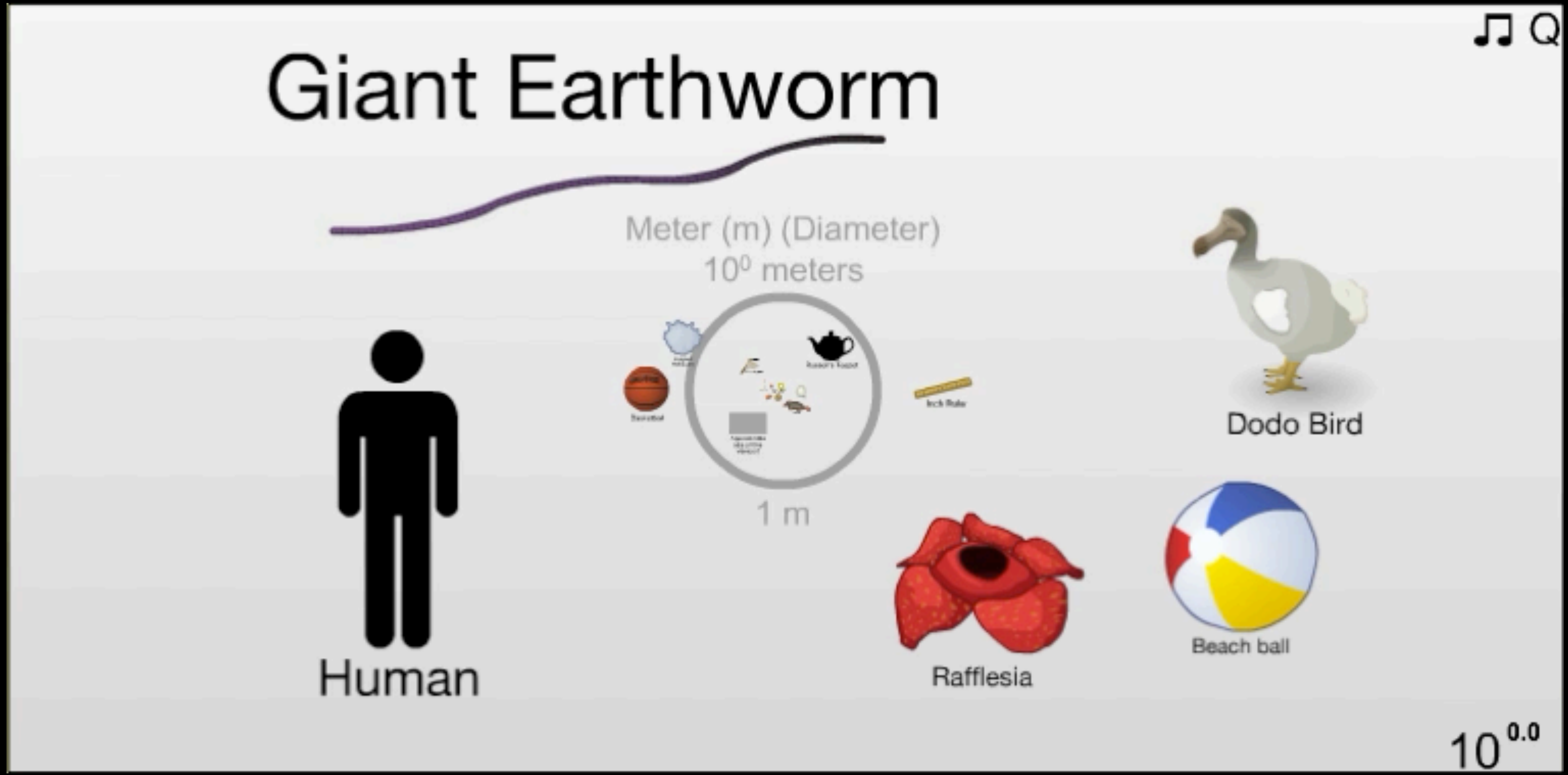
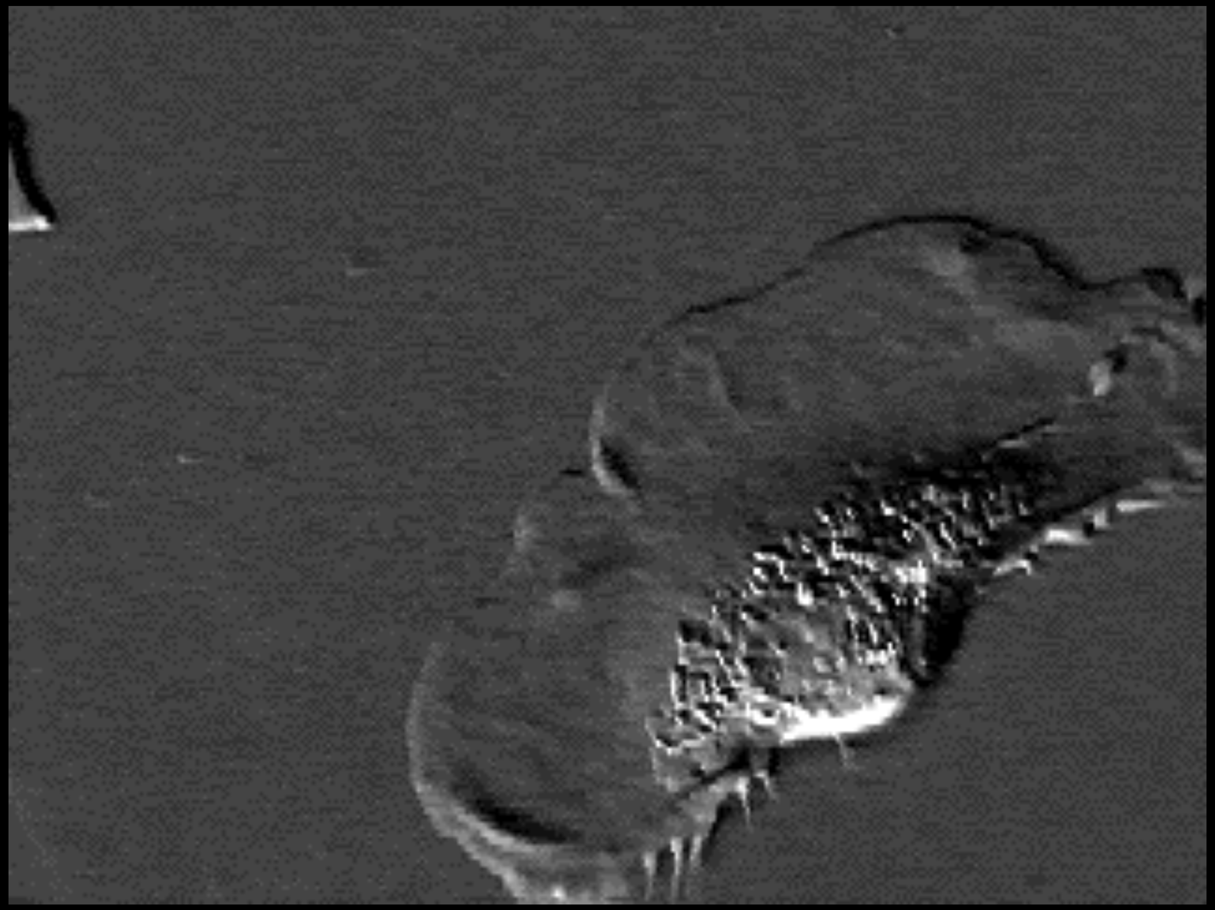
Single molecule biological activity

Kellermayer Miklós

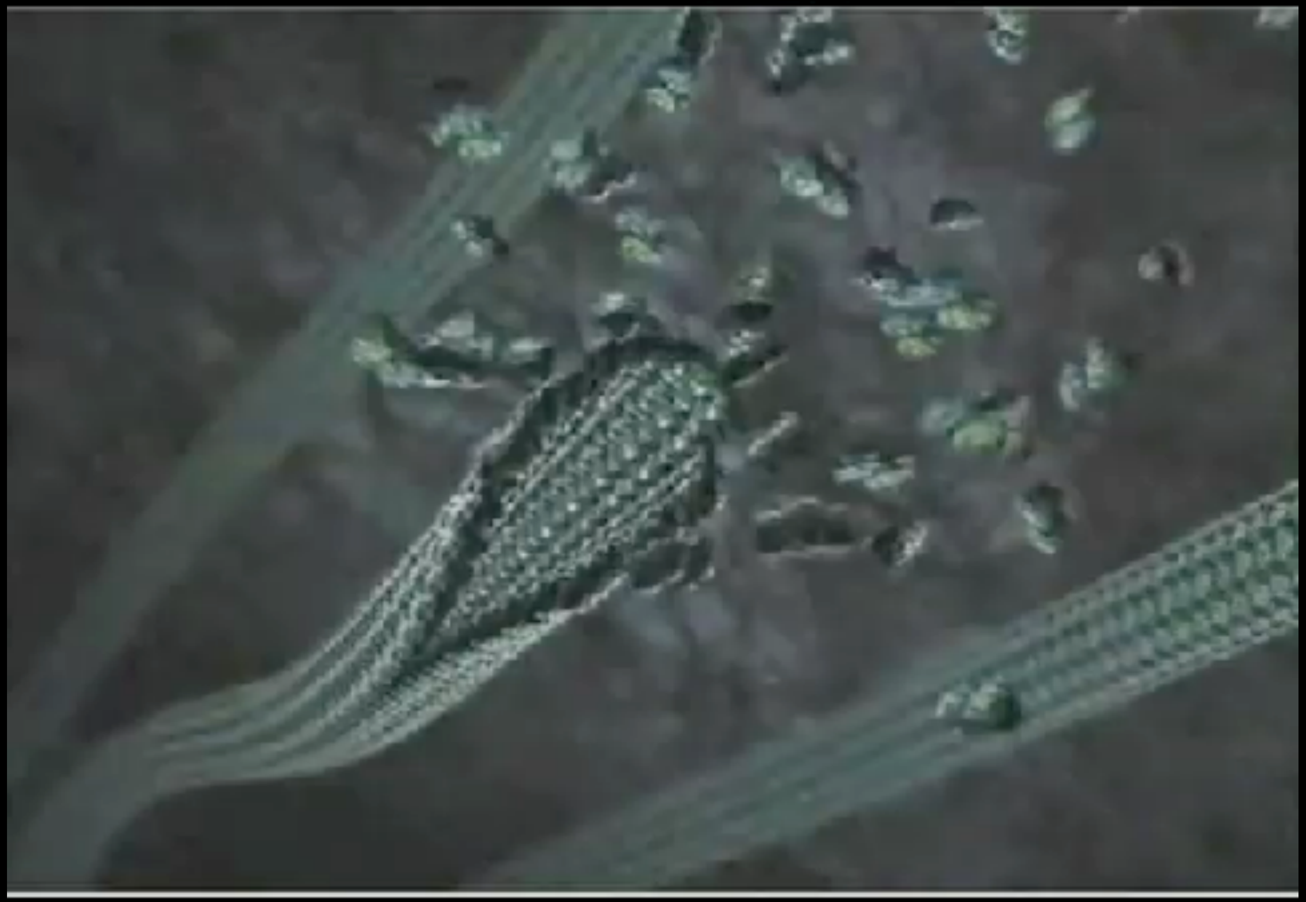


Cary and Michael Huang (http://htwins.net)

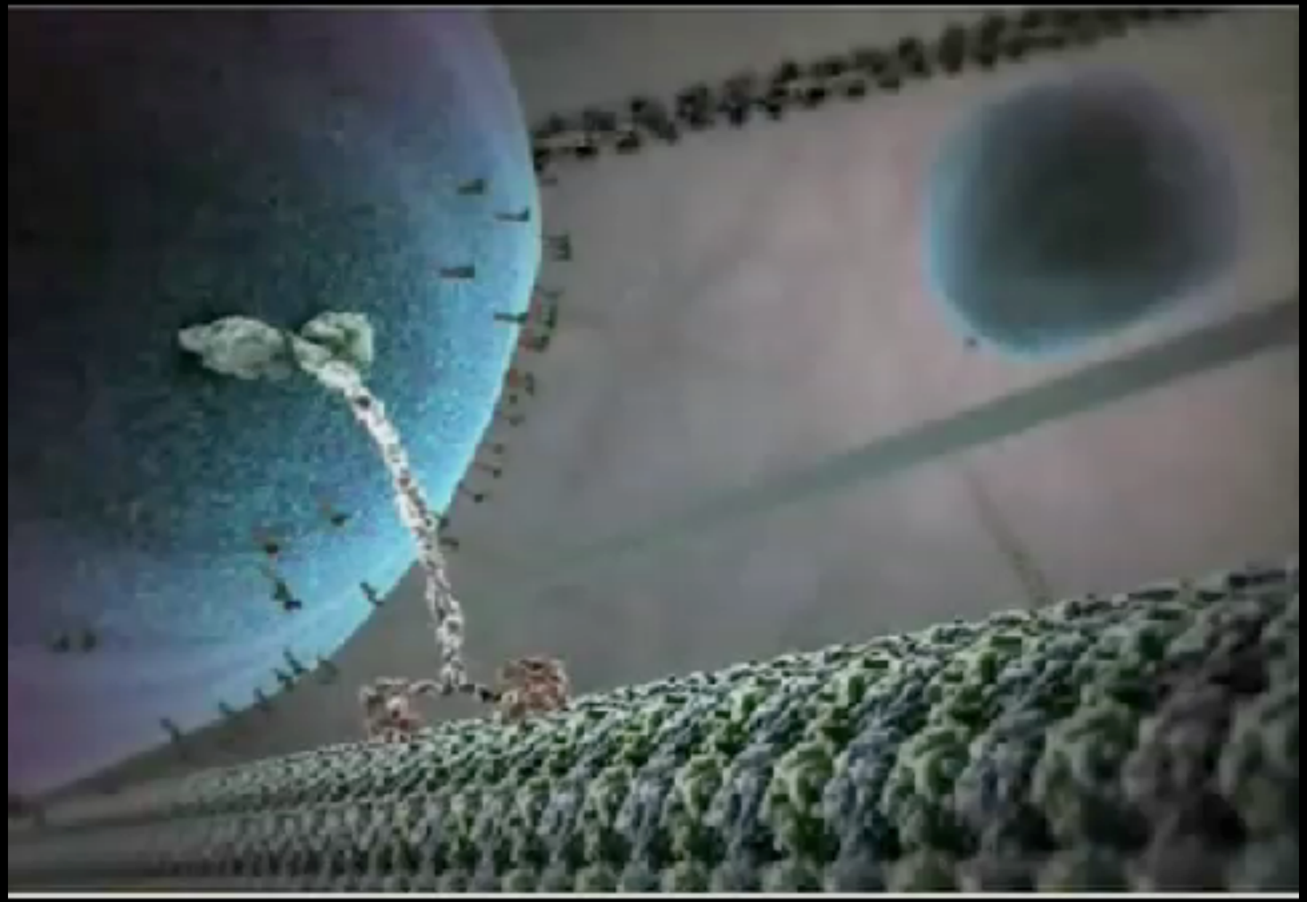
The living cell is a complex network of nanoscale machines



Crawling keratinocyte



Microtubule dynamic instability



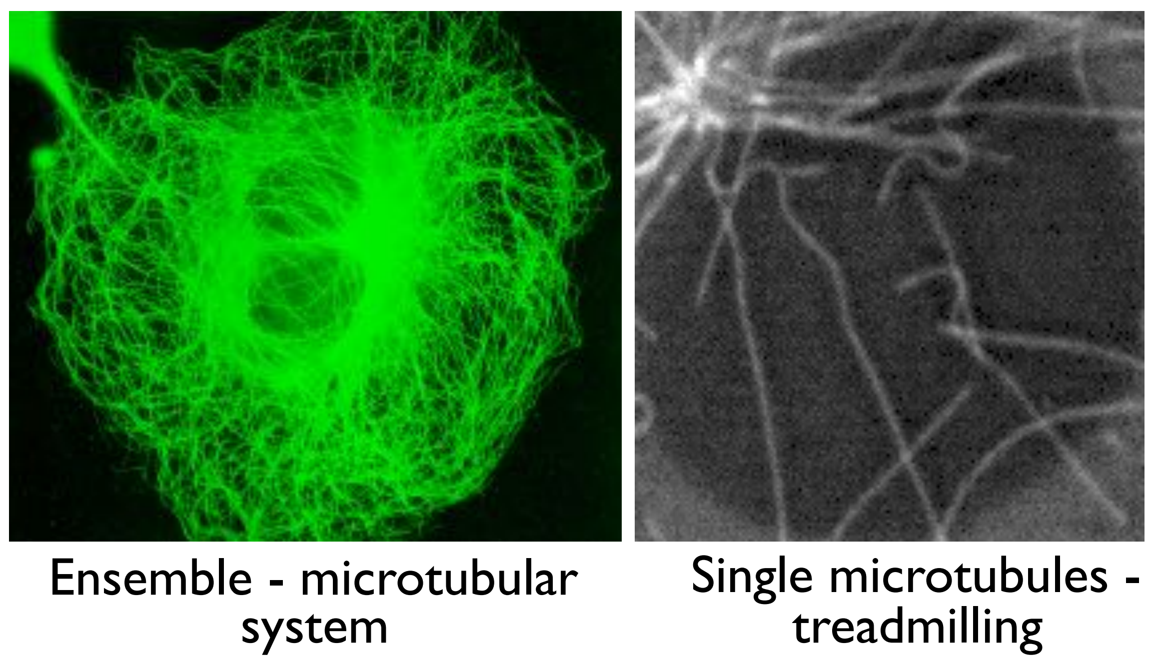
Kinesin-driven vesicular transport

Protein synthesis on the ribosome

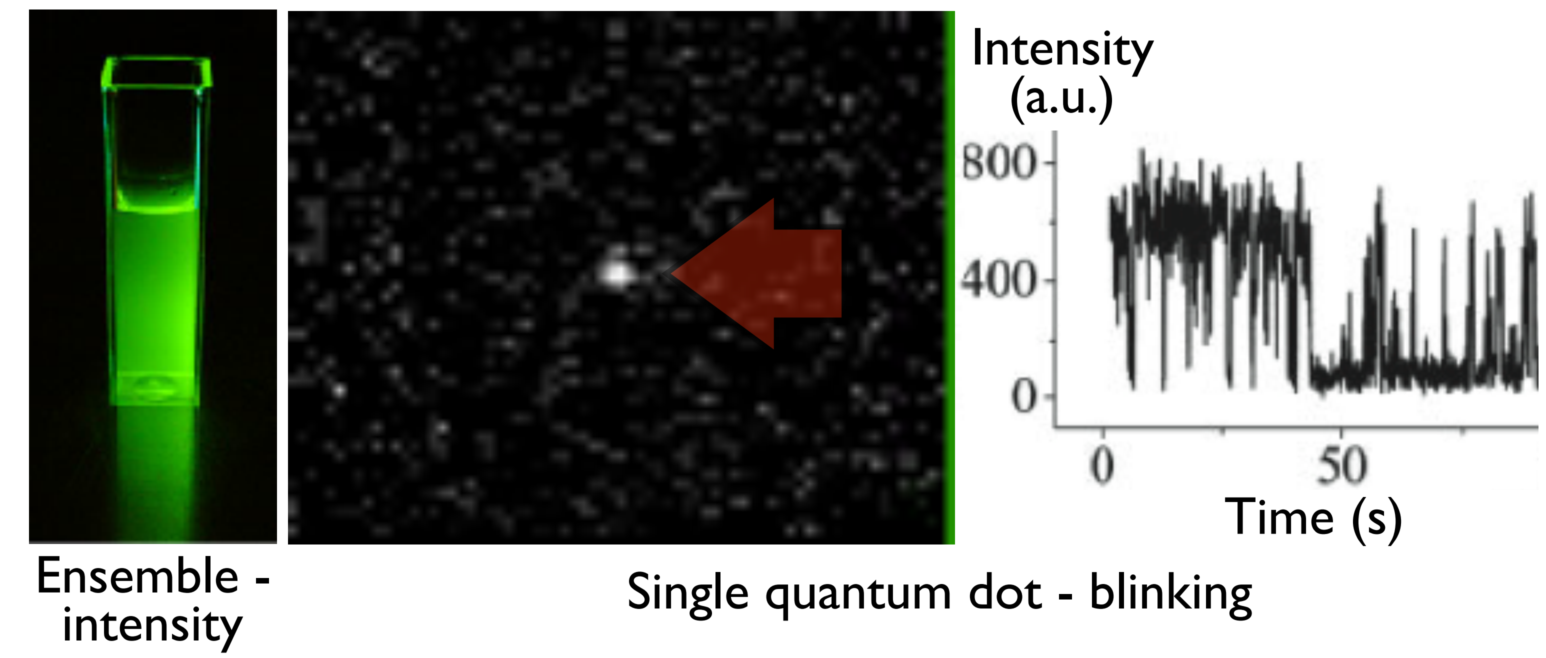
http://multimedia.mcb.harvard.edu

Why single molecules?

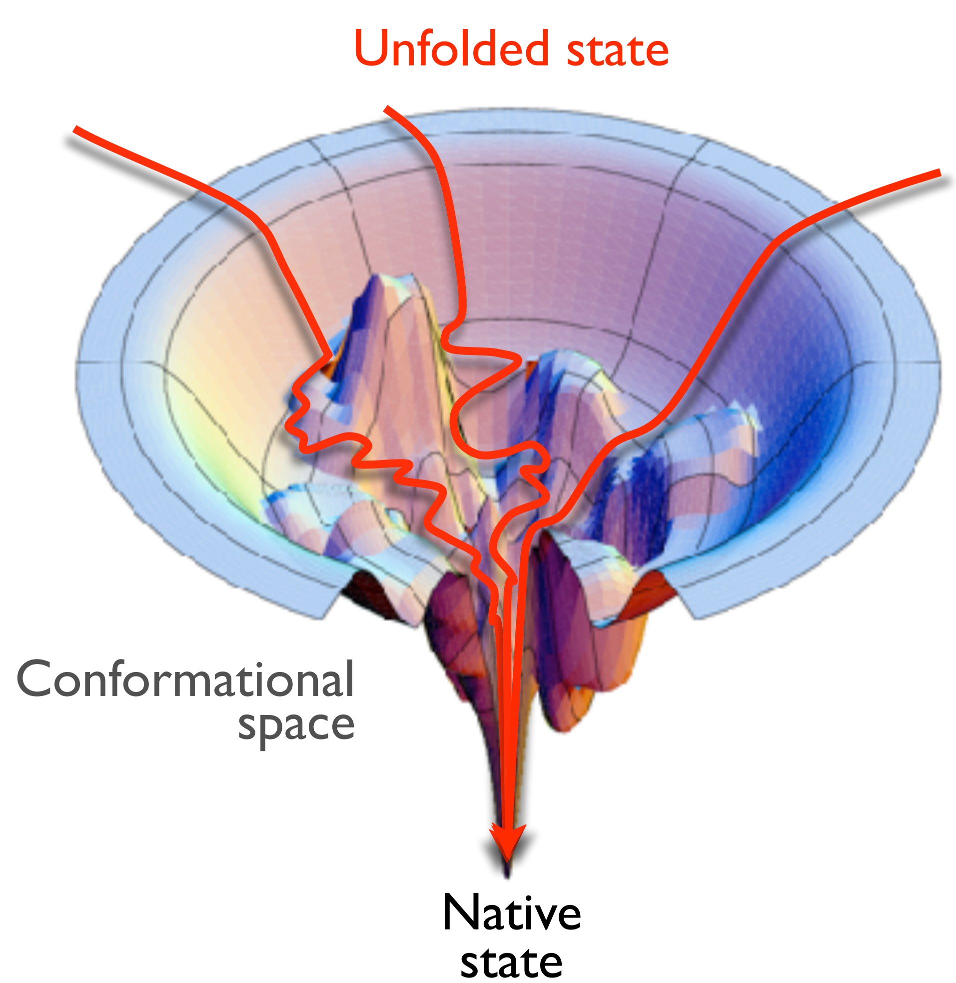
1. Individuals (spatial and temporal trajectories) may be identified in a crowd



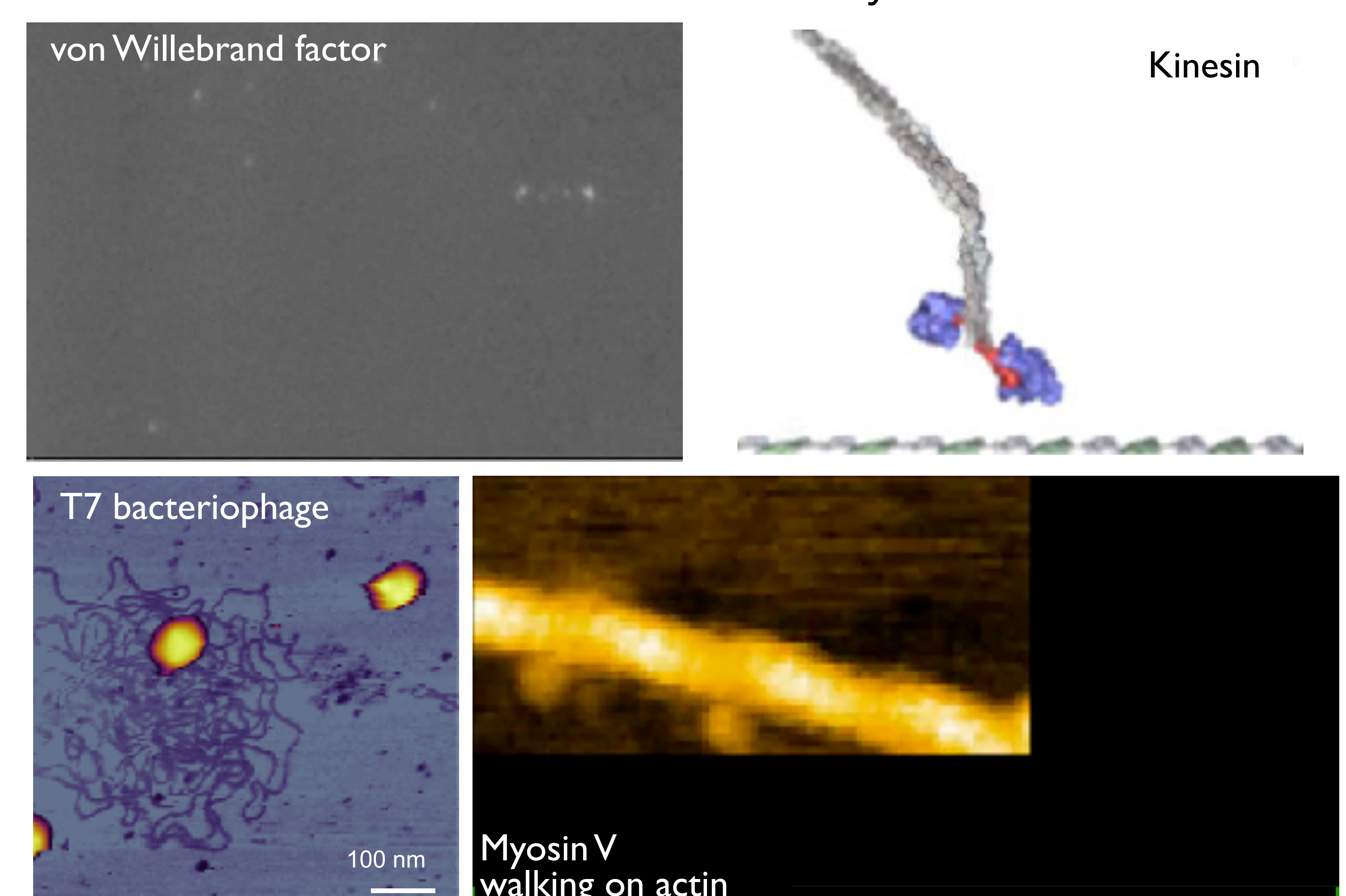
2. Stochastic processes may be uncovered



3. Parallel-pathway events may be identified



4. Mechanics of biomolecules may be characterized



Brief history of single-molecule science

1976: Fluorescence microscopy of a single antibody molecule

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

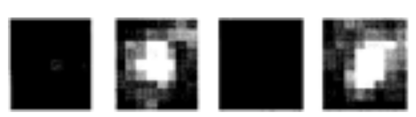
1991: J.Spudich, T.Yanagida, J.Molloy, single myosin mechanics

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

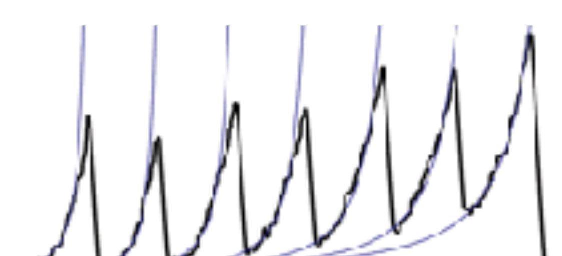


1997: W.E. Moerner, GFP blinking

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



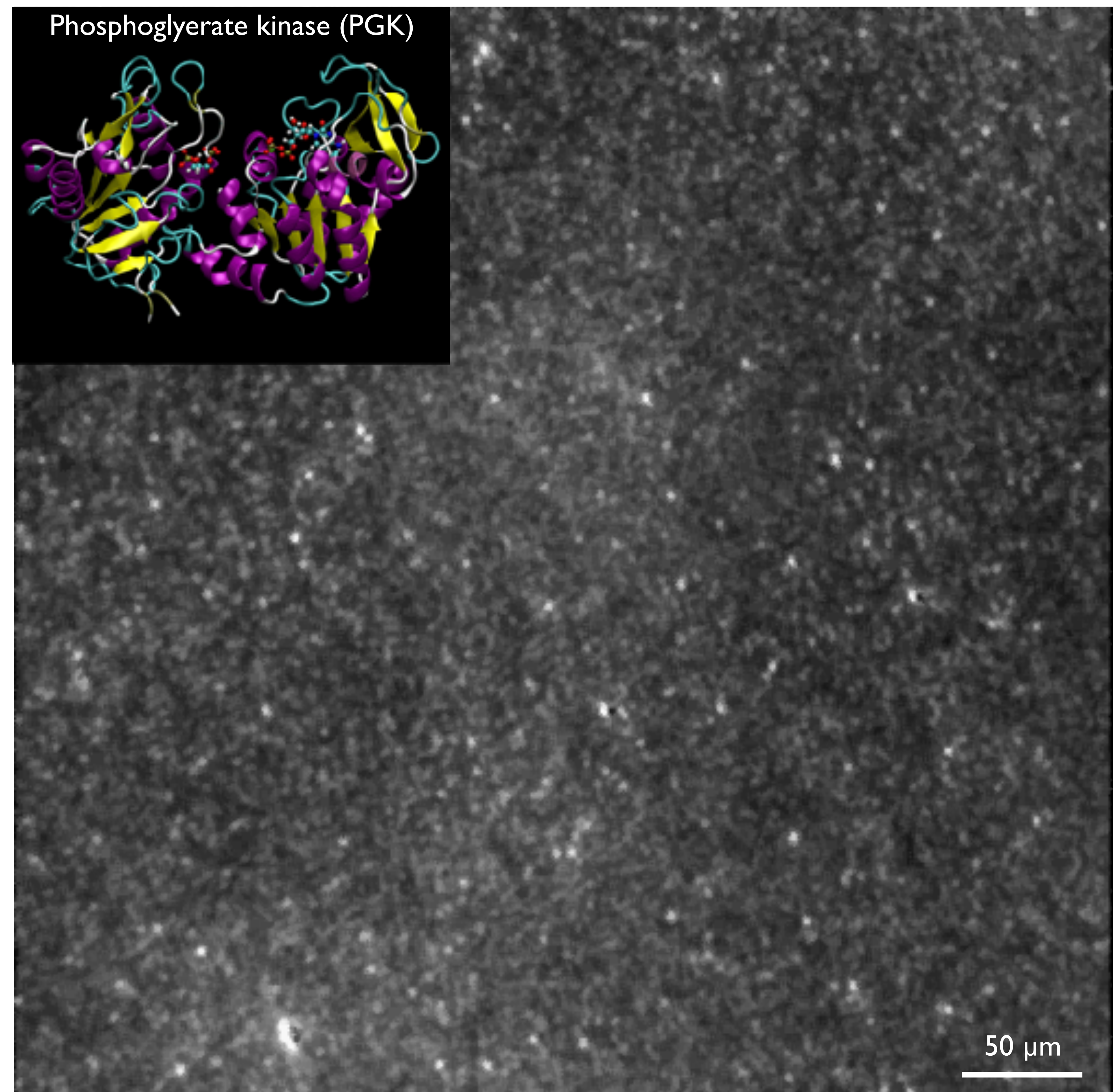
2001: J.Liphardt, C.Bustamante, single RNA manipulation

2004: J.Fernandez, single protein molecule folding

2008: Bustamante, Tinoco: ribosome mechanics

Measurable parameters I. Fluorescence

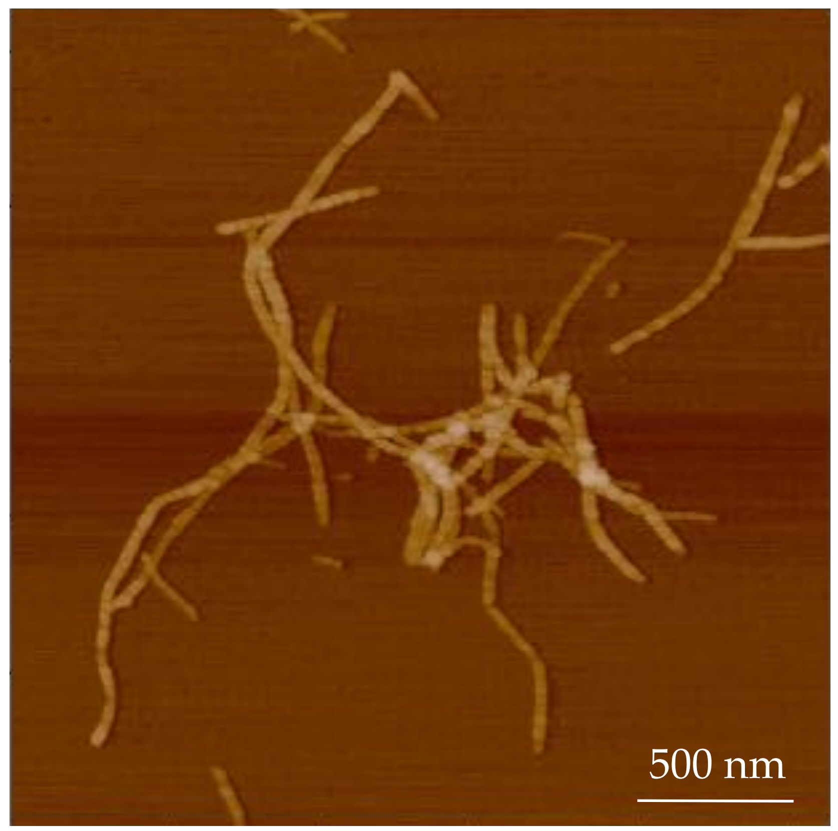
What are the conformational states of a molecule?



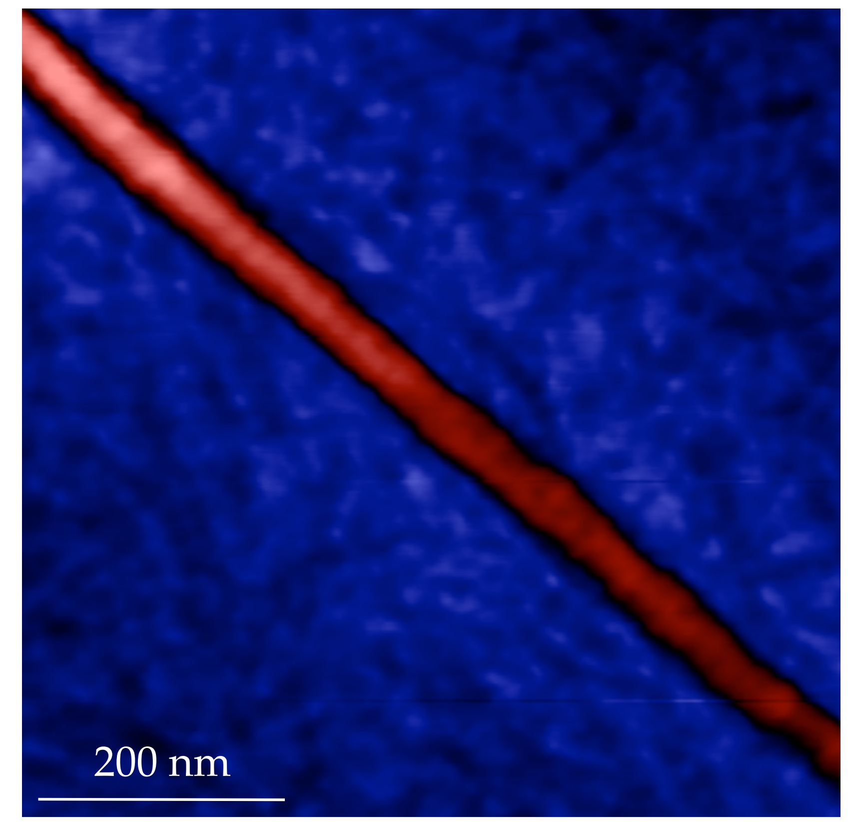
PGK molecules labeled with Alexa488, TIRF microscopy

Measurable parameters II.
Structure - topography

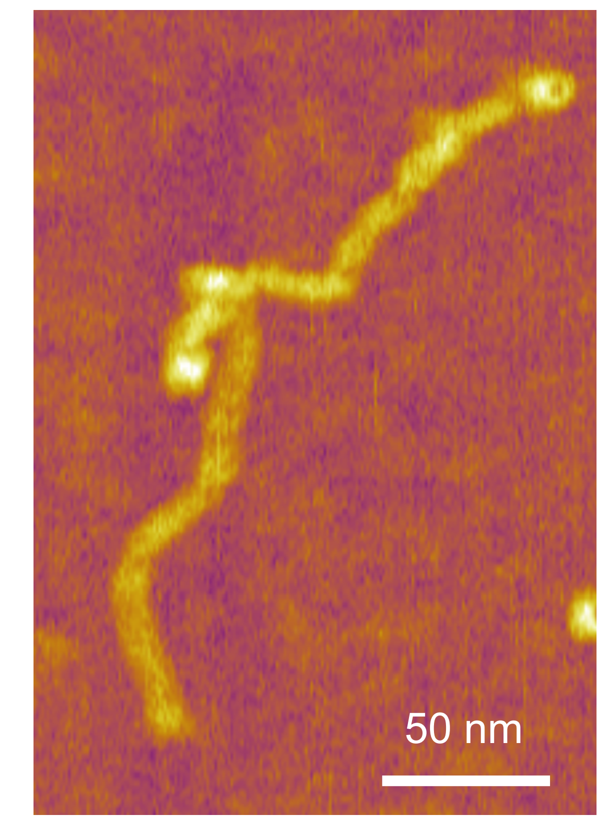
What are the conformational states of a molecule?



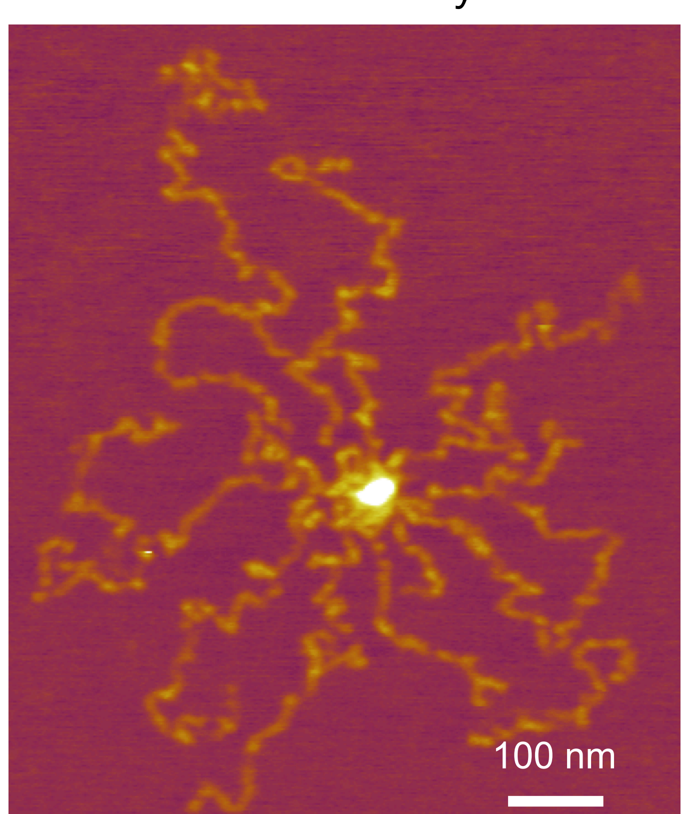
Amyloid ß1-42



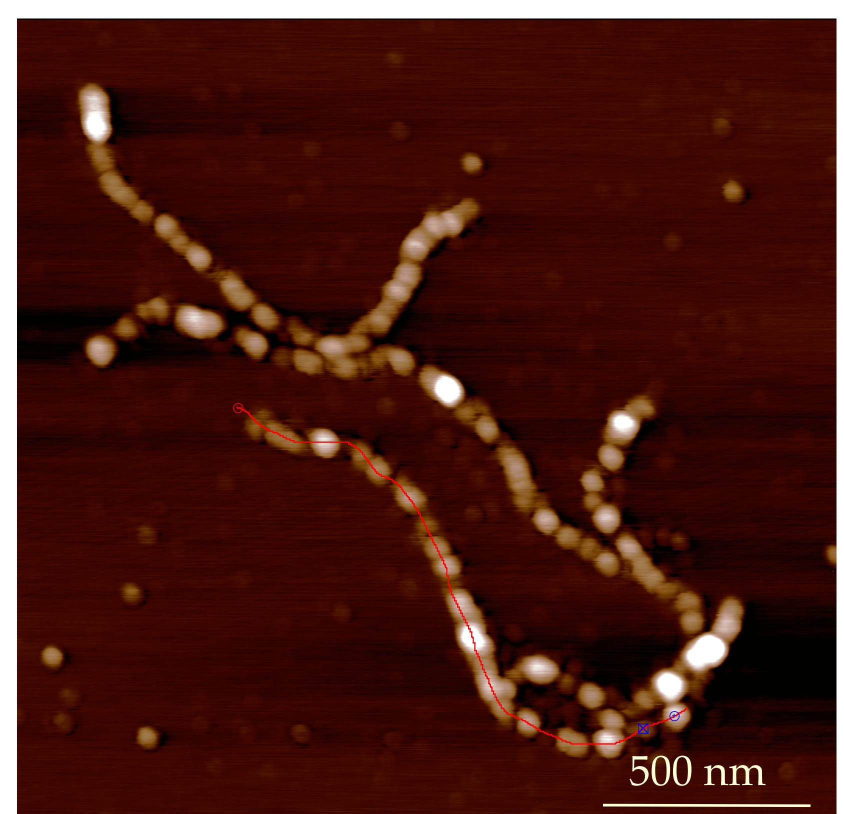
Fibrin protofibril



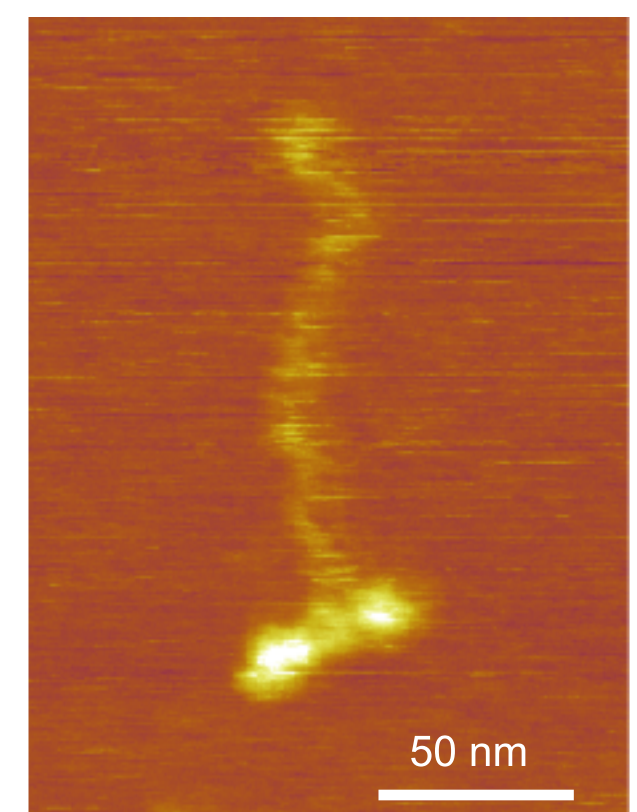
RNA-DNA hybrid



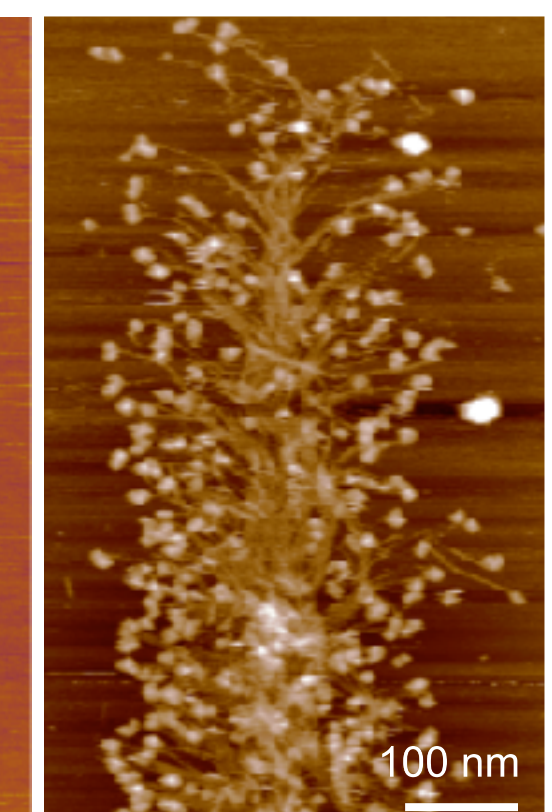
Titin molecule



Desmin filament



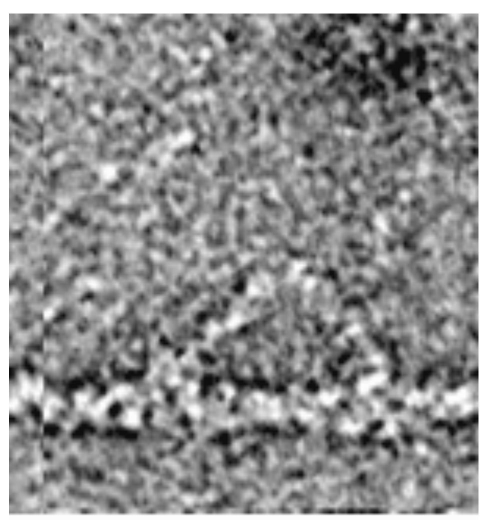
Myosin molecule



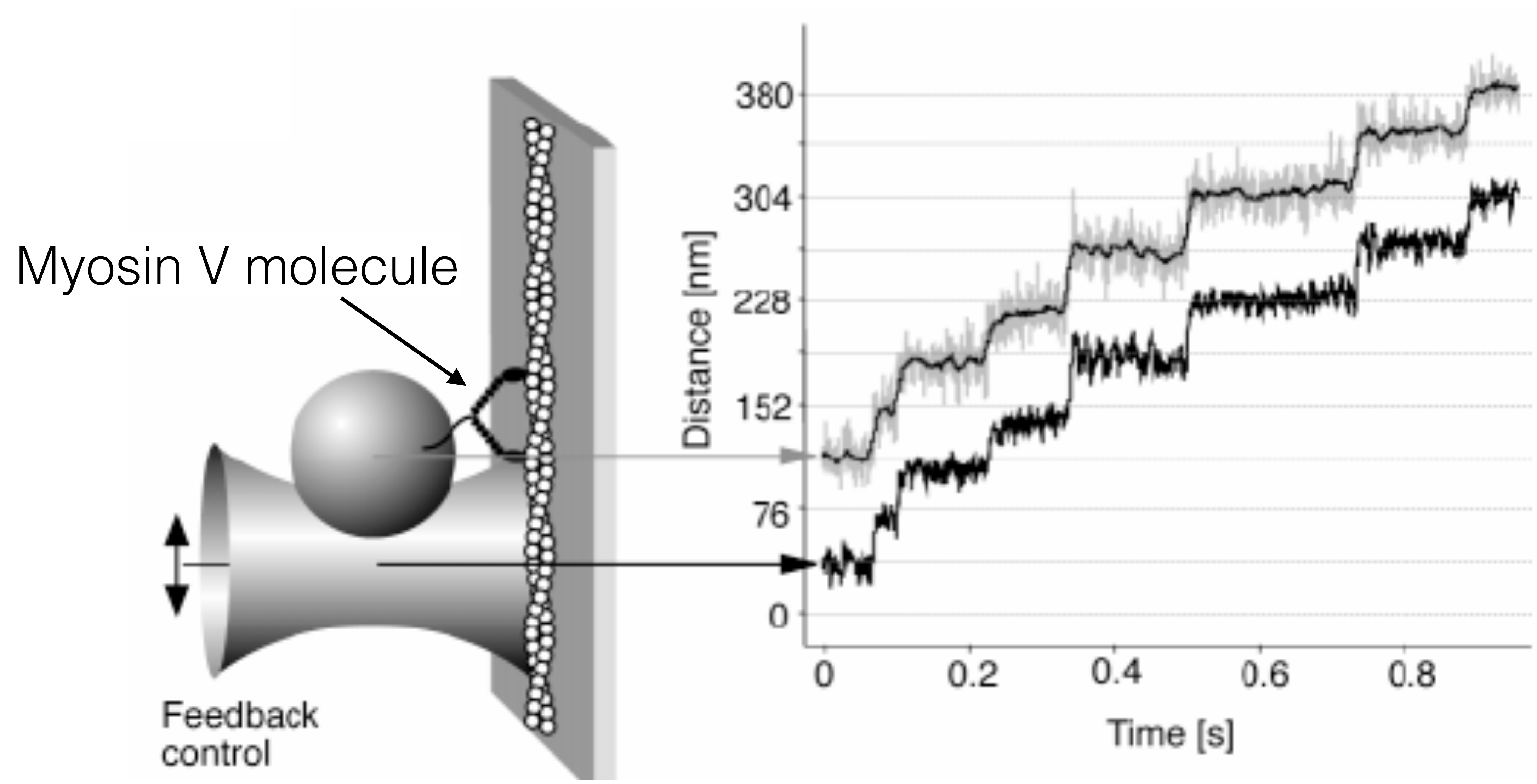
Myosin thick filament

Measurable parameters III. Distance

What is the step size of a motor protein?

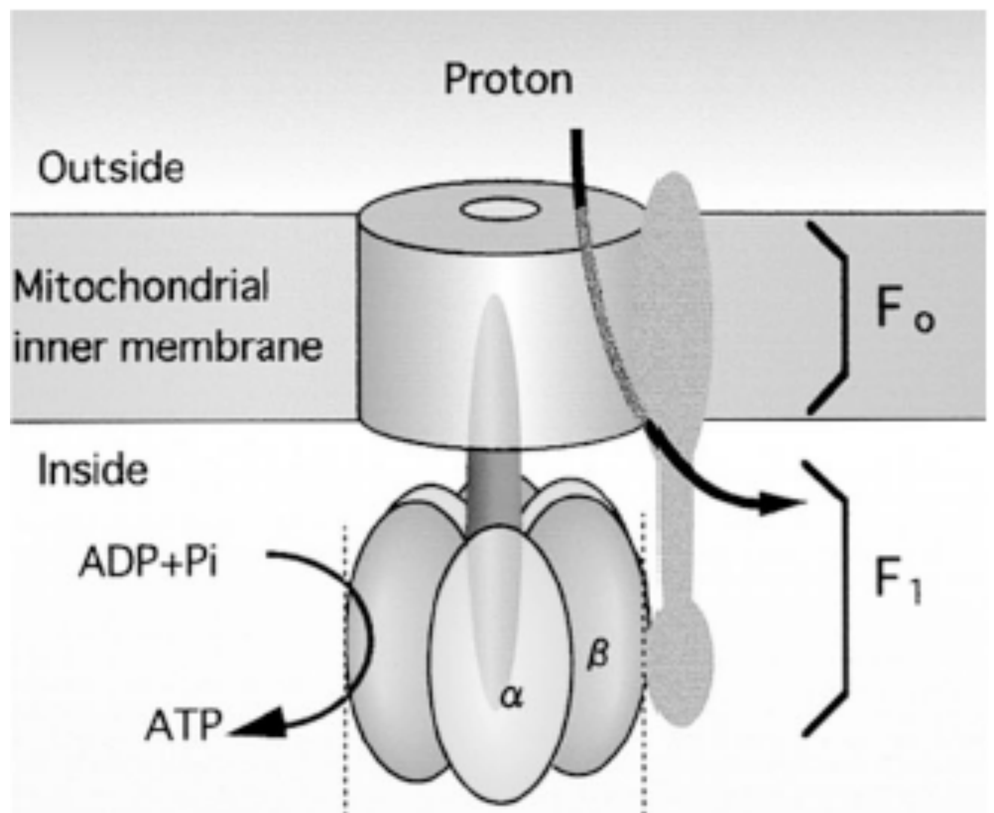


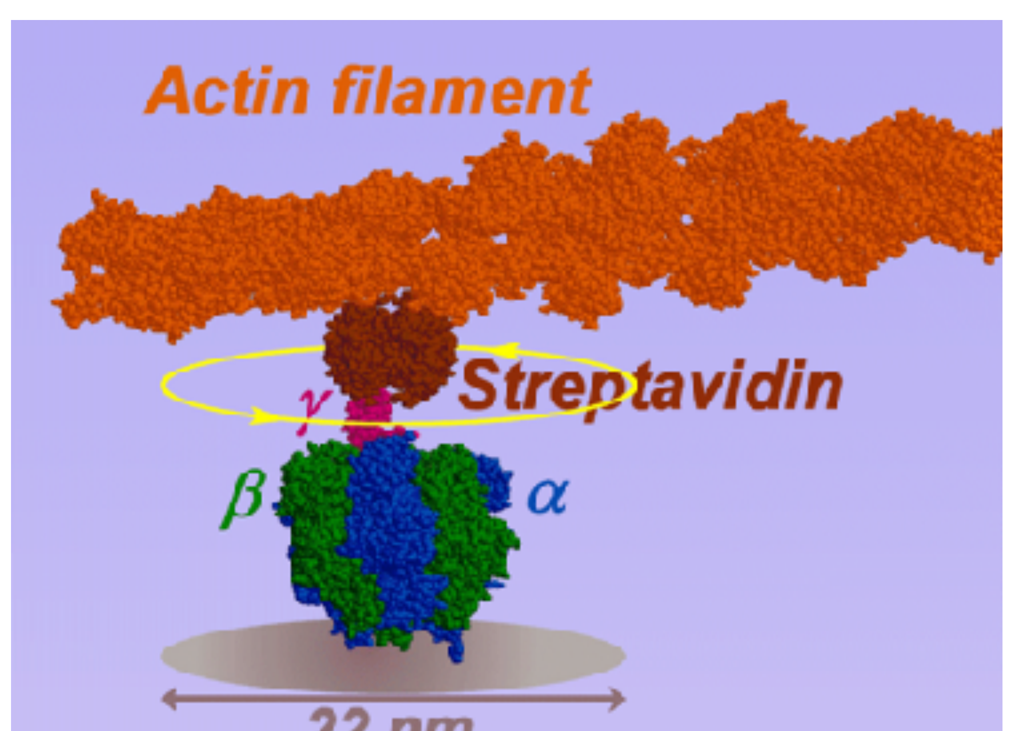
Myosin V cryoelectron-microscopic image series

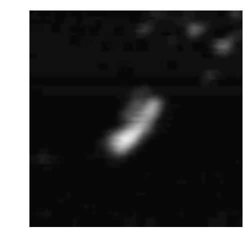


Measurable parameters IV. Rotational angle

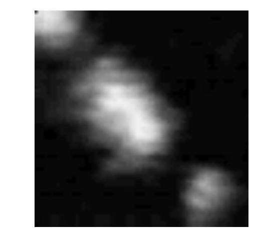
How does the ATP synthase work?



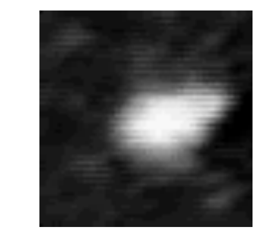




1 mM ATP

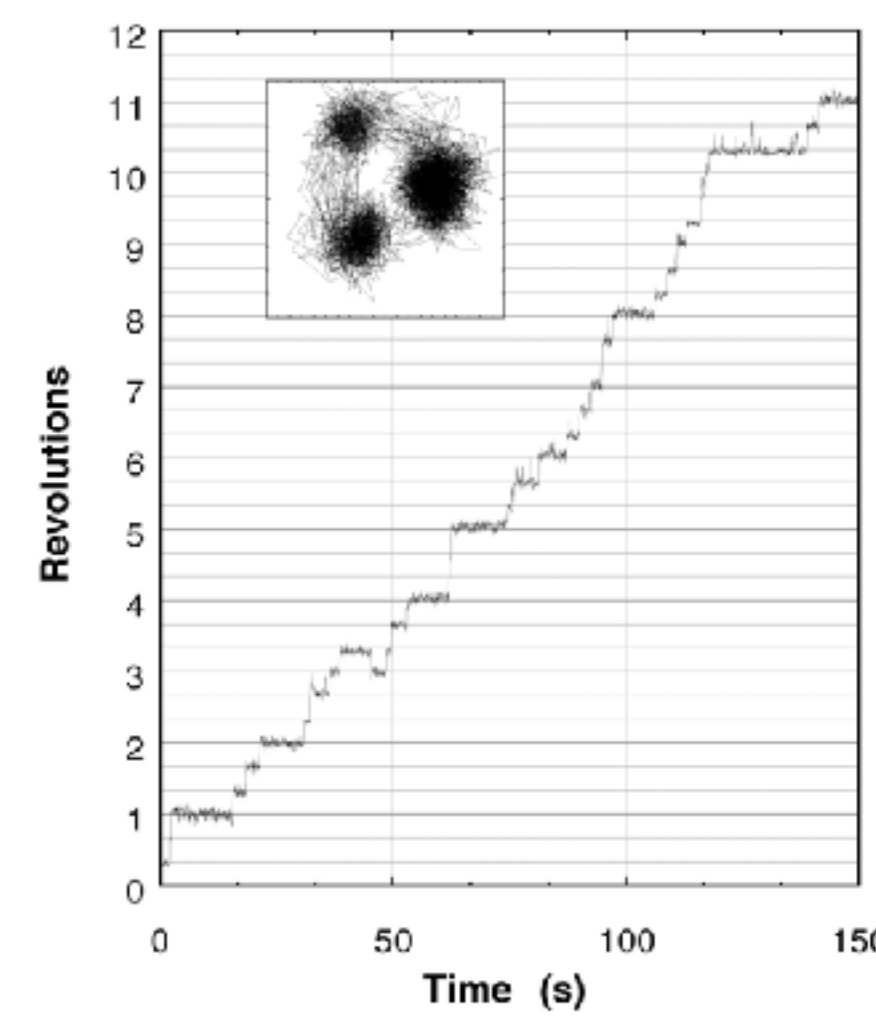


200 nM ATP



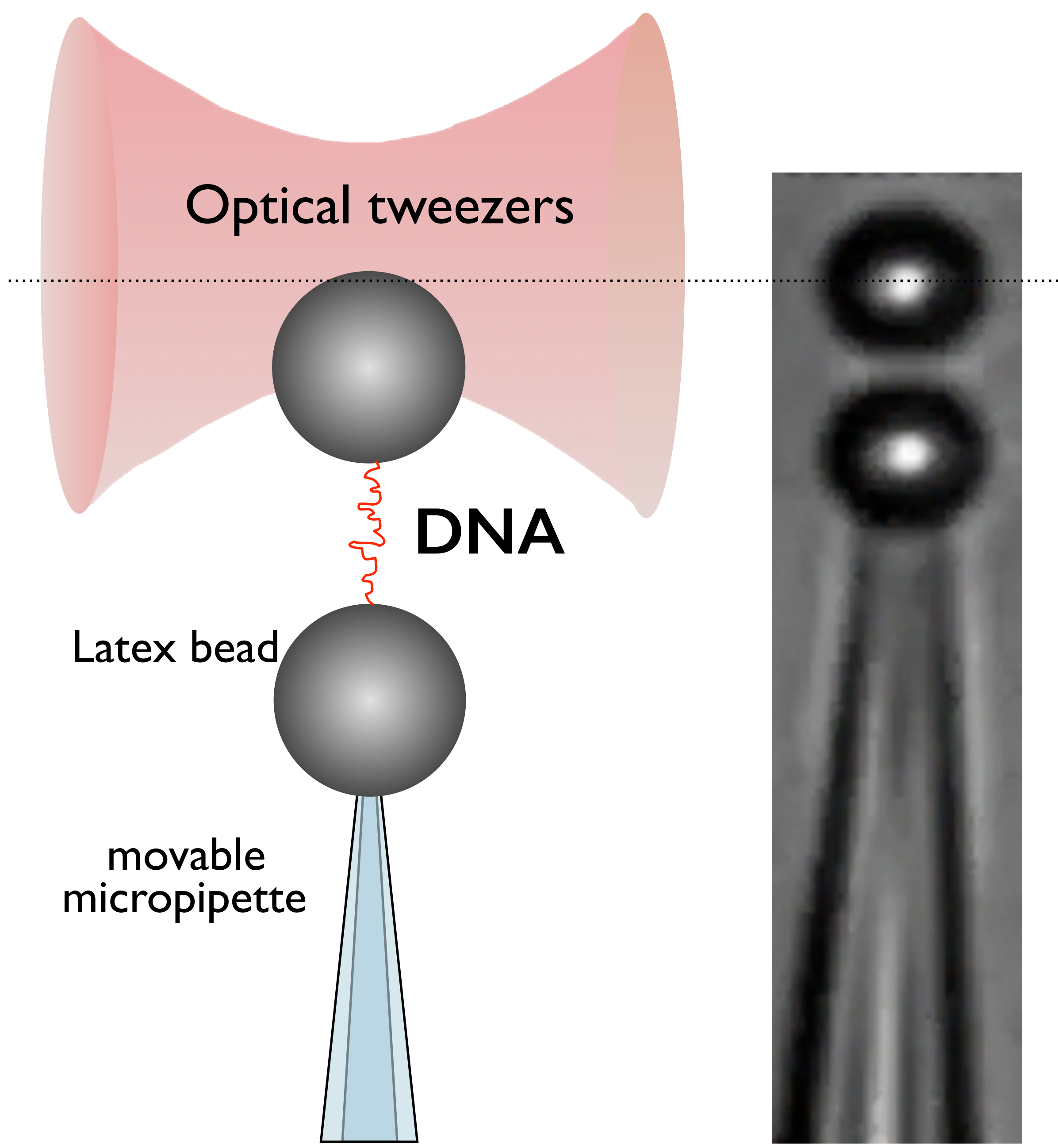
20 nM ATP

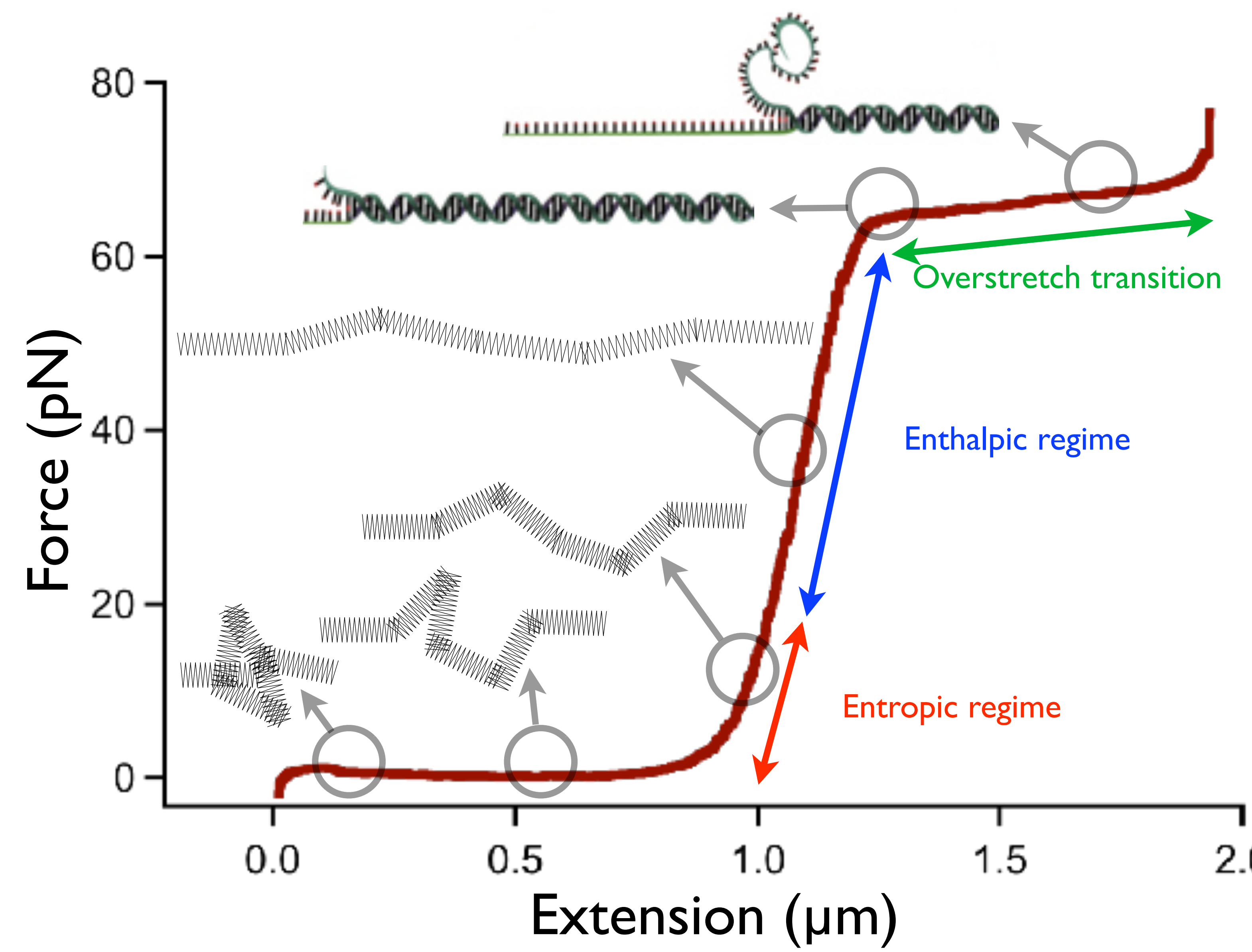
Discrete rotational steps of 120˚



Measurable parameters V. Force

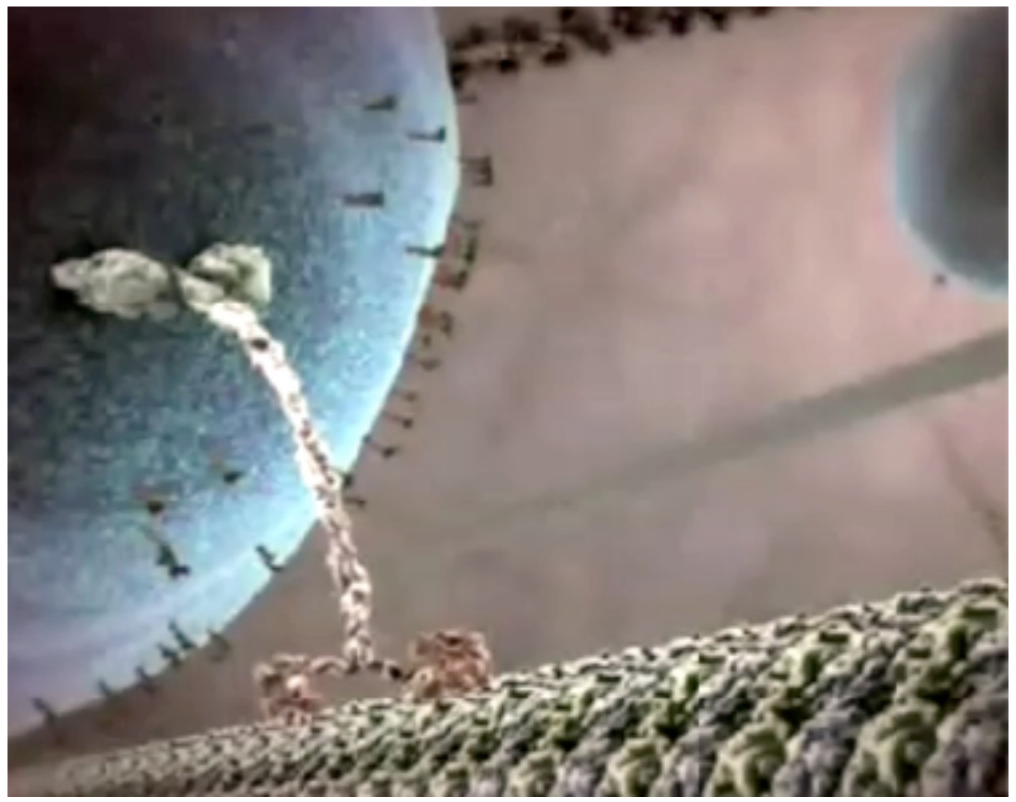
How much force develops during the stretch of a single dsDNA molecule?



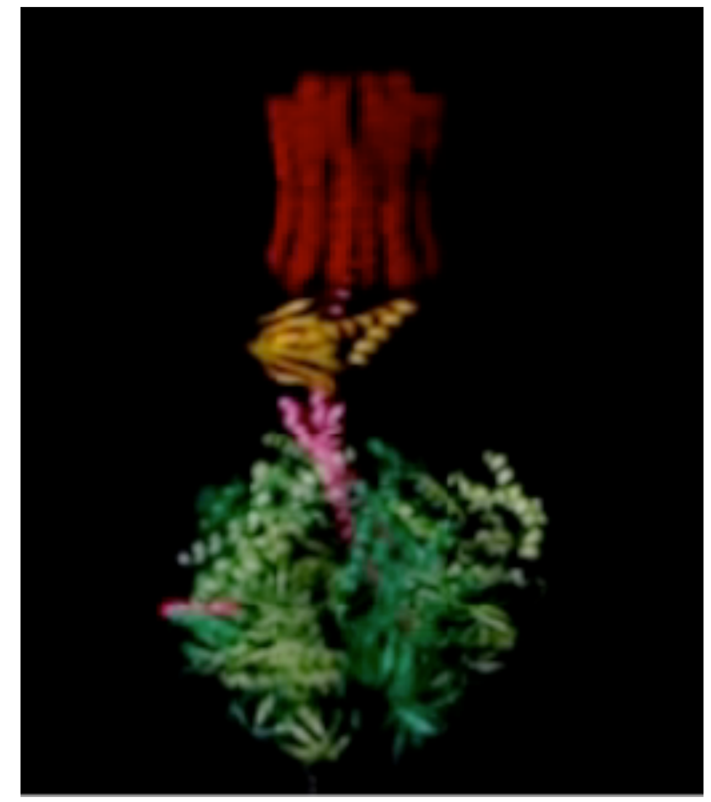


1. Force: develops

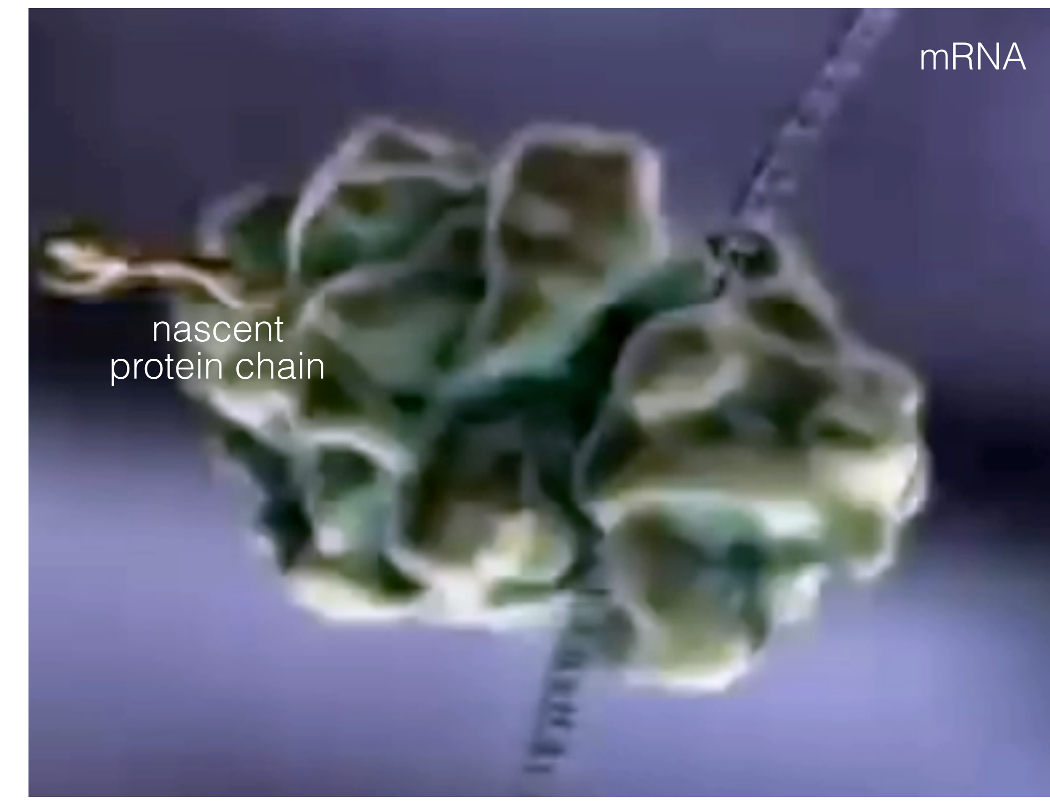
Kinesin



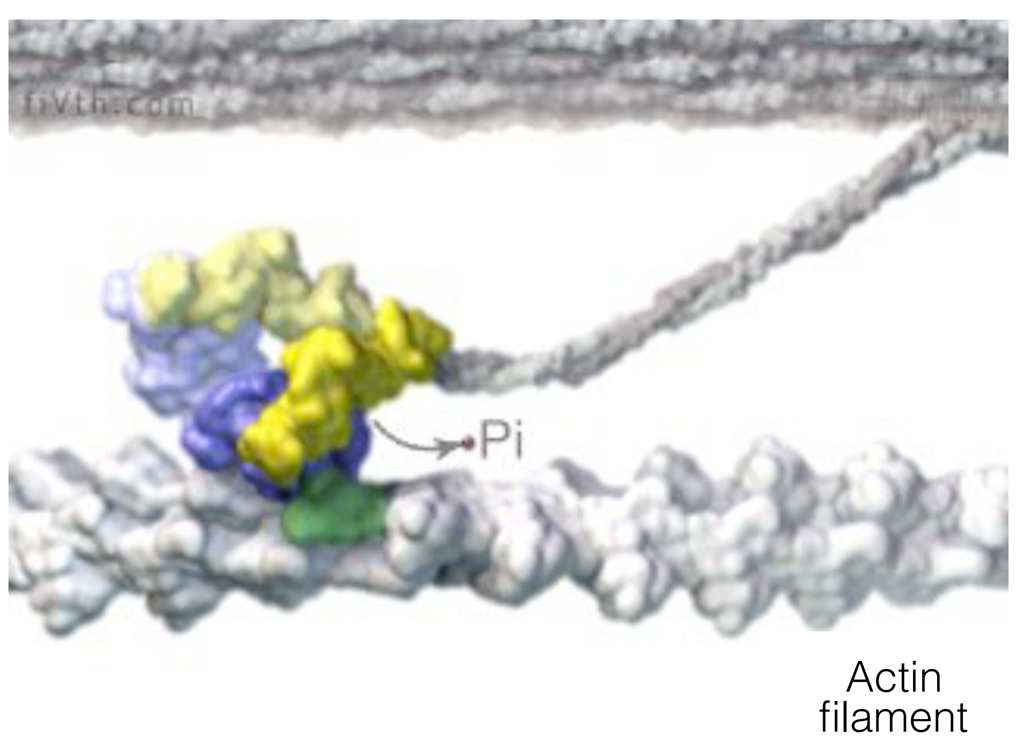
F1F0 ATPase



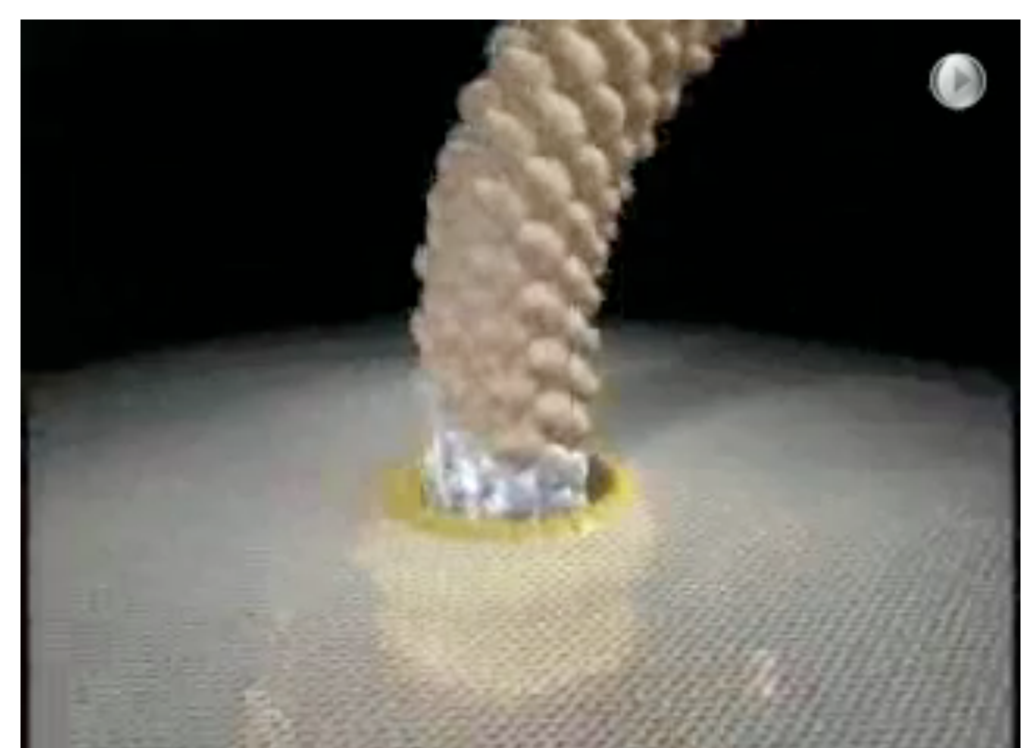
Ribosome



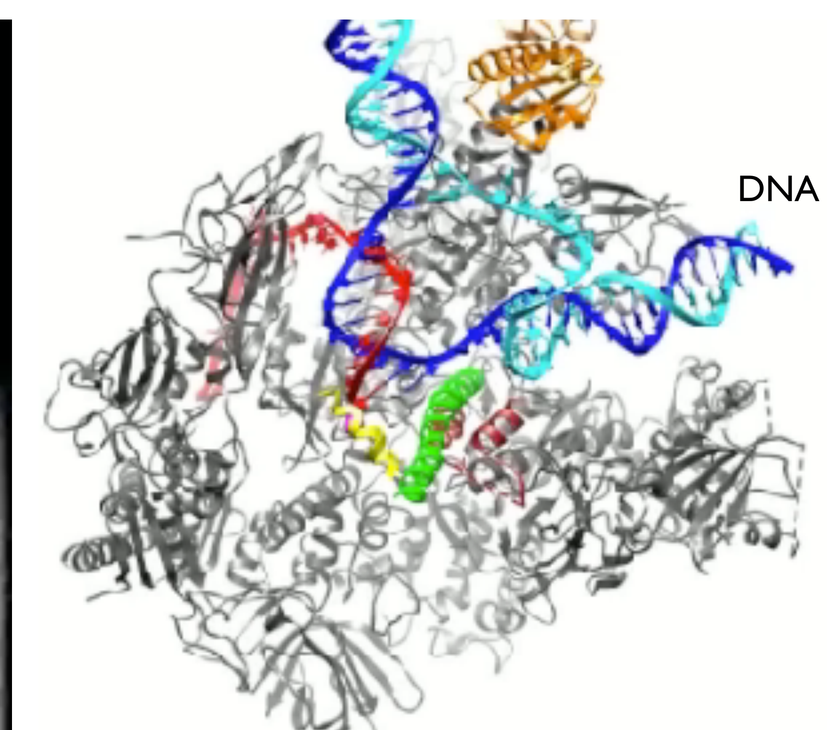
Myosin II



Bacterial flagellar motor

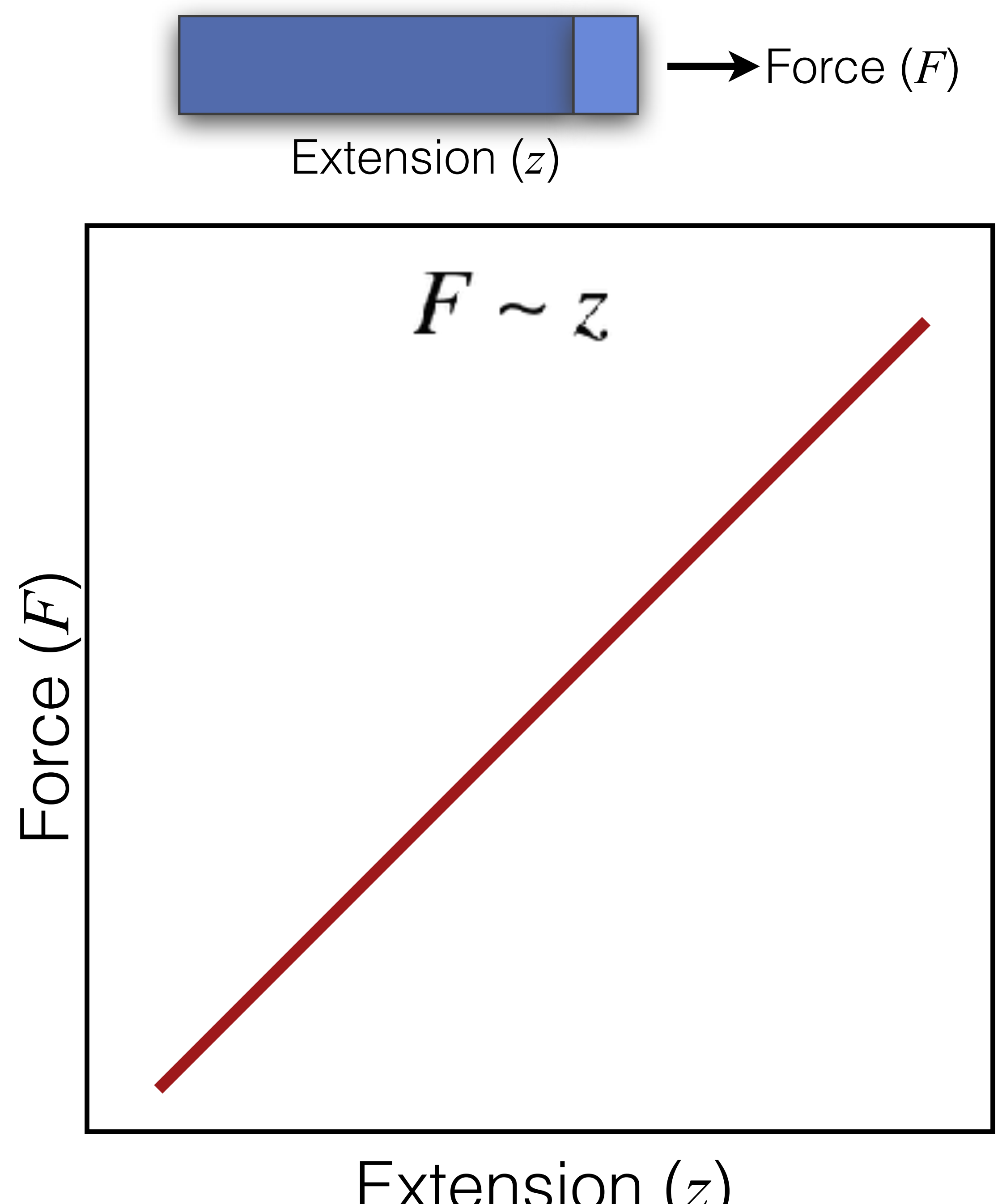


RNA polymerase

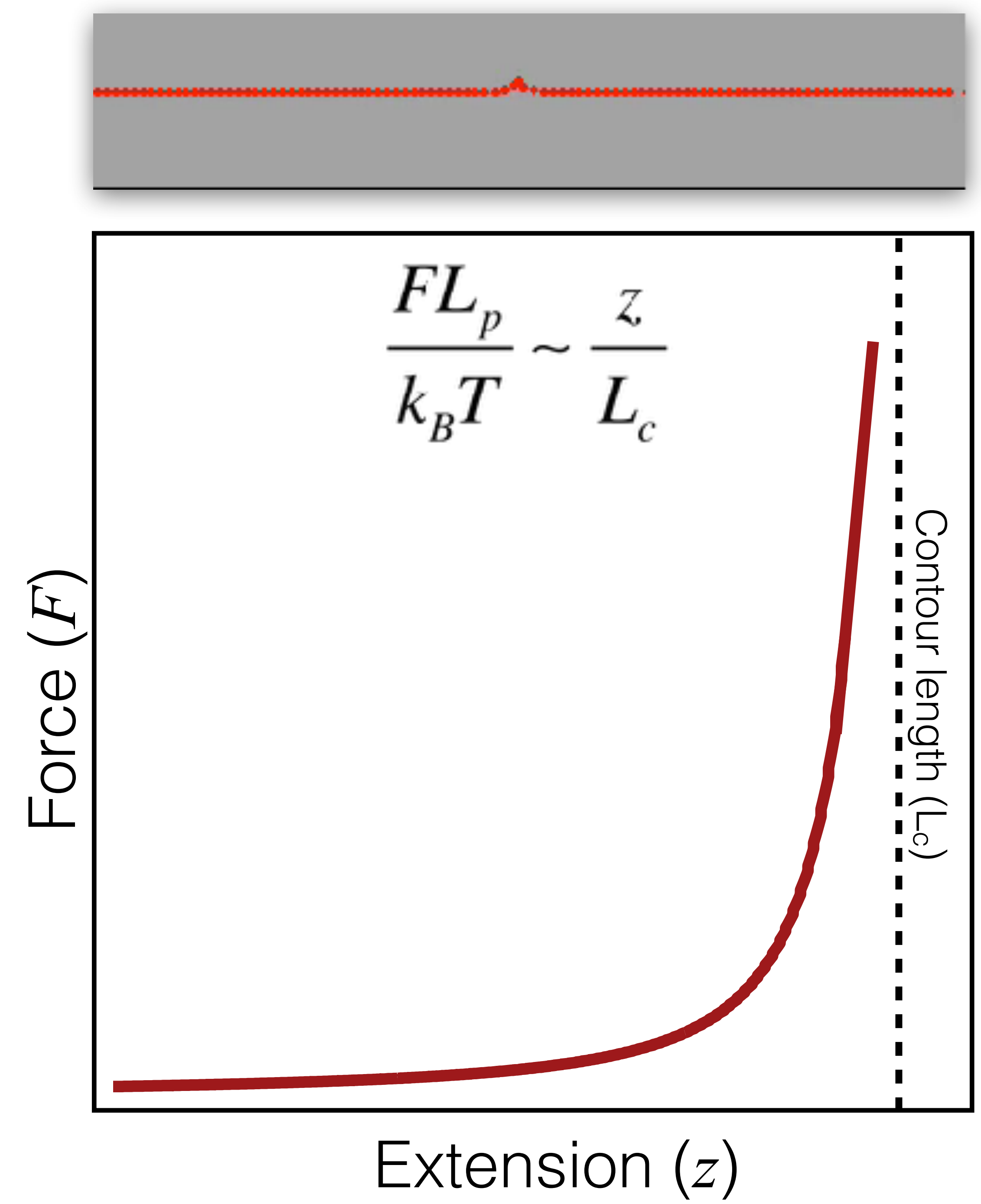


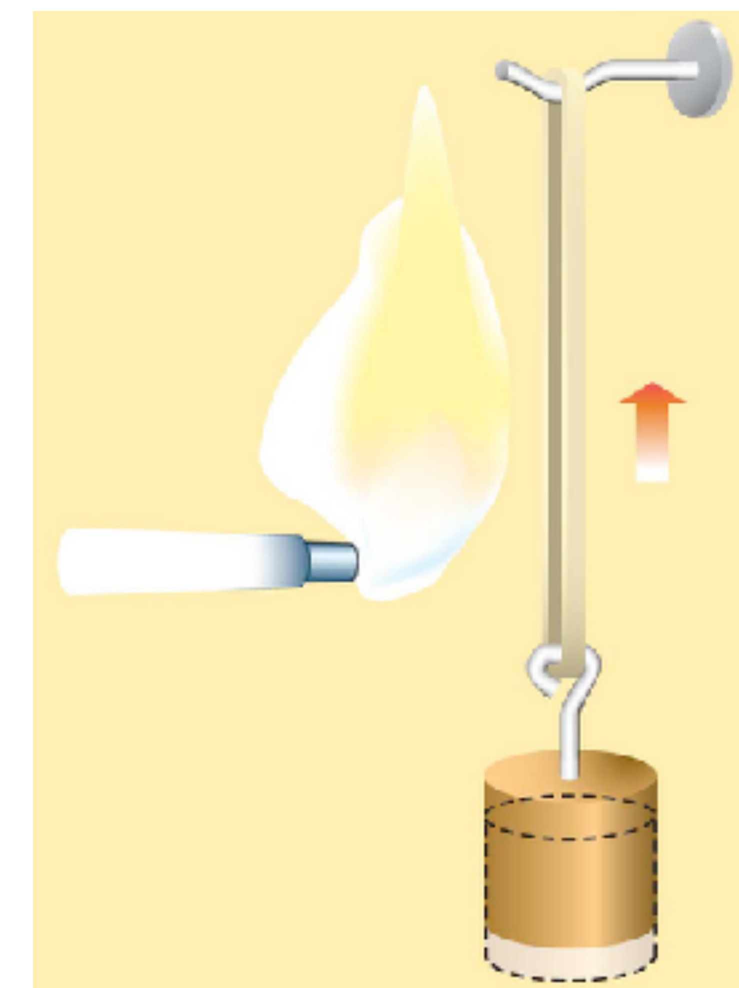
2. Force: deforms shape

Rigid body:
Hooke's law



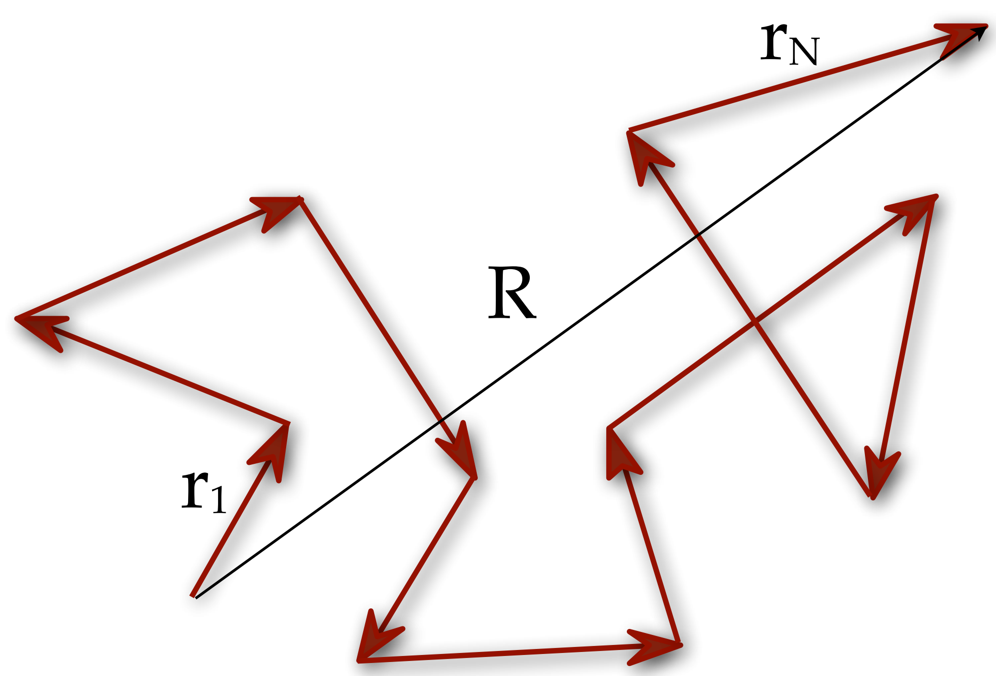
Polymer chain:
fluctuations, configurational entropy





Macroscopic manifestation
of entropic elasticity:
Gough-Joule effect

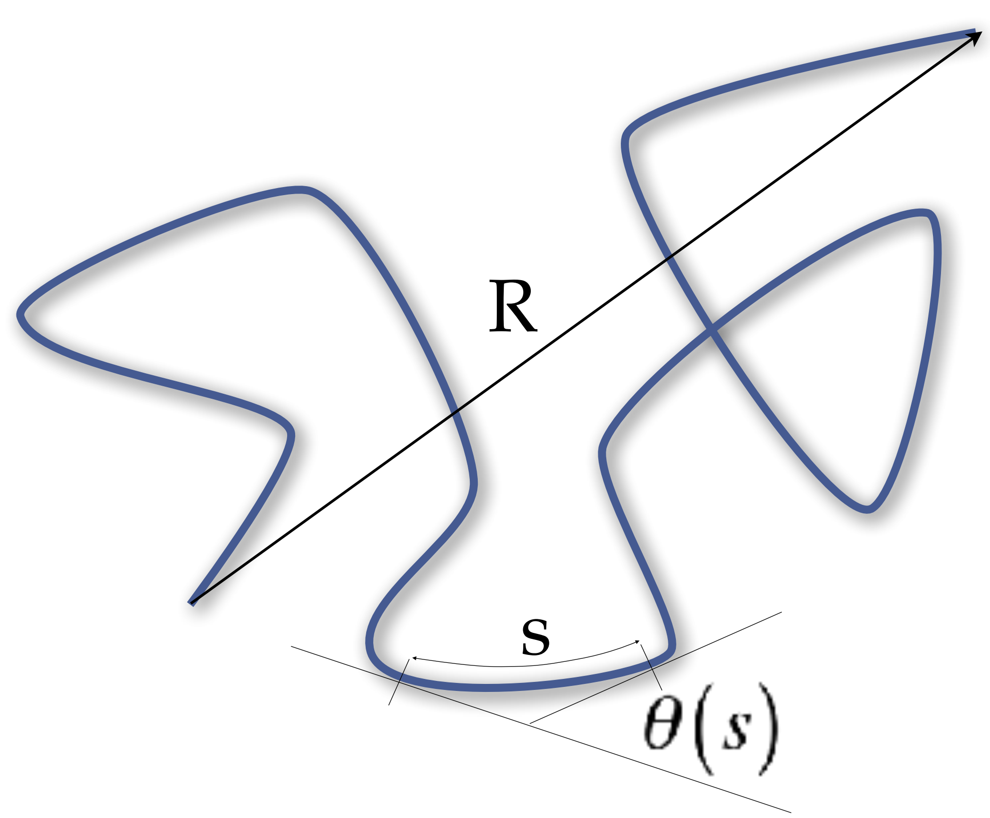
Models of entropic elasticity



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

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

L_P = persistence length
EI = bending rigidity
$l_K = 2L_P$

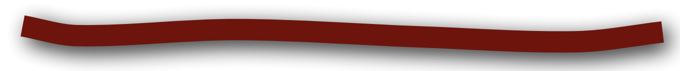
Equilibrium shape and bending rigidity of a polymer chain are related

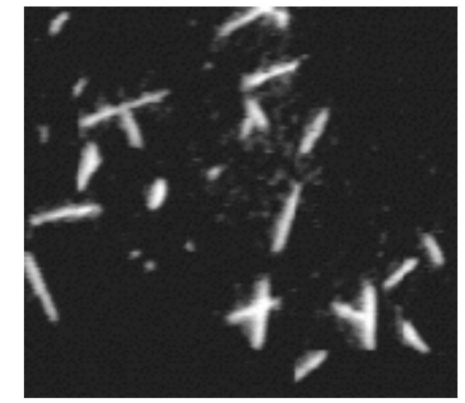
Rigid chain

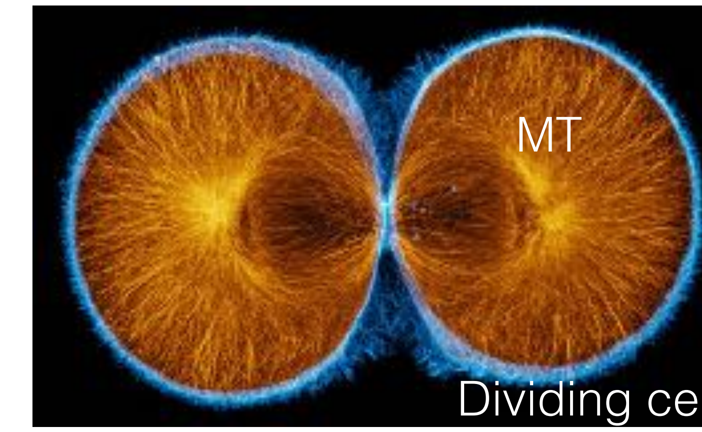
$L_p >> L_c$

(mm >> 10 μm)

Microtubule





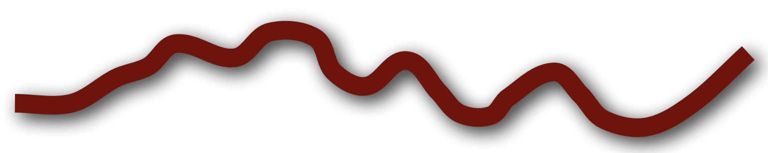


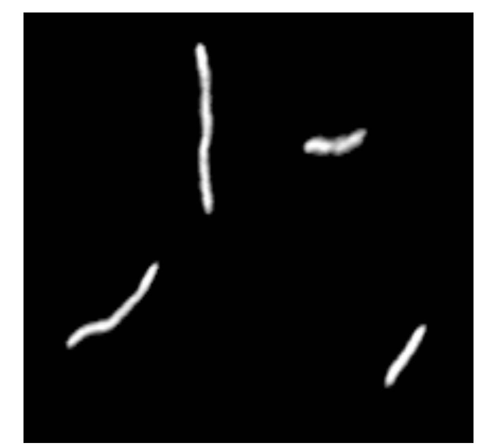
Semiflexible chain

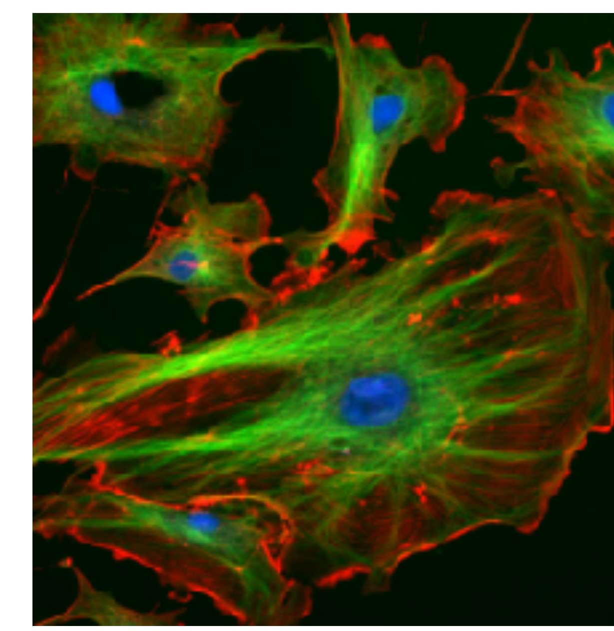
$L_p \approx L_c$

(μm $\approx$ μm)

Microfilament (actin)







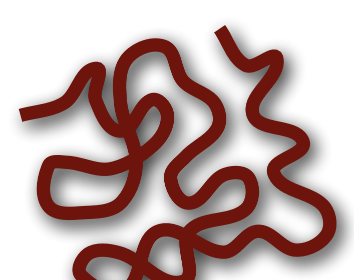
actin
tubulin

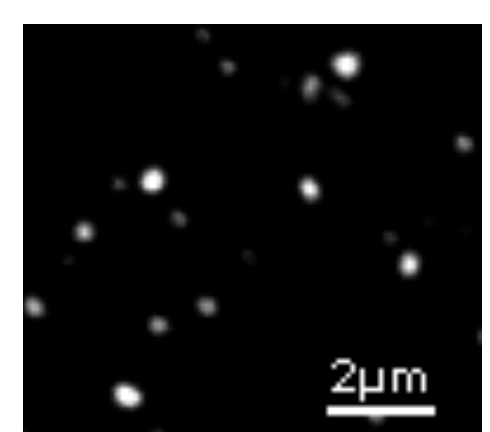
Flexible chain

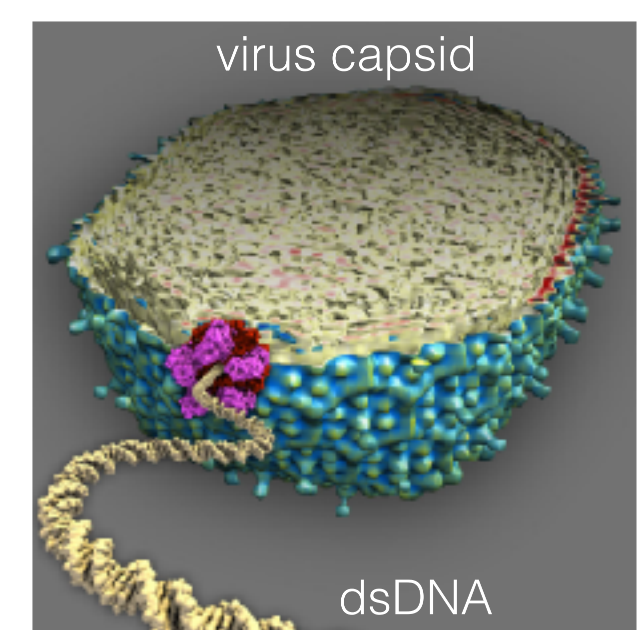
$L_p << L_c$

(50 nm << cm)

DNA



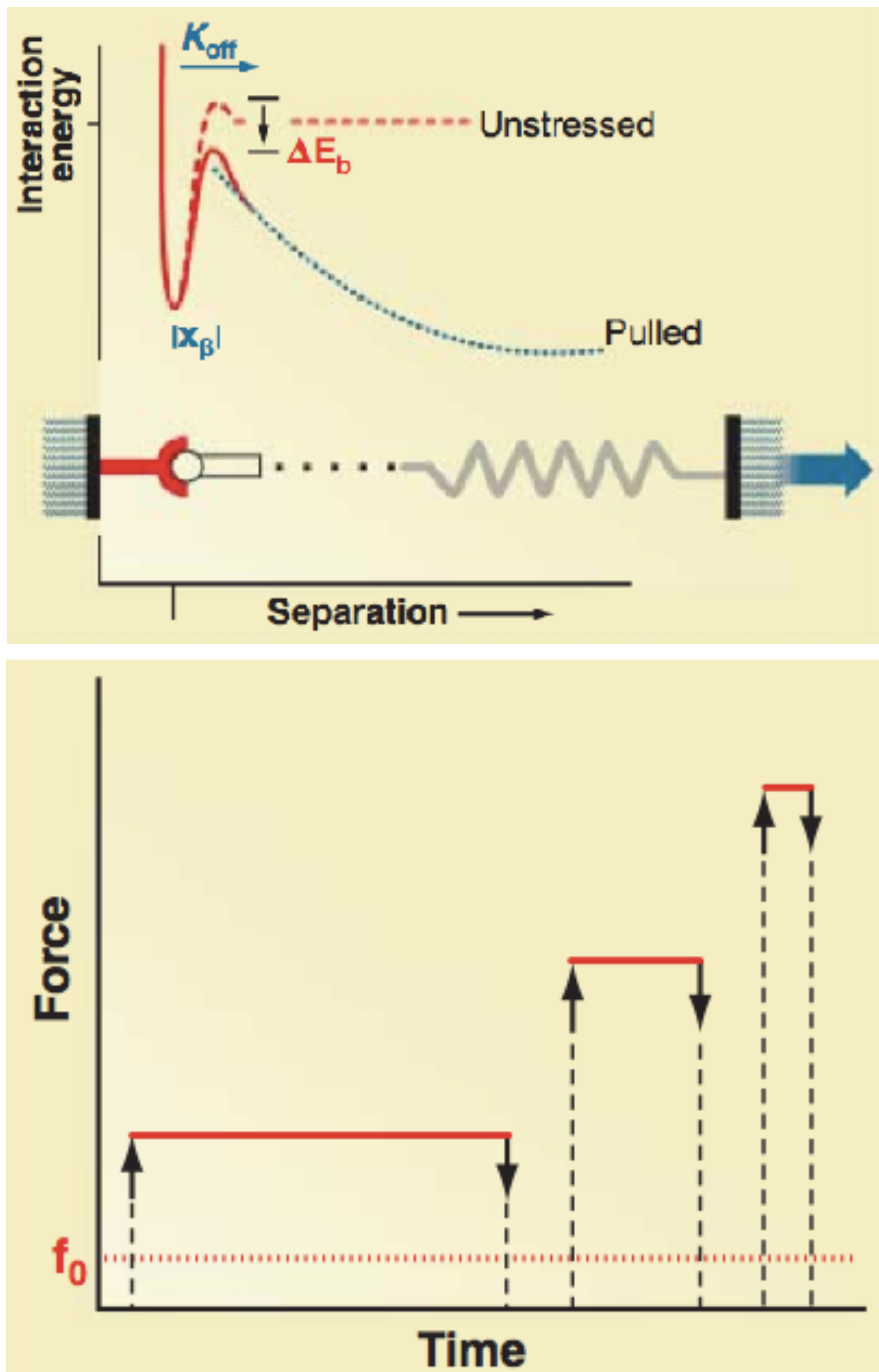




L_p = persistence length

L_c = contour length

3. Force: reduces bond lifetime

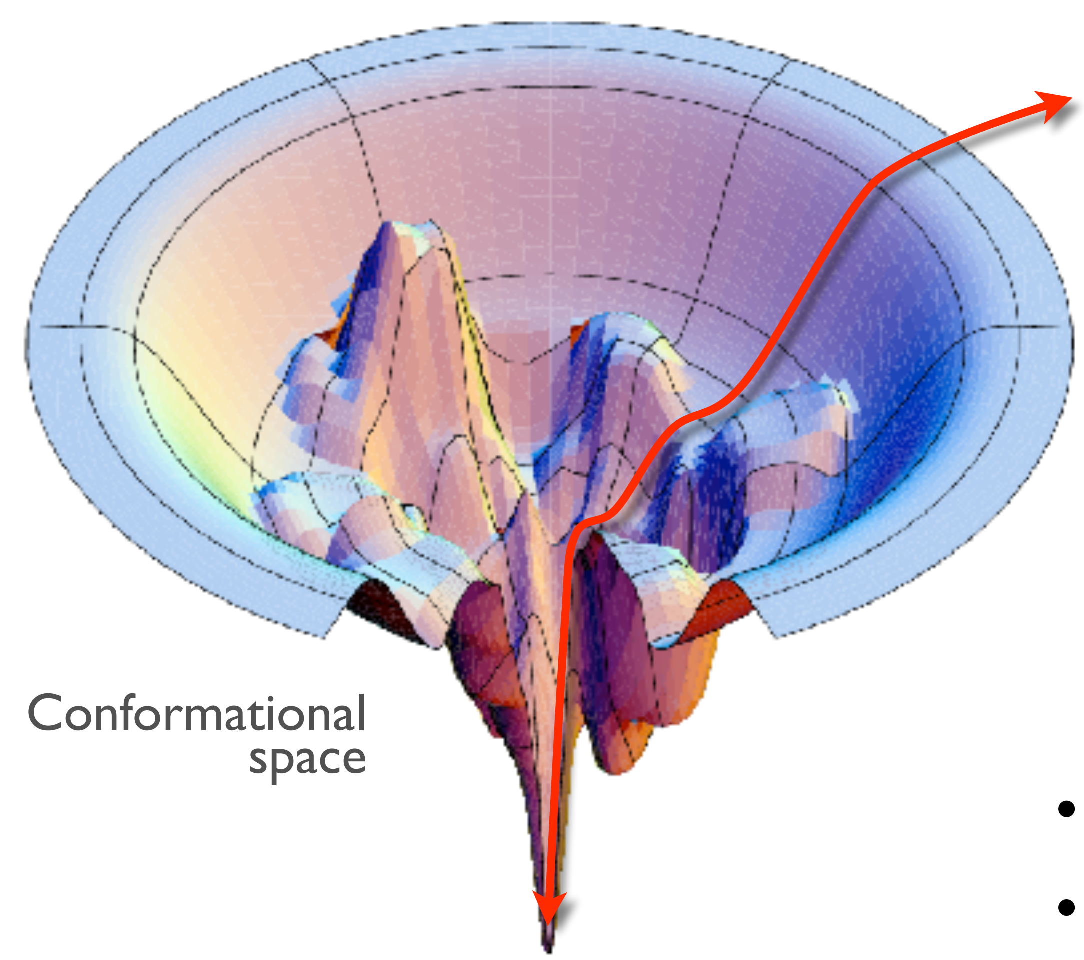


Evan A. Evans and David A. Calderwood Science 316, 1148 (2007)

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

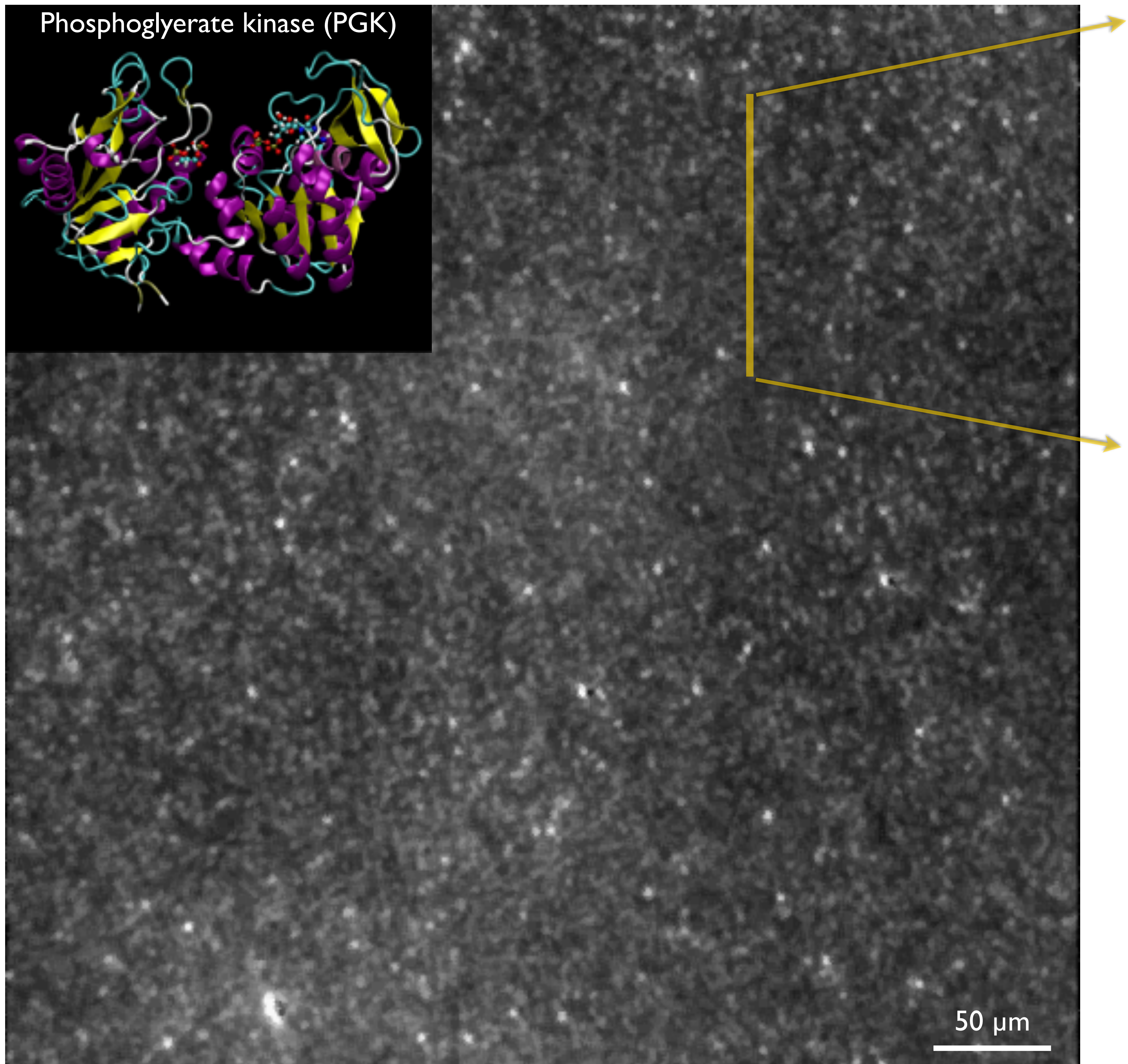
Under mechanical load: $\tau(F) = \omega e^{\frac{E_a - F\Delta x}{k_B T}} = \tau(0) e^{-\frac{F\Delta x}{k_B T}}$

ω = characteristic time
E_a = activation energy
Δx = distance between bound and transition states

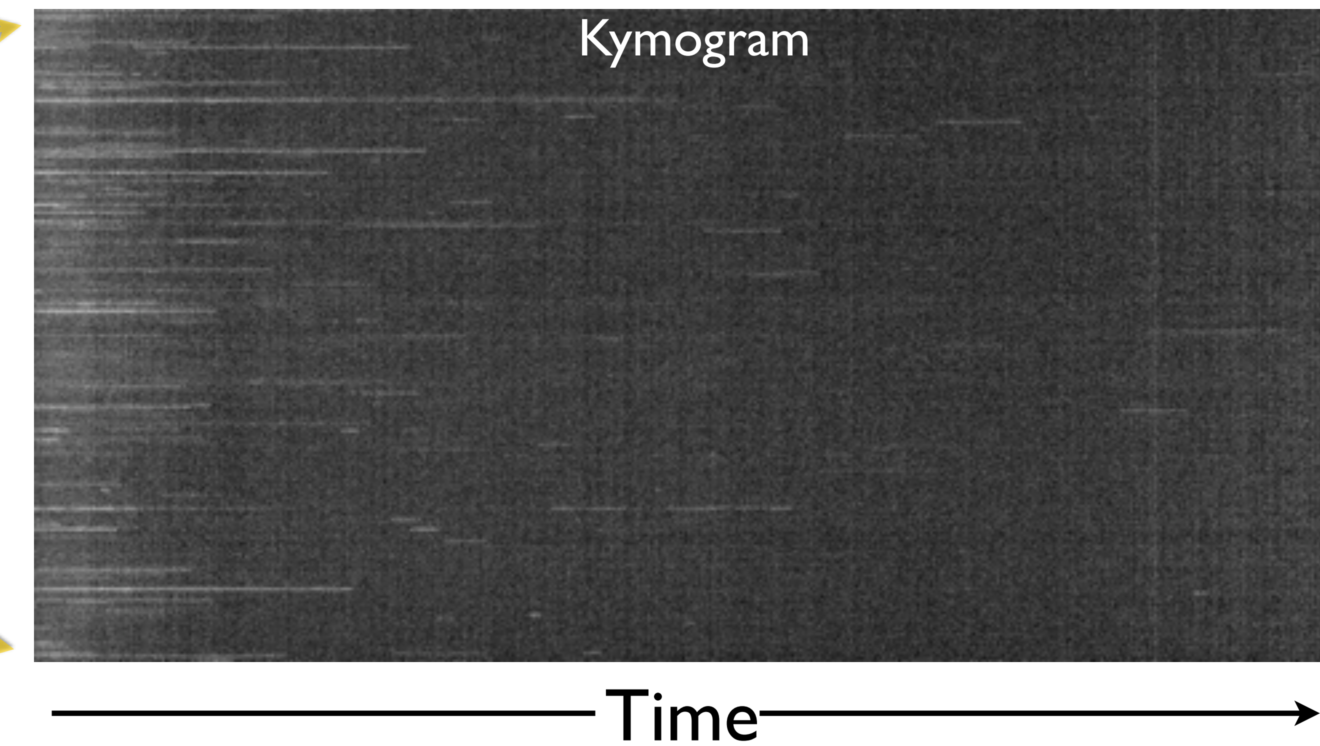


- Covalent bonds break at *~nN*
- H-bonds, electrostatic interactions break at *~tens of pN*

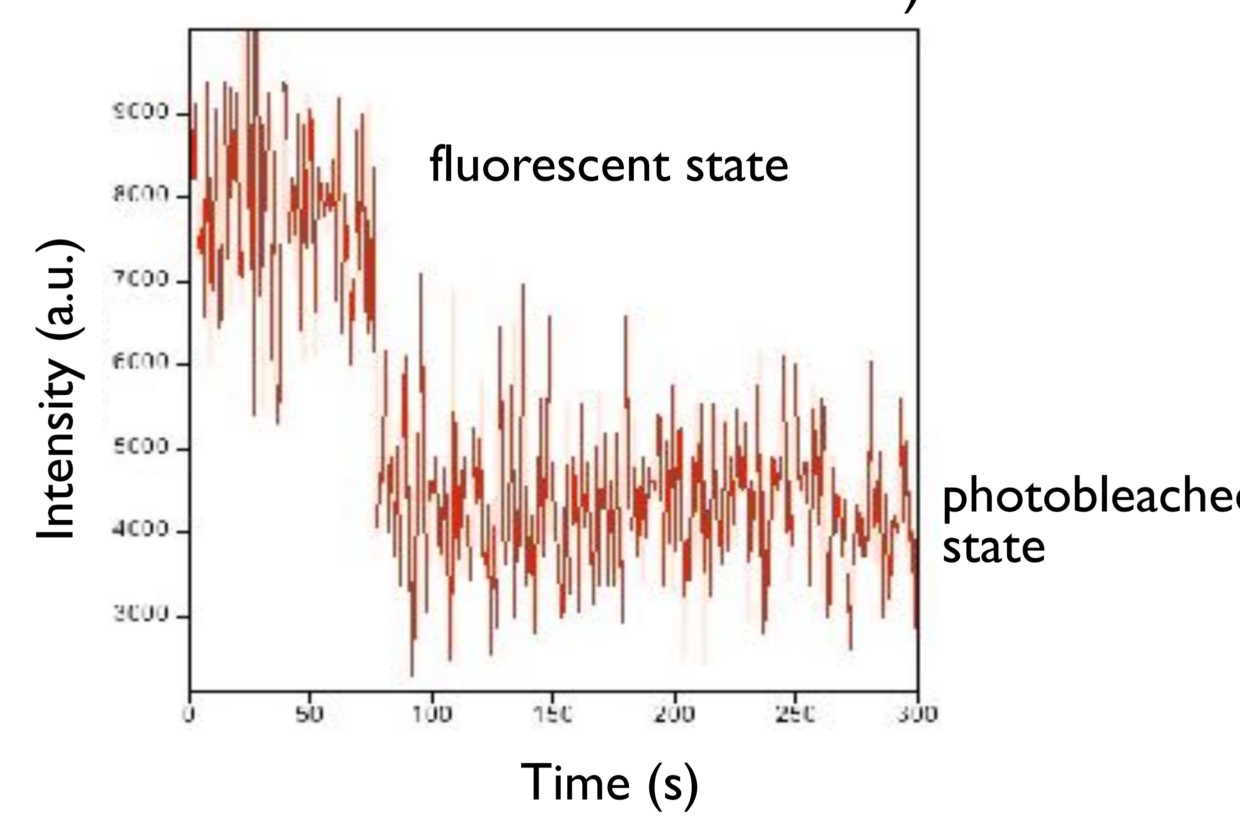
Molecular singularity I.
Single-step photobleaching



PGK molecules labeled with Alexa488, TIRF microscopy

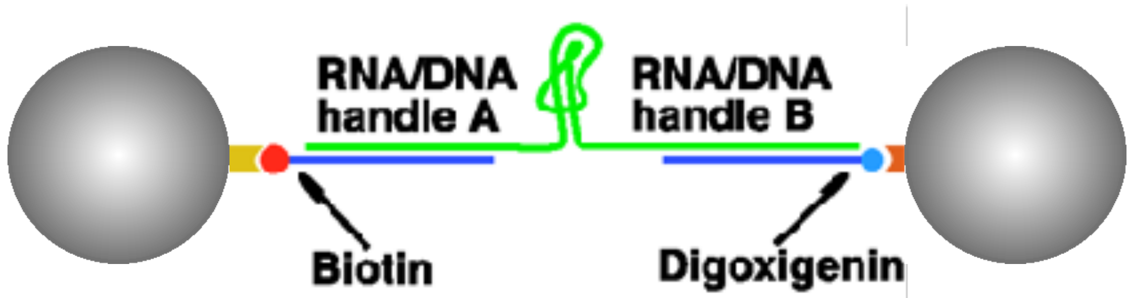


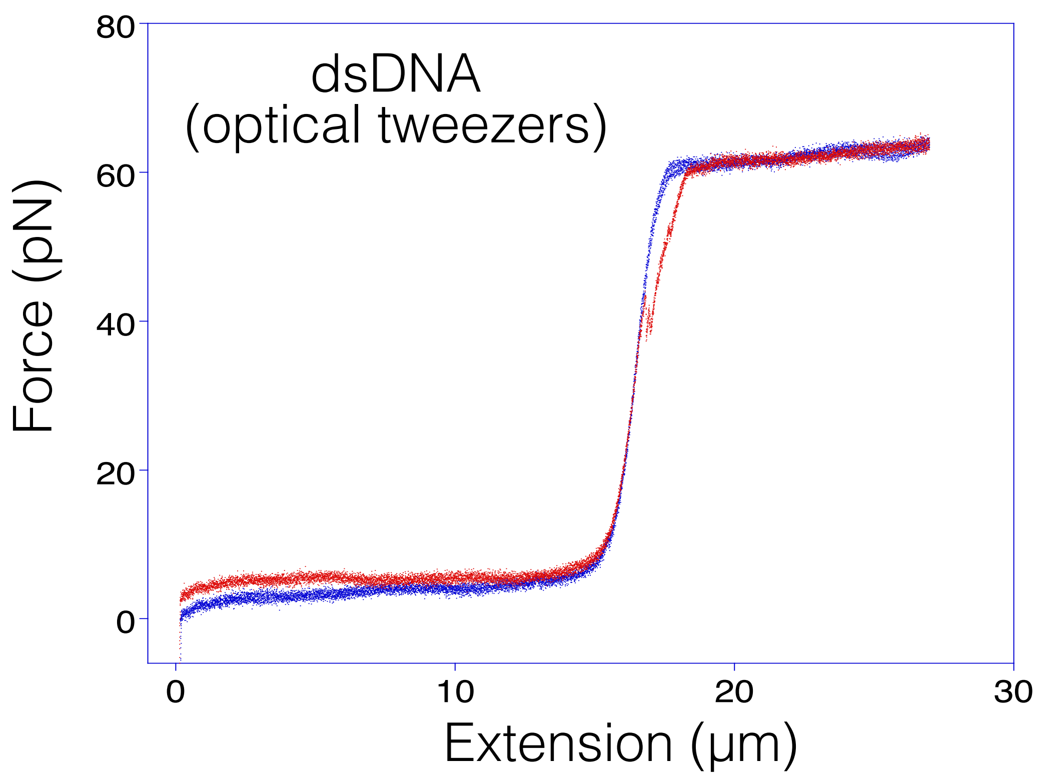
Single-step photobleaching (exponential function in an ensemble!)



Molecular singularity II. Nanomechanical fingerprint

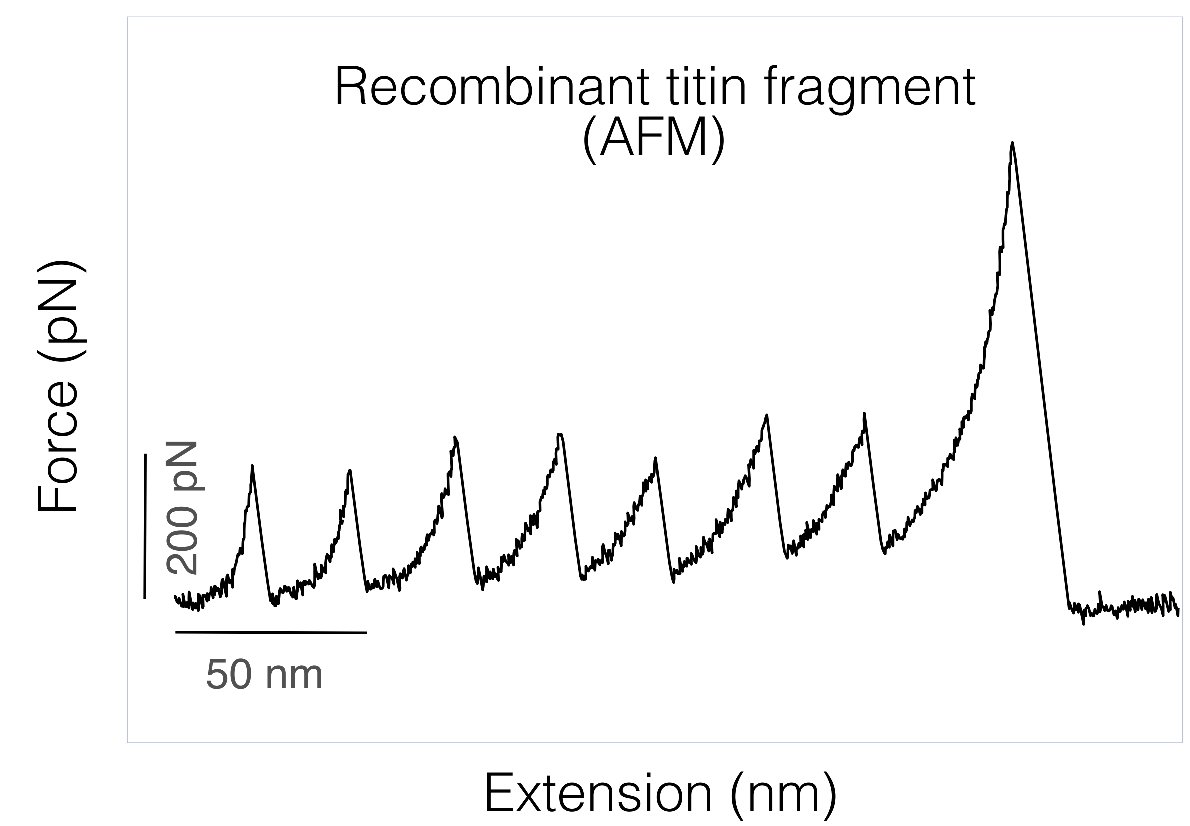
DNA handle





Cooperative transition at 65 pN

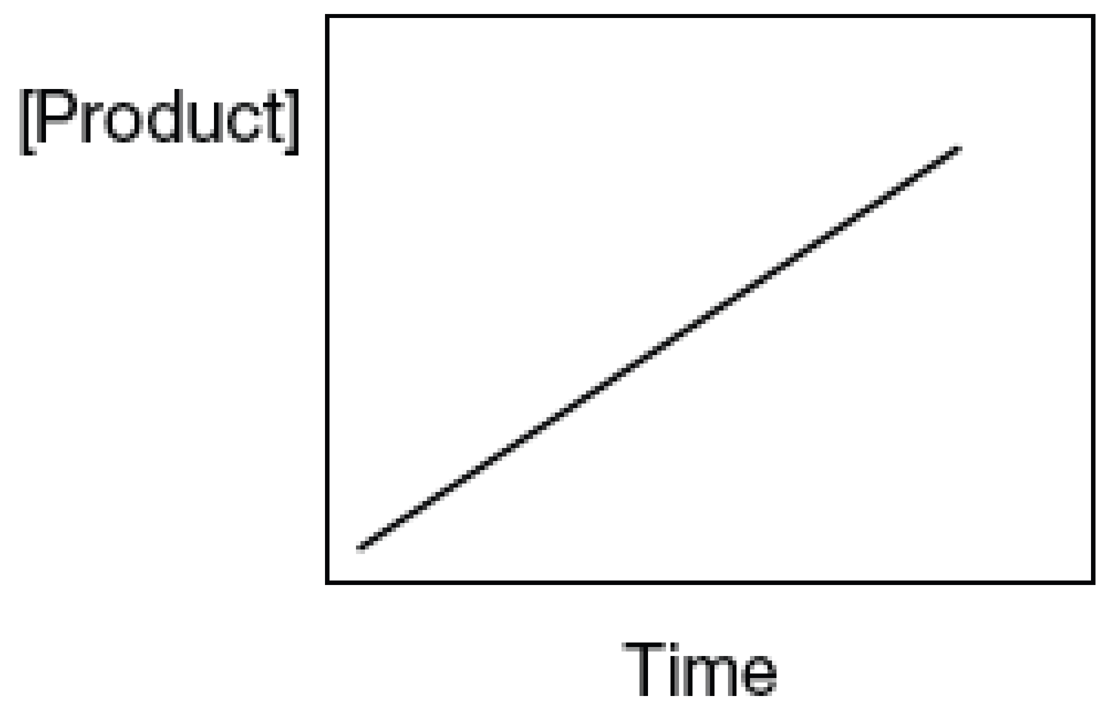
Recombinant polyprotein

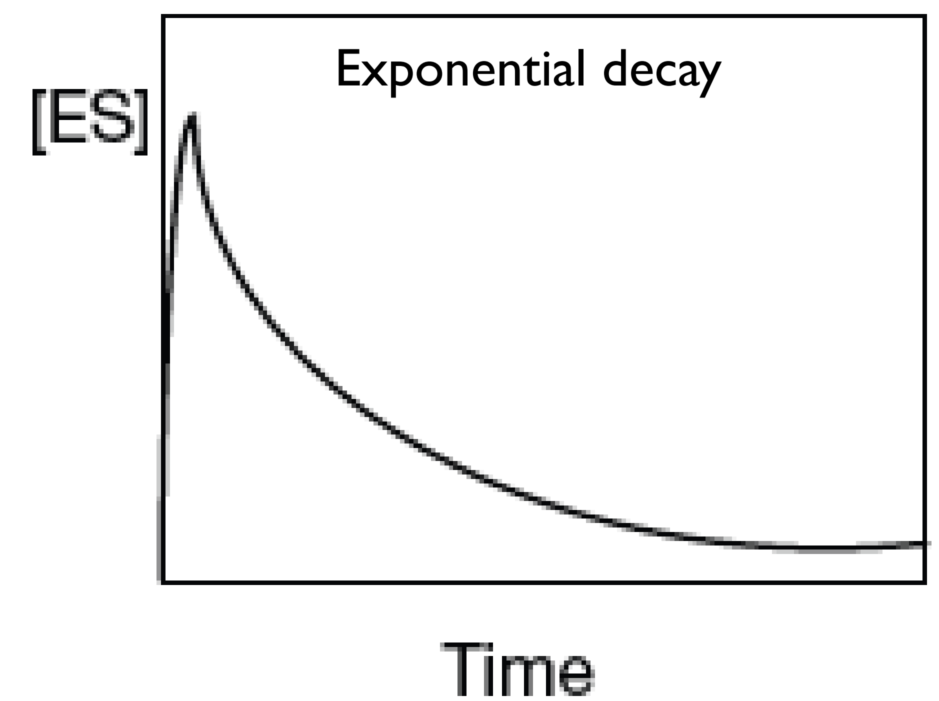


Sawtooth-shaped, equidistant transitions

Ensemble *versus* single molecule behavior

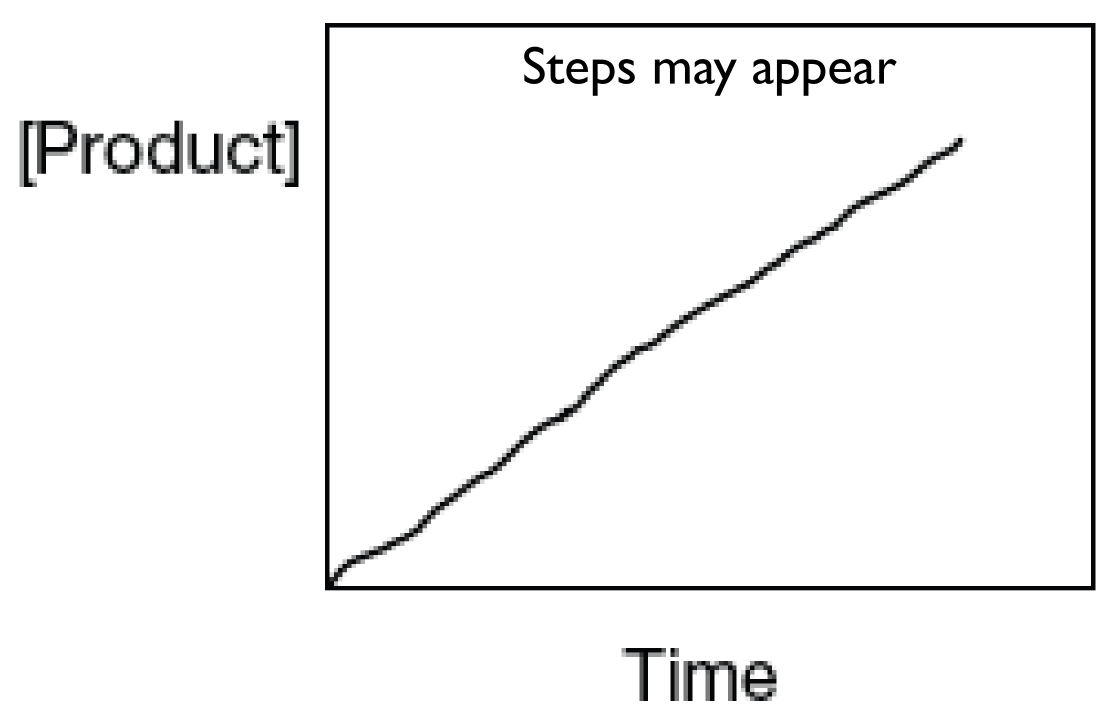
Ensemble:

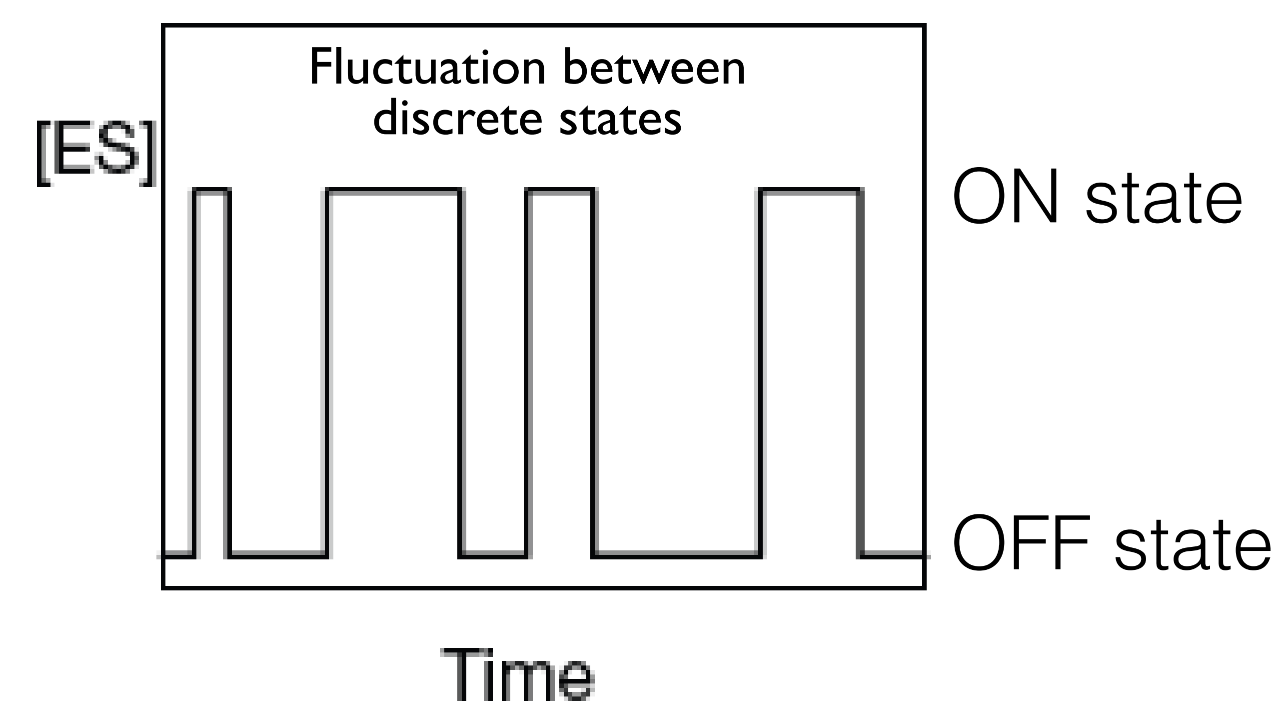




[ES] = [Enzyme-substrate complex]

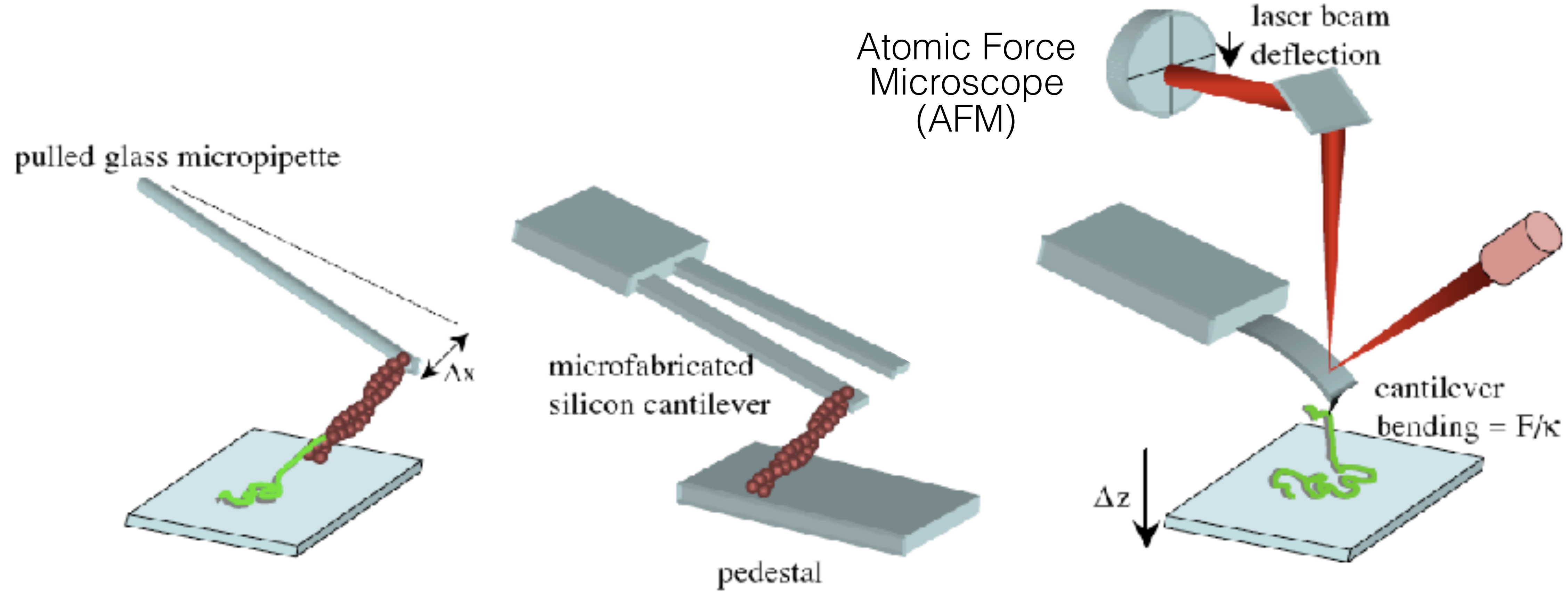
Single molecule:



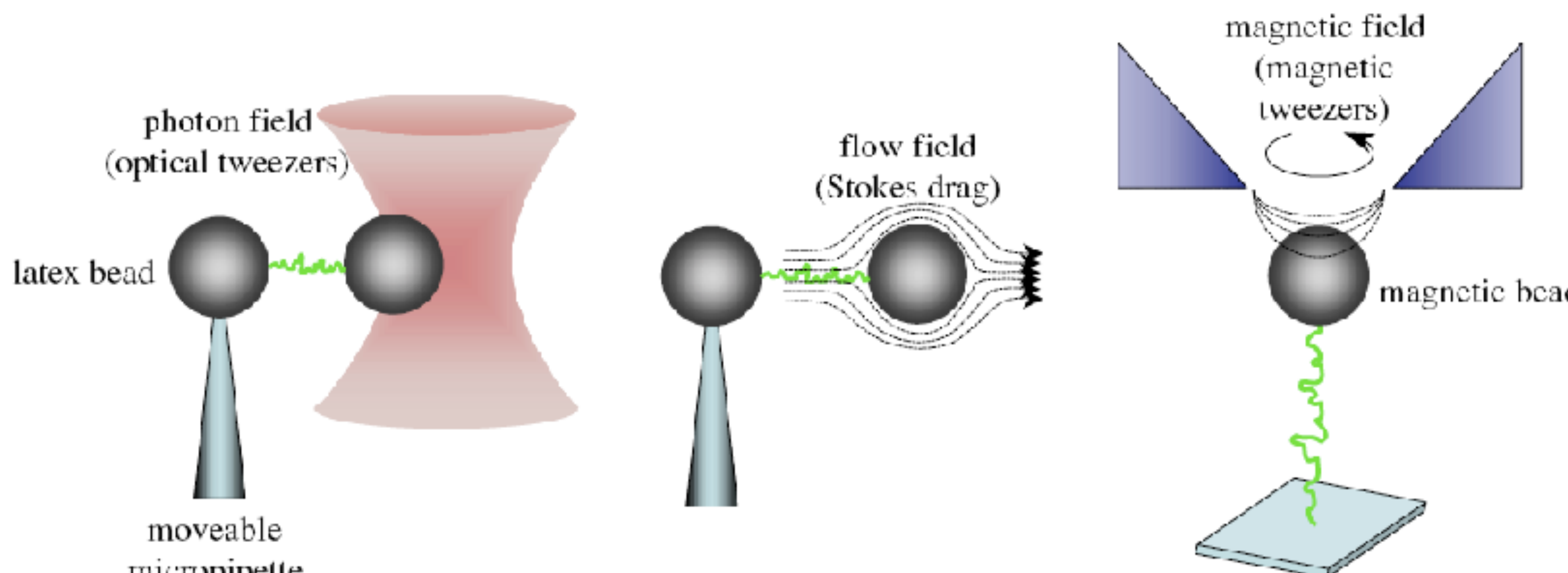


Methods of manipulation

Cantilever methods

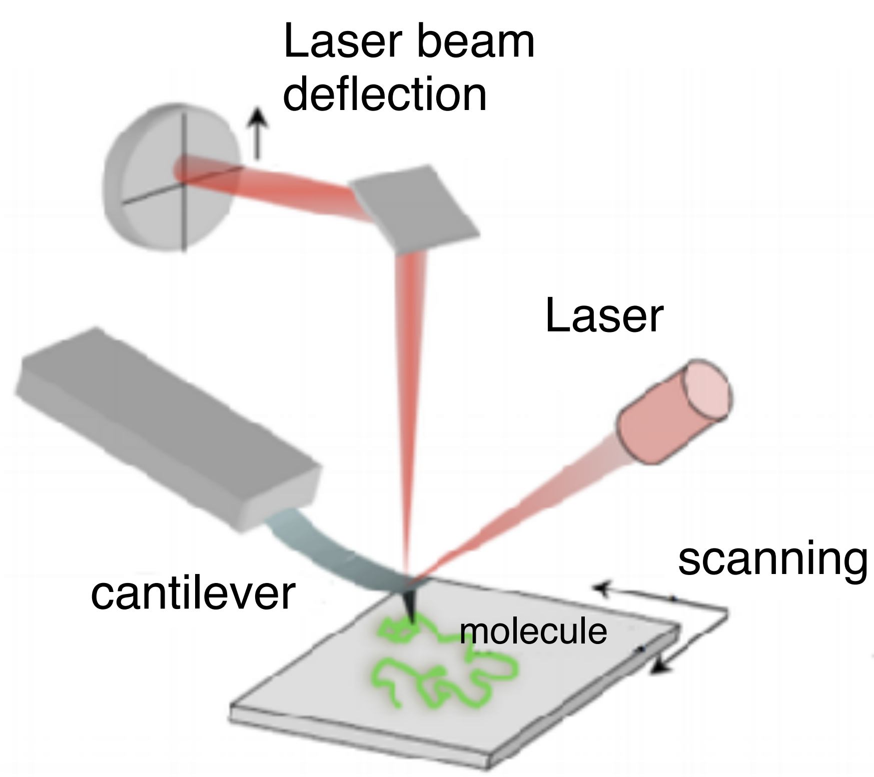


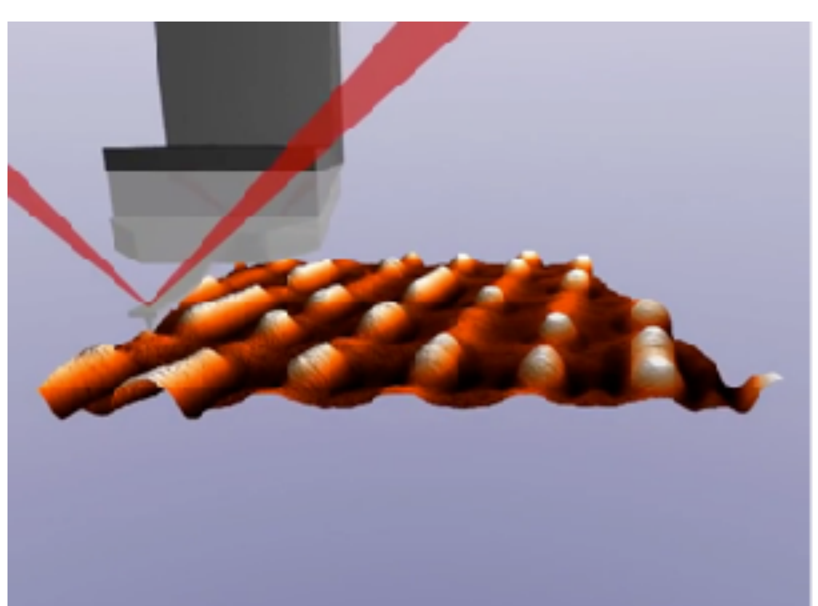
Field-based methods

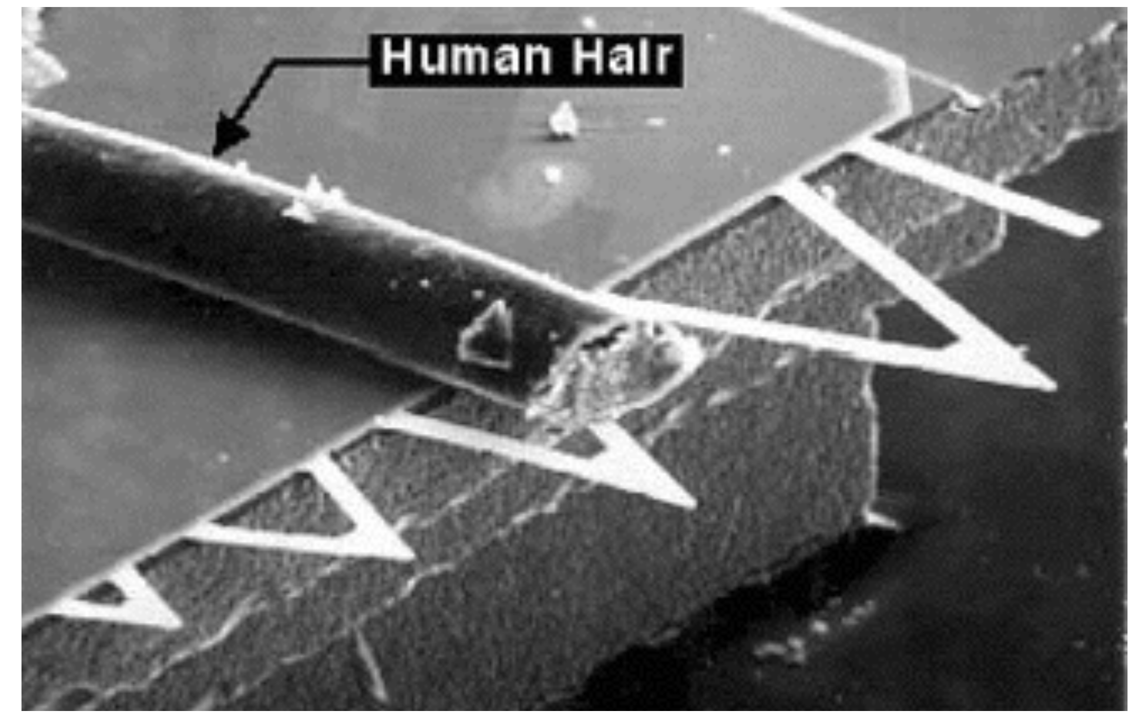


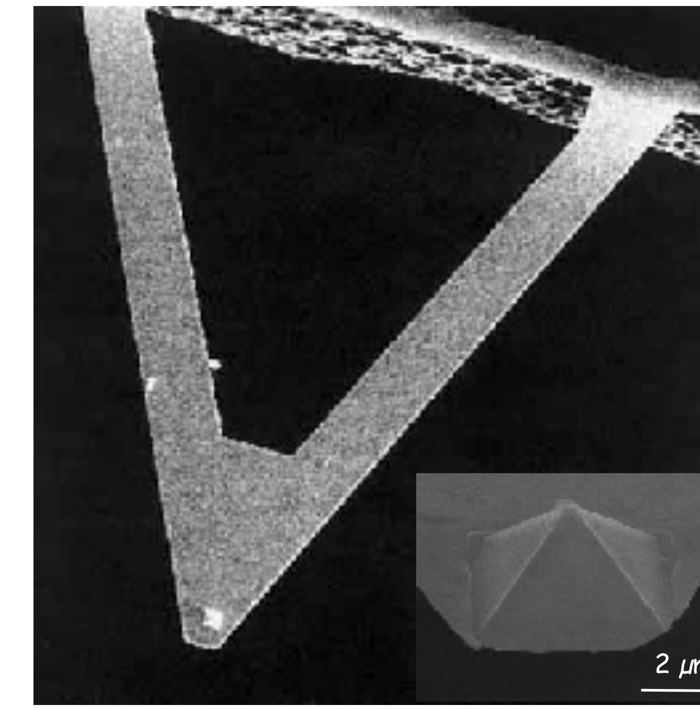
Manipulation with AFM

Atomic Force Microscopy

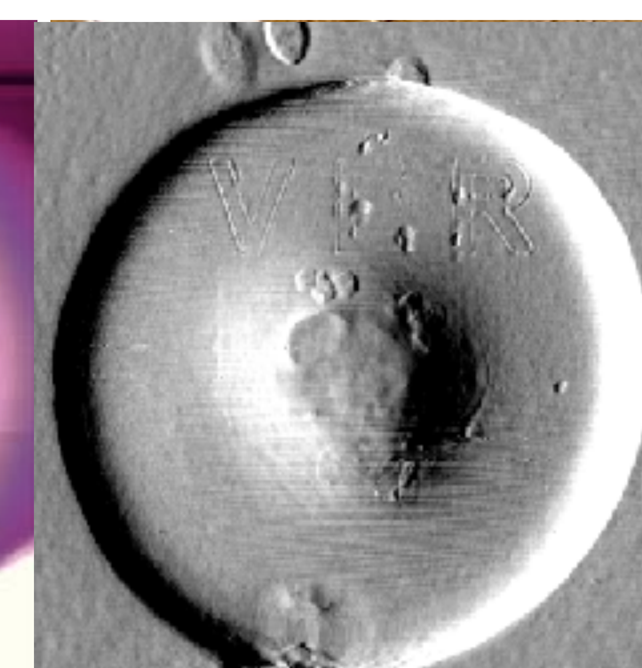




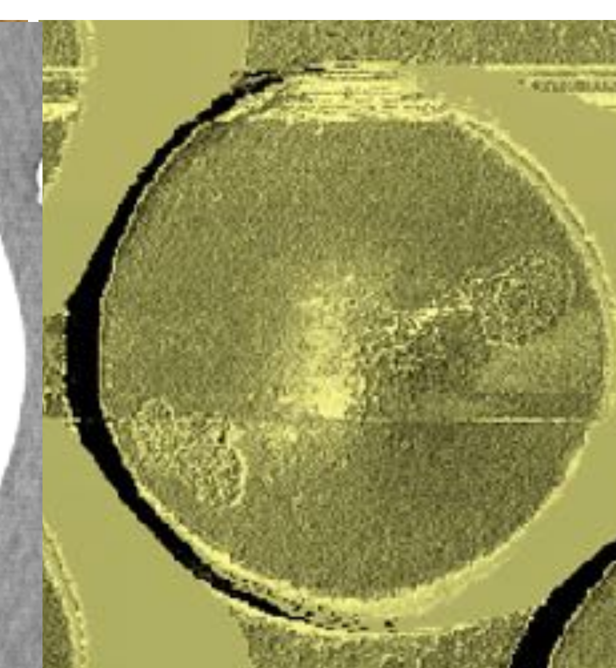




Height contrast



Amplitude contrast



Phase contrast

Molecular manipulation with AFM

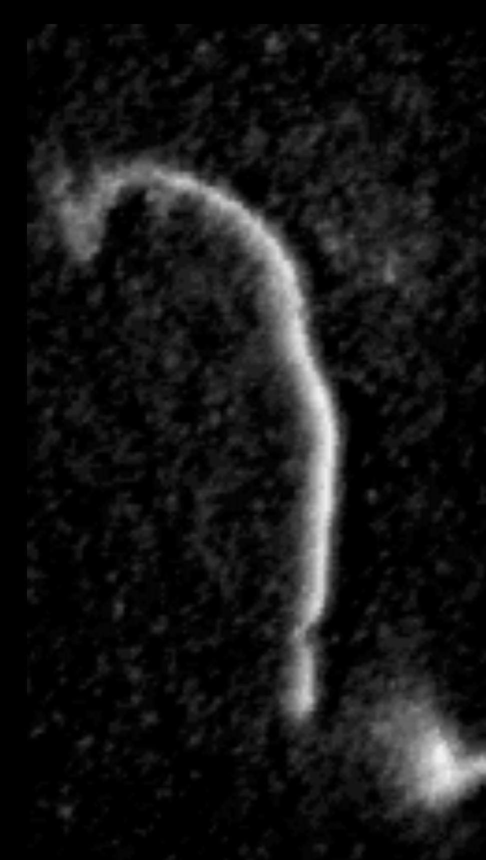
Manipulation with light

Optical tweezers

Arthur Ashkin, Nobel-prize 2018

E. coli bacterium

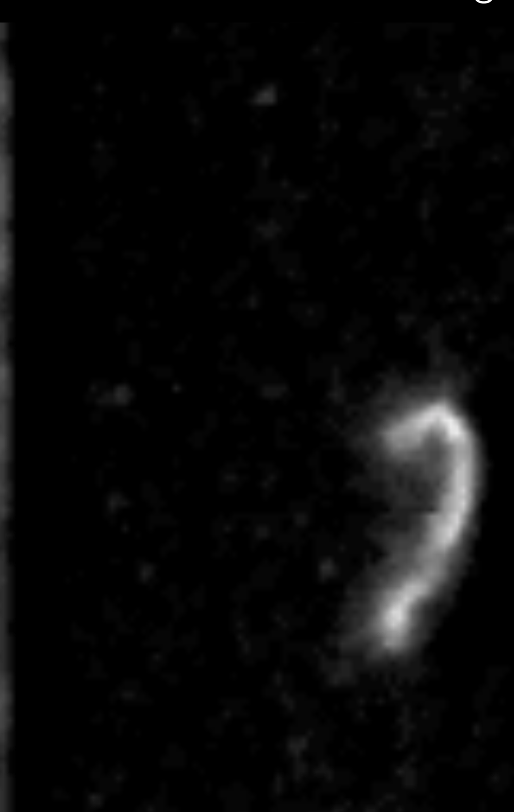
Actin filament



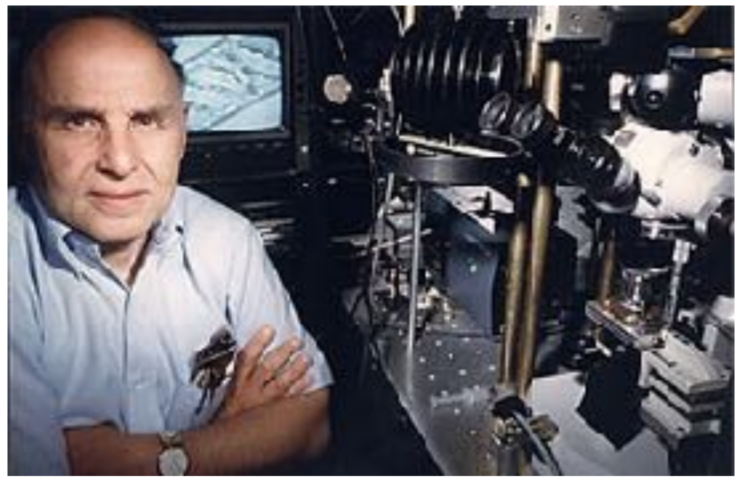
DNA

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

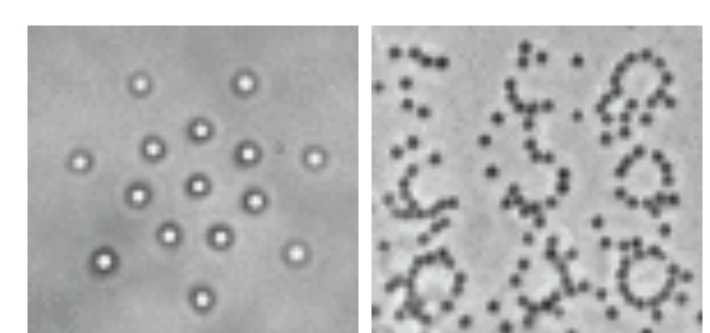
C.Bustamante

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

J.Molloy

S. Block

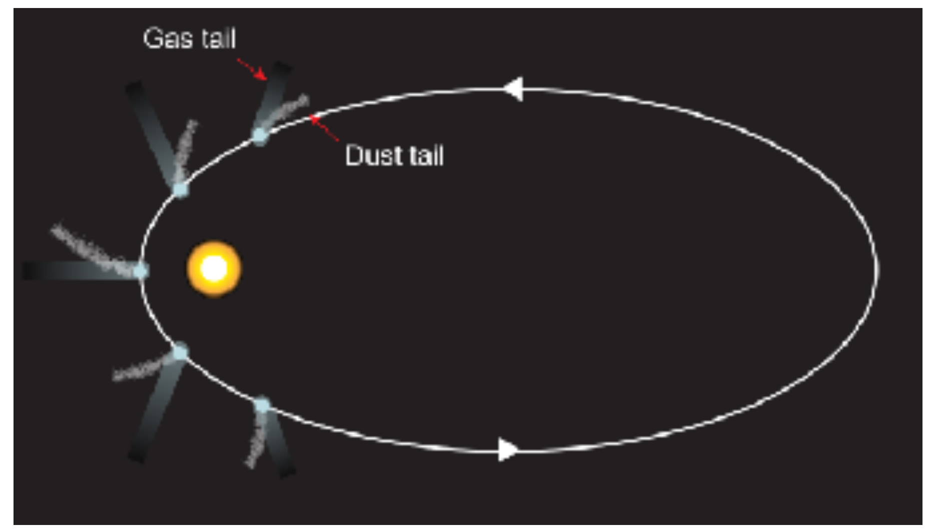
Microfabricated propeller



Simultaneous manipulation of many particles with holographic optical tweezers

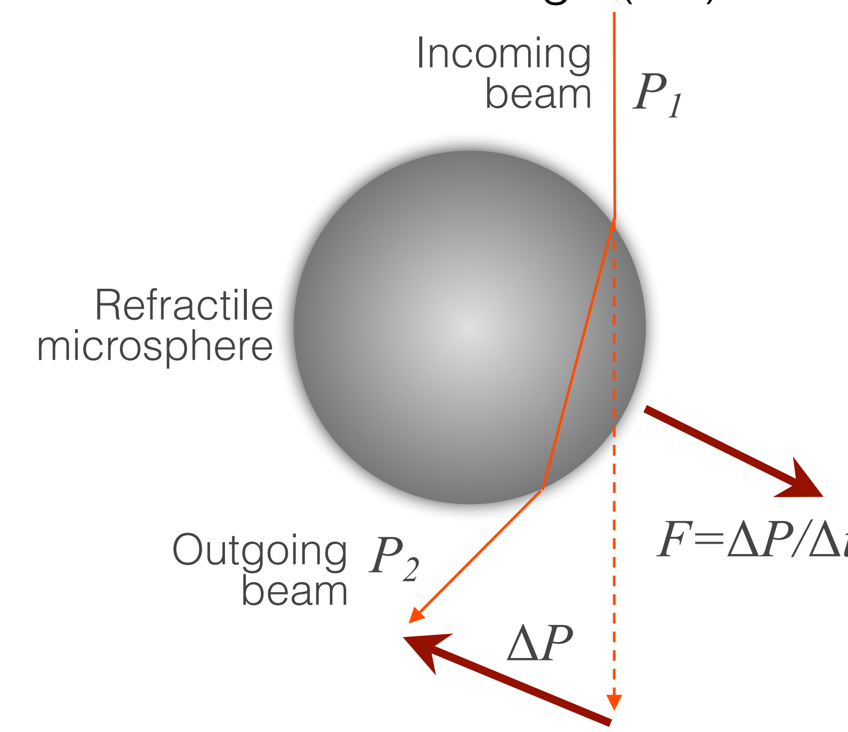
Photonic momentum changes upon interaction with particles

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

$$c = \lambda f$$

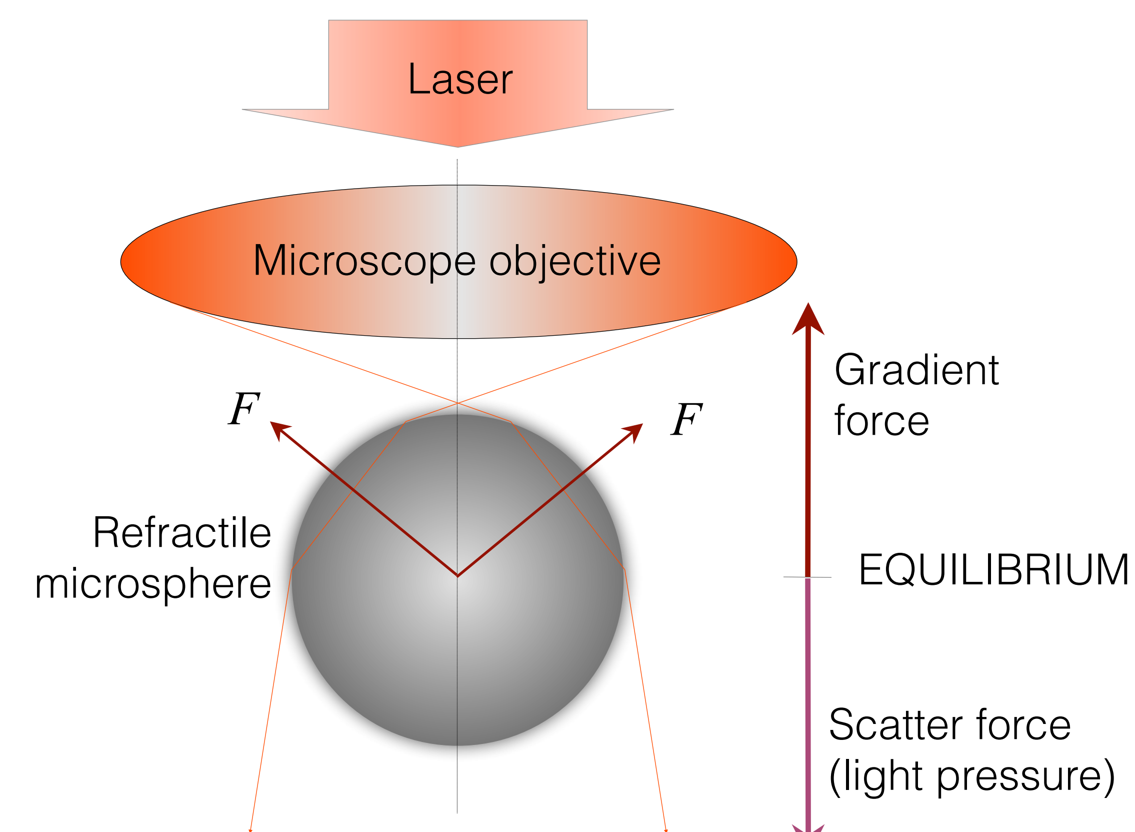
Louis-Victor-Pierre-Raymond, 7th duc de Broglie (1892-1987)

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

Momentum of photon:

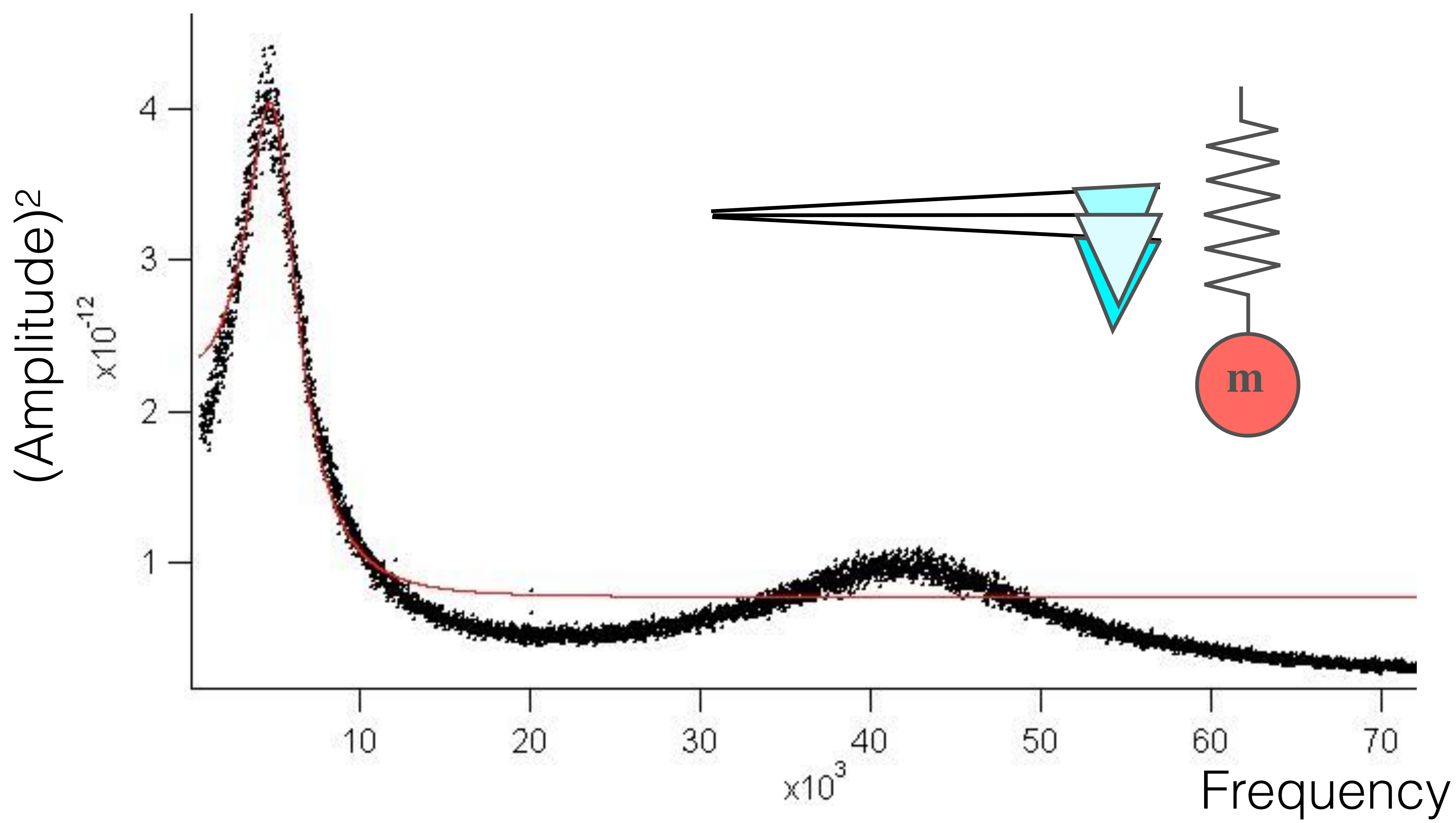
$$P = \frac{h}{\lambda}$$

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)



$$\kappa = m\omega^2$$

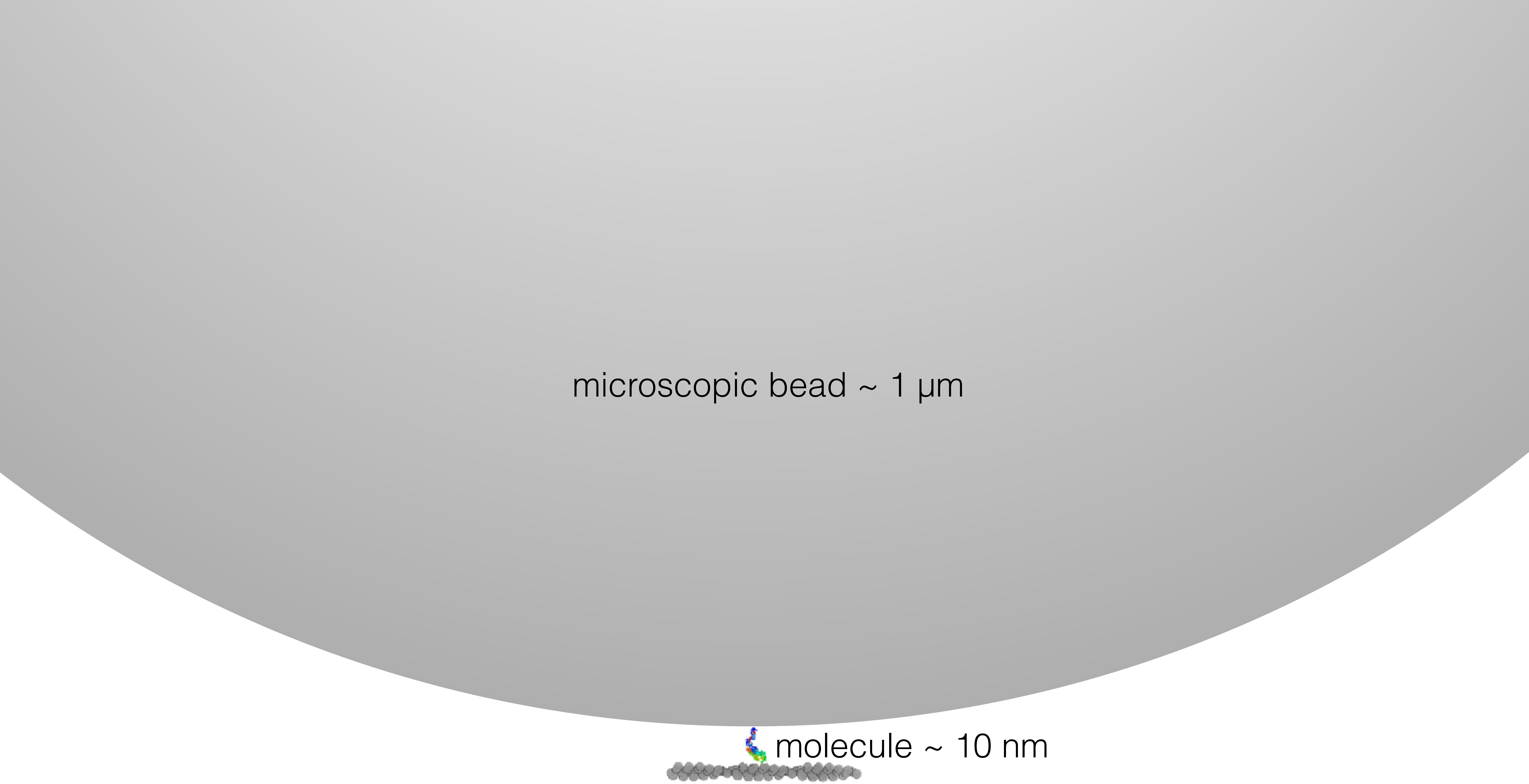
$$\frac{1}{2}\kappa\left\langle x^2 \right\rangle = \frac{1}{2}k_B T$$

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

AFM cantilever stiffness ~ 10-1000 pN/nm

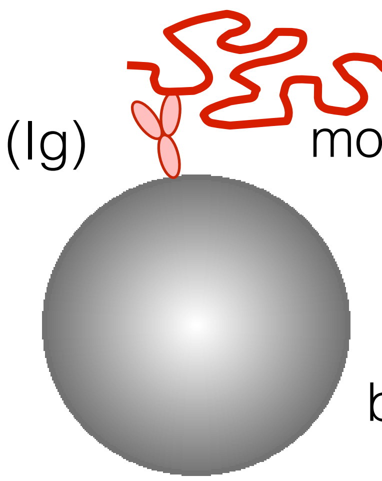
Optical trap stiffness ~ 0.1-1.0 pN/nm

Molecule - handle geomety

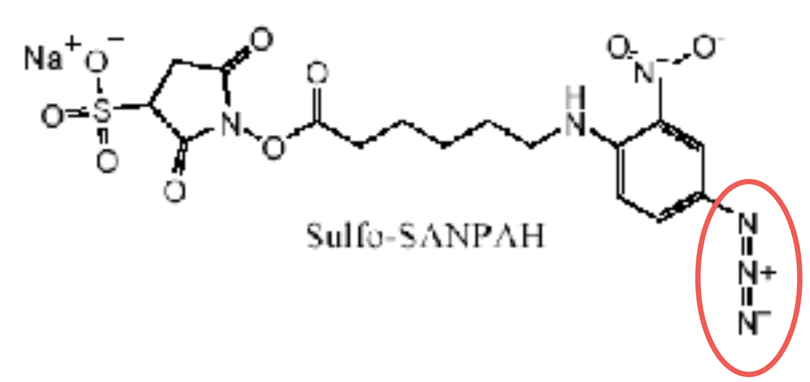


Attaching the molecule to the handle

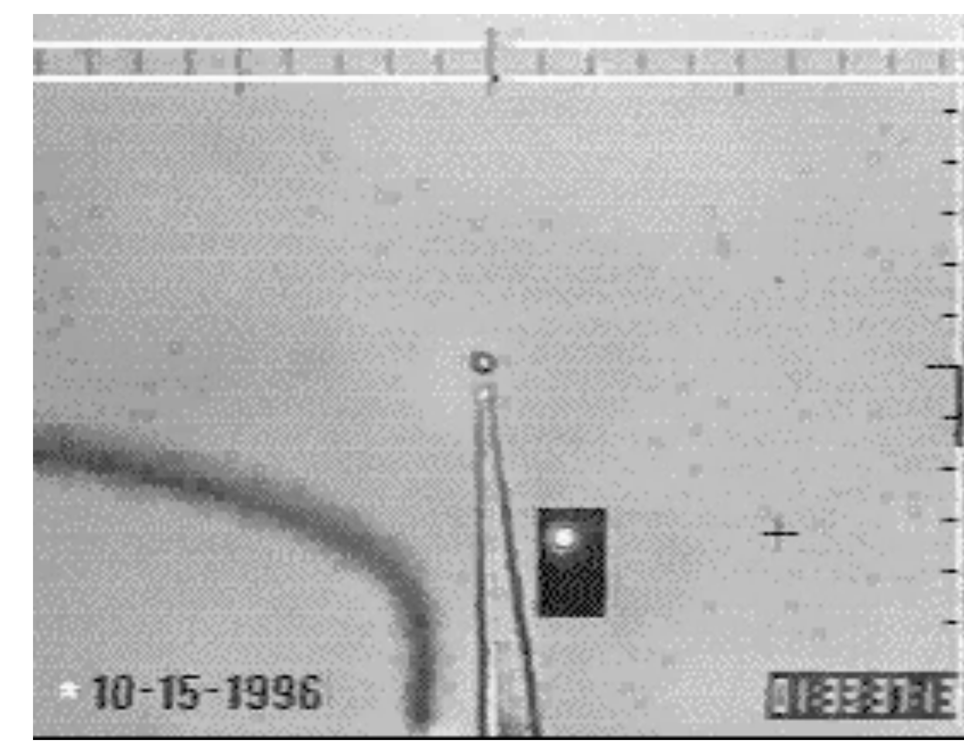
Sequence-specific antibodies



Photoreactive cross-linker - "molecular welding"

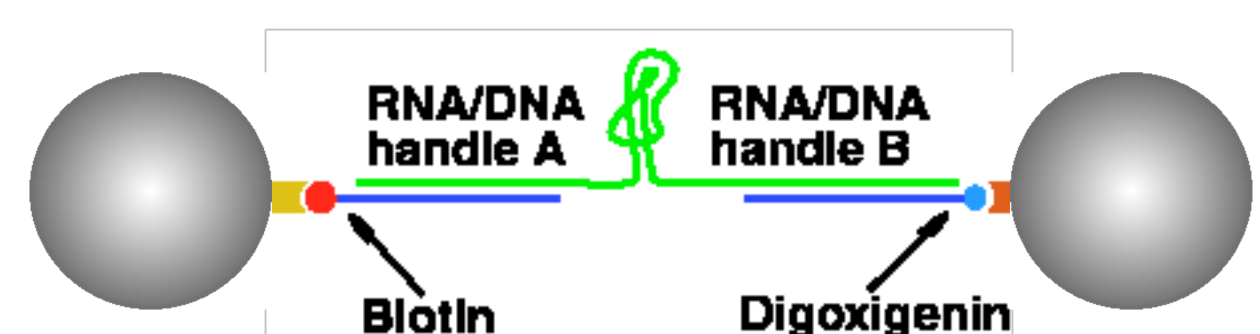


- Non-specific
- Photoreactive N_3- (azido) group (reacts with amines)
- UV illumination

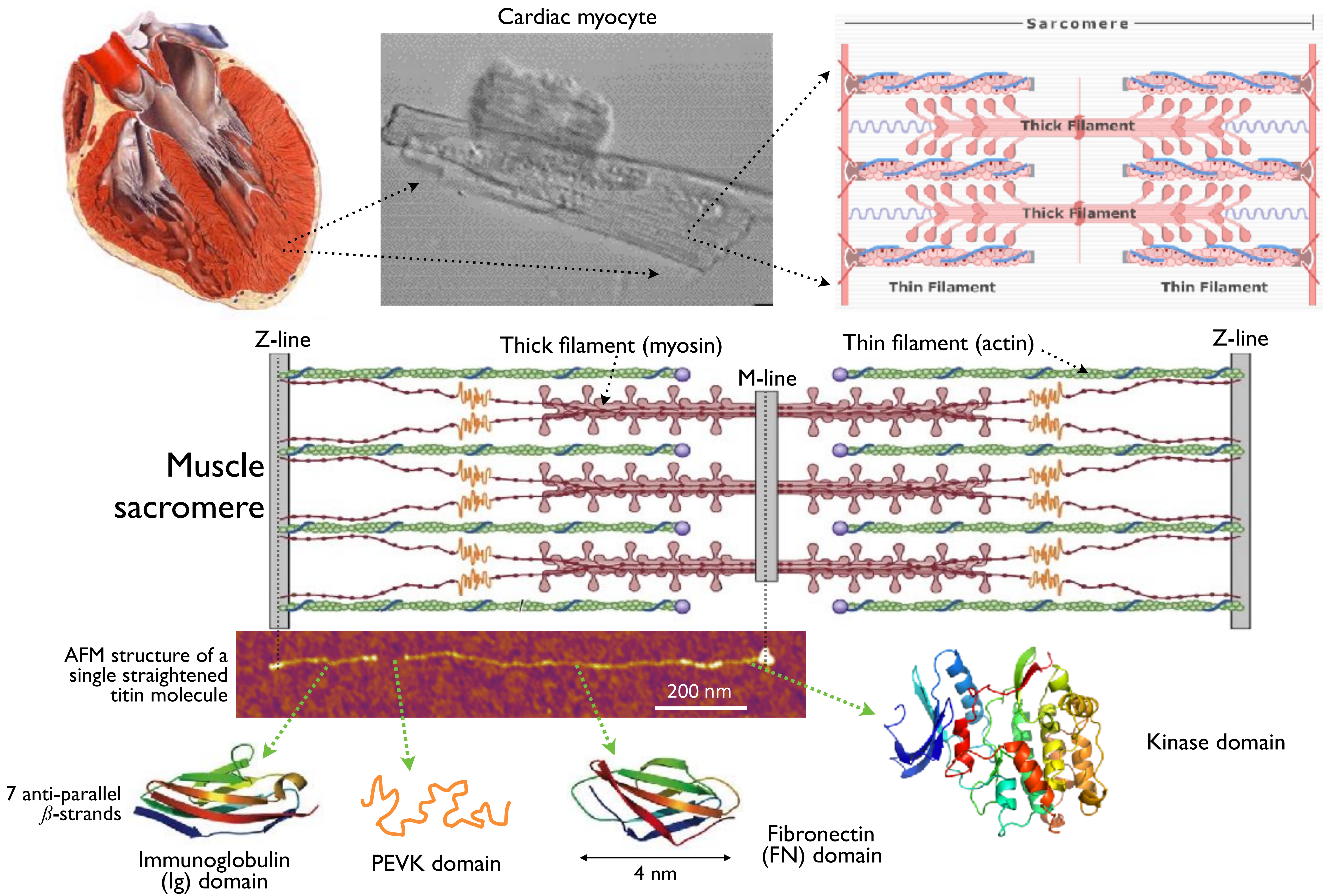


DNA handle

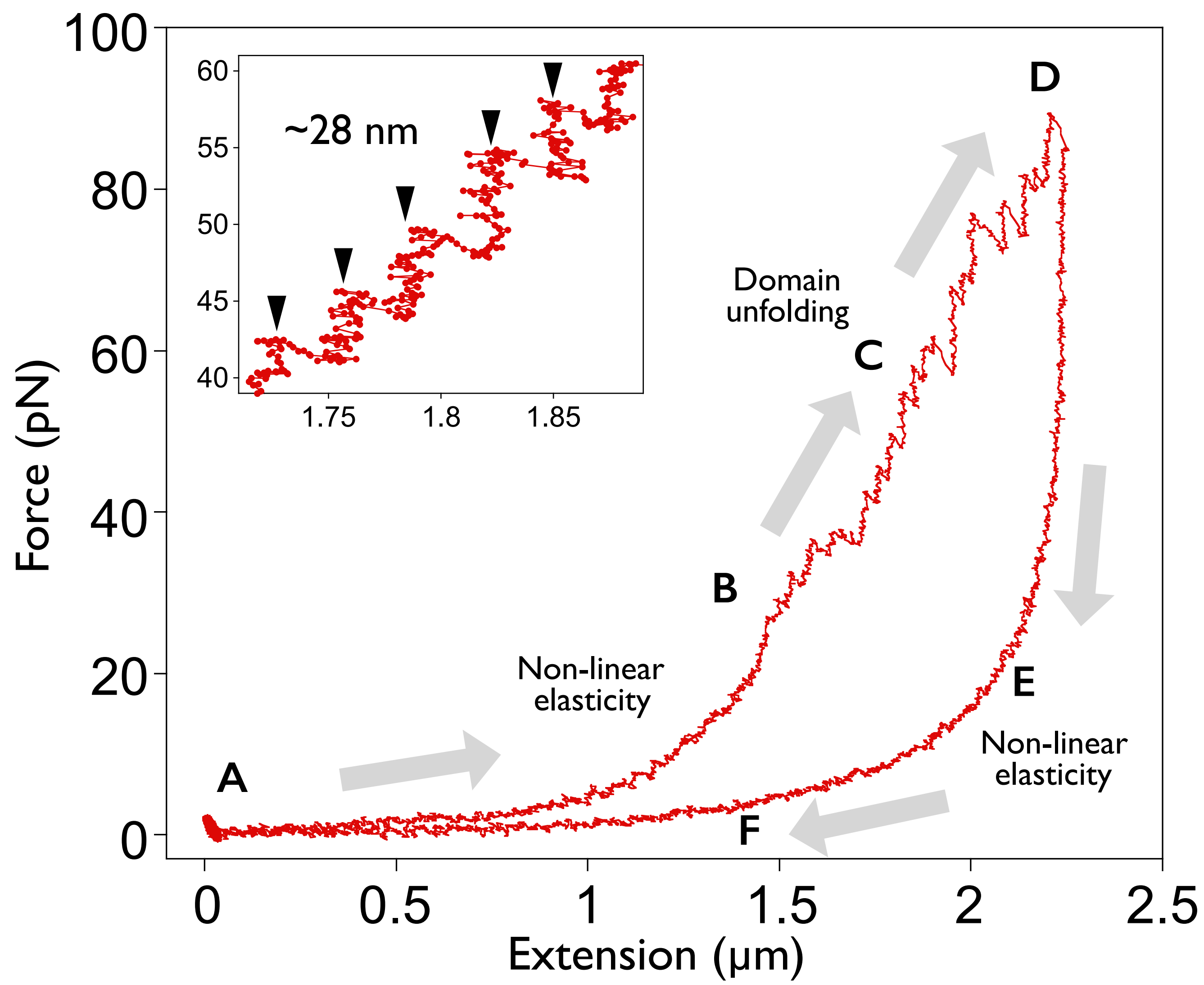
- Molecular dimension
- Can be made specific with cloning techniques
- Provides mechanical fingerprint

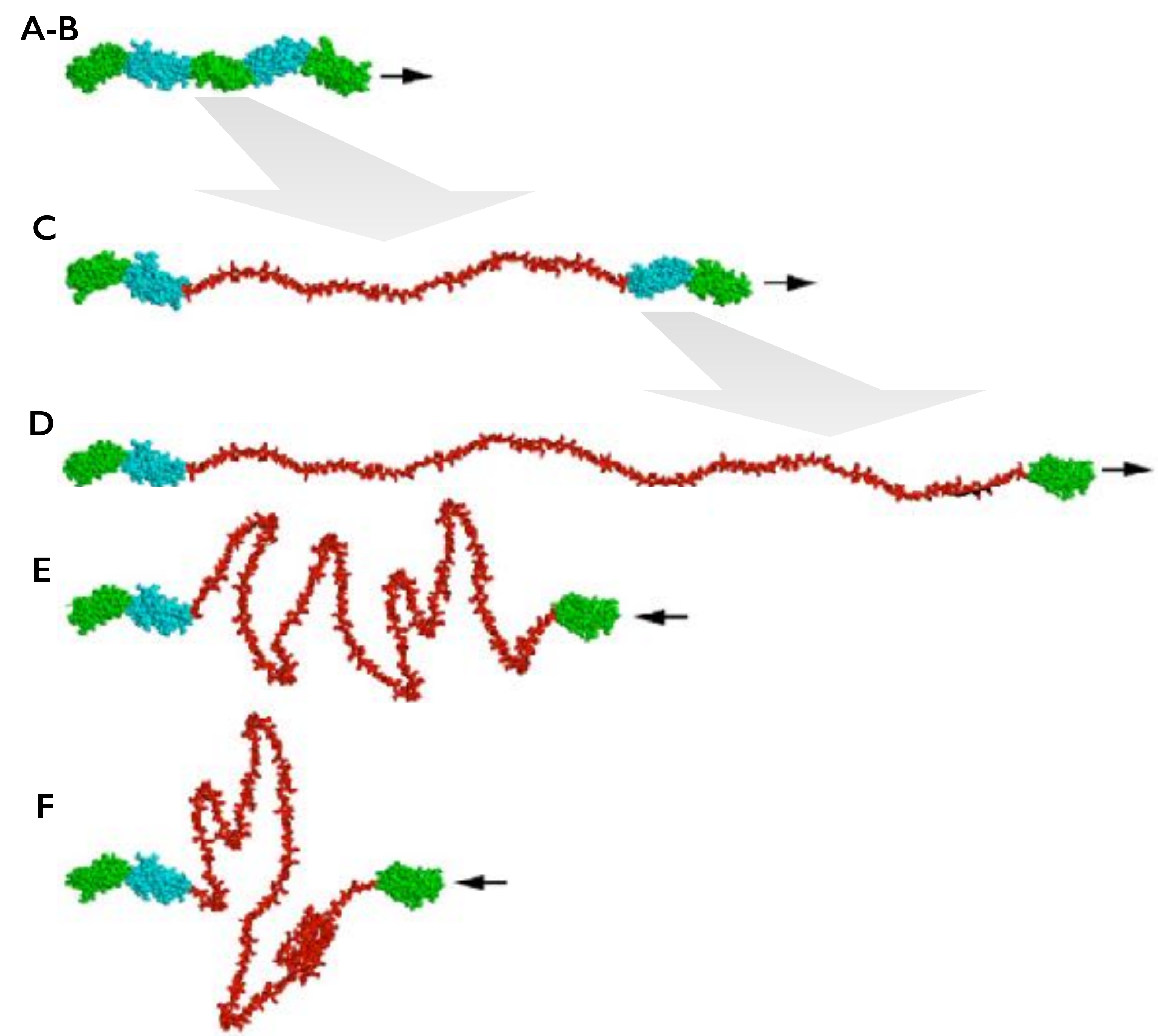


1. Titin: giant elastic muscle protein

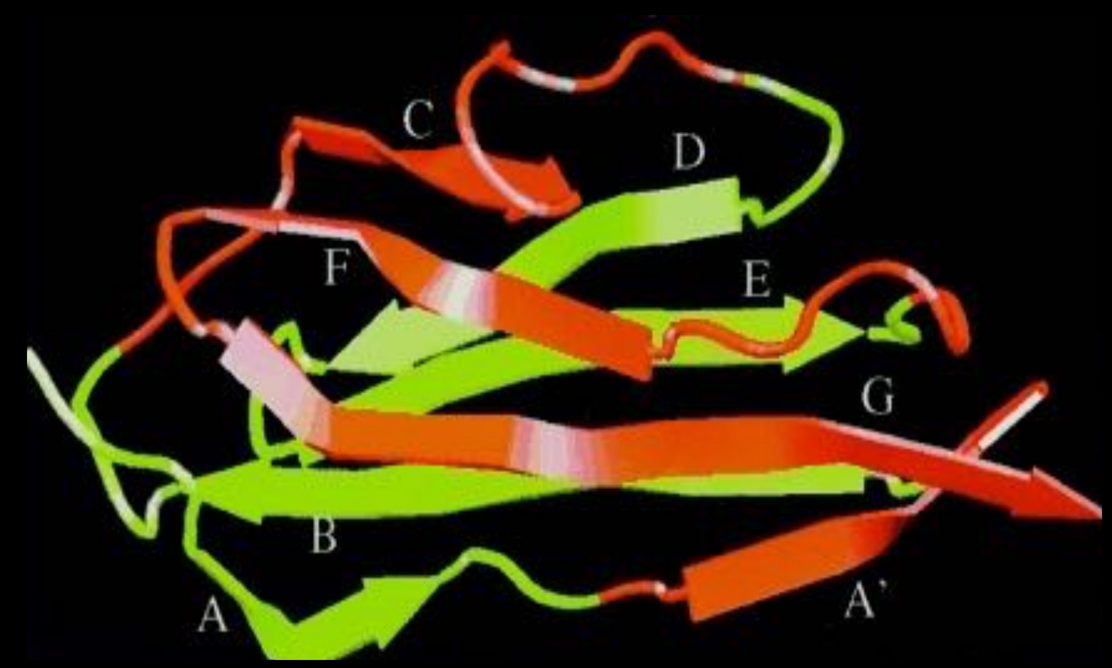


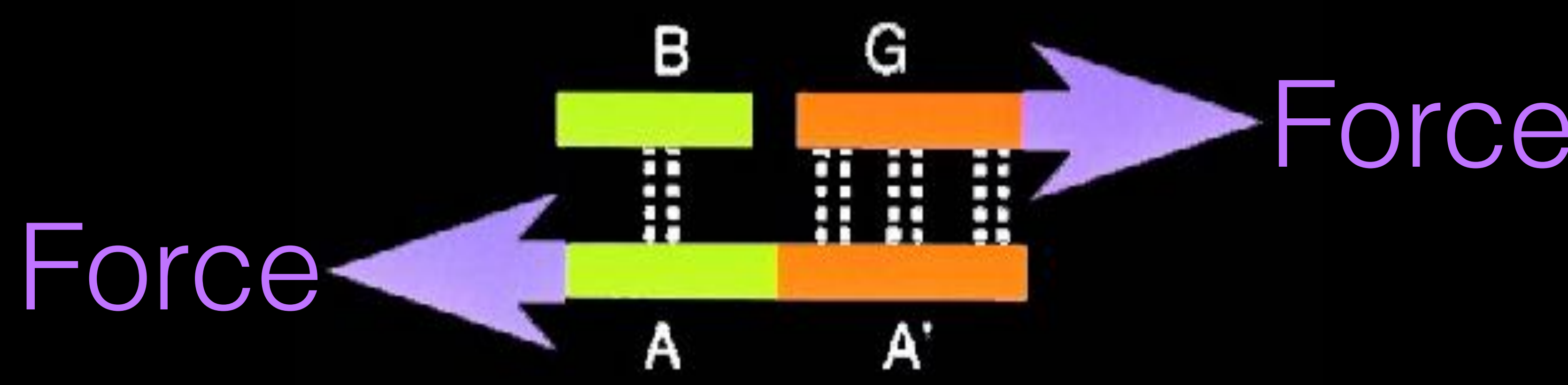
Titin mechanics - constant-velocity experiment: non-linear elasticity + domain unfolding

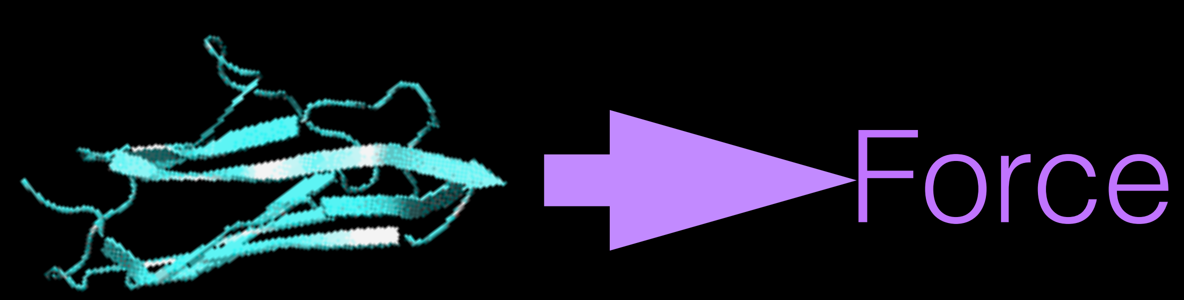




Structural basis of mechanical stability

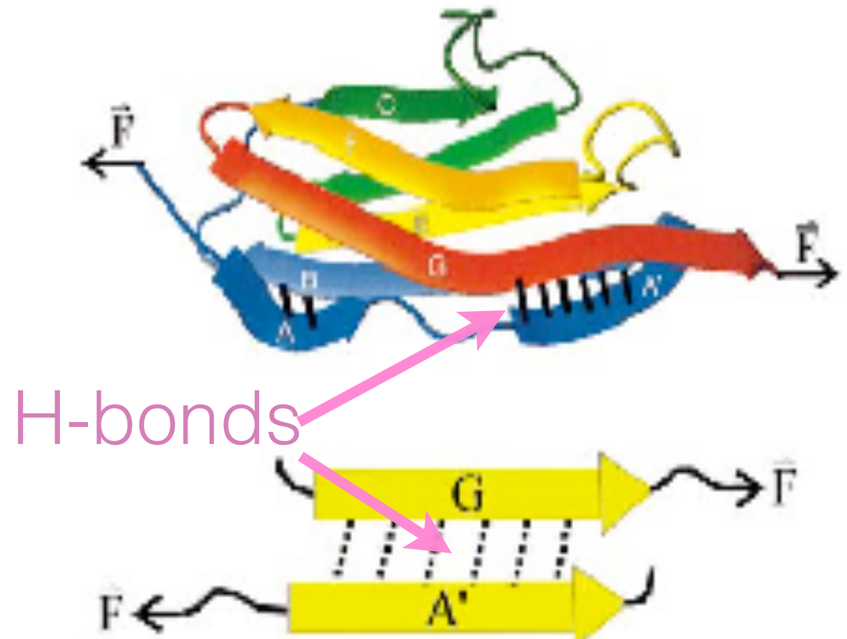




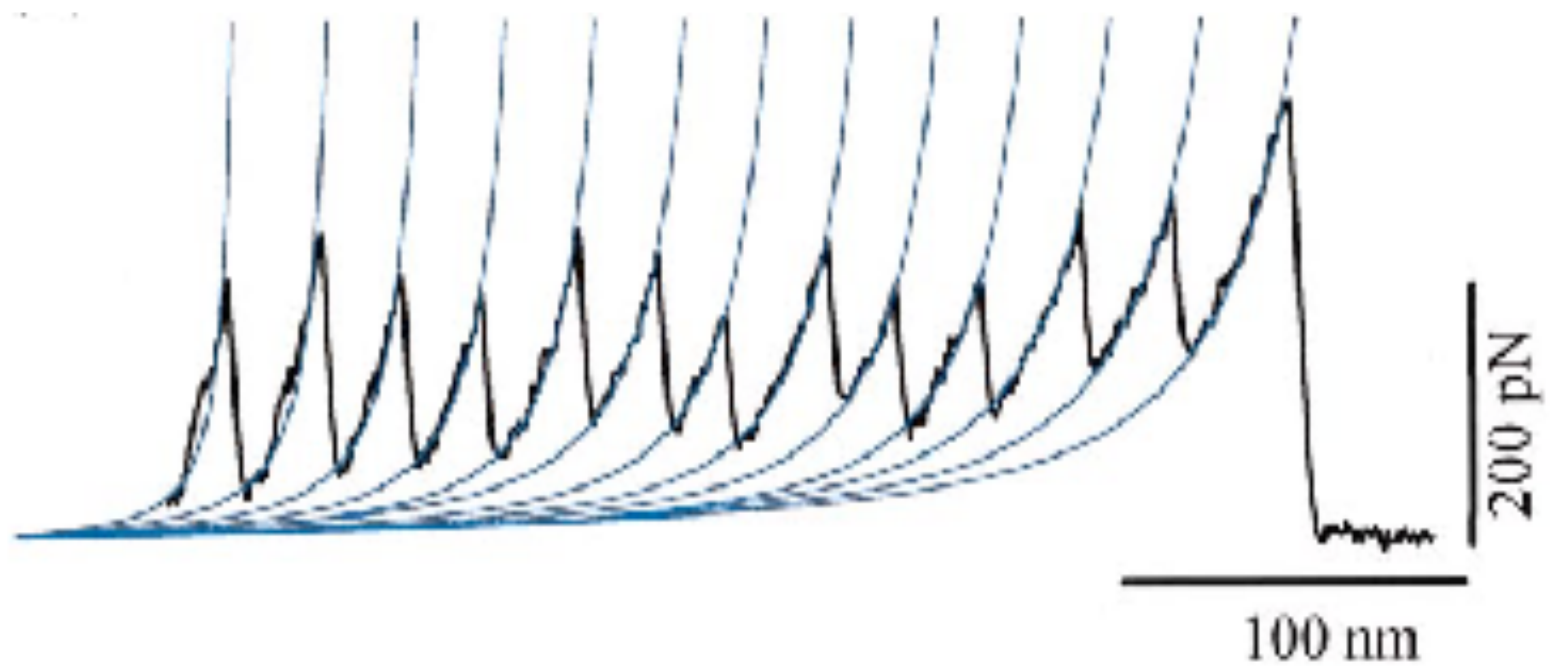


Biological logic of mechanical stability

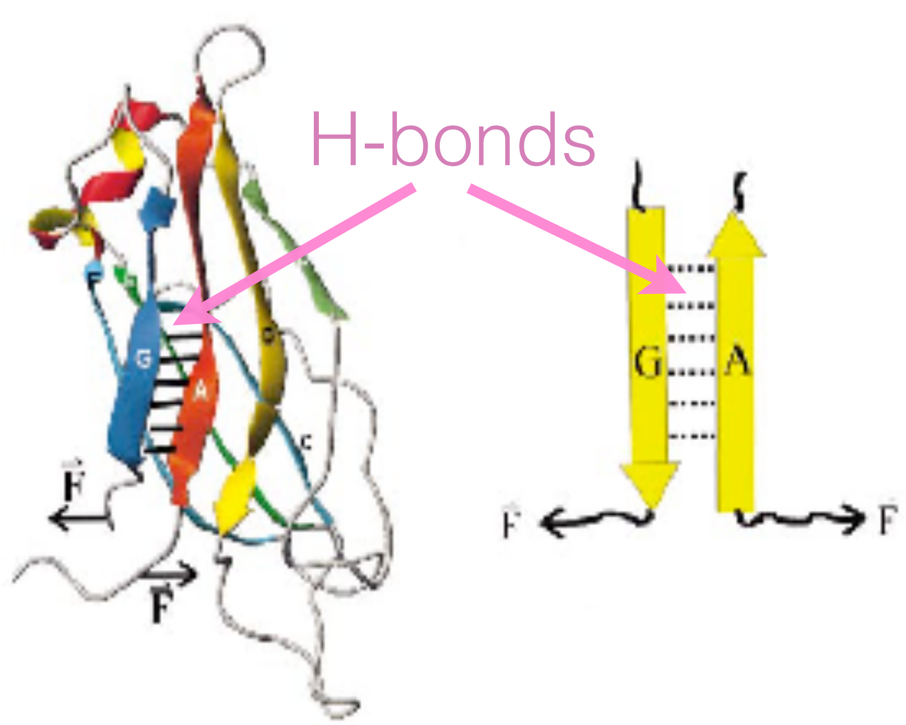
Parallel coupling of structure-stabilizing H-bonds



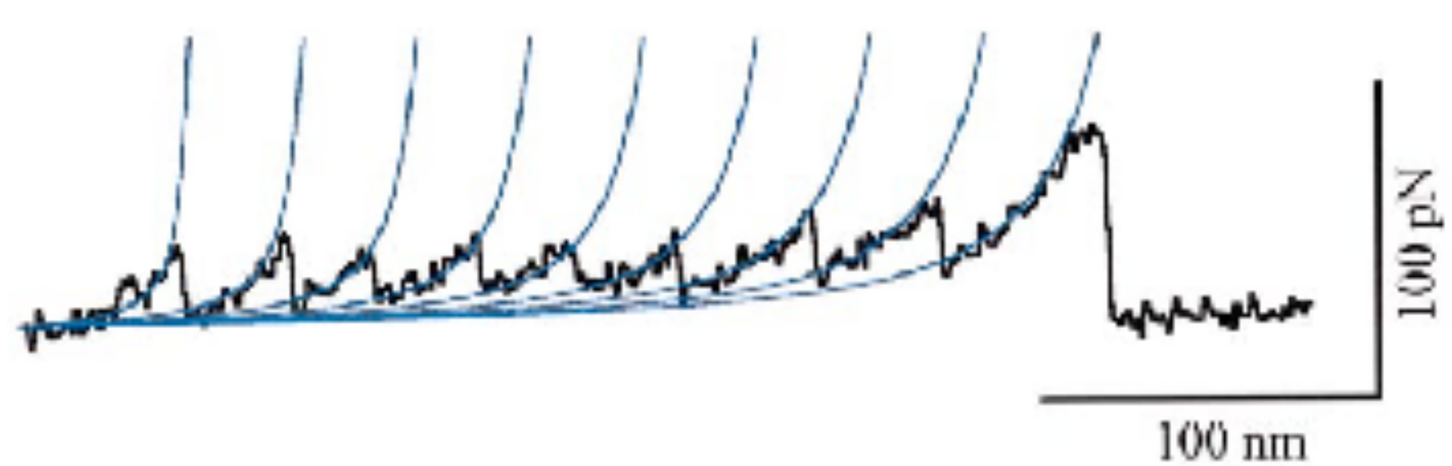
High unfolding forces



Serial coupling of structure-stabilizing H-bonds



Low unfolding forces

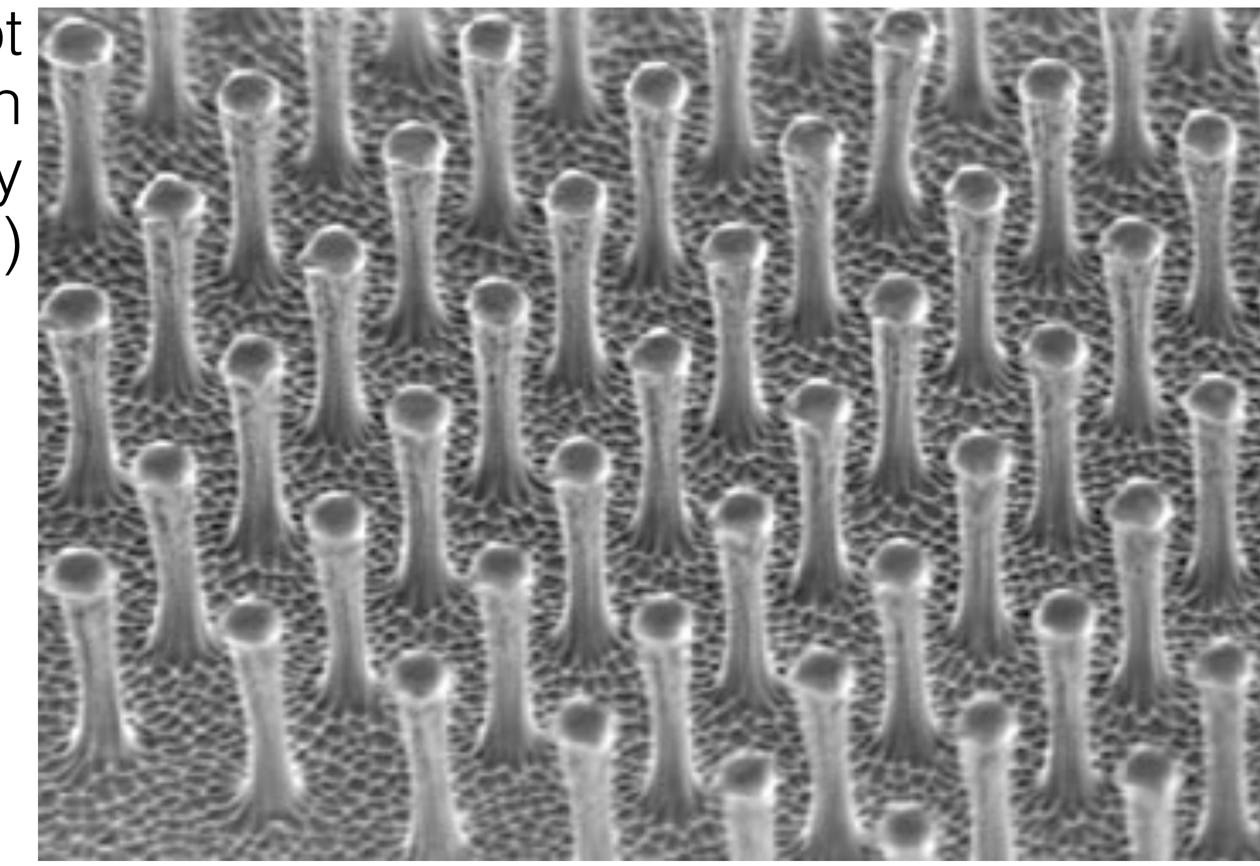


Macroscopic mechanical stability

Highly efficient glue based on the principle or parallel coupling

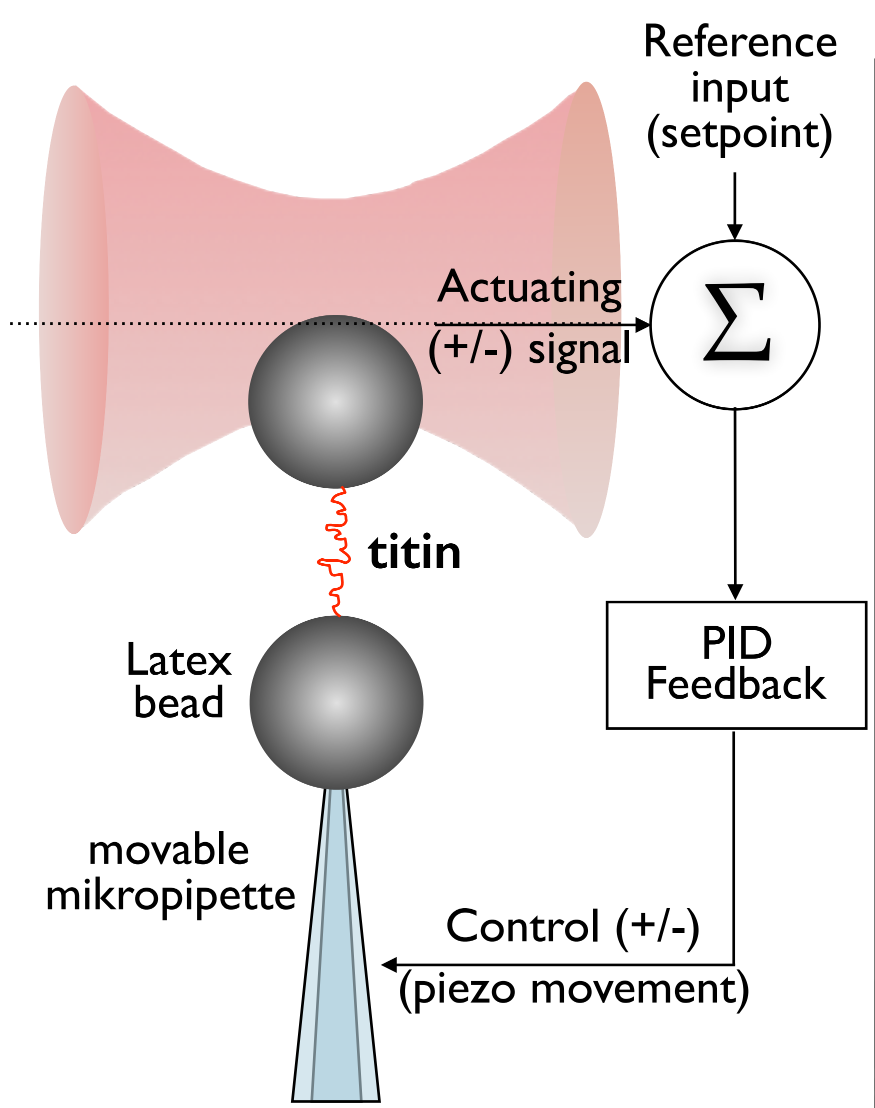
Surface attachment of the gecko foot: Numerous Van der Waals interactions - between bristles and surface - coupled in parallel

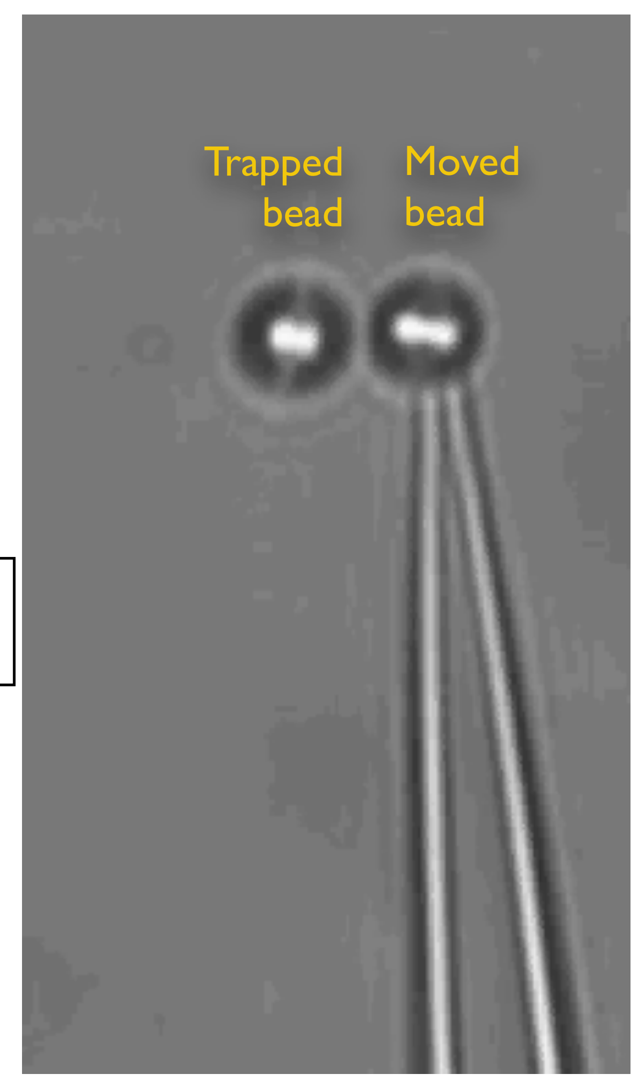
Artificial gecko foot (prepared with nanotechnology methods)



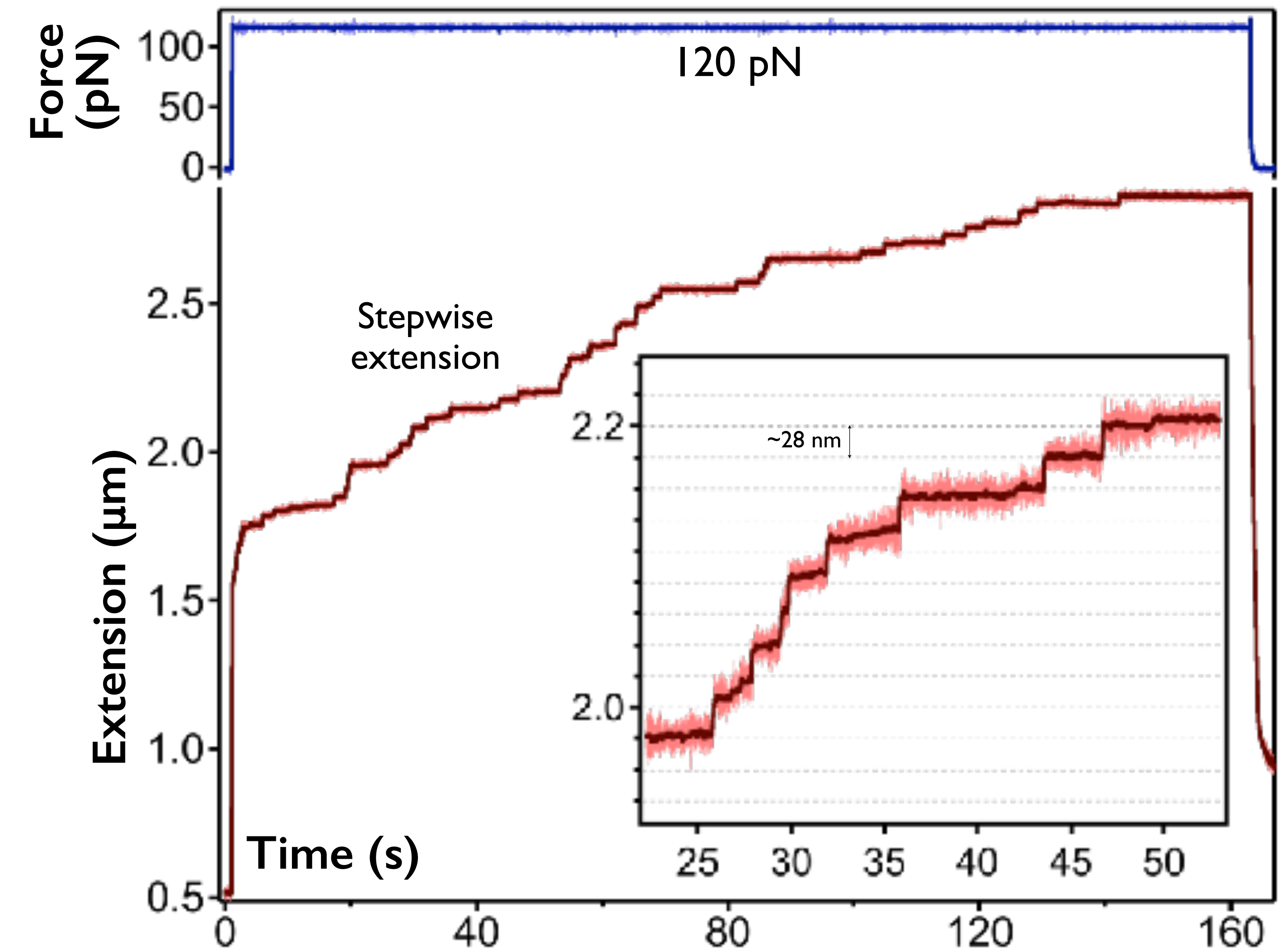
Titin mechanics - constant-force experiment: extension *via* stepwise domain unfolding

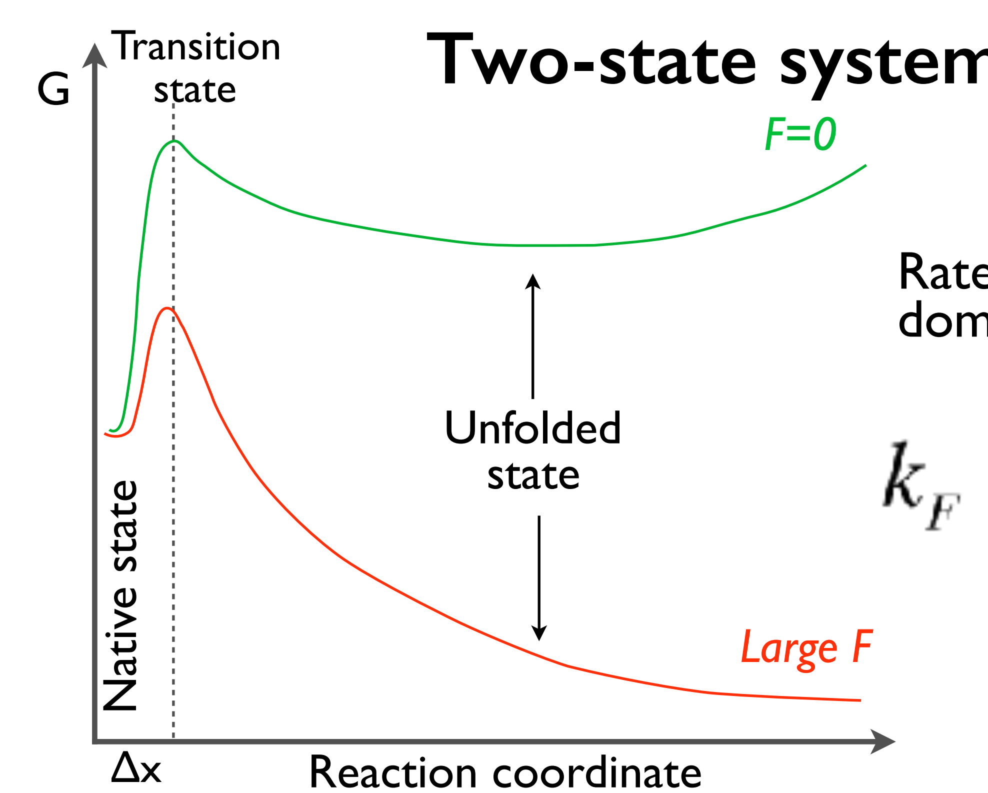
Manipulation with force-clamp optical tweezers





Bianco et al. Biophys J. 109:340-5, 2015.

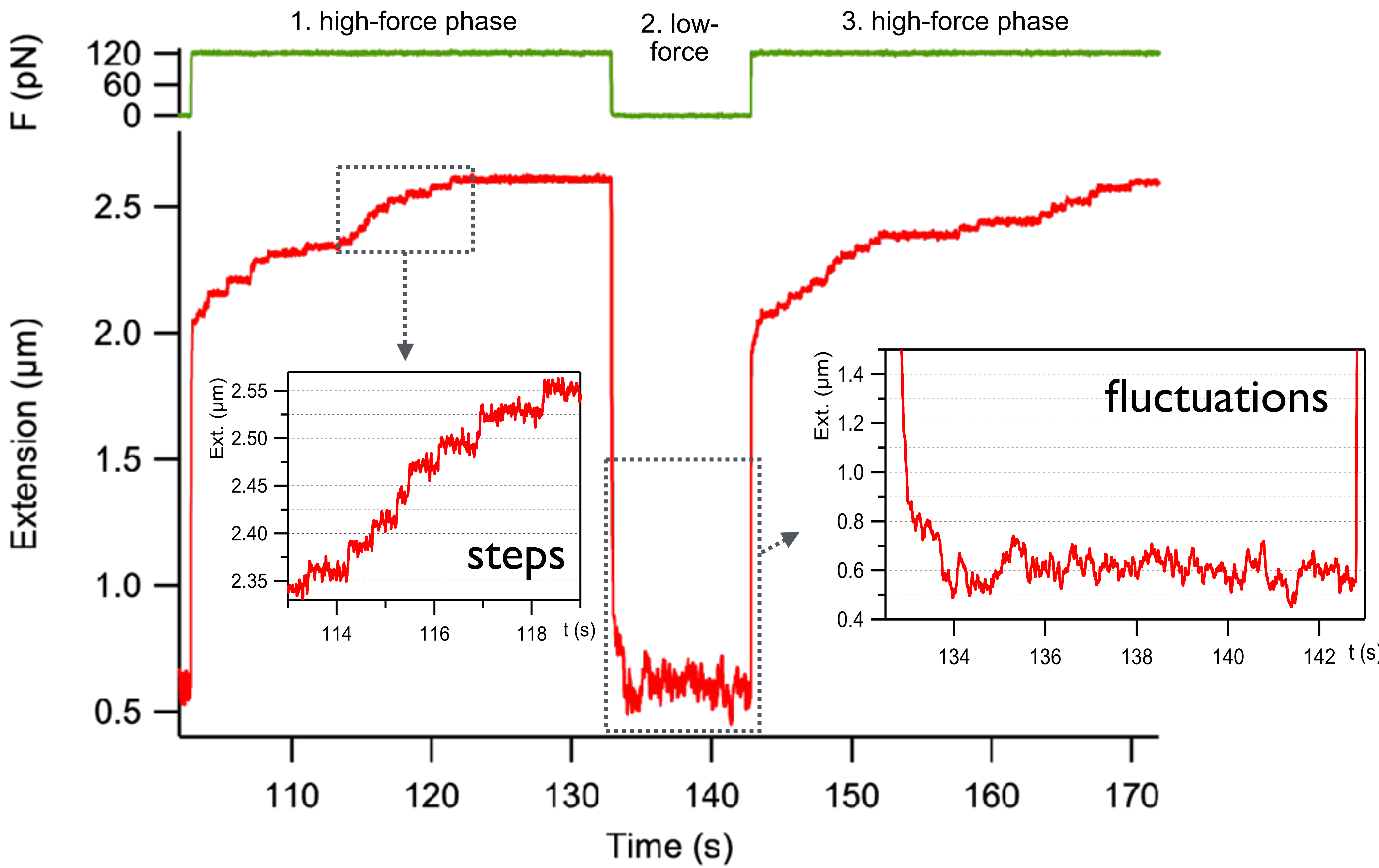




Rate of force-driven domain unfolding:

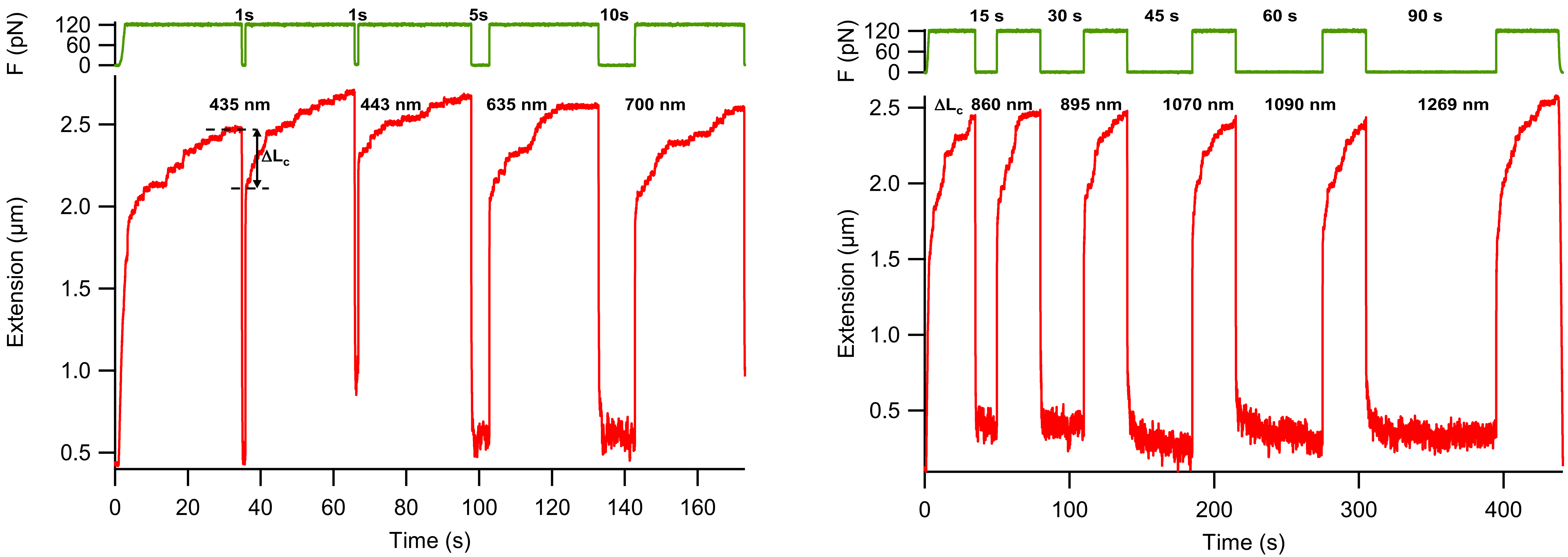
$$k_F = k_0 e^{F\Delta x/k_BT}$$

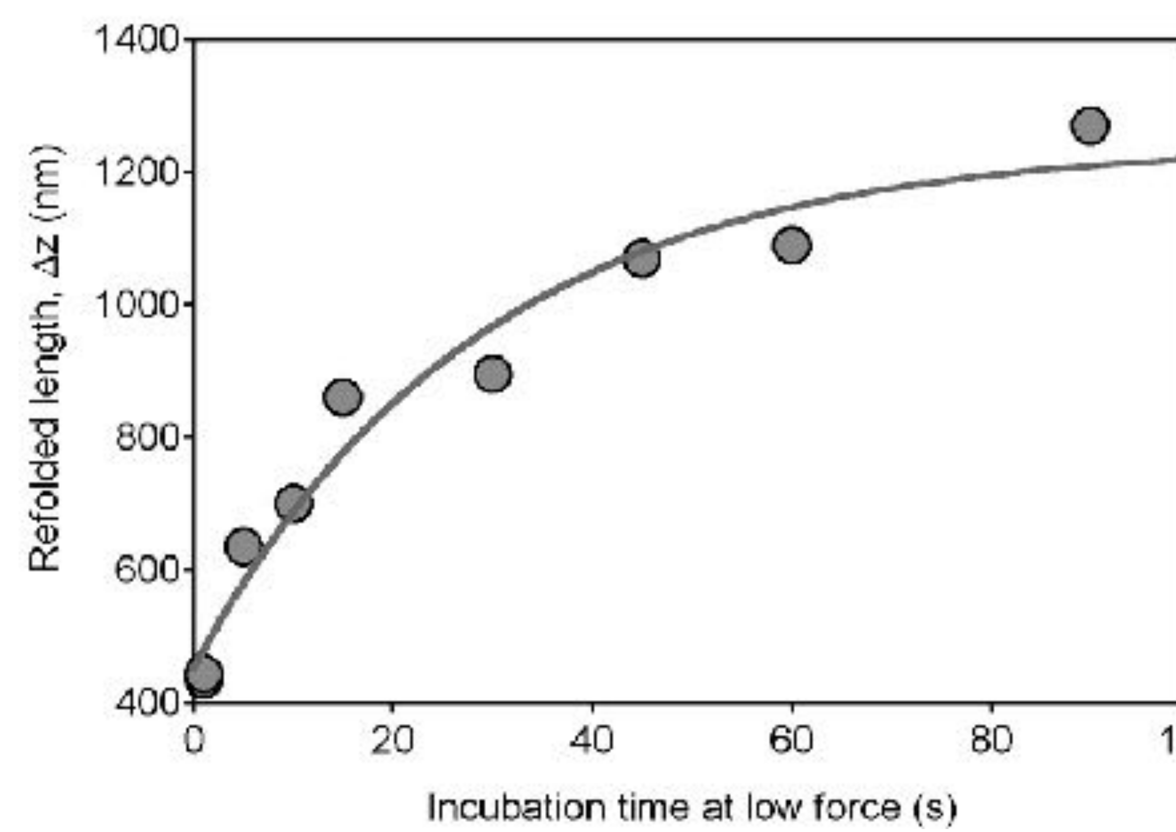
No steps, only fluctuations during refolding



Mártonfalvi et al. Prot. Sci. 2017. DOI 10.1002/pro.3117

Refolding follows first-order kinetics





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

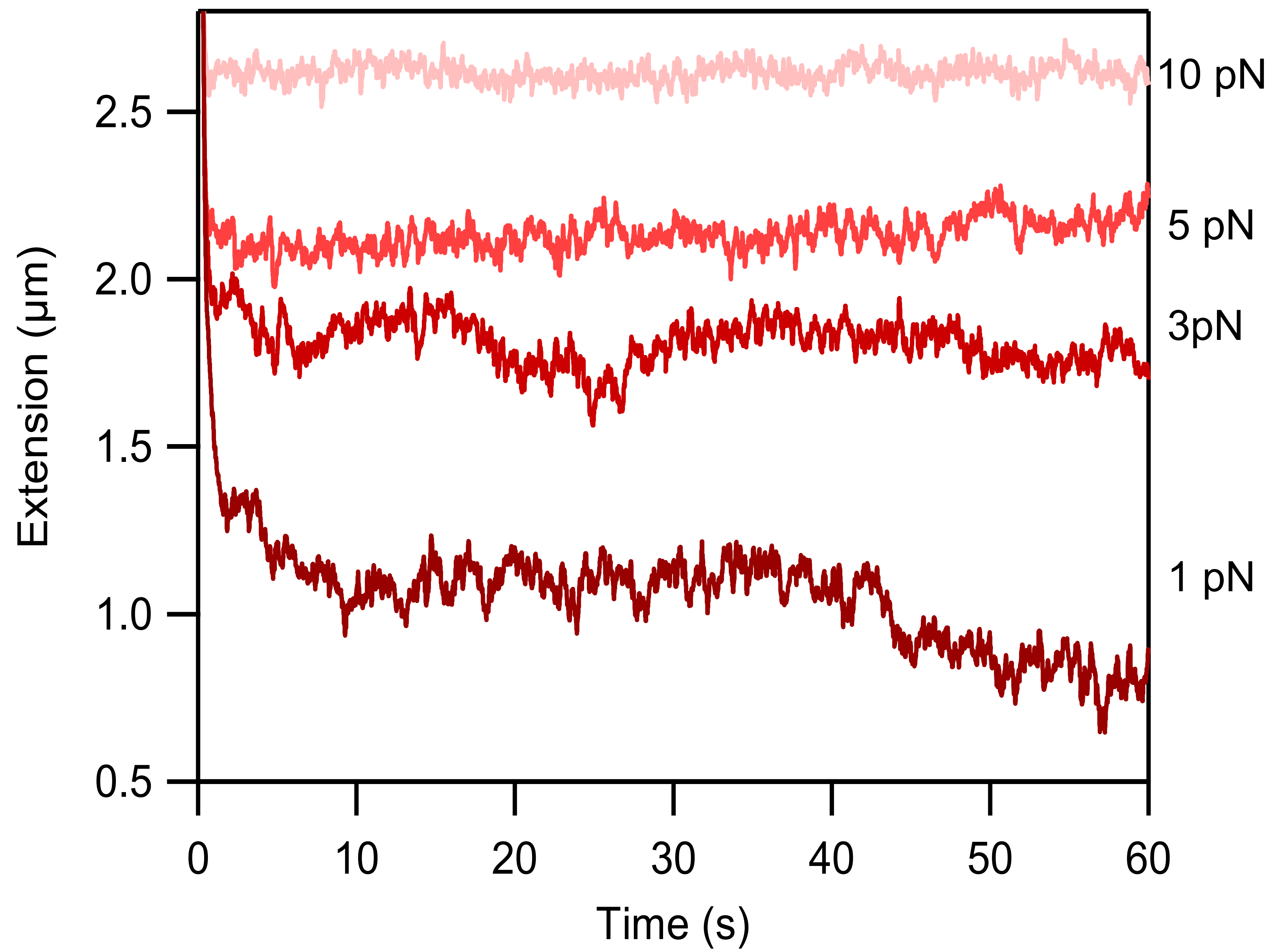
ΔZ_0 : maximal refolded length

τ : time constant

Mártonfalvi et al. Prot. Sci. 2017. DOI 10.1002/pro.3117

Titin refolds against force

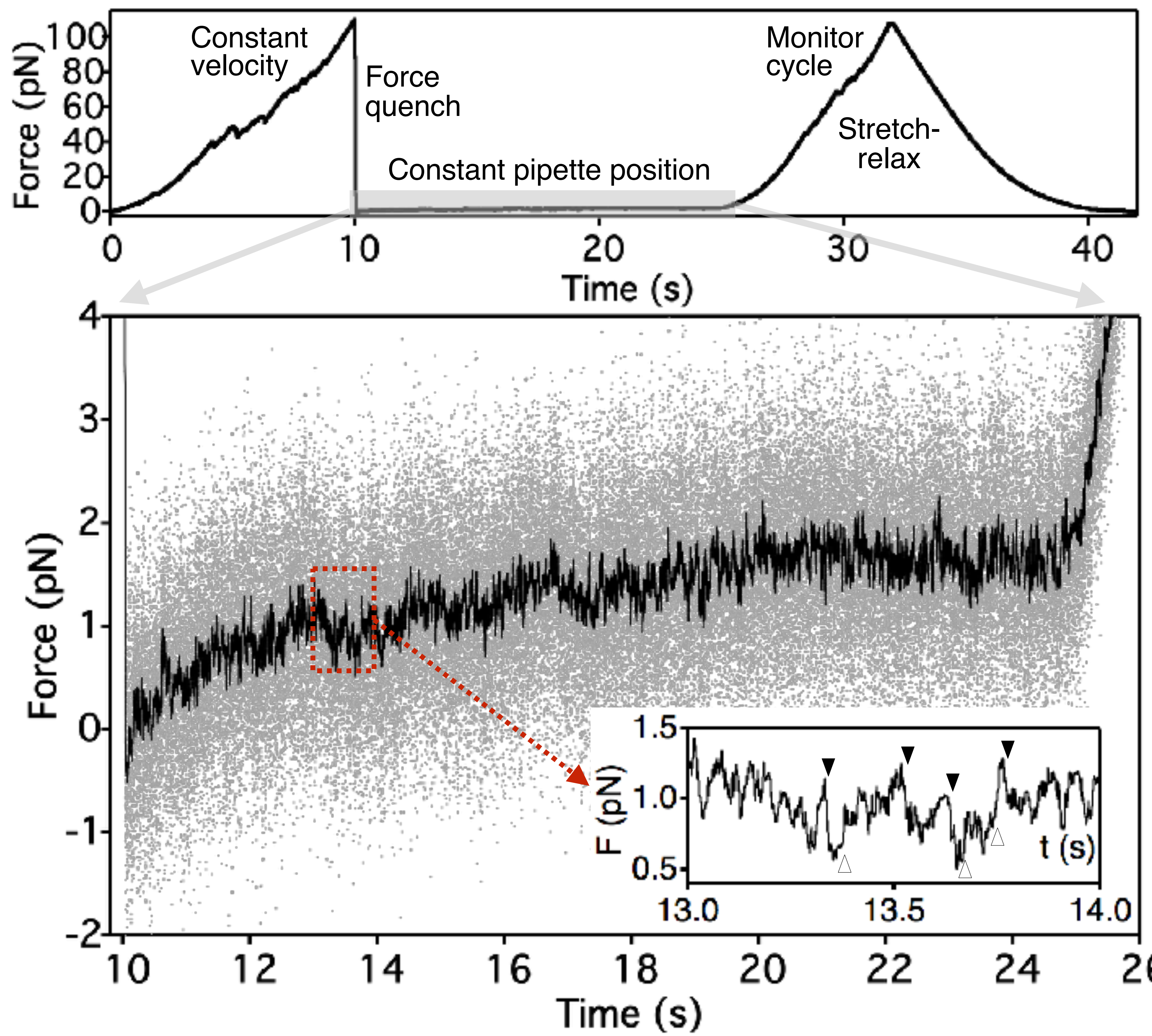
Large length fluctuations occur during refolding



Mártonfalvi et al. Prot. Sci. 2017. DOI 10.1002/pro.3117

Force is generated during refolding

Position clamp experiment



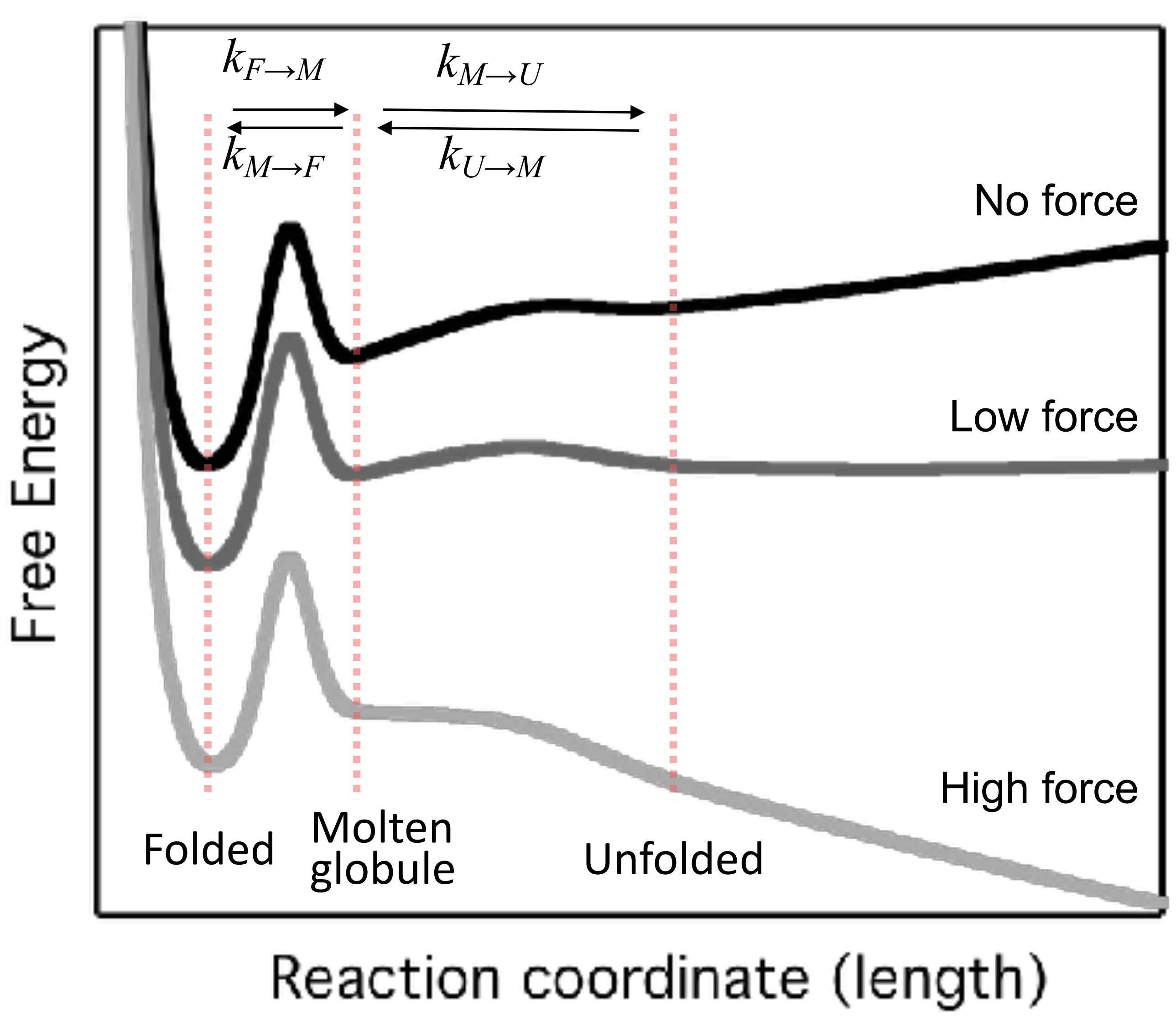
Accompanying contraction (extension change): 10 nm

Fluctuations between contracted (▼) and extended (△) states

Mártonfalvi et al. Prot. Sci. 2017. DOI 10.1002/pro.3117

Fluctuations are explained by molten-globule dynamics

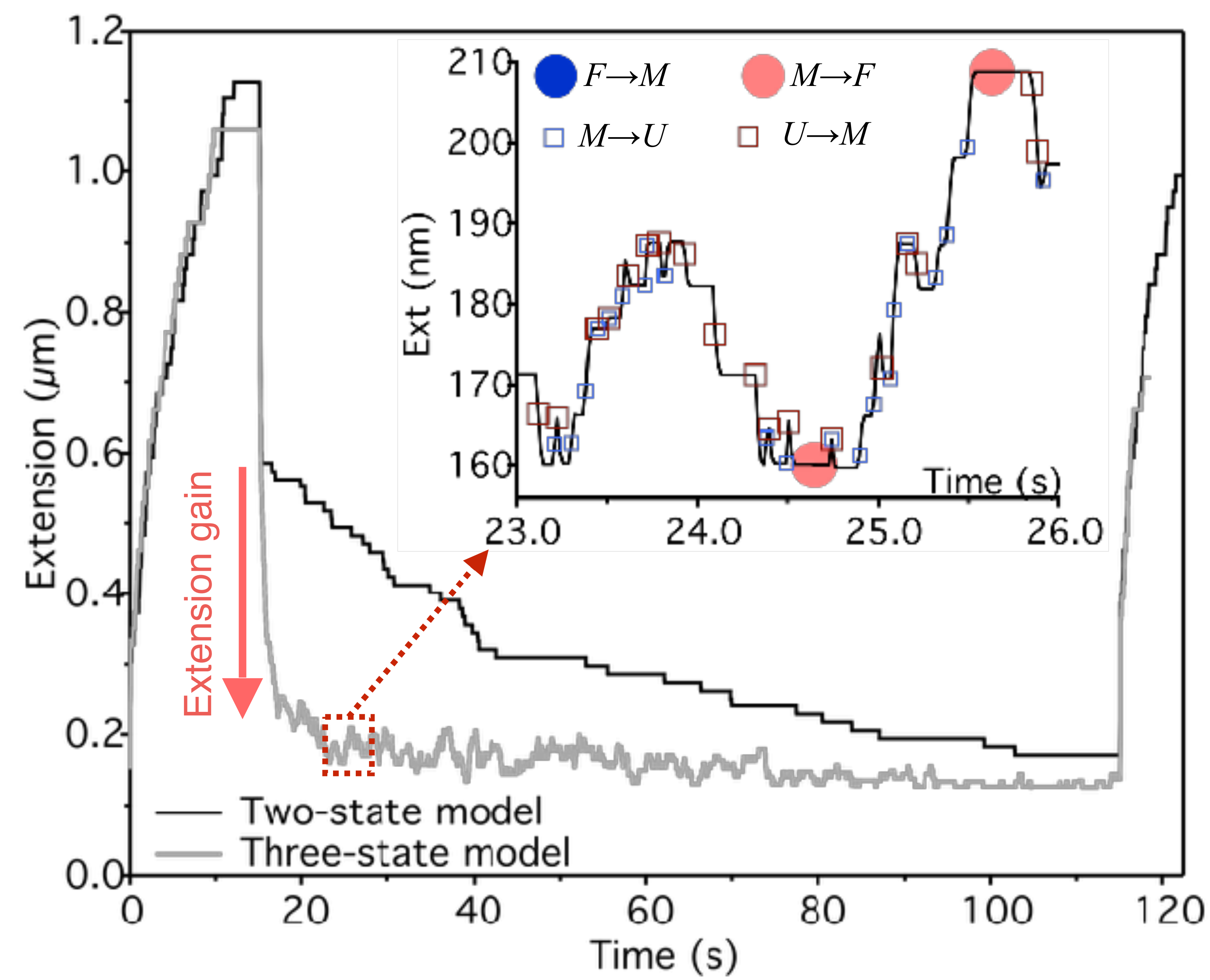
Three-state folding model



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

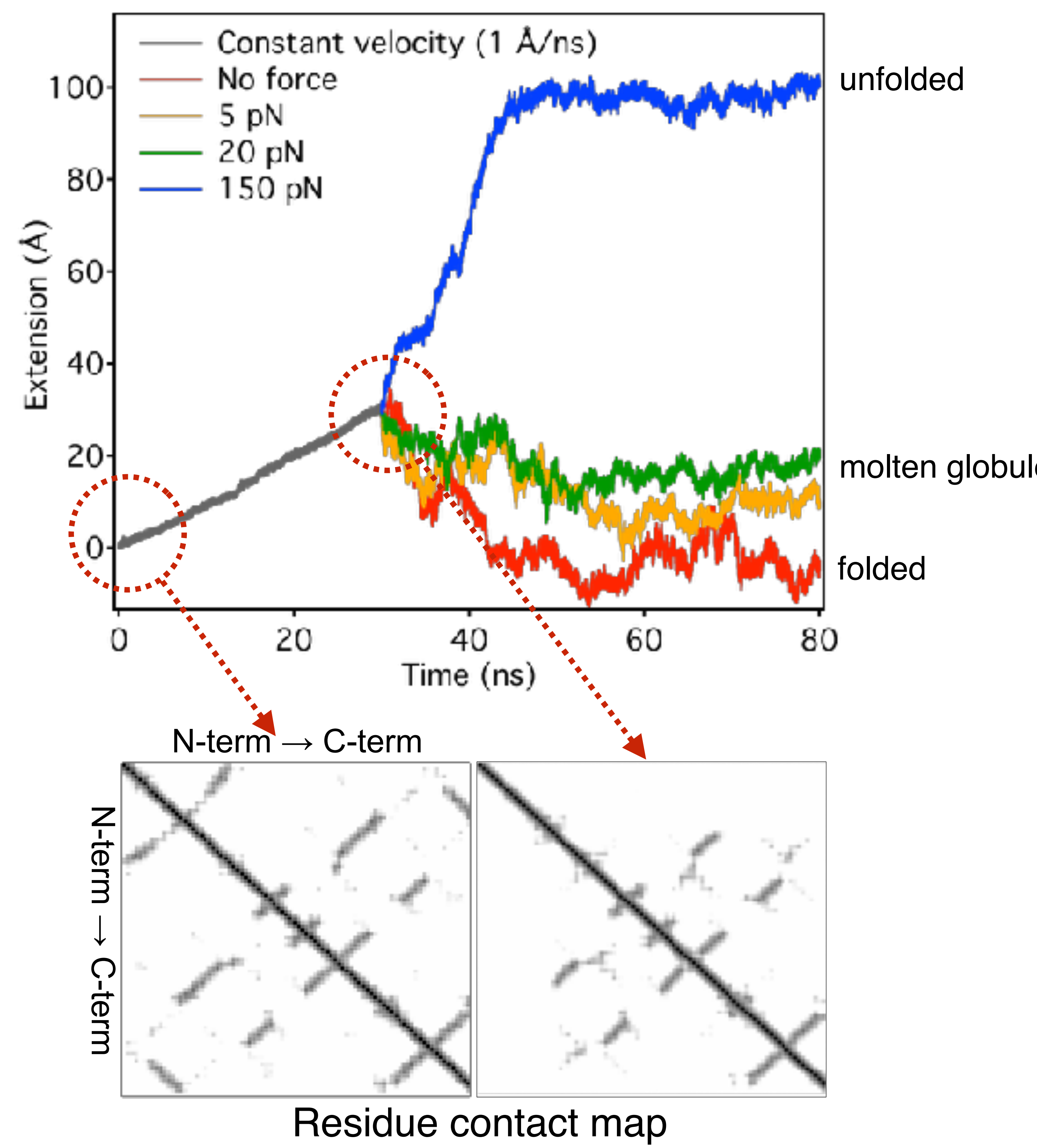
k_0 : spontaneous unfolding/refoldig rate

Monte-Carlo simulation



Mártonfalvi et al. Prot. Sci. 2017. DOI 10.1002/pro.3117

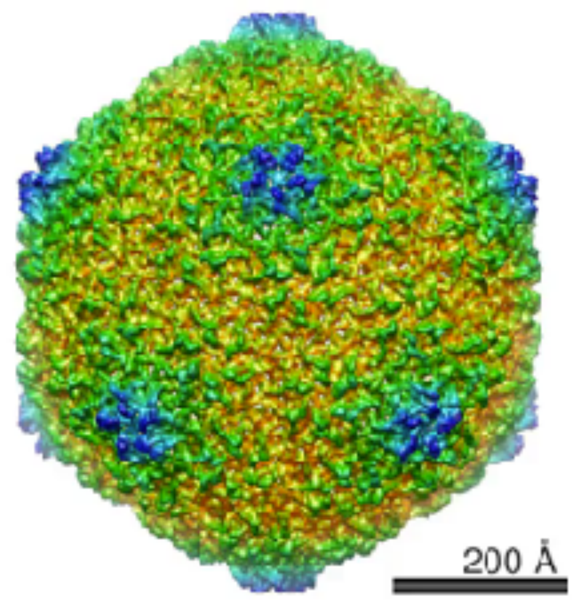
Molten-globule structure explored with sMDS

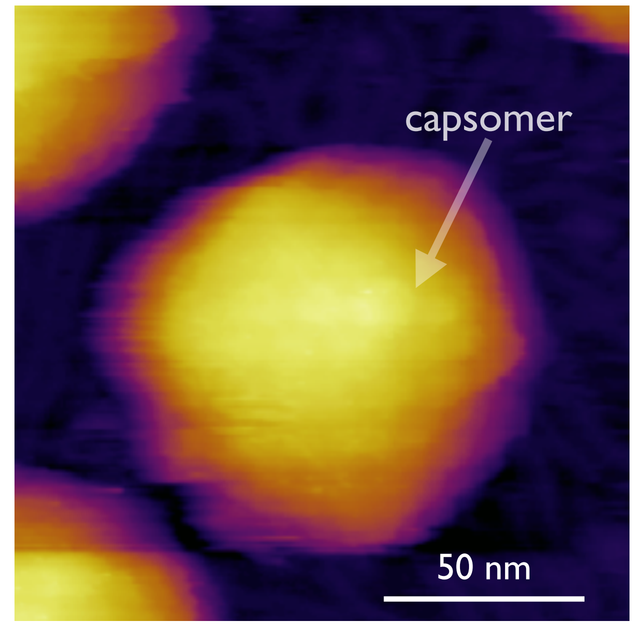


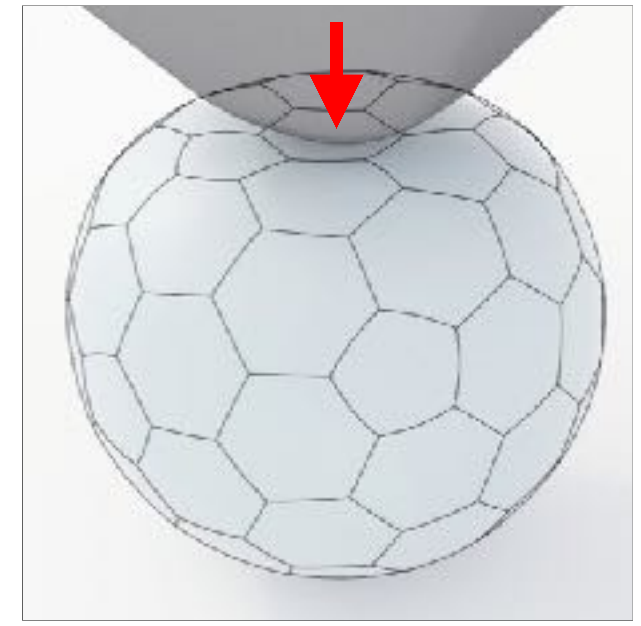
Mártonfalvi et al. Prot. Sci. 2017. DOI 10.1002/pro.3117

2. T7 phage nanomechanics

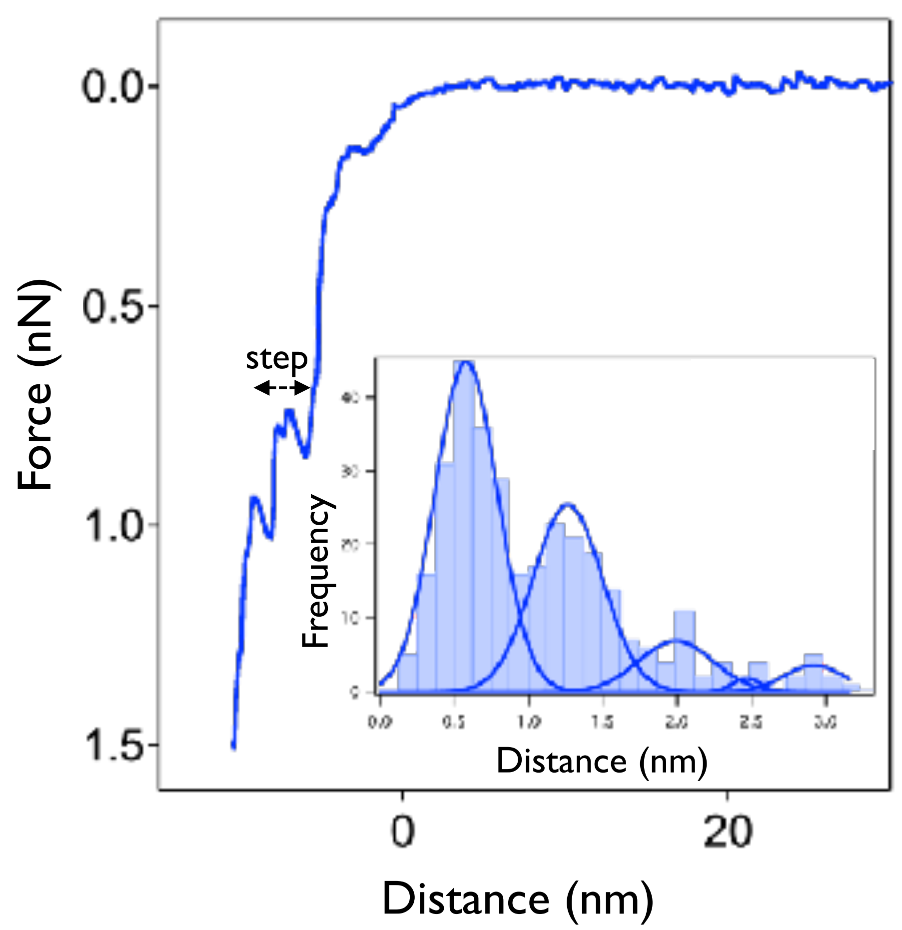
Short-tailed icosaherdal virus

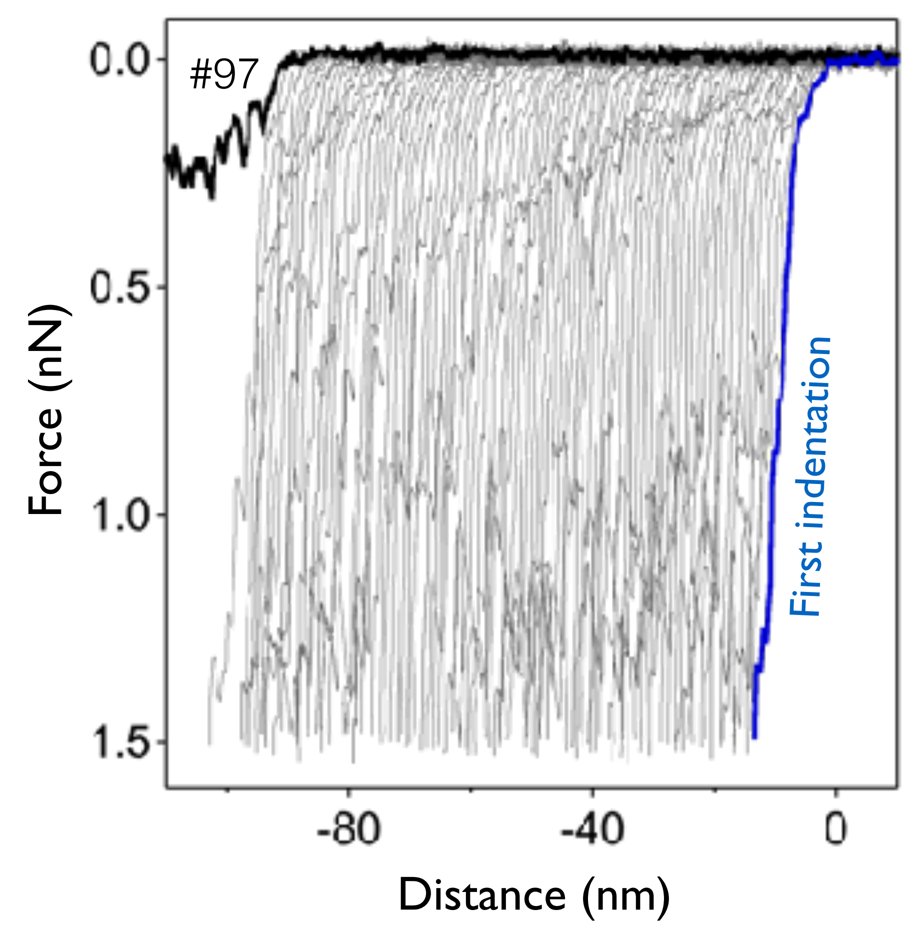


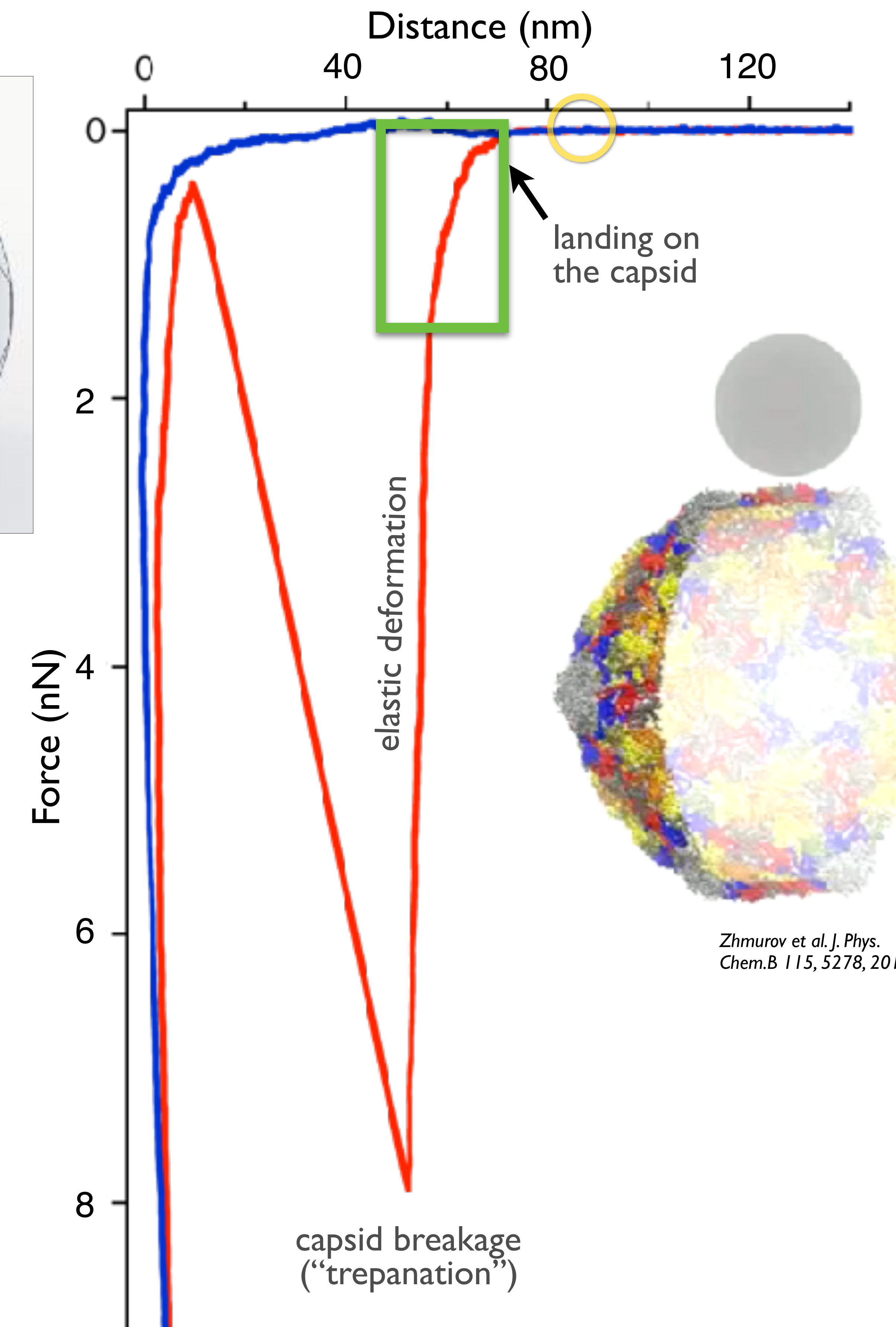




Ionel et al. J.Biol.Chem. 286, 234, 2010.







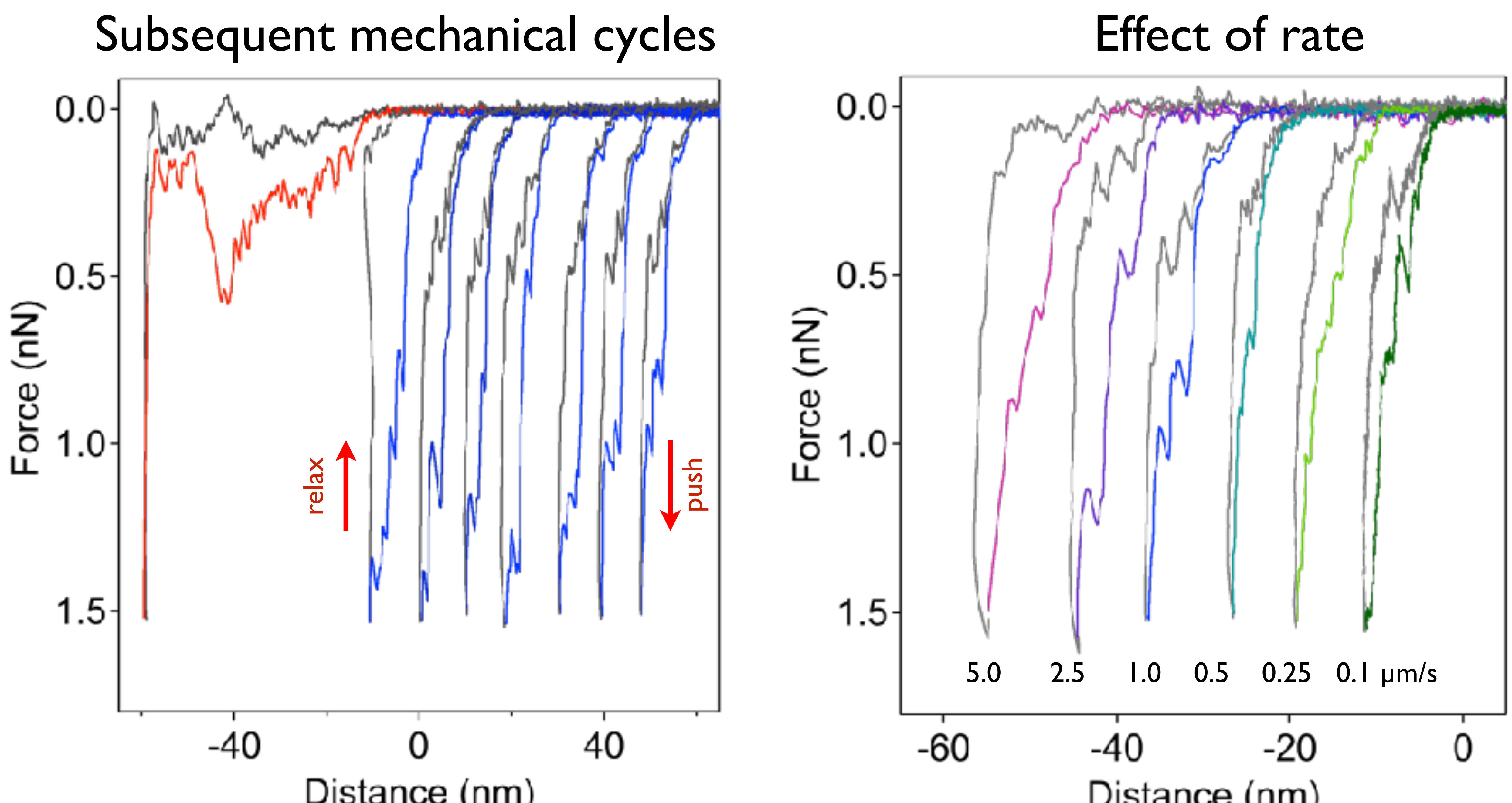
Zhmurov et al. J. Phys. Chem.B 115, 5278, 2011.

Barsegov's

Reversible 6 Å buckling steps, followed by rapid relaxation.

Vörös, Z.…Kellermayer, M. Nanoscale 9, 1136, 2017.

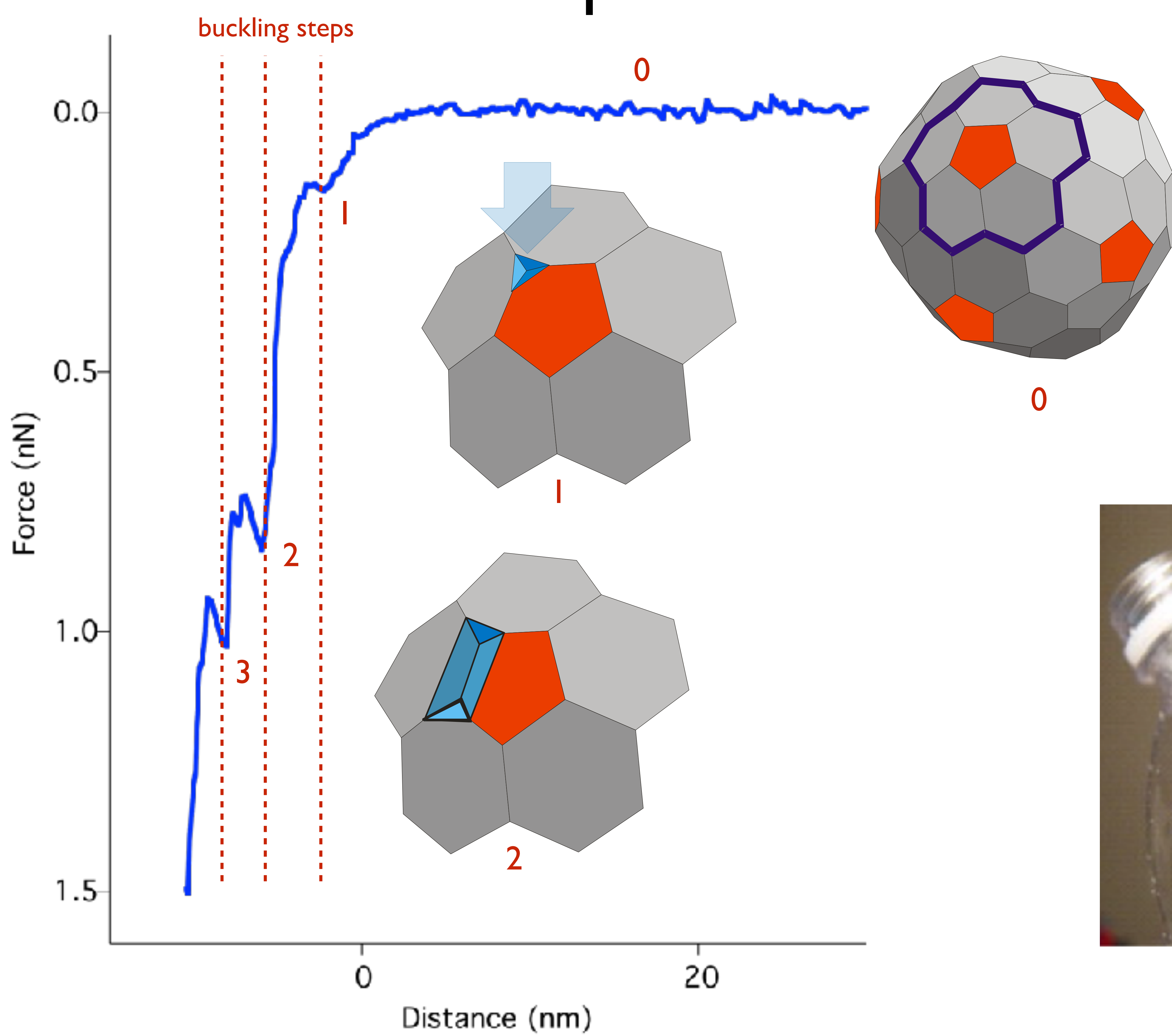
Similar discrete steps occur during mechanical relaxation



- Hysteresis increases
- Push force increases
- Relaxation force decreases
- At low instantaneous forces reversibility is seen

Vörös et al. Nanoscale 9, 1136-1143, 2017.

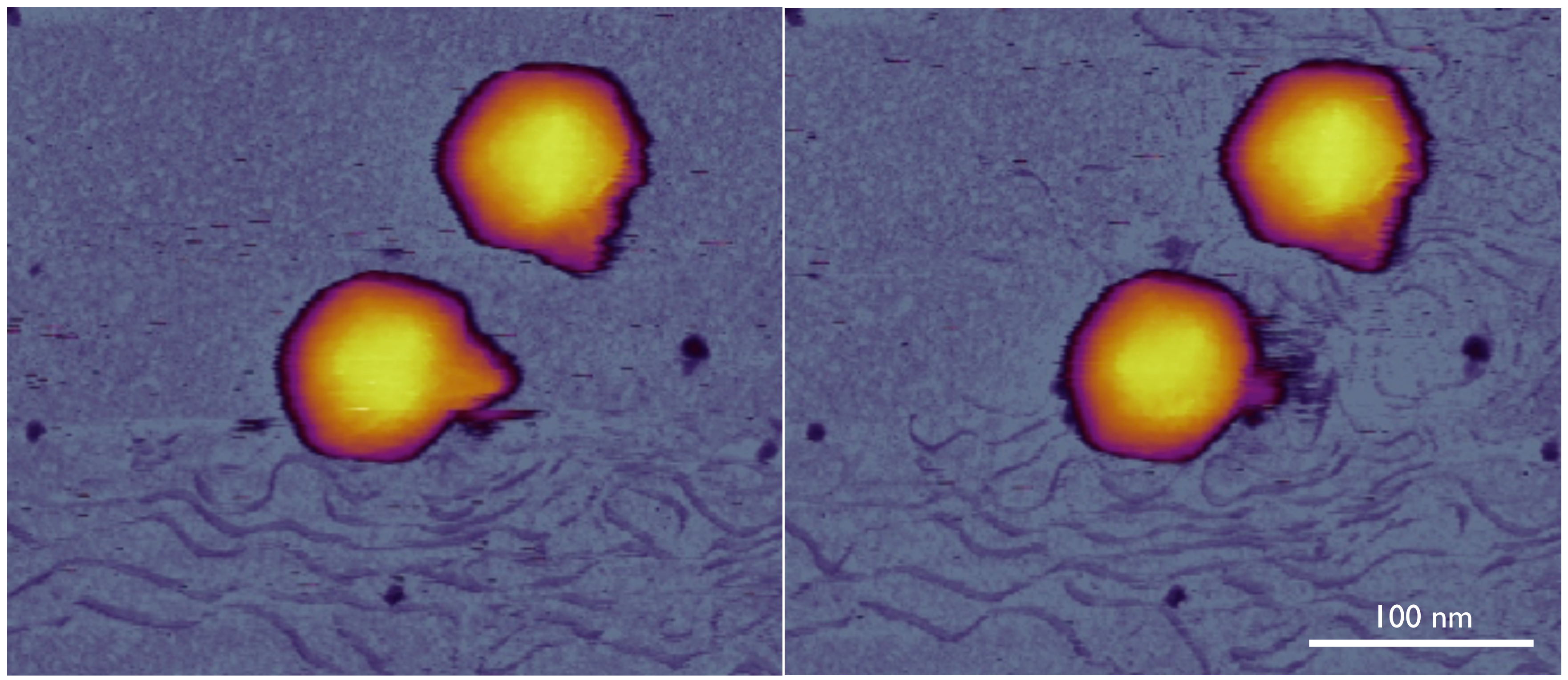
Mechanical buckling of the T7 capsid

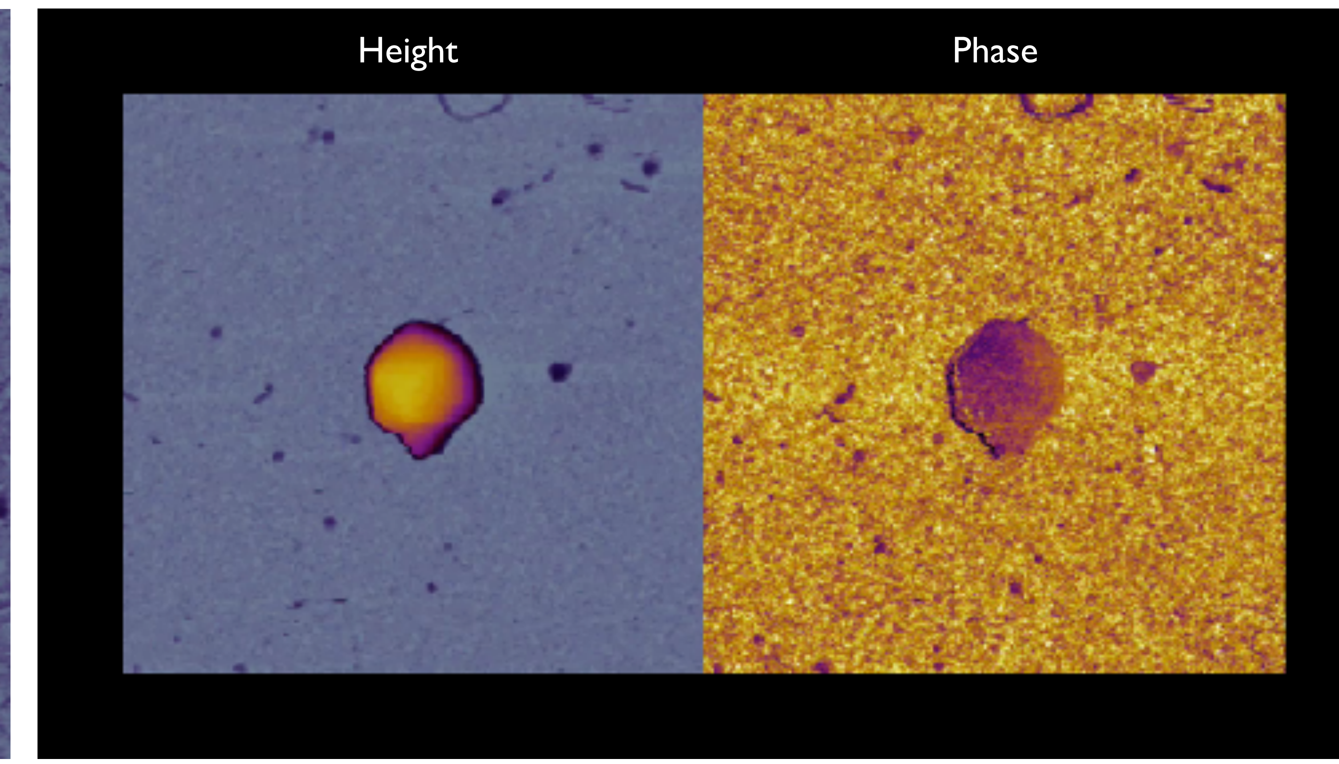


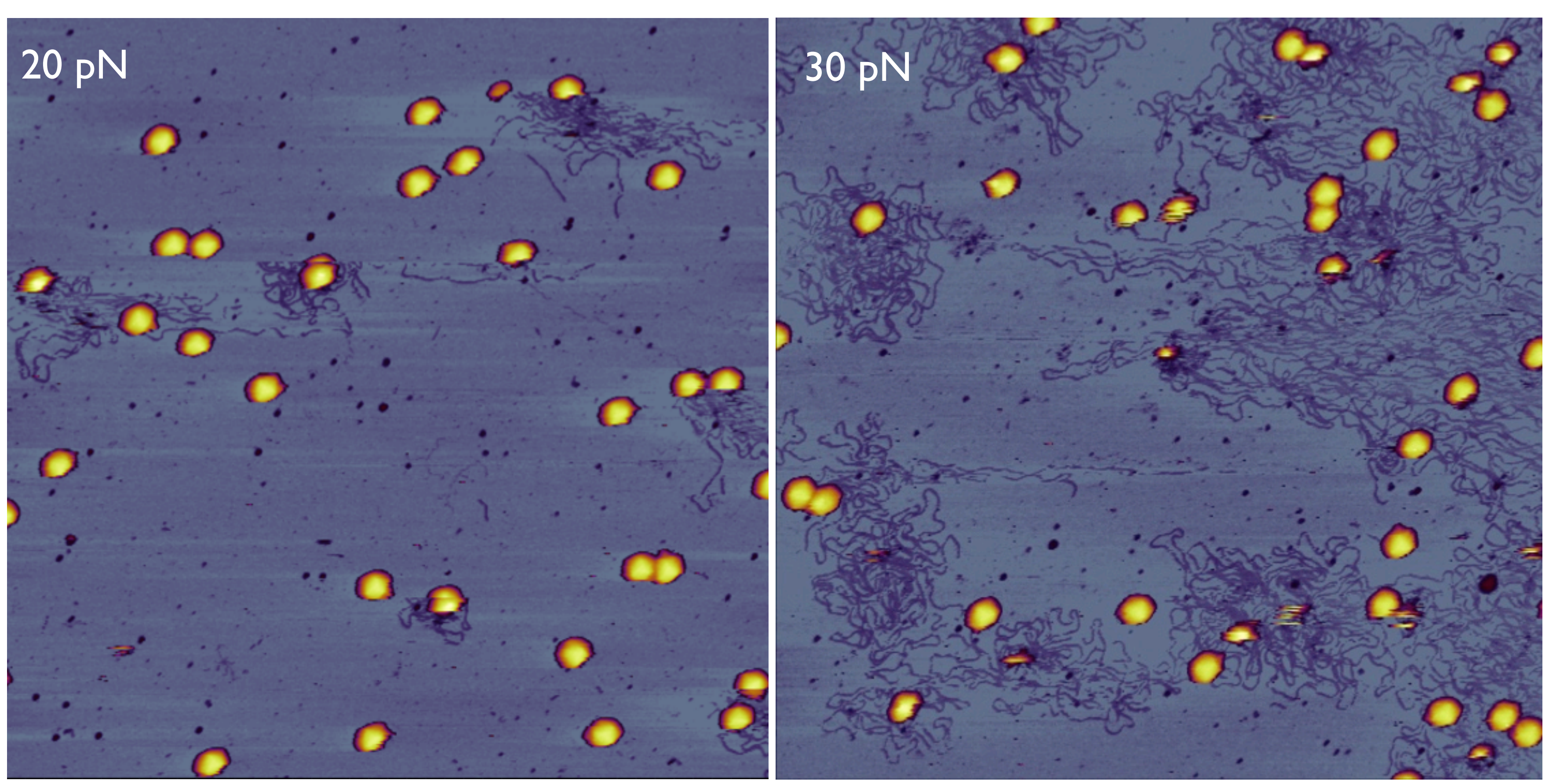
buckling

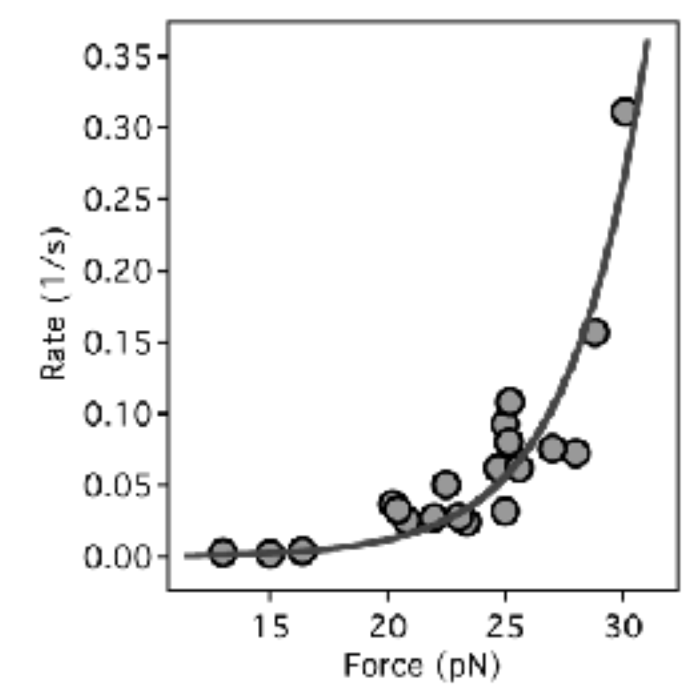
Vörös et al. Nanoscale 9, 1136-1143, 2017.

Force triggers T7 DNA ejection

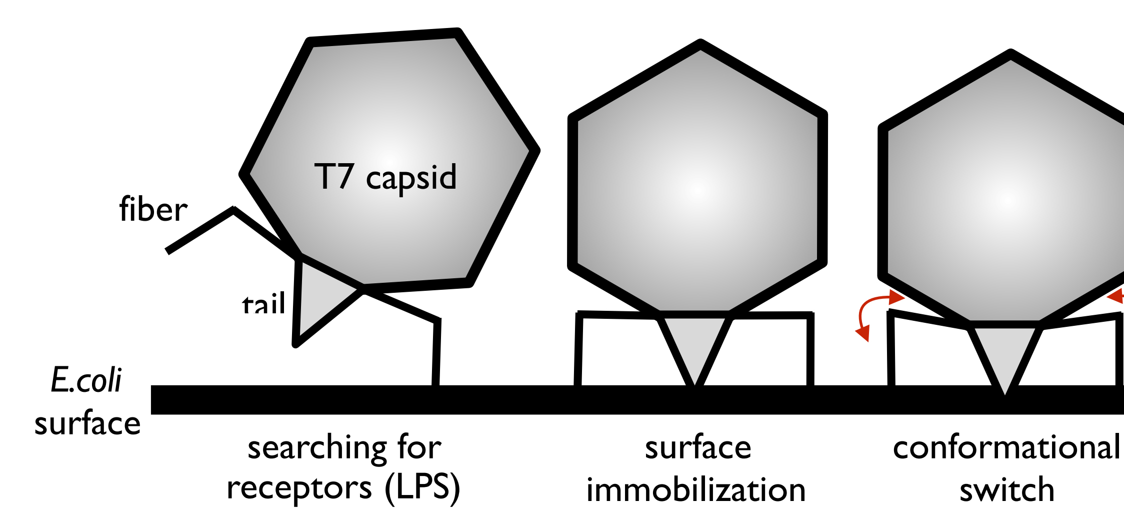




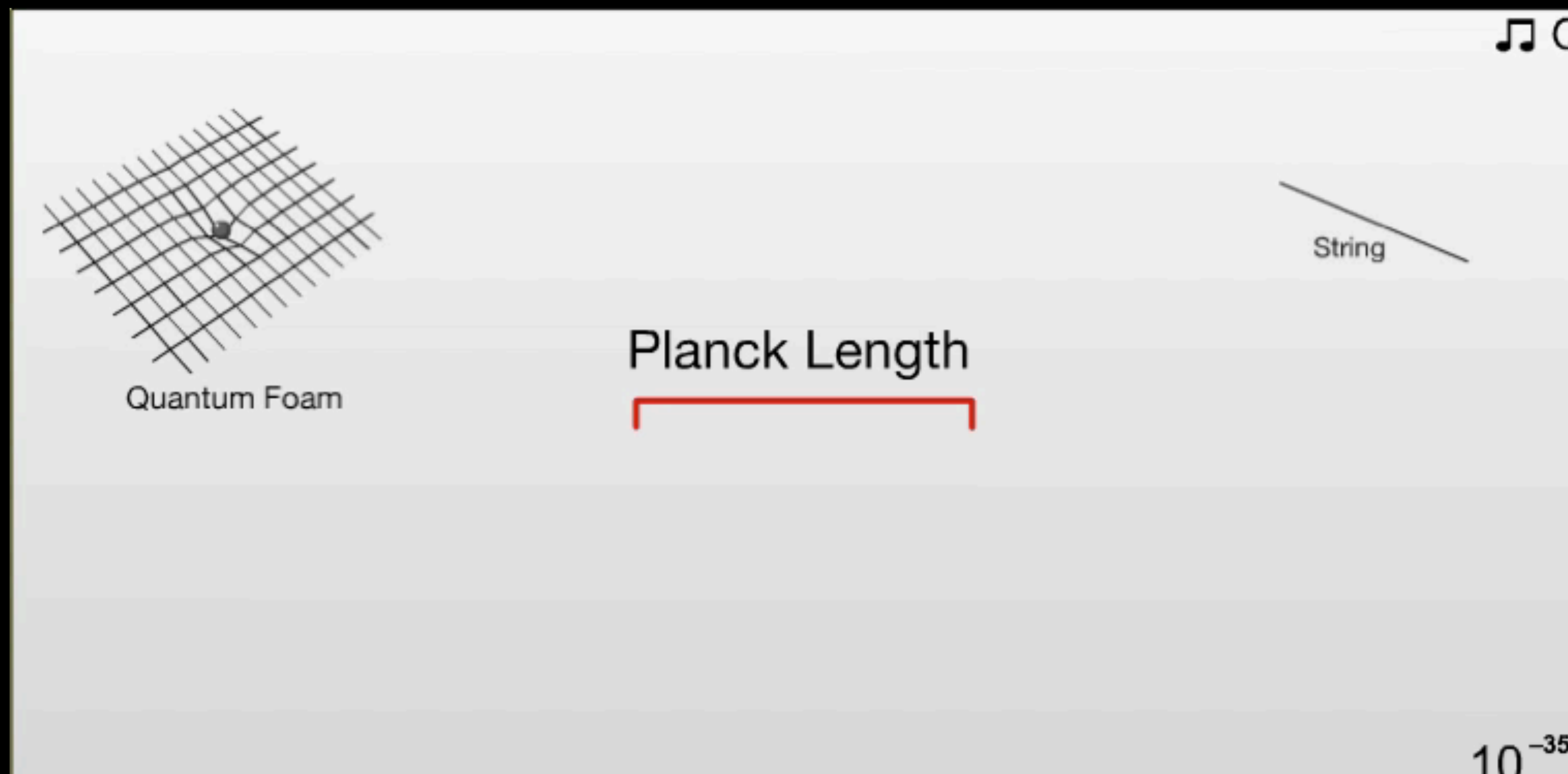




Switch-like
trigger response



Kellermayer, M. et al Nanoscale ***10****, 11898, 2018*



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