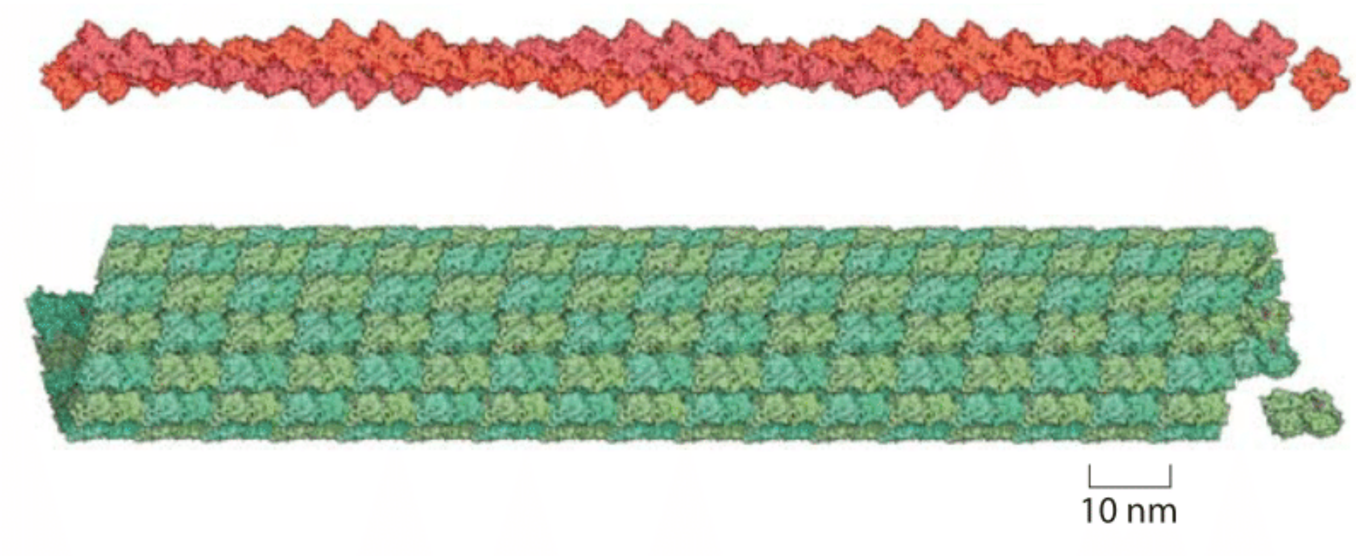
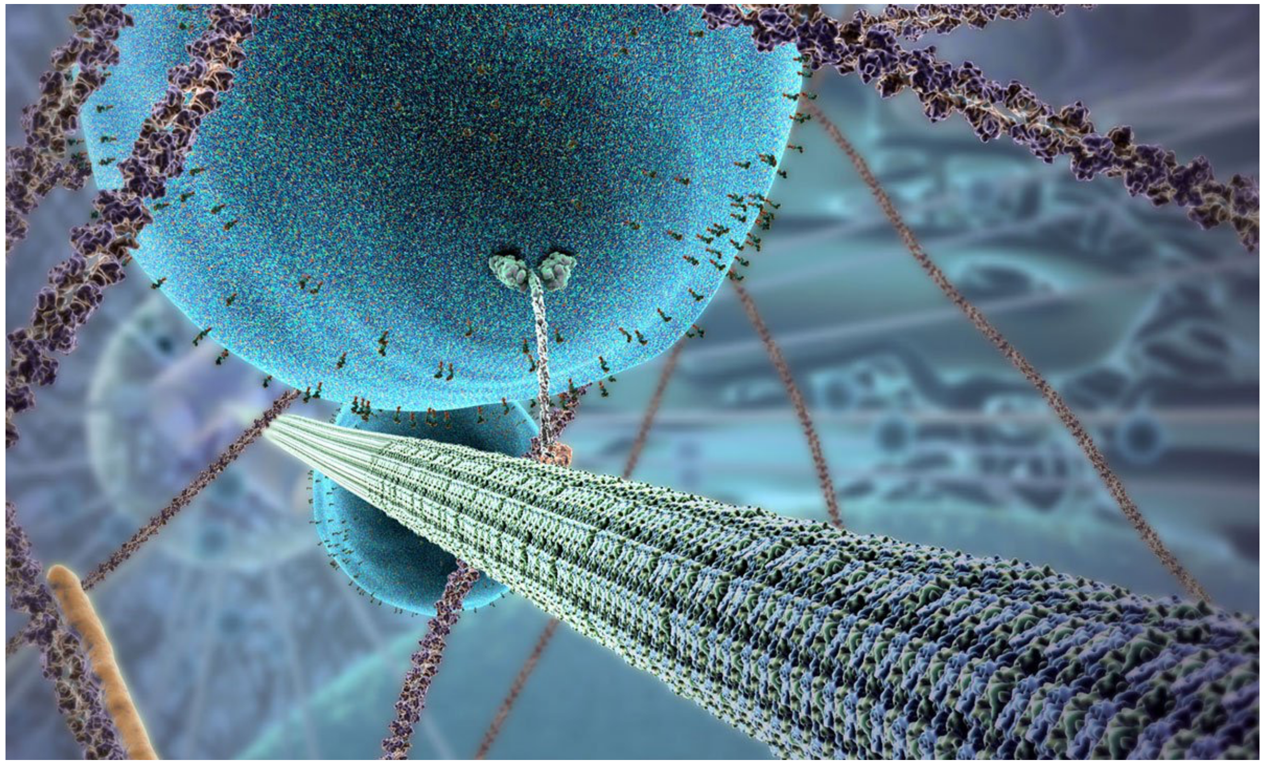
MAE 545: Lecture 20 (5/2)

Growth dynamics of actin filaments and microtubules



Dynamics of molecular motors



Actin filaments

$7\mathrm{nm}$

actin monomer

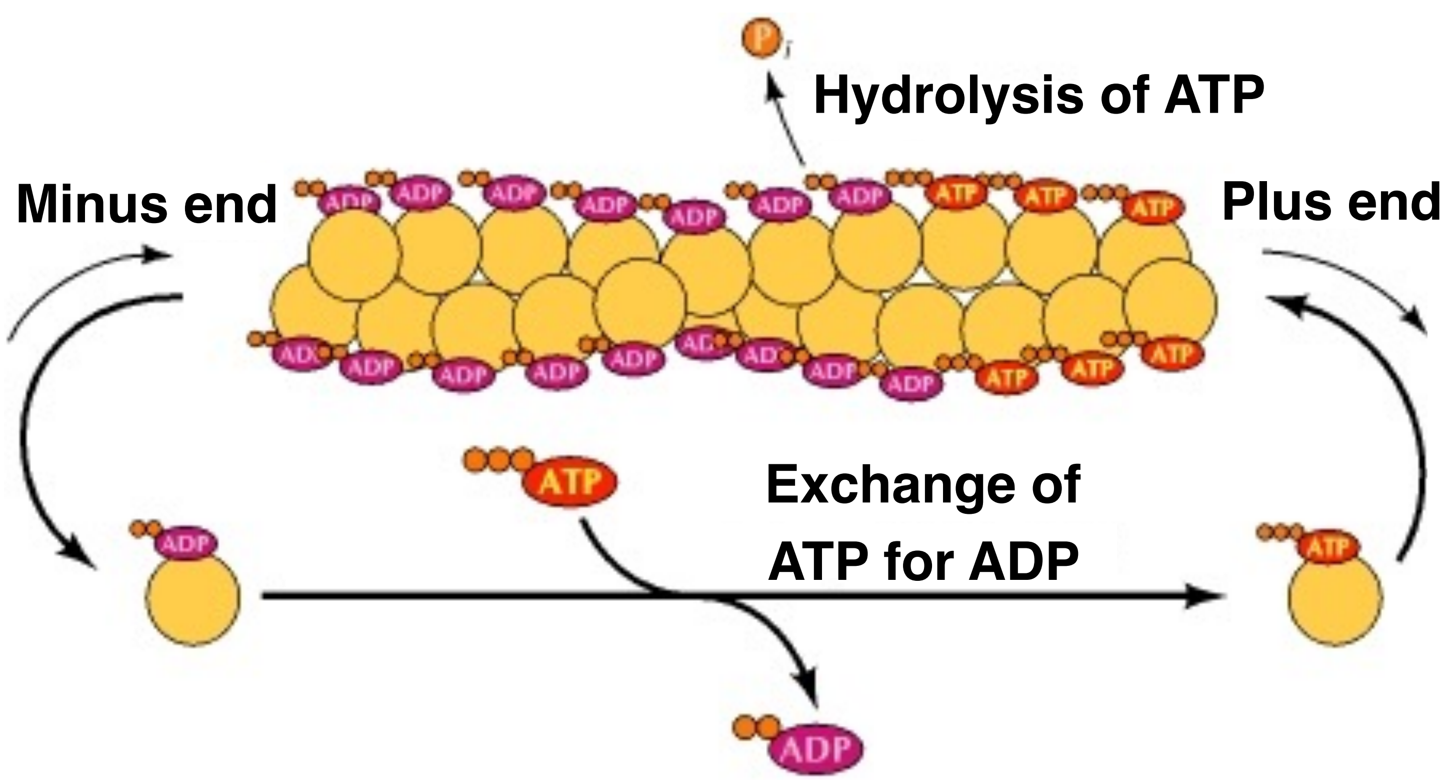
Minus end (pointed end)

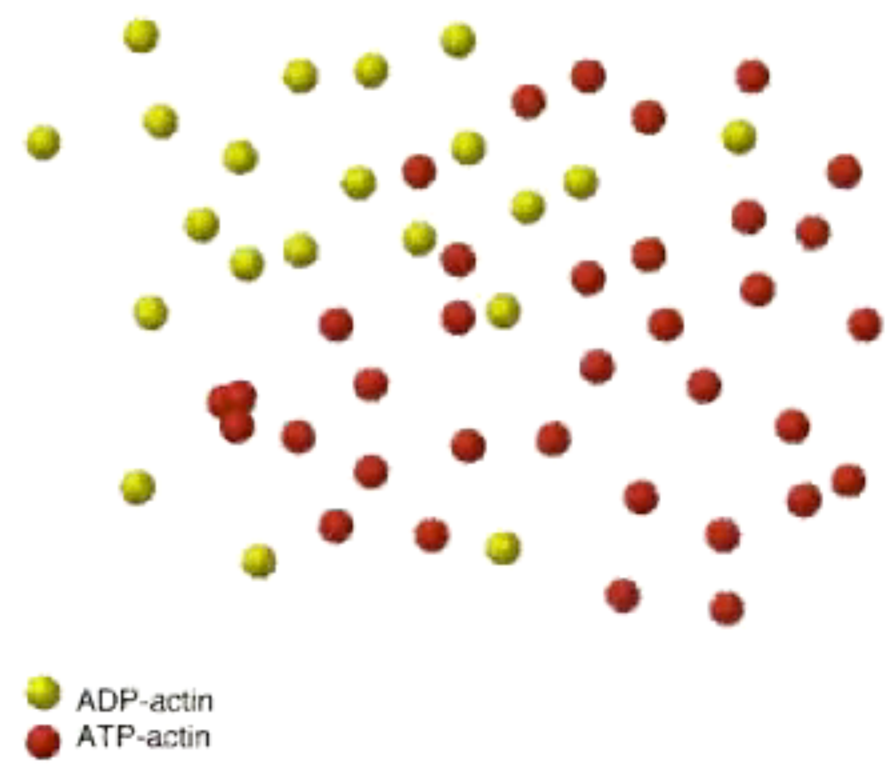
Plus end (barbed end)

Persistence length $\ell_p \sim 10\mu\mathrm{m}$

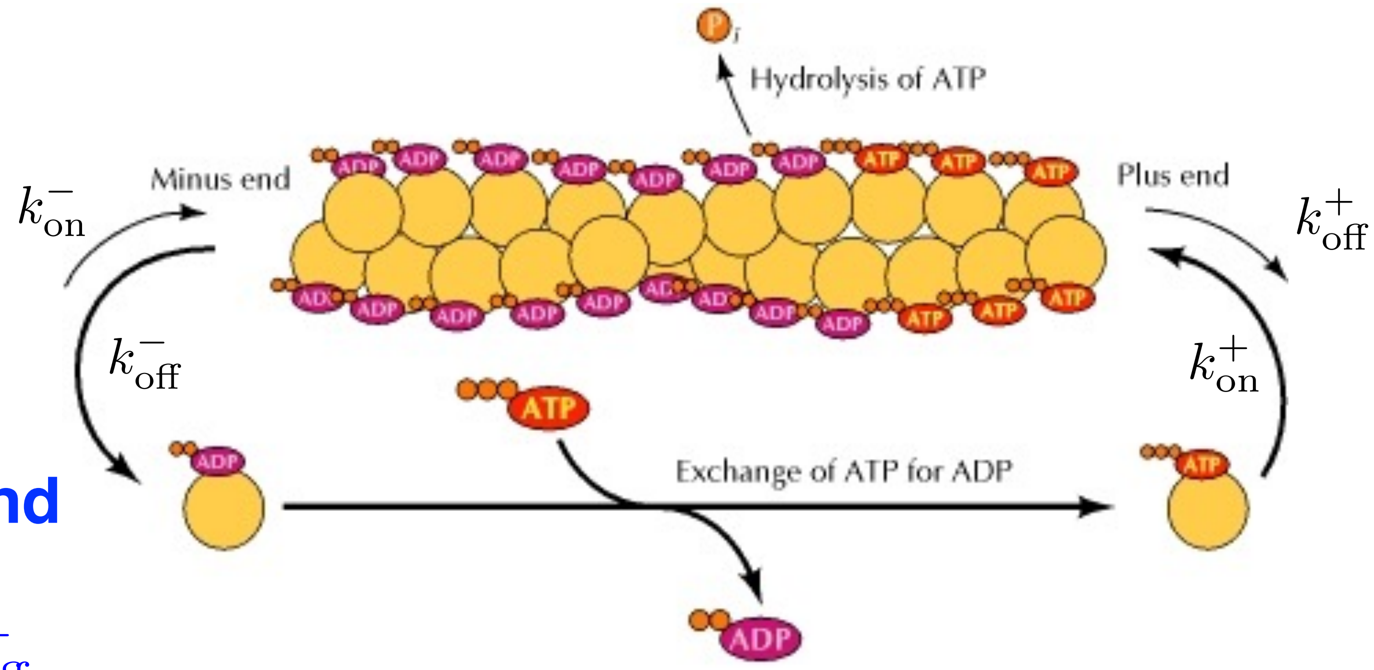
Typical length $L \lesssim 10\mu\mathrm{m}$

Actin treadmilling





Actin growth



growth of minus end

$$\frac{dn^-}{dt} = k_{\text{on}}^-[M] - k_{\text{off}}^-$$

no growth at

$$[M]_c^- = \frac{k_{\text{off}}^-}{k_{\text{on}}^-}$$

growth of plus end

$$\frac{dn^+}{dt} = k_{\text{on}}^+[M] - k_{\text{off}}^+$$

no growth at

$$[M]_c^+ = \frac{k_{\text{off}}^+}{k_{\text{on}}^+}$$

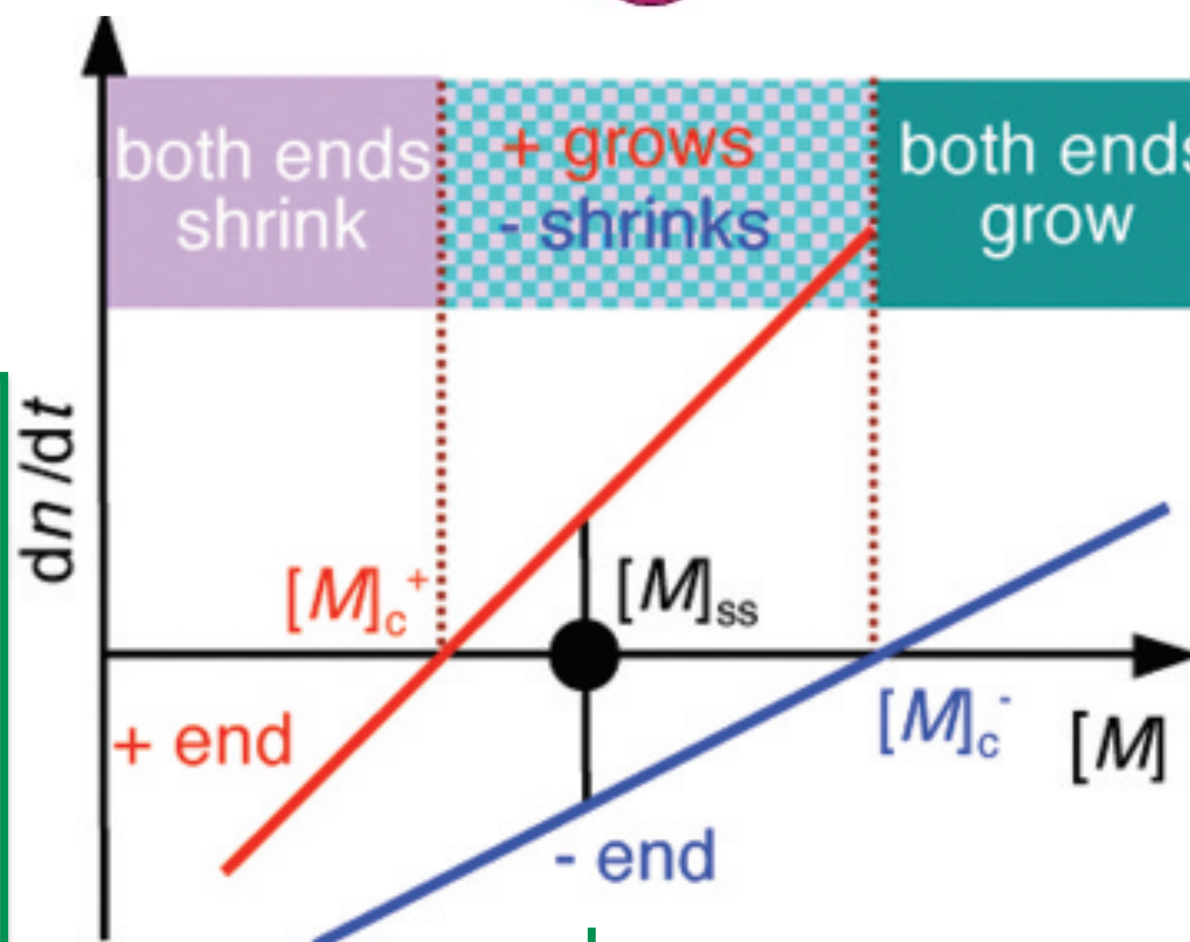
Steady state regime

$$\frac{dn^+}{dt} = -\frac{dn^-}{dt}$$

$$[M]_{\text{ss}} = \frac{k_{\text{off}}^+ + k_{\text{off}}^-}{k_{\text{on}}^+ + k_{\text{on}}^-} \approx 0.17\mu\text{M}$$

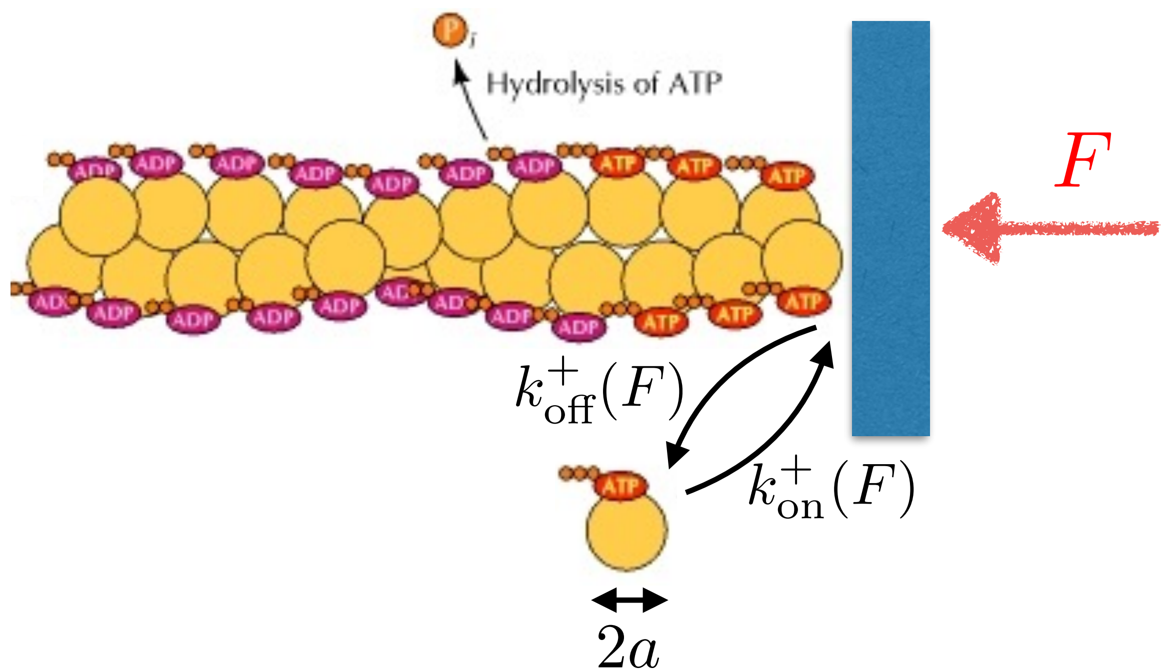
front speed

$$\frac{dn^+}{dt} = \frac{k_{\text{on}}^+ k_{\text{off}}^- - k_{\text{on}}^- k_{\text{off}}^+}{k_{\text{on}}^+ + k_{\text{on}}^-} \approx 0.6\text{s}^{-1}$$



actin shrinks | **actin grows**

Actin filament growing against the barrier



work done against the barrier for insertion of new monomer

$$W = Fa$$

effective monomer free energy potential without barrier

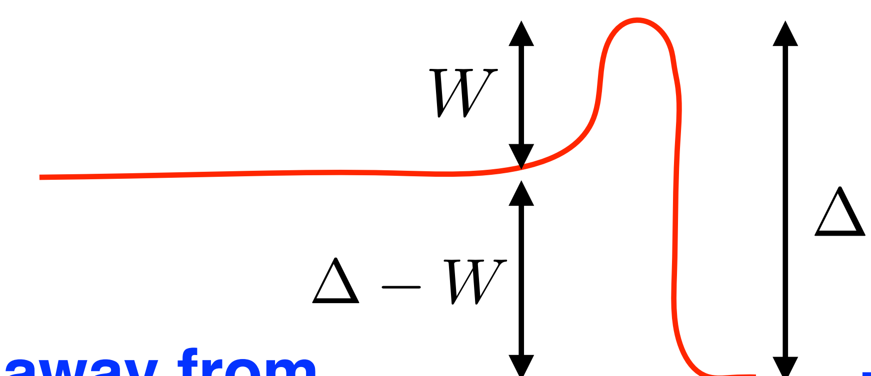
away from filament

attached to the tip

$$k_{\text{on}}^{+} \sim 4\pi D_3 a$$

$$k_{\text{off}}^{+} \propto e^{-\Delta/k_B T}$$

effective monomer free energy potential with barrier



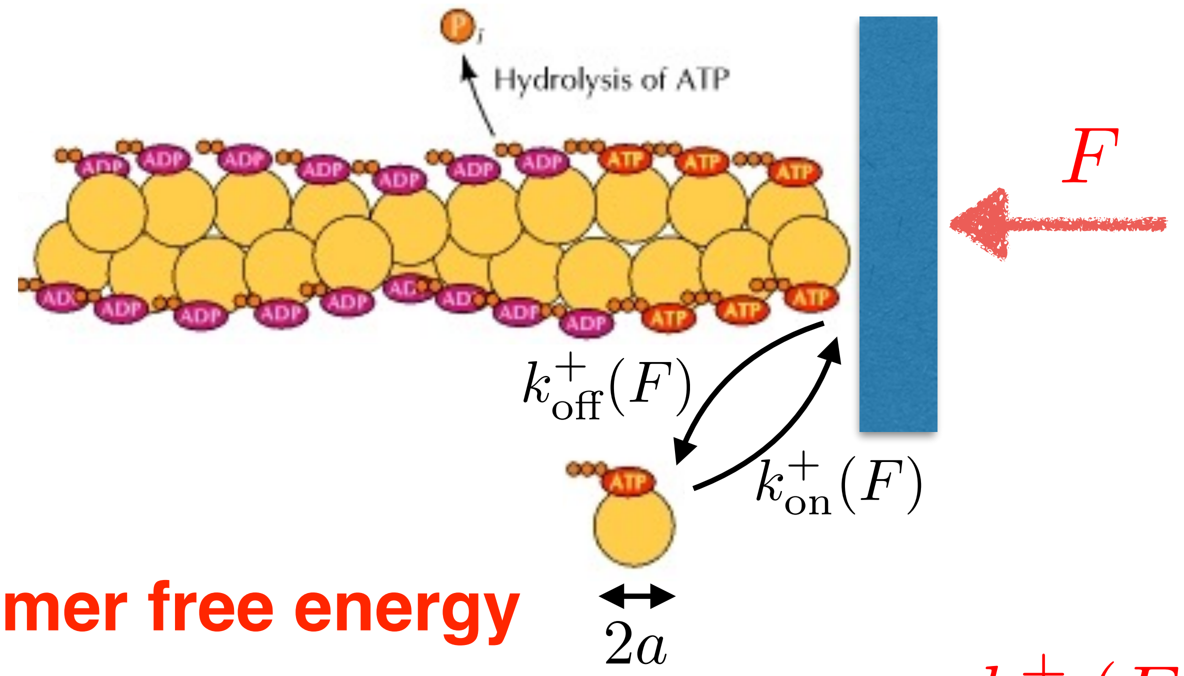
away from filament

attached to the tip

$$k_{\text{on}}^{+}(F) \sim k_{\text{on}}^{+} e^{-Fa/k_B T}$$

$$k_{\text{off}}^{+}(F) \sim k_{\text{off}}^{+}$$

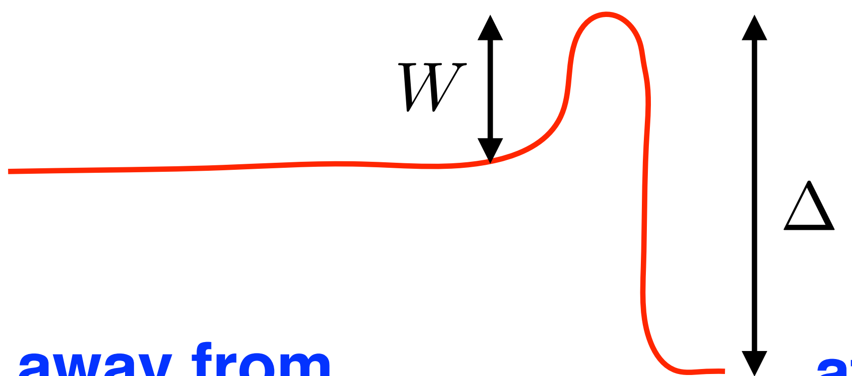
Actin filament growing against the barrier



work done against the barrier for insertion of new monomer

$$W = Fa$$

effective monomer free energy potential with barrier



away from filament

attached to the tip

$$k^+_{\text{on}}(F) \sim k^+_{\text{on}} e^{-Fa/k_B T}$$

$$k^+_{\text{off}}(F) \sim k^+_{\text{off}}$$

Growth speed of the tip

$$\boxed{v^+(F) = \frac{dn^+(F)}{dt} = k^+_{\text{on}}[M]e^{-Fa/k_B T} - k^+_{\text{off}}}$$

Maximal force that can be balanced by growing filament

(stall force)

$$\boxed{v^+(F_{\max}) = 0 \longrightarrow F_{\max} = \frac{k_B T}{a} \ln\left(\frac{k^+_{\text{on}}[M]}{k^+_{\text{off}}}\right)}$$

$k^+_{\text{on}} \sim 10\mu\text{M}^{-1}\text{s}^{-1}$

$k^+_{\text{off}} \sim 1\text{s}^{-1}$

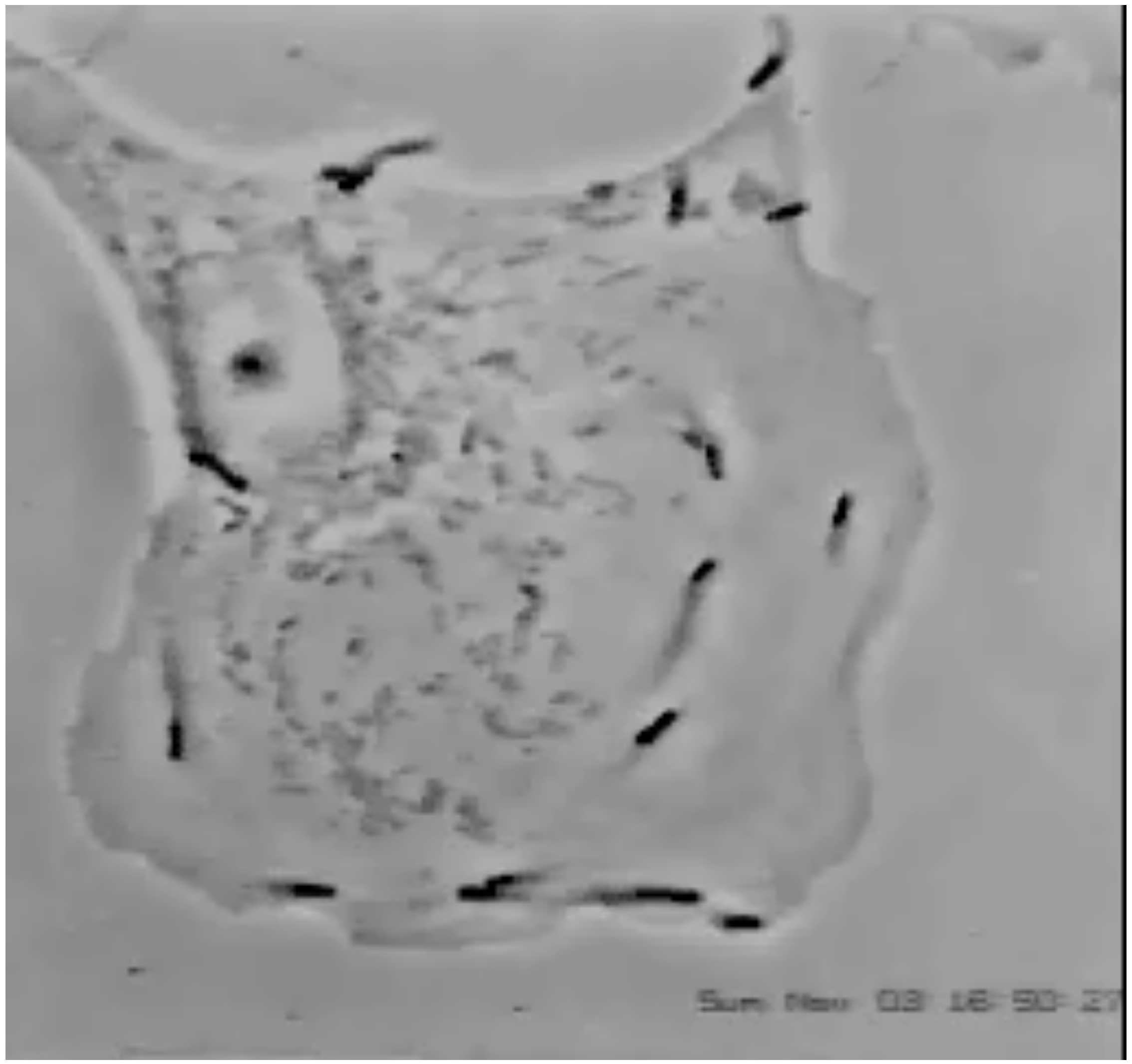
$[M] \sim 10\mu\text{M}$

$a \approx 2.5\text{nm}$

$F_{\max} \sim 8\text{pN}$

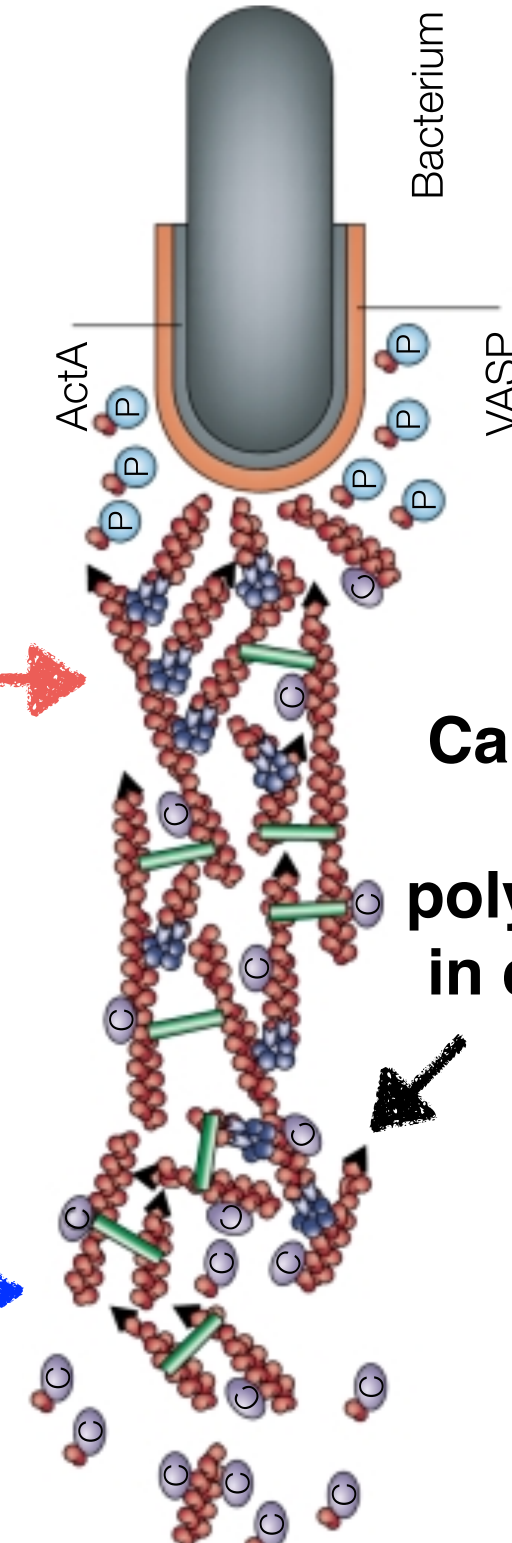
Movement of bacteria

Listeria monocytogenes
moving in infected cells



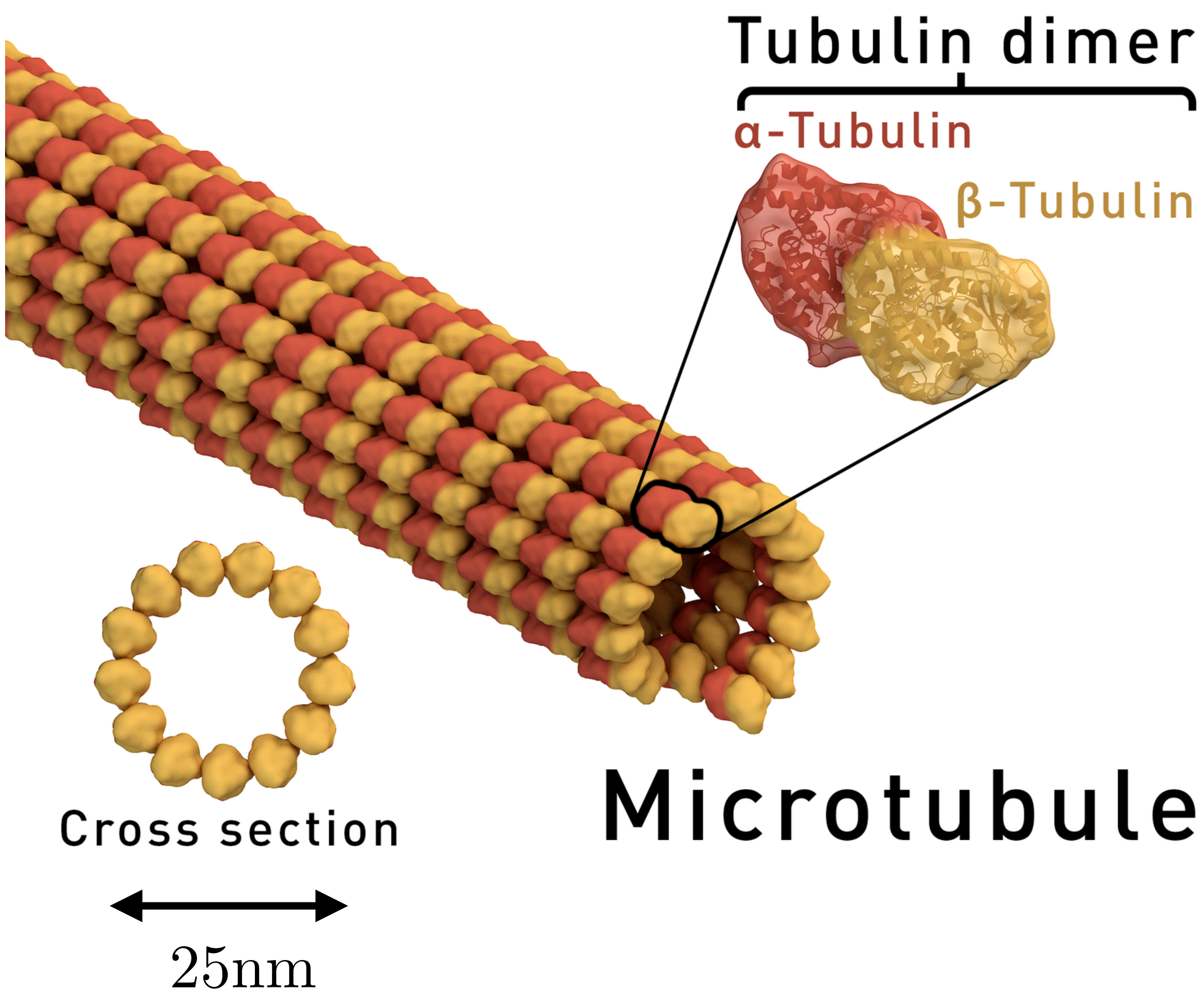
Julie Theriot (speeded up 150x)

$$v \sim 0.1 - 0.3 \mu\mathrm{m/s}$$



L. A. Cameron *et al.*,
Nat. Rev. Mol. Cell Biol. **1**, 110 (2000)

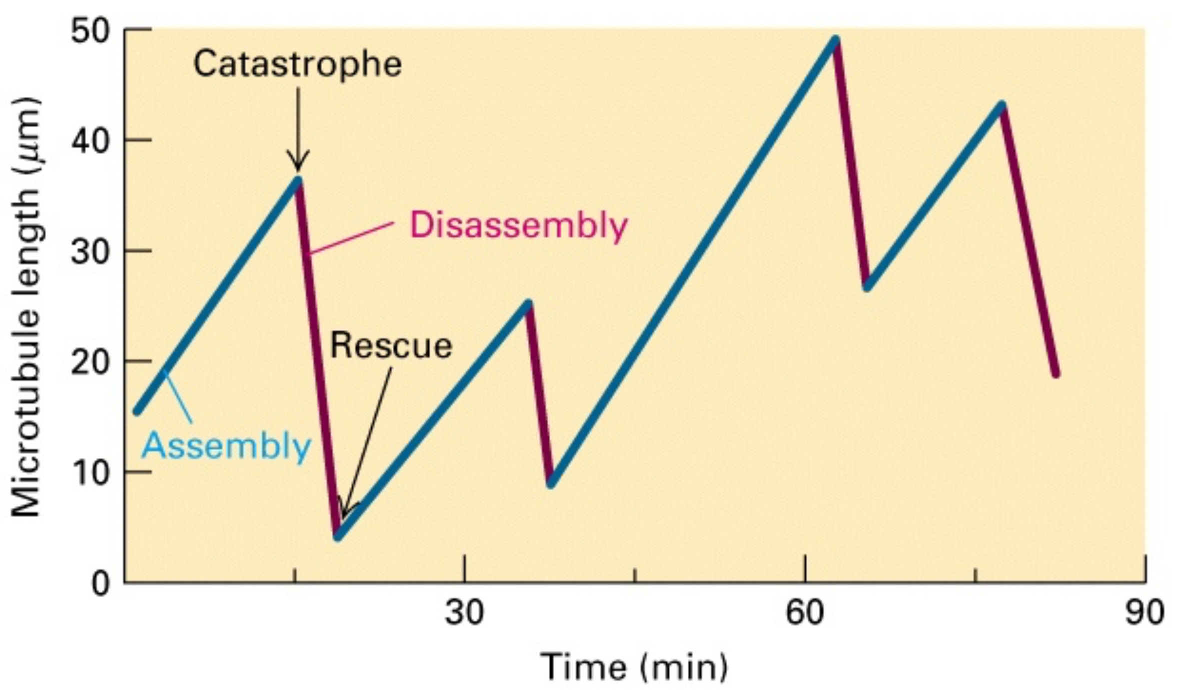
Microtubules

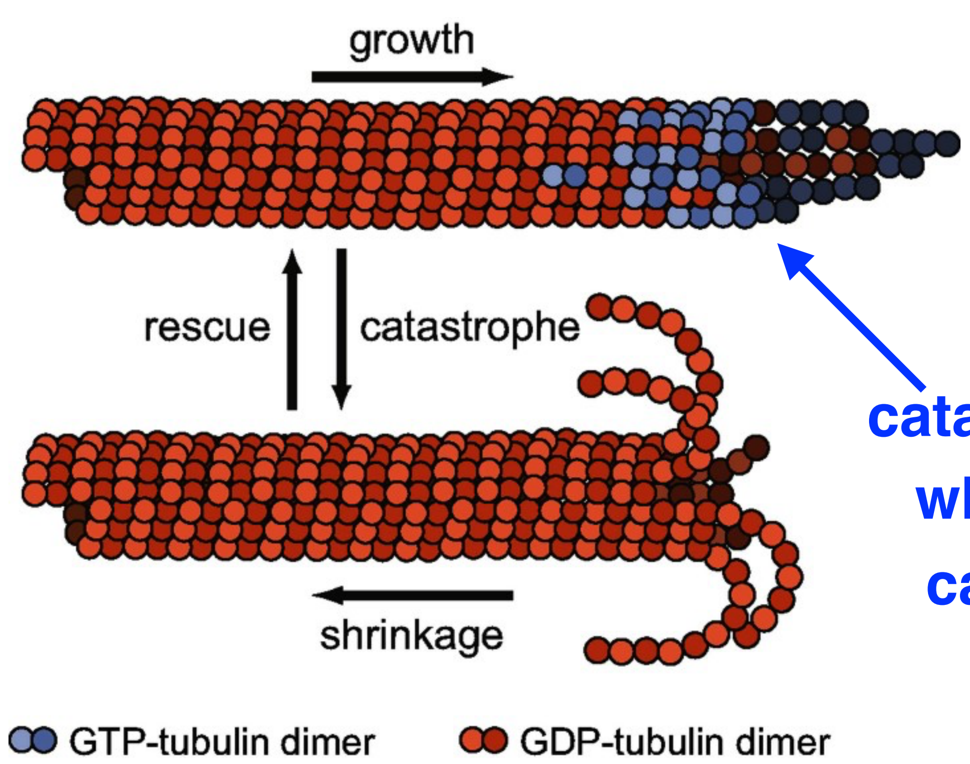


Persistence length $\ell_p \sim 1\text{mm}$

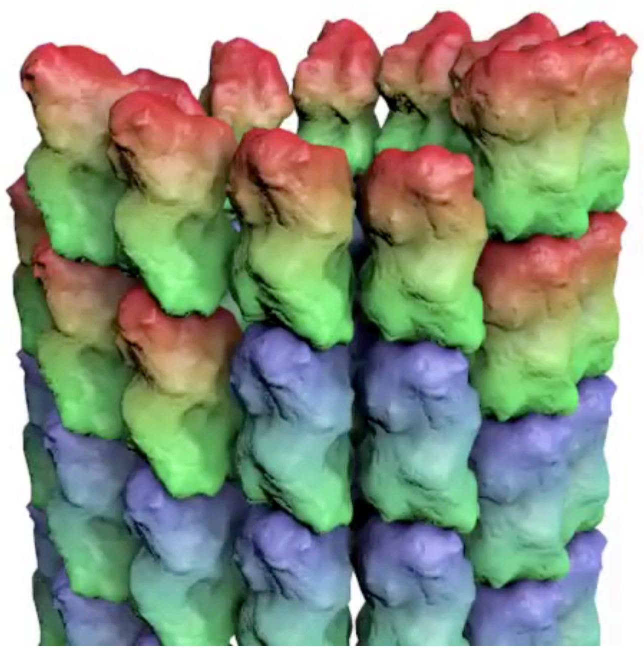
Typical length $L \lesssim 50\mu\text{m}$

Microtubule dynamic instability



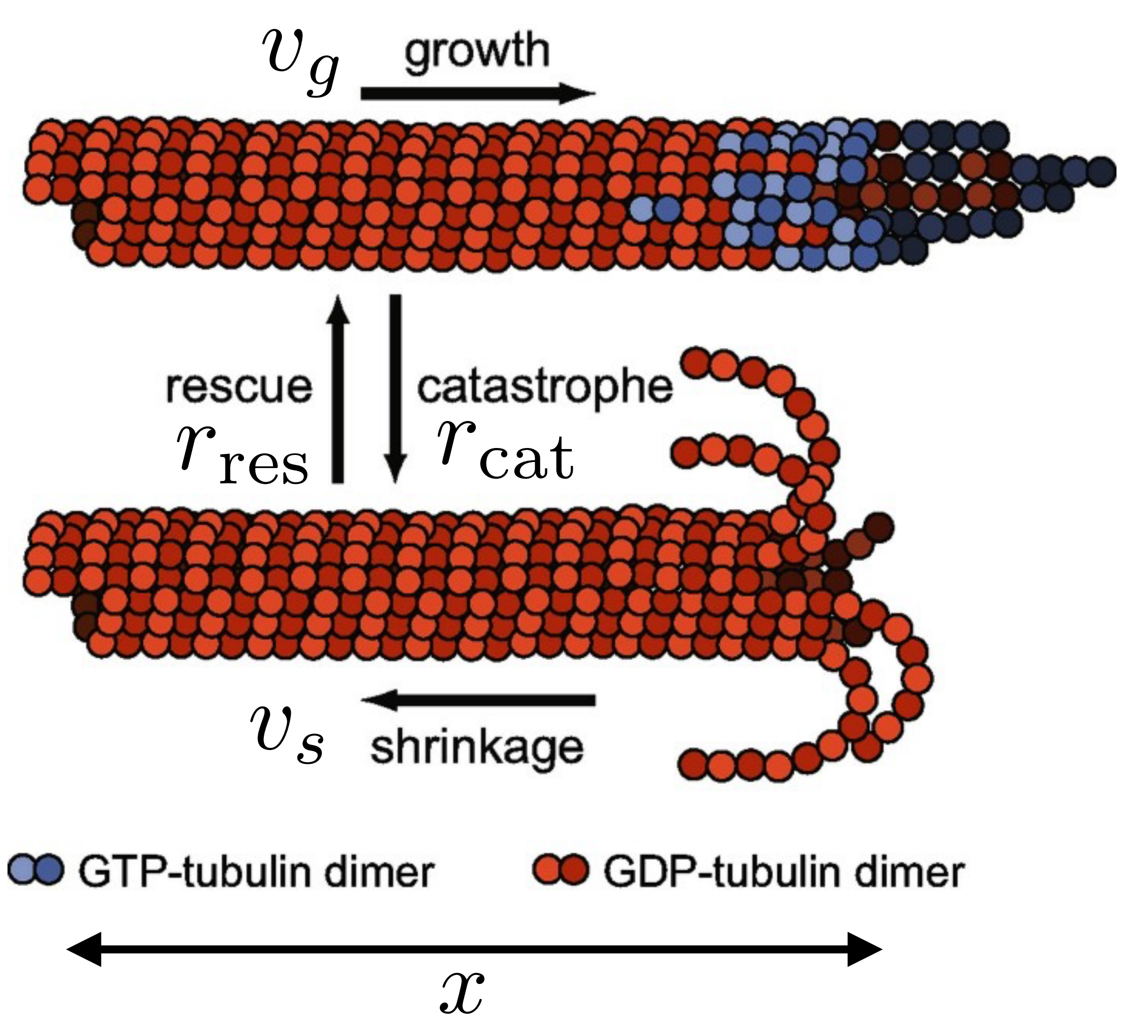


catastrophe occurs when protective cap disappears



Wikipedia

Simple model of microtubule growth



Let's ignore all molecular details and assume that microtubules switch at fixed rates between growing and shrinking phases

Master equation:

$$\frac{\partial p_{\text{growth}}}{\partial t} = -r_{\text{cat}}\, p_{\text{growth}} + r_{\text{res}}\, p_{\text{shrinking}}$$

$$\frac{\partial p_{\text{shrinking}}}{\partial t} = +r_{\text{cat}}\, p_{\text{growth}} - r_{\text{res}}\, p_{\text{shrinking}}$$

$$p_{\text{growth}} + p_{\text{shrinking}} = 1$$

Steady state ($\partial p / \partial t \equiv 0$):

$$\boxed{p^*_{\text{growth}} = \frac{r_{\text{res}}}{r_{\text{res}} + r_{\text{cat}}} \qquad p^*_{\text{shrinking}} = \frac{r_{\text{cat}}}{r_{\text{res}} + r_{\text{cat}}}}$$

Typical values in a tubilin solution of concentration $[\mathrm{T}] \approx 10\mu\mathrm{M}$:

$$v_g \approx 2\mu\mathrm{m/min} \qquad \propto [\mathrm{T}]$$

$$v_s \approx 20\mu\mathrm{m/min} \qquad \sim \mathrm{const}$$

$$r_{\text{cat}} \approx 0.24\mathrm{min}^{-1} \qquad \sim \mathrm{const}$$

$$r_{\text{res}} \approx 3\mathrm{min}^{-1} \qquad \propto [\mathrm{T}]$$

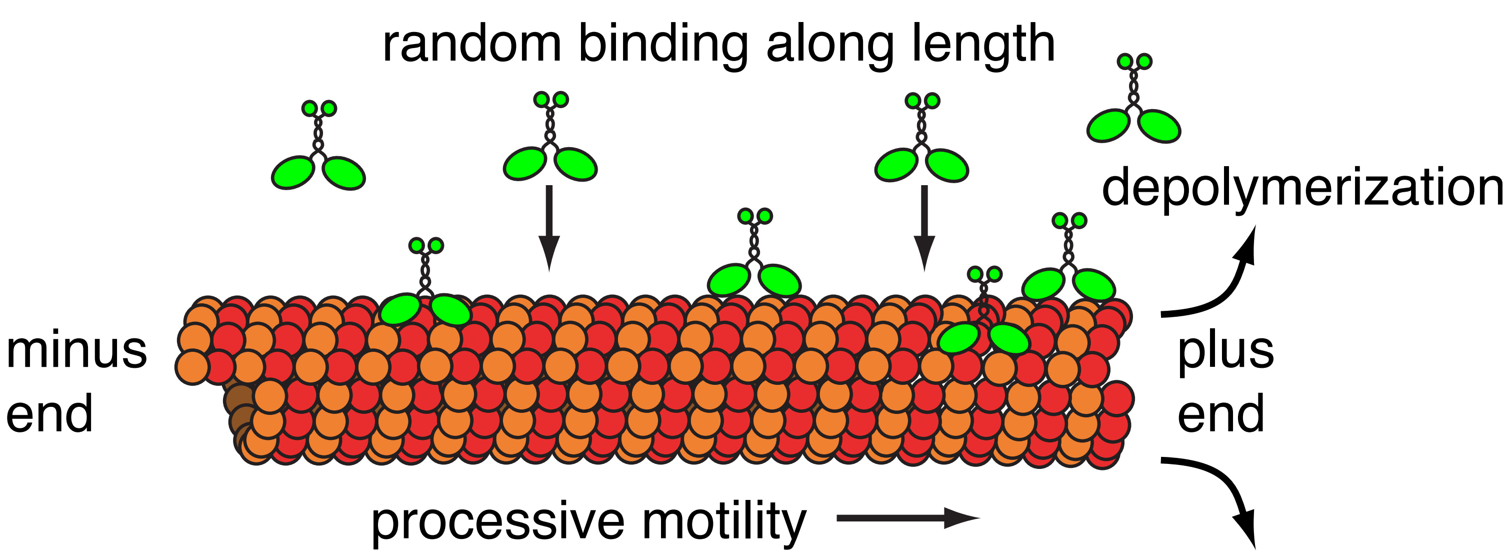
Average growth speed of microtubules

$$\boxed{\bar{v} = p^*_{\text{growth}} v_g - p^*_{\text{shrinking}} v_s}$$

$$\bar{v} \approx 0.4\,\mu\mathrm{m/min}$$

How cells control the total length of microtubules

Special kinesin-8 motors bind to microtubules and then walk towards the plus end, where they help detach (depolymerize) tubulin dimers

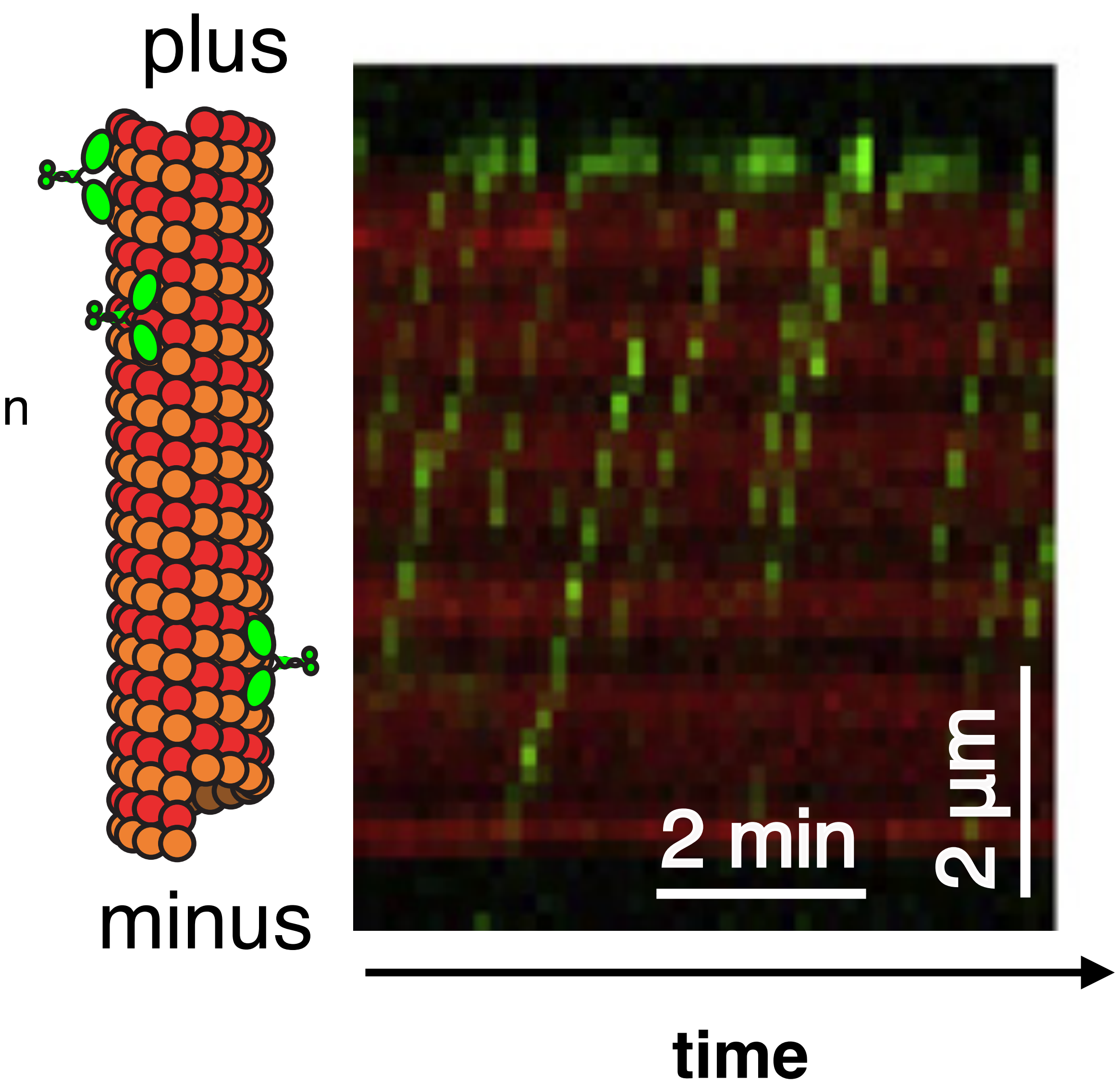


Motors walk at speed

$$v_{\mathrm{mot}} \approx 3\mu\mathrm{m}/\mathrm{min}$$

kymograph

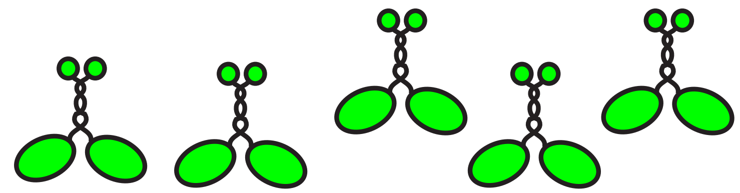
$$v_{\mathrm{mot}} \approx 3\mu\mathrm{m}/\mathrm{min}$$

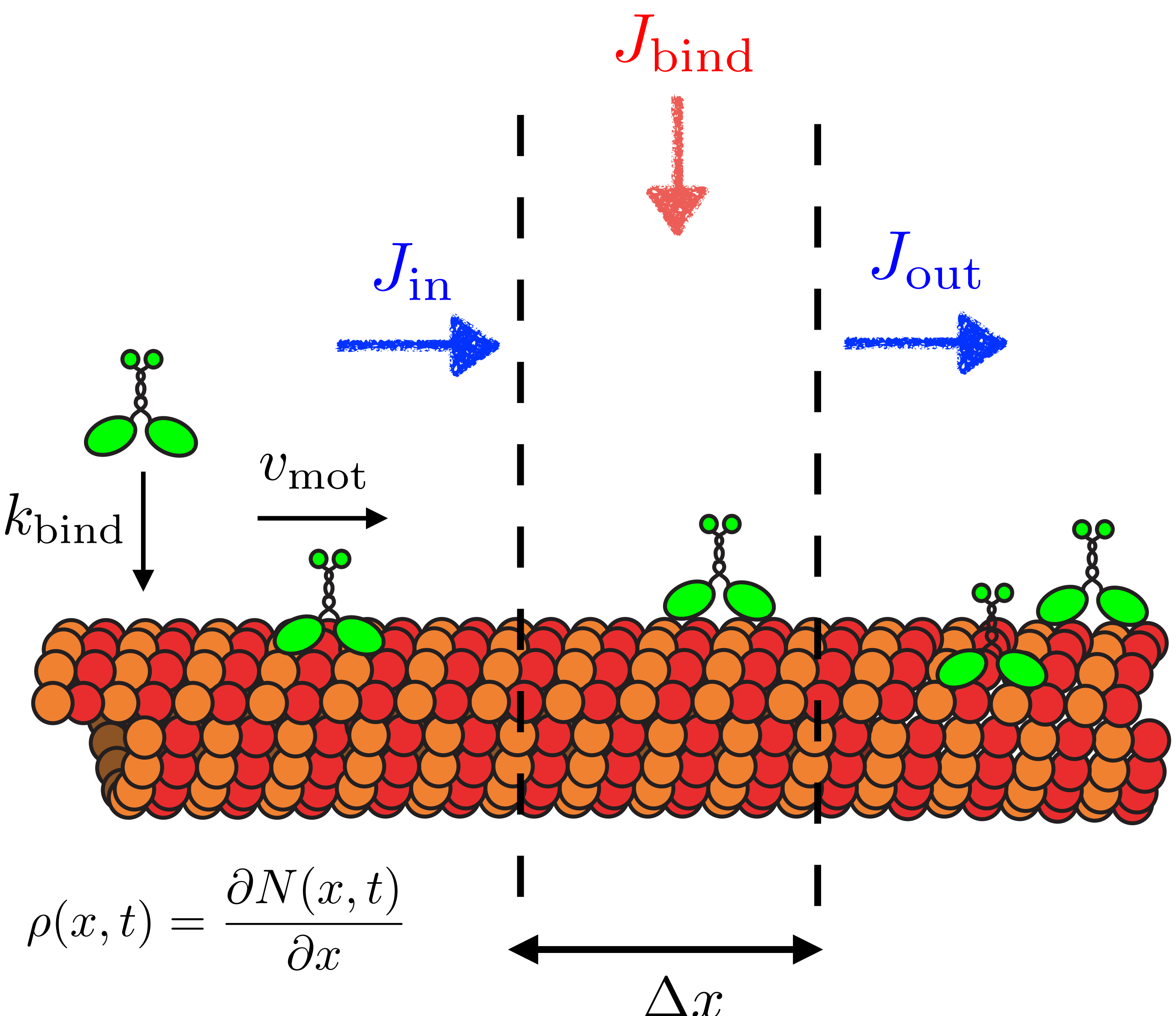


V. Varga *et al.*, Cell 138, 1174-1183 (2009)

Density of motors bound to microtubules

[*M*] concentration of unbound motors





$$\rho(x,t) = \frac{\partial N(x,t)}{\partial x}$$

density of bound motors

Conservation law for the number of bound motors

$$\frac{\Delta N}{\Delta t} = J_{\text{bind}} - J_{\text{out}} + J_{\text{in}}$$

$$\frac{\Delta N(x,t)}{\Delta t} = k_{\text{bind}}[M]\Delta x - \big(\rho(x+\Delta x,t) - \rho(x,t)\big)v_{\text{mot}}$$

$$\boxed{\frac{\partial \rho(x,t)}{\partial t} = k_{\text{bind}}[M] - v_{\text{mot}}\frac{\partial \rho(x,t)}{\partial x}}$$

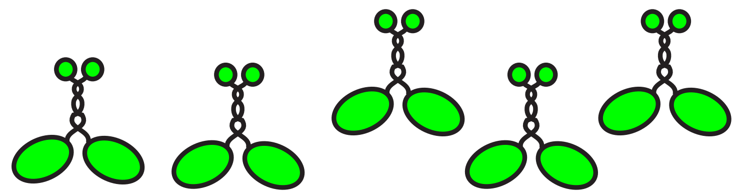
Generalized Fick's law

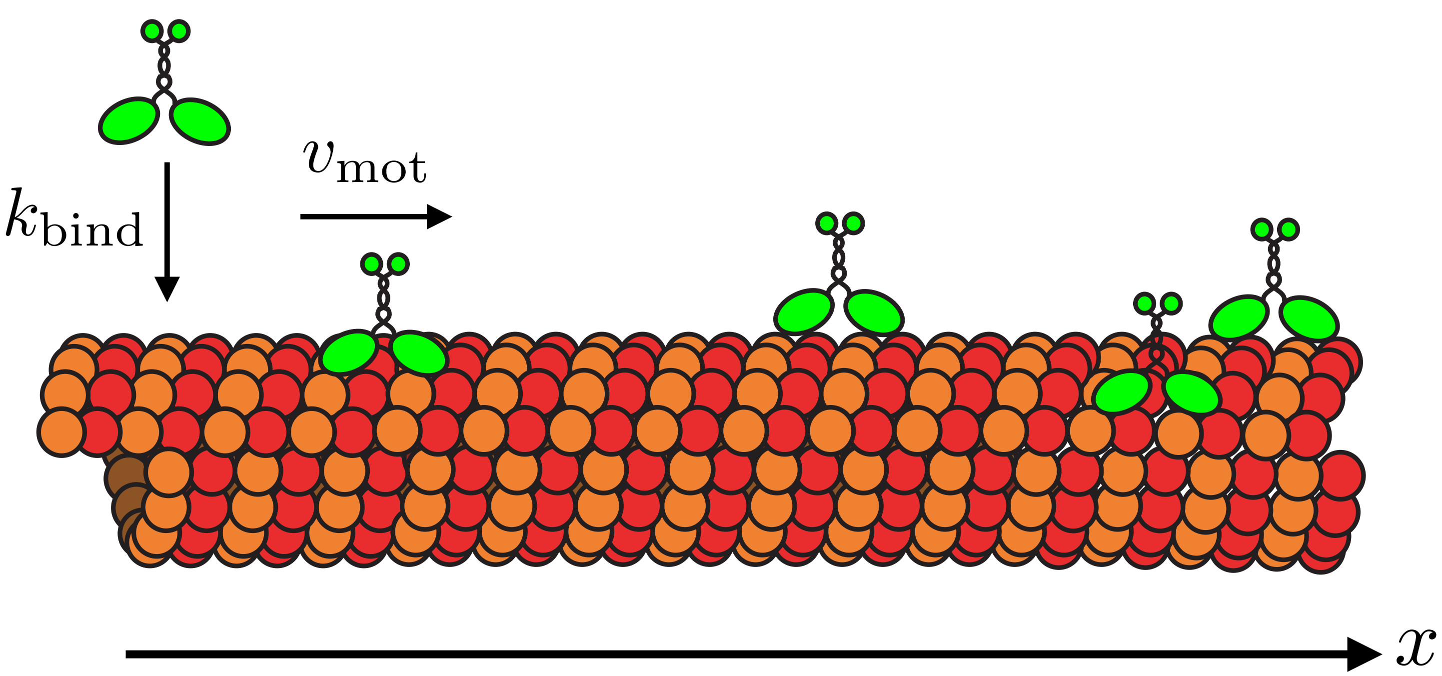
$$\boxed{\frac{\partial \rho(x,t)}{\partial t} = r(x,t) - \frac{\partial j(x,t)}{\partial x}}$$

creation/removal of particles

Density of motors bound to microtubules

[*M*] concentration of unbound motors





Time evolution for density of bound motors

$$\frac{\partial \rho(x,t)}{\partial t} = k_{\text{bind}}[M] - v_{\text{mot}} \frac{\partial \rho(x,t)}{\partial x}$$

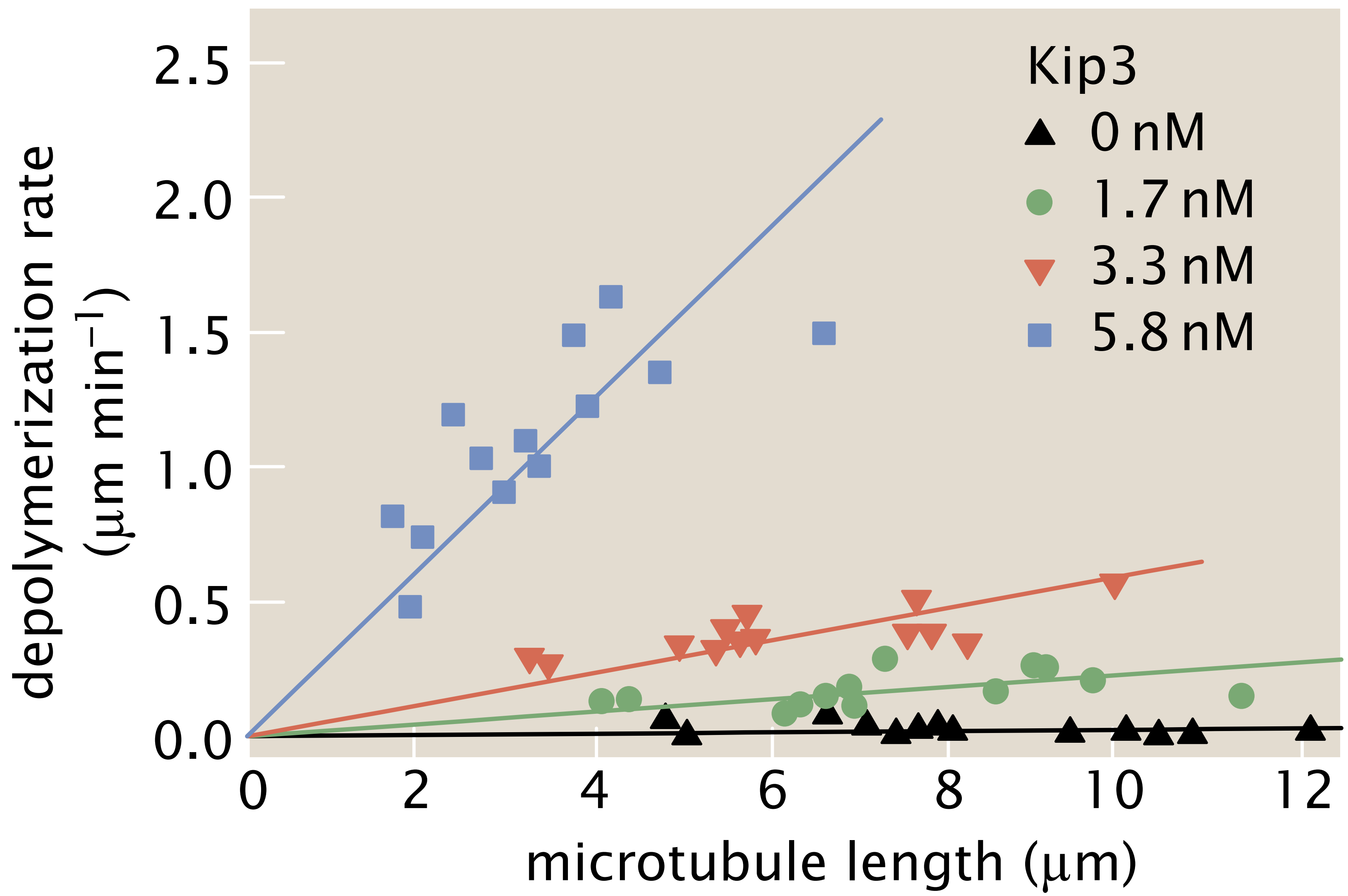
For initially empty microtubule

$$\rho(x,t) = \begin{cases} \frac{k_{\text{bind}}[M]}{v_{\text{mot}}}\, x, & 0 < x < v_{\text{mot}} t \\ k_{\text{bind}}[M] t, & x > v_{\text{mot}} t \end{cases}$$

Stationary density of bound motors

$$\boxed{\rho^*(x) = \frac{k_{\text{bind}}[M]}{v_{\text{mot}}}\, x}$$

Length dependent depolymerization rate

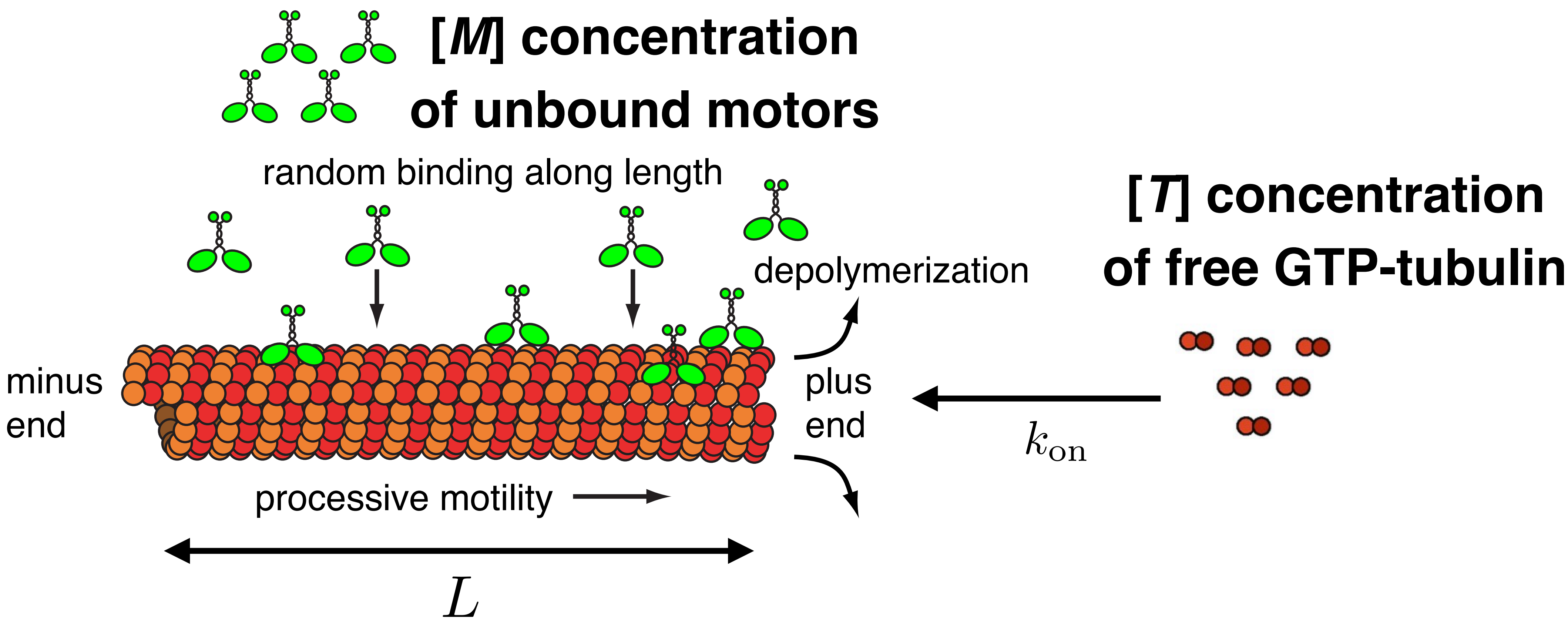


Depolymerization rate is proportional to density of Kip3 motors

$$\boxed{\rho^*(L) = \frac{k_{\mathrm{bind}}[M]}{v_{\mathrm{mot}}} L}$$

V. Varga *et al.*, Nat. Cell Biol. **8**, 957-962 (2006)

Controlled length of microtubules



$$\frac{dL}{dt} = ak_{\mathrm{on}}[T] - a\rho^*(L)\overbrace{\left[v_{\mathrm{mot}} - \frac{dL}{dt}\right]}^{\text{relative velocity of motors arriving to the tip}}$$

$$\frac{dL}{dt} = \frac{(ak_{\mathrm{on}}[T] - a\rho^*(L)v_{\mathrm{mot}})}{1 - a\rho^*(L)}$$

$$\rho^*(L) = \frac{k_{\mathrm{bind}}[M]}{v_{\mathrm{mot}}}\,L$$

Stationary length of microtubules

$$\boxed{L^* = \frac{k_{\mathrm{on}}[T]}{k_{\mathrm{bind}}[M]}}$$

$[T] \approx 10\mu\mathrm{M}$

$k_{\mathrm{on}} \approx 9\mu\mathrm{M}^{-1}\mathrm{s}^{-1}$

$[M] \approx 3\mathrm{nM}$

$k_{\mathrm{bind}} \approx 24\mathrm{nM}^{-1}\mathrm{min}^{-1}\mu\mathrm{m}^{-1}$

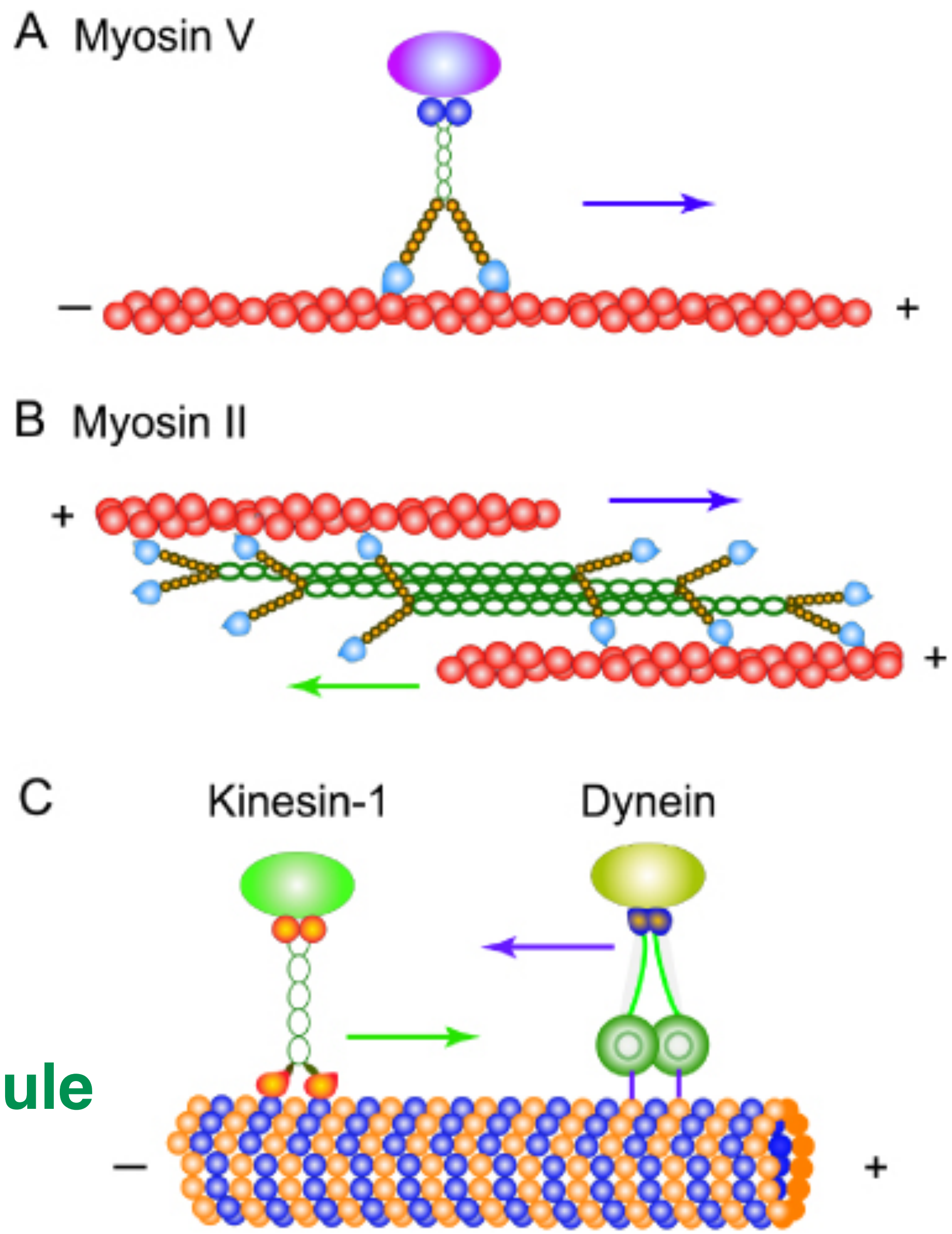
$$L^* \sim 75\mu\mathrm{m}$$

Molecular motors

Actin

Actin

Microtubule

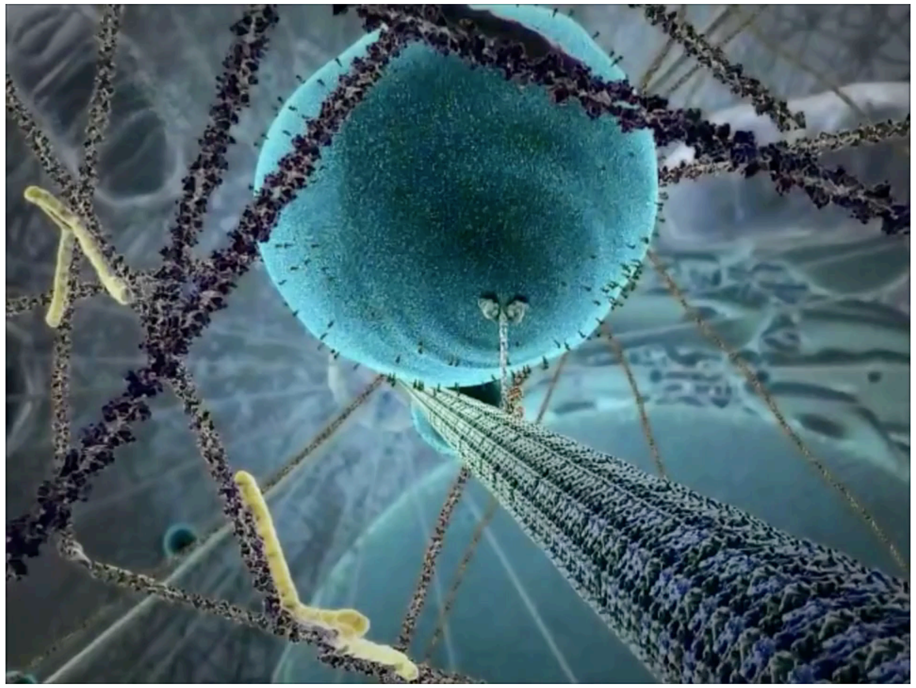


A.B. Kolomeisky, <u>J. Phys.: Condens. Matter</u> **25**, 463101 (2013)

Transport of large molecules around cells (diffusion too slow)

$v \sim 1 \mu\mathrm{m/s}$

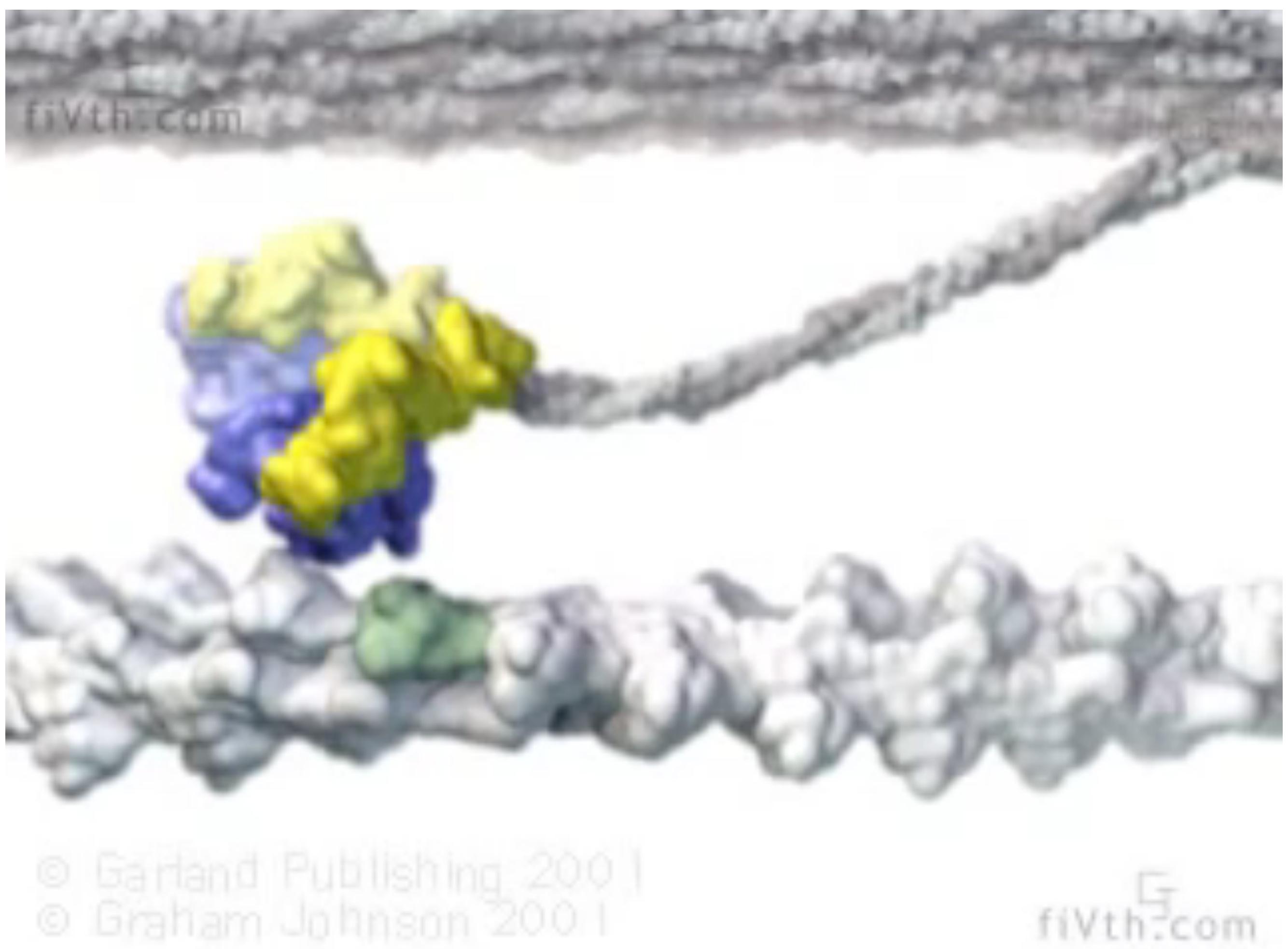
Contraction of muscles



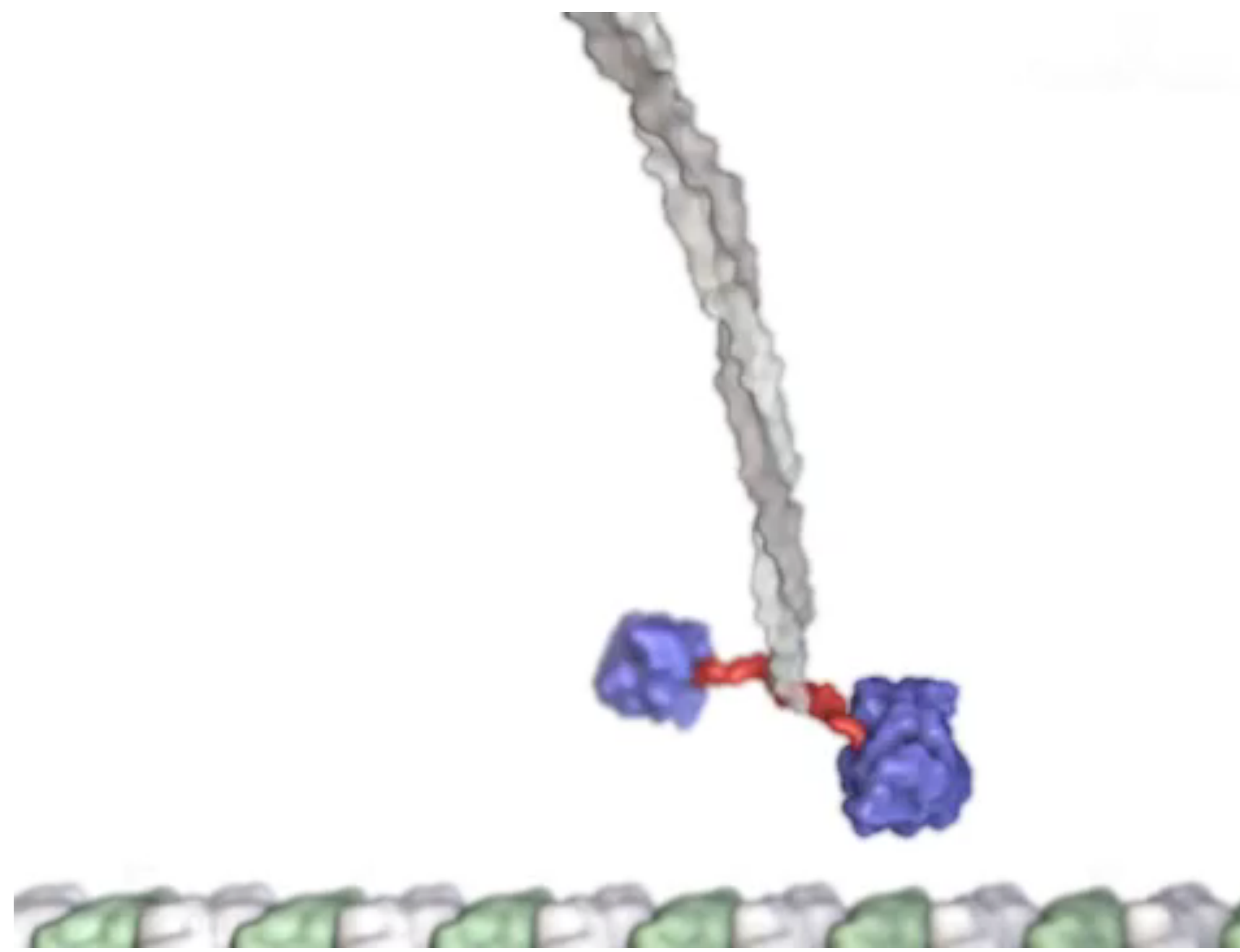
Harvard BioVisions

Movement of molecular motors is powered by ATP molecules

Myosin motor walking on actin in muscles



Kinesin motor walking on microtubule

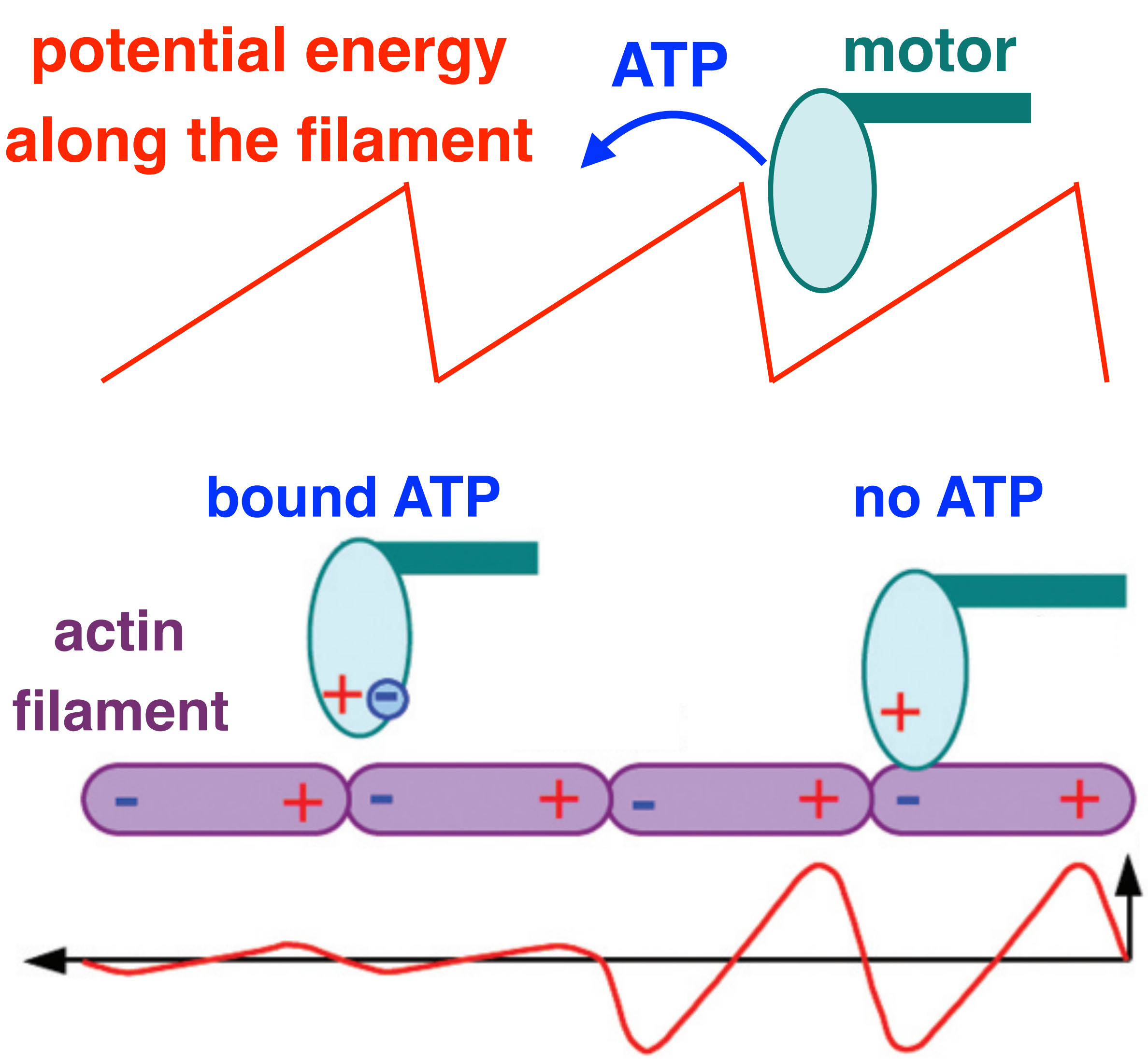


Graham Johnson

Molecular motors vs Brownian ratchets

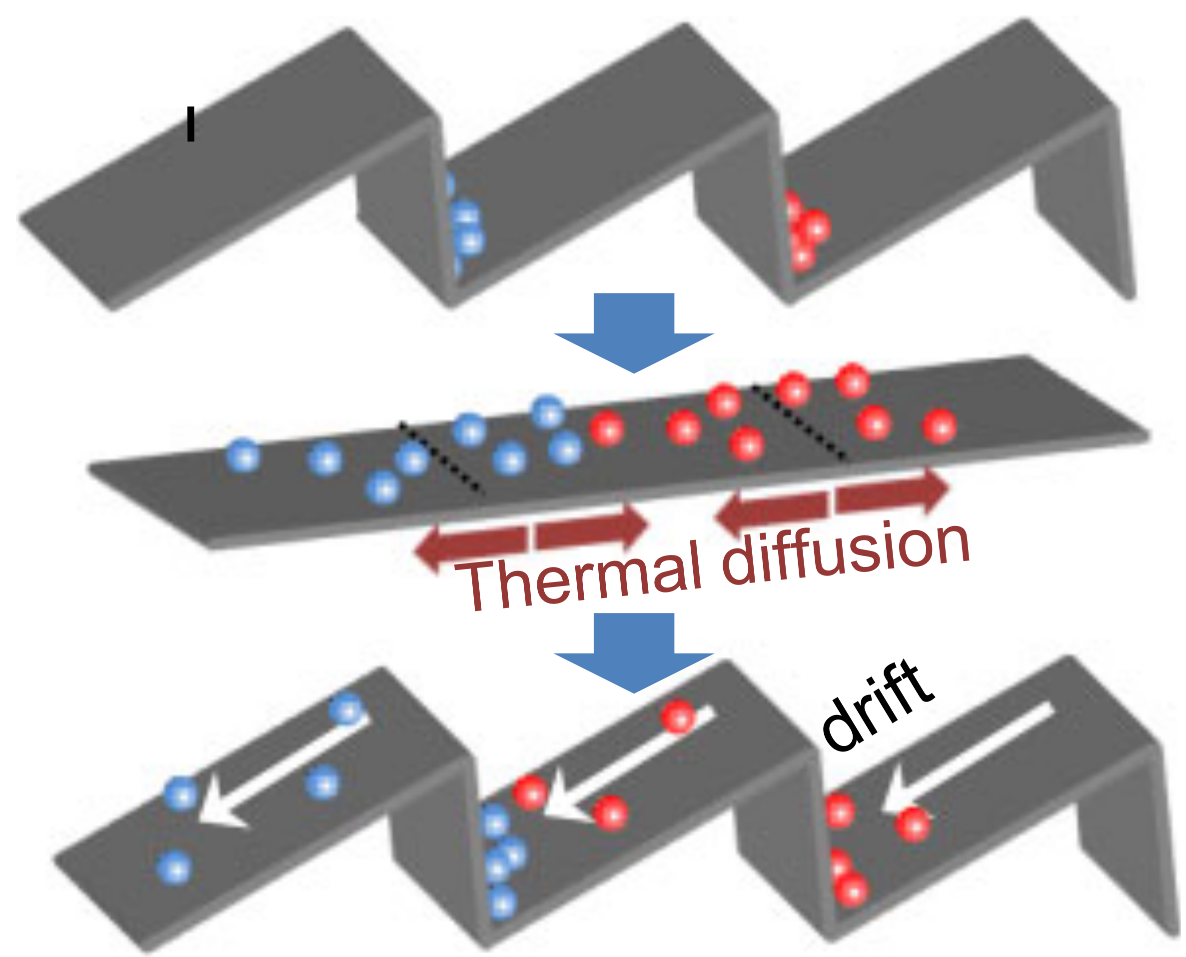
Myosin motor

ATP driven process drives molecular motors along the filaments



Brownian ratchet

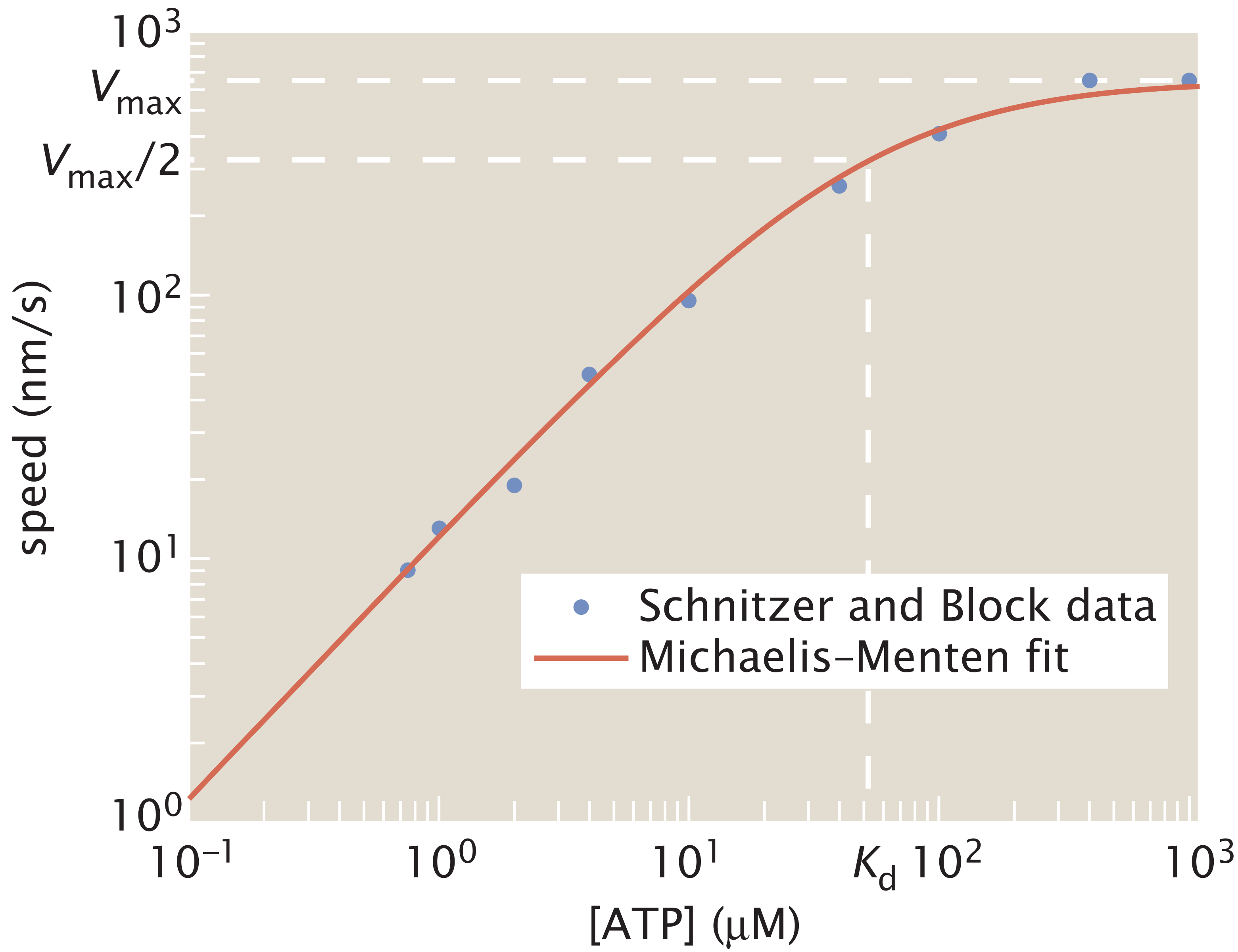
net movement of particles is achieved by periodic modulation of asymmetric external potential



ATP concentration dependent speed of motors

$$v \approx v_{\mathrm{max}} \frac{[\mathrm{ATP}]}{[\mathrm{ATP}] + K_d}$$

Kinesin motor on microtubules



Maximal speed

$$v_{\mathrm{max}} \approx 0.6\ \mu\mathrm{m/s}$$

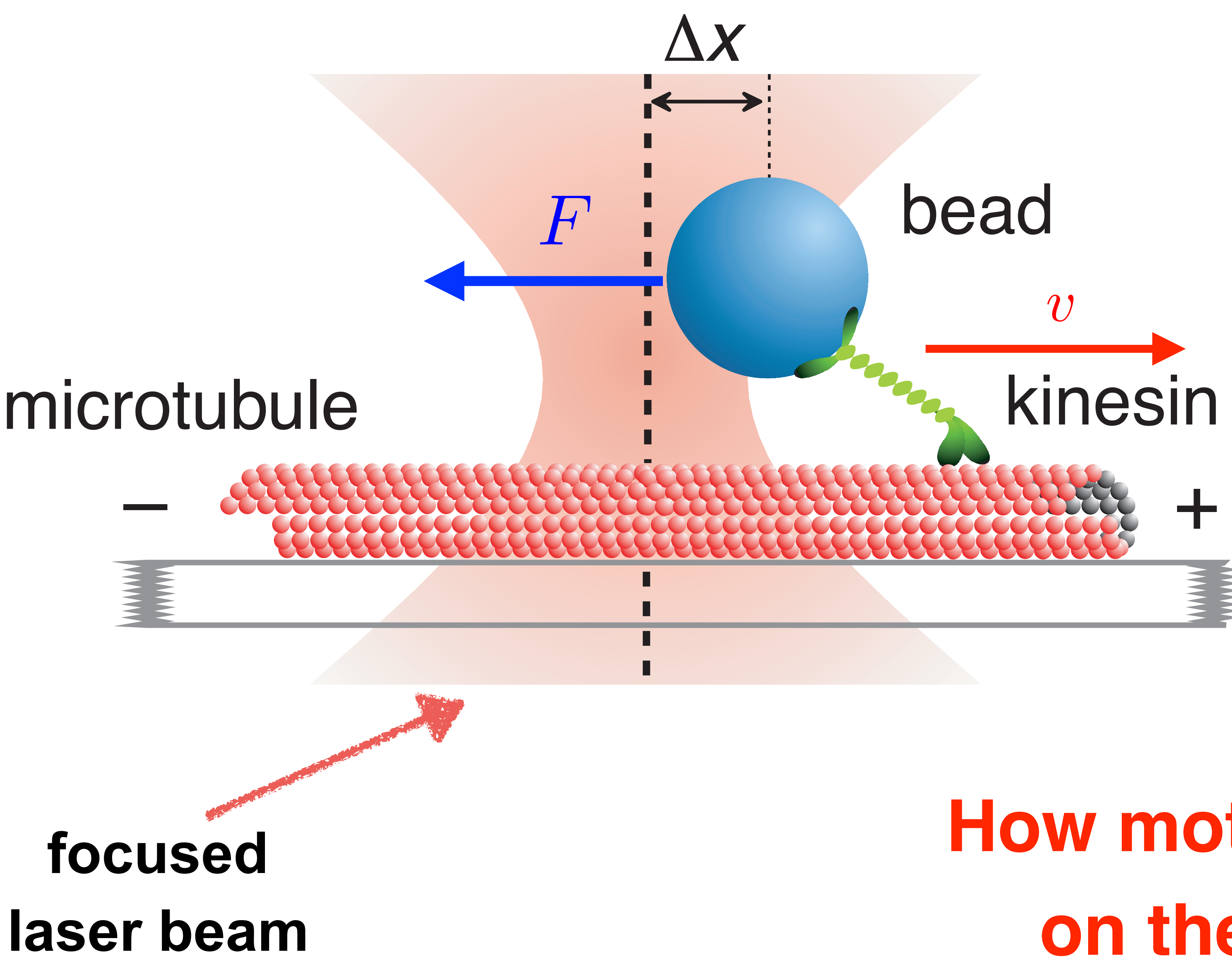
ATP concentration at half the maximal speed

$$K_d \approx 50\ \mu\mathrm{M}$$

R. Phillips et al., Physical Biology of the Cell

Motors carrying the load

Force exerted on kinesin motors carrying plastic beads can be controlled with optical tweezers



$$F \approx k\Delta x$$

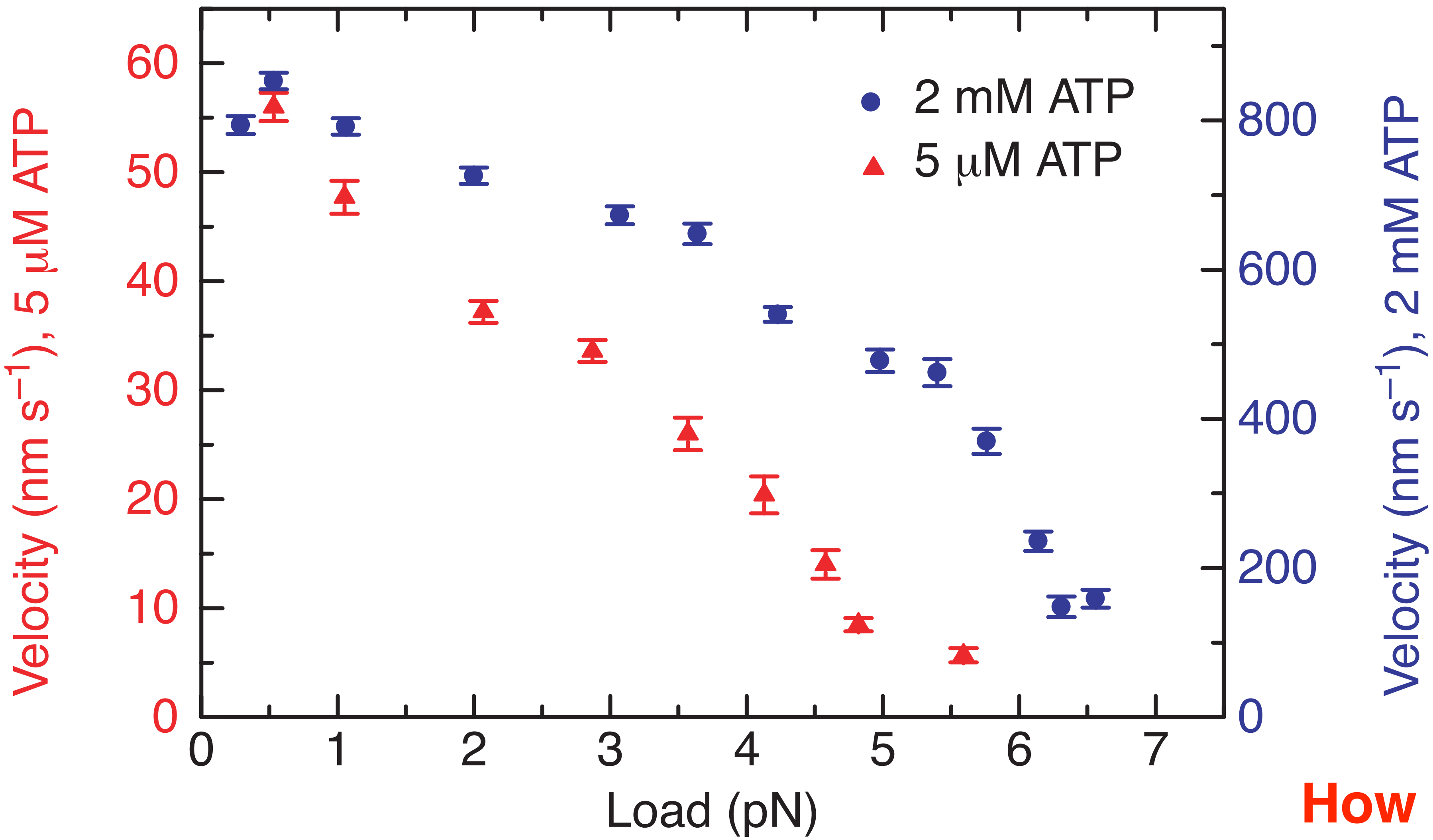
Effective spring constant *k* depends on the bead size, refractive indices of the bead and surrounding medium, and the gradient of laser intensity

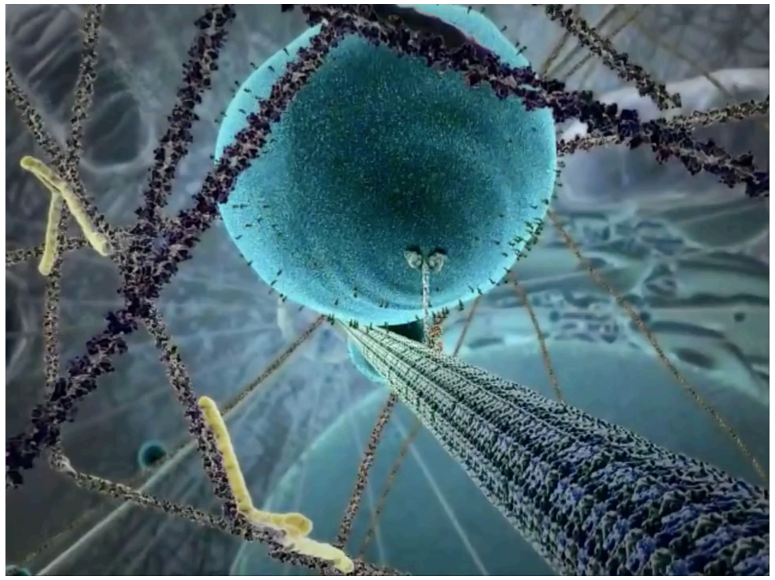
How motor speed depends on the loading force?

K. Visscher et al., <u>Nature</u> **400**, 184-189 (1999)

Motor velocity dependence on the load

kinesin walking on microtubules





How important is viscous drag for motors carrying vesicles?

stall force

$$v(F_s) = 0$$

$$F_{\mathrm{drag}} = 6\pi\eta R v$$

$$F_{\mathrm{drag}} \sim 6\pi 10^{-3}\mathrm{kgm}^{-1}\mathrm{s}^{-1} \cdot 1\mu\mathrm{m} \cdot 1\mu\mathrm{m/s}$$

$$F_{\mathrm{drag}} \sim 10^{-2}\,\mathrm{pN}$$

K. Visscher et al., Nature **400**, 184-189 (1999)

Note: viscous drag is negligible

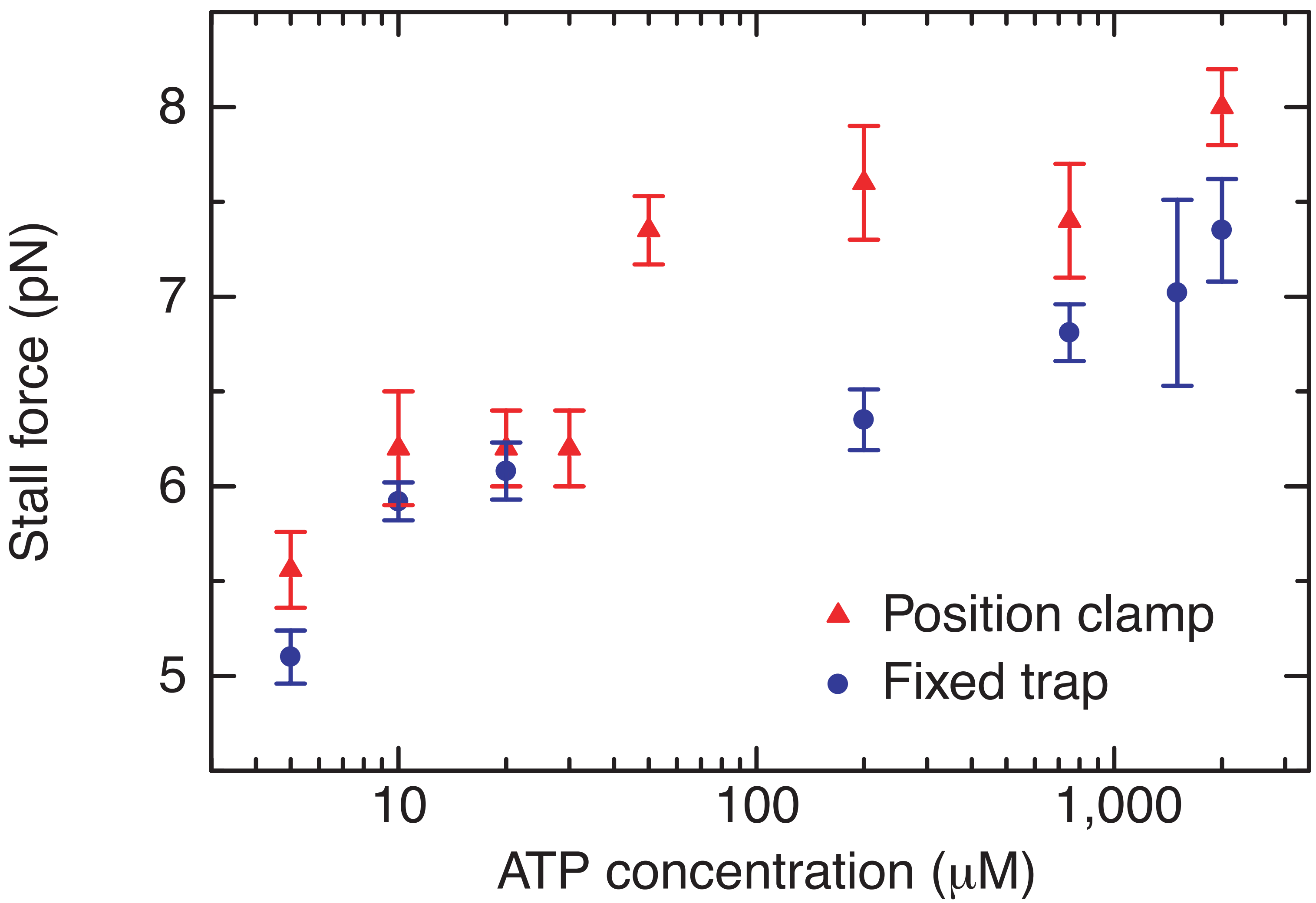
ATP concentration dependent stall force

$$F_s \sim \frac{k_B T}{a} \ln[\mathrm{ATP}]$$

motor step length

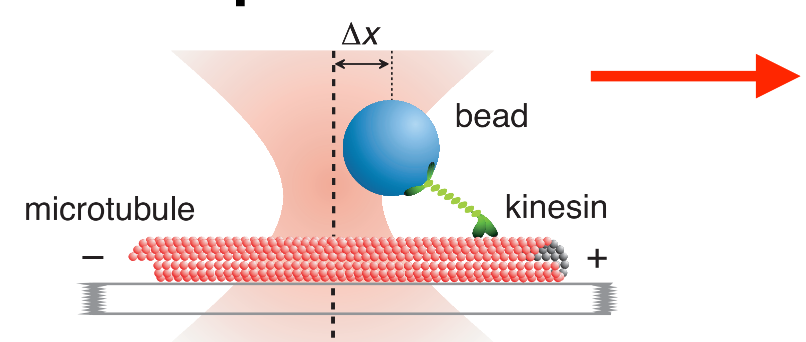
$$a \approx 8\,\mathrm{nm}$$

kinesin walking on microtubules



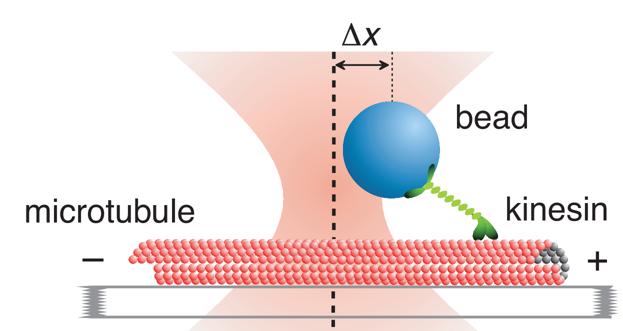
Position clamp

laser follows the bead and keeps fixed force



Fixed trap

laser position is is fixed

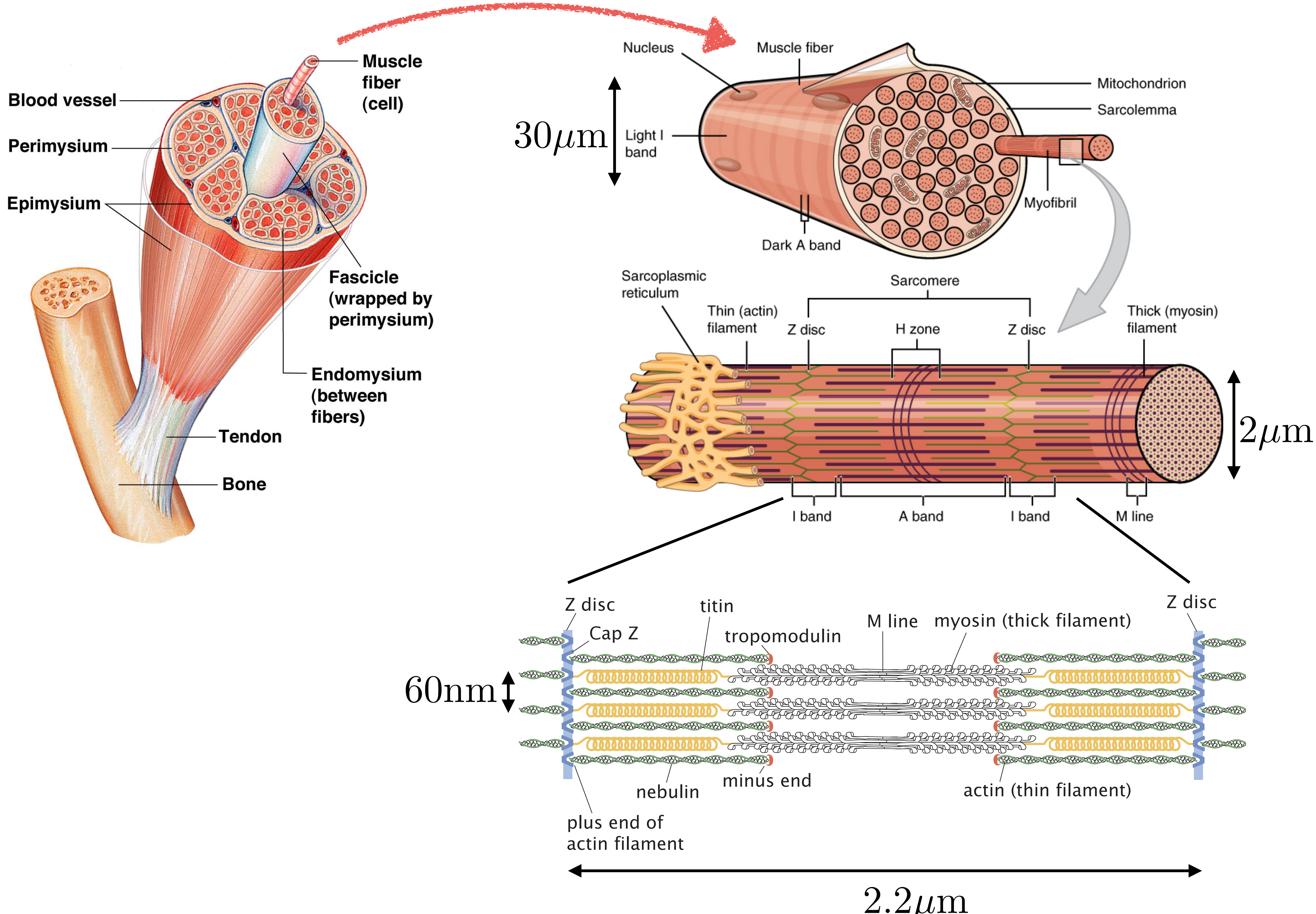


maximal possible force exerted by motors can be estimated from energy conservation

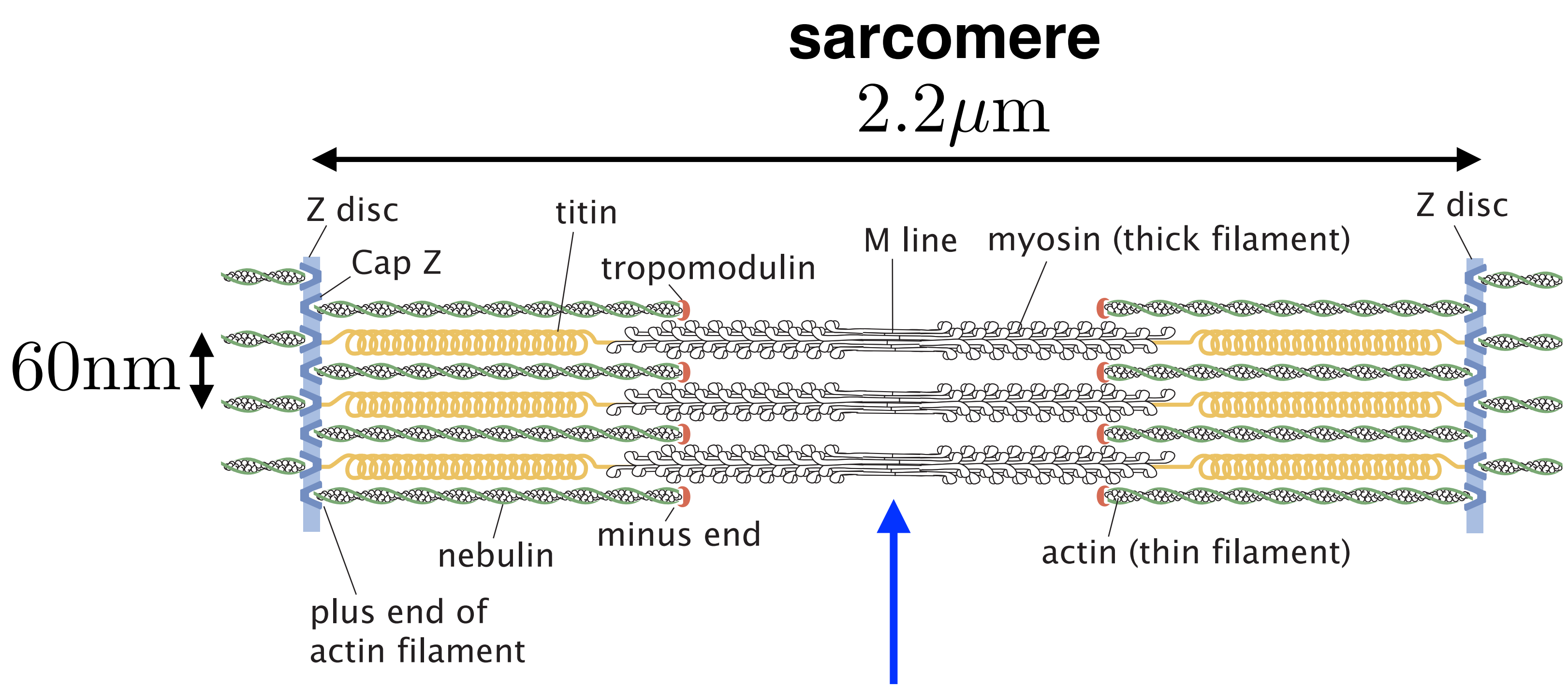
$$F_{\max} = \frac{\Delta G_{\mathrm{ATP}}}{a} \approx \frac{20 k_B T}{8\mathrm{nm}} \sim 10\mathrm{pN}$$

K. Visscher et al., Nature **400**, 184-189 (1999)

Skeletal muscle contraction by myosin motors



Skeletal muscle contraction by myosin motors



~300 myosin

motors per bundle

Estimated force generated by myosin motors

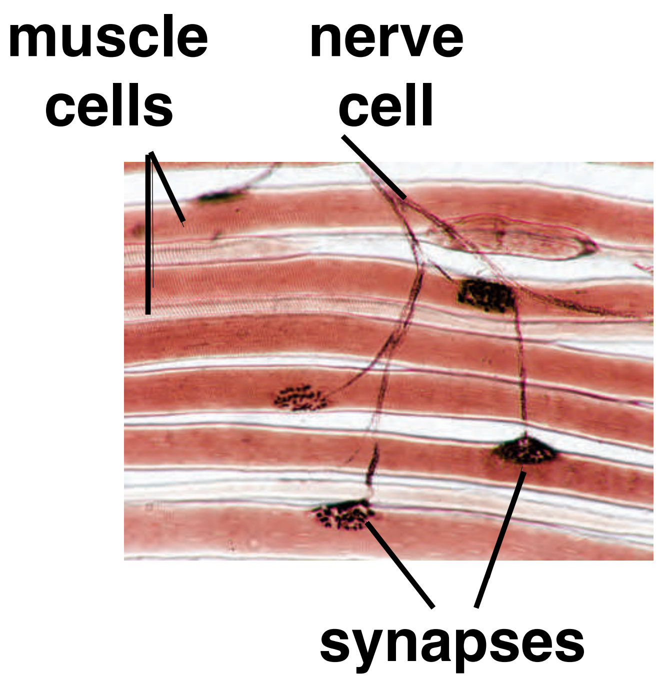
$$300 \times \frac{2\mathrm{pN}}{\pi(30\mathrm{nm})^2} \sim 20\mathrm{N}/\mathrm{cm}^2$$

Muscles contract at twice the speed of myosin motors

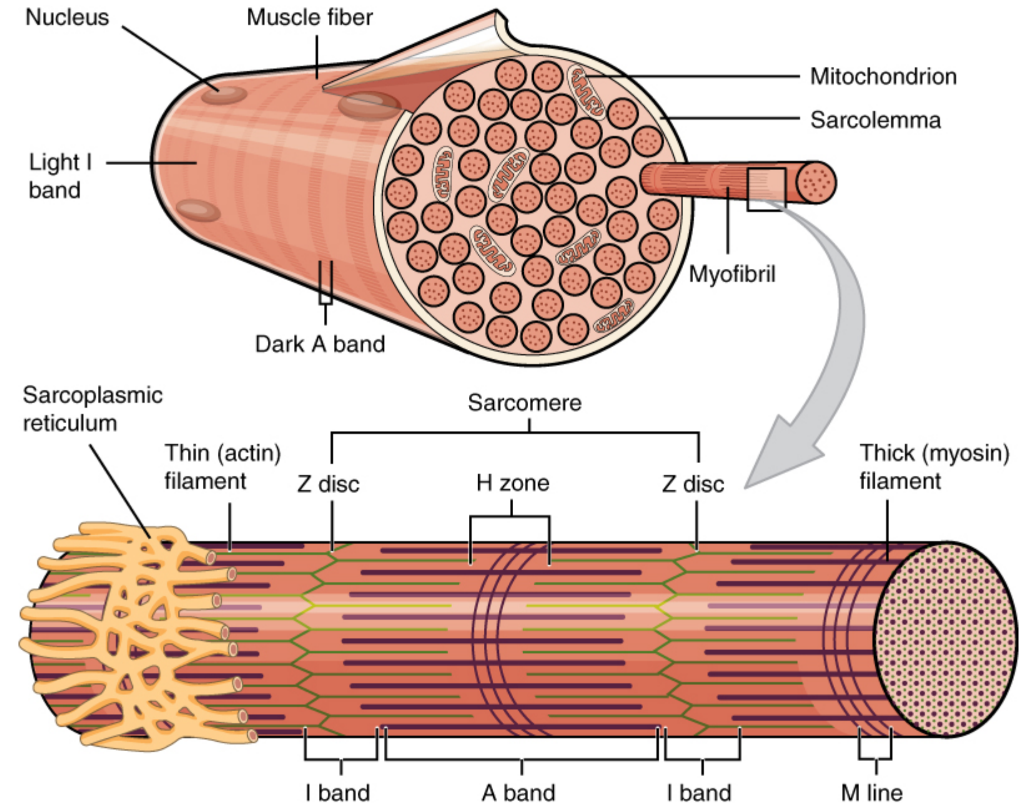
$$\sim 0.1\text{-}1\mu\mathrm{m/s}$$

Muscles may contract by 5%-45% per second!

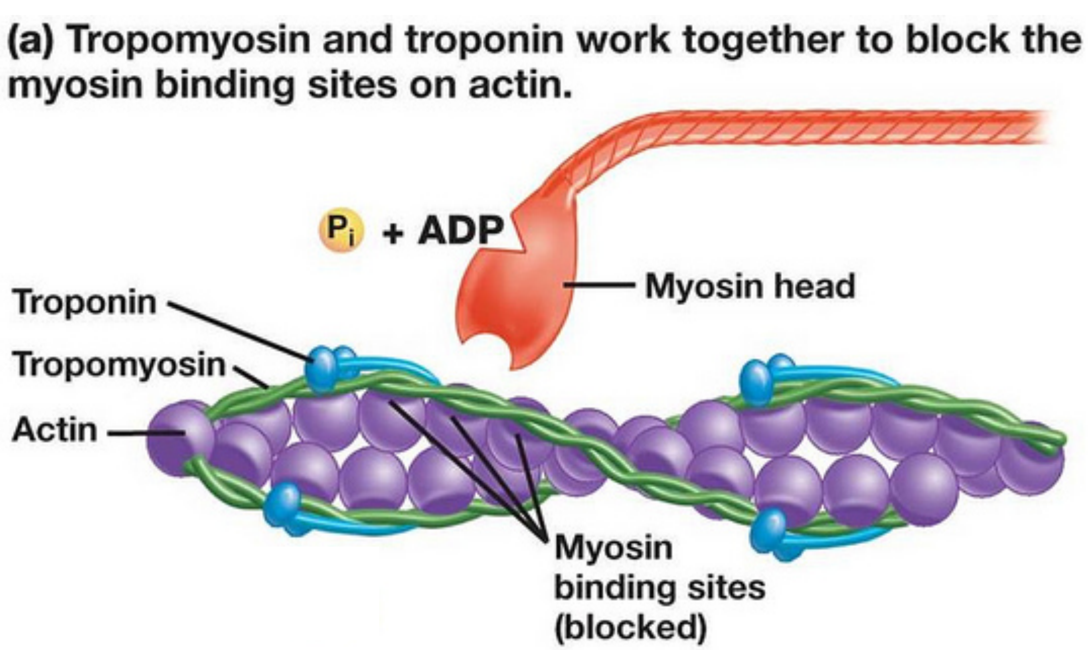
Skeletal muscle contraction is controlled by nerve cells



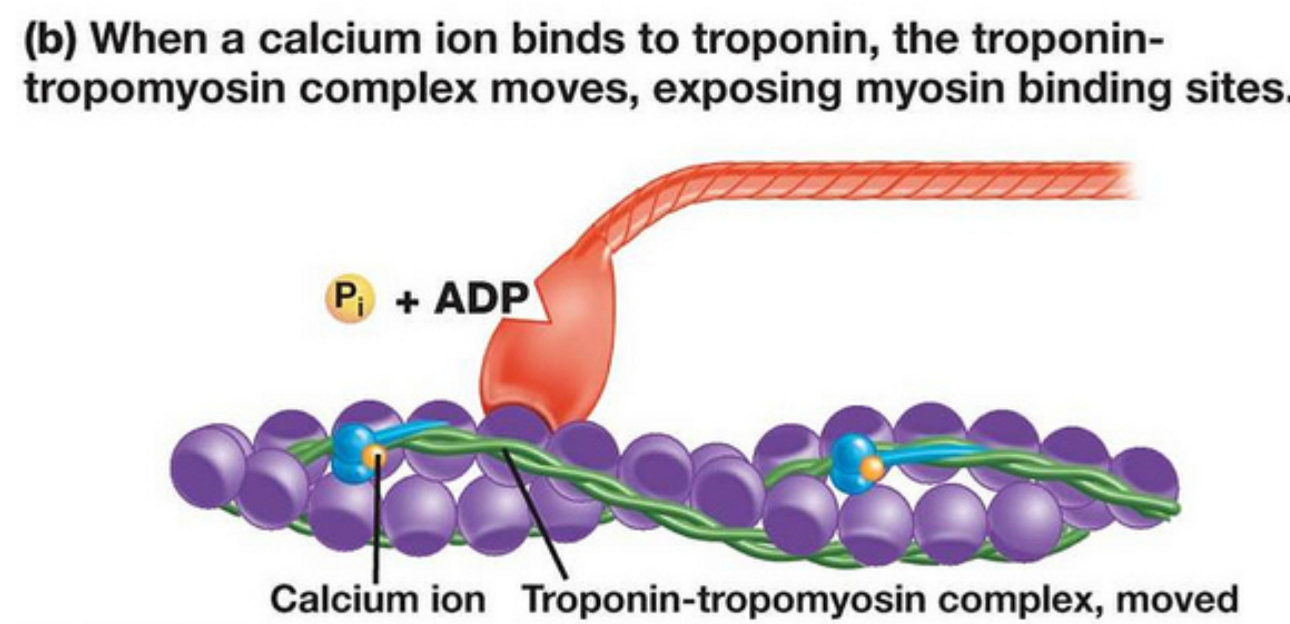
Electric signal from nerve cells releases Ca^{2+} from sarcoplasmic reticulum



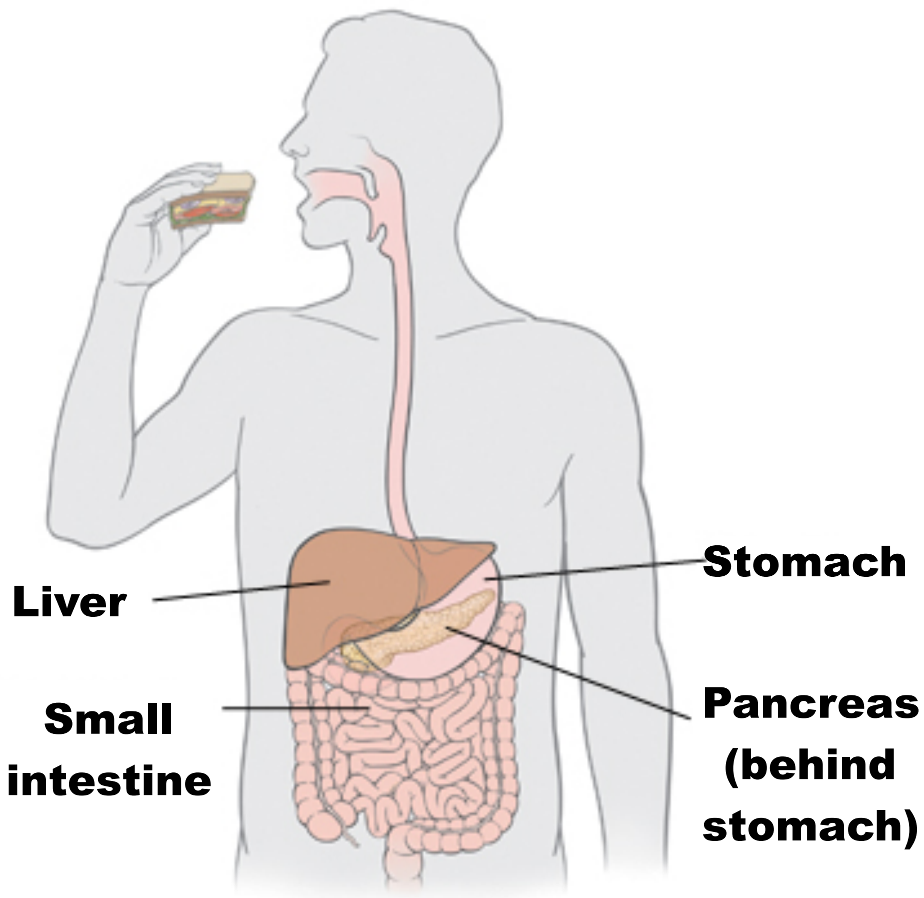
Low Ca^{2+}, muscles are relaxed

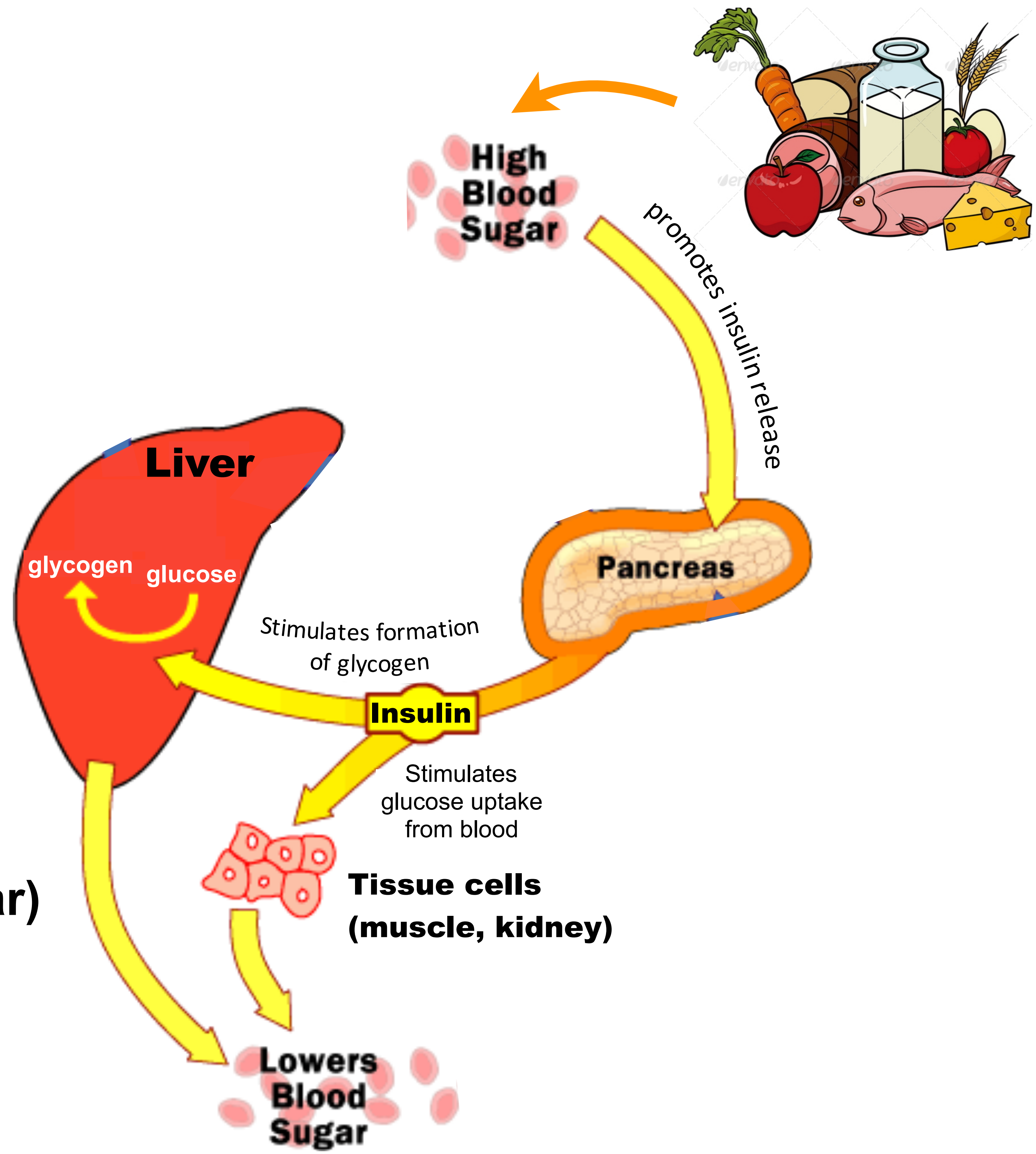


High Ca^{2+}, muscles are contracted

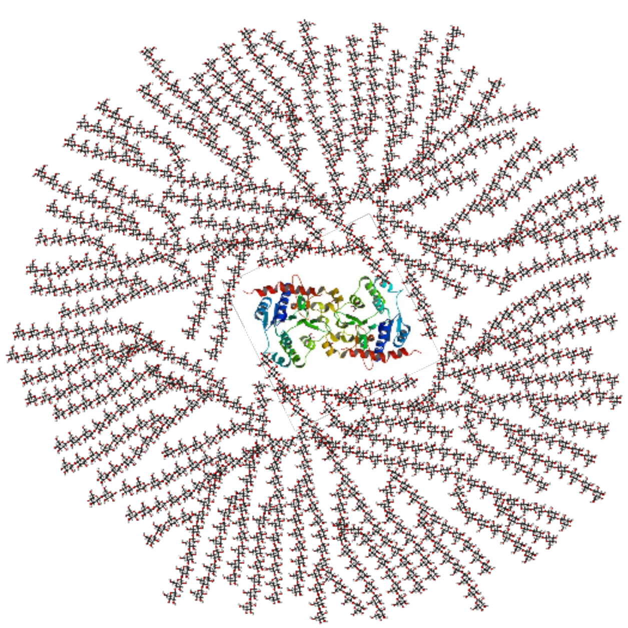


How muscles get ATP energy?

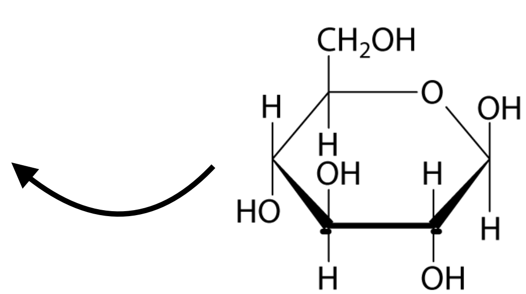




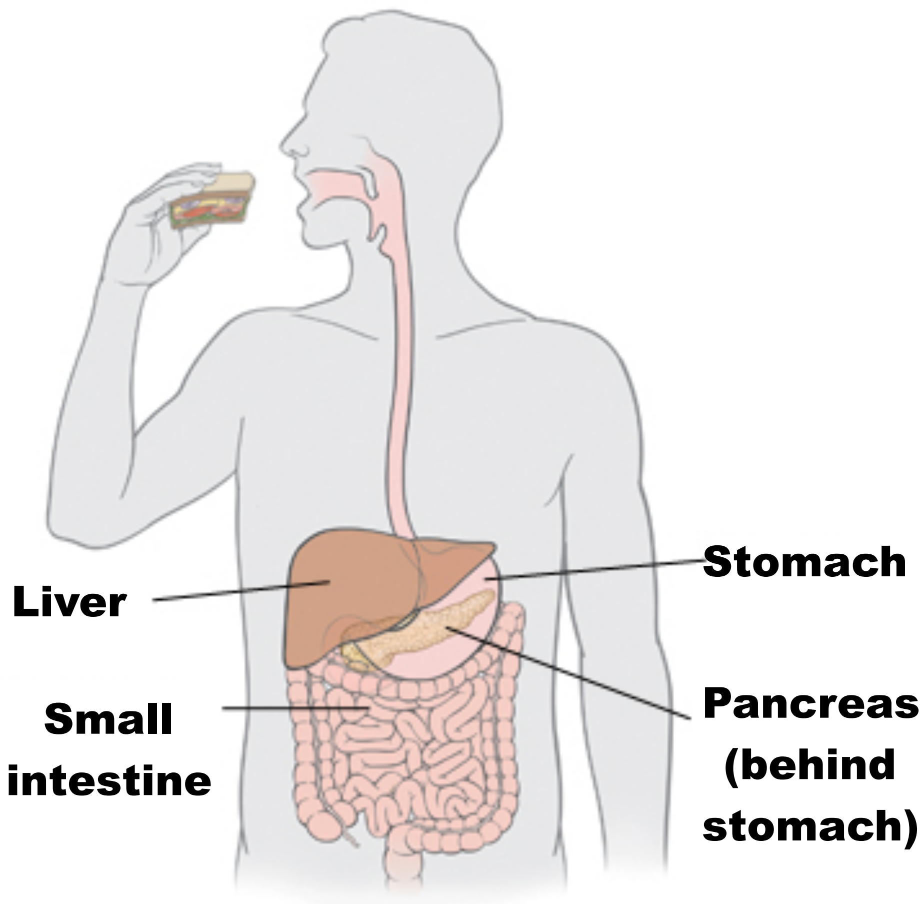
glycogen
(polysaccharide
of glucose)

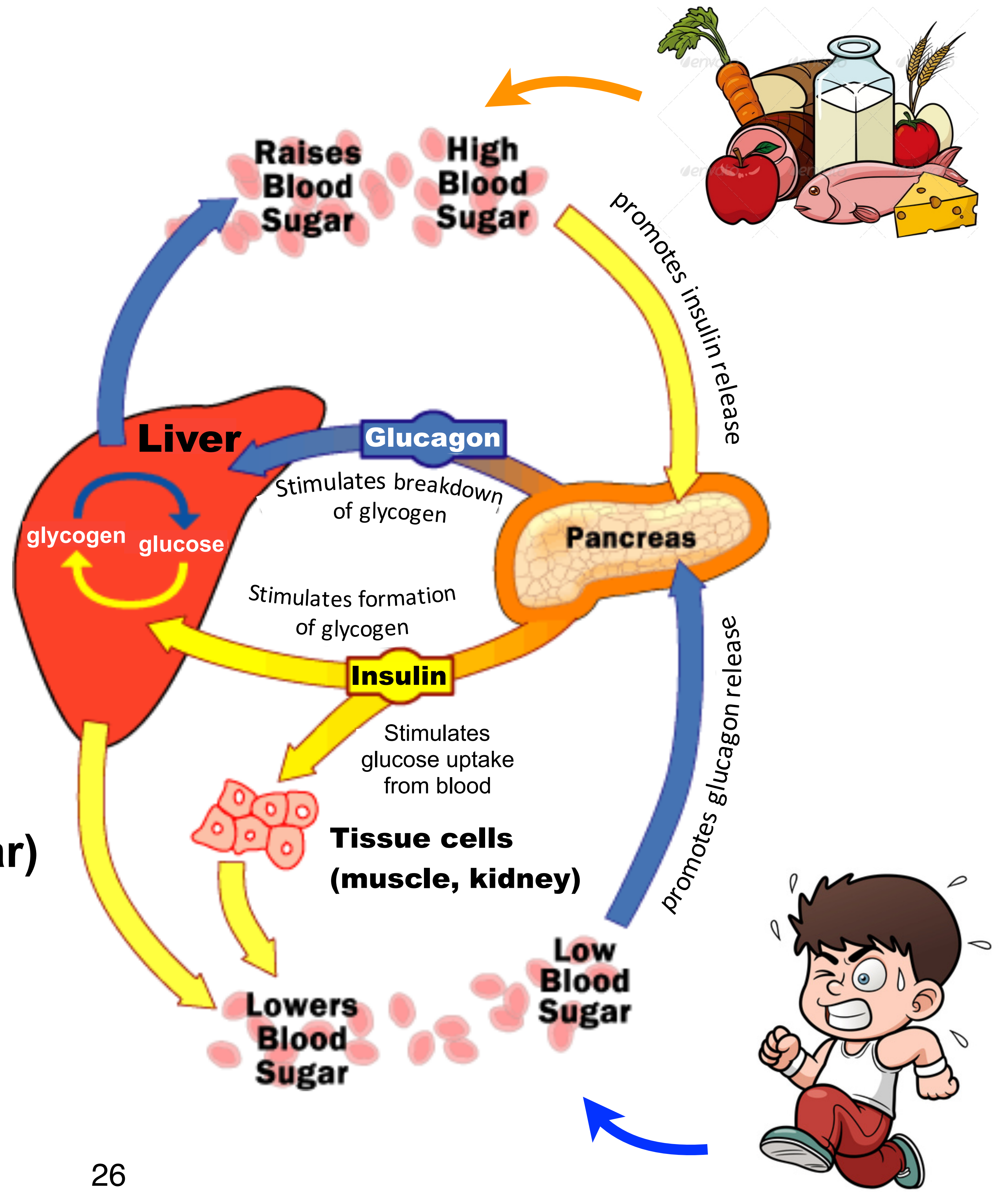


glucose
(blood sugar)

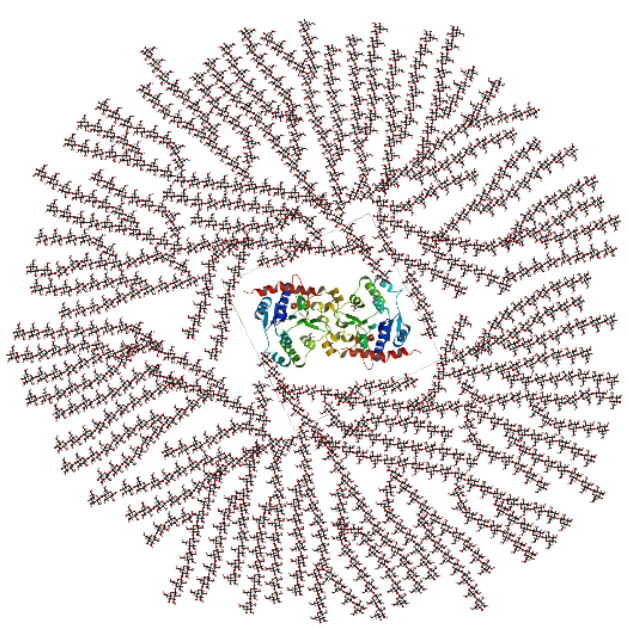


How muscles get ATP energy?

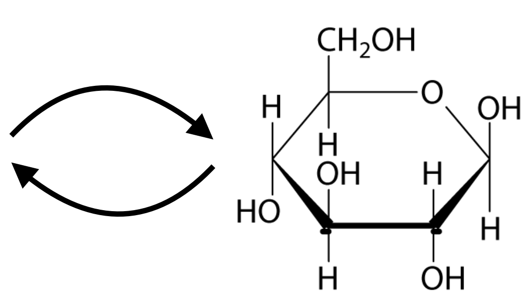




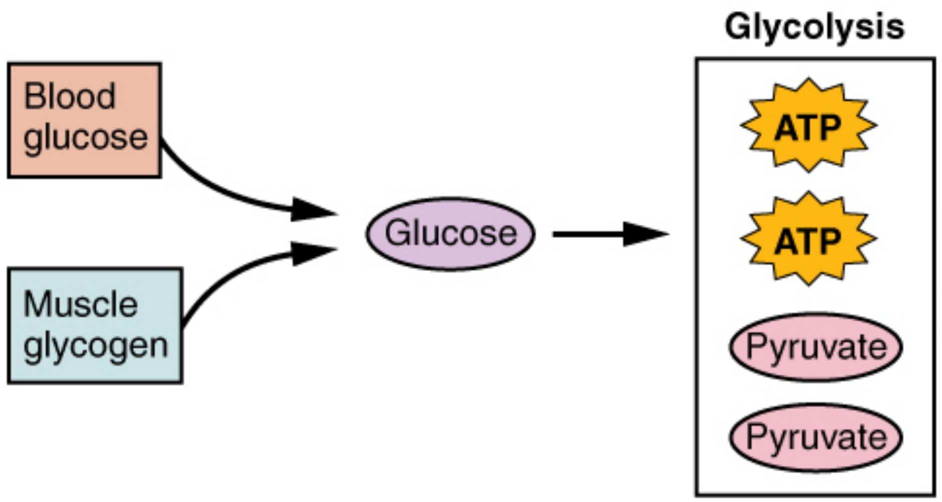
glycogen
(polysaccharide of glucose)



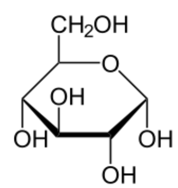
glucose
(blood sugar)



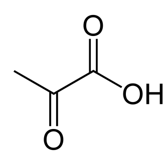
How muscles get ATP energy?



glucose



pyruvate



O_2

Aerobic respiration

$$C_6H_{12}O_6 + 6O_2 \Longrightarrow 6CO_2 + 6H_2O + 36ATP$$

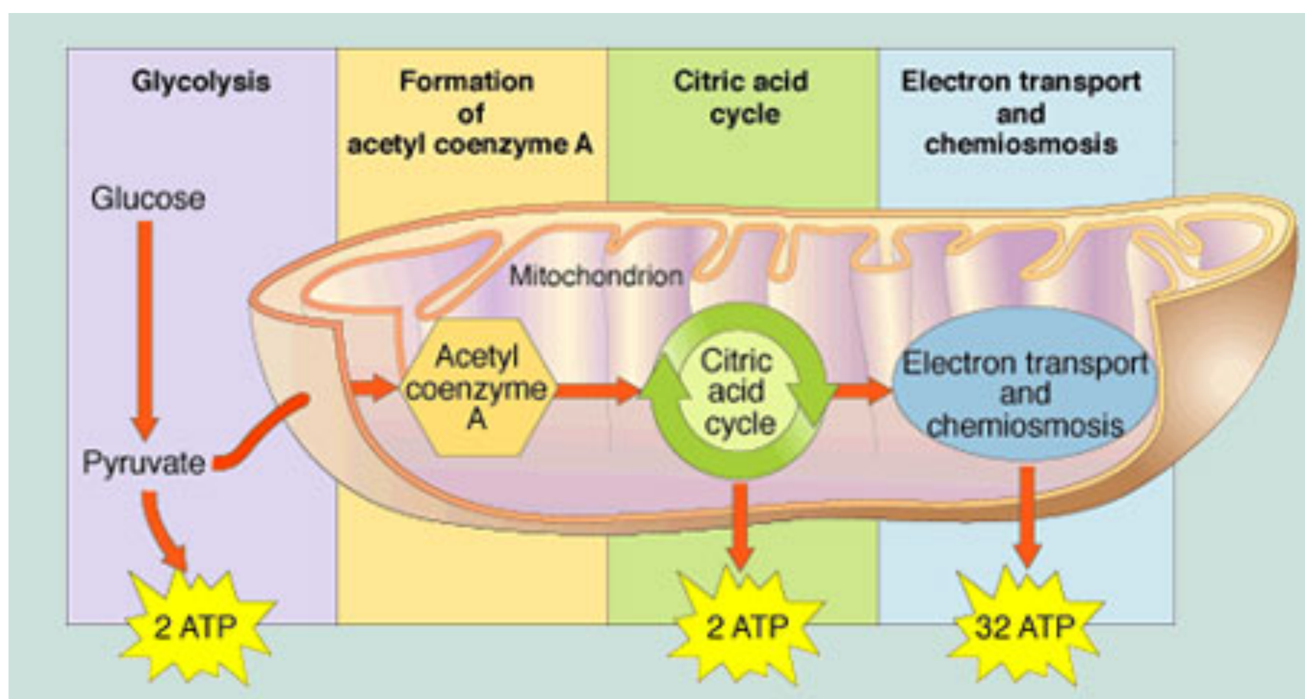
no O_2

Anaerobic respiration

$$C_6H_{12}O_6 \Longrightarrow 2 \text{ Lactic Acids} + 2ATP$$

(muscle fatigue)

Aerobic respiration



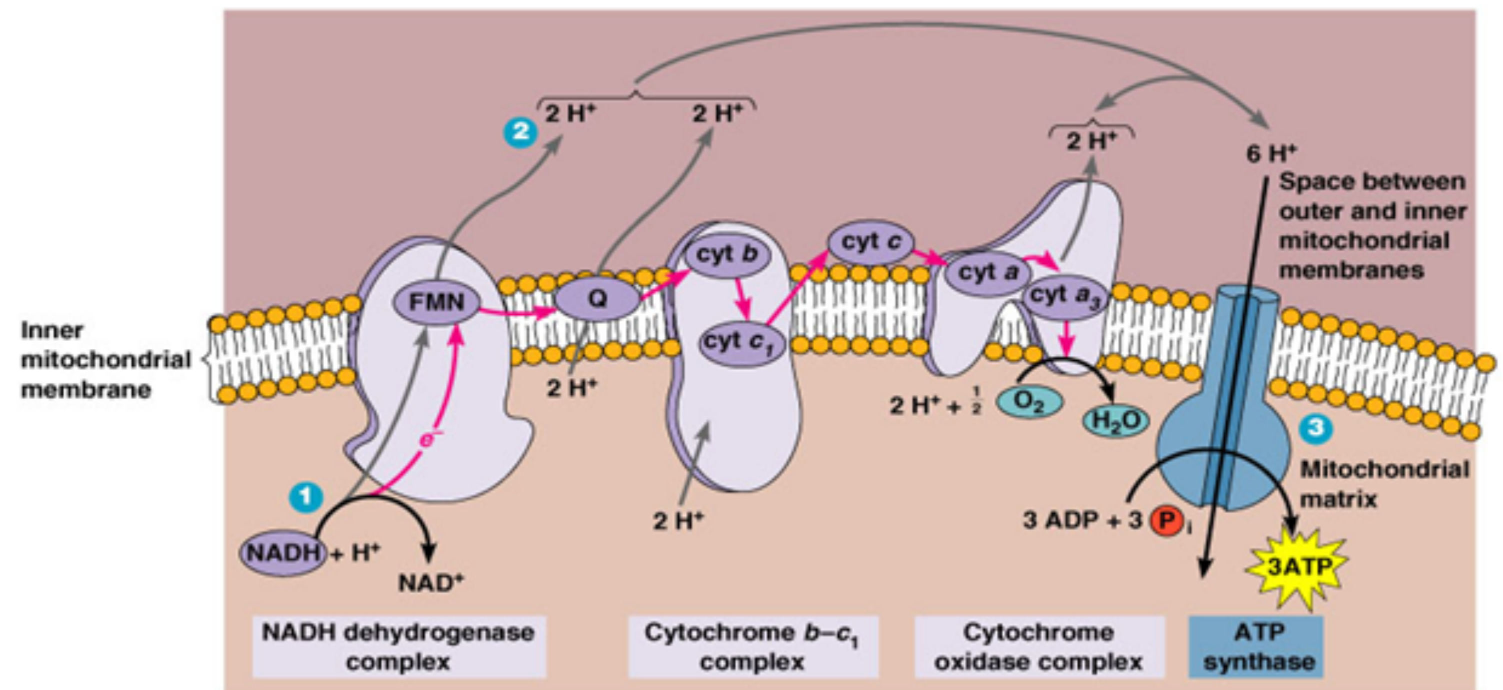
Note:

Citric acid cycle

= Krebs cycle

Electron transport chain

Mitochondrion

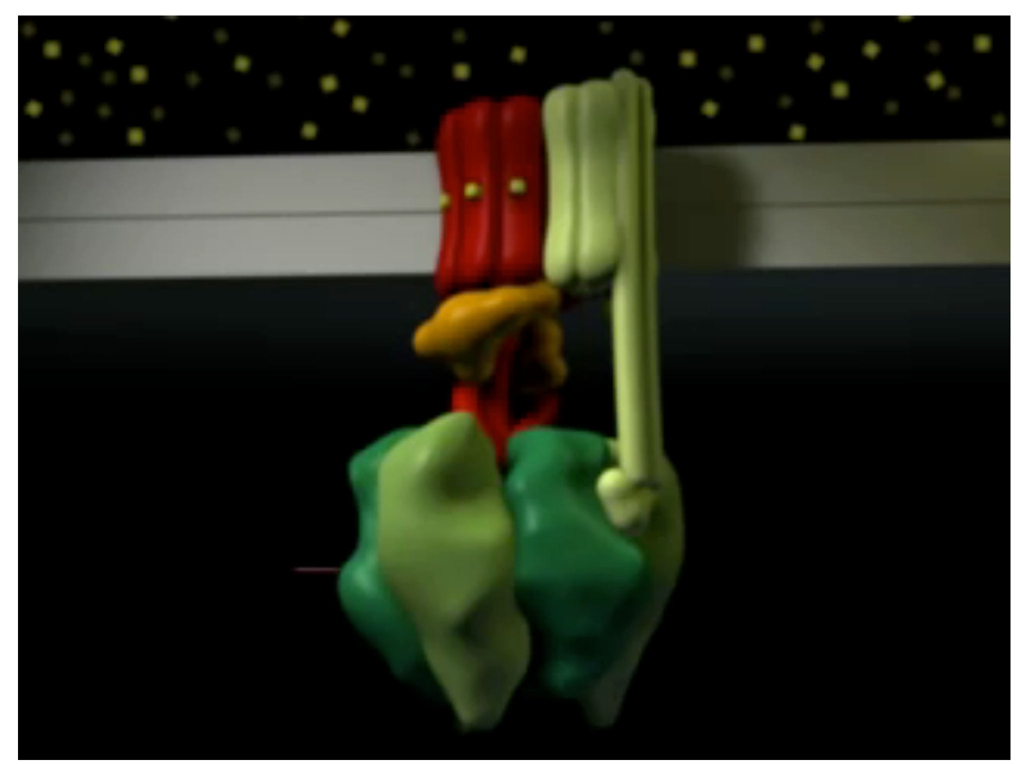


NADH products of the Cytric acid cycle are used to pump H$^+$ to the space between outer and inner mitochondrial membrane.

Gradient of H$^+$ concentration drives the ATP synthase motor that converts ADP to ATP.

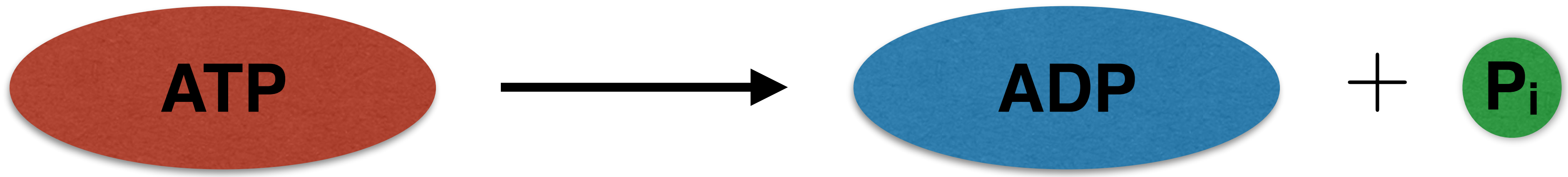
Note: ATP synthase can run in reverse and use ATP to pump H$^+$ at low concentrations.

ATP synthase



Energetics of ATP hydrolysis

How much energy is released during ATP hydrolysis?



$$\Delta G = \mu_{\mathrm{ADP}} + \mu_{\mathrm{P}} - \mu_{\mathrm{ATP}}$$

$$\boxed{\Delta G = \underbrace{\mu^0_{\mathrm{ADP}} + \mu^0_{\mathrm{P}} - \mu^0_{\mathrm{ATP}}}_{-12.5 k_B T} + k_B T \ln\left(\frac{[\mathrm{ADP}][\mathrm{P_i}]}{[\mathrm{ATP}]c_0}\right)}$$

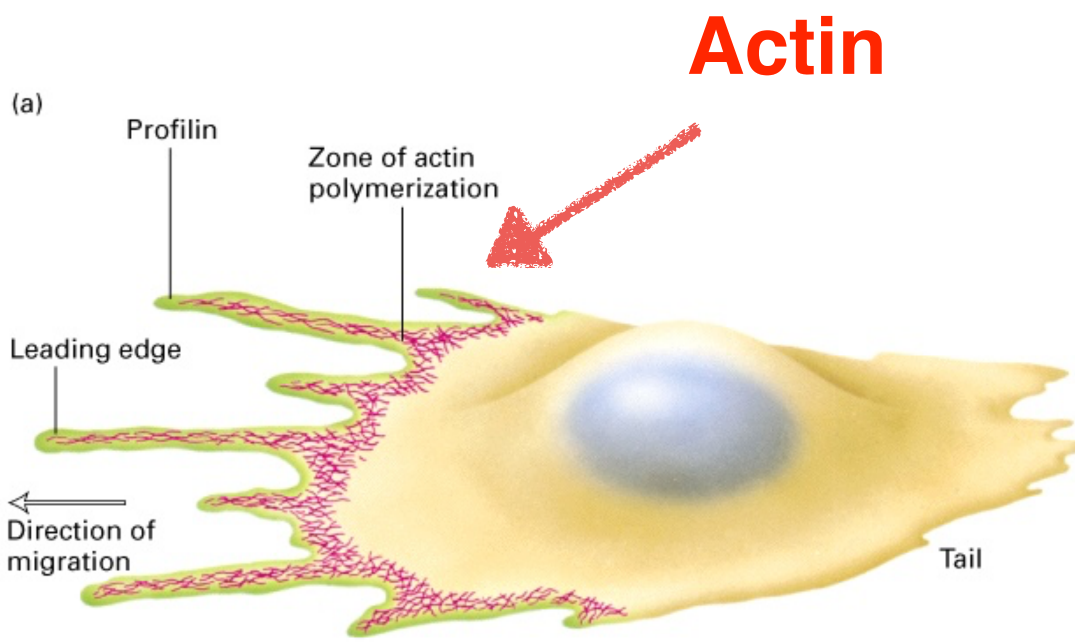
Under physiological conditions: $\Delta G \sim -20 k_B T$

$([\mathrm{ATP}], [\mathrm{ADP}], [\mathrm{P}_i] \sim 1\mathrm{mM})$

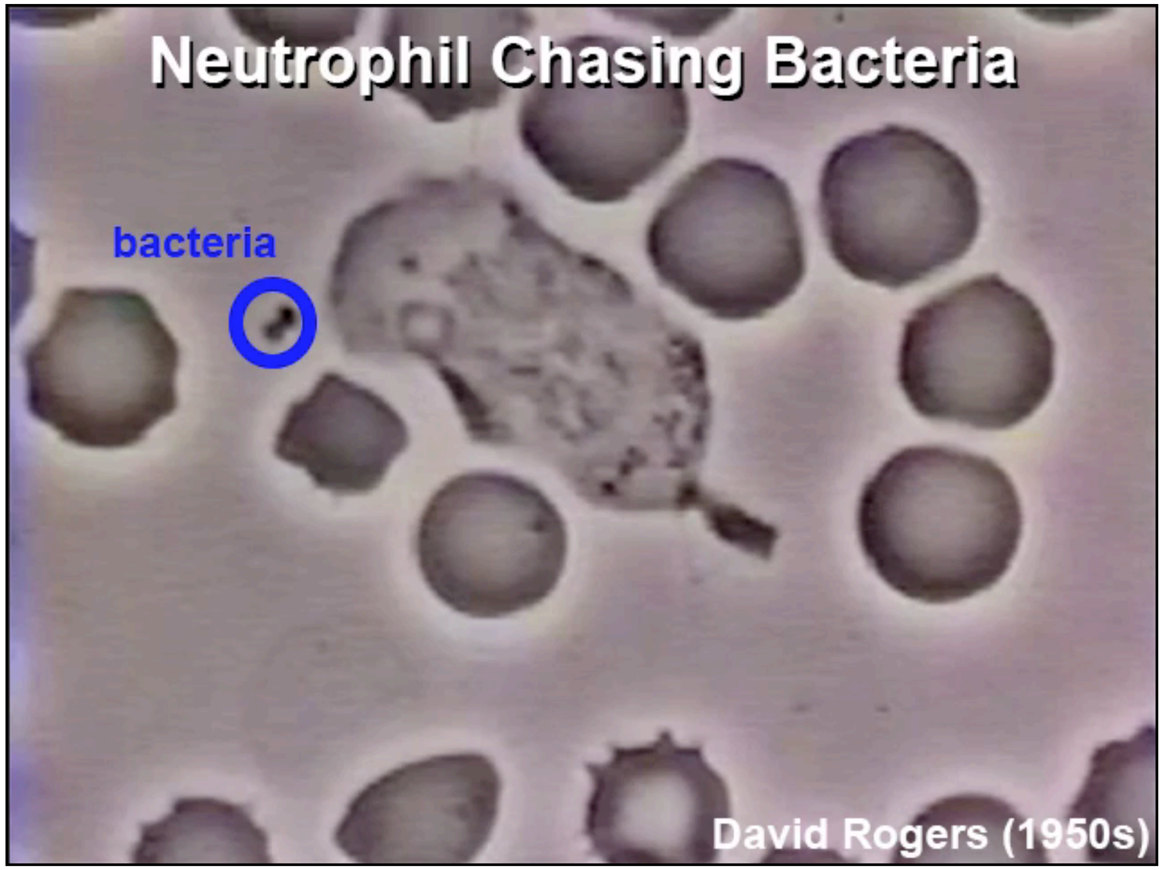
Chemical potentials are typically defined relative to concentration c_0 ~ 1 M.

$$\mu_s(c_s) = \mu_s(c_0) + k_B T \ln(c_s/c_0)$$

Crawling of cells



Immune system:
neutrophils chasing bacteria



David Rogers, 1950s

$v \sim 0.1 \mu\mathrm{m/s}$

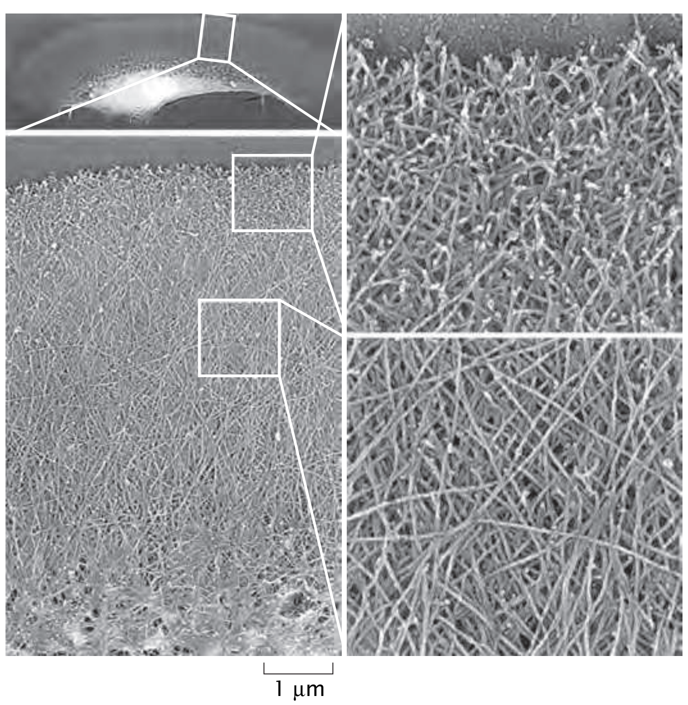
migration of skin cells during wound healing

spread of cancer cells during metastasis of tumors

amoeba searching for food

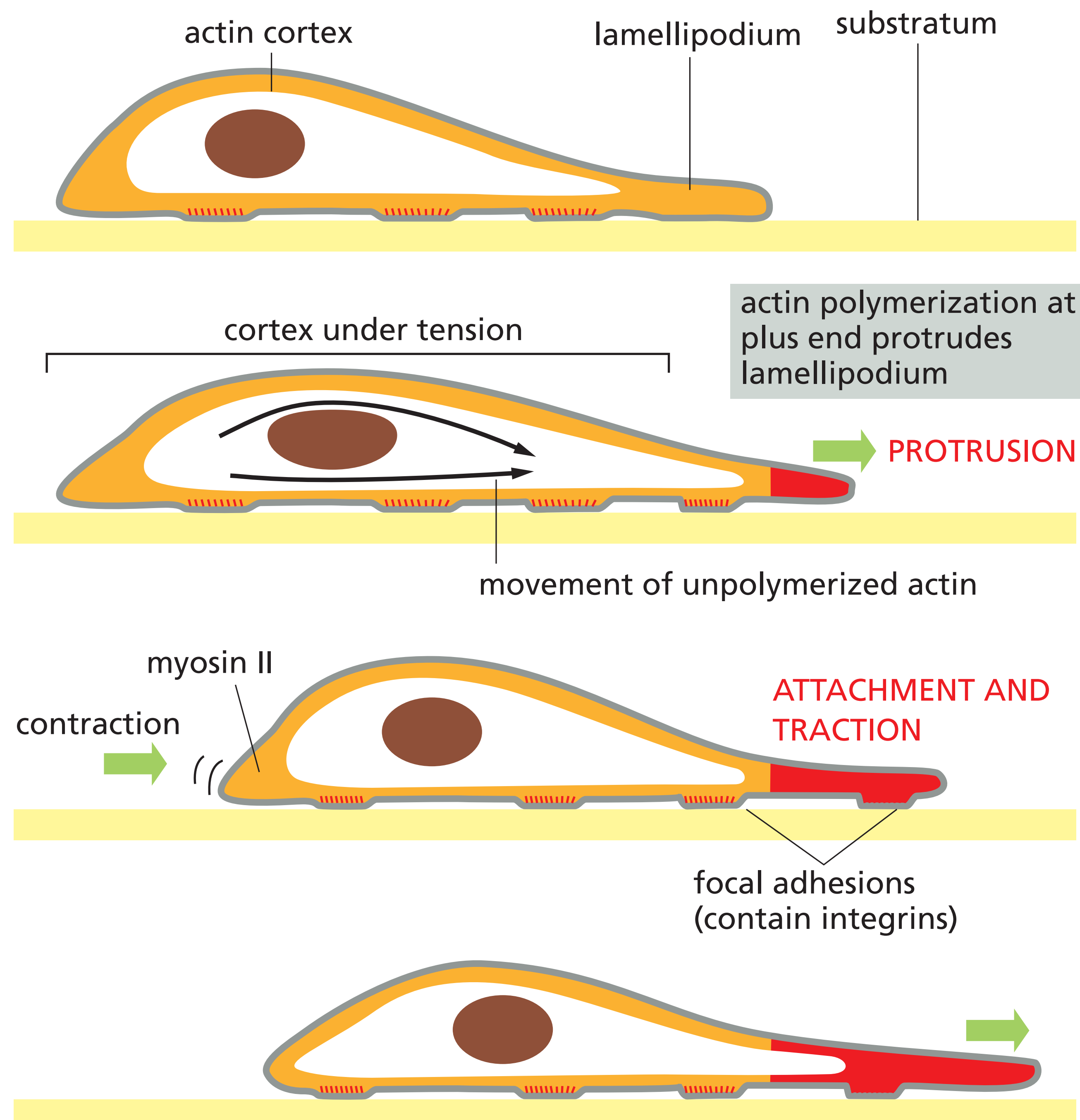
Crawling of cells

fish skin cell $v = 0.2\mu\mathrm{m/s}$



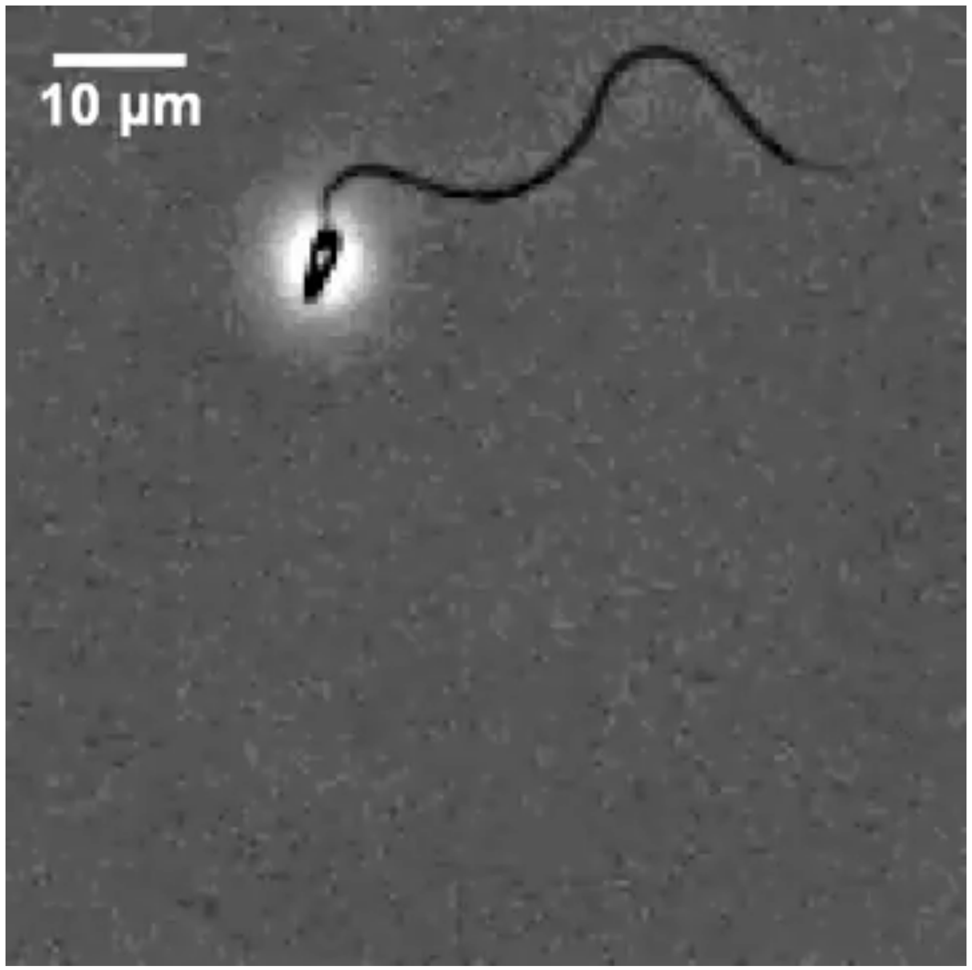
actin

R. Phillips et al., Physical Biology of the Cell



Alberts et al., Molecular Biology of the Cell

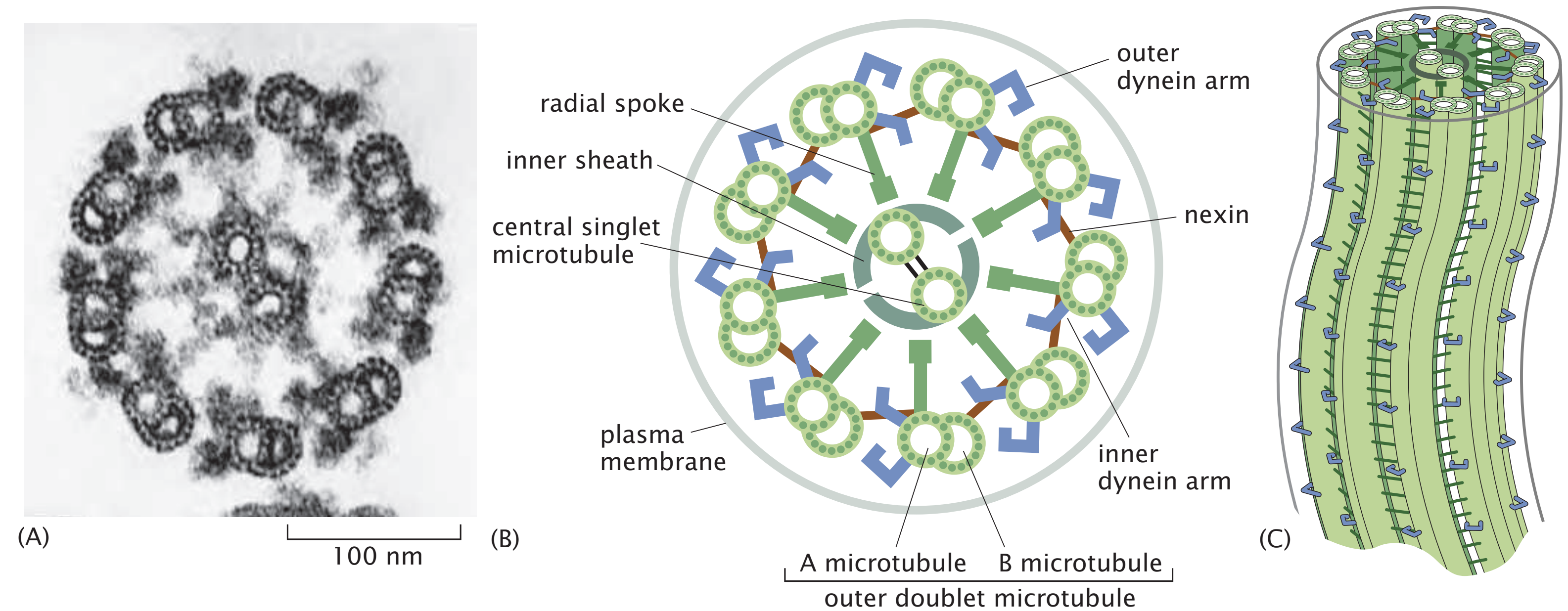
Swimming of sperm cells



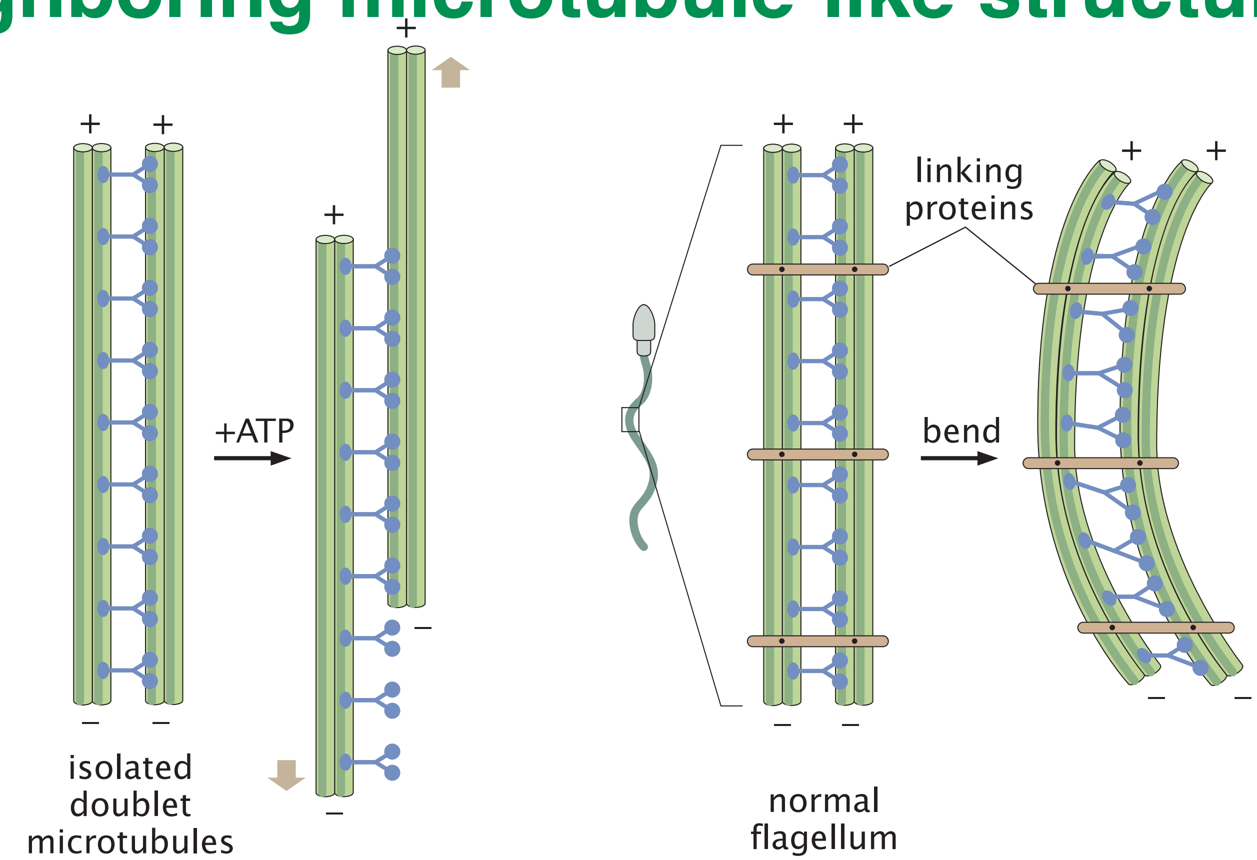
Jeff Guasto

$v \sim 50 \mu\mathrm{m/s}$

Sperm flagellum is constructed from microtubules



Bending is produced by motors walking on neighboring microtubule-like structures



R. Phillips et al., Physical Biology of the Cell

Further reading