de losse eindjes van ons DNA

NCP-1

NCP-2
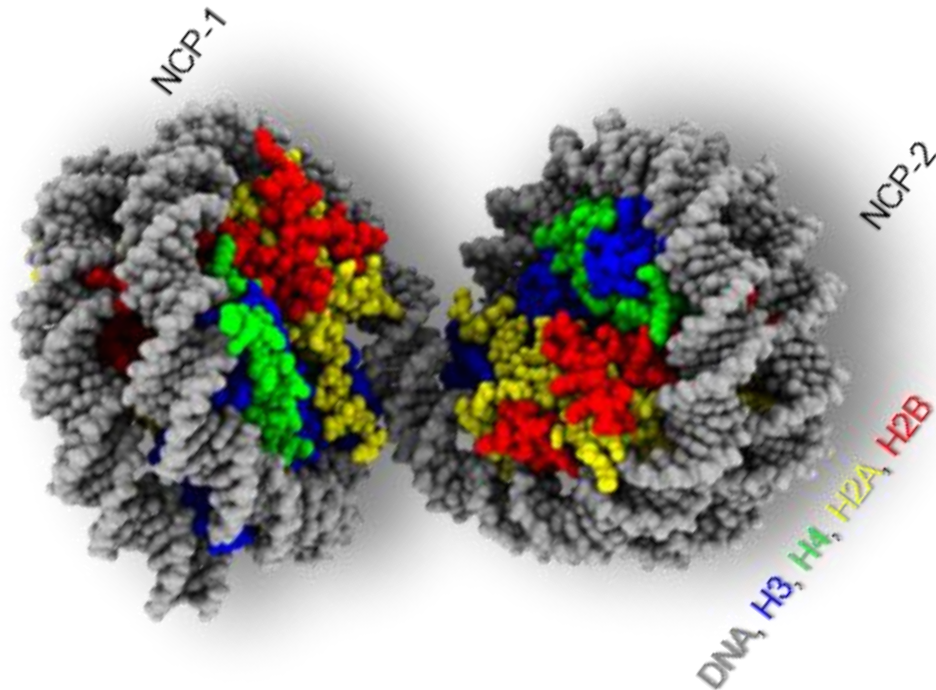
Soman et al. 2022 Nature

DNA, H3, H4, H2A, H2B

, en hoe dat zit opgevouwen …

Voor filmpjes, mail: noort@physics.leidenuniv.nl

John van Noort

Universiteit Leiden

cell division sets the pace of life
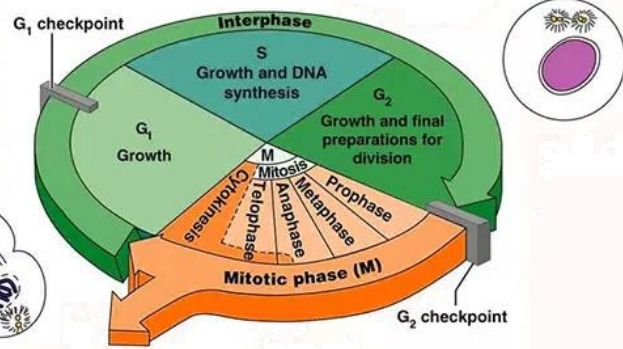
all DNA is replicated in in S-phase

https://www.youtube.com/watch?v=N97cgUqV0Cg

the end replication problem

last Okazaki fragment
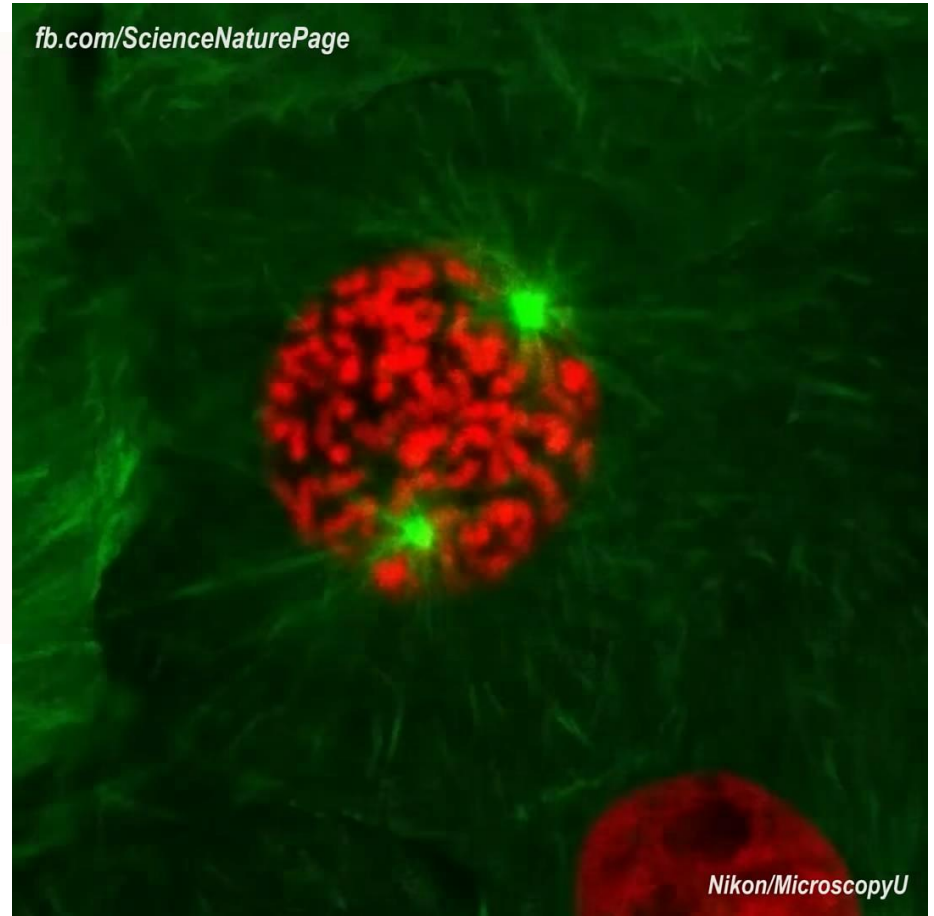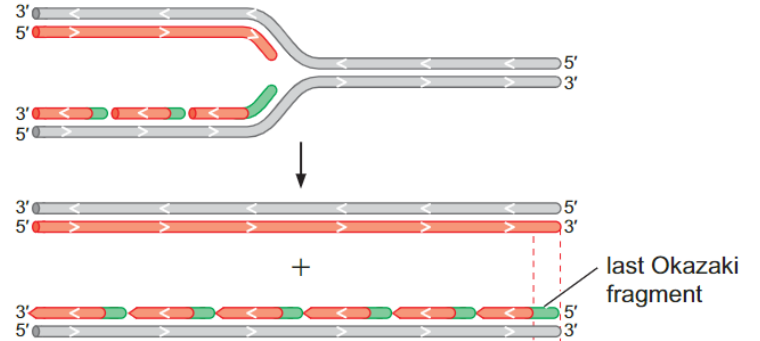
replication shortens the chromosome

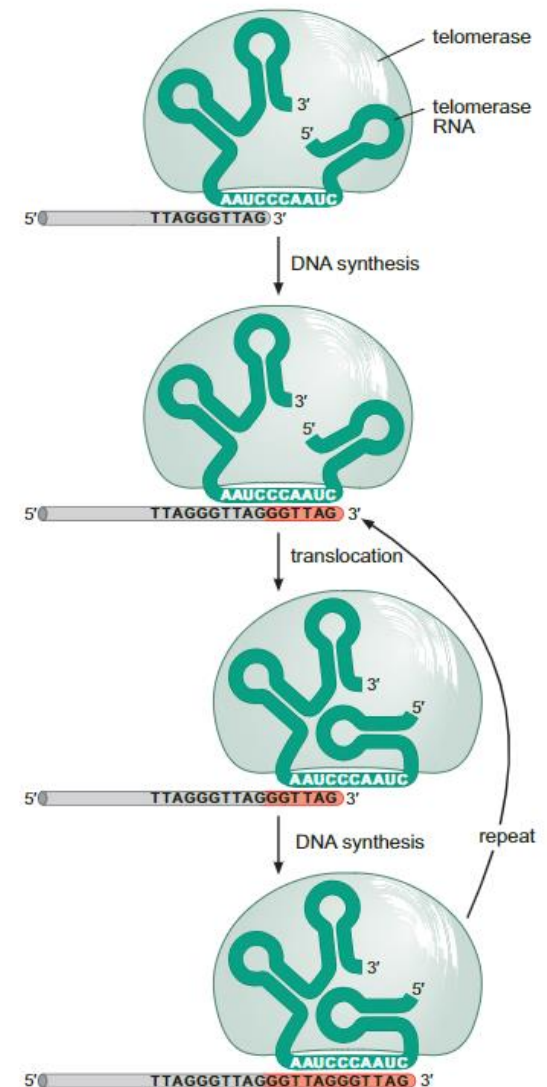# *telomerase solves the end replication problem*

ends of eukaryotic chromosomes are called telomeres

human telomeres consist of many head-to-tail repeats of the sequence 5'-TTAGGG-3'

the 3' end of each chromosome extends beyond the 5' end as ssDNA

it recruits a specialized DNA polymerase called **telomerase**

discovered in 1985 by Elizabeth Blackburn and Carol Greider, who shared the 2009 Nobel Prize in physiology or medicine (with Jack Szostak)

Watson et al. 2013 Molecular Biology of the Gene

telomerase solves the end replication problem

Telomerase Enzyme (17)

Uses external data. Price when purchased online ⓘ

$**34**^{95}

Liposomal Telomerase Enzyme Anti Aging Formula 4 oz

★★★☆☆ 2

$**20**^{99}

Enzymedica Telomere Plus, 30 Capsules

★★★★⯪ 4

$**42**^{99}

Enzyme Science, Telomere Pro 30 capsules

Save with W+

$**49**^{00}

SuperSmart - Telomeres Maintenance Formula | Non-GMO & Gluten Free - 60 Vegetarian Capsules

★★★★★ 2

Showing 1-3 of 3 reviews

Nov 5, 2024

★★★★★ **Verified Purchase** ⓘ

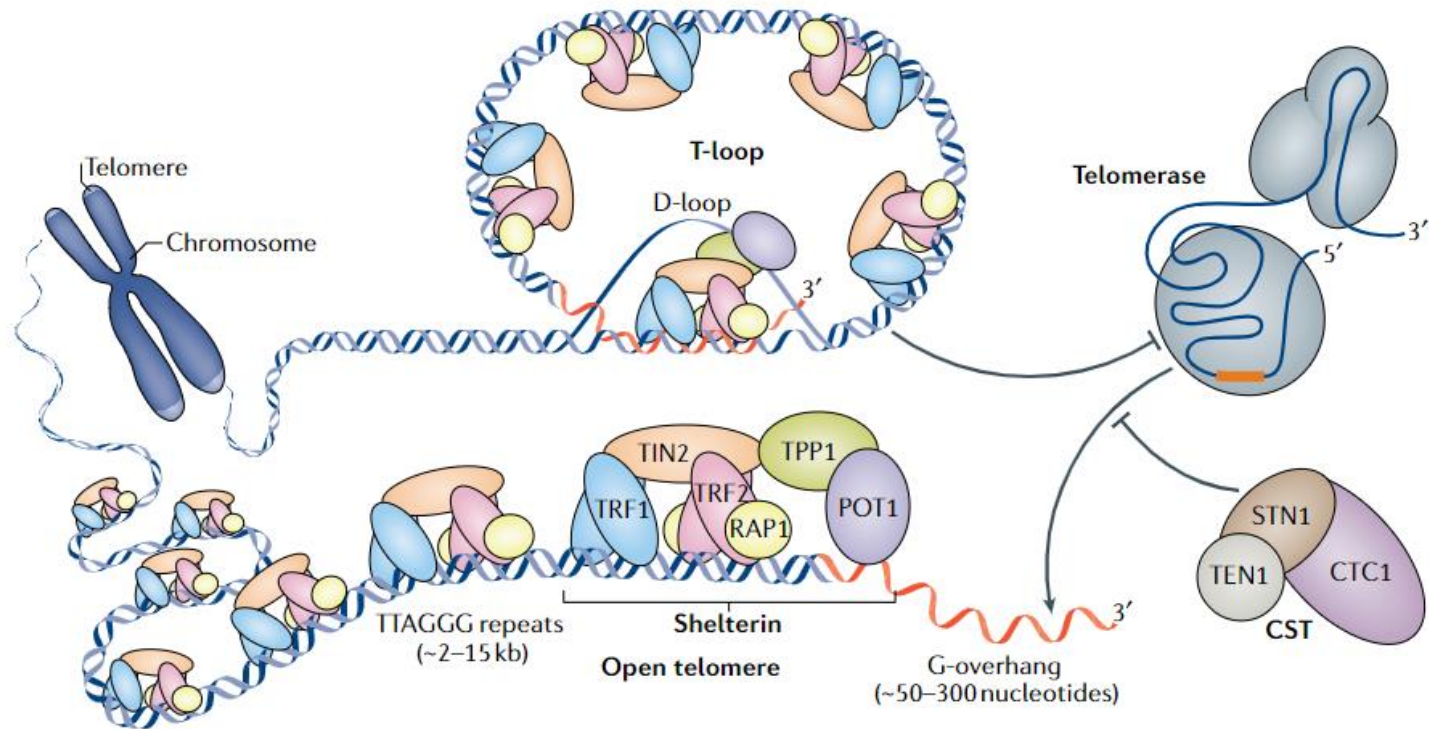Q Tammy

Sold by
Enzymedica, Inc.

this is my third time order. i am very satisfied with it. I give it to my 68 yr old husband. He is doing so much better. Thinking and walking. He starting to have more good days than bad.

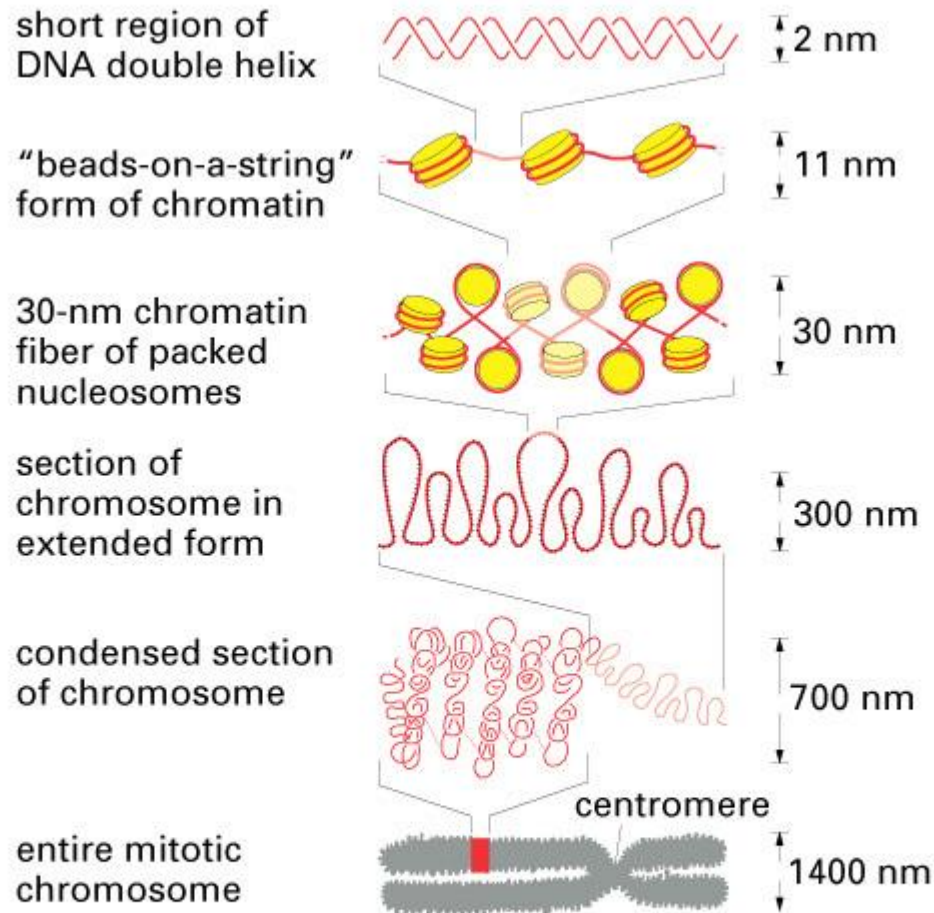Helpful? 👍 (0)   👎 (0)   Report

https://www.walmart.com

what stops telomerase?

Lim and Chech 2021 Nature Reviews

DNA folding into T-loops, assisted by shelterin complex

DNA organization in eukaryotes

short region of DNA double helix — 2 nm

"beads-on-a-string" form of chromatin — 11 nm

30-nm chromatin fiber of packed nucleosomes — 30 nm

section of chromosome in extended form — 300 nm

condensed section of chromosome — 700 nm

centromere

entire mitotic chromosome — 1400 nm

Alberts et al. 2012 Essential Cell Biology

Net result: each DNA molecule in a mitotic chromosome is 10.000 fold shorter than its extended length

telomeric chromatin and the shelterin complex
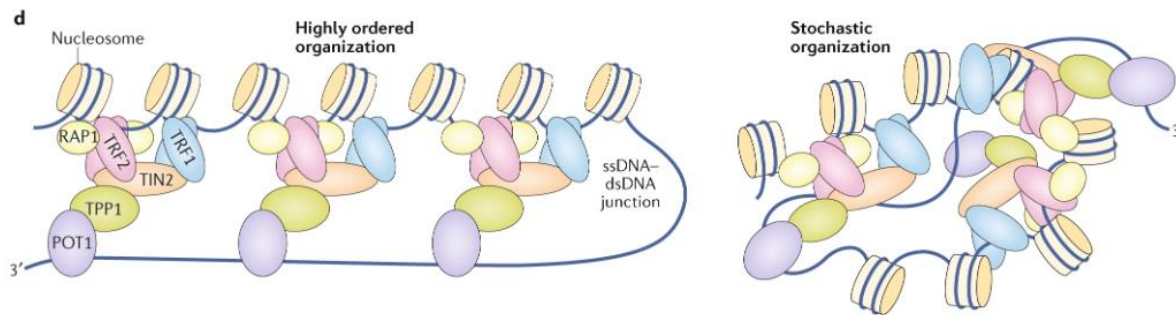
'two possible and polar opposite outcomes of telomere organization driven by shelterin complexes. A highly ordered shelterin array could result in **a zipper-like folding of the telomere**. On the other hand, a random deposition of shelterin would result in a **disordered telomere architecture**.'

other regulatory roles of chromatin
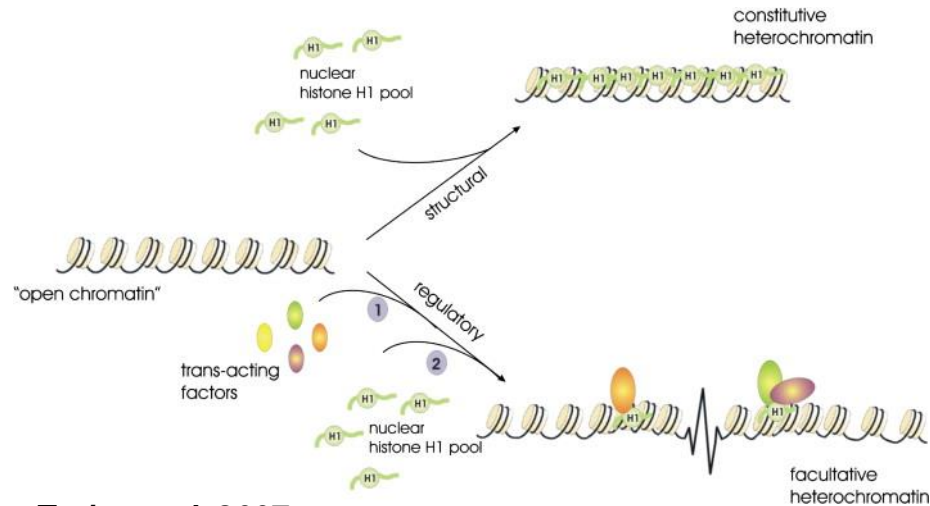
Trojer et al. 2007

- Structural and regulatory roles of chromatin are tightly connected

- Chromatin structure is poorly defined on the molecular scale
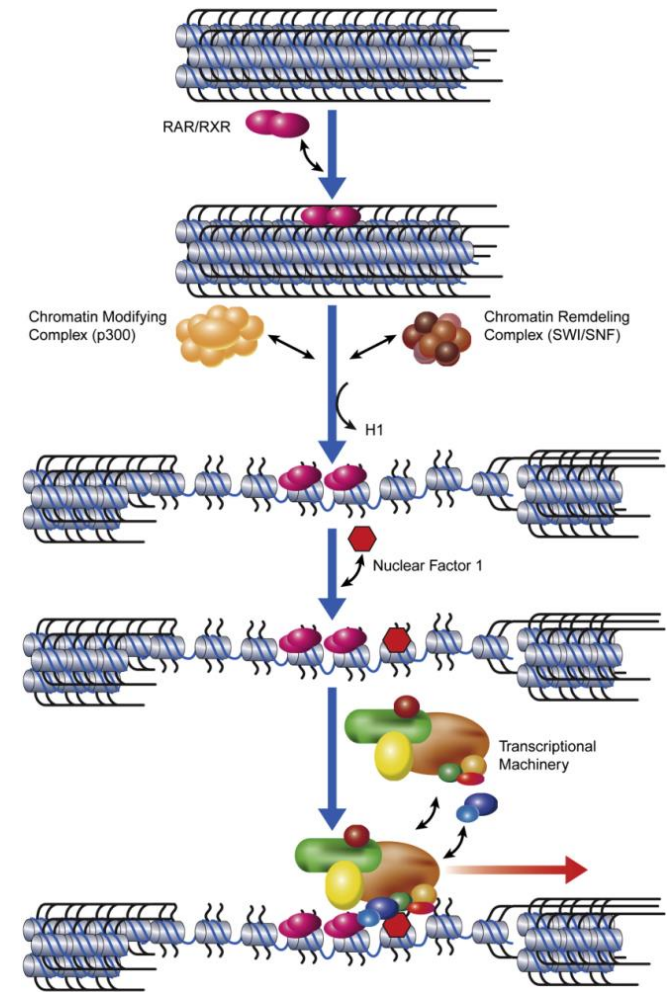
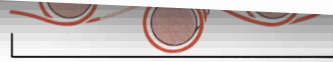**Li et al. 2010 Mol. Cell**

35 years of chromatin structure

STRUCTURAL BIOLOGY

The 30-nm Fiber Redux

Andrew Travers[1,2]

Do alternating stacking modes of nucleosomes underlie the compaction of chromatin?

25 APRIL 2014   VOL 344   **SCIENCE**   www.sciencemag.org

DNA he

nucleos

Epigenetics 9:5, 653–657; May 2014; © 2014 Landes Bioscience

Chromatin without the 30-nm fiber
Constrained disorder instead of hierarchical folding

Sergey V Razin[1,2,3,*] and Alexey A Gavrilov[1,3]

[1]Institute of Gene Biology of the Russian Academy of Sciences; Moscow, Russia; [2]Faculty of Biology; M.V. Lomonosov Moscow State University; Moscow, Russia; [3]LIA 1066 French-Russian Joint Cancer Research Laboratory; Moscow, Russia

Keywords: chromatin fiber, DNA packaging, histone modifications, nucleosome mobility, chromatin domain, 3D genome organization

Cell

Feb 2007
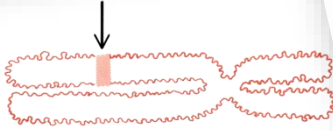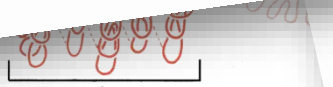
Higher-Order Structures of Chromatin:
The Elusive 30 nm Fiber

David J. Tremethick[1,*]
[1]The John Curtin School of Medical Research, The Australian National University, P.O. Box 334, Canberra, The Australian Capital Territory, Australia, 2601
*Correspondence: david.tremethick@anu.edu.au
DOI 10.1016/j.cell.2007.02.008

Despite progress in understanding chromatin function, the structure of the 30 nm chromatin fiber has remained elusive. However, with the recent crystal structure of a short tetranucleosomal array, the 30 nm fiber is beginning to come into view.

mitotic
chromo-
some

Alberts et al. 1983

Alberts et al. 2007

*Compilation by **Karsten Rippe***

partially decondensed mitotic chromosomes, tomography

"The **lengths of linker DNA in regions resembling the tetrameric unit** were similar but not identical, and we find **extensive variation of linker length,** whereas all published coiling motifs require uniformity."
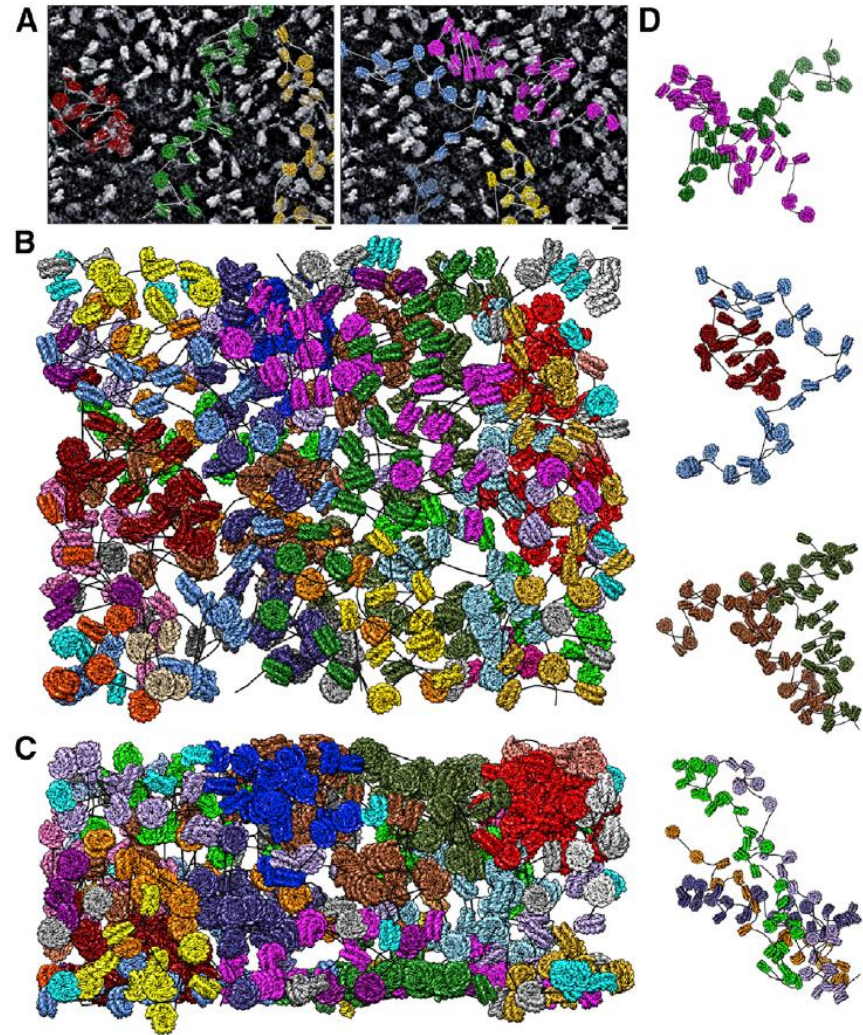
Beel et al. 2021 Mol. Cell

Figure 6. Chromatin fibers in a partially decondensed mitotic chromosome
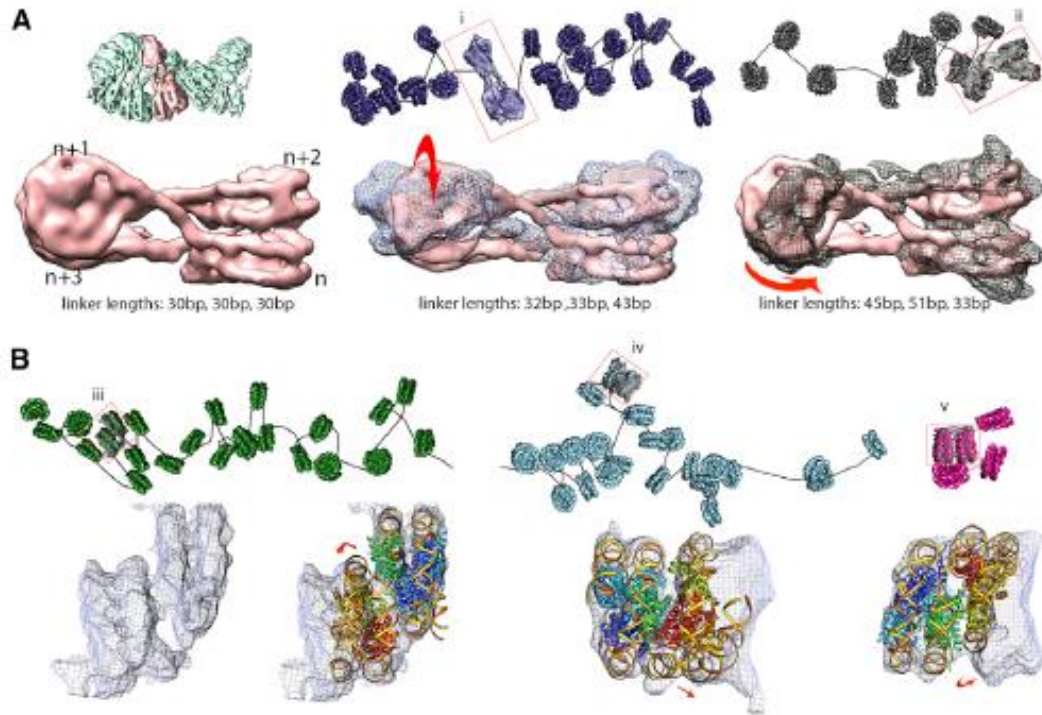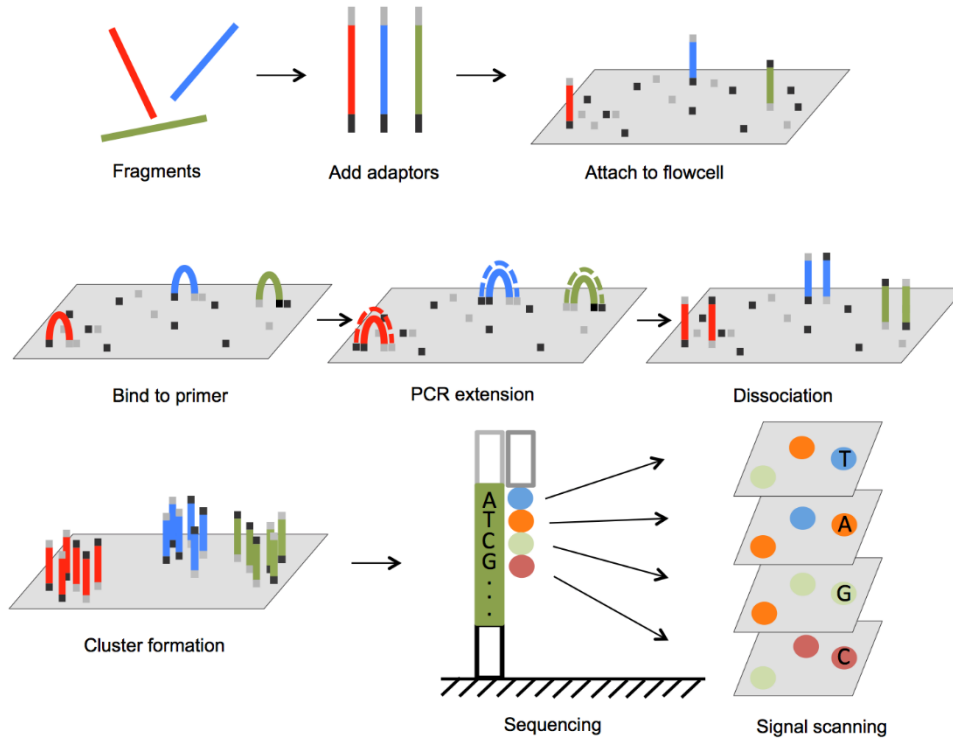
partially decondensed mitotic chromosomes, tomography

Figure 7. Short folding motifs, similar, but not identical, to those found in structures of reconstituted chromatin, related to Figure 5

Take home:

1) Nucleosome stacking defines higher order folding

2) Linker DNA defines nucleosome stacking

Beel et al. 2021 Mol. Cell

Illumina HiSeq

Fragments → Add adaptors → Attach to flowcell

Bind to primer → PCR extension → Dissociation

Cluster formation → Sequencing → Signal scanning

Furlani et al.2021 ActChimSlov.

Decreasing Genome Sequencing Costs

Cost of Whole Genome Sequencing ($)

Genome sequencers announced, costs dramatically reduced

Illumina released HiSeqX Ten

Illumina announced NovaSeqX Series

Ultima Genomics announced UG100

genomize

Source: National Human Genome Research Institute
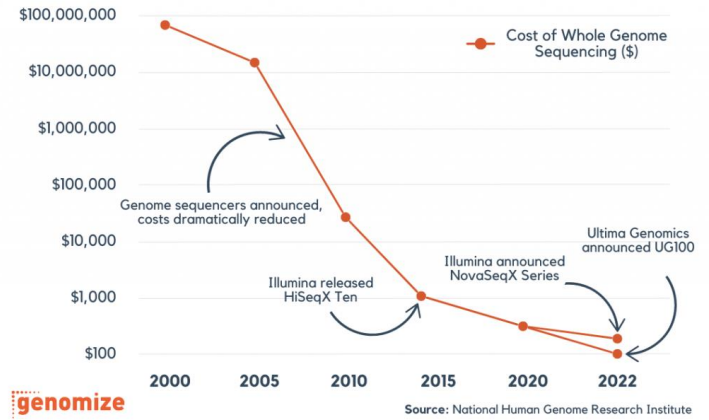
DNA sequencing has revolutionized DNA research

MNase-seq
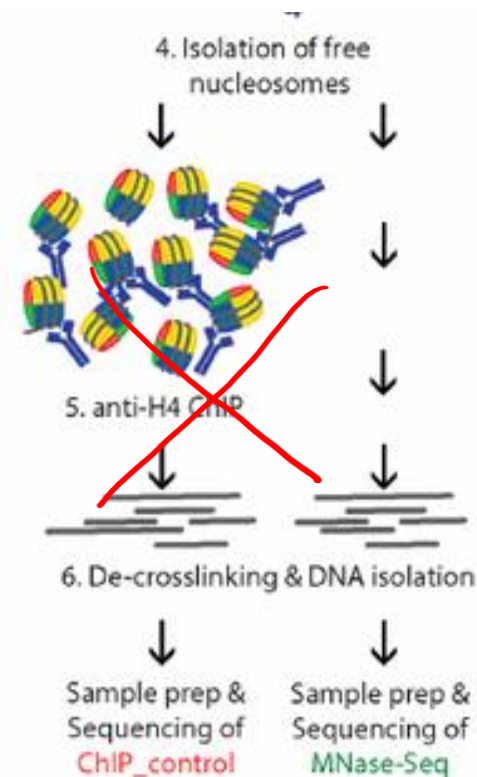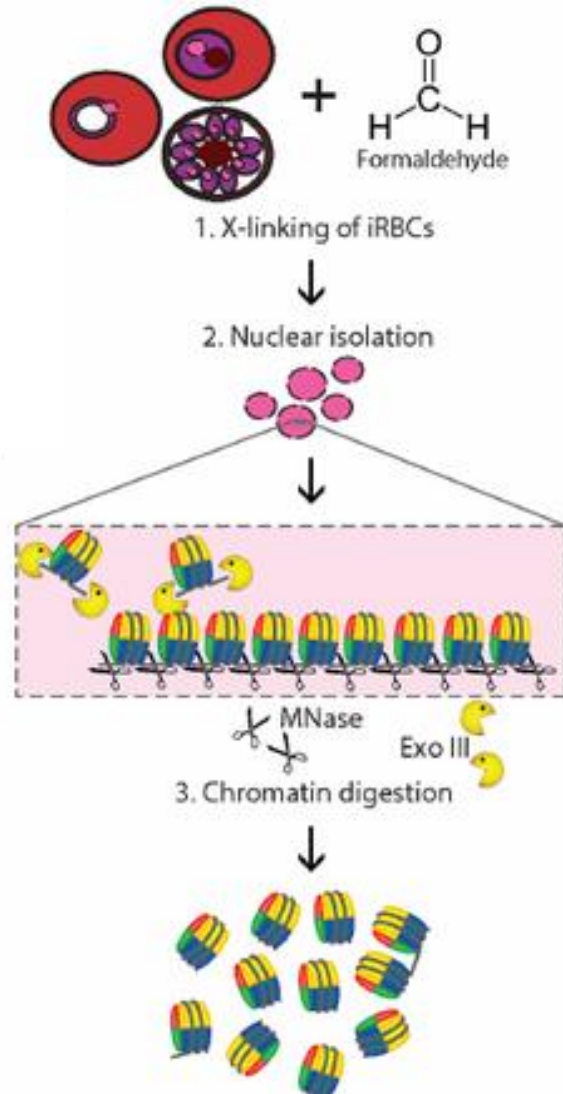
Adapted from Kensche NAR 2015

Sequencing of nucleosomal DNA maps their positions on the genome

genome wide nucleosome mapping
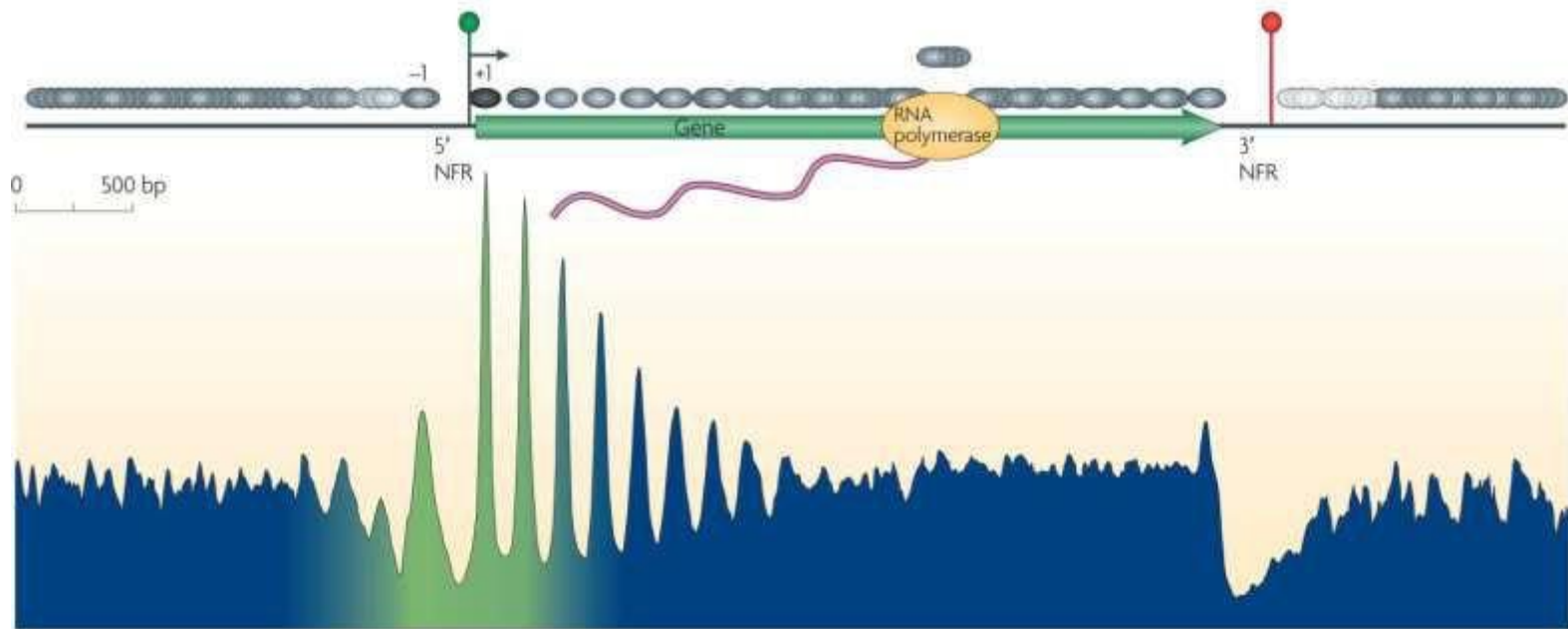
Jiang and Pugh 2009 Nature Rev. Gen.

Nucleosomes can be well-positioned,
especially around transcription start sites

chromatin remodeling

- Host of dedicated enzymes facilitate sliding, (dis-)assembly, modification, etc.

- In yeast: ~1 remodeller per 7 nucleosomes!

- But how do they know where to position the nucleosome?
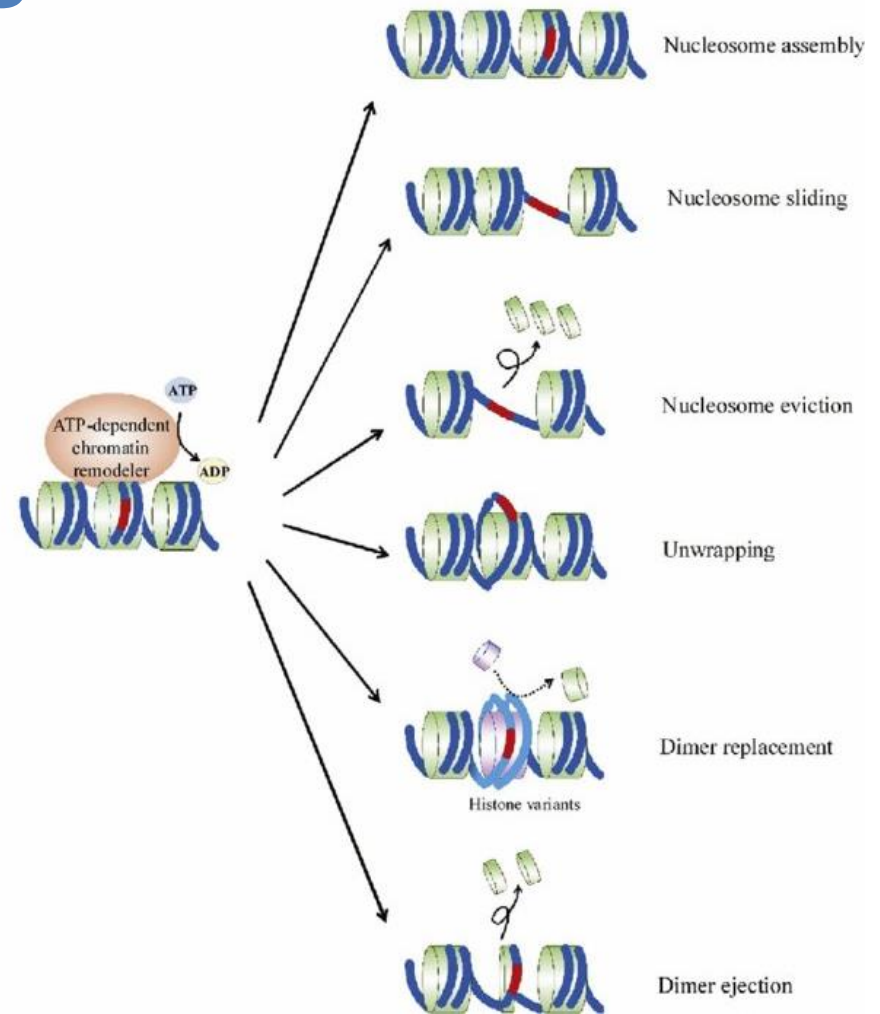
Does DNA sequence guide nucleosome positions?

Nucleosome assembly

Nucleosome sliding

Nucleosome eviction

Unwrapping

Dimer replacement

Dimer ejection

DNA wrapping into nucleosomes
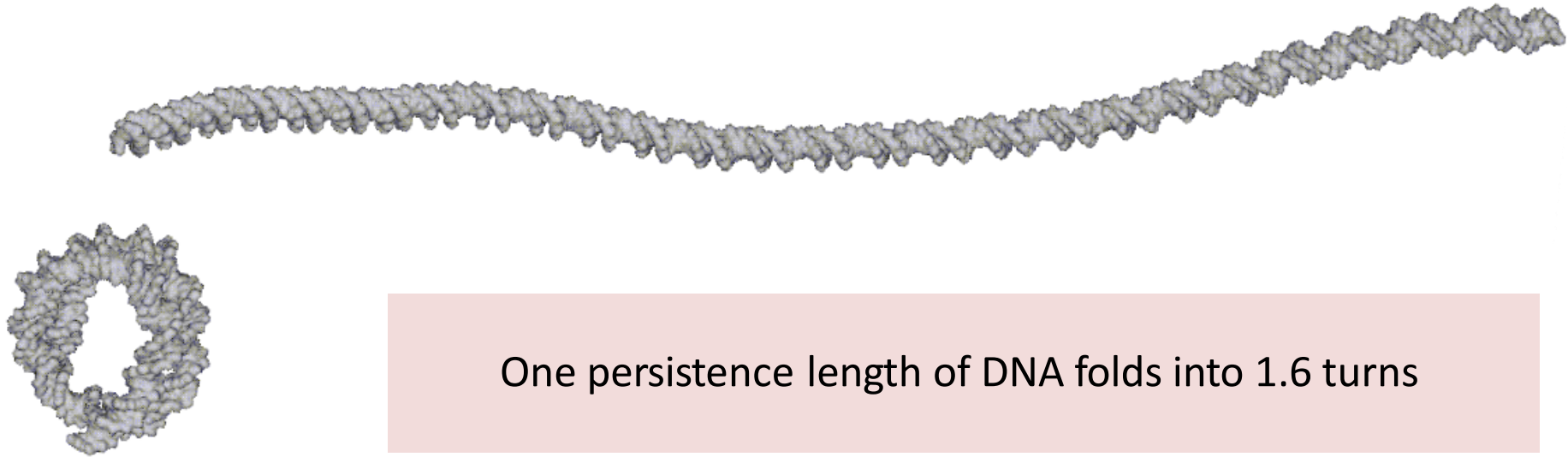
One persistence length of DNA folds into 1.6 turns

Dyad axis

DNA entry-exit
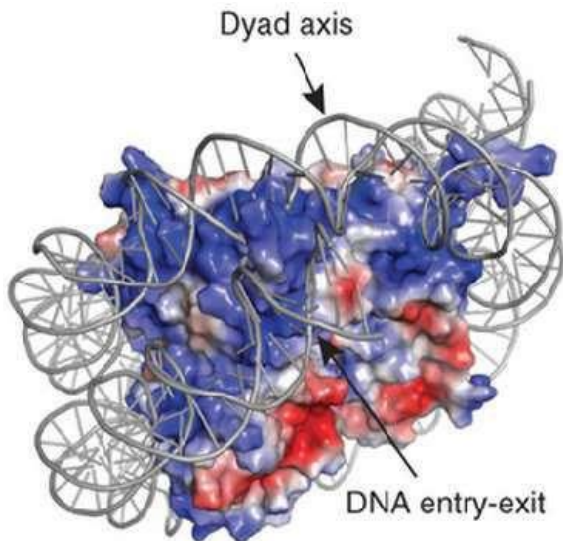
Approximation:
pure adsorption   -85 k_bT
bending   58 k_bT
free energy   -27 k_bT

Schiessel 2003 J. Phys. Condens. Matter

DNA sequence

"short runs of (A, T) are preferentially positioned with minor grooves facing in, while runs of (G, C) tend to have their minor grooves facing out."

"the periodicity of this modulation in sequence content (10.17 base-pairs)"

Drew and Travers 1985 JMB
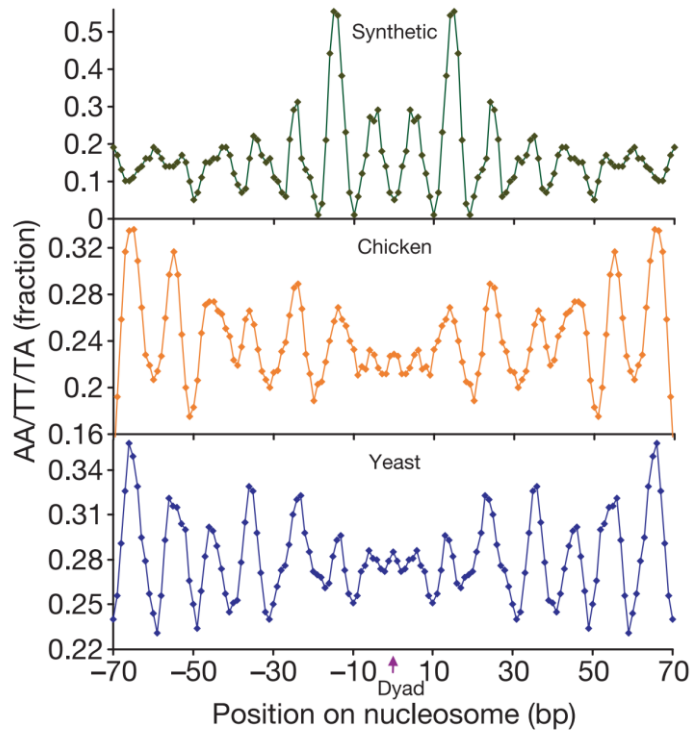
Segal et al. 2004 Nature
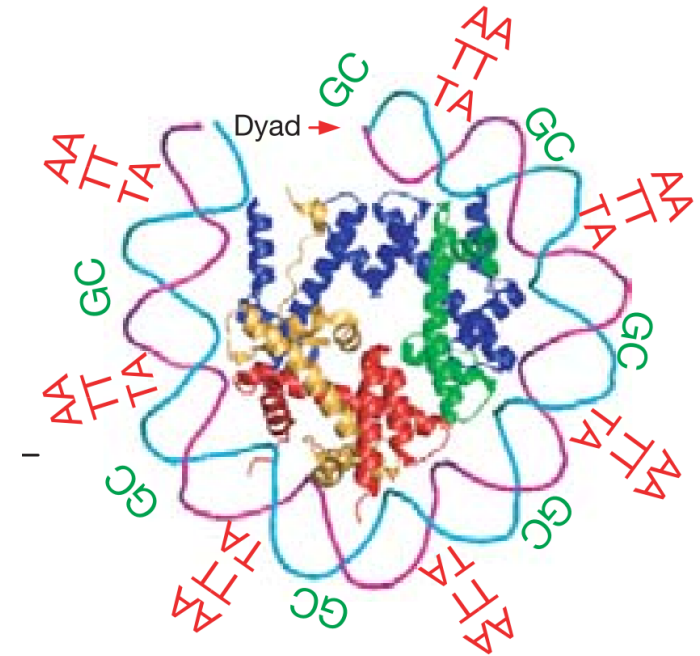
SELEX experiment

Lowary and Widom 1998 JMB

1) reconstitute nucleosomes with large pool of random DNA sequence

1) select properly folded nucleosomes

1) remove histone proteins, PCR

1) iterate steps 1-3 multiple times

1) sequence remaining DNA: clone 601, ….
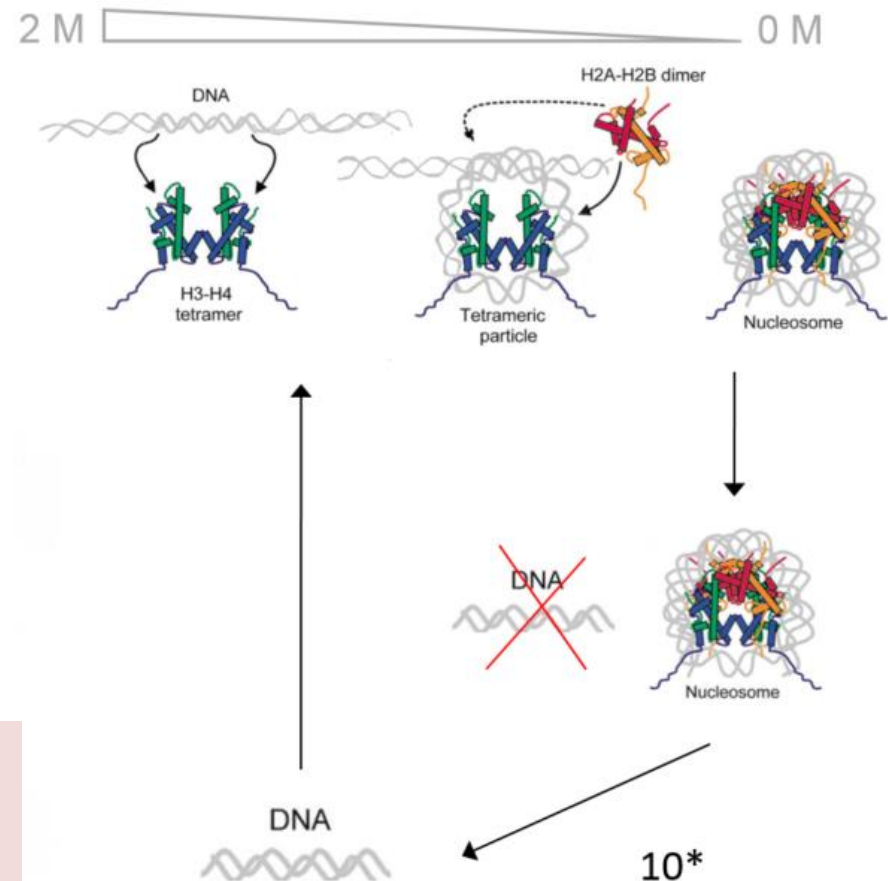
nucleosomes are typically reconstituted on non-natural, synthetic DNA

statistical physics
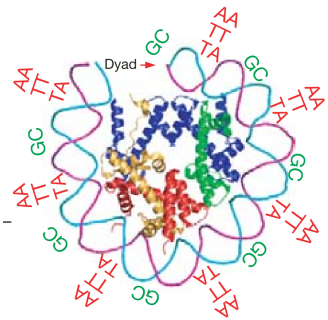
Remove position dependence within the nucleosome
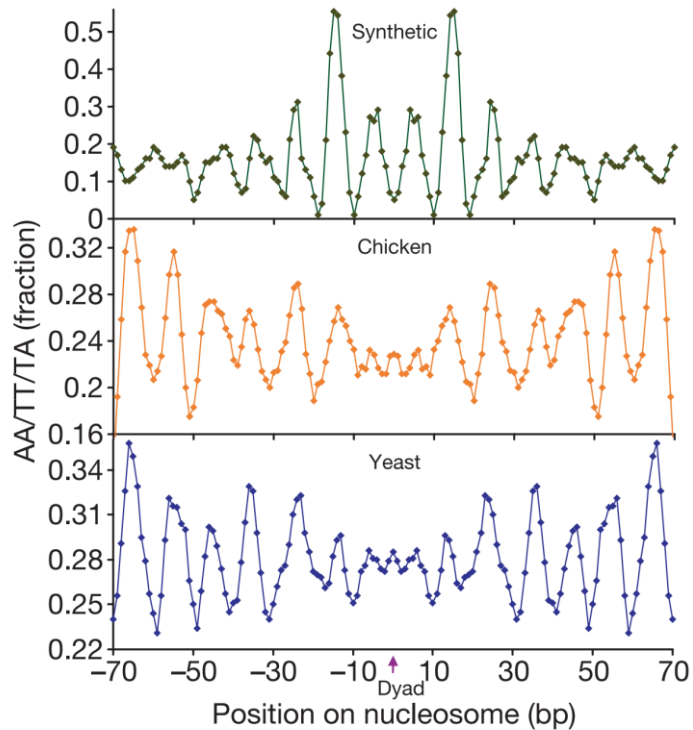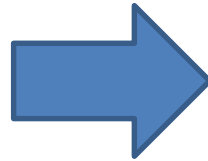
$$P_{YY}(S_i|S_{i-1}) = \frac{1}{4} + B\, sin\left(2\pi\left(\frac{i}{p} + \frac{1}{2}\delta_{GC}\right)\right)$$

Segal et al. 2004 Nature
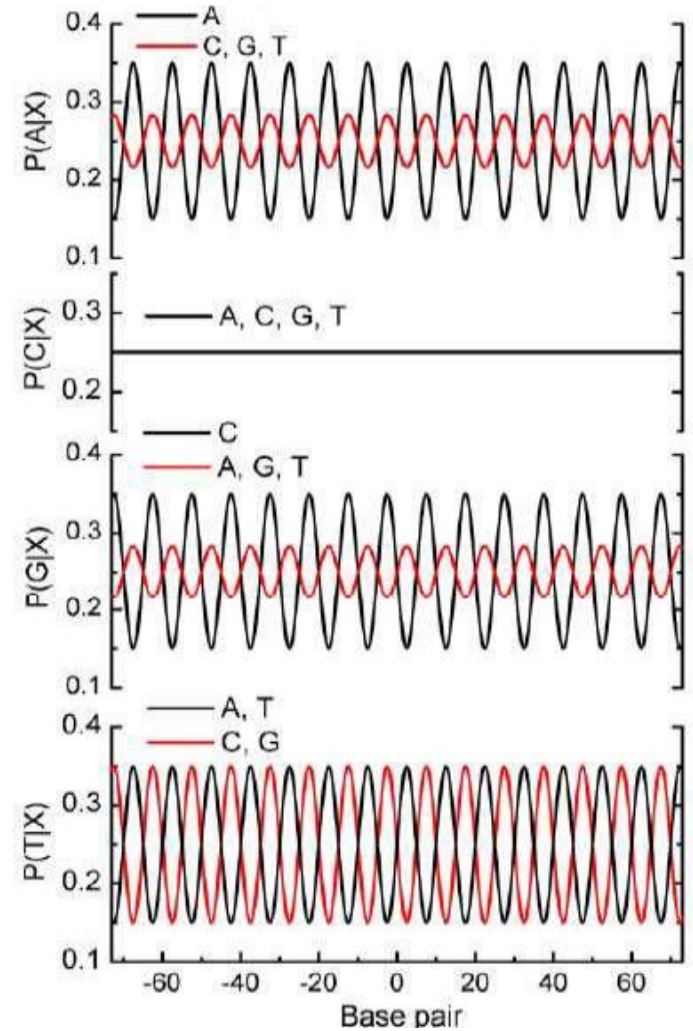
Van der Heijden, JvN et al. 2012 PNAS

in vivo sequence dependence
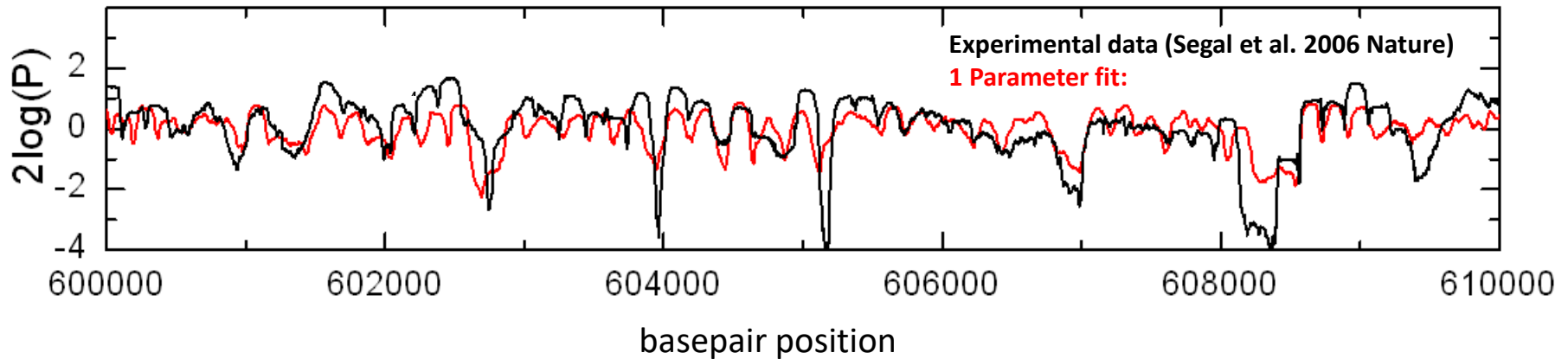
$$\Delta\Delta G(i) = -k_B T \ln P(i)$$

probability that bp is occupied:

$$\ln P(i) = -\frac{\mu + \Delta\Delta G(i)}{k_B T} - \ln\left[1 - \int_x^{x+l} P(x)dx + \int_{x-l}^{l} \frac{P(x)dx}{1 - \int_x^{x+l} P(x')dx'}\right]$$

Vanderlick, et al. 1986 PR A, Vaillant et al. 2007 PRL

Nucleosome positions are largely defined by DNA sequence

Van der Heijden, van Vugt , Logie, JvN 2012 PNAS

DNA sequence and chromatin

- Specific di-nucleotides facilitate DNA bending into a nucleosome

- DNA sequence can largely explain nucleosome positioning in vivo ('the mechanical genome')

But how does this affect the structure of chromatin?

Frank and Ernest

FIGURING OUT THE HUMAN GENOME MAP WAS EASY -- THE HARD PART IS REFOLDING IT.

www.frankandernest.com

THAVES

Copyright (c) by Thaves. Distributed from www.thecomics.com.

magnetic tweezers

- fiber is tethered between glass slide and a paramagnetic bead

- By moving a magnet above the bead the force can be varied

- (Brownian) motion of the bead is measured by video microscopy/image processing

- Bead position represents the end-to-end distance of the fiber
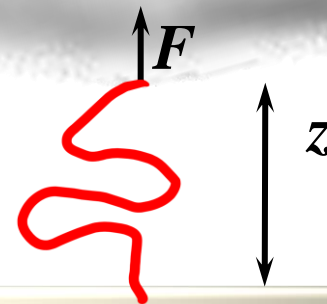
F

z

dynamic force spectrocopy
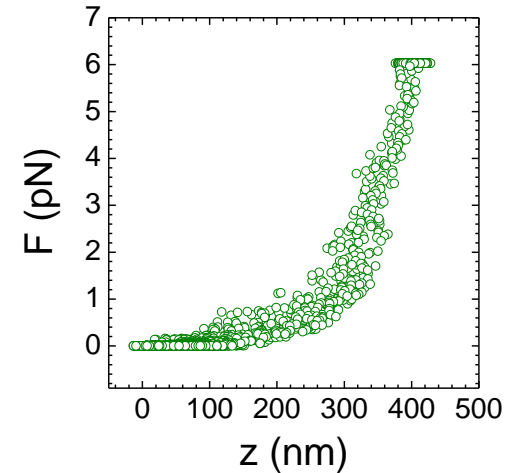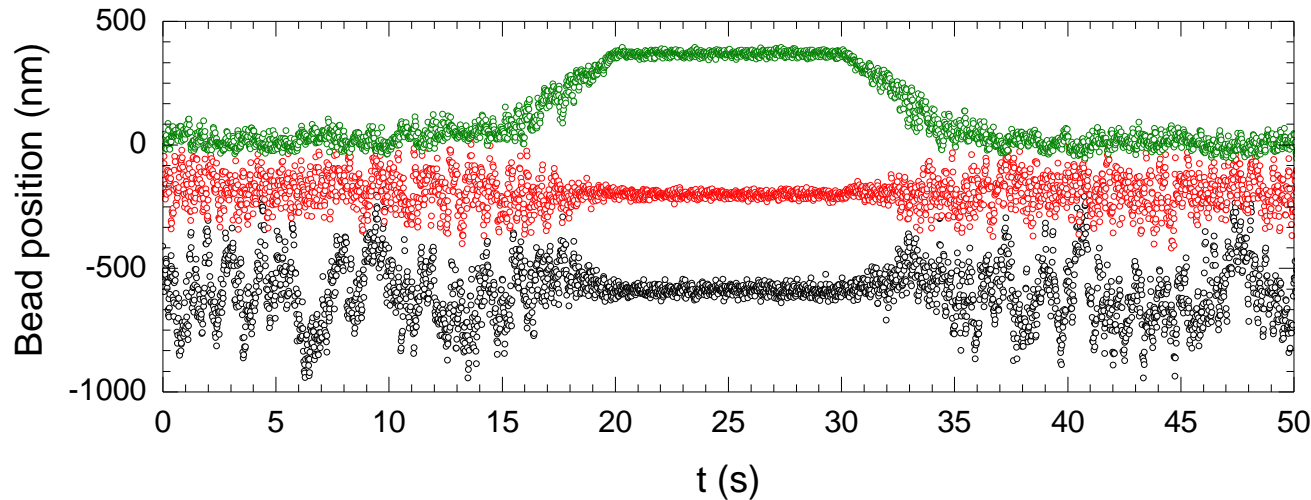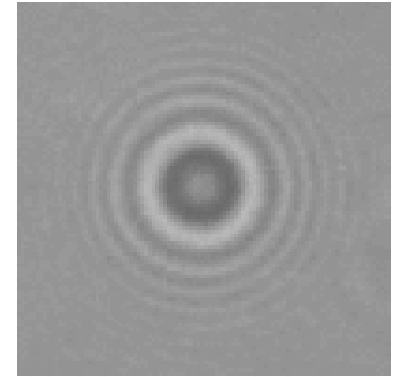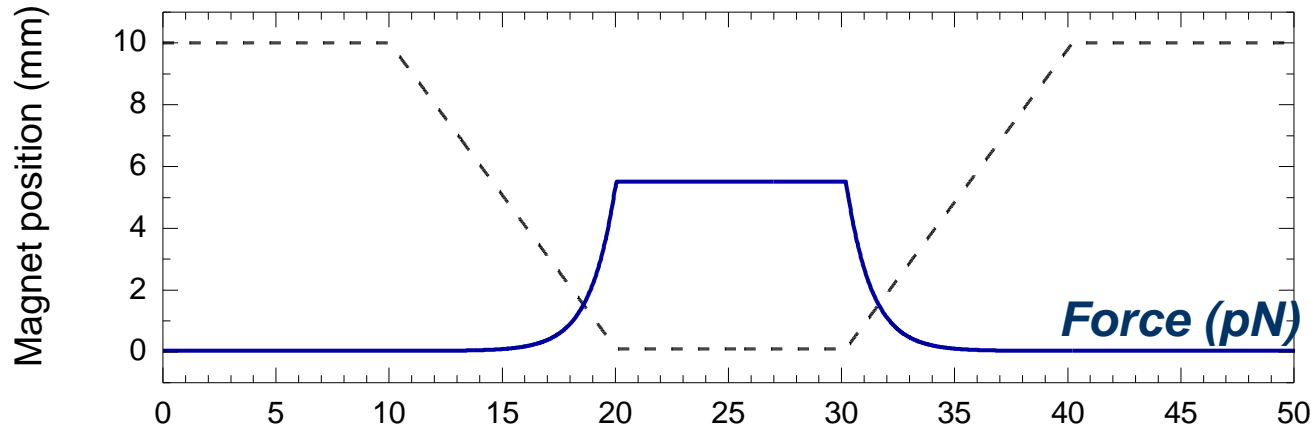
- Magnet is moved down and up
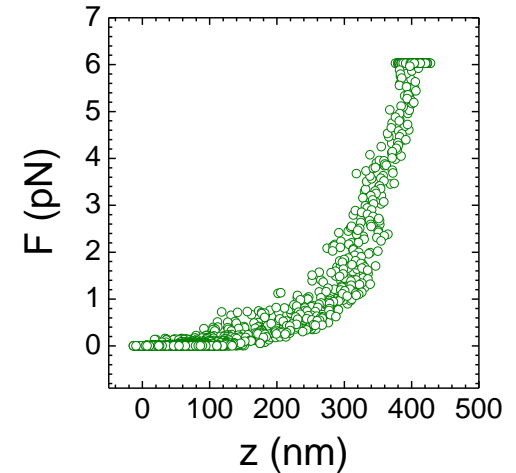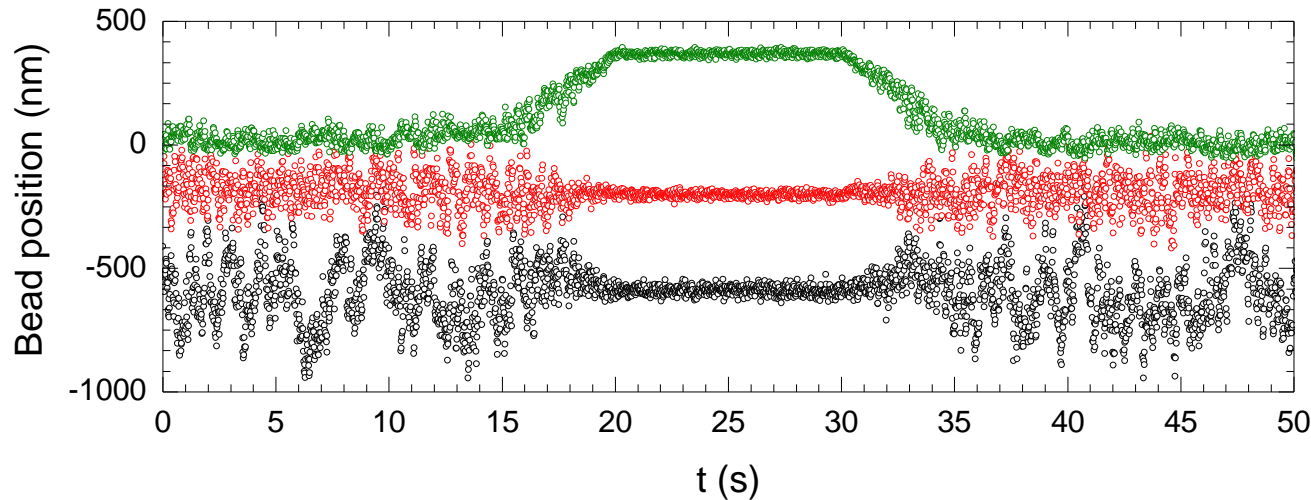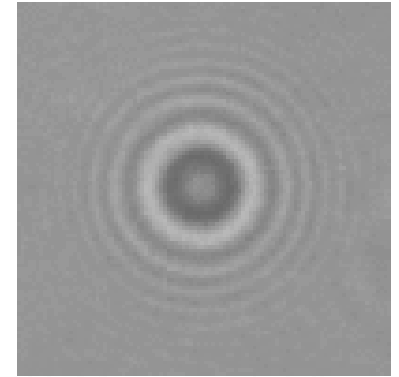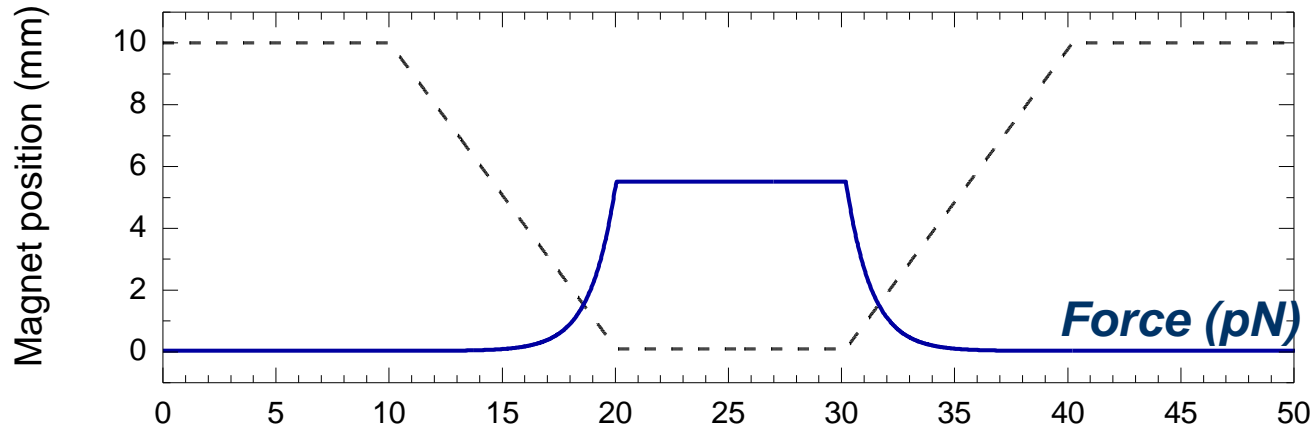- Image processing to recover bead position

force measurements

$$F_{rest} = F_{DNA} \frac{\delta x}{z}$$

$$= \frac{F_{DNA}}{z} \delta x = k \delta x,$$

$$k = \frac{F_{DNA}}{z}$$
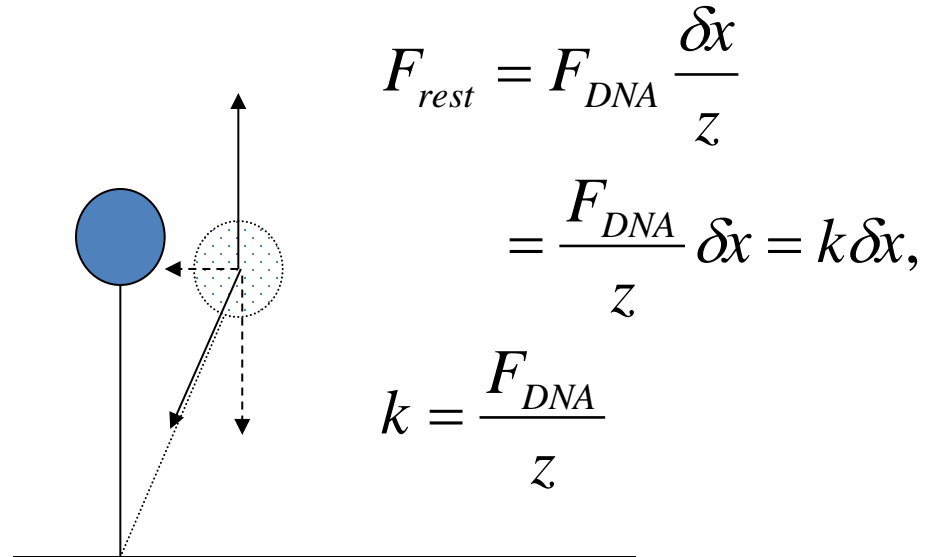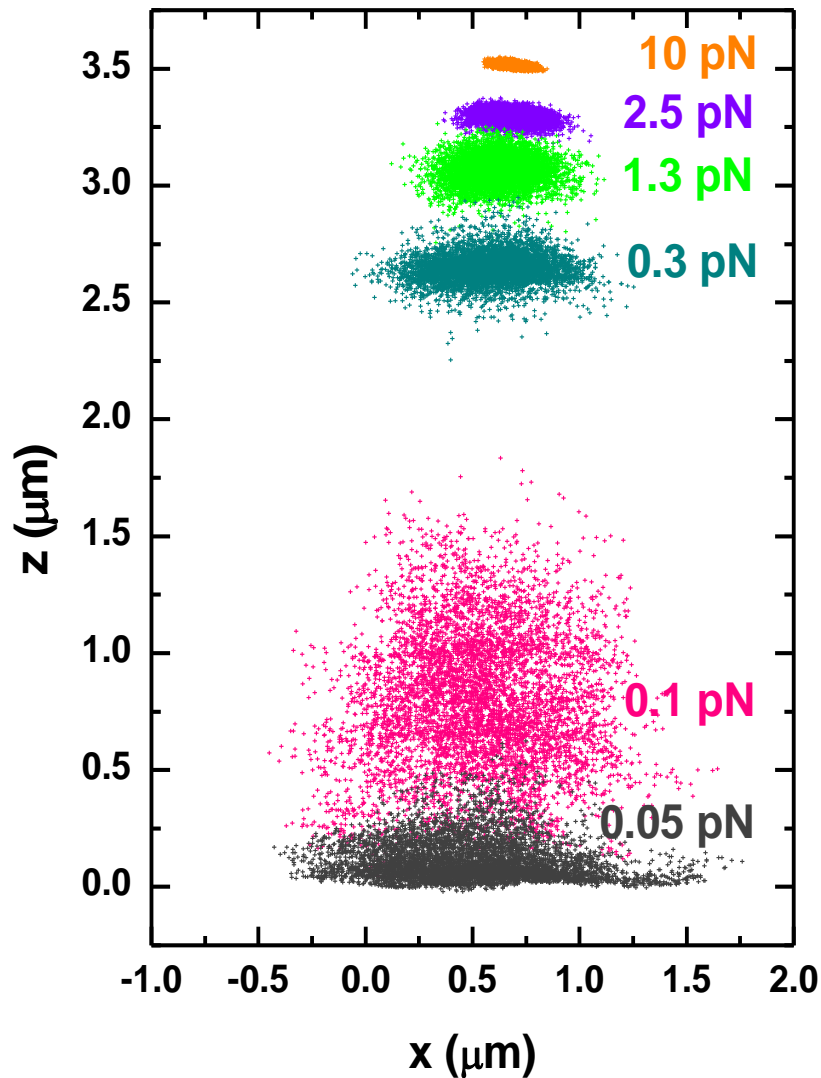
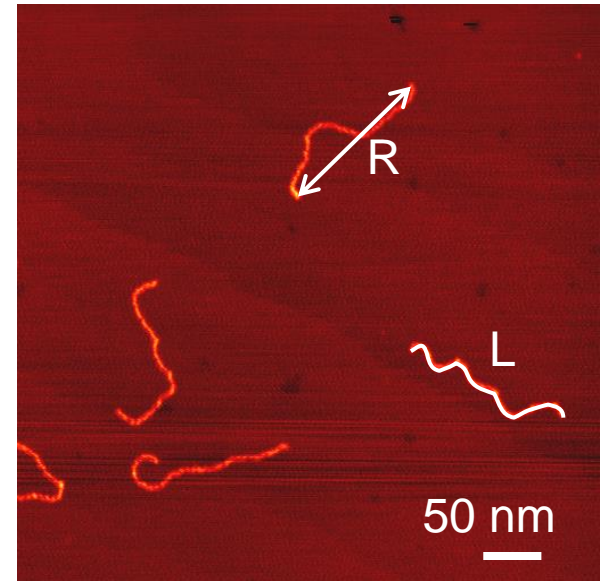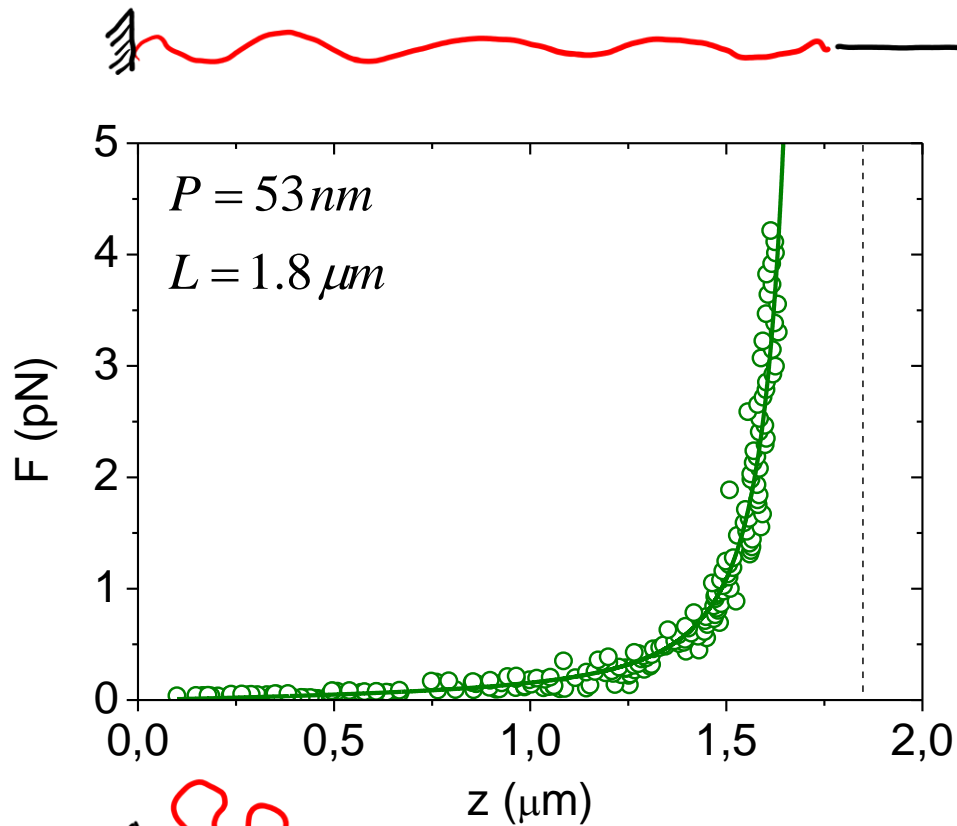- Equipartition theorem:

$$\tfrac{1}{2} k_b T = \tfrac{1}{2} k x^2$$

$$F_{DNA} = \frac{k_b T z}{\delta x^2}$$

Strick et al. 1996 Science

dynamic force spectrocopy

- Magnet is moved down and up
- Image processing to recover bead position

force-extension curve

$P = 53\,nm$

$L = 1.8\,\mu m$

Worm-Like Chain: $F(z) = \dfrac{k_B T}{P}\left[\dfrac{1}{4(1 - z/L)^2} - \dfrac{1}{4} + \dfrac{z}{L}\right]$

Marko and Siggia 1995 Macromolecules

forced unfolding of single nucleosomes

ΔG_1

ΔG_3
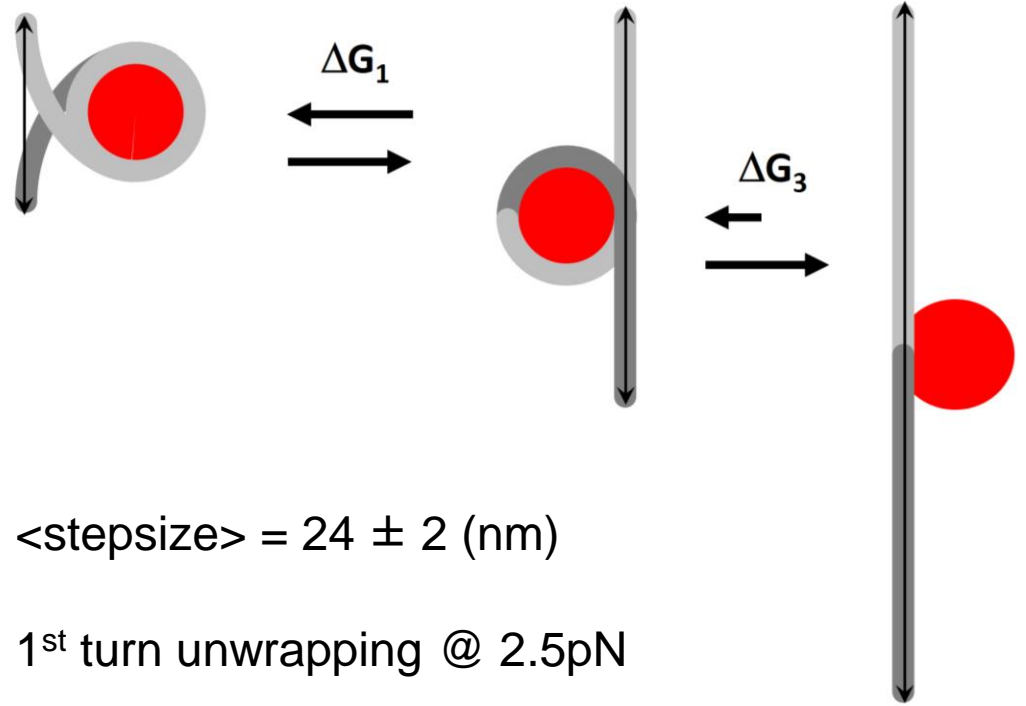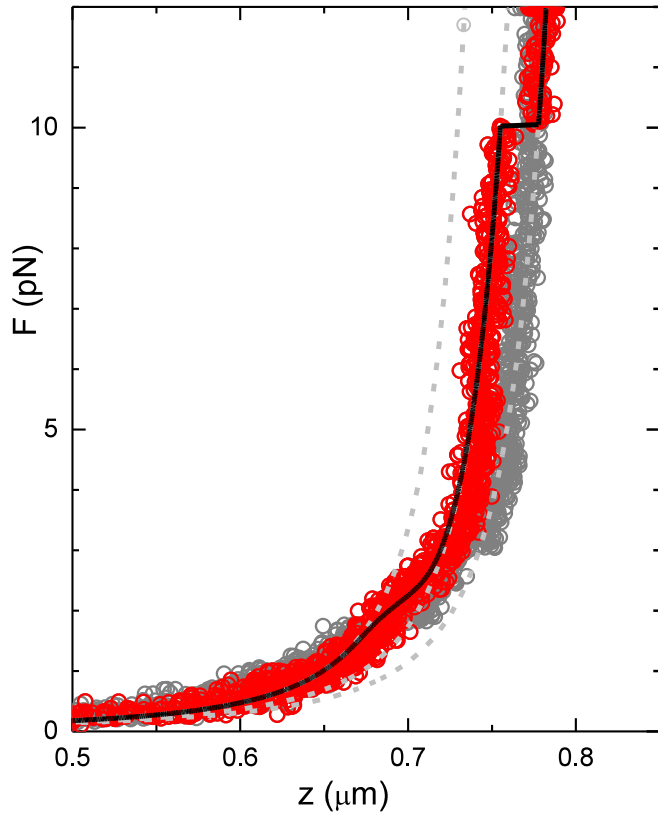
<stepsize> = 24 ± 2 (nm)

1st turn unwrapping @ 2.5pN

Forced nucleosome unwrapping features two transitions
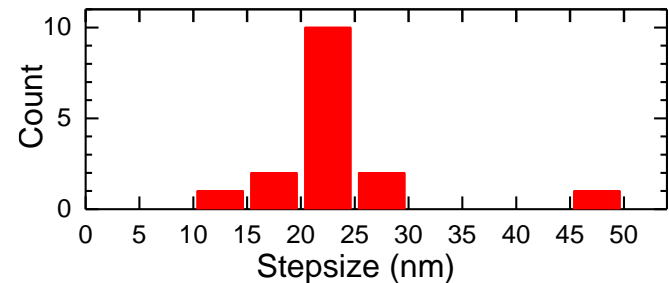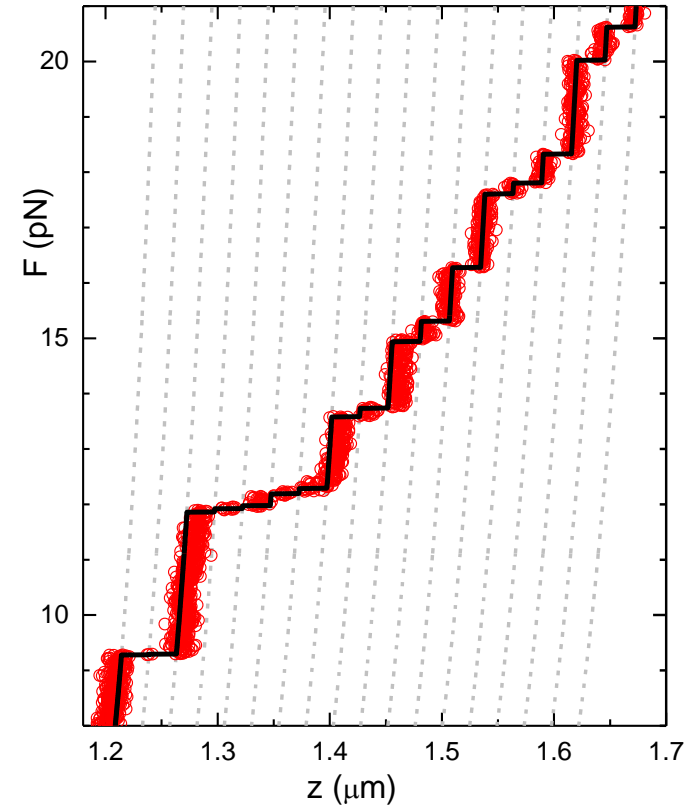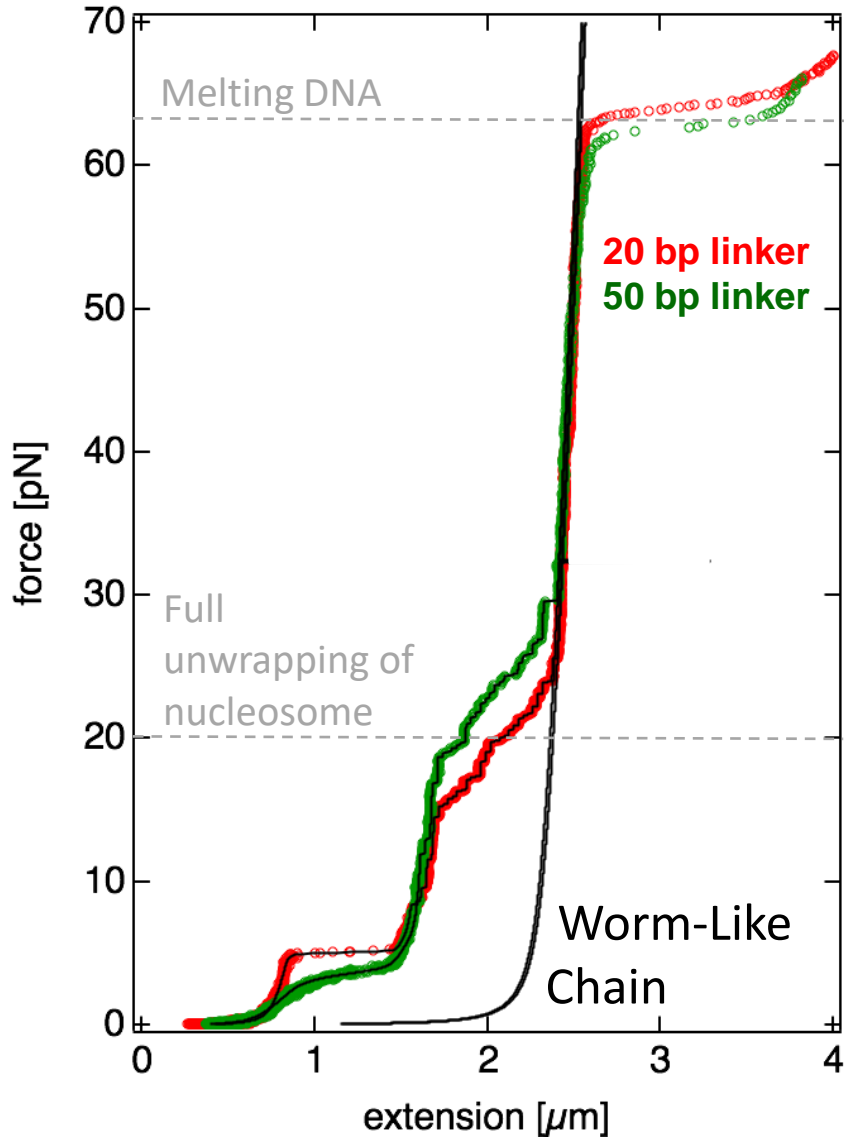
Meng, JvN et al. 2015 NAR

time traces at constant force
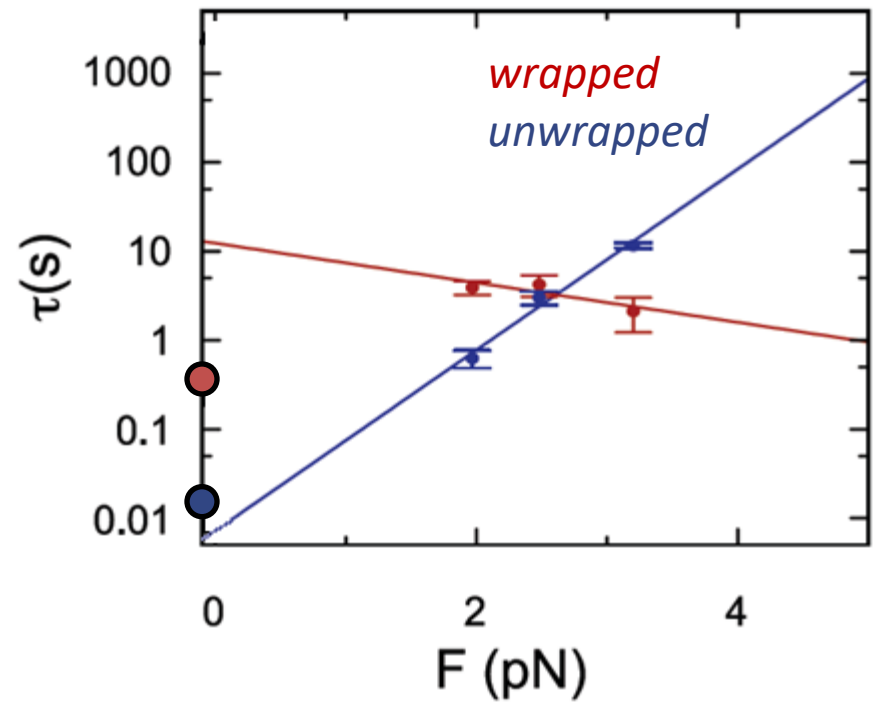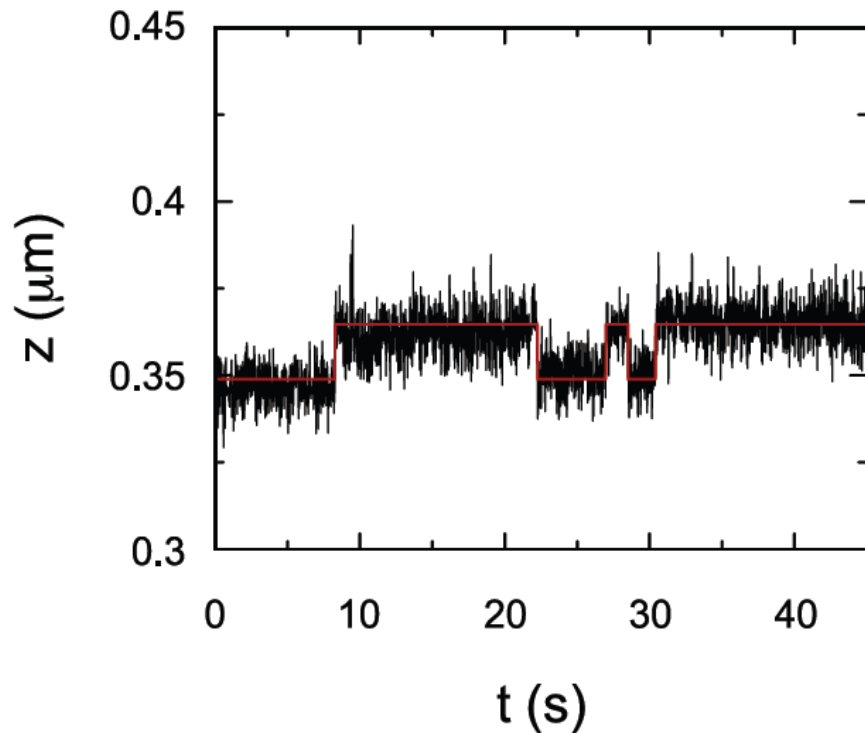
- Lifetimes decay/increase exponentially with force

- Lifetimes do not match spFRET measurements

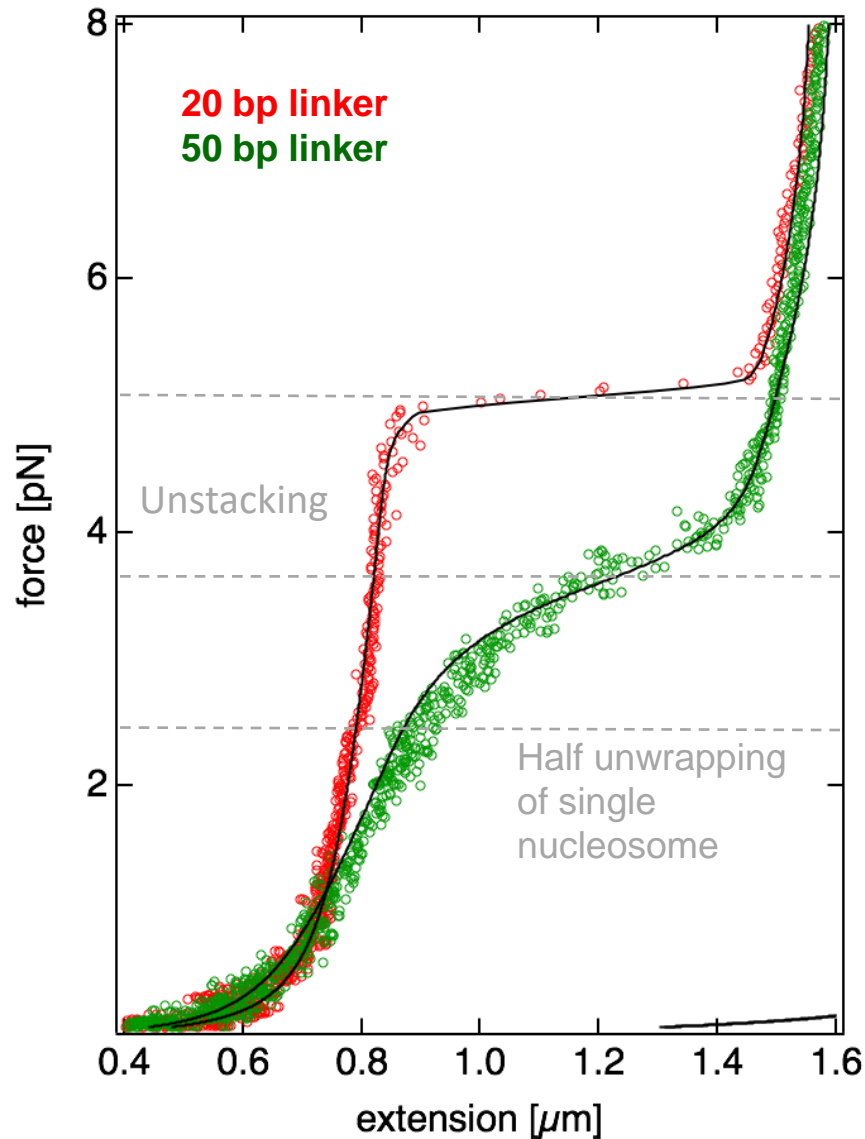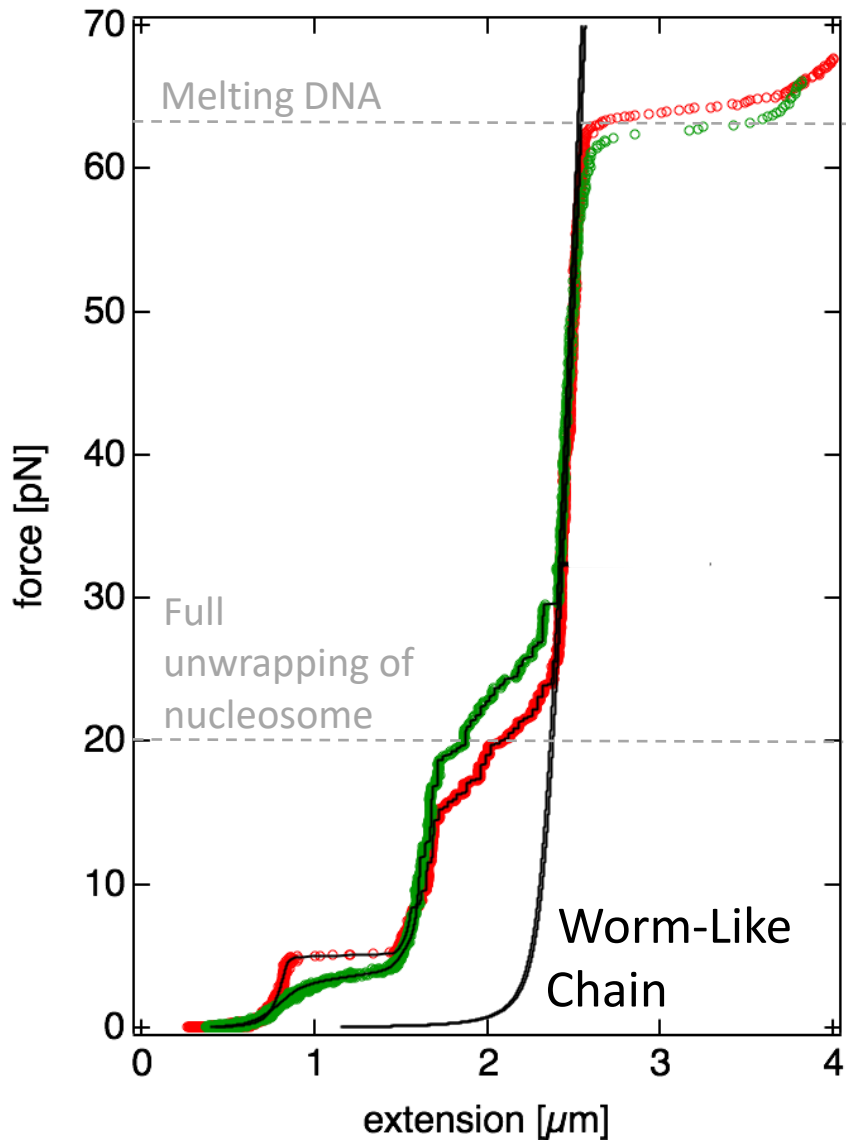# *chromatin unfolding*

100 mM KAc, 2 mM MgAc$_2$, 10 mM Tris.HCl pH 8, 0.1 mg/ml BSA, 0.03 % Nonidet-P40
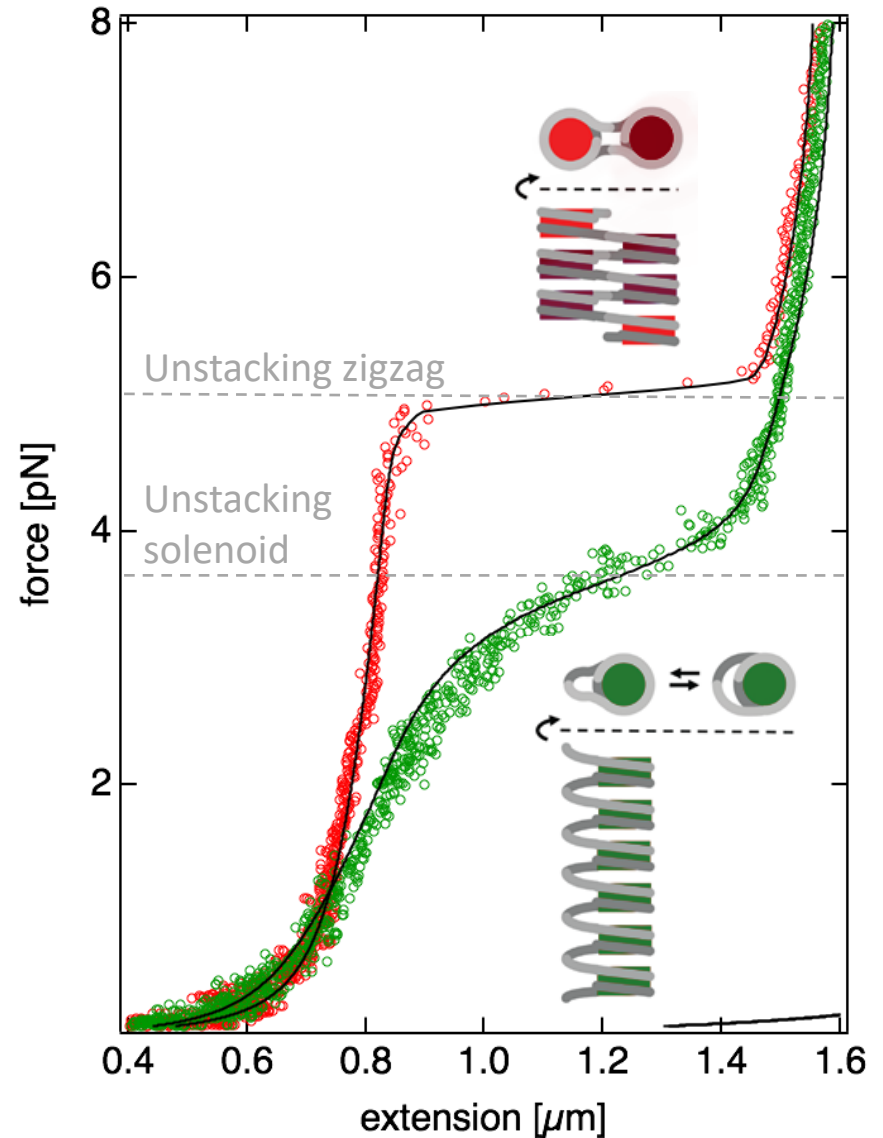
fiber unfolding

100 mM KAc, 2 mM MgAc$_2$, 10 mM Tris.HCl pH 8,
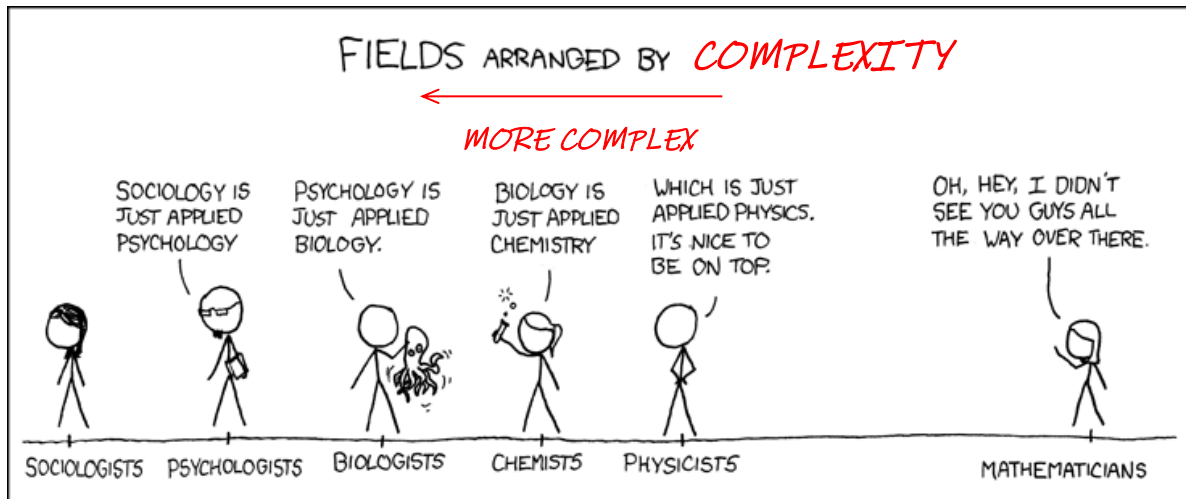0.1 mg/ml BSA, 0.03 % Nonidet-P40

fiber unfolding

NRL (bp)	20 bp	50 bp
Stiffness (pN/nm)	1.1	0.3
ΔG_1 (k$_B$T)	19	17
Cooperativity	yes	no
Z$_{rupture}$(nm)	5	10

197 NRL fibers feature a non-cooperative unfolding mechanism, consistent with a one-start solenoid structure

Artur Kaczmarczyk

intermezzo

order in disciplines?

https://imgs.xkcd.com/comics/purity.png