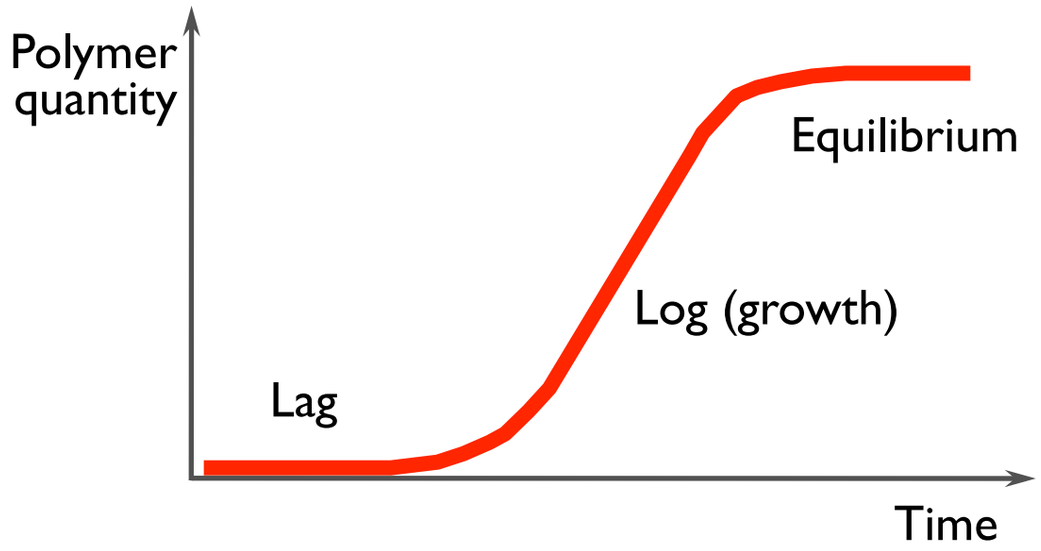
Polymerization, self-assembly, mechanoenzymes, protein folding, irreversible processes

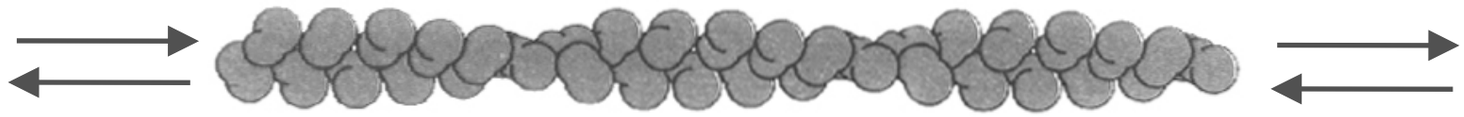
Miklós Kellermayer

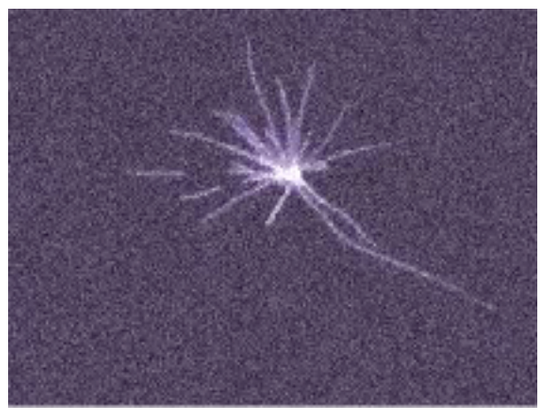
Polymerization



Polymerization equilibria

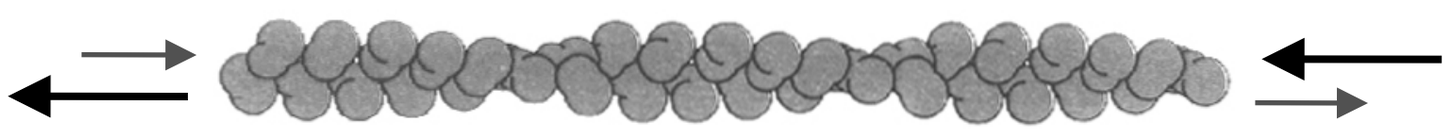
1. true equilibrium

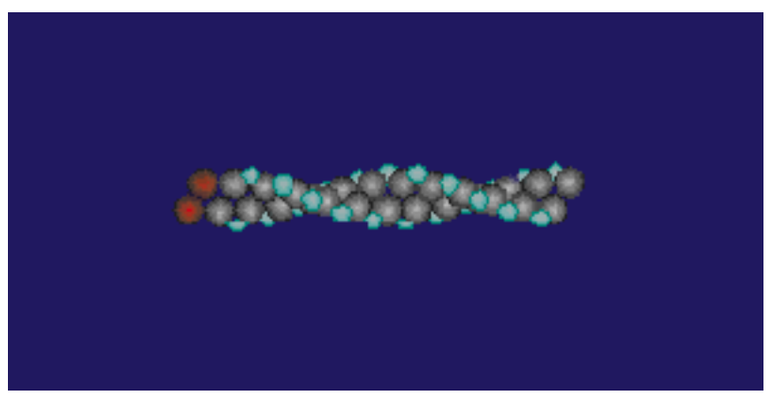




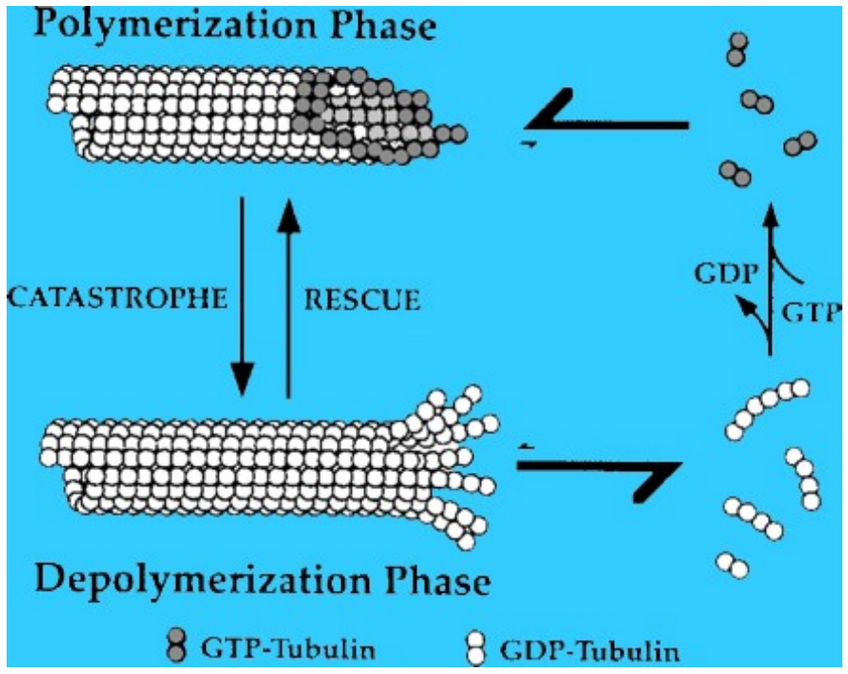
2. dynamic instability: slow growth followed by catastrophic depolymerization

3. Treadmilling



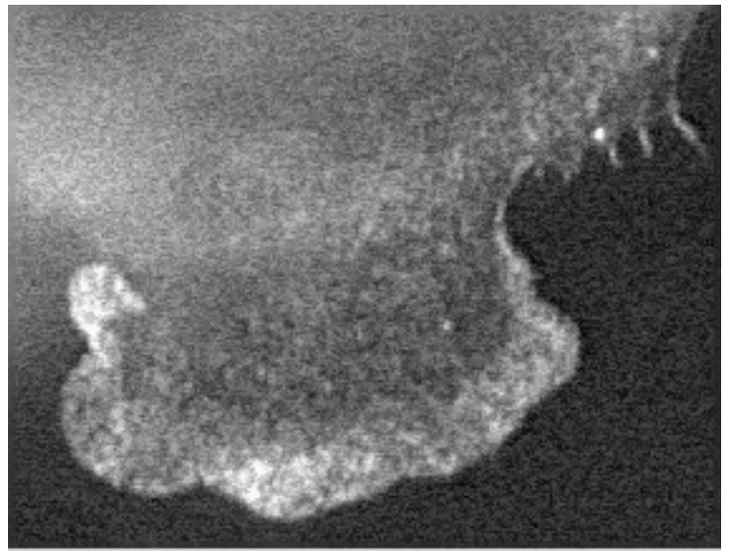


In vivo dynamic instability
Microtubules



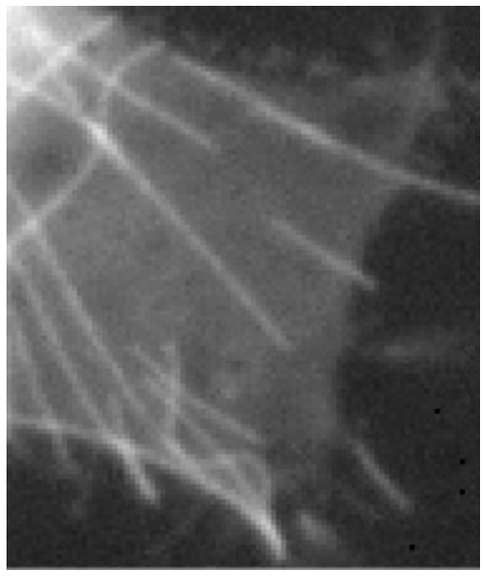
CHO Cytoplast with Centrosome

In vivo treadmilling

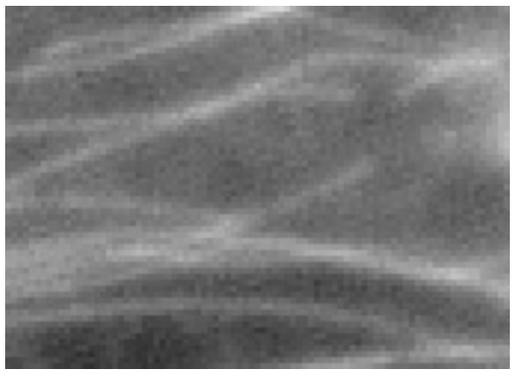


Actin

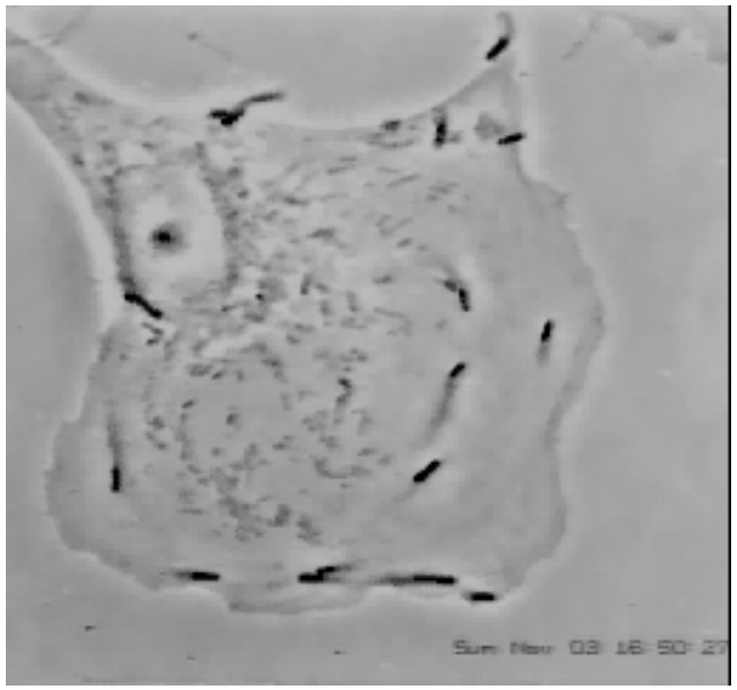
GFP-actin Speckle microscopy



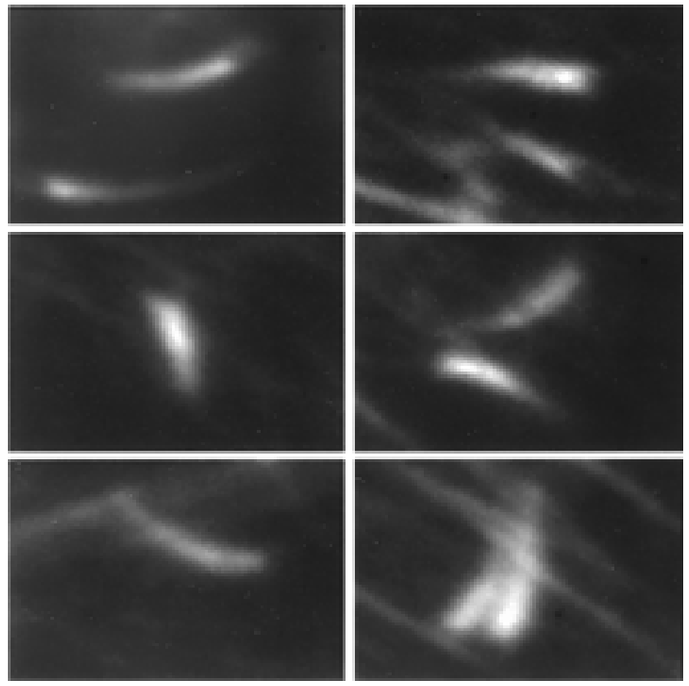
Microtubules



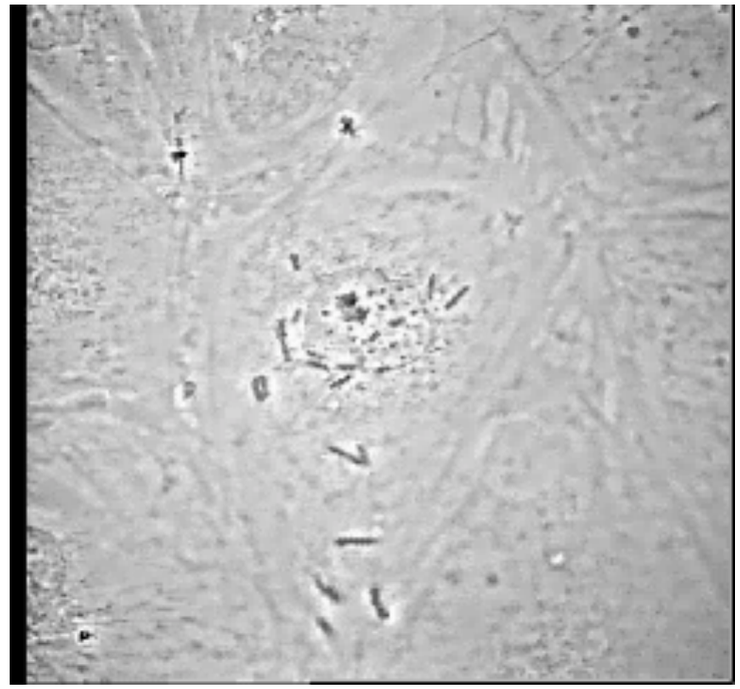
Motility with actin polymerization
Intracellular pathogen motion



Listeria monocytogenes



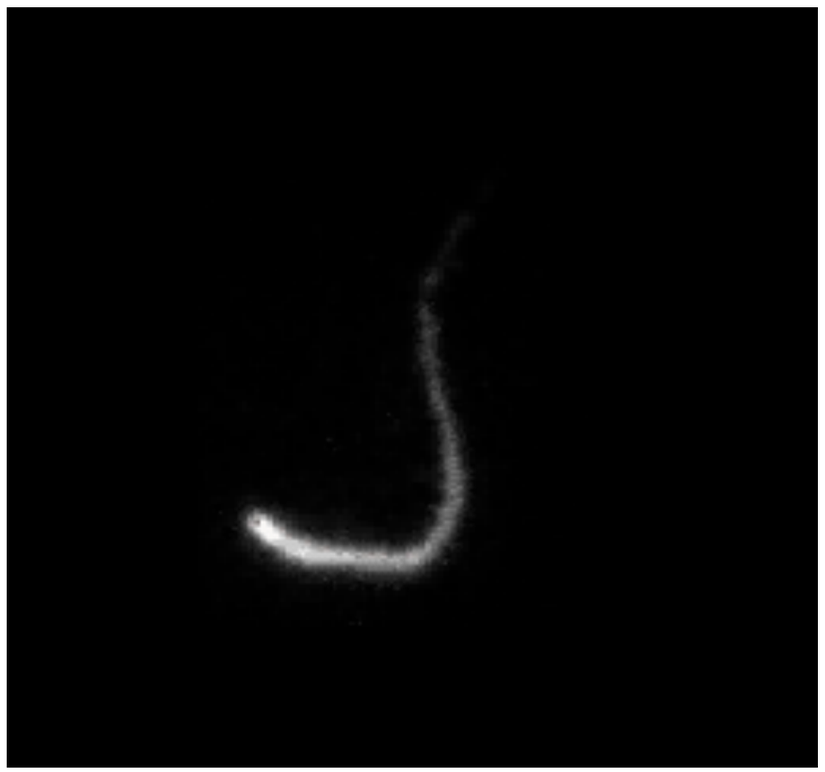
F-actin labeled with phalloidin



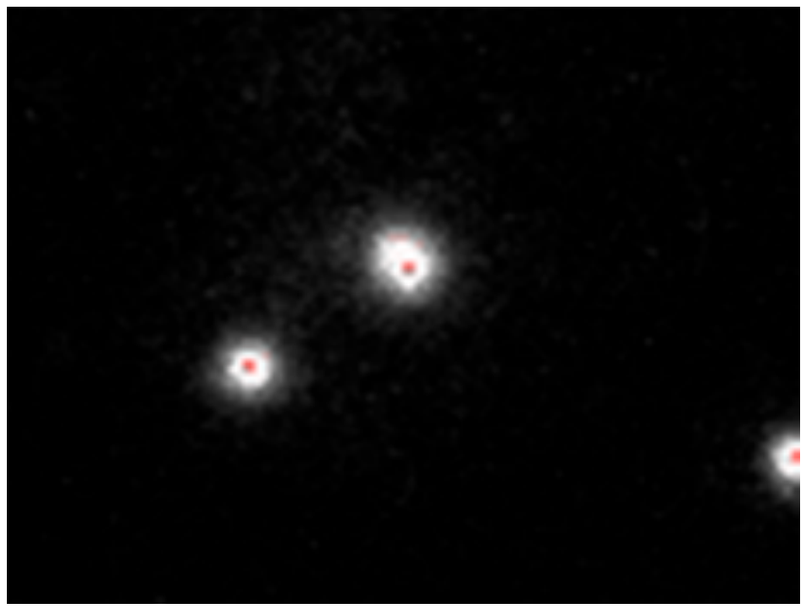
Shigella flexneri

Motility with actin polymerization
In vitro experiments

In Listeria Xenopus extract



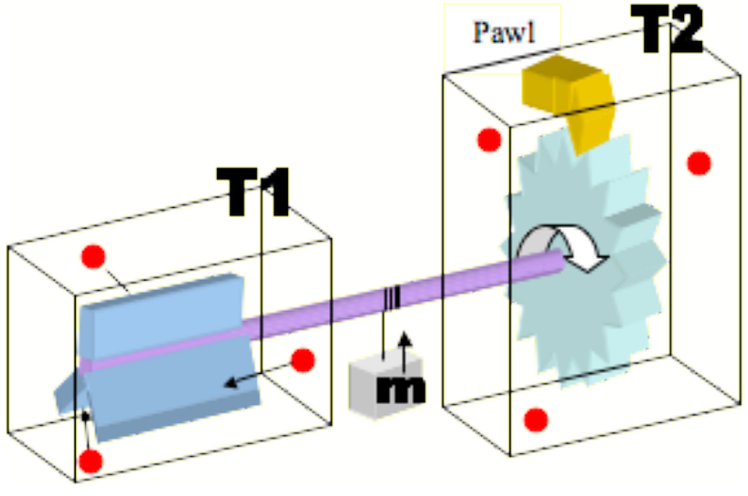
*Microbead **asymmetrically** coated with ActA in Xenopus extract*

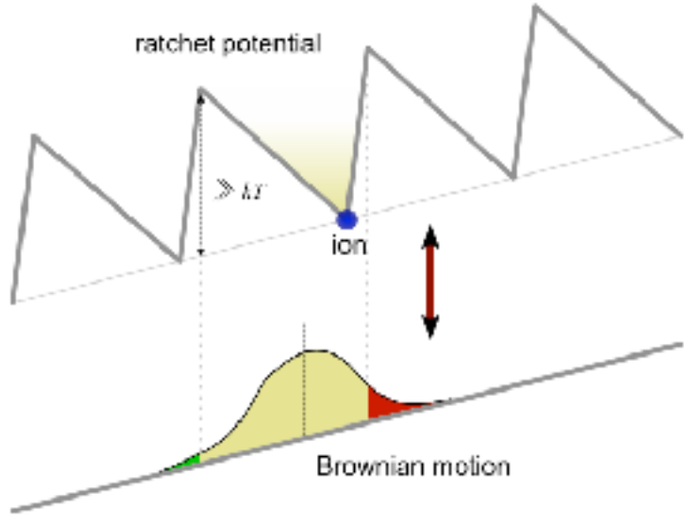


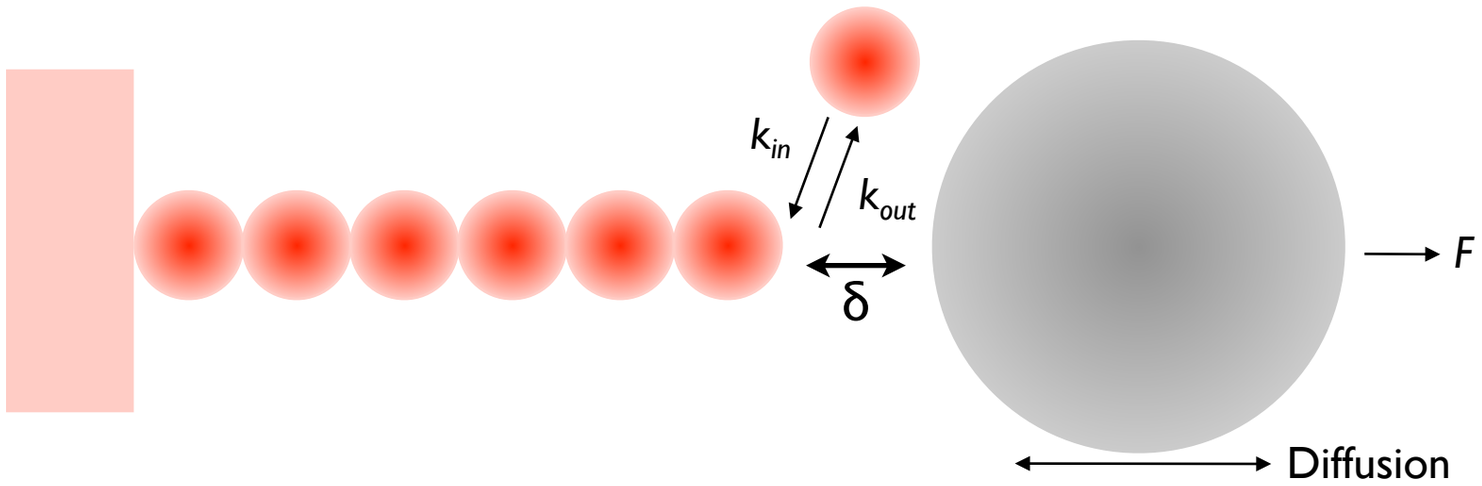
*Microbead **symmetrically** coated with ActA in Xenopus extract*

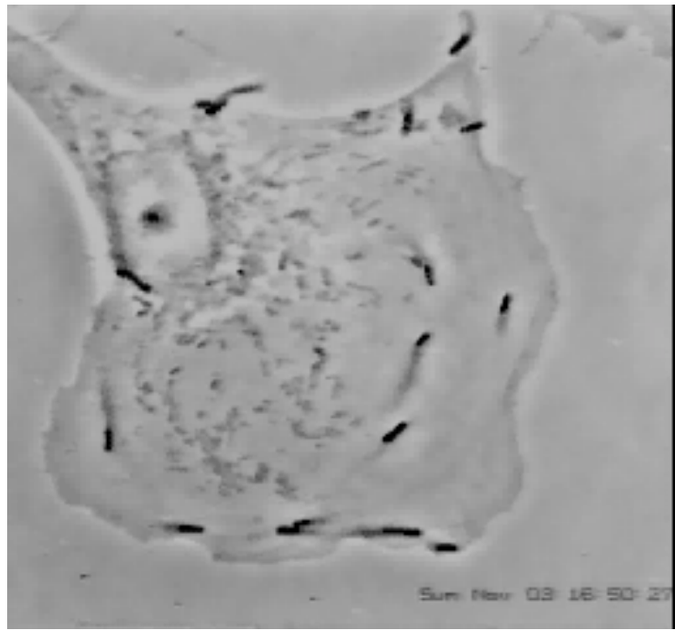
ActA: A protein expressed by the bacterium *Listeria monocytogenes* that is responsible for the "rocketing" motility of the bacterium throughout the eukaryotic host cell. In addition to other host proteins, ActA binds actin directly.

A special case of diffusion: the Brownian ratchet







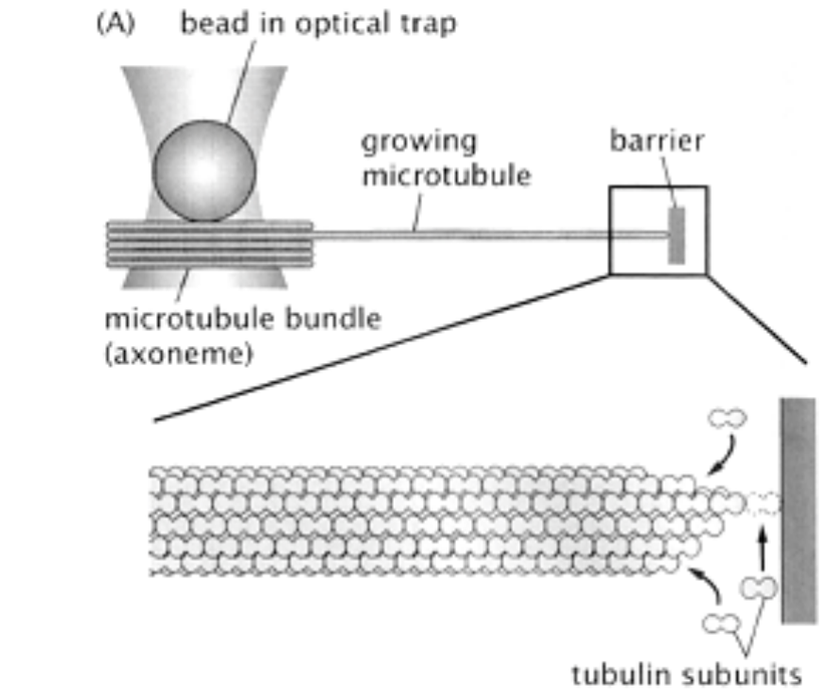


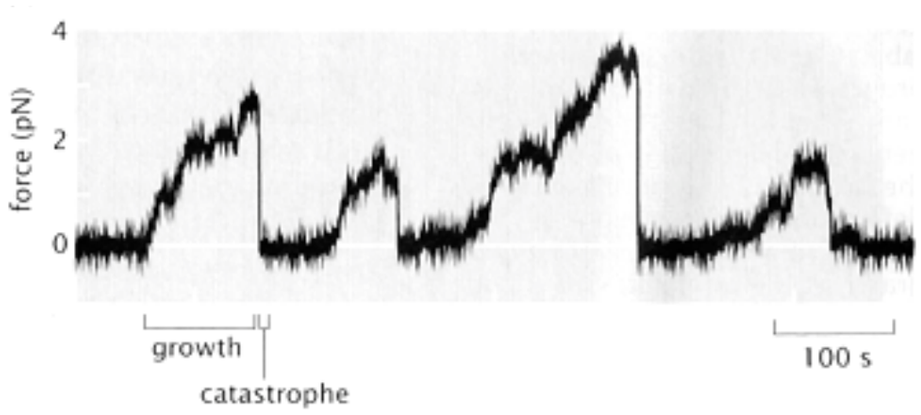
Listeria monocytogenes intracellular motion with actin polymerization

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

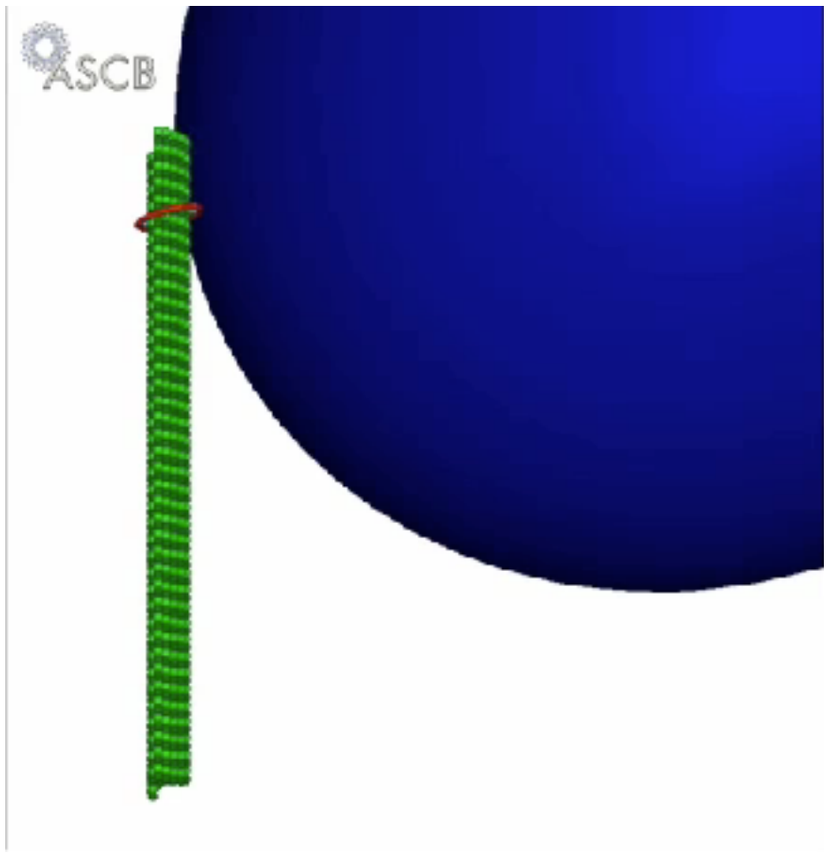
$K(F)$ = dissociation constant in the presence of force - monomer concentration at a net filament growth of 0.
K_c = critical concentration (at 0 force); F = force; δ = discrete growth upon the binding of one monomer. k_BT = thermal energy.
N.B.: F may be + or -. The process may be ***reaction driven*** (too fast diffision for k_{in}) or ***diffusion driven*** (too slow diffusion for k_{in}).

Force generation with polymerization

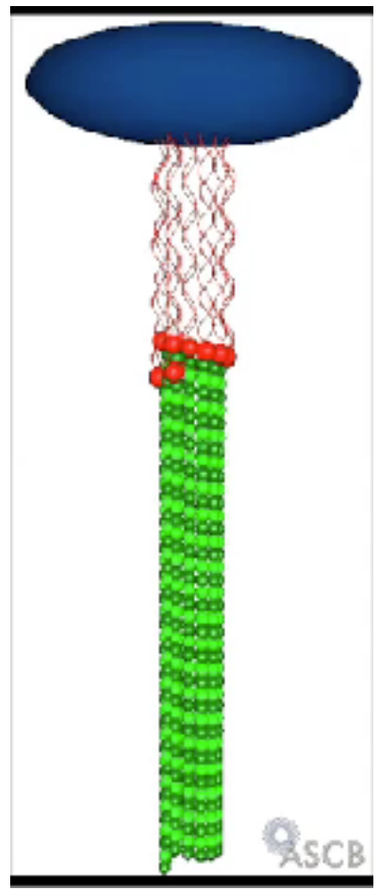




Force generation with depolymerization



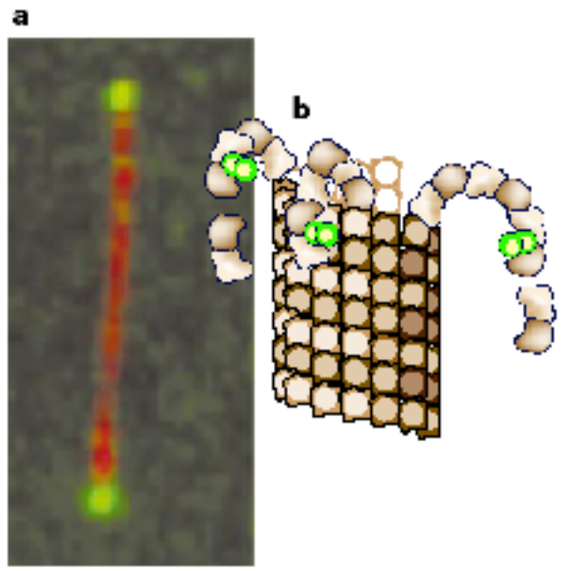
Vesicle transport with MT depolymerization

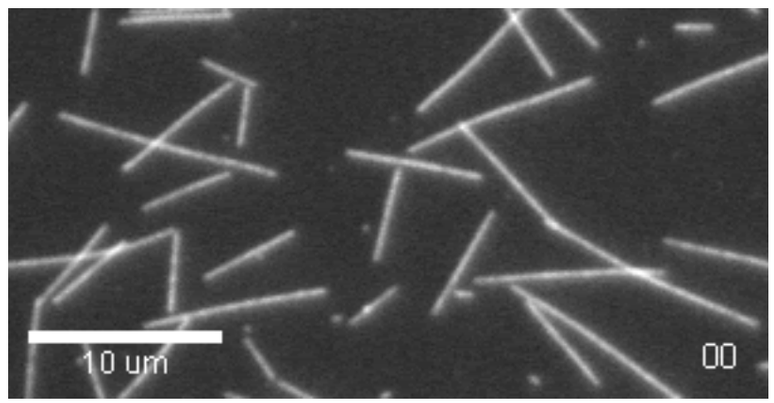


Chromosome (kinetochore) movement with MT depolymerization

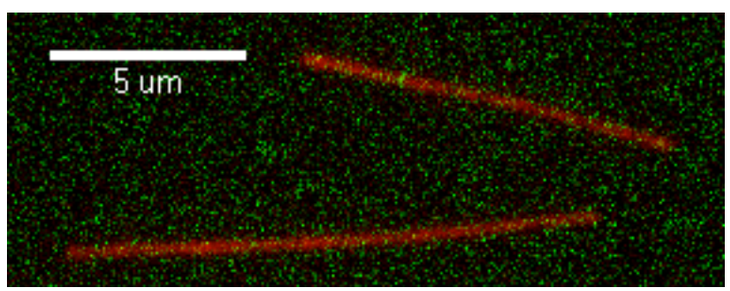
MCAK: MT-depolymerizing kinesin

MCAK:
"Kinesin-13"
Binds to the plus (+) end of MT
Finds the + end with a diffusional search
Hydrolyses ATP
Controls MT depolymerization ("catastrophy factor")





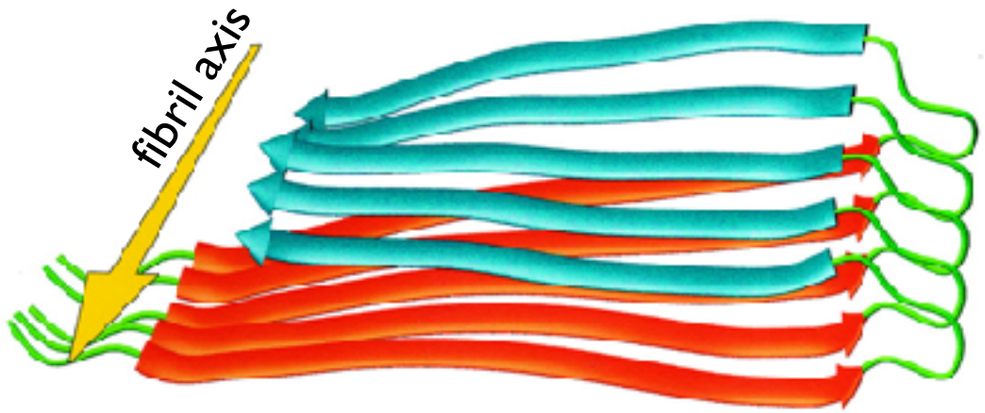
MCAK-induced MT depolymerization

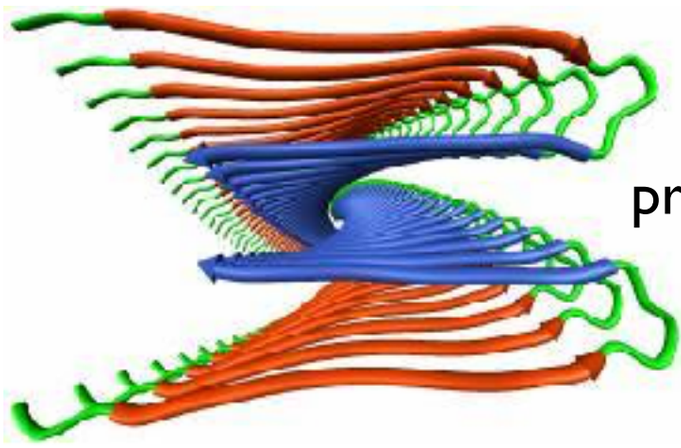


Diffusional search along MT (GFP-MCAK)

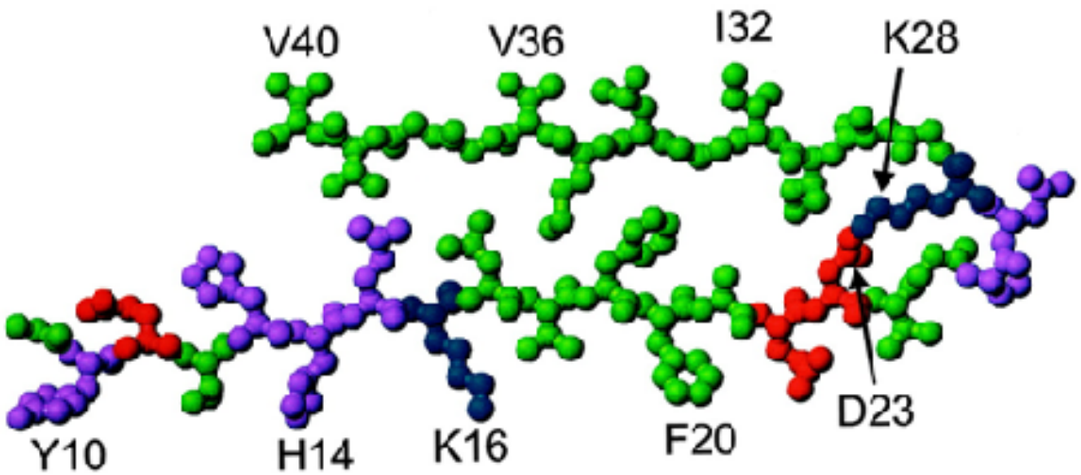
Self-assembly, catalyzed polymerization

Amyloid ß-fibrils: components of Alzheimer plaques

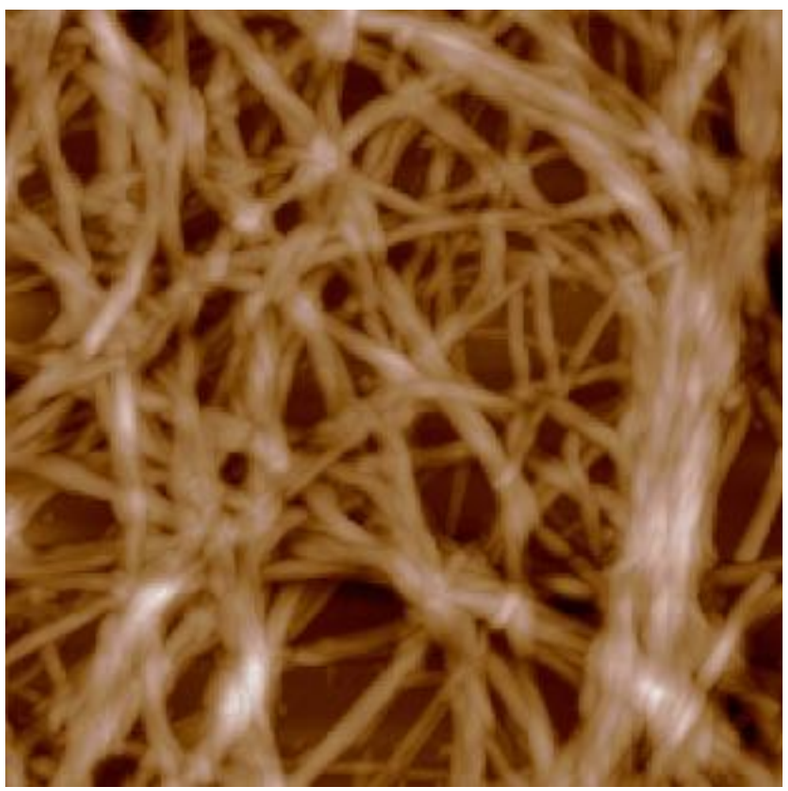




Aß1-40
protofilament



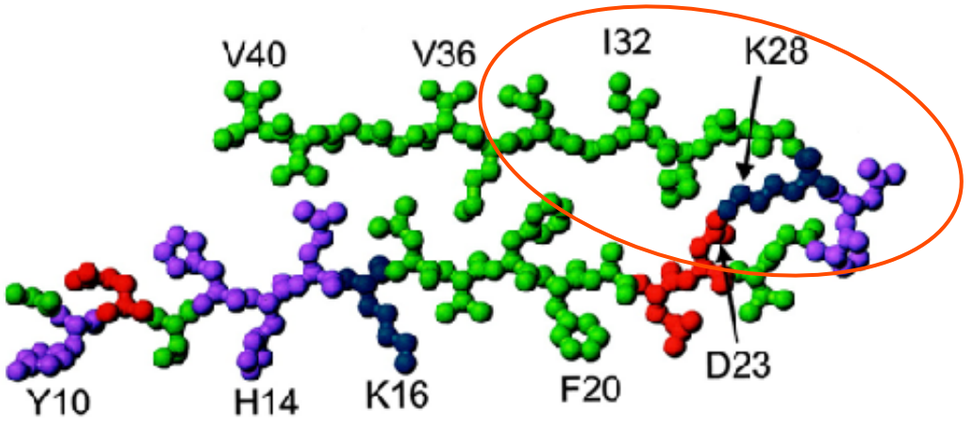
Amyloid ß1-40 peptide



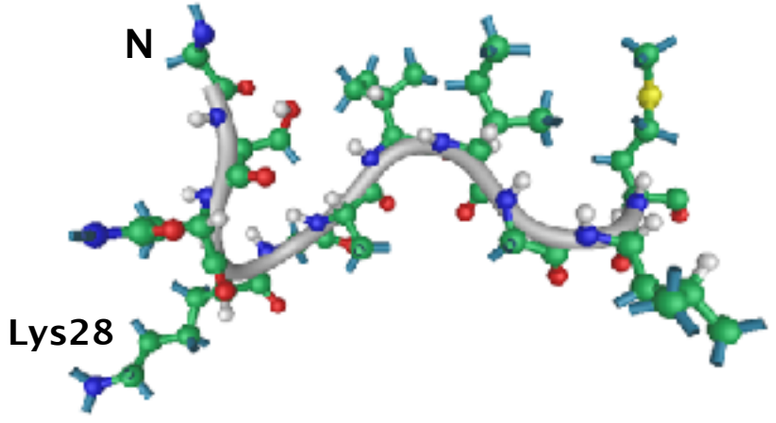
Fibrils grown from Aß1-40 peptide *in vitro* (AFM)

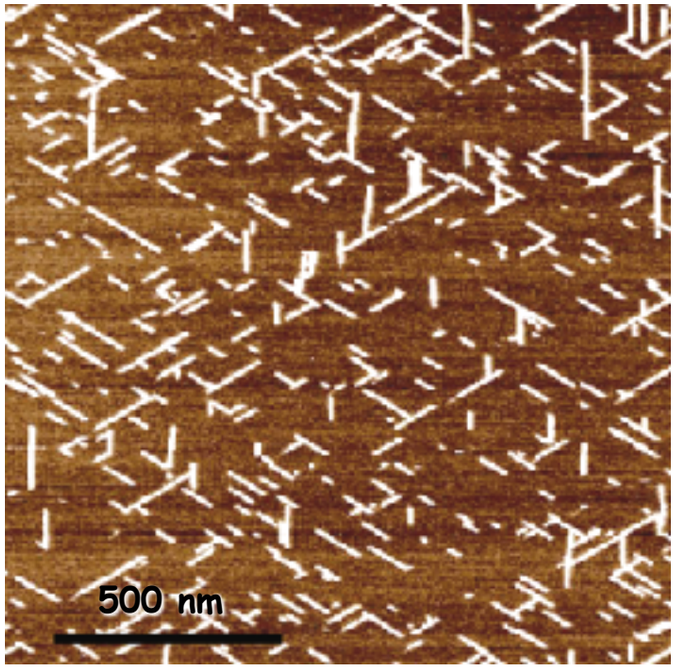
Epitaxial growth of amyloid fibrils

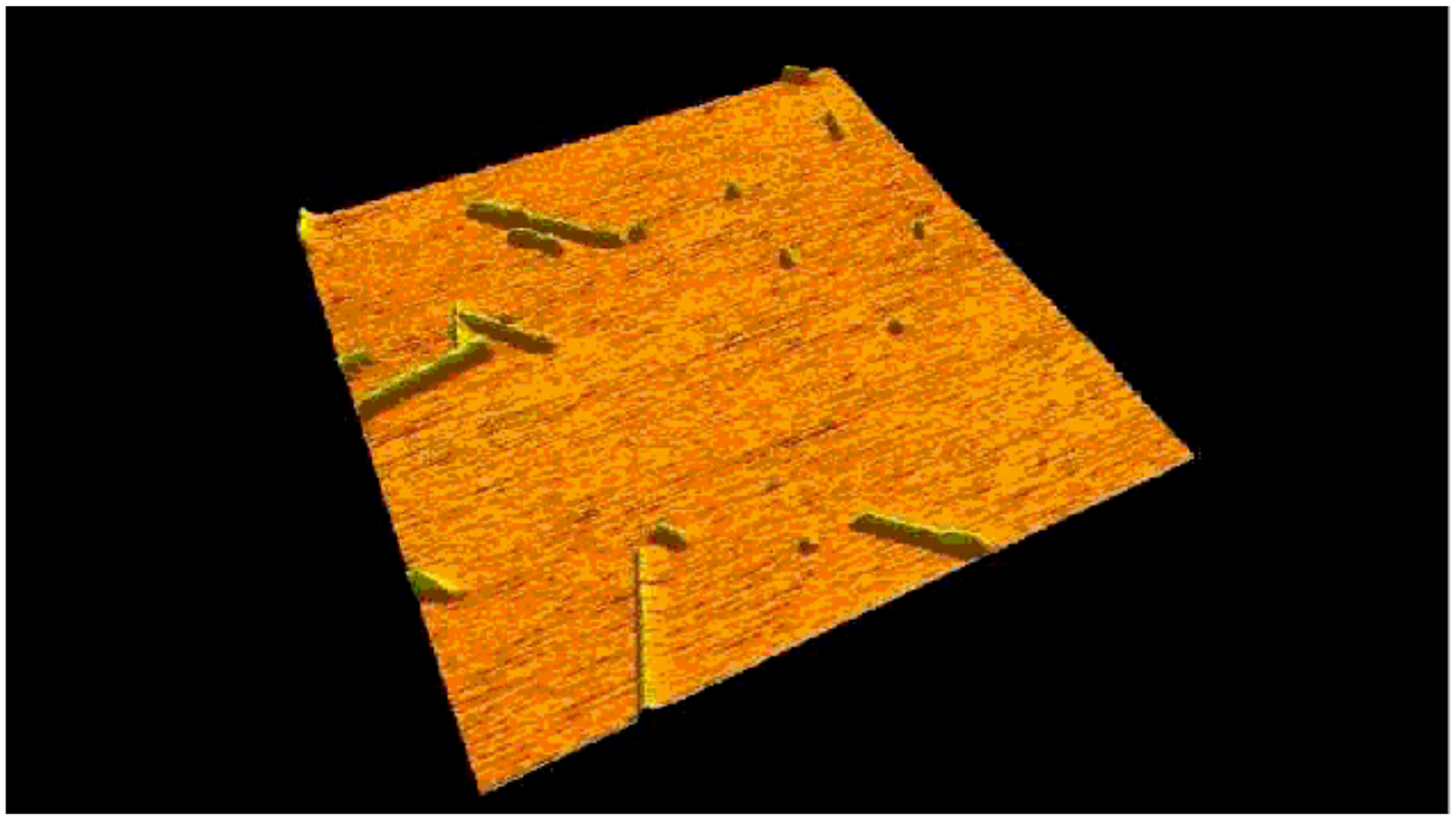
Aß1-40



Aß25-35

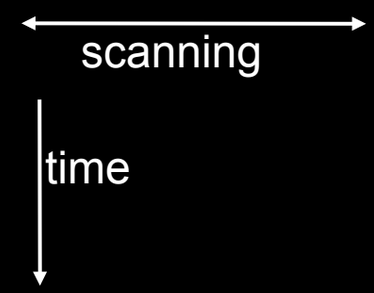


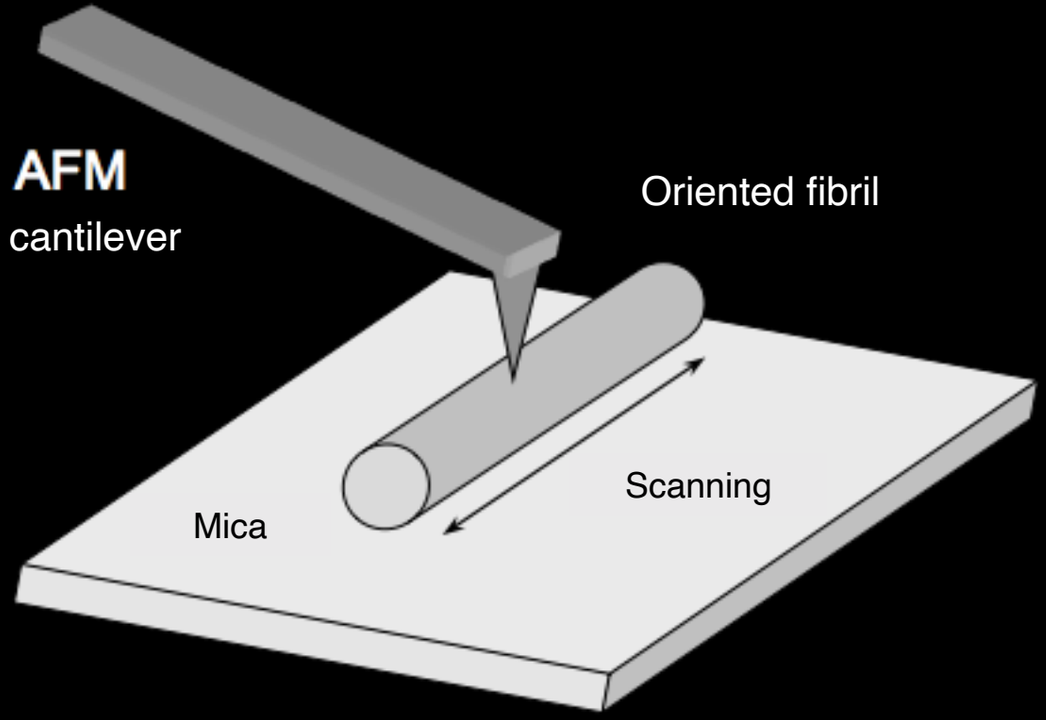




Epitaxial growth of Aß25-35 fibrils on mica surface

Nanoscale mechanism of catalyzed fibril growth:
scanning probe kymography

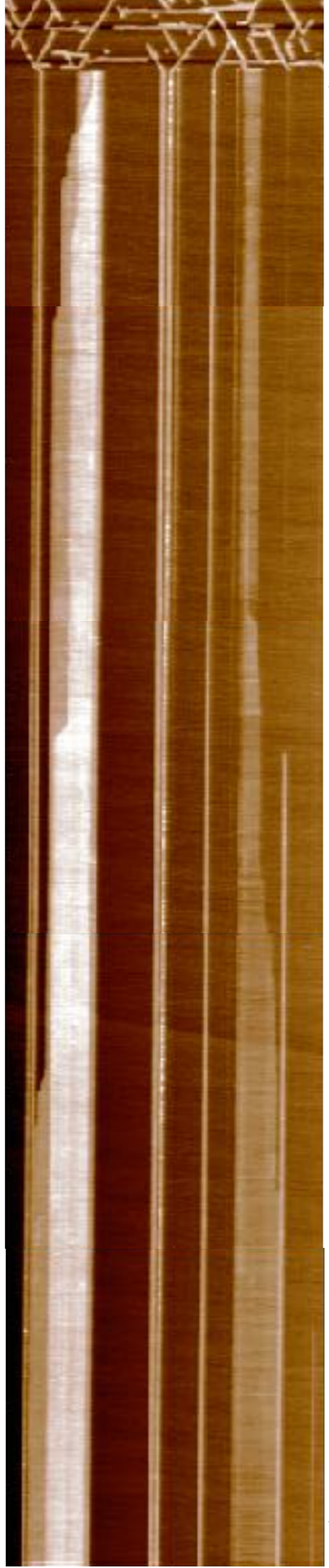




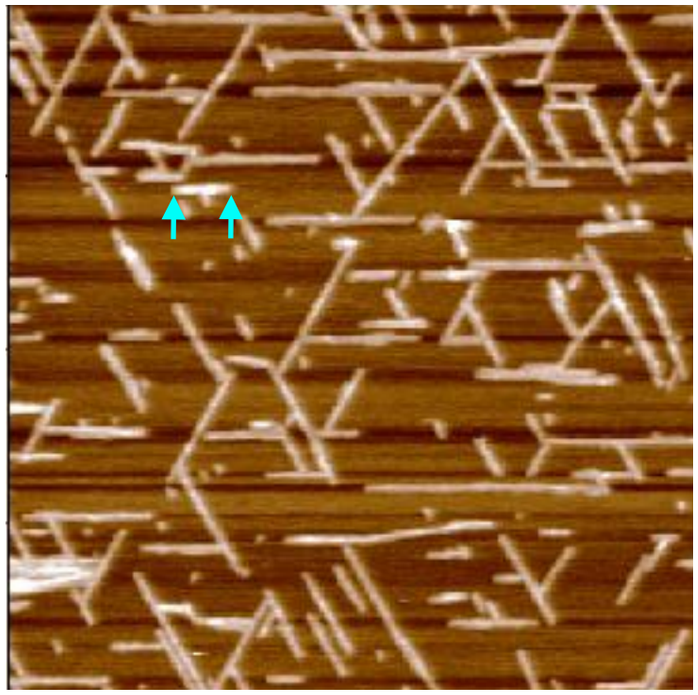
Spatial resolution:
1 nm

Temporal resolution:
300 ms

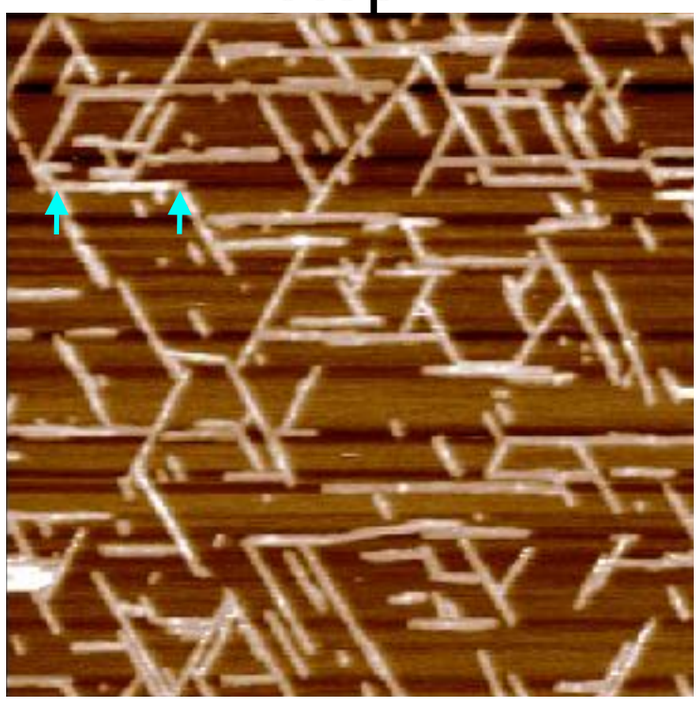
Scanning probe kymography

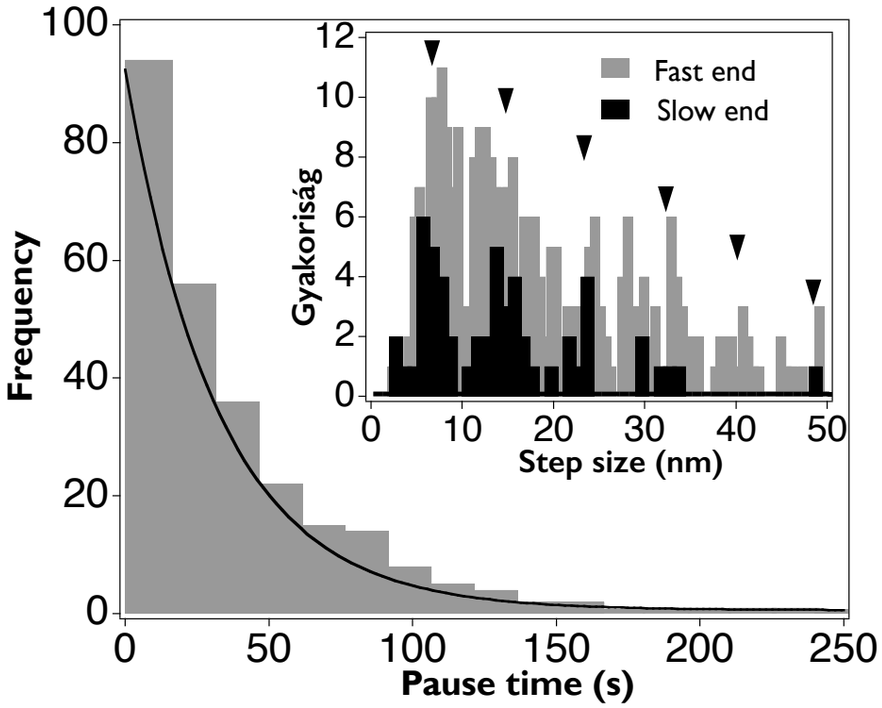


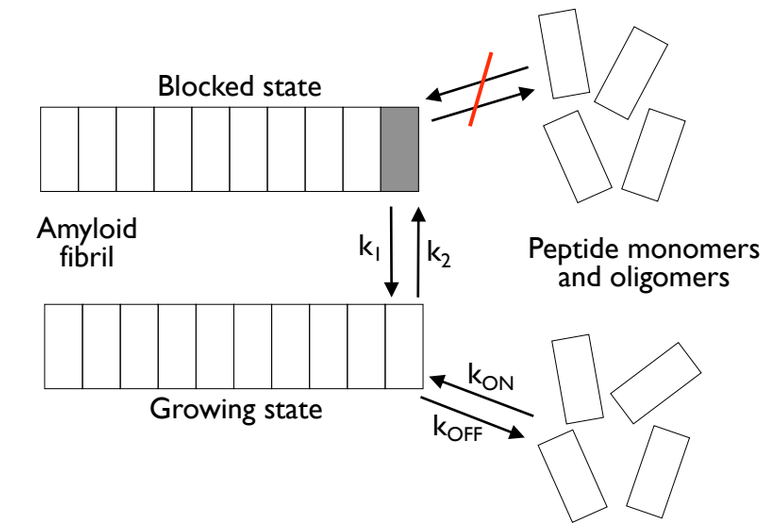
Start

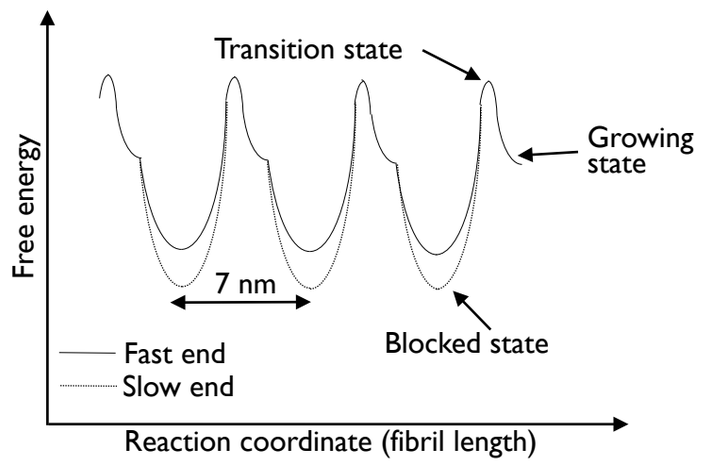


Stop









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

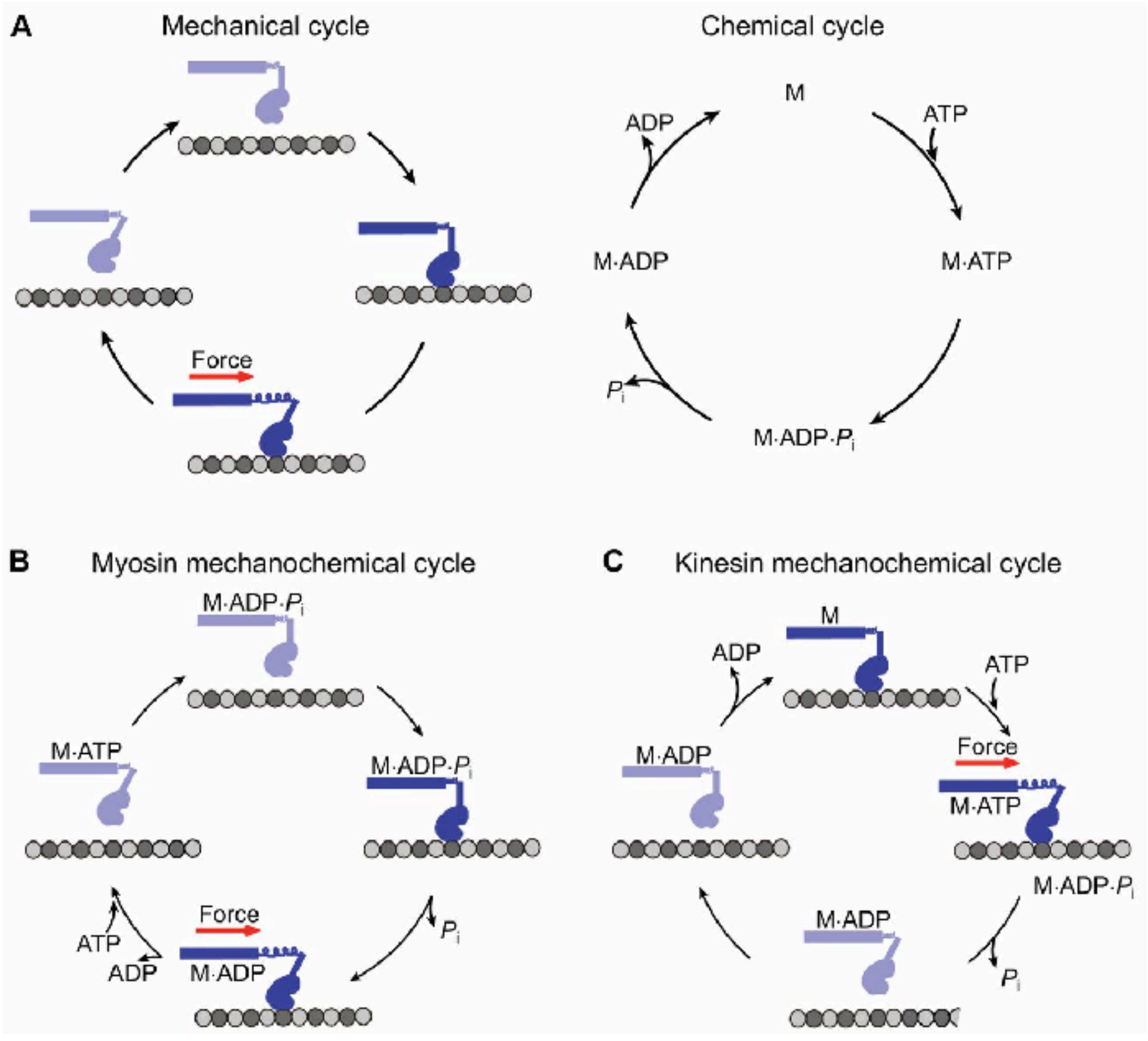
4. Rotary motors

F1F0-ATP synthase
Bacterial flagellar motor

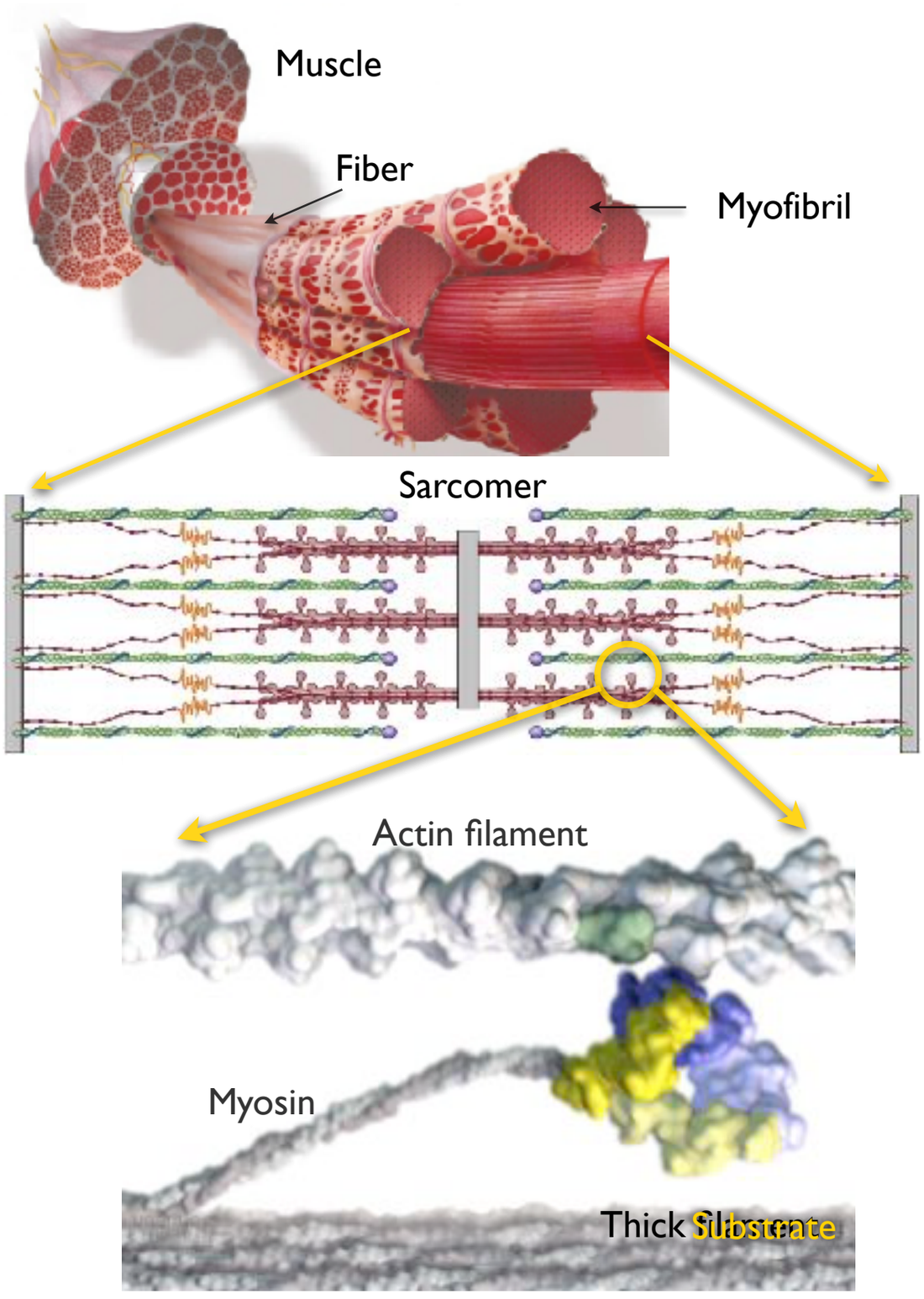
5. Mechanoenzyme complexes

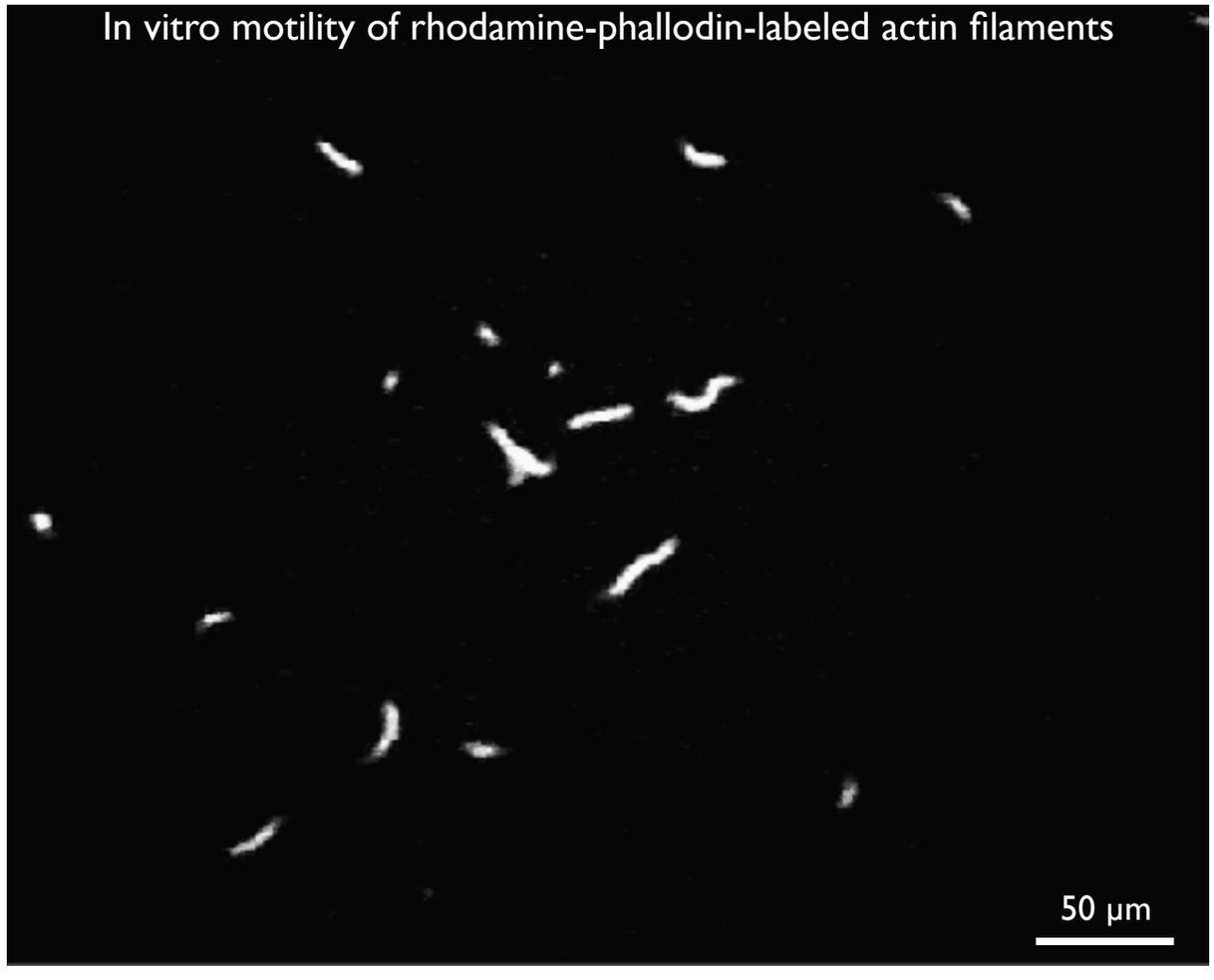
Ribosome

Cyclic mechanism - “duty cycle”

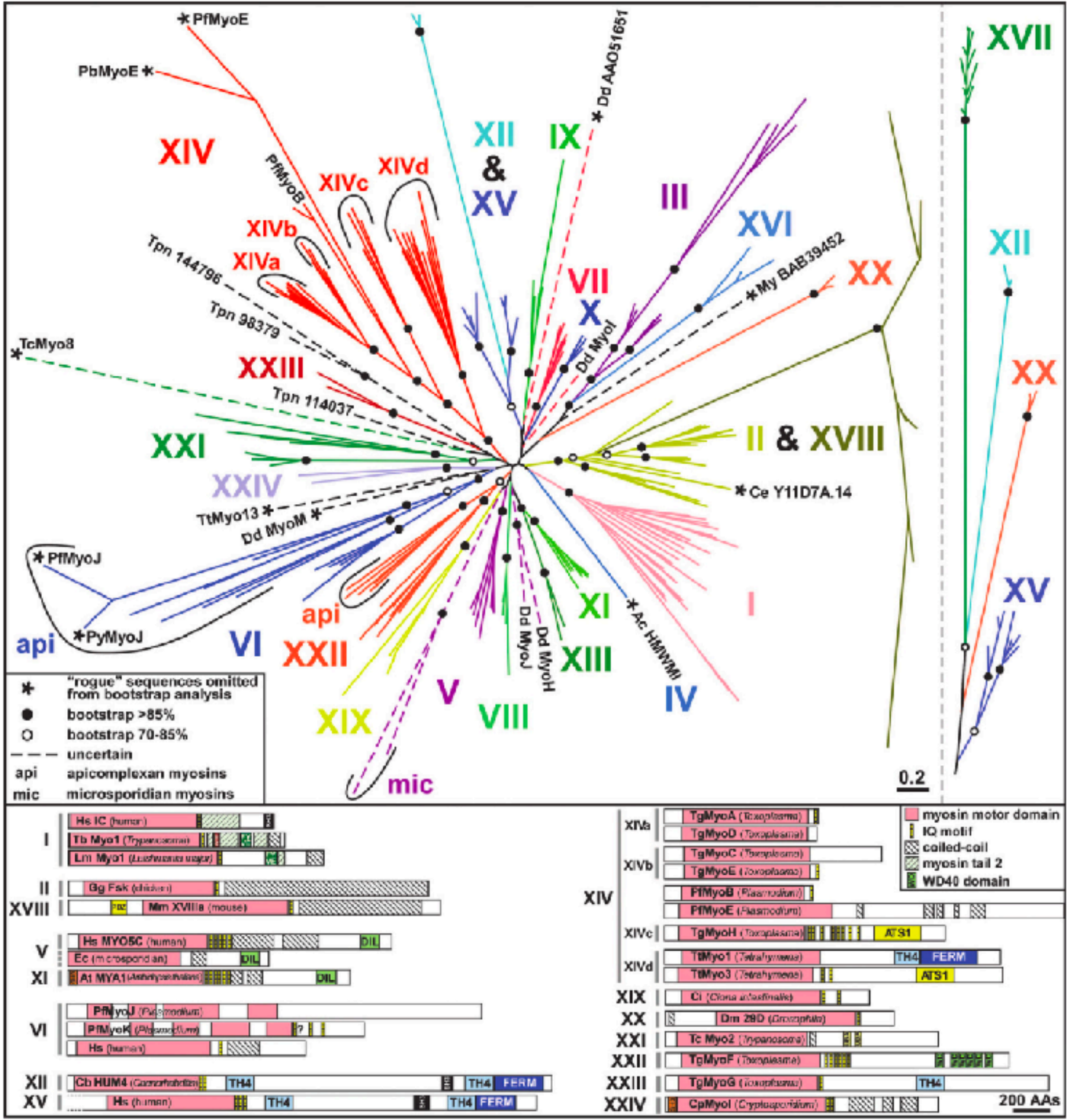


Myosin


Muscle
Fiber
Myofibril
Sarcomer
Actin filament
Myosin
Thick Filament
Substrate

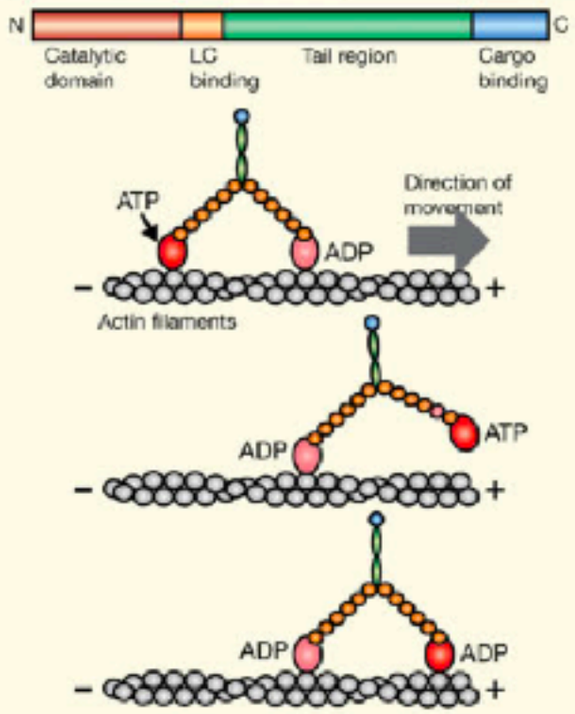

In vitro motility of rhodamine-phallodin-labeled actin filaments
50 µm

The myosin superfamily

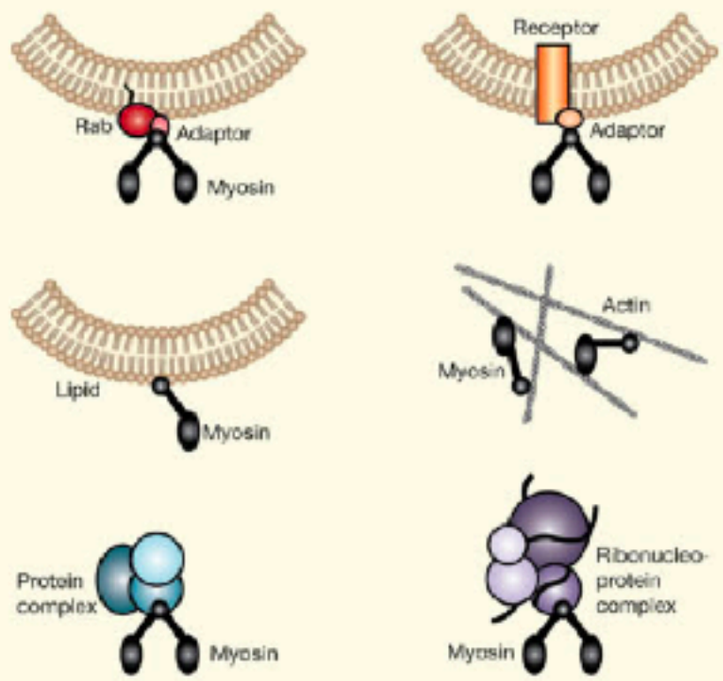


Functions of the myosin superfamily

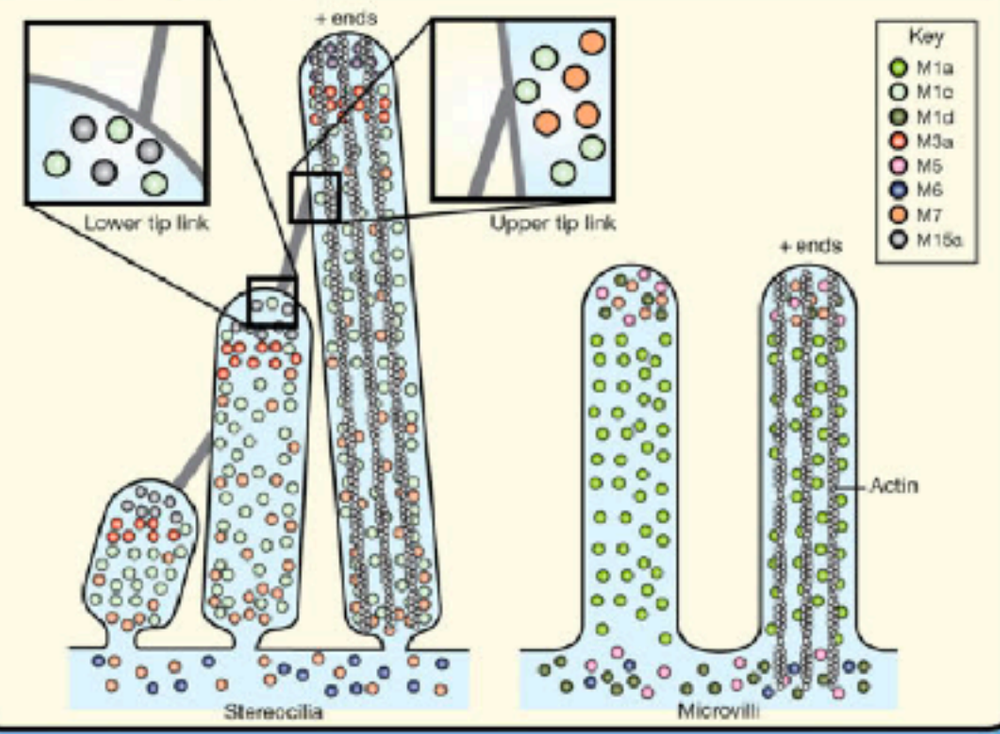
Myosin structure and motility



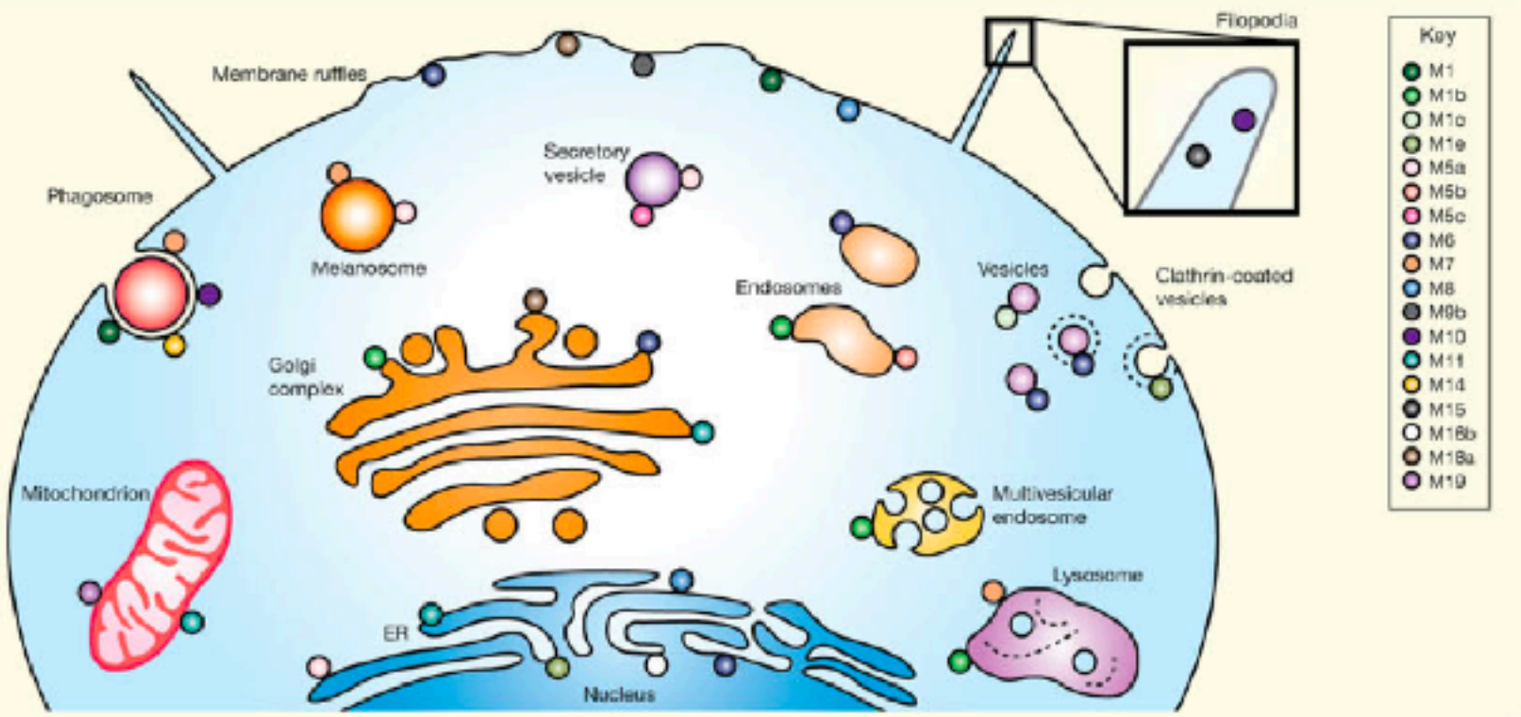
Cargo interactions



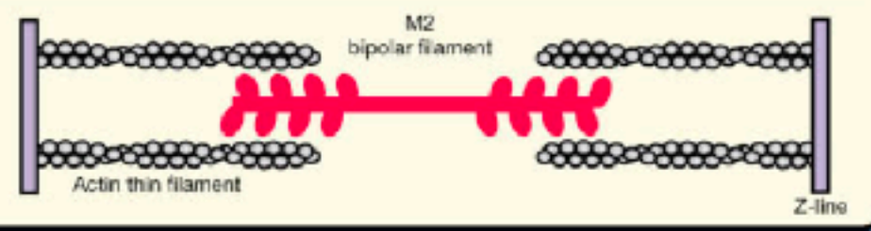
Actin-based projections



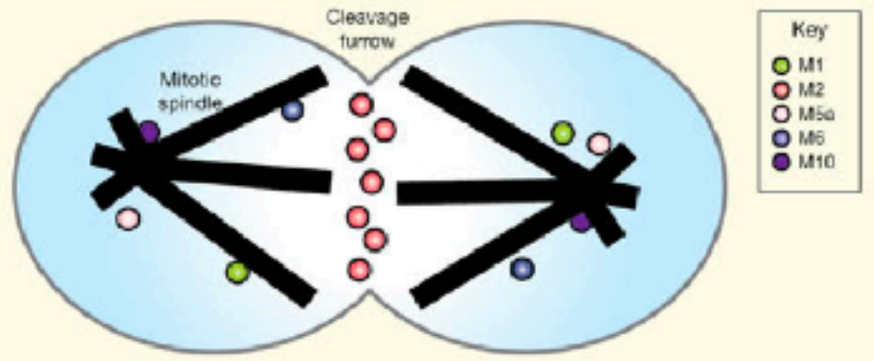
Membrane compartments



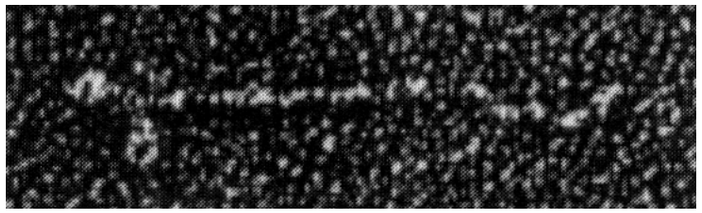
Muscle contraction

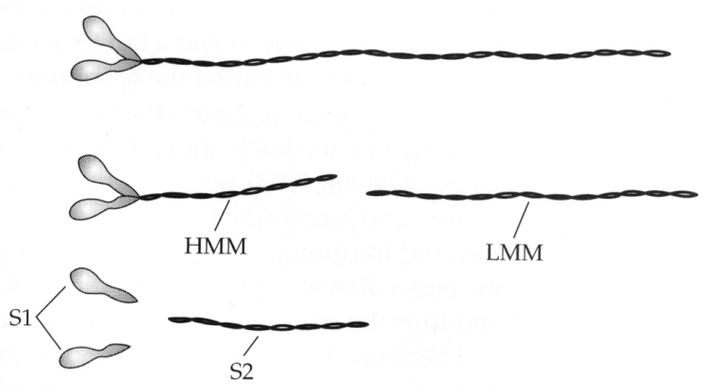


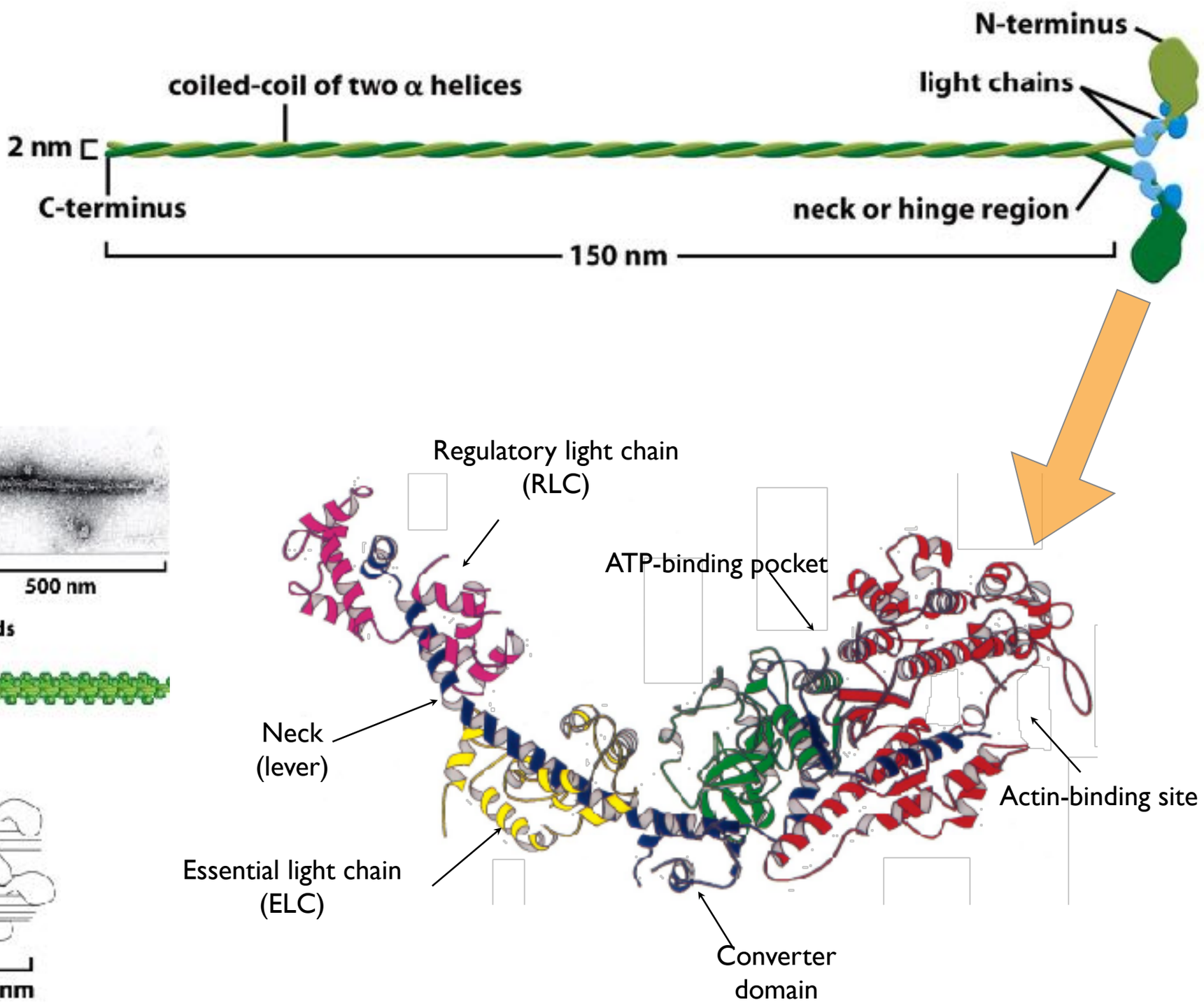
Cell division



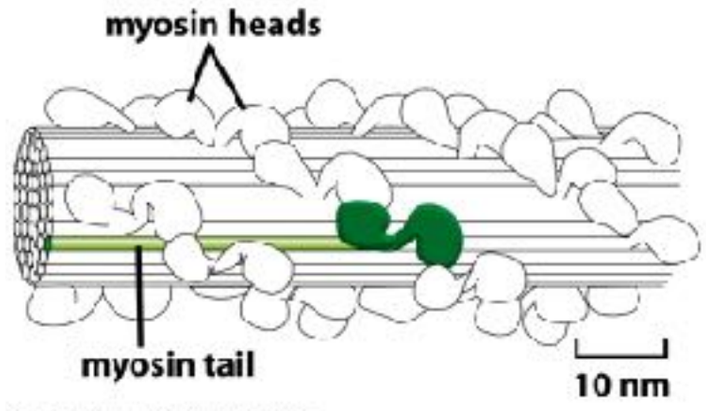
Myosin II




HMM
LMM
S1
S2

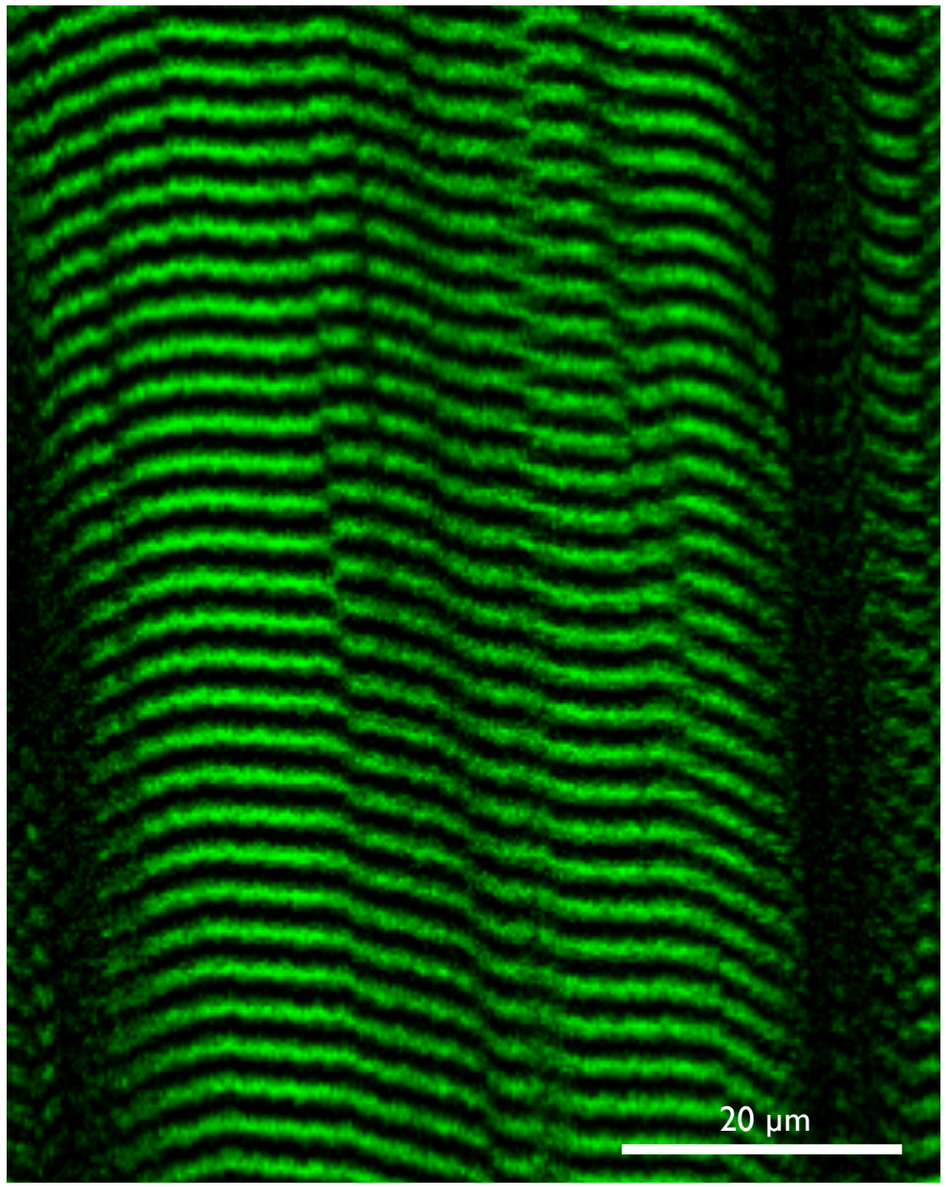

N-terminus
light chains
coiled-coil of two α helices
2 nm
C-terminus
neck or hinge region
150 nm
Regulatory light chain
(RLC)
ATP-binding pocket
Neck
(lever)
Actin-binding site
Essential light chain
(ELC)
Converter
domain


500 nm
bare zone
myosin heads


myosin heads
myosin tail
10 nm

Myosin II assembles into thick filaments

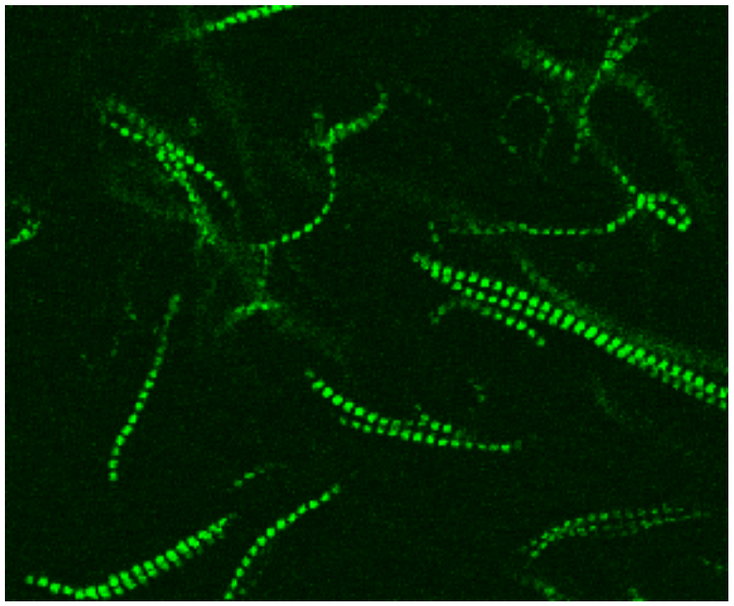
Two-photon microscopic image of a muscle fiber



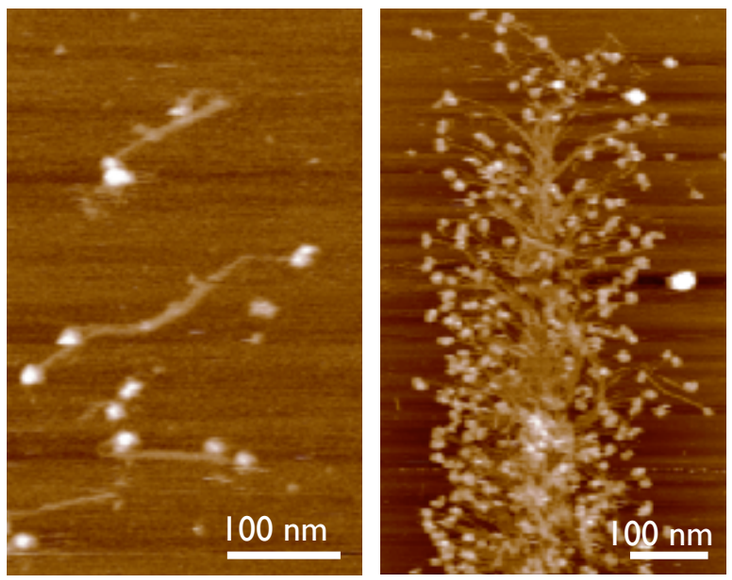
Signal source: helical structure of myosin filaments

Excitation: 1000 nm

Second harmonic generation (SHG): 500 nm



Unlabeled myofibrils. 2P microsopic image

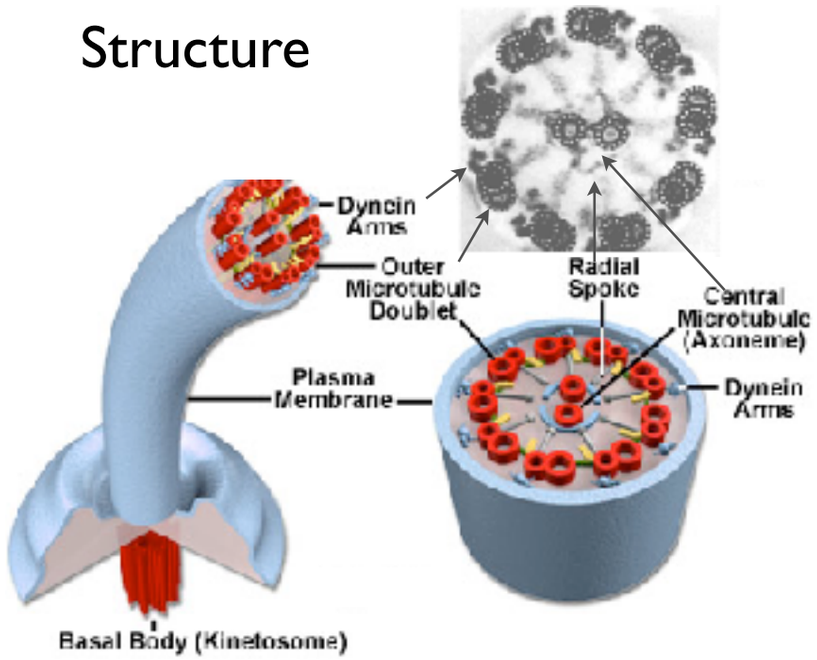


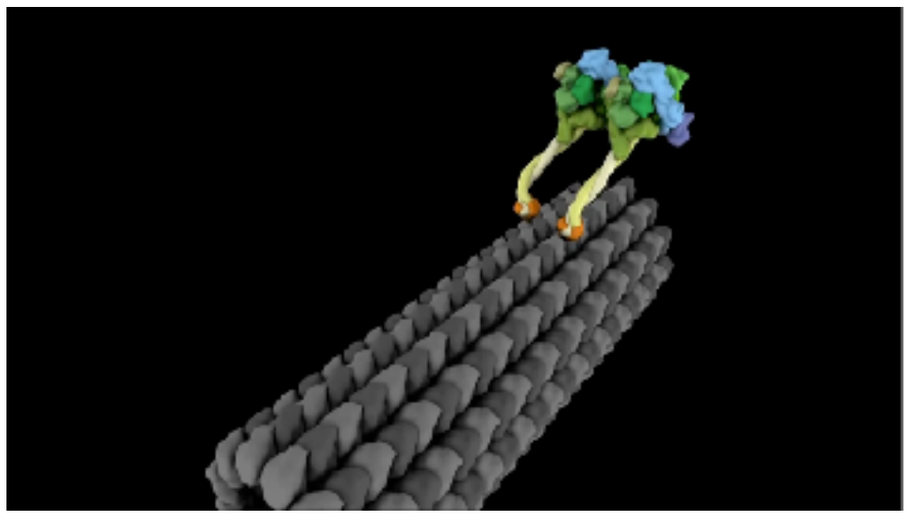
Myosin molecules and thick filament. AFM image

Dyneins

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

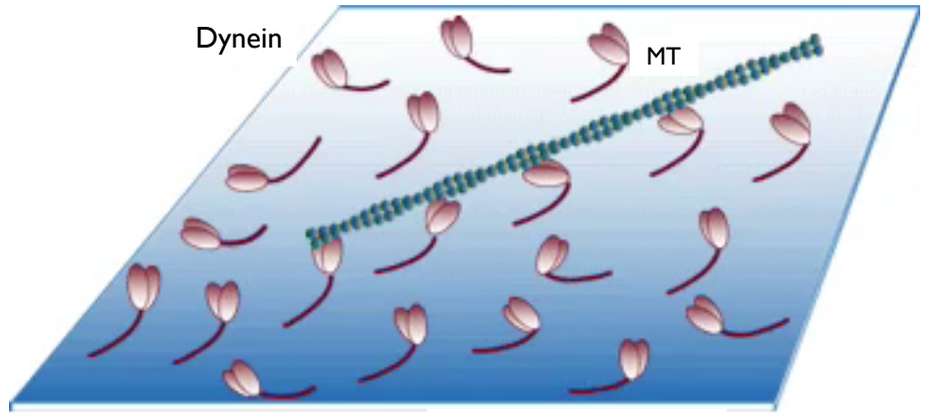
Structure





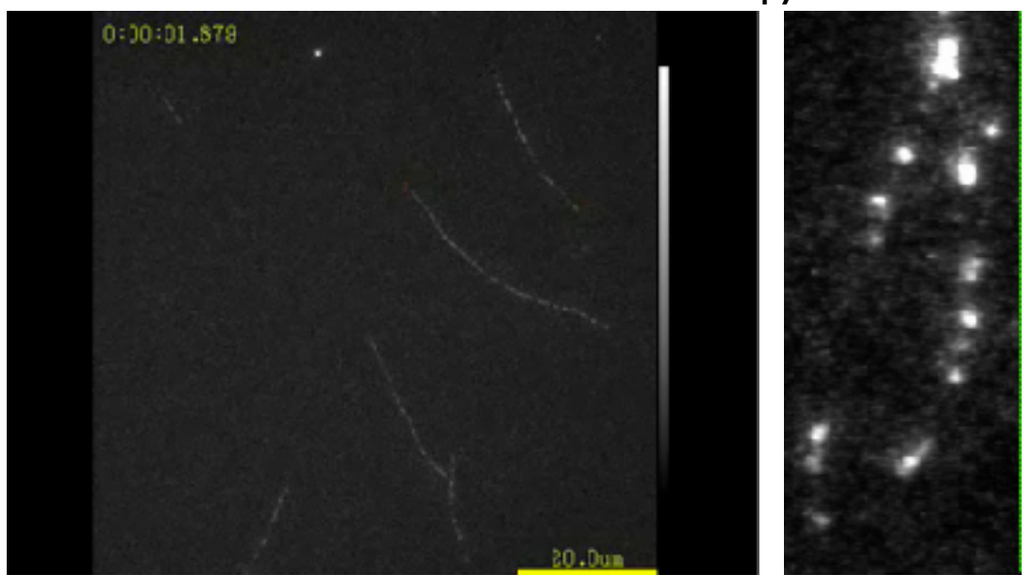
"Drunken sailor" stepping mechanism

Biomolecular *functional model:* "*In vitro* motility assay"



Microscope coverslip

Fluorescence video microscopy

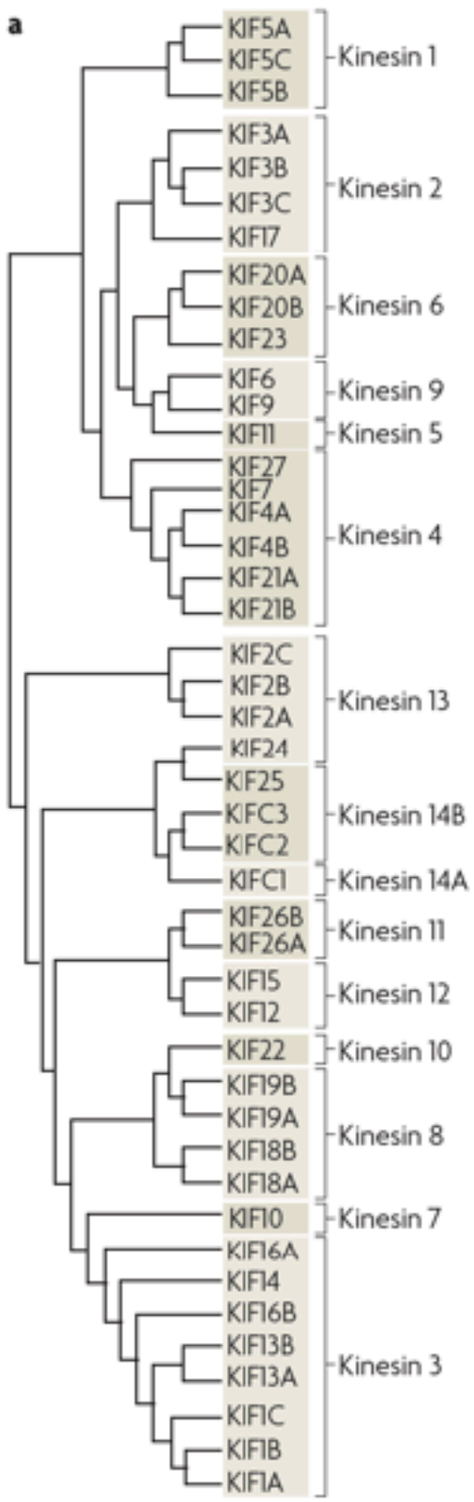


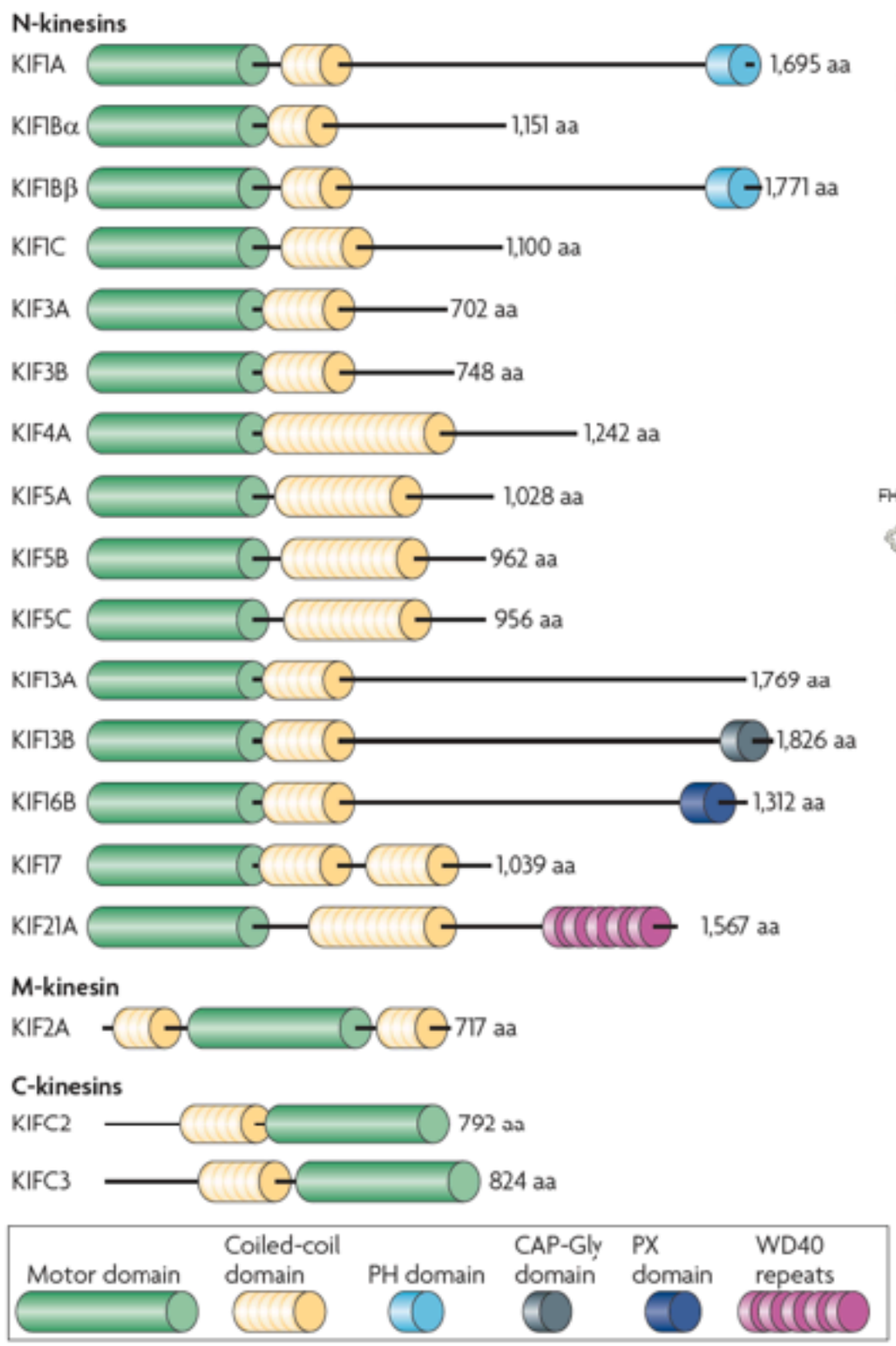
Microtubule moves on dynein

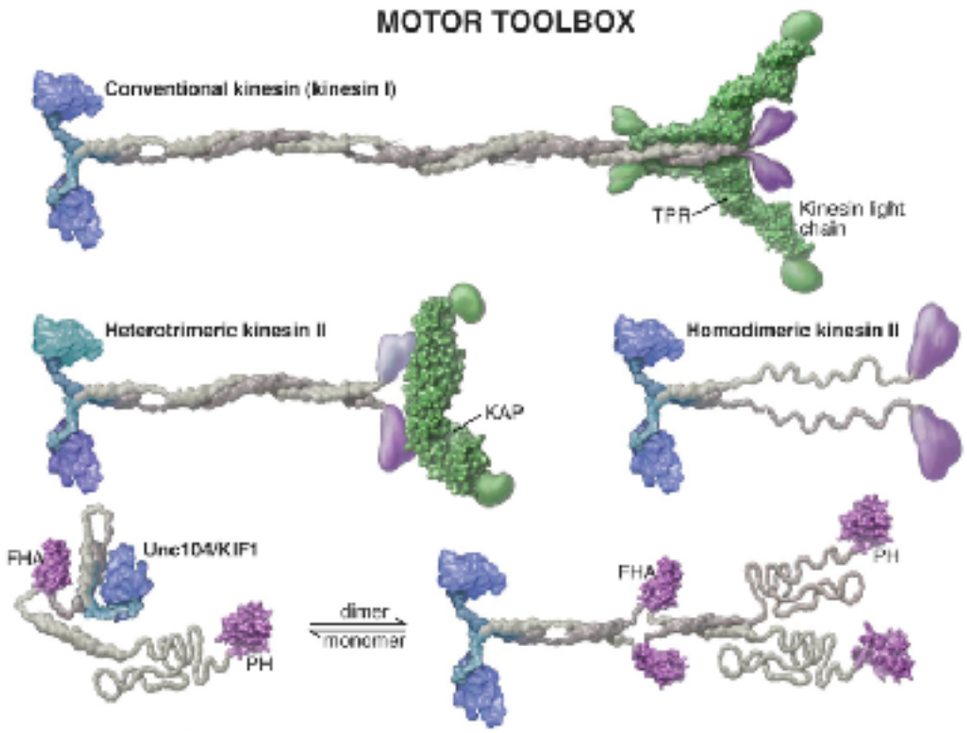
Dynein moves on microtubule

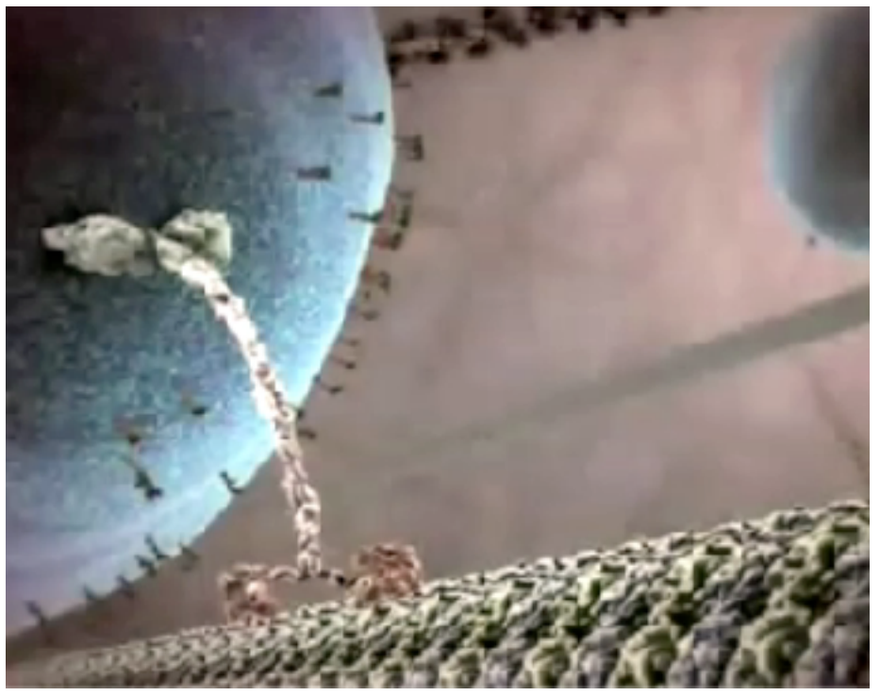
Kinesin superfamily

Processive motors, move mostly towards the plus end of MT



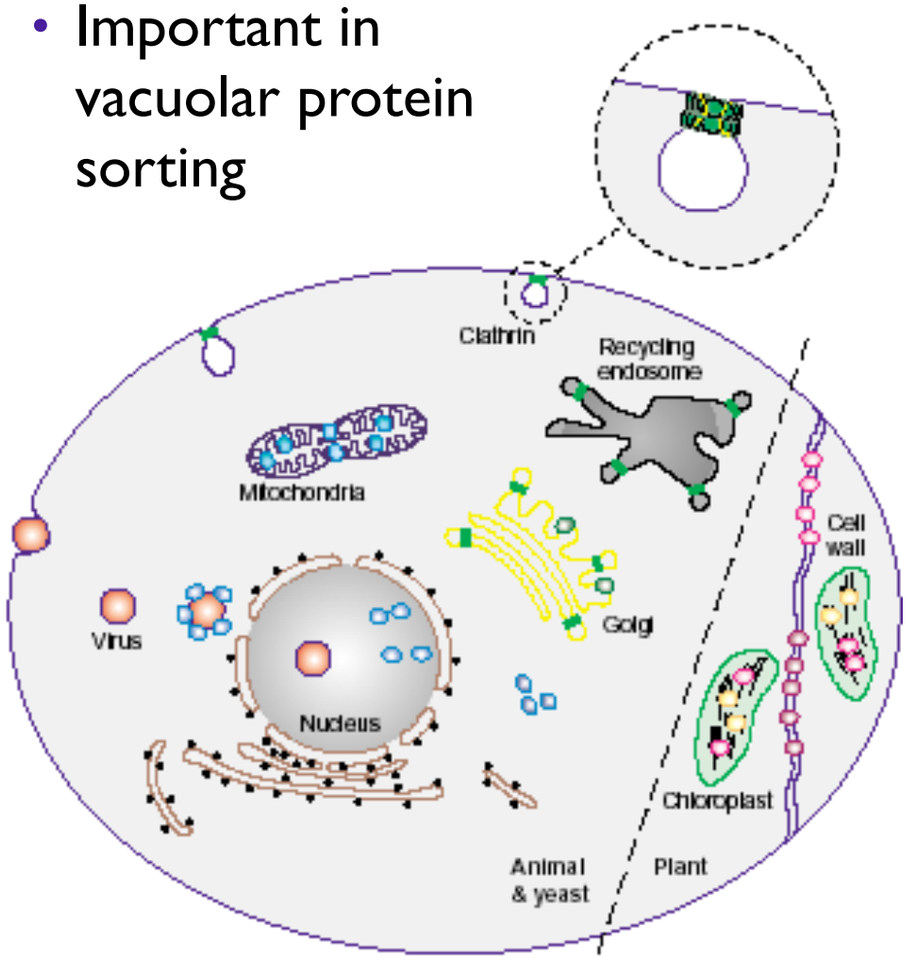


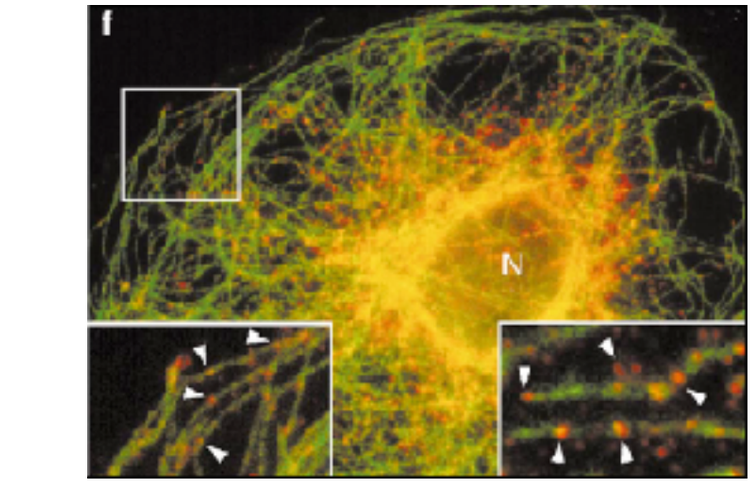


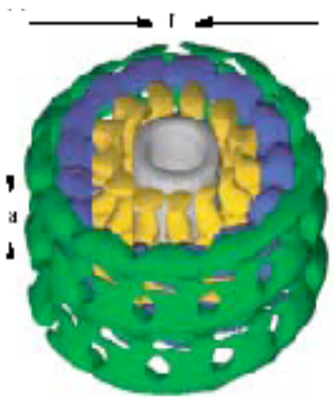


Dynamins

- GTPases
- Important in vacuolar protein sorting



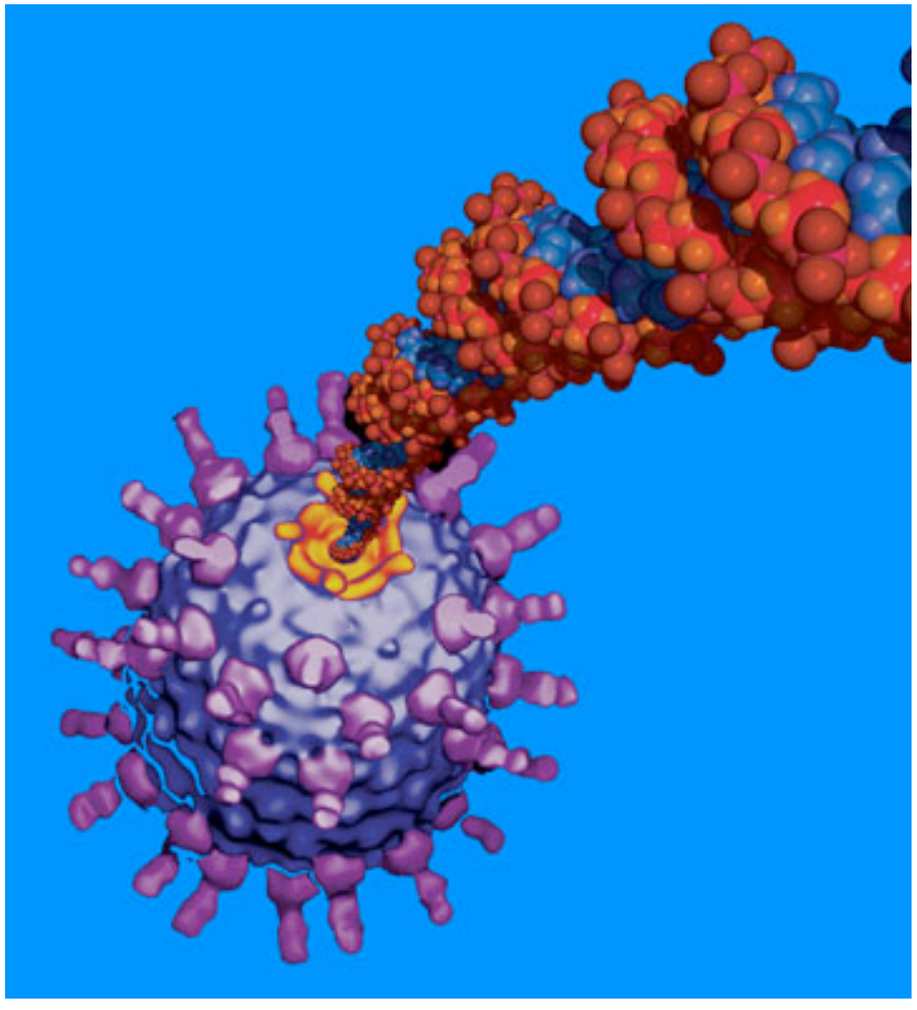


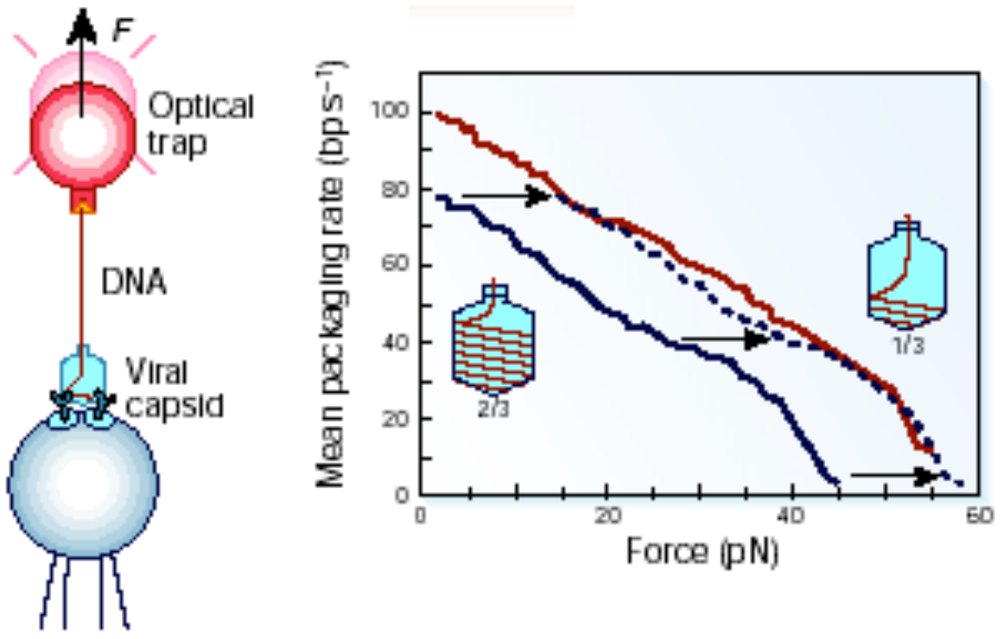


Protein	Localization	Function	Self-assembly
Dynamin	Plasma membrane (clathrin coated, caveolae), Golgi, endosomes	Vesicle formation, fission	+
Vps1	Golgi	Vesicle formation and transport	Unknown
Dnm1/Drp1/DRP-1	Mitochondria outer membrane	Mitochondrial fission & morphology	+
Mgm1/Msp1/OPA1	Mitochondria 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

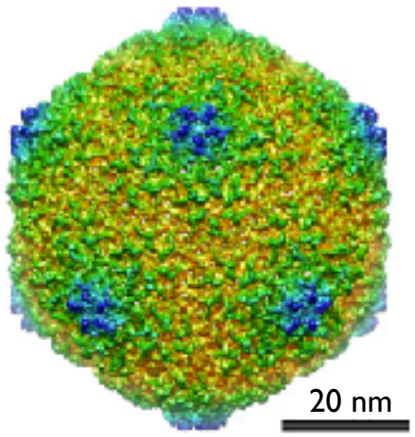
Viral portal motor
Unique DNA motor



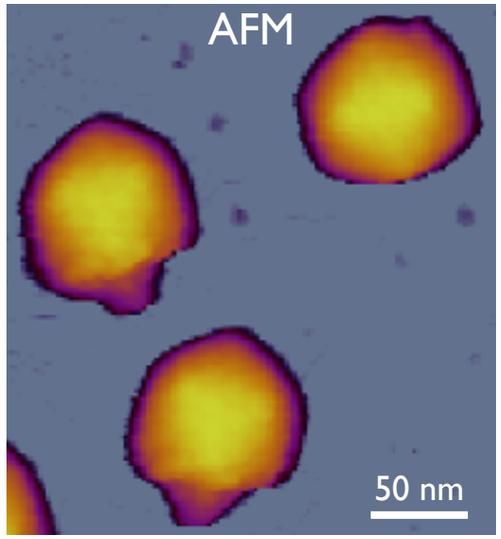


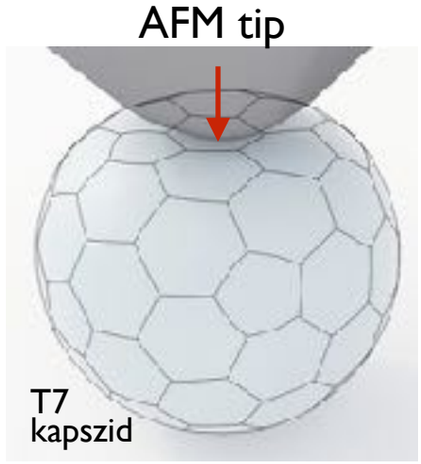
φ29 bacteriophage portal motor

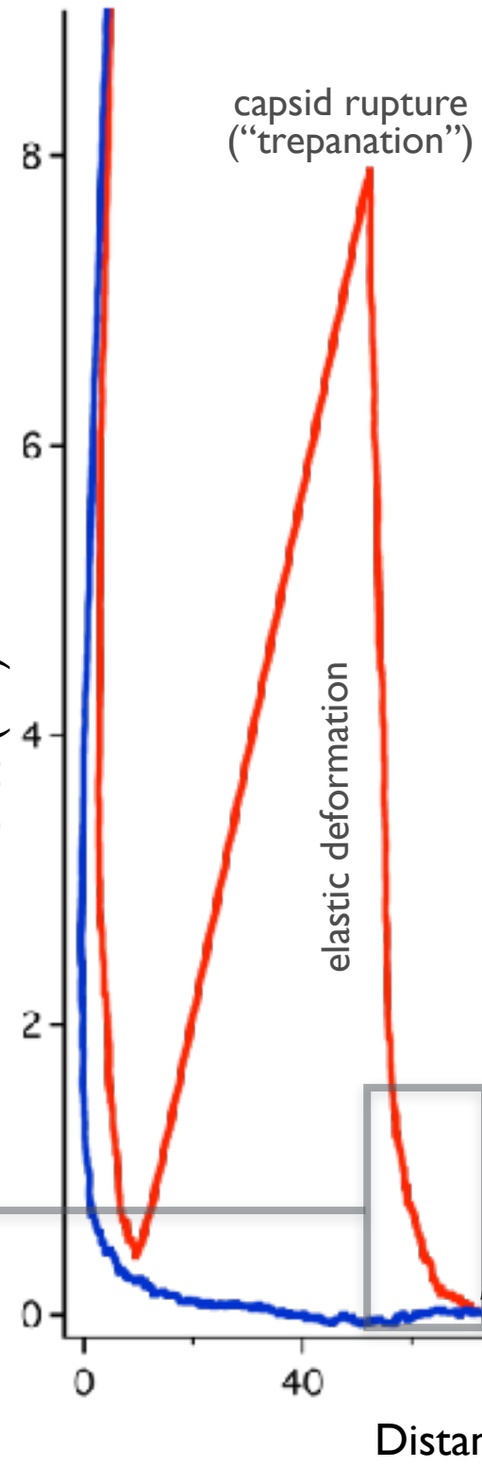
Viral capsid mechanical rupture - irreversible process

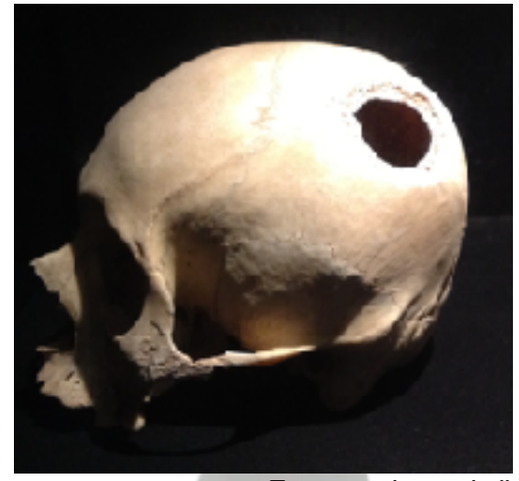


Ionel et al. J.Biol.Chem. 286, 234, 2010.

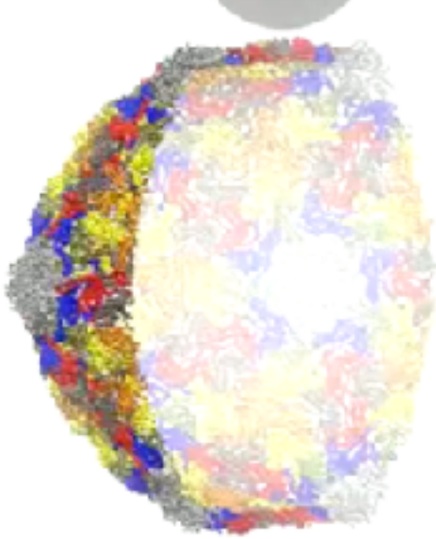




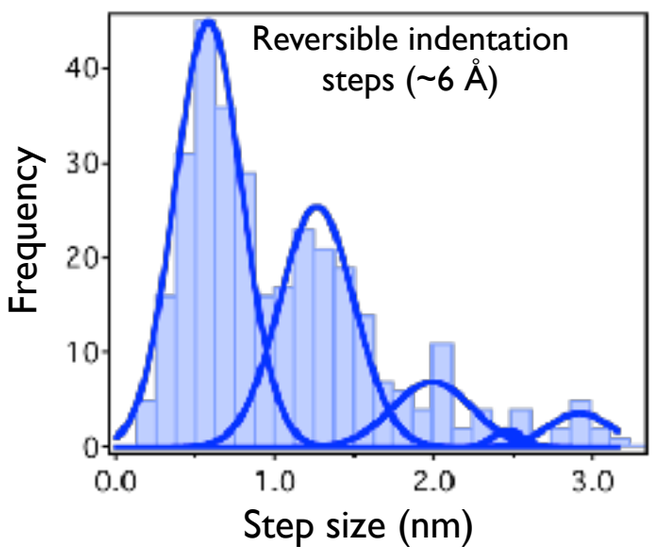


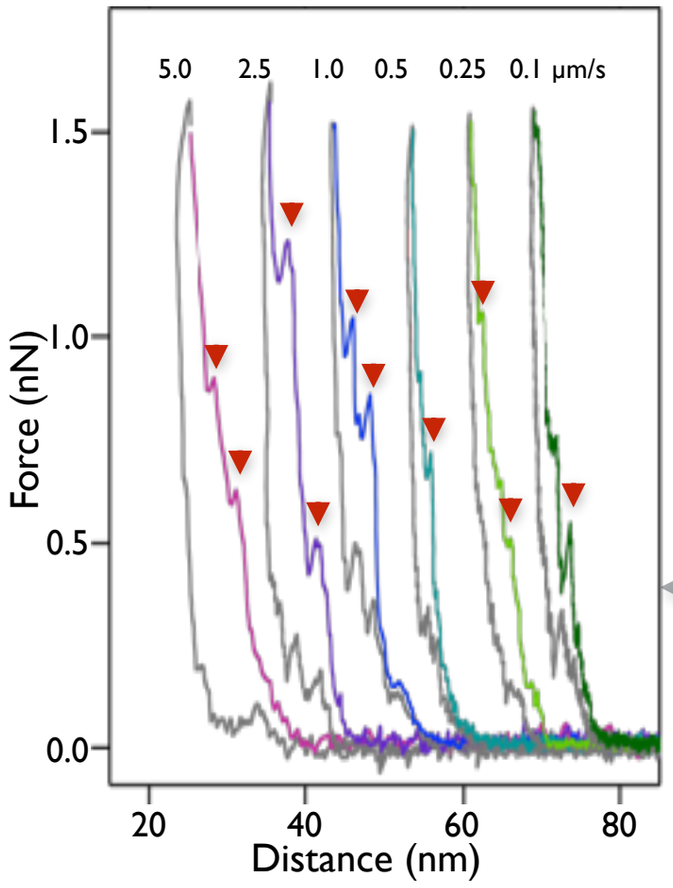


Trepanated inca skull,
Museo Rafael Larco Herrera, Lima

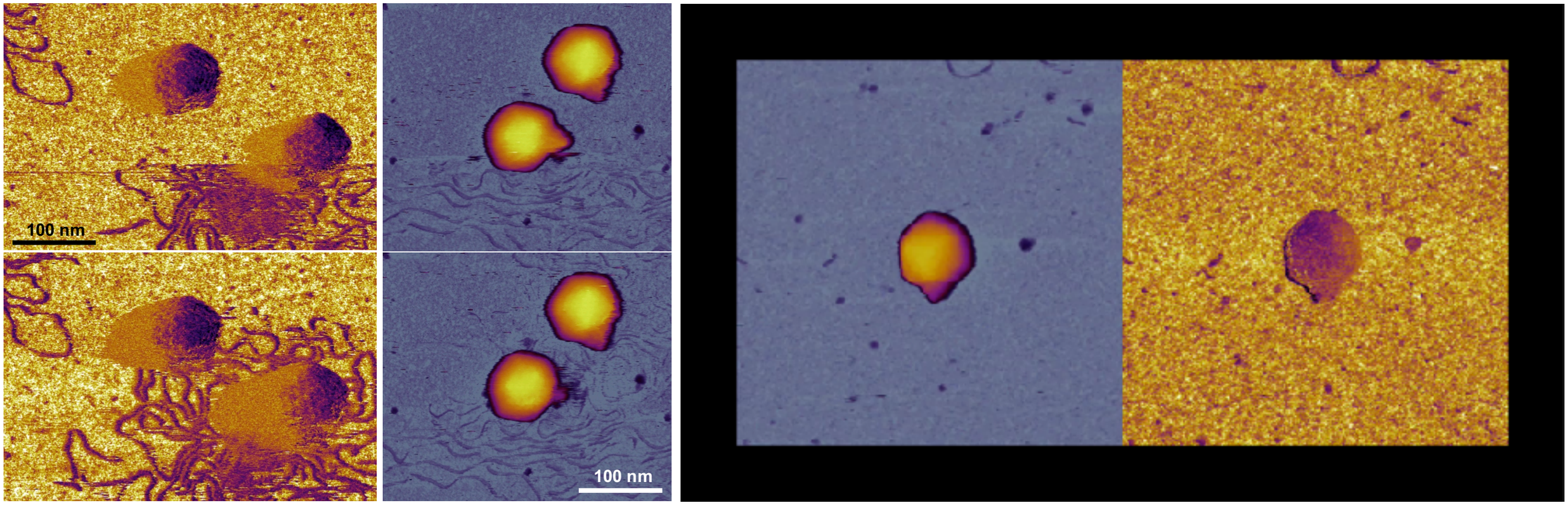


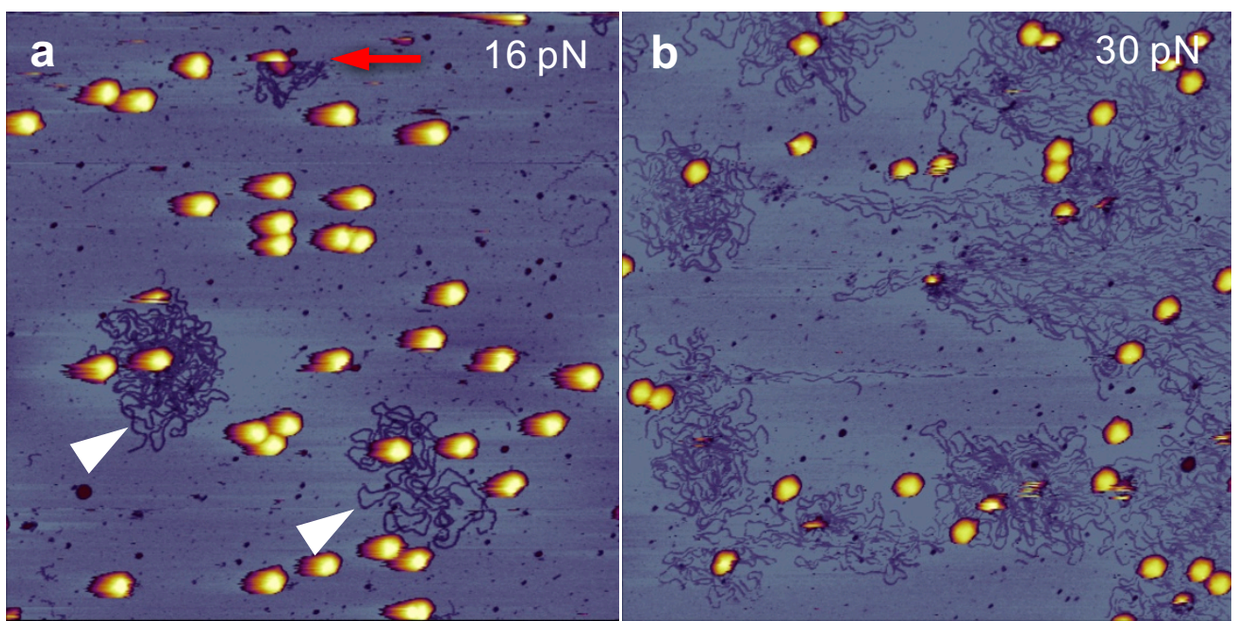
Zhmurov et al. J.Phys.Chem.B
115, 5278, 2011.

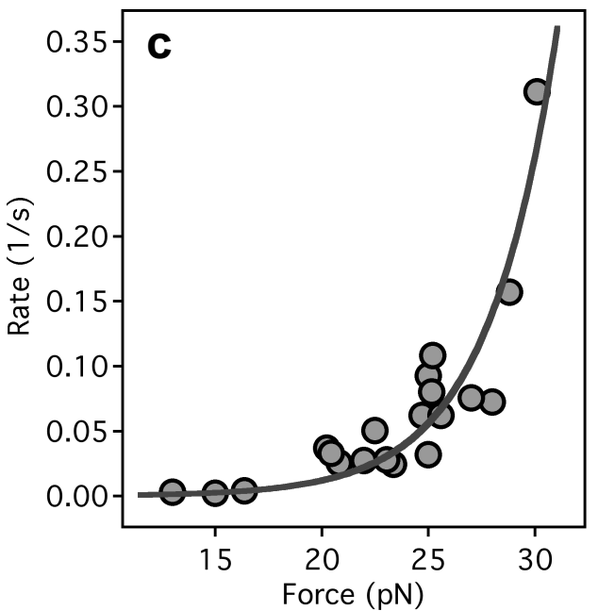




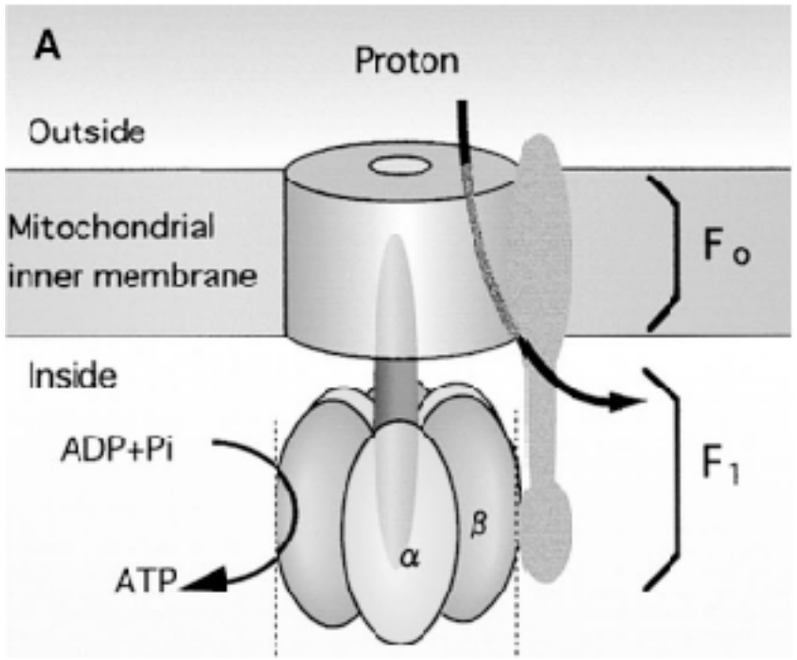
Force-induced DNA ejection from T7 phage

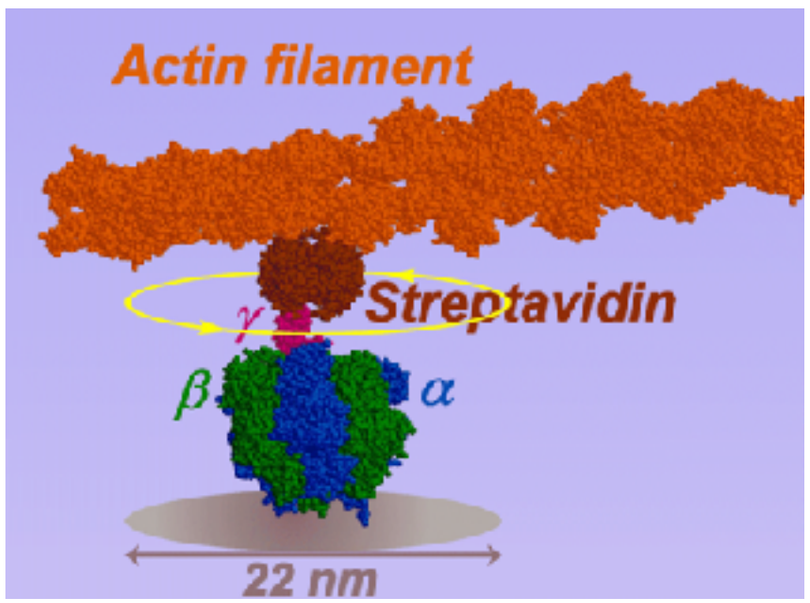


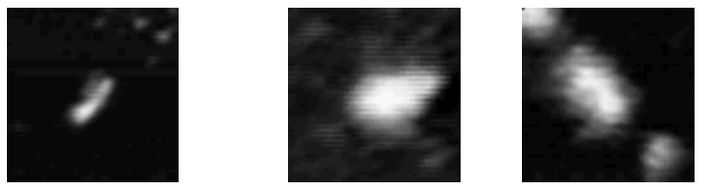




Rotary motors I:
F1F0-ATP Synthase

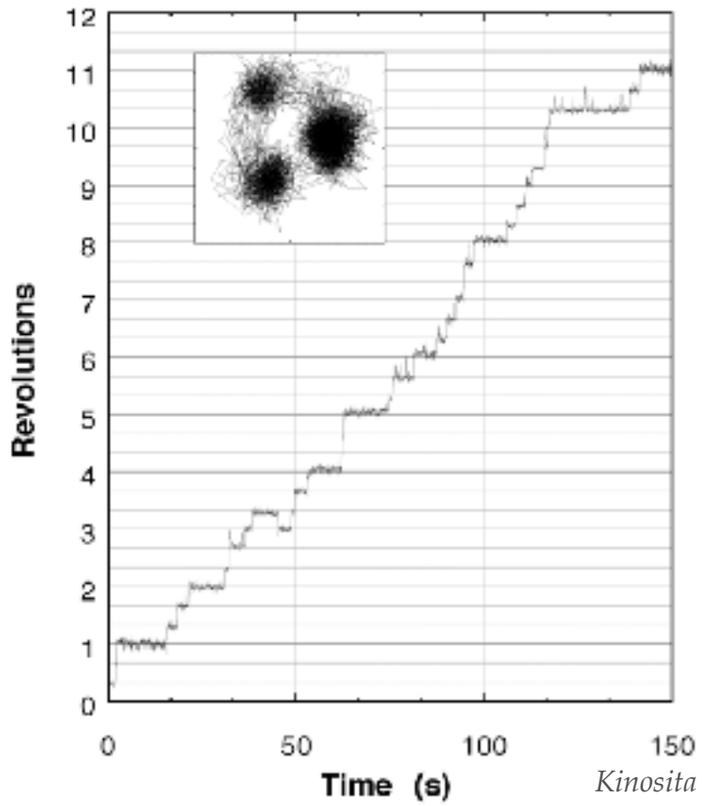






20 nM ATP 200 nM ATP

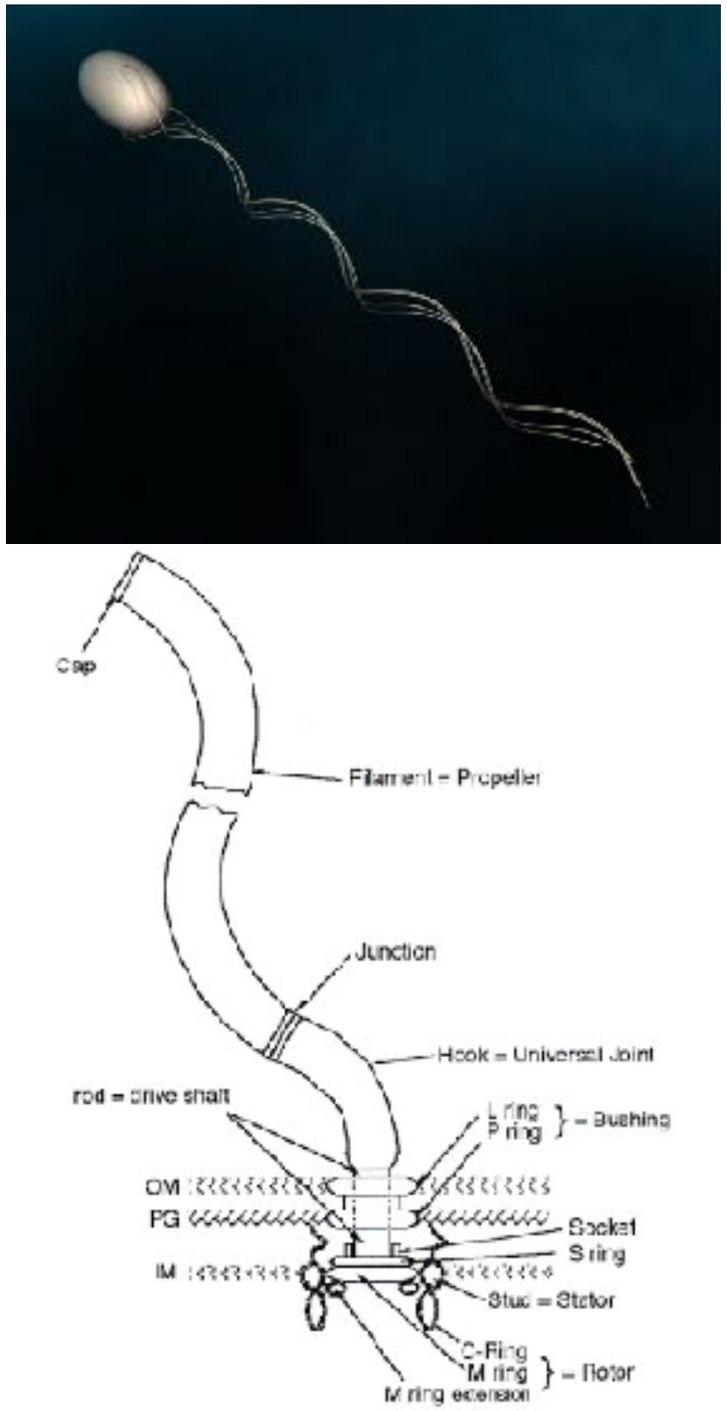
Discrete 120° rotational steps

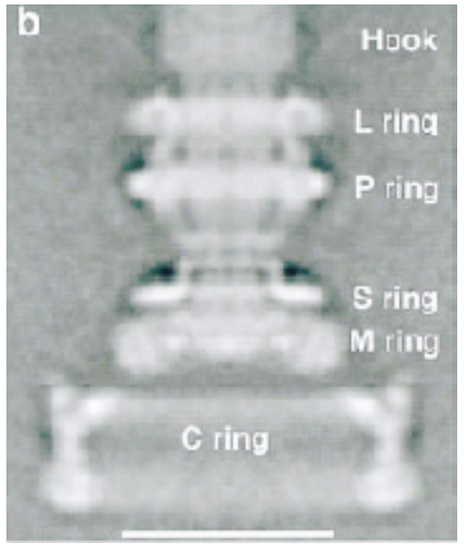


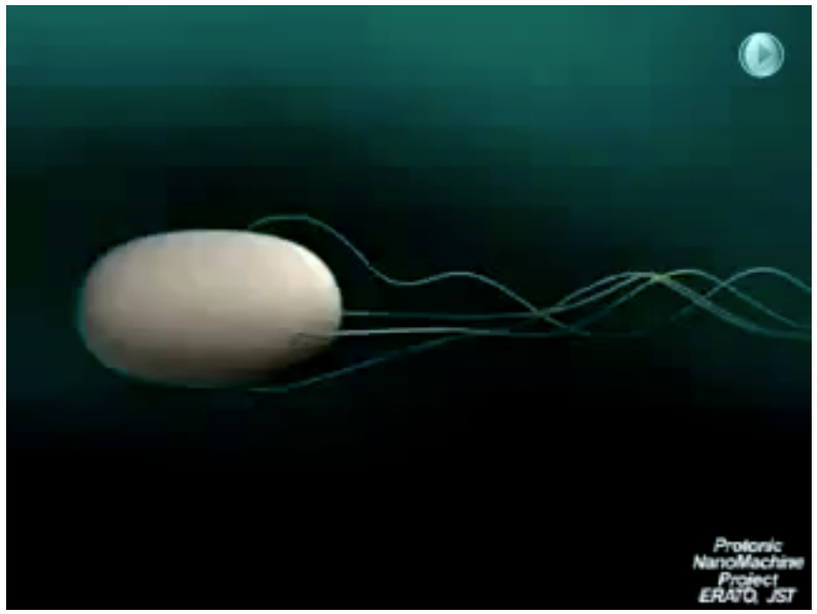
Kinosita

Rotary motors II:

Bacterial flagellar motor

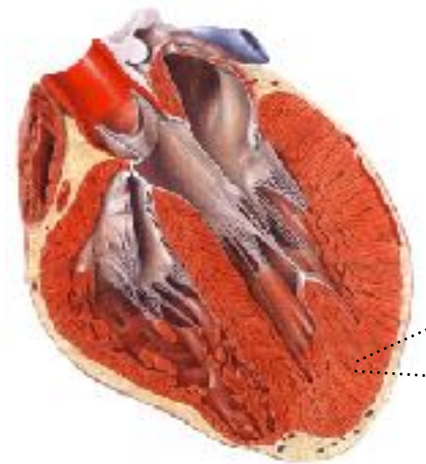




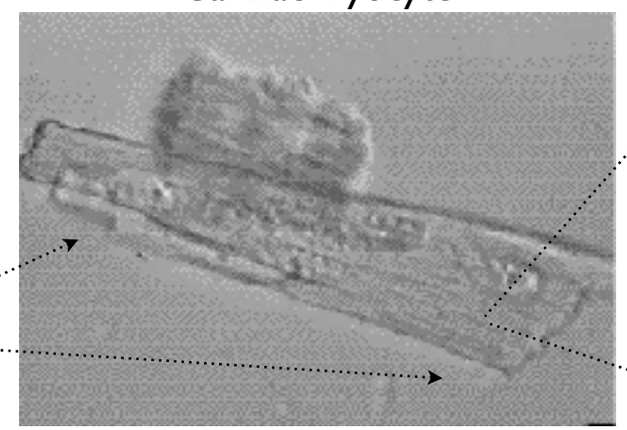


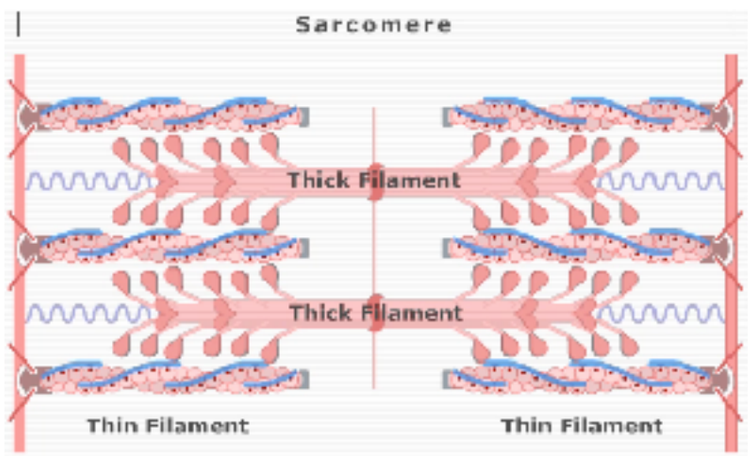
Rotational frequency: > 20000 rpm
Power consumption: 10^{-16} W
Efficiency: > 80%
Energy source: protons

Force-driven protein folding
Nanomanipulation of titin

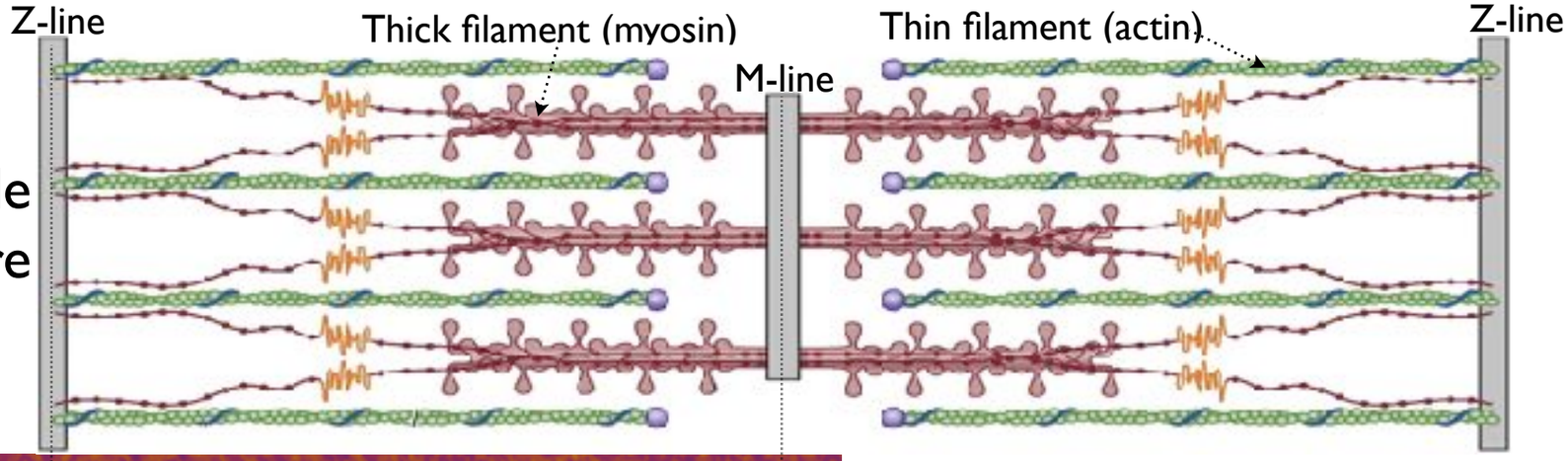


Cardiac myocyte

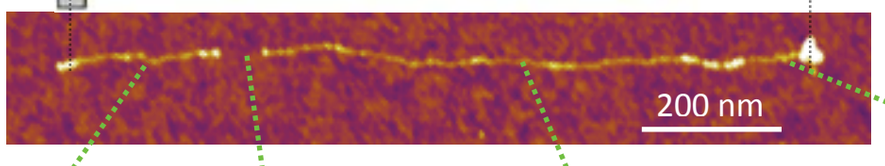




Muscle sacromere



AFM structure of a single straightened titin molecule

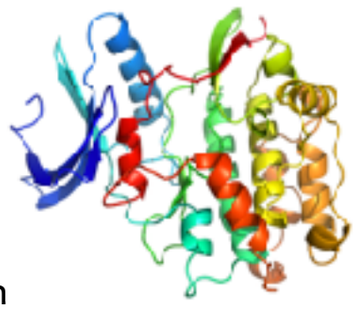


7 anti-parallel β-strands

Immunoglobulin (Ig) domain

PEVK domain

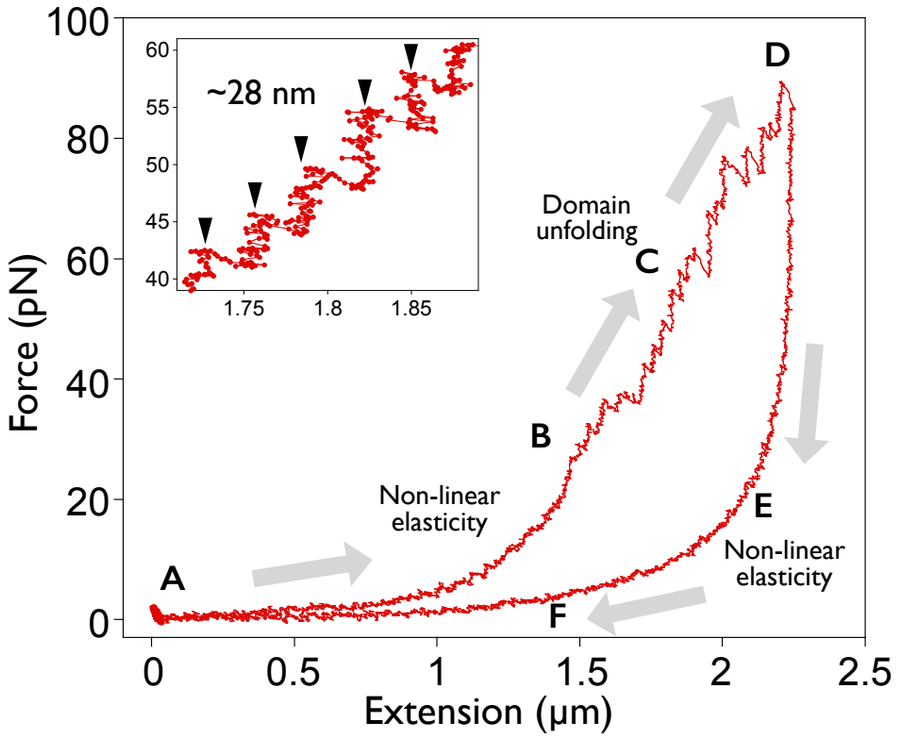
Fibronectin (FN) domain

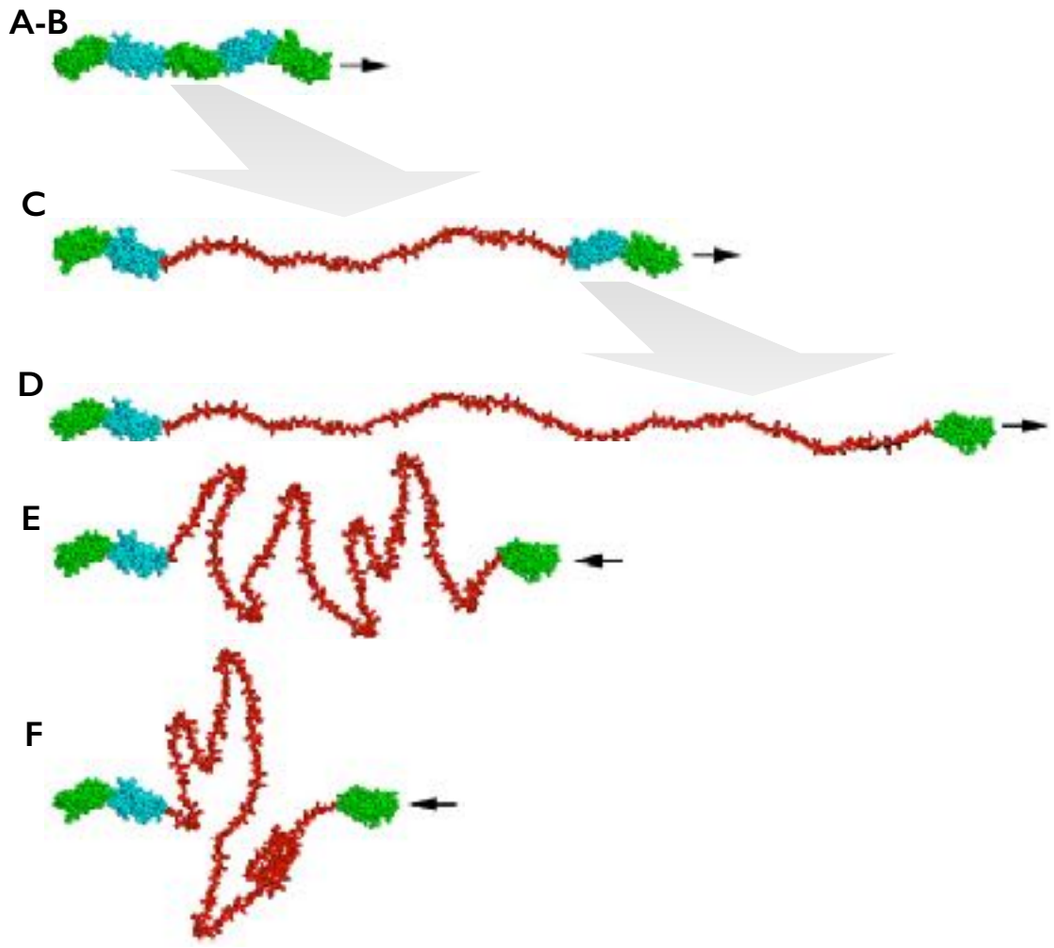


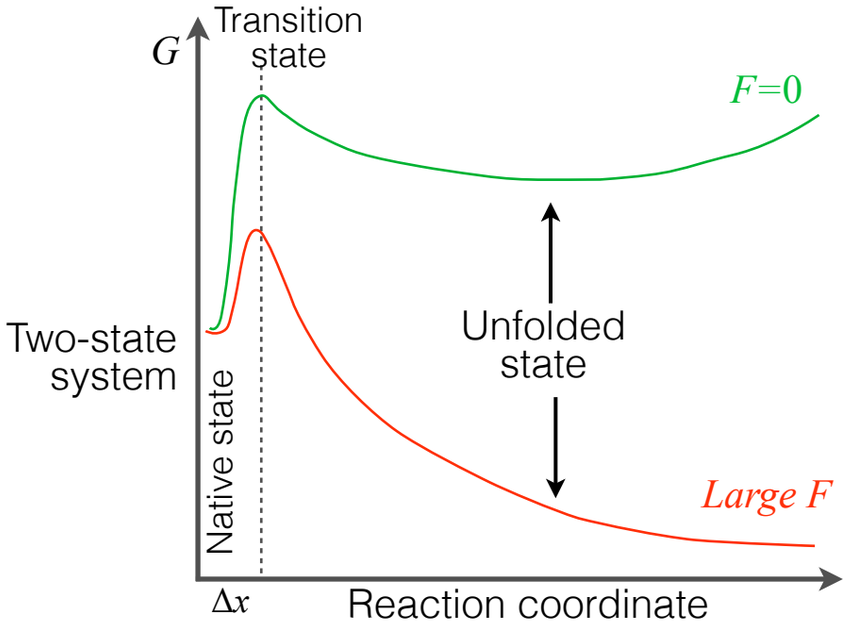
Kinase domain

Titin functions:
1. muscle elasticity
2. structural template
3. force sensing

Upon stretching with force, titin unfolds







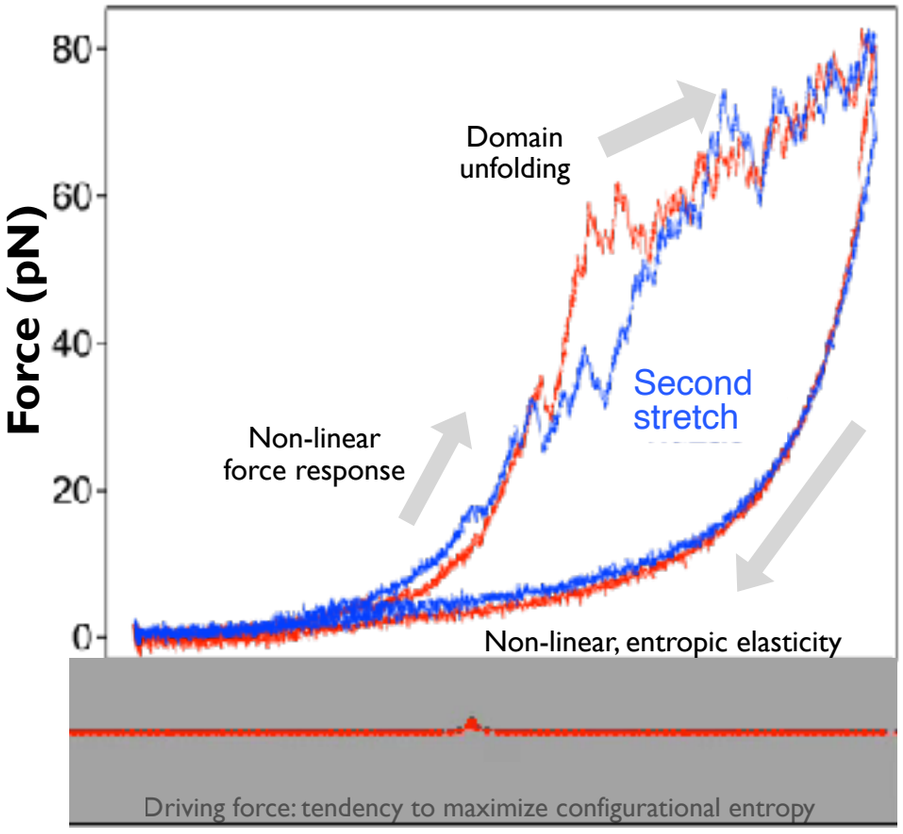
Force-driven domain unfoling rate:

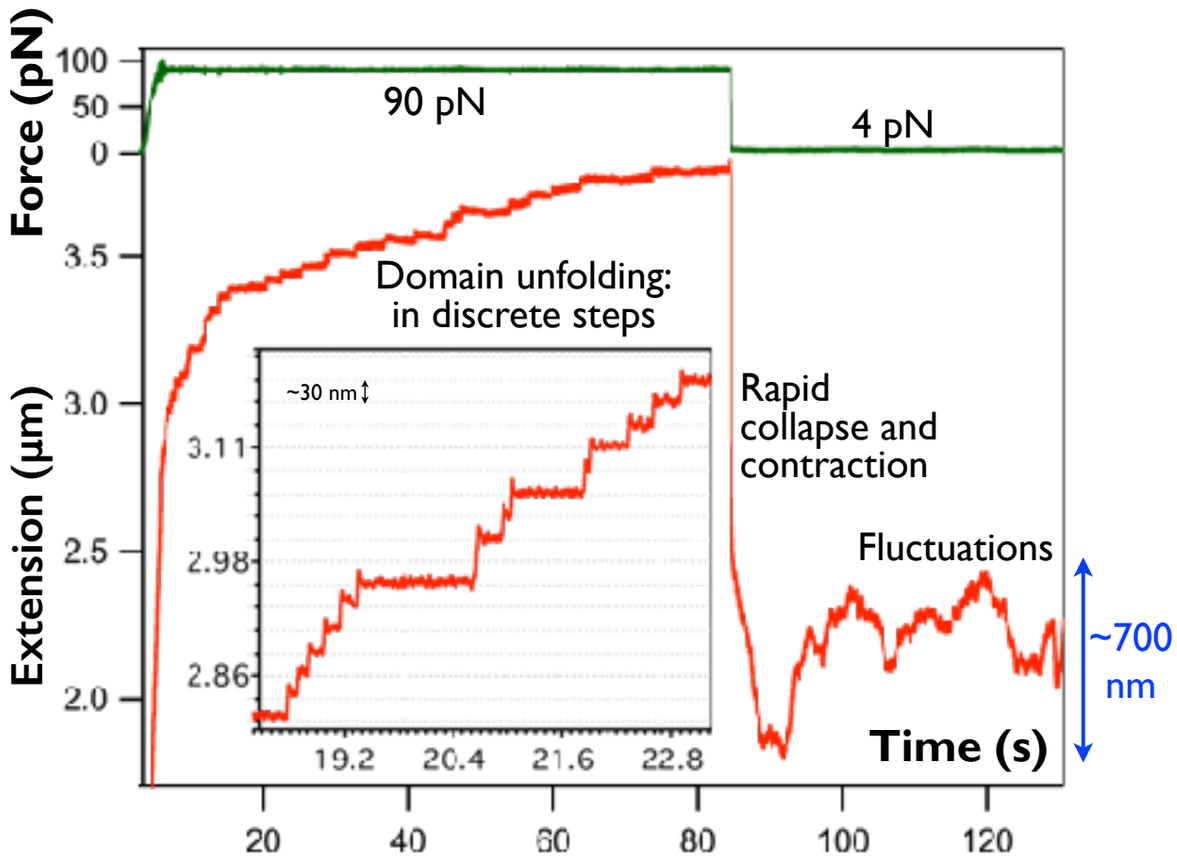
$$k_F = k_0 e^{F\Delta x / k_B T}$$

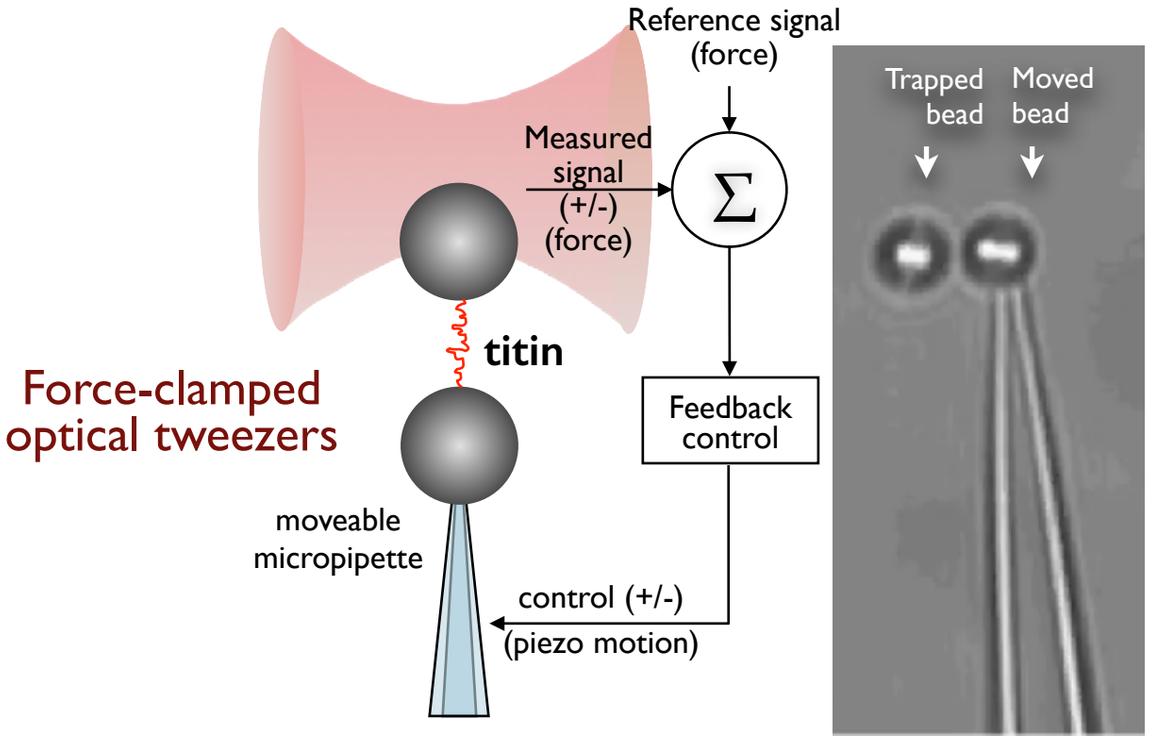
k_0 : spontaneous unfolding rate

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

How does titin contract?

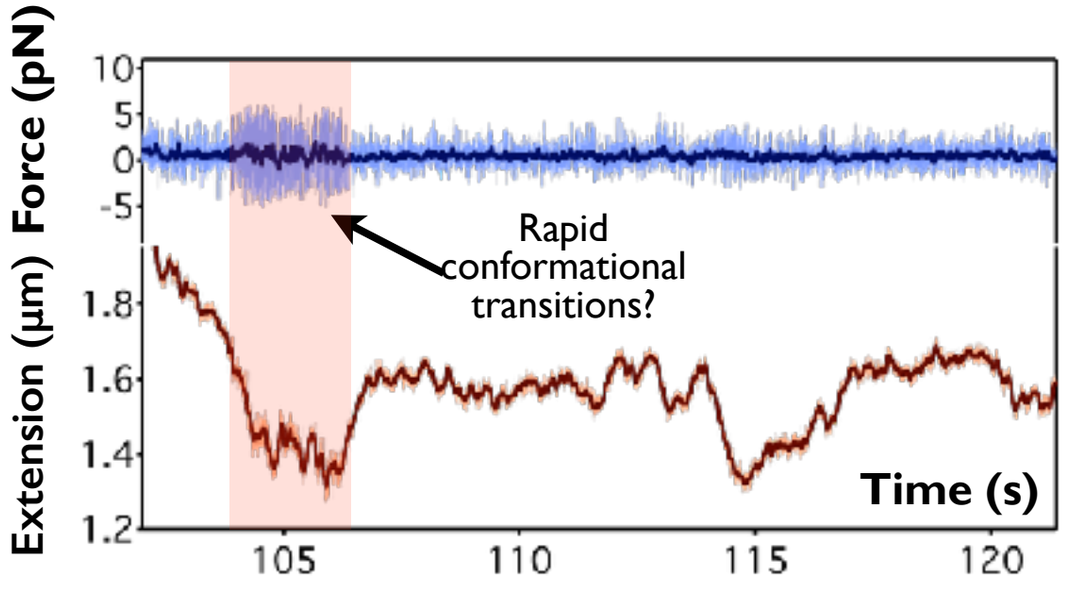






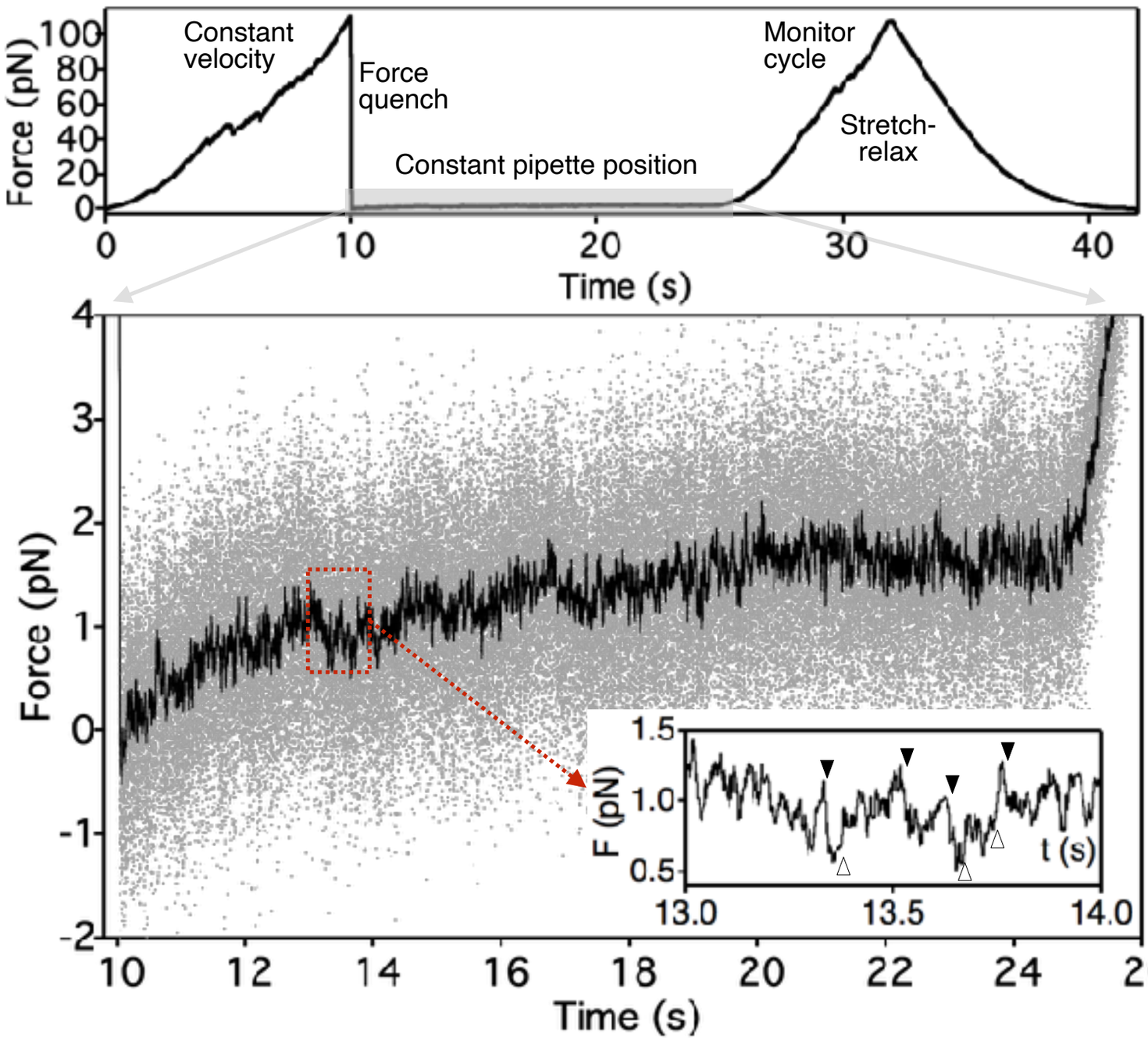
Force-clamped optical tweezers

Fluctuations cannot be explained with a two-state system



Force is generated during refolding

Position clamp experiment

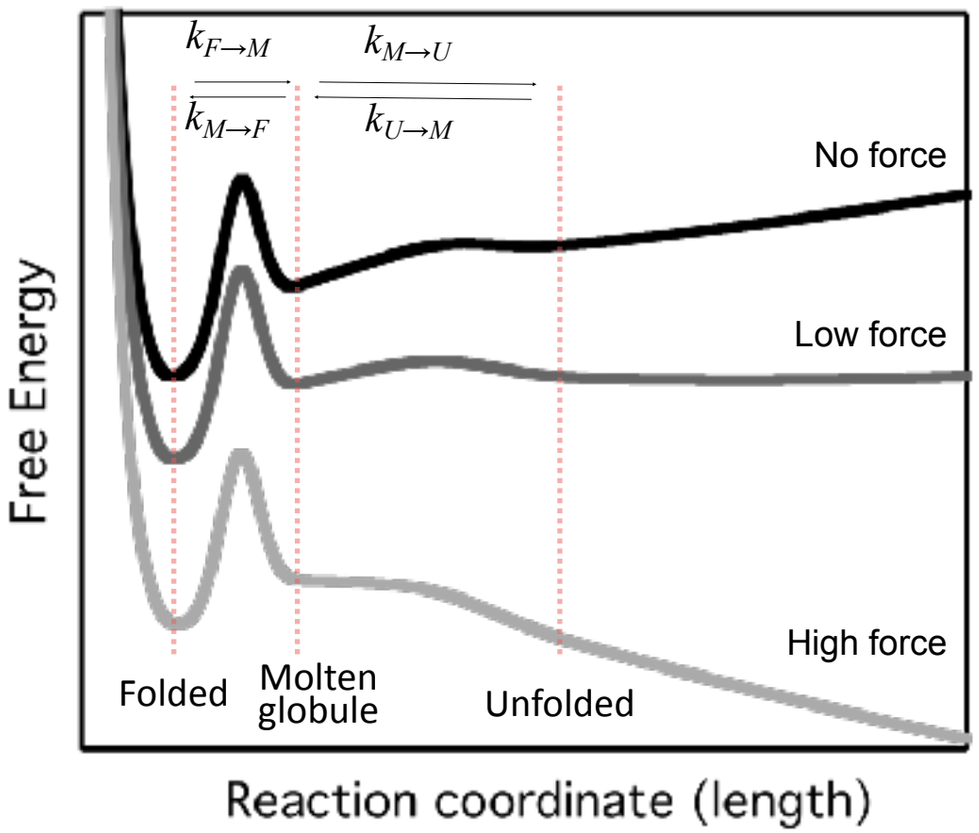


Accompanying contraction (extension change): 10 nm

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

Fluctuations are explained by molten-globule dynamics

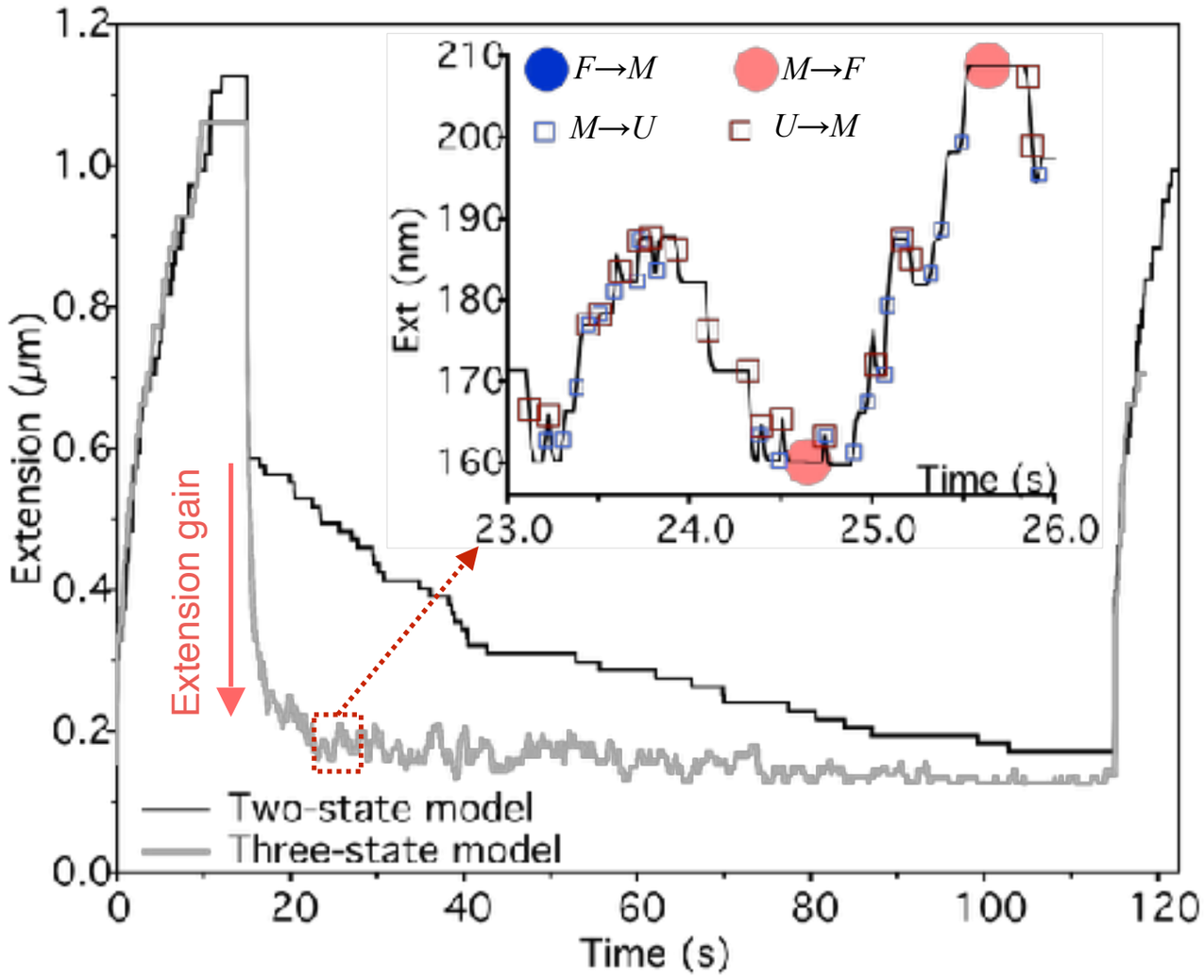
Three-state folding model



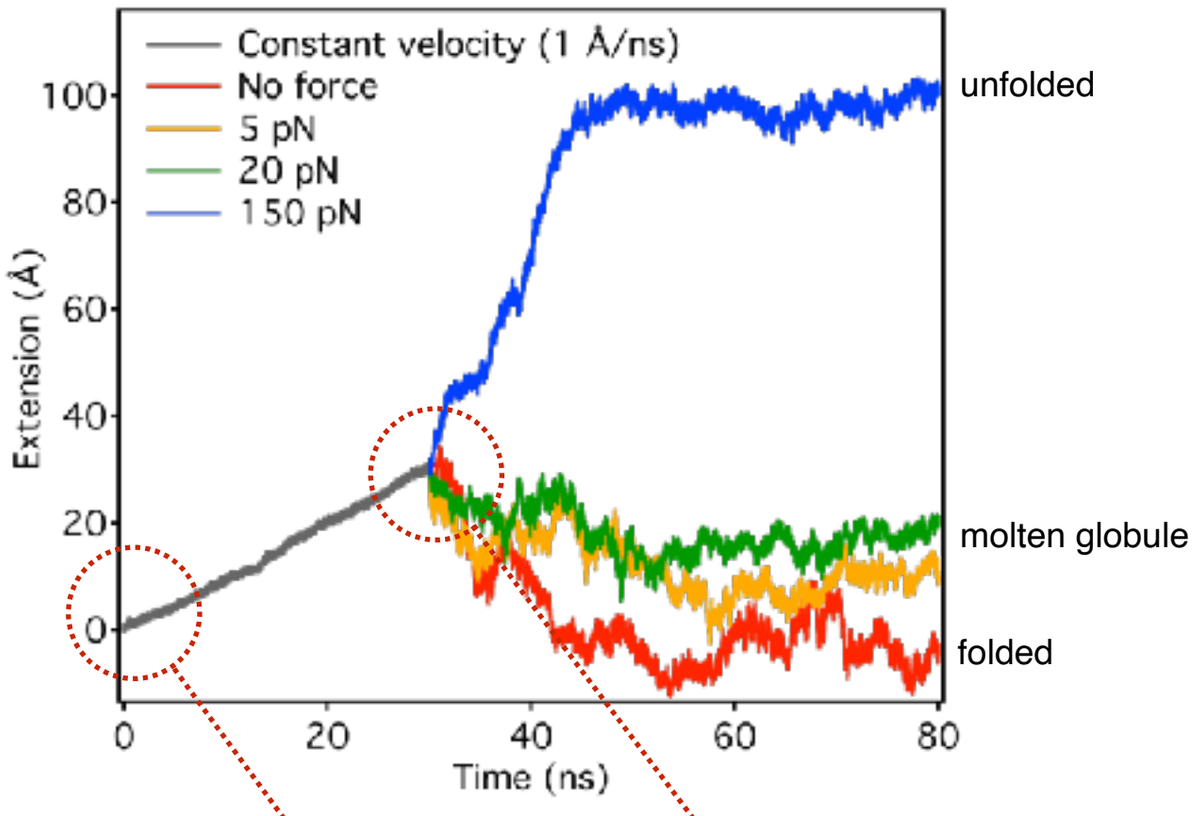
$$k_F = k_0 e^{\pm F\Delta x / k_B T}$$

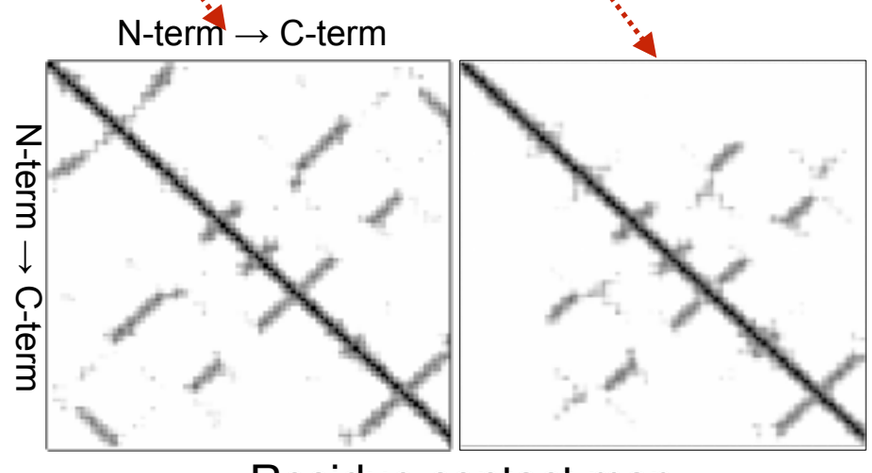
k_0 : spontaneous unfolding/refoldig rate

Monte-Carlo simulation



Molten-globule structure explored with sMDS





Residue contact map