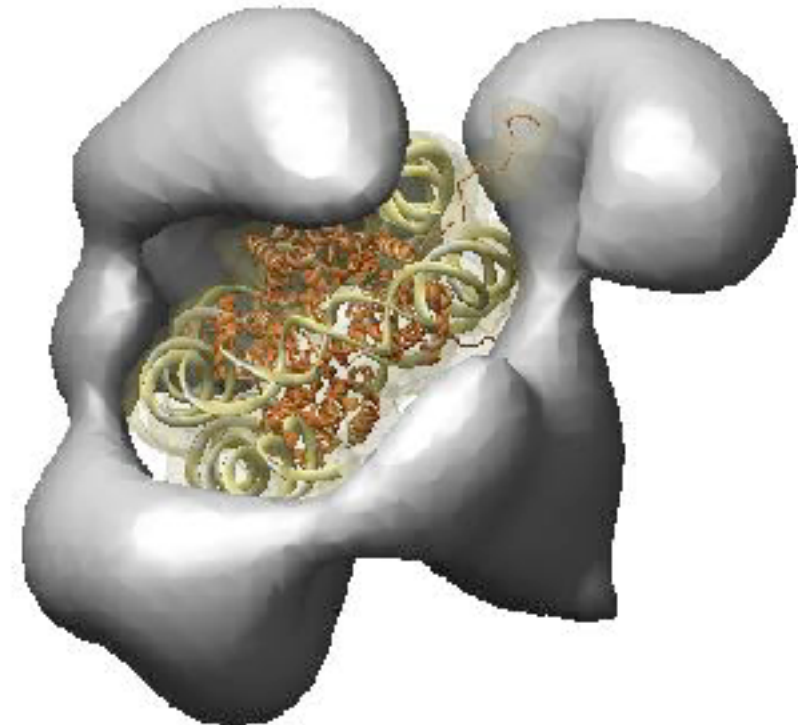


# Molecular machines use ATP, and rearrange nucleosome positions



ATP-dependent  
sliding

(candidates: yeast ISWI,  
ACF etc — ISWI family)

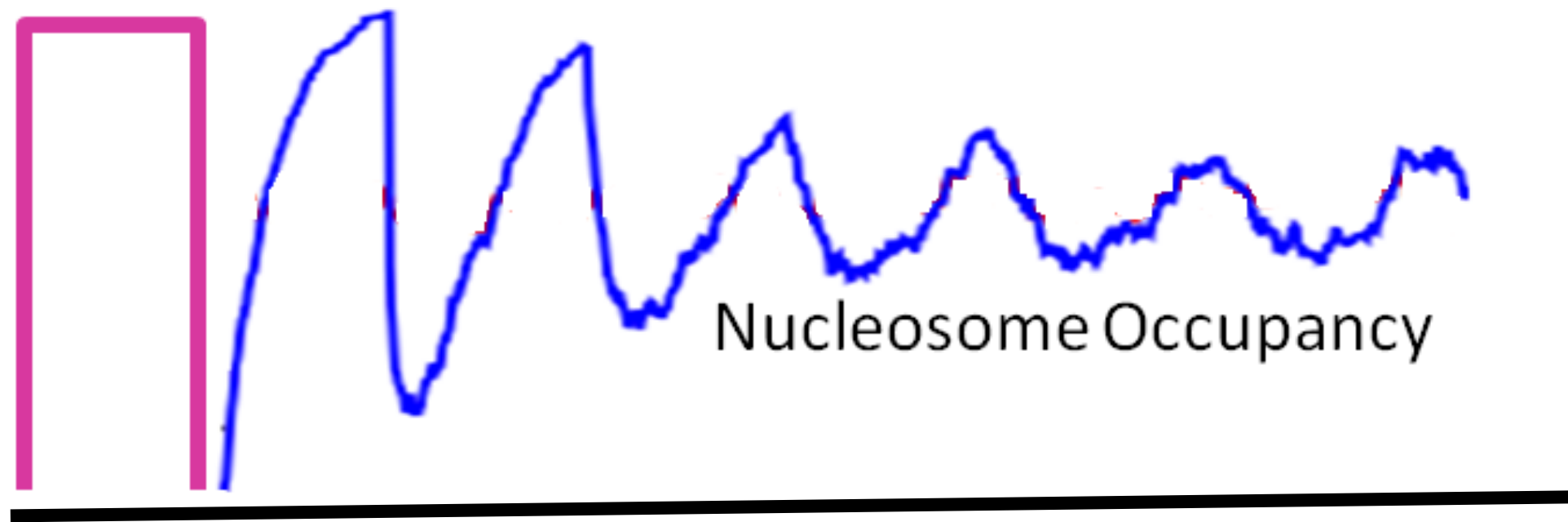


ATP-dependent  
disassembly  
(candidate: RSC,  
SWI/SNF family)

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



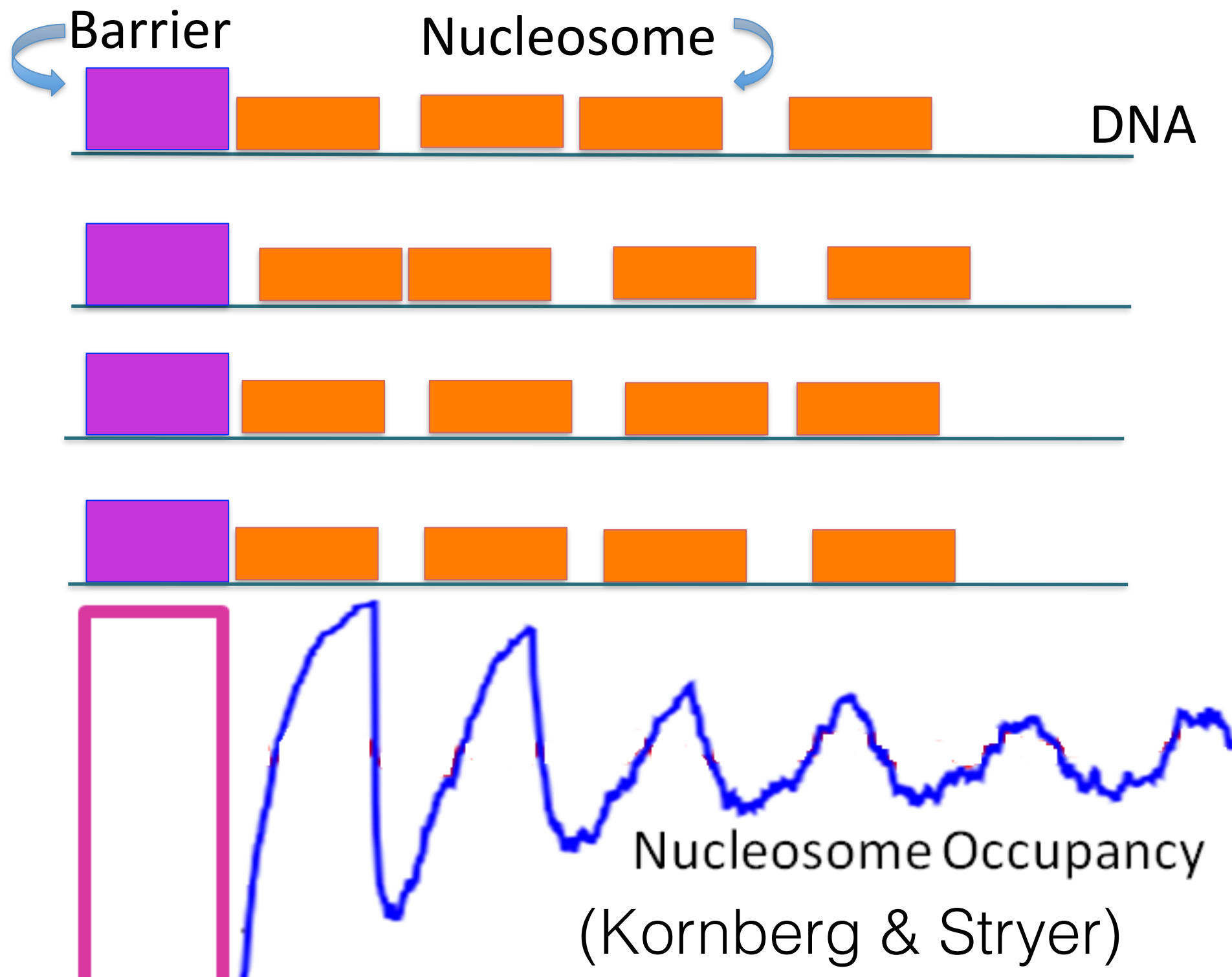
Nucleosome occupancy: Probability that a DNA site  $j$  is covered by a nucleosome

---

**Statistical distributions of nucleosomes: nonrandom locations by a stochastic mechanism**

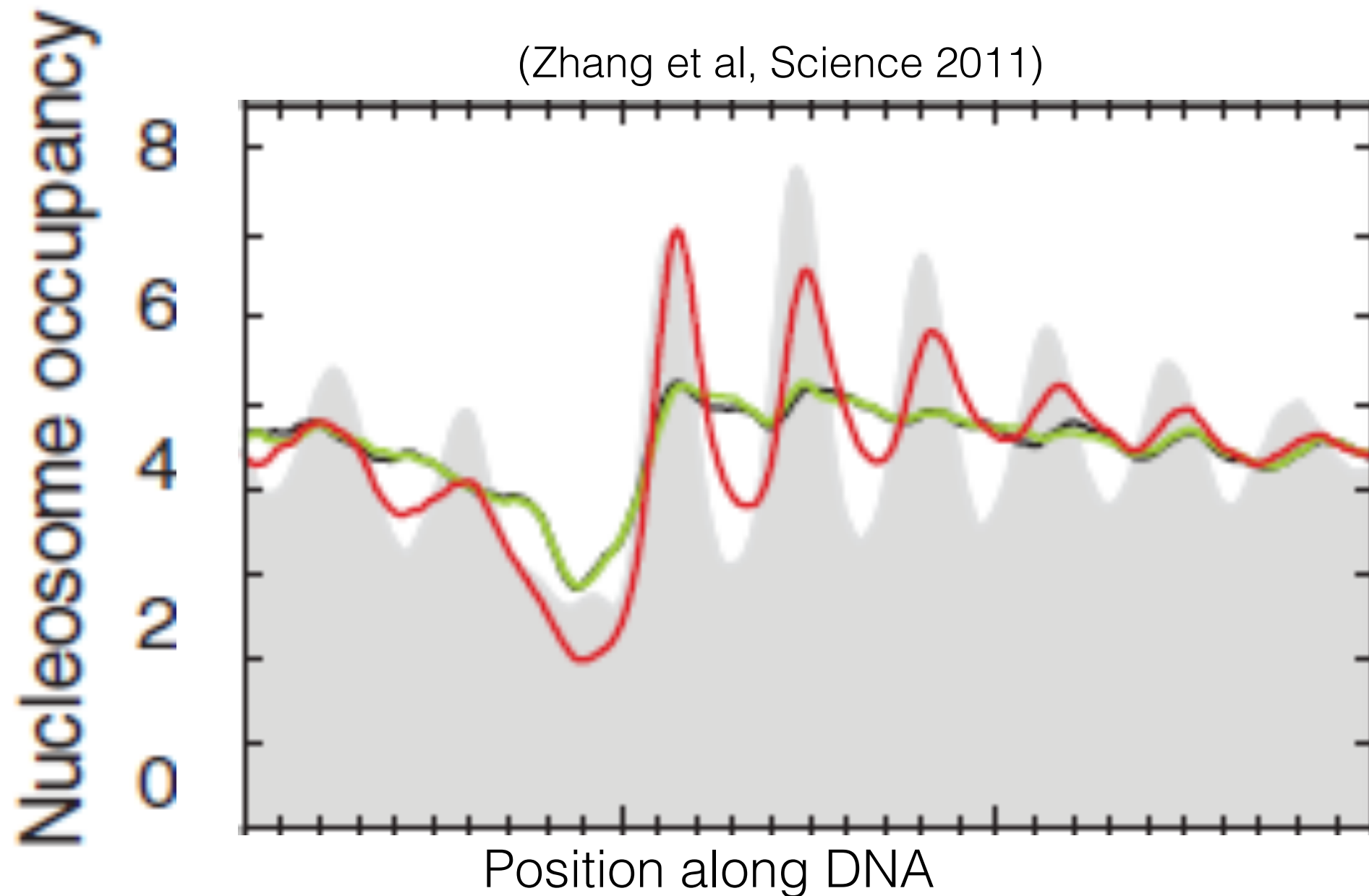
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Roger D. Kornberg and Lubert Stryer





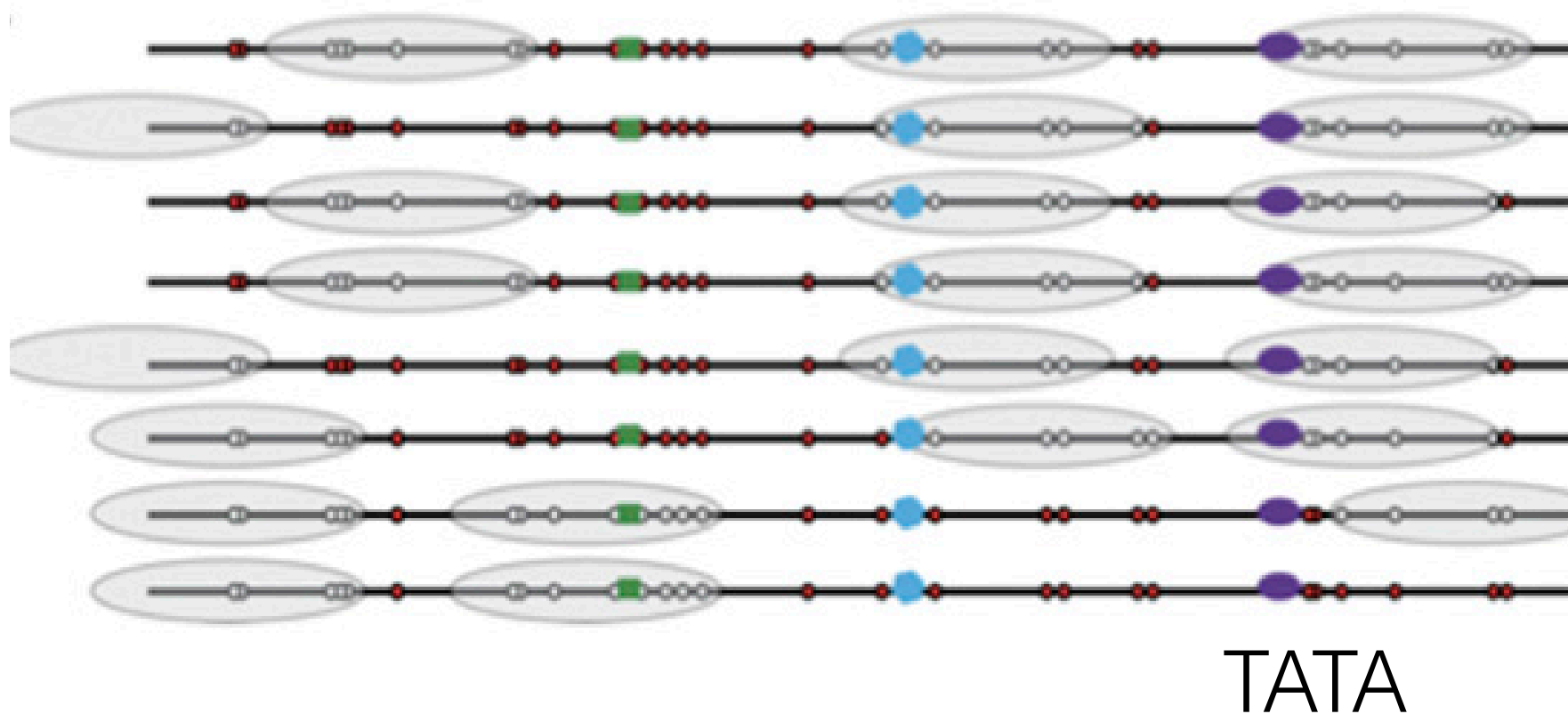
# ATP-dependent activity is necessary for maintaining nucleosome positioning



Red: Cell extract with ATP

Green: Cell extract with no ATP

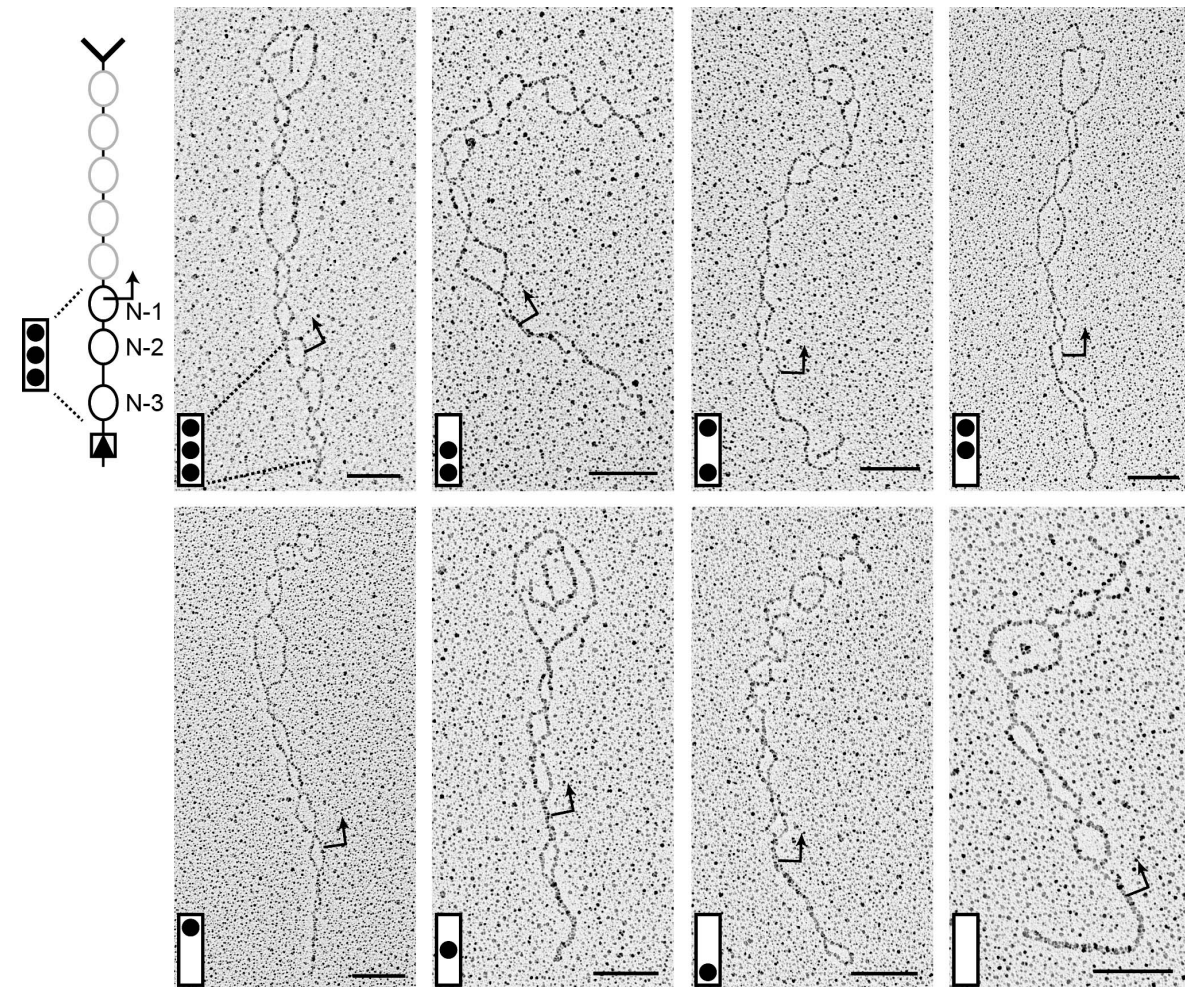
# Stochasticity in nucleosome organization in promoters



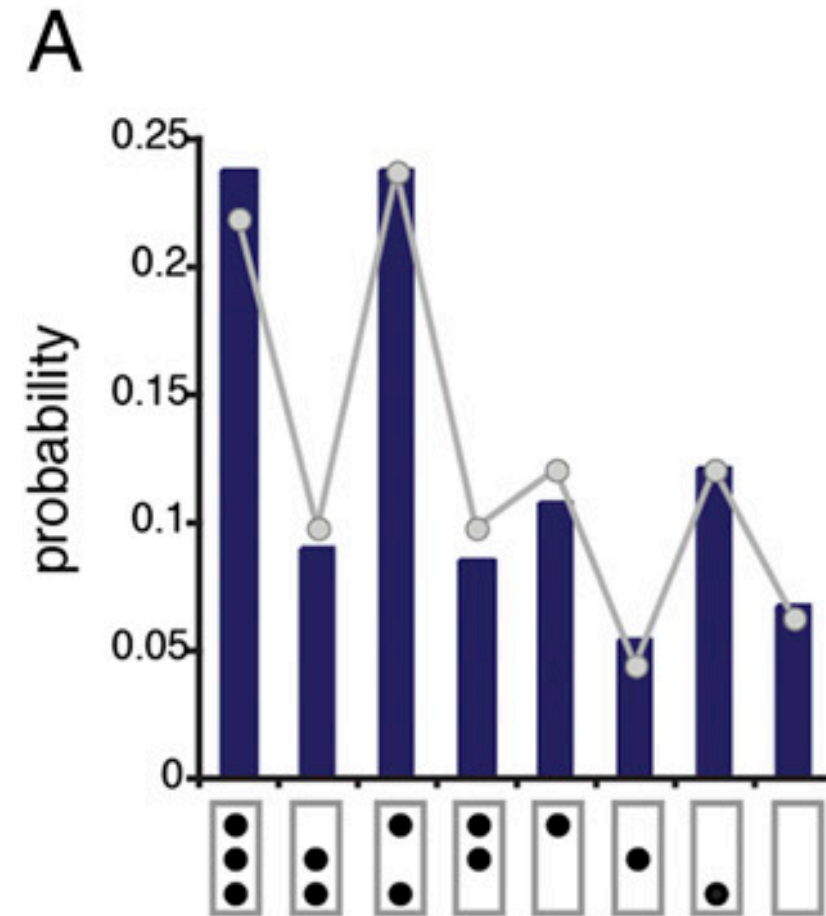
Different cells (under exactly the same condition) have different nucleosome organization

(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)



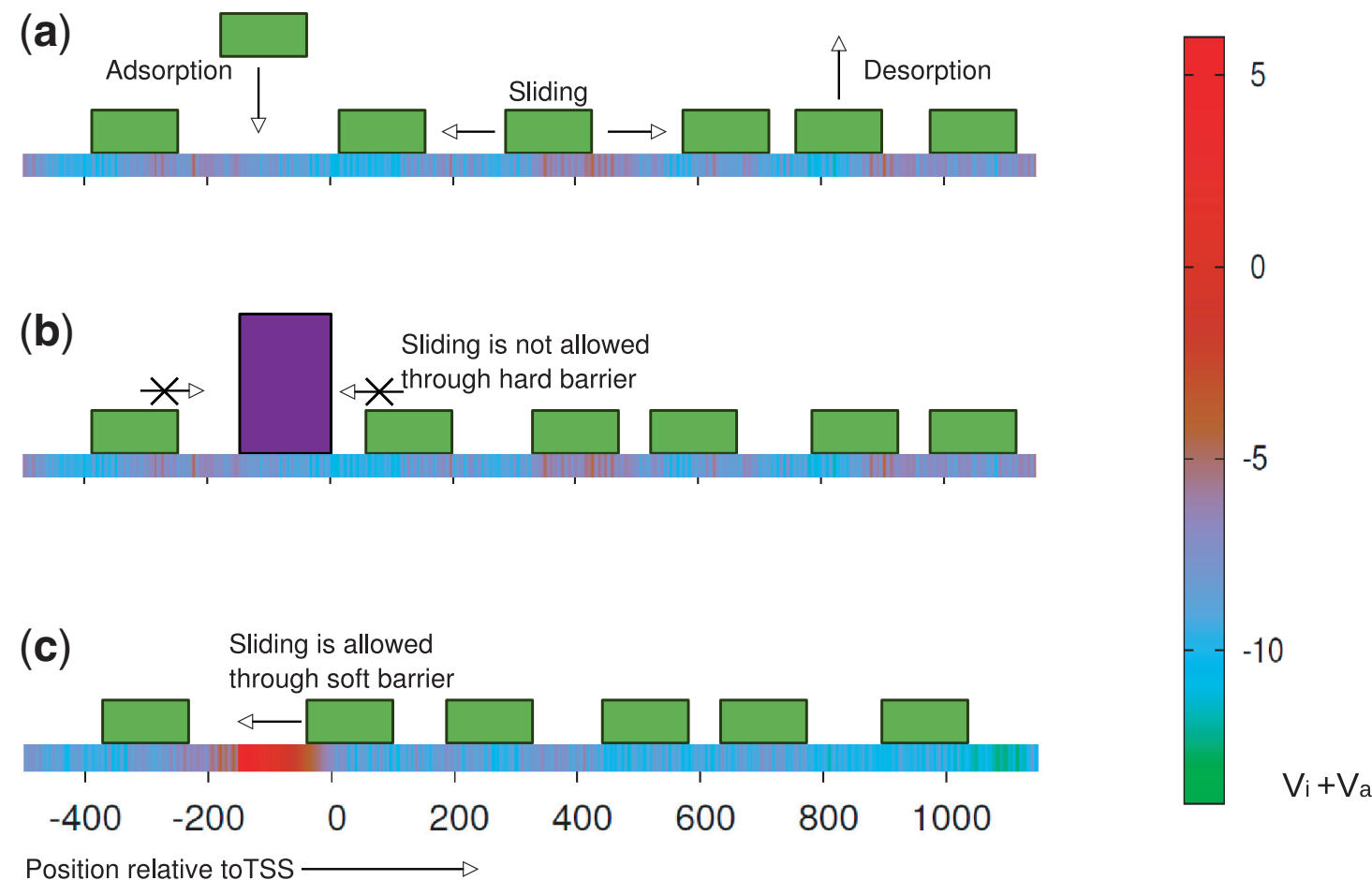
Brown & Beoger PNAS (2014)

# 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

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

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

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

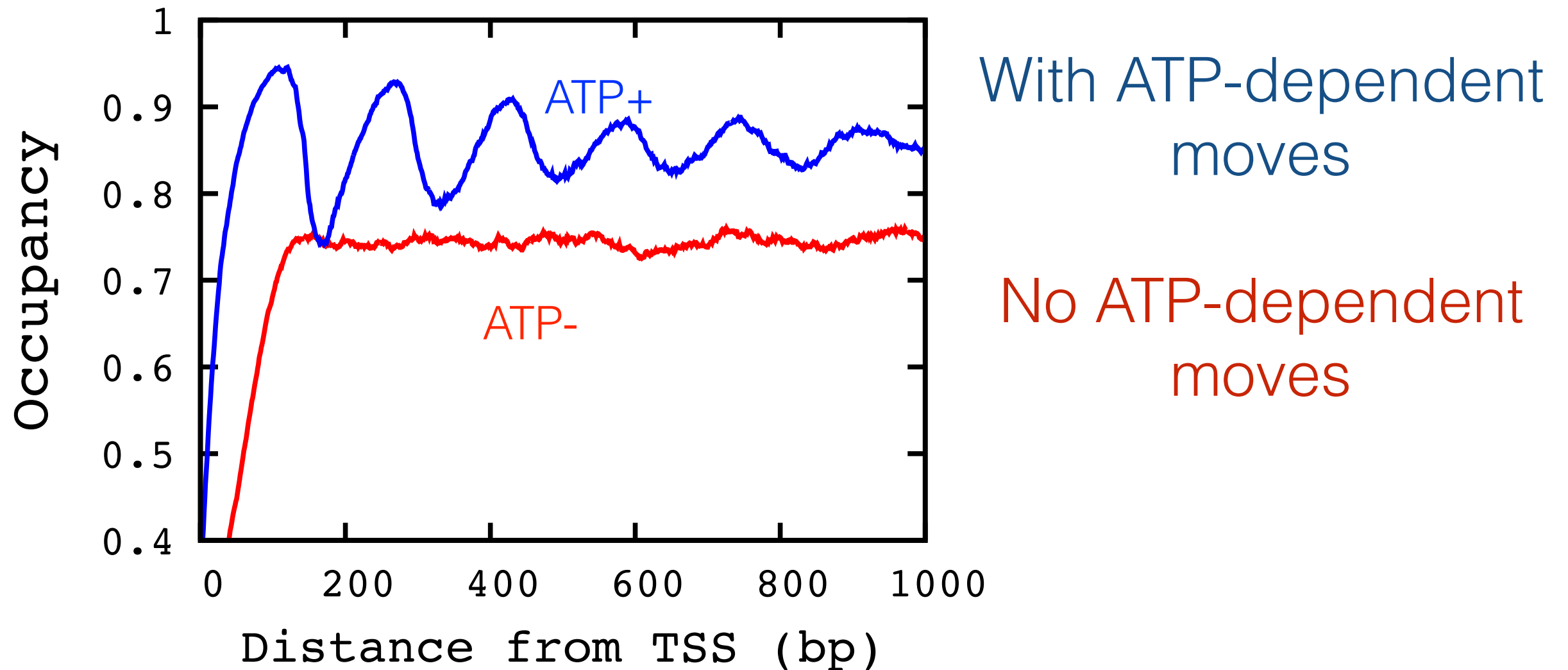
$$r_{\text{slide}} = r_s \text{ (with ATP)}$$

(with some sliding rule)

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

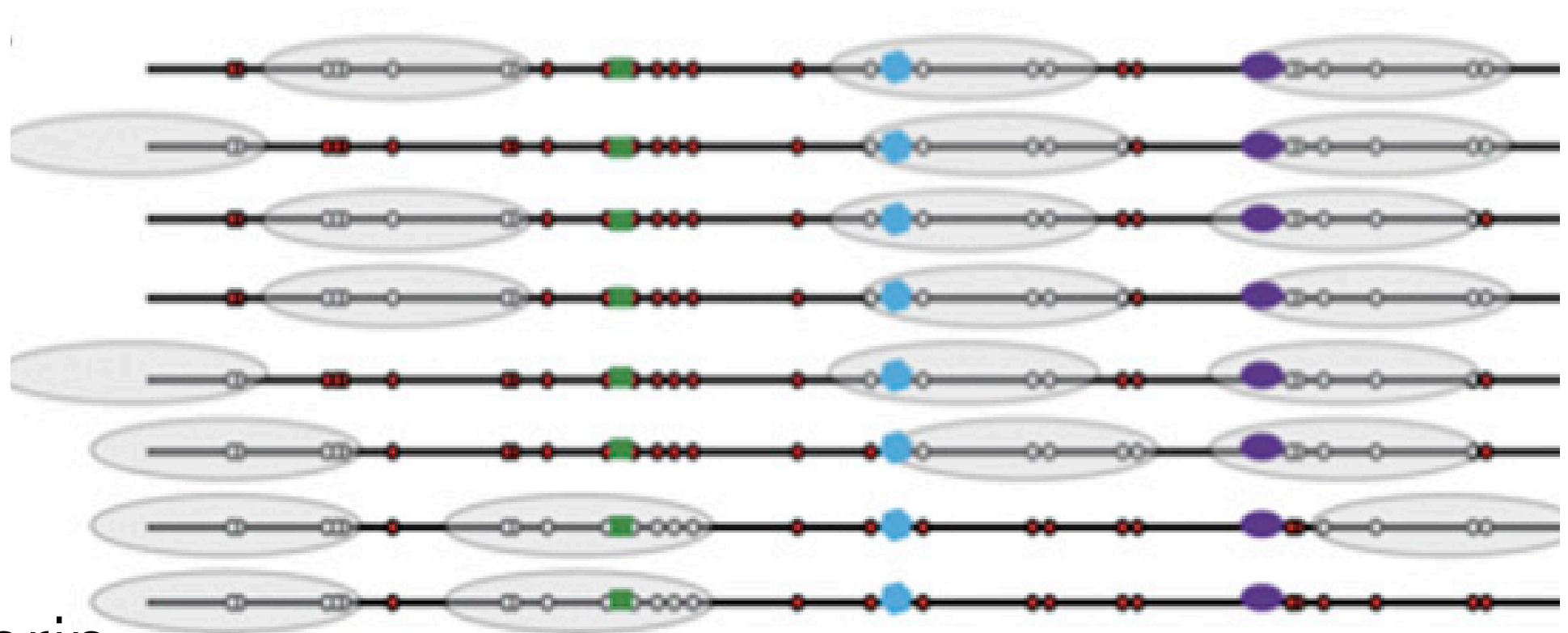
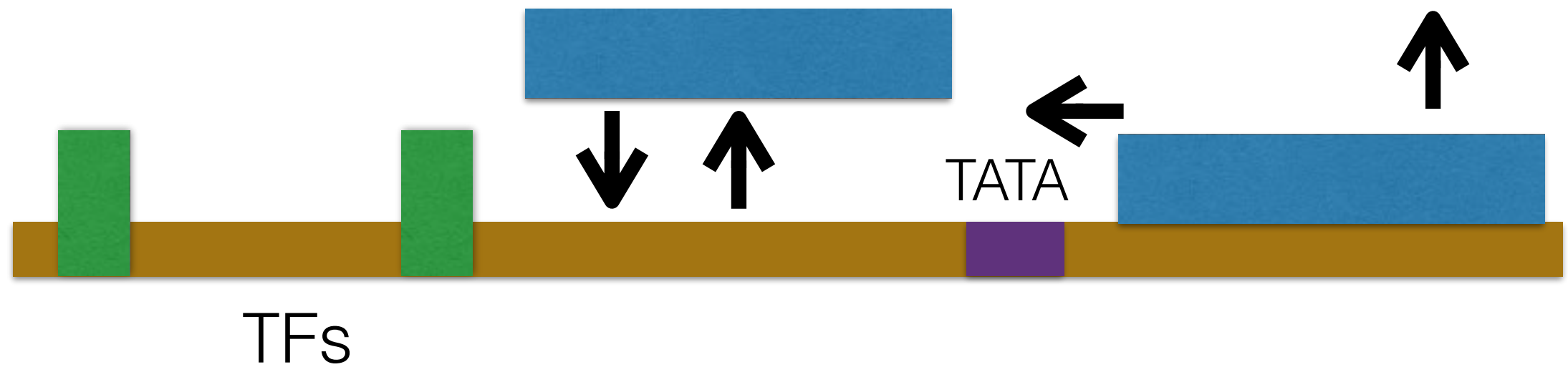
Jyotsana Parmar et al NAR (2014), and NAR (2016)

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



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

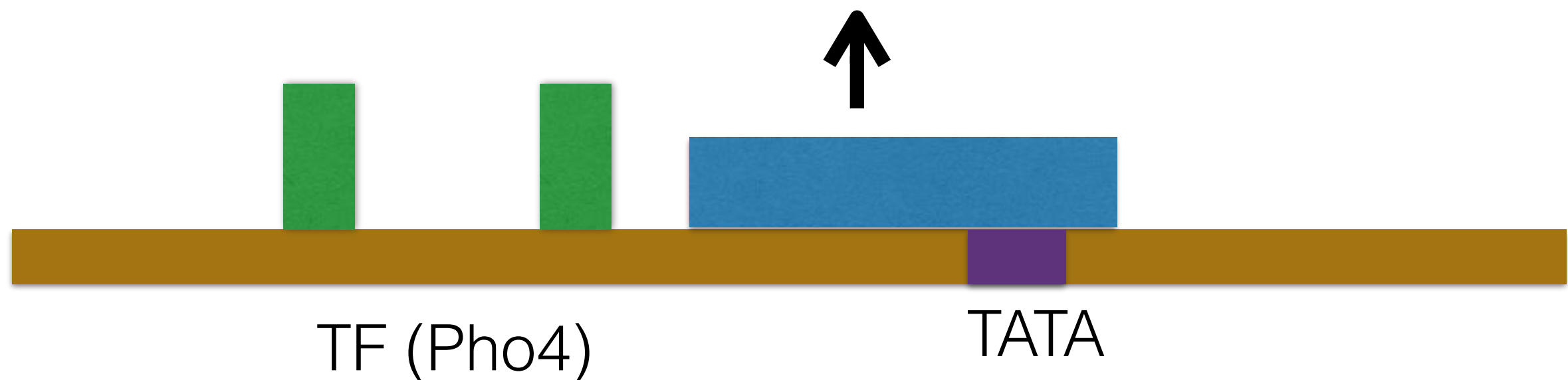
What could be the underlying kinetics that will lead to the experimentally seen nucleosome organization?



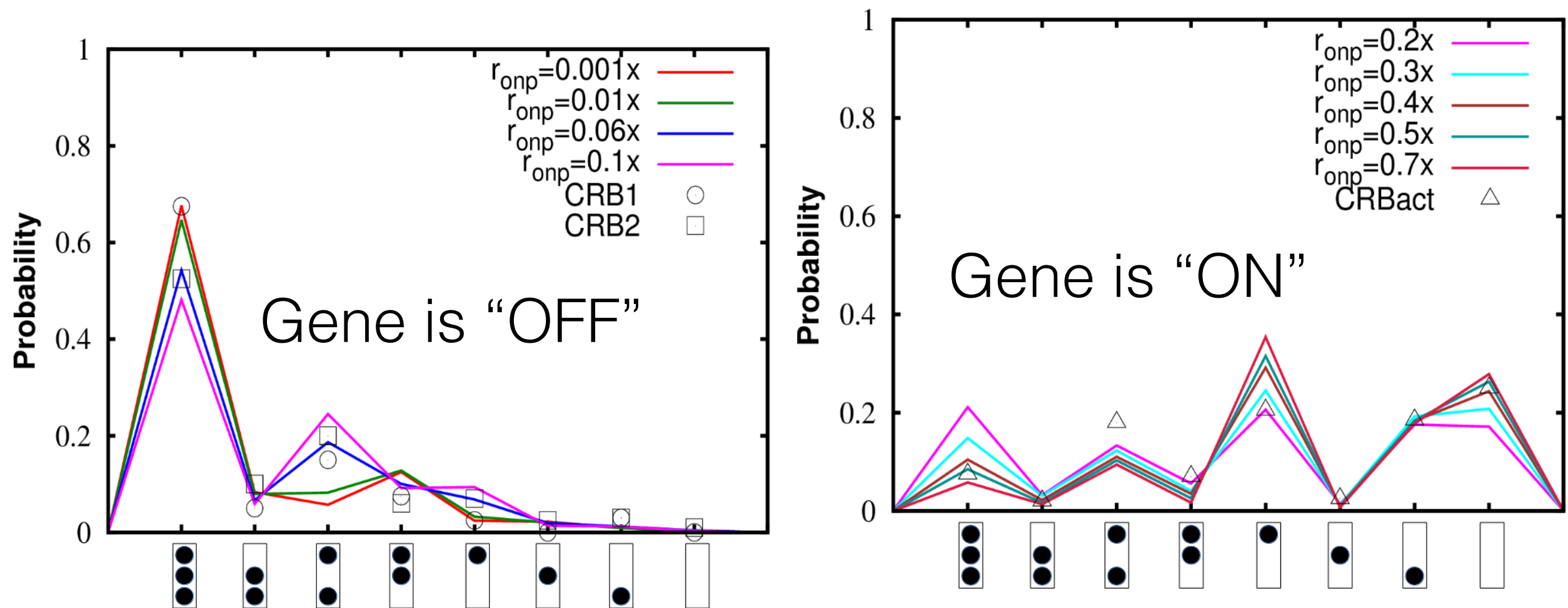
Hungyo Kharerin

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

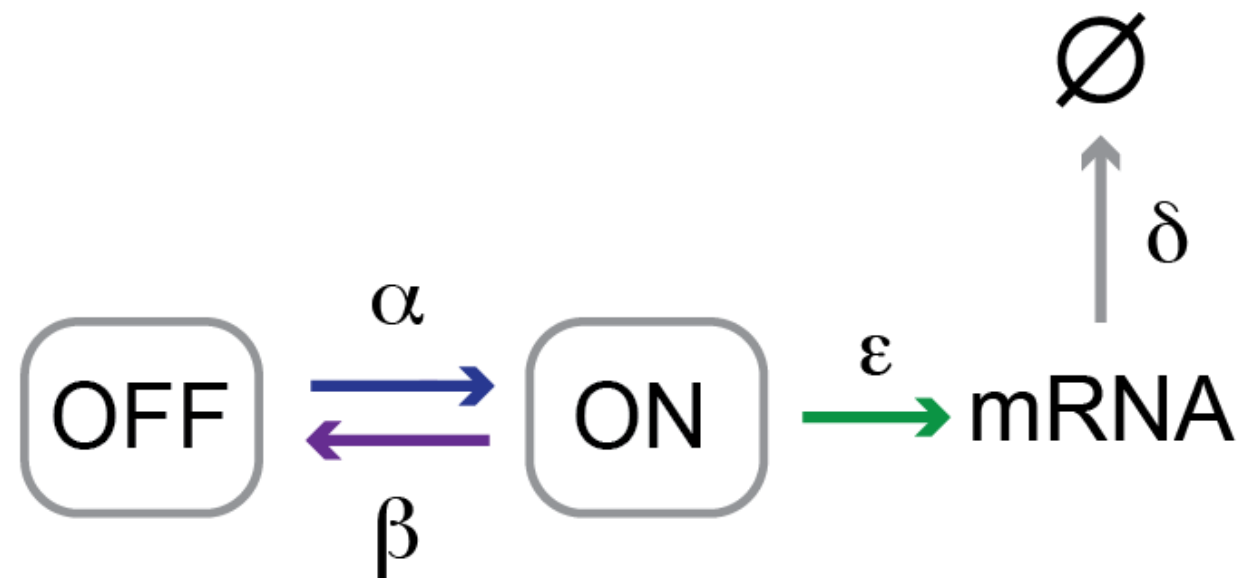


Off state: Nucleosome kinetics + TF binding

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

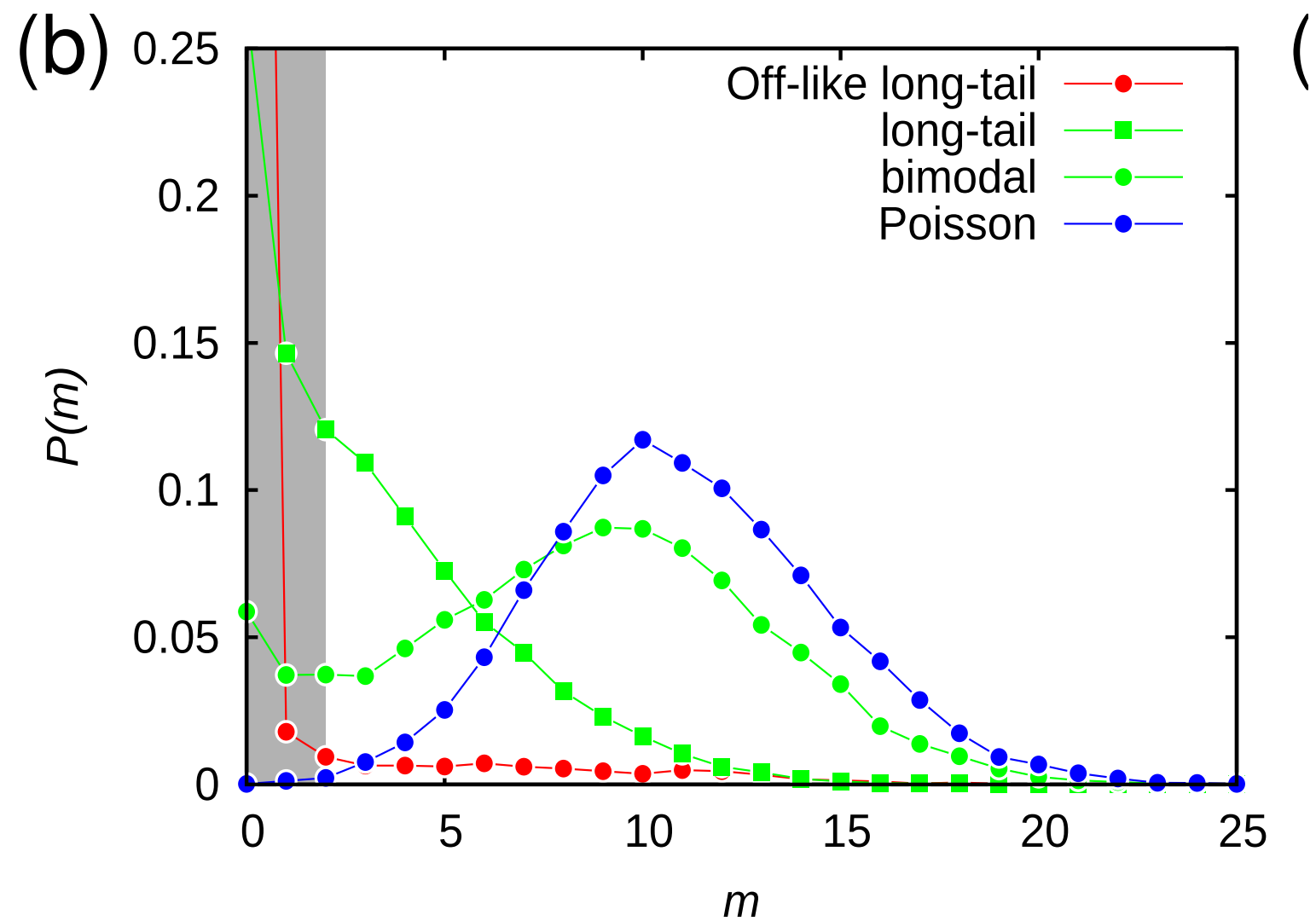
When the gene is “on”, we introduce mRNA production

**A**

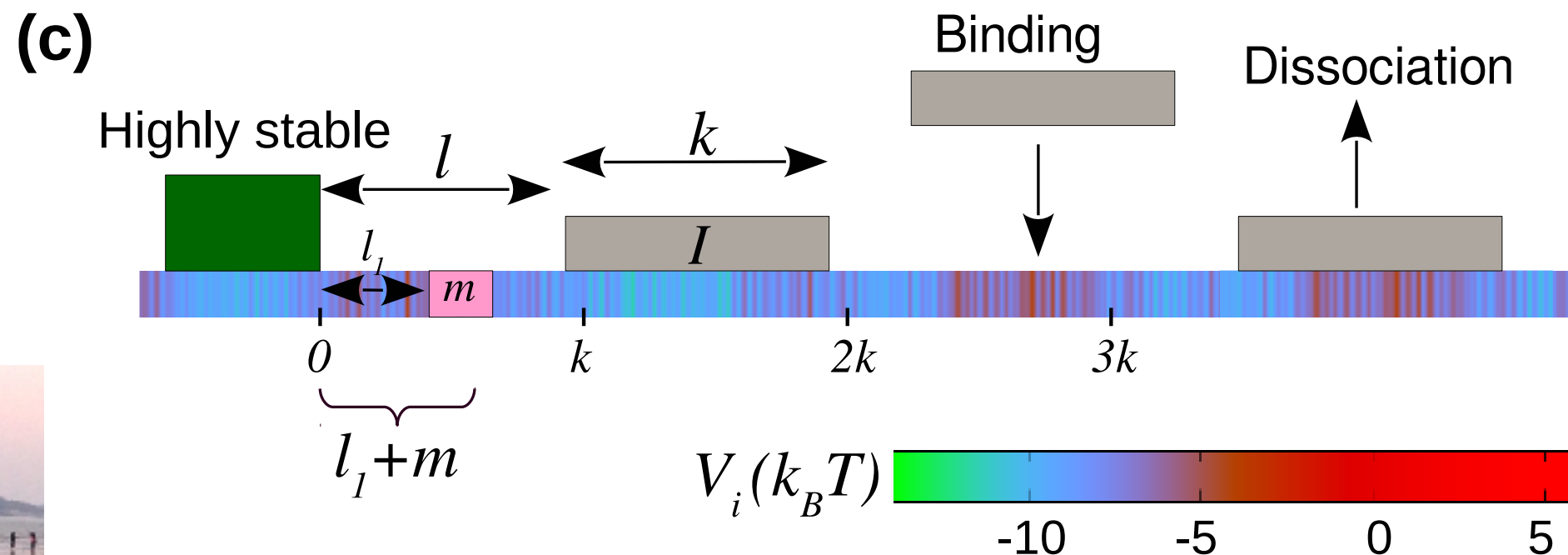




# mRNA distribution



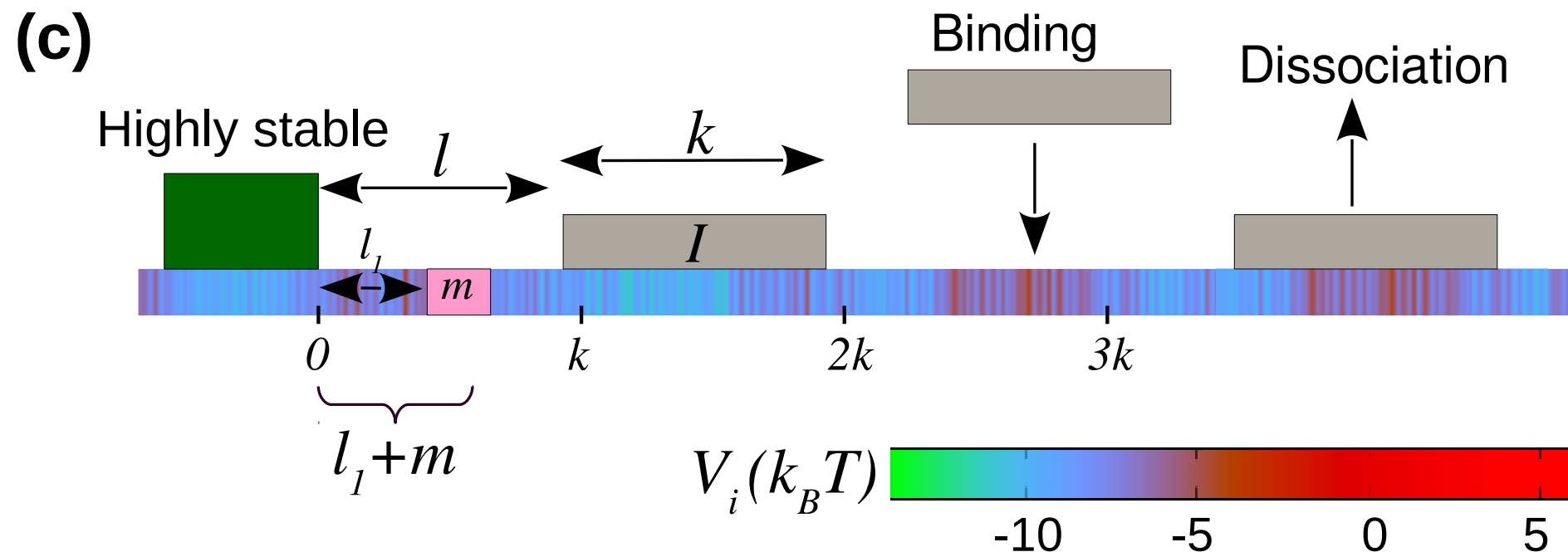
Given nucleosome dynamics, we can compute, how long a particular region of interest will remain “open” (exposed) before it getting covered by another nucleosome



(Jyotsana Parmar, Dibyendu Das)



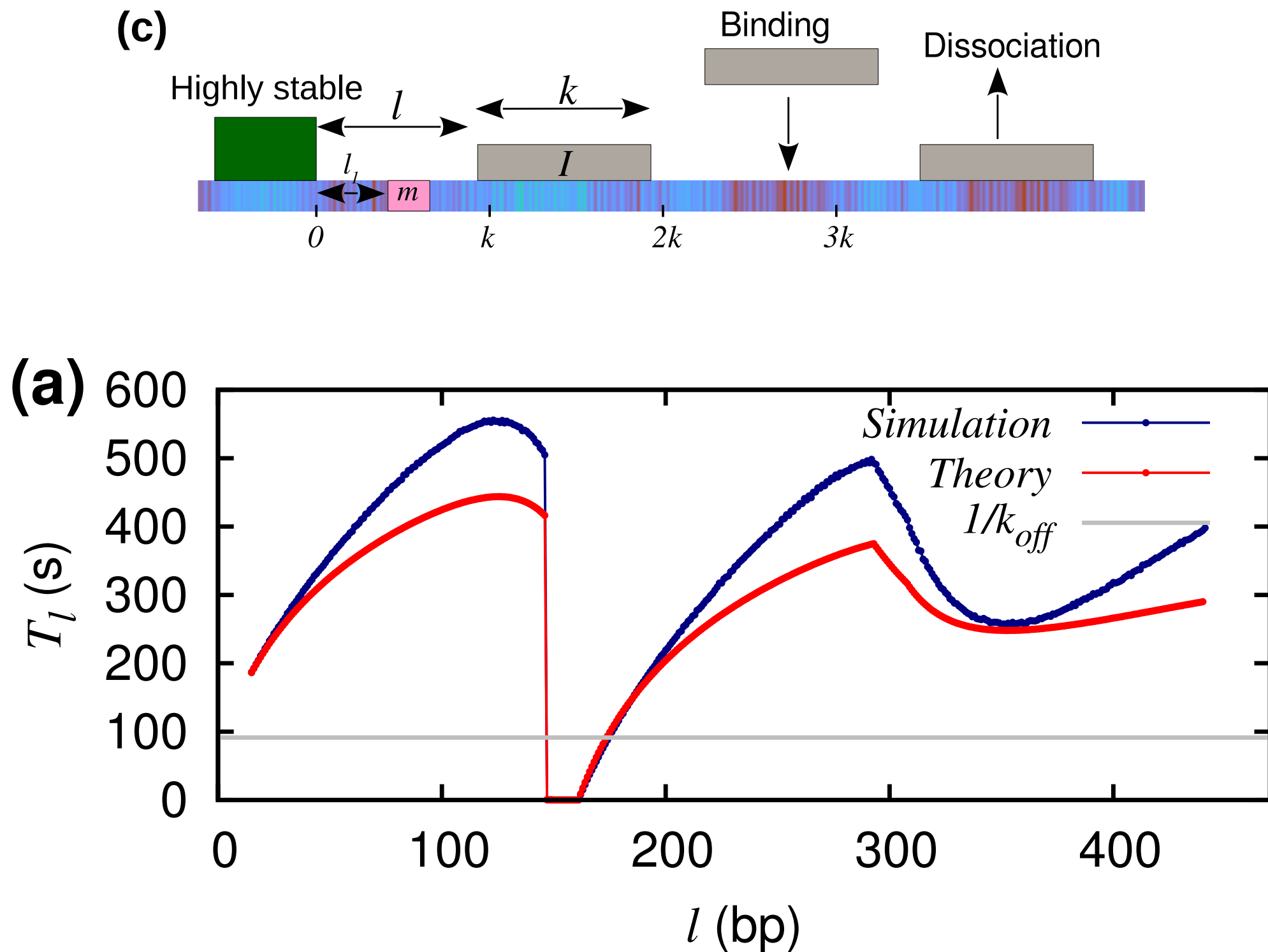
We compute “first passage time” of covering the patch of size “m”



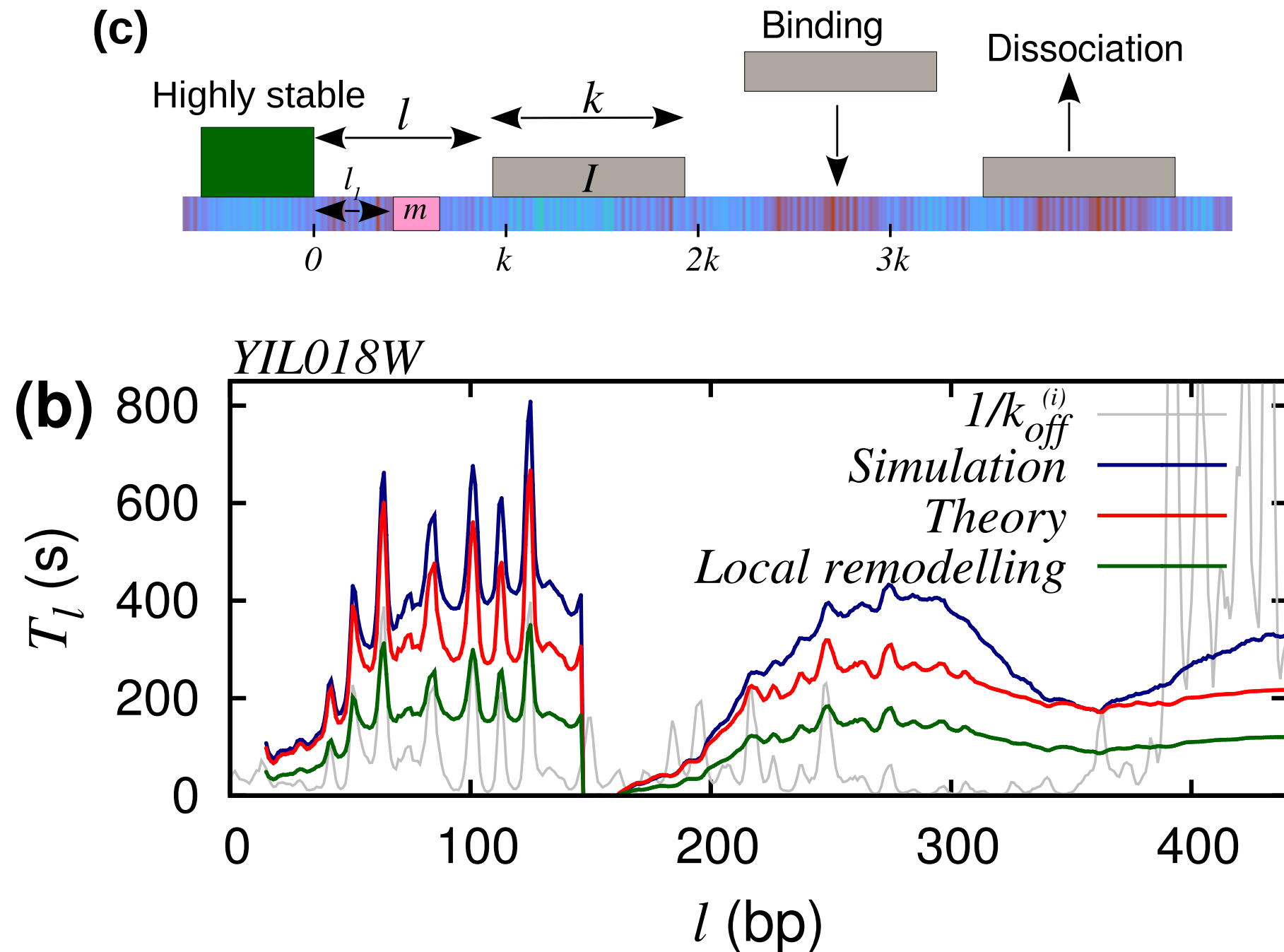
$$T_l - \sum_{\tilde{l}=l+k}^{3k-1} P_{ss}(\tilde{l} - l - k) T_{\tilde{l}} = \frac{1}{k_{\text{off}}}.$$

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

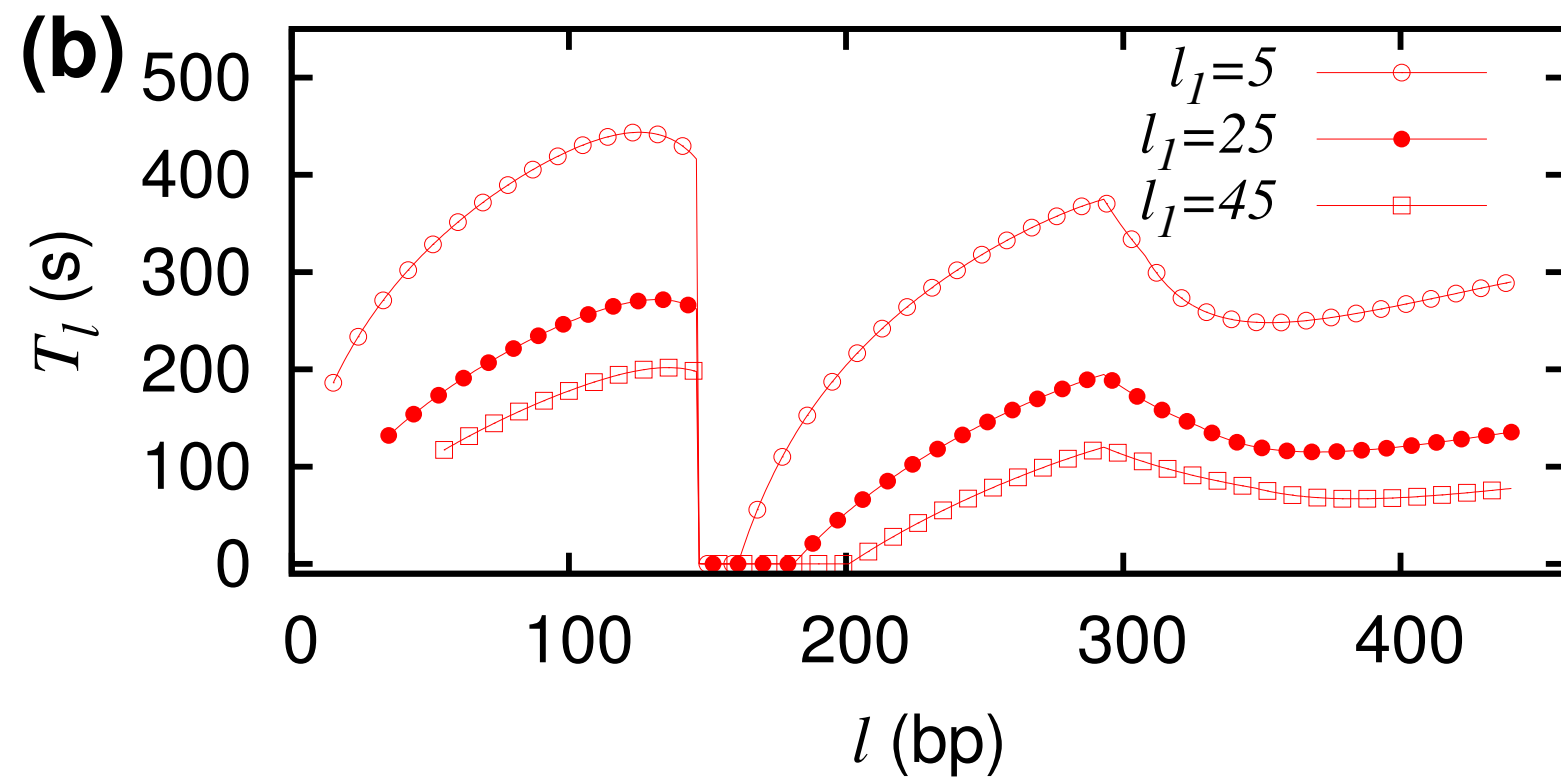
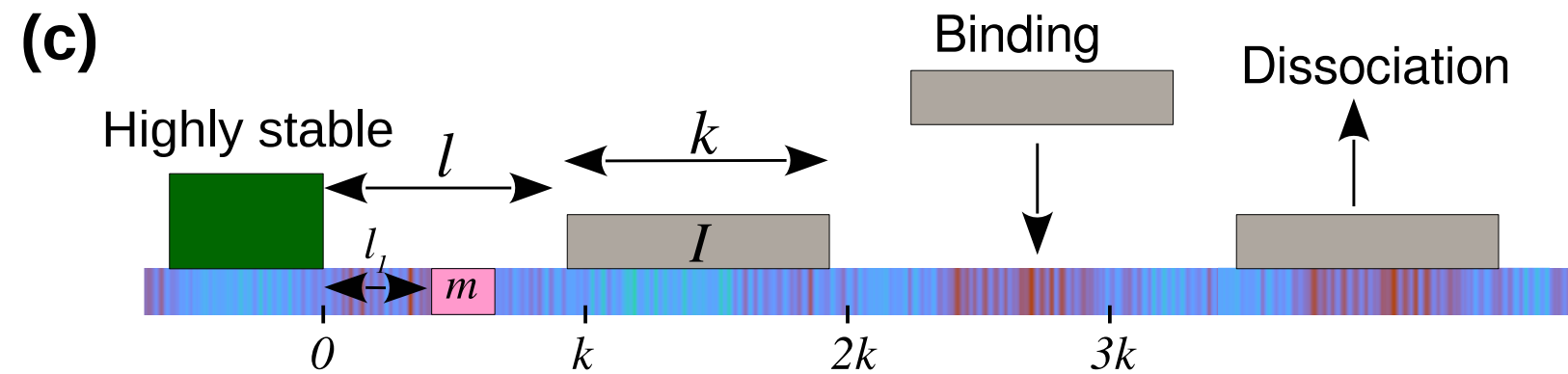
Mean exposure time is very different from any of the known timescales in the problem.



We can compute the same with sequence-dependent rates

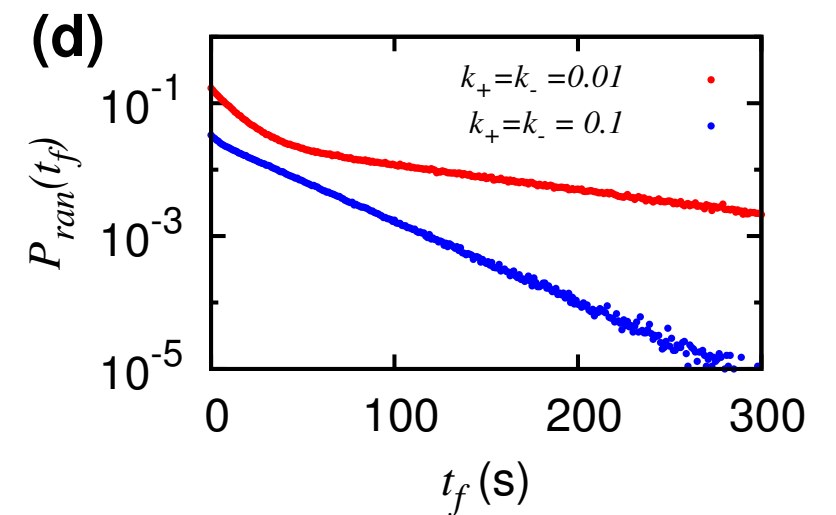
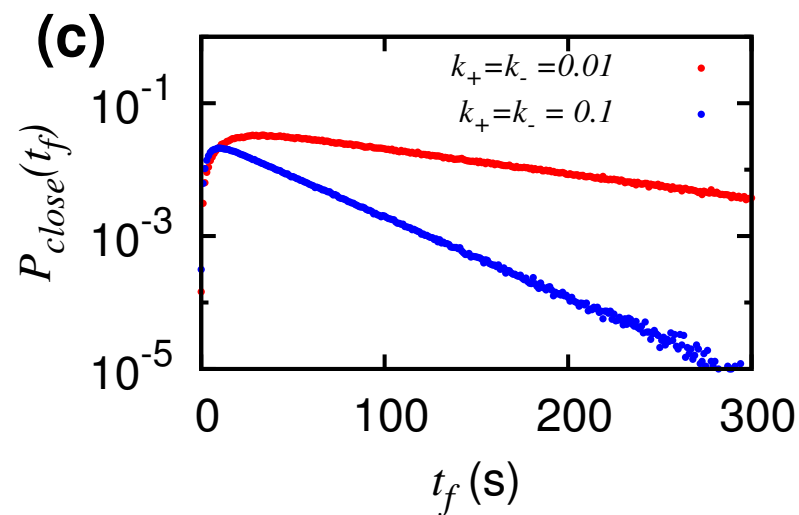
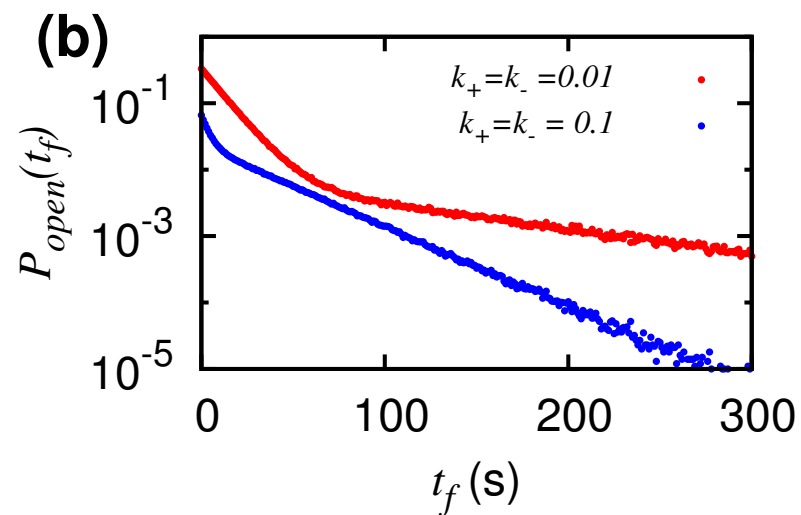
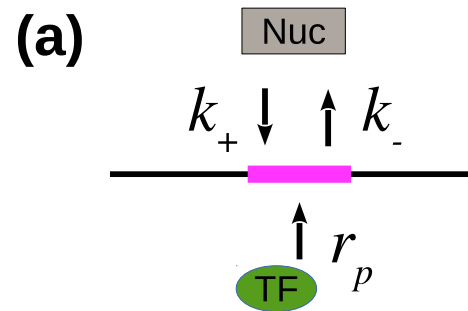


# Promoter architecture matters





# Distribution of TF binding times

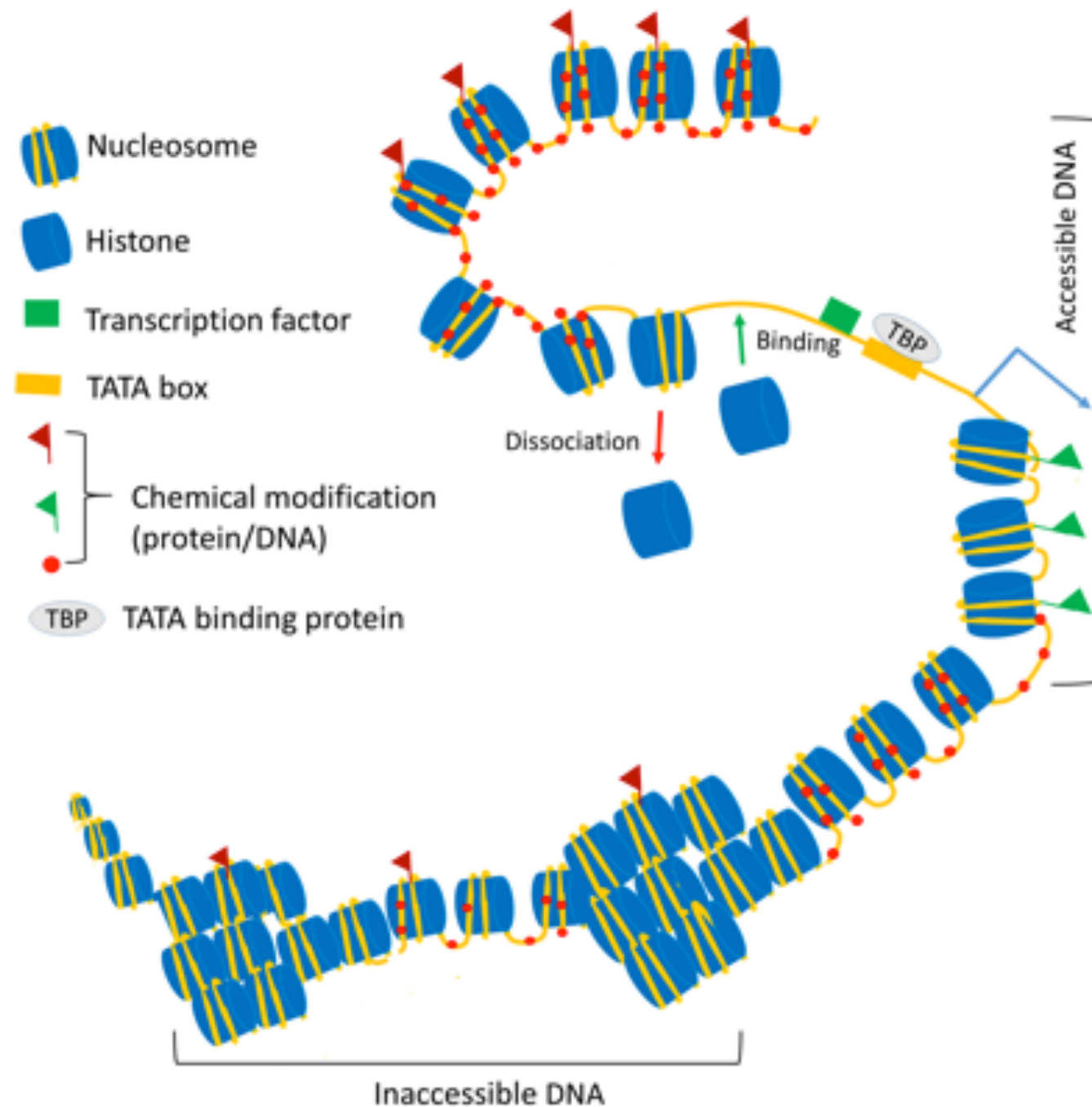


$$r_p = 1/15s$$

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

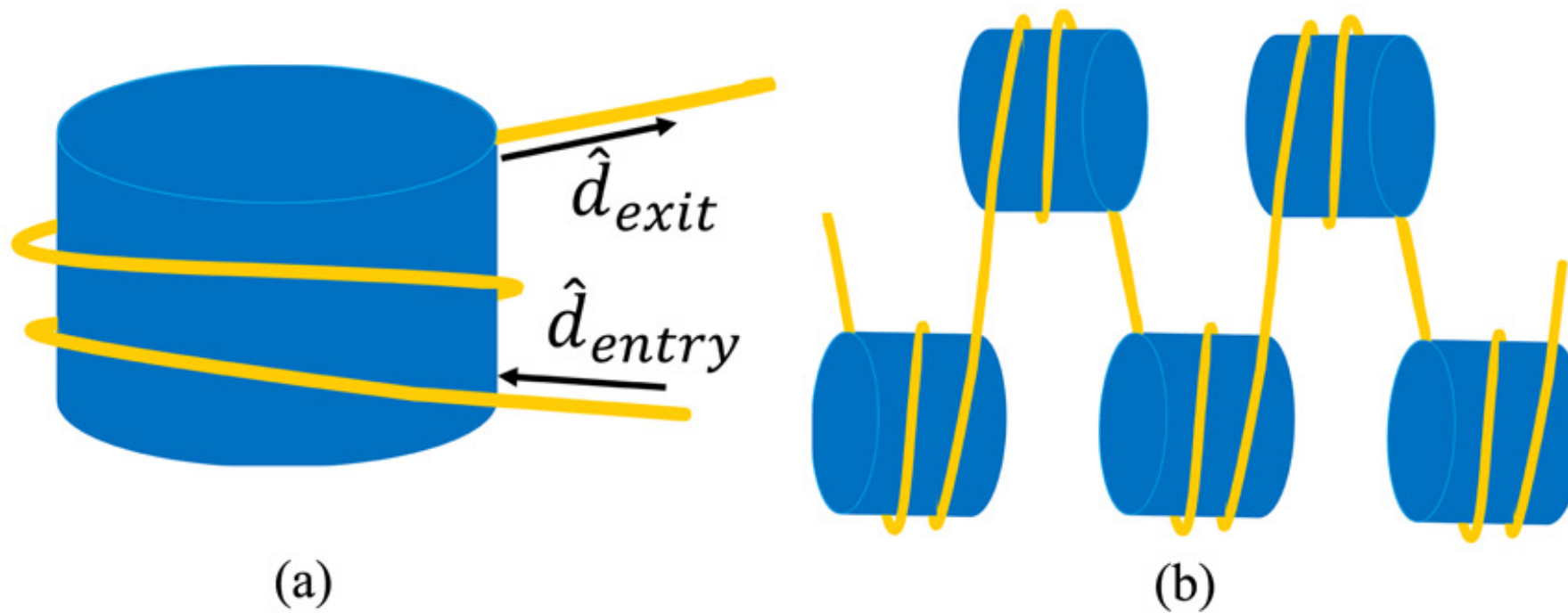
Same nucleosome occupancy; but different TF binding times

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

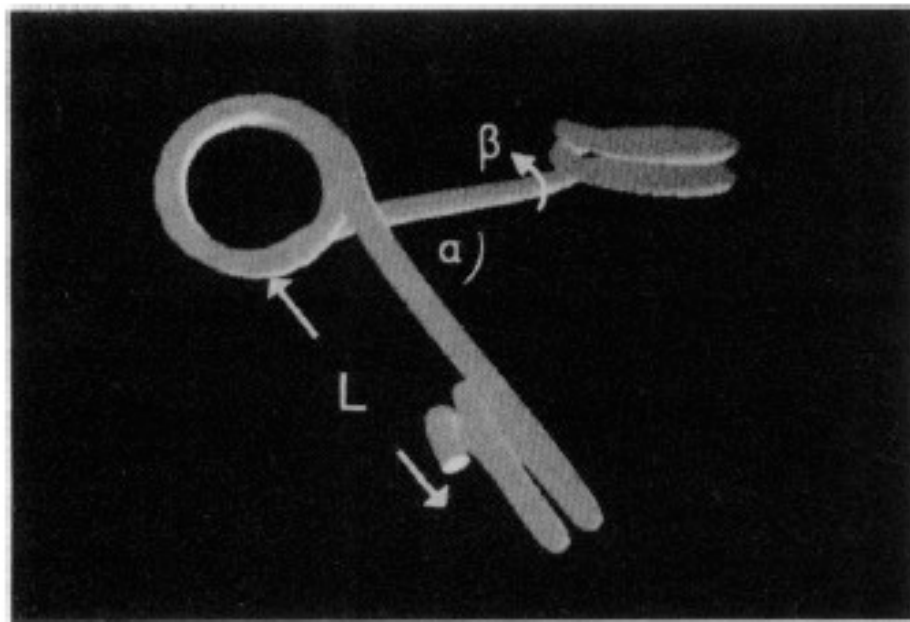
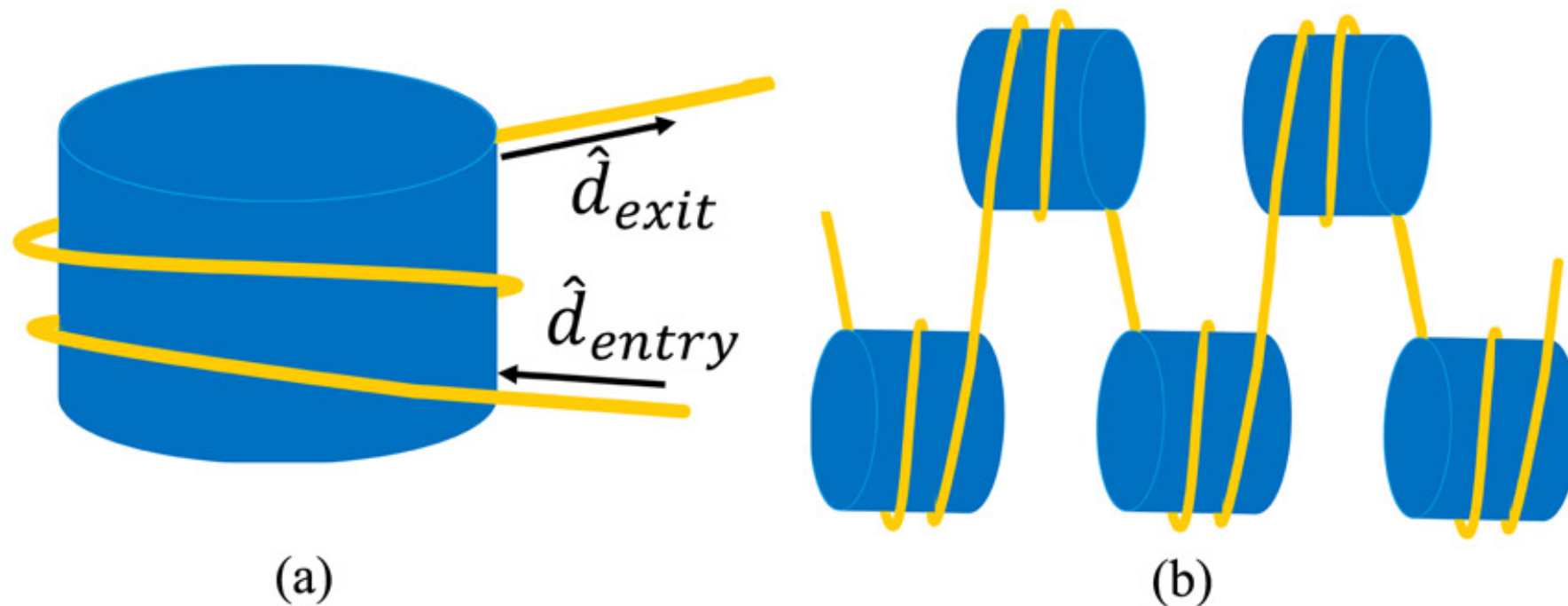


Gaurav Bajpai

# Prevalent theory: zig-zag



# Physical basis of zig-zag



- \* Entry/exit angle of DNA
- \* Stiffness of linker region

Woodcock et al PNAS (1993)

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



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

Mikhail Eltsov<sup>a,b,1,2</sup>, Kirsty M. MacLellan<sup>a,c,1</sup>, Kazuhiro Maeshima<sup>d,1</sup>, Achilleas S. Frangakis<sup>b,e</sup>, and Jacques Dubochet<sup>a,f</sup>

<sup>a</sup>Laboratoire d'Analyse Ultrastructurale, Université de Lausanne, Biophore, CH-1015, Lausanne, Switzerland; <sup>d</sup>Cellular Dynamics Laboratory, RIKEN, 2-1, Hirosawa, Wako-shi, Saitama, 351-0198, Japan; <sup>b</sup>European Molecular Biology Laboratory, Meyerhofstrasse 1, D-69117 Heidelberg, Germany; <sup>c</sup>Institut de Minéralogie et de Physique des Milieux Condensés, Université Pierre et Marie Curie, IMPMC-UMR7590, Paris F-75005, France; and <sup>f</sup>Département d'Ecologie et d'Evolution, Université de Lausanne, Biophore, CH-1015, Lausanne, Switzerland; and <sup>e</sup>Cluster of Excellence Macromolecular Complexes, Johann Wolfgang Goethe University, Max-von-Laue-Strasse 1, Frankfurt D-60438, Germany

Communicated by Nancy Kleckner, Harvard University, Cambridge, MA, October 10, 2008 (received for review August 5, 2008)

The EMBO Journal (2012) 31, 1644–1653 | © 2012 European Molecular Biology Organization | All Rights Reserved 0261-4189/12  
www.embojournal.org

THE  
EMBO  
JOURNAL

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

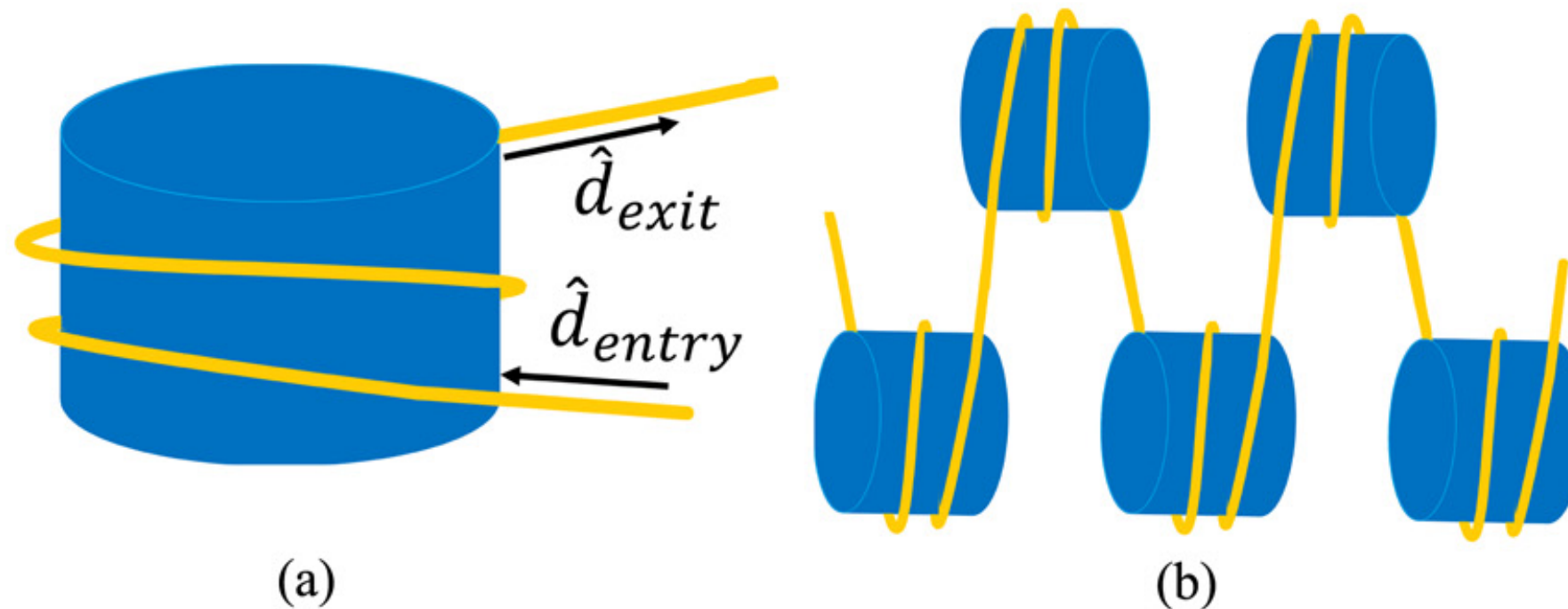
Yoshinori Nishino<sup>1,2,9</sup>, Mikhail Eltsov<sup>3,9</sup>,  
Yasumasa Joti<sup>4,9</sup>, Kazuki Ito<sup>1,9</sup>,  
Hideaki Takata<sup>5</sup>, Yukio Takahashi<sup>1,6</sup>,  
Saera Hihara<sup>5,7</sup>, Achilleas S Frangakis<sup>3</sup>,  
Naoko Imamoto<sup>8</sup>, Tetsuya Ishikawa<sup>1,4</sup>,  
and Kazuhiro Maeshima<sup>1,5,7,8,\*</sup>

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, 2002; Kiyono *et al*, 2004), although the folding processes

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



# Models so far: DNA+histones



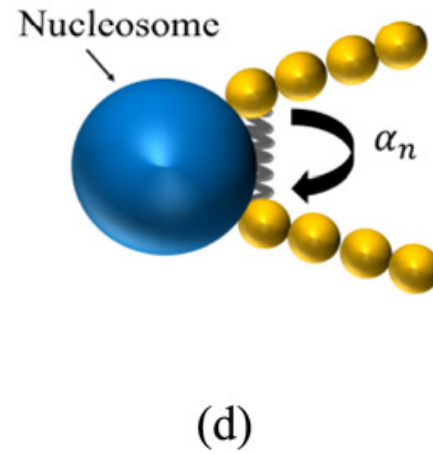
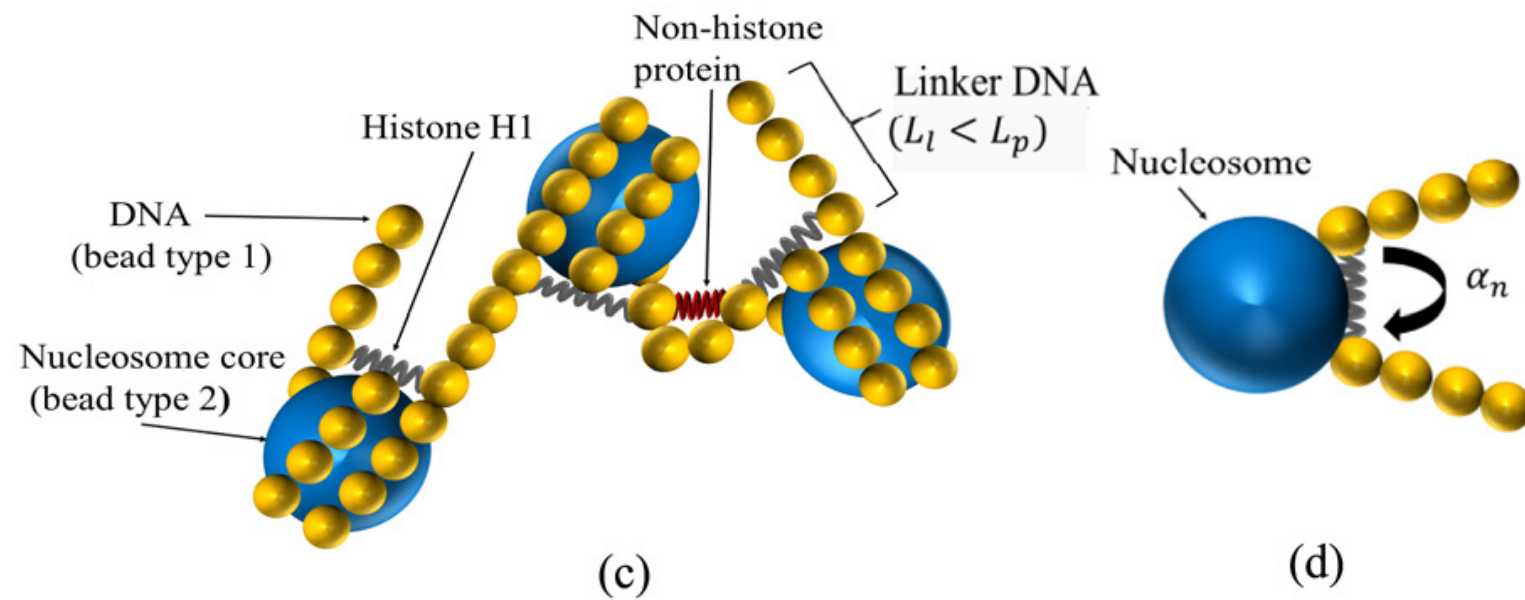
Models so far only consider histones (core histones+H1)  
That too mostly regular organization of histones!

Woodcock et al,  
Tamar Schlick lab, Langowski lab

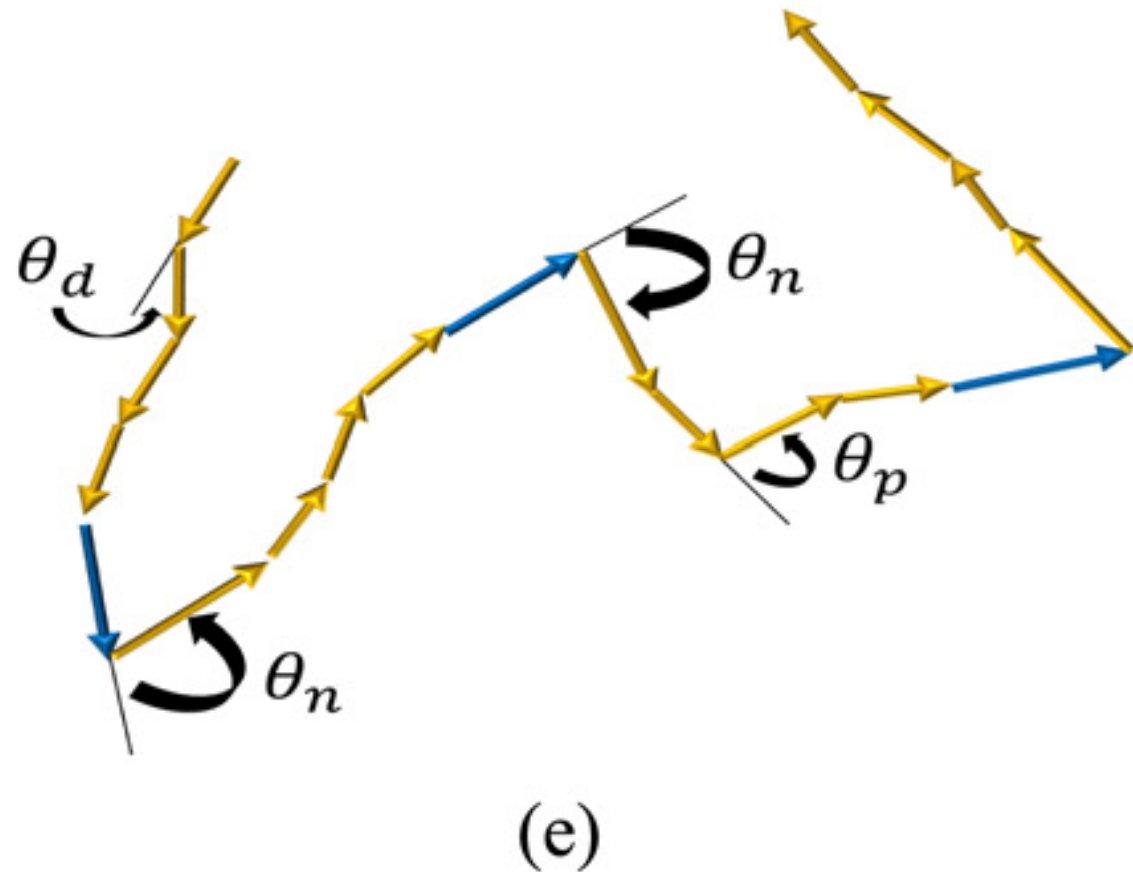


- No model so far has accounted for the role of DNA-bending non-histone proteins (eg. HMG/nhnp6)
- What would be the chromatin organization in the length-scale of a gene (a few genes) given a concentration of DNA-bending proteins and a given nucleosome organization?

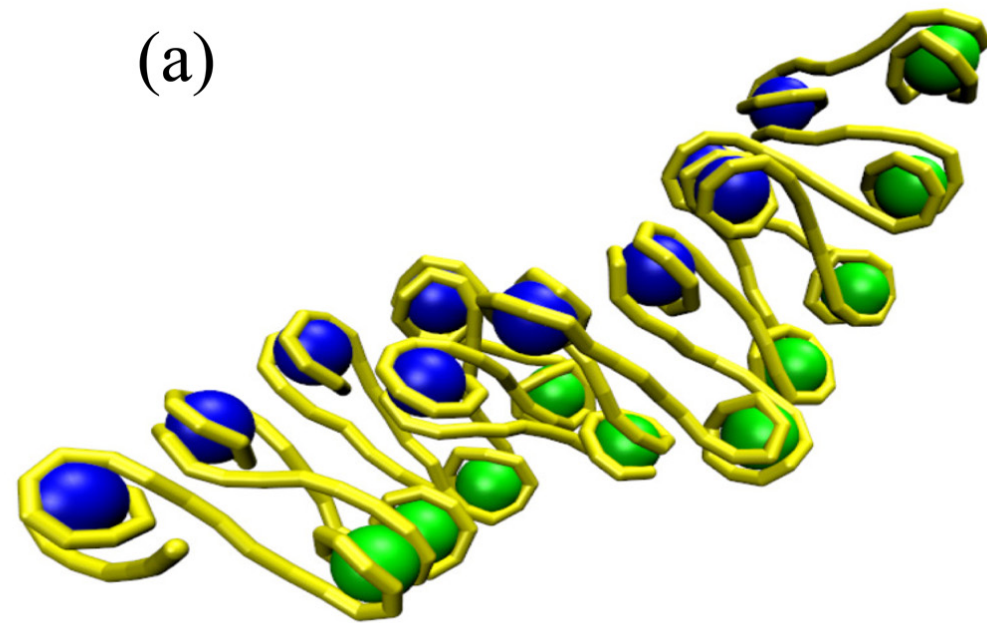
We do polymer simulations accounting for nucleosomes and DNA-bending proteins



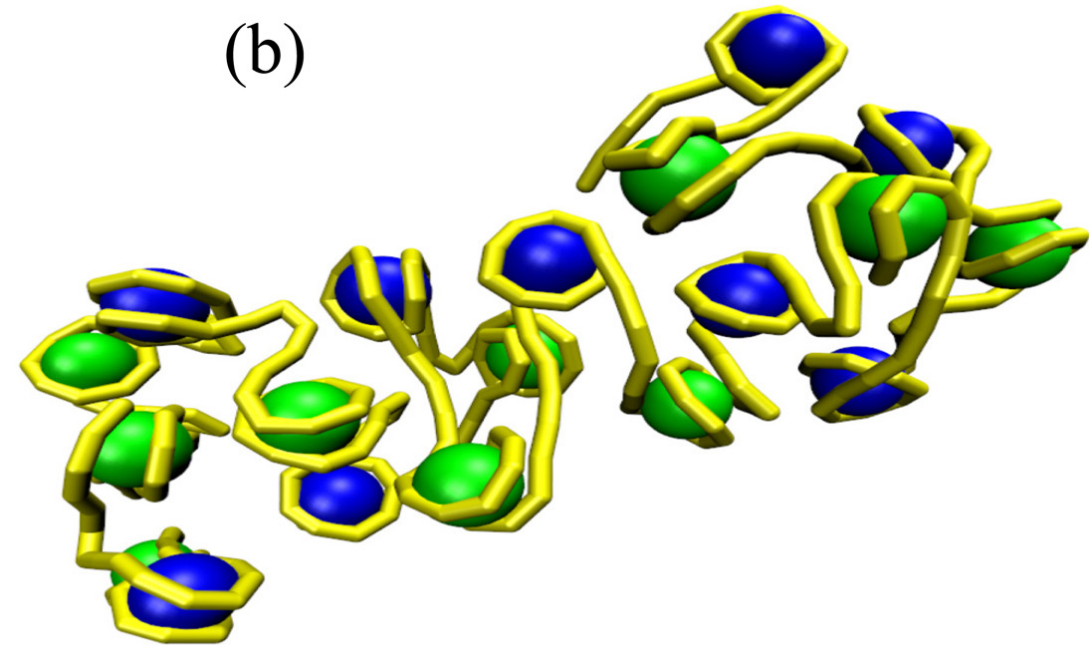
Brownian dynamics simulation



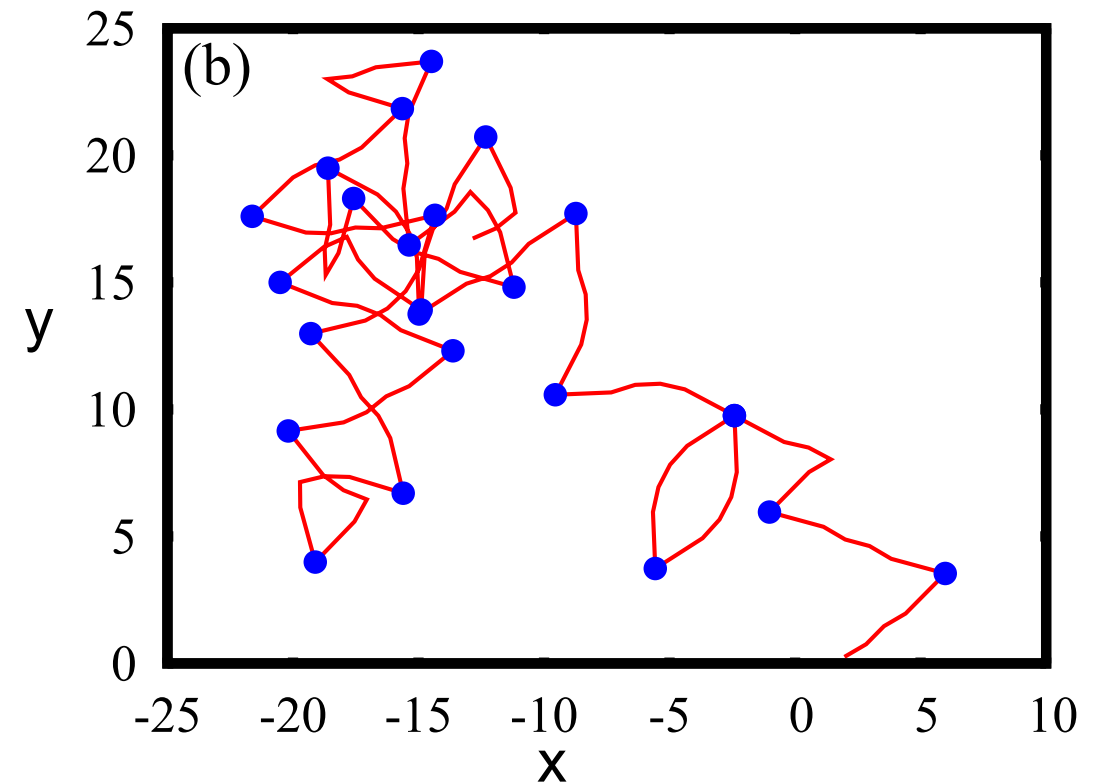
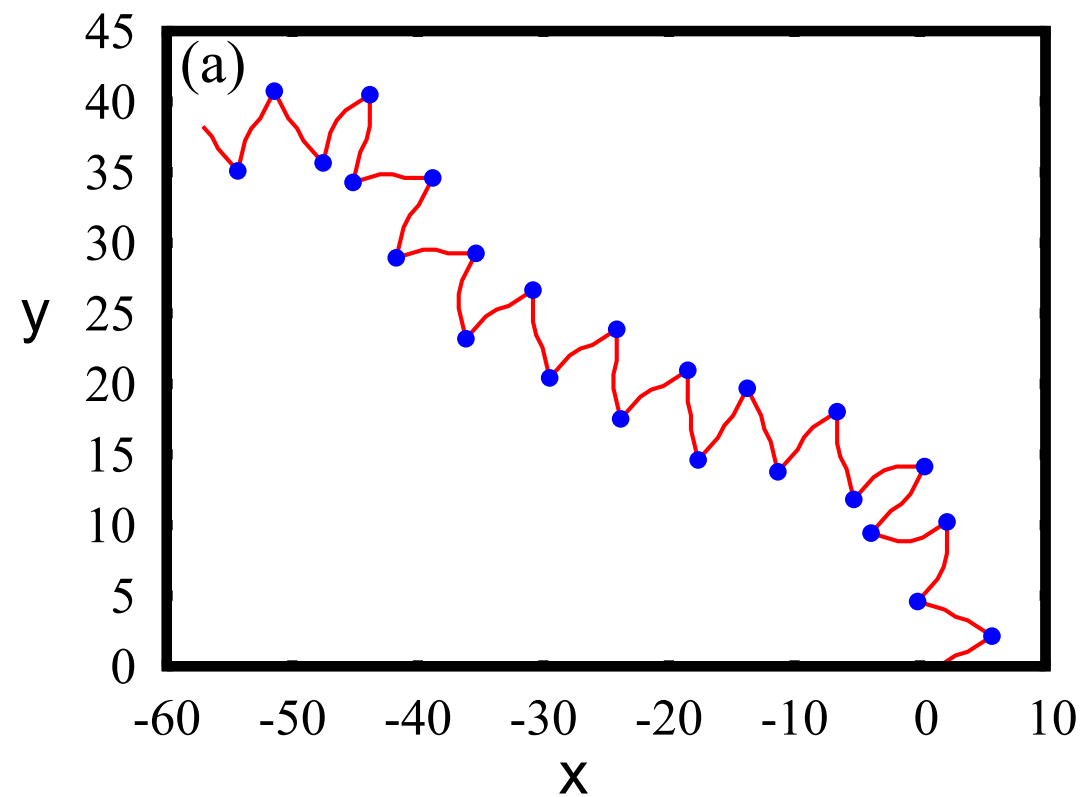
Freely rotating Chain model for polymers



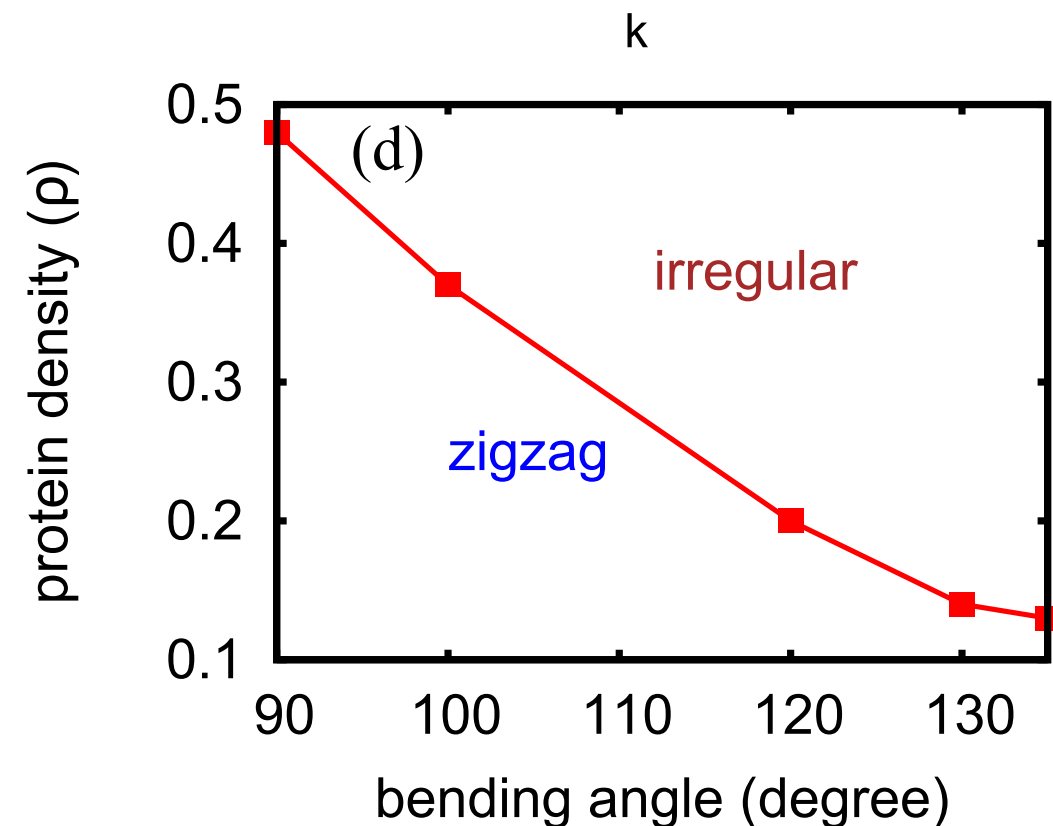
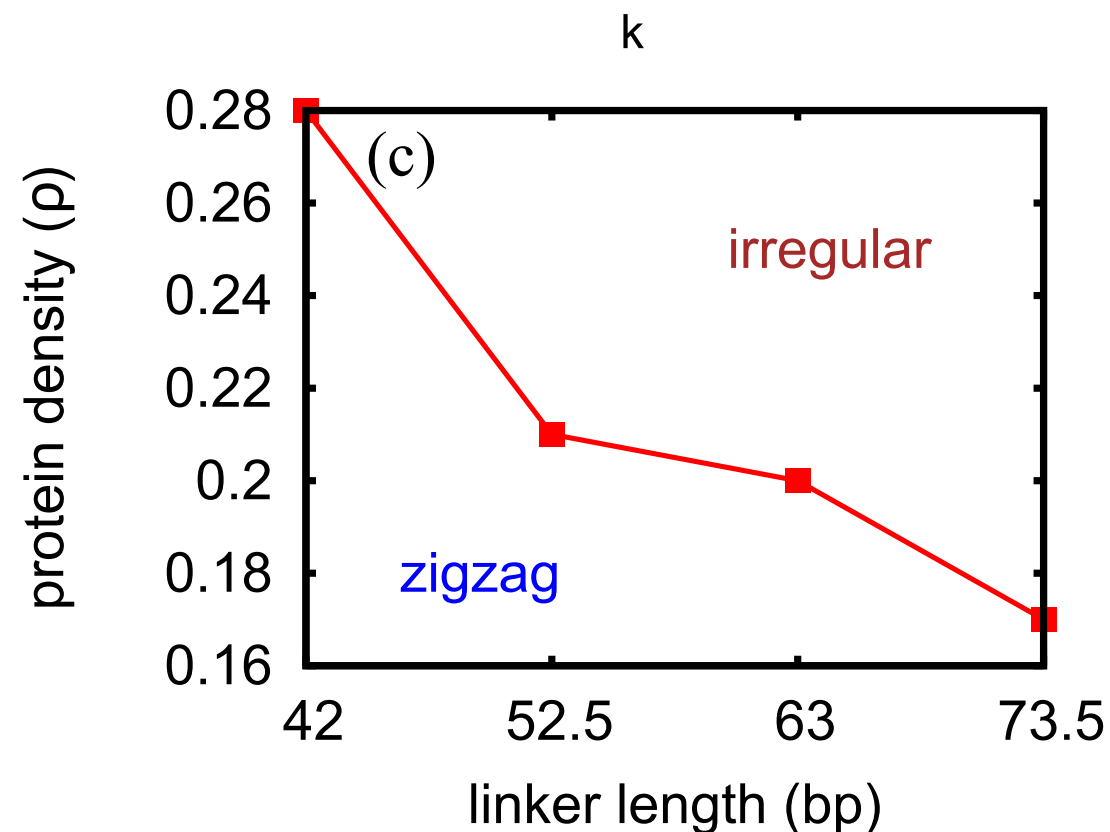
No non-histone protein



With non-histone protein



# Under what conditions will one observe “irregular” chromatin?

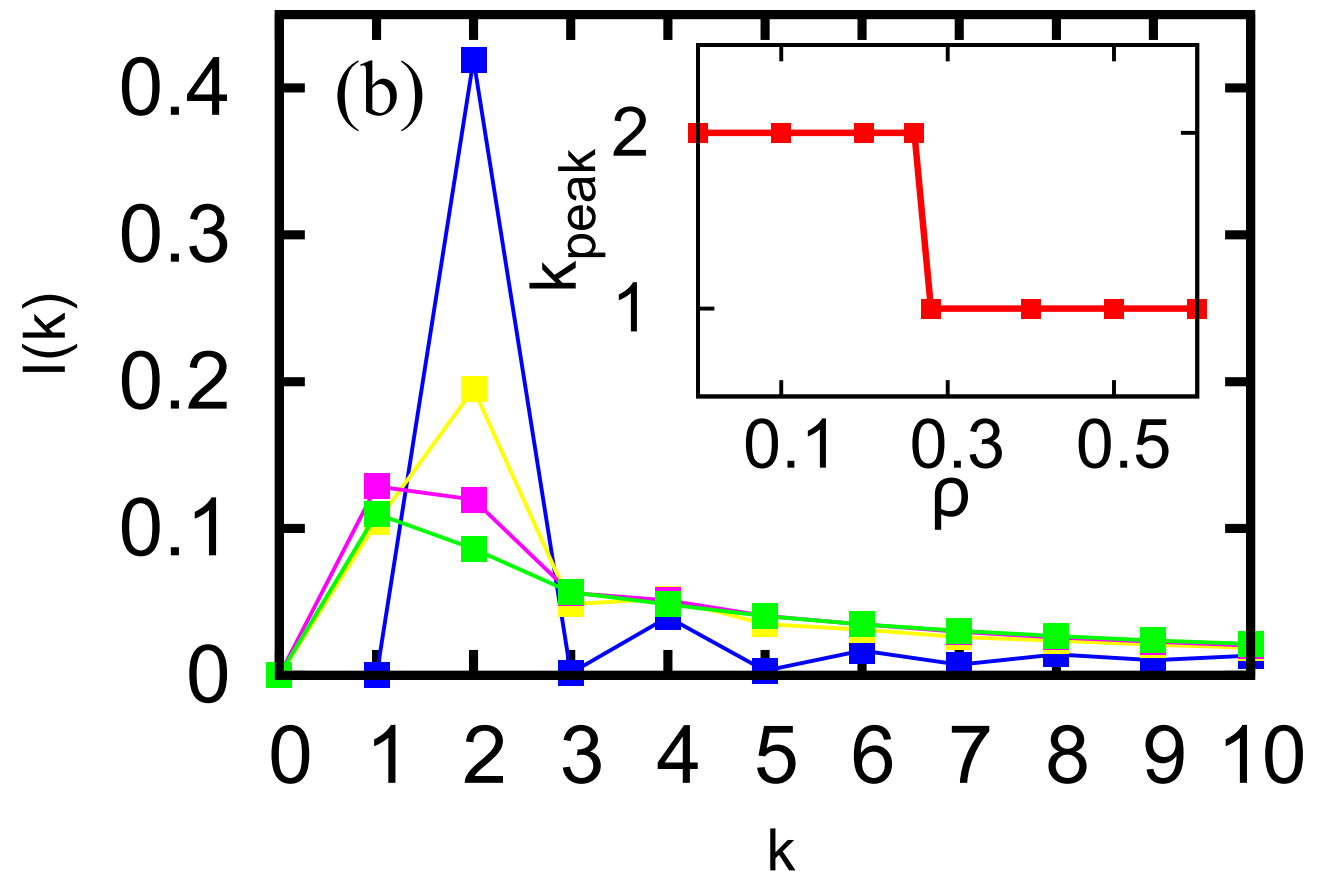
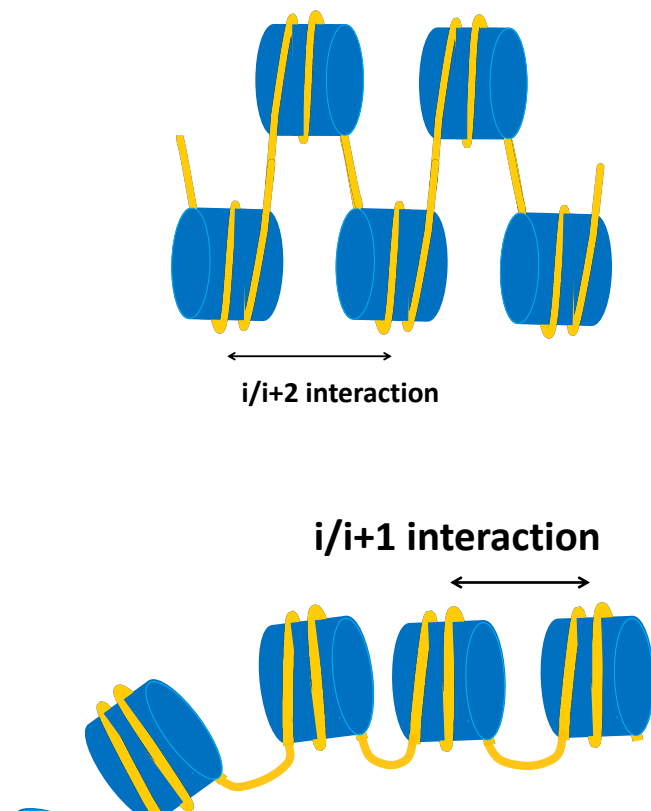


Bajpai et al (2017) PLOS Comp. Bio.

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

Is that density biologically relevant?

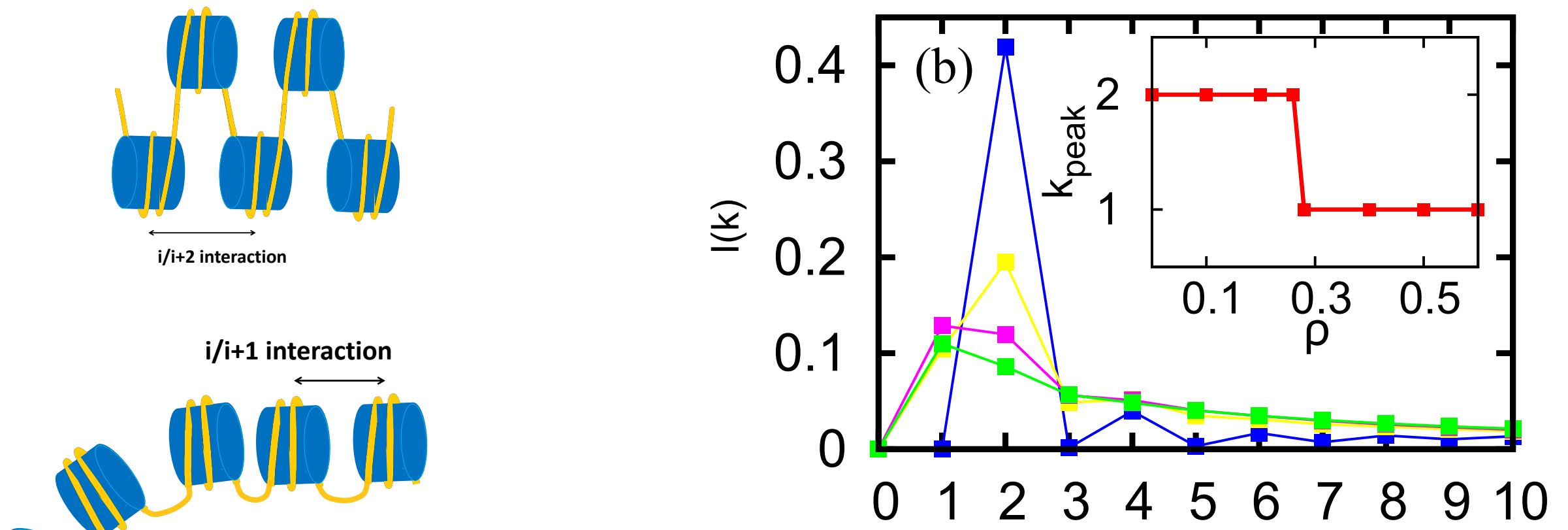
# Probability of neighboring nucleosomes to interact



When NHP density  $\sim 30\%$ ,  $I(1)=I(2)$



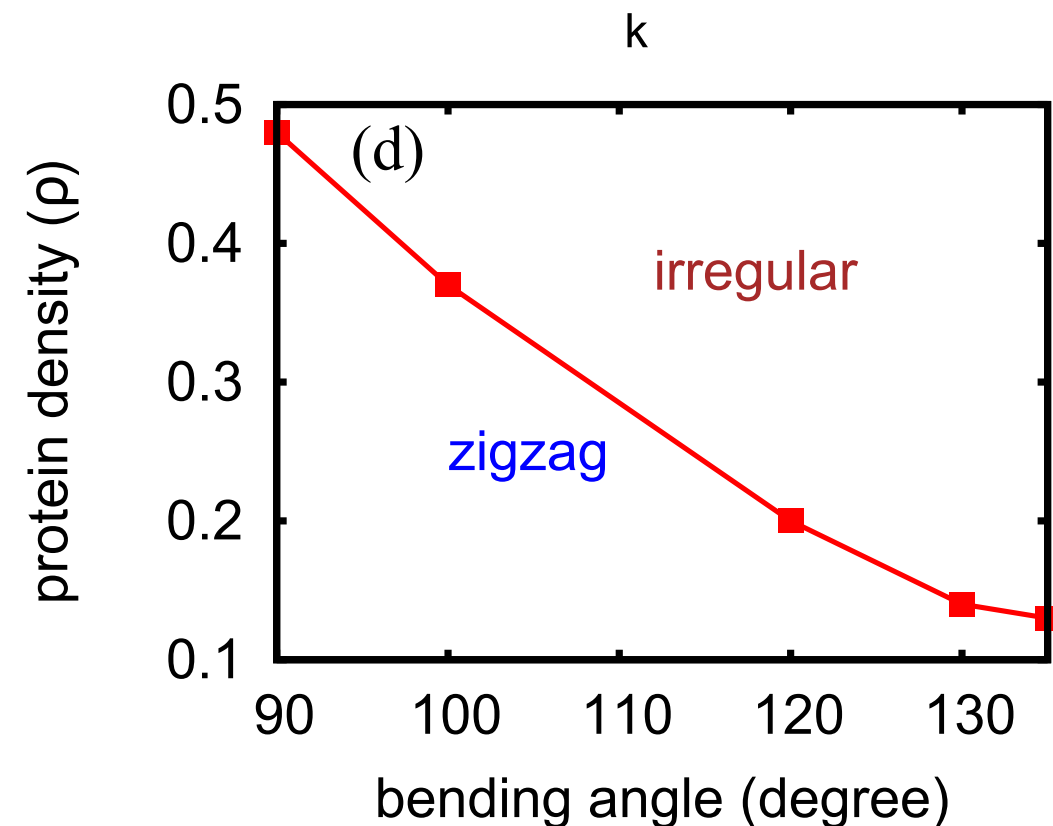
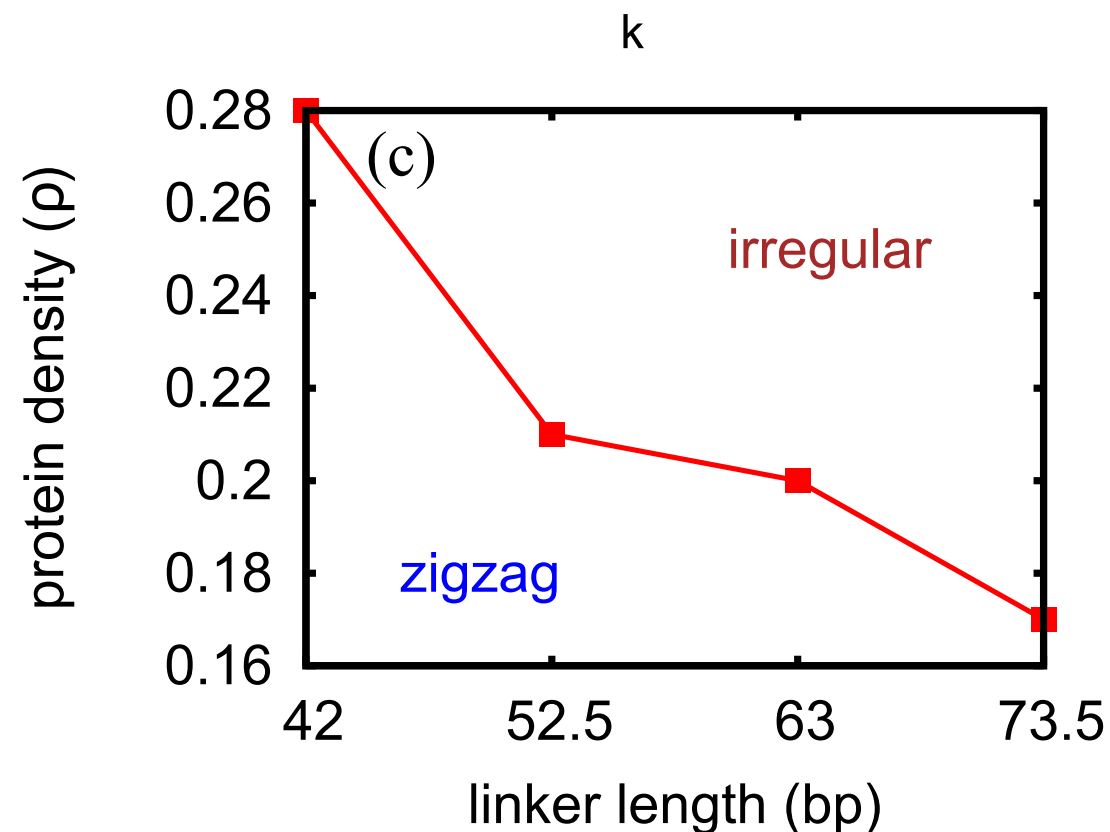
# Probability of neighboring nucleosomes to interact



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

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

# 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

# Acknowledgement

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