17. Directed and Active Transport

Motor Proteins

Many proteins act as molecular motors using an energy source to move themselves or cargo in space. They create directed motion by coupling energy use to conformational change.

Motor Classes

Translational

- Cytoskeletal motors that step along filaments (actin, microtubules)
- Helicase translation along DNA

Rotary

- ATP synthase
- Flagellar motors

Polymerization

- Cell motility

Translocation

- DNA packaging in viral capsids
- Transport of polypeptides across membranes

Translational Motors

Processivity

- Some motors stay on fixed track for numerous cycles
- Others bind/unbind often—mixing stepping and diffusion

Cytoskeletal motors

- Used to move vesicles and displace one filament relative to another
- Move along filaments—tracks have polarity (±)
- Steps of fixed size

Classes

- Dynein moves on Microtubules (+ → –)
- Kinesin Microtubules (mostly – → +)
- Myosin Actin

Molecular Motors

We can make a number of observations about common properties of translational and rotational motor proteins.

Molecular motors are cyclical

- They are "processive" involving discrete stepping motion
- Multiple cycles lead to directional linear or rotary motion

Molecular motors require an external energy source

- Commonly this energy comes from ATP hydrolysis
    - ~50 kJ/mol or ~20 k_BT or ~80 pN/nm
    - ATP consumption correlated with stepping
- Or from proton transfer across a transmembrane proton gradient

Protein motion is strongly influenced by thermal fluctuations and Brownian motion

- Molecular motors work at energies close to k_BT
- Short range motions are diffusive—dominated by collisions
- Inertial motion does not apply

Passive vs Active Transport

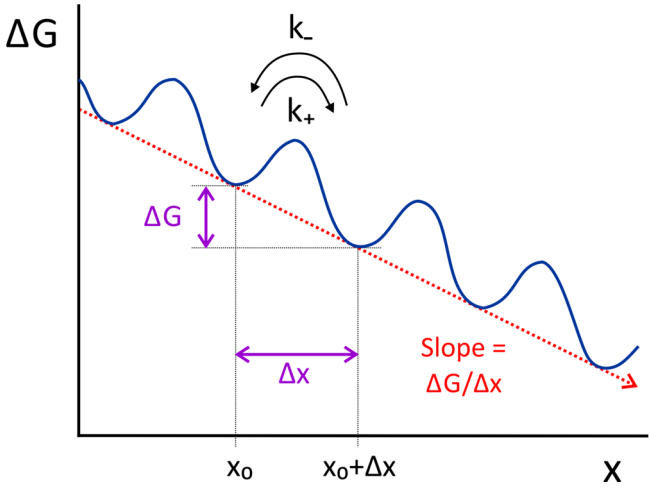
Directed motion of molecules in a statistically deterministic manner (i.e., $\bar{x}(t) = \bar{v}\,t$) in a thermally fluctuating environment cannot happen spontaneously. It requires a free energy source, which may come from chemical bonds, charge transfer, and electrochemical gradients. From one perspective, displacing a particle requires work, and the force behind this work originates in free energy gradients along the direction of propagation

$$w = -\int_{path} f\,dx \qquad f_{rev} = \frac{\partial G}{\partial x}$$

An example of this is steady-state diffusion driven by a spatial difference in chemical potential, for instance the diffusion of ions through a membrane channel driven by a transmembrane potential. This problem is one of passive transport. Although an active input of energy was required to generate the transmembrane potential and the net motion of the ion is directional, the ion itself is a passive participant in this process. Such processes can be modeled as diffusion within a potential.

Active transport refers to the direct input of energy into the driving the moving object in a directional manner. At a molecular scale, even with this input of energy, fluctuations and Brownian motion remain very important.

Even so, there are multiple ways in which to conceive of directed motion. Step-wise processive motion can also be viewed as a series of states along a free energy or chemical potential gradient. Consider this energy landscape:



Under steady state conditions, detailed balance dictates that the ratio of rates for passing forward or reverse over a barrier is dictated by the free energy difference between the initial and final states:

$$\frac{k_+}{k_-} = e^{-\Delta G/k_B T}$$

and thus the active driving force for this downhill process is

$$f \approx -\frac{\Delta G}{\Delta x} = \frac{k_B T}{\Delta x} \ln \frac{k_+}{k_-}$$

This perspective is intimately linked with a biased random walk model when we remember that

$$\frac{k_+}{k_-} = \frac{P_+}{P_-}$$

If our free energy is the combination of a chemical process (ΔG_0) and an external force, then we can write

$$\frac{k_+}{k_-} = \exp\left[-(\Delta G_0 + f\Delta x)/k_B T\right]$$

Feynman's Brownian Ratchet

Feynman used a thought experiment to show you cannot get work from thermal noise.[1] Assume you want to use the thermal kinetic energy from the molecules in a gas, and decide to use the collisions of these molecules with a vane to rotate an axle. The direction or rotation will be based on the velocity of the molecules hitting the vane, so to assure that this rotation proceeds only one way, we use a ratchet with a pawl and spring to catch the ratchet when it advances in one direction. This is the concept of rectified Brownian motion.

At a microscopic level, this reasoning does not hold, because the energy used to rotate the ratchet must be enough to lift the pawl against the force of the spring. If we match the thermal energy of gas $T = \frac{1}{2} m\langle v_x^2 \rangle$ to the energy needed to raise the pawl $U = \frac{1}{2}\kappa x^2$ we find that the pawl will also be undergoing fluctuations in x with similar statistics to the bombardment of the vane $\kappa = \sqrt{mk_B T/\langle x^2 \rangle}$. Therefore, the ratchet will instead thermally diffuse back and forth as a random walk. Further, Feynman showed that if you imbedded the vane and ratchet in reservoirs of temperature T_1 and T_2, respectively, then the ratchet will advance as desired if $T_1 > T_2$, but will move in reverse if $T_1 < T_2$. Thus, one cannot extract useful work from thermal fluctuations alone. You need some input of energy—any source of free energy.

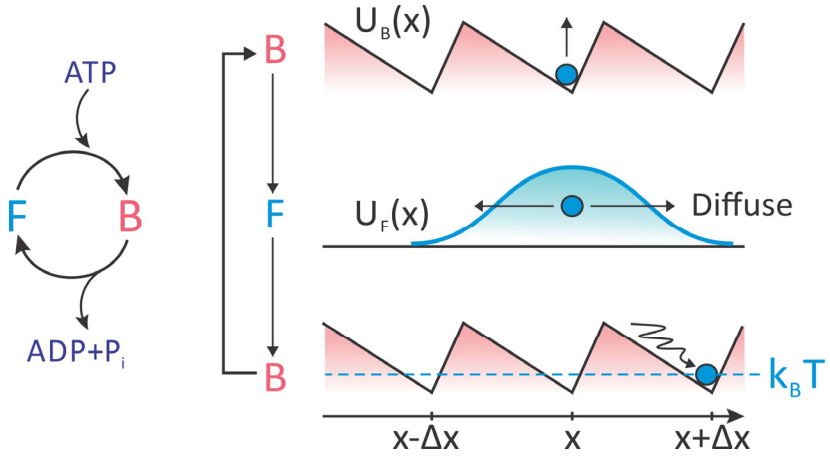
1. http://www.feynmanlectures.caltech.edu/I_46.html

Brownian Ratchet[2]

The Brownian ratchet refers to a class of models for directed transport using Brownian motion that is rectified through the input of energy. For a diffusing particle, the energy is used to switch between two states that differ in their diffusive transport processes. This behavior results in biased diffusion. It is broadly applied for processive molecular motors stepping between discrete states, and it therefore particularly useful for understanding translational and rotational motor proteins.

One common observation we find is that directed motion requires the object to switch between two states that are coupled to its motion, and for which the exchange is driven by input energy. Switching between states results in biased diffusion. The interpretation of real systems within the context of this model can vary. Some people consider this cycle as deterministic, whereas others consider it quite random and noisy, however, in either case, Brownian motion is exploited to an advantage in moving the particle.

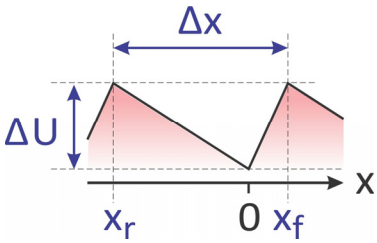
We will consider an example relevant to the ATP-fueled stepping of cytoskeletal motors along a filament. The motor cycles between two states: (1) a bound state (B), for which the protein binds to a particular site on the filament upon itself binding ATP, and (2) a free state (F) for which the protein freely diffuses along the filament upon ATP hydrolysis and release of ADP + P_i. The bound state is described by a periodic, spatially asymmetric energy profile $U_B(x)$, for which the protein localizes to a particular energy minimum along the filament. Key characteristics of this potential are a series of sites separated by a barrier $\Delta U > k_B T$, and an asymmetry in each well that biases the system toward a local minimum in the direction of travel. In the free state, there are no barriers to motion and the protein diffuses freely. When the free protein binds another ATP, it returns to $U_B(x)$ and relaxes to the nearest energy minimum.



2. K. Dill and S. Bromberg, *Molecular Driving Forces: Statistical Thermodynamics in Biology, Chemistry, Physics, and Nanoscience*. (Taylor & Francis Group, New York, 2010); R. Phillips, J. Kondev, J. Theriot and H. Garcia, *Physical Biology of the Cell*, 2nd ed. (Taylor & Francis Group, New York, 2012).

Let's investigate the factors governing the motion of the particle in this Brownian ratchet, using the perspective of a biased random walk. The important parameters for our model are:

- The distance between adjacent binding sites is Δx.
- The position of the forward barrier relative to the binding site is x_f. A barrier for reverse diffusion is at $-x_r$, so that



$$x_f + x_r = \Delta x$$

  The asymmetry of U_B is described by

$$\alpha = (x_f - x_r)/\Delta x$$

- The average time that a ratchet stays free or bound are τ_F and τ_B. Therefore, the average time per bind/release cycle is

$$\Delta t = \tau_F + \tau_B$$

- We define a diffusion length ℓ_0 which is dependent on the time that the protein is free

$$\ell_0(\tau_F) = \sqrt{4D\tau_F}$$

Conditions For Efficient Transport

Let's consider the conditions to maximize the velocity of the Brownian ratchet.

1) While in *F*: the optimal period to be diffusing freely is governed by two opposing concerns. We want the particle to be free long enough to diffuse past the forward barrier, but not so long that it diffused past the reverse barrier. Thus we would like the diffusion length to lie between the distances to these barriers:

$$\ell_0 = \sqrt{4D\tau_F}$$
$$x_r > \ell_0 > x_F$$

   Using the average value as a target:

$$\ell_0 \approx \frac{x_r + x_F}{2} = \frac{\Delta x}{2}$$
$$\tau_F \approx \frac{\Delta x^2}{16D}$$

2) While in *B*: After the binding ATP, we would like the particle to stay with ATP bound long enough to relax to the minimum of the asymmetric energy landscape. Competing with this consideration, we do not want it to stay bound any longer than necessary if speed is the issue.

We can calculate the time needed to relax from the barrier at x_r forward to the potential minimum, if we know the drift velocity v_d of this particle under the influence of the potential.

$$\tau_B \approx x_r / v_d$$

The drift velocity is related to the force on the particle through the friction coefficient, $v_d = f / \xi$, and we can obtain the magnitude of the force from the slope of the potential:

$$|f| = \frac{\Delta U}{x_r}$$

So the drift velocity is $v_d = \frac{f\,D}{k_B T} = \frac{\Delta U\,D}{x_r k_B T}$ and the optimal bound time is

$$\tau_B \approx \frac{x_r^2 k_B T}{\Delta U\,D}$$

Now let's look at this a bit more carefully. We can now calculate the probability of diffusing forward over the barrier during the free interval by integrating over the fraction of the population that has diffused beyond x_f during τ_F. Using the diffusive probability distribution with $x_0 \rightarrow 0$,

$$\begin{aligned} P_+ &= \frac{1}{\sqrt{4\pi D \tau_F}} \int_{x_f}^{\infty} e^{-x^2/4D\tau_F}\, dx \\ &= \frac{1}{2} erfc\left(\frac{x_f}{\ell_0}\right) \end{aligned}$$

Similarly, the probability for diffusing backward over the barrier at $x = -x_r$ is

$$P_- = \frac{1}{2} erfc\left(\frac{x_r}{\ell_0}\right)$$

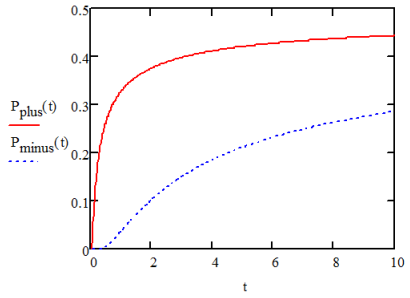
Now we can determine the average velocity of the protein by calculating the average displacement in a given time step. The average displacement is the difference in probability for taking a forward versus a reverse step, times the step size. This displacement occurs during the time interval Δt. Therefore,

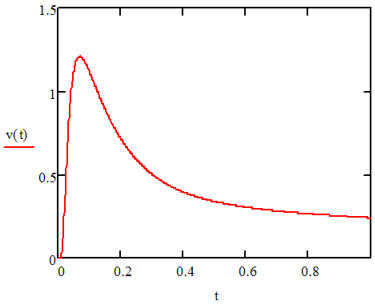
$$\begin{aligned} v &= \frac{\Delta P \Delta x}{\Delta t} \\ &= \frac{(P_+ - P_-)\Delta x}{(\tau_B + \tau_F)} \\ &= \frac{\Delta x}{2\Delta t}\left[erf\left(\frac{x_r}{\ell_0(\tau_F)}\right) - erf\left(\frac{x_f}{\ell_0(\tau_F)}\right)\right] \end{aligned}$$

It is clear from this expression that the velocity is zero when the asymmetry of the potential is zero. For asymmetric potentials, P_+ and P_- are dependent on τ_F, with one rising in time faster than the other. As a result, the velocity, which depends on the difference of these reaches a maximum in the vicinity of $\tau_F = x_f^2 / D$.

$$P_{plus}(t) := \frac{1}{2}\,\mathrm{erfc}\left(\frac{a}{\sqrt{4 \cdot D \cdot t}}\right) \qquad P_{minus}(t) := \frac{1}{2}\,\mathrm{erfc}\left(\frac{b}{\sqrt{4 \cdot D \cdot t}}\right)$$

$$v(t) := \frac{-1}{1 - P_{plus}(t) - P_{minus}(t)} \cdot \left[\frac{d}{dt}\left(1 - P_{plus}(t) - P_{minus}(t)\right)\right]$$





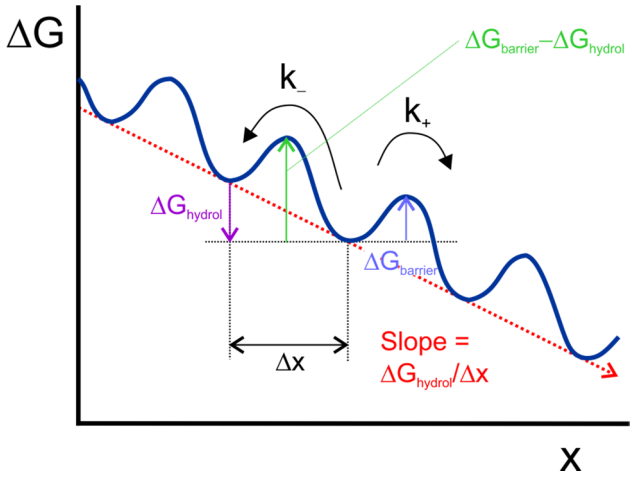
So how does the ATP hydrolysis influence the free energy gradient? Here free energy gradient is

$$\frac{\Delta G_{\mathrm{Hyd.}}}{\Delta x}$$

$$k_+ = A_+ e^{-\left(\Delta G_{barrier} - \Delta G_{hydrolysis}\right)/kT}$$

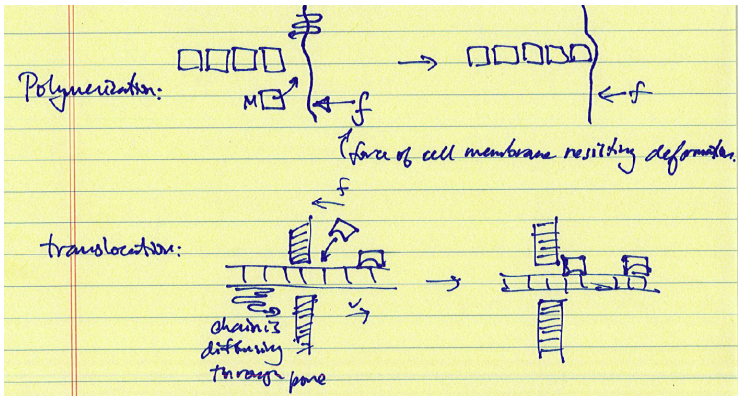
$$k_- = A_- e^{-\left(\Delta G_{barrier}\right)/kT}$$

$$v = \left(k_+ - k_-\right)\Delta x$$



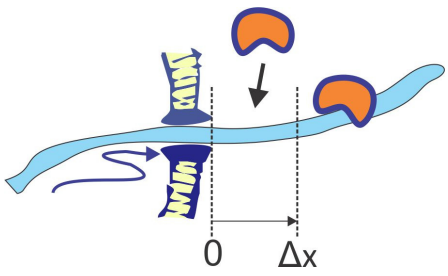
Polymerization Ratchet and Translocation Ratchet

Polymerization and translocation ratchets refer to processes that result in directional displacements of a polymer or oligomer chain rather than a specific protein. The models for these ratchets also involve rectified Brownian motion, in which a binding unit is added to a diffusing chain to bias the diffusive motion in a desired direction. Once the displacement reaches a certain diffusion length, a monomer or binding protein can add to the chain, locking in the forward diffusion of the chain. In this case, it is the binding or attachment of protein units that consumes energy, typically in the form of ATP or GTP hydrolysis.



Translocation Ratchet[3]

Protein translocation across cell membranes is a ubiquitous process for transporting polypeptide chains across bacterial and organelle membranes through channels with the help of chaperone proteins on the inner side of the membrane. The translocation ratchet refers to a model in which the transport of the chain occurs through Brownian motion which is rectified by the binding of proteins to the chain on one side of the pore as it is displaced. Once the chain diffuses through the pore for a distance Δx, a protein can bind to the chain, stopping backward diffusion. At each step, energy is required to drive the binding of the chaperone protein.



The translocation ratchet refers to a continuum model for the diffusion of the chain. It is possible to map this diffusion problem onto a Smoluchowski equation, but it would be hard to solve for the probability density. It is easier if we are just interested in describing the average velocity of the chain under steady state conditions, we can solve for the steady-state chain flux across the pore:

3. C. S. Peskin, G. M. Odell and G. F. Oster, Cellular motions and thermal fluctuations: the Brownian ratchet, Biophys. J. **65** (1), 316–324 (1993).

$$J(x) = -D\left(\frac{\partial P}{\partial x} + \frac{f}{k_B T} P\right) \tag{1}$$

where f is the force acting against the chain displacement. Steady state behavior corresponds to $\partial P/\partial t = 0$, so from the continuity equation

$$\frac{\partial P}{\partial t} = -\frac{\partial J}{\partial x}$$

we know that $\partial J/\partial x = 0$. Therefore J is a constant. To find P, we want to solve

$$\frac{\partial P}{\partial x} + \frac{f}{k_B T} P + \frac{J}{D} = 0$$

for which the general solution is $P = A_1 e^{-fx/k_B T} + A_2$. We find the integration constants using the boundary condition $P(\Delta x, t) = 0$, which reflects that a protein will immediately and irreversibly bind once the diffusing chain reaches an extension Δx. (No back-stepping is allowed.) And we use the conservation statement:

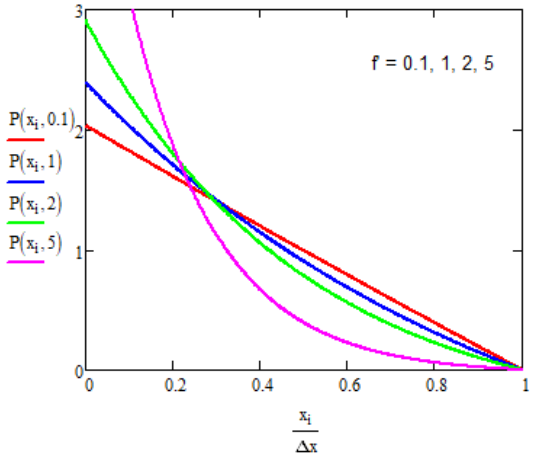
$$\int_0^{\Delta x} dx\, P(x) = 1$$

which says that a protein must be bound within the interval 0 to Δx. The steady-state probability distribution with these two boundary conditions is

$$P(x) = \frac{\underset{\sim}{f}\left[\exp\left(\underset{\sim}{f}\left(1 - x/\Delta x\right)\right) - 1\right]}{\Delta x\left(1 + \underset{\sim}{f} - e^{\underset{\sim}{f}}\right)} \tag{2}$$

$$\underset{\sim}{f} = \frac{f \Delta x}{k_B T}$$

$\underset{\sim}{f}$ is a dimensionless constant that expresses the load force in units of $k_B T$ opposing ratchet displacement by Δx.

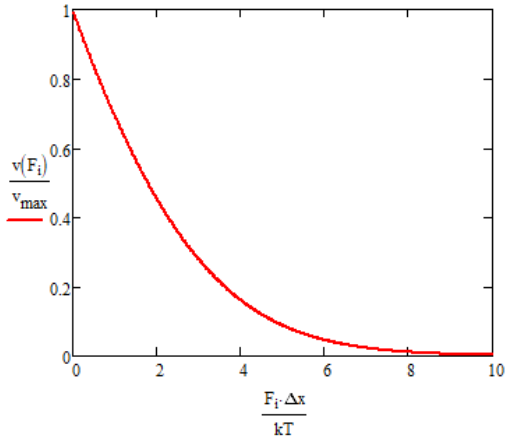


Substituting eq. (2) into eq. (1) allows us to solve for J.

$$J(x) = \frac{-D\tilde{f}^2}{\Delta x^2\left(1+\tilde{f}-e^{\tilde{f}}\right)}\left(1-2\exp\left[\tilde{f}\left(\frac{x}{\Delta x}-1\right)\right]\right)$$

Now, the average velocity can be determined from $\langle v\rangle = J\Delta x$. Evaluating the flux at $x = \Delta x$:

$$\langle v\rangle = \frac{2D}{\Delta x}\left[\frac{\tilde{f}^2/2}{e^{\tilde{f}}-\tilde{f}-1}\right]$$



Now look at low force limit $f \to 0$. Expand $e^{\tilde{f}} = 1+\tilde{f}+\tilde{f}^2/2$:

$$\langle v\rangle \to \frac{2D}{\Delta x} = v_{\max}$$

Note that this is the maximum velocity for ideal ratchet, and it follows the expected behavior for pure diffusive motion.

Now consider probability of the protein binding is governed by equilibrium between free and bound forms:

$$F \underset{k_d}{\overset{k_a}{\rightleftharpoons}} B \qquad\qquad K = \frac{k_a}{k_d} = \frac{\tau_B}{\tau_F}$$

Here k_a refers to the effecting quasi-first-order rate constant for binding at a chaperone concentration [chap]: $k_a = k_a'\,[chap]$.

Fast kinetics approximation

$$\langle v \rangle = \frac{2D}{\Delta x}\left[\frac{\tilde{f}^2/2}{\frac{e^{\tilde{f}}-1}{1-K(e^{\tilde{f}}-1)}-\tilde{f}}\right]$$

$$\langle v \rangle_{\max} = \frac{2D}{\Delta x}\left(\frac{1}{1+2K}\right)$$

Stall load

$$f_0 = \frac{k_B T}{\Delta x}\ln\left(1+\frac{1}{K}\right)$$