How proteins find target sites on DNA?

**Figure 1.**

(A) Schematic representation of the protein–DNA search problem. The protein (yellow) must find its target site (red) on a long DNA molecule confined within the cell nucleoid (in bacteria) or cell nucleus (in eukaryotes). Compare with figure 9 (A) which shows confined DNA.

(B) The target site must be recognized with 1 base-pair (0.34 nm) precision, as displacement by 1 bp results in a different sequence and consequently a different site.

**Figure 2.**

(A) The mechanism of facilitated diffusion. The search process consists of alternating rounds of 3D and 1D diffusion, each with average duration \( \tau_{3D} \) and \( \tau_{1D} \), respectively.

(B) The antenna effect. During 1D diffusion (sliding) along DNA, a protein visits on average \( \bar{n} \) sites. This allows the protein to associate some distance \( \sim \bar{n} \) away from the target site and reach it by sliding, effectively increasing the reaction cross-section from 1bp to \( \sim \bar{n} \).

The antenna effect is responsible for the speed-up by facilitated diffusion.

1.3. History of the problem: theory

To resolve this discrepancy, one possible mechanism of facilitated diffusion that includes both 3D diffusion and effectively 1D diffusion of protein along DNA (the 1D/3D mechanism) was suggested. This mechanism was first proposed and dismissed by Riggs et al [1] but was soon revived and rigorously studied by Richter and Eigen [3], then further expanded and corrected by Berg and Blomberg [4] and finally developed by Berg et al [5]. The basic idea of the 1D/3D mechanism is that while searching for its target site, the protein repeatedly binds and unbinds DNA and, while bound non-specifically, slides along the DNA, undergoing one-dimensional (1D) Brownian motion or a random walk. Upon dissociation from the DNA, the protein diffuses three dimensionally in solution and binds to the DNA in a different place for the next round of one-dimensional searching (figure 2 (A)).

During 1D sliding the protein is kept on DNA by the binding energy to non-specific DNA. This energy has been measured for several DNA-binding proteins and has a range of 10–15 \( k_B T \) (at physiological salt concentration), was shown to be driven primarily by screened electrostatic interactions between charged DNA and protein molecules [6].

**Statistical mechanics of polymers**

**Growth dynamics of actin filaments and microtubules**

**Dynamics of molecular motors**
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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

Translation
Protein-DNA interactions

Binding to specific target sequence is strong

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

Binding to nonspecific sequence is weak

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

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

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

**on rates are diffusion limited**

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

**off rates depend on binding strengths**

$$k_{off}^S = A_se^{-\Delta G^S/k_B T} \ll k_{off}^{NS} = A_se^{-\Delta G^{NS}/k_B T}$$

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

---

protein

...ATTATGCATGACGATGTGGACAAACACCTGCGT...

target sequence

DNA sequence

---


3D diffusion and effectively 1D diffusion of protein along DNA.

**1.3. History of the problem: theory**

Screened electrostatic interactions between charged DNA and protein molecules diffuses three dimensionally in solution and binds to the DNA in a different place for the next (1D) Brownian motion or a random walk. Upon dissociation from the DNA, the protein mechanism is that while searching for its target site, the protein repeatedly binds and unbinds by Berg and Blomberg, then further expanded and corrected by Richter and Eigen. This energy has been measured for several DNA-binding proteins and has a range from $10^{-15}$ to $10^{-16}$.

During 1D sliding the protein is kept on DNA by the binding energy to non-specific sites. The antenna effect resists the speed-up by facilitated diffusion. These search processes consist of alternating rounds of 3D and 1D diffusion, each with average duration $\tau$. During 1D diffusion (sliding) along DNA, a protein visits on average $n \gg 1$ sites. The 1D/3D mechanism was finally developed by Berg and his group. The basic idea of the 1D/3D mechanism is that while searching for its target site, the protein repeatedly binds and unbinds by Berg and Blomberg, then further expanded and corrected by Richter and Eigen. This energy has been measured for several DNA-binding proteins and has a range from $10^{-15}$ to $10^{-16}$.

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Figures from referring papers are credited accordingly.
How long proteins remain bound on DNA?

Probability that protein unbinds in a small time interval $\Delta t$:

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

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

$$k_{\text{off}} \Delta t \times \left(1 - k_{\text{off}} \Delta t\right)^{t/\Delta t}$$

Limit $\Delta t \rightarrow 0$

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

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

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

\[ b = 0.34\text{nm} \]

c(\( r \rightarrow \infty \)) = \([ P ]\)

rate of absorption (see slide 6)

\[ I_0 = 4\pi D_3 b [P] \equiv k_{on} [P] \]

Kinetics of protein binding/unbinding

\[ [P] + [T] \xrightleftharpoons[k_{off}]{k_{on}} [P-T] \]

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

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

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

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

Characteristic search time

\[ t_s = (k_{on}[T])^{-1} \]
How quickly proteins find target sites on DNA?

**Characteristic search time via 3D diffusion**

\[ k_{\text{on}} = 4\pi D_3 b \hspace{1cm} t_s = (k_{\text{on}}[T])^{-1} \]

1917 Smoluchowski theory

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

\[ b \approx 0.34\text{nm} \hspace{1cm} D_3 \approx 30\mu\text{m}^2/\text{s} \]

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

\[ k_{\text{on}} \sim 10^8 M^{-1} s^{-1} \hspace{1cm} t_s \sim 10 s \]

**in vitro experiments (1970)**

\[ k_{\text{on}}^{\text{exp}} \sim 10^{10} M^{-1} s^{-1} \hspace{1cm} t_s \sim 0.1 s \]

**Why is experimentally observed rate 100 times larger?**

(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.

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

Berg - von Hippel theory (1980s)

First assume fixed sliding time $\tau_{1d}$

Number of distinct sites visited during each sliding event

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

(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

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

Average search time

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

$\tau_{1d}$ - characteristic jumping time
$\tau_{3d}$ - characteristic jumping time

$b = 0.34\text{nm}$ $L$ - DNA length
$D_3$ - diffusion constant in space
$D_1$ - diffusion constant along the DNA

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

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

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

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

\[ \langle N_R \rangle = 1/\langle q \rangle \]

Average search time

\[ \langle t_s \rangle = \langle N_R \rangle (\langle \tau_{1d} \rangle + \tau_{3d}) \]

\[ \langle t_s \rangle = \frac{L}{2\sqrt{D_1 \langle \tau_{1d} \rangle}} (\langle \tau_{1d} \rangle + \tau_{3d}) \]

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

\( D_3 \) - diffusion constant in space

\( D_1 \) - diffusion constant along the DNA

\( \tau_{3d} \) - characteristic jumping time
Facilitated diffusion

Average search time
\[ \langle t_s \rangle = \frac{L}{\langle \ell_{sl} \rangle} \left( \langle \tau_{1d} \rangle + \tau_{3d} \right) \]

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

Optimal search time
\[ \frac{d\langle t_s \rangle}{d \langle \tau_{1d} \rangle} = 0 \quad \Rightarrow \quad \langle \tau_{1d} \rangle_{opt} = \tau_{3d} \]
\[ \langle t_s \rangle_{opt} = L \sqrt{\frac{\tau_{3d}}{D_1}} \]

Search time for jumps alone

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

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

average number of jumps needed to find the target
\[ \bar{N}_{\text{jumps}} = \frac{L}{b} \]
\[ \bar{t}_{s,\text{jumps}} = \bar{N}_{\text{jumps}} \tau_{3d} = \frac{V}{4\pi D_3 b} \]

Search time for sliding alone
\[ \langle t_s \rangle_{\text{sliding}} \sim \frac{L^2}{D_1} \]

Search time speed up for facilitated diffusion
\[ \frac{\bar{t}_{s,\text{jumps}}}{\langle t_s \rangle} = \frac{\langle \ell_{sl} \rangle}{b} \frac{\tau_{3d}}{(\langle \tau_{1d} \rangle + \tau_{3d})} \]
Example: search time for target site in bacteria on DNA with $10^6$ base pairs

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

**search time for jumps alone**
\[ \bar{t}_{s, \text{jumps}} = (L/b)\tau_{3d} \approx 300 \text{s} \]

**average search time**
\[ \langle t_s \rangle = \frac{L}{\langle \ell_{sl} \rangle} (\langle \tau_{1d} \rangle + \tau_{3d}) \]

**average sliding length**
\[ \langle \ell_{sl} \rangle = 2\sqrt{D_1 \langle \tau_{1d} \rangle} \]

Graph showing the relative contributions of sliding and jumping to the overall search time.

- **Jumping**
- **Sliding + Jumping**
- **Sliding**

\[ \langle \ell_{sl} \rangle \ll b \]
\[ \langle \ell_{sl} \rangle \gg L \]
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?

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

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

\[ Z = \sum_c e^{-E_c/k_BT} \]

\[ \langle \mathcal{O} \rangle = \sum_c \mathcal{O}_c \frac{e^{-E_c/k_BT}}{Z} \]

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)

expected value of observables

\[ E_c \] energy of a given configuration

\[ T \] temperature

\[ k_B \] Boltzmann constant

\[ k_B = 1.38 \times 10^{-23} \text{JK}^{-1} \]
Persistence length

correlations between tangents

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

tangents become uncorrelated beyond persistence length!

Long filaments perform self-avoiding random walk

$$L \gg \ell_p$$

Short filaments remain straight

$$L \ll \ell_p$$

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

$B$ - filament bending rigidity

$T$ - temperature

$L$ - filament length
Examples: persistence length

Polyethylene
\[ \ell_p = 2.6 \text{ nm} \]

Persistence length for polymers is on the order of nm

Actin
\[ \ell_p \approx 17 \mu\text{m} \]

Microtubule
\[ \ell_p \approx 1.4 \text{ mm} \]

double stranded DNA
\[ \ell_p \approx 50 \text{ nm} \]

Single stranded DNA
\[ \ell_p \approx 2 \text{ nm} \]

Uncooked spaghetti
\[ \ell_p \approx 10^{18} \text{ m} \]
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!

\[
\langle \vec{R}_{AB} \rangle = 0
\]

\[
\langle \vec{R}^2_{AB} \rangle \approx L^2
\]

Exact result

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

Polymers shrink, when temperature is increased!
Negative thermal expansion of rubber.
**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 \( C \):
  \[ E_c = \frac{B}{2} \int_0^L ds \left( \frac{d^2 r}{ds^2} \right)^2 \]

- 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

![Diagram of Ideal Chain](image)

\[ \langle \vec{R}_{AB}^2 \rangle = Na^2 = aL \]

**Worm-like chain**

- Continuous unstretchable rod

![Diagram of Worm-like Chain](image)

\[ \langle \vec{R}_{AB}^2 \rangle \approx 2\ell_pL = \frac{2BL}{k_BT} \]

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

\[ a = 2\ell_p \]

\[ L = Na - \text{chain length} \]
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 \( Fa \ll k_BT \)

\[
\langle x \rangle \approx \frac{FN\alpha^2}{3k_BT} = \frac{2FL\ell_p}{3k_BT}
\]

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 $F\ell_p \ll k_BT$

large force $F\ell_p \gg k_BT$

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

entropic spring constant

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

$B$ - 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,
Experimental results for stretching of DNA

\[ L = 32.8 \mu m \]

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

For DNA

\[ \ell_p = 50 \text{nm} \]

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

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,
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

\( T < \Theta \)

\[ R \sim (d^2 L)^{1/3} \]

d - diameter of polymer chain

at low temperature

attraction between polymer chains dominates

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.
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

\[ v \sim 0.1 \mu m/s \]
**Movement of bacteria**

*L. monocytogenes* moving in infected cells

*Image of bacteria moving within a cell.*

Julie Theriot (speeded up 150x)

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

Molecular motors

Contraction of muscles

Transport of large molecules around cells (diffusion too slow)

\[ v \sim 1\mu m/s \]


Harvard BioVisions

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

Segregation of chromosomes

- Spindle pole
- Replicated chromosome with sister chromatids
- Kinetochore
- Interpolar microtubules
- Astral microtubules

Microtubules

Contractile ring divides the cell in two

- Contractile ring
- Actin
Swimming of sperm cells

Swimming of Chlamydomonas (green alga)

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

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

Jeff Guasto

*v* \(\sim 50\mu m/s\)

Jeff Guasto

*v* \(\sim 60\mu m/s\)
Actin filaments

7 nm

actin monomer

Minus end
(pointed end)

Plus end
(barbed end)

Persistence length $\ell_p \sim 10 \mu m$

Typical length $L \lesssim 10 \mu m$

Actin treadmilling

Hydrolysis of ATP

Exchange of ATP for ADP

ADP-actin

ATP-actin
**Fig. 11.12**

(a) If $[M]_c^+ + [M]_c^- = [M]_c^-$, both filament ends grow or shrink simultaneously. (b) If $[M]_c^+ \neq [M]_c^-$, there is a region where one end grows while the other shrinks. The vertical line indicates the steady-state concentration $[M]_{ss}$ where the filament length is constant.

**Steady state regime**

\[
\frac{dn^+}{dt} = \frac{dn^-}{dt} = - \frac{n^-}{n^+}
\]

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

**Front speed**

\[
\frac{dn^+}{dt} = \frac{k_{on}^+ k_{off}^- - k_{on}^- k_{off}^+}{k_{on}^- + k_{on}^+} \approx 0.68 s^{-1}
\]
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

effective monomer free energy potential with barrier

away from filament

attached to the tip

\[ k_{on}^+ \sim 4\pi D_3 a \]
\[ k_{off}^+ \propto e^{-\Delta/k_B T} \]

\[ k_{on}^+(F) \sim k_{on}^+ e^{-Fa/k_B T} \]
\[ k_{off}^+(F) \sim k_{off}^+ \]
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

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

\[ v^+(F_{\text{max}}) = 0 \quad \Rightarrow \quad F_{\text{max}} = \frac{k_BT}{a} \ln \left( \frac{k_{\text{on}}[M]}{k_{\text{off}}} \right) \]

\[ k_{\text{on}} \sim 10 \mu M^{-1}s^{-1} \]

\[ k_{\text{off}} \sim 1s^{-1} \]

\[ [M] \sim 10 \mu M \]

\[ a \approx 2.5 \text{nm} \]

\[ F_{\text{max}} \sim 8\text{pN} \]
Movement of bacteria

*Listeria monocytogenes* moving in infected cells

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

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.

growth
rescue catastrophe shrinkage

GTP-tubulin dimer GDP-tubulin dimer
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 (\(\frac{\partial p}{\partial t} \equiv 0\)):

\[
p^*_{\text{growth}} = \frac{r_{\text{res}}}{r_{\text{res}} + r_{\text{cat}}} \quad p^*_{\text{shrinking}} = \frac{r_{\text{cat}}}{r_{\text{res}} + r_{\text{cat}}}
\]

Average growth speed of microtubules:

\[
\overline{v} = p^*_{\text{growth}} v_g - p^*_{\text{shrinking}} v_s
\]

\[
\overline{v} \approx 0.4 \mu m/min
\]
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

\[ v_{\text{mot}} \approx 3 \mu\text{m}/\text{min} \]

Kymograph

\[ v_{\text{mot}} \approx 3 \mu\text{m}/\text{min} \]

V. Varga et al., Cell 138, 1174-1183 (2009)
Density of motors bound to microtubules

\[ [M] \text{ concentration of unbound motors} \]

Conservation law for the number of bound motors

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

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

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

Generalized Fick’s law

\[
\frac{\partial \rho(x, t)}{\partial t} = r(x, t) - \frac{\partial j(x, t)}{\partial x}
\]

density of bound motors

\[ \rho(x, t) = \frac{\partial N(x, t)}{\partial x} \]
Density of motors bound to microtubules

\[ [M] \text{ concentration of unbound motors} \]

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

\[
\rho^*(x) = \frac{k_{\text{bind}} [M]}{v_{\text{mot}}} x
\]
Depolymerization rate is proportional to density of Kip3 motors

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

To understand how Kip3p shortens microtubules, we needed to determine an average lifetime, which we did by using the formulation of the antenna model. This allowed us to account quantitatively for Kip3p molecules that bind randomly along the microtubule (increasing toward the plus end), as has been observed in studies of the behavior of individual Kip3p molecules on microtubules over long periods of time using total-internal-reflection-fluorescence (TIRF) microscopy to critically test the antenna model.

One possible model for length-dependent depolymerization is that Kip3p molecules bind to the microtubule lattice and ends over a wide range of Kip3p concentrations. Incorporation of this mechanism into a mathematical formulation allowed us to account quantitatively for Kip3p molecules that bind randomly along the microtubule (increasing toward the plus end), as has been observed in studies of the behavior of individual Kip3p molecules on microtubules over long periods of time using total-internal-reflection-fluorescence (TIRF) microscopy to critically test the antenna model.

Controlled length of microtubules

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

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

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

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

$$[T] \approx 10\mu M$$
$$k_{on} \approx 9\mu M^{-1}s^{-1}$$
$$L^* \sim 75 \mu m$$

$$[M] \approx 3nM$$
$$k_{bind} \approx 24nM^{-1}min^{-1}\mu m^{-1}$$
Molecular motors

A. Myosin V

B. Myosin II

C. Kinesin-1, Dynein

Actin

Microtubule

Contraction of muscles

Transport of large molecules around cells
diffusion too slow

\[ v \sim 1\mu m/s \]


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=oHDRIwRZRVl

https://www.youtube.com/watch?v=YAv4g3Pk6k
Molecular motors vs Brownian ratchets

Myosin motor
ATP driven process drives molecular motors along the filaments

potential energy along the filament

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

ATP
motor

bound ATP
no ATP

actin filament

- + - + - + - +

ATP

Thermal diffusion

drift
ATP concentration dependent speed of motors

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

Kinesin motor on microtubules

Maximal speed

\[ v_{\text{max}} \approx 0.6 \, \mu\text{m/s} \]

ATP concentration at half the maximal speed

\[ K_d \approx 50 \, \mu\text{M} \]
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?

Motor velocity dependence on the load

kinesin walking on microtubules

How important is viscous drag for motors carrying vesicles?

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

\[ F_{\text{drag}} \approx 6\pi 10^{-3} \text{kgm}^{-1}\text{s}^{-1} \cdot 1\mu\text{m} \cdot 1\mu\text{m/s} \]

\[ F_{\text{drag}} \approx 10^{-2} \text{pN} \]

stall force

\[ v(F_s) = 0 \]


Note: viscous drag is negligible
ATP concentration dependent stall force

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

**motor step length**
\[ a \approx 8 \text{ nm} \]

**Position clamp**
laser follows the bead and keeps fixed force

**Fixed trap**
laser position is is fixed

**kinesin walking on microtubules**

<table>
<thead>
<tr>
<th>Stall force (pN)</th>
<th>ATP concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>1000</td>
</tr>
<tr>
<td>6</td>
<td>1000</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
</tr>
</tbody>
</table>

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

\[ F_{\text{max}} = \frac{\Delta G_{\text{ATP}}}{a} \approx \frac{20k_B T}{8 \text{nm}} \approx 10 \text{pN} \]

Skeletal muscle contraction by myosin motors

Figure 16.7: The structure of muscle. (A) Thin-section electron micrograph showing the organization of a single sarcomere when a muscle is stretched. The dark band in the middle represents the location of the aligned myosin thick filaments and the light bands on the sides show the position of actin. The diagrams below show the change in sarcomere length during muscle contraction. (B) The regular structure of the sarcomere depends on the precise arrangement and alignment of many structural proteins. The long proteins titin and nebulin help to set the length of the actin thin filaments and determine the overall length of the sarcomere. The Z disc serves as an anchor point for the actin thin filaments, and ensures that they are all oriented in the same direction, so that the myosin heads walking toward the actin filament plus ends will cause the sarcomere to shorten. (C) A quick-freeze deep-etch electron micrograph shows the extremely regular spacing of thick myosin filaments alternating with thin actin filaments and the myosin heads bridging the gap between them. (A, courtesy of Roger Craig; B, adapted from B. Alberts et al., Molecular Biology of the Cell, 5th ed. Garland Science, 2008; C, courtesy of J. Heuser.)

Properties of individual motors, how much force might we estimate can be applied by an array of motors such as are found in muscle? We can evaluate an estimate of this force by examining the structure and function of muscles. Figure 16.7(B) shows a cartoon of a muscle cell and muscle fibers. The myofibrils are themselves composed of contractile units called sarcomeres. Myosin molecules are arranged in a cylindrically symmetric structure called the thick filament, and exert forces on the outer actin filaments. We can estimate the net force per myosin molecule by appealing to a simple picture in which muscles are thought of as arrays of springs in series and parallel as shown in Figure 16.8. The number of myosins in a cross-section of muscle is roughly

\[
N_{\text{myosin}} \approx \frac{\text{cross-sectional area of muscle}}{\text{cross-sectional area of thick filament}} \times N_{\text{myosin/thick filament}} \approx \pi (3 \text{ cm})^2 \times \pi (60 \text{ nm})^2 \times 300 \approx 10^{14}.
\]
Muscles contract at twice the speed of myosin motors

\[ \sim 0.1-1 \mu m/s \]

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

Estimated force generated by myosin motors

\[
300 \times \frac{2pN}{\pi (30nm)^2} \sim 20N/cm^2
\]
Skeletal muscle contraction is controlled by nerve cells

Low \( \text{Ca}^{2+} \), muscles are relaxed

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

High \( \text{Ca}^{2+} \), muscles are contracted

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

Electric signal from nerve cells releases \( \text{Ca}^{2+} \) from sarcoplasmic reticulum
How muscles get ATP energy?

1. **Liver**
   - Stimulates glucose uptake from blood
   - Tissue cells (muscle, kidney)

2. **Stomach**
   - Small intestine

3. **Pancreas**
   - (behind stomach)

4. **Insulin**
   - High blood sugar
   - Stimulates formation of glycogen
   - Stimulates glucose uptake from blood
   - Tissue cells (muscle, kidney)
   - Lowers blood sugar

**glycogen**
- (polysaccharide of glucose)

**glucose**
- (blood sugar)
How muscles get ATP energy?

**glycogen** (polysaccharide of glucose)

**glucose** (blood sugar)

Liver

Stomach

Pancreas (behind stomach)

Small intestine

**Liver**

Stimulates uptake of glucose from blood

Tissue cells (muscle, kidney)

Insulin

Glucagon

Promotes insulin release

Promotes glucagon release

How muscles get ATP energy?

Liver

Stomach

Pancreas (behind stomach)

Small intestine

**Liver**

Stimulates breakdown of glycogen

Stimulates formation of glycogen

Stimulates glucose uptake from blood

Tissue cells (muscle, kidney)

Insulin

Glucagon

Promotes insulin release

Promotes glucagon release

High Blood Sugar

Low Blood Sugar

How muscles get ATP energy?

Liver

Stomach

Pancreas (behind stomach)

Small intestine

**Liver**

Stimulates breakdown of glycogen

Stimulates formation of glycogen

Stimulates glucose uptake from blood

Tissue cells (muscle, kidney)

Insulin

Glucagon

Promotes insulin release

Promotes glucagon release

High Blood Sugar

Low Blood Sugar

How muscles get ATP energy?
How muscles get ATP energy?

Aerobic respiration

\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 36ATP \]

Anaerobic respiration

\[ C_6H_{12}O_6 \rightarrow 2 \text{ Lactic Acids} + 2\text{ATP} \]

(muscle fatigue)

Note:
Citric acid cycle
= Krebs cycle
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.

https://www.youtube.com/watch?v=CN2XOe_c0iM
Energetics of ATP hydrolysis

How much energy is released during ATP hydrolysis?

\[ \Delta G = \mu_{ADP} + \mu_P - \mu_{ATP} \]

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

\[ \Delta G \sim -12.5k_B T \]

**Under physiological conditions:** \( \Delta G \sim -20k_B T \)

([ATP], [ADP], [P\textsubscript{i}] \sim 1\text{mM})

Chemical potentials are typically defined relative to concentration \(c_0 \sim 1\text{ M} \).

\[ \mu_s(c_s) = \mu_s(c_0) + k_B T \ln(c_s/c_0) \]
Crawling of cells

Immune system: neutrophils chasing bacteria

Direction of migration

Immune system: neutrophils chasing bacteria

migration of skin cells during wound healing

spread of cancer cells during metastasis of tumors

amoeba searching for food

Actin

David Rogers (1950s)

$\nu \sim 0.1 \mu m/s$
**Crawling of cells**

**fish skin cell**  \( v = 0.2 \mu \text{m/s} \)

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

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Alberts et al., Molecular Biology of the Cell
Swimming of sperm cells

Sperm flagellum is constructed from microtubules

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

Jeff Guasto

\[ v \sim 50 \mu m/s \]
Further reading