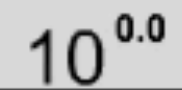


SINGLE MOLECULE BIOLOGICAL ACTIVITY

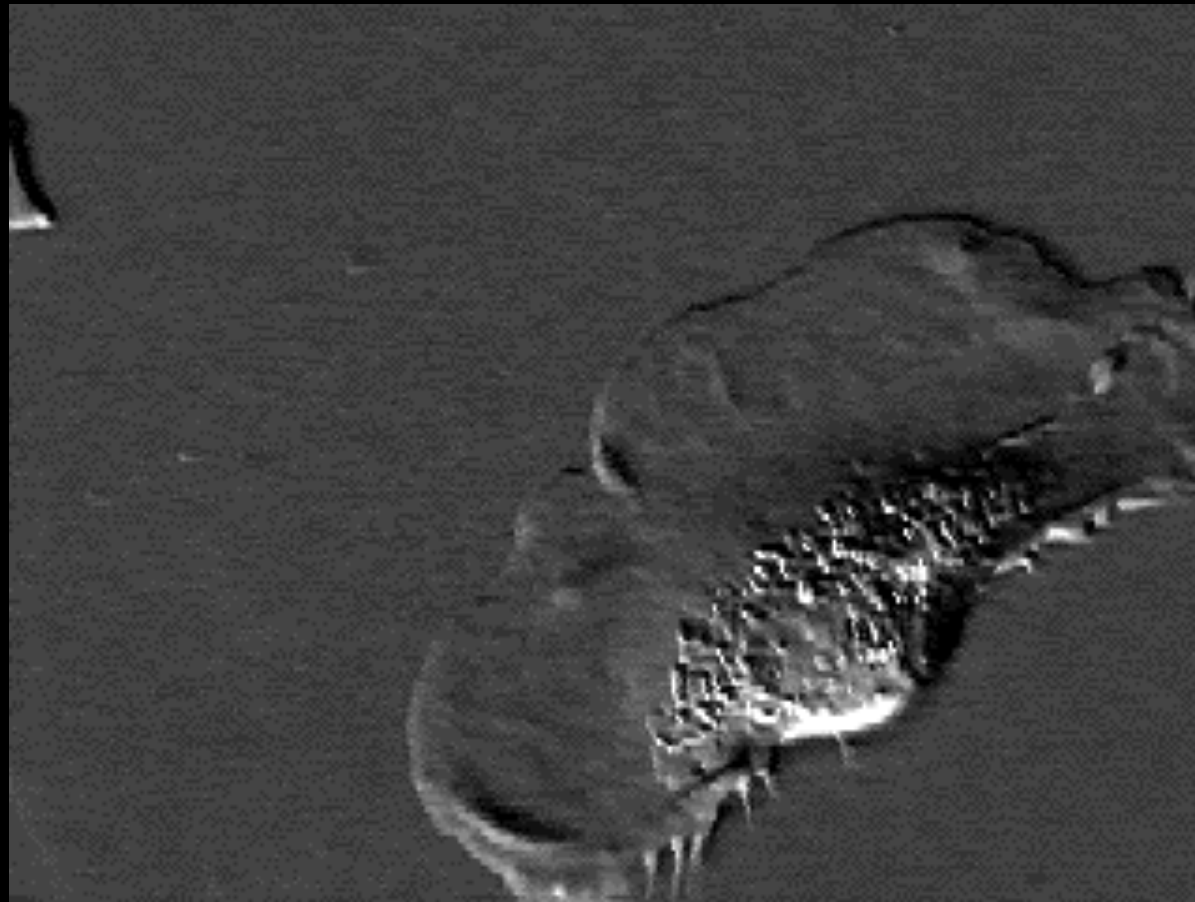
KELLERMAYER MIKLÓS

♪ Q

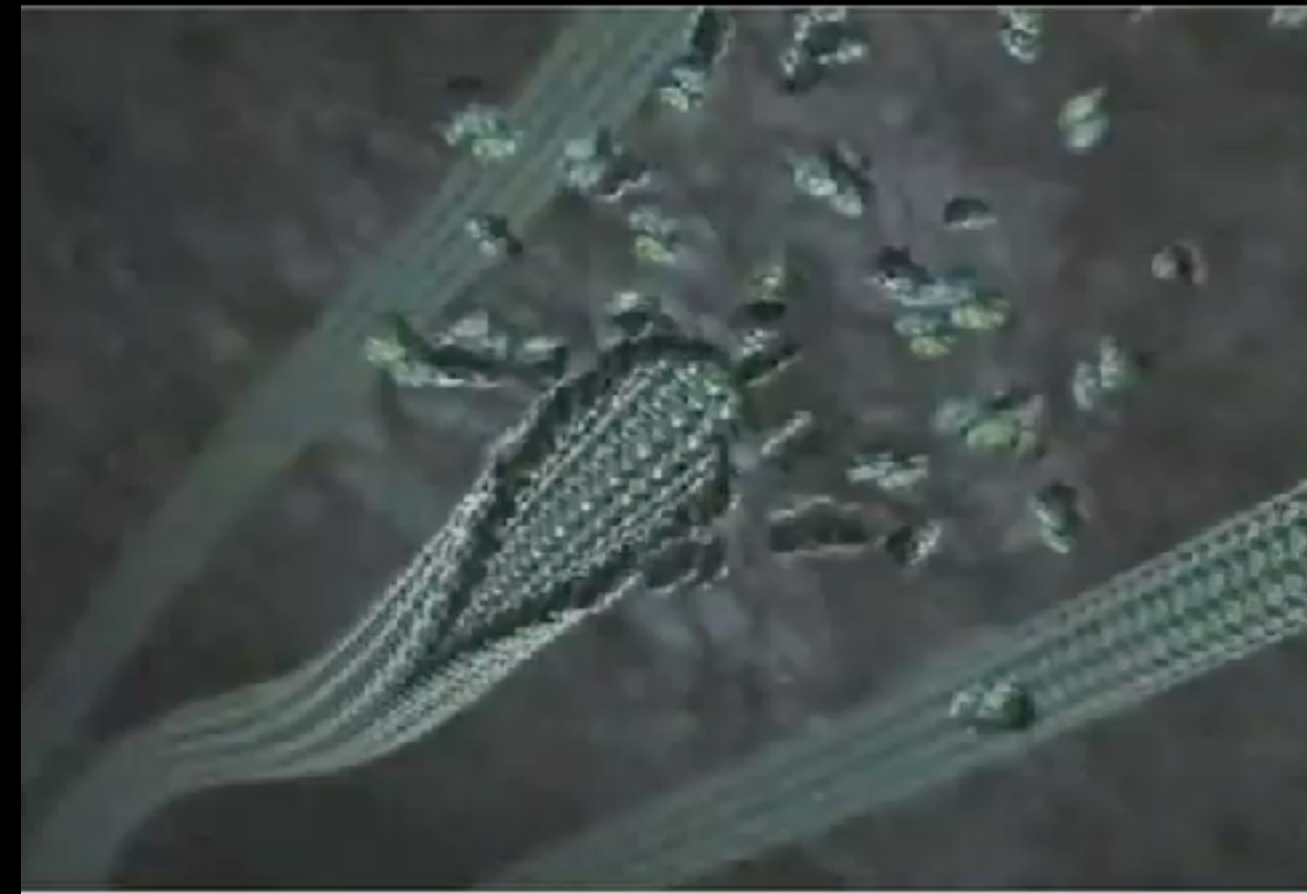


Cary and Michael Huang (<http://htwins.net>)

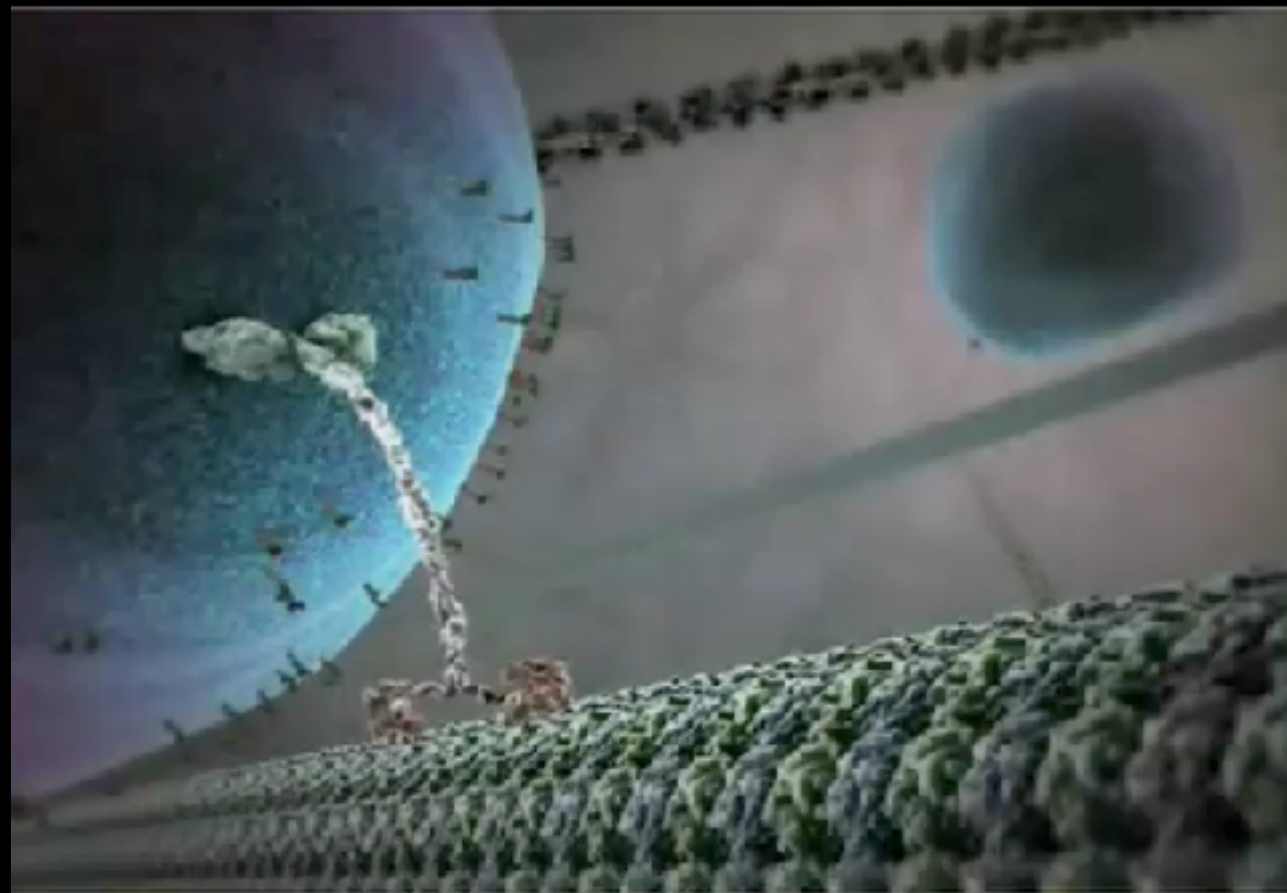
The living cell is a complex network of nanoscale machines



Crawling keratinocyte



Microtubule dynamic instability



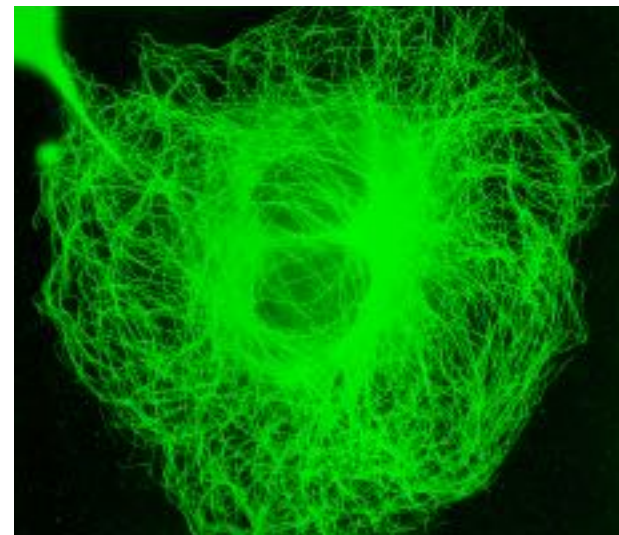
Kinesin-driven vesicular transport



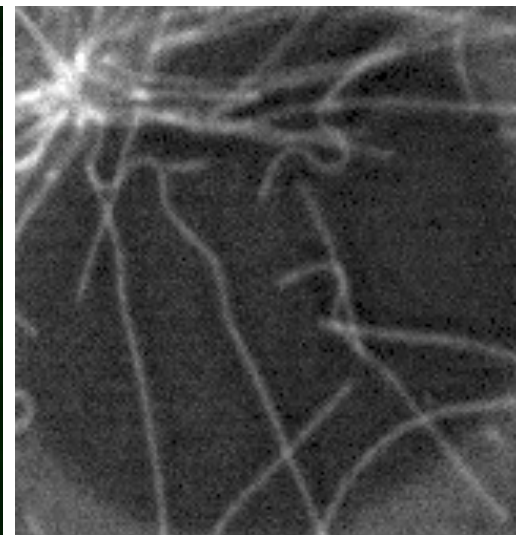
Protein synthesis on the ribosome

Why single molecules?

1. Individuals (spatial and temporal trajectories) may be identified in a crowd



Ensemble - microtubular system

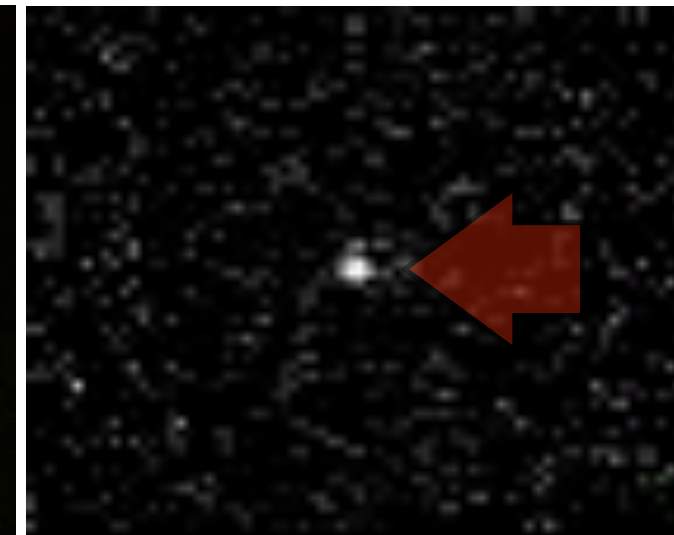


Single microtubules - treadmilling

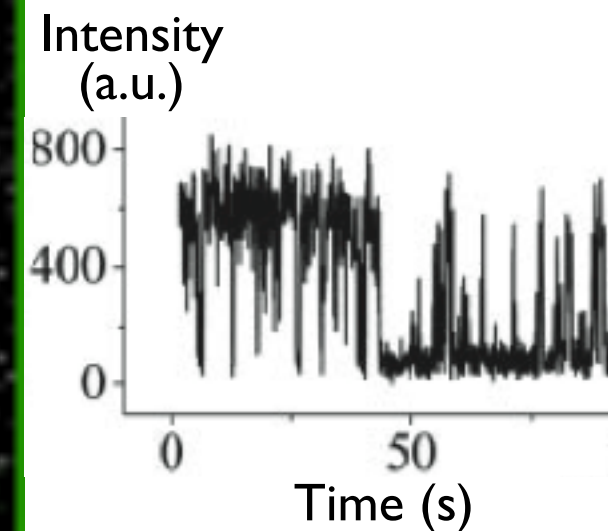
2. Stochastic processes may be uncovered



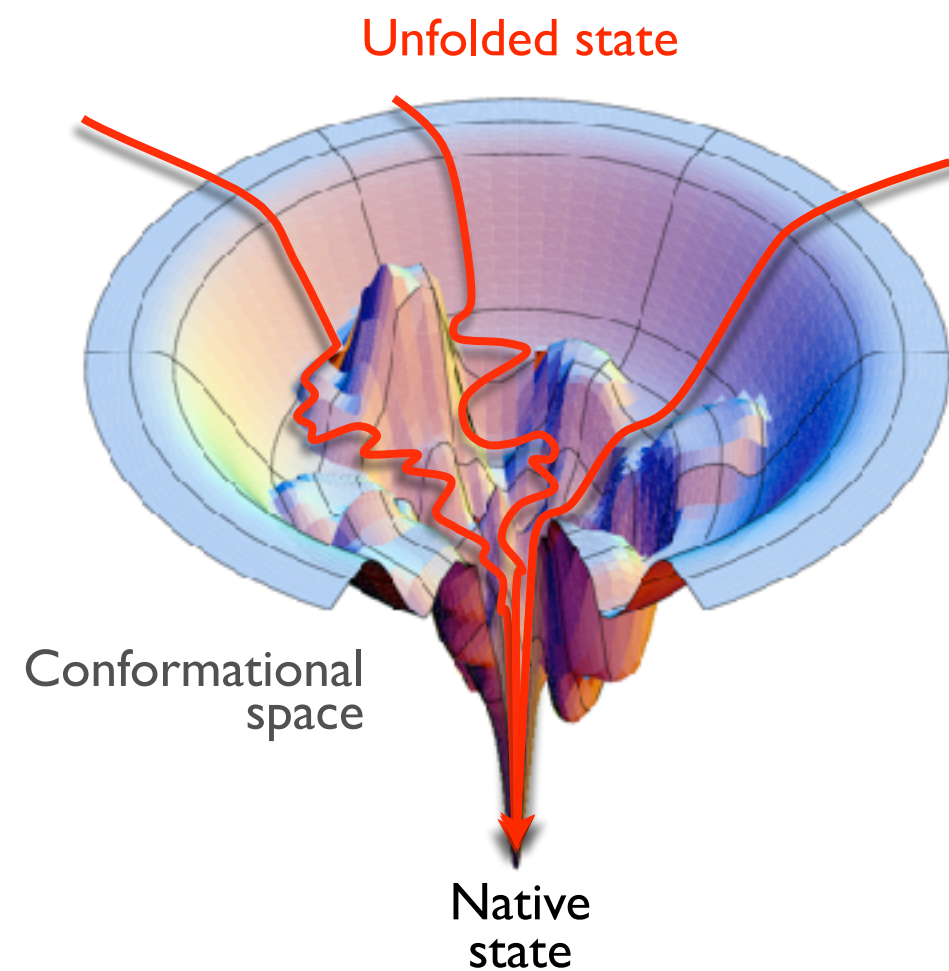
Ensemble - intensity



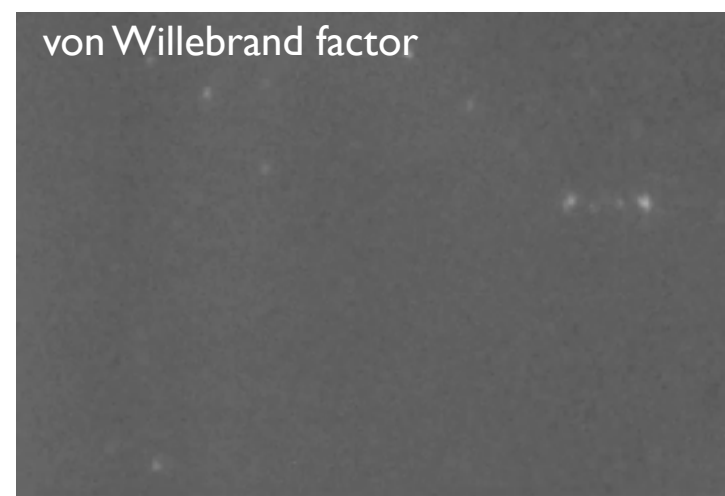
Single quantum dot - blinking



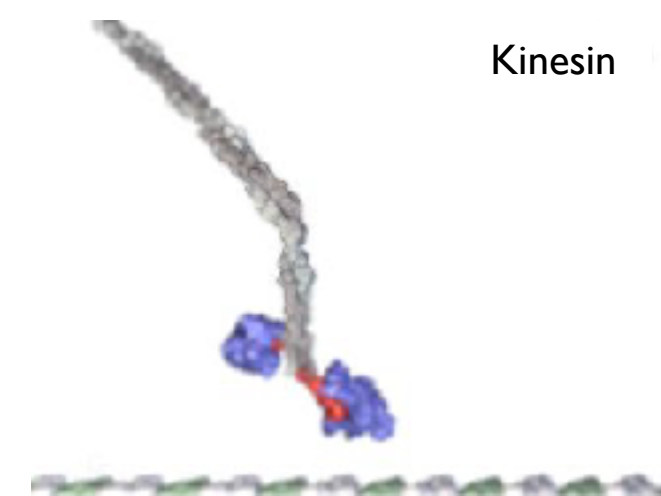
3. Parallel-pathway events may be identified



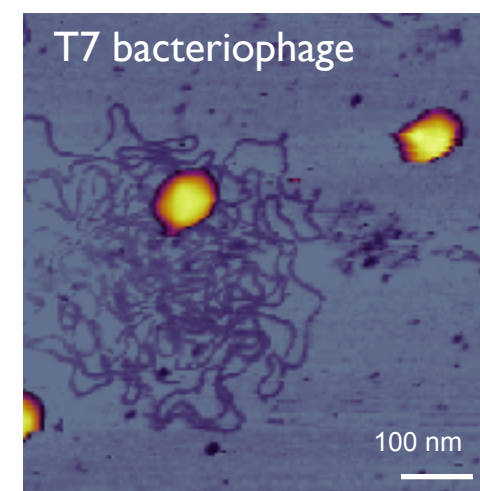
4. Mechanics of biomolecules may be characterized



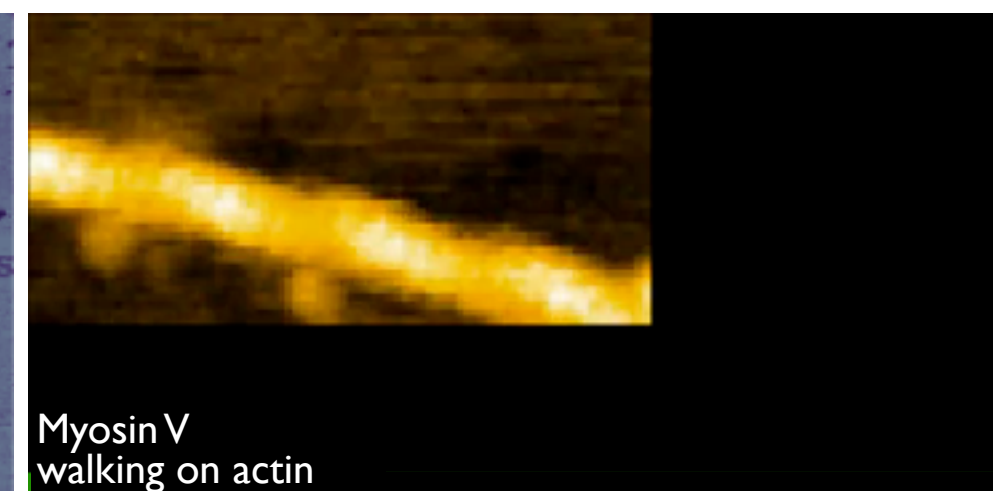
von Willebrand factor



Kinesin



T7 bacteriophage



Myosin V walking on actin

Brief history of single-molecule science



1976: Fluorescence microscopy of a single antibody molecule

1986: J. Spudich, T. Yanagida, *in vitro* motility assay

1991: J. Spudich, T. Yanagida, J. Molloy, single myosin mechanics

1994: T. Yanagida, single ATP turnover on myosin

1994: K. Svoboda, S. Block, single kinesin mechanics

1996: C. Bustamante, D. Bensimon, mechanical stretch of a single dsDNA molecule

1996: T. Ha, S. Weiss, single-pair FRET



1997: W.E. Moerner, GFP blinking

1997: M. Kellermayer, M. Rief, L. Tskhovrebova, mechanical stretch of a single protein (titin)



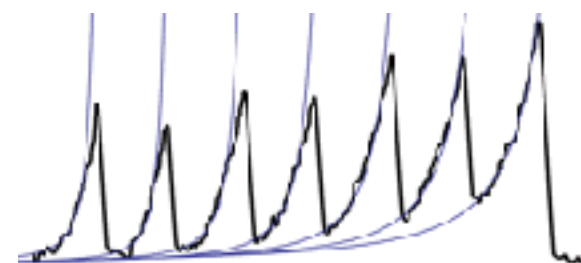
1998: Kinosita, F1F0 ATPase stepping kinetics

1998: J. Fernandez, nanomechanics of a genetic polymer

2001: J. Liphardt, C. Bustamante, single RNA manipulation

2004: J. Fernandez, single protein molecule folding

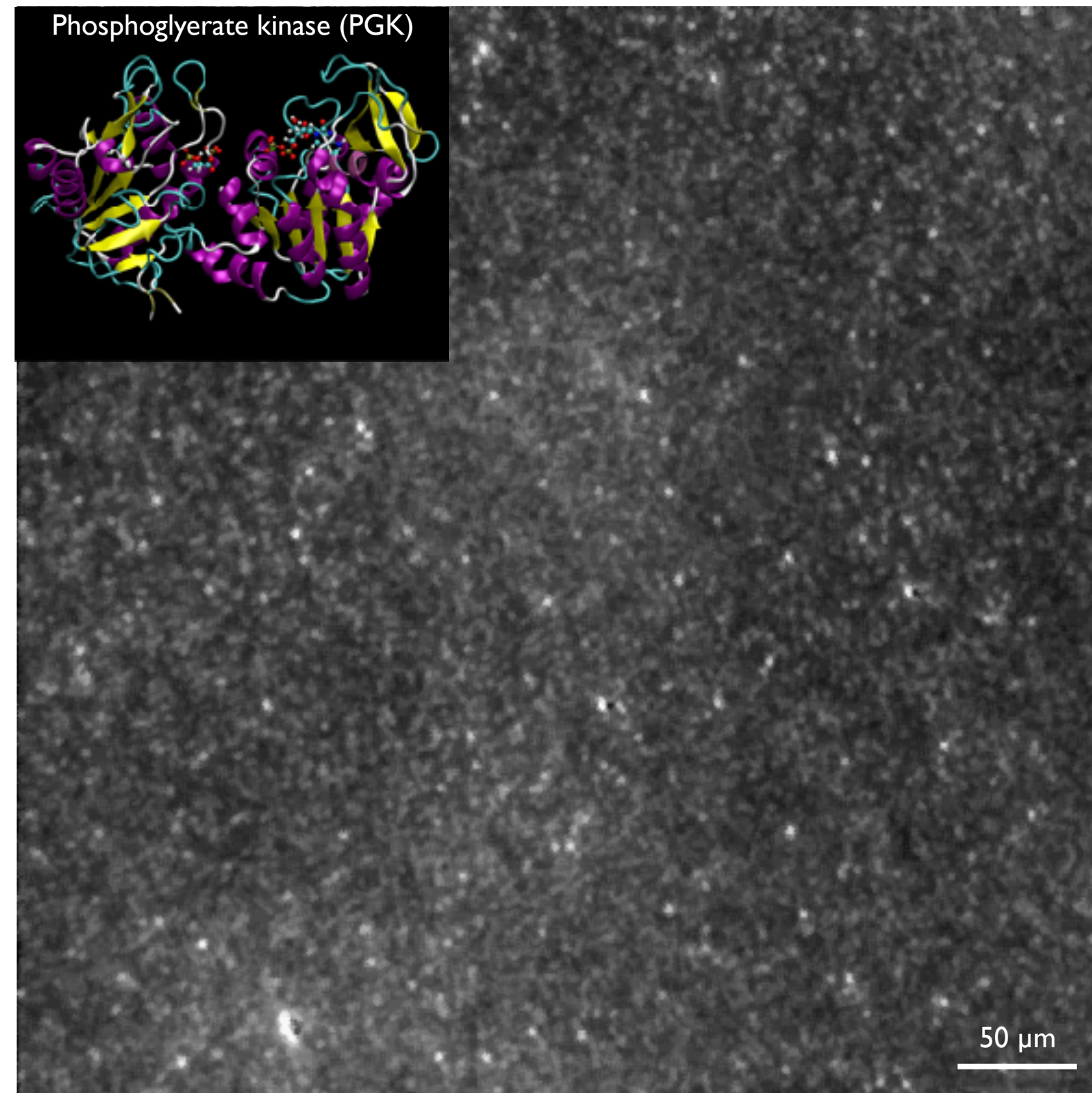
2008: Bustamante, Tinoco: ribosome mechanics



Measurable parameters I.

Fluorescence

What are the conformational states of a molecule?

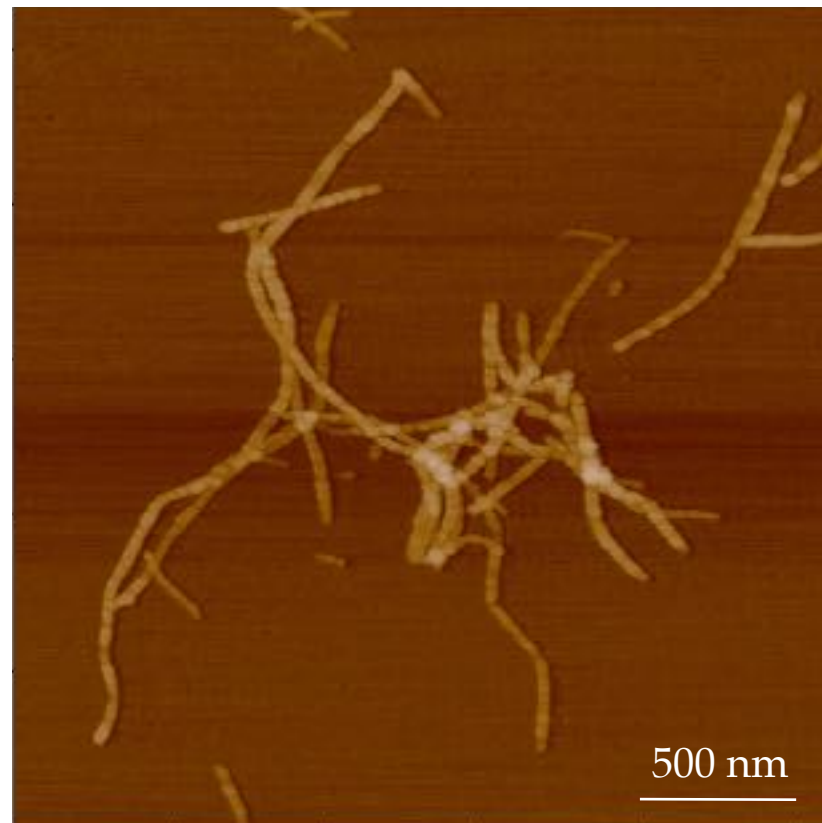


PGK molecules labeled with Alexa488, TIRF microscopy

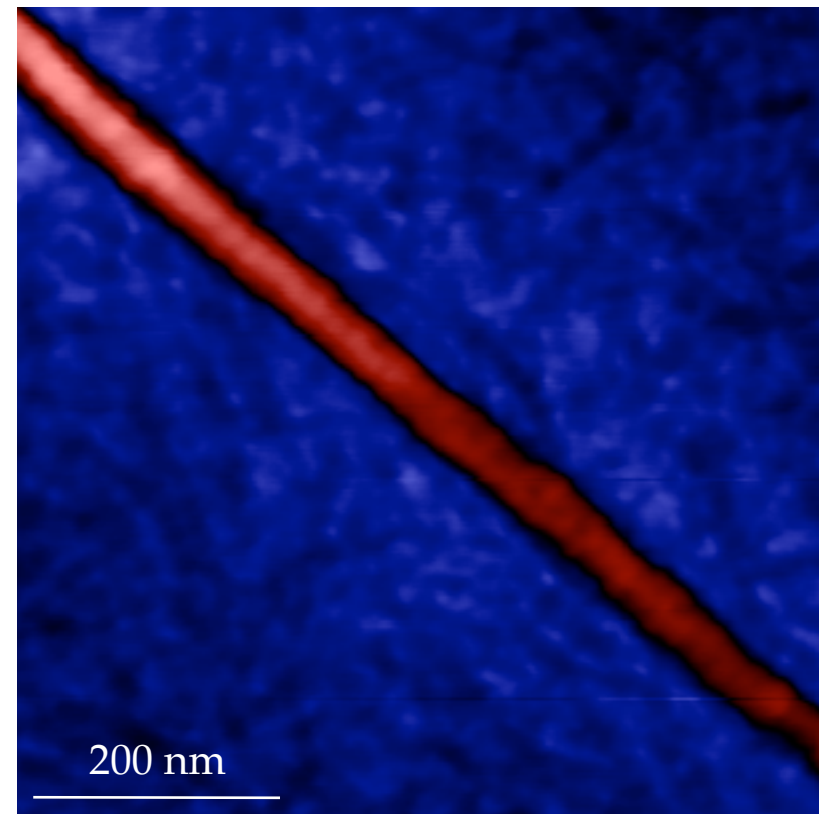
Measurable parameters II.

Structure - topography

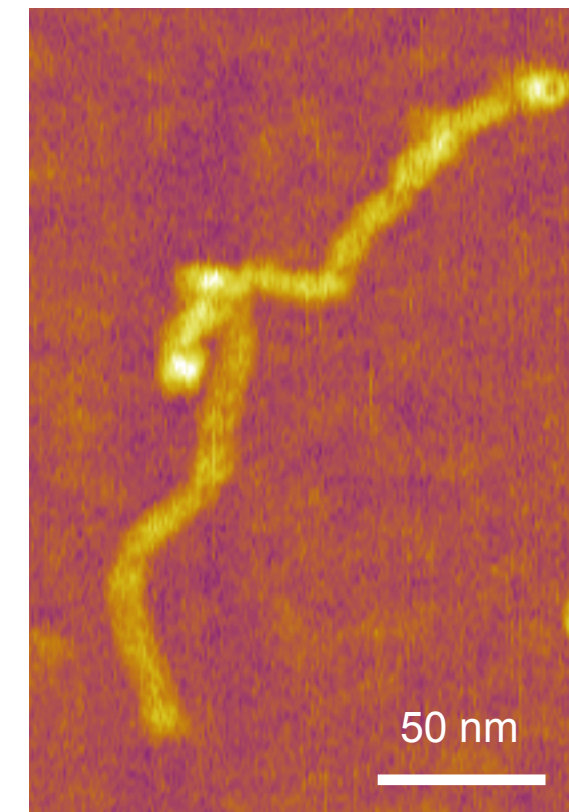
What are the conformational states of a molecule?



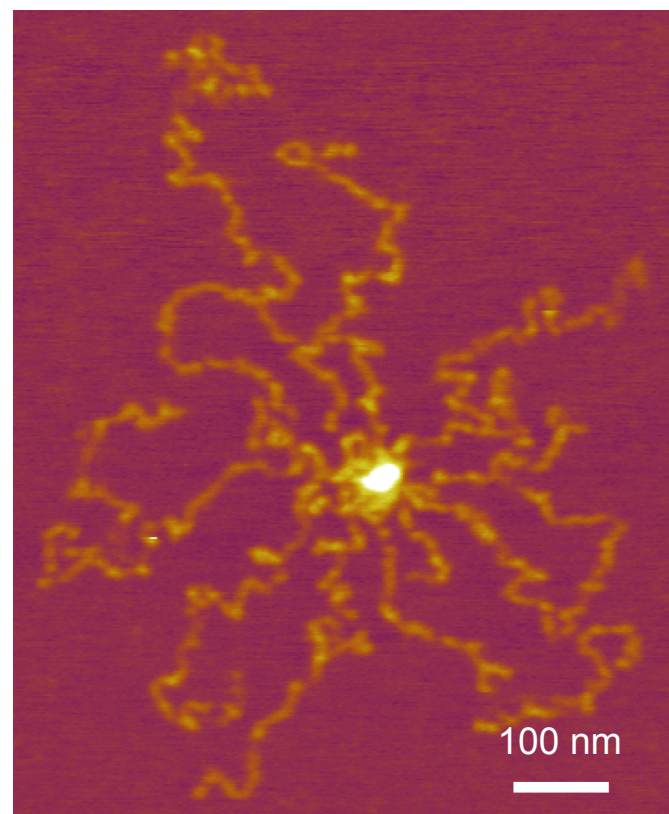
Amyloid β 1-42



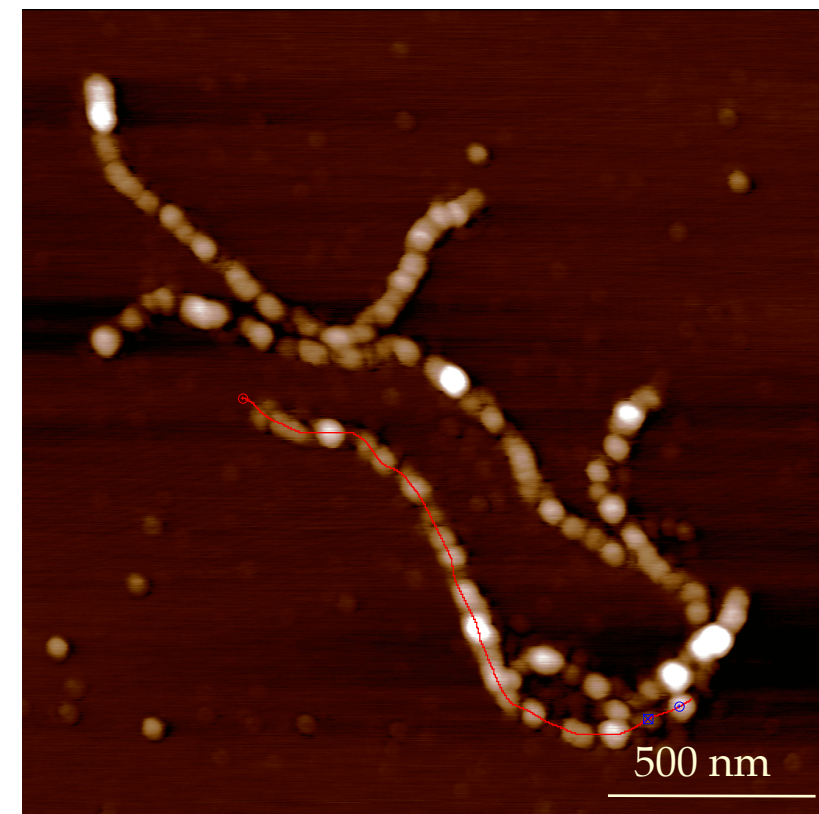
Fibrin protofibril



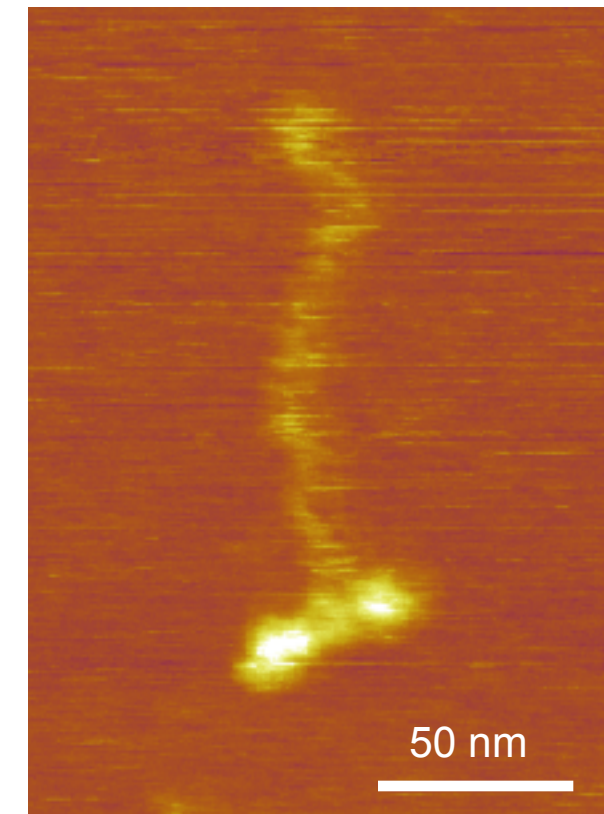
RNA-DNA hybrid



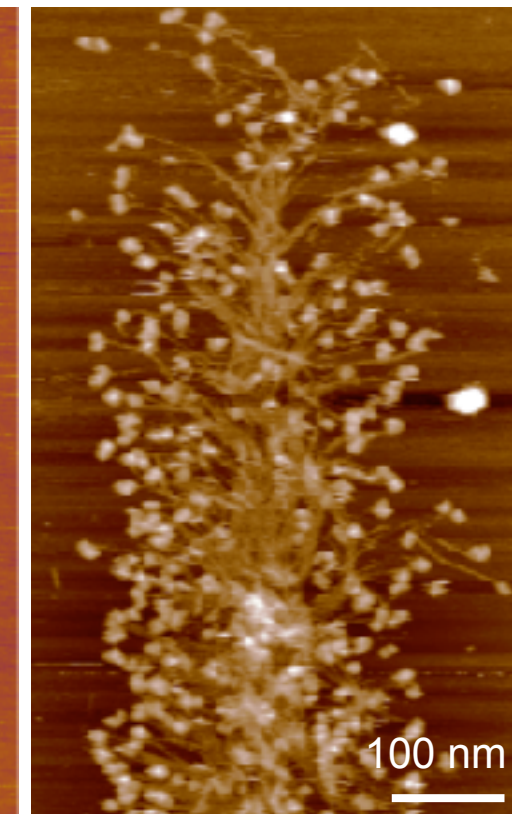
Titin molecule



Desmin filament



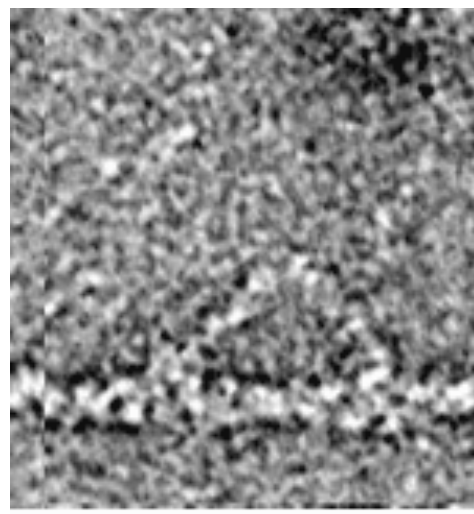
Myosin molecule



Myosin thick filament

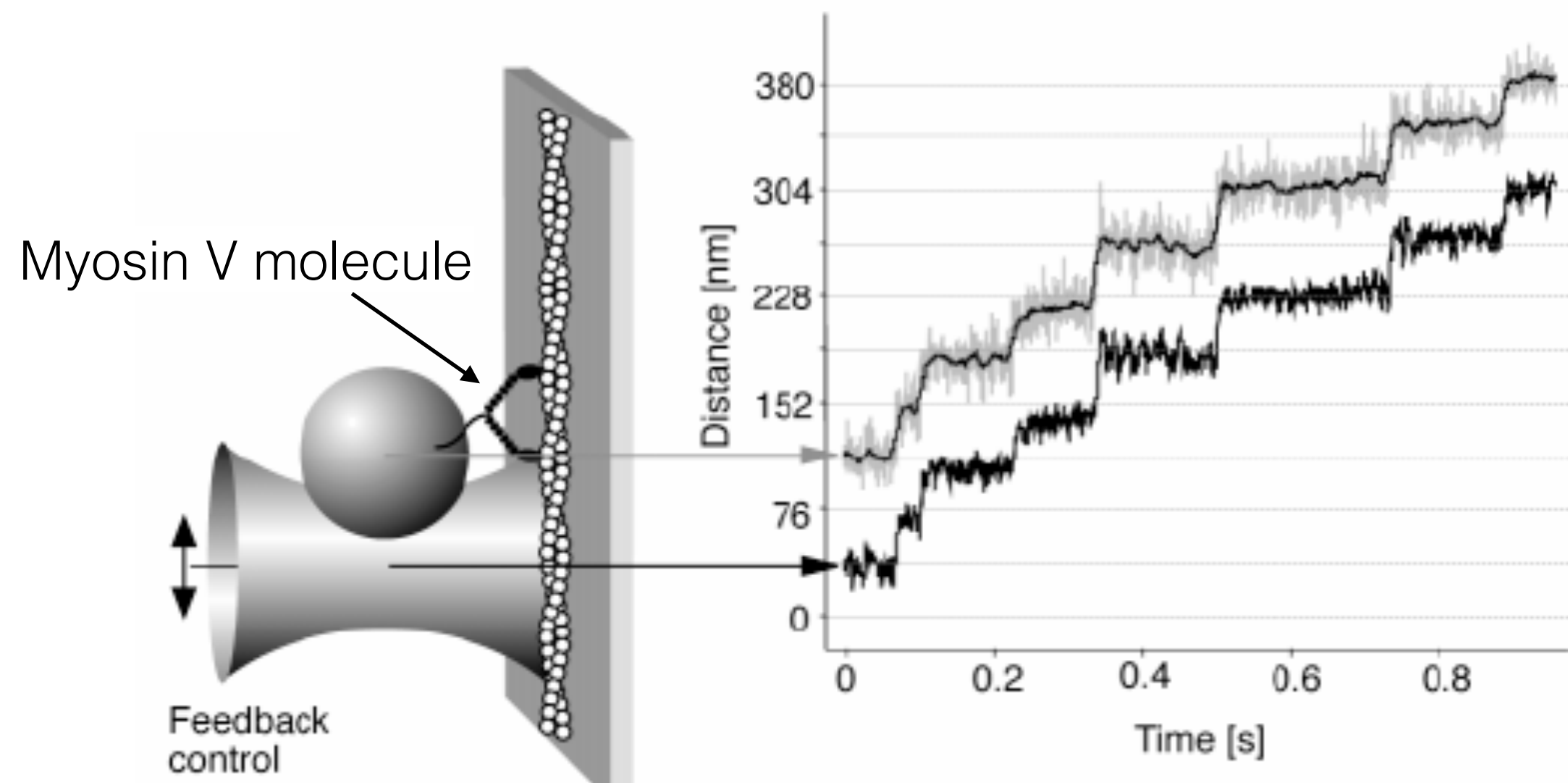
Measurable parameters III. Distance

What is the step size of a motor protein?



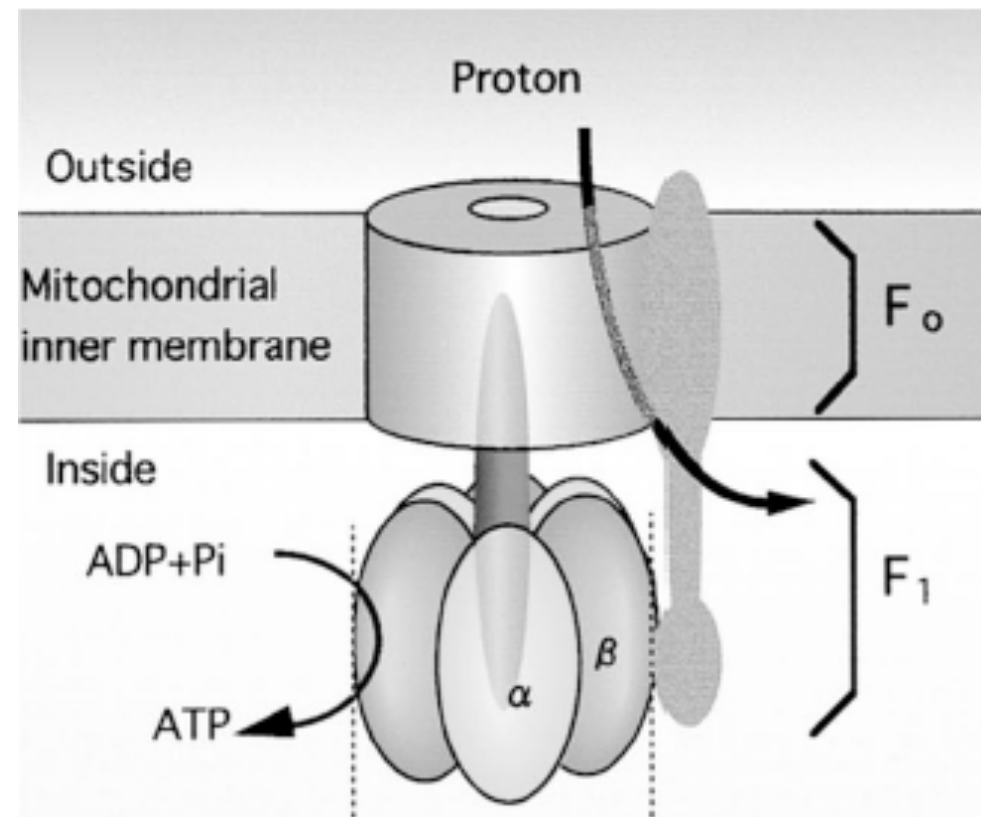
The Muscle Group, Leeds 2000

Myosin V
cryoelectron-
microscopic image
series

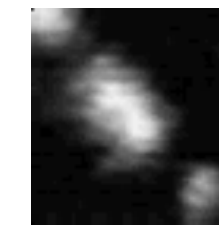


Measurable parameters IV. Rotational angle

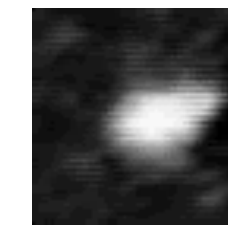
How does the ATP synthase work?



1 mM ATP

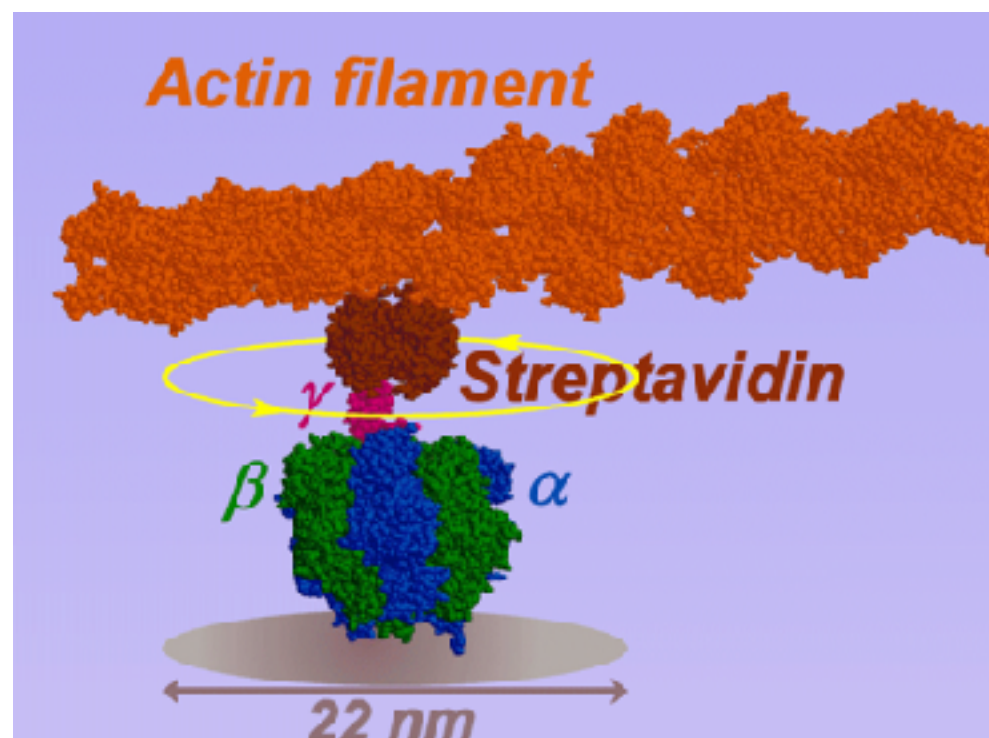
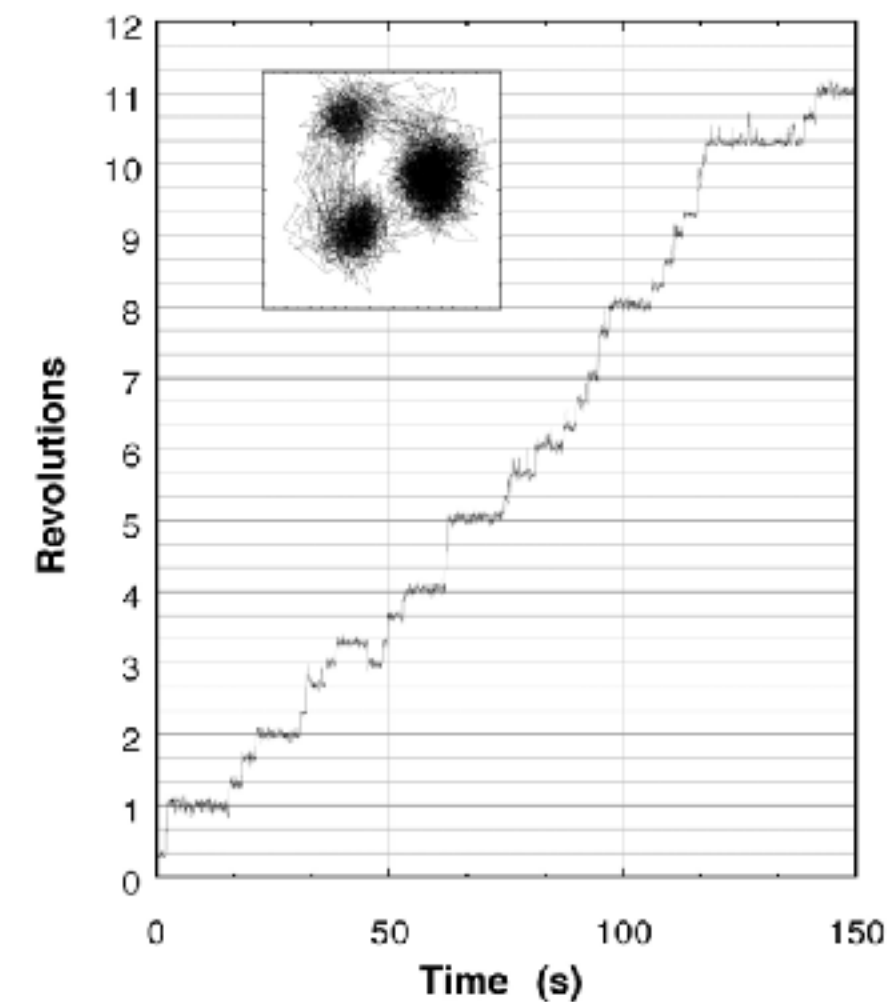


200 nM ATP



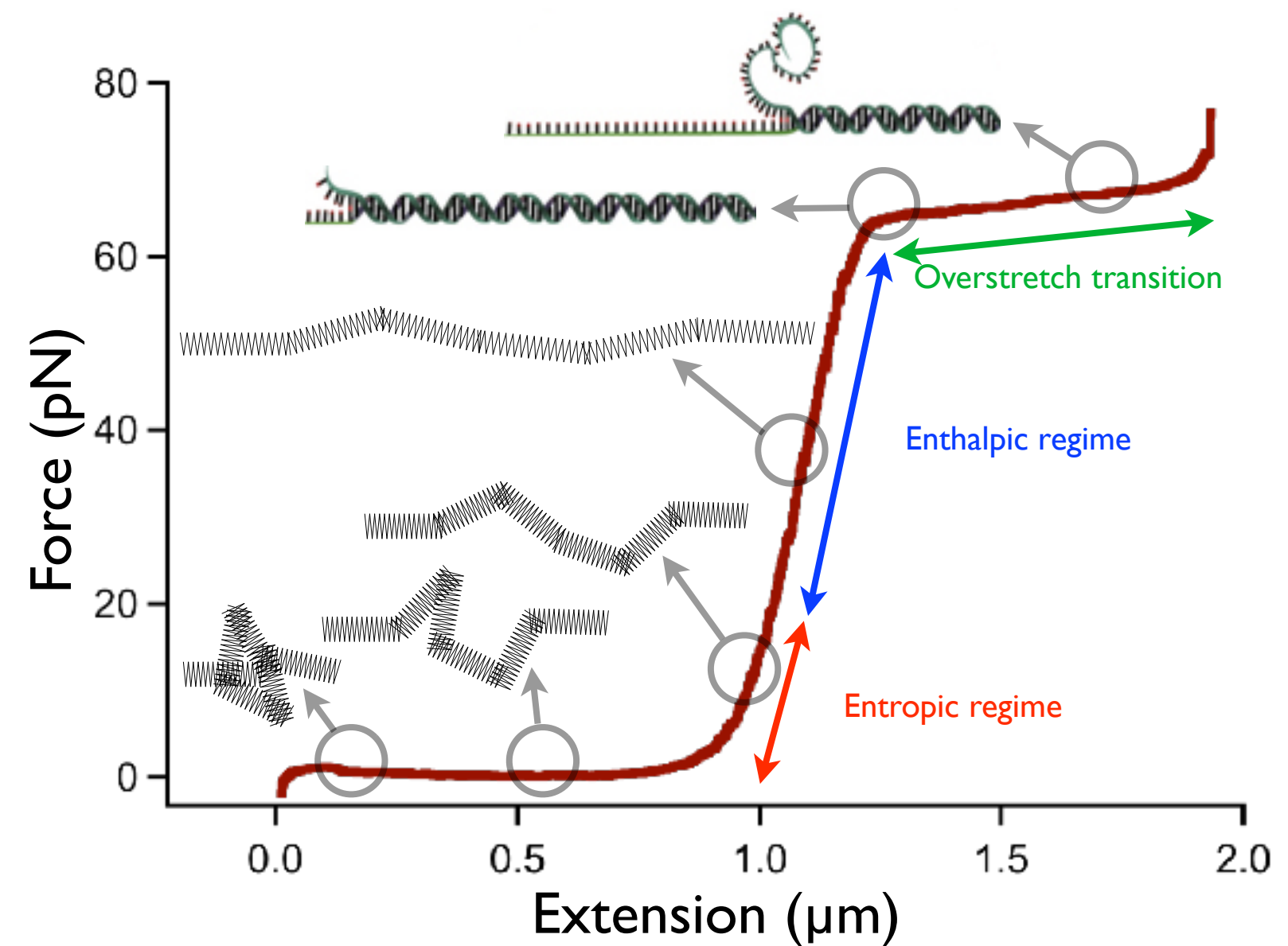
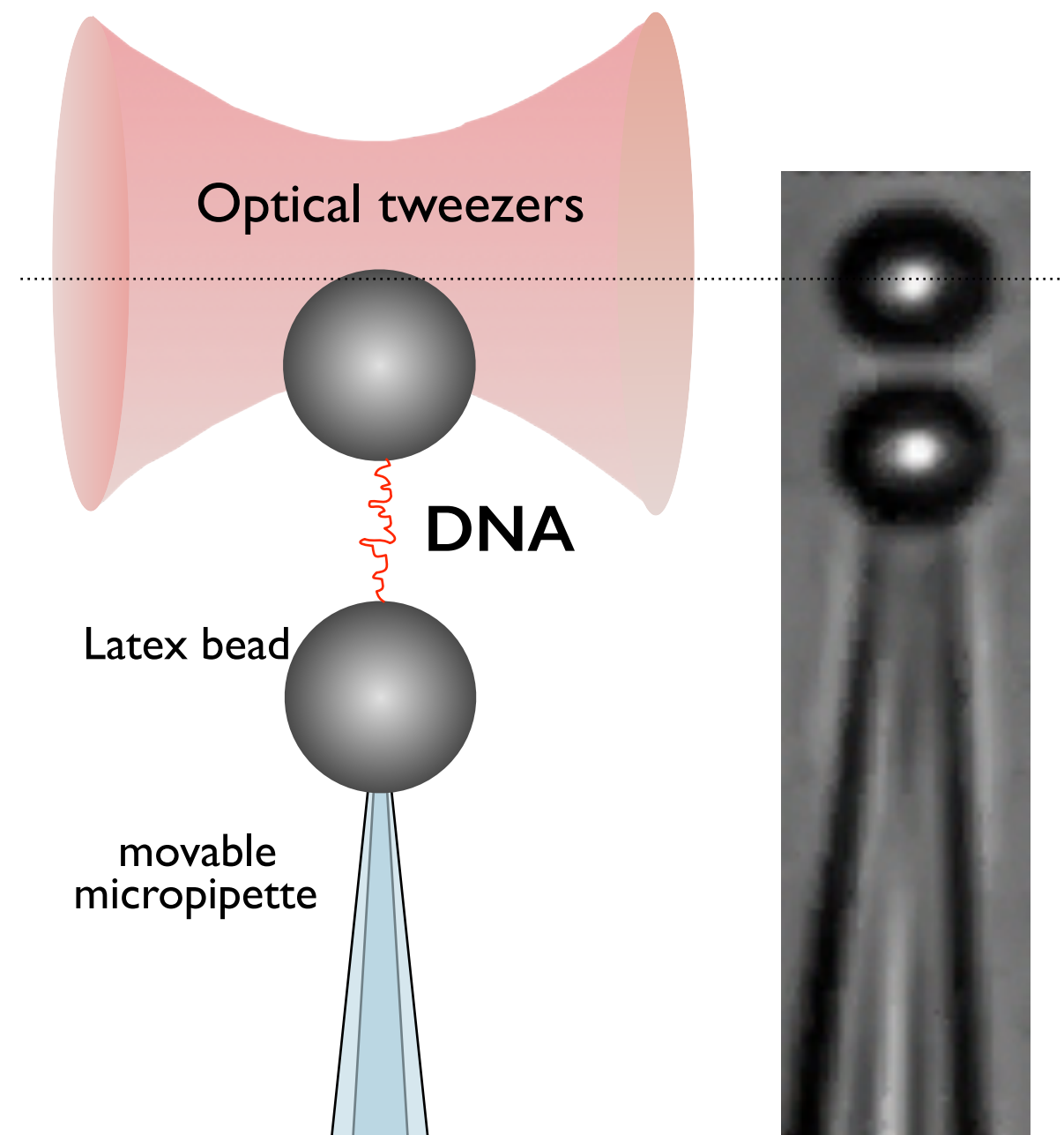
20 nM ATP

Discrete rotational steps of 120°



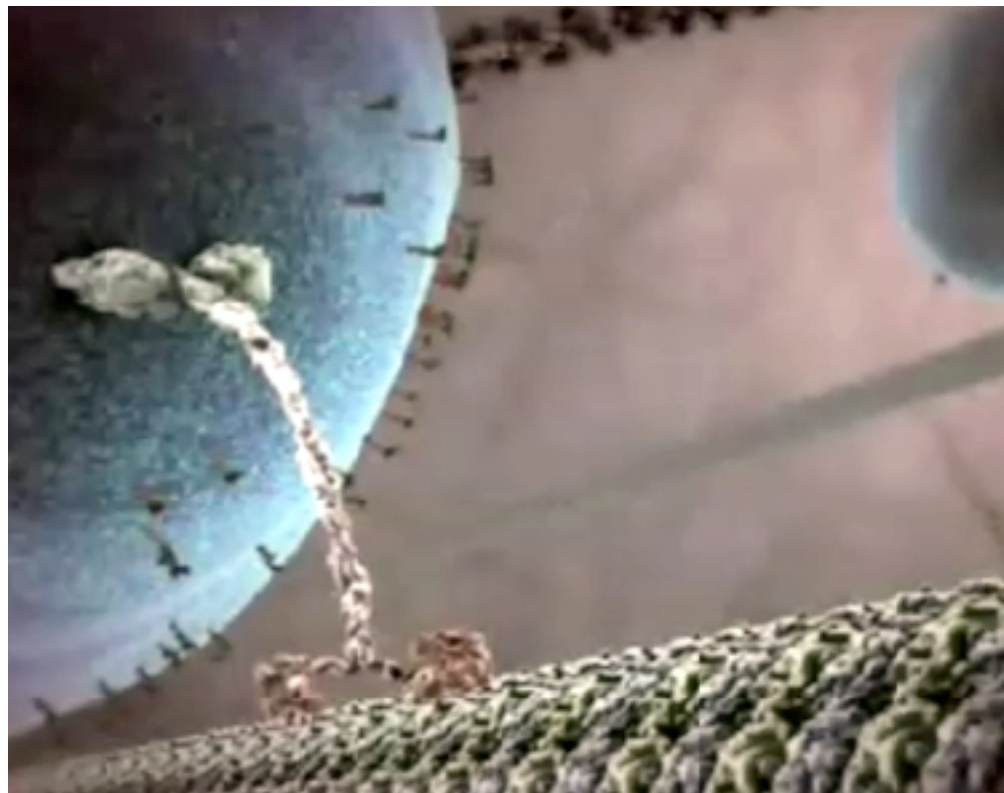
Measurable parameters V. Force

How much force develops during the stretch of a single dsDNA molecule?

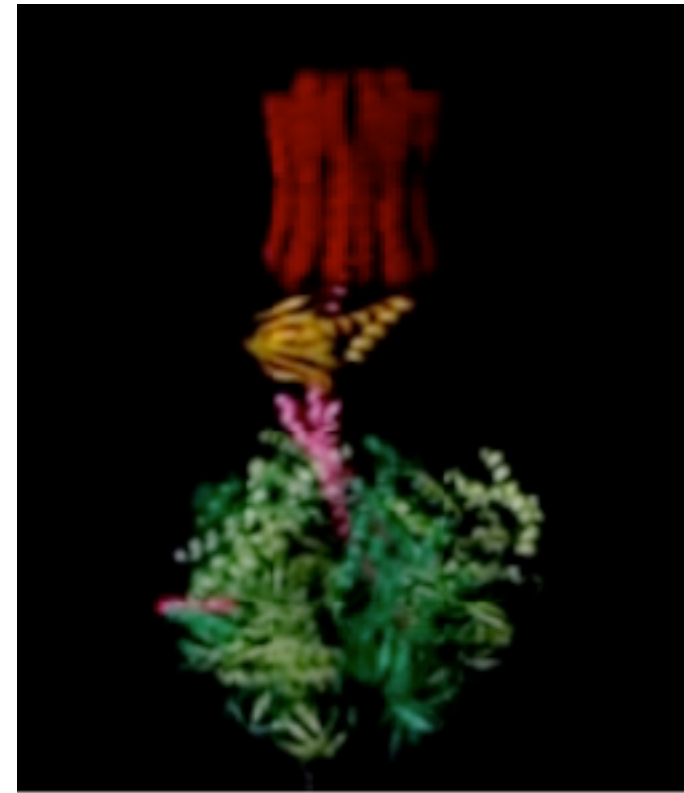


1. Force: develops

Kinesin



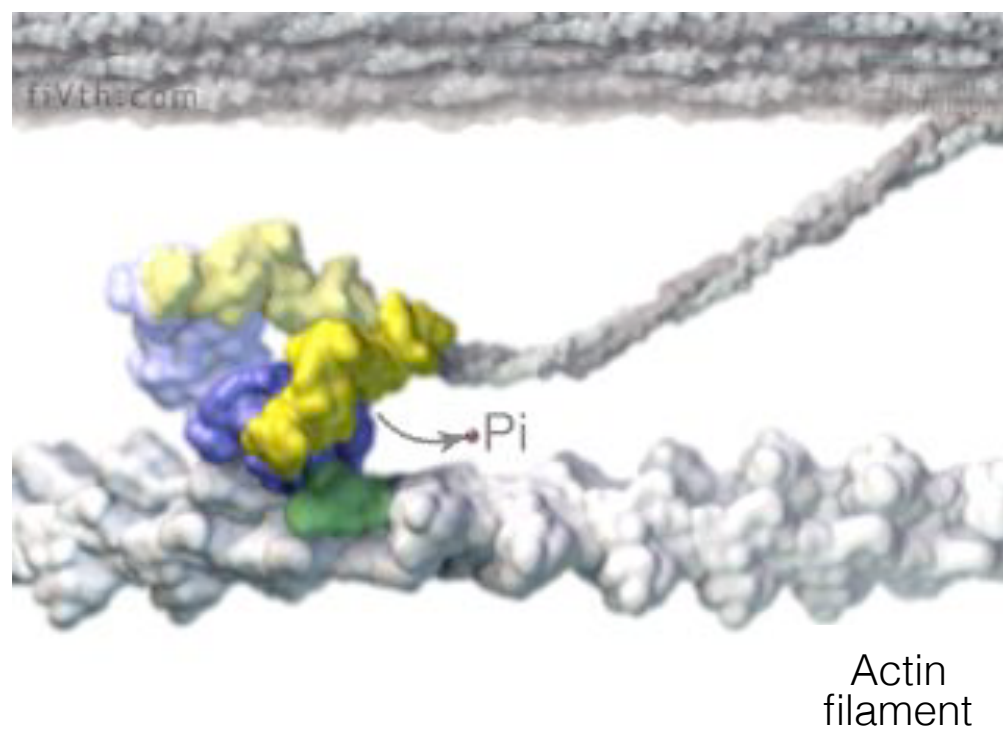
F1F0 ATPase



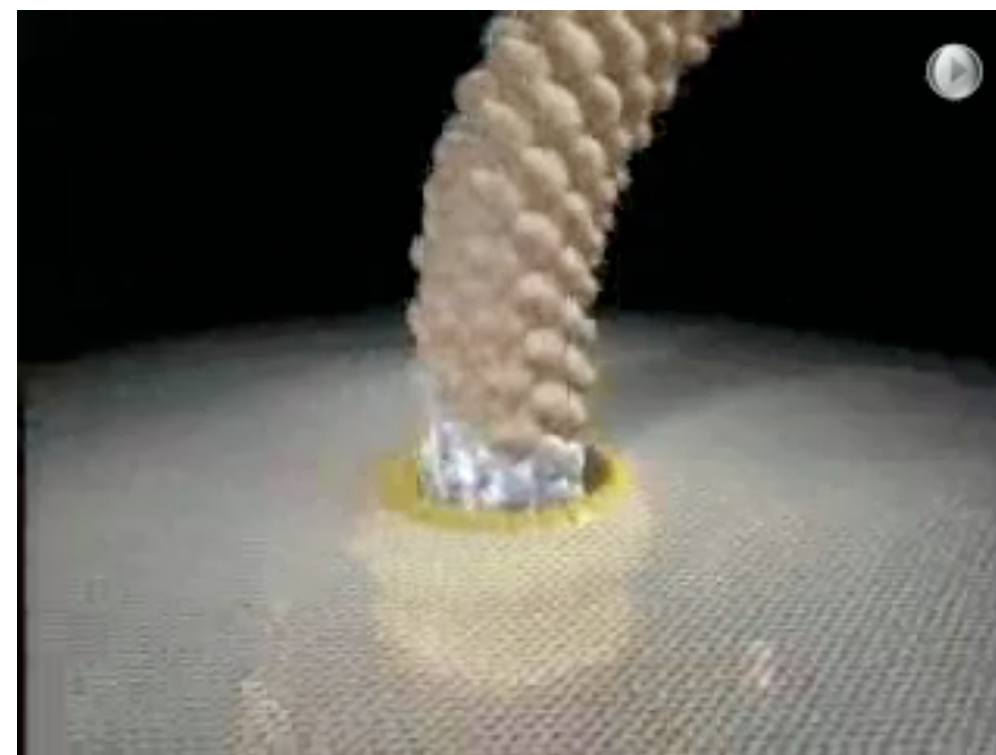
Ribosome



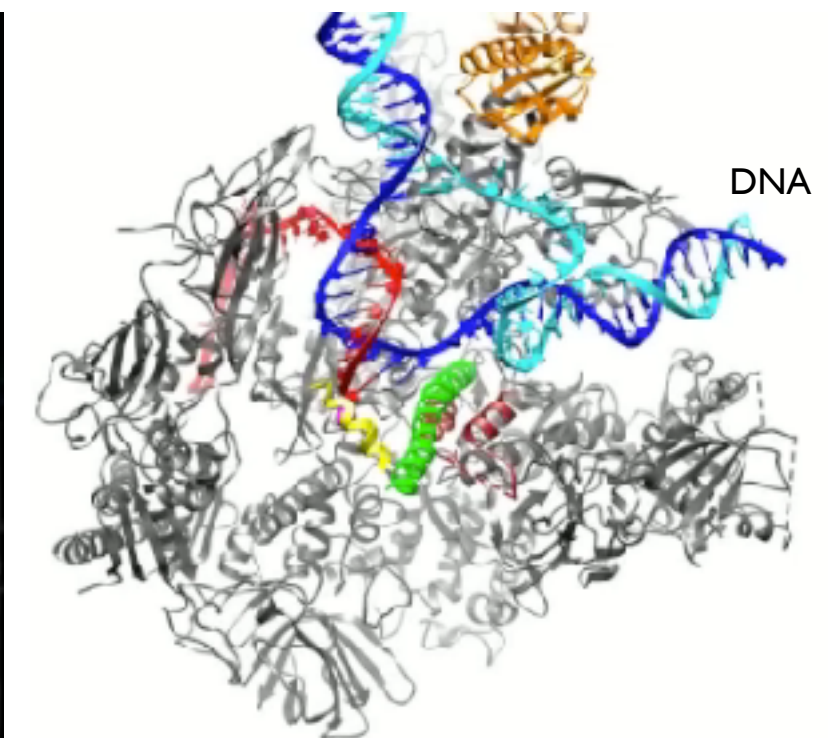
Myosin II



Bacterial flagellar motor

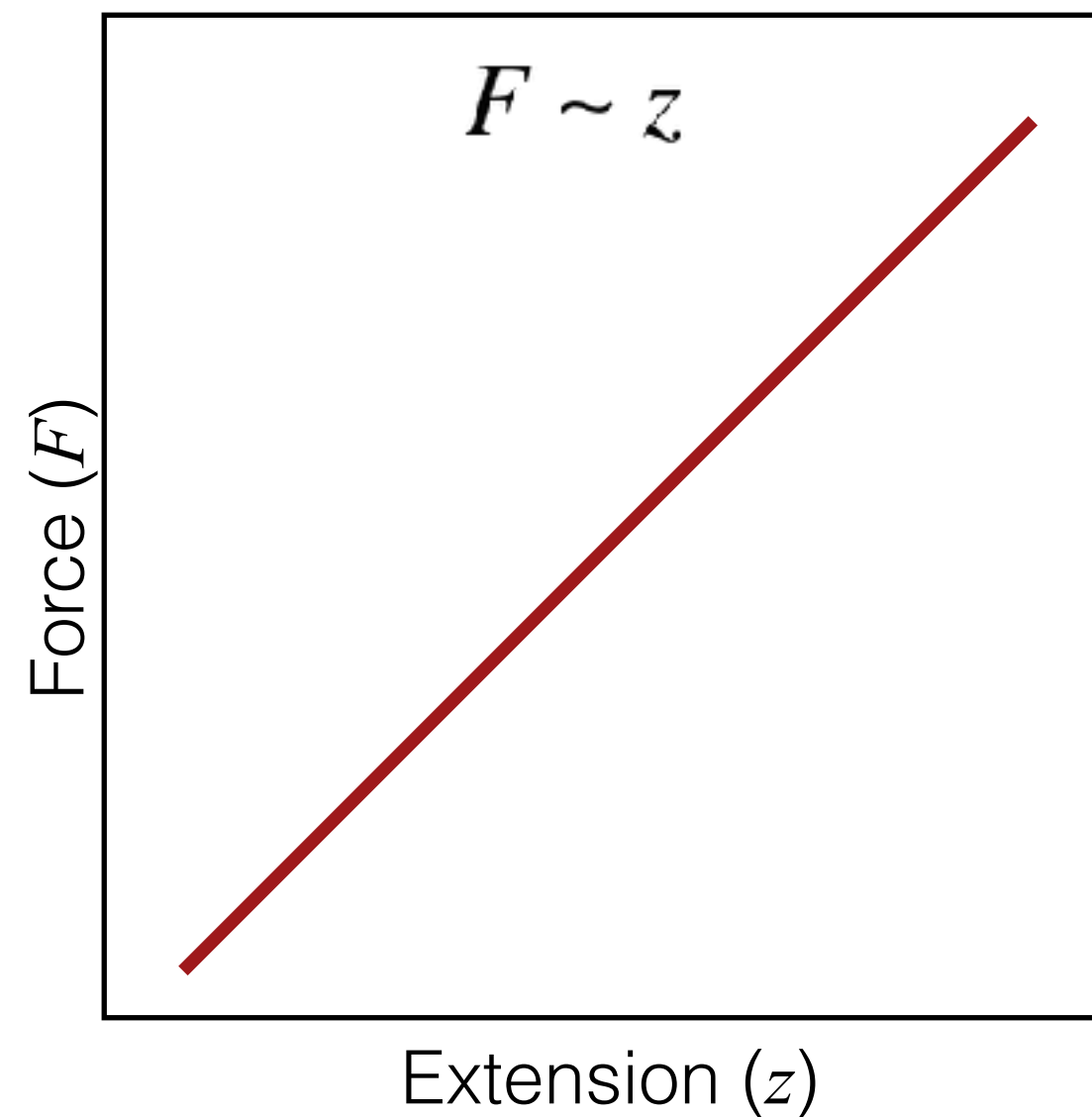
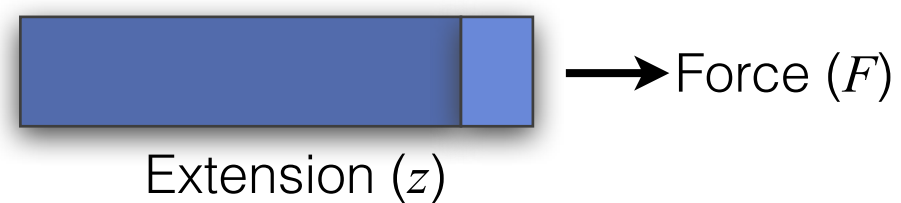


RNA polymerase

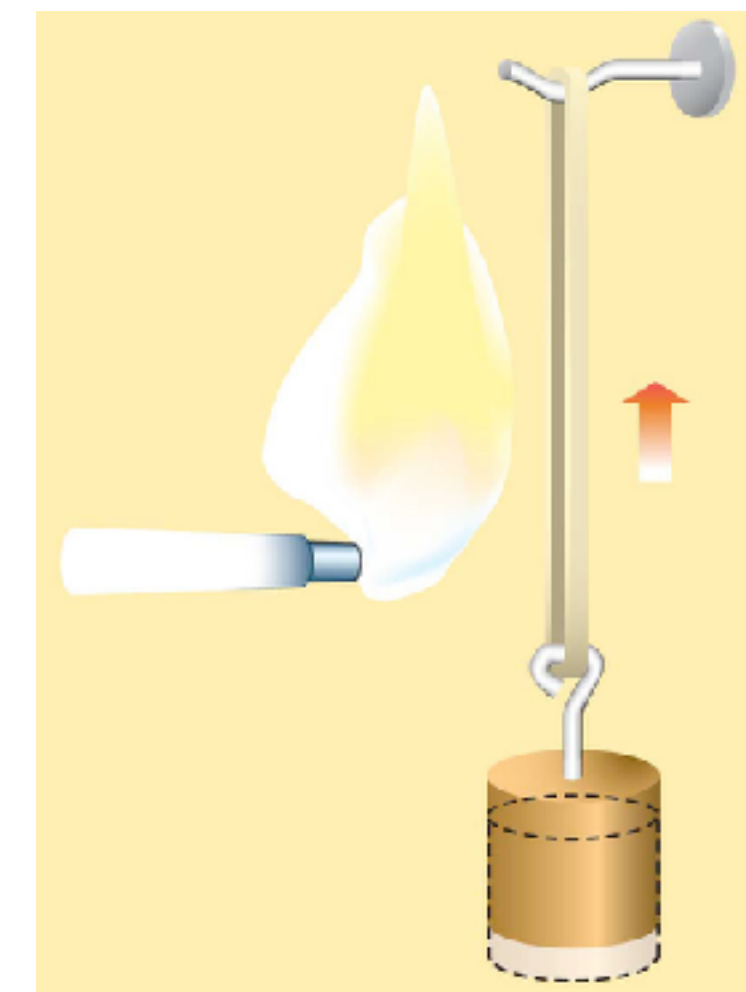
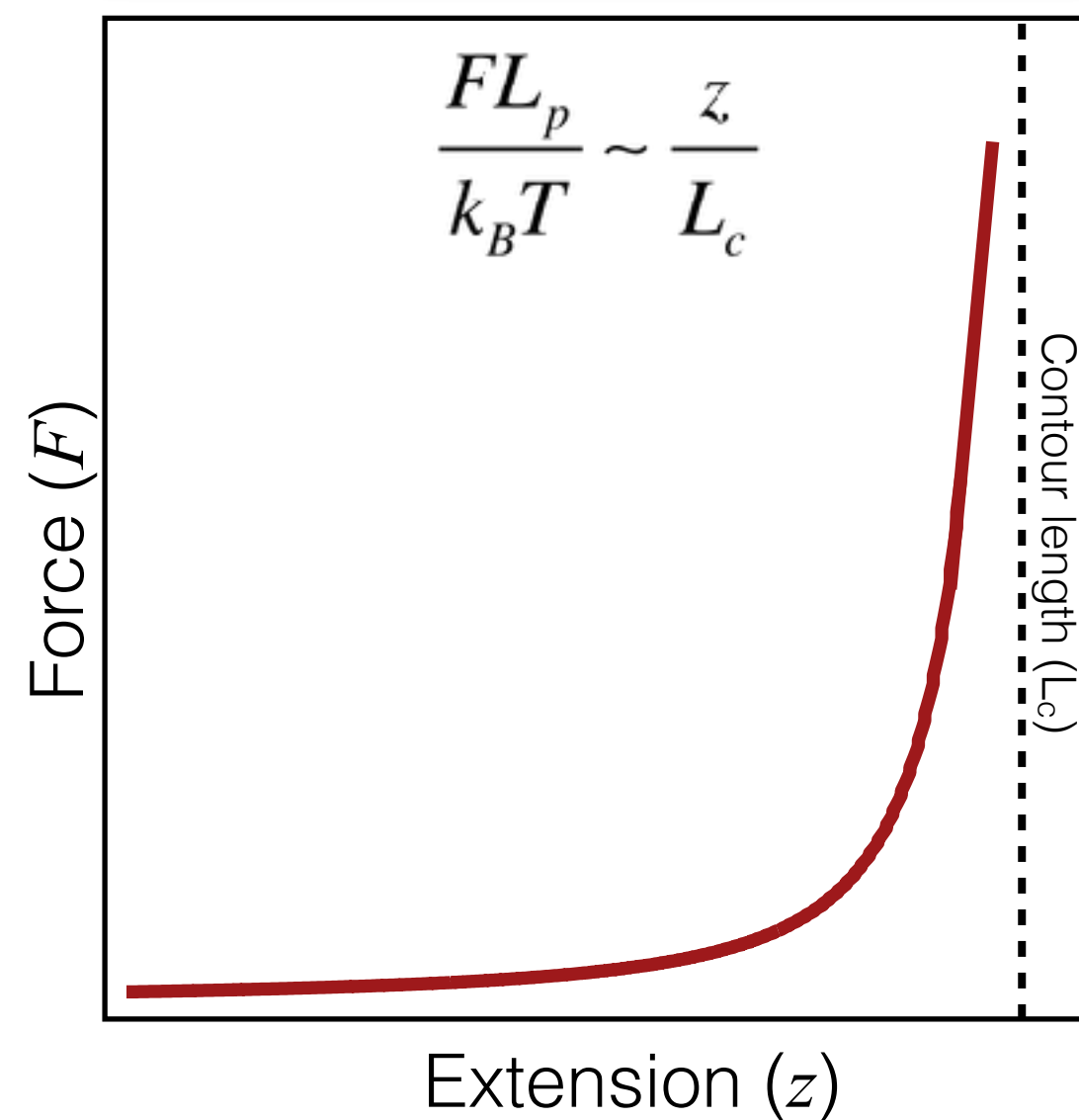
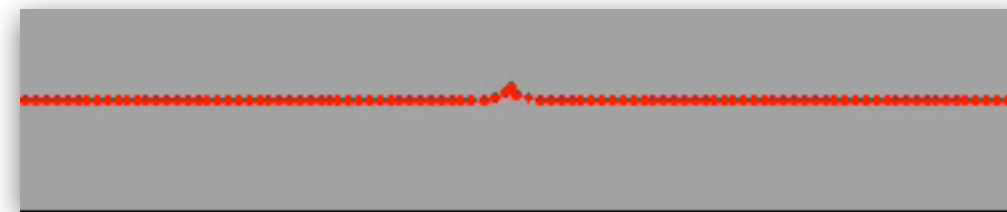


2. Force: deforms shape

Rigid body:
Hooke's law

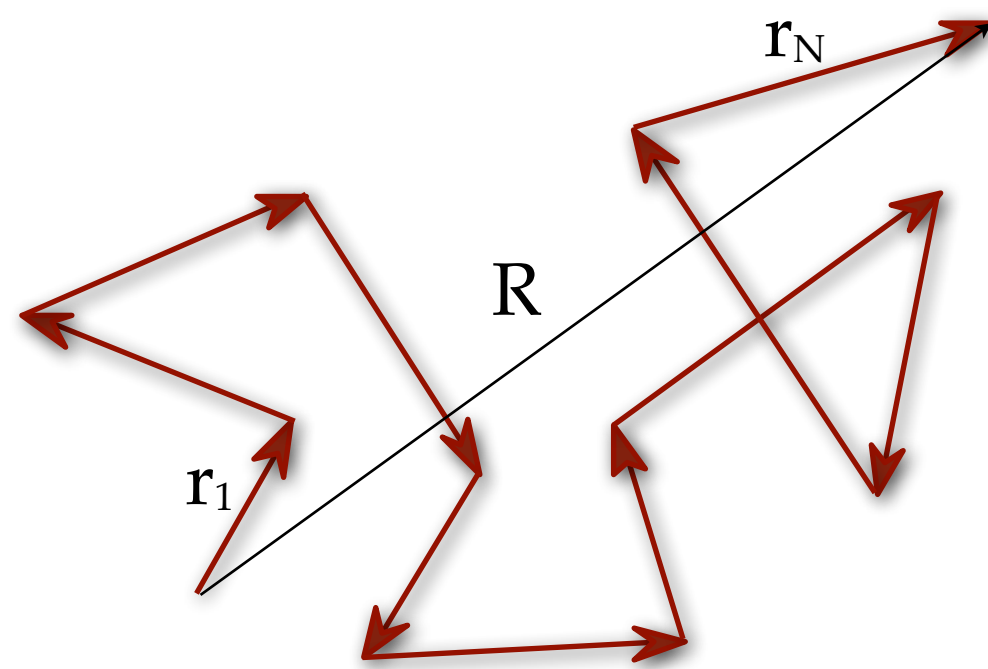


Polymer chain:
fluctuations, configurational entropy



Macroscopic manifestation
of entropic elasticity:
Gough-Joule effect

Models of entropic elasticity



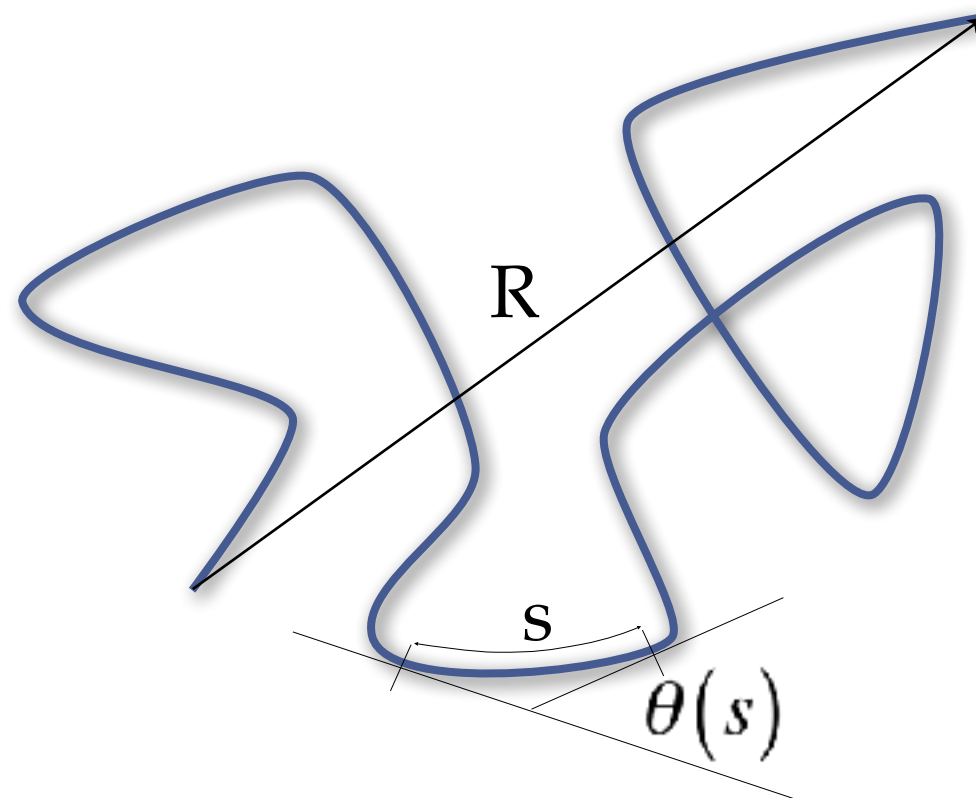
Freely jointed chain

$$\langle R^2 \rangle = N \langle \vec{r}_i \rangle^2 = N l_K^2 = L_C l_K$$

R = end-to-end distance

L_C = contour length

l_K = Kuhn segment length



Wormlike chain

$$\langle \cos \theta(s) \rangle = e^{-\frac{s}{L_P}} \quad L_P = \frac{EI}{k_B T}$$

L_P = persistence length

EI = bending rigidity

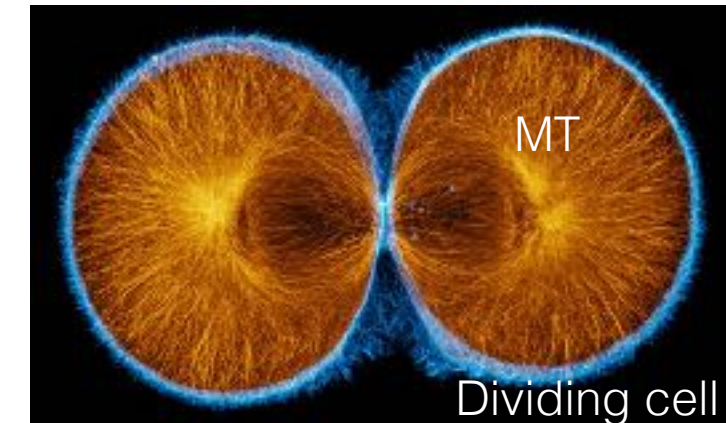
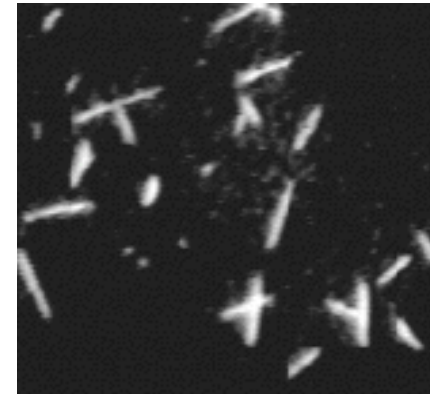
$l_K = 2L_P$

Equilibrium shape and bending rigidity of a polymer chain are related

Rigid chain

$$L_p \gg L_c$$

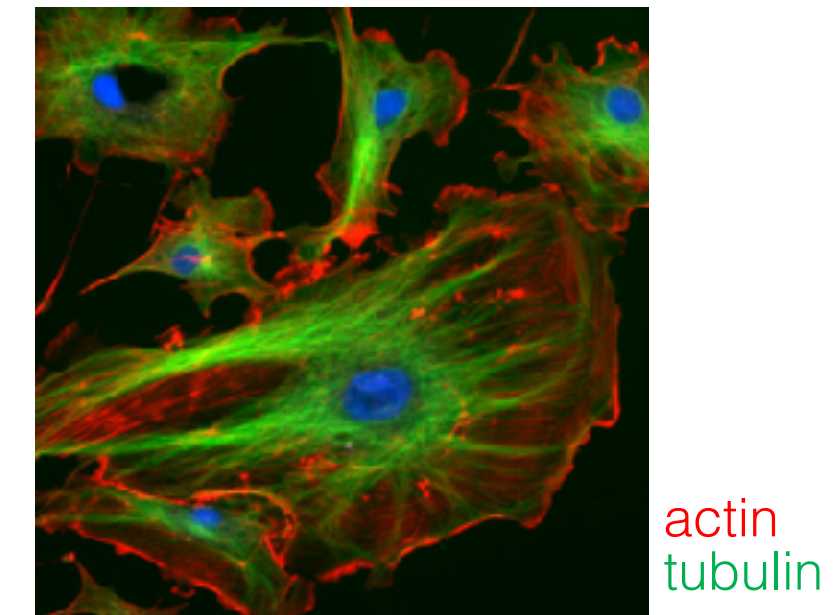
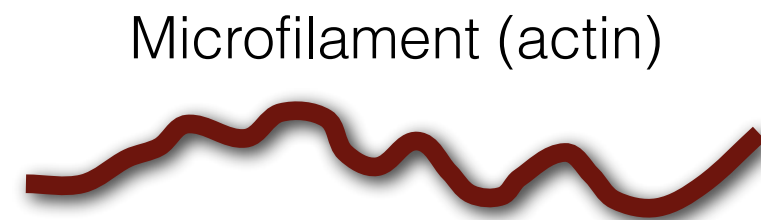
(mm \gg 10 μ m)



Semiflexible chain

$$L_p \approx L_c$$

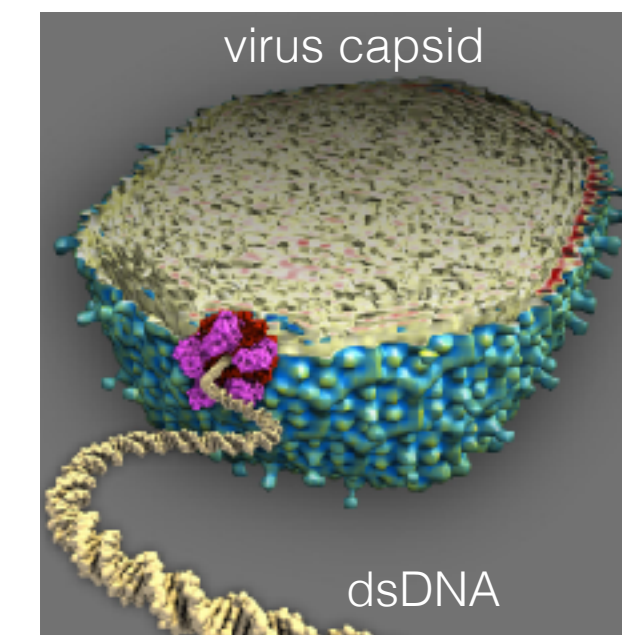
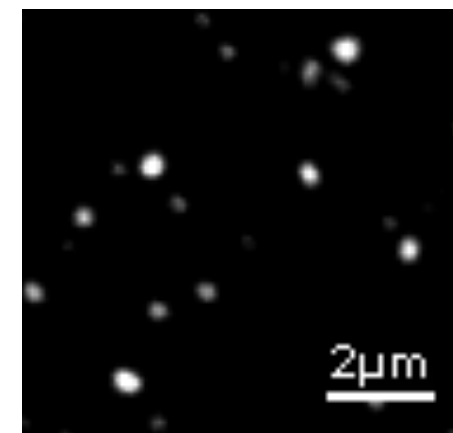
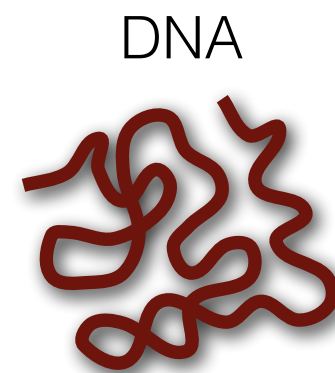
(μ m \approx μ m)



Flexible chain

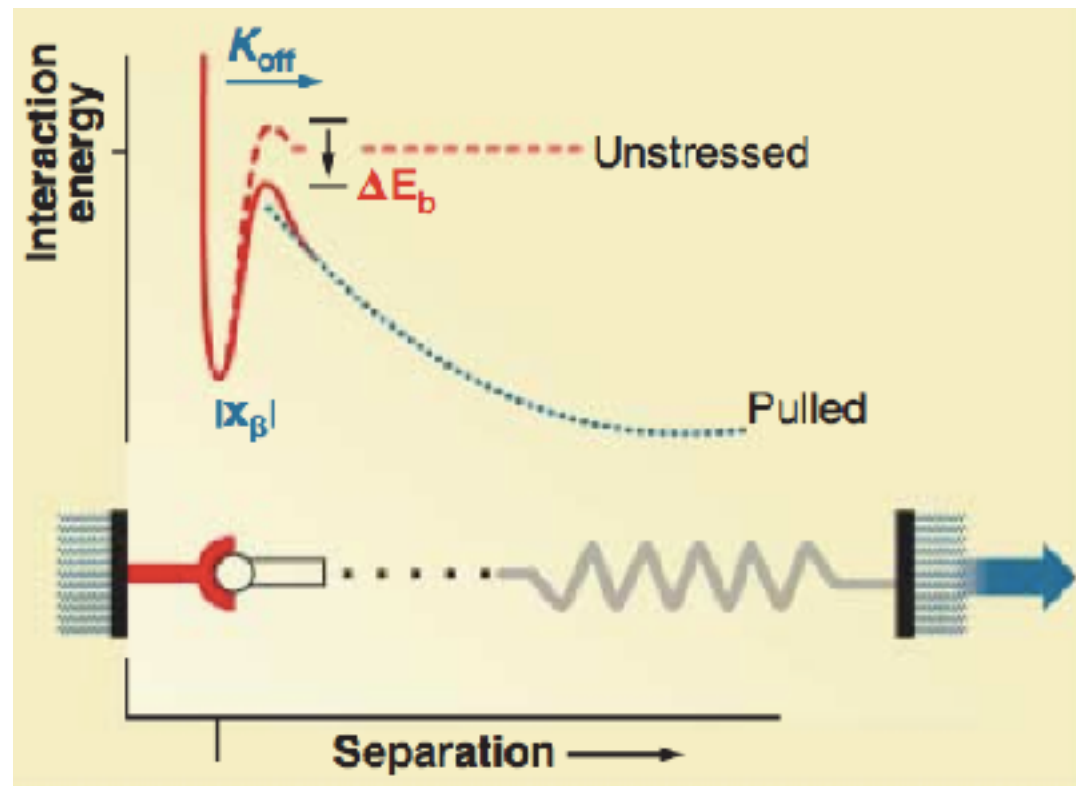
$$L_p \ll L_c$$

(50 nm \ll cm)



L_p = persistence length
 L_c = contour length

3. Force: reduces bond lifetime



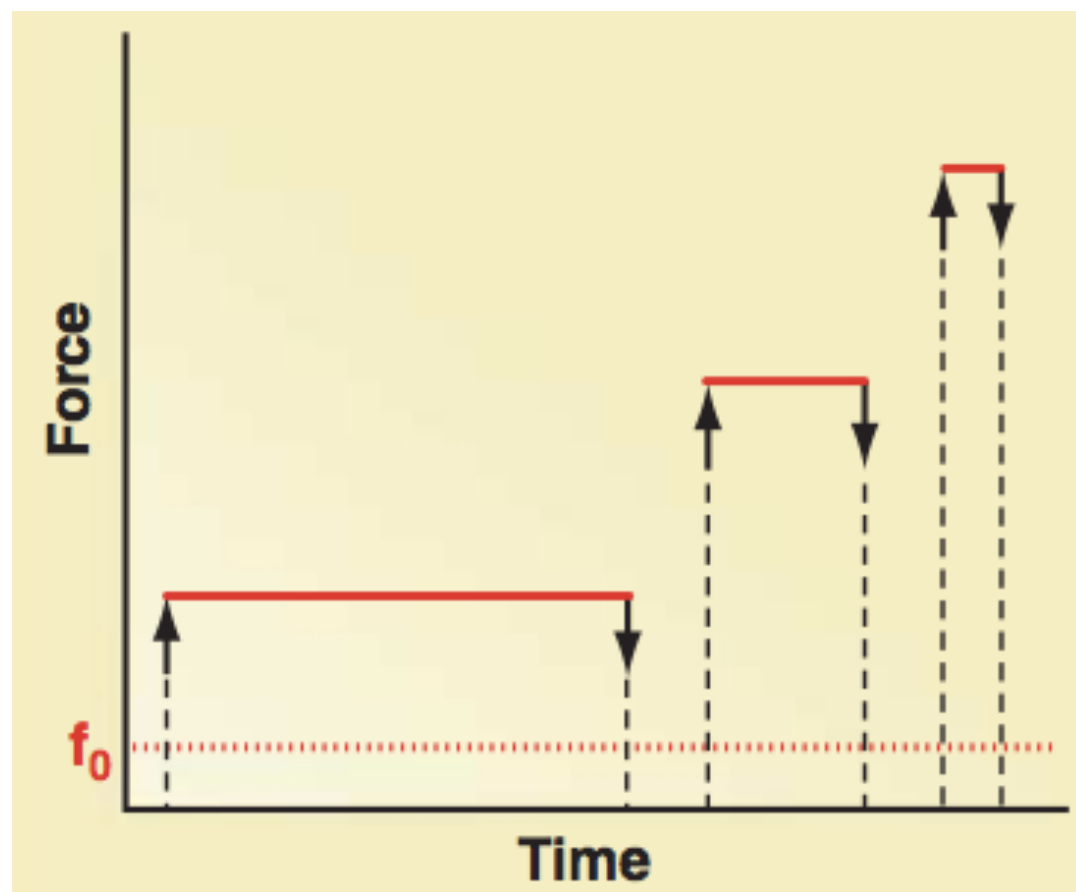
Under thermal activation: $\tau(0) = \omega e^{\frac{E_a}{k_B T}}$

Under mechanical load: $\tau(F) = \omega e^{\frac{E_a - F\Delta x}{k_B T}} = \tau(0) e^{-\frac{F\Delta x}{k_B T}}$

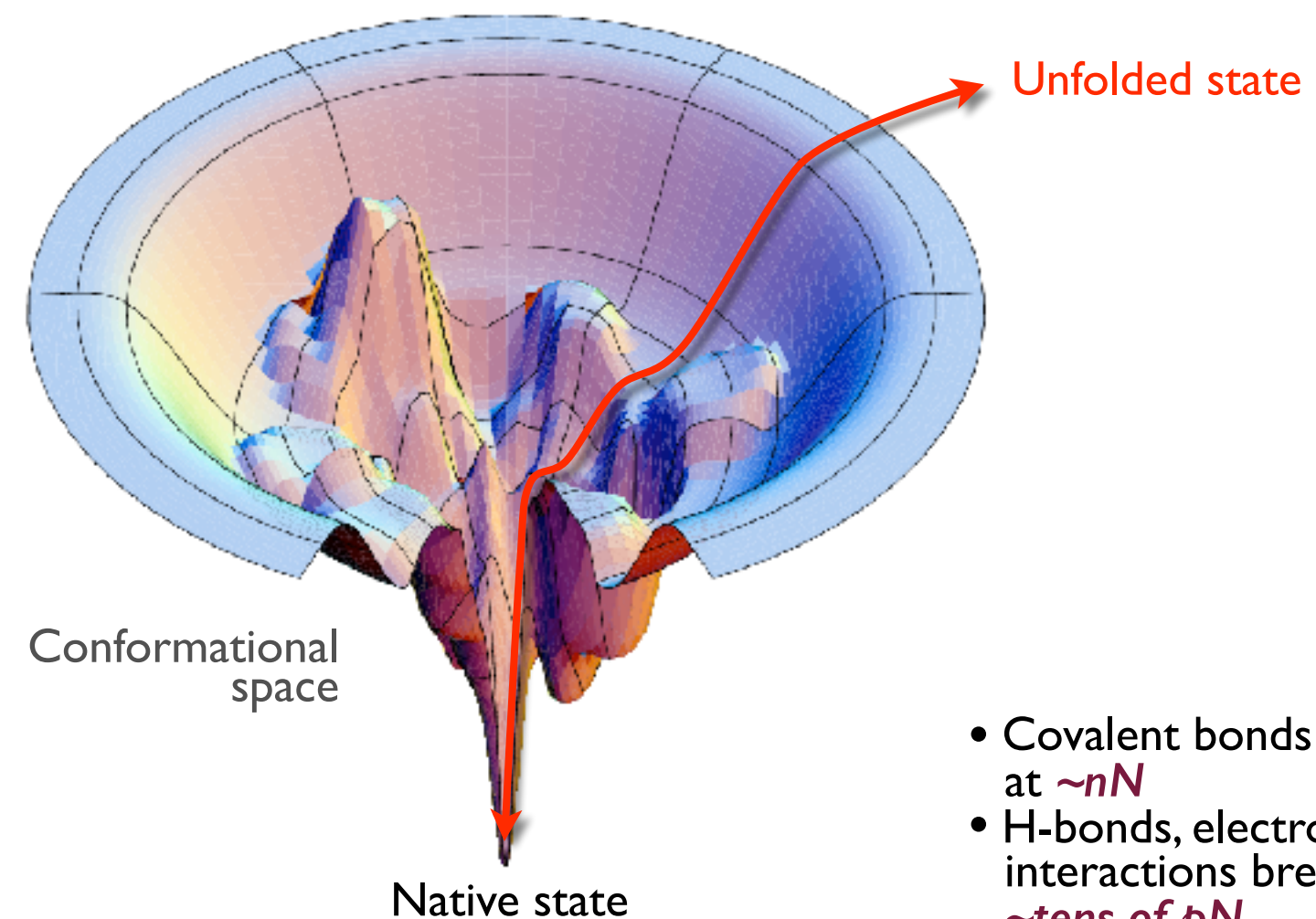
ω = characteristic time

E_a = activation energy

Δx = distance between bound and transition states



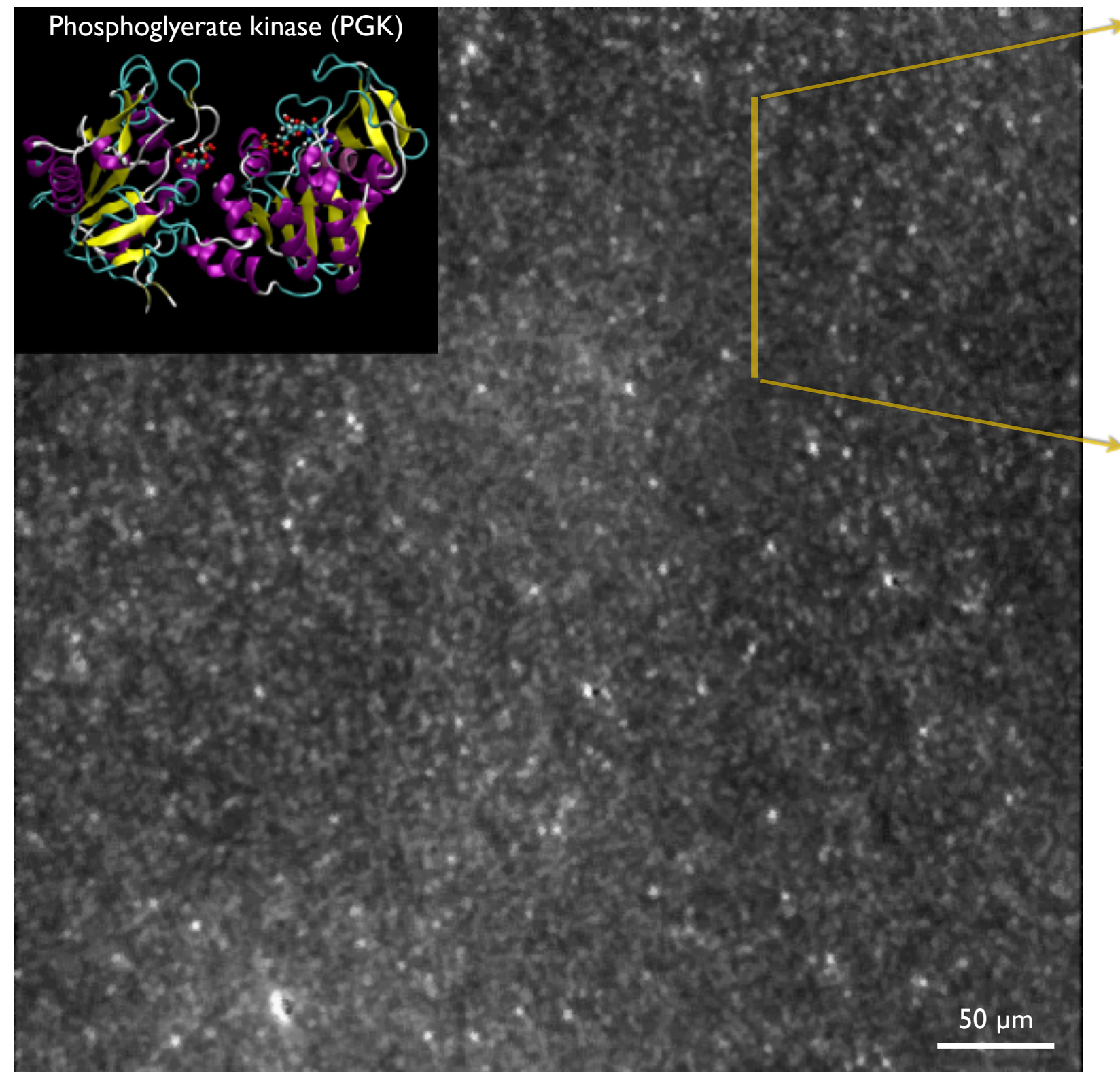
Evan A. Evans and David A. Calderwood Science 316, 1148 (2007)



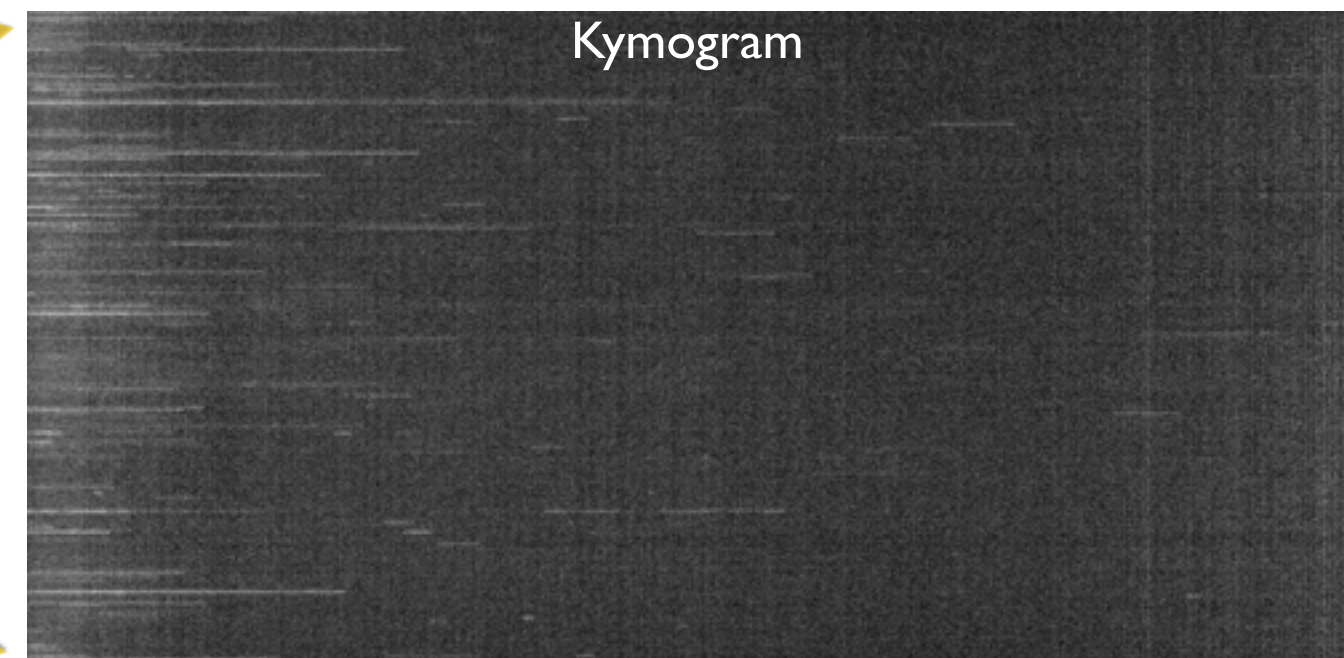
- Covalent bonds break at $\sim nN$
- H-bonds, electrostatic interactions break at $\sim \text{tens of } pN$

Molecular singularity I.

Single-step photobleaching

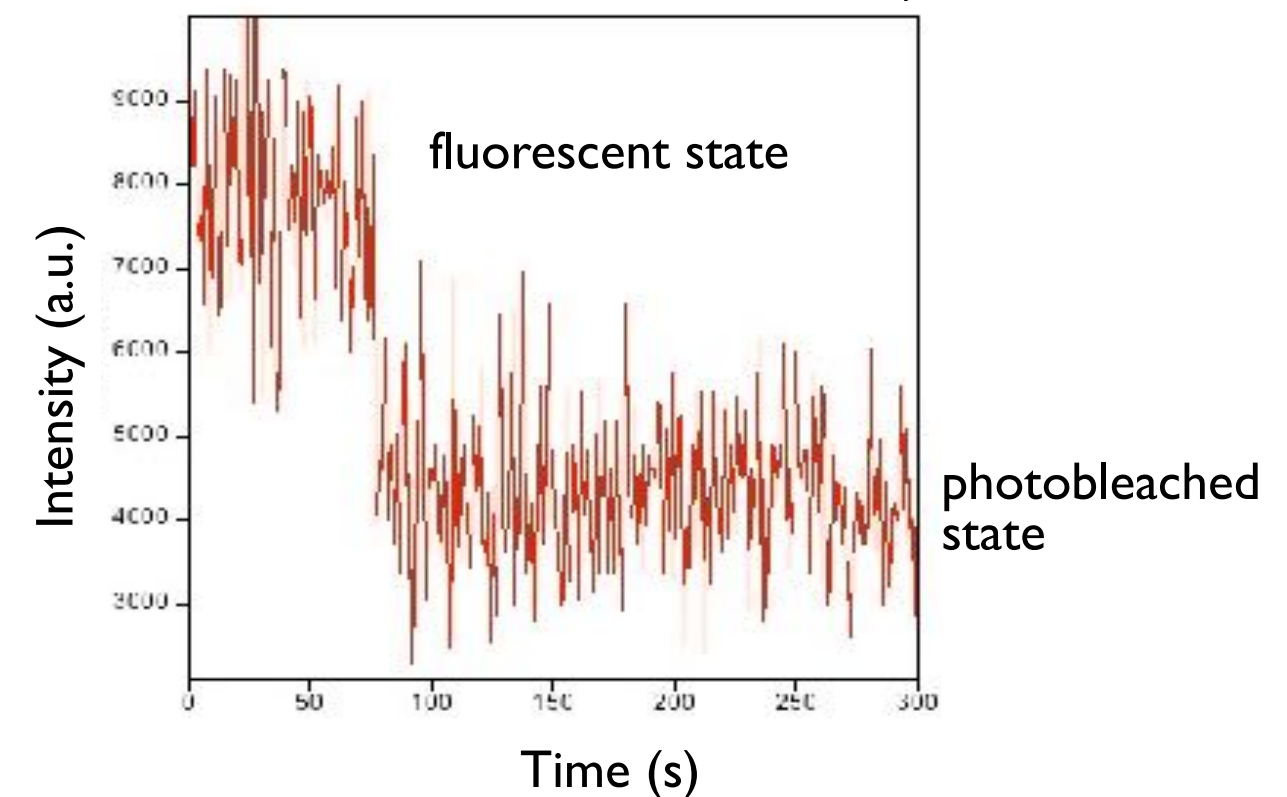


PGK molecules labeled with Alexa488, TIRF microscopy



Time

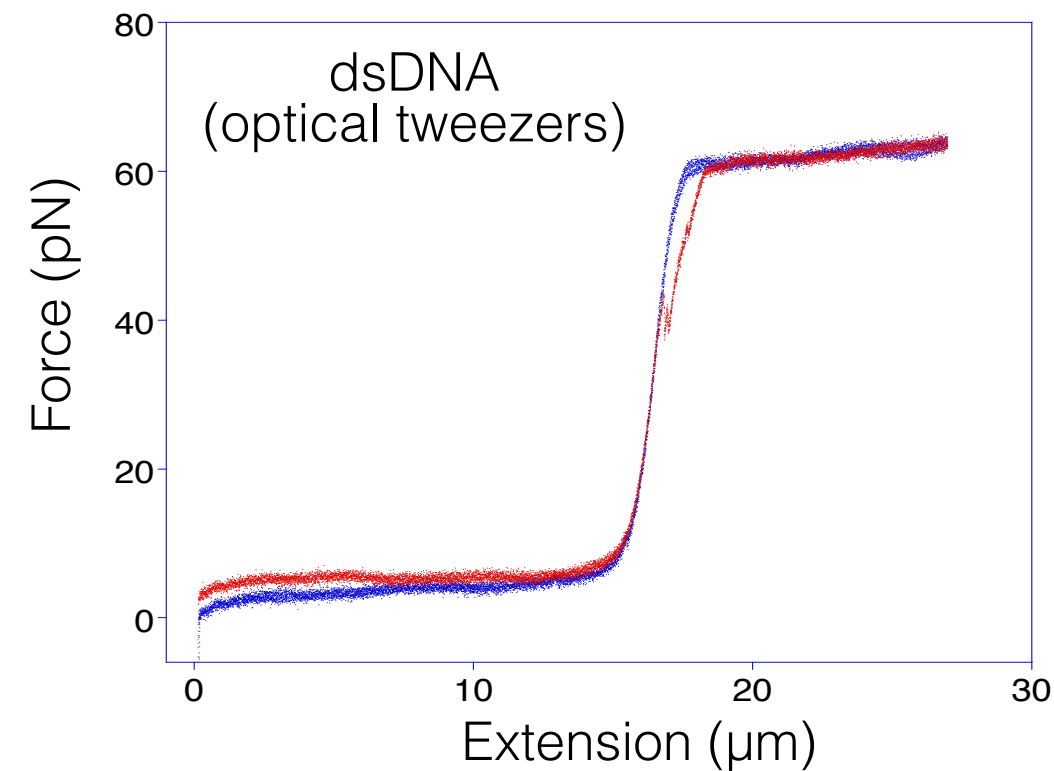
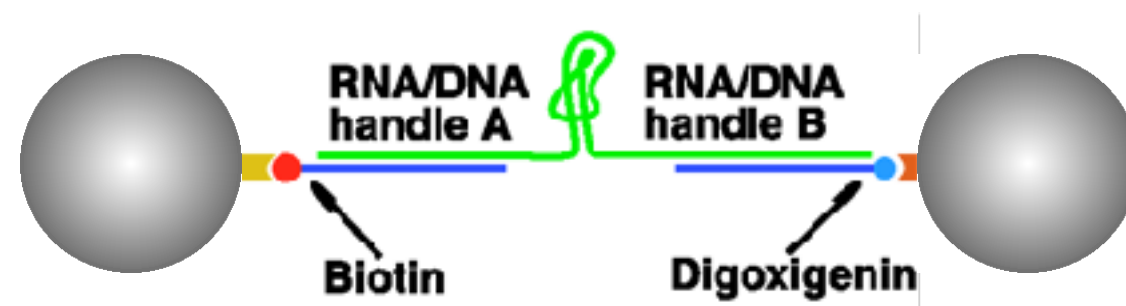
Single-step photobleaching (exponential function in an ensemble!)



Molecular singularity II.

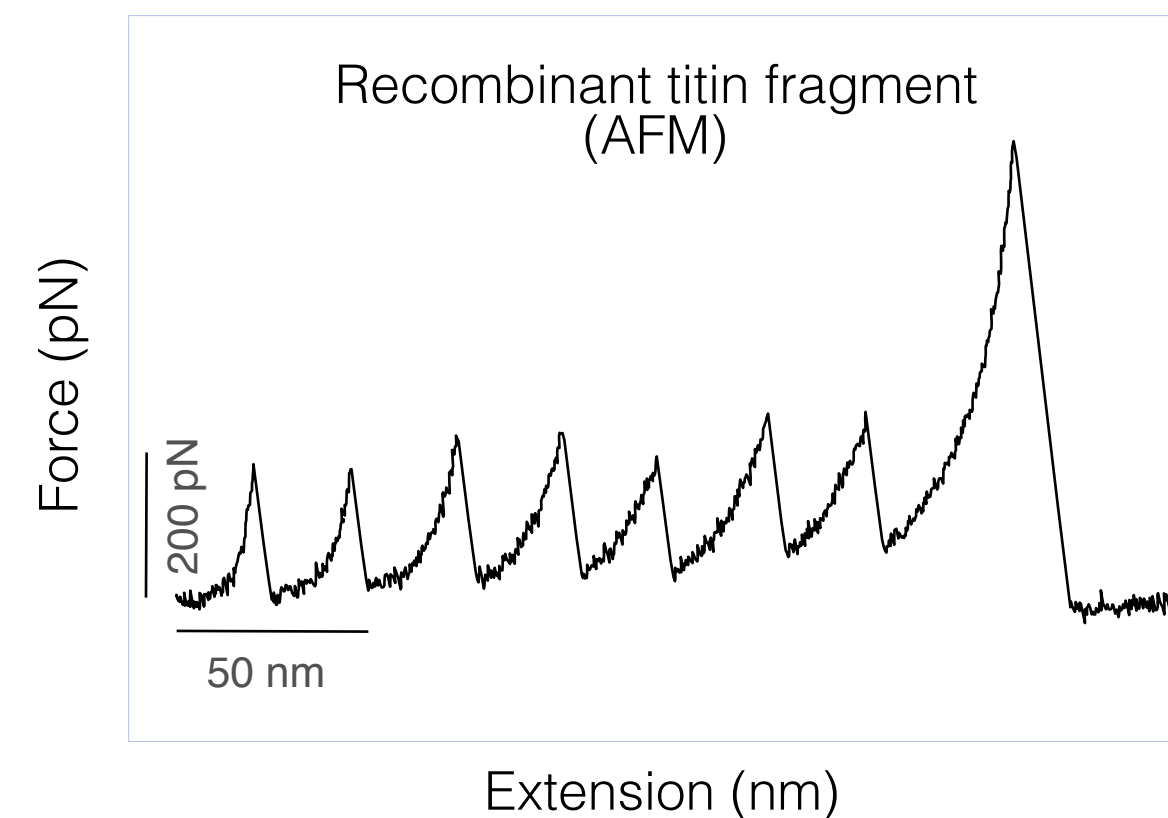
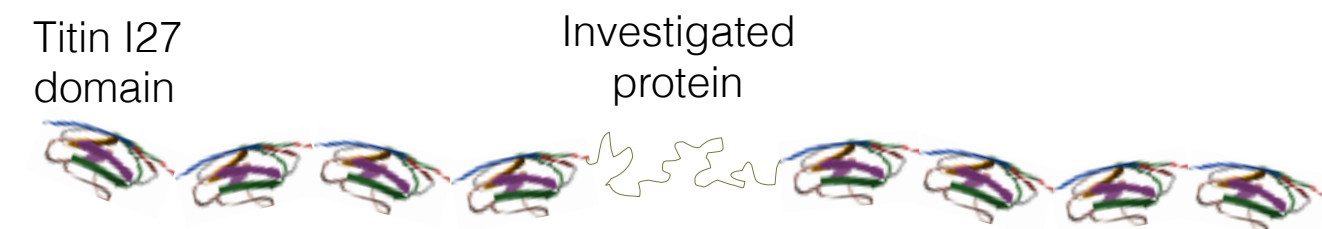
Nanomechanical fingerprint

DNA handle



Cooperative transition at 65 pN

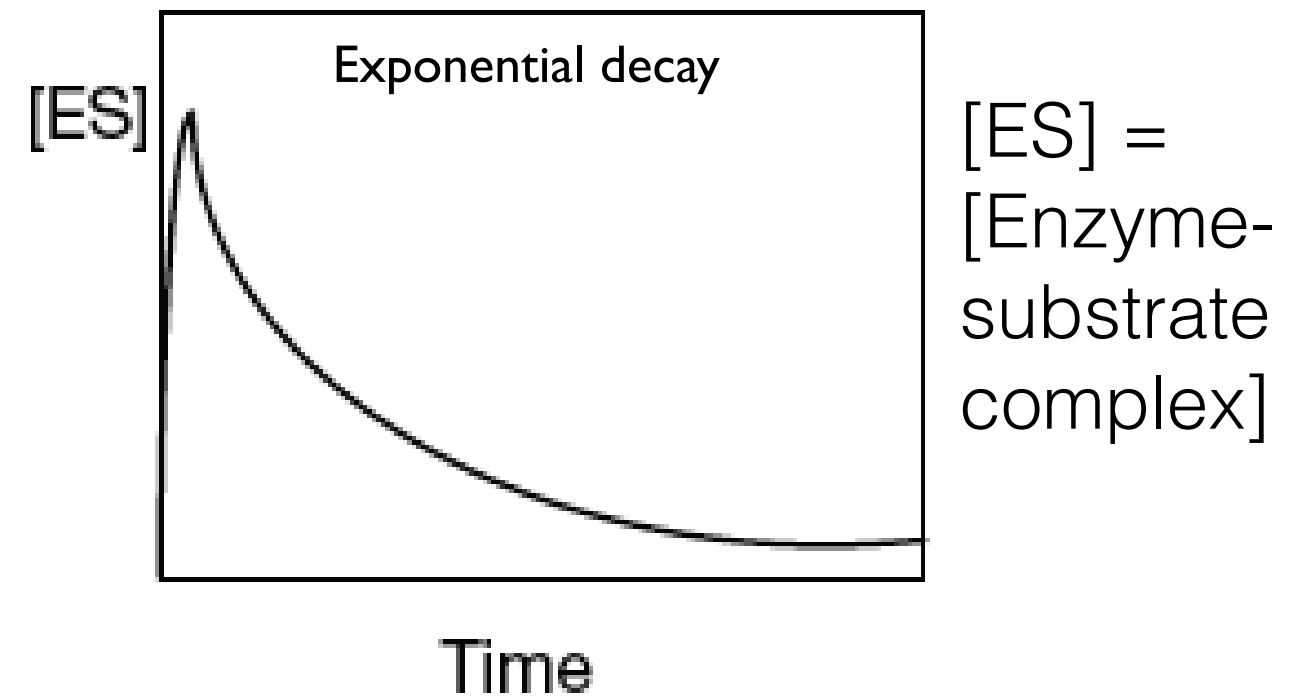
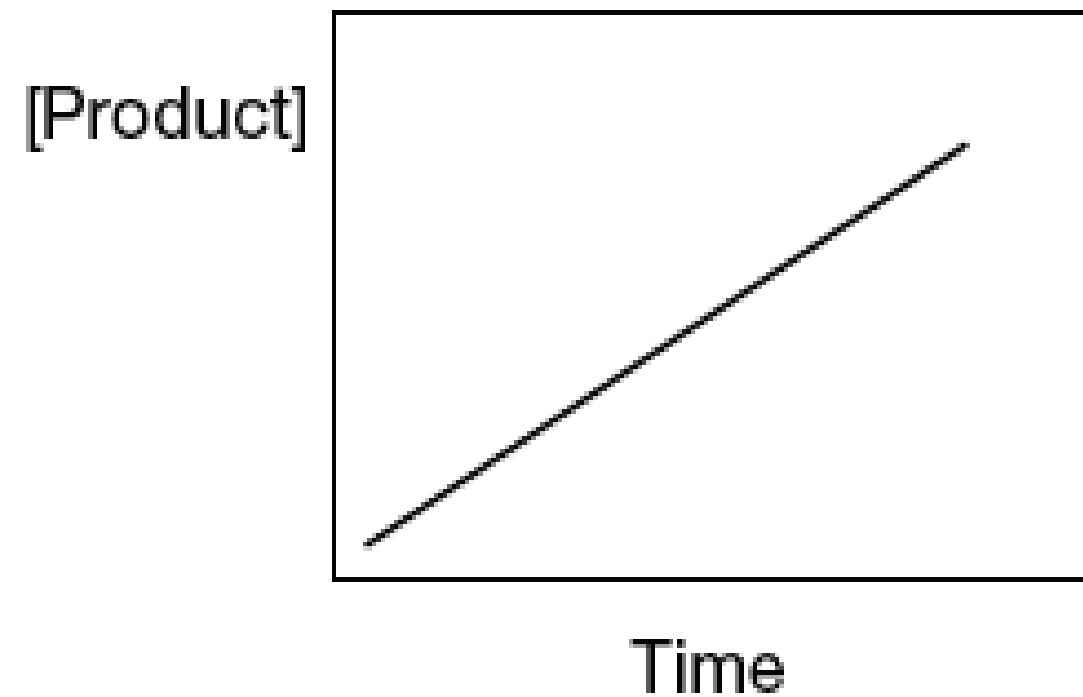
Recombinant polyprotein



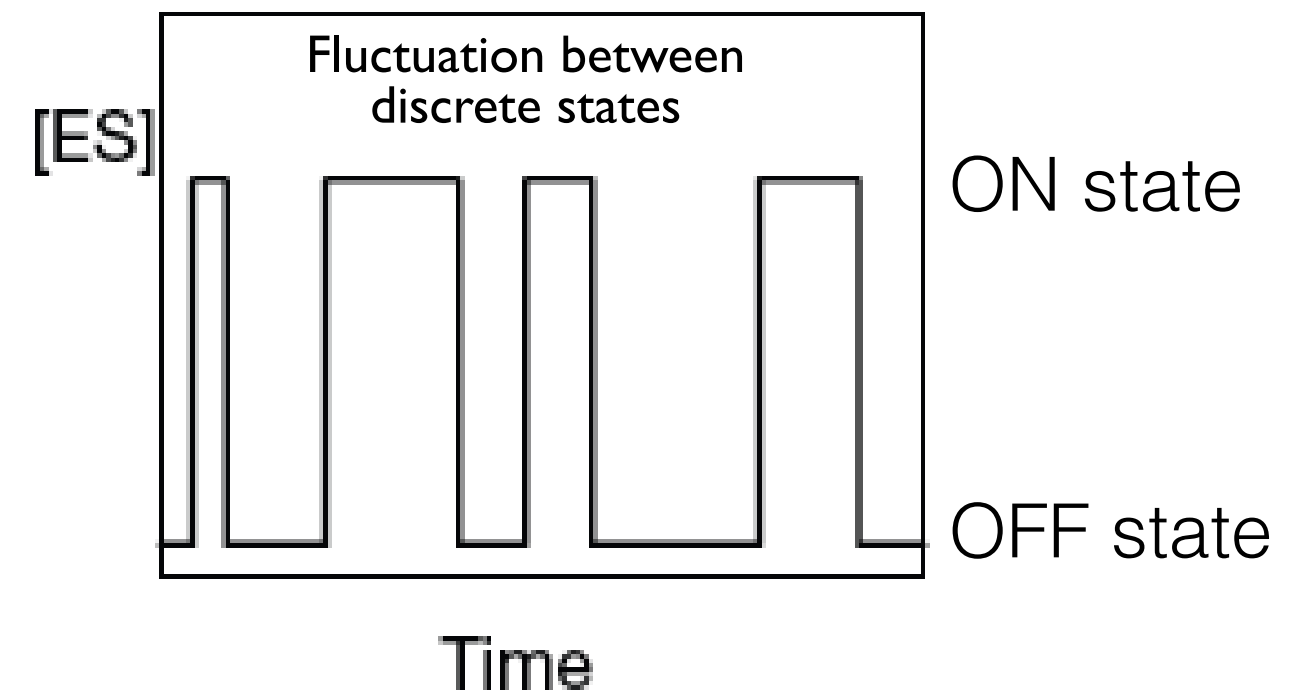
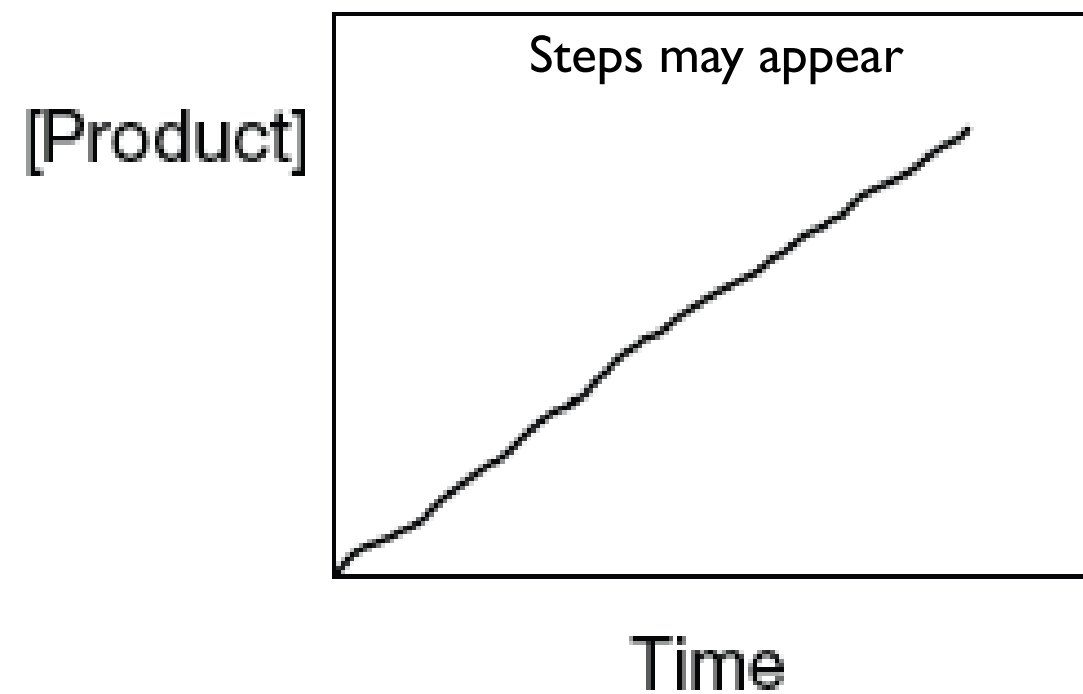
Sawtooth-shaped, equidistant transitions

Ensemble *versus* single molecule behavior

Ensemble:

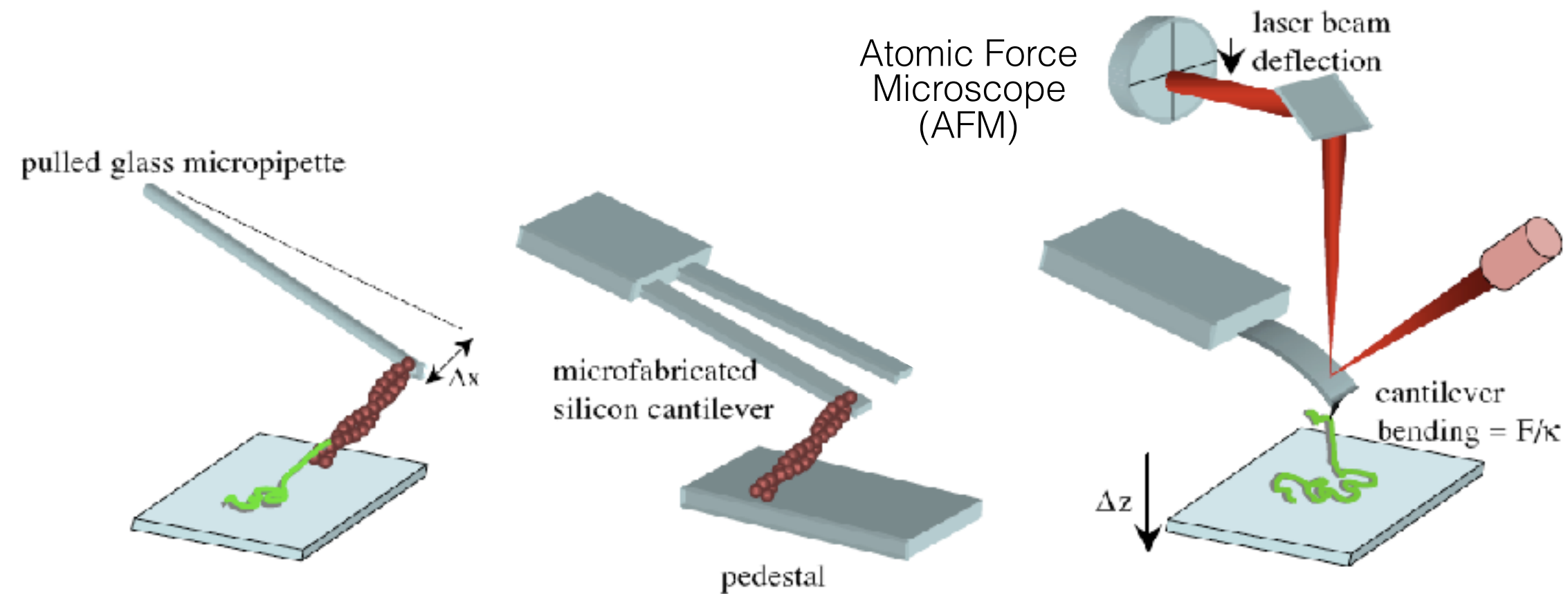


Single molecule:

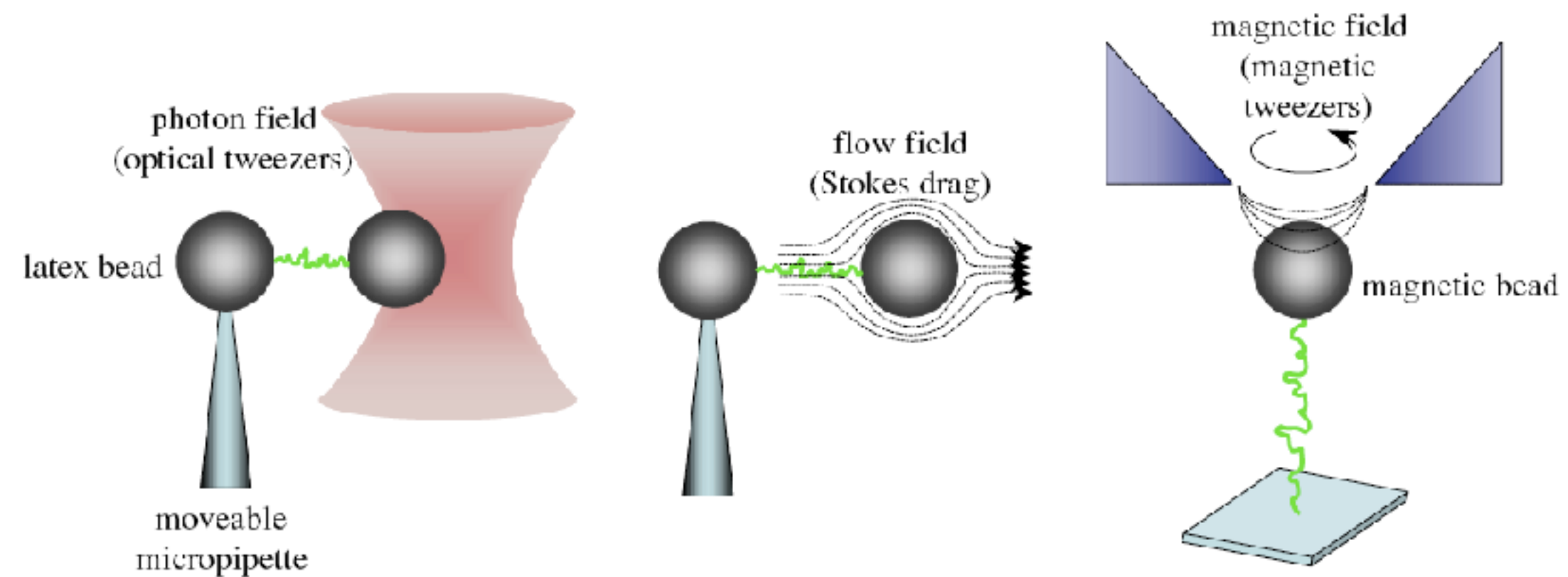


Methods of manipulation

Cantilever methods

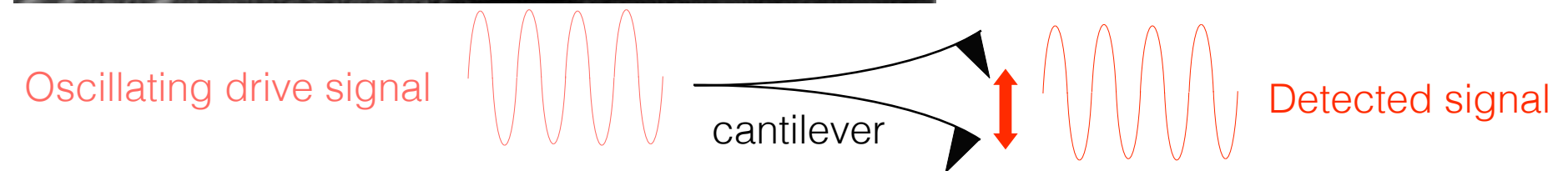
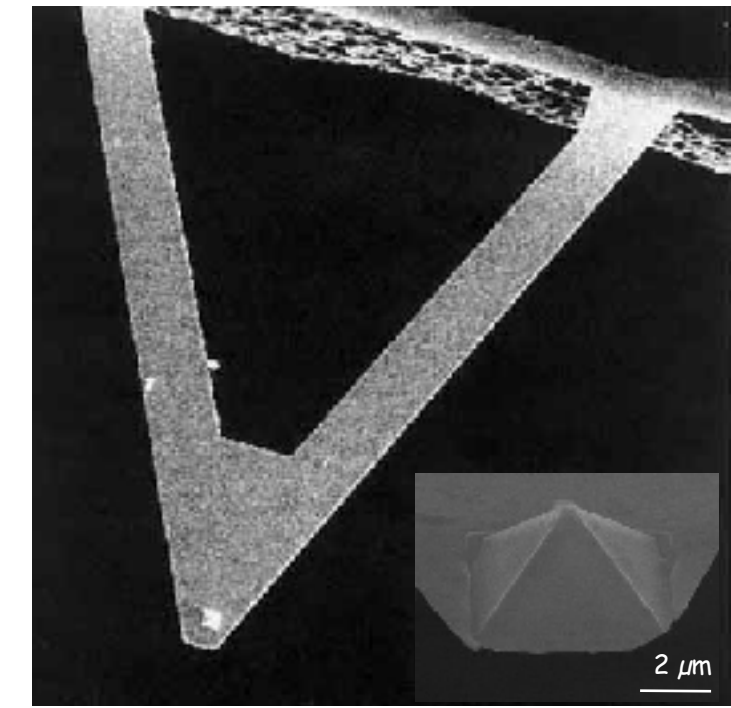
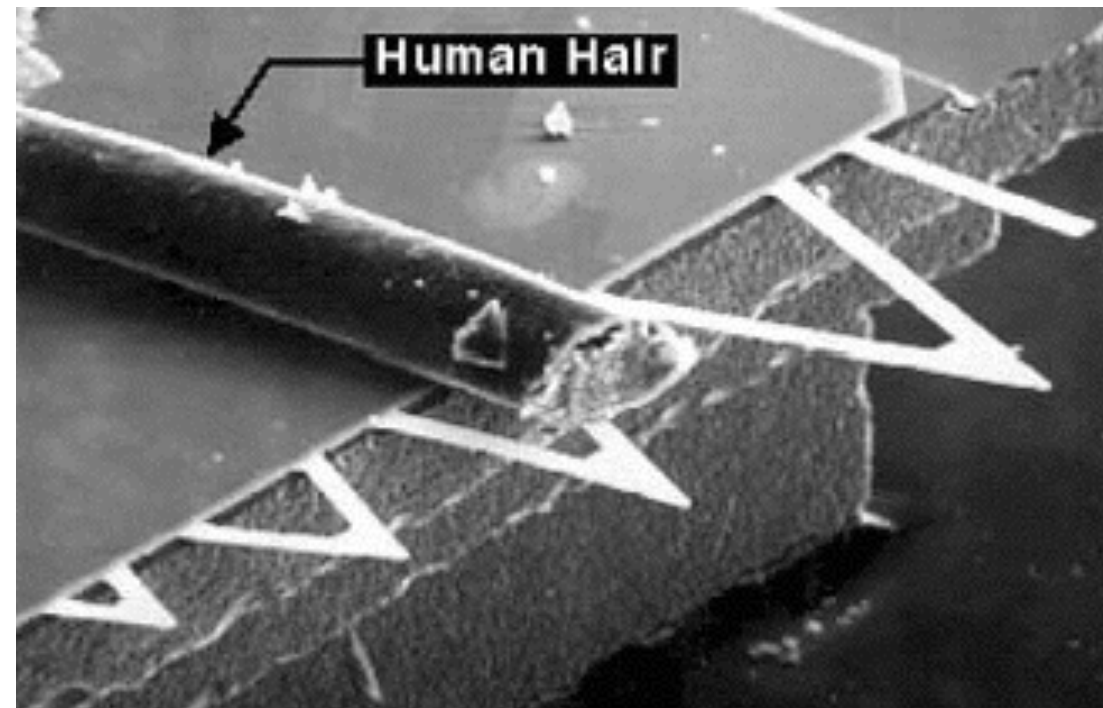
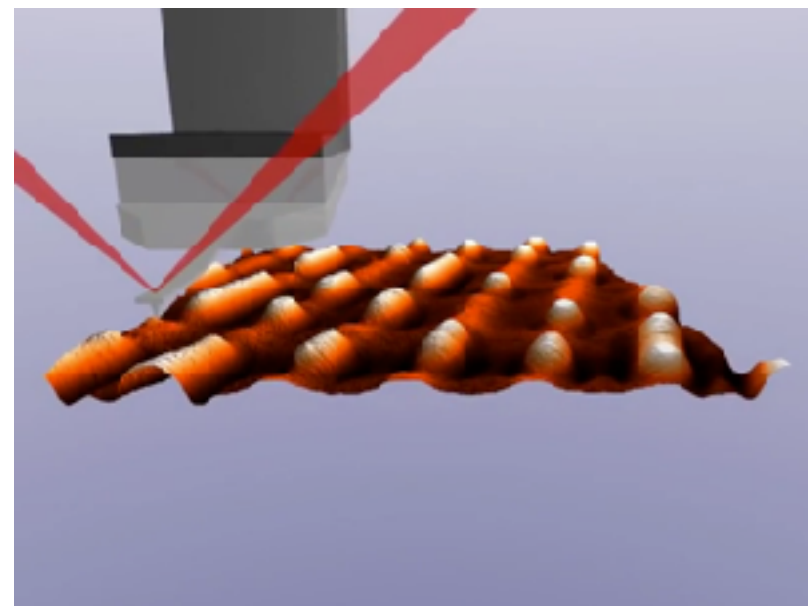
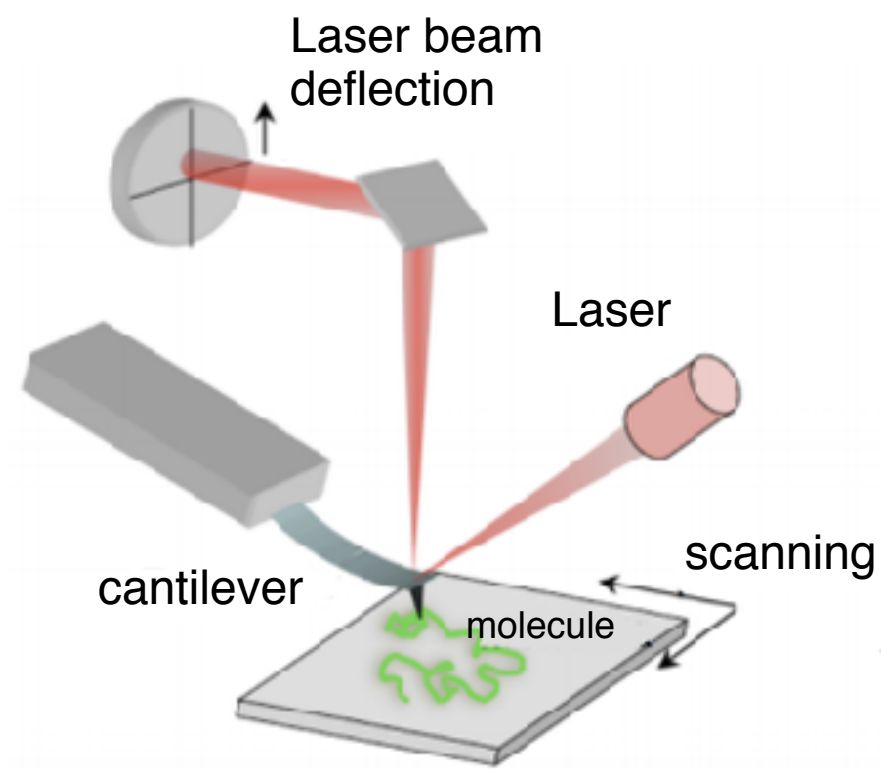


Field-based methods

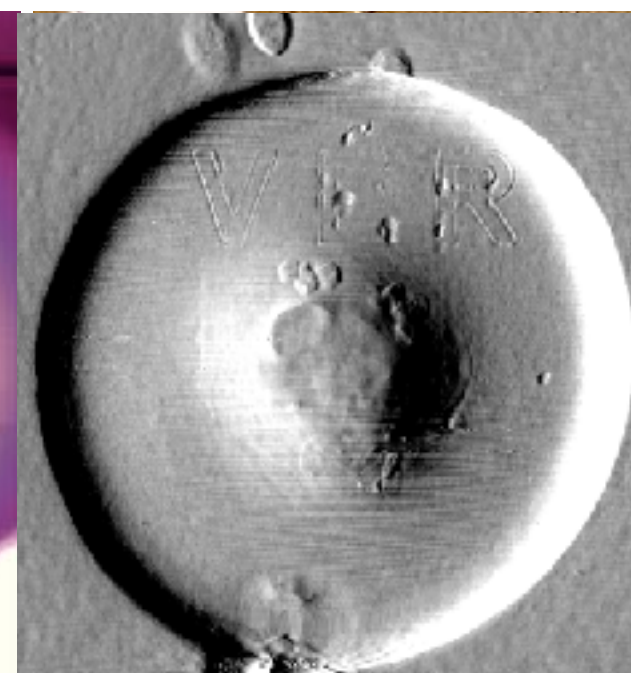


Manipulation with AFM

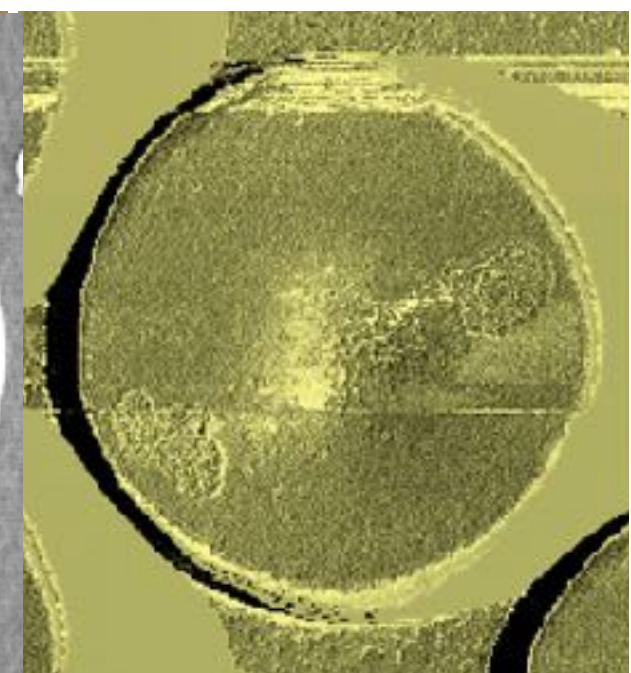
Atomic Force Microscopy



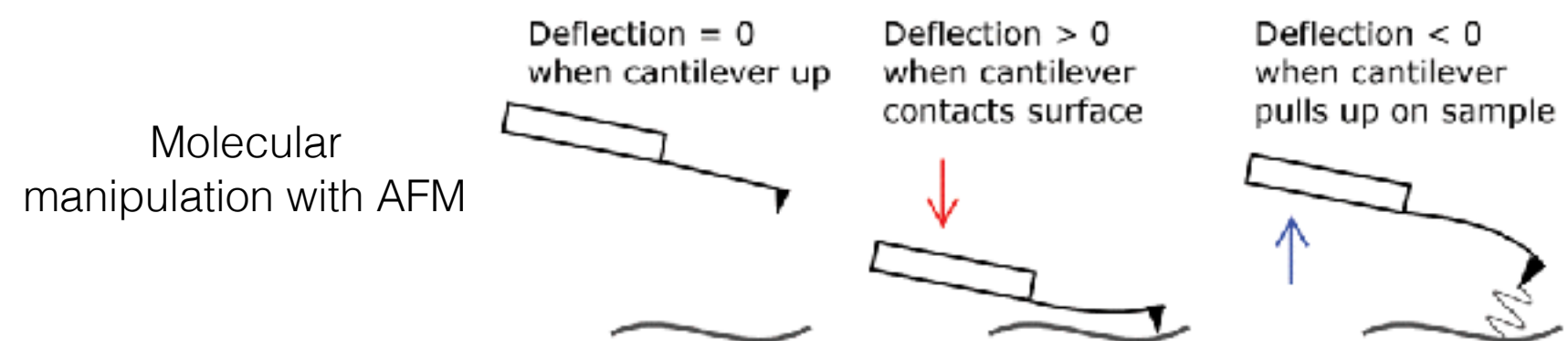
Height contrast



Amplitude contrast



Phase contrast



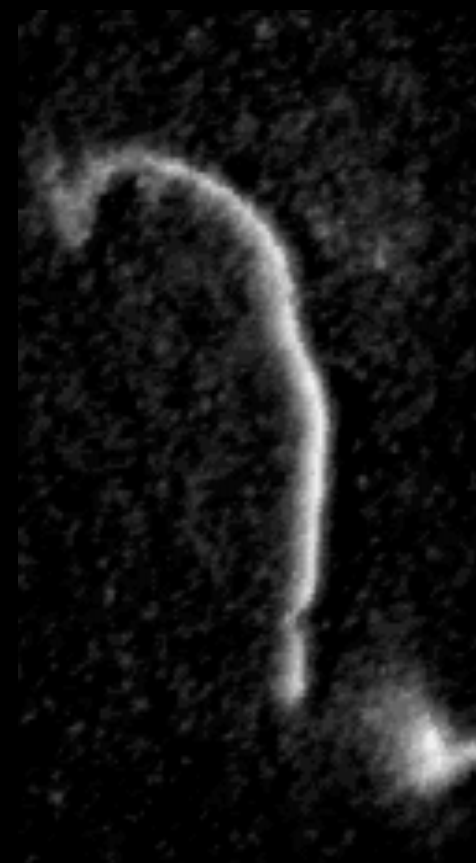
Manipulation with light



E. coli bacterium



Actin filament



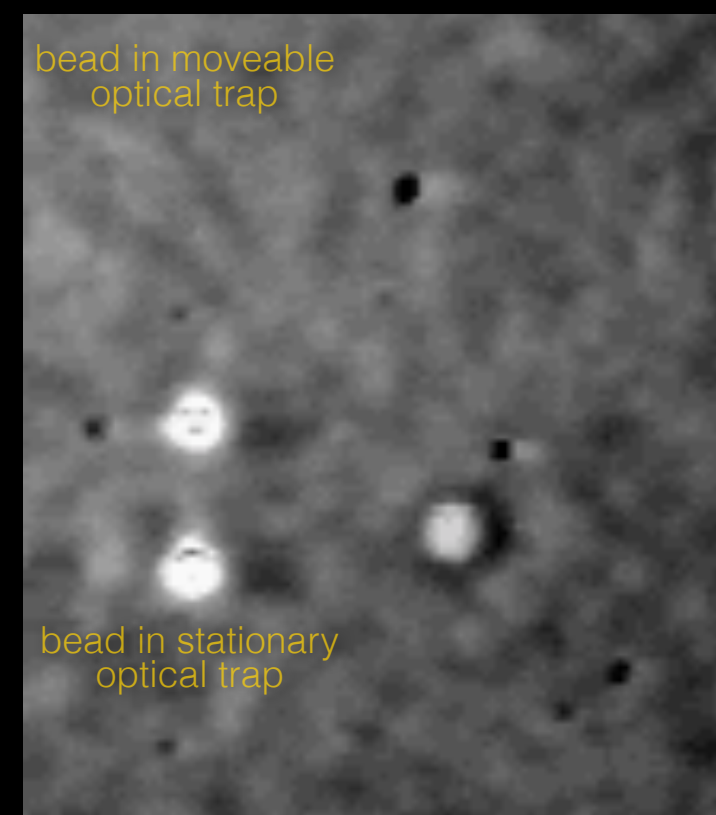
Optical tweezers



Arthur Ashkin, Nobel-prize 2018

DNA

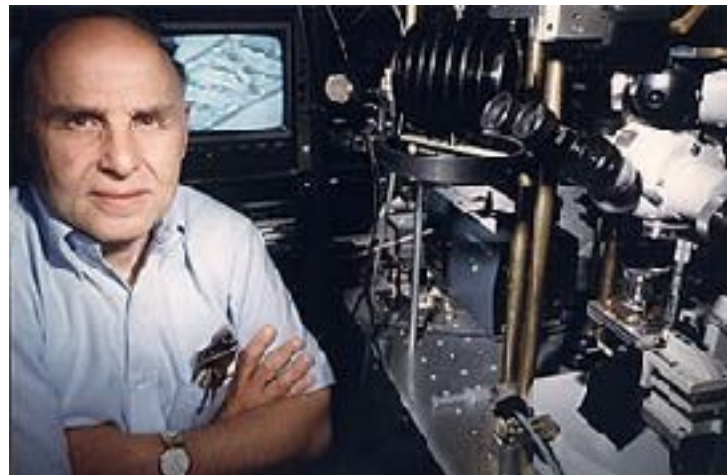
Phase contrast image



Fluorescence image



Optical tweezers - brief history



Arthur Ashkin

1970: Arthur Ashkin: optical tweezers (Nobel-prize, 2018)

1991: J.Spudich, T.Yanagida, J.Molloy, single myosin mechanics

1994: T.Yanagida, single ATP turnover on myosin

1994: K.Svoboda, S. Block, single kinesin mechanics

1996: C.Bustamante, D.Bensimon, DNS molecule manipulation

1997: S. Chu, W.D. Phillips and C. Cohen-Tanoudji (Nobel-prize, 1997): atom cooling with optical tweezers.

1997: M.Kellermayer, M.Rief, L.Tskhovrebova, titin manipulation

2000: Galajda P., Ormos O., microfabrication and optomechanical manipulation of artificial motors

2001: J.Liphardt, C.Bustamante, RNA stretch

2002: Holographic optical tweezers (spatial light modulator, SLM)

2008: Bustamante, Tinoco: ribosome mechanics



J.Spudich



J.Finer



C.Bustamante



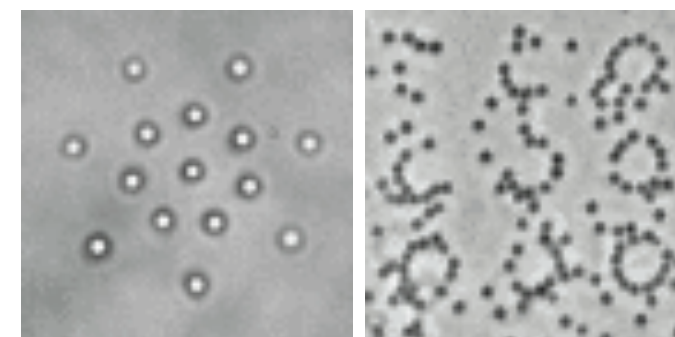
S. Chu, W.D. Phillips és C. Cohen-Tanoudji



J.Molloy



Microfabricated propeller



Simultaneous manipulation of many particles with holographic optical tweezers

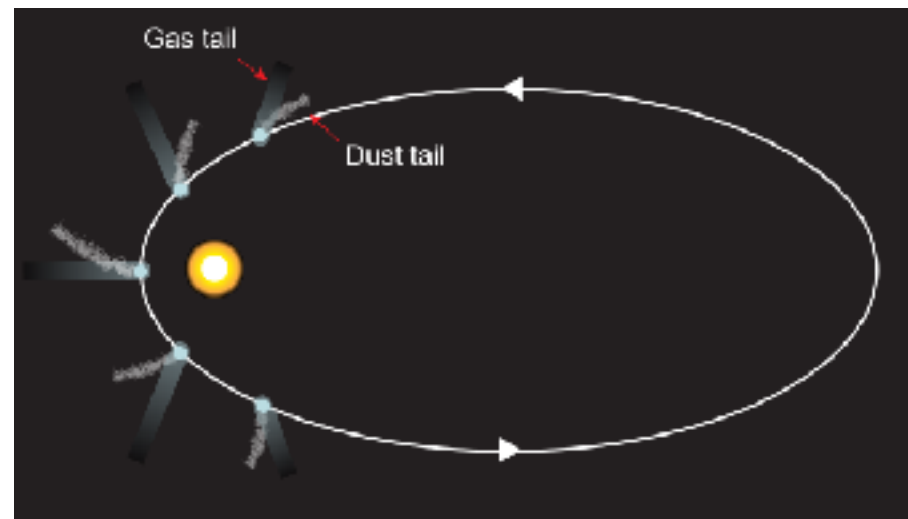


S. Block

Photonic momentum changes upon interaction with particles

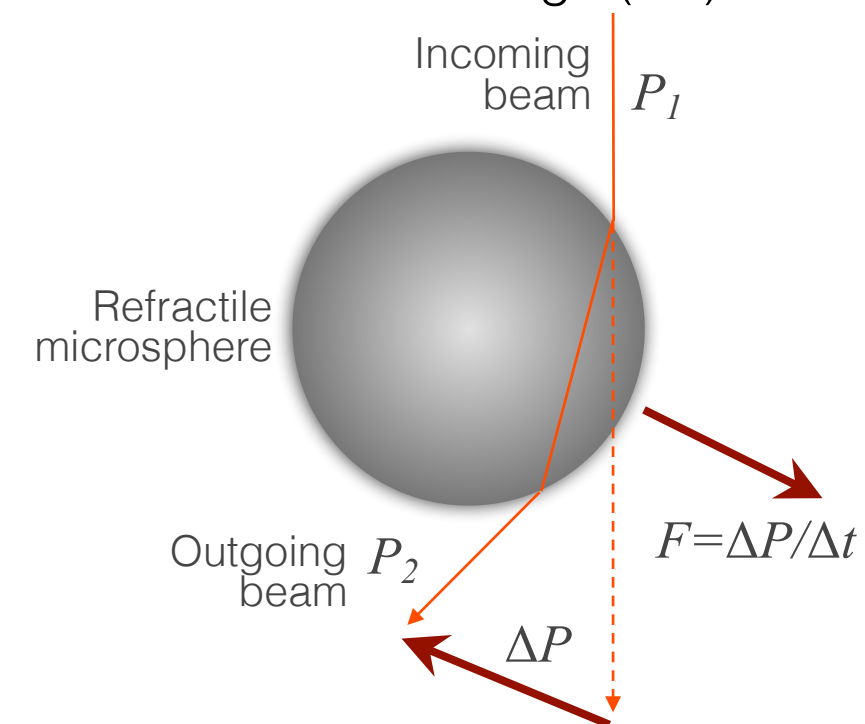


Johannes Kepler
(1571-1630)



Comet tail stands away from the sun: mechanical effect of the light rays?

Refraction is accompanied by photonic momentum change (ΔP):



Einstein:
mass-energy
equivalence
 $E = mc^2$

Planck:
radiation
law
 $E = hf$

Maxwell:
speed of light
 $c = \lambda f$



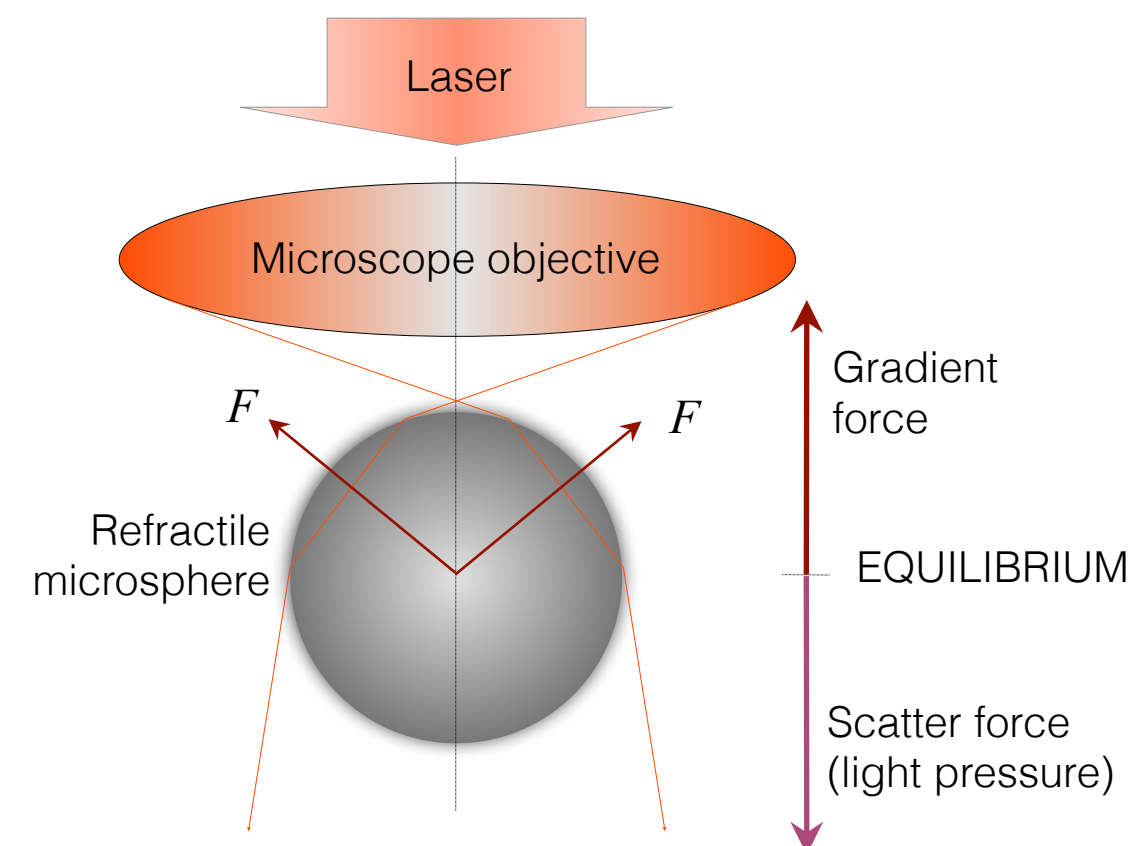
Louis-Victor-Pierre-Raymond,
7th duc
de Broglie (1892-1987)

$$mc^2 = h \cdot \frac{c}{\lambda}$$

Momentum of photon:

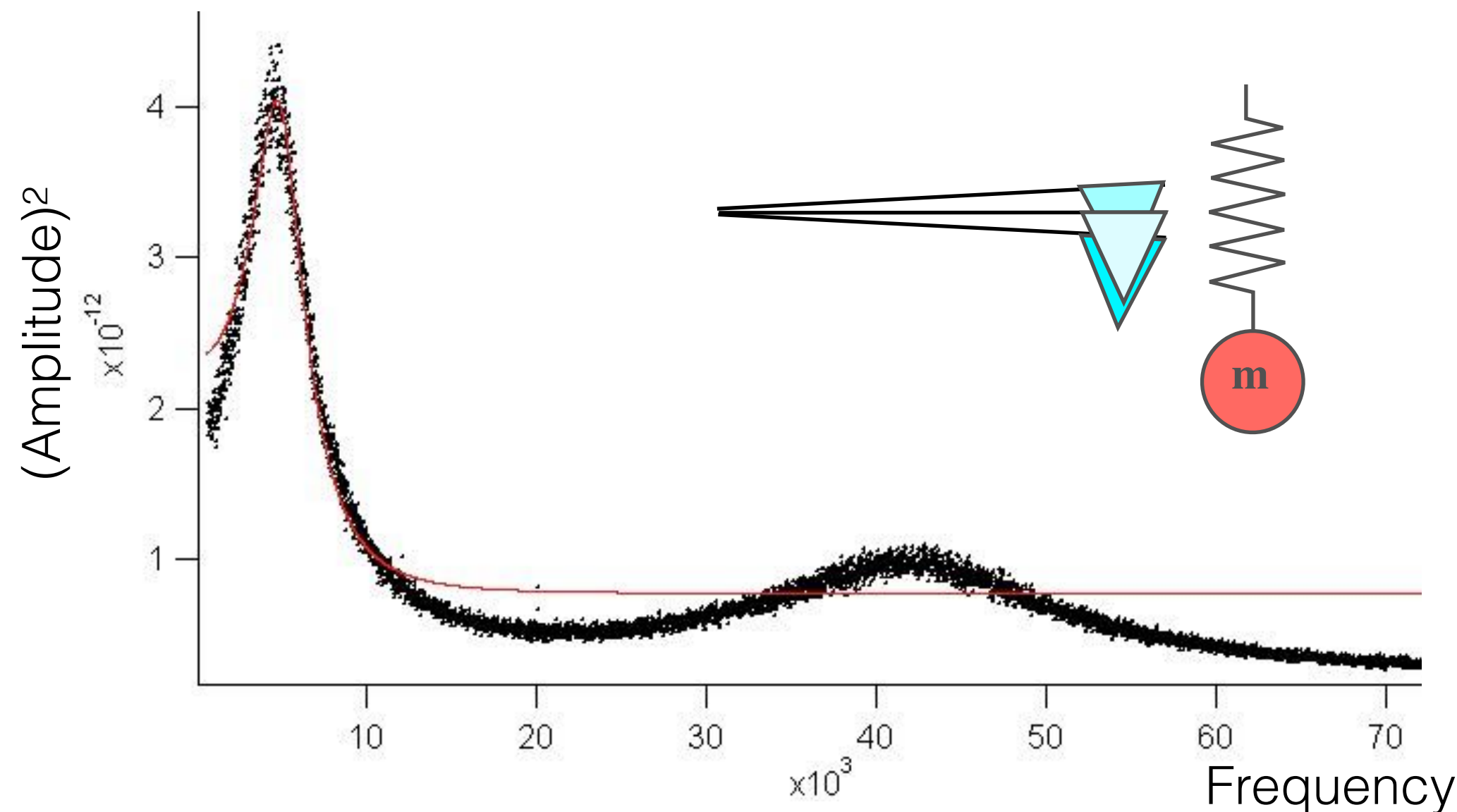
$$P = \frac{h}{\lambda}$$

In the optical trap a momentum change occurs between the photons and the trapped particle:



Calibrating the probe

- Direct force measurement (photonic momentum change)
- Application of known forces (added weight, Stokes drag)
- Thermal method (equipartition theorem)



$$\kappa = m\omega^2$$
$$\frac{1}{2}\kappa \langle x^2 \rangle = \frac{1}{2}k_B T$$

κ = stiffness
 m = mass
 ω = resonance frequency
 x = deflection

AFM cantilever stiffness ~ 10 -1000 pN/nm

Optical trap stiffness ~ 0.1 -1.0 pN/nm

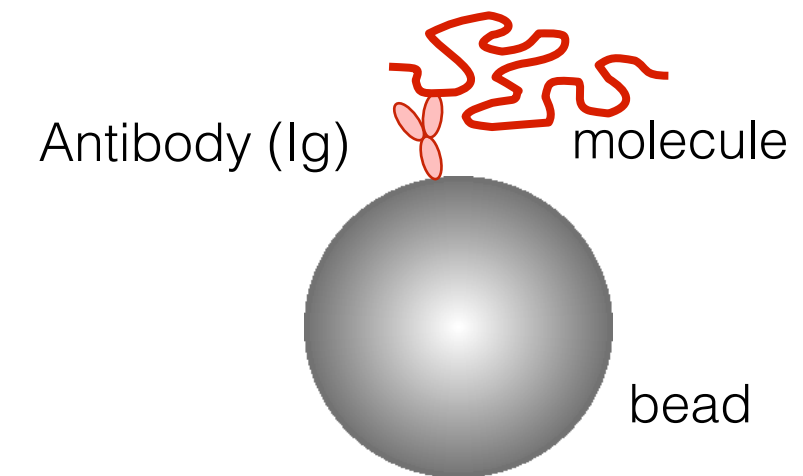
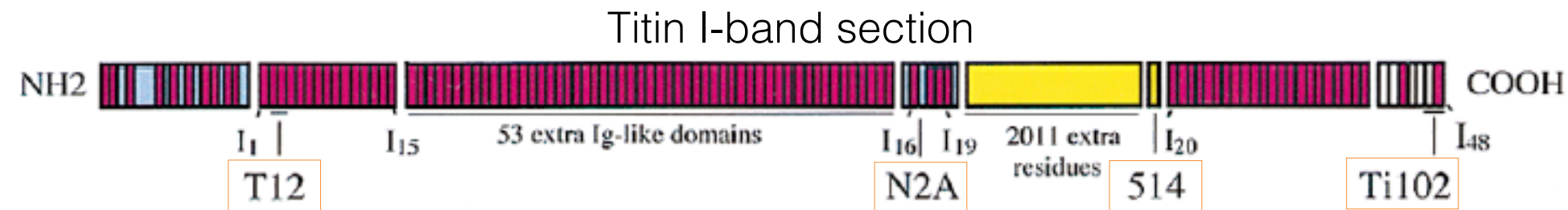
Molecule - handle geometry

microscopic bead $\sim 1\ \mu\text{m}$

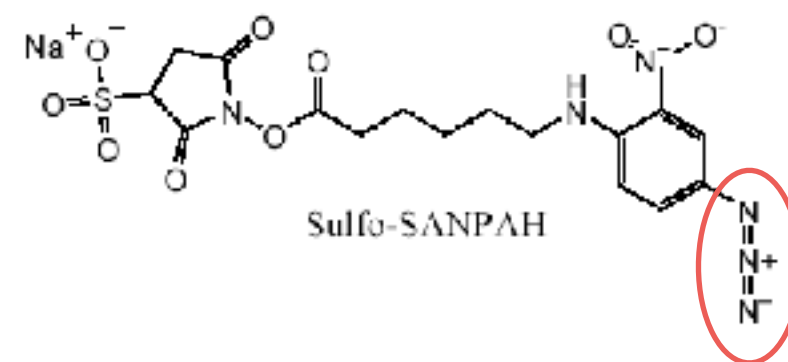
 molecule $\sim 10\ \text{nm}$

Attaching the molecule to the handle

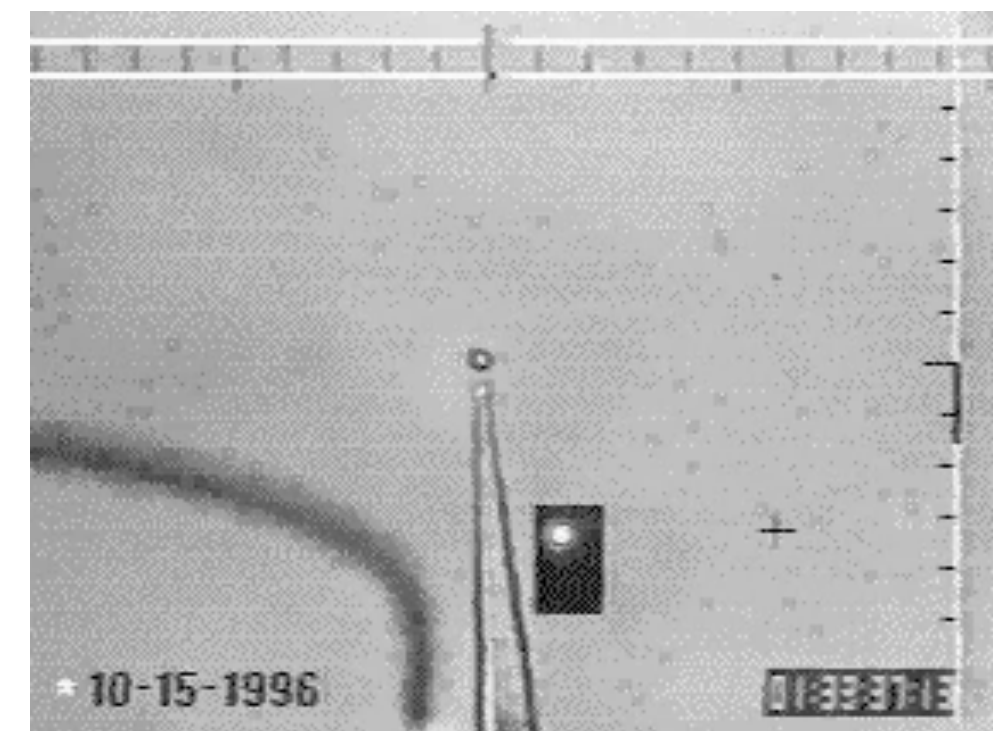
Sequence-specific antibodies



Photoreactive cross-linker - “molecular welding”

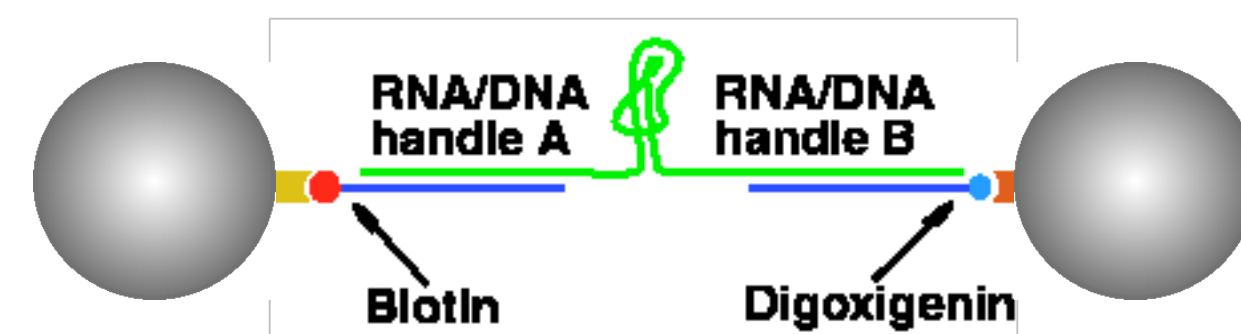


- Non-specific
- Photoreactive N₃- (azido) group (reacts with amines)
- UV illumination

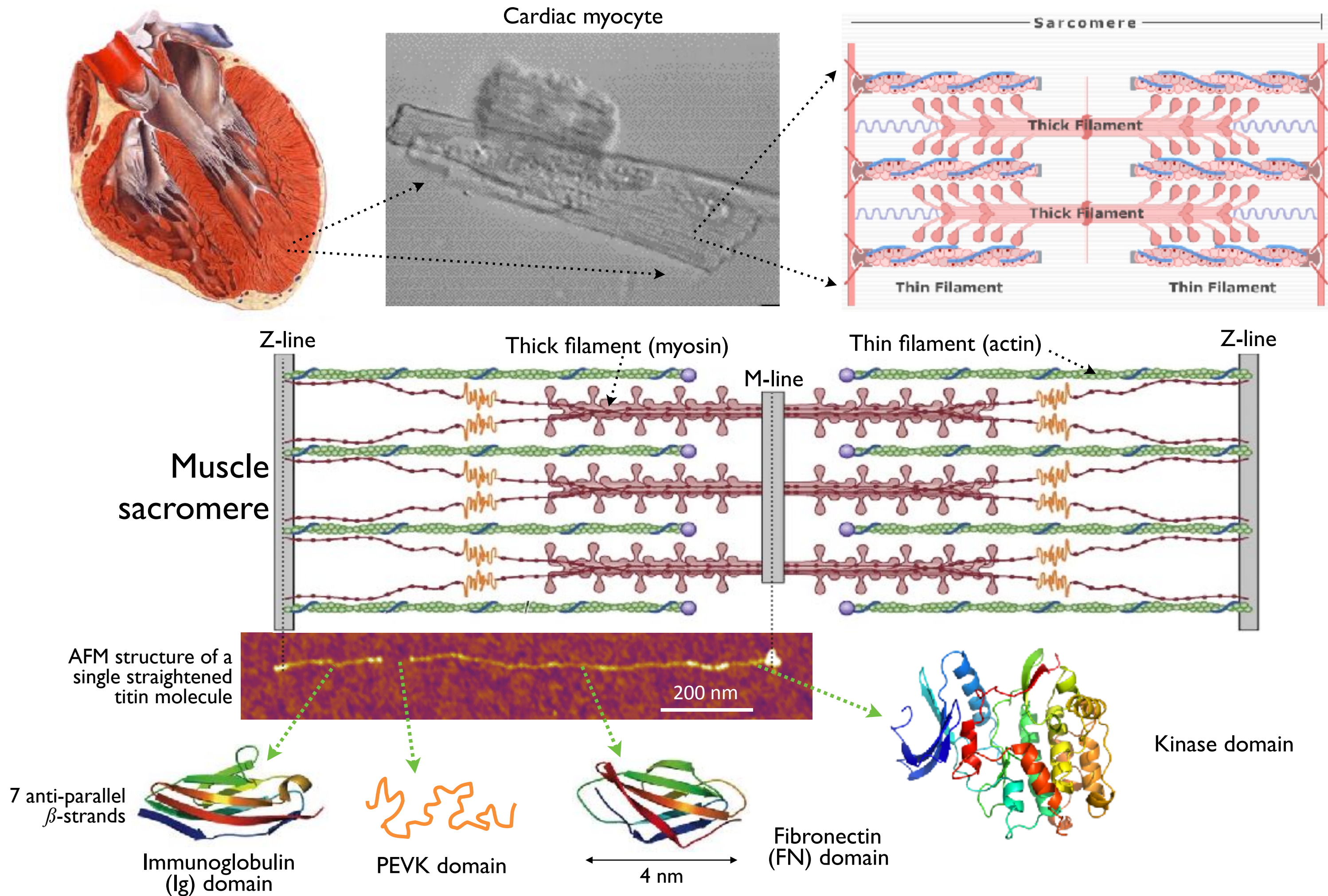


DNA handle

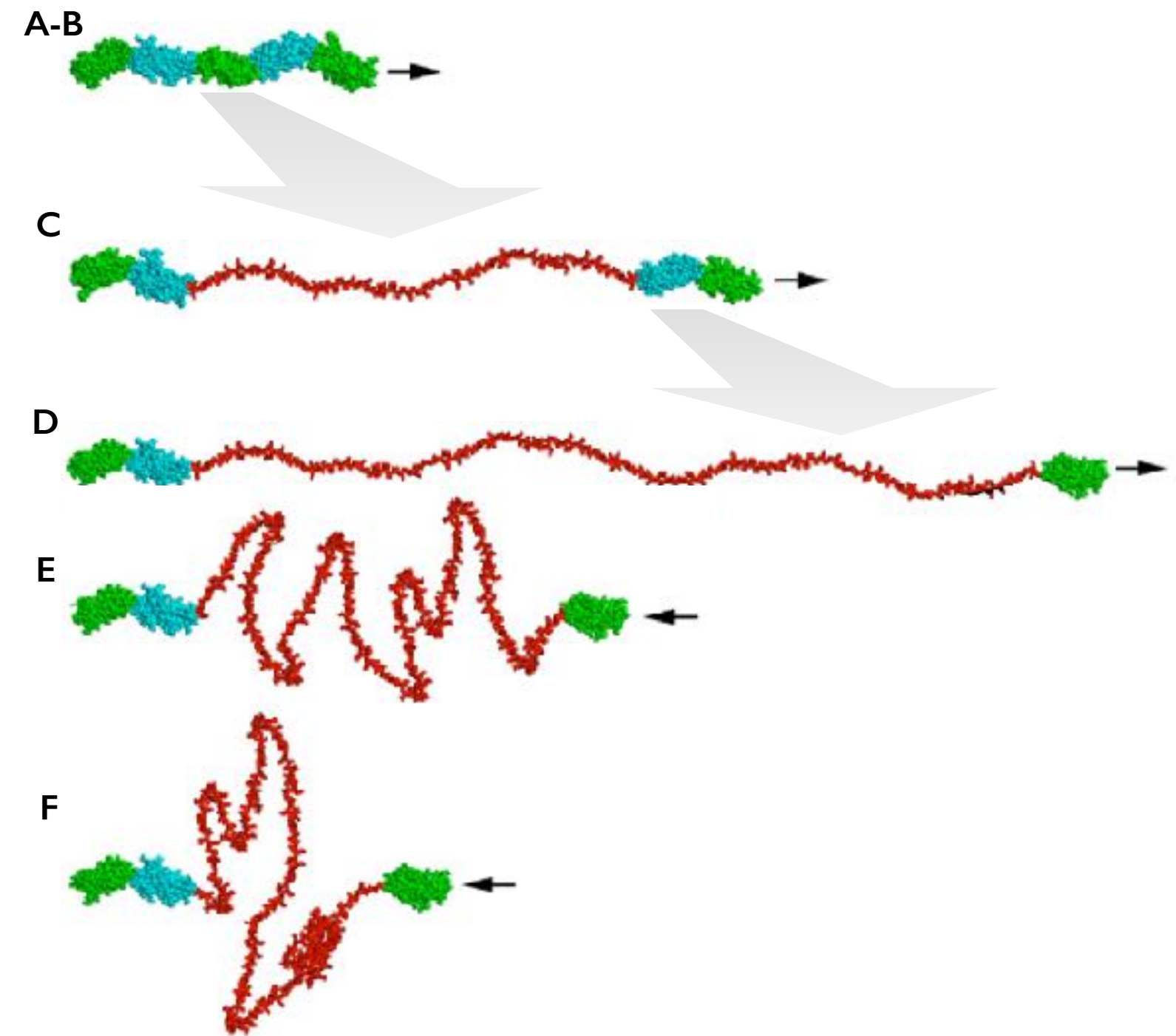
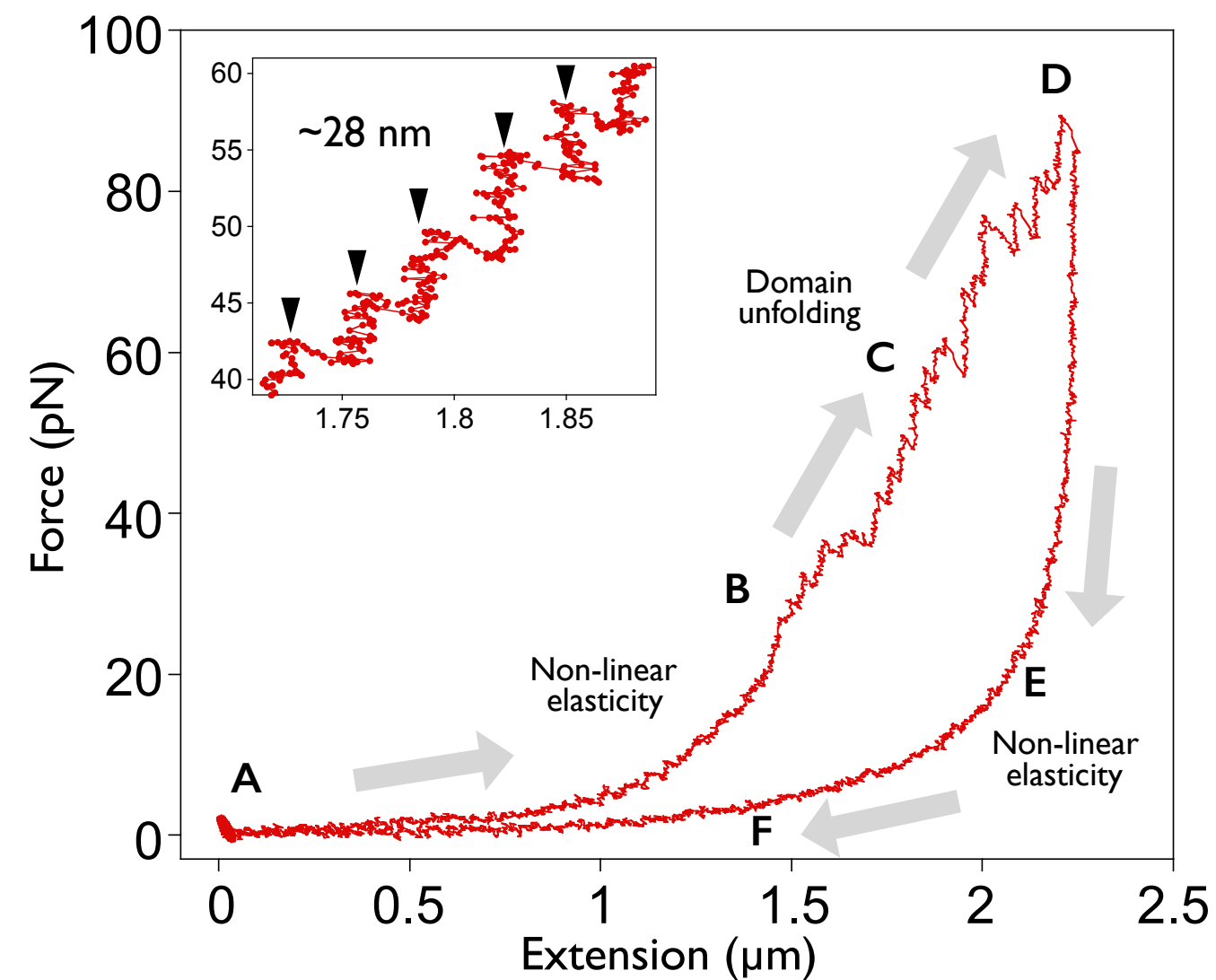
- Molecular dimension
- Can be made specific with cloning techniques
- Provides mechanical fingerprint



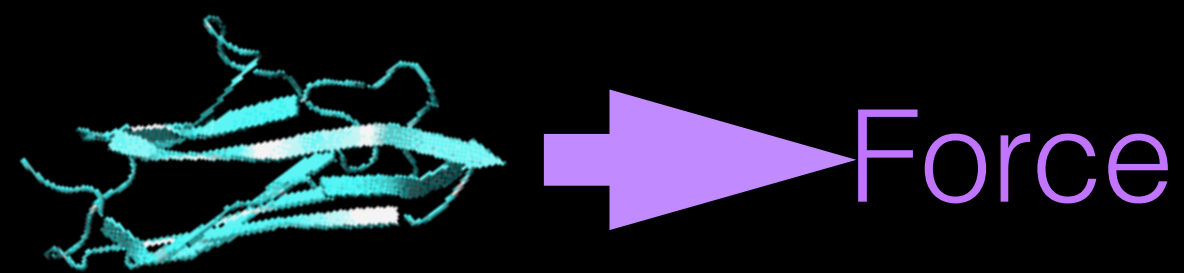
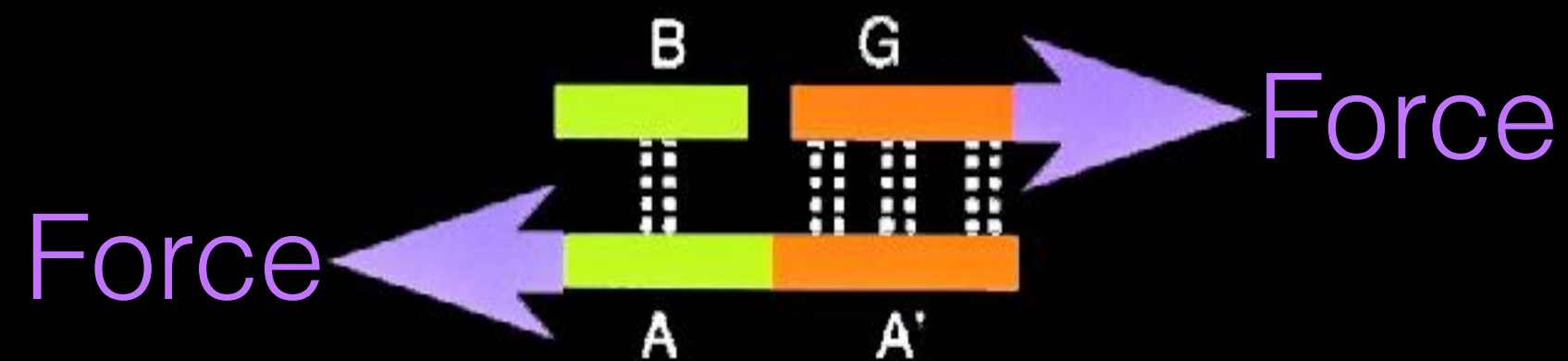
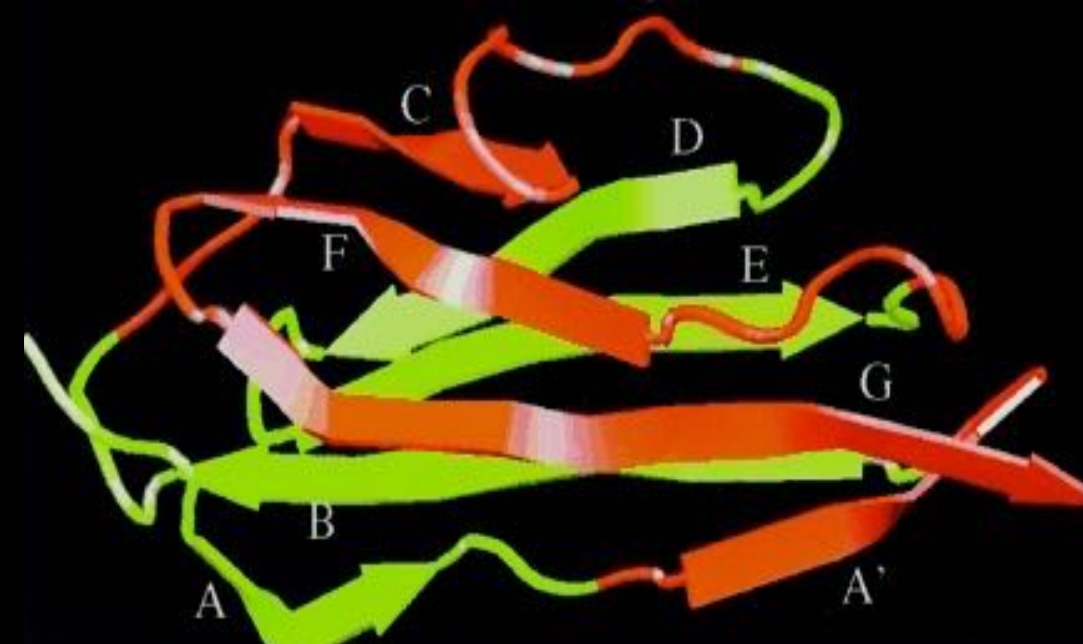
1. Titin: giant elastic muscle protein



Titin mechanics - constant-velocity experiment: non-linear elasticity + domain unfolding

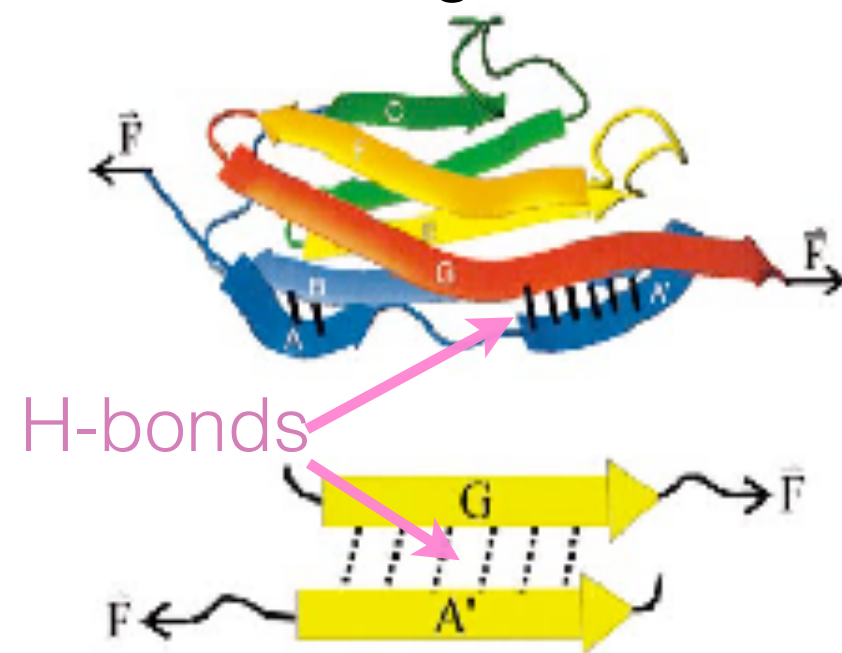


Structural basis of mechanical stability

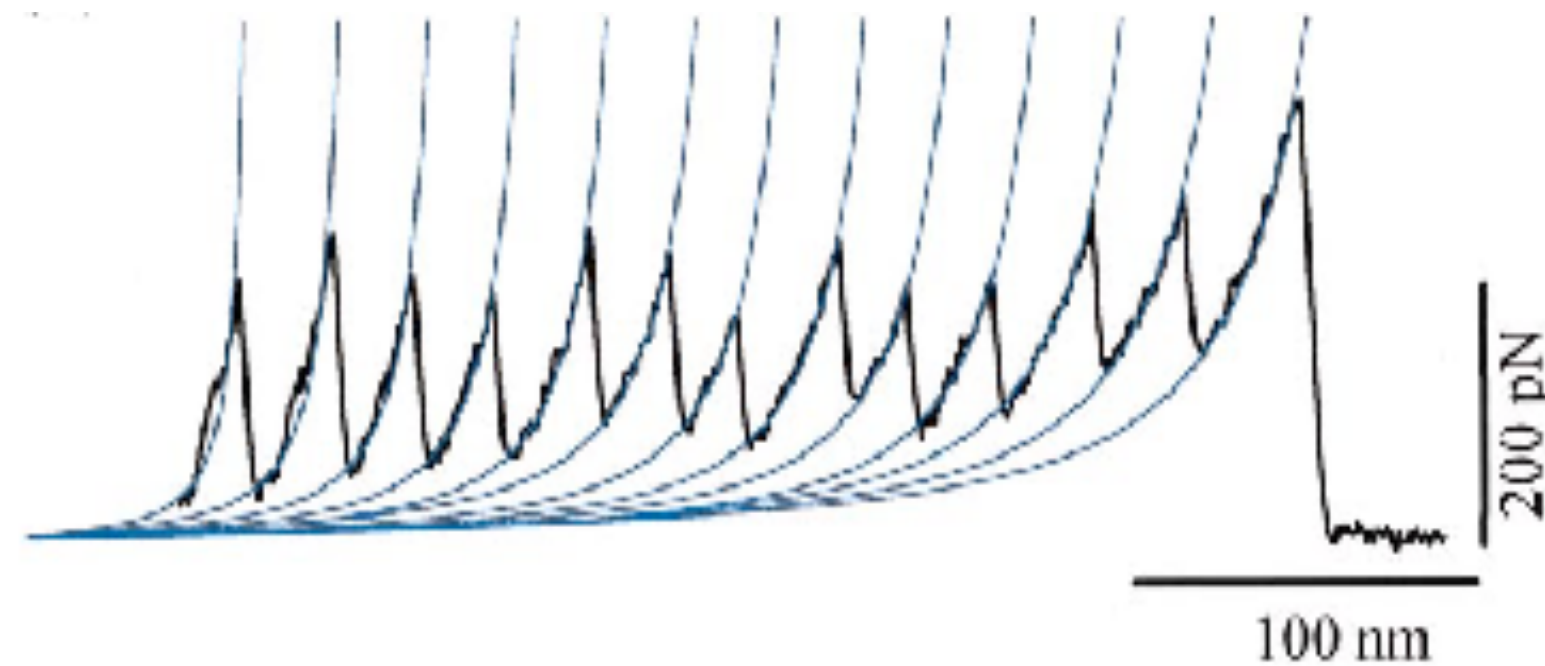


Biological logic of mechanical stability

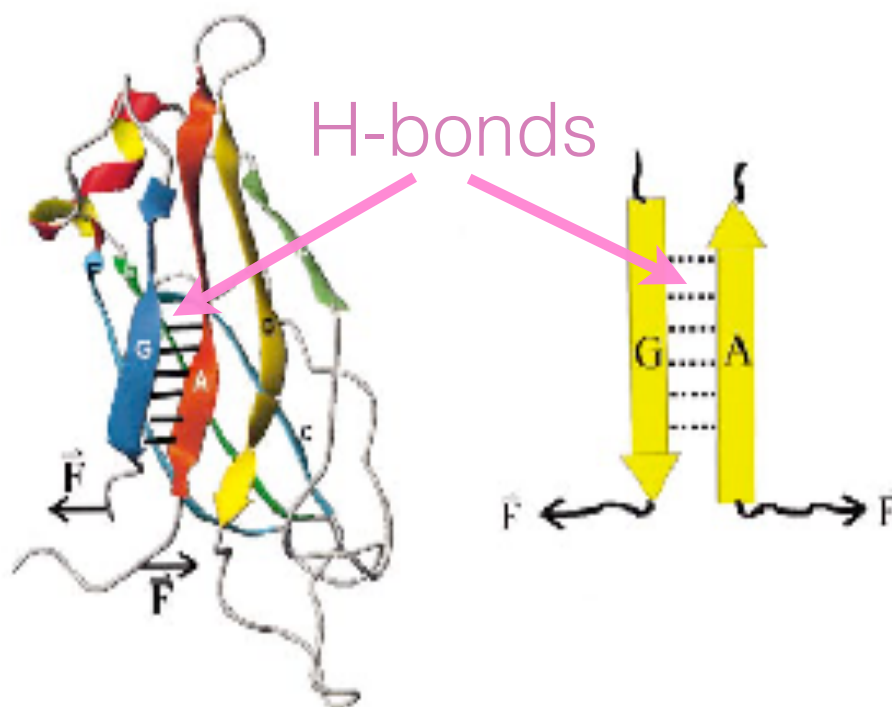
Parallel coupling of structure-stabilizing H-bonds



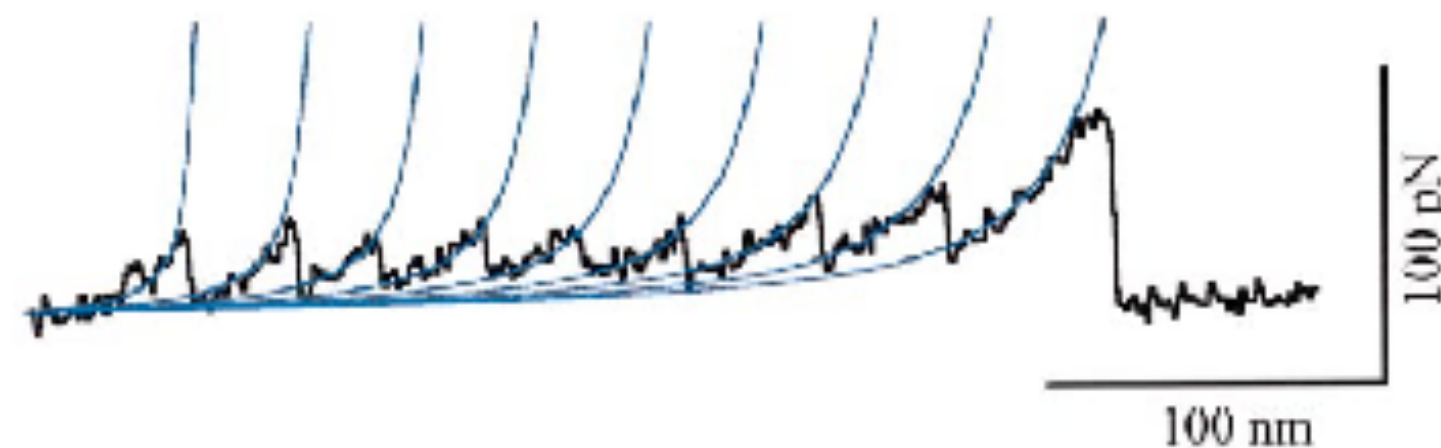
High unfolding forces



Serial coupling of structure-stabilizing H-bonds



Low unfolding forces

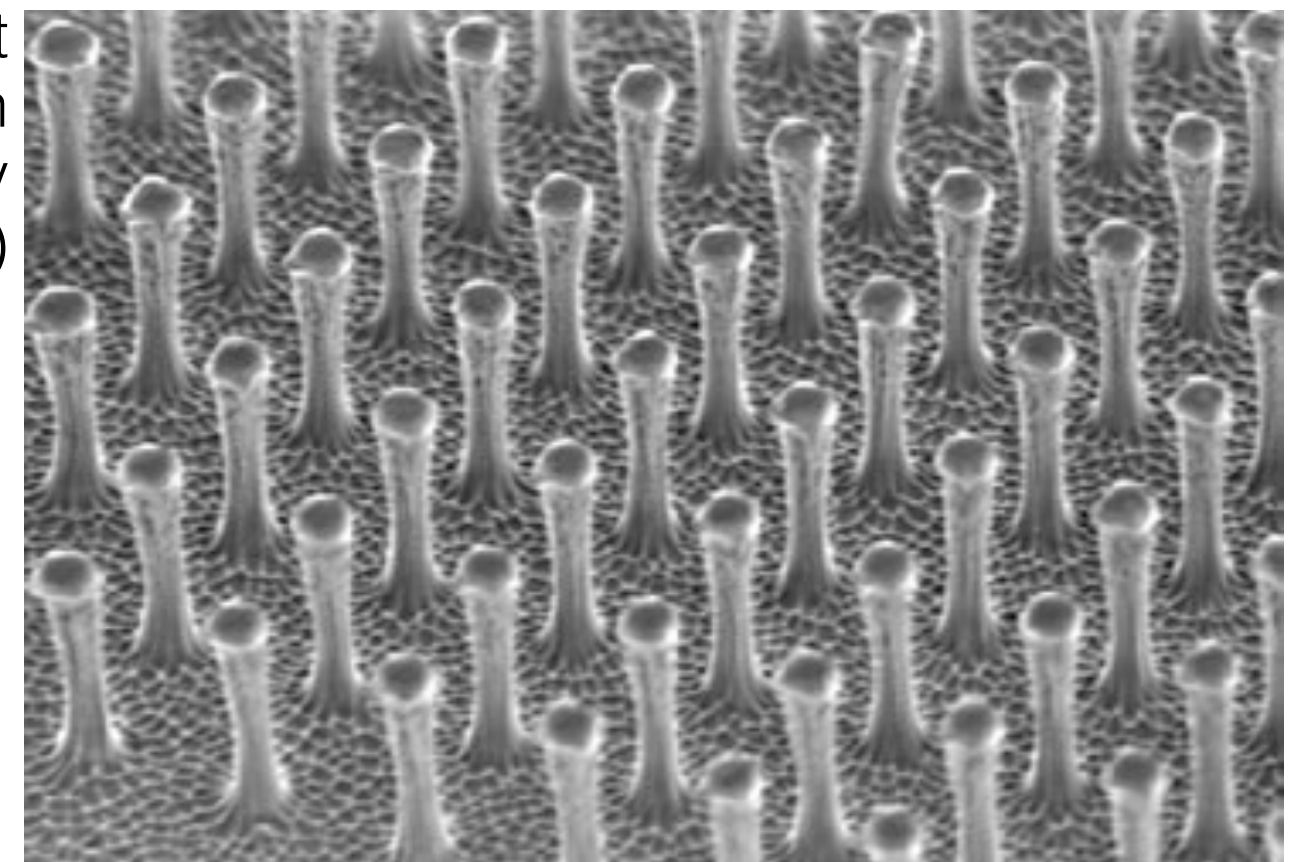


Macroscopic mechanical stability

Highly efficient glue based on the principle of parallel coupling



Artificial gecko foot
(prepared with
nanotechnology
methods)

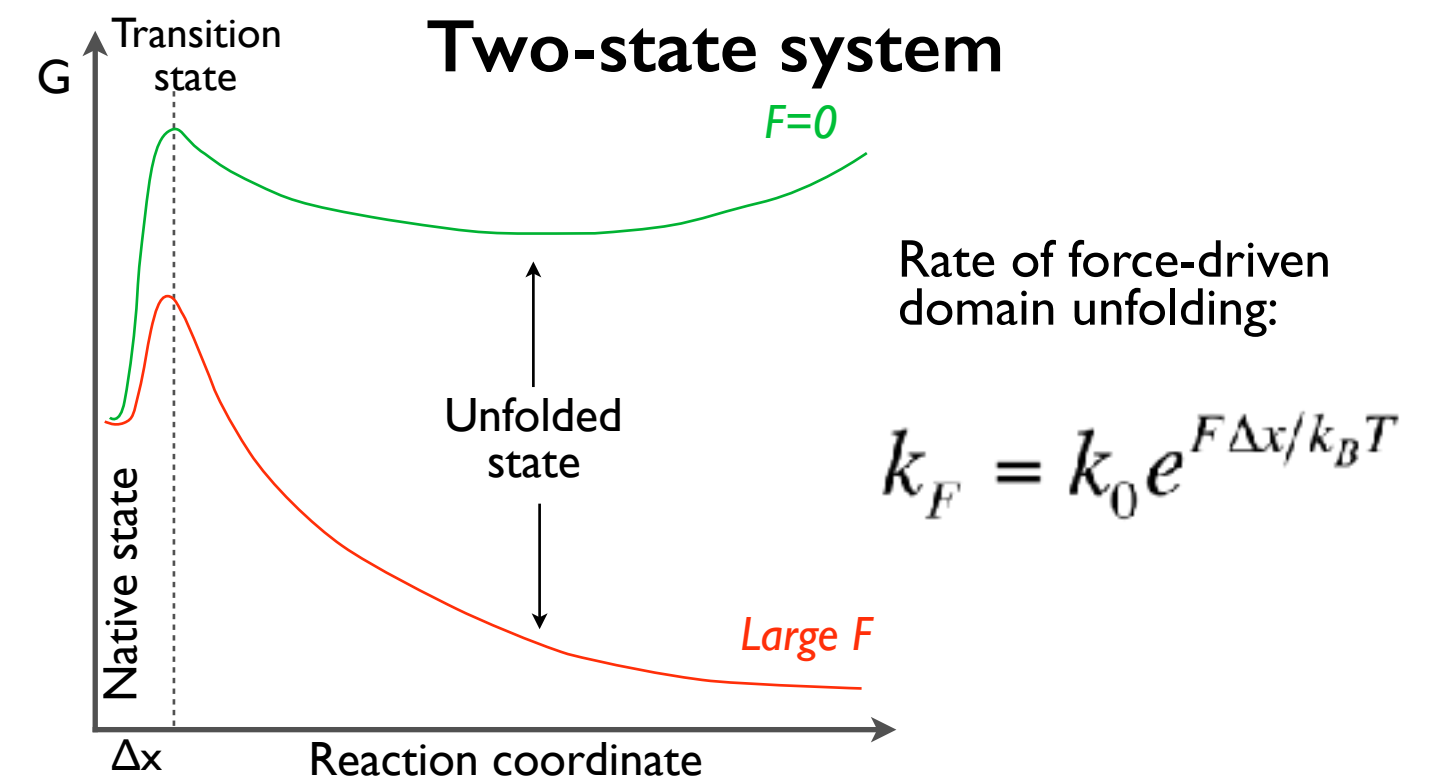
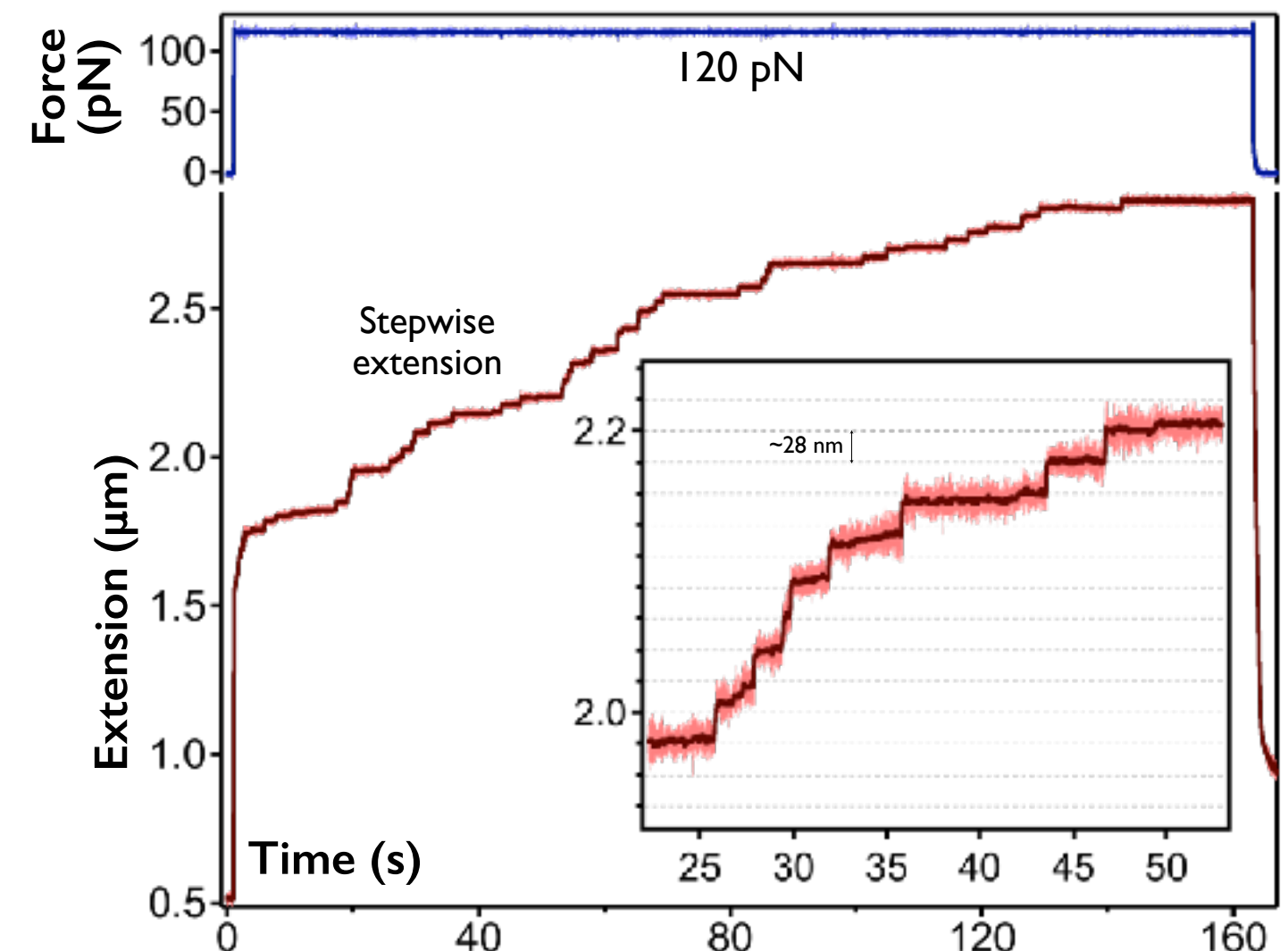
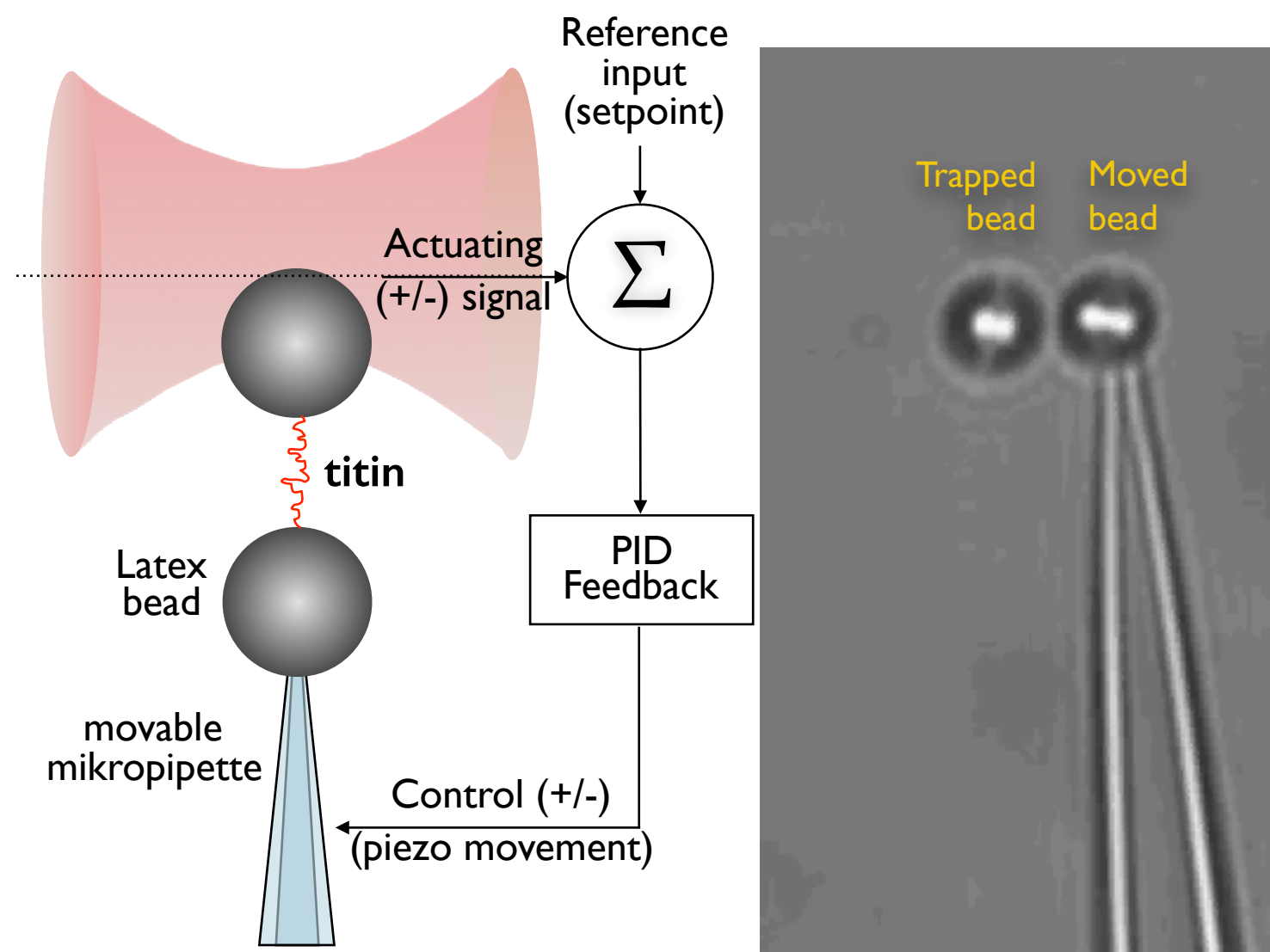


Surface
attachment of the
gecko foot:
Numerous Van der
Waals interactions
- between bristles
and surface -
coupled in parallel

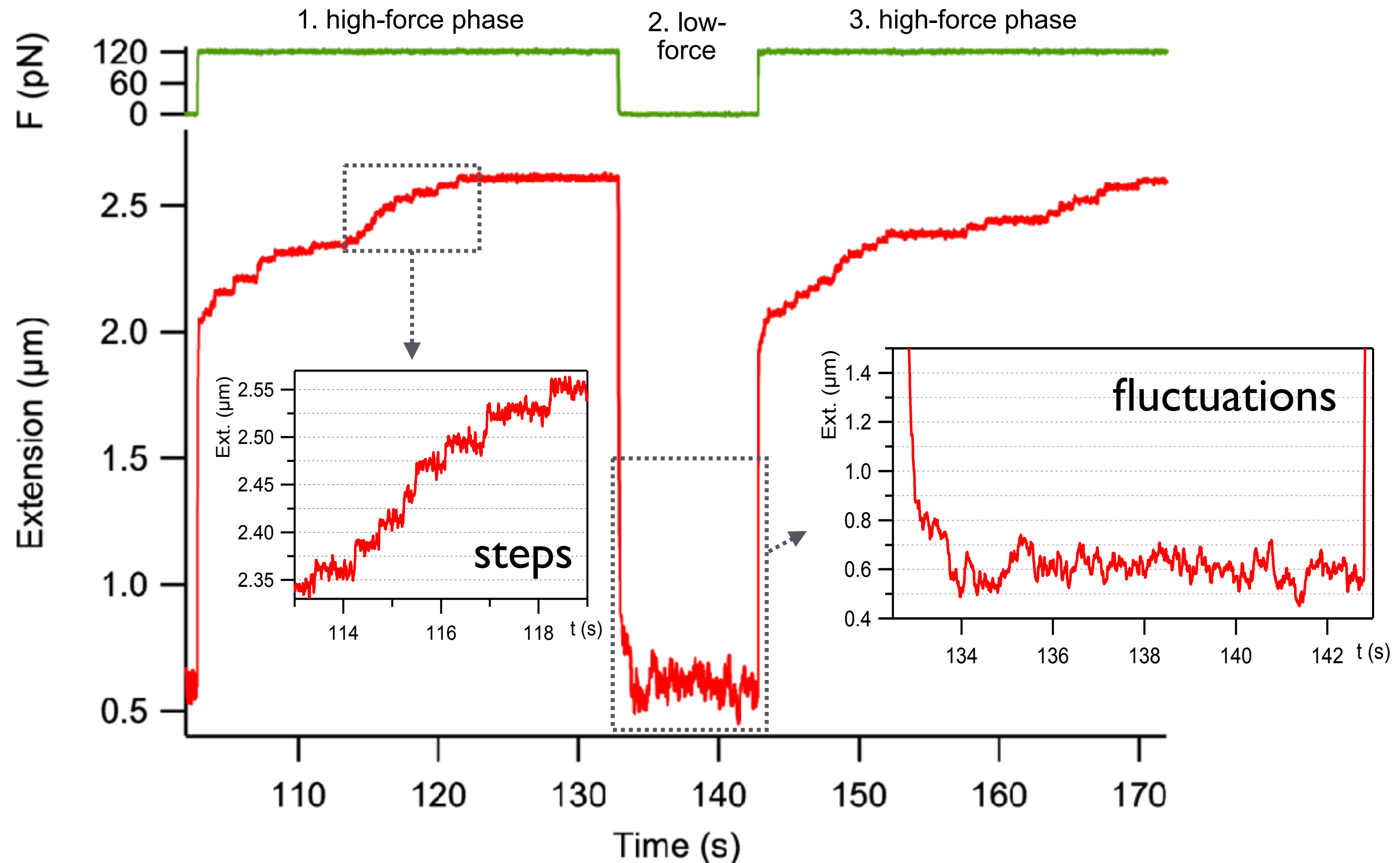


Titin mechanics - constant-force experiment: extension *via* stepwise domain unfolding

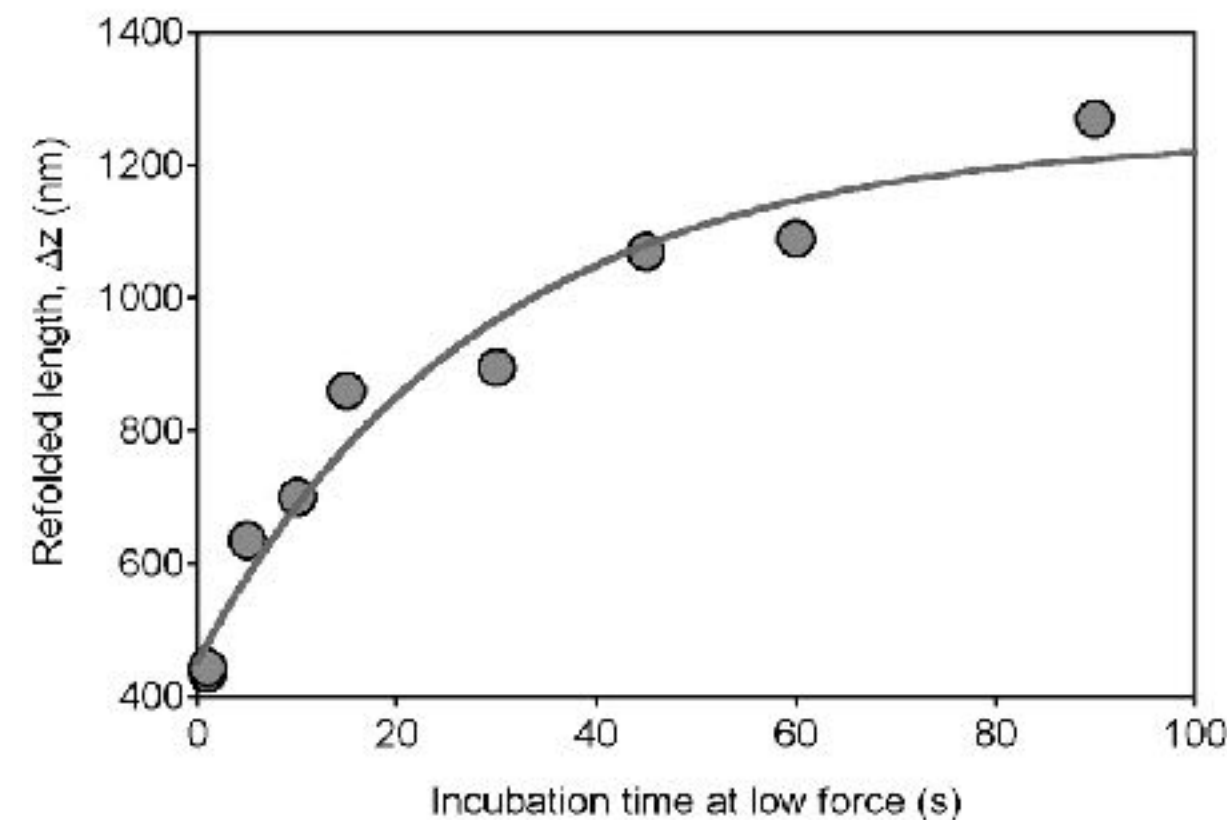
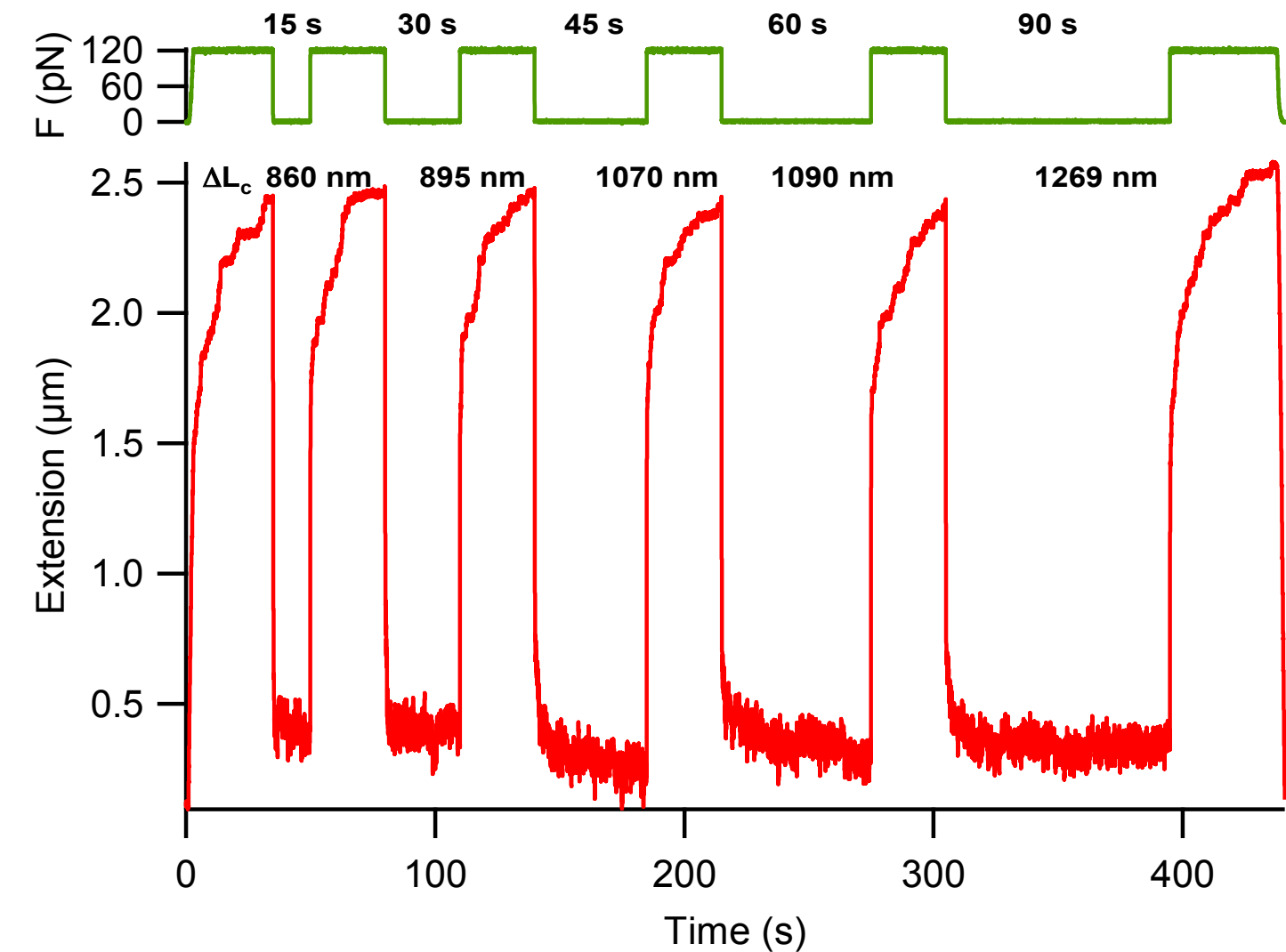
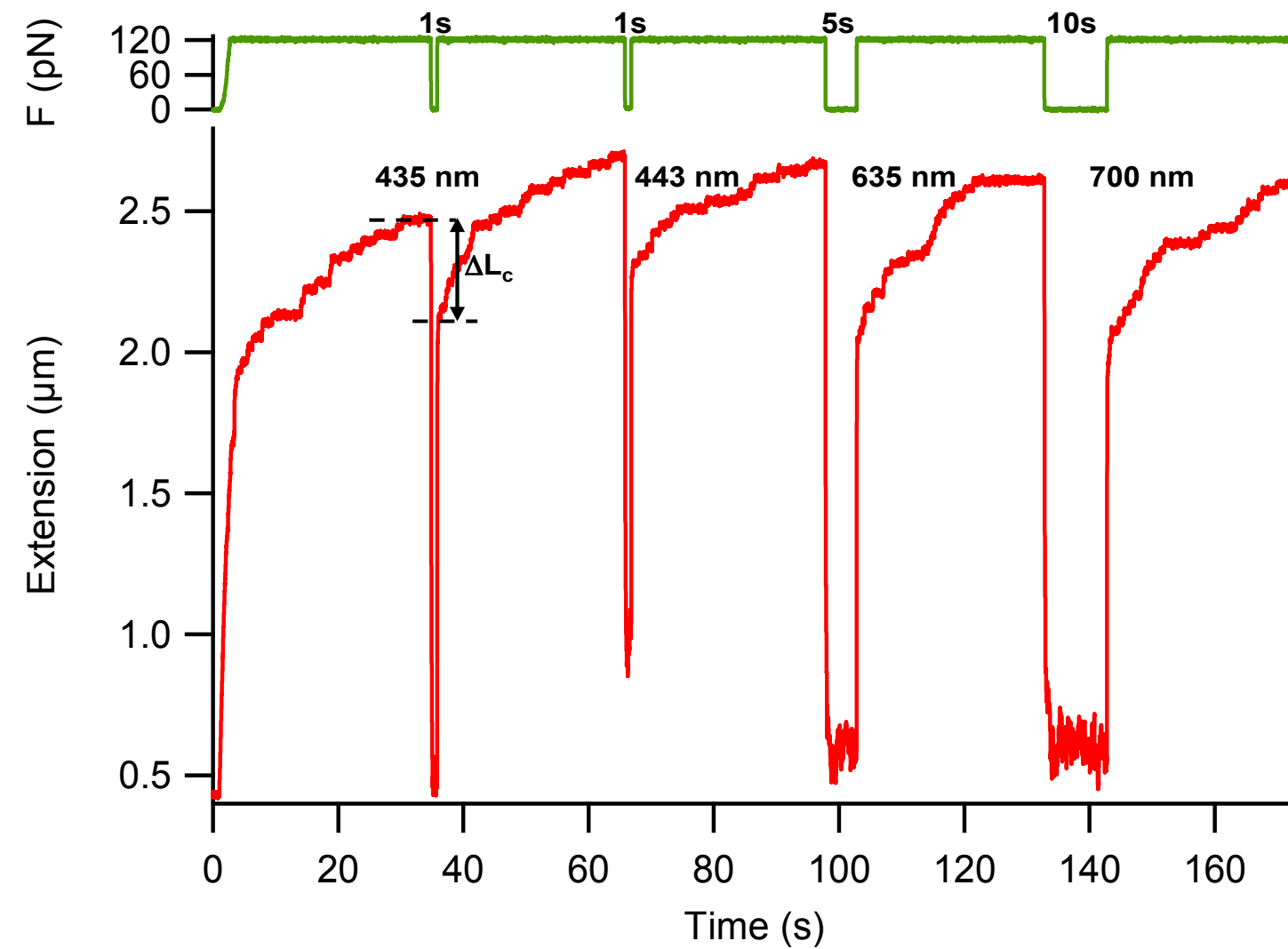
Manipulation with force-clamp optical tweezers



No steps, only fluctuations during refolding



Refolding follows first-order kinetics

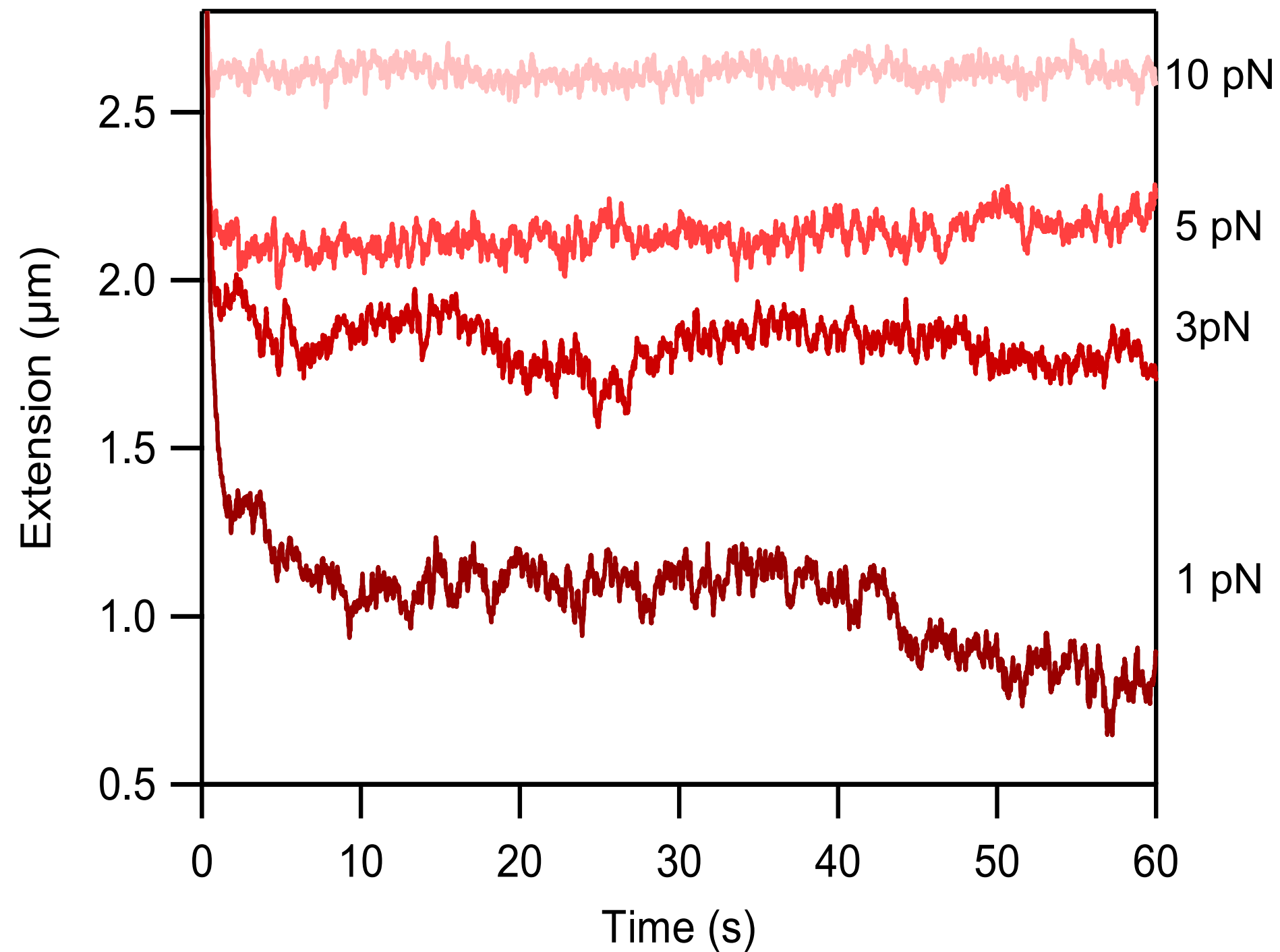


$$\Delta Z = \Delta Z_0 - A e^{-t/\tau}$$

ΔZ_0 : maximal refolded length
 τ : time constant

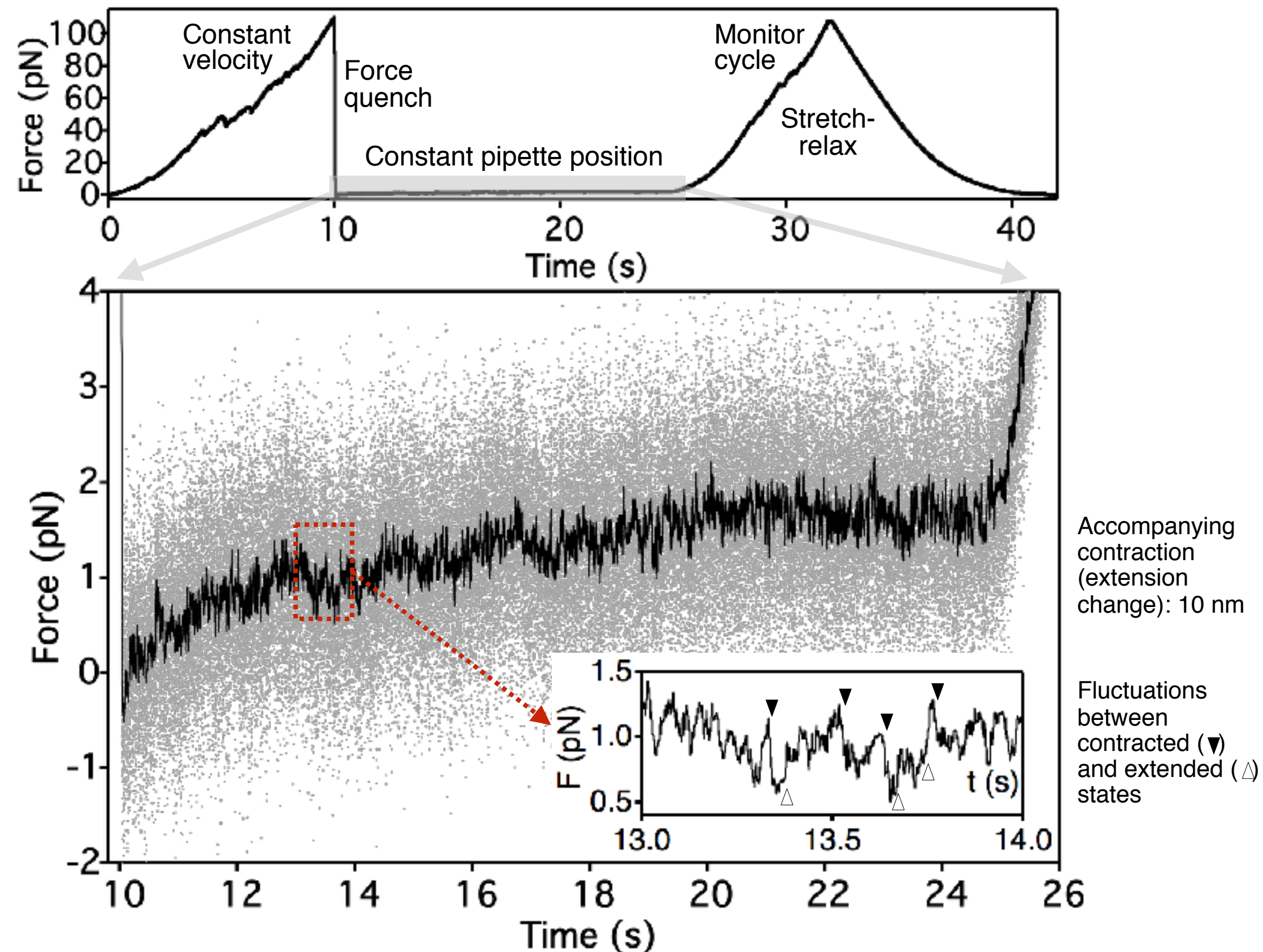
Titin refolds against force

Large length fluctuations occur during refolding



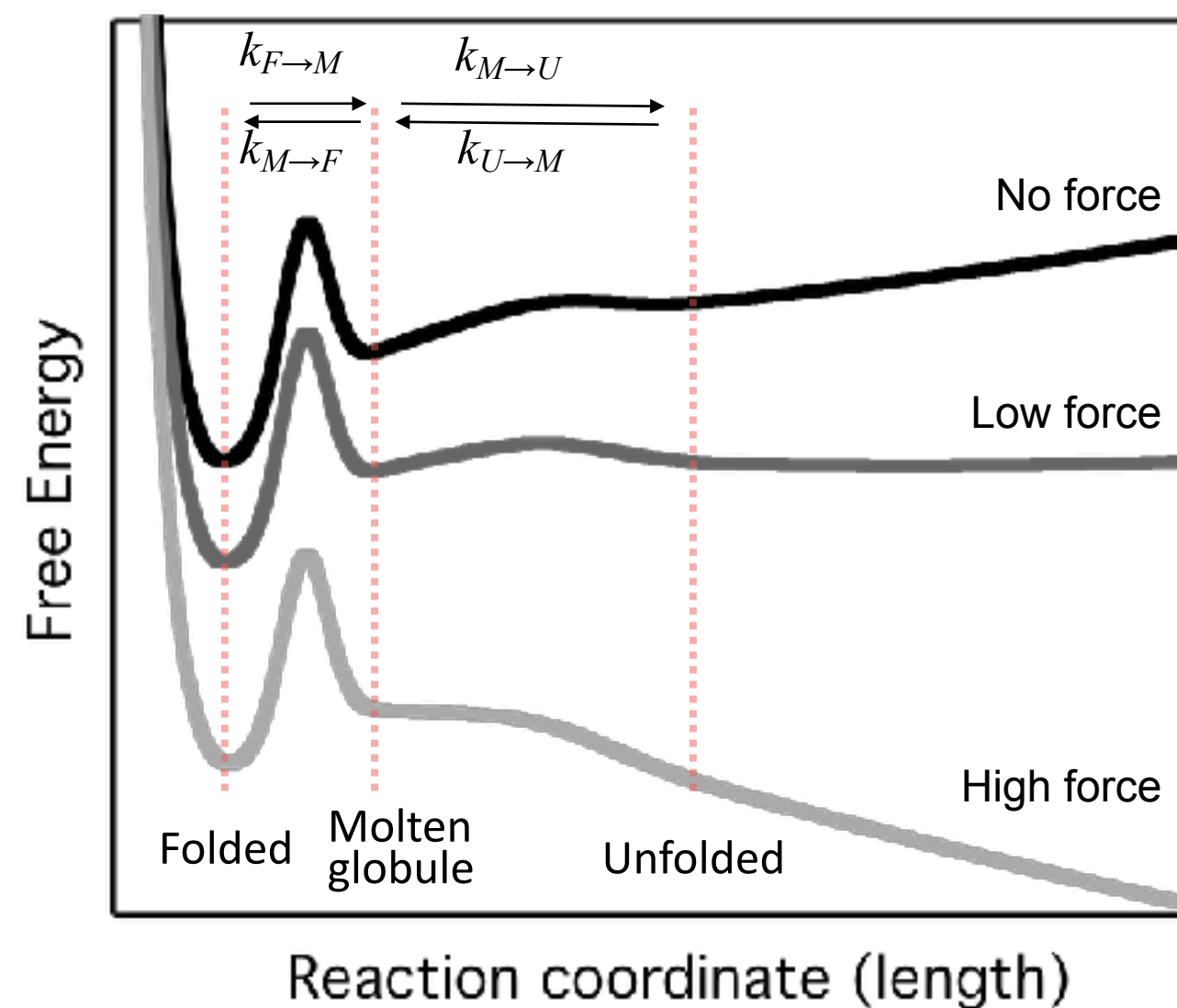
Force is generated during refolding

Position clamp experiment



Fluctuations are explained by molten-globule dynamics

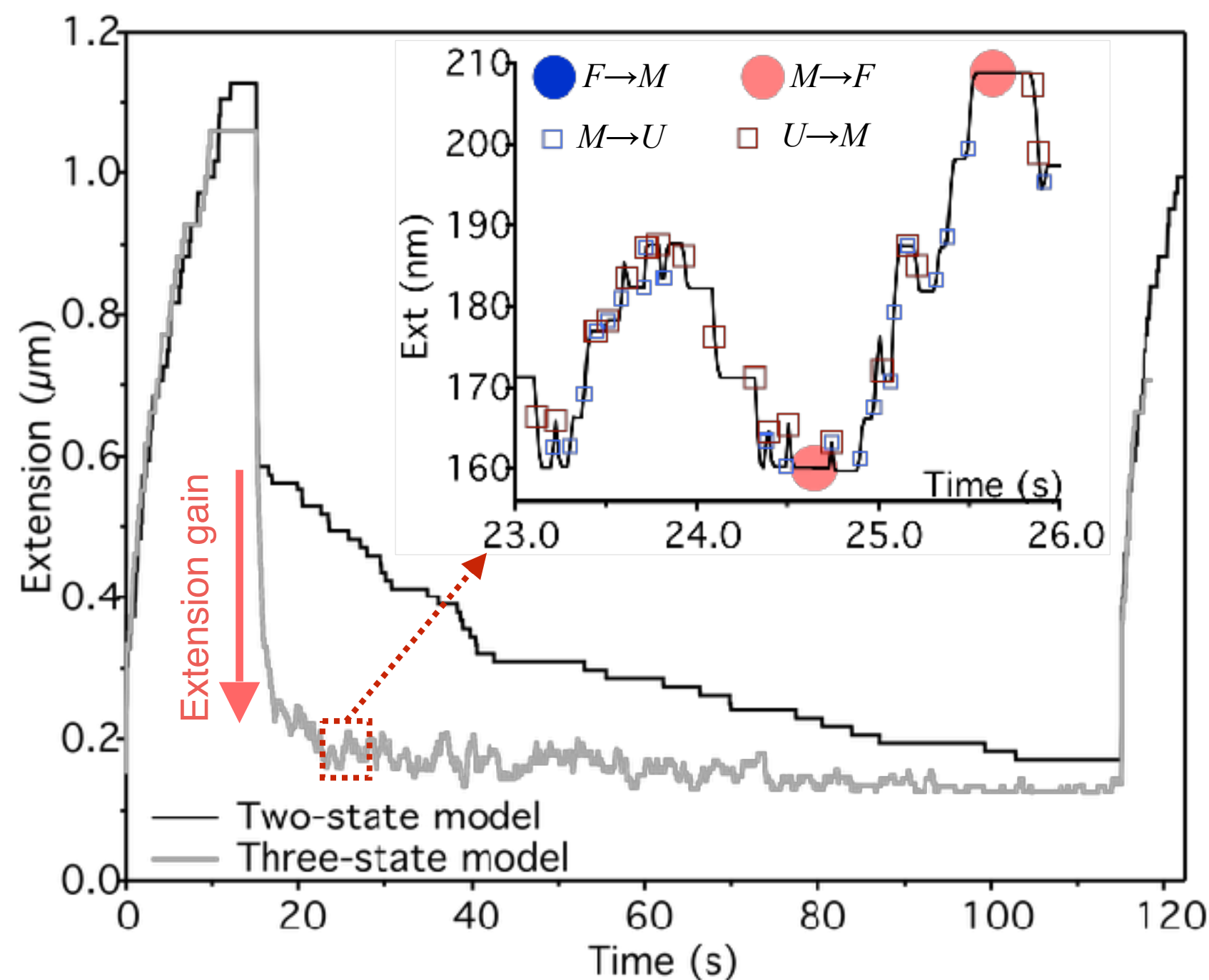
Three-state folding model



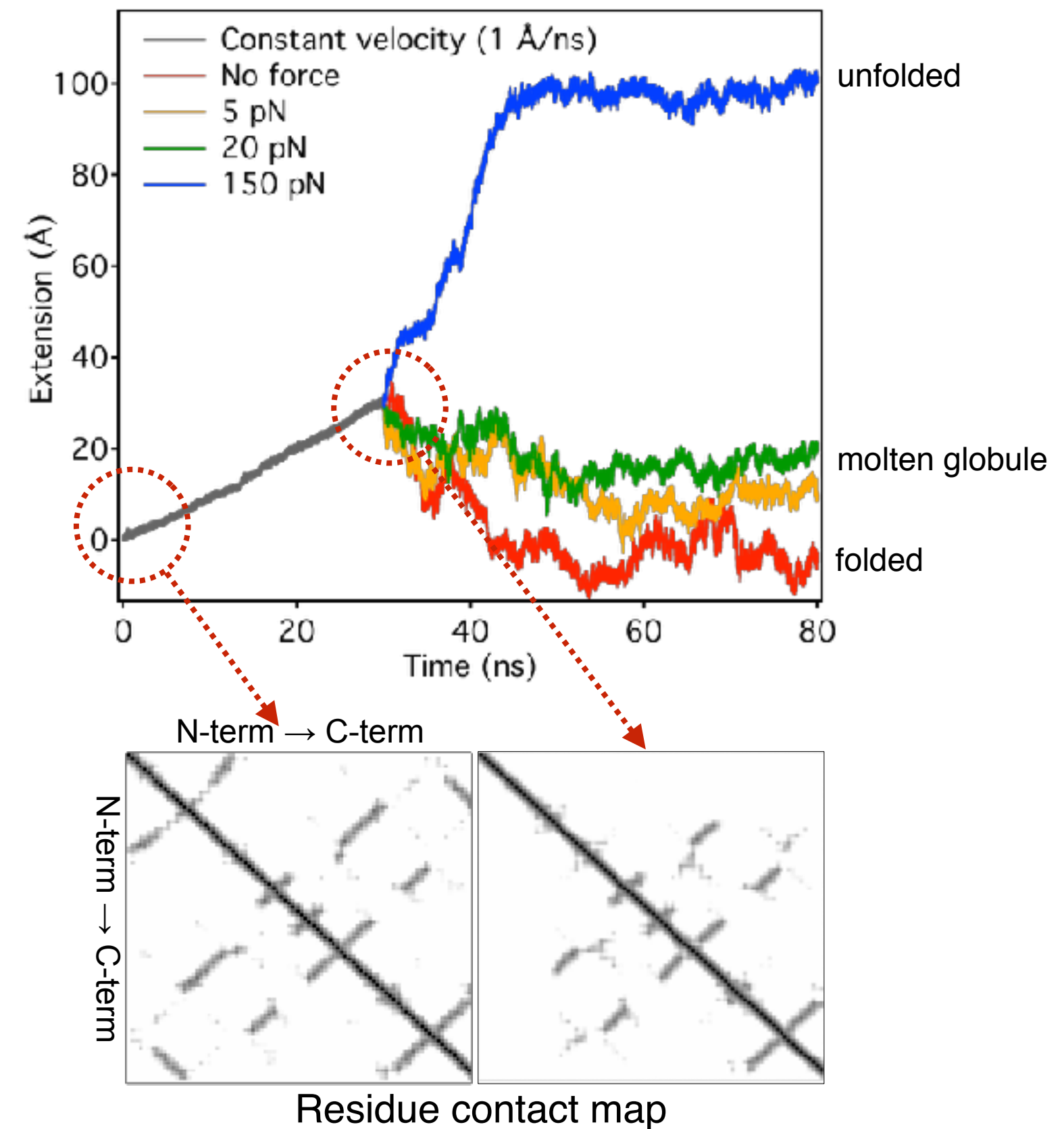
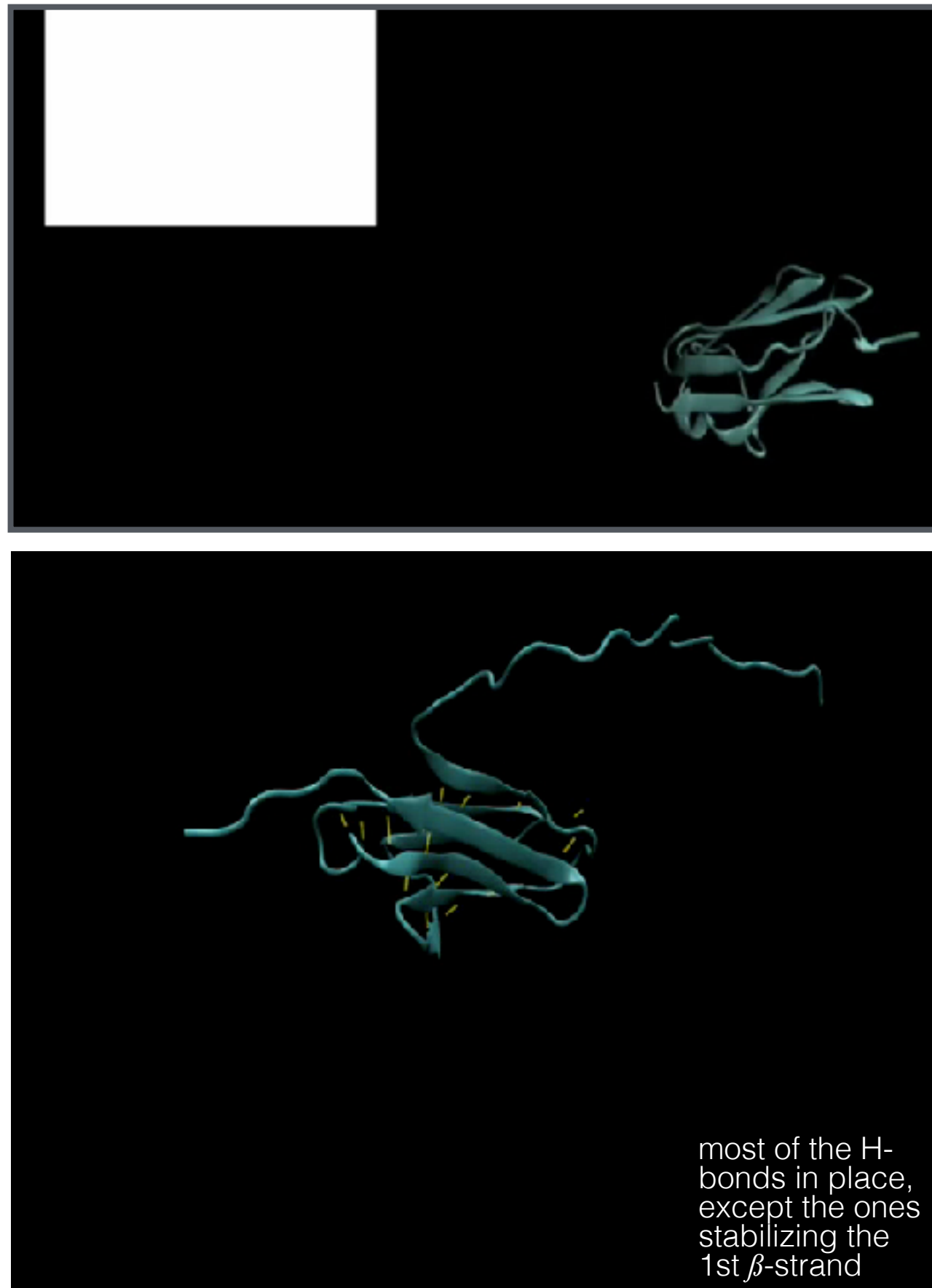
$$k_F = k_0 e^{\pm F \Delta x / k_B T}$$

k_0 : spontaneous unfolding/refolding rate

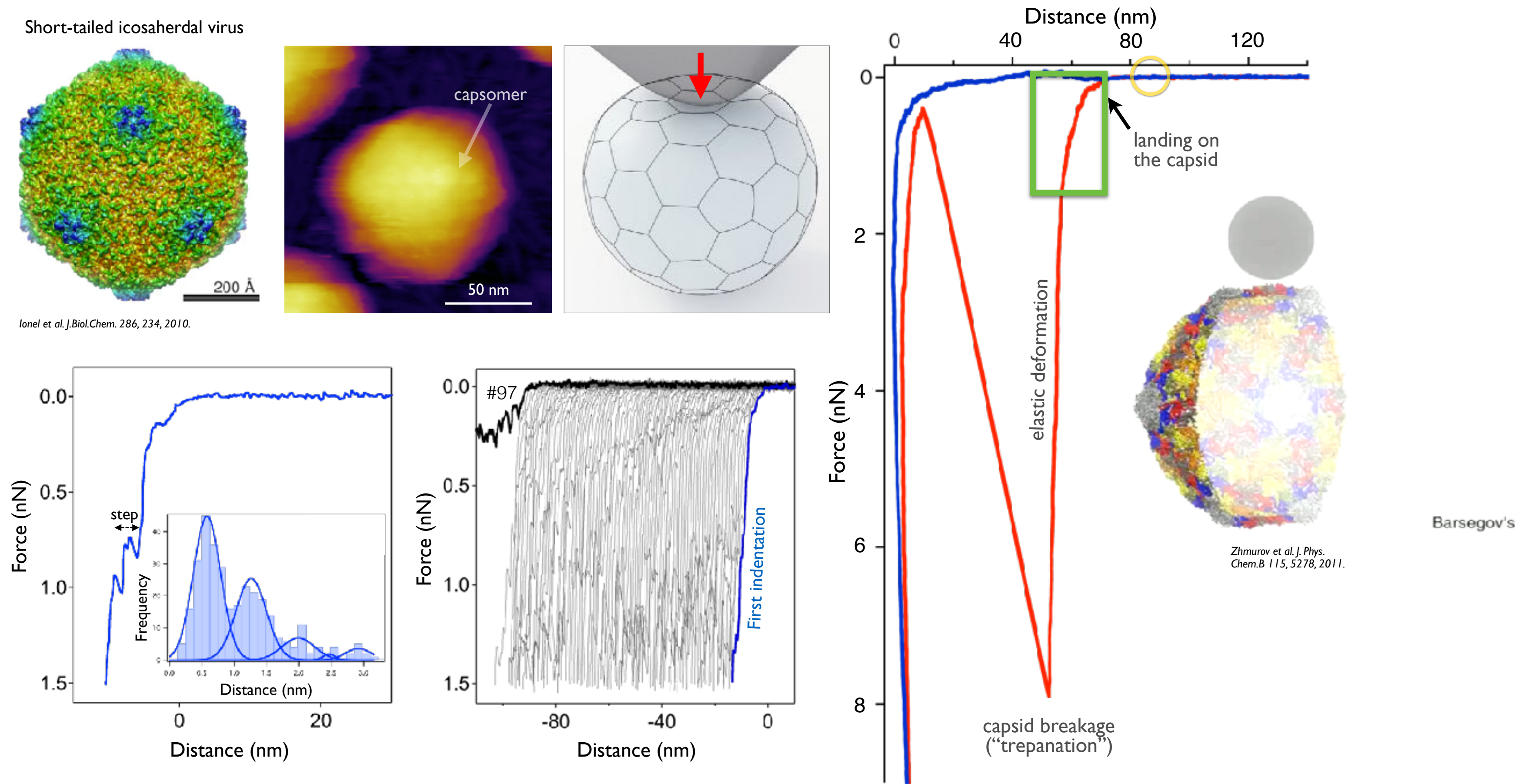
Monte-Carlo simulation



Molten-globule structure explored with sMDS

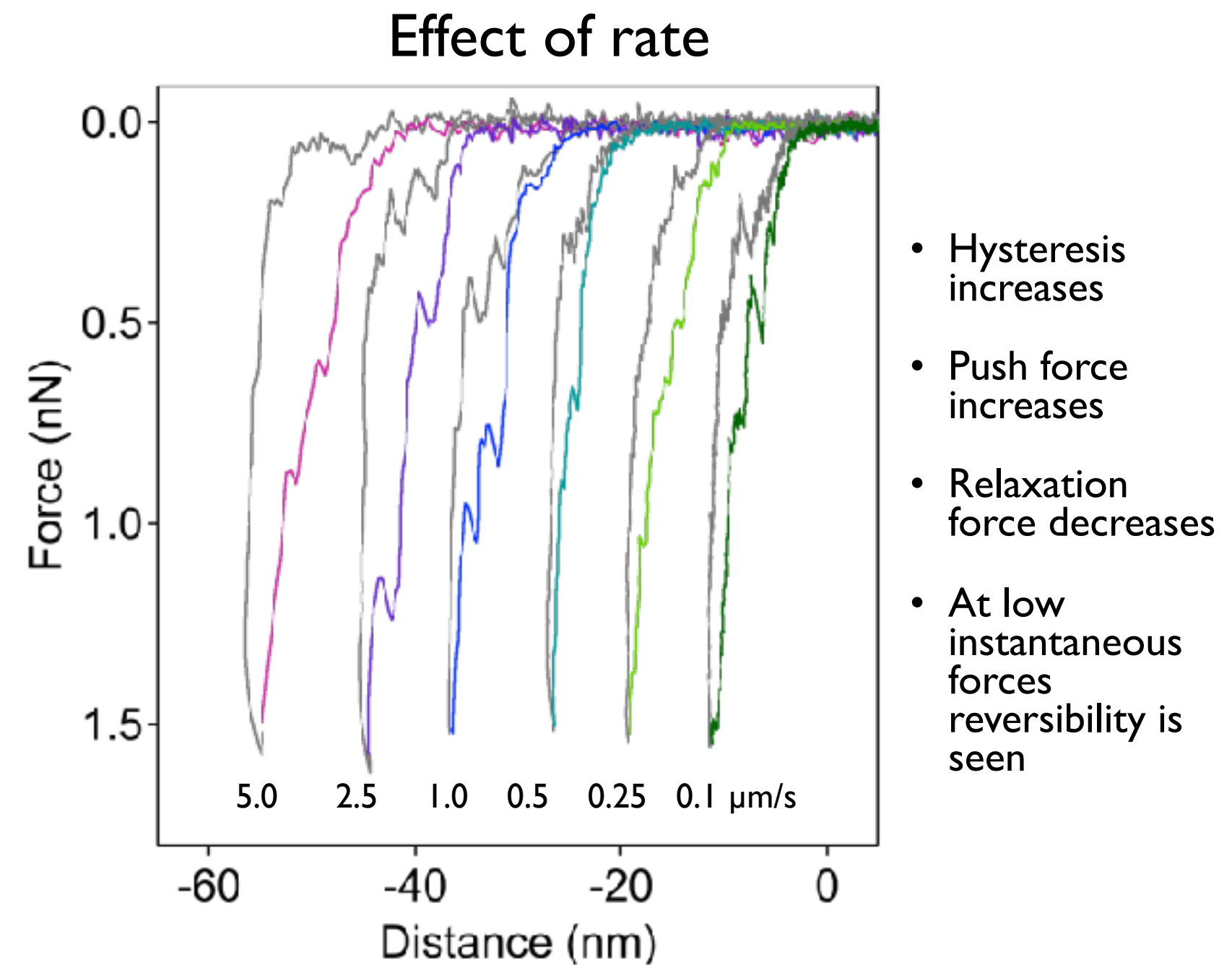
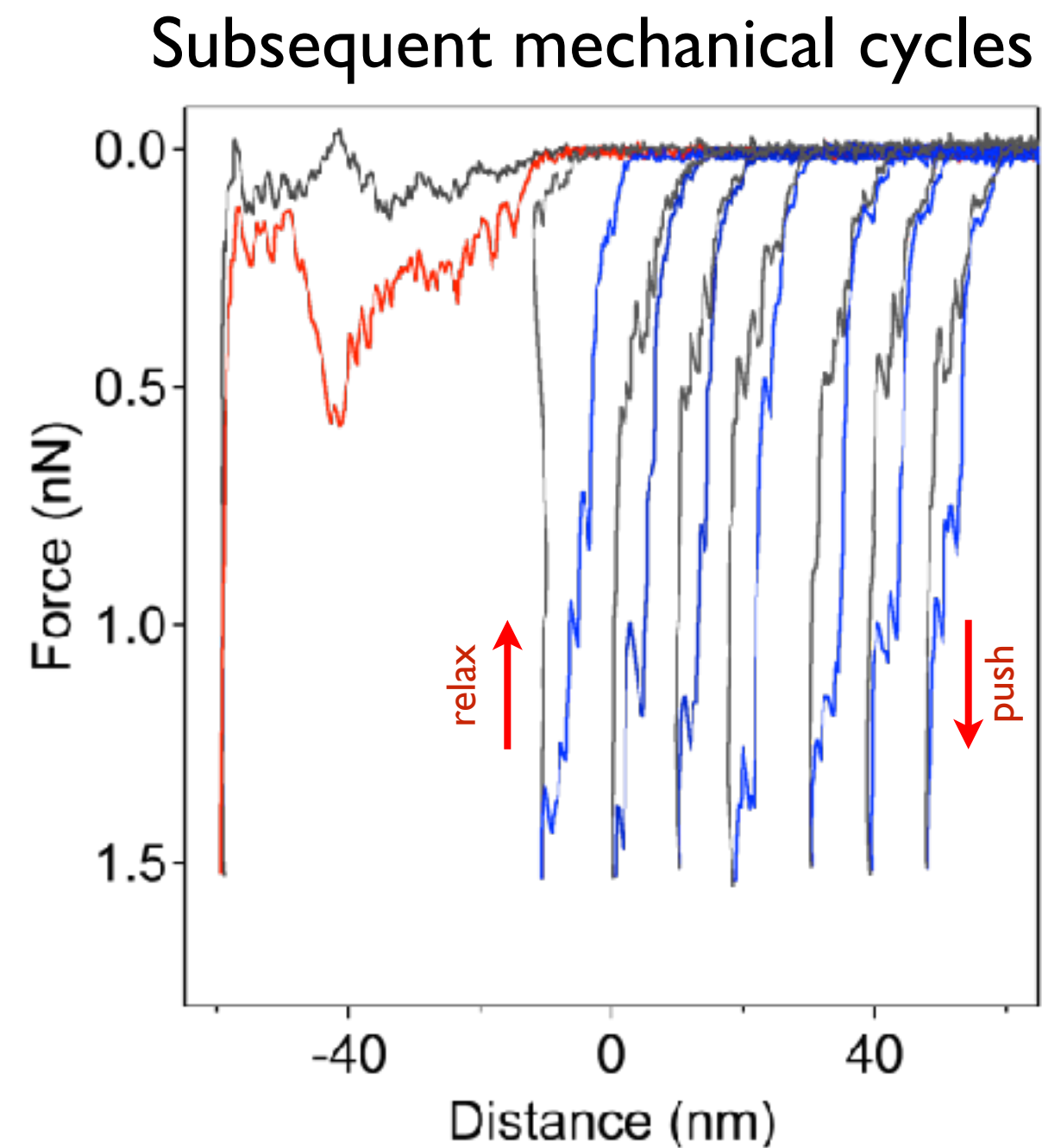


2. T7 phage nanomechanics

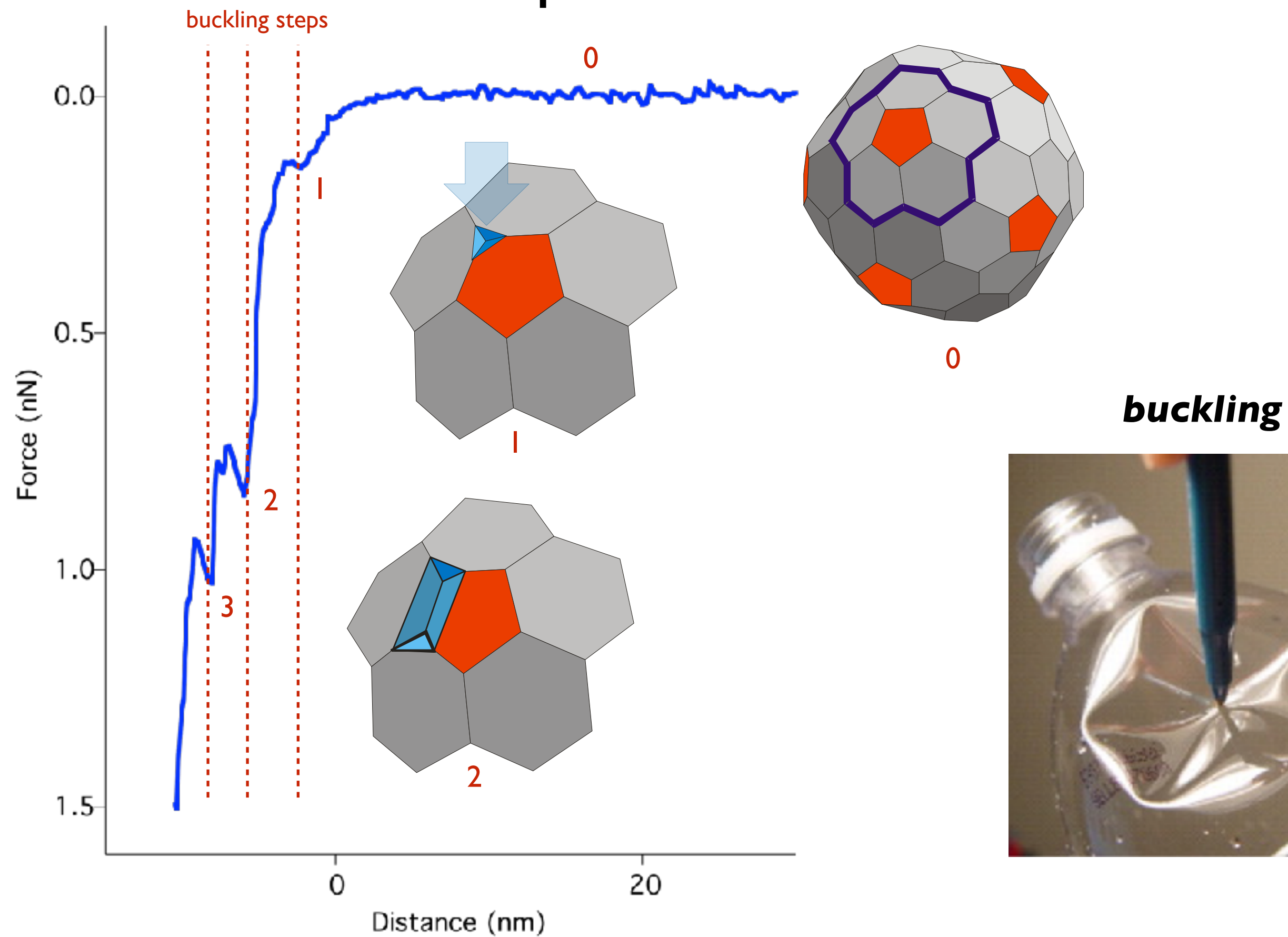


Reversible 6 Å buckling steps, followed by rapid relaxation.

Similar discrete steps occur during mechanical relaxation



Mechanical buckling of the T7 capsid



Force triggers T7 DNA ejection

