Molecular cell and tissue biomechanics: BE 410

Pulling on single molecules

Matthew Lang

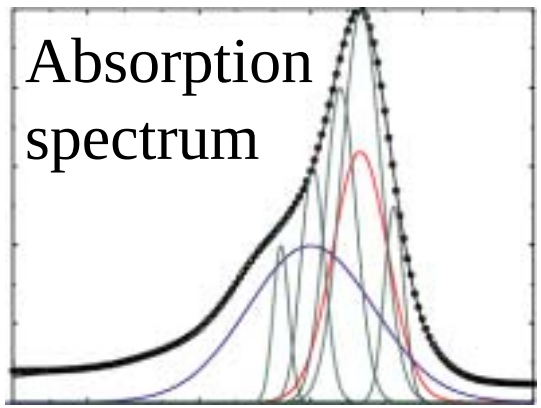
Some things that you can learn from nanomechanical measurements

- Key forces: unbinding, stall
- Distances: mechanical transitions, stepping, pauses
- Processivity
- Work/efficiency
- Timing/dynamics
- Biochemical: output, kinetics, models
- Inhomogeneous distributions

Single molecule measurements

- Directly observe protein distributions, inhomogeneity
- Populations and kinetics are inherently separable
- Able to orient or determine the orientation of the molecule

Hole burning
Ensemble echo



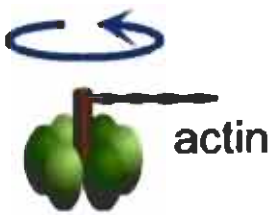
"There's plenty of room at the bottom"

Nanotechnology has a lot to learn from Biology

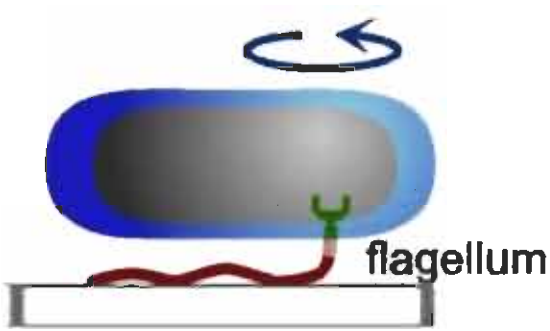
From "Real engines of creation" sblock

Rotary motors

a ATP synthase

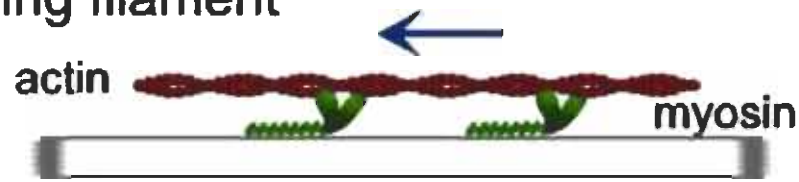


b tethered bacterium

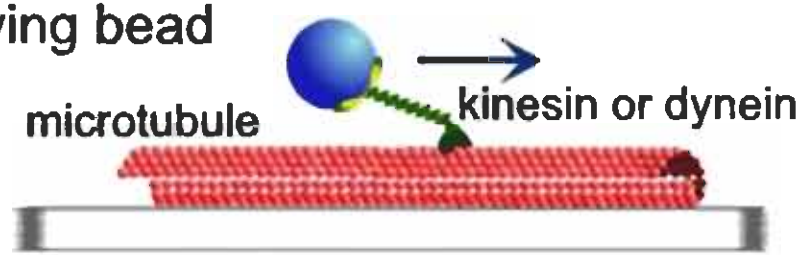


Linear motors

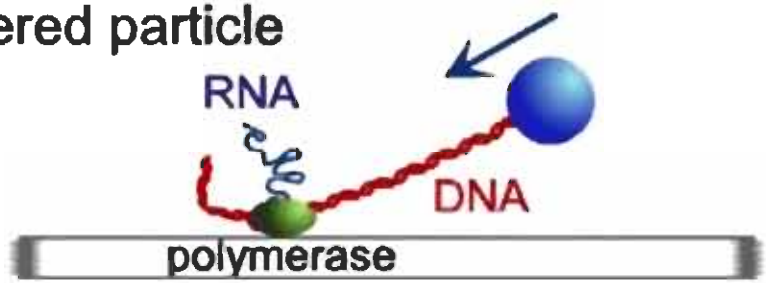
c gliding filament



d moving bead



e tethered particle



forces

- Protein protein interactions: 1-10pN
- Protein unfolding: ~100pN
- Covalent Bonds: ~lots of pN

- Stalls:
- Kinesin ~5pN
- Virus ~50pN
- RNAP ~20-30pN

FORCE EFFECTS ON BIOCHEMICAL KINETICS

Shahid Khan
Department of Physiology and Biophysics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461

Michael P. Sheetz
Department of Cell Biology, Duke University Medical Center, Durham, North Carolina 27710

Annu. Rev. Biochem. 1997. 66:785-805

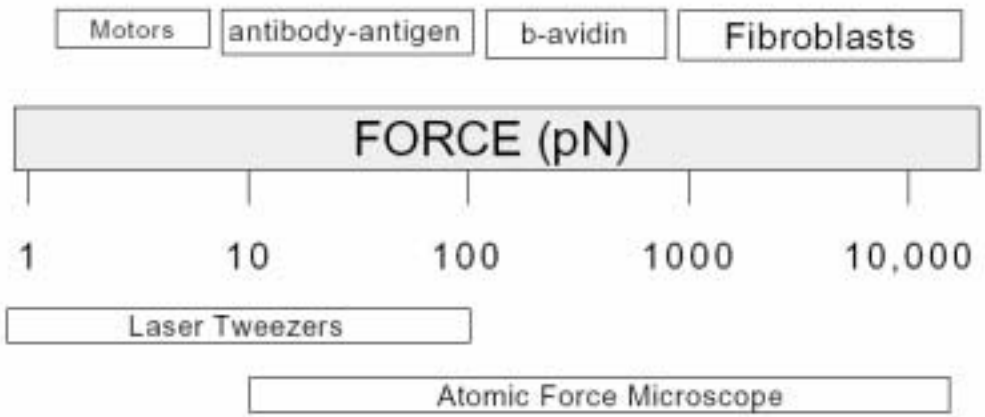
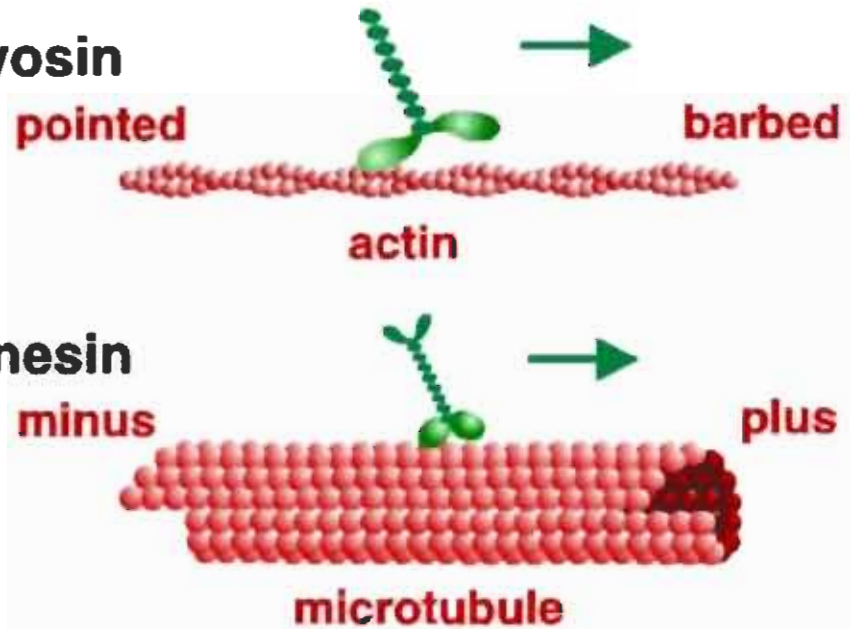


Figure 1 Force diagram showing the approximate ranges of the forces generated by motors (1, 3, 63) needed to break antigen-antibody bonds (8) and avidin-biotin bonds (5) and produced by fibroblasts on substrata (64). Below the force bar are the approximate ranges of the forces measured by the laser tweezers and the atomic force microscope.

Molecular motors

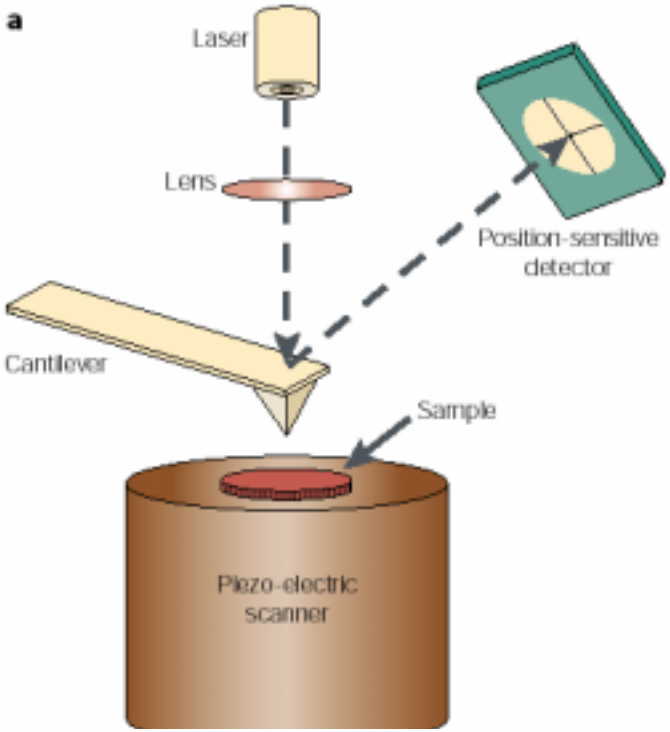


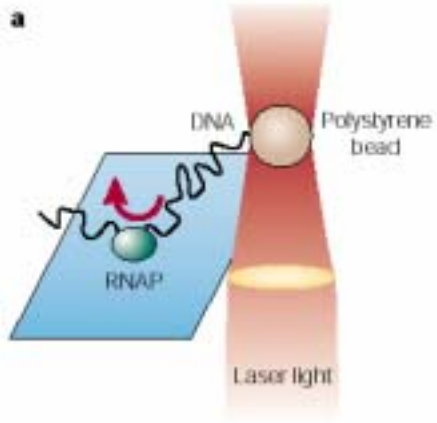
	F(pN)	v(nm/s)
Myosin	~ 2–5	~ 6000
Kinesin	~ 6-7	~ 800
RNA Polymerase	~ 25	~ 5

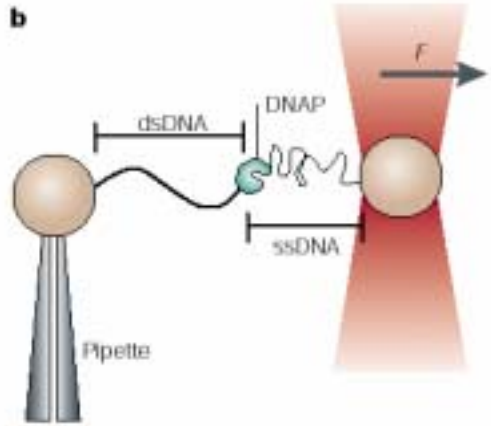
Some single molecule force measurement methods

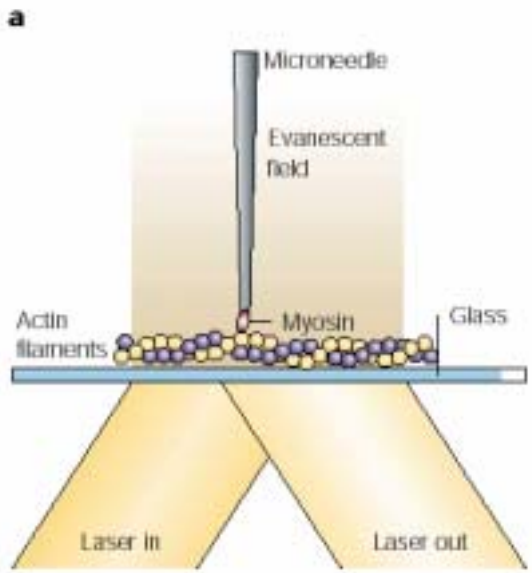
- Optical tweezers
- Magnetic traps
- Atomic force microscopy
- microneedles
- Loading using flow

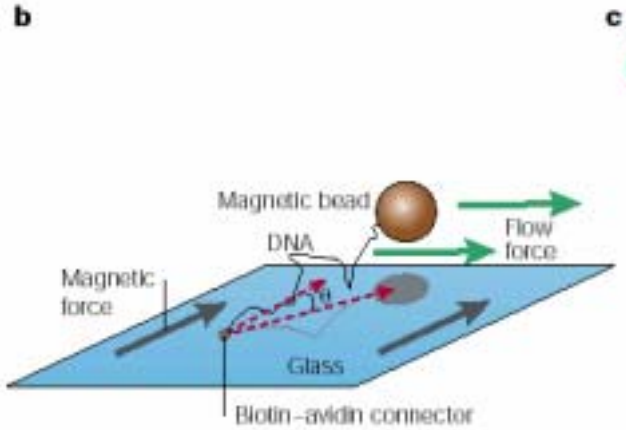
geometries

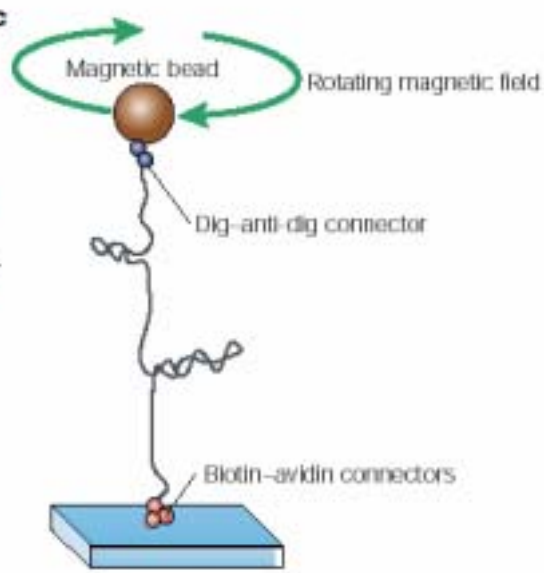

a
Laser
Lens
Position-sensitive detector
Cantilever
Sample
Piezo-electric scanner


a
DNA
Polystyrene bead
RNAP
Laser light


b
F
dsDNA
DNAP
ssDNA
Pipette


a
Microneedle
Evanescent field
Actin filaments
Myosin
Glass
Laser in
Laser out


b
Magnetic bead
DNA
Flow force
Magnetic force
Glass
Biotin–avidin connector


c
Magnetic bead
Rotating magnetic field
Dig–anti-dig connector
Biotin–avidin connectors

More on tools for measuring

- AFM: 0.01-100nN
- Optical tweezers: 0.01-150pN
- Magnetic tweezers: 0.01-150pN

- Deformations on the order of few angstroms or a few nm.
- Protein sizes 5-50nm

GRABBING THE CAT BY THE TAIL: MANIPULATING MOLECULES ONE BY ONE

Carlos Bustamante‡§, Jed C. Macosko‡ and Gijs J. L. Wuite§*

Methods for manipulating single molecules are yielding new information about both the forces that hold biomolecules together and the mechanics of molecular motors. We describe here the physical principles behind these methods, and discuss their capabilities and current limitations.

Table 1 | **Overview of single-molecule manipulation methods**

Methods	$F_{min-max}$ (N)[a]	X_{min} (m)[a]	Stiffness (N m^{-1})	Applications	Practical advantages
Cantilevers[c]	10^{-11}–10^{-7}	10^{-10}	0.001–100	Protein/polysaccharides[5,54] Bond strength[55,56]	High spatial resolution Commercially available
Microneedles[c]	10^{-12}–10^{-10}	10^{-9}	10^{-6}–1	Myosin motor force[12] DNA/titin strength[26,28]	Good operator control Soft spring constant
Flow field[d]	10^{-13}–10^{-9}	10^{-8}	n.a.	DNA dynamics[29] RNA polymerase[30]	Rapid buffer exchange Simplicity of design
Magnetic field[d]	10^{-14}–10^{-11}	10^{-8}	n.a.	DNA entropic elasticity[6] Topoisomerase activity[31]	Specificity to magnets Ability to induce torque
Photon field[d]	10^{-13}–10^{-10}	10^{-9}	10^{-10}–10^{-3}	Protein motors[13,14] Protein unfolding[32]	Specific manipulation High force resolution

[a]Mechanical transducers: probes are bendable beams; spatial location is by beam deflection. [d]External field manipulators: probes are microscopic beads; spatial location is by bead displacement. [e]These numbers represent only empirical, not absolute limits. ($F_{min-max}$, force range; X_{min}, minimum displacement.)

Energy Barriers

Force: an experimentalists time machine

$$K_{\mathrm{eq}}(f) = K_{\mathrm{eq}}^{0} \exp\left(-\frac{fx}{2k_{\mathrm{B}}T}\right),$$

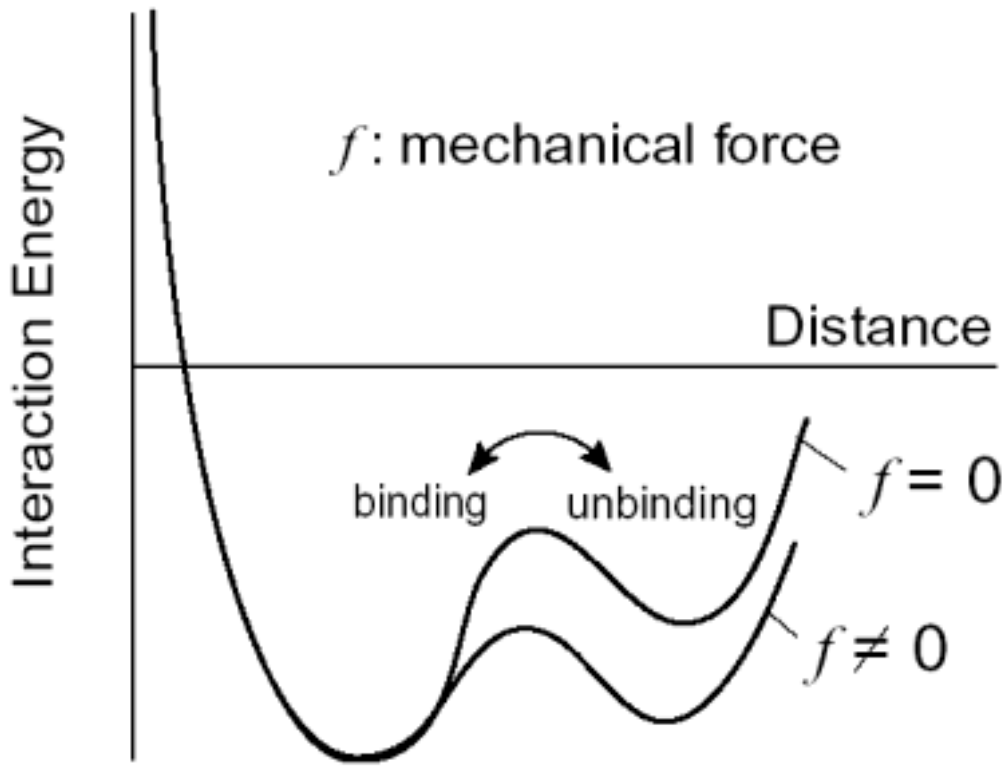


Fig. 14. The applied mechanical force can lower the energy barrier of molecular unbinding, thus influencing receptor–ligand reaction kinetics.

Probing the Relation Between Force—Lifetime—and Chemistry in Single Molecular Bonds

Annu. Rev. Biophys. Biomol. Struct. 2001. 30:105–28

Evan Evans
Physics and Pathology, University of British Columbia, Vancouver, Canada V6T 2A6; Biomedical Engineering, Boston University, Boston, Massachusetts 02215; e-mail: evans@physics.ubc.ca

Intermediate transition states can be investigated

infrequent reactions can be sped up.

“soft” forces are needed to probe the intermediates

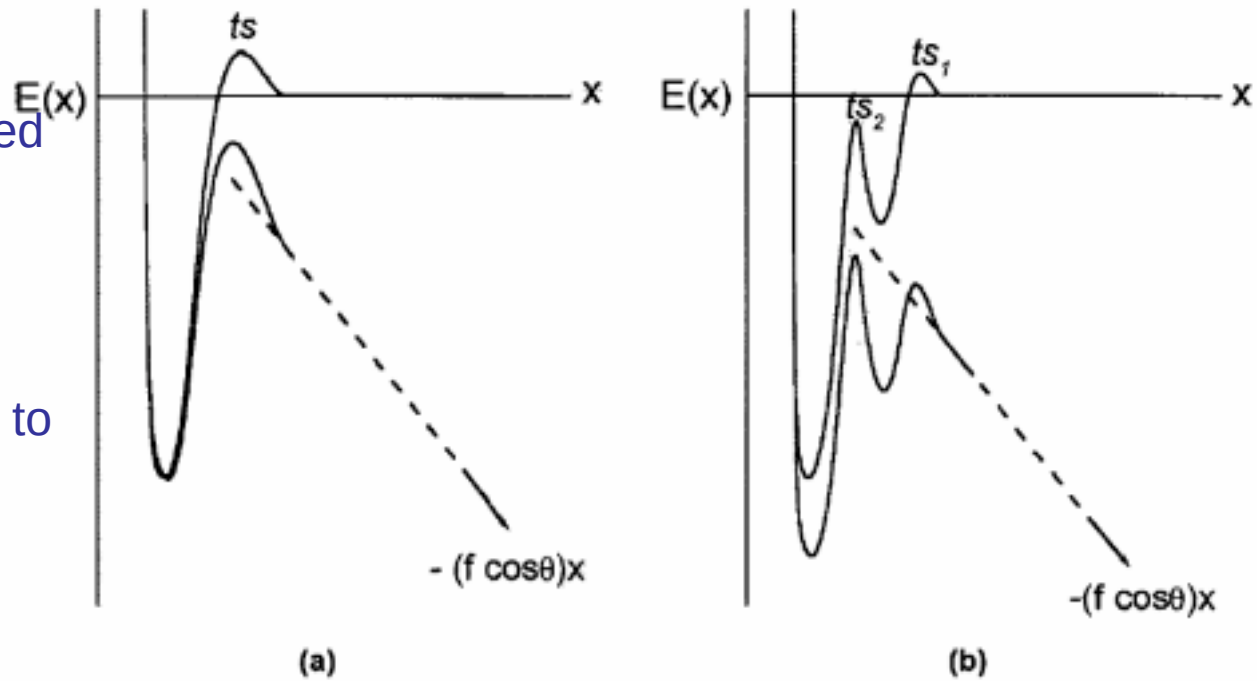


Figure 2 Conceptual energy landscapes for bonds confined by sharp activation barriers—transition states (ts). Oriented at an angle θ to the molecular coordinate x, external force f adds a mechanical potential $-(f \cdot \cos\theta)x$ that tilts the landscape and lowers barriers. (*a*) A single barrier under force. (*b*) A cascade of barriers under force where an inner barrier emerges to dominate kinetics when the outer barrier falls below by $\sim k_B T$.

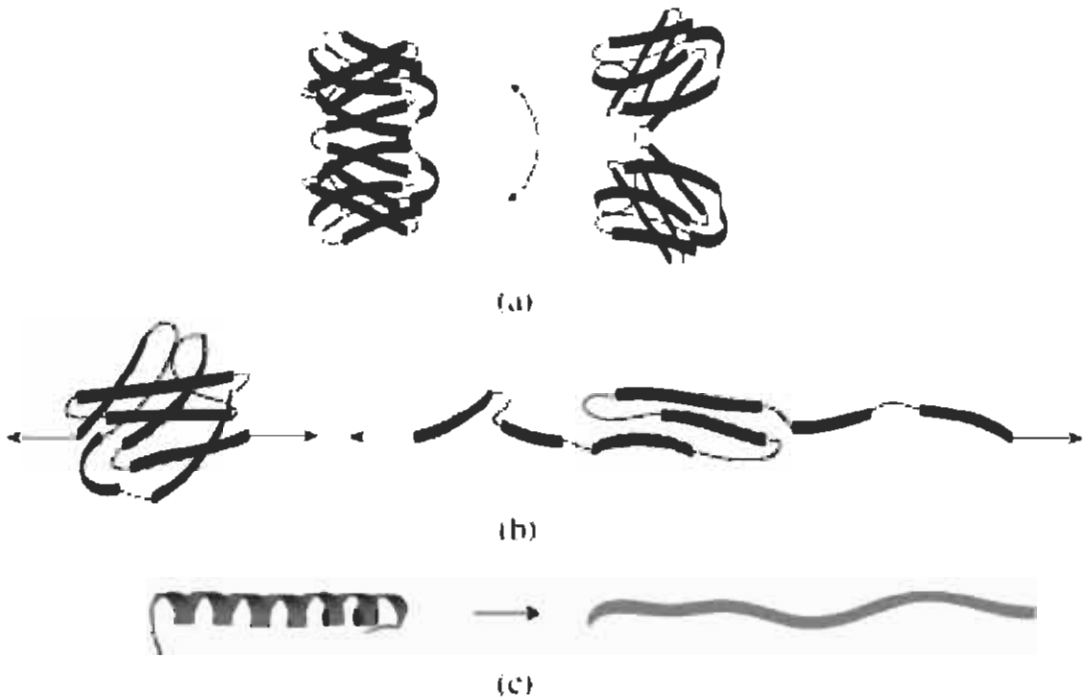


Fig. 7. Modes of protein deformation: (a) domain hinge motion, (b) domain deformation and unfolding, (c) unfolding of secondary structures.

Models for proteins under applied force:

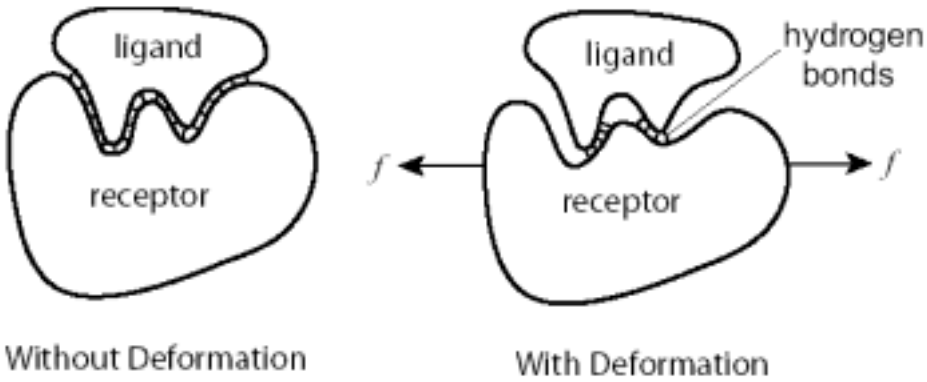


Fig. 10. Receptor–ligand binding can be affected by protein deformation. (a) A good conformational match between the receptor and ligand leads to strong binding and reaction. (b) When the receptor deforms under force, the binding affinity decreases due to poor conformational match.

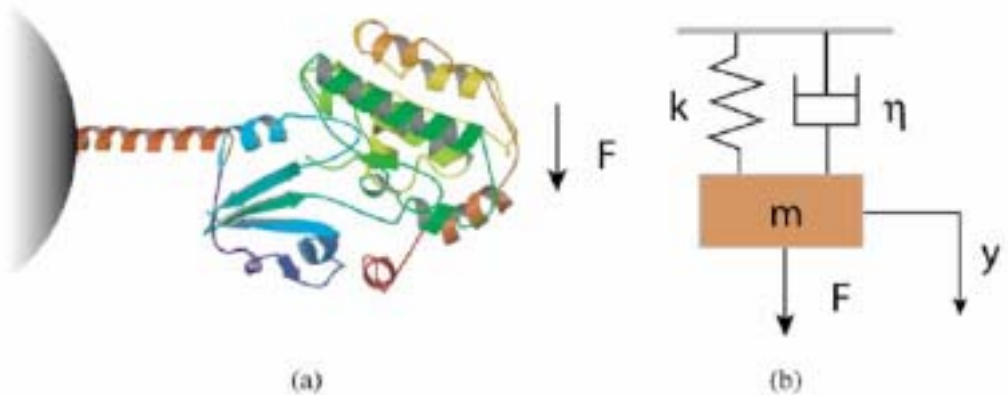


Fig. 6. The motion of a protein under applied force F. (a) A globular protein immobilized on a surface through an α-helix. (b) The mass-spring-dashpot system as a model for protein motion.

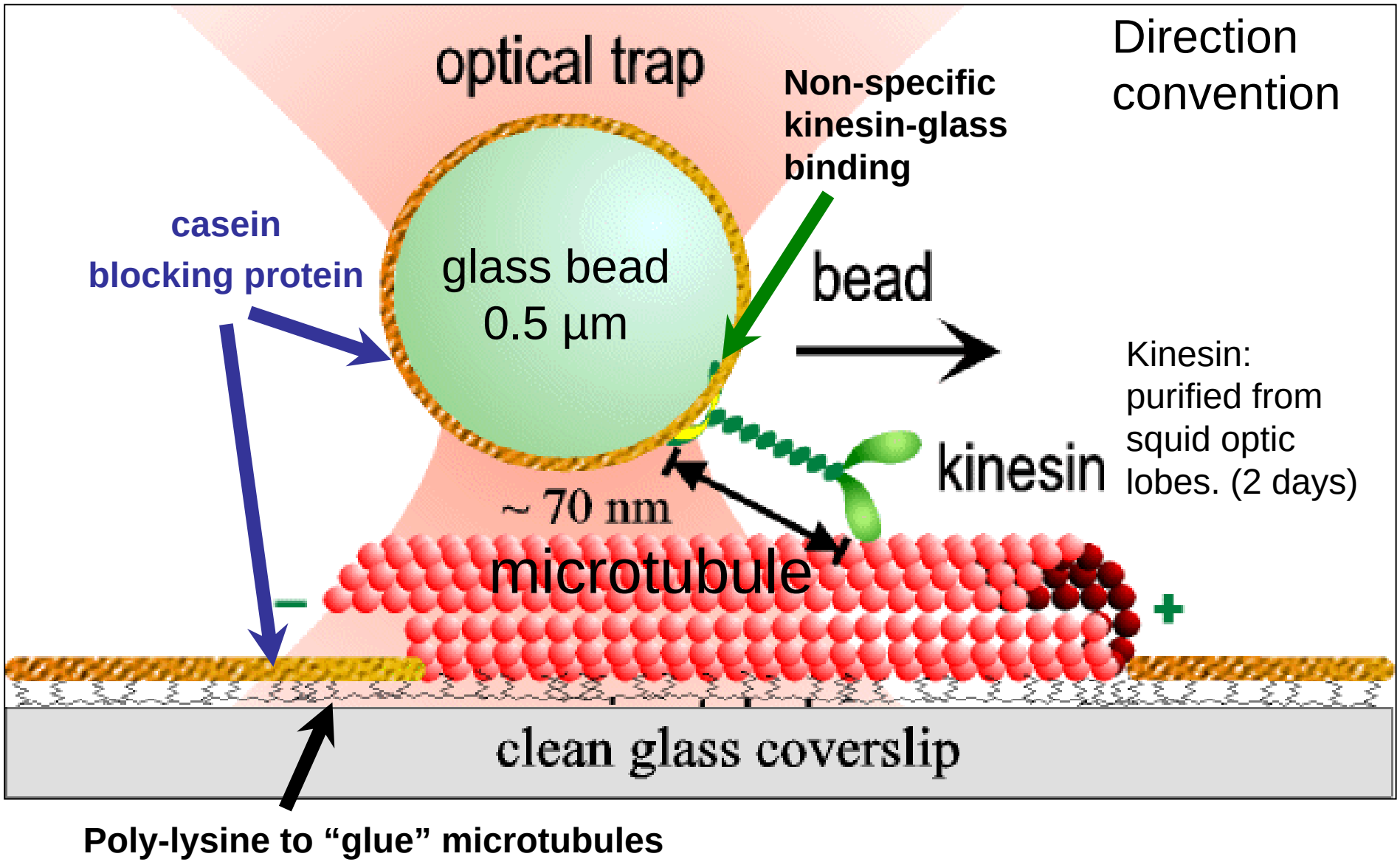
Challenges in constructing SM assays

- Linkages
- Tethers
- Pulling geometry
- Acquisition speed
- Noise
- Biology

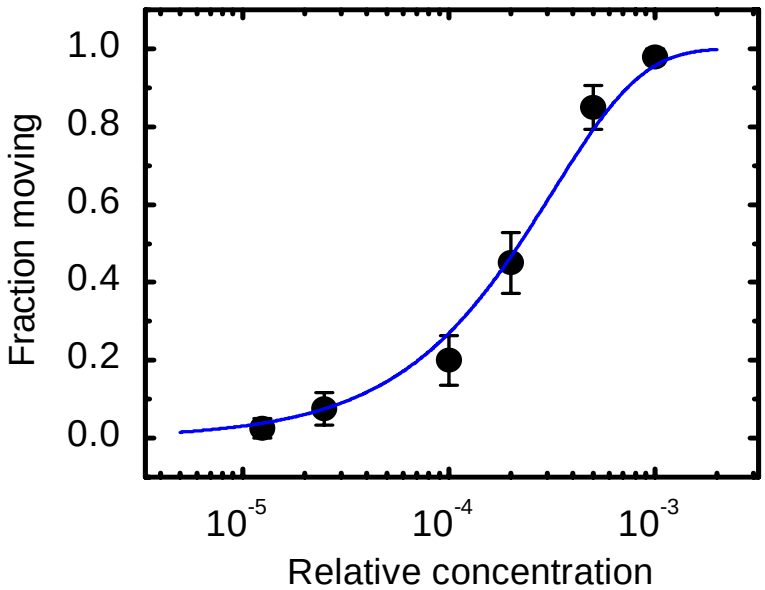
Linkage chemistries/ strategies, constructing the assay.

- Poly-lycene
- BSA/ casein
- His-tag
- Epitope tags
- Biotin-avadin
- Dig-antidig
- Nucleotide overlap
- Non-specific binding

The single molecule assay



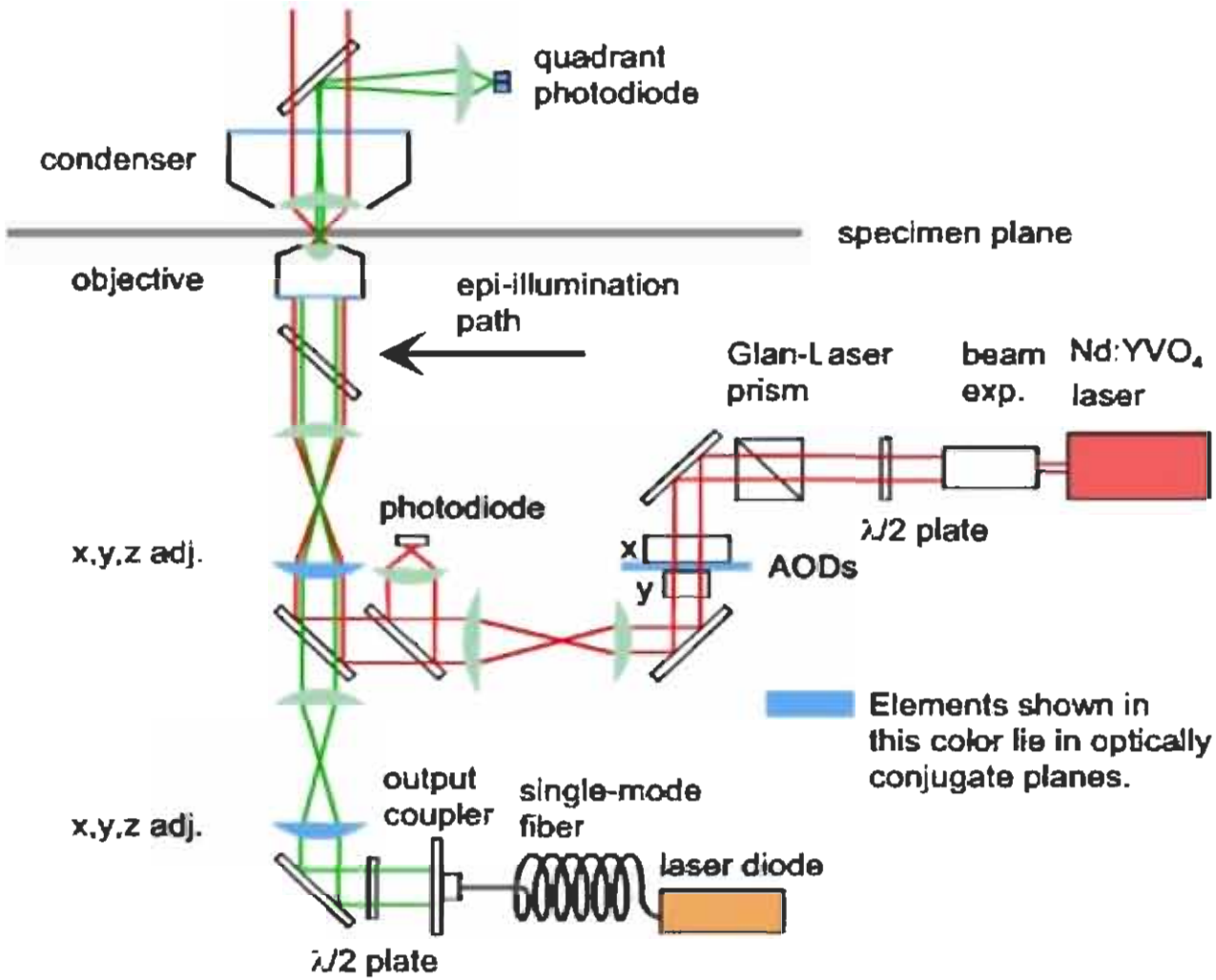
Getting to the single molecule limit



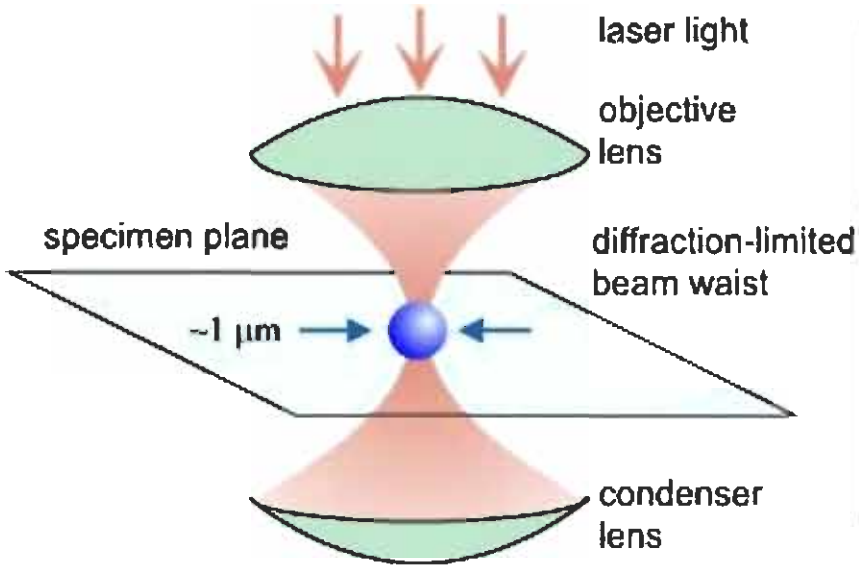
$$f = \left(1 - e^{-\lambda C}\right)$$

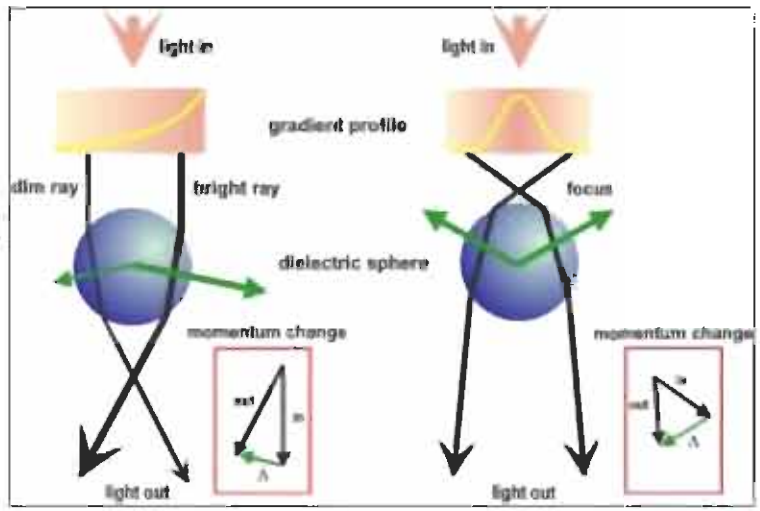
Change the relative concentrations of reagents and monitor the activity.

Other signals are available. To do it right you need to take your time.


quadrant photodiode
condenser
specimen plane
objective
epi-illumination path
Glan-Laser prism
beam exp.
Nd:YVO4 laser
λ/2 plate
photodiode
x,y,z adj.
x
y
AODs
Elements shown in this color lie in optically conjugate planes.
output coupler
single-mode fiber
x,y,z adj.
laser diode
λ/2 plate

Gradient force – ray optic picture

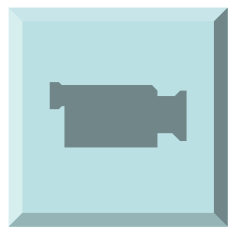




Single molecule mechanical measurements with optical tweezers

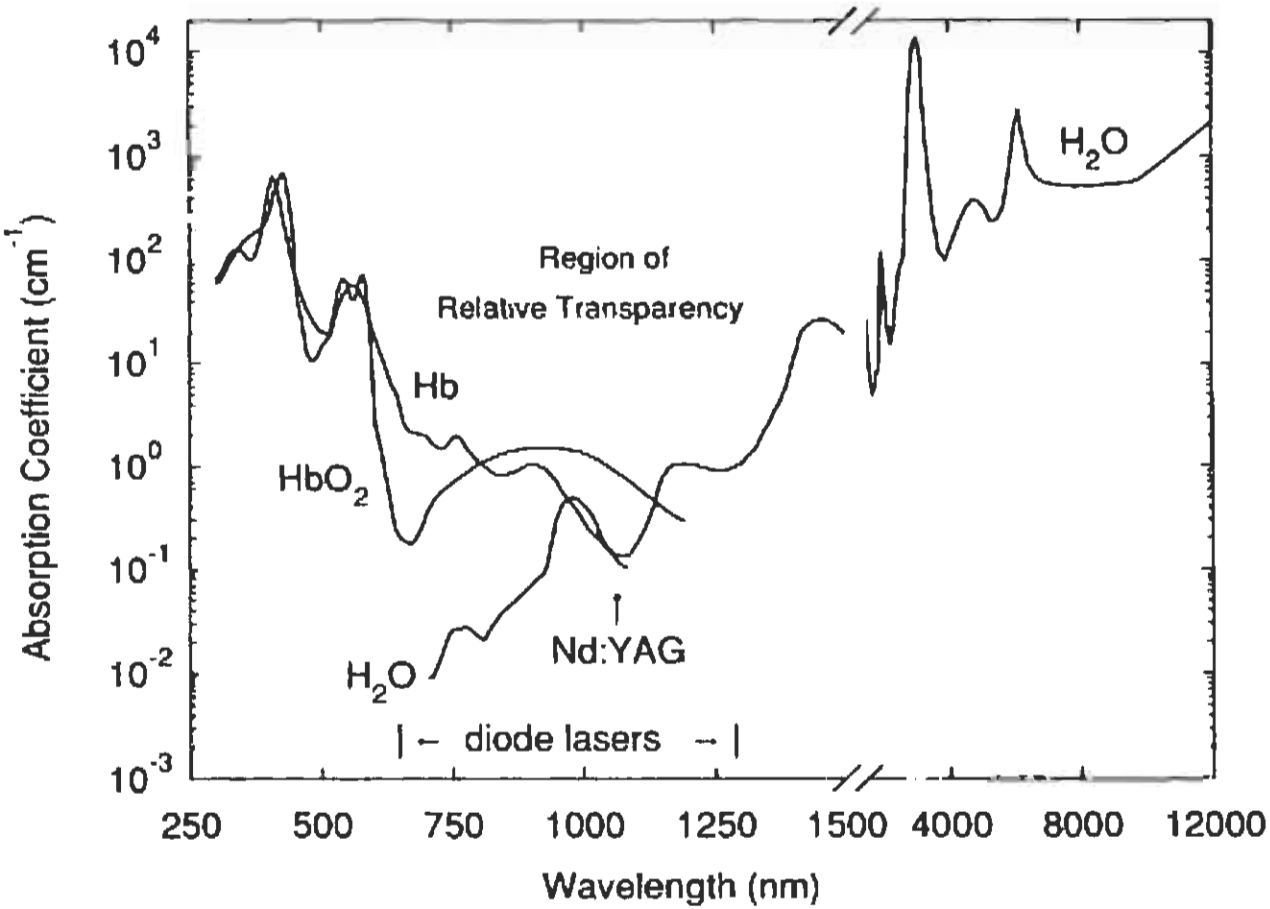
- Force resolution sub pN
- Force range -200pN
- Position resolution ~1nm
- Self-orienting
- Manipulate with light
- Non-invasive infrared light
- Can synthesize multiple traps

tetris

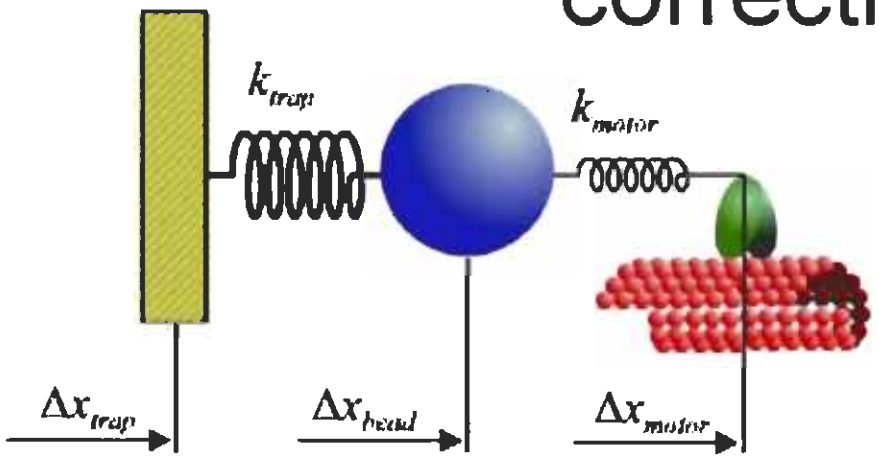


Christoph Schmidt

Window of optical transparency

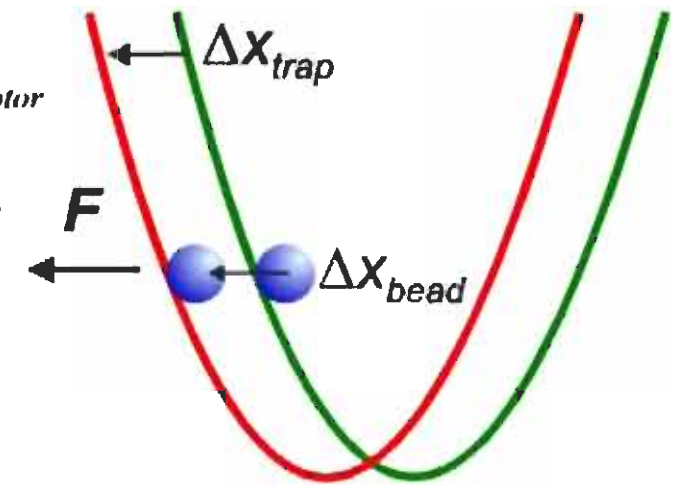


Force clamping: No compliance corrections



Fixed trap: $\Delta x_{bead} - \frac{k_{motor}}{k_{trap} + k_{motor}} \Delta x_{motor}$

Force clamp: $\Delta x_{bead} = \Delta x_{motor} = \Delta x_{trap}$

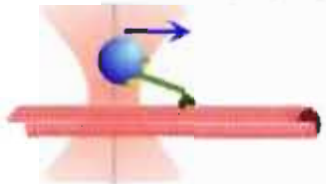


$\Delta x_{trap} = \Delta x_{bead} \rightarrow F = constant$

Coppin, et al. (1997) Forward load results

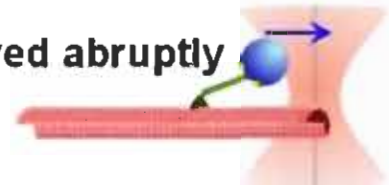
initial state

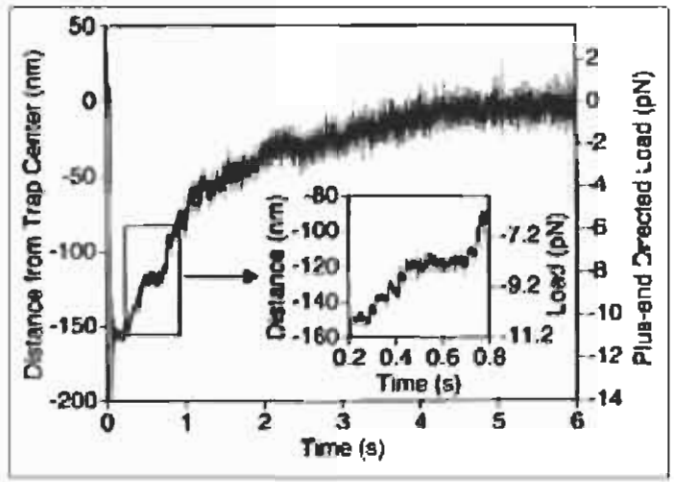
tension bead & trap

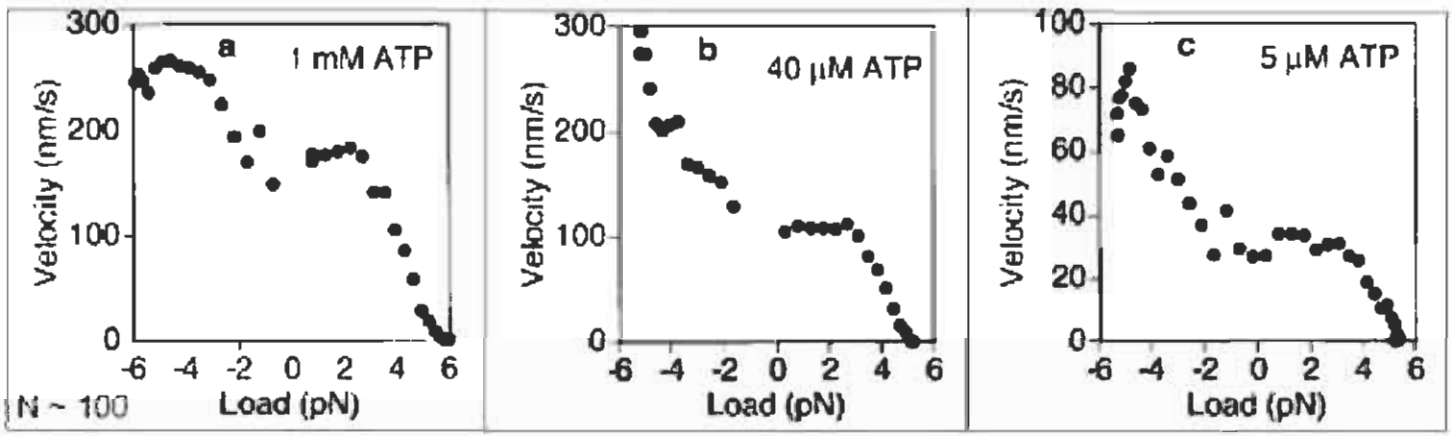


final state

stage moved abruptly to the left

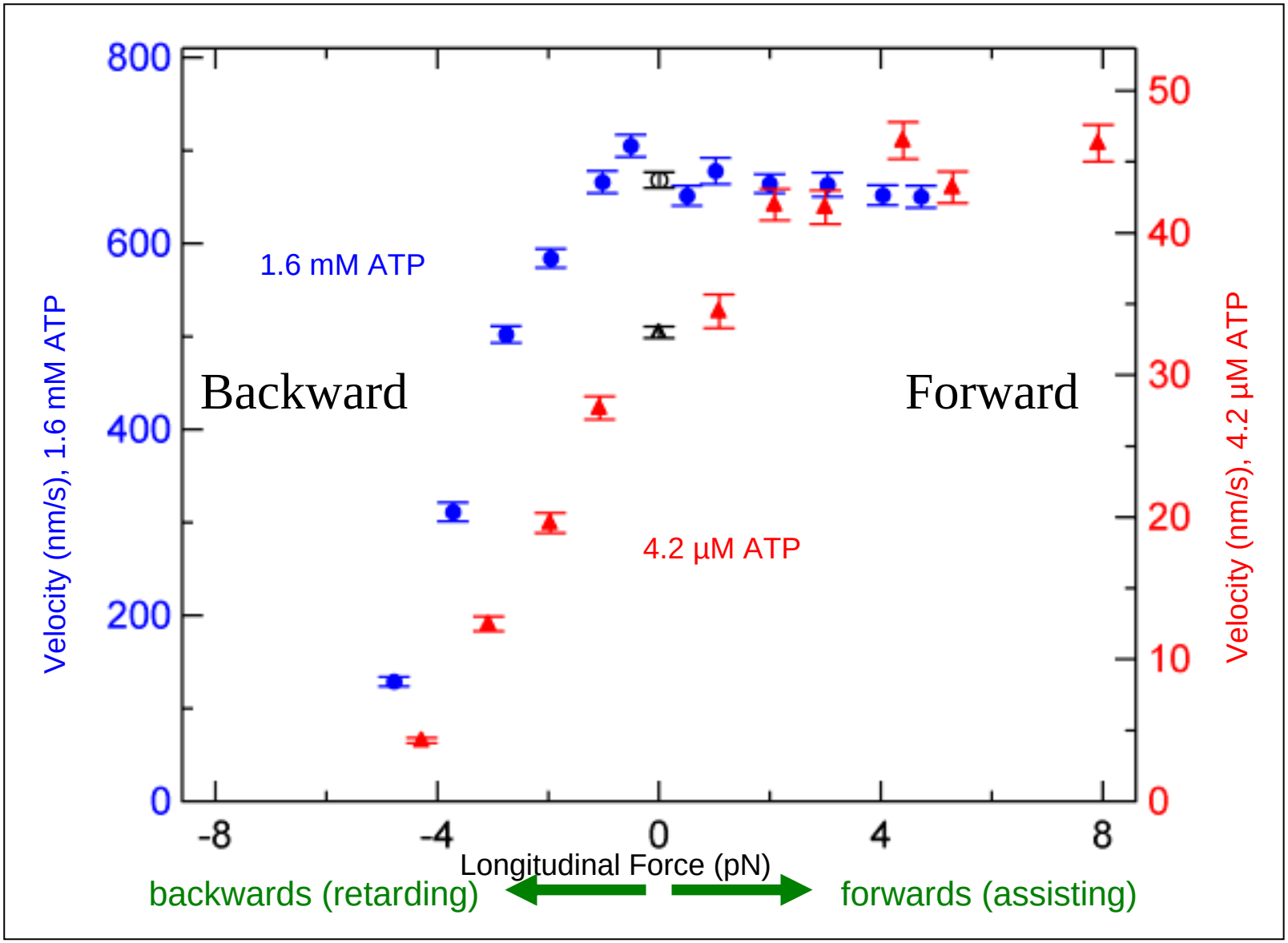






Coppin, Pierce, Hsu, and Vale (1997) *PNAS* 94: 8539-8544

LONGITUDINAL FORCE-VELOCITY CURVES



Magnetic Tweezers: Micromanipulation and Force Measurement at the Molecular Level

Charlie Gosse and Vincent Croquette
Laboratoire de Physique Statistique, École Normale Supérieure, Unité de Recherche 8550 associée au Centre National de la Recherche Scientifique et aux Universités Paris VI et VII, 75231 Paris, France

Change the force by stepping the magnet or decreasing the current in the coils

0.05pN resolution
Rotation ability
Limited position resolution

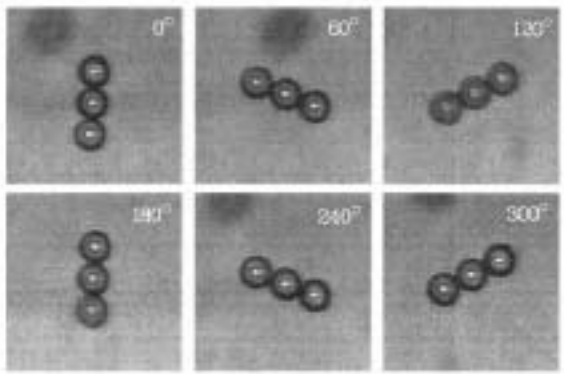


FIGURE 14 Counter-clockwise rotation of three aggregated beads linked to a surface by a double-stranded DNA molecule. This manipulation could also be done with a single locked particle, but we chose these images because of their higher visual impact.

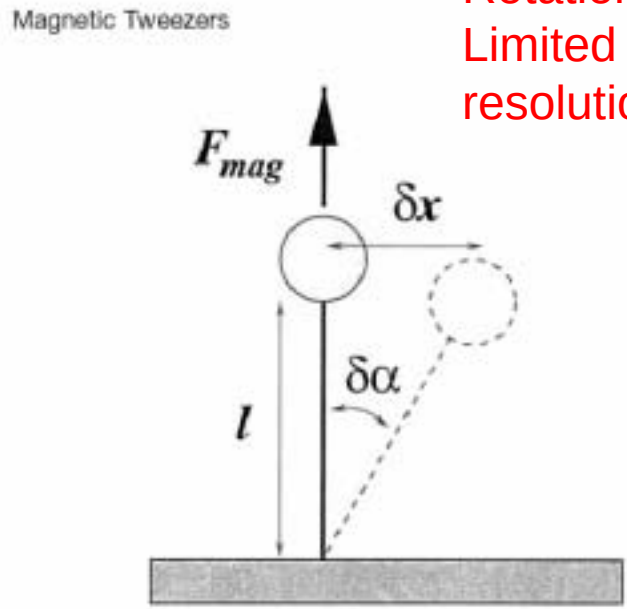


FIGURE 5 Principle of force measurement. The vertical magnetic force F_{mag} applied to the bead stretches the DNA molecule. The transverse Brownian fluctuations $\langle \delta x^2 \rangle$ of this inverted pendulum are then used to evaluate its rigidity F_{mag}/l and thus the pulling force F_{mag}.

Force Spectroscopy of Single Biomolecules

Matthias Rief*[a] and Helmut Grubmüller*[b]

Tethers at the very tip?

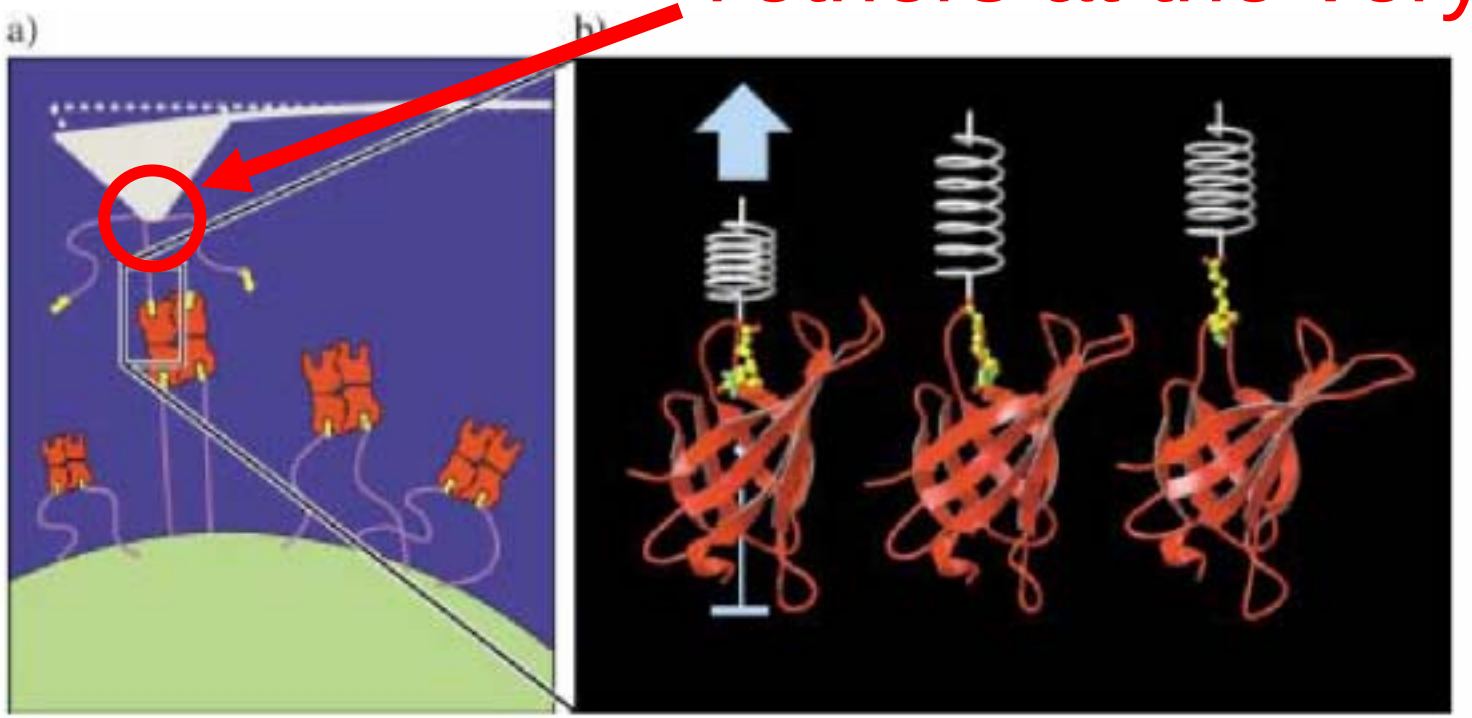
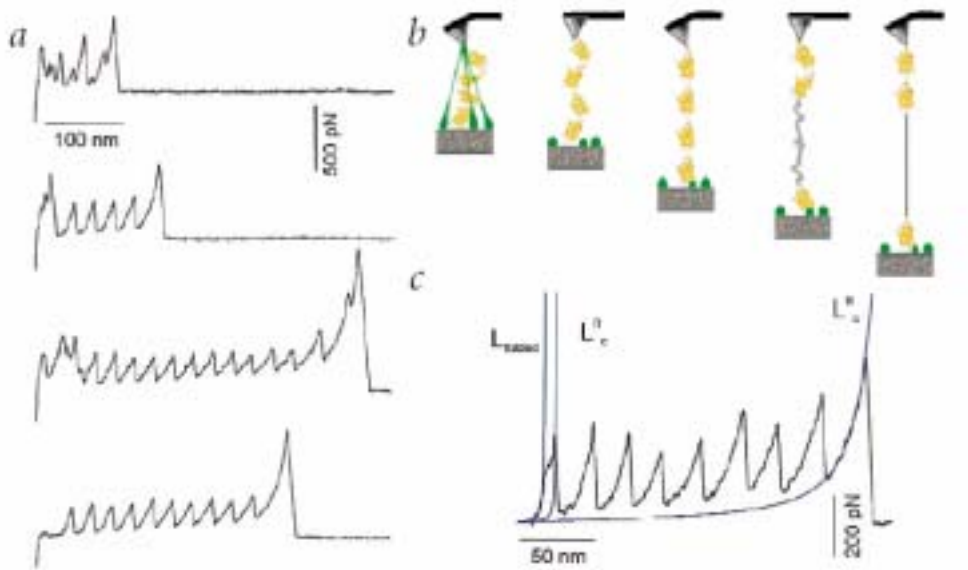


Figure 1. *With an atomic force microscope, single molecules can be subjected to a controlled load and the acting forces can be measured. Ligands (yellow) and receptors (red) are attached to the cantilever tip (gray) and the surface (green) via linker molecules (magenta). Both in the experiment (a) and in the simulation (b), the ligand is subject to an increasing pulling force, and the rupture force is measured.*

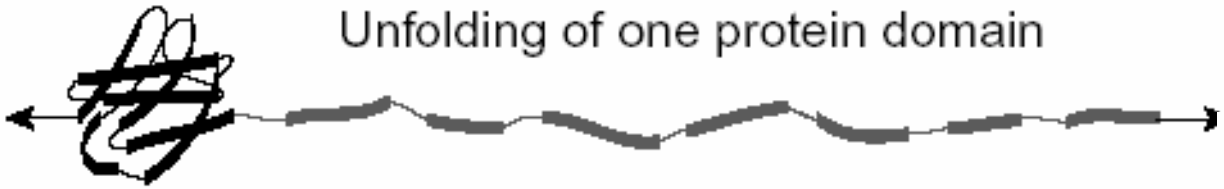
Stretching single molecules into novel conformations using the atomic force microscope

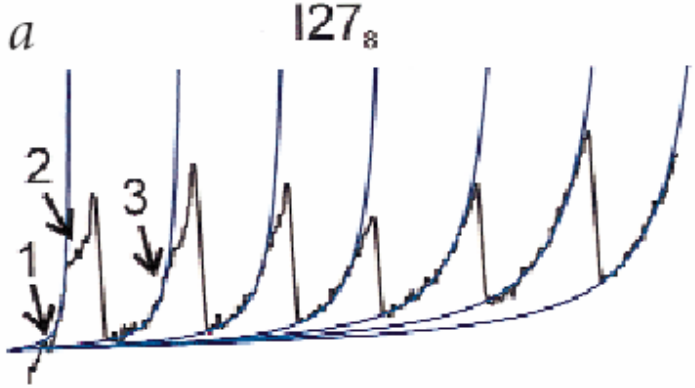
Thomas E. Fisher, Piotr E. Marszalek and Julio M. Fernandez



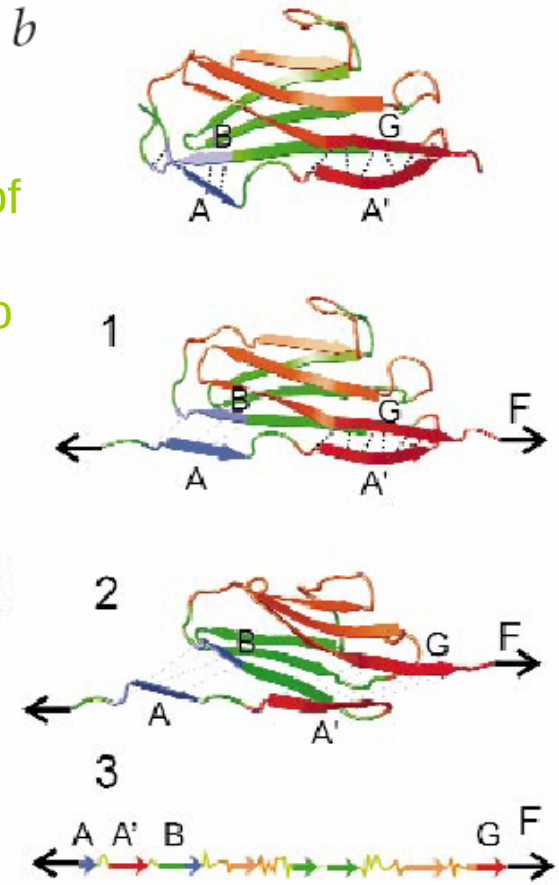
Typical sawtooth structure

Bao / J. Mech. Phys. Solids 50 (2002) 2237–2274





release force on unbinding
steady increase of successive domains: tough to measure “soft” transitions



review

Stretching single molecules into novel conformations using the atomic force microscope

Thomas E. Fisher, Piotr E. Marszalek and Julio M. Fernandez

Geometry Matters!

Biophysical Journal Volume 79 December 2000 3267–3281

Biomolecular Interactions Measured by Atomic Force Microscopy

Oscar H. Willemsen,* Margot M. E. Snel,*† Alessandra Cambi,† Jan Greve,* Bart G. De Grooth,* and Carl G. Figdor†

*Department of Applied Physics, Biophysical Techniques Group, University of Twente, Enschede, and †Department of Tumor Immunology, University Hospital Nijmegen, Nijmegen, The Netherlands

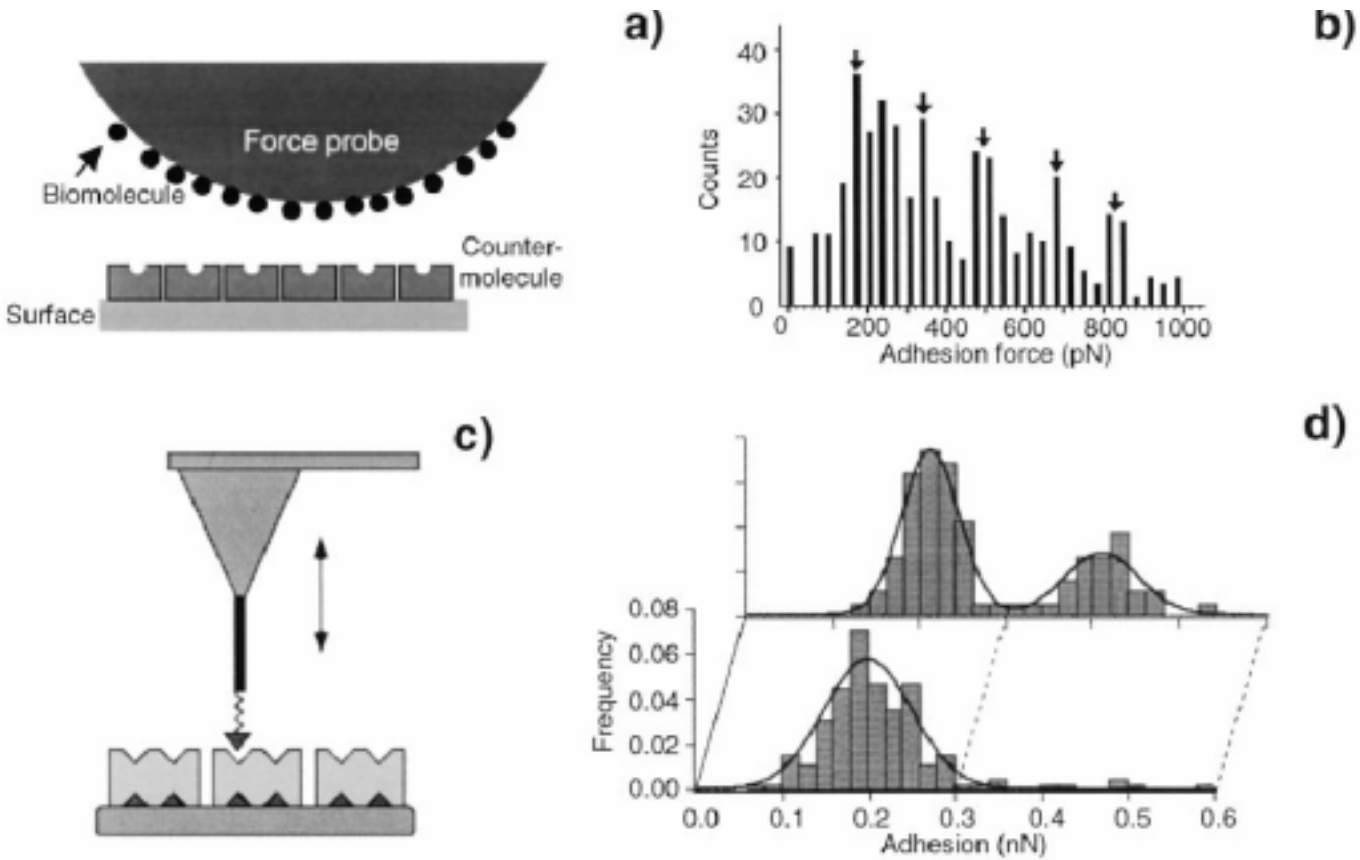
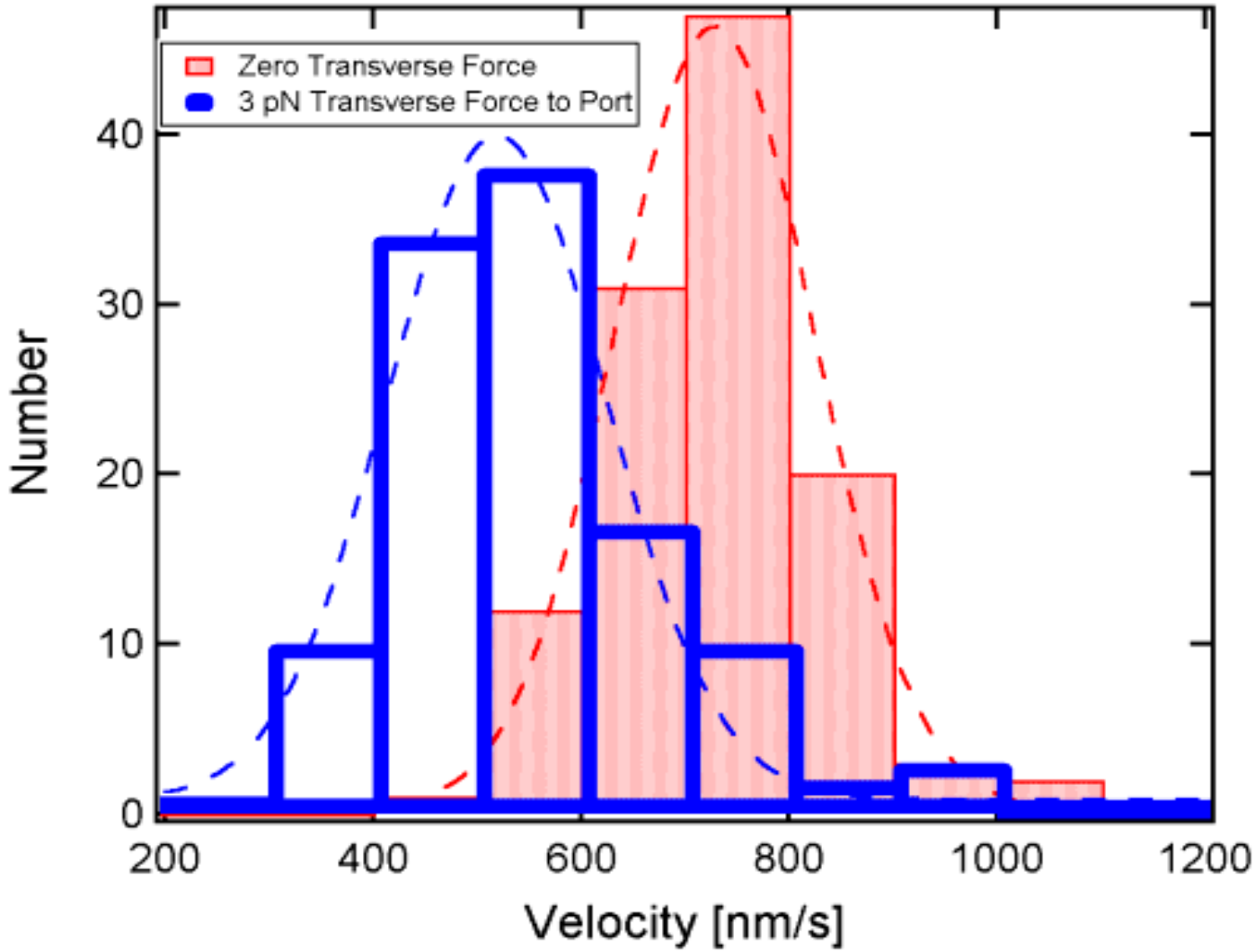


TABLE 1 AFM measurements on biomolecular forces

System	Method			
	Measurement of Disruption Force	Disruption Force versus Loading Rate	Adhesion Mode Imaging	Force Spectroscopy
Biotin-(strept)avidin	Lee et al., 1994a; Florin et al., 1994; Moy et al., 1994a, b; Chilkoti et al., 1995; Allen et al., 1996; Wong et al., 1998; Lo et al., 1999	Merkel et al., 1999*	Ludwig et al., 1997	
Antibody-antigen	Hinterdorfer et al., 1995–1997; Stuart and Hlady, 1995, 1999; Dammer et al., 1996; Allen et al., 1997; Ros et al., 1998		Willemsen et al., 1998, 1999	
Receptor-ligand				
Proteoglycans	Dammer et al., 1995			
P-selectin		Fritz et al., 1998		
Tenascin		Oberhauser et al., 1998†		Oberhauser et al., 1998
$\alpha_v\beta_3$ Integrin	Lehenkari and Horton, 1999			
VE-cadherin	Baumgartner et al., 2000	Baumgartner et al., 2000		
Acetylcholinesterase				Ymgge et al., 1999
Myelin basic protein				Mueller et al., 1999
Intramolecular				
Titin	Carrion-Vazquez et al., 1999†	Rief et al., 1997b, 1998a†		Rief et al., 1997b, 1998a; Marszalek et al., 1999, Oberhauser et al., 1999; Li et al., 2000†
Bacteriorhodopsin				Oesterhelt et al., 2000†
T4 lysozyme				Yang et al., 2000†
DNA	Lee et al., 1994b; Boland and Ratner, 1995; Strunz et al., 1999	Strunz et al., 1999		Rief et al., 1999; Clausen-Schaumann et al., 2000
Shell-protein				Smith et al., 1999
Polysaccharides				Rief et al., 1997a; Li et al., 1998; Marszalek et al., 1998
Covalent bonds				Grandbois et al., 1999

*Measured with a biomembrane force probe.
†Measurement of unfolding force.

Velocity distributions “N” matters

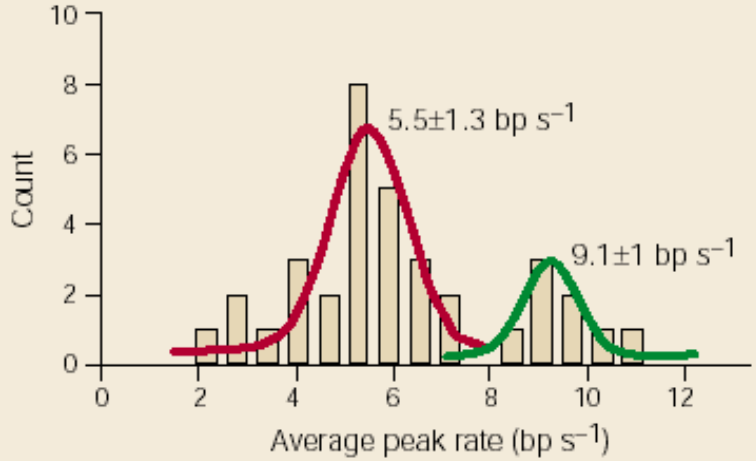


Measure unbinding distributions

careful!

Box 2 | **What can single-molecule manipulation tell us about biology?**

Two recent studies have demonstrated the ability of single-molecule techniques to elucidate new aspects of enzyme kinetics. First, Davenport *et al.*[36], by using a single RNA polymerase (RNAP) molecule moving along a DNA strand attached to a bead in a flow field, determined that RNAP can operate in at least two modes, one slow and one fast. The figure illustrates the averaged peak rates of single RNAP molecules, showing that they can be in a slow or a fast transcription state.



Need large "N"

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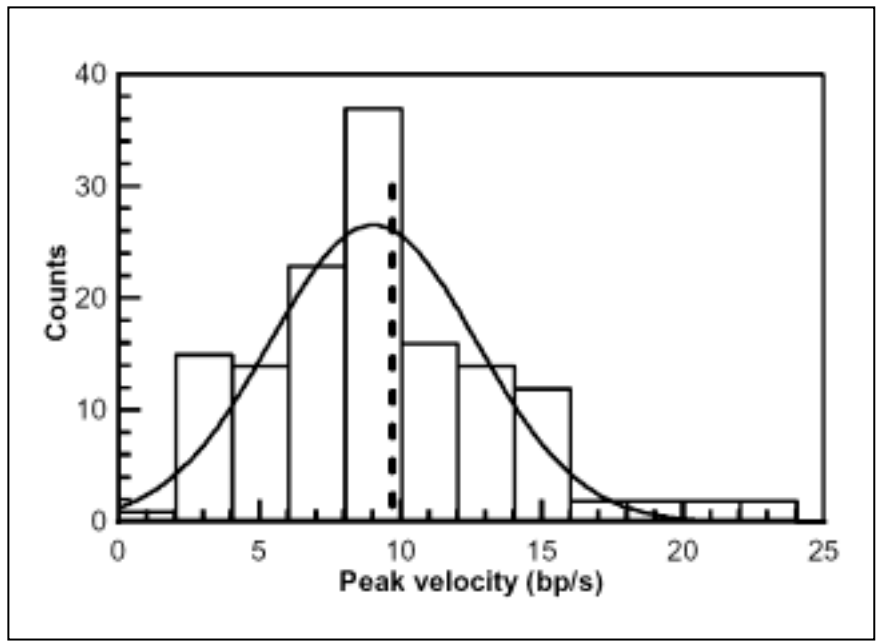
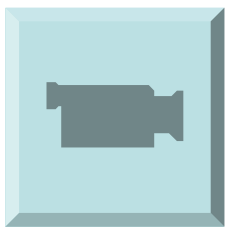


Kinesin motility cycle

Motility cycle events:

- Binding of ATP
- Hydrolysis of ATP
- Release of phosphate
- Release of ADP
- Release of the lagging head
- Binding of the forward head
- Conformational changes, stepping under load
- One-head and two-head bound states

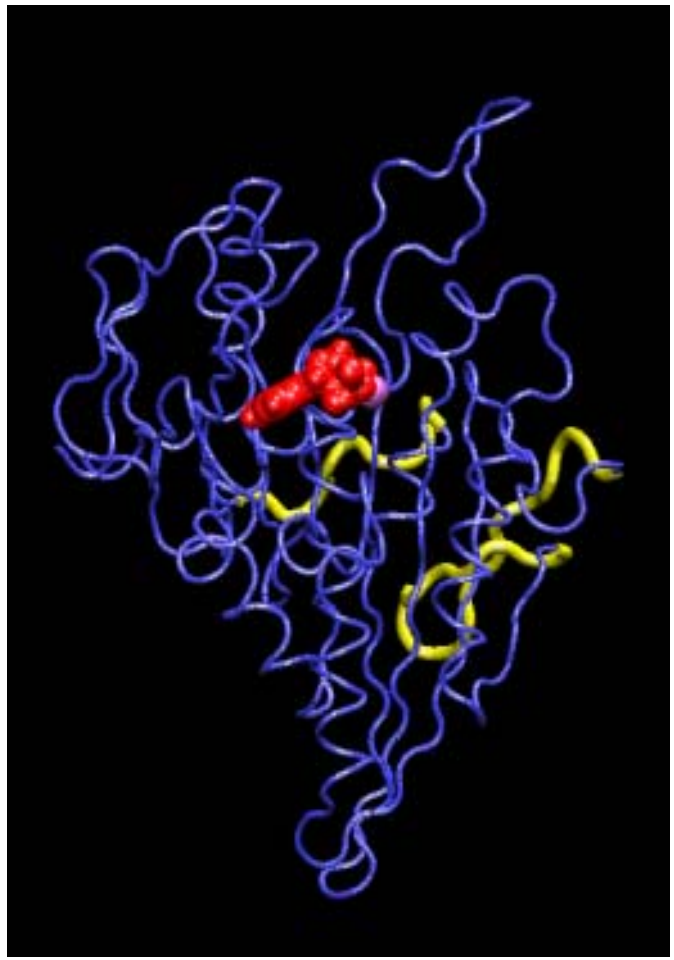


movie: Vale, Milligan and coworkers

www.scripps.edu/milligan/

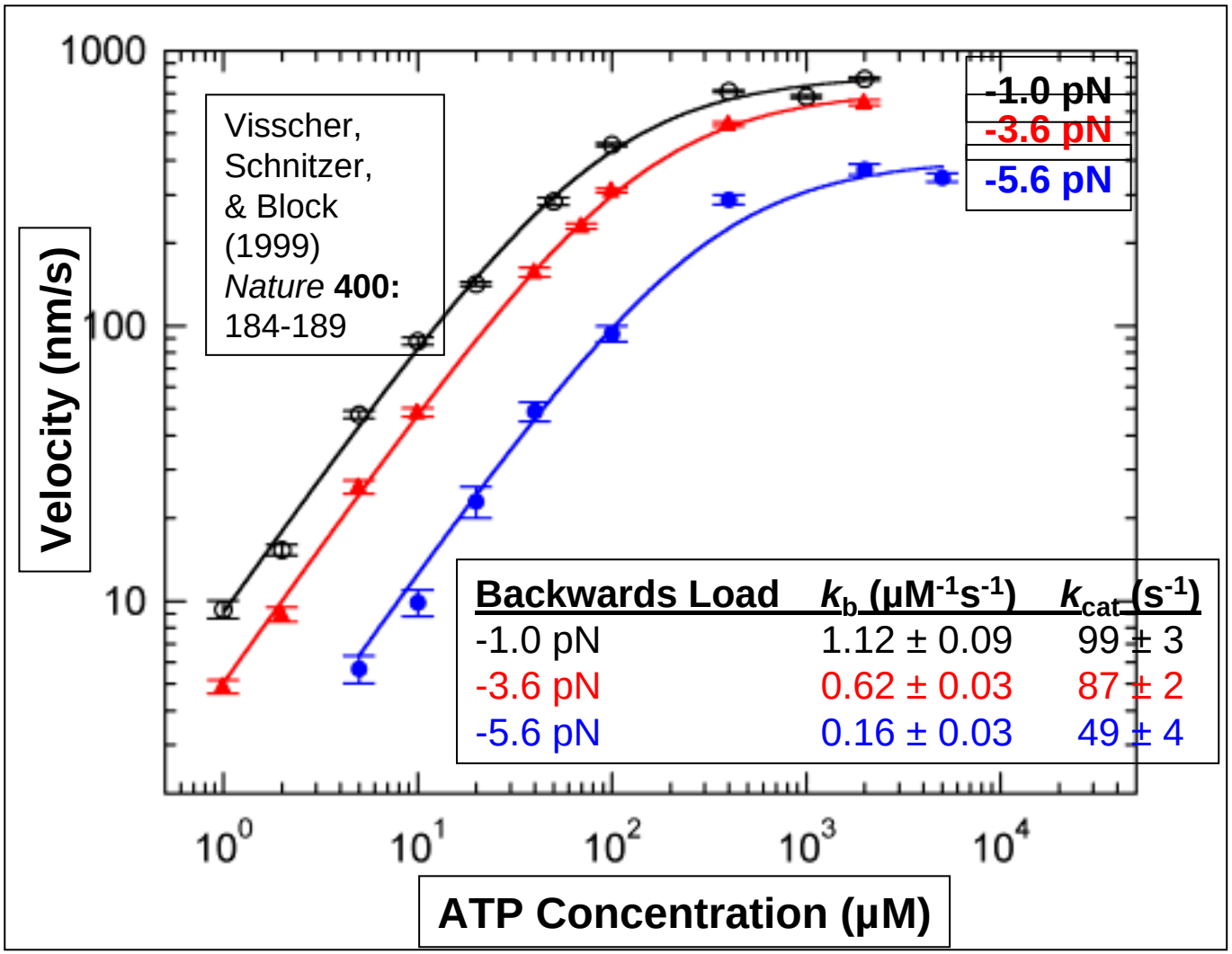
ATP hydrolysis structures

- ATP hydrolysis vs
- ADP structure
- Simulated annealing
- Changes the structure of the binding site



(Wriggers, Schulten Biophys. J., 75 p646 1998)

Longitudinal force vs. [ATP]



heterogeneity

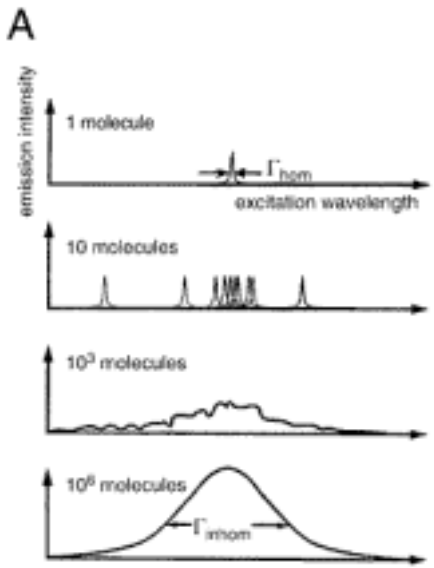
- Proteins have personality…

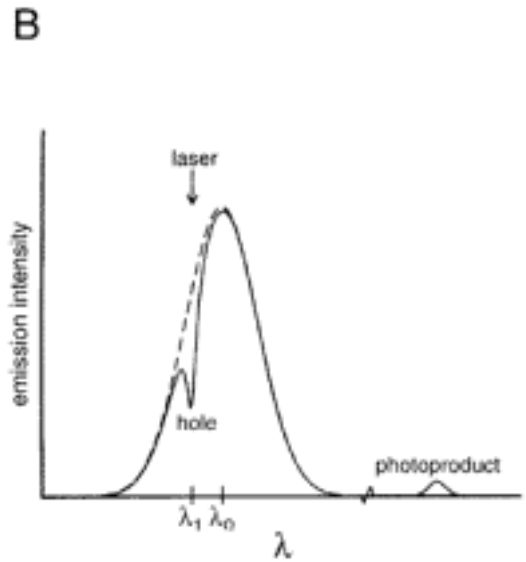
Ensemble methods for looking at distributions

Hole-burning spectroscopy

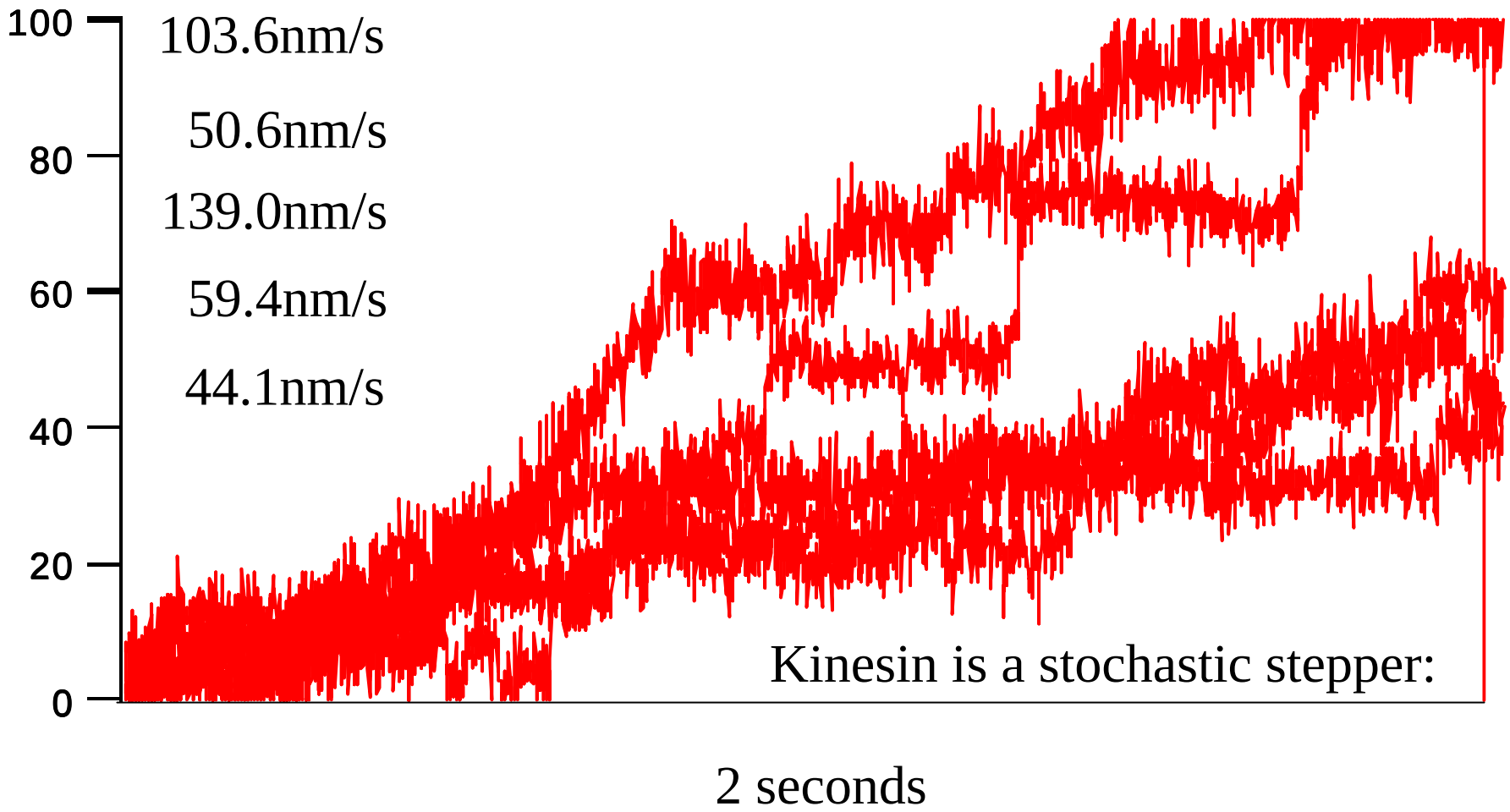
Photon Echo Spectroscopy

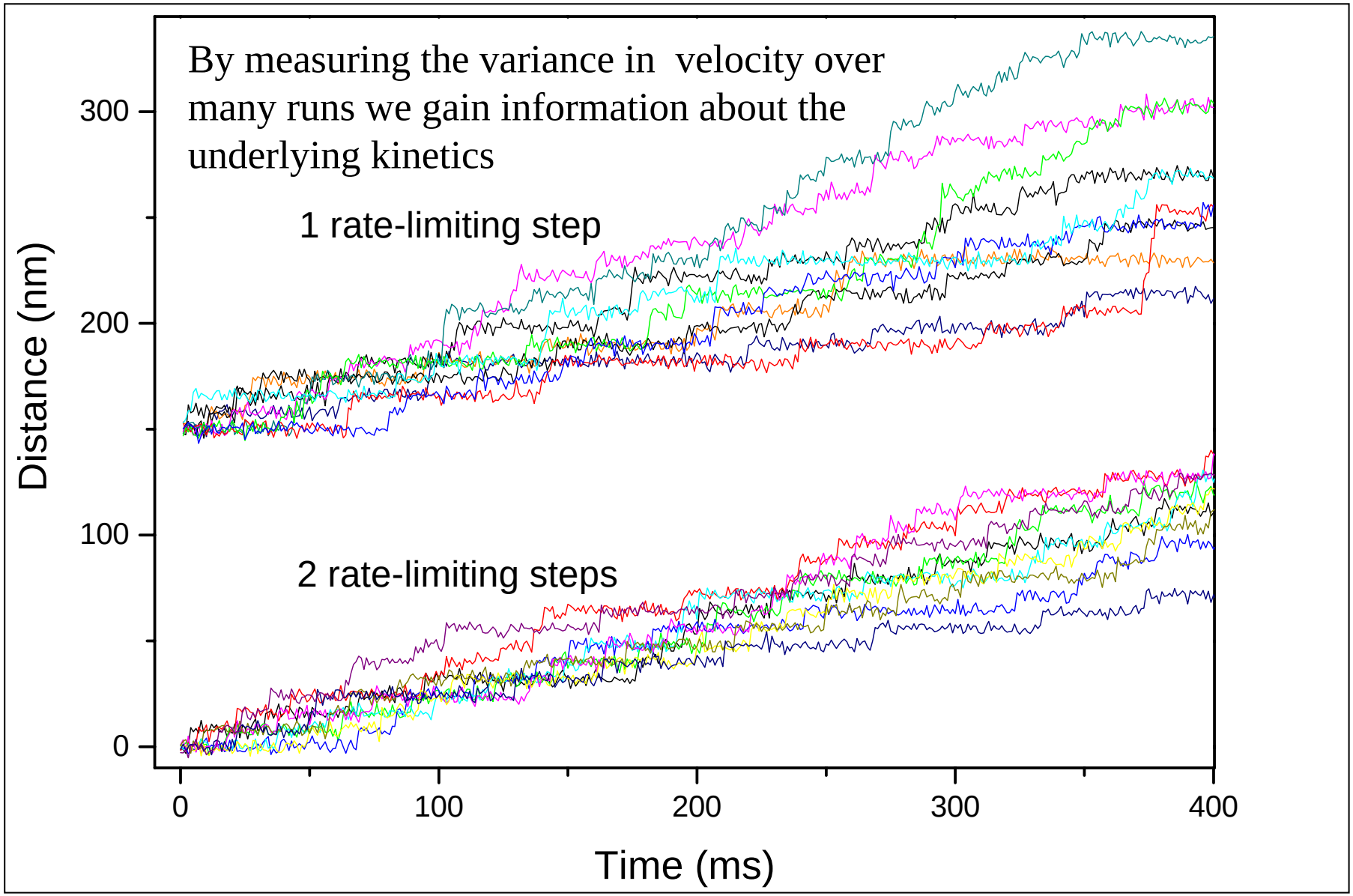
NMR
X-ray crystallography





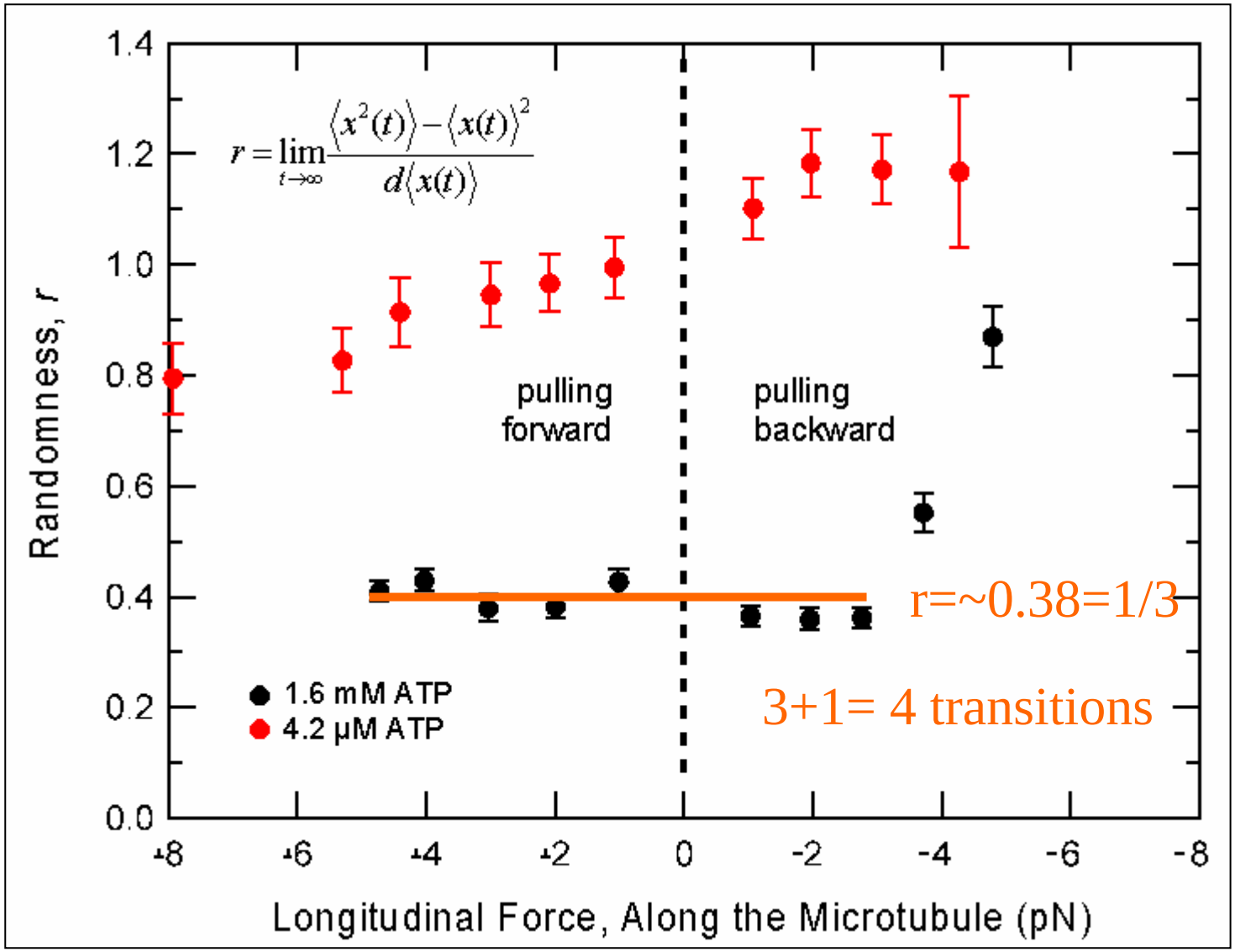
Single molecule records





Mark Schnitzer: randomness parameter

Randomness analysis

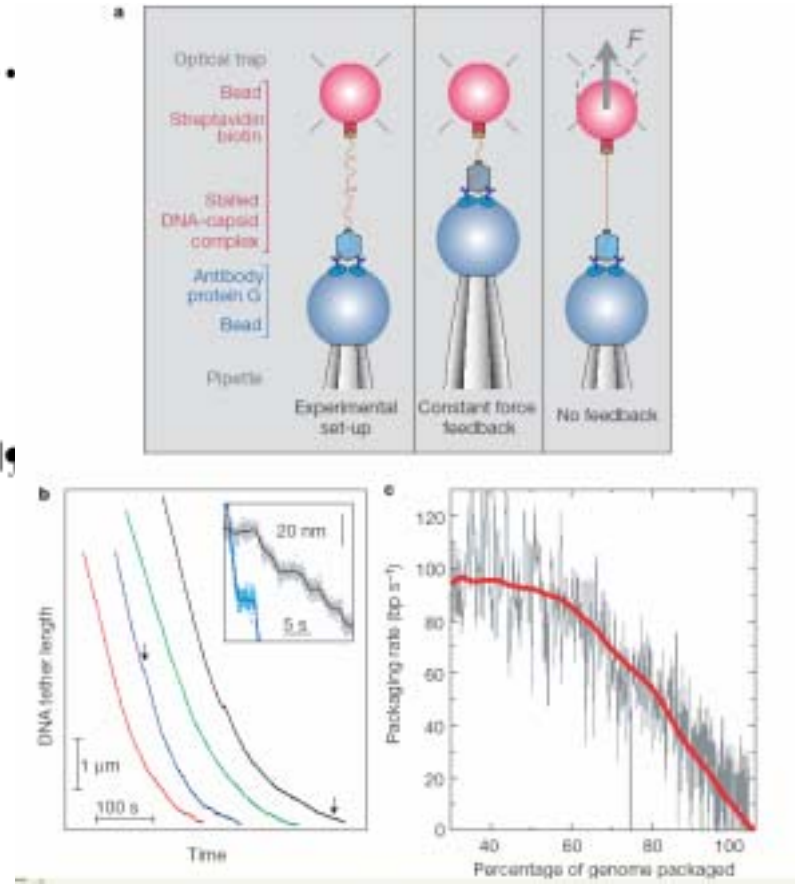


Phage packing experiments

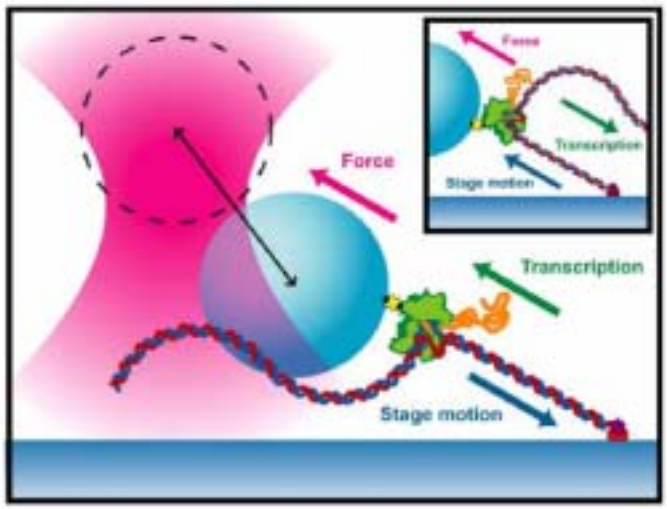
letters to nature

The bacteriophage ϕ29 portal motor can package DNA against a large internal force

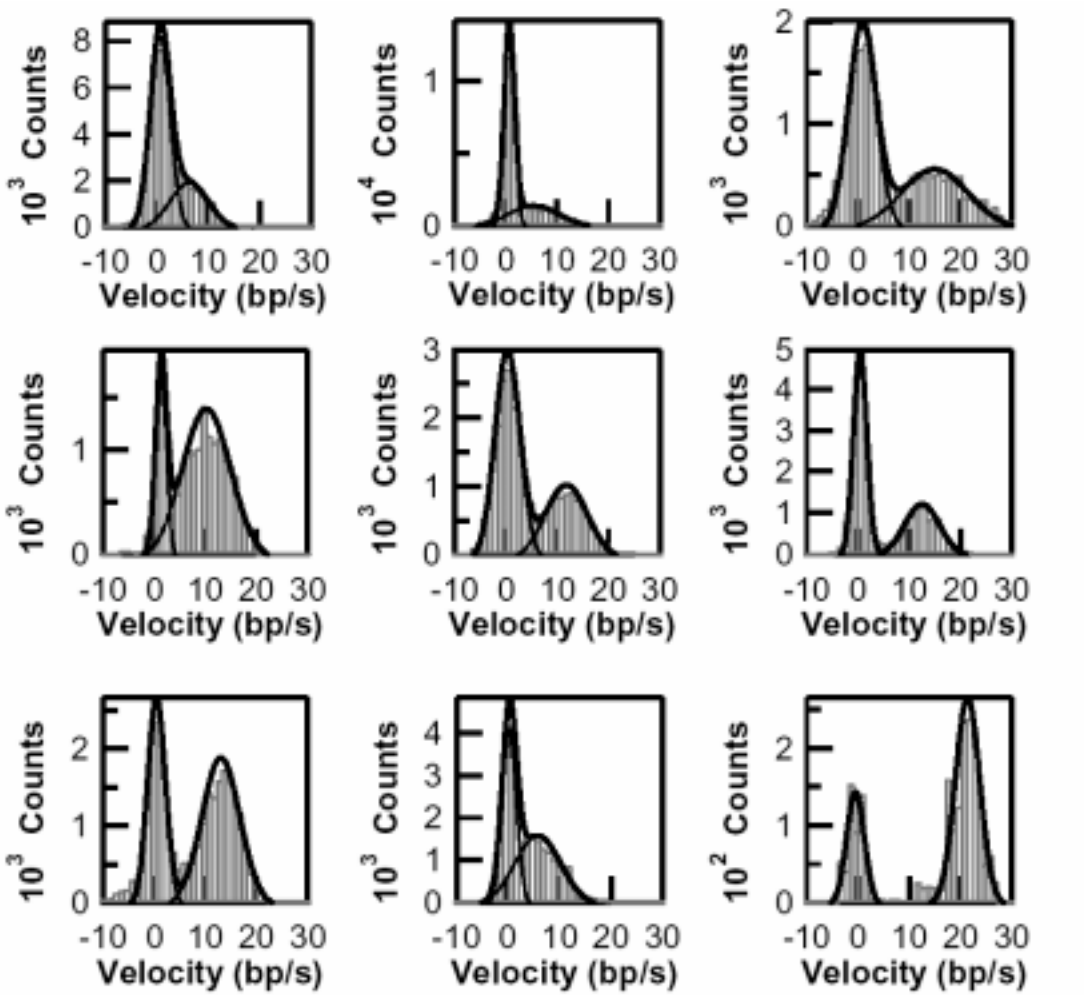
Douglas E. Smith*†, Sander J. Tans*†, Steven B. Smith‡, Shelley Grimes§, Dwight L. Anderson§ & Carlos Bustamante†‡‖¶



RNAP



Heterogeneity in RNA polymerase



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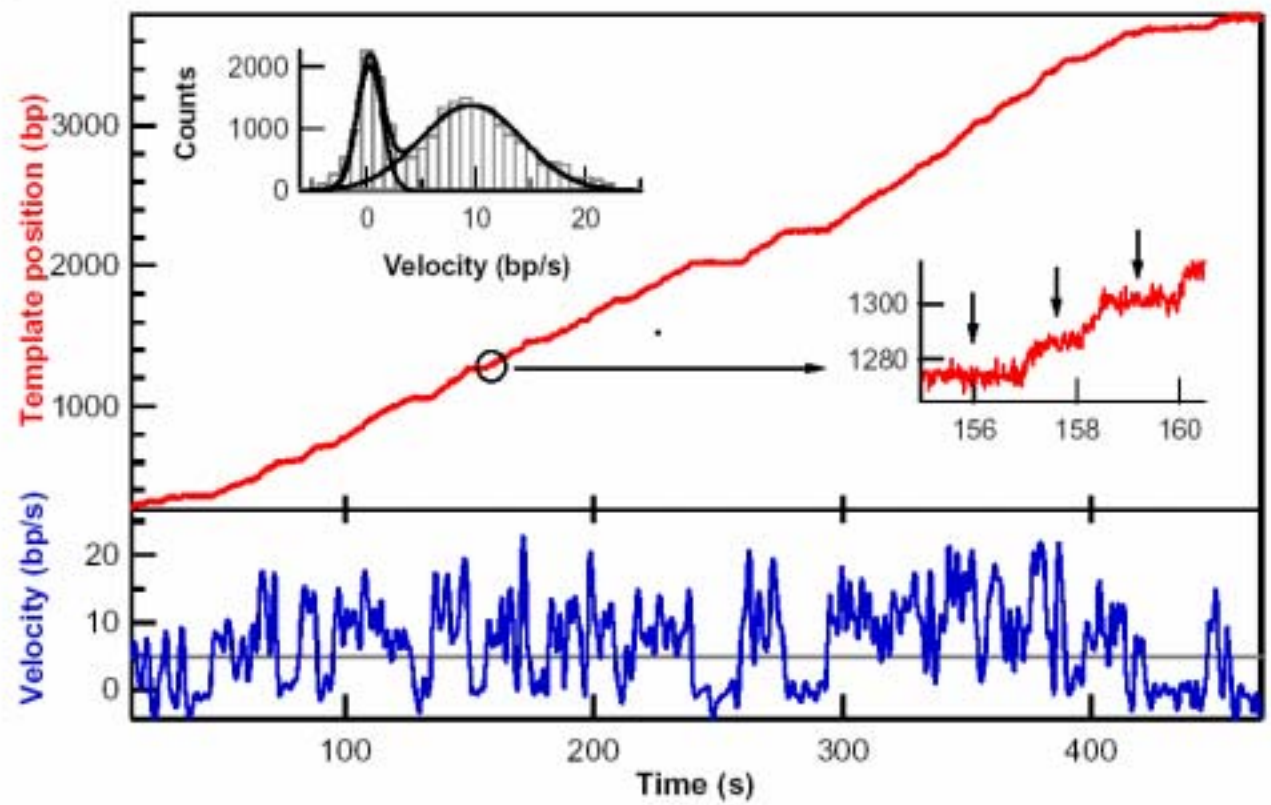

Figure 3.1 B. RNA polymerase translocation and pausing under load. Representative record of elongation for a single polymerase molecule transcribing 3.5 kb of DNA (1 mM

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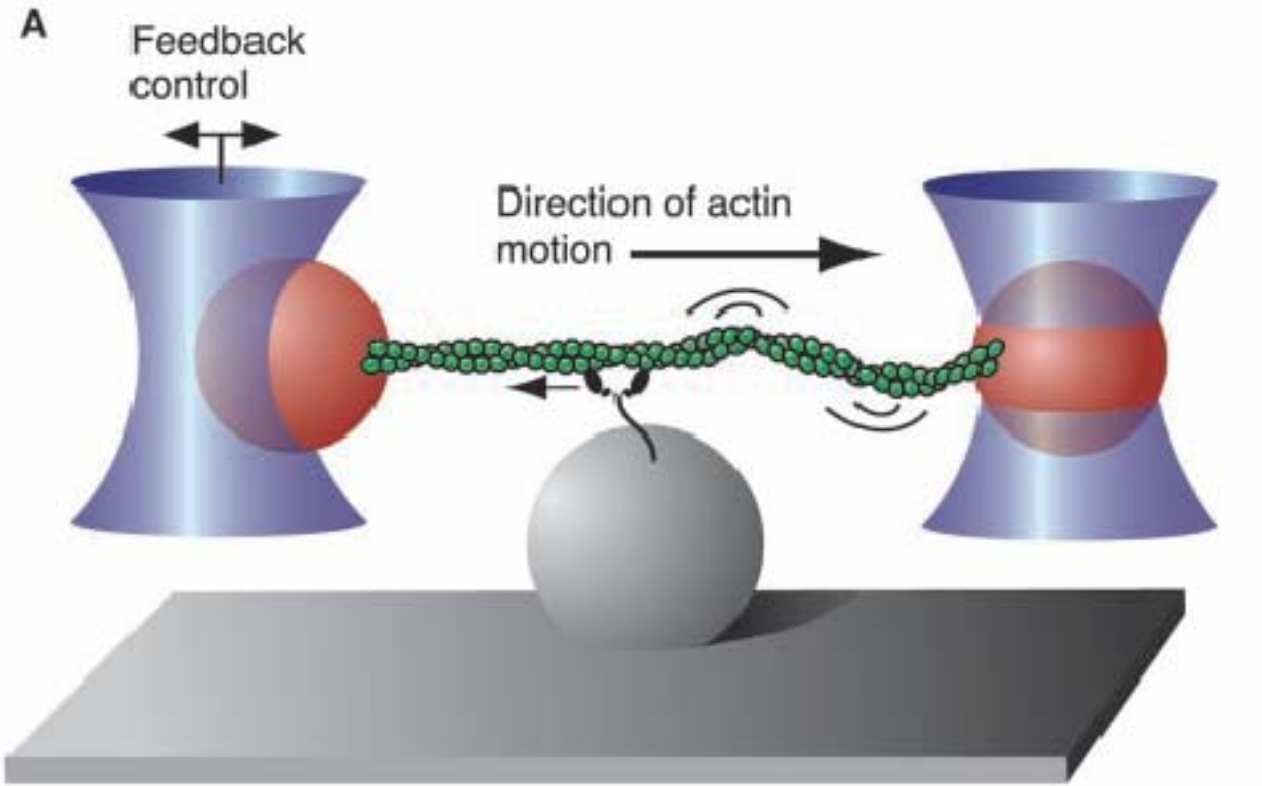
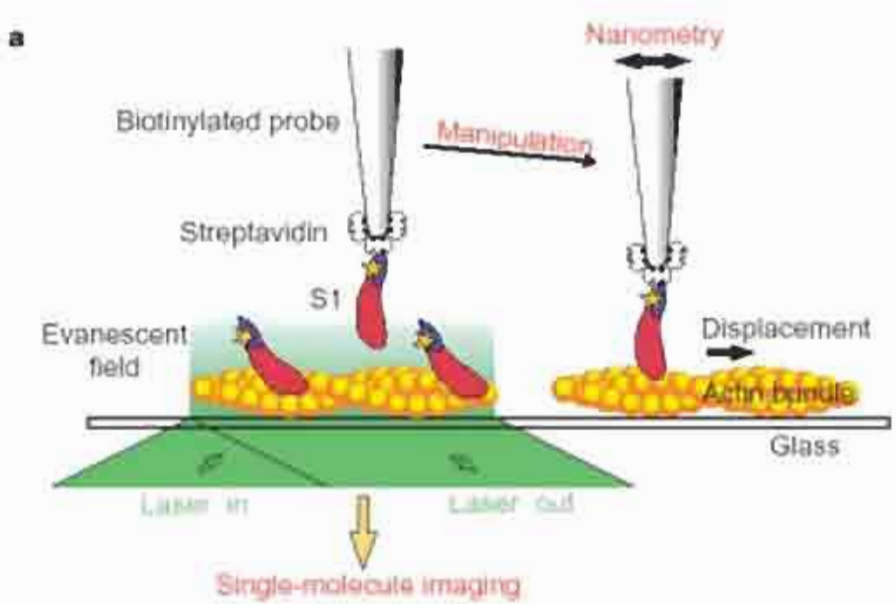
Myosin experiments, dumbell geometry

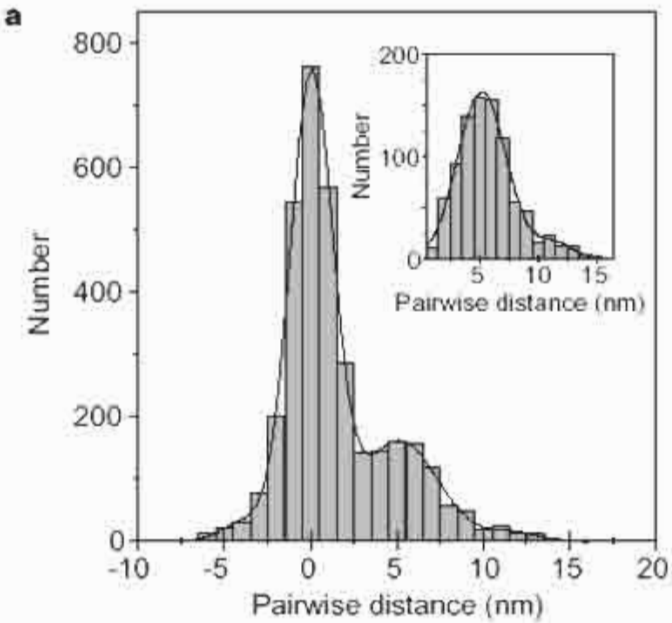


A single myosin head moves along an actin filament with regular steps of 5.3 nanometres

Kazuo Kitamura*†‡, Makio Tokunaga*§, Atsuko Hikikoshi Iwane†‖ & Toshio Yanagida*†‖¶

* *Yanagida BioMotron Project, ERATO, JST, 2-4-14 Senba-Higashi, Mino, Osaka 562-0035, Japan*
† *Department of Biophysical Engineering, Osaka University, 1-3 Machikaneyama, Toyonaka, Osaka 560-8531, Japan*
‡ *Department of Physiology I, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
§ *Structural Biology Center, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan*
‖ *Single Molecule Processes Project, ICORP, JST, 2-4-14 Senba-Higashi, Mino, Osaka 562-0035, Japan*

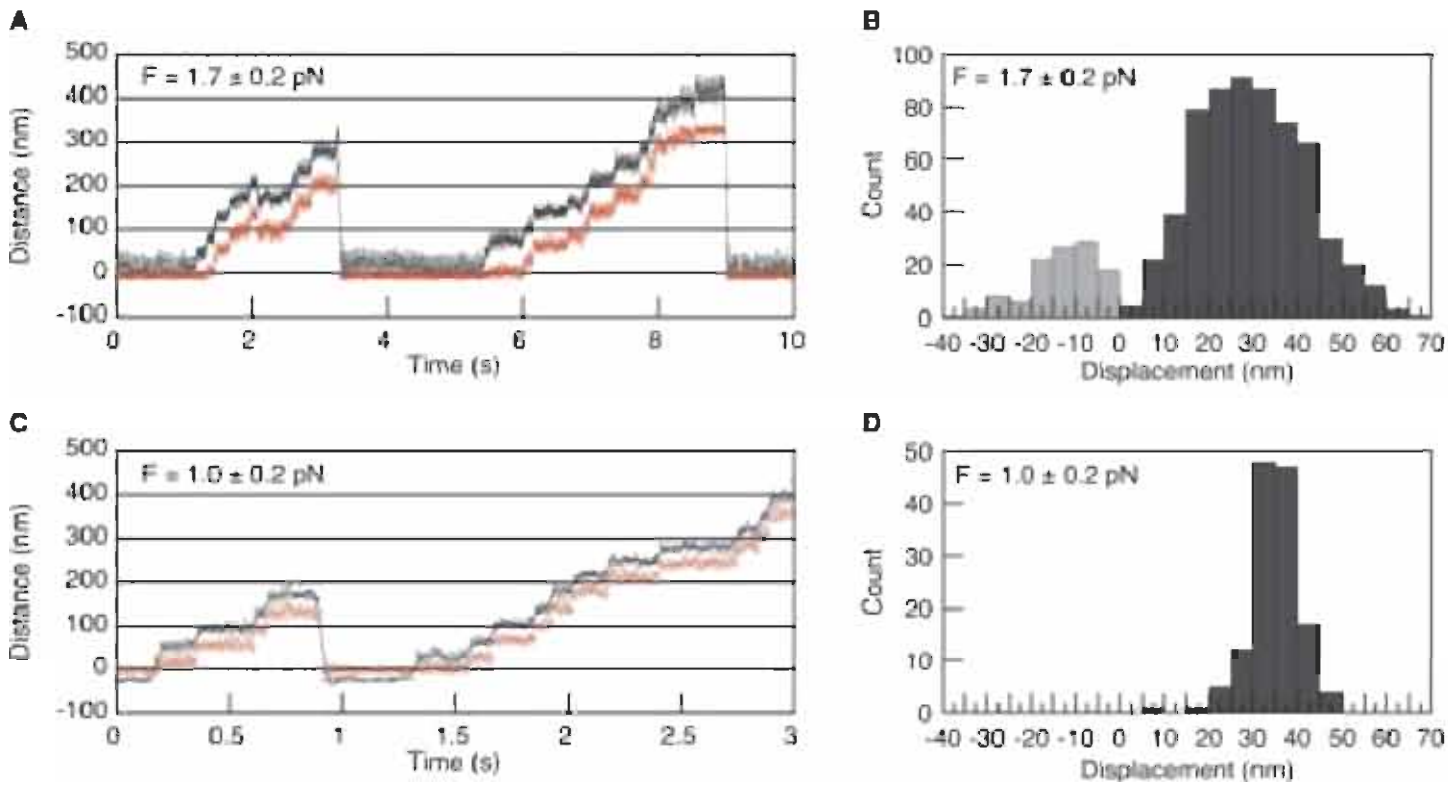




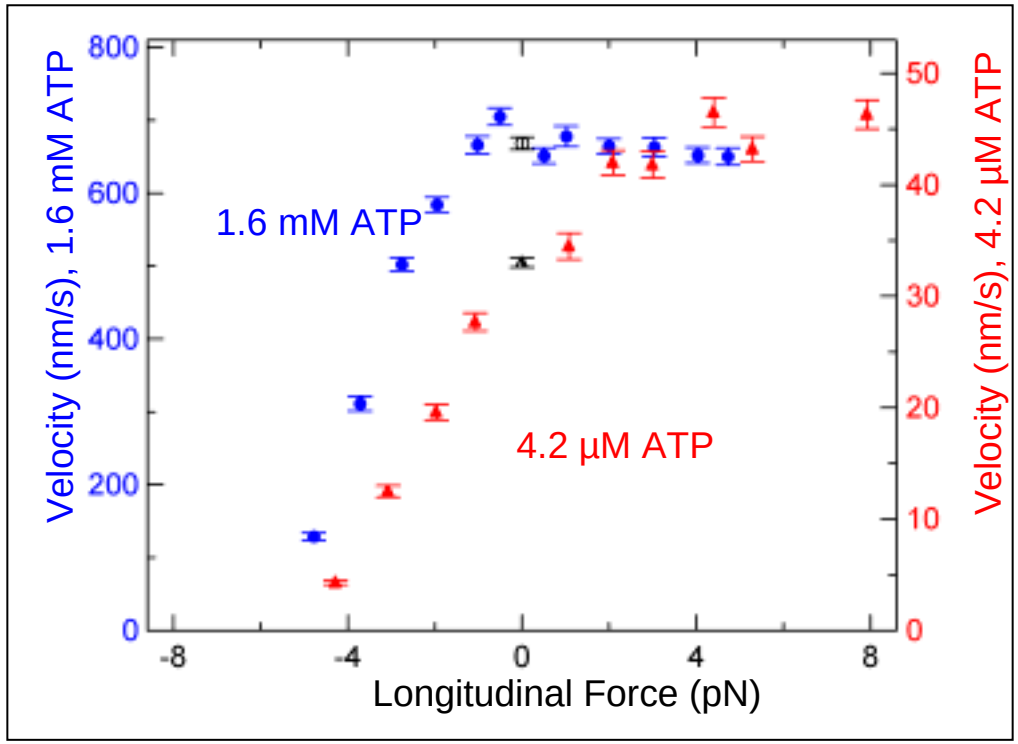
Myosin VI is a processive motor with a large step size

Ronald S. Rock*, Sarah E. Rice*, Amber L. Wells†, Thomas J. Purcell*, James A. Spudich*‡, and H. Lee Sweeney†

*Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305; and †Department of Physiology, University of Pennsylvania School of Medicine, 3700 Hamilton Walk, Philadelphia, PA 19104-6085



Force Velocity Curves



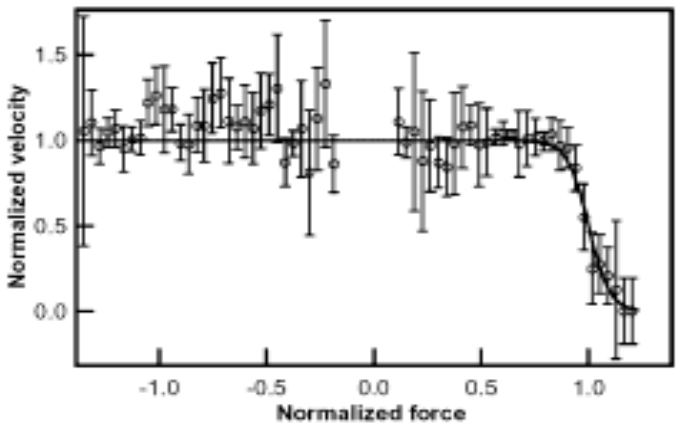


Figure 3.2. Force-velocity relationship for RNA polymerase. Normalized velocity (mean

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How does a system respond when driven away from thermal equilibrium?

C. Jarzynski*

Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM 87545

Distribution:

$$p(x) \propto e^{-E(x,\lambda_A)/k_BT},$$

$$\exp[-\beta\Delta G(z)] = \lim_{N\to\infty}\langle \exp[-\beta w_i(z,r)]\rangle_N \quad (1)$$

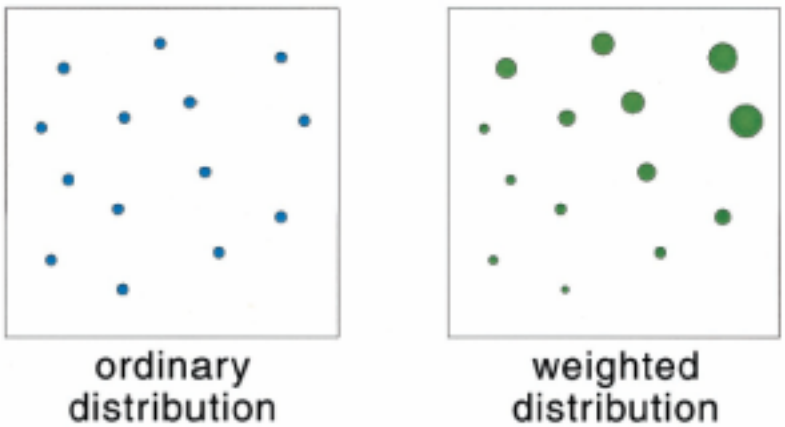


Fig. 2. A schematic representation of the ordinary and weighted distributions of molecule configurations. (*Left*) A simple snapshot of the ensemble at a given moment in time, for instance when the handle reaches the position λ_0; each circle represents the current configuration of a specific realization. (*Right*) Each realization is additionally assigned a statistical weight, depicted by the size of the size of the circle.

Free energy reconstruction from nonequilibrium single-molecule pulling experiments

Gerhard Hummer* and Attila Szabo

Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0520

Communicated by David Chandler, University of California, Berkeley, CA, January 28, 2001 (received for review November 28, 2000)

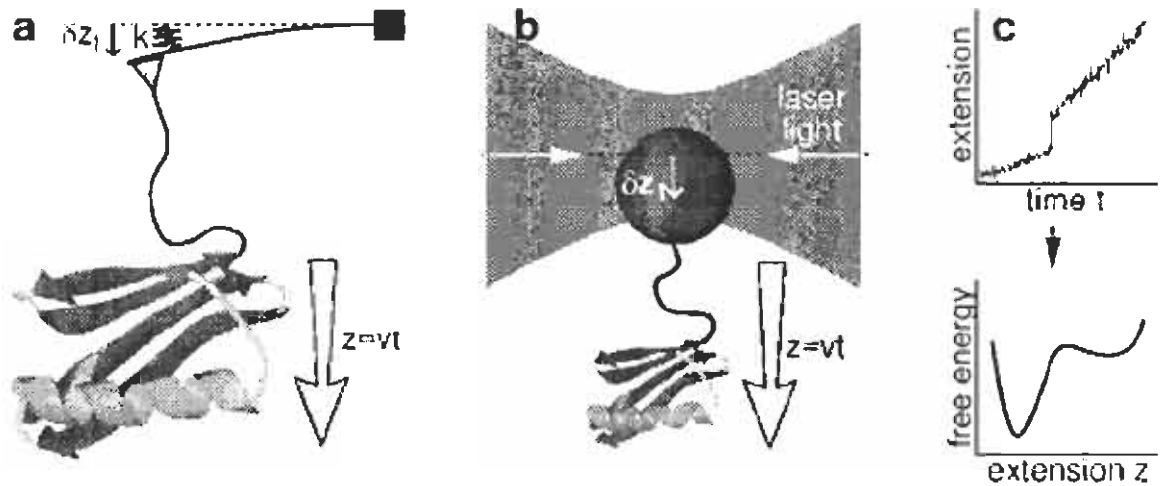


Fig. 1. Single-molecule force measuring experiments by using AFM (*a*) and laser tweezers (*b*). In the AFM experiment (*a*), the sample is moved at a constant speed v relative to the cantilever with spring constant k. The position $z_t = vt + \delta z_t$ of the cantilever tip with respect to the sample is recorded, where δz_t is the displacement of the cantilever tip. From repeated measurements of z_t, the free energy profile $G_0(z)$ of the unperturbed system can be determined exactly (*c*).

Equilibrium Information from Nonequilibrium Measurements in an Experimental Test of Jarzynski's Equality

Jan Liphardt,[1,4] Sophie Dumont,[2] Steven B. Smith,[3] Ignacio Tinoco Jr.,[1,4] Carlos Bustamante[1,2,3,4]*

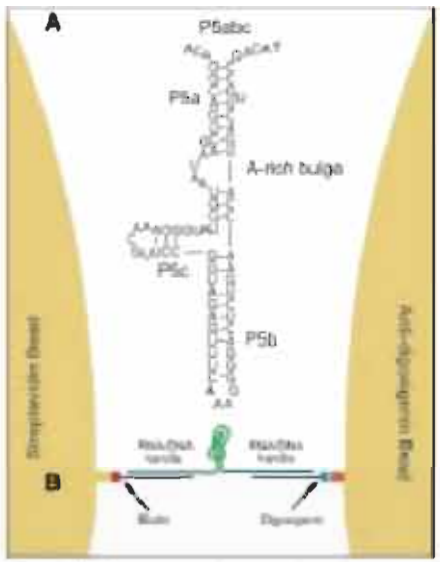


Fig. 1. (A) Sequence and secondary structure of the P5abc RNA. (B) RNA molecules were attached between two beads with RNA-DNA hybrid handles.

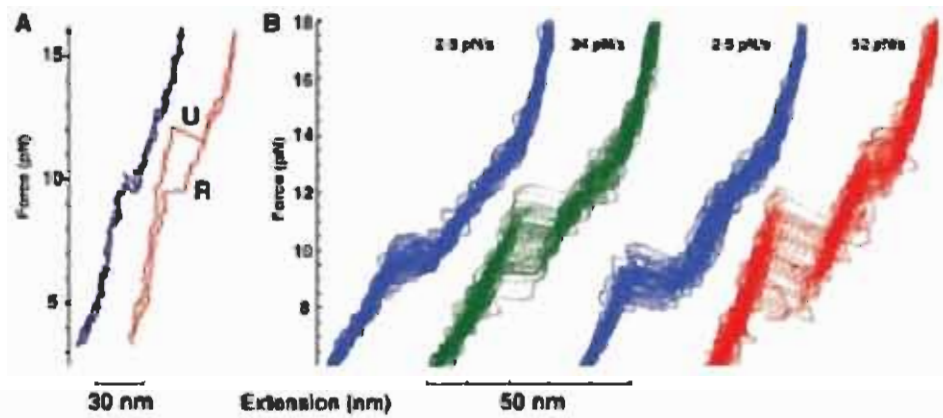
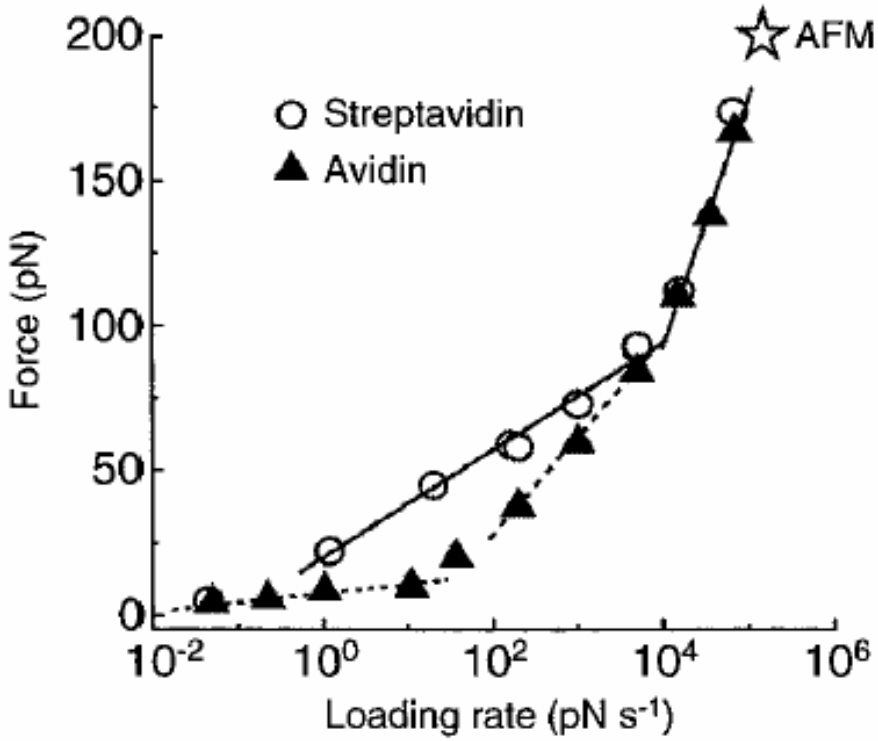


Fig. 2. Force-extension unfolding curves of P5abc at three different switching rates. (A) Typical force-extension unfolding (U) and refolding (R) curves of the P5abc RNA in 10 mM EDTA in reversible (blue, 2 to 5 pN/s) and irreversible (red, 52 pN/s) switching conditions. (B) Two experiments are shown: one in which a molecule was unfolded at rates of 2 to 5 pN/s and 34 pN/s (left pair, blue and green), and another in which the molecule was unfolded at rates of 2 to 5 pN/s and 52 pN/s (right pair, blue and red). Curves (superposition of about 40 curves per experiment) were smoothed by convolution with a Gaussian kernel.

Loading rate matters



A two-state kinetic model for the unfolding of single molecules by mechanical force

F. Ritort*, C. Bustamante[†§], and I. Tinoco, Jr.[‡¶]

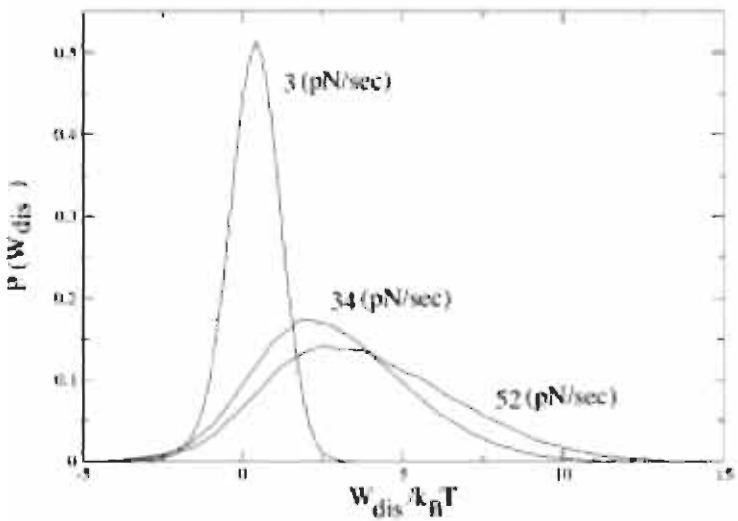


Fig. 3. Dissipated work probability distributions at pulling rates of 3, 34, and 52 pN/sec. They compare reasonably well with those reported in ref. 8.

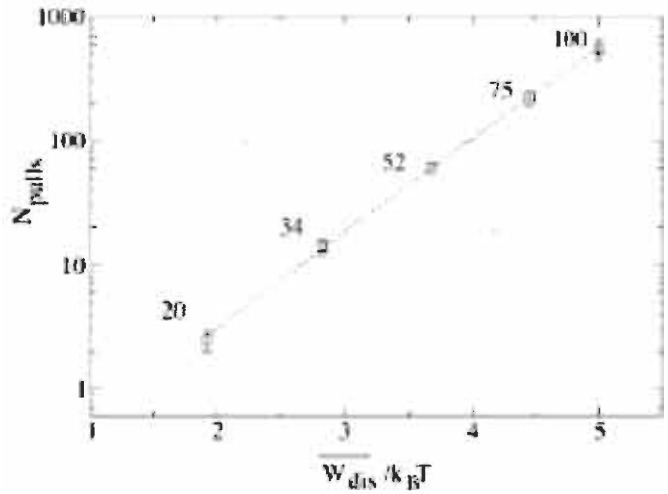
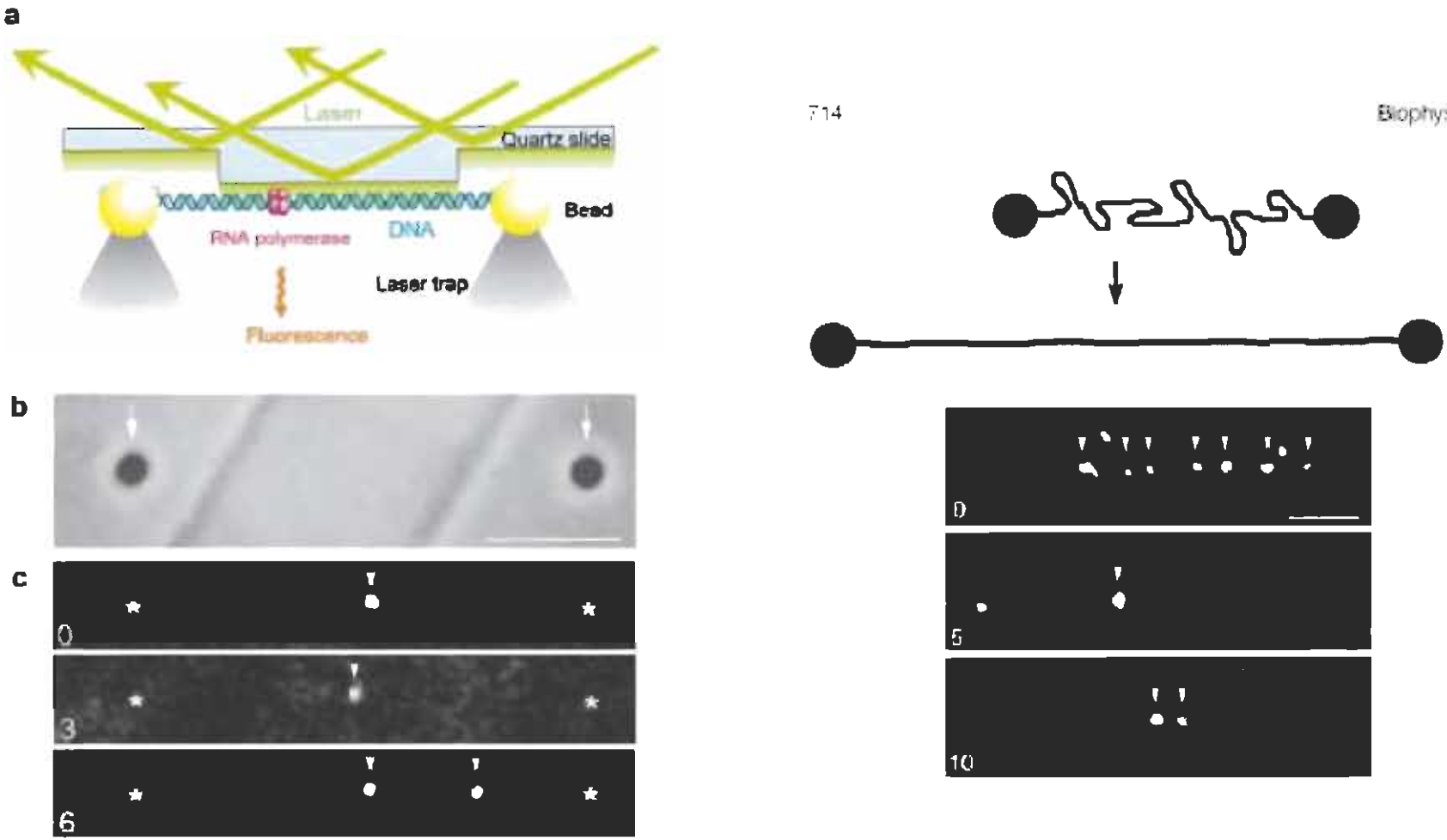


Fig. 5. Comparison of the number of pulls necessary to obtain an estimate for the Jarzynski average within k_BT for five pulling rates in pN/sec (squares) fit to the theoretical expression, Eq. **11**. Each point corresponds to 100 sets of calculations with each set having the same number of pulls, N_{pulls}. The error bars show the variation among the sets. The fit to Eq. **11** yields $R \sim 1.5$ in good agreement with the value obtained by analyzing the left Gaussian tail of the distributions shown in Fig. 3. The dotted box indicates the dynamical regime explored in the experiments (8).

Biophysical Journal Volume 76 February 1999 709–715

Single-Molecule Imaging of RNA Polymerase-DNA Interactions in Real Time

Yoshie Harada,* Takashi Funatsu,* Katsuhiko Murakami,# Yoshikazu Nonoyama,§ Akira Ishihama,# and Toshio Yanagida*§¶



Kinesin–microtubule binding depends on both nucleotide state and loading direction

Sotaro Uemura*, Kenji Kawaguchi*, Junichiro Yajima†, Masaki Edamatsu†, Yoko Yano Toyoshima†, and Shin'ichi Ishiwata*‡§¶

In PNAS

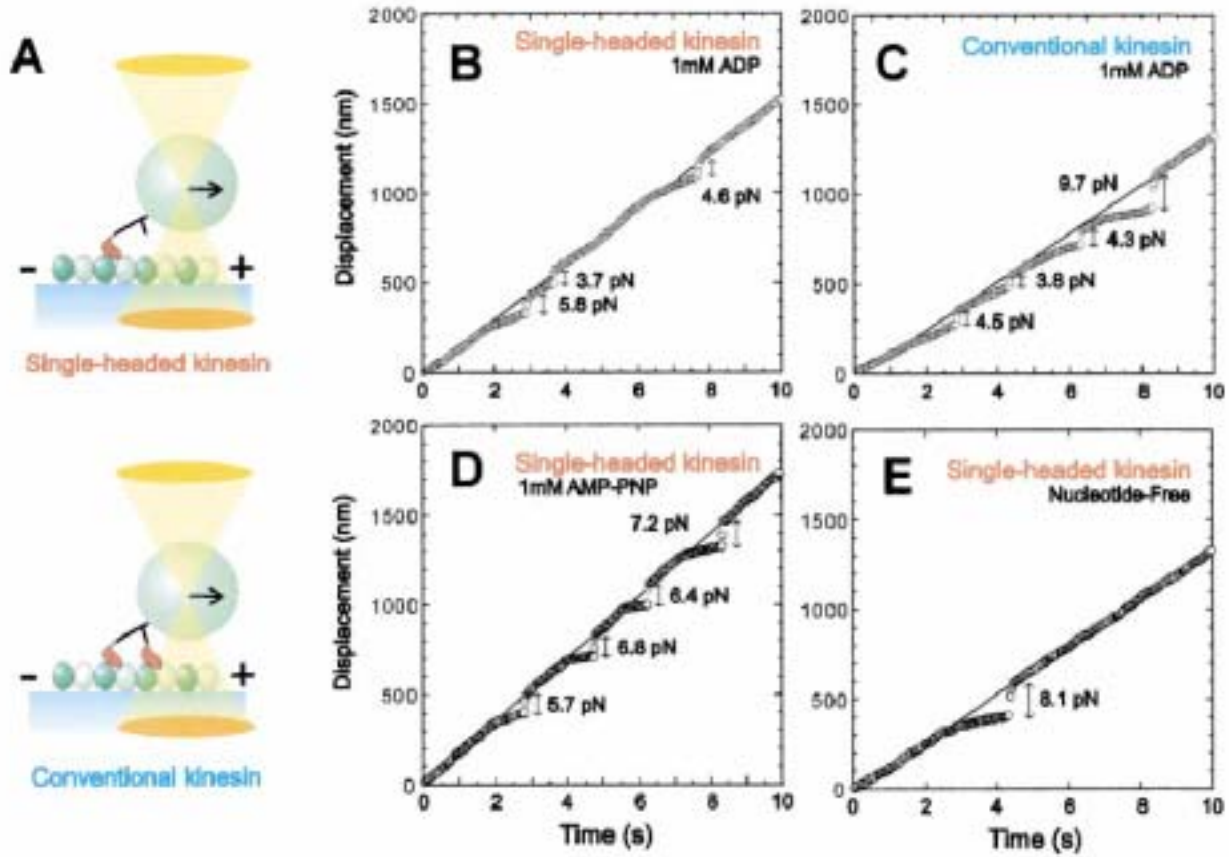


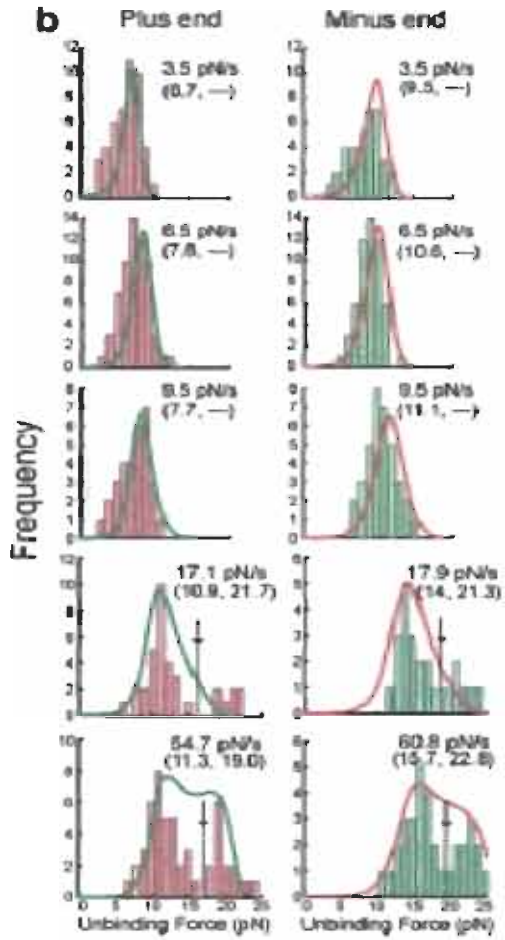
Fig. 1. Measurement of unbinding force. (*A*) Schematic illustration showing the method of application of external load to one-headed or two-headed kinesin-coated bead by using optical tweezers. The relative size of the bead to kinesin is reduced to about 1/10 of the actual scale. In this illustration, the load is applied toward the plus-end of a microtubule. (*B* and *C*) Examples in the ADP state showing the time course of movement of the trap center (thin lines) and the bead (circles) on which one-headed (*B*) or conventional two-headed (*C*) kinesin was attached. The trap center was moved at a constant rate toward the plus-end of a microtubule. The unbinding force was estimated from the abrupt displacement of the bead. (*D* and *E*) Examples showing the behavior of the bead in the AMP-PNP state (*D*) or in the nucleotide-free state (*E*). In all examples, the load was applied toward the plus end.

Biophysical Journal Volume 84 February 2003 1103–1113

Equilibrium and Transition between Single- and Double-Headed Binding of Kinesin as Revealed by Single-Molecule Mechanics

Kenji Kawaguchi,* Sotaro Uemura,* and Shin'ichi Ishiwata*†

*Department of Physics, School of Science and Engineering, †Advanced Research Institute for Science and Engineering, and Materials Research Laboratory for Bioscience and Photonics, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan



Journal of Biology

BioMed Cent

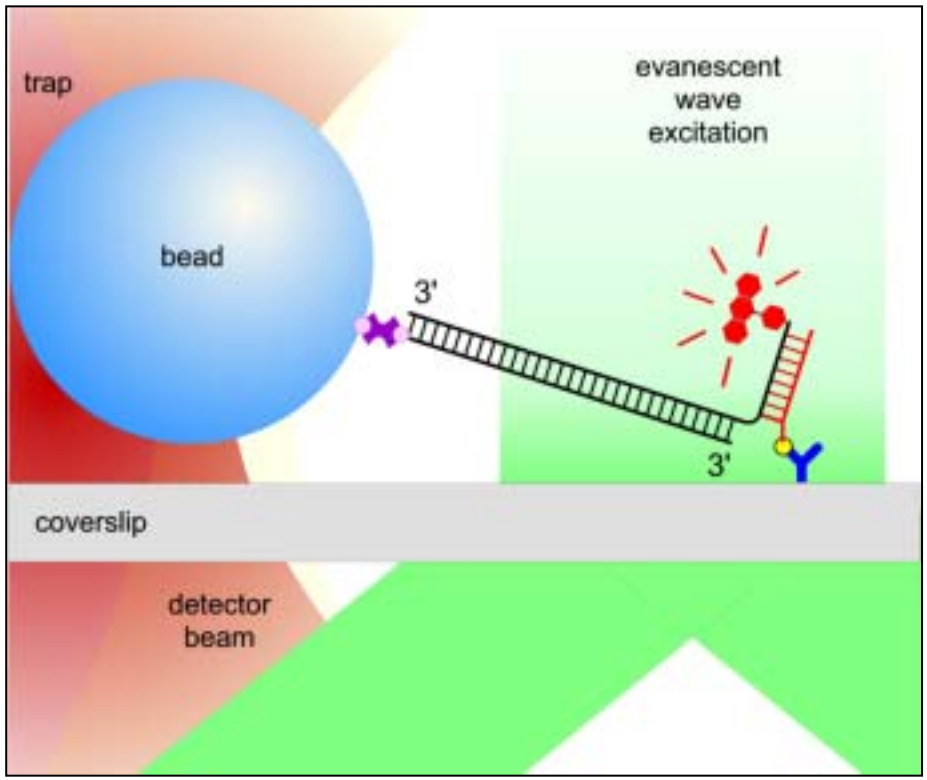
Research article

Combined optical trapping and single-molecule fluorescence

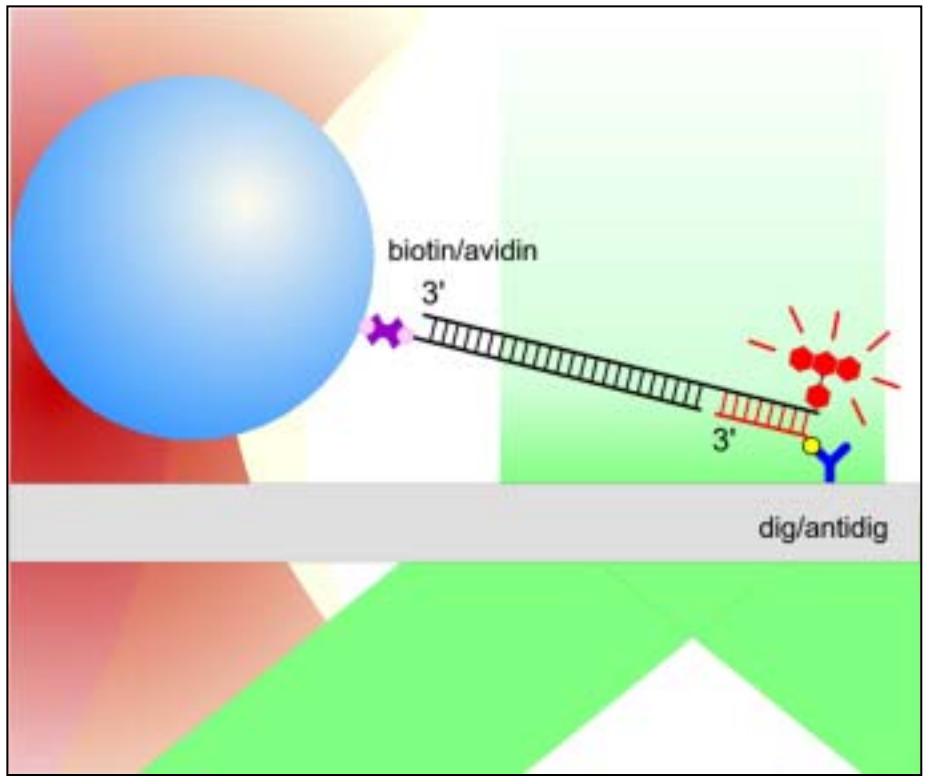
Matthew J Lang*†‡, Polly M Fordyce§ and Steven M Block*†

Force-induced strand separation of ds DNA

Geometry for "Unzipping" Force

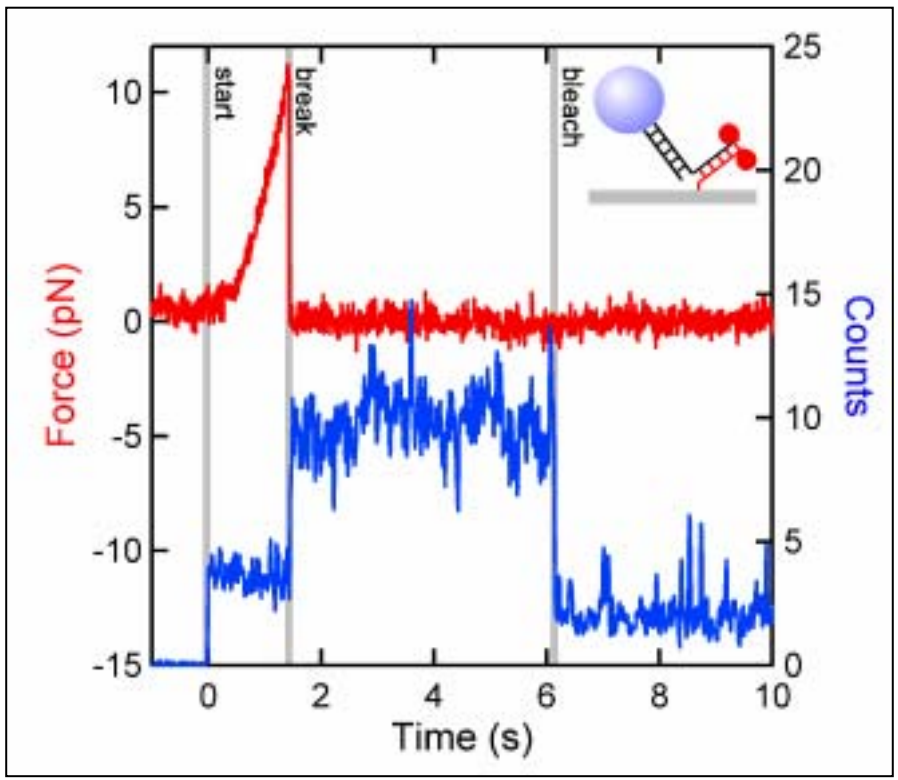


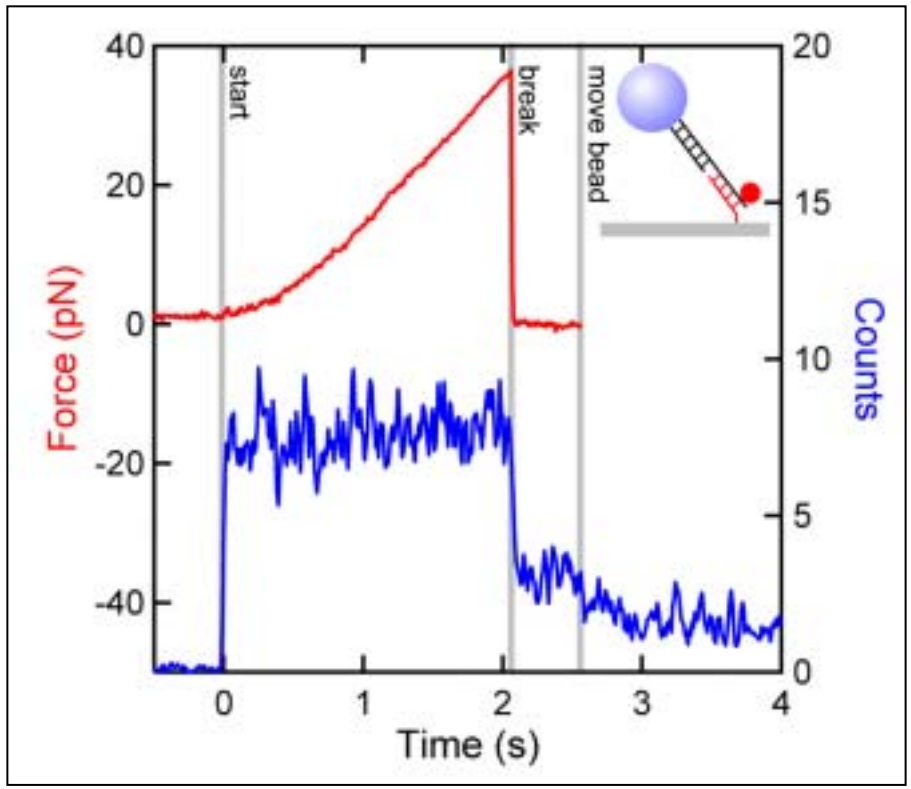
Geometry for "Shearing" Force



Chromophores on adjacent base pairs unquench at the mechanical break.

HIGHER RUPTURE FORCES FOR SHEARING





COMPARING SHEARING AND UNZIPPING