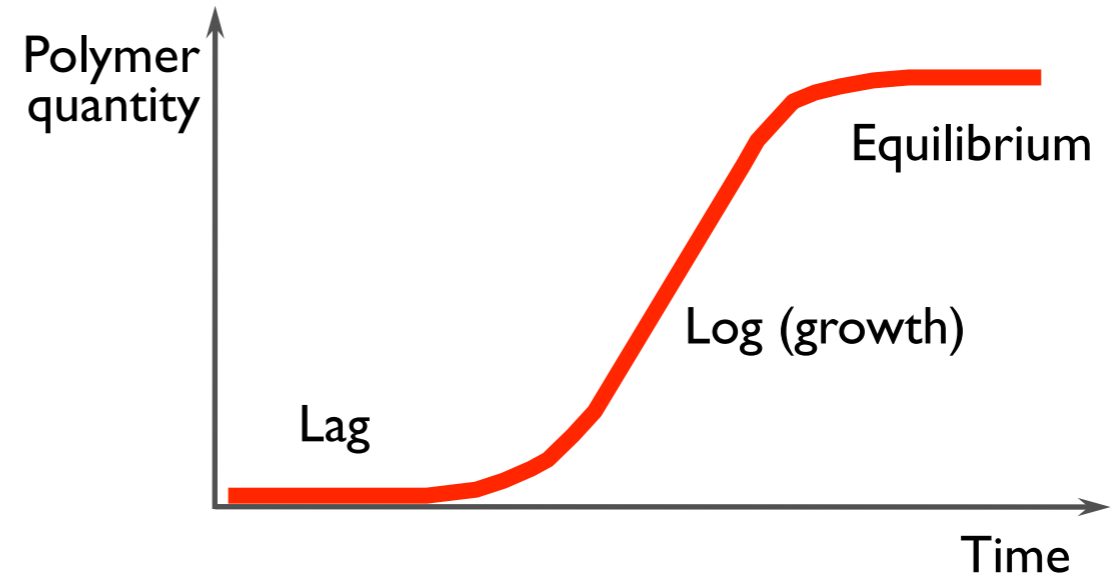


**POLYMERIZATION, SELF-ASSEMBLY,
MECHANOENZYMES, PROTEIN FOLDING,
IRREVERSIBLE PROCESSES**

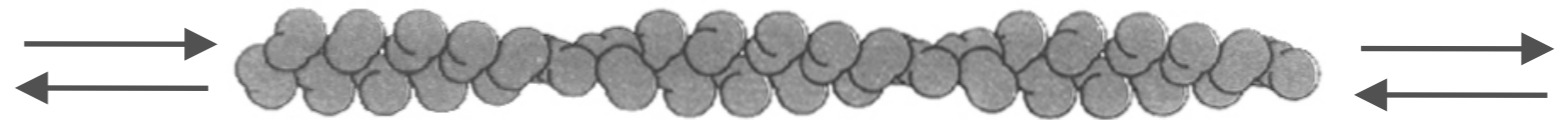
MIKLÓS KELLERMAYER

Polymerization

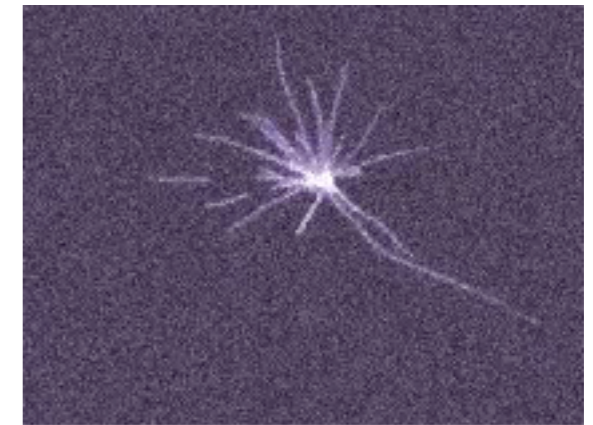


Polymerization equilibria

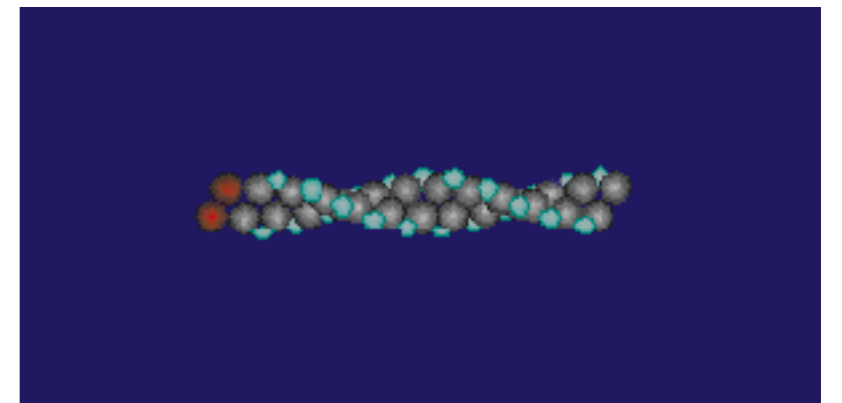
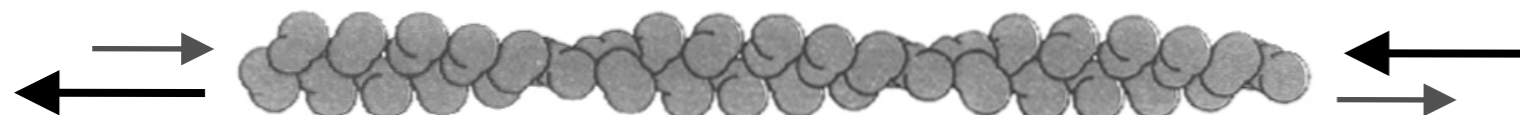
1. true equilibrium



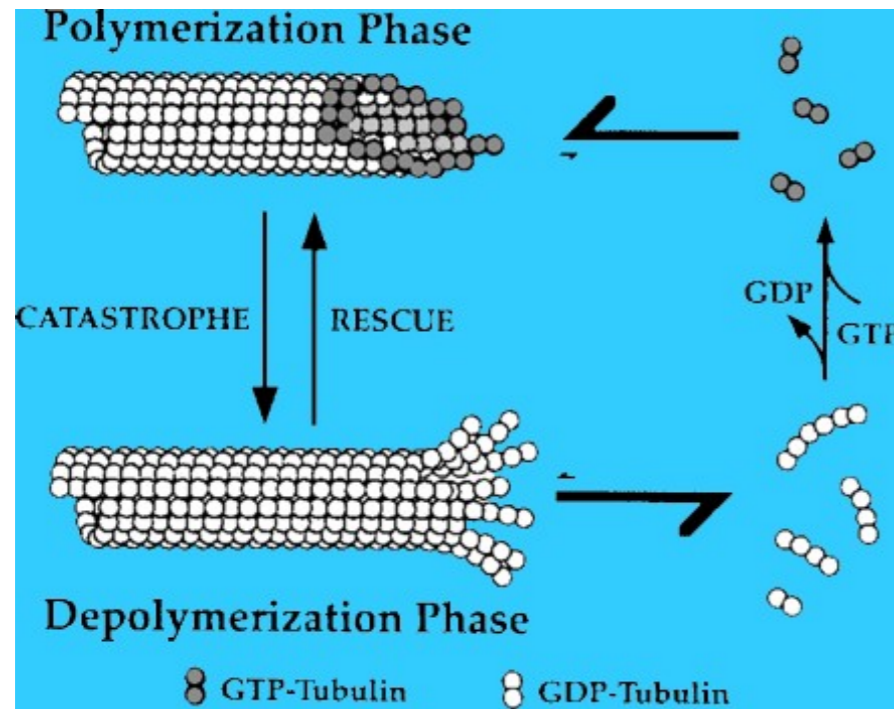
2. dynamic instability: slow growth followed by catastrophic depolymerization



3. Treadmilling

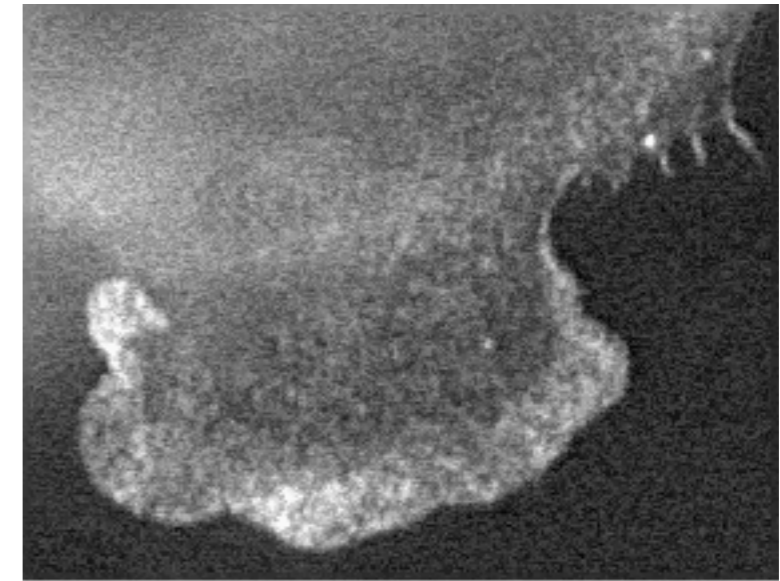


In vivo dynamic instability Microtubules

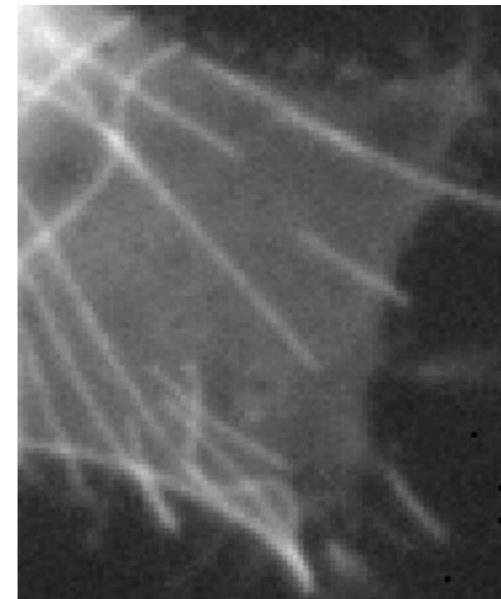


**CHO Cytoplasm
with
Centrosome**

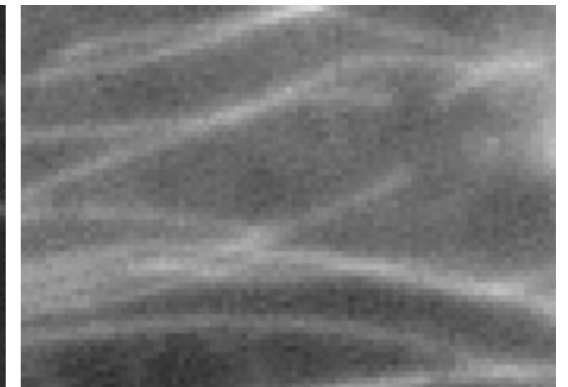
In vivo treadmilling



Actin
GFP-actin Speckle microscopy



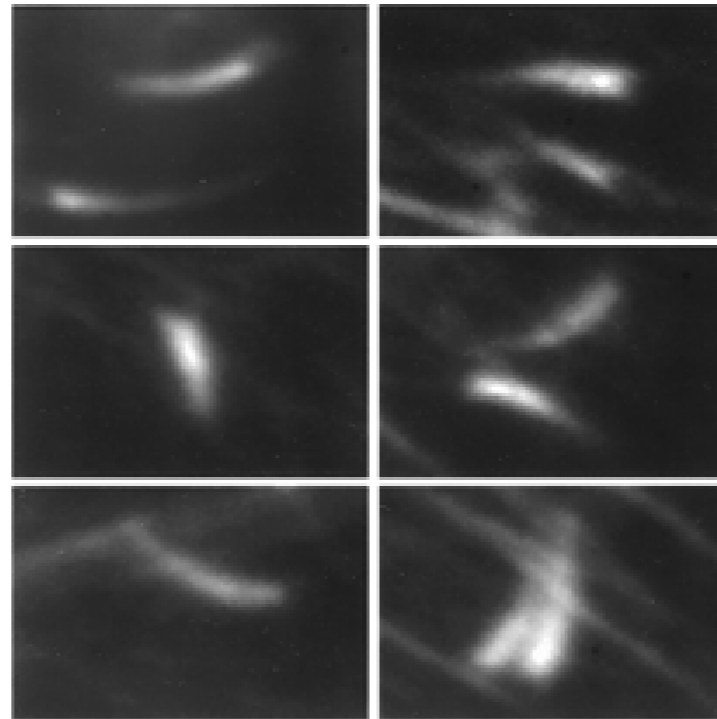
Microtubules



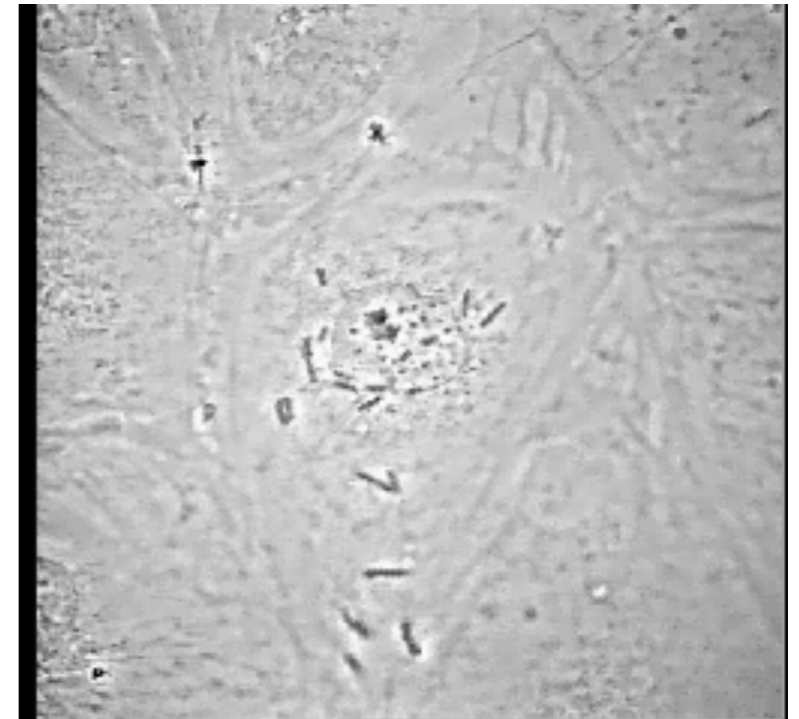
Motility with actin polymerization Intracellular pathogen motion



Listeria monocytogenes



F-actin labeled with phalloidin



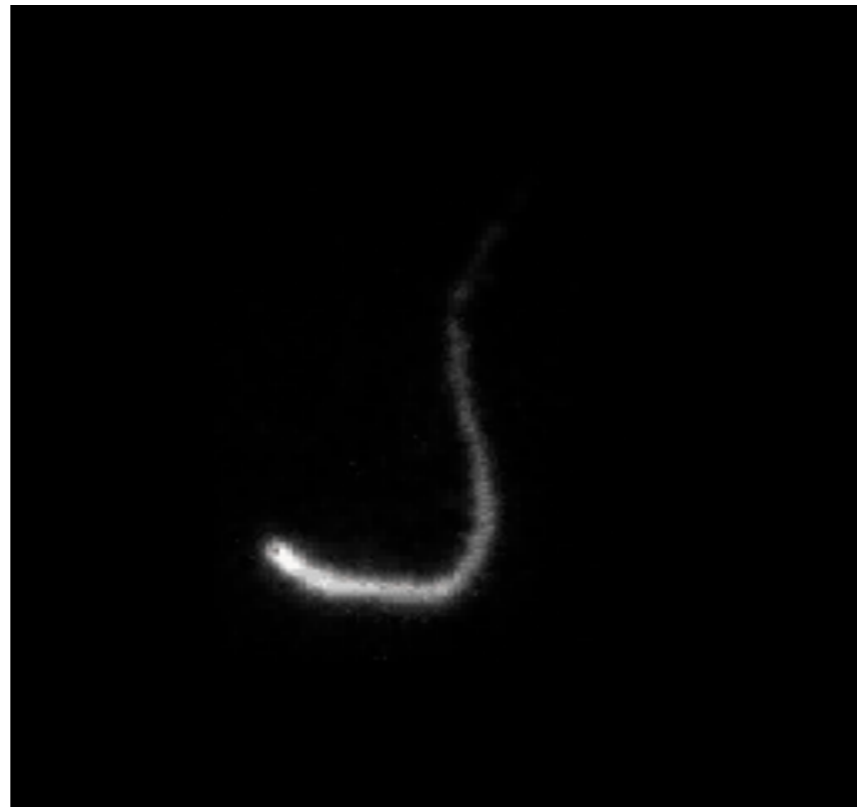
Shigella flexneri

Motility with actin polymerization

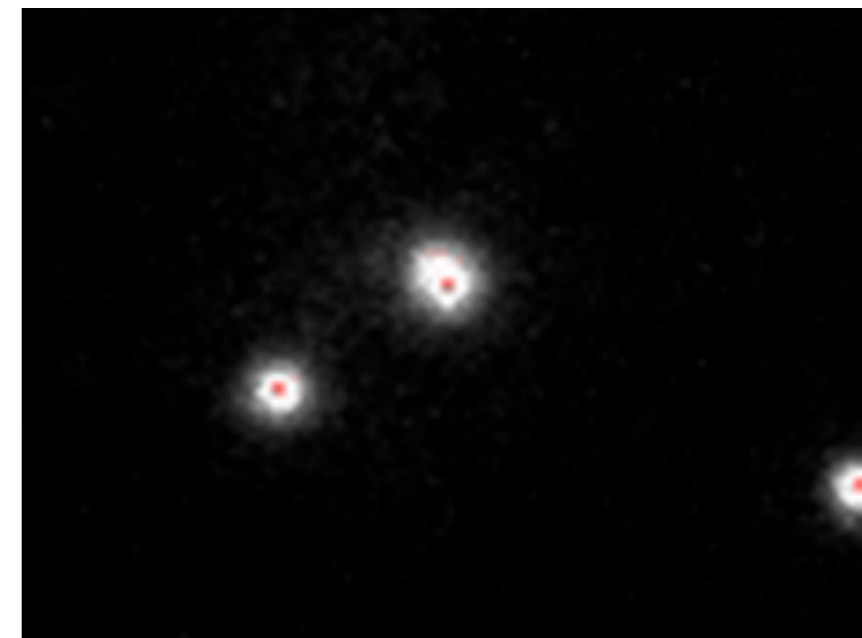
In vitro experiments



In Listeria Xenopus extract



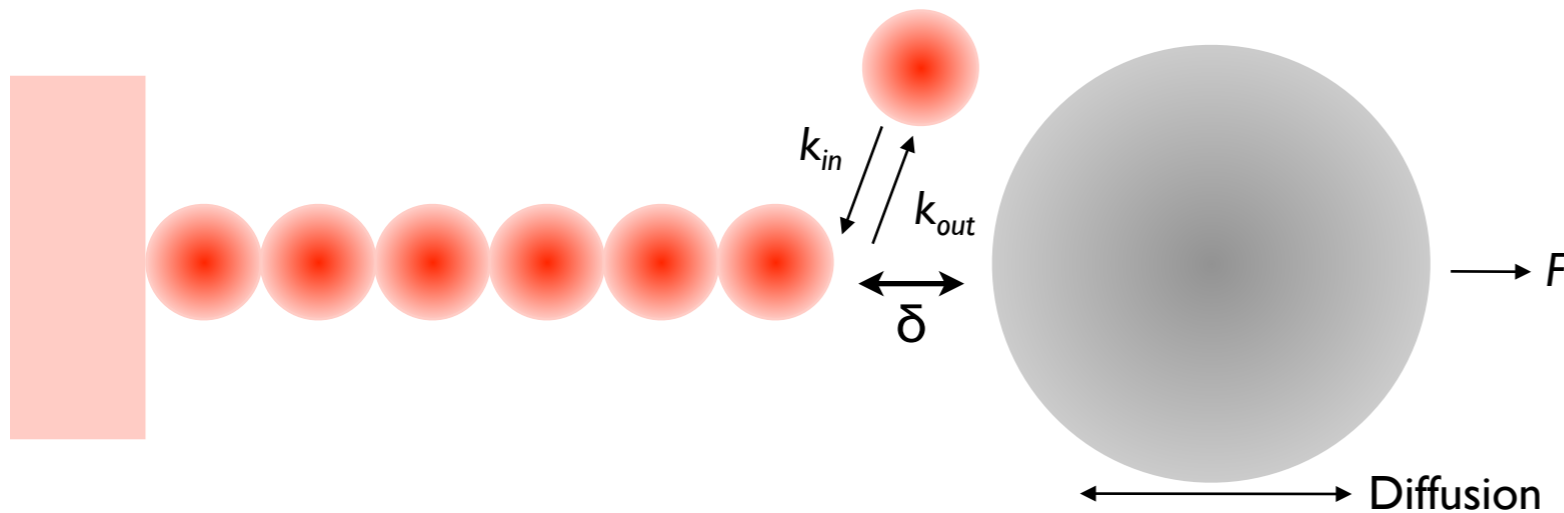
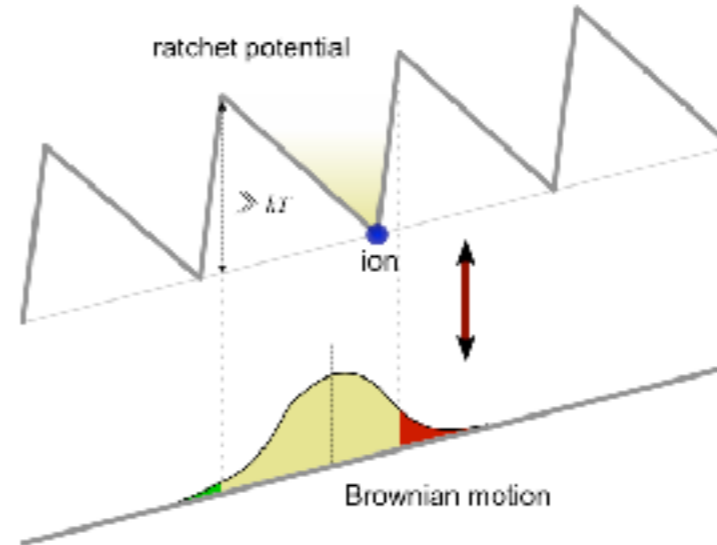
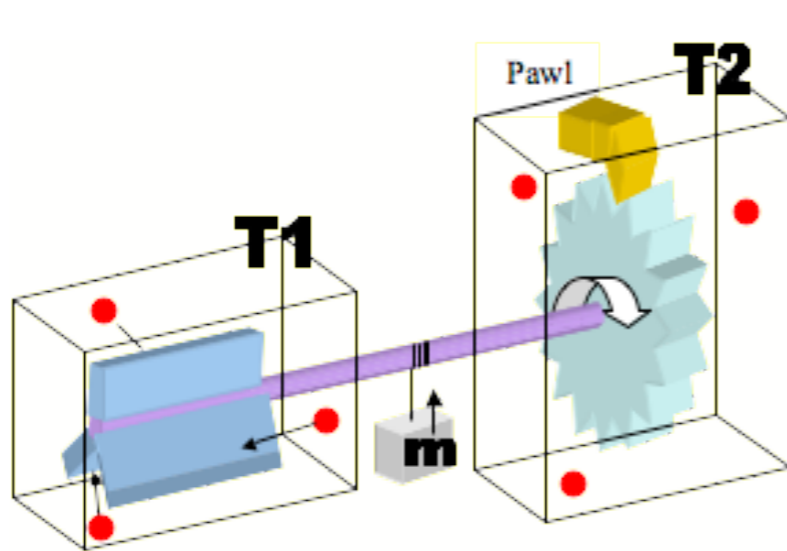
Microbead **asymmetrically** coated
with ActA in Xenopus extract



Microbead **symmetrically** coated
with ActA in Xenopus extract

ActA: A protein expressed by the bacterium *Listeria monocytogenes* that is responsible for the "rocketing" motility of the bacterium throughout the eukaryotic host cell. In addition to other host proteins, ActA binds actin directly.

A special case of diffusion: the Brownian ratchet



Listeria monocytogenes intracellular motion with actin polymerization

$$K(F) = K_c e^{\frac{F\delta}{k_B T}}$$

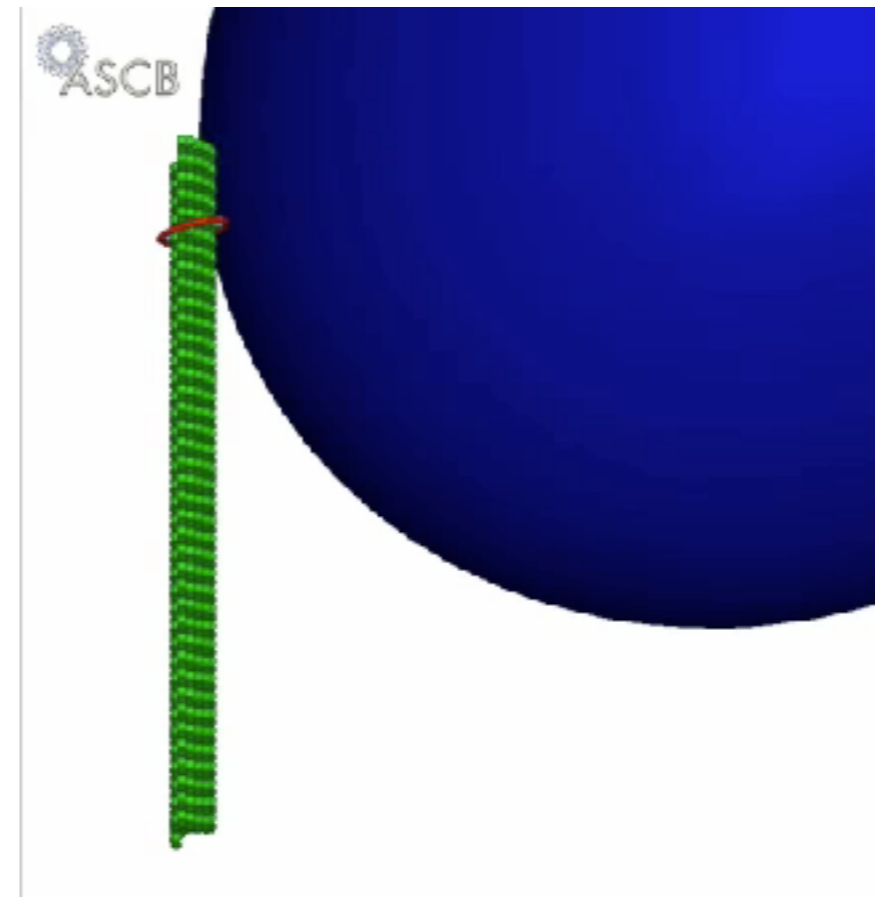
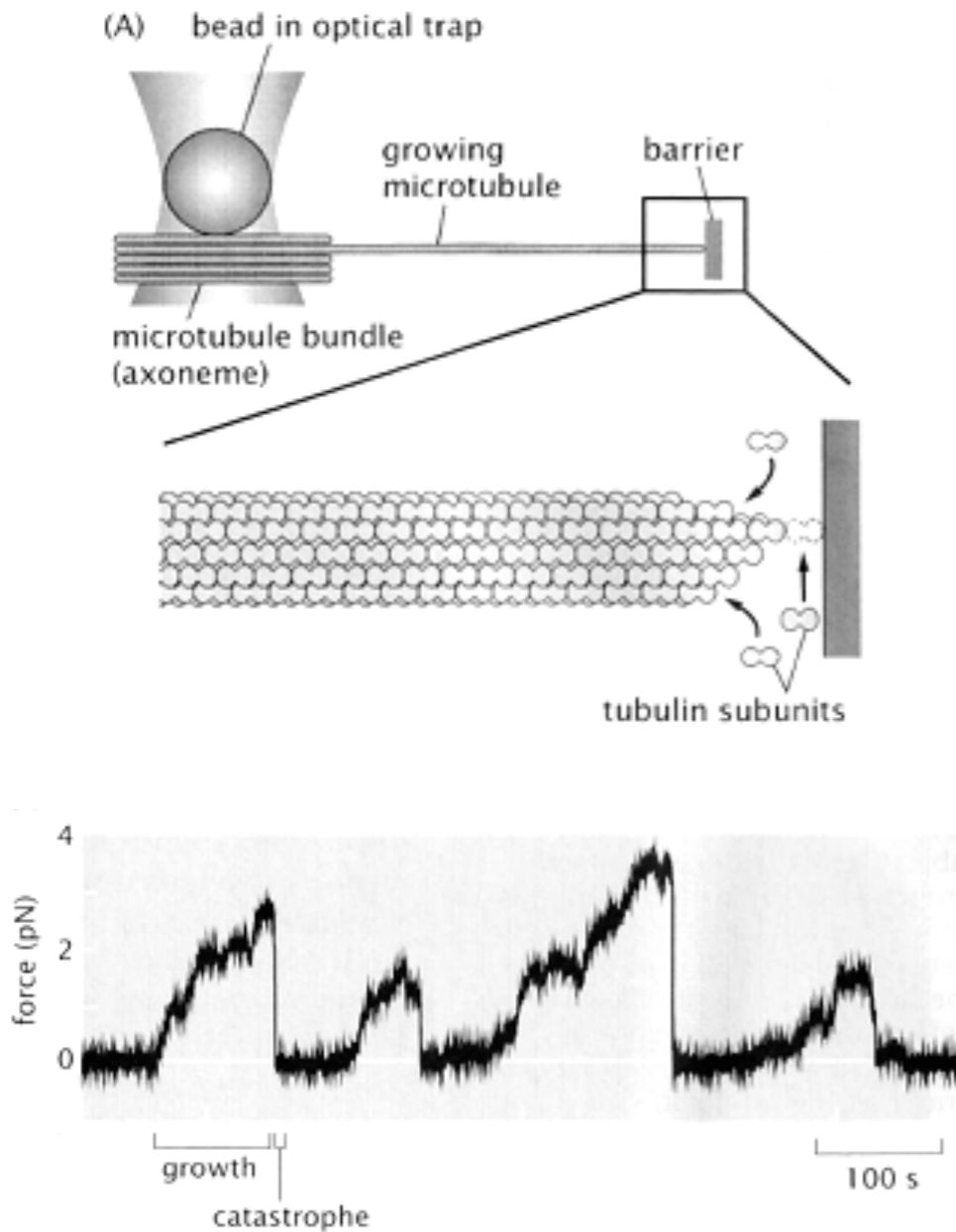
$K(F)$ = dissociation constant in the presence of force - monomer concentration at a net filament growth of 0.

K_c = critical concentration (at 0 force); F = force; δ = discrete growth upon the binding of one monomer. $k_B T$ = thermal energy.

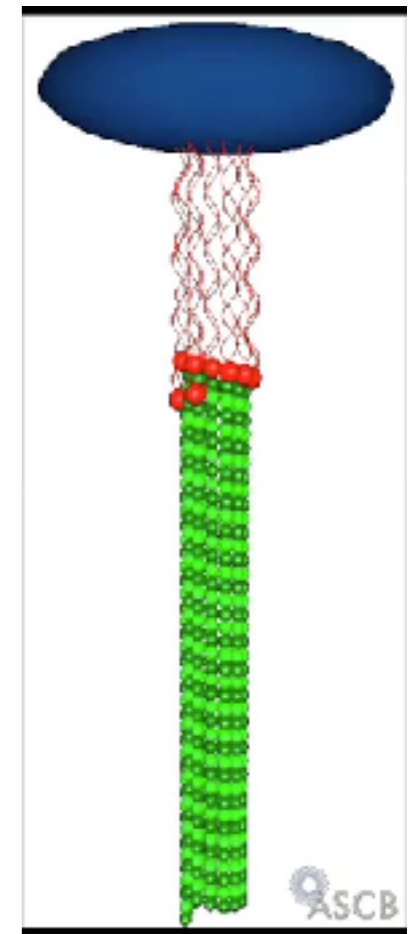
N.B.: F may be + or -. The process may be **reaction driven** (too fast diffusion for k_{in}) or **diffusion driven** (too slow diffusion for k_{in}).

Force generation with polymerization

Force generation with depolymerization



Vesicle transport with MT depolymerization



Chromosome (kinetochore) movement with MT depolymerization

MCAK: MT-depolymerizing kinesin

MCAK:

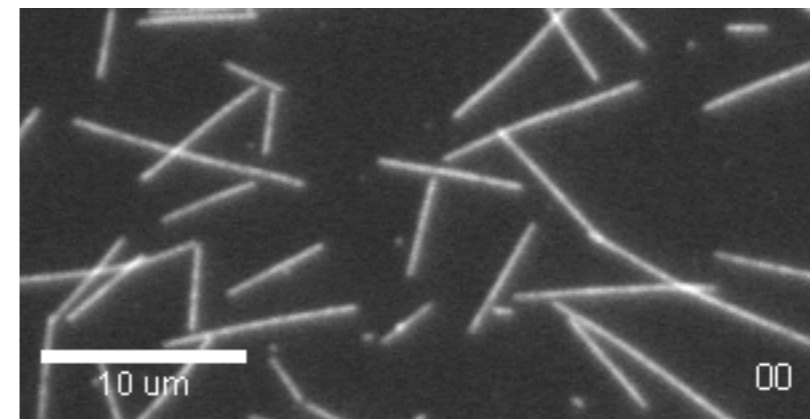
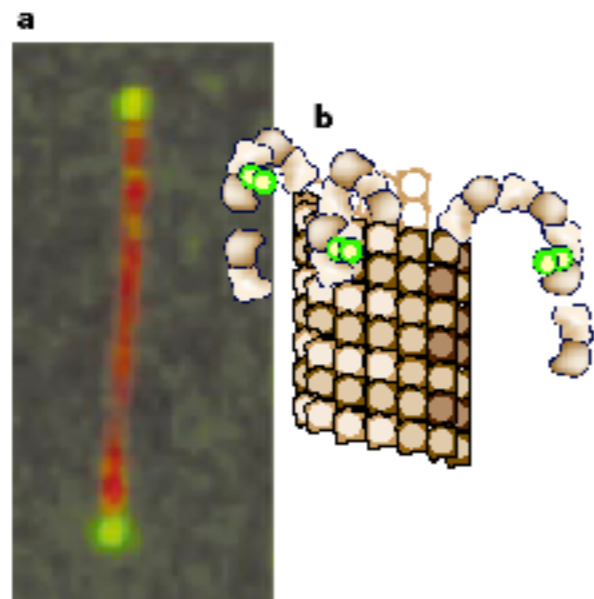
“Kinesin-13”

Binds to the plus (+) end of MT

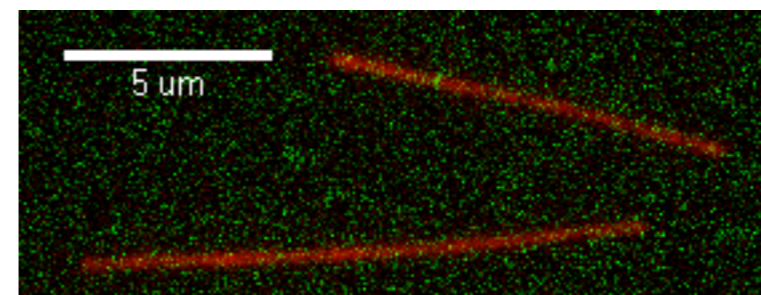
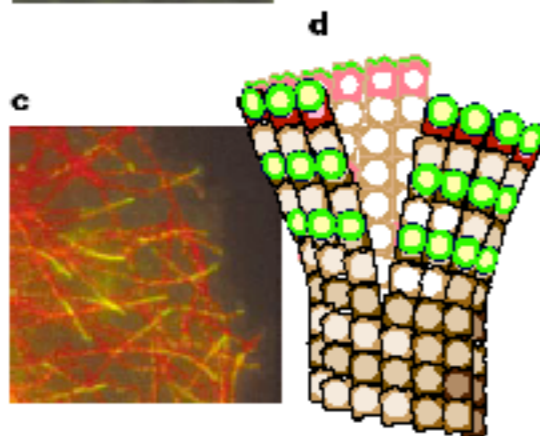
Finds the + end with a diffusional search

Hydrolyses ATP

Controls MT depolymerization (“catastrophe factor”)



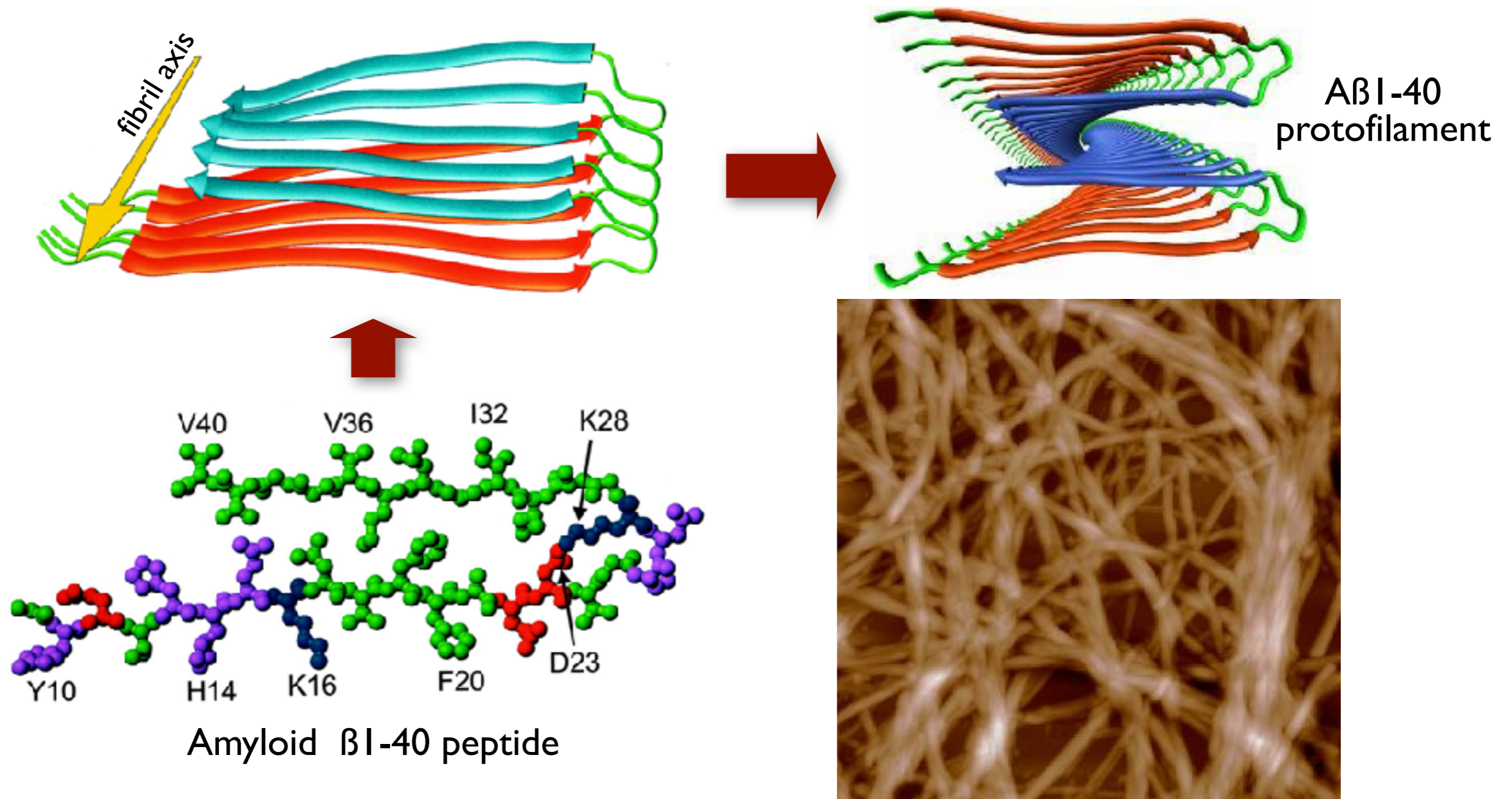
MCAK-induced MT depolymerization



Diffusional search along MT (GFP-MCAK)

Self-assembly, catalyzed polymerization

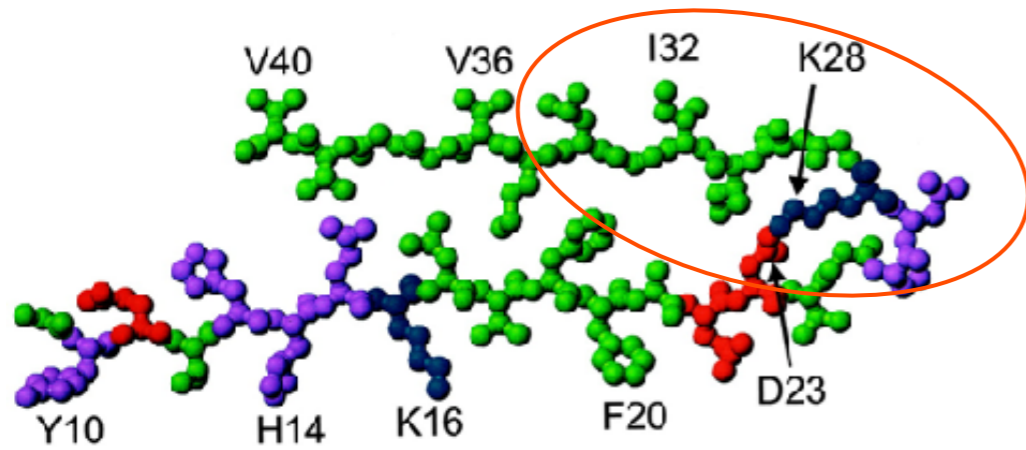
Amyloid β -fibrils: components of Alzheimer plaques



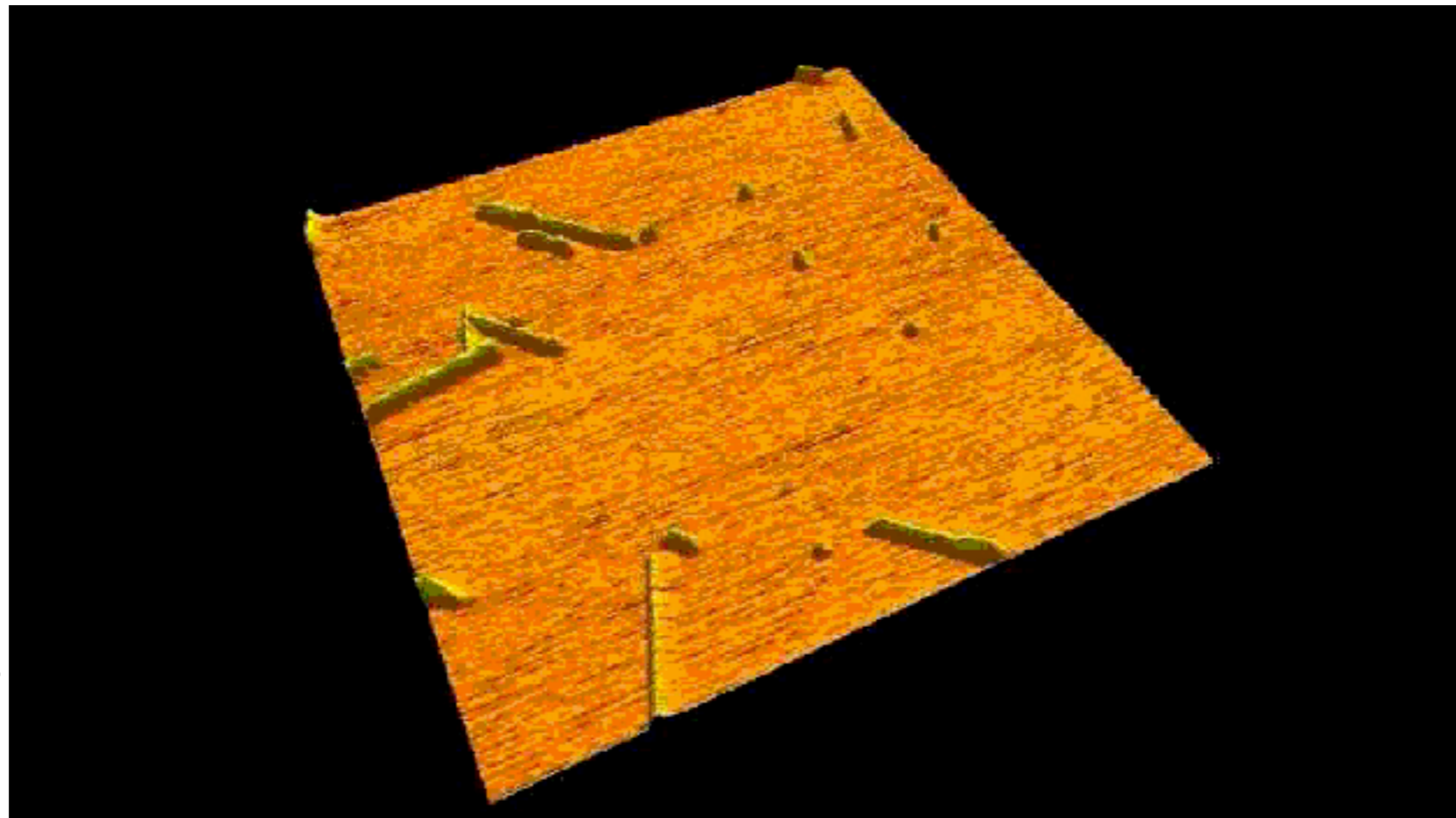
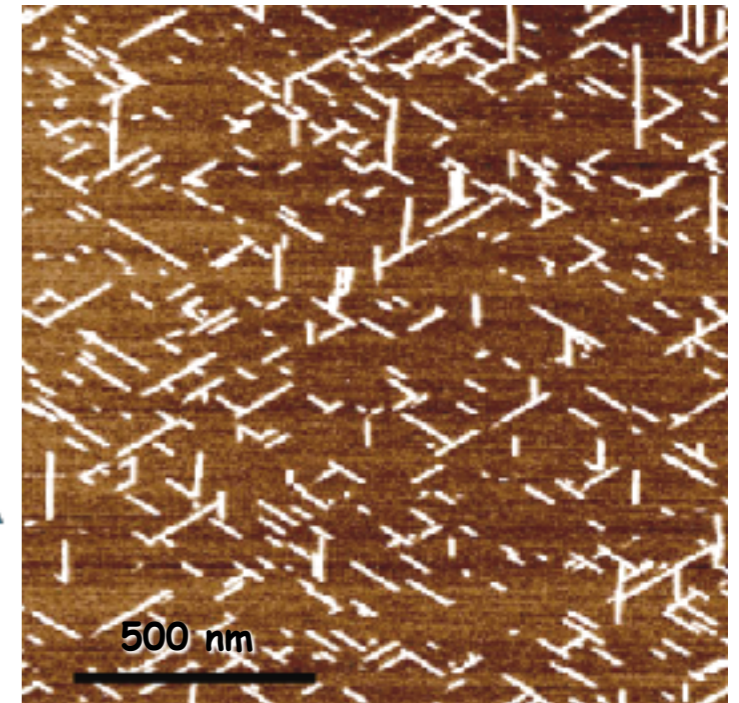
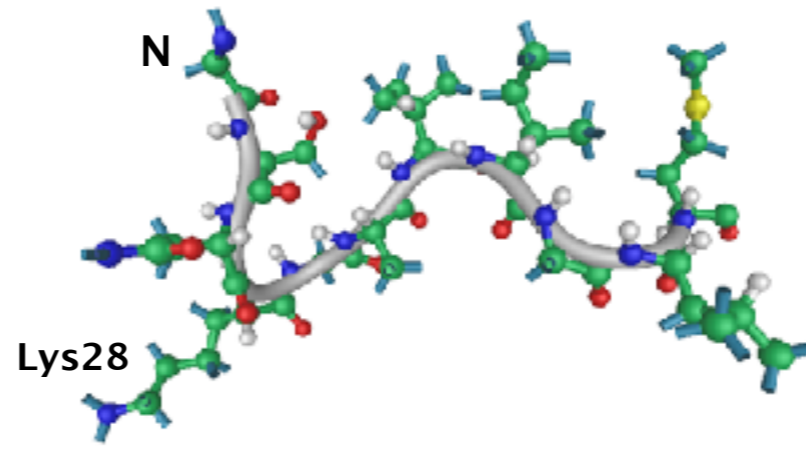
Fibrils grown from A β 1-40 peptide *in vitro* (AFM)

Epitaxial growth of amyloid fibrils

A β 1-40

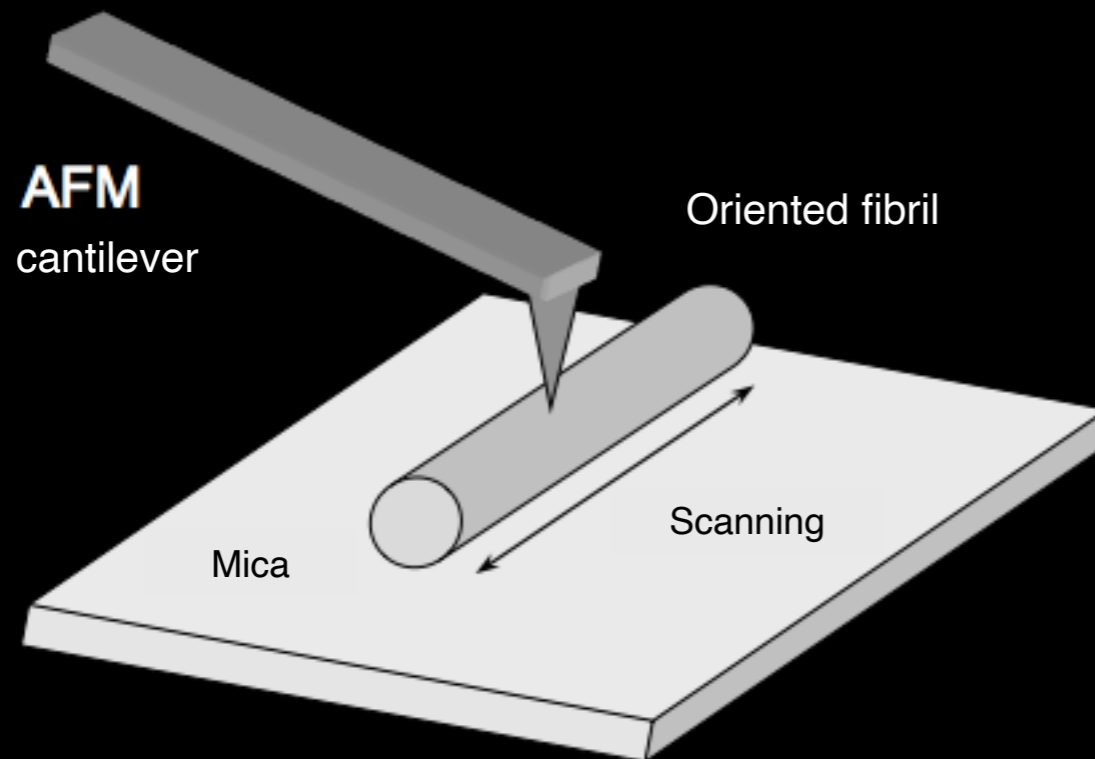
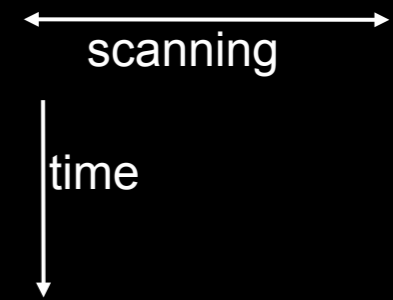


A β 25-35



Epitaxial growth of
A β 25-35 fibrils on mica
surface

Nanoscale mechanism of catalyzed fibril growth: scanning probe kymography



Spatial
resolution:
1 nm

Temporal
resolution:
300 ms

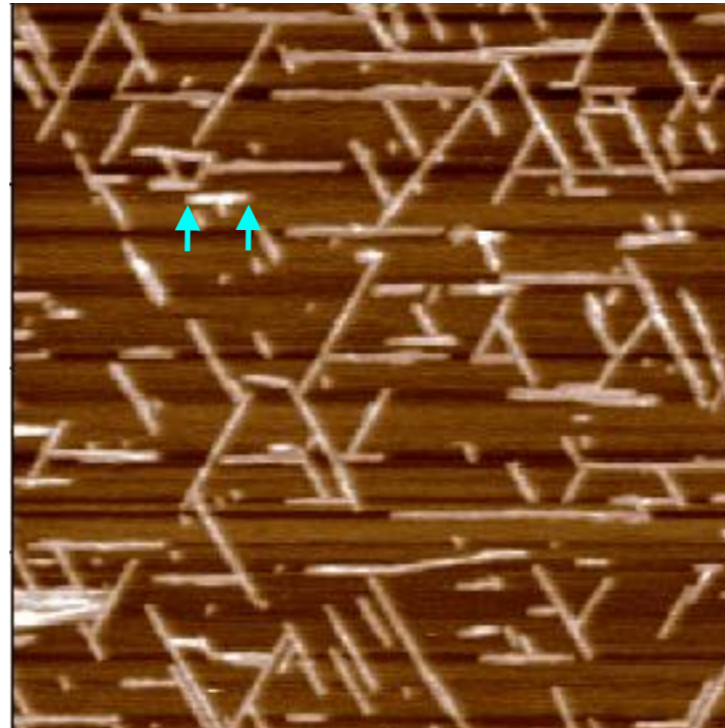
Scanning probe kymography

Space domain

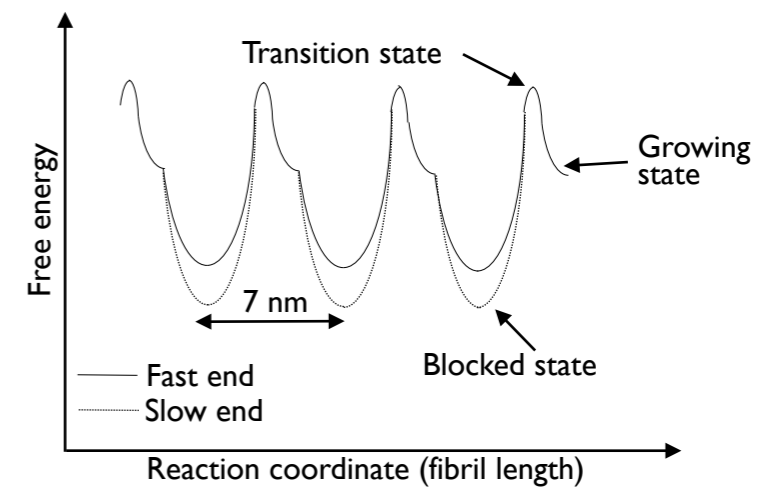
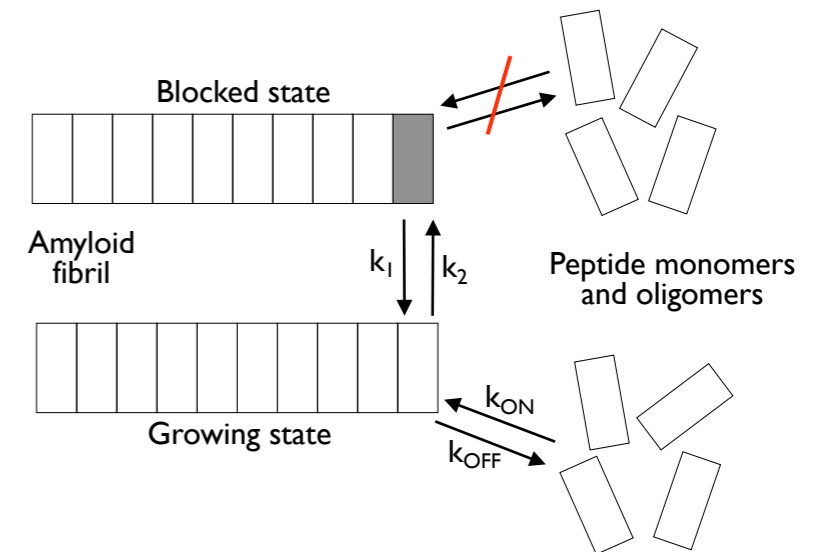
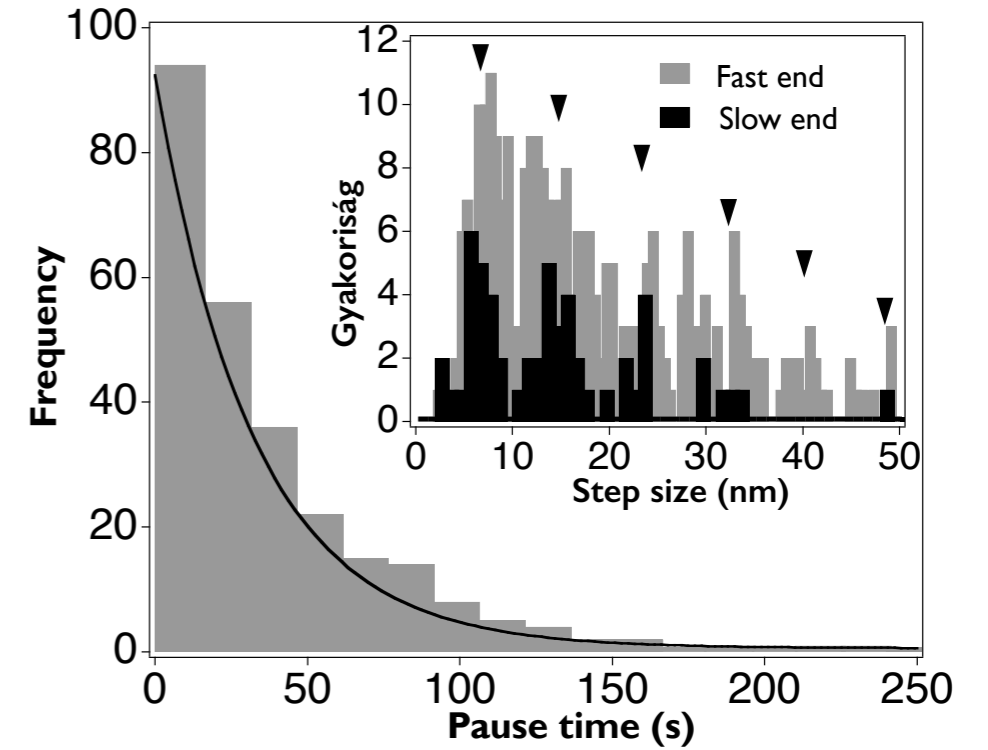
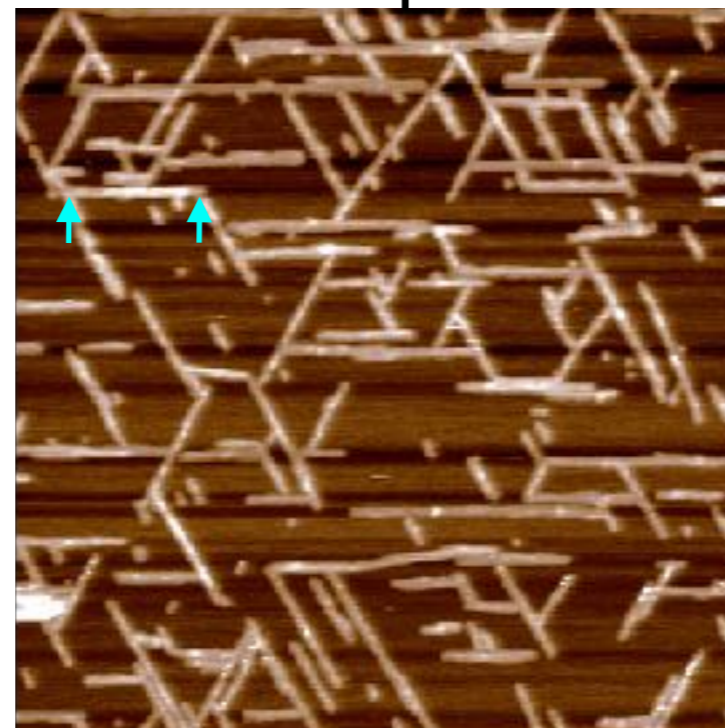
Vertical scanning turned off

Time domain (20 min)

Start



Stop



Motor proteins

(introduced last semester)

1. Bind to specific filaments
(cytoskeleton, DNA, RNA)
2. Generate force and displacement (or torque)
3. Convert chemical energy to mechanical (directly, not through heat conversion)

Types of motor proteins

1. Actin based

Myosins: Conventional (myosin II) and non-conventional Myosin superfamily (I-XXIV classes). Move towards plus end.

2. Microtubule based

a. Dyneins: Ciliary (flagellar) and cytoplasmic dyneins. Move towards the minus end along the microtubule.

b. Kinesins: Kinesin superfamily: conventional and non-conventional. Move towards the plus end along the microtubule.

c. Dynamins: MT-dependent GTPase activity
Biological role: vacuolar protein sorting (pinchase enzymes)?

3. DNA based motors

DNA and RNA polymerases, virus capsid packaging motor, condensins
Produce force and displacement along the DNA strand

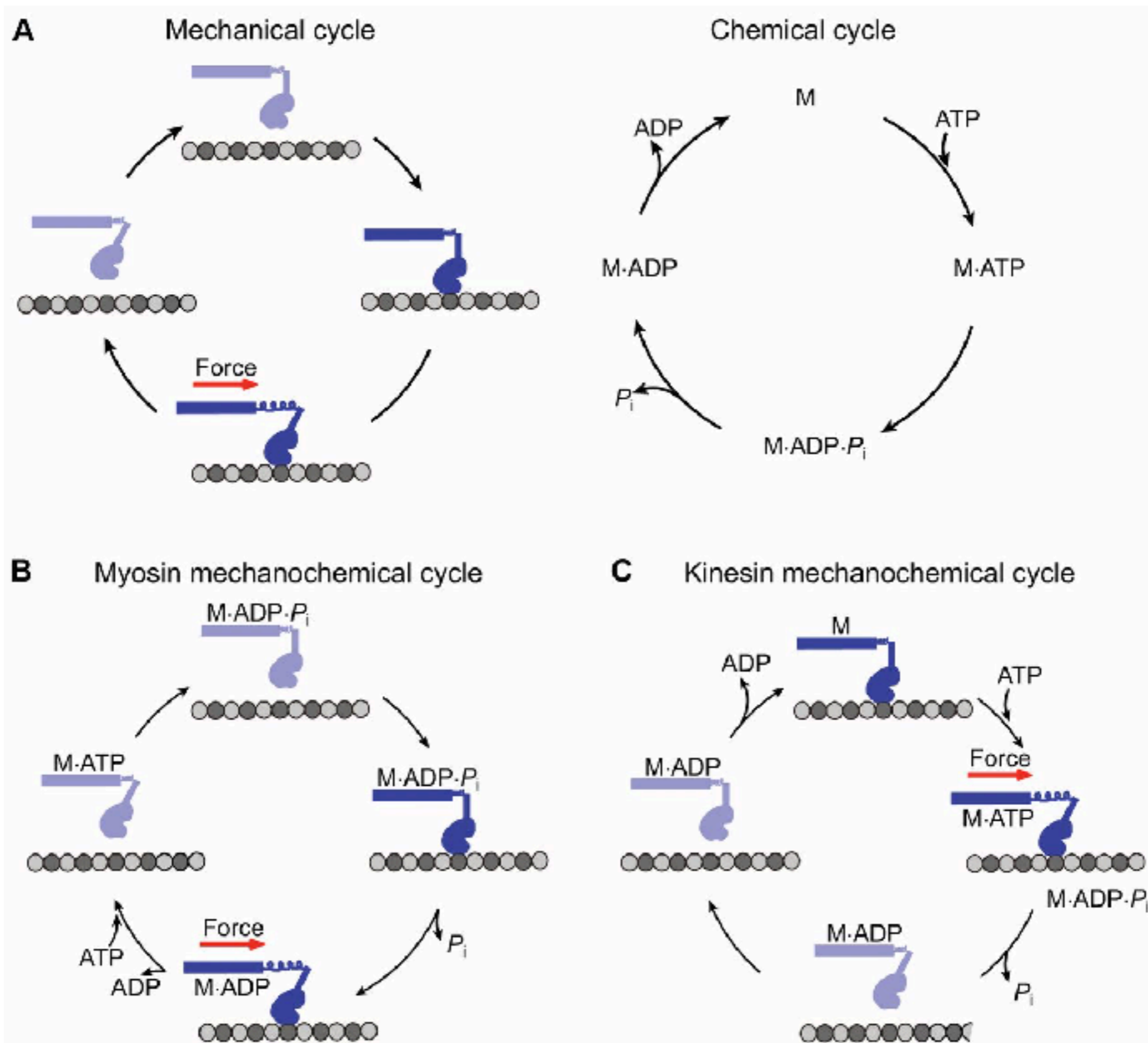
4. Rotary motors

F1F0-ATP synthase
Bacterial flagellar motor

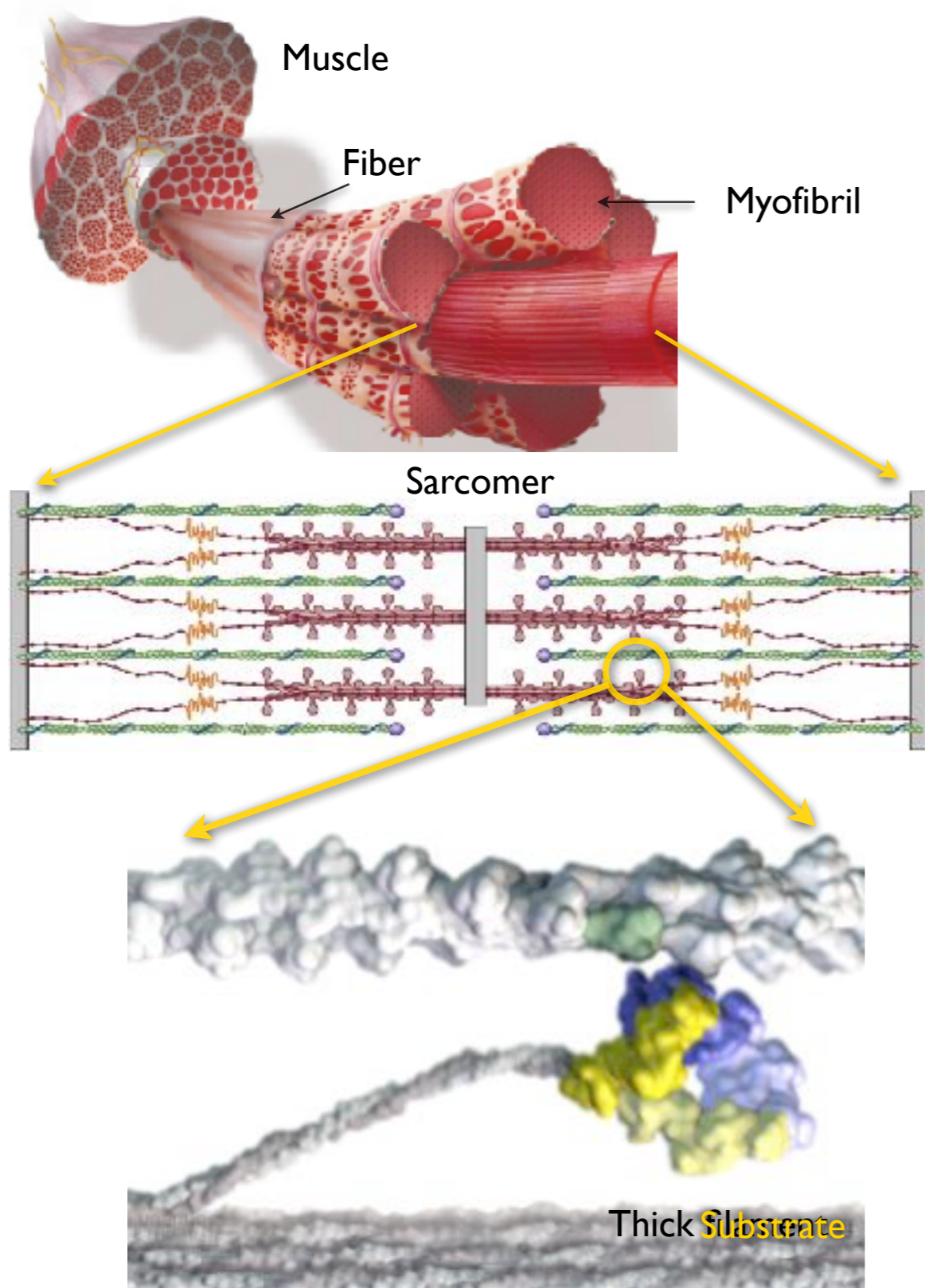
5. Mechanoenzyme complexes

Ribosome

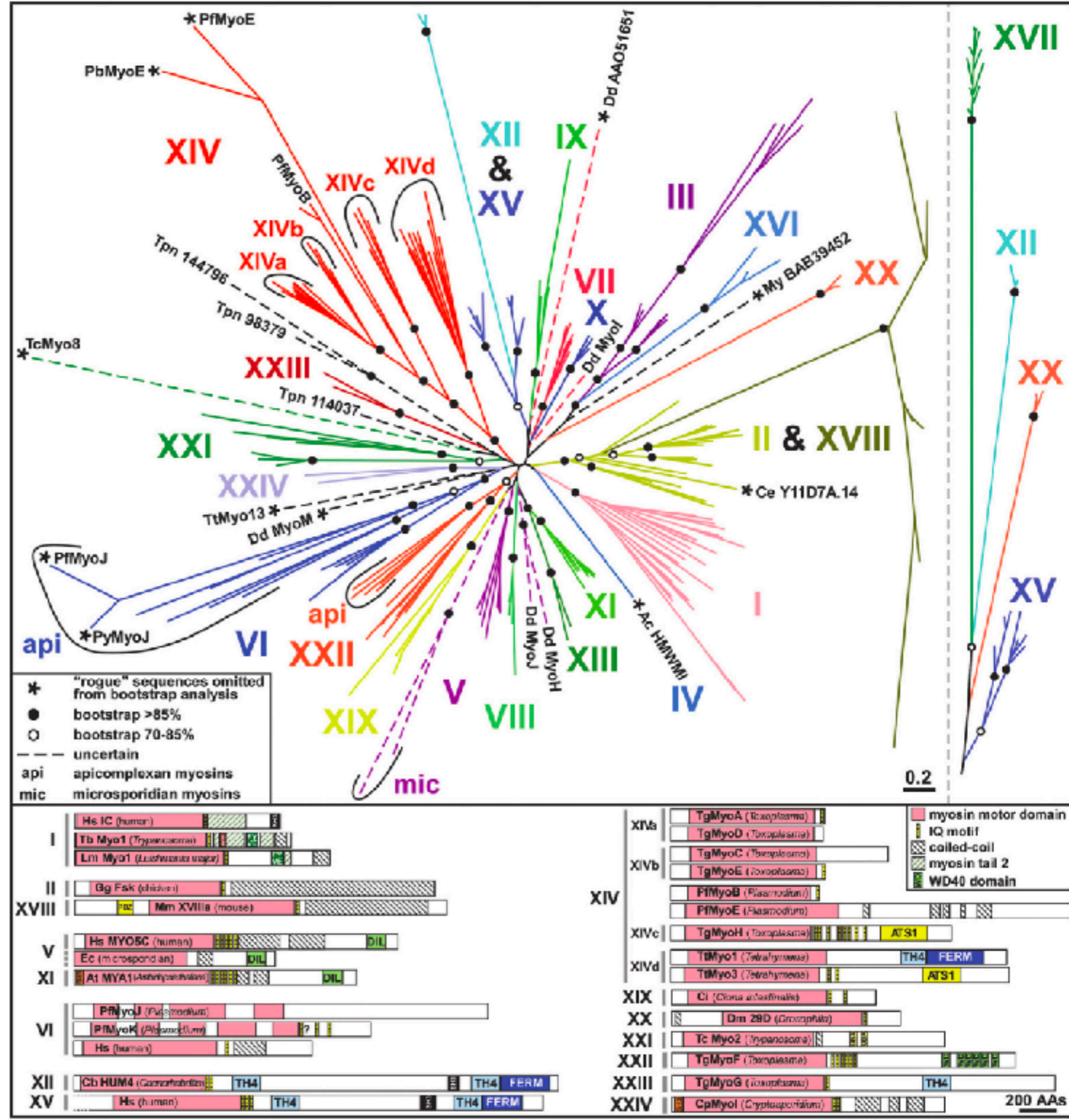
Cyclic mechanism - “duty cycle”



Myosin

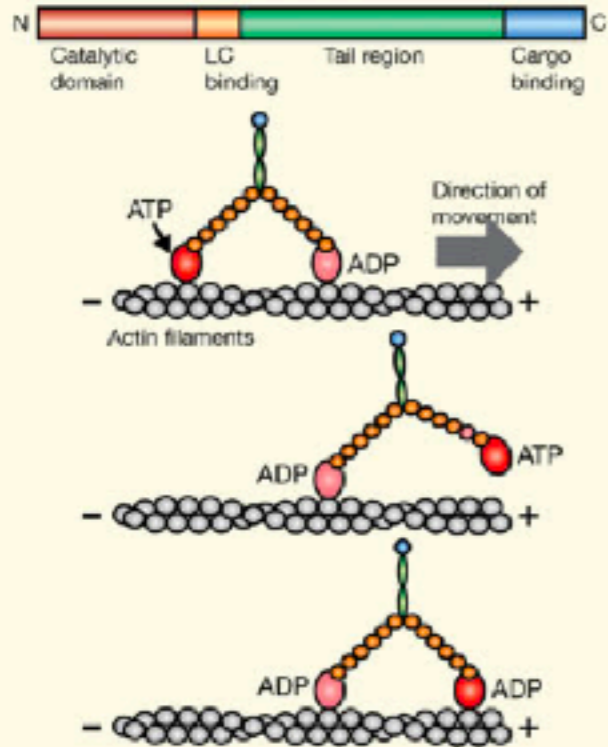


The myosin superfamily

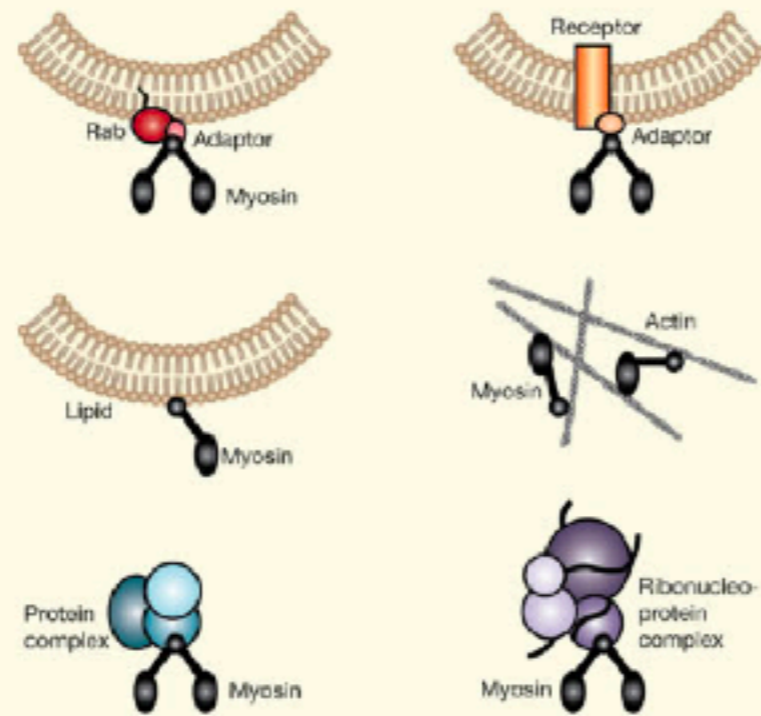


Functions of the myosin superfamily

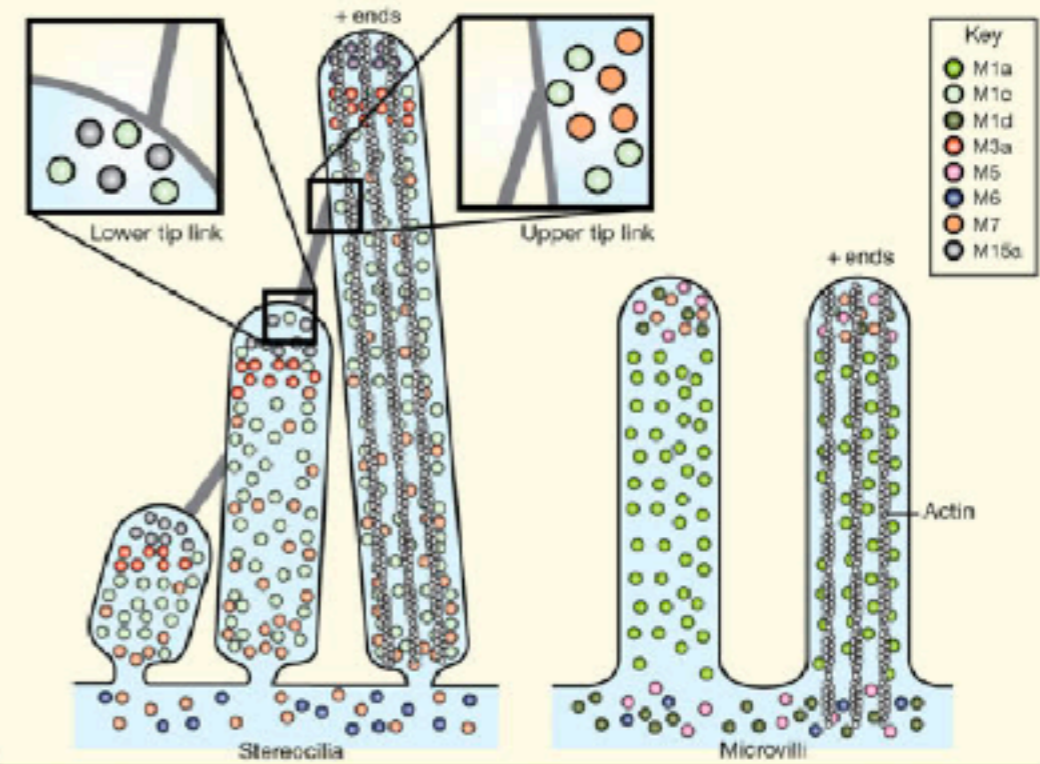
Myosin structure and motility



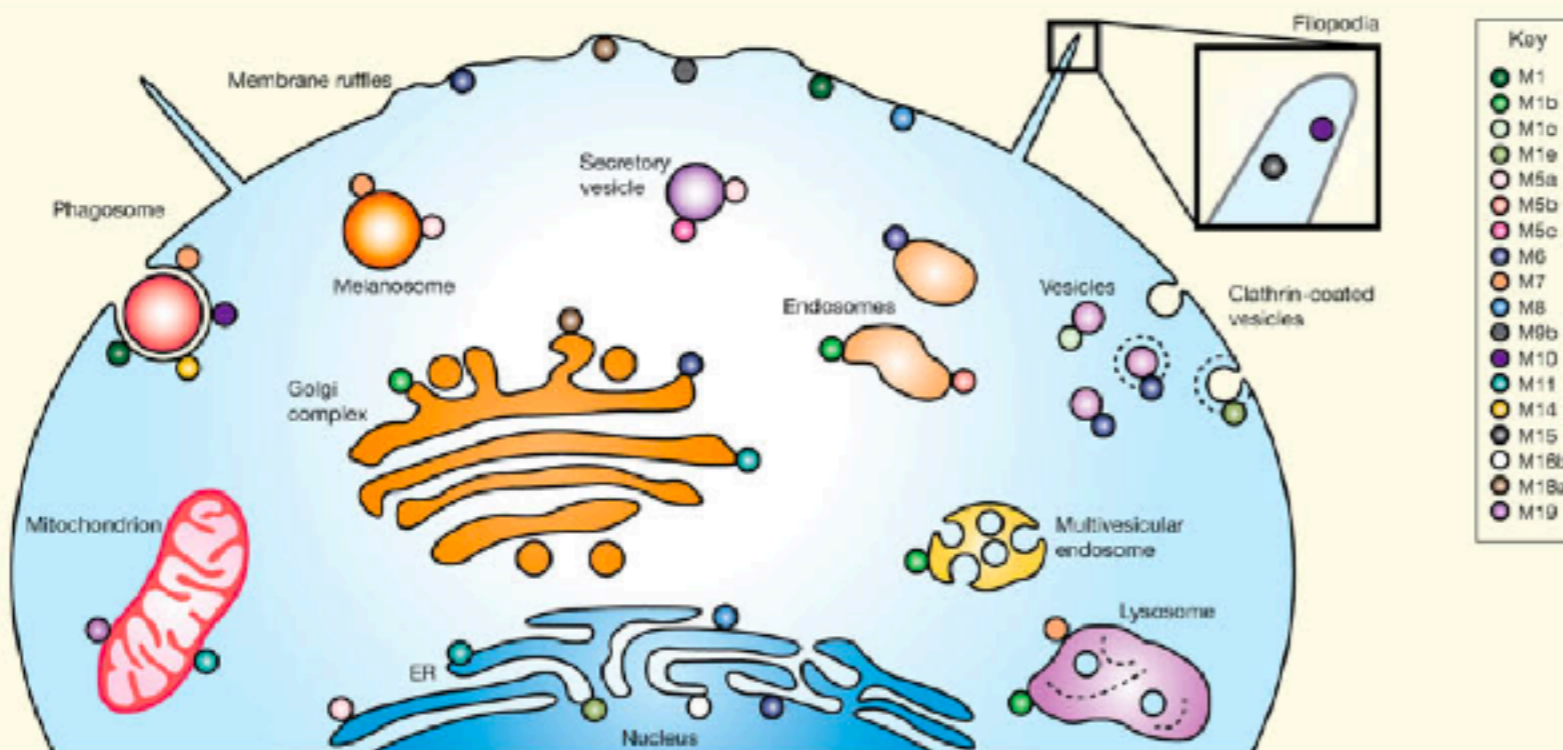
Cargo interactions



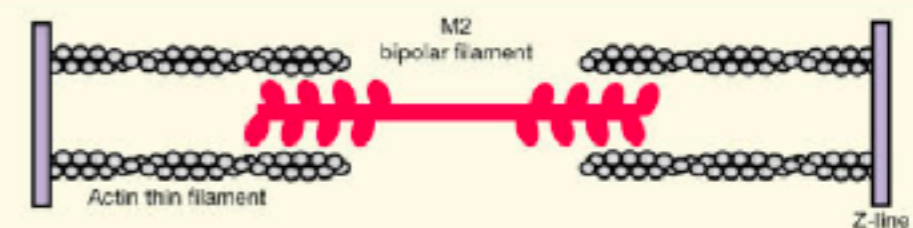
Actin-based projections



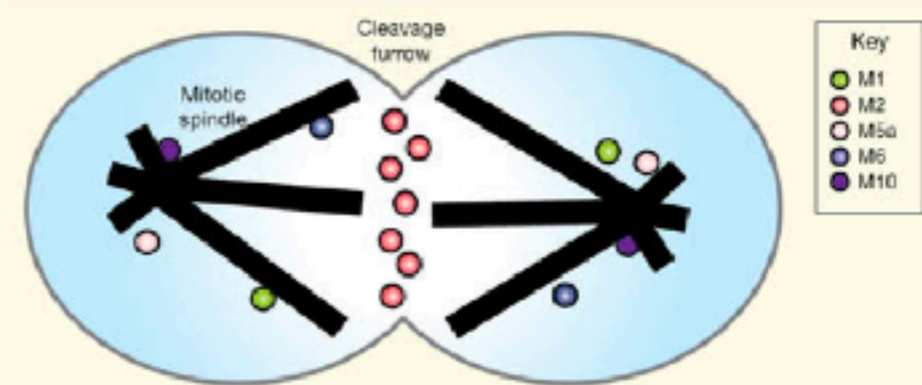
Membrane compartments



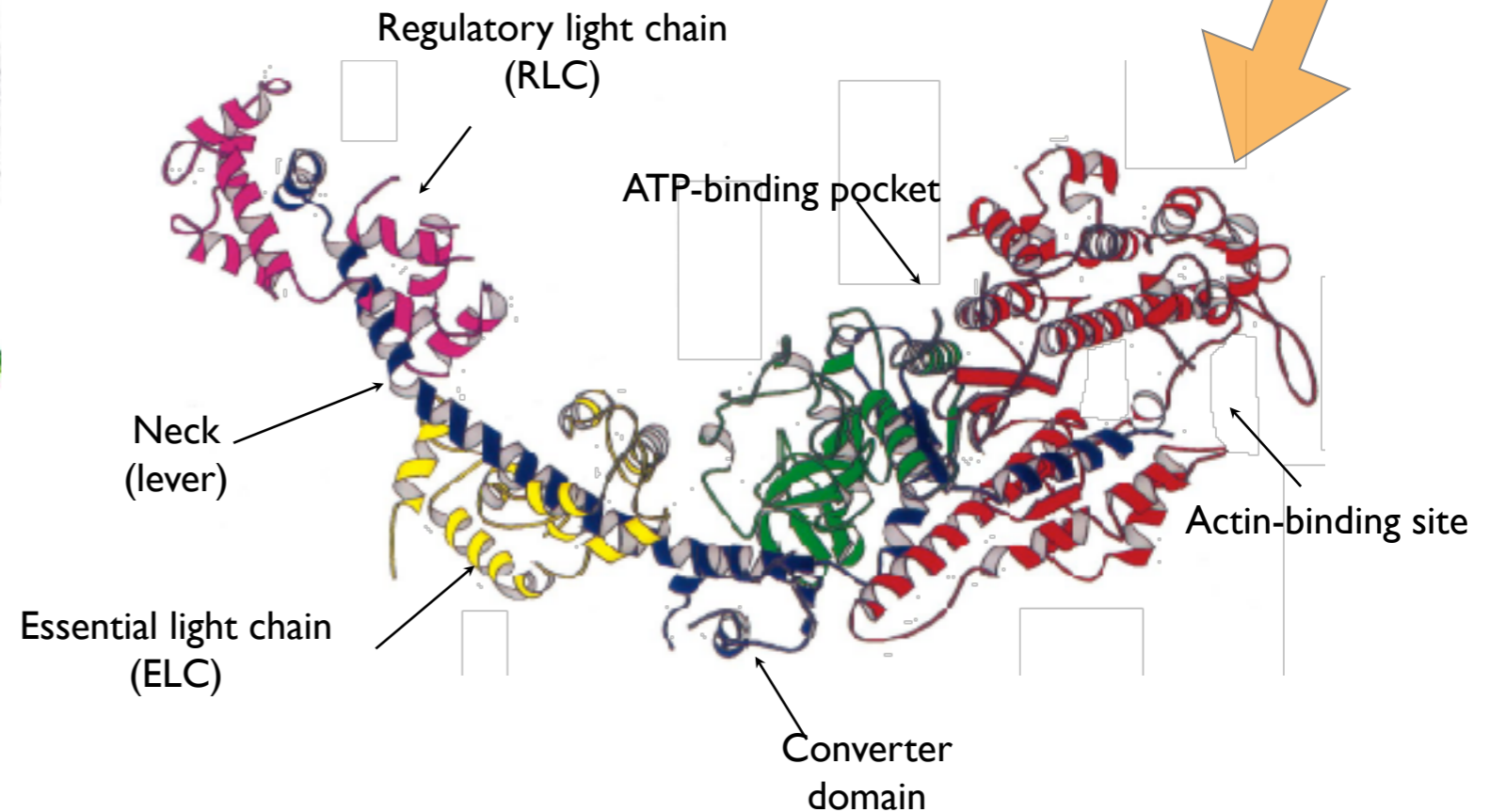
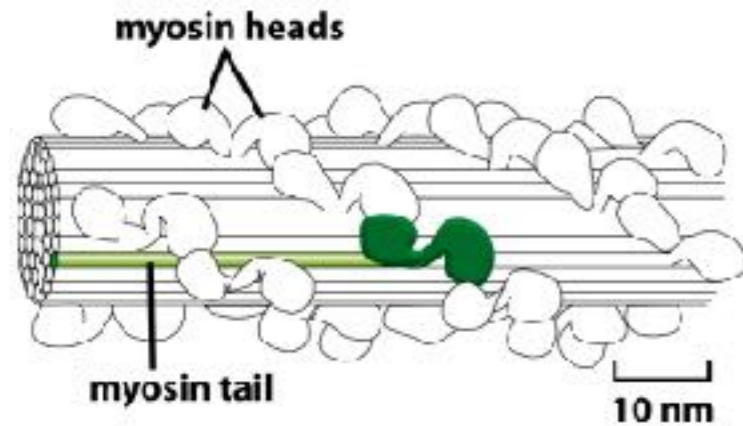
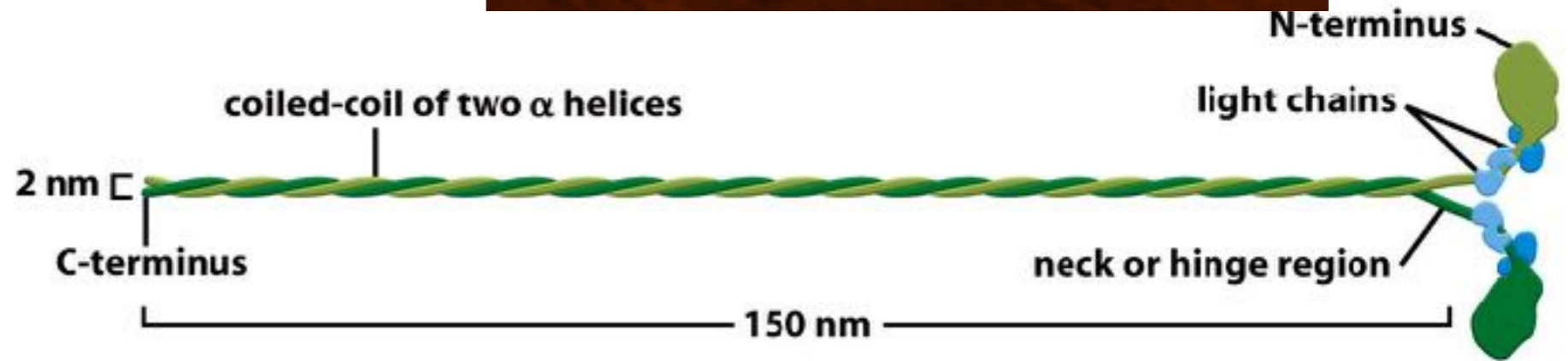
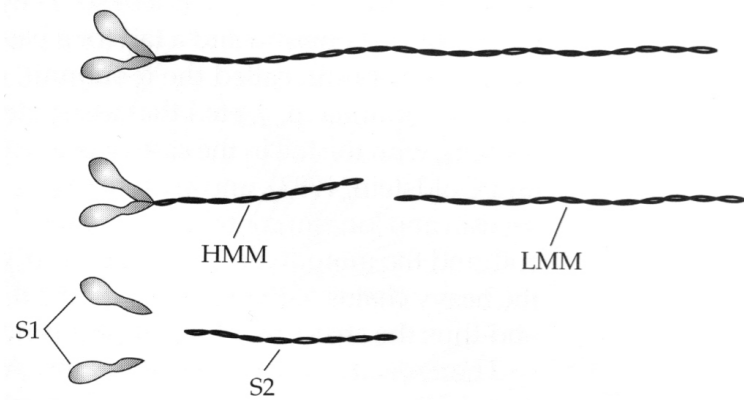
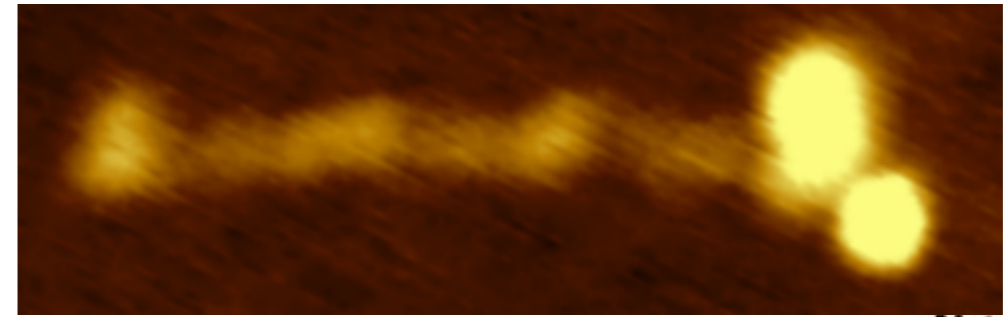
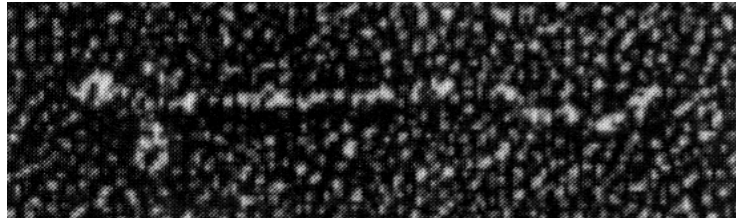
Muscle contraction



Cell division

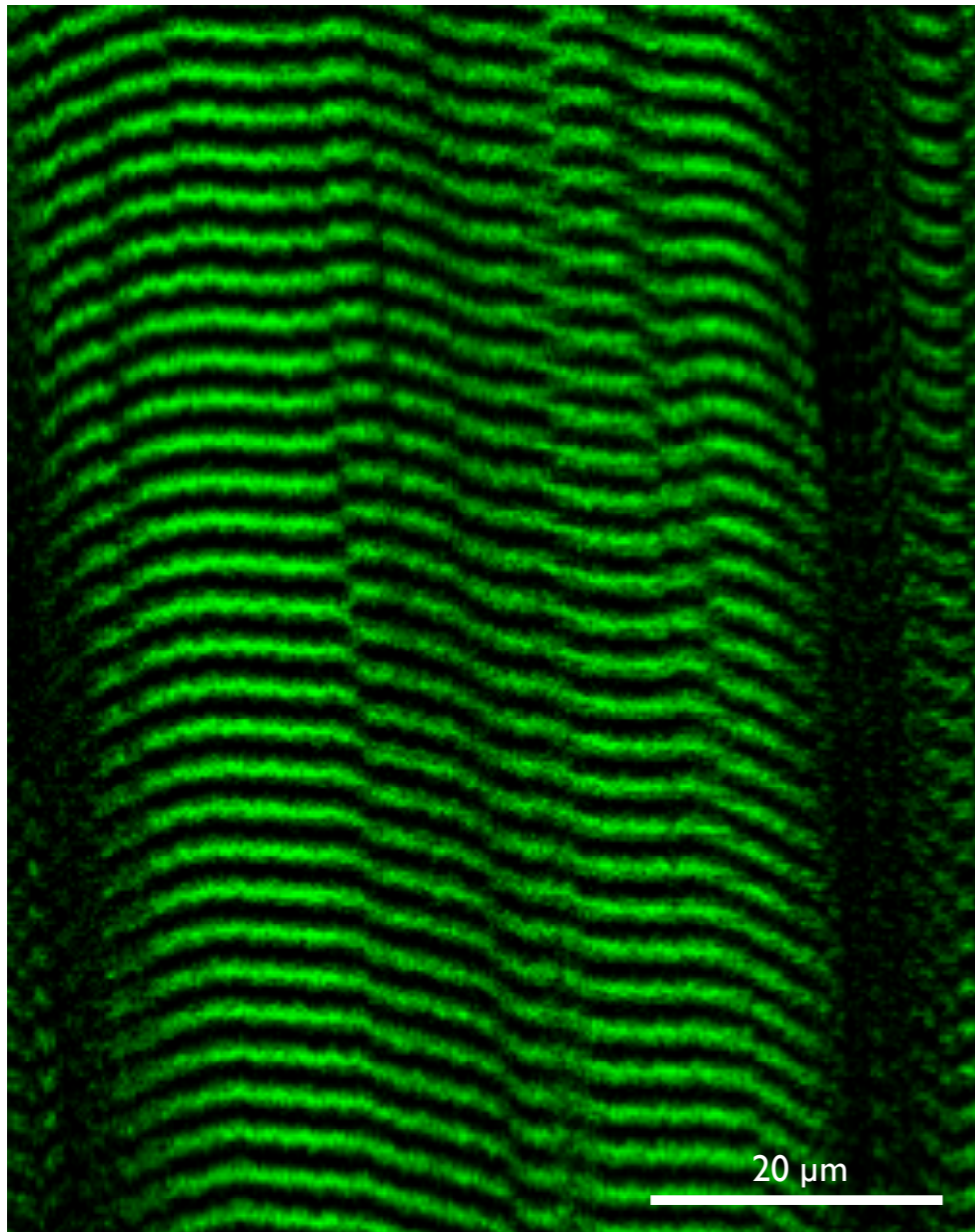


Myosin II



Myosin II assembles into thick filaments

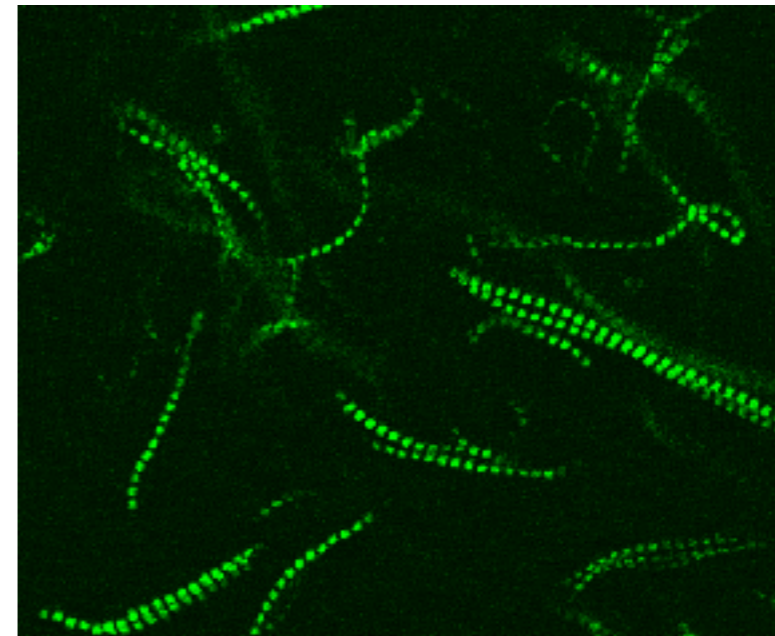
Two-photon microscopic image of a muscle fiber



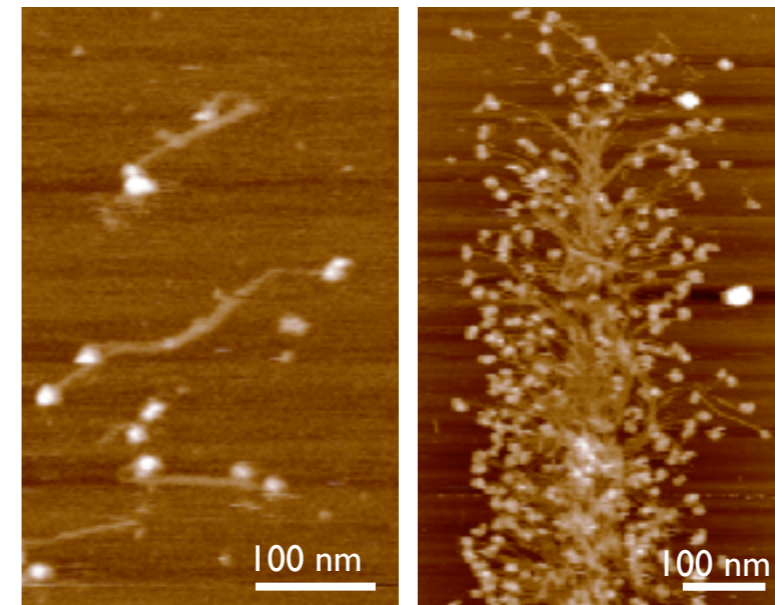
Signal source:
helical
structure of
myosin
filaments

Excitation:
1000 nm

Second
harmonic
generation
(SHG):
500 nm



Unlabeled myofibrils.
2P microscopic image

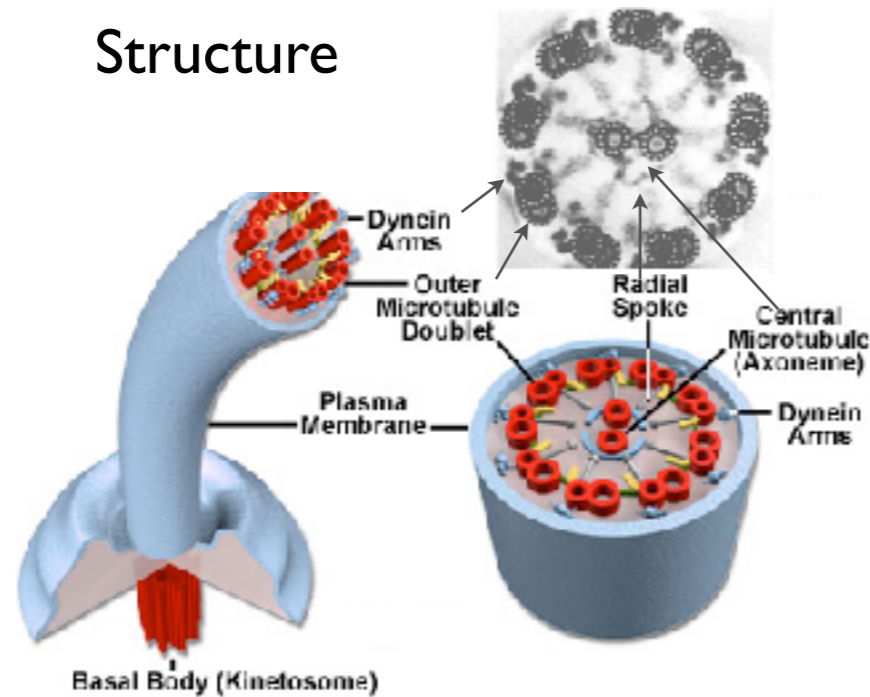


Myosin
molecules
and thick
filament.
AFM image

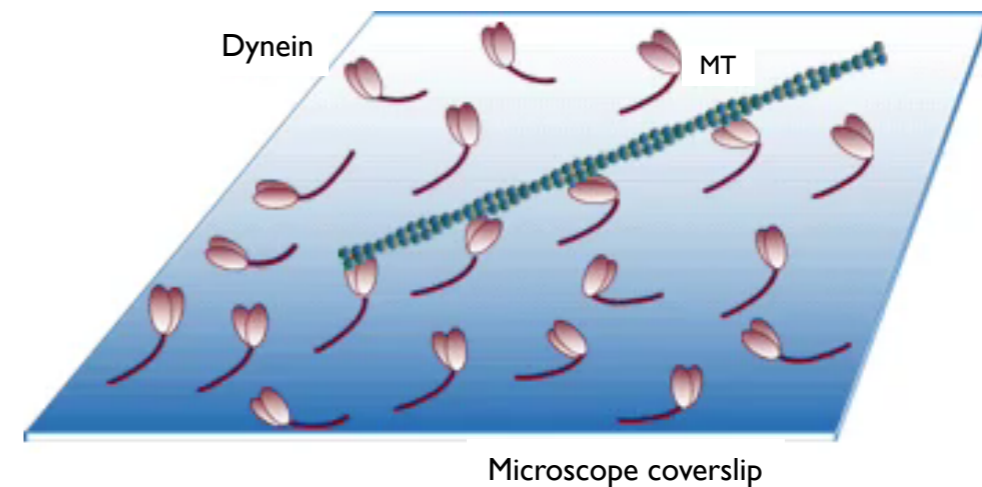
Dyneins

Types: axonemal and cytoplasmic. Numerous subunits ($M_r \sim 500$ kDa). Move towards the MT minus end. Their coordinated action bends the cilium.

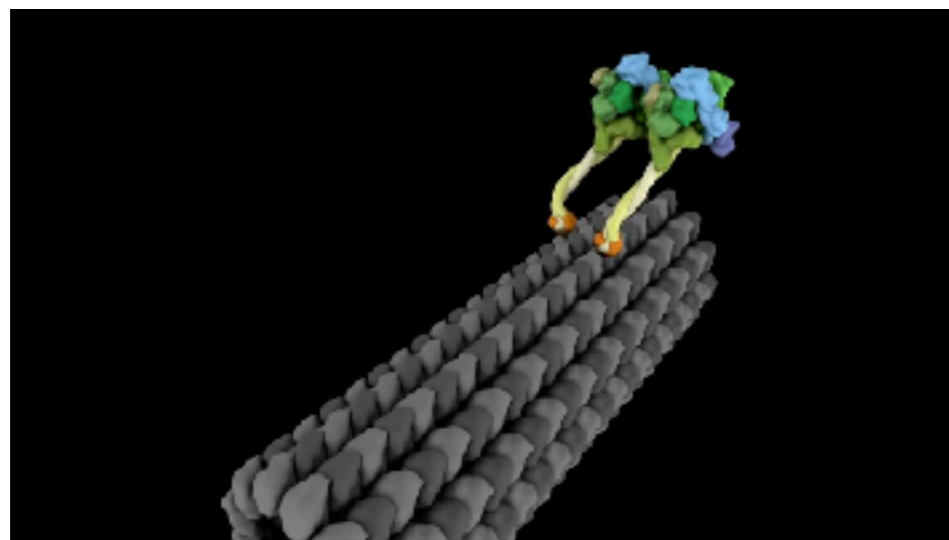
Structure



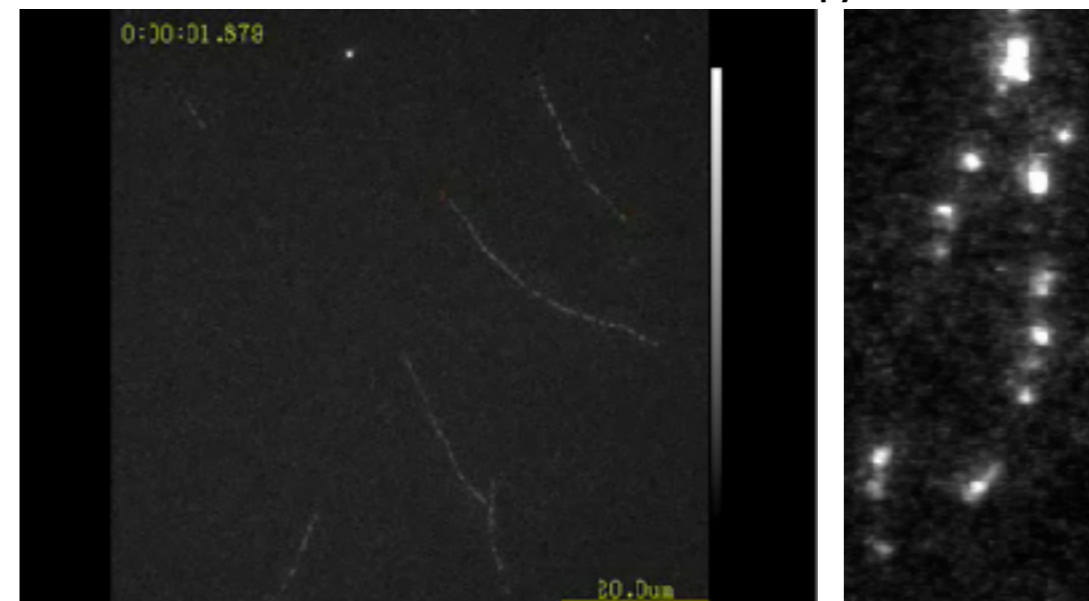
Biomolecular *functional model*:
“*In vitro motility assay*”



Fluorescence video microscopy



“Drunken sailor” stepping mechanism

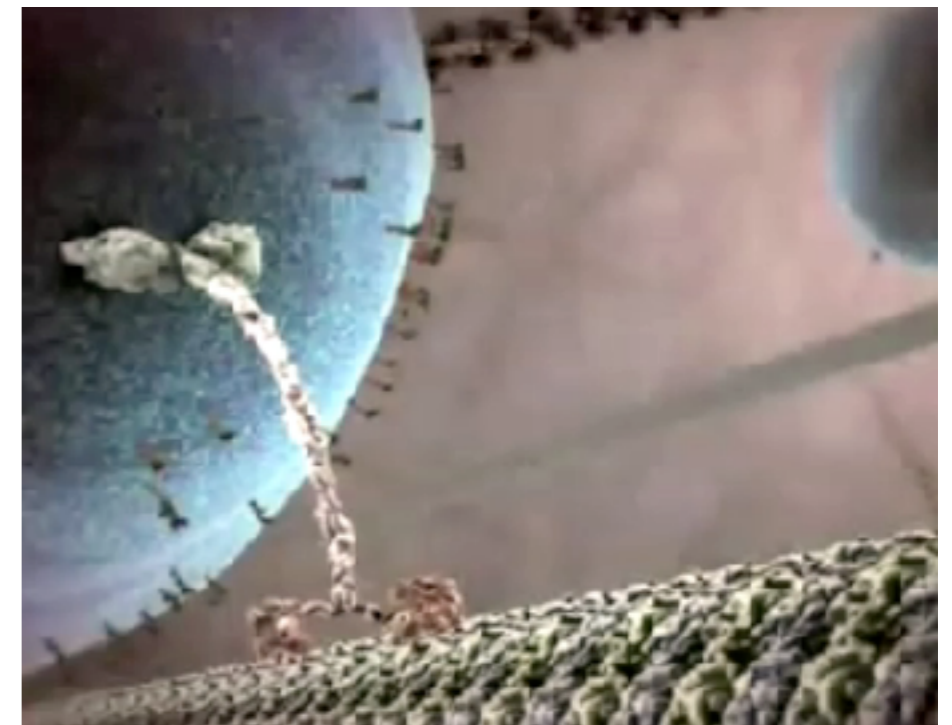
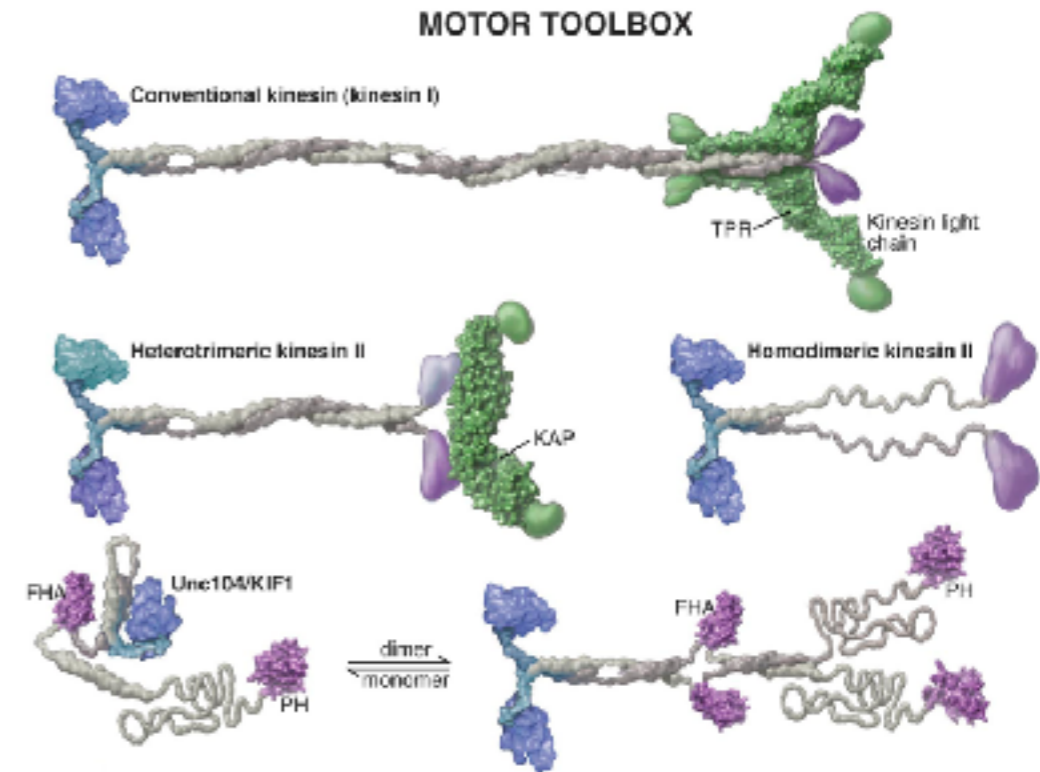
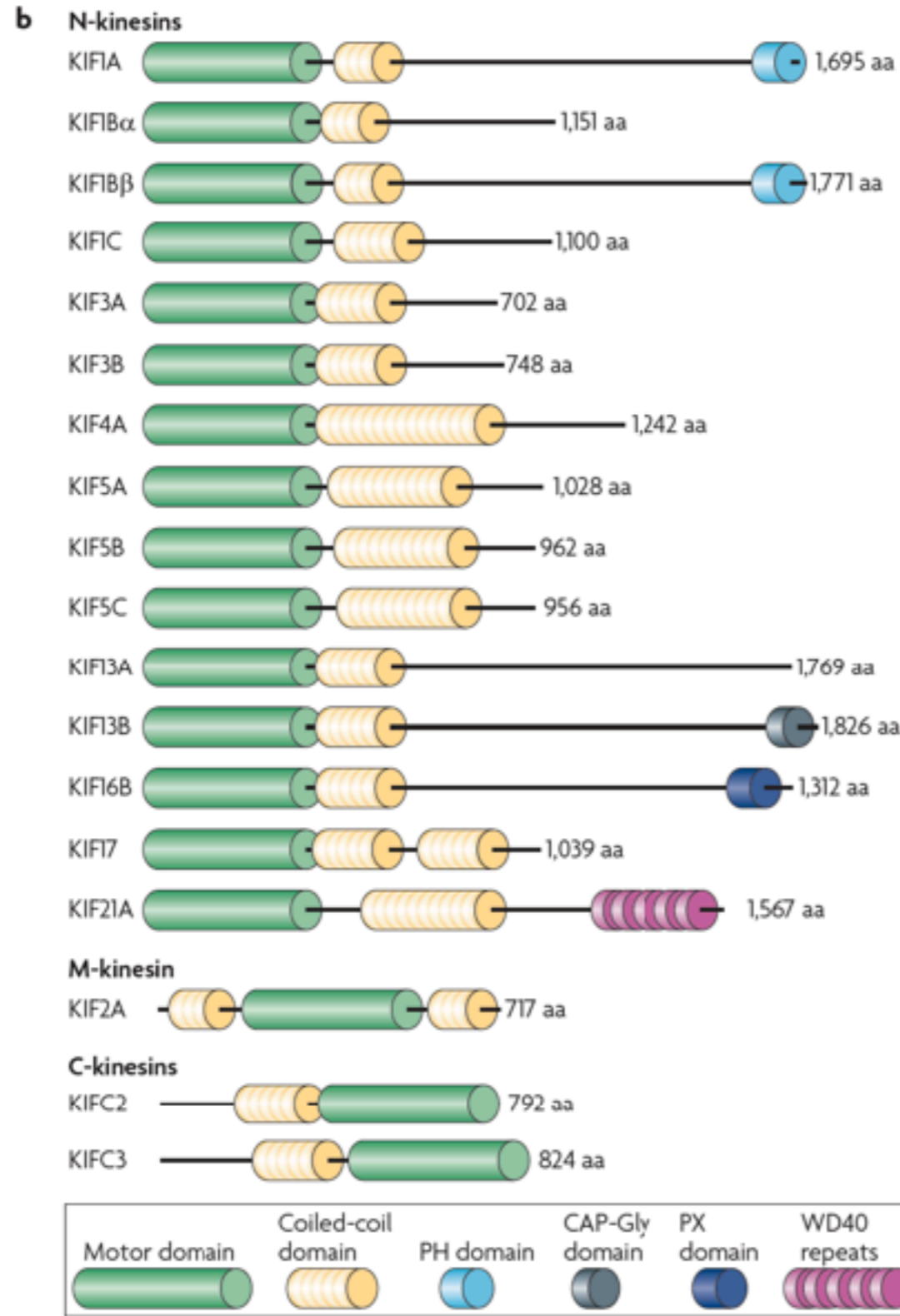
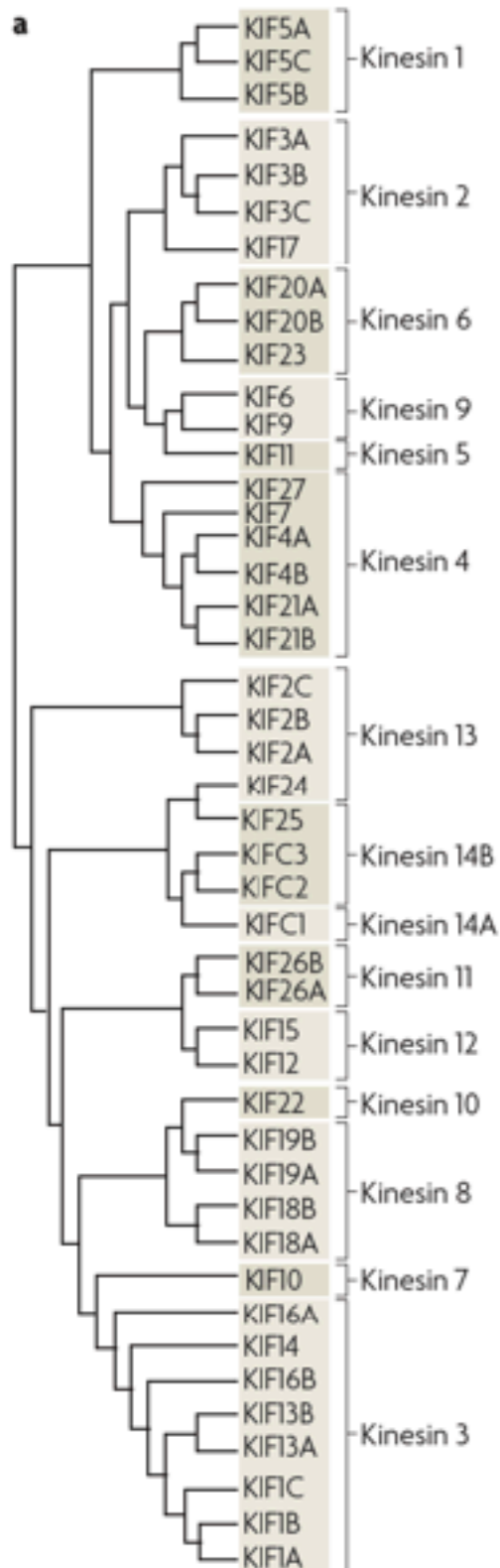


Microtubule moves on dynein

Dynein moves on microtubule

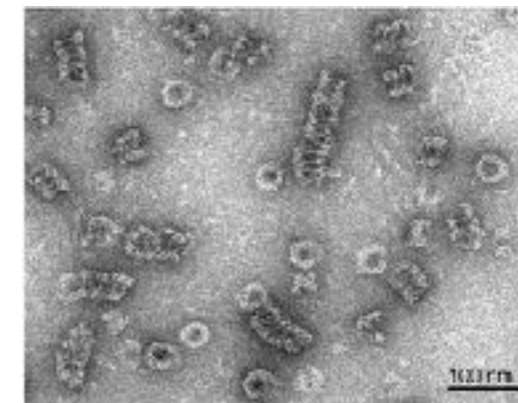
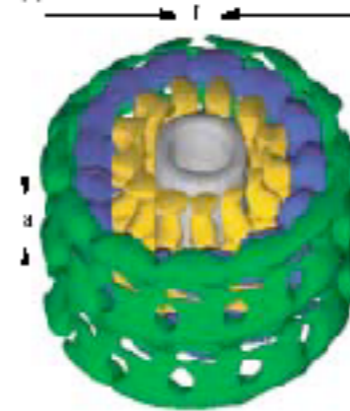
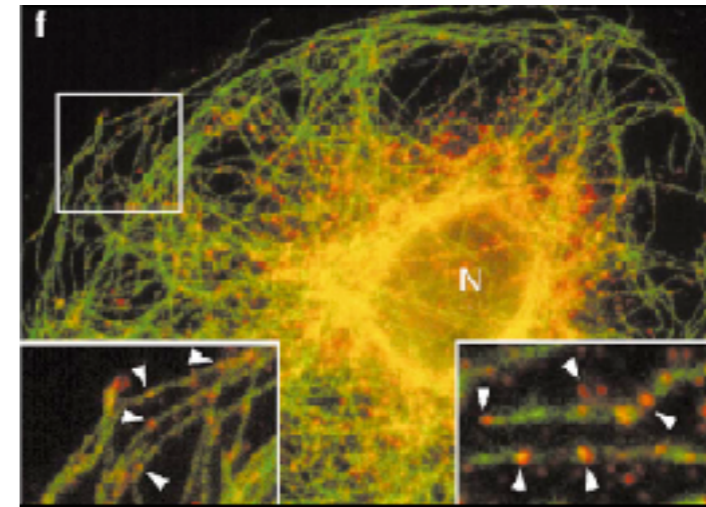
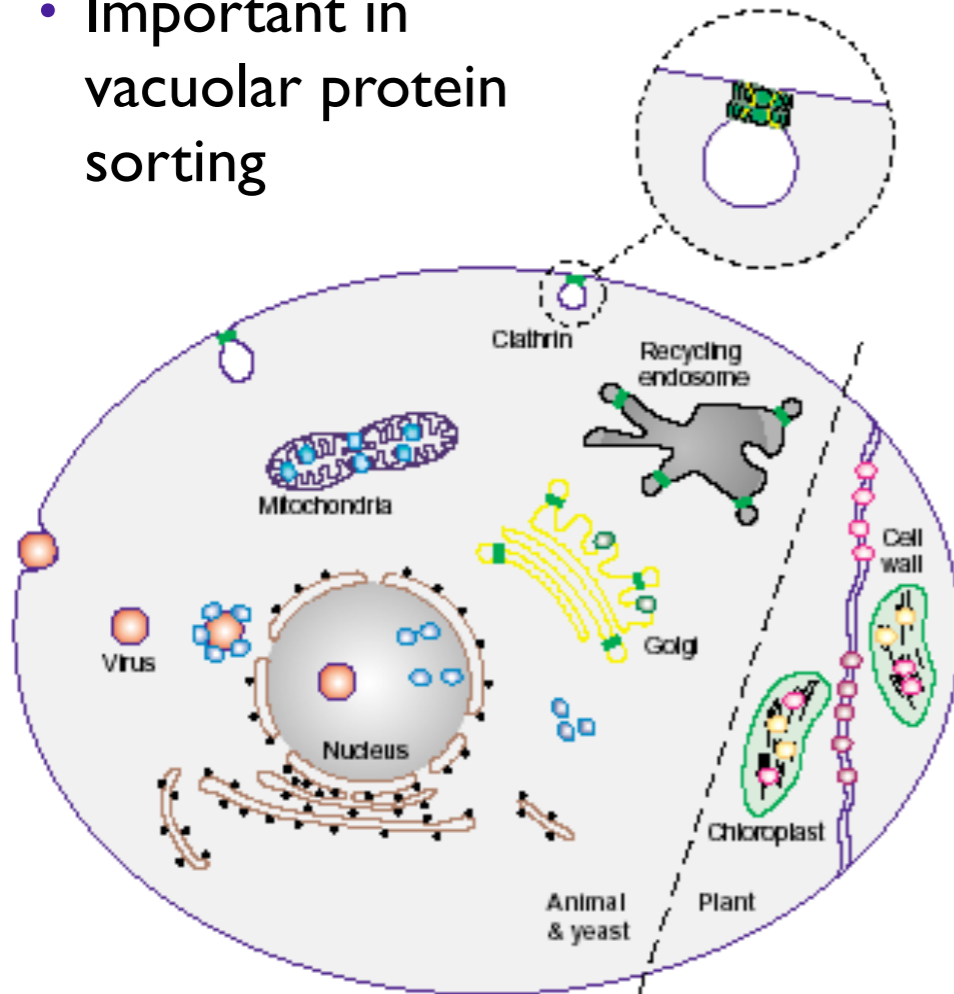
Kinesin superfamily

Processive motors, move mostly towards the plus end of MT



Dynamins

- GTPases
- Important in vacuolar protein sorting



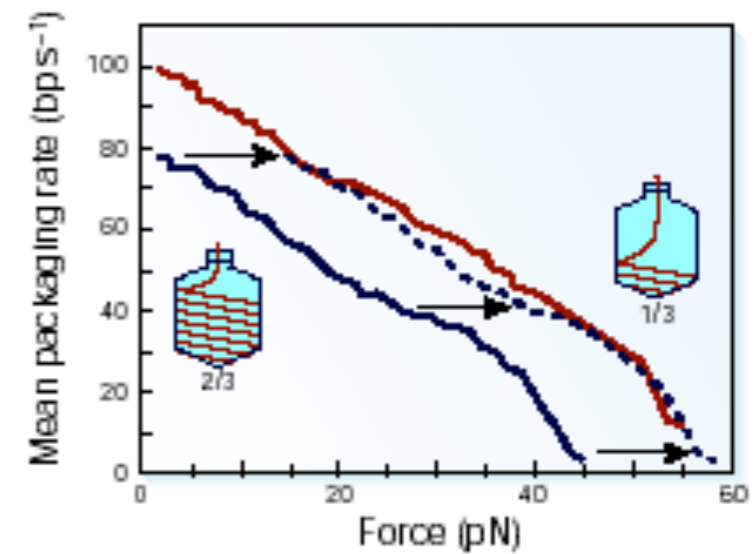
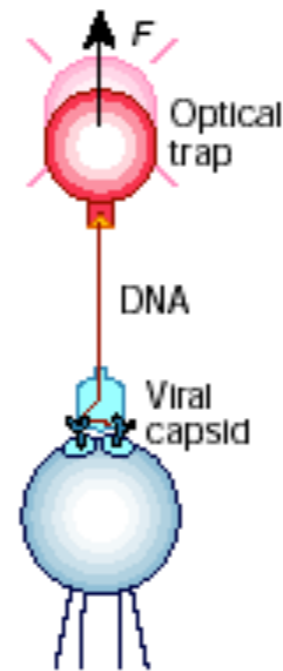
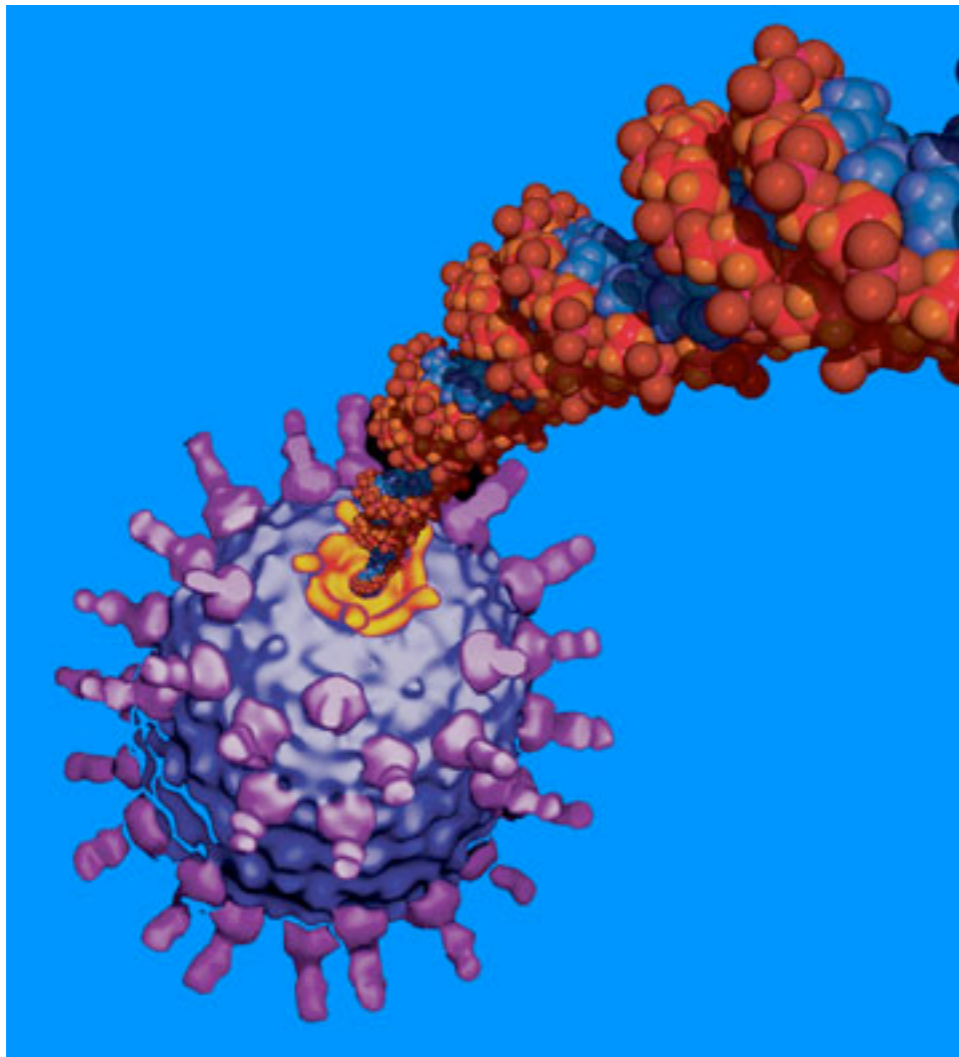
Protein	Localization	Function	Self-assembly
Dynamin	Plasma membrane (clathrin coated, caveolae), Golgi, endosomes	Vesicle formation, fission	+
Vps1	Golgi	Vesicle formation and transport	Unknown
Dnm1/Drp1/DRP-1	Mitochondria outer membrane	Mitochondrial fission & morphology	+
Mgm1/Msp1/OPA1	Mitochondria inner or outer membrane, or matrix	Mitochondrial morphology	Unknown
Phragmoplastin	Cell wall	Membrane morphology	+
ADL1	Cell wall, chloroplast	Membrane biogenesis	+
ADL2	Chloroplast	Unknown	Unknown
hGBP1	Cytoplasm	Anti-viral activity	+
Mx	Cytoplasm, nucleus	Anti-viral activity	+



“pinchase” function

Viral portal motor

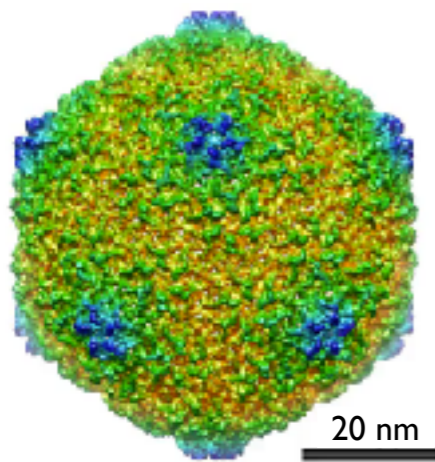
Unique DNA motor



$\phi 29$ bacteriophage portal motor

Viral capsid mechanical rupture - irreversible process

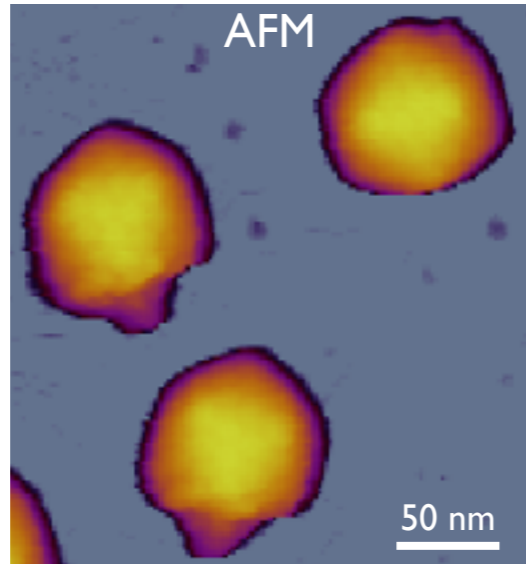
T7 phage



20 nm

lonel et al. *J.Biol.Chem.* 286, 234, 2010.

AFM



50 nm

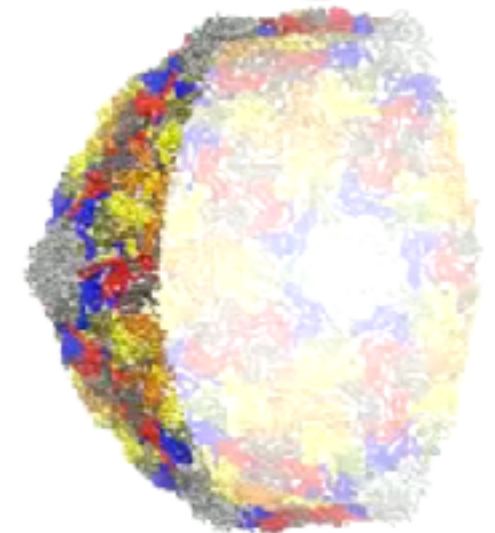
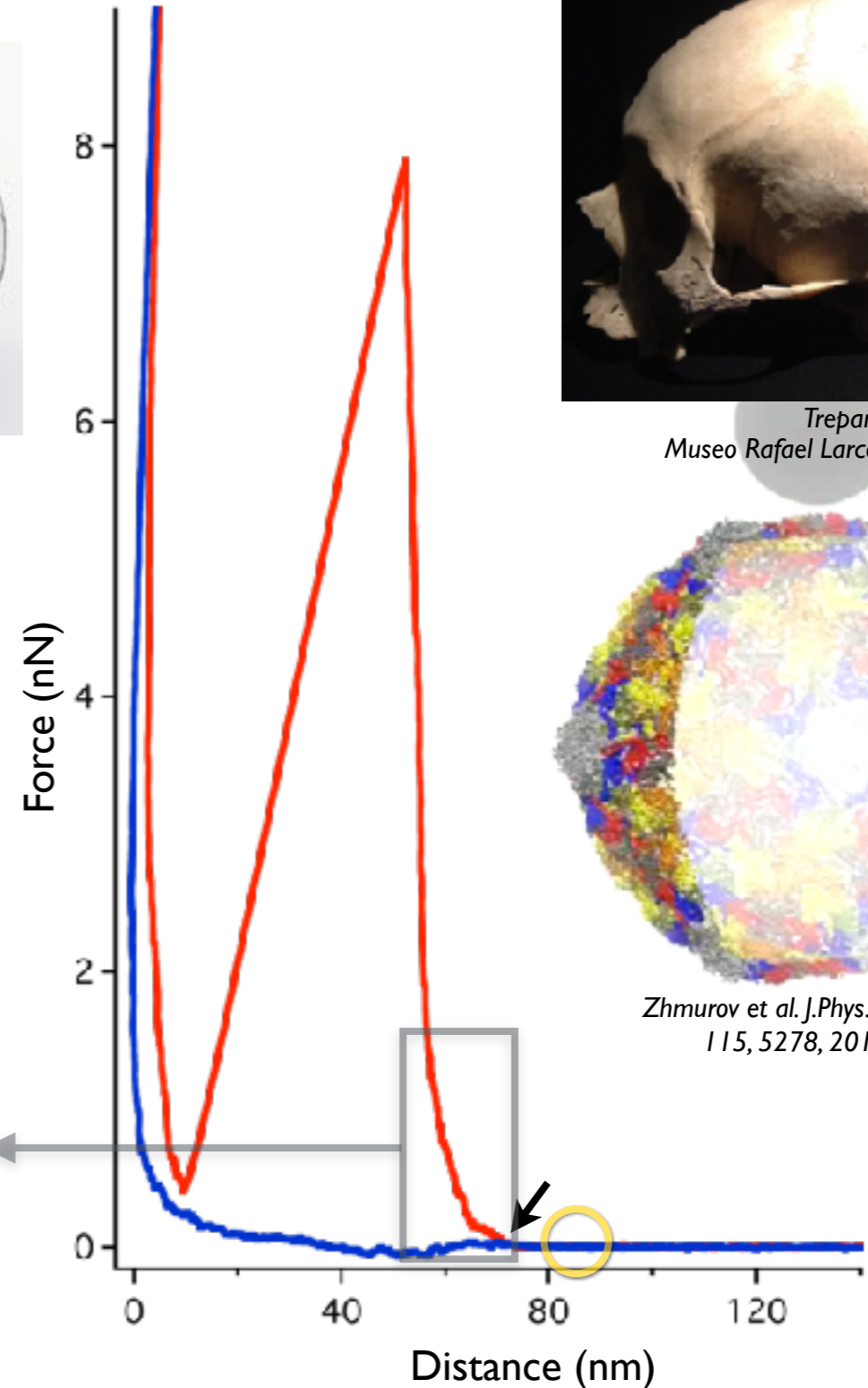
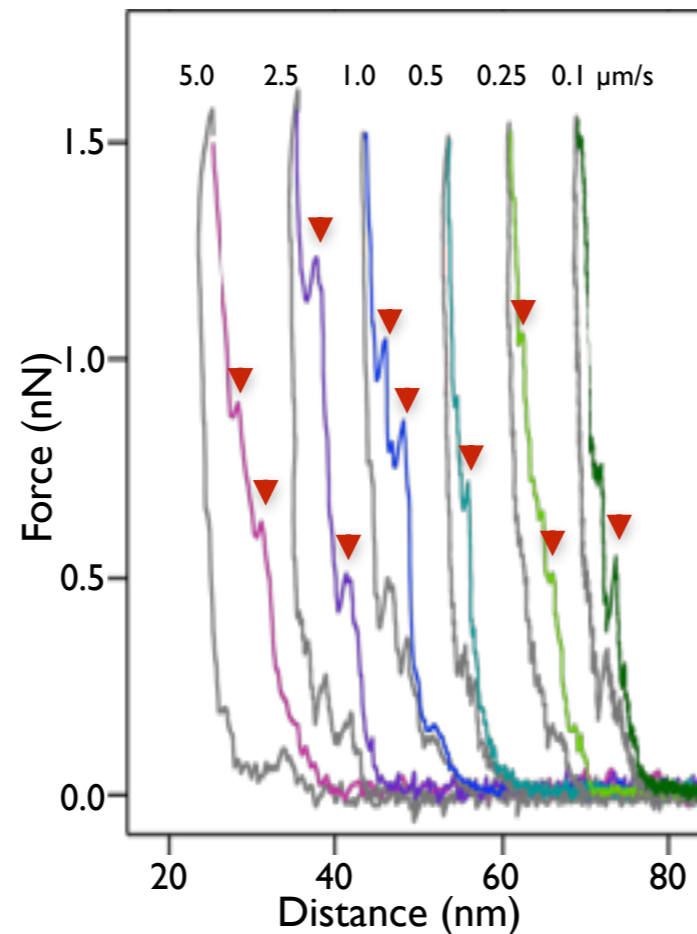
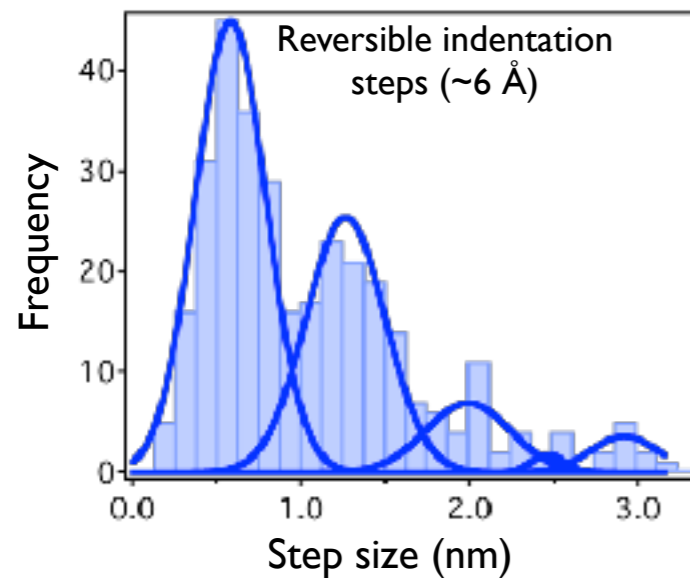
AFM tip



T7 kapszid

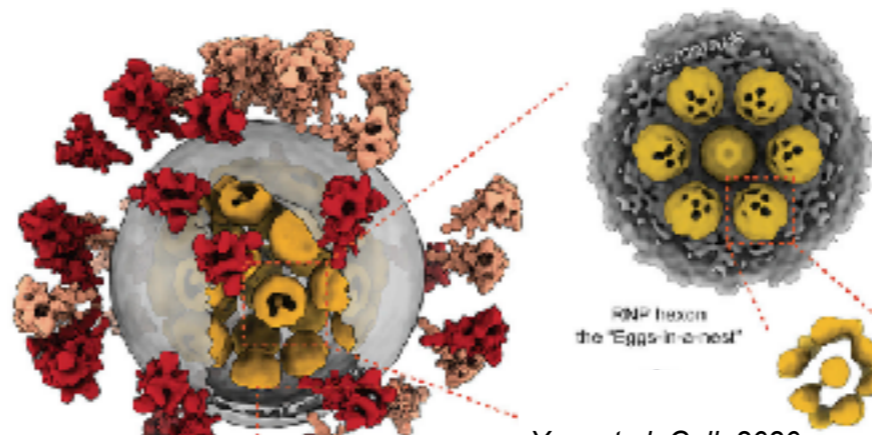
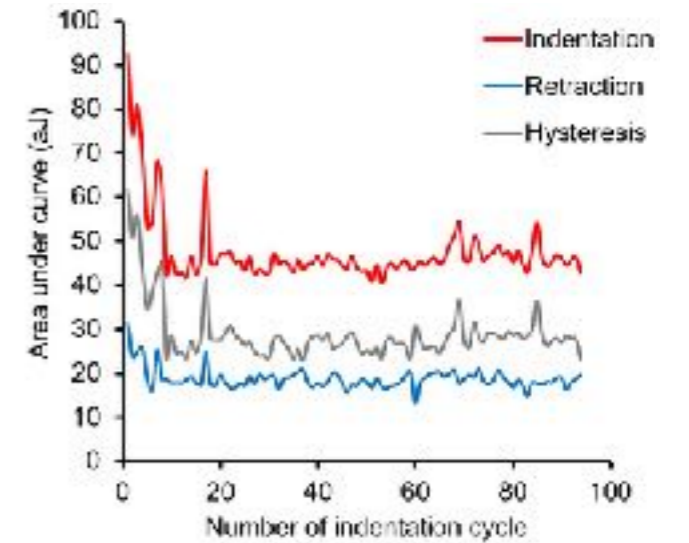
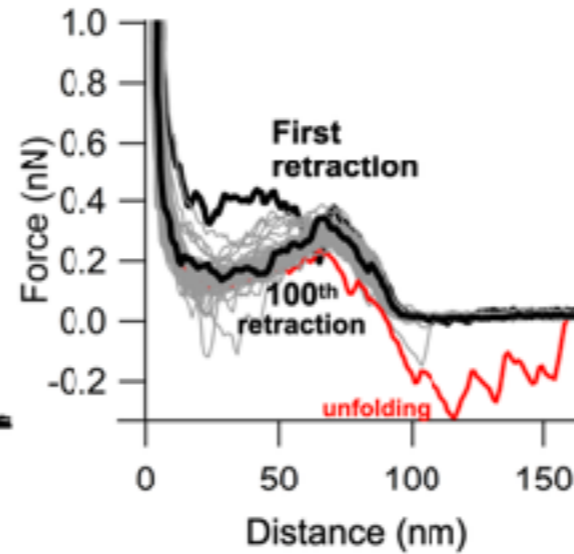
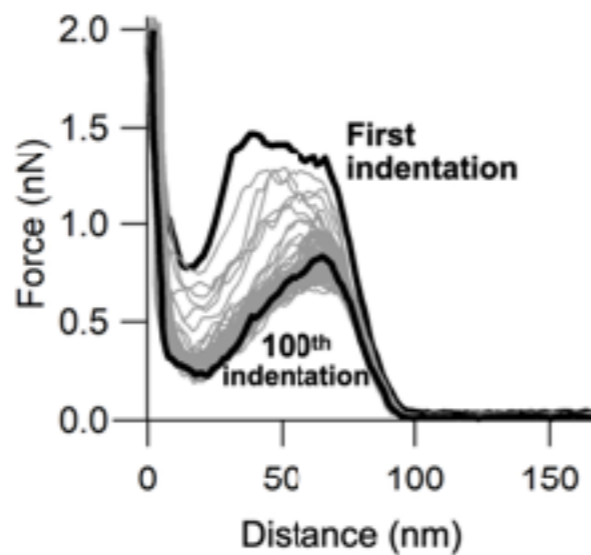
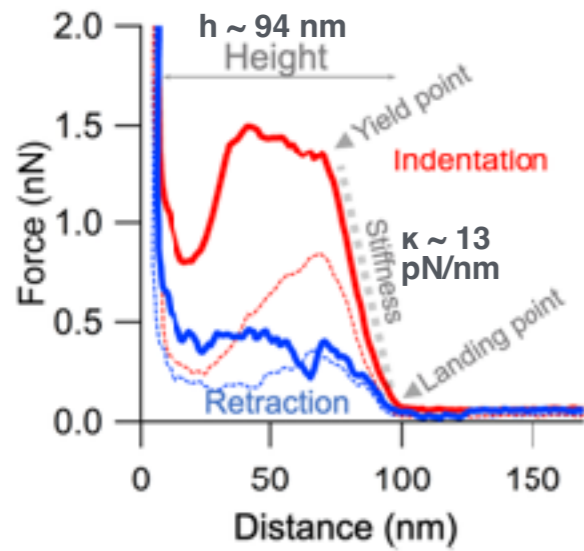
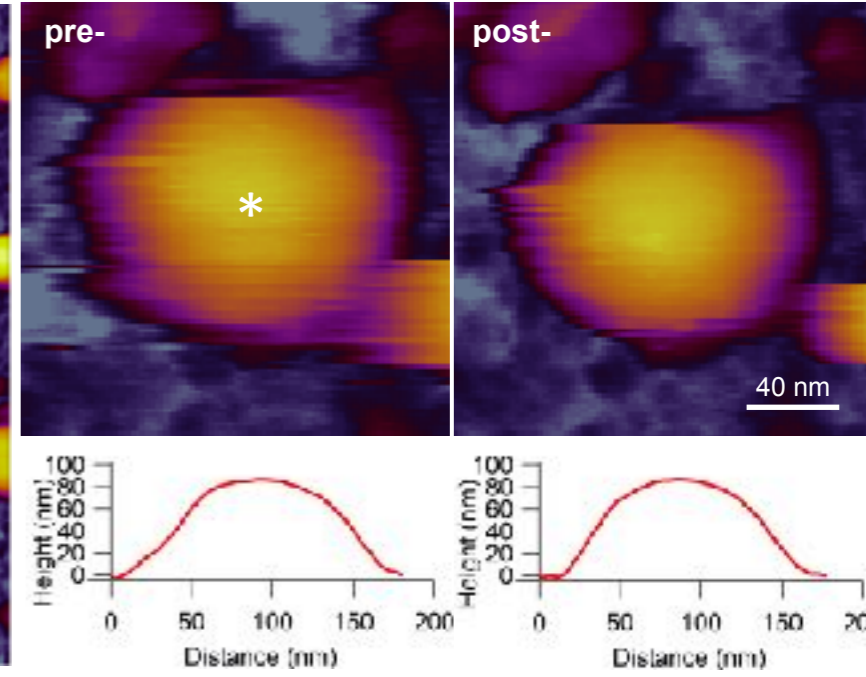
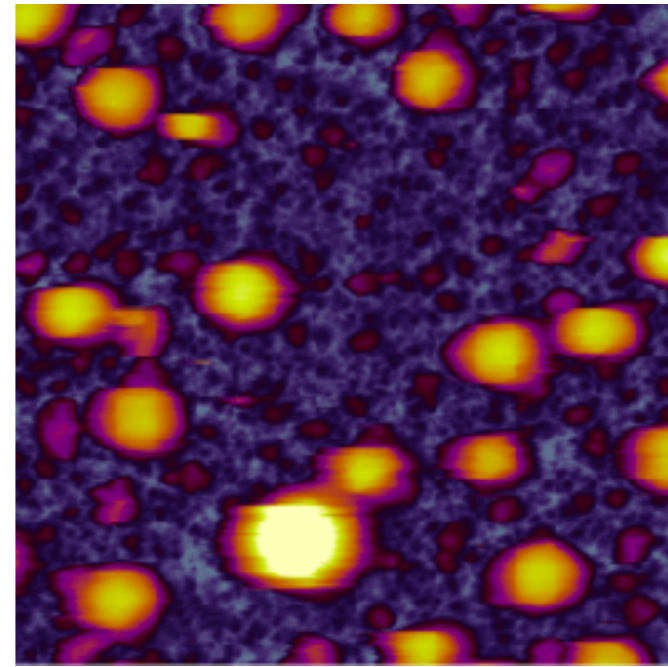
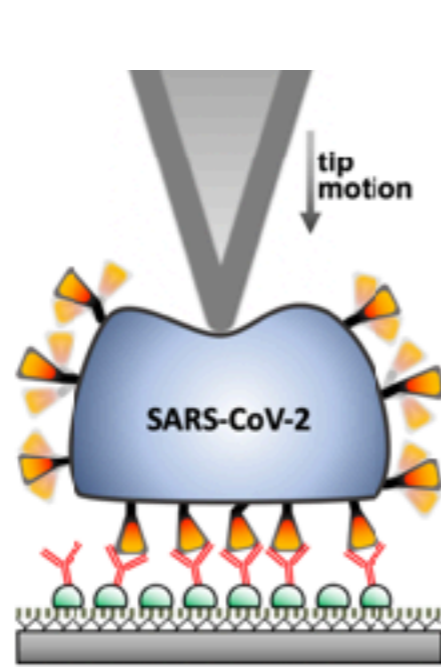
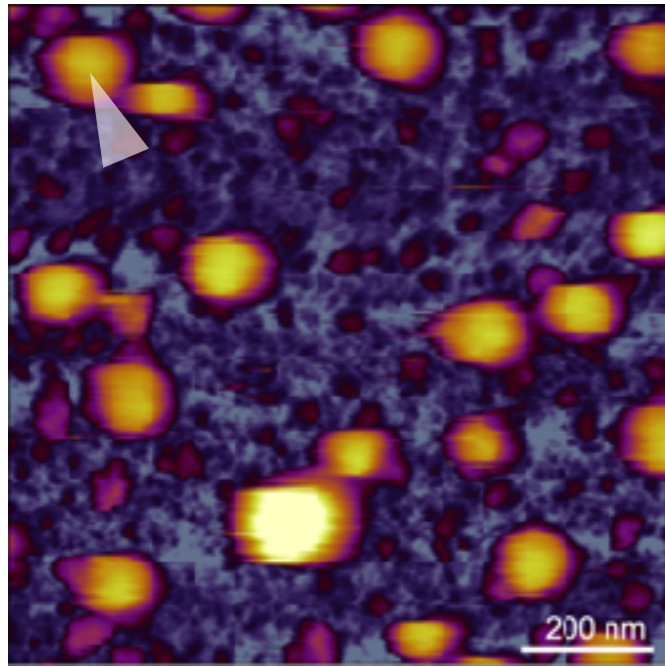


Trepanated inca skull,
Museo Rafael Larco Herrera, Lima



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

SARS-CoV-2 is highly resilient



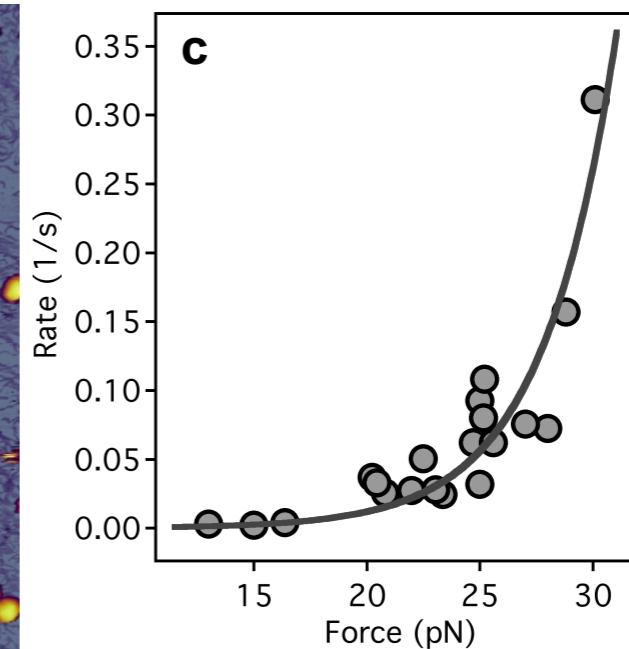
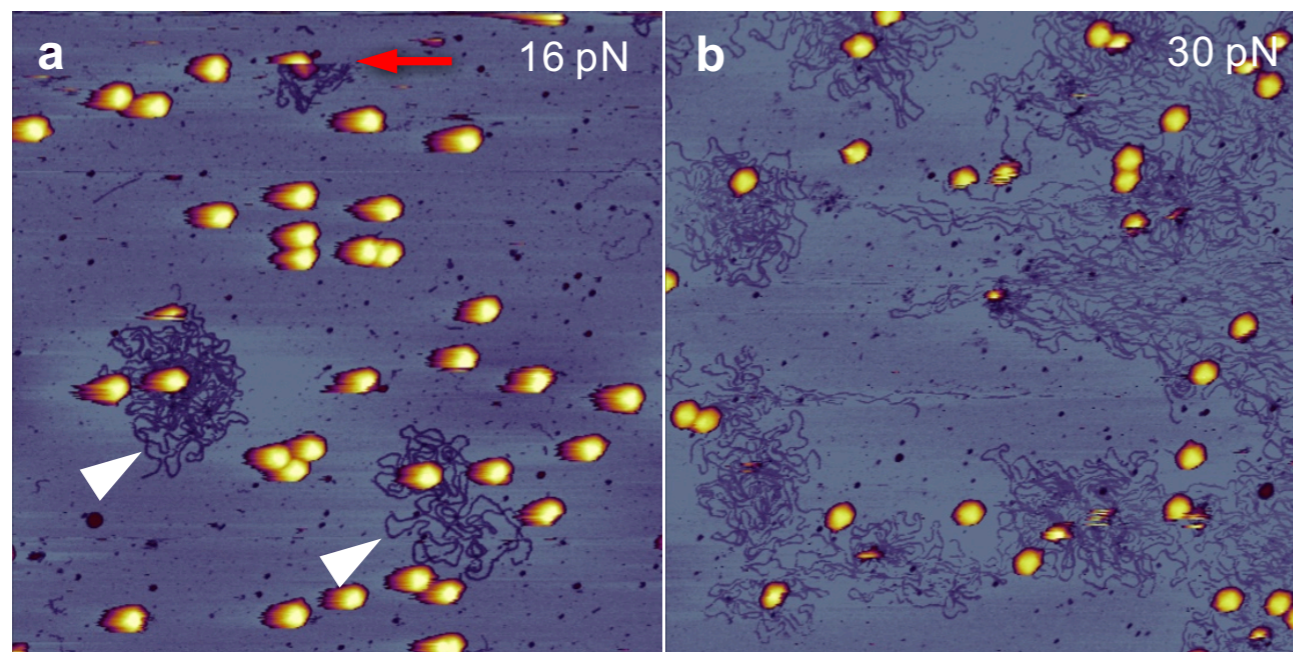
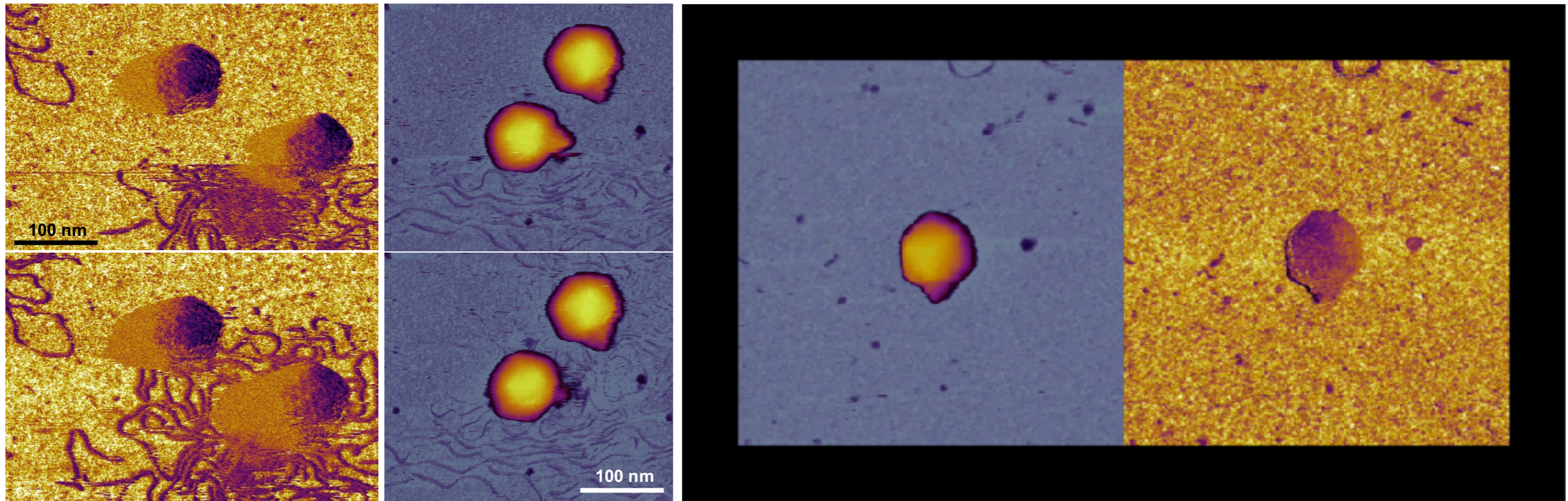
Nest-like organization of RNP in SARS-CoV-2 may yield to nanoindentation

Video by Scixel

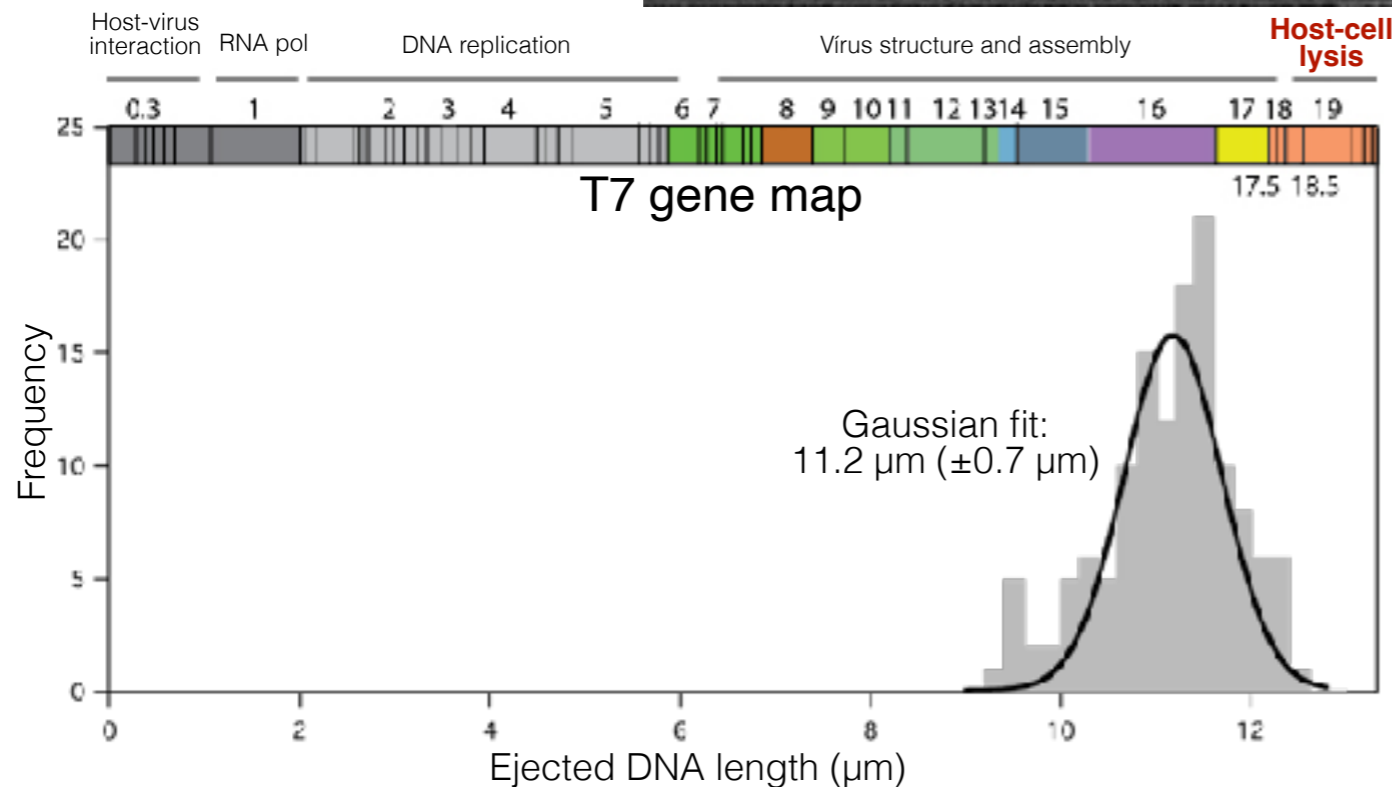
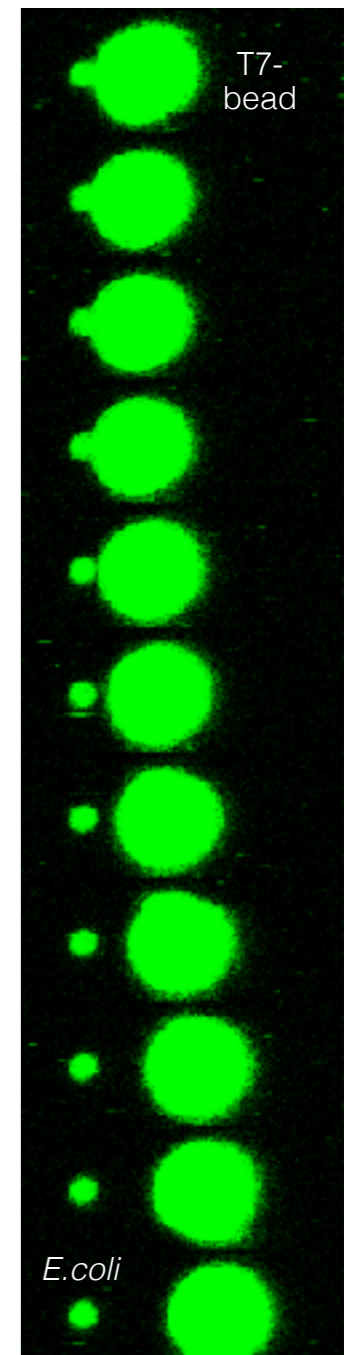
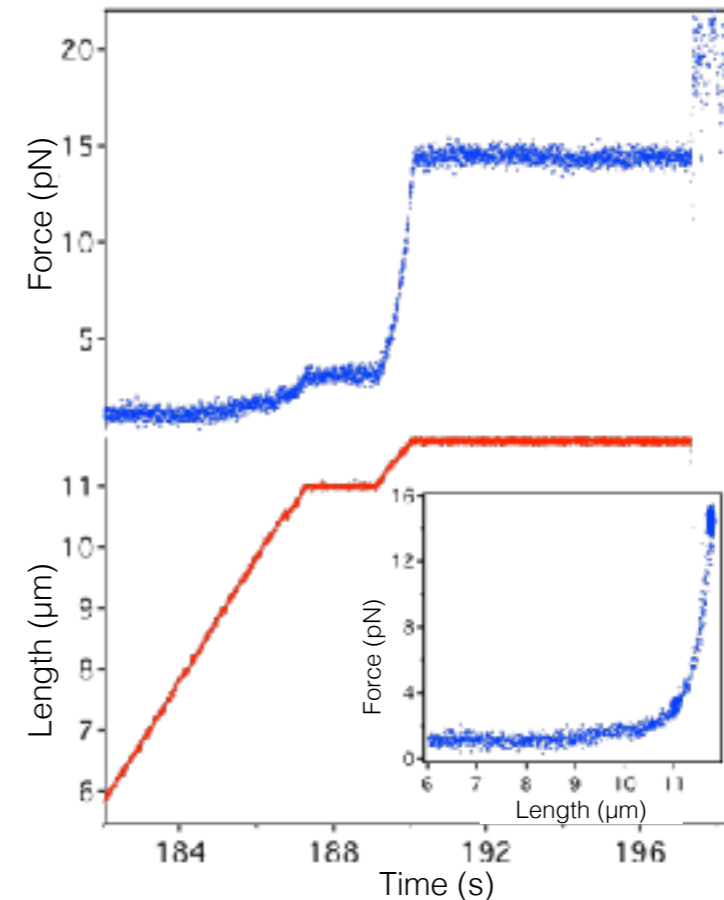
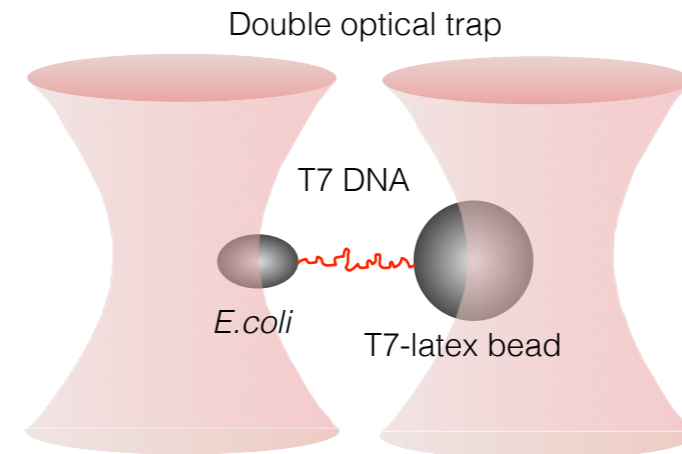
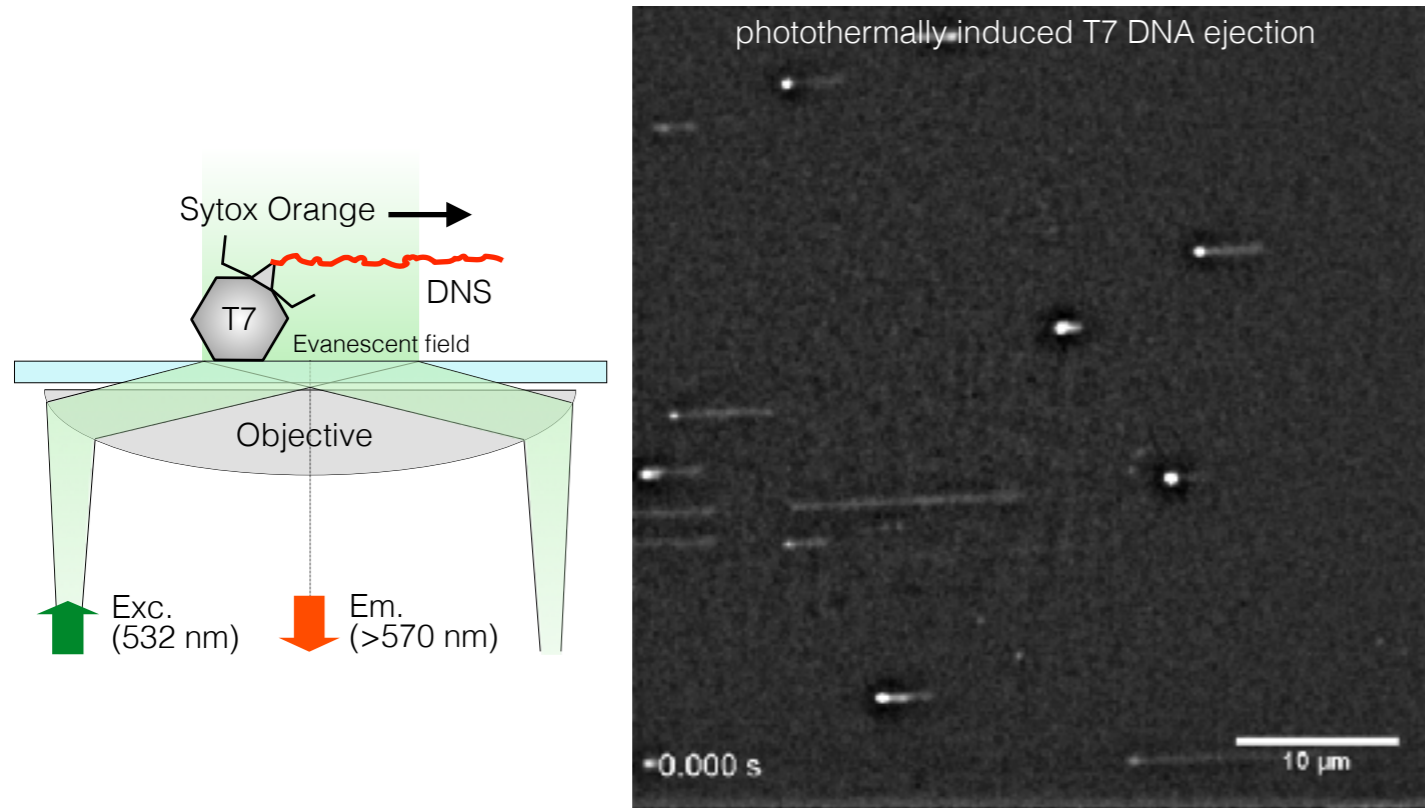
Yao, et al. Cell, 2020

Kiss et al. Nano Lett 2021

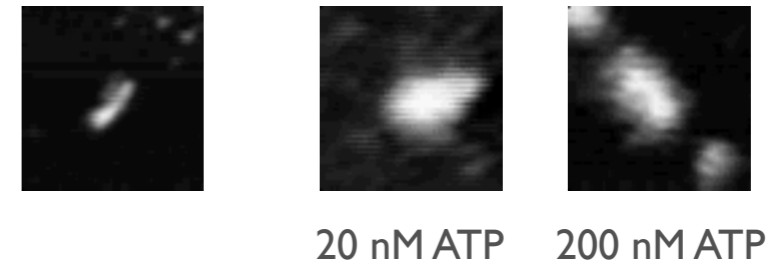
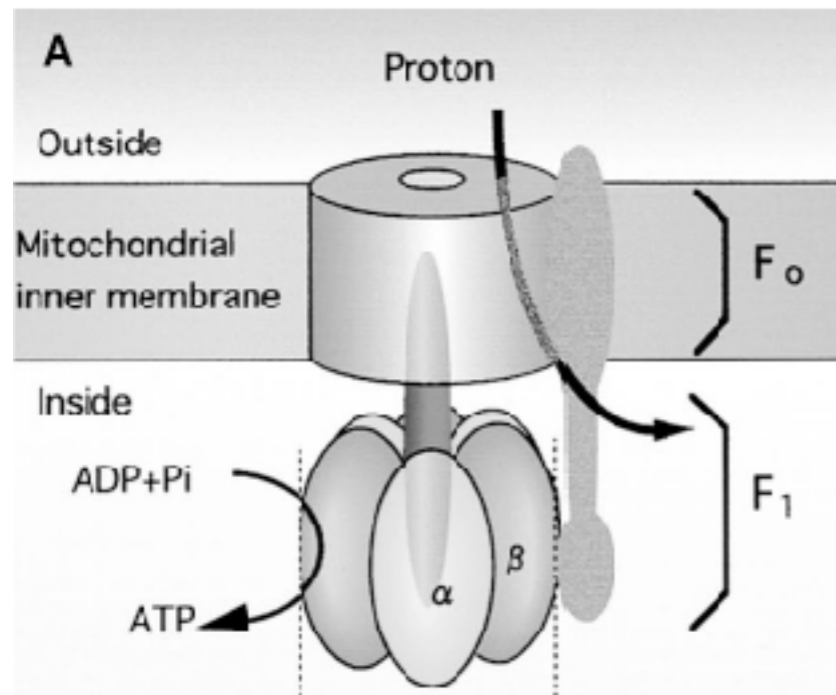
Force-induced DNA ejection from T7 phage



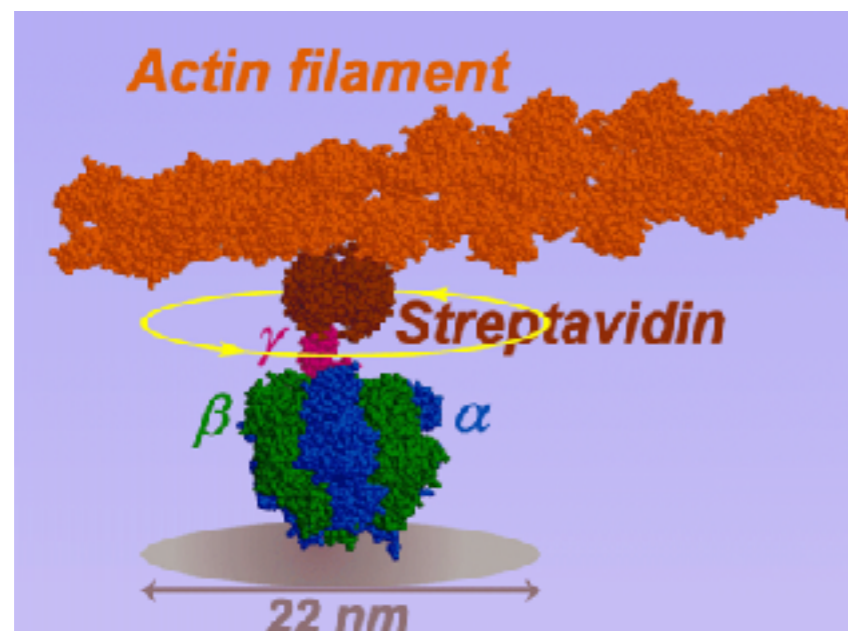
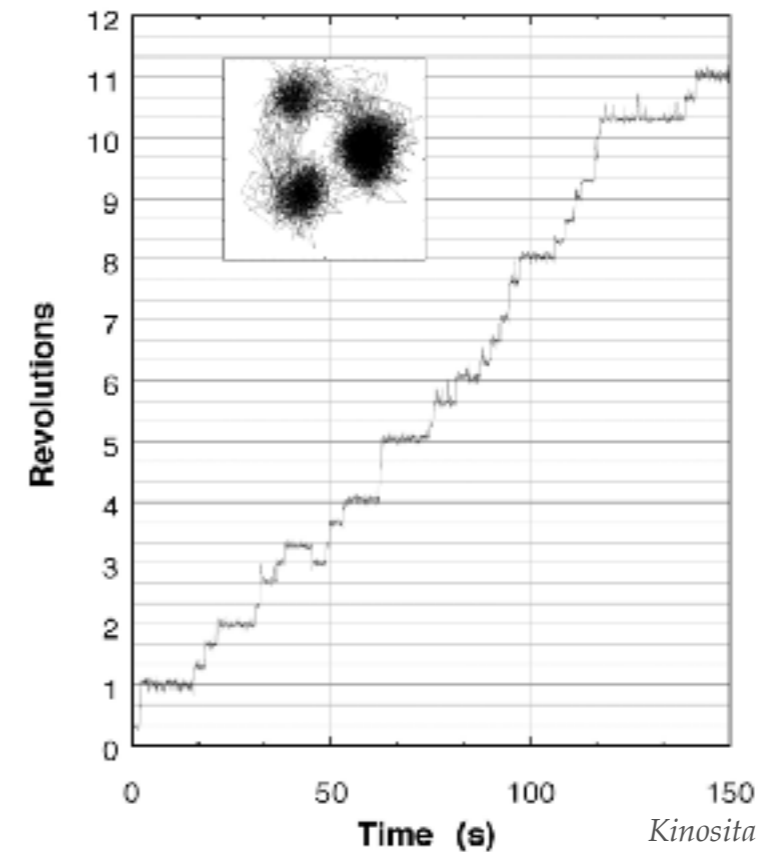
T7 DNA release may be under mechanical control



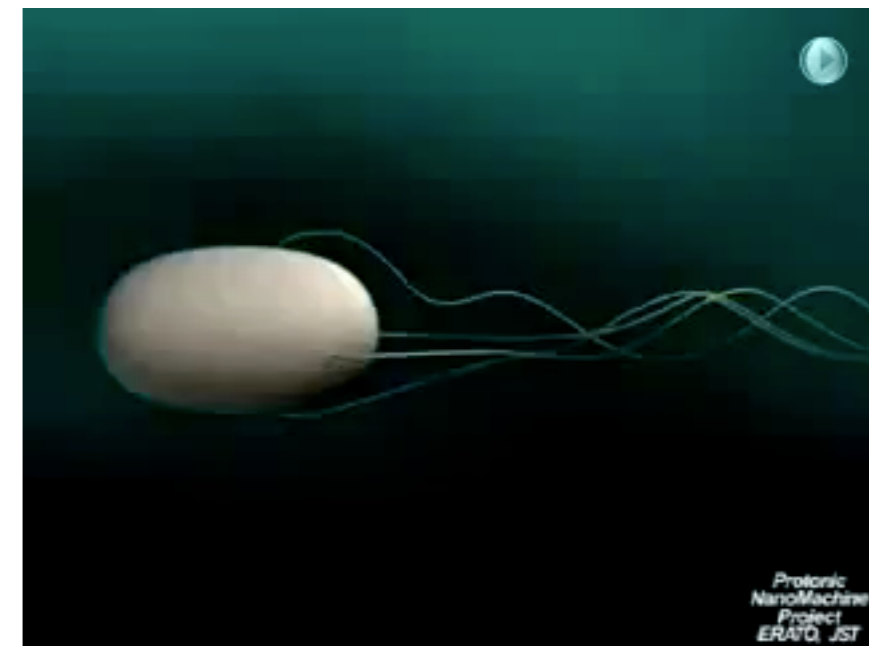
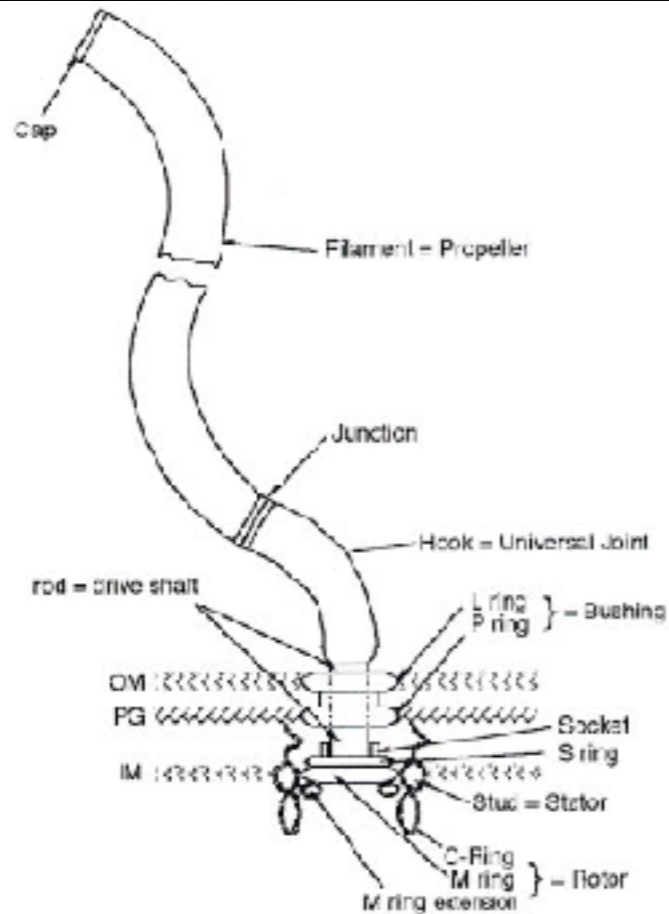
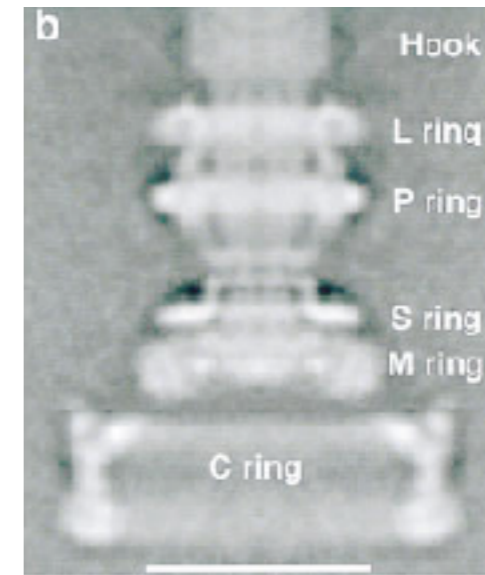
Rotary motors I: F1F0-ATP Synthase



Discrete 120° rotational steps



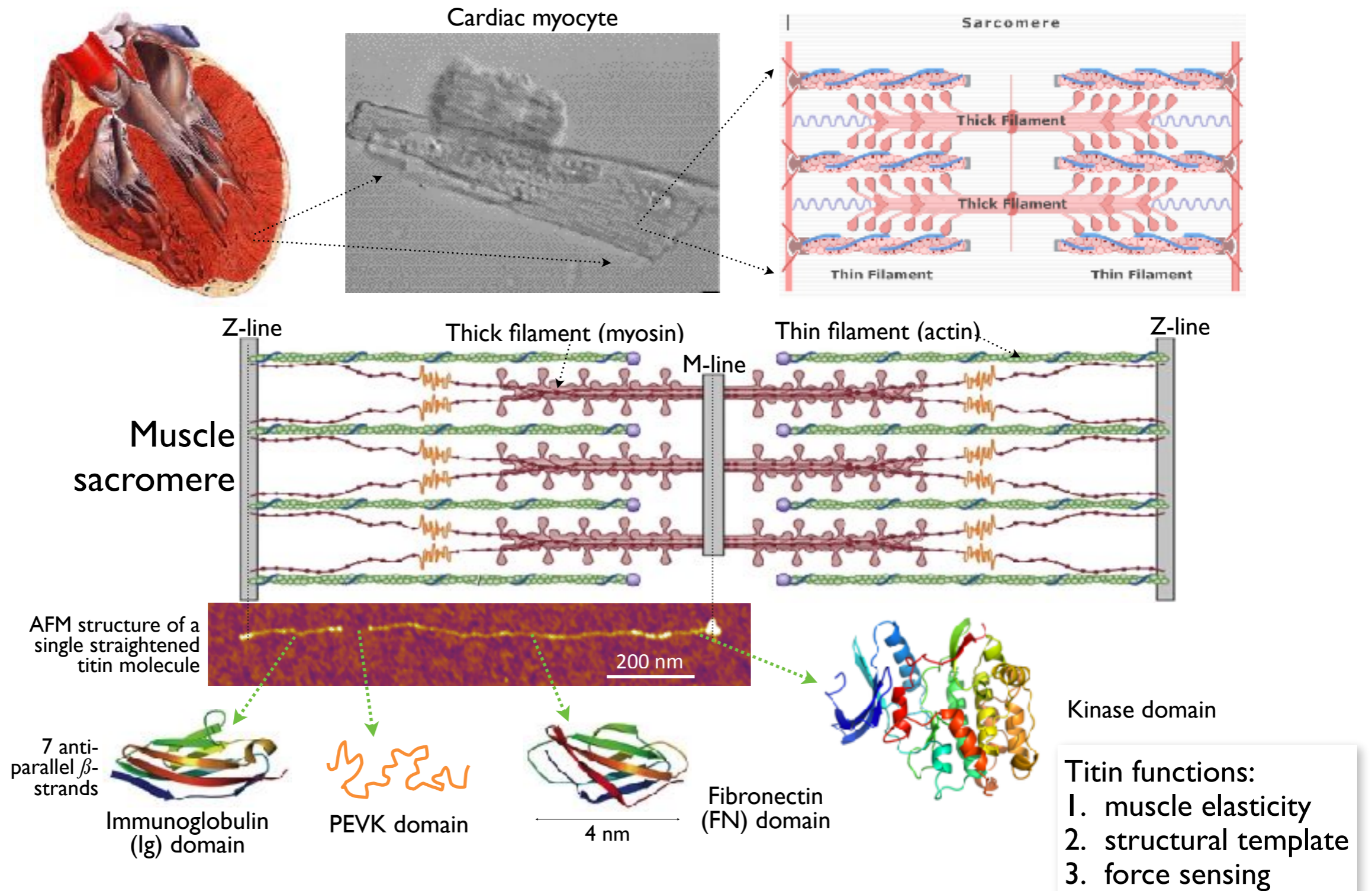
Rotary motors II: Bacterial flagellar motor



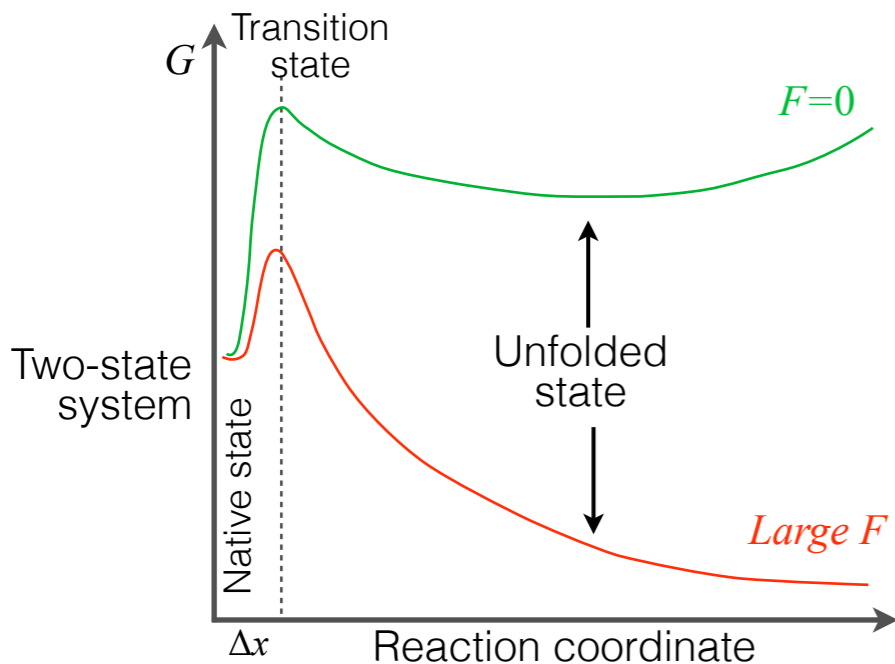
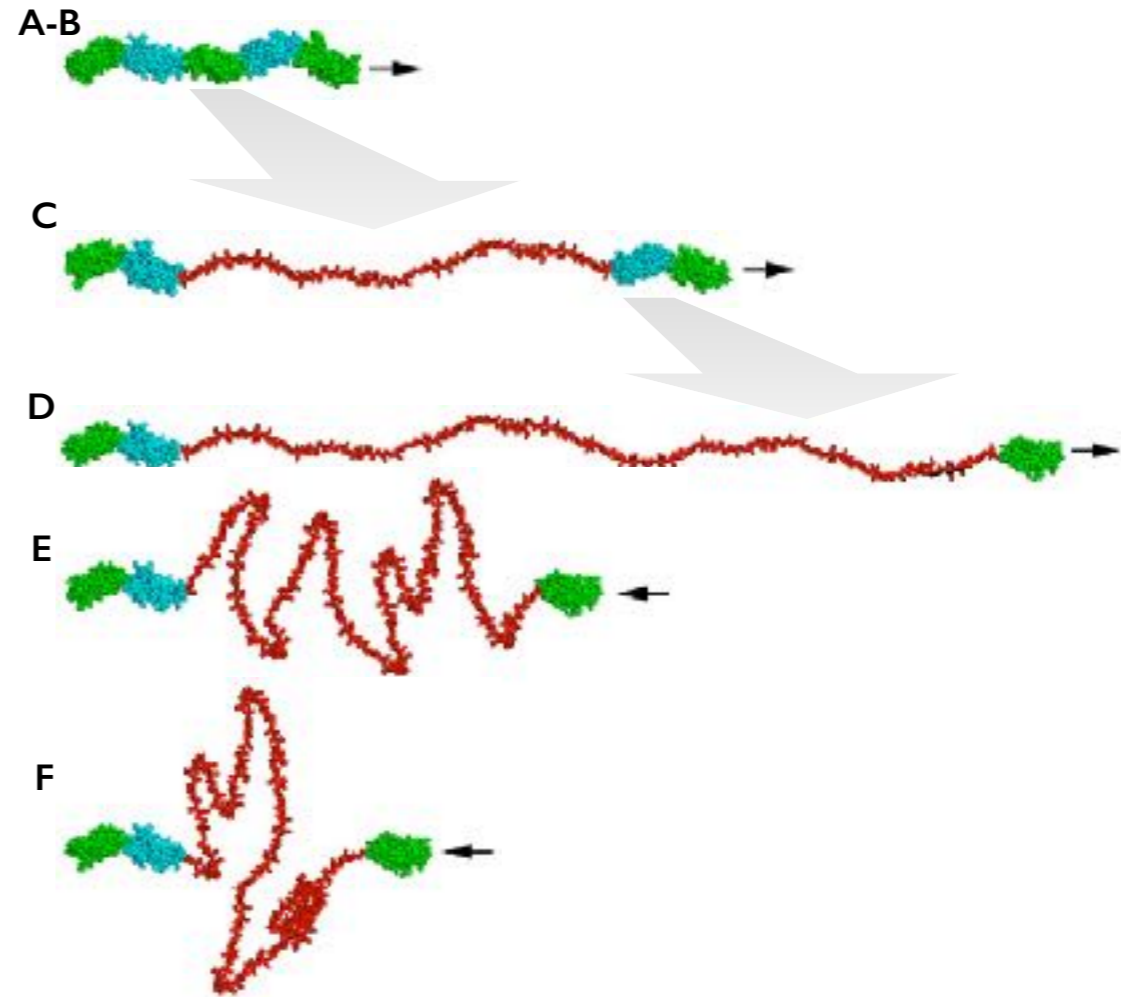
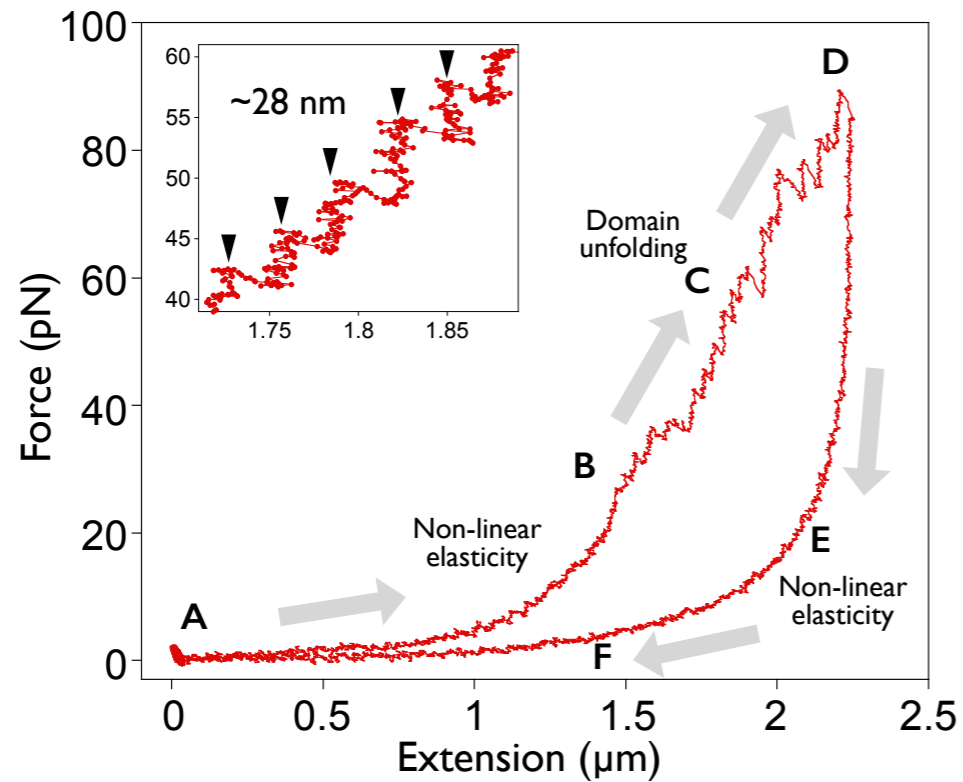
Rotational frequency: > 20000 rpm
Power consumption: 10^{-16} W
Efficiency: > 80%
Energy source: protons

Force-driven protein folding

Nanomanipulation of titin



Upon stretching with force, titin unfolds



Force-driven domain unfolding rate:

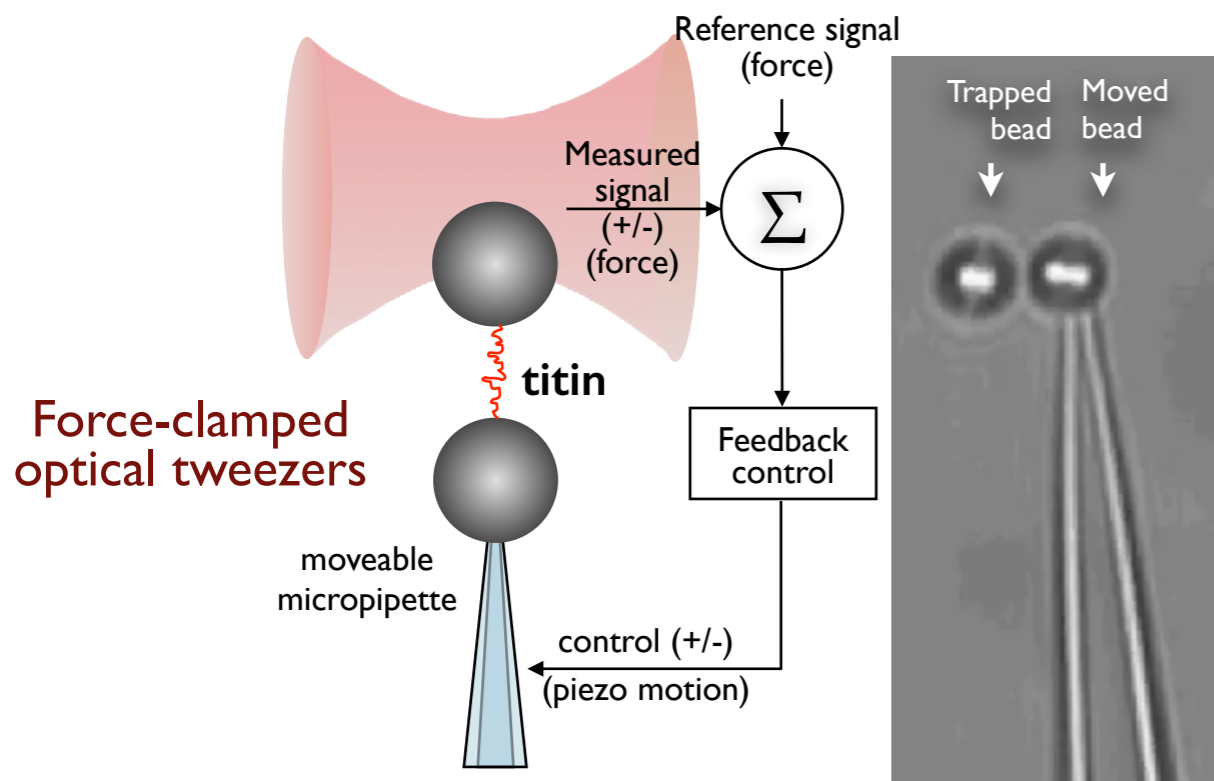
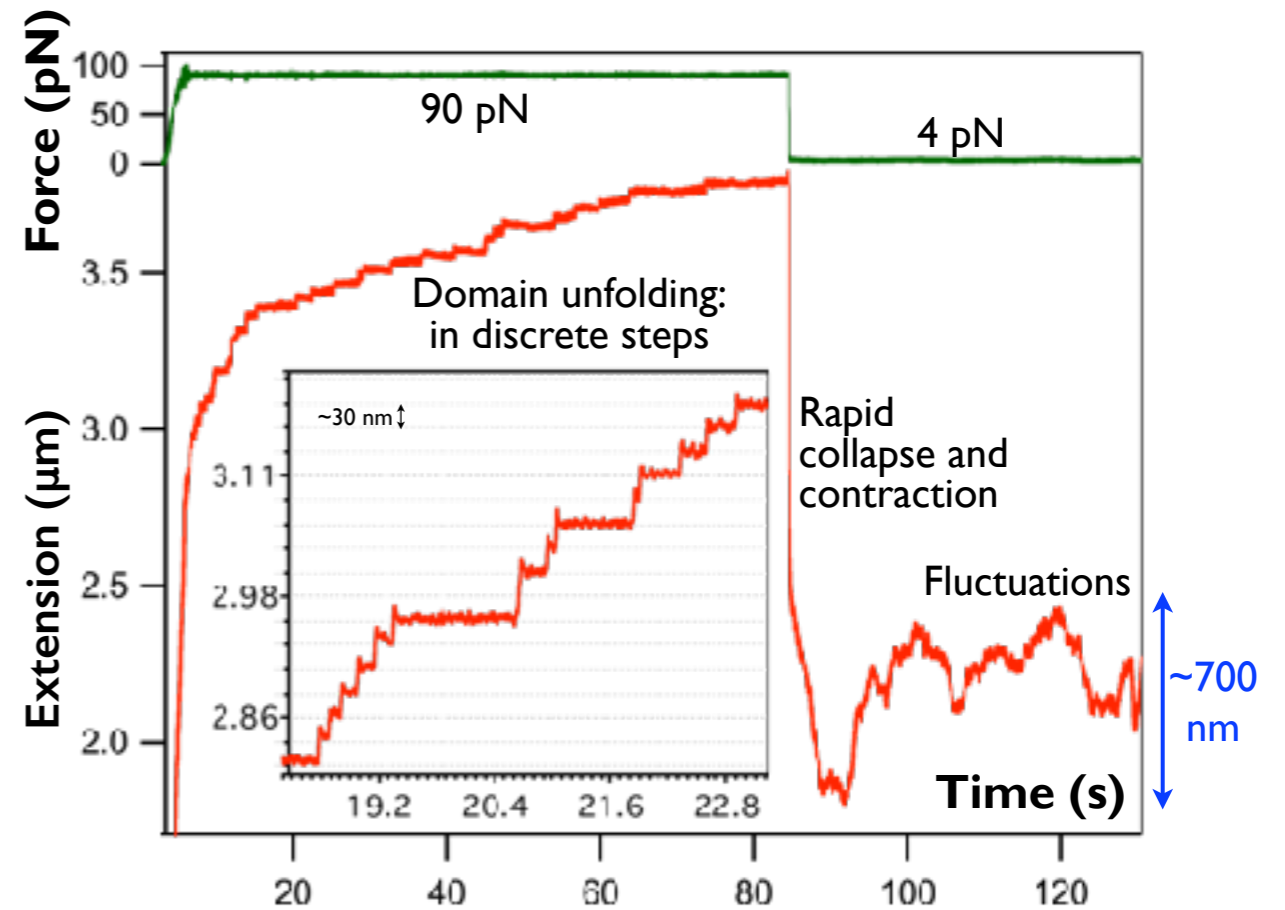
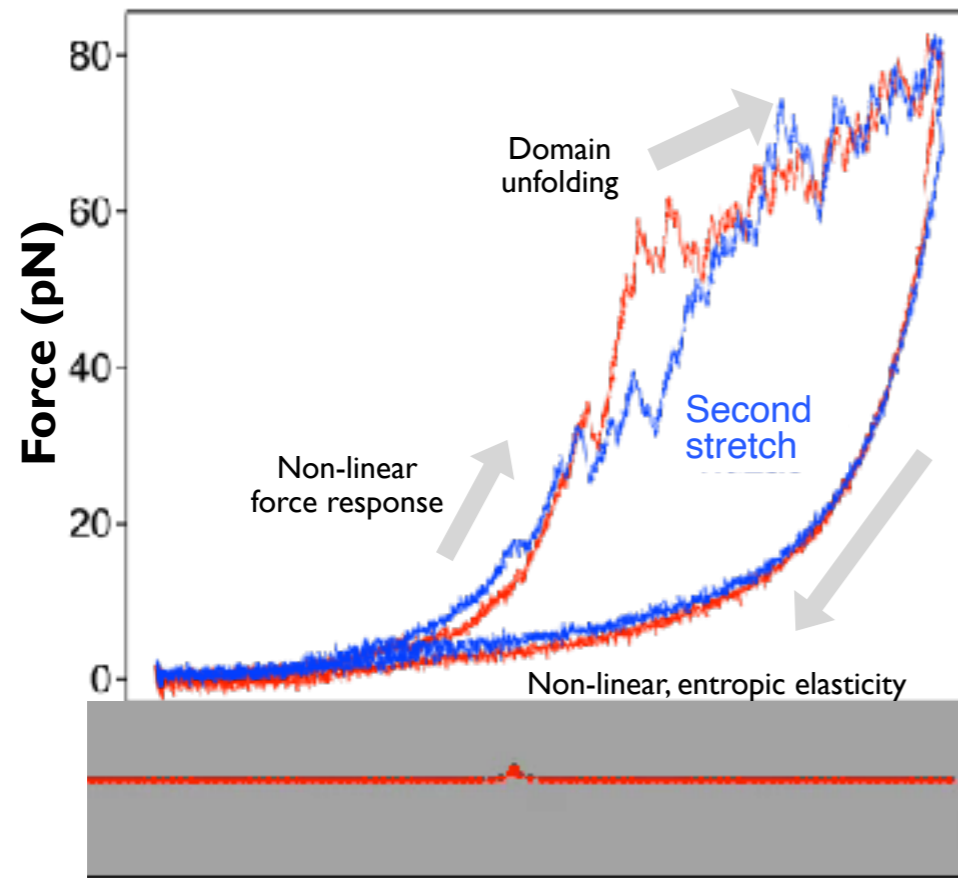
$$k_F = k_0 e^{F\Delta x/k_B T}$$

k_0 : spontaneous unfolding rate

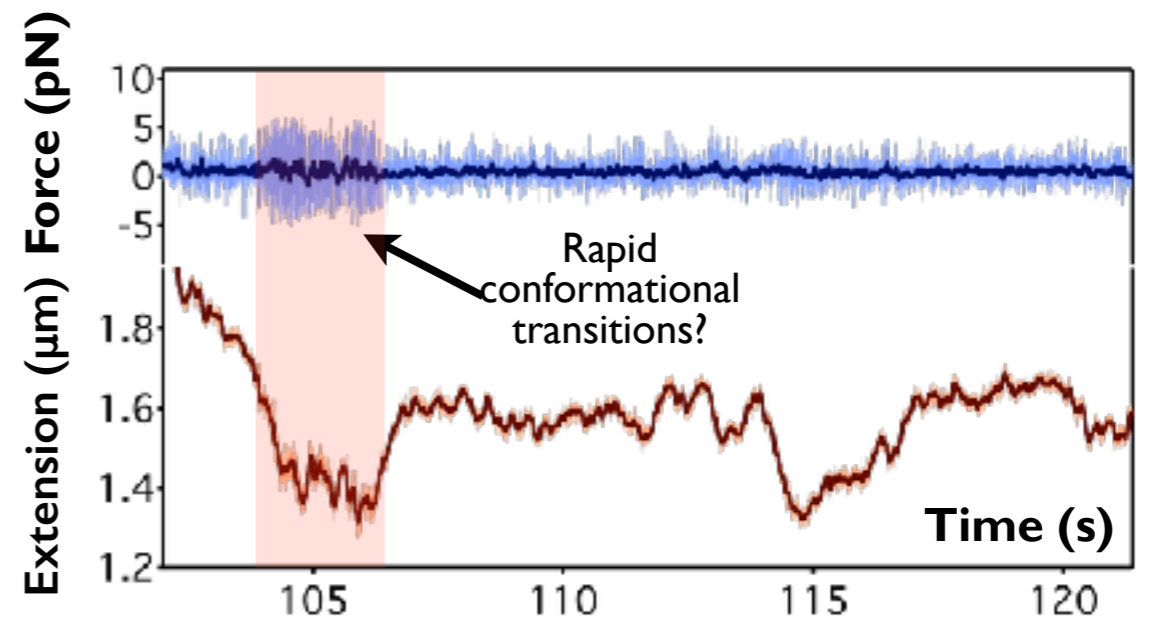
Domain unfolding:

1. all-or-none
2. no intermediates
3. dictated by the hierarchy of mechanical stabilities of domains

How does titin contract?

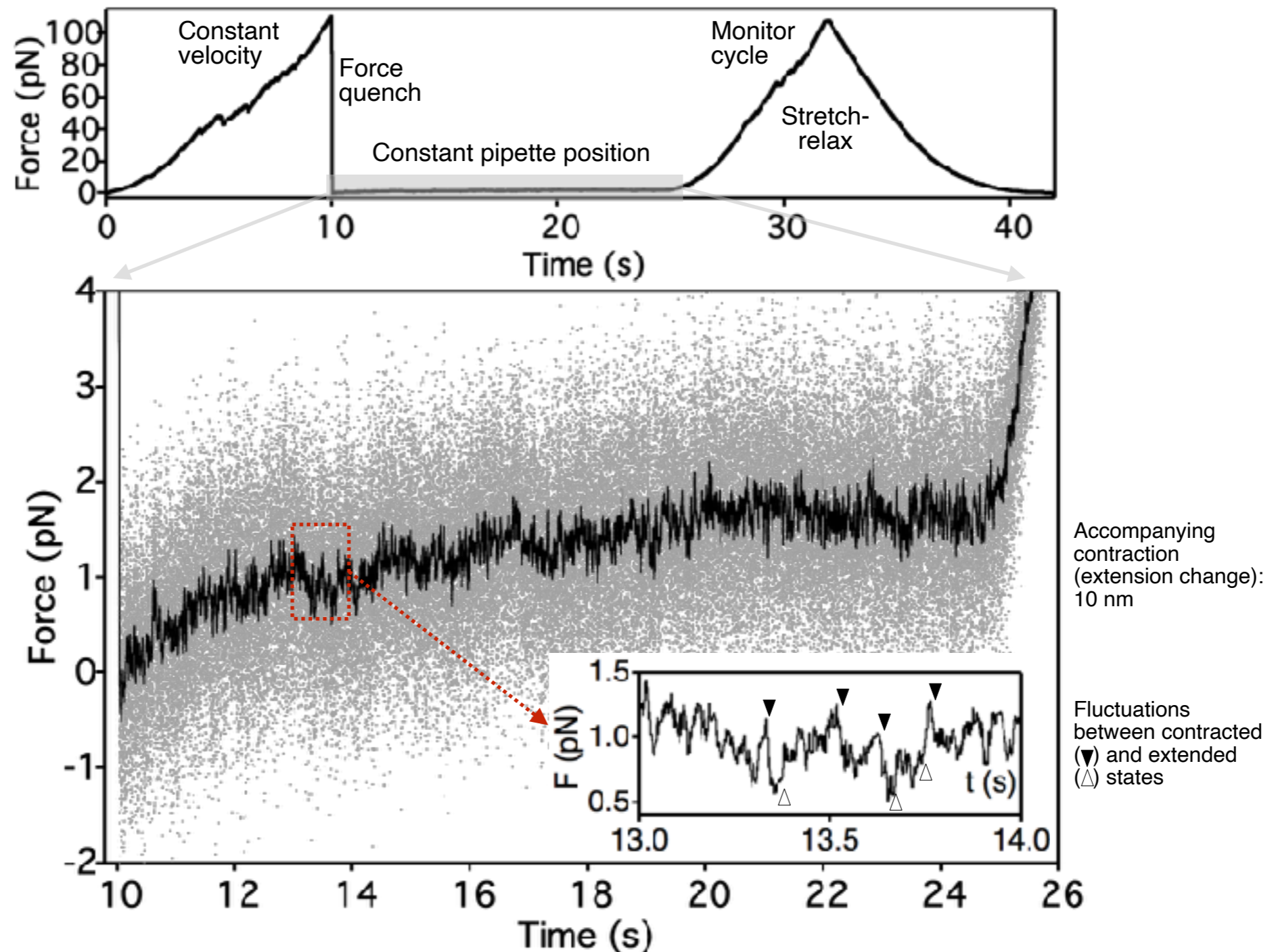


Fluctuations cannot be explained with a two-state system



Force is generated during refolding

Position clamp experiment



Molten-globule structure explored with sMDS

