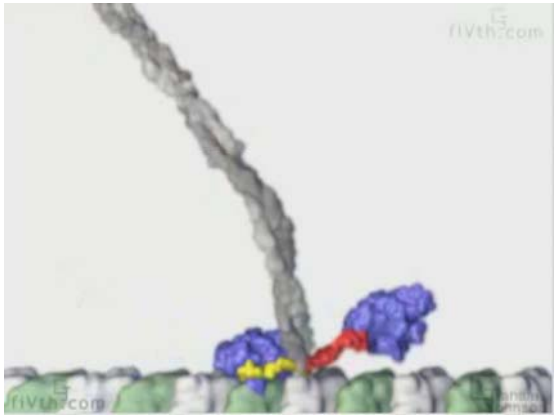
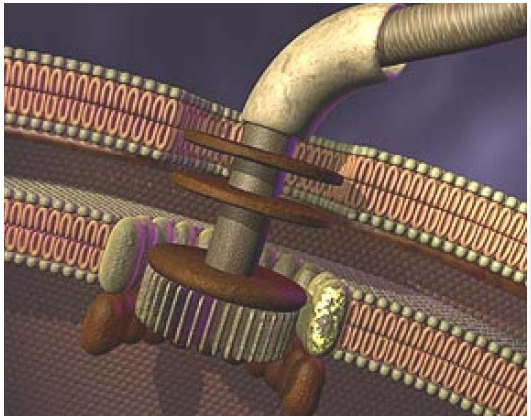
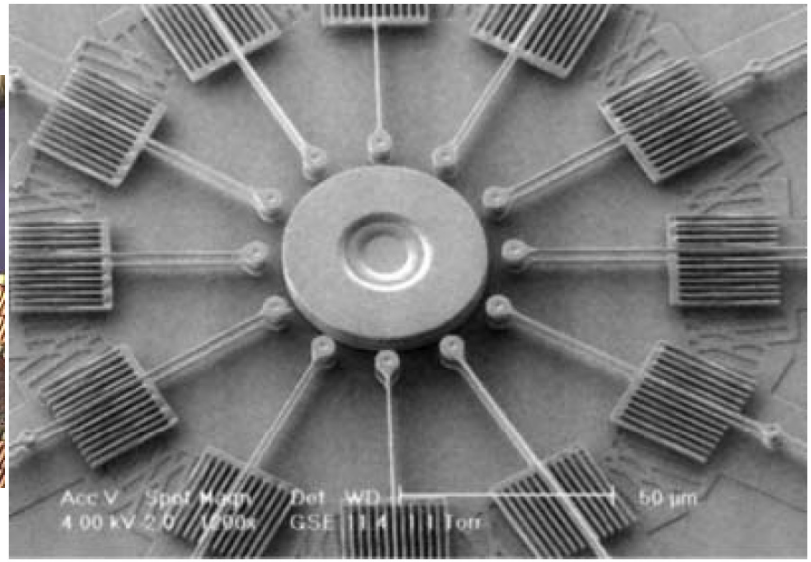
Biological Machines, Cell Mechanics and Nanotechnology







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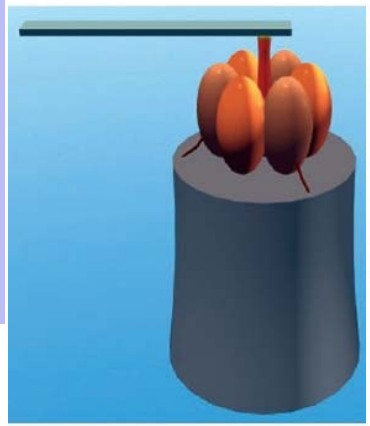
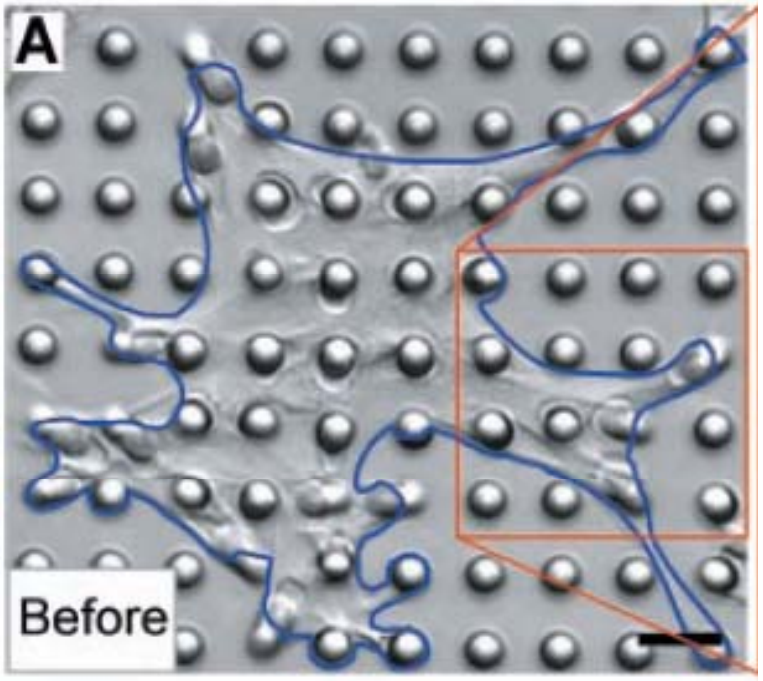
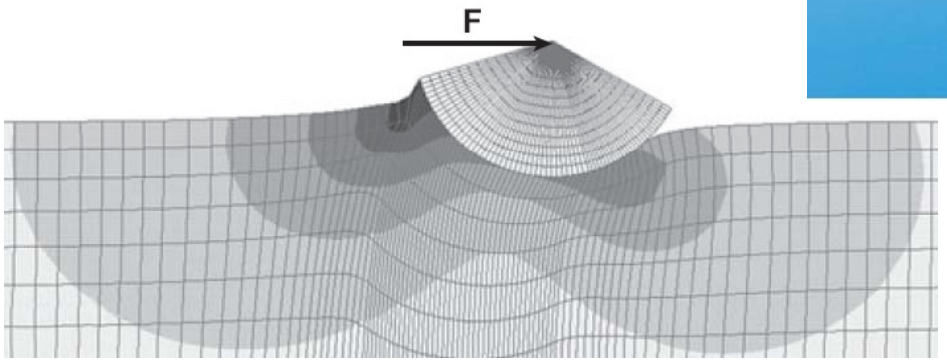
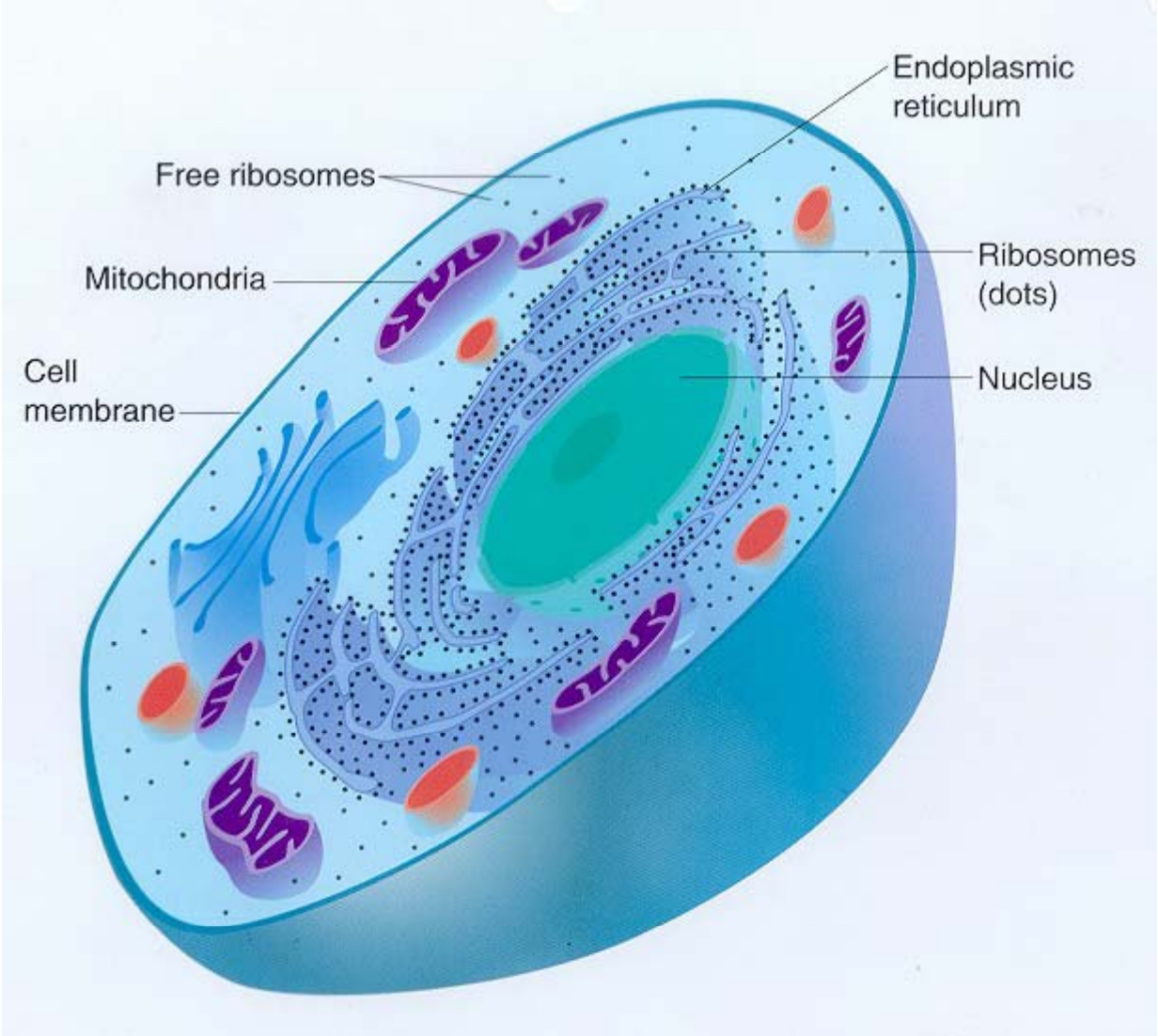


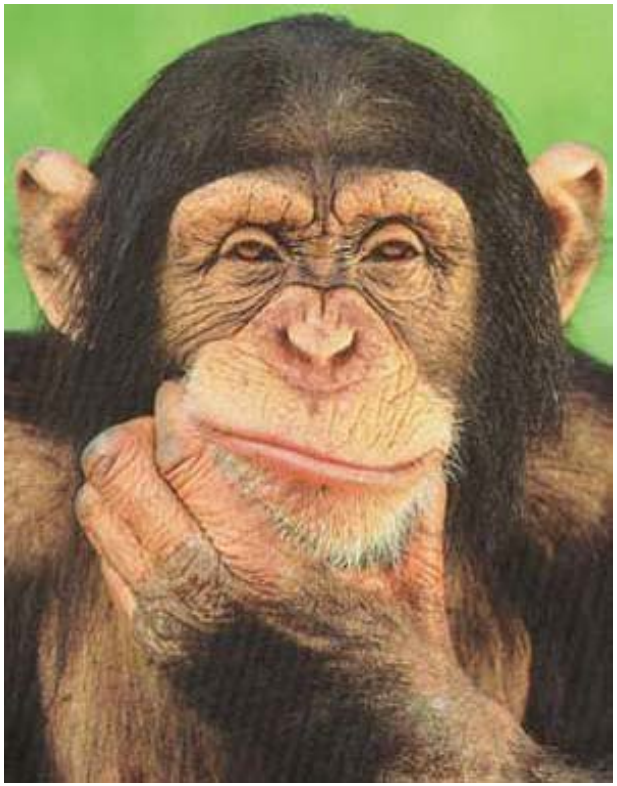




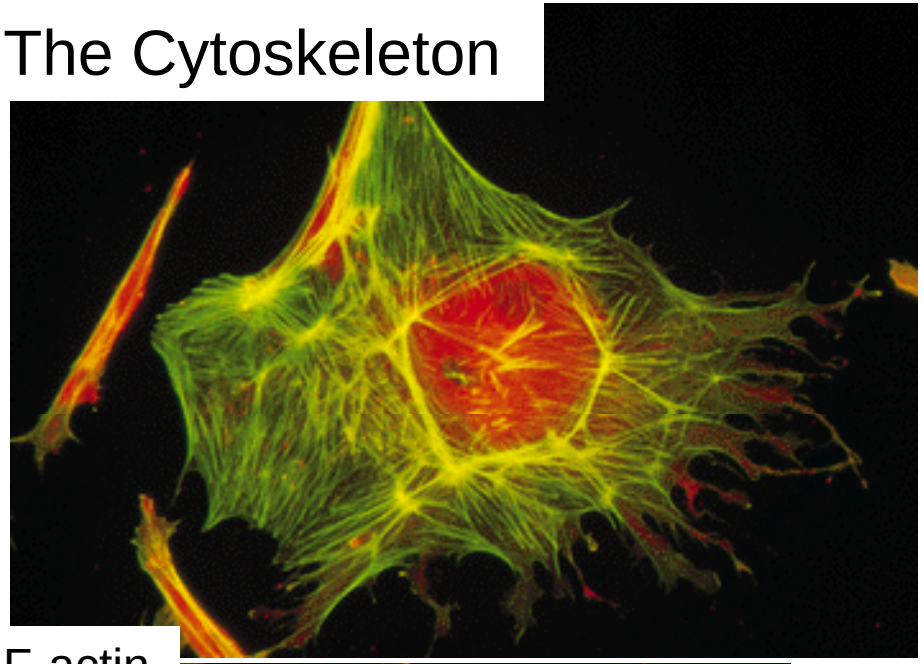
The eukaryotic cell



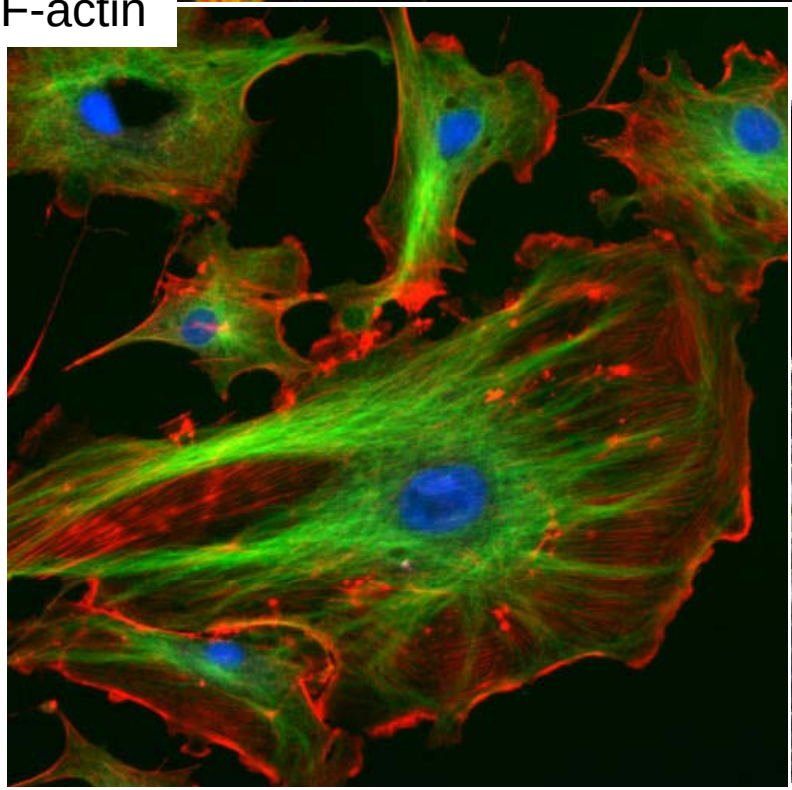
Um... something is missing?



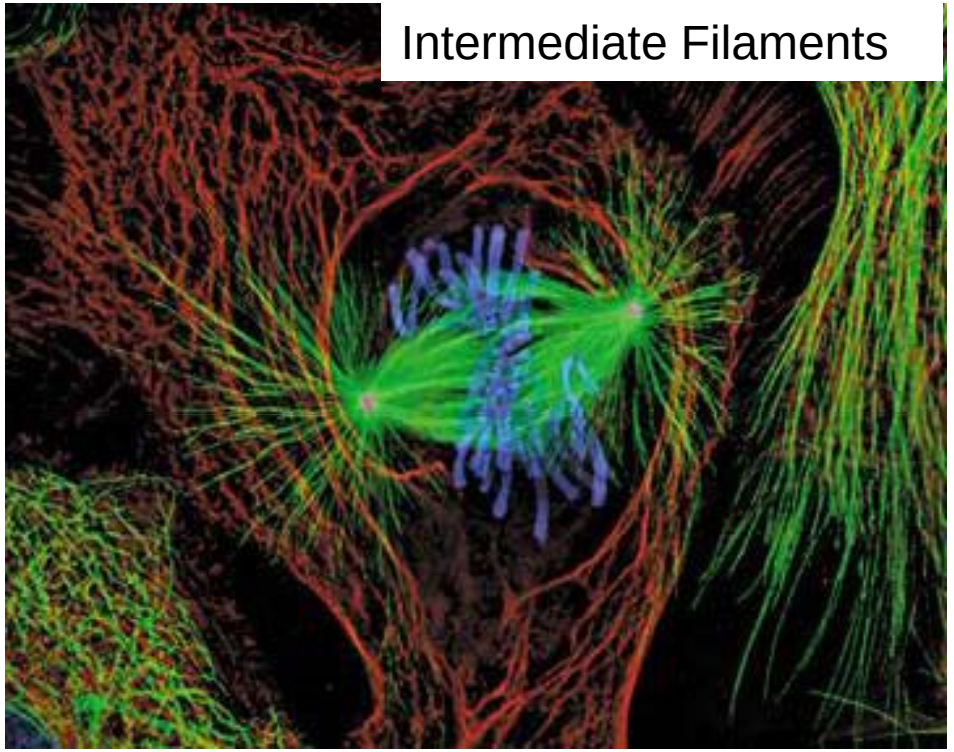
The Cytoskeleton



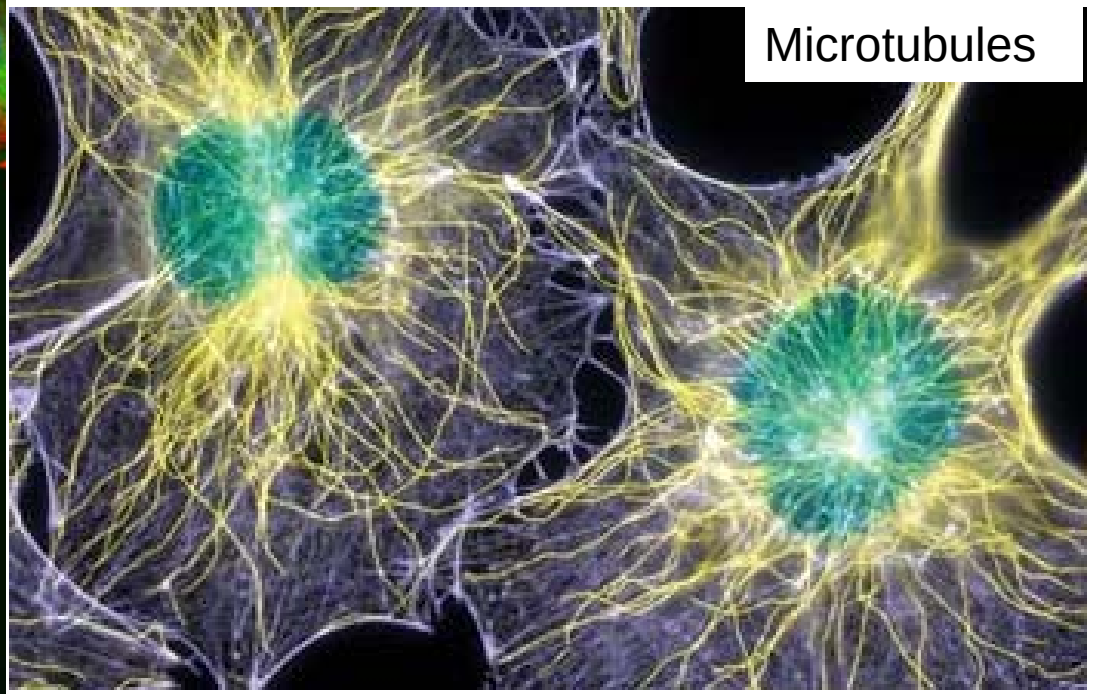
F-actin



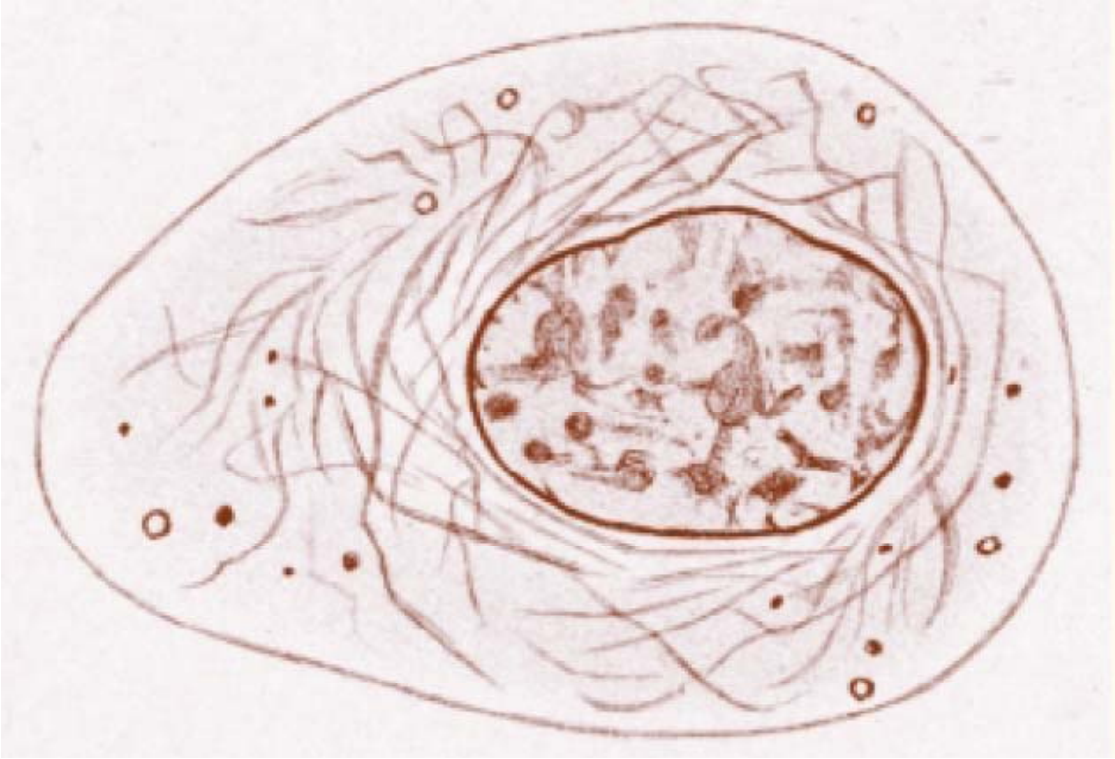
Intermediate Filaments



Microtubules



An early view of the cytoskeleton by W. Flemming (1879)

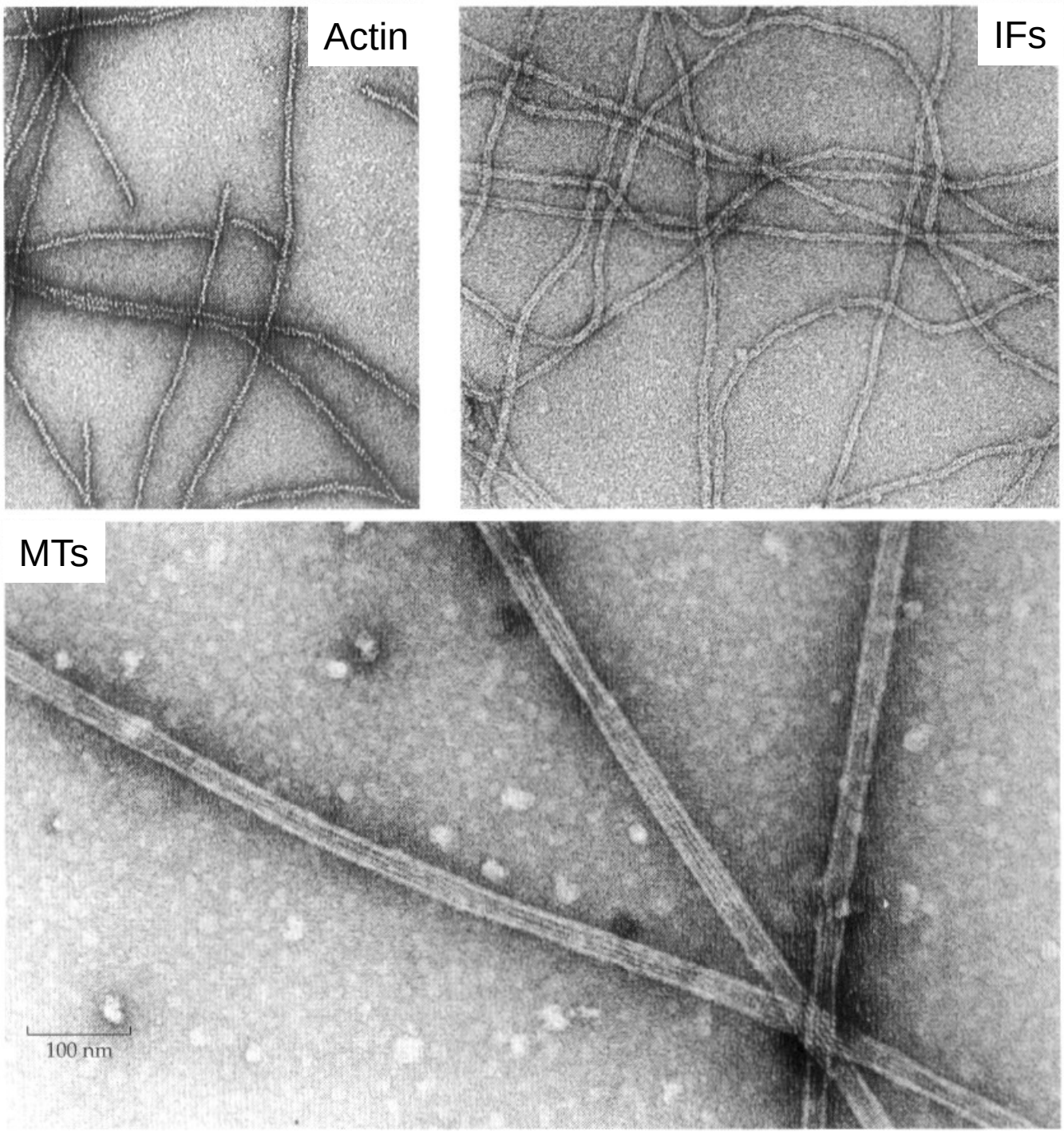


Flemming, W., *Arch. Mikrosk. Anat.* 16, 302–436 (1879)

"What are those wispy structures in the cytoplasm?"

Cytoskeleton of **cartilage cells** described as "threads" (*'Fäden')*

Flemming (when he looked at **epithelia cells**): "On reaching the plasma, one sees nothing at all."



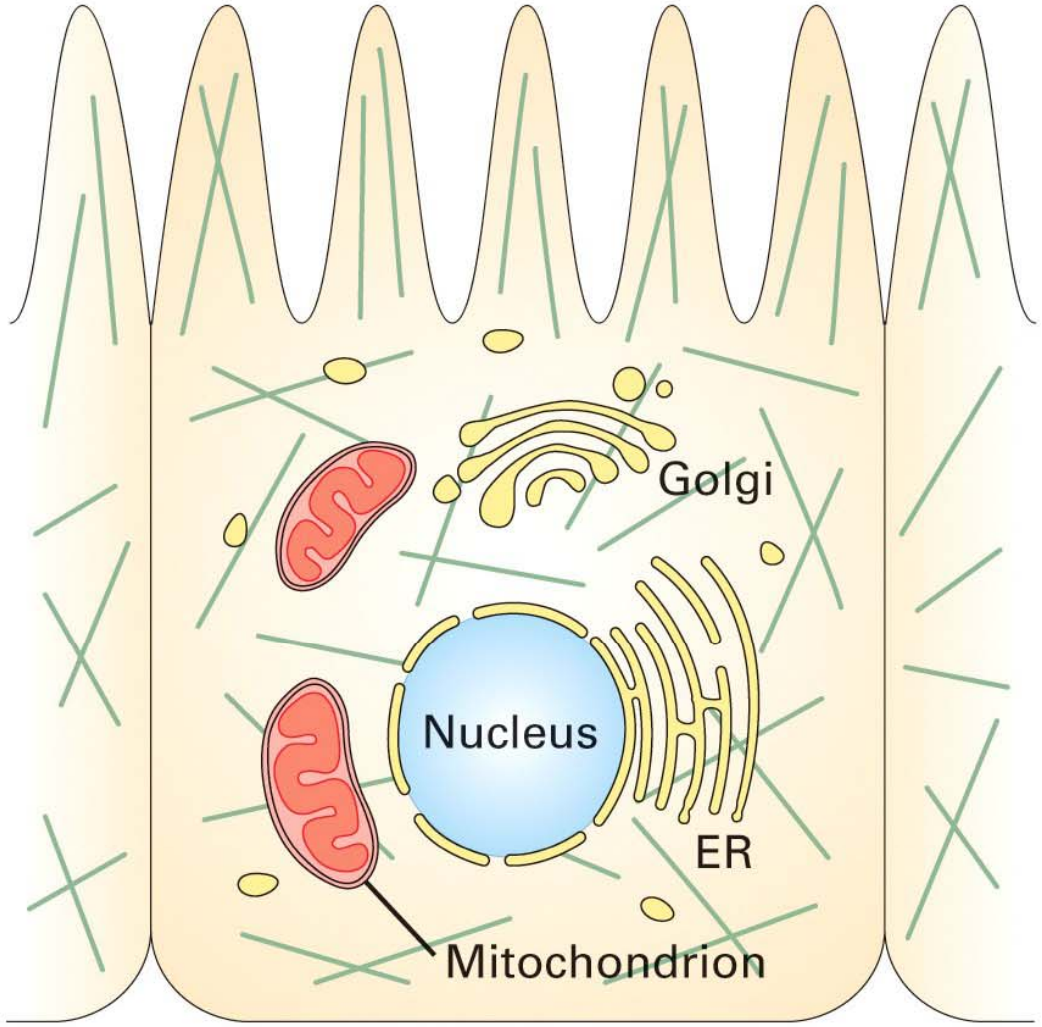
Cytoskeletal elements drawn to scale

Howard, Mechanics of Motor Proteins, 1st Ed.

The Cytoskeleton

- Important for **cell shape** and **cell stiffness**
- Brings organelles into their correct positions
- **Highway** for molecular motors

$\Rightarrow$ occupies lots of space!



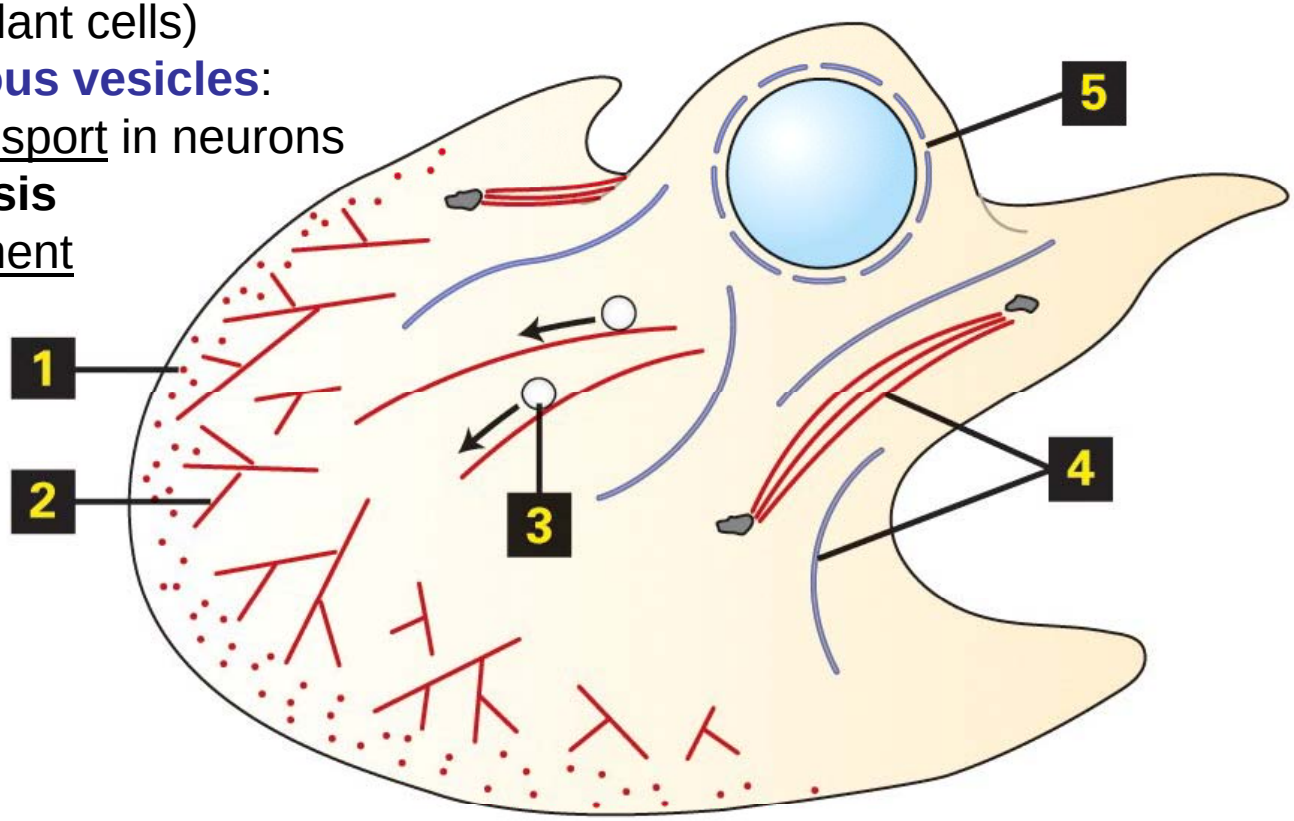
Plasma membrane ($700\ \mu m^2$)

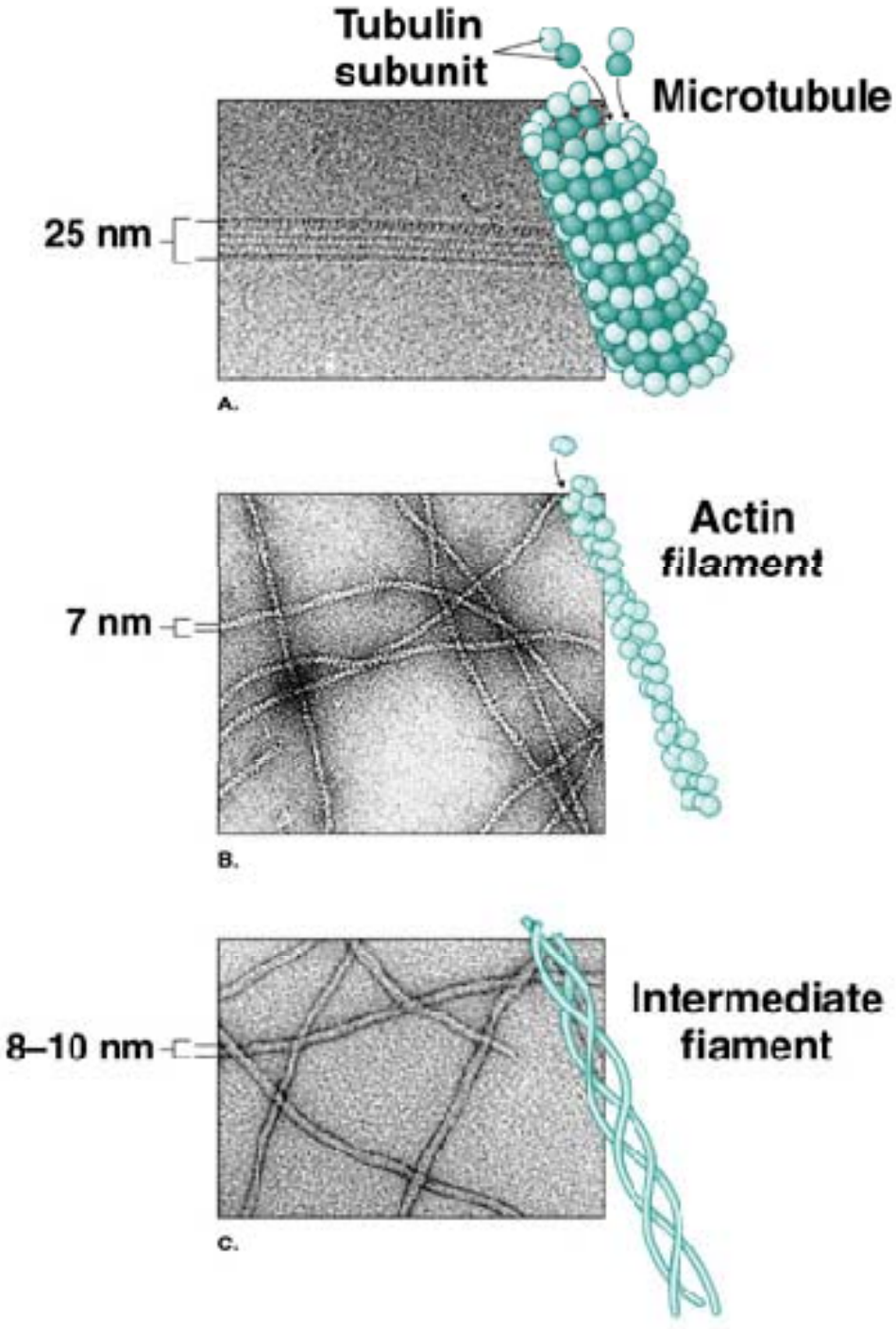
Internal membranes ($7000\ \mu m^2$)

Cytoskeleton ($94{,}000\ \mu m^2$)

The cytoskeleton drives internal movements

- **Separation of chromosomes**
- **Streaming of cytosol** (plant cells)
- **Transport of membranous vesicles**:
  - Synaptic vesicle transport in neurons
  - **Endo- and exocytosis**
  - Mitochondria movement
  - **RNA-granules**
  - Other “cargo”





3 basic cytoskeletal elements

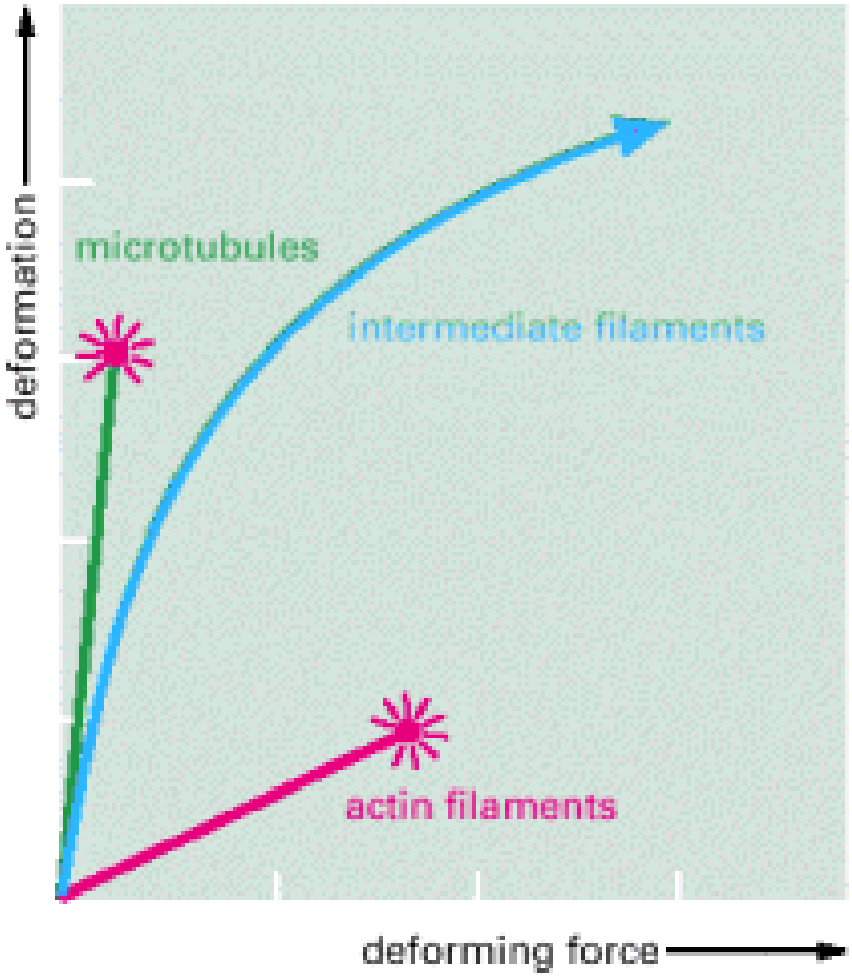
- Cytoskeleton is composed of **3 types of fibers** which are all polymers built from **globular protein subunits**
- The fibers can be **distinguished by** their **diameter**

Actin: twisted, two-stranded (pearl-string like) structure
⇒ **cell shape** and **highway** for molecular motors (cargo transport)
MT: hollow cylinder formed by proto-filaments made of tubulin-subunits
⇒ positioning of organelles; form **flagella**; chromosome separation; **highway**
IFs: rope-like structure
⇒ cell shape and **cell elasticity**

Movie
v20-01-microtubules.mov

Cytoskeletal fibers also differ in their mechanical properties

Based on their specific structures, the 3 types of cytoskeletal polymers exhibit also different elastic properties



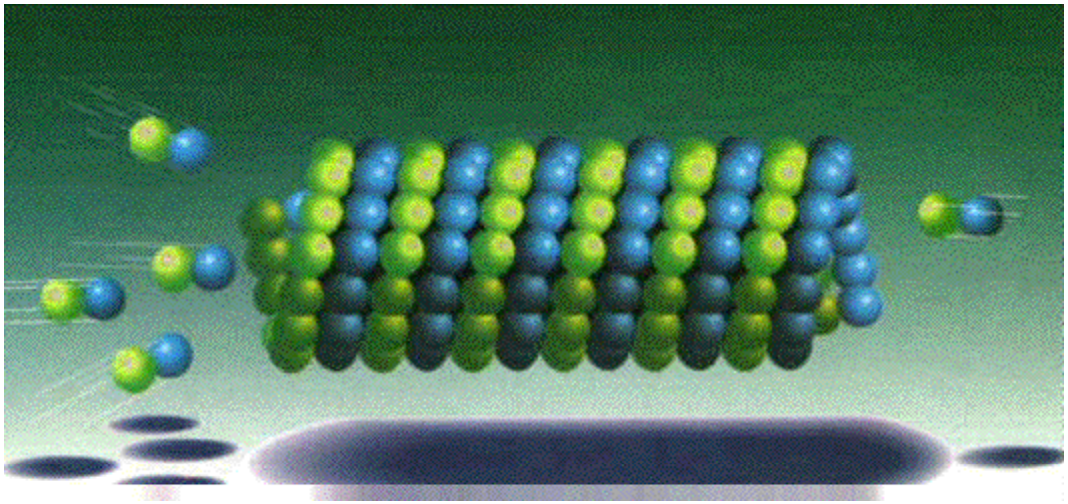
P. Janmey, *JCB*, 1991

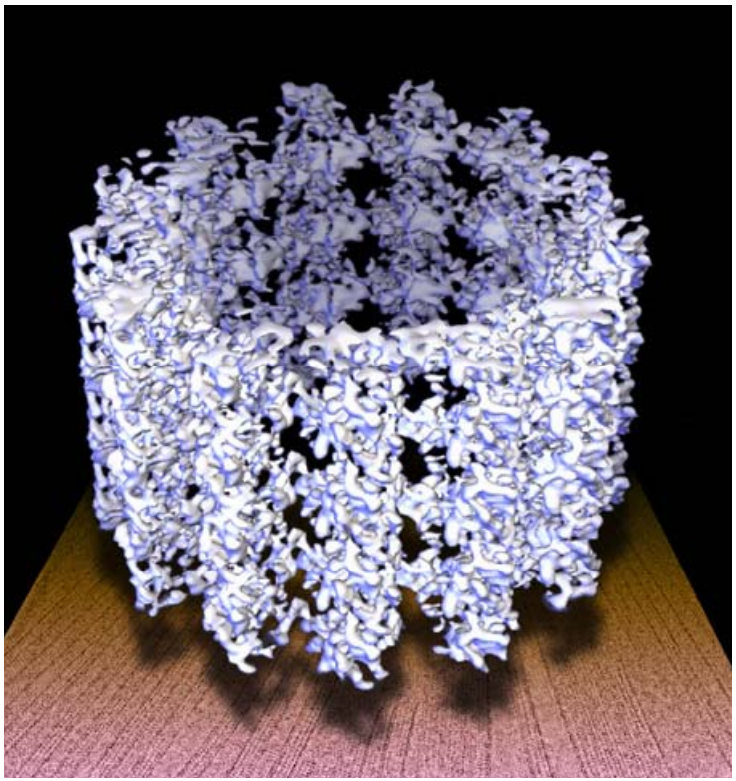
- **Microtubules**, **actin** and **intermediate filaments** (all the same concentrations) were exposed to shear force in a *elastometer* and the resulting degree of stretch was measured

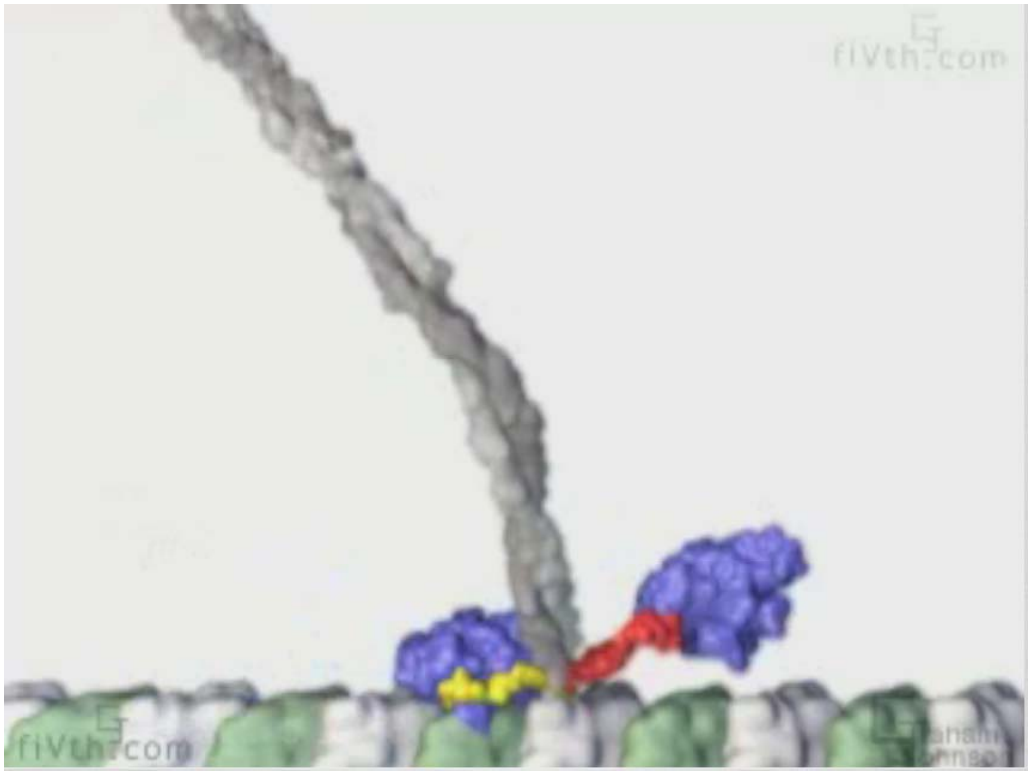
- With increasing deforming force, micro-tubules are the first which cannot resist the strain and start to break following actin

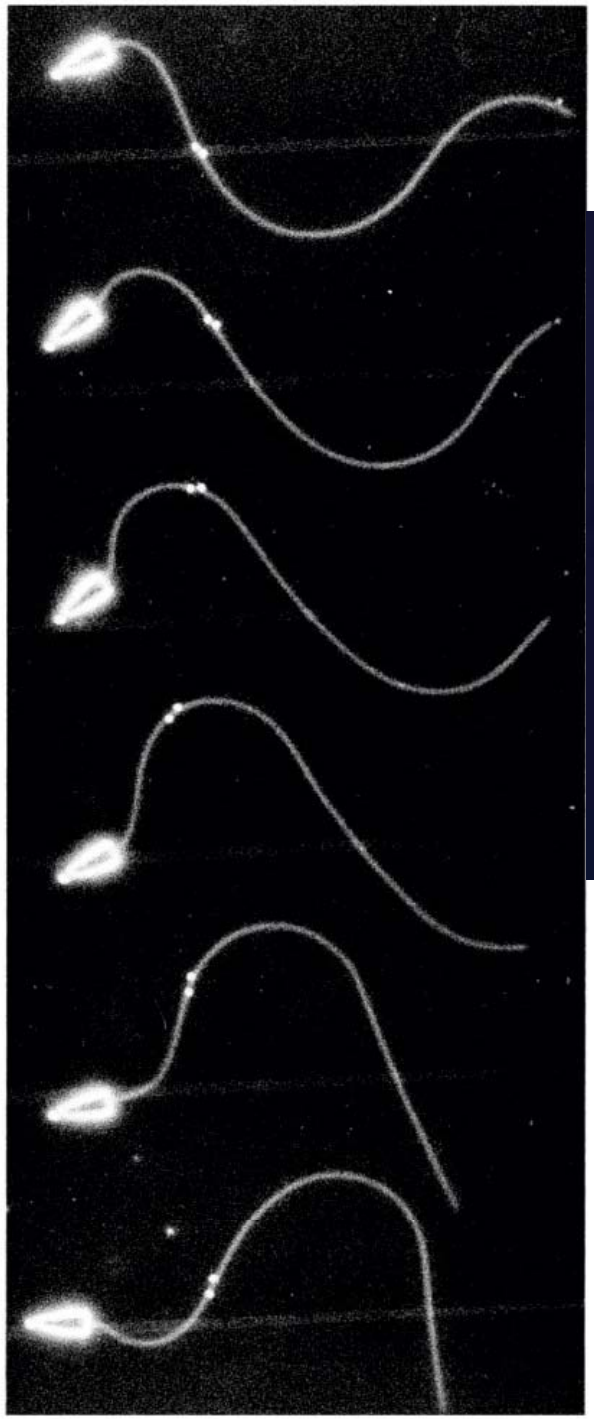
- IFs are the most flexible filaments which resist large deformations

Microtubule-based motors



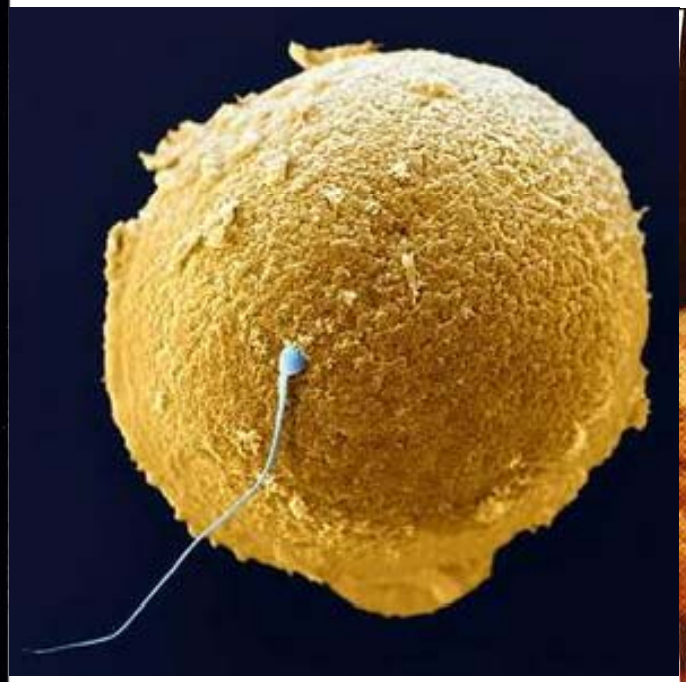


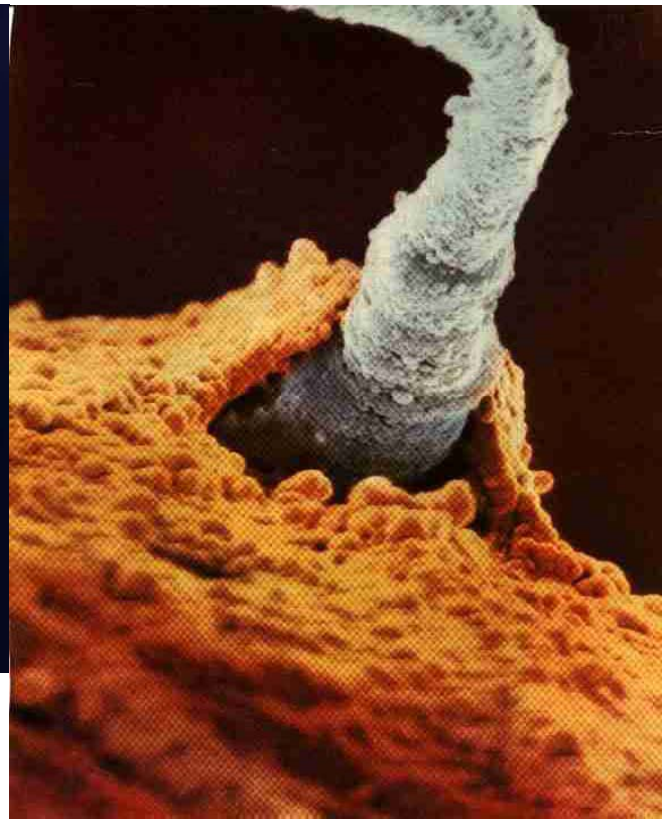




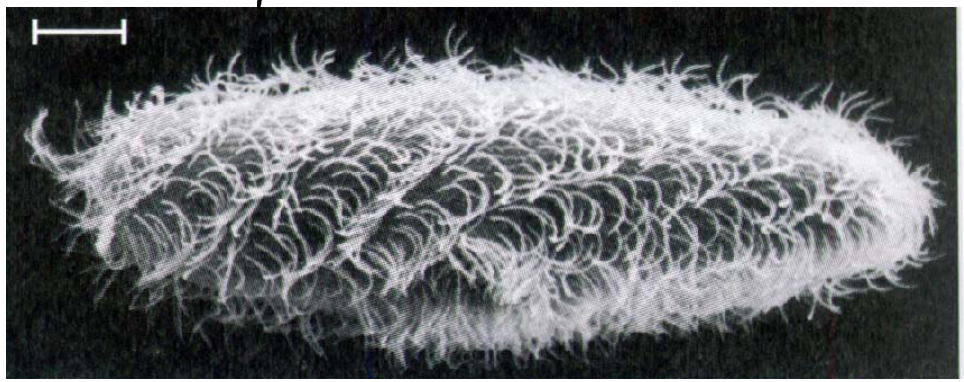
“External motors”: flagella and cilia

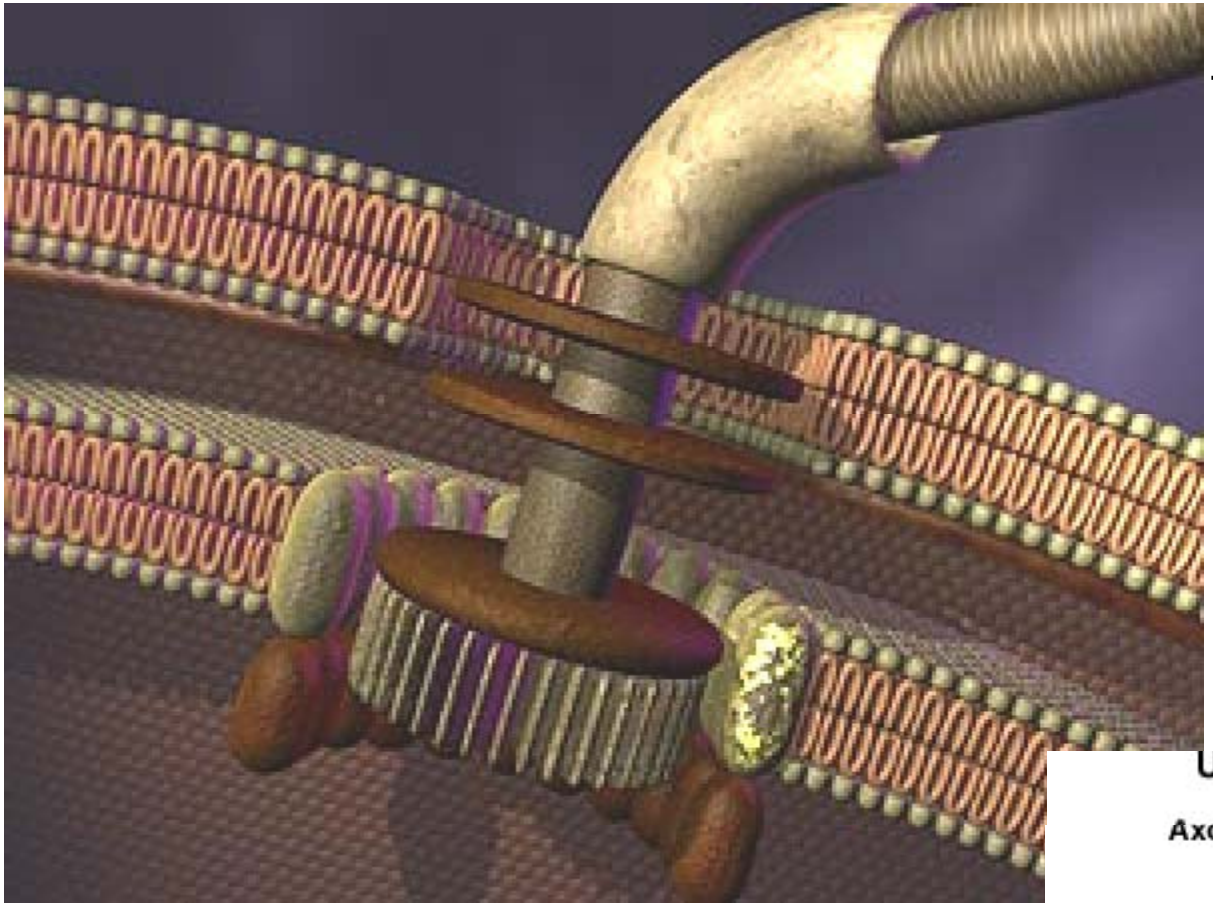
The bending sperm flagella pushes against the surrounding fluid propelling the cell forward





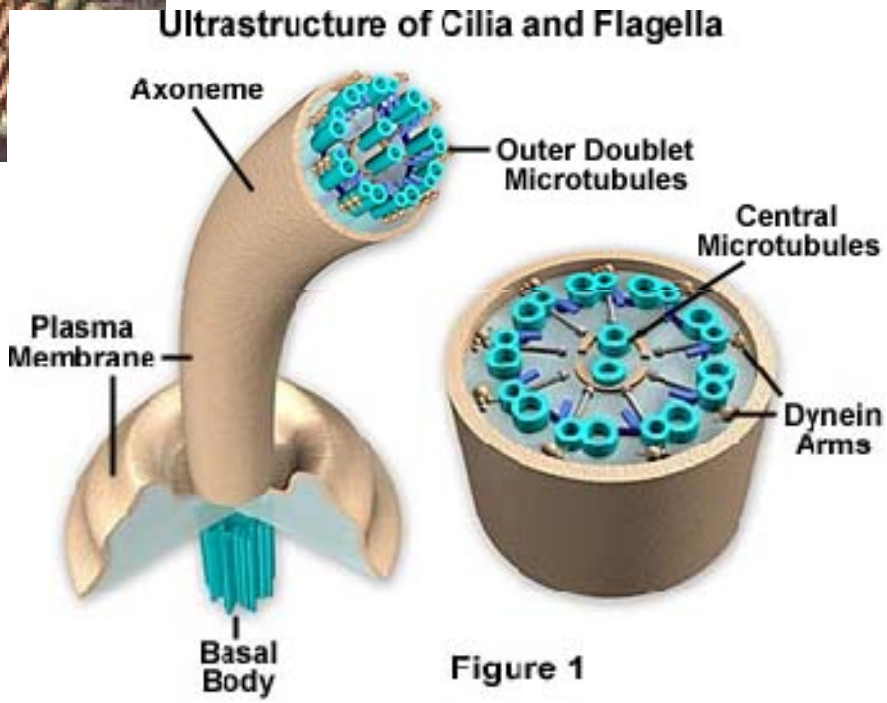
Cilia on a *paramecium*

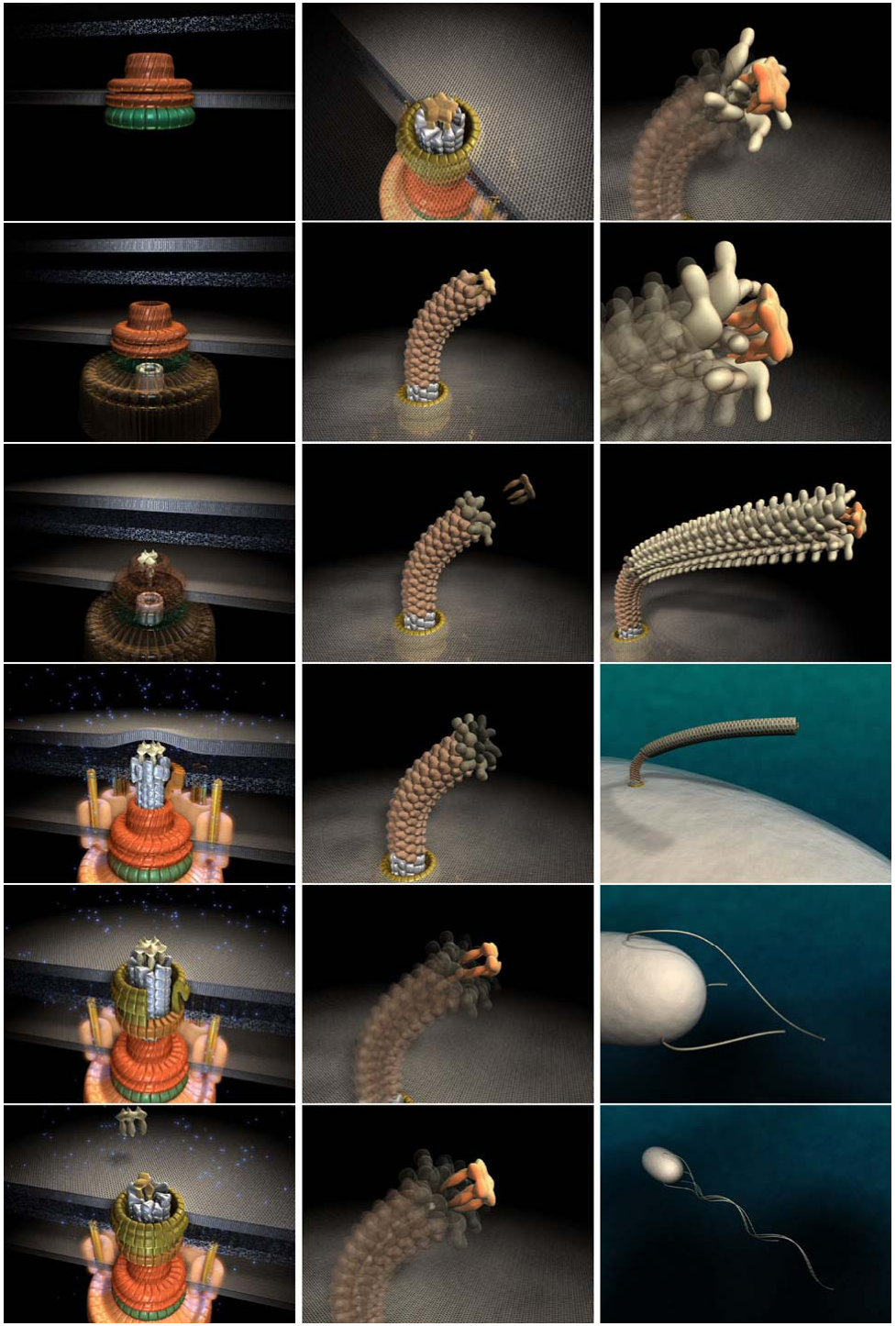




The bacterial flagellum motor

... very different from flagella found in eukaryotes



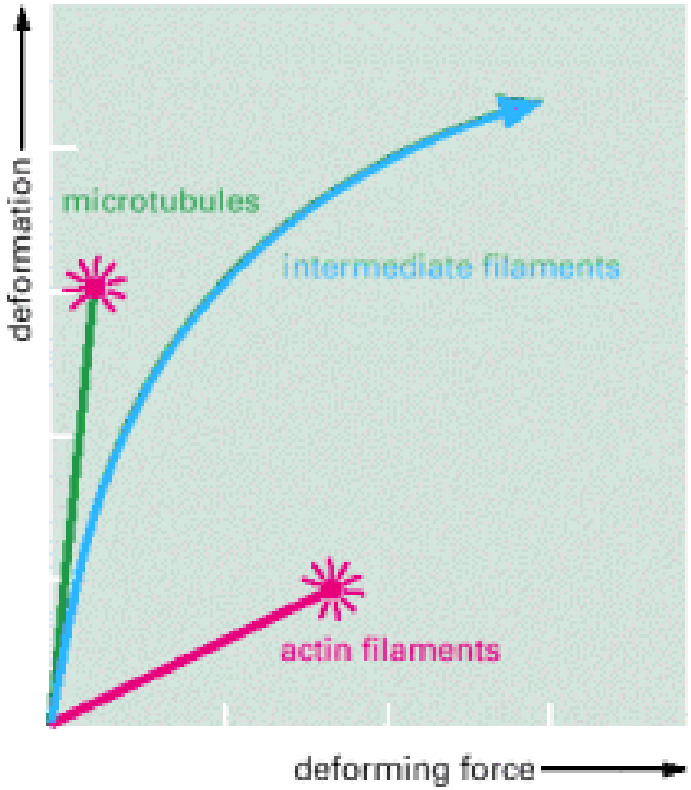


The bacterial flagellum motor

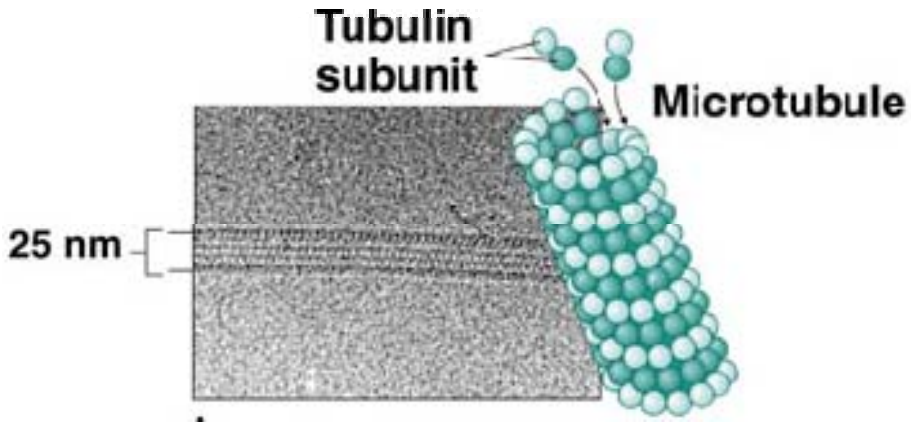
Composed of **20 proteins** while
40 genes needed to make the motor
and its flagellum

Microtubules are stiff and breakable

• Microtubules are polymers composed of **globular tubulin** subunits forming a stiff and hollow cylinder
• This stiff and inflexible mechanical property allows microtubules to push chromosomes apart or to form and move long flagella



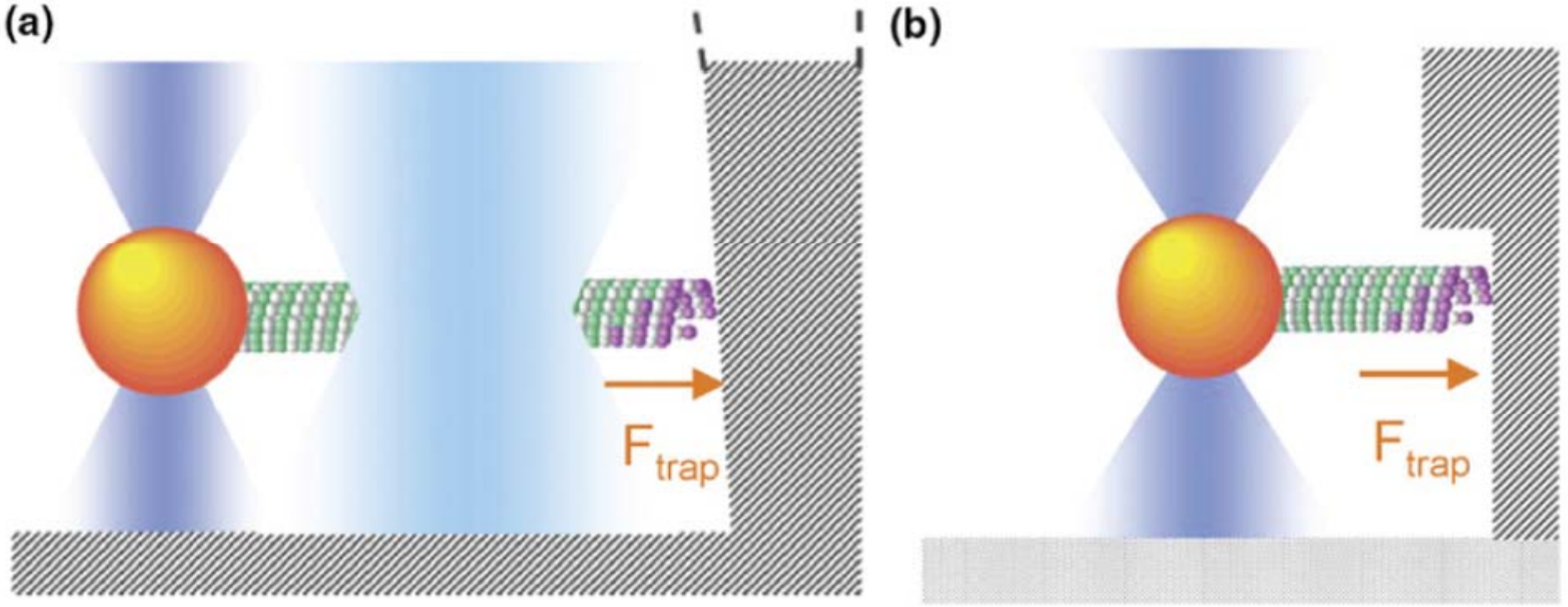
In the rheometer, microtubules are the first that cannot resist the strain and start to **break**



P. Janmey, *JCB*, 1991

Measuring nanoscale MT dynamics

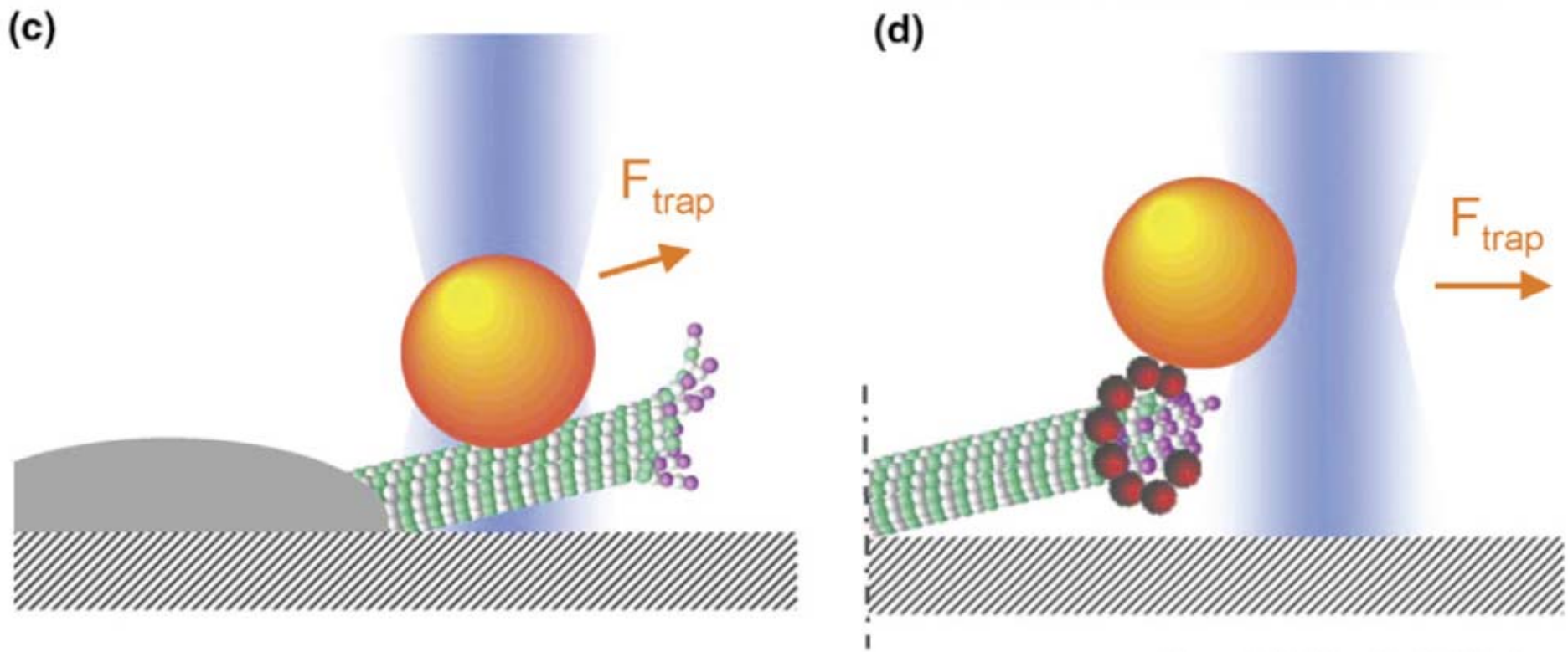
- MT-attached bead is centered via an **optical trap** (highly focused infrared laser beam) keeping the plus-end in contact with a **microfabricated barrier**
- The light blue trap serves to orient the MT perpendicular to the barrier wall
- Deflection of the bead reflects protofilament length fluctuations at the MT plus-end
- A different setup similarly measures MT plus-end fluctuations via bead deflections
- Here the MT is held in position by the **microfabricated structure**



Gardner et al., 2008, Curr. Opin. Cell Biol.

Measuring nanoscale MT dynamics

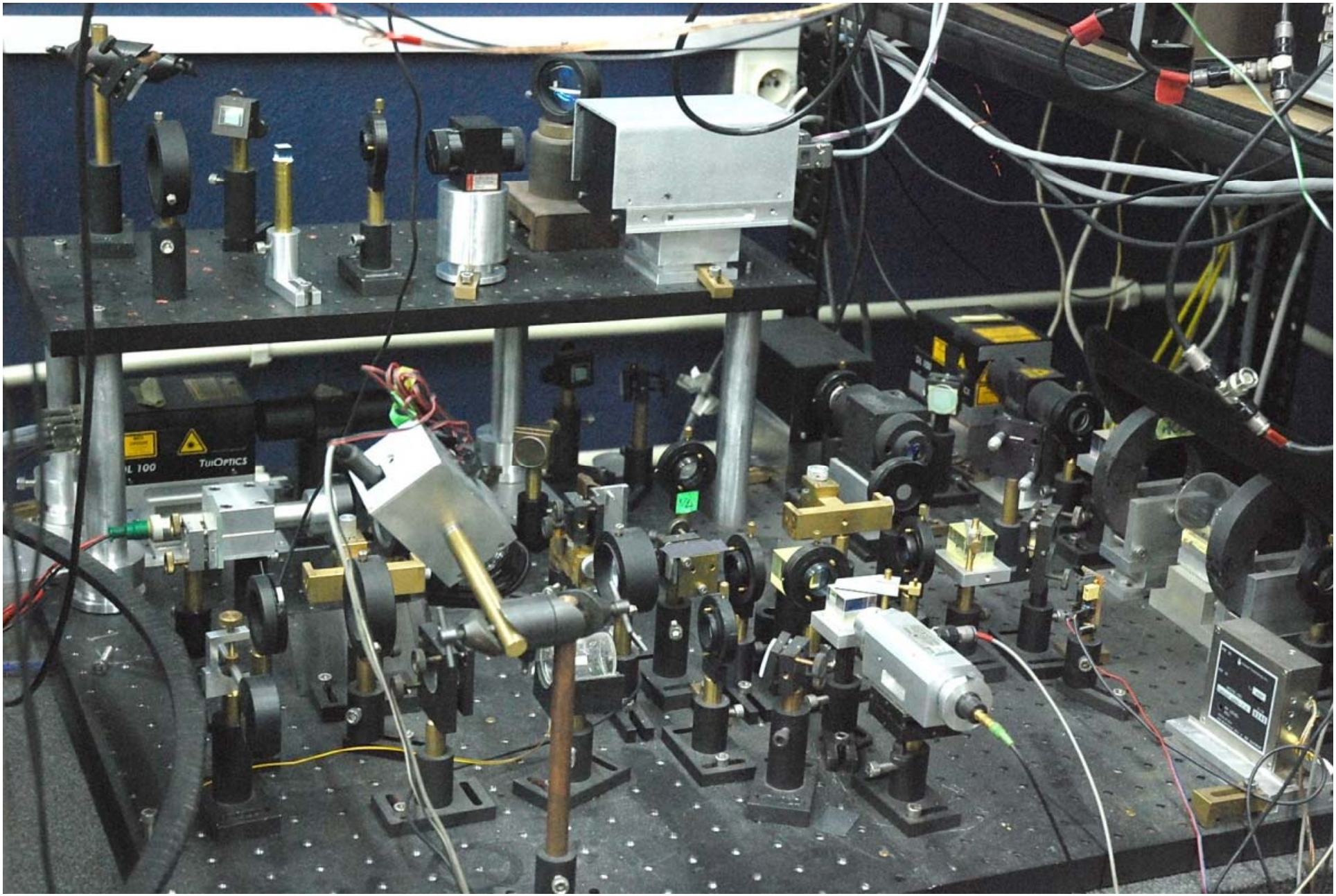
- Bead attached to the **microtubule lattice**
- As MT depolymerizes bead deflection is measured (arrow: resisting force of bead)
- Bead linked to the plus-end via a **specific MT-binding protein**
- During MT fluctuations, the bead is pulled away while bead deflection is measured



Gardner et al., 2008, Curr. Opin. Cell Biol.

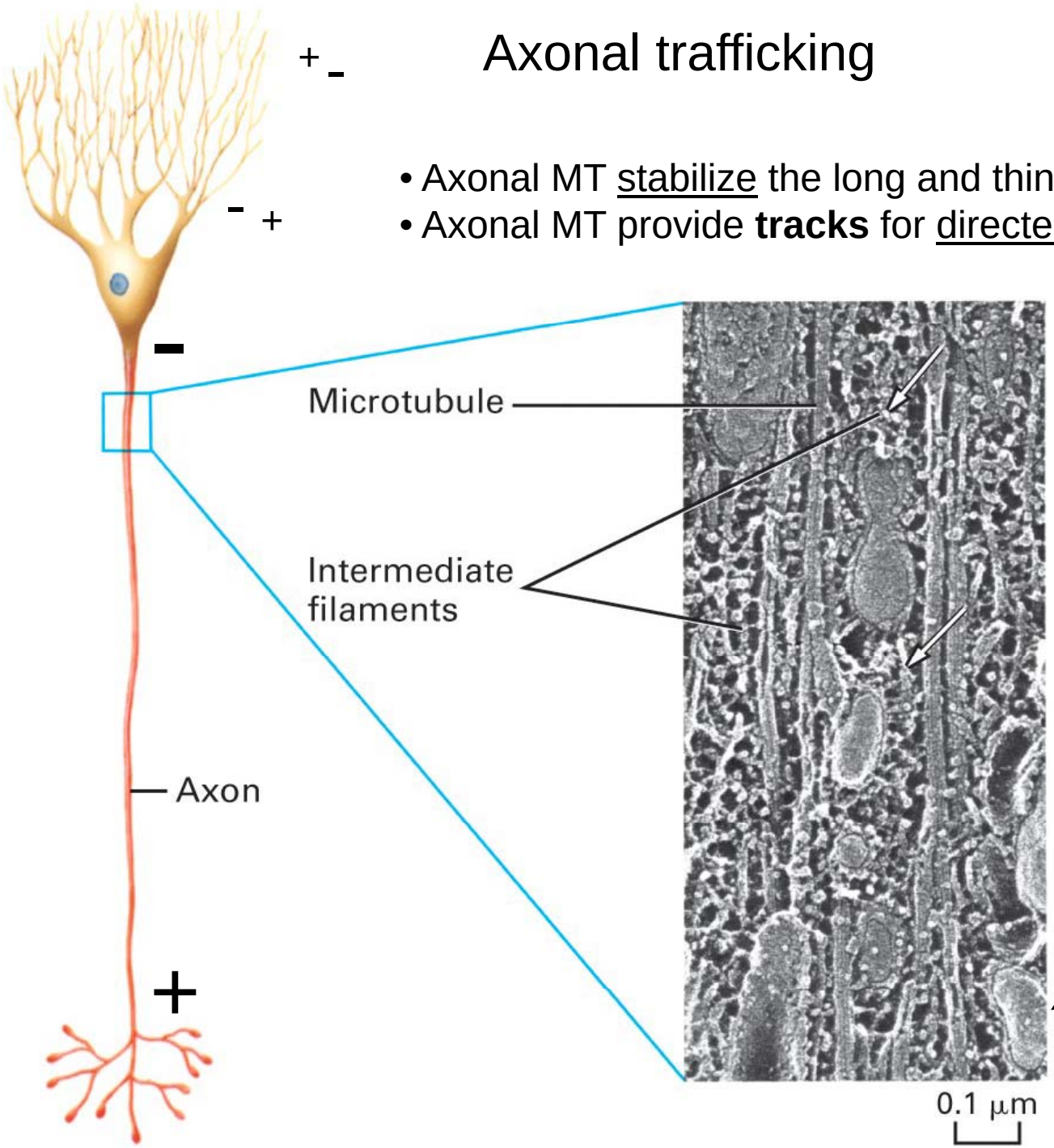
Optical trap setups are indeed sophisticated

Optical trap setups are indeed sophisticated



Axonal trafficking

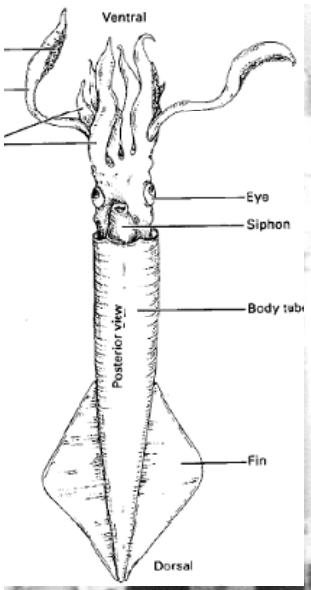
- Axonal MT <u>stabilize</u> the long and thin <u>neuronal extension</u>
- Axonal MT provide **tracks** for <u>directed synaptic vesicle transport</u>



Frog axon ("deep-etching quick freeze" technique)

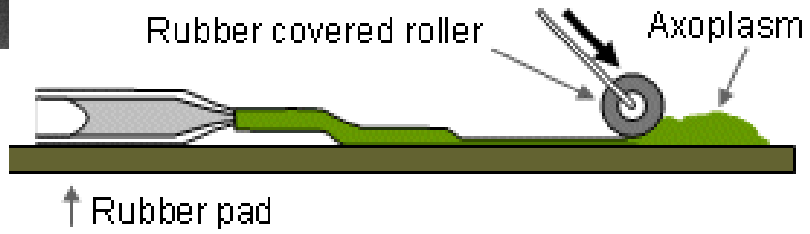
synaptic vesicles and mitochondria

Working with the giant squid axon

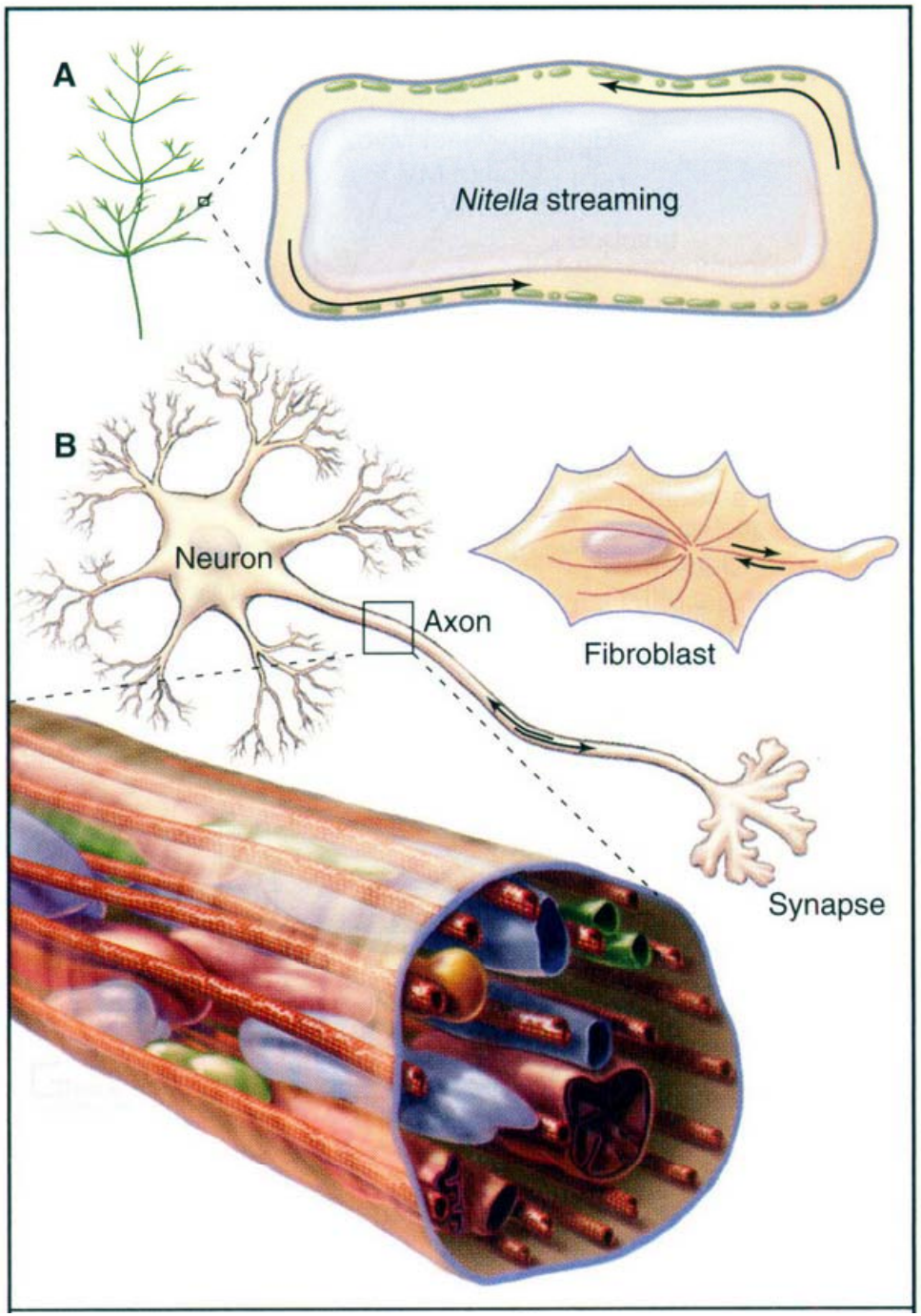


Movie
v20-02-squid_axon.mov

Cytoplasm squeezed out of a squid giant axon and observed with a microscope

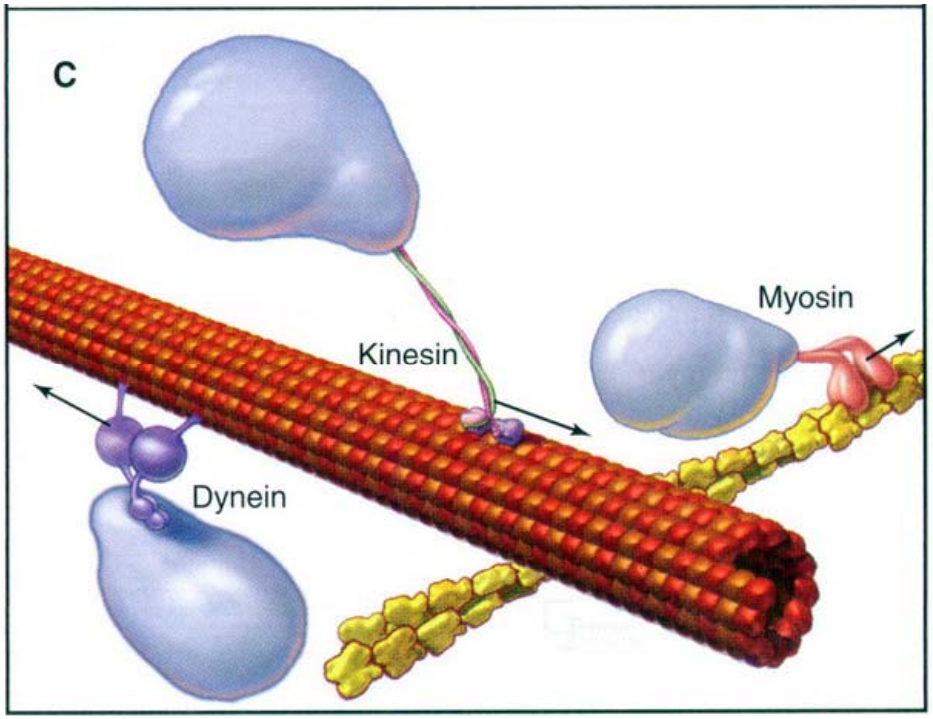


Axonal trafficking



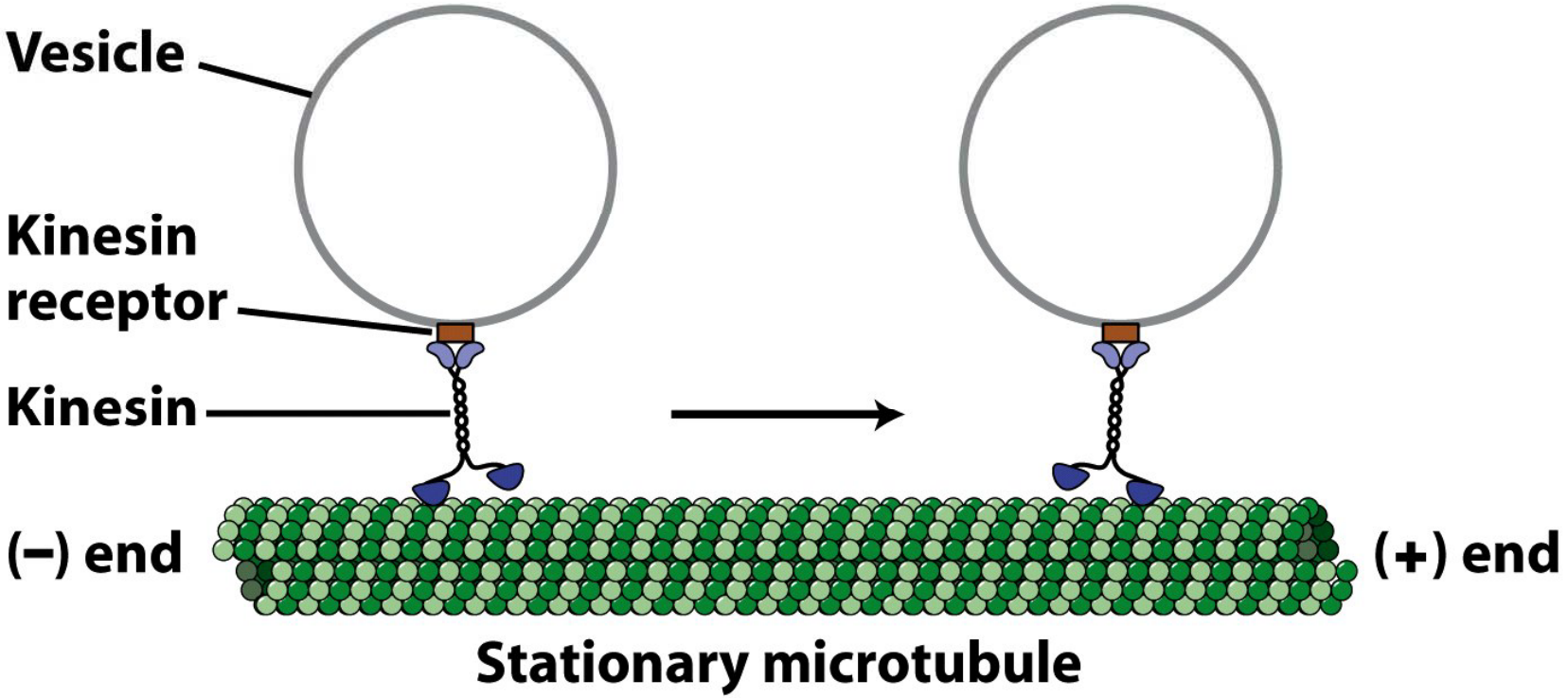
Synaptic vesicles, **dense core vesicles** (neuropeptides), mitochondria, RNA-granules etc. move along microtubule tracks attached to molecular motors

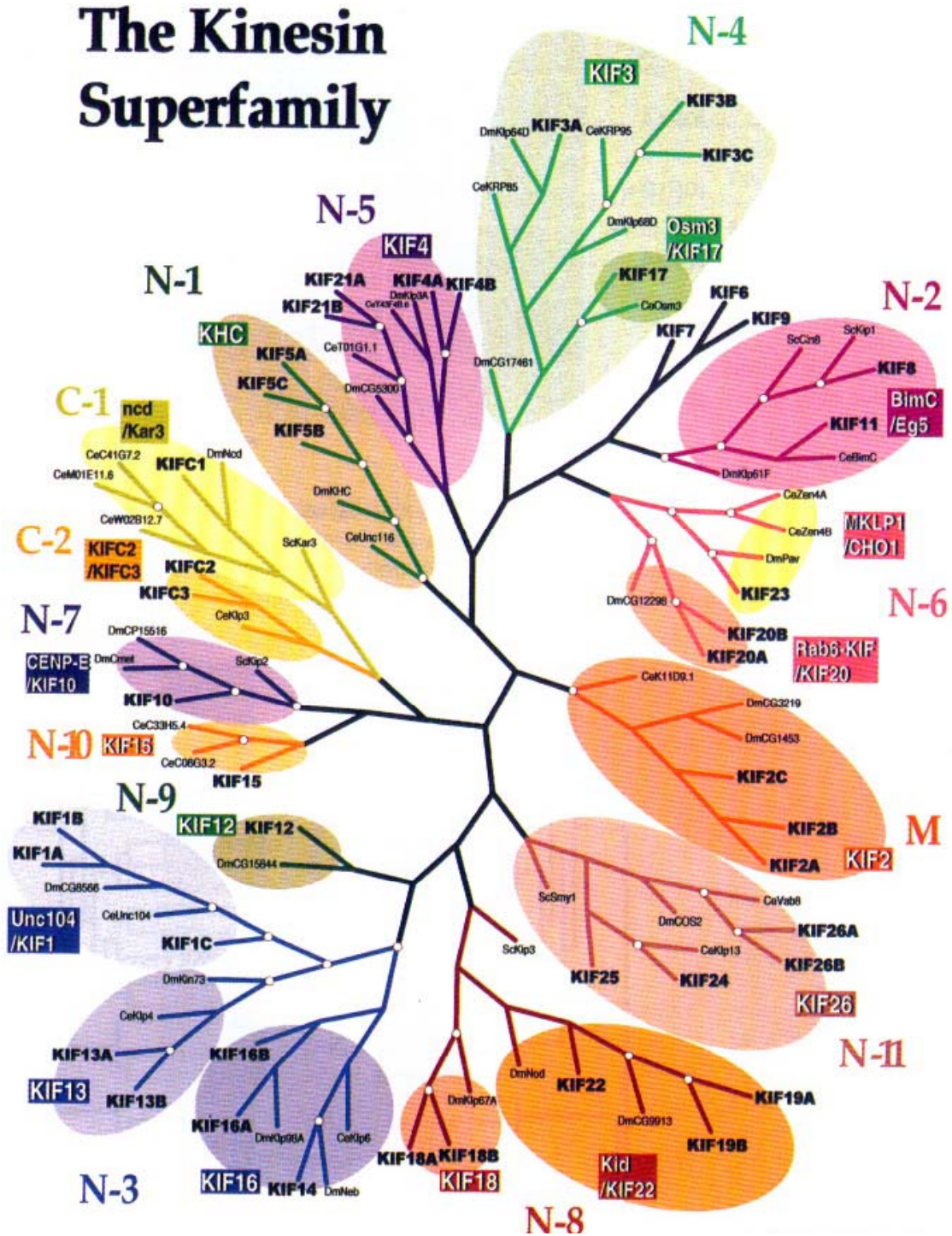
Movie
v20-02-vesicle_transport.mov



Model of kinesin-based vesicle transport

- Kinesins bind via their **globular *motor* domain** to microtubules while the **globular *tail* domain** is connected to the vesicle
- The vesicle connection is mediated by **kinesin receptor proteins** (linker proteins)





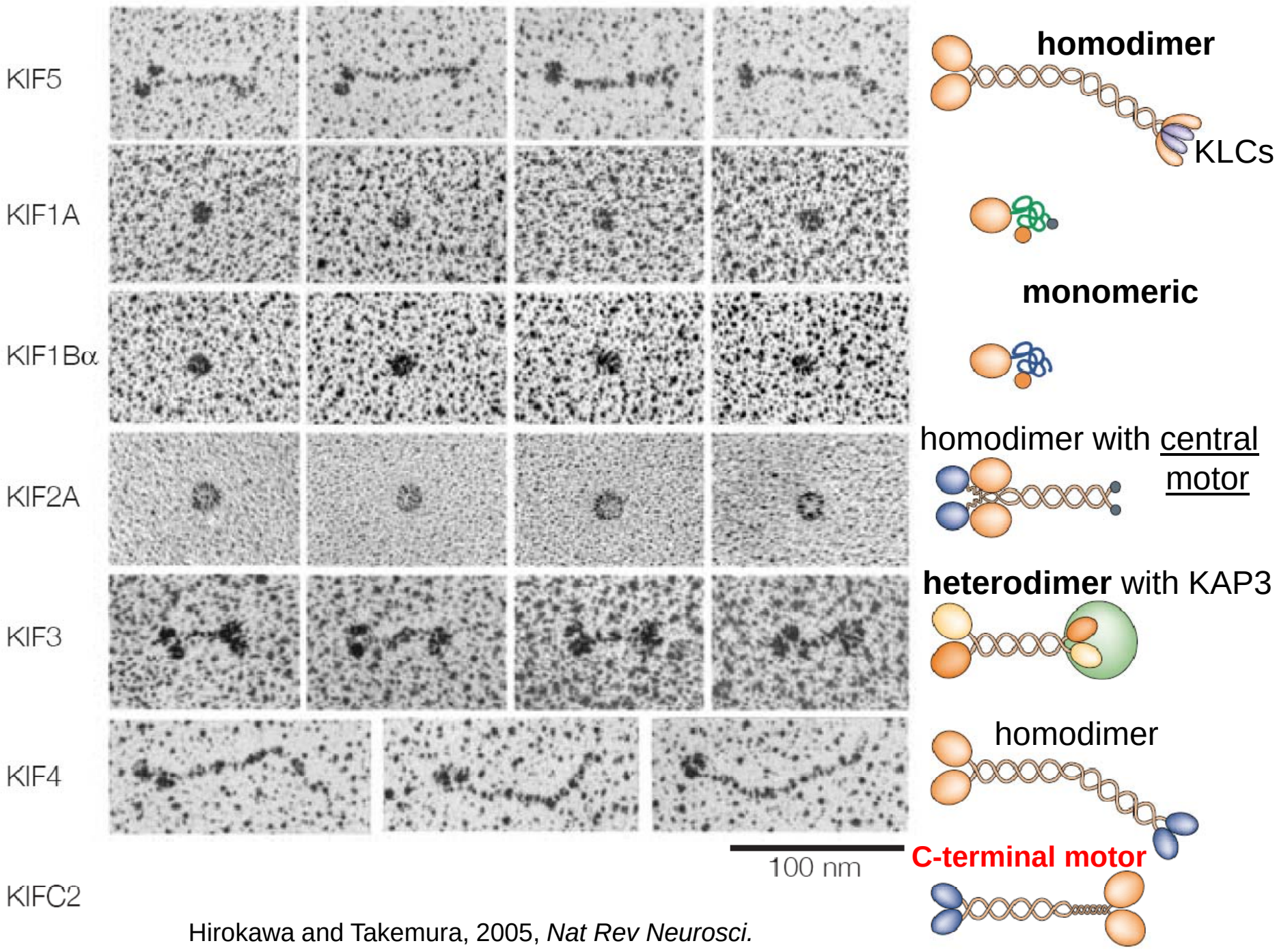
3 major types of **KIFs** (kinesin superfamily proteins) exist based on the position of the motor domain:

1) **NH_2**-terminal motor domain type
2) **Middle** motor domain type
3) **COOH**-terminal motor domain

14 classes exist:

- 11 classes for **N-kinesins** (16 family members)
- 2 classes of **C-kinesins**
- 1 **M-kinesin** class (KIF2)

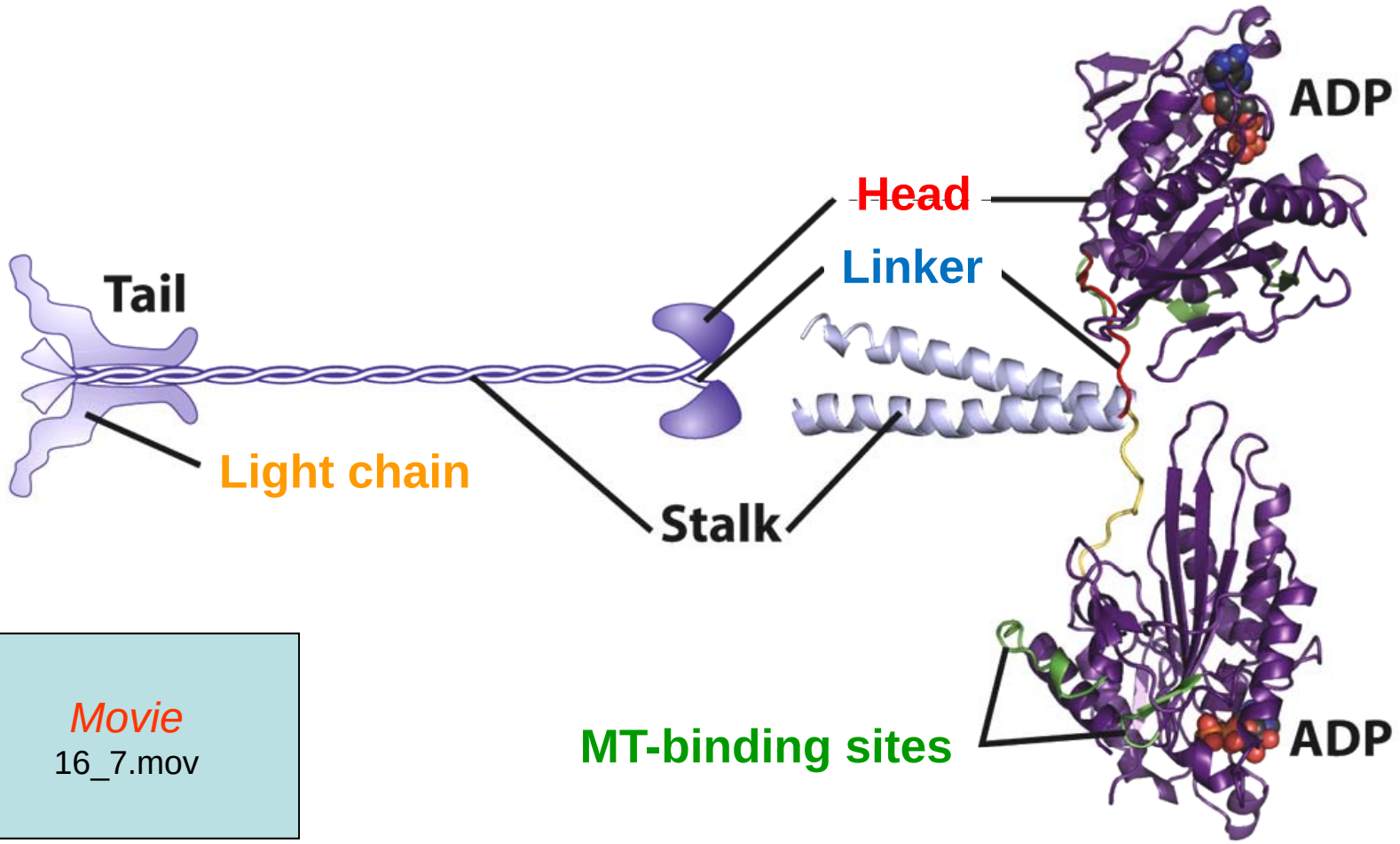
Schliwa, Molecular Motors, 1st Ed.



Hirokawa and Takemura, 2005, *Nat Rev Neurosci.*

Vesicle movement requires the motor protein kinesin and ATP

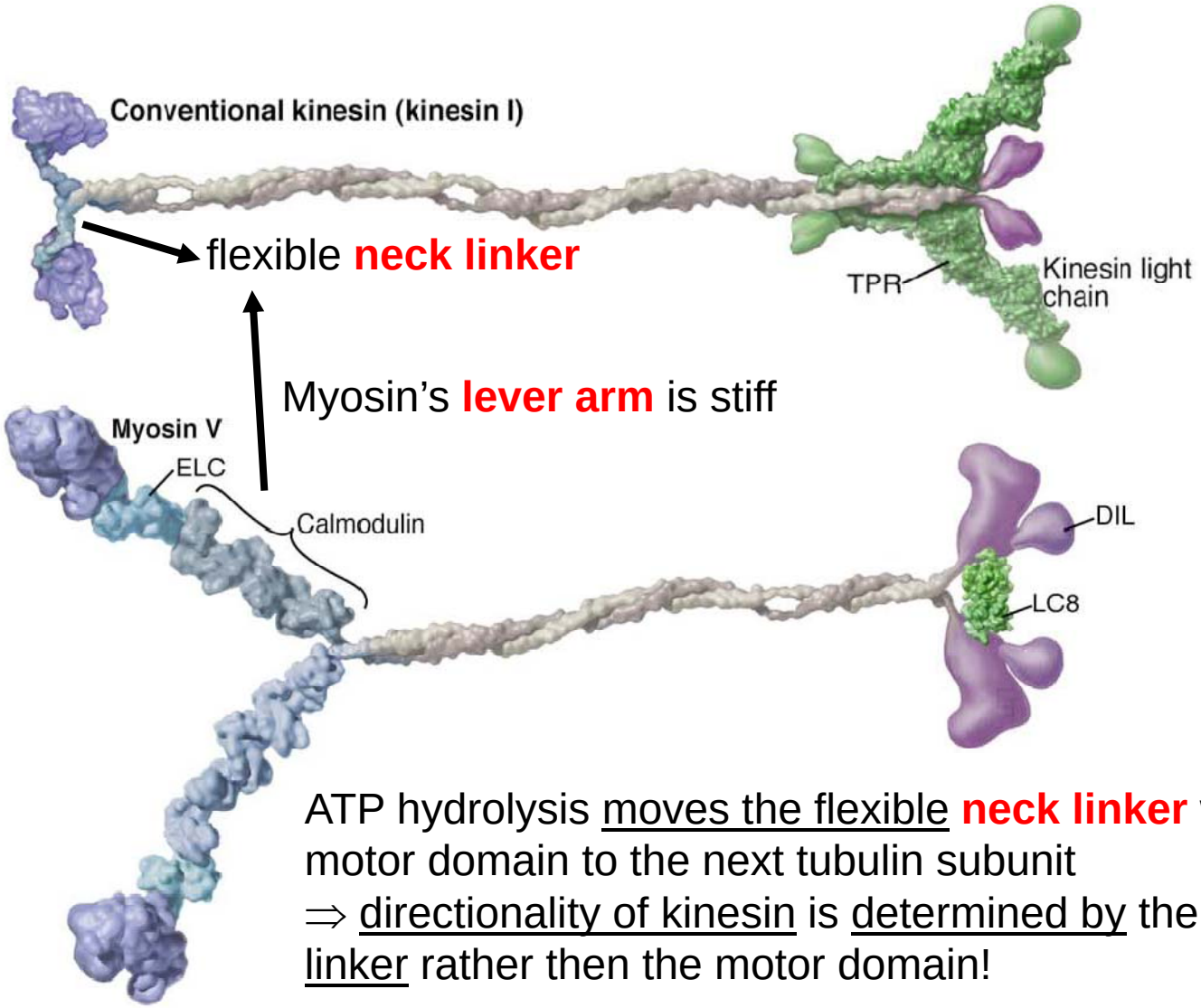
- **Kinesin I** is a 380 kDa dimer composed of **two heavy chains** and **one light chain**
- The **globular head domain** binds to the microtubule and converts chemical energy (from ATP hydrolysis) into mechanical energy (to move along the MT)
- The **globular tail domain** binds to the vesicle via adaptor proteins



Movie
16_7.mov

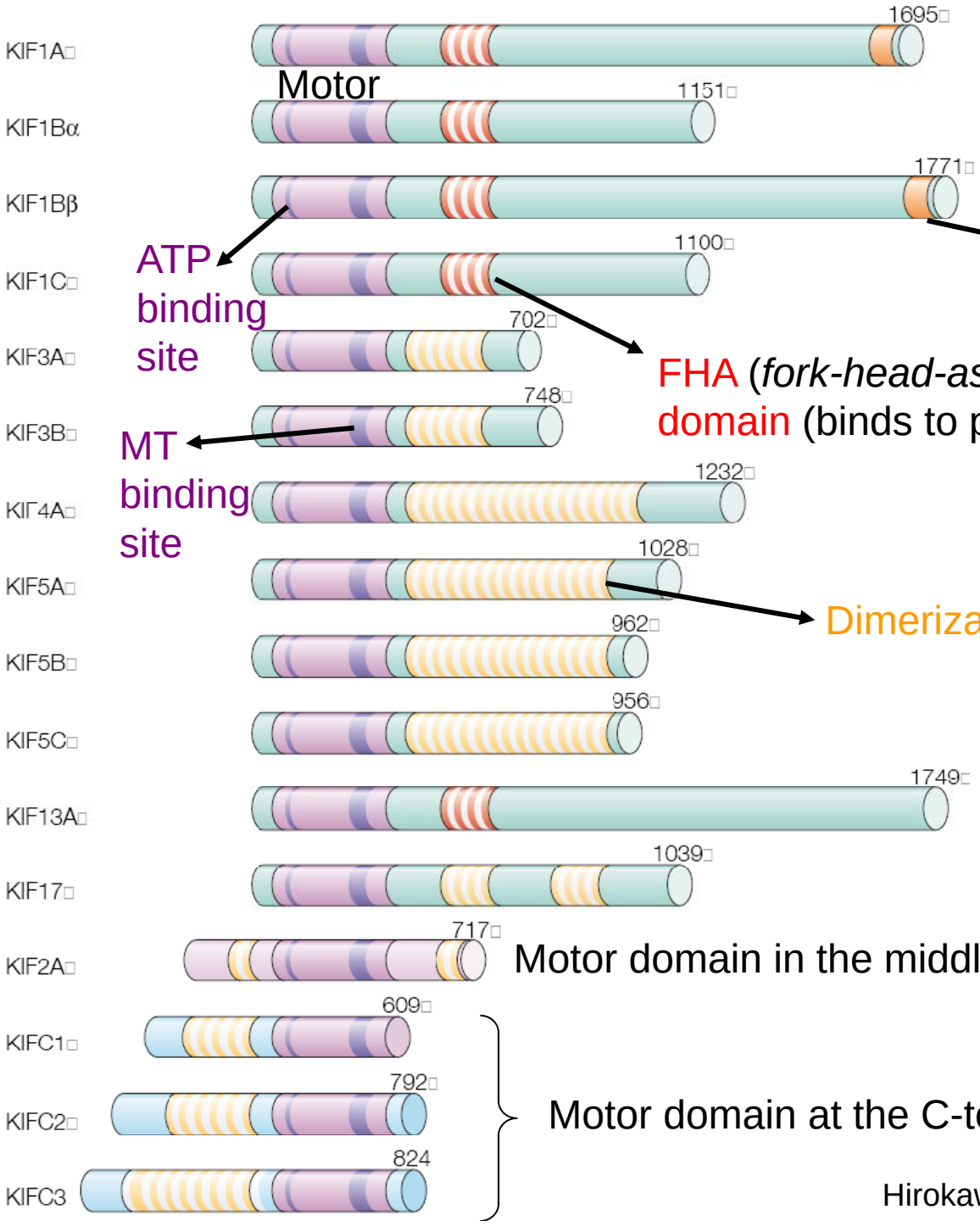
Kinesin's directionality is controlled by a flexible neck linker

- Kinesin I exerts a force of 6 pN (piconewton) to pull a vesicle thru the cytoplasm
- Step size is 8 nm which matches the distance between two tubulin subunits in the MT
- *Processivity* is a term defining the distance a kinesin walks without detaching



ATP hydrolysis moves the flexible **neck linker** which positions the motor domain to the next tubulin subunit
⇒ directionality of kinesin is determined by the function of the neck linker rather then the motor domain!

Vale, 2003, *Cell*



Domain structures of the principal KIFs

ATP binding site

PH (*pleckstrin homology*) domain (binds to lipids)

FHA (*fork-head-associated*) domain (binds to phosphoproteins)

MT binding site

Dimerization site

Motor domain in the middle = M-kinesin

Motor domain at the C-terminus = C-kinesin

Hirokawa and Takemura, 2005, *Nat Rev Neurosci.*

New family names

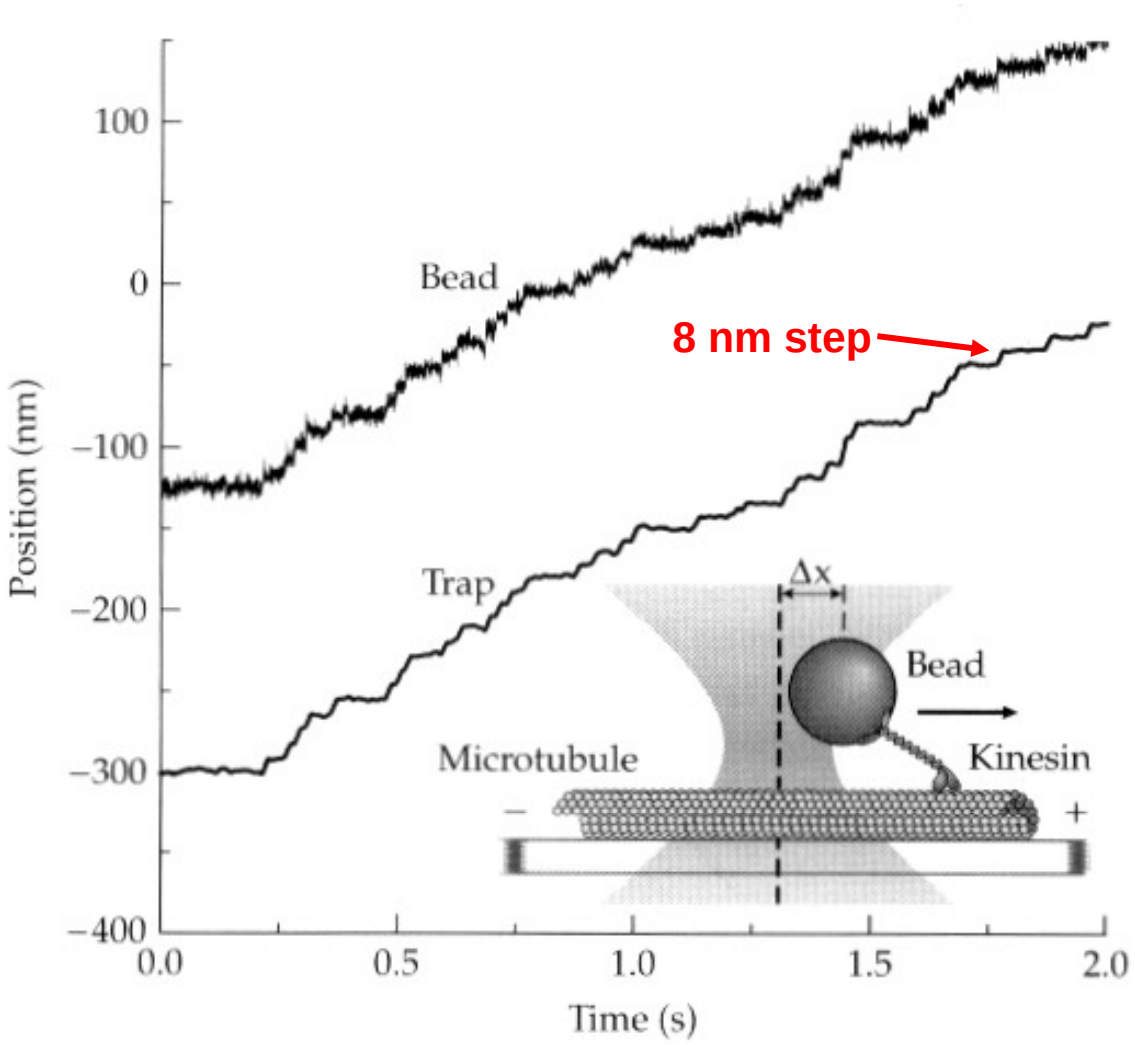
KIF5 = Kinesin 1
KIF3 and KIF17 = Kinesin 2
KIF1A = Kinesin 3

Table 1 | **Family, subfamily and member names of kinesin superfamily proteins**

Family name[19]	Class	Previous family name[14,147]	Subfamily name	Member names in mammals	Examples of nonmammalian members*
Kinesin 1	N-1	KIF5 (KHC, Kinesin I, Conventional kinesin)	KIF5	KIF5A, KIF5B KIF5C	‡KHC, §UNC-116
Kinesin 2	N-4	KIF3 (Kinesin II)	KIF3	KIF3A, KIF3B, KIF3C	§KRP-85/95, ||KRP85/95
		Osm3/KIF17	KIF17	KIF17	§OSM-3
Kinesin 3	N-3	Unc104/KIF1	KIF1	KIF1A, KIF1Bα, KIF1Bβ, KIF1C	§UNC-104
		KIF13	KIF13	KIF13A, KIF13B	
Kinesin 4	N-5	KIF4	KIF4	KIF4A, KIF4B, KIF21A, KIF21B	¶Chromokinesin
Kinesin 13	M	KIF2	KIF2	KIF2A, KIF2B, KIF2C	#XKCM1
Kinesin 14	C-1	Ncd/Kar3/KIFC1	KIFC1	KIFC1	‡NCD, **Kar3
	C-2	KIFC2/C3	KIFC2/C3	KIFC2, KIFC3	

Hirokawa and Takemura, 2005, *Nat Rev Neurosci.*

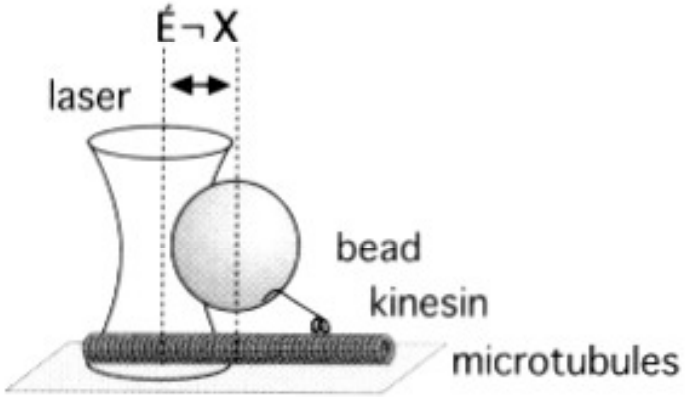
Using the optical trap to determine kinesins stepping behavior

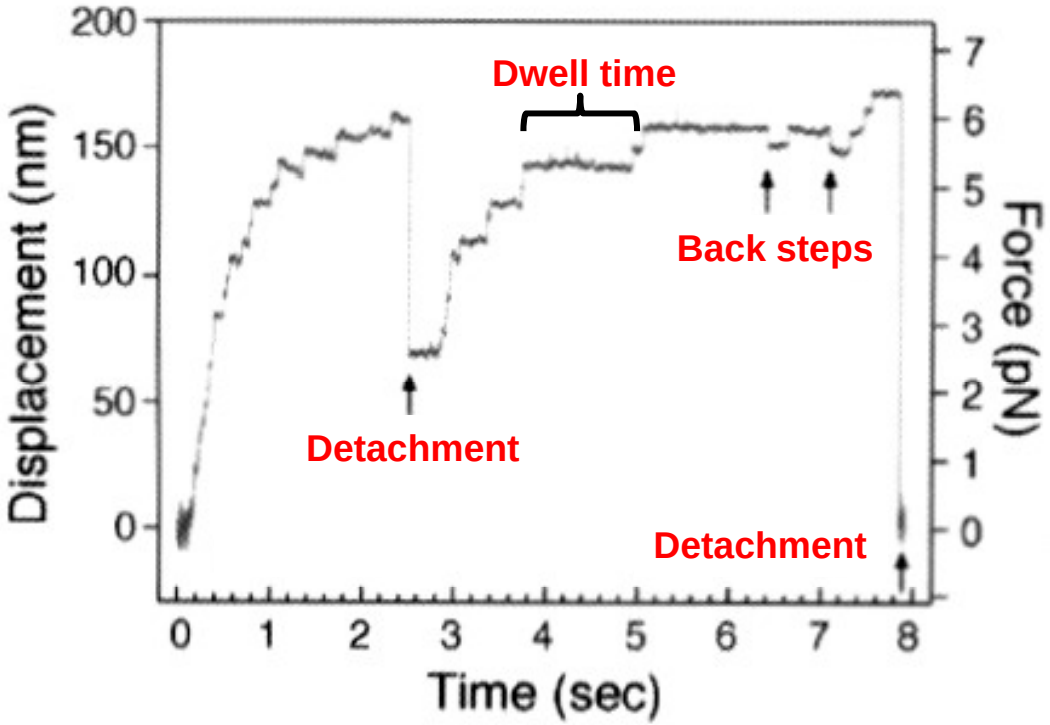


- Kinesin bound to a bead
- Bead kept in position by an **optical trap** (focused infrared laser-beam)
- Bead position determined by photodiode detector (upper trace)
- Opposing and constant force (6.5 pN) applied just behind the bead (by optical trap)
- After kinesin moves, **feedback loop** adjusts the bead position to its original position in the trap (lower trace)
- **Step size** of kinesin is **8 nm** reflecting the spacing of tubulin dimers in the protofilament

Howard, Mechanics of Motor Proteins, 1st Ed.

Force dependent kinesin stepping

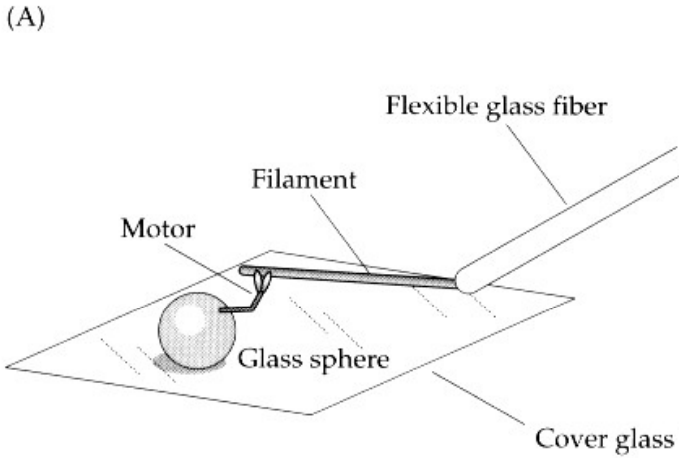




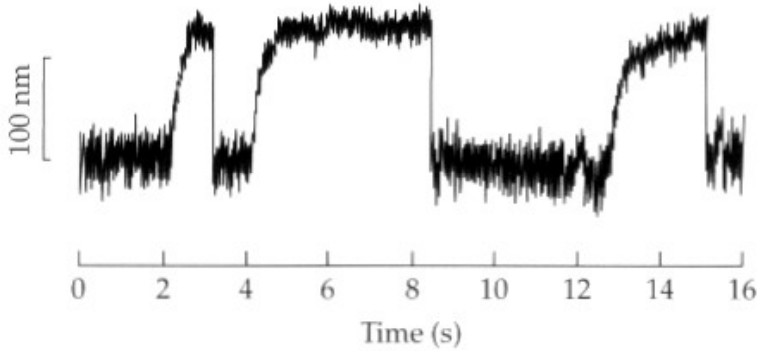
- Under <u>zero</u> to very low <u>load</u> kinesin exhibits <u>Brownian motion</u> only and turns around its own axis (no stepping measurable)
- **Discrete 8 nm steps** occur under <u>moderate loads</u>
- <u>Increasing loads</u> lead to occasional **detachments**
- **Dwell time** (or limp factor) is the <u>pausing time at which no steps occur</u>

Schliwa, Molecular Motors, 1st Ed.

A crossbridge model for kinesin



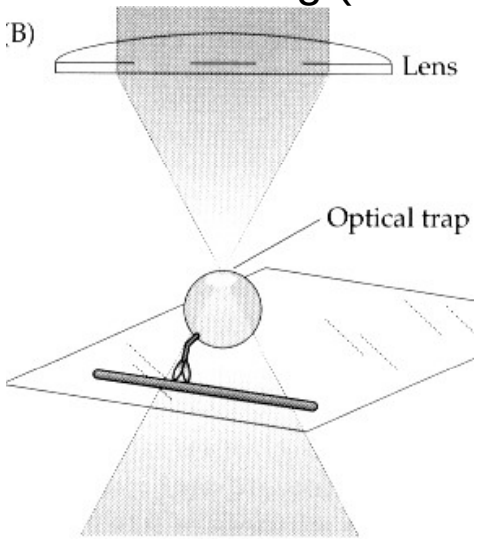
Proof for a kinesin crossbridge-mechanism model came from evidence that single kinesin makes discrete steps

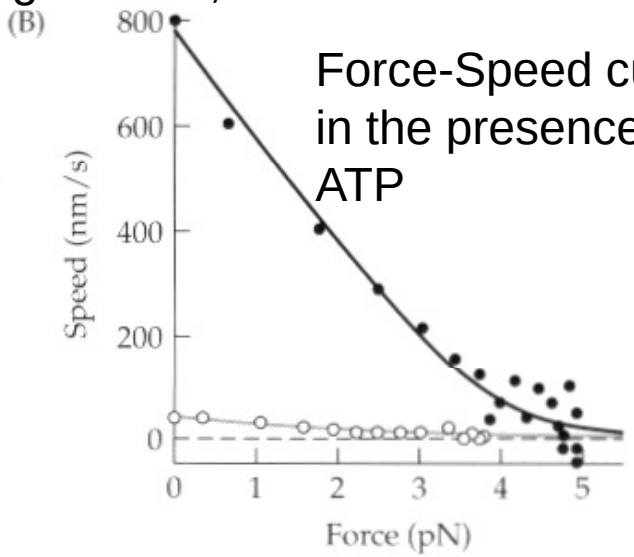


A single kinesin pulls three times on the MT <u>but keep being attached</u>

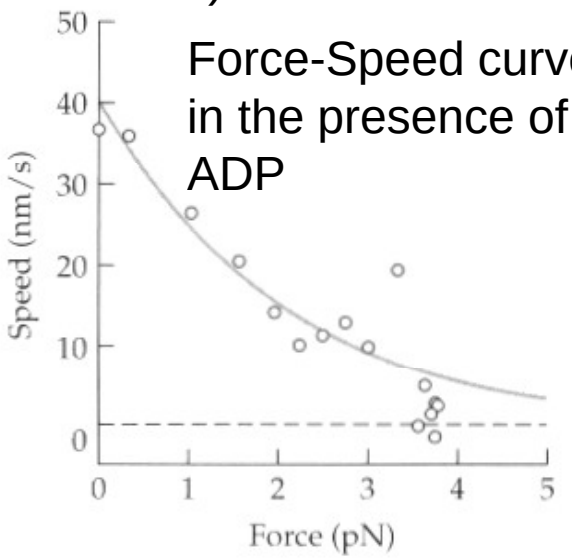
Kinesin's speed depends on the applied force and the presence of ATP:

- At (low) load, the speed slows down => there is a **force rate-limiting step**
- The motor should not dissociate from the filament: <u>one head must be always attached</u>
- Speed/Force curve is <u>almost linear</u> that can explain that a second step (at higher load) is rate-limiting (crossbridge model; now called hand-over hand model)



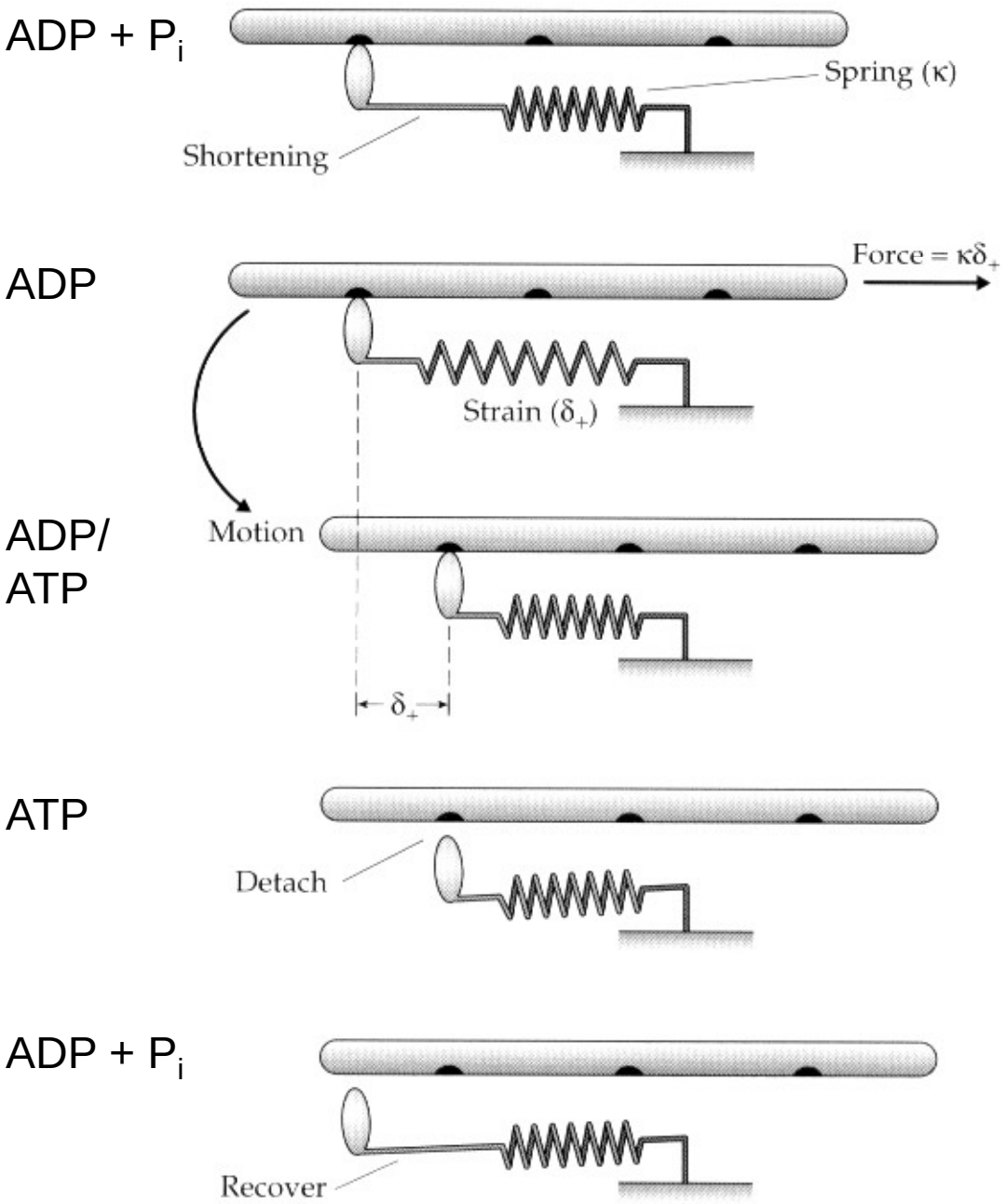


Force-Speed curve in the presence of ATP



Force-Speed curve in the presence of ADP

Powerstroke model



- In the powerstroke model the motor has an **elastic element** (a spring) which can store mechanical energy

- For kinesins this spring might be the **neck domain** or for myosin the light chain domain or **lever arm**

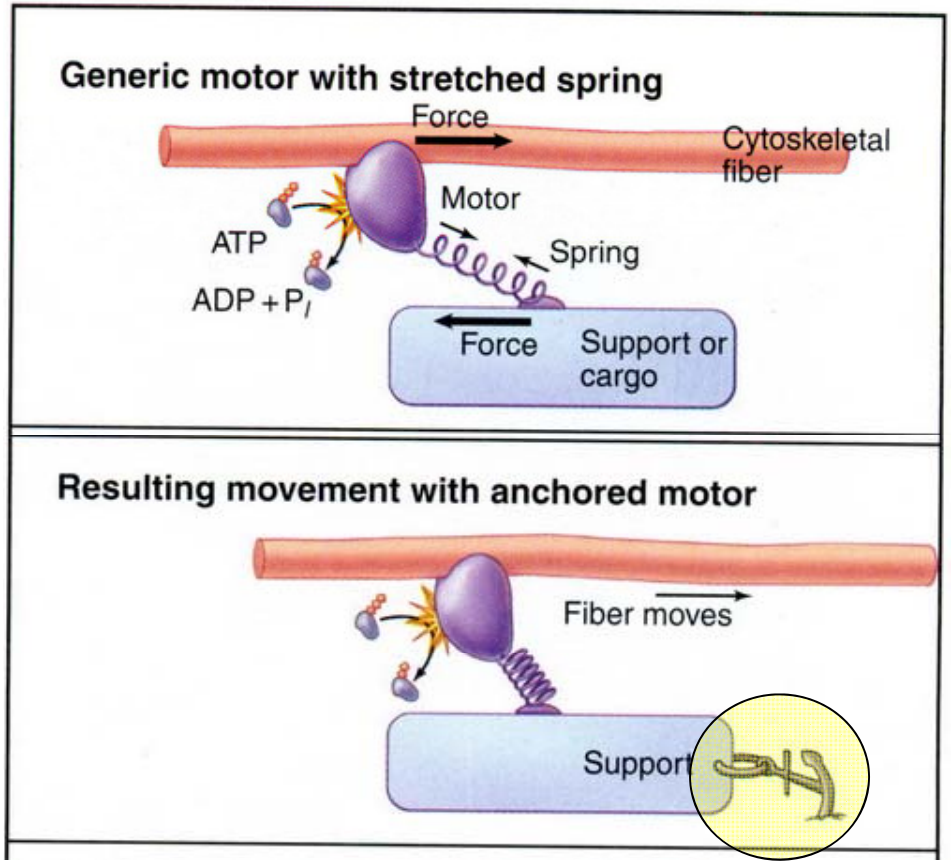
- Strain is produced by a conformational change in the crossbridge

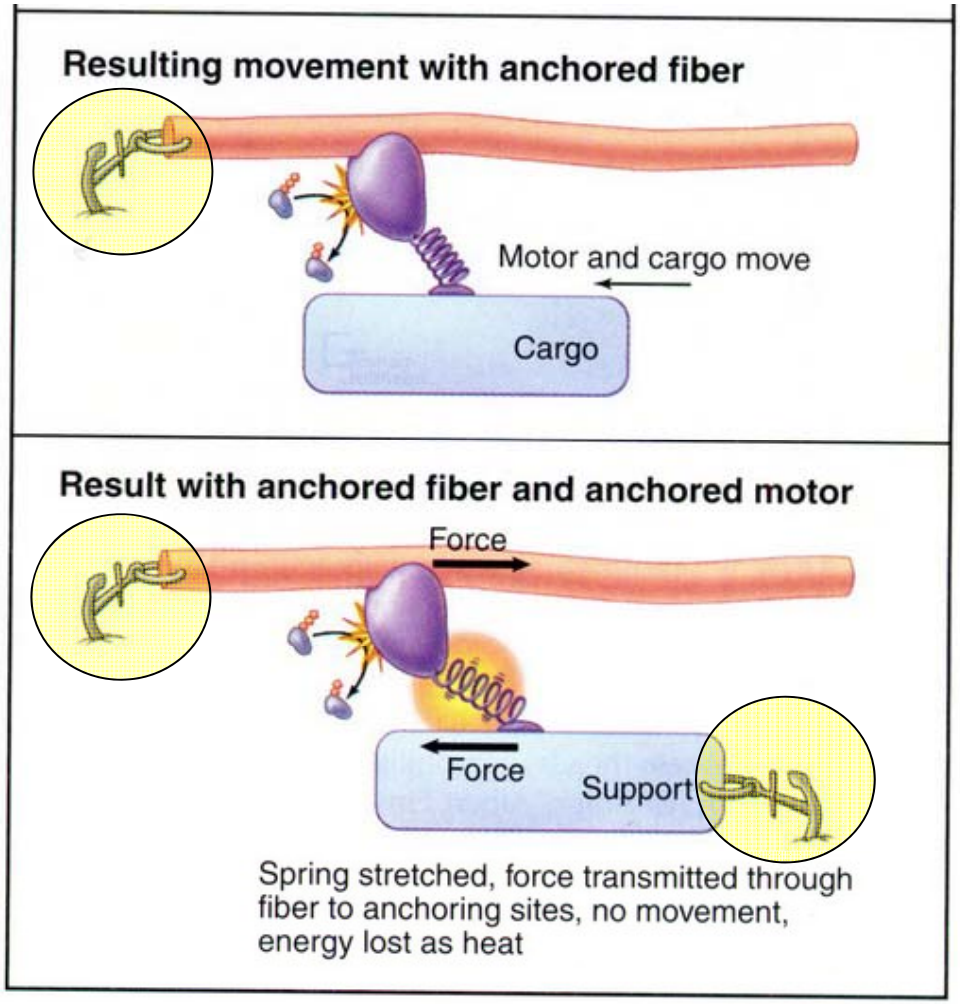
- The motor's **force** is the tension in the spring while the **relaxation** is the driving force for the motion

- ATP-binding detaches the motor

Force generation upon motor-filament interaction

- Energy released by ATP hydrolysis leads to stretching of an **elastic element** between cargo and fiber
- Resulting motion depends on the **resistance of** the cargo or fiber



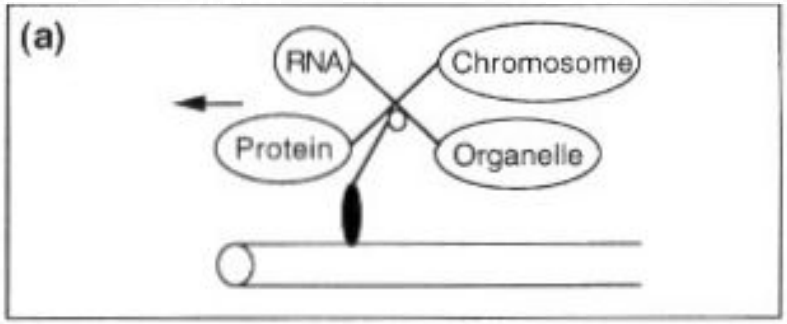


Pollard, 1st ed.

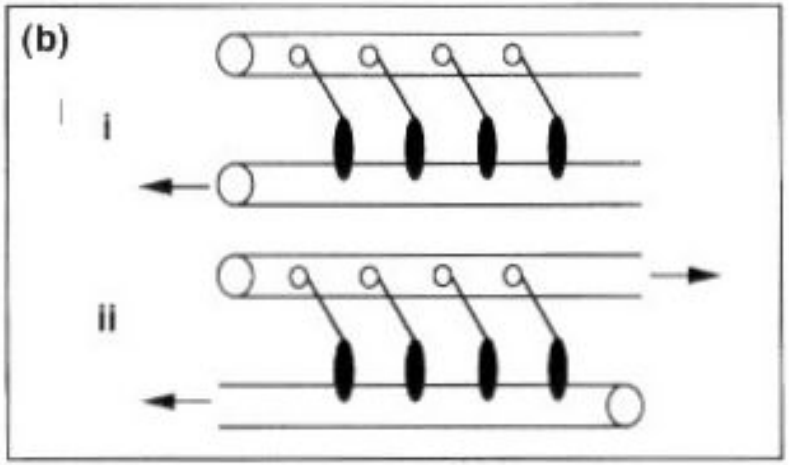
Motor-filament interactions in cells

In order to generate force or tension, a motor must be fixed to an “attachment” domain

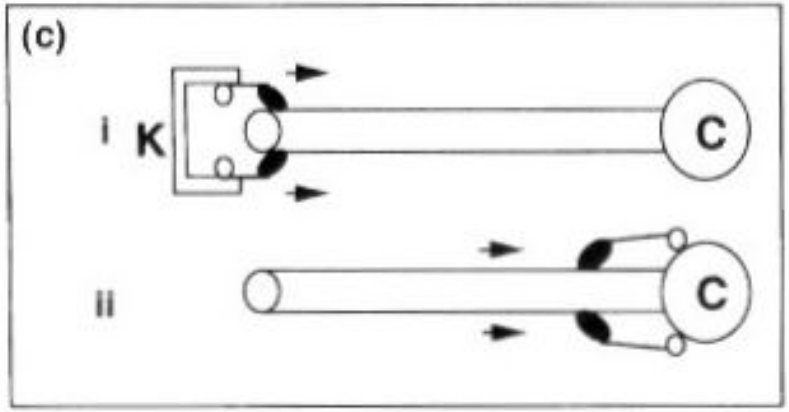
Motor **attached to** its **cargo** (= cargo moves)



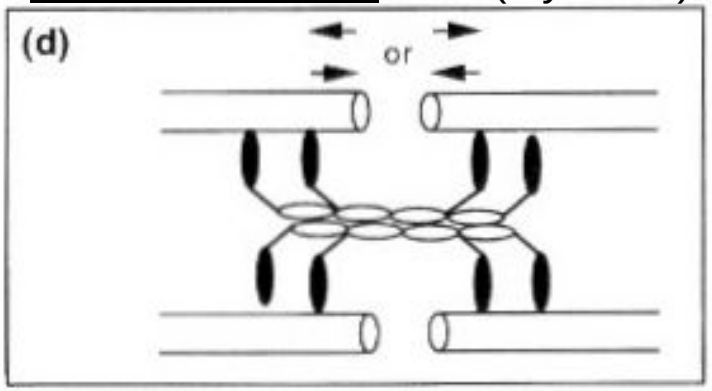
<u>Parallel</u> (i) or <u>antiparallel</u> (ii) cross-bridging = generate **sliding forces** (flagella beating or chromosome separation)

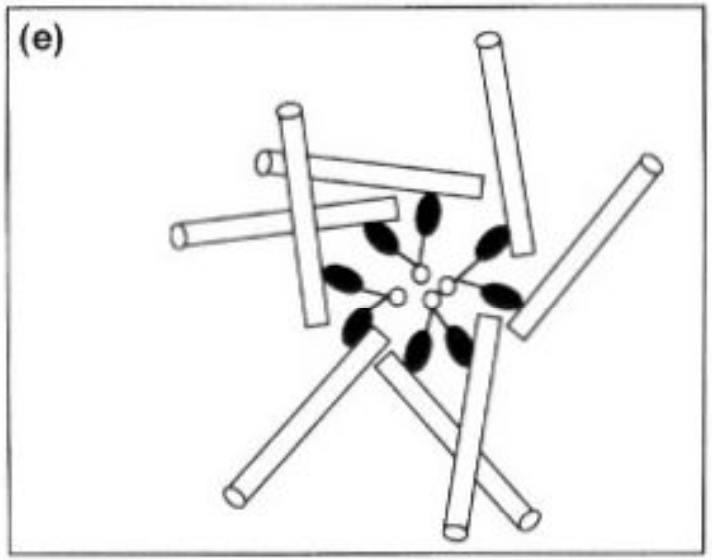


Motor <u>attached to</u> **filament ends** (**k**inetochore motors, **c**entrosome pulling)



Motors **attached to each other** generating <u>sliding forces</u> to form <u>stretch/contraction</u> units (myosin II)

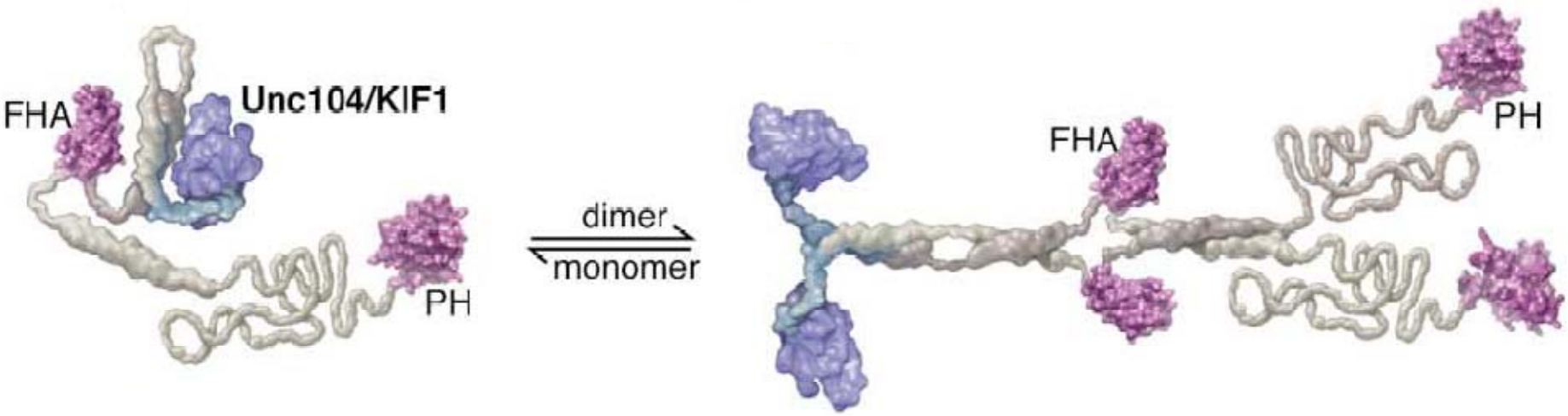


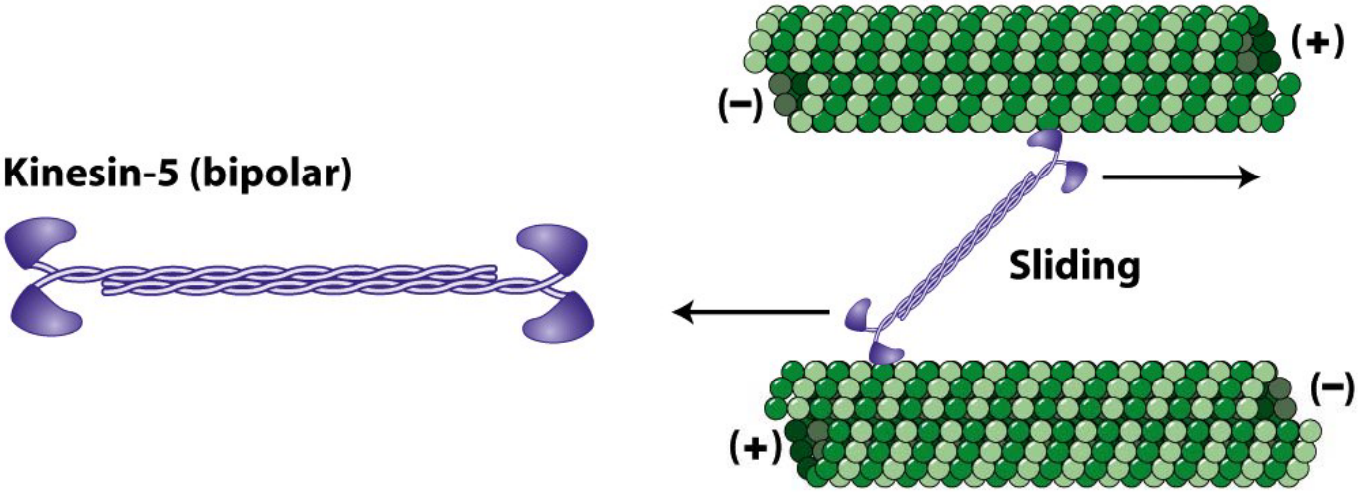


Dimeric motors cross-bridging <u>randomly organized</u> filaments (spindle formation)

Special types of kinesins: monomeric and bipolar kinesins

• KIF1A is a monomeric kinesin: <u>main synaptic vesicle transporter in neurons</u>

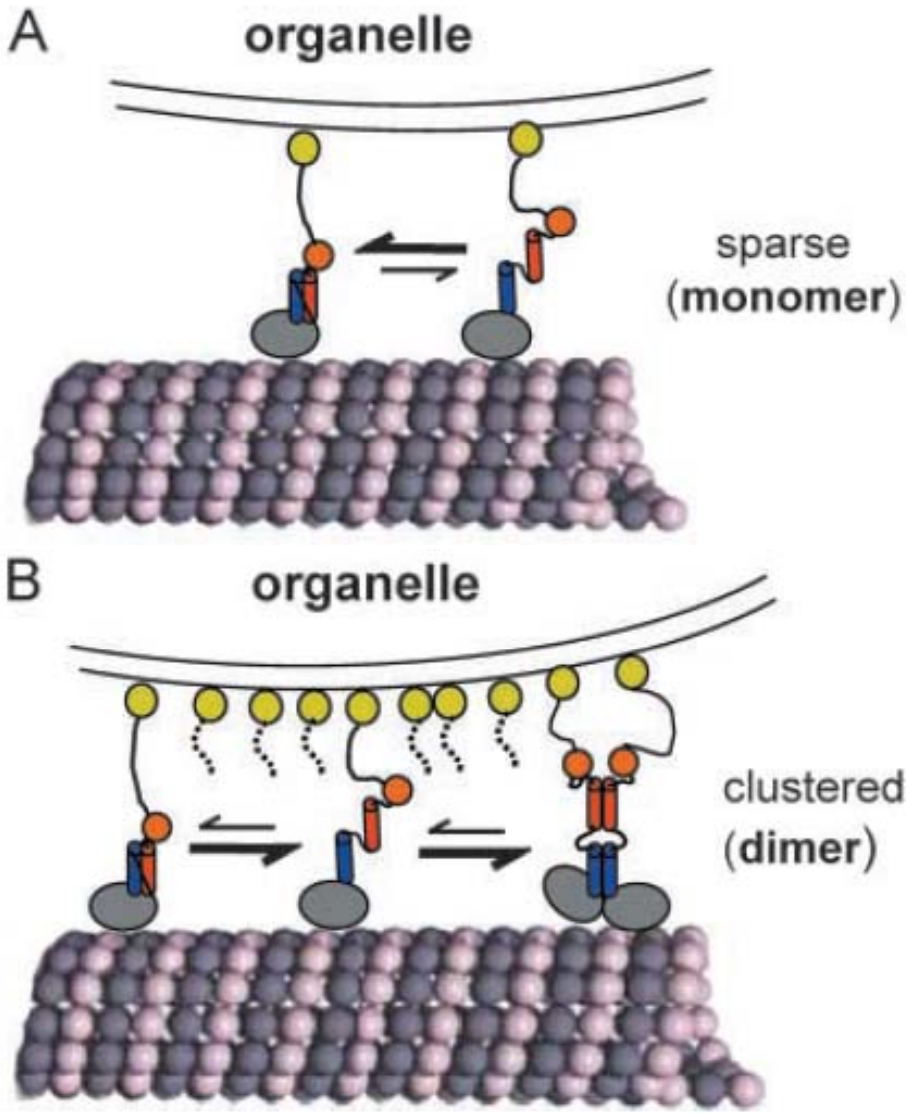




• **Bipolar** kinesin-5 (BimC) <u>assembles MTs into antiparallel dimers</u>

• **Mediates sliding of MT** relative to one another

Model for KIF1A monomer to dimer transition by self-folding of neck-linkers



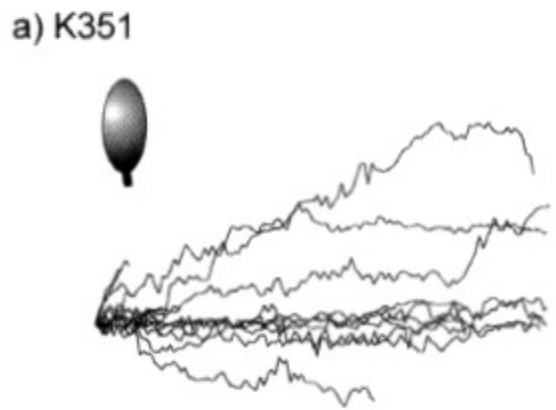
- When UNC-104/KIF1A is sparsely distributed on an organelle membrane, the self-folded state is favored over the unfolded state
- Self-folded UNC-104/KIF1A monomers move very slowly and **non-processively** with some plus end–directed biased diffusion

- When UNC-104/KIF1A is clustered in lipid rafts, **unfolded** monomers are recruited into dimers (shifting the previous equilibrium)
- Dimerized UNC-104/KIF1A undergo fast processive motility and generate maximal force

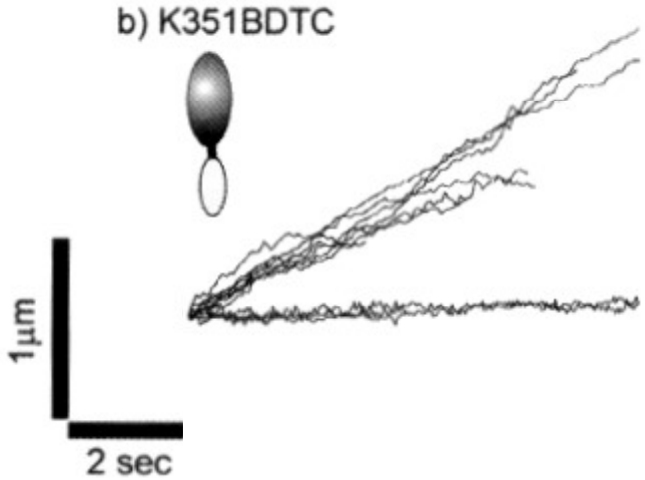
Al-Bassam et al., 2003, JCB

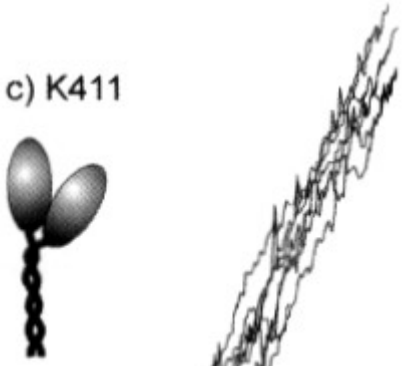
Protein engineering for functional analysis of motor domains

KIF1A lacking the MT-interacting domain (K loop) only **moves** driven **by diffusion**



Adding an artificial MT-binding domain (BDTC) single headed KIF1A **moves processive**, but only short distances

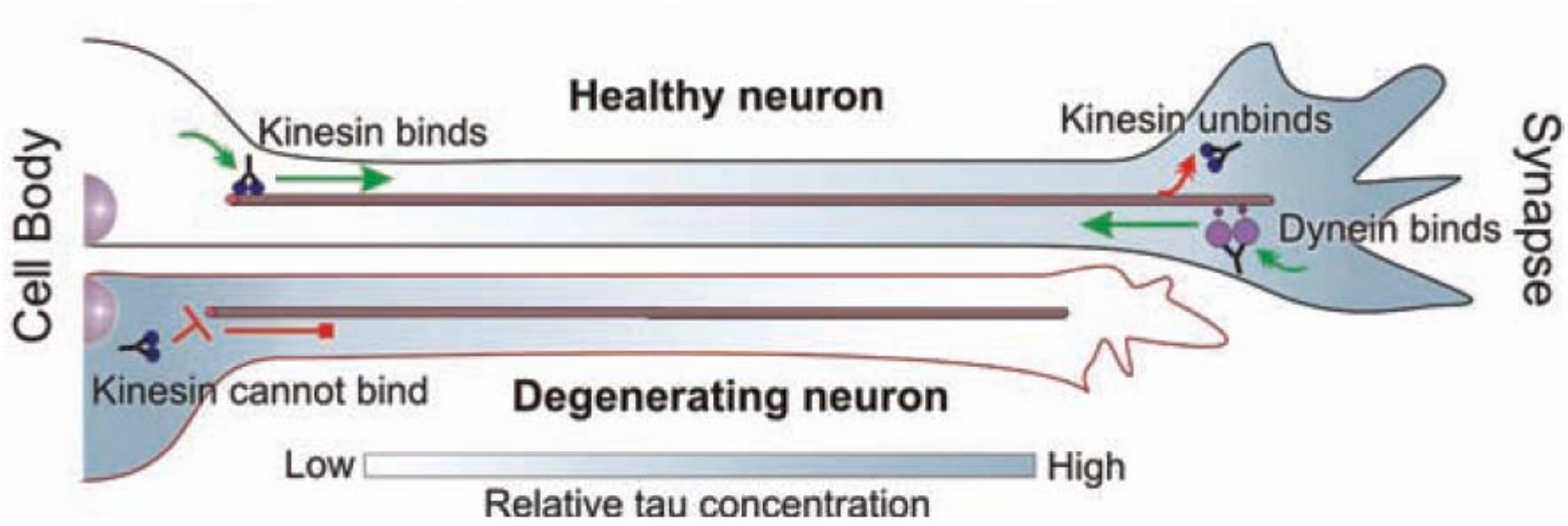




Dimeric KIF1A moves processive for long distances

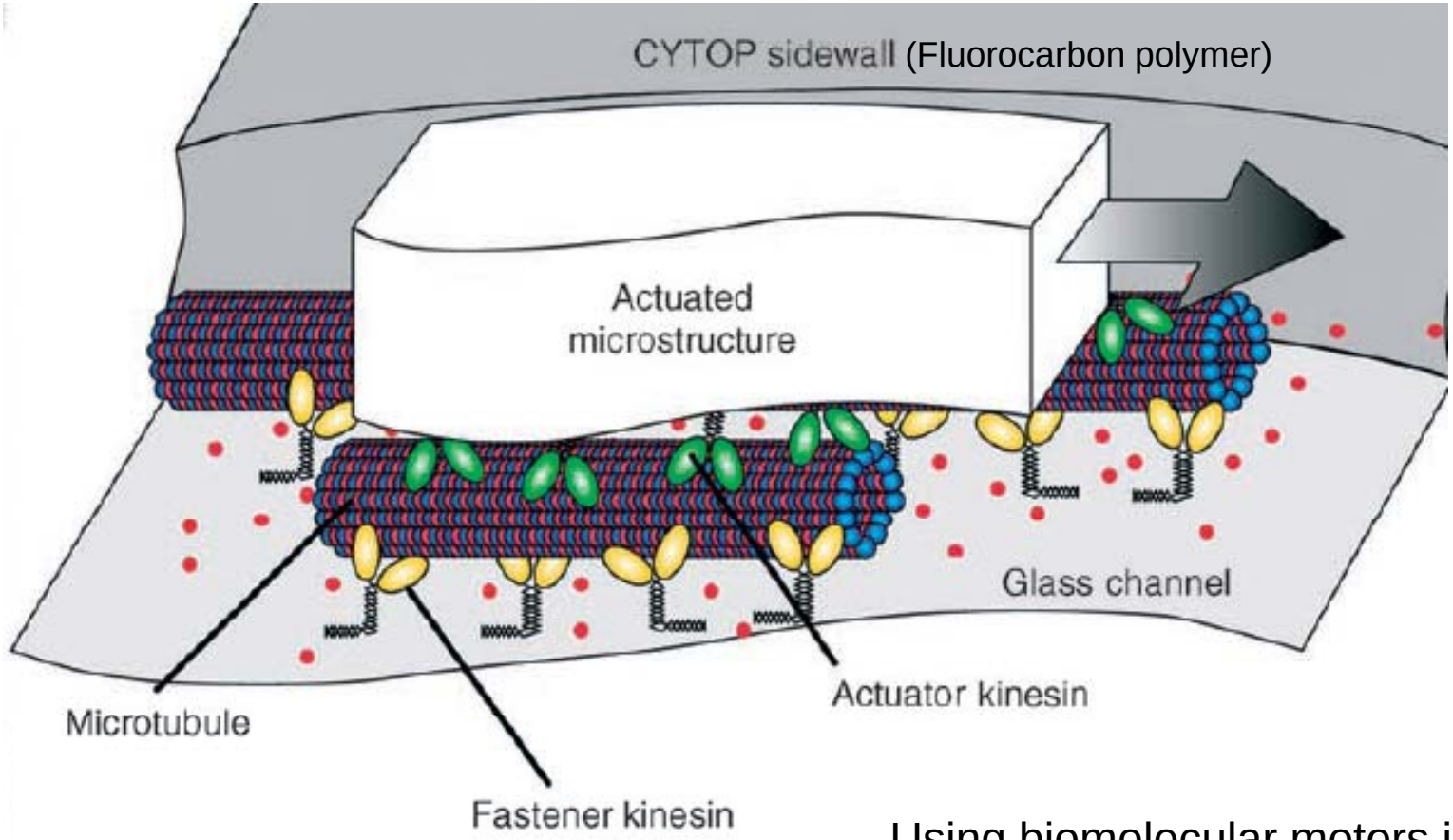
Kinesins and neurodegenerative diseases

• Tau is important for binding of kinesins to MT (**low tau conc.**) and unbinding from MT (**high tau**)
• **High tau** facilitates directional reversal of dynein
• In degenerating neurons, the gradient of tau is reversed that binding of kinesins to proximal MTs is inhibited **leading to neurodegeneration** (inhibited cargo transport)



Dixit et al., 2008, *Science*

Cytoskeleton-based molecular motors and nanotechnology

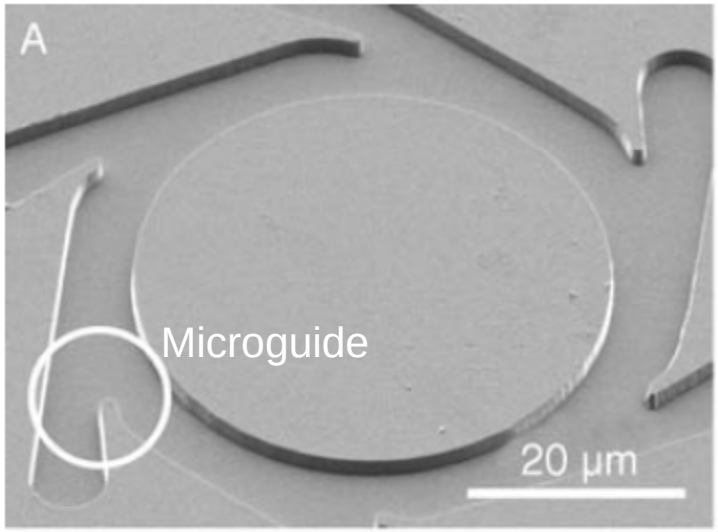


Using biomolecular motors in MEMS/NEMS allows for controlled material transport on the nanometer scale

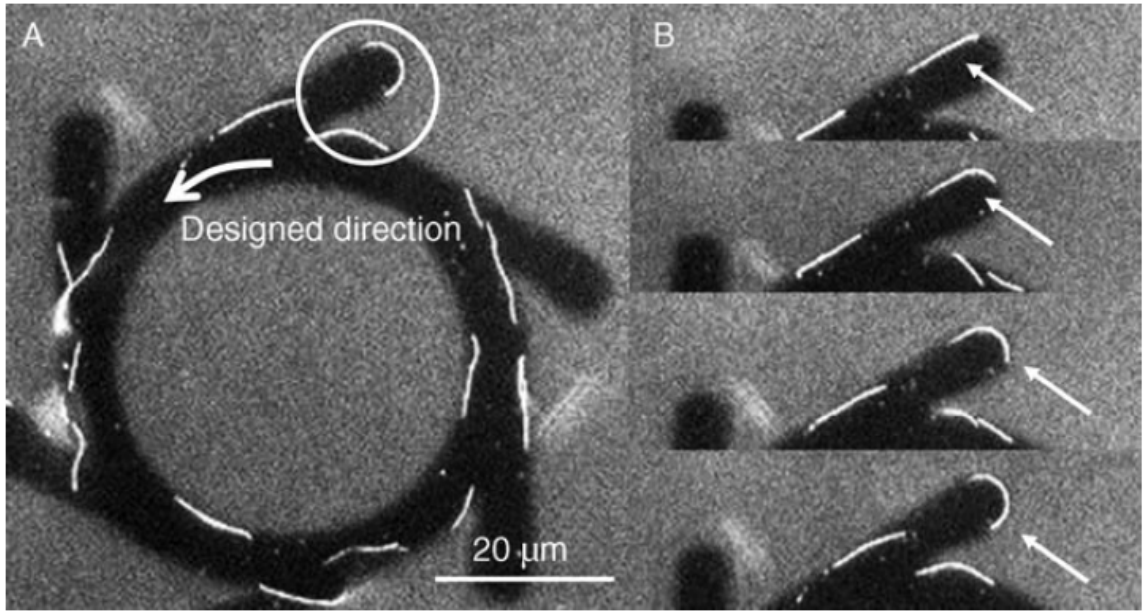
(MEMS = **M**icro**e**lectro**m**echanical **s**ystems)

Molecular sorting, concentrating and purification using biomolecular motors

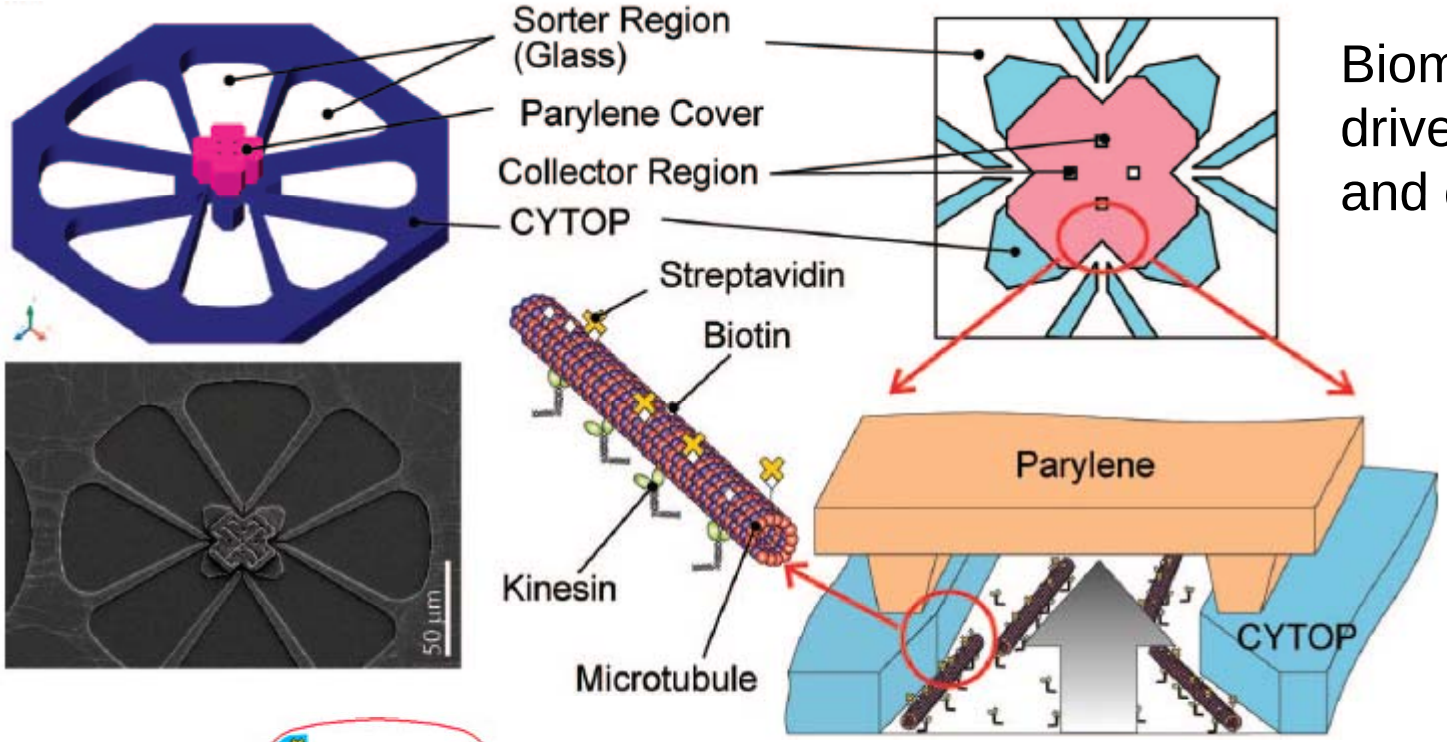
Problems for autonomous nanoscale transport of materials along nanochannels:
Microtubules frequently change directions => need for **rectifier** (direction adjuster)



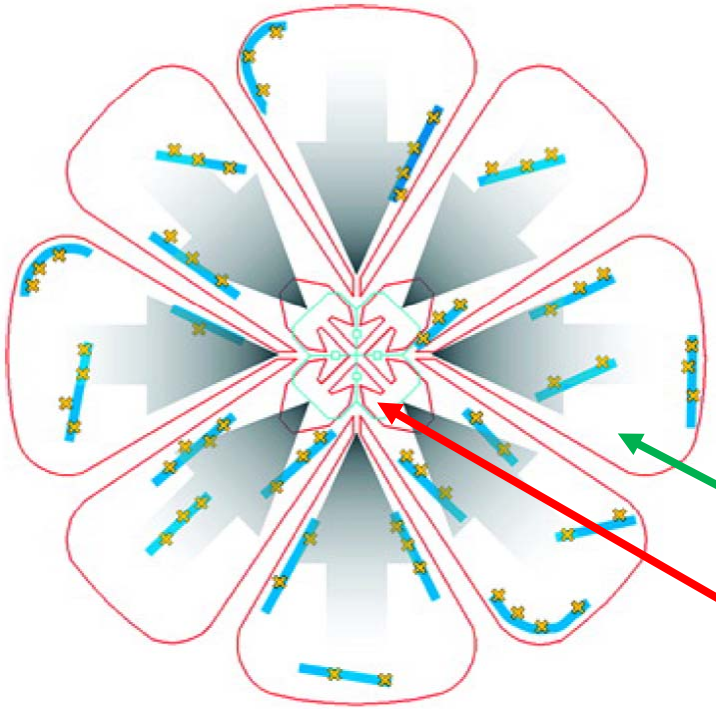
Rectifier system for autonomous transport of microfabricated structures in microfluidics system



Lin et al., 2006, *Small*



Biomolecular motor-driven protein sorting and concentrating



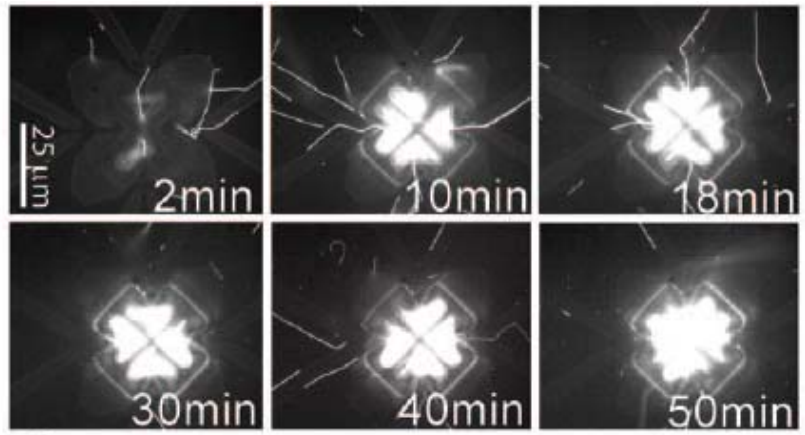
How to separate ***two*** (or more) substance(s) from each other and concentrate one of them?

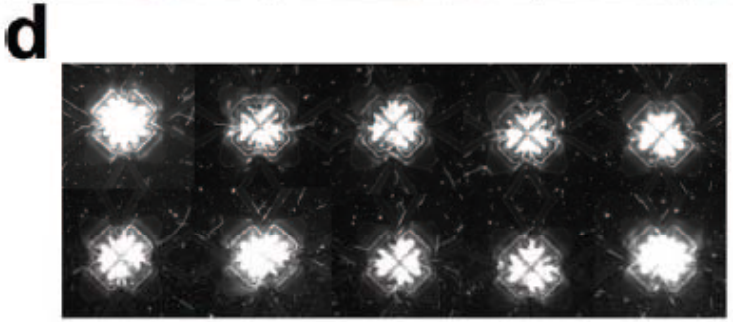
- Let <u>one substance specifically bind to MTs</u>
- Let MTs direct from the **sorter region** out into the **collector region**

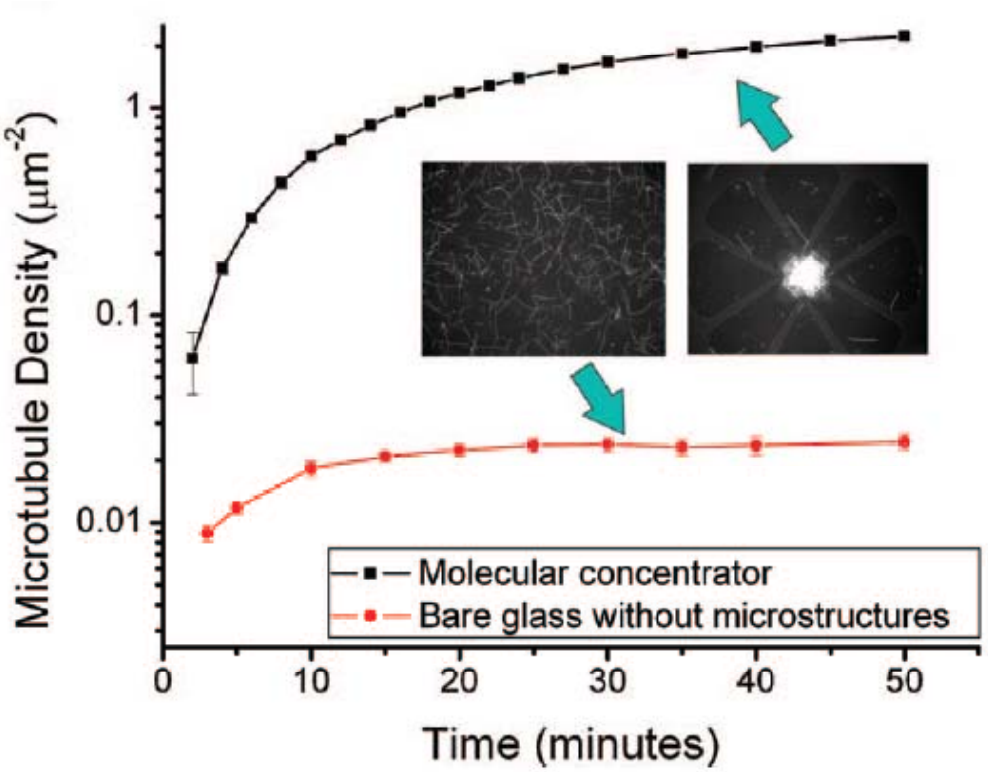
sorter region

collector region

Biomolecular motor-driven protein sorting and concentrating





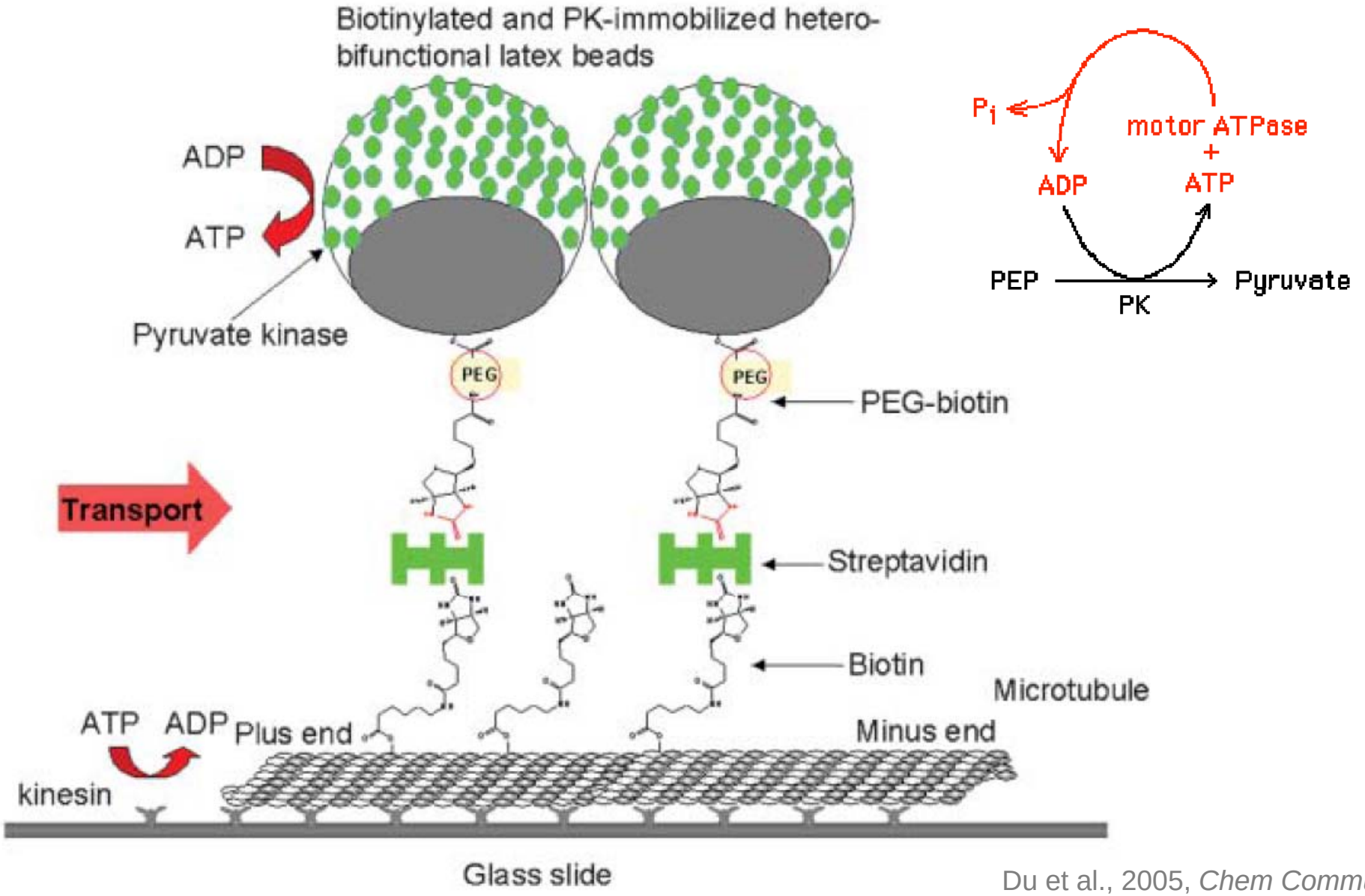


Applications:

- **Protein purification processes** on the nano- to micrometer scale level
- Analyte concentrations in the nM to pM range
- Ultrasensitive screening and **bio-detection** for **diagnostic applications**

Nano-biomachine powered by self-supplying ATP

- ATP can be generated from ADP by the enzyme **pyruvate kinase** (PK)
- P_i from PEP (phosphoenol pyruvate) transferred to ATP (PEP => pyruvate)

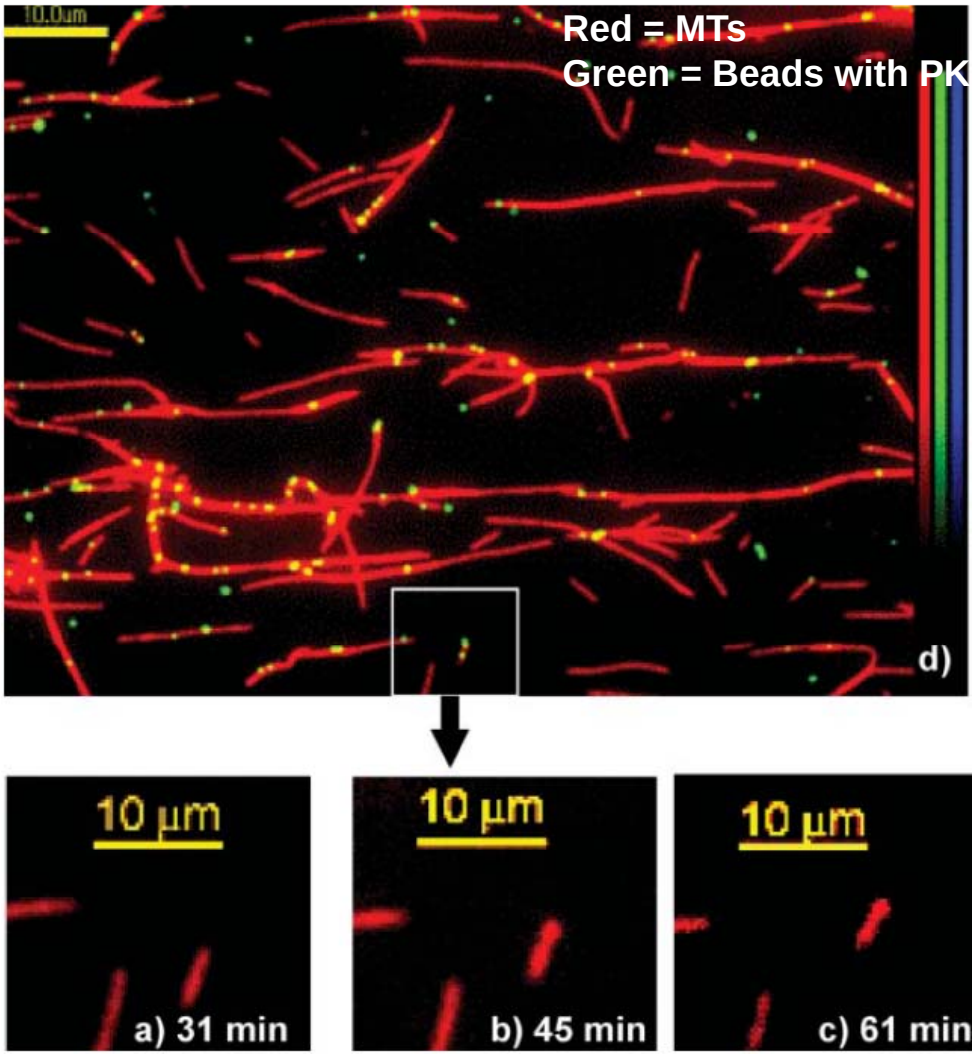


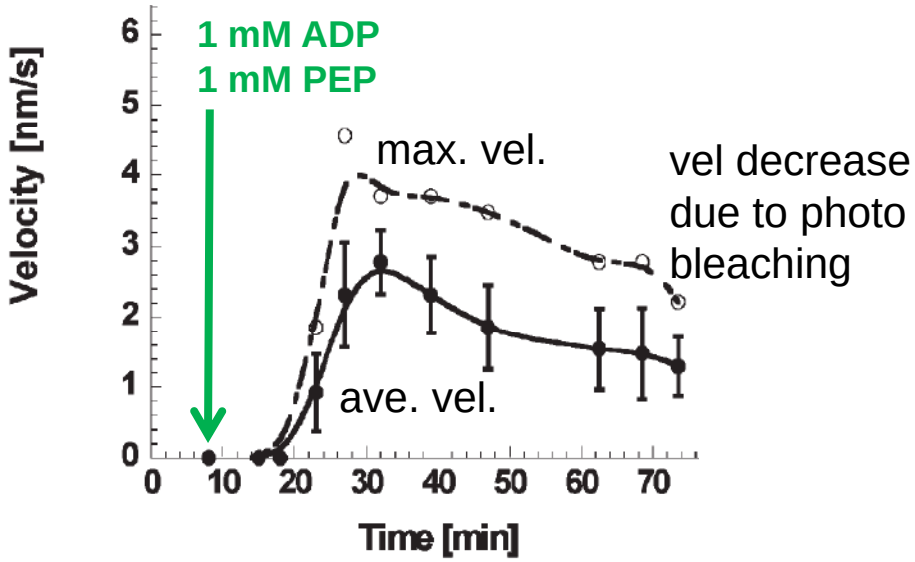
Du et al., 2005, *Chem Commun.*

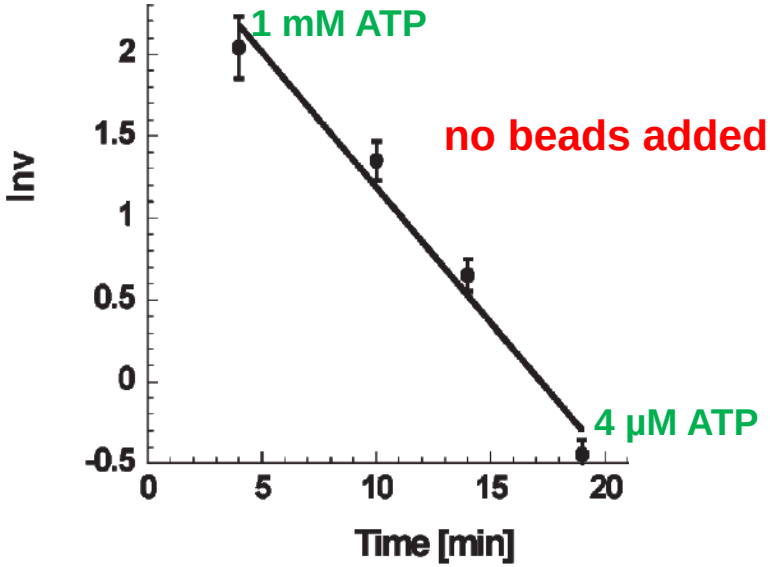
Nano-biomachine powered by self-supplying ATP

- Nano-biomachined **moved** for **75 minutes** at (almost) constant velocity
- Without the self-supplying system the speed of MTs decreased to zero after **20 min**

Movie
ATP self-suppl bio nanomachine.mov

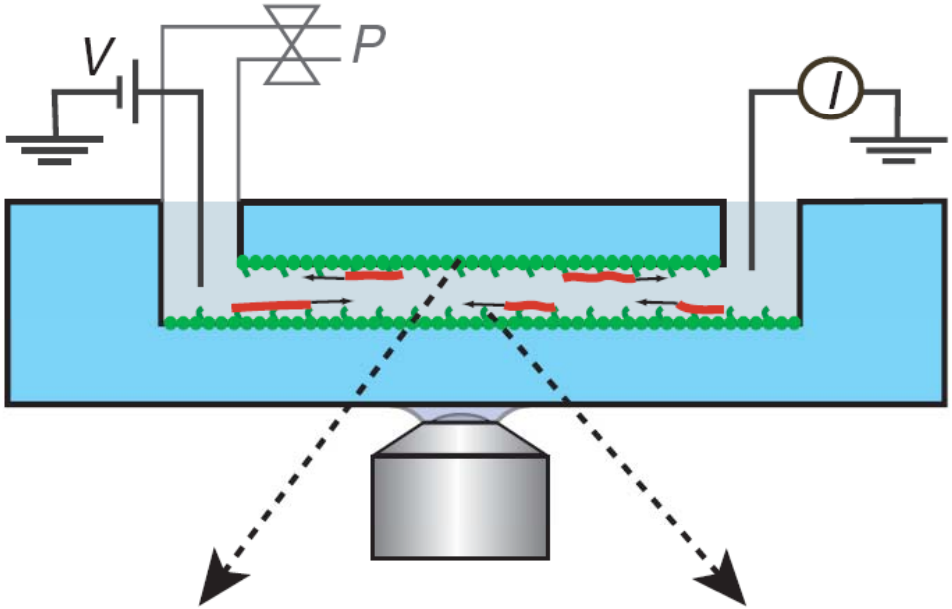




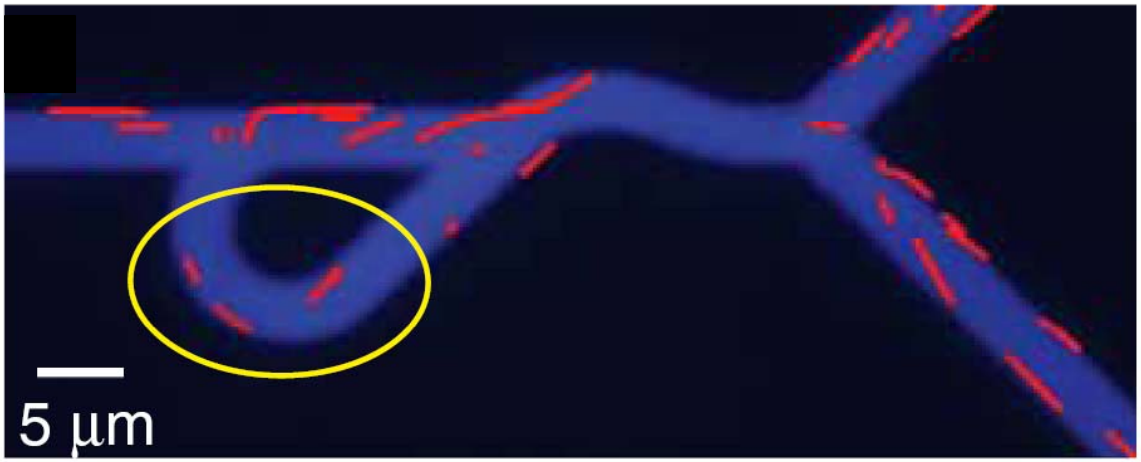


Molecular sorting, concentrating and purification using biomolecular motors

Using **electric fields** to control the direction of material transport in MEMS



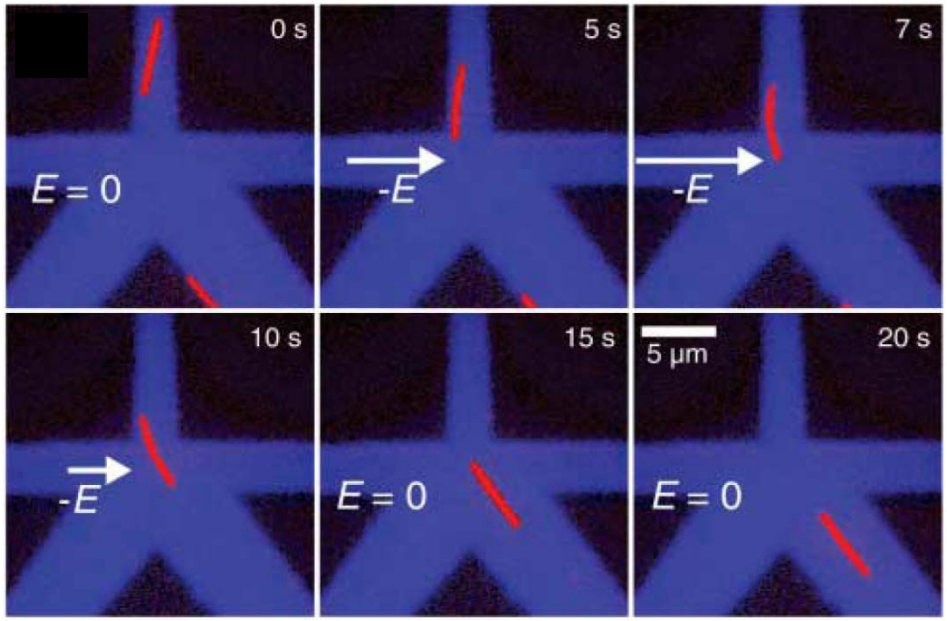
- 800 nm deep **nanochannels** made by E-beam lithography and wet etching techniques
- Channels are coated with **kinesin** (green) and **microtubules** (red) flown inside
- An electrical field (35 kV/m) can be applied

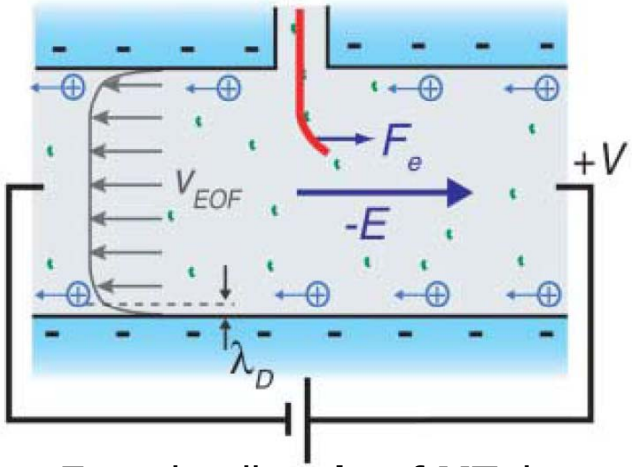


Microscopic image of fluorescently labeled MTs in nanochannels

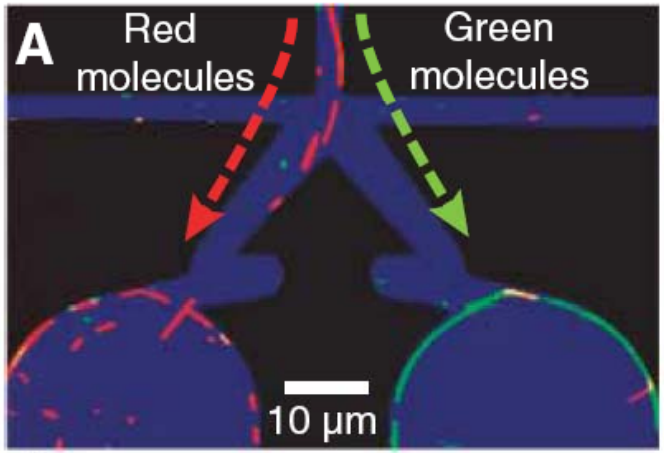
Heuvel at al., 2006, Science

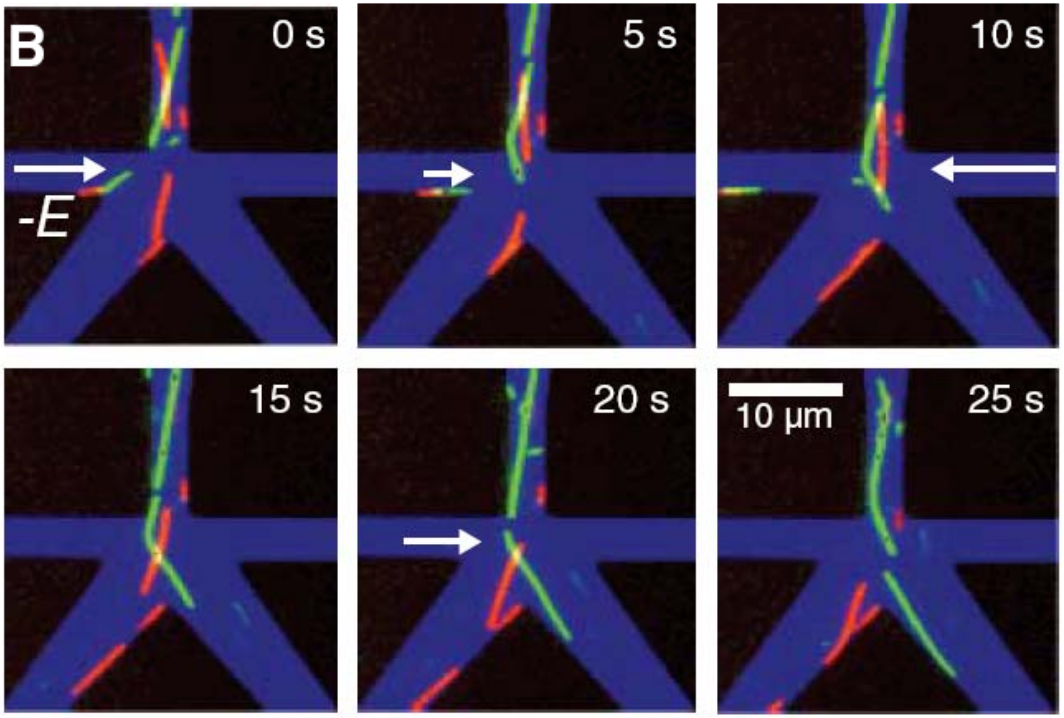
Molecular sorting, concentrating and purification using biomolecular motors





- Free, leading **tip of MT**: brownian motion
- **Electric field**: bending of the free tip occurs
- MT travels into the direction of bending



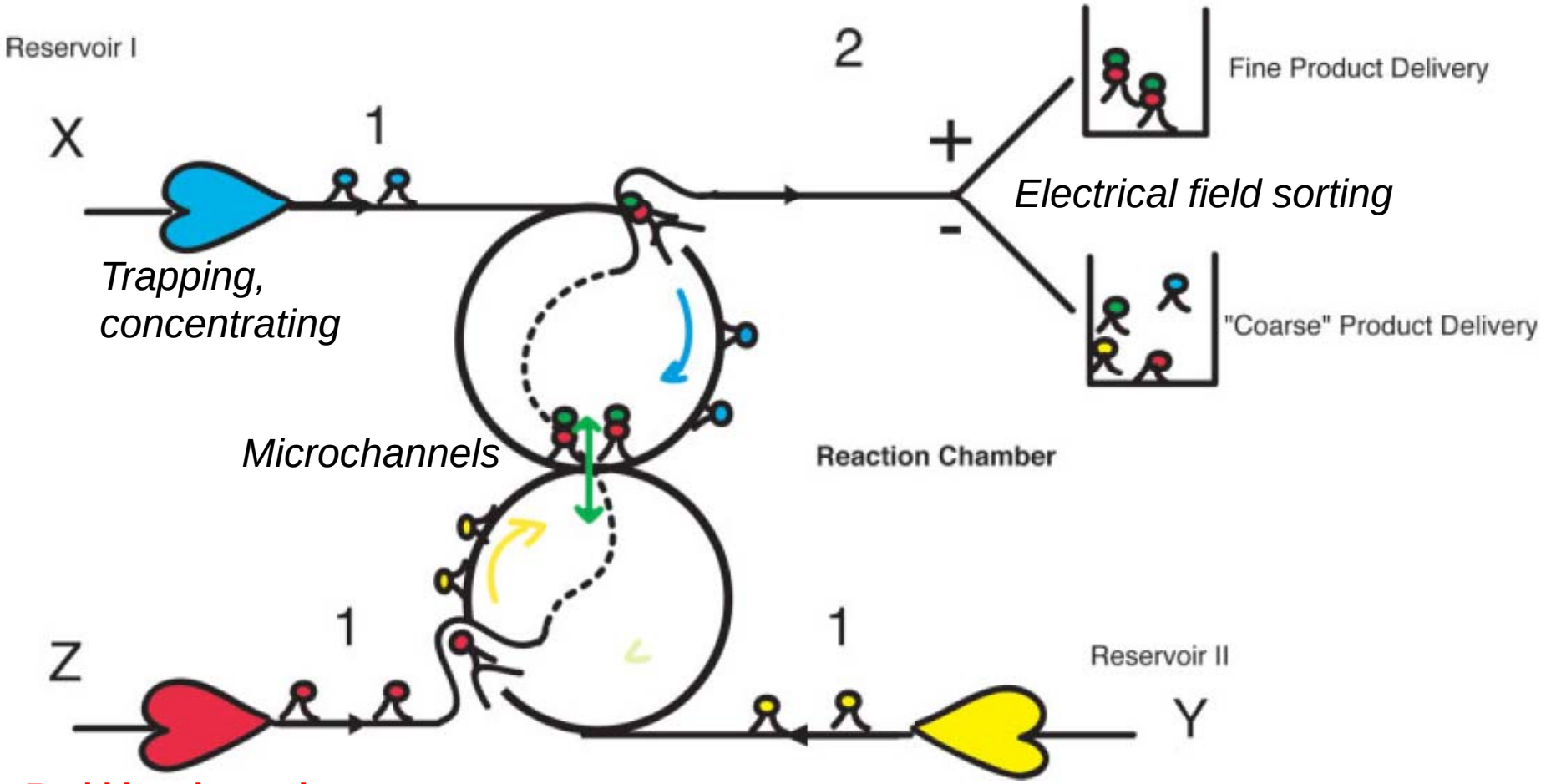


A simple nano-factory

- Nano-factory for **product synthesis**, **sorting** and **quality control**
- Goal: **blue cargo X** should react with **yellow cargo Y**

to become a **new product (green)** that will be transported by the **red motor**

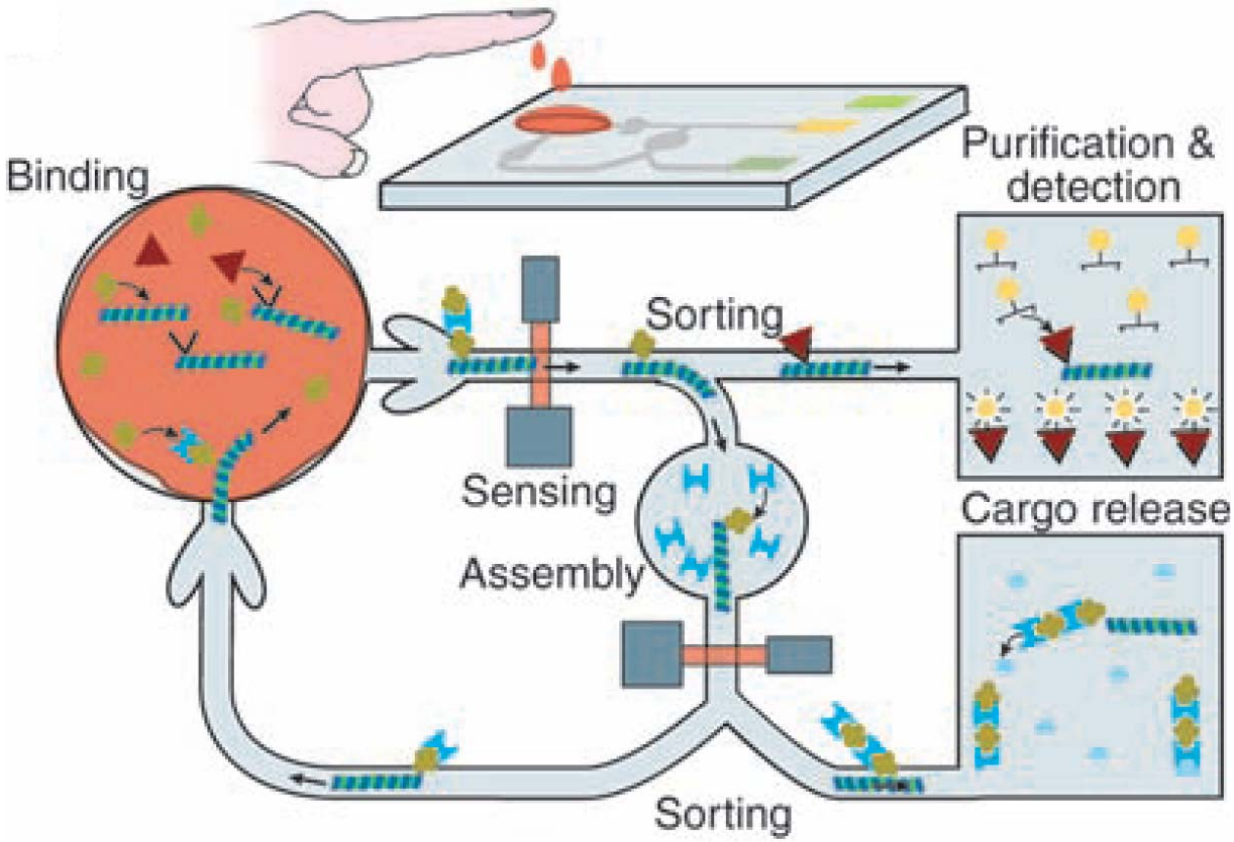
- Cargo: Protein, biochemical substance, DNA oligomer etc.



Red kinesins only transport newly reacted cargo

Medical applications for autonomous nano-factories

Lab-on-a-chip device (powered by molecular motors) for autonomous sorting and purification of blood components

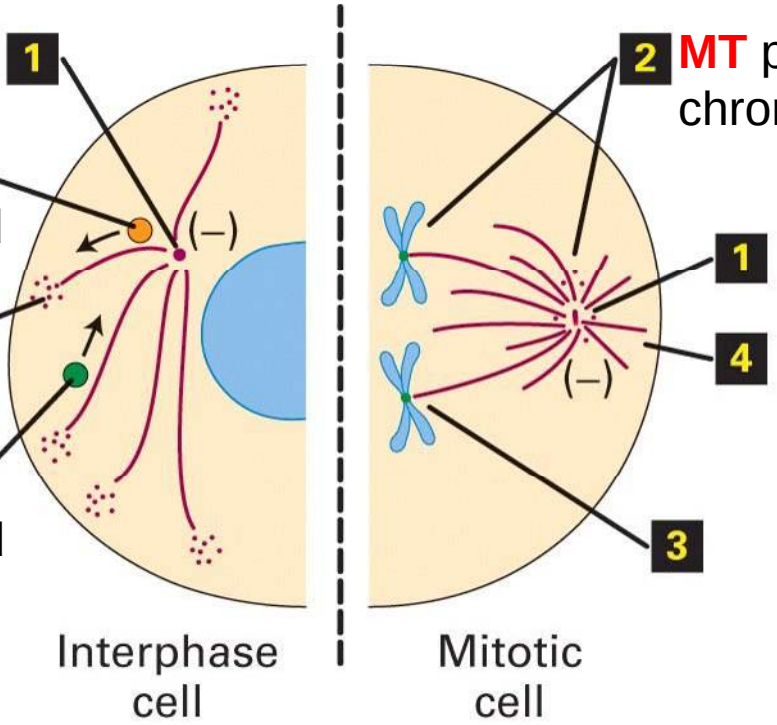


Antibody-tagged shuttles **capture** and **separate target molecules** in <u>otherwise undetectable low quantities</u> in an analyte

Molecular motors play different roles in interphase and mitotic cells

Kinesins move from the (-) end to the (+) end

Dyneins move from the (+) end to the (-) end



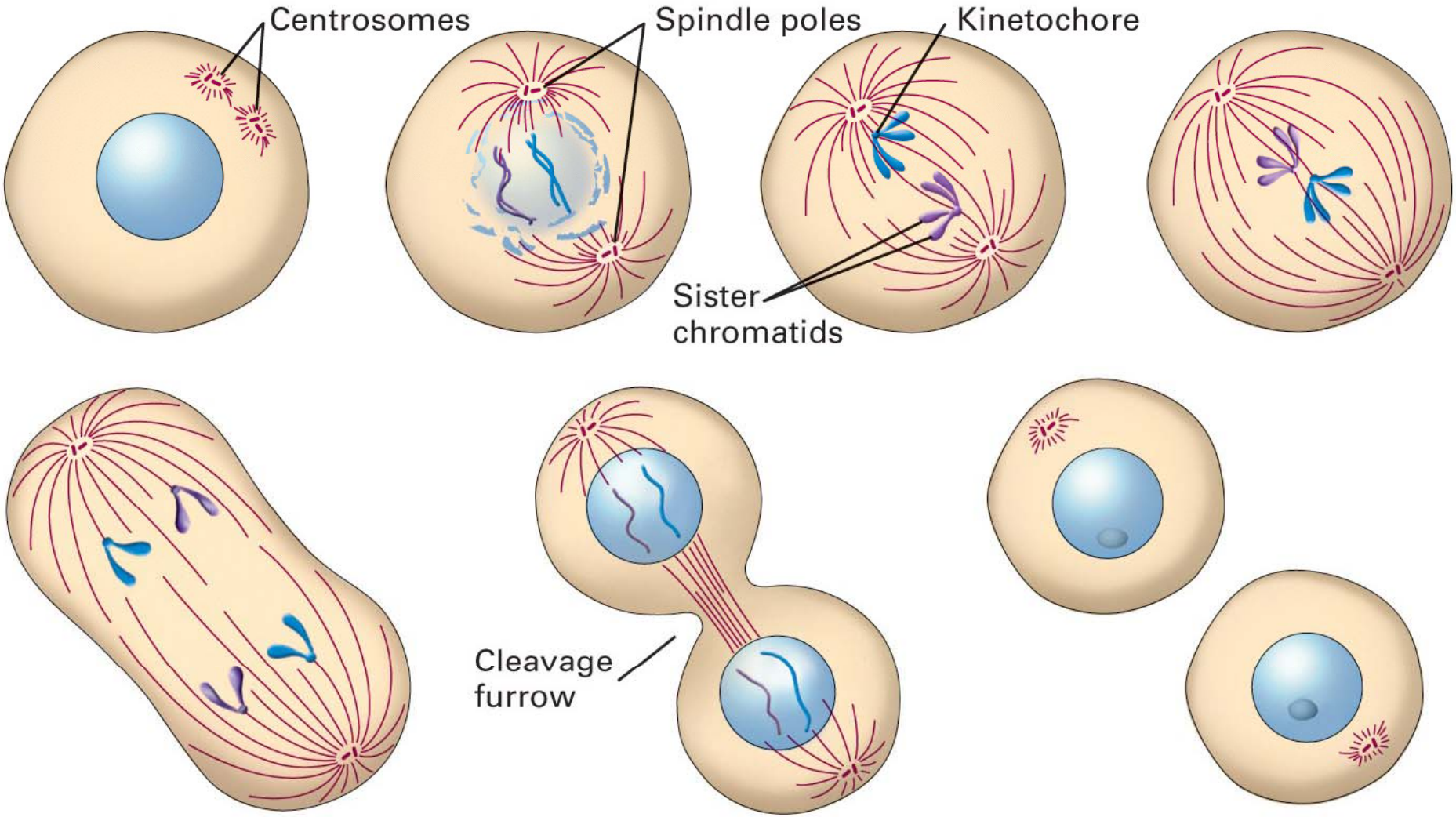
MTs are the highway for molecular motors

MTs push chromosomes to the daughter cells

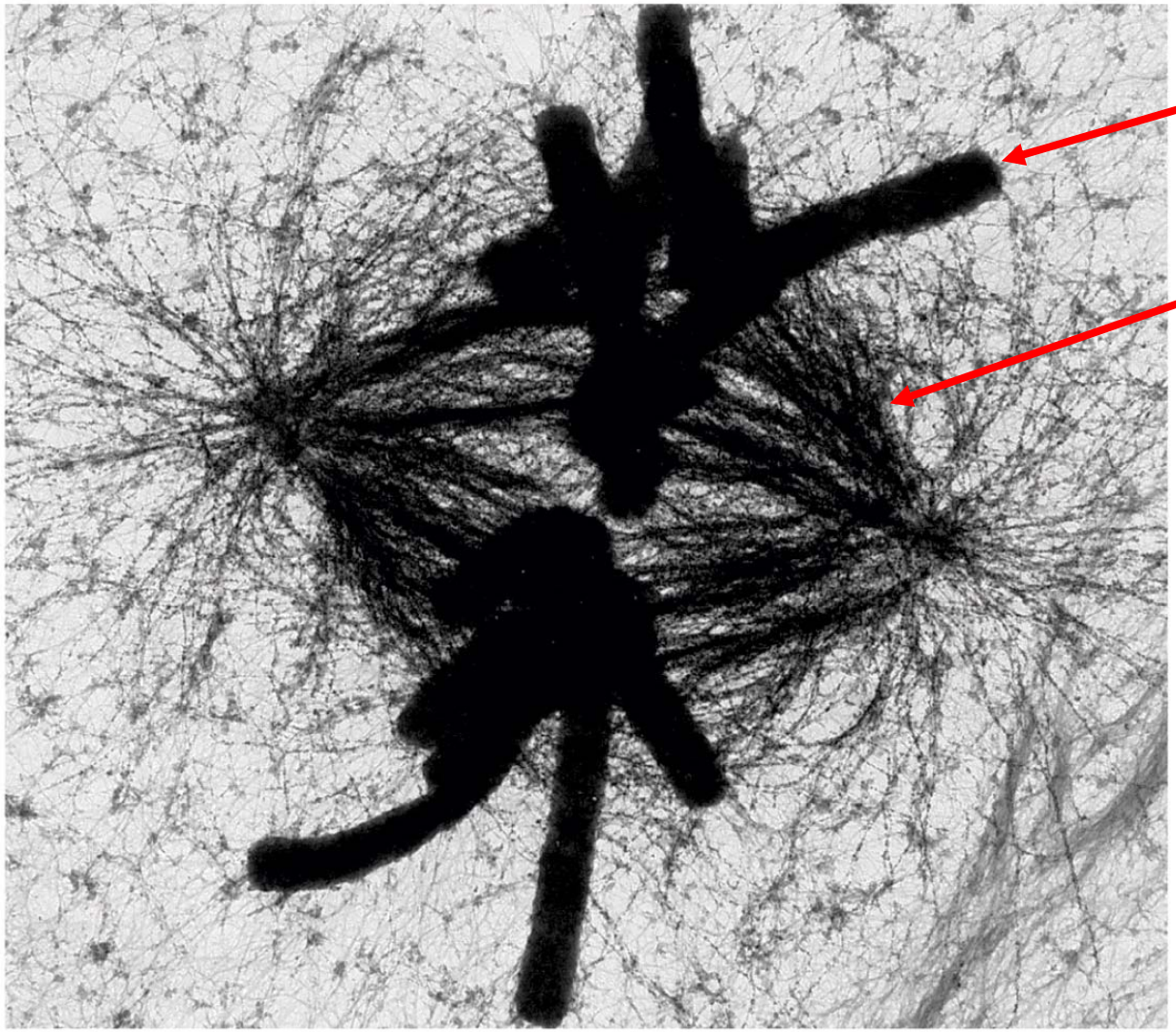
Mitosis

Microtubules can attach to chromosomes and pull them apart

During cell division the complete DNA material has to be duplicated and than distributed into two cells



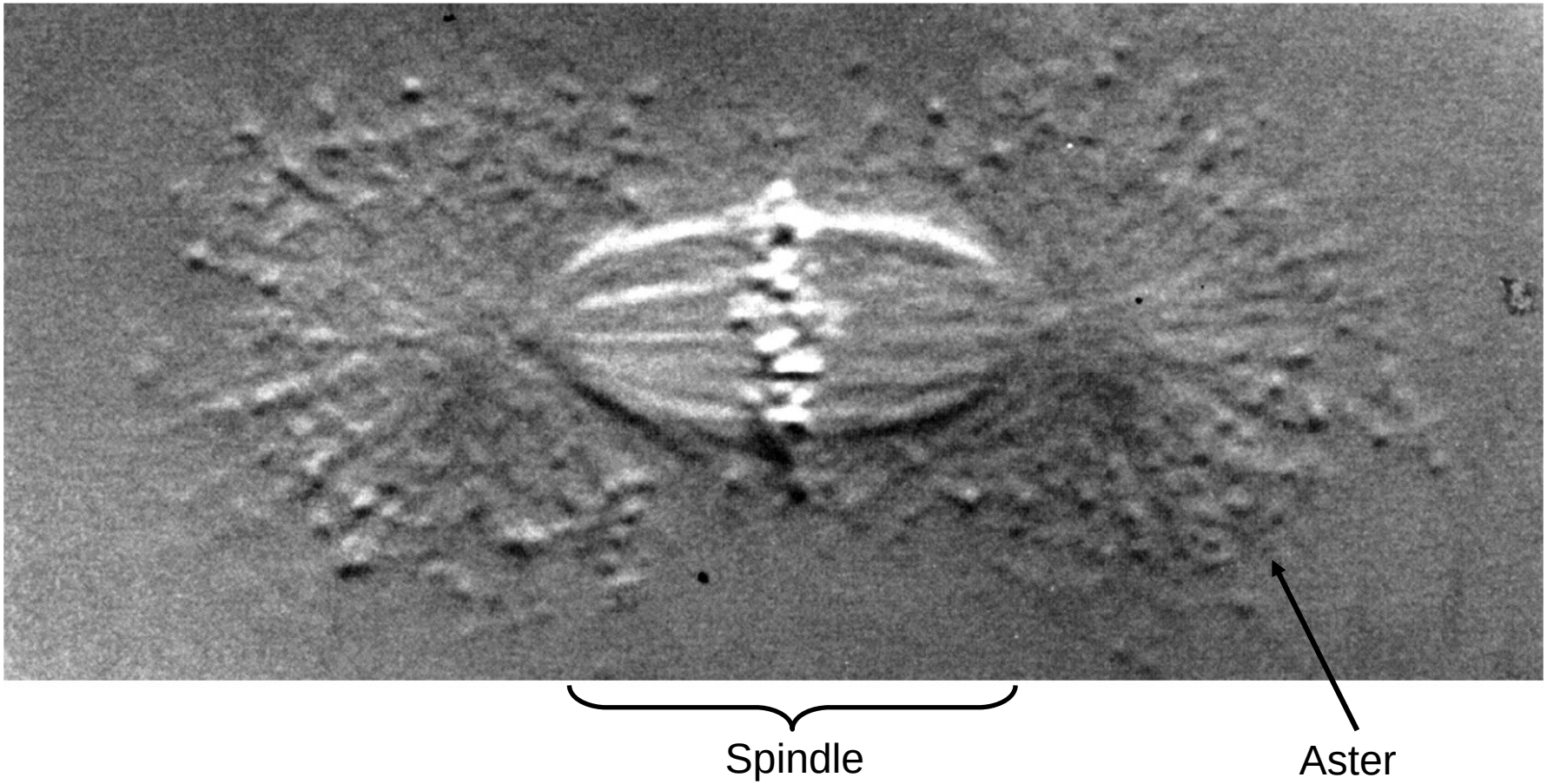
Microtubules can attach to chromosomes and pull them apart



EM image of an isolated mitotic apparatus

Microtubules can attach to chromosomes and pull them apart

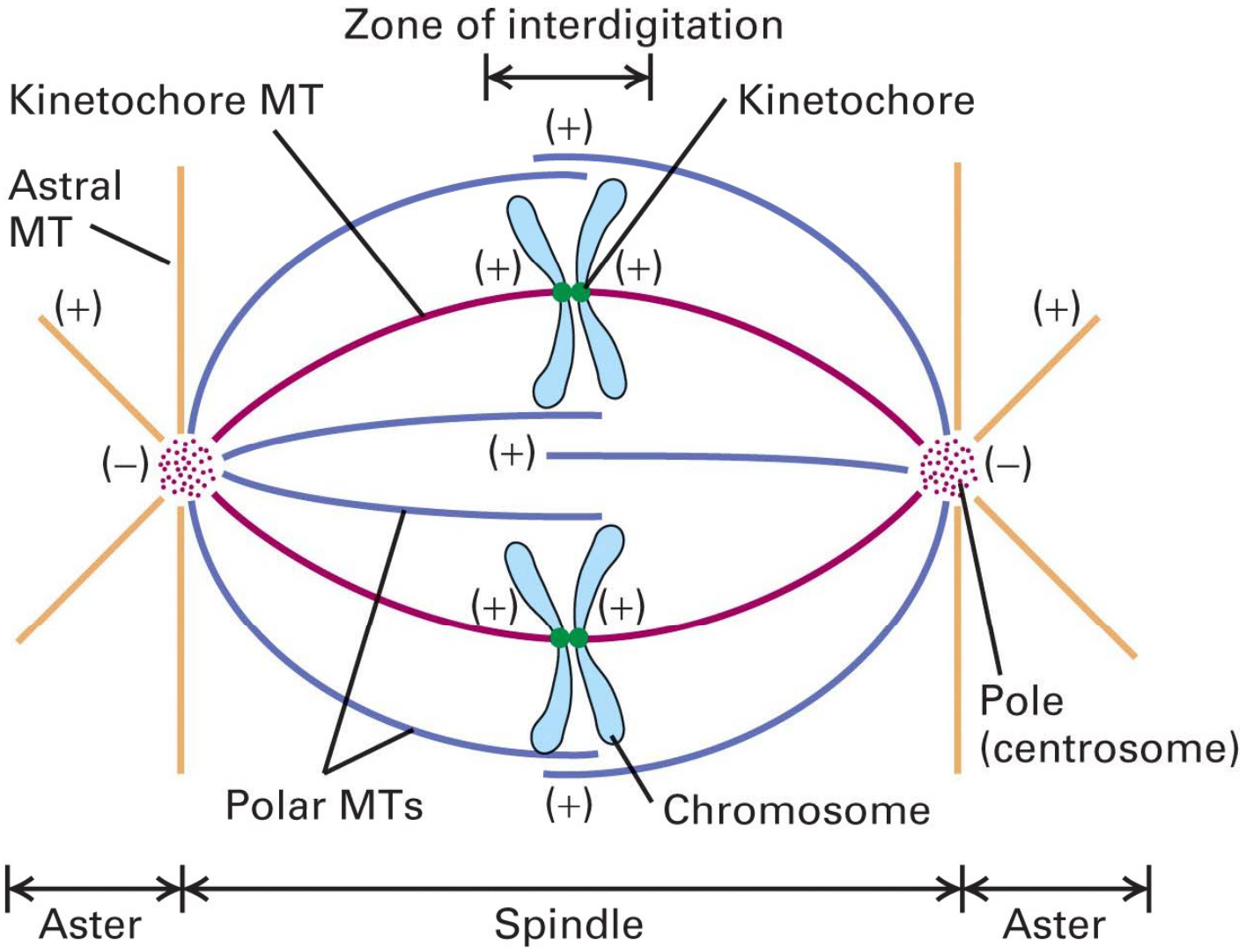
Animation	*Movie*
a20-03-mitosis.swf	18_2.mov



Isolated mitotic spindle apparatus

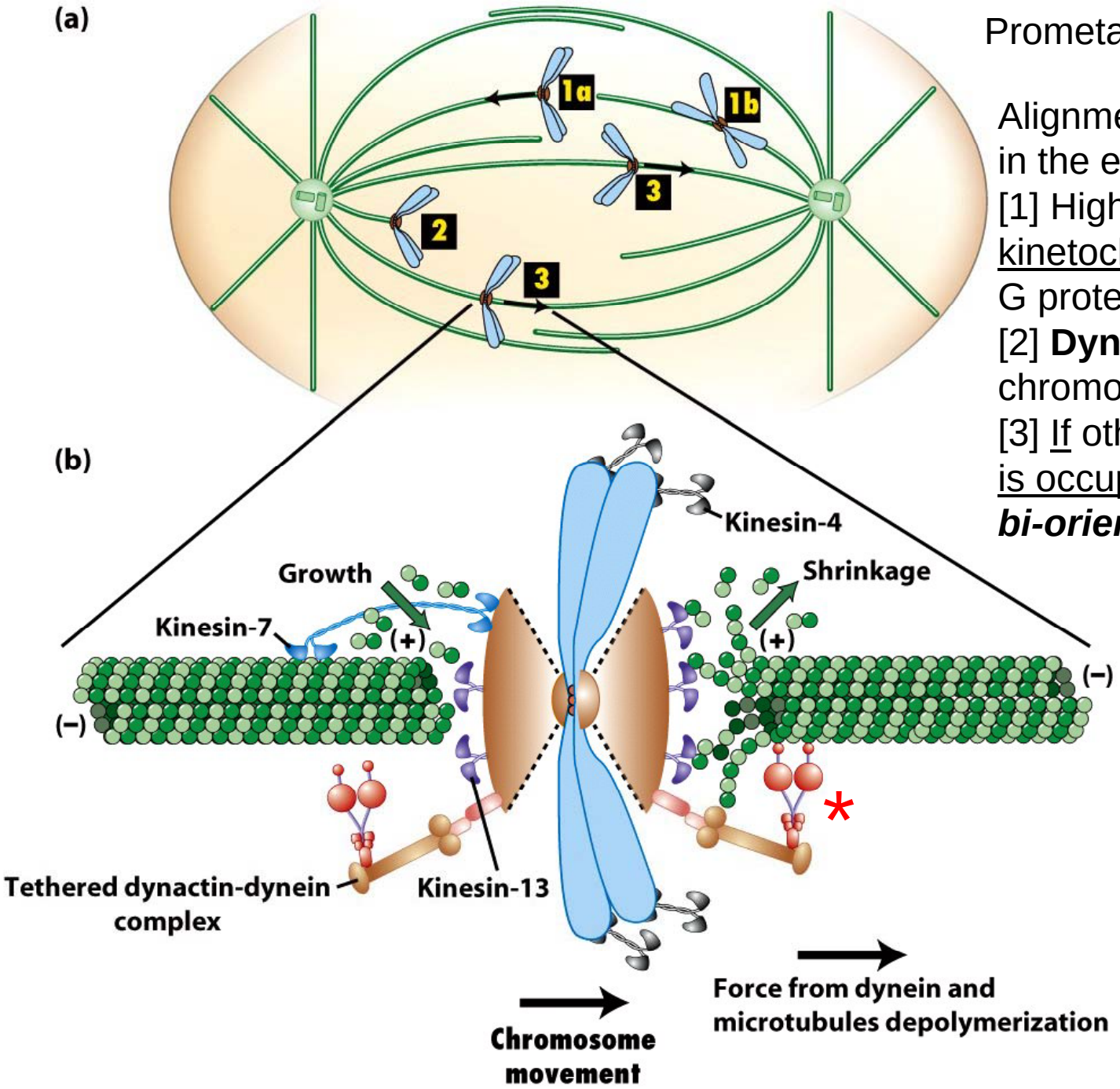
Three distinct sets of MT in the mitotic apparatus

- **Aster MT**: radiate out of the centrosome up to the cell cortex: **position the spindle**
- **Kinetochore MT**: part of the spindle; **attach to** centromeres of **chromosomes**
- **Polar MT**: overlap with opposite polar MT; do not connect to kinteochores



Cytosolic and mitotic kinesins

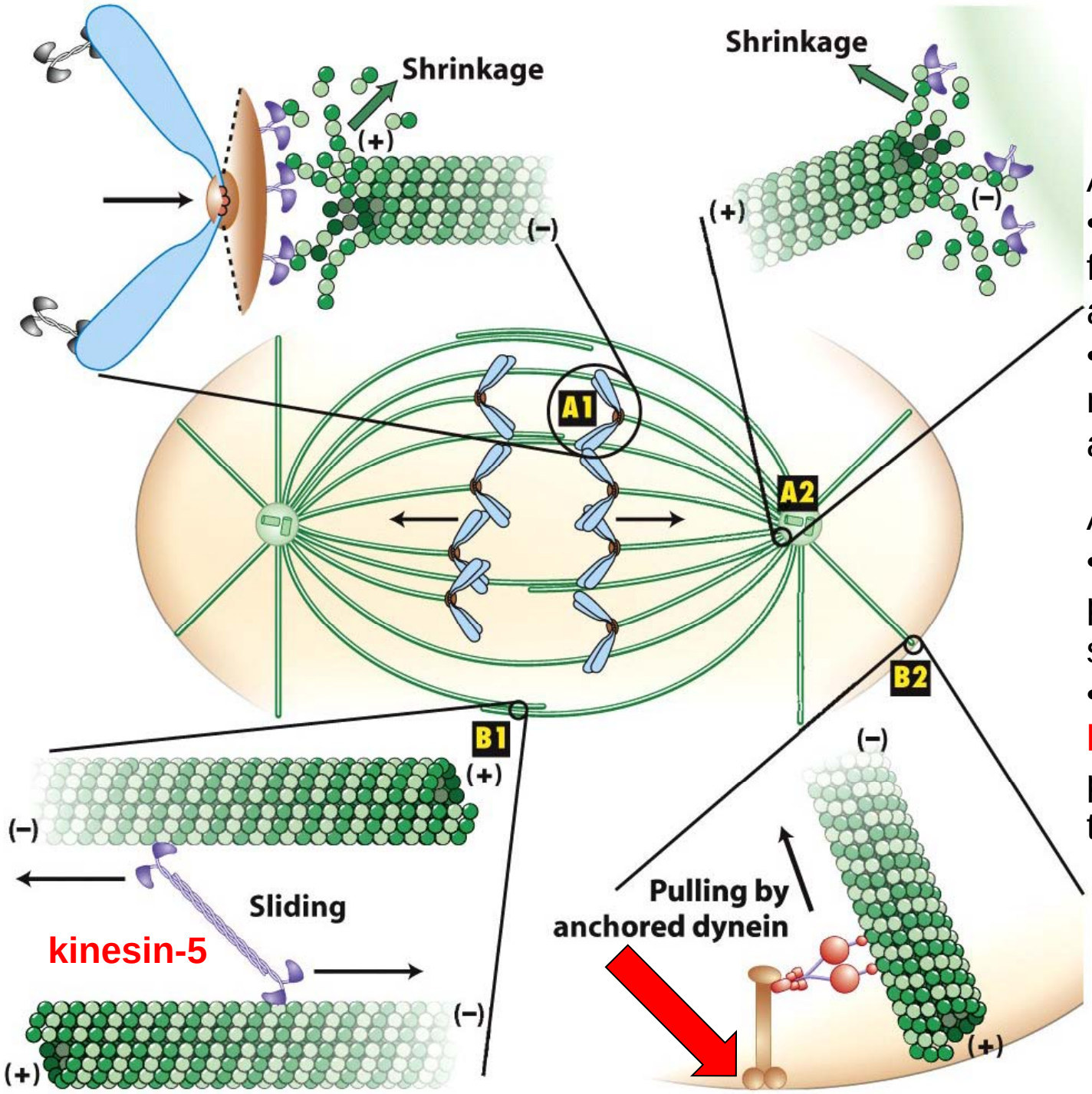
- Cytosolic **Kinesin I** (conventional kinesin) transports **organelles** (as lysosomes) while **KIF1A** transports **synaptic vesicles** and **KIF1B** **mitochondria** in axons
- Mitotic kinesins:
    - Kinesin-**4** (Chromokinesin): links chromosome *arms* to polar MTs
    - Kinesin-**5** (BimC): involved in spindle pole separation
    - Kinesin-**7** (CENP-E): links chromosome *centromeres* to astral MTs
    - Kinesin-**13** (MCAK): depolymerizes mitotic MTs



Prometaphase events

Alignment of chromosomes in the equatorial plate:
[1] Highly dynamic MTs hit kinetochore (promoted by G protein Ran-GTP)
[2] **Dynein/dynactin** moves chromosome to spindle pole
[3] If other free kinetochore is occupied chromosome is ***bi-oriented*** (strong tension!)

- While **dynein** * pulls, **kinesin-13** (MCAK) induces depolymer-ization
- **Kinesin-7** (CENP-E) keeps growing (+) end attached
- **Kinesin-4** (chro-mokinesin) anchors chromosome to the polar MTs



Anaphase A
and B events

Anaphase A:
• **Dynein** releases from the centromere and moves to the pole
• **Kinesin-13** depolymerizes **spindel MTs** at (+) end ***and*** (-) end

Anaphase B:
• [B1] *Anchored* dynein pulls on **astral MTs** to separate spindle poles
• [B2] Bipolar **kinesin-5** (BimC) pushes on **polar MTs** to separate the poles

Animation
a20-03-microtubule
_dynamics.swf