Seeing Biological Interactions At the Nanometer Scale

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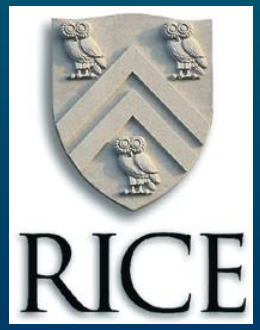


Outline

- I DNA Melting
  - Background and motivation
  - Experimental procedures
  - Effects of disorder on the melting temperature
  - Effects of defects
- II Single-molecule manipulation
  - Background and motivation
  - Experimental techniques
  - The muscle protein titin
  - Information from nonequilibrium measurements
  - Free energy surfaces of stretching and unfolding
  - Other applications
- Conclusion

DNA Melting

DNA Based Nanosensors

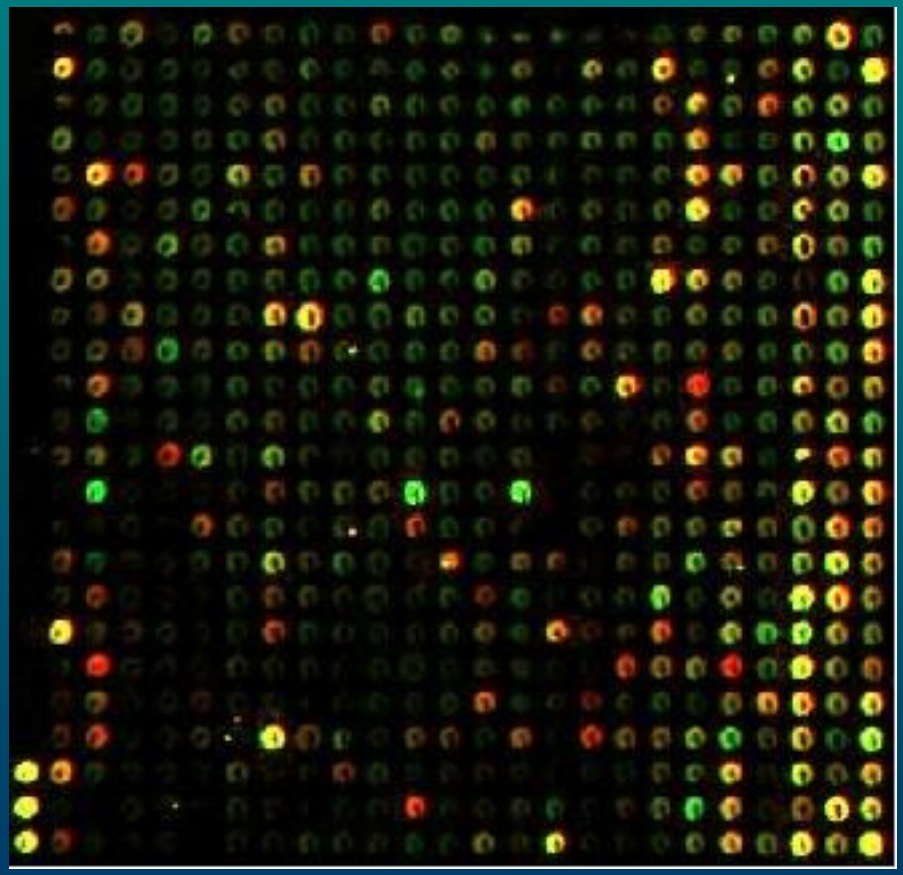
- Colloidal gold covered with oligonucleotide for DNA detection

  Mirkin et. al. Nature 382 (1996)

  Jin, Wu, Li, Mirkin, and Schatz, J. Amer. Chem. Soc. 125,1643 (2003)
- Used to detect Anthrax toxin
- An alternative technology to DNA microarray
- Understanding surface-bound DNA interactions

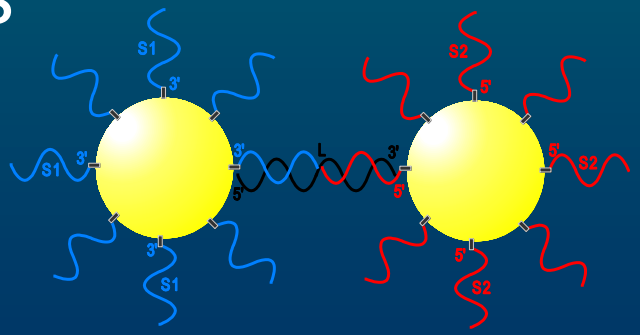
DNA Microarray

- DNA sensor
- Gene discovery
- Disease diagnosis
- Drug discovery



DNA-Linked Gold Nanoparticles

- Gold nanoparticle capped with ssDNA complementary to target (linker) ssDNA
- Probe particles self-assemble upon mixing with proper target DNA
- Color change upon phase transition
- New class of complex fluids



Sample Preparation

- Thiol modified DNA synthesis
- DNA-gold conjugation
- Excess DNA removal
- Target and probe DNA hybridization
- Aggregation kinetics and melting monitored by optical spectroscopy

Phase Transition of DNA-Linked Gold Nanoparticles

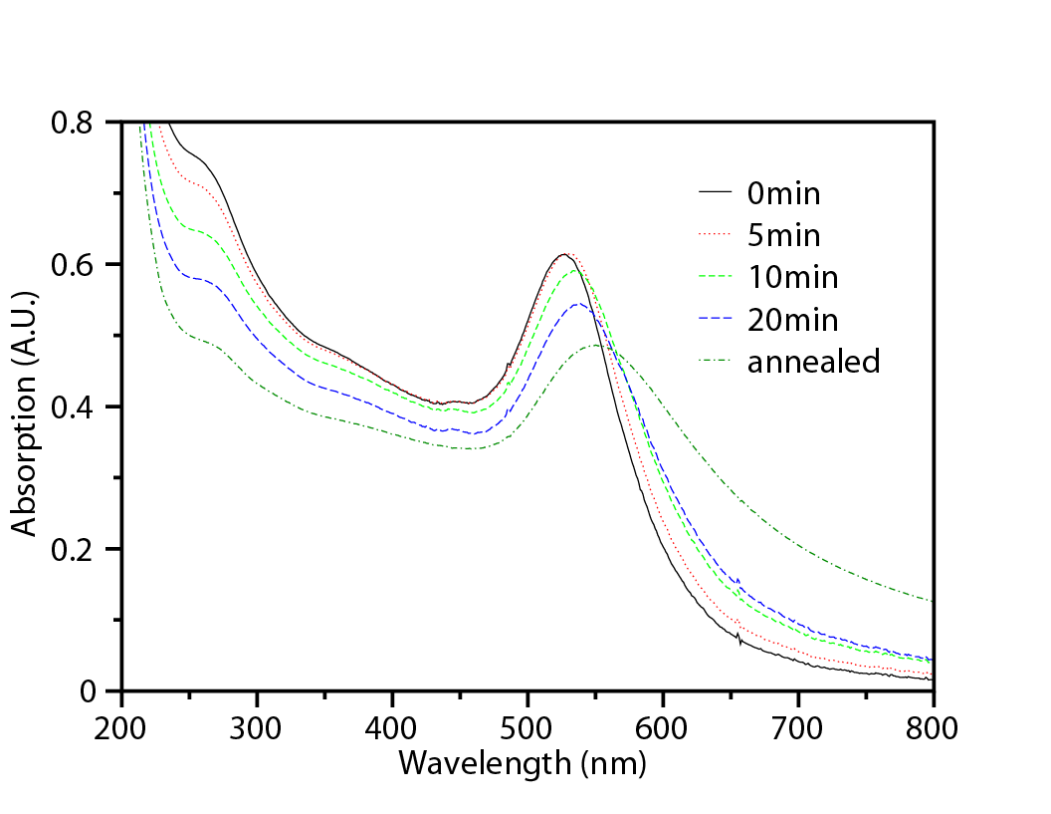
- Unique phase diagram
- Mapping microscopic DNA sequences onto the macroscopic phase behavior of colloids

  Lukatsky and Frenkel, Phys. Rev. Lett. 92, 068302 (2004)
- Optical properties and cluster aggregation thermodynamics and kinetics.

  Storhoff et. al., J. Amer. Chem. Soc. 122, 4640 (2000)

  Park and Stroud, Phys. Rev. B 68, 224201 (2003)

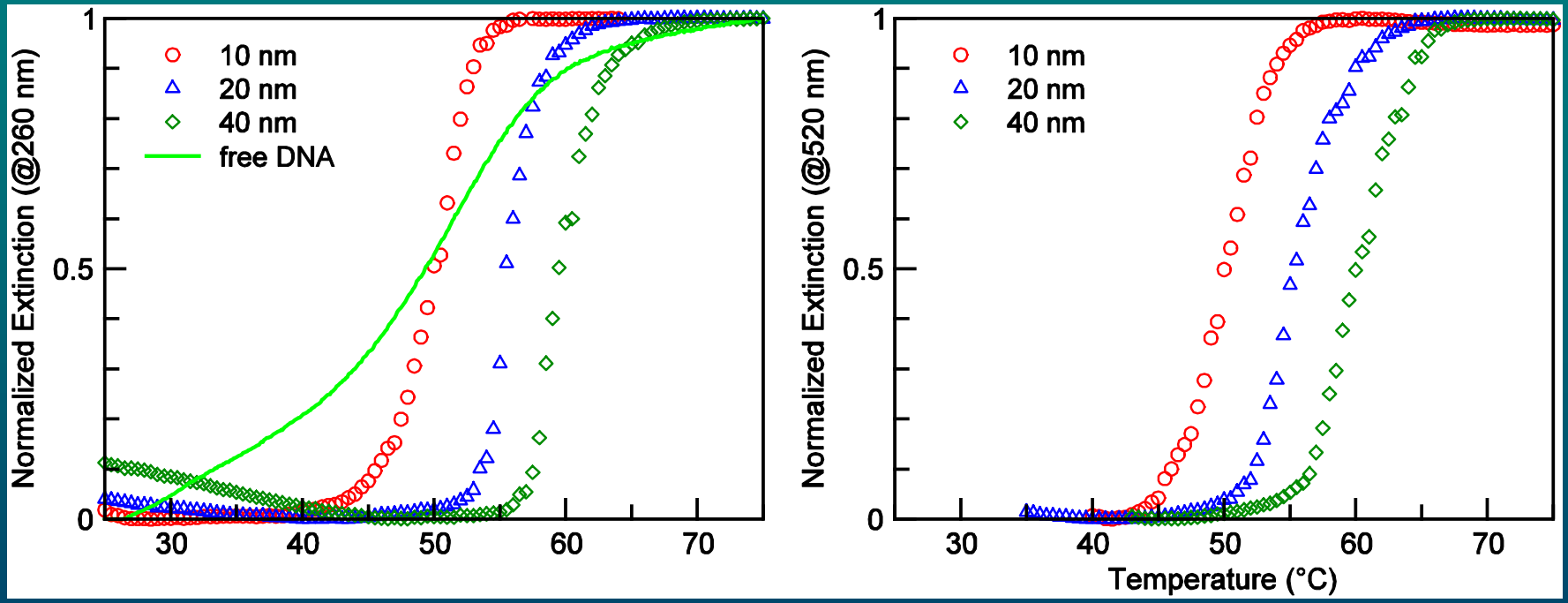
Kinetics of Aggregation



Kiang, Physica A, 321, 164 (2003)

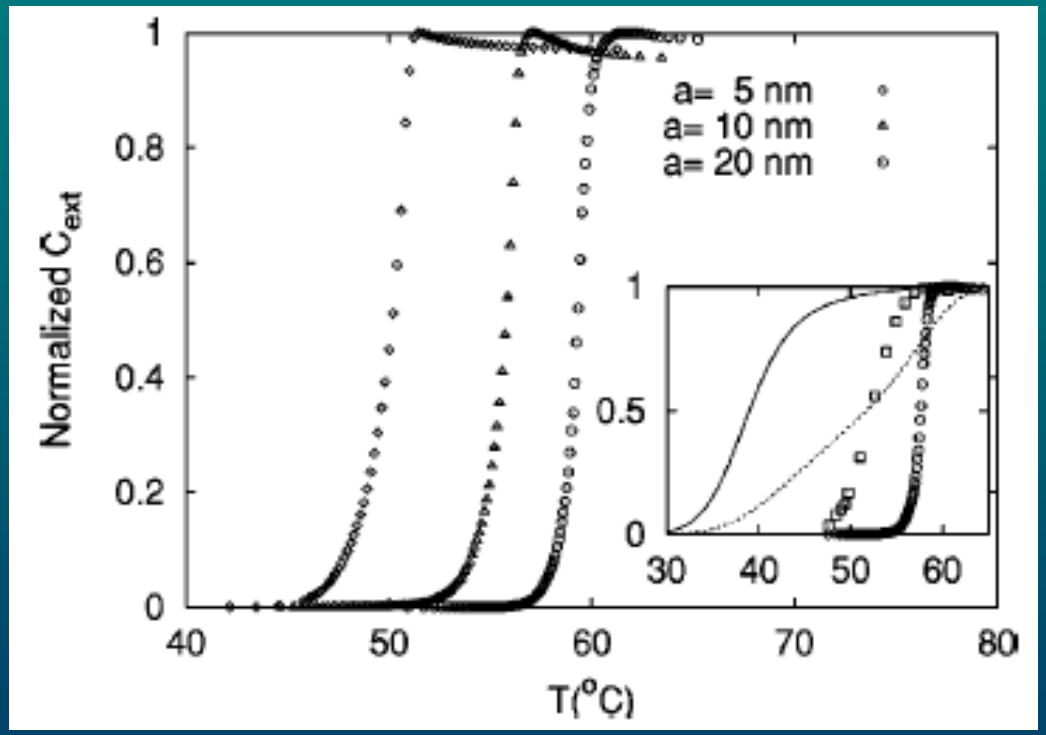
Sun and Kiang, in Handbook of Nanostructured Biomaterials and Their Applications in Nanobiotechnology, **Vol.2**, Ch.7 *(2005)*

Melting Curves



Sun, Harris, and Kiang, Physica A, 354, 1 (2005)

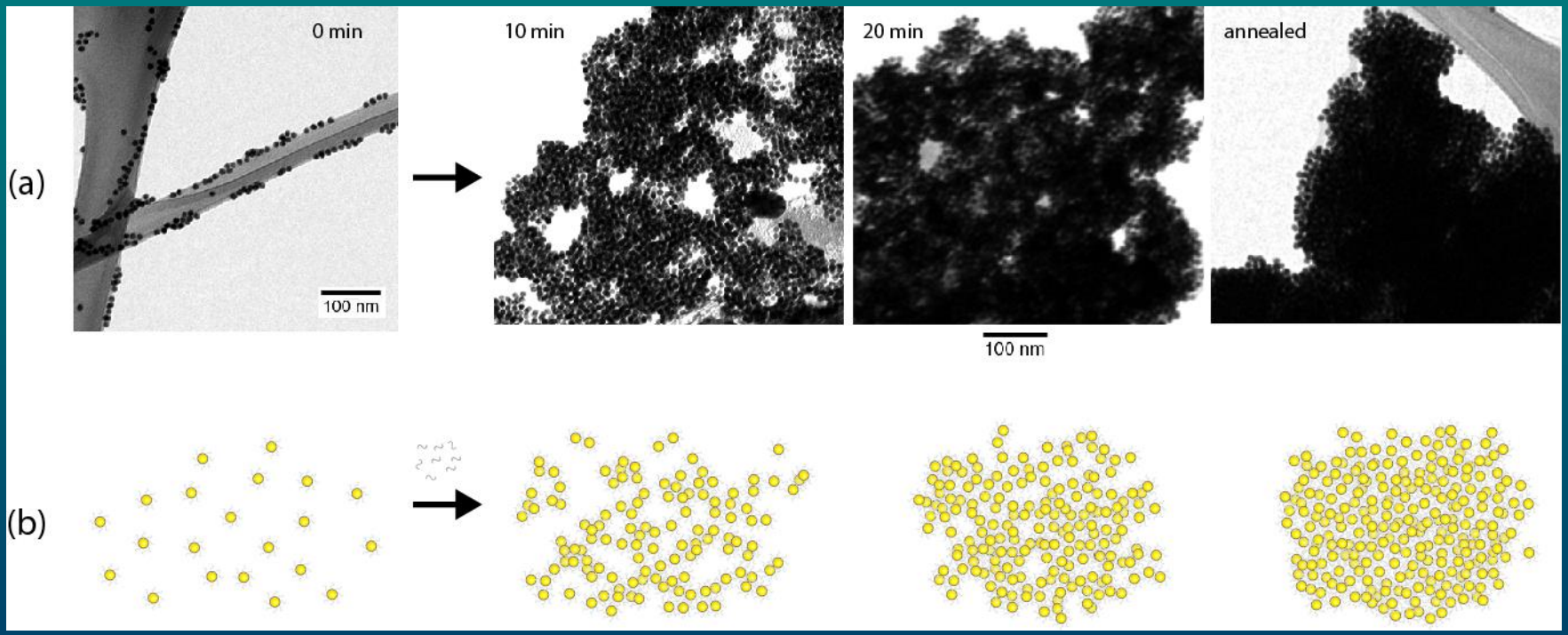
Melting Temperature as a Function of Gold Nanoparticle Size



Simulation results agree with experiments

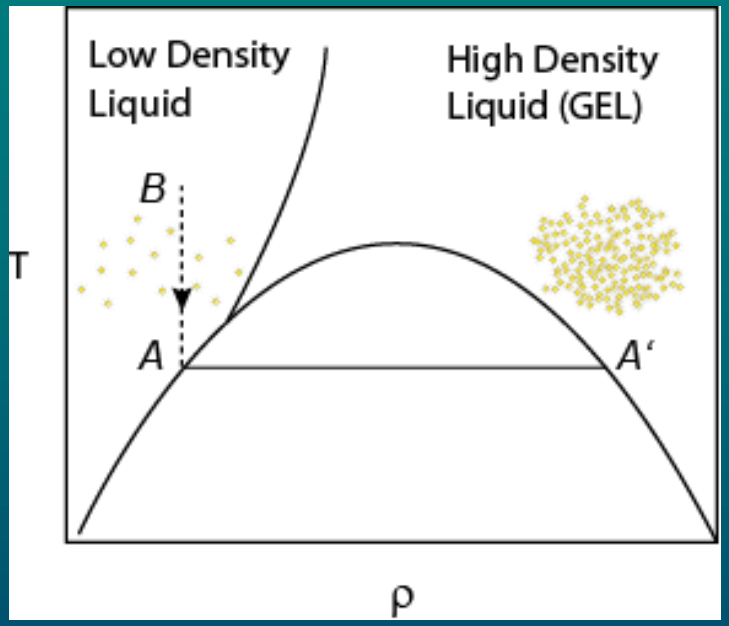
Park and Stroud, Phys. Rev. B 67, 212202 (2003)

Structural Phase Transition



Sun, Harris, and Kiang, Physica A, 354, 1 (2005)

Sol-Gel Transition



- Gelation in the presence of solvent – segregation of the gelating species
- Sol-gel transition with suitable concentration
- Critical exponents of the percolation type

De Gennes, "Scaling Concepts in Polymer Physics." (1979)

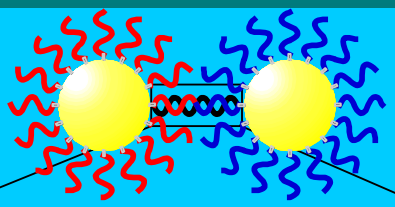
Sun, Harris, and Kiang, Physica A, 354, 1 (2005)

Disorder

Using Simple DNA Sequences

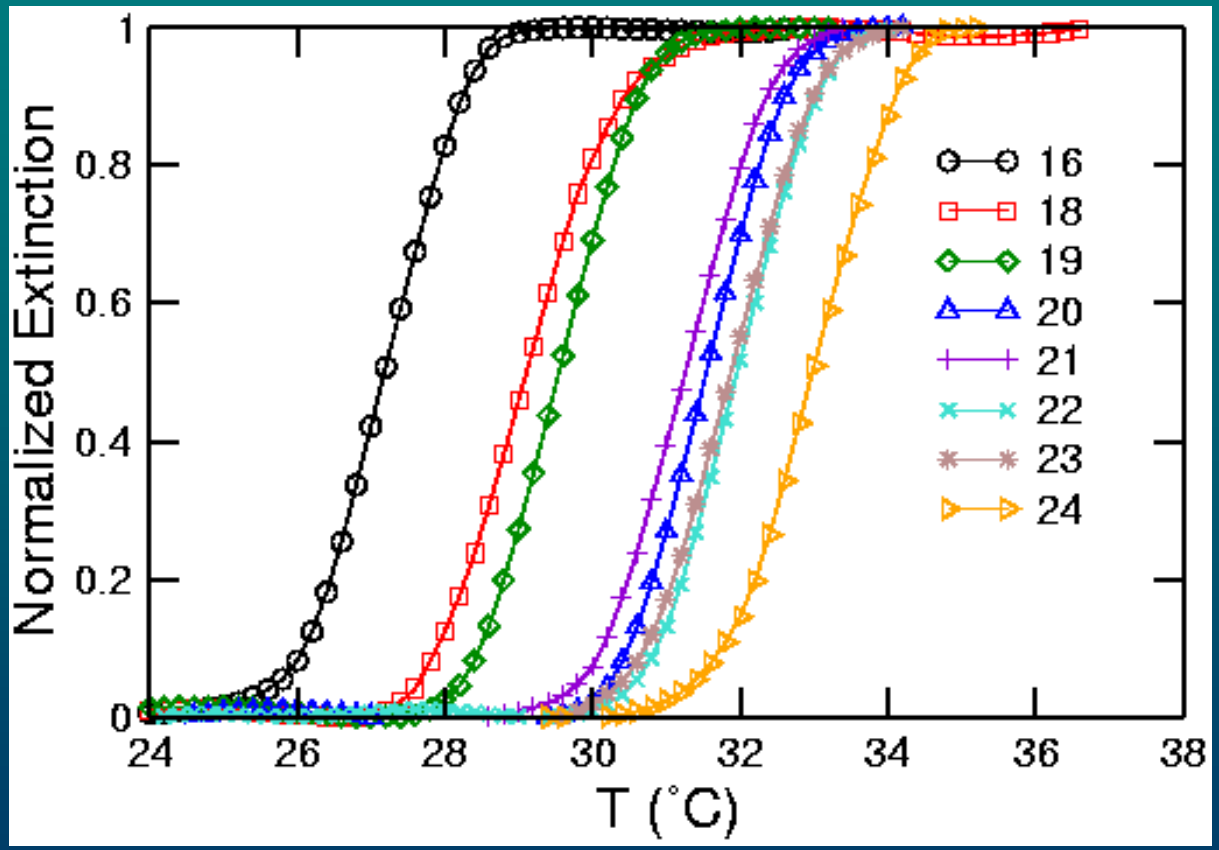
- Eliminate sequence dependent phase transition properties
- Smooth and reproducible melting curves resulting in more accurate T_m determination
- Well-defined variables for isolating key effects
- Designing DNA-gold nanoparticles with specific interaction strength

Experimental Design



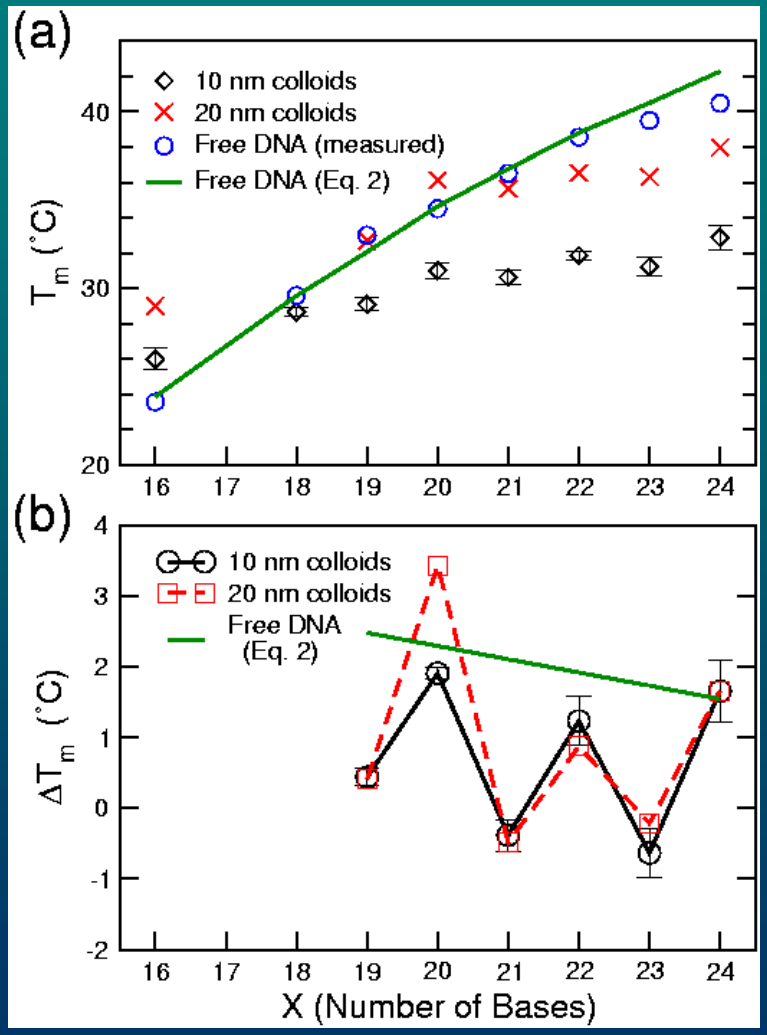
(a)	3'-AAAAAAAAAAAG|GAAAAAAAAAAA-5'	Probes
	5'-TTTTTTTTTTTCCTTTTTTTTTTT-3'	Linker (12-12)
	5'-TTTTTTTTTTTCCTTTTTTTTTTT-3'	Linker (12-11)
	5'-TTTTTTTTTTCCTTTTTTTTTTT-3'	Linker (11-11)
	5'-TTTTTTTTTTCCTTTTTTTTTT-3'	Linker (11-10)
	5'-TTTTTTTTTTCCTTTTTTTTTT-3'	Linker (10-10)
	5'-TTTTTTTTTCCTTTTTTTTT-3'	Linker (10-9)
	5'-TTTTTTTTTCCTTTTTTTTT-3'	Linker (9-9)
	5'-TTTTTTTCCTTTTTTT-3'	Linker (8-8)
(b)	3'-AAAAAAAAAAAG|GAAAAAAAAAAA-5'	Probes
	5'-TTTTTTTTTTCCTTTTTTTTTT-3'	Linker (11-11)
	5'-TTTTTTTTTTTCCTTTTTTTTTT-3'	Linker (12-10)
	5'-TTTTTTTTTTCCTTTTTTTTTT-3'	Linker (10-10)
	5'-TTTTTTTTTTCCTTTTTTTTT-3'	Linker (11-9)
	5'-TTTTTTTTTTTCCTTTTTTTT-3'	Linker (12-8)

Effect of DNA Linker Length



Harris and Kiang, Phys. Rev. Lett., 95, 0461101 (2005)

Effect of Disorder



$$T_m = \left(\frac{\Delta H^0 + 3.4 \ \frac{\text{kcal}}{\text{mol}}}{\Delta S^0 - R \ln(\frac{1}{[\text{DNA}]})} \right) + 16.6 \log_{10}([\text{Na}^+])$$

-8.0 -8.0 -8.0 -8.8 -6.6 -8.0 -8.0 -8.0

3′–AAAAA–A–A–A–A–A–G G–A–A–A–A–A–A–AAAAA–5′

5′–T–T–T–T–T–T–T–C–C–T–T–T–T–T–T–T–3′

-8.0 -8.0 -8.0 -5.45 -8.0 -8.0 -8.0

$5' : \Delta H^0 = 6(-8.0) + 1(-8.8) + 1(-5.45) = -62.25 \ (\text{kcal/mol})$

$3' : \Delta H^0 = 6(-8.0) + 1(-6.6) + 1(-5.45) = -60.05 \ (\text{kcal/mol})$

Disorder: Asymmetric Connection Energy

- In free DNA, T_m increases linearly with number of linker DNA bases
- Odd number of linker DNA bases results in lower T_m than expected in the nanoparticle systems

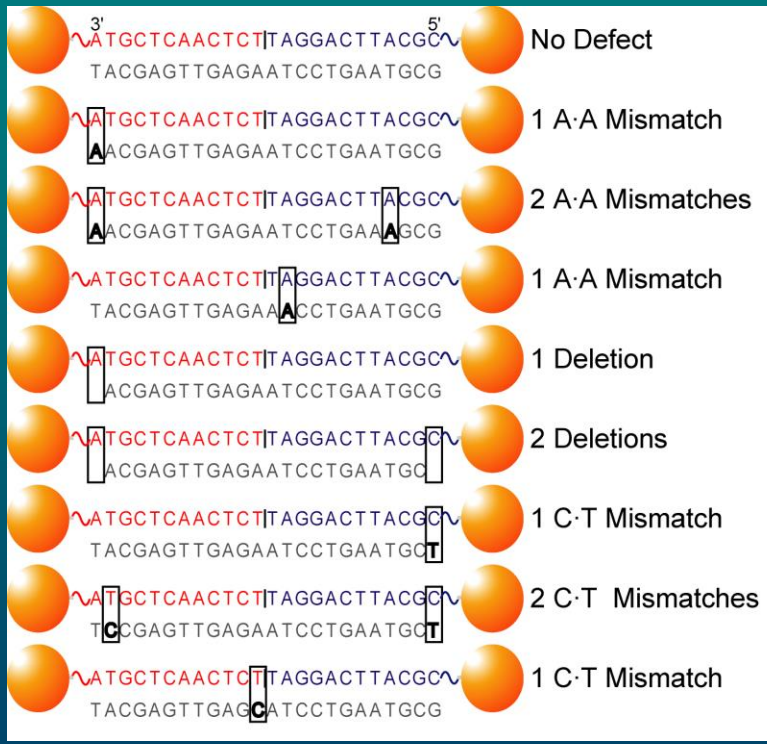
Harris and Kiang, Phys. Rev. Lett. 95, 0461101 (2005)

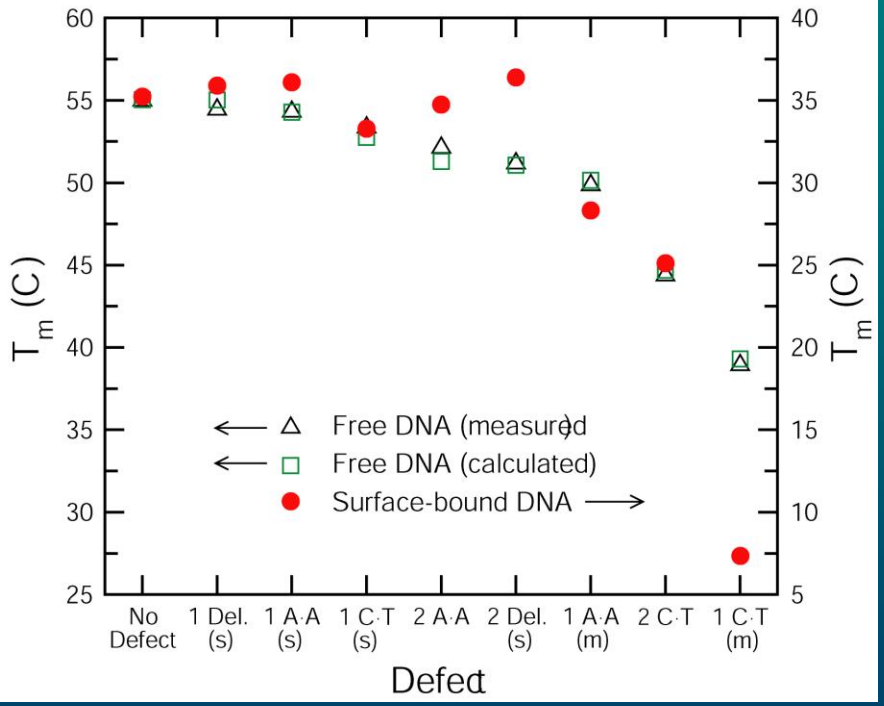
Defects

Mismatches and Deletions

- Present in DNA-linked gold nanoparticle system and DNA microarray
- Introducing error in DNA data
- Unexpected melting behavior
- Critical in interpreting data but poorly understood

T_m Trends in Bound vs Free DNA



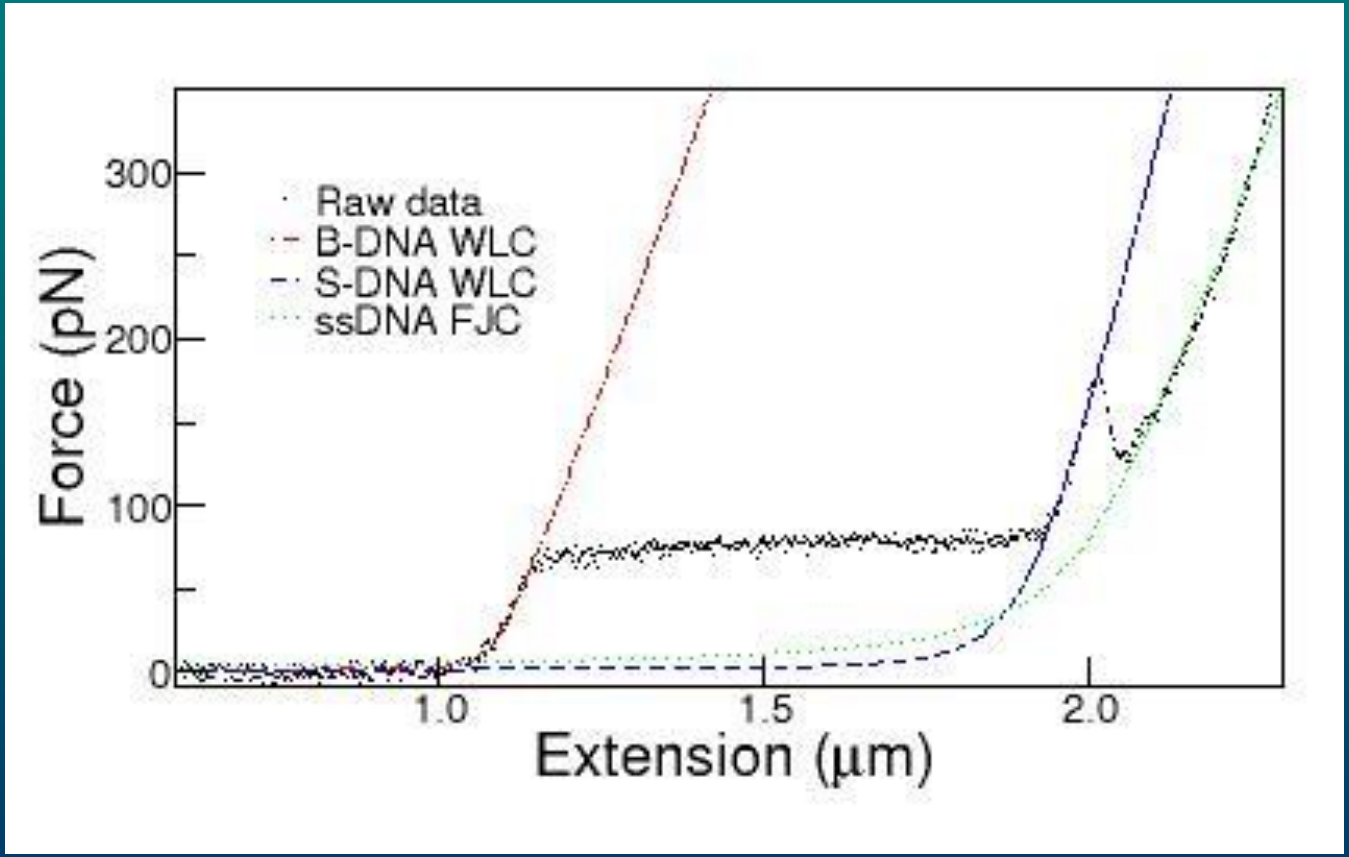


Harris and Kiang, J. Phys. Chem. B, 110, 16393 (2006)

Defects: Can Increase T_m

- Different from free DNA
- May increase melting temperature T_m
- Mismatches and deletions on or near surfaces are likely to increase T_m
- AA mismatches usually increase T_m, while CT mismatches decrease T_m
- Depending on factors such as base, sequence, and location
- May be used to increase detection sensitivity

Mechanical Melting of DNA



Summary

- DNA-linked gold nanoparticle assemblies represents a new class of complex fluids, with tunable interaction between particles
- Introducing disorder and defects to the system results in melting temperature changes not explainable with free DNA thermodynamics

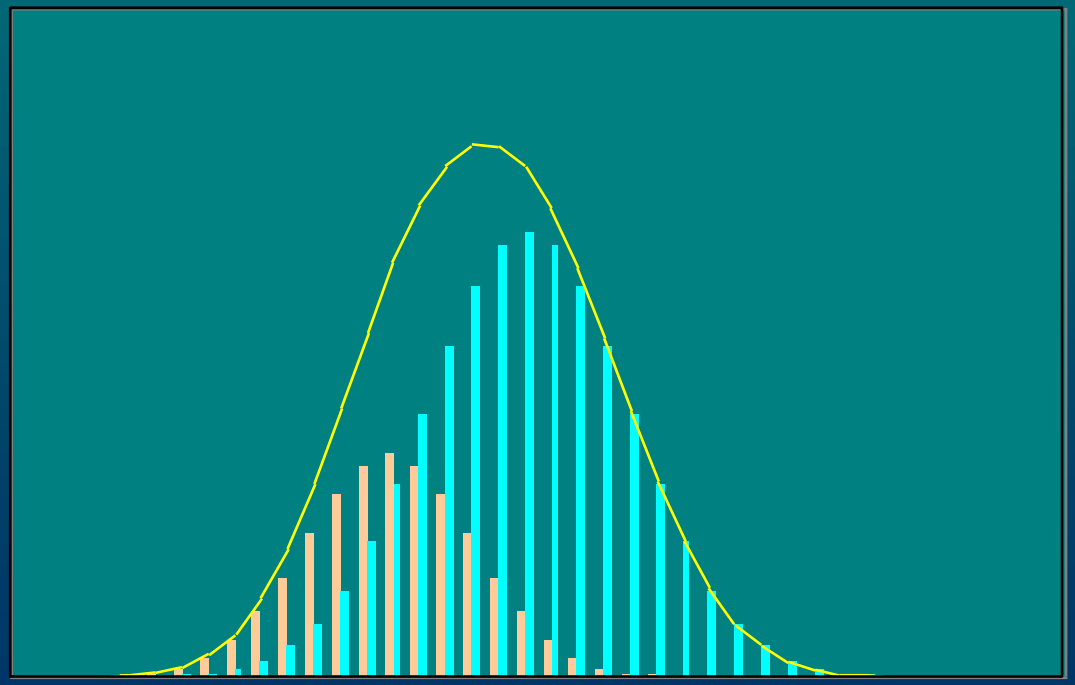
Single-Molecule Manipulation

Applications for Single-Molecule Manipulation

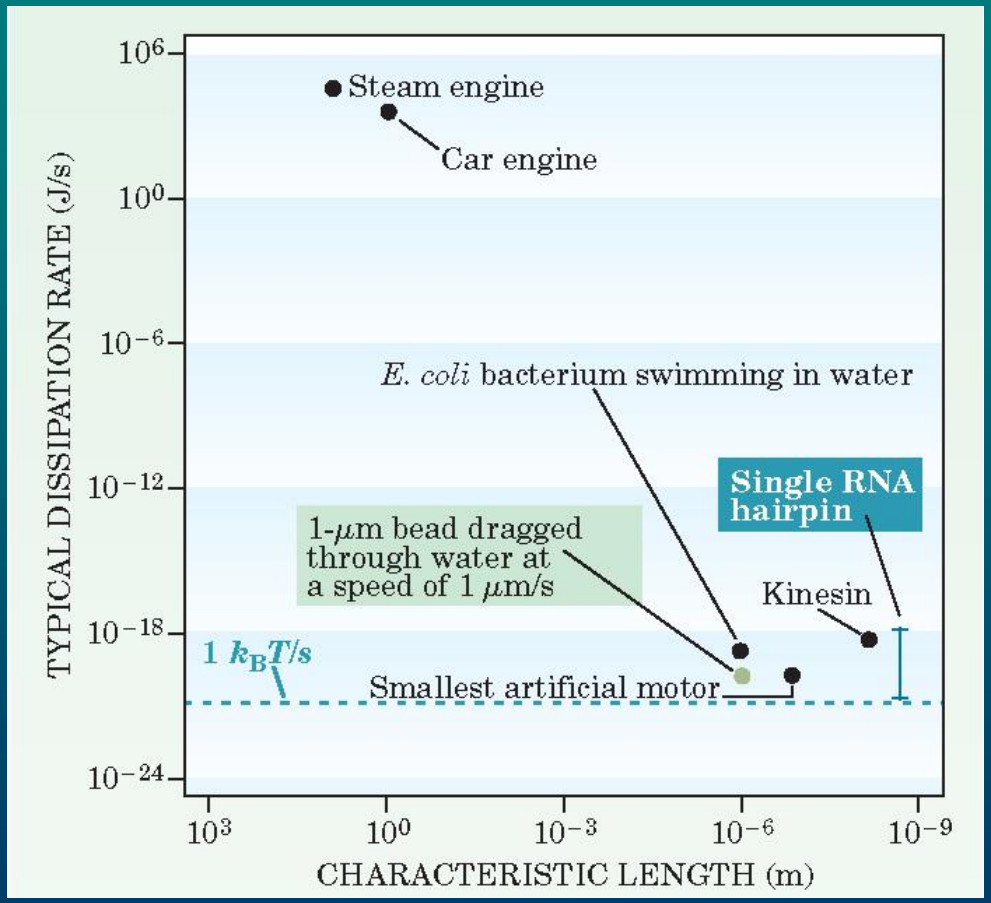
	Force (N)	X_{min}(m)	Stiffness (force const. N/m)	Applications	Advantages
Cantilevers (SFM/AFM)	10^{-12}-10^{-7}	10^{-10}	0.001-100	Protein Polysaccharides Bond strength	High spatial resolution Large dynamic range Strong interactions
Microneedles	10^{-12}-10^{-10}	10^{-9}	10^{-5}-1	Myosin motor force DNA/titin strength	Good operator control Soft spring constant
Flow field	10^{-13}-10^{-9}	10^{-8}	--	DNA dynamics RNA polymerase	Rapid buffer exchange Simple design
Magnetic field	10^{-14}-10^{-11}	10^{-8}	--	DNA entropic elasticity Topoisomerases activity	Specificity to magnets Ability to induce torque
Photon field	10^{-13}-10^{-10}	10^{-9}	10^{-10}-10^{-3}	Protein motors RNA unfolding	Specific manipulation High force resolution
Electric field	0-10^{-12}	--	--	Electrophoresis	

Why Single-Molecule Experiments

- Manipulating objects and measuring properties at the single-molecule level
- Eliminating confusion from ensemble averaging
- Observing reaction occurring at real time



Nonequilibrium Statistical Mechanics of Single-Molecules

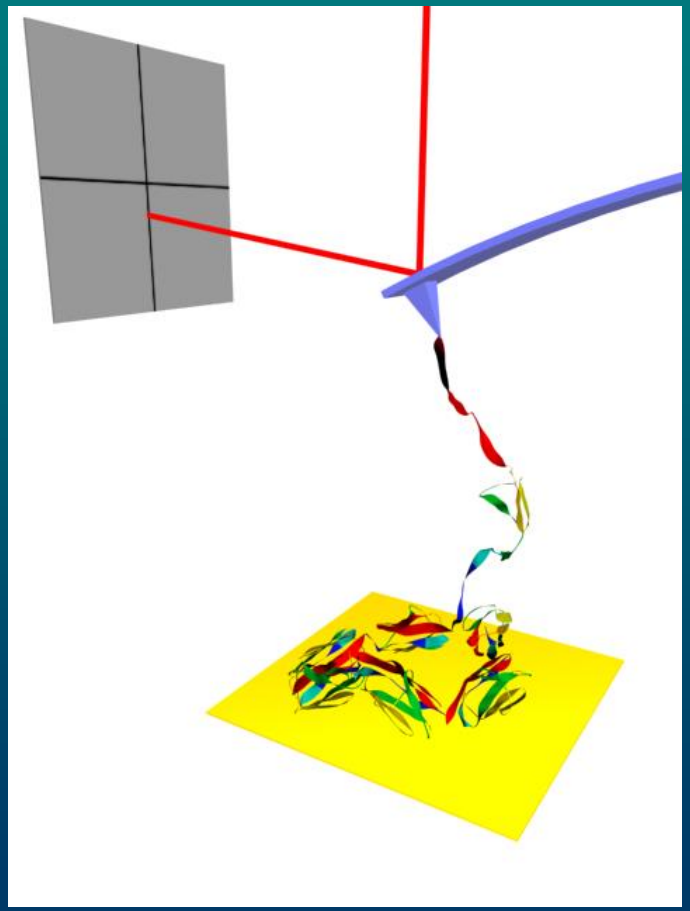


Length scales and energy dissipation rates of various thermodynamic systems. The two systems in the boxes have been used to test fluctuation theorems and the Jarzynski's equality

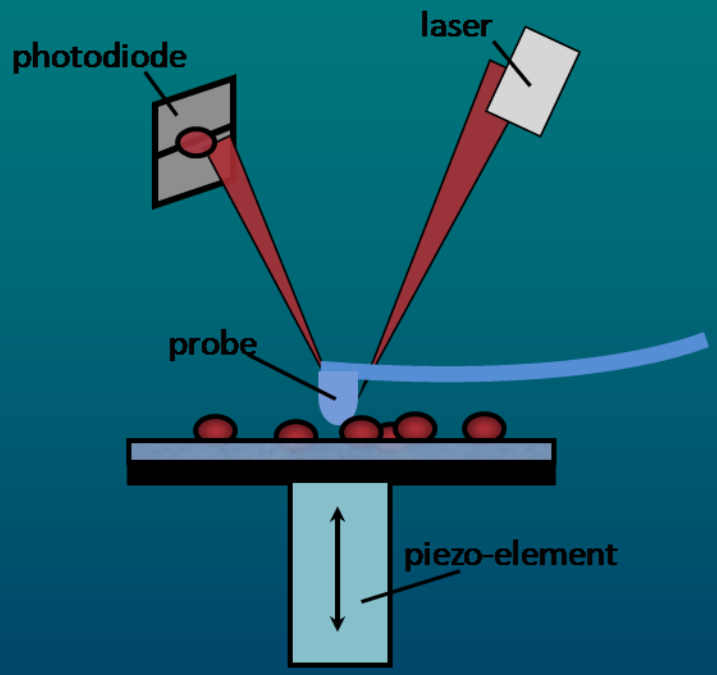
Bustamante et. al., Physics Today 43 (2005)

Pulling Single-Molecules

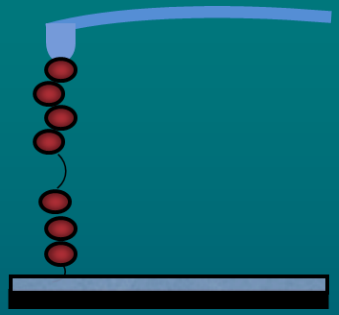
- Nanobiology approach to probe biomolecular interactions
- Manipulation and measurements at the single-molecule level
- The end-to-end distance (*z*) and the force (*f*) on the trapped bead were measured



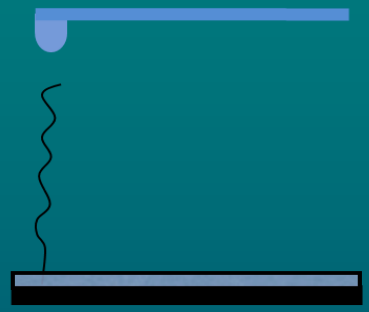
Atomic Force Microscopy



A single molecule is attached between the AFM tip and the substrate surface.

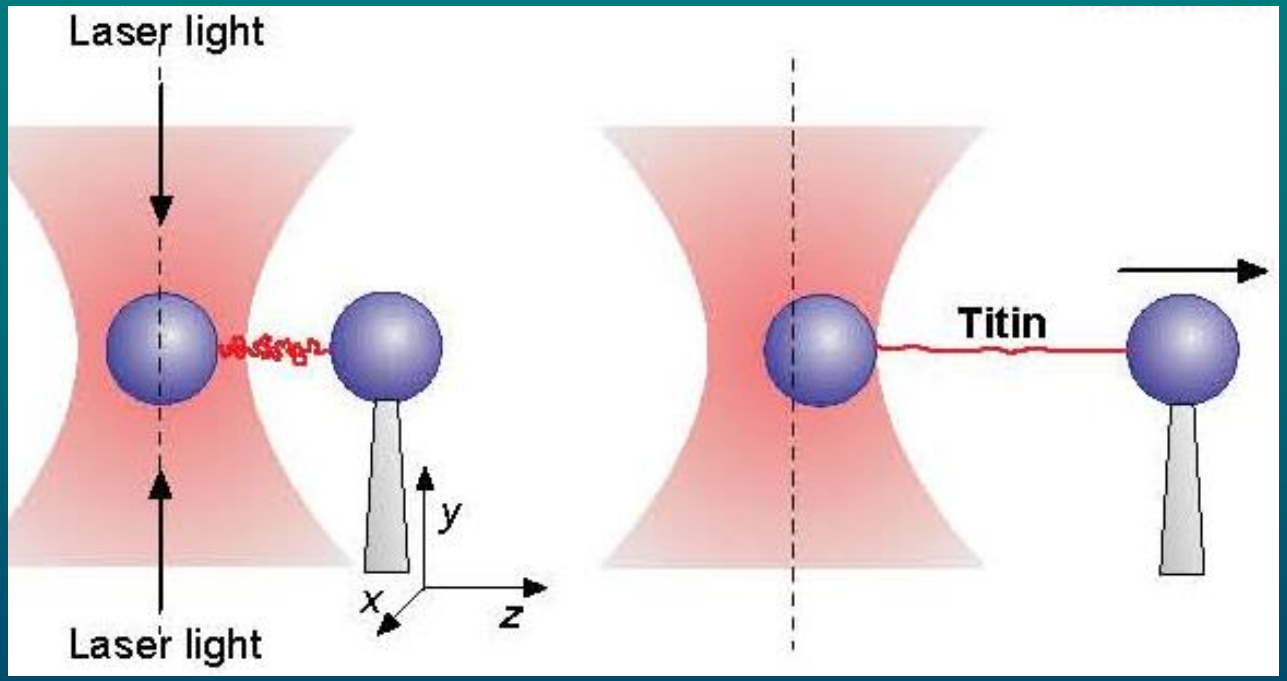


Pulling the molecule causes the cantilever to bend, the cantilever restoring force is recorded as a function of time, which is converted to extension.



Continued pulling causes the protein to unfold, and eventually detached from the tip.

Optical Tweezers

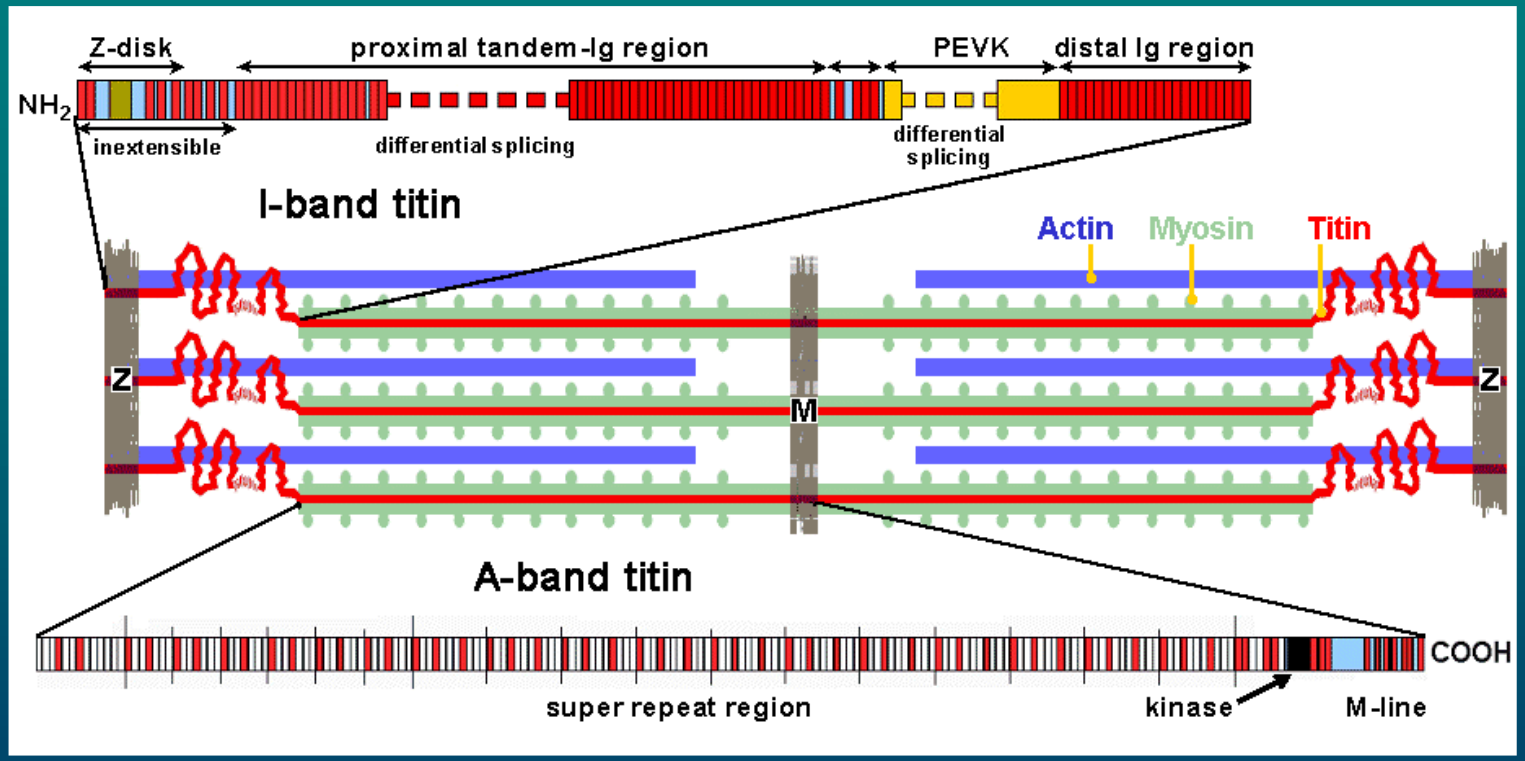


Molecules were stretched by moving the micropipette away from the optical trap. The end-to-end distance (*z*) and the force (*f*) on the trapped bead were measured

Kellermayer et. al., Science 276, 1116 (1997)

Titin

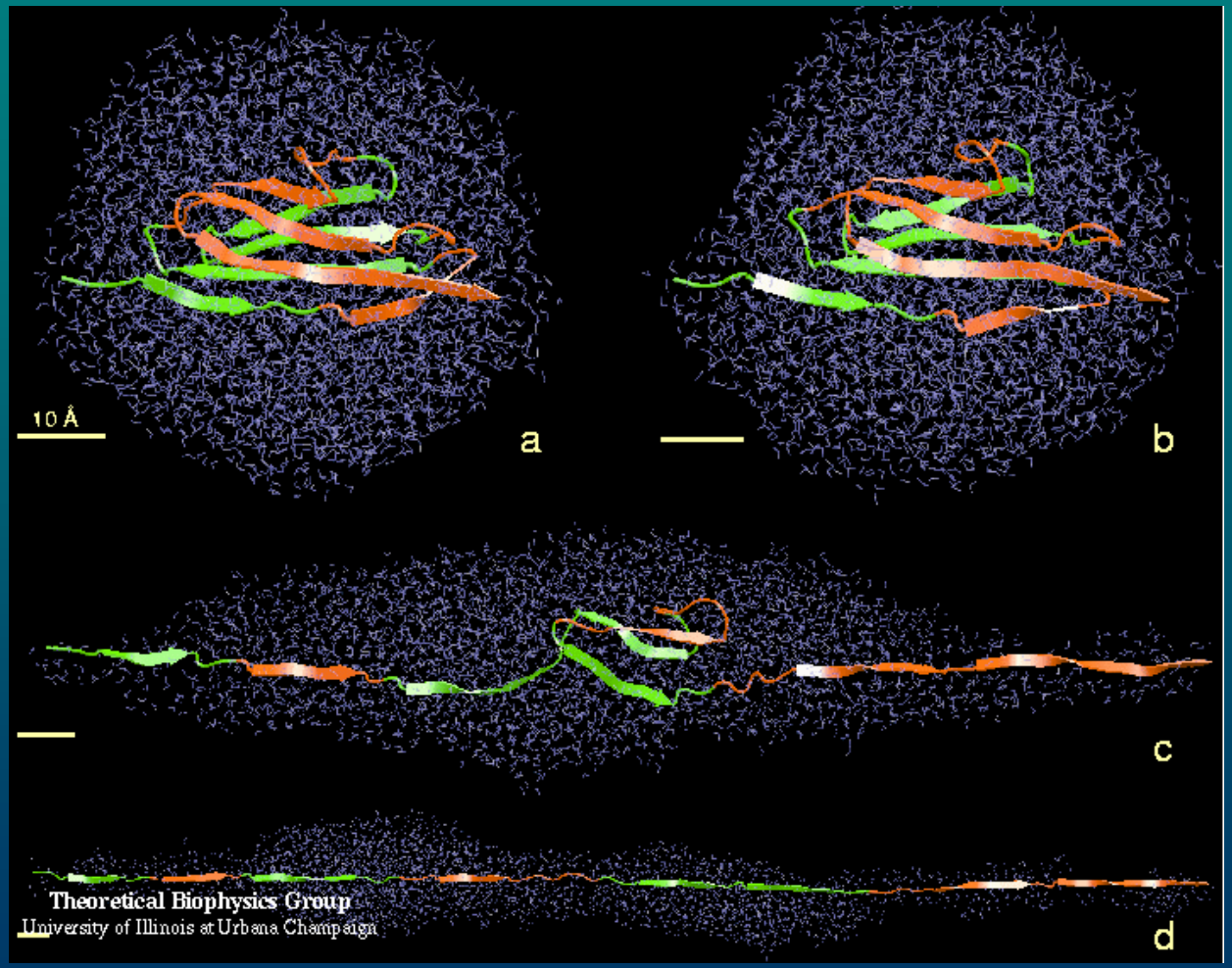
Titin in the sarcomere



The giant muscle protein titin (connectin), is a roughly 30,000 amino acid long filament which plays a number of important roles in muscle contraction and elasticity

www.uni-muenster.de/Biologie.AllgmZoo/AGLinke/PAGES/GENERAL/RESEARCH/research3.htm

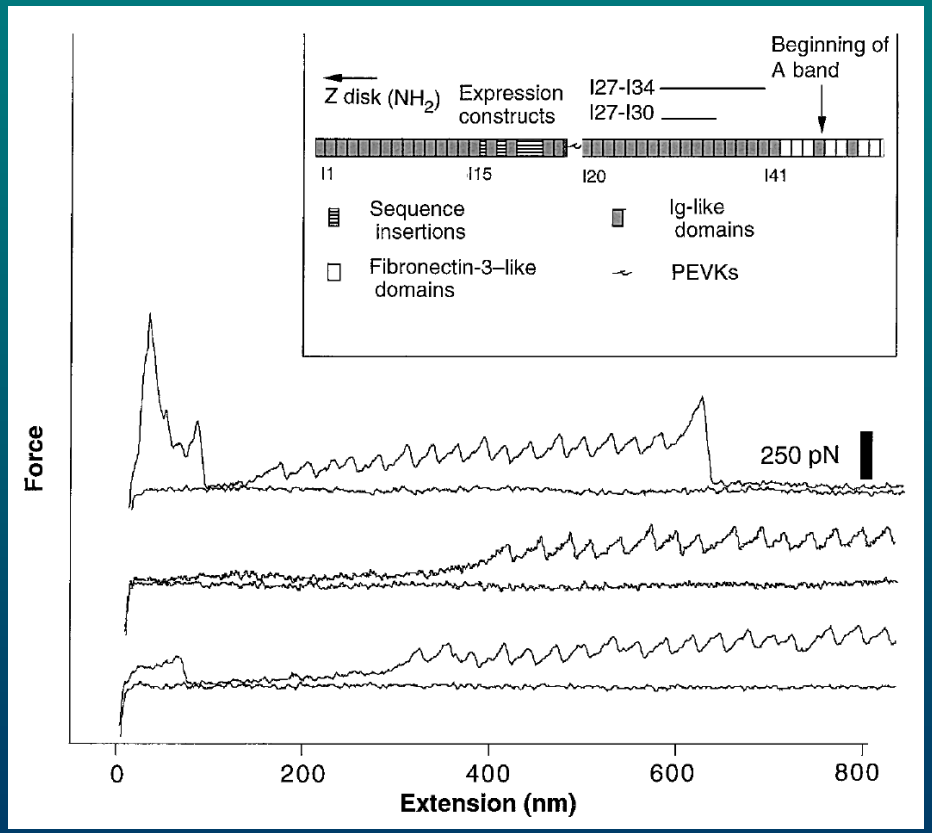
Molecular Dynamic Simulations of Titin Unfolding



http://www.ks.uiuc.edu/Research/smd_imd/titin

Stretching Single Titin Molecule with Atomic Force Microscopy

- First demonstrate in 1997 to stretching native titin
- Force-extension curves show sawtooth pattern
- Domain unfolds under mechanical force



Rief et al., Science (1997)

Nonequilibrium Work Theorem

Protein Folding Thermodynamics

- Energy profile for a two state system

A: native state

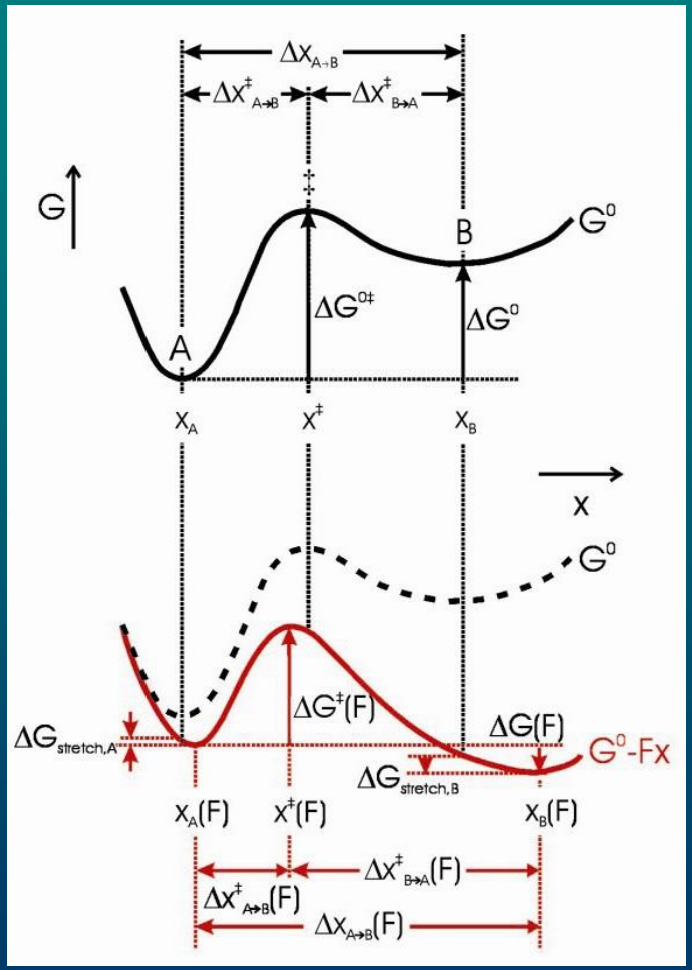
B: denatured state

‡: transiton state

$X^{‡}_{A->B}$: distance between native and transition states

ΔG^0: stability of the protein

- The rate constant for unfolding is related to $\Delta G^{0‡}$
- Application of force changes the free energy profile



Bustanamte *et. al, Annu. Rev. Biochem.* (2004)

Jarzynski's Equality

$$\langle e^{-\beta W_\lambda} \rangle_N \equiv \int dW_\lambda \rho(W_\lambda) e^{-\beta W_\lambda} = e^{-\beta \Delta G}$$

$\rho(w)$ Work distribution

- Relates equilibrium properties from nonequilibrium measurements
- Thermodynamics $W \geq \Delta G$

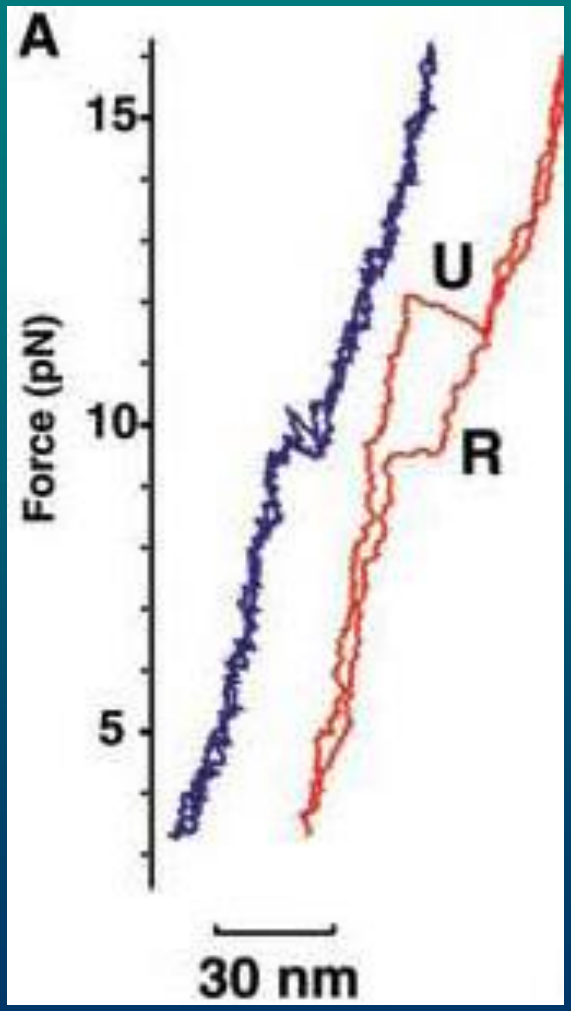
Jarzynski, Phys. Rev. Lett. 78, 2690 (1997)

Experimental Test of Jarzynski's Equality: RNA Folding Experiment

- Force-extension curves of RNA folding/unfolding.
  - Red: 52 pN switching rate (irreversible)
  - Blue: 2-5 pN switching rate (reversible)
- Integrate from 341 to 371 nm

$\Delta G = 60.2\ k_B T$ (error within 1 $k_B T$)

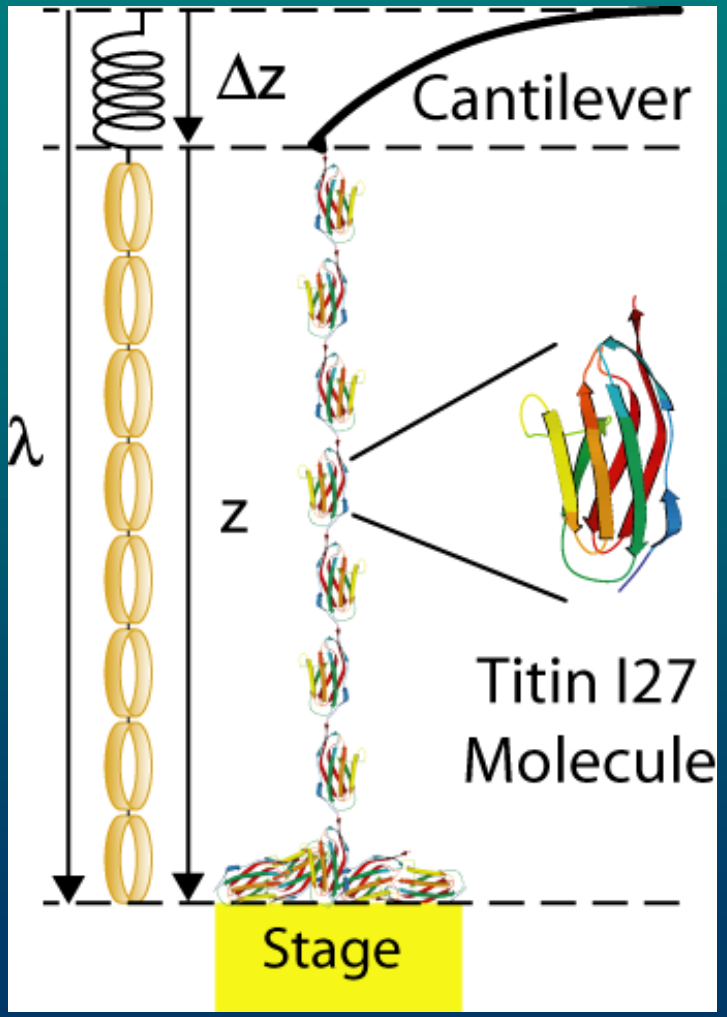
Liphardt et al., Science (2002)



Free Energy Surface Reconstruction

Experimental Procedures

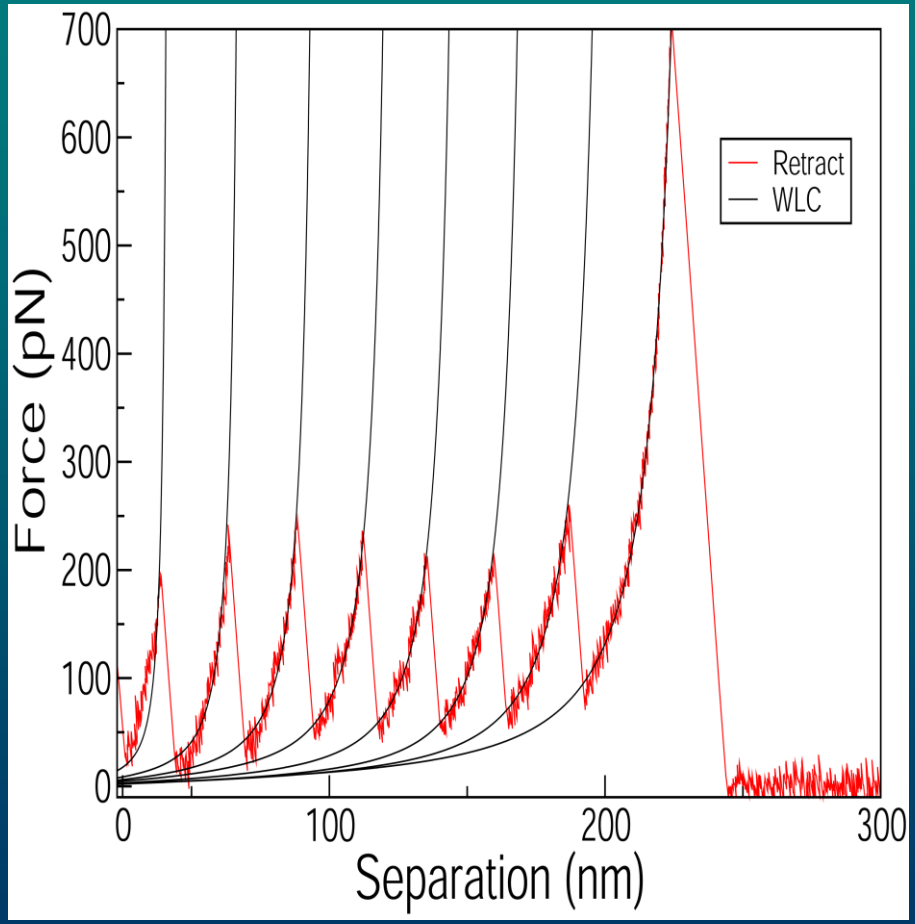
- Pulling engineered 8mer of the I27 domain of the human cardiac titin protein
- Dynamic force spectroscopy done at constant pulling velocities of 0.05, 0.1, and 1.0 μm/s
- 144, 266, 820 titin force-extension curves at each speed were used for calculations
- Determining the entire free energy curve of stretching including free energy barrier of unfolding



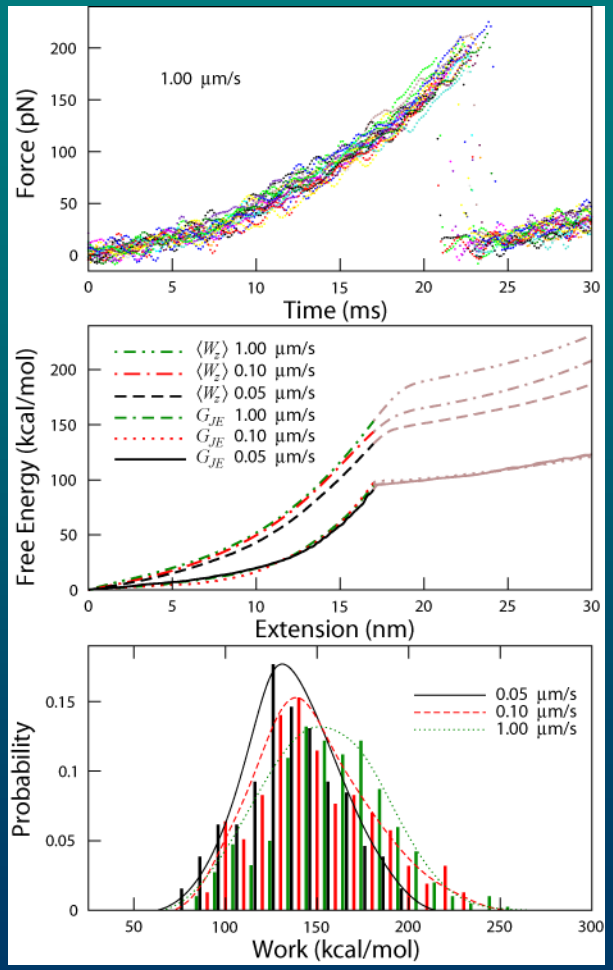
Force-Extension Curves

- Typical sawtooth pattern of the force–extension curve of $(I27)_8$
- Force peaks near 200 pN: Ig-domain unfolding
- Last peak: rupture of the polymer from the sites of attachment
- Fits worm-like-chain (WLC) model

$$F(x) = \frac{k_B T}{p}\left(\frac{1}{4(1 - x/L)^2} - \frac{1}{4} + \frac{x}{L}\right)$$



Mechanical Unfolding of Titin I27



- Align force-extension curves at the transition state
- Jarzynski's equality averages same z
- Shown are 20 curves taken at 1 μm/s pulling velocity
- Work distribution depends on pulling velocity

Histogram Method

$$e^{-\beta G(z)} = \langle \delta(z - z_t) e^{-\beta[W_z(t) - U_0(z_0, \lambda_A)]} \rangle$$

$$\exp[-\beta G(z^{(m)})] \approx \frac{1}{NT} \sum_{s=1}^{T} \sum_{n=1}^{N} \delta_\epsilon(z^{(m)} - z_{n,s}) \exp(-\beta[W_{n,s} - U(z_{n,0}, \lambda_A)])$$

$z^{(m)}$: z from the mth bin

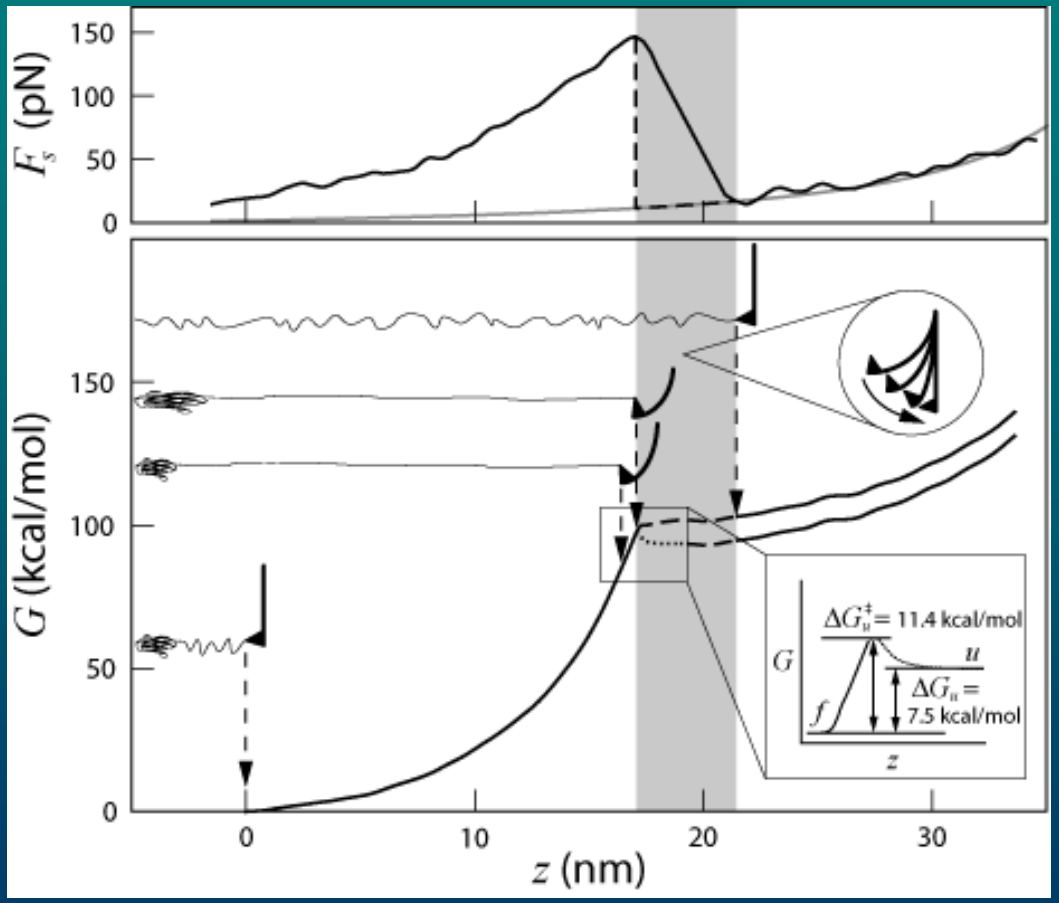
N: number of realizations

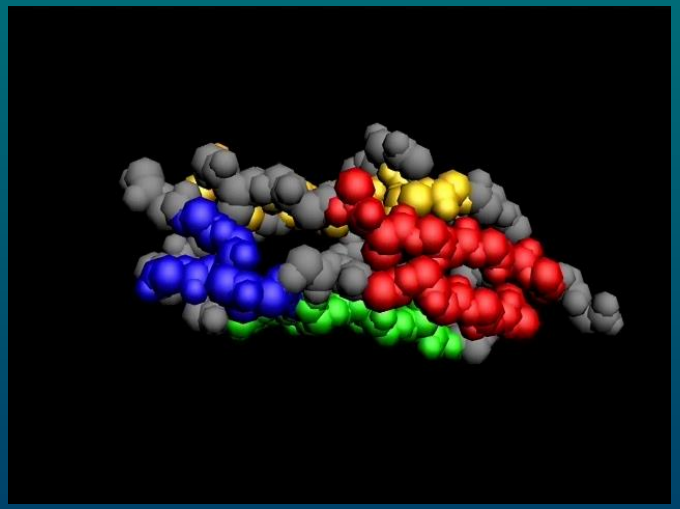
T: time

U: potential energy stored in the cantilever

Hummer and Szabo, Proc. Nat. Acad. Sci. 98, 3658 (2001)

Free Energy Surface of I27 Stretching and Unfolding





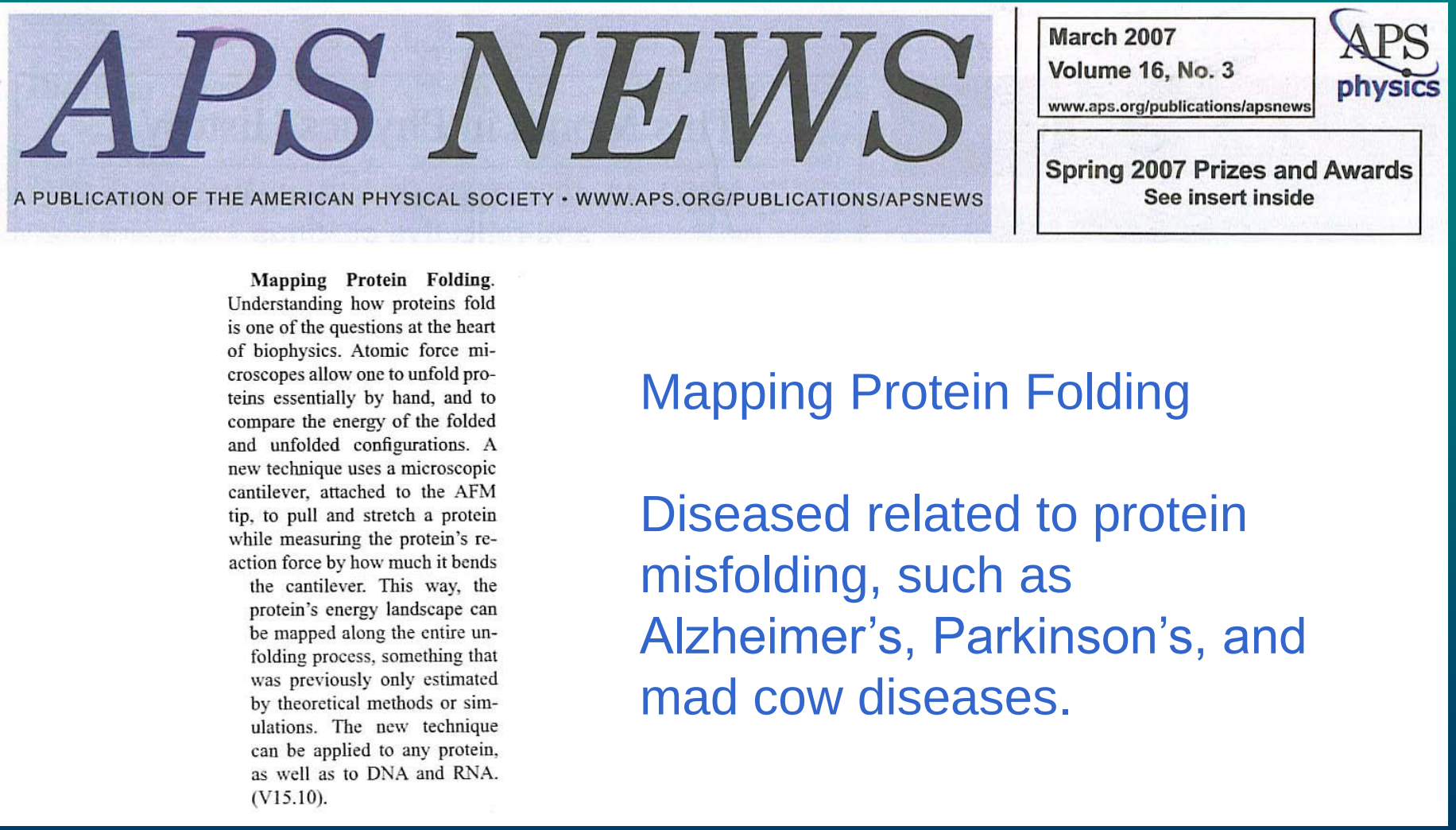
Harris, Song, and Kiang, ***Phys. Rev. Lett.*** , *99*, 068101 (2007).

Free Energy Curves of Stretching

- Using 6 Å as the distance between the native and the transition state, we determine the free energy barrier of unfolding I27 to be 11 kcal/mol
- The result compares favorably with previous estimates using chemical denaturation and other force-peak distribution methods,

  10-22 kcal/mol using $k = k_o \, exp(-\Delta G / k_B T)$

William et. Al., Nature, 422, 449 (2003)
Hummer and Szabo, Proc. Nat. Acad. Sci. 98, 3658 (2001)
Vasquez and Fernandez, Proc. Nat. Acad. Sci. 96, 3694 (1999)



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APS physics

Spring 2007 Prizes and Awards
See insert inside

Mapping Protein Folding. Understanding how proteins fold is one of the questions at the heart of biophysics. Atomic force microscopes allow one to unfold proteins essentially by hand, and to compare the energy of the folded and unfolded configurations. A new technique uses a microscopic cantilever, attached to the AFM tip, to pull and stretch a protein while measuring the protein's reaction force by how much it bends the cantilever. This way, the protein's energy landscape can be mapped along the entire unfolding process, something that was previously only estimated by theoretical methods or simulations. The new technique can be applied to any protein, as well as to DNA and RNA. (V15.10).

Mapping Protein Folding

Diseased related to protein misfolding, such as Alzheimer's, Parkinson's, and mad cow diseases.

SCIENCE NEWS
This Week

Pulling Strings

Stretching proteins can reveal how they fold

Proteins, long strings of amino acids, spontaneously fold into intricate shapes that enable them to perform a cell's dazzling variety of functions. To better understand the forces that determine these shapes, scientists have developed a technique for stretching a protein to follow in reverse the path it took when folding.

"The basic idea is to pull the molecule at both ends to stretch it and see what happens," says Ching-Hwa Kiang, a biological physicist at Rice University in Houston.

When a cell builds a protein, it links amino acids that pivot around each other and interlock. These movements are dictated by electrostatic forces between the amino acids and by their tendency to hide their water-repelling sides while leaving their water-loving sides exposed.

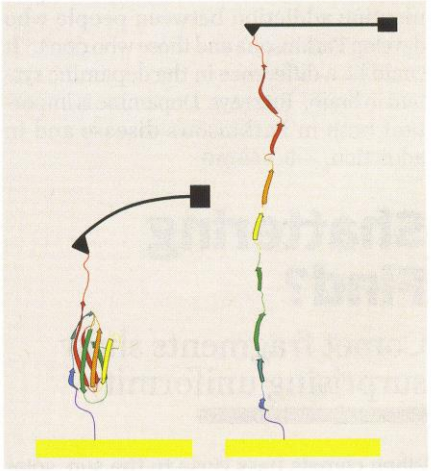
A fully folded protein is in a state of minimum energy because force must be applied to pull it apart. Kiang and her Rice collaborators devised a technique to measure that force. They placed water droplets containing proteins on a movable surface below a microcantilever akin to a tiny diving board. The researchers fished for proteins by varying the distance between the surface and the cantilever. When the cantilever snagged one end of a protein, the scientists could pull back the surface, slowly unfolding the protein.

The bending of the cantilever indicated the force required to stretch the protein. The researchers tested their technique on a synthetic version of the muscle protein titin, consisting of a chain of eight identical amino acid strings. As the researchers stretched the protein, the strings unfolded one after the other, generating the same sequence of force measurements each time. The team reports its findings in an upcoming issue of *Physical Review Letters*.

Unfolding a protein requires energy to overcome friction between molecules in addition to the energy needed to counter molecular forces. To tease apart these effects, the team used a mathematical technique invented in 1997 by Christopher Jarzynski, now at the University of Maryland at College Park. That analysis took into account the reductions in measured force due to random molecular jiggling that sometimes kicked the protein into an unfolded state.

The researchers plan to apply their technique to other proteins. They also hope to measure the energy required to unzip the double helix of DNA. Kiang says that researchers could also use the technique to test whether environmental conditions such as acidity or temperature affect folding. Scientists believe that misfolded proteins may cause certain diseases, including Alzheimer's.

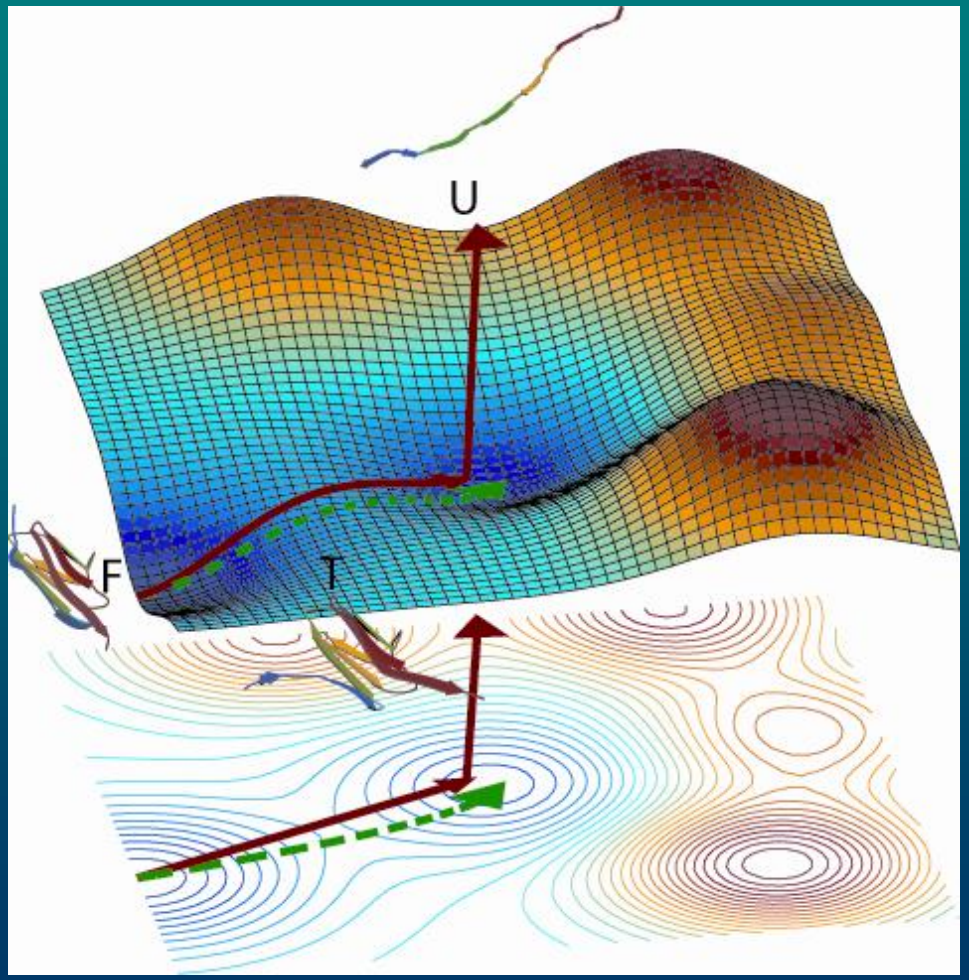
Kevin Plaxco of the University of California, Santa Barbara says that scientists are eager to find methods for mapping the energy of proteins. While the new technique traces only one possible way that a protein unfolds, as opposed to the full range of a protein's possible states, "it's the most concrete example I've seen," of such a measurement, he says. —D. CASTELVECCHI



SCIENCE; KIANG/RICE UNIV.

FISHING FOR MOLECULES The bending of a microscopic cantilever reveals the force required to unfold a protein.

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

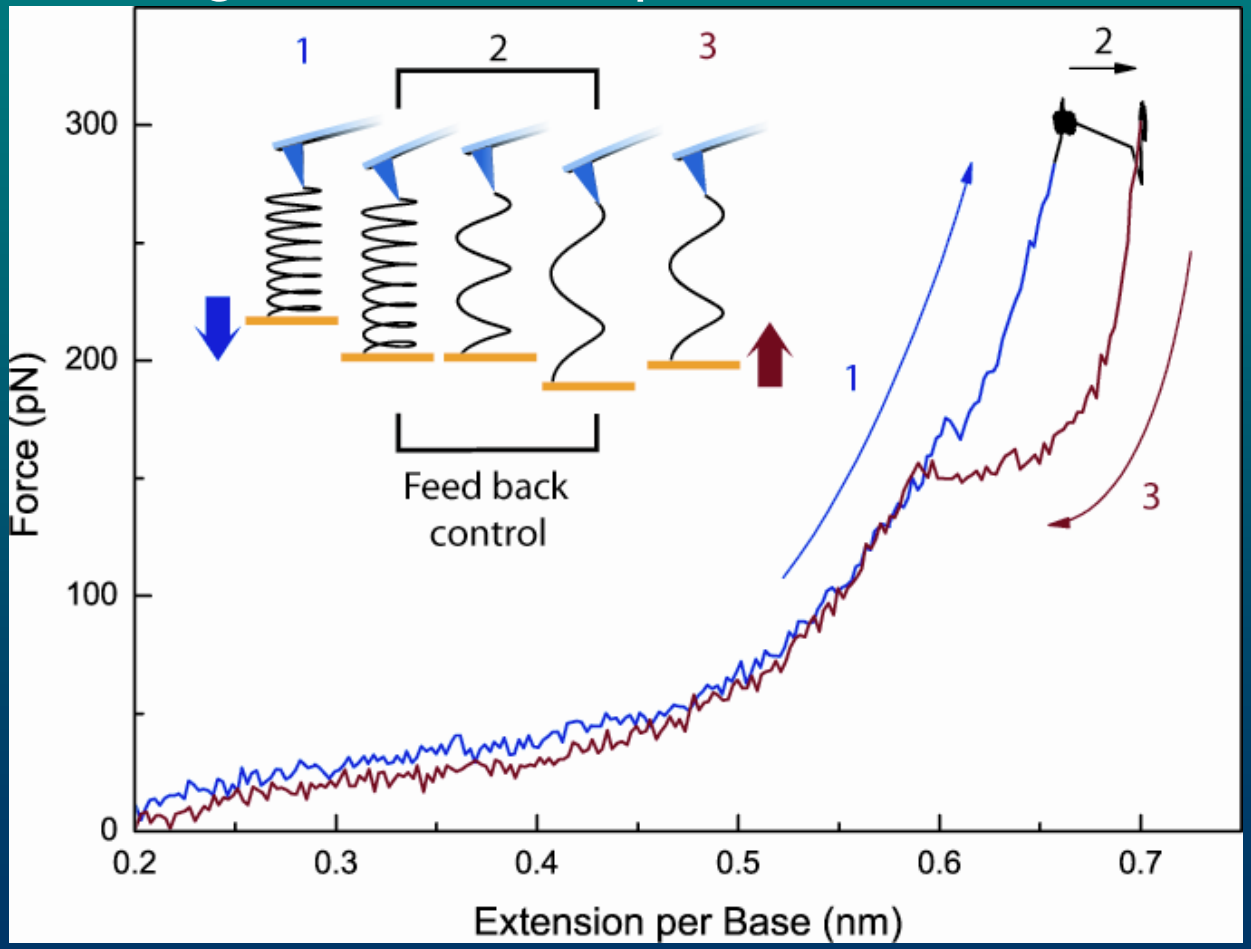


Other Applications

- DNA mechanics
- Protein-nucleic acid interactions in Virus
- Molecular adhesion

DNA Mechanics

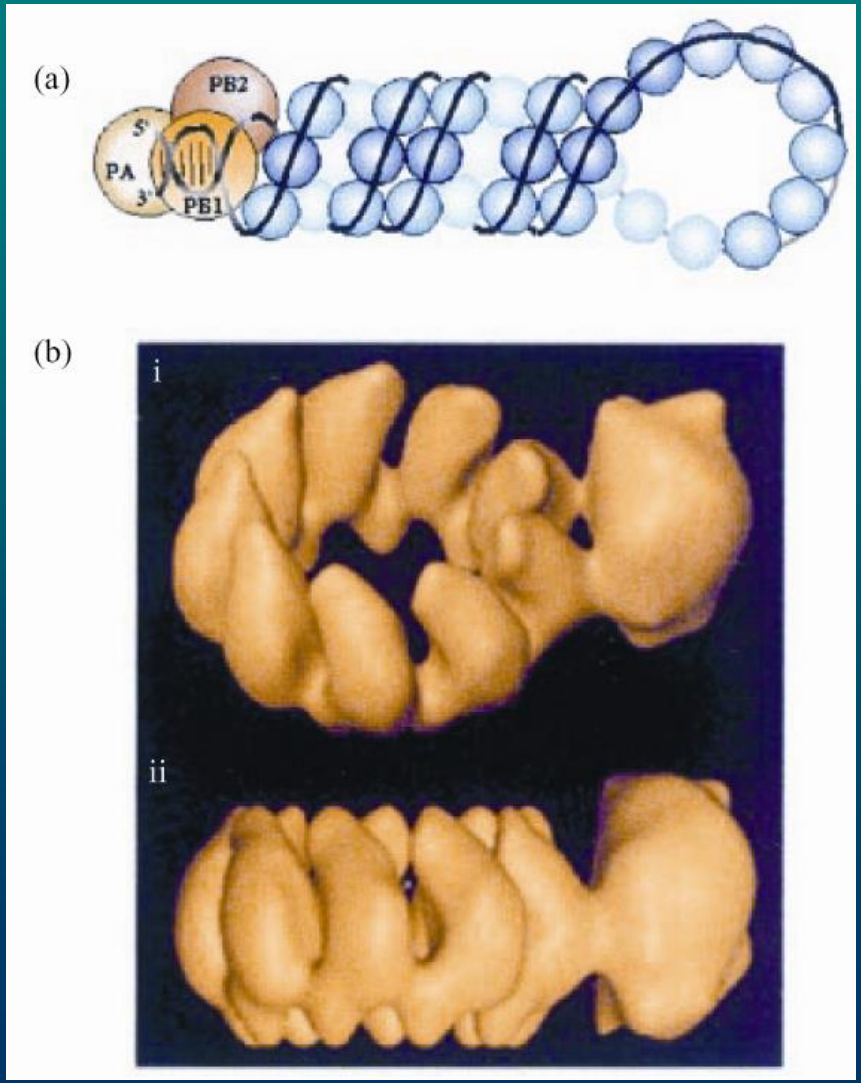
- Understanding the nature of protein-DNA interactions.



Protein-Nucleic Acid Interactions

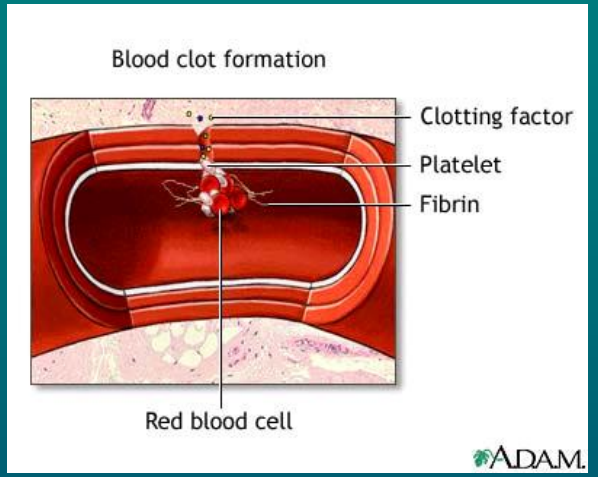
Influenza Virus

Ribonucleoprotein complex



Von Willebrand Factor (VWF)

- A large multimeric protein circulating in blood
- Critical for bleeding arrest at sites of vascular injury.
- Serving as a protective carrier for Factor VIII and mediating platelet adhesion and aggregation
- Hemostatically inactive unless exposed to high fluid shear stress
- The ultra-large form of VWF is hyperactive
- Undergoes conformational change when activated
- Inactivity may results in bleeding disorder while hyperactivity may causes thrombosis



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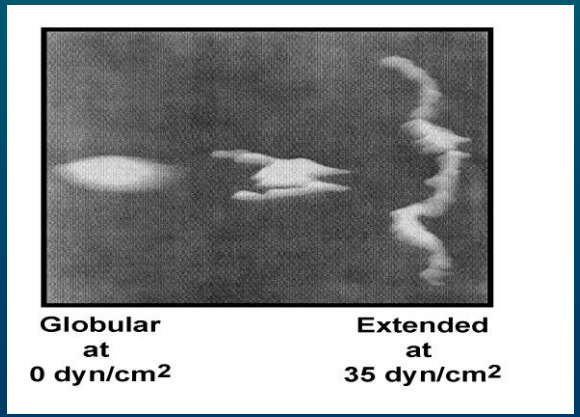
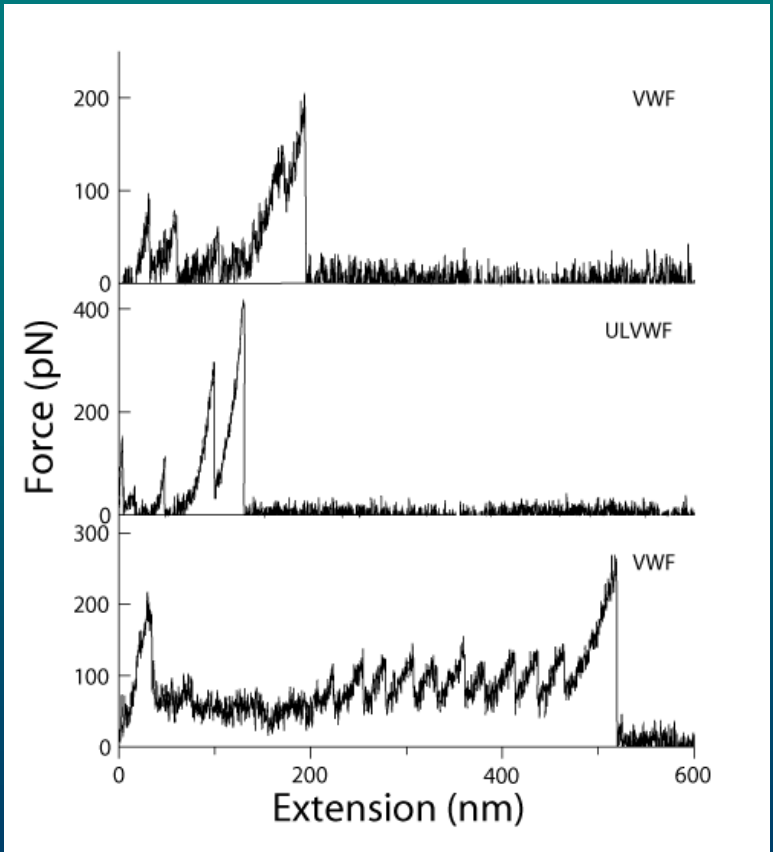


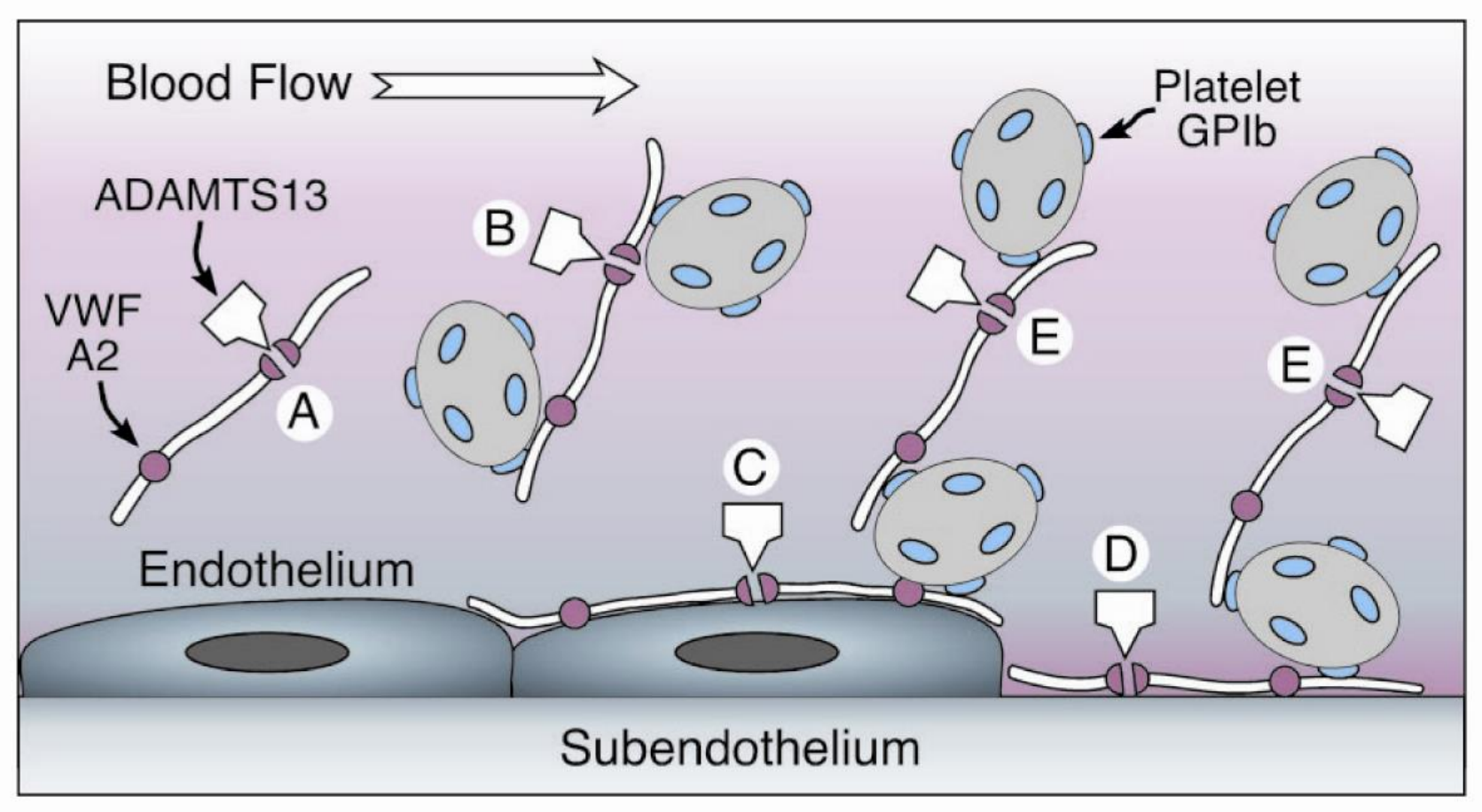
Siedlecki, C. et. Al. Blood 1996: 2939

Force-Extension of VWF



- Single-molecule pulling using AFM shows different force signature in characteristic force-extension curves for plasma VWF, ULVWF, and sheared VWF.
- The force peaks are attributed to unfolding of the domain in VWF, and the force peak is related to its mechanical resistance to unfolding.
- Ultra-large form and sheared VWF shows higher mechanical resistance to unfolding, consistent with the adhesion activities.

Molecular Adhesion



Conclusion

I DNA Melting

- DNA-gold is a new class of complex fluids with unusual phase behavior
- Understanding and developing strategy for obtaining quantitative data for proper interpretation of sensor results
- Mechanical manipulation of DNA provides new insight into the melting behavior of DNA

II Single-molecule manipulation

- Single-molecule manipulation opens a door for observation of events not previously accessible
- Jarzynski's equality allows free energy surface reconstruction and barrier height determination
- Can be used to investigating complex interactions such as protein-cell interactions, genome packaging, and virus assemblies.

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