Single-Molecule Manipulation Experiments of Biological Molecules I: Introduction and Techniques

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Outline

- Single-molecule manipulation experiments of biological molecules I :

  Introduction and Techniques
- Single-molecule manipulation experiments of biological molecules II :

  Principles Involved in Interpreting Force Measurements
- Single-molecule manipulation experiments of biological molecules III :

  Applications to Biological Systems

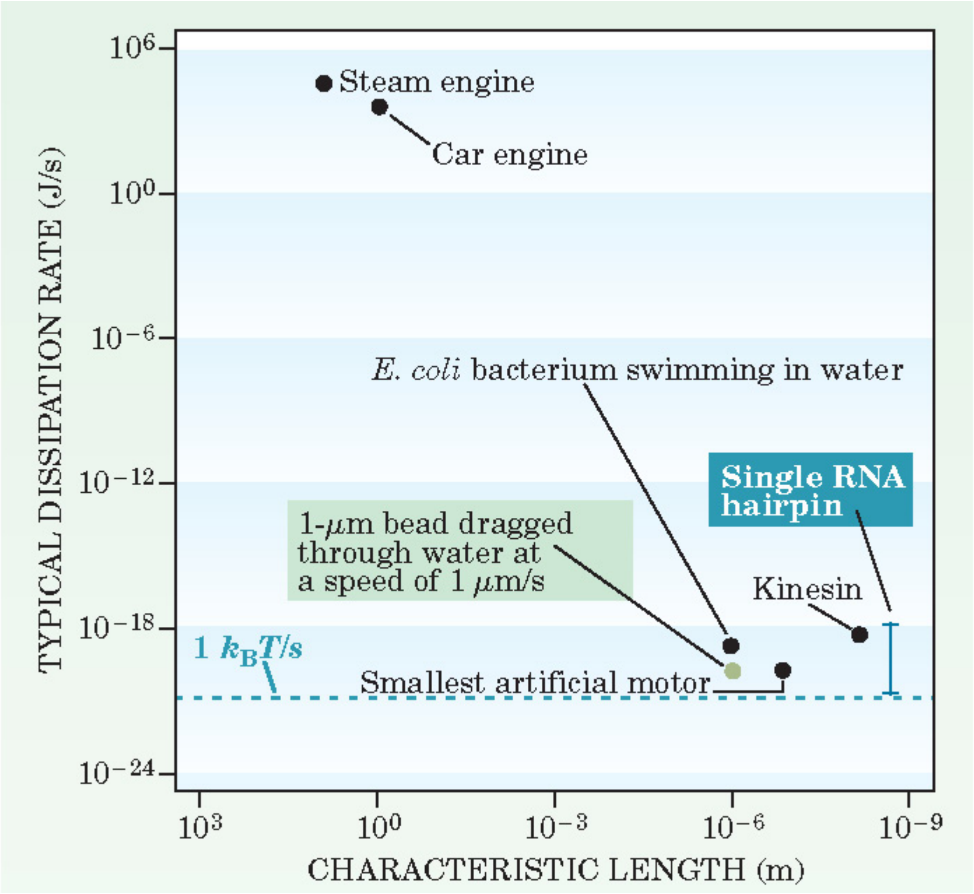
Single-molecule manipulation experiments of biological molecules I : Introduction and Techniques

- Introduction
  - Background
  - Motivation
- Experimental techniques
  - Atomic force microscopy
  - Optical tweezers
  - Magnetic tweezers

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	

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)

TABLE 1 Mechanical properties of selected molecules

Molecule	ΔG^0 (kcal/mol)[a]	F_u (pN)[b]	$\Delta X^{\ddagger}_{f\rightarrow u}$ (nm)	$k^0_{f\rightarrow u}$ (s^{-1})	Structure at "breakpoint"	Reference
dsDNA	1.5–3 (per base pair)	9–20[c]	—	—	DNA base pairs	(110, 111)
P5ab hairpin	37.5 ± 4.8	14.5 ± 0.4[d]	11.9	—	RNA base pairs	(4)
P5abcΔA three-helix junction − Mg^{2+}	34.4 ± 4.8	11.4 ± 0.5[d]	~12	—	RNA base pairs	(4)
P5abc three-helix junction + Mg^{2+}	36.3[e]	19 ± 3 (at 3 pN/s with optical tweezers)	1.6 ± 0.1	2×10^{-4}	Hairpin-bulge	(4)
127 domain from titin (unfolding from native state)	7.6	~100	0.59	1×10^{-6}	Parallel β-sheet shear	(27)
127 domain from titin (unfolding from intermediate)	—	204 ± 26	0.25	3.3×10^{-4}	Parallel β-sheet shear	(112)
128 domain from titin	3.0	257 ± 27	0.25	2.8×10^{-5} or 1.9×10^{-6}	Parallel β-sheet shear	(23, 46)
^{10}FNIII domain from fibronectin	—	74 ± 20	0.38	2.0×10^{-2}	Antiparallel β-sheet shear	(14, 35)
C2A domain	—	60	—	—	β-sheet zipper	(42)
Spectrin	4.8 ± 0.5[f]	25–35 (at 300 nm/s)	1.7 ± 0.5	3.3×10^{-5}	α-helix bundle	(37)
Ubiquitin (N-C linked)	6.7[g]	203 ± 35 (at 400 nm/s)	0.25	4×10^{-4}	β-sheet shear	(38)
Ubiquitin (K48-C linked)	6.7[g]	85 ± 20 (at 300 nm/s)	0.63	4×10^{-4}	β-sheet shear	(38)

[a]From solution measurements, where available. Note that thermodynamic stability ΔG^0 is not a mechanical property, nor does it correlate with mechanical stability, Fu.

[b]All unfolding forces are quoted for pulling speeds in the AFM of 600 nm/s, unless otherwise noted.

[c]Forces obtained vary from 9 pN for AT base pairs to 20 pN for GC base pairs and were the same for optical tweezers and AFM experiments, suggesting that unfolding occurs at equilibrium.

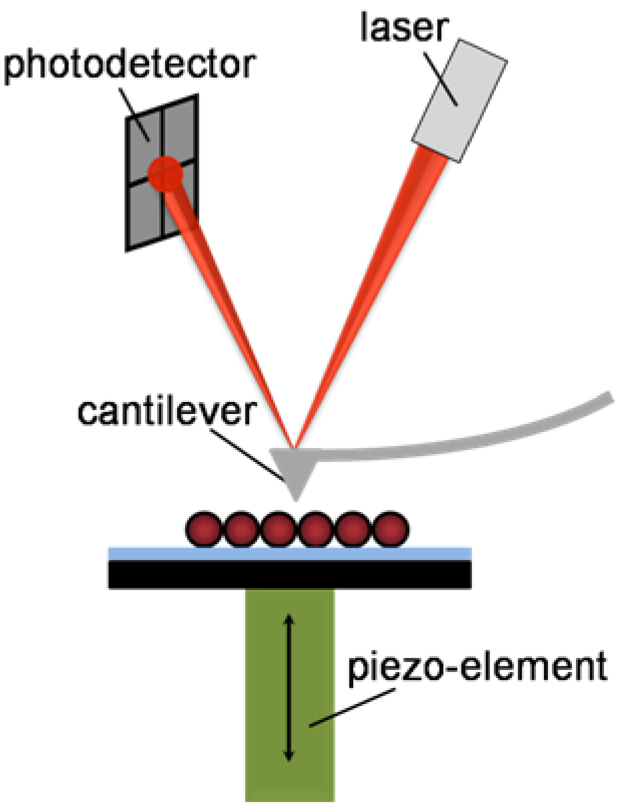
[d]F_u quoted for these secondary structures represents $F_{1/2}$, the force at which the molecule was equally likely to populate the folded and unfolded states. These experiments were done in the optical tweezers under equilibrium, constant force conditions.

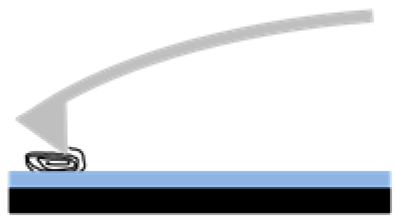
[e]Theoretical prediction from Reference 4.

[f]For the α16 domain at 25°C (113).

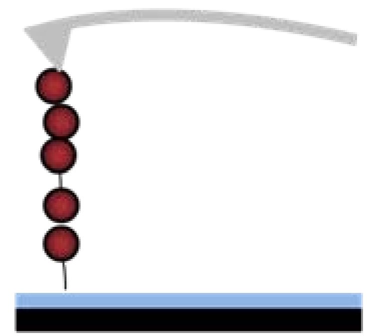
[g]Reference 114.

Stretching Single Molecules with Atomic Force Microscope

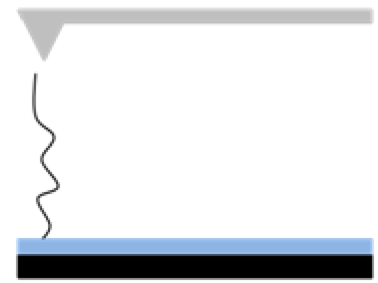




A single molecule is attached between the AFM tip and the substrate surface.

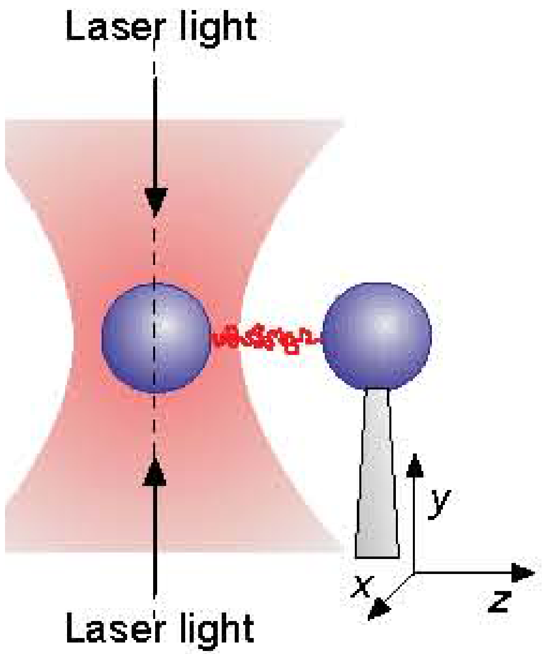


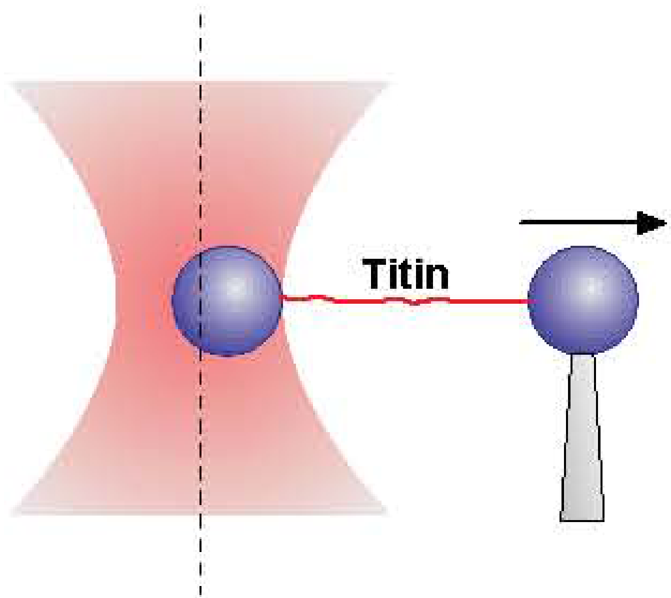
Pulling the molecule causes the cantilever to bend, the cantilever restoring force is recorded as a function of time, which is converted to extension.



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

Optical Traps

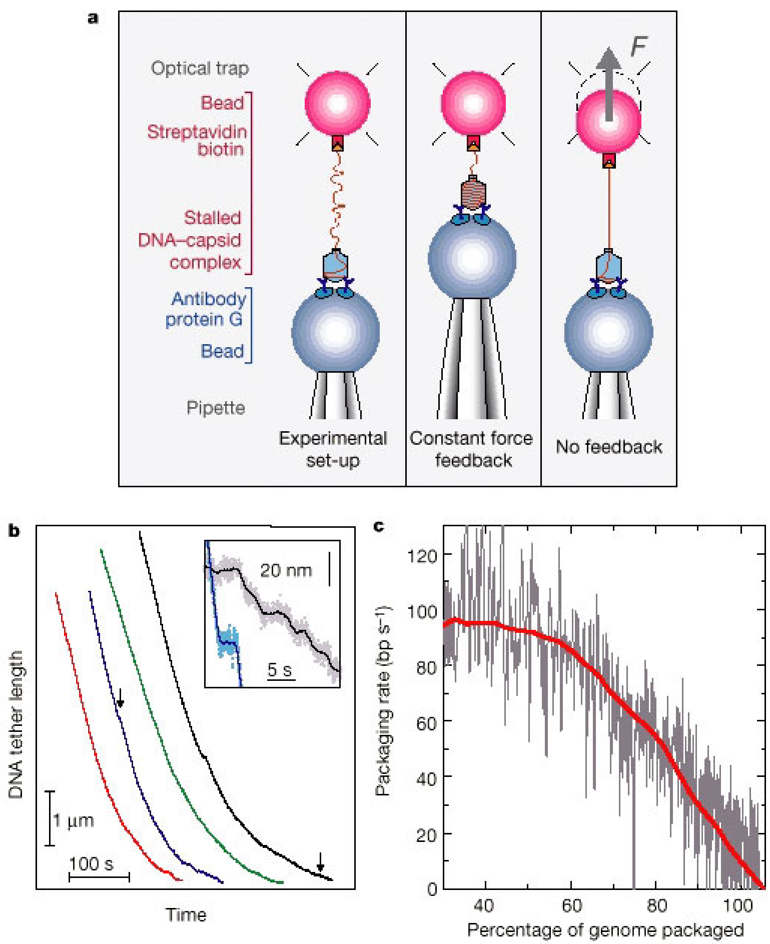




Molecules were stretched by moving the micropipette away from the optical trap. The distance that the micropipette has traveled (*z*) and the force (*f*) on the trapped bead were measured as a function of time.

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

Laser Optical Tweezers

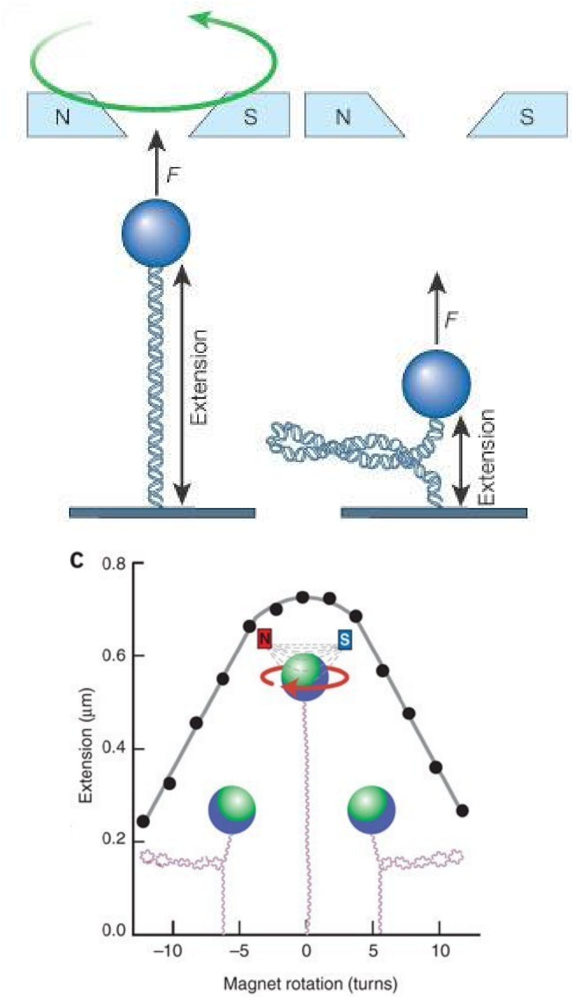


Bustamante, et al. Nature 413, 748 (2001).

- Dielectric microsphere (bead) trapped by focused laser beam, restoring force near center obeys Hooke's law
- Second microsphere attached to micropipette using suction, with position controlled by piezo
- Bead positions measured by optical imaging
- Spring constant of the trap measured thermal fluctuations of the trapped bead
- 1 pN resolution, force up to 300 pN; single base-pair resolution possible in unzipping experiments

Magnetic Tweezers

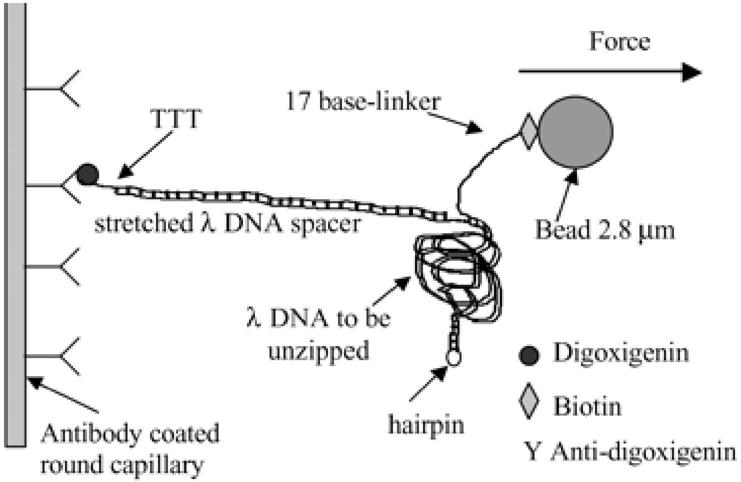
- A force is exerted on a magnetic microsphere using an applied B field
- Single molecule attached between bead and surface
- Bead is free to rotate, torque can be applied by rotating the B field
- Force measured by imaging thermal fluctuations in bead position
- 0.1 pN resolution, force up to 30 pN
- Can measure twist, writhe, and stretch at same time

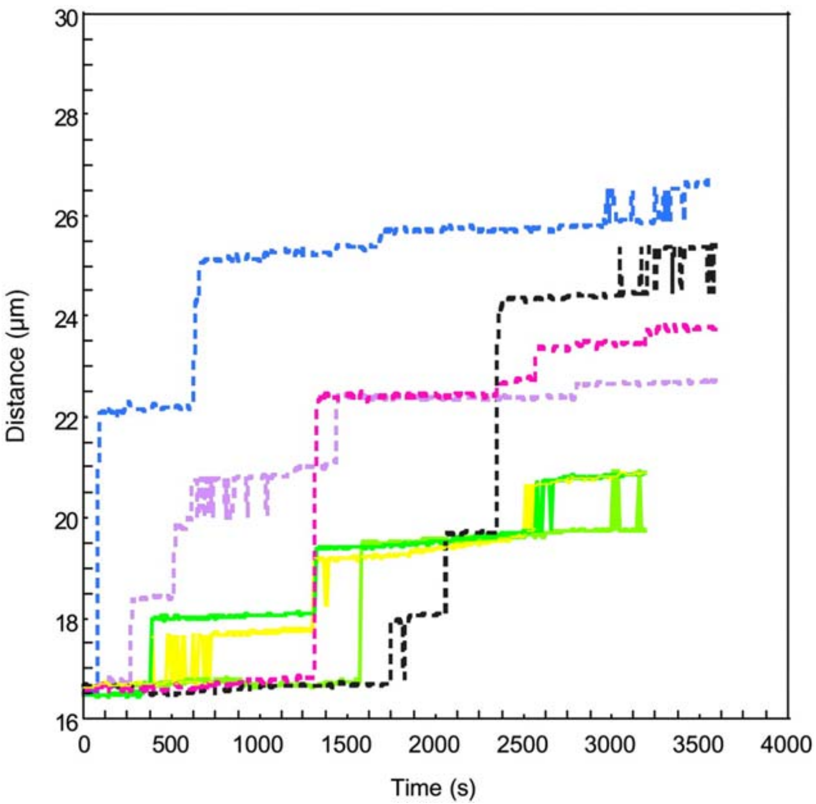


Bustamante, et al. (2003) Nature 421, 423-427.

Keir C Neuman & Attila Nagy. (2008) Nature Methods 5, 491-505.

Magnetic Tweezers for DNA Unzipping





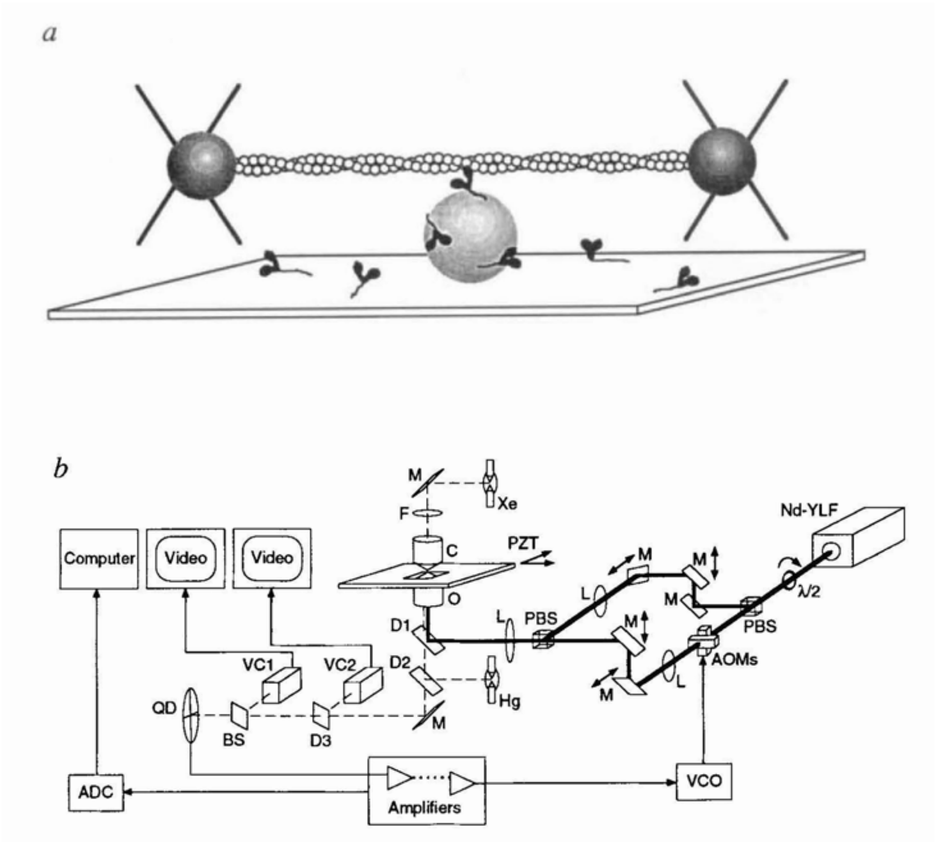
Measurement of the sequence-dependent pauses in DNA unzipping at constant forces

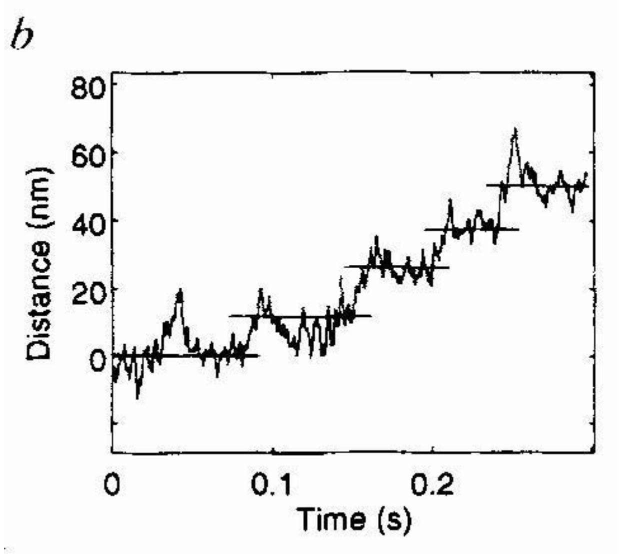
Danilowicz et al. PNAS 100, 1694 (2003).

Systems Studied with Single-Molecule Manipulation

- Molecular motors
- DNA mechanics
- RNA folding
- Protein folding
- Virus packaging
- Protein-DNA interactions

Molecular Motors

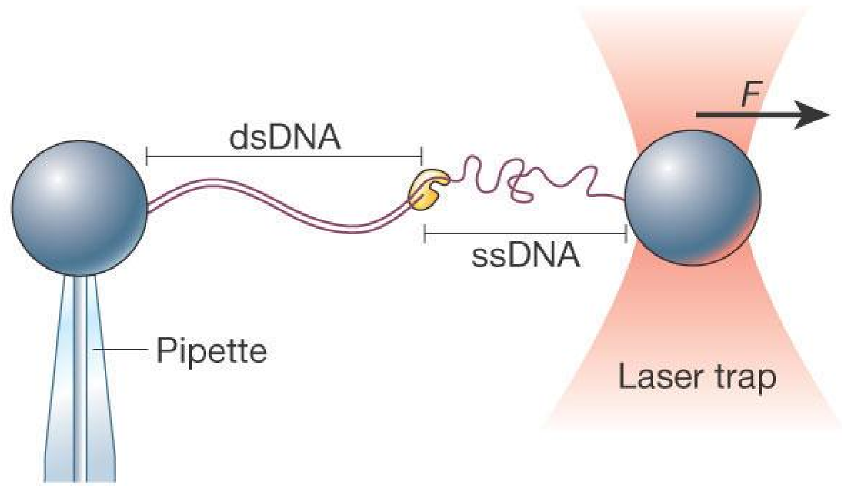


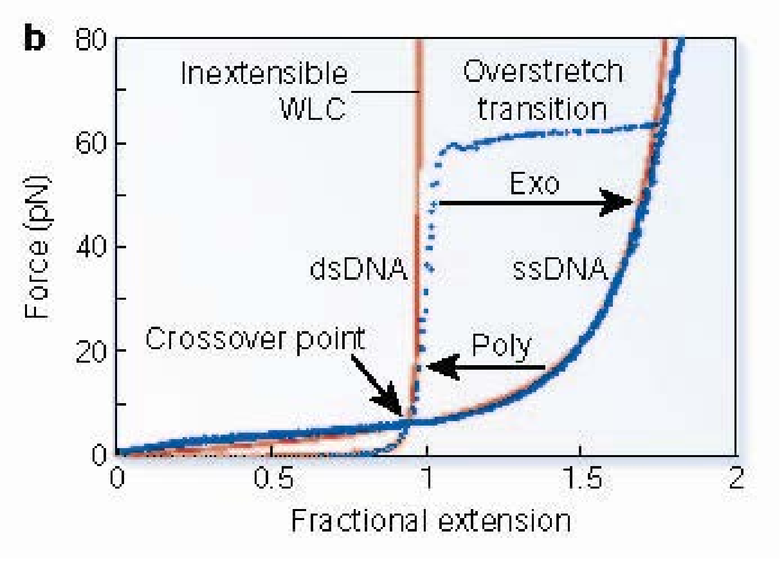


- Observing motor motion using optical trap

Finer *et. al., Nature 368,113* (1994)

DNA Mechanics

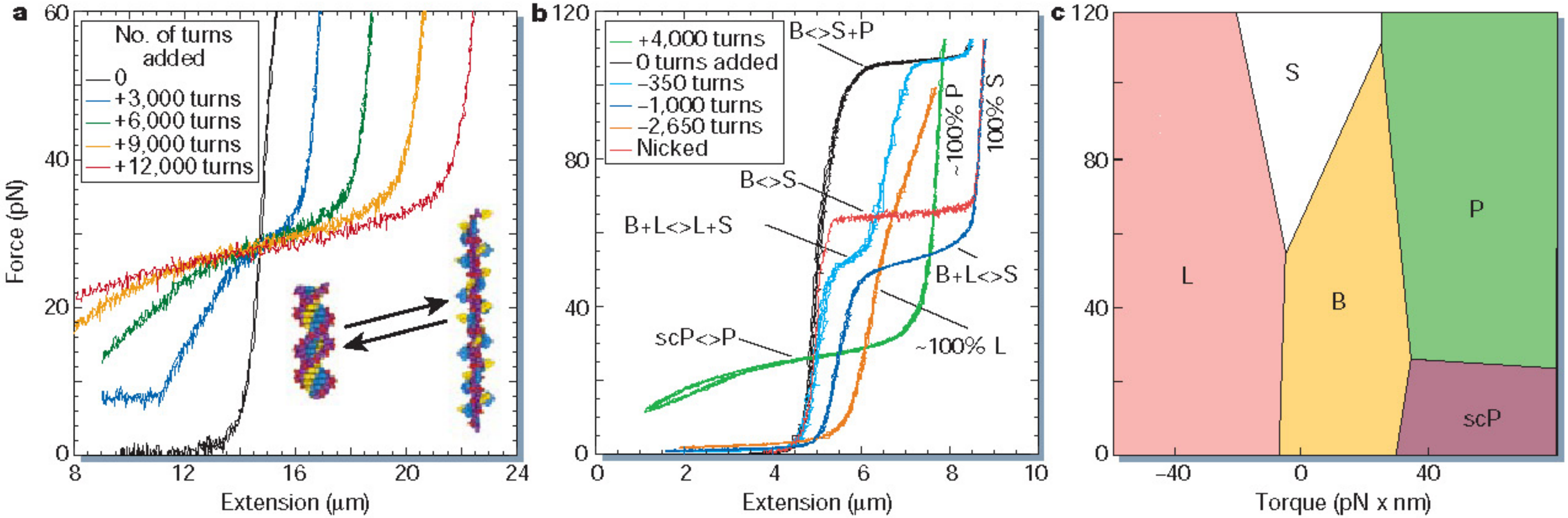




DNA replication observed with single-molecule technique

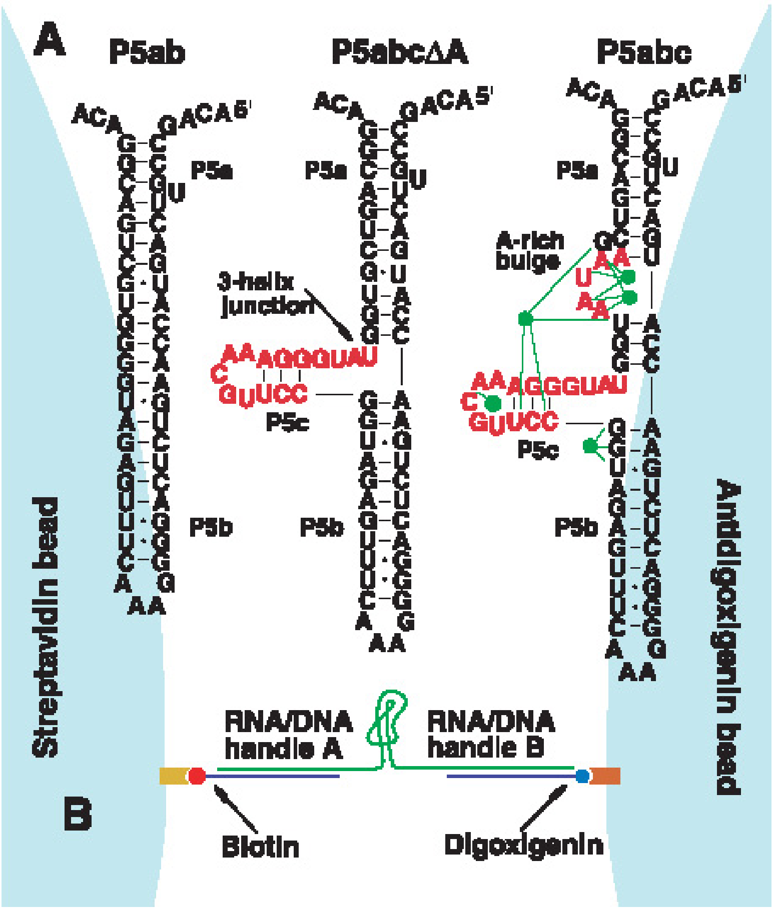
Bustamante *et. al. Nature* **421**, 423 (2003).

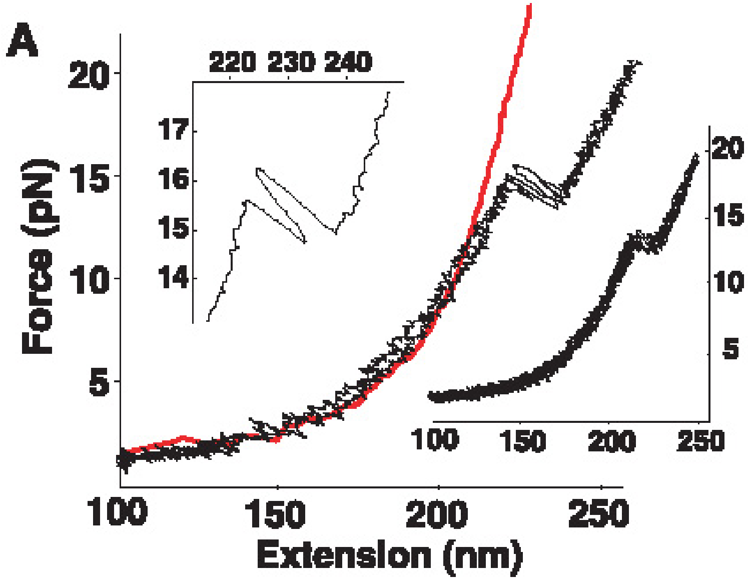
DNA Phase Diagram



Bustamante *et. al. Nature* 421, 423 (2003).

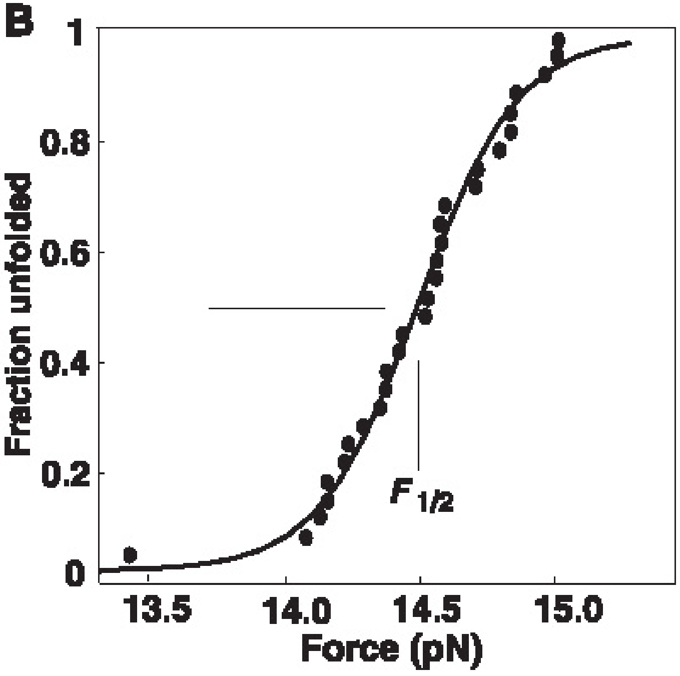
RNA Unfolding

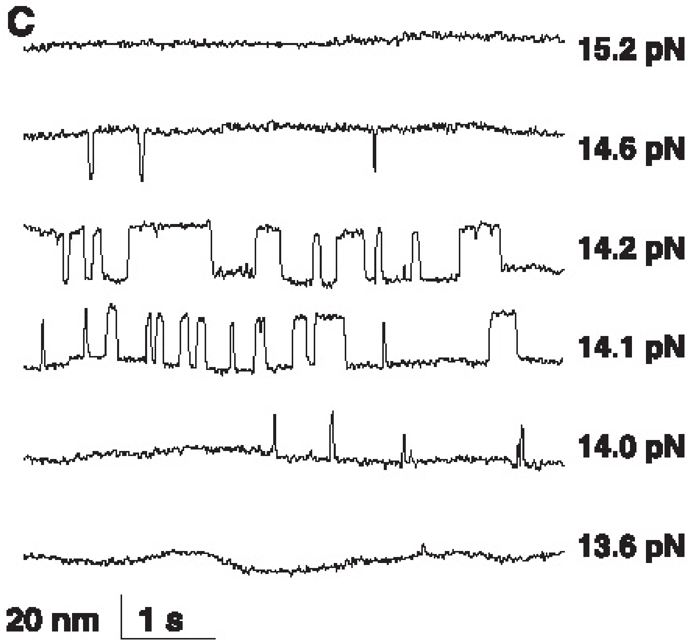




Liphardt *et. al.*, *Science* **292**, 733 (2001)

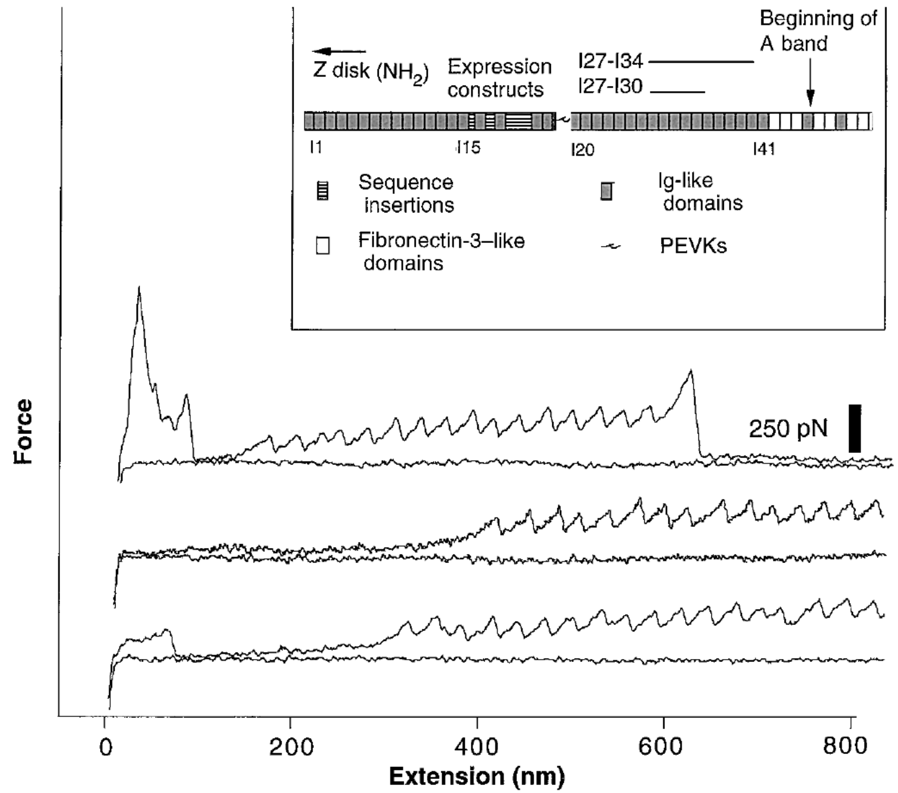
RNA Folding Kinetics





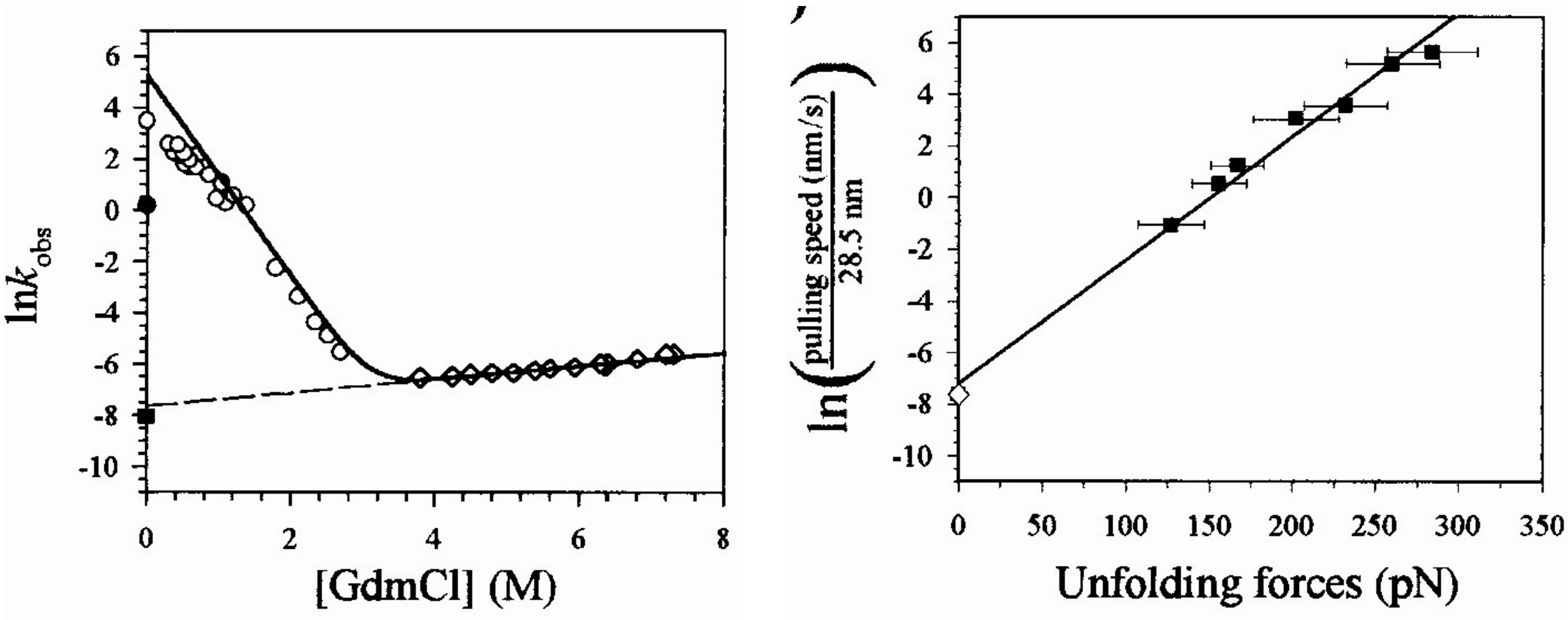
Protein Folding

- First demonstrated in 1997 to stretching native titin
- Force-extension curves show sawtooth pattern
- Domains unfold under mechanical force



Rief *et al., Science 276, 1109 (1997).*

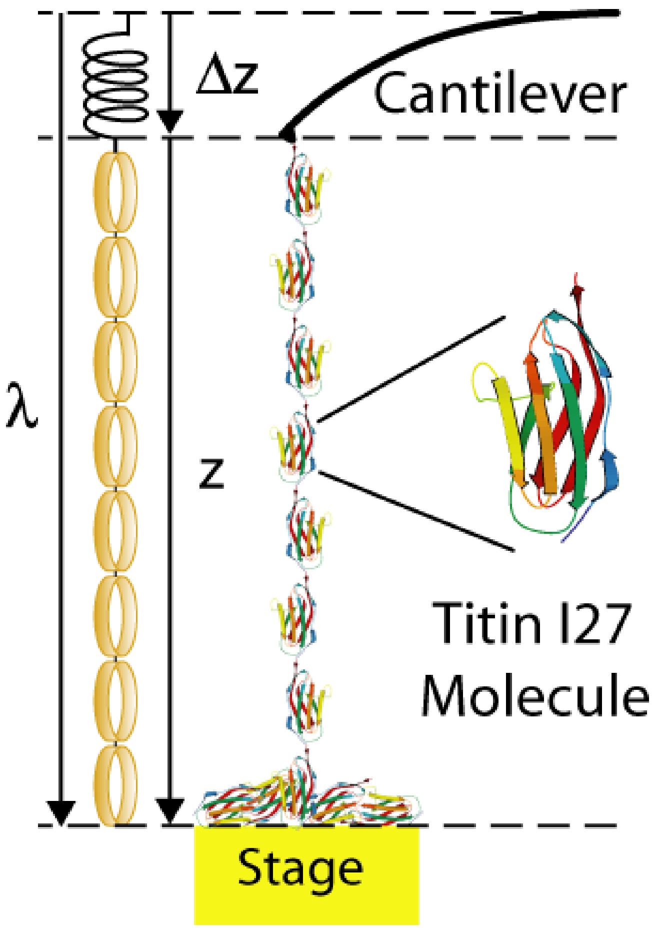
Protein Folding

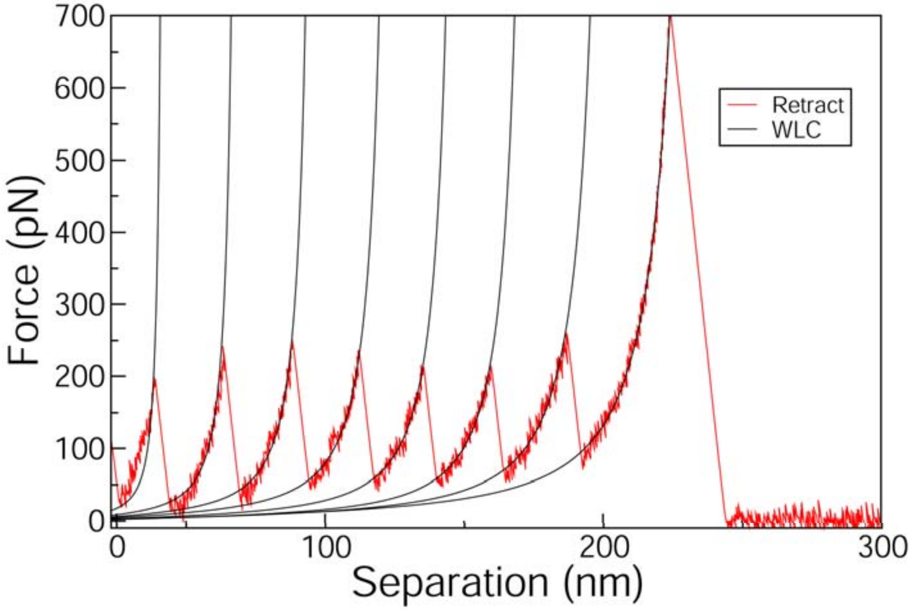


Kinetics obtained from forced unfolding compares favorably with that from bulk chemical denaturant studies

Carrion-Vazouez et. al., *PNAS* **96**, 3694 (1999)

Nonequilibrium Measurements





- Distance between the peaks: the length of one unfolded domain
- Height: force exerted on molecule

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