

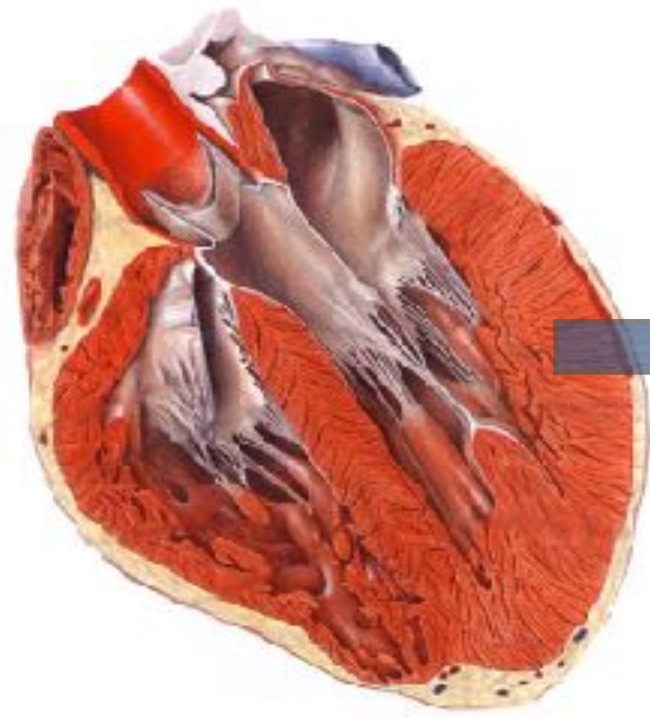
# DYNAMIC INTRACELLULAR PROTEIN SYSTEMS

MIKLÓS KELLERMAYER

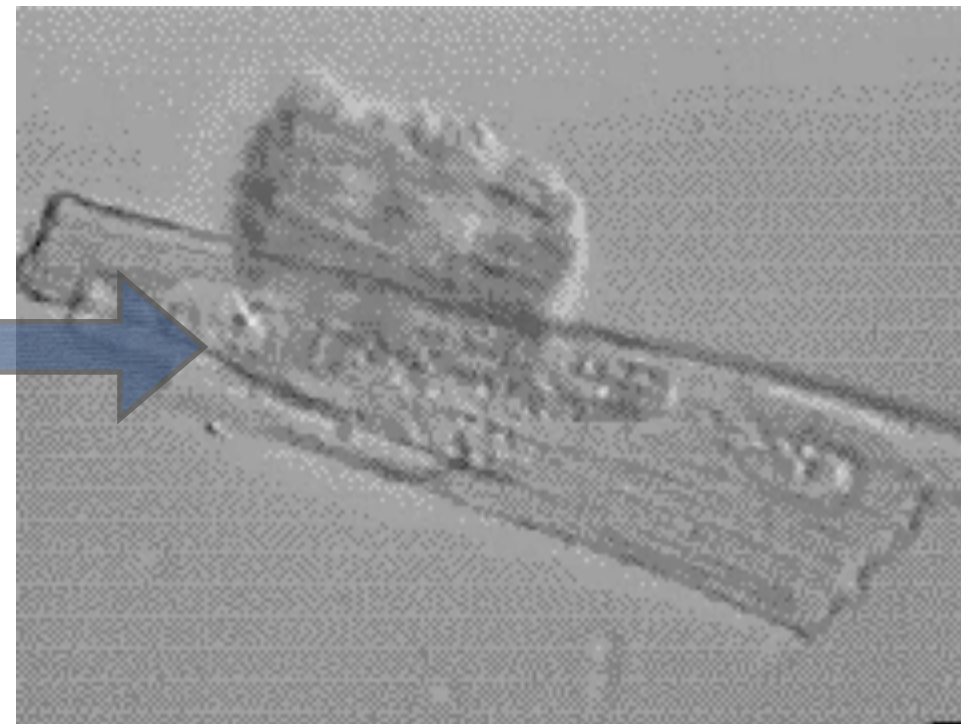


# Types of biological motion

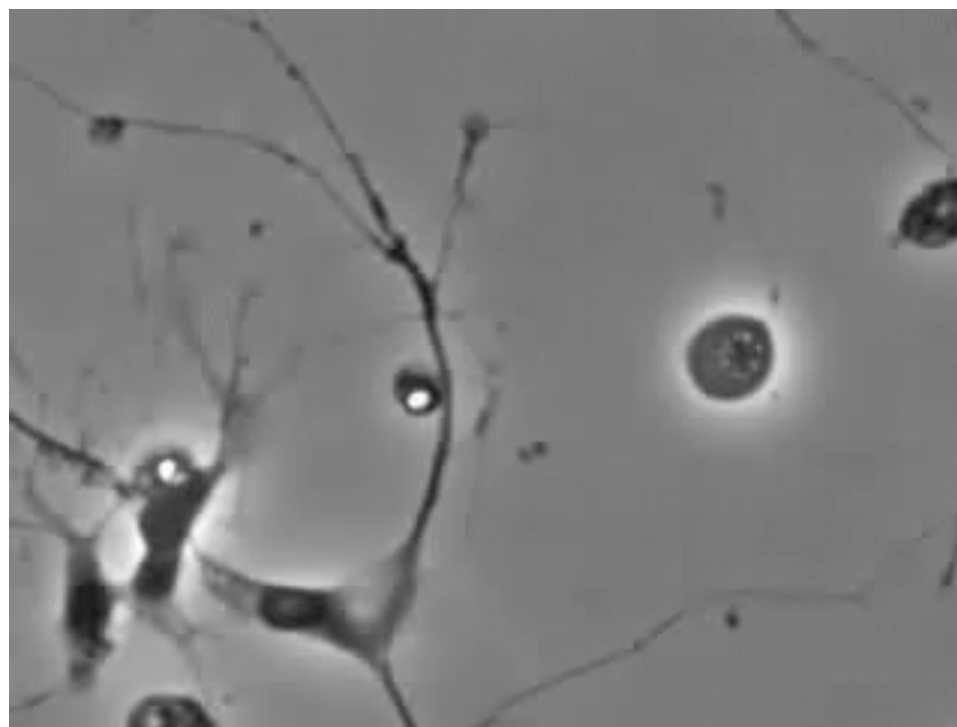
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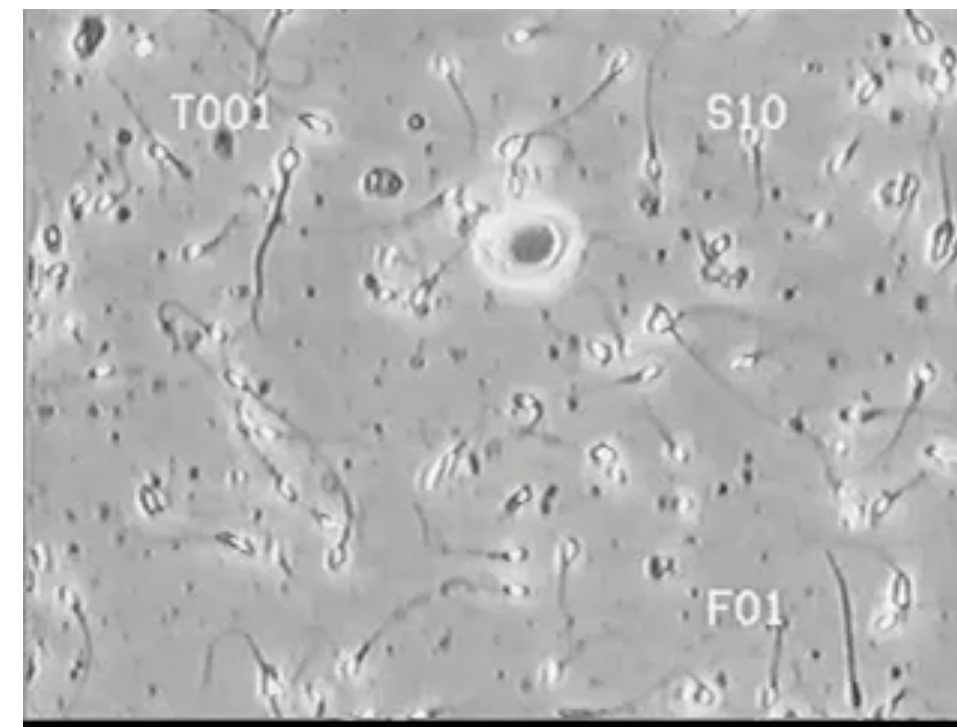
Autonomous cardiomyocyte



Dividing cell



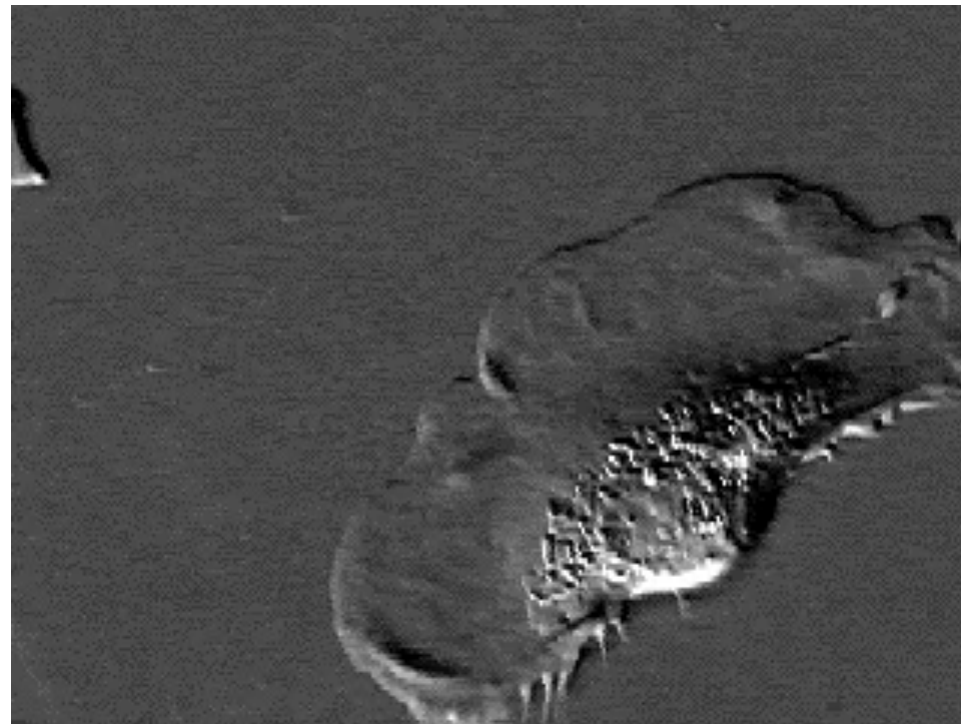
Axonal (neurite) growth



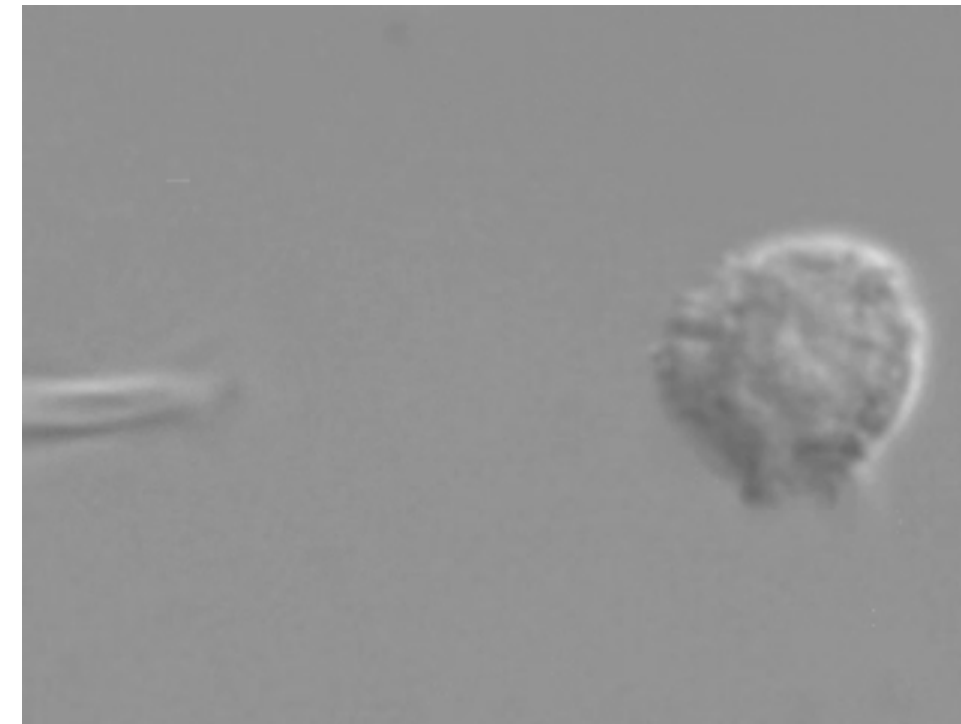
Moving spermatozoa

# Types of biological motion

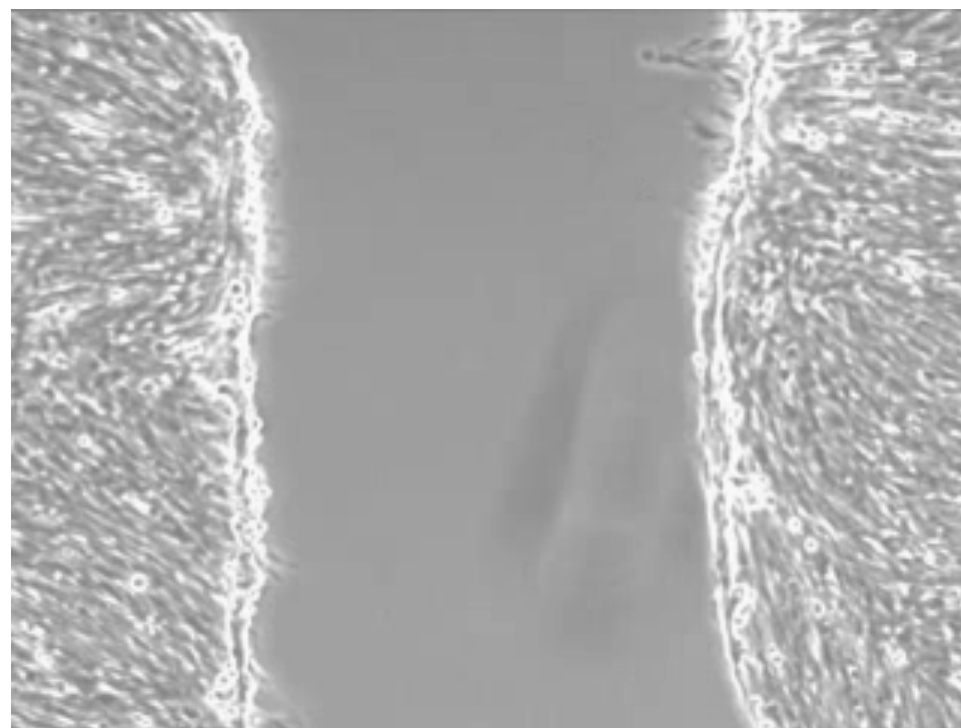
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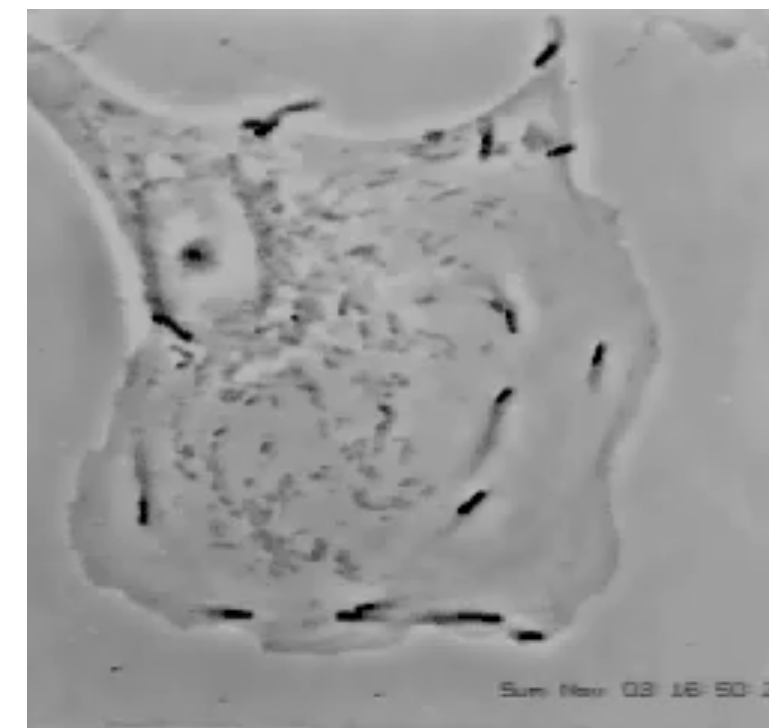
Crawling keratinocyte



Chemotaxis



Wound healing model - collective  
fibroblast movement

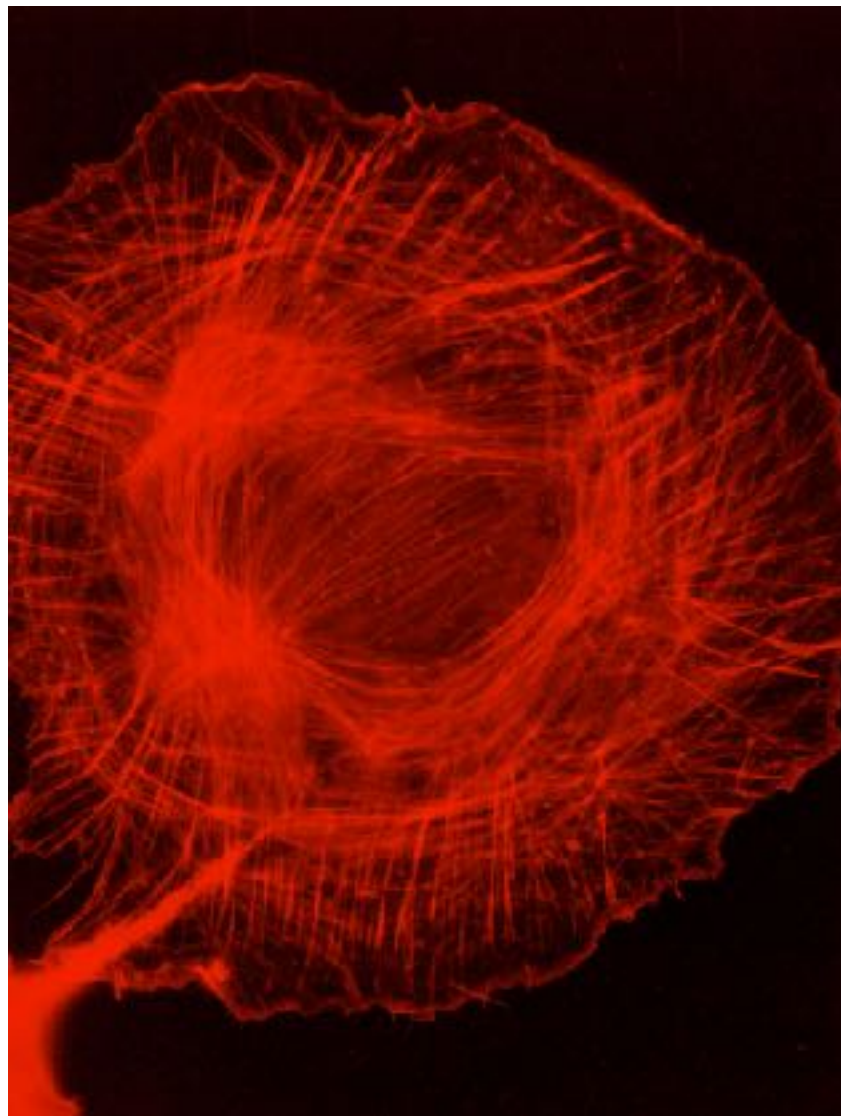


Intracellular movement of  
pathogenic Listeria bacteria

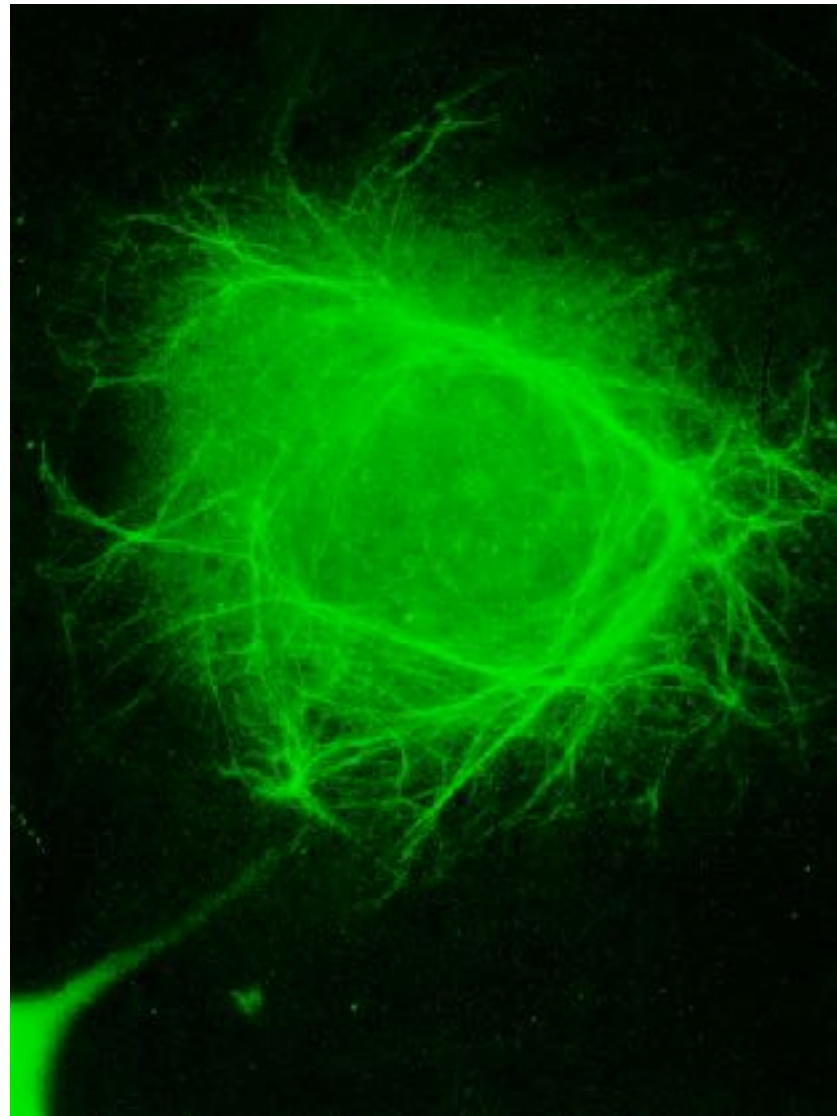


# The cytoskeletal system

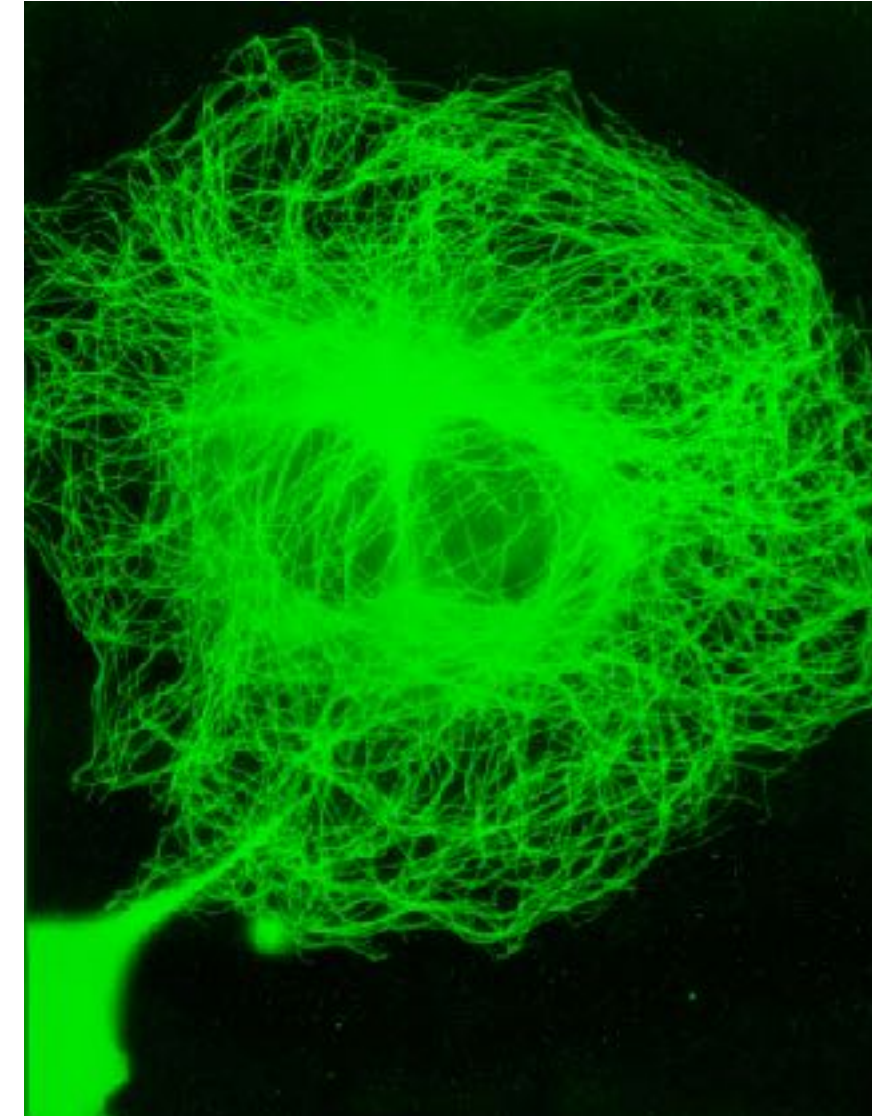
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Actin  
(rodamine-phalloidin)



Vimentin  
(anti-vimentin)



Microtubules  
(GFP-tubulin)

1. Polymerization (from “smart brick” building blocks)
2. Mechanics (see following lecture)

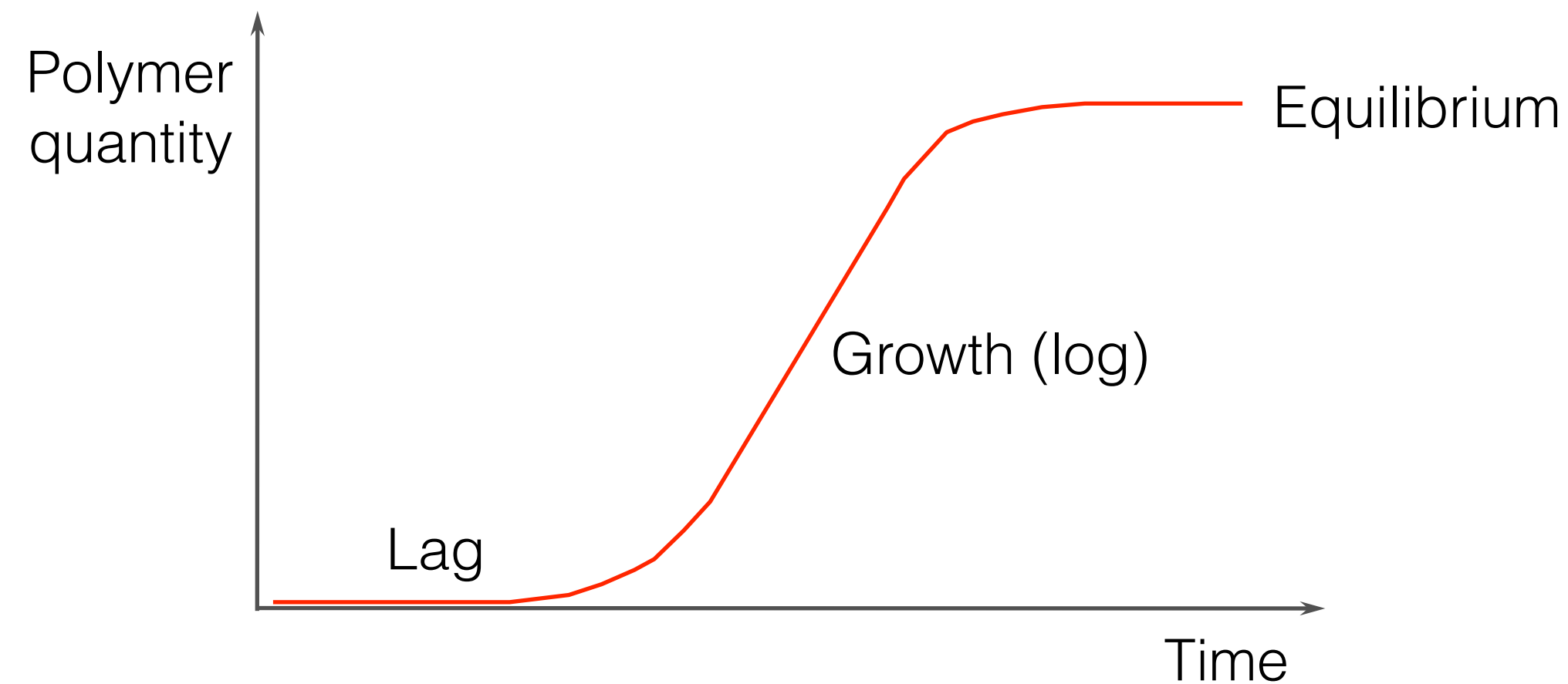
# Polymerization

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## Process of the assembly of monomers

Phases of polymerization:

1. Lag phase: nucleation
2. Growth phase
3. Equilibrium phase



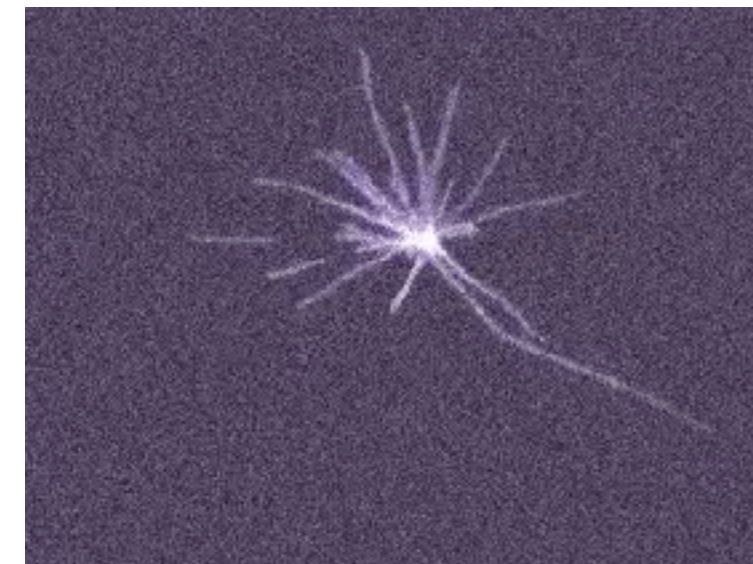
# Polymerization equilibria

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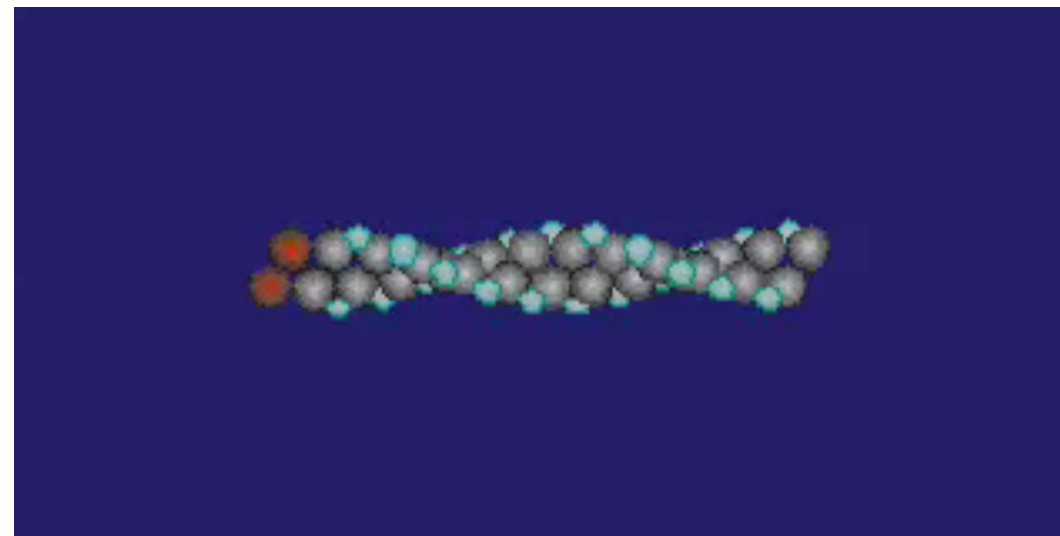
1. True equilibrium



2. Dynamic instability: slow growth followed by “catastrophic” depolymerization



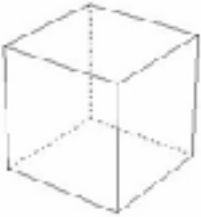
3. Treadmilling





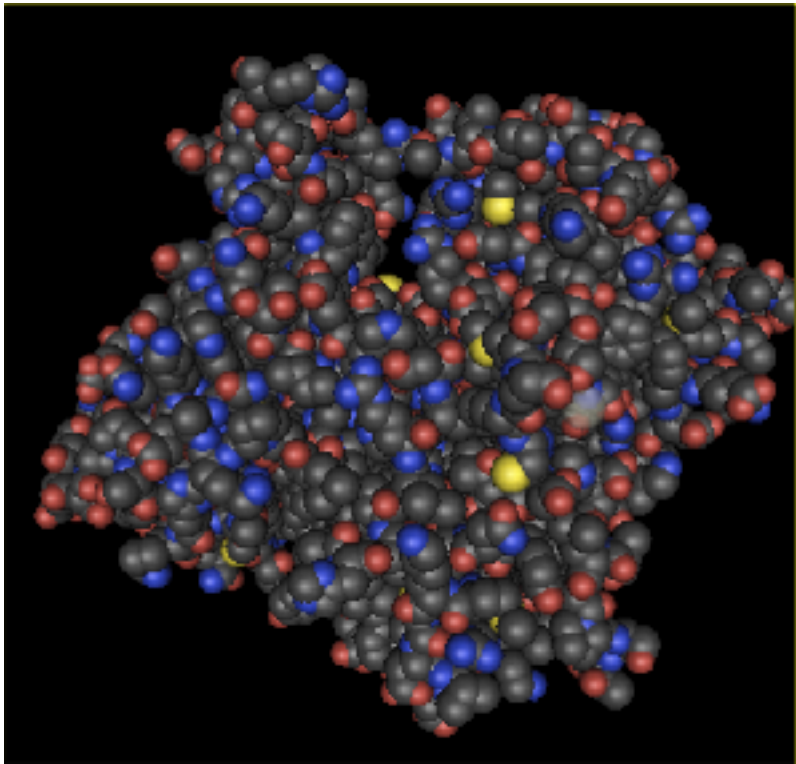
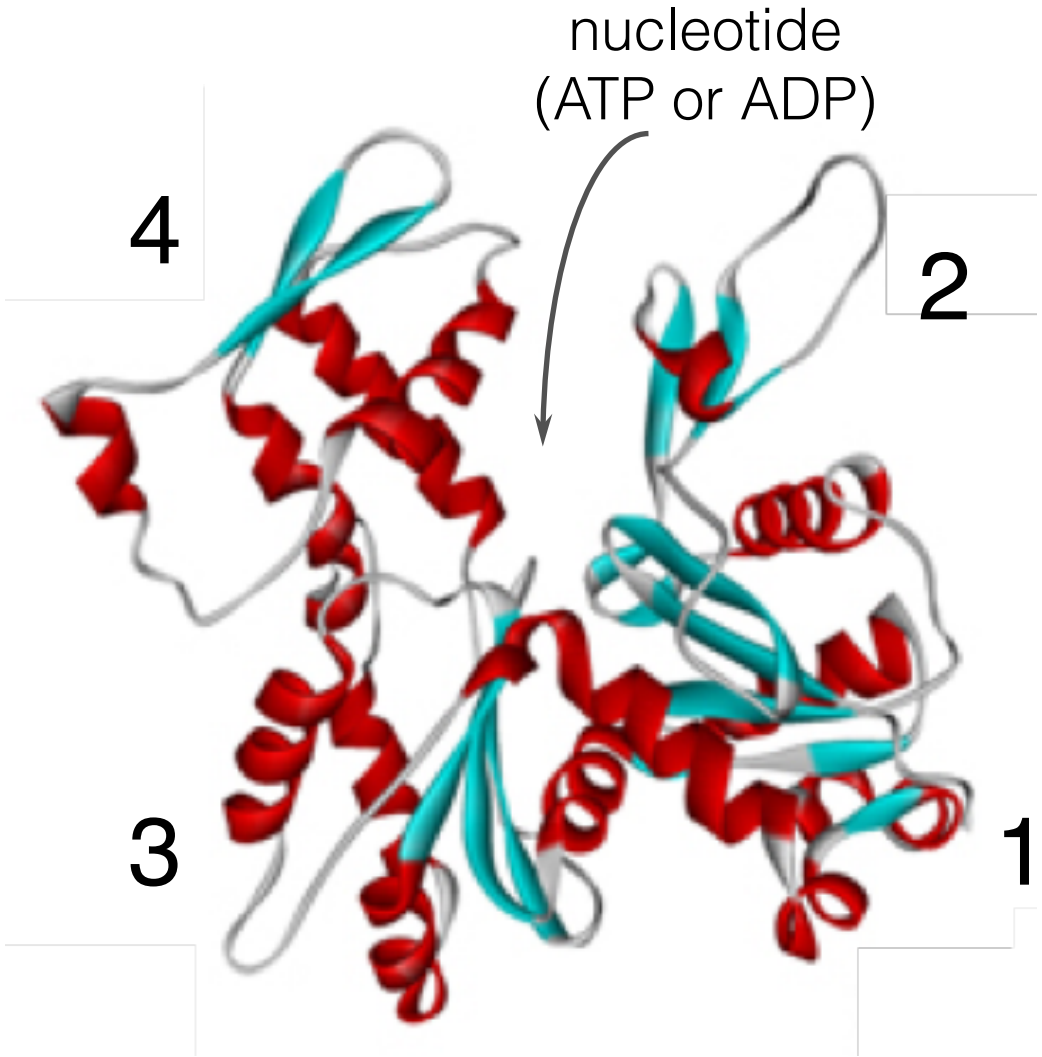
# Actin monomer (G-actin)

- Protein of largest quantity in the eukaryotic cell (5% of total protein)
- Concentration in the cell: 2-8 mg/ml (50-200  $\mu$ M).
- This is equivalent to ~25 nm mean nearest neighbor distance.

Simplified cube model of the cell 	Cell: cube with 20 $\mu$ m edge	Analogue - Lecture hall: cube with 20 m edge (for appreciation)
Size of actin molecule	5 nm	5 mm
Number of actin molecules	~500 million	~500 million
Average distance between actins	~25 nm	~25 mm

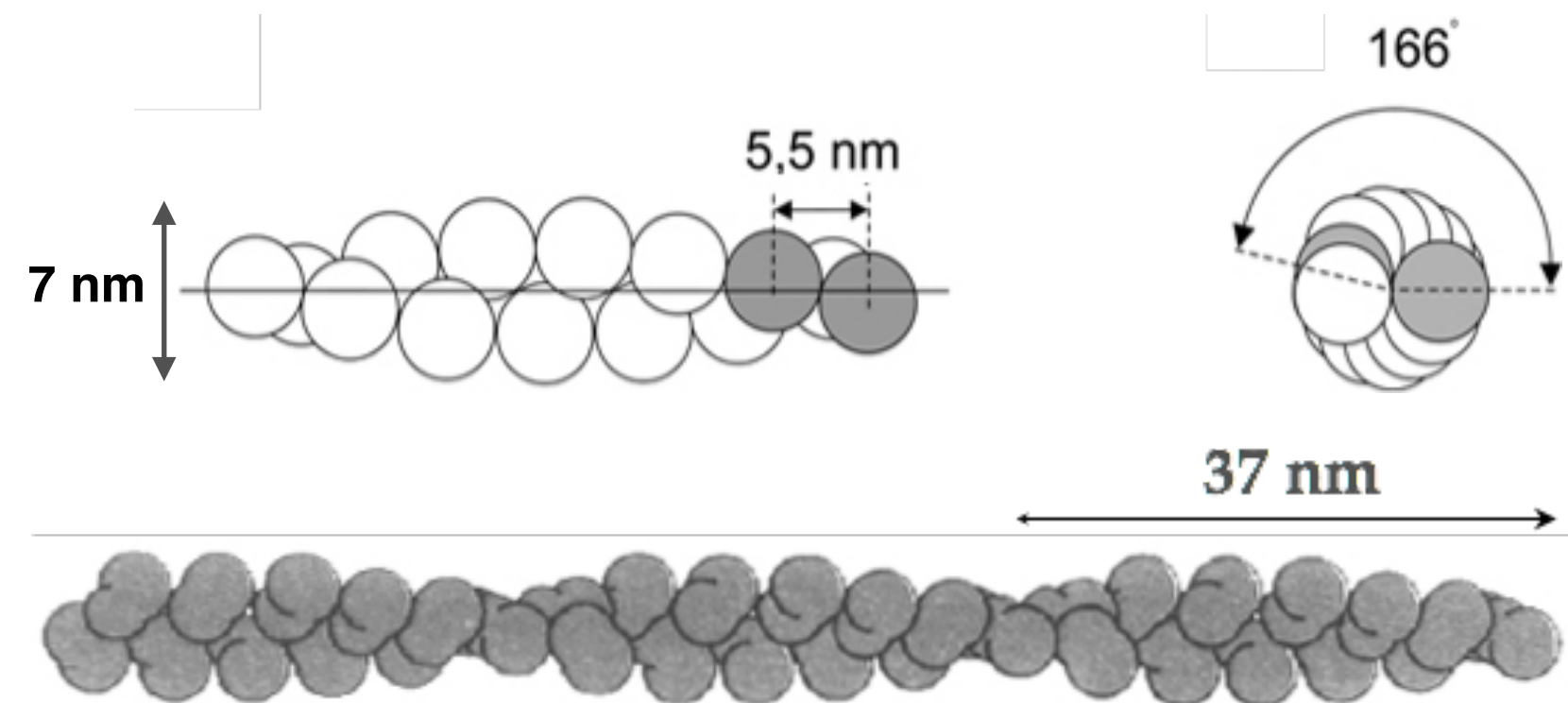
### Subunit:

- Globular (G-) actin
- MW: 43 kDa, 375 amino acid residues,
- 1 molecule bound nucleotide (ATP or ADP)
- Subdomains (4)
- Genetic variability: in mammals, 6 different actins in 3 different families ( $\alpha$  muscle-type,  $\beta$ ,  $\gamma$  non-muscle type)

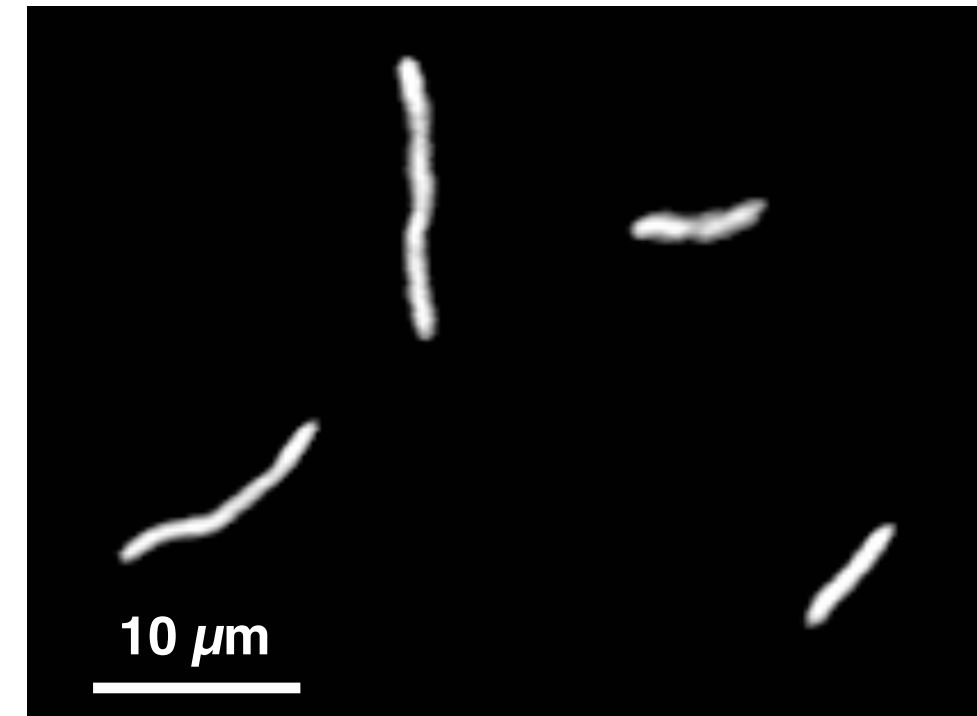
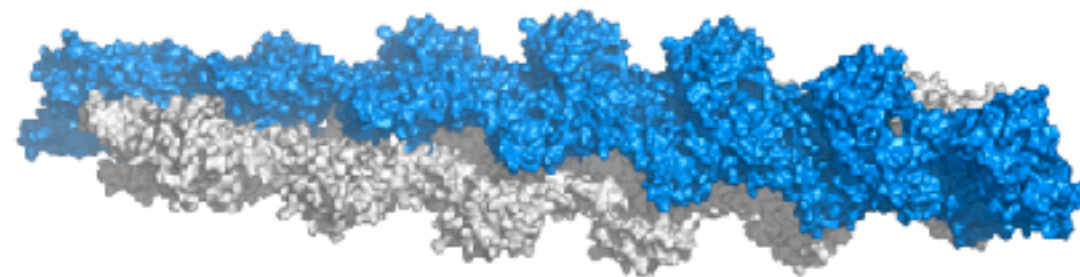


G-actin (d=5 nm,  
CC<sub>cell</sub>~100  $\mu$ M)

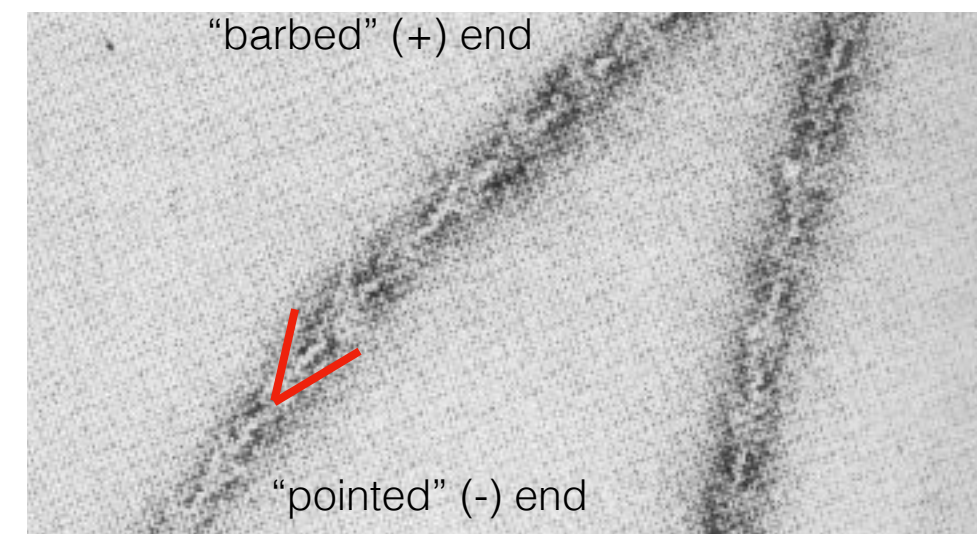
# The actin filament (F-actin)



High-resolution structure of one pitch of F-actin

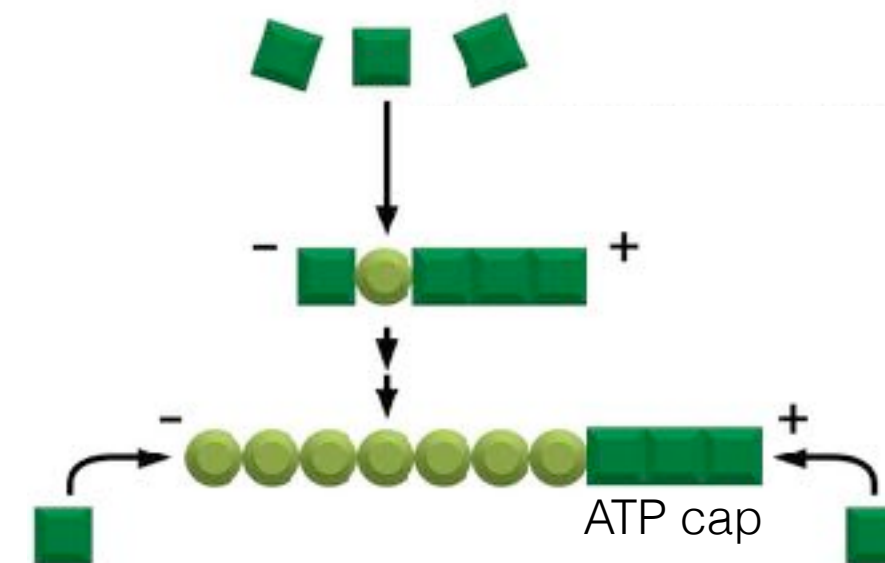


Fluorescence microscopic image of rhodamine-phalloidine-labeled F-actin



Electron microscopic image of myosin S1-labeled F-actin

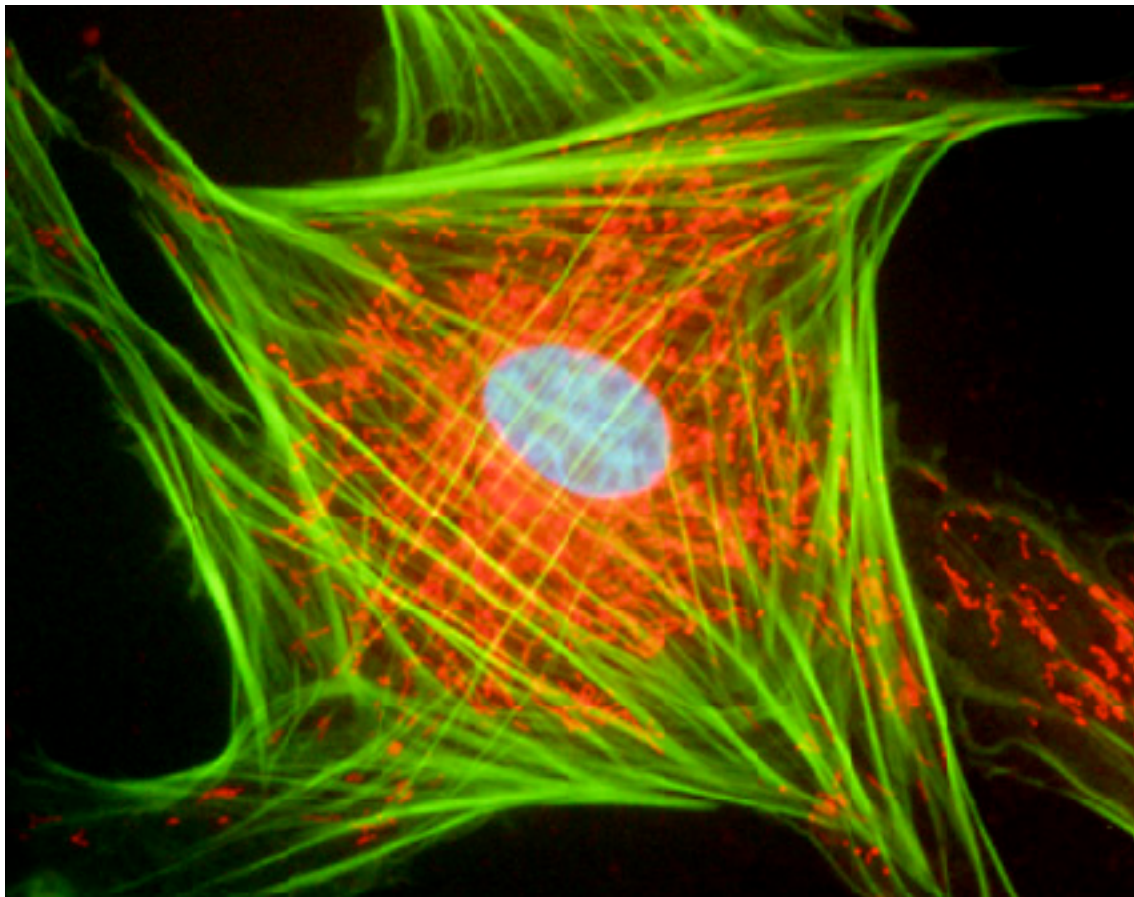
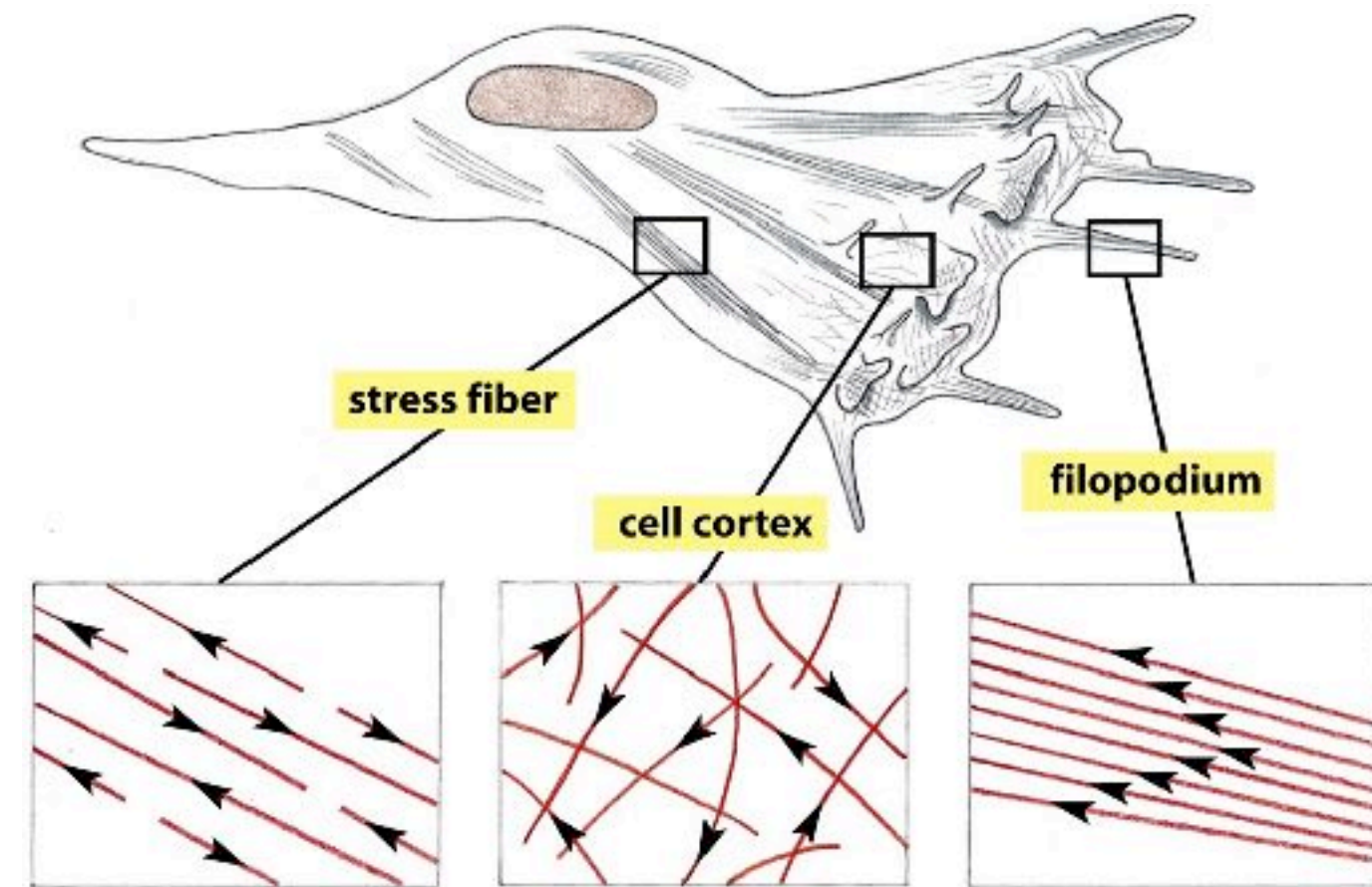
- ~7 nm thick, length in vitro exceeds 10 μm, in vivo 1-2 μm
- Semiflexible chain (persistence length 1-2 μm)
- Right-handed double helix.
- Structural polarity ("barbed", "pointed" ends)
- Asymmetric polymerization: ATP cap



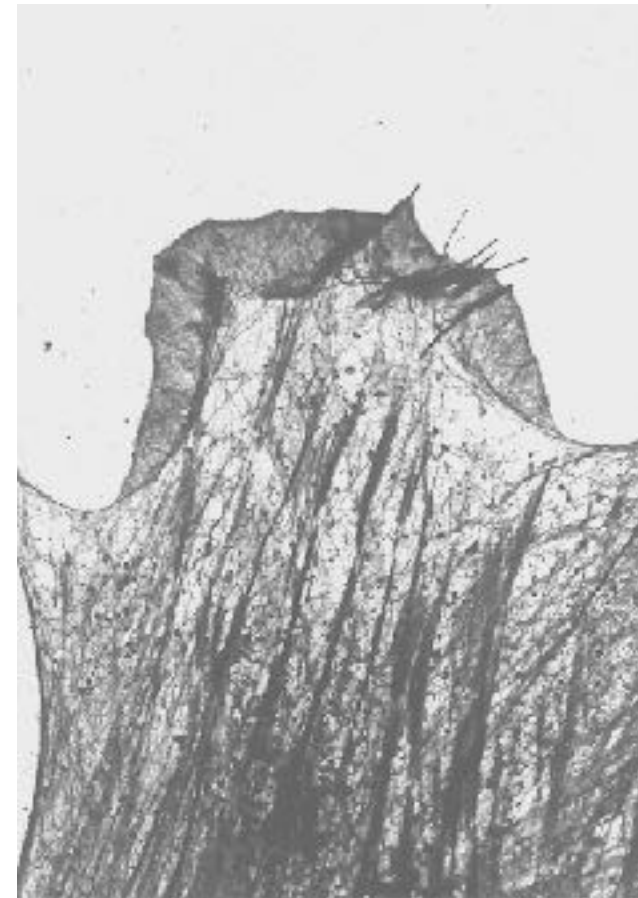


# Actin in the cell

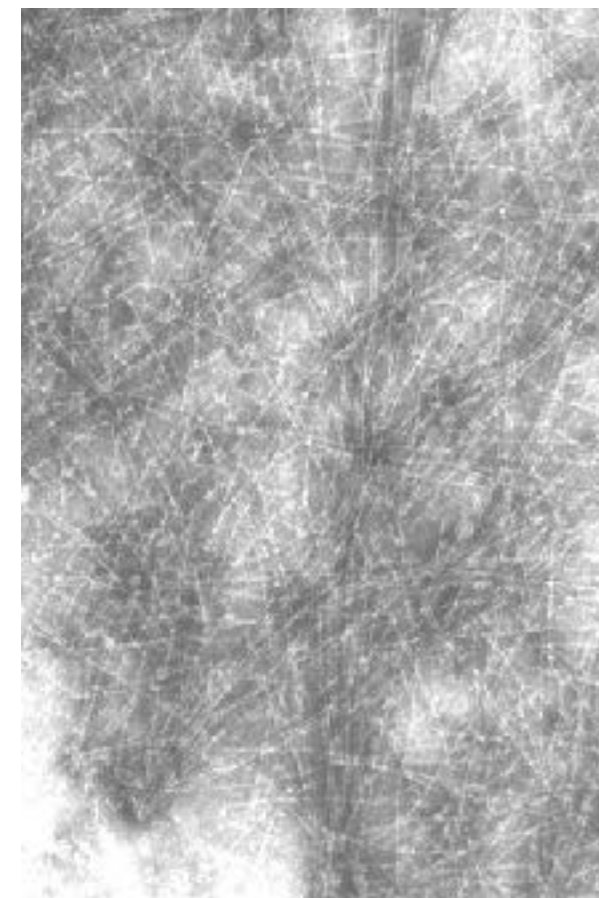
- cortex
- stress fibers,
- cellular processes (lamellipodia, filopodia, microspikes, focal contacts, invagination)
- microvillus



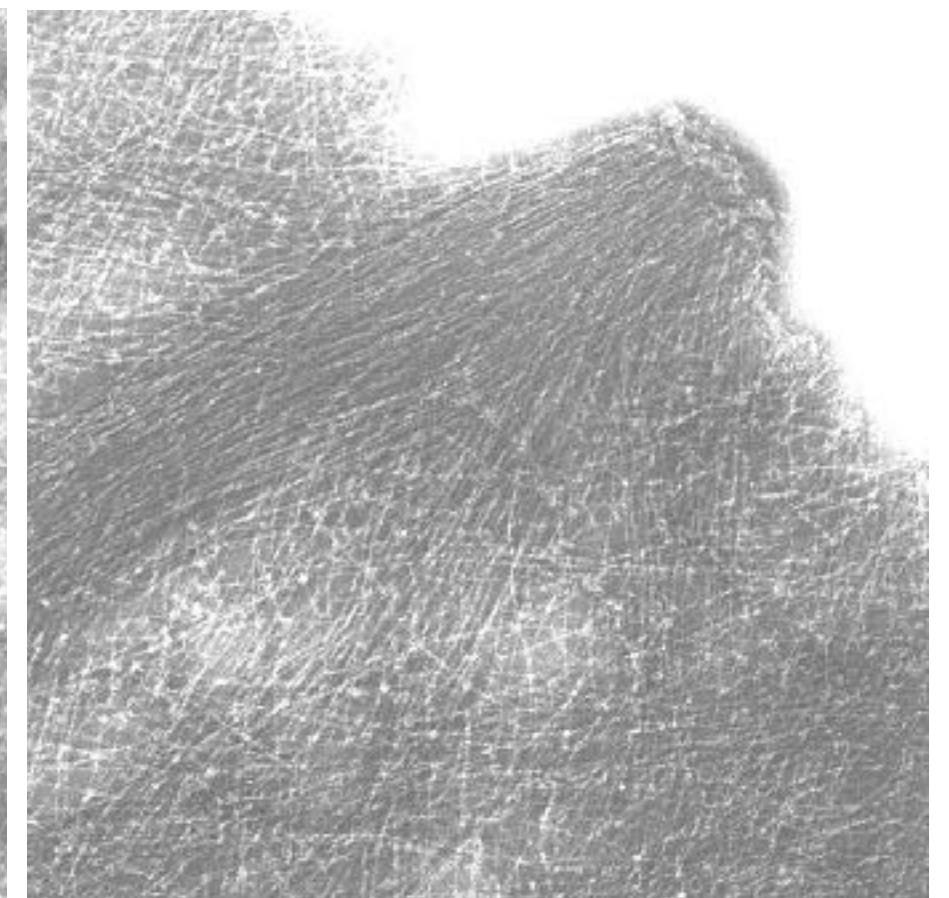
Fluorescence microscopic image (green: F-actin)



Stress fibers



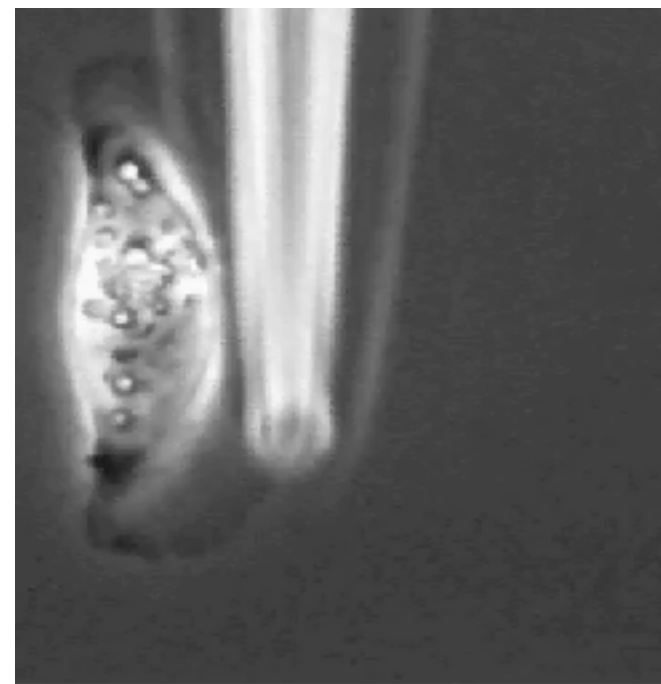
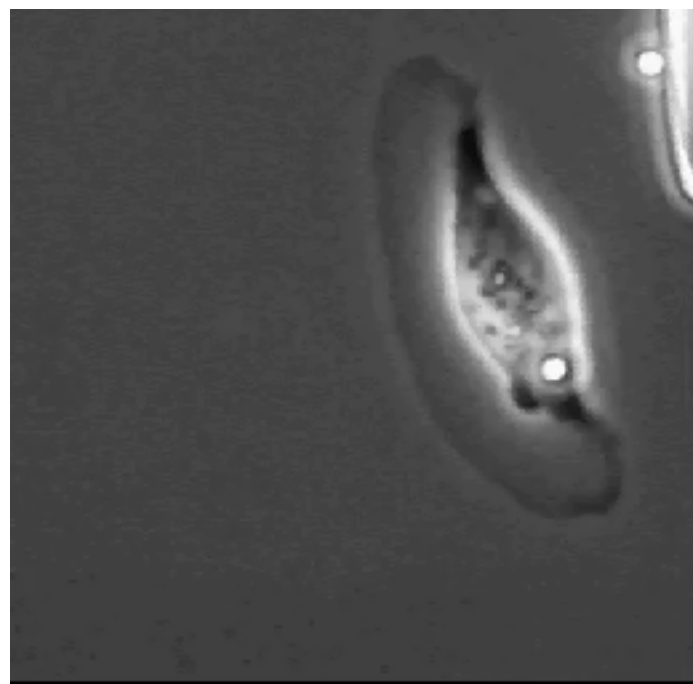
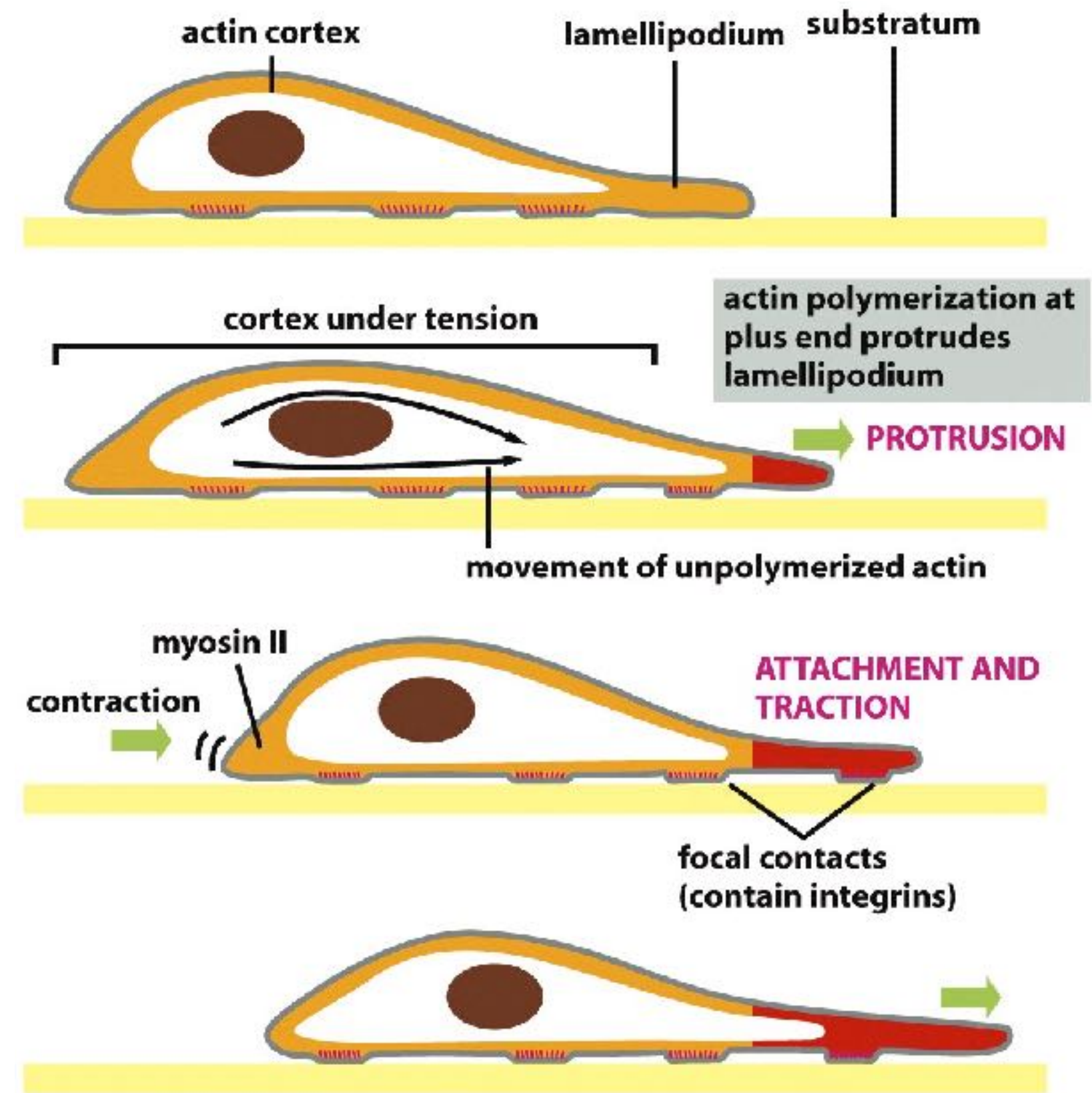
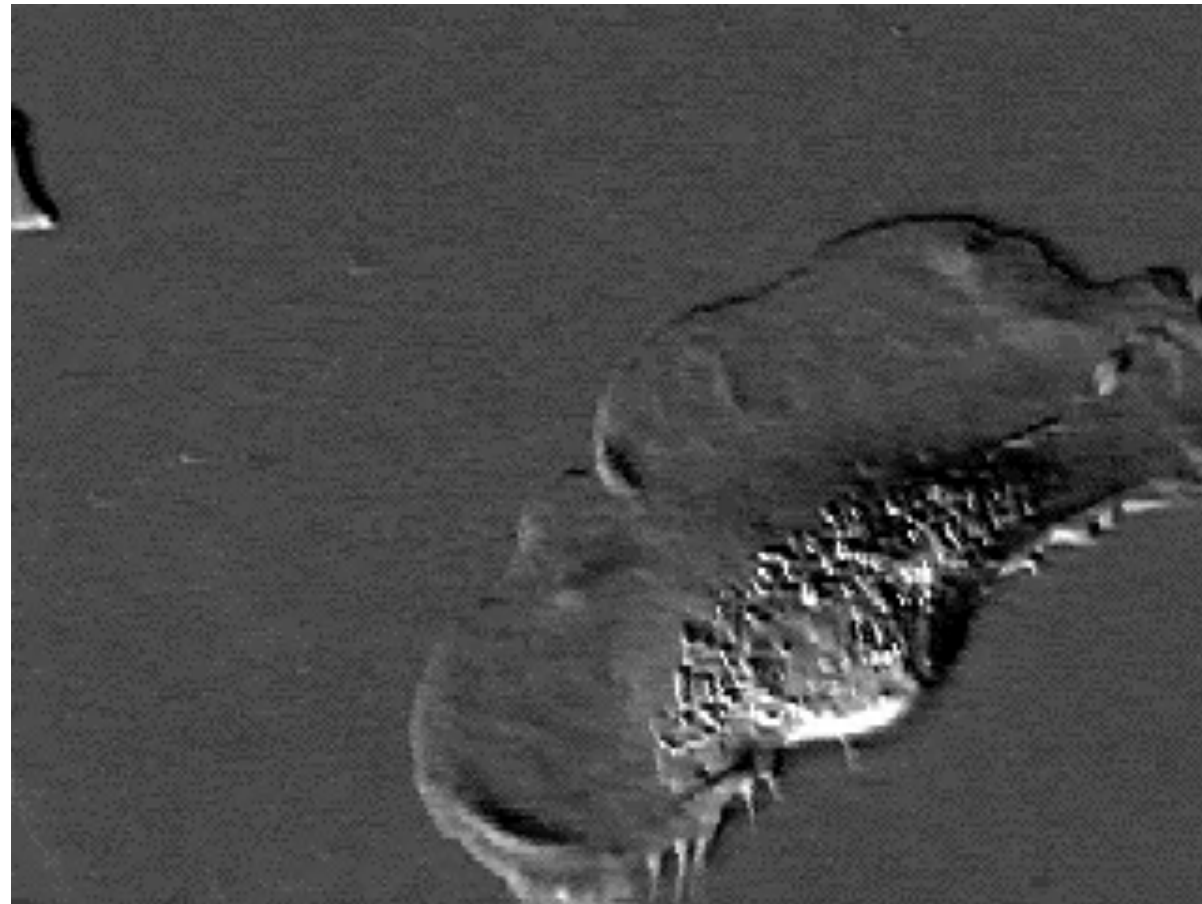
cortex



filopodium



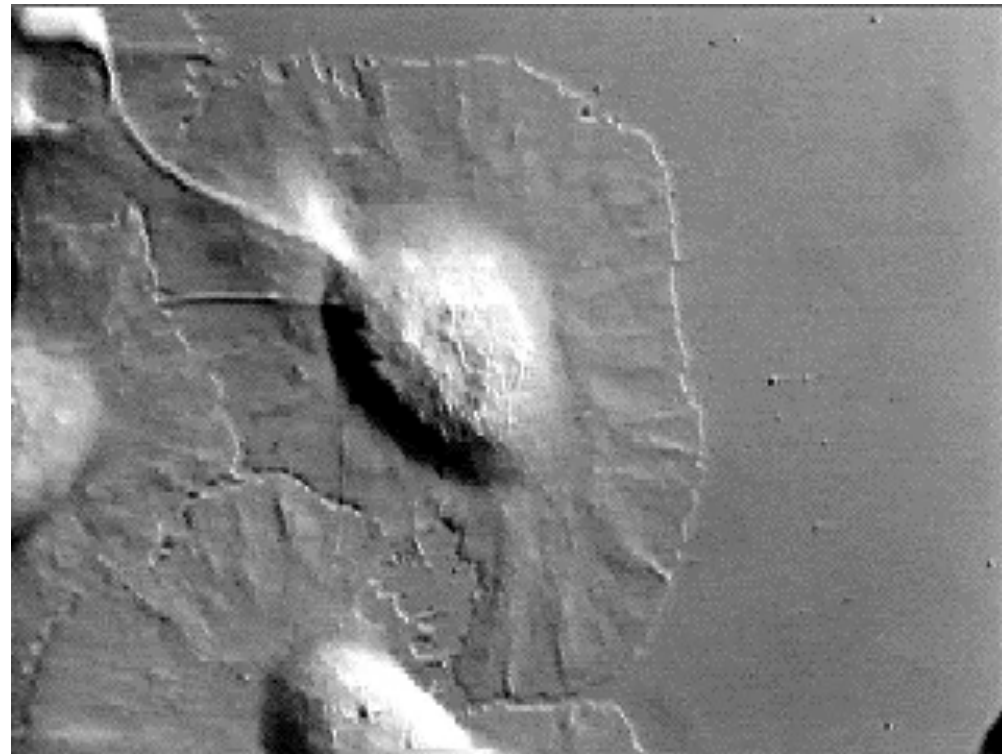
# Actin-dependent cell movement



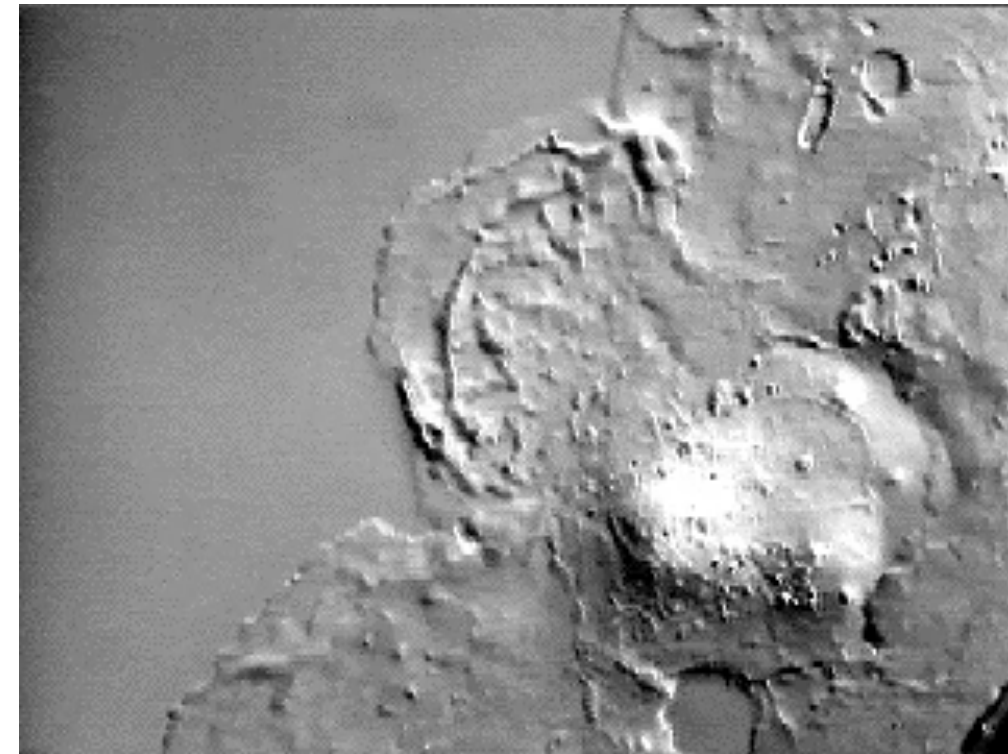


# Manifestations of actin-dependent movement

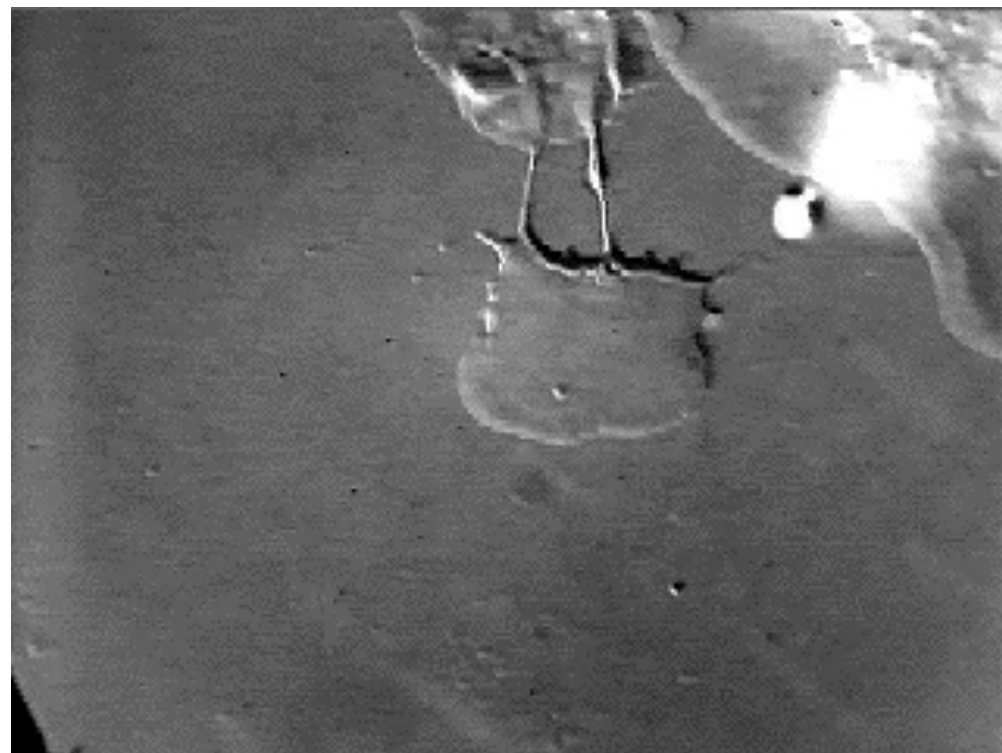
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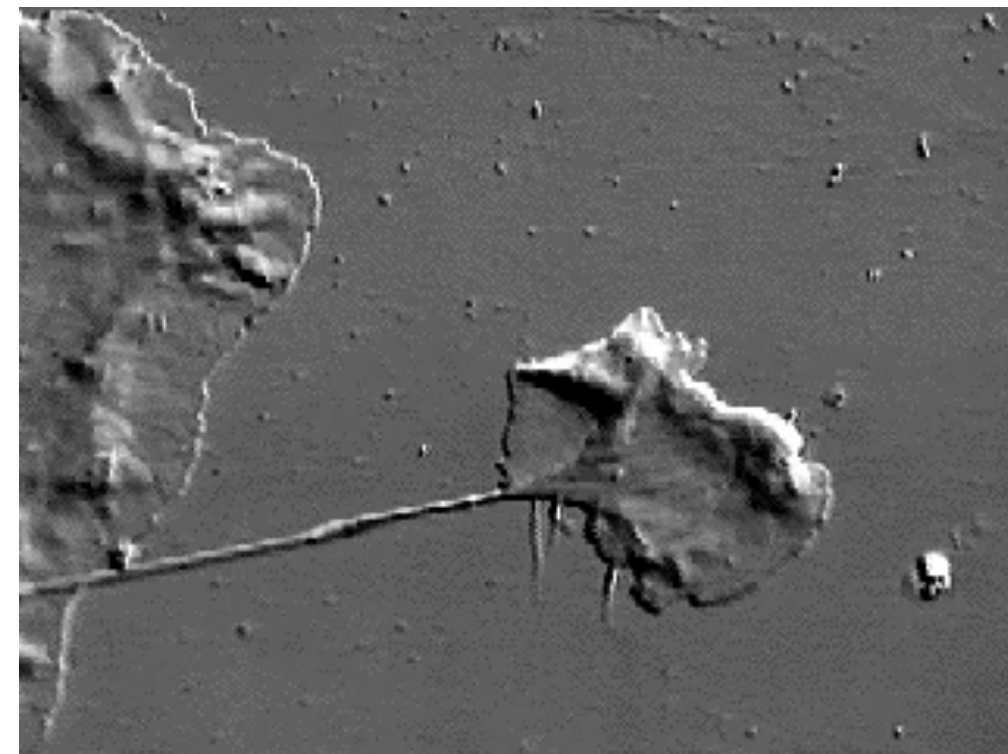
Retrograde flow



Filopodial dynamics



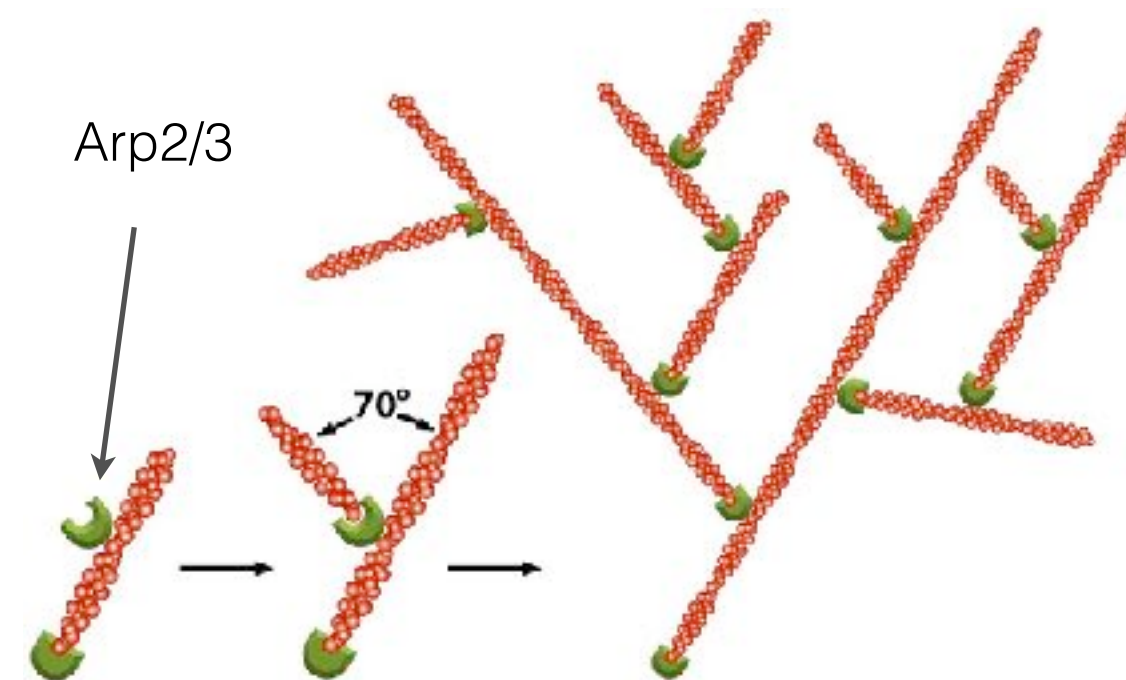
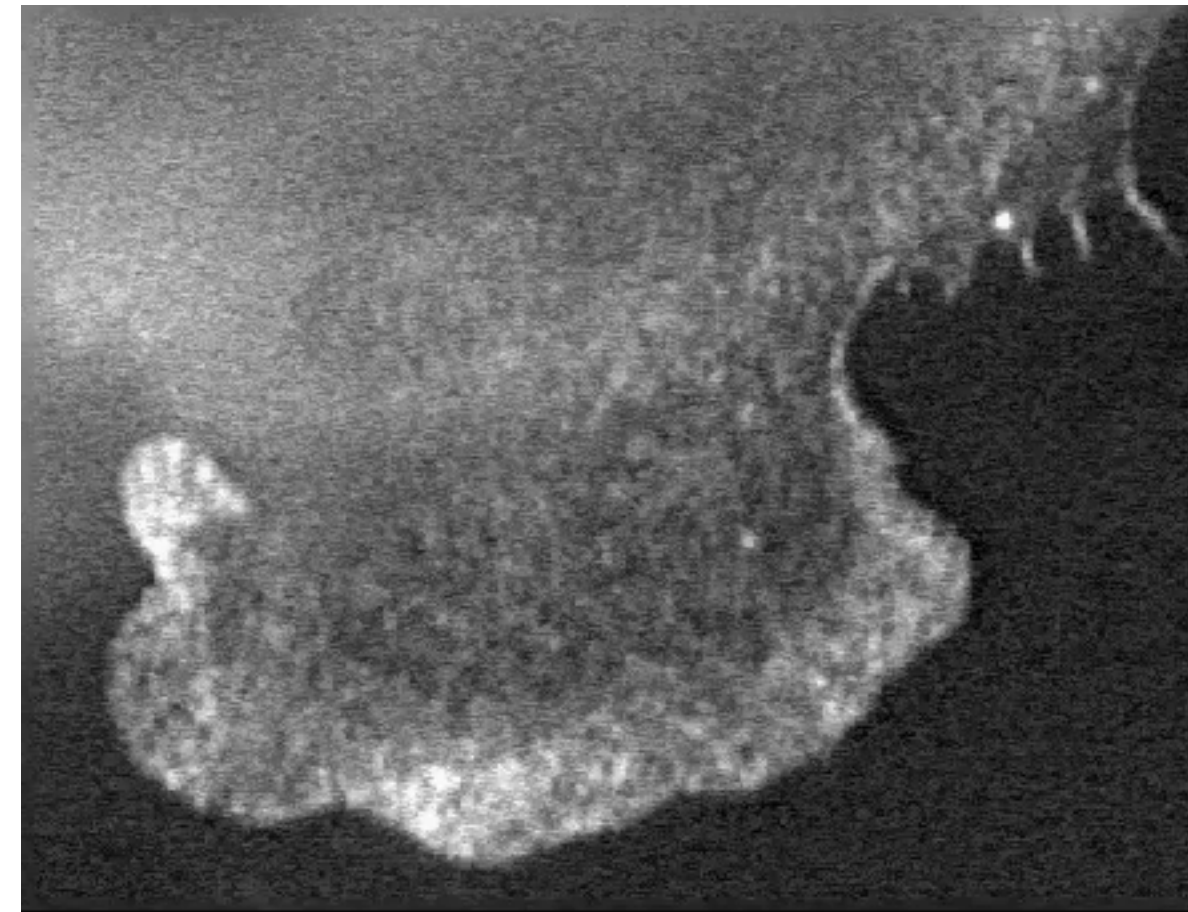
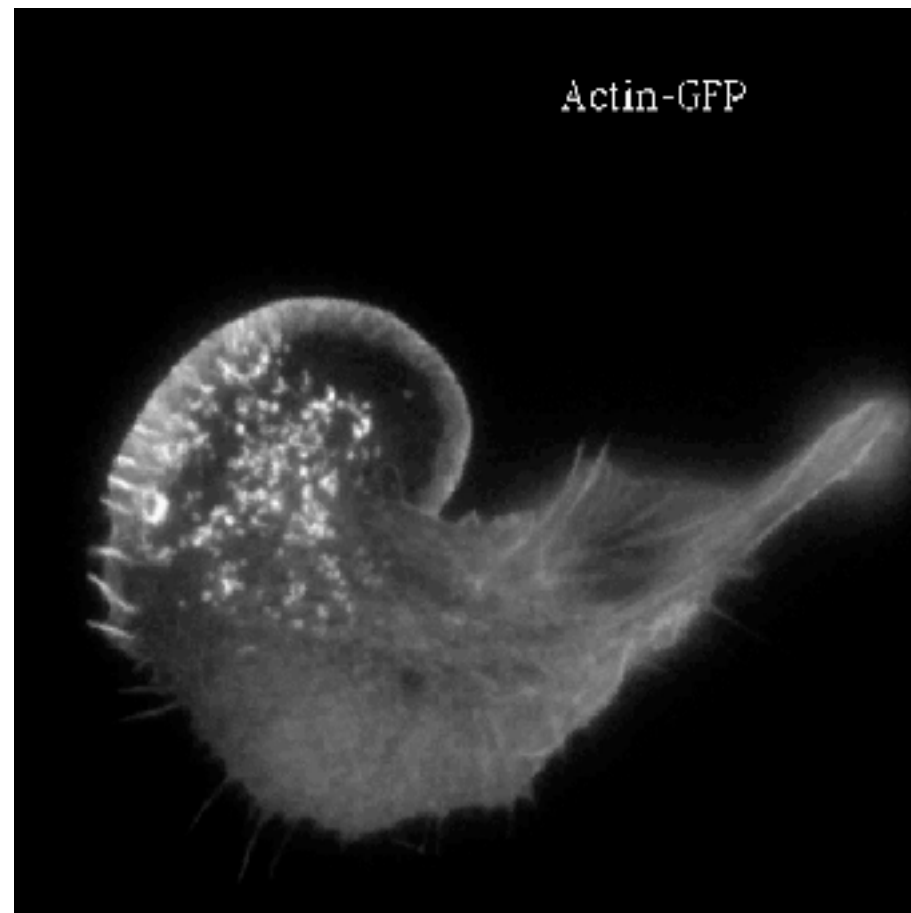
Autonomous movement of cytoplasm  
(anuclear cell fragment)



Membrane ruffling

# Actin dynamics in the lamellipodium

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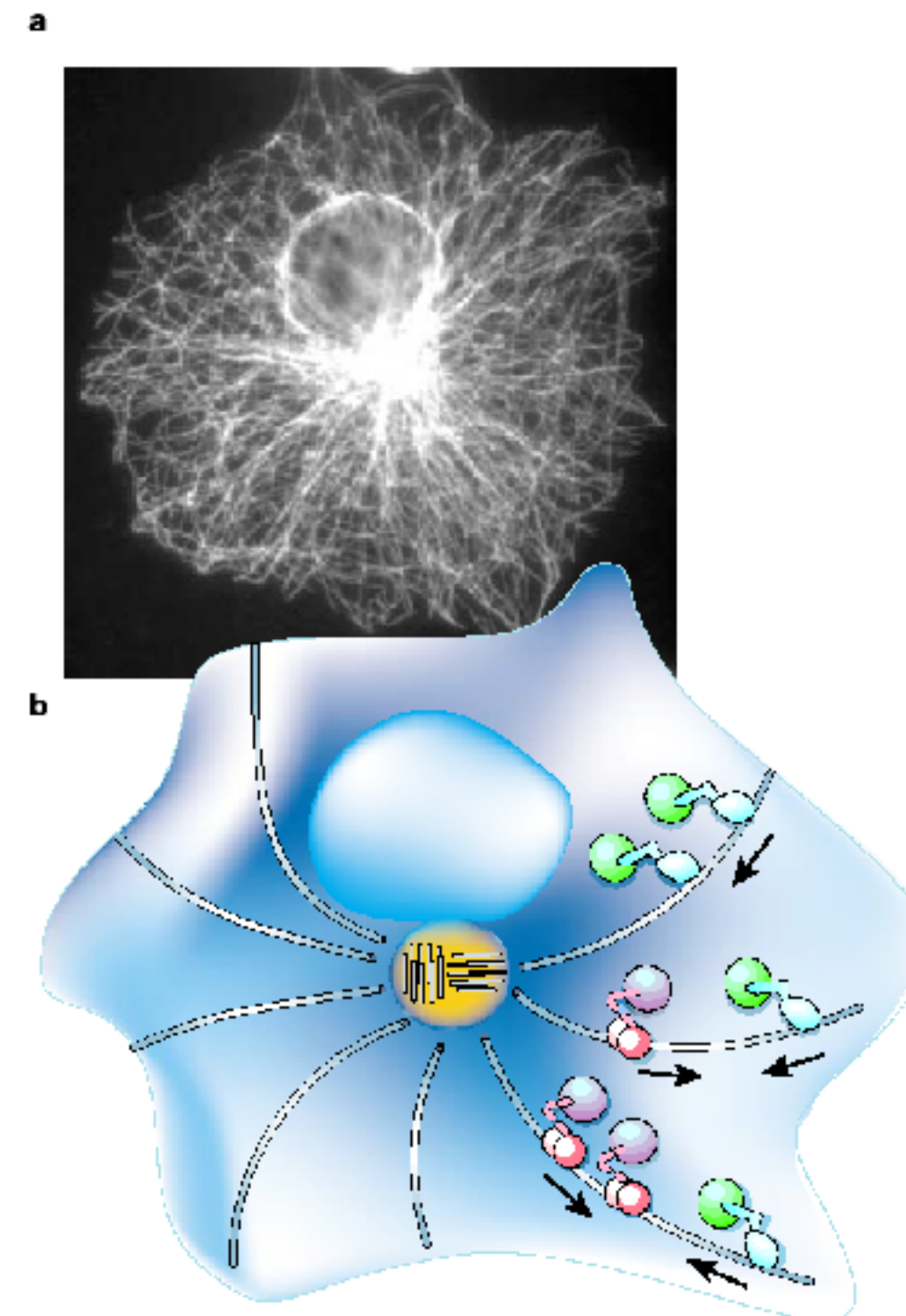
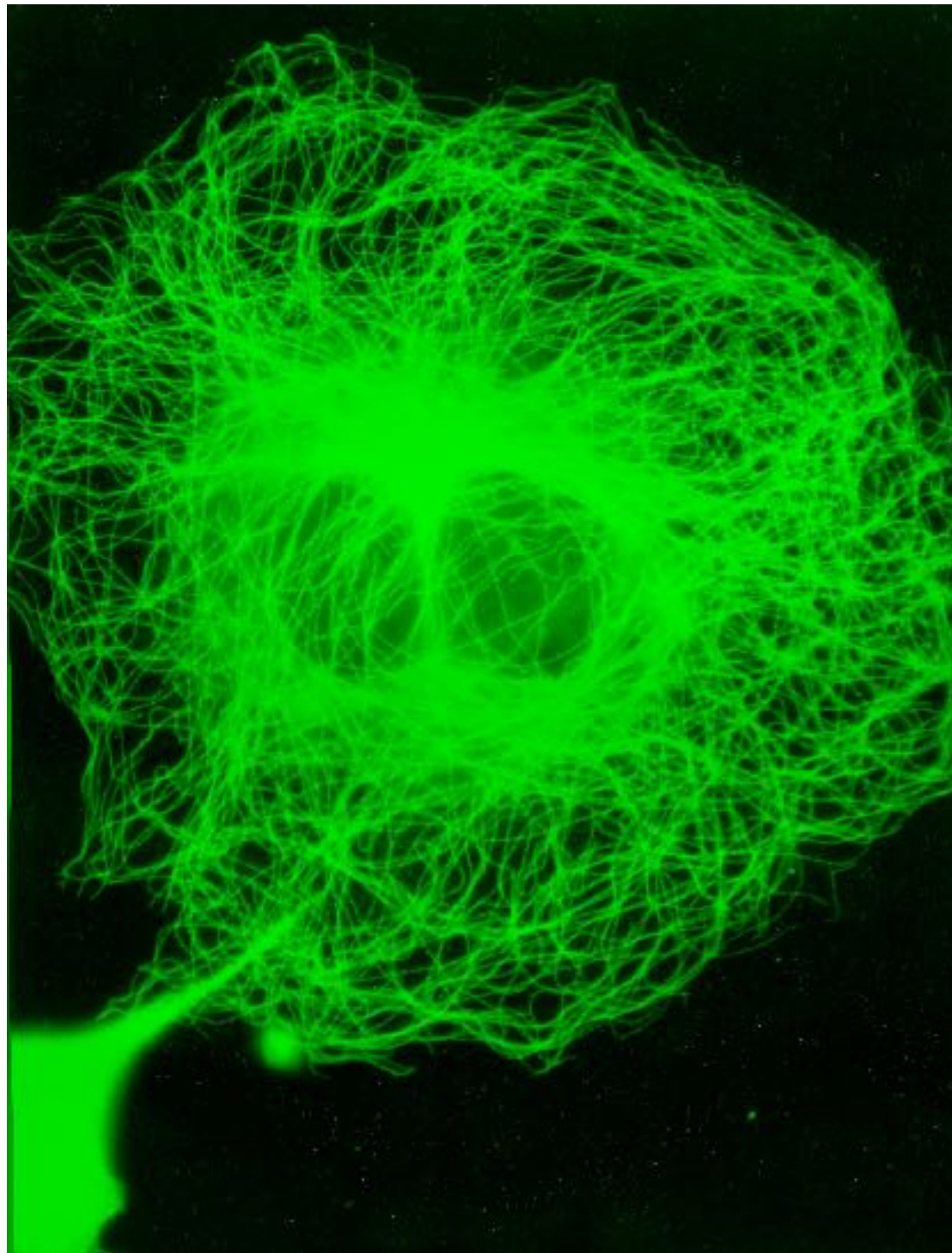




# Microtubular system

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Filamentous system of eukaryotic cells composed of tubulin and its associated proteins



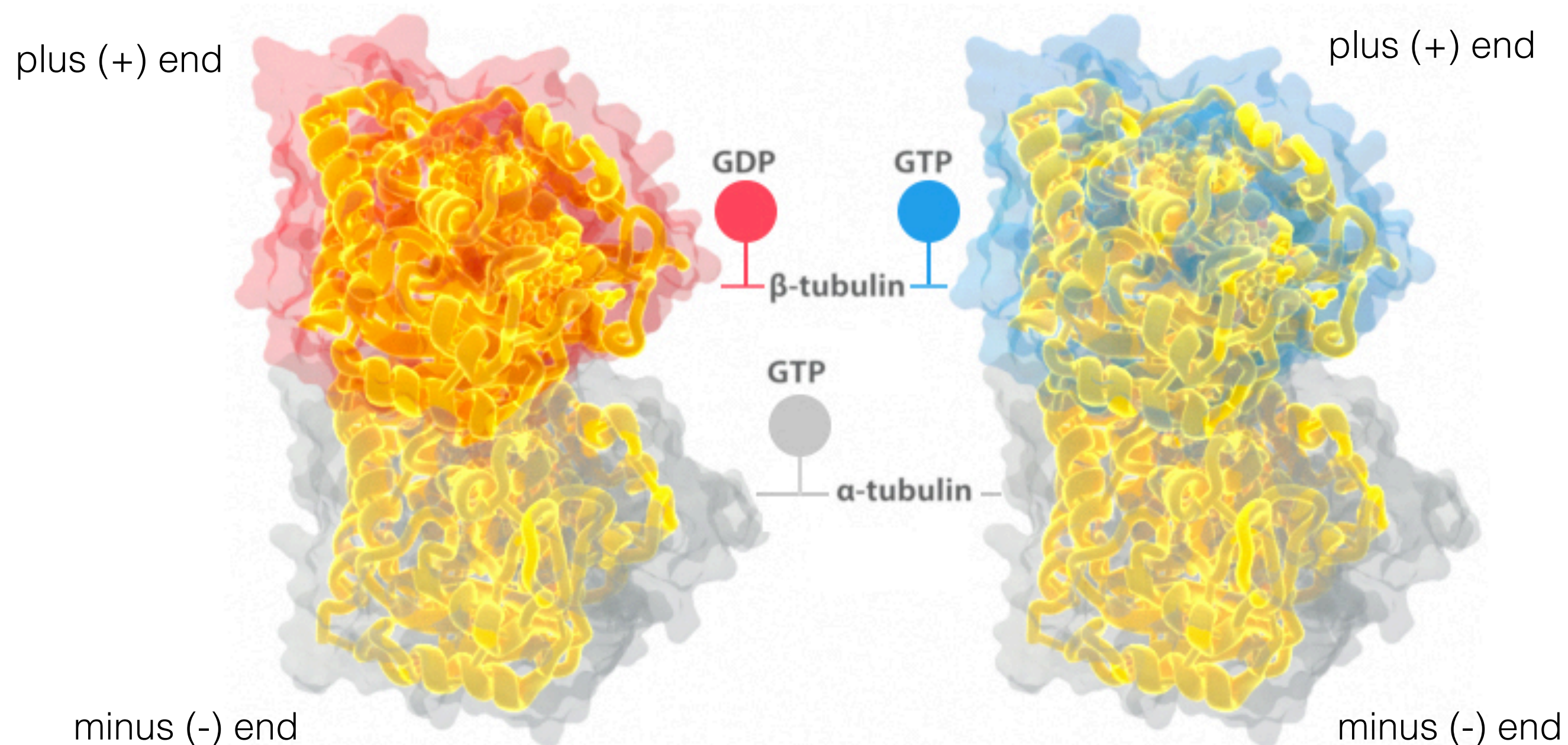


# Microtubule building block: tubulin

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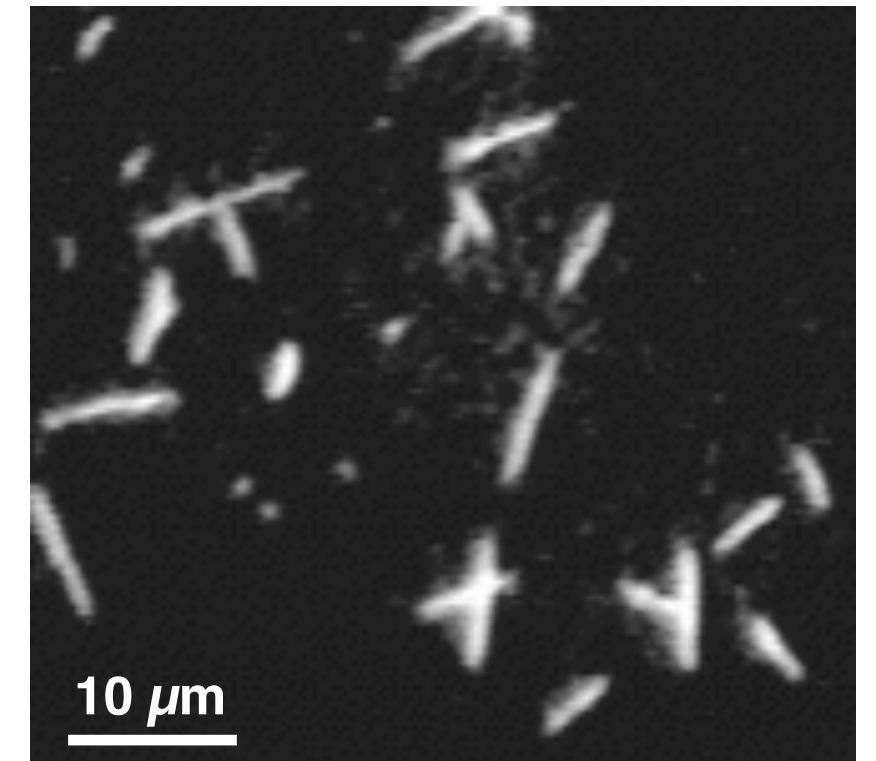
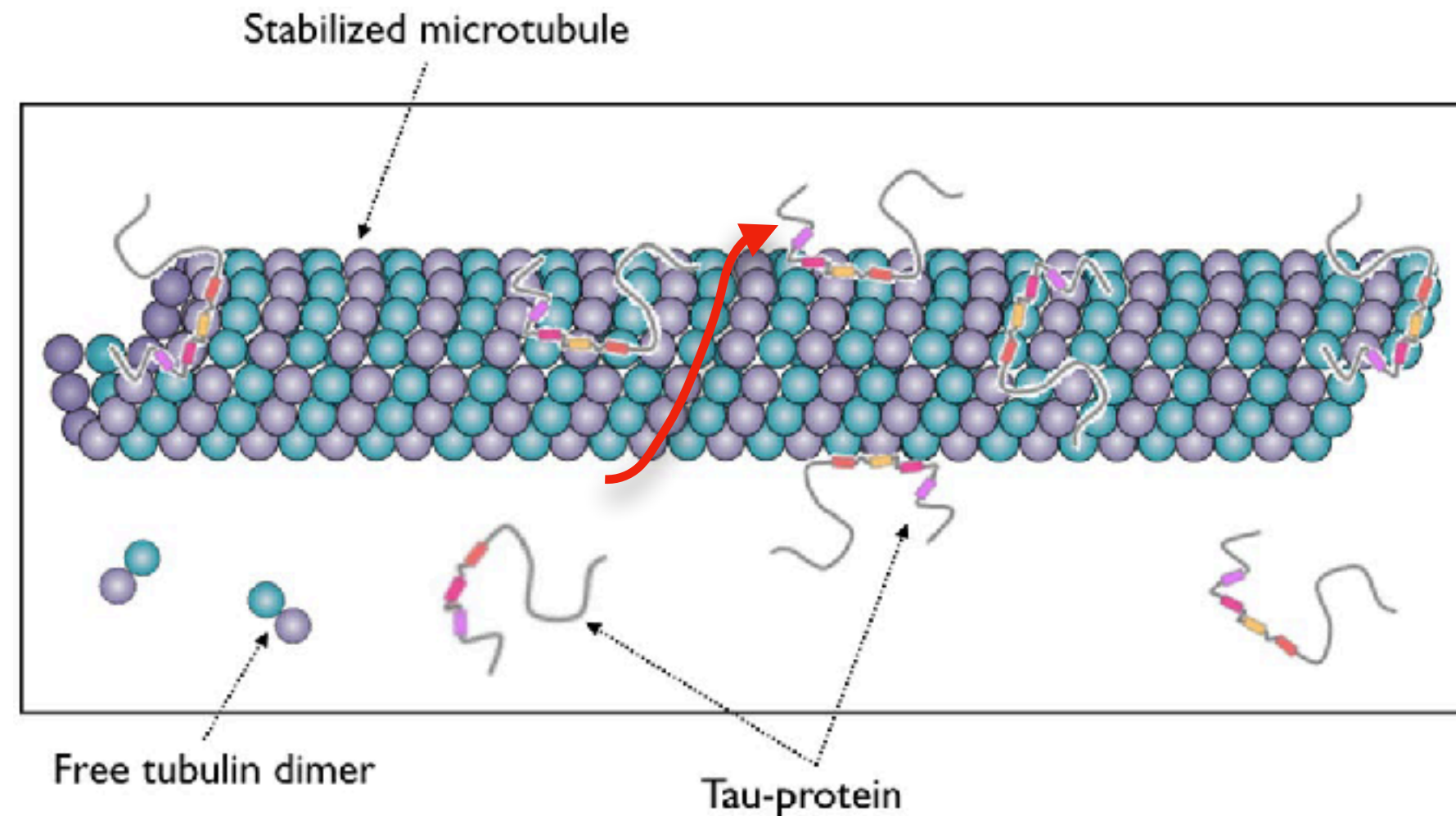
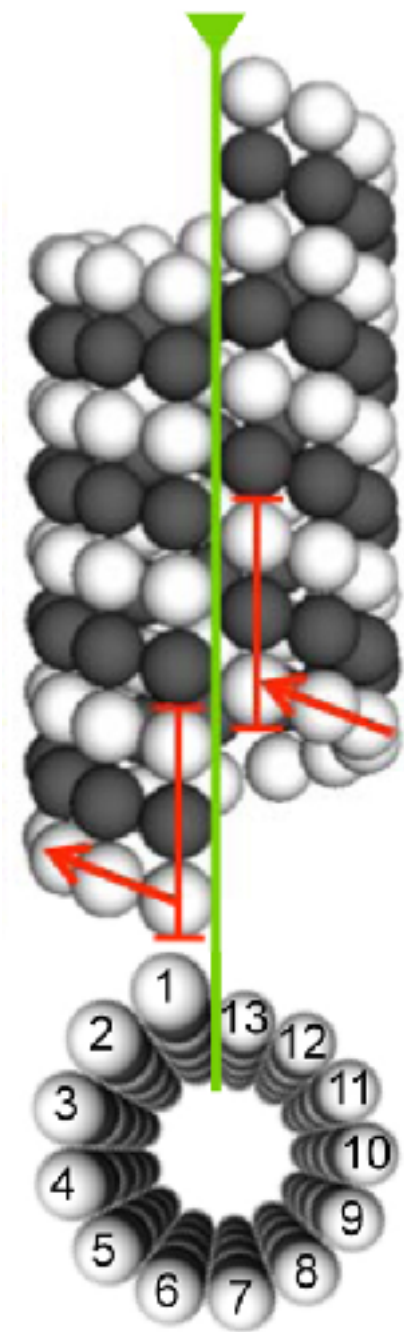
Subunit: tubulin

- 10-20% of total protein in neural tissue
- MW: ~50 kD:  $\alpha$ - and  $\beta$ -tubulin  $\rightarrow$  heterodimer
- 1 molecule bound guanosine nucleotide (GTP or GDP); exchangeable ( $\beta$ ), and non-exchangeable ( $\alpha$ )
- Structural polarity (plus/minus ends)
- Genetic variability: at least 6 different  $\alpha$  and  $\beta$  tubulins





# The microtubule



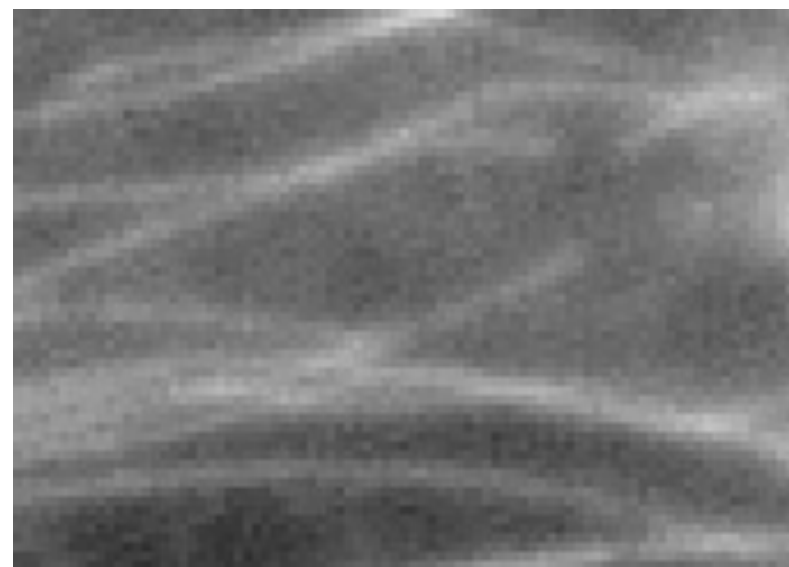
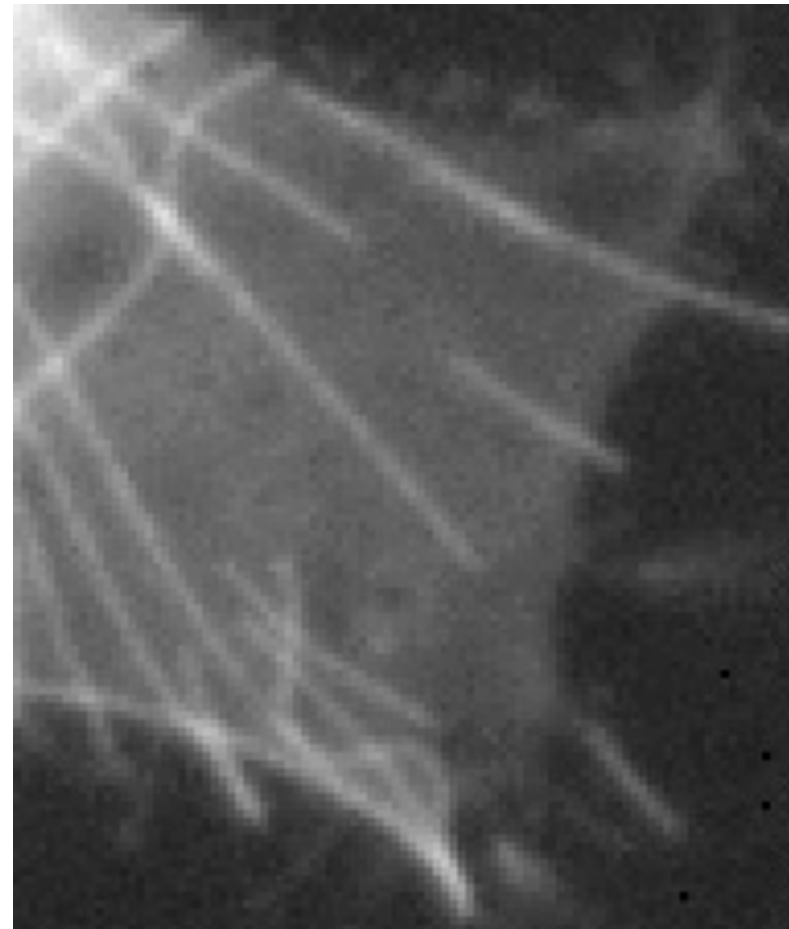
Fluorescence microscopic image of taxol-stabilized, rhodamine-labeled microtubules

- ~25 nm in diameter, tubular structure
- Rigid polymer chain (persistence length ~mm)
- 13 protofilaments
- Same monomers link up into a left-handed short-pitch helix
- Structural polarity:
  - +end: rapid polymerization, terminated by  $\beta$ -subunit
  - end: slow polymerization, terminated by  $\alpha$ -subunit
- GTP-cap

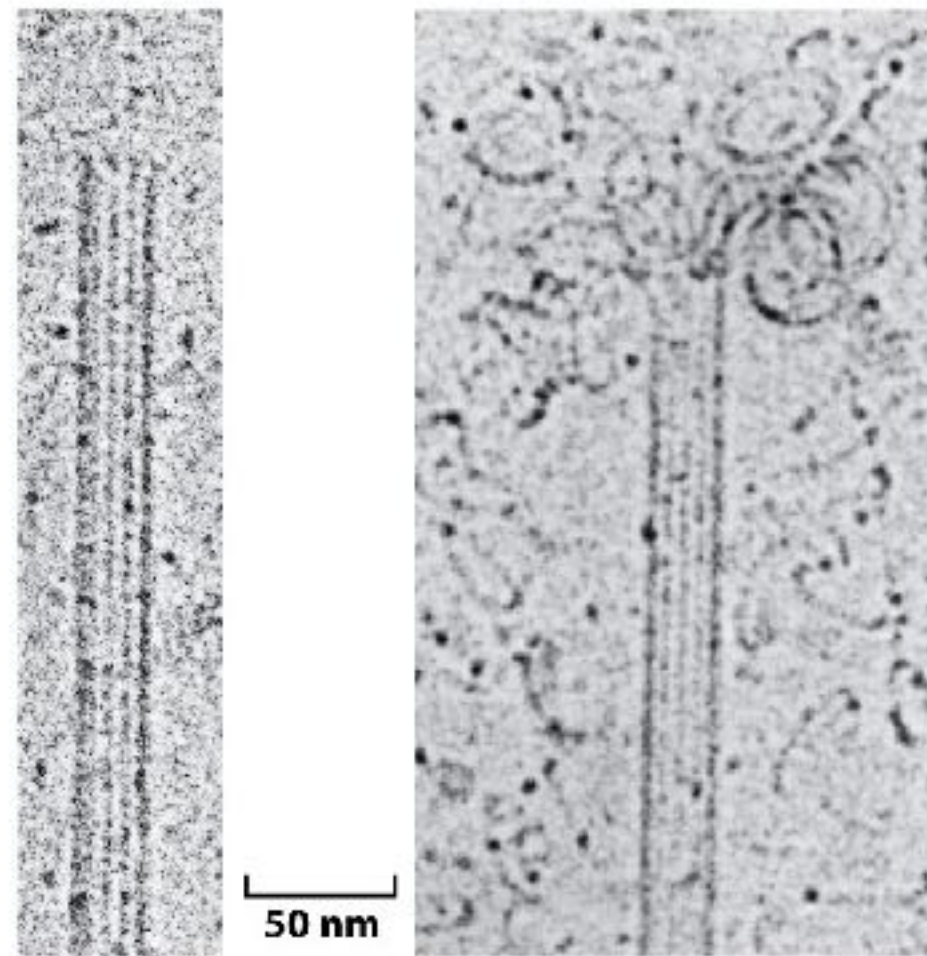


# Polymerization equilibria in microtubules

## Treadmilling

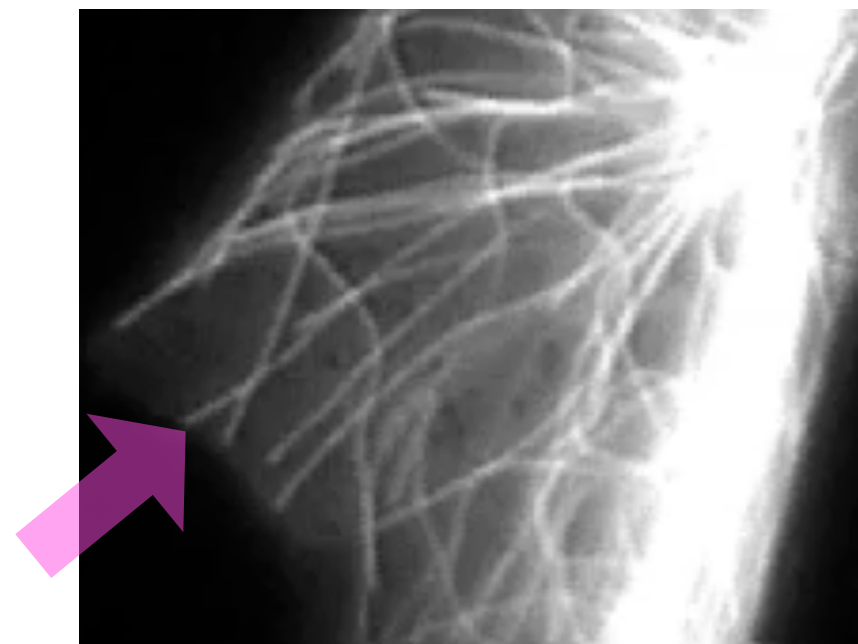


Treadmilling demonstrated with  
lase-induced photobleaching



## Dynamic instability

Once the GTP cap is gone (due to GTP hydrolysis), MT protofilaments dissociate and curve up due to mechanical stress





# Microtubular system in the eukaryotic cell

## Where?

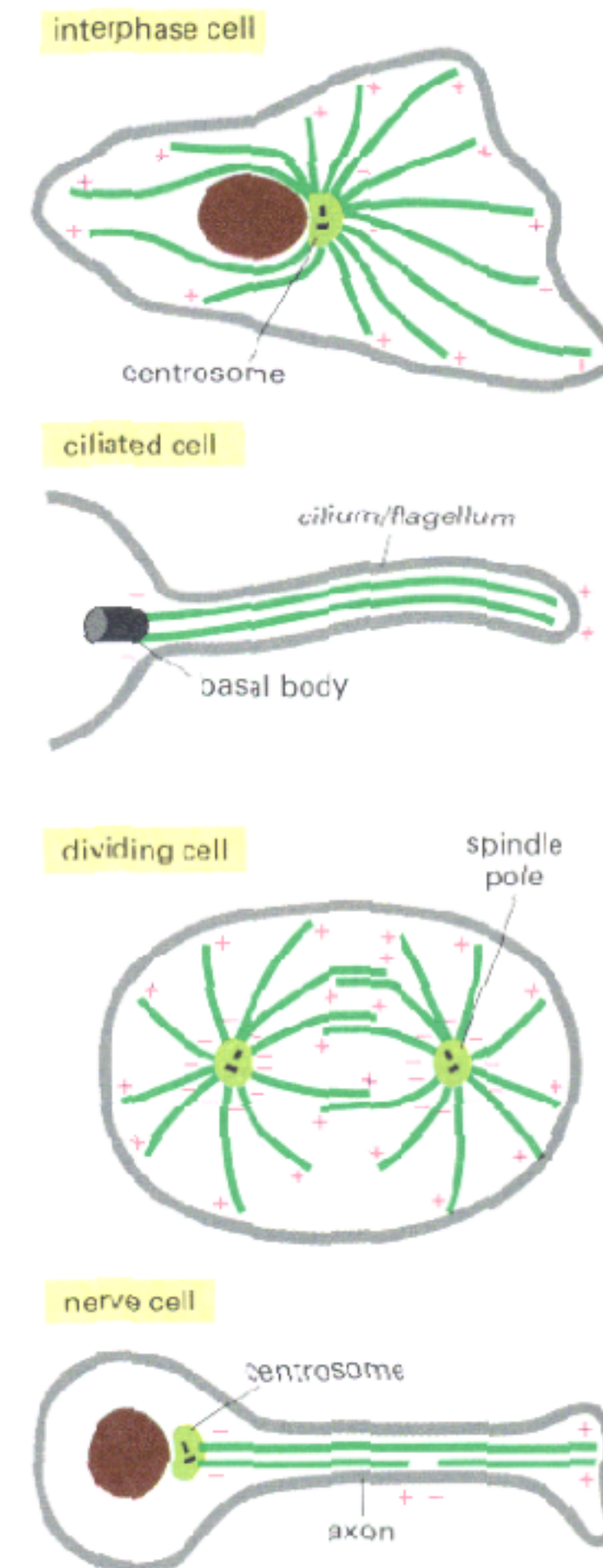
Cytoplasm of interphase cell, axon, cilia, flagella, mitotic spindle.

## Polarity within the cell

-end in centrosome, +end in periphery.

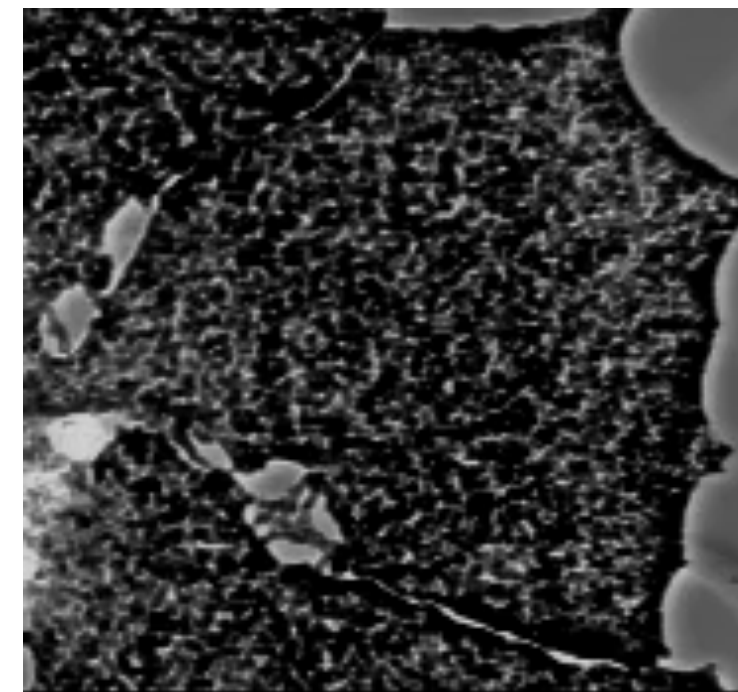
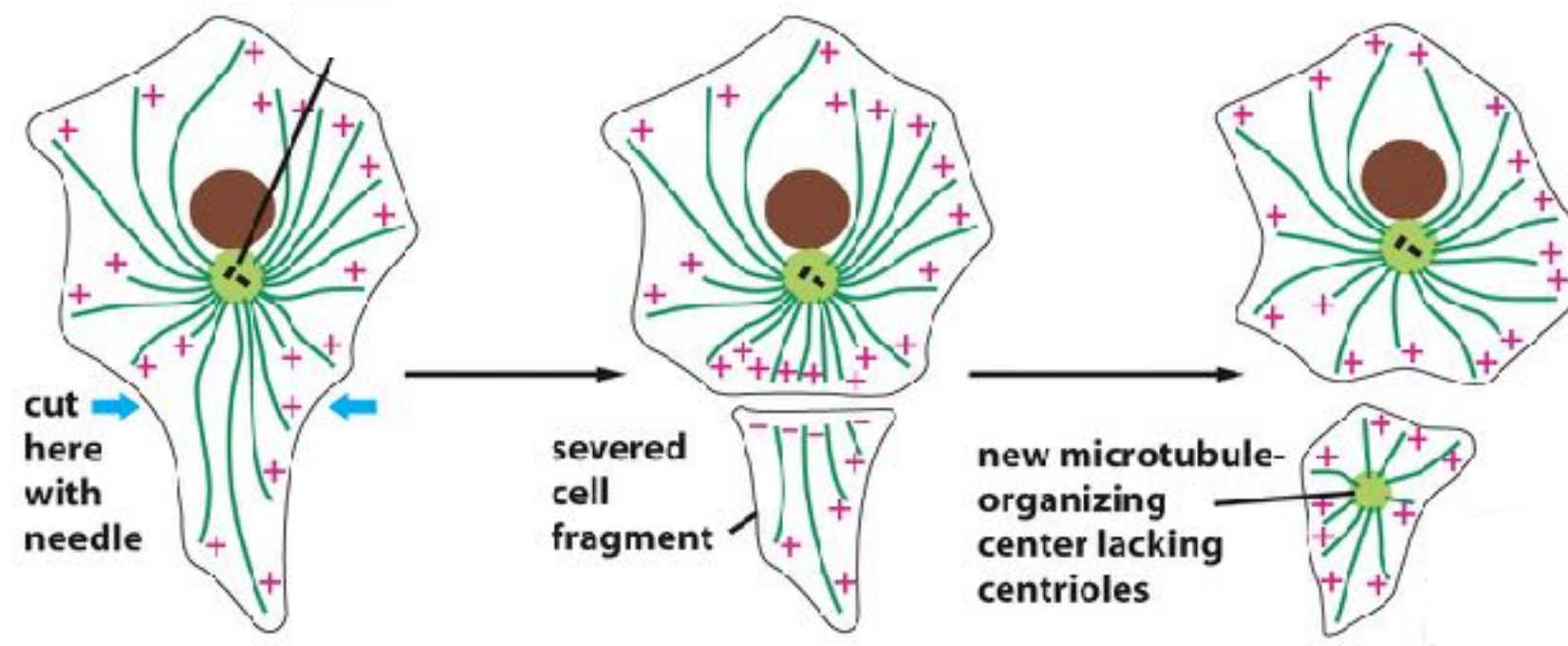
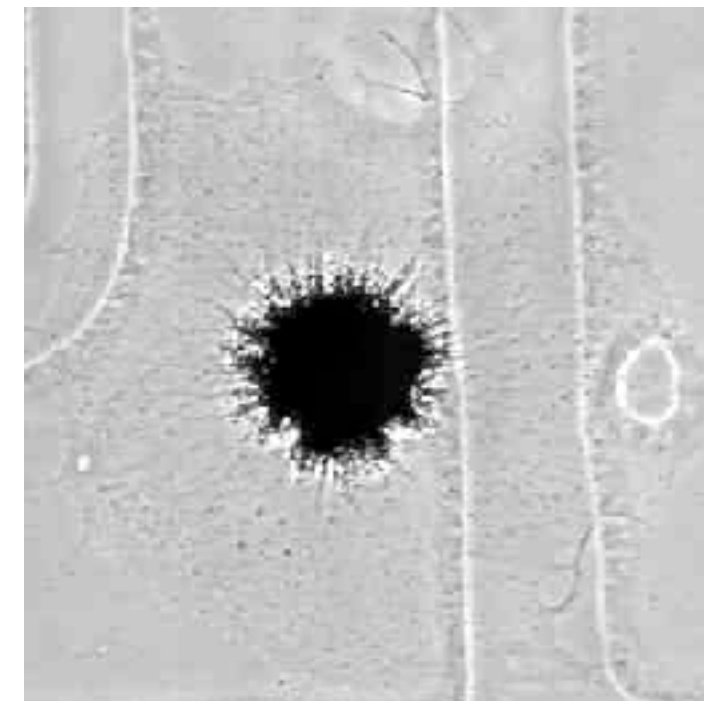
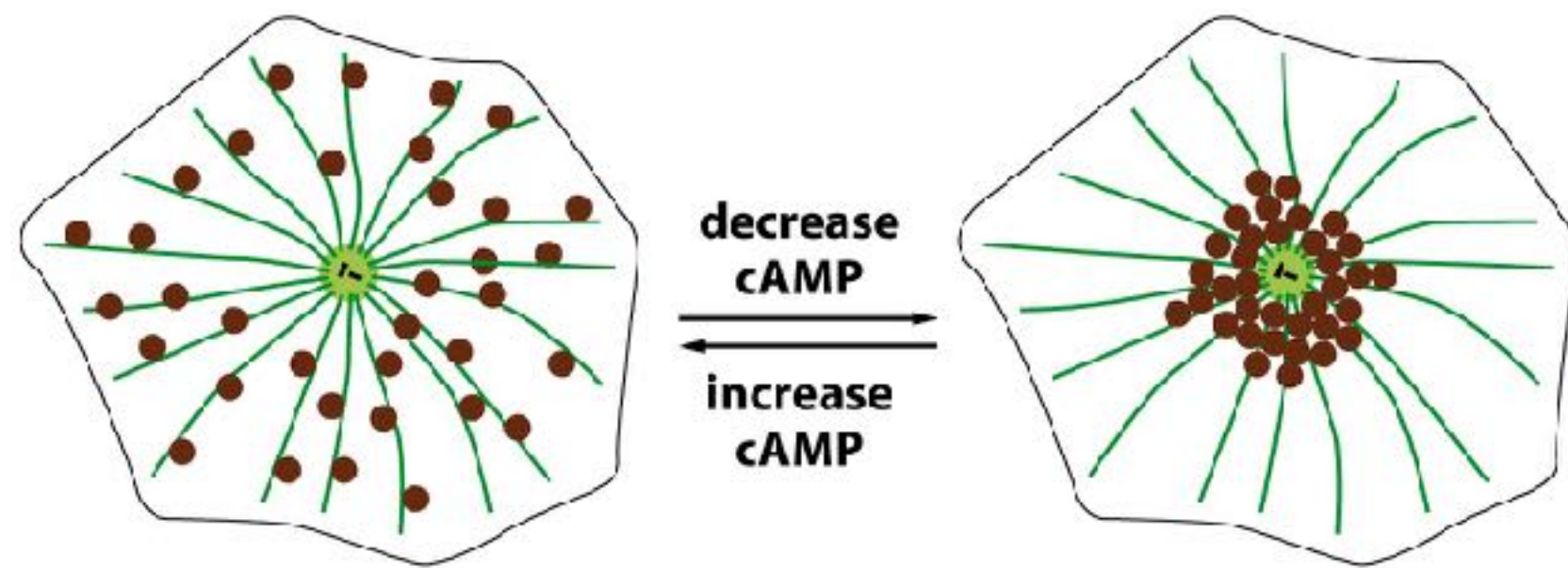
Centrosome: 2 centrioles, centrosome matrix with  $\gamma$ -tubulin.

Microtubules might be involved in the commitment and fixation of cell polarity with the help of associated (capping) proteins.



# Functions of the microtubular system

1. “Highways” for motor proteins
2. Senses, monitors and finds the geometric center of the cell.
3. Motility functions (e.g., cell division)

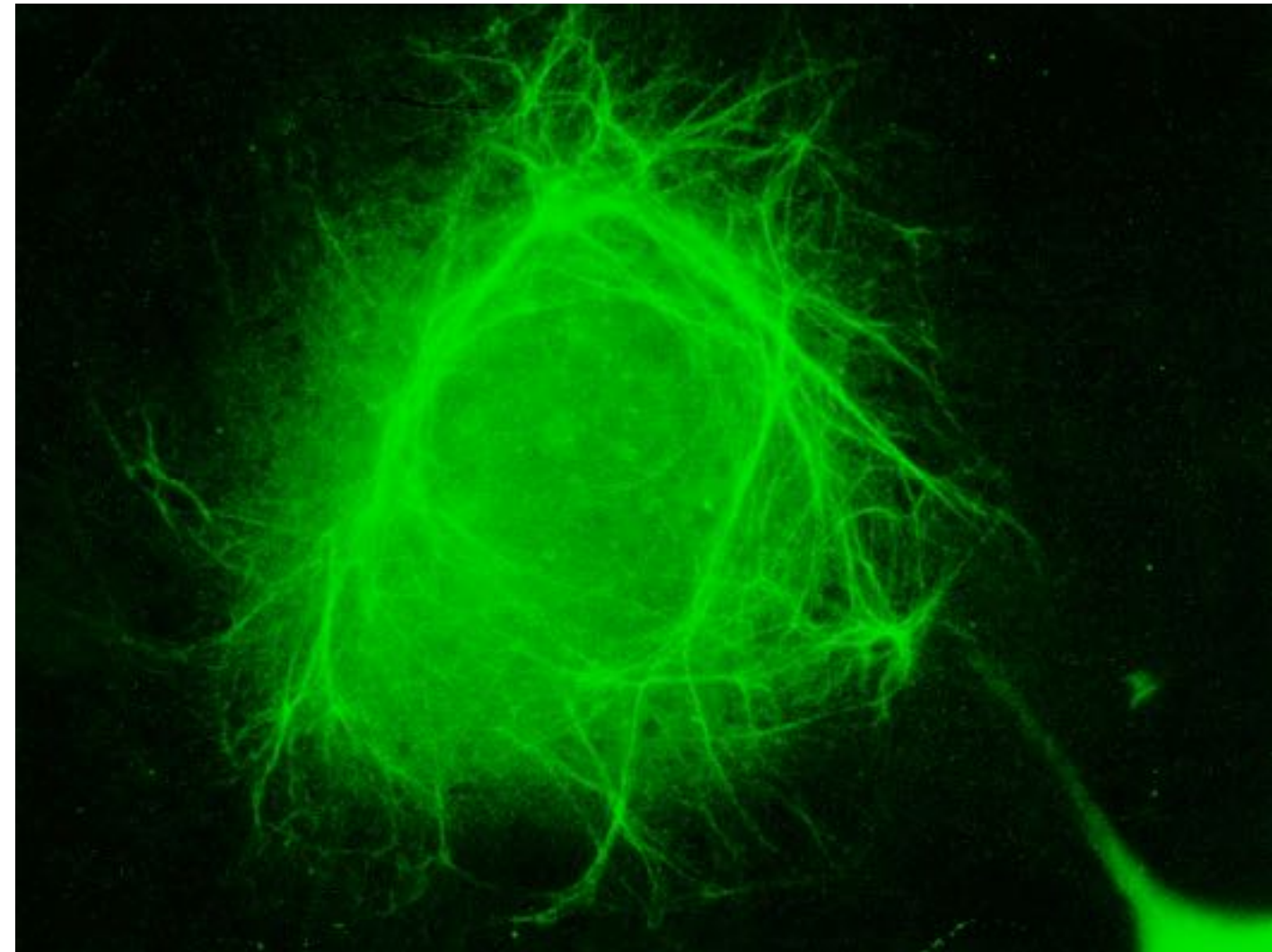
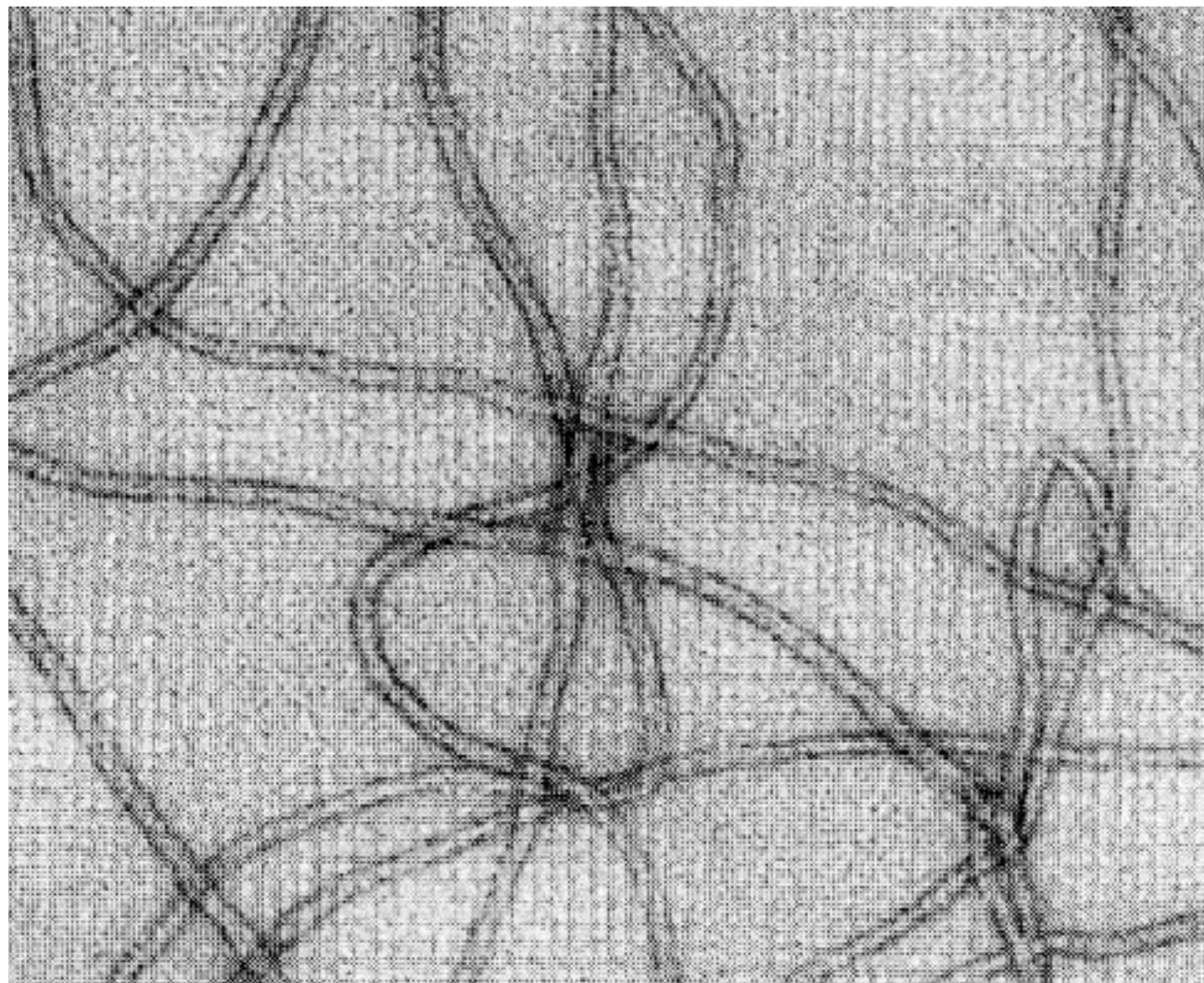




# Intermediate filament system

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- Tissue-specific filamentous protein system composed of 8-10-nm filaments,
- found in most animal cell types.
- Fundamental biological function is providing mechanical stability.



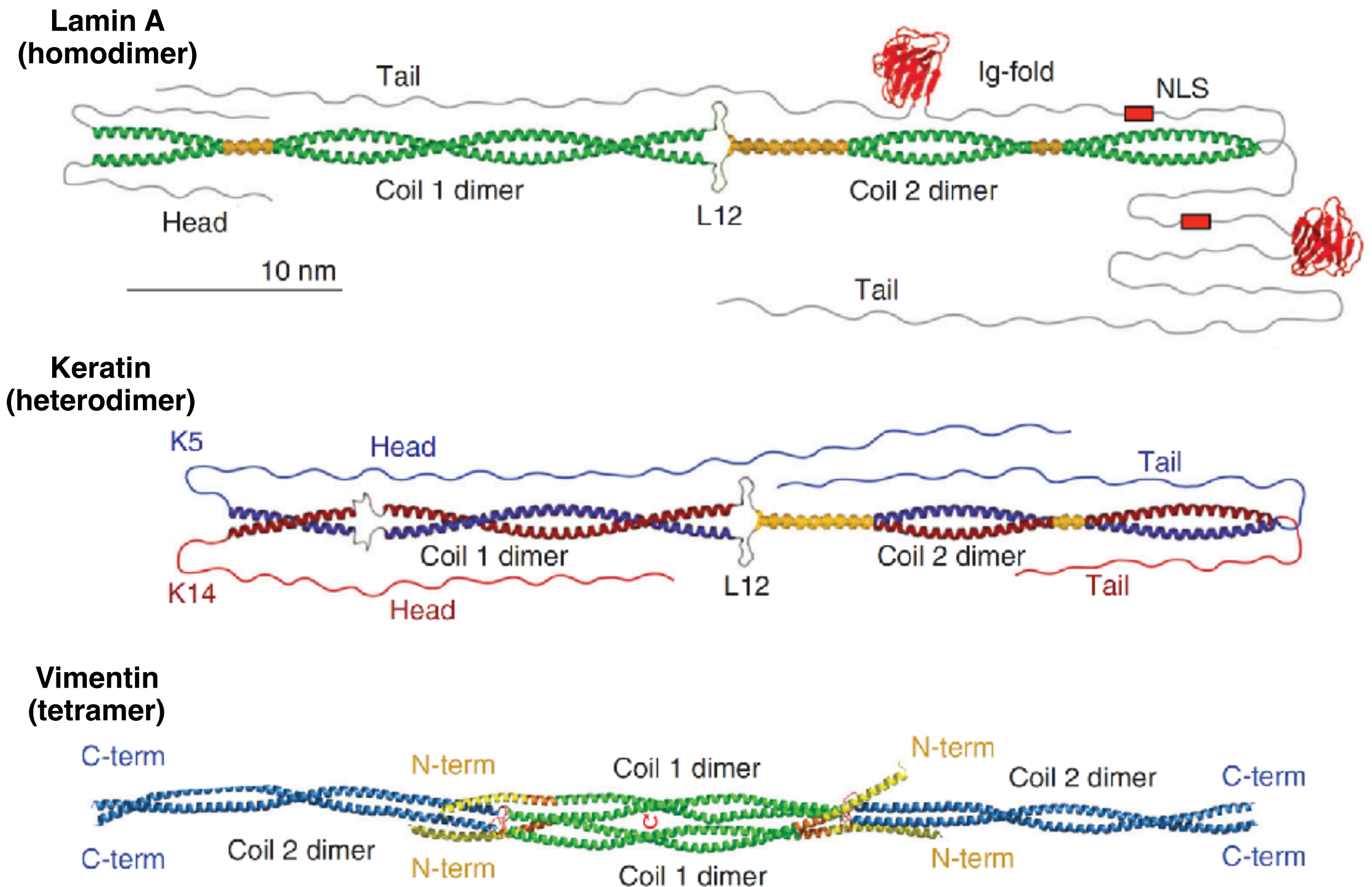
Vimentin, Vic Small



# Intermediate filament building block: the intermediate filament dimer

## Properties:

- Chemically resistant (detergents, high ionic strength)
- Can be extracted with denaturants (e.g., urea)
- Fibrous monomer (not globular as actin or tubulin)
- Amino-terminal head
- Central rod ( $\alpha$ -helix, heptad repeat)
- Carboxy-terminal tail
- Tissue-specific monomers differ in their terminal sequences

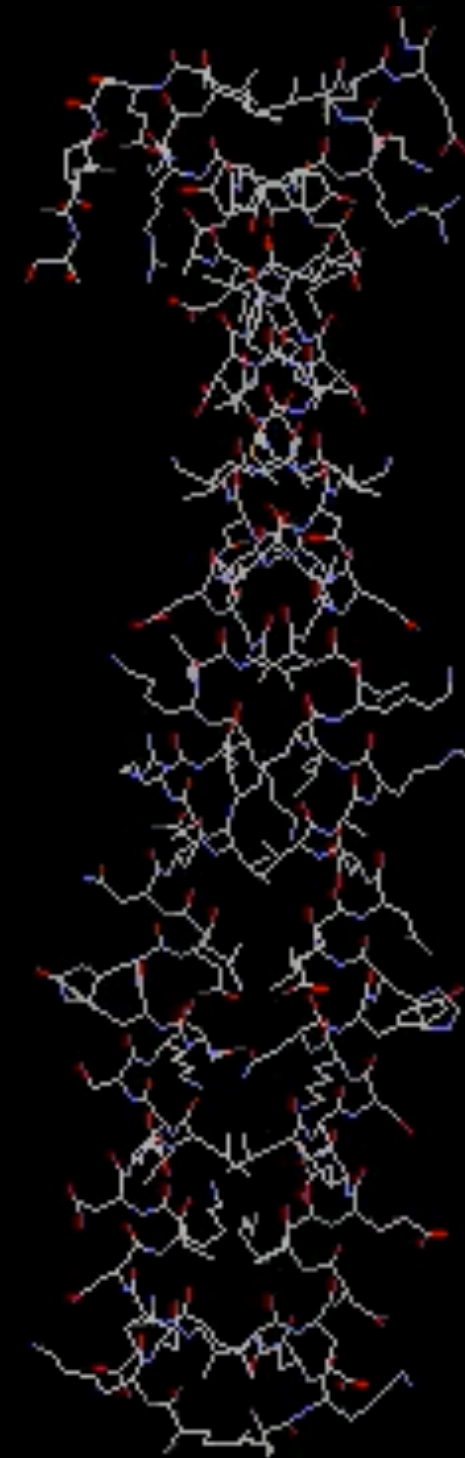




# Structural unit of intermediate filaments: „coiled-coil” dimer Heptad repeat, hydrophobic residues



Vimentin 1B domain dimer ribbon diagram



Vimentin 1B domain dimer wireframe diagram

# Classification of intermediate filaments

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Based on tissue specificity  
(Classical categories)

Tissue type	Intermediate filament
Epithelium	Keratins
Muscle	Desmin
Mesenchyme	Vimentin
Glia	Glial fibrillar acidic protein (GFAP)
Nerve	Neurofilaments (NF-L, NF-M, NF-H)
Cell nucleus	Lamins



# Polymerization of intermediate filaments

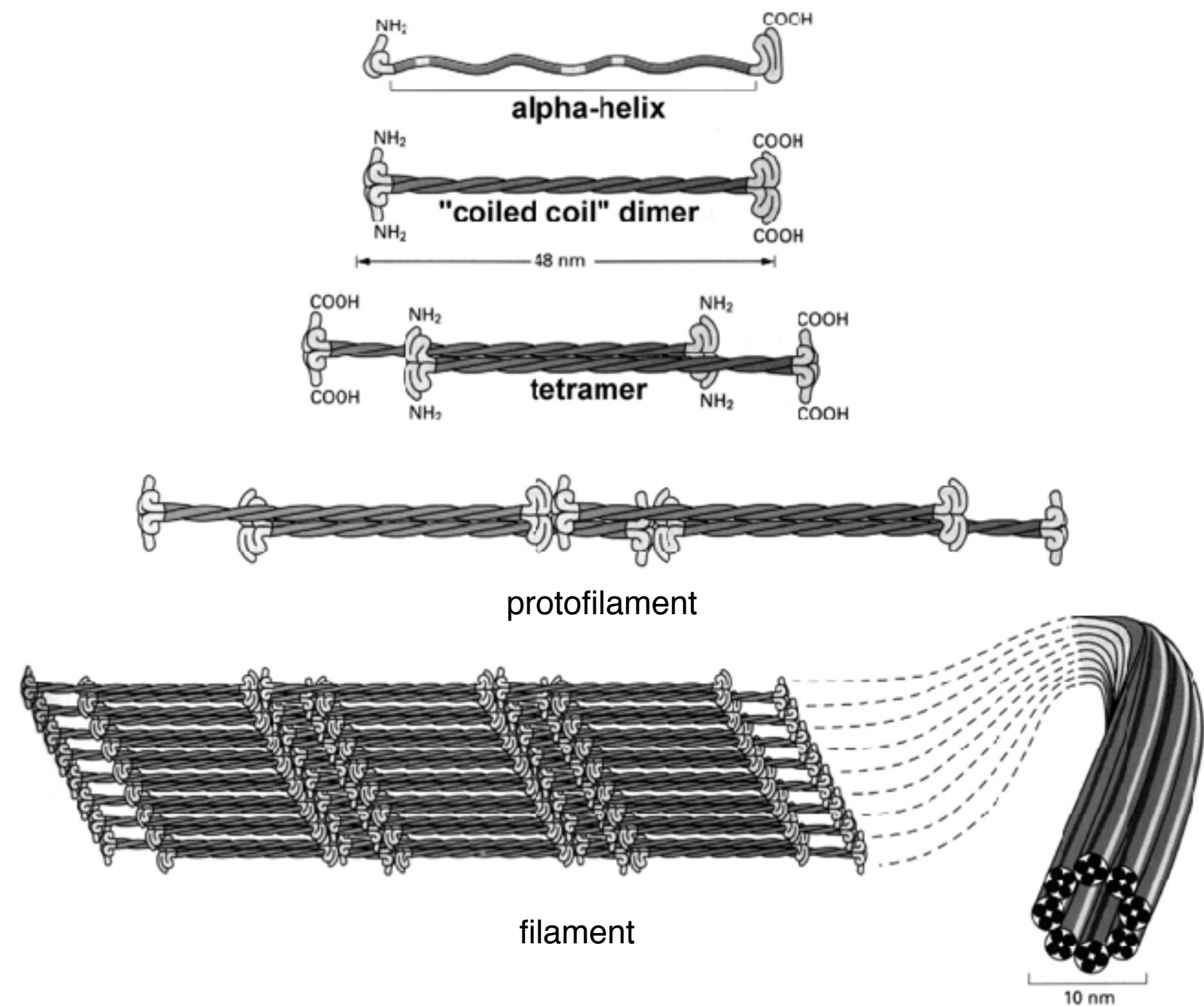
Fully polymerized state in the cell  
(not dynamic equilibrium)

Central rods ( $\alpha$ -helix)  
hydrophobic interactions  
-> coiled-coil dimer

2 dimers -> tetramer  
(antiparallel arrangement,  
structural apolarity)

Longitudinal association of tetramers  
-> protofilament

8 protofilaments -> filament

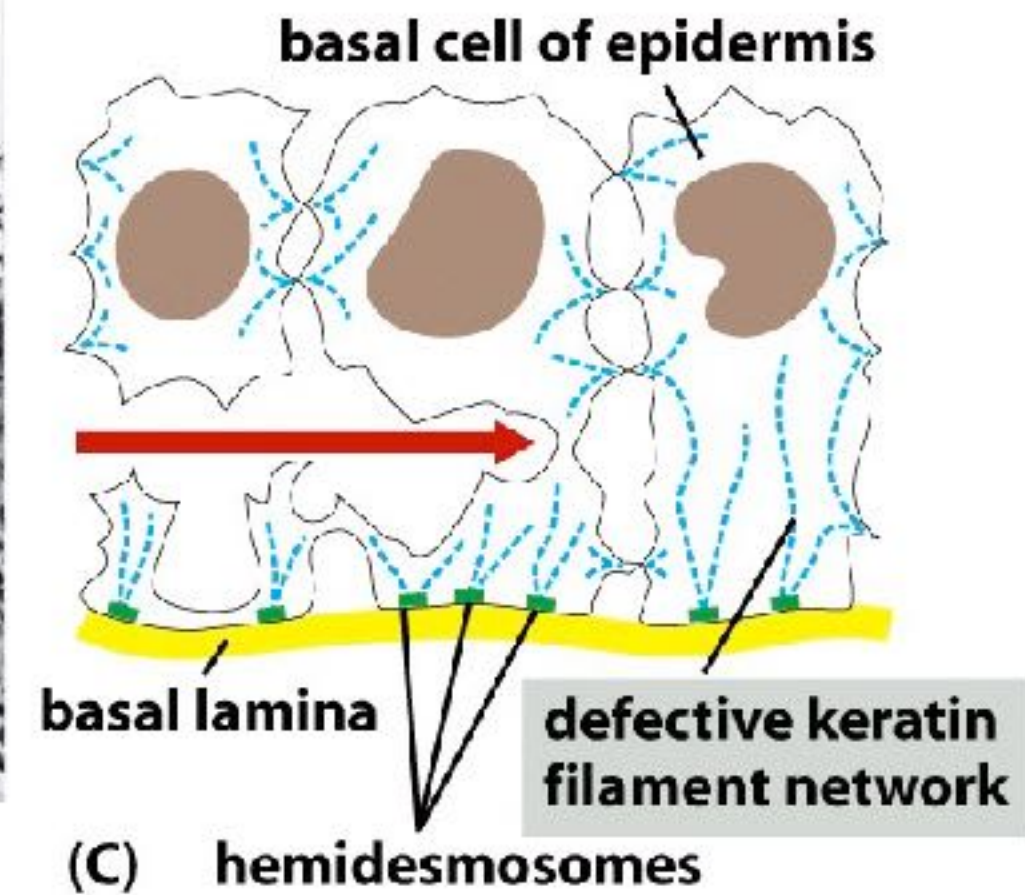
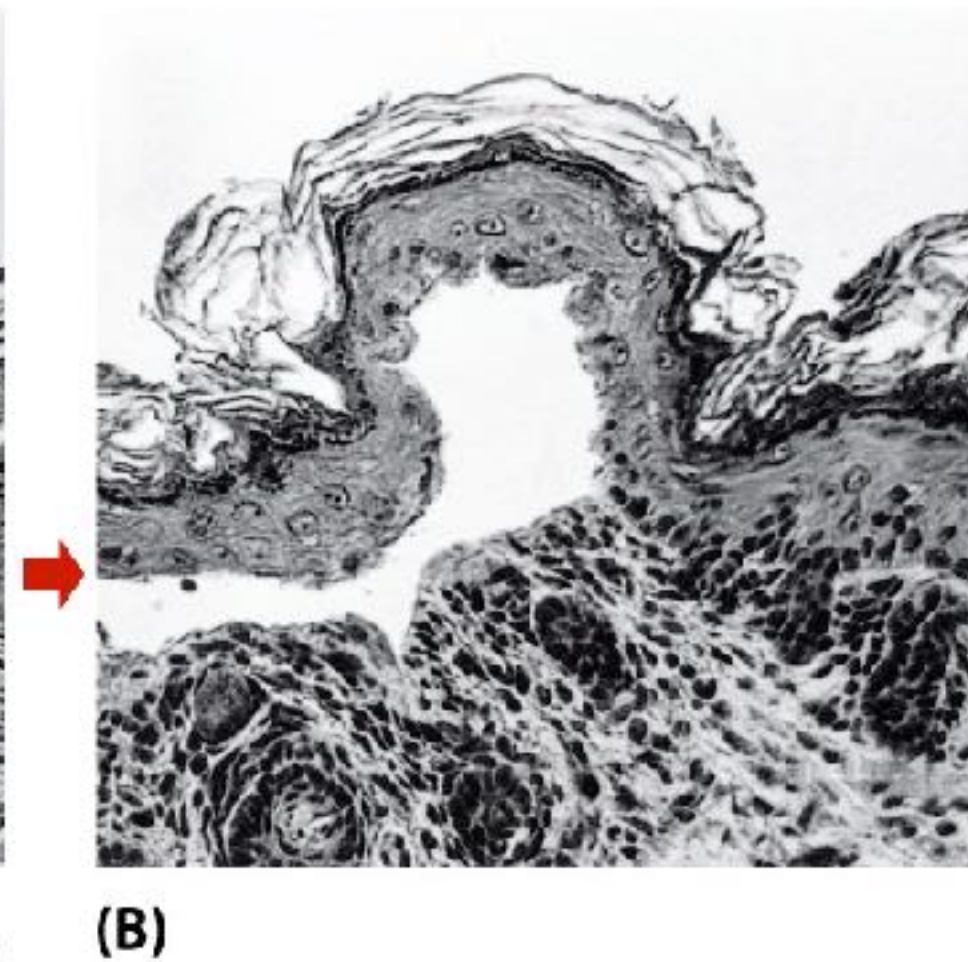
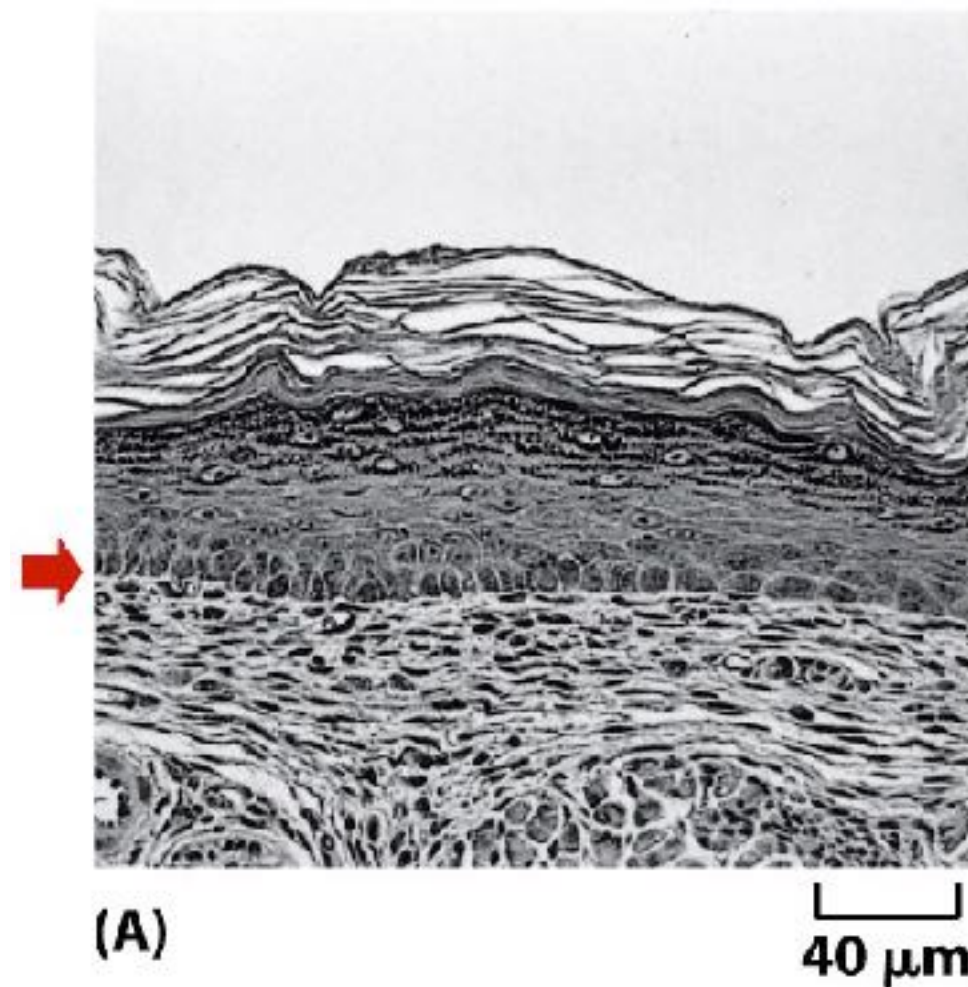


# Tissue functions of intermediate filaments

## Providing mechanical stability

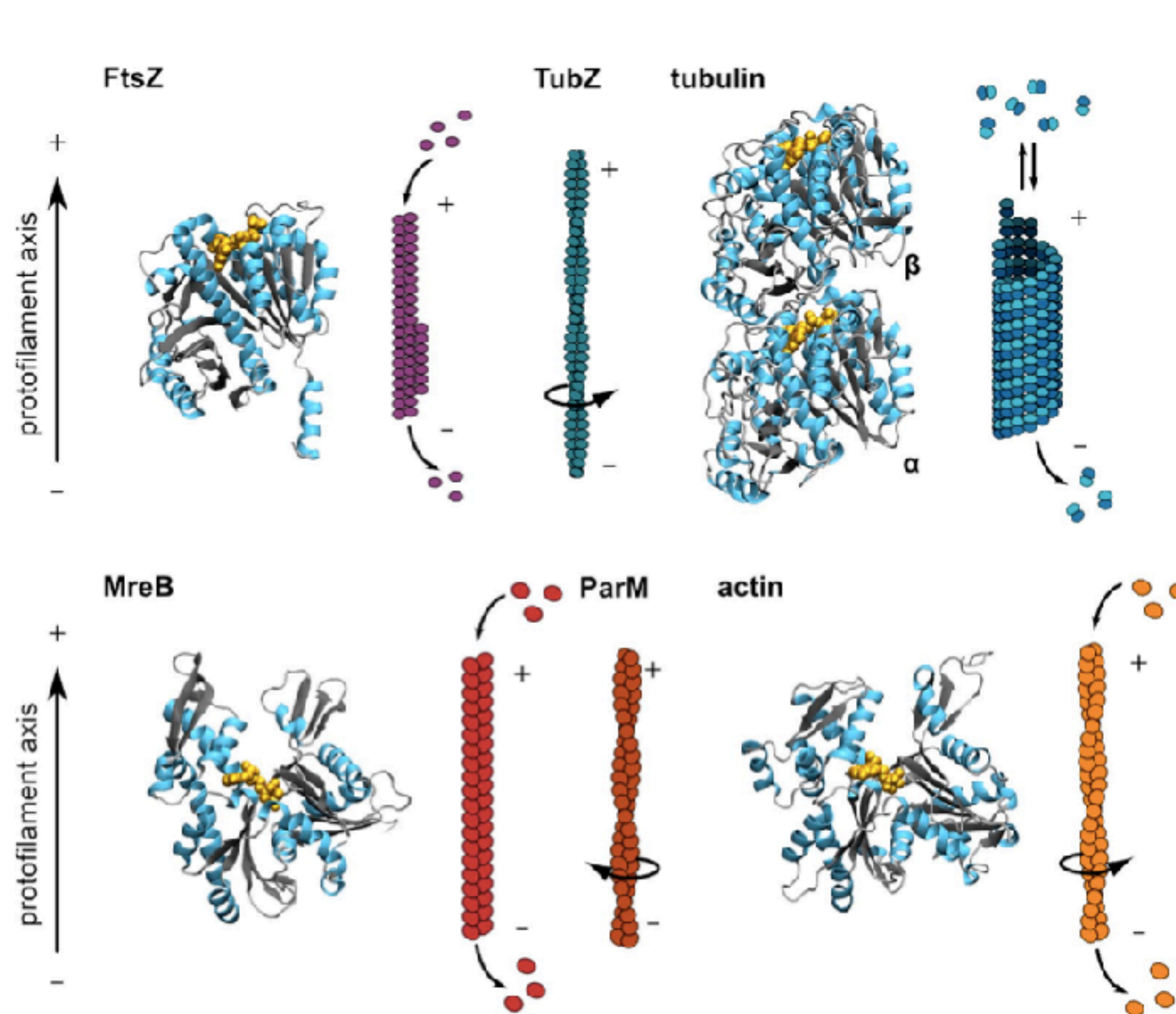
### Epithelial cells:

- Pathology: *epidermolysis bullosa simplex*.
- Mutation in the keratin gene.
- Bullous epithelial destruction upon minor mechanical effects.

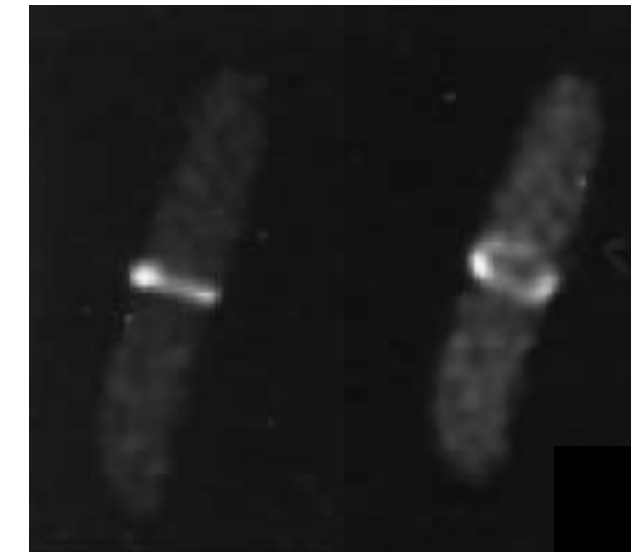




# Prokaryotic cytoskeleton



FtsZ: tubulin homolog

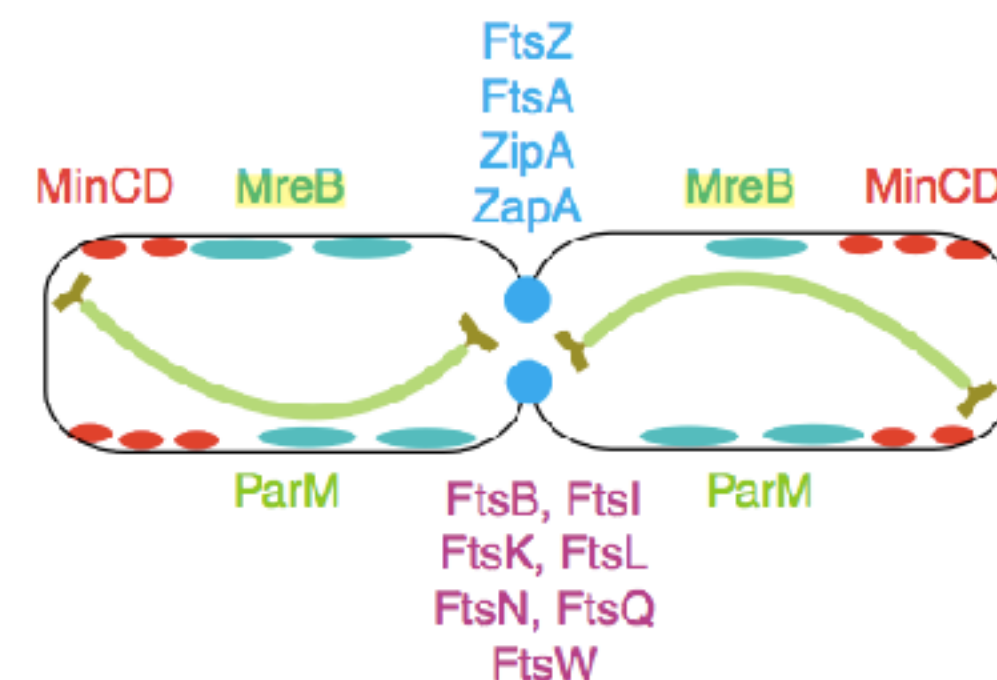


- Main component of the Z-ring
- Important role in cytokinesis
- Dynamic rearrangement

MreB: actin homolog



- Discovery based on sequence homology
- Helical filaments underneath cell membrane
- Role in chromosome segregation



# MOTOR PROTEINS

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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

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## 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

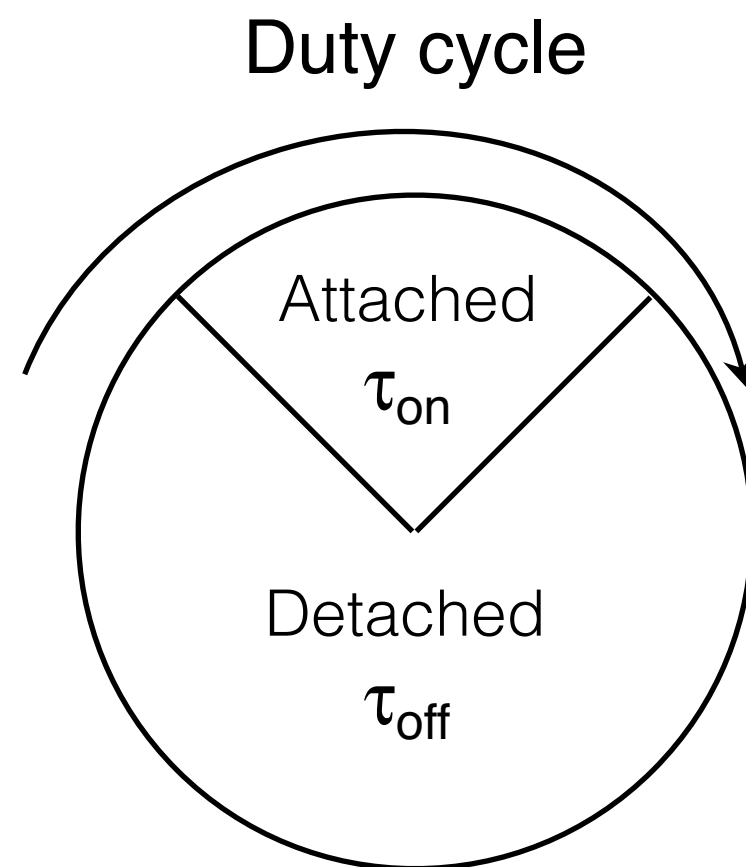
Ribosome

# Duty cycle of motor proteins

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“Duty ratio”:  $r = \frac{\delta V}{v}$

$\delta$ =working distance  
 $V$ =ATPase rate  
 $v$ =sliding velocity



**Processive motor:**  $r \rightarrow 1$

E.g., kinesin, DNA-, RNA-polymerase.

Remains attached throughout most of the duty cycle.

Carries its load by itself.

**Non-processive motor:**  $r \rightarrow 0$

E.g., myosin.

Remains detached throughout most of the duty cycle.

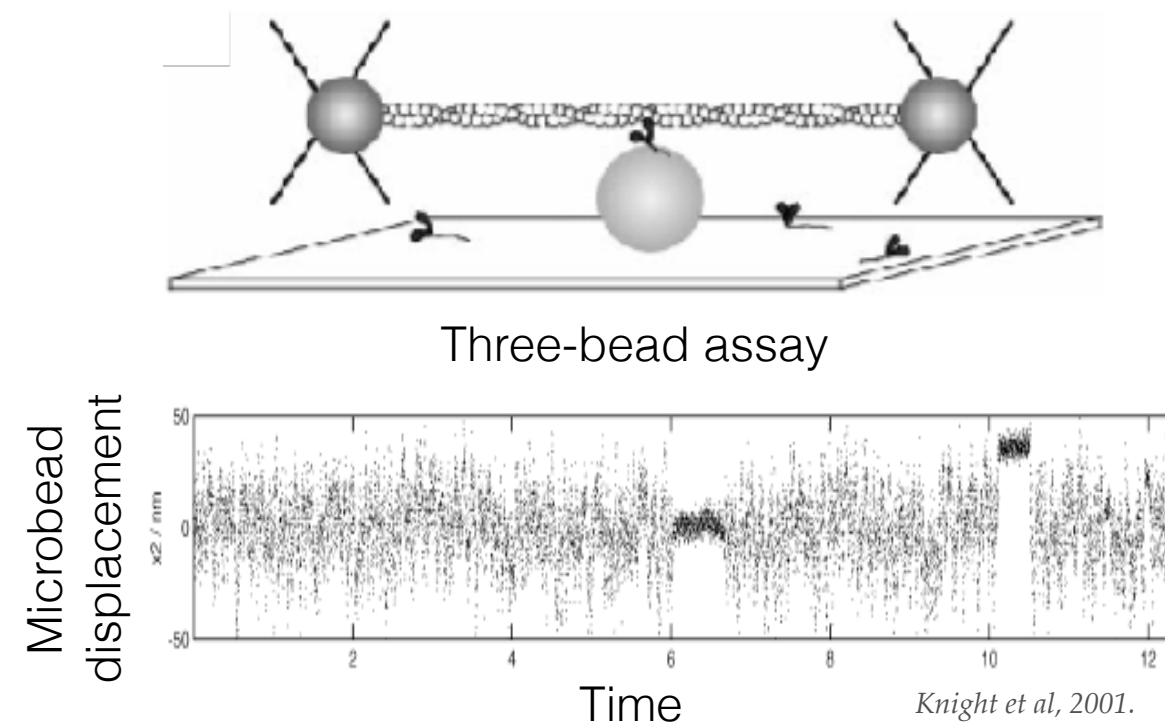
Works in ensembles.

Force generated by a single motor protein: **few pN.**

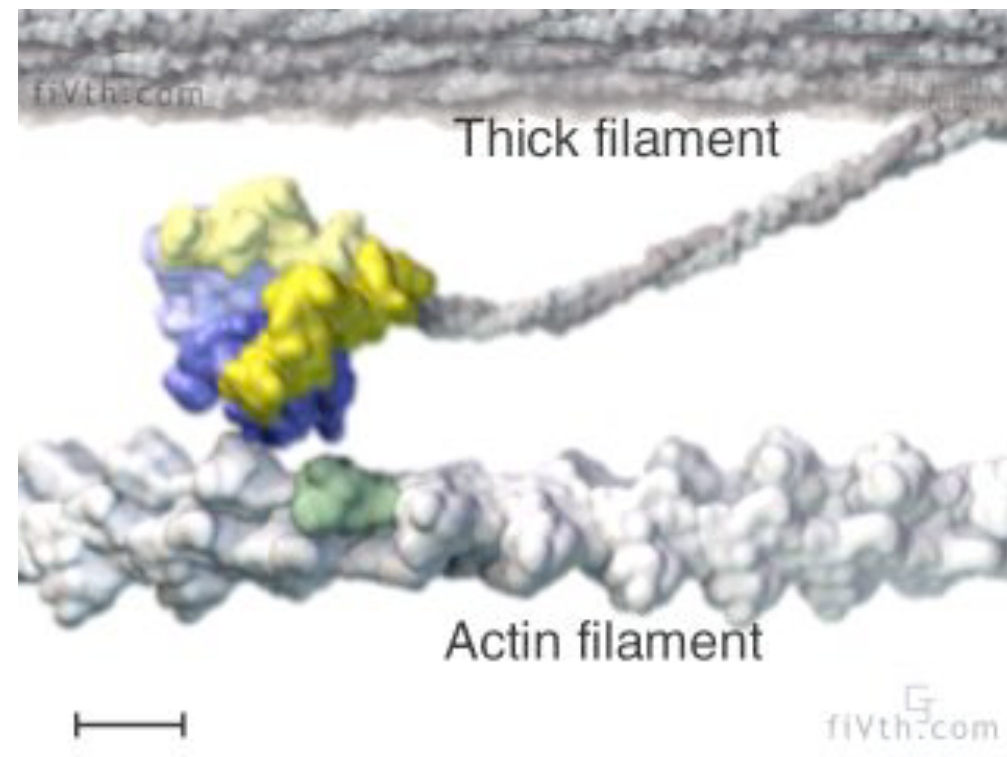
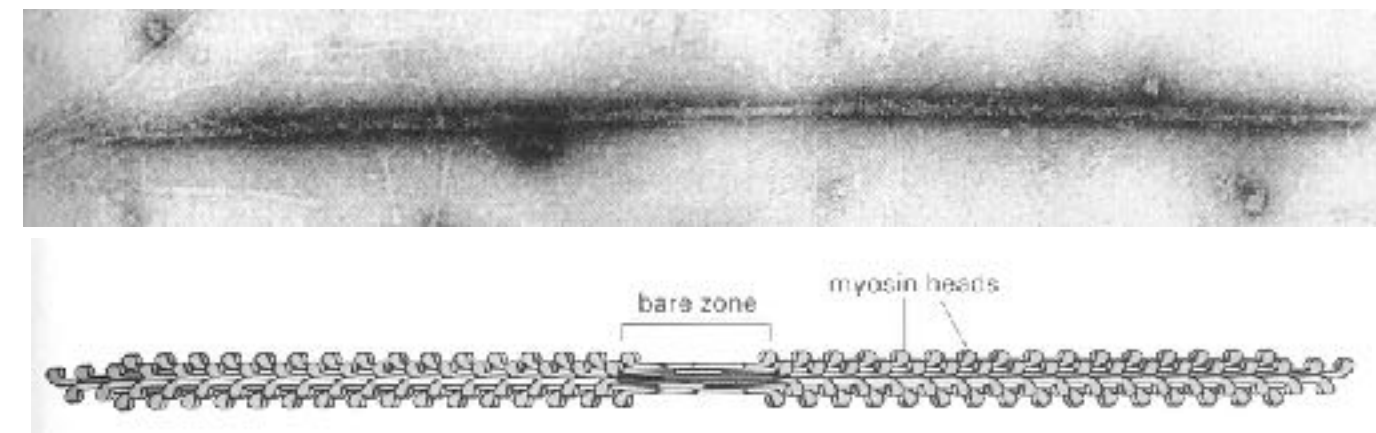


# Non-processive motor proteins

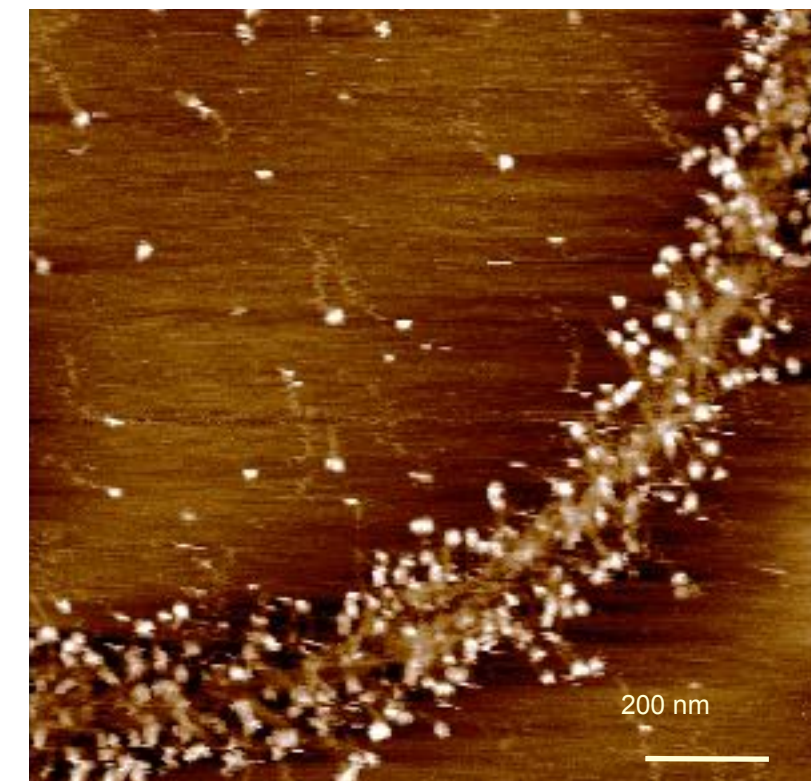
## Muscle myosin



Non-processive motors work in ensembles



Step size: 5.5 nm  
(distance between neighboring actin subunits)

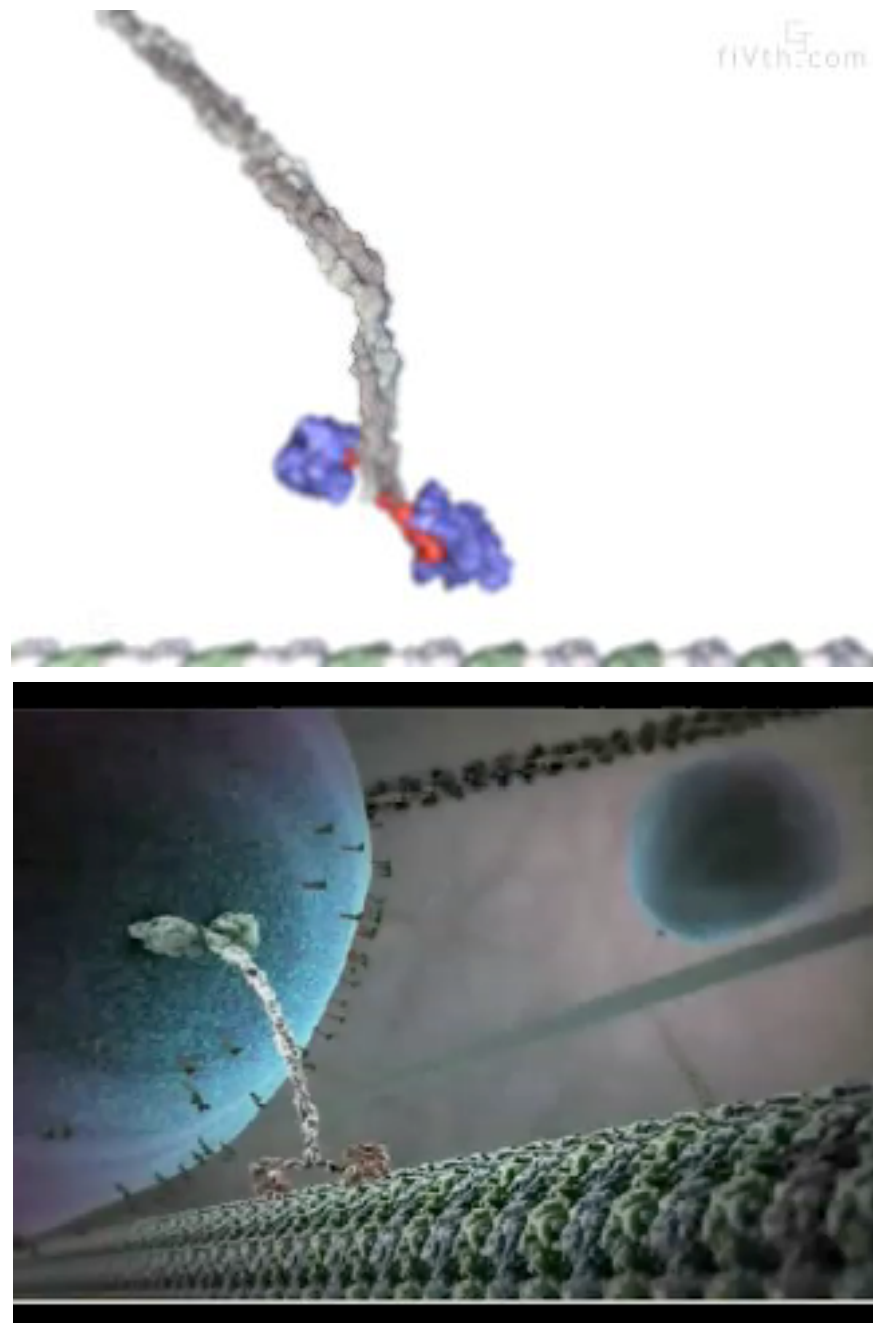


Synthetic thick filament  
AFM image

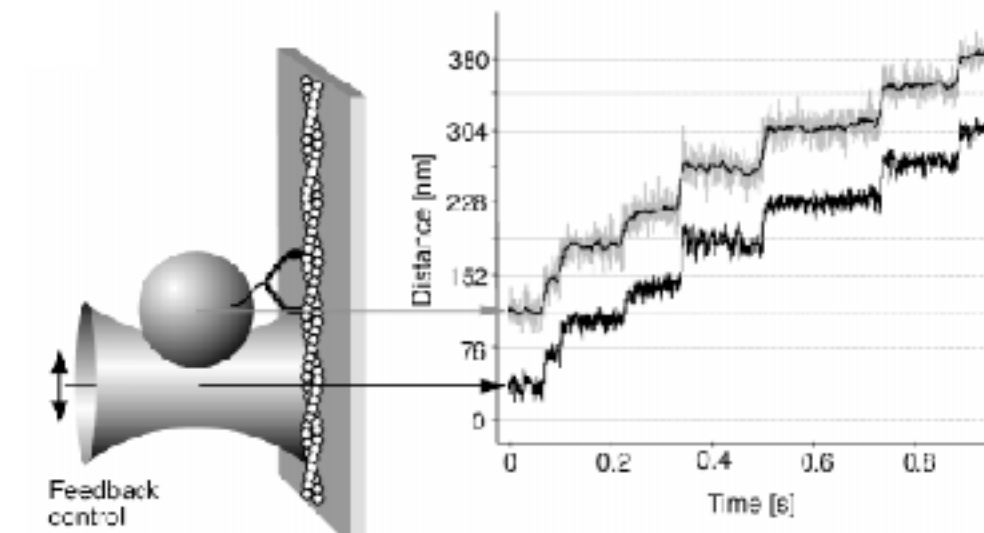
# PROCESSIONAL MOTOR PROTEINS

## Kinesin

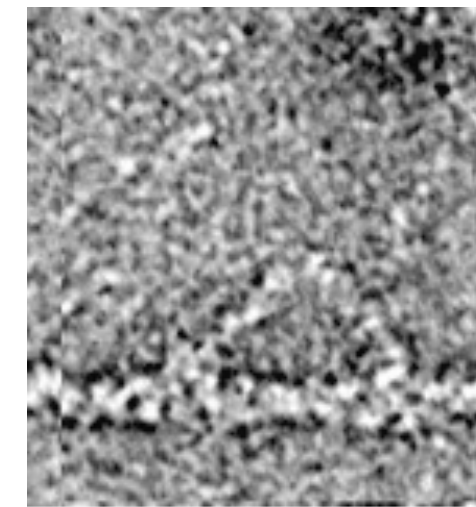
Step size: 8 nm  
(distance between every other tubulin subunit)



## Myosin V



Step size: ~36 nm  
(half pitch along actin helix)



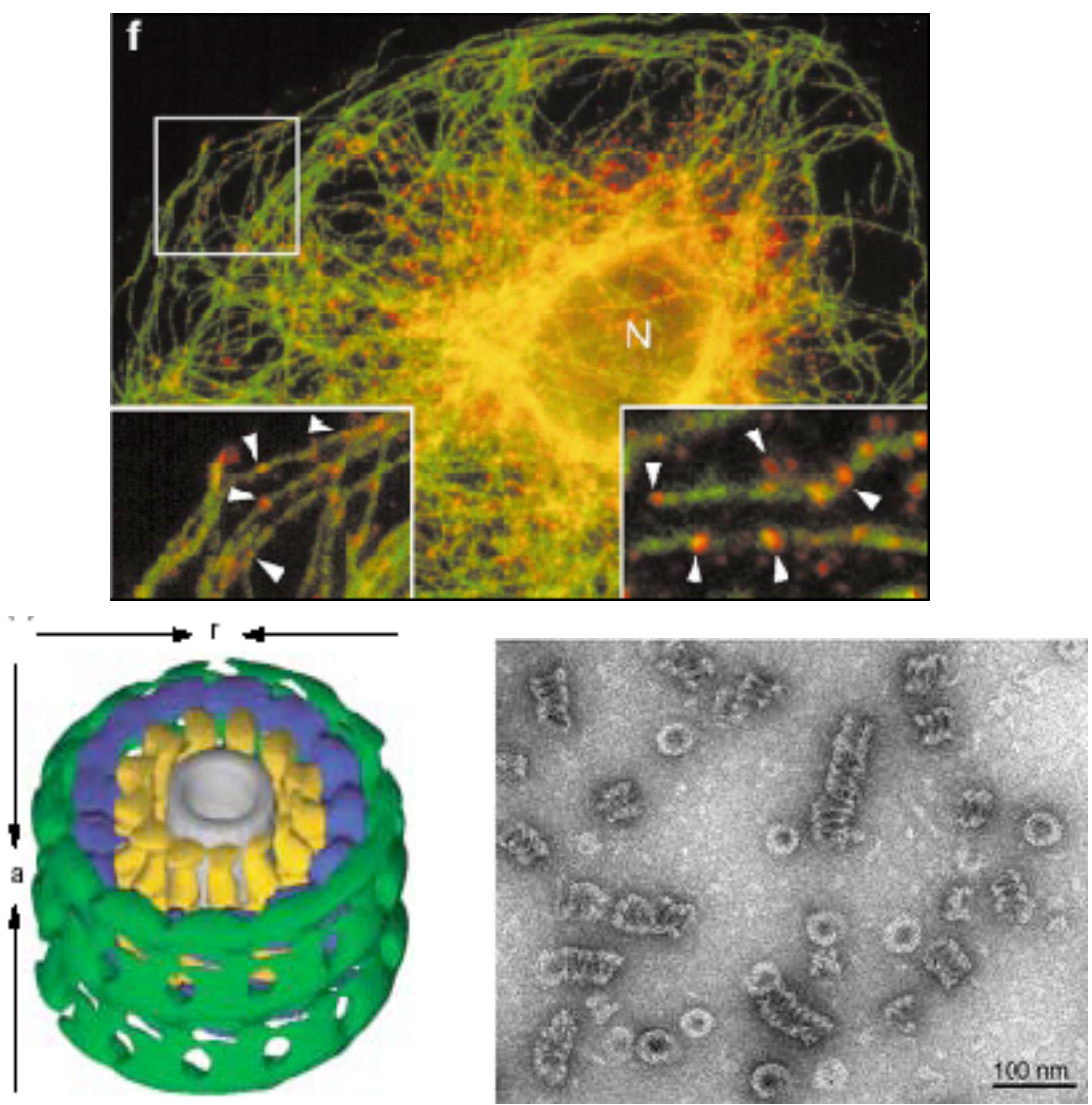
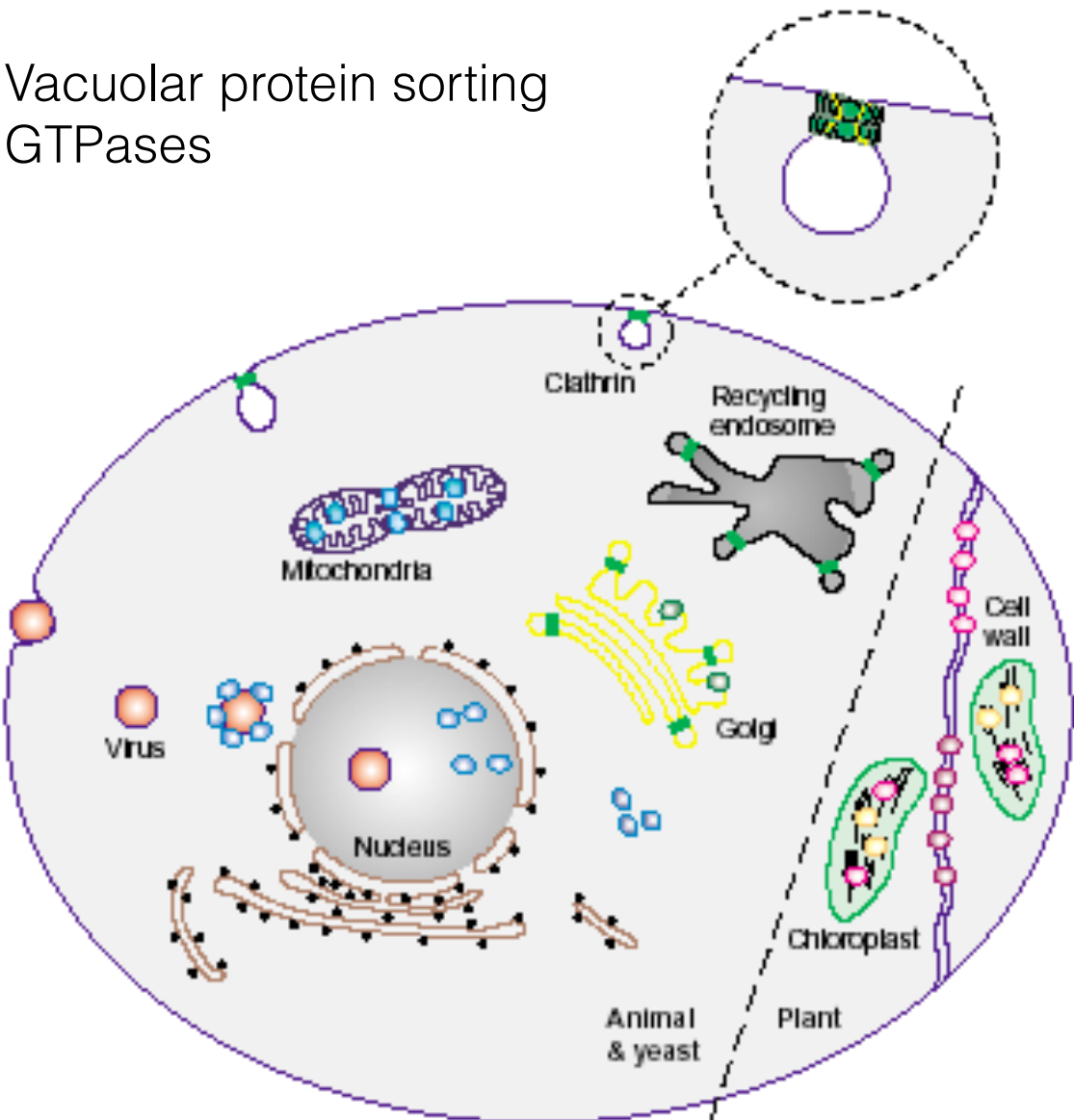
The Muscle Group, Leeds 2000

Processive motors work alone.



# Dynamins

Vacuolar protein sorting  
GTPases

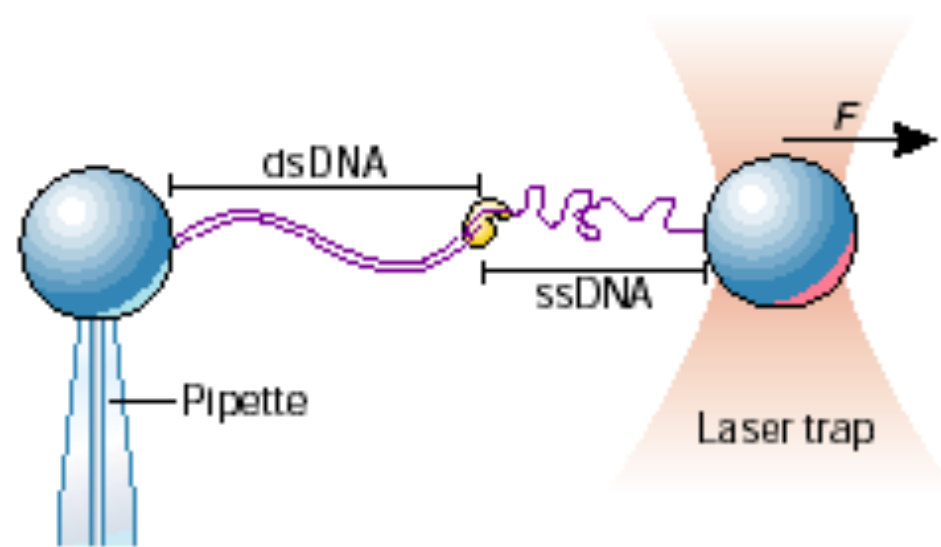


Protein	Localization	Function	Self-assembly
<span style="color: green;">■</span> Dynamin	Plasma membrane (clathrin coated, caveolae), Golgi, endosomes	Vesicle formation, fission	+
<span style="color: blue;">○</span> Vps1	Golgi	Vesicle formation and transport	Unknown
<span style="color: blue;">○</span> Drm1/Drp1/DRP-1	Mitochondria outer membrane	Mitochondrial fission & morphology	+
<span style="color: blue;">○</span> Mgm1/Msp1/OPA1	Mitochondria inner or outer membrane, or matrix	Mitochondrial morphology	Unknown
<span style="color: pink;">○</span> Phragmoplastin	Cell wall	Membrane morphology	+
<span style="color: pink;">○</span> ADL1	Cell wall, chloroplast	Membrane biogenesis	+
<span style="color: yellow;">○</span> ADL2	Chloroplast	Unknown	Unknown
<span style="color: blue;">○</span> hGBP1	Cytoplasm	Anti-viral activity	+
<span style="color: blue;">○</span> Mx	Cytoplasm, nucleus	Anti-viral activity	+

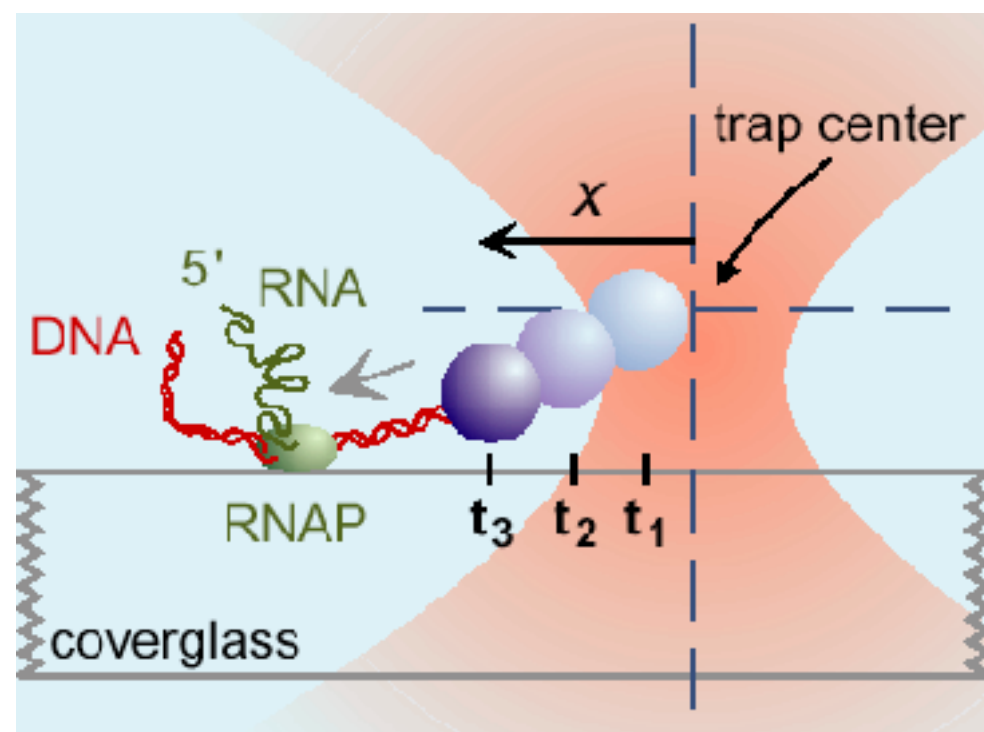


“pinchase” function

# DNA Motors

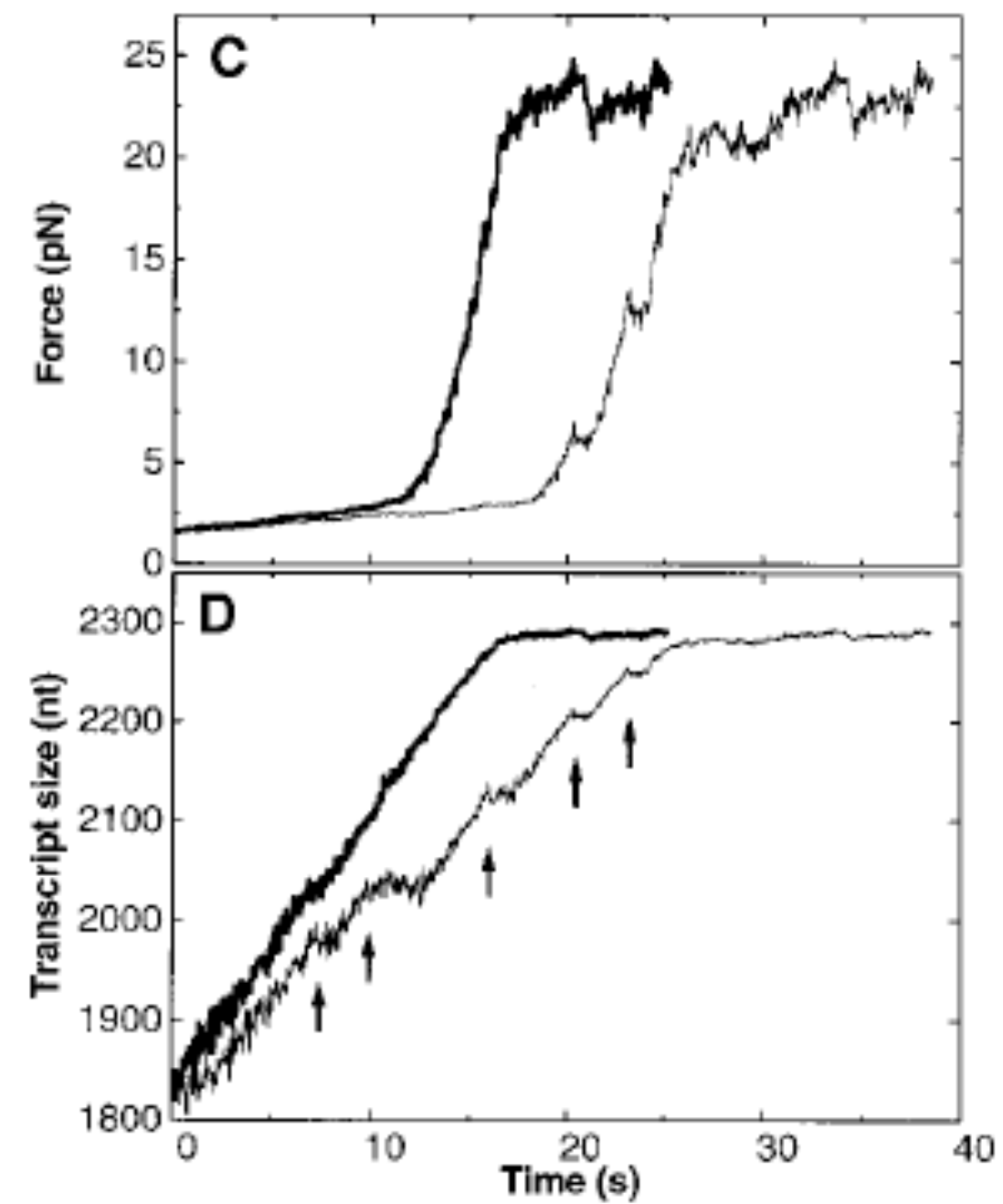


T7 DNA Polymerase



RNA Polymerase

Processive motors

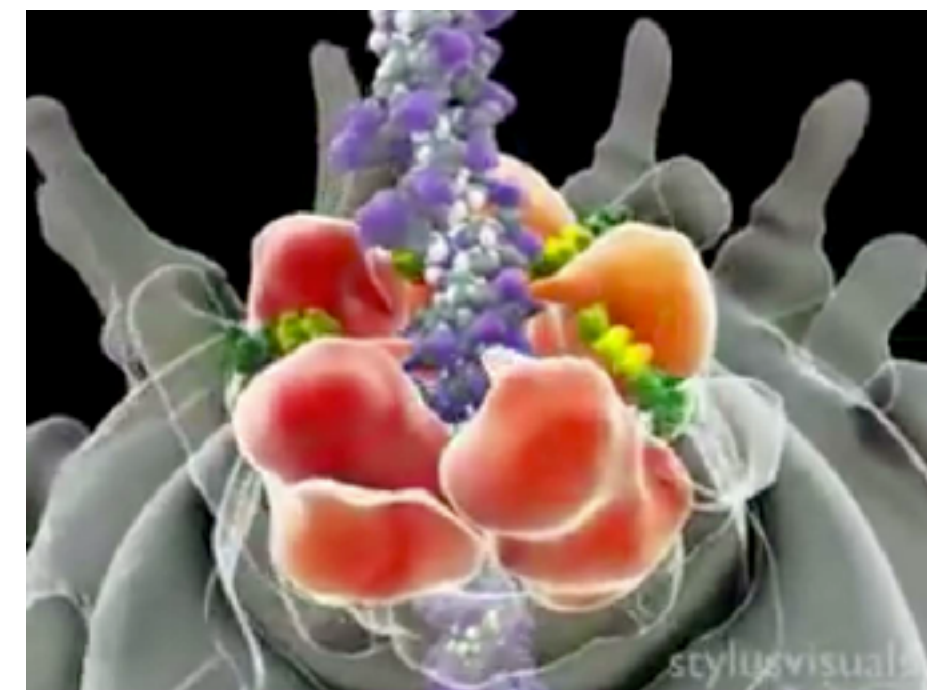
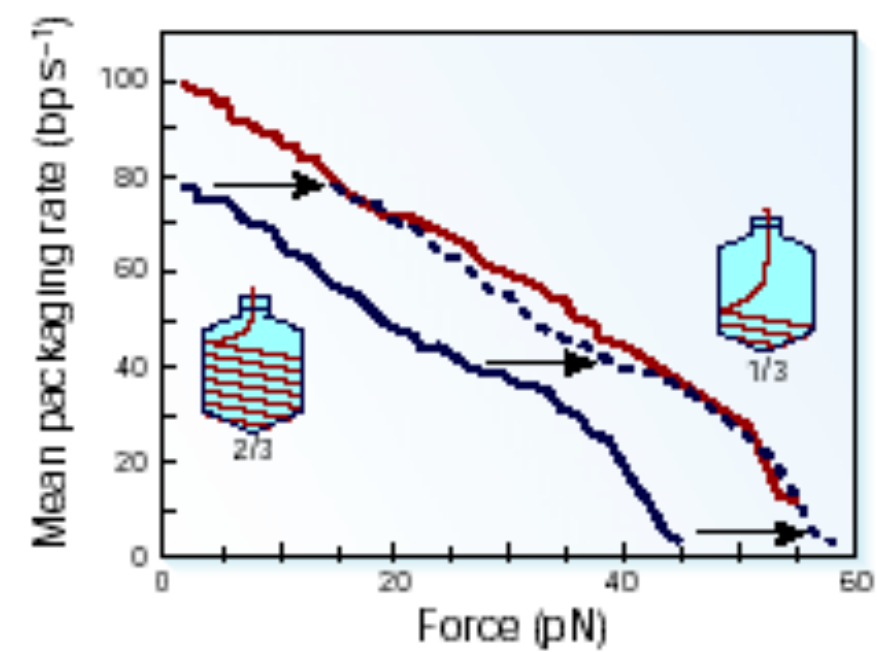
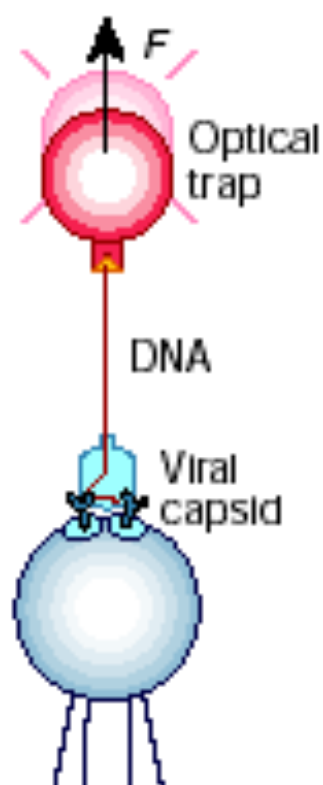
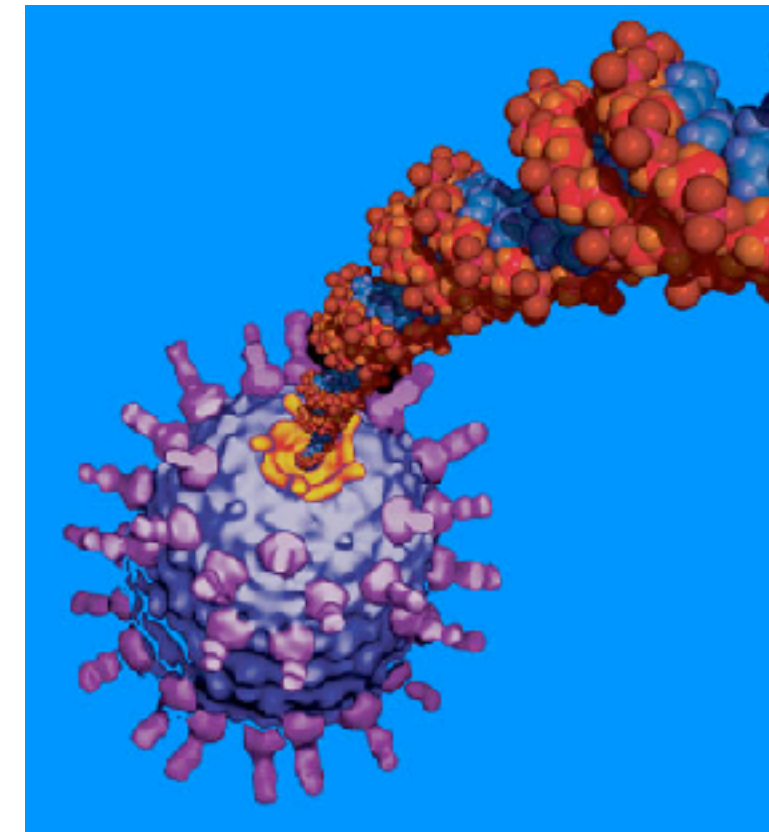
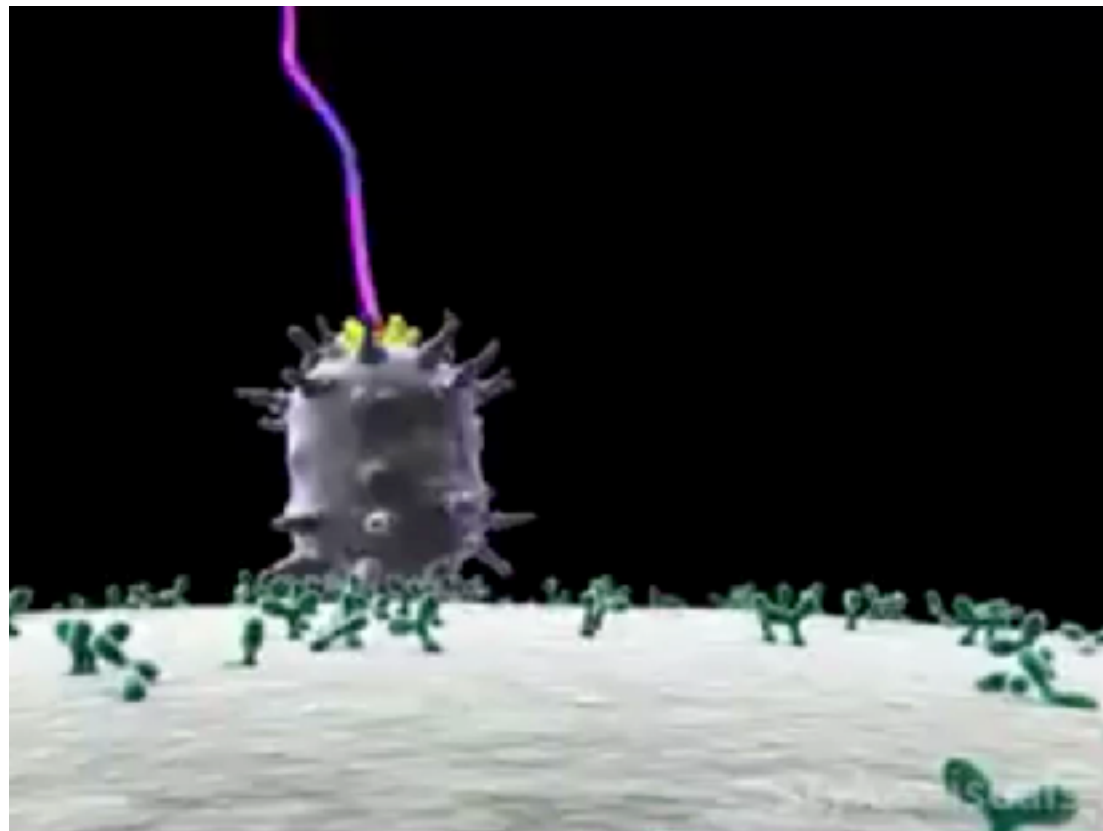


*RNA Polymerase, Wang et al. 1998.*



# Virus portal motor

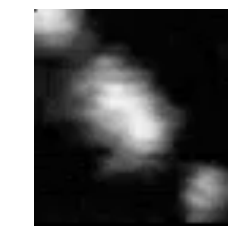
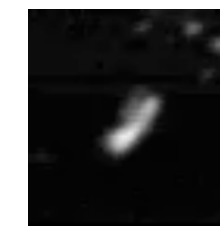
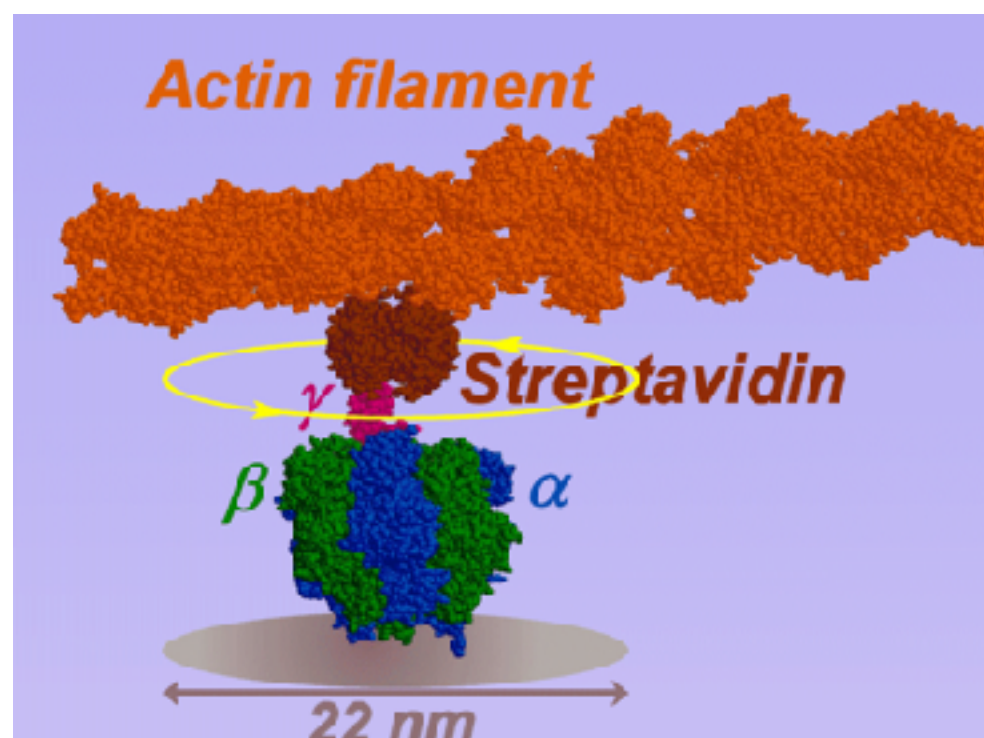
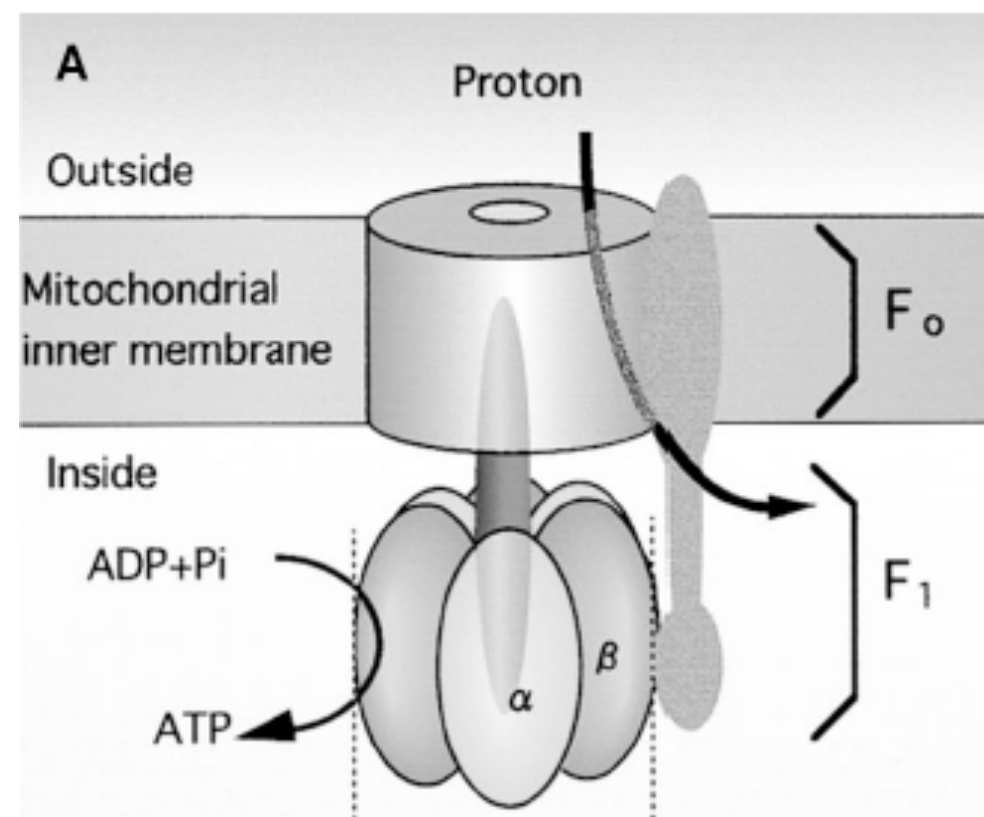
## Special DNA motor



$\phi$ 29 bacteriophage portal motor

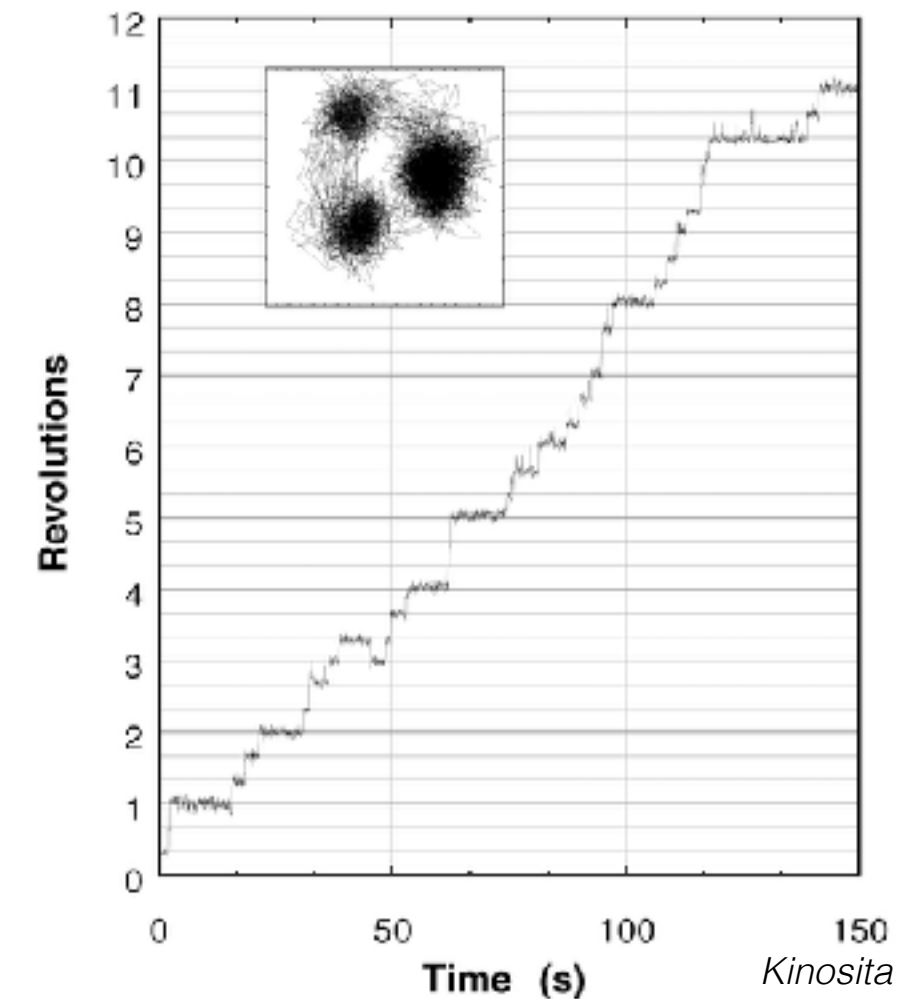
# ROTARY MOTORS I:

## F<sub>1</sub>F<sub>0</sub>-ATP Synthase



20 nM ATP    200 nM ATP

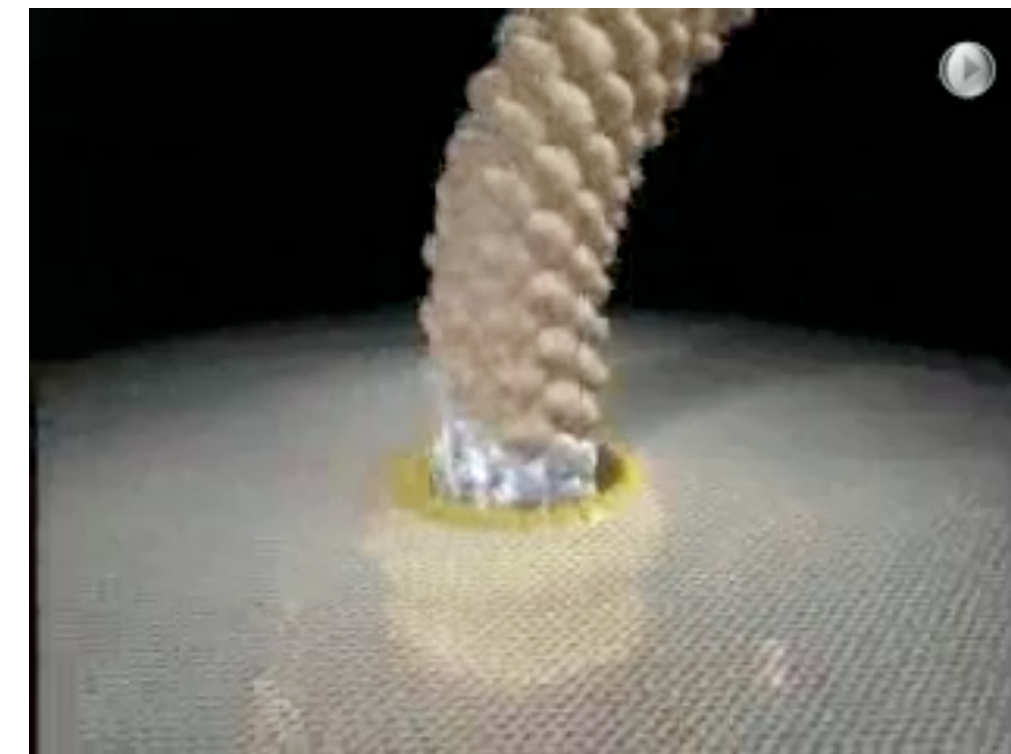
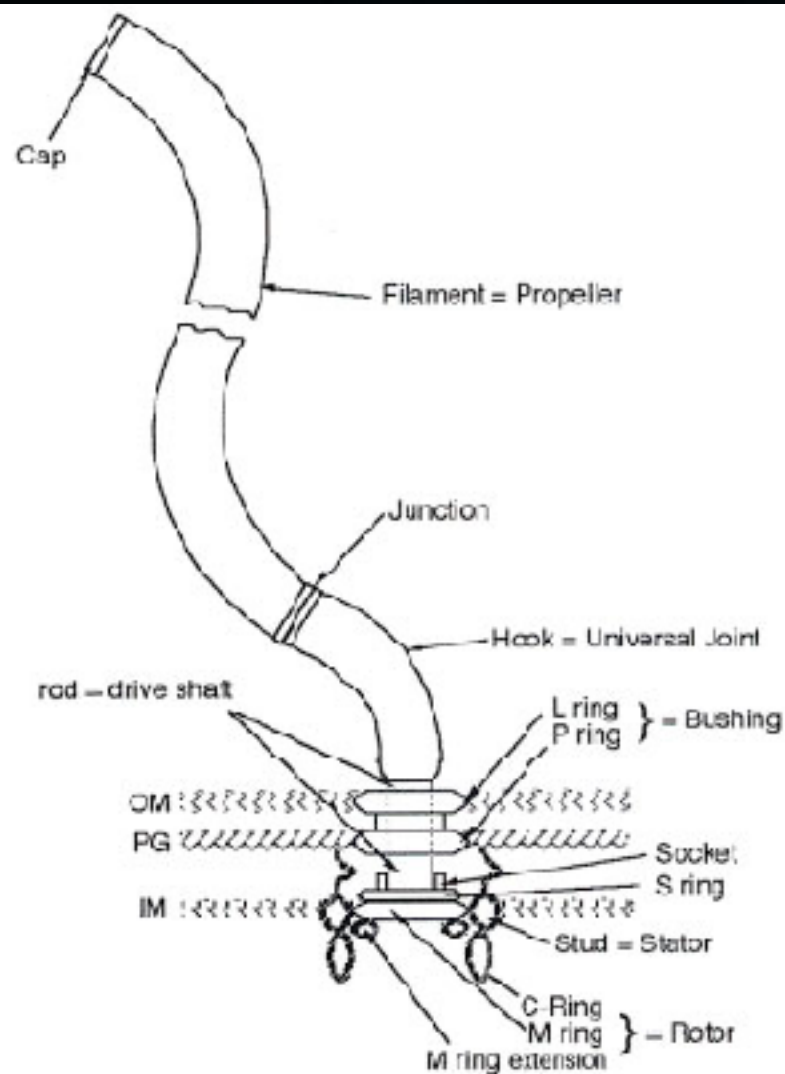
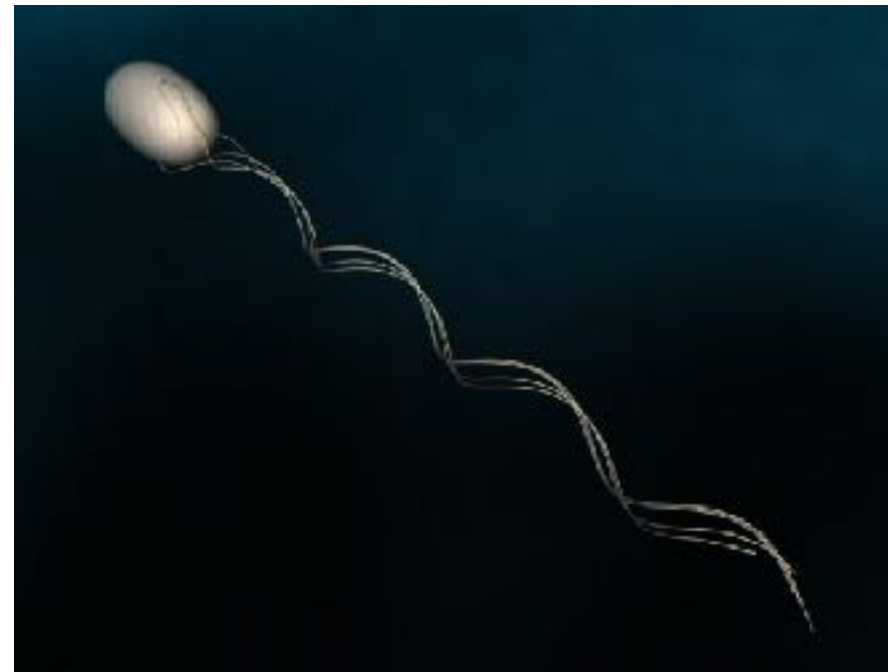
Discrete 120° rotational steps





# ROTARY MOTORS II:

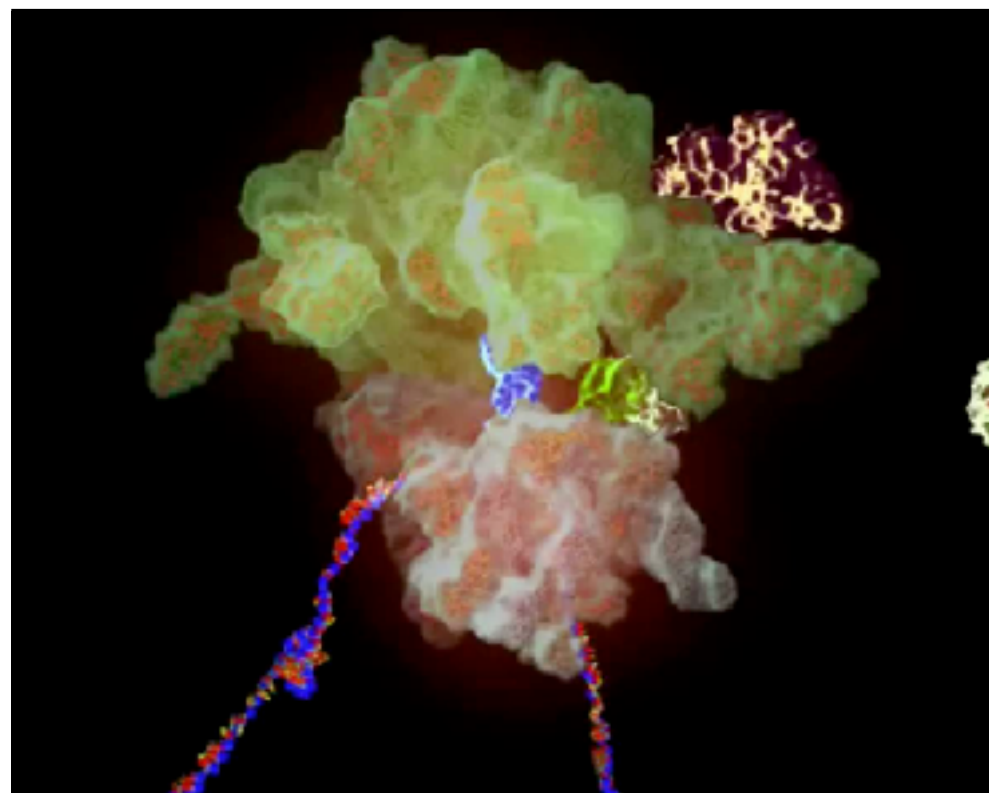
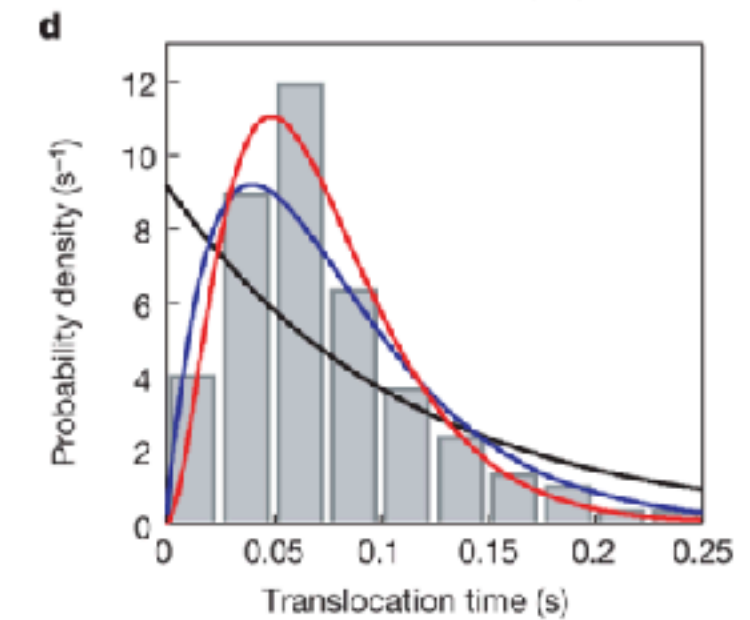
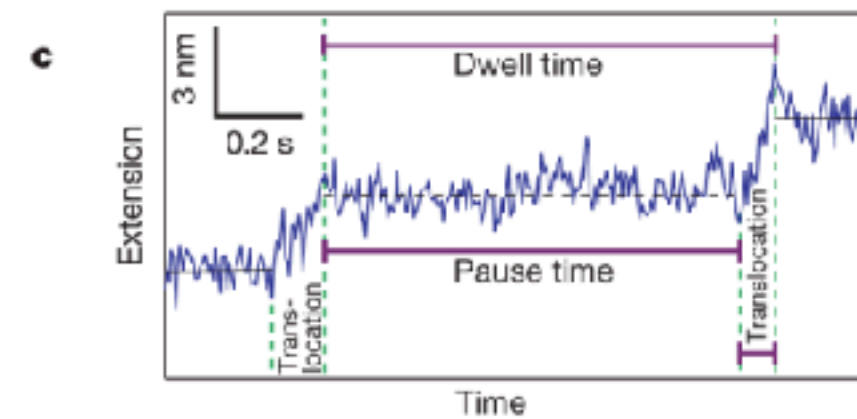
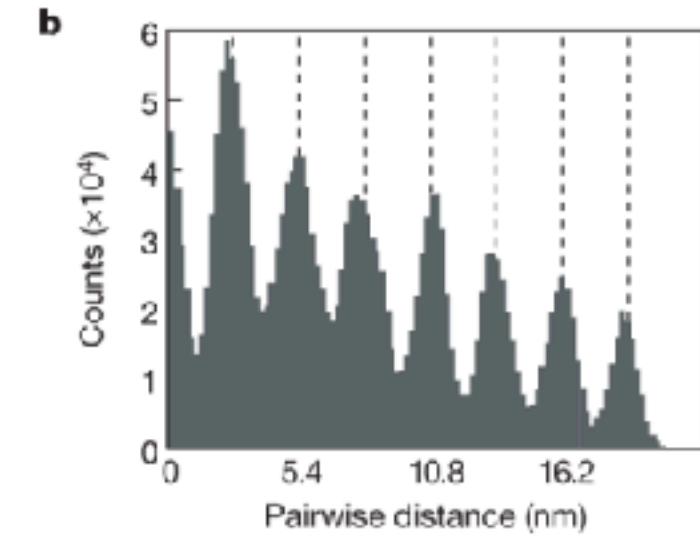
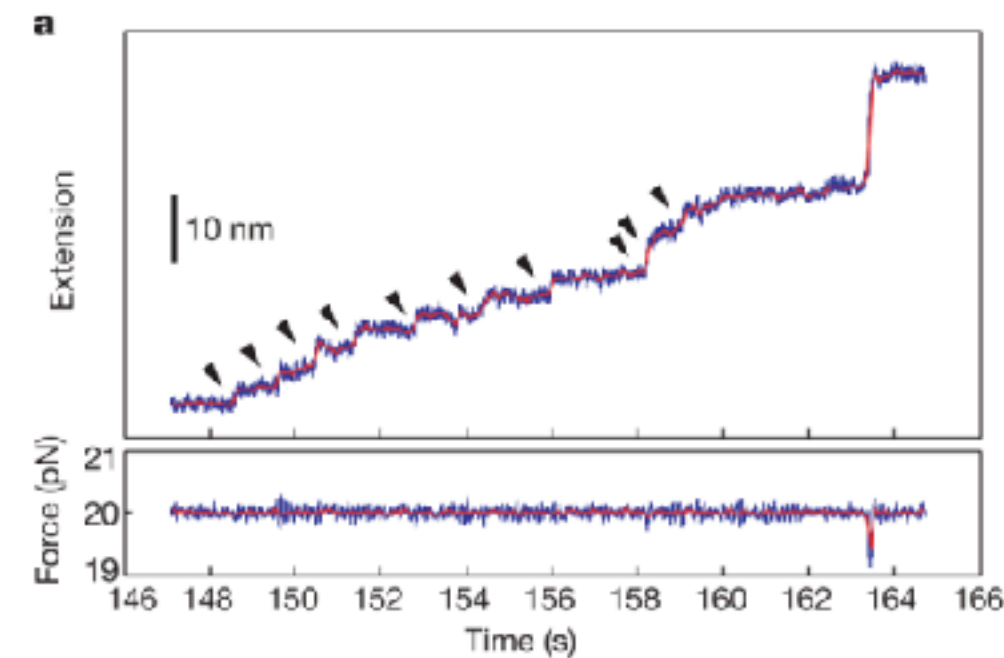
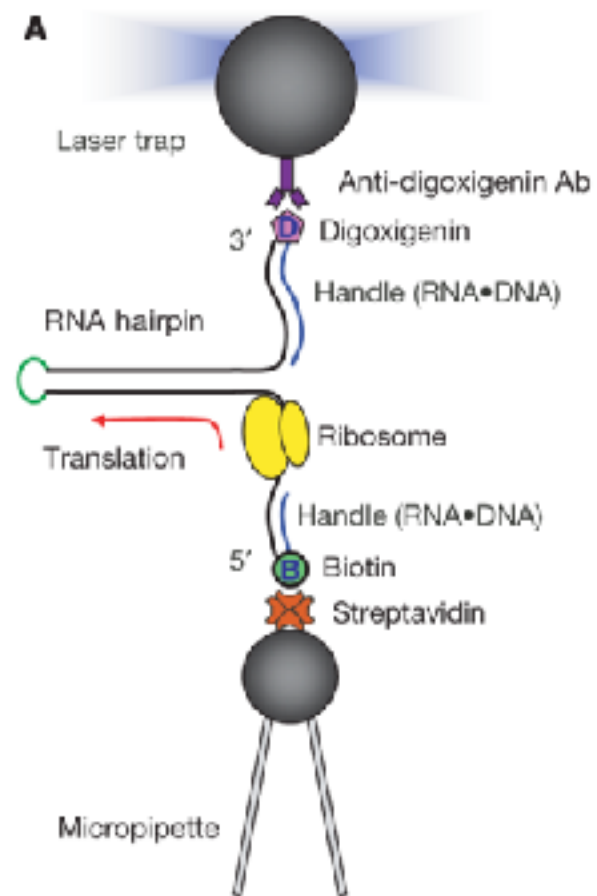
## Bacterial flagellar motor



Speed: > 20000 rpm  
 Energy consumption:  $10^{-16}$  W  
 Efficiency: > 80%  
 Energy source: protons

# Mechanoenzyme complex

## Ribosome



Wen et al. Nature 2008

- 2.7 nm steps (one triplet)
- 0.078 s translocation time
- Helicase activity coupled with translocation