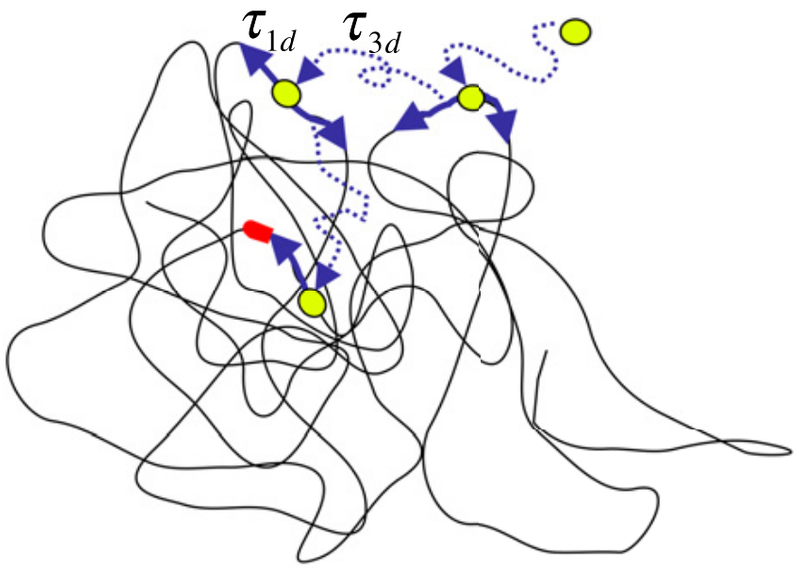
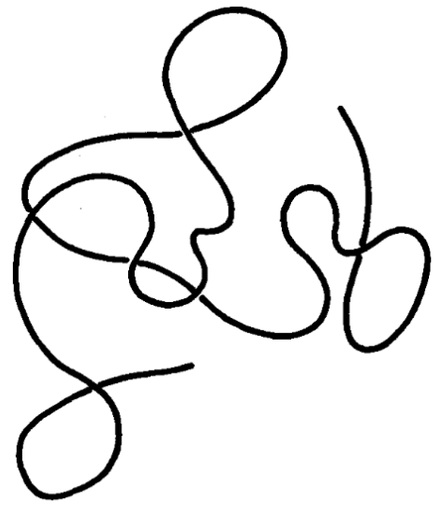
MAE 545: Lecture 19 (4/27)

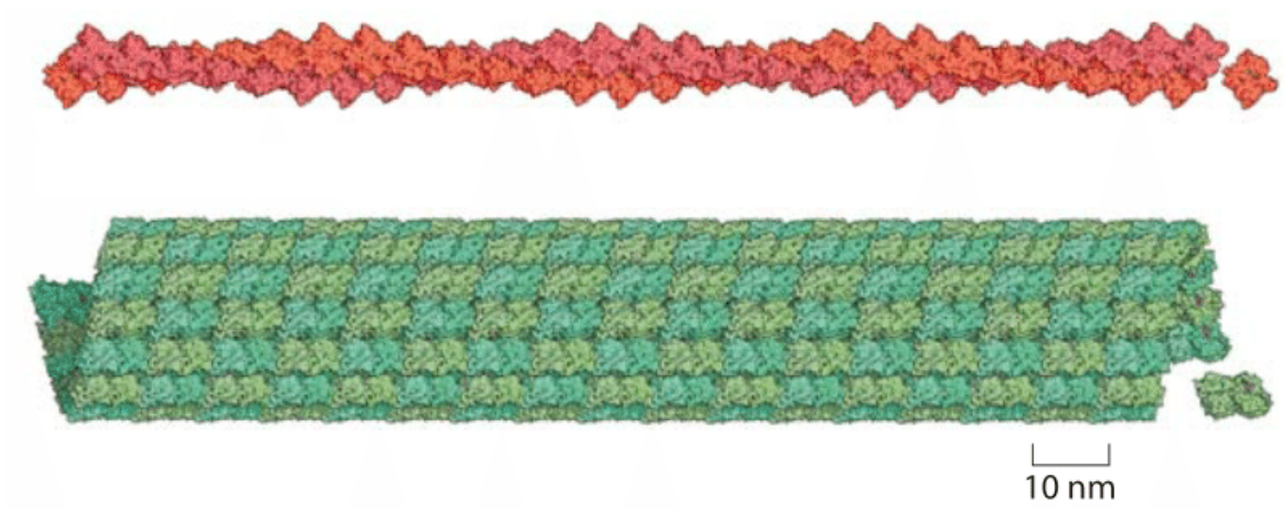
How proteins find target sites on DNA?



Statistical mechanics of polymers



Growth dynamics of actin filaments and microtubules



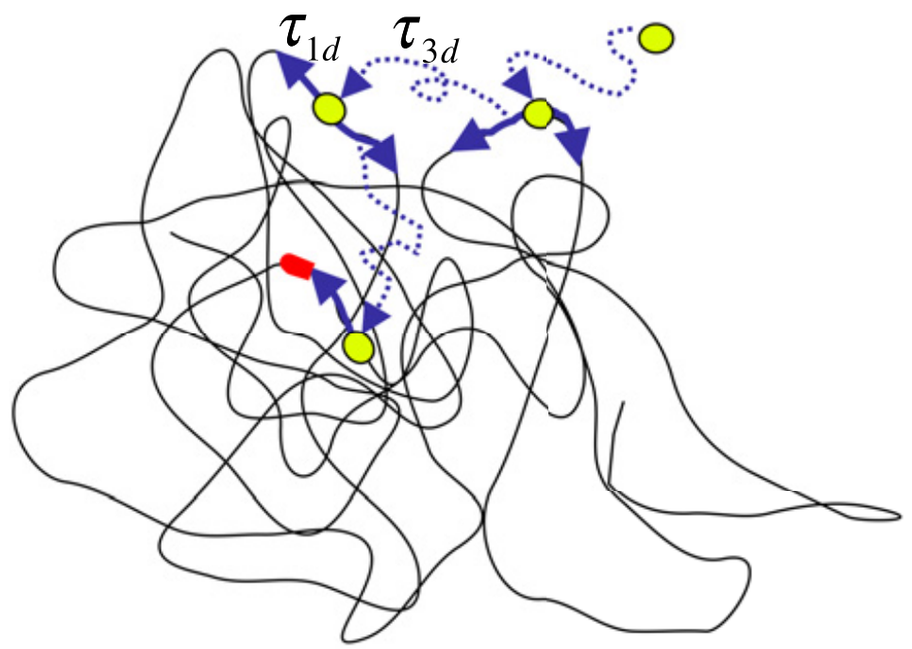
Berg - von Hippel theory (1980s)

(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

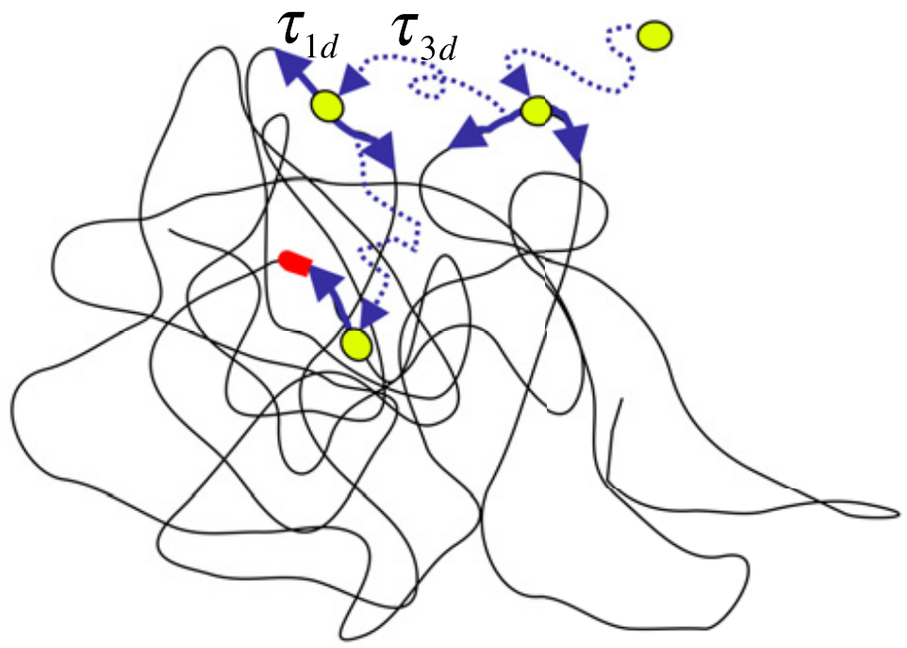
$$q = nb/L$$

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

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

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

Average number of distinct sites visited during each sliding

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

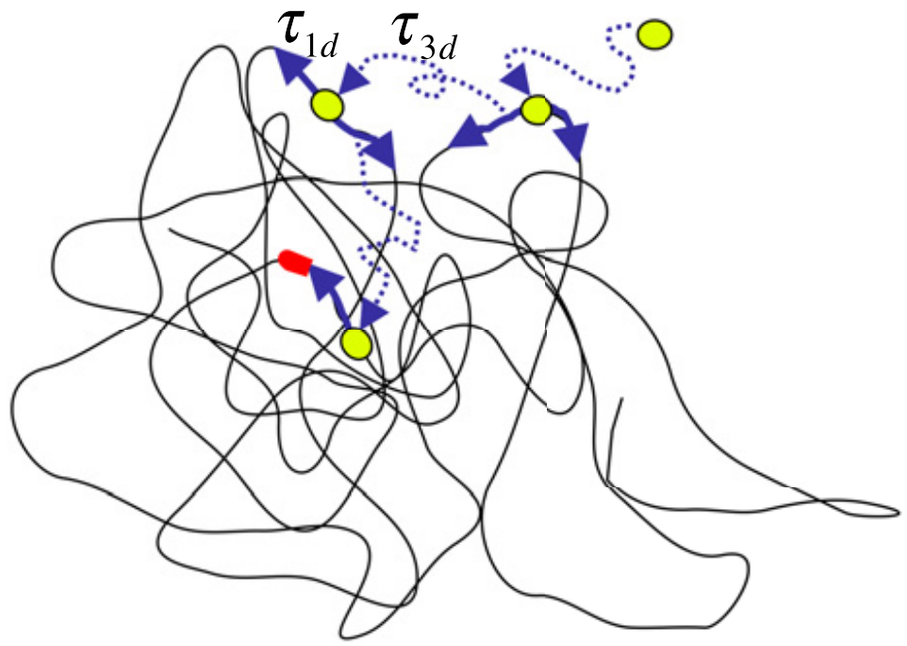
$$\langle n \rangle = 2\sqrt{D_1 \langle \tau_{1d} \rangle / (b^2)}$$

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

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



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

D_3 **- diffusion constant in space**

D_1 **- diffusion constant along the DNA**

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

Average search time

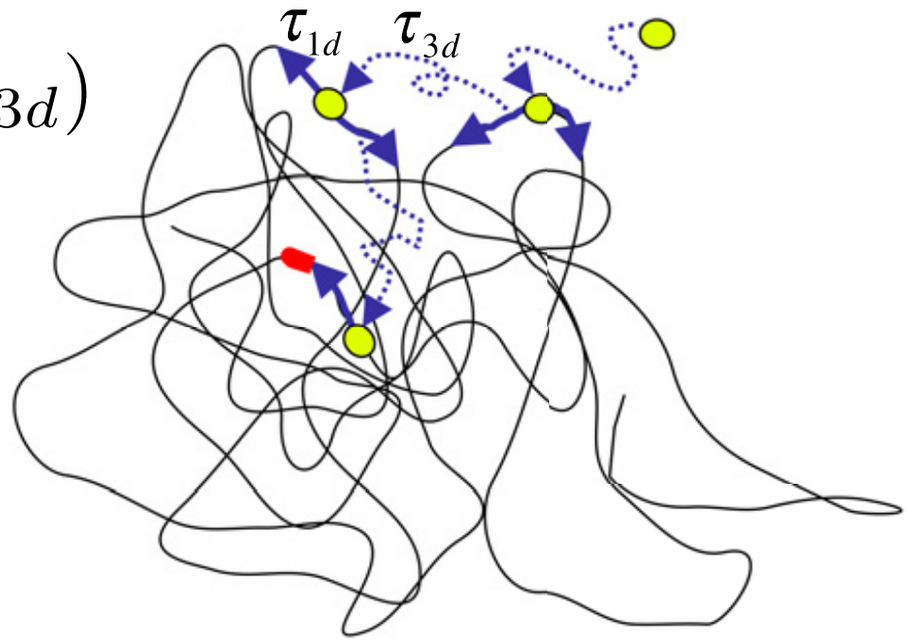
$$\overline{\langle t_s \rangle} = \overline{\langle N_R \rangle} \left(\langle \tau_{1d} \rangle + \tau_{3d} \right)$$

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

Facilitated diffusion

Average search time $\overline{\langle t_s \rangle} = \dfrac{L}{\langle \ell_{\rm sl} \rangle} \left(\langle \tau_{1d} \rangle + \tau_{3d} \right)$

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



Optimal search time

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

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

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

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

D_3 **- diffusion constant in space**

D_1 **- diffusion constant along the DNA**

Search time for jumps alone

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

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

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

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

Search time for sliding alone

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

Search time speed up for facilitated diffusion

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

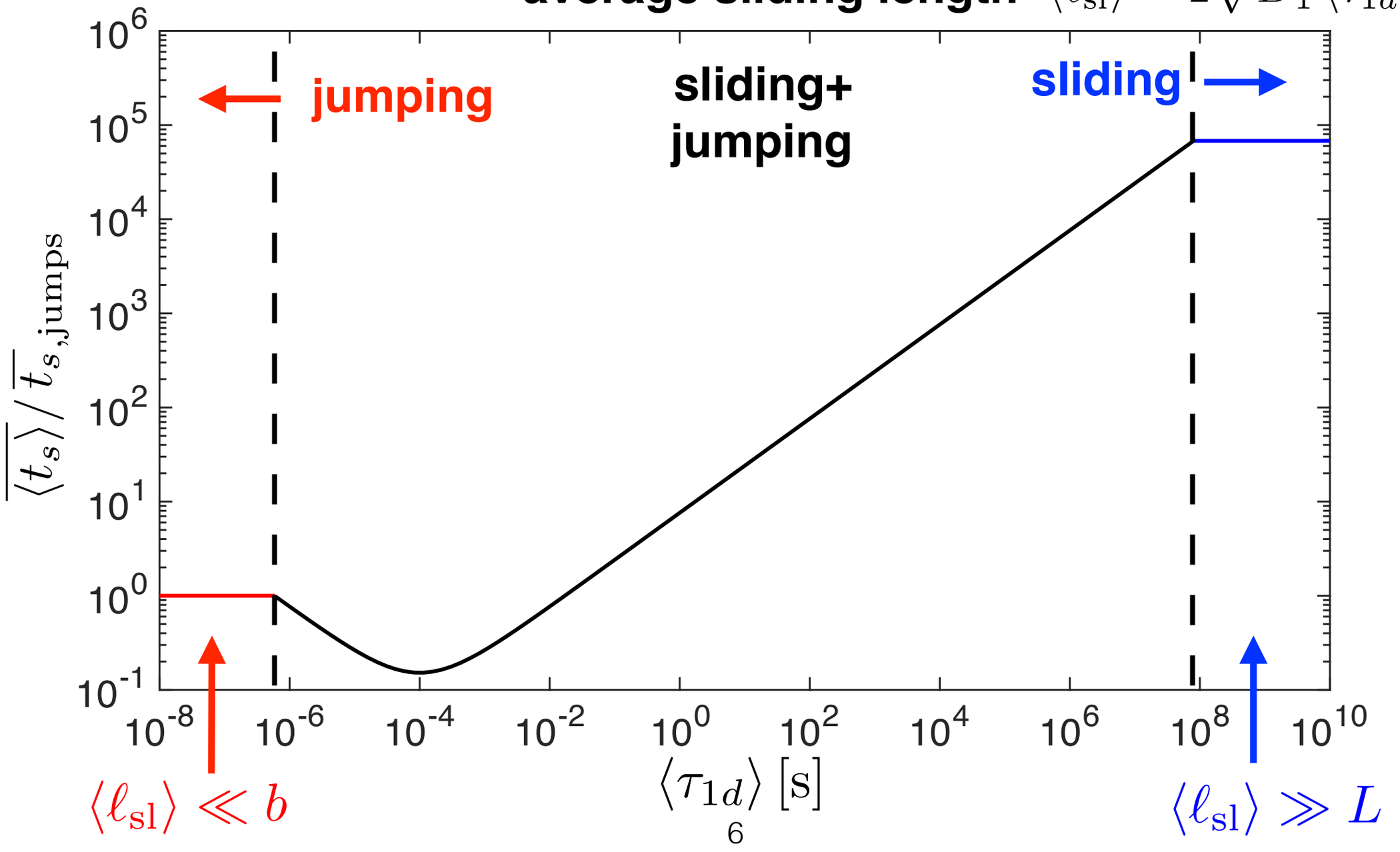
Example: search time for target site in bacteria on DNA with 10^6 base pairs

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

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

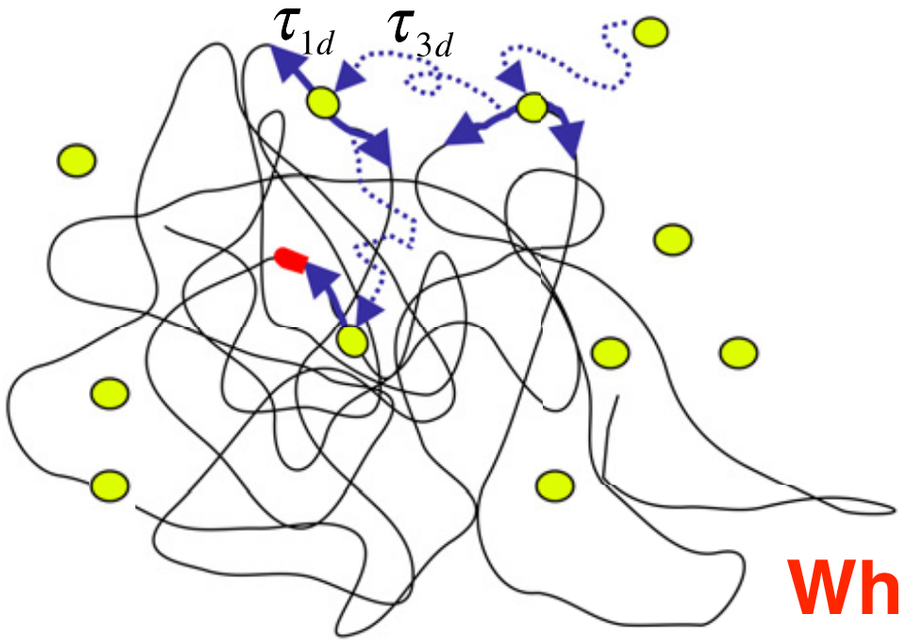
average search time $\overline{\langle t_s \rangle} = \dfrac{L}{\langle \ell_{\mathrm{sl}} \rangle}\left(\langle \tau_{1d} \rangle + \tau_{3d}\right)$

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

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

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

(Extreme value distributions)

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

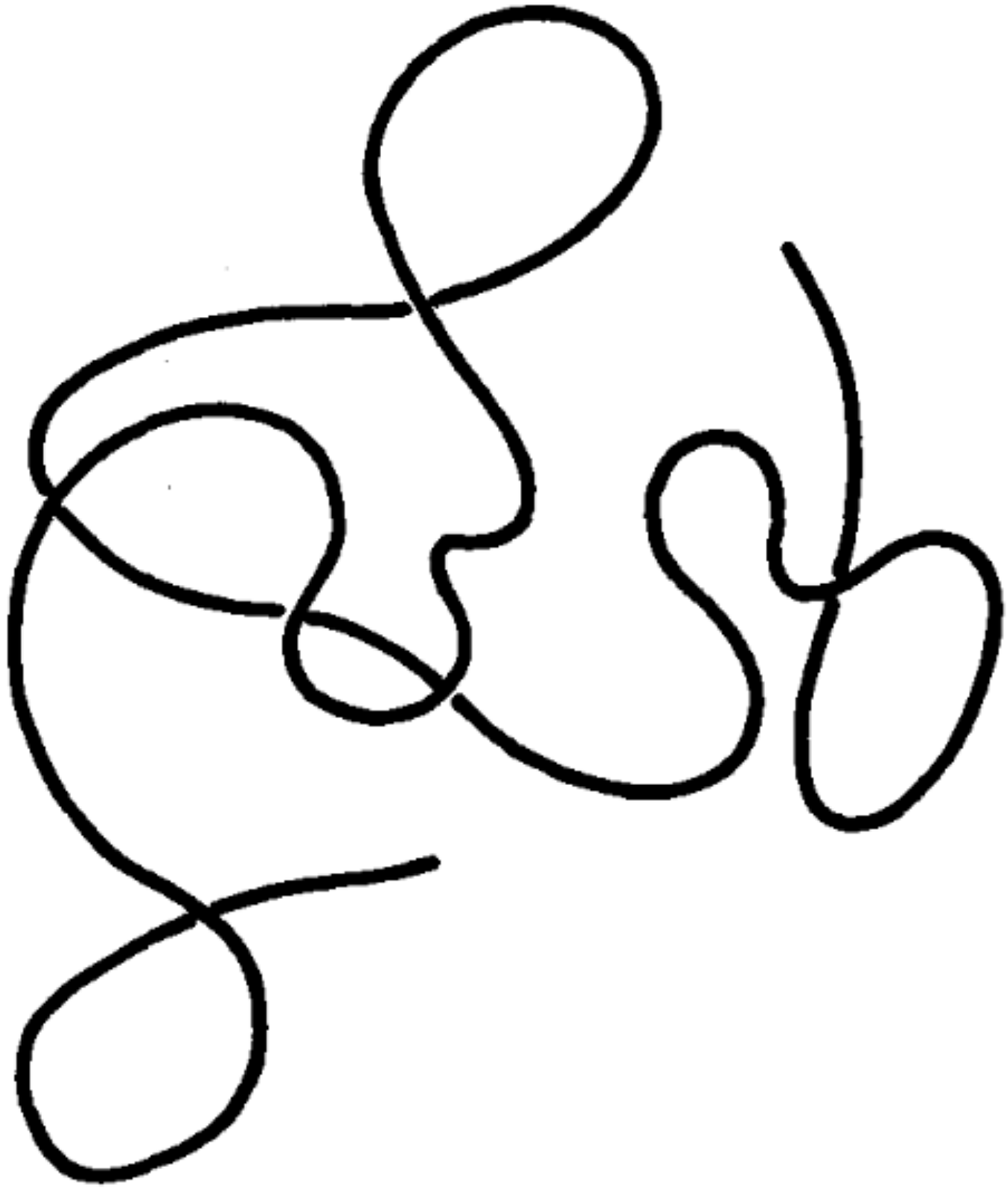
probability that one of *n* proteins finds the target site at time t_s

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

Average search time is reduced by factor *n*

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

Statistical mechanics of polymers and filaments



Statistical mechanics of polymers and filaments

molecular dynamics simulation

Note: 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**

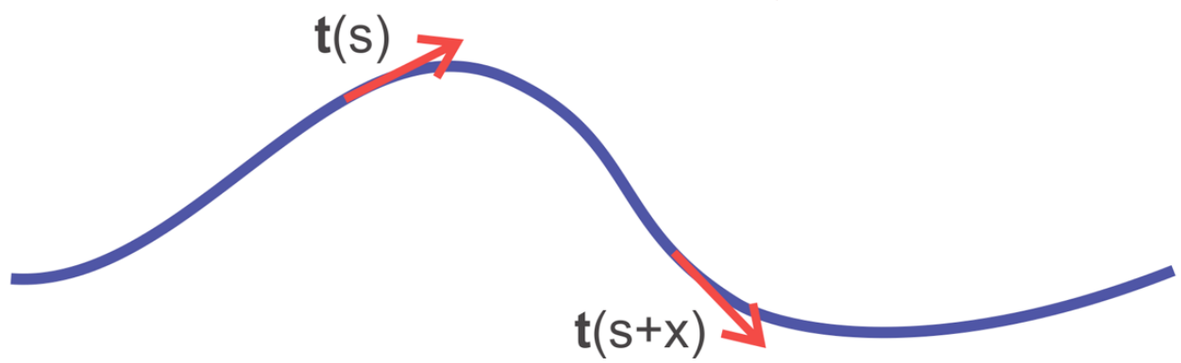
k_B **Boltzmann constant**

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

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**

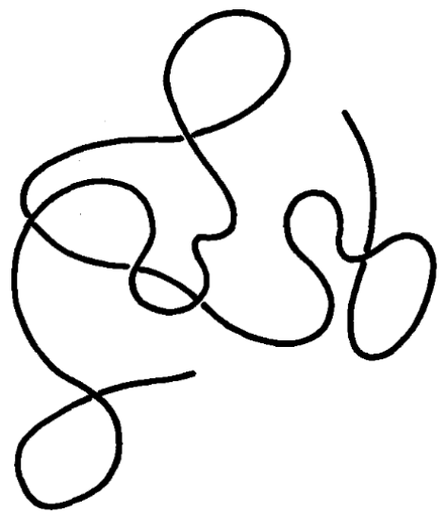
***L* - filament length**

Short filaments remain straight

$L \ll \ell_p$

Long filaments perform self-avoiding random walk

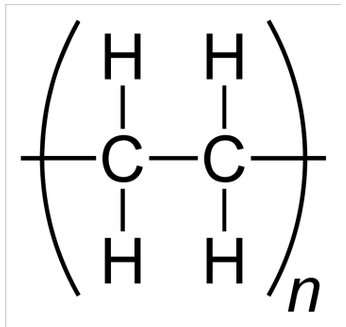
$L \gg \ell_p$



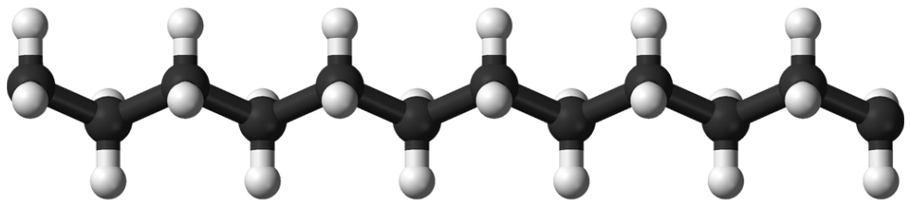
Examples: persistence length

polyethylene

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



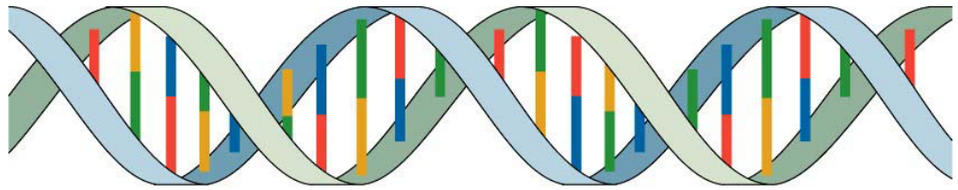
$$\ell_p = \frac{B}{k_B T}$$



Persistence length for polymers is on the order of nm

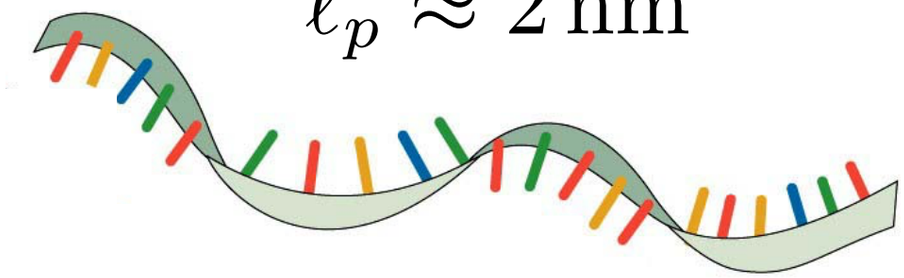
double stranded DNA

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



single stranded DNA

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

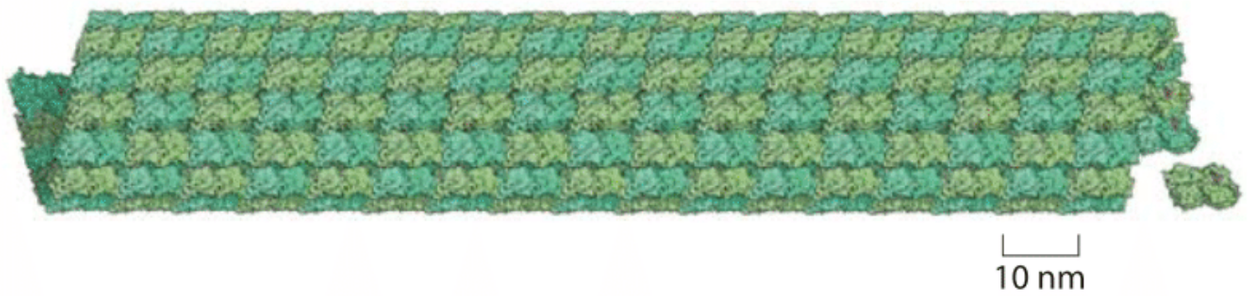


actin $\ell_p \approx 17\,\mu\mathrm{m}$

uncooked spagetthi

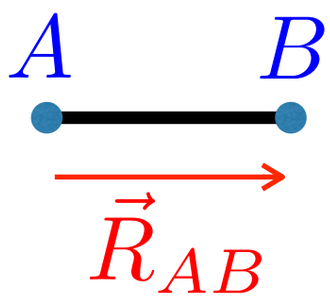
$\ell_p \approx 10^{18}\,\mathrm{m}$

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

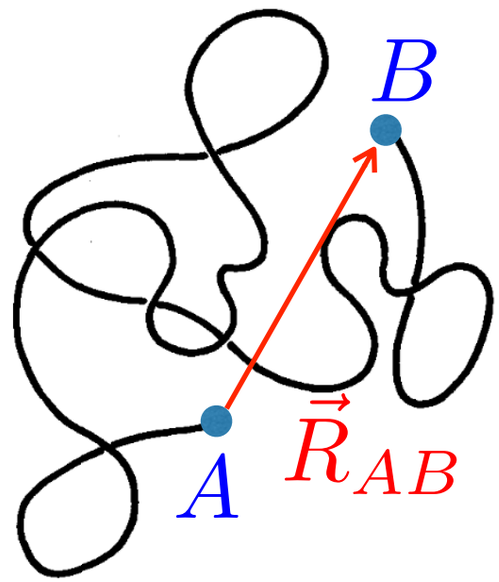


End-to-end distance

Short filaments $L \ll \ell_p$



Long filaments $L \gg \ell_p$



Over time thermal fluctuations reorient filaments in all possible directions!

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

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

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

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

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

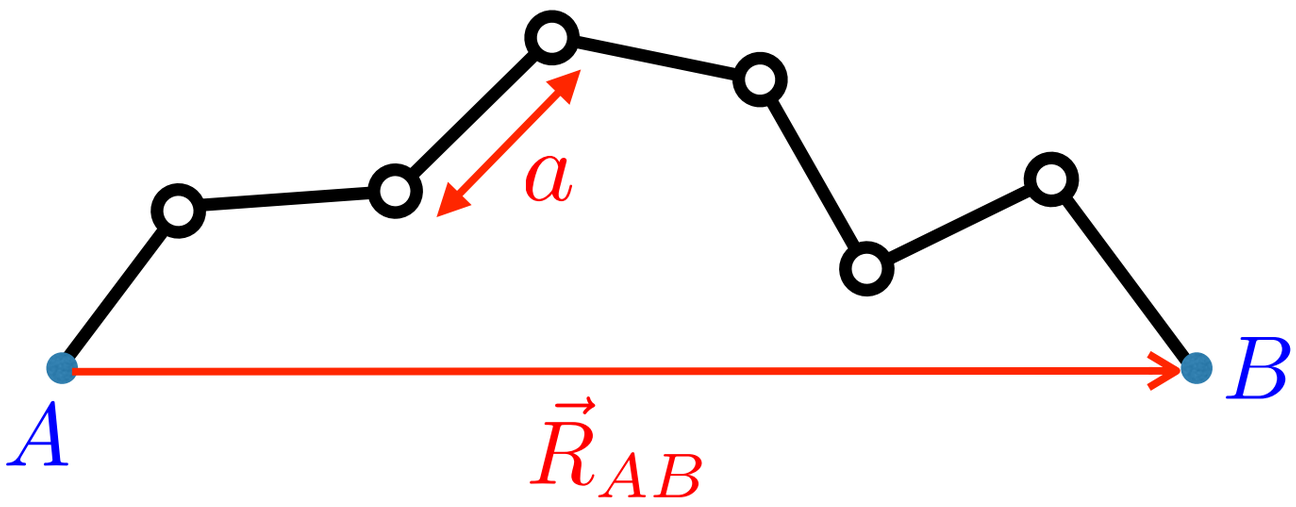
Exact result

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

Ideal chain vs worm-like chain

Ideal chain

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

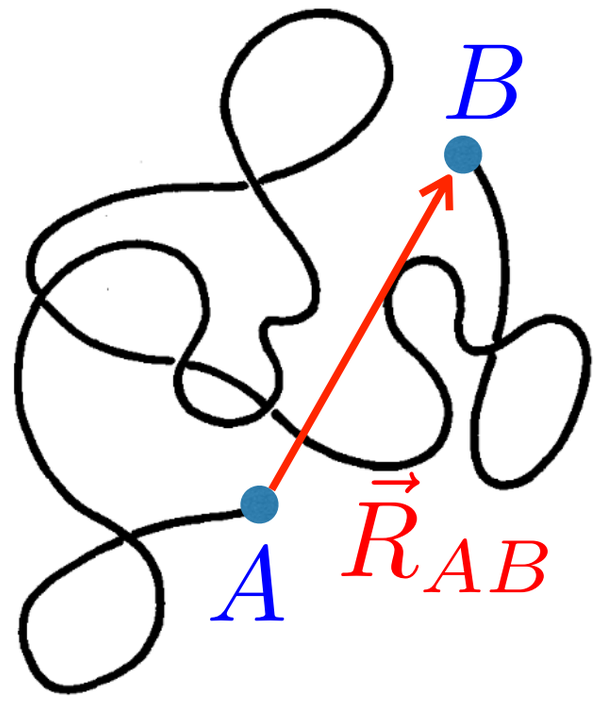


Each configuration *C* has zero energy cost.

$$E_c = 0$$

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

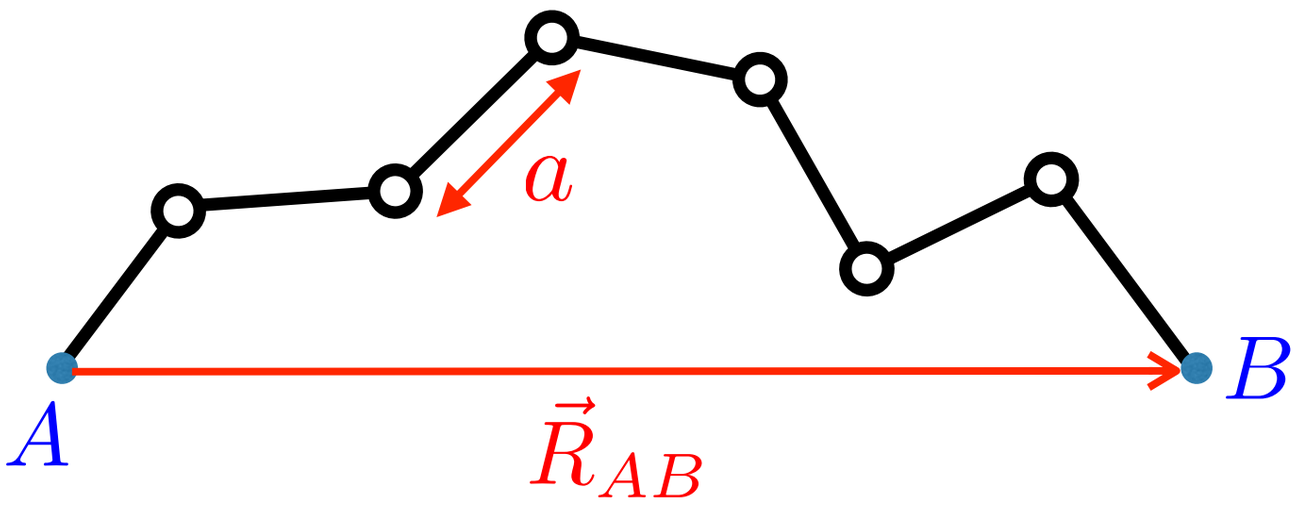
$$p_c \propto e^{-E_c/k_BT}$$

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

Ideal chain vs worm-like chain

Ideal chain

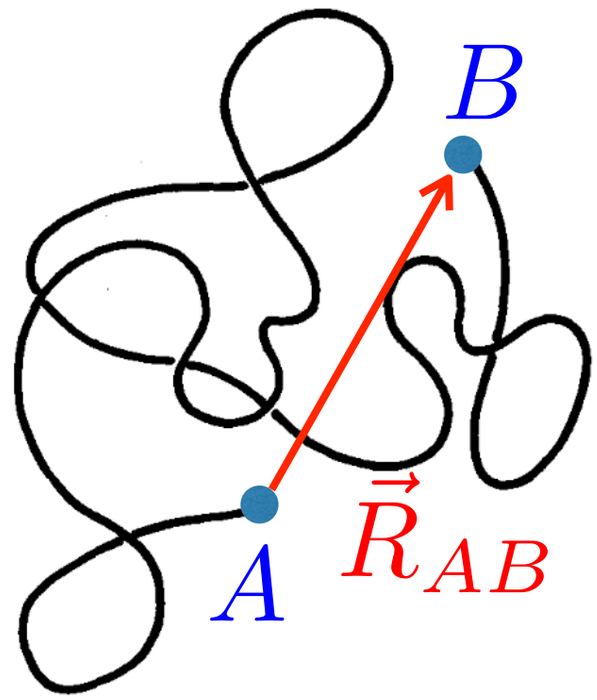
***N* identical unstretchable links (Kuhn segments) of length *a* with freely rotating joints**



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

Worm-like chain

Continuous unstretchable rod



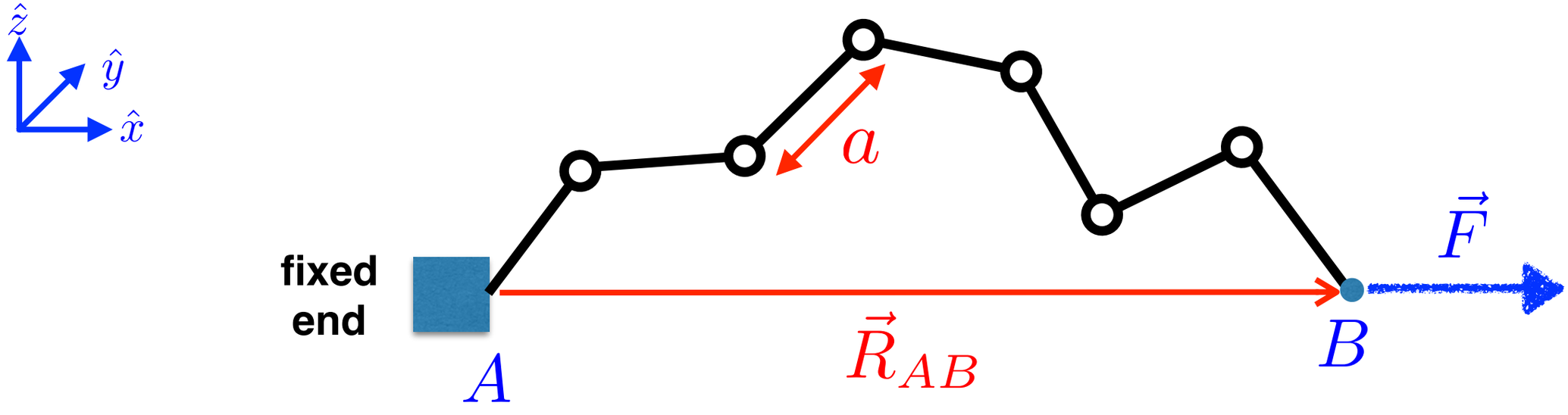
$$\left\langle \vec{R}_{AB}^2 \right\rangle \approx 2\ell_p L = \frac{2BL}{k_B T}$$

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

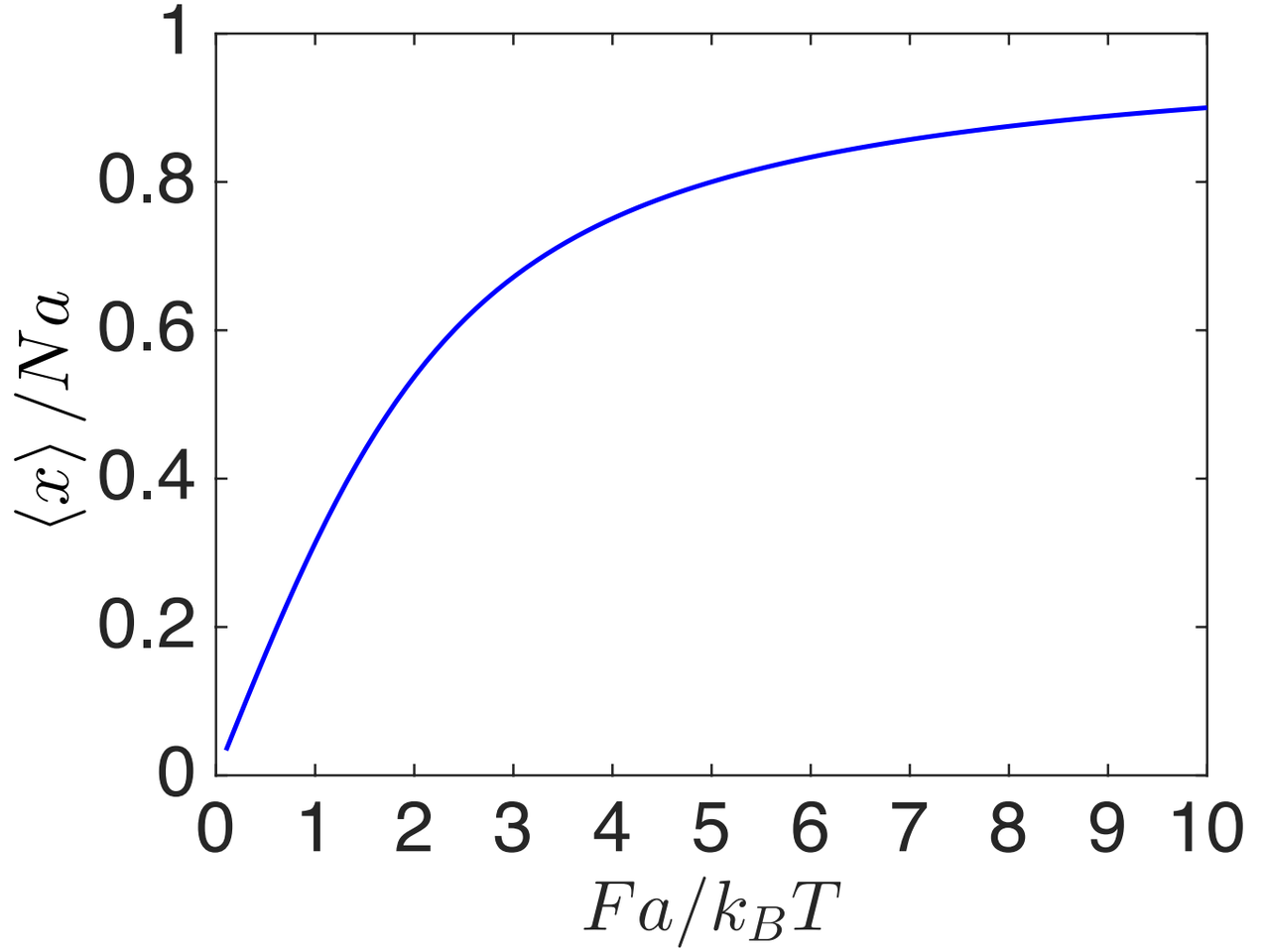
$$\langle x \rangle = Na \left(\coth \left[\frac{Fa}{k_B T} \right] - \frac{k_B T}{Fa} \right)$$

small force $Fa \ll k_B T$

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

large force $Fa \gg k_B T$

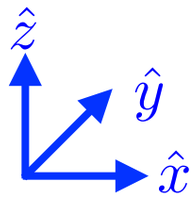
$$\langle x \rangle \approx Na \left(1 - \frac{k_B T}{Fa} \right) = L \left(1 - \frac{k_B T}{2F\ell_p} \right)$$

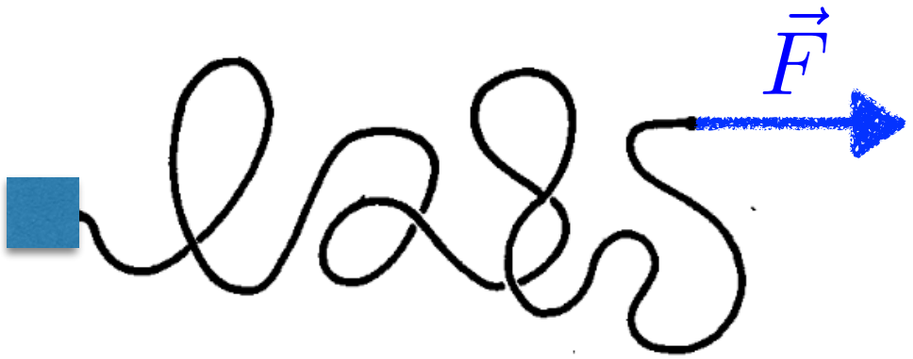


Stretching of worm-like chains

Assume long chains $L \gg \ell_p$

small force $F\ell_p \ll k_BT$





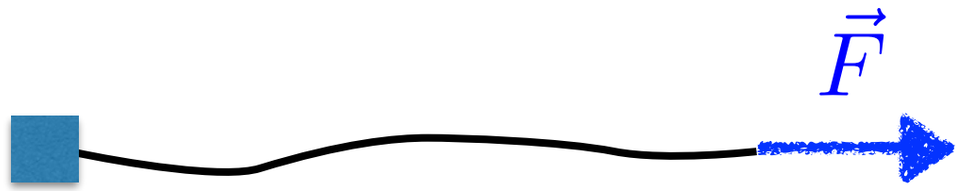
$$\langle x \rangle \approx \frac{2FL\ell_p}{3k_BT} \equiv \frac{F}{k}$$

entropic spring constant

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

B **- filament bending rigidity**

large force $F\ell_p \gg k_BT$



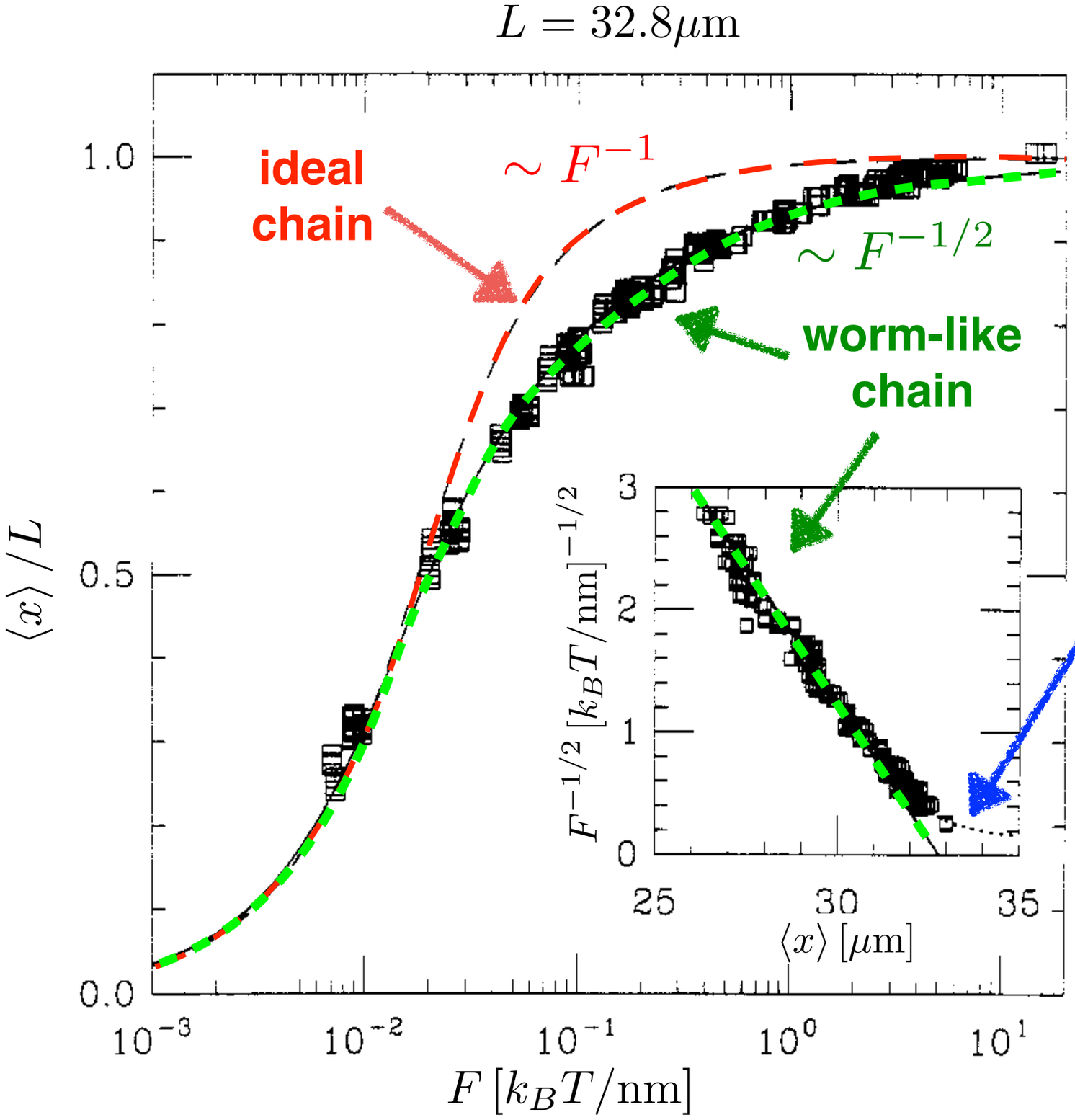
$$\langle x \rangle \approx L\left[1 - \sqrt{\frac{k_BT}{4F\ell_p}}\right]$$

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



$1k_BT/\mathrm{nm} \approx 4\mathrm{pN}$

Stretching of the DNA backbone

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

For DNA

$$\ell_p = 50\mathrm{nm}$$

$$\gamma \approx 500k_BT/\mathrm{nm} \approx 2\mathrm{nN}$$

Improved interpolation formula

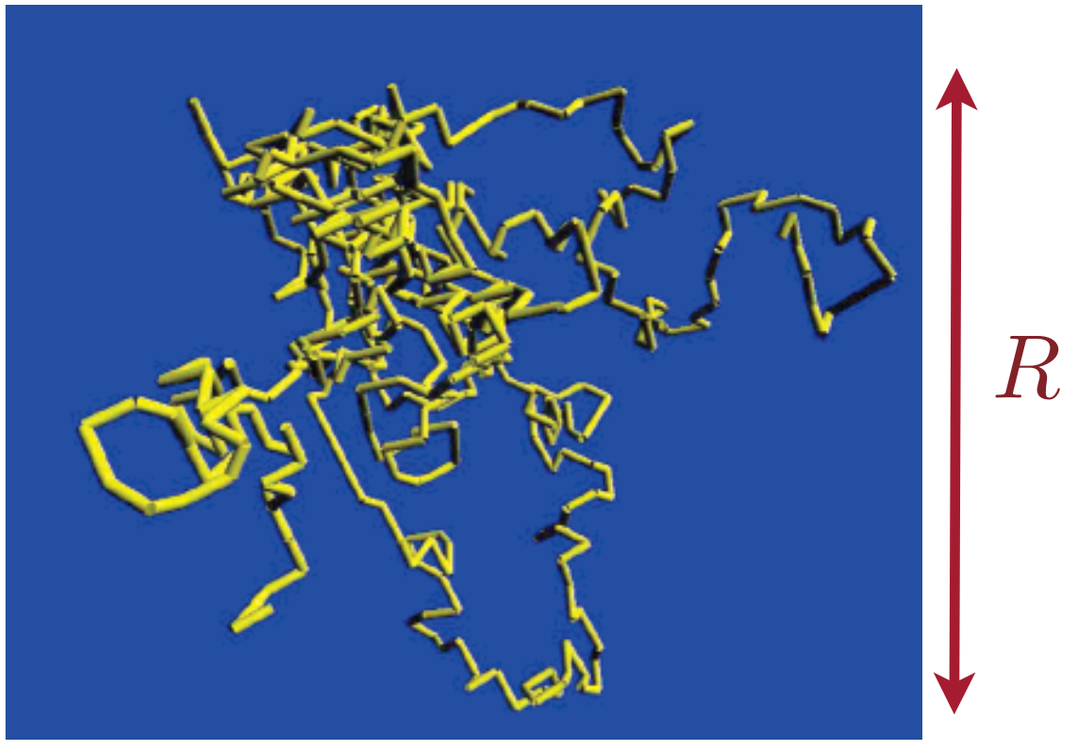
$$\frac{F\ell_p}{k_BT} = \frac{1}{4}\left(1-\frac{\langle x\rangle}{L}+\frac{F}{\gamma}\right)^{-2}-\frac{1}{4}+\frac{\langle x\rangle}{L}-\frac{F}{\gamma}$$

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

Random coil to globule transition in polymers

random coil

$T > \Theta$

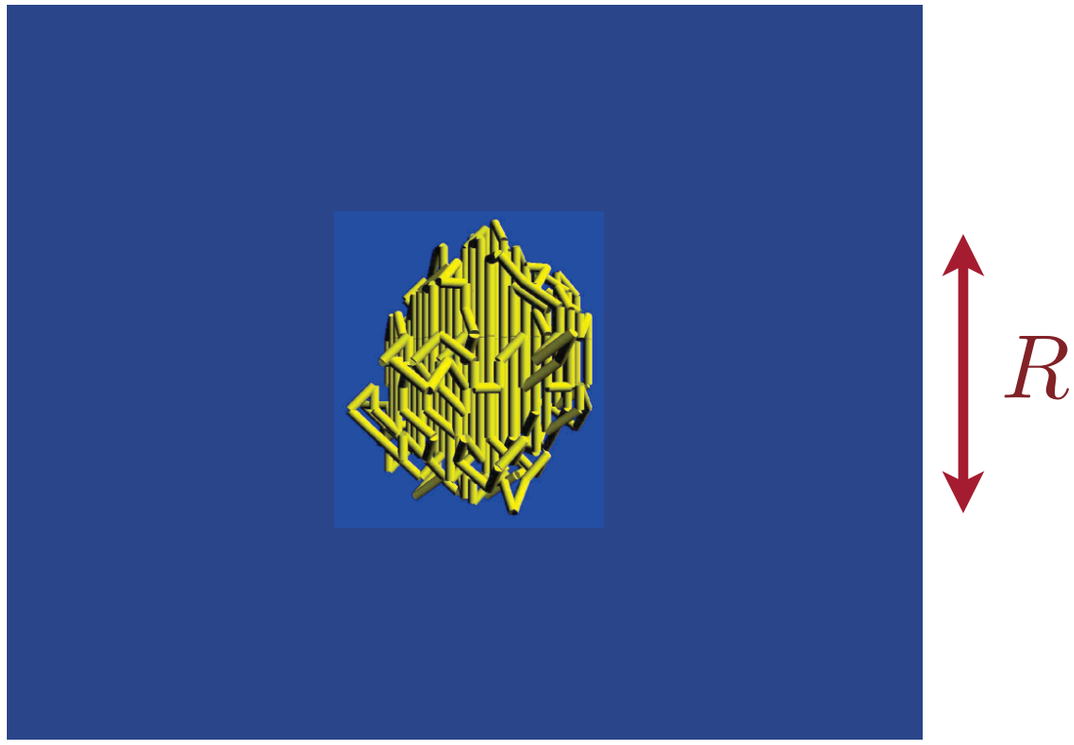


$$R \sim \sqrt{L\ell_p}$$

at high temperature entropic contributions dominate

compact globule

$T < \Theta$



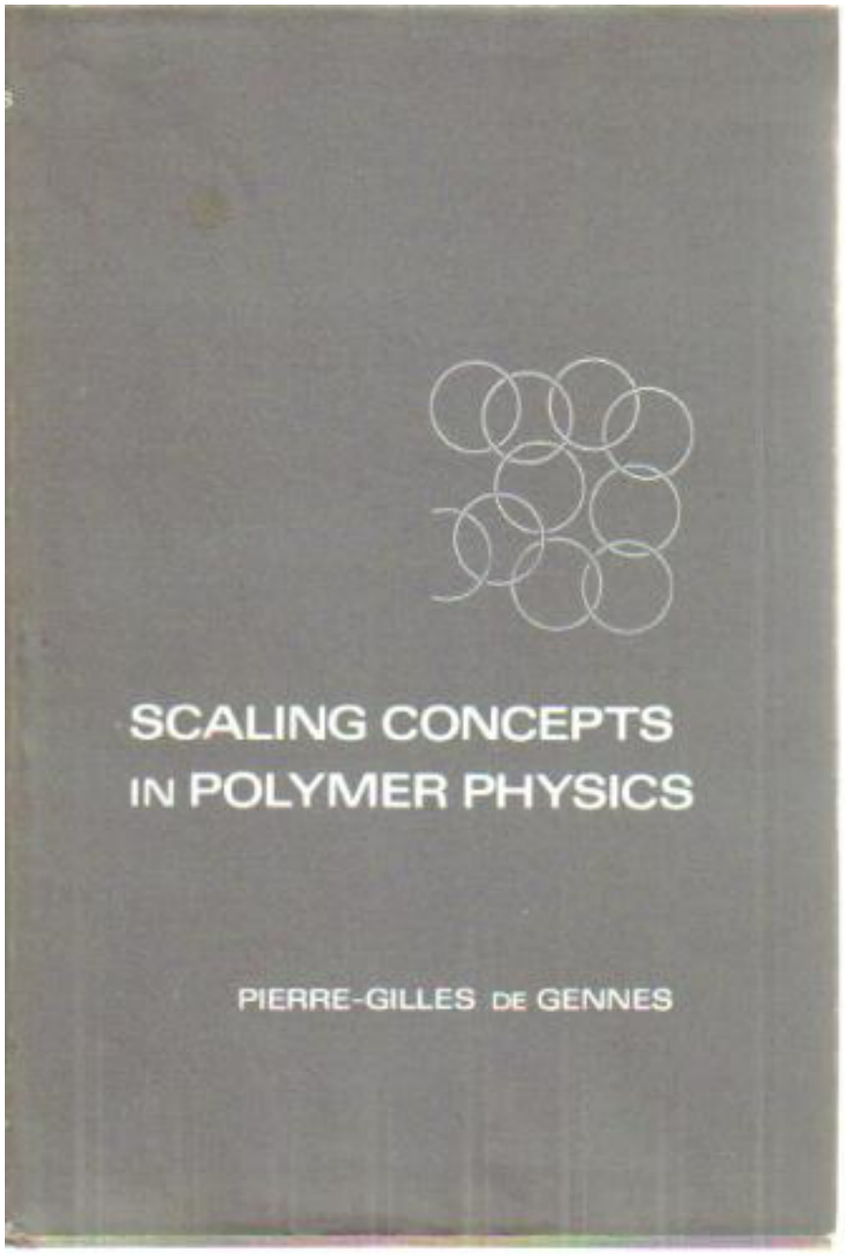
$$R \sim \left(d^2 L\right)^{1/3}$$

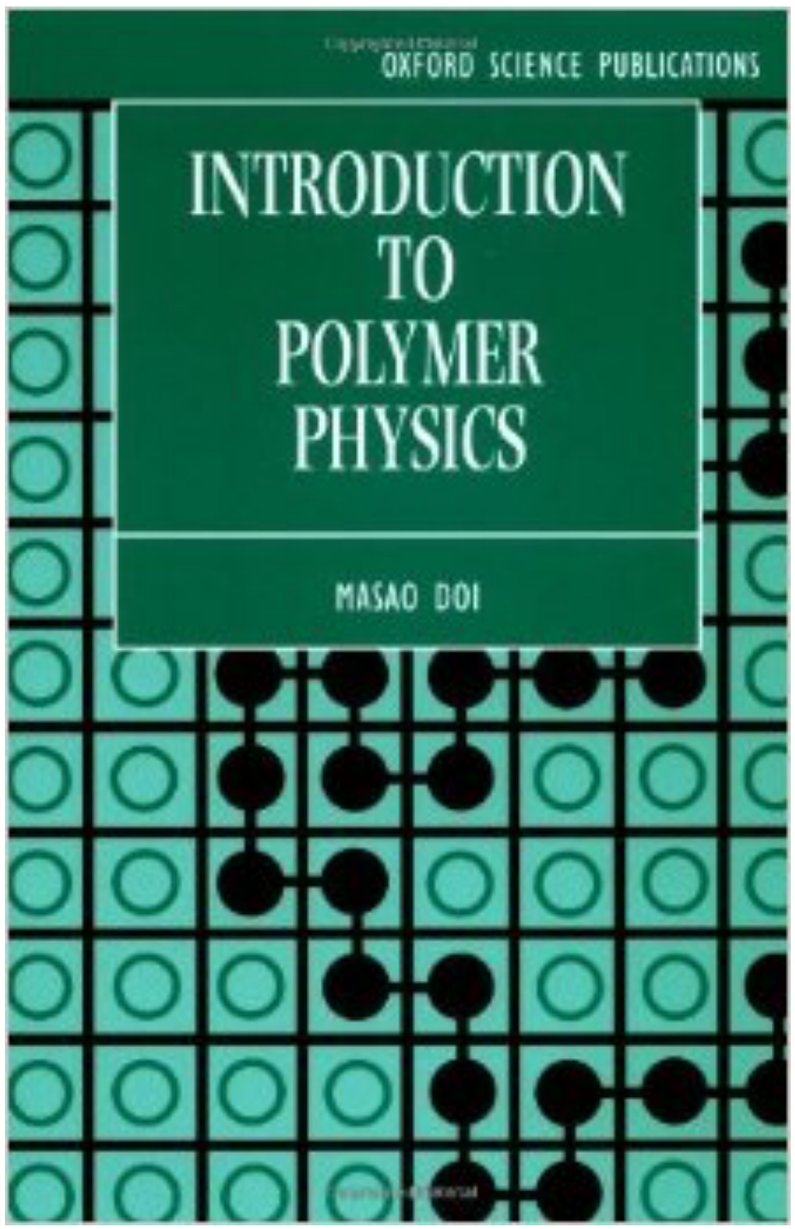
d **- diameter of polymer chain**

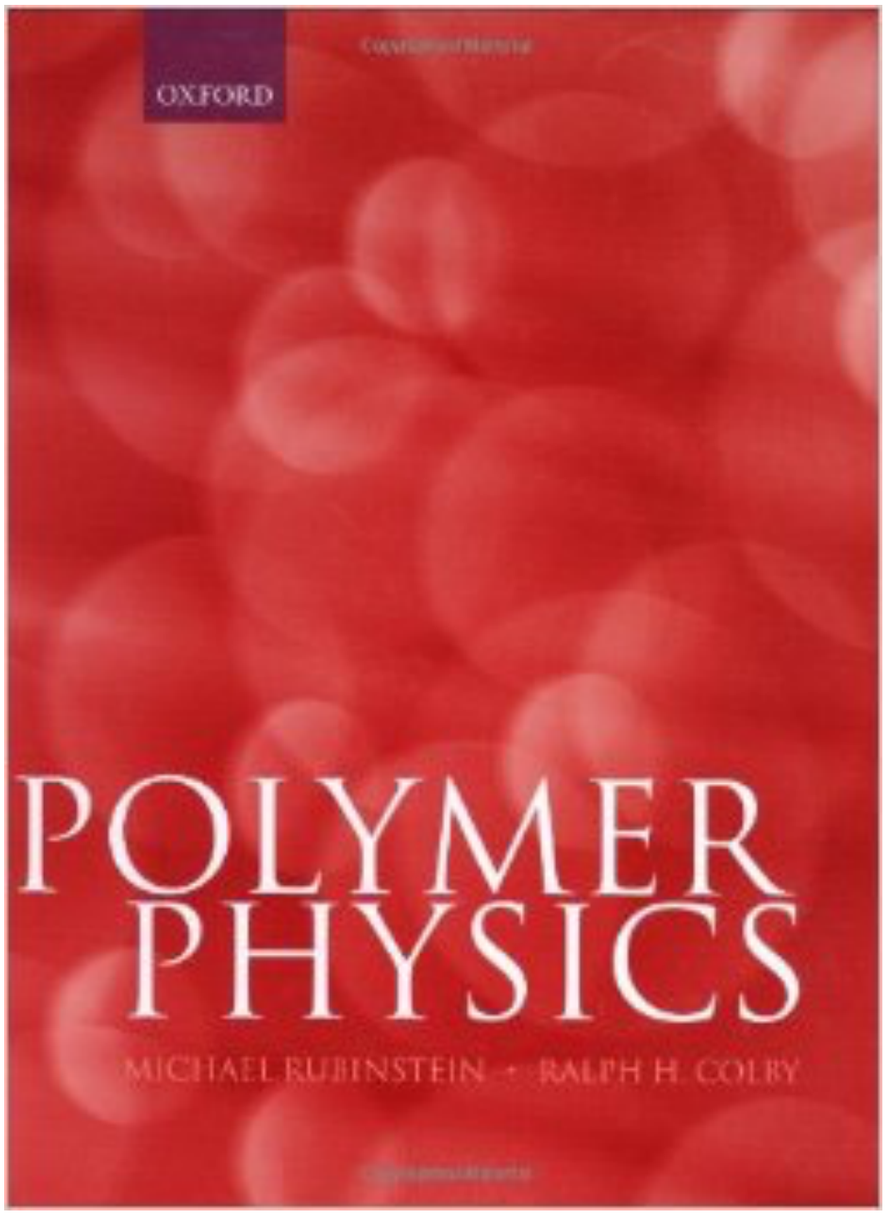
at low temperature attraction between polymer chains dominates

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

Further reading



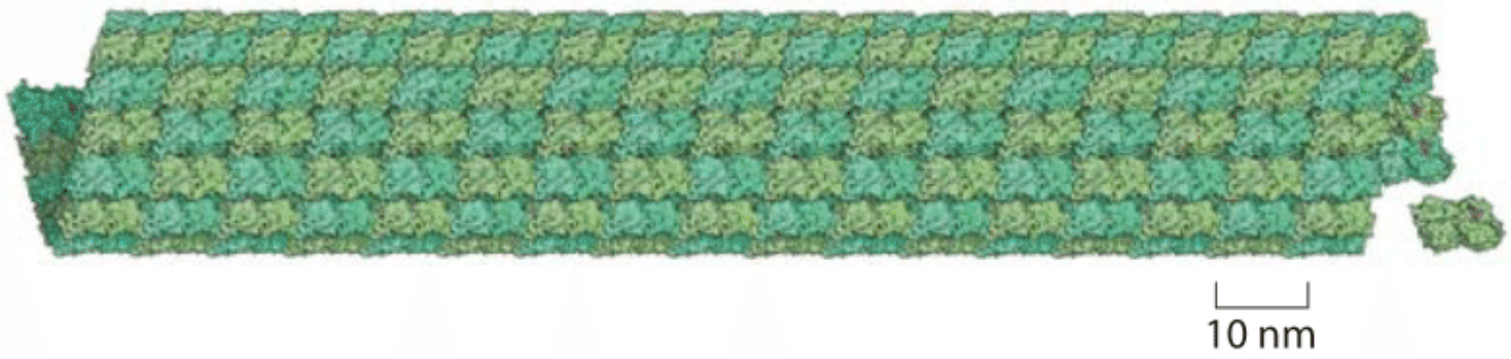




Dynamics of actin filaments and microtubules

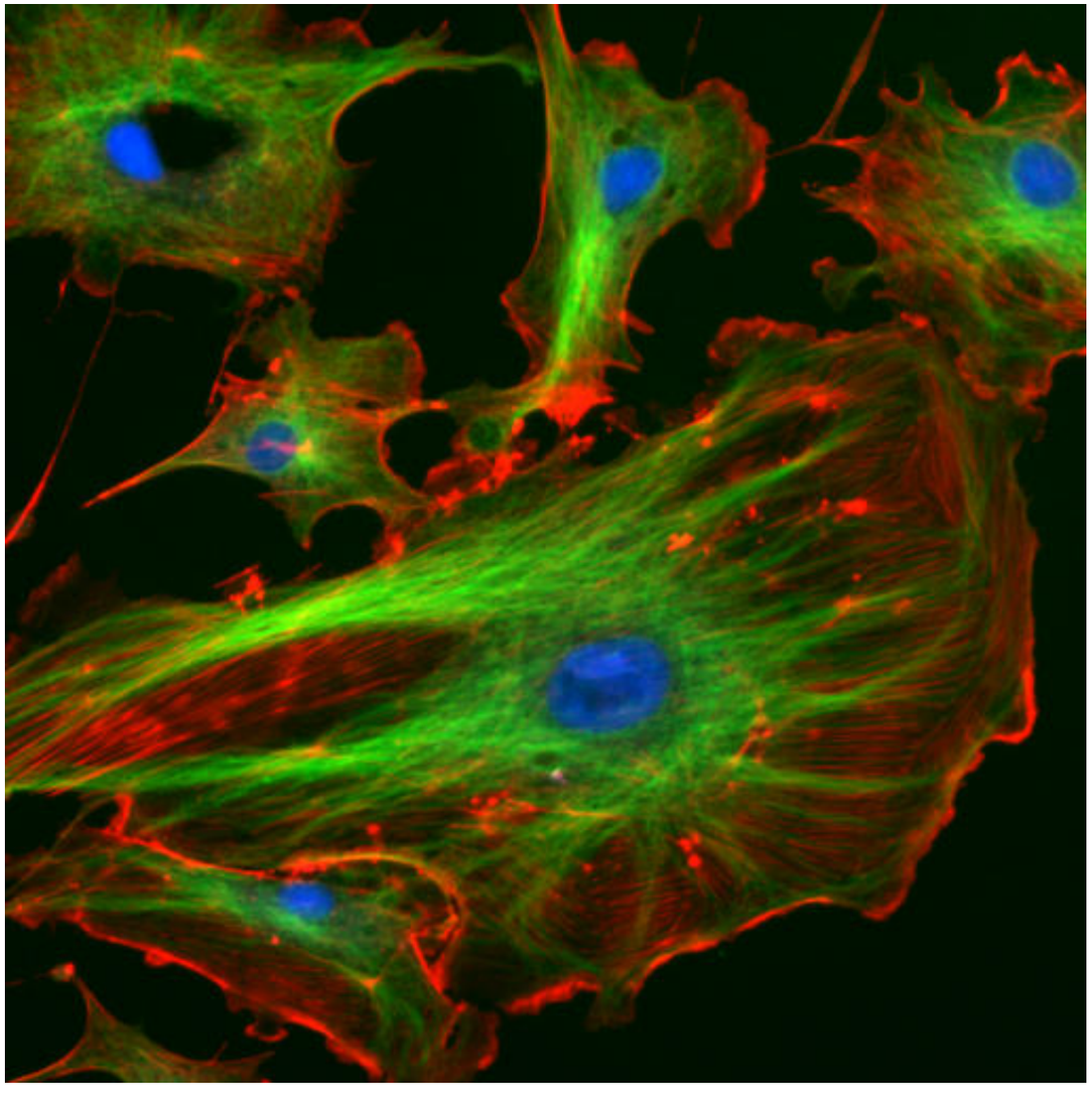
Actin filament

Microtubule



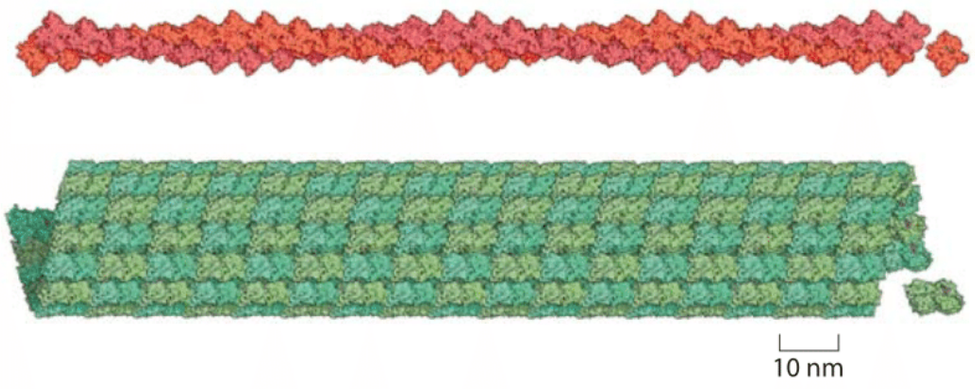
Cytoskeleton in cells

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



(wikipedia)

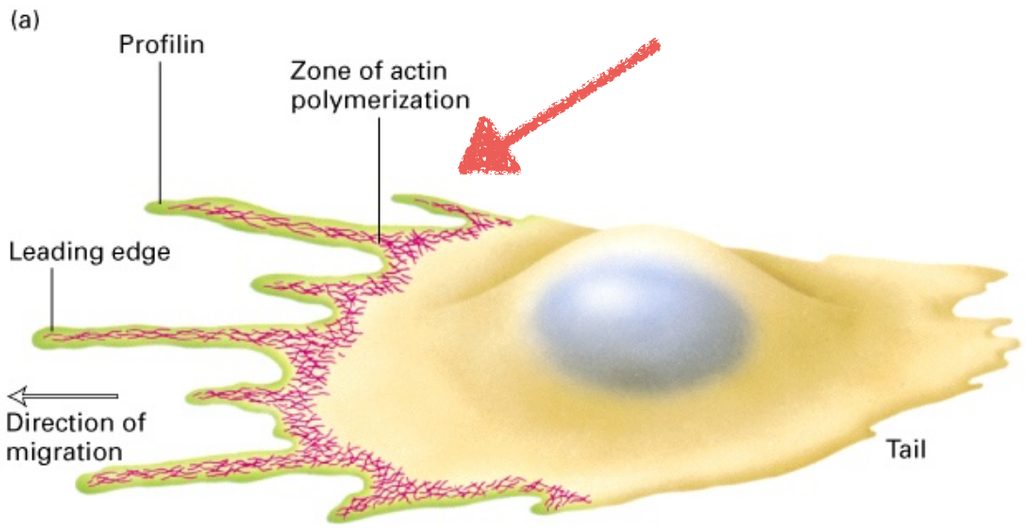
Actin filament



Microtubule

Crawling of cells

Actin

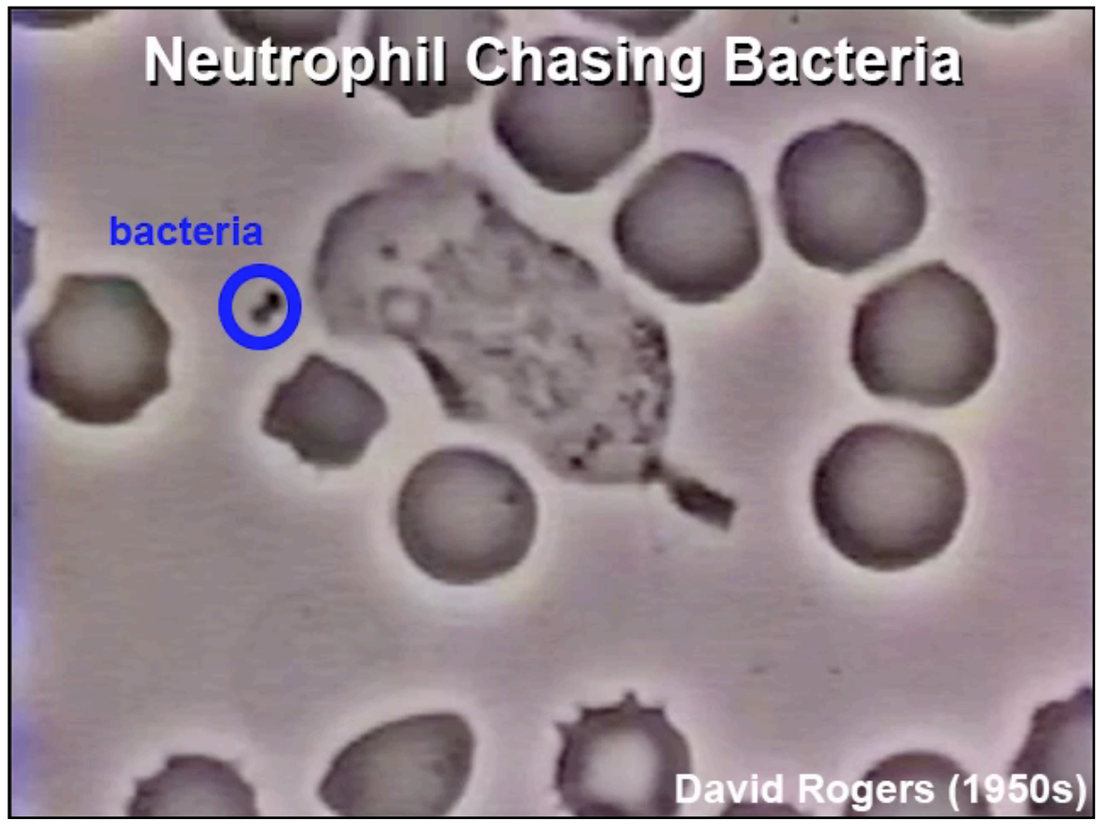


migration of skin cells during wound healing

spread of cancer cells during metastasis of tumors

amoeba searching for food

Immune system: neutrophils chasing bacteria

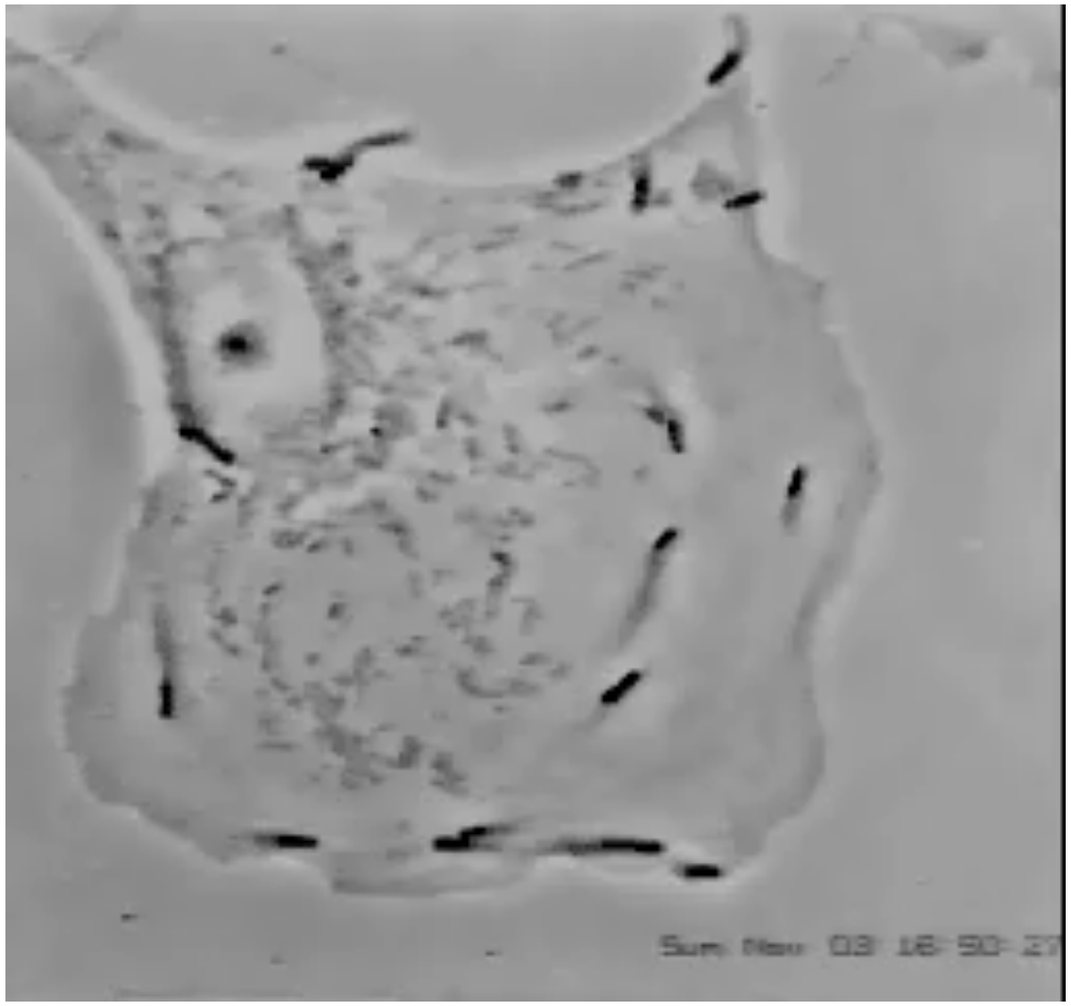


David Rogers, 1950s

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

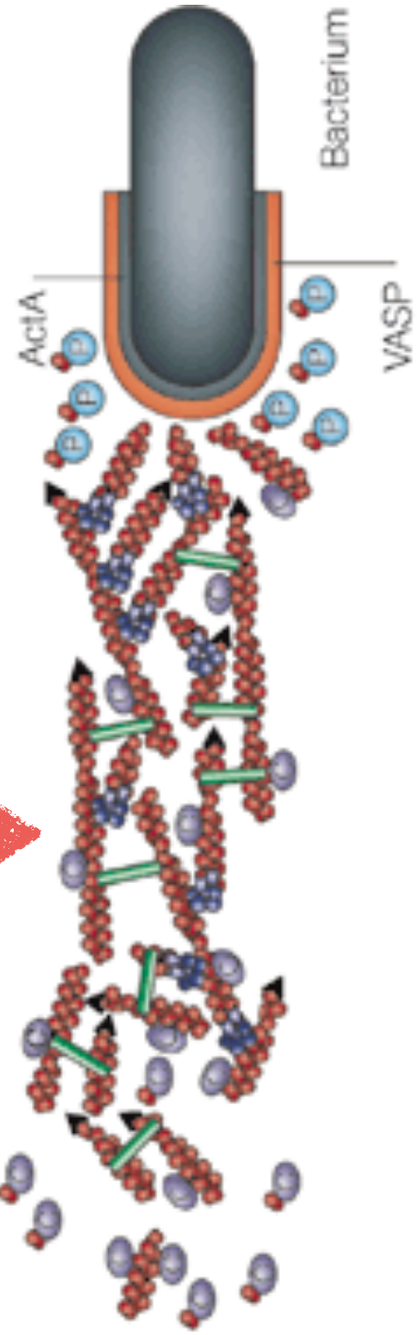
Movement of bacteria

Listeria monocytogenes
moving in infected cells



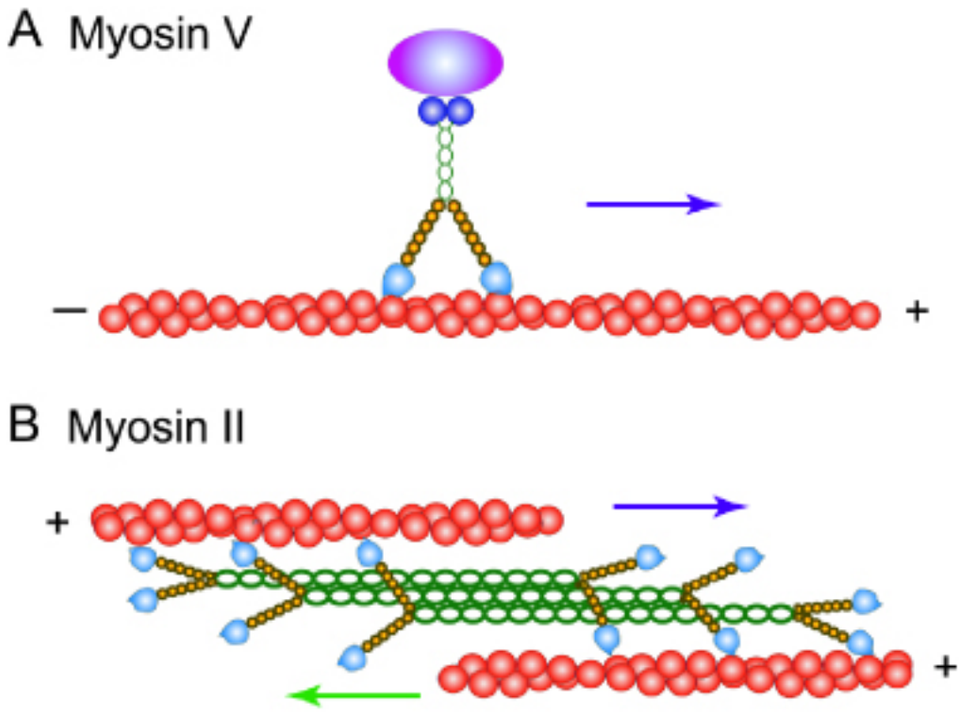
Julie Theriot (speeded up 150x)

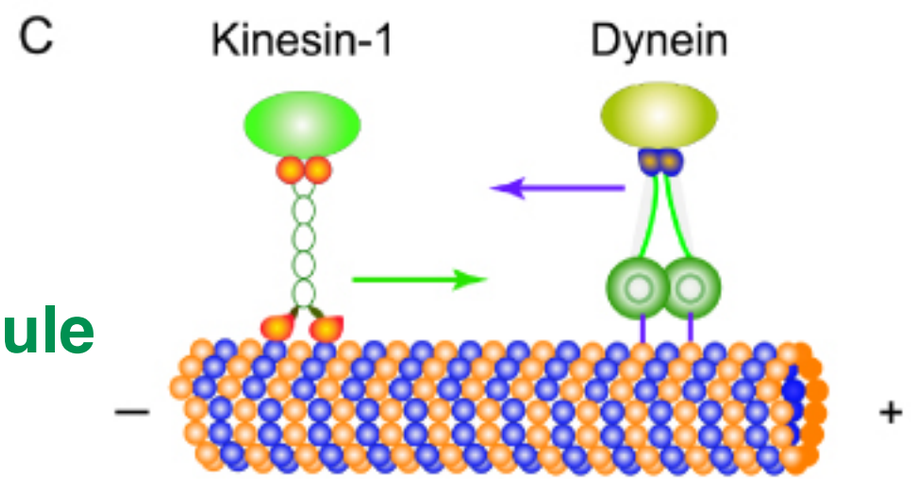
$v \sim 0.1 - 0.3 \mu\mathrm{m/s}$



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

Molecular motors



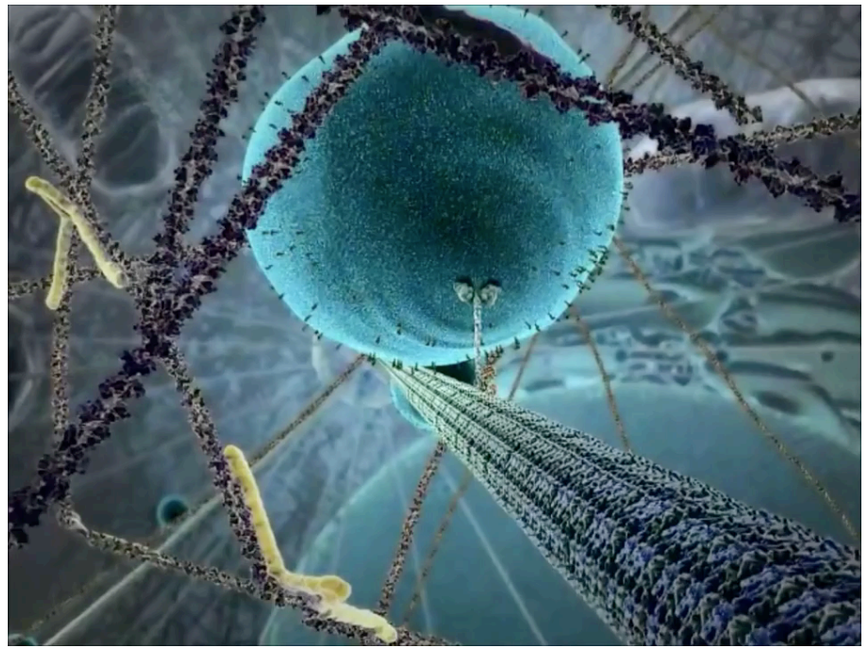


A.B. Kolomeisky, J. Phys.: Condens. Matter **25**, 463101 (2013)

Transport of large molecules around cells (diffusion too slow)

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

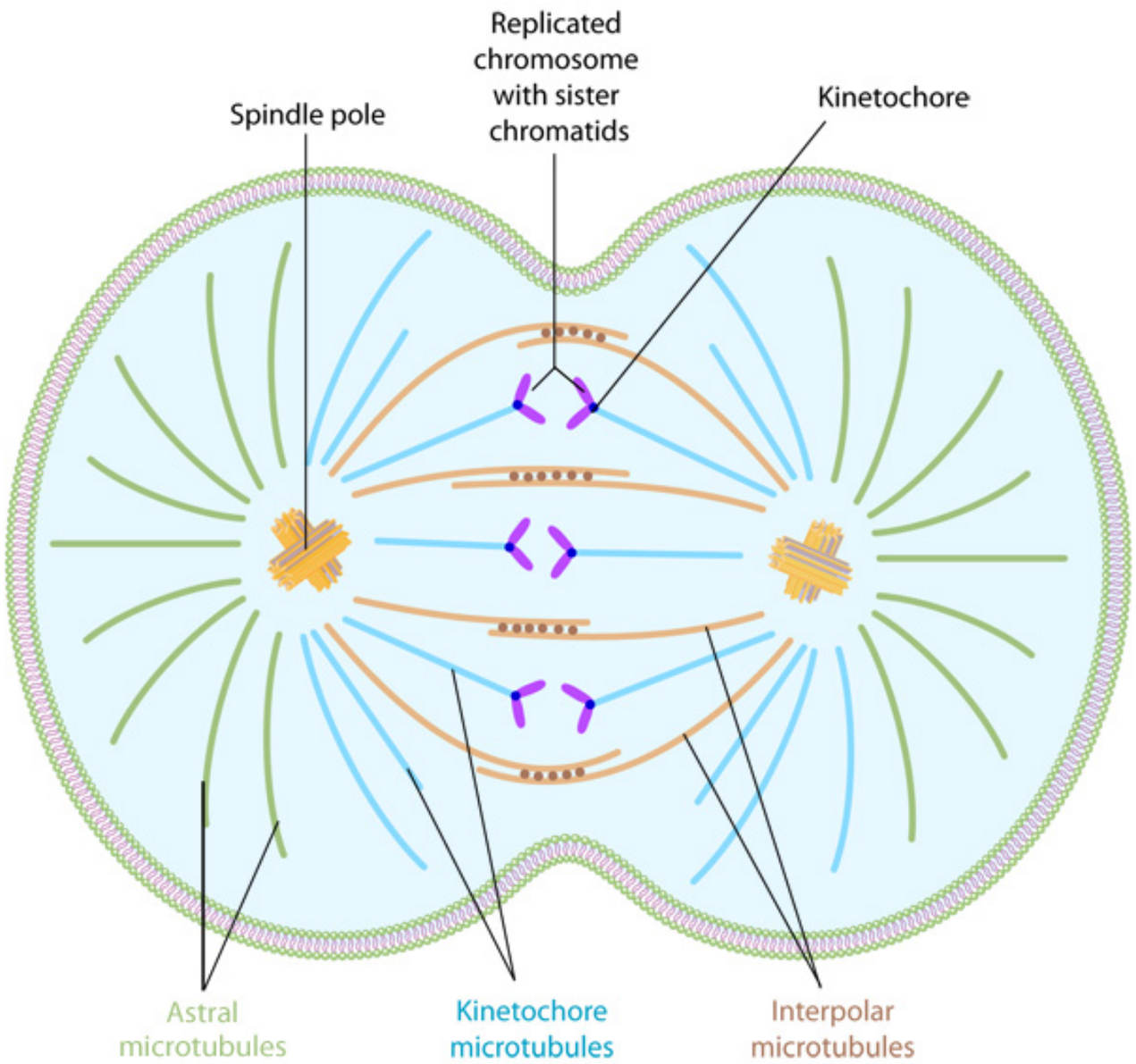
Contraction of muscles



Harvard BioVisions

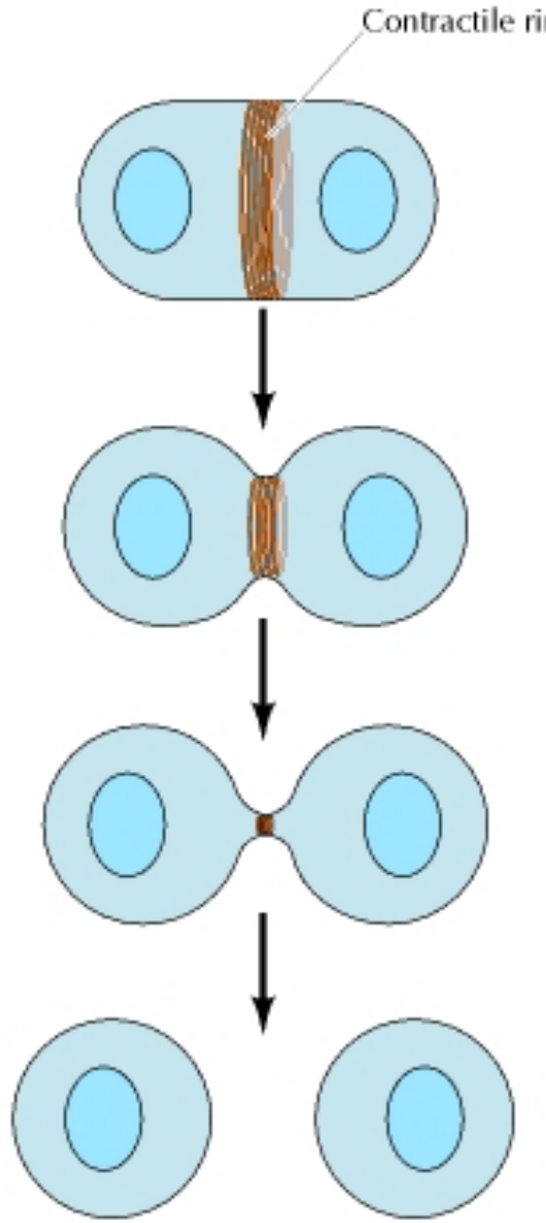
Cell division

Segregation of chromosomes



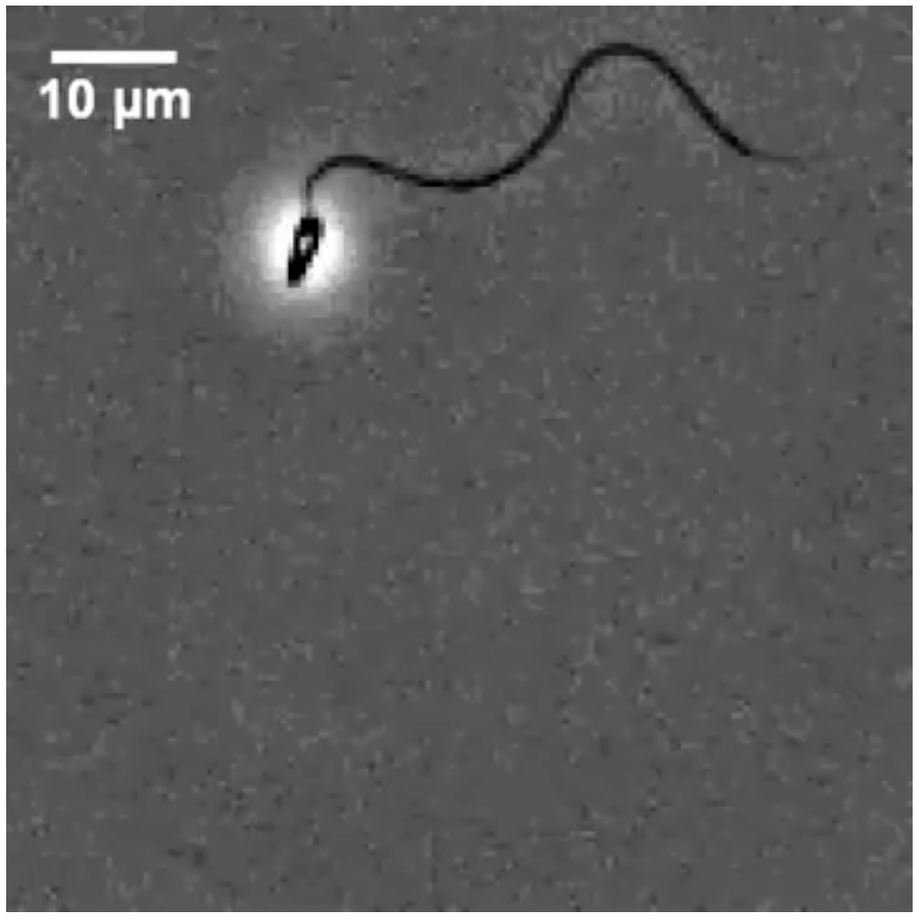
Microtubules

Contractile ring divides the cell in two



Actin

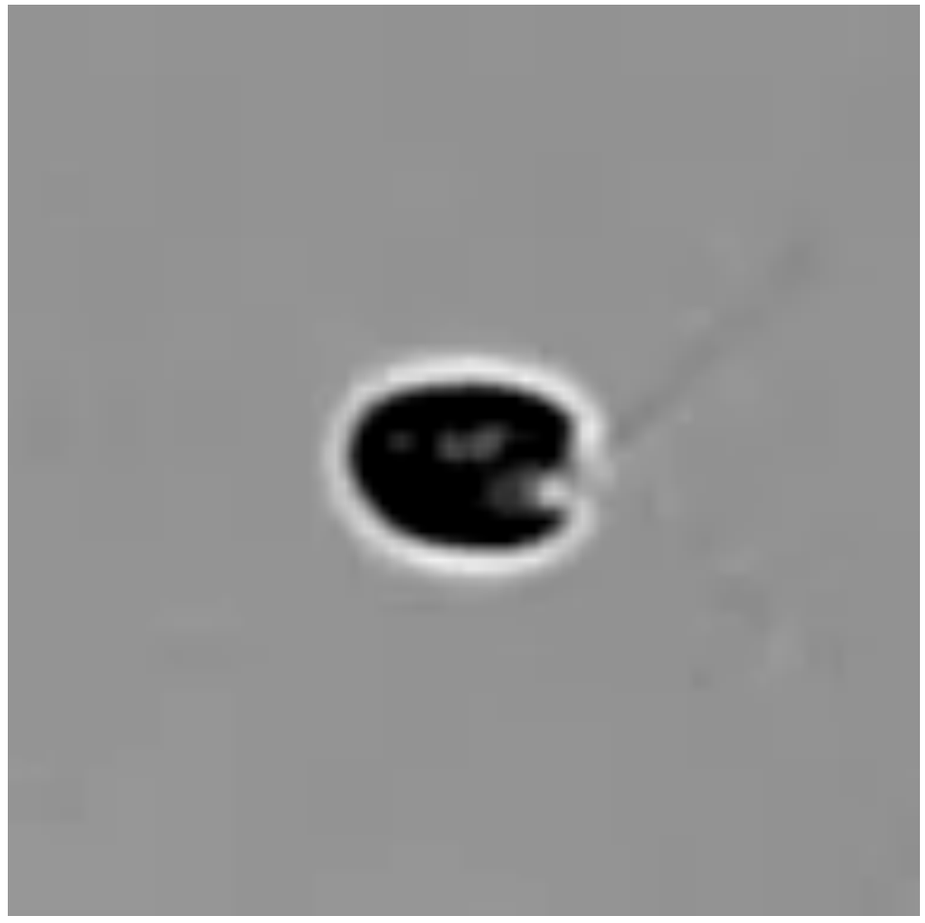
Swimming of sperm cells



Jeff Guasto

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

Swimming of Chlamydomonas (green alga)



Jeff Guasto

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

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

Actin filaments

actin monomer

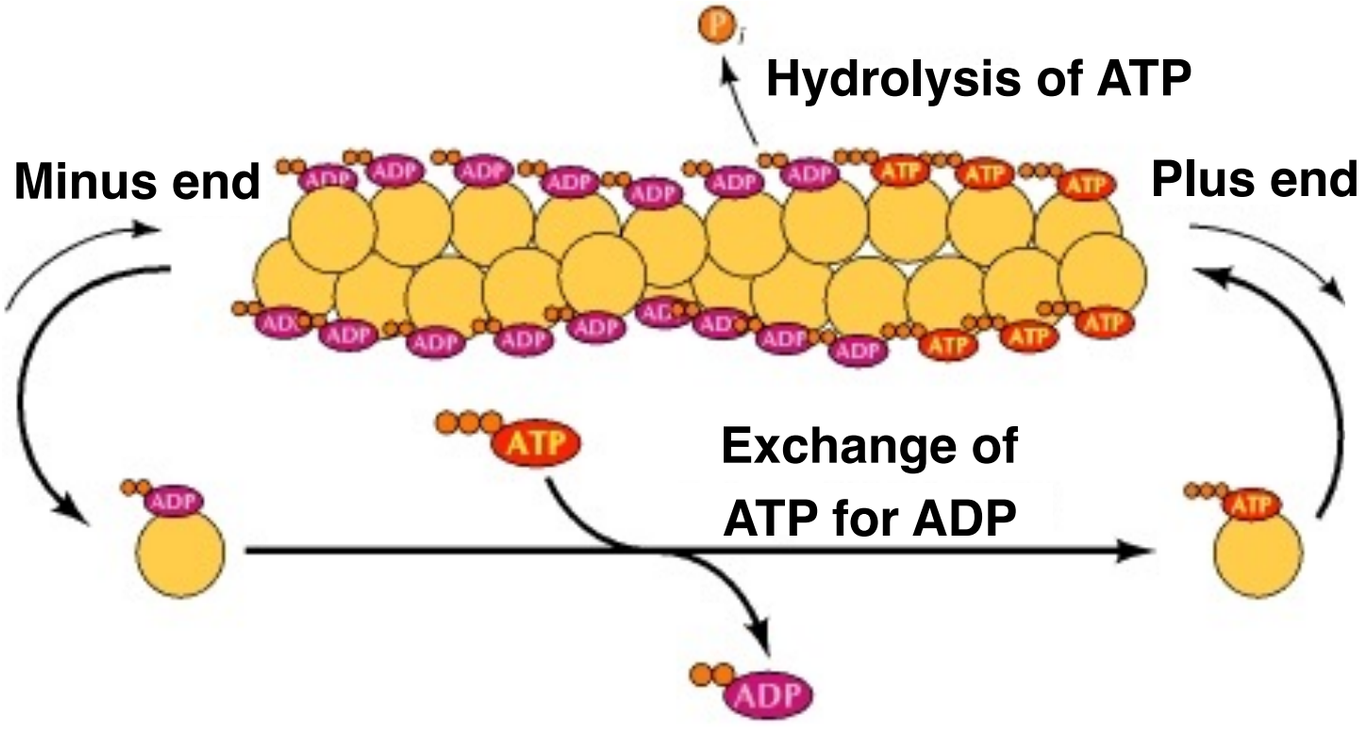
Minus end (pointed end)

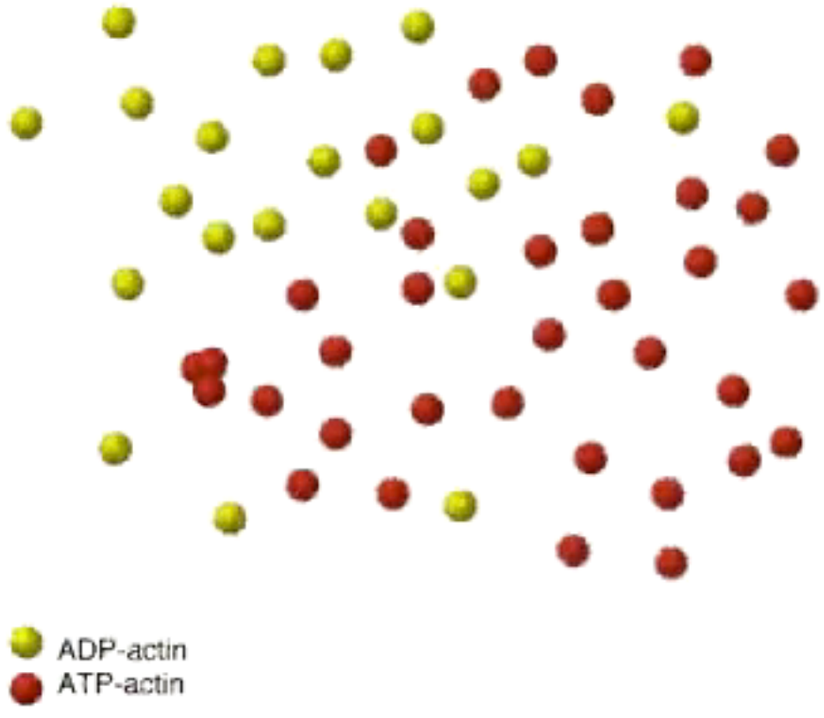
Plus end (barbed end)

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

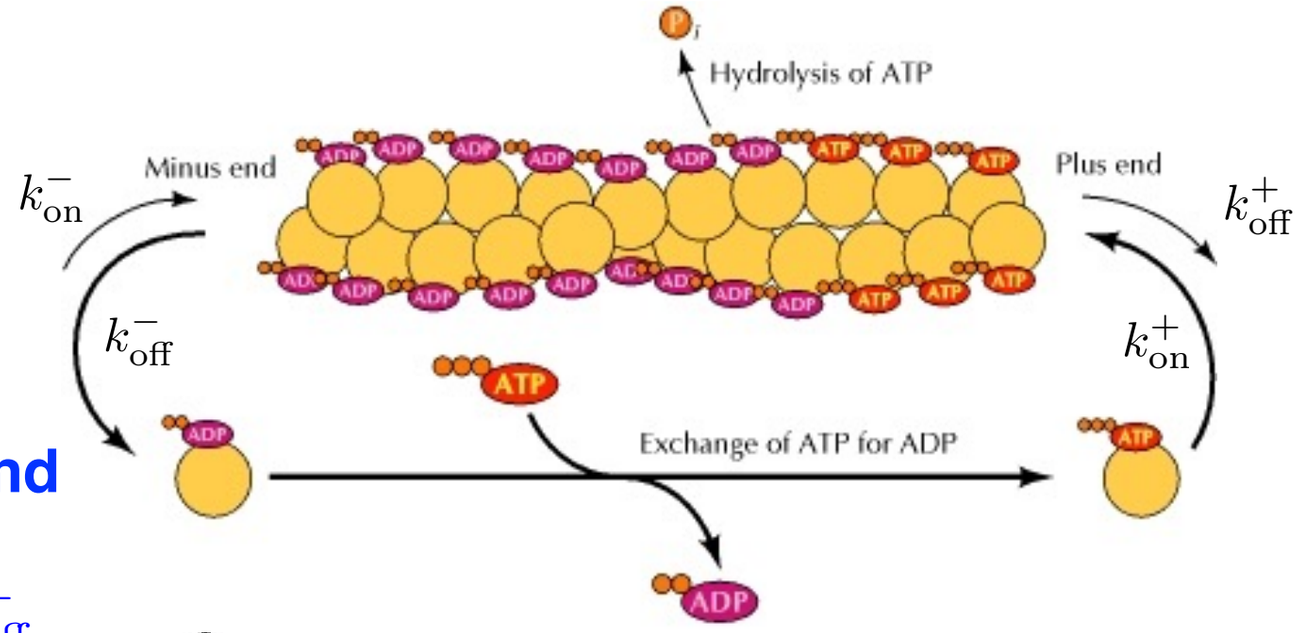
Typical length $L \lesssim 10\mu\mathrm{m}$

Actin treadmilling





Actin growth



growth of minus end

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

no growth at

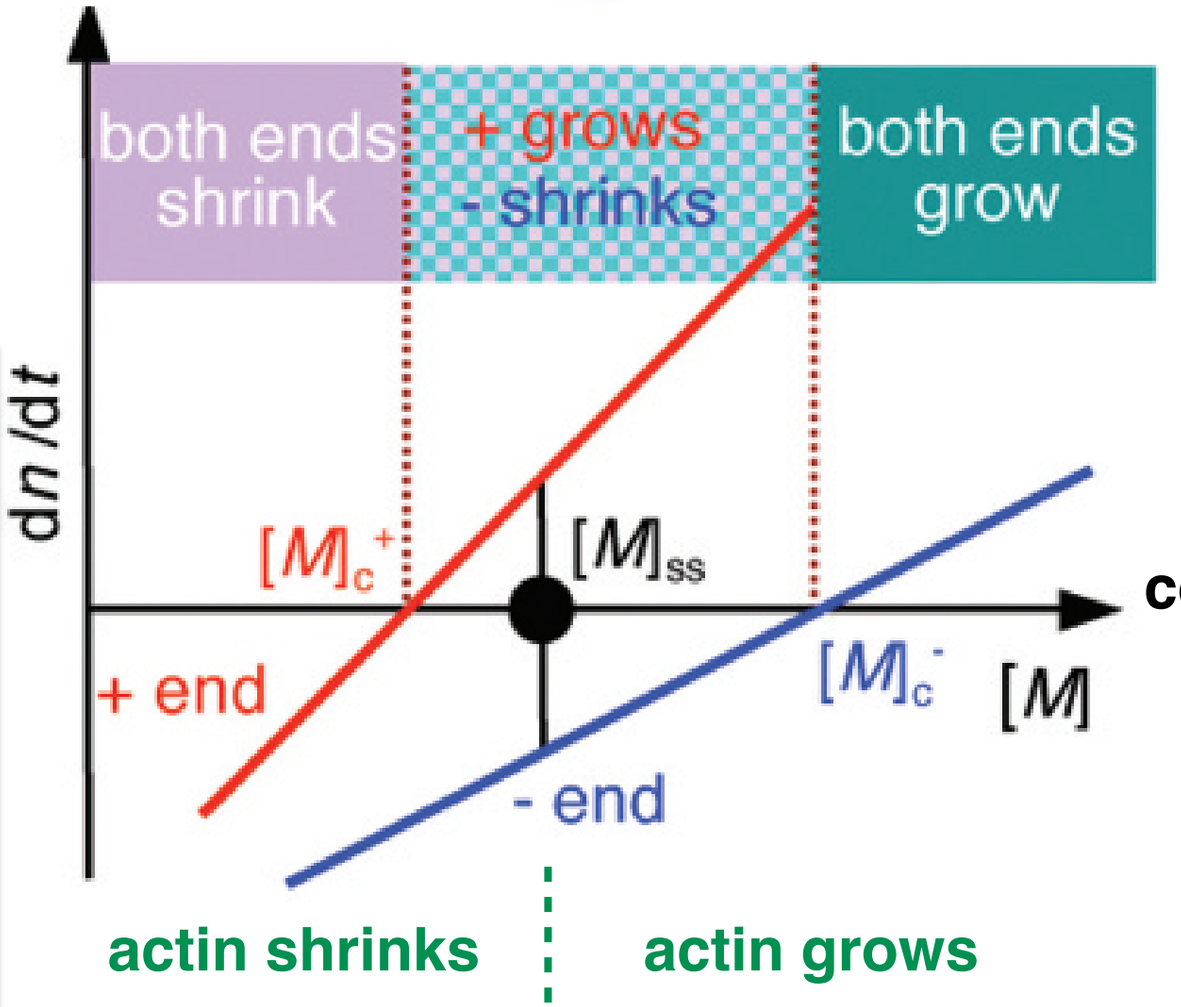
$$[M]_c^- = \frac{k_{\text{off}}^-}{k_{\text{on}}^-}$$

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

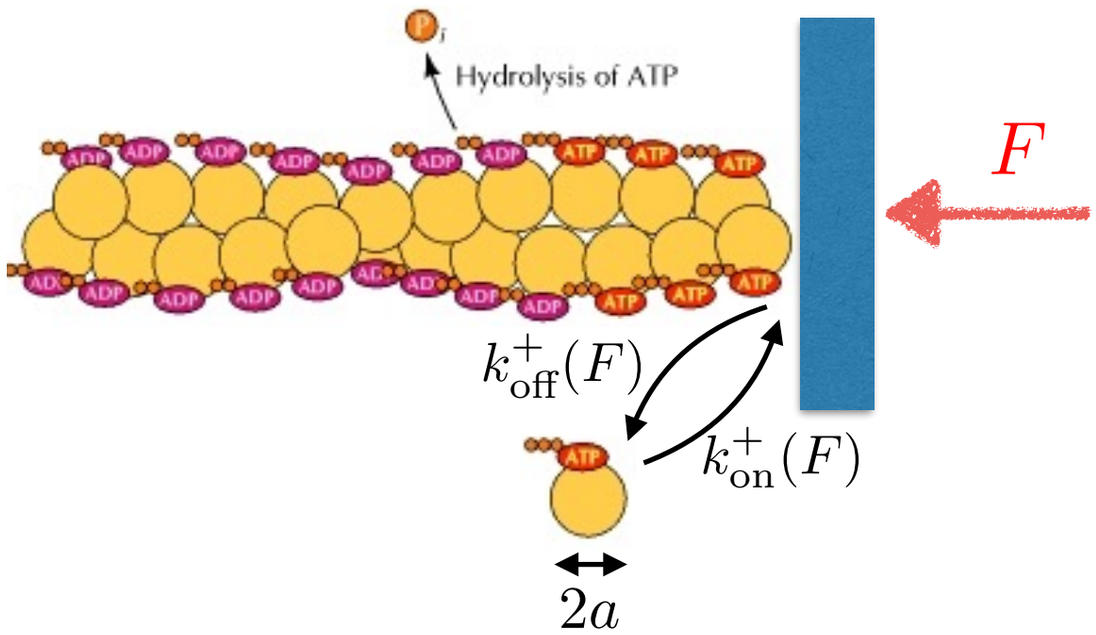
$$\frac{dn^+}{dt} = -\frac{dn^-}{dt}$$

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

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

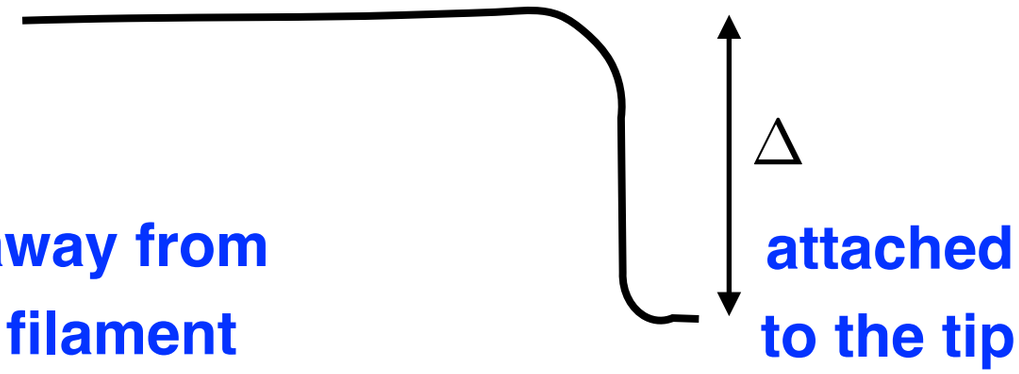


work done against the barrier for insertion of new monomer

$$W = Fa$$

effective monomer free energy

potential without barrier

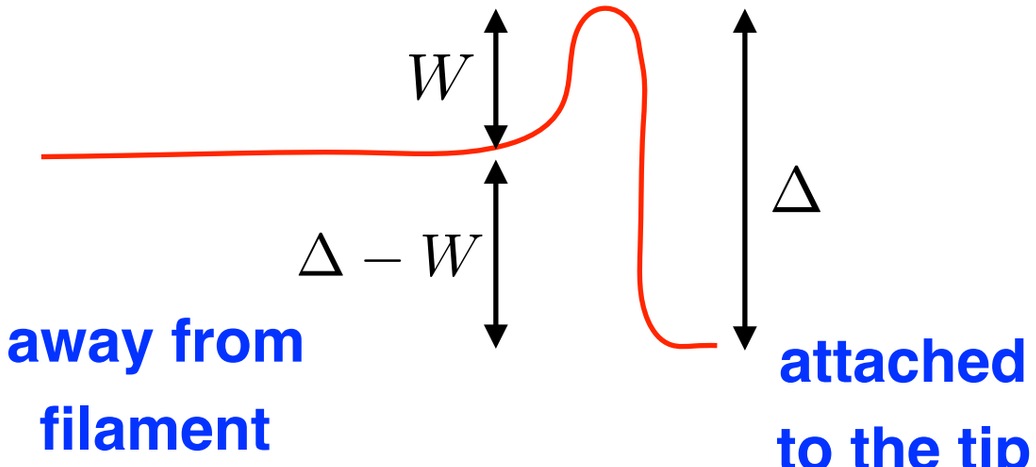


$$k_{\rm on}^+ \sim 4\pi D_3 a$$

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

effective monomer free energy

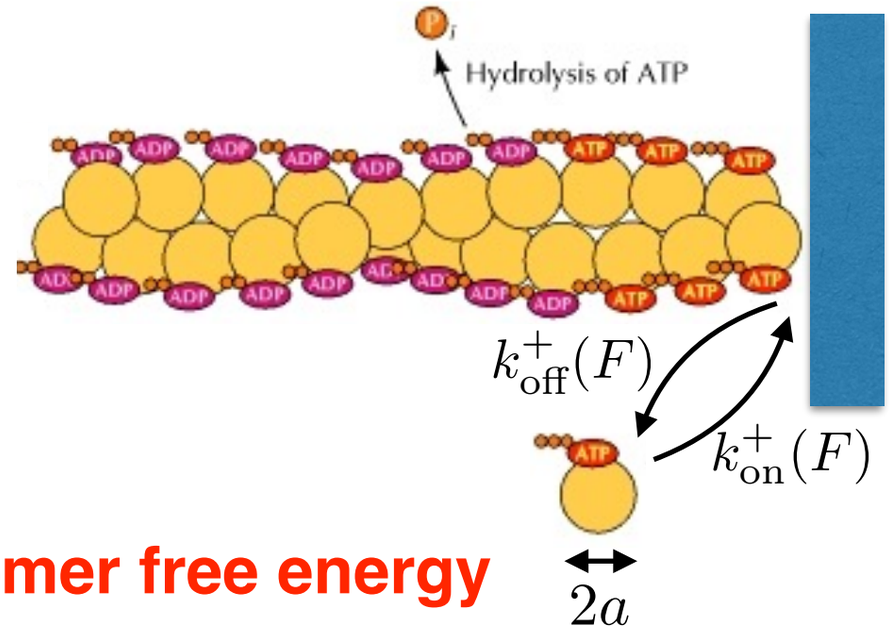
potential with barrier



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

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

Actin filament growing against the barrier

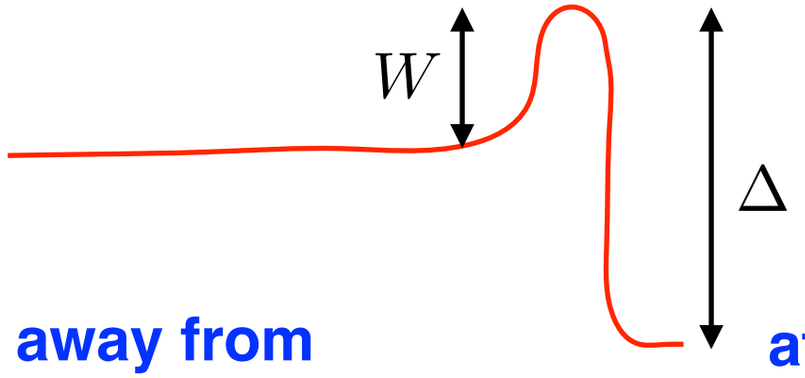


F

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

$$k^+_{\rm on}(F) \sim k^+_{\rm on} e^{-Fa/k_BT}$$

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

Growth speed of the tip

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

Maximal force that can be balanced by growing filament

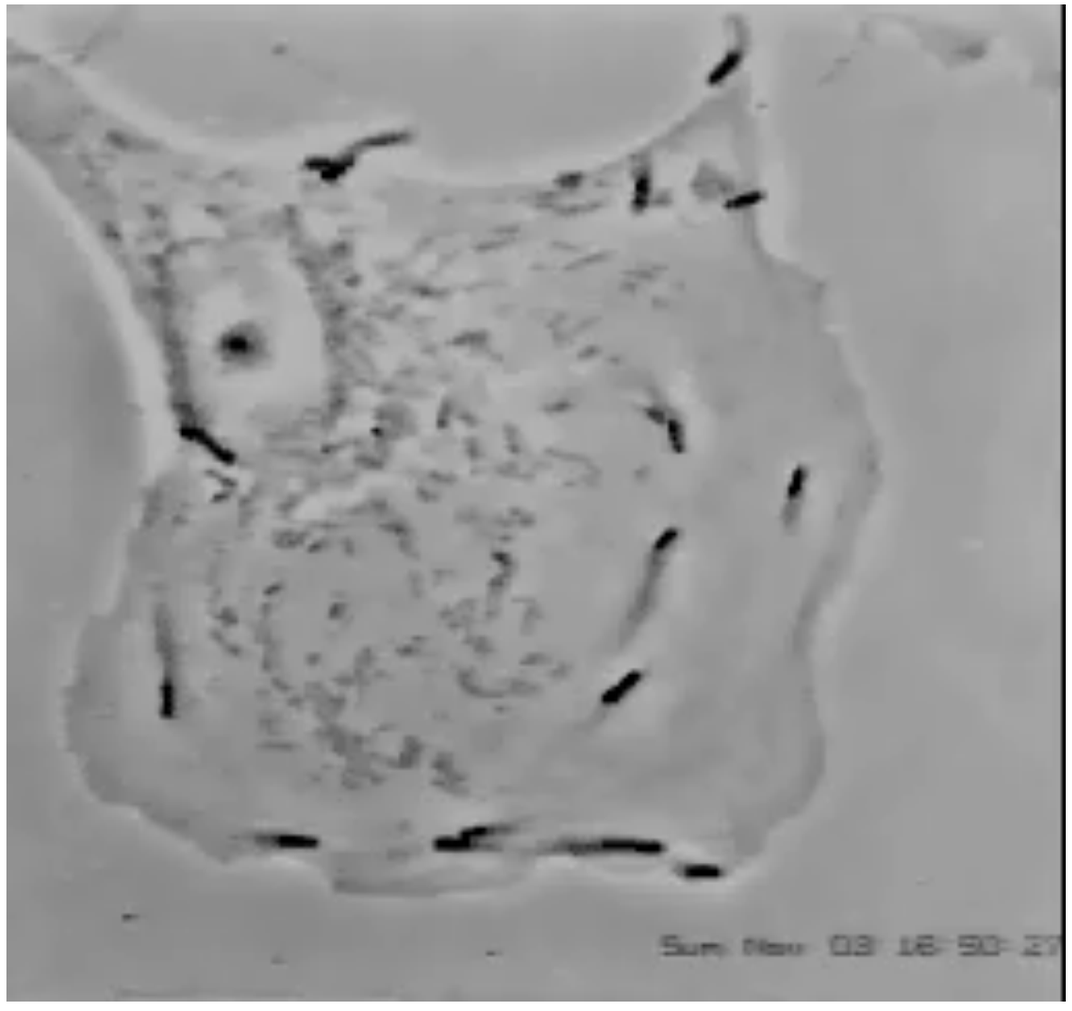
(stall force)

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

$k^+_{\rm on} \sim 10\mu{\rm M}^{-1}{\rm s}^{-1}$
$k^+_{\rm off} \sim 1{\rm s}^{-1}$
$[M] \sim 10\mu{\rm M}$
$a \approx 2.5{\rm nm}$
$F_{\rm max} \sim 8{\rm pN}$

Movement of bacteria

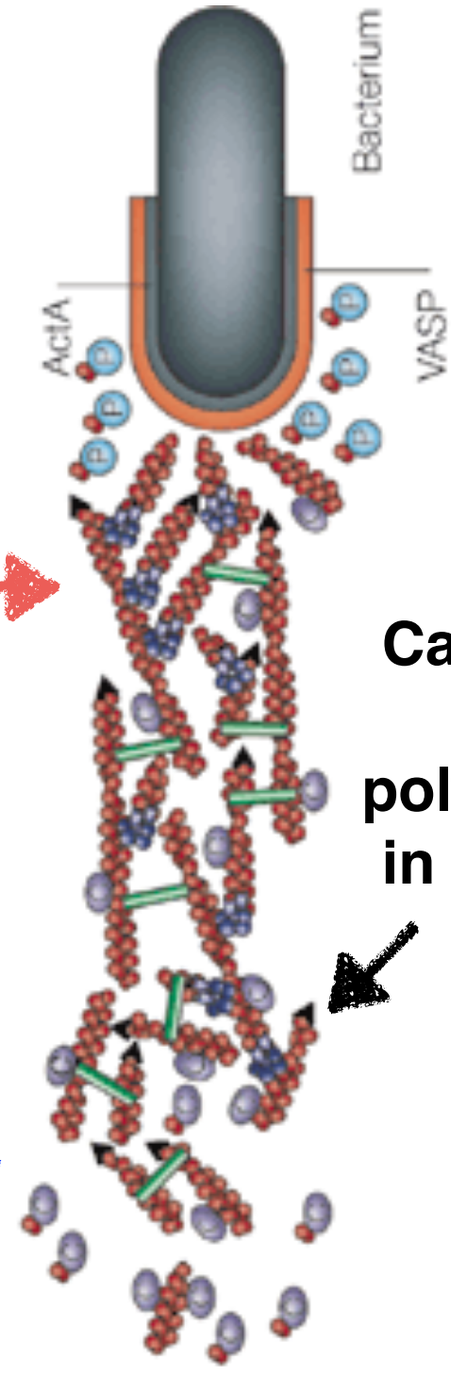
Listeria monocytogenes
moving in infected cells



Julie Theriot (speeded up 150x)

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

Actin polymerization is pushing bacteria



Caps prevent further polymerization in comet tails

Actin is randomly depolymerized in comet tails

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