Single-Molecule Manipulation Experiments of Biological Molecules II: Principles Involved in Interpreting Force Measurements

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Acknowledgment

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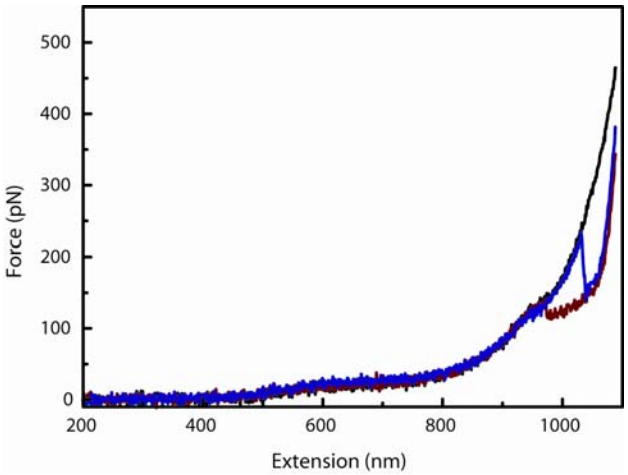
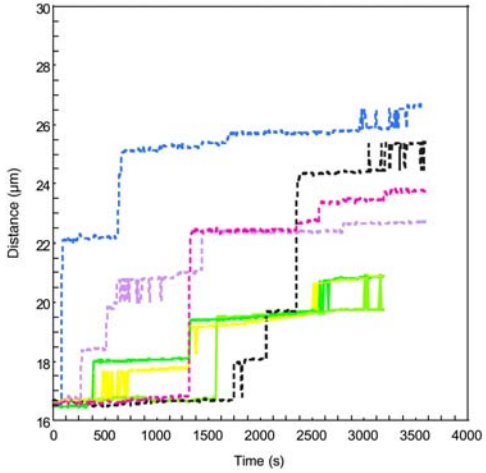
- Wenshi Chen
- Sithara Wijeratne
- Eric Frey
- Nolan Harris
- Wei-Hung Chen
- Eric Botello
- Young Sun
- Jacob Sargent
- Eric Rowe

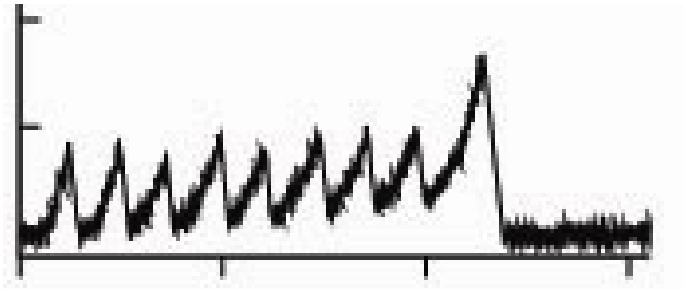
Funding

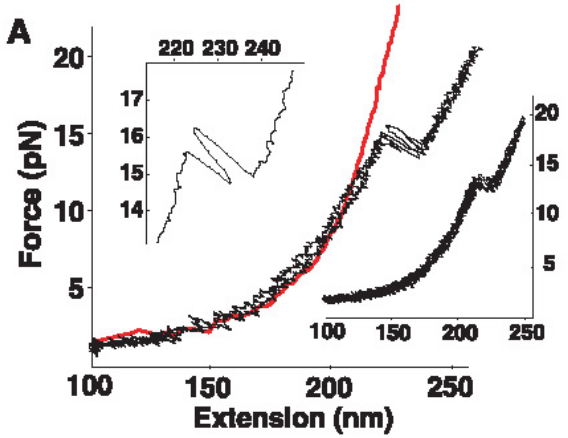
- National Science Foundation
- National Institutes of Health
- The Welch Foundation
- Hamill Foundation

Examples of Single-Molecule Manipulation Data









Danilowicz et al. ***PNAS*** 100, 1694 (2003).
Liphardt *et. al.*, ***Science*** 292, 733 (2001).
Botello *et. al*, ***J. Phys. Chem. B*** (2009) in press.

Outline

- Equilibrium information from nonequilibrium measurements
- Jarzynski's equality
- Experimental free energy curve reconstruction

Protein Folding Thermodynamics

- Energy profile for a two state system

A: native state

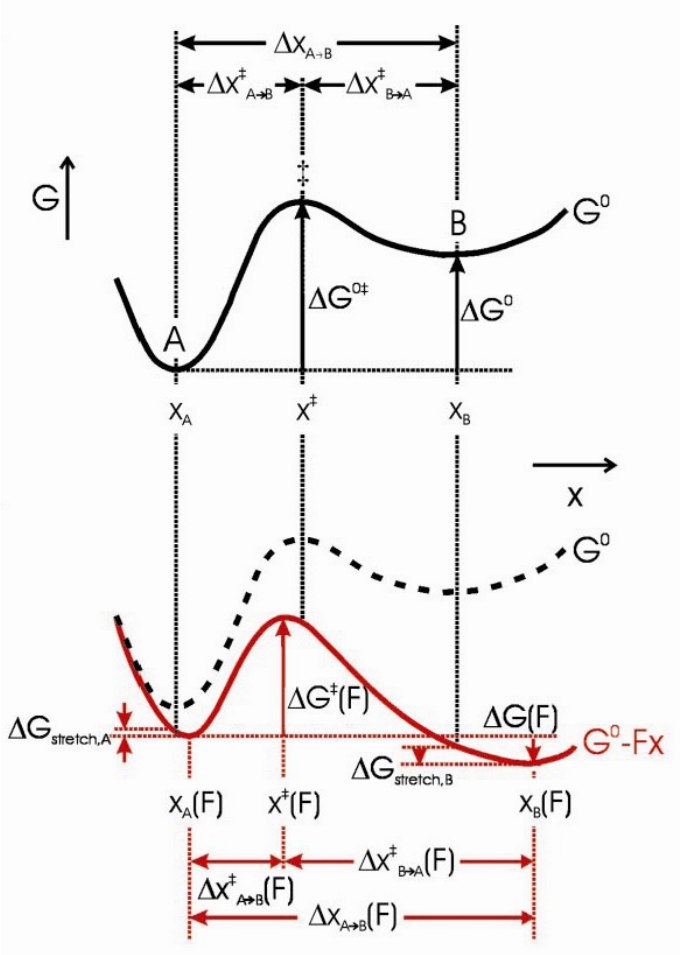
B: denatured state

‡: 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

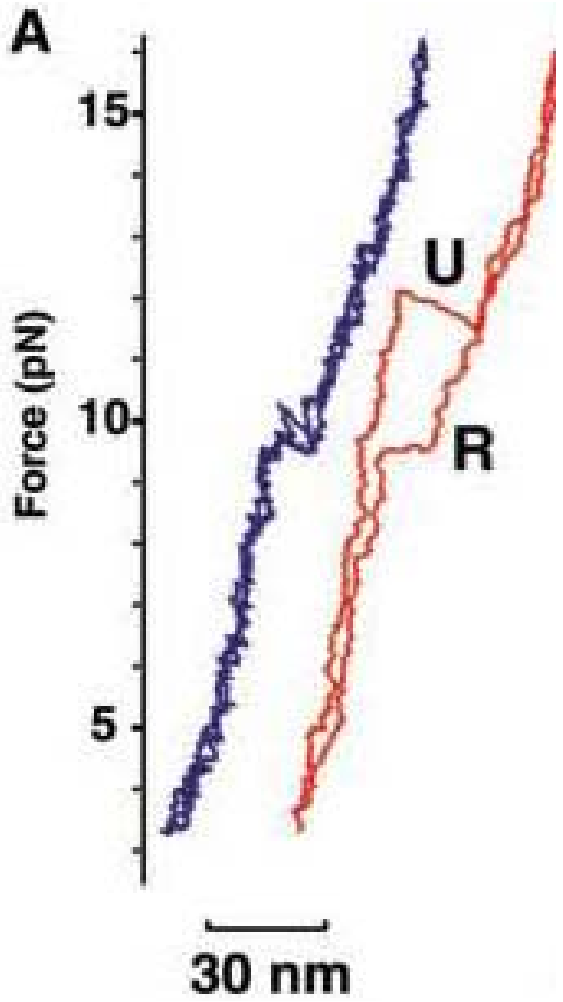
Jarzynski, ***Phys. Rev. Lett.*** *78, 2690 (1997)*

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

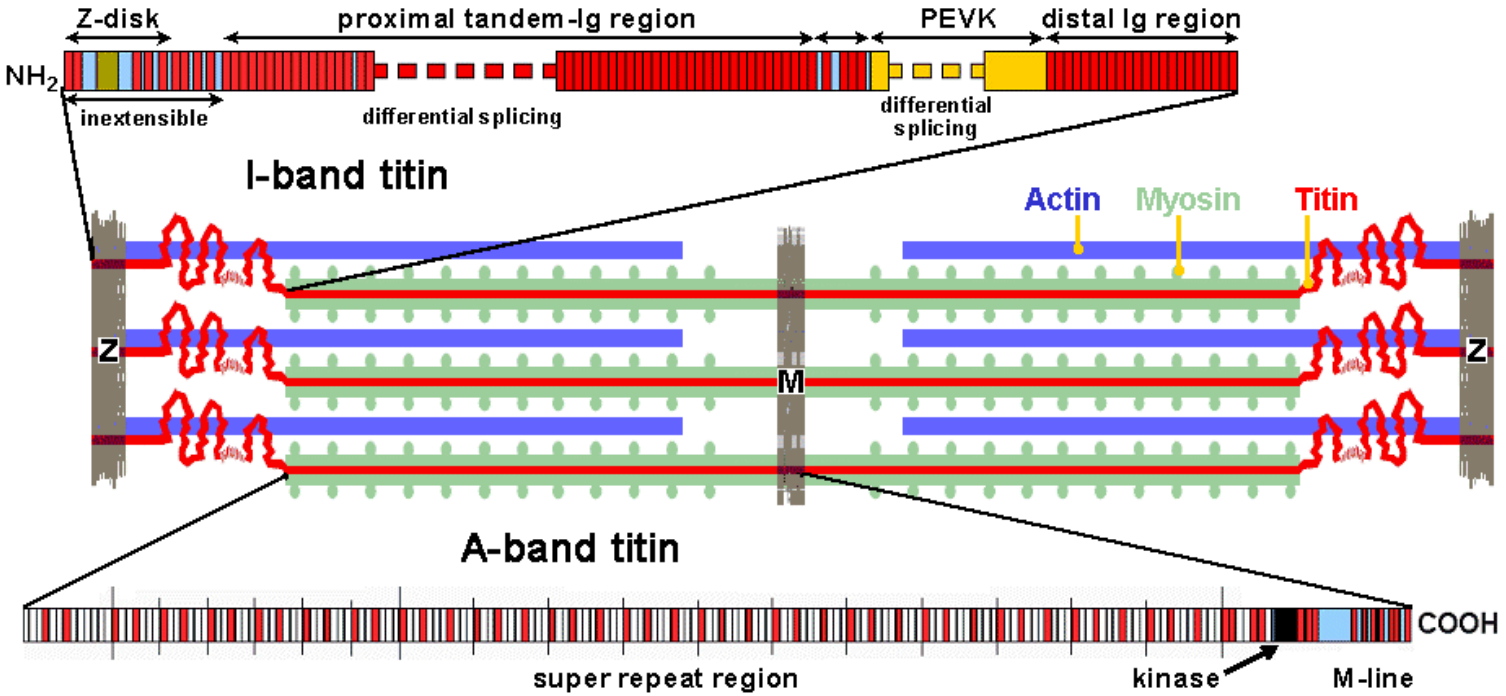
$\Delta G = 60.2\ k_B T$ (error within 1 $k_B T$)

Liphardt *et al.*, ***Science***, 296, 1832 (2002).



Ching-Hwa Kiang

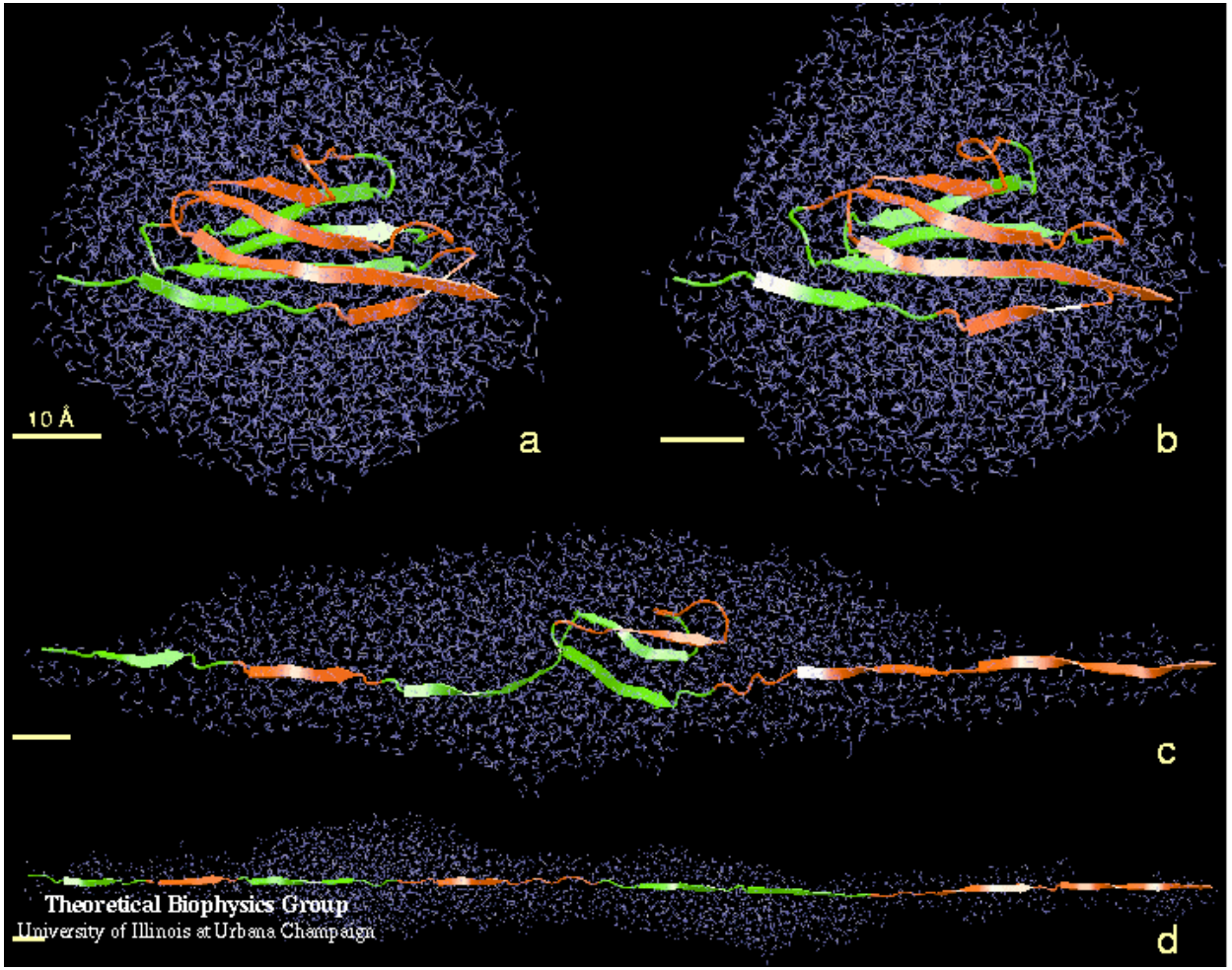
Titin in the sarcomere



- The giant muscle protein titin (connectin), is 30,000 amino acid long
- Titin play an important role in muscle contraction and elasticity

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

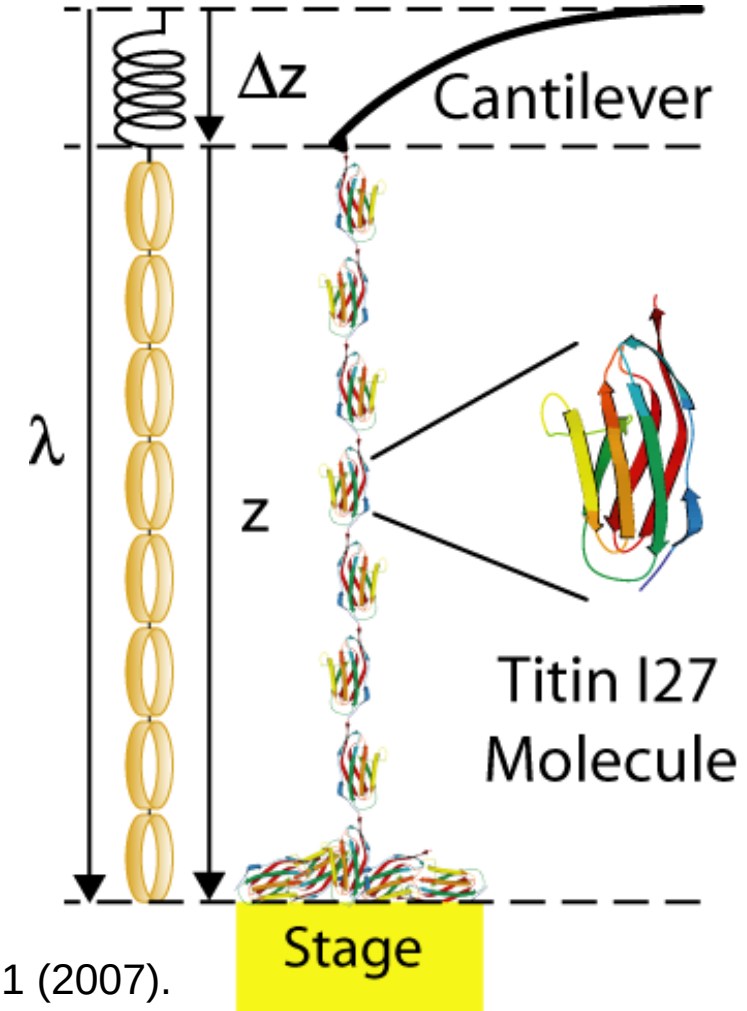
Molecular Dynamic Simulations of Titin Unfolding



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

Experimental Procedures

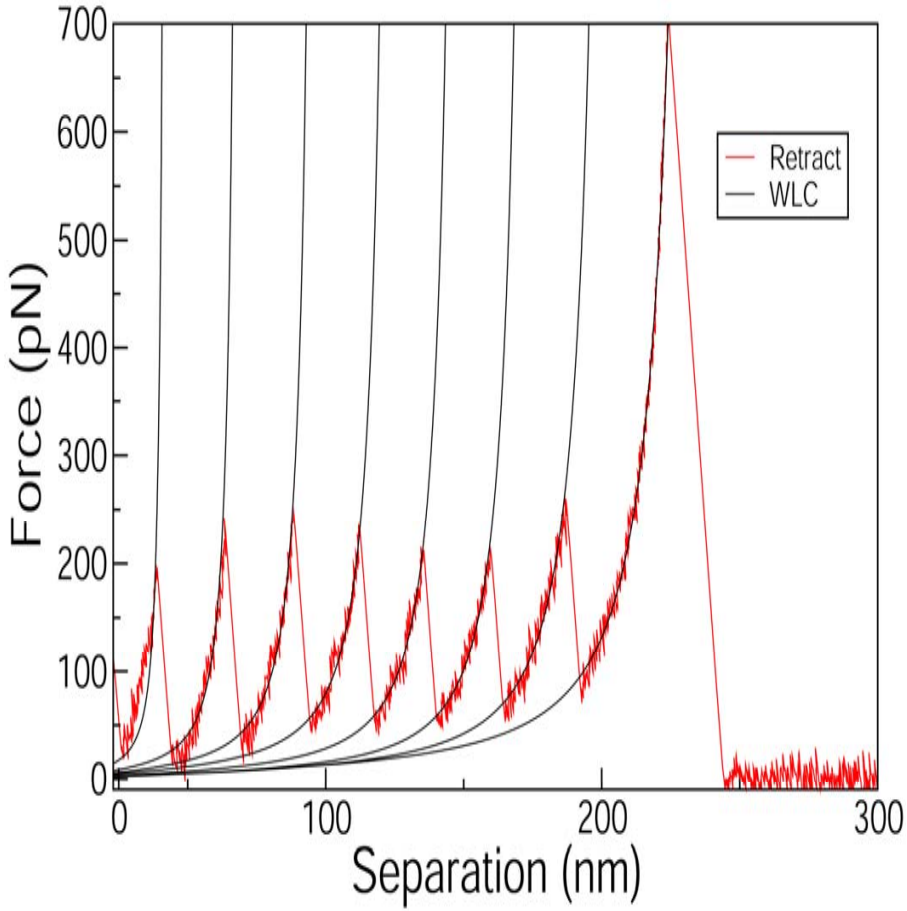
- Pulling engineered 8mer of the I27 domain of the human cardiac titin protein
- Several hundred force-extension curves at each speed were used for calculations
- Determining the entire free energy curve of stretching including free energy barrier of unfolding



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

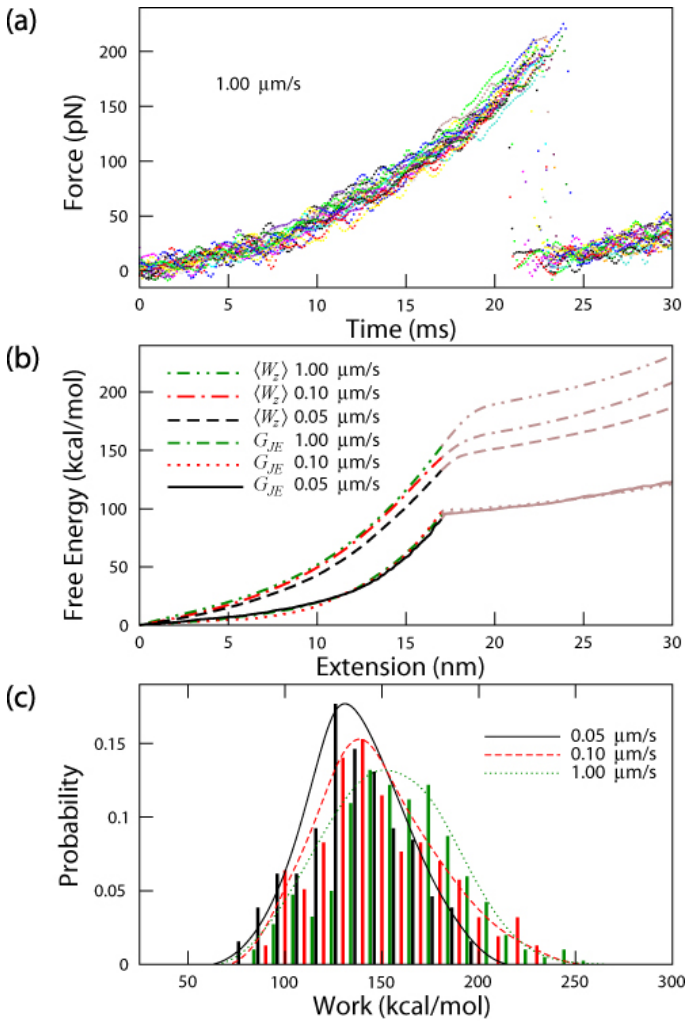
Force-Extension Curves

- 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

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

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

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

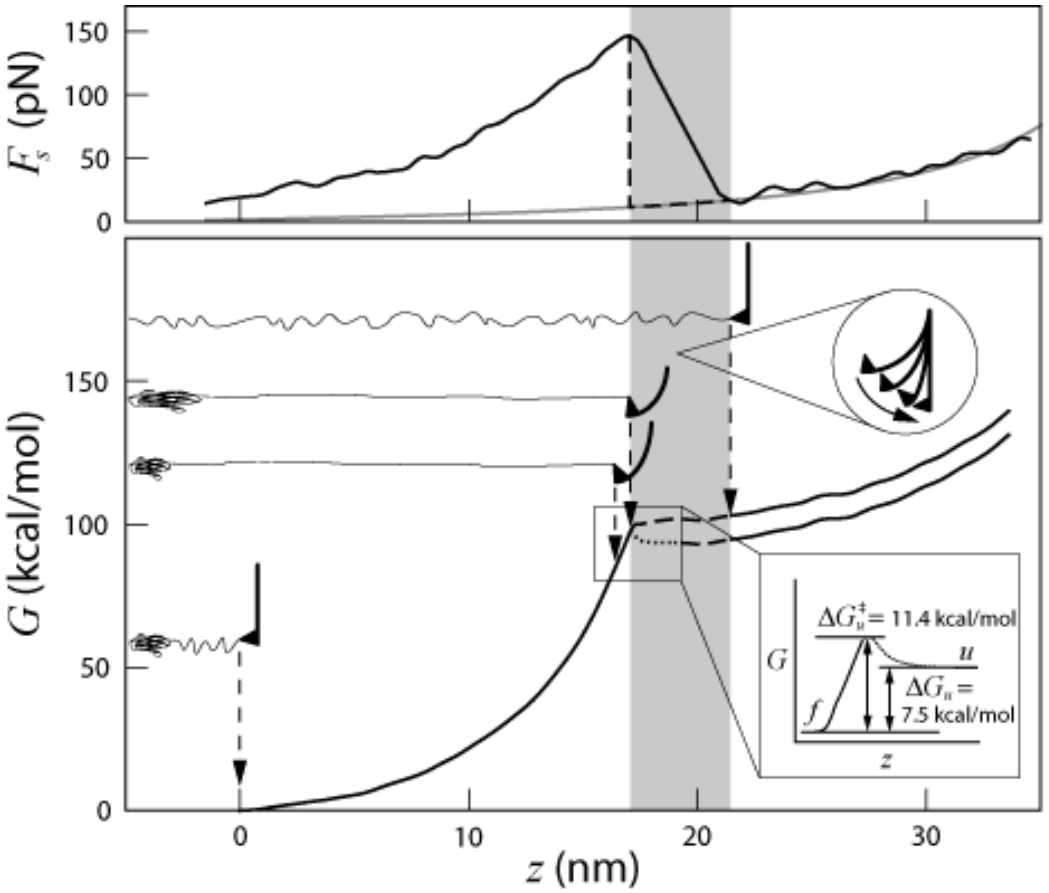
N: number of realizations

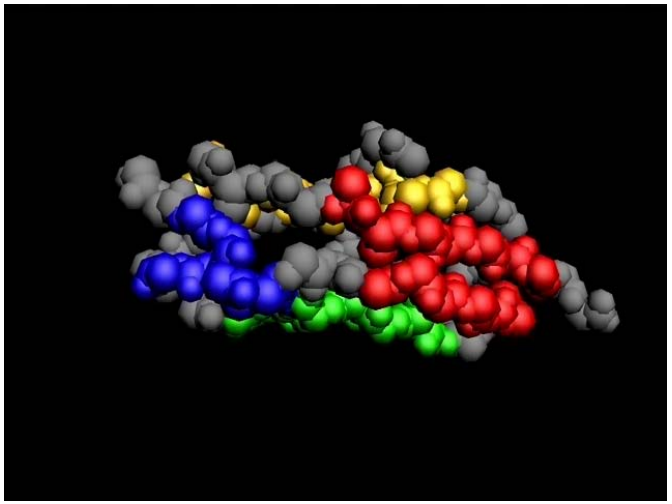
T: time

U: potential energy stored in the cantilever

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

Free Energy Surface of I27 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)

Experimental Free Energy Surface Reconstruction from Single-Molecule Force Spectroscopy using Jarzynski's Equality

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Department of Physics and Astronomy, Rice University, Houston, Texas 77005, USA

(Received 25 July 2006; published 6 August 2007)

We used the atomic force microscope to manipulate and unfold individual molecules of the titin I27 domain and reconstructed its free energy surface using Jarzynski's equality. The free energy surface for both stretching and unfolding was reconstructed using an exact formula that relates the nonequilibrium work fluctuations to the molecular free energy. In addition, the unfolding free energy barrier, i.e., the activation energy, was directly obtained from experimental data for the first time. This Letter demonstrates that Jarzynski's equality can be used to analyze nonequilibrium single-molecule experiments, and to obtain the free energy surfaces for molecular systems, including interactions for which only nonequilibrium work can be measured.

DOI: 10.1103/PhysRevLett.99.068101 PACS numbers: 87.15.He, 87.14.Ee, 87.64.Dz

- Harris, Song, and Kiang, ***Phys. Rev. Lett.*** 99, 068101 (2007).
- Nome *et. al.*, ***Proc. Natl. Acad. Sci. USA***, 104, 20799 (2007).
- Preiner *et. al.* ***Biophys. J.*** 93, 930 (2007).

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.

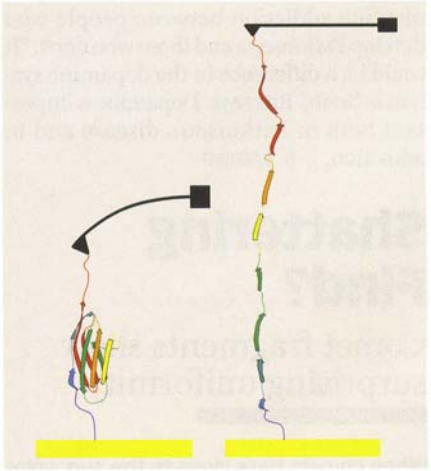
A fully folded protein is in a state of minimum energy because force must be applied to pull it apart. Kiang and her Rice collaborators devised a technique to measure that force. They placed water droplets containing proteins on a movable surface below a microcantilever akin to a tiny diving board. The 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.

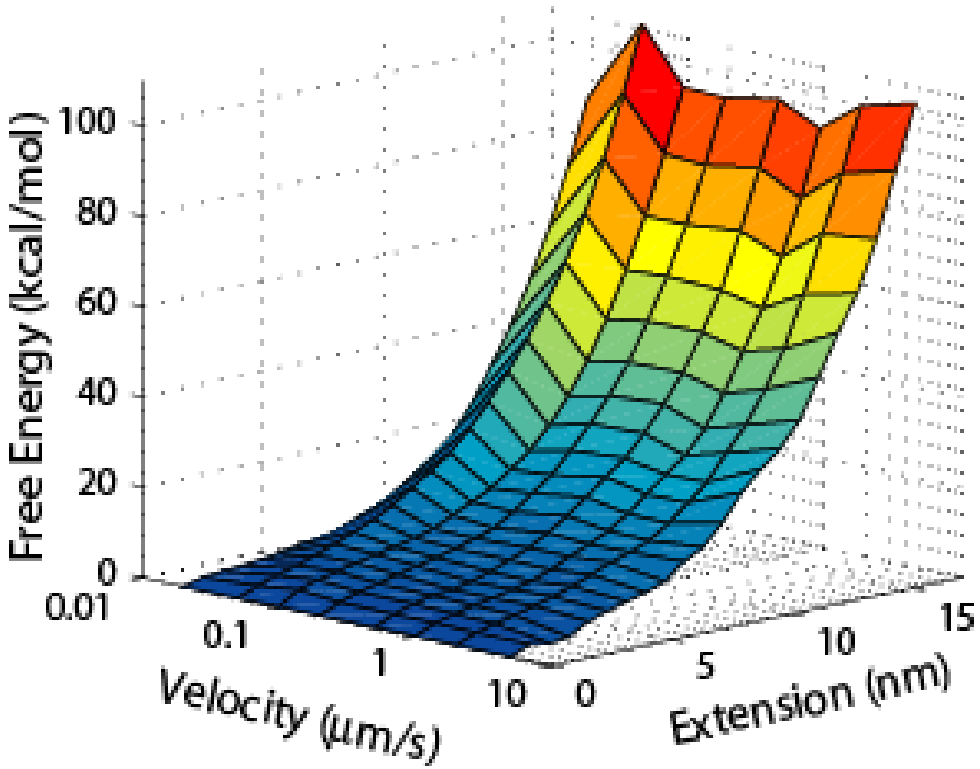
Kevin Plaxco of the University of California, Santa Barbara says that scientists are eager to find methods for mapping the energy of proteins. While the new technique traces only one possible way that a protein unfolds, as opposed to the full range of a protein's possible states, "it's the most concrete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



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

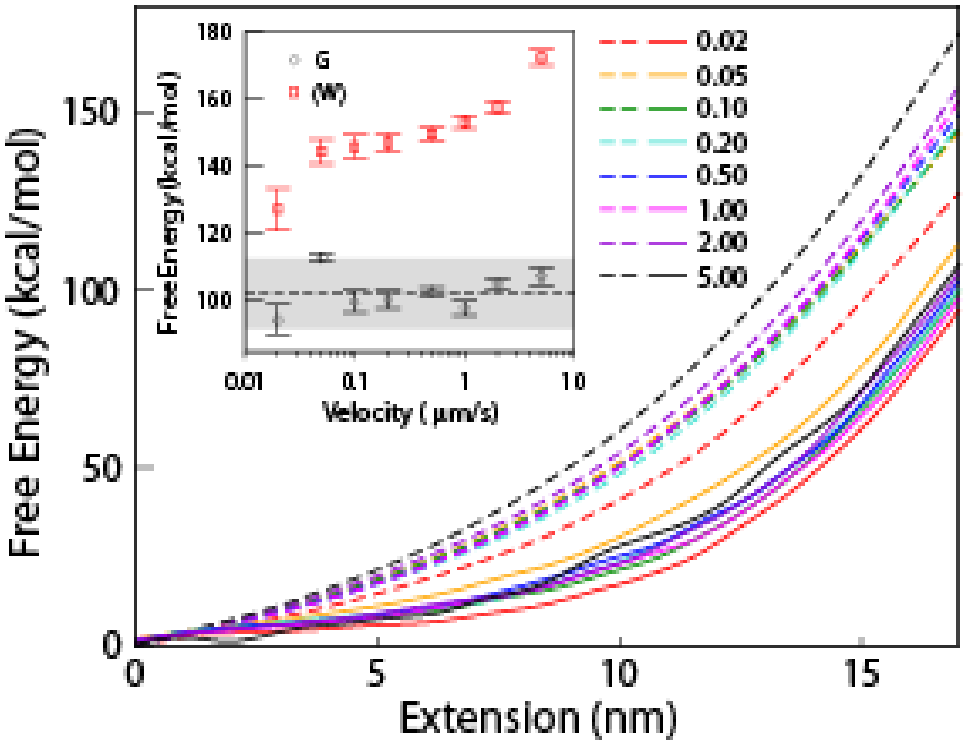
SCIENCE; KIANG/RICE UNIV.

Velocity Convergence of Free Energy Surfaces



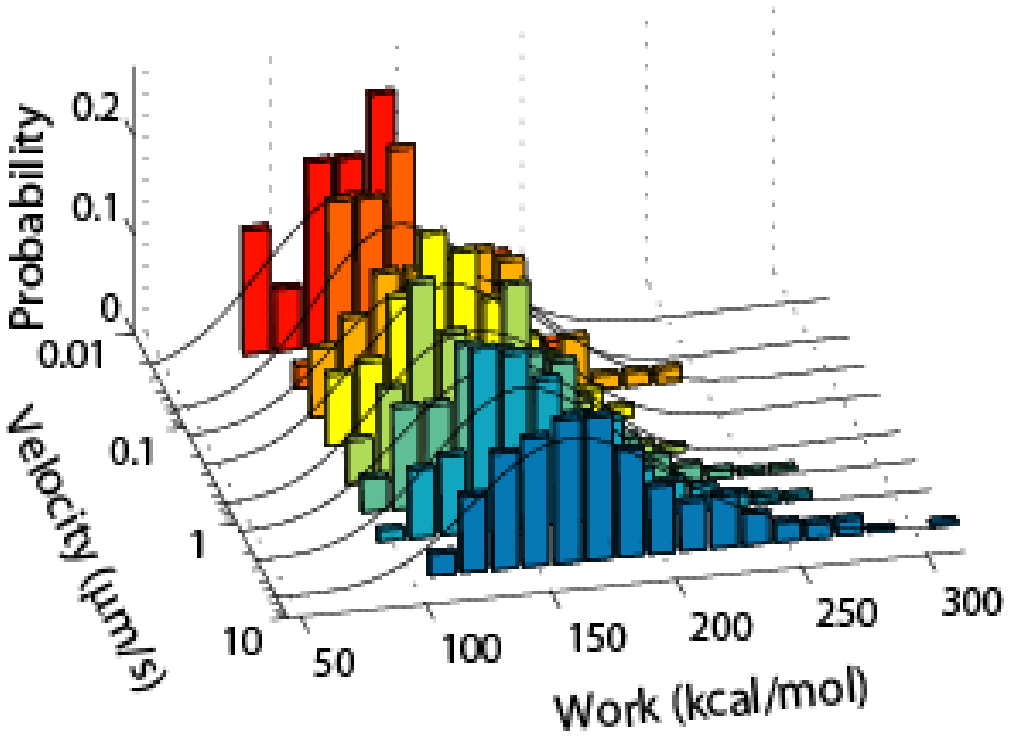
Free energy curves from Jarzynski's equality are independent of velocity

Comparing Results from Jarzynski's Equality with Regular Averaging



Both the free energy curves and the barrier are independent of velocity when using Jarzynski's equality

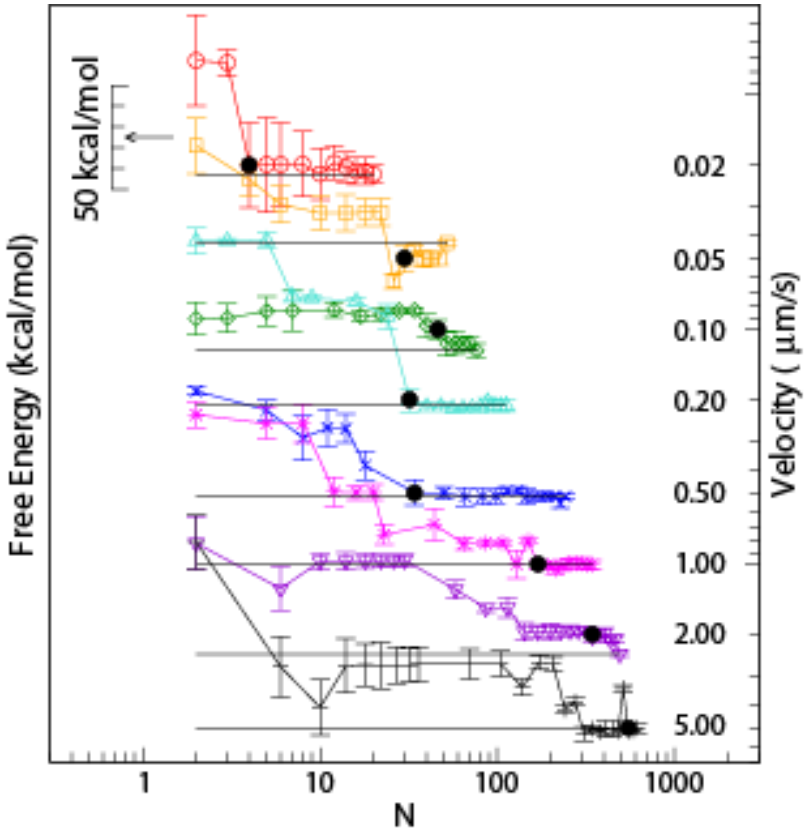
Work as a Function of Velocity



Distribution is non-Gaussian, indicating process nonequilibrium

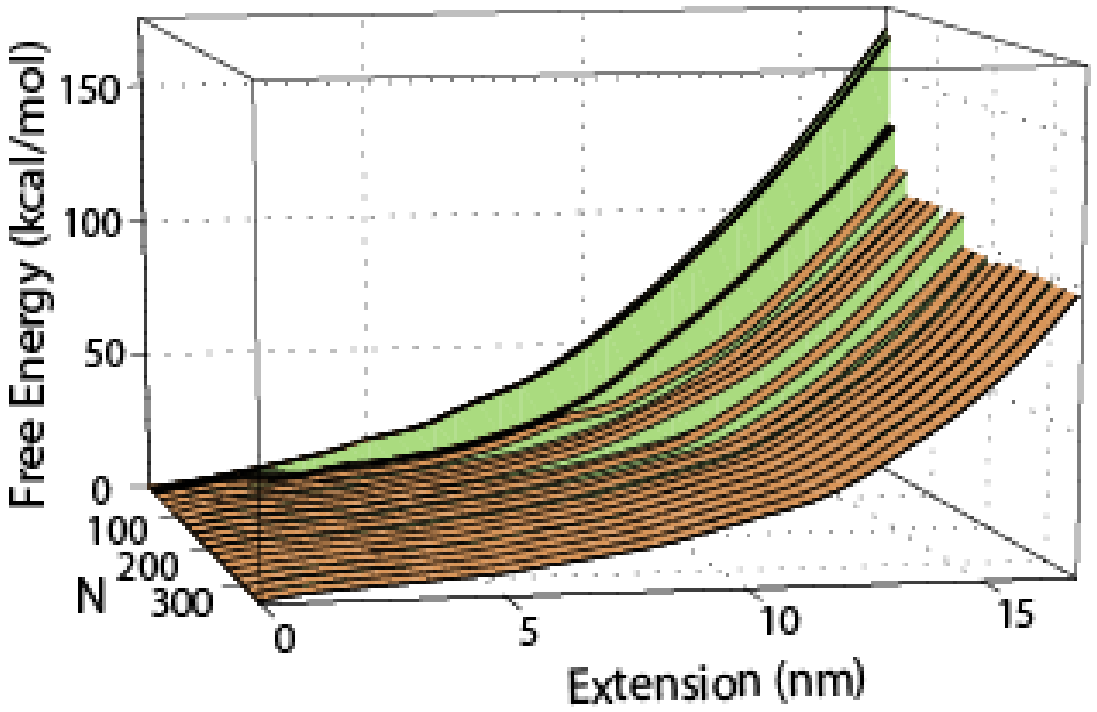
Fast or Slow Pulling?

- Low pulling velocity
  - requires fewer data
  - instrument drift becomes the major source of error
- High pulling velocity
  - Data needed grows exponentially
  - Limited accuracy and resolution



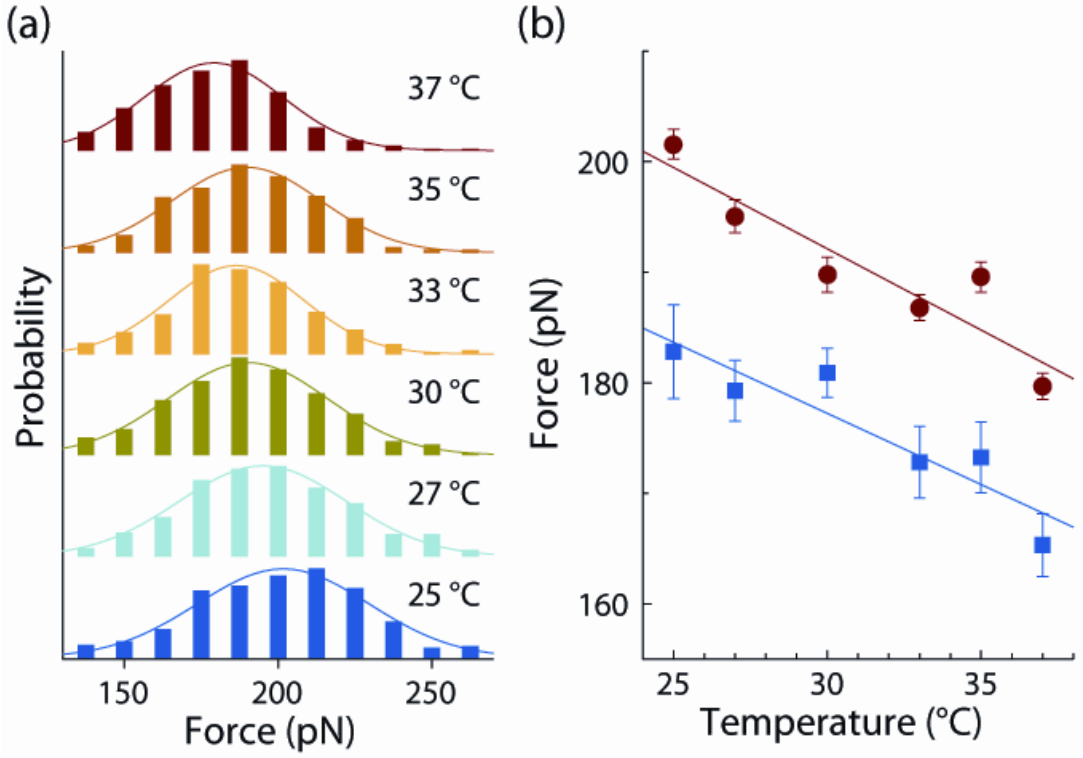
Harris and Kiang, Phys. Rev. E 79, 041912 (2009).

Velocity Convergence



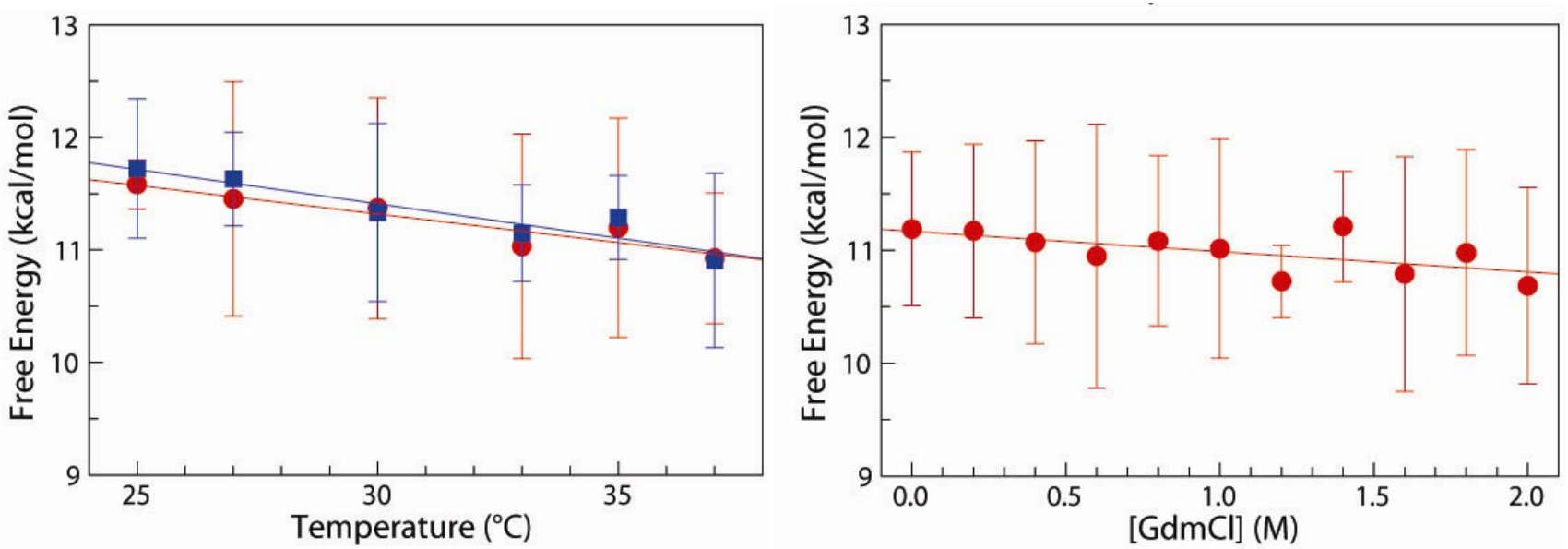
For 0.5 μm/s pulling velocity, the results converge after averaging 200 data points

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.

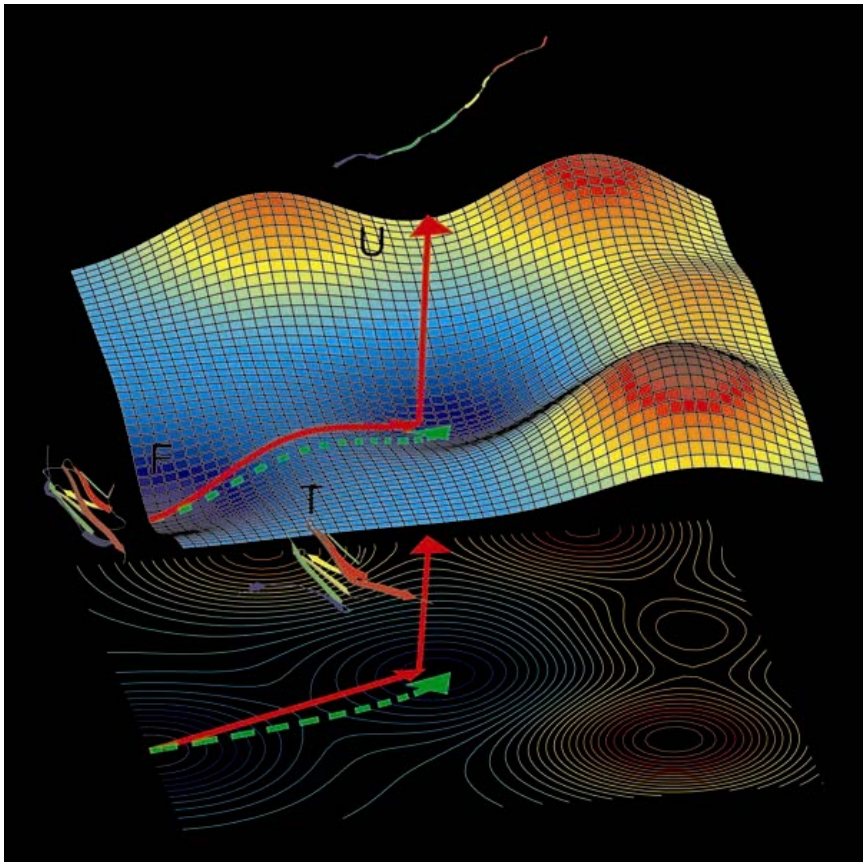
Temperature and Chemical Denaturant Dependent of Unfolding Free Energy Barrier of Titin I27



- 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.*

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



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