Single-Molecule Manipulation Experiments of Biological Molecules III: Applications to Biological Systems

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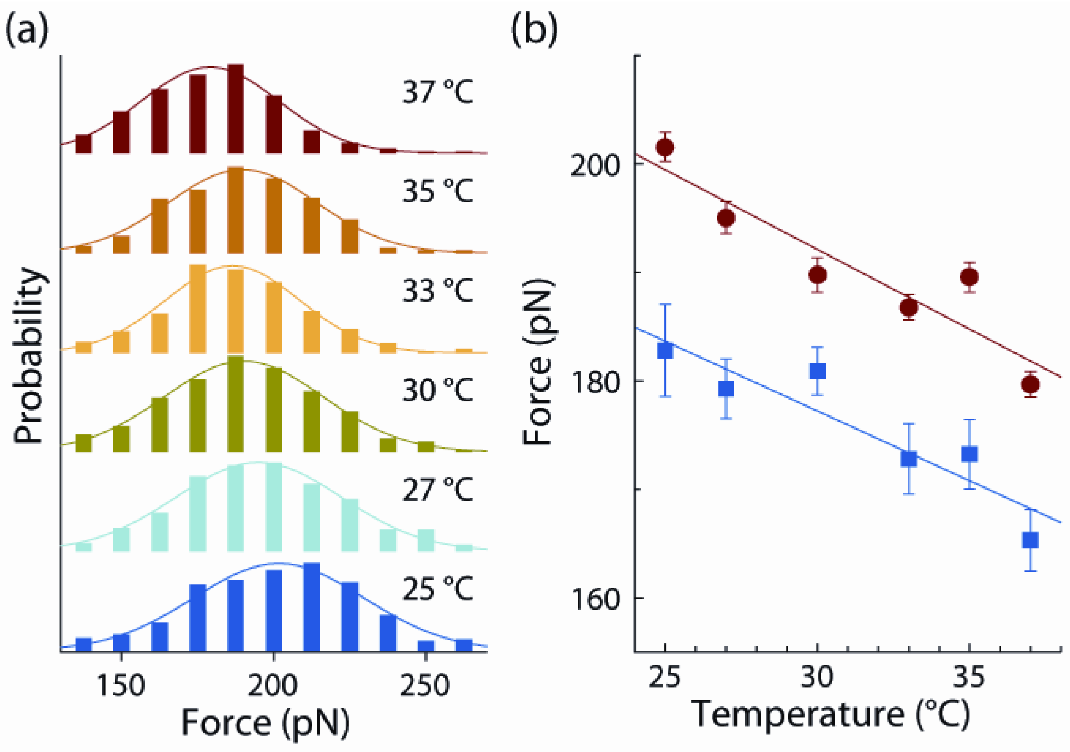
Temperature Dependence of Free Energies



- Titin free energies depends on temperature and denaturant concentration linearly, similar to bulk chemical unfolding.
- Trends consistent for different pulling velocities.

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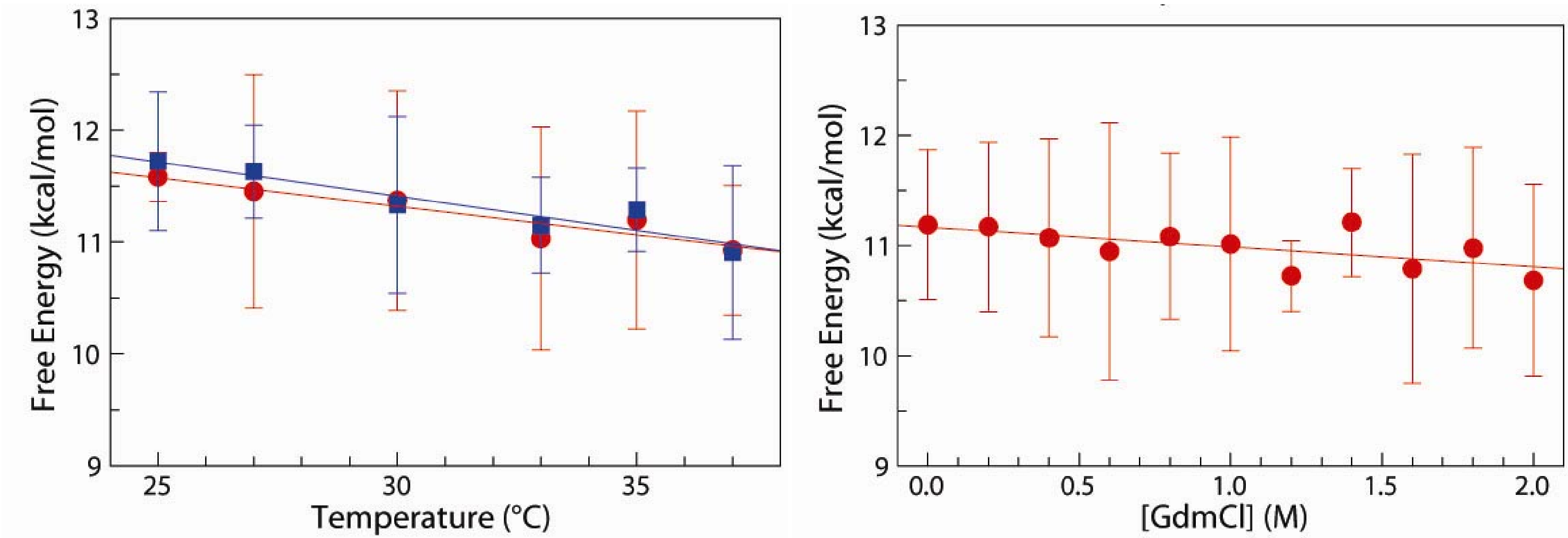
Temperature and Chemical Denaturant Dependent of Unfolding Free Energy Barrier of Titin I27



- Trends are consistent with bulk chemical denaturant experiments. $\Delta H^{\ddagger}$=29 kcal/mol, $\Delta S^{\ddagger}$=0.06 kcal/mol-T.

*Botello, Harris, Sargent, Chen, Lin, and Kiang, **J. Phys. Chem. B** (2009) in press.*

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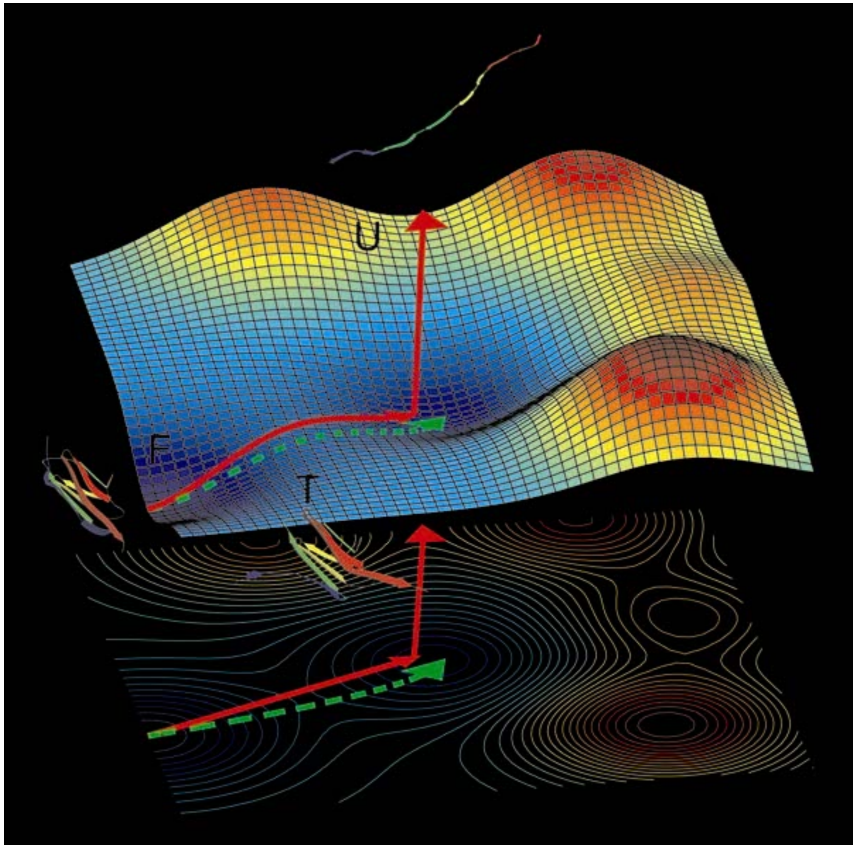
Is End-To-End Distance a Good Reaction Coordinate?



Botello, Harris, Sargent, Chen, Lin, and Kiang, ***J. Phys. Chem. B*** *(2009) in press.*

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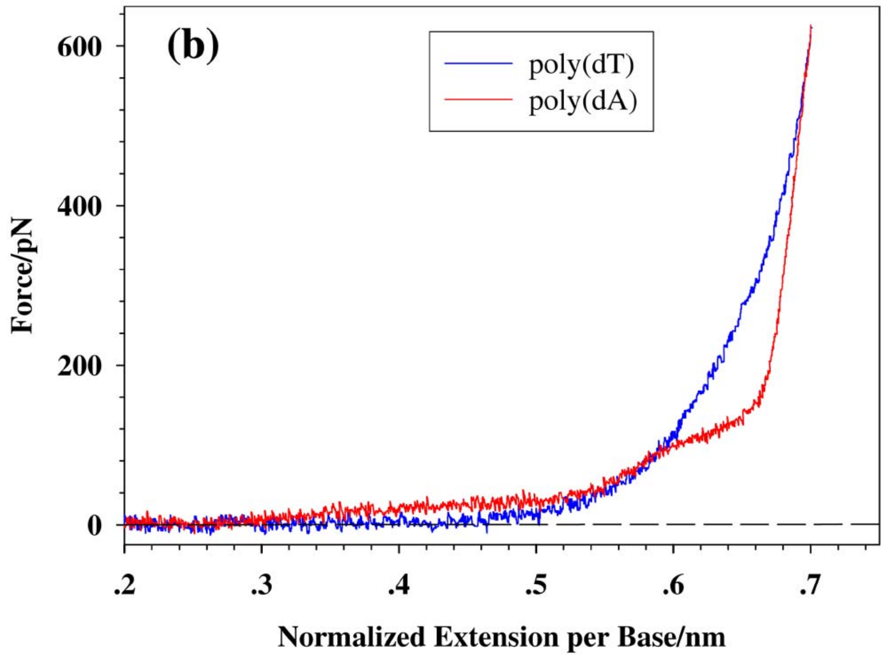
Applications

- Double-stranded DNA melting and overstretching
- Single-stranded DNA unstacking
- RNA folding kinetics
- Protein folding – nanospring
- Protein-protein interactions
- Protein-nucleic acid interactions
- Genome packaging

DNA Melting and Overstretching Transitions

- Conformational and energetic changes of stretched DNA are relevant to biological functions
- During processes such as replication, transcription, and repair, ssDNA is stretched and stabilized by coupling with proteins to serve as an intermediate state
- Ribonucleoprotein complexes in virus (Influenza A, H1N1 virus that causes the “Swine Flu”)
- Implication in microarray analysis
- Base stacking energetics without interacting molecules or melting
- PolydA has two transitions during overstretching transitions
- Elasticity of overstretched ssDNA

PolydA Exhibits Unique Stacking Behavior



Ke *et. al.*, **Phys. Rev. Lett**., 99 (2007) 018302.

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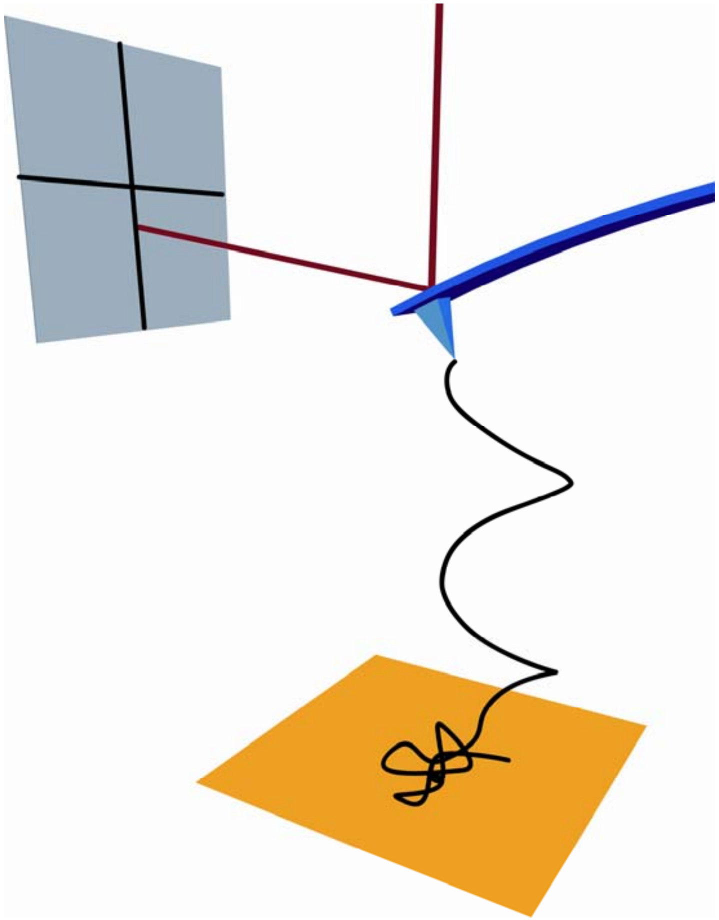
Pulling Single-Molecules Using Atomic Force Microscopy

- Nanobiology approach to probe biomolecular interactions
- Manipulation and measurements at the single-molecule level
- The end-to-end distance (z) and the force (f) on the molecule were measured



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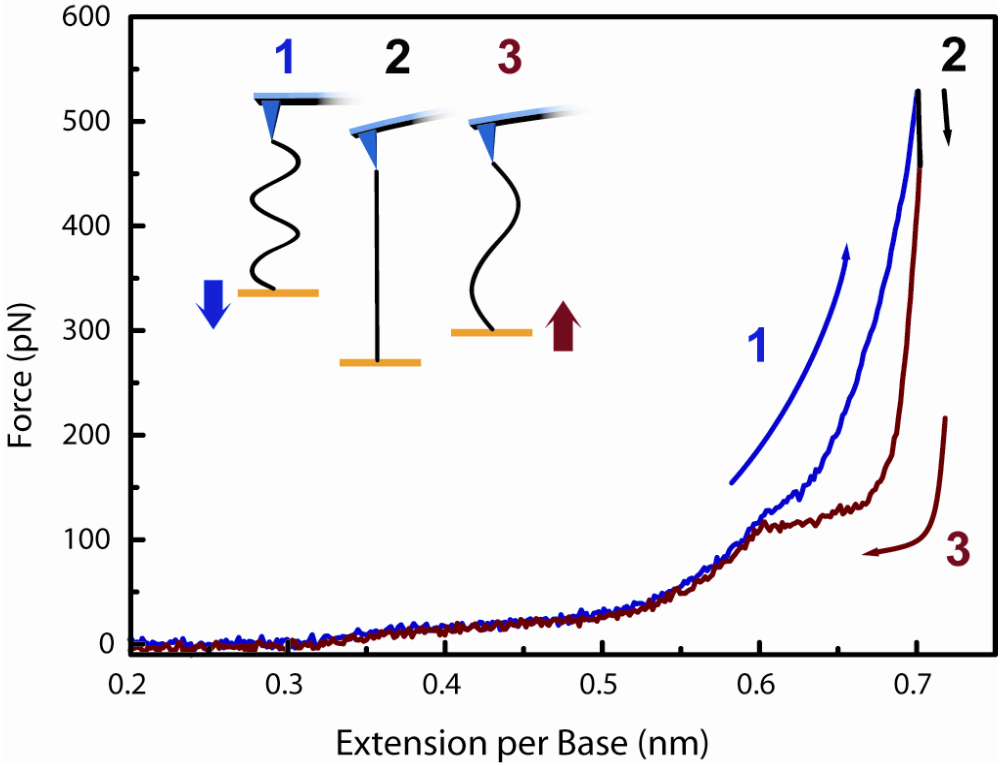
Stacking and Unstacking



Forward and reverse takes different pathways

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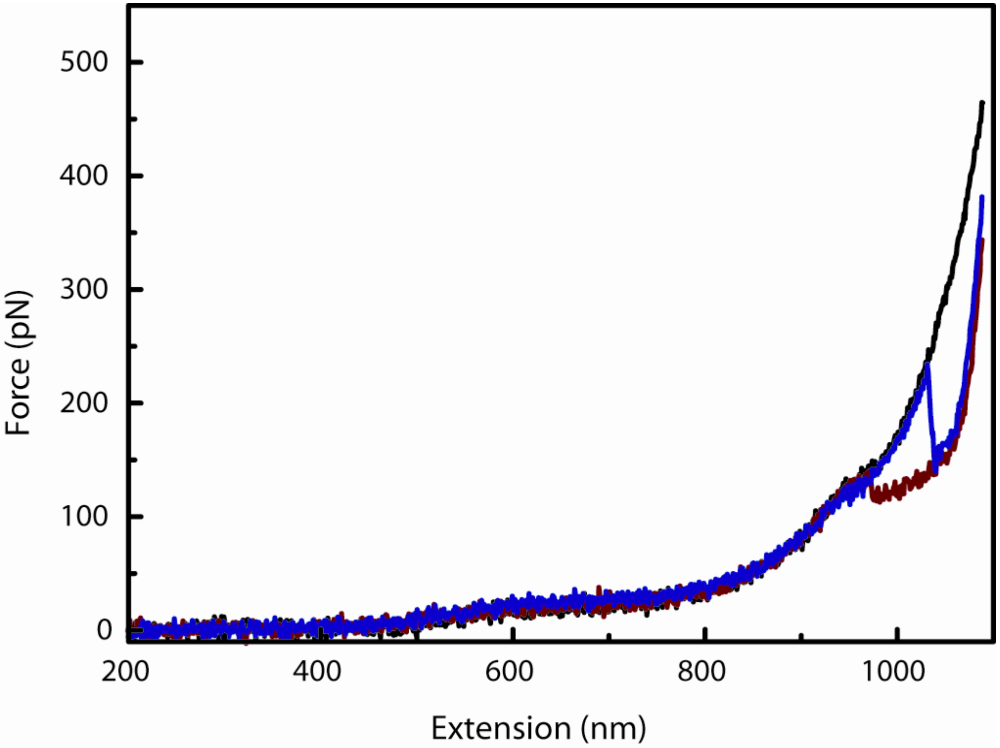
Multiple Pathways of PolydA Unstacking



Occasional hopping between two pathways

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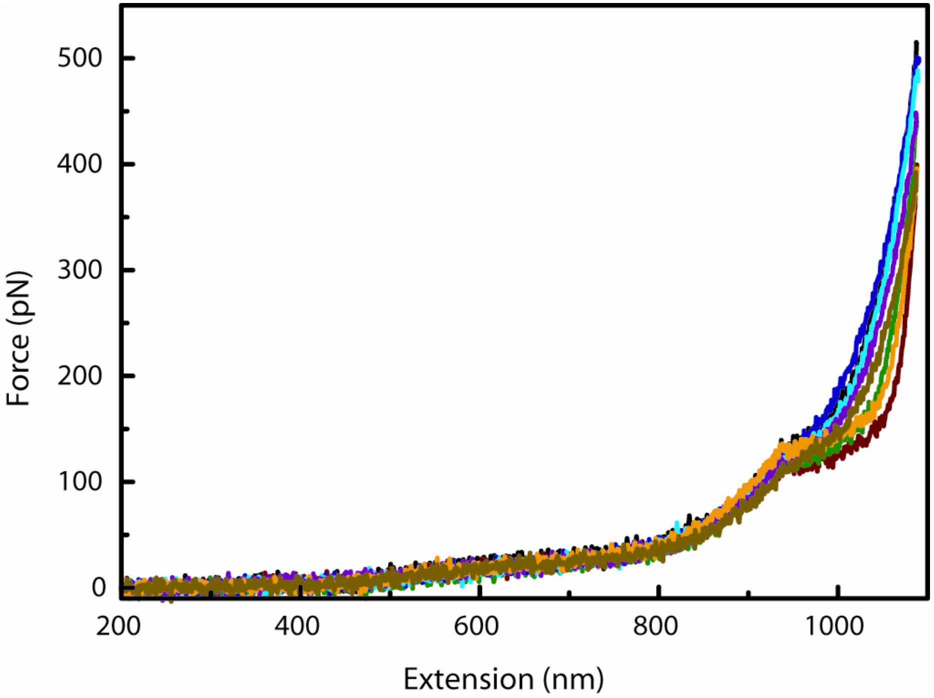
Multiple Stacking Pathways



Different persistence length

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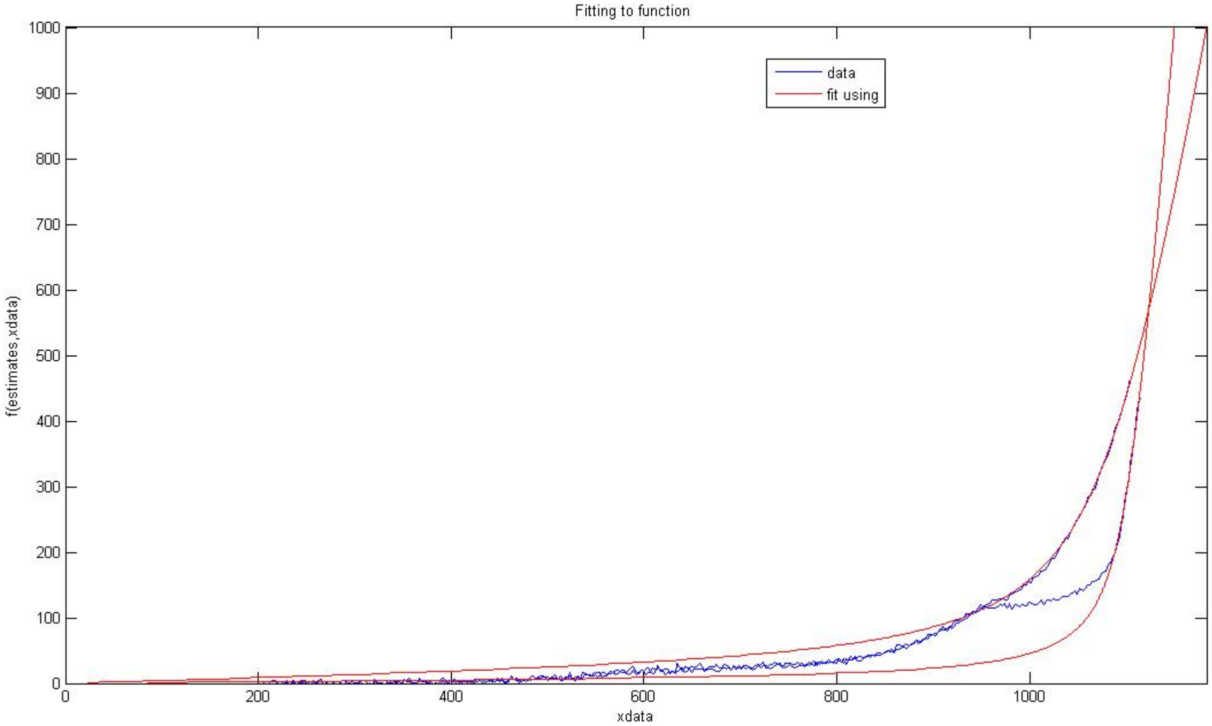
Two Pathways Intersect

- The two pathways intersects at 600 pN, and the high energy pathway becomes the low energy pathway.



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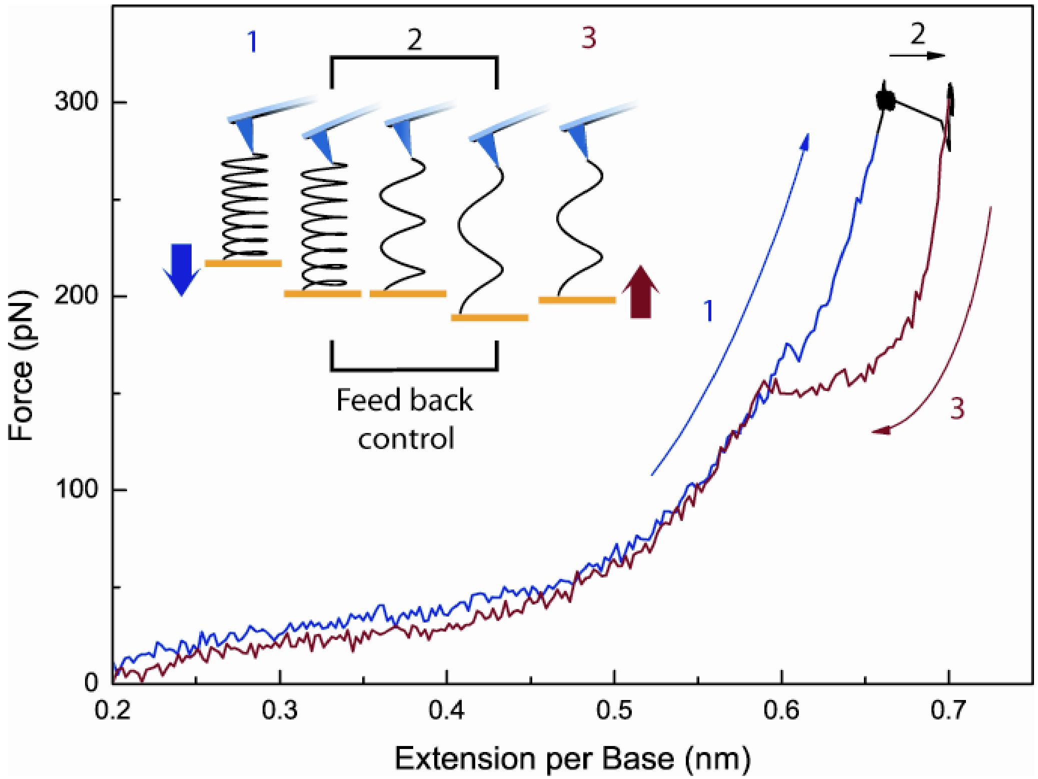
Constant Force Measurements



ssDNA was pulled to 300 pN and kept at constant force

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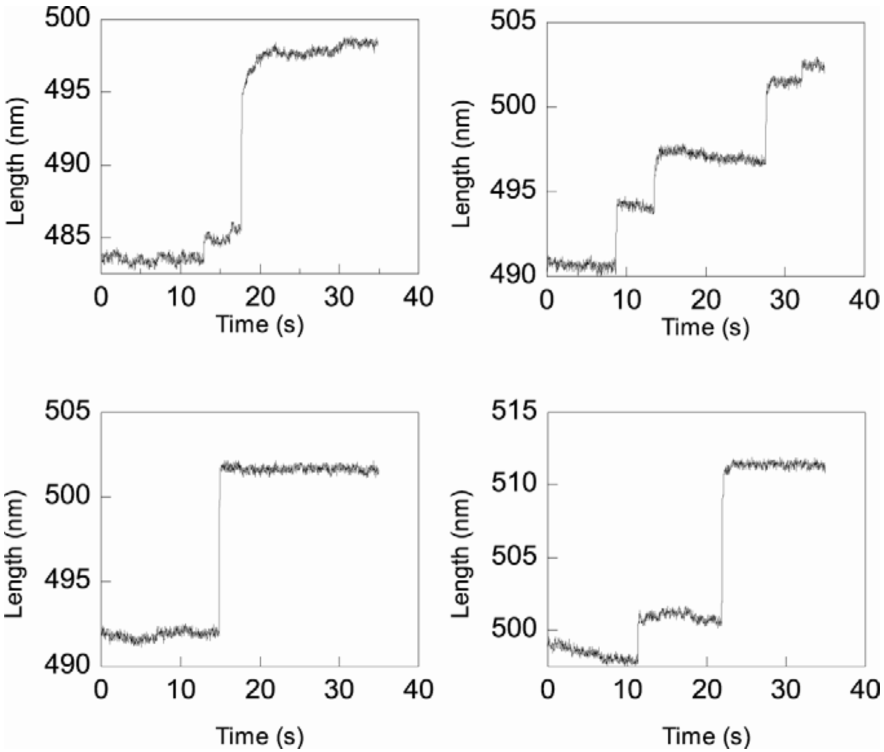
Constant Force Measurements



- Cooperative transitions?
- Ground state configurations?
- dsDNA unzipping metastable states? *Danilowica et.al. PNAS 100, 1694 (2003).*

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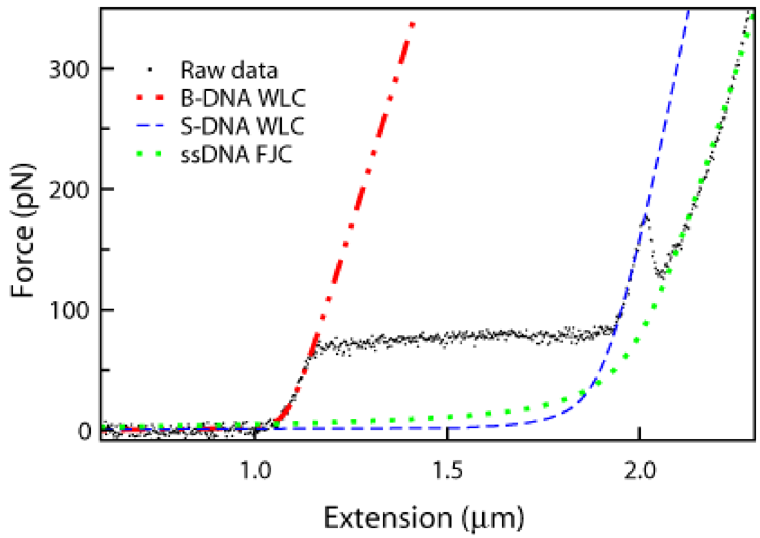
Mechanical Melting of Double-Stranded DNA



Extensible Worm-like-chain (WLC)

$$x = b_{\mathrm{ds}}\left[1 - \frac{1}{\sqrt{4\beta P_{\mathrm{ds}} F}} + \frac{F}{K_{\mathrm{ds}}}\right]$$

Extensible Freely-Jointed-Chain (FJC)

$$x = b_{\mathrm{ss}}\left[\coth(2\beta P_{\mathrm{ss}} F) - \frac{1}{2\beta P_{\mathrm{ss}} F}\right]\left[1 + \frac{F}{K_{\mathrm{ss}}}\right]$$

	P persistence Length (nm)	*b* contour length (nm)	*K* stretch modulus (pN)
B-DNA	50	1100	1200
S-DNA	10	2000	3200
ssDNA	0.75	2000	2200

Calderon, Chen, Lin, Harris, and Kiang, J. Phys.: Condens. Matter, 21 (2009) 034114.

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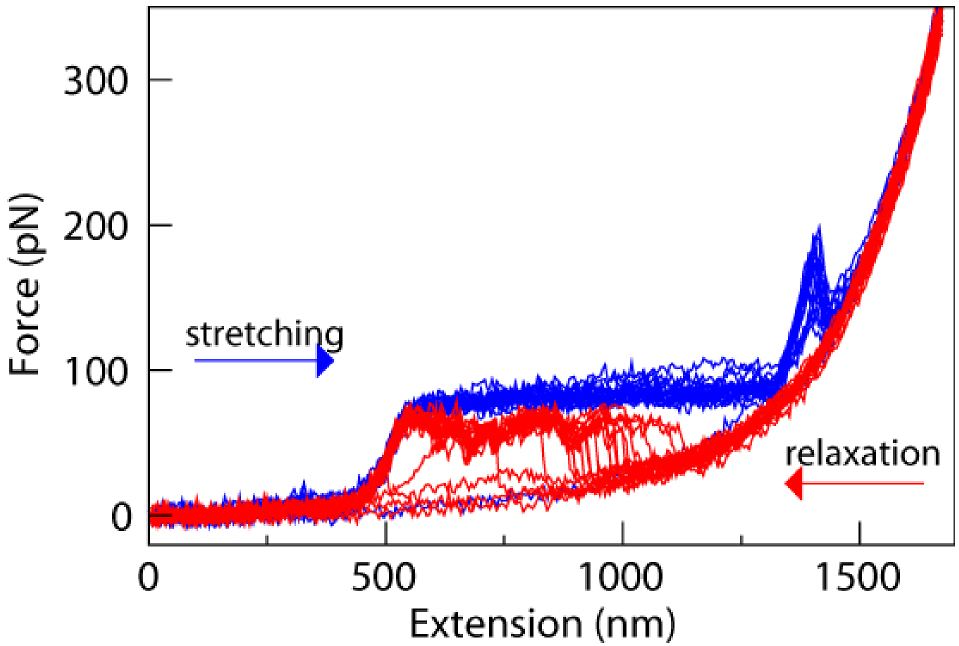
B-S Transition of *dsDNA*



- Repeated stretch/relaxation cycle reproducible
- Significant hysteresis during ssDNA relaxation cycle

Calderon, Harris, Kiang, and Cox, J. Phys. Chem. B 113, 138 (2009).
Calderon, Harris, Kiang, and Cox, J. Mol. Recognit., (2009) in press.

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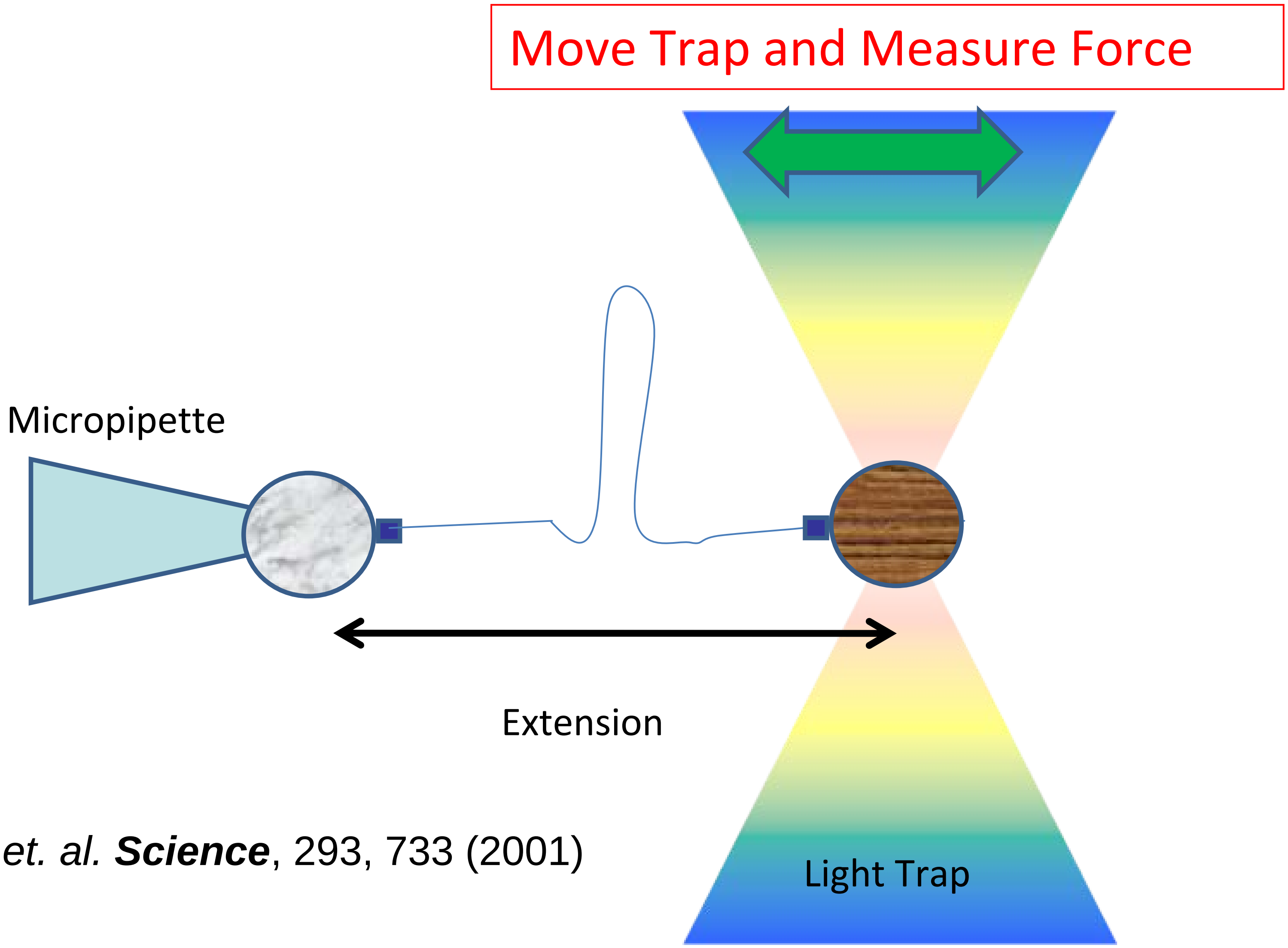
Force Curve Setup



Lipardt *et. al.* ***Science***, 293, 733 (2001)

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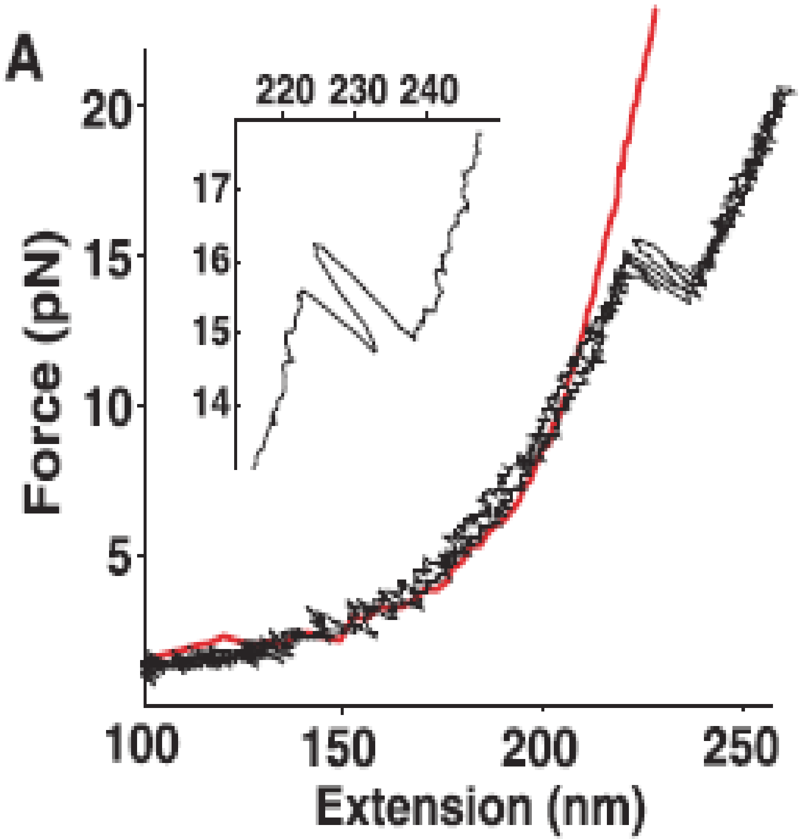
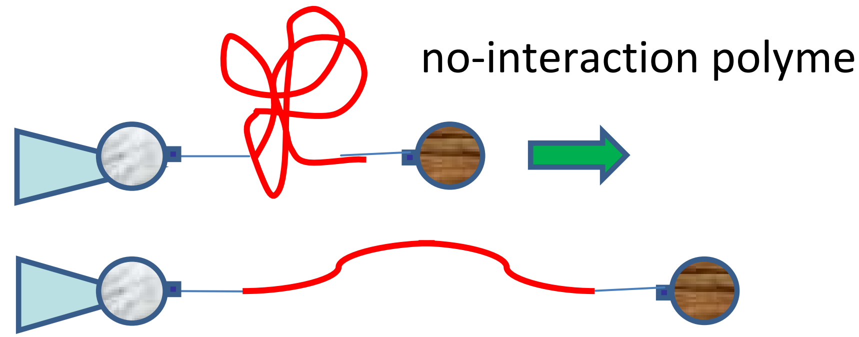
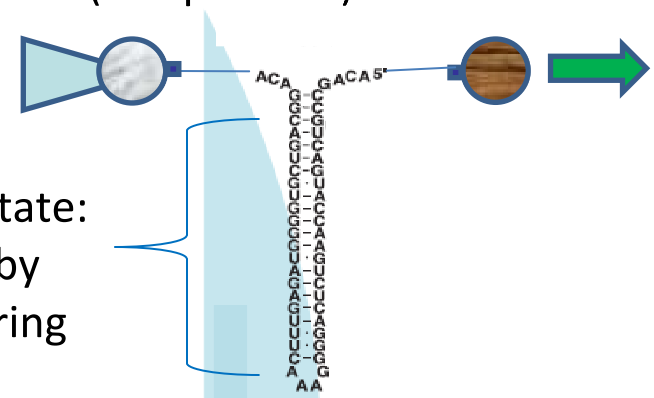


Red Line (Satisfies WLC):



Black Line (Hairpin RNA):



Folded State:
Formed by
base pairing

Unfolded State:
Hydrogen Bonds Broken by Force

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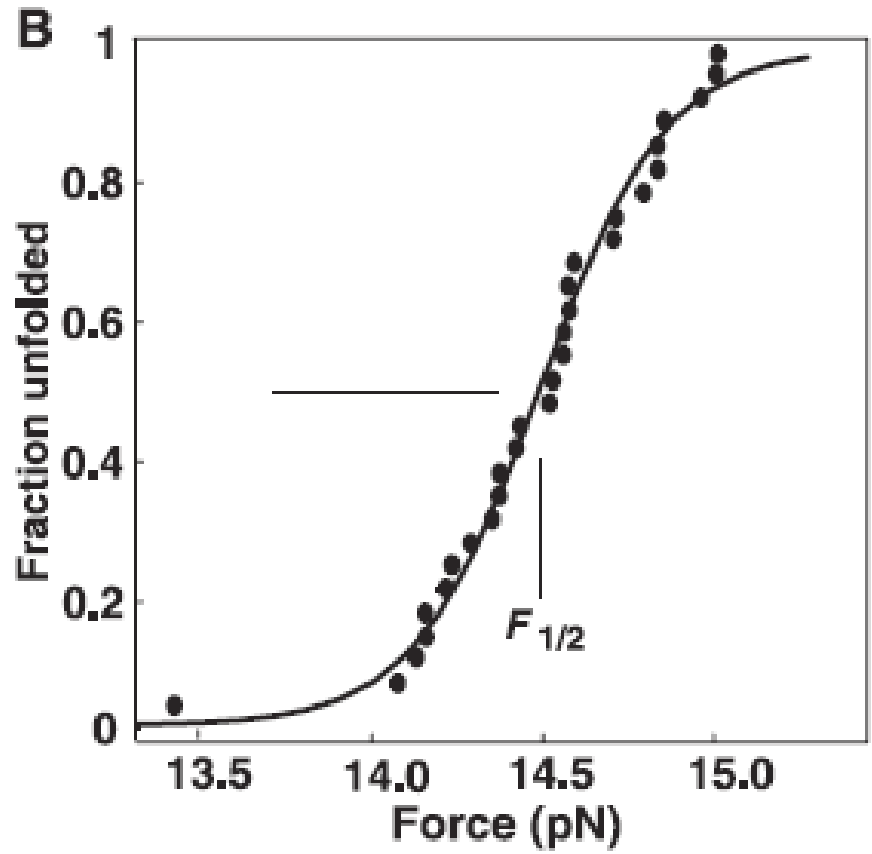
Equilibrium Approaches

- By repeated foldng/ unfolding, one can get a graph of fraction unfolded RNA versus force and fit into a two-state equation:

$$p(E) = 1/(1 + e^{E/k_B T})$$

- The free energy of unfolding of RNA

$$\Delta G = 157 \pm 20 kJ / mole$$



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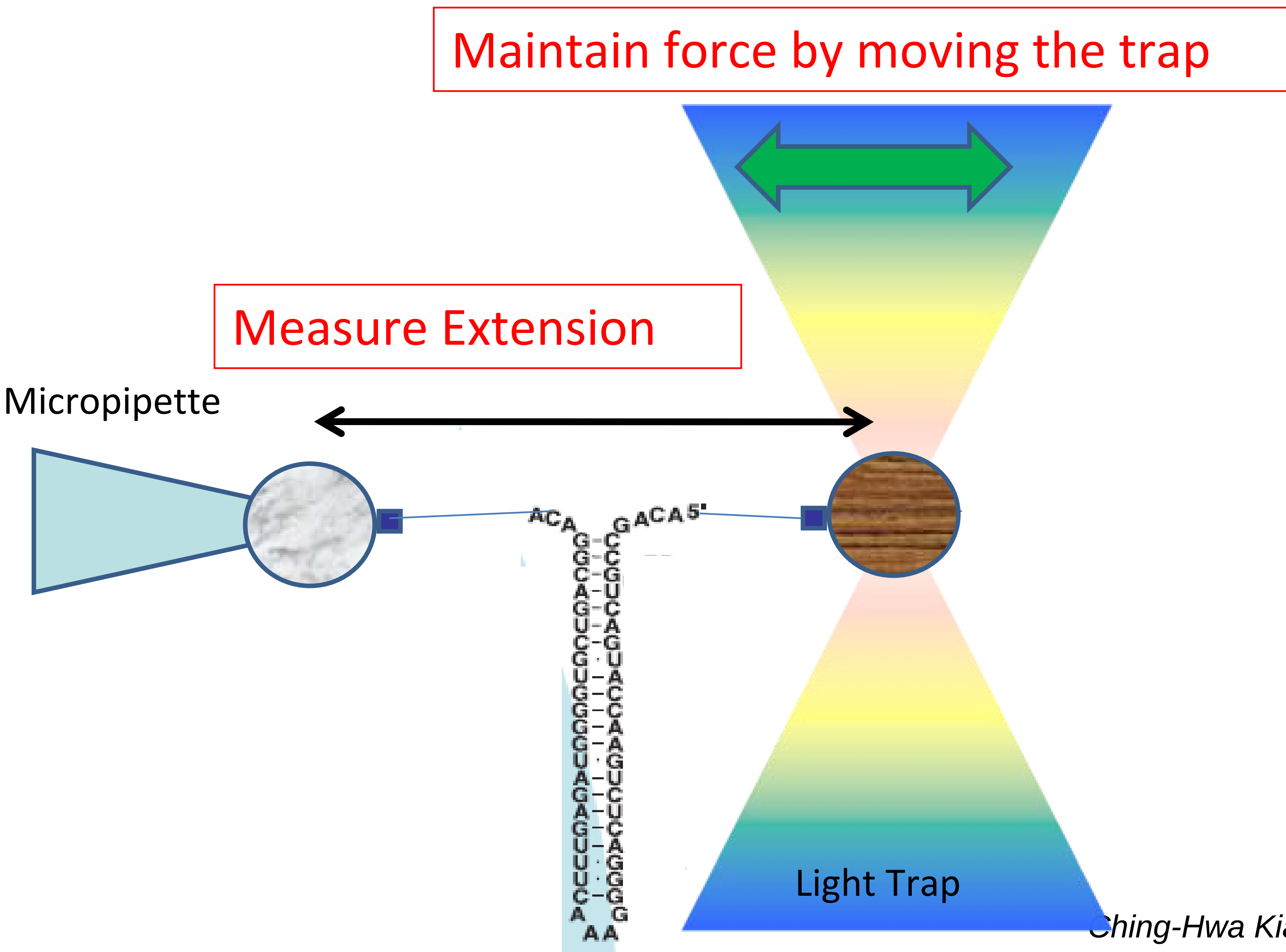
Constant Force Setup



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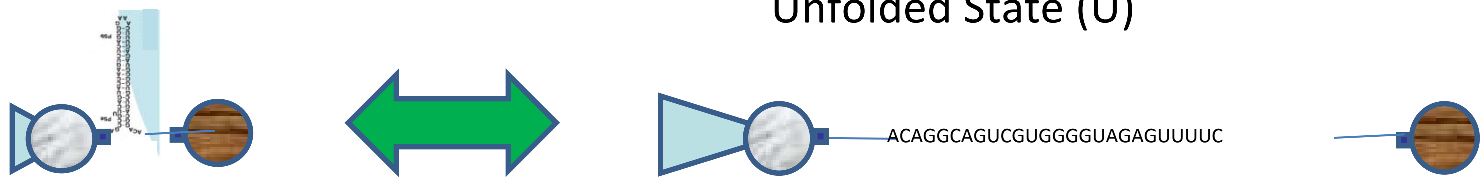
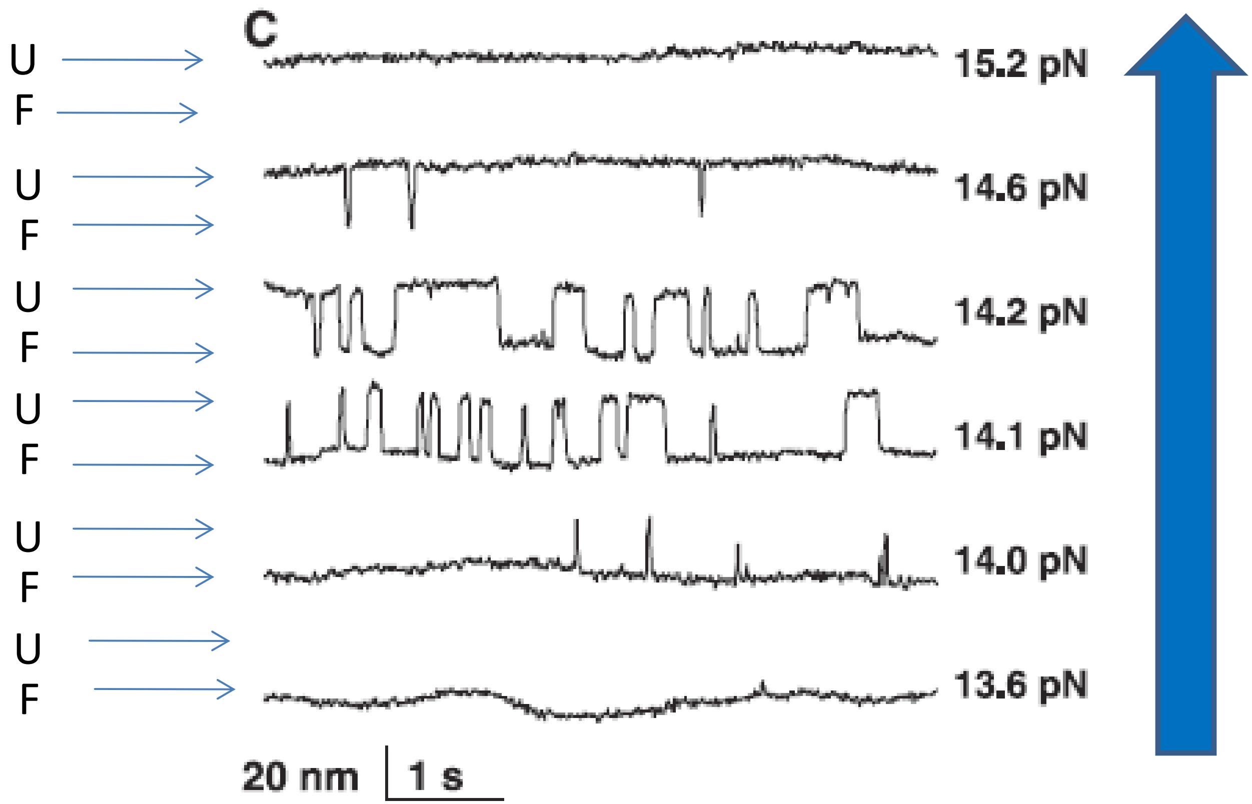
Folded State (F)

Unfolded State (U)





High force favors open state

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Free Energy Estimate

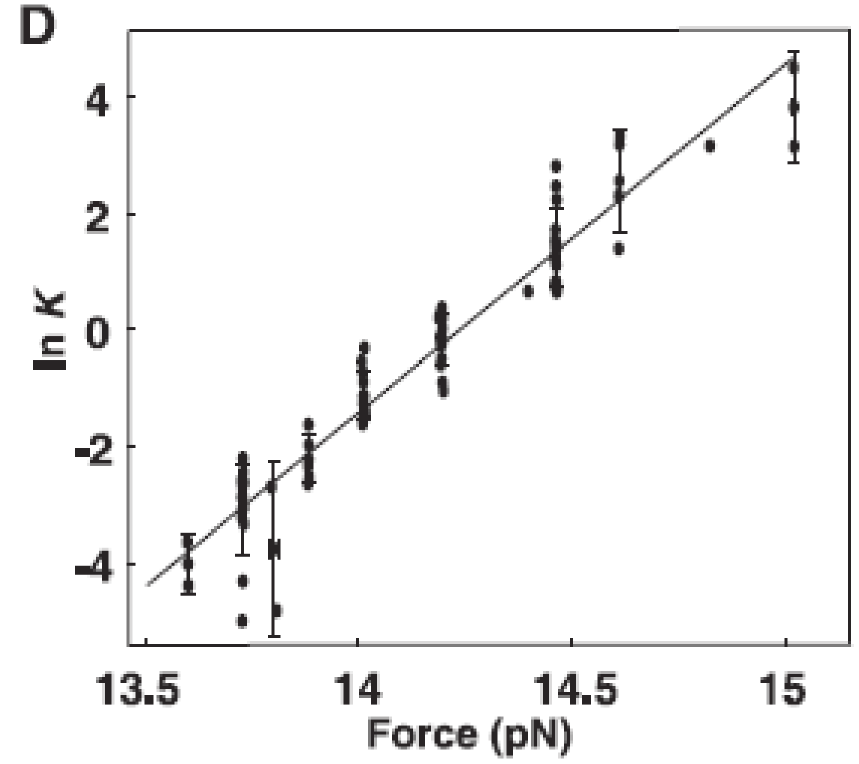
- The ratio of the average life time results in K. By plotting ln K versus force, we can get the free energy between the unfold and folded state using:

  $$\Delta G = \Delta G^o + RT \ln K$$

  They obtained

  $$\Delta G = 156 \pm 8 kJ / mole$$

  consistence with bulk studies.



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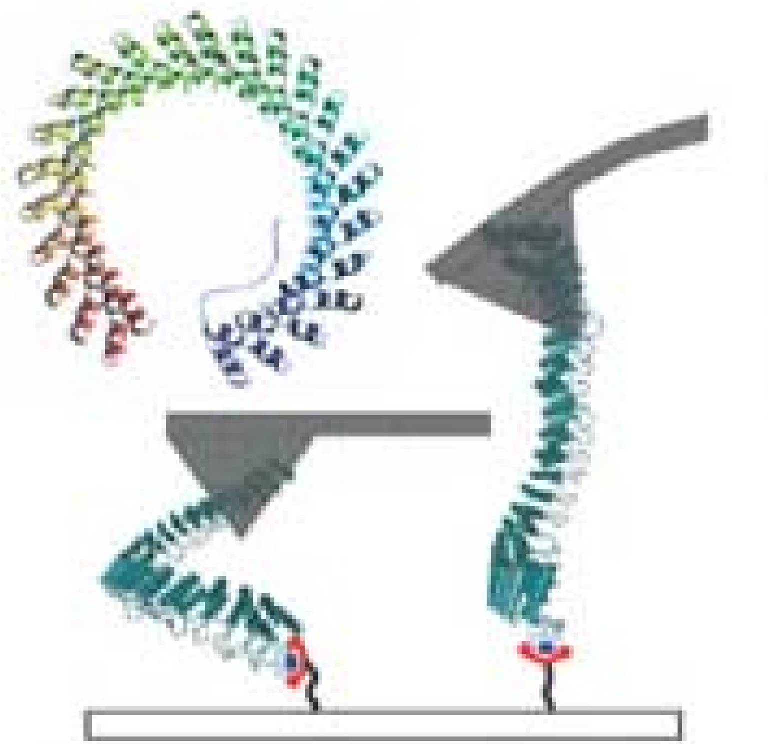
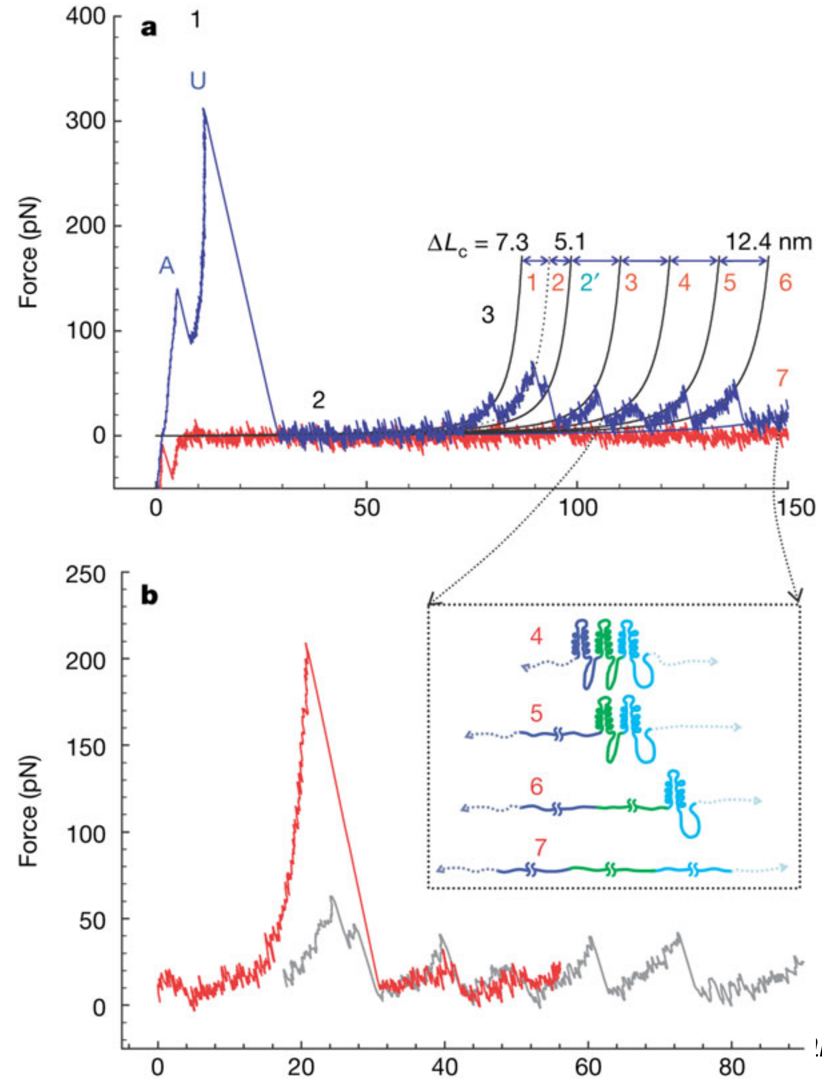
Protein Nanosprings

Linear Elasticity



His-tagged ankyrin fragment bound to NTA (red handles)

Unfolding Ankyrin Repeats



Lee *et. al., Nature* ***440, 246 (2006)***