Chromatin Journal Club presentation, Feb 2007

Effect of Force on Mononucleosomal Dynamics

Mihardja, Shirley et al. (2006) Proc. Natl. Acad. Sci. USA 103, 15871-15876

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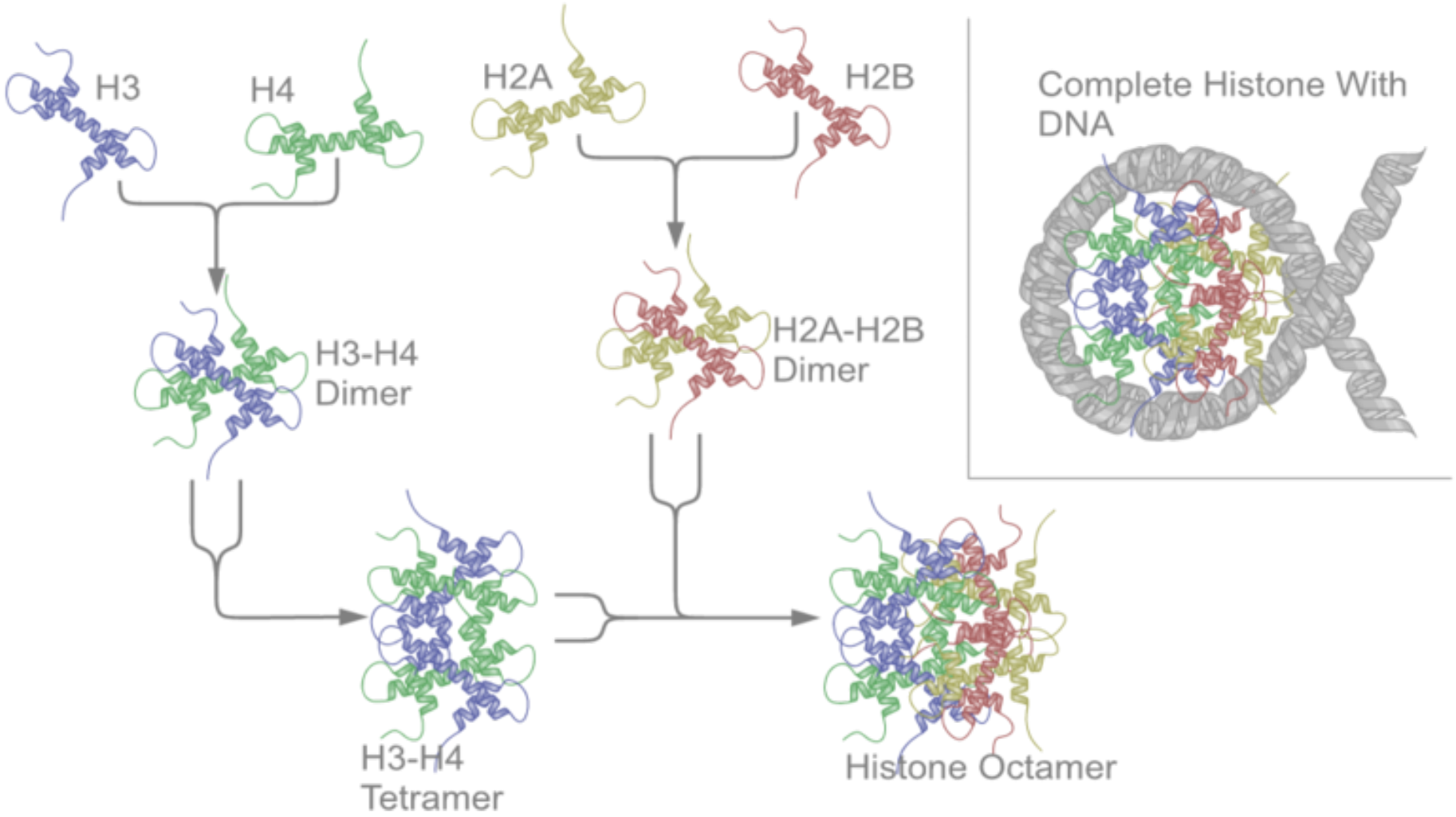
Histones; schematic



http://en.wikipedia.org/wiki/Histone

Histones; the basics

- Histones form dimers: H2A-H2B, H3-H4
- Dimers complex to form octamers
- Basic, water soluble, positively charged
- Histone tails are subject to modifications:
    - Acetylation, associated with expression
    - Phosphorylation, associated with repair
    - Methylation, ubiquitination, sumoylation etc....
- Function:

    - Pack DNA into the nucleus
    - Chromatin regulation & modificaton in various processes (e.g. condensation in mitosis)
    - Histone code?

On histone-DNA interactions

- Basic, positively charged histones act as spools around which negatively charged DNA wraps; 146 base pairs, or ~1.7 times
- Post-translationall modification of histone tails may serve as a “histone code”, further regulating DNA accessibility, expression.
- It's believed that “site exposure” can be mediated by nucleosome dynamics and modifications (Widom et al., 2002)
  - One such example is a “Brownian ratchet mechanism” (Astumian, Bier, 1994); rather than pulling bound DNA from histones, proteins may trap fluctuations already present in the dynamics of the nucelosome to access to DNA.

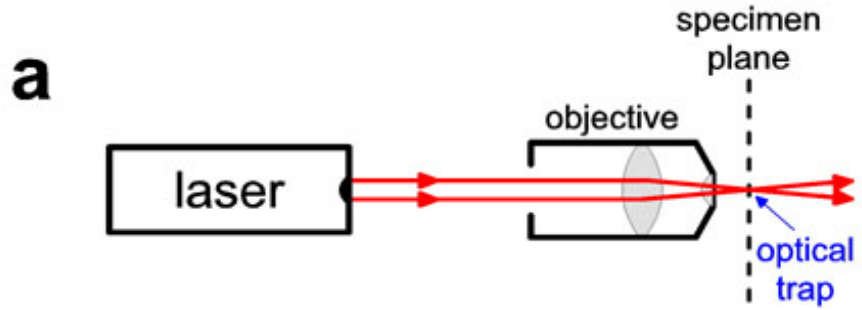
Some thoughts on the subject:

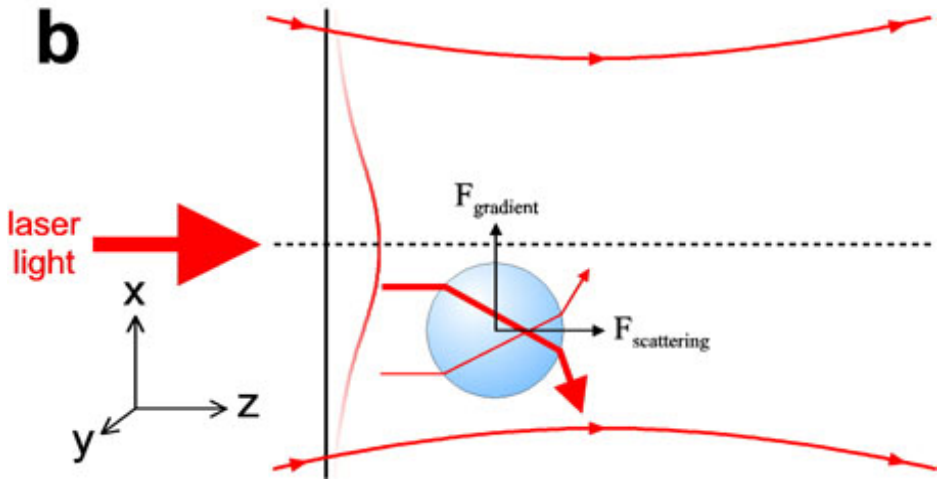
- Why is this interesting?
- Ques:
  - How Can protein expression be controlled by histone activity and ΔG values?
  - Is it reasonable to say that protein expression is controlled largely by free-energy relationships (e.g. Histone tail modifications based on DNA sequence-histone interactions such as sliding, spontaneous wrapping/unwrapping, hopping and other fluctuations?
  - How can DNA be accessed if it is coiled?
- Spontaneous wrapping, unwrapping and sequence-specific affinities, measured in free energy.

Motivation

- Understand mononucleosomal dynamics in real time (at pN resolution)
- Understand how proteins, (RNA polymerase, chromatin remodeling factors, DNA translocases), gain access to occluded DNA, important for chromatin remodeling, gene expression.
- Ques: How does the addition of force affect the dynamics of nucleosomes?
- Extend previous studies that characterized 2-state force model
    - Nucleosome-nucleosome interactions (from nucleosomal arrays) could affect kinetics. Can't study individual nucleosome dynamics

Note on optical trapping technique

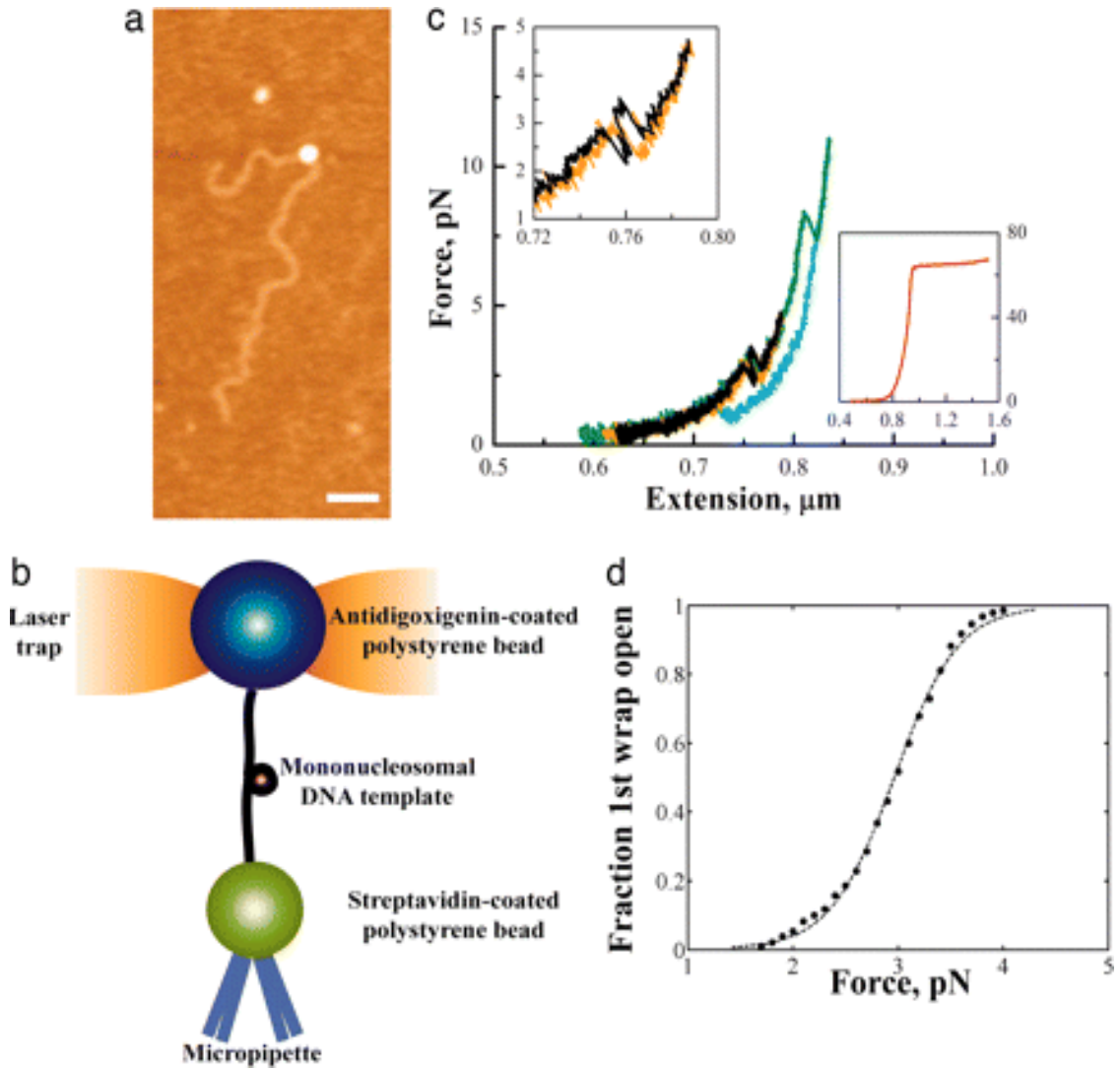




- Trap a particle in 3 dimensions using light.
- Use momentum transfer associated with bending light
- How much the molecule bends “Gaussian” laser is therefore detectable by conservation of momentum, i.e. Equal & opposite reaction

http://www.stanford.edu/group/blocklab/Optical%20Tweezers%20Introduction.htm

Fig 1. Pulling the mononucleosome revealed two distinct rips



- **1a & b.**
  - AFM illustrating proper binding of histone to 3547-bp piece of DNA
  - Mononucleosome is tethered between 2 beads, one is in the laser trap, the other held by micropipette
  - Bead tension is controlled by computer & held constant

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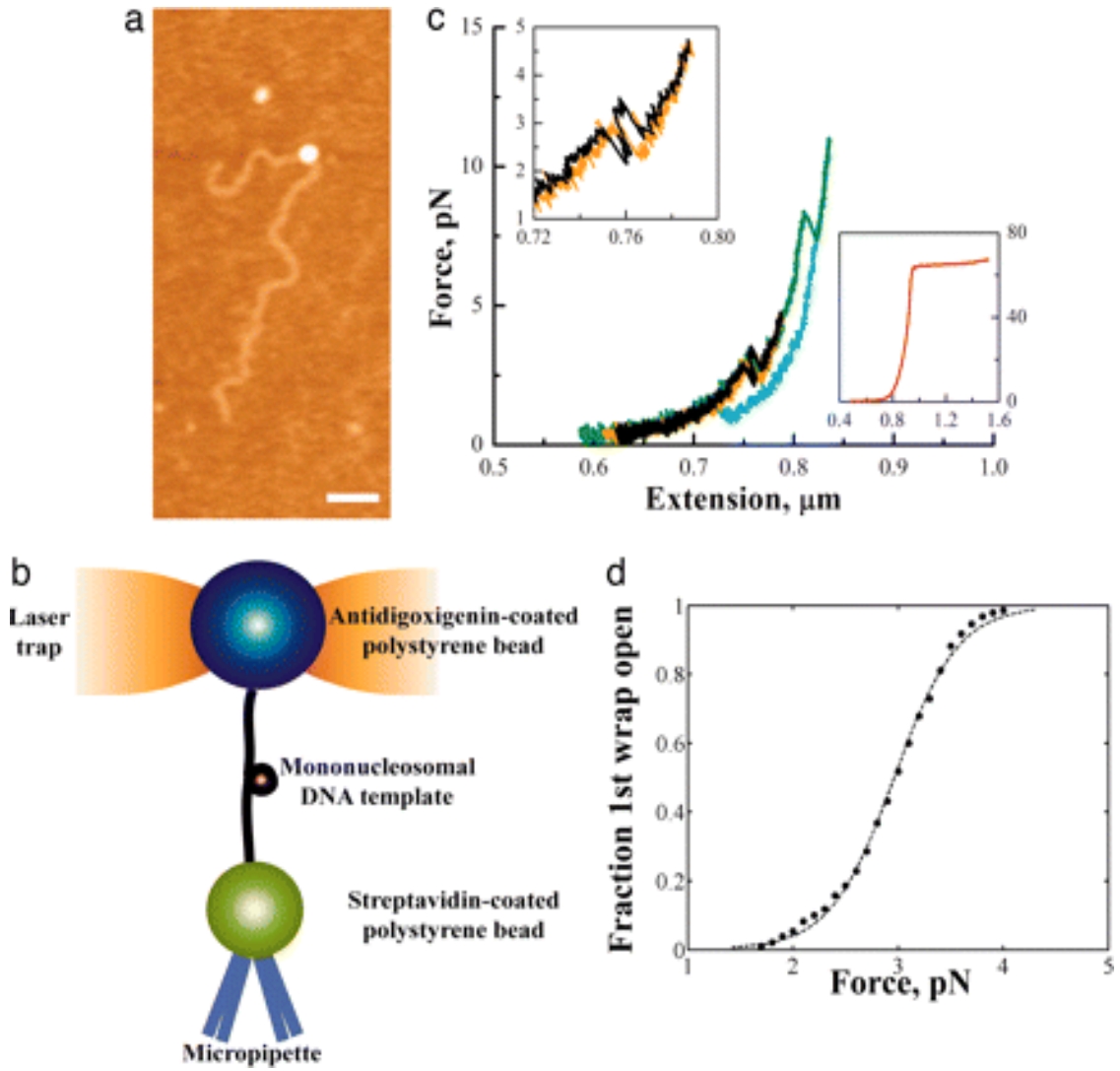

- **1c.**
  - 2 distinct states emerge; low and high-force transitions
  - Hops are evidenced; represent open and closed states
  - Control kinetics by adding tension using a force-feedback algorithm
  - Determine Keq by ratio of low force open:closed states

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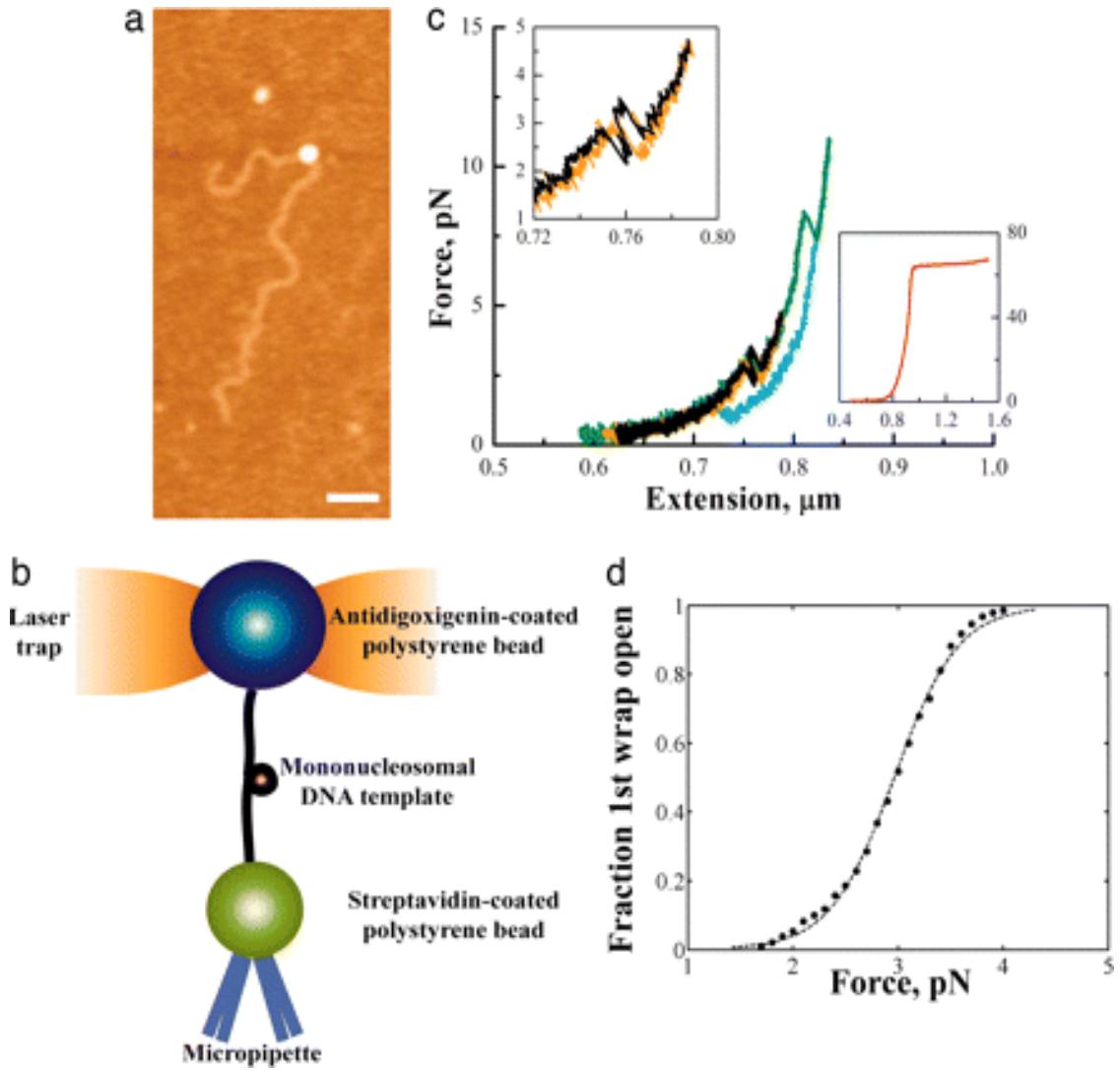
Fig 1. Pulling the mononucleosome revealed two distinct rips



- **1d.**
  - Fraction of unwrapping as related to force during the low force transition
  - Note that by 4 pN all are unwrapped
  - Each point represents a unique experiment

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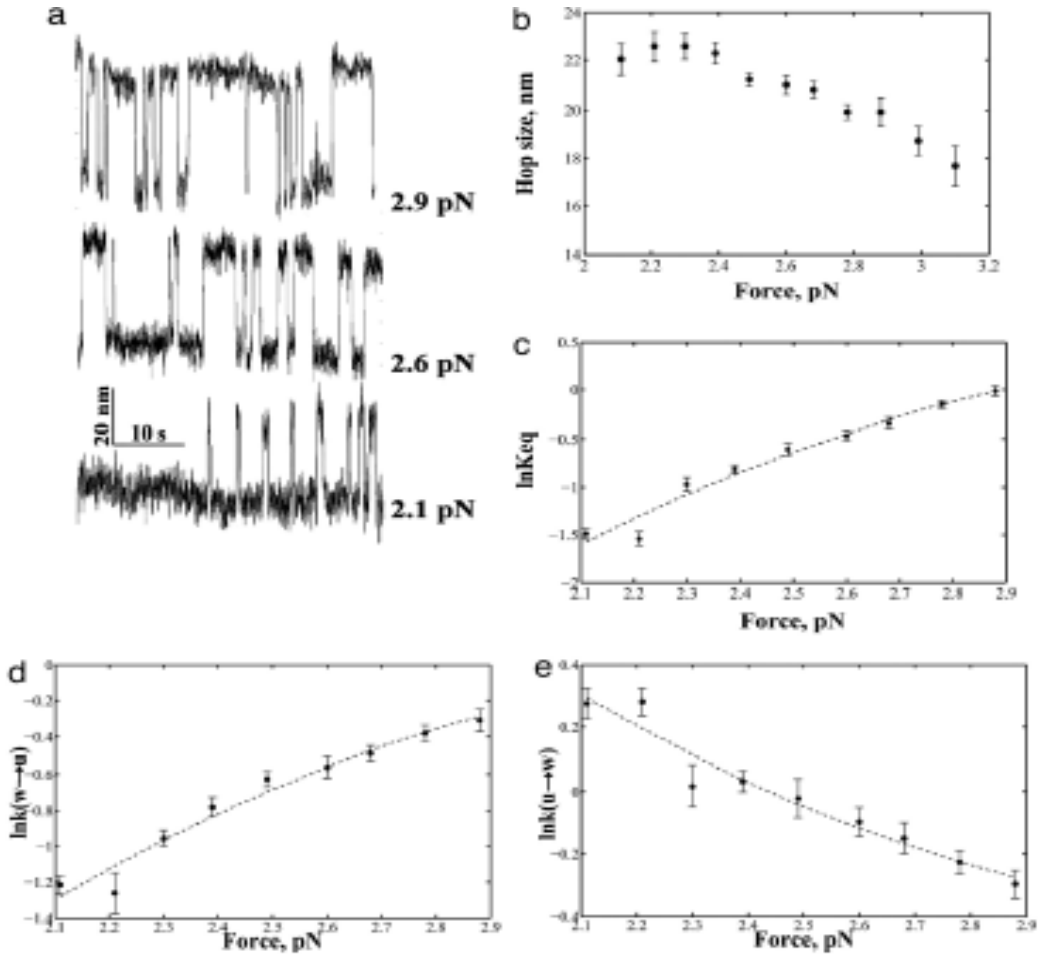
Fig 2. two state hopping of the low-force transition



- 2a.
  - Elaboration of 1d.
  - Brownian noise is the flux
  - Two fractions indicated: that of mostly unraveling (top) or mostly not-unraveling (bottom) as related to force applied
  - 2.1pN is less likely to unravel than is 2.9pN
  - This correlates with ΔG values for a reaction's favorability

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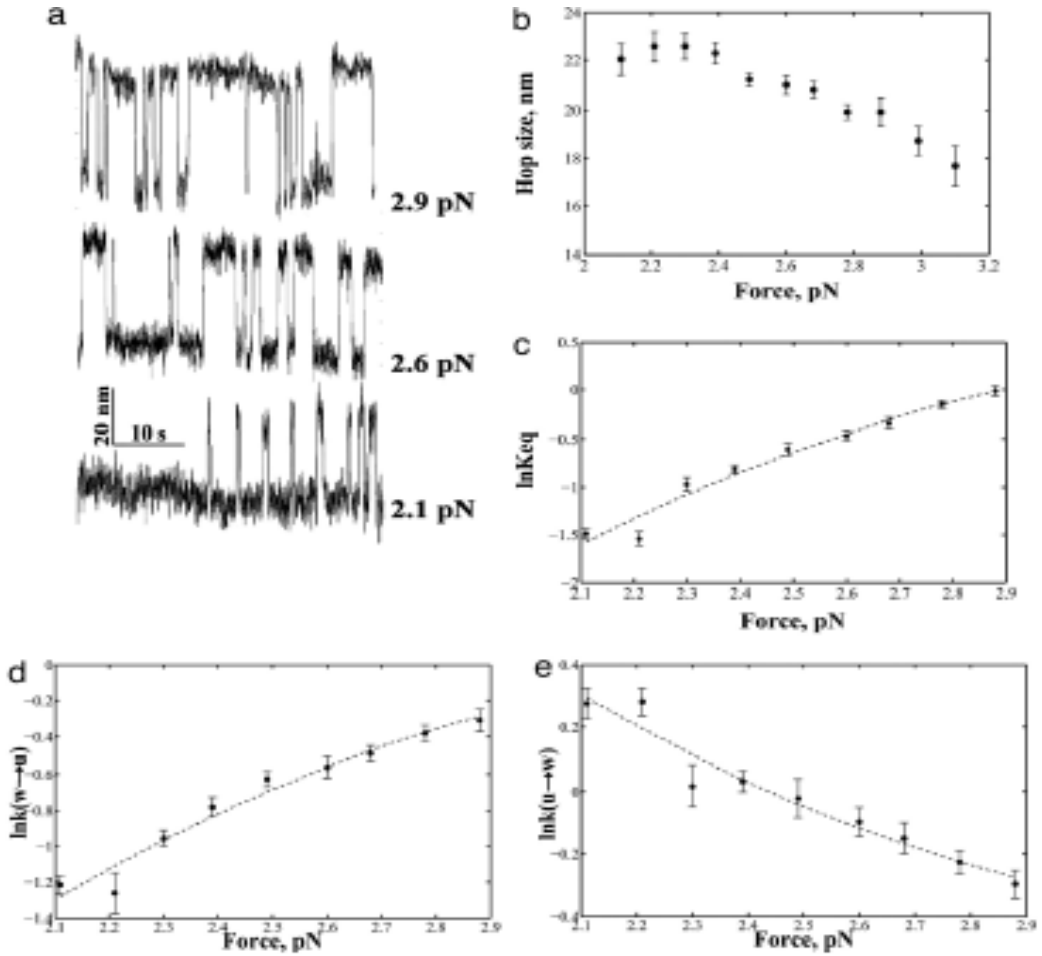

- 2b.
  - Greater force means a smaller hop size, (nm) in the low force transition (1st unwrap)
  - Question: What is hop size actually representing?

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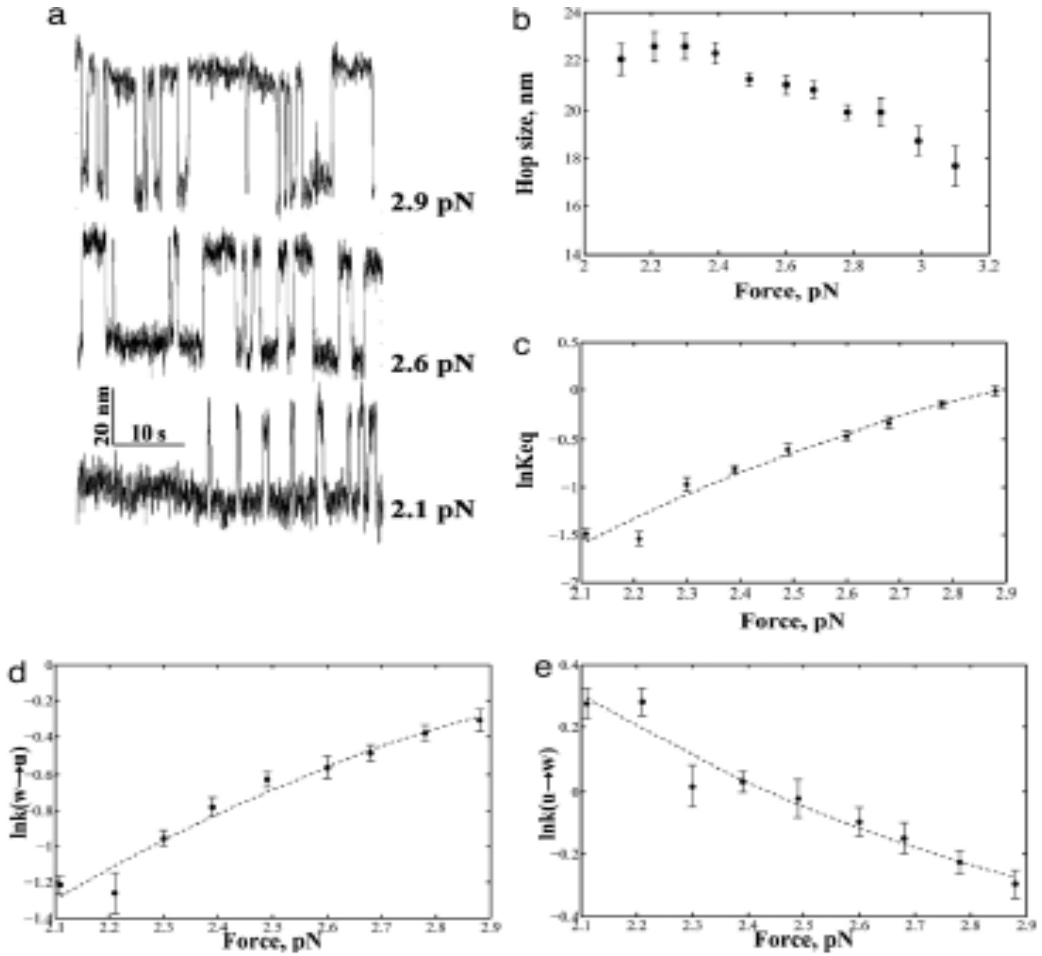

- 2c.
  - Log transform of Keq
  - Negative value = bound
  - Positive value = unbound
  - Similar point to 1d.; unwrapping frequency increases with force
  - Question: why do a log transform?

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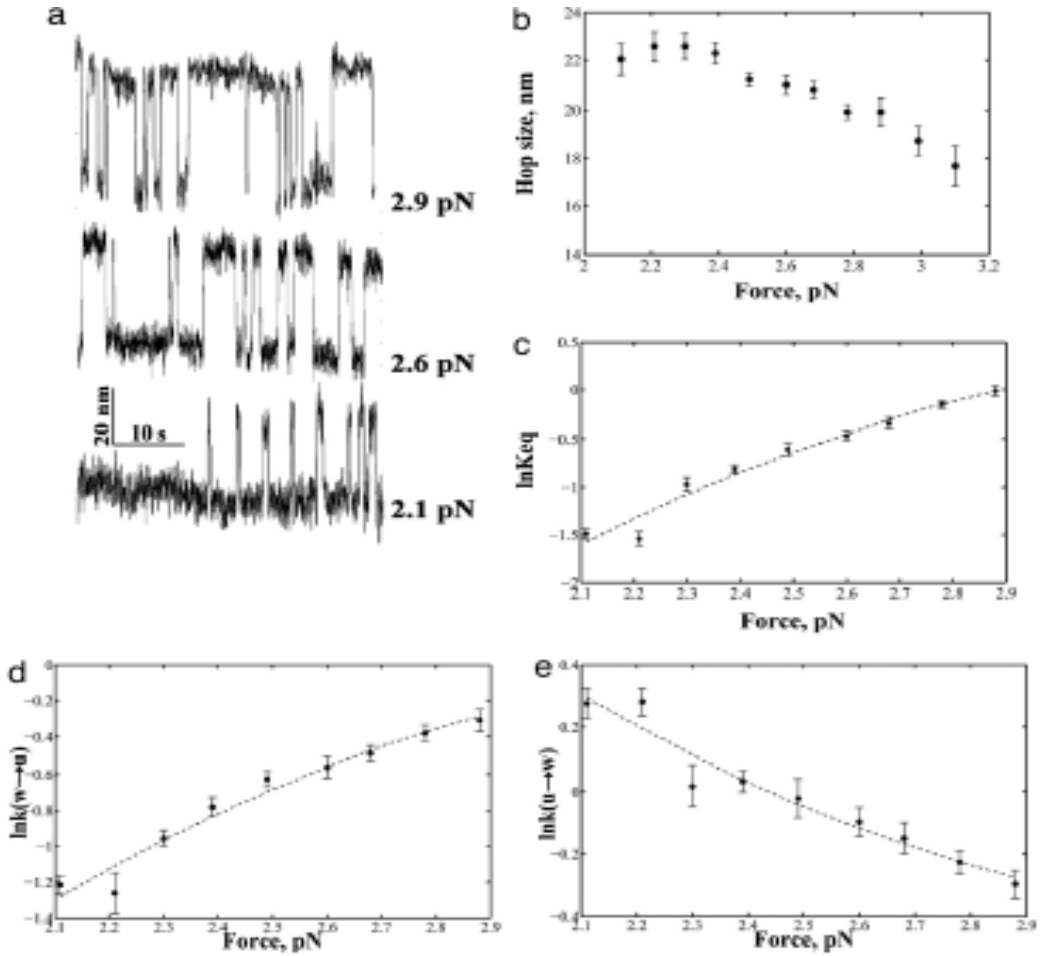

- 2d.
  - Shows the rate of transition from wrapped to unwrapped state
  - Ques: why end at 2.9 pN?

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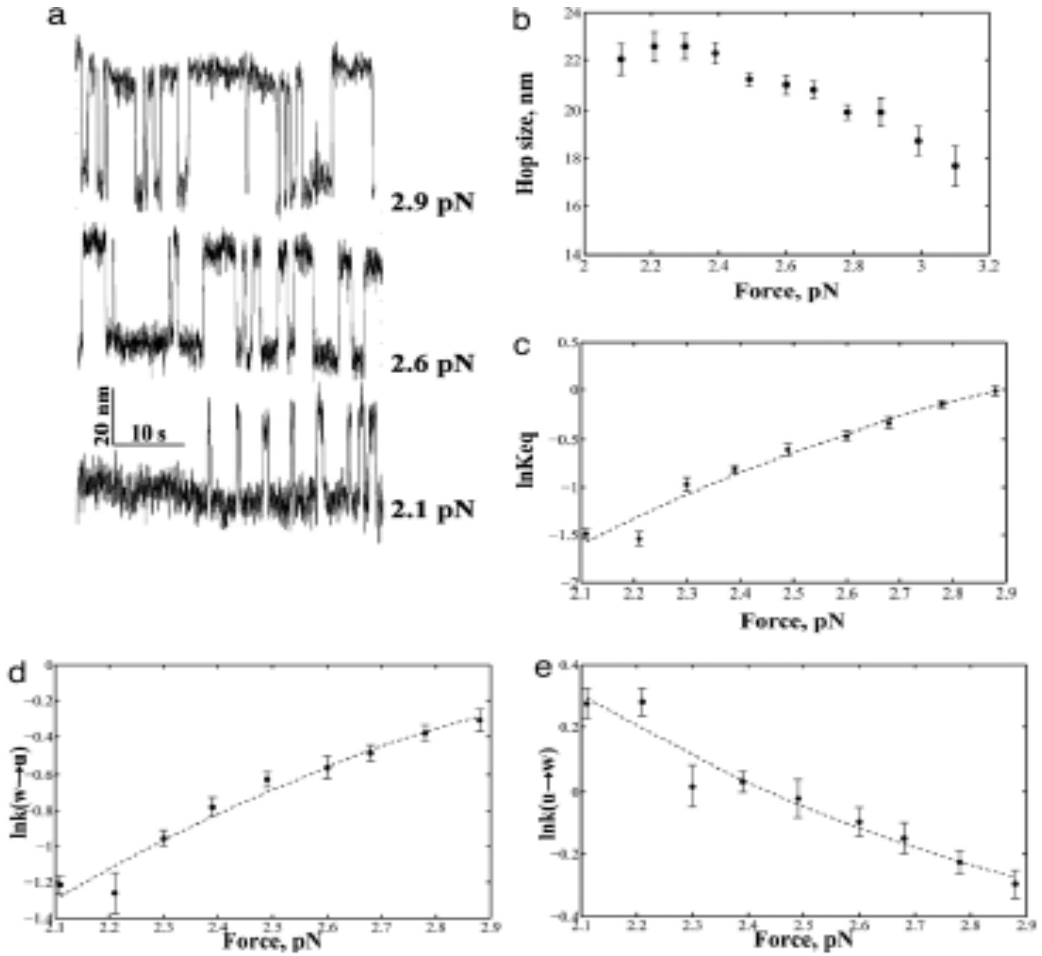
Fig 2. two state hopping of the low-force transition



- 2e.
  - Shows the rate of transition from unwrapped to wrapped state

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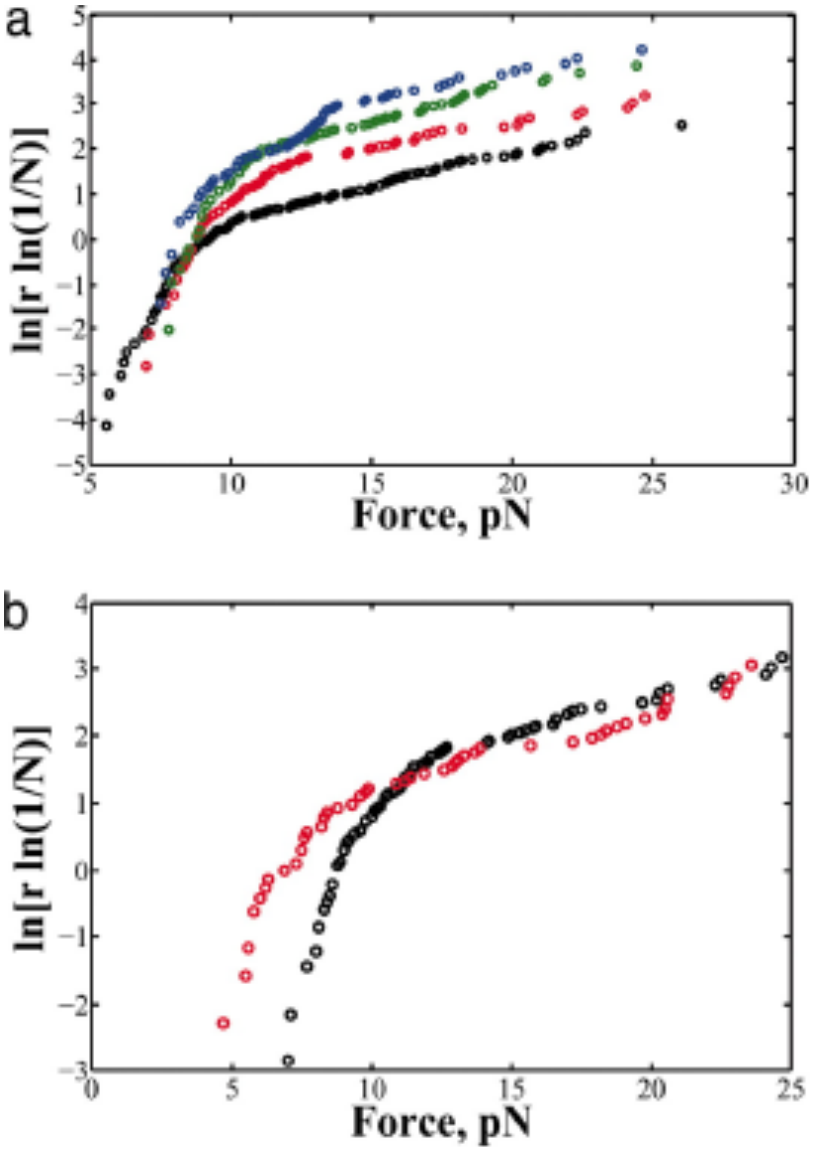
Fig 3. Behavior of the high-force transition



- 3a.
  - Shows the high-force transition (inner DNA wrap) unraveling at varied loading rates
    - blue represents a high load rate.
    - Black represents a low load rate

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Fig 4. Effect of high salt on the mononucleosome

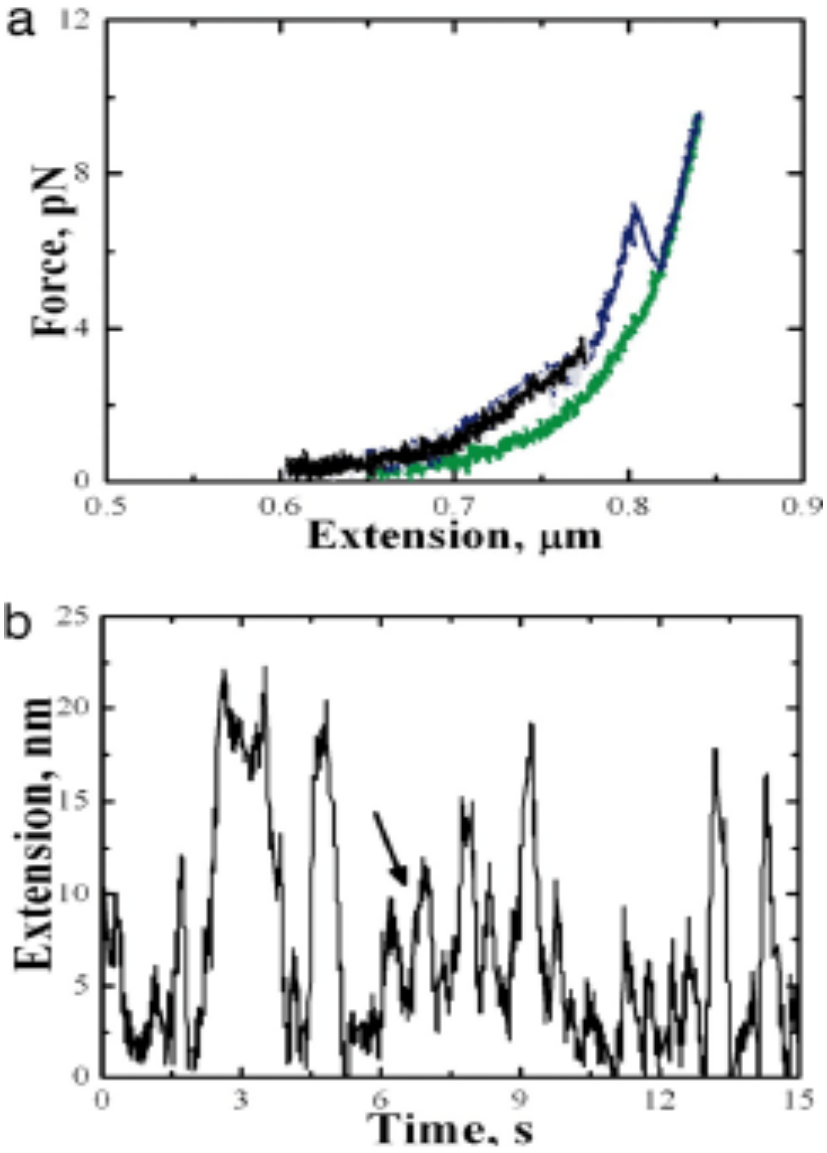

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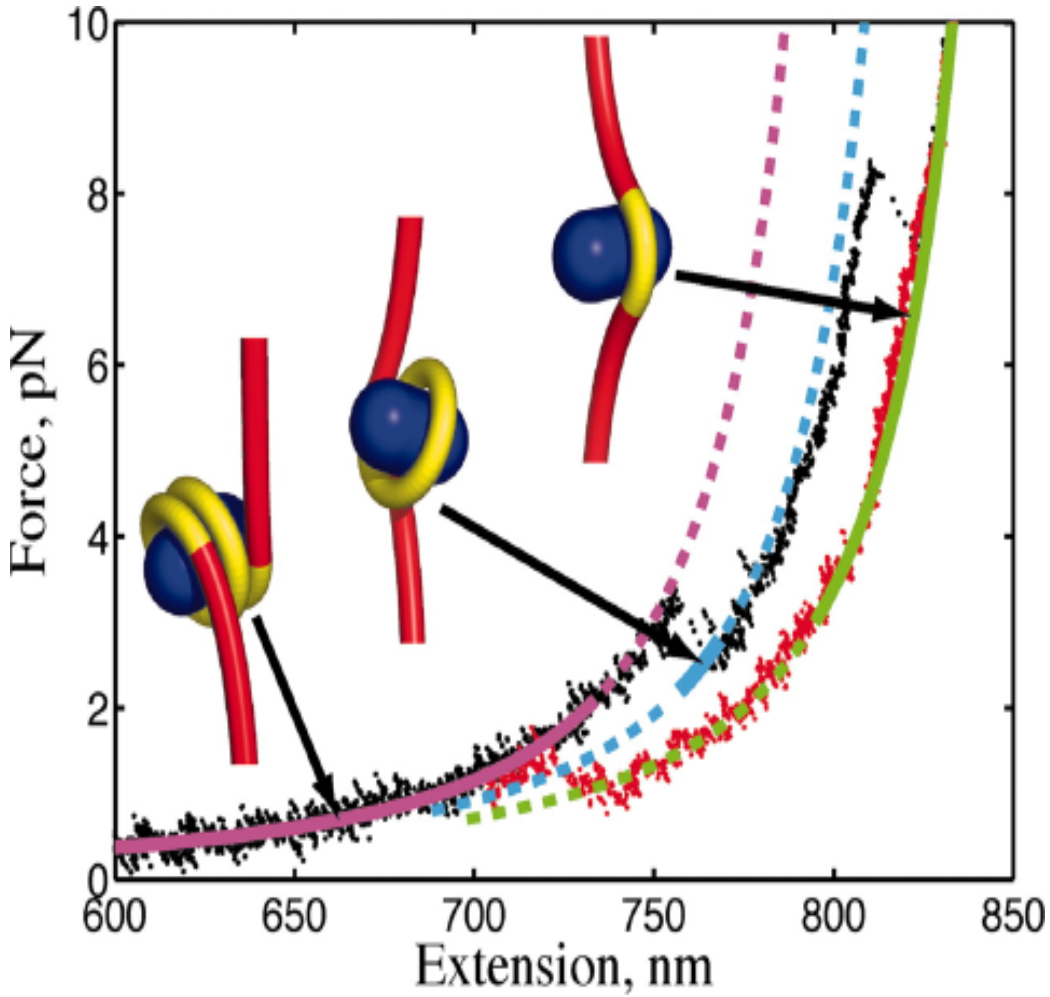
Fig. 5. Force-extension curve of a spool model for the nucleosome



- Less extension requires greater force

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Unraveling stages and forces

- Note; {-ΔG = unwrapping favorability}
- First transition: -ΔG = 3 pN
- 2^{nd} transition: -ΔG = ~8-9 pN
- Low force rip = 21nm; {stochastic process}
- High-force rip = 22nm
- Each rip corresponds to one DNA wrap around histone octamer
- Histone octamers can reform one DNA wrap at a time

The low-force transition

- Their Keq value (~6.6 x 10^-6) is comparable to values obtained by enzyme accessibility assays

Effect of high salt concentration on mononucleosome dynamics

- Mainly electrostatic interactions hold DNA and histone octamer together
- By increasing buffer's ionic strength they lower electrostatic interactions – alter behavior
- Test buffer of double the ionic strength of previous experiment
  - The high & low transitions are still observable
- Low force transition is still reversible for < 3 pN
- No longer a simple two state process
- Conclude that 1st wrap is sensitive to ionic conditions

Effect of high salt concentration on mononucleosome dynamics

- Increased ionic conditions shield the histone's positive charge, thus lessening affinity for DNA
- During transcriptional activation histones associated with particular genes become acetylated, dampening positive charges (Wolffe, 1999; vanHolde, 1989)

Theoretical model

- Attempt an independent theoretical model to explain biological data: validity by matching biological data

Questions, comments, ideas

- Does this correlate to larger scale dynamics; e.g. Energetics associated with accessing a whole gene?
- Site-specific affinity for DNA to octamer