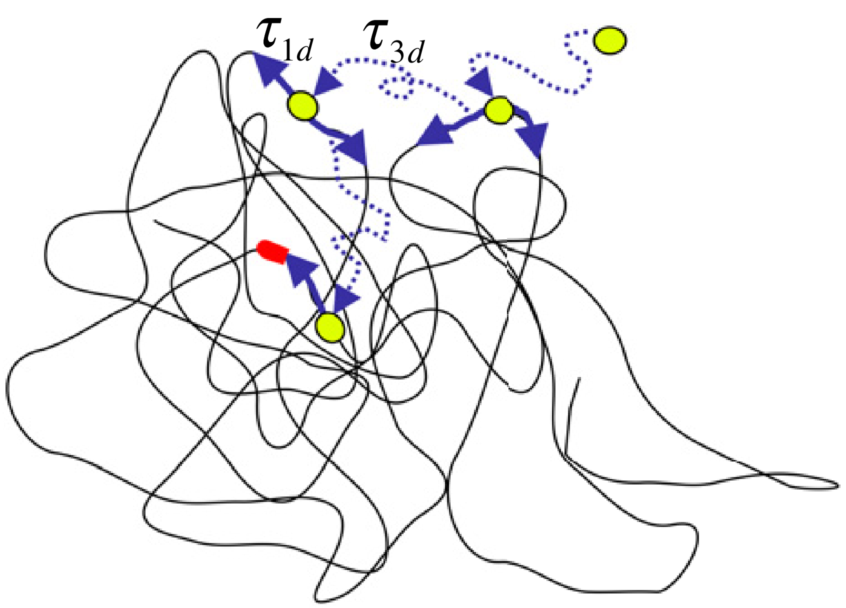
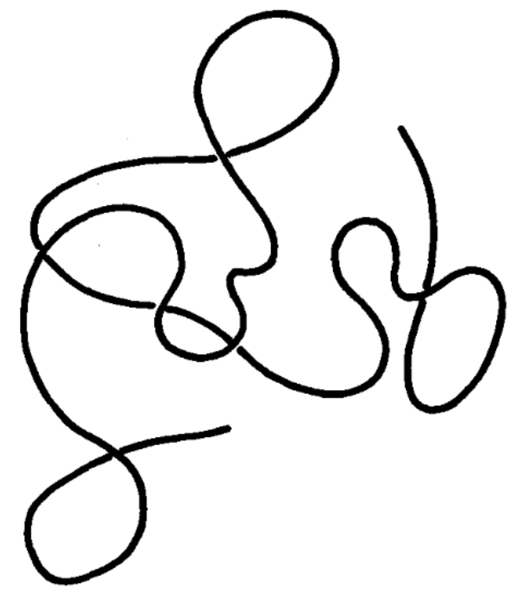
MAE 545: Lecture 21,22 (4/24)

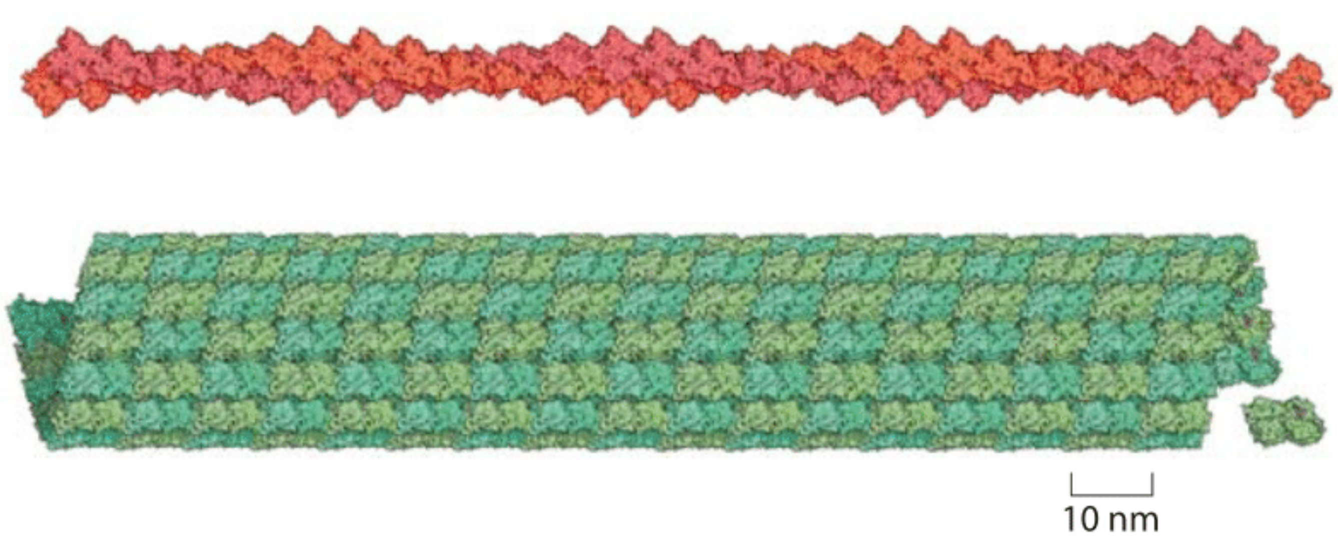
How proteins find target sites on DNA?



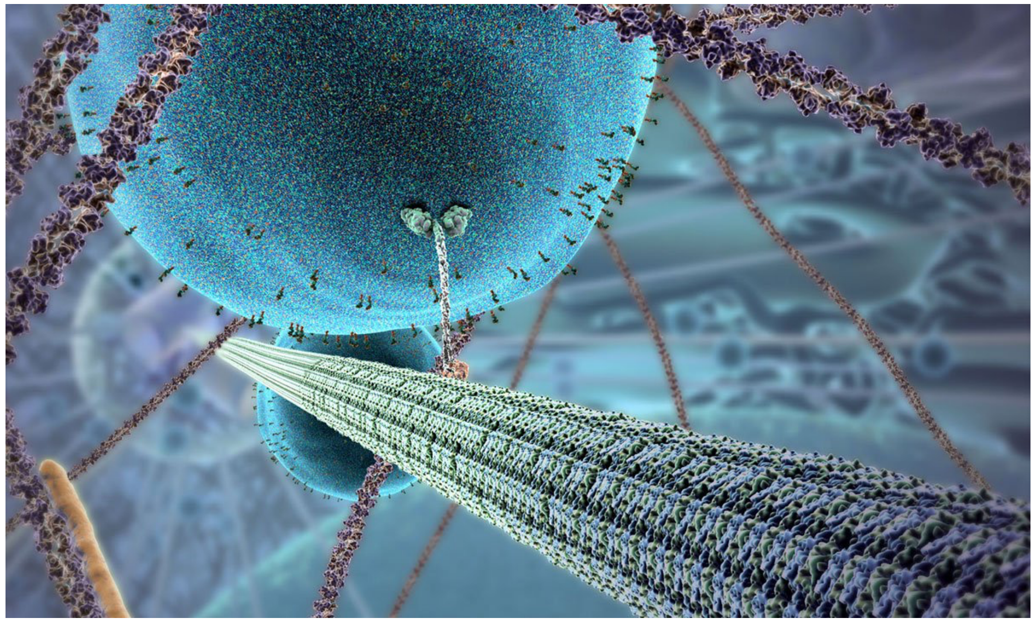
Statistical mechanics of polymers



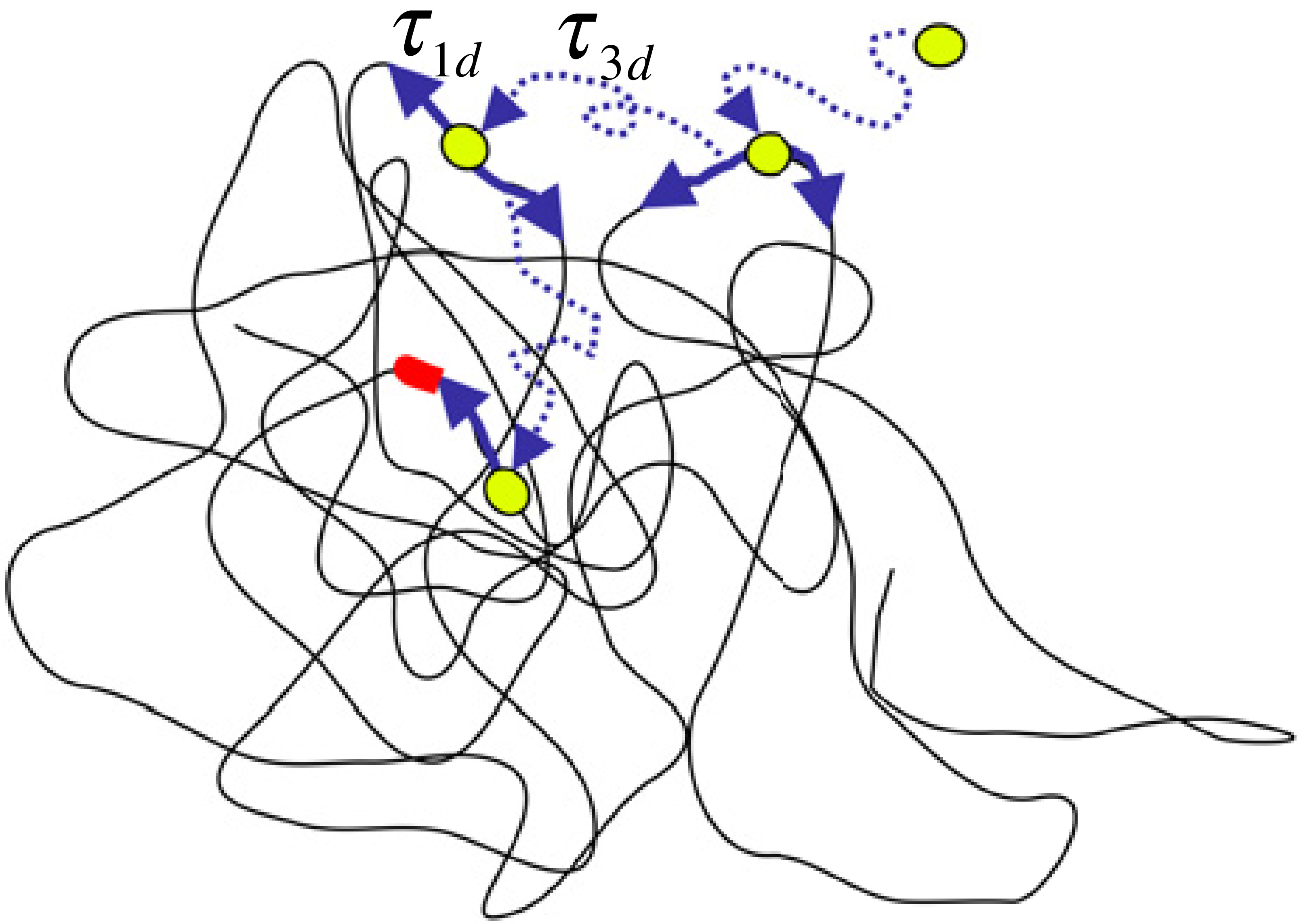
Growth dynamics of actin filaments and microtubules



Dynamics of molecular motors

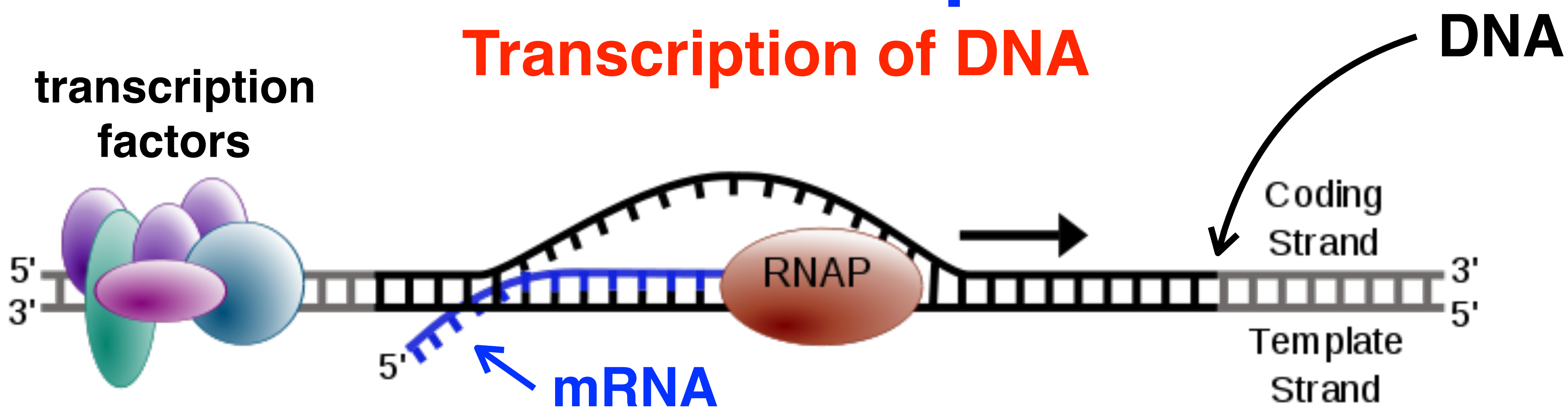


How proteins find target sites on DNA?



Production of new proteins

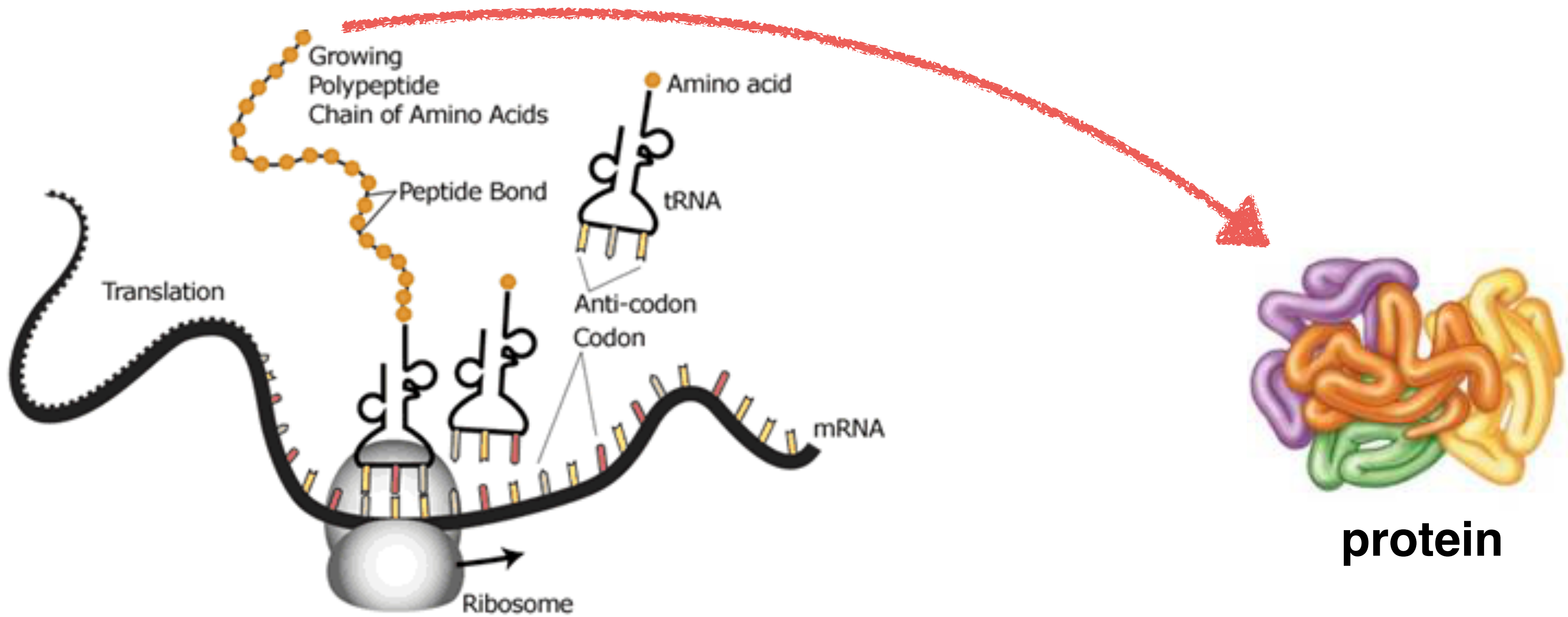
Transcription of DNA



Transcription factors are proteins, which bind to specific locations on DNA, and they help recruiting RNA polymerase (RNAP) that makes a messenger RNA (mRNA) copy of certain DNA segment.

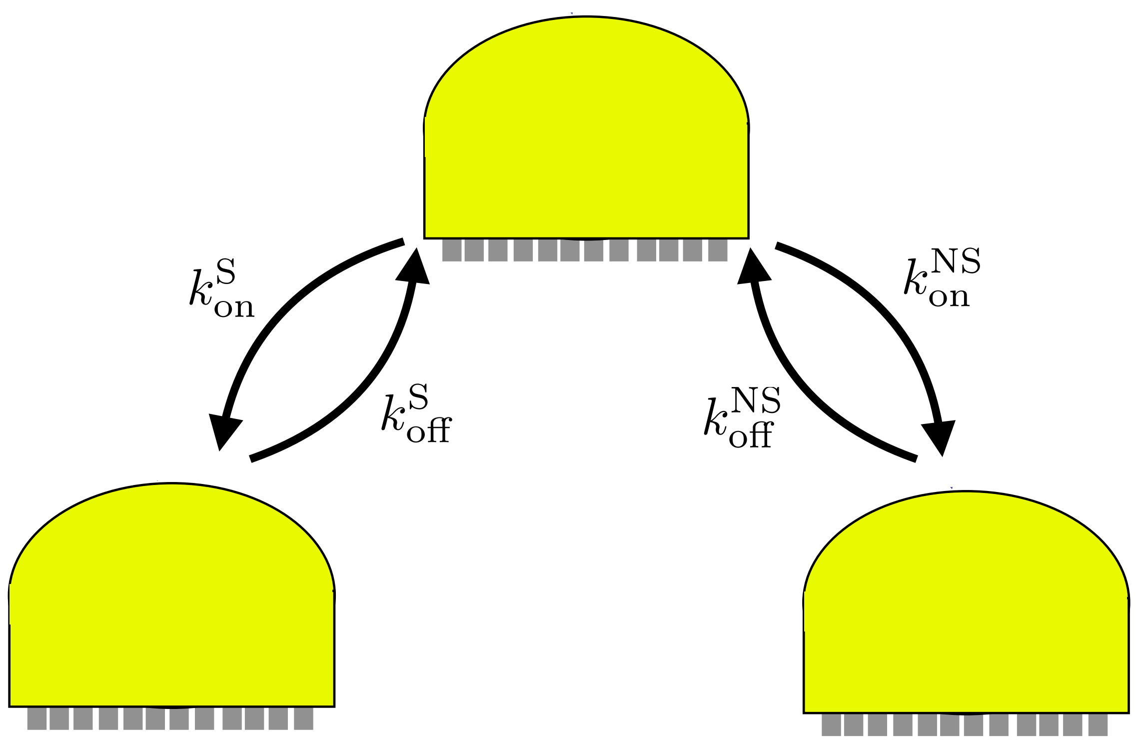
Note: some transcription factors (repressors) also prevent transcription.

Translation of mRNA



Protein-DNA interactions

protein



…ATTATGCATGACGATGTGGACAAACACCTGCGT…

target sequence

DNA sequence

$b = 0.34\text{nm}$

Binding to specific target sequence is strong

$$\Delta G^{\text{S}} \sim 20 - 25 k_B T$$

Binding to nonspecific sequence is weak

$$\Delta G^{\text{NS}} \sim 5 - 10 k_B T$$

(Binding free energies can be modified by changing salt concentration, etc.)

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on rates are diffusion limited

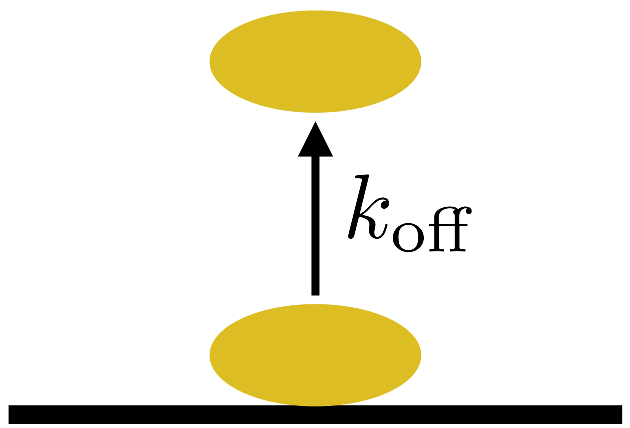
$$k_{\text{on}}^{\text{S}} \approx k_{\text{on}}^{\text{NS}} \approx 4\pi D_3 b$$

off rates depend on binding strengths

$$k_{\text{off}}^{\text{S}} = A_{\text{s}} e^{-\Delta G^{\text{S}}/k_B T} \ll k_{\text{off}}^{\text{NS}} = A_{\text{s}} e^{-\Delta G^{\text{NS}}/k_B T}$$

$$\frac{k_{\text{off}}^{\text{S}}}{k_{\text{off}}^{\text{NS}}} \sim 10^{-6}$$

How long proteins remain bound on DNA?



Probability that protein unbinds in a small time interval Δt :

$$k_{\rm off}\Delta t$$

Probability that protein remains bound for time t and then it unbinds between time t and $t+\Delta t$:

$$k_{\rm off}\Delta t \times (1 - k_{\rm off}\Delta t)^{t/\Delta t}$$

limit $\Delta t \to 0$

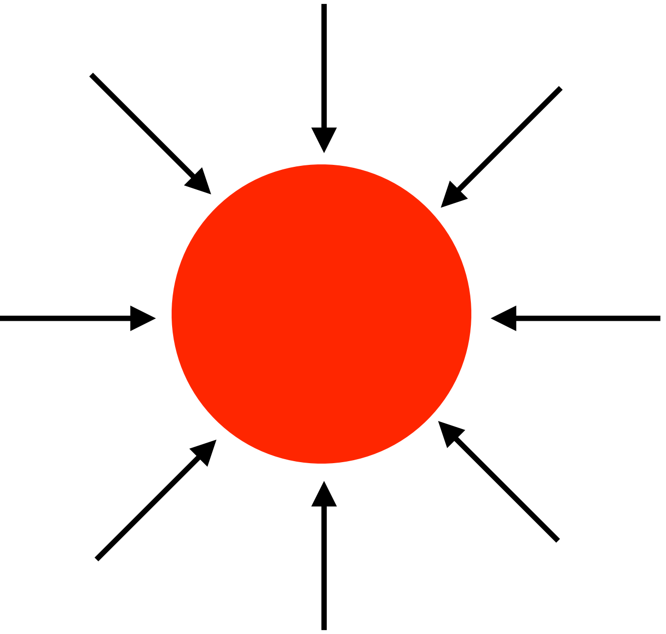
$$p(t) = k_{\rm off} e^{-k_{\rm off} t}$$

Average binding time $\langle t \rangle = \int_0^{\infty} t\, p(t) dt = \frac{1}{k_{\rm off}}$

Proteins remain bound to specific target sites for minutes to hours, while they unbind from nonspecific sites after milliseconds to seconds.

How quickly proteins find target sites on DNA?

Characteristic search time via 3D diffusion

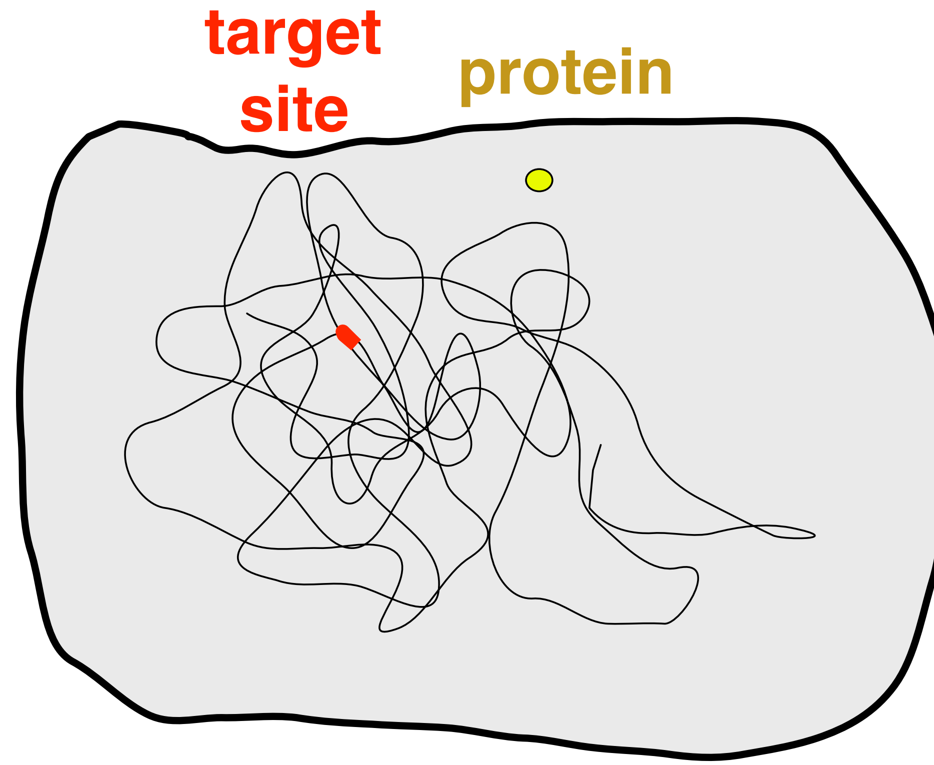


Approximate target site as absorbing sphere of radius

$$b = 0.34\text{nm}$$

concentration of free proteins far from the target site

$$c(r \to \infty) = [P]$$



rate of absorption (see slide 6)

$$I_0 = 4\pi D_3 b[\text{P}] \equiv k_{\text{on}}[\text{P}] \longrightarrow \boxed{k_{\text{on}} = 4\pi D_3 b}$$

Kinetics of protein binding/unbinding

$$[\text{P}] + [\text{T}] \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} [\text{P-T}]$$

$$\frac{d[\text{P-T}]}{dt} = k_{\text{on}}[\text{P}][\text{T}] - k_{\text{off}}[\text{P-T}]$$

[P-T] concentration of proteins bound to target sites
[P] concentration of free proteins
[T] concentration of empty target sites

short time binding kinetics for initially empty target sites [P-T]=0

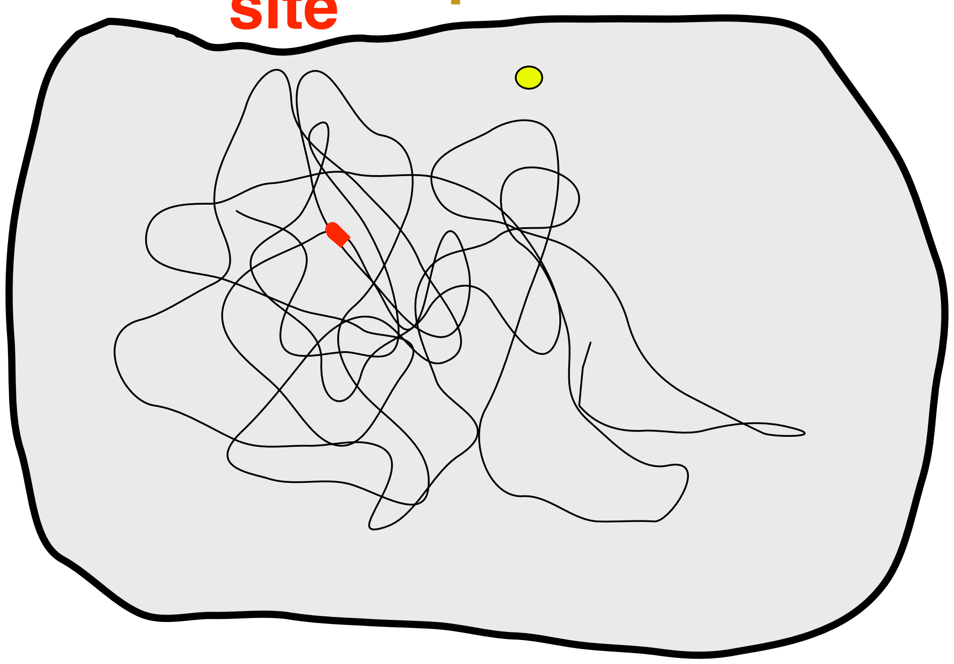
$$\frac{d[\text{P-T}]}{dt} = (k_{\text{on}}[\text{T}])[\text{P}] \equiv \frac{[\text{P}]}{t_s}$$

characteristic search time

$$\boxed{t_s = (k_{\text{on}}[\text{T}])^{-1}}$$

How quickly proteins find target sites on DNA?

Characteristic search time via 3D diffusion



$$k_{\rm on} = 4\pi D_3 b \qquad t_s = (k_{\rm on}[{\rm T}])^{-1}$$

1917 Smoluchowski theory

Example: characteristic search time for lac repressor protein in E. coli

$$b \approx 0.34{\rm nm} \qquad D_3 \approx 30\mu{\rm m}^2/{\rm s}$$

$$[{\rm T}] \sim 1 \text{ per cell} \sim 10^{-9}M$$

$$k_{\rm on} \sim 10^8 M^{-1} s^{-1} \qquad t_s \sim 10s$$

Molar concentration

$$1M = 6 \times 10^{26}{\rm m}^{-3}$$

in vitro experiments (1970)

$$k_{\rm on}^{\rm exp} \sim 10^{10} M^{-1} s^{-1} \qquad t_s \sim 0.1s$$

A.D.Riggs *et al.*,
J. Mol. Biol. **53**, 401-417 (1970)

Why is experimentally observed rate 100 times larger?

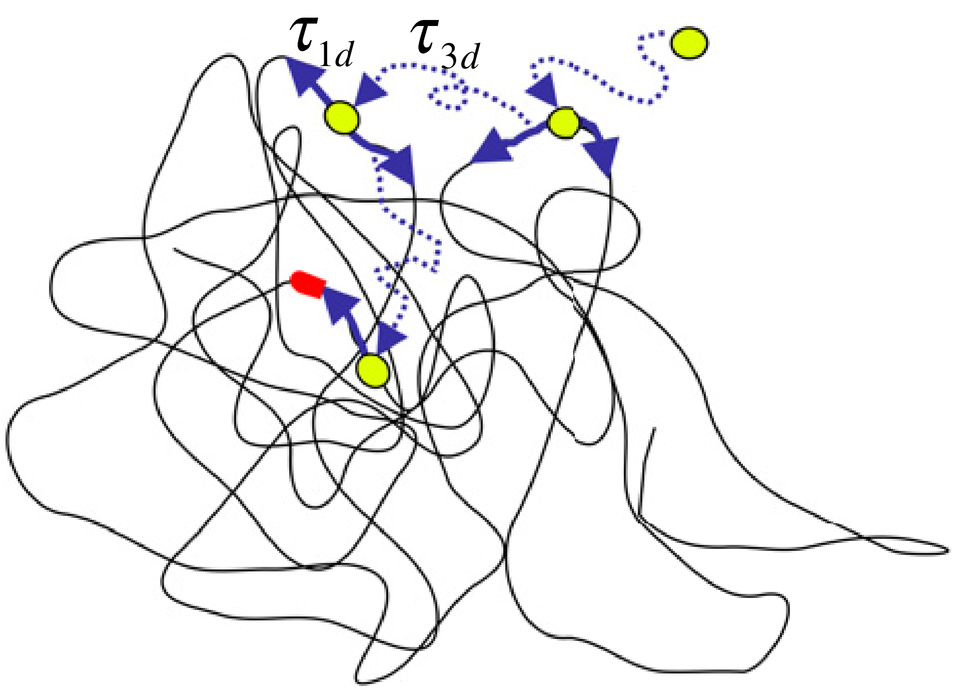
Berg - von Hippel theory (1980s)

(facilitated diffusion)

1. Proteins diffuse in space and non-specifically bind to a random location on DNA.

2. Proteins slide (diffuse) along the DNA.

3. Proteins jump (diffuse) to another random location on DNA and continue this sliding/ jumping process until the target site is found.



$b = 0.34\text{nm}$ L - DNA length

D_3 - diffusion constant in space

D_1 - diffusion constant along the DNA

How long that is it take to find a target site in this process?

O.G.Berg et al.,
Biochemistry **20**, 6929-48 (1981)

Berg - von Hippel theory (1980s)

First assume fixed sliding time τ_{1d}

Number of distinct sites visited during each sliding event

$$n = \sqrt{16 D_1 \tau_{1d}/(\pi b^2)}$$

(valid for $n >> 1$)

Probability that target site is found during a sliding event

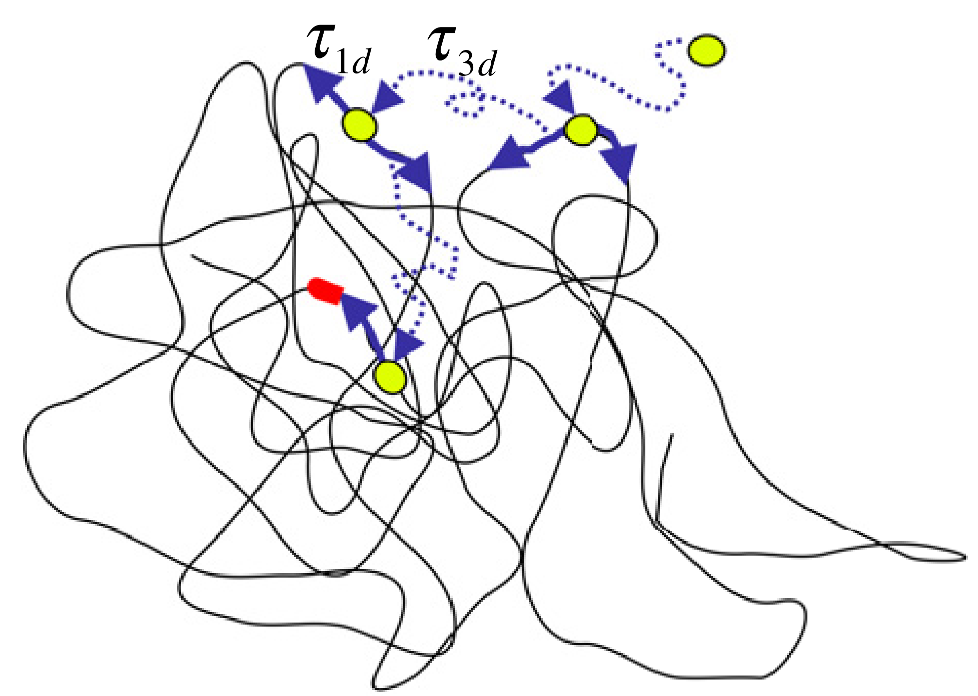
$$q = nb/L$$

Probability that target site is found exactly after N_R rounds

$$p(N_R) = q(1-q)^{N_R - 1}$$

Average number of rounds needed to find the target

$$\overline{N_R} = \sum_{N_R=1}^{\infty} N_R \, p(N_R) = 1/q$$



$b = 0.34\text{nm}$ L **- DNA length**

D_3 **- diffusion constant in space**

D_1 **- diffusion constant along the DNA**

τ_{3d} **- characteristic jumping time**

Average search time

$$\overline{t_s} = \overline{N_R}\,(\tau_{1d} + \tau_{3d})$$

O.G.Berg et al., Biochemistry **20**, 6929-48 (1981)

Facilitated diffusion

In reality sliding times are exponentially distributed

$$p(\tau_{1d}) = k_{\rm off}^{\rm NS}\; e^{-k_{\rm off}^{\rm NS}\tau_{1d}}$$

$$\langle \tau_{1d} \rangle = \int_0^\infty d\tau_{1d}\, \tau_{1d}\, p(\tau_{1d}) = 1/k_{\rm off}^{\rm NS}$$

Average number of distinct sites visited during each sliding

$$\langle n \rangle = \int_0^\infty d\tau_{1d}\, p(\tau_{1d}) \sqrt{16 D_1 \tau_{1d} / \left(\pi b^2\right)}$$

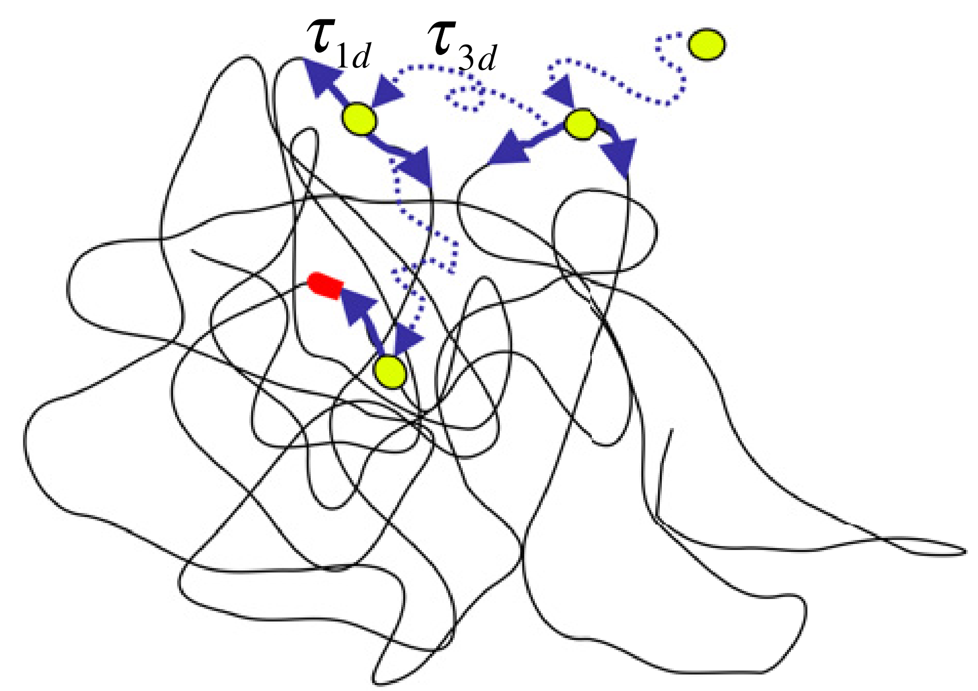
$$\langle n \rangle = 2\sqrt{D_1 \langle \tau_{1d} \rangle / \left(b^2\right)}$$

Average probability that target site is found during a sliding event

$$\langle q \rangle = \langle n \rangle\, b/L$$

Average number of rounds N_R needed to find the target site

$$\overline{\langle N_R \rangle} = 1/\langle q \rangle$$



$b = 0.34\mathrm{nm}$ L **- DNA length**

D_3 **- diffusion constant in space**

D_1 **- diffusion constant along the DNA**

τ_{3d} **- characteristic jumping time**

Average search time

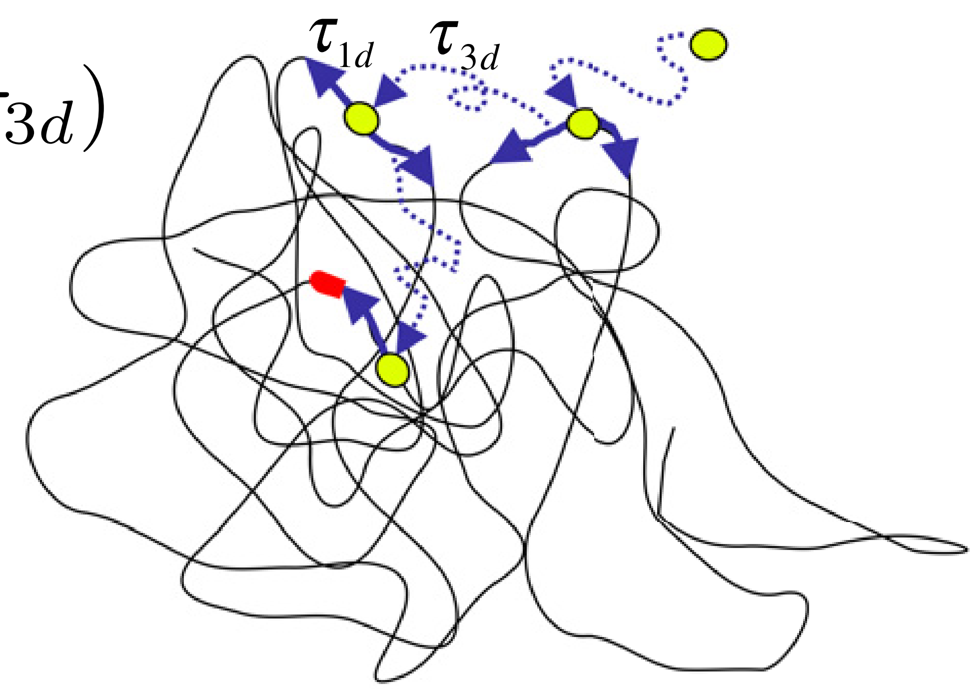
$$\overline{\langle t_s \rangle} = \overline{\langle N_R \rangle} \left(\langle \tau_{1d} \rangle + \tau_{3d}\right)$$

$$\overline{\langle t_s \rangle} = \frac{L}{2\sqrt{D_1 \langle \tau_{1d} \rangle}} \left(\langle \tau_{1d} \rangle + \tau_{3d}\right)$$

Facilitated diffusion

Average search time $\overline{\langle t_s \rangle} = \frac{L}{\langle \ell_{\text{sl}} \rangle} (\langle \tau_{1d} \rangle + \tau_{3d})$

Average sliding length $\langle \ell_{\text{sl}} \rangle = 2\sqrt{D_1 \langle \tau_{1d} \rangle}$



Optimal search time

$$\frac{d\overline{\langle t_s \rangle}}{d\langle \tau_{1d} \rangle} = 0 \rightarrow$$

$$\langle \tau_{1d} \rangle_{\text{opt}} = \tau_{3d}$$

$$\overline{\langle t_s \rangle}_{\text{opt}} = L\sqrt{\frac{\tau_{3d}}{D_1}}$$

$b = 0.34\text{nm}$ L - DNA length

D_3 - diffusion constant in space

D_1 - diffusion constant along the DNA

Search time for jumps alone

Typical jump time $\tau_{3d} = \frac{1}{k_{\text{on}} [\text{NS}]} = \frac{V}{4\pi D_3 L}$

Concentration of non-specific sites $[\text{NS}] = \frac{L/b}{V}$

average number of jumps needed to find the target $\overline{N}_{\text{jumps}} = \frac{L}{b}$

$$\overline{t}_{s,\text{jumps}} = \overline{N}_{\text{jumps}} \tau_{3d} = \frac{V}{4\pi D_3 b}$$

Search time for sliding alone

$$\langle t_s \rangle_{\text{sliding}} \sim \frac{L^2}{D_1}$$

Search time speed up for facilitated diffusion

$$\frac{\overline{t}_{s,\text{jumps}}}{\overline{\langle t_s \rangle}} = \frac{\langle \ell_{\text{sl}} \rangle}{b} \frac{\tau_{3d}}{(\langle \tau_{1d} \rangle + \tau_{3d})}$$

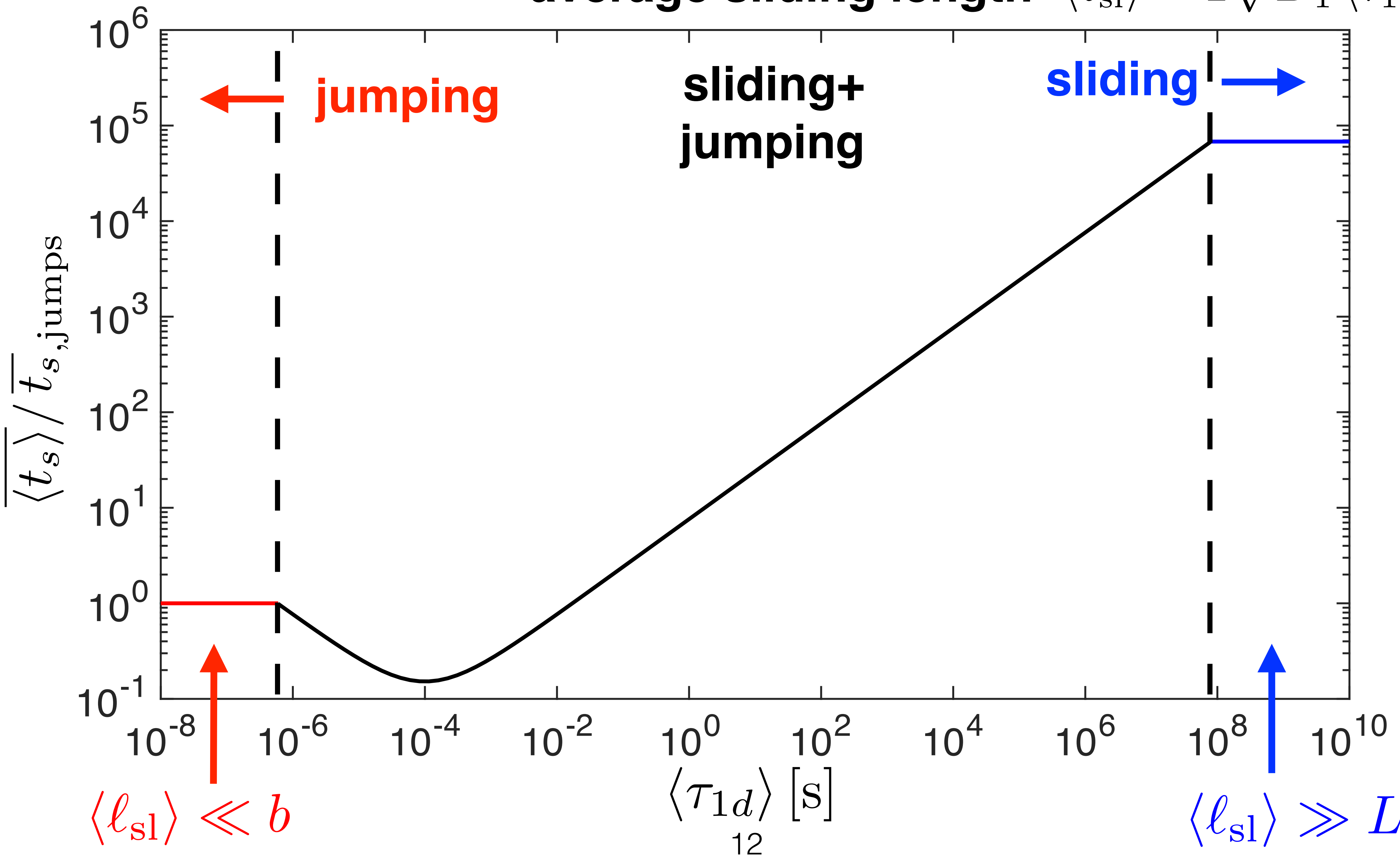
Example: search time for target site in bacteria on DNA with 10^6 base pairs

$\tau_{3d} = 10^{-4}\mathrm{s}$
$D_1 = 0.05\mu\mathrm{m}^2/s$
$L = 1\mathrm{mm}$
$b = 0.34\mathrm{nm}$

search time for jumps alone $\overline{t_s}_{,\mathrm{jumps}} = (L/b)\tau_{3d} \approx 300s$

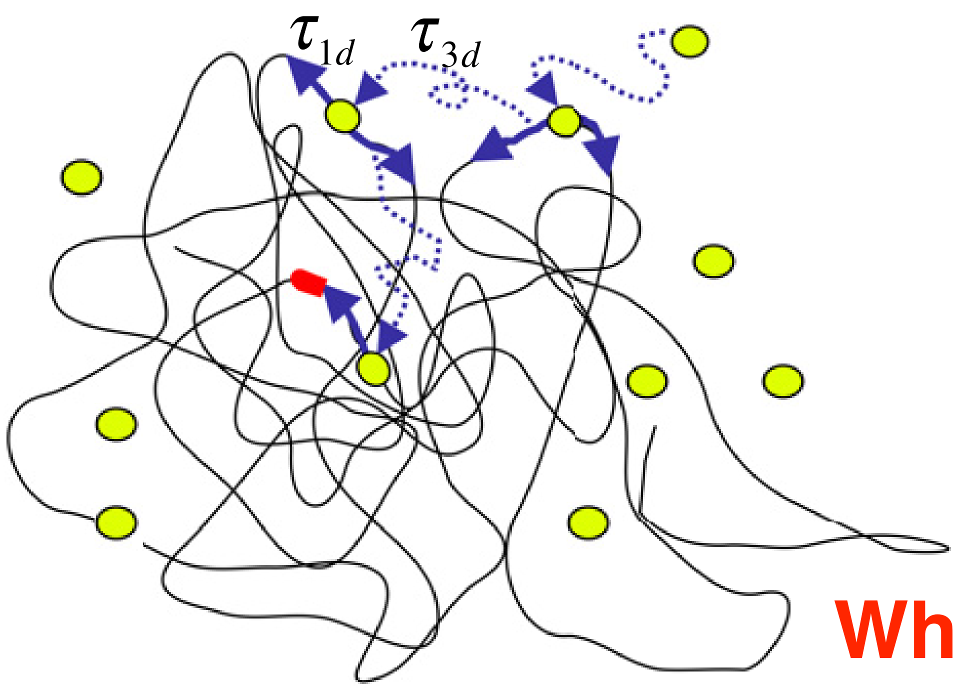
average search time $\overline{\langle t_s \rangle} = \frac{L}{\langle \ell_{\mathrm{sl}} \rangle}\left(\langle \tau_{1d} \rangle + \tau_{3d}\right)$

average sliding length $\langle \ell_{\mathrm{sl}} \rangle = 2\sqrt{D_1 \langle \tau_{1d} \rangle}$



Simultaneous search for target site by multiple proteins

Interactions and collisions between proteins are ignored



Search times for target site by individual proteins are exponentially distributed

$$p_1(t_s) = \frac{1}{\overline{\langle t_s \rangle}} e^{-t_s/\overline{\langle t_s \rangle}}$$

What is the typical search time for the fastest of *n* independently searching proteins?

(Extreme value distributions)

$$p_n(t_s) = n \times p_1(t_s) \times \left(\int_{t_s}^{\infty} dt' \; p_1(t') \right)^{n-1} = \frac{n}{\overline{\langle t_s \rangle}} e^{-n t_s/\overline{\langle t_s \rangle}}$$

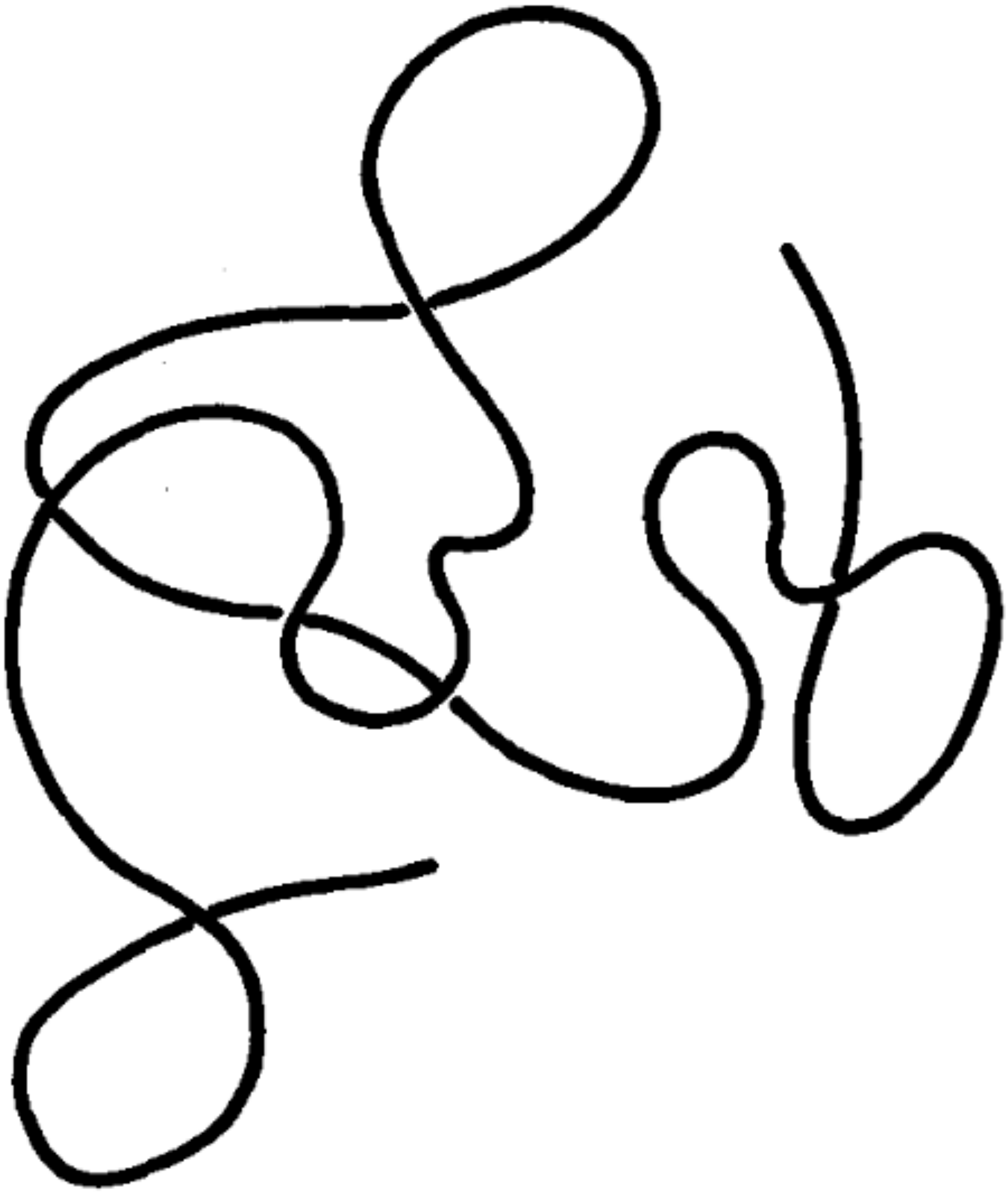
probability that one of *n* proteins finds the target site at time t_s

probability that other *n*-1 proteins take longer time to find the target site

Average search time is reduced by factor *n*

$$\int_0^{\infty} dt_s \, t_s \, p_n(t_s) = \frac{\overline{\langle t_s \rangle}}{n}$$

Statistical mechanics of polymers and filaments



Statistical mechanics of polymers and filaments

molecular dynamics simulation

Note: in equilibrium averaging over time is equivalent to averaging over all possible configurations weighted with Boltzmann weights!

partition function (sum over all possible configurations)

$$Z = \sum_c e^{-E_c/k_B T}$$

expected value of observables

$$\langle \mathcal{O} \rangle = \sum_c \mathcal{O}_c \frac{e^{-E_c/k_B T}}{Z}$$

E_c **energy of a given configuration**

T **temperature**

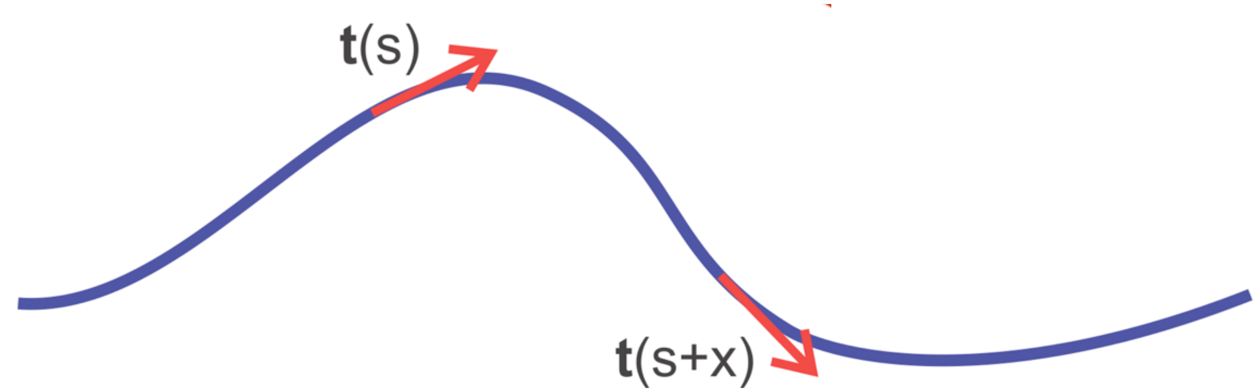
k_B **Boltzmann constant**

$$k_B = 1.38 \times 10^{-23} \mathrm{JK}^{-1}$$

Persistence length

correlations between tangents

$$\langle \mathbf{t}(s) \cdot \mathbf{t}(s+x) \rangle = e^{-x/\ell_p}$$



tangents become uncorrelated beyond persistence length!

persistence length

$$\ell_p = \frac{B}{k_B T}$$

B **- filament bending rigidity**

T **- temperature**

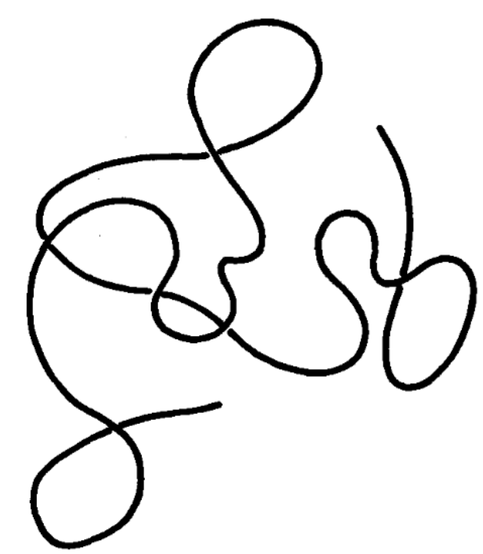
L **- filament length**

Short filaments remain straight

$$L \ll \ell_p$$

Long filaments perform self-avoiding random walk

$$L \gg \ell_p$$

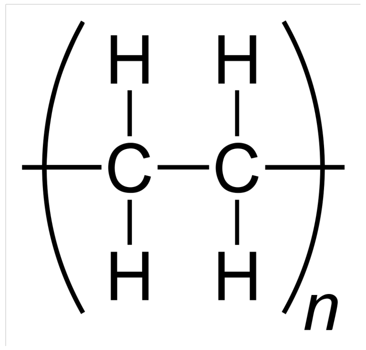


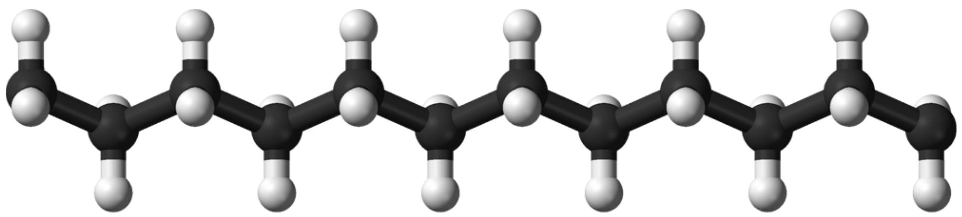
Examples: persistence length

$$\ell_p = \frac{B}{k_B T}$$

polyethylene

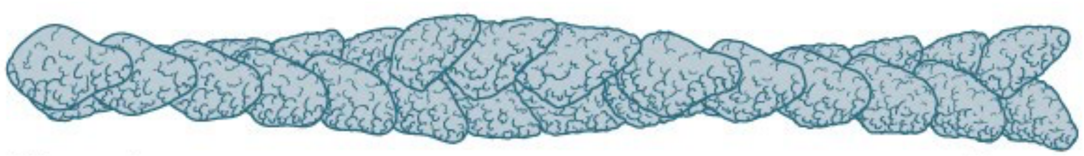
$\ell_p = 2.6\,\mathrm{nm}$



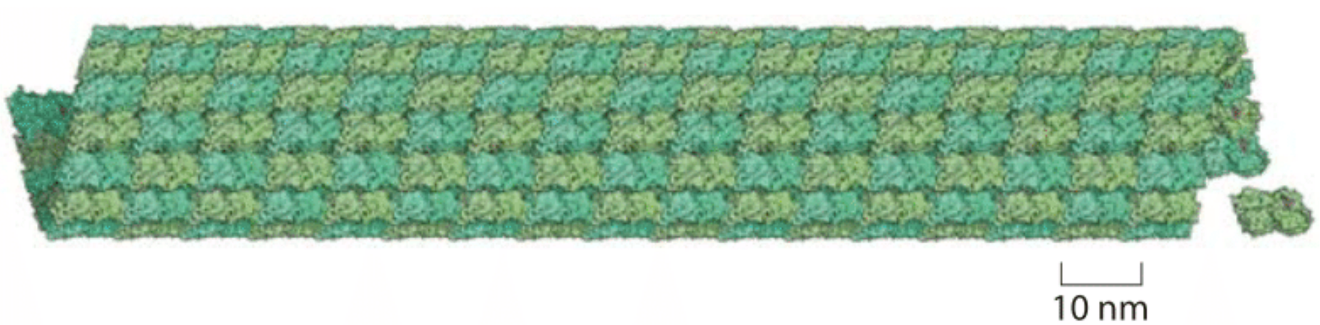


Persistence length for polymers is on the order of nm

actin $\ell_p \approx 17\,\mu\mathrm{m}$

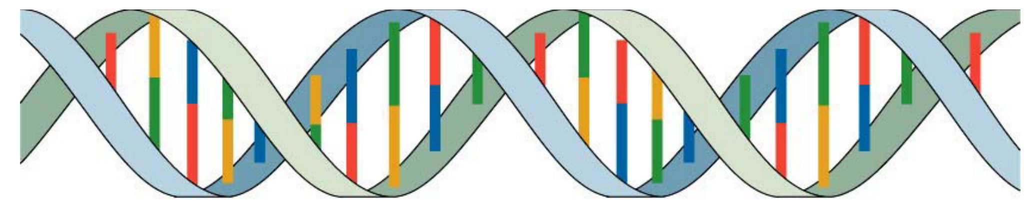


microtubule $\ell_p \approx 1.4\,\mathrm{mm}$



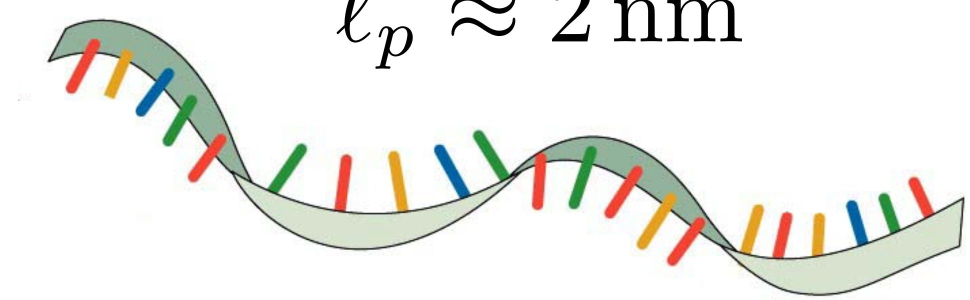
double stranded DNA

$\ell_p \approx 50\,\mathrm{nm}$



single stranded DNA

$\ell_p \approx 2\,\mathrm{nm}$

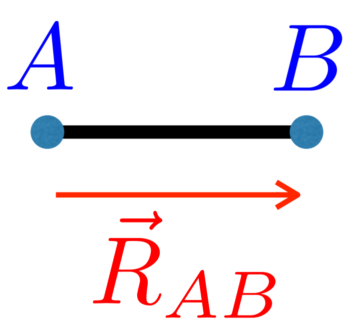


uncooked spagetthi

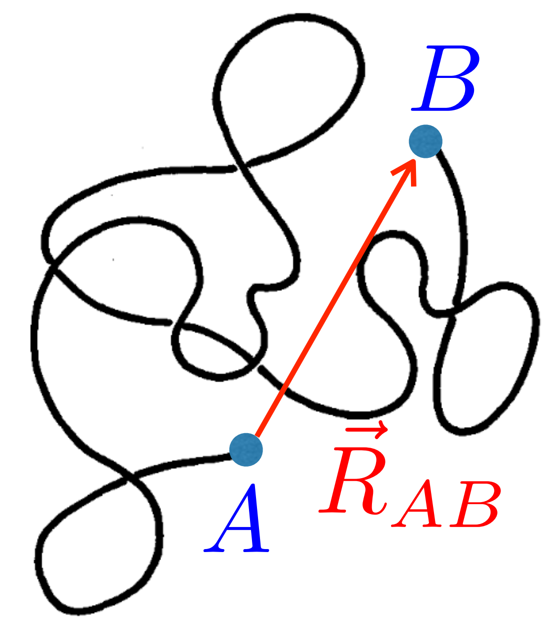
$\ell_p \approx 10^{18}\,\mathrm{m}$

End-to-end distance

Short filaments $L \ll \ell_p$



Long filaments $L \gg \ell_p$



Over time thermal fluctuations reorient filaments in all possible directions!

$$\left\langle \vec{R}_{AB} \right\rangle = 0$$

$$\left\langle \vec{R}^2_{AB} \right\rangle \approx L^2$$

$$\left\langle \vec{R}_{AB} \right\rangle = 0$$

$$\left\langle \vec{R}^2_{AB} \right\rangle \approx 2\ell_p L = \frac{2BL}{k_B T}$$

Polymers shrink, when temperature is increased! Negative thermal expansion of rubber.

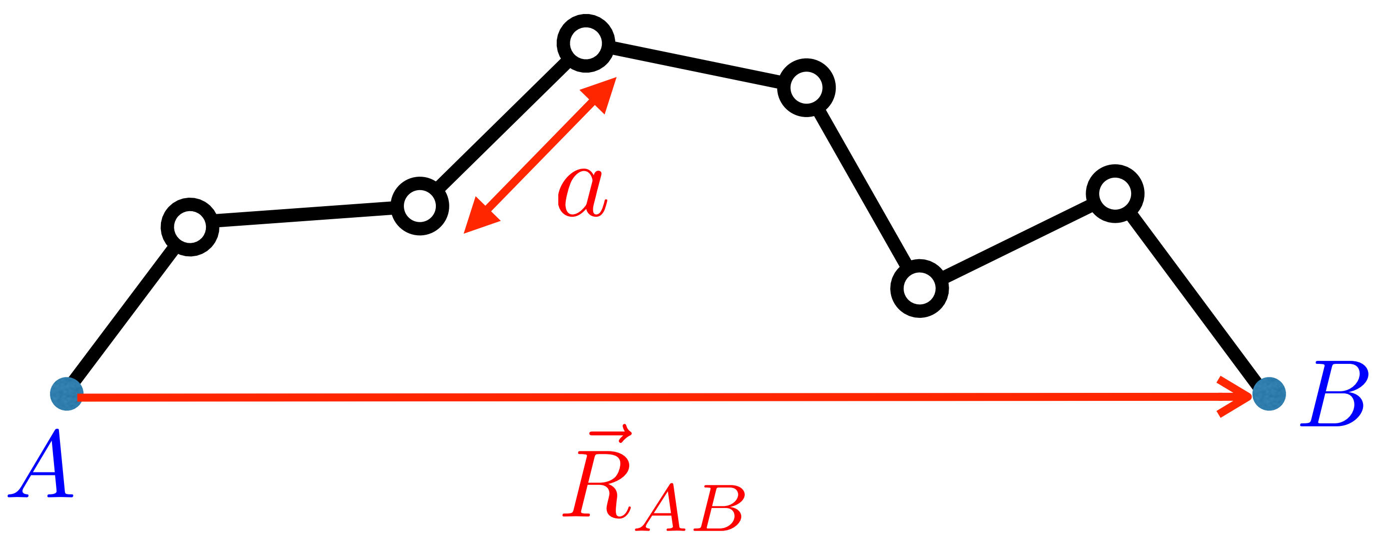
Exact result

$$\left\langle \vec{R}^2_{AB} \right\rangle = 2\ell_p L \left[1 - \frac{\ell_p}{L} \left(1 - e^{-L/\ell_p} \right) \right]$$

Ideal chain vs worm-like chain

Ideal chain

***N* identical unstretchable links (Kuhn segments) of length *a* with freely rotating joints**

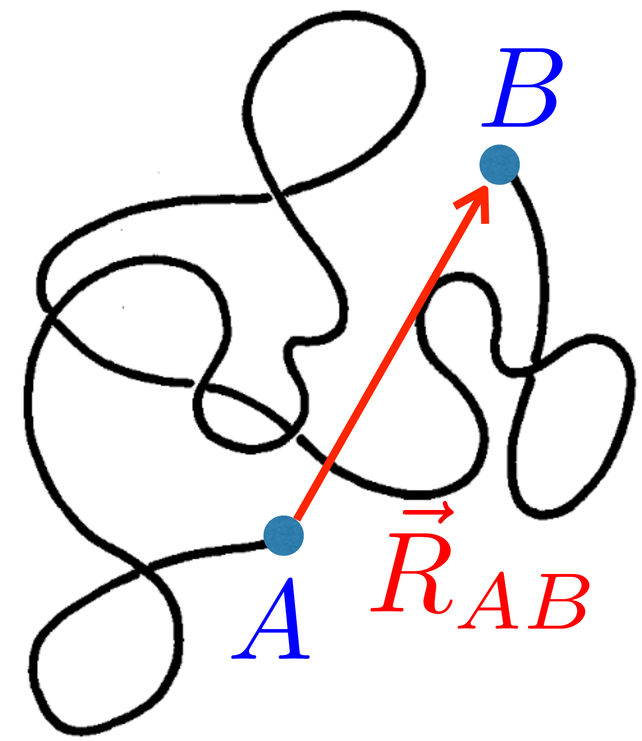


Each configuration *C* has zero energy cost.

$$E_c = 0$$

Worm-like chain

Continuous unstretchable rod



Bending energy cost of configuration *C*:

$$E_c = \frac{B}{2}\int_0^L ds \left(\frac{d^2\vec{r}}{ds^2}\right)^2$$

Each configuration *C* appears with probability

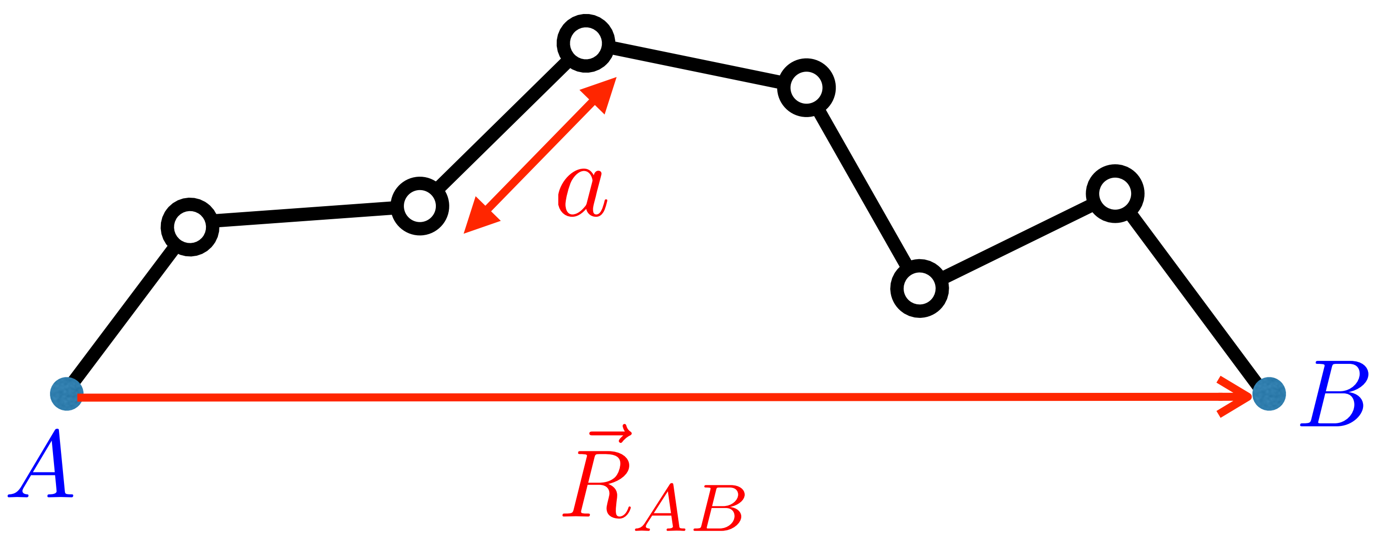
$$p_c \propto e^{-E_c/k_B T}$$

***L* = *Na* - chain length**

Ideal chain vs worm-like chain

Ideal chain

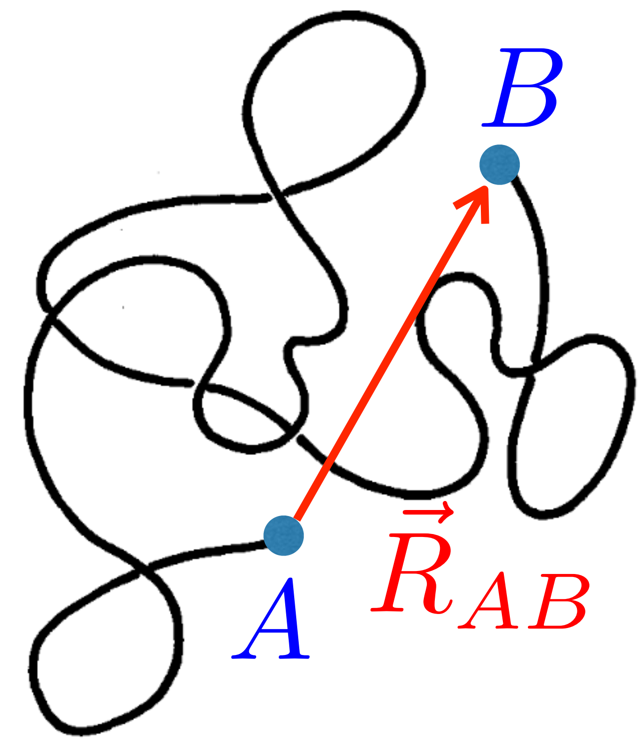
_N_ identical unstretchable links (Kuhn segments) of length _a_ with freely rotating joints



$$\left\langle \vec{R}_{AB}^2 \right\rangle = Na^2 = aL$$

Worm-like chain

Continuous unstretchable rod



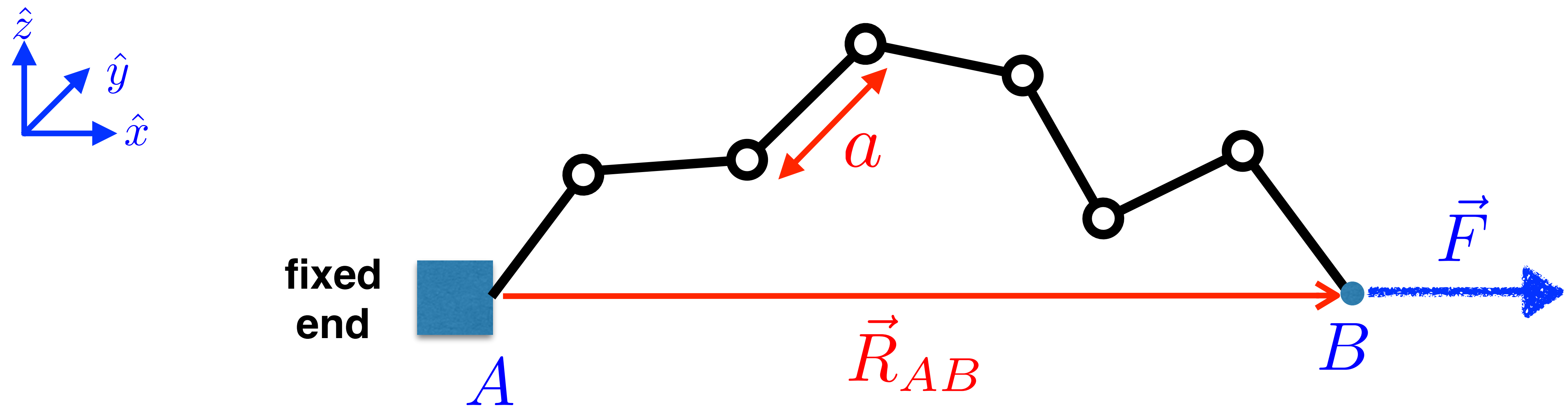
$$\left\langle \vec{R}_{AB}^2 \right\rangle \approx 2\ell_p L = \frac{2BL}{k_B T}$$

End-to-end distance fluctuations can be made identical if one choses the segment length to be

$$a = 2\ell_p$$

***L* = *Na* - chain length**

Stretching of ideal freely jointed chain



Exact result for end-to-end distance

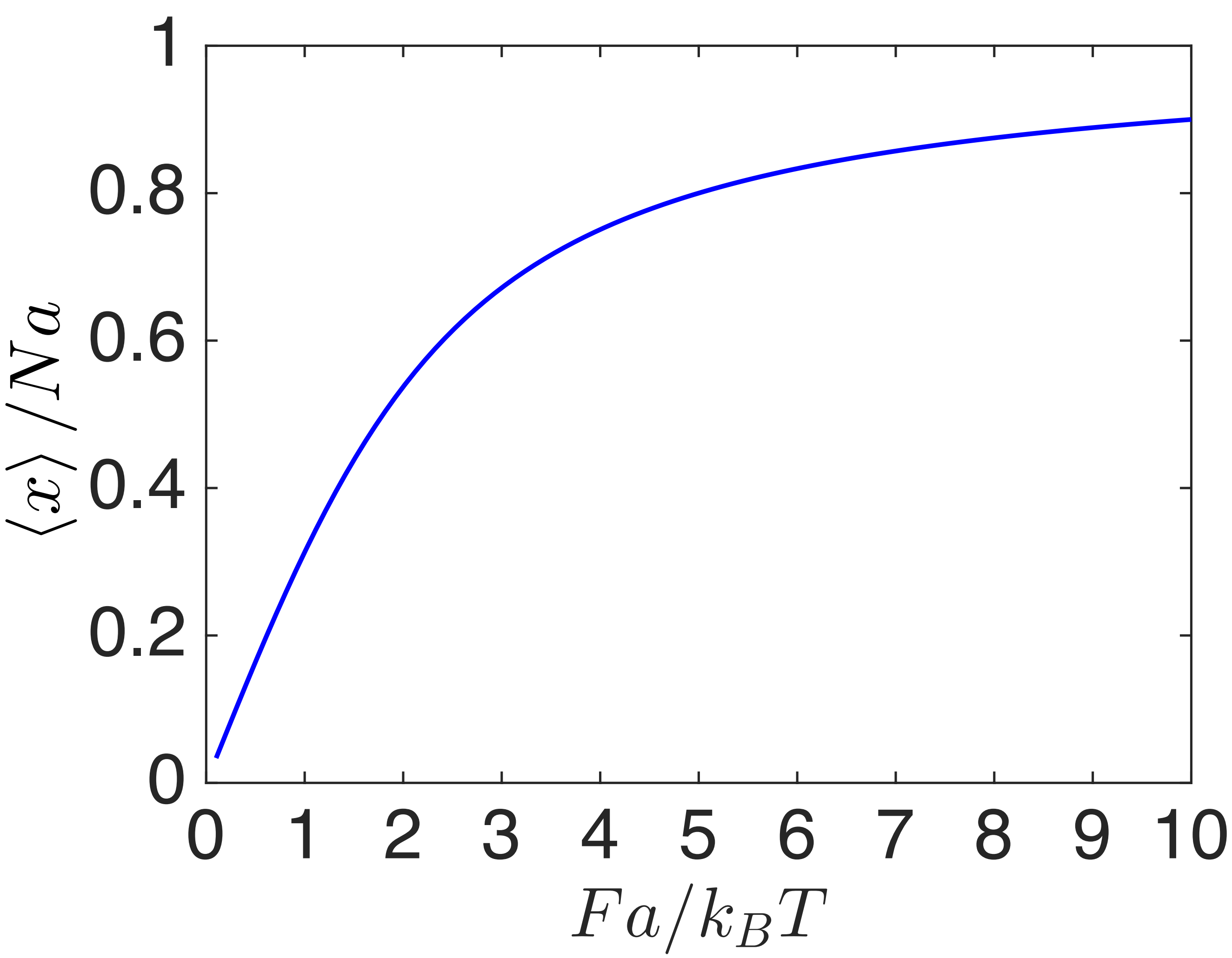
$$\langle x \rangle = Na\left(\coth\left[\frac{Fa}{k_BT}\right] - \frac{k_BT}{Fa}\right)$$

small force $Fa \ll k_BT$

$$\langle x \rangle \approx \frac{FNa^2}{3k_BT} = \frac{2FL\ell_p}{3k_BT}$$

large force $Fa \gg k_BT$

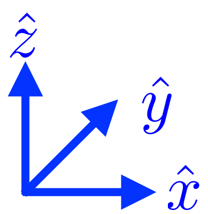
$$\langle x \rangle \approx Na\left(1 - \frac{k_BT}{Fa}\right) = L\left(1 - \frac{k_BT}{2F\ell_p}\right)$$

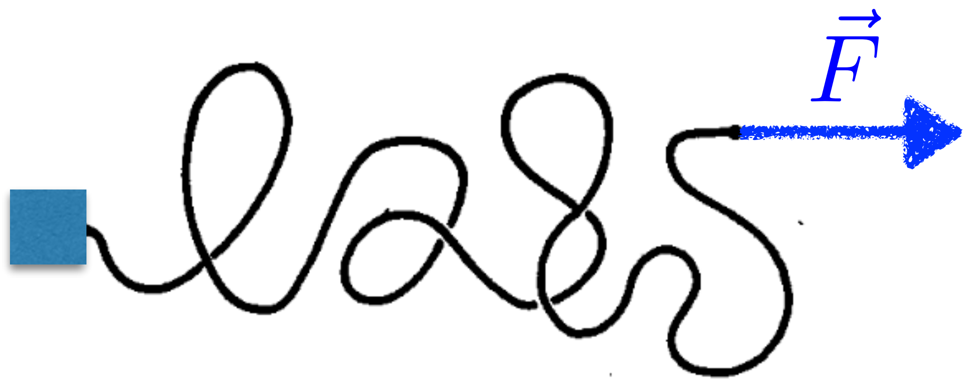


Stretching of worm-like chains

Assume long chains $L \gg \ell_p$

small force $F\ell_p \ll k_B T$



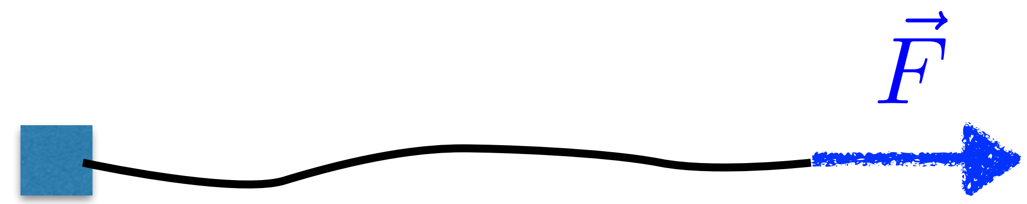


$$\langle x \rangle \approx \frac{2FL\ell_p}{3k_BT} \equiv \frac{F}{k}$$

entropic spring constant

$$k = \frac{3k_BT}{2L\ell_p} = \frac{3k_B^2T^2}{2LB}$$

large force $F\ell_p \gg k_B T$



$$\langle x \rangle \approx L\left[1 - \sqrt{\frac{k_BT}{4F\ell_p}}\right]$$

B **- filament bending rigidity**

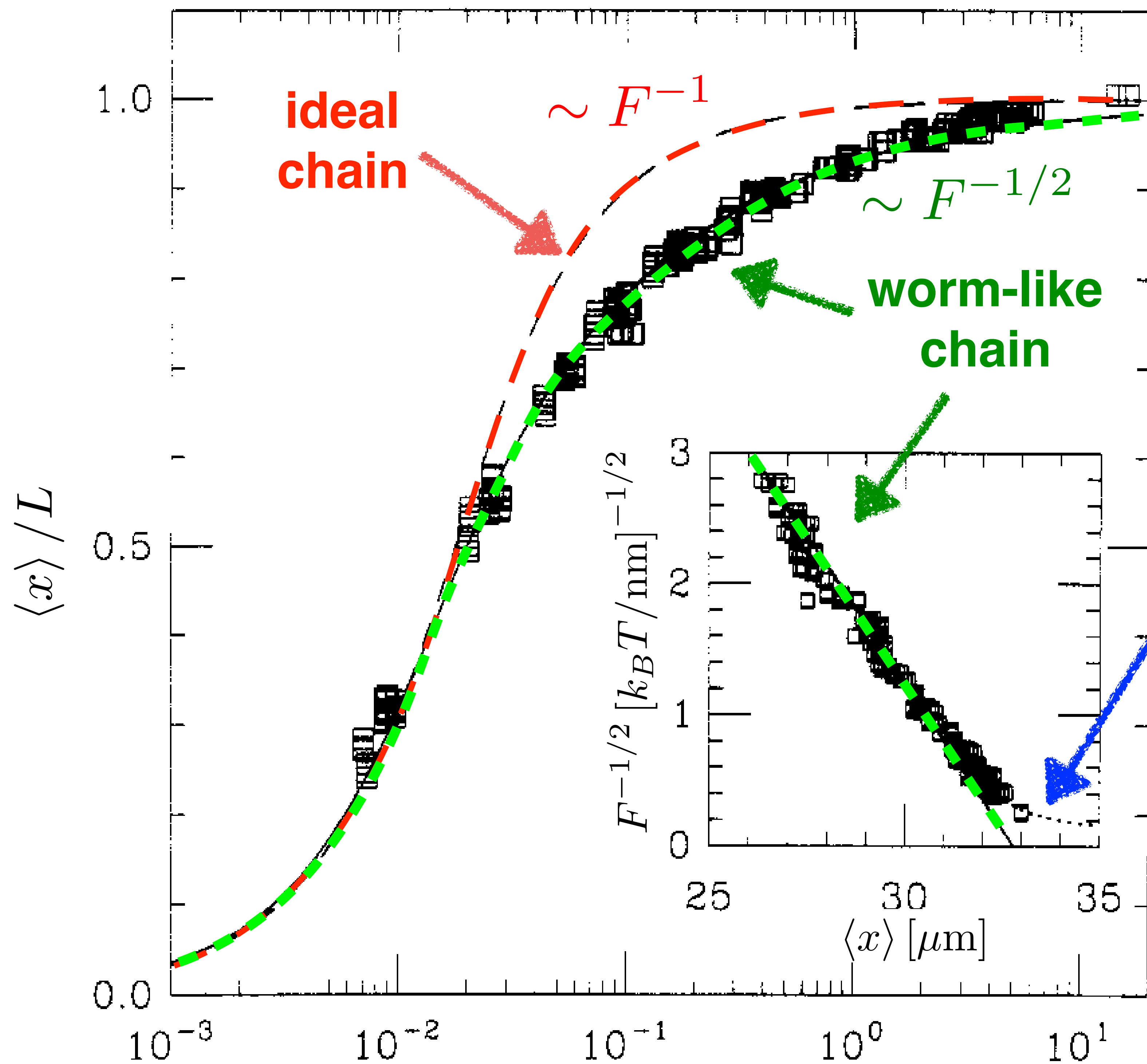
Approximate expression that interpolates between both regimes

$$\frac{F\ell_p}{k_BT} = \frac{1}{4}\left(1 - \frac{\langle x \rangle}{L}\right)^{-2} - \frac{1}{4} + \frac{\langle x \rangle}{L}$$

J.F. Marko and E.D. Siggia, Macromolecules **28**, 8759-8770 (1995)

Experimental results for stretching of DNA

$L = 32.8\mu\text{m}$



$1 k_B T/\text{nm} \approx 4\text{pN}$

Stretching of the DNA backbone

$$\langle x \rangle \approx L \left[1 - \sqrt{\frac{k_B T}{4 F \ell_p}} \right] + \frac{FL}{\gamma}$$

For DNA

$\ell_p = 50\text{nm}$

$\gamma \approx 500 k_B T/\text{nm} \approx 2\text{nN}$

Improved interpolation formula

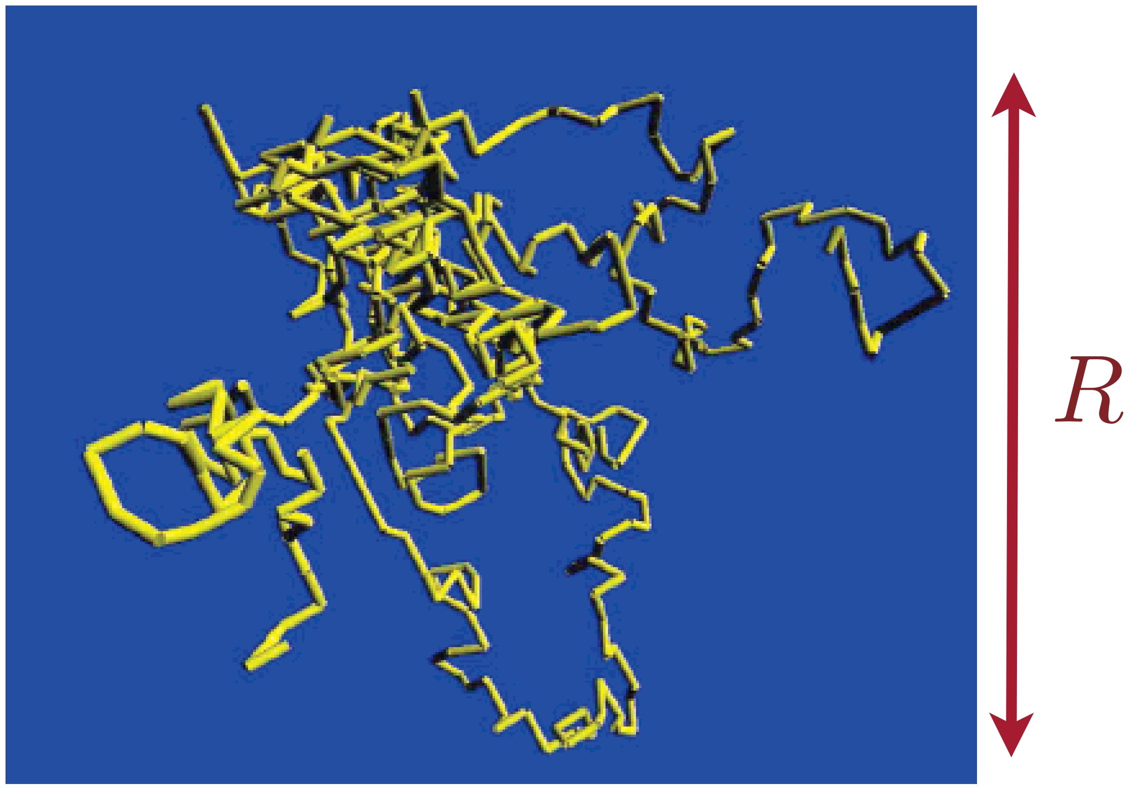
$$\frac{F\ell_p}{k_B T} = \frac{1}{4}\left(1 - \frac{\langle x \rangle}{L} + \frac{F}{\gamma}\right)^{-2} - \frac{1}{4} + \frac{\langle x \rangle}{L} - \frac{F}{\gamma}$$

J.F. Marko and E.D. Siggia, Macromolecules **28**, 8759-8770 (1995)

Random coil to globule transition in polymers

random coil

$$T > \Theta$$

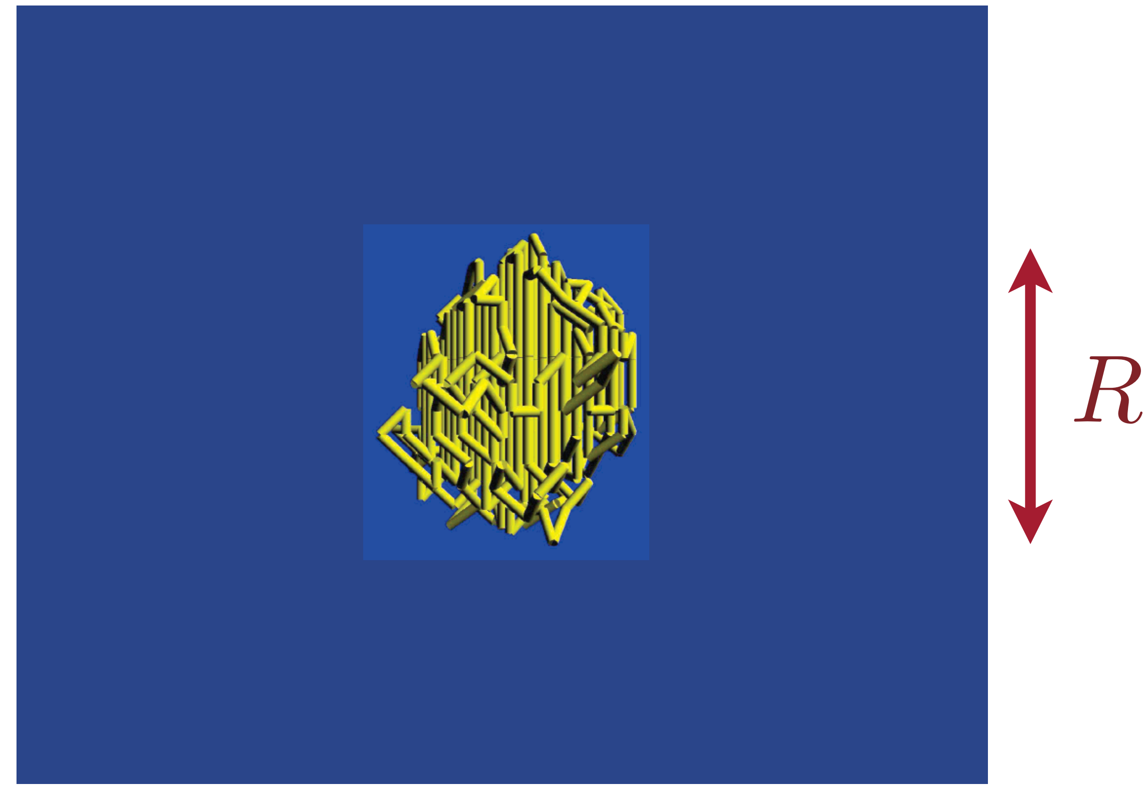


$$R \sim \sqrt{L \ell_p}$$

at high temperature entropic contributions dominate

compact globule

$$T < \Theta$$



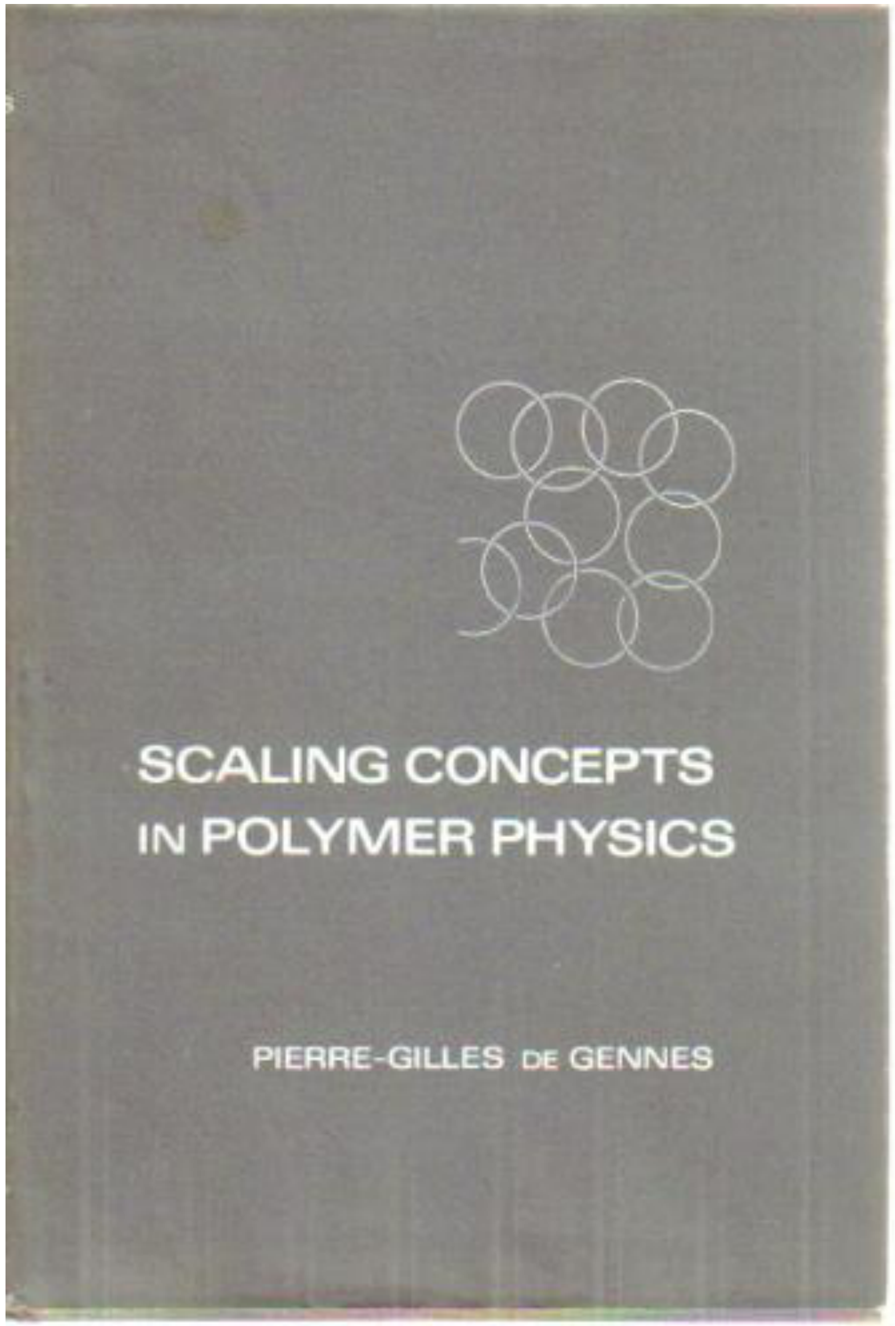
$$R \sim \left(d^2 L\right)^{1/3}$$

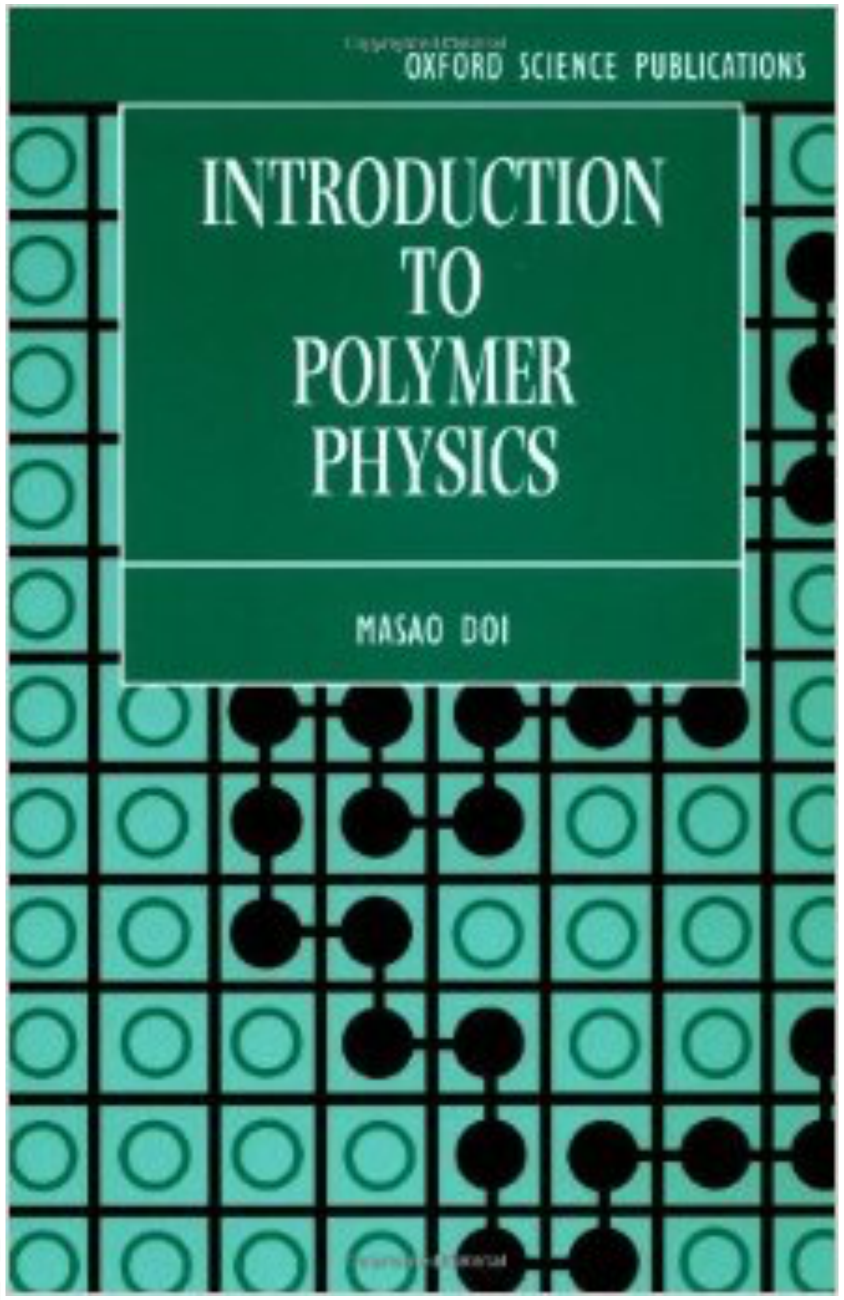
d **- diameter of polymer chain**

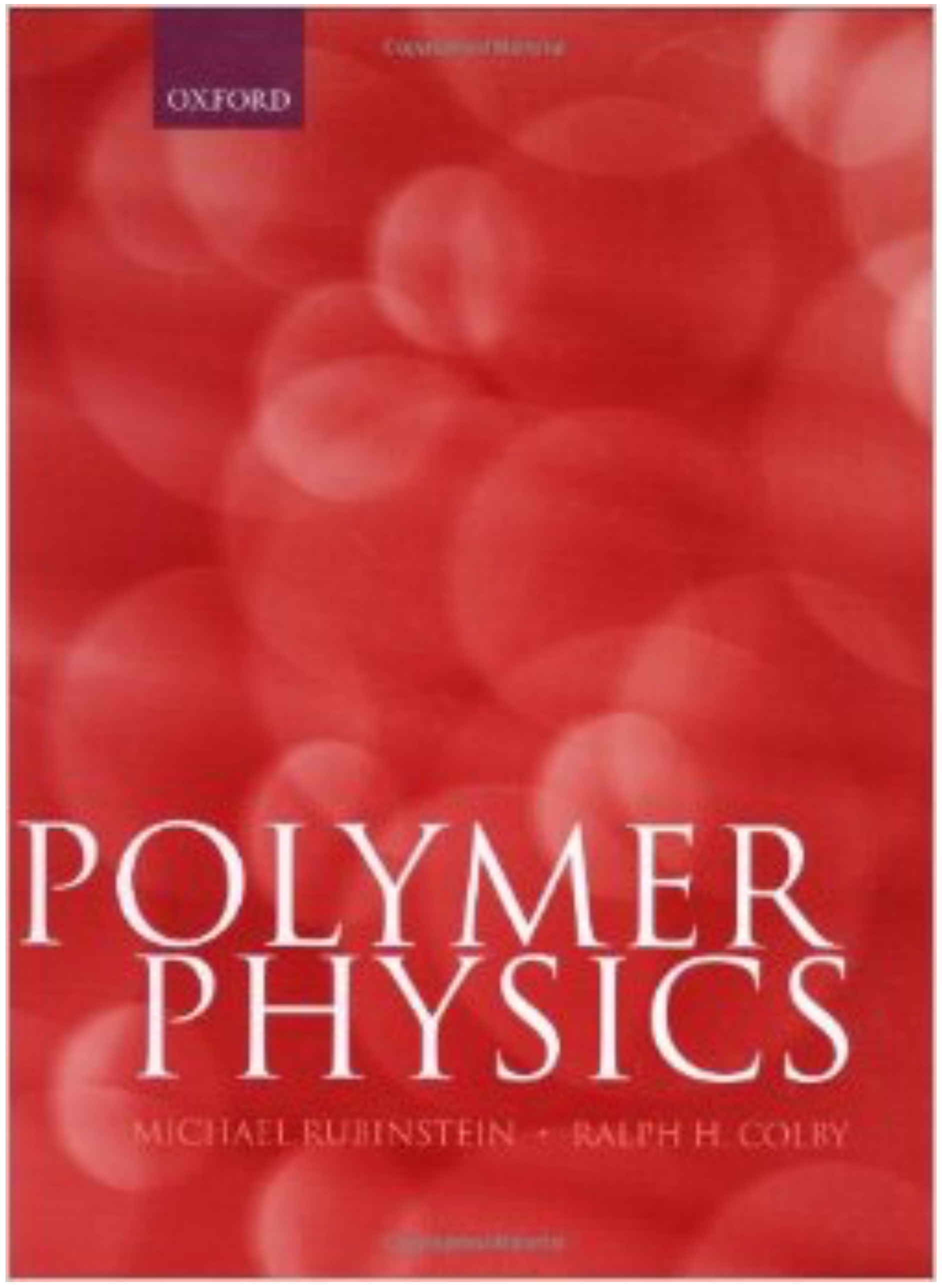
at low temperature attraction between polymer chains dominates

Figures from: W.B. Hu and D. Frenkel, J. Phys. Chem. B **110**, 3734 (2006)

Further reading



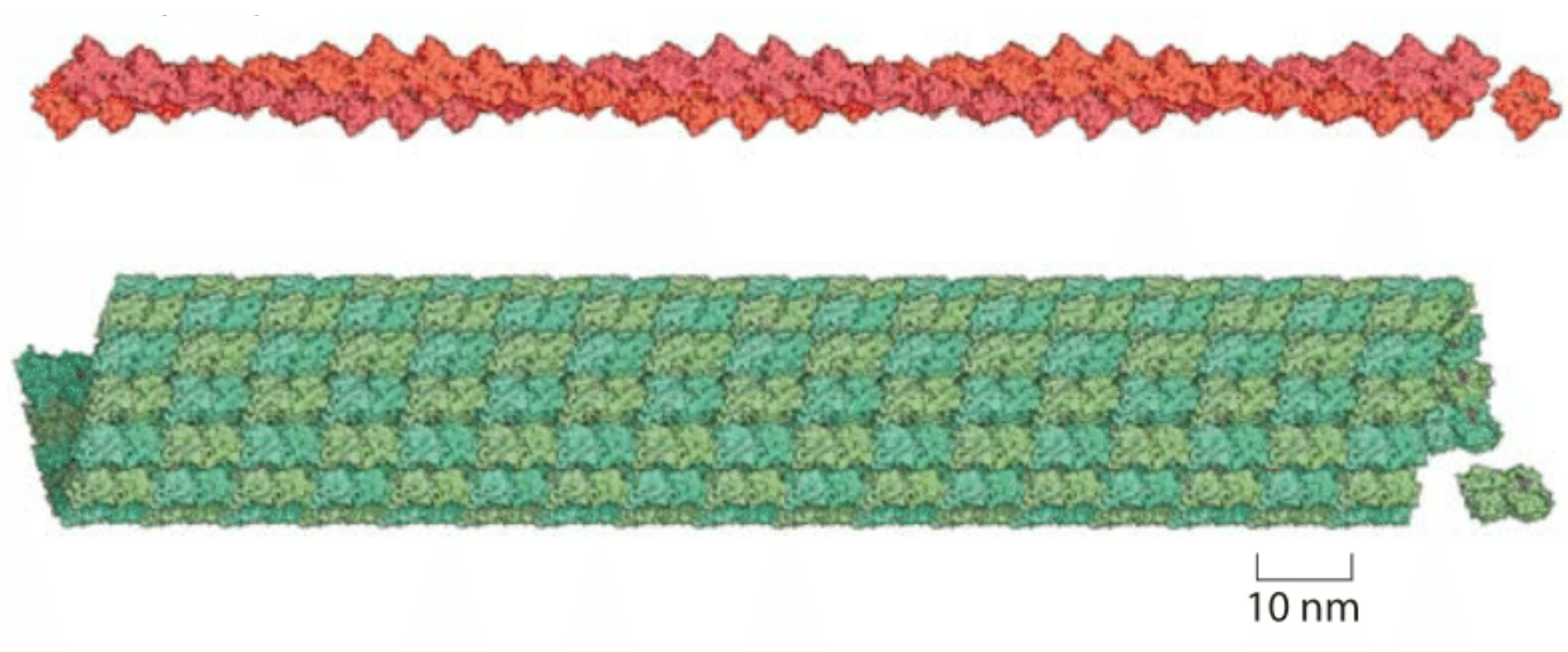




Dynamics of actin filaments and microtubules

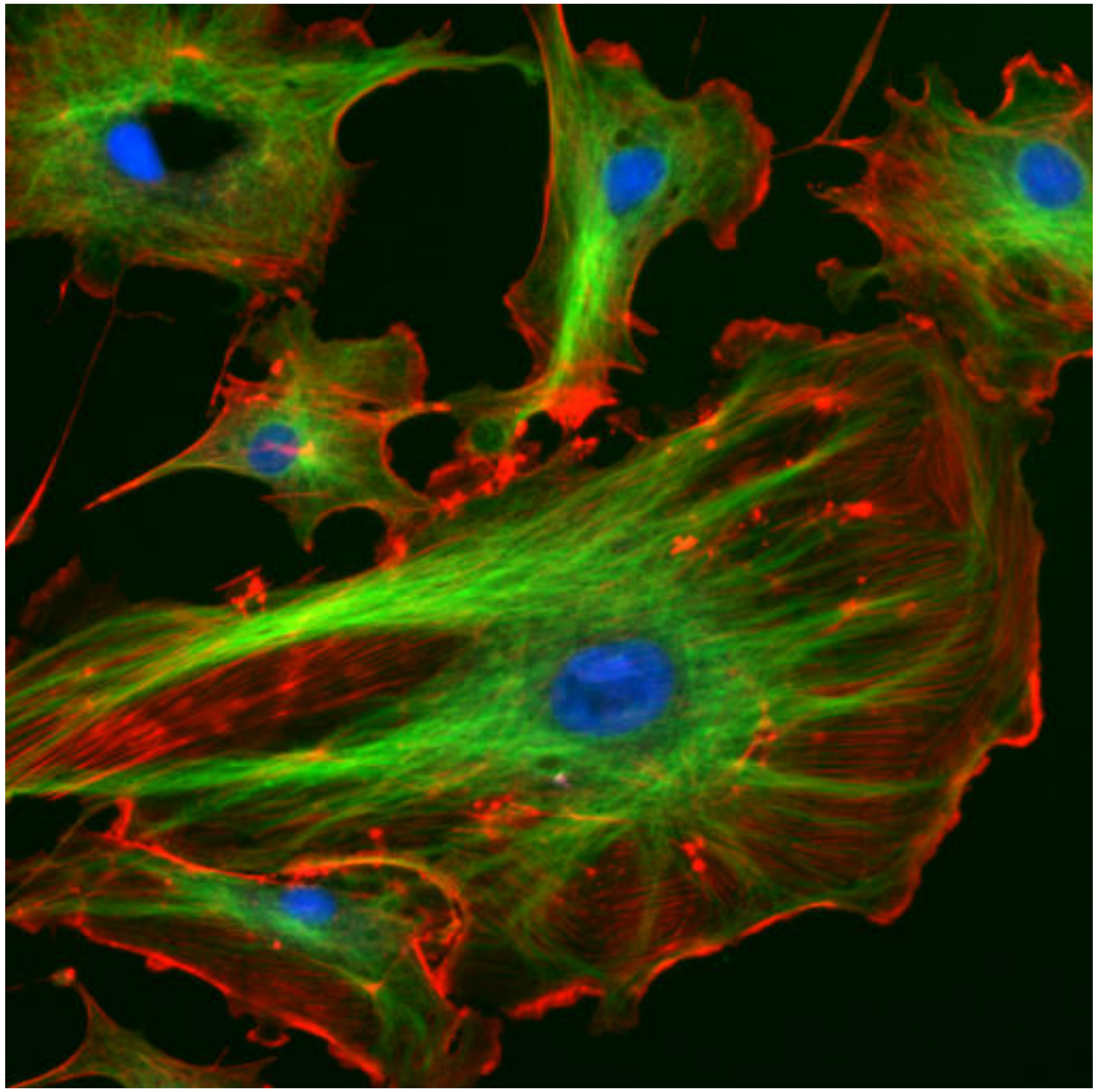
Actin filament

Microtubule



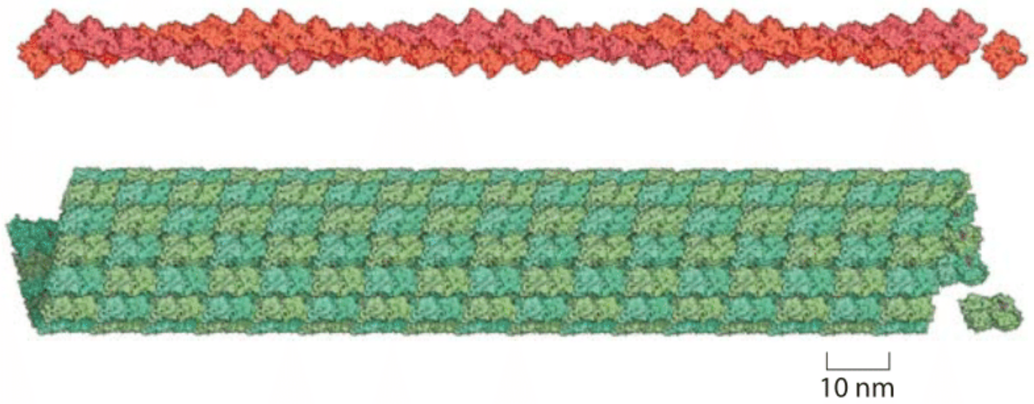
Cytoskeleton in cells

Cytoskeleton matrix gives the cell shape and mechanical resistance to deformation.



(wikipedia)

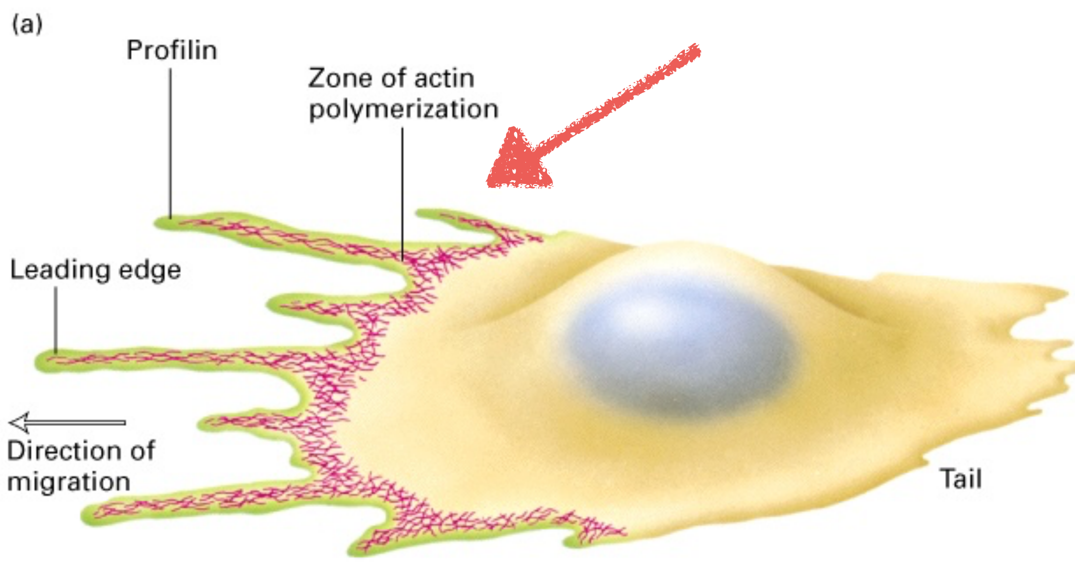
Actin filament



Microtubule

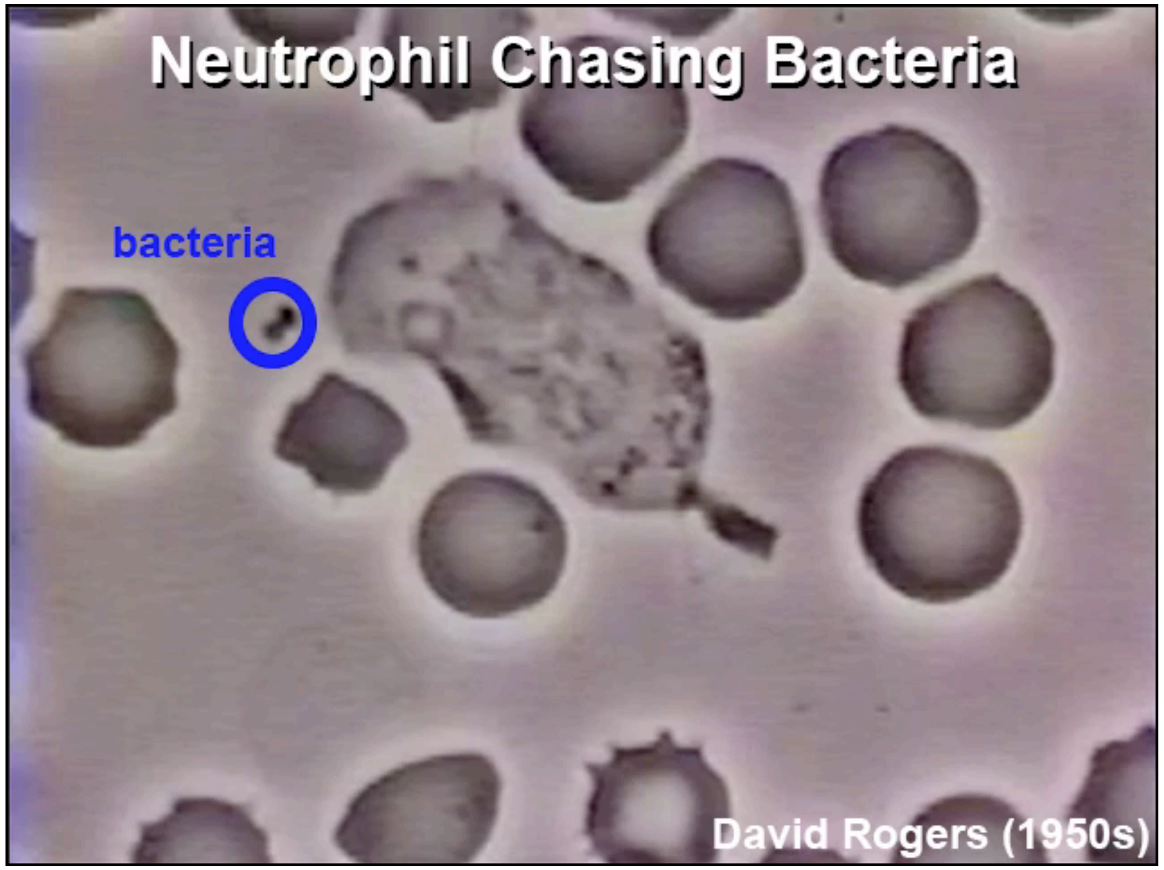
Crawling of cells

Actin



Immune system:

neutrophils chasing bacteria



migration of skin cells during wound healing

spread of cancer cells during metastasis of tumors

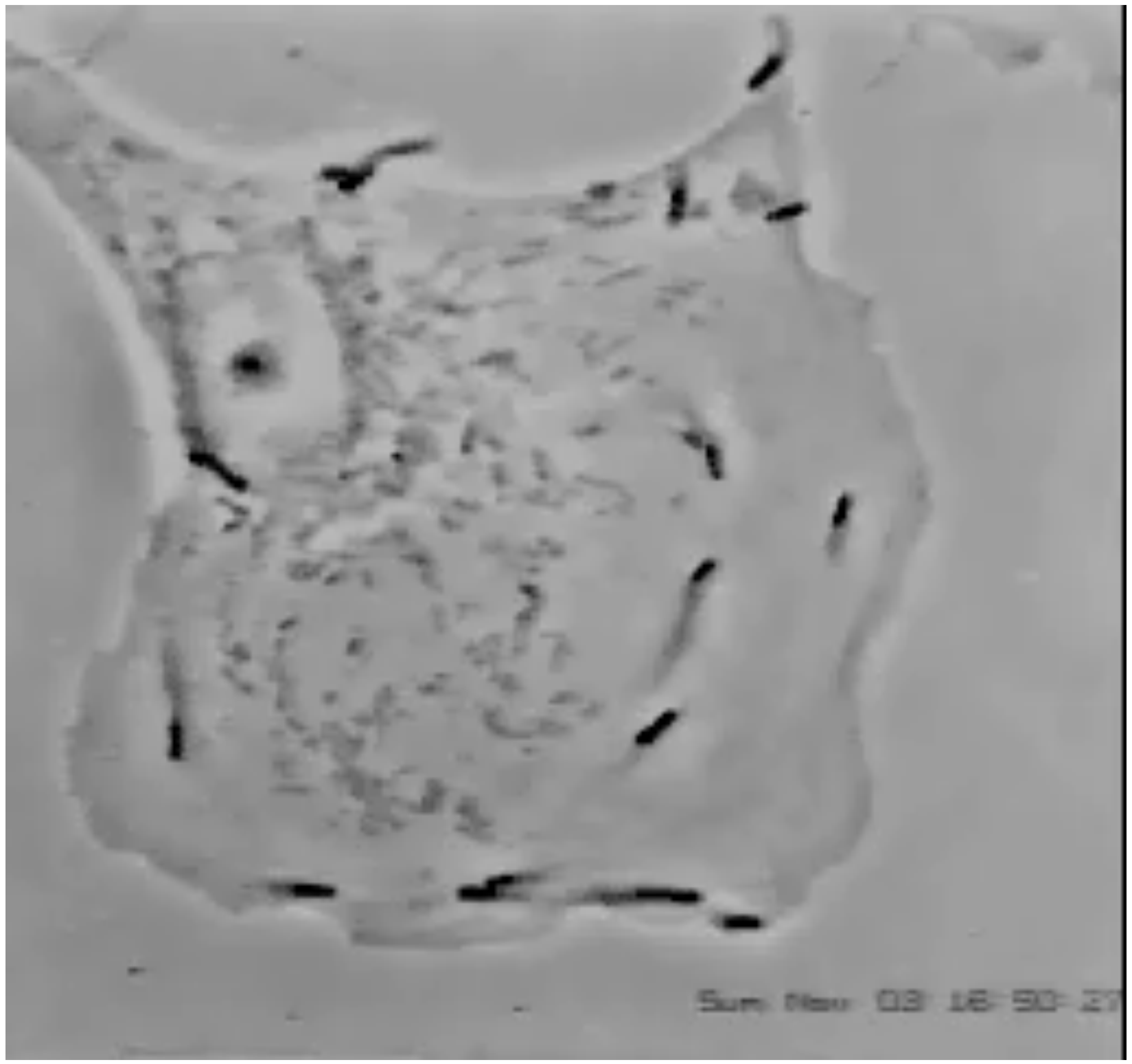
amoeba searching for food

David Rogers, 1950s

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

Movement of bacteria

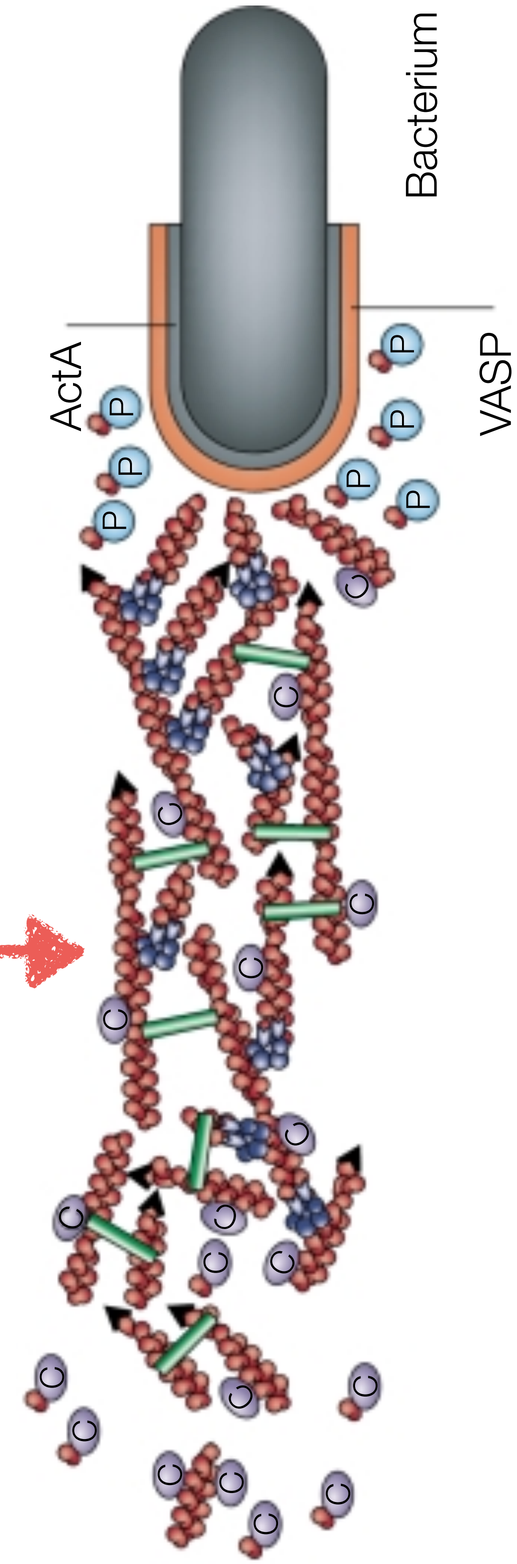
Listeria monocytogenes
moving in infected cells



Julie Theriot (speeded up 150x)

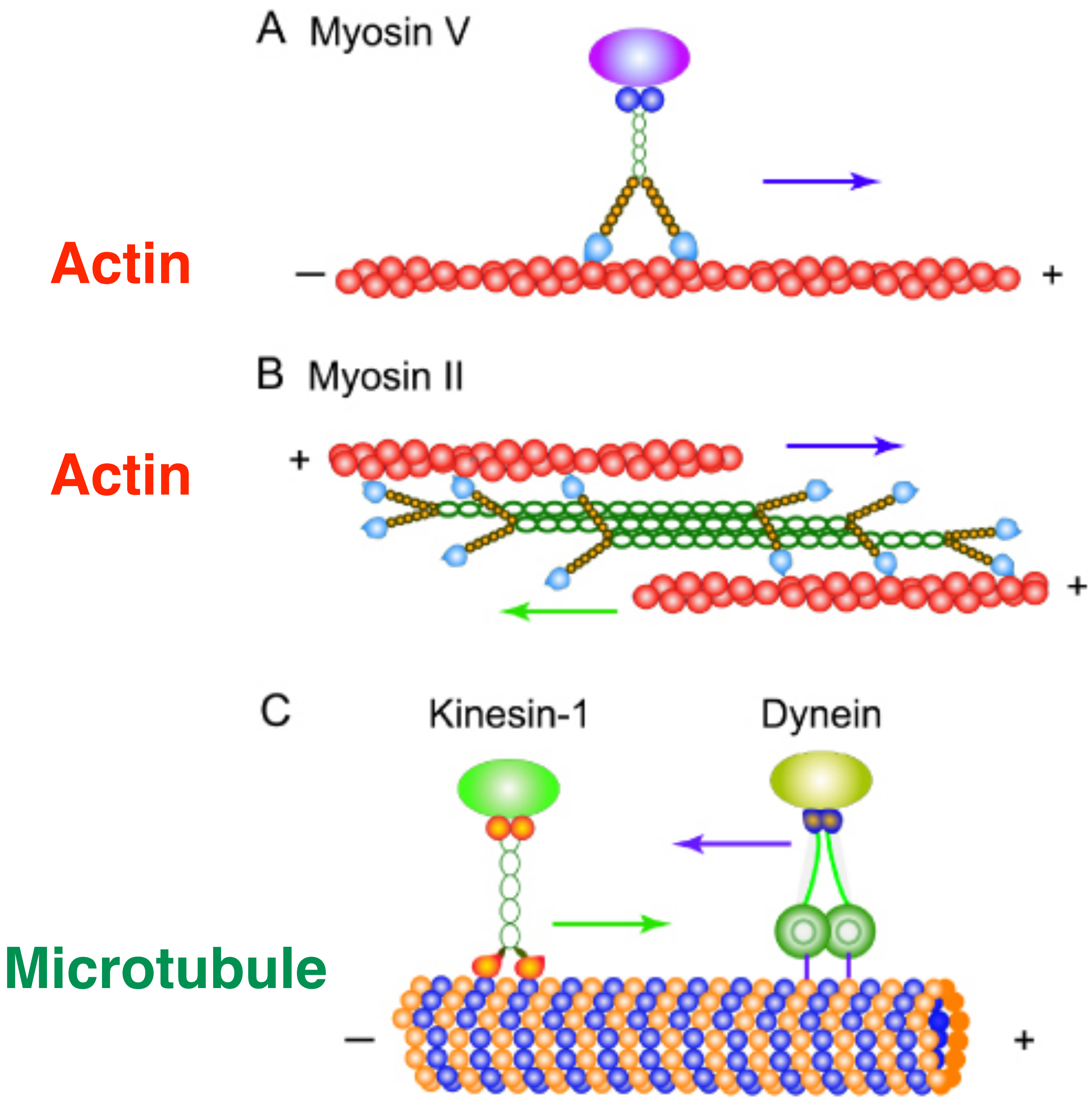
$$v \sim 0.1 - 0.3 \mu\mathrm{m/s}$$

Actin



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

Molecular motors

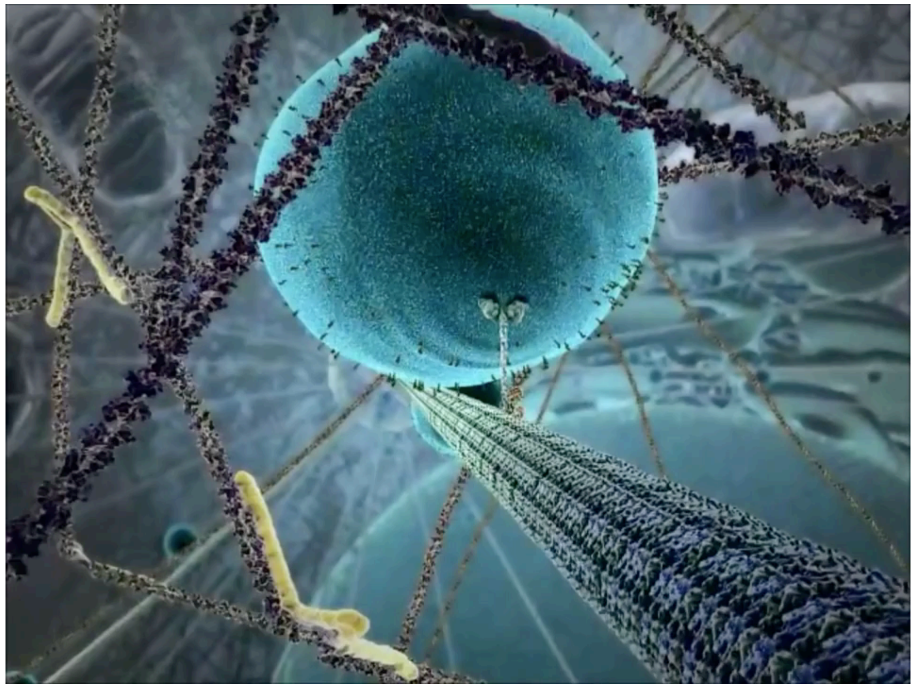


A.B. Kolomeisky, J. Phys.: Condens. Matter **25**, 463101 (2013)

Contraction of muscles

Transport of large molecules around cells (diffusion too slow)

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

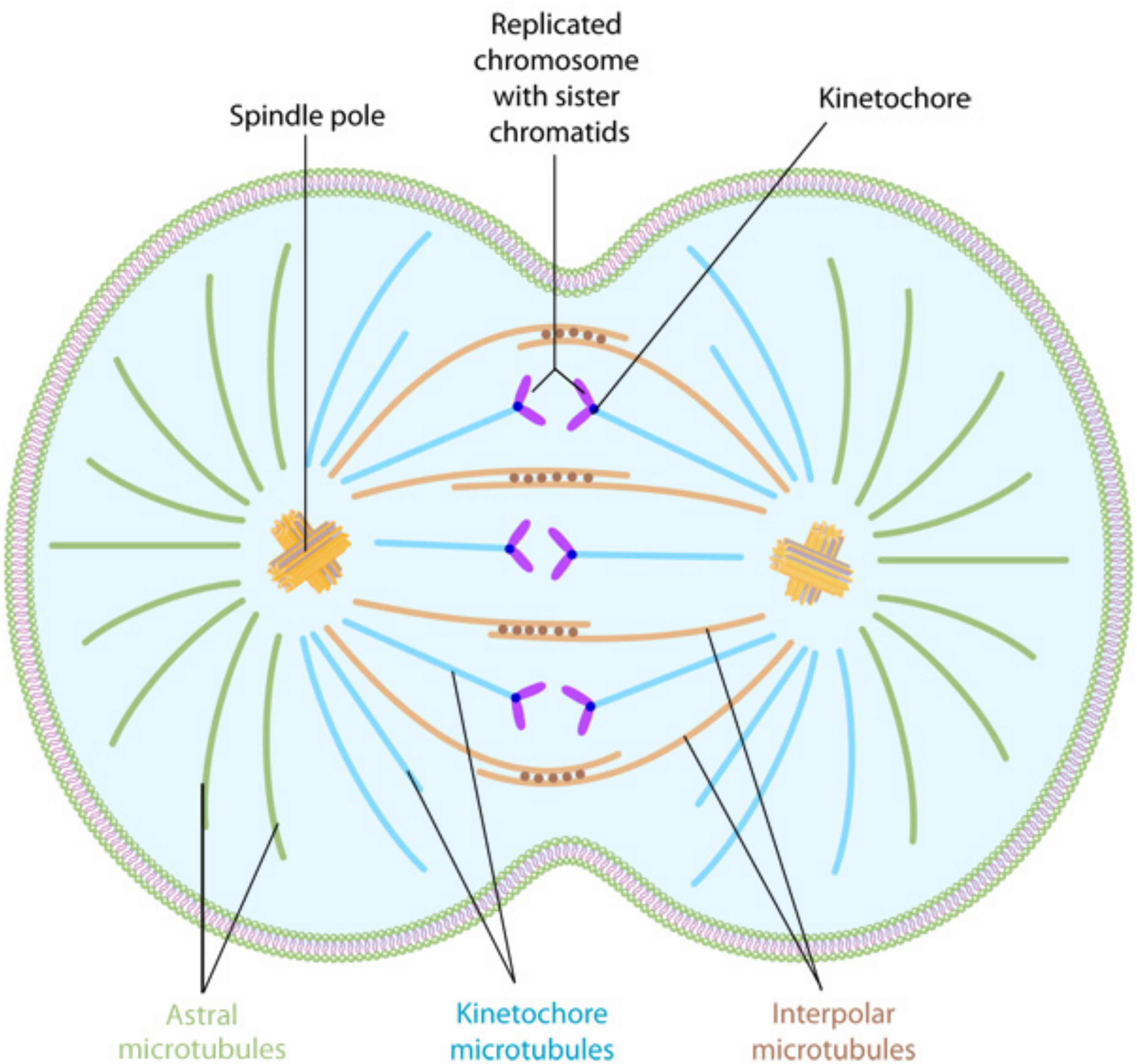


Harvard BioVisions

https://www.youtube.com/watch?v=FzcTgrxMzZk

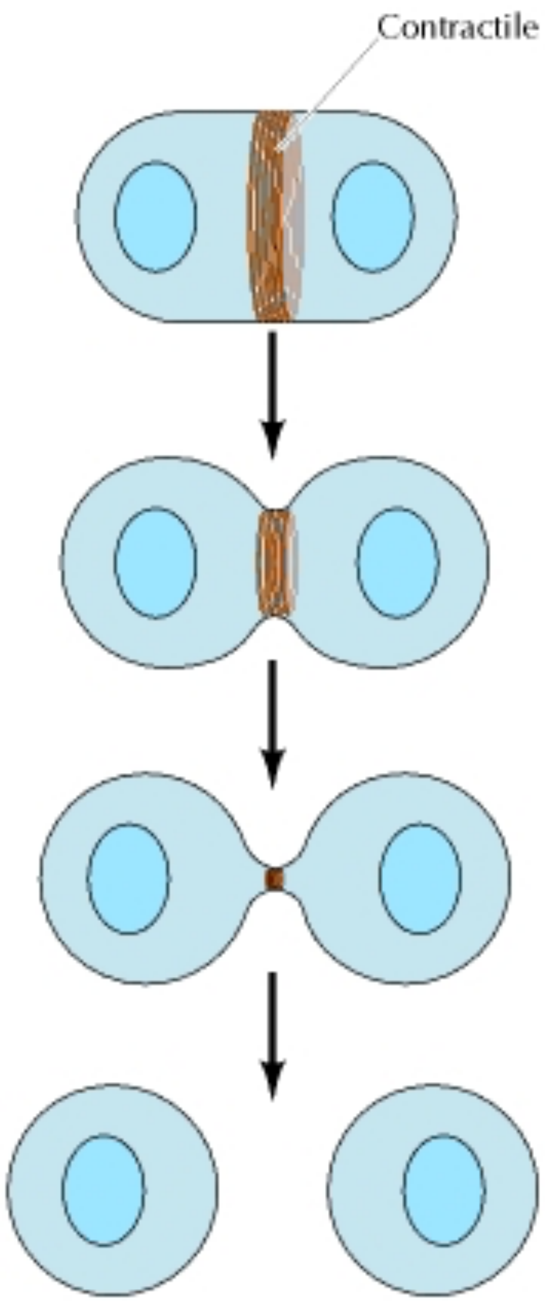
Cell division

Segregation of chromosomes



Microtubules

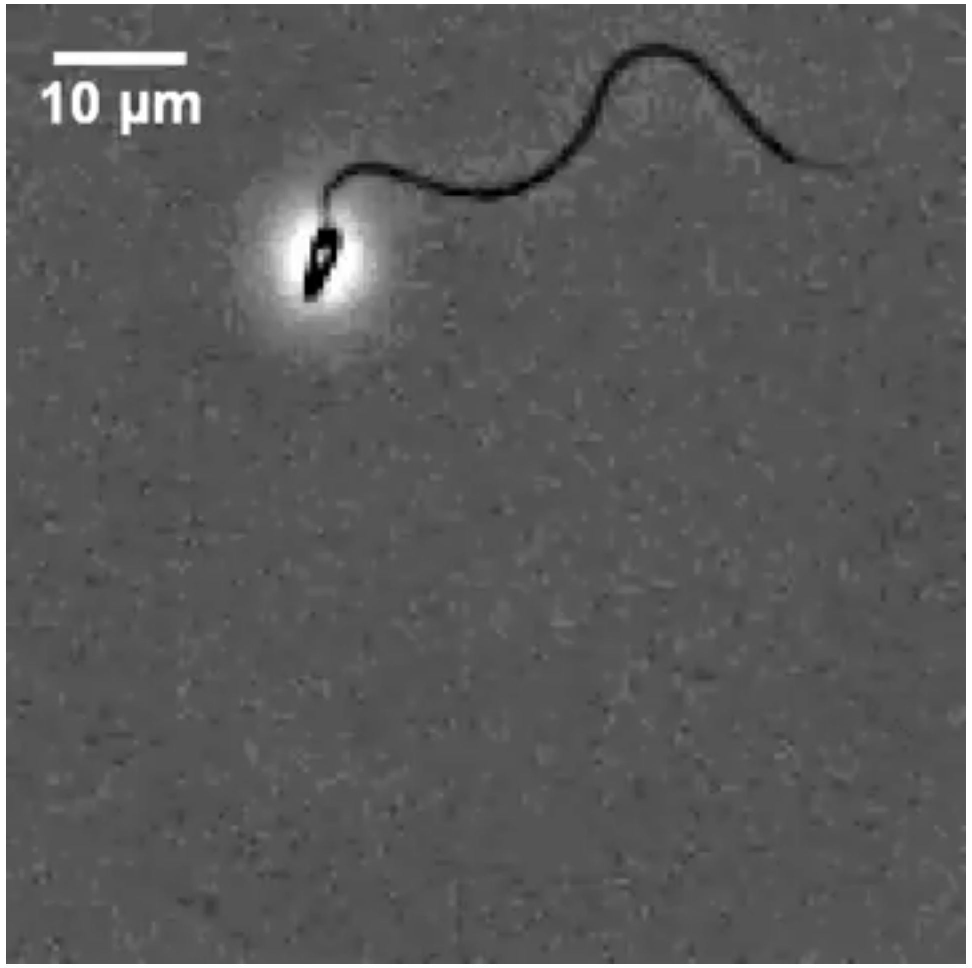
Contractile ring divides the cell in two

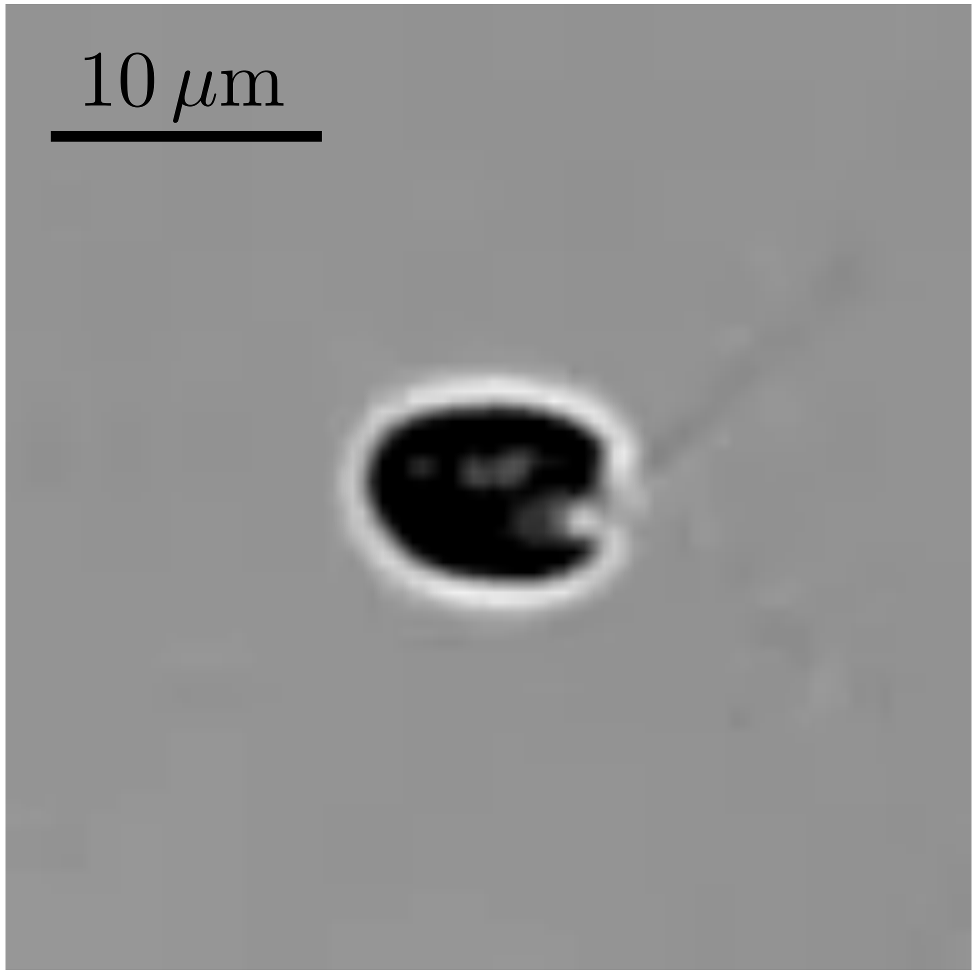


Actin

Swimming of sperm cells

Swimming of Chlamydomonas (green alga)





https://sites.tufts.edu/guastolab/movies/

Jeff Guasto

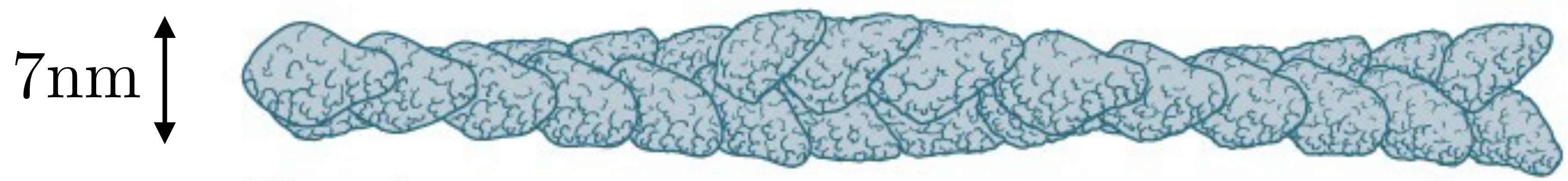
$v \sim 50 \mu\text{m/s}$

Jeff Guasto

$v \sim 60 \mu\text{m/s}$

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

Actin filaments



actin monomer

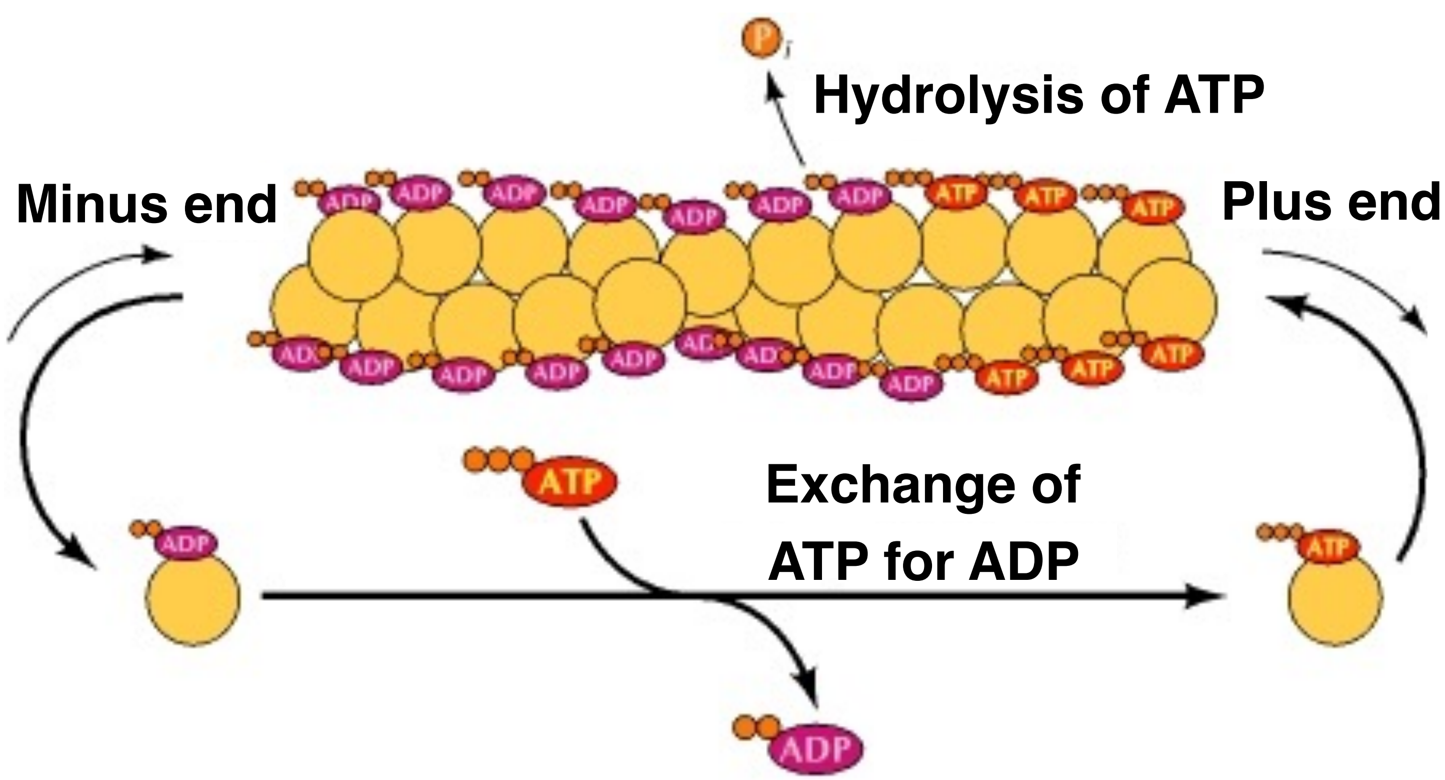
Minus end (pointed end)

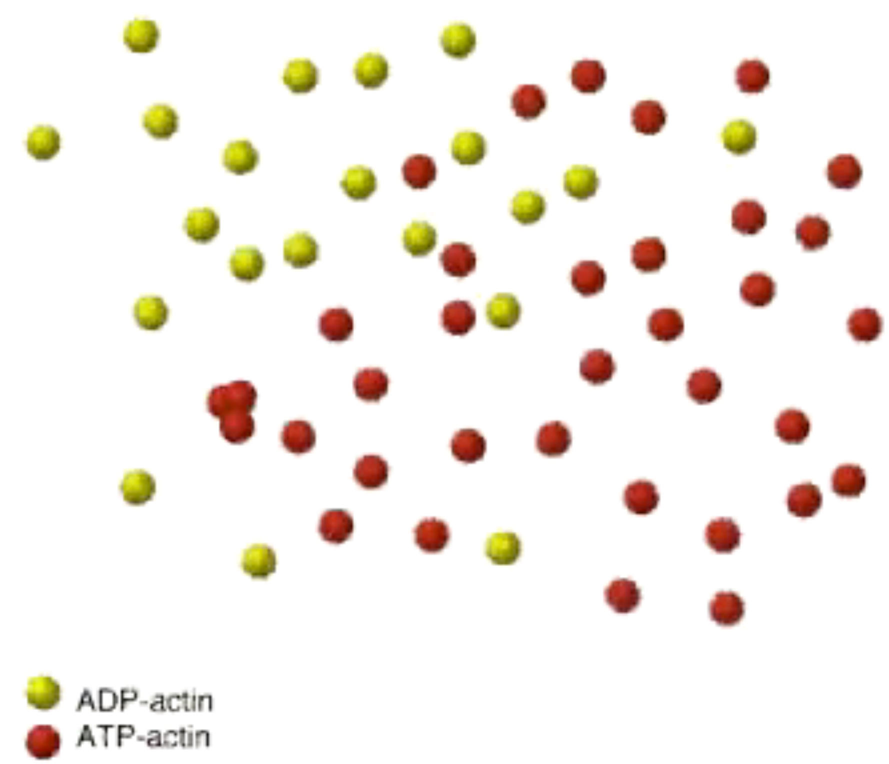
Plus end (barbed end)

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

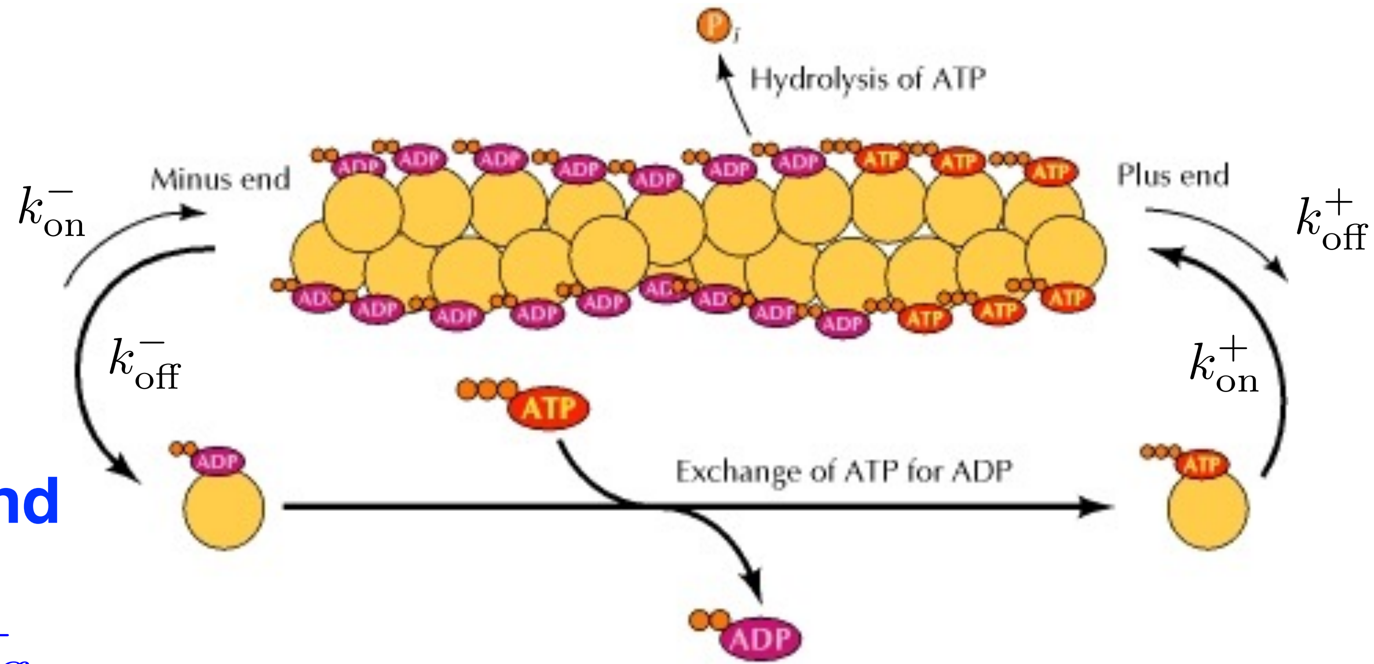
Typical length $L \lesssim 10\mu\mathrm{m}$

Actin treadmilling





Actin growth



growth of minus end

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

no growth at

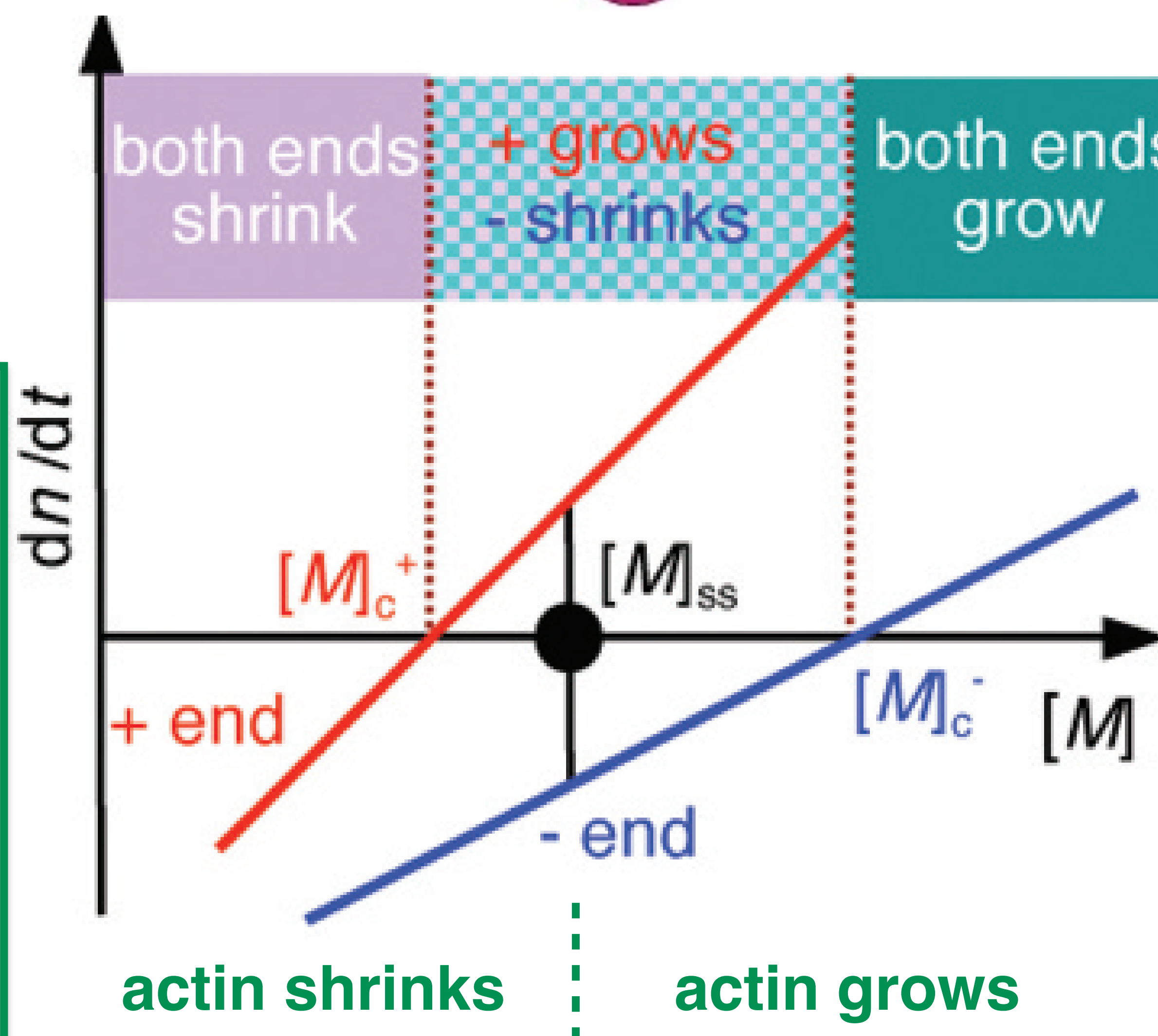
$$[M]_c^- = \frac{k_{\text{off}}^{-}}{k_{\text{on}}^{-}}$$

growth of plus end

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

no growth at

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



concentration of free actin monomers

Steady state regime

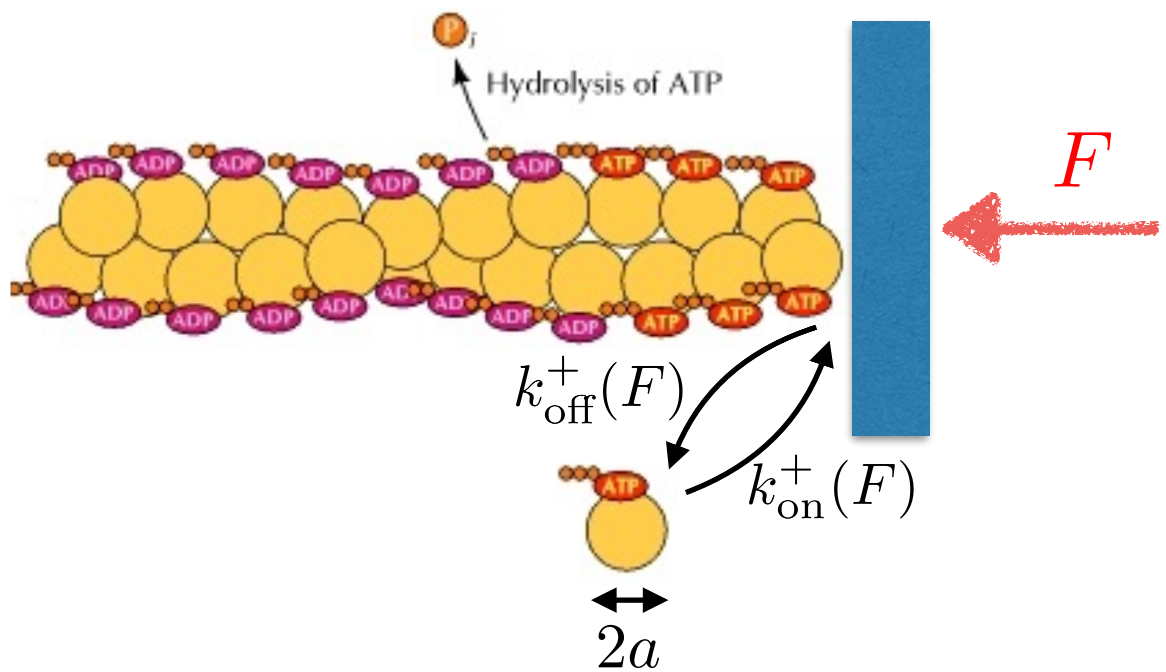
$$\frac{dn^+}{dt} = -\frac{dn^-}{dt}$$

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

front speed

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

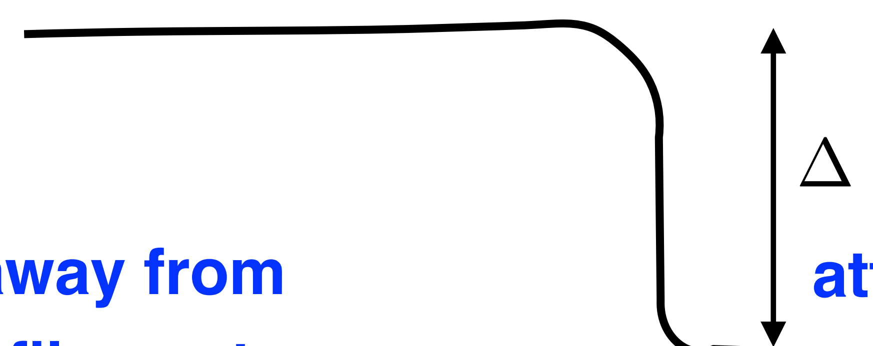
Actin filament growing against the barrier



work done against the barrier for insertion of new monomer

$$W = Fa$$

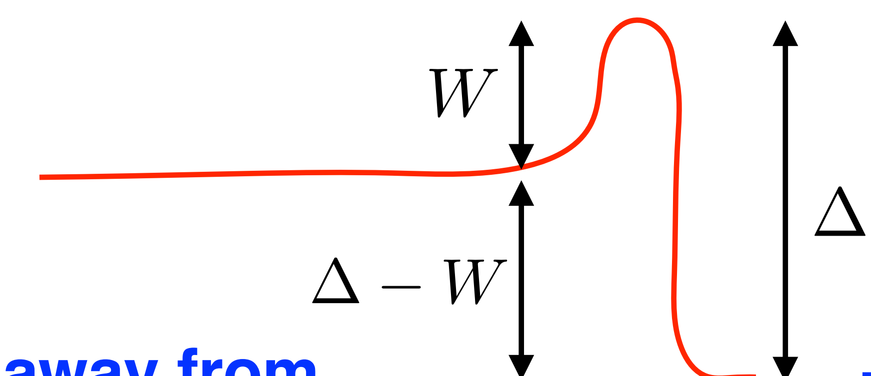
effective monomer free energy potential without barrier



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

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

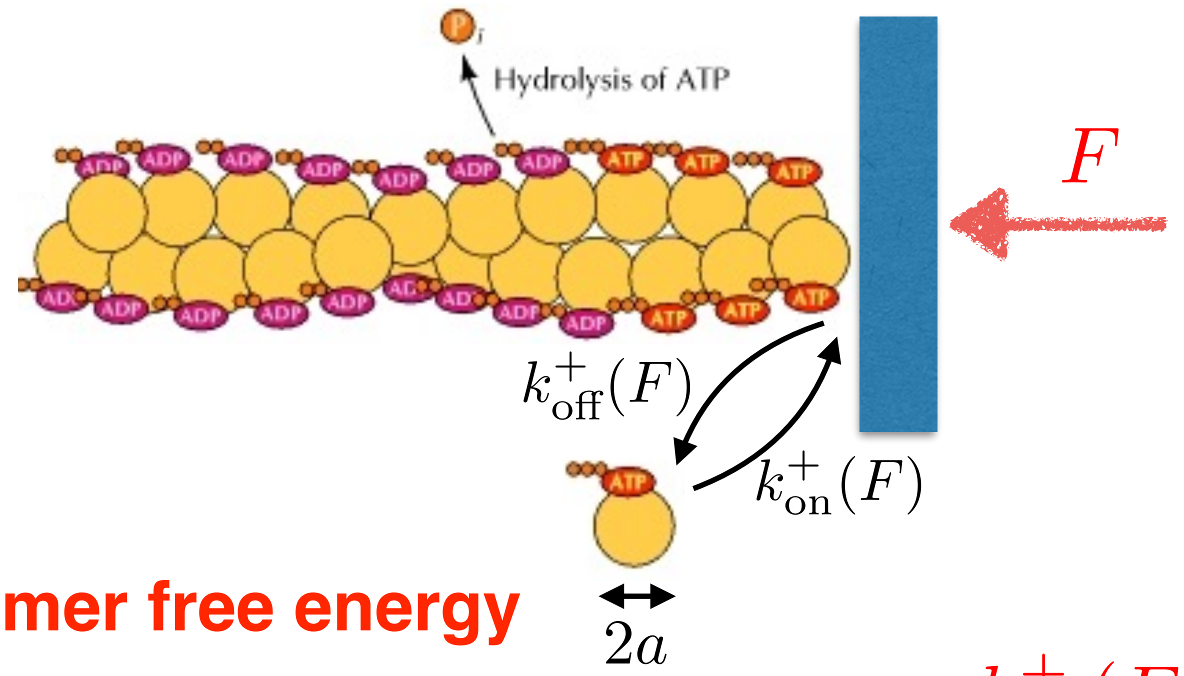
effective monomer free energy potential with barrier



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

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

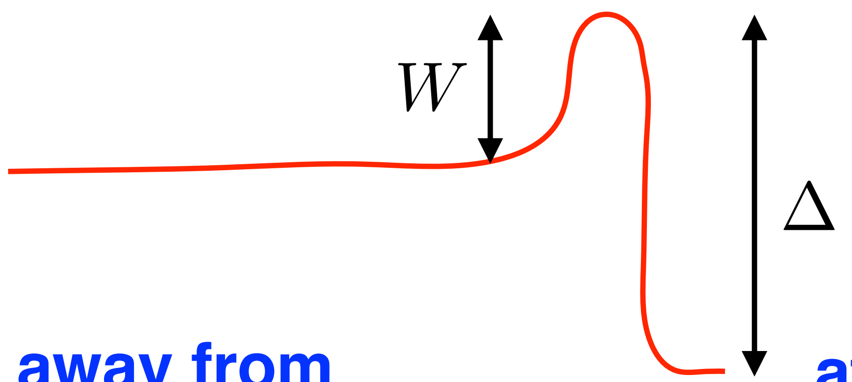
Actin filament growing against the barrier



work done against the barrier for insertion of new monomer

$$W = Fa$$

effective monomer free energy potential with barrier



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

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

Growth speed of the tip

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

Maximal force that can be balanced by growing filament (stall force)

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

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

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

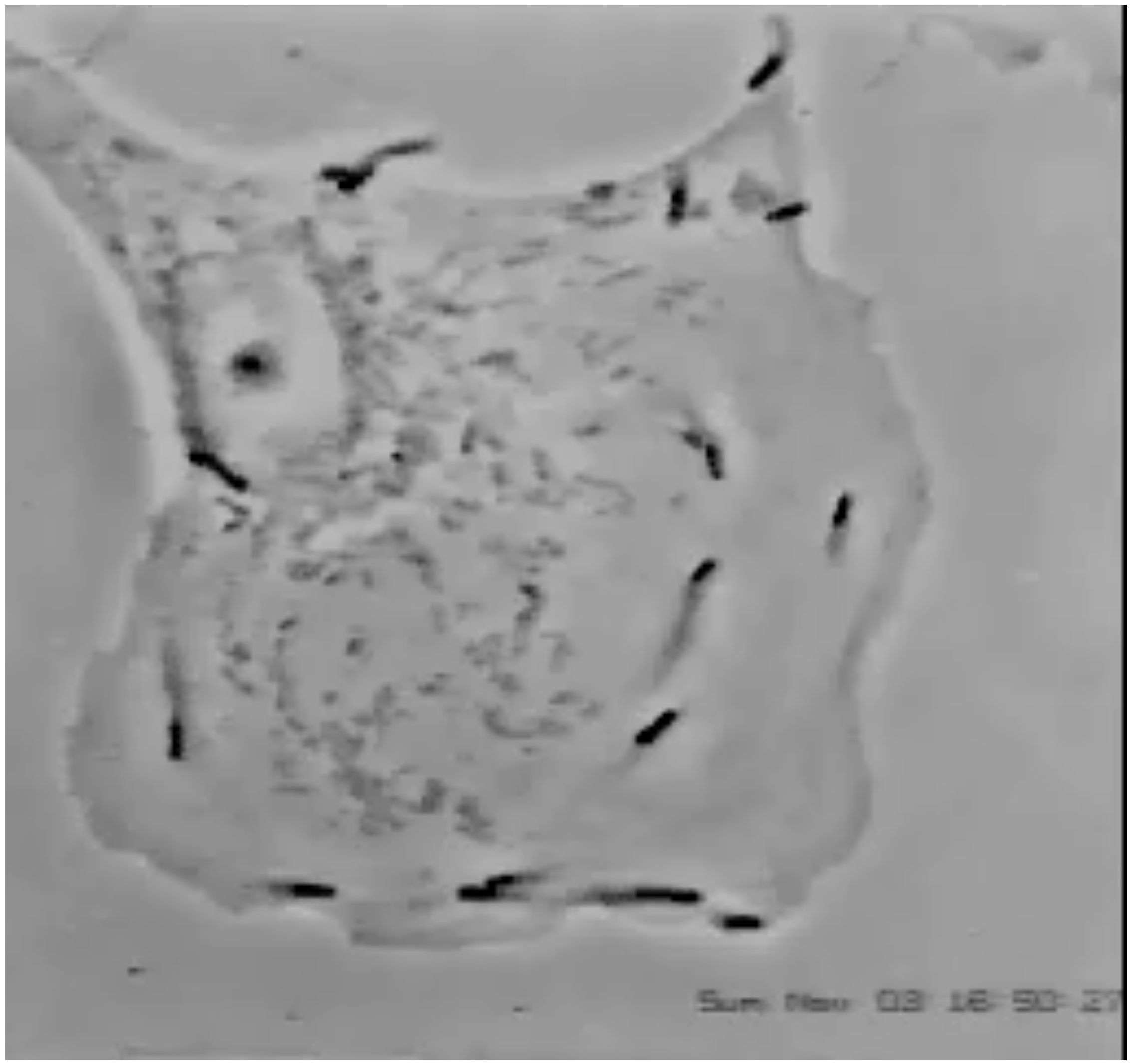
$[M] \sim 10\mu\mathrm{M}$

$a \approx 2.5\mathrm{nm}$

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

Movement of bacteria

Listeria monocytogenes
moving in infected cells

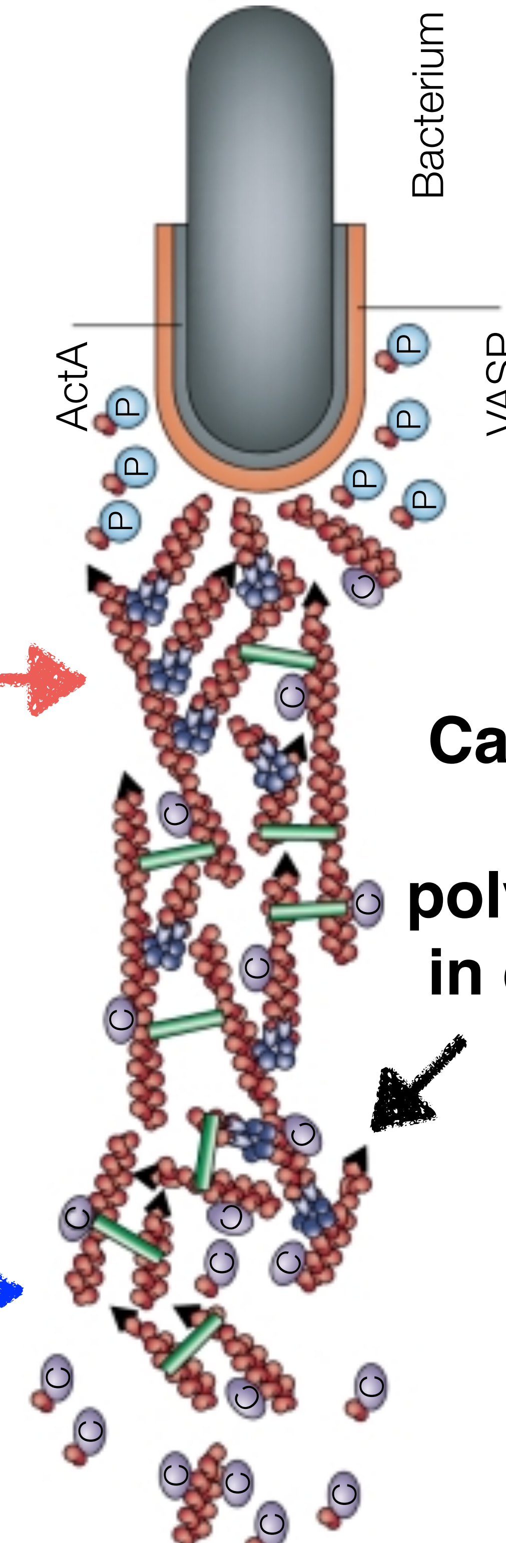


Julie Theriot (speeded up 150x)

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

Actin polymerization is pushing bacteria

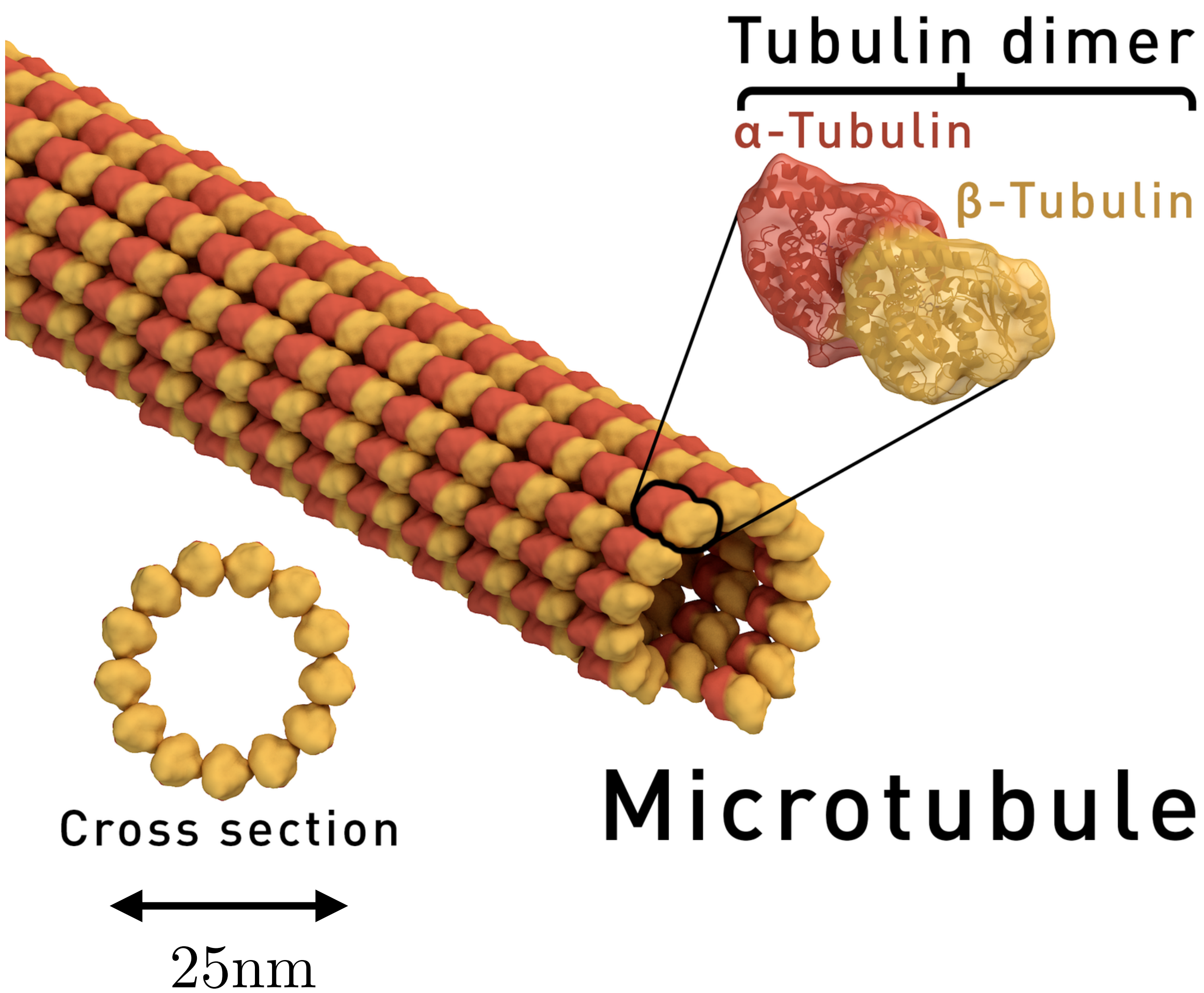
Actin is randomly depolymerized in comet tails



Caps prevent further polymerization in comet tails

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

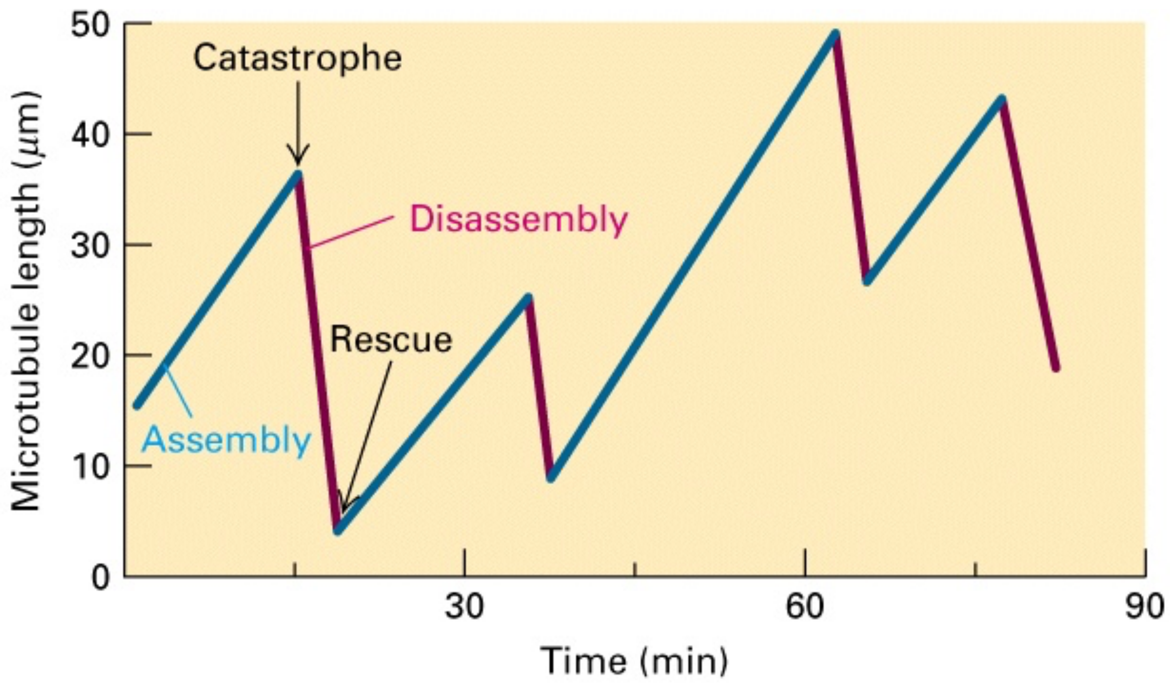
Microtubules

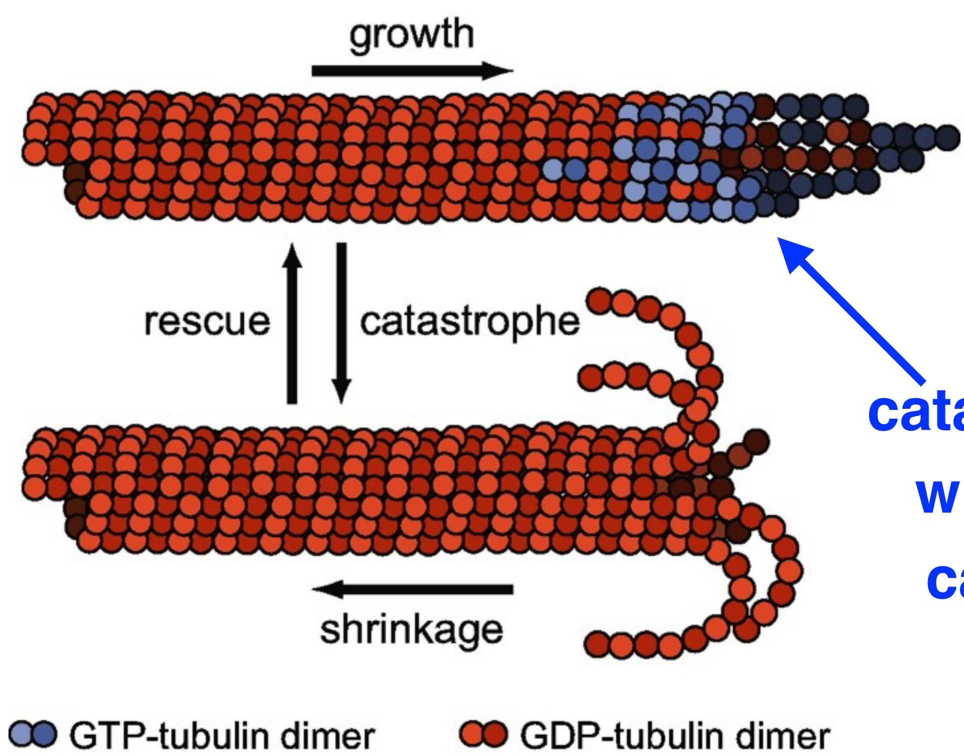


Persistence length $\ell_p \sim 1\text{mm}$

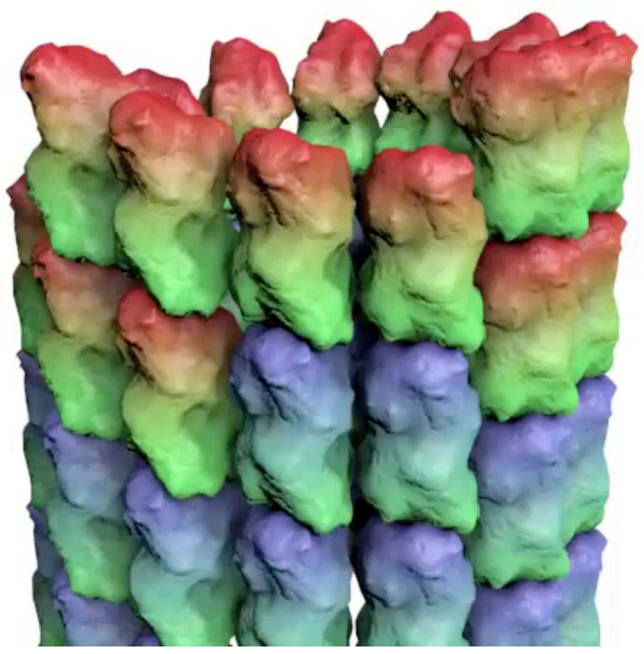
Typical length $L \lesssim 50\mu\text{m}$

Microtubule dynamic instability



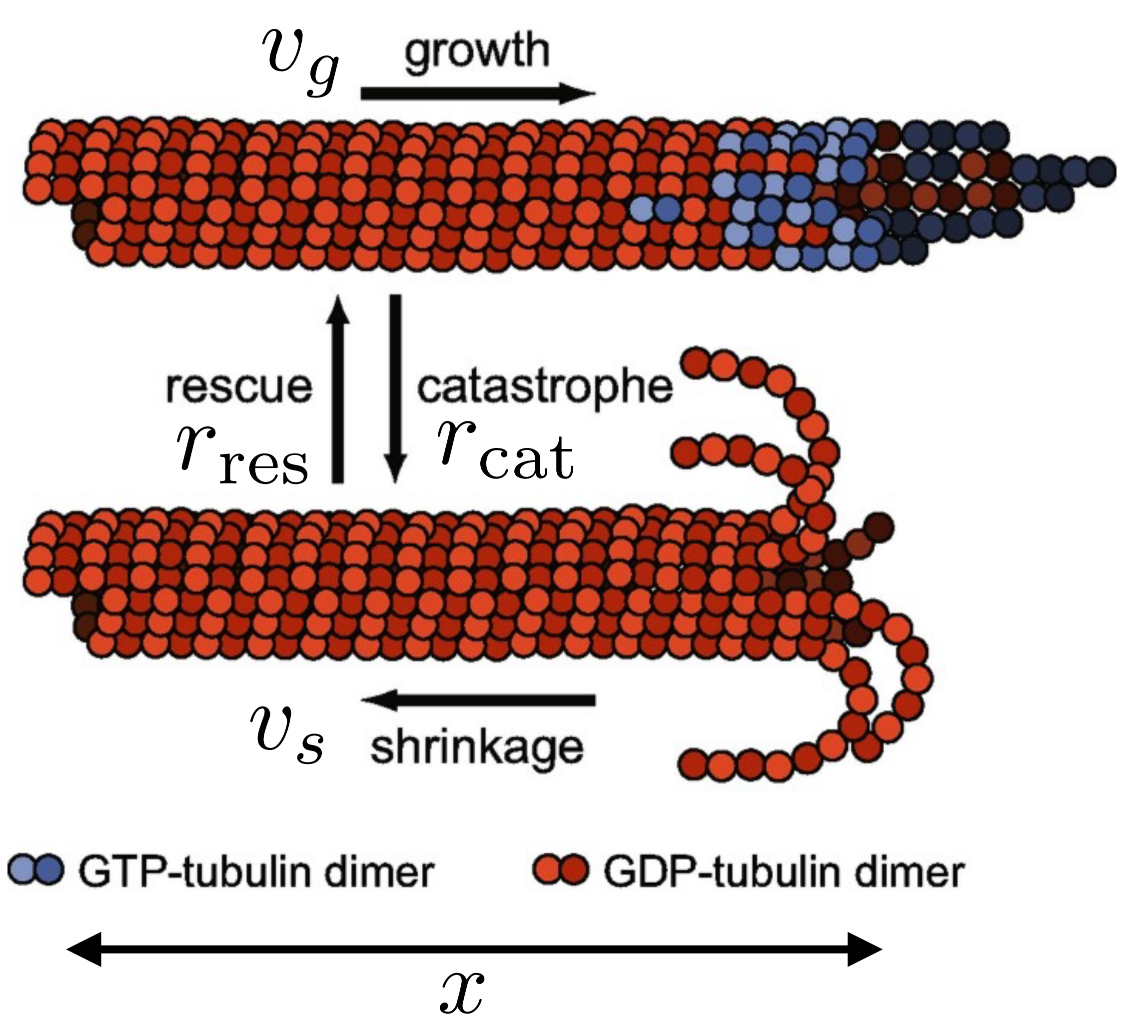


catastrophe occurs when protective cap disappears



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

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

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

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

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

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

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

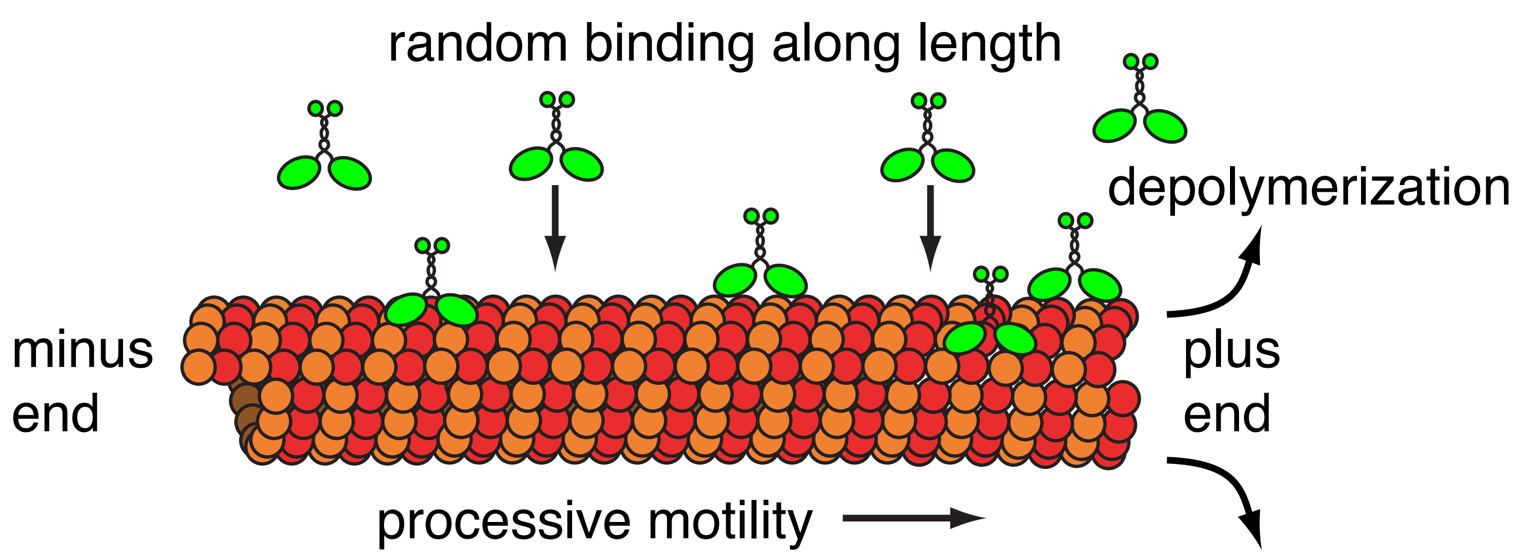
Average growth speed of microtubules

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

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

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

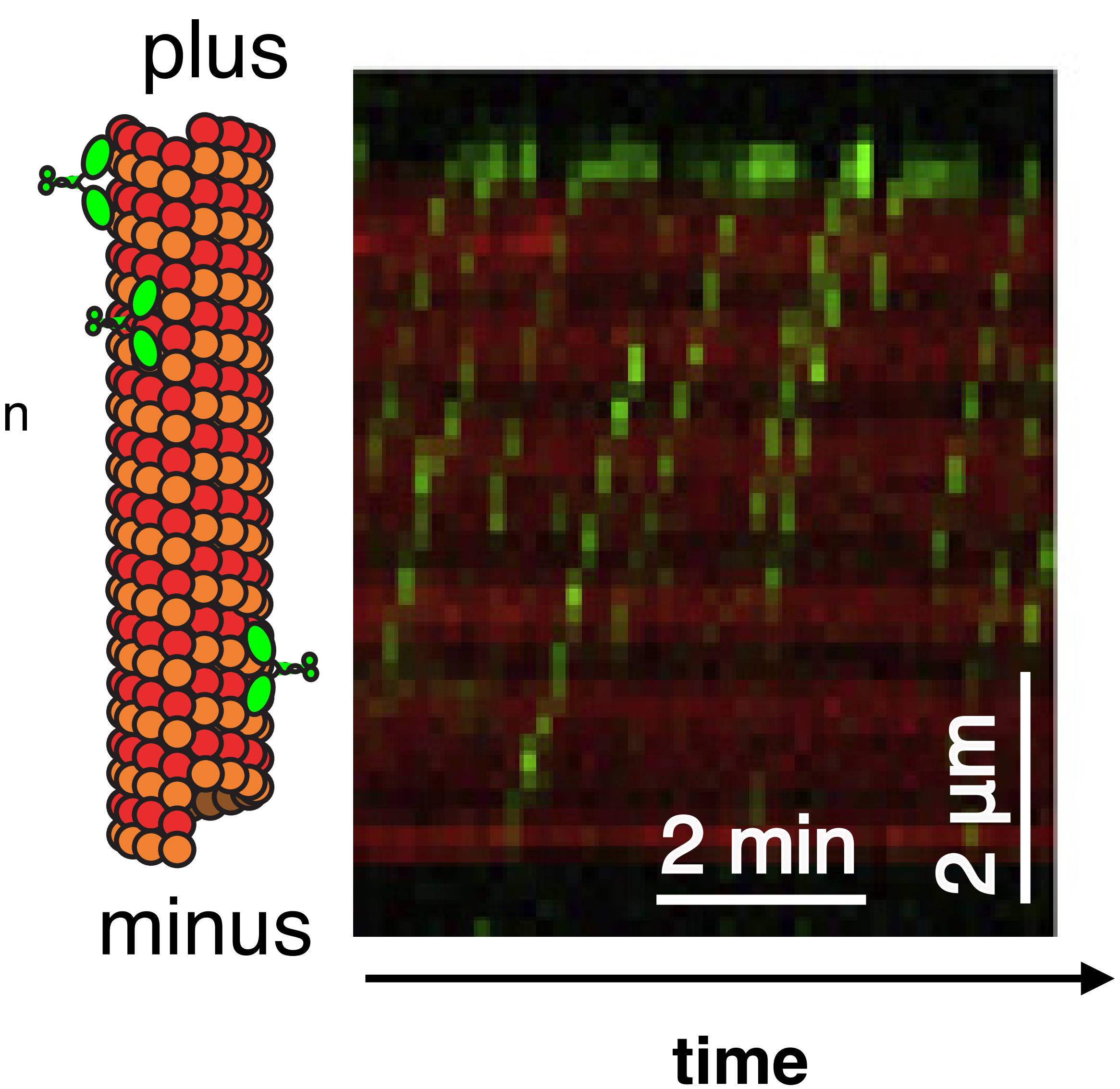


Motors walk at speed

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

kymograph

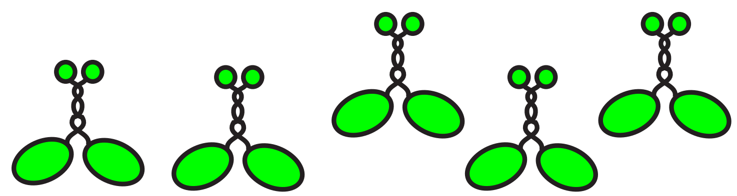
$$v_{\mathrm{mot}} \approx 3\mu\mathrm{m}/\mathrm{min}$$

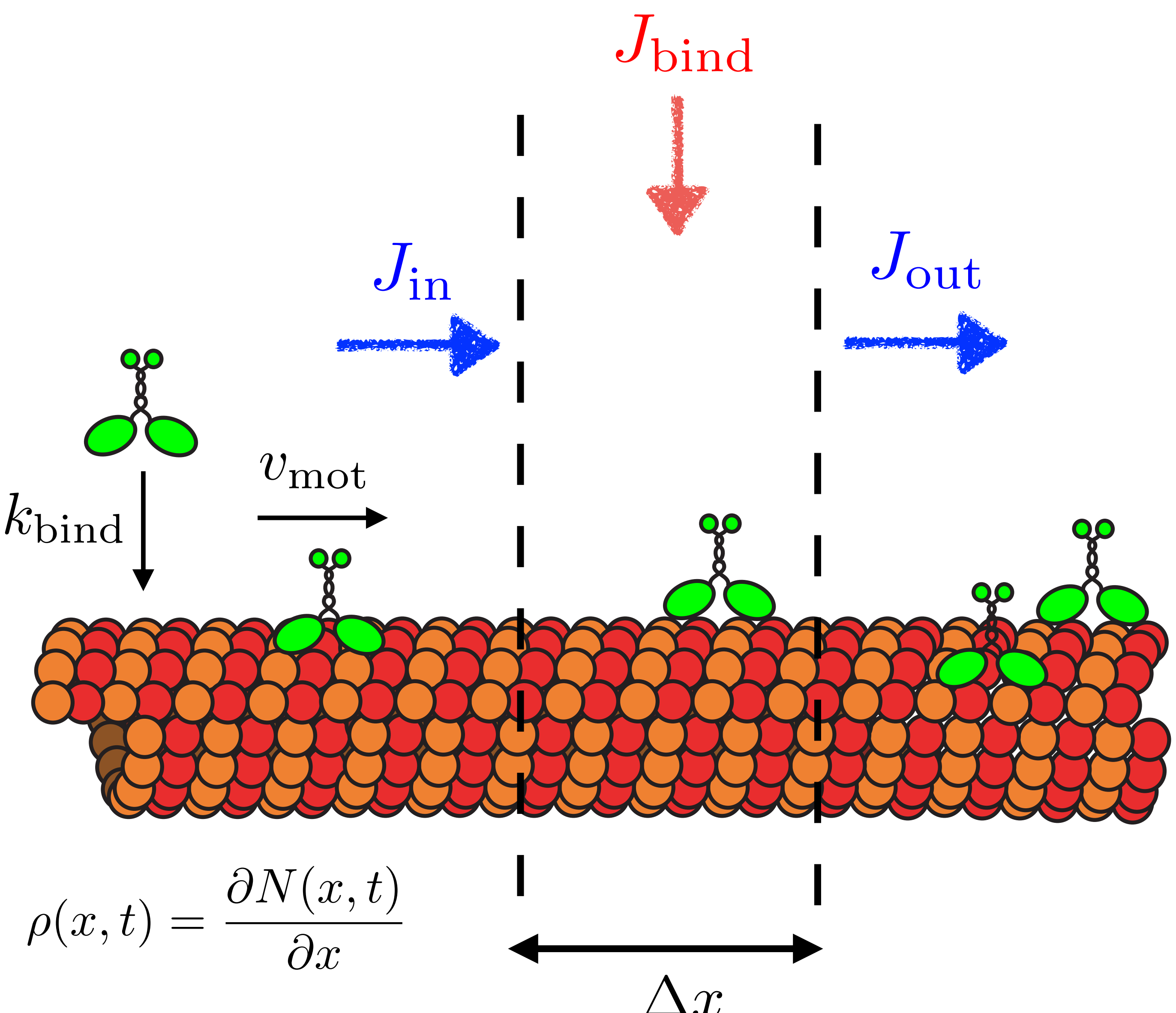


V. Varga *et al.*, Cell **138**, 1174-1183 (2009)

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

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

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

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

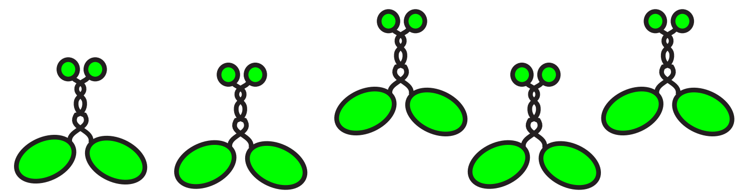
Generalized Fick's law

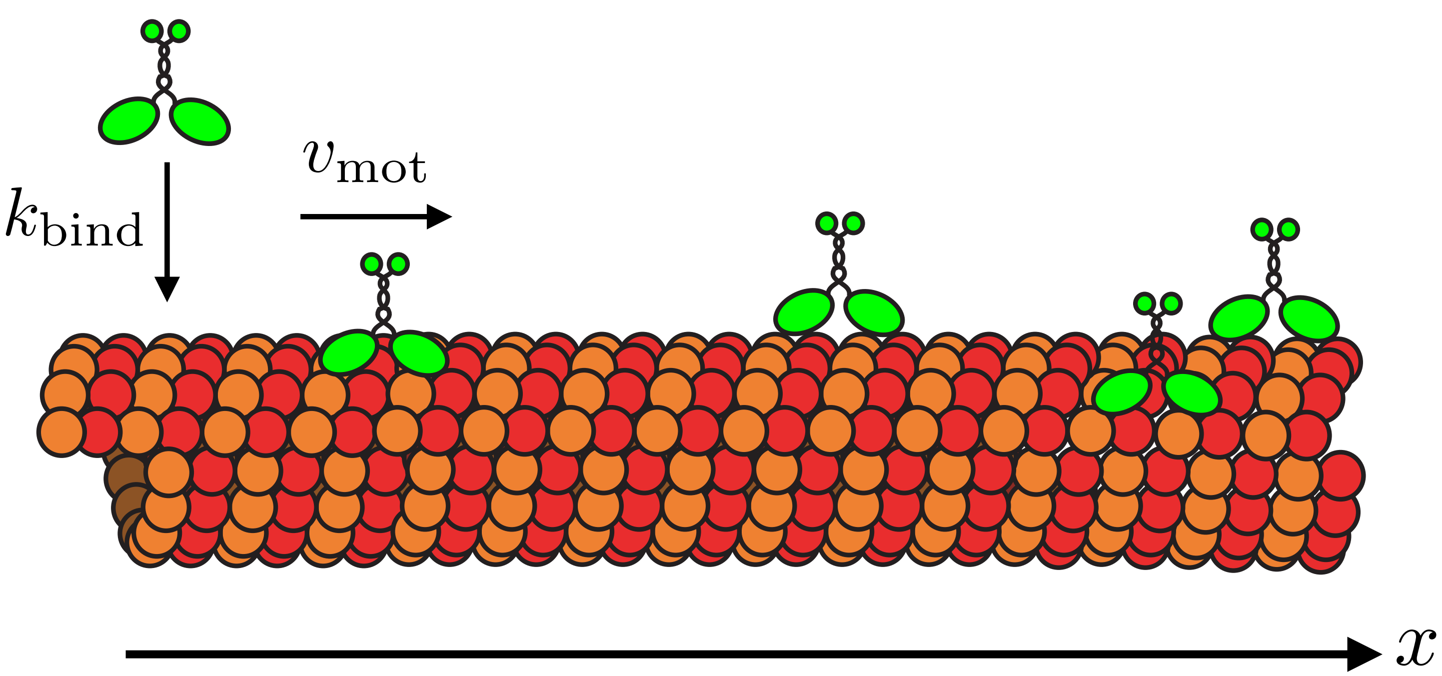
$$\boxed{\frac{\partial \rho(x,t)}{\partial t} = r(x,t) - \frac{\partial j(x,t)}{\partial x}}$$

creation/removal of particles

Density of motors bound to microtubules

[*M*] concentration of unbound motors





Time evolution for density of bound motors

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

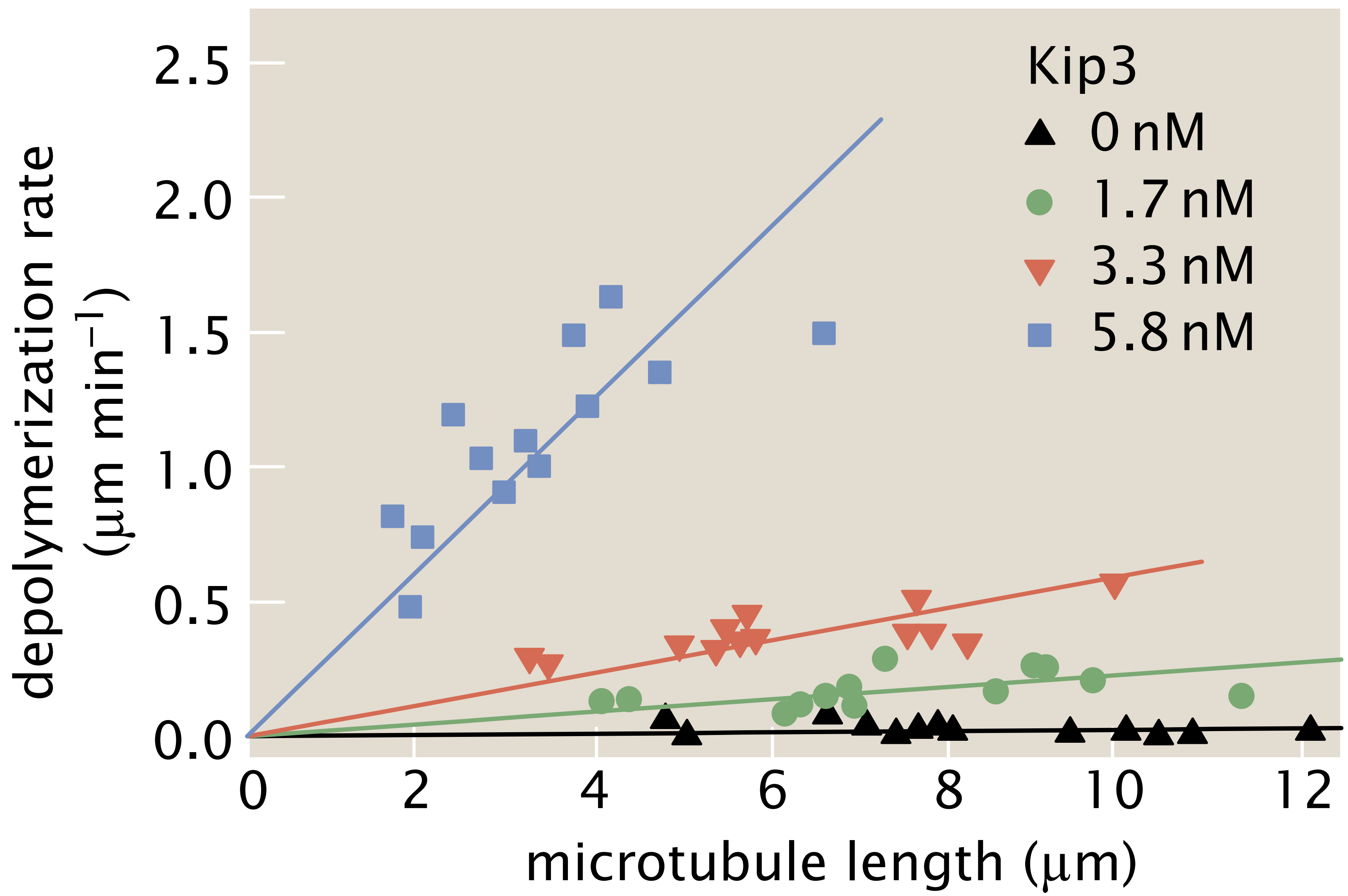
For initially empty microtubule

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

Stationary density of bound motors

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

Length dependent depolymerization rate

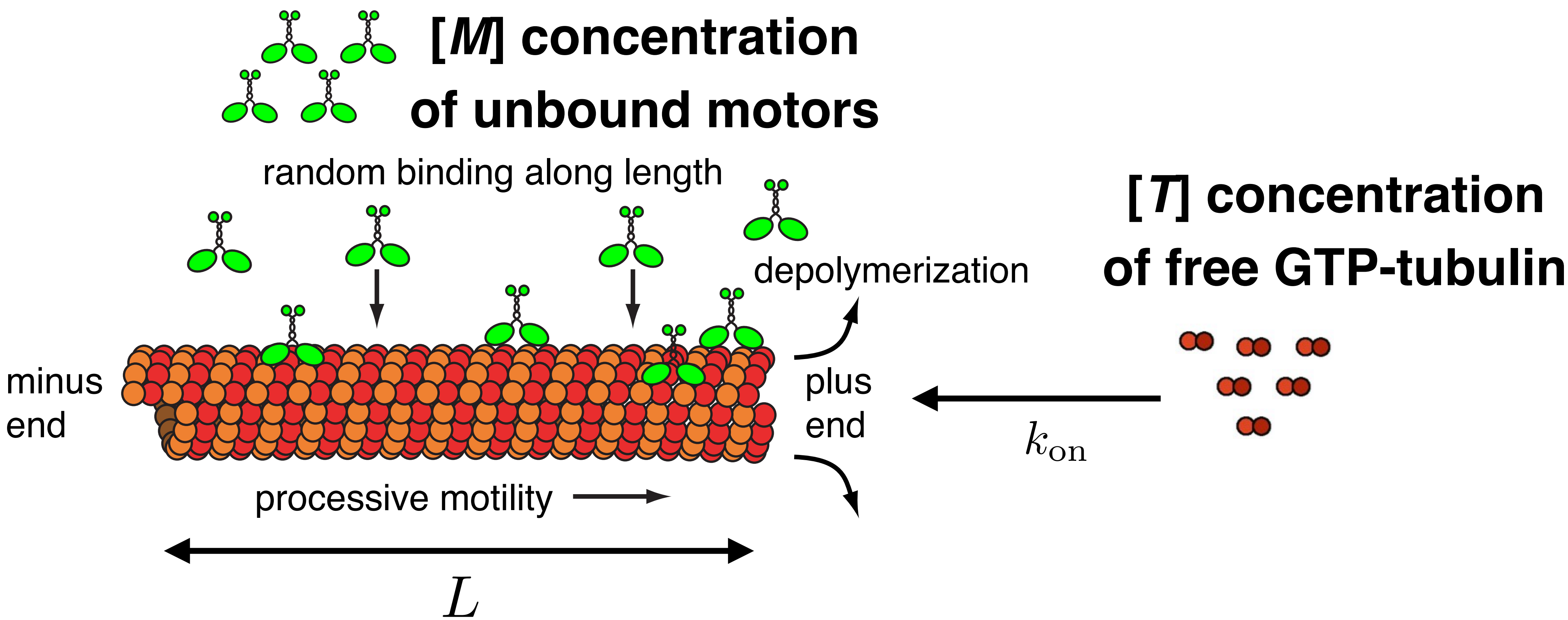


Depolymerization rate is proportional to density of Kip3 motors

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

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

Controlled length of microtubules



relative velocity of motors arriving to the tip

$$\frac{dL}{dt} = a k_{\text{on}}[T] - a\rho^*(L)\left[v_{\text{mot}} - \frac{dL}{dt}\right]$$

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

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

Stationary length of microtubules

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

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

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

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

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

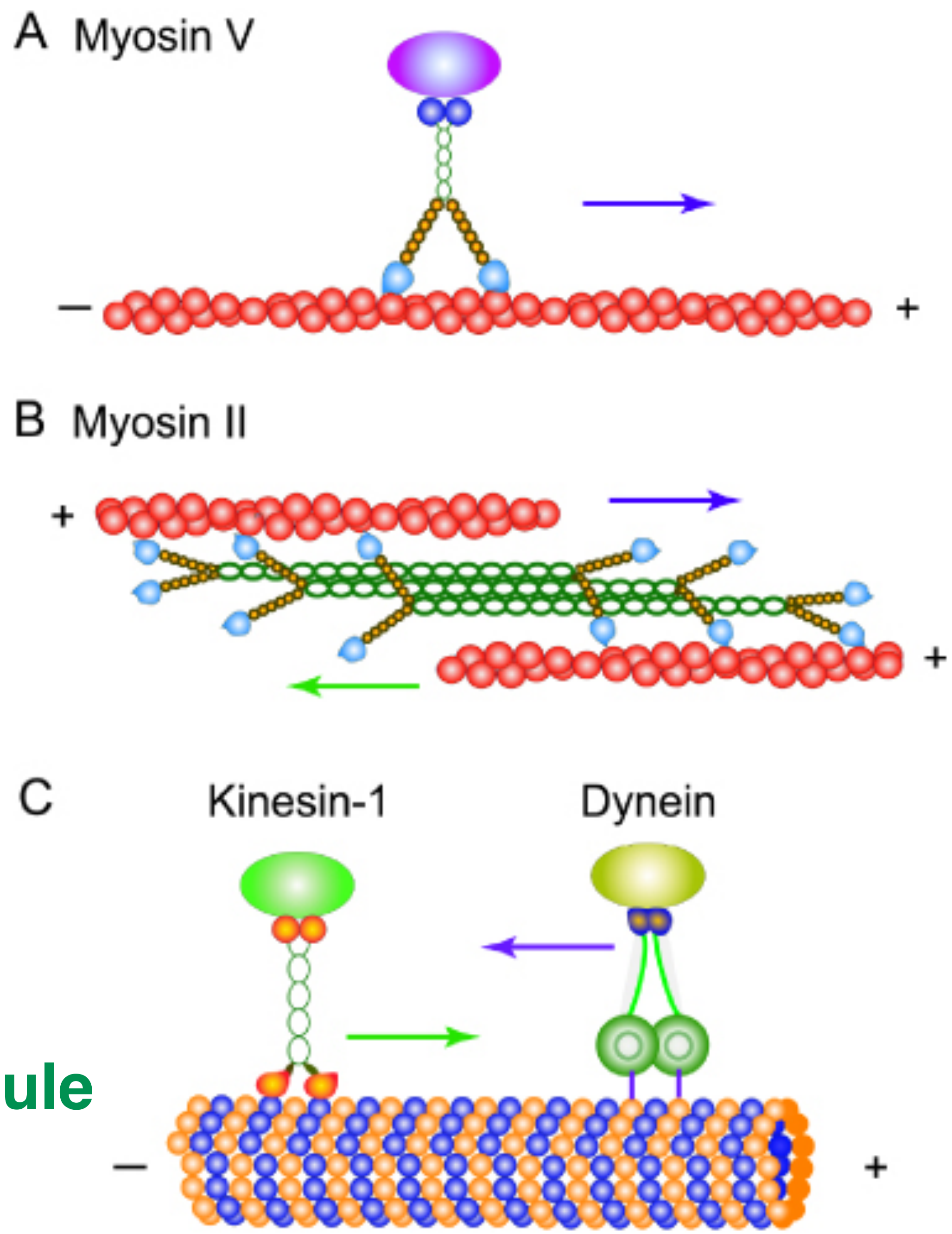
$$L^* \sim 75\mu\text{m}$$

Molecular motors

Actin

Actin

Microtubule

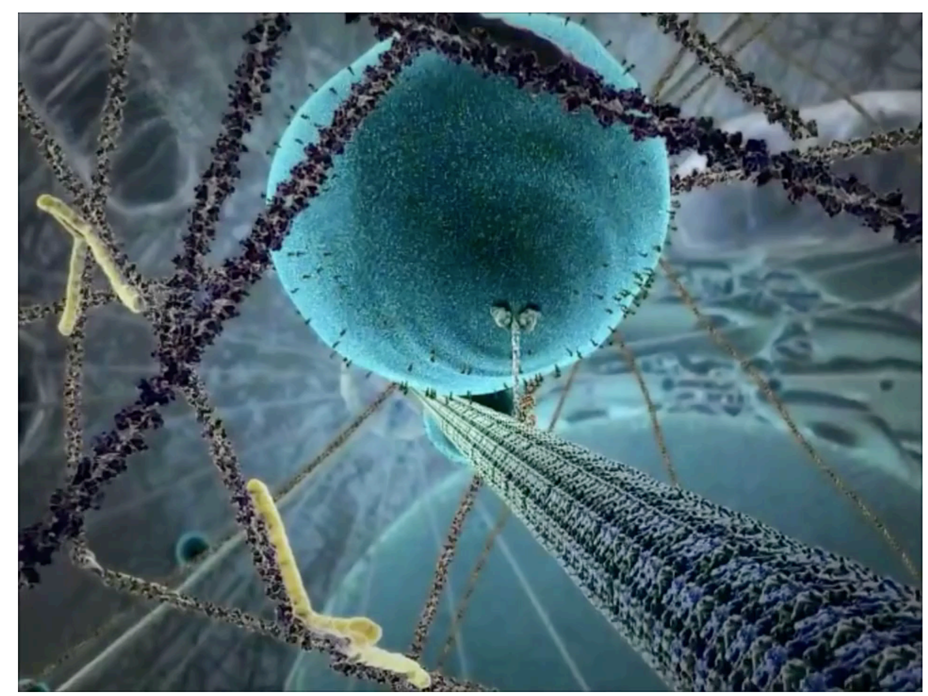


A.B. Kolomeisky, J. Phys.: Condens. Matter **25**, 463101 (2013)

Contraction of muscles

Transport of large molecules around cells (diffusion too slow)

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



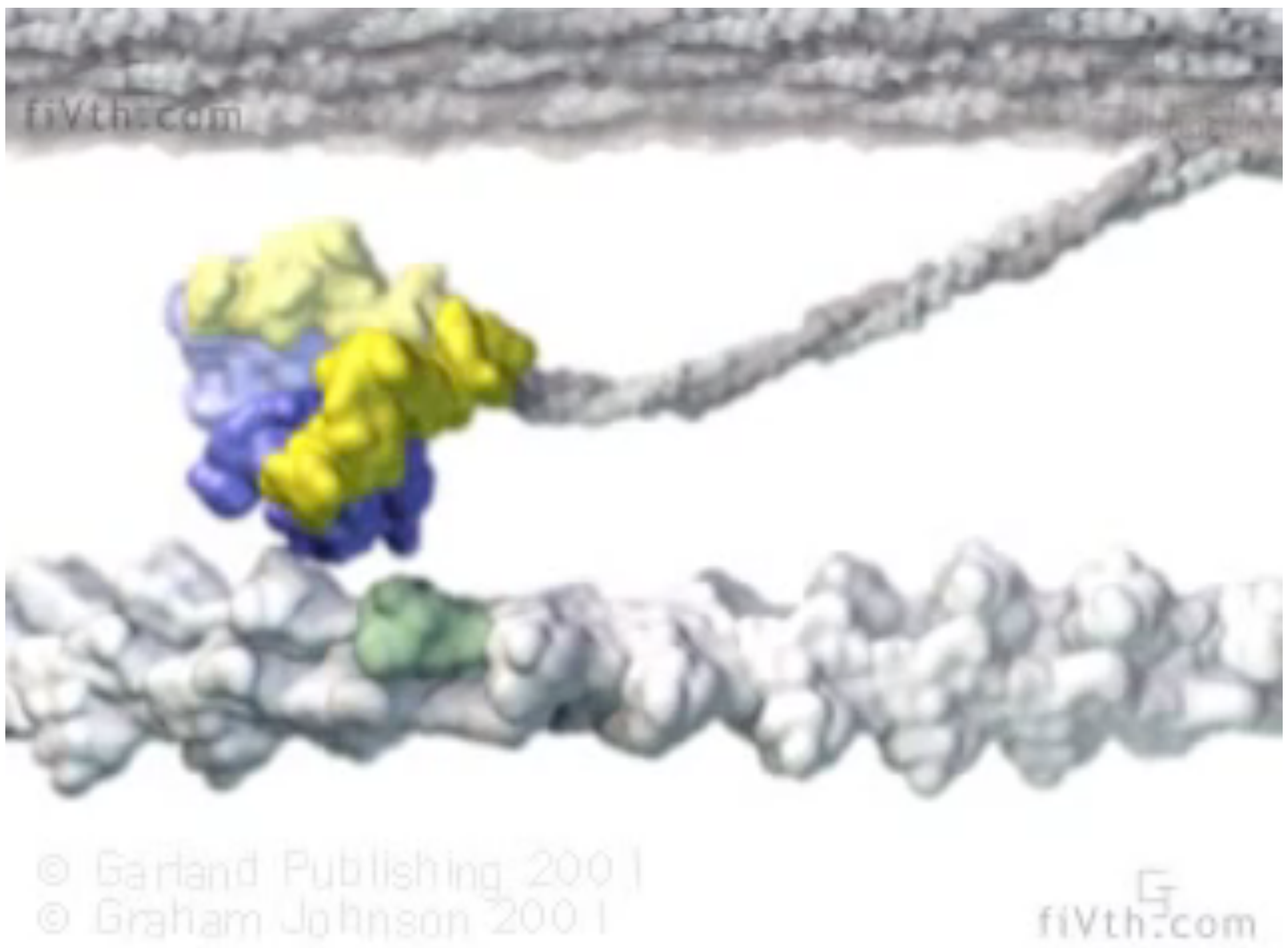
Harvard BioVisions

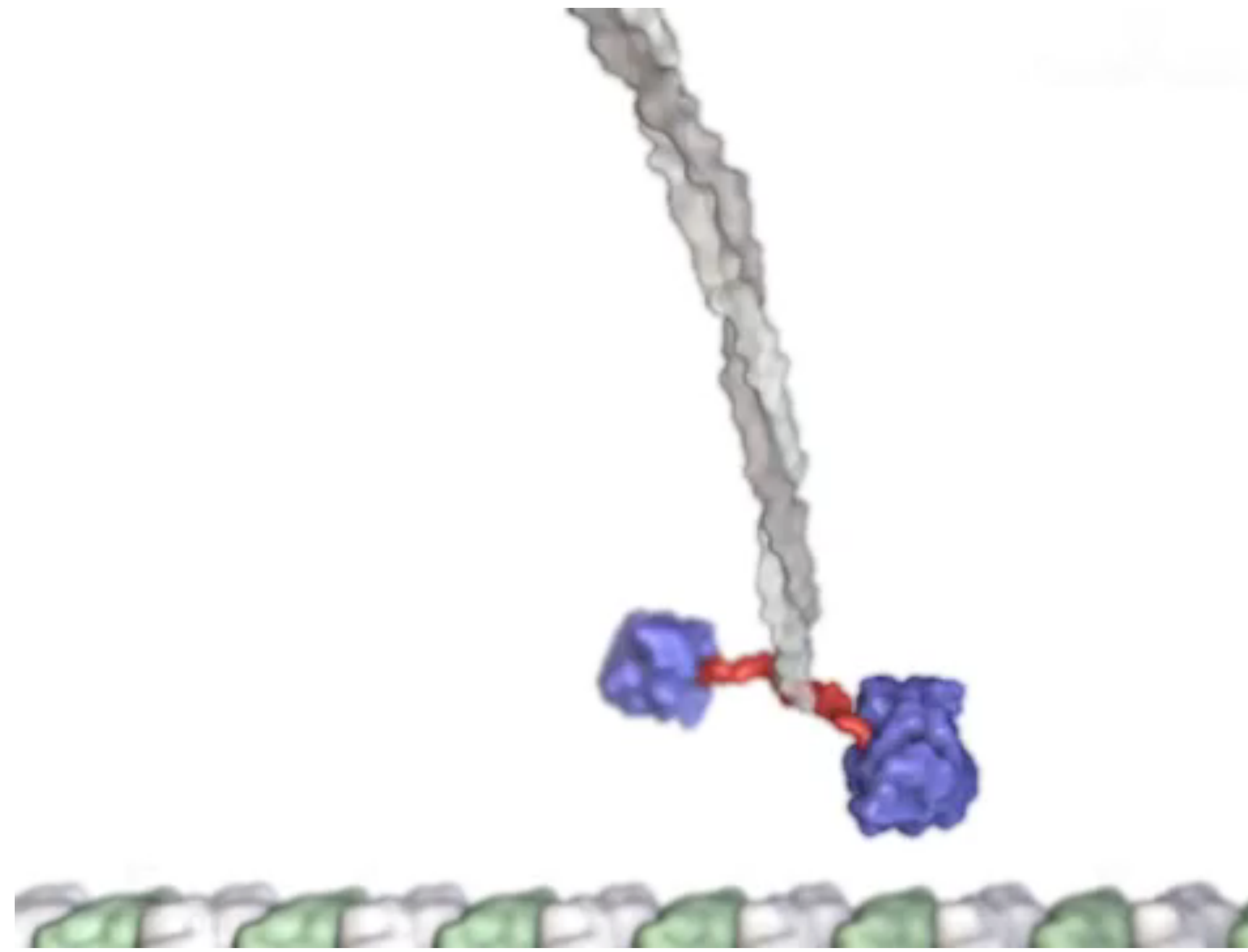
https://www.youtube.com/watch?v=FzcTgrxMzZk

Movement of molecular motors is powered by ATP molecules

Myosin motor walking on actin in muscles

Kinesin motor walking on microtubule





Graham Johnson

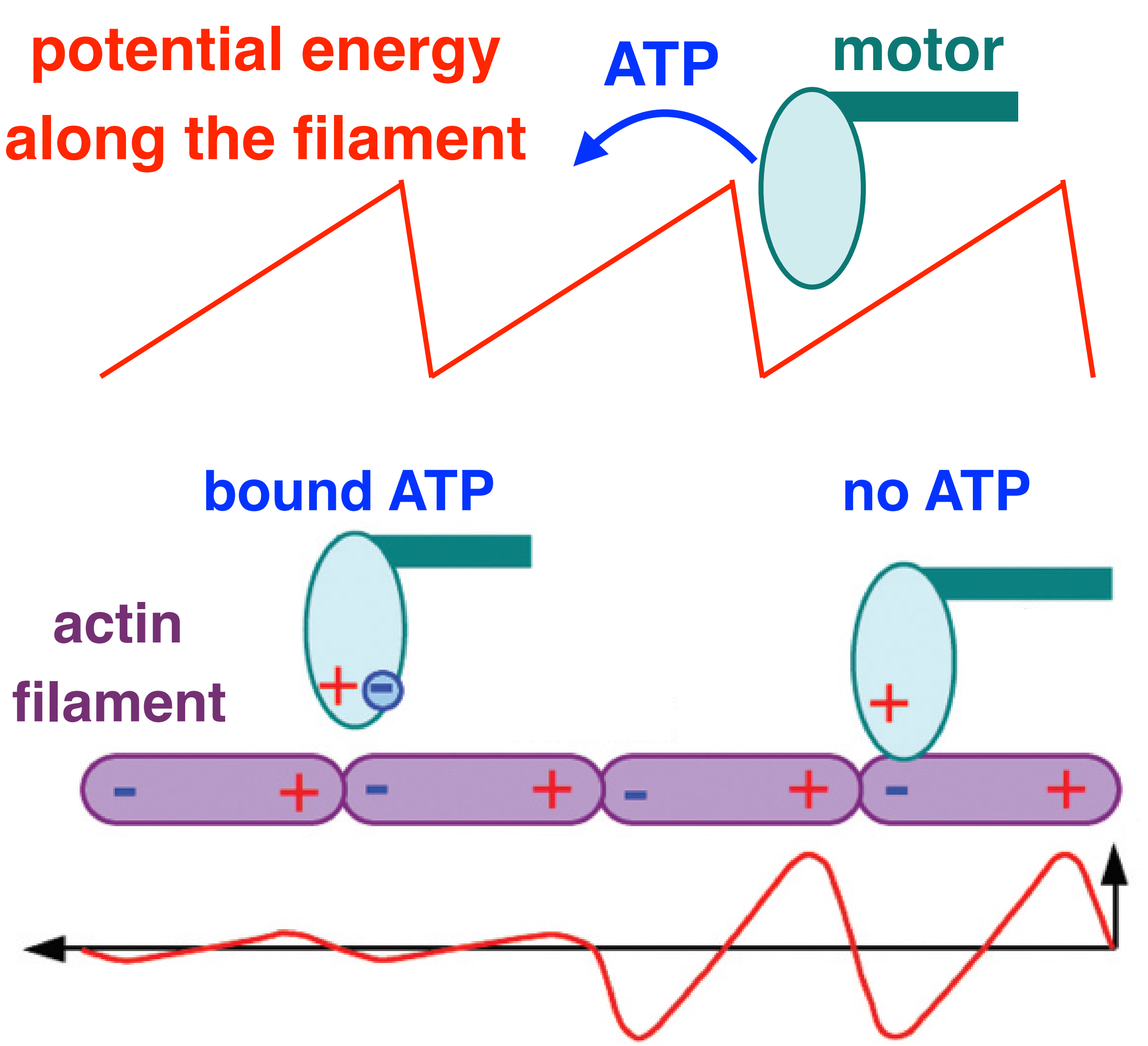
https://www.youtube.com/watch?v=oHDRIwRZRVI

https://www.youtube.com/watch?v=YAva4g3Pk6k

Molecular motors vs Brownian ratchets

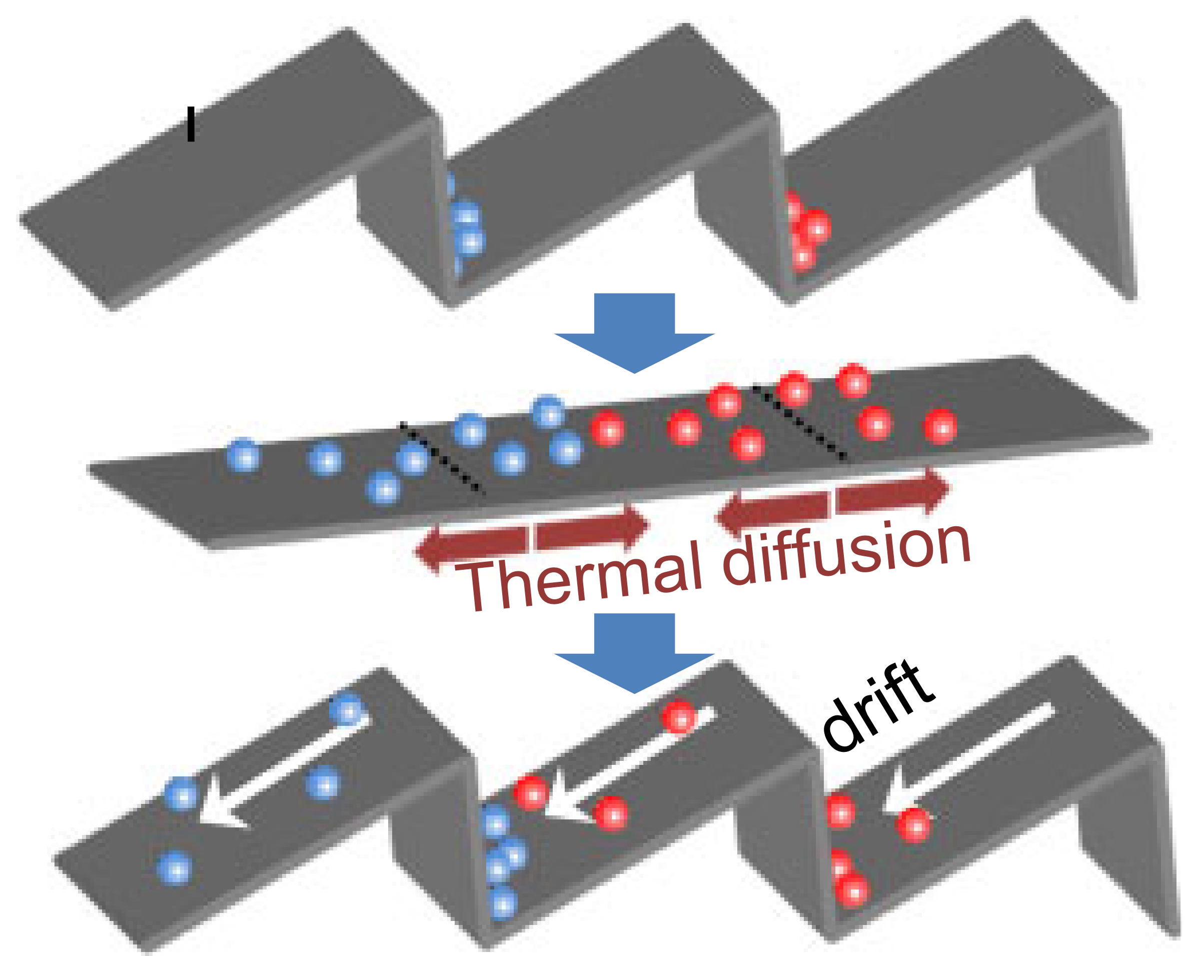
Myosin motor

ATP driven process drives molecular motors along the filaments



Brownian ratchet

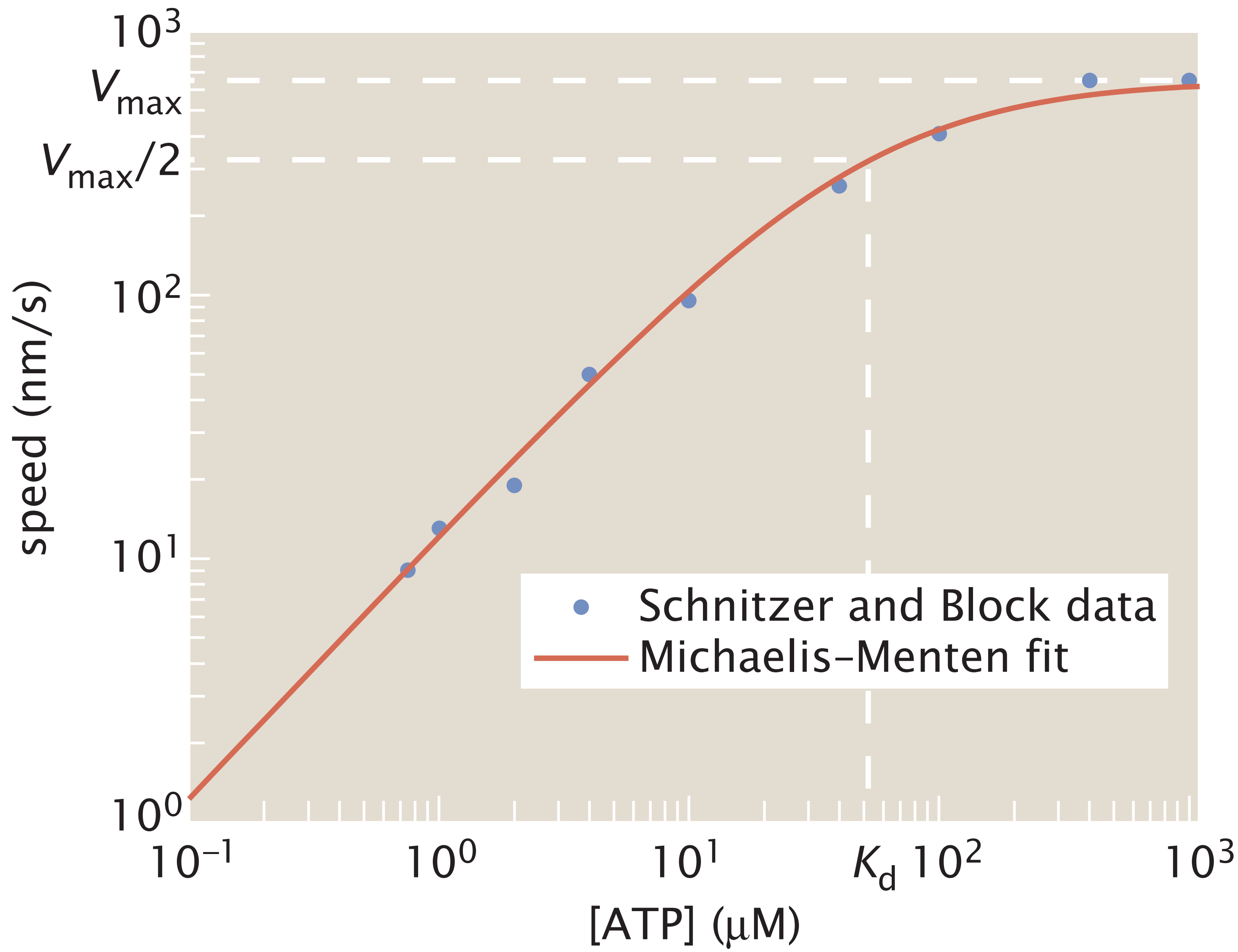
net movement of particles is achieved by periodic modulation of asymmetric external potential



ATP concentration dependent speed of motors

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

Kinesin motor on microtubules



Maximal speed

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

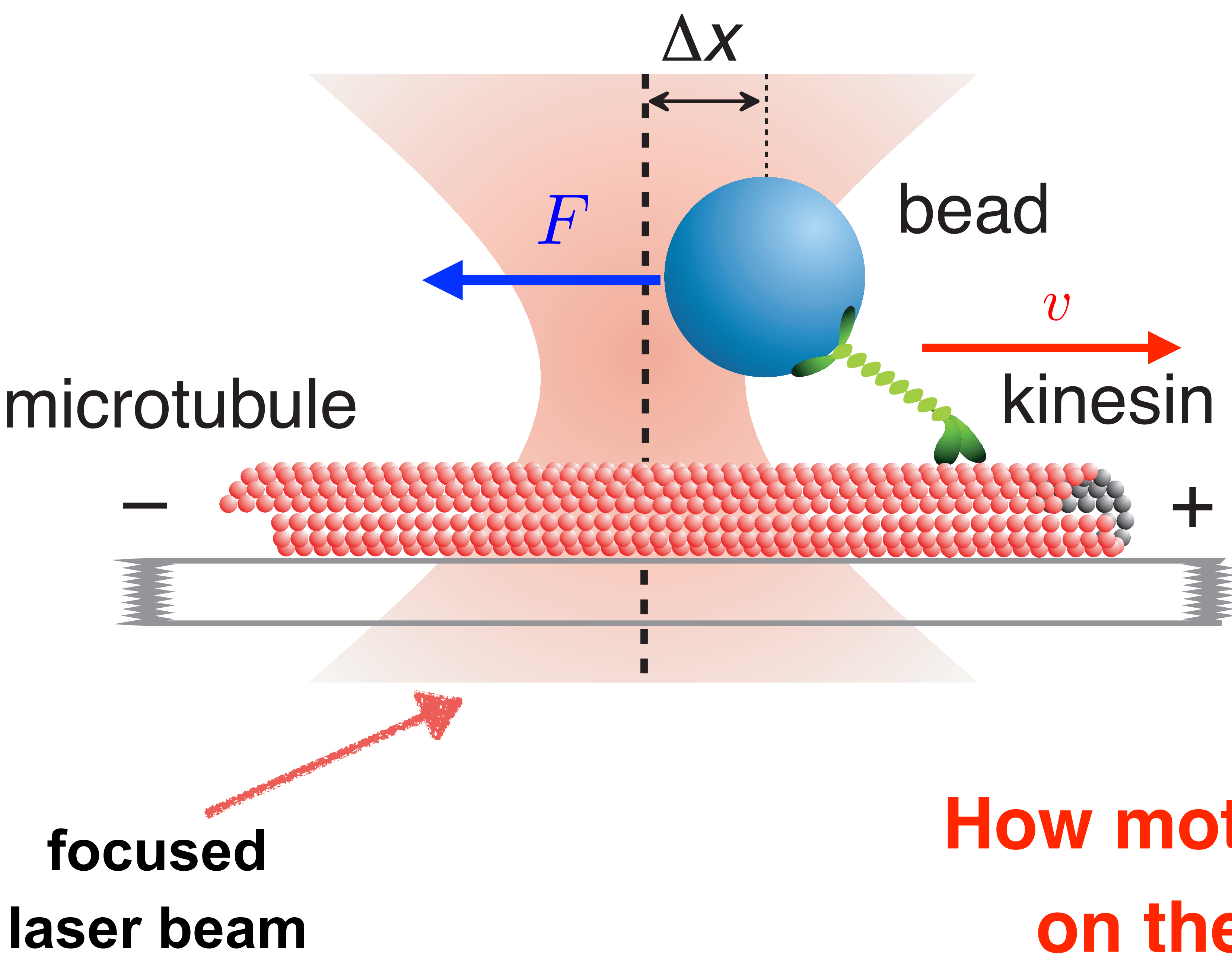
ATP concentration at half the maximal speed

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

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



$$F \approx k\Delta x$$

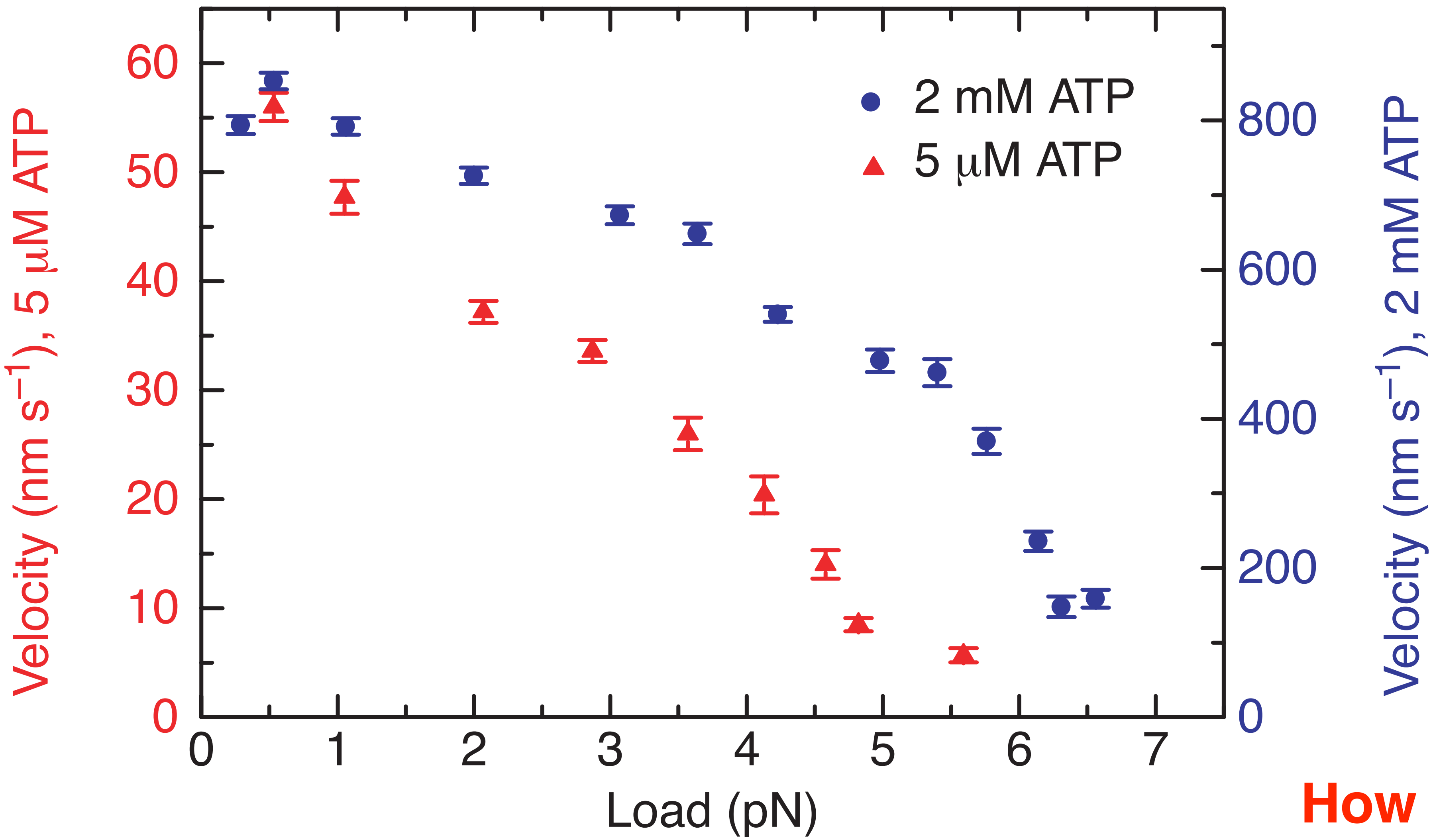
Effective spring constant *k* depends on the bead size, refractive indices of the bead and surrounding medium, and the gradient of laser intensity

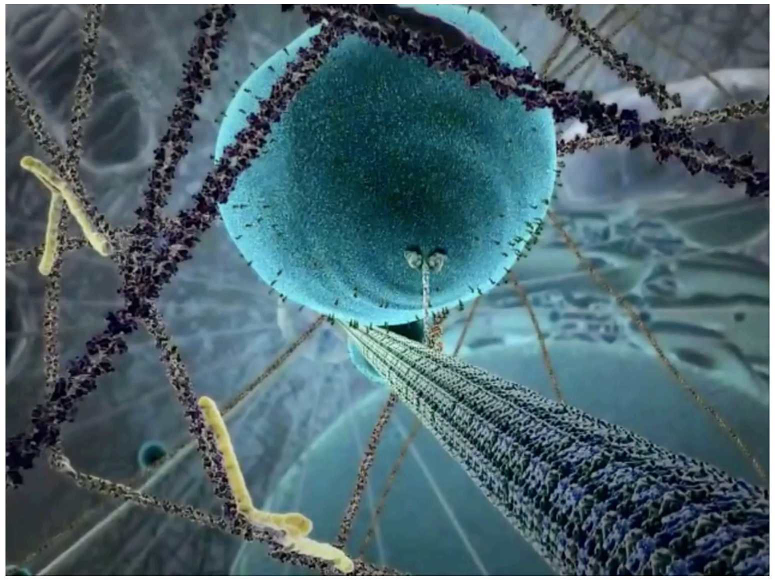
How motor speed depends on the loading force?

K. Visscher et al., Nature **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

$$v(F_s) = 0$$

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

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

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

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

Note: viscous drag is negligible

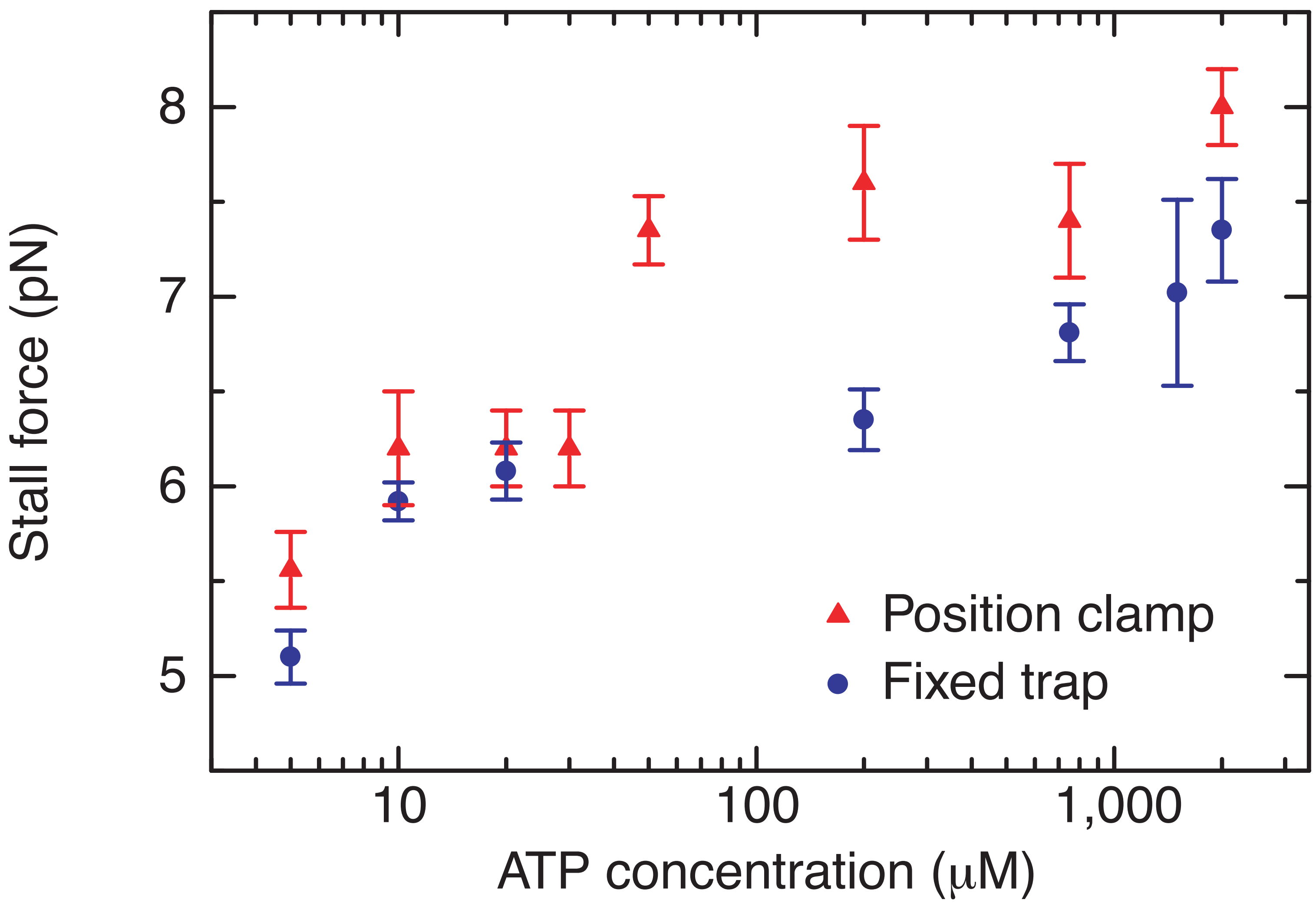
ATP concentration dependent stall force

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

motor step length

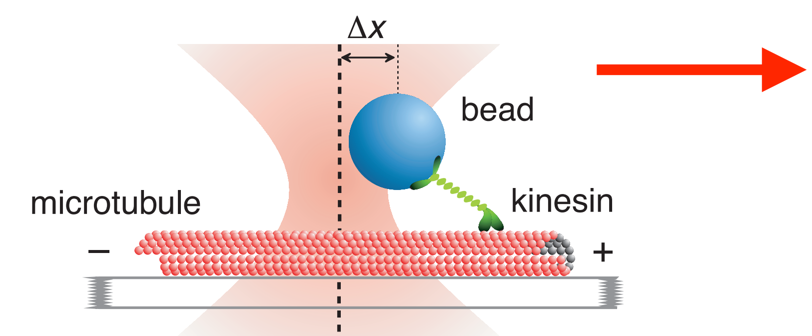
$$a \approx 8\,\mathrm{nm}$$

kinesin walking on microtubules



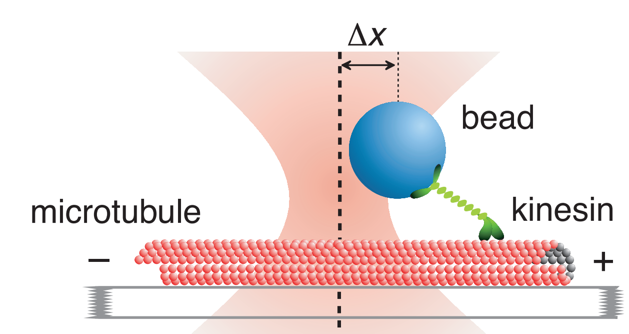
Position clamp

laser follows the bead and keeps fixed force



Fixed trap

laser position is is fixed

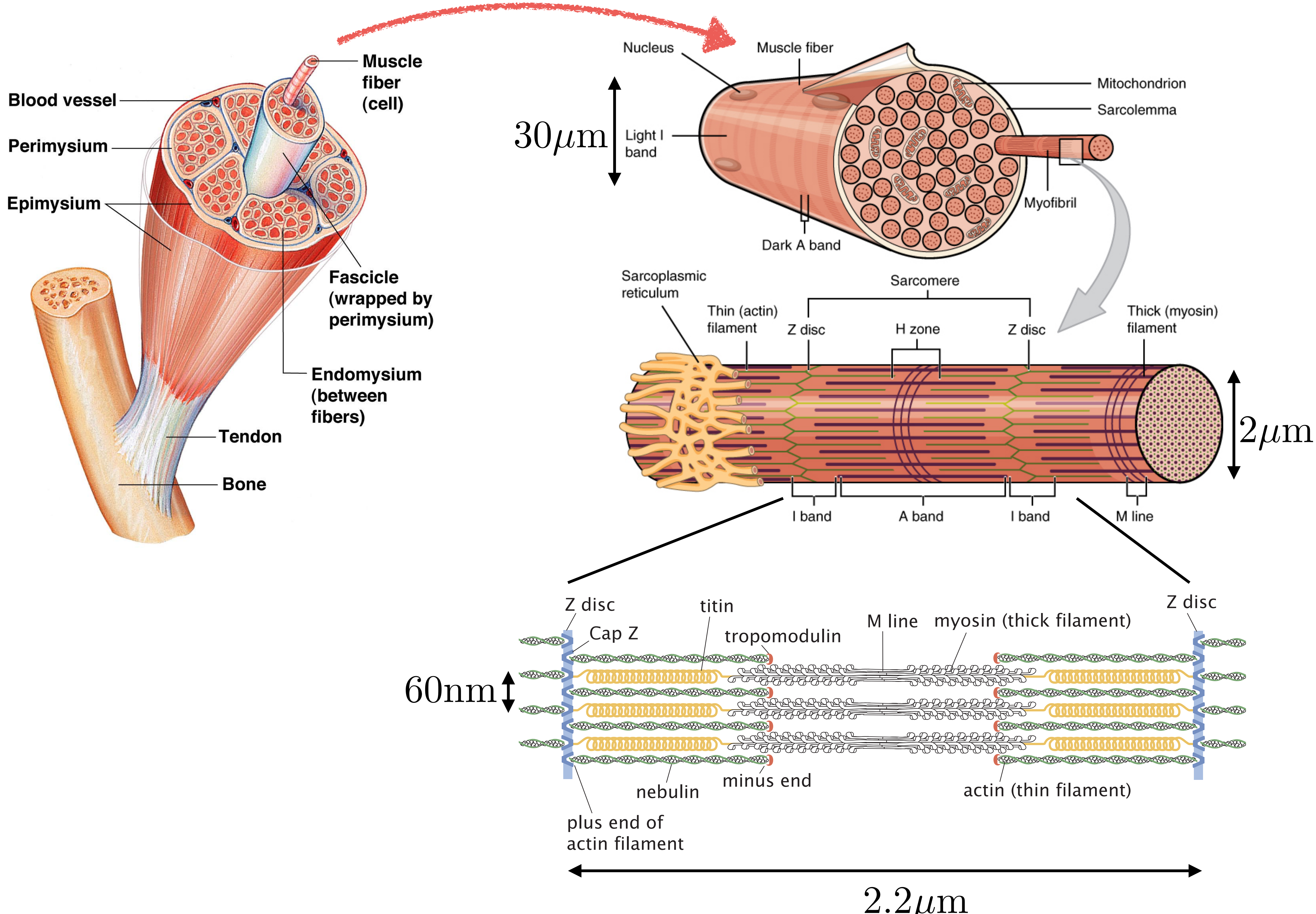


maximal possible force exerted by motors can be estimated from energy conservation

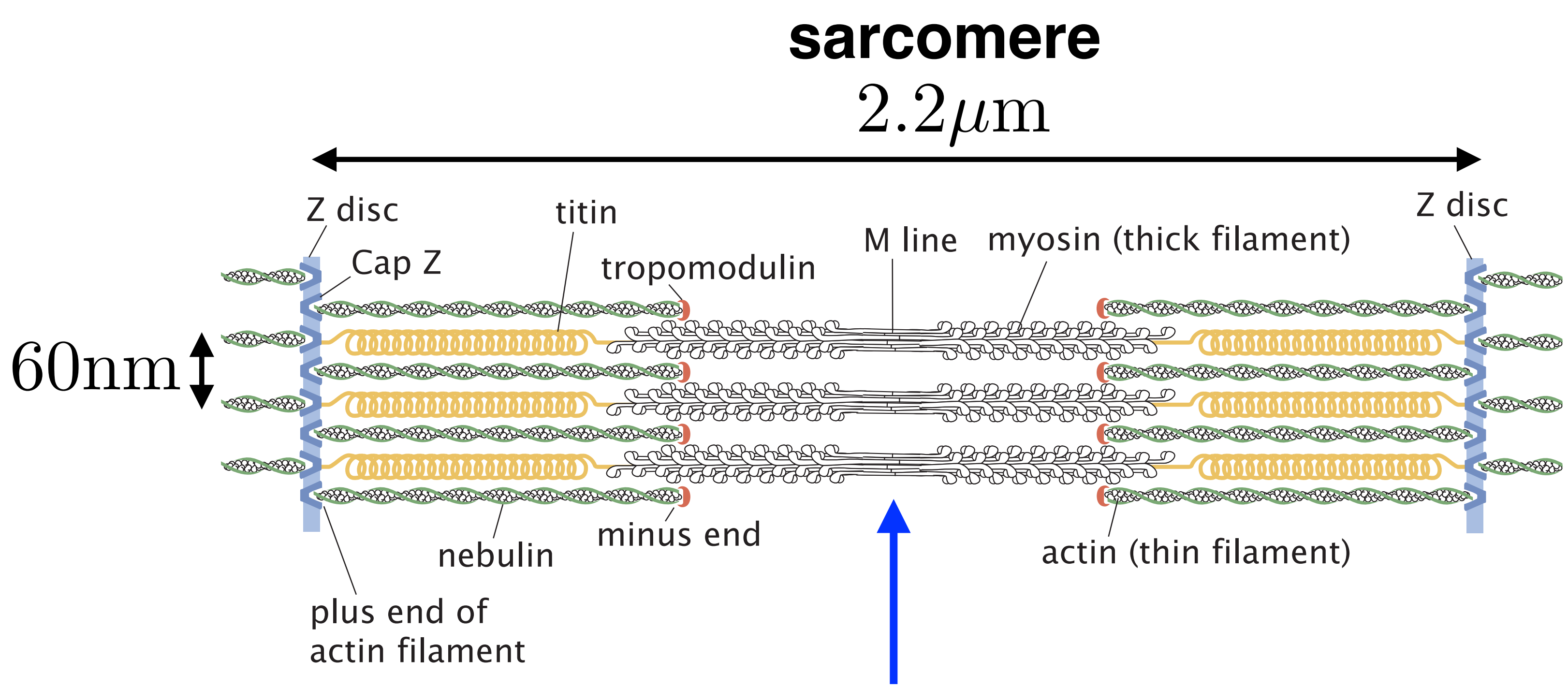
$$F_{\max} = \frac{\Delta G_{\mathrm{ATP}}}{a} \approx \frac{20 k_B T}{8\mathrm{nm}} \sim 10\mathrm{pN}$$

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

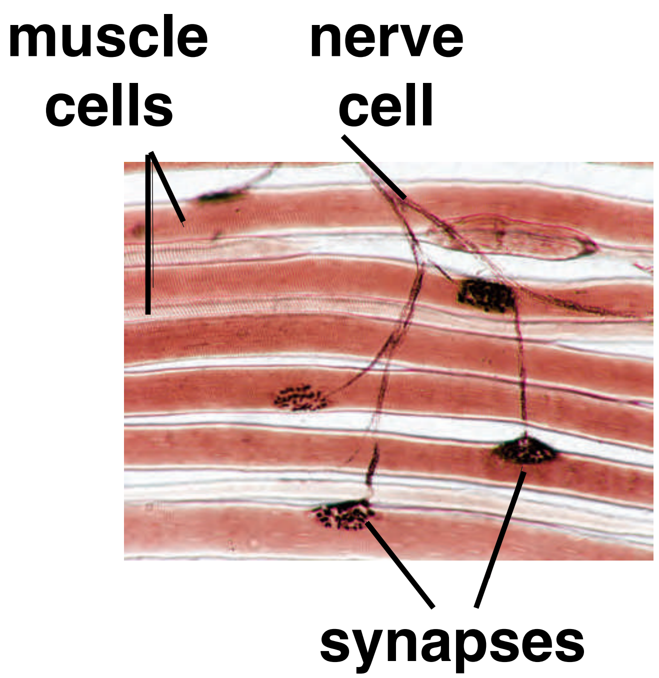
$$300 \times \frac{2\text{pN}}{\pi(30\text{nm})^2} \sim 20\text{N/cm}^2$$

Muscles contract at twice the speed of myosin motors

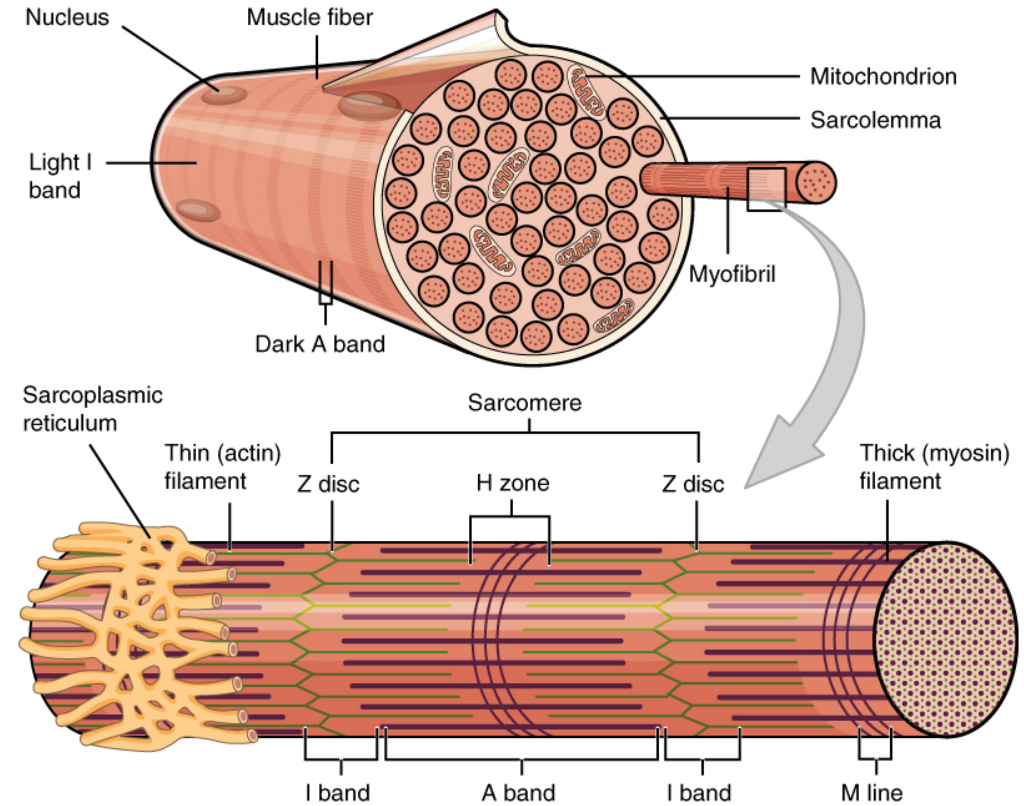
$$\sim 0.1\text{-}1\mu\text{m/s}$$

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

Skeletal muscle contraction is controlled by nerve cells

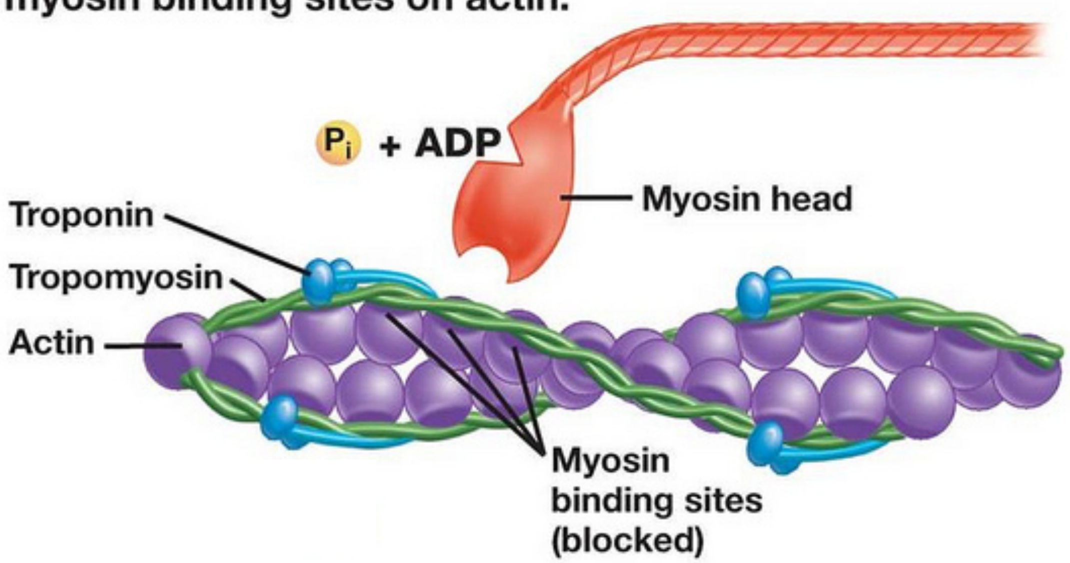


Electric signal from nerve cells releases Ca^{2+} from sarcoplasmic reticulum



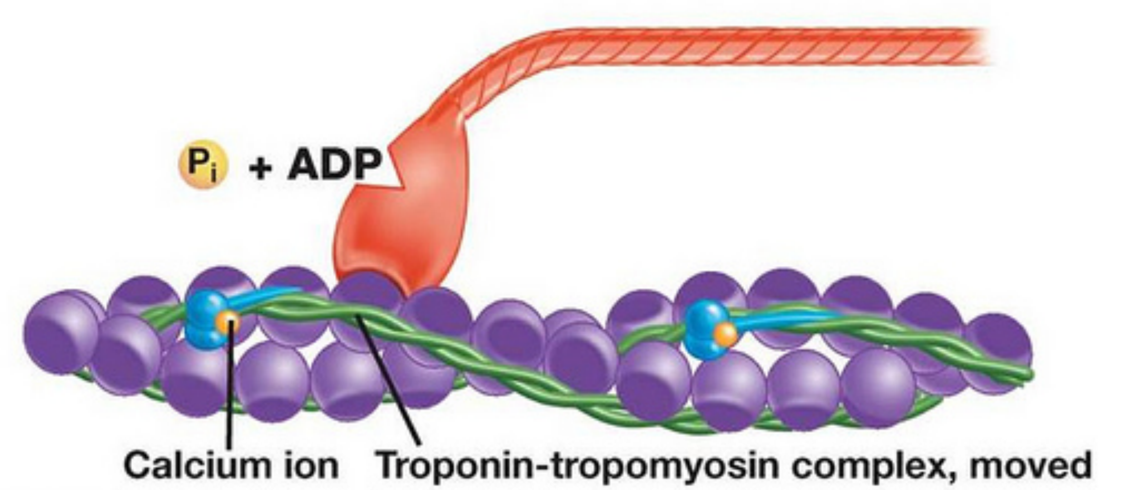
Low Ca^{2+}, muscles are relaxed

(a) Tropomyosin and troponin work together to block the myosin binding sites on actin.

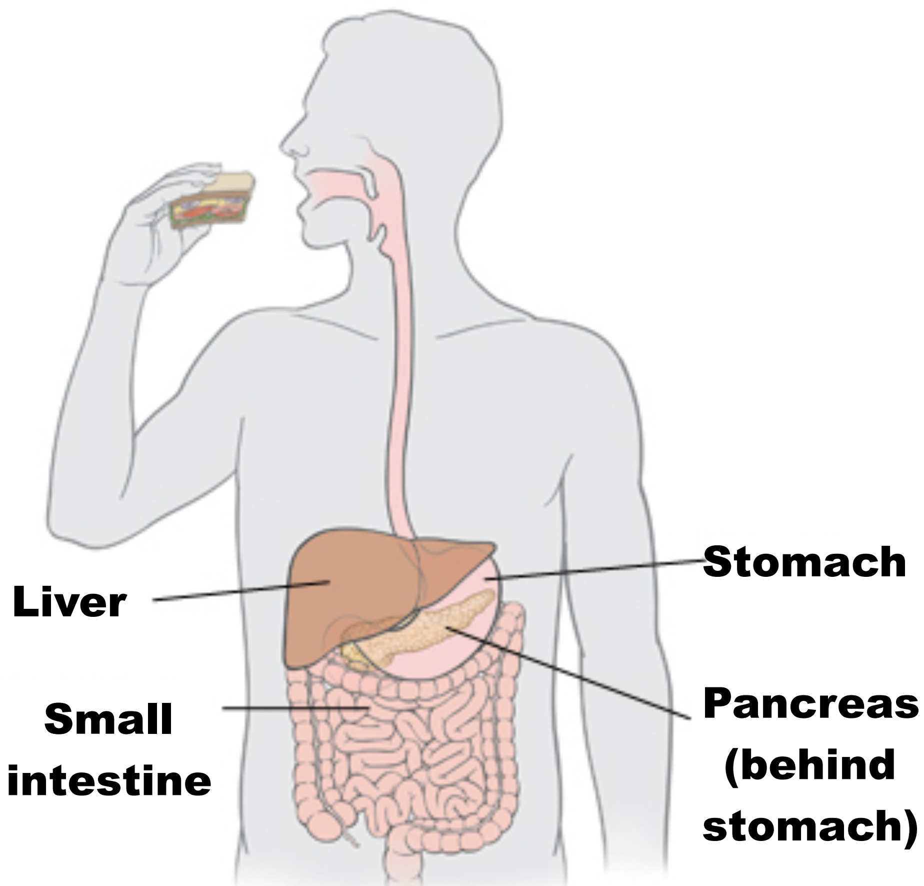


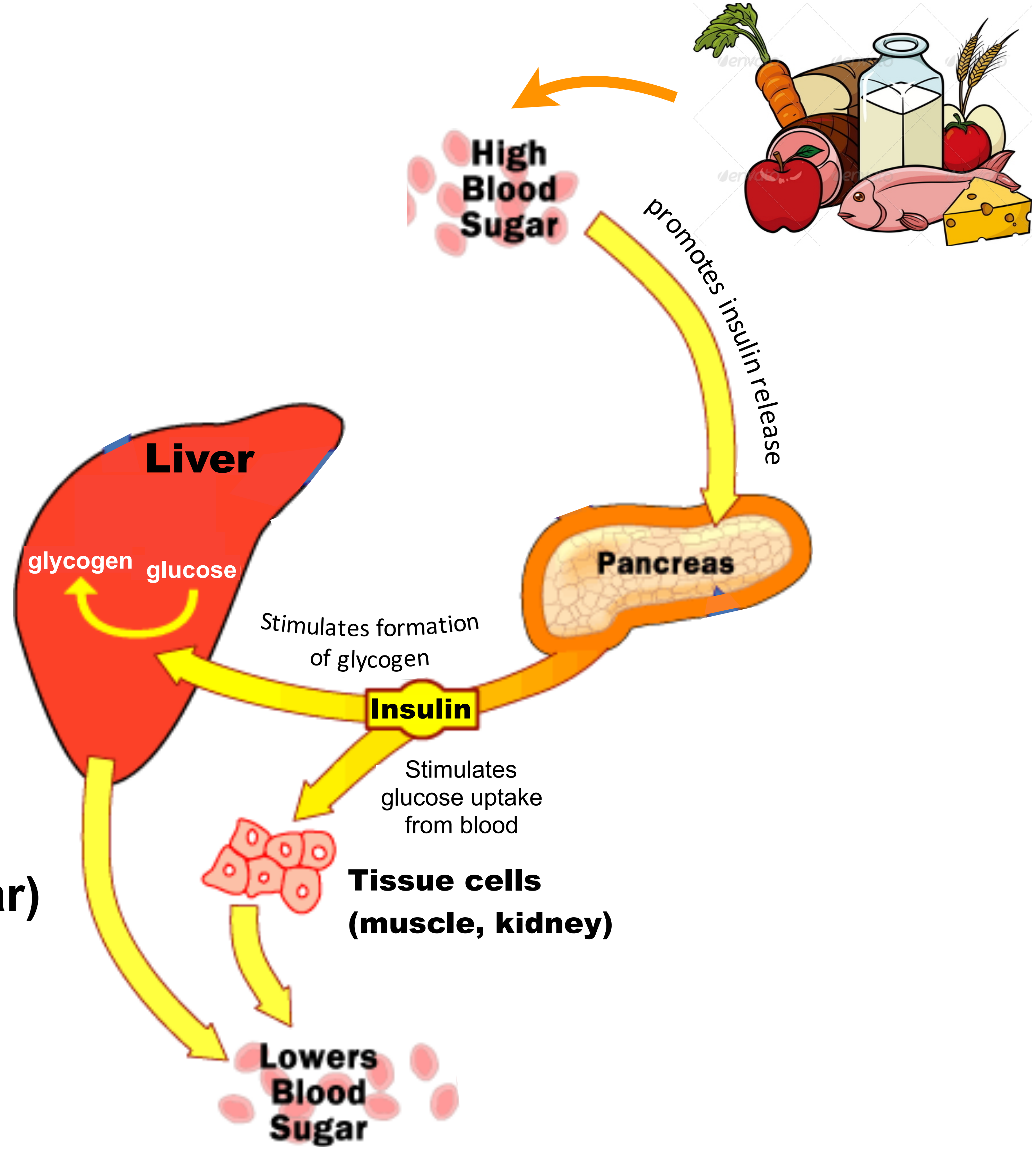
High Ca^{2+}, muscles are contracted

(b) When a calcium ion binds to troponin, the troponin-tropomyosin complex moves, exposing myosin binding sites.

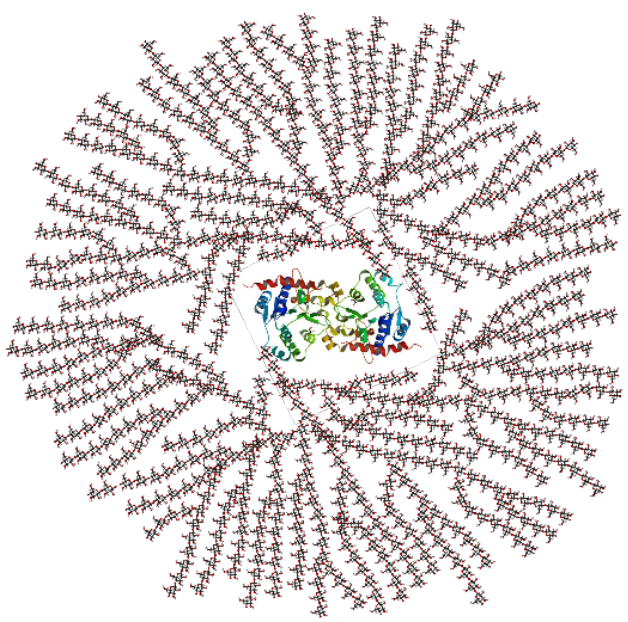


How muscles get ATP energy?

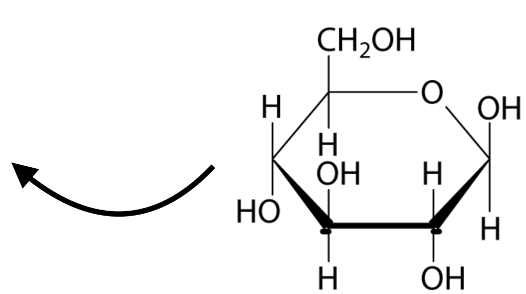




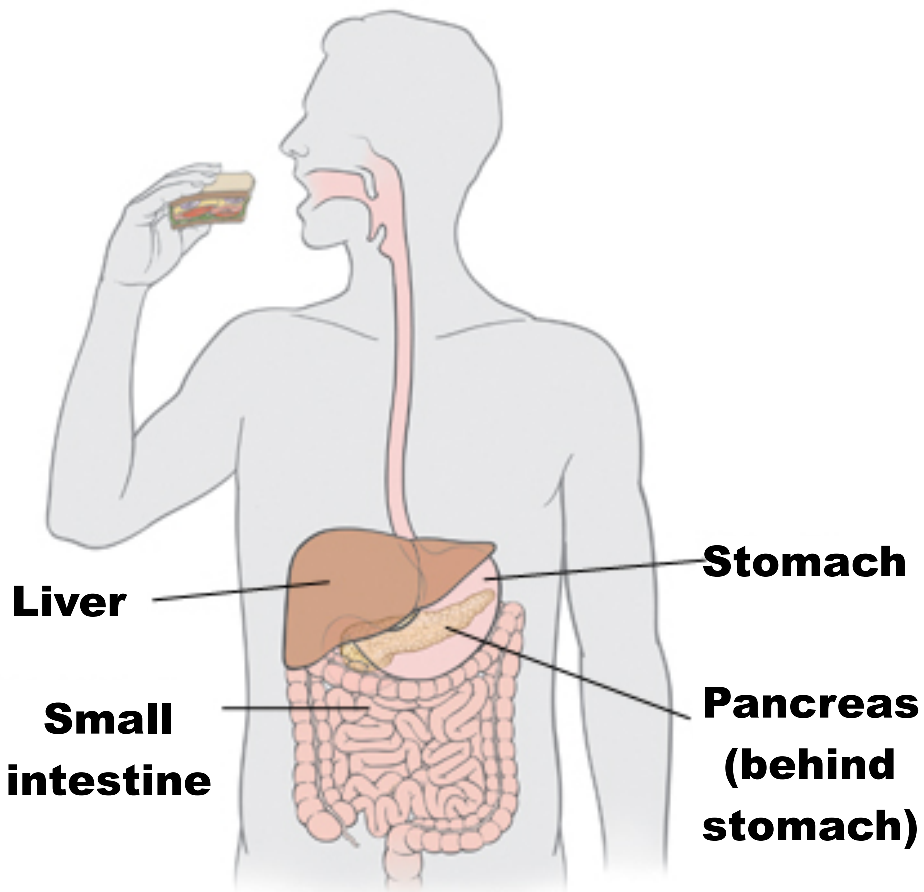
glycogen
(polysaccharide of glucose)



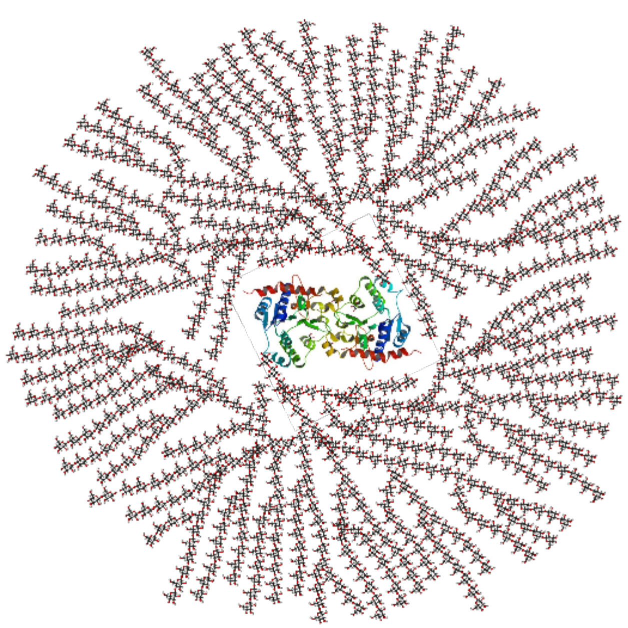
glucose
(blood sugar)



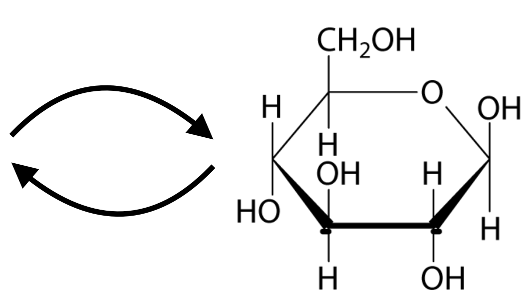
How muscles get ATP energy?

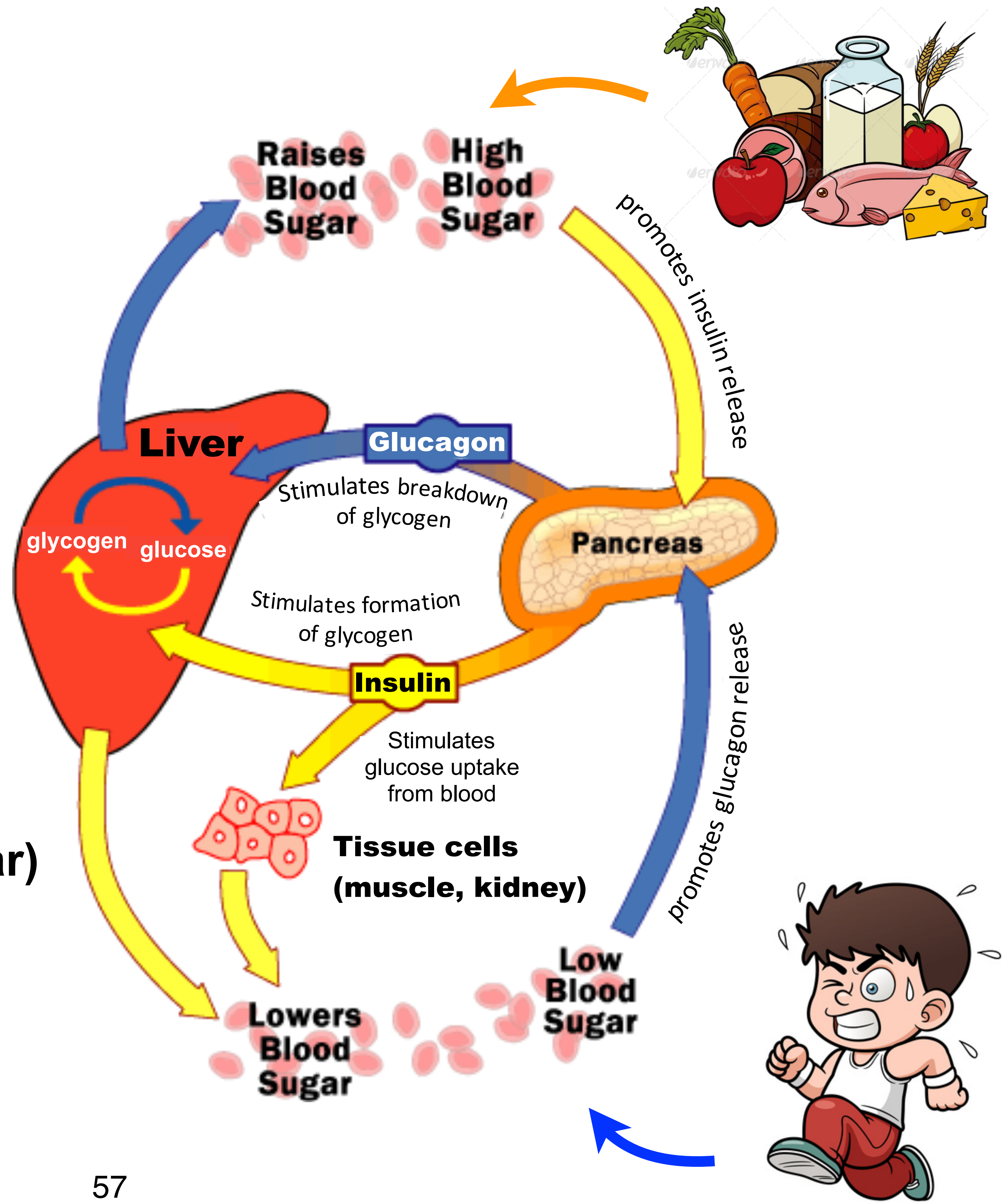


glycogen
(polysaccharide of glucose)

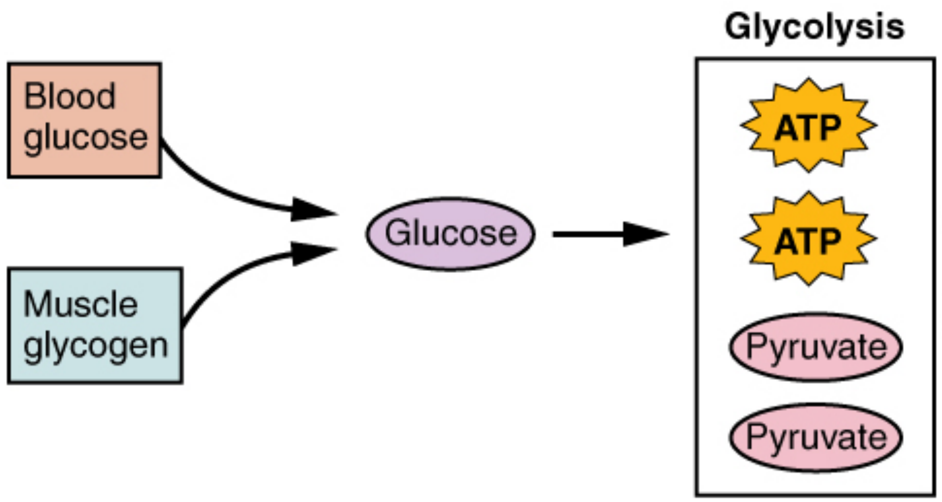


glucose
(blood sugar)





How muscles get ATP energy?



O_2

no O_2

Aerobic respiration

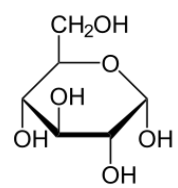
$$C_6H_{12}O_6 + 6O_2 \Longrightarrow 6CO_2 + 6H_2O + 36ATP$$

Anaerobic respiration

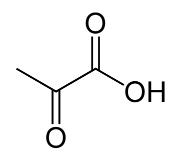
$$C_6H_{12}O_6 \Longrightarrow 2 \text{ Lactic Acids} + 2ATP$$

(muscle fatigue)

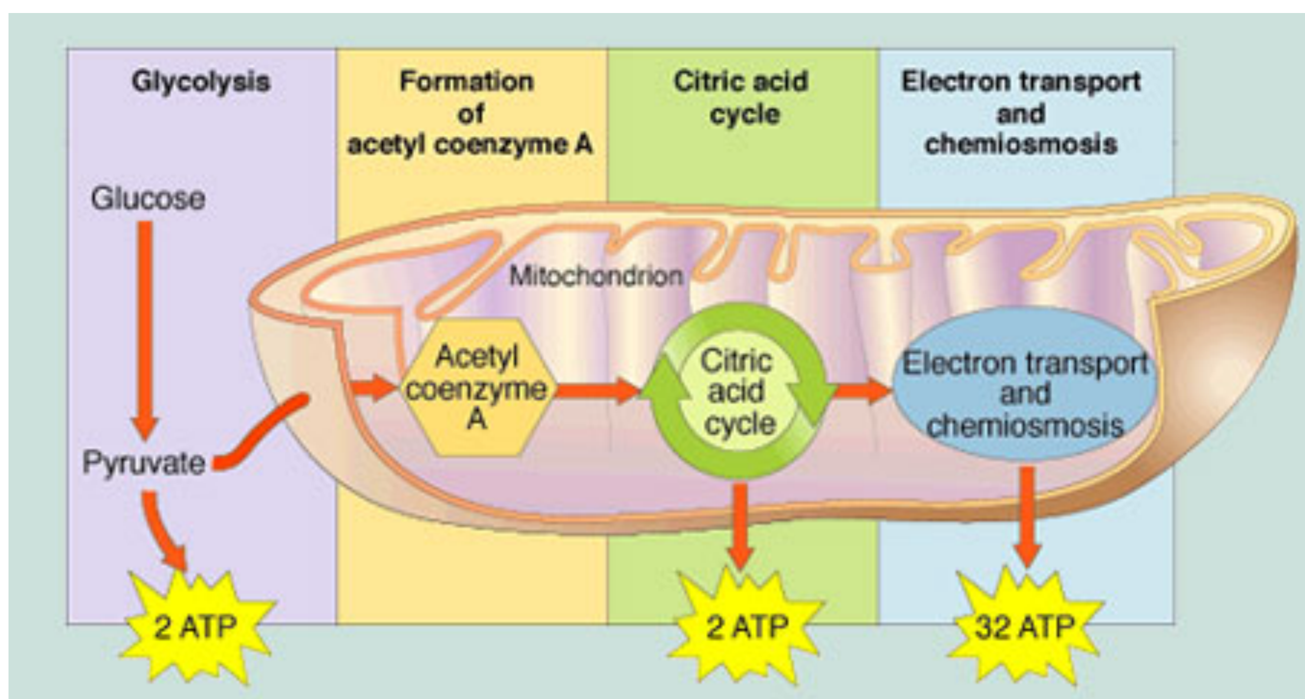
glucose



pyruvate



Aerobic respiration



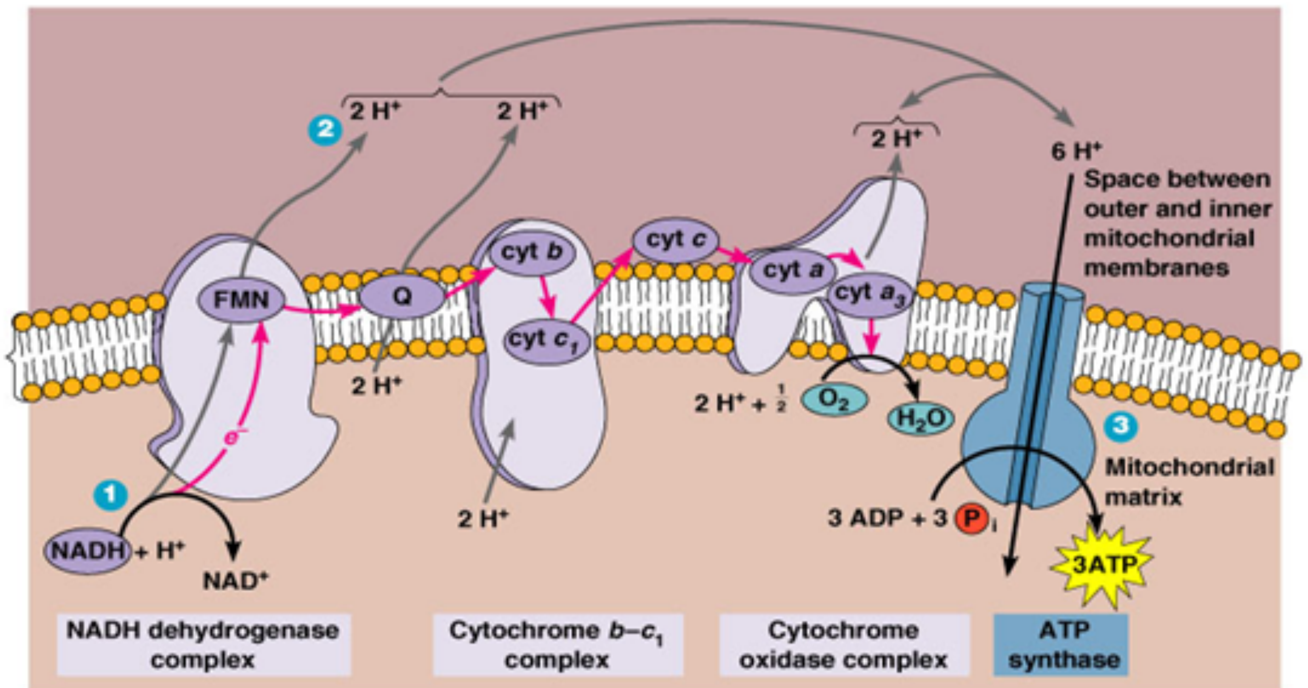
Note:

Citric acid cycle

= Krebs cycle

Electron transport chain

Mitochondrion

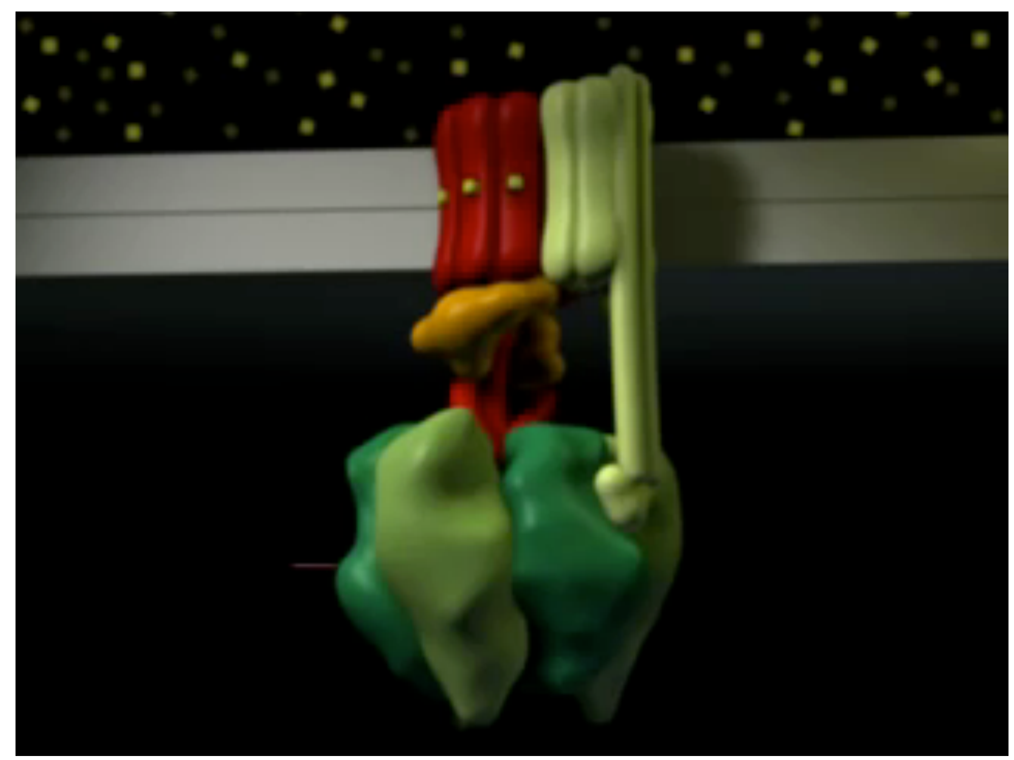


NADH products of the Cytric acid cycle are used to pump H+ to the space between outer and inner mitochondrial membrane.

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.

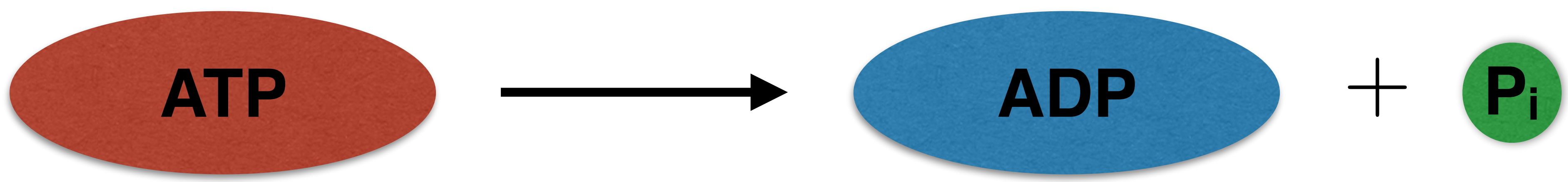
ATP synthase



https://www.youtube.com/watch?v=CN2XOe_c0iM

Energetics of ATP hydrolysis

How much energy is released during ATP hydrolysis?



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

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

$-12.5 k_B T$

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

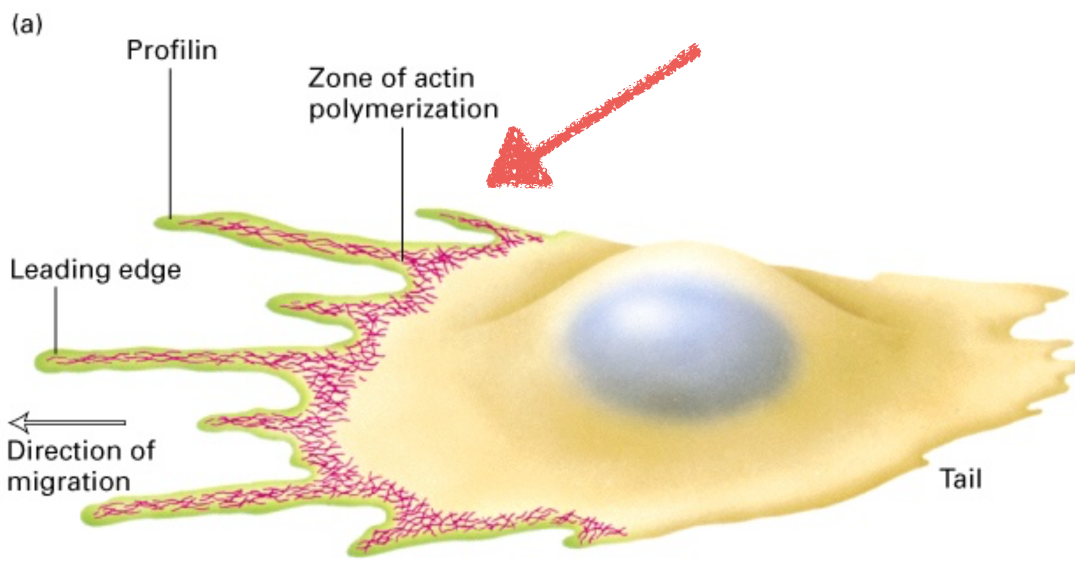
$([\mathrm{ATP}], [\mathrm{ADP}], [\mathrm{P}_i] \sim 1\mathrm{mM})$

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

Actin

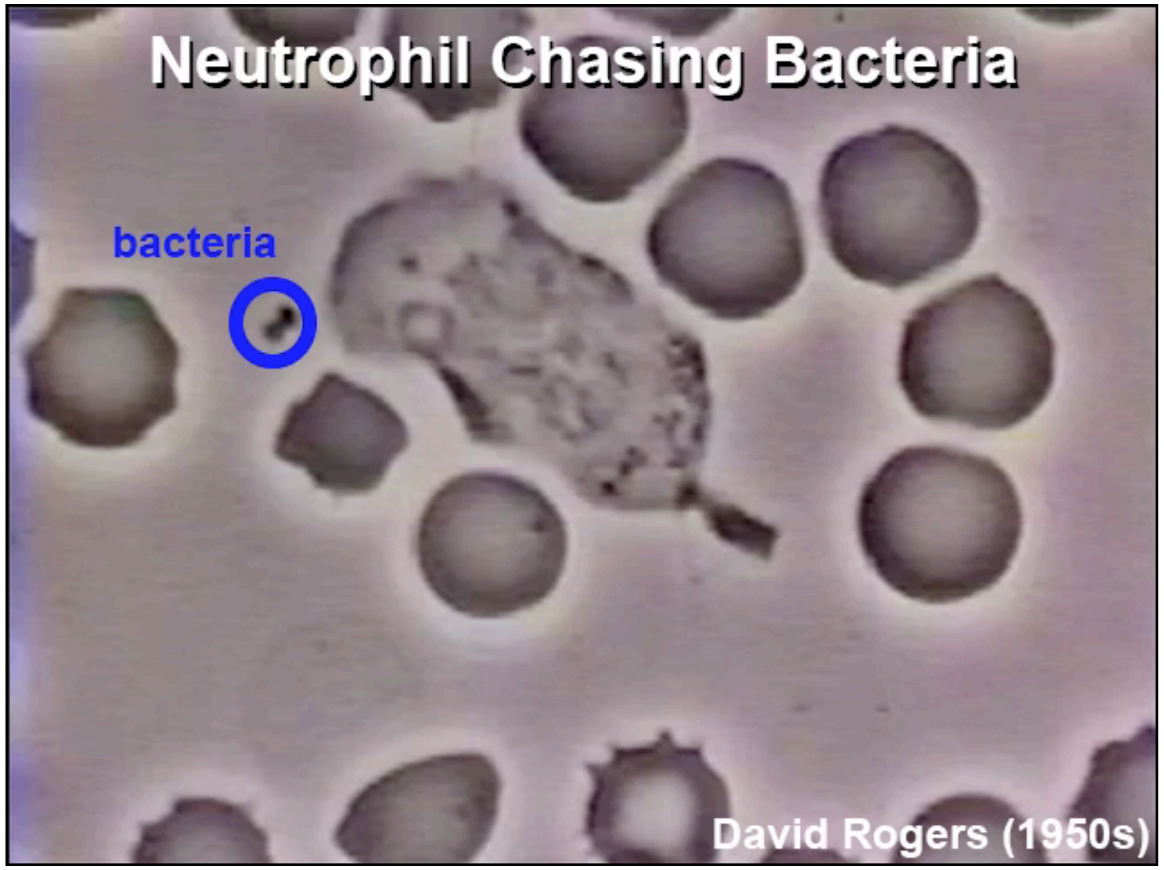


migration of skin cells during wound healing

spread of cancer cells during metastasis of tumors

amoeba searching for food

Immune system:
neutrophils chasing bacteria

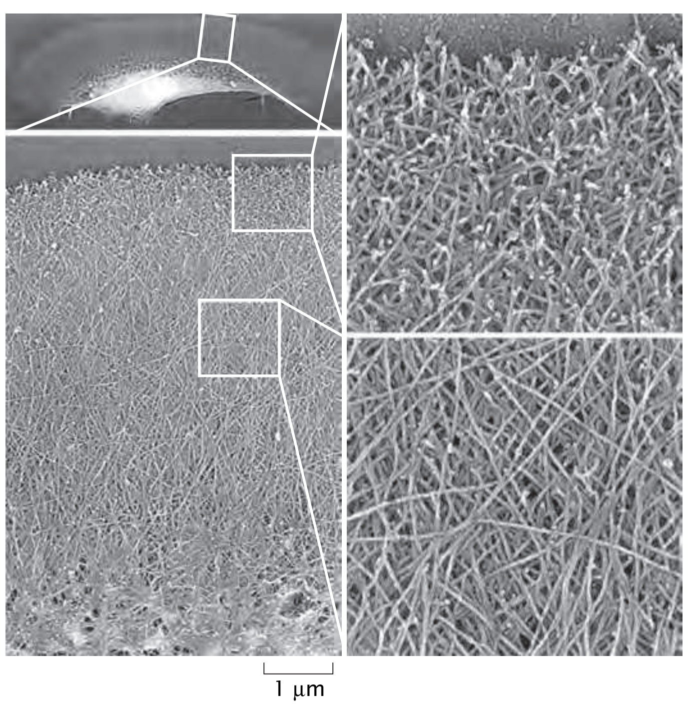


David Rogers, 1950s

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

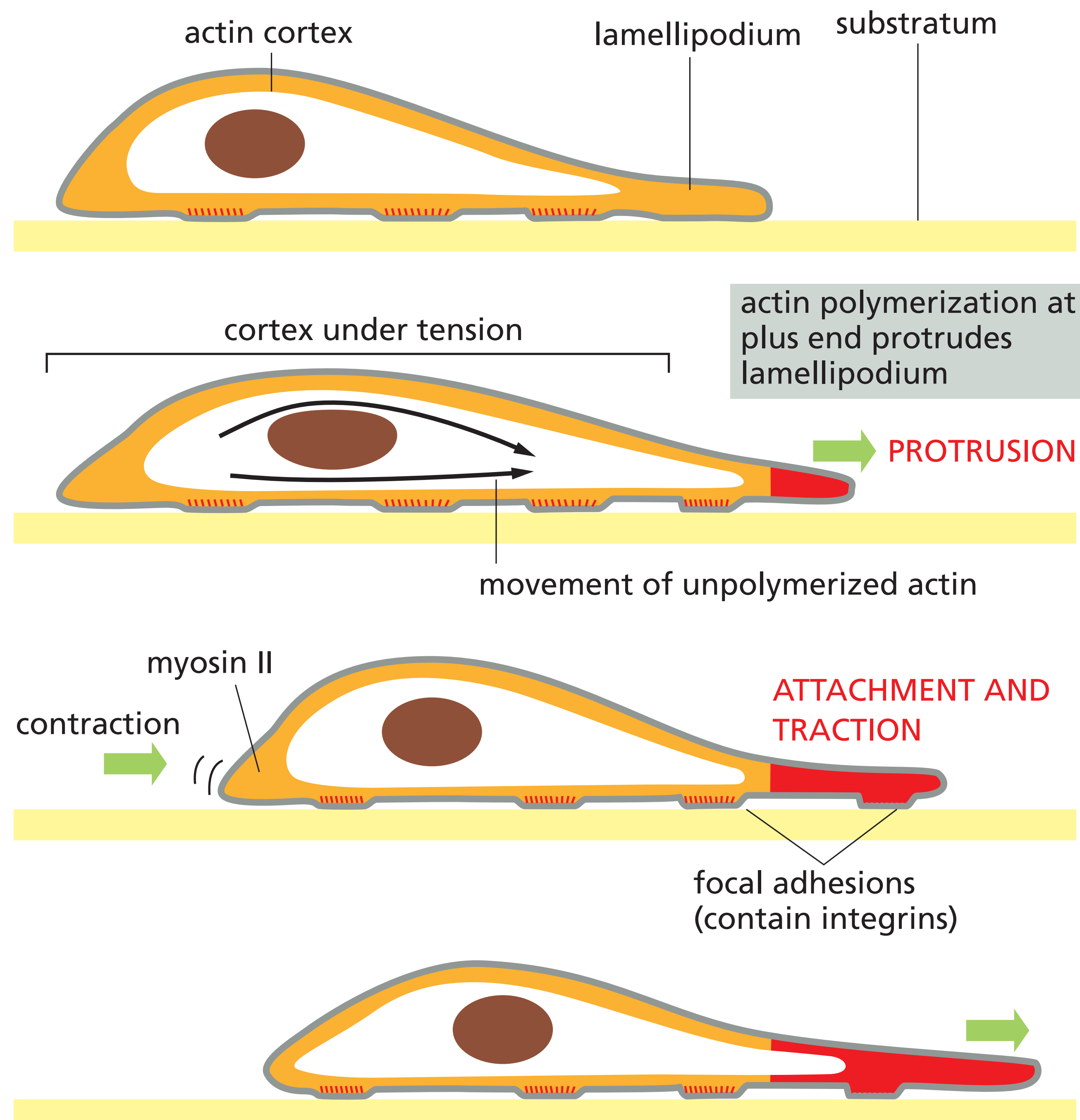
Crawling of cells

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



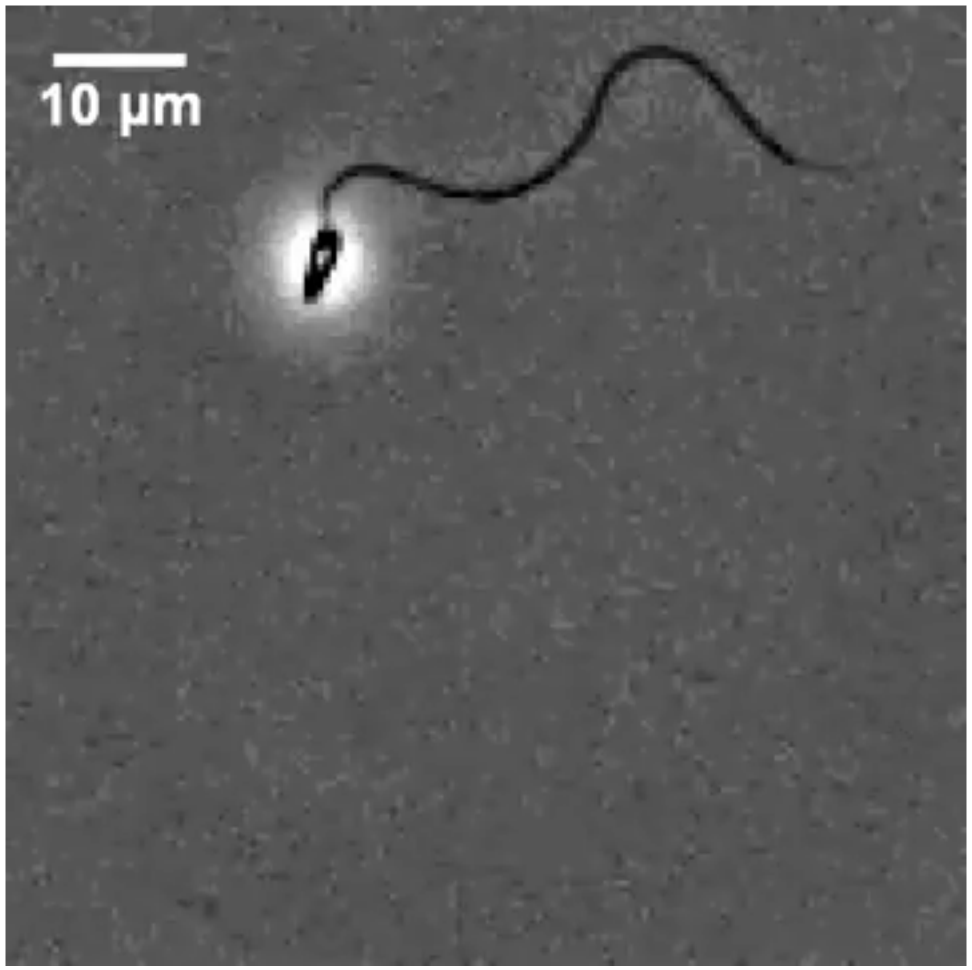
actin

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



Alberts et al., Molecular Biology of the Cell

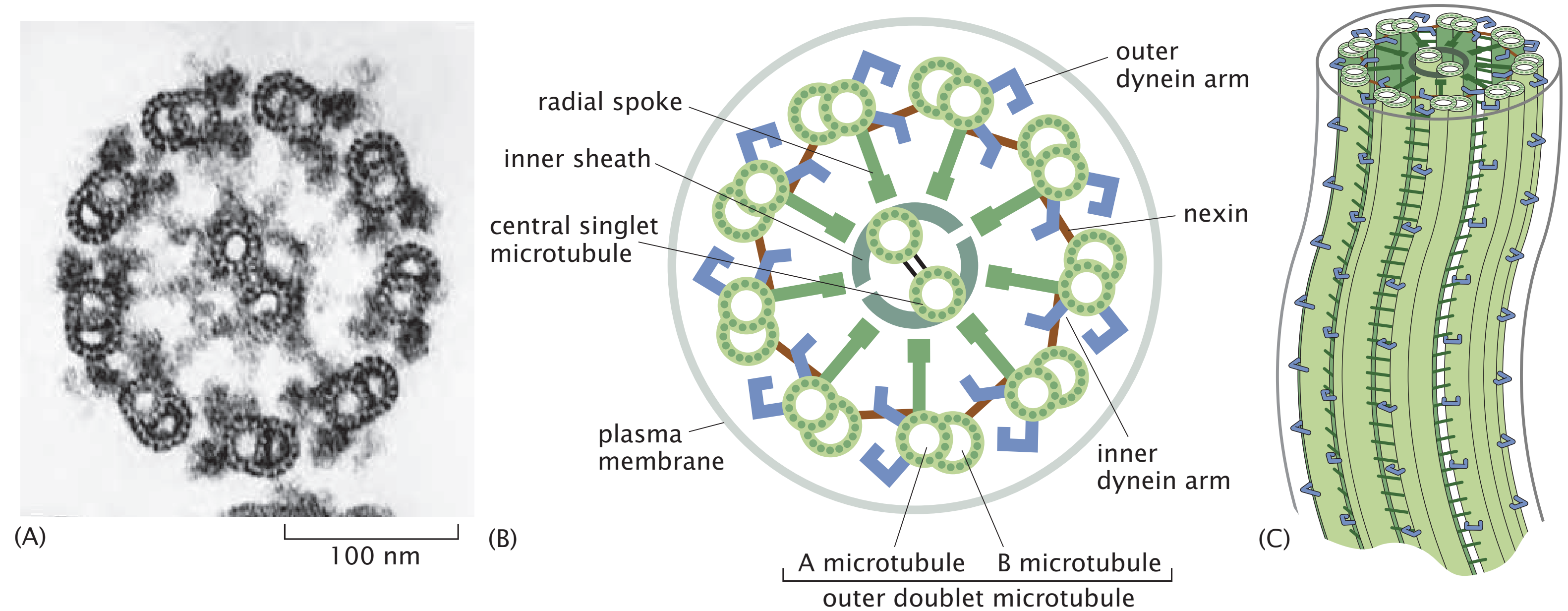
Swimming of sperm cells



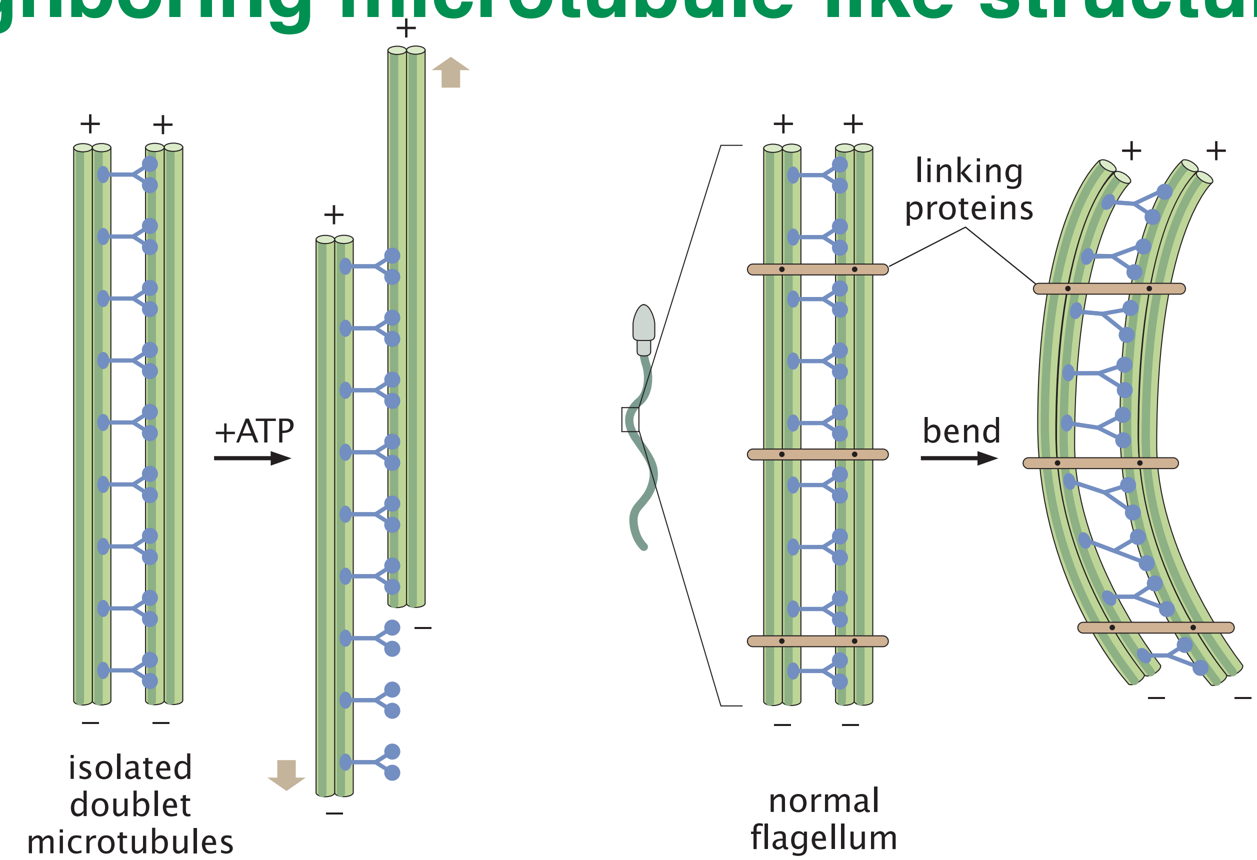
Jeff Guasto

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

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