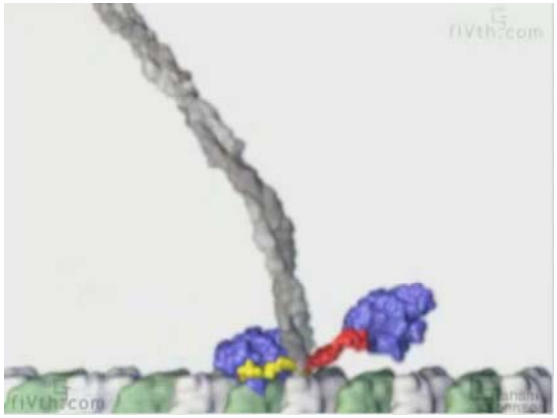
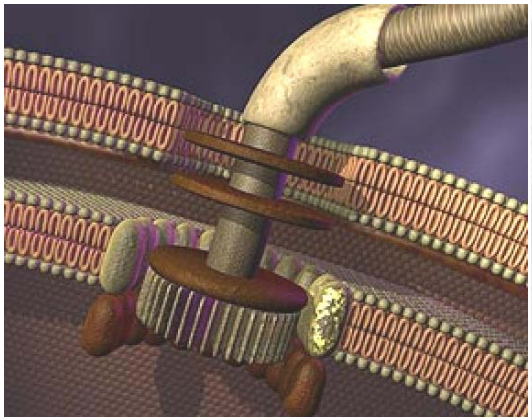
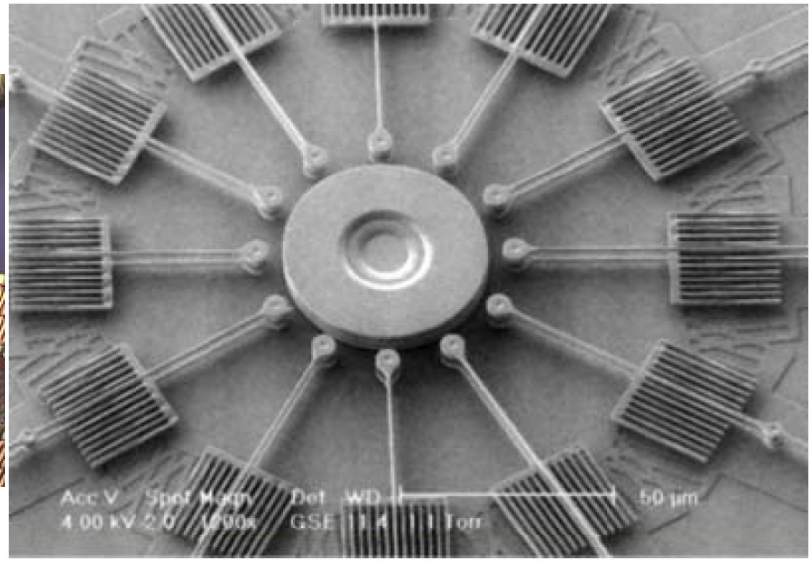
Biological Machines, Cell Mechanics and Nanotechnology Part II







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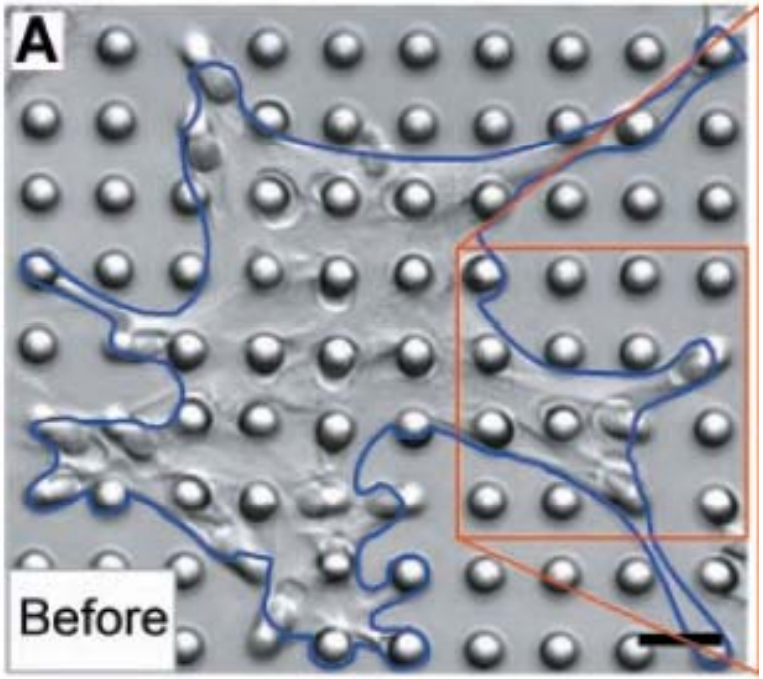
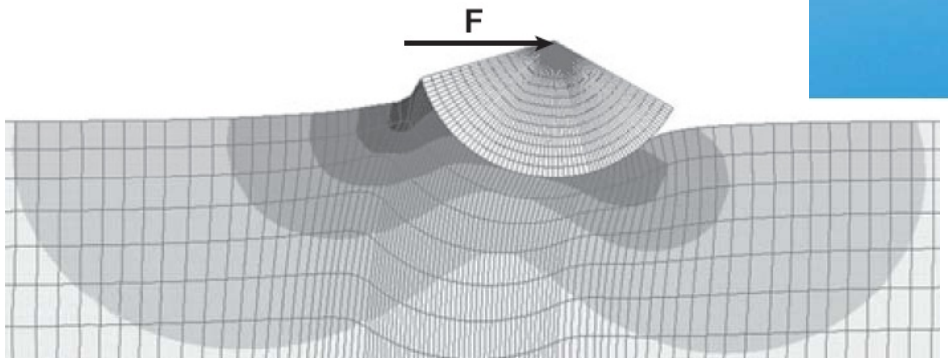
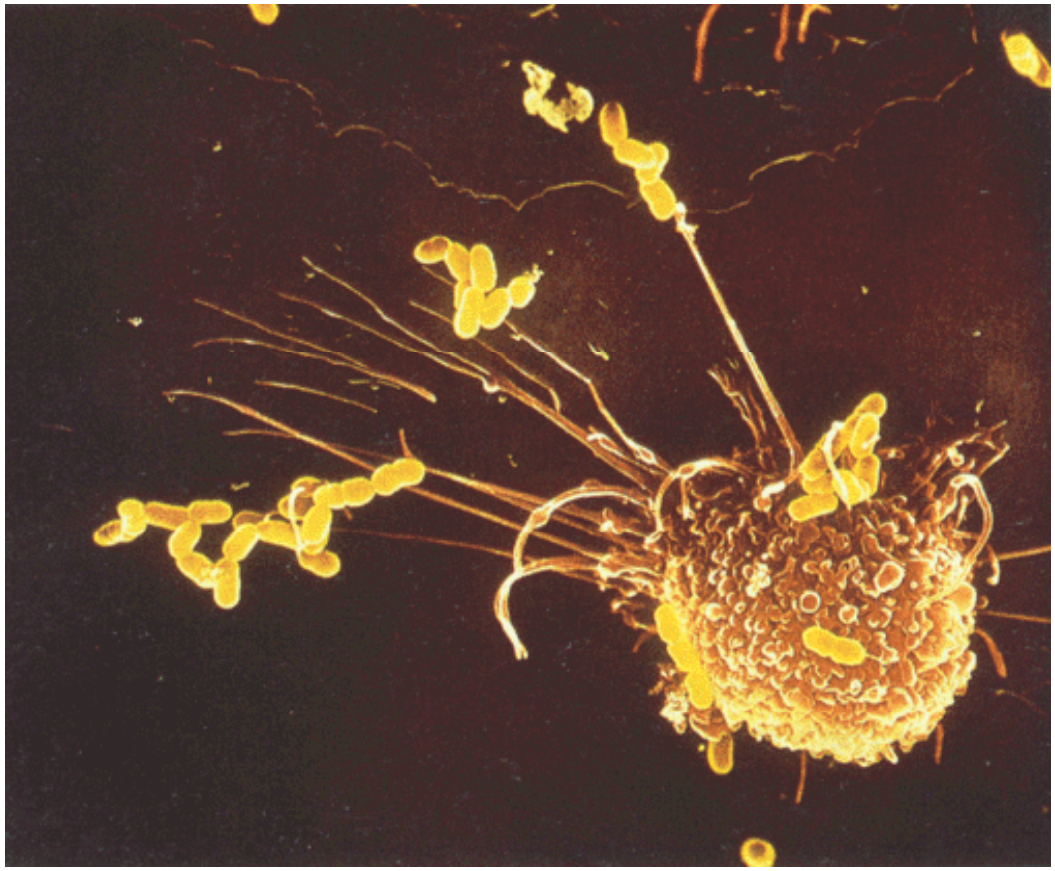
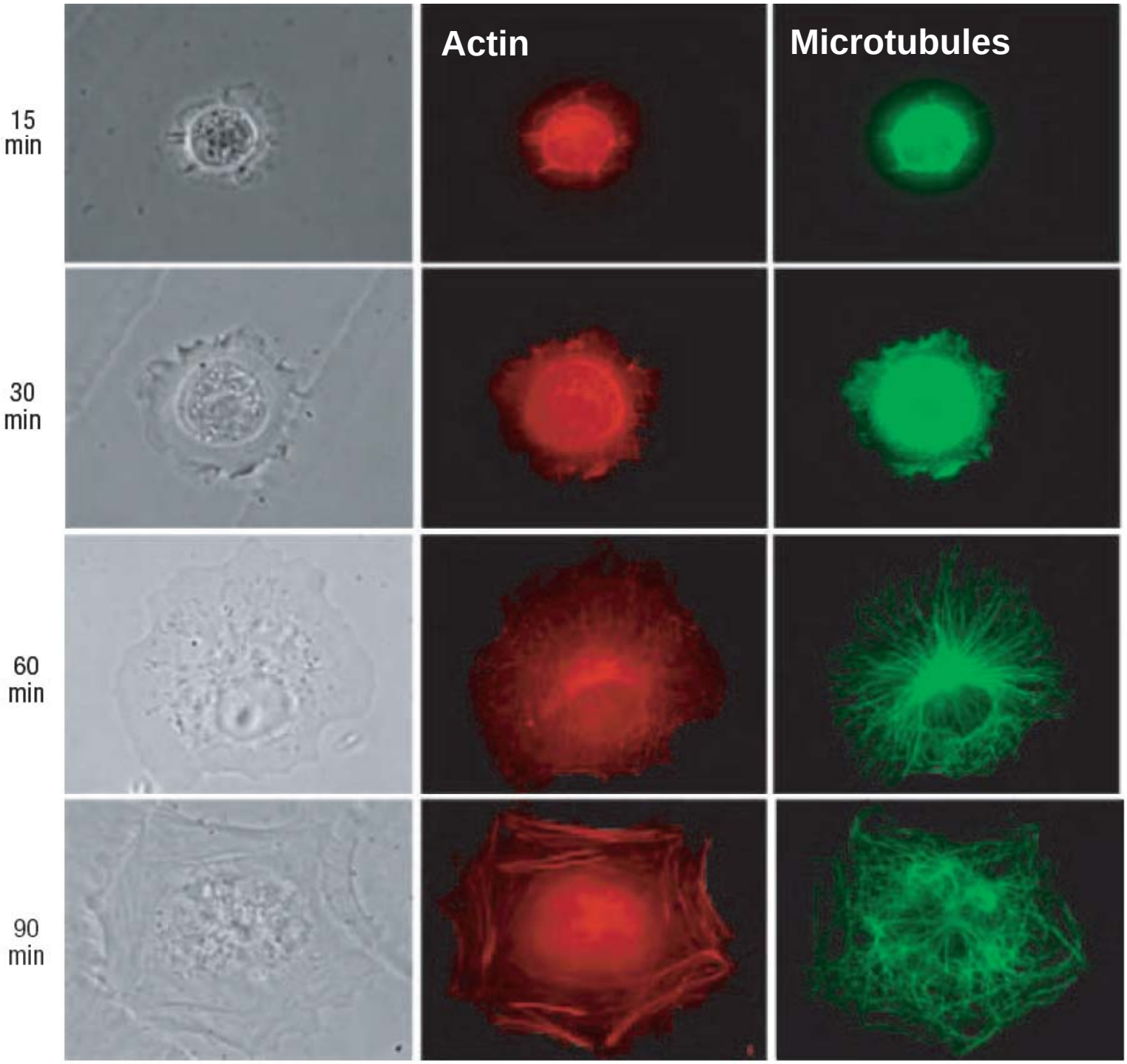




Importance of cytoskeleton and cytomechanics in environmental cell responses



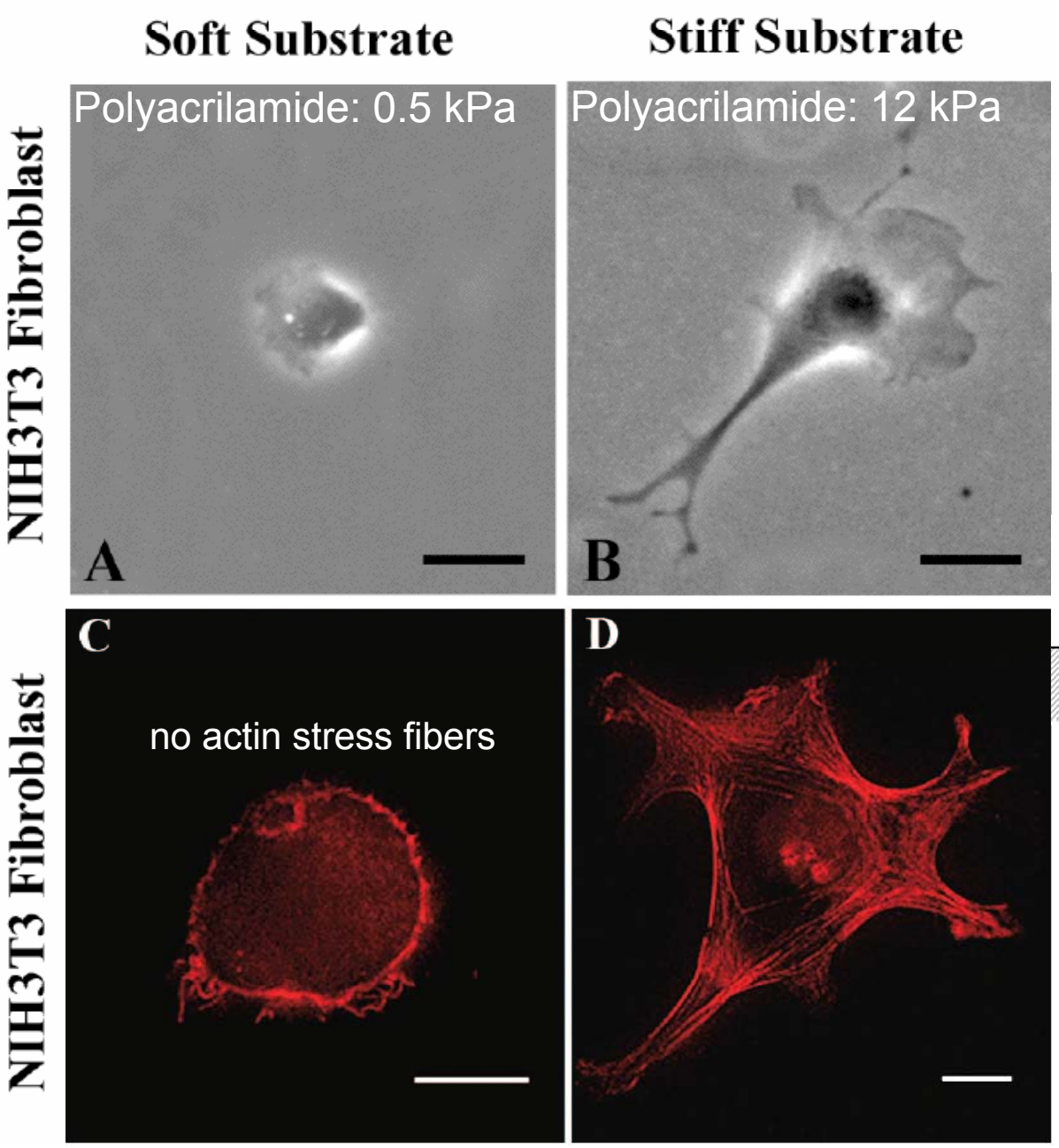
Filopodia (made of thick actin bundles) of white blood cells catching bacteria for digestion



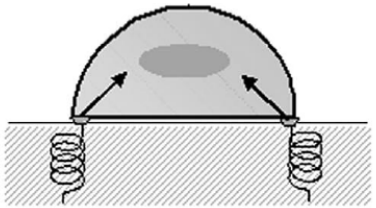
Cytoskeletal response to cell spreading

Actin bundle (stress fiber) increase during spreading of a cell (fibroblast) on glass

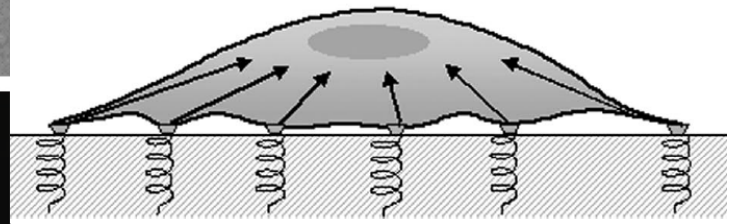
Cellular response to substrate stiffness



Model



Soft substrate with **small spring constant** (k):
Cell can easily pull on the gel
(no need for stress fibers)

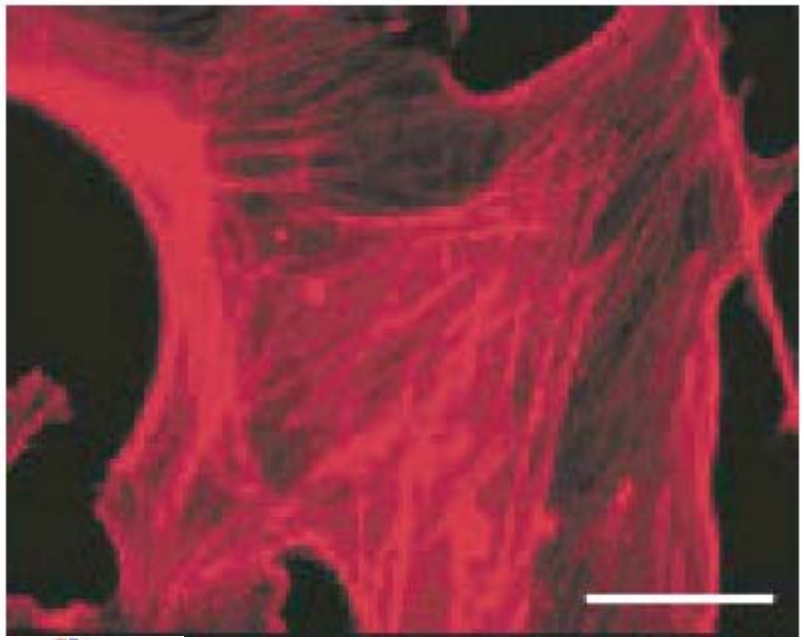


Stiff substrate with **larger spring constant** (k):
Cell need greater force to displace the polyacrilamide gel
(cell need power of stress fibers)

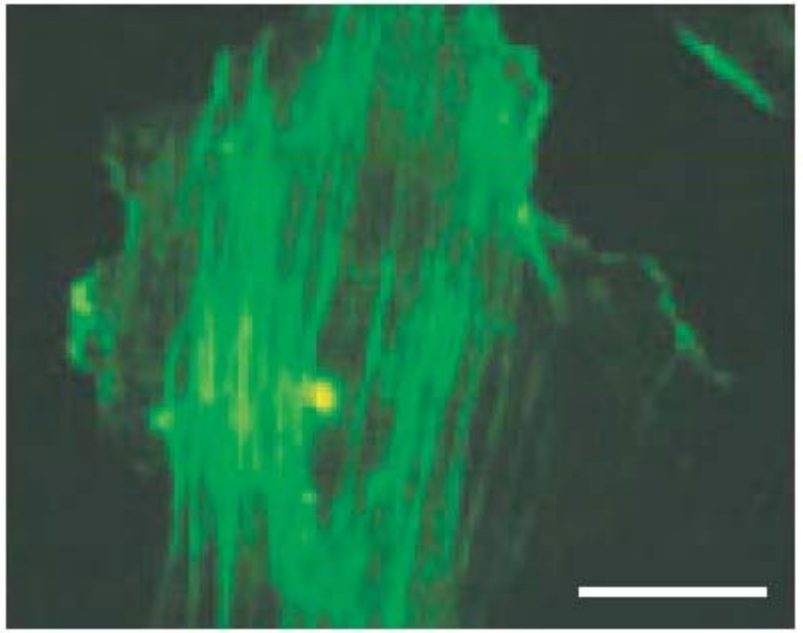
Rearrangement of stress fibers after cyclic cell stretching

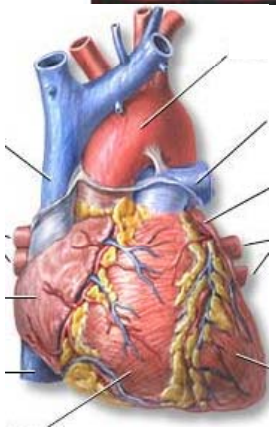
How do cells handle mechanical forces generated in organs? For example, the heart or the blood pressure in vessels?

Unstretched human aortic endothelial cell: random distributed stress fibers

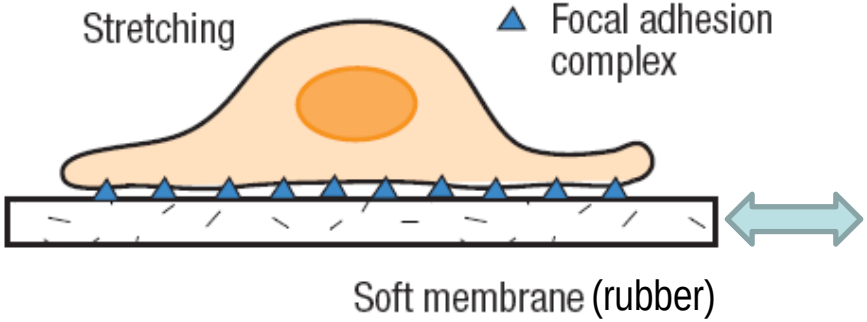


After 3 hours of stretching: stress fibers are oriented into direction of stretching

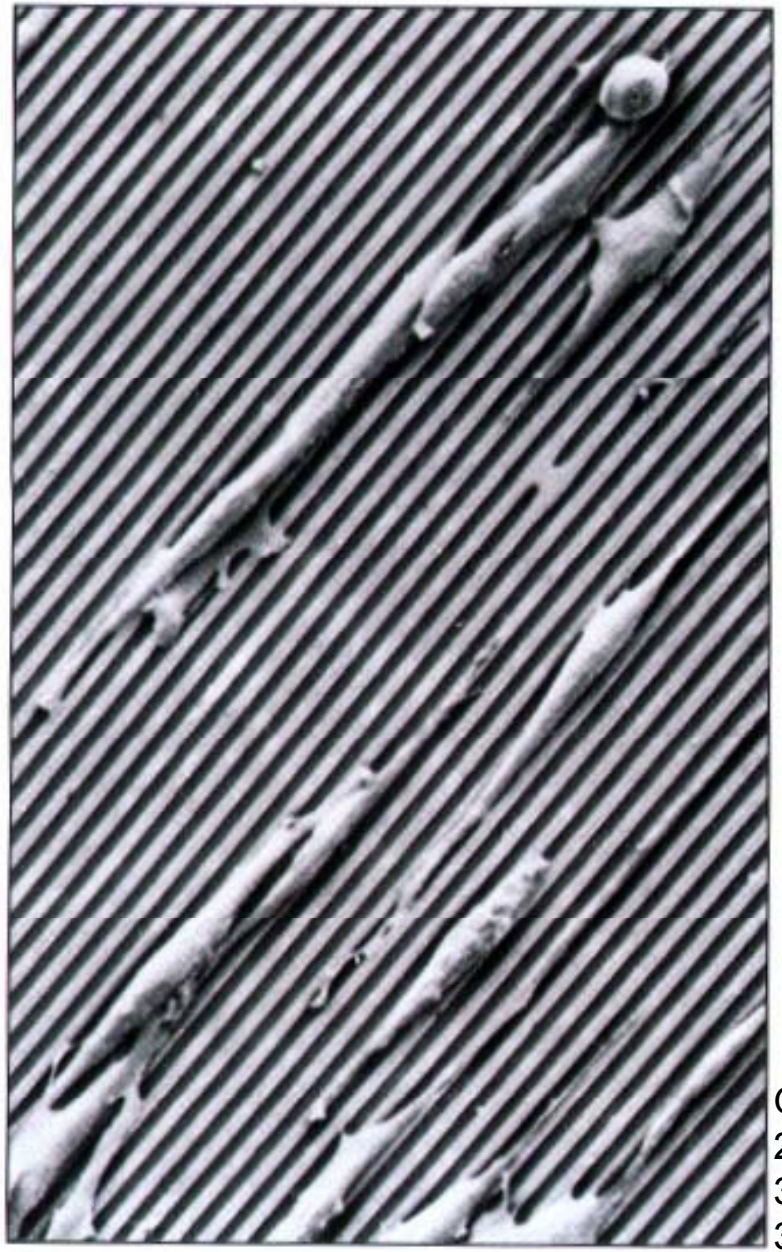




Very dynamic features of stress fibers are critical for **force sensing** and **force transduction**

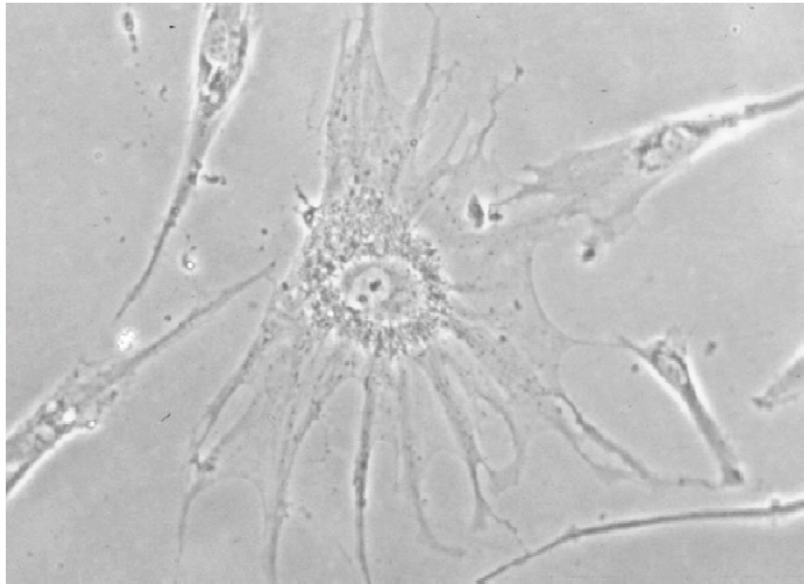


Design your own cell: Cellular response to substrate composition



Cultured cell (fibroblast) aligns on a furrowed surface in the direction of the grooves

Preference of the substrate coating is obvious since **growing does not occur across the furrows**

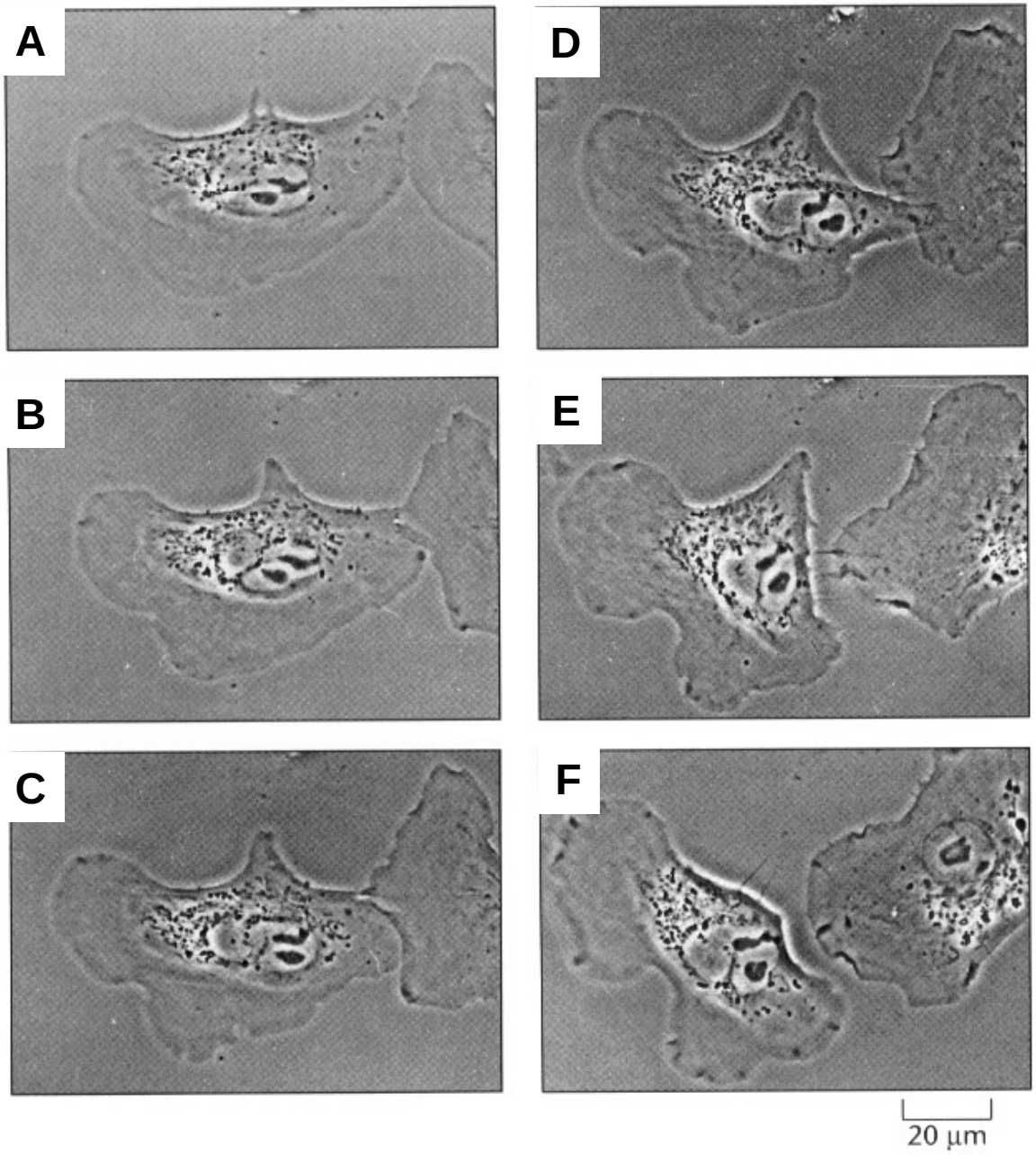


Normal fibroblast cells

Groove dimensions:
2 μm deep
3 μm wide
3 μm spaced apart

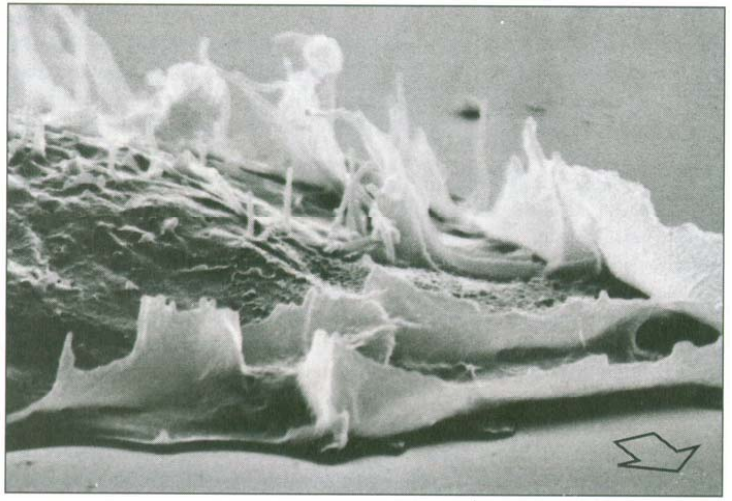
Bray, Cell Movements, 2nd Ed.

Cellular response to “cell traffic”: contact inhibition



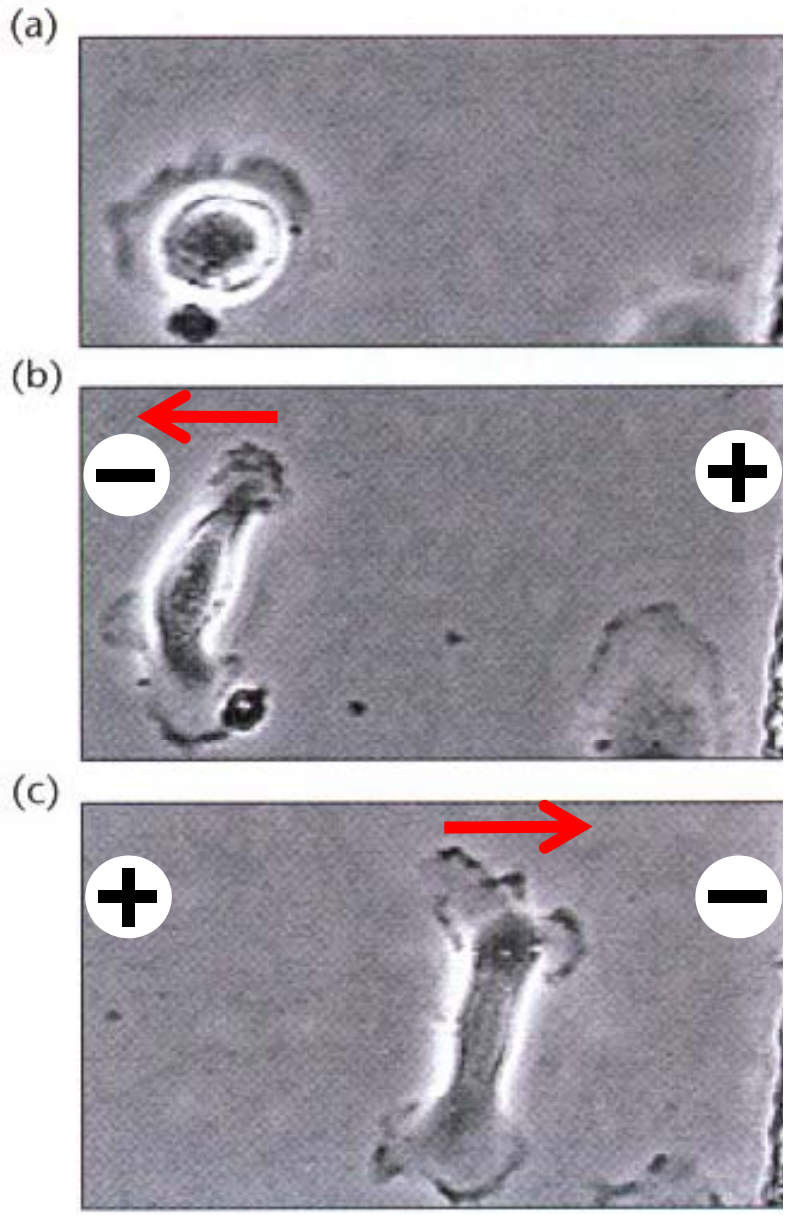
When one <u>cell collides with another</u> a phenomenon named **contact inhibition** occurs:

- At the region of contact (cell’s ruffles) a **stationary (quiet) zone** is formed in which cells seemed to form **contact by filopodia**
- **Ruffling** now occurs in the **opposite direction**
- Cells are moving away from each other



Bray, Cell Movements, 2nd Ed.

Cellular response to an electric field



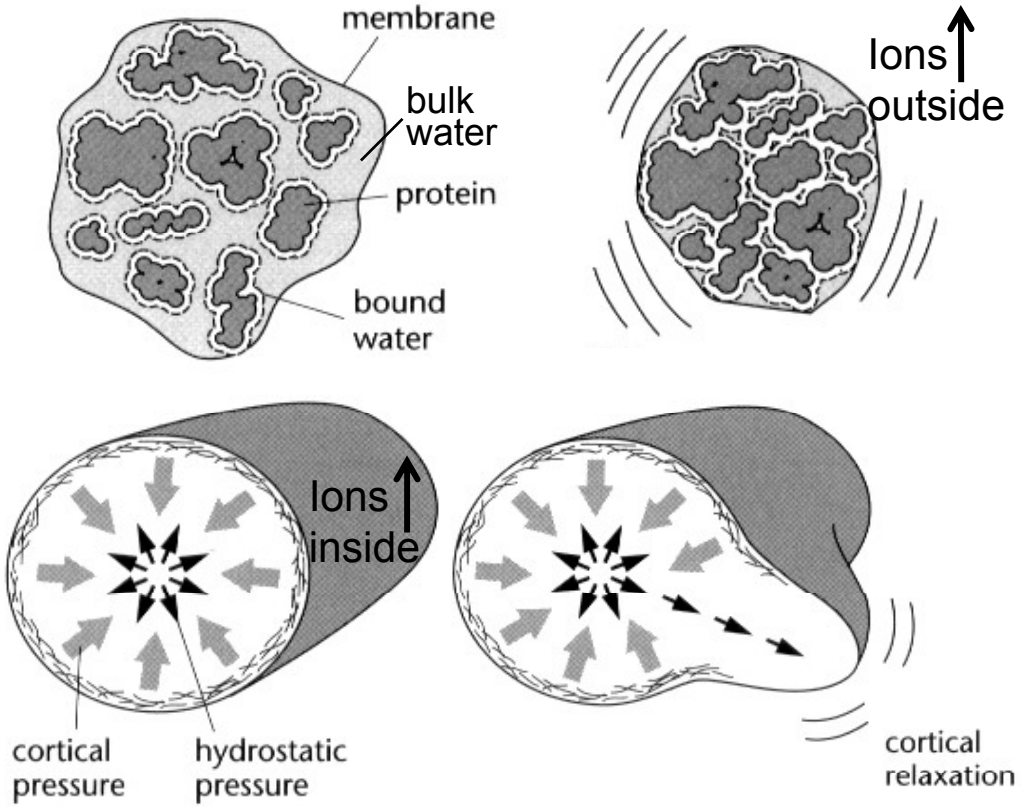
Before the field, the epithelial **cell rounded**

After 1 hour exposure to an electric field of 150 mV/mm **cell becomes elongated** (90° to the field) and starts to move to the minus-pole

Switching the polarity of the field results in a movement to the preferred minus-pole (the cathode)

Bray, Cell Movements, 2nd Ed.

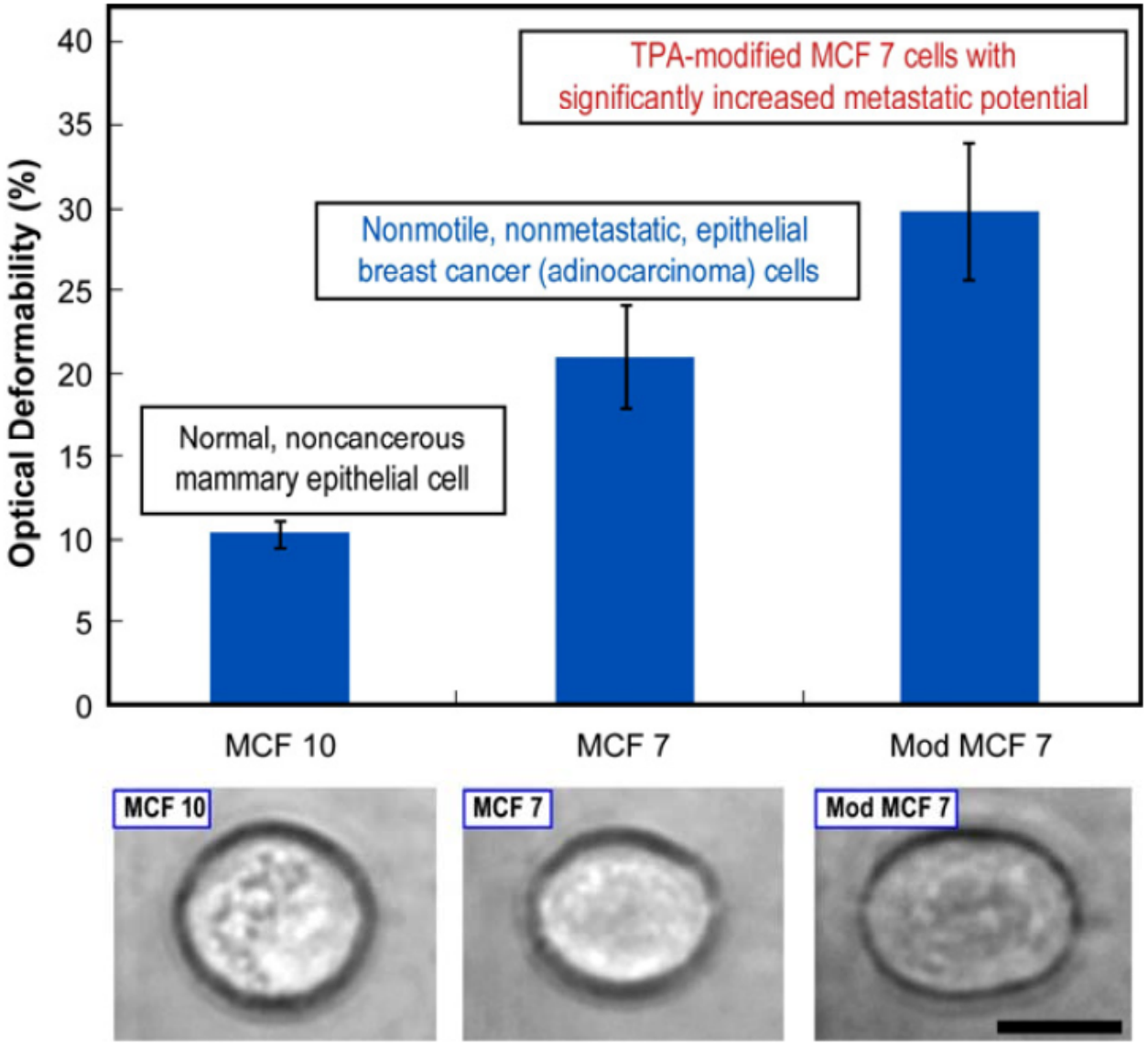
What about the role of internal hydrostatic pressure?



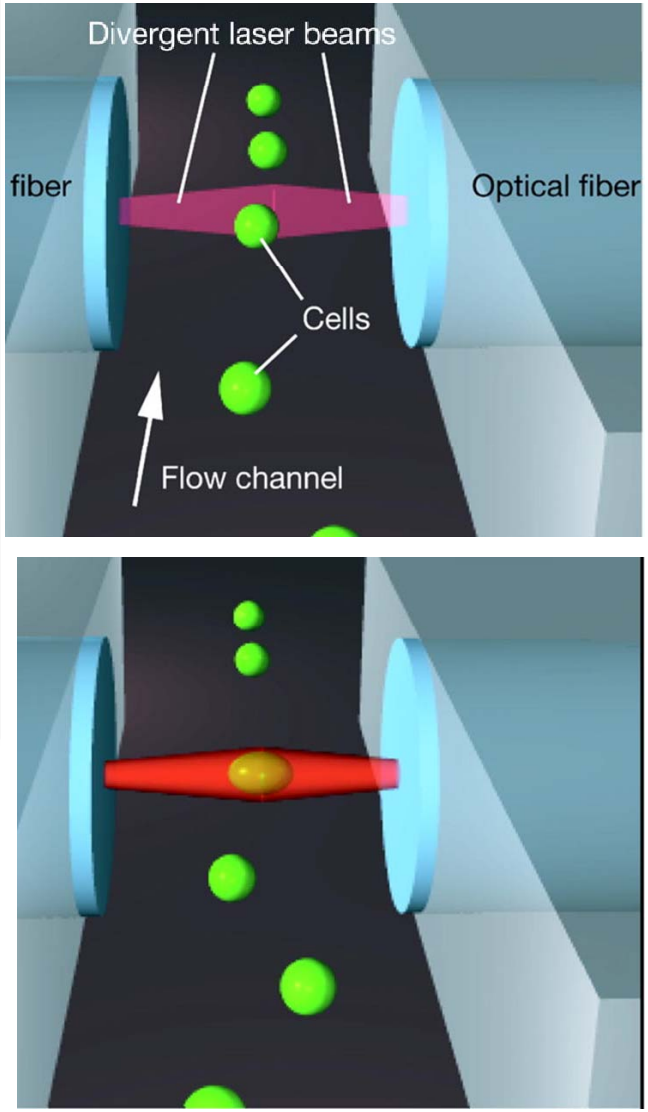
- Cell contains **bulk water** (free water) and **bound water** (bound by proteins)
- Under hyperosmotic conditions, only the bulk water will be lost
- On the other hand, the **high ionic content *inside* the cell** might lead to a **constant flow of water inside** the cell => cell swelling
- To avoid this, the cell develops and maintains a constant hydrostatic pressure to stop water flowing in/outside
- Some plant cells and bacteria can develop internal pressures up to 10^6Pa (car tire: 2×10^5Pa)
- Relaxation of cortical tension result in redirecting internal pressure that may also drives cell membrane extension
- How much does hydrostatic pressure contribute to cell mechanics?

Biomechanics and biophysics of cancer cells

Deformability of **breast cancer cell** is increased (due to f-actin reduction) that also increases metastatic potential



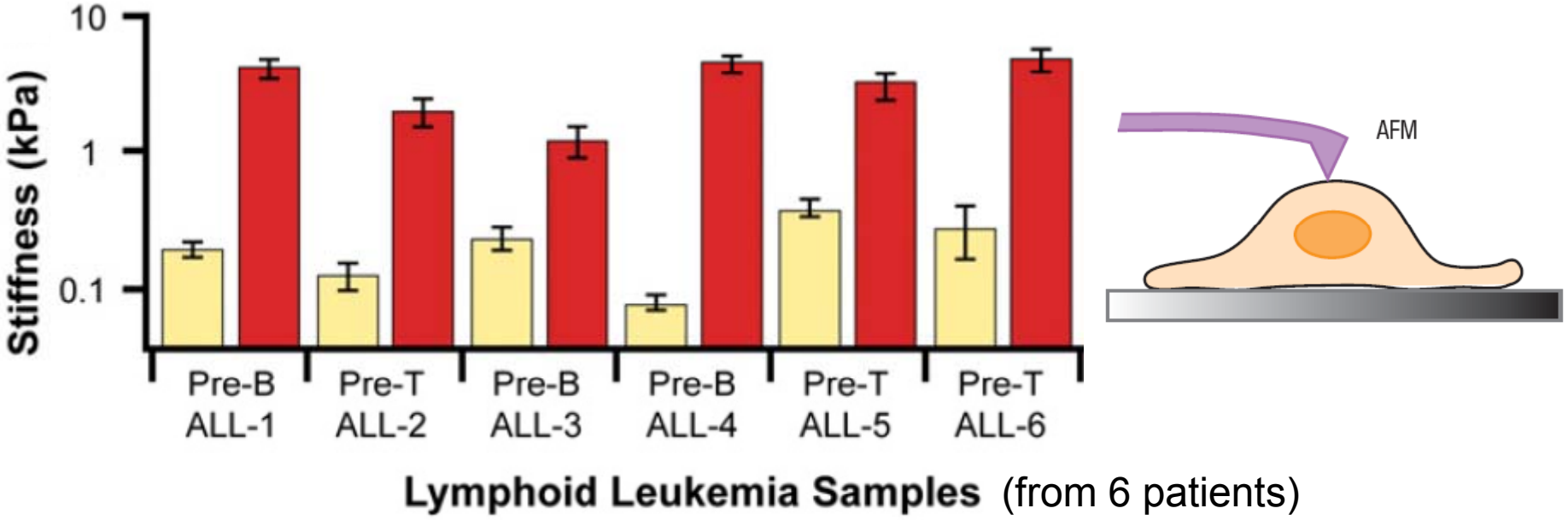
Microfluidic optical stretcher: trapping and stretching cells with two laser beams



Guck et al., Biophys. J, 2005

Effects of chemotherapy on the elastic properties of cancer cells

- Chemotherapy to treat leukemia leads to stiffening of the dead cells
(that might explain observed vascular compilations as atherosclerosis etc.)
- Parallel treatment with cytochalasin D (a drug that weakens the actin-network)
helped to make the dead cells softer (for better “dead-cell recycling”)



Yellow bars: blood cells **before** chemotherapy
Red bars: dead blood cells **after** chemotherapy (drug: daunorubicin)

Motor Proteins at Work for Nanotechnology

Martin G. L. van den Heuvel and Cees Dekker*

The biological cell is equipped with a variety of molecular machines that perform complex mechanical tasks such as cell division or intracellular transport. One can envision employing these biological motors in artificial environments. We review the progress that has been made in using motor proteins for powering or manipulating nanoscale components. In particular, kinesin and myosin biomotors that move along linear biofilaments have been widely explored as active components. Currently realized applications are merely proof-of-principle demonstrations. Yet, the sheer availability of an entire ready-to-use toolbox of nanosized biological motors is a great opportunity that calls for exploration.

A huge amount of biological research in recent decades has spurred the realization that the living cell can be viewed as a miniature factory that contains a large collection of dedicated protein machines (*1*). Consider the complicated tasks that a single cell can perform: It can create a full copy of itself in less than an hour; it can proofread and repair errors in its own DNA, sense its environment and respond to it, change its shape and morphology, and obtain energy from photosynthesis or metabolism, using principles that are similar to solar cells or batteries. All this functionality derives from thousands of sophisticated proteins, optimized by billions of years of evolution. At the moment, we can only dream of constructing machines of similar size that possess just a fraction of the functionality of these natural wonders.

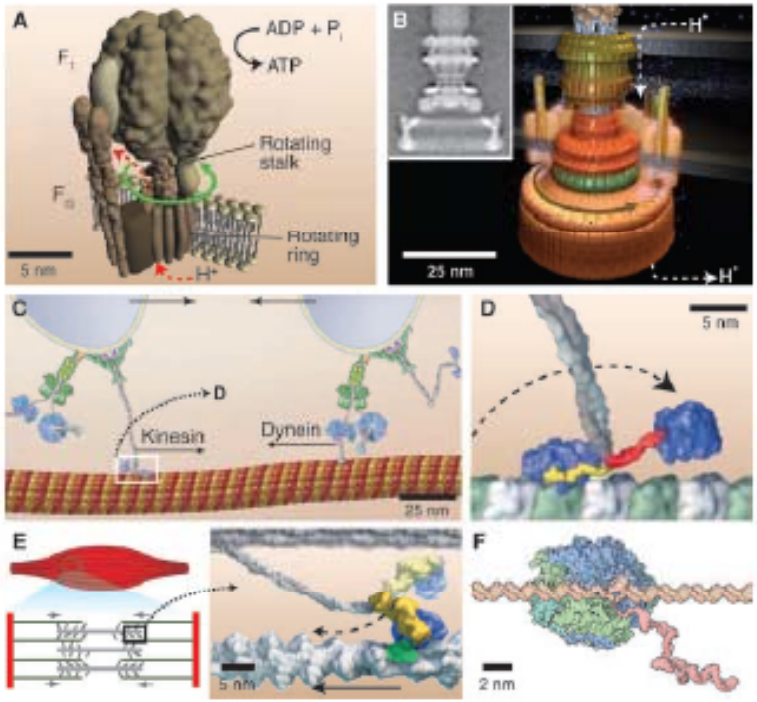


Fig. 1. Motor proteins in the cell. (**A**) Representation of F_OF_1-ATPase [reprinted with permission from (*45*); copyright 2006, Wiley-VCH]. (**B**) Representation of the

It is of interest to ponder whether we can employ these biological nanomachines in artificial environments outside the cell to perform tasks that we design to our benefit (*2*, *3*). Or, at the very least, can these proteins provide us with the inspiration to mimic biocomponents or design artificial motors on comparable scales?

Nature's Workhorses in the Cell

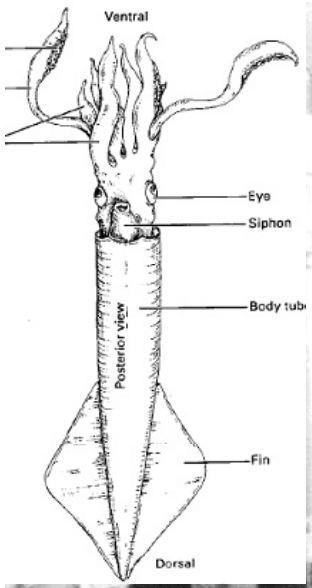
In contrast to macroscopic machines, motor proteins operate in a world where Brownian motion and viscous forces dominate. The relevant energy scale here is k_BT, the product of Boltzmann's constant and temperature, which amounts to 4 pN·nm. This may be compared to the ~80 pN·nm of energy derived from hydrolysis of a single ATP molecule at physiological conditions. Thermal, nondeterministic motion is thus an important aspect of the dynamics of motor proteins.

Let's briefly consider some examples of biomotors. The rotary engine F_OF_1-ATP synthase (Fig. 1A) synthesizes ATP from adenosine diphosphate (ADP) and phosphate (*4*). The flow of protons along an electrochemical gradient through the membrane-bound F_O motor drives rotation of the F_O ring and the central stalk connecting the F_O and F_1 motors. This induces conformational changes of the F_1 motor that drives the catalytic formation of ATP. Remarkably, the complex can also work in reverse, using the energy of ATP hydrolysis to drive the reverse rotation of the F_1 motor and subsequently pump protons against their electrochemical gradient.

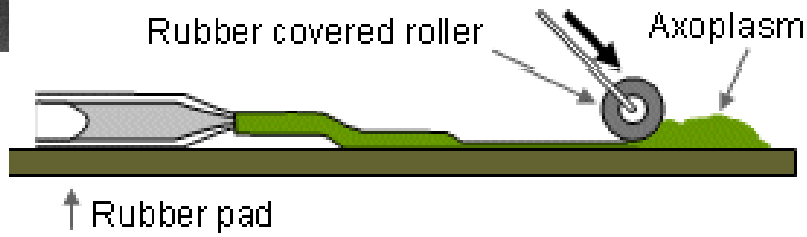
The rotary bacterial flagellar motor (Fig. 1B) is used by bacteria such as *Escherichia coli* as a propulsion mechanism by spinning a helical flagellum (*5*). This

Working with the giant squid axon

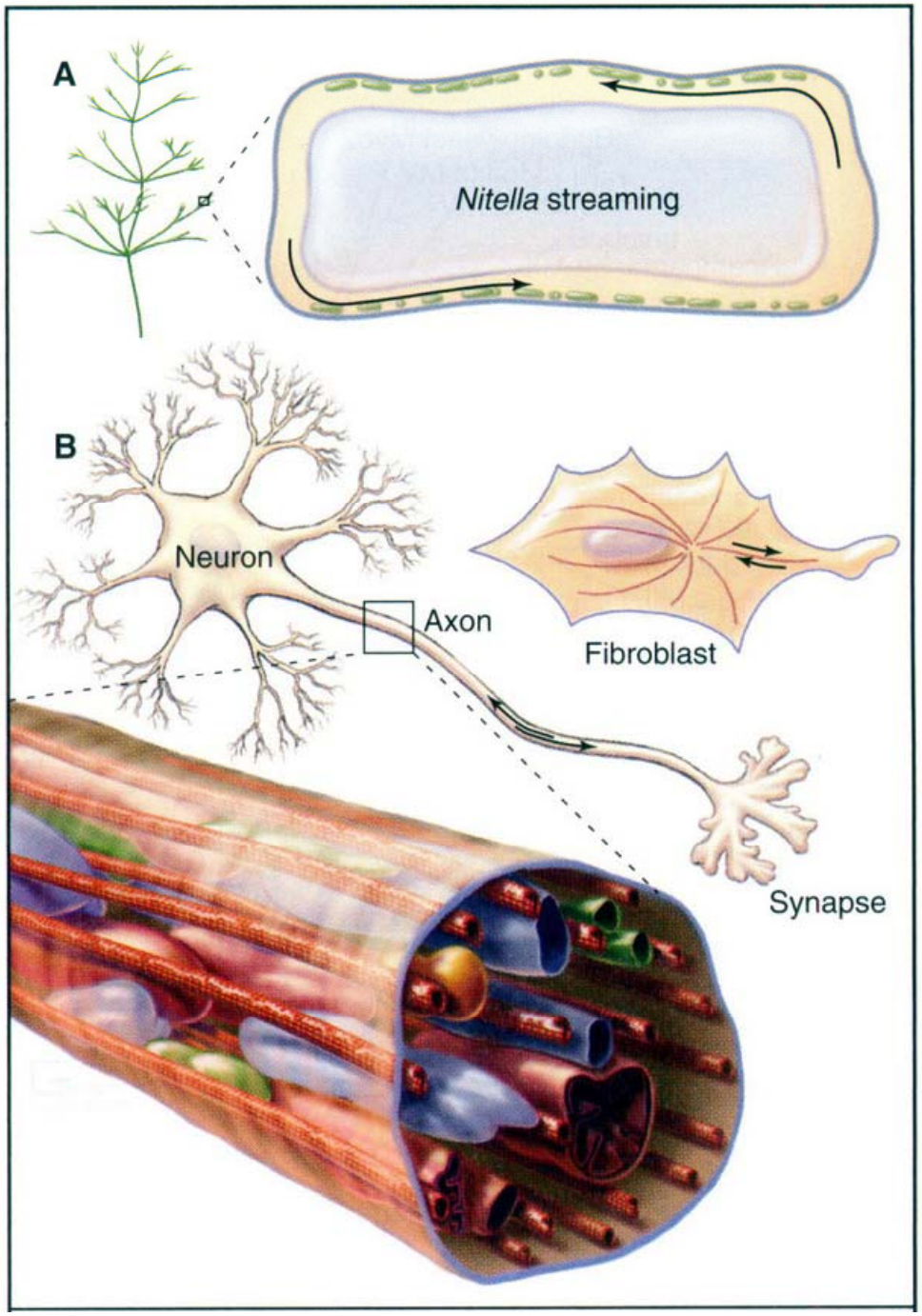
Movie
v20-02-vesicle_transport.mov



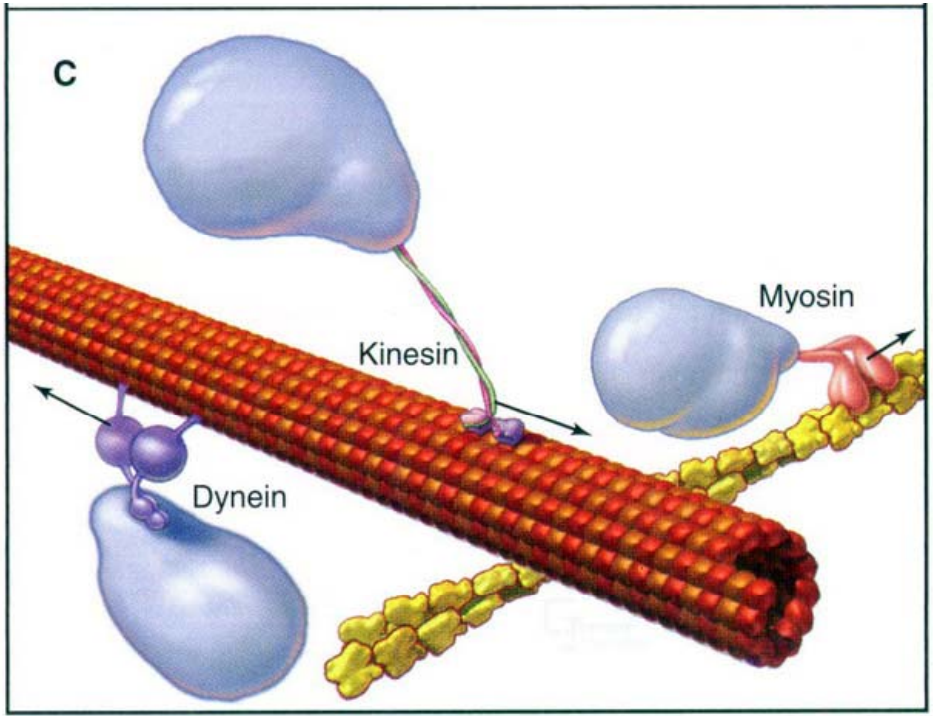
Cytoplasm squeezed out of a squid giant axon and observed with a microscope



Molecular Motors

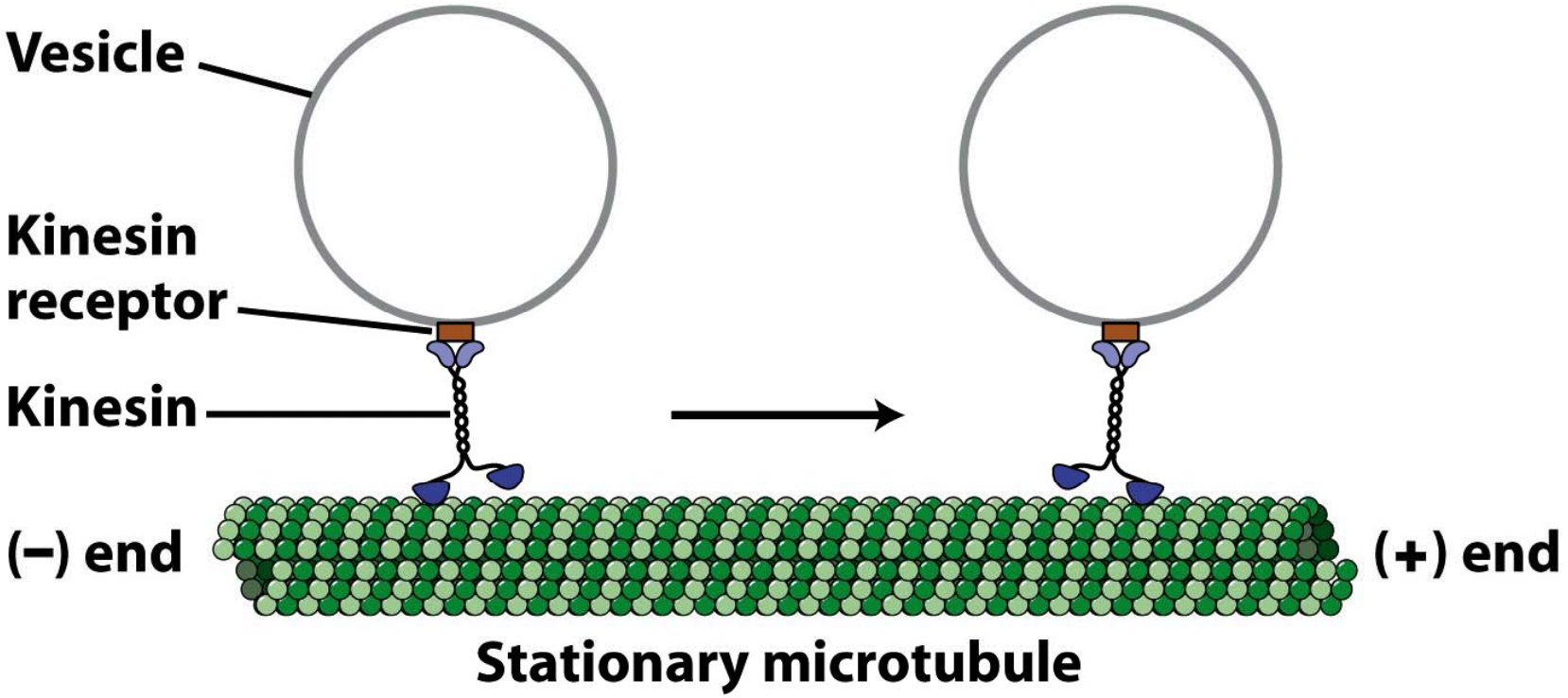


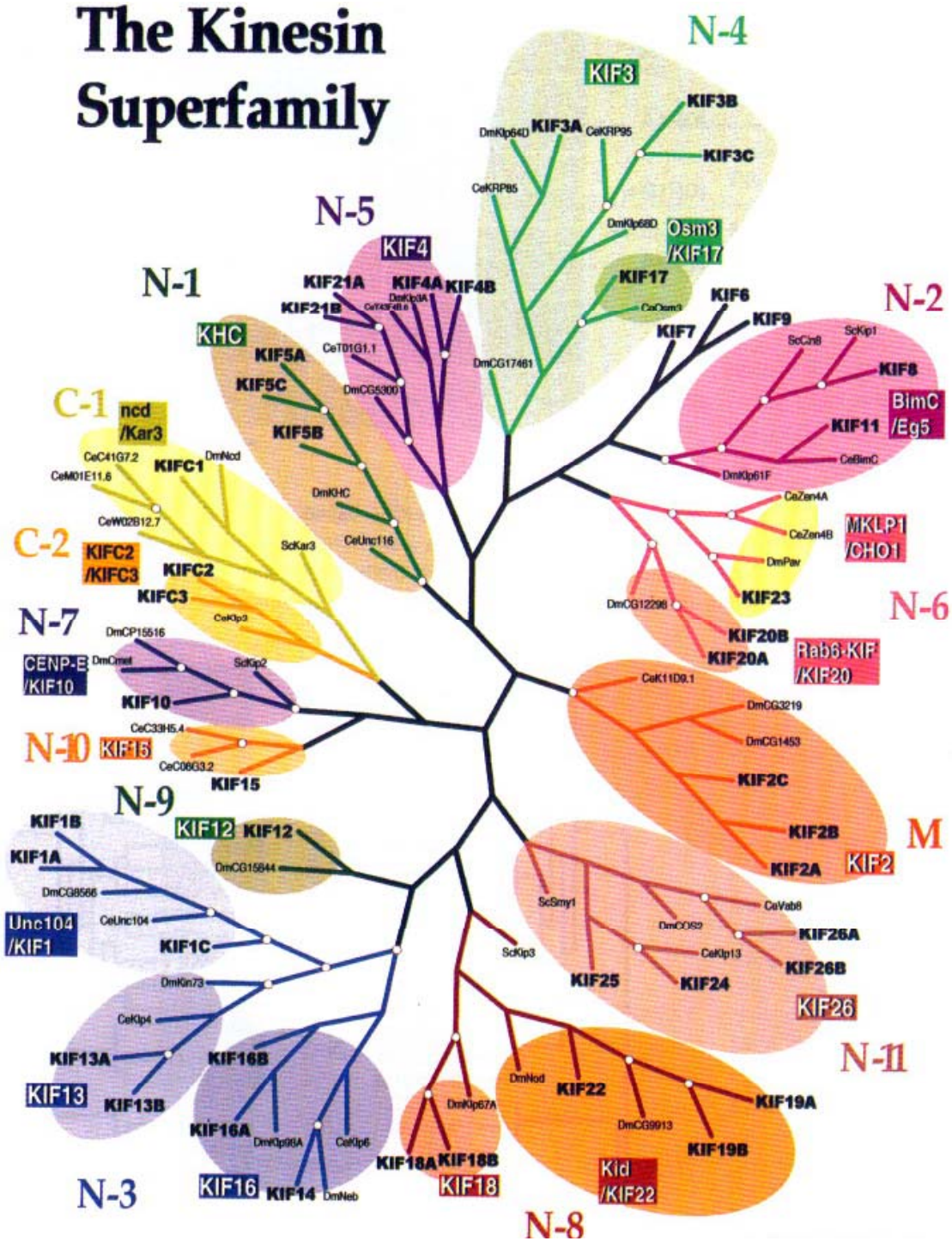
Cargo moves along actin or microtubule tracks attached to molecular motors as **myosins**, **kinesins** and **dynein**



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)





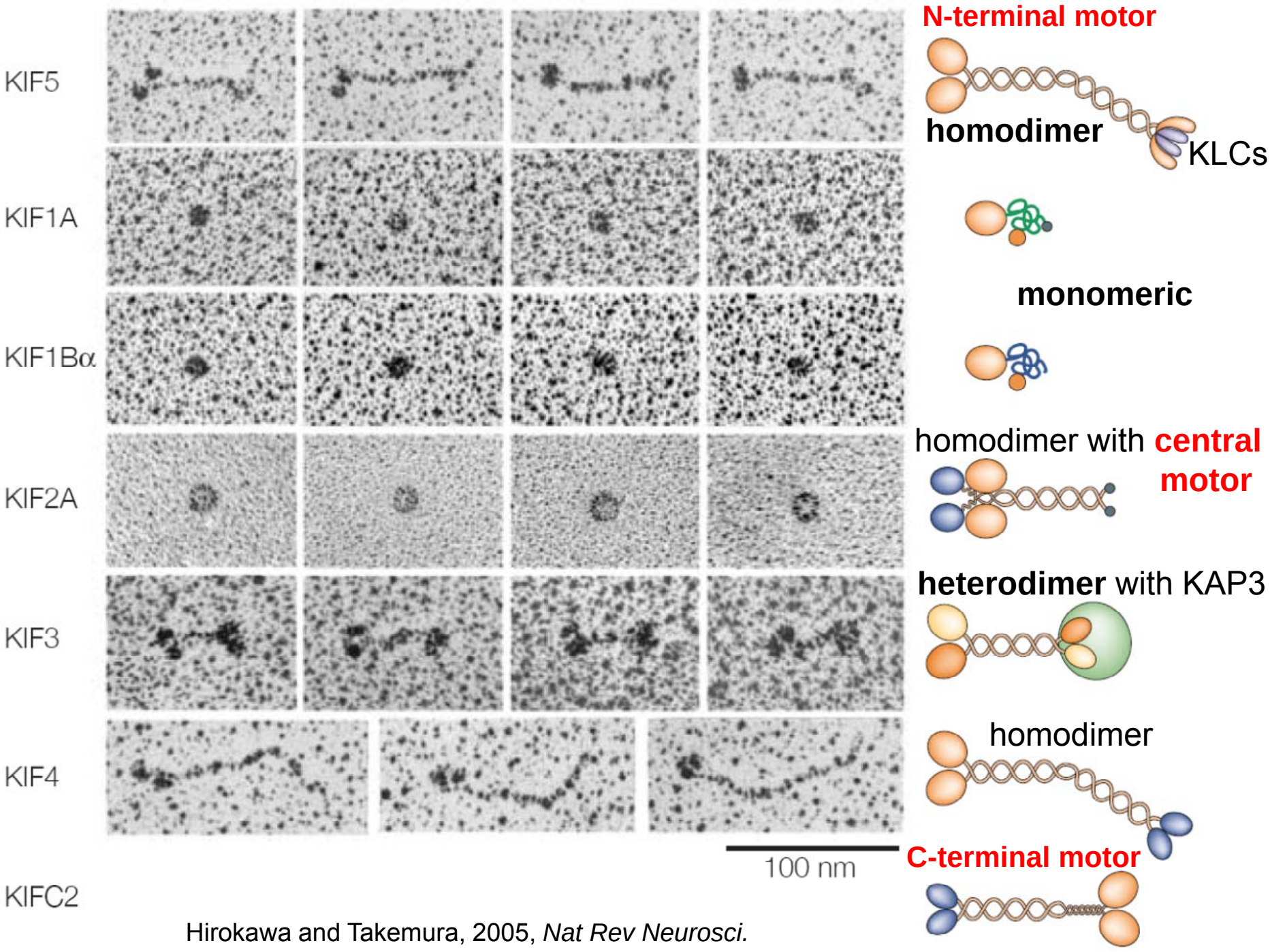
3 major types of **KIFs** (**ki**nesin super**f**amily proteins) exist based on the position of the motor domain:

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.

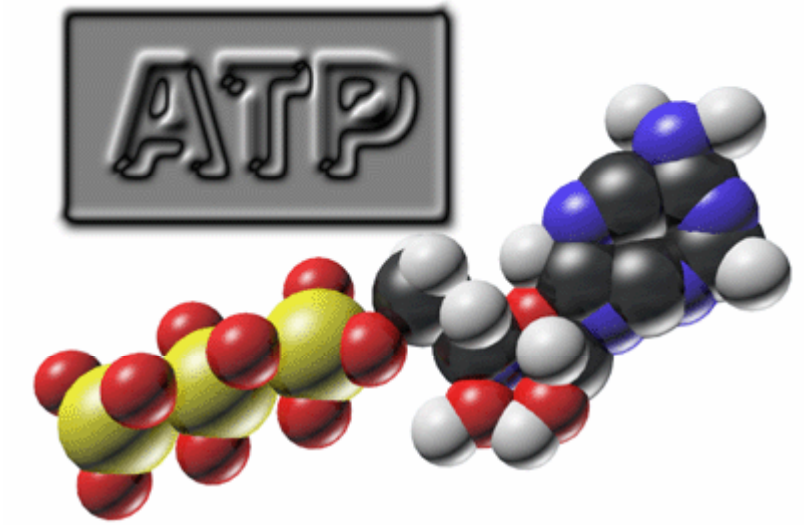


Hirokawa and Takemura, 2005, *Nat Rev Neurosci.*

What is ATP?

• Sunlight or nutrients (as glucose) are converted in the cell to a biologically universal energy carrier **ATP** (adenosine triphosphate)
=> the **fuel of the cell**
• During hydrolysis of ATP to ADP+Pi the cell can use the released energy to power many energetically unfavorable processes as:

- **Protein synthesis** (from amino acids)
- **DNA synthesis** (from nucleotides)
- Molecule transport along a membrane via **ATP-powered pumps**
- Muscle contraction
- Cytoskeleton-based **molecular motors**
- Beating of **cilia and flagella** (moving of sperm and bacteria)

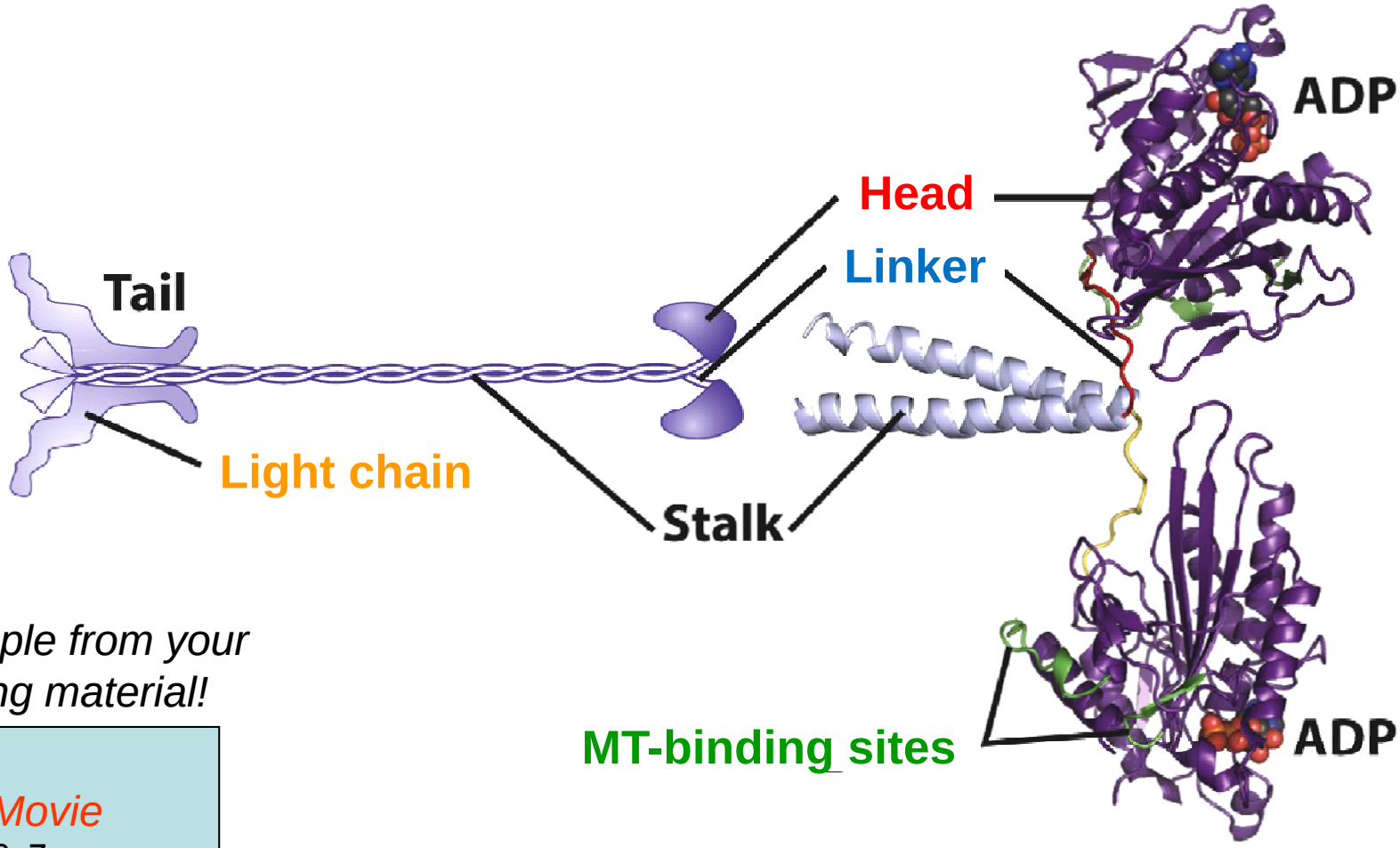


This guy was dreaming about?

Molecular machines

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

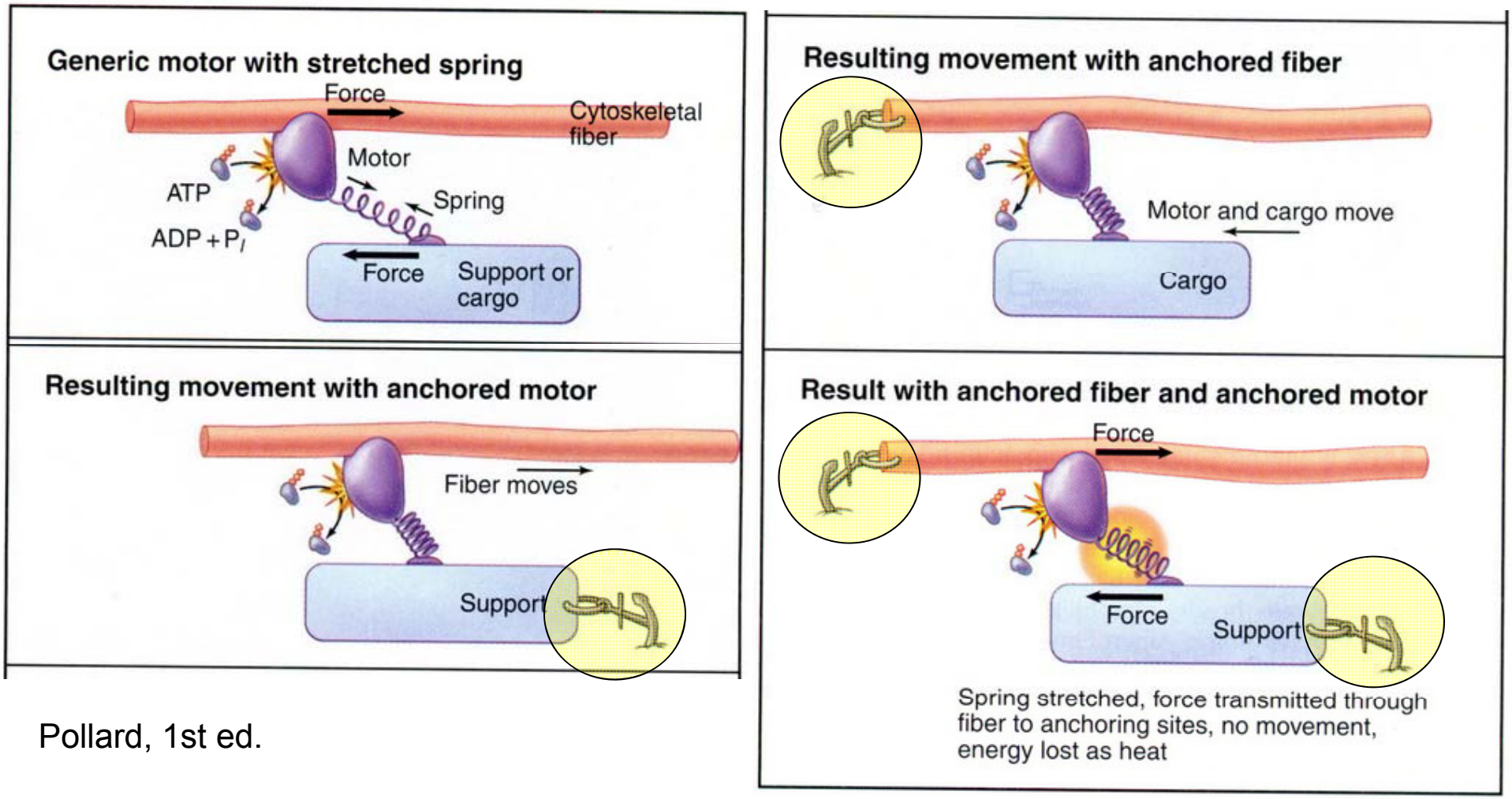


Example from your reading material!

Movie
16_7.mov

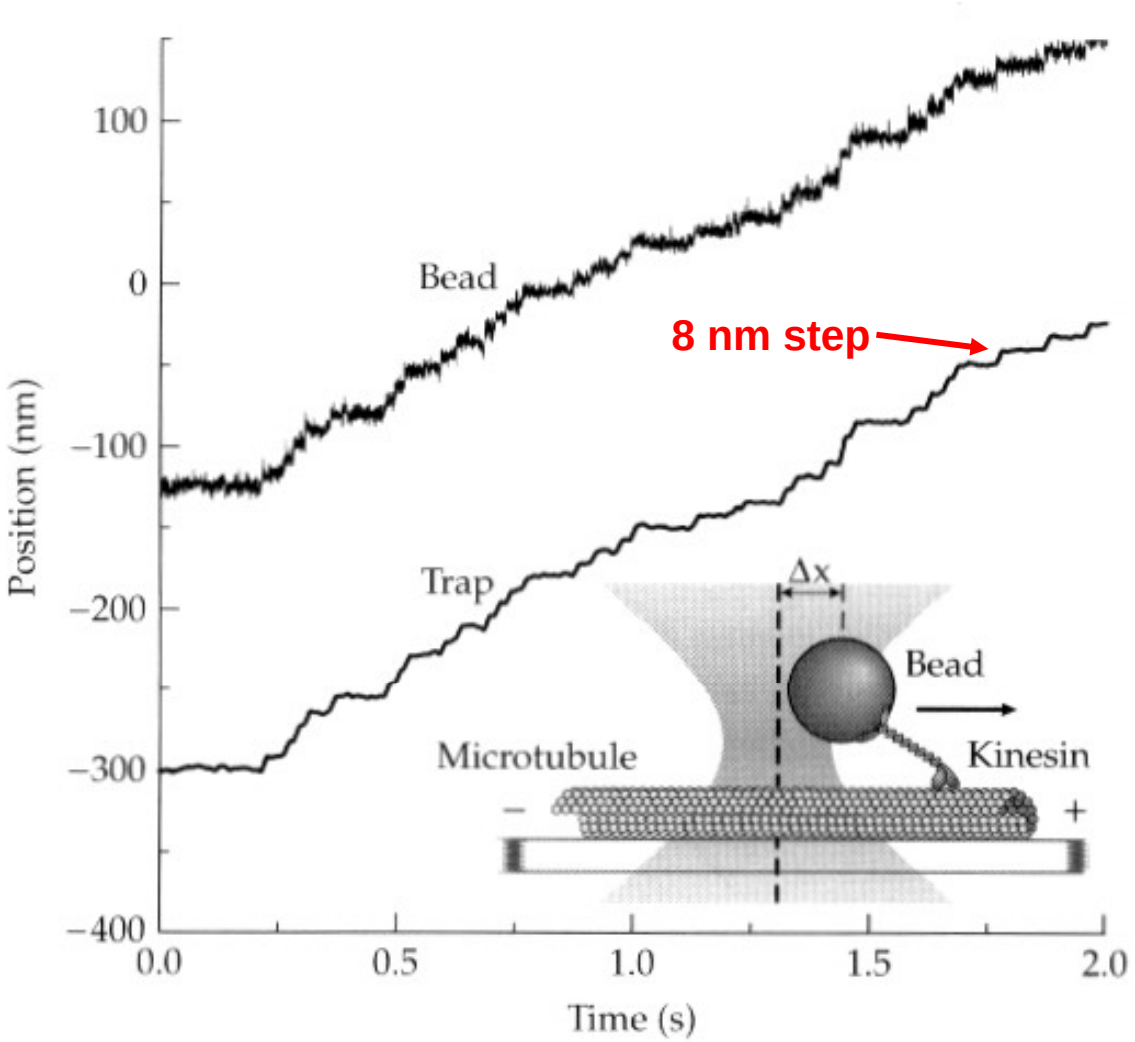
Force generation upon motor-filament interaction

- 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.

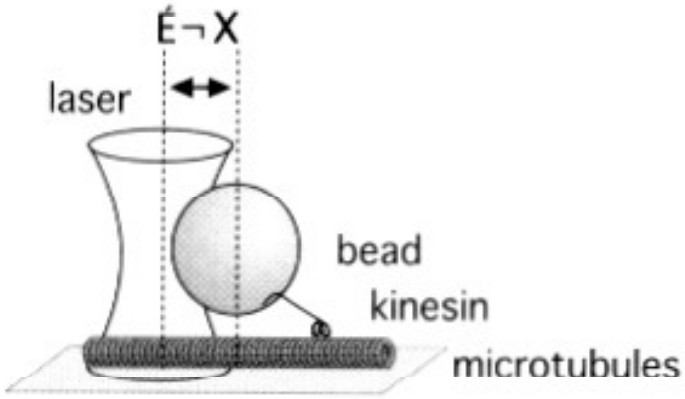
Using the optical trap to determine kinesins stepping behavior

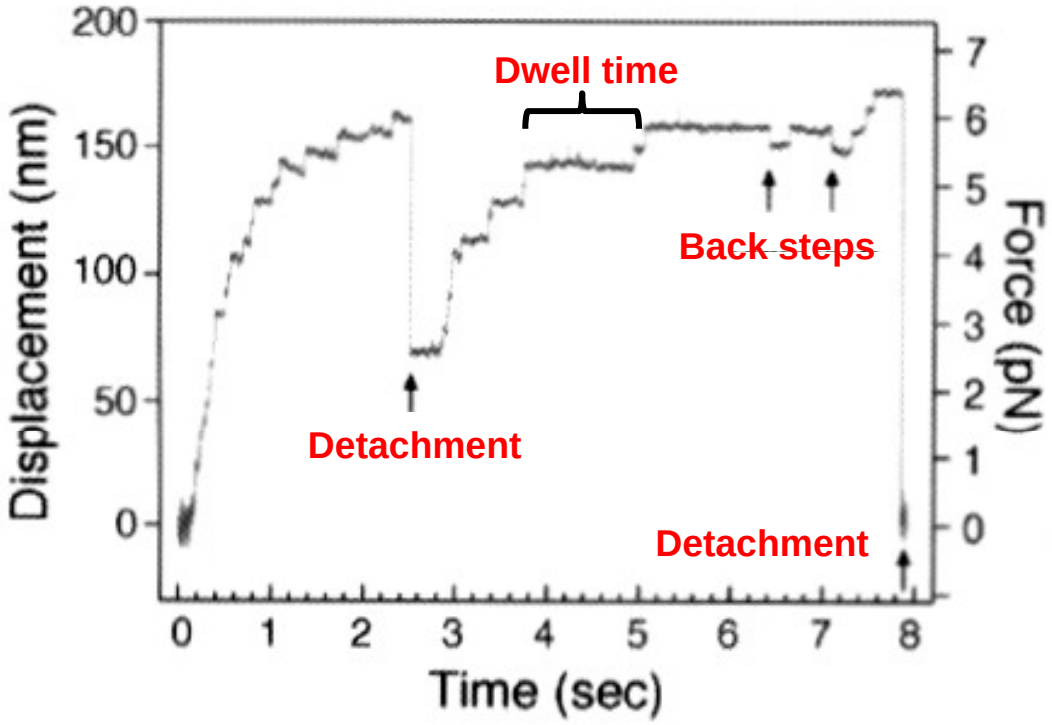


- Kinesin bound to a bead
- Bead kept in position by an **optical trap** (focused infrared laser-beam)
- Bead position determined by photodiode detector (upper trace)
- Opposing and constant force (6.5 pN) applied just behind the bead (by optical trap)
- After kinesin moves, **feedback loop** adjusts 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.

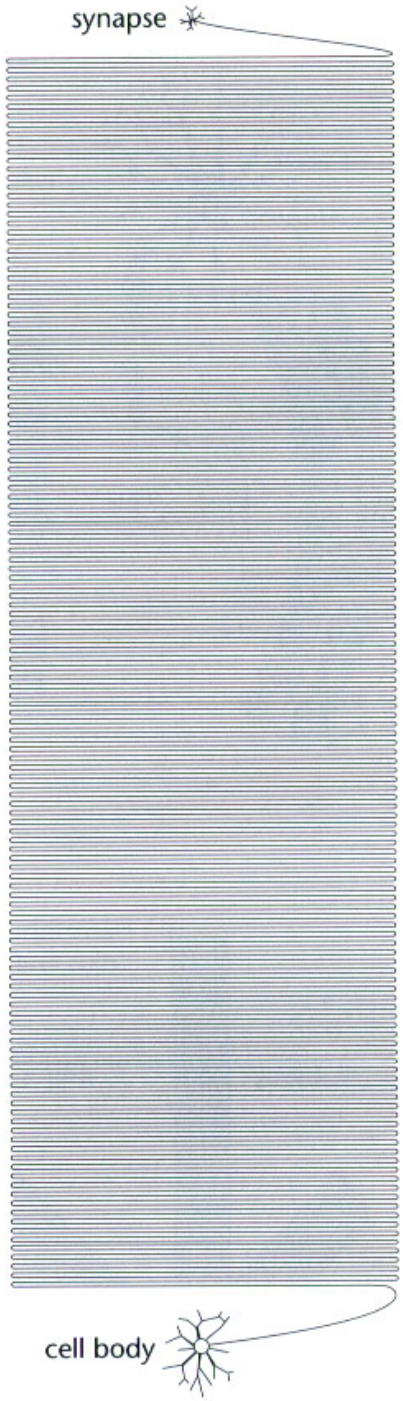
Kinesins need a certain loading force to start moving





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

Schliwa, Molecular Motors, 1st Ed.



Importance of transport in neurons

• The neuron consists of a cell body (soma), an axon (a long cell extension) and a synapse

• Axons are between less than 1mm and up to more than 1m in length

• Transport of “cargo” is important for **neuronal development**, **synaptic plasticity** and **transmission**

Anterograde transport (via **kinesins**):

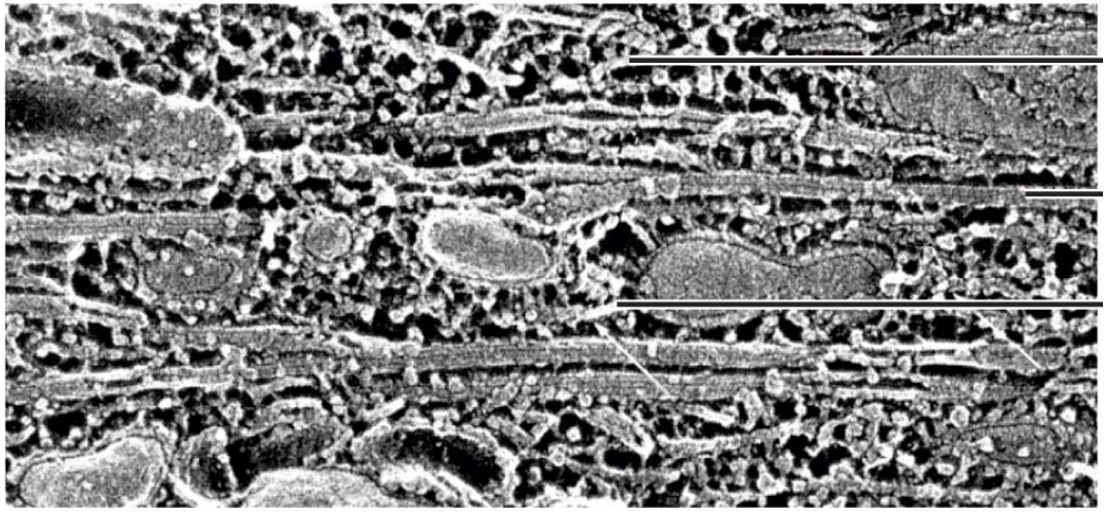
⇒ from the cell body to the terminals

Retrograde transport (via **dynein**)

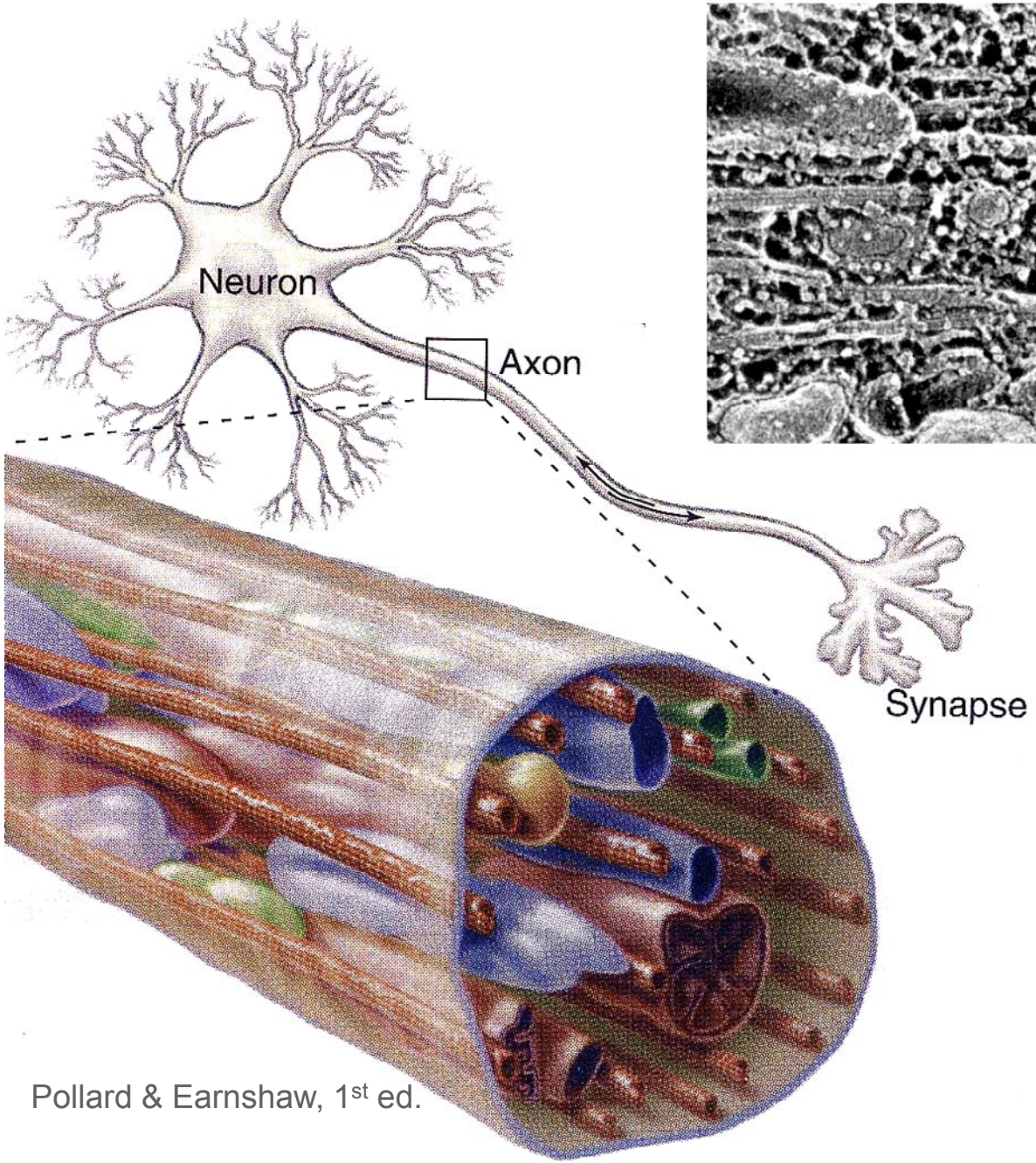
⇒ from the termini back to the cell body

30 cm long human motor neuron drawn to scale

EM image of an axon



Hirokawa, Science, 1998

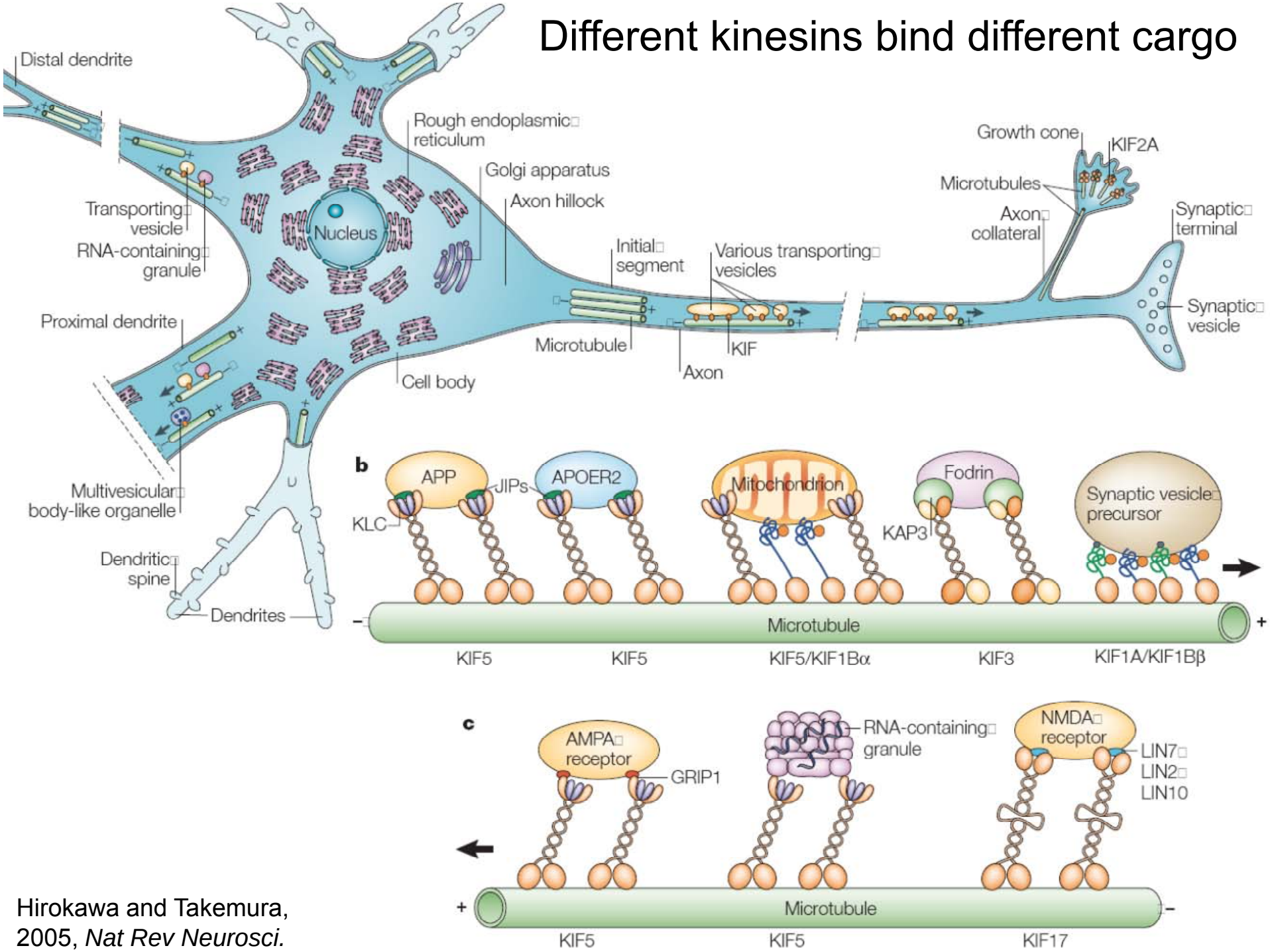


Pollard & Earnshaw, 1st ed.

Crowded axon:

- microtubules
- neurofilaments
- mitochondria
- synaptic vesicles
- motors…

Different kinesins bind different cargo

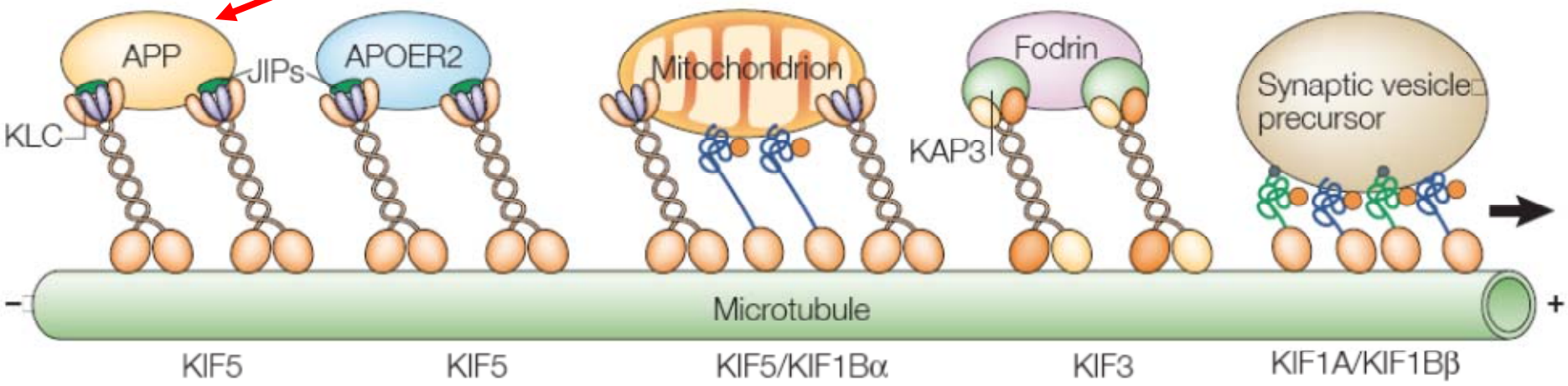


Hirokawa and Takemura, 2005, *Nat Rev Neurosci.*

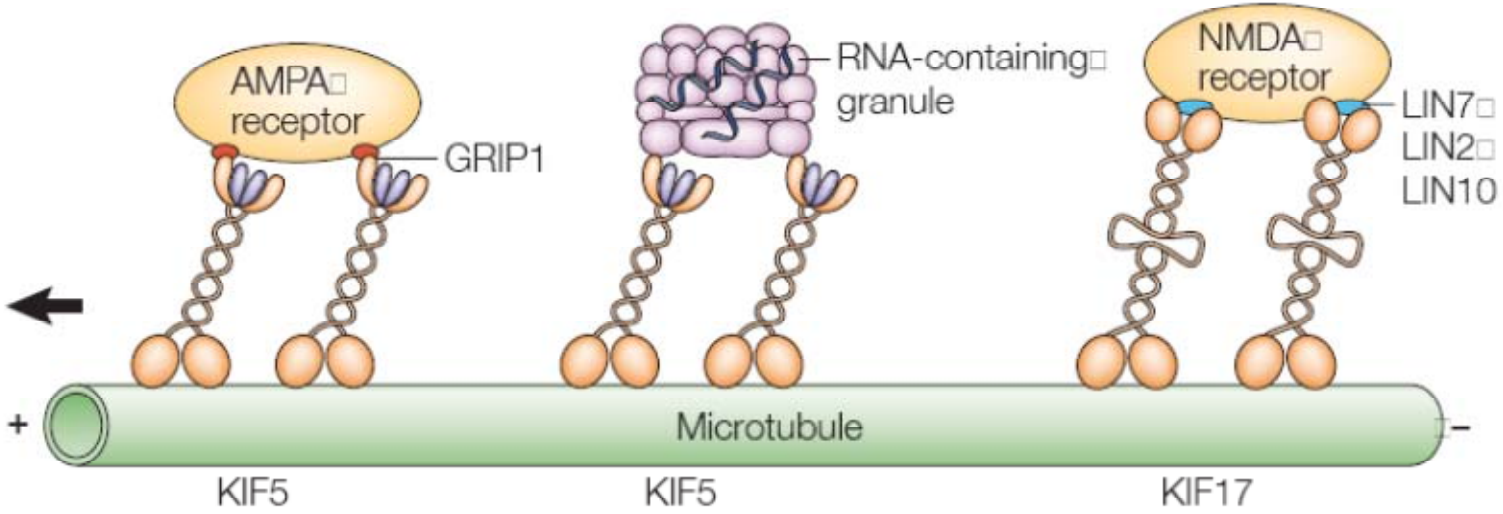
Different kinesins bind different cargo

Axon

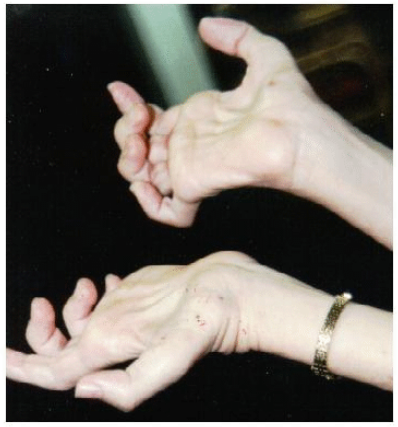
APP = amyloid precursor protein: involved in **Alzheimer's disease**

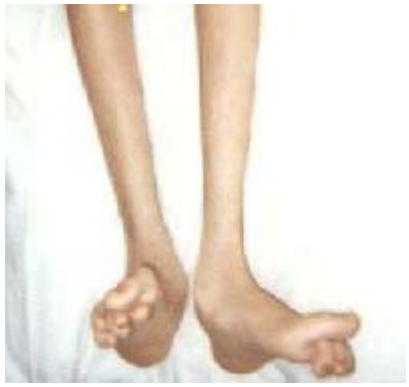


Dendrites



• **Proper** regulation of motor activity is critical in this highly overloaded axon
• **Incorrect** motor regulation leads to **accumulation of cargo** => a symptom for many neurodegenerative diseases (brain diseases)





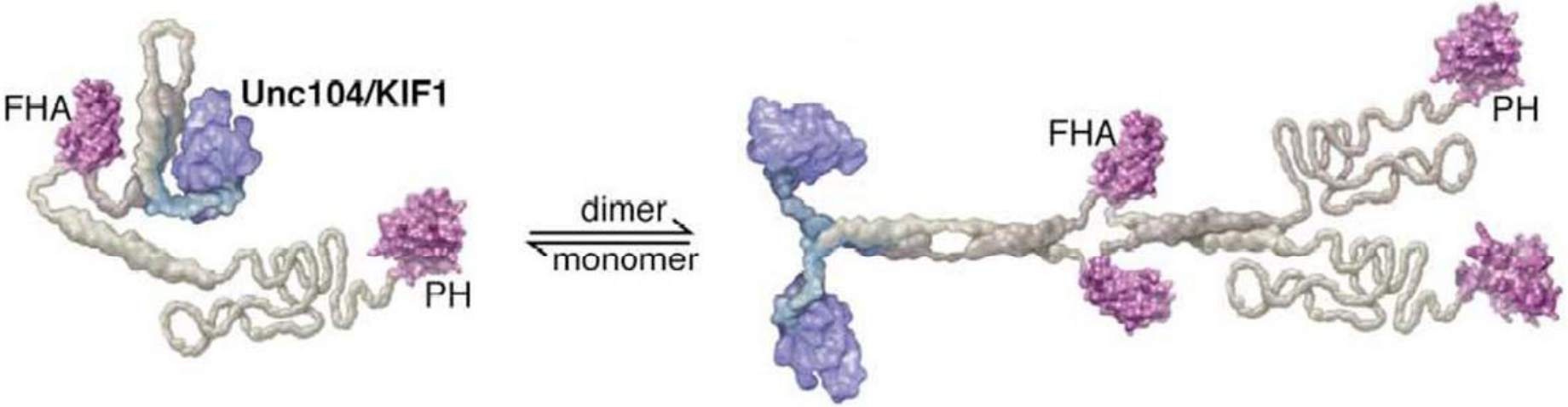
Charcot-Marie-Tooth disease

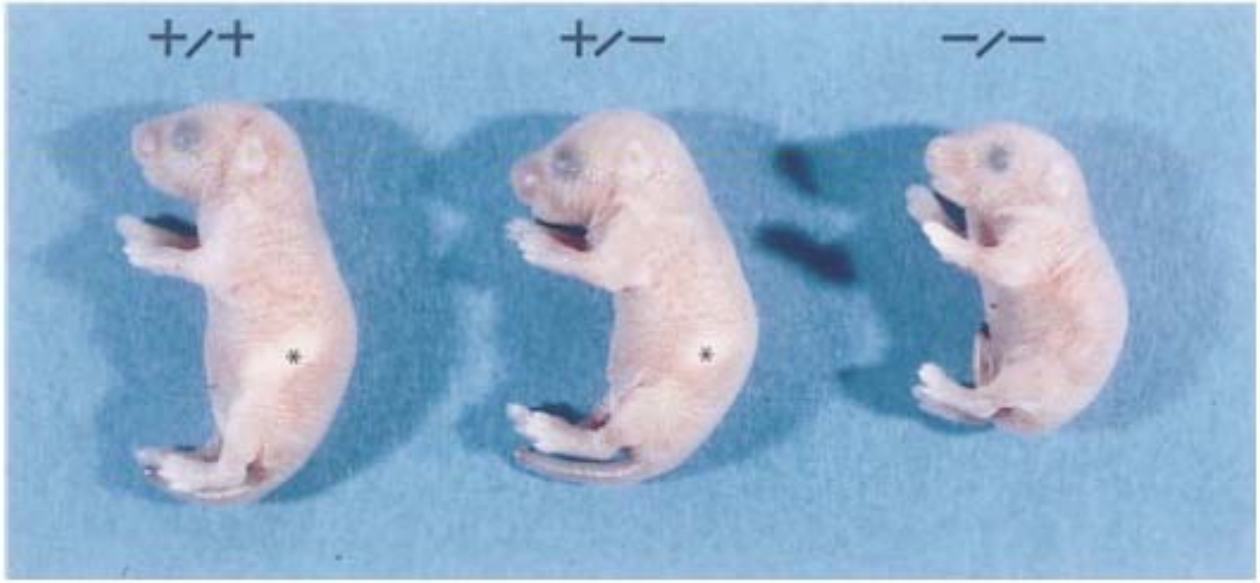
Neurodegenerative disease	Involved motors
Alzheimer's disease	kinesin I/KIF5A
ALS (Amyotrophic lateral sclerosis)	dynein
Lissencephaly	dynein
Charcot-Marie-Tooth disease	KIF1Bβ
LMN (lower motor neuron disease)	dynactin
Senile dementia	KIF1A

Amyotrophic lateral sclerosis, ALS

Special types of kinesins: monomeric and bipolar kinesins

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

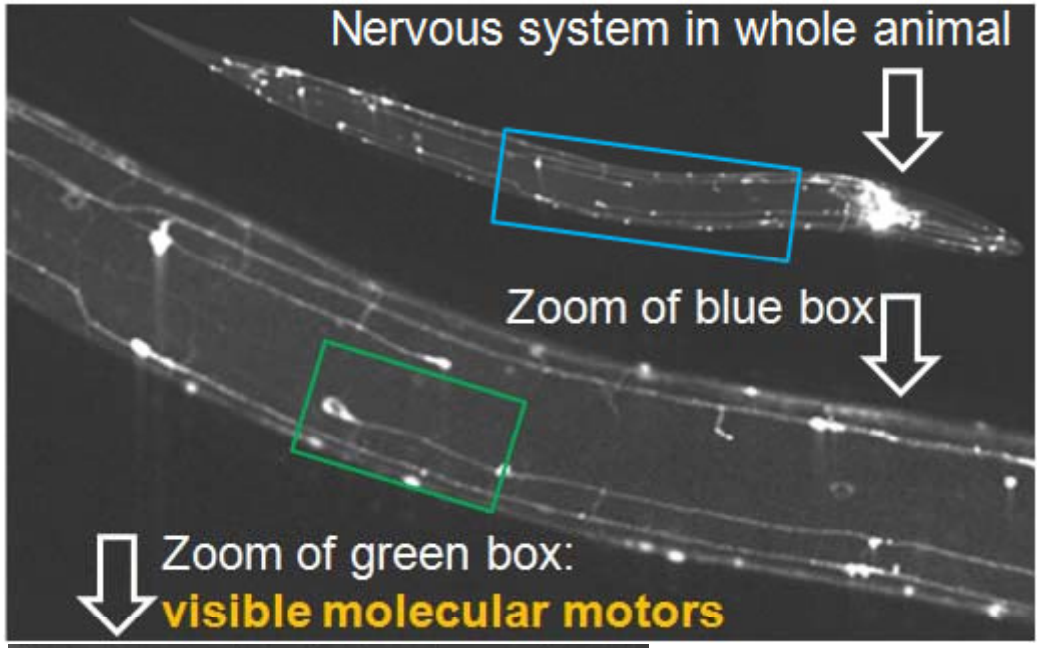


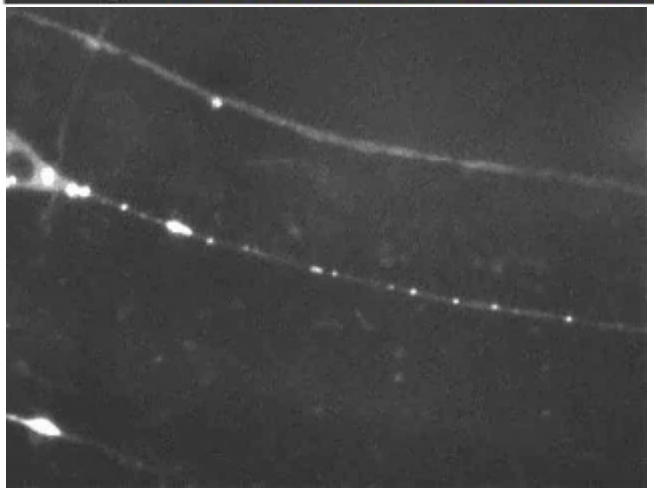


WT *wt/kif1a* *kif1a/kif1a*

Neuropathies with severe <u>developmental defects</u> occur in KIF1A knockout mice (based on **impaired axonal transport**)

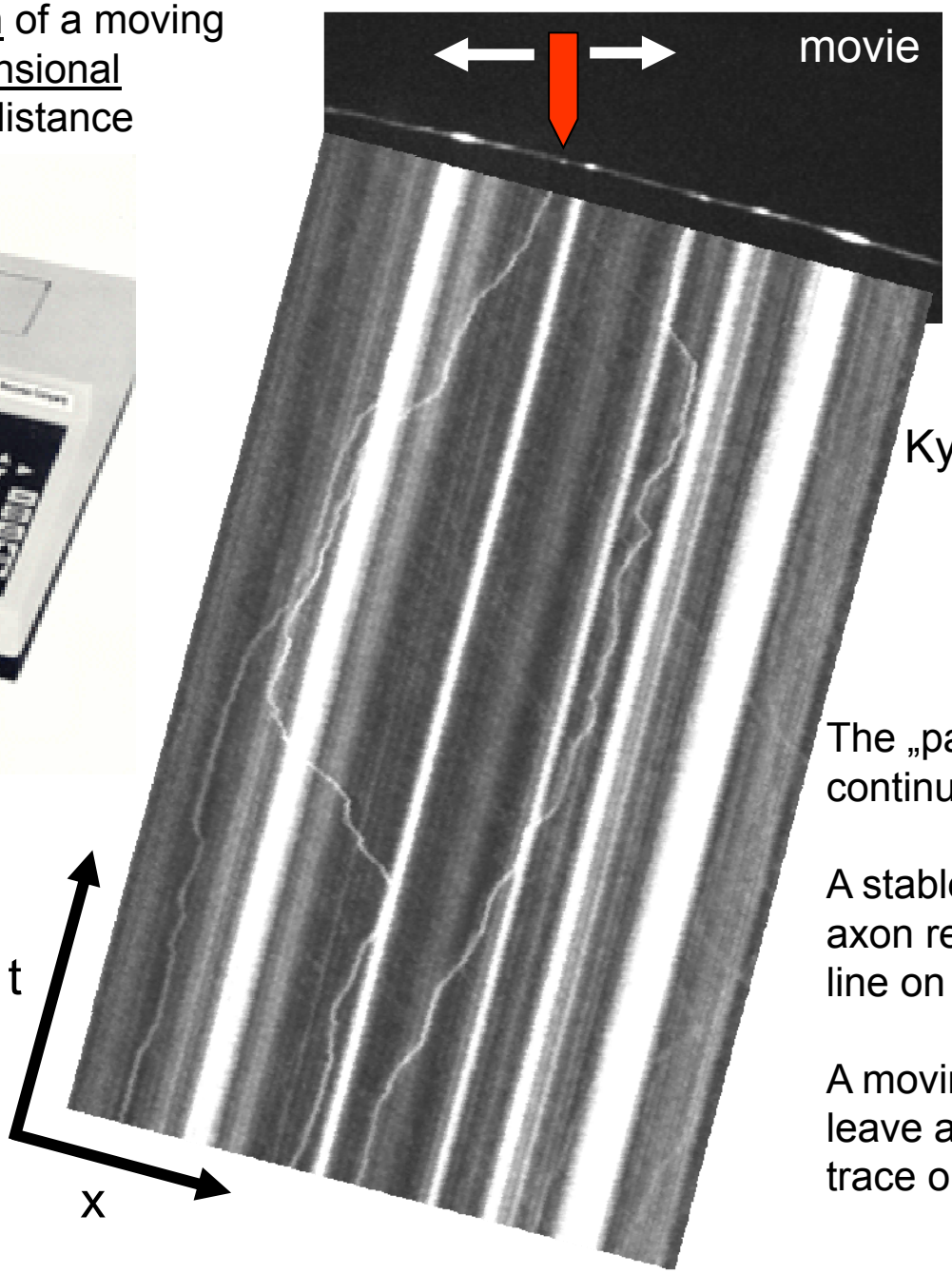
Tracking molecular motors in the neurons of an living animal





Even though the motor moves bidirectional, net movement (anterograde minus retrograde) is anterograde

A Kymograph is the translation of a moving spot on a line, into a two-dimensional projection area with time and distance



Kymograph

The „paper“ is continuously moving

A stable spot in the axon remains as a line on the “paper”

A moving spot will leave an individual trace on the „paper“

=> with time and distance we can calculate **velocity**, **pausing**, **run length** etc.

Example of data evaluation using the kymograph technique

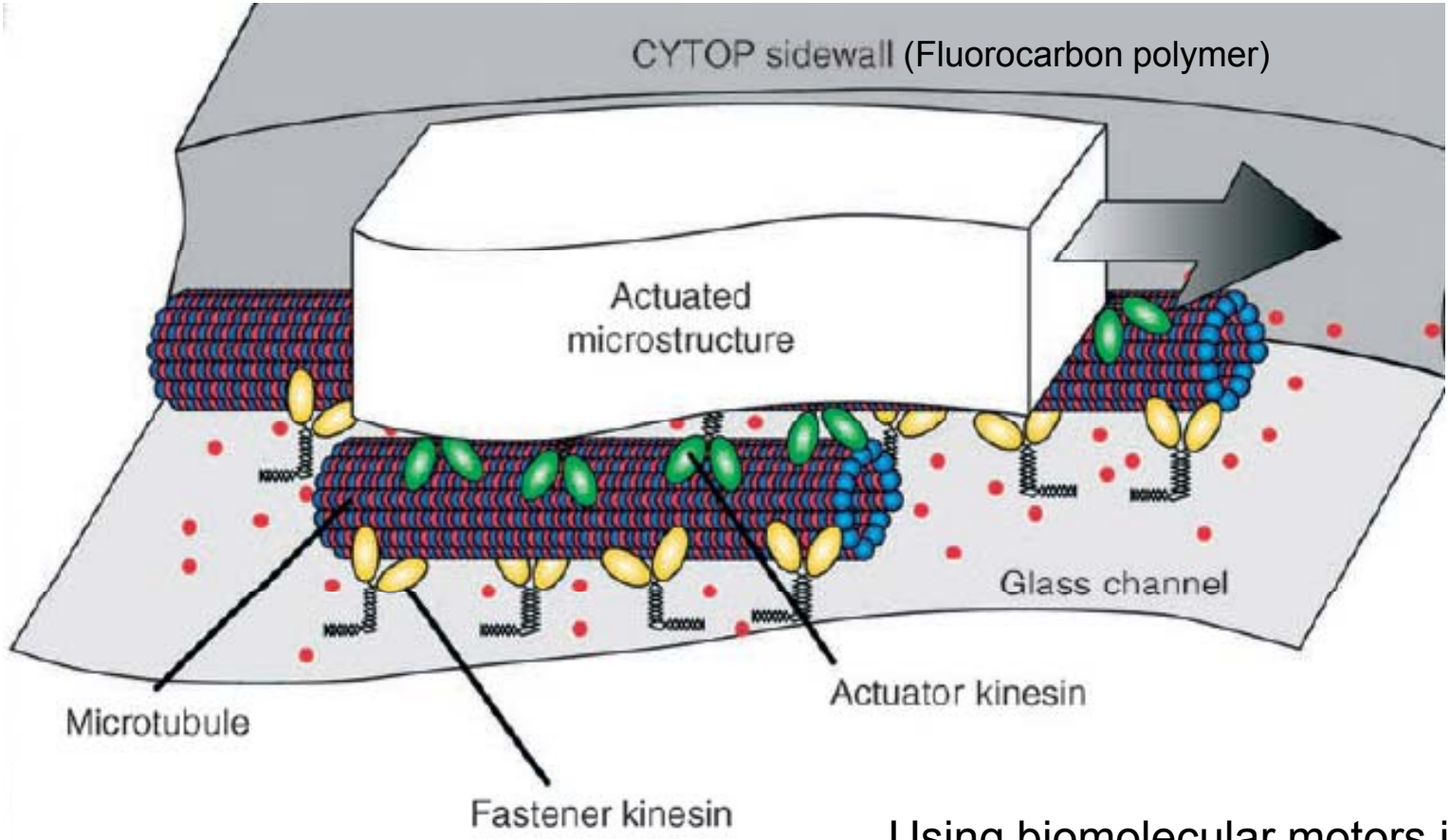
All Particles	***unc104(ok217) cells***	STDEV+/-	Antero	STDEV+/-	Retro	STDEV+/-
Velo. w/o pauses (µm/s)	**0,43**	0,19	**0,32**	0,13	**0,47**	0,17
Total run length (µm)	**5,47**	3,56	**5,19**	3,00	**5,94**	3,84
Change direction per 100 s	**4,12**	2,53	**4,37**	2,37	**4,09**	2,79
Change direction per 10 µm	**2,06**	1,26	**2,34**	1,18	**1,94**	1,37
Pausing per 100 s	**2,19**	1,16	**1,98**	0,73	**2,54**	1,61
Pausing per 10 µm	**1,44**	1,03	**1,36**	0,87	**1,68**	1,31
Pausing duration (s)	**16,45**	7,16	**19,37**	9,78	**14,68**	7,90
Persis. of mov. at uni. velo.(s)	**9,00**	3,37	**7,85**	2,56	**9,62**	3,69
# Events	**490**		**245**		**186**	
# Anterograde movements	**25**	Events neither antero nor retro:		59		
# Retrograde movements	**30**		**45**	**% antero**	**57**	**% antero events**
# Unidentified movements	**7**		**55**	**% retro**	**43**	**% retro events**
# Axons	**25**					
# Dendrites	**0**					
# Commissures	**0**					
# Unidentified	**0**					
# Particles	**62**					
# Movies	**25**					
Pause Calibration Ave. (s)	**0,062**					

Velocity due to particle size						
Counts:	**7**		**21**		**32**	
	Large	STDEV+/-	**Medium**	STDEV+/-	**Small**	STDEV+/-
Aver.	**0,39**	0,13	**0,30**	0,14	**0,48**	0,22
	L versus M		**L vers. S**		**M vers. S**	
T-Tests	0,19		0,18		0,001	

Velos of particles with no CD and one event only (linear and directed movements)						
Aver.	**0,62**	STDEV+/-	0,26	**Aver.**	**0,34**	STDEV+/- 0,12
T-Test	0,0017					

Wagner-Lab

Cytoskeleton-based molecular motors and nanotechnology

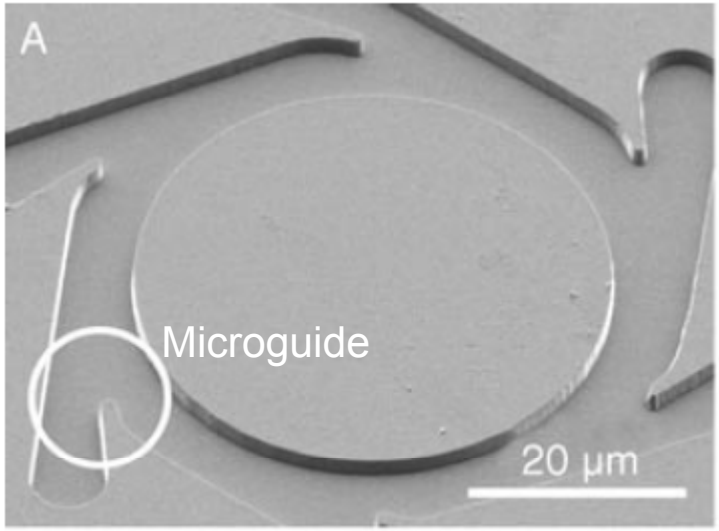


Using biomolecular motors in MEMS/NEMS allows for controlled material transport on the nanometer scale

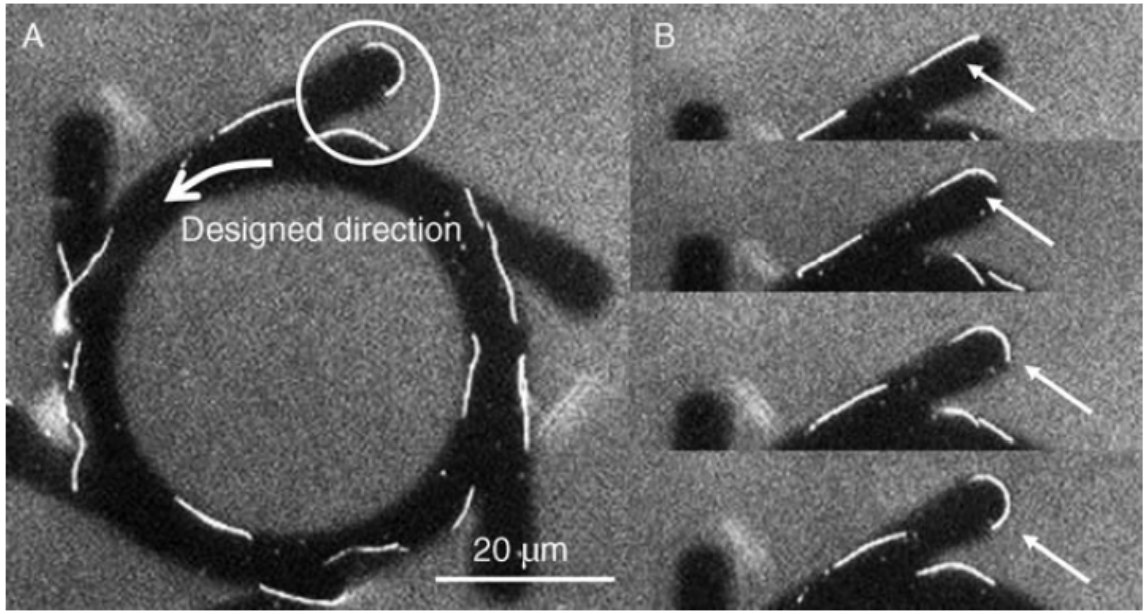
(MEMS = **M**icro**e**lectro**m**echanical **s**ystems)

Molecular sorting, concentrating and purification using biomolecular motors

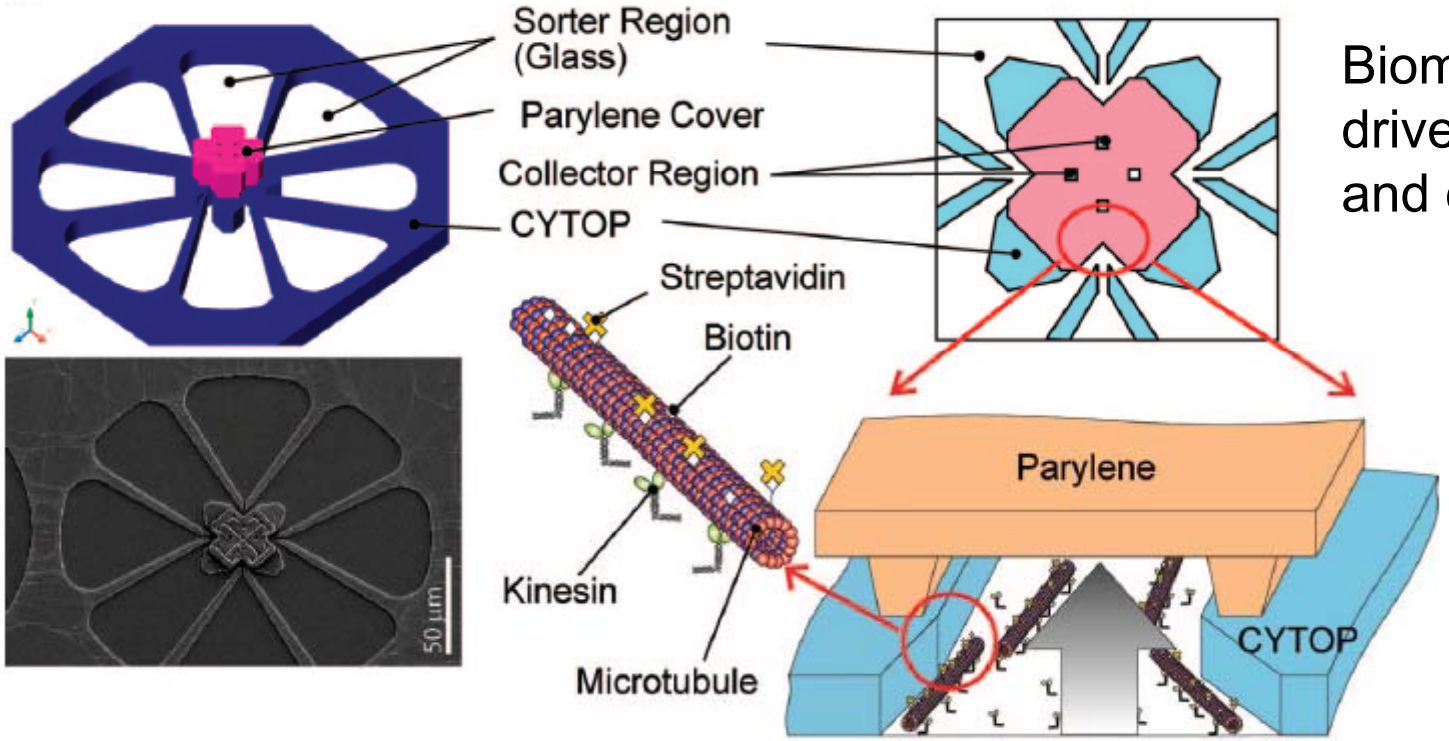
Problems for autonomous nanoscale transport of materials along nanochannels:
Microtubules frequently change directions => need for **rectifier** ("direction adjuster")



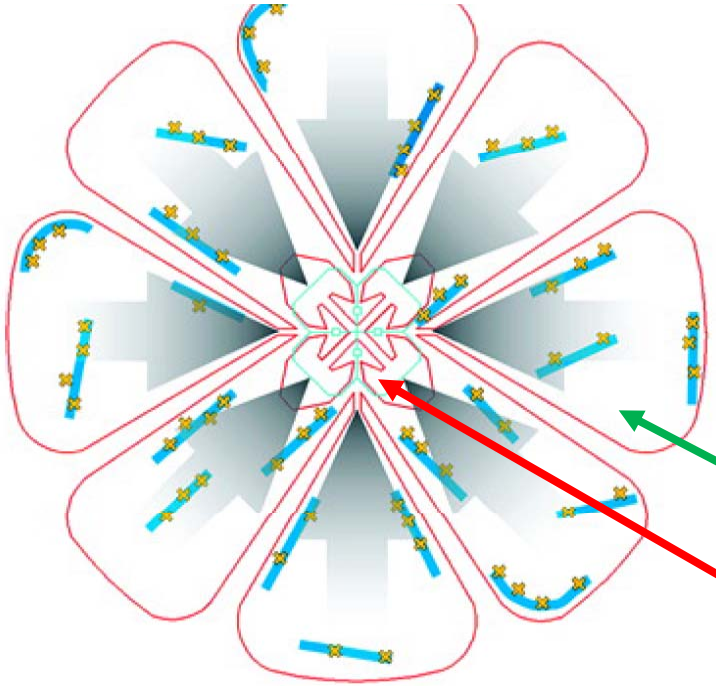
Rectifier system for autonomous transport of microfabricated structures in microfluidics system



Lin et al., 2006, *Small*



Biomolecular motor-driven protein sorting and concentrating



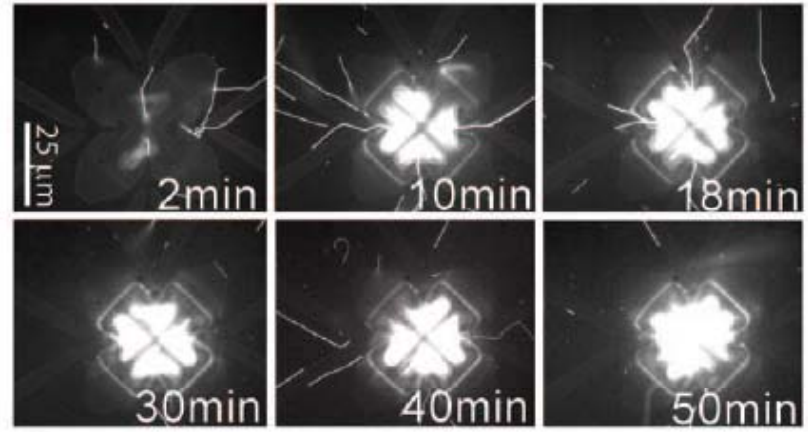
How to <u>automatically separate</u> ***two*** (or more) substance(s) from each other and concentrate one of them?

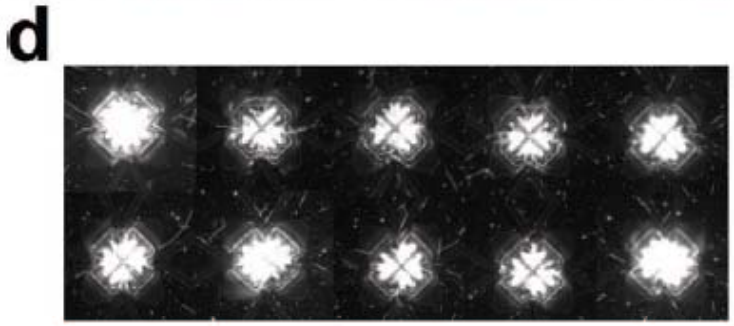
- Let <u>one substance specifically bind to MTs</u>
- Let MTs direct from the **sorter region** out into the **collector region**

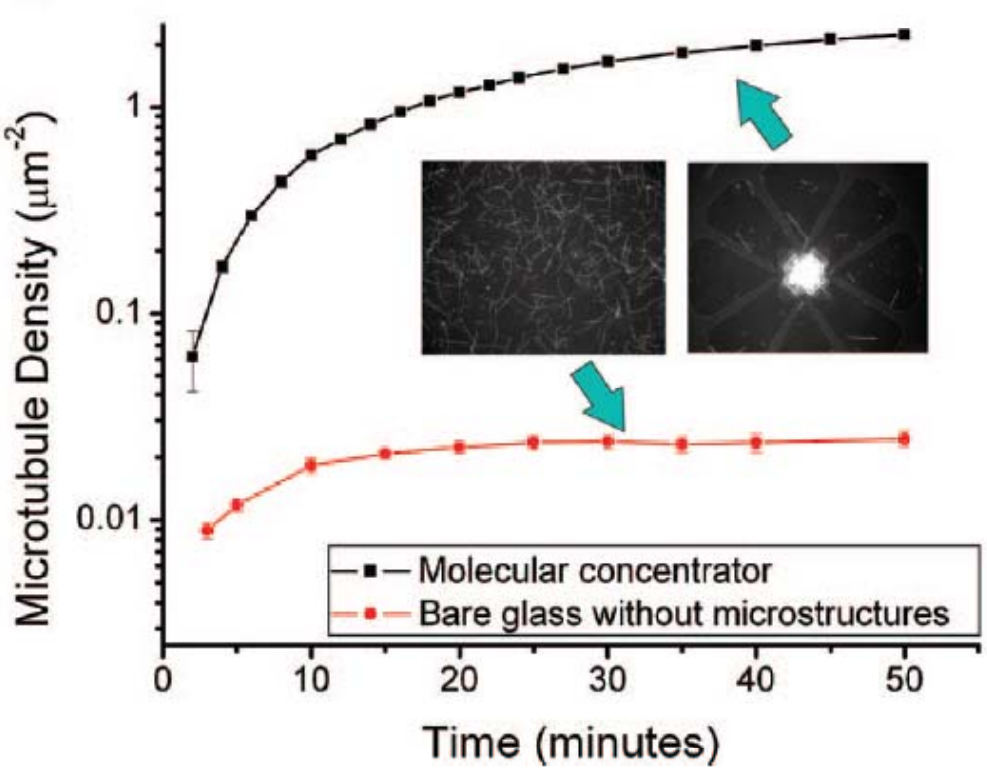
sorter region

collector region

Biomolecular motor-driven protein sorting and concentrating





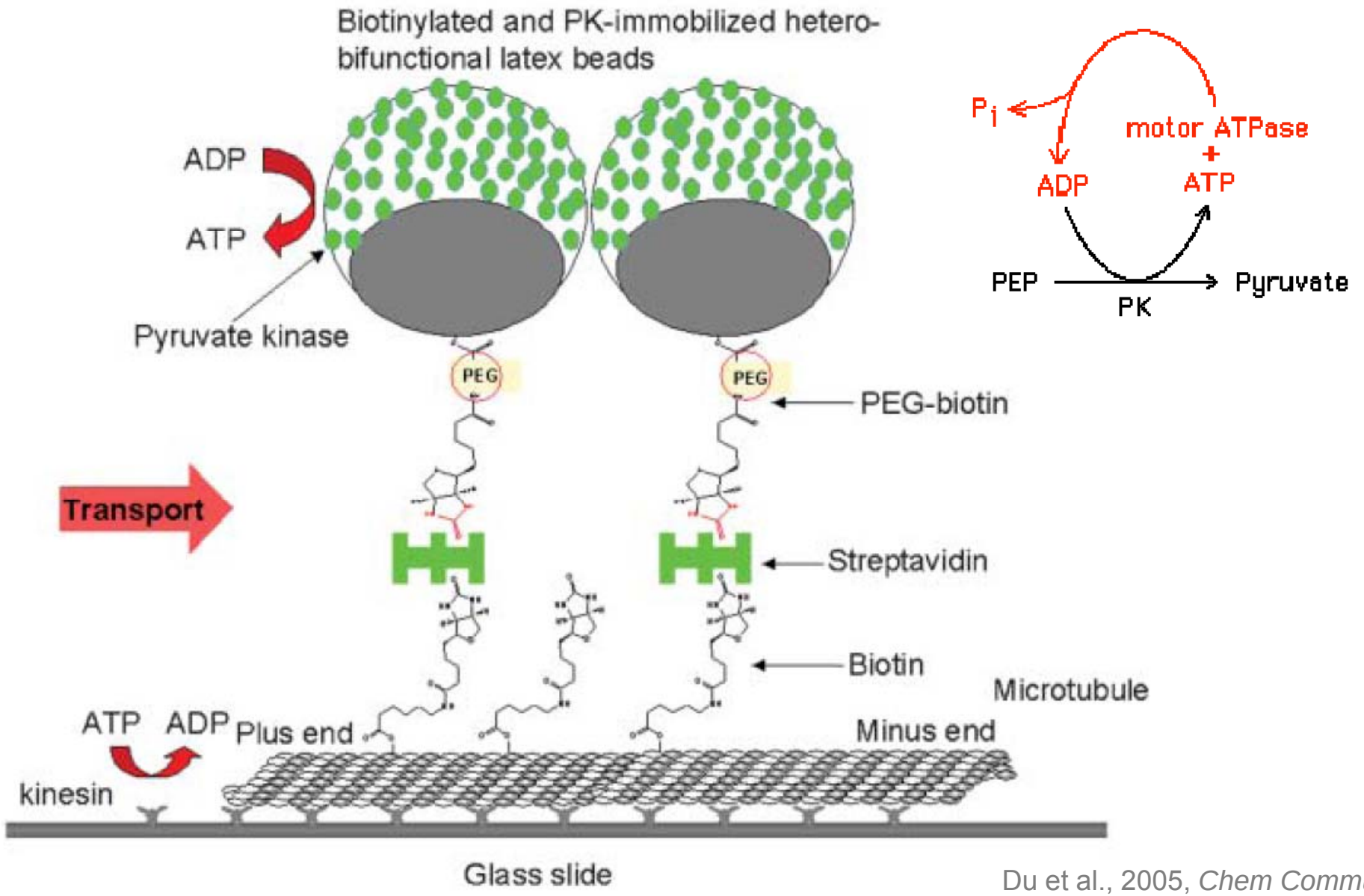


Applications:

- **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)

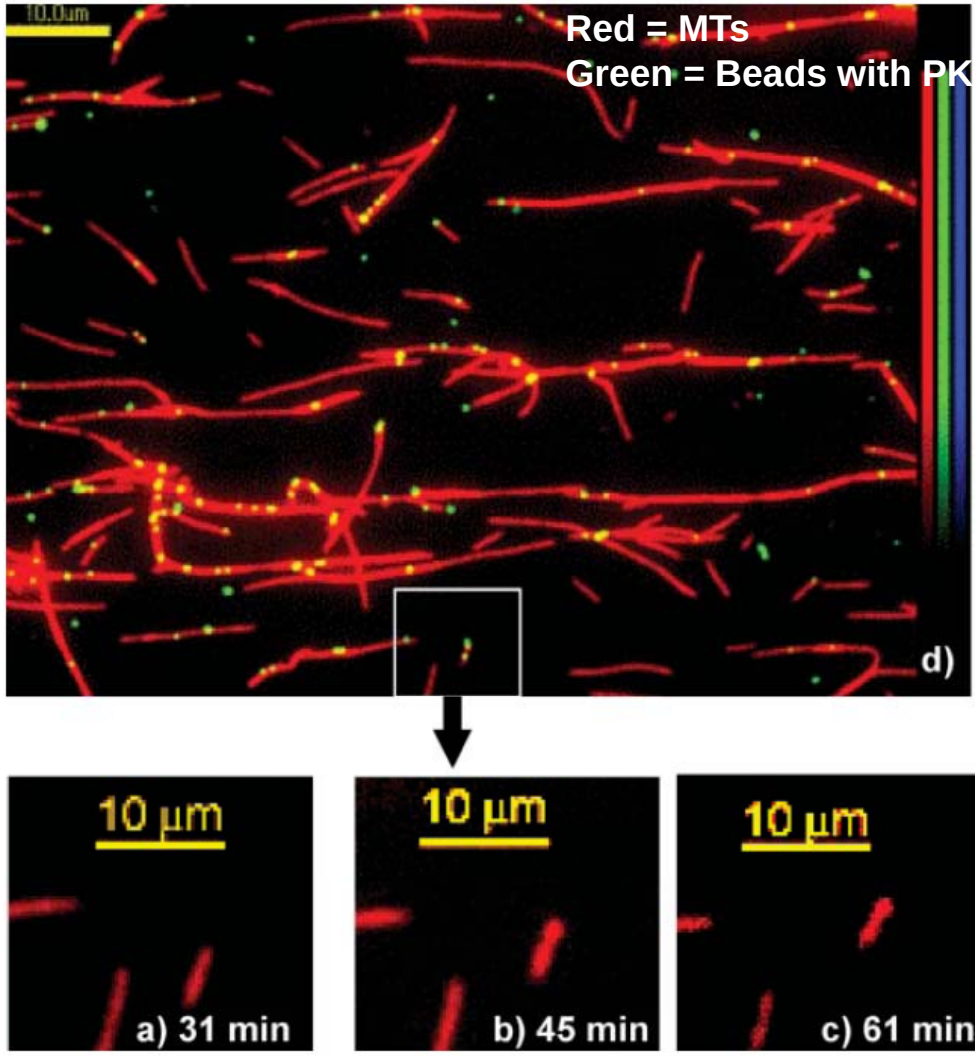


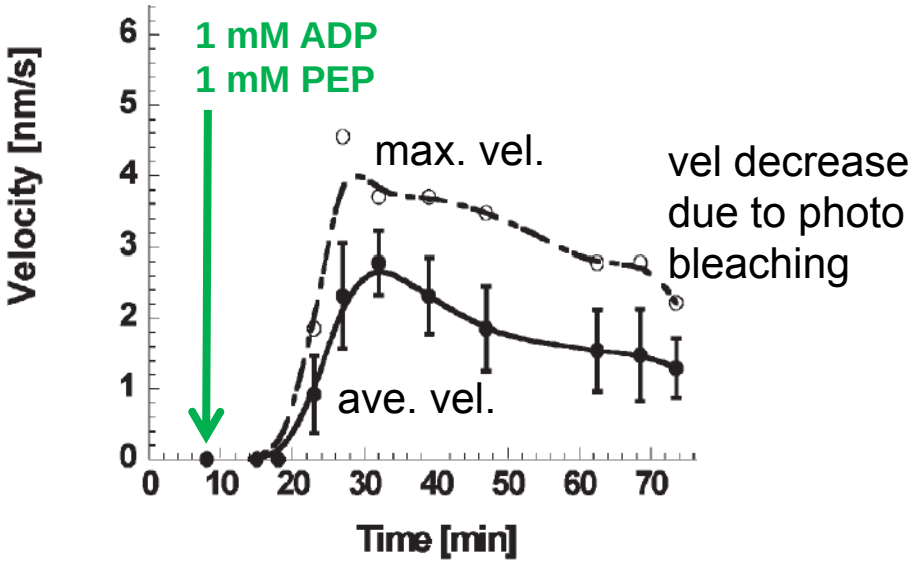
Du et al., 2005, *Chem Commun.*

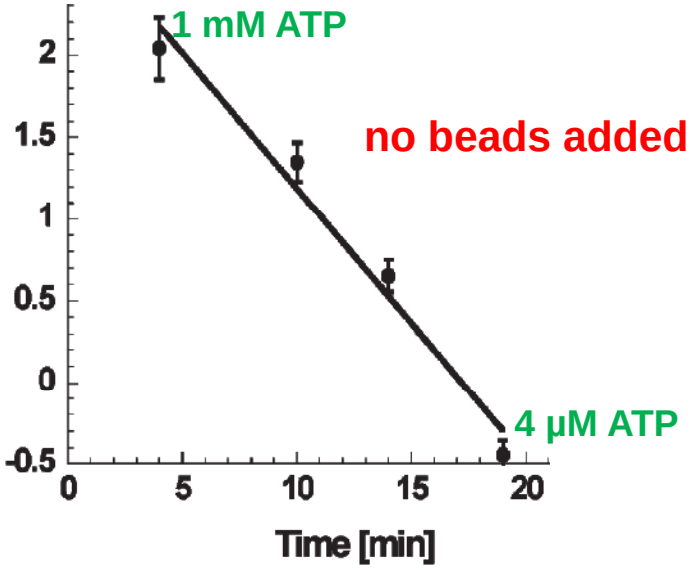
Nano-biomachine powered by self-supplying ATP

- Nano-biomachine **moved** for **75 minutes** (*time of a HBO movie!*) at constant velocity
- Without the self-supplying system the speed of MTs decreased to zero after 15 min

Movie
ATP self-suppl bio nanomachine.mov



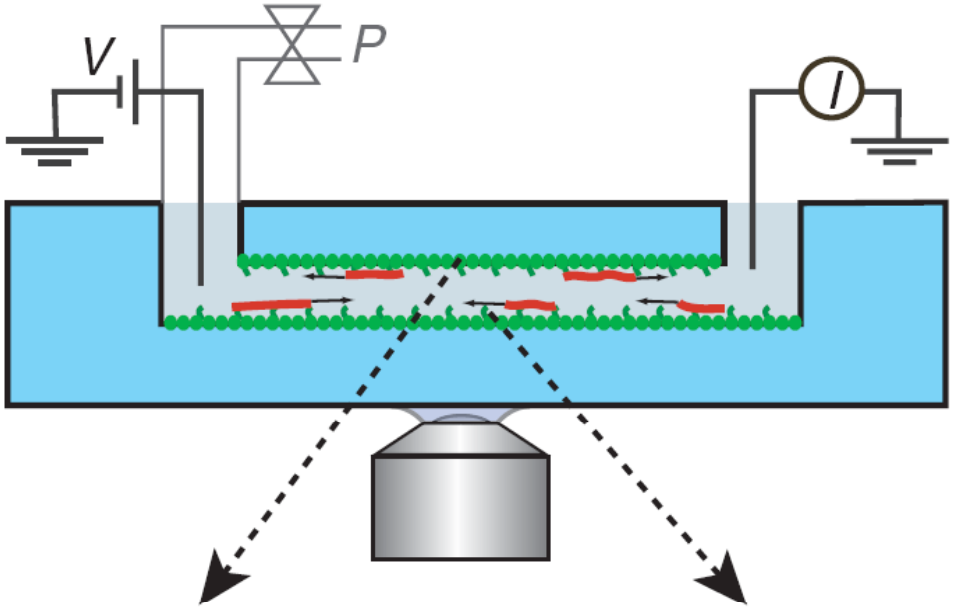




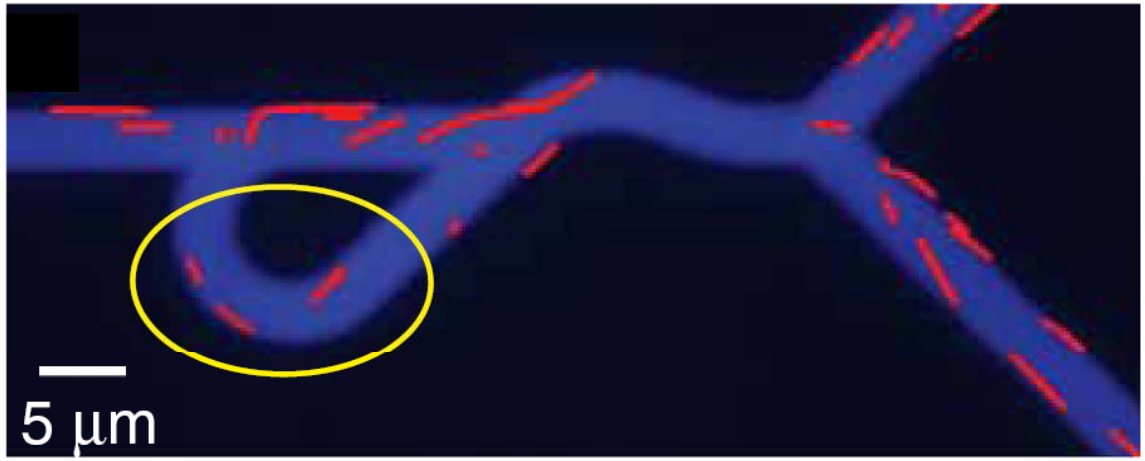
Molecular sorting, concentrating and purification using biomolecular motors

Example from your reading material!

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



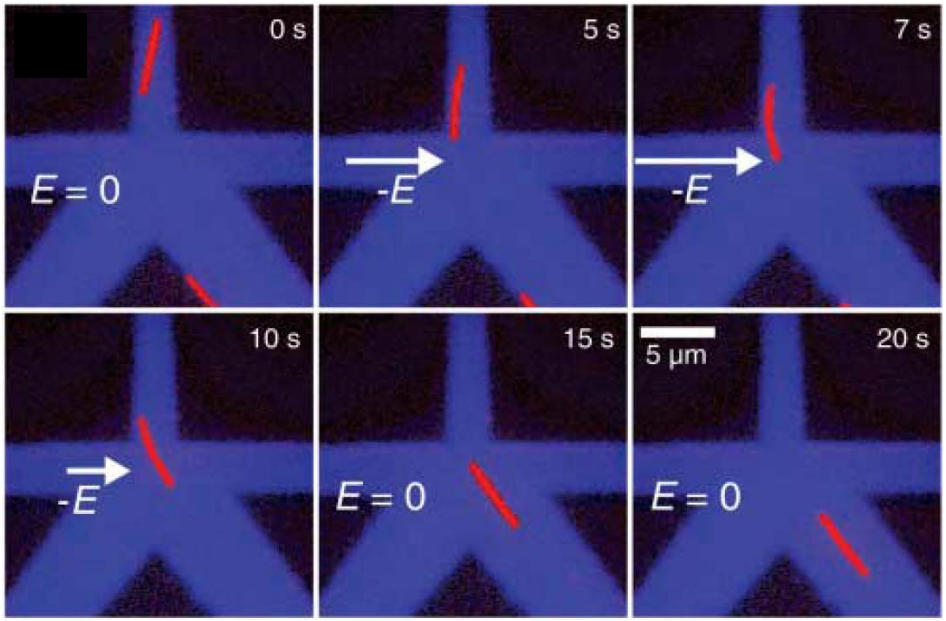
- 800 nm deep **nanochannels** made by E-beam lithography and wet etching techniques
- Channels are coated with **kinesin** (green) and **microtubules** (red) flown inside
- An electrical field (35 kV/m) can be applied

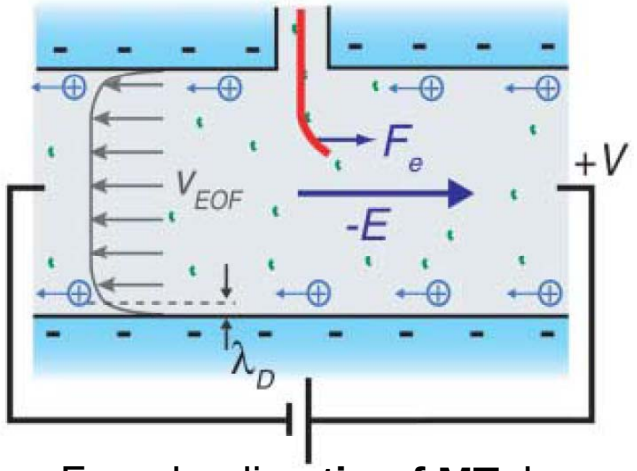


Microscopic image of fluorescently labeled MTs in nanochannels

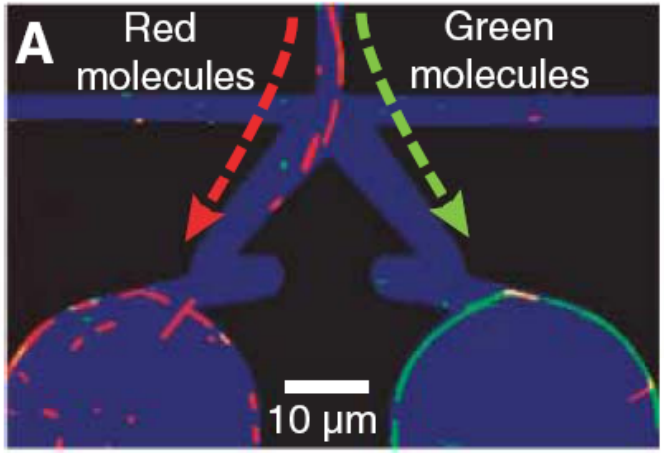
Heuvel at al., 2006, Science

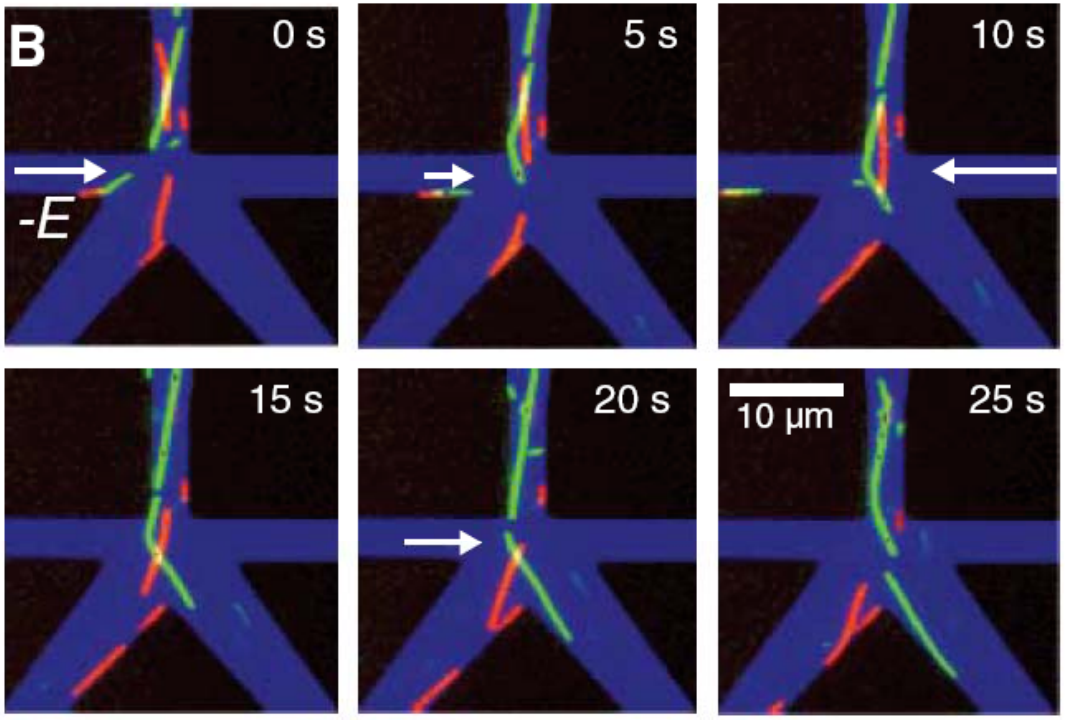
Molecular sorting, concentrating and purification using biomolecular motors





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



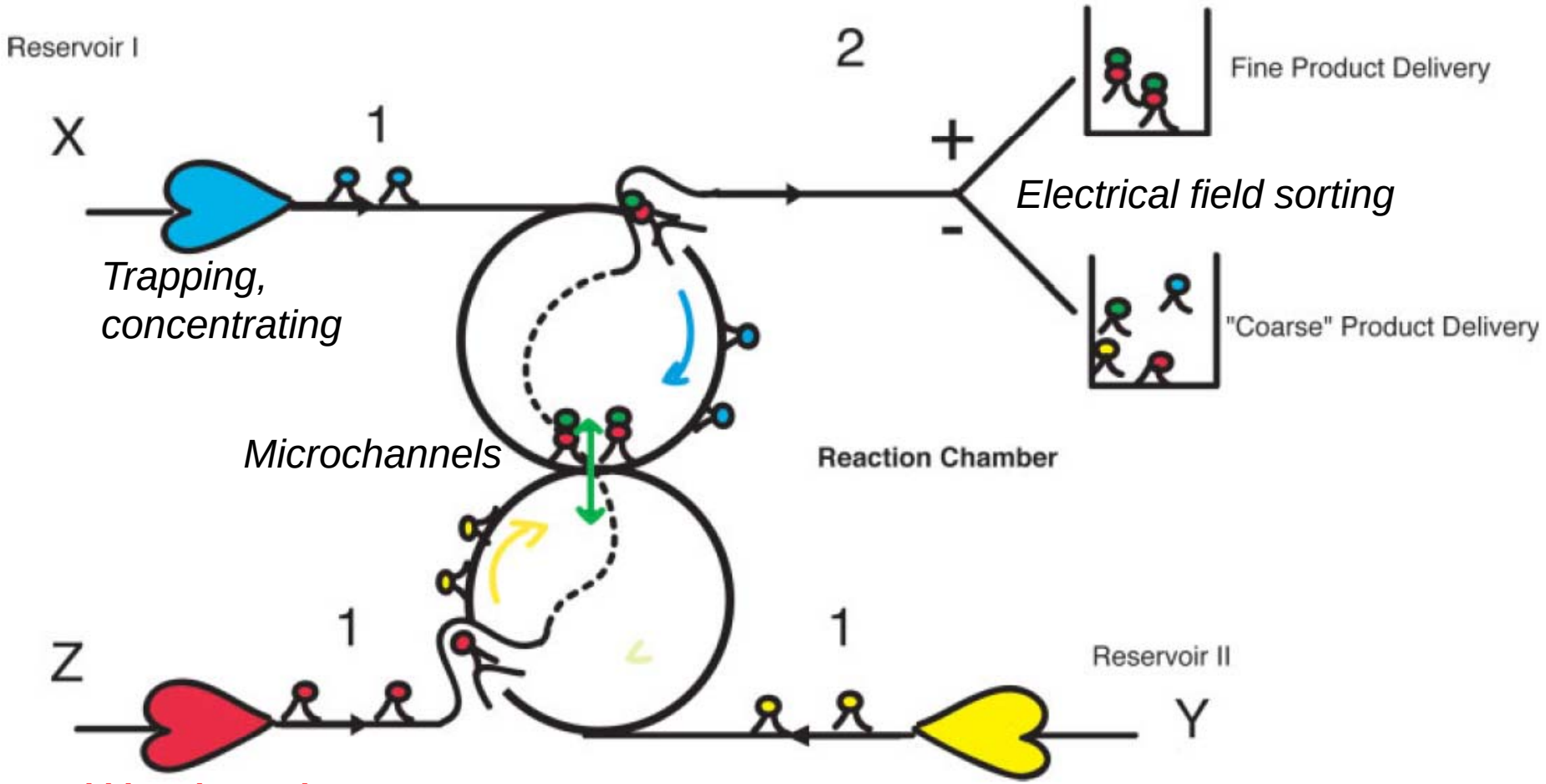


A simple nano-factory

- 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 could be: protein, biochemical substance, DNA oligomer etc.

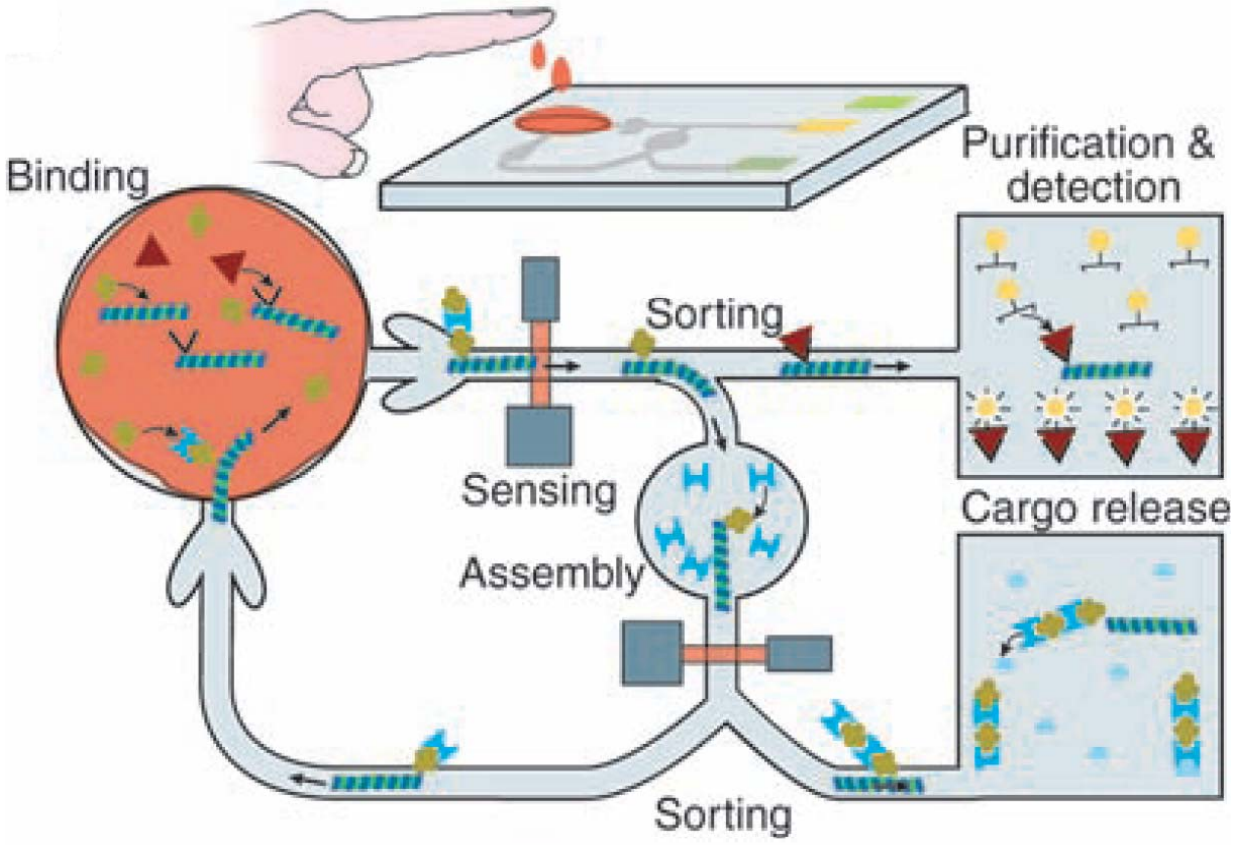


Red kinesins only transport newly reacted cargo

Medical applications for autonomous nano-factories

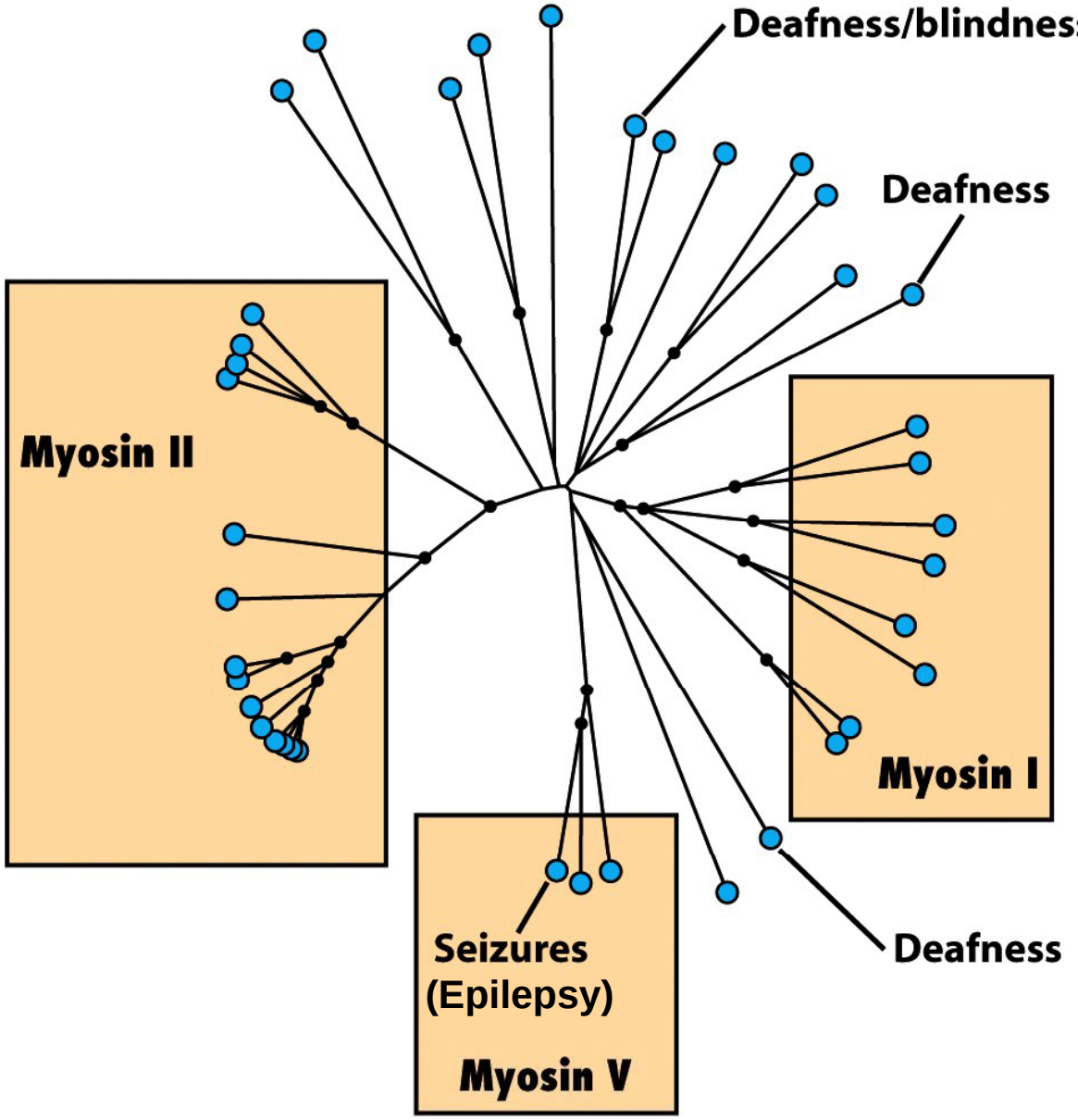
Example from your reading material!

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

Myosins make up a large family of actin-based motor proteins



- **40 myosin genes** found in the human genome
- Loss of specific myosins can cause severe diseases
- 3 important classes:
  - Myosin I (**endocytosis**)
  - Myosin II (**muscle contract.**)
  - Myosin V (**cargo transport**)

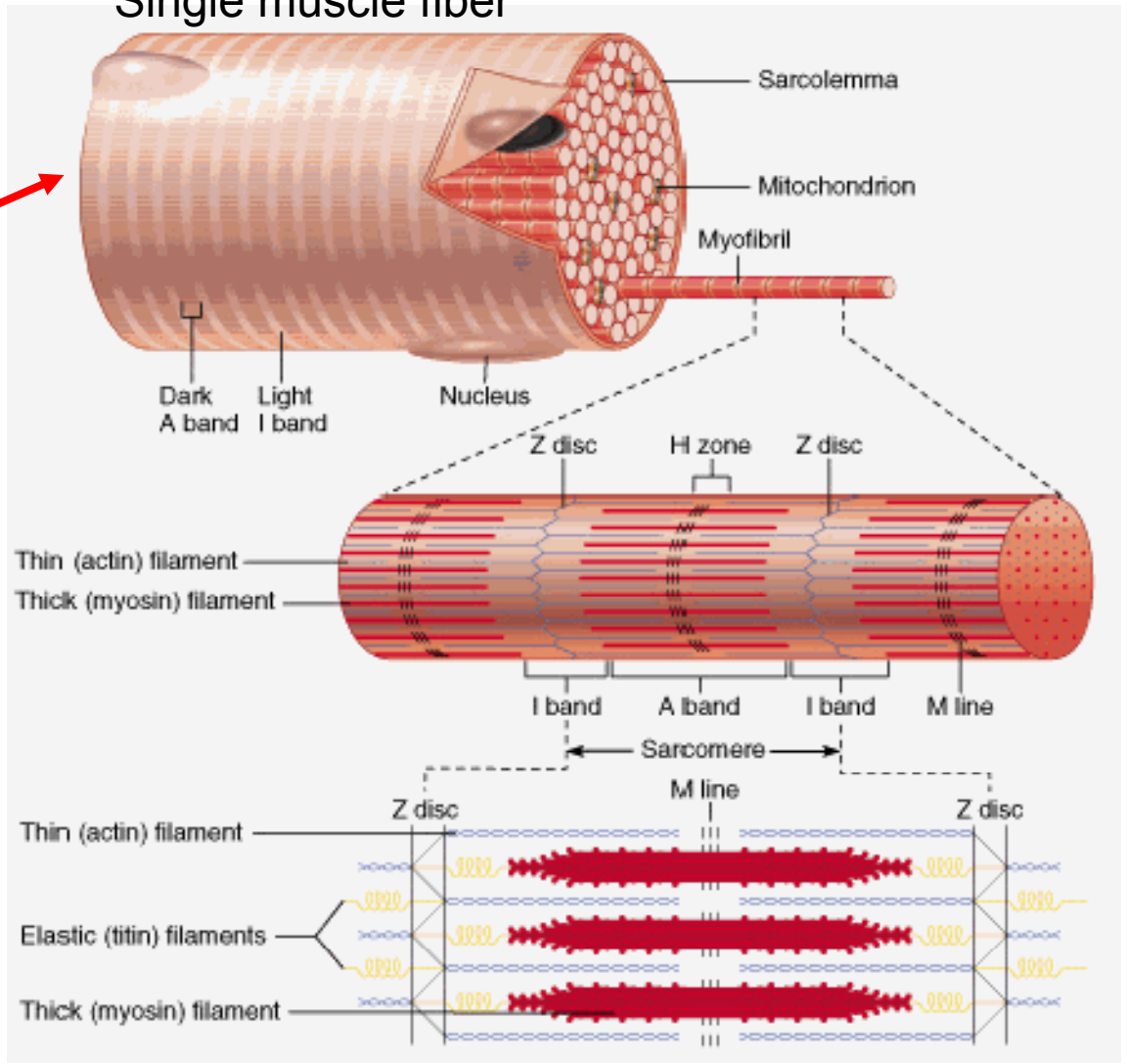
Myosin and actin is the smallest contraction unit of skeletal muscles

“If you don’t listen to 王歐力
I will come to his classroom”

Actin filament

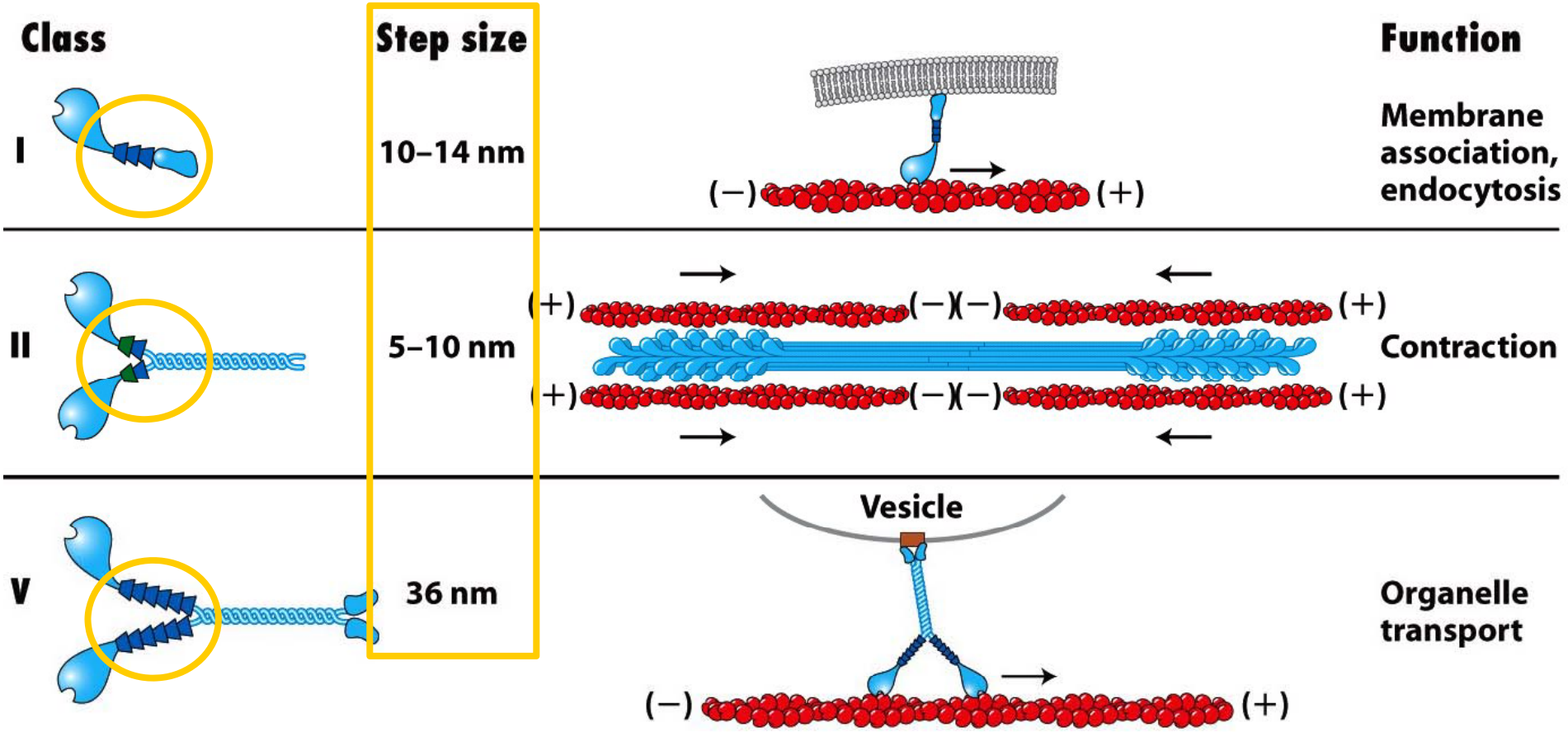
Myosin bundles

Single muscle fiber



Cellular and intracellular movements depend on myosins

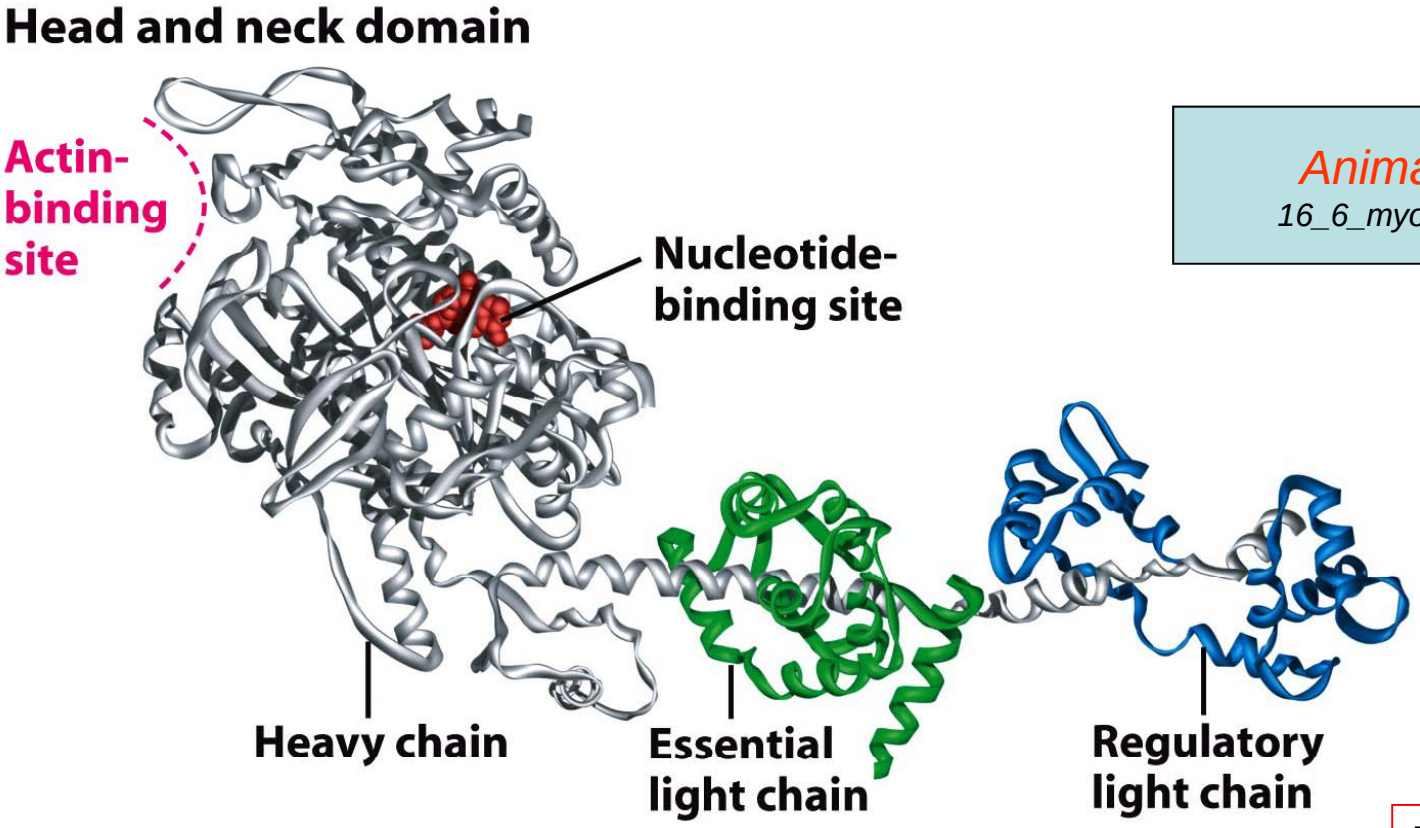
- **Muscle cells**: <u>contraction</u> based on filament sliding between F-actin and **myosin II**
- **Vesicle transport**: **myosin V**
- **Endocytosis (membrane invagination)**: monomeric **myosin I**



Myosins walk on actin filaments: *Example from your reading material!*

Myosins consists of **several light** and **heavy chains**
⇒ **light chains** have regulatory function
⇒ **heavy chains** form the motor head (**actin-binding** and **ATP-binding**),
a flexible neck and a tail domain (with “cargo-binding” function)

Tail consists of α-**helical coiled-coils** that **form** (the rod-like) myosin **dimer**

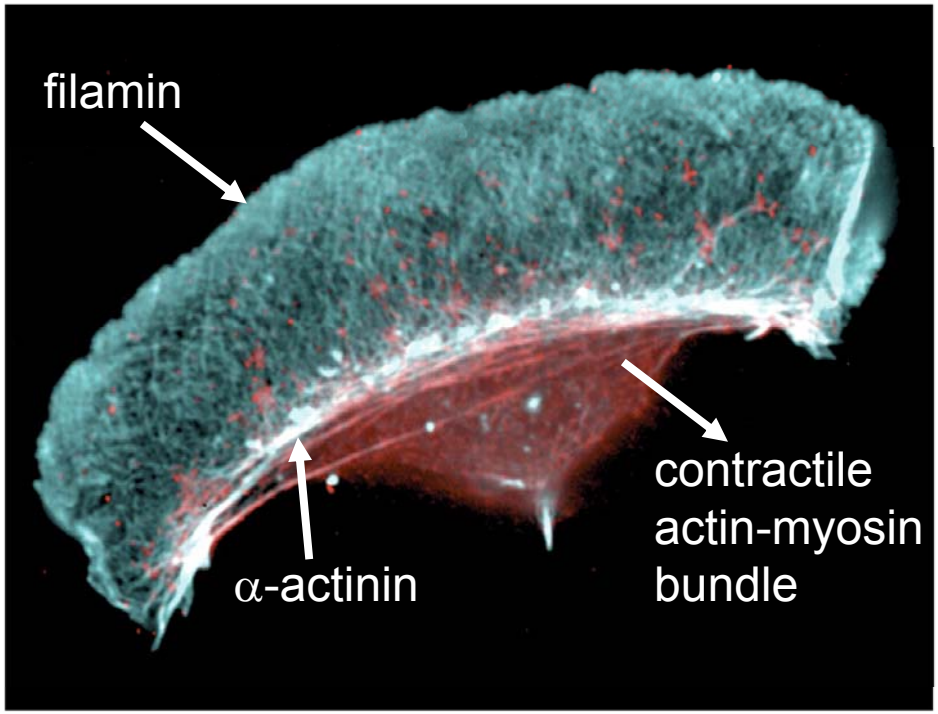


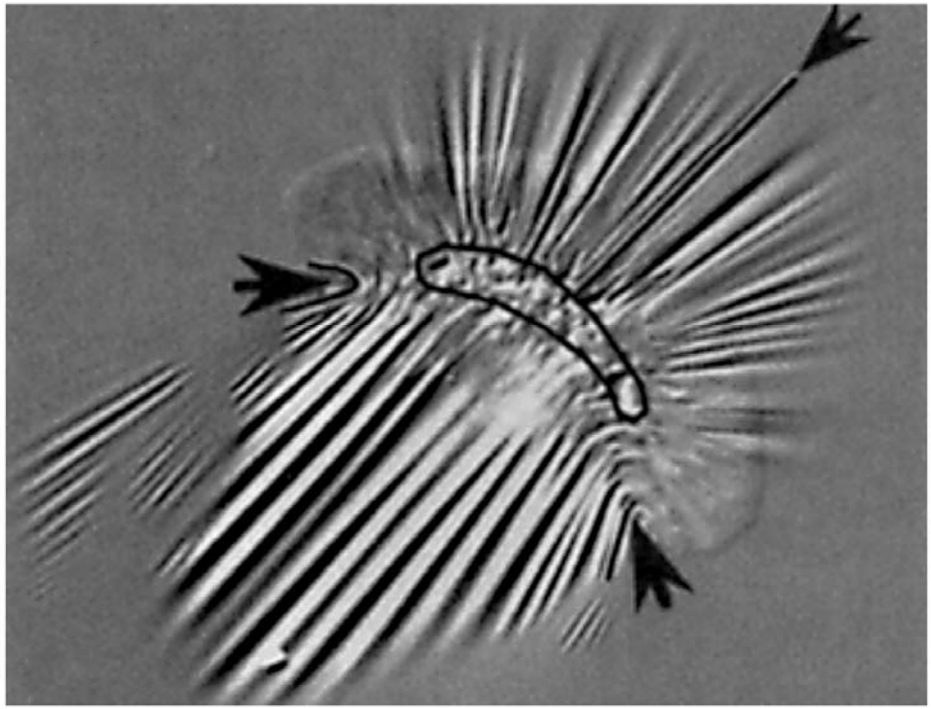
Animation
16_6_myosin.mov

Myosin monomer

Forces generated by contractile bundles made visible on thin silicon substratum

As a keratinocyte moves forward, the generated force by the actin-myosin bundle in the center of the cell causes the silicon rubber to wrinkle



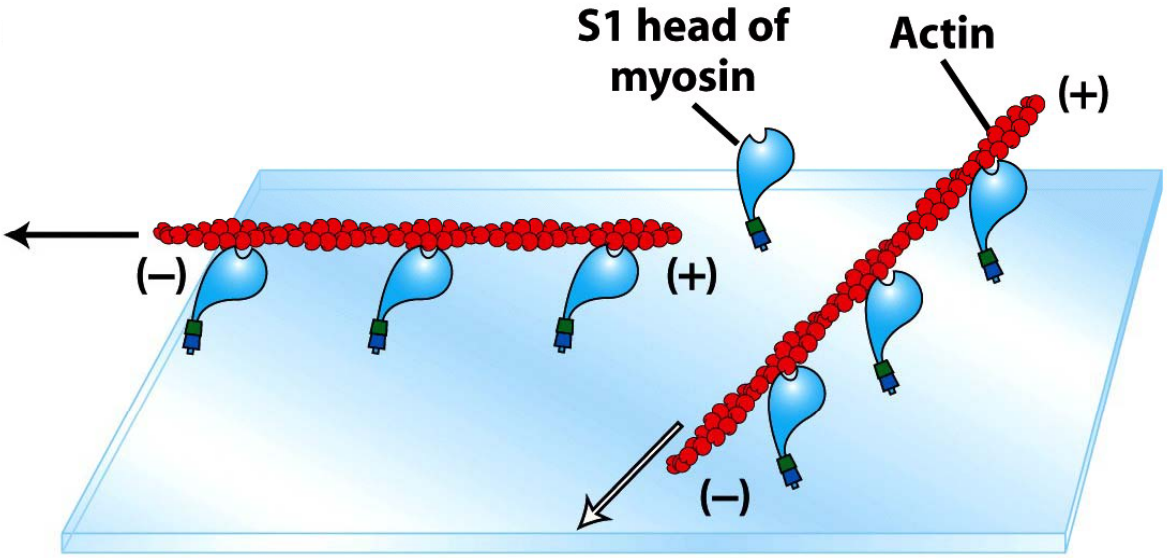


Movie
16_4_heart muscle cell on rubber substrate.mov

"beating" heart muscle cell on thin silicon substratum

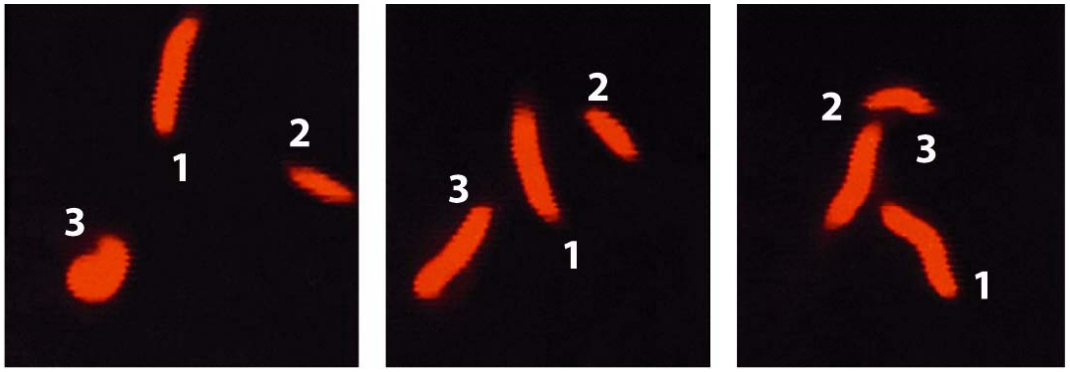
In vitro motility assay to determine forces and motor steps in the nano-range

- Movement of fluorescently-labeled **actin filaments** on immobilized **myosin heads** on a cover glass is observed using a fluorescence microscope
- Addition of ATP triggers the **movement of actin filaments** along the fixed myosins
- Upon ATP-binding, myosin heads dissociate from the filament while the head tilts towards the (+) end (no power-stroke yet)
- The **upcoming power-stroke** pushes the filament with the (-) end in the lead



Animation 1
a19-03-in_vitro_motility.swf

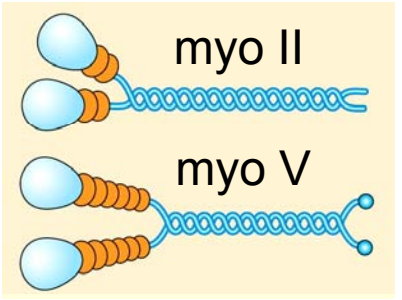
Animation 2
16_8_actin myosin in vitro assay.mov

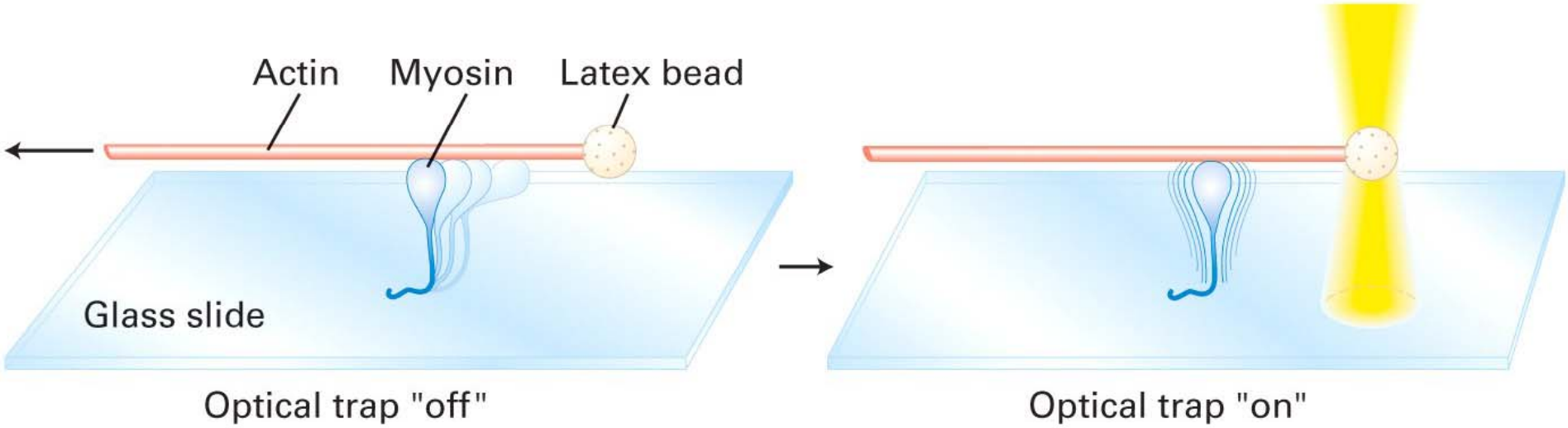


Rhodamine-phalloidin labeled actin filaments. One frame each 30s.

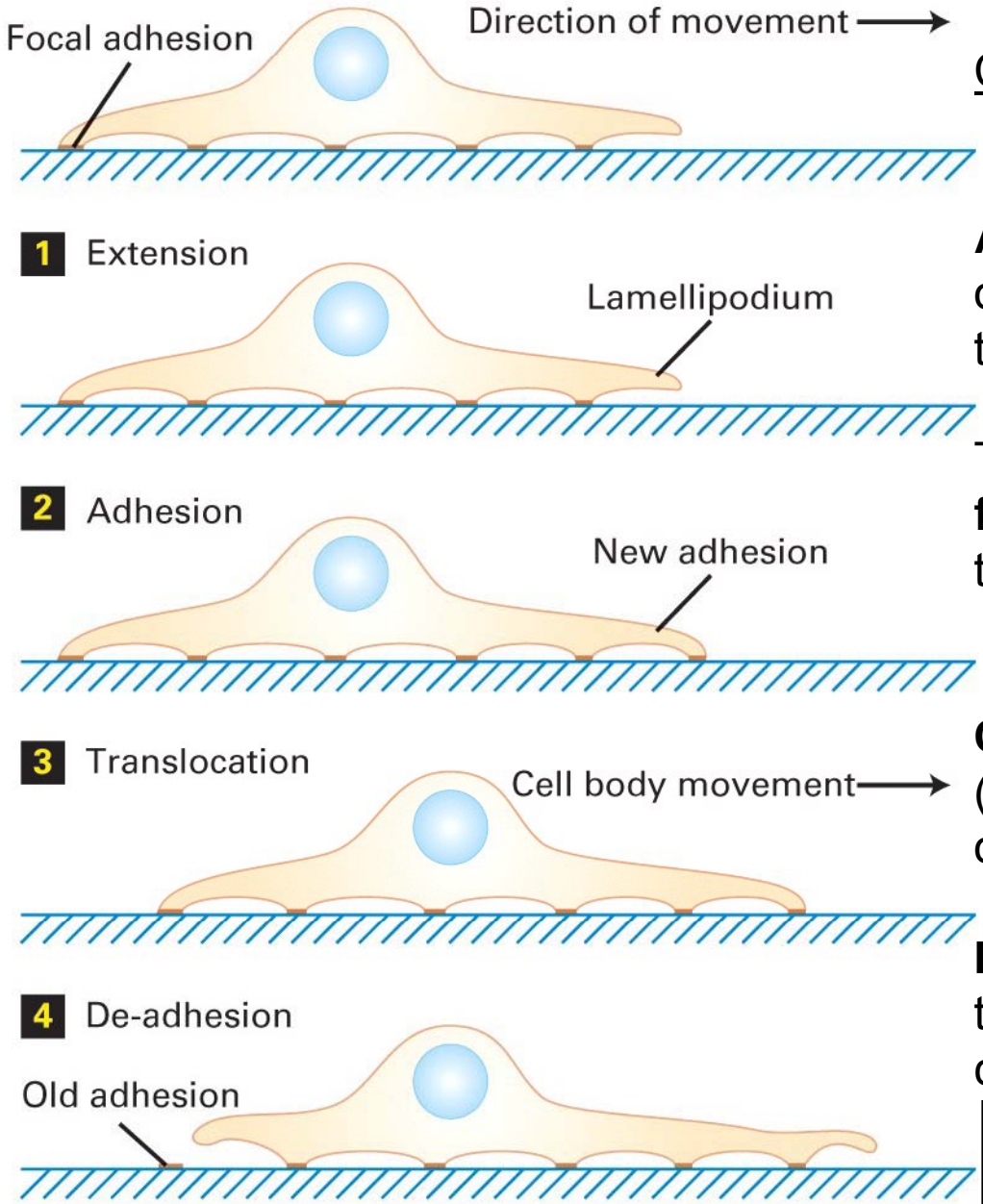
Measuring the force generated by single myosin heads using the optical trap

- An highly focused infrared beam is used to immobilize a bead attached to **actin**
- During the myosin's **power stroke** the actin filament is hold in position
- The force generated by the myosin head is determined by measuring the bead displacement => for myosin II about **3-5 pN** (piconewton)
- The **step-size** and force depends on the length of the **lever arm**:
  - myosin II = **5-10 nm**
  - myosin V = **36 nm**





Cell movement requires contractile bundles and cell adhesions



Cell movement occurs in 4 discrete steps

Actin polymerization and **cross linking** of new filaments forms a lamellipodium at the *leading edge*

The lamellipodium (or filopodia) form new **focal adhesions** to fix the leading edge to the substratum

Contraction of the **actin-myosin** cortex (near the cell body) leads to translocation of the tail

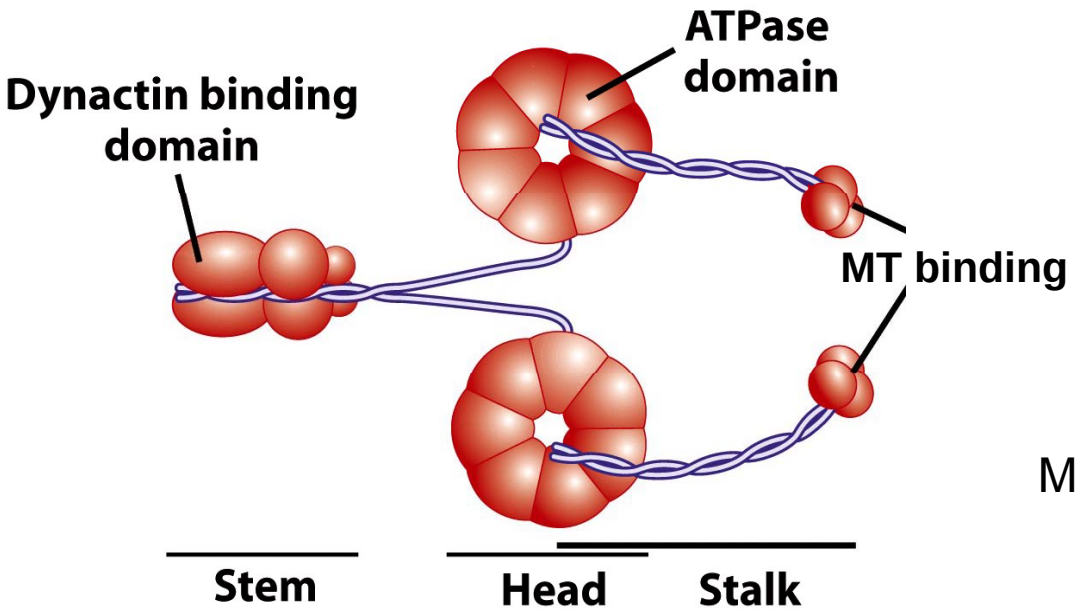
De-adhesion of focal adhesions at the tail releases the stress caused by contraction of the actin-myosin network

Animation 1
a19-04-cell_motility.swf

Animation 2
v19-04-macrophage.mov

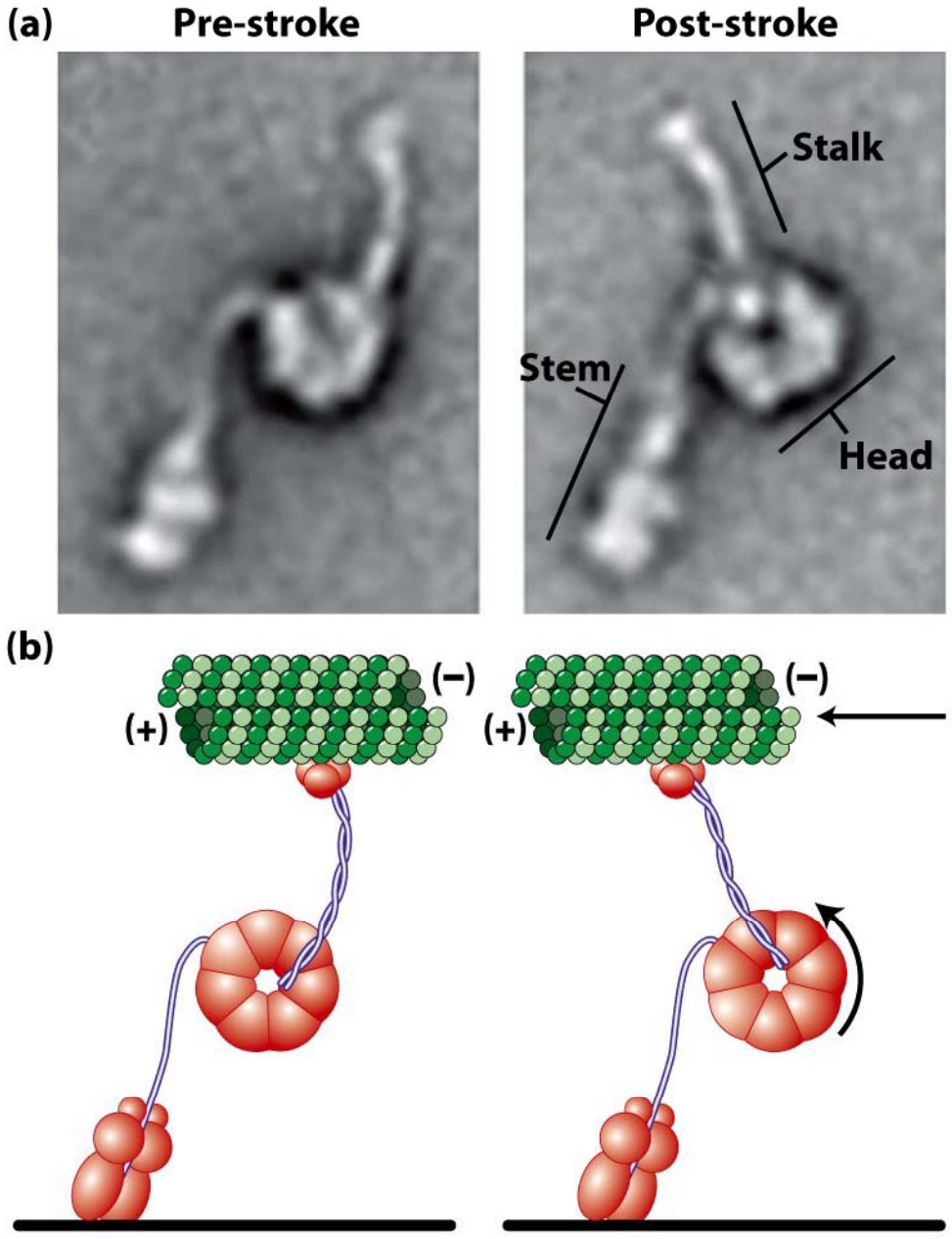
Dyneins move retrograde (backwards) on microtubules

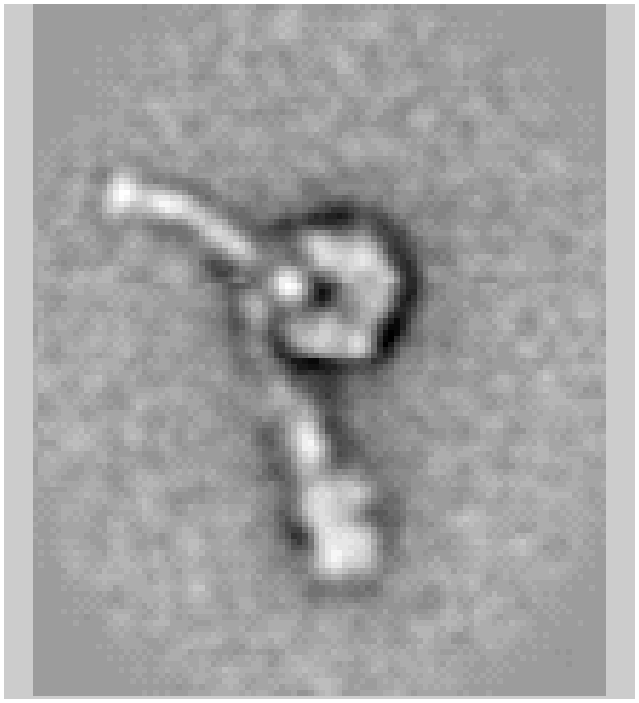
- **Dyneins** move cargo retrograde (backwards)
- In axons: from plus-end to minus-ends



Model of Dynein

Force generation of dynein revealed by electron microscopy

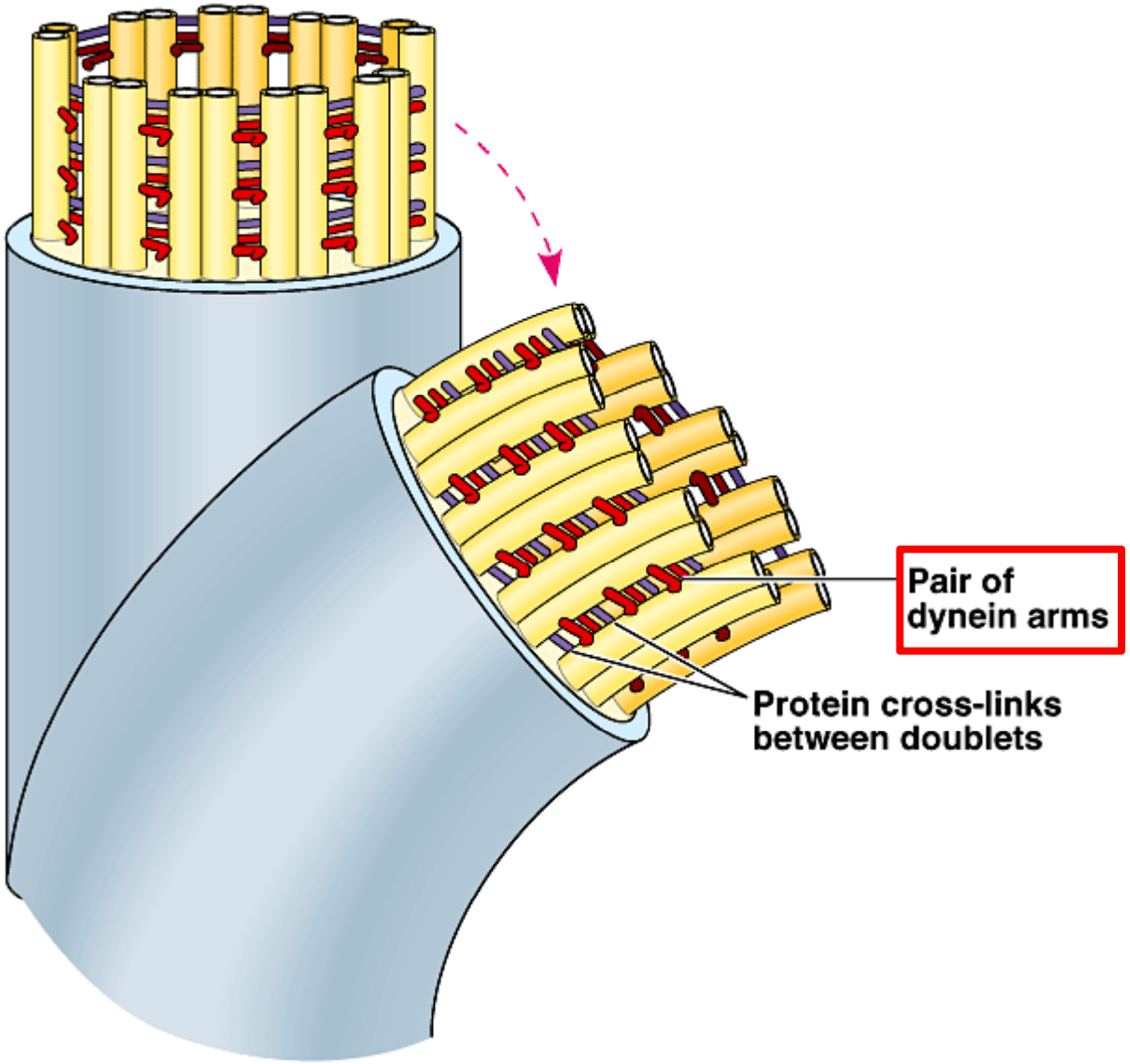


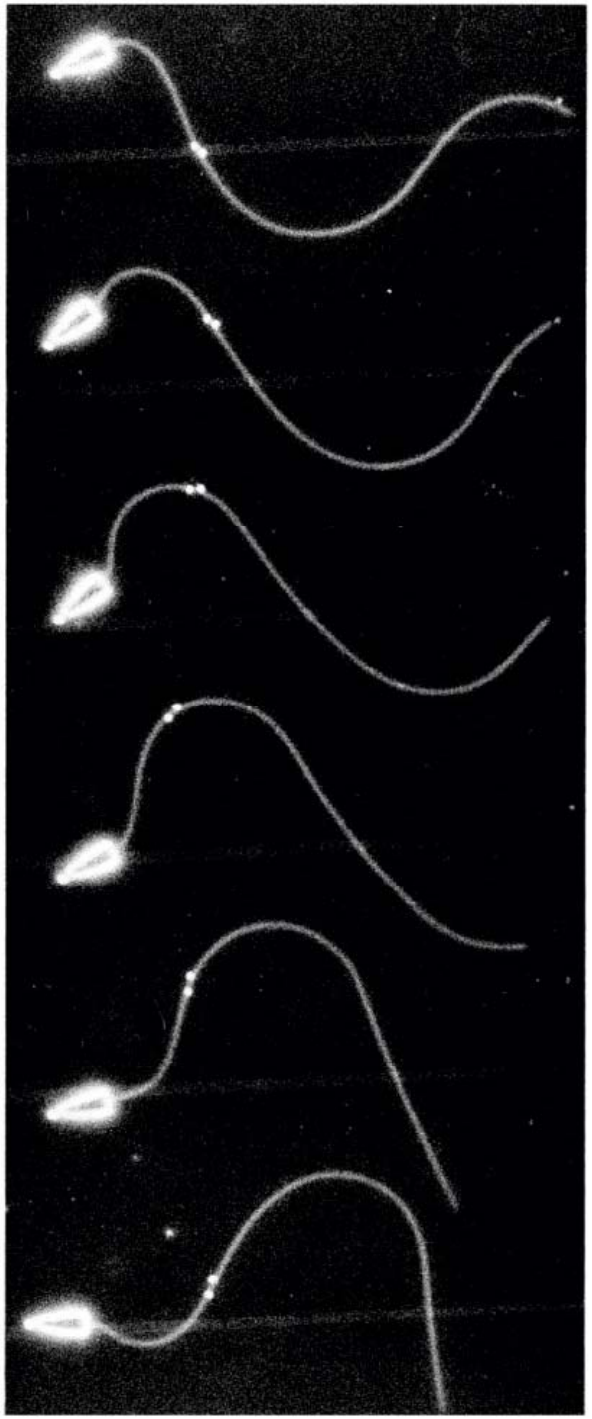


animation

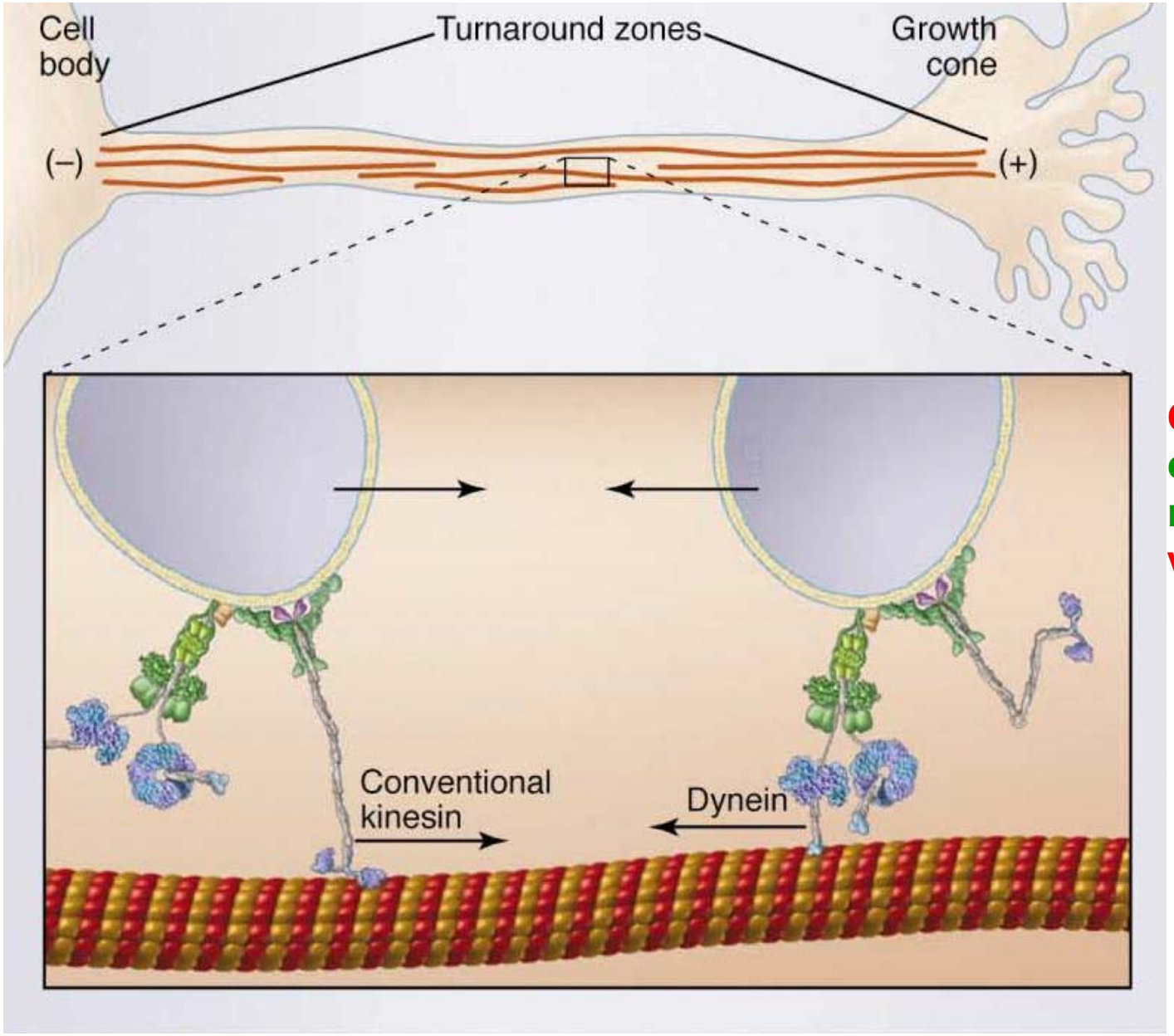
During ATP hydrolysis, the dynein head undergoes a conformational change showing that a poststroke follows a prestroke

Flagella bending generated by MTs sliding past each other powered by dynein





How can vesicles move bidirectional? *Example from your reading material!*

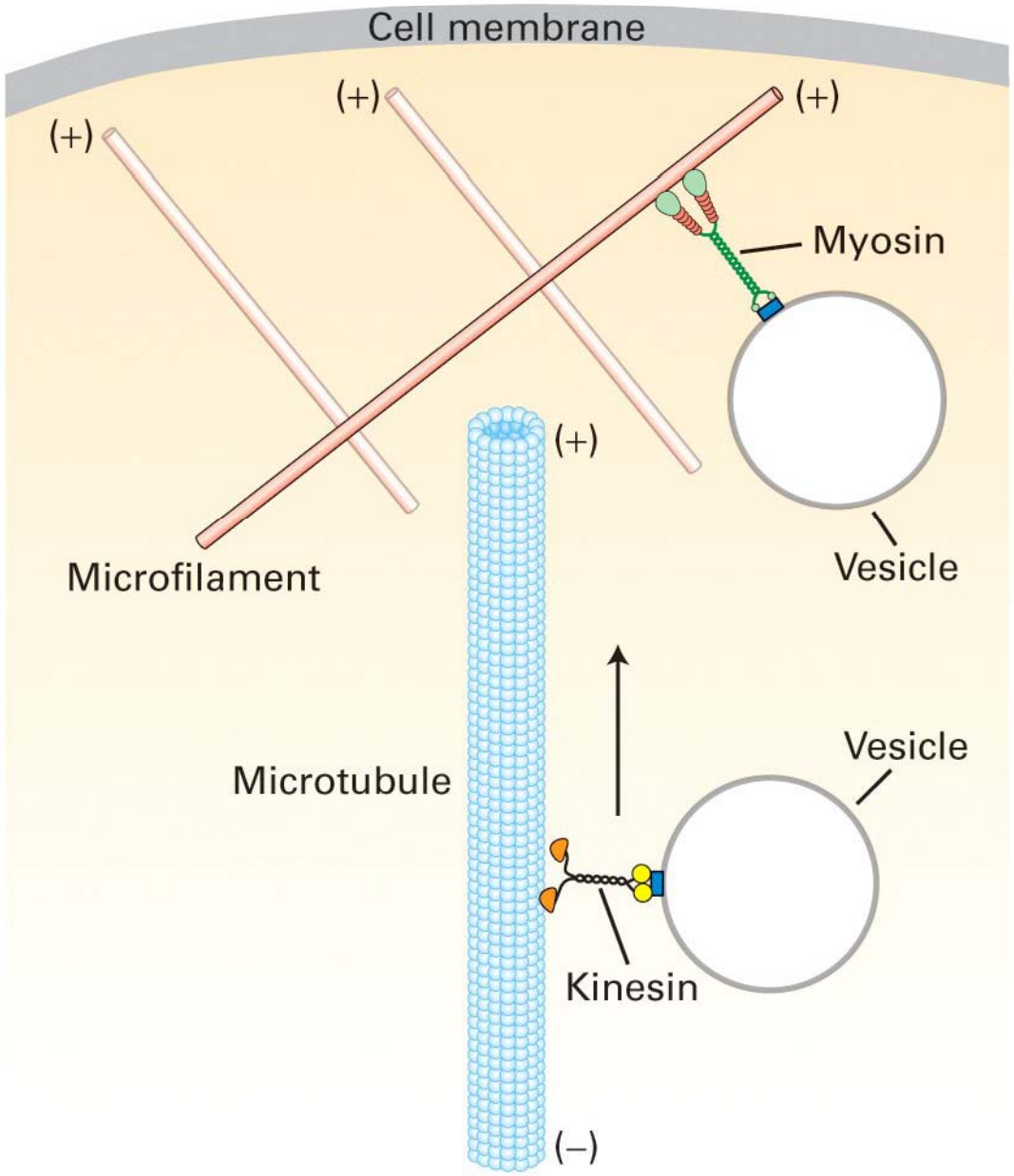


Coordinated activity of two types of motors on the same vesicle?

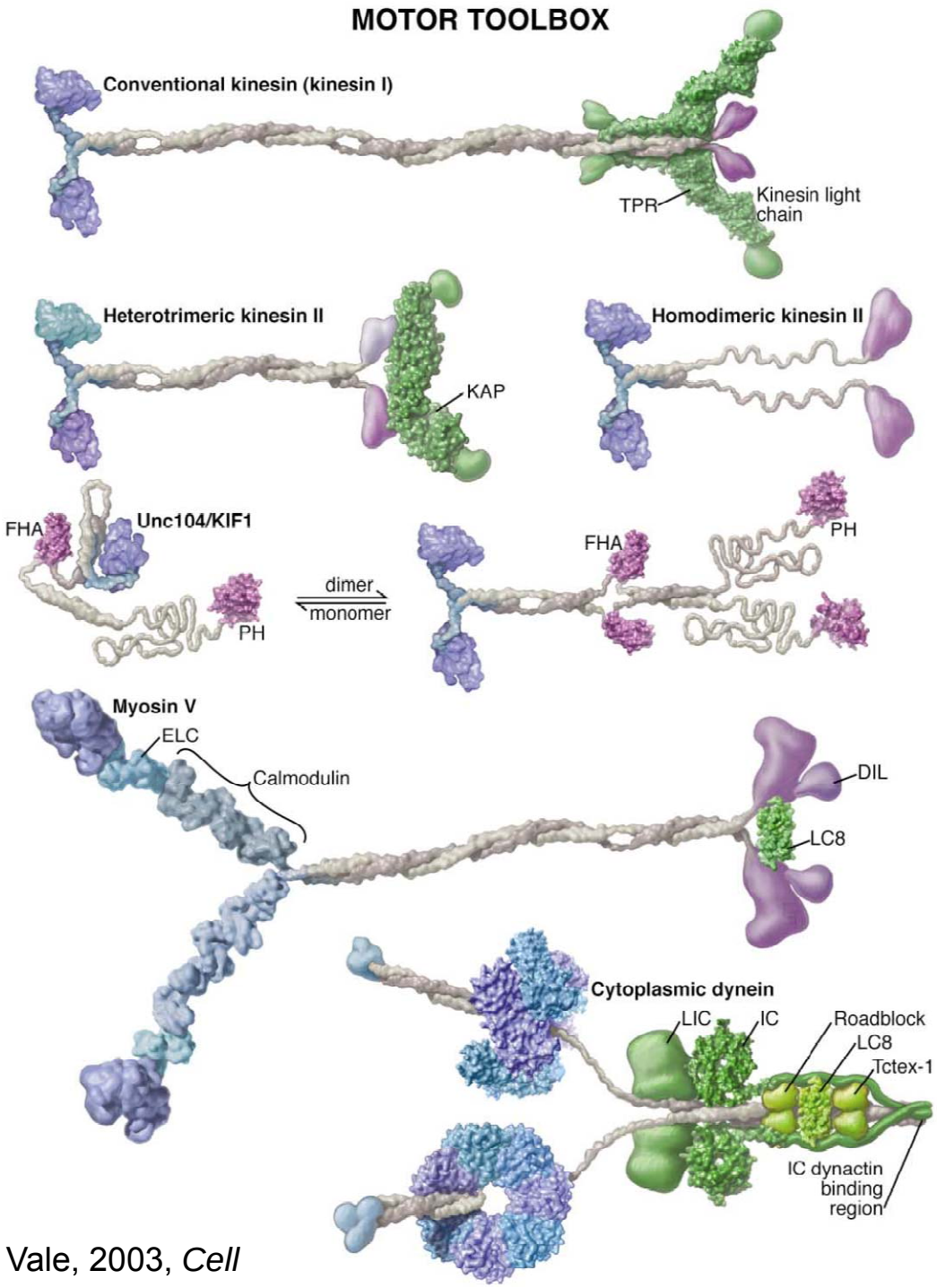
Pollard, 1st ed.

Bidirectional movement: “tug-of-war” between opposing motors?

Changing tracks: myosins and kinesins may work together



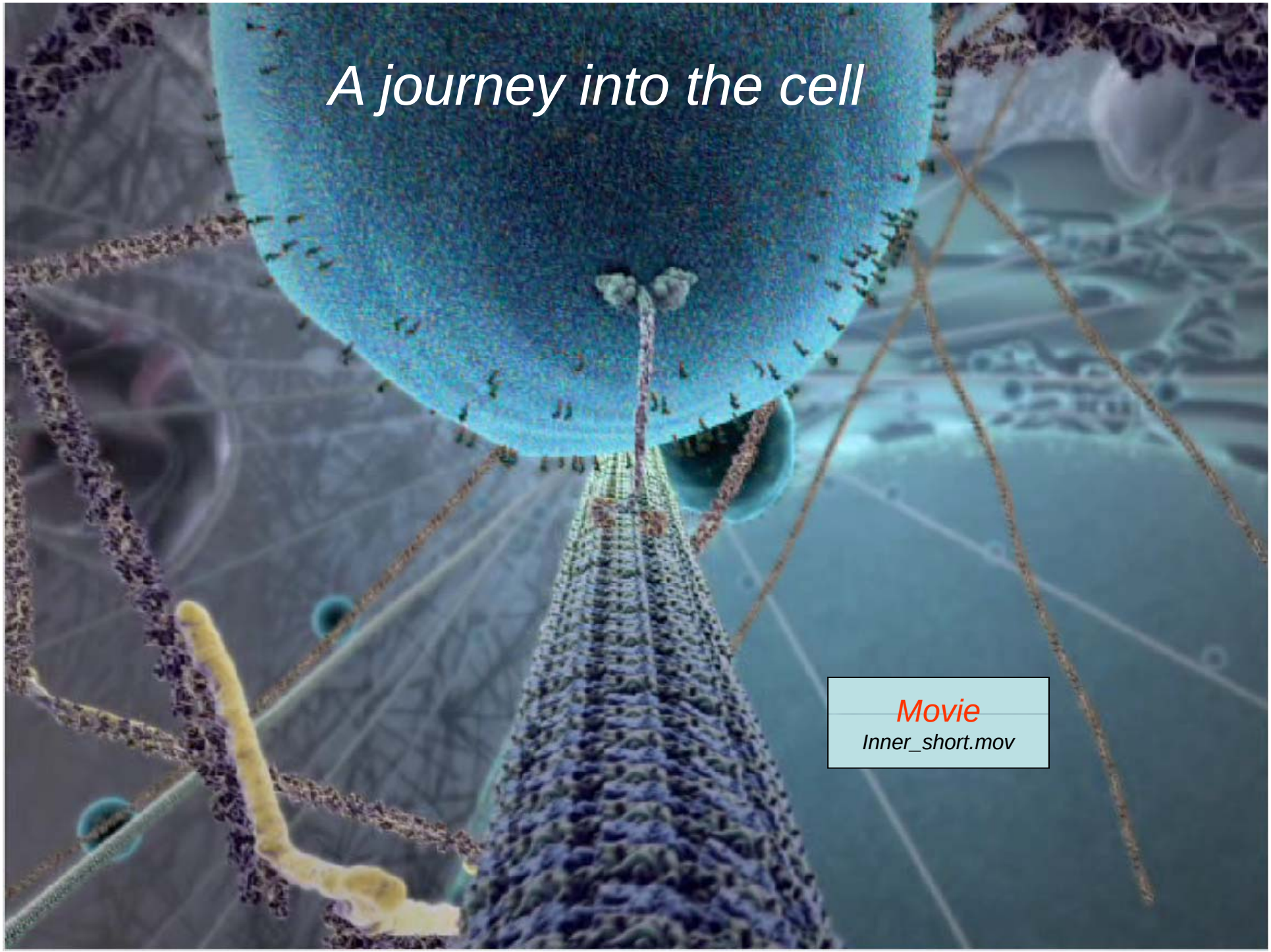
A vesicle might be moved from deep inside the cell to the periphery by a **kinesin** while at the cell periphery a **myosin** takes over this vesicle



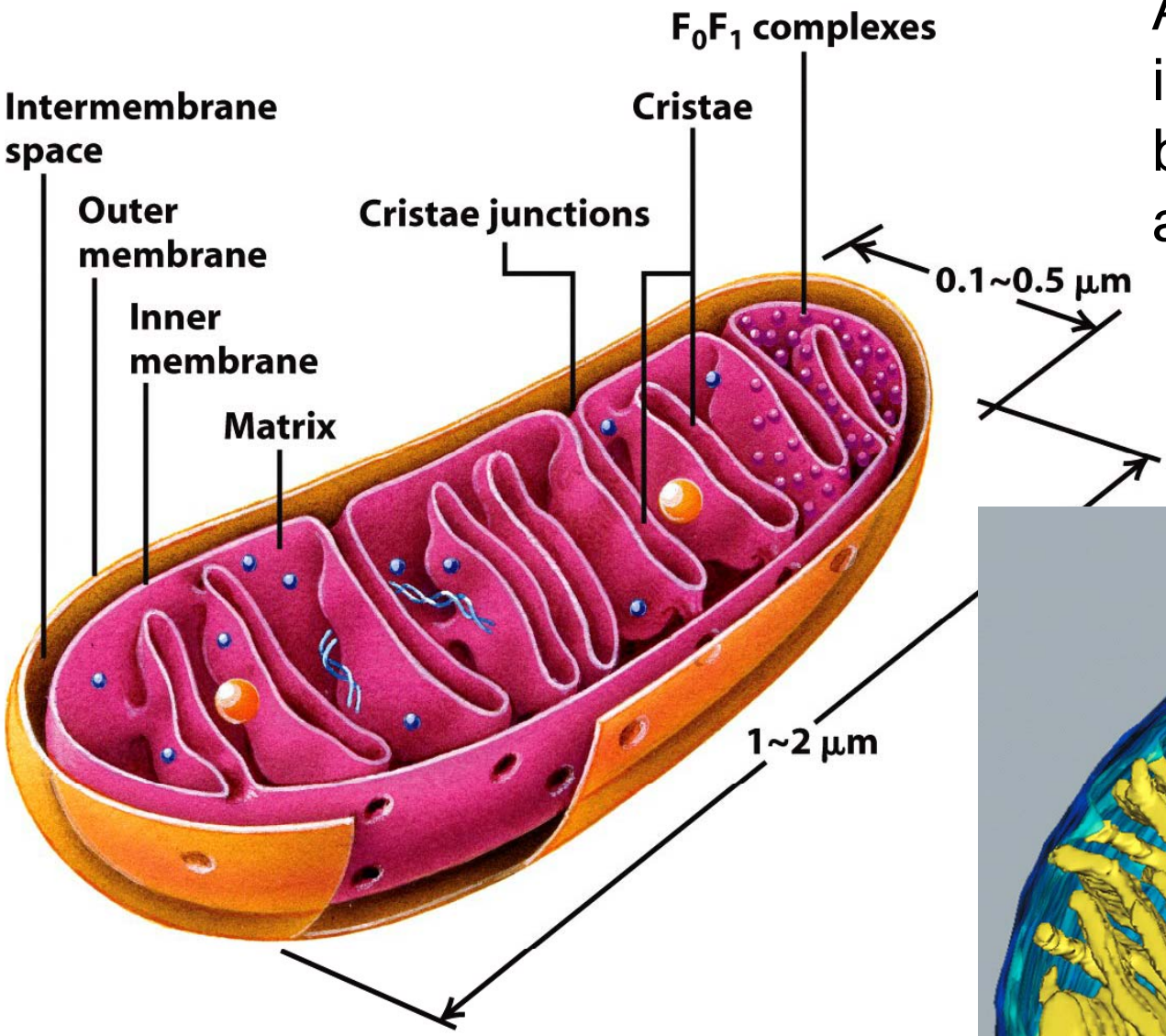
Vale, 2003, *Cell*

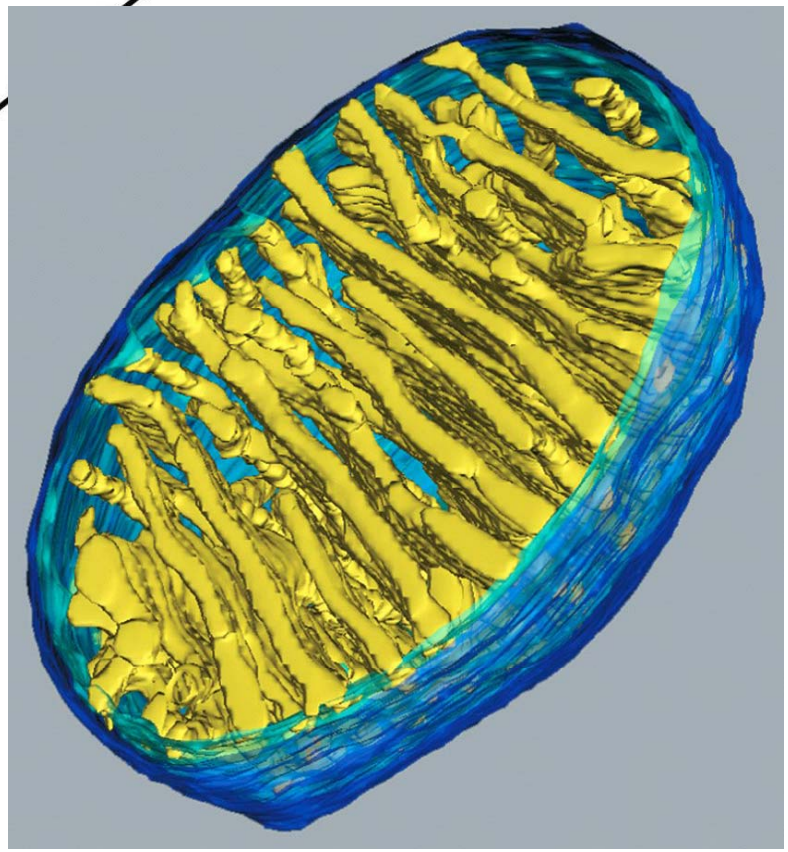
The cell has many choices of different motors depending on the specific need

- **Motor domains** = blue
- **Mechanical amplifiers** (neck linkers, lever arms) = light blue
- **Tail domains** = purple
- **Regulatory subunits** = green


A journey into the cell
Movie
Inner_short.mov

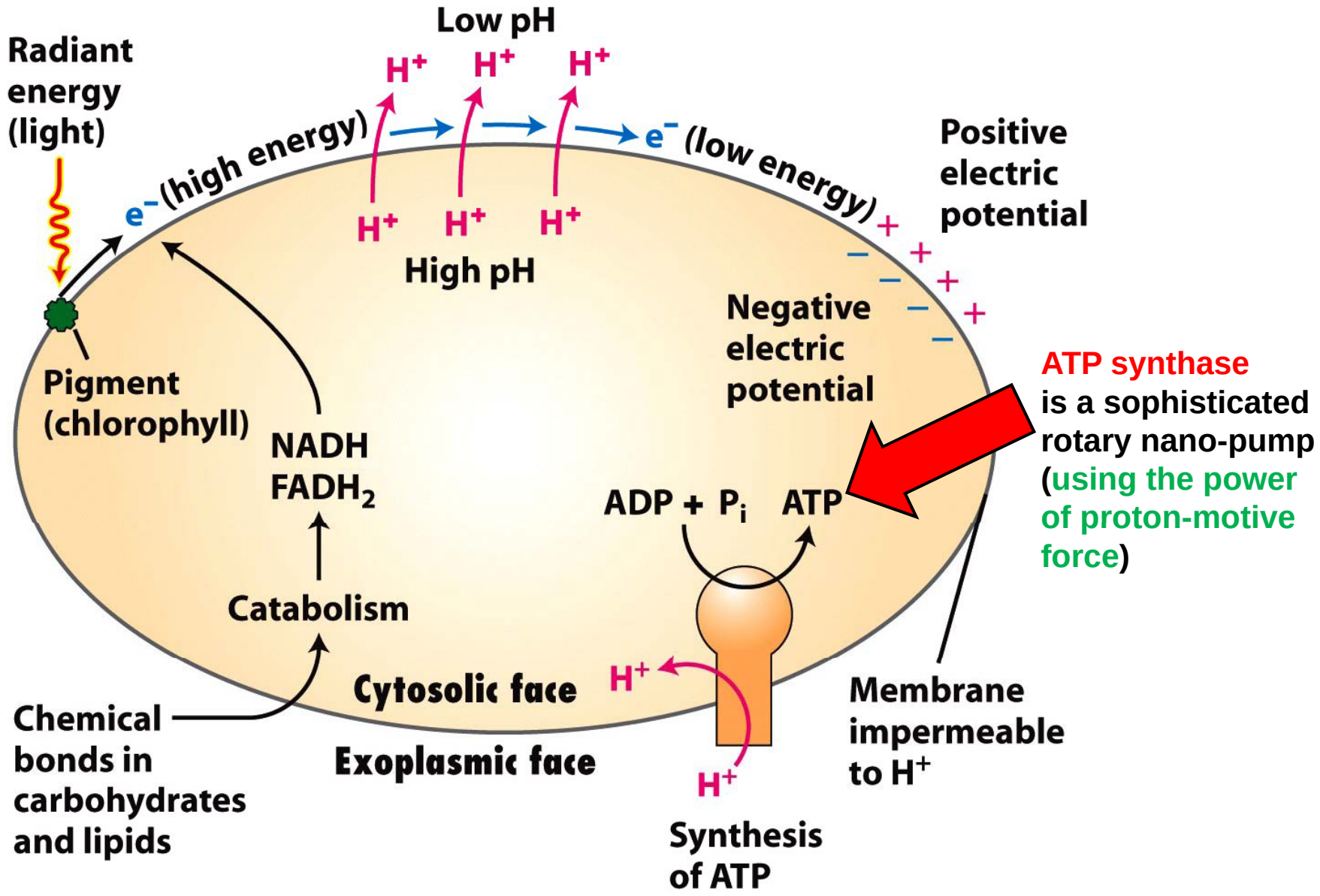
ATP is synthesized in the mitochondrion by degrading sugar and lipids



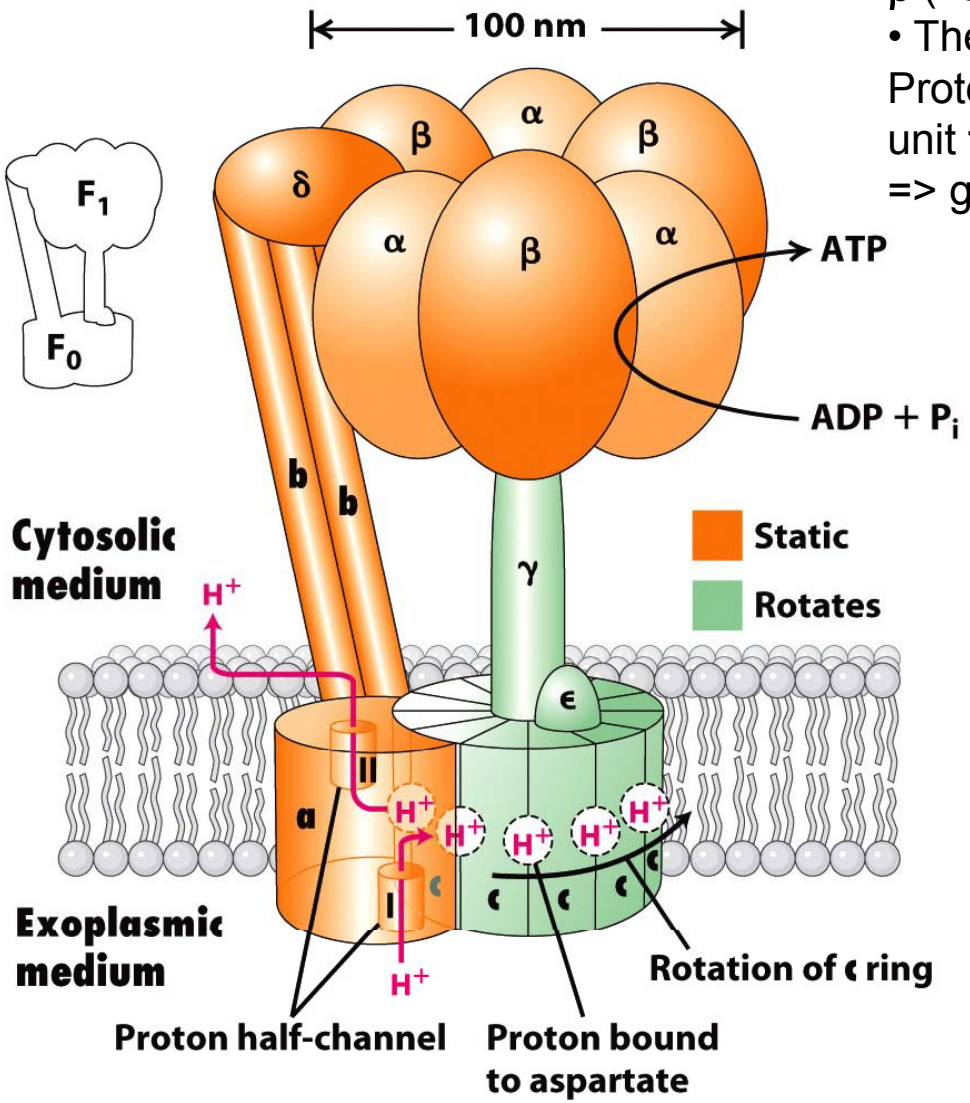


3D EM image of a mitochondrion (computer-generated from series of 2D EM images)

ATP is synthesized by a **rotary nano-pump** using the power of an proton gradient along the membrane (**proton-motive force**)



How does the ATP synthase (F_0F_1) work? *Example from your reading material!*



- ATPase consists of two major units: **F_0** and **F_1**
- **F_0** consists of subunits **a** (x1), **b** (x2) and **c** (x10)
- **F_1** consists of a **hexamer** composed of α (x3) and β (x3) subunits as well as of a γ, δ and ε subunits
- The **F_0** a-subunit contains two proton half-channels: Proton **channel I** guides a proton to a **c**-subunit => unit **turns** => proton of a preceding unit is released => guided thru half-**channel II** (released into cytosol)

- The δ **subunit** permanently links the hexamer to the F_0 unit
- Rotation of the c-subunit (and thus the connected γ subunits) causes a conformational change in the β subunits that **catalyzes ATP synthesis**
- The ATPase can make 400 ATPs per second! (134 rotations per second; one rotation needs 10 protons)

Animation
14_1_ATP_synthase.mov

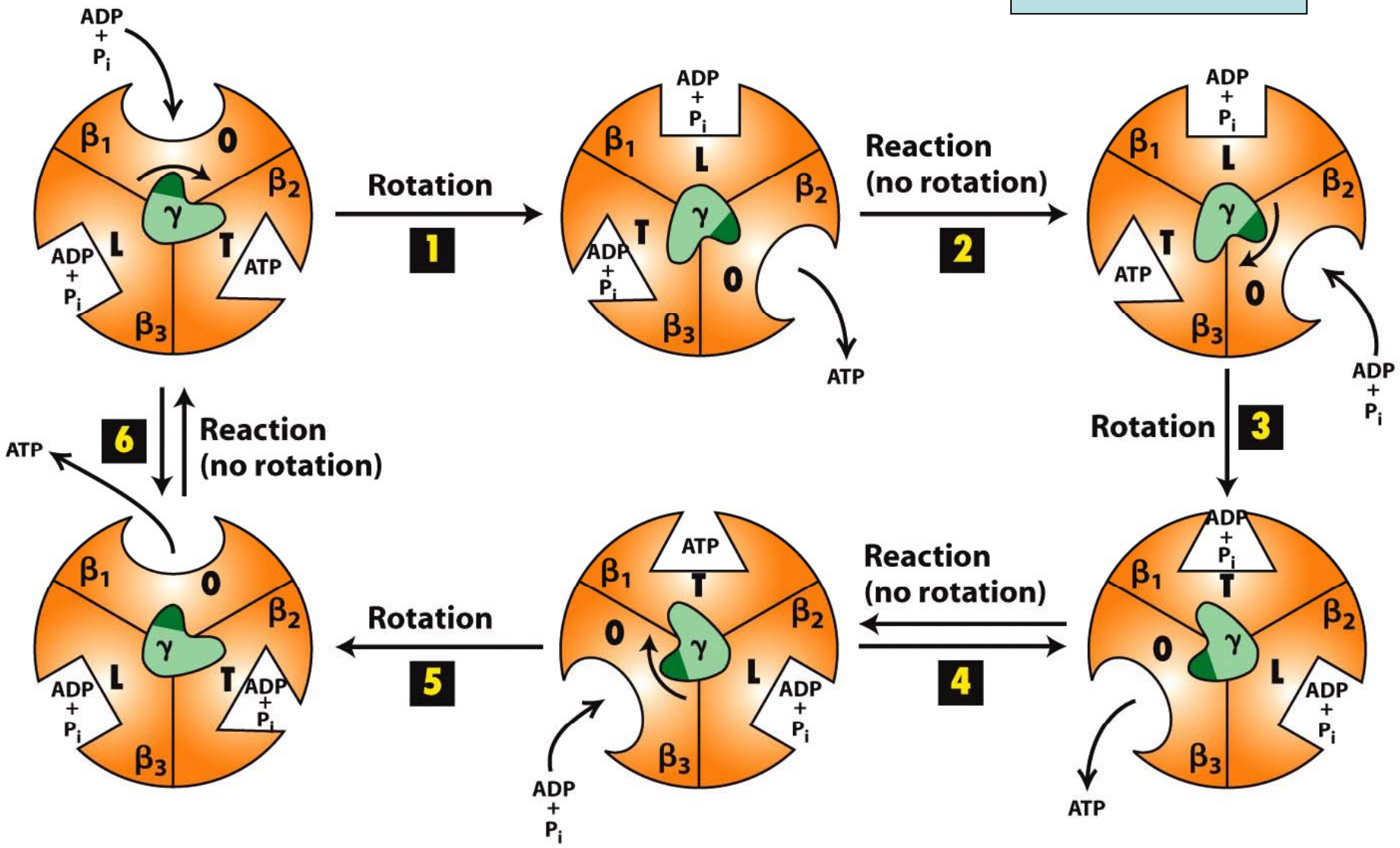
• Because the rotating F_0 γ subunit is **asymmetric**, it pushes differently to the F_1 β subunit which thus can appear in **3 different conformations**: O, L and T

• **O (*open*) stage** binds **weakly** either ADP+Pi (or ATP)

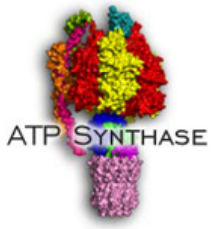
• **L (*loose*) stage** binds **strongly** ADP+Pi

• **T (*tight*) stage** favors the **chemical reaction** ADP+Pi => ATP

Animation
1203_ATP_synthesis.swf



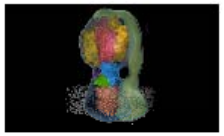
http://www.mrc-dunn.cam.ac.uk/research/atp_synthase/movies.php



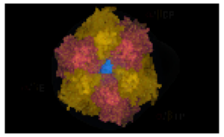
Animation
2_spheretop.mov

Movies

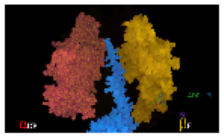
These movies were created by Said Sannuga in collaboration with John Walker and Andrew Leslie.



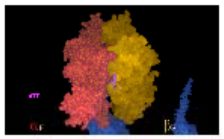
The rotary mechanism of mitochondrial ATP synthase. (12 Mb)



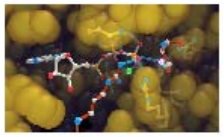
View from above and then below the F_1 domain along the rotating γ-subunit. (8.2 Mb)



How the rotating γ-subunit imposes the conformational states on a β-subunit required for substrate binding, ATP formation and ATP release. (4.5 Mb)



Three conformations of a catalytic β-subunit produced by 120º rotations of the central γ-subunit. (2.5 Mb)



Changes in the positions of sidechains in the catalytic site of F_1-ATPase bringing about binding and subsequent hydrolysis of ATP. (8.9 Mb)

> ATP Synthase Home
> Subunit Composition
> Rotary mechanism
> Structural analysis
> Current projects
> Group Leader - Sir John Walker
> Collaborators
> Current members
> Vacancies
> Recent publications

MRC Dunn Human Nutrition Unit

14.2 ATP SYNTHASE—DISCO

Subunits:
- Center (gamma subunit): **Toyoki Amano**
- Left (beta subunit 1): **Hiroyuki Noji**
- Right (beta subunit 2): **Satoshi P. Tsunoda**
- Back (beta subunit 3): **Masaki Shibata**

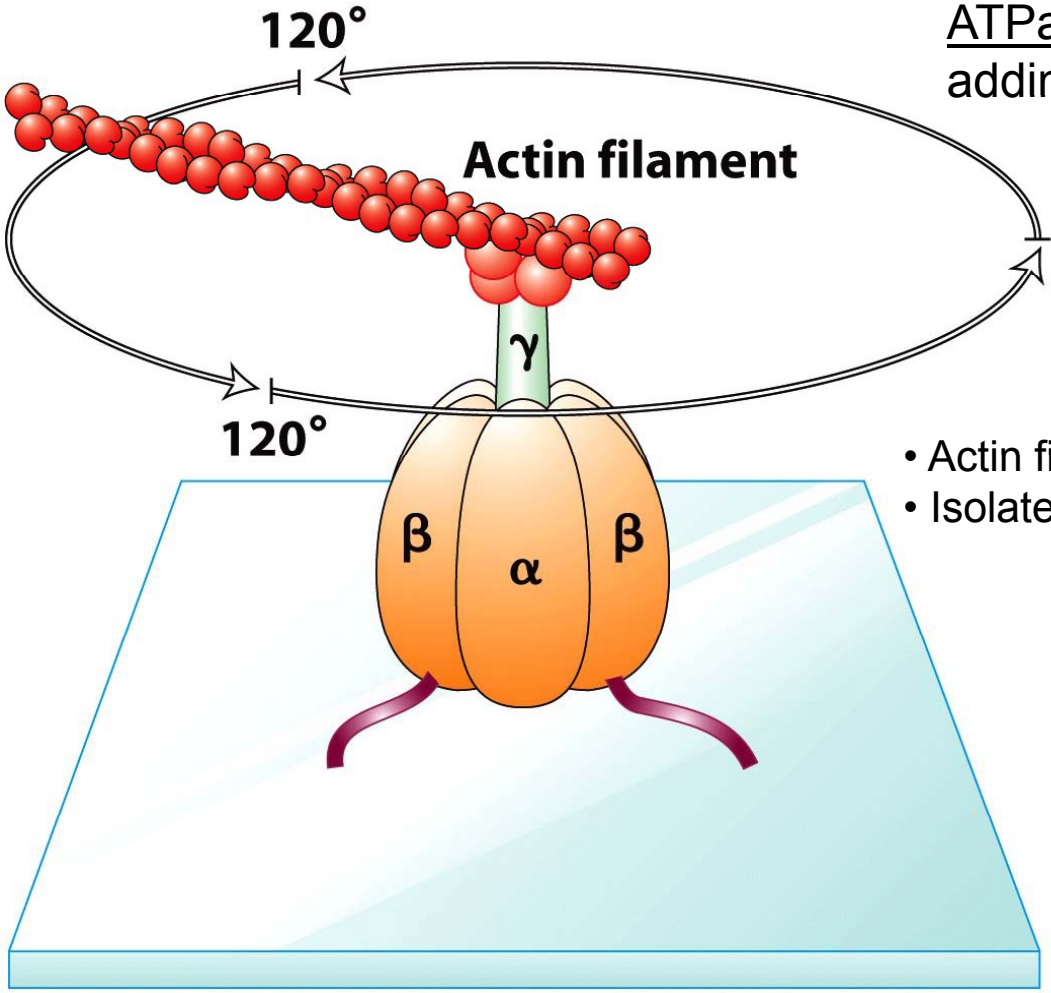
Dance direction:
- **Nagatsuta Bon-Odori**

Camera work and production:
- **Hiroyuki Noji**

Movie
14_2_ATP_synthase_disco.mov

Noji et al., 1997, ***Nature***
Yasuda et al., 1998, ***Cell***

Simple, but amazing experiment: **Making the rotation** of the ATPase **visible** (in nature and <u>real-time</u>) by sticking an actin polymer to the γ-subunit of the F_1 complex.

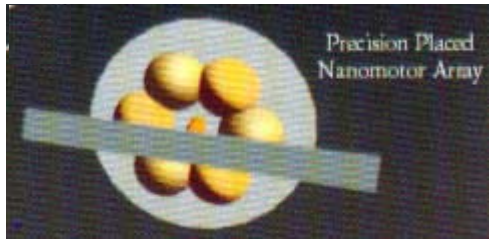


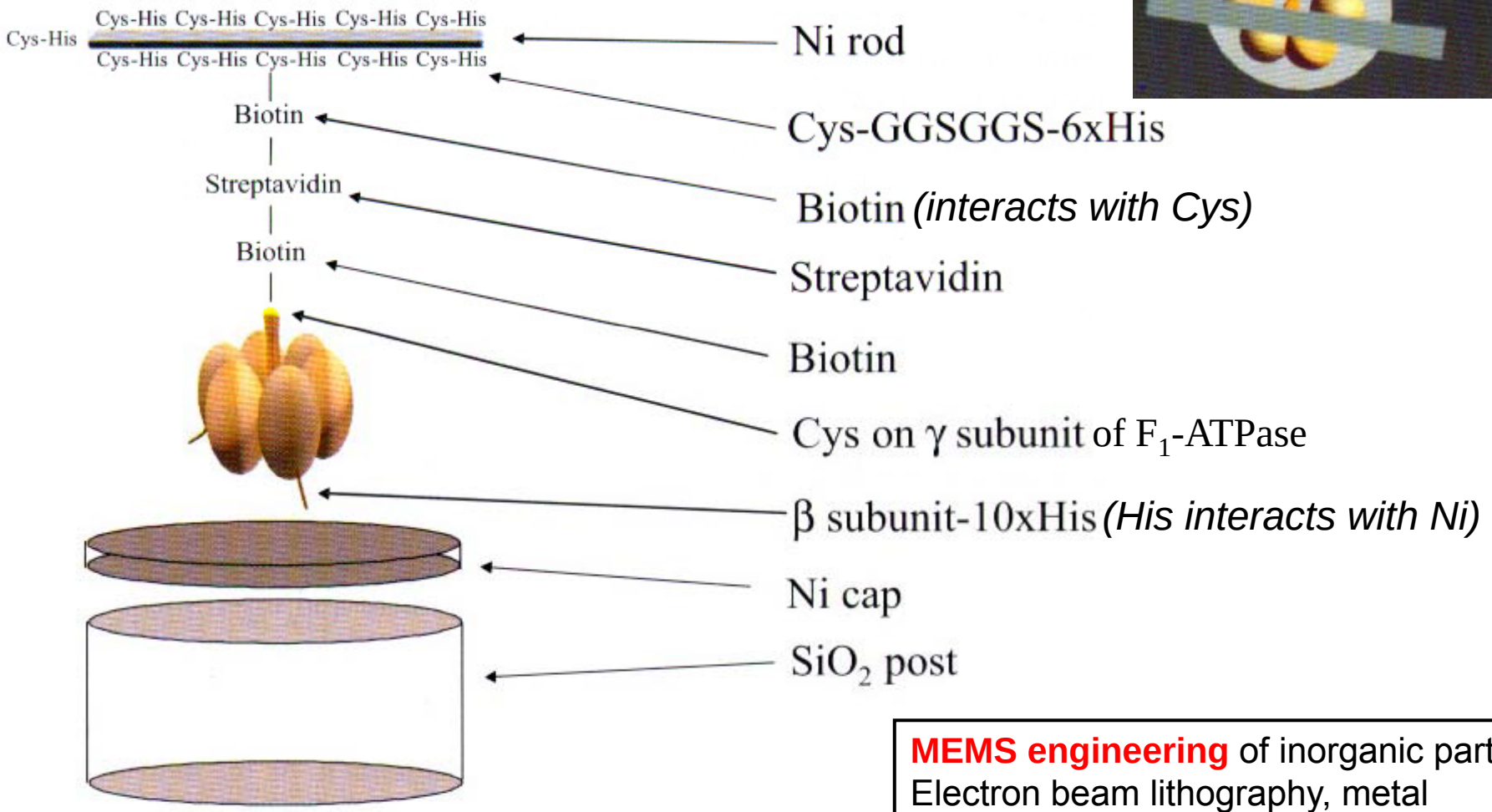
<u>ATPase works reversible</u>:
adding ATP makes it rotate

- Actin filament was fluorescently labeled
- Isolated F_1 complex adheres to a glass slide

Movie
1203_ATP_synthase_actin.mov

A hybrid nanodevice (nanopropeller)



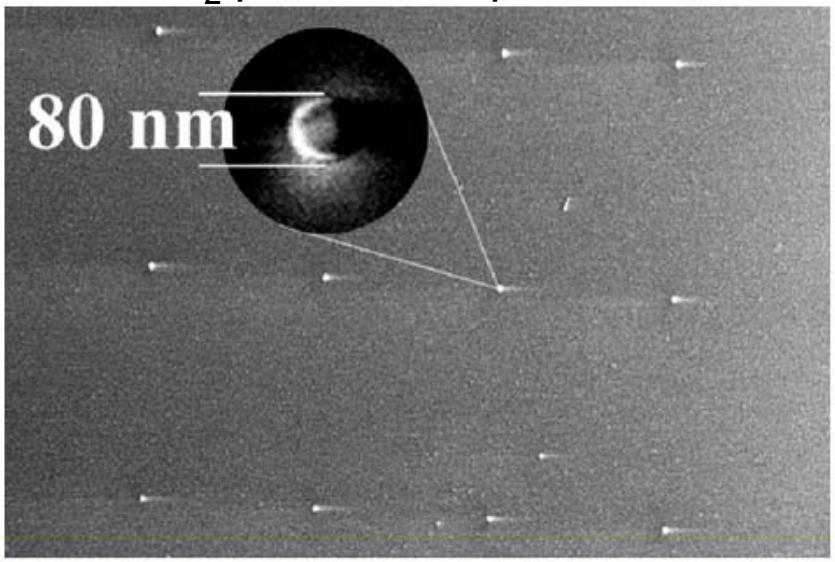


- **Biotin** (= Vitamin H) **binds strongly to** the protein **streptavidin** (strongest known ligand-protein interaction: K_D 10^{-15} = *almost covalent properties*)
- Negatively charged His binds to positively charged Ni
- **Biotin** strongly **interacts with cys-residues**

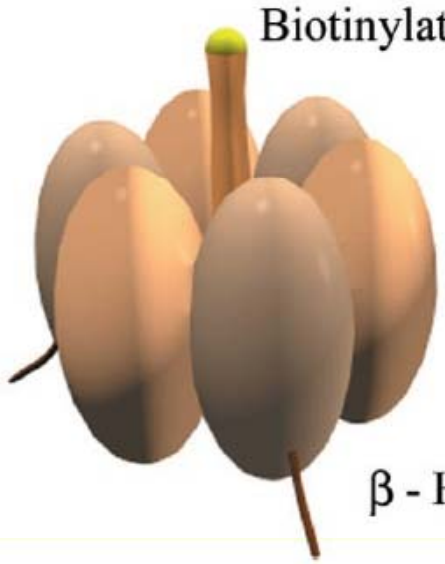
MEMS engineering of inorganic parts: Electron beam lithography, metal evaporation, reactive ion etching
Protein engineering: Recombinant DNA technology to add 10x His on β subunit and Cys on γ subunit of F_1-ATPase

Nanofabrication of single parts for the motor: *Example from your reading material!*

A SiO_2 post + Ni cap

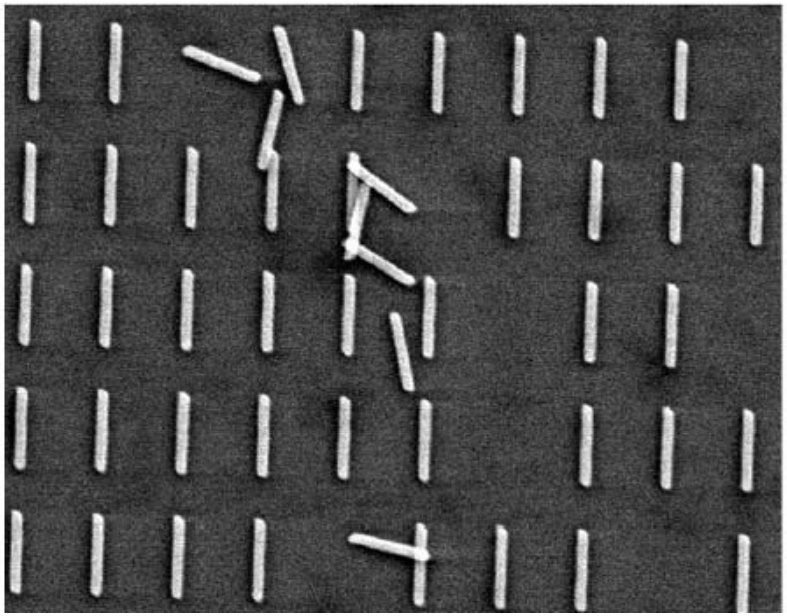


B

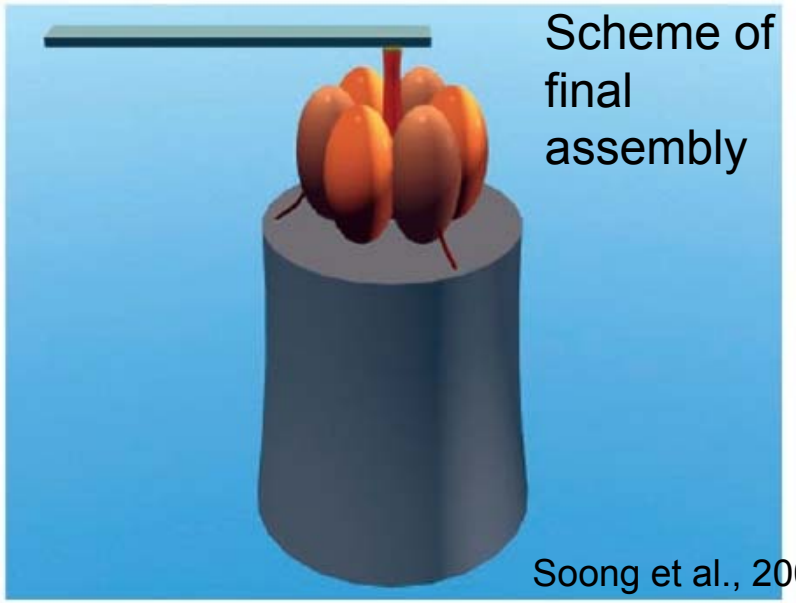


With tags (interfaces) modified motor

C Nanopropeller

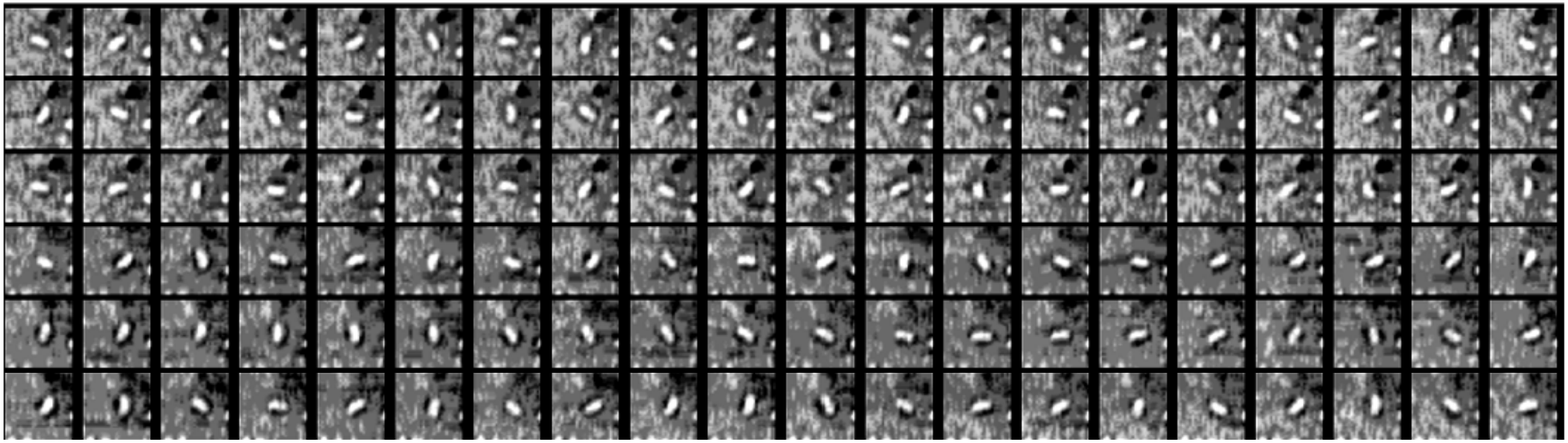


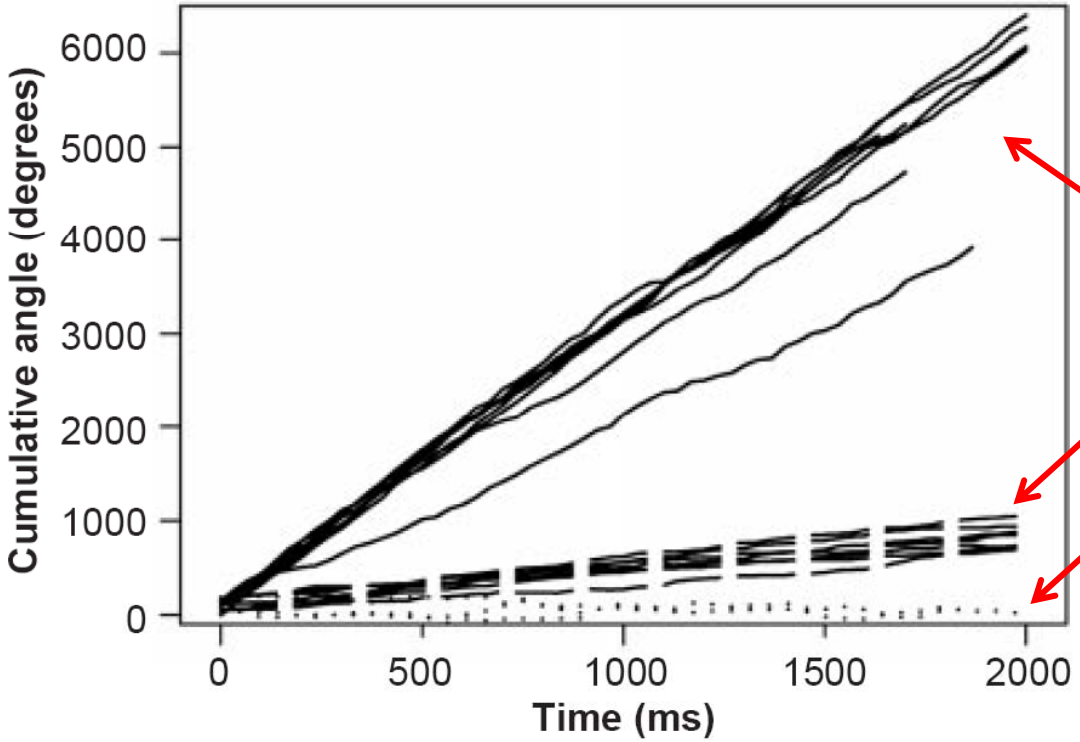
D



Soong et al., 2000, *Science*

Real-time recording of nanopropeller rotation





Propellers rotated for almost 2.5 hours

750 nm long propeller

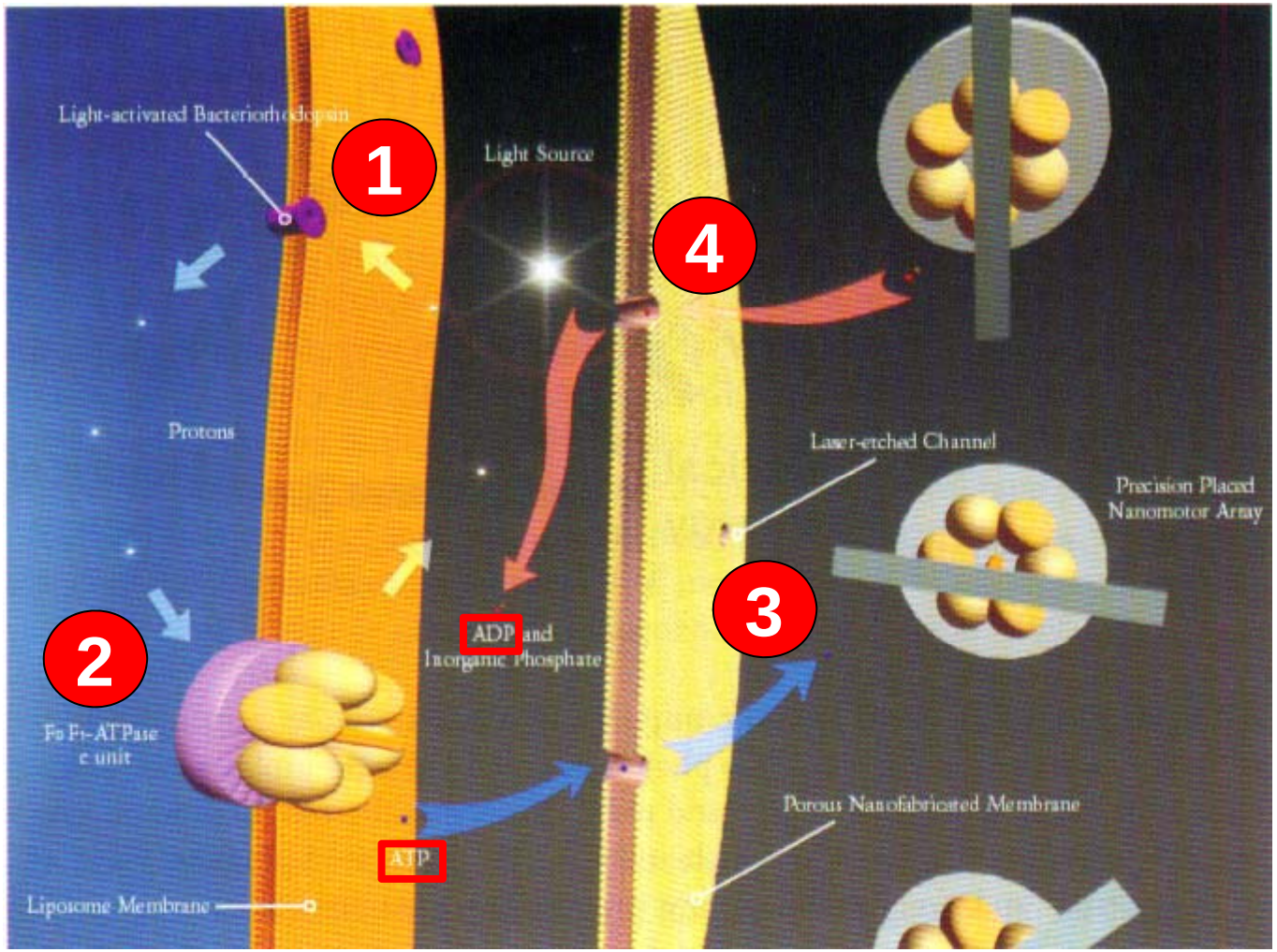
1400 nm long propeller

1400 nm long propeller + sodium azide (NaN_3)

Soong et al., 2000, *Science*

A self-fueled hybrid nano-device: *Example from your reading material!*

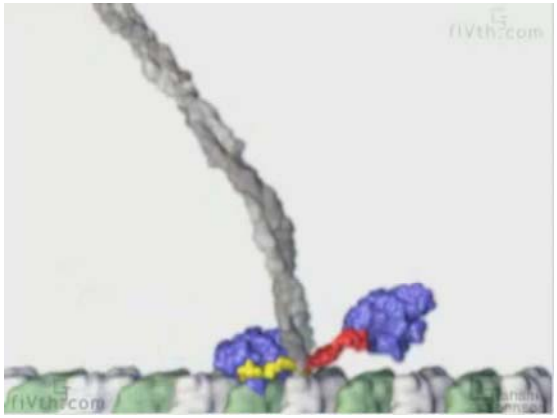
ATP-regenerating system using **bacteriorhodopsin** (BR), **light** and a **ATP-synthase**

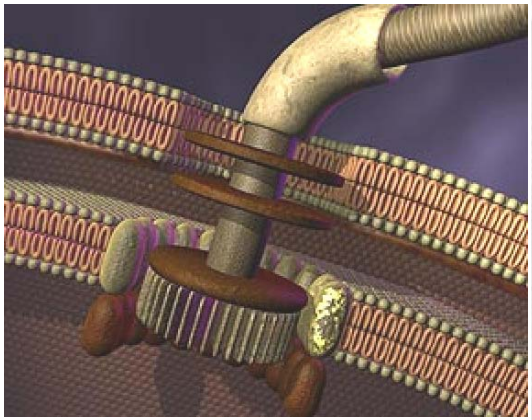


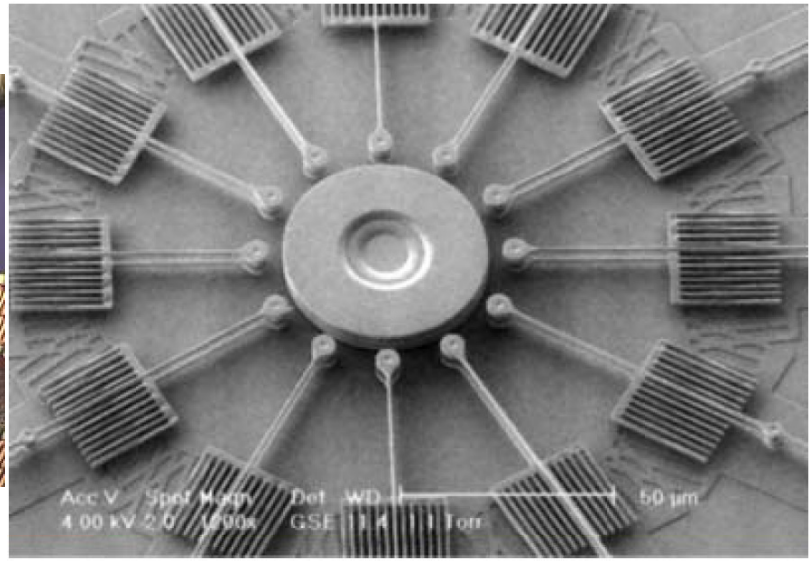
1 BR pumps H^+ after absorption of *photons*
2 ATPase uses proton-gradient to produce ATP (from $ADP{\cdot}P_i$)
3 ATP powers **hybrid nanodevice**
4 $ADP{\cdot}P_i$ diffuses thru porous nanofabricated membrane back to ATPase

Schliwa, Molecular Motors, 1st Ed.

Thank you for your attention!







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