PHYSICAL LENS ON THE CELL

Basic Principles Underlying Cellular Processes

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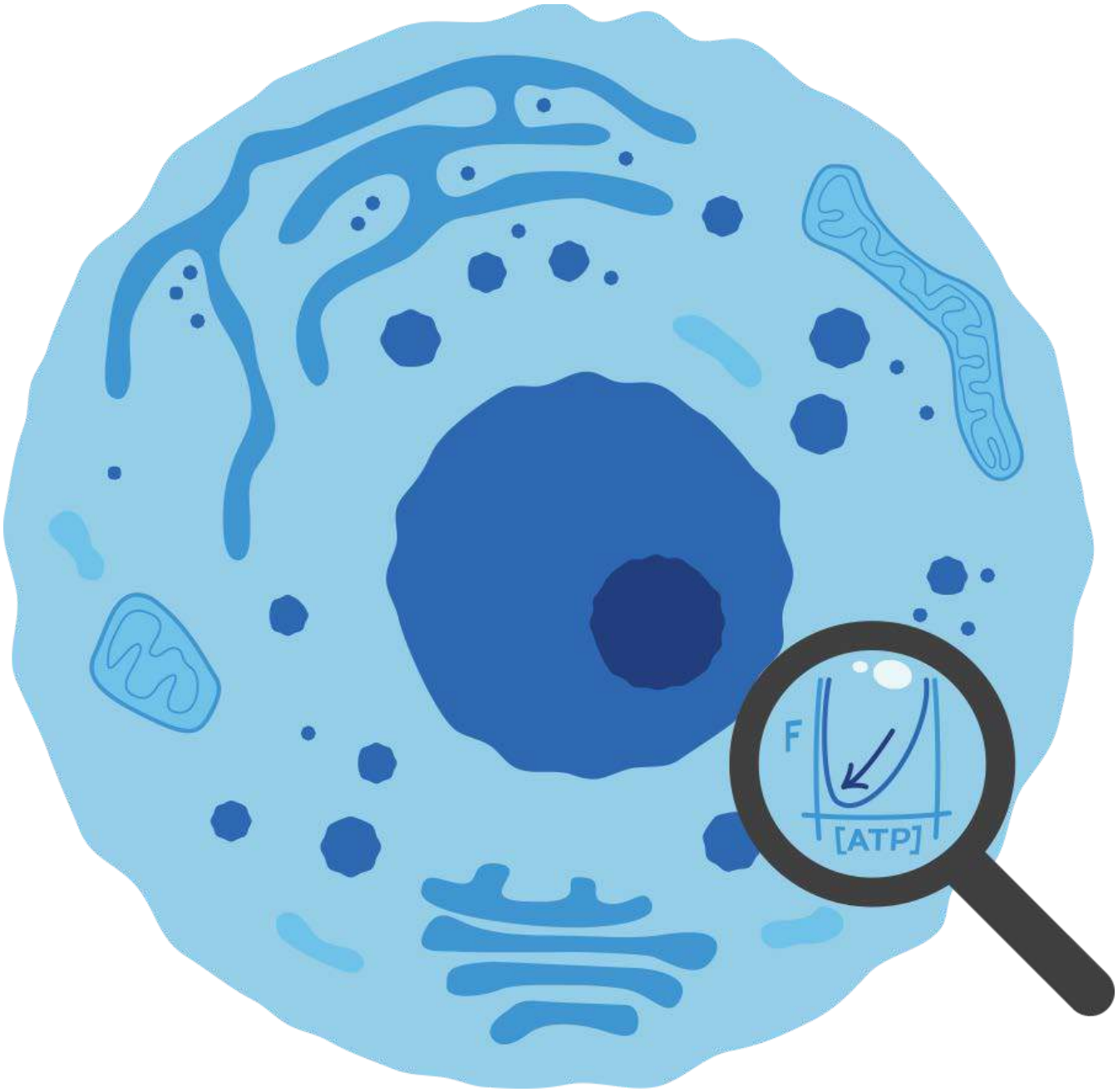


Goals

- Understand the physical driving forces behind cellular processes
- Organize cellular processes into physical categories to facilitate trans standing
- Focus on deep understanding and specific examples because learnir ogy is not an option

Philosophy

- All processes in the cell necessarily are driven by physical rules. Otherwise, they wouldn't happen.
- Biologists *and* physicists should understand how and why.
- The key physical principles can be understood through a relatively simple mass-action picture of non-equilibrium kinetics.

Notation

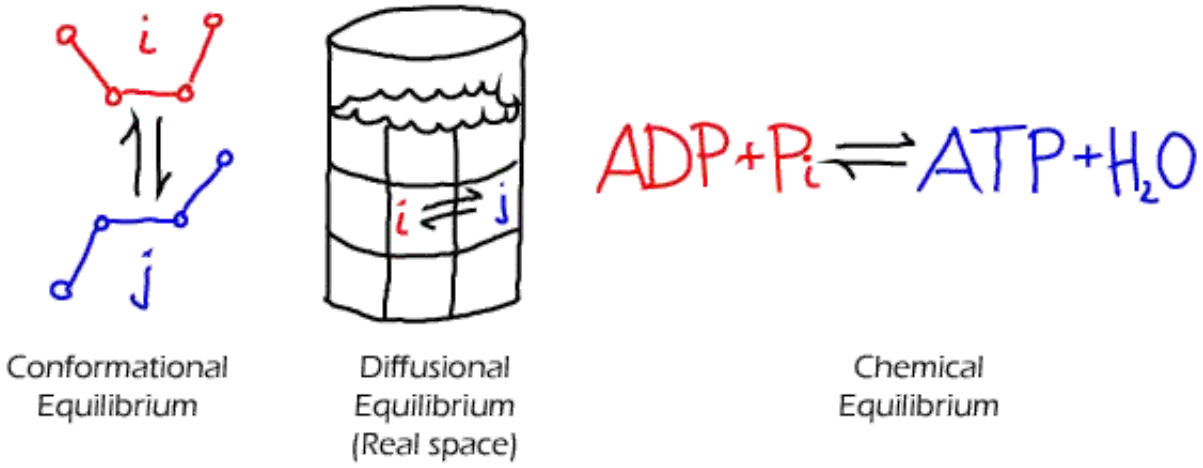
Notation and Technical Terminology

Symbol or Term	Definition
Φ	The electrostatic potential, which generally will vary in space and different cellular compartments. See Ion Gradients.
$[\mathrm{X}]$	The concentration of species (or state) X, generally equal to N_X/V. See States & Kinetics, Equilibrium.
$\langle A \rangle$	Thermodynamic average of the quantity A (under specified conditions). See Free Energy & Work.
F	Helmholtz free energy, $\langle E \rangle - TS$. See Free Energy & Work.
k_B	Boltzmann's constant. $k_B T \sim RT$ is the natural scale of thermal energy at temperature T. See The Ideal Gas.
k_{ij}	Rate constant for transitions from i to j. Probability per unit time to transition from i to j. See Mass-Action Kinetics, States & Kinetics, Catalysis, Equilibrium.
k_{off}	Rate constant for unbinding - i.e., for bi-molecular dissociation. Probability per s for a bi-molecular complex to dissociate. See Mass-Action Kinetics, Activated Carriers.
k_{on}	Rate constant for binding - i.e., for bi-molecular association. Probability per s for a single molecule to bind one molecule of another species, with the latter at the reference 1M concentration. See Mass-Action Kinetics, Activated Carriers.
k_X	Rate constant for a process "X", which might be a chemical reaction, isomerization, or (un)binding process - and should be determined by the context. See Mass-Action Kinetics, States & Kinetics, Catalysis, Synthesis.
N_i	Number of systems or molecules in state i. See States & Kinetics, Equilibrium.
N_X	Number of copies of species (e.g., molecule) X. See States & Kinetics, Equilibrium.
p_i	Probability of state i, generally equal to N_i/N. See States & Kinetics, Equilibrium.

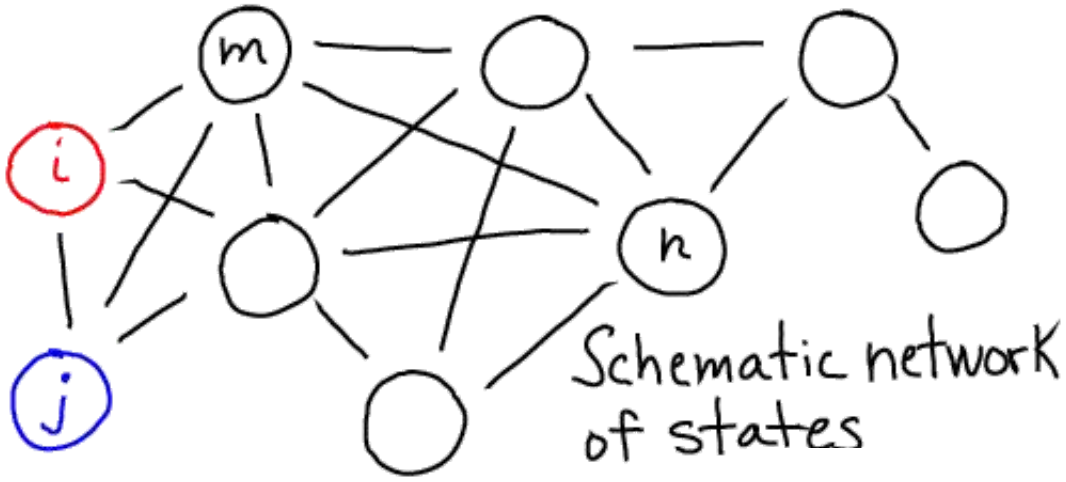
R	The gas constant. $k_B\,T = RT$ is the natural scale of thermal energy at temperature T. See The Ideal Gas.
$\mathbf{r}^N$	The configuration of a system of N atoms or molecules, it is short-hand for the full set of Cartesian coordinates: $\mathbf{r}^N = \{x_1, y_1, z_1,\ x_2, y_2, z_2,\ \ldots,\ x_N, y_N, z_N\}$. See The Ideal Gas, Free Energy Storage in a Concentration Gradient.
T	Absolute temperature, in degrees Kelvin. See Free Energy & Work, The Ideal Gas.
V	Volume of system. See The Ideal Gas, Free Energy Storage in a Concentration Gradient.

Basics: State & Kinetics

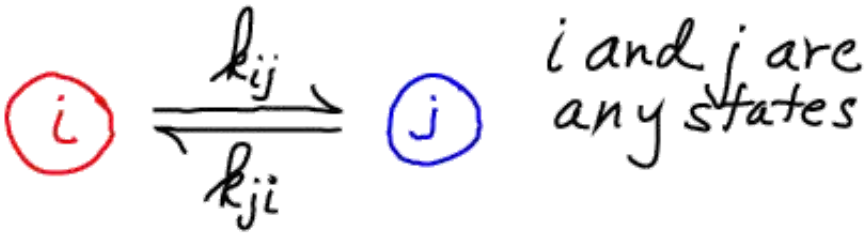
Biological systems can be described by the states which can be occupied, and the way in which the syste switches from one state to another.



- **States** may be defined in many different ways. For example, a conformational state of a protein typically refers to a collection of similar conformations. The state of larger scale-system (e.g., an entire cell) could be described by the number of copies of each type of molecule. States of a diffusing system could refer to different physical locations.
- **Transitions** may occur directly between some states, as suggested by the lines in the figure below. These transitions dictate the temporal behavior of the system.
- Transitions are generally assumed to occur with a certain **rate constant**, or probability per unit time, based solely on the present state and independent of prior history (i.e., in a Markovian way).
  See Mass Action Kinetics.



Transition rates (rate constants)

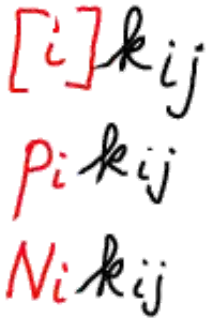


- The transition rate k_{ij} is the (conditional) probability per unit time of making a transition from state i to state j. This is the probability given the condition that the system is already in state i. Sometimes k_{ij} is called the rate constant to distinguish it from the overall rate or flux, below. See the discussion of different types of mass action rates.
- Every physical process is reversible, so the reverse rate (j to i) must exceed zero if the forward rate (i to j) does. Thus, you should always expect to see double arrows as in the diagram above. In some cases, one direction is so slow that its effect is negligible, only a single arrow is shown - but the reverse process is still possible.

Fluxes, or Overall Rates

The flux (sometimes called overall rate or, confusingly, just the rate) is defined to be the total number of events per unit time, though in some cases this will be normalized to represent the total probability making a certain transition per unit time.

- The flux from i to j is calculated, as the number of systems in state i (N_i) multiplied by the rate constant. Several notations, shown below, can be used.
- The rate constant, as the name suggests, is assumed constant, but the state population can vary with time. See the mass action page for the conditions on the assumption of rate constancy.



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- R. Phillips et al., *Physical Biology of the Cell,* (Garland Science, 2009).
- D.M. Zuckerman, *Statistical Physics of Biomolecules: An Introduction,* (CRC Press, 2010).

Advanced

- Chodera, J. D.; Singhal, N.; Pande, V. S.; Dill, K. A. & Swope, W. C. "Automatic discovery of metastable

states for the construction of Markov models of macromolecular conformational dynamics," J. Chemical Physics, 2007, 126, 155101-17

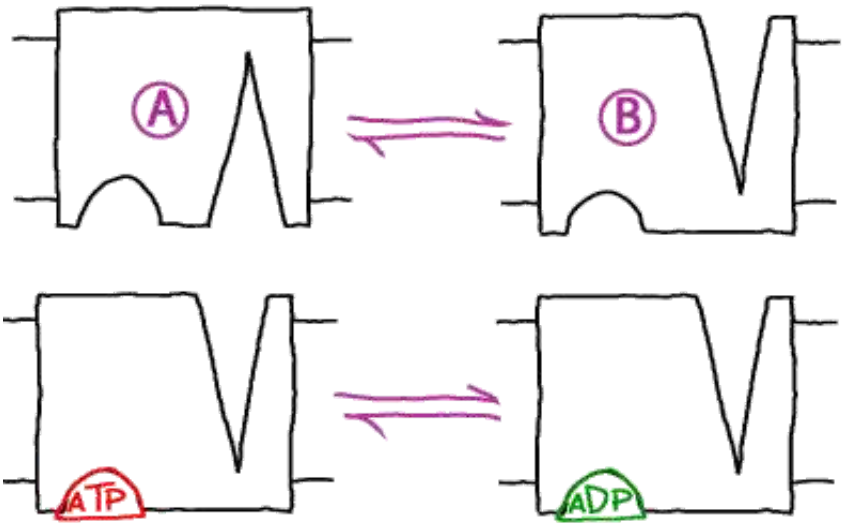
- Zhang, X.; Bhatt, D. & Zuckerman, D. M. "Automated sampling assessment for molecular simulations using the effective sample size," J. Chemical Theory Comp., 2010, 6, 3048-3057. Despite its title, this paper has a detailed discussion on the meaning of states.

Basics: Mass-Action Kinetics

Mass-Action Kinetics

The "mass action" picture is a specific, simple version of how kinetic processes (binding, catalysis, conformational transitions, ...) occur in chemical systems. In essence, the mass-action picture describes an ideal-gas situation: molecules interact strictly according to their concentrations without regard to the size or shape of the species. There are no interactions except those explicitly described by the mass-action rate laws.

First-order Kinetics: Catalysis, Conformational transitions, *Un*binding



In first-order kinetic processes, transitions occur in proportion to the quantity of (reactant) molecules or states present. Thus a rate constant k_{ij} describes the probability per unit time for molecule (or state) i to convert to j. This implies:

- $N_i k_{ij}$ = the number of molecules (or states) i transitioning to j per unit time, with N_i the number of molecules/states i at the time of interest.
- $[\mathrm{i}]\, k_{ij}$ = the number of molecules (or states) i transitioning to j *per unit volume* per unit time, where $[\mathrm{i}]$ is the concetration of i. This is the same as above except in concentration units.
- IMPORTANT: Although enzyme-catalyzed reactions will exhibit first-order kinetics, this applies *only* to the chemical reaction of the bound reactants/products - *not* to the binding process. Binding processes typically are second order: see below.

Rate constants, such as k_{ij}, are assumed not to change with time, although populations (e.g., N_i or $[\mathrm{i}]$) tend to change in time except under steady-state conditions. This holds for mass-action kinetics of all orders.

First-order examples

- **Conformational transition.** A system undergoing first-order mass-action kinetics between two states, perhaps a conformational transition

$$A \rightleftharpoons B$$

has two overall rates or fluxes

$$\text{rate of A to B transitions, per unit volume} = [\text{A}]\, k_{AB}$$
$$\text{rate of B to A transitions, per unit volume} = [\text{B}]\, k_{BA}$$

These can be combined to characterize the overall time-dependent behavior by differential equations:

$$\frac{d[\text{A}]}{dt} = -[\text{A}]\, k_{AB} + [\text{B}]\, k_{BA} \quad (1)$$

$$\frac{d[\text{B}]}{dt} = -[\text{B}]\, k_{BA} + [\text{A}]\, k_{AB} \quad (2)$$

The first equation, for example, says that the concentration of A changes by transitions out to B (in proportion to the concentration of A) as well as by transitions from B to A (in proportion to the concentration of B). These mass-action kinetic equations determine how the concentrations of A and B change in time. A and B could be conformational states or chemical states if it's a catalytic process.

- **Enzyme catalysis.** The catalysis $ES \rightleftharpoons EP$ of an enzyme/substrate complex, ES, into the enzyme-product complex, EP, at a rate k_{cat} is quantified by

$$\text{rate of EP production, per unit volume} = [\text{ES}]\, k_{\text{cat}}, \quad (3)$$

which indicates the concentration of EP is increasing in proportion to the concentration of ES. As a concrete example, S could be ATP and P could be ADP (ignoring phosphate).

- **Unbinding.** Although binding is a second-order process because the two binding partners must "find" each other (see below), *un*binding is a first-order processes. Continuing the example above, we can quantify the rate of substrate unbinding from an enzyme via

$$\text{rate of E, P unbinding, per unit volume} = [\text{EP}]\, k_{\text{off}}, \quad (4)$$

which indicates that the rate of concentration change due to unbinding is simply proportional to the number of EP complexes. In other words, a given complex has fixed probability per unit time (namely, k_{off}) to unbind.

Second-order (Binding) Kinetics

Second-order kinetics is the highest order that we really need to understand (see below), but it's a bit trickier than first-order. Second-order, or binding kinetics depends on the concentrations of two species. There is a greater chance for collision/complexation as the concentrations of the two binding partners increase. In the mass-action picture, the probability of binding is simply proportional to the product of the two concentrations, so that if A and B can bind to form AB according to the scheme

$$A + B \rightleftharpoons AB \quad (5)$$

then

- $[\text{A}][\text{B}]\, k_{\text{on}}$ = the number of binding events per unit time per unit volume
- The second-order rate k_{on} is a probabilty per unit time *per molar concentration* - in contrast to first-order rates which are simple probabilities per unit time.
- In essence, a second-order rate is the probability per molar concentration of one species (say, A) to bind the other species (B) held at some fixed reference concentration (typically, 1M). If B is at a different concentration, the product $[\text{A}][\text{B}]k_{\text{on}}$ corrects for values above or below the reference.

Second-order/binding example

Given the binding process (5) if we include both binding and unbinding (a first-order process), we have

$$\frac{d[\mathrm{AB}]}{dt} = [\mathrm{A}][\mathrm{B}]\, k_{\mathrm{on}} - [\mathrm{AB}]\, k_{\mathrm{off}} \tag{6}$$

Note that we could equally have used the $E + S \rightleftharpoons ES$ process. Including both binding and catalysis (from above: first-order) for ES, we can write

$$\frac{d[\mathrm{ES}]}{dt} = [\mathrm{E}][\mathrm{S}]\, k_{\mathrm{on}} - [\mathrm{ES}]\, k_{\mathrm{cat}} \tag{7}$$

where we have assumed catalysis is essentially irreversible, so that EP never converts to ES. (In truth, irreversibility is only an approximation for a very slow reverse process, as discussed in the context of cycles.)

Higher-order Kinetics

Although higher-order kinetics (e.g., the simultaneous binding of three molecules) can be described mathematically, such kinetics largely represent an unnecessary approximation. Of course, numerous biological complexes consist of many more than three molecules, but it is not reasonable to expect that the binding needs to occur *simultaneously* - which would be extremely improbable. We expect, rather, that sequential pairwise binding events (described by second-order kinetics) will be a more physically realistic description for the buildup of multi-component complexes.

"Zero-order" Kinetics

Zero-order kinetics apparently represent something coming from nothing (a constant rate of creation or destruction of a species, *independent of concentration*). Really, however, zero-order kinetics are meant to stand in for processes that are not explicitly modeled. For example, if you were studying a molecular machine powered by ATP hydrolysis, to keep ATP and ADP at steady values (as the cell does viaevascript:changeTo('massAction','atpCycle')''>complex machinery), a steady input and output of these molecules could be modeled by differential equations including zero-order temrms:

$$\frac{d[\mathrm{ATP}]}{dt} = k_{\mathrm{in}} + \cdots \tag{8}$$

$$\frac{d[\mathrm{ADP}]}{dt} = k_{\mathrm{out}} + \cdots \tag{9}$$

Note the absence of a molecular concentration multiplying the "in" and "out" rates on the right-hand-sides of the equations.

Limitations of the mass-action assumption

The mass action picture assumes ideal gas behavior: no concentration-dependence to interactions (except binding), and no volumetric effects. Of course, both these assumptions are wrong. For example, at higher concentrations, ions will interact differently with one another than at lower concentrations, due to the strong distance-dependence of Coulombic forces. Also, *any* molecules, at high enough concentrations, will not bind by simple proportionality rules: as sketched in the figure above, once the environment becomes sufficiently crowded, the number of neighbors of a given molecule will reach a maximum, independent of concentration.

An explicit example of the mass-action picture breaking down is given at the end of the discussion of ionic gradients.

References:

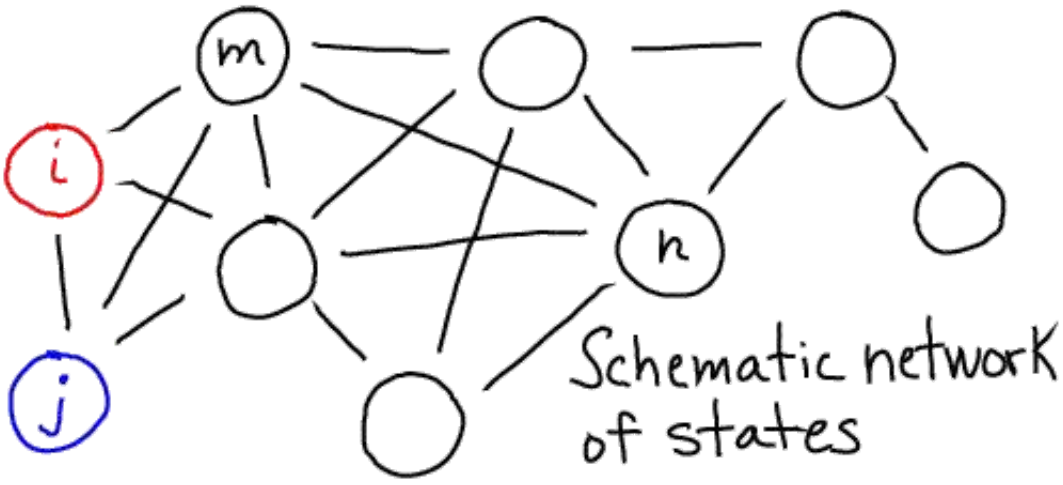
- J. Kuriyan, B. Konforti, and D. Wemmer, *The Molecules of Life: Physical and Chemical Principles* (Garland Science, 2013).
- D.M. Zuckerman, *Statistical Physics of Biomolecules: An Introduction* (CRC Press, 2010).

Equilibrium Means Detailed Balance

Equilibrium and Detailed Balance

Equlibrium has a very precise meaning in statistical physics, which also applies to biology. Equilibrium describes the *average* behavior (averaged over many systems under identical conditions) in which there is no net flow of material, probability or reactions.

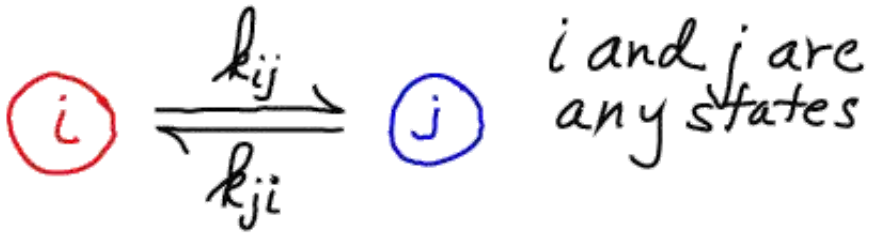
- Equilibrium is *not static* because each individual system undergoes its ordinary behavior/dynamics.
- There will be no net flow in any "space" examined: real-space, conformation space, or population space.
- Equilibrium can only occur under fixed conditions (e.g., constant temperature and total mass) when there is no addition or removal of energy or material from the system.
- The requirement for "no net flow of material, probability or reactions" is embodied in the condition for **detailed balance**.



Detailed Balance

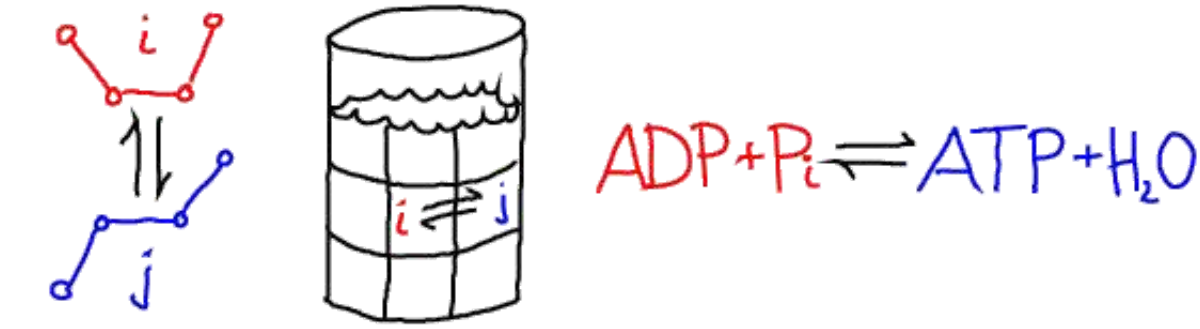
Detailed balance is the balance of flows between any (and every) pair of states you care to define. Typically, one thinks of "detailed" infinitesimal states, but a balance of flows among tiny states implies a balance of flows among global states which are groups of tiny states.

"Flow" refers to the motion of material or trajectories/probability, depending on the situation at hand.



In this schematic, i and j are any states, and the k's are the rates between them. If we have $N = \sum_i N_i$ equilibrium systems with N_i systems in each state i, then detailed balance is formulated as

$$N_i \, k_{ij} = N_j \, k_{ji} \tag{1}$$



Conformational Equilibrium | Diffusional Equilibrium (Real space) | Chemical Equilibrium

- **In a solution.** As always in equilibrium, we have (at least conceptually) a large number of copies of our system. If we consider any two sub-volumes (i and j) in just *one* of these systems, some set of molecules will move from region to the other in a given time interval. However, in our equilibrium set of systems, there will be other systems in which the opposite flow occurs. *Averaging over all systems*, there is no net flow of any type of molecule between any two sub-volumes in equilibrium. This is detailed balance. (See also the time-averaging perspective, below.)
- **In a chemical reaction.** Normally, we distinguish "products" and "reactants", but equilibrium largely abolishes this distinction. As described above for the solution case, if we have an equilibrium set of many chemical-reaction systems, an equal number will be proceeding in the forward (say, i to j) and reverse (j to i) directions. Although, nominally, a reaction may seem to prefer a certain direction, in equilibrium that just means that the products of the favored direction will be present in greater quantity (e.g., $N_j \gg N_i$) - even though the forward and reverse flows stay the same as in (1) because the rates would be very different ($k_{ji} \ll k_{ij}$).
- **In a conformation space of a single molecule.** In an equilibrium set of molecules with, say, two conformational states A and B, there will be an equal number of A-to-B as B-to-A transitions in any given time interval. If there are many states, then there will be a balance between all pairs of states i and j as given in (1).

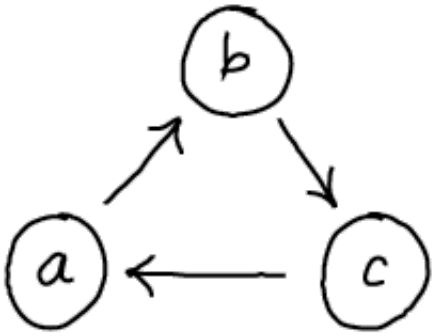
Time vs. Ensemble Averaging

It is useful to consider the relation between "ensemble averaging" (e.g., averaging over the set of equilibrium systems described above) and "time averaging". Time-averaging is just what you would guess: averaging behavior (e.g., values of a quantity of interest) over a long period of time.

In equilibrium, *time averaging and ensemble averaging will yield the same result.* To see this, consider a solution containing many molecules diffusing around and perhaps exhibiting conformational motions as well. Assume the system has been equilibrating for a time much longer than any of its intrinsic timescales (inverse rates). Because finite-temperature motion in a finte system is inherently stochastic, over a long time each molecule will visit different regions of the container and also different conformations - in the same proportion as every other molecule. If we take a snapshot at any given time of this equilibrium system, the "ensemble" of molecules in the system will exhibit the same distribution of positions and conformations as a long single trajectory of any individual molecule. This has to be true because the snapshot itself results from the numerous stochastic trajectories of the molecules that have evolved over a long time.

Unphysical Models Cannot Equilibrate

Standard Michaelis-Menten process



Schematic cycle of motor protein conformations

Although every physical system that is suitably isolated will reach a state of equilibrium, that does not mean that every *model* made by scientists can properly equilibrate. In fact, many common models of biochemistry exhbit "irreversible" steps - in which the reverse of some step *never* occurs - and could never satisfy detailed balance. The Michaelis-Menten model of catalysis (above left) is an irrervsible model. Such model irreversibility typically represents the observation that the forward rate exceeds the reverse rate so greatly that the reverse process can safely be ignored. This may be true in some cases, but here are numerous cases in biochemistry where reversibility is critical, such as typical binding processes (unbinding is needed to terminate a signal) and in ATP synthase (which can make ATP or pump protons depending on conditions).

For corrected (physically possible) versions of the cycles depicted above, see the discussion of cycles.

References:

- R. Phillips et al., *Physical Biology of the Cell,* (Garland Science, 2009).

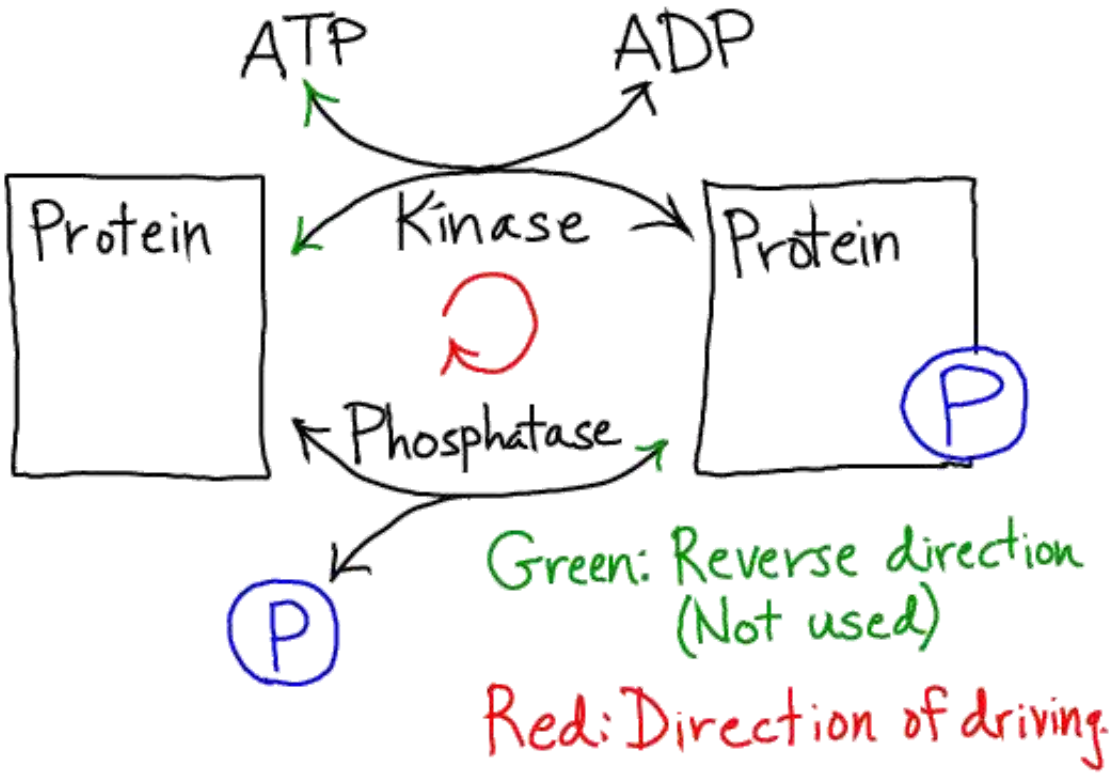
- D.M. Zuckerman, *Statistical Physics of Biomolecules: An Introduction,* (CRC Press, 2010).

Cycles and Constraints

Cycles: The Cell's Working Units

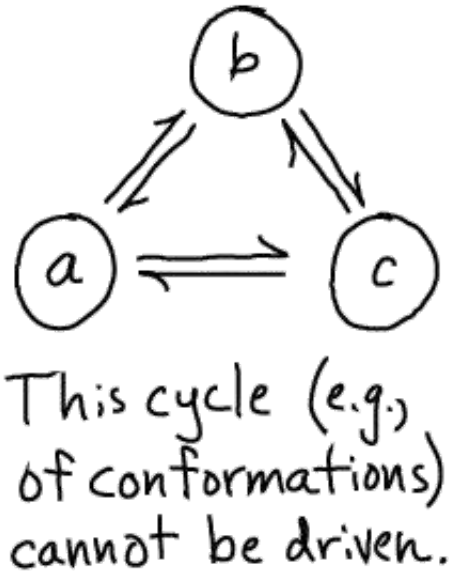
A **cycle** is a series of states, connected by transitions, in which the first and last state are the same.

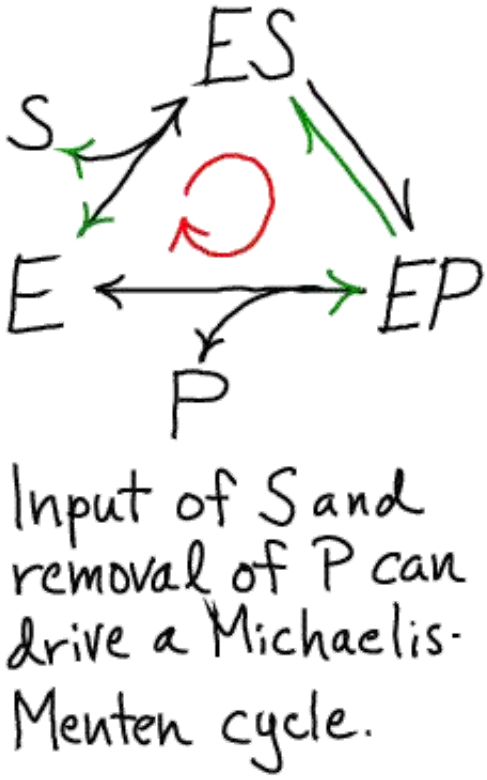
- Because the cell re-uses its materials (proteins, poly-nucleic acids, and molecular building blocks), cycles arise naturally. Accordingly, *ycles describe almost all catalytic activity, signalling and molecular machines.*
- Although all molecular processes are reversible, the cell functions by driving processes in one direction in order to ensure "processivity" (moving with high probability in a single direction).
- The phosphorylation cycle below, driven by the relatively high ratio of [ATP] to [ADP] (compared to equilibrium) is typical.



Binding processes provide "handles" for driving cycles

As sketched below, and discussed in detail in Cycle Logic, a cycle without binding cannot be forced to move consistently in a desired direction.

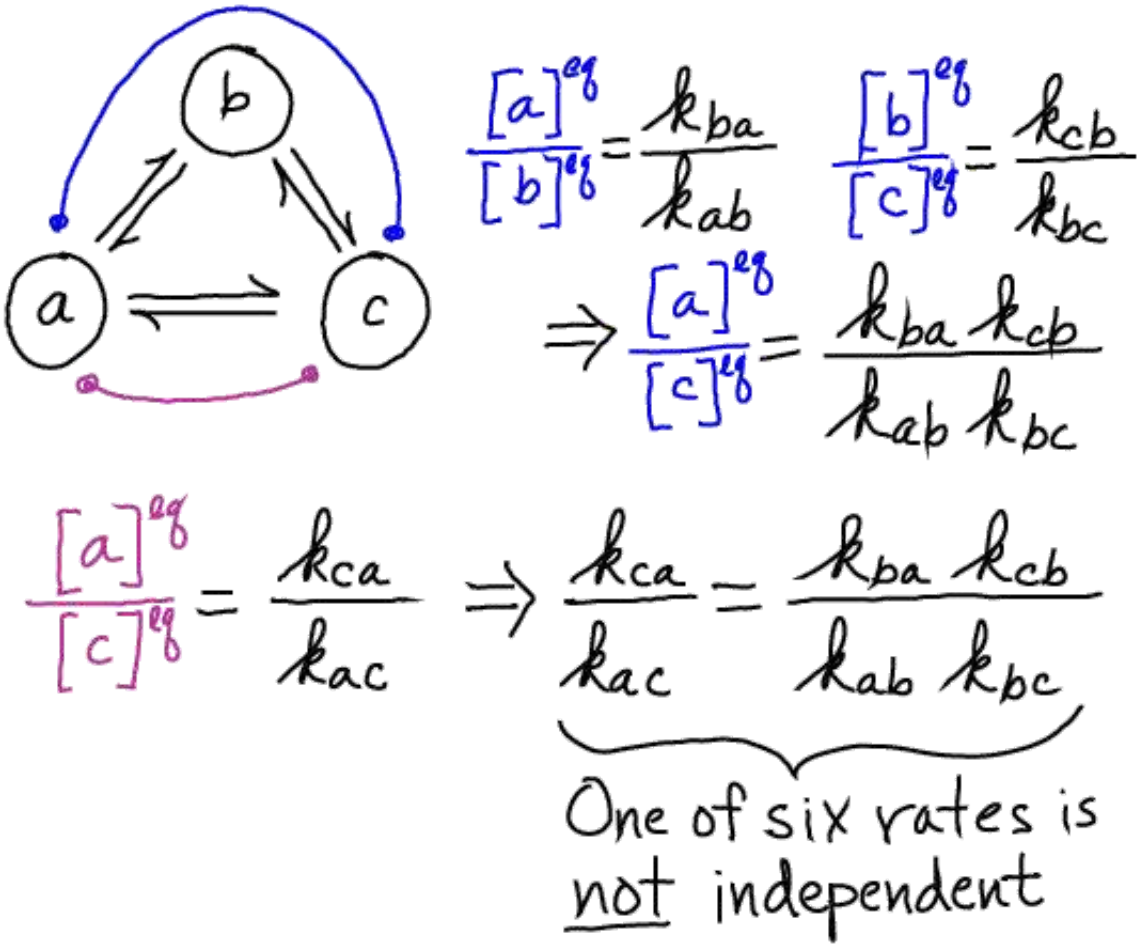




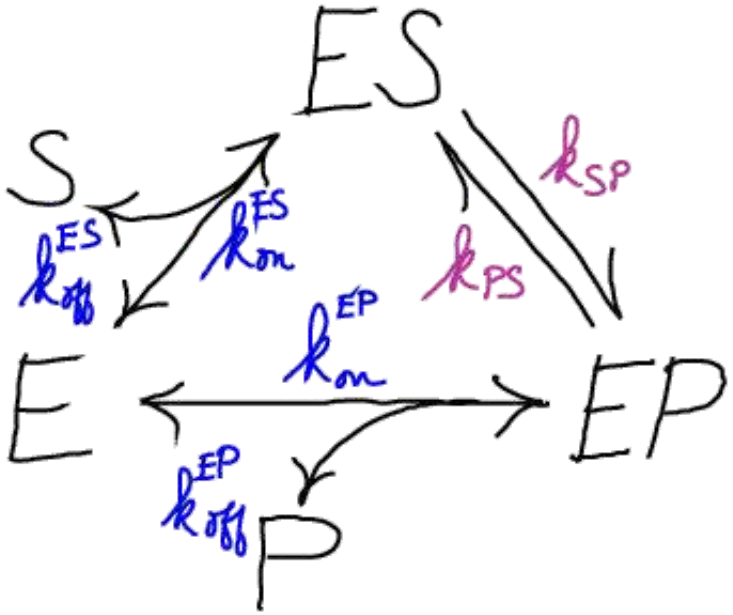
The Michaelis-Menten cycle on the right typically is driven clockwise by an excess of substrate (compared to equilibrium) but it equally well could be driven in the other direction with an excess of product.

Cycles impose constaints on rates

Because correct models must be consistent with the principle of detailed balance, not all rate constants in a model are independent. This fact can prevent (some) mistakes in model construction.



This same principle can be used to correct - fully specify - the Michaelis-Menten model.



The needed constraint on the rates can be obtained by solving for the equilibrium ratio [ES]/[EP] in two ways:

$$[ES]^{eq} k_{SP} = [EP]^{eq} k_{PS} \Rightarrow \frac{[ES]^{eq}}{[EP]^{eq}} = \frac{k_{PS}}{k_{SP}}$$

$$\left.\begin{aligned} [E]^{eq}[S]^{eq} k_{on}^{ES} &= [ES]^{eq} k_{off}^{ES} \\ [E]^{eq}[P]^{eq} k_{on}^{EP} &= [EP]^{eq} k_{off}^{EP} \end{aligned}\right\} \Rightarrow$$

$$\frac{[ES]^{eq}}{[EP]^{eq}} = \frac{[S]^{eq}}{[P]^{eq}} \frac{k_{on}^{ES} k_{off}^{EP}}{k_{on}^{EP} k_{off}^{ES}}$$

Equating the two results for $[ES]^{eq}/[EP]^{eq}$ yields

$$\frac{[S]^{eq}}{[P]^{eq}} \frac{k_{on}^{ES} k_{off}^{EP}}{k_{on}^{EP} k_{off}^{ES}} = \frac{k_{PS}}{k_{SP}}$$

Noting that $[S]^{eq}/[P]^{eq}$ is a constant determined by the chemistry (relative stability) of the two compounds *independent of the catalytic process or model rates,* indicates that the preceding result is indeed a constraint on the rate constants of the model. Only five of the six rates are independent.

More on cycles

To learn more about cycles in a qualitative way, visit the Cycle Logic page.

References:

- T.L. Hill, "Free Energy Transduction and Biochemical Cycle Kinetics," (Dover, 2005). Absolutely **the** book on cycles. Describes the effects of including additional states and transitions. Beyond Chapter 1 is difficult reading.
- D.A. Beard and H. Qian, "Chemical Biophysics: Quantitative analysis of cellular systems," (Cambridge, 2008).

Cycle Logic

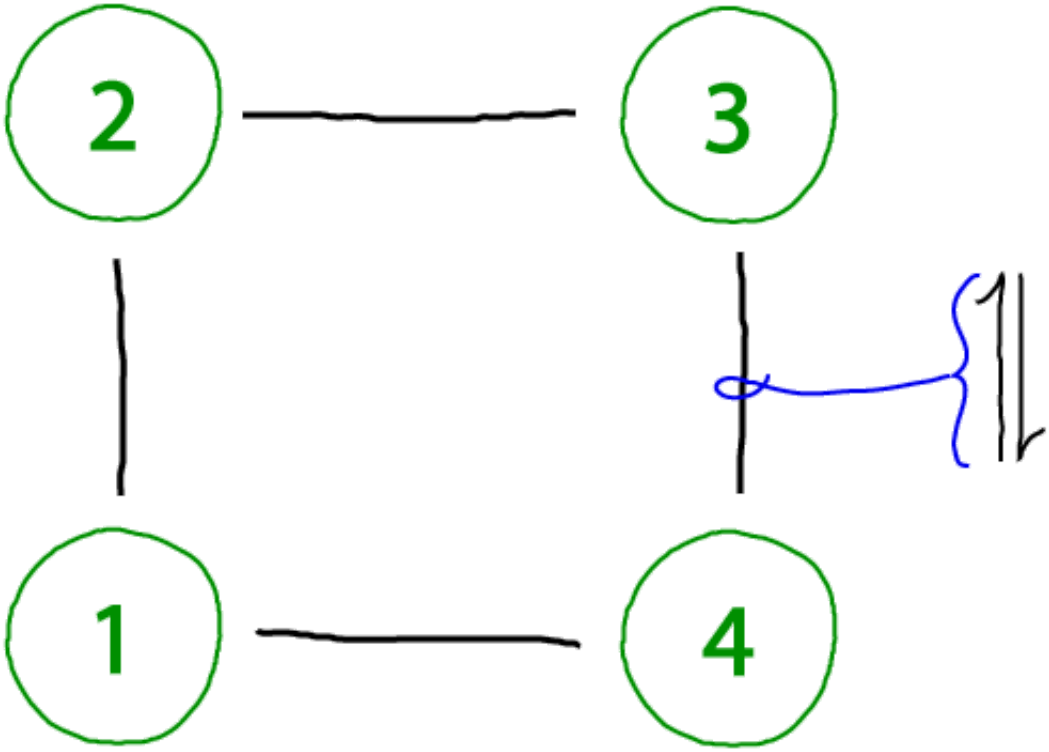
Cycle Logic: How they work

Cycles are essential to biochemistry and cell biology for the simple reason that the cell re-uses molecular components like enzymes.

Drawing a cycle connecting a series of states is straightforward, but what are the key ingredients that make cycles interesting and useful? What features should we look for? We'll look through a set of cycles to identify key functional features - as well as warning signs for problematic cycles.

Read all the way through from the beginning to pick up the essential concepts.

Simple conformational cycle

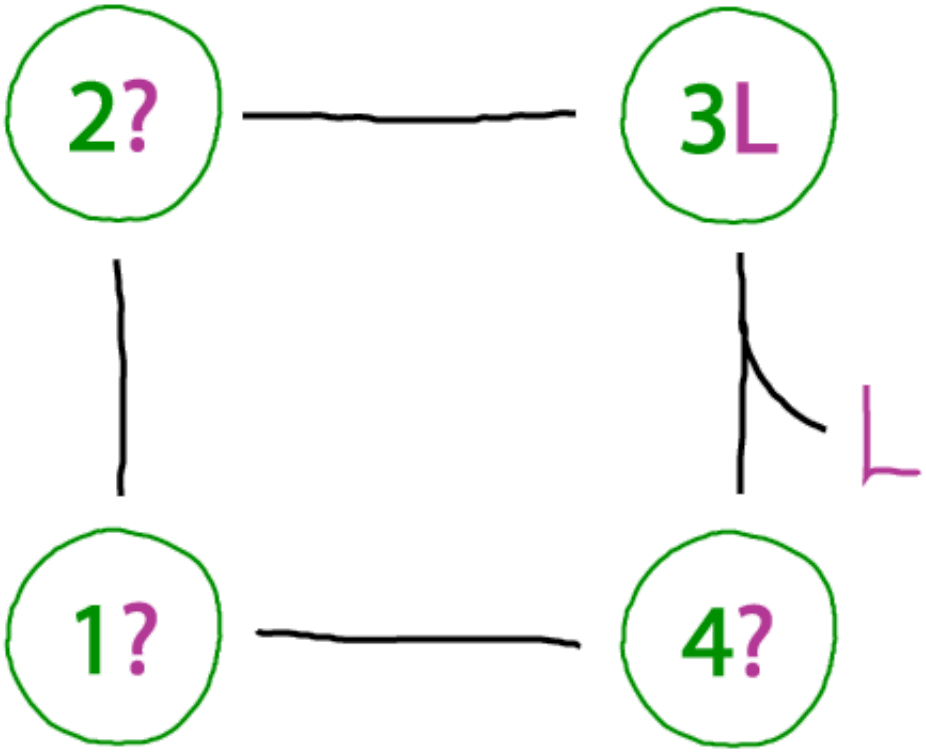


In this cycle, each numbered circle represents the conformational state of some protein. It seems boring but we can learn important lessons already.

- First, because we know that all microscopic processes must be reversible (see Equilibrium discussion), all states are *implicitly* connected by bi-directional arrows. Whether we draw a line, a single arrow (misleadingly) or two arrows, all the processes are reversible even if some are much slower than others. *We will not be concerned with the specific values of rates in this discussion.*
- Second, although perhaps not obvious at first, *there is no way to drive this cycle*. Although any non-

equilibrium condition (e.g., all systems in state 1) will lead to a net flow toward equilibrium, there is no way to cause that flow to occur in a desired direction (e.g., clockwise) because neither binding nor catalysis are part of this process. This makes a simple conformational cycle - by itself - fairly useless to the cell, because the cell's cycles have evolved to function in a specific direction due to external driving.

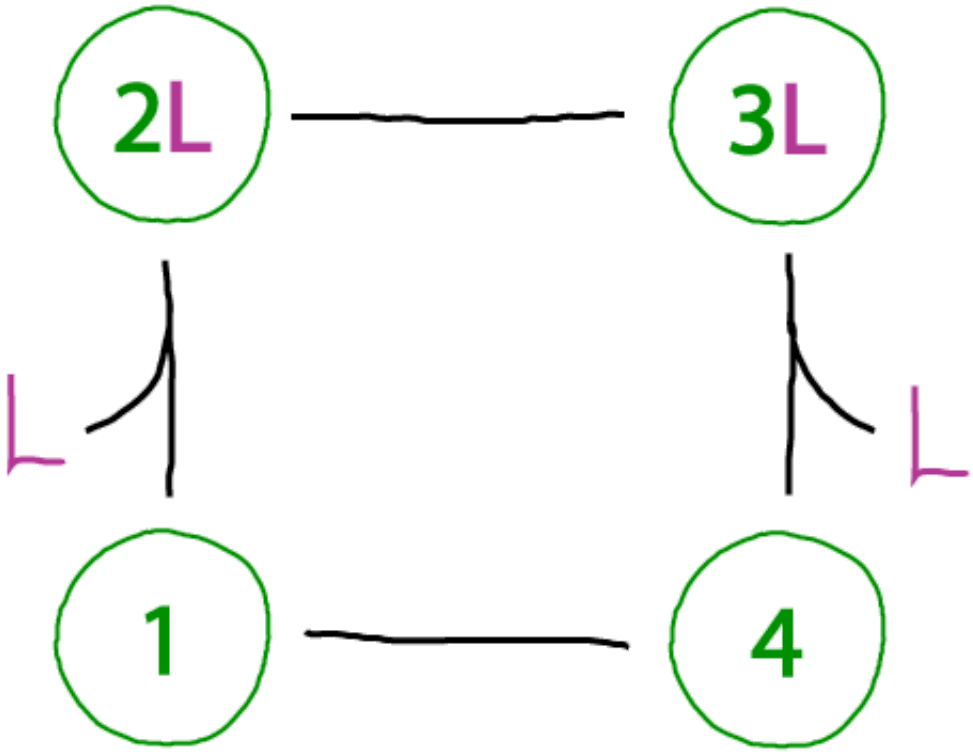
A binding cycle that doesn't make sense



We want to be able to recognize when something essential is omitted from a cycle. In this case, the binding process occuring from state 4 to 3 - even though it's reversible - must be balanced by another unbinding process. To see this, imagine proceding counter-clockwise around the cycle from state "3L", which is state 3 of the protein with ligand L bound. One then gets, in sequence, states 2L, 1L, and 4L. But, unless our protein can bind an arbitrary number of ligands, it doesn't makes sense that state 4L binds another ligand: if it did, we would get 4L2, then 4L3 the next time around, and so on.

Below, a possible corrected cycle with binding is shown.

A working binding cycle - which can't be driven

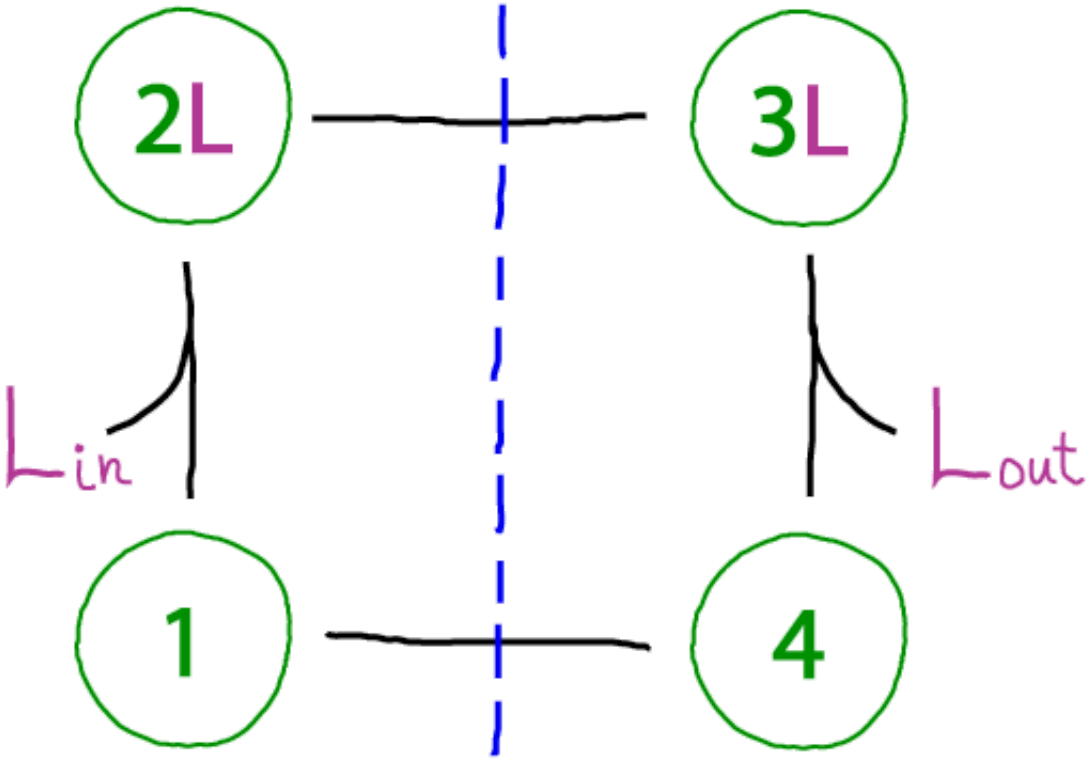


This cycle lacks the problem we saw above. We can go around (and around) the cylce any way we want, and we'll end up in the same state. The states are well defined, and that's how a cycle should be.

However, if the all the ligand is in the same compartment - i.e., all the L molecules are in one pool and can bind to either state 1 (yielding 2L) or state 4 (yielding 3L) - then this cycle cannot be driven in a particular direction. There is no way to favor binding of one state over the other based on the cycle shown. (It's possible some external process could favor state 1 over 4, but no such process in indicated in the cycle.)

In sum, this cycle exhibits no physical inconsistency but it cannot driven.

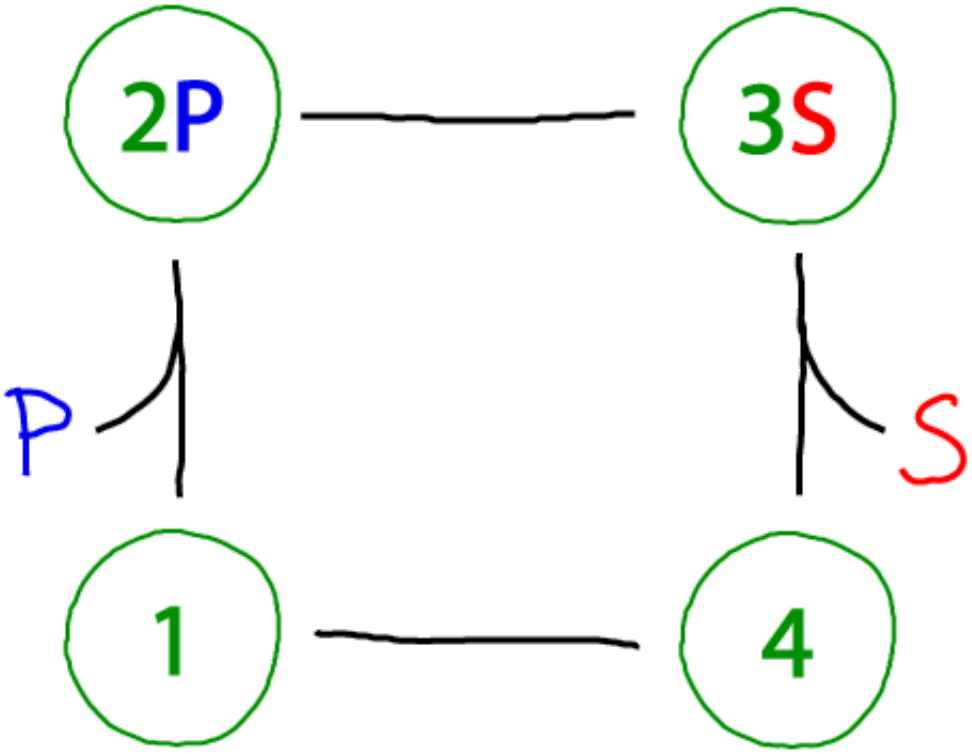
A functional binding cycle connecting two compartments



This cycle portrays a protein connecting two compartments (e.g., inside and outside an organelle), so that ligands must be classified as "in" or "out". The blue dashed line is meant to suggest that states on a given side of the line only allow binding with a single compartment, such as occurs in *alternating access* mechanisms utilized by transporter proteins. (Note that the numbered states do *not* indicate physical location - rather, the protein will be located at a membrane separating the compartments and change conformations according to the cycle.)

The cycle is functional in the sense that it can be driven - made to move in a specified direction, whether clockwise or counter-clockwise. Once a cycle can be driven, it can be used to perform work - as transporters do. Functional machines like transporters require more elaborate state spaces and cycles, but the basic ideas are illustrated here.

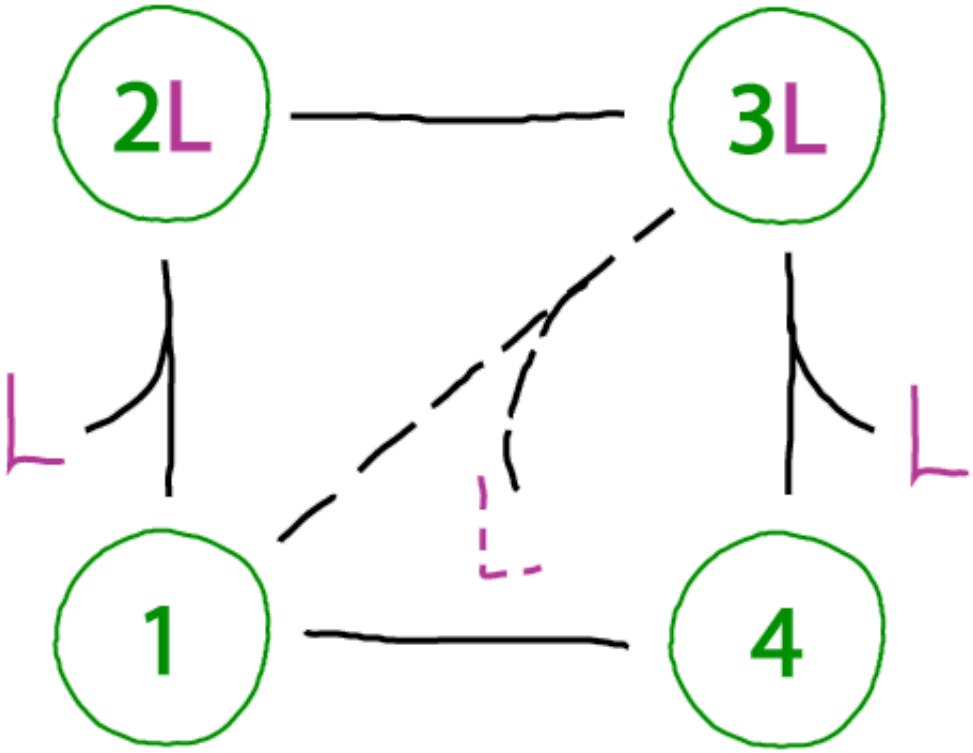
A catalytic cycle (Michaelis-Menten-ish)



The beauty of the catalytic cycle is that it can always be driven. The cycle above converts "substrate" S to "product" P - or the reverse, of course, depending on whether there is an excess of S or P. The term 'excess' is relative to the equilibrium state in which a system would have an equal probability to move clockwise as counter-clockwise. Excess P, for example, would drive the cycle clockwise.

Compared to the standard Michaelis-Menten cycle, there are two differences here. Most obviously, our enzyme has an extra conformational state (which may be more realistic as many enzymes exhibit significantly different open and closed states - e.g., adenylate kinase). Secondly, as noted in our discussion of the first cycle above, all processes here are reversible. By contrast, the Michaelis-Menten cycle assumes the catalytic step is irreversible and only proceeds in a single direction. See further discussion of this point in the main cycles page and equilibrium page.

A realistic complication



Not all cycles will function in an ideal fashion. A key issue of concern is "slippage," where states are connected by undesirable transitions or where undesirable states are accessible. The cycle above shows a toy example of slippage connecting two states not in our original model.

In the cell, we can presume that such inefficiencies have evolved to be minimal - via low rate constants. Nevertheless, do not assume that simple models will account for every process occuring in nature!

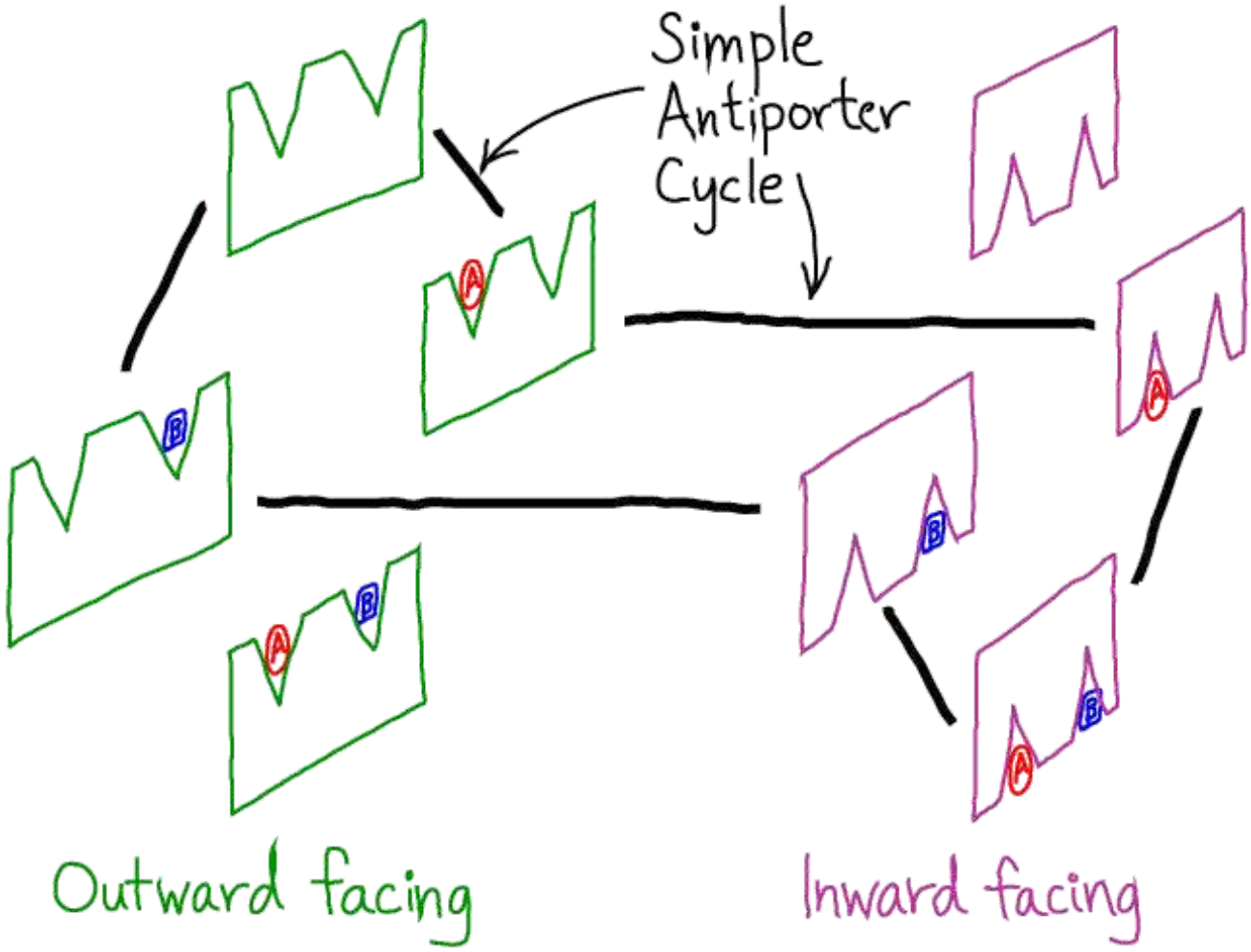
Advanced Cycle Logic

Cycles are essential to biochemistry and cell biology for the simple reason that the cell re-uses molecular components like enzymes. The basic "logic" of cycles has been described.

Here, we want to build on the example of the antiporter / exchanger to understand the "space" of states in which machine cycles tend to exist and also how those states are "wired" together.

Seeing the antiporter in an extended space

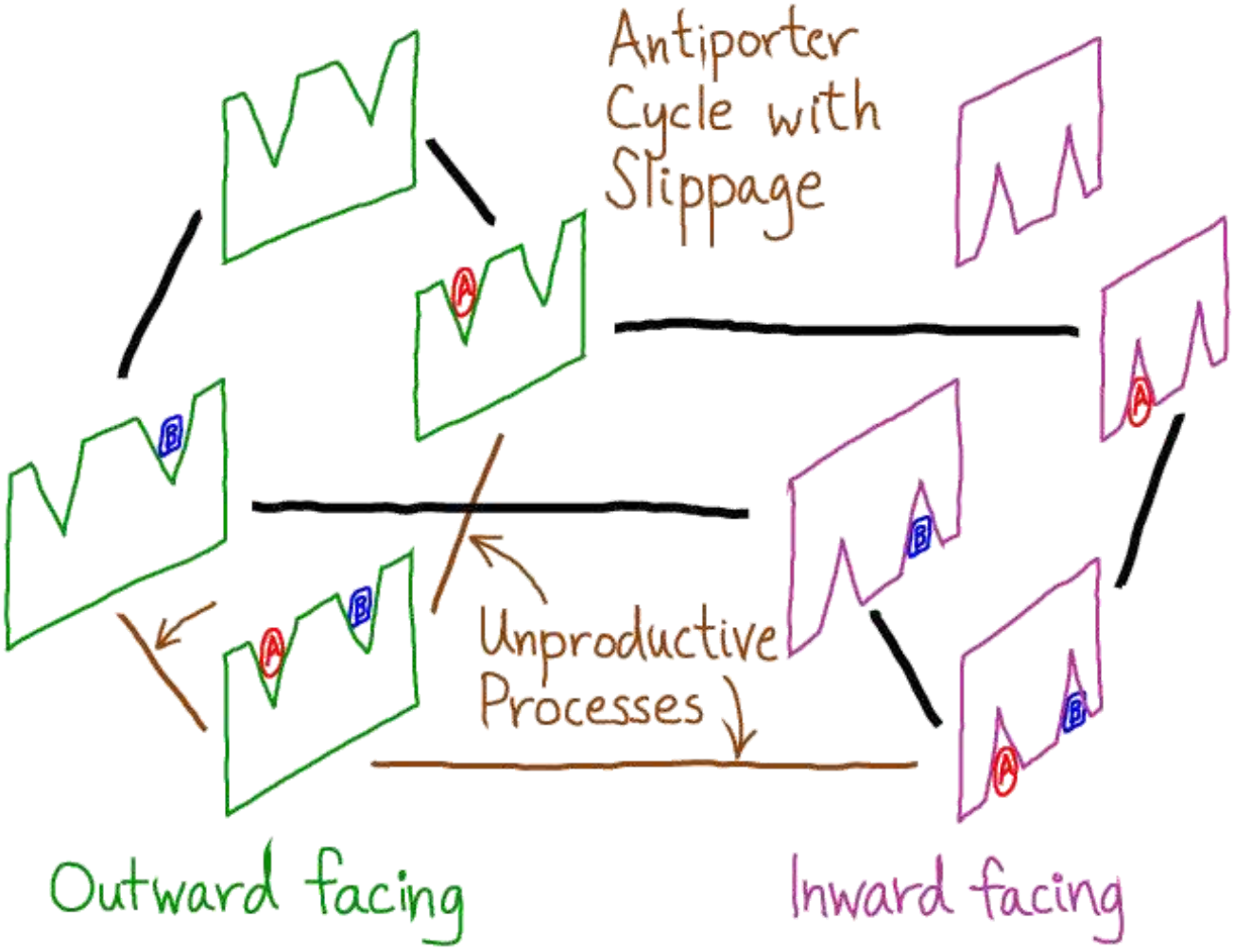
Compare the original antiporter cycle to the equivalent representation below. Here, the binding events are "implicit" - if a new molecule appears along a given line (process) then a binding event must occur.



We have categorized the states as inward and outward facing to emphasize additional states which are not visited in the original antiporter cycle. Indeed, further states might occur, which are not shown - for example, mixed states including one binding site facing inward and the other outward.

Adding "slippage" to the antiporter cycle

Our original antiporter cycle is a perfect 1:1 transporter - each time one A molecule moves in one direction (e.g., out-to-in) a B molecule moves in the opposite direction (e.g., in-to-out). But there is no reason to expect that molecular machines, which function in a thermal environment, function so precisely. Moreover, there may be functional (regulatory) reasons why slippage, or inefficiency, could be helpful.

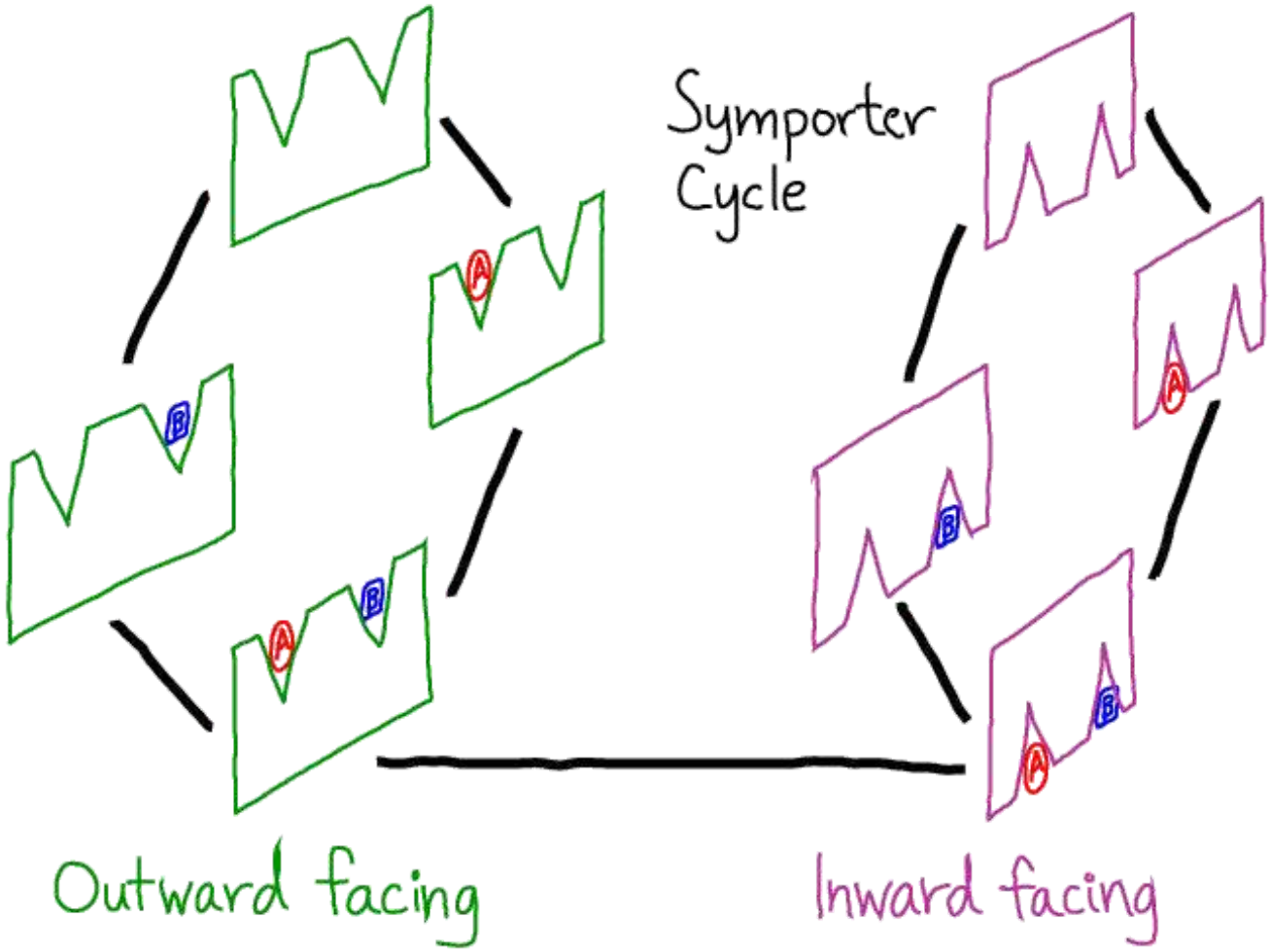


The added brown connections in this cycle do not contribute to the 1:1 exchange of molecules. You should follow the cycle around various possible pathways from an initial state (e.g., top left) and all the way back to the same state to see that the unproductive processes cause the molecule to act as a simple channel.

The relative magnitudes of the rate constants will determine the effectiveness of this molecule as an antiporter. Generally, you should expect that slippage processes can occur.

A symporter cycle in the same space

Using the same set of states shown, we can wire together a "symporter" (co-transporter) which transports one A and one B molecule together.



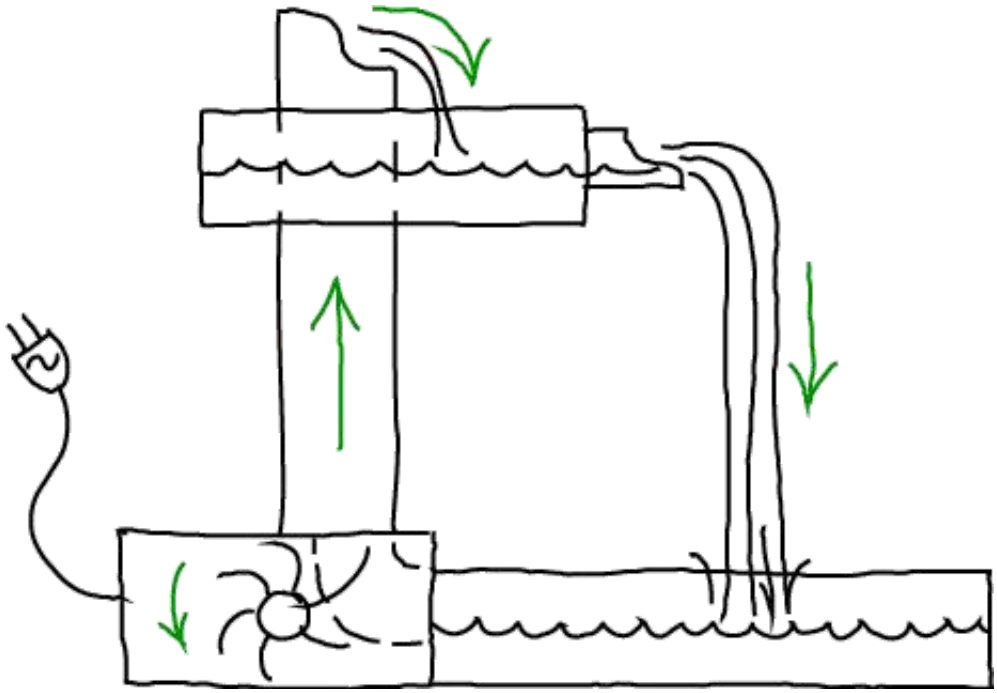
Comparing this diagram to the previous two, it is not difficult to imagine one system evolving from the others.

Exercises:

1. Sketch a symporter wiring diagram that includes a slippage process, and describe what happens during the slippage.
2. Using the principles explained for general cycles, determine the constraints that would apply for processes that include slippage.

Non-equilibrium: Steady States

The Steady State: A Key Description of Biology

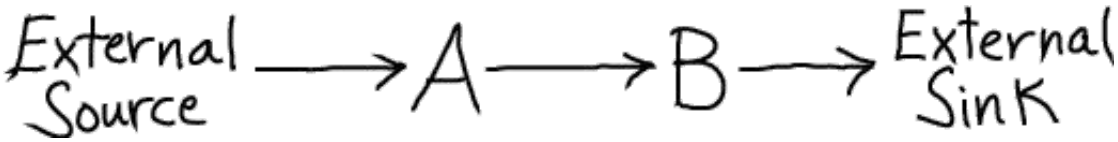


Background

- A steady state is characterized by unchanging probabilities / concentrations, but matter or probability may be flowing.
- Mathematically, all time derivatives are zero.
- Although the cell is not strictly in a steady state, many processes can be modeled reasonably as steady: think "homeostasis".
- Equilibrium is a special steady state in which no net flows of material or probability occur.
- Steady states with flows (i.e., those out of equilibrium) require input of matter or energy. They are not self-sustaining. The "desktop waterfall" shown above must be plugged in for the flow to be maintained.
- Steady states with flows typically are amenable to a simple mathematical treatment. They also are convenient modules for connecting to other parts of a larger system - the sources and sinks of flows.

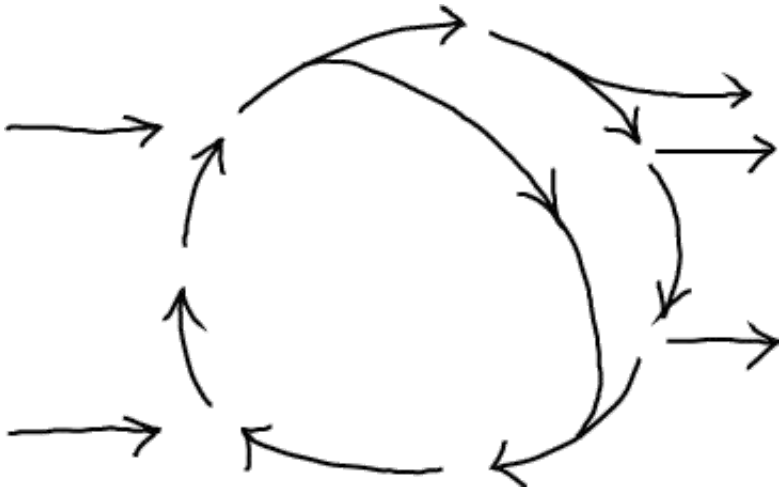
Schematically

A steady state consists of one or more inputs and one or more outputs, with each component unchanging in time.



$$\frac{d[\mathrm{A}]}{dt} = 0 \qquad \frac{d[\mathrm{B}]}{dt} = 0 \tag{1}$$

A more typical (and complex) case includes multiple inputs/outputs and an internal cycle



Key Biological Examples

- Michaelis-Menten catalytic cycle
- Citric acid cycle
- Molecular locomotion
- Active transport

Steady-state analysis of a Michaelis-Menten (MM) process

A standard MM process models conversion of a substrate (S) to a product (P), catalyzed by an enzyme (E) after formation of a bound-but-uncatalyzed complex (ES).

The simple MM model can also be viewed as a cycle because the enzyme E is re-used. Blue arrows indicate steady net flows.

(The standard MM process here can be contrasted with the corrected MM cycle that allows for reverse events and physical single-step processes.)

A steady state will occur if P is removed at the same rate as S is added. Mathematically, for steady state, we set the time derivative of the ES complex to zero.

$$\frac{d[\mathrm{ES}]}{dt} = [\mathrm{E}][\mathrm{S}]\, k_{\mathrm{on}}^{\mathrm{ES}} - [\mathrm{ES}]\, k_{\mathrm{off}}^{\mathrm{ES}} - [\mathrm{ES}]\, k_{\mathrm{cat}} = 0 \tag{2}$$

The result yields what looks like a dissociation constant in terms of the steady-state (SS) concentrations:

$$\frac{[\mathrm{E}]^{\mathrm{SS}}[\mathrm{S}]^{\mathrm{SS}}}{[\mathrm{ES}]^{\mathrm{SS}}} = \frac{k_{\mathrm{off}}^{\mathrm{ES}} + k_{\mathrm{cat}}}{k_{\mathrm{on}}^{\mathrm{ES}}} \equiv K_M \tag{3}$$

In words, in the steady state, the ratio of concentrations on the left assumes the constant value given by the particular ratio of rate constants in the middle. The effective "equilibrium" constant KM is conventionally defined but not strictly needed.

The basic steady state result (3) can be used to calculate other quantities of interest, such as the overall rate of product production

$$k_{\mathrm{cat}}[\mathrm{ES}]^{\mathrm{SS}} = [\mathrm{E}]^{\mathrm{SS}}[\mathrm{S}]^{\mathrm{SS}} \frac{k_{\mathrm{cat}}}{K_M} \tag{4}$$

now given in terms of the steady-state E and S concentrations, which should be known.

The standard MM model is unphysical

All molecular processes are reversible, so any model with a uni-directional arrow is necessarily approximate: see the discussion of cycles. The full MM cycle, allowing for reverse events and permitting only single-step processes, is subjected to a (more complicated) steady-state analysis in an advanced section.

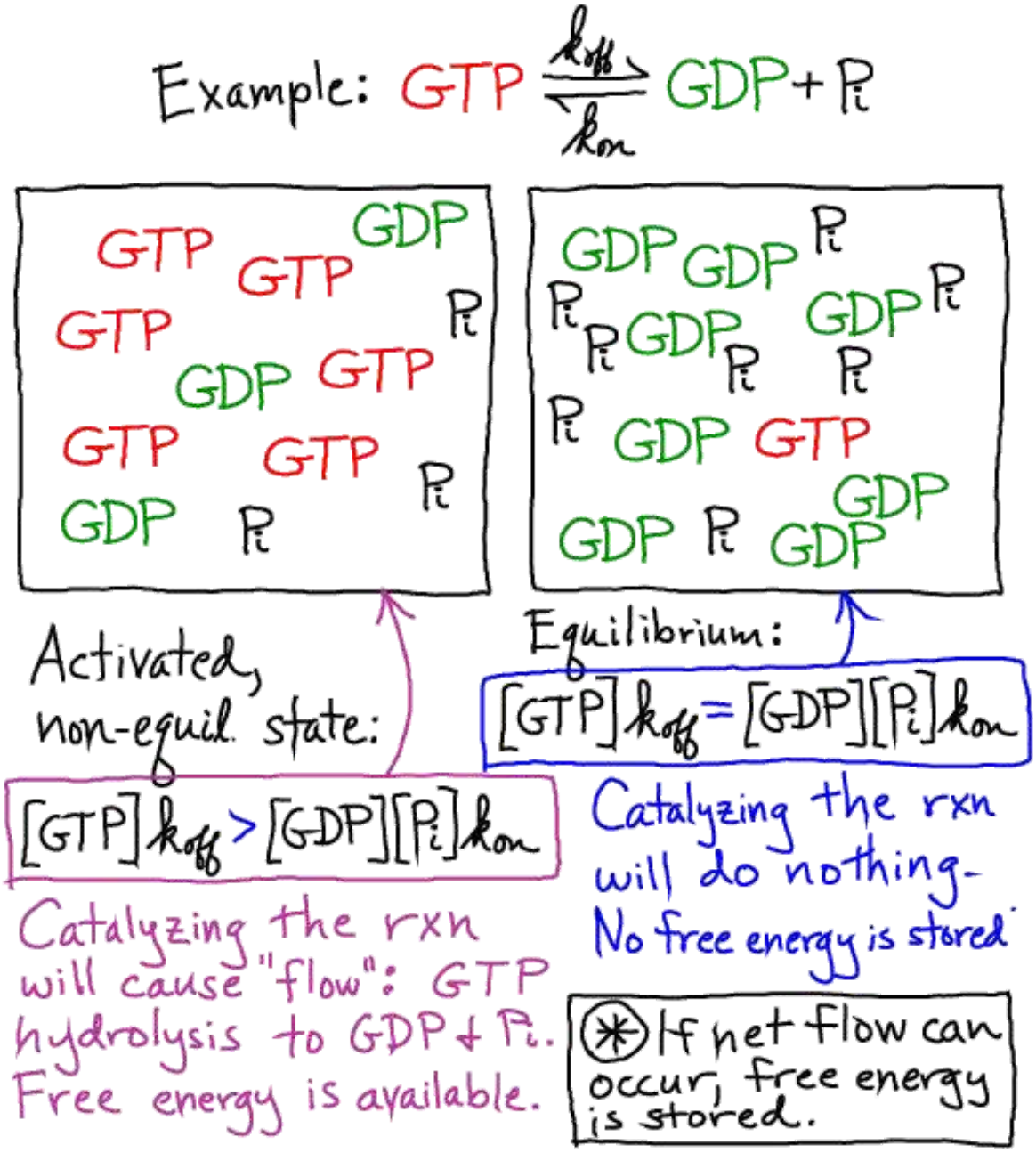
Free Energy is the Energy Available for Work

Free Energy is the Cell's Available Energy

Free Energy is energy avaliable to do work.

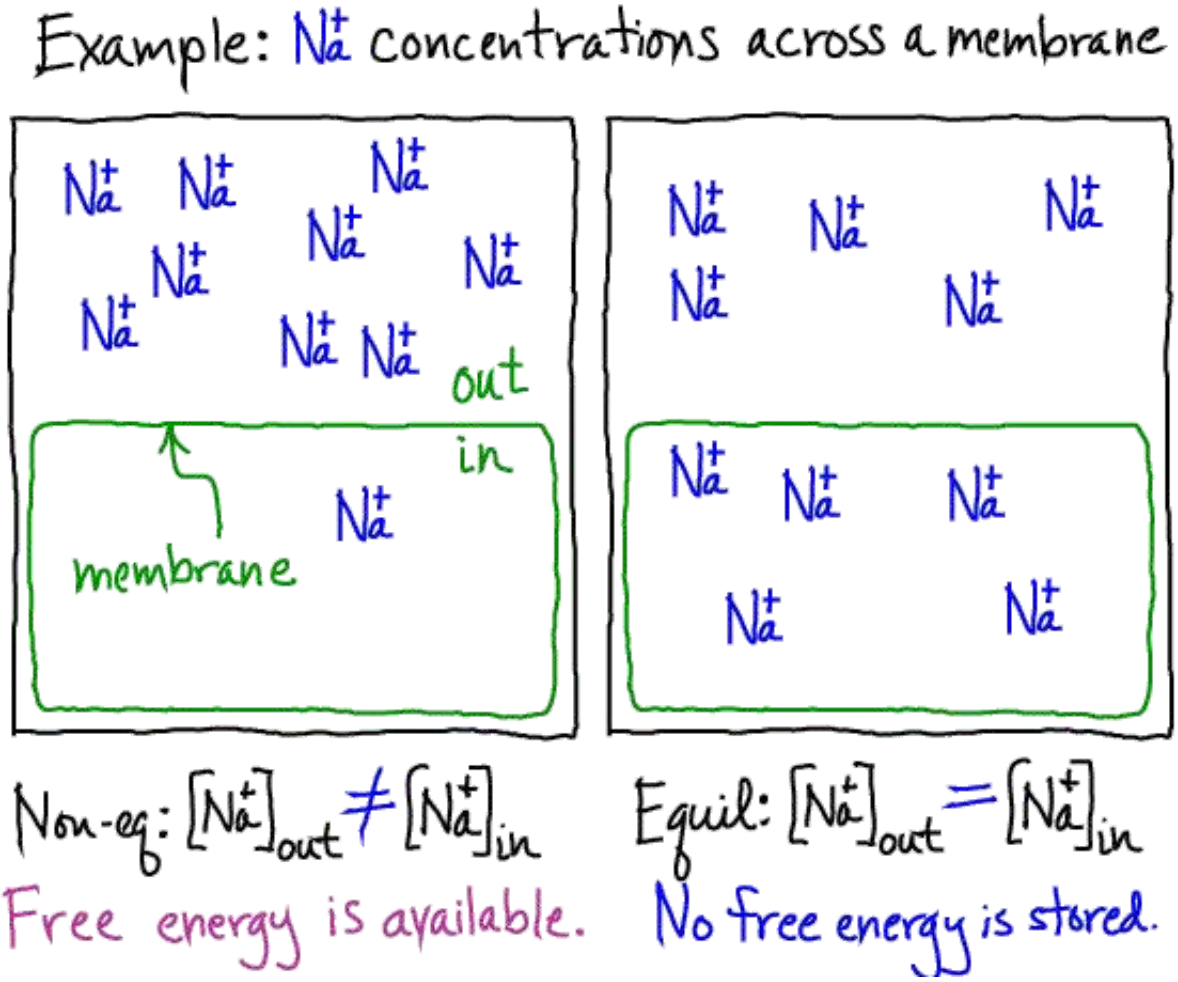
- It can be considered the analog of potential energy in a "thermal environment" (where molecular collisions substantially alter the potential and / or kinetic energies of objects of interest - i.e., proteins, solvent, etc.).
- Systems will move from a condition of high to low free energy if it is possible: a ball will roll downhill in the absence of a barrier.
- The cell stores free energy in two primary ways:
  - (1) a gradient - i.e., differing concentrations of ions or small molecules across a membrane, as is the case for some transporters.
  - (2) activated carriers - i.e., molecules capable of dissociating, but which are maintained at a concentration well above the equilibrium value, such as ATP and GTP.
- Any system that is out of equilibrium stores free energy that can be used for work - e.g., to drive cellular processes such as transport, locomotion, synthesis - or signaling processes. Conversely *an equilibrium system stores no free / usable energy* unless the conditions are changed.

Activated Carriers Store Free Energy



The cell maintains the concentration of an activated carrier (e.g., GTP or ATP) well above its equilibrium value so that there is always a driving force toward equilibrium (via the decomposition reaction). Because there is a drive toward equilibrium, there is free energy which can be harnessed for work - as in the example of ATP-driven transporters.

Concentration Gradients across Membranes Store Free Energy



As in the case of activated carriers, gradients are also out of equilibrium: there is a driving force to equalize the concentrations of species across a membrane (assuming for simplicity no coupling among species). A more quantitative discussion of this phenomenon is available. The cell can use gradient-stored free energy, for instance in the case of active transport.

The Cell Uses Free Energy in Different Ways

In general, the cell uses free energy it stores in gradients and activated carriers to accomplish two types of things:

- (1) Work in the usual sense - i.e., energetically unfavorable tasks such as transport (against a gradient), locomotion, and chemical synthesis.
- (2) Energy-neutral signaling processes, such as phosphorylation, which involve a specific sequence of events that may leave the signal carriers (e.g., proteins) unchanged.

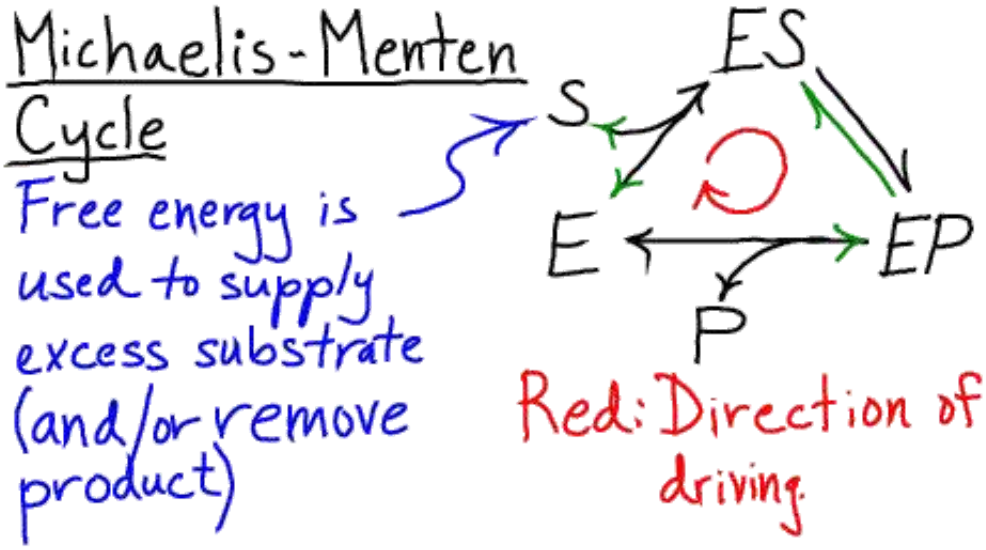
How does the free energy remain stored without "running downhill"?

Free energy in the cell will not dissipate unless the transition from the high-free-energy non-equilibrium state toward a lower free-energy state is catalyzed: for activated carriers, enzymes are required; for gradients across membranes, flow is enabled by channels or transporters.

How does the cell get energy?

The cell maintains its supply of ATP and other stored energy by metabolizing nutrients in an ongoing cycle of ATP synthesis.

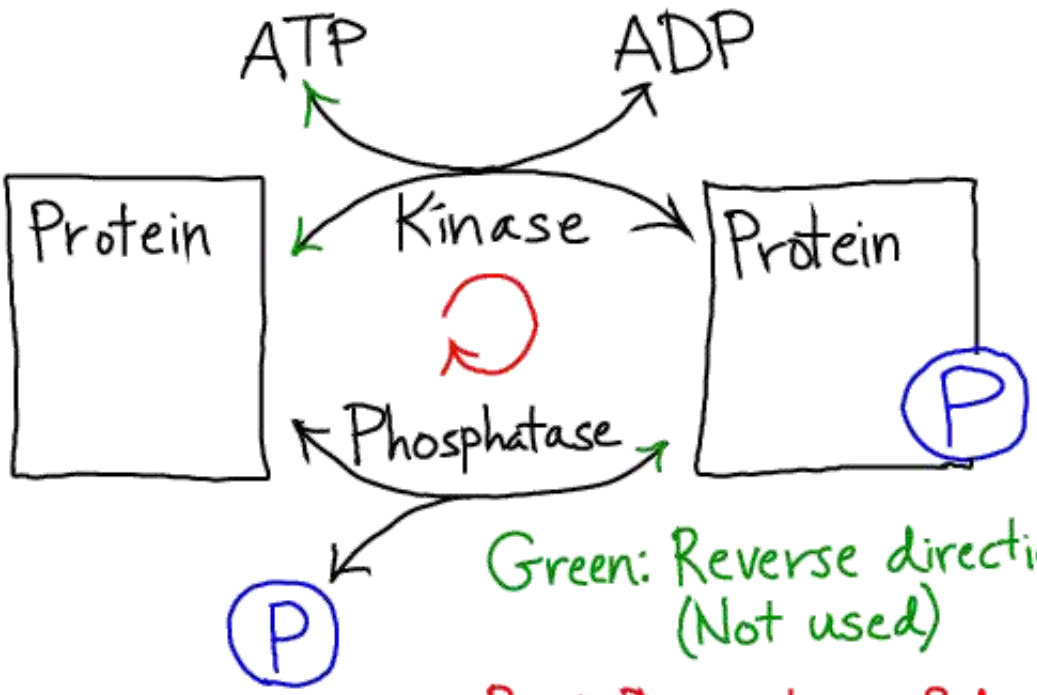
Cycles and the Cell's Non-equilibrium Use of Free Energy



Cellular processes typically function in cycles to re-use molecular components, and free energy is used to drive such cycles in a single direction by maintaining some components out of equilibrium - e.g., supplying excess ATP or other substrates for catalysis.

Phosphorylation Cycle

Free energy is used to synthesize excess ATP, which drives the cycle



Green: Reverse direction (Not used)

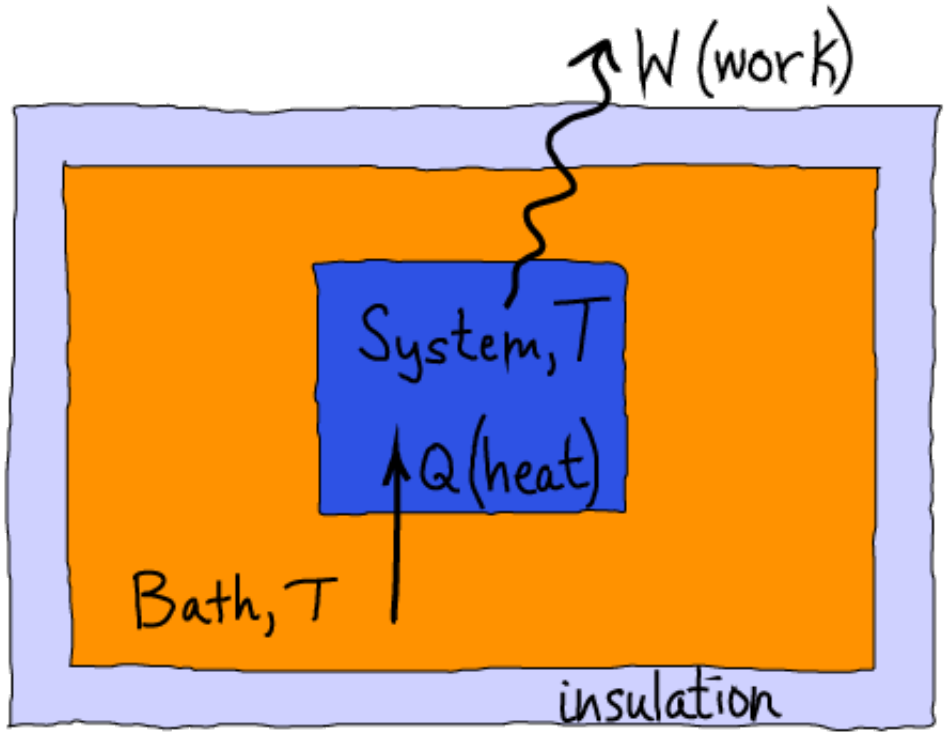
Red: Direction of driving.

Thermodynamic Connection Between Free Energy and Work

From Free Energy to Work, Thermodynamically

The amount of work a system can do is limited by the the change in its free energy. Work is done during spontaneous processes in which the free energy decreases. Spontaneous processes, roughly speaking, amount to systems in non-equilibrium states either relaxing toward equilibrium, or systems "trying" to relax to equilibrium without being able to because of external constraints. An example of external constraints is the continual addition of substrate and removal of product from an enzyme in steady state: the system never reaches the natural equilibrium between substrate and product because of (externally driven) addition/removal processes.

A quick thermodynamic derivation



We will use the schematic above to aid in deriving the limits on work that can be performed in a thermal environment.

Our derivation will be based on the following **assumptions and notation**, which are explained in most basic physics texts.

- A system is separated from a much larger "bath" in such a way that the system is maintained at the same constant temperature T as the bath.

- Q is the heat transferred from bath to system - could be negative.
- W is the work done *by* the system on the environment.
- The entropy and average energy of the system are $S_{\rm sys}$ and $\langle E\rangle_{\rm sys}$.
- The *changes* in these quantities during the spontaneous process are deoted by $\Delta S_{\rm sys}$ and $\Delta\langle E\rangle_{\rm sys}$.
- Our derivation will assume the system's volume remains constant and therefore use the Helmholtz free energy $F = \langle E\rangle - T\,S$ but an analogous result is valid for the Gibbs free energy G.
- The first law of thermodynamics: $Q = \Delta\langle E\rangle_{\rm sys} + W$, which just states that energy is conserved.
- The second law of thermodynamics: $\Delta S_{\rm tot} = \Delta S_{\rm bath} + \Delta S_{\rm sys} > 0$, which says that the *total* entropy (of the universe) increases in any spontaneous process.
- $\Delta S_{\rm bath} = -Q/T$ because the only change to the bath is the addition or removal of heat.

The derivation itself starts with the second law:

$$0 < \Delta S_{\rm bath} + \Delta S_{\rm sys} = -\frac{Q}{T} + \Delta S_{\rm sys}\,, \quad (1)$$

where $\Delta S_{\rm sys}$ is unknown because a complex process may be occurring in the system.

Substituting the first law into (1) leads to

$$\Delta E_{\rm sys} - T\Delta S_{\rm sys} < -W \quad (2)$$

Now, multiplying both sides by -1 (noting that the direction of inequality therefore switches) and using the definition of F yields

$$W < -\Delta F_{\rm sys} \quad (3)$$

This is the key result: the work done in a spontaneous process is limited by the change in free energy. The negative sign in front of F reflects that $\Delta F < 0$ in a spontaneous process, so that the work can be positive.

Biologically the most important type of work is chemical (i.e., shifting a chemical system further from equilibrium than it was previously) although mechanical work clearly is important for cytoskeletal processes and muscle contraction. Not surprisingly, free energy typically is stored by chemical means.

References:

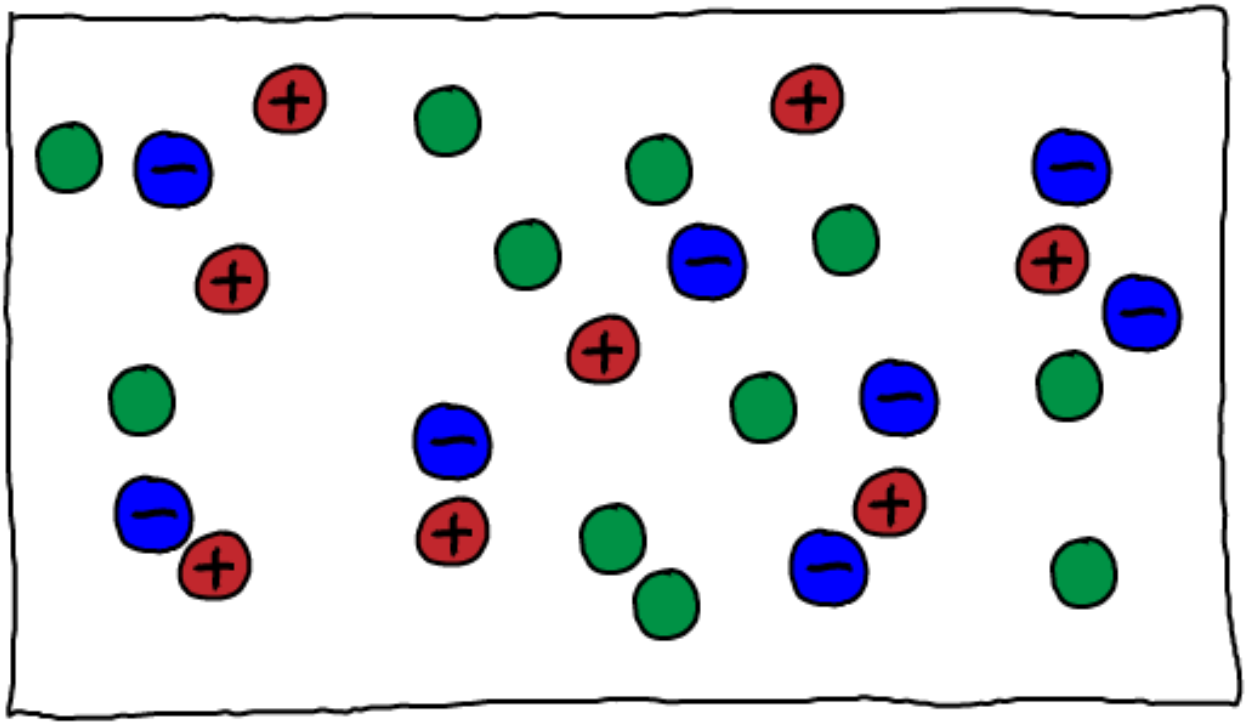
- Any thermodynamics or basic physics textbook.
- D.M. Zuckerman, "Statistical Physics of Biomolecules: An Introduction," (CRC Press, 2010), Chapter 7.
- T.L. Hill, "Free Energy Transduction and Biochemical Cycle Kinetics," (Dover, 2005). Fantastic first chapter - easily worth the price of the book.

Chemical Potential

The Chemical Potential: Simple Thermodynamics of Chemical Processes

The chemical potential μ, which is simply the free energy per molecule, is probably the most useful thermodynamic quantity for describing and thinking about chemical systems. Because μ represents an energy for one molecule, it is easy to think about concretely. In fact, for a system consisting of molecule types A, B, C, etc., each occurring with N_A , N_B , ... copies, we can write the total free energy at constant pressure exactly as

$$G = N_A\mu_A + N_B\mu_B + \cdots \quad (1)$$



To focus on the chemical potential itself, we can say that μ_X is the energy required to add a molecule of type X to a system at temperature T and pressure P, which consists of N_A molecules of type A, N_B molecules of type B, and so on. Importantly, the chemical potential is not a physical constant: implicit in μ are its many dependences

$$\mu_X = \mu_X\left(T, P, N_A, N_B, \ldots\right) \quad (2)$$

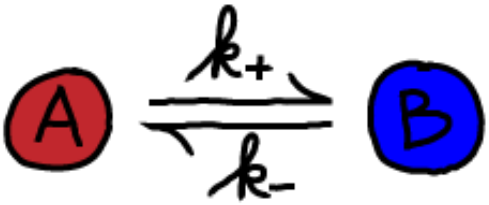
which often may not be written out. The key point is that, in general, the chemical

depends on everything about the system. To give a simple example of such dependence, molecule type A could represent protons in solution (implying a certain pH), which would affect the energy required to add an acidic molecule of type B. As another example, in a solution of charged molecules of type A, the chemical potential μ_A will depend on how many A molecules are already present (i.e., the concentration [A]) because charged molecules repel one another. Note that the apparently simple form of Eq. (1) does not imply the molecule types are independent of one another because the μ values change as the N values do.

To understand chemical equilibrium, a fundamental reference point in biochemistry, it turns out to be important to write the chemical potential as a derivative of the free energy. From the definition above, we want to know the incremental change in free energy ΔG based on adding a single molecule of type X - i.e., for $\Delta N_X = 1$. Using basic calculus ideas, we can write

$$\mu_X = \Delta G(\text{adding one X molecule}) = \frac{\partial G}{\partial N_X}\Delta N_X = \frac{\partial G}{\partial N_X} \quad (3)$$

Chemical reactions: Equilibrium and beyond



The chemical potential, by design, is the perfect tool for analyzing chemical behavior. Let us first consider the very simplest chemical reaction:

$$A \rightleftharpoons B \quad (4)$$

When getting started with a new system, it's always a good plan to examine the equilibrium point. Because the free energy will be minimized with respect to adjustable paramters (N_A and N_B in this case) at equilibrium, we should differentiate G with respect to either N_A or N_B. In fact, there is really only one adjustable parameter in this system since $N_B = N - N_A$ and N is assumed constant. Using the basic rules of calculus and the definition of μ (3), we find

$$\frac{dG}{dN_A} = \frac{\partial G}{\partial N_A} + \frac{\partial G}{\partial N_B}\frac{dN_B}{dN_A} = \mu_A - \mu_B \ , \quad (6)$$

because $dN_B/dN_A = -1$ at fixed total N. The equilibrium point itself is obtained by setting the G derivative to zero (to minimize G), yielding the key chemical result that chemical potentials will match in equilibrium:

$$\mu_A = \mu_B \ (\text{equilibrium}) \quad (8)$$

This equality of chemical potentials does not imply that $N_A = N_B$ at equilibrium: in general, the equilibrium point will imply quite different reactant and product numbers (and hence concentrations). After all, most reactions substantially favor either products or reactants under a given set of conditions.

Connection to reaction rates, free energy, and standard free energy

The chemical potential must be intimately related to reaction rates and standard free energies, which also can be said to "control" chemical reactions. We will begin to clarify those connections now. Briefly, the standard free energy indicates where the equilibrium point of a reaction will be, as does the ratio of reaction rate constants. The difference in chemical potentials, like the free energy difference (compared to the standard free energy) tells us about the displacement from equilibrium.

We'll start with the standard free energy change of reaction $\Delta G^{\circ'}$, where the "$\circ$" indicates standard temperature and pressure and the prime (') denotes pH = 7. From any chemistry or biochemistry book, the definition (for our simple $A \rightleftharpoons B$ reaction) is

$$\begin{aligned} \Delta G^{\circ'} &= -RT \ln \frac{[\mathrm{B}]^{\mathrm{eq}}}{[\mathrm{A}]^{\mathrm{eq}}} \\ &= -RT \ln K'_{\mathrm{eq}}, \quad (9) \end{aligned}$$

where it's essential to realize that this applies to the equilibrium concentrations at standard conditions. The second line defines the equilibrium constant $K^{\mathrm{eq}} = [\mathrm{B}]^{\mathrm{eq}}/[\mathrm{A}]^{\mathrm{eq}}$.

Eq. (9) tells us the precise relation between the equilibrium concentrations and the standard free energy. When the product (B) is "favored" (i.e., when $[\mathrm{B}]^{\mathrm{eq}} > [\mathrm{A}]^{\mathrm{eq}}$), then $\Delta G^{\circ'}$ is

negative, but when reactant A is favored then $\Delta G^{\circ'}$ is positive. Turning that around, $\Delta G^{\circ'}$ merely tells us the relative A and B concentrations that will occur at equilibrium. As we will see below, $\Delta G^{\circ'}$ absolutely does not indicate whether a given reaction will tend to proceed in the forward direction, because that depends on the immediate (starting) species concentrations - which typically are not in equilibrium. Again, $\Delta G^{\circ'}$ only indicates what the ratio of concentrations will be at equilibrium.

From the detailed-balance condition of equilibrium, we know that the ratio of rate constants also yield the equilibrium ratio of concentrations.

$$[\mathrm{A}]^{\mathrm{eq}} k_+ = [\mathrm{B}]^{\mathrm{eq}} k_- \qquad \Rightarrow \qquad \frac{k_+}{k_-} = \frac{[\mathrm{B}]^{\mathrm{eq}}}{[\mathrm{A}]^{\mathrm{eq}}} = K'_{\mathrm{eq}}; \tag{10}$$

where k_+ is the rate constant for the A to B direction and k_- is for the reverse reaction. This enables to write the standard free energy change in term of rates constants:

$$\Delta G^{\circ'} = -RT \ln \left(k_+ \,/\, k_- \right) \tag{11}$$

Using the explicit dependence of chemical potential on concentration, namely $\mu_X = \mu_X^\circ + RT \ln[\mathrm{X}]$ derived below in Eq. (27) assuming independent molecules, we can also re-write the equality of chemical potentials (8) in equilibrium as

$$\mu_A^\circ + RT \ln \left([\mathrm{A}]^{\mathrm{eq}} / 1\mathrm{M} \right) = \mu_B^\circ + RT \ln \left([\mathrm{B}]^{\mathrm{eq}} / 1\mathrm{M} \right). \tag{12}$$

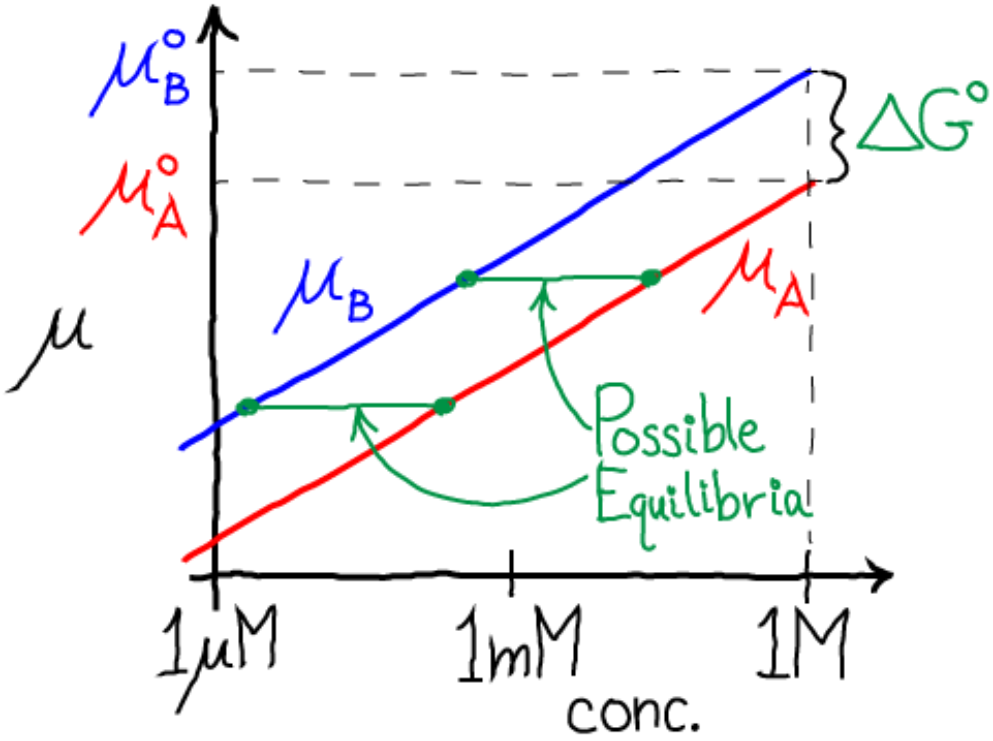
Rearranging and comparing to the defintion of $\Delta G^{\circ'}$ from Eq. (9), we have

$$-RT \ln \frac{[\mathrm{B}]^{\mathrm{eq}}}{[\mathrm{A}]^{\mathrm{eq}}} = \mu_B^{\circ'} - \mu_A^{\circ'} = \Delta G^{\circ'} \tag{13}$$

where again the prime (') indicates we are considering pH = 7. It is very useful to also be familiar with exponentiated form of this same relation:

$$\frac{[\mathrm{B}]^{\mathrm{eq}}}{[\mathrm{A}]^{\mathrm{eq}}} = e^{-(\mu_B^\circ - \mu_A^\circ)/RT} = e^{-\Delta G^{\circ'} \big/ RT} = K'_{\mathrm{eq}}; \tag{14}$$

which makes it simpler to grasp that higher standard free energy of B (relative to A) decreases the relative equilibrium concentration of B, as shown in the figure by the green equilibrium horizontal tie lines.



We have now seen the full set of equilibrium connections among the chemical potential, standard free energy difference, and reaction rates. Most importantly, the standard free energy difference (and standard chemical potentials) merely set the equilibrium point. A lower standard chemical potential means a larger equilibrium concentration, as we see from Eq. (13).

Below, we will generalize these results for more complicated reactions.

What μ tells us about out-of-equilibrium systems

Biological systems are rarely in equilibrium, so it is essential to understand non-equilibrium behavior. The result (8) that chemical potentials match in equilibrium is equally valuable for what it tells us about systems which are out of equilibrium, namely, that the chemical potentials are not equal. Such non-equilibrium systems will always tend to be driven toward equilibrium.

Consider a case where $\mu_A > \mu_B$ (so $\Delta\mu = \mu_B - \mu_A < 0$), and assume the chemical potential depends on concentration according to Eq. (), derived below. Then we have

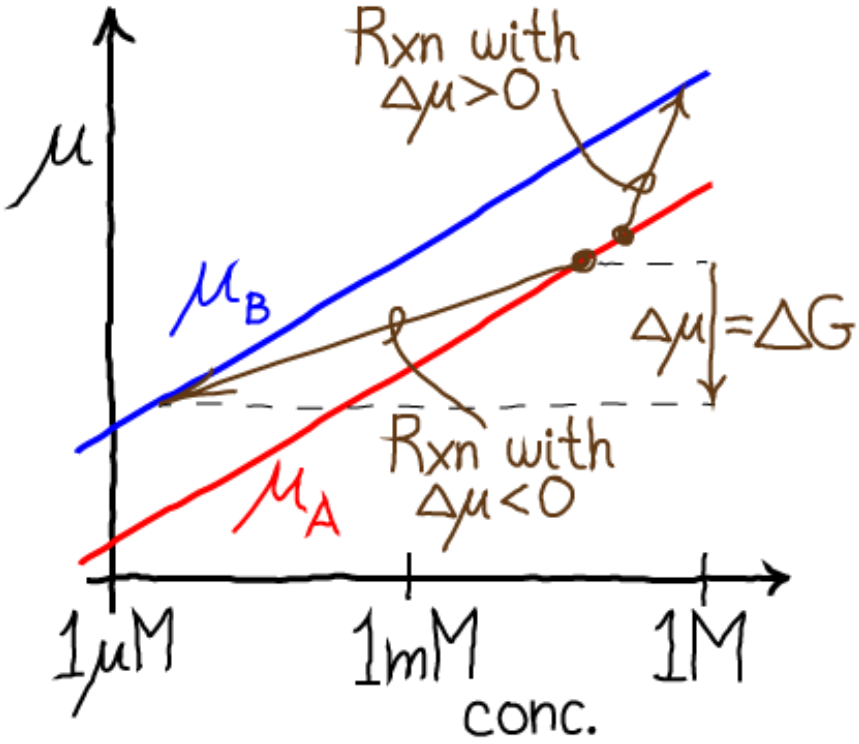
$$\mu_A = \mu_A^\circ + RT \ln\left([\mathrm{A}]/1\mathrm{M}\right) > \mu_B^\circ + RT \ln\left([\mathrm{B}]/1\mathrm{M}\right) = \mu_B, \quad (15)$$

which can be re-written as

$$\frac{[\mathrm{B}]}{[\mathrm{A}]} < e^{-(\mu_B^\circ - \mu_A^\circ)/RT}. \quad (16)$$

That is, in this particular non-equilibrium example, the concentration of A is (relatively) higher than its equilibrium value: compare to Eq. (14). As shown in the figure below, for a non-equilibrium reaction with $\Delta\mu < 0$, the concentrations of A and B differ from the equilibrium values which would be described by a horizontal tie line (equal μ values).

steady state.



Importantly, the chemical potential difference $\Delta\mu = \mu_B - \mu_A$ tells us the actual free energy required (or released, if negative) when a reaction occurs at a specific set of conditions - which is absolutely critical for understanding cellular reactions such as the hydrolysis of ATP under cellular conditions. (Remember that the standard free energy change $\Delta G^{\circ'}$ only tells us the equilibrium point and not the free energy change for a realistic reaction.) When $\Delta\mu = 0$, we have equilibrium, and no free energy is required to push the reaction. The figure shows two examples of non-equilibrium reactions: the vertical components of the brown arrows represent the $\Delta\mu$ values in each case.

Using Eq. (), we can write the independent-molecule approximation for the change in chemical potential which is precisely equal to ΔG for the reaction, which can be seen from

Eq. (1) for a single reaction which corresponds to $\Delta N_B = -\Delta N_A = 1$ since one A molecule is converted to B.

$$\Delta G = G(N_A - 1, N_B + 1) - G(N_A, N_B) = \Delta\mu = \mu_B - \mu_A \quad (17)$$

You can probably guess that $\Delta\mu$ for ATP hydrolysis is large and negative under typical cellular conditions though it can take on any value - even positive - under different conditions. Of course, the cell has tricks for making unfavorable reactions ($\Delta\mu > 0$) occur - by coupling them to other reactions that are more favorable still, so that the overall $\Delta\mu$ is negative.

More complex reactions

The book by Hill or any chemical thermodynamics text will explain the full generalization of the preceding results. Here we consider a somewhat more general reaction that will be useful for nucleotides, namely,

$$A + B \rightleftharpoons C + D, \quad (18)$$

with forward and reverse rate constants k_+ and k_-. A key example is ATP + H_2O $\rightleftharpoons$ ADP + Pi.

By applying mass-action kinetic rules to the reaction (18), we obtain the equilibrium balance condition $[\mathrm{A}]^{\mathrm{eq}}[\mathrm{B}]^{\mathrm{eq}}k_+ = [\mathrm{C}]^{\mathrm{eq}}[\mathrm{D}]^{\mathrm{eq}}k_-$, that in turn can be rewritten as

$$\frac{[\mathrm{C}]^{\mathrm{eq}}[\mathrm{D}]^{\mathrm{eq}}}{[\mathrm{A}]^{\mathrm{eq}}[\mathrm{B}]^{\mathrm{eq}}} = \frac{k_+}{k_-} = K'_{\mathrm{eq}} = e^{-\Delta G^{\circ\prime}/RT} \quad (19)$$

The free energy per reaction under non-equilibrium conditions (typical of the cell) can be derived following Eqs. (1) and (17). For the reaction (18), we have

$$\begin{aligned}\Delta G = \Delta\mu &= \mu_C + \mu_D - \mu_A - \mu_B \\ &= RT \ln\left(\frac{[\mathrm{C}][\mathrm{D}]}{[\mathrm{A}][\mathrm{B}]}\right) + \Delta G^{\circ\prime},\end{aligned} \quad (20)$$

which is the free energy change per reaction in the forward direction. Thus, for example, if

the concentrations of C and D exceed their equilibrium values, then the free energy change is positive - take the log of Eq. (19) to see this. A positive free energy change for a reaction implies that free energy is required for the reaction to occur. In other words, on average, the reaction will occur spontaneously only in reverse, which makes sense since the level of "products" (C and D) is elevated.

ATP free energy under cellular conditions

ATP is famously the fuel of the cell, but to understand this quantitatively - or even in a qualitatively accurate way - we need to use the free energy and chemical potential concepts developed here. The ATP hydrolysis reaction is

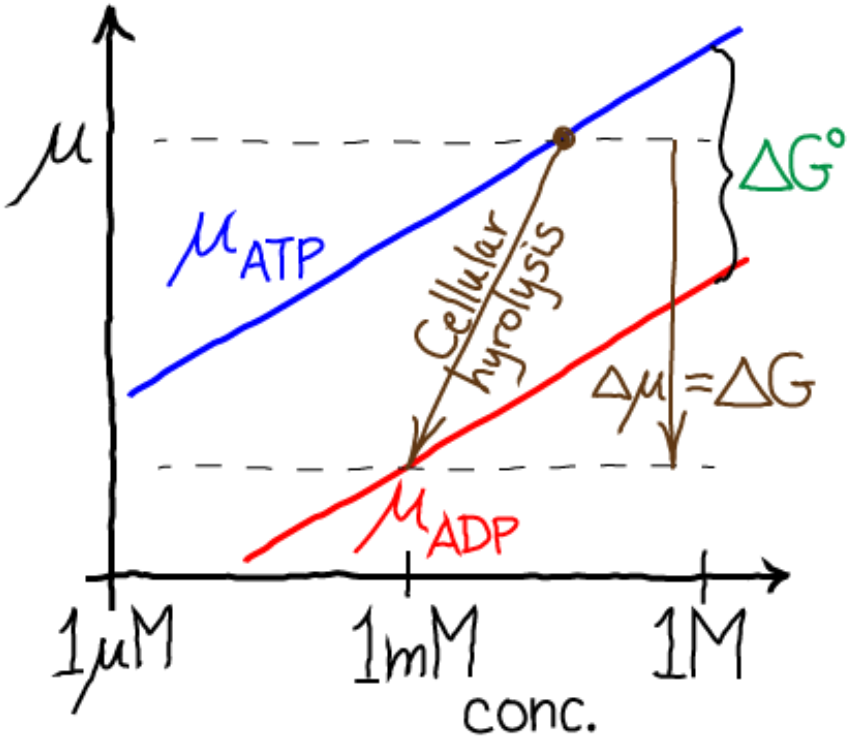
$$\mathrm{ATP} + \mathrm{H_20} \rightleftharpoons \mathrm{ADP} + \mathrm{Pi}, \tag{21}$$

with $\Delta G^{\circ'} = -7.3$ kcal/mol (which refers to the forward/hydrolysis direction) according to Berg's textbook. That is, under standard conditions ADP is favored over ATP - i.e., in equilibrium the concentration of ADP will greatly exceed that of ATP - given typical water and phosphate concentrations. In the cell, however, there is often more ATP than ADP, leading to an even larger (more negative) $\Delta G \simeq -12$ kcal/mol via Eq. (20).

To understand this mathematically, we can re-cast Eq. (20) for ATP hydrolysis using Eq. (19) as

$$\begin{aligned} \Delta G &= RT \ln \left(\frac{[\mathrm{ADP}][\mathrm{Pi}]/\left([\mathrm{ATP}][\mathrm{H_2O}]\right)}{[\mathrm{ADP}]^{\mathrm{eq}}[\mathrm{Pi}]^{\mathrm{eq}}/\left([\mathrm{ATP}]^{\mathrm{eq}}[\mathrm{H_2O}]^{\mathrm{eq}}\right)} \right) \\ &\simeq RT \ln \left(\frac{[\mathrm{ADP}][\mathrm{Pi}]/[\mathrm{ATP}]}{[\mathrm{ADP}]^{\mathrm{eq}}[\mathrm{Pi}]^{\mathrm{eq}}/[\mathrm{ATP}]^{\mathrm{eq}}} \right), \end{aligned} \tag{22}$$

where we have used the fact that the concentration of water will change only negligibly due the reaction. If $\Delta G \simeq -12$ kcal/mol, that means that the cellular concentrations (numerator) differ from the equilibrium concentrations (denominator) by more than seven orders of magnitude! It is in this sense only that ATP is a highly activated molecule: the concentrations are kept far from equilibrium. Even though $\Delta G^{\circ'}$ is large and negative, if the cellular concentrations matched the equilibrium values there would be no usable ΔG.



The figure shows the free energy considerations for ATP hydrolysis in a very schematic way - assuming the concentrations of water and phosphate are constant (though [Pi] certainly would change significantly). Qualitatively, the figure gives a correct sense of hydrolysis free energetics: the red ADP chemical potential is well below that for ATP (blue), and for cellular hydrolysis the actual free energy change per reaction is greater than the vertical distance ($\Delta G^{\circ'}$ for standard conditions) because cellular [ATP] typically exceeds [ADP].

Deriving the (approximate) anayltical form for the chemical potential

Building on our statistical mechanics discussion of the ideal gas, we can derive an equation for the chemical potential of a system of molecules, under the assumption they do not interact with one another. In this sense, the molecules are "ideal" in the same way as ideal gas particles. To fully appreciate the brief derivation below, readers should first review the partition function calculation in the ideal gas section.

Without writing down all the details, for N non-interacting molecules of type X in a fixed volume V, the partition function becomes

$$Z_X(N,V,T) = \frac{\lambda_X^{-3N}}{N!} V^N q_X^N, \quad (23)$$

where the dimensionless factor q_X (which does not appear in the simple ideal gas partition function) represents integration of the Boltzmann factor over all degrees of freedom internal

to the molecule - bond lengths, angles, and dihedrals. Thus, q_X is an internal partition function which may include substantial intra-molecular interactions, but any inter-molecular interactions are neglected. Note that the factorized form (23) is expected for non-interacting/independent species because the energy in the Boltzmann factor does not connect different molecules; separating the center-of-mass behavior as the usual ideal gas factors is simply a mathematical trick: see, e.g., books by Hill and Zuckerman for further details.

We can derive the free energy itself, using the relation $F = -k_B T \ln Z$ along with Stirling's approximation, which leads to

$$F_X(N,V,T) = N\,k_B T \ln\left(\frac{N}{V q_X\,/\lambda_X^3}\right) - N k_B T \quad (24)$$

Although we will not prove it here, the chemical potential can be derived either by differentiating the Gibbs free energy G or the Helmholtz free energy F. The two will be equivalent when volume fluctuations are not important, which is what we expect for systems of biological interest, which tend to contain a large number of reactant and product molecules. (See the book by Zuckerman for more detailed discussion of volume fluctuations.) Assuming this equivalence, we have

$$\mu_X = \frac{\partial G}{\partial N_X} \simeq \frac{\partial F}{\partial N_X} = k_B T \ln\left(\frac{N_X}{V q_X\,/\lambda_X^3}\right) \quad (25)$$

It is convenient to separate this out into two terms using the rules of logarithms:

$$\mu_X = k_B T \ln\left(1\mathrm{M}\lambda_X^3/q_X\right) + k_B T \ln\left([\mathrm{X}]/1\mathrm{M}\right), \quad (26)$$

where we have explicitly shown the standard one molar ("1M") concentration which makes arguments of the logs dimensionless and also makes the first term the standard chemical potential $\mu_X^\circ = k_B T \ln\left(1\mathrm{M}\lambda_X^3/q_X\right)$. The full chemical potential is then, in molar energy units

$$\mu_X = \mu_X^\circ + RT \ln\left([\mathrm{X}]/1\mathrm{M}\right), \quad (27)$$

References:

- T.L. Hill, An Introduction to Statistical Thermodynamics, Dover, 1986.
- J. M. Berg et al., "Biochemistry", W. H. Freeman. The 2002 edition is online for free.
- D.M. Zuckerman, Statistical Physics of Biomolecules: An Introduction (CRC Press, 2010).

Exercises:

1. Explain the quantitative relationship between k_BT which uses the Boltzmann constant and RT which uses the gas constant. To do this, convert k_BT in MKS units for T = 300K to units of kcal/mole.
2. For the reaction A + B $\rightleftharpoons$ C, starting from the explicit form of the Gibbs free energy (1), derive the equilibrium relation $\mu_A + \mu_B = \mu_C$. Use the fact that N_C must increase when N_A and N_B decrease.

Underpinnings of Mass Action: The Ideal Gas

The Ideal Gas: The basis for "mass action" and a window into free-energy/work relations

The simplest possible multi-particle system, the ideal gas, is a surprisingly valuable tool for gaining insight into biological systems - from mass-action models to gradient-driven transporters. The word "ideal" really means non-interacting, so in an ideal gas all molecules behave as if no others are present. The gas molecules only feel a force from the walls of their container, which merely redirects their momenta like billiard balls. Not surprisingly, it is possible to do exact calculations fairly simply under such extreme assumptions. What's amazing is how relevant those calculations turn out to be, particularly for understanding the basic mechanisms of biological machines and chemical-reaction systems.

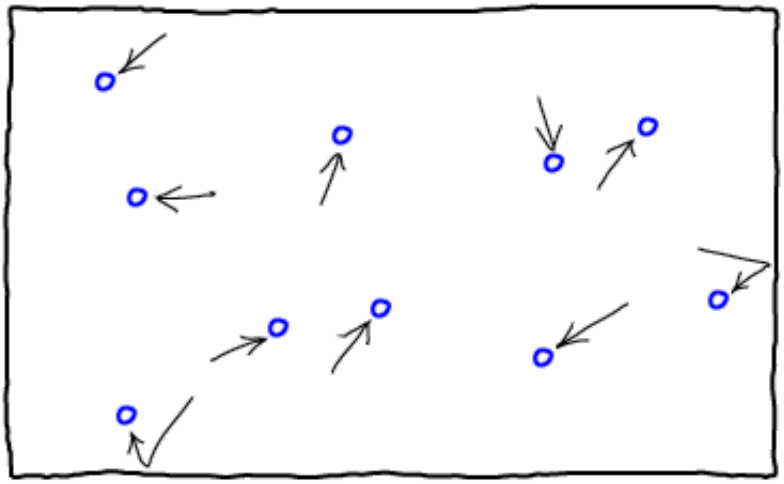
Although ideal particles do not react or bind, their statistical/thermodynamic behavior in the various states (e.g., bound or not, reacted or not) can be used to build powerful models - e.g., for transporters.

Mass-action kinetics are ideal-gas kinetics

The key assumption behind mass-action models is that events (binding, reactions, ...) occur precisely in proportion to the concentration(s) of the participating molecules. This certainly cannot be true for *all* concentrations, because all molecules interact with one another at close enough distances - i.e., at high enough concentrations. In reality, beyond a certain concentration, simple crowding effects due to steric/excluded-volume effects mean that each molecule can have only a maximum number of neighbors.

But in the ideal gas - and in mass-action kinetics - no such crowding effects occur. All molecules are treated as point particles. They do not interact with one another, although virtual/effective interactions occur in a mass-action picture. (We can say these interactions are "virtual" because the only effect is to change the number of particles - no true forces or interactions occur.)

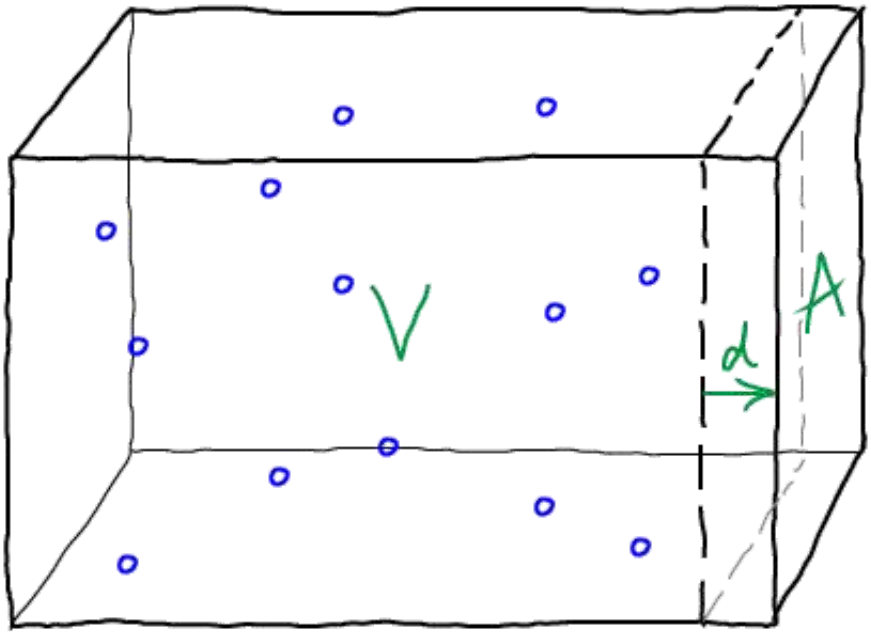
Pressure and work in an ideal gas



Ideal gases can perform work directly using pressure. The molecules of an ideal gas exert a pressure on the walls of the container holding them due to collisions, as sketched above. The amount of this pressure depends on the number of molecules colliding with each unit area of the wall per second, as well as the speed of these collisions. These quantities can be calculated based on the mass m of each molecule, the total number of molecules, N, the total volume of the container V and the temperature, T. In turn, T determines the average speed via the relation $(3/2)\, N\, k_B T = \langle (1/2)\, m\, v^2 \rangle$. See the book by Zuckerman for more details.

All these facts can be combined - consult a physics book - to yield the ideal gas law relating **pressure** P to the other quantities:

$$P^{\mathrm{idl}}\, V = N\, k_B\, T \tag{1}$$



We can calculate the **work** done by an ideal gas to change the size of its container by pushing one wall a distance d as shown above. We use the basic rule of physics that work is force (f) multiplied by distance and the definition of pressure as force per unit area. If we denote the area of the wall by A, we have

$$W = f\,d = (f/A)\,(A\,d) = P\,\Delta V \tag{2}$$

If d is small enough so that the pressure is nearly constant, we can calculate P using (1) at either the beginning or end of the expansion. More generally, for a volume change of arbitrary size (from V_i to V_f) in an ideal gas, we need to integrate:

$$W^{\text{idl}} = \int_{V_i}^{V_f} P^{\text{idl}}\,dV = \int_{V_i}^{V_f} \frac{N\,k_B\,T}{V}\,dV = N\,k_B\,T \ln \frac{V_f}{V_i} \tag{3}$$

which assumes the expansion is performed slowly enough so that (1) applies throughout the process.

Free energy and work in an ideal gas

The free energy of the ideal gas can be calculated exactly in the limit of large N (see below). We will see that it does, in fact, correlate precisely with the expression for work just derived. The free energy depends on temperature, volume, and the number of molecules; for large N, it is given by

$$F^{\text{idl}}(N,V,T) = N\,k_B T \ln\left(\frac{N}{V/\lambda^3}\right) \tag{4}$$

where λ is a constant for fixed temperature. For reference, it is given by $\lambda = h/\sqrt{2\pi m k_B T}$ with h being Planck's constant and m the mass of an atom. See the book by Zuckerman for full details.

Does the free energy tell us anything about work? If we examine the **free energy change** occurring during the same expansion as above, from V_i to V_f at constant T, we get

$$\begin{aligned} F^{\text{idl}}(N, V_f, T) - F^{\text{idl}}(N, V_i, T) & \\ = N k_B T \left(\ln \frac{N \lambda^3}{V_f} - \ln \frac{N \lambda^3}{V_i} \right) & \\ = -N k_B T \left(\ln \frac{V_f}{V_i} \right) & \end{aligned} \tag{5}$$

Comparing to (3), **this is exactly the negative of the work done!** In other words, the free energy of the ideal gas decreases by exactly the amount of work done (when the expansion is performed slowly). More generally, the work can be no greater than the free energy decrease. The ideal gas has allowed us to demonstrate this principle concretely.

The ideal gas free energy from statistical mechanics

The free energy is derived from the "partition function" Z, which is simply a sum/integral over Boltzmann factors for all possible configurations/states of a system. Summing over all possibilities is why the free energy encompasses the full thermodynamic behavior of a system.

The mathematical relation is

$$F(N, V, T) = -k_B T \ln Z(N, V, T) \tag{6}$$

where Z is defined by

$$Z(N, V, T) = \frac{\lambda^{-3N}}{N!} \int_V d\mathbf{r}^N \, e^{-U(\mathbf{r}^N)/k_B T}, \tag{7}$$

where $\lambda(T) \propto 1/\sqrt{(T)}$ is the thermal de Broglie wavelength (which is not important for the phenomena of interest here), $\mathbf{r}^N$ is the set of (x, y, z) coordinates for all molecules and U is the potential energy function. The factor $1/N!$ accounts for interchangeability of identical molecules, and the integral is over all volume allowed to each molecule. For more information, see the book by Zuckerman, or any statistical mechanics

book.

The partition function can be evaluated exactly for the case of the ideal gas because the non-interaction assumption can be formulated as $U(\mathbf{r}^N) = 0$ for all configurations - in other words, the locations of the molecules do not change the energy or lead to forces. This makes the Boltzmann factor exactly 1 for all $\mathbf{r}^N$, and so each molecule's inegration over the full volume yields a factor of V, making the final result

$$\text{Ideal Gas:} \quad Z^{\text{idl}}(N,V,T) = \frac{\lambda^{-3N}}{N!} V^N. \tag{8}$$

Although (8) assumes there are no degrees of freedom internal to the molecule - which might be more reasonable in some cases (ions) than others (flexible molecules) - the expression is sufficient for most of the biophysical explorations undertaken here.

The combination of (6) and (8) can be used to derive (4) in conjunction with Stirling's approximation for large N. See a statistical mechanics book for details.

References:

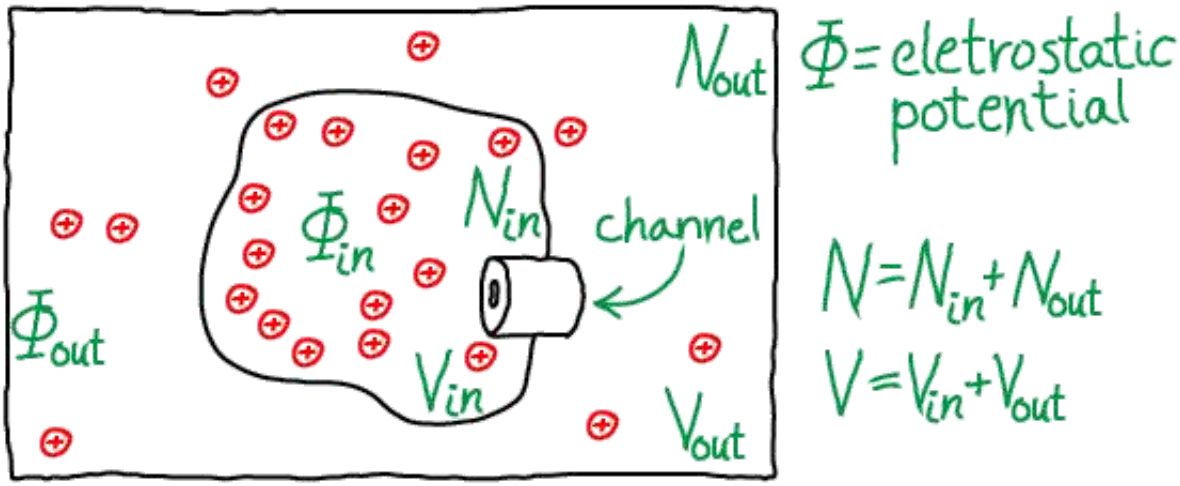
- Any basic physics textbook.
- D.M. Zuckerman, "Statistical Physics of Biomolecules: An Introduction," (CRC Press, 2010), Chapters 5, 7.

The Ideal Gas in a Field: Transmembrane Ionic Gradients

A Half-Step Beyond Ideal: Ion Gradients and Transmembrane Potentials

One key way the cell stores free energy is by having different concentrations of molecules in different "compartments" - e.g., extra-cellular vs. intracellular or in an organelle compared to cytoplasm. The molecules playing this role are charged molecules, or ions, such as sodium (Na^+), chloride (Cl^-), potassium (K^+), calcium (Ca^{++}), and numerous nucleotide species. A brief overview of trans-membrane ion physiology is available.

Although the simplest way to study the physics of free energy storage in such a gradient is by considering ideal particles all with zero potential energy, the reality of the cell is that electrostatic interactions are critical. Fortunately, the most important non-ideal effects of charge-charge interactions can be understood in terms of the usual ideal particles (which do not interact with one another) that do, however, feel the effects of a "background" electrostatic field. Such a mean-field picture is a simple approximation to the electrostatic effects induced primarily by having an excess of one or more charged species on a given side of a membrane - for example, the excess of Na^+ ions in the extracellular environment.



Two semi-ideal gases of ions in different potentials

Unlike our examination of two membrane-separated ideal gases, the particles here are explicitly charged and they "feel" the electrostatic potential Φ. The positively charged anions schematically represent K^+ ions interacting with the field generated by the imbalance of Na^+ ions - the extracellular or "outside" concentration of sodium is maintained at a high value relative to the cytoplasm or "inside" by constant ATP-driven pumping. However, *the effects of the* Na^+ *concentration gradient will only be treated* ***implicitly*** *via the different values for* $\Phi_{\mathrm{in}} < \Phi_{\mathrm{out}}$.

To be precise, the model consists of N particles that do not interact with one another, but which interact with the external potential Φ as if each had a charge of q, leading to potential energy $q \cdot \Phi_{\mathrm{X}}$ for each particle, where X = "in" or "out". The total volume V is divided into inside and outside so that $V_{\mathrm{in}} + V_{\mathrm{out}} = V$, with $N_{\mathrm{in}} + N_{\mathrm{out}} = N$ ions populating the two compartments. The whole system is maintained at constant temperature T. Particles can pass through the channel shown in the figure, but we assume it is closed so that V_{in} and V_{out} are constants: as shown in our discussion of two membrane-separated ideal gases, the assumption is a convenience and not an approximation because the total system volume V and particle-number N are truly constant.

Deriving the free energy

We have two gases of ideal "ions" (that interact with the external potential but not with other ions). Mathematically, we can largely follow our discussion of two membrane-separated ideal gases. The total free energy is the sum of the two ideal gas free energies and the two electrostatic potential energies:

$$\begin{aligned} F(N_{\mathrm{in}}, N_{\mathrm{out}}) =& F^{\mathrm{idl}}(N_{\mathrm{in}}, V_{\mathrm{in}}) + F^{\mathrm{idl}}(N_{\mathrm{out}}, V_{\mathrm{out}}) \\ &+ N_{\mathrm{in}} q \, \Phi_{\mathrm{in}} + N_{\mathrm{out}} q \, \Phi_{\mathrm{out}} \end{aligned} \tag{1}$$

where F^{idl} is defined in the ideal gas page and q is the ionic charge.

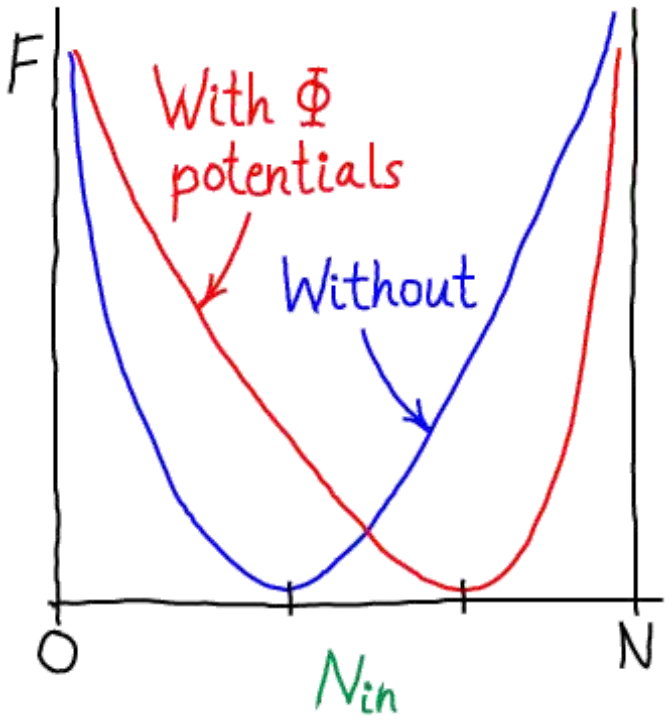
Substituting for F^{idl} and noting that $N_{\mathrm{out}} = N - N_{\mathrm{in}}$, we have

$$\begin{aligned} F(N_{\mathrm{in}}, N - N_{\mathrm{in}}) =& N_{\mathrm{in}} \, k_B T \ln \frac{N_{\mathrm{in}} \, \lambda^3}{V_{\mathrm{in}}} \\ &+ (N - N_{\mathrm{in}}) \, k_B T \ln \frac{(N - N_{\mathrm{in}}) \, \lambda^3}{V_{\mathrm{out}}} \\ &+ N_{\mathrm{in}} \, q \, \Phi_{\mathrm{in}} + (N - N_{\mathrm{in}}) \, q \, \Phi_{\mathrm{out}}. \end{aligned} \tag{2}$$

Because it is really the *difference* in electrostatic potential which governs the ionic behavior, we define $\Delta\Phi = \Phi_{\mathrm{in}} - \Phi_{\mathrm{out}}$. In terms of this quantity, we can rewrite the total free energy as

$$F(N_{\text{in}}, N - N_{\text{in}}) = N_{\text{in}}\, k_B T \ln \frac{N_{\text{in}}\, \lambda^3}{V_{\text{in}}} + (N - N_{\text{in}})\, k_B T \ln \frac{(N - N_{\text{in}})\, \lambda^3}{V_{\text{out}}} + N q\, \Phi_{\text{out}} + N_{\text{in}} q\, \Delta\Phi, \tag{3}$$

Eq. (3) is the free energy as a function of the number of particles inside the membrane (in volume V_{in}). Inclusion of the electrostatic effects shifts the location of the most probable state, or free energy minimum.



The most probable concentrations: The Nernst equation

If we open the channel and allow exchange of atoms between the compartments, the value of N_{in} can change. The probability of having N_{in} atoms in V_{in} is proportional to the Boltzmann factor of the free energy:

$$p(N_{\text{in}}) \propto e^{-F(N_{\text{in}},\, N - N_{\text{in}})/k_B T} \tag{4}$$

The most probable N_{in} value therefore can be found by determining the minimum of F. This will represent the equilibrium point in the thermodynamic limit (very large N - when fluctuations about the most probable N_{in} will be very small compared to N_{in} itself). We set $\partial F/\partial N_{\text{in}} = 0$ in Eq. (3), then re-arrange and cancel terms to find

$$0 = k_B T \ln \frac{N_{\text{in}} \lambda^3}{V_{\text{in}}} - k_B T \ln \frac{N_{\text{out}} \lambda^3}{V_{\text{out}}} + q\Delta\Phi / k_B T \qquad (5)$$

Combining the terms using the rules of logarithms, followed by exponentiation, we find the **Nernst equation**,

$$\text{Equilibrium: } \frac{N_{\text{in}}/V_{\text{in}}}{N_{\text{out}}/V_{\text{out}}} = e^{-q\,\Delta\Phi/k_B T} \qquad (6)$$

where you should recognize the left-hand side as the ratio of concentrations.

In words, Eq. (6) shows that *the concentrations inside and outside vary according to the Boltzmann factor of the ionic charge times the potential difference*. Such an equilibrium is called a Donnan equilibrium. It should be comforting that when $\Delta\Phi = 0$, we recover equal concentrations.

Comparison to cellular behavior

As the exercise below will show, for some ions (Cl^-, K^+) the Nernst equation is a reasonable approximation. This suggests that such ions permeate the membrane passively. For some ions (Na^+, Ca^{++}), the concentration ratios are very different from what would be predicted from the Nernst equation because the cell uses active transport to control them.

Mass action and its limitations

It is always worthwhile to pursue both thermodynamic *and* kinetic analyses of any system you really care about, or just to train yourself to consider a problem from multiple perspectives. By comparison to the present case, some of the results from the truly ideal (uncharged) two-compartment system may seem puzzling.

In contrast to the uncharged system, we can see that the transport rates through the channel *cannot* be equal in the two directions. Let k_{io} be the inside-to-outside rate constant and k_{oi} be the reverse rate constant. Starting from detailed balance, which says that the overall flows must be equal and opposite, and substituting the Nernst relation (6), we find that

$$\frac{k_{oi}}{k_{io}} = \frac{N_{\text{in}}/V_{\text{in}}}{N_{\text{out}}/V_{\text{out}}} = e^{-q\,\Delta\Phi/k_BT} \tag{7}$$

In other words, the ratio of rates for an ion channel depends on the potential difference. By itself, this does not contradict the mass action viewpoint (that rate constants are independent of concentrations) ... so long as $\Delta\Phi$ is truly constant. But if, more generally, $\Delta\Phi$ depends on the relative concentrations of the ion species moving through the channel, then the mass-action picture breaks down. Such a breakdown would occur, for example, if there were two species of ions, one of which could not permeate the membrane and hence was maintained at fixed inside and outside concentrations: in this case, flow of the permeable ion would change $\Delta\Phi$ and, in turn, change the rate "constants".

The brief overview of trans-membrane ion physiology may help to clarify the bigger picture of ion/membrane behavior.

References:

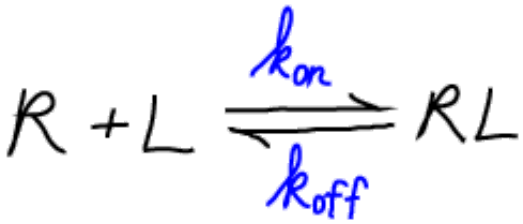
- R. Phillips et al., "Physical Biology of the Cell," (Garland Science, 2009).
- B. Alberts et al., "Molecular Biology of the Cell", Garland Science (many editions available).

Exercises:

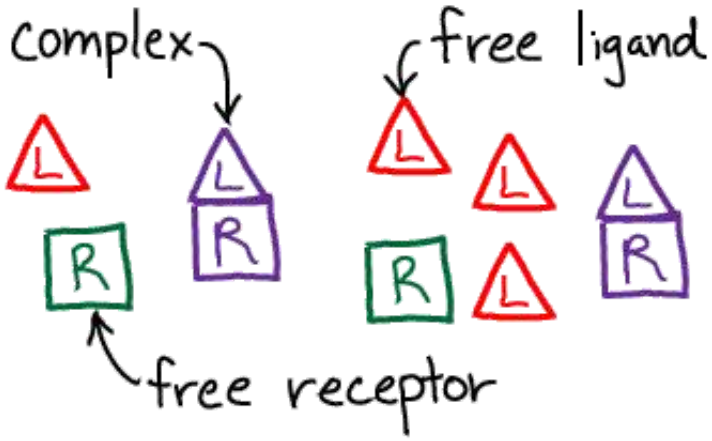
1. Derive Eqs. (5) and (6).
2. Use Eq. (6) to derive a concentration ratio for Cl^- using $\Delta\Phi = -90\,\text{mV}$ (typical for skeletal muscle) and compare your result to the experimental value of $\sim 1/30$. This will require careful consideration of units when multiplying together physical constants.

Binding: Kinetics & Thermodynamics

Binding is one of **the** essential processes in the cell. It is key to signaling and to the function of molecular machines. Because most biochemistry is catalyzed by enzymes, binding of substrate to enzyme precedes most chemical reactions. Further, the mass-action formulation, of binding is directly applicable to the analysis of chemical reactions and facilitates understanding energy storage in activated carriers such as ATP.



We will use the symbol "R" for receptor, "L" for ligand, and "RL" for the bound complex. Although it is often the case that R is a protein and L a small molecule, the analysis we develop could apply equally well if L were a second protein.



We will use the standard [...] notation for concentration. Note that, by convention, the concentration of a species refers *only* to the specific form named. Thus, for the receptor R, which can be either "free" (unbound) or bound, we have

$$[\mathrm{R}]_{\mathrm{tot}} = [\mathrm{R}] + [\mathrm{RL}]$$
$$[\mathrm{L}]_{\mathrm{tot}} = [\mathrm{L}] + [\mathrm{RL}], \qquad (1)$$

with a similar expression for ligand L.

Binding Kinetics

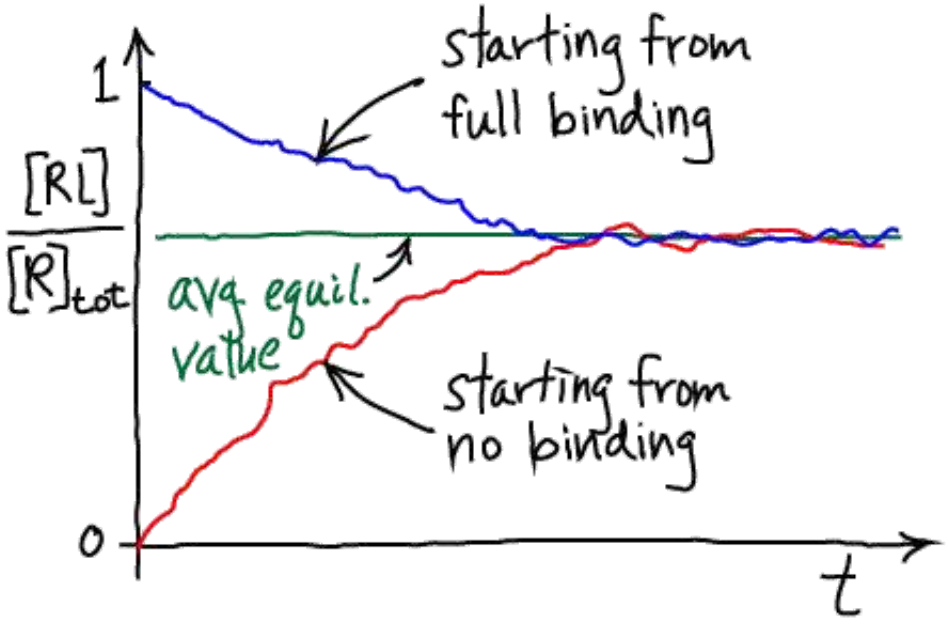
In a kinetic picture, we study the time evolution of a binding system using differential equations. We will use

the simple (but standard) mass-action assumptions that (i) binding is proportional to the product of R and L concerntrations, and (ii) unbinding occurs in proportion RL concentration.

This leads to the basic equation

$$\frac{d[\mathrm{RL}]}{dt} = [\mathrm{R}][\mathrm{L}]\, k_{\mathrm{on}} - [\mathrm{RL}]\, k_{\mathrm{off}}, \tag{2}$$

where k_{on} is the number of binding events per second per unit volume which would occur (nominally) if both $[\mathrm{R}] = [\mathrm{L}] = 1M$, and k_{off} is the probability to unbind per second. Note that k_{on} has to have "funny units", which reference a standard concentration, because a binding event depends on the concentrations of two molecules.



The figure sketches sample solutions of the behavior described by (2). The time evolution of the fraction of bound receptors is shown for two different initial conditions - but a given system will always relax to the same equilibrium. Note that noisy lines are purposely shown: even though solutions to (2) are perfectly smooth, actual behavior is stochastic as sketched.

Binding Equilibrium

From the definition of equilibrium, we expect the number of binding events per second will exactly balance the number of unbinding events. In a mass-action picture, this condition amounts to

$$[\mathrm{R}]^{\mathrm{eq}}\,[\mathrm{L}]^{\mathrm{eq}}\,k_{\mathrm{on}} = [\mathrm{RL}]^{\mathrm{eq}}\,k_{\mathrm{off}}, \tag{3}$$

(In this case setting $d[\mathrm{RL}]/dT = 0$ leads to equilibrium *only* because there are no inputs to, or outputs from, our binding system. In general, setting a time-derivative to zero leads only to a steady state - which could be in or out of equilibrium.)

The equilibrium (3) is usually re-written so that all concentrations are collected together, yielding

$$\frac{[\mathrm{R}]^{\mathrm{eq}}\,[\mathrm{L}]^{\mathrm{eq}}}{[\mathrm{RL}]^{\mathrm{eq}}} = \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} \equiv K_{\mathrm{d}} \tag{4}$$

where we have defined the dissociation constant K_{d} in the last equality. Note that a *smaller* K_{d} implies *stronger* binding.

The equilibrium point - the equilibrium concentrations $[\mathrm{RL}]^{\mathrm{eq}}$, $[\mathrm{R}]^{\mathrm{eq}}$, and $[\mathrm{L}]^{\mathrm{eq}}$ - depends on $[\mathrm{R}]_{\mathrm{tot}}$ and $[\mathrm{L}]_{\mathrm{tot}}$. That is, even for a given type of receptor and ligand, the fraction of bound complexes will depend on total concentrations of R and L. One consequence is that a weaker-binding ligand (higher K_{d}) could result in more bound complexes than a stronger-binding ligand (lower K_{d}) - if enough of the weaker binder is placed in solution.

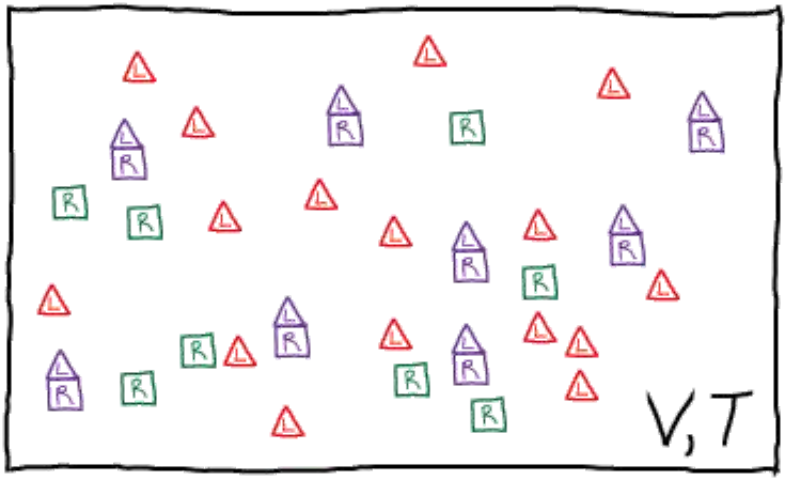
For reference, we note that the dissociation constant is the basis for defining the "standard" free energy change of binding:

$$\frac{[\mathrm{R}]^{\mathrm{eq}}\,[\mathrm{L}]^{\mathrm{eq}}}{[\mathrm{RL}]^{\mathrm{eq}}} = K_{\mathrm{d}} = 1\mathrm{M}\,e^{\Delta G_0^{\mathrm{bind}}/k_B T}. \tag{5}$$

$\Delta G_0^{\mathrm{bind}}$ refers to a standard state in which concentrations are measured in molar units. Note that a different choice of units (and pre-factor on the right) would lead to a different $\Delta G_0^{\mathrm{bind}}$ value for the same system.

Binding Thermodynamics

The thermodynamic equivalent of mass-action kinetics for binding is a mixture of ideal gases. In mass-action kinetics, after all, particles interact only depending on their concentrations: specific interactions - e.g., electrostatic - are not accounted for.



In our model, we will have ideal gases of R, L, and RL particles. We will assume, however, that there is a (free) energy change of $\Delta\mu$ for every bound complex, or $N_{RL}\,\Delta\mu$ total. Presumably $\Delta\mu < 0$ for favorable binding, although our formalism does not require that. The total free energy thus consists of the three ideal gas free energies, plus the binding term.

$$F(N_R, N_L, N_{RL}) = F^{\text{idl}}(N_R) + F^{\text{idl}}(N_L) + F^{\text{idl}}(N_{RL}) + N_{RL}\Delta\mu \quad (6)$$

Note that all three "gases" are in the same volume at the same temperature. The explicit form for F^{idl} has been derived separately.

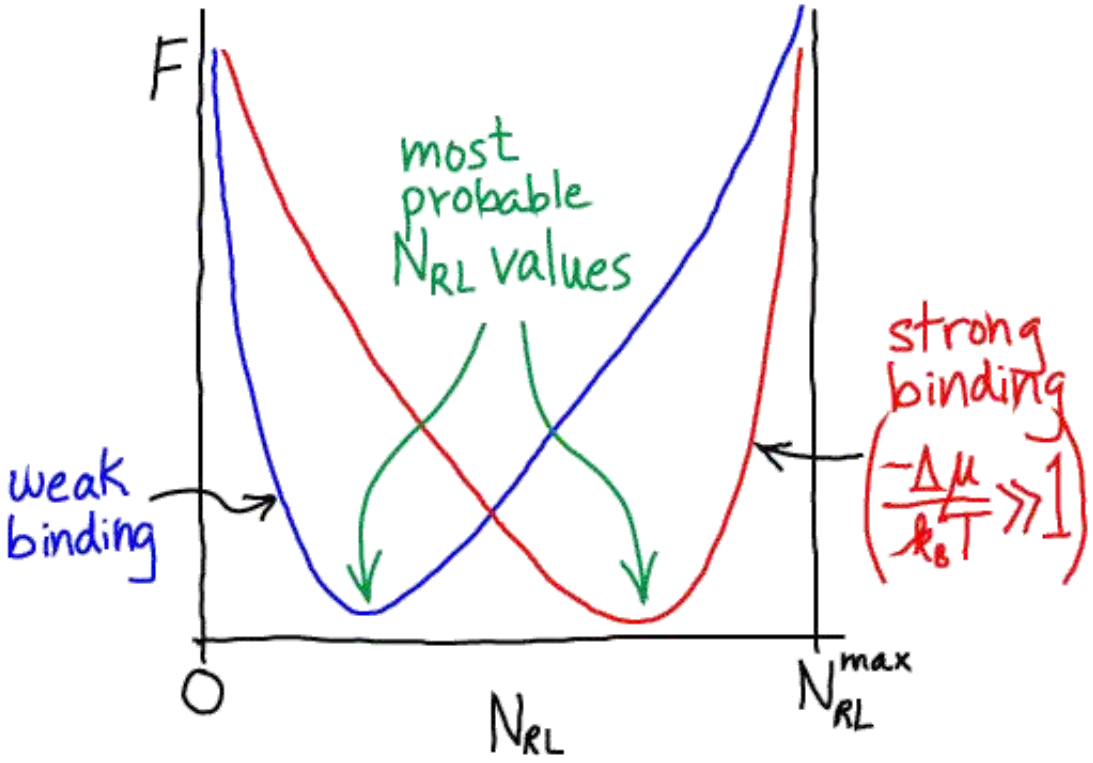
In analogy to Eqs. (1), the numbers of particles are not independent because a binding event changes the identity of a molecule.

$$\begin{aligned} N_R^{\text{tot}} &= N_R + N_{RL} \\ N_L^{\text{tot}} &= N_L + N_{RL} \end{aligned} \quad (7)$$

The free energy then gives us **the probability to observe a given N_{RL} value** via

$$p(N_{RL}) \propto e^{-F(N_R^{\text{tot}} - N_{RL},\, N_L^{\text{tot}} - N_{RL},\, N_{RL})/k_B T}. \quad (8)$$

The reason for this is explained in our discussion of free energy in a concentration gradient. In brief, the Boltzmann factor of a free energy is *defined* to be the sum of all probability consistent with the specified condition - the N_{RL} value in our case.



In the limit of many molecules, the equilibrium point of the system is well approximated by the *most probable value*, which we can find by minimizing F in Eq. (6). Some algebra is required to get the derivative, but setting it to zero, collecting logs, and exponentiating, yields

$$\frac{[\mathrm{R}]^{\mathrm{eq}}\,[\mathrm{L}]^{\mathrm{eq}}}{[\mathrm{RL}]^{\mathrm{eq}}} = \frac{1}{\lambda^3 e}\, e^{\Delta\mu/k_BT}. \tag{9}$$

This is the same form as Eq. (4) or (5): the particular ratio of concentrations on the left is seen to depend only on constant parameters of the system. We can see further that the ratio is proportional to the Boltzmann factor of the free energy change per complex formed. In other words, $\Delta\mu = \Delta G_0^{\mathrm{bind}} + \mathrm{const.}$

References:

- D.M. Zuckerman, *Statistical Physics of Biomolecules: An Introduction* (CRC Press, 2010).
- J. Kuriyan, B. Konforti, and D. Wemmer, *The Molecules of Life: Physical and Chemical Principles* (Garland Science, 2013).
- R. Phillips et al., *Physical Biology of the Cell,* (Garland Science, 2009).
- K. Dill and S. Bromberg, *Molecular Driving Forces* (Garland Science, 2010).

Exercises:

1. Using Eqs. (1) and (2), derive the time-dependence of $[\mathrm{RL}]$ in terms of the initial concentration(s) and the rate constants. *Hint: The solution is exponential.*

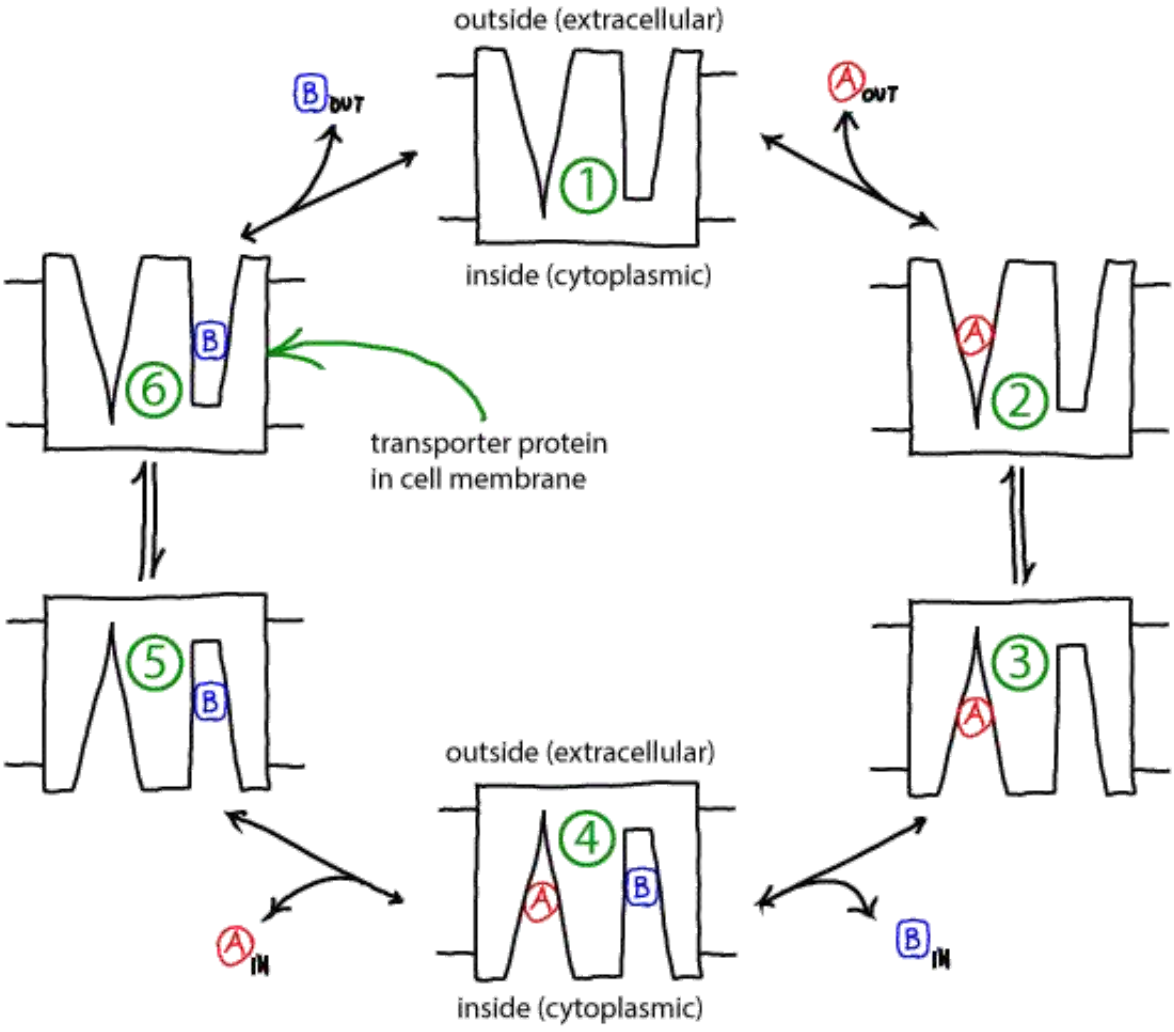
2. Using Eq. (4), show that K_{d} is equal to the equilibrium ligand concentration at which precisely half the receptors are bound. This fact enables K_{d} to be measured by titrating ligand in any experiment in which the measured signal is proportional to concentration.
3. Derive Eq. (9).

Binding: Allostery (in a transport cycle)

Active transport in an alternating-access antiporter model

Free energy stored in a non-equilibrium gradient of concentrations across a membrane can be used by an antiporter ("exchanger") to pump another molecule against its gradient.

- Membrane-embedded transporter proteins can function as machines because binding events are coupled to conformational changes.
- The cycle below is a simplified, schematic model of a transporter for which binding events are coupled to "eversion" - transition between inward-facing and outward-facing conformations.
- The alpha helices in membrane proteins achieve eversion in roughly the same manner as you might re-adjust the orientations of a group of irregular pens and pencils held in your hand.



Basics of the antiporter cycle

- The antiporter schematized above can perform active transport using the gradient of one molecule or

ion (e.g., A) to pump the other (B) in the opposite direction. Free energy is supplied by the gradient(s) -- *no other source of free energy, such as ATP, is needed.*

- If the ratio of outside to inside concentrations of one molecule (e.g., A) is higher than the other, the cycle will be driven in a particular direction (e.g., clockwise).
- A transporter such as the antiporter shown can be considered a "passive element": it does not supply any energy itself, but only transduces free energy stored in gradients. The transduction occurs due to the coordination of binding and conformational changes.
- Because it is a passive element, the antiporter does not "know" which molecule's gradient is meant to drive the transport of the other. Thus, if the outside/inside concentration ratio is greater for B, then the cycle will run counter-clockwise and A will be pumped by B.
- The model as shown is a simplified version that omits some possible states and connections among states - e.g., both binding sites are assumed to always face the same direction. Additional states and connections could reduce the efficiency of pumping. See also discussion in Hill's book. The presence of additional states or connections would have to be verified on a system-by-system basis.

Understanding how the cycle is driven

- A simple but powerful qualitative analysis starts by considering the condition of equilibrium. Equilibrium describes a (hypothetical) very large set or ensemble of identical systems of which - on average - an equal number are executing the forward and reverse of every process. Thus, for example, between steps 1 and 2, if there are N_{12} systems in which extracellular ("outside") A binds every second, there is an equal number ($N_{21} = N_{12}$) for which A unbinds to the outside.
- In the perfectly balanced state of equilibrium, there will be equal numbers of systems performing full clockwise and full counter-clockwise cycles per unit time. Thus, although individual cycles may pump A and B, there is no net pumping.
- Because the balance of equilibrium is so perfect, however, it can be disturbed at almost any point in the cycle. For example, adding an excess of A outside will cause more 1-to-2 transitions, which will cause 2-to-3 transitions and so on, leading to clockwise cycling. It is the binding process that provides directionality. *Binding events can be considered the "handles" used to drive a cycle in a given direction.*
- Importantly, the cycle can also be driven by imbalancing the detailed equilibrium at *any* point in the cycle:
    - Excess B added inside will also drive the cycle clockwise.
    - Excess A added inside will drive the cycle *counter*-clockwise.
    - Excess B will also drive the cycle counter-clockwise.

A quantitative model: Simulation and analysis

- We will employ a chemical-kinetics model, which is formulated solely in terms of state populations and rate constants for transitions among states, assumed to obey mass-action behavior.

- To keep the model simple, we will assume all rates are the same:
  - k_{on} will be the on-rate (see Notation) for all A and B binding -- for transitions from state 1 to 2, from 1 to 6, from 3 to 4, and from 5 to 4.
  - k_{off} will be the off-rate for all unbinding of A or B -- reversals of the transitions noted above.
  - k_{conf} will be the rate for all conformational transitions -- both directions between states 2 and 3, and between 5 and 6.
  - We will assume that the outside and inside volumes are the same -- so that the numbers of A and B molecules tell us the concentrations in a simple way.

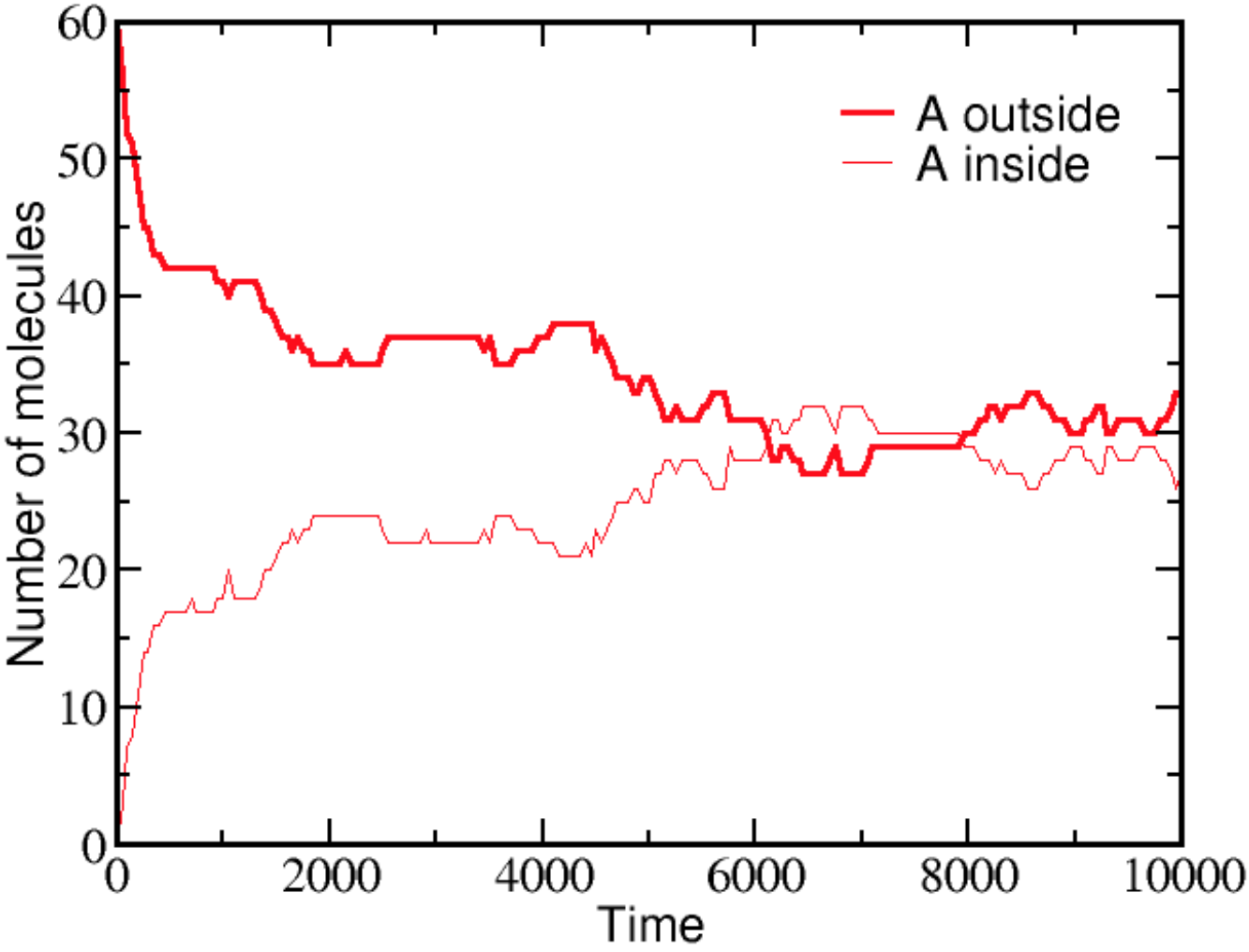
- The model is now fully specified. For example, the differential equation governing the population of state 1 (denoted [1]) is given by

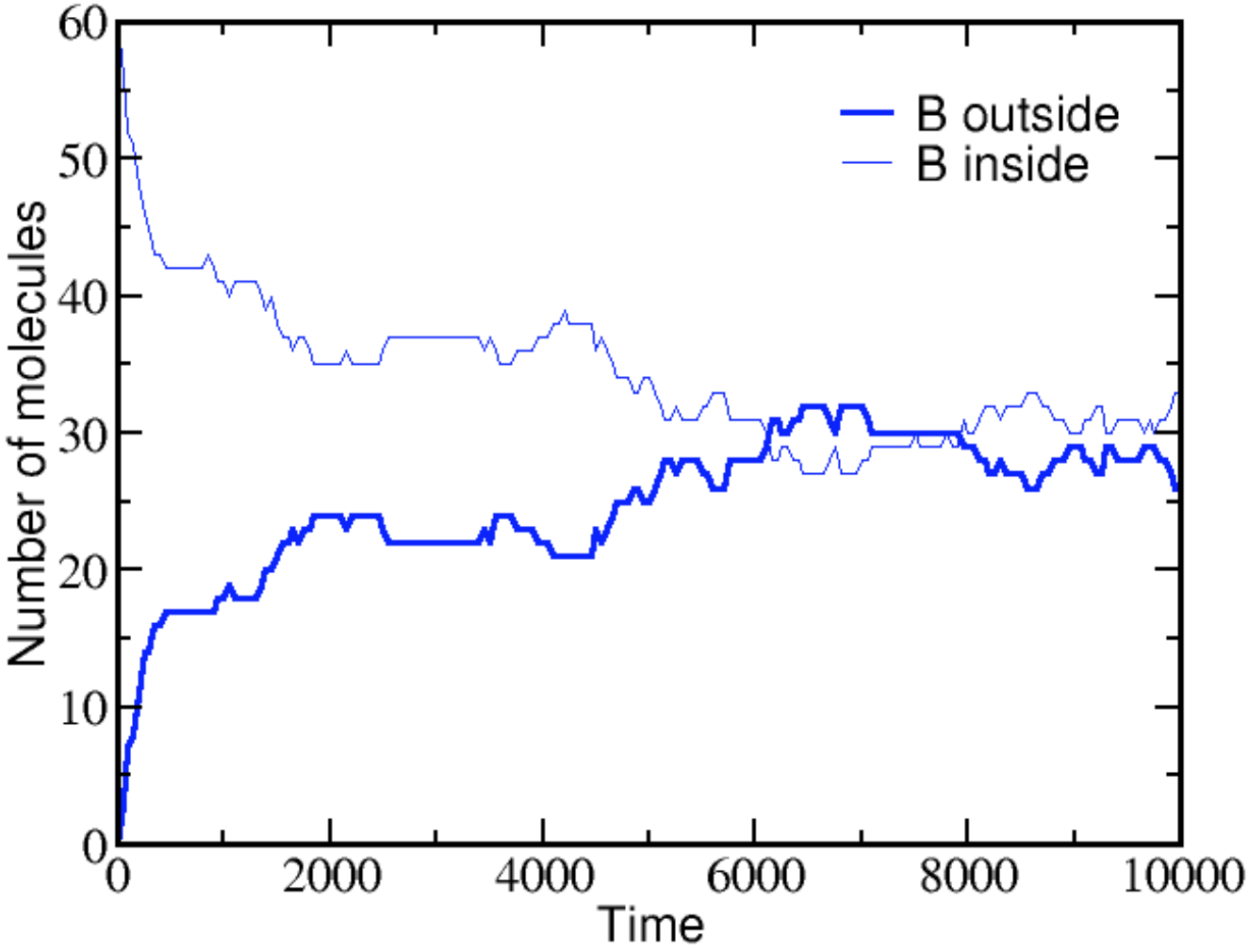
$$\frac{d[1]}{dt} = -[1][\text{A}]_{\text{out}}\, k_{\text{on}} - [1][\text{B}]_{\text{out}}\, k_{\text{on}} + [2]\, k_{\text{off}} + [6]\, k_{\text{off}}$$

  - In words, the equation means that the population of the unbound state 1 decreases due to binding of A or B (from the outside) with rate k_{on} and increases due to unbinding from either of states 2 or 6 with rate k_{off}.

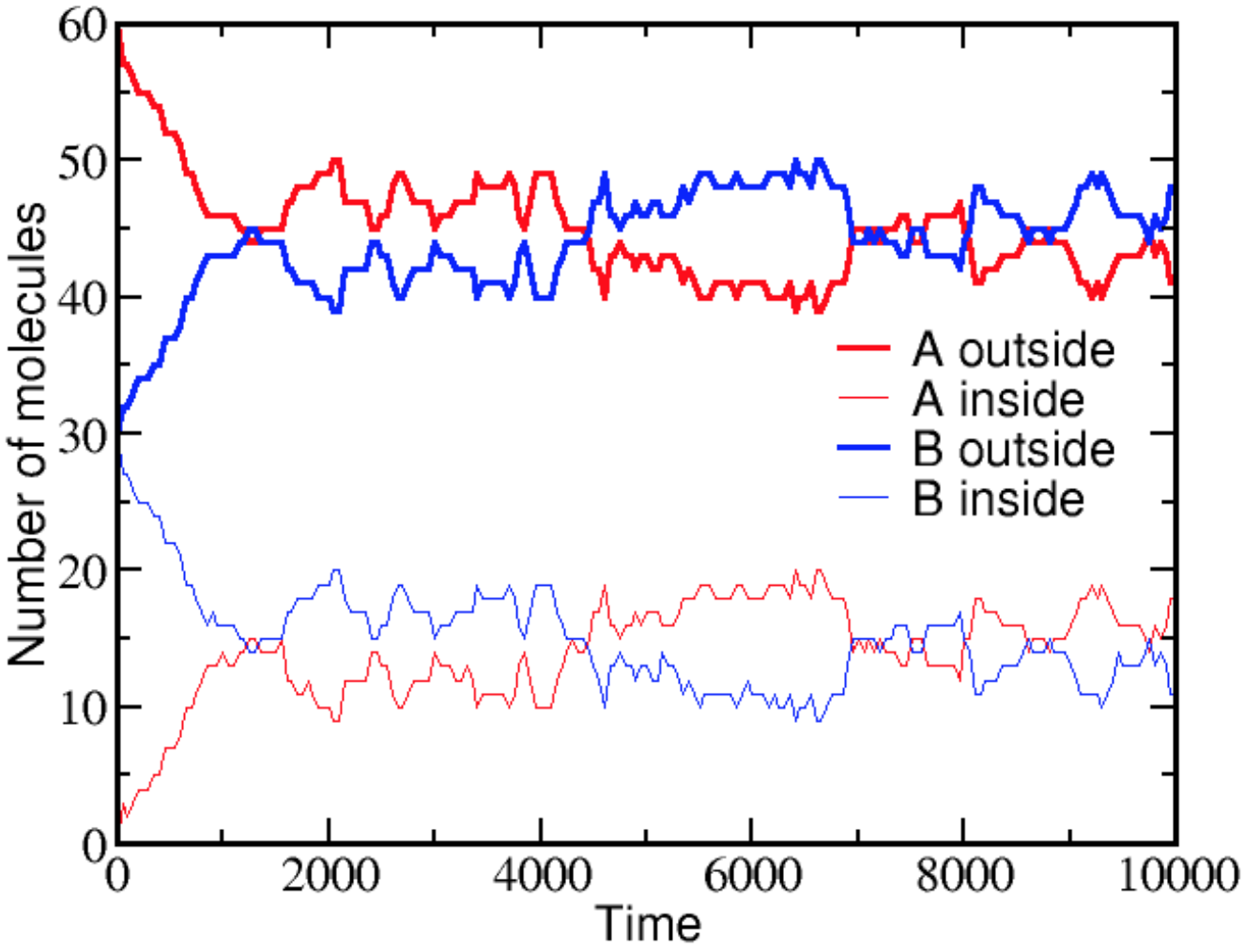
Simulating the model

If we start a simulation with all A molecules on the outside and an equal number of B molecules solely on the inside, the system equilibrates to equal concentrations on both sides -- for both species (A and B).


A outside
A inside
Number of molecules
Time
0
10
20
30
40
50
60
0
2000
4000
6000
8000
10000



However, if we start a simulation with B molecules equally distributed between outside and inside, and the A molecule all outside as before, there is a very different outcome. The greater outside concentration of A tends to drive A molecules inward, but doing so requires "pumping" B molecules to the outside. Because the B molecules "resist" having different outside and inside concentrations, the final equilibrium is one where the ratio of outside/inside concentrations equalizes between the two species.



These simulations were performed using BioNetGen, a rule-based platform for kinetic modeling. The brief source code for the model (a .bngl file) can be downloaded by right-clicking here.

Analyzing the model

Perhaps the simplest and most important analysis of the model is deriving and understanding the equilibrium behavior. In equilibrium, every process and its reverse will be in exact (detailed) balance -- the flow in both directions will match. Hence, we need to write down balance equations for each set of arrows (connecting states 1 and 2, 2 and 3, ...). Using $[\mathrm{X}]^{\mathrm{eq}}$ to refer to the equilibrium concentration of component X, we write down the equilibrium equations for the three processes on the right side of the cycle.

$$[1]^{\mathrm{eq}}\,[\mathrm{A}]^{\mathrm{eq}}_{\mathrm{out}}\,k_{\mathrm{on}} = [2]^{\mathrm{eq}}\,k_{\mathrm{off}} \qquad (1)$$

$$[2]^{\mathrm{eq}}\,k_{\mathrm{conf}} = [3]^{\mathrm{eq}}\,k_{\mathrm{conf}} \qquad (2)$$

$$[3]^{eq}\,[B]_{in}^{eq}\,k_{on} = [4]^{eq}\,k_{off} \tag{3}$$

The equations for the left side mirror these.

Solving the full set of equations (by going around the cycle and eliminating one of the numbered-state concentrations at a time) yields the result

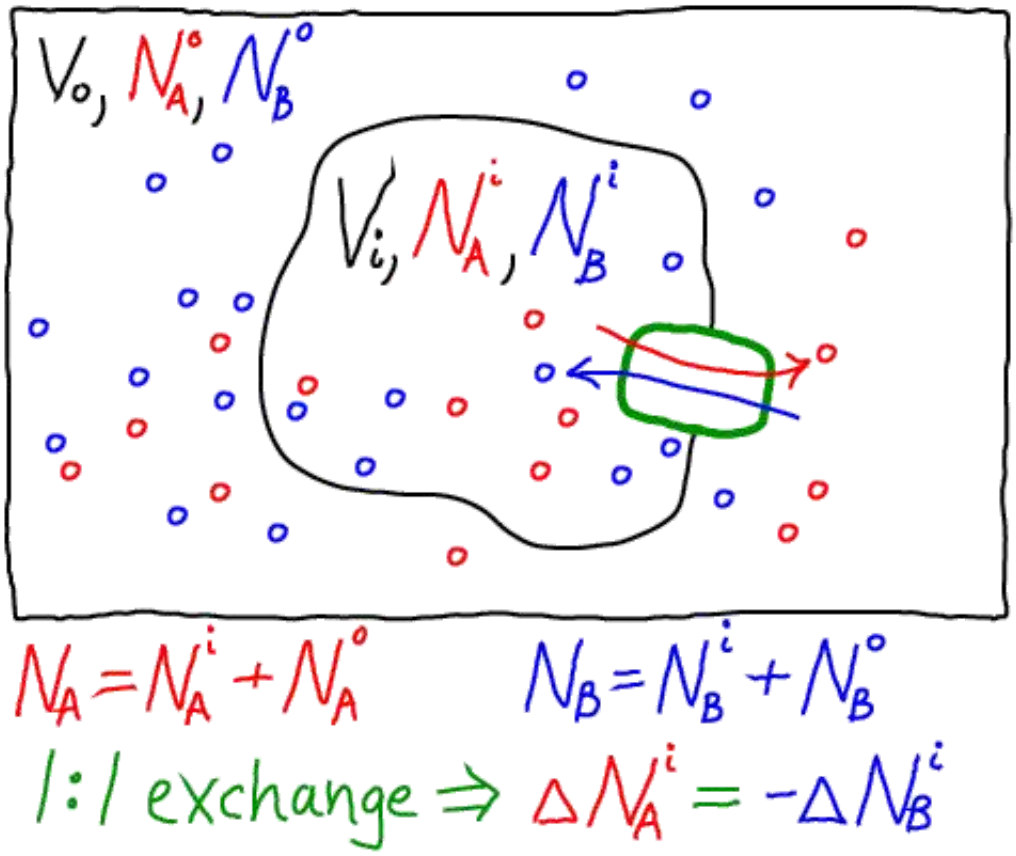
$$\frac{[A]_{out}^{eq}}{[A]_{in}^{eq}} = \frac{[B]_{out}^{eq}}{[B]_{in}^{eq}} \tag{4}$$

As we saw in the simulations, equilibrium is reached when the outside-to-inside concentration ratios match for A and B. In fact, because our transporter is a passive element, this result is independent of the rates chosen -- even if the rates are not symmetric (but be careful that the set of rates in a cycle has to be physically consistent).

Note that, perhaps surprisingly, the equilibrium point will depend on the initial condition. This is true because our simple antiporter model does not allow "slippage" (where only an A or a B is transported in some cycles). If there were some mechanism for A and/or B to switch compartments independently of the other, then the equilibrium point would not only have equal concentration ratios, but the ratios would be 1 -- equal inside and outside concentrations. A more advanced discussion of the antiporter cycle describes further possibilities and the relation to a symporter cycle.

Thermodynamic analysis

In contrast to the kinetic analysis above, which includes a description of specific states of the antiporter, we can perform a thermodynamic analysis of the same system. Using an ideal gas description, in analogy to what was done for binding and for understanding free energy storage in a gradient, arguably leads to a simpler description than we encountered using kinetics. The thermodynamic analysis will directly lead to the equilibrium condition (4).



The thermodynamic model consistent with the mass-action picture above consists of ideal gases for each of the components: A molecules inside (i) and out (o), as well as B molecules inside and out. The combined free energy F for the whole system is the sum of the free energies for each of these ideal gases.

$$\begin{aligned} F =& F^{\text{idl}}(N_A^i, V_i) + F^{\text{idl}}(N_B^i, V_i) \\ &+ F^{\text{idl}}(N_A^o, V_o) + F^{\text{idl}}(N_B^o, V_o), \end{aligned} \tag{5}$$

where we have omitted the temperature dependence that will not be pertinent here. The total free energy is the simple sum of the component free energies because there are no (energetic) interactions between the components. In an ideal gas, by definition, there are no interactions among molecules.

To obtain concrete results, we must specify one specific state of our system - something like an initial condition. After all, the 1:1 exchange of A and B means that not every possible set of populations ($N_A^i, N_A^o, N_B^i, N_B^o$) is possible. For simplicity, we shall assume that our reference state has all A molecules inside ($N_A^i = N_A, N_A^o = 0$) and all B molecules outside ($N_B^o = N_B, N_B^i = 0$).

Further, because we are considering a 1:1 antiporter (where one A always exchanges for one B), there is really only one variable that can change. To see this, we can express all the species numbers in terms of N_A^o, the number of A molecules outside, and the *constant* species totals N_A and N_B:

- because an A molecule must be either inside or out, we have $N_A^i = N_A - N_A^o$;
- because of the 1:1 exchange, $N_B^i = N_A^o$; and
- because a B molecule must be either inside or out, $N_B^o = N_B - N_B^i = N_B - N_A^o$.

Using these relations and noting that $F^{\text{idl}}(N, V, T) = Nk_BT \ln[N/(V/\lambda^3)]$, we can now re-write the total free energy from (5) as

$$\begin{aligned}\frac{F}{k_BT} =& \left(N_A - N_A^o\right) \ln\left(\frac{N_A - N_A^o}{V_i/\lambda_a^3}\right) \\ &+ N_A^o \ln\left(\frac{N_A^o}{V_o/\lambda_a^3}\right) + N_A^o \ln\left(\frac{N_A^o}{V_i/\lambda_b^3}\right) \\ &+ \left(N_B - N_A^o\right) \ln\left(\frac{N_B - N_A^o}{V_o/\lambda_b^3}\right)\end{aligned} \tag{6}$$

We find the most probable state (which is equilibrium in the large N limit) by setting to zero the derivative of F with respect to N_A^o. After some algebra, the result is

$$\frac{N_A^o/V_o}{N_A^i/V_i} = \frac{N_B^o/V_o}{N_B^i/V_i}, \tag{7}$$

which is equivalent to (4) because N_X/V is the concentration of species X.

Importantly, *the thermodynamic analysis does not require knowledge of the system's substates, but only the 1:1 stoichiometry*.

More on antiporters

The stoichiometry of transport has very interesting effects: see the discussion of 2:1 exchange. Also, all the analysis above assumed perfect 1:1 exchange. In reality slippage could be important, and is a critical general phenomenon in complex molecular machines.

References:

- B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).
- T.L. Hill, "Free Energy Transduction and Biochemical Cycle Kinetics," (Dover, 2005). Absolutely **the** book on cycles. Describes the effects of including additional states and transitions.

Exercises:

1. Derive (4).
2. Derive (4) using arbitrary rates $\{k_{12}, k_{21}, k_{23}, \dots\}$. In doing so, you will want to be sure your cycle is "thermodynamically consistent" with equilibrium principles. In other words, you will need to determine a constraint, which can be done by assuming the system starts at the state of minimum global free energy in which each species has equal concentrations on both sides.
3. Derive (7).

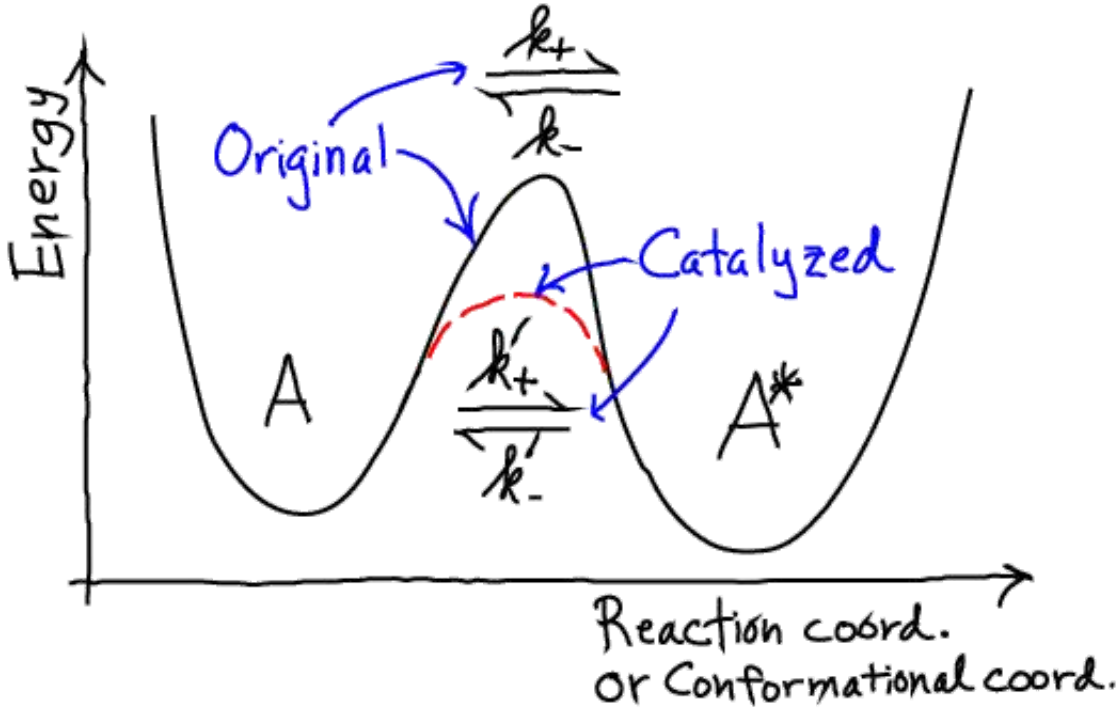
Chemical Reactions: Catalysis

Essentials of Catalysis (Enzyme Action)

Key points

- **Catalysis** is the acceleration of a molecular process, such as a chemical reaction (i.e., covalent change), isomerization (i.e., conformational change), or a binding process. The catalyst remains unchanged after the process.
- **It goes both ways:** All molecular processes are reversible, and a given catalyst (e.g., an enzyme) necessarily catalyzes both directions of the reaction equally well, as explained below. This key point is sometimes overlooked.
- **In the cell:** Very few chemical reactions necessary for cellular activity (e.g., phosophorylation, chemical synthesis) occur spontaneously during the lifetime of a cell. This necessitates catalysis, but more importantly, *provides a means for the cell to regulate its processes.* In essence, reactions only happen when the necessary enzyme is present and active, and the presence/activity of enzymes is tightly regulated through control of protein expression, degradation, and post-translational modifications such as phosphorylation.

Basic Catalysis: Isomerization or Unimolecular Chemical Change



- Isomerization is a conformational change in a small molecule or a macromolecule like protein, RNA, or DNA.

- A unimolecular reaction is one affecting only a single molecule, perhaps by forming or breaking a single covalent bond within the molecule.
- Either situation can be schematized using a simple energy landscape, in which high energy barriers are rarely overcome (i.e., characterized by low rates).

In catalysis, rates increase. Denoting catalyzed rates with primes, we have

$$k'_+ > k_+ \quad \text{and} \quad k'_- > k_-. \tag{1}$$

However, catalysis cannot change the equilibrium ratio of the state populations $[\mathrm{A}]/[\mathrm{A}*]$. Mathematically,

$$[A]^{eq} k_+ = [A^*]^{eq} k_- \Rightarrow$$

$$\frac{[A]^{eq}}{[A^*]^{eq}} = \underbrace{\frac{k_+}{k_-}}_{\text{Original}} = \underbrace{\frac{k'_+}{k'_-}}_{\text{Catalyzed}}$$

In words, the catalyzed rates *increase together* so as to maintain the proper equilibrium - which is unaffected by the presence of a catalyst.

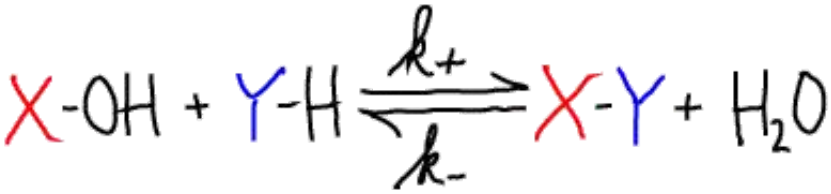
There is a nice way to convince yourself that catalysis cannot change the equilibrium point - it is a worthwhile **exercise**. Consider a cycle in which the two states of interest are connected by both a catalyzed and uncatalyzed process. You can show that, if the ratio of forward and reverse rates does not agree for the two cases, the cycle will spontaneously circulate - violating the second law of thermodynamics and enabling the construction of (imaginary) energy-creating processes.

References:

- J. M. Berg et al., "Biochemistry", W. H. Freeman. The 2002 edition is online for free.
- B. Alberts et al., "Molecular Biology of the Cell", Garland Science (many editions available).
- Any bioenergetics or biochemistry book will discuss catalysis.

Reaction coupling

Biosynthesis is Driven by Free Energy



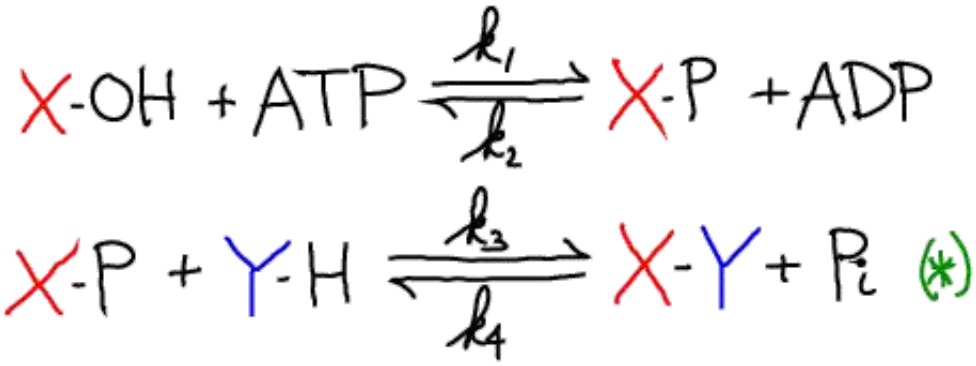
Chemical syntheses in the cell, such as the formation of X-Y above, typically require an input of free energy. Free energy could be supplied directly if there were an over-abundance of reactants (compared to equilibrium) and/or a dearth of products. More commonly, there is a complex sequence of reactions required for synthesis, and free energy drives these intermediate reactions. Below, we see how the hydrolysis of ATP can be coupled to a series of two synthesis reactions. Other carriers could also supply the free energy to drive synthetic reactions.

Note that if a synthetic reaction were favorable (did not require free energy), it would only be necessary for the cell to supply a catalyst.

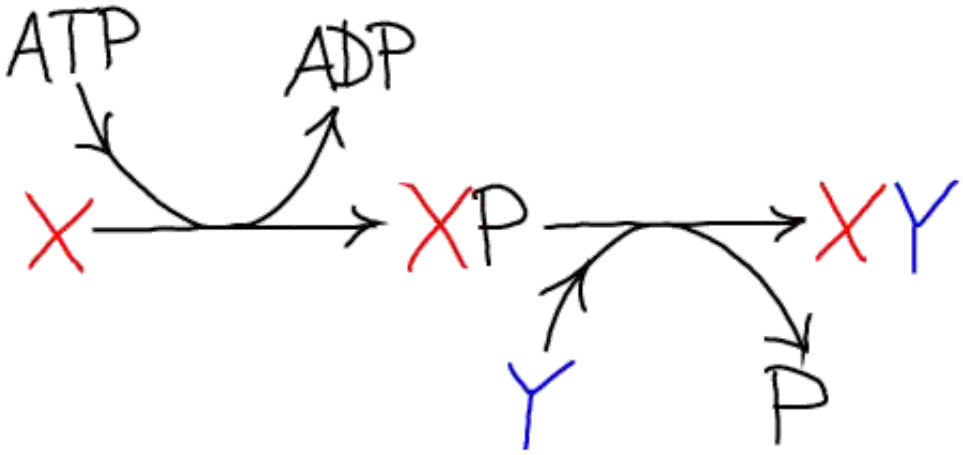
Reaction coupling, driven by ATP

An example of the mechanism here is the synthesis of glutamine from glutamic acid, where X-OH = glutmatic acid, Y-H = NH_3. Below, -P = -O-PO_3.

The synthesis of X-Y can be coupled to ATP hydrolysis by the following two reactions



The net result of the two reactions is the synthesis of X-Y *and* the hydrolysis of ATP. Graphically the process looks like this



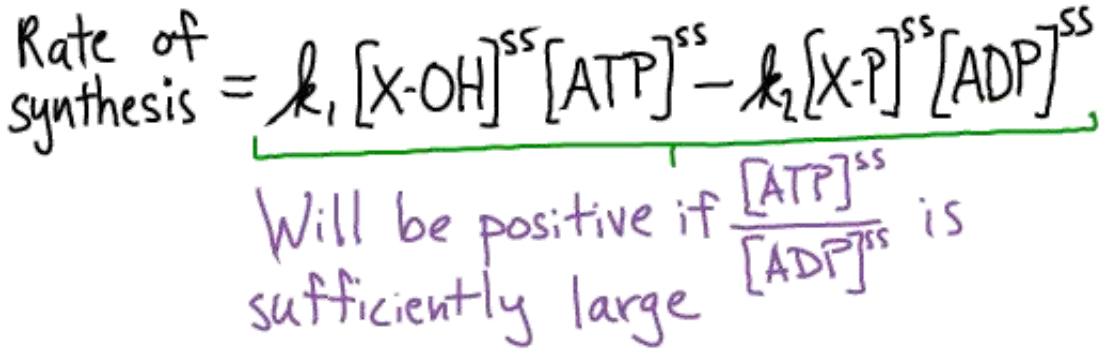
Steady-state analysis of the coupled reactions

Will the two-step synthesis above really occur under typical cellular conditions, even if the direct synthesis ($X + Y \rightarrow XY$) is unfavorable? We can answer this question using a steady-state analysis of the equations above in a mass action picture. Specifically, we can calculate the rate of formation of XY (and Pi) in the second of the two reactions, which is marked with a green (*). We use a steady-state condition on the intermediate XP:

$$0 = \frac{d[X\text{-}P]}{dt} = k_1[X\text{-}OH][ATP] - k_2[X\text{-}P][ADP] - \{k_3[X\text{-}P][Y\text{-}H] - k_4[X\text{-}Y][P_i]\}$$

Precisely net flow to right in (*) = rate of synthesis

Because the last two terms are precisely the net rate of XY and Pi synthesis - based on Eq (*) - and the four terms together sum to zero in a steady state, we can rewrite the synthesis rate to see the dependence on ATP and ATP:



So long as the ratio $[ATP]/[ADP]$ is sufficiently large, the rate of XY synthesis will be positive. And because ATP is an activated carrier that ratio *is* large.

Bottom line: XY is synthesized at a positive rate if ATP is sufficiently activated.

References:

- B. Alberts et al., "Molecular Biology of the Cell", Garland Science (many editions available). See the 2nd chapter's discussion of ATP-driven synthesis.
- J. M. Berg et al., "Biochemistry", W. H. Freeman. The 2002 edition is online for free.
- Any biochemistry or cell biology book will discuss synthesis.

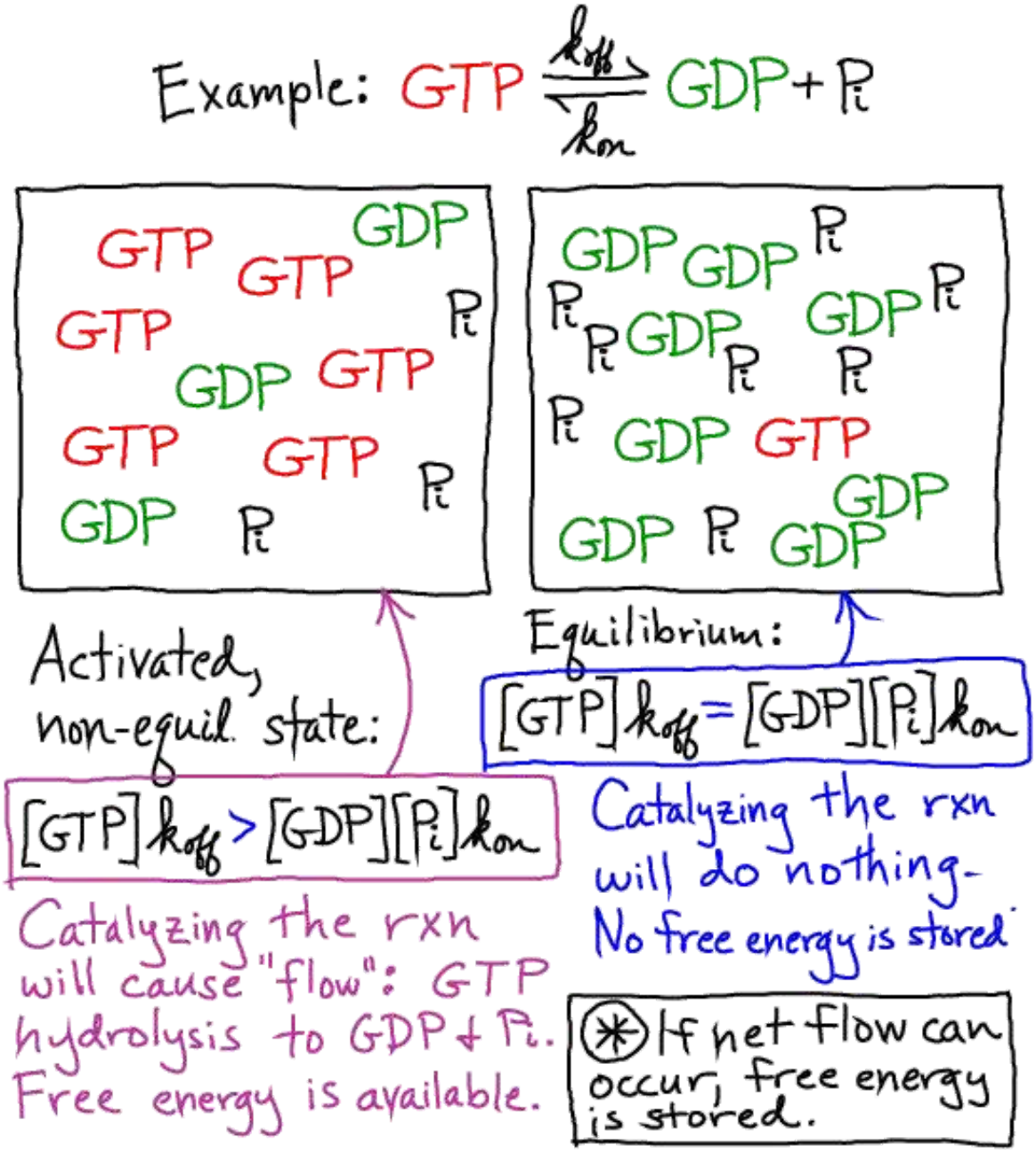
Energy Storage in Metastable Conditions

Free Energy is the Cell's Available Energy

Free energy is energy available to do work.

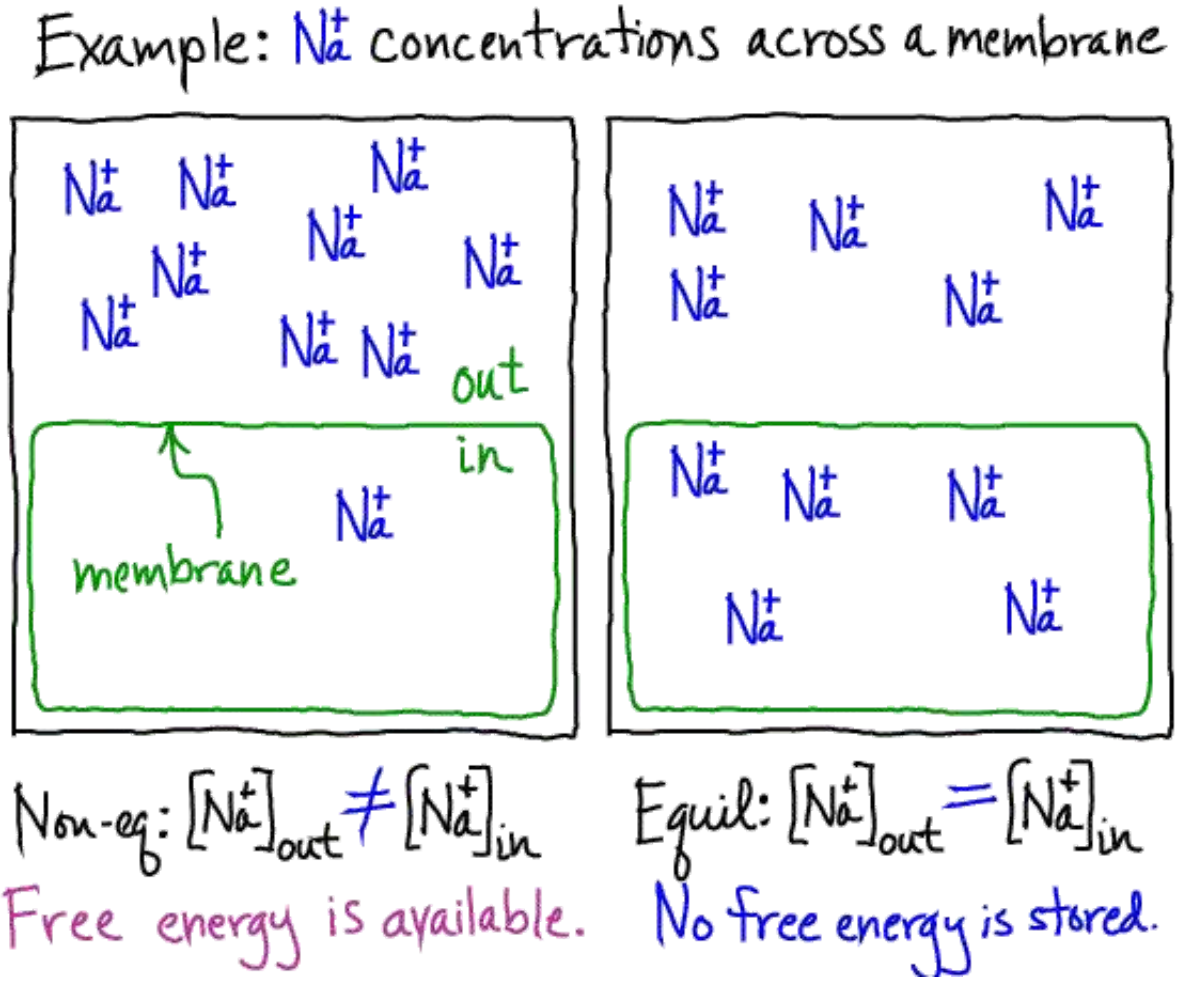
- It can be considered the analog of potential energy in a "thermal environment" (where molecular collisions substantially alter the potential and/or kinetic energies of objects of interest - i.e., proteins, solvent, etc.).
- Systems will move from a condition of high to low free energy if it is possible: a ball will roll downhill in the absence of a barrier.
- The cell stores free energy in two primary ways:
  - (1) a gradient - i.e., differing concentrations of ions or small molecules across a membrane, as is the case for some transporters.
  - (2) activated carriers - i.e., molecules capable of dissociating, but which are maintained at a concentration well above the equilibrium value, such as ATP and GTP.
- Any system that is out of equilibrium stores free energy that can be used for work - e.g., to drive cellular processes such as transport, locomotion, synthesis - or signaling processes. Conversely, *an equilibrium system stores no free/usable energy* unless the conditions are changed.

Activated Carriers Store Free Energy



The cell maintains the concentration of an activated carrier (e.g., GTP or ATP) well above its equilibrium value so that there is always a driving force toward equilibrium (via the decomposition reaction). Because there is a drive toward equilibrium, there is free energy which can be harnessed for work - as in the example of ATP-driven transporters.

Concentration Gradients across Membranes Store Free Energy



As in the case of activated carriers, gradients are also out of equilibrium: there is a driving force to equalize the concentrations of species across a membrane (assuming for simplicity no coupling among species). A more quantitative discussion of this phenomenon is available. The cell can use gradient-stored free energy, for instance in the case of active transport.

The Cell Uses Free Energy in Different Ways

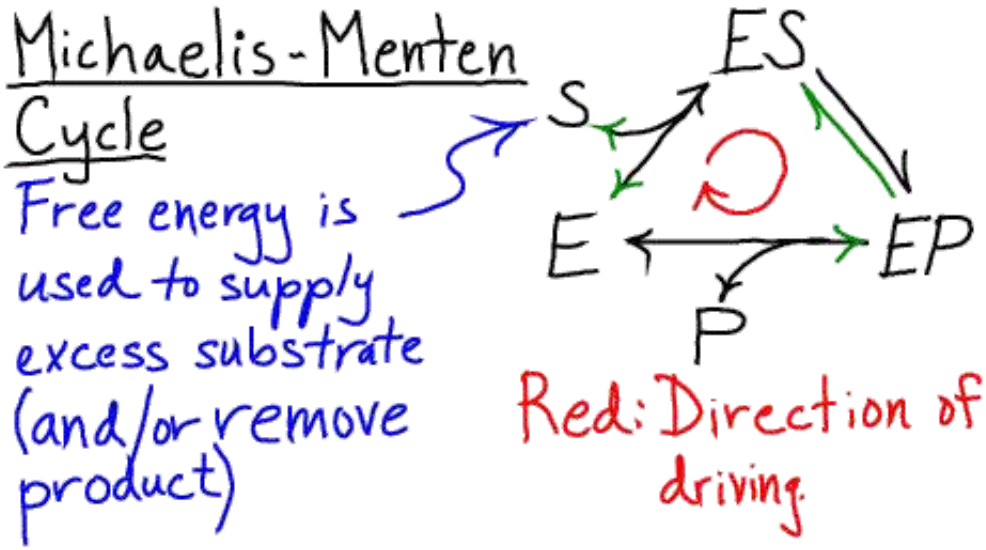
In general, the cell uses free energy it stores in gradients and activated carriers to accomplish two types of things:

- (1) Work in the usual sense - i.e., energetically unfavorable tasks such as transport (against a gradient), locomotion, and chemical synthesis.
- (2) Energy-neutral signaling processes, such as phosphorylation, which involve a specific sequence of events that may leave the signal carriers (e.g., proteins) unchanged.

How does the free energy remain stored without "running downhill"? Free energy in the cell will not dissipate unless the transition from the high-free-energy non-equilibrium state toward a lower free-energy state is catalyzed: for activated carriers, enzymes are required; for gradients across membranes, flow is enabled by channels or transporters.

How does the cell get energy? The cell maintains its supply of ATP and other stored energy by metabolizing nutrients in an ongoing cycle of ATP synthesis.

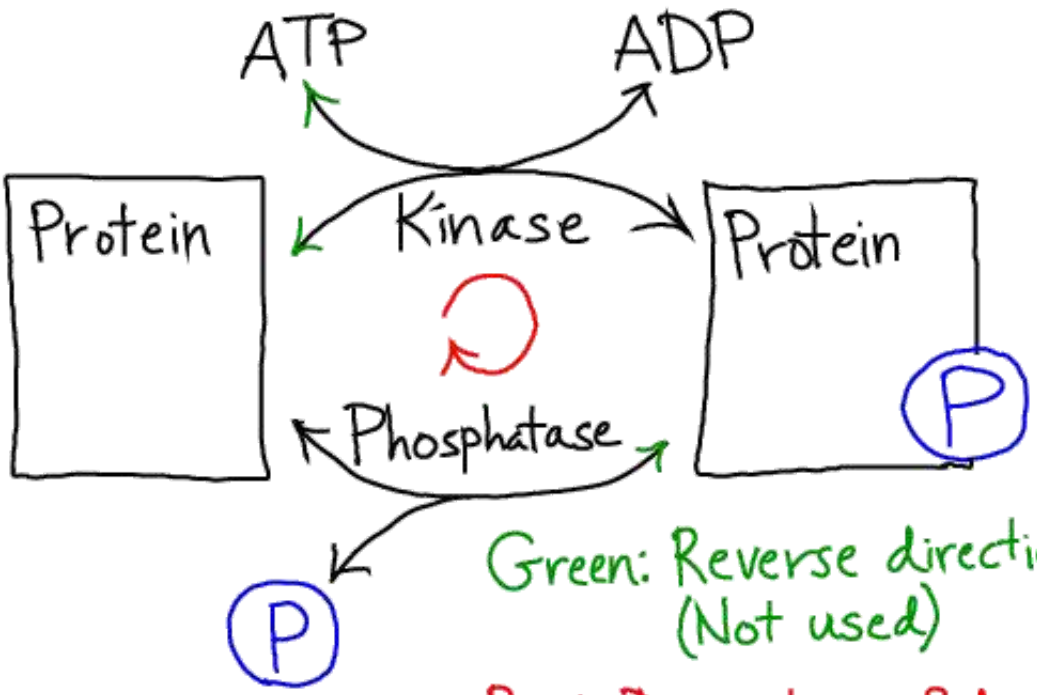
Cycles and the Cell's Non-equilibrium Use of Free Energy



Cellular processes typically function in cycles to re-use molecular components, and free energy is used to drive such cycles in a single direction by maintaining some components out of equilibrium - e.g., supplying excess ATP or other substrates for catalysis.

Phosphorylation Cycle

Free energy is used to synthesize excess ATP, which drives the cycle



Green: Reverse direction (Not used)

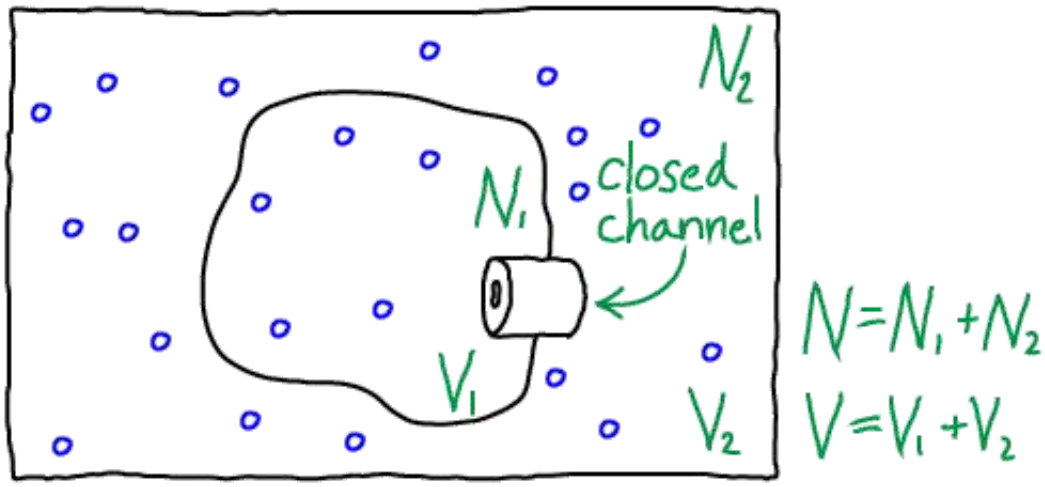
Red: Direction of driving.

Simple Concentration Gradient Across a Bilayer

The Free Energy in a Concentration Gradient

One key way the cell stores free energy is by having different concentrations of molecules in different "compartments" - e.g., extra-cellular vs. intracellular or in an organelle compared to cytoplasm. Here, we will study the simplest example of such a *gradient*, differing concentrations of ideal gas molecules across an idealized rigid membrane. Elsewhere, we consider a simple model of ion concentration gradient across a membrane.

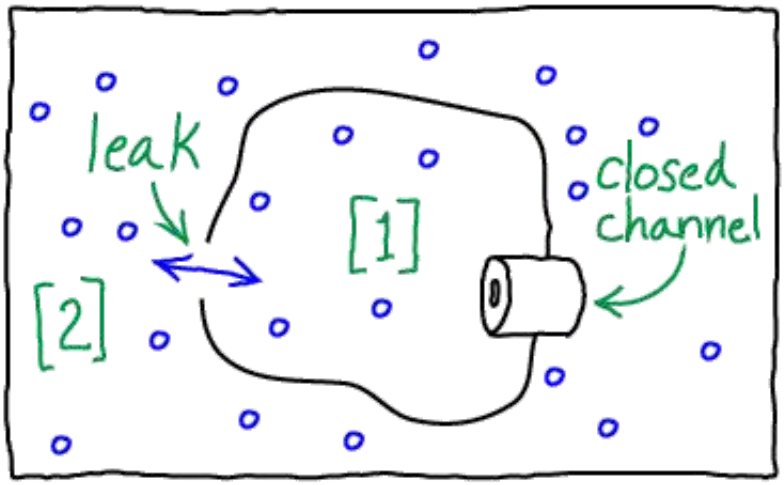
Two ideal gases separated by a barrier



Analyzing the model depicted above will enable us to understand free energy storage in a concentration gradient, but the basic ideas generalize to activated carriers as well.

To be precise, the model consists of N non-interacting atoms in the volume V maintained at constant temperature T. Beyond the simple ideal gas studied elsewhere, our present system is divided into two compartments by a rigid "membrane," with V_1 the volume of the inner compartment and V_2 the outer volume such that $V_1 + V_2 = V$. Similarly, there are N_1 atoms in the inner compartment and N_2 outside, with $N_1 + N_2 = N$. Although particles could pass through the channel shown in the figure, we assume it is closed so that N_1 and N_2 are constant.

A quick mass-action analysis



We can derive the key result for this system very quickly using a mass action "thought experiment". Our simple kinetic analysis will provide a key reference when we delve into some specific limitations of the mass-action picture in the context of ionic gradients.

Instead of our original system, we consider the leaky cell or organelle shown above. The leak should be considered a simple hole (unlike a channel, which is expected to interact strongly with molecules passing through it). Hence the inside-to-outside rate constant k_{12} for the leak/hole must be equal to the outside-to-inside rate constant k_{21}. After all, if the hole is large enough the "atoms" will not interact with the membrane at all - or if they do, the effects should be symmetric.

In equilibrium, we know that the total number of events in each direction will match: $[1]\,k_{12} = [2]\,k_{21}$. Cancelling the equal rates on both sides of this relation, we find that the **equilibrium concentrations must be equal inside and outside**:

$$[1]^{\mathrm{eq}} = [2]^{\mathrm{eq}}. \tag{1}$$

Deriving this result thermodynamically in a careful way requires more effort (see below).

This equilbrium result for the leaky condition actually tell us something about the channel rates. Because the channel is a passive element that uses no energy, it cannot change the (equal) equilibrium concentrations just derived. Hence, applying the principle of detailed balance to the channel (which implies the flows through the channel must be equal and opposite) we see that the *channel rate constants must be equal in both directions*. This perhaps obvious result only holds for a channel separating two systems with no driving force or external field applied - a condition which breaks down in the case of trans-membrane ionic gradients.

Deriving the free energy

Because we have non-interacting ("ideal") particles which cannot exchange across the membrane, the total free energy for the combined systems F^{comb} is simply the sum of the two free energies calculated

independently for the two systems. (From the probability point of view embedded in the calculation of a partition function - see below - the lack interactions implies statistical independence and hence factorizability of the full-system partition function into those for the two systems.) We have

$$F^{\text{comb}}(N_1, N_2) = F^{\text{idl}}(N_1, V_1, T) + F^{\text{idl}}(N_2, V_2, T), \tag{2}$$

where F^{idl} is defined in the ideal gas page. We have omitted the V_1 , V_2 , and T dependence in F^{comb} because these will be held constant throughout.

Substituting in for F^{idl} , we have

$$F^{\text{comb}}(N_1, N_2) = N_1 \, k_B T \ln \frac{N_1 \, \lambda^3}{V_1} + N_2 \, k_B T \ln \frac{N_2 \, \lambda^3}{V_2}. \tag{3}$$

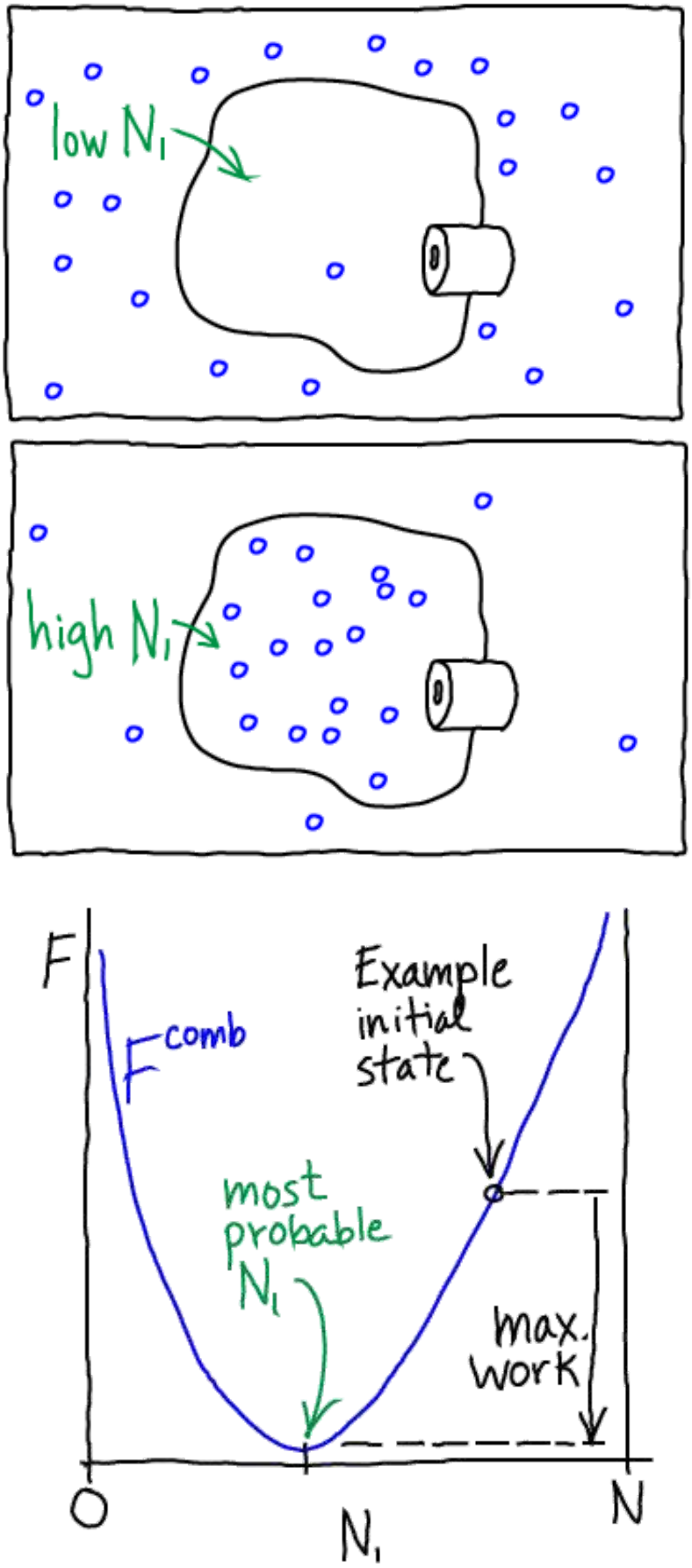
Noting that

$$N_2 = N - N_1,$$

we can rewrite this further as

$$\begin{aligned} F^{\text{comb}}(N_1, N - N_1) = & N_1 \, k_B T \ln \frac{N_1 \, \lambda^3}{V_1} \\ & + (N - N_1) \, k_B T \ln \frac{(N - N_1) \, \lambda^3}{V_2}. \end{aligned} \tag{4}$$

Eq. (4) is the free energy as a function of the number of particles inside the membrane (volume V_1).



If we open the channel and allow exchange of atoms between the compartments, the value of N_1 can change. **The probability of having N_1 atoms in V_1 is proportional to the Boltzmann factor of the free energy**:

$$p(N_1) \propto e^{-F^{\text{comb}}(N_1, N-N_1)/k_BT} \tag{5}$$

The most probable N_1 value therefore can be found by determining the minimum of F^{comb}. This will represent the equilibrium point in the thermodynamic limit (very large N - when fluctuations about the most probable N_1 will be very small compared to N_1 itself). We set $\partial F^{\text{comb}}/\partial N_1 = 0$ in Eq. (4), then re-arrange and cancel terms to find

$$0 = k_BT \ln \frac{N_1 \lambda^3}{V_1} - k_BT \ln \frac{(N - N_1)\lambda^3}{V_2} \tag{6}$$

Combining the terms using the rules of logarithms, followed by exponentiation, we find that

$$\text{Equilibrium: } \frac{N_1}{V_1} = \frac{N - N_1}{V_2} = \frac{N_2}{V_2}, \tag{7}$$

where we substitute $N_2 = N - N_1$ to obtain the last equality.

In words, Eq. (7) shows that *the concentrations inside and outside the membrane must match in equilibrium*. You probably knew that already, but we have derived it from statistical/thermodynamic principles.

The next step: Considering ions

Ion concentration gradients can also be analyzed in a similar way.

Work that can be performed

As we move from higher to lower free energy, the system can perform work - if it is coupled to a suitable mechanism for harvesting the work. The maximum amount of work that can be extracted is equal to the decrease in free energy as sketched above. In a simple gas system, work could be extracted by placing a turbine at the "channel"/nozzle as the gas flows toward equilibrium. Models of work extraction which are more pertinent to cell biology are discussed in the transport section.

Passive Transport

In the simplest kind of passive transport, molecules flow down a gradient (from high to low concentration) and that flow is not coupled other processes. In our technical language, such a process would involve moving

from a state of higher to lower free energy (see sketch above) or from lower to higher probability - see Eq. (5).

A deeper look at partition functions and probabilities

A partition function Z is simply a sum of Boltzmann factors for all possible states (configurations - and velocities if considered) of a system. Because a Boltzmann factor represents a weight (an un-normalized probability), Z is the sum of weights. Many times, partition functions are easier to work with mathematically, compared to free energies. Our system is such a case.

Factorizability

Our combined system consists of two independent sub-systems. That is, the state of one system will not affect the other. Hence the probability for a configuration of the combined system is simply the *product* of the probabilities for the individual system configurations, and this also holds for the Boltzmann weights summed in Z.

If U_1 is the potential energy of the configuration of system 1 and U_2 is the energy of system 2, these two are independent, so we have

$$e^{-U_{\text{tot}}/k_BT} = e^{-(U_1+U_2)/k_BT} = e^{-U_1/k_BT}\, e^{-U_2/k_BT} \tag{8}$$

We can extend this reasoning to calculate the partition function of the combined system, building on what was done for a simple ideal gas. Denoting the configuration of the combined system by $(\mathrm{r}_1^N, \mathrm{r}_2^N)$, the partition function is

$$\begin{aligned} Z^{\text{comb}}(N_1, N_2) &= \frac{\lambda^{-3N_1}}{N_1!}\frac{\lambda^{-3N_2}}{N_2!}\int_{V_1} d\mathrm{r}_1^N \int_{V_2} d\mathrm{r}_2^N\, e^{-U_{\text{tot}}\left(\mathrm{r}_1^N, \mathrm{r}_2^N\right)/k_BT} \\ &= \left[\frac{\lambda^{-3N_1}}{N_1!}\int_{V_1} d\mathrm{r}_1^N\, e^{-U_1\left(\mathrm{r}_1^N\right)/k_BT}\right]\left[\frac{\lambda^{-3N_2}}{N_2!}\int_{V_2} d\mathrm{r}_2^N\, e^{-U_2\left(\mathrm{r}_2^N\right)/k_BT}\right] \\ &= \frac{\lambda^{-3N_1}}{N_1!}V_1^{N_1}\,\frac{\lambda^{-3N_2}}{N_2!}V_2^{N_2} = Z^{\text{idl}}(N_1, V_1)\, Z^{\text{idl}}(N_2, V_2) \end{aligned} \tag{9}$$

where we evaluated the integrals in the last line, noting $U_1 = U_2 = 0$ for ideal particles, so that each atom's integration yields a factor of V_i depending on which volume i is occupied.

Connecting Z and F to probability

A partition function is the sum of all probability (weights) consistent with the conditions/constraints - such as constant T or V. This allows us to compare the (summed) probabilities consistent with different constraints, such as different N_1 values in Z^{comb}. More specifically, recalling that $N_2 = N - N_1$, we have

$$p(N_1) \propto Z^{\text{comb}}(N_1, N - N_1). \tag{10}$$

Because the free energy is nothing other than the log of the partition function ($F = -k_B T \ln Z$ or $Z = \exp(-F/k_B T)$), we see that Eqs. (10) and (5) are equivalent.

The total partition function

Advanced readers may have considered the possibility of the *total* partition function Z^{tot}, which not only sums over all configurations consistent with a given N_1 value - *but which also sums over all possible N_1 values from 0 to N*. The outcome is rather neat. We write

$$\begin{aligned} Z^{\text{tot}} &= \sum_{N_1=0}^{N} Z^{\text{comb}}(N_1, N - N_1) \\ &= \sum_{N_1=0}^{N} \frac{1}{N_1!}\left(\frac{V_1}{\lambda^3}\right)^{N_1} \frac{1}{(N-N_1)!}\left(\frac{V_2}{\lambda^3}\right)^{(N-N_1)} \\ &= \frac{1}{N!}\sum_{N_1=0}^{N} \frac{N!}{N_1!\,(N-N_1)!}\left(\frac{V_1}{\lambda^3}\right)^{N_1}\left(\frac{V_2}{\lambda^3}\right)^{(N-N_1)} \\ &= \frac{1}{N!}\left(\frac{V}{\lambda^3}\right)^{N} \end{aligned} \tag{11}$$

where the last line derives from recognizing the binomial expansion for $(V_1 + V_2)^N$ implicit in the previous line.

You should recognize Eq. (11) as the partition function for a simple ideal gas of N atoms in volume

$V = V_1 + V_2$. Indeed, once the channel is open, all atoms can access both volumes and this is the correct result.

References:

- J. Kuriyan, B. Konforti, and D. Wemmer, *The Molecules of Life: Physical and Chemical Principles* (Garland Science, 2013).
- R. Phillips et al., *Physical Biology of the Cell,* (Garland Science, 2009).
- B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).

Exercises:

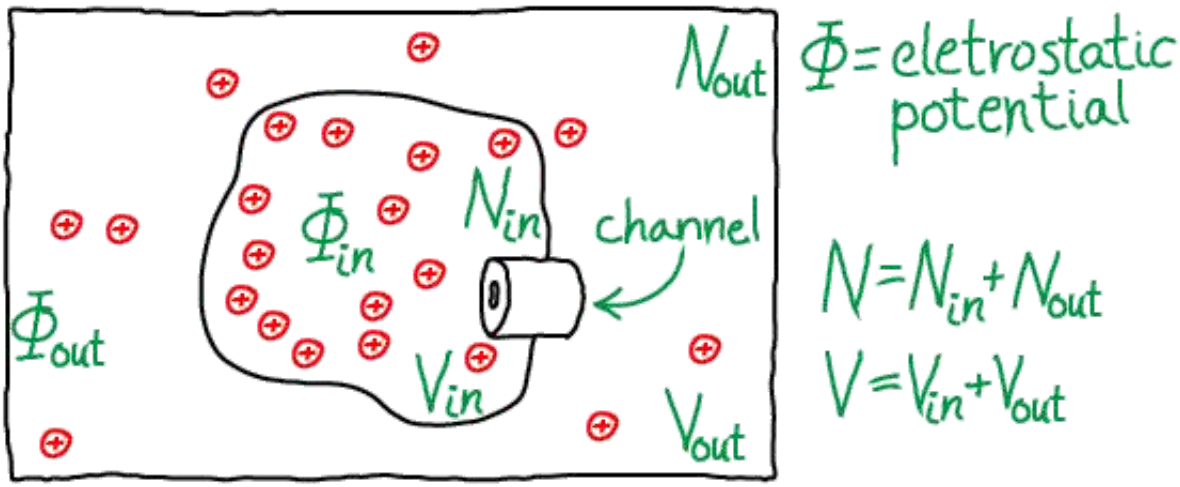
1. Derive Eqs. (6) and (7).

Ionic Concentration Gradient Across a Bilayer

A Half-Step Beyond Ideal: Ion Gradients and Transmembrane Potentials

One key way the cell stores free energy is by having different concentrations of molecules in different "compartments" - e.g., extra-cellular vs. intracellular or in an organelle compared to cytoplasm. The molecules playing this role are charged molecules, or ions, such as sodium (Na^+), chloride (Cl^-), potassium (K^+), calcium (Ca^{++}), and numerous nucleotide species. A brief overview of trans-membrane ion physiology is available.

Although the simplest way to study the physics of free energy storage in such a gradient is by considering ideal particles all with zero potential energy, the reality of the cell is that electrostatic interactions are critical. Fortunately, the most important non-ideal effects of charge-charge interactions can be understood in terms of the usual ideal particles (which do not interact with one another) that do, however, feel the effects of a "background" electrostatic field. Such a mean-field picture is a simple approximation to the electrostatic effects induced primarily by having an excess of one or more charged species on a given side of a membrane - for example, the excess of Na^+ ions in the extracellular environment.



Two semi-ideal gases of ions in different potentials

Unlike our examination of two membrane-separated ideal gases, the particles here are explicitly charged and they "feel" the electrostatic potential Φ. The positively charged anions schematically represent K^+ ions interacting with the field generated by the imbalance of Na^+ ions - the extracellular or "outside" concentration of sodium is maintained at a high value relative to the cytoplasm or "inside" by constant ATP-driven pumping. However, *the effects of the* Na^+ *concentration gradient will only be treated* ***implicitly*** *via the different values for* $\Phi_{\mathrm{in}} < \Phi_{\mathrm{out}}$.

To be precise, the model consists of N particles that do not interact with one another, but which interact with

the external potential Φ as if each had a charge of q, leading to potential energy $q \cdot \Phi_{\mathrm{X}}$ for each particle, where X = "in" or "out". The total volume V is divided into inside and outside so that $V_{\mathrm{in}} + V_{\mathrm{out}} = V$, with $N_{\mathrm{in}} + N_{\mathrm{out}} = N$ ions populating the two compartments. The whole system is maintained at constant temperature T. Particles can pass through the channel shown in the figure, but we assume it is closed so that V_{in} and V_{out} are constants: as shown in our discussion of two membrane-separated ideal gases, the assumption is a convenience and not an approximation because the total system volume V and particle-number N are truly constant.

Deriving the free energy

We have two gases of ideal "ions" (that interact with the external potential but not with other ions). Mathematically, we can largely follow our discussion of two membrane-separated ideal gases. The total free energy is the sum of the two ideal gas free energies and the two electrostatic potential energies:

$$\begin{aligned} F(N_{\mathrm{in}}, N_{\mathrm{out}}) = & F^{\mathrm{idl}}(N_{\mathrm{in}}, V_{\mathrm{in}}) + F^{\mathrm{idl}}(N_{\mathrm{out}}, V_{\mathrm{out}}) \\ & + N_{\mathrm{in}} q \, \Phi_{\mathrm{in}} + N_{\mathrm{out}} q \, \Phi_{\mathrm{out}} \end{aligned} \tag{1}$$

where F^{idl} is defined in the ideal gas page and q is the ionic charge.

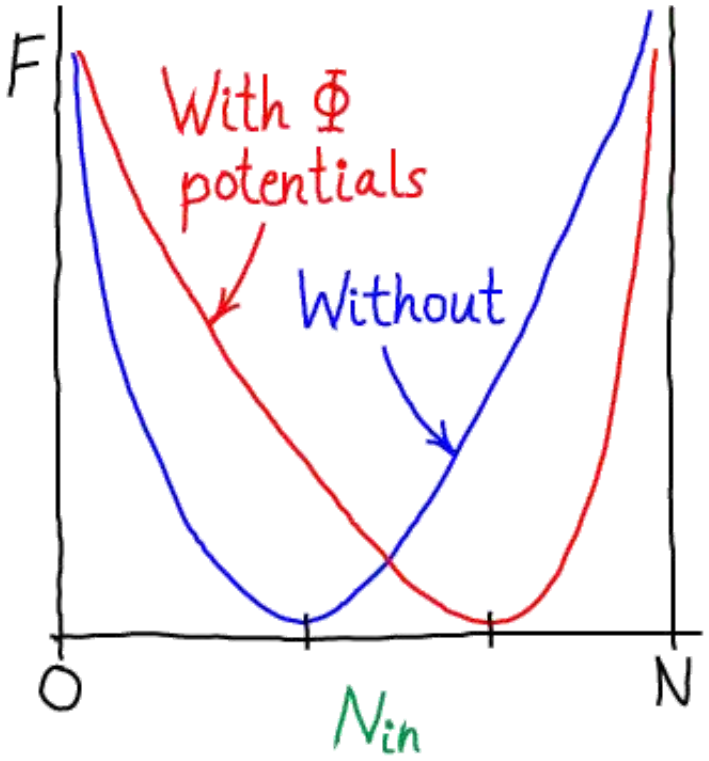
Substituting for F^{idl} and noting that $N_{\mathrm{out}} = N - N_{\mathrm{in}}$, we have

$$\begin{aligned} F(N_{\mathrm{in}}, N - N_{\mathrm{in}}) = & N_{\mathrm{in}} \, k_B T \ln \frac{N_{\mathrm{in}} \, \lambda^3}{V_{\mathrm{in}}} \\ & + (N - N_{\mathrm{in}}) \, k_B T \ln \frac{(N - N_{\mathrm{in}}) \, \lambda^3}{V_{\mathrm{out}}} \\ & + N_{\mathrm{in}} \, q \, \Phi_{\mathrm{in}} + (N - N_{\mathrm{in}}) \, q \, \Phi_{\mathrm{out}}. \end{aligned} \tag{2}$$

Because it is really the *difference* in electrostatic potential which governs the ionic behavior, we define $\Delta\Phi = \Phi_{\mathrm{in}} - \Phi_{\mathrm{out}}$. In terms of this quantity, we can rewrite the total free energy as

$$F(N_{\text{in}}, N - N_{\text{in}}) = N_{\text{in}}\, k_B T \ln \frac{N_{\text{in}}\, \lambda^3}{V_{\text{in}}} + (N - N_{\text{in}})\, k_B T \ln \frac{(N - N_{\text{in}})\, \lambda^3}{V_{\text{out}}} + N q\, \Phi_{\text{out}} + N_{\text{in}} q\, \Delta\Phi, \quad (3)$$

Eq. (3) is the free energy as a function of the number of particles inside the membrane (in volume V_{in}). Inclusion of the electrostatic effects shifts the location of the most probable state, or free energy minimum.



The most probable concentrations: The Nernst equation

If we open the channel and allow exchange of atoms between the compartments, the value of N_{in} can change. The probability of having N_{in} atoms in V_{in} is proportional to the Boltzmann factor of the free energy:

$$p(N_{\text{in}}) \propto e^{-F(N_{\text{in}},\, N - N_{\text{in}})/k_B T} \quad (4)$$

The most probable N_{in} value therefore can be found by determining the minimum of F. This will represent the equilibrium point in the thermodynamic limit (very large N - when fluctuations about the most probable N_{in} will be very small compared to N_{in} itself). We set $\partial F/\partial N_{\text{in}} = 0$ in Eq. (3), then re-arrange and cancel terms to find

$$0 = k_B T \ln \frac{N_{\text{in}}\,\lambda^3}{V_{\text{in}}} - k_B T \ln \frac{N_{\text{out}}\,\lambda^3}{V_{\text{out}}} + q\Delta\Phi \big/ k_B T \qquad (5)$$

Combining the terms using the rules of logarithms, followed by exponentiation, we find the **Nernst equation**,

$$\text{Equilibrium: } \frac{N_{\text{in}}/V_{\text{in}}}{N_{\text{out}}/V_{\text{out}}} = e^{-q\,\Delta\Phi/k_B T} \qquad (6)$$

where you should recognize the left-hand side as the ratio of concentrations.

In words, Eq. (6) shows that *the concentrations inside and outside vary according to the Boltzmann factor of the ionic charge times the potential difference*. Such an equilibrium is called a Donnan equilibrium. It should be comforting that when $\Delta\Phi = 0$, we recover equal concentrations.

Comparison to cellular behavior

As the exercise below will show, for some ions (Cl^-, K^+) the Nernst equation is a reasonable approximation. This suggests that such ions permeate the membrane passively. For some ions (Na^+, Ca^{++}), the concentration ratios are very different from what would be predicted from the Nernst equation because the cell uses active transport to control them.

Mass action and its limitations

It is always worthwhile to pursue both thermodynamic *and* kinetic analyses of any system you really care about, or just to train yourself to consider a problem from multiple perspectives. By comparison to the present case, some of the results from the truly ideal (uncharged) two-compartment system may seem puzzling.

In contrast to the uncharged system, we can see that the transport rates through the channel *cannot* be equal in the two directions. Let k_{io} be the inside-to-outside rate constant and k_{oi} be the reverse rate constant. Starting from detailed balance, which says that the overall flows must be equal and opposite, and substituting

the Nernst relation (6), we find that

$$\frac{k_{oi}}{k_{io}} = \frac{N_{\text{in}}/V_{\text{in}}}{N_{\text{out}}/V_{\text{out}}} = e^{-q\,\Delta\Phi/k_BT} \tag{7}$$

In other words, the ratio of rates for an ion channel depends on the potential difference. By itself, this does not contradict the mass action viewpoint (that rate constants are independent of concentrations) ... so long as $\Delta\Phi$ is truly constant. But if, more generally, $\Delta\Phi$ depends on the relative concentrations of the ion species moving through the channel, then the mass-action picture breaks down. Such a breakdown would occur, for example, if there were two species of ions, one of which could not permeate the membrane and hence was maintained at fixed inside and outside concentrations: in this case, flow of the permeable ion would change $\Delta\Phi$ and, in turn, change the rate "constants".

The brief overview of trans-membrane ion physiology may help to clarify the bigger picture of ion/membrane behavior.

References:

- R. Phillips et al., "Physical Biology of the Cell," (Garland Science, 2009).
- B. Alberts et al., "Molecular Biology of the Cell", Garland Science (many editions available).

Exercises:

1. Derive Eqs. (5) and (6).
2. Use Eq. (6) to derive a concentration ratio for Cl^- using $\Delta\Phi = -90\,\text{mV}$ (typical for skeletal muscle) and compare your result to the experimental value of $\sim 1/30$. This will require careful consideration of units when multiplying together physical constants.

ATP and Other Activated Carriers

Activated Carriers: Why chemical energy storage is "statistical"

Any system that is out of equilibrium stores free energy. The cell stores free energy using out-of-equilibrium chemically reacting systems involving molecules known as "activated carriers," as well as non-equilibrium concentration differences - gradients - across membranes.

Activated carriers are molecules that can be split (C → A + B) to release free energy *but only if there is an excess of C relative to its equilibrium concnetration*. Key examples are ATP, GTP, NADH, $FADH_2$, and NADPH.

In order for carriers like ATP to be a stable source of free energy, they must stay "activated" when not being used for cellular purposes. In other words, the decomposition/splitting reaction C → A + B must be slow compared to cellular timescales and unlikely to occur without catalysis.

For completeness, we emphasize that free energy is stored because the decompostion/synthesis reactions are maintained away from equilibrium - not because bond-breakage is involved. Free energy could equally well be stored in an isomerization "reaction" $A \rightleftharpoons A'$, in which the species populations were maintained away from equilibrium.

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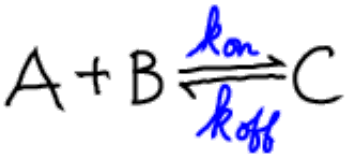

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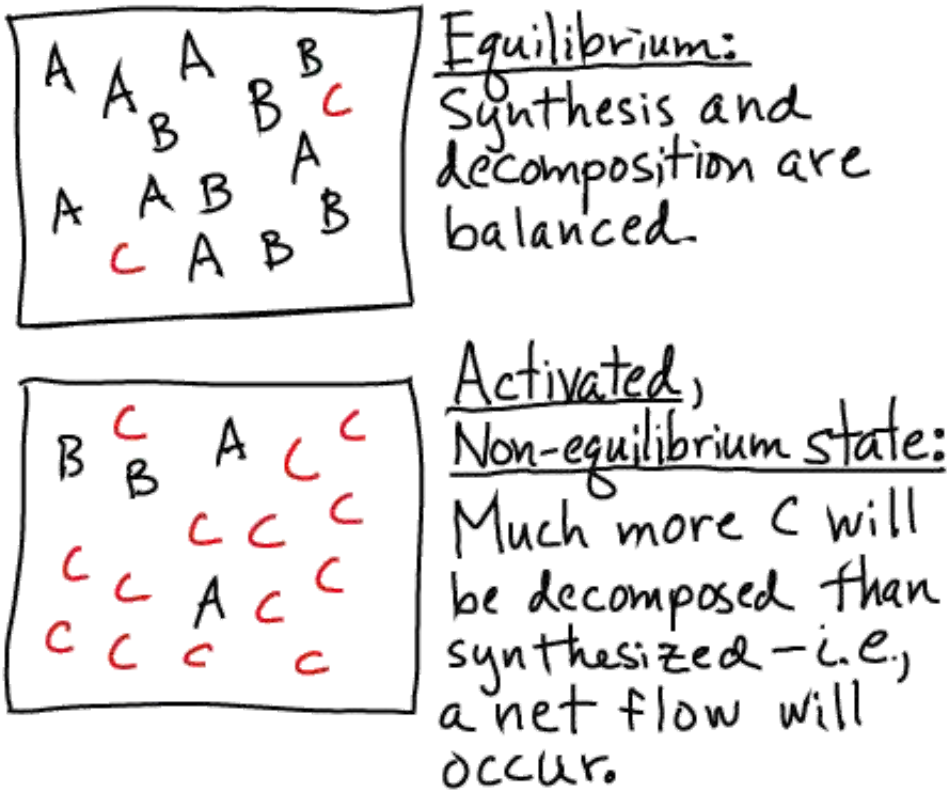

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Other carriers

The physical explanation just given suggests that ATP is not the only molecule that can be used as an activated carrier. Indeed, it is well known that GTP is used as a carrier for signaling processes and for translation. The logic here also explains energy storage in electron carriers like NADH, which participate in the oxidative phosphorylation process that "activates" ATP in the first place by synthesizing it well beyond its

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A Simple, Quantitative Model for Free Energy Storage in Activated Carriers

We can use the same approach taken to analyze binding to see how free energy is stored in a very simple model. In particular, we can model the A, B, and C components (e.g., ADP, Pi, and ATP) as non-interacting except for their probilities to react or decompose. This is nothing other than a mass-action picture - or, thermodynamically, treatment of the components as ideal gases.

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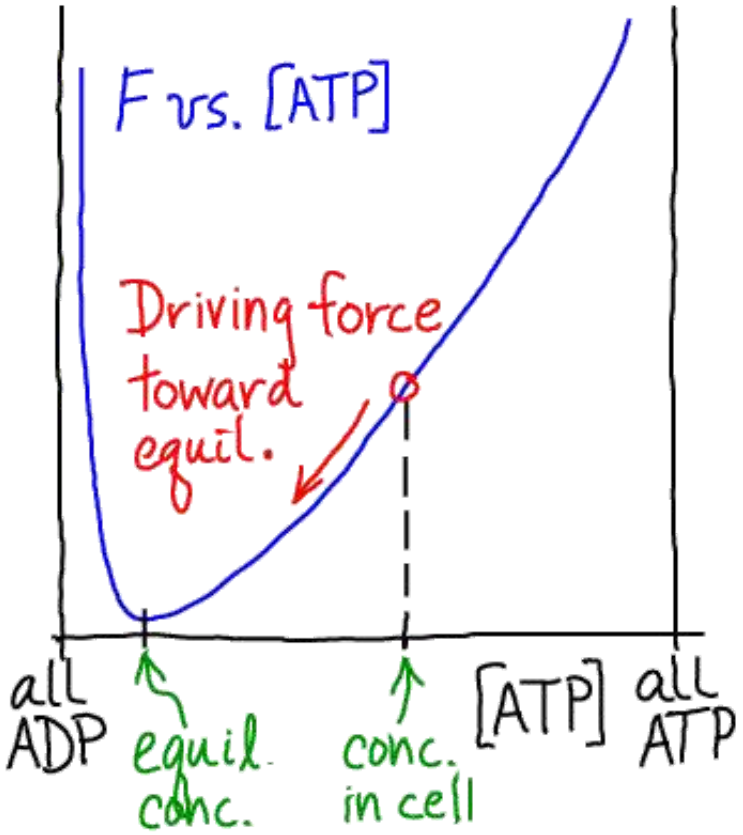

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Activated Carriers

Activated Carriers: Why chemical energy storage is "statistical"

Any system that is out of equilibrium stores free energy. The cell stores free energy using out-of-equilibrium chemically reacting systems involving molecules known as "activated carriers," as well as non-equilibrium concentration differences - gradients - across membranes.

Activated carriers are molecules that can be split (C → A + B) to release free energy *but only if there is an excess of C relative to its equilibrium concnetration*. Key examples are ATP, GTP, NADH, $FADH_2$, and NADPH.

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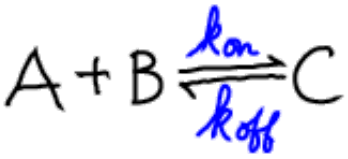

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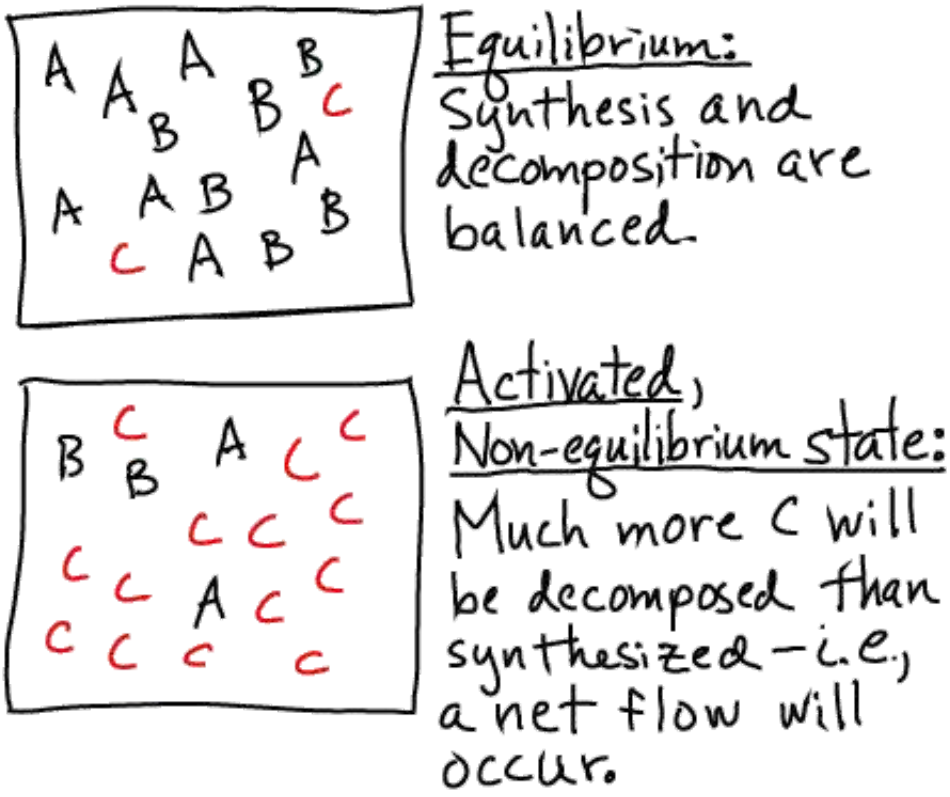

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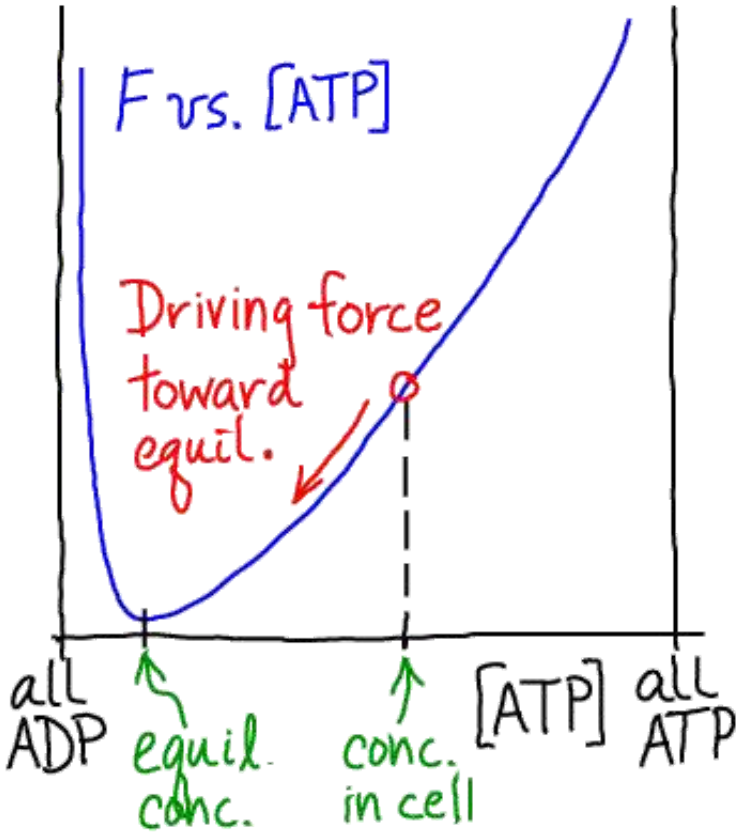

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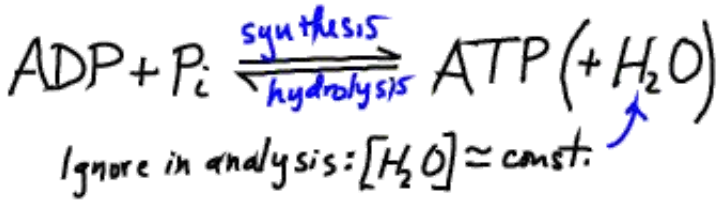
ATP: The Great Carrier

"ATP is the fuel of the cell." We have all heard this many times, but the reason why ATP (adenosine tri-phosphate) stores energy is not well understood. In essence, ATP is a standard activated carrier. It stores free energy **only** because the cell keeps $[\mathrm{ATP}]$ well above its equilibrium value.



The ATP/ADP equilibrium greatly favors ADP

ATP does *not* store free energy because of a "high energy phosphate bond" or because heat is released upon ATP hydrolysis - even though these descriptors themselves may be accurate. Rather, such details of the ATP molecule (which, presumably, are dominated by the proximity of like-charged phosphate groups) lead to an equilibrium which greatly favors ADP formation. That is, if both ATP and ADP are present in a solution, then after enough time very little ATP will remain. (How much time is 'enough' will depend on whether a catalyst is present.)

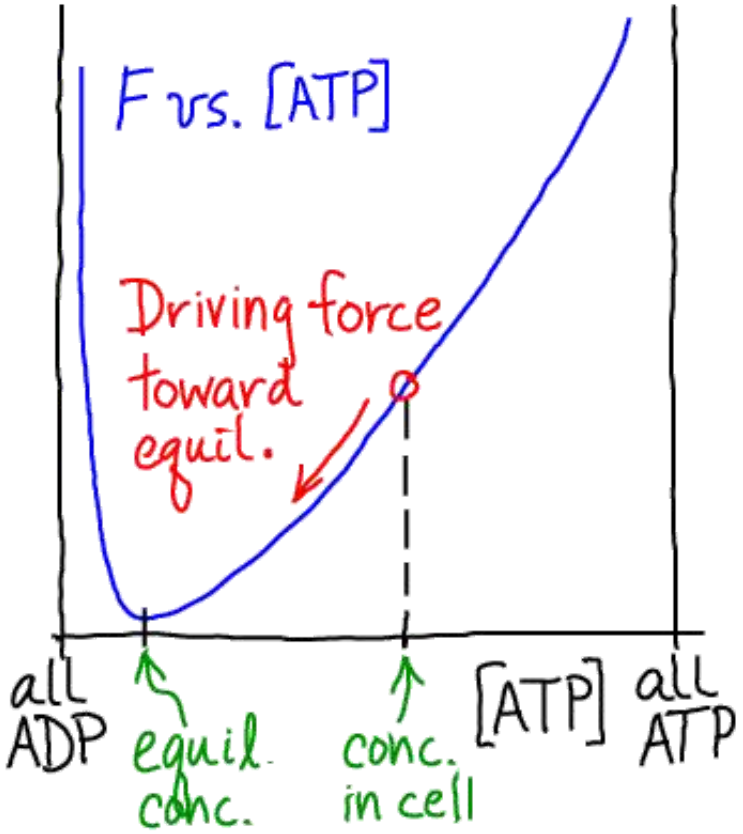
As with any activated carrier, to understand ATP we first examine the equilibrium point - which will serve as a reference point for understanding the non-equilibrium conditions prevailing in the cell. In equilibrium, the decomposition and synthesis of ATP will exactly balance.

$$[\mathrm{ADP}][\mathrm{Pi}]\, k_{\mathrm{on}} = [\mathrm{ATP}]\, k_{\mathrm{off}}, \qquad (1)$$

where k_{off} should be understood as an effective rate constant incorporating water's effects (see Exercises, below). In exact analogy with a binding analysis, we can re-write this as

$$\frac{[\mathrm{ADP}]^{\mathrm{eq}}\,[\mathrm{Pi}]^{\mathrm{eq}}}{[\mathrm{ATP}]^{\mathrm{eq}}} = \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} \tag{2}$$

ATP in the cell: The skewed equilibrium point makes ATP an effective carrier



ATP is an ideal carrier because a lot of free energy is stored when both ADP and ATP are present in reasonable cellular concentrations. That is, at typical cellular concentrations $[\cdots]^{\mathrm{cell}}$, we have

$$\frac{[\mathrm{ADP}]^{\mathrm{cell}}\,[\mathrm{Pi}]^{\mathrm{cell}}}{[\mathrm{ATP}]^{\mathrm{cell}}} \ll \frac{[\mathrm{ADP}]^{\mathrm{eq}}\,[\mathrm{Pi}]^{\mathrm{eq}}}{[\mathrm{ATP}]^{\mathrm{eq}}}. \tag{3}$$

As sketched above, this non-equilibrium condition creates a driving force toward equilibrium.

The cell does not allow ATP and ADP to mutually equilibrate (i.e., reach the free energy minimum). Instead the cell is perpetually synthesizing new ATP from nutrients, creating a flow of free energy through the system in what we could call the big energy cycle of life.

Note that, as suggested in the sketch above, ADP could act as a carrier of free energy if it were maintained at sufficiently high concentration (beyond the equilibrium point, to the left in the sketch). Needless to say, the cell is not set up to use energy stored in ADP!

Kinetics and Catalysts

Like any carrier, ATP is "kinetically stable" - it is overwhelmingly decomposed by enzymatic catalysis because spontaneous hydrolysis in solution is very slow compared to cellular processes. Thus, ATP fulfills the necessary criteria for a carrier: (1) it is maintained out of equilibrium, and (2) it only decomposes enzymatically as part of cellular processes: transport, phosphorylation, and myriad other tasks.

Appreciating ATP as an ordinary activated carrier enables us to re-envision molecular motors as simple enzymes that happen to couple catalysis to translation. You can consider a thought experiment studying motor motion near the ADP/ATP equilibrium.

References:

- J. M. Berg et al., "Biochemistry", W. H. Freeman. The 2002 edition is online for free.
- B. Alberts et al., "Molecular Biology of the Cell", Garland Science (many editions available). The 2nd chapter has an oustanding discussion of energy use. Consider it required reading.
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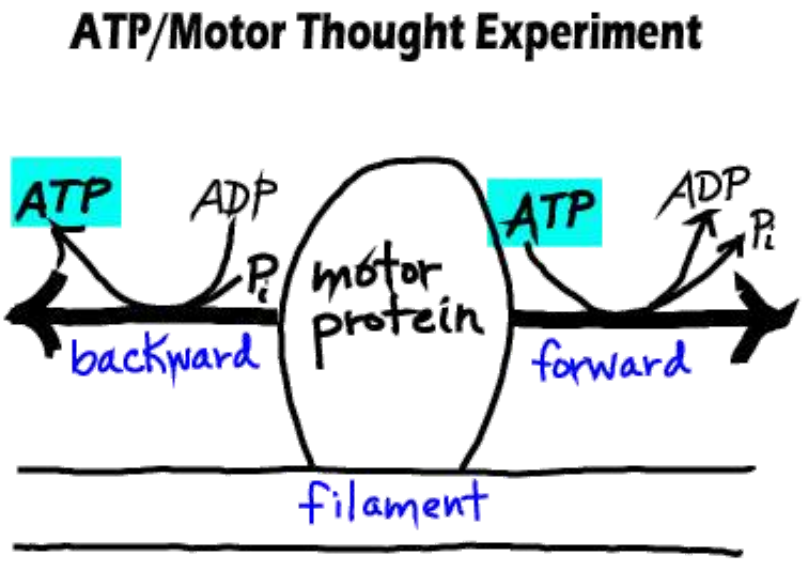
Exercises:

- The full chemical equation for ATP hydrolysis of course includes water. Explain why a mass-action description including water's concentration would be inappropriate by considering the limitations described for mass action.

ATP/Motor Thought Experiment

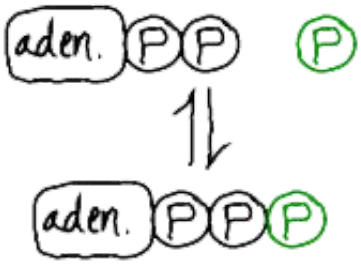


- Motor Protein (without load/resistance) can be viewed as a simple catalyst – effective in both directions
- "Regular" /forward step: ATP is hydrolyzed
- Backwards steps: ATP is synthesized
- Equilibrium of ADP + Pi <-> ATP: Equal mix of forward and reverse steps
- KEY: ATP binding typically results in a forward step, but in equilibrium, [ADP] >>[ATP] and ATP rarely binds.
- For details of balance among binding/catalytic rates, study the ATP enzymatic cycle.
- ATP really is synthesized by a motor protein complex.

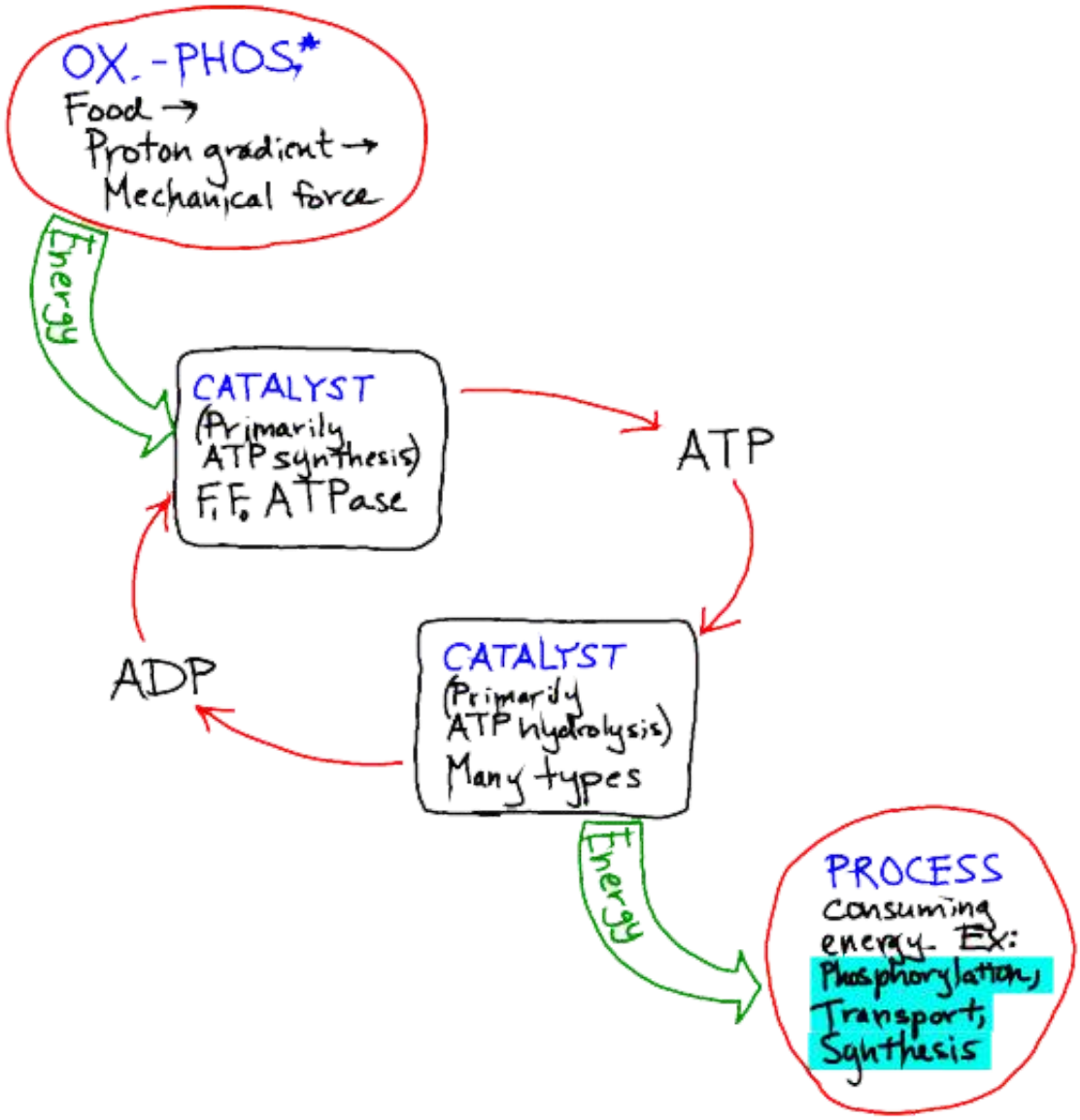
Recycling of ATP and Coupling to Cellular Processes

ATP: The Big Picture

- How do ATP hydrolysis (decomposition) and synthesis fit into the overall picture energy use in the cell?
- ATP and its components follow a cycle, as do most (perhaps all) molecules in the cell, which are recycled.



- Oxidative phosphorylation (OX-PHOS) extracts energy from food molecules via complex chemical processes that ultimately lead to (re)synthesis of ATP.
- The input of energy drives this cycle in the clockwise direction.

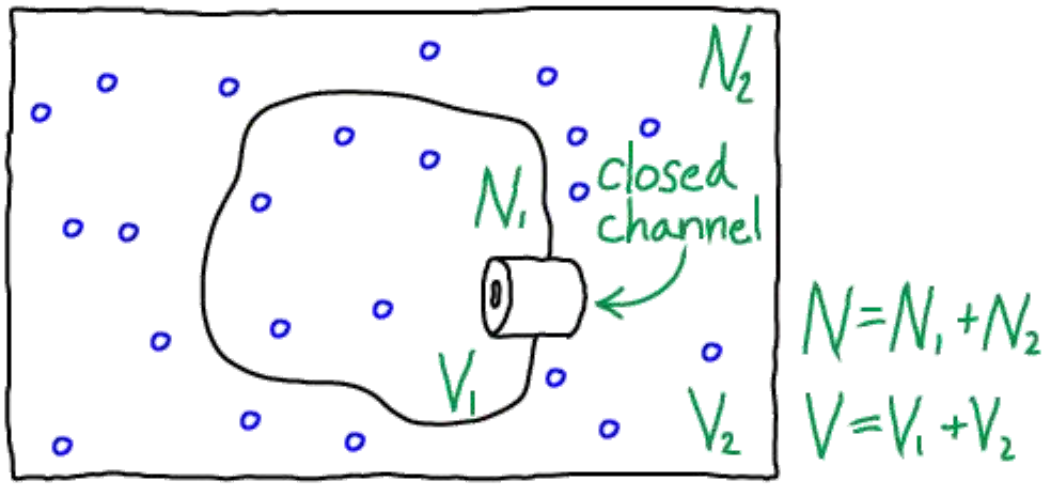

OX.-PHOS*
Food →
Proton gradient →
Mechanical force
Energy
CATALYST
(Primarily
ATP synthesis)
F_1F_0 ATPase
ATP
CATALYST
(Primarily
ATP hydrolysis)
Many types
ADP
Energy
PROCESS
consuming
energy. EX:
Phosphorylation,
Transport,
Synthesis

Energy Storage in Gradients

The Free Energy in a Concentration Gradient

One key way the cell stores free energy is by having different concentrations of molecules in different "compartments" - e.g., extra-cellular vs. intracellular or in an organelle compared to cytoplasm. Here, we will study the simplest example of such a *gradient*, differing concentrations of ideal gas molecules across an idealized rigid membrane. Elsewhere, we consider a simple model of ion concentration gradient across a membrane.

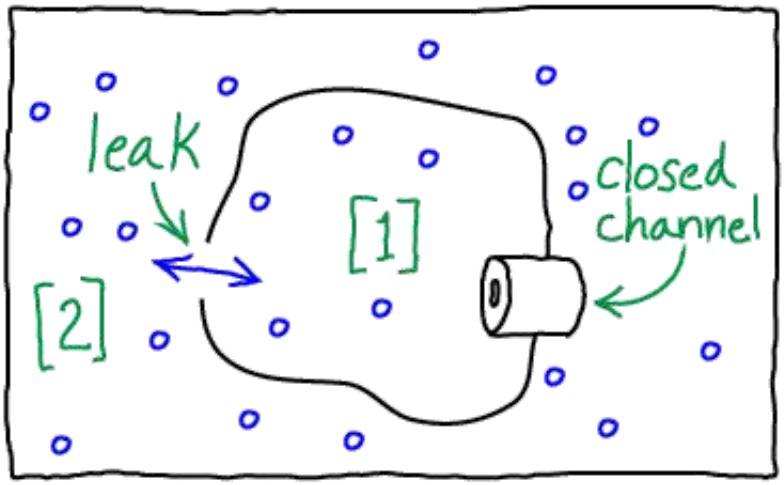
Two ideal gases separated by a barrier



Analyzing the model depicted above will enable us to understand free energy storage in a concentration gradient, but the basic ideas generalize to activated carriers as well.

To be precise, the model consists of N non-interacting atoms in the volume V maintained at constant temperature T. Beyond the simple ideal gas studied elsewhere, our present system is divided into two compartments by a rigid "membrane," with V_1 the volume of the inner compartment and V_2 the outer volume such that $V_1 + V_2 = V$. Similarly, there are N_1 atoms in the inner compartment and N_2 outside, with $N_1 + N_2 = N$. Although particles could pass through the channel shown in the figure, we assume it is closed so that N_1 and N_2 are constant.

A quick mass-action analysis



We can derive the key result for this system very quickly using a mass action "thought experiment". Our simple kinetic analysis will provide a key reference when we delve into some specific limitations of the mass-action picture in the context of ionic gradients.

Instead of our original system, we consider the leaky cell or organelle shown above. The leak should be considered a simple hole (unlike a channel, which is expected to interact strongly with molecules passing through it). Hence the inside-to-outside rate constant k_{12} for the leak/hole must be equal to the outside-to-inside rate constant k_{21}. After all, if the hole is large enough the "atoms" will not interact with the membrane at all - or if they do, the effects should be symmetric.

In equilibrium, we know that the total number of events in each direction will match: $[1]\,k_{12} = [2]\,k_{21}$. Cancelling the equal rates on both sides of this relation, we find that the **equilibrium concentrations must be equal inside and outside**:

$$[1]^{\mathrm{eq}} = [2]^{\mathrm{eq}}. \tag{1}$$

Deriving this result thermodynamically in a careful way requires more effort (see below).

This equilbrium result for the leaky condition actually tell us something about the channel rates. Because the channel is a passive element that uses no energy, it cannot change the (equal) equilibrium concentrations just derived. Hence, applying the principle of detailed balance to the channel (which implies the flows through the channel must be equal and opposite) we see that the *channel rate constants must be equal in both directions*. This perhaps obvious result only holds for a channel separating two systems with no driving force or external field applied - a condition which breaks down in the case of trans-membrane ionic gradients.

Deriving the free energy

Because we have non-interacting ("ideal") particles which cannot exchange across the membrane, the total free energy for the combined systems F^{comb} is simply the sum of the two free energies calculated

independently for the two systems. (From the probability point of view embedded in the calculation of a partition function - see below - the lack interactions implies statistical independence and hence factorizability of the full-system partition function into those for the two systems.) We have

$$F^{\text{comb}}(N_1, N_2) = F^{\text{idl}}(N_1, V_1, T) + F^{\text{idl}}(N_2, V_2, T), \qquad (2)$$

where F^{idl} is defined in the ideal gas page. We have omitted the V_1 , V_2 , and T dependence in F^{comb} because these will be held constant throughout.

Substituting in for F^{idl} , we have

$$F^{\text{comb}}(N_1, N_2) = N_1 \, k_B T \ln \frac{N_1 \, \lambda^3}{V_1} + N_2 \, k_B T \ln \frac{N_2 \, \lambda^3}{V_2}. \qquad (3)$$

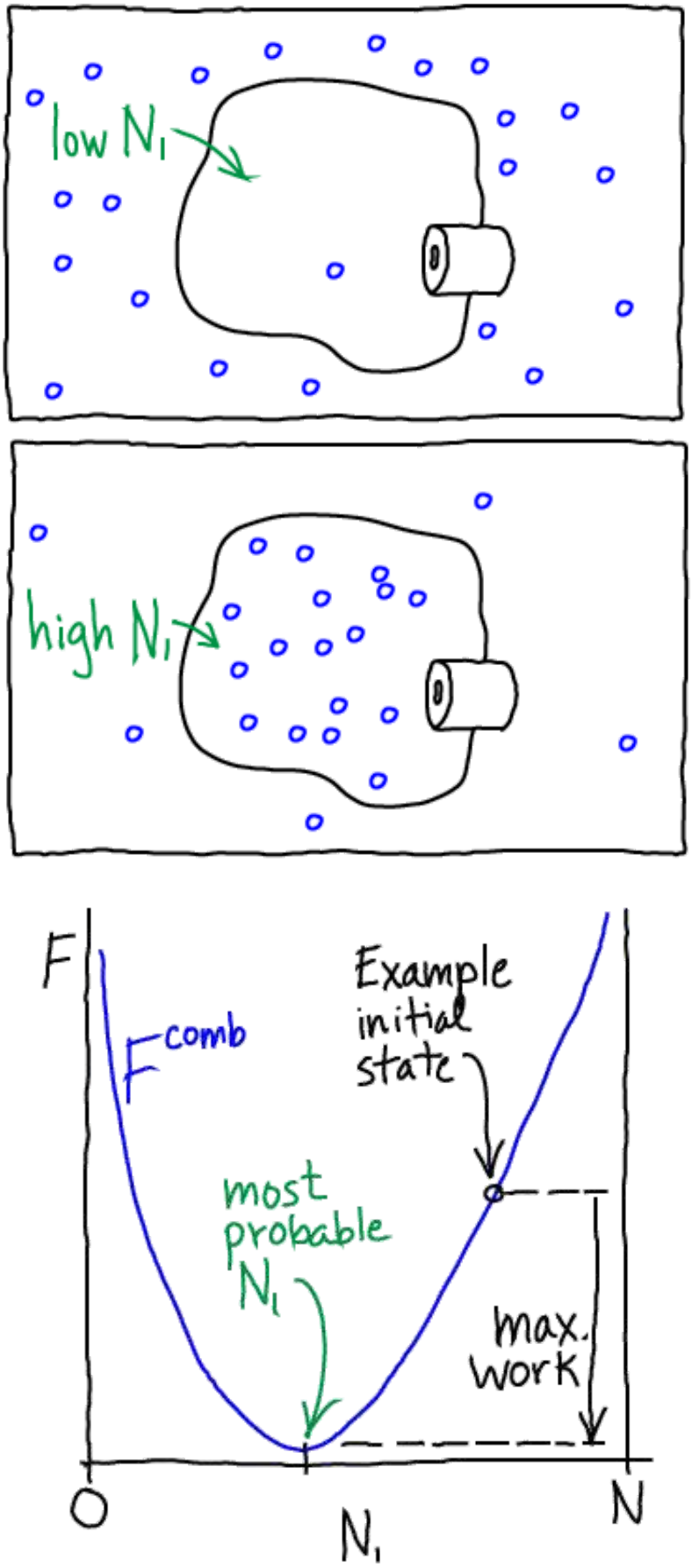
Noting that

$$N_2 = N - N_1,$$

we can rewrite this further as

$$F^{\text{comb}}(N_1, N - N_1) = N_1 \, k_B T \ln \frac{N_1 \, \lambda^3}{V_1} + (N - N_1) \, k_B T \ln \frac{(N - N_1) \, \lambda^3}{V_2}. \qquad (4)$$

Eq. (4) is the free energy as a function of the number of particles inside the membrane (volume V_1).



If we open the channel and allow exchange of atoms between the compartments, the value of N_1 can change. **The probability of having N_1 atoms in V_1 is proportional to the Boltzmann factor of the free energy**:

$$p(N_1) \propto e^{-F^{\text{comb}}(N_1, N-N_1)/k_BT} \tag{5}$$

The most probable N_1 value therefore can be found by determining the minimum of F^{comb}. This will represent the equilibrium point in the thermodynamic limit (very large N - when fluctuations about the most probable N_1 will be very small compared to N_1 itself). We set $\partial F^{\text{comb}}/\partial N_1 = 0$ in Eq. (4), then re-arrange and cancel terms to find

$$0 = k_BT \ln \frac{N_1 \lambda^3}{V_1} - k_BT \ln \frac{(N - N_1)\lambda^3}{V_2} \tag{6}$$

Combining the terms using the rules of logarithms, followed by exponentiation, we find that

$$\text{Equilibrium: } \frac{N_1}{V_1} = \frac{N - N_1}{V_2} = \frac{N_2}{V_2}, \tag{7}$$

where we substitute $N_2 = N - N_1$ to obtain the last equality.

In words, Eq. (7) shows that *the concentrations inside and outside the membrane must match in equilibrium*. You probably knew that already, but we have derived it from statistical/thermodynamic principles.

The next step: Considering ions

Ion concentration gradients can also be analyzed in a similar way.

Work that can be performed

As we move from higher to lower free energy, the system can perform work - if it is coupled to a suitable mechanism for harvesting the work. The maximum amount of work that can be extracted is equal to the decrease in free energy as sketched above. In a simple gas system, work could be extracted by placing a turbine at the "channel"/nozzle as the gas flows toward equilibrium. Models of work extraction which are more pertinent to cell biology are discussed in the transport section.

Passive Transport

In the simplest kind of passive transport, molecules flow down a gradient (from high to low concentration) and that flow is not coupled other processes. In our technical language, such a process would involve moving

from a state of higher to lower free energy (see sketch above) or from lower to higher probability - see Eq. (5).

A deeper look at partition functions and probabilities

A partition function Z is simply a sum of Boltzmann factors for all possible states (configurations - and velocities if considered) of a system. Because a Boltzmann factor represents a weight (an un-normalized probability), Z is the sum of weights. Many times, partition functions are easier to work with mathematically, compared to free energies. Our system is such a case.

Factorizability

Our combined system consists of two independent sub-systems. That is, the state of one system will not affect the other. Hence the probability for a configuration of the combined system is simply the *product* of the probabilities for the individual system configurations, and this also holds for the Boltzmann weights summed in Z.

If U_1 is the potential energy of the configuration of system 1 and U_2 is the energy of system 2, these two are independent, so we have

$$e^{-U_{\rm tot}/k_BT} = e^{-(U_1+U_2)/k_BT} = e^{-U_1/k_BT}\, e^{-U_2/k_BT} \tag{8}$$

We can extend this reasoning to calculate the partition function of the combined system, building on what was done for a simple ideal gas. Denoting the configuration of the combined system by $(\mathrm{r}_1^N, \mathrm{r}_2^N)$, the partition function is

$$\begin{aligned} Z^{\rm comb}(N_1,N_2) &= \frac{\lambda^{-3N_1}}{N_1!}\frac{\lambda^{-3N_2}}{N_2!}\int_{V_1} d\mathrm{r}_1^N \int_{V_2} d\mathrm{r}_2^N\, e^{-U_{\rm tot}\left(\mathrm{r}_1^N,\,\mathrm{r}_2^N\right)/k_BT} \\ &= \left[\frac{\lambda^{-3N_1}}{N_1!}\int_{V_1} d\mathrm{r}_1^N\, e^{-U_1\left(\mathrm{r}_1^N\right)/k_BT}\right]\left[\frac{\lambda^{-3N_2}}{N_2!}\int_{V_2} d\mathrm{r}_2^N\, e^{-U_2\left(\mathrm{r}_2^N\right)/k_BT}\right] \\ &= \frac{\lambda^{-3N_1}}{N_1!}V_1^{N_1}\,\frac{\lambda^{-3N_2}}{N_2!}V_2^{N_2} = Z^{\rm idl}(N_1,V_1)\,Z^{\rm idl}(N_2,V_2) \end{aligned} \tag{9}$$

where we evaluated the integrals in the last line, noting $U_1 = U_2 = 0$ for ideal particles, so that each atom's integration yields a factor of V_i depending on which volume i is occupied.

Connecting Z and F to probability

A partition function is the sum of all probability (weights) consistent with the conditions/constraints - such as constant T or V. This allows us to compare the (summed) probabilities consistent with different constraints, such as different N_1 values in Z^{comb}. More specifically, recalling that $N_2 = N - N_1$, we have

$$p(N_1) \propto Z^{\text{comb}}(N_1, N - N_1). \tag{10}$$

Because the free energy is nothing other than the log of the partition function ($F = -k_B T \ln Z$ or $Z = \exp(-F/k_B T)$), we see that Eqs. (10) and (5) are equivalent.

The total partition function

Advanced readers may have considered the possibility of the *total* partition function Z^{tot}, which not only sums over all configurations consistent with a given N_1 value - *but which also sums over all possible N_1 values from 0 to N*. The outcome is rather neat. We write

$$\begin{aligned} Z^{\text{tot}} &= \sum_{N_1=0}^{N} Z^{\text{comb}}(N_1, N - N_1) \\ &= \sum_{N_1=0}^{N} \frac{1}{N_1!}\left(\frac{V_1}{\lambda^3}\right)^{N_1} \frac{1}{(N-N_1)!}\left(\frac{V_2}{\lambda^3}\right)^{(N-N_1)} \\ &= \frac{1}{N!}\sum_{N_1=0}^{N} \frac{N!}{N_1!\,(N-N_1)!}\left(\frac{V_1}{\lambda^3}\right)^{N_1}\left(\frac{V_2}{\lambda^3}\right)^{(N-N_1)} \\ &= \frac{1}{N!}\left(\frac{V}{\lambda^3}\right)^{N} \end{aligned} \tag{11}$$

where the last line derives from recognizing the binomial expansion for $(V_1 + V_2)^N$ implicit in the previous line.

You should recognize Eq. (11) as the partition function for a simple ideal gas of N atoms in volume

$V = V_1 + V_2$. Indeed, once the channel is open, all atoms can access both volumes and this is the correct result.

References:

- J. Kuriyan, B. Konforti, and D. Wemmer, *The Molecules of Life: Physical and Chemical Principles* (Garland Science, 2013).
- R. Phillips et al., *Physical Biology of the Cell,* (Garland Science, 2009).
- B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).

Exercises:

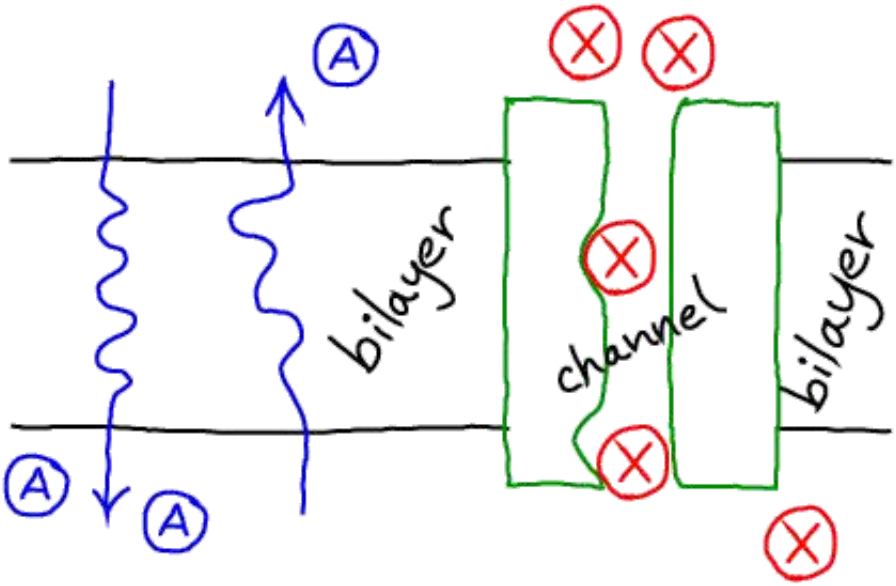
1. Derive Eqs. (6) and (7).

Channels

Passive Transport: No Energy Required

Passive "transport" is really just unbiased diffusion through some kind medium or object - as opposed to diffusion through a simple solution, or active transport requiring (free) energy. But passive transport is key to cellular functioning due to its role in, for example:

- Diffusion of small molecules (e.g., drugs) across lipid bilayers.
- Flow of water through channels.
- Flow of ions through channels including for propagation of the action potential in nerve cells.



Basics of Passive Diffusion

- It refers to diffusion across a membrane or through a membrane-embedded object (a "channel").
- Molecules will tend to move "downhill" according to the concentration gradient - from high to low concentration.
- The flow will tend to continue until equilibrium or another state (of lower free-energy than the initial condition) is reached. See the thermodynamic/statistical description of a concentration gradient. Note that the equilibrium point might not exhibit exactly equal concentrations on both sides of the membrane if the transported species interacts strongly with other species present (e.g., electrostatically).

Binding and gating in passive transport

- **Binding.** In general, if an ion or small molecule crosses the membrane or passes through a channel, the passage will not happen instantaneously, and we can expect there will be some degree of binding to the

membrane or channel. *Binding will **not** affect the ultimate equilibrium (or steady state) that is reached, however, only the rate at which the state is approached.* We don't expect binding to be too strong, otherwise transport could be prohibitively slow.

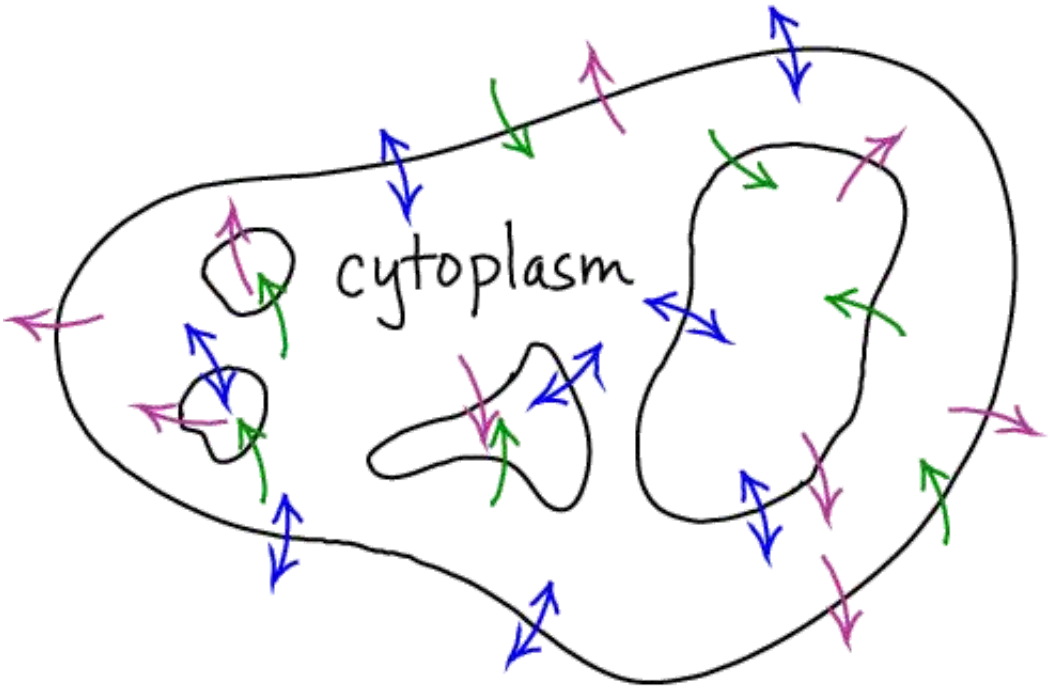
- **Gating.** Gating just means switching, on or off. Thus, even though passive transport may require no free energy, the cell will tightly control any gating processes (e.g., ion-channel gating in an action potential: see above) typically using energy. But once the gate on a channel is open, the channel is a passive bystander, like a faucet allowing water flow.

References:

- B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).
- Wikipedia: Passive transport

Pumps and Transporters

Transport in the Cell



Basics

- **Transport** refers to the cell transporting molecules and ions across membranes - cellular or organelle membranes.
  - Transport is critical to the cell, as it must obtain nutrients, sort molecules, and export waste.
  - Transport can be active or passive.

- Passive transport occurs when molecules flow from a region of high to low concentration. Such flow requires no free energy, but the cell may use free energy to regulate the flow, by turning it on or off as needed.
  - Example: The action potential in a neuron is gated passive transport.
  - See the discussion of ion concentration gradients to learn the basics of electrostatic influences in passive transport.

- **Active transport** uses free energy to pump a molecule against its concentration gradient (i.e., from low to high concentration). This can occur by two generic mechanisms depending on the type of free energy expended to do the pumping:
  1. ATP-driven transport, or
  2. Transport driven by the gradient of another molecule or ion.

Symporters (Co-transporters)

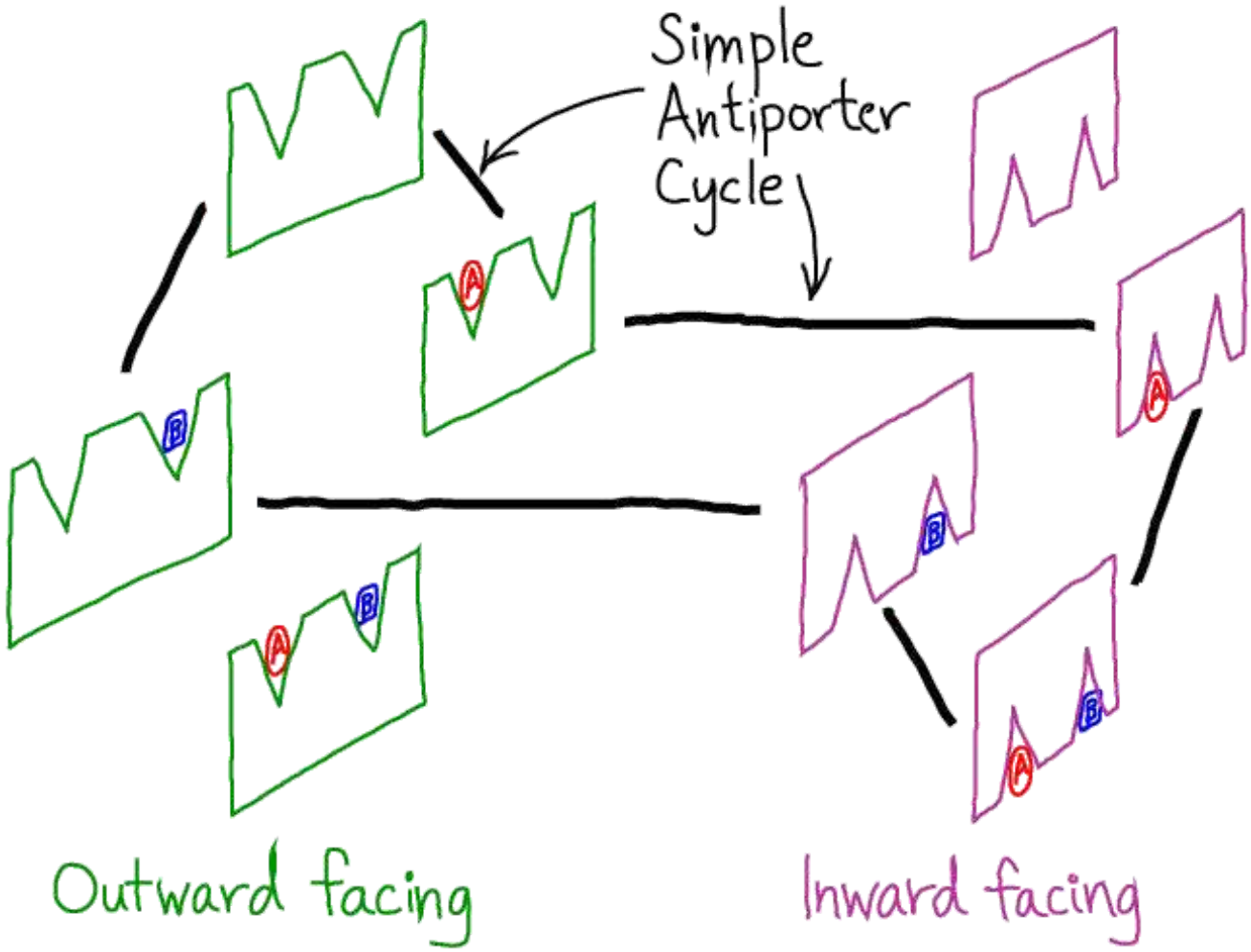
Advanced Cycle Logic

Cycles are essential to biochemistry and cell biology for the simple reason that the cell re-uses molecular components like enzymes. The basic "logic" of cycles has been described.

Here, we want to build on the example of the antiporter/exchanger to understand the "space" of states in which machine cycles tend to exist and also how those states are "wired" together.

Seeing the antiporter in an extended space

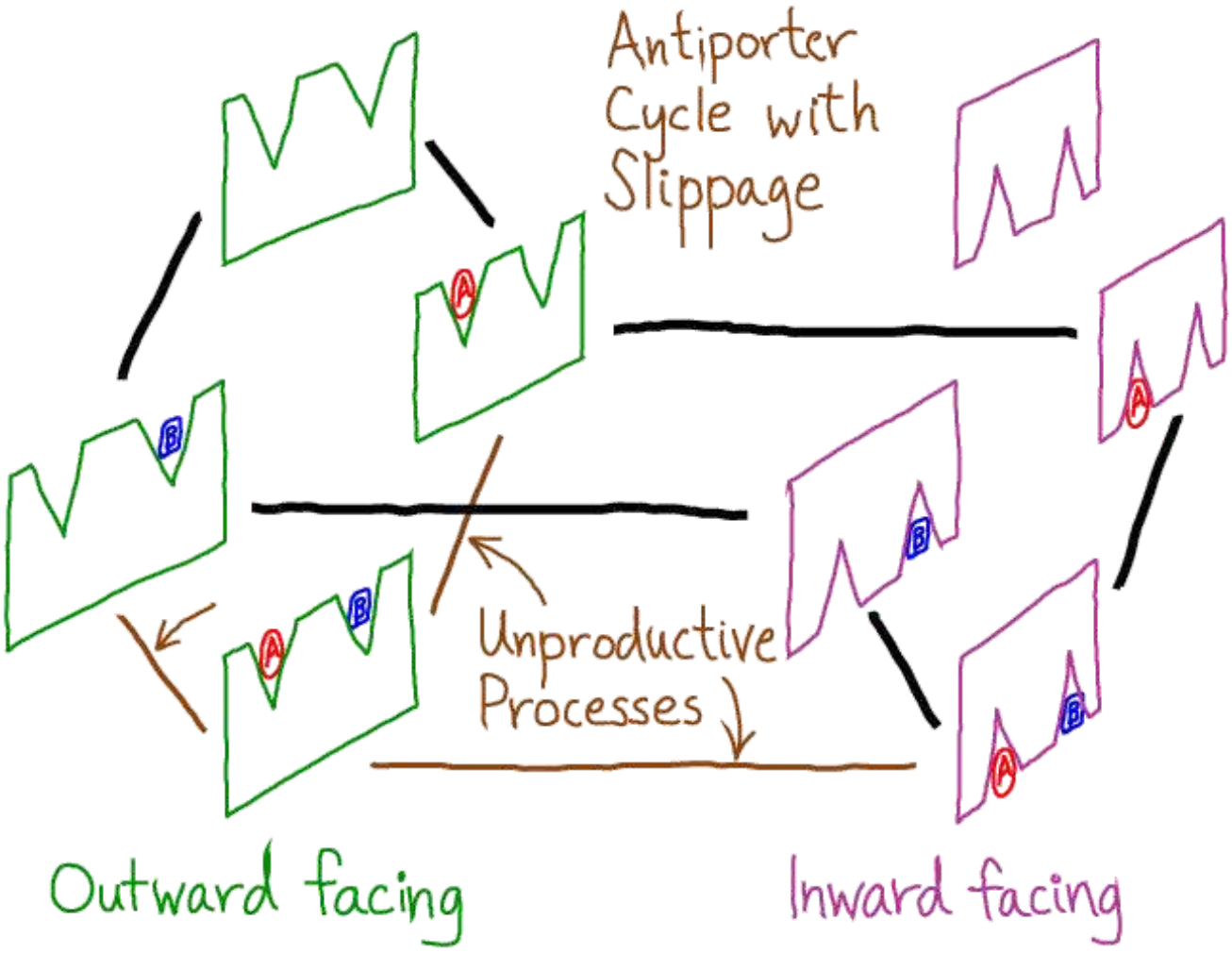
Compare the original antiporter cycle to the equivalent representation below. Here, the binding events are "implicit" - if a new molecule appears along a given line (process) then a binding event must occur.



We have categorized the states as inward and outward facing to emphasize additional states which are not visited in the original antiporter cycle. Indeed, further states might occur, which are not shown - for example, mixed states including one binding site facing inward and the other outward.

Adding "slippage" to the antiporter cycle

Our original antiporter cycle is a perfect 1:1 transporter - each time one A molecule moves in one direction (e.g., out-to-in) a B molecule moves in the opposite direction (e.g., in-to-out). But there is no reason to expect that molecular machines, which function in a thermal environment, function so precisely. Moreover, there may be functional (regulatory) reasons why slippage, or inefficiency, could be helpful.



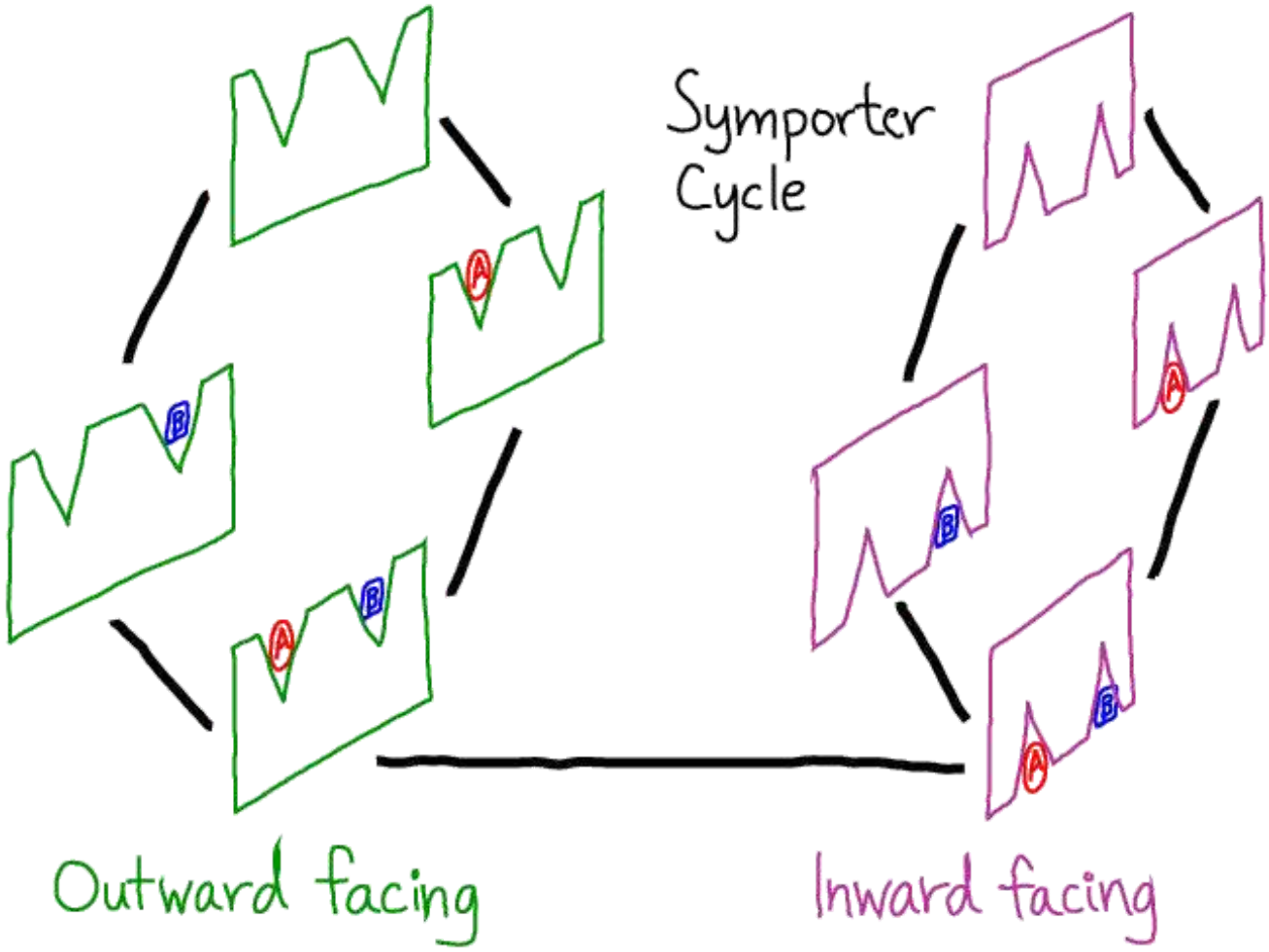
The added brown connections in this cycle do not contribute to the 1:1 exchange of molecules. You should follow the cycle around various possible pathways from an initial state (e.g., top left) and all the way back to the same state to see that the unproductive processes cause the molecule to act as a simple channel.

The relative magnitudes of the rate constants will determine the effectiveness of this molecule as an antiporter. Generally, you should expect that slippage processes can occur.

A symporter cycle in the same space

Using the same set of states shown, we can wire together a "symporter" (co-transporter) which transports

one A and one B molecule together.



Comparing this diagram to the previous two, it is not difficult to imagine one system evolving from the others.

References:

- T.L. Hill, "Free Energy Transduction and Biochemical Cycle Kinetics," (Dover, 2005). Absolutely **the** book on cycles. Describes the effects of including additional states and transitions. Beyond Chapter 1 is difficult reading.
- D.A. Beard and H. Qian, "Chemical Biophysics: Quantitative analysis of cellular systems ," (Cambridge, 2008).

Exercises:

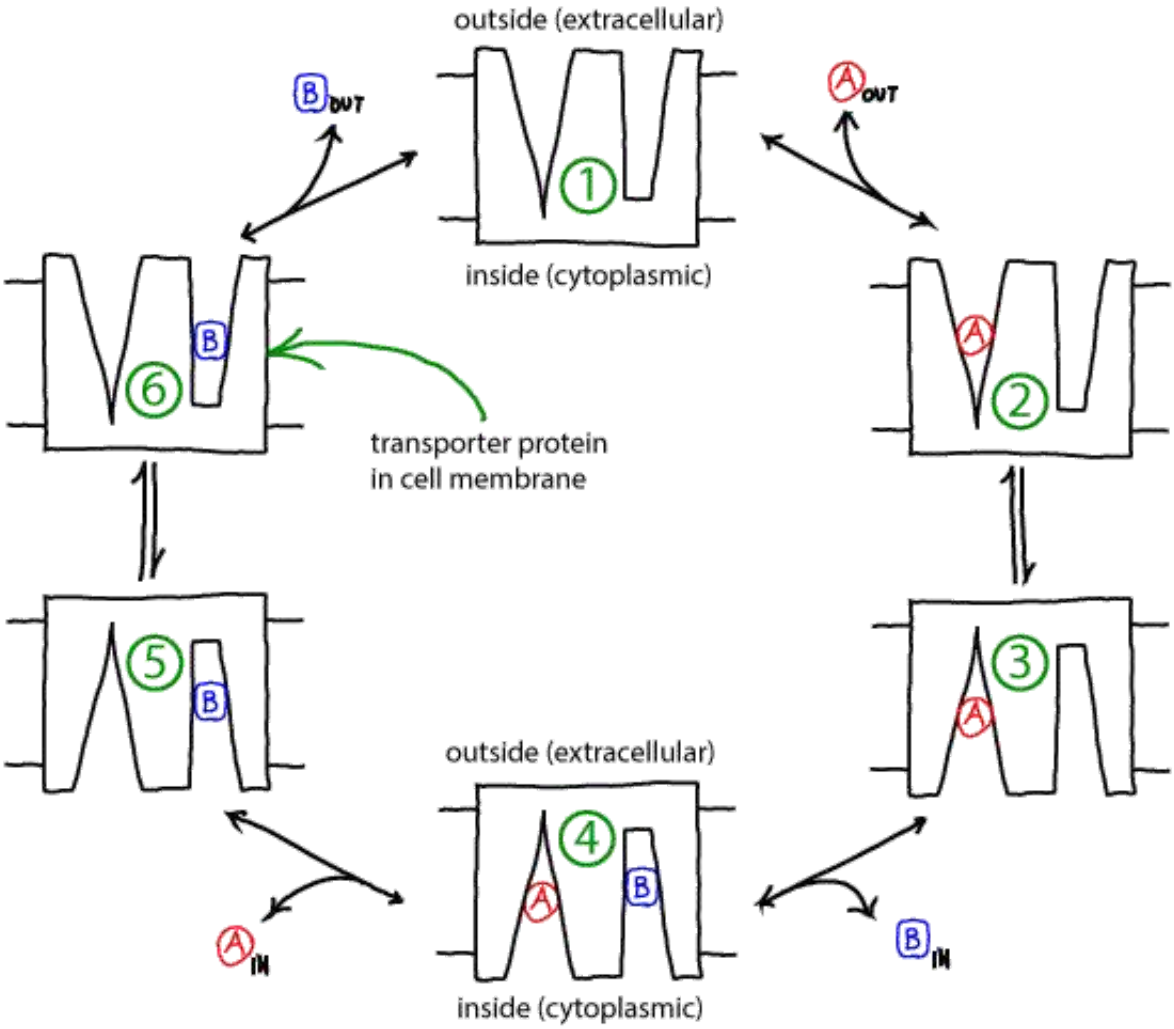
1. Sketch a symporter wiring diagram that includes a slippage process, and describe what happens during the slippage.
2. Using the principles explained for general cycles, determine the constraints that would apply for processes that include slippage.

Antiporters (Exchanger or Counter-transporter)

Active transport in an alternating-access antiporter model

Free energy stored in a non-equilibrium gradient of concentrations across a membrane can be used by an antiporter ("exchanger") to pump another molecule against its gradient.

- Membrane-embedded transporter proteins can function as machines because binding events are coupled to conformational changes.
- The cycle below is a simplified, schematic model of a transporter for which binding events are coupled to "eversion" - transition between inward-facing and outward-facing conformations.
    - The alpha helices in membrane proteins achieve eversion in roughly the same manner as you might re-adjust the orientations of a group of irregular pens and pencils held in your hand.



Basics of the antiporter cycle

- The antiporter schematized above can perform active transport using the gradient of one molecule or

ion (e.g., A) to pump the other (B) in the opposite direction. Free energy is supplied by the gradient(s) -- *no other source of free energy, such as ATP, is needed.*

- If the ratio of outside to inside concentrations of one molecule (e.g., A) is higher than the other, the cycle will be driven in a particular direction (e.g., clockwise).
- A transporter such as the antiporter shown can be considered a "passive element": it does not supply any energy itself, but only transduces free energy stored in gradients. The transduction occurs due to the coordination of binding and conformational changes.
- Because it is a passive element, the antiporter does not "know" which molecule's gradient is meant to drive the transport of the other. Thus, if the outside/inside concentration ratio is greater for B, then the cycle will run counter-clockwise and A will be pumped by B.
- The model as shown is a simplified version that omits some possible states and connections among states - e.g., both binding sites are assumed to always face the same direction. Additional states and connections could reduce the efficiency of pumping. See also discussion in Hill's book. The presence of additional states or connections would have to be verified on a system-by-system basis.

Understanding how the cycle is driven

- A simple but powerful qualitative analysis starts by considering the condition of equilibrium. Equilibrium describes a (hypothetical) very large set or ensemble of identical systems of which - on average - an equal number are executing the forward and reverse of every process. Thus, for example, between steps 1 and 2, if there are N_{12} systems in which extracellular ("outside") A binds every second, there is an equal number ($N_{21} = N_{12}$) for which A unbinds to the outside.
- In the perfectly balanced state of equilibrium, there will be equal numbers of systems performing full clockwise and full counter-clockwise cycles per unit time. Thus, although individual cycles may pump A and B, there is no net pumping.
- Because the balance of equilibrium is so perfect, however, it can be disturbed at almost any point in the cycle. For example, adding an excess of A outside will cause more 1-to-2 transitions, which will cause 2-to-3 transitions and so on, leading to clockwise cycling. It is the binding process that provides directionality. *Binding events can be considered the "handles" used to drive a cycle in a given direction.*
- Importantly, the cycle can also be driven by imbalancing the detailed equilibrium at *any* point in the cycle:
    - Excess B added inside will also drive the cycle clockwise.
    - Excess A added inside will drive the cycle *counter*-clockwise.
    - Excess B will also drive the cycle counter-clockwise.

A quantitative model: Simulation and analysis

- We will employ a chemical-kinetics model, which is formulated solely in terms of state populations and rate constants for transitions among states, assumed to obey mass-action behavior.

- To keep the model simple, we will assume all rates are the same:
  - k_{on} will be the on-rate (see Notation) for all A and B binding -- for transitions from state 1 to 2, from 1 to 6, from 3 to 4, and from 5 to 4.
  - k_{off} will be the off-rate for all unbinding of A or B -- reversals of the transitions noted above.
  - k_{conf} will be the rate for all conformational transitions -- both directions between states 2 and 3, and between 5 and 6.
  - We will assume that the outside and inside volumes are the same -- so that the numbers of A and B molecules tell us the concentrations in a simple way.

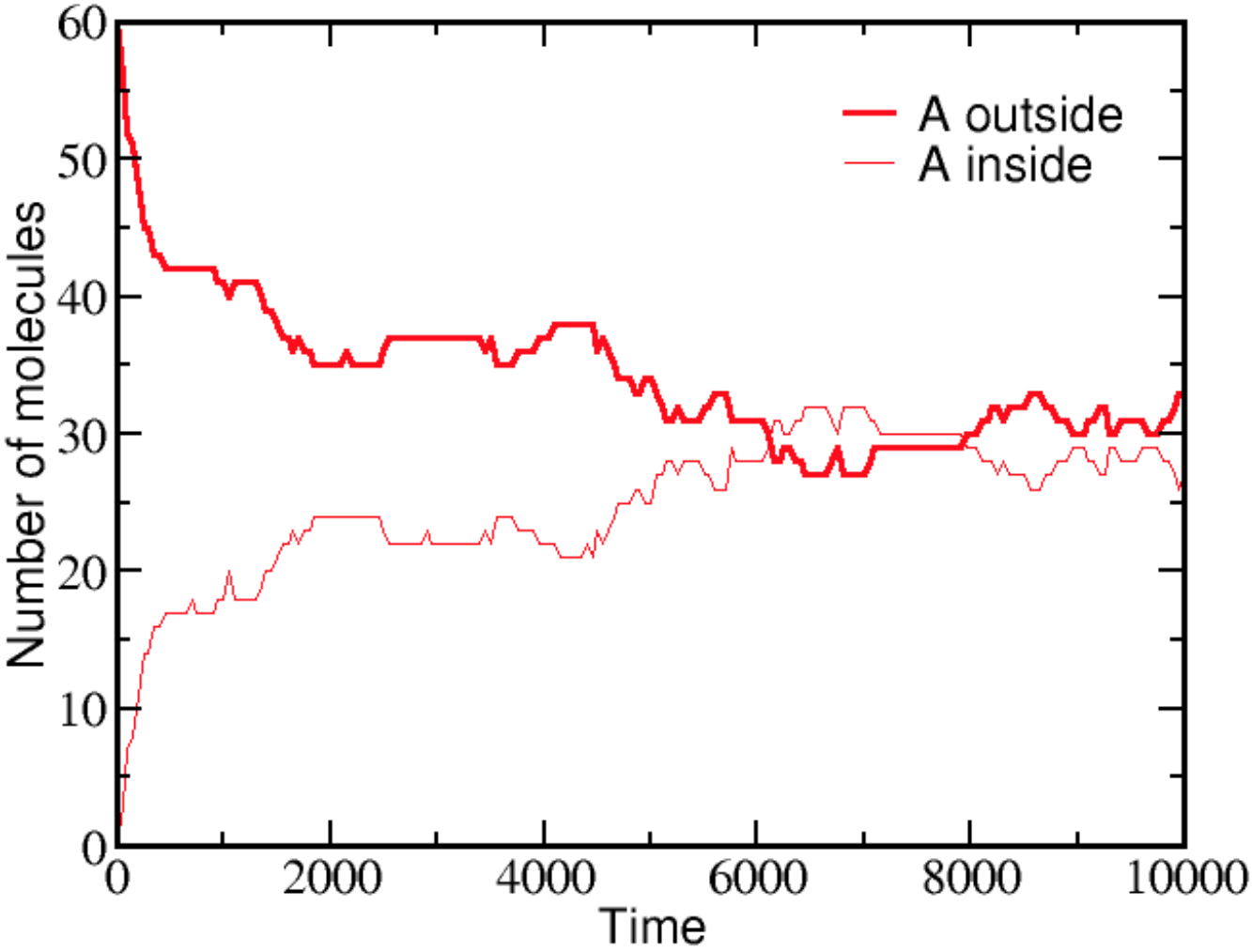
- The model is now fully specified. For example, the differential equation governing the population of state 1 (denoted [1]) is gievn by

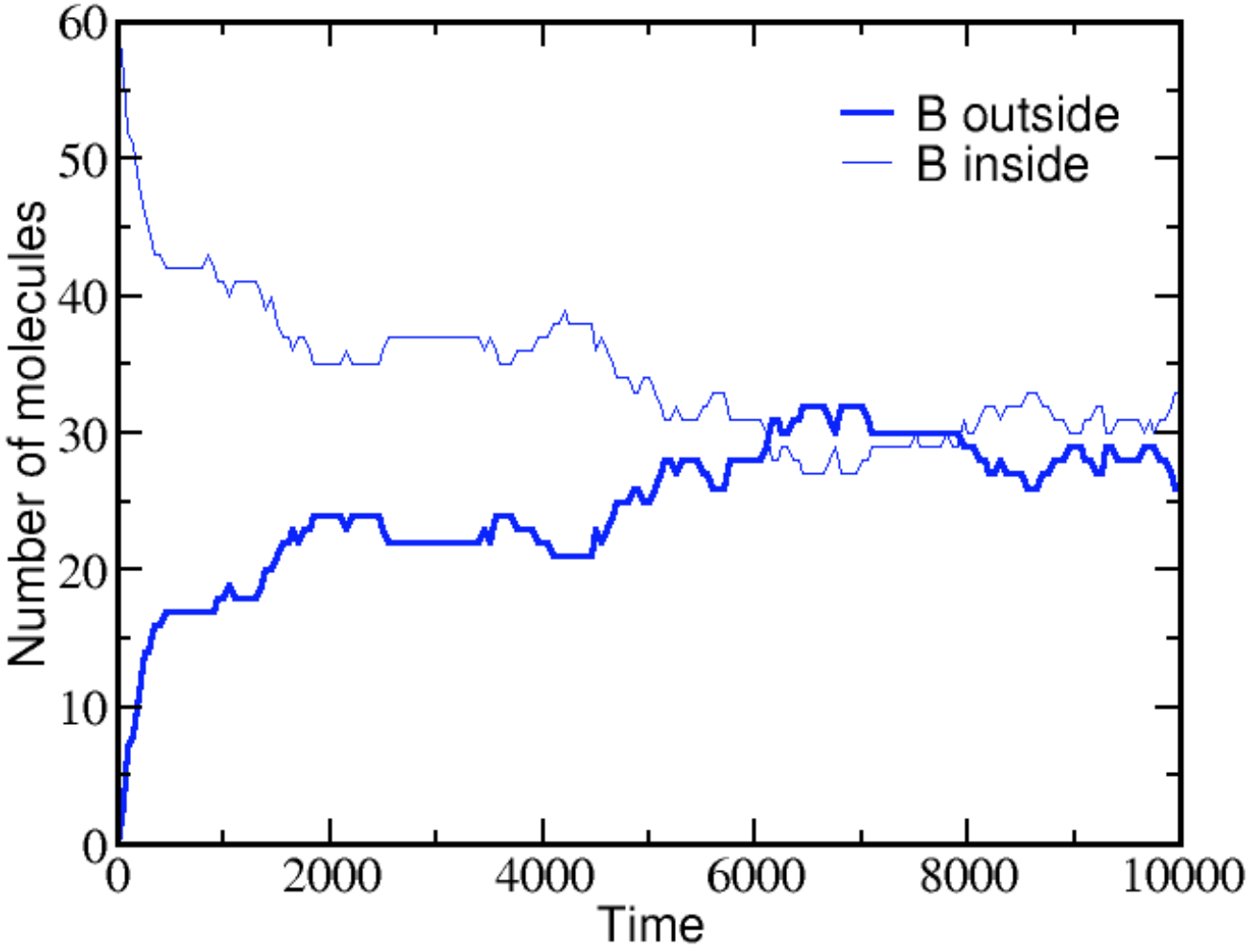
$$\frac{d[1]}{dt} = -[1][\text{A}]_{\text{out}}\, k_{\text{on}} - [1][\text{B}]_{\text{out}}\, k_{\text{on}} + [2]\, k_{\text{off}} + [6]\, k_{\text{off}}$$

  - In words, the equation means that the population of the unbound state 1 decreases due to binding of A or B (from the outside) with rate k_{on} and increases due to unbinding from either of states 2 or 6 with rate k_{off}.

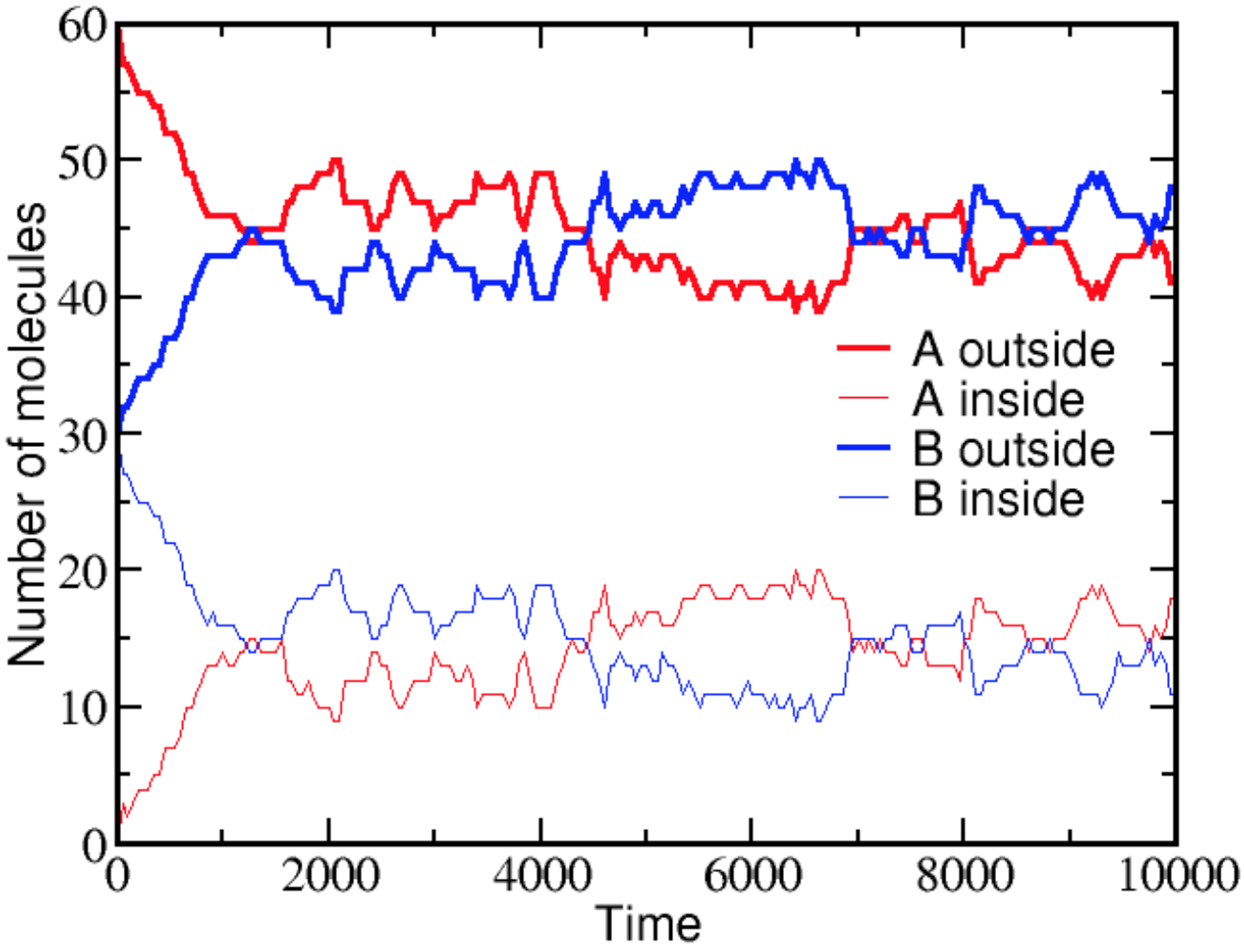
Simulating the model

If we start a simulation with all A molecules on the outside and an equal number of B molecules solely on the inside, the system equilibrates to equal concentrations on both sides -- for both species (A and B).


A outside
A inside
Number of molecules
Time
0
10
20
30
40
50
60
0
2000
4000
6000
8000
10000



However, if we start a simulation with B molecules equally distributed between outside and inside, and the A molecule all outside as before, there is a very different outcome. The greater outside concentration of A tends to drive A molecules inward, but doing so requires "pumping" B molecules to the outside. Because the B molecules "resist" having different outside and inside concentrations, the final equilibrium is one where the ratio of outside/inside concentrations equalizes between the two species.



These simulations were performed using BioNetGen, a rule-based platform for kinetic modeling. The brief source code for the model (a .bngl file) can be downloaded by right-clicking here.

Analyzing the model

Perhaps the simplest and most important analysis of the model is deriving and understanding the equilibrium behavior. In equilibrium, every process and its reverse will be in exact (detailed) balance -- the flow in both directions will match. Hence, we need to write down balance equations for each set of arrows (connectings states 1 and 2, 2 and 3, ...). Using $[\mathrm{X}]^{\mathrm{eq}}$ to refer to the equilibrium concentration of component X, we write down the equilibrium equations for the three processes on the right side of the cycle.

$$[1]^{\mathrm{eq}}\,[\mathrm{A}]_{\mathrm{out}}^{\mathrm{eq}}\,k_{\mathrm{on}} = [2]^{\mathrm{eq}}\,k_{\mathrm{off}} \tag{1}$$

$$[2]^{\mathrm{eq}}\,k_{\mathrm{conf}} = [3]^{\mathrm{eq}}\,k_{\mathrm{conf}} \tag{2}$$

$$[3]^{eq}\,[B]_{in}^{eq}\,k_{on} = [4]^{eq}\,k_{off} \tag{3}$$

The equations for the left side mirror these.

Solving the full set of equations (by going around the cycle and eliminating one of the numbered-state concentrations at a time) yields the result

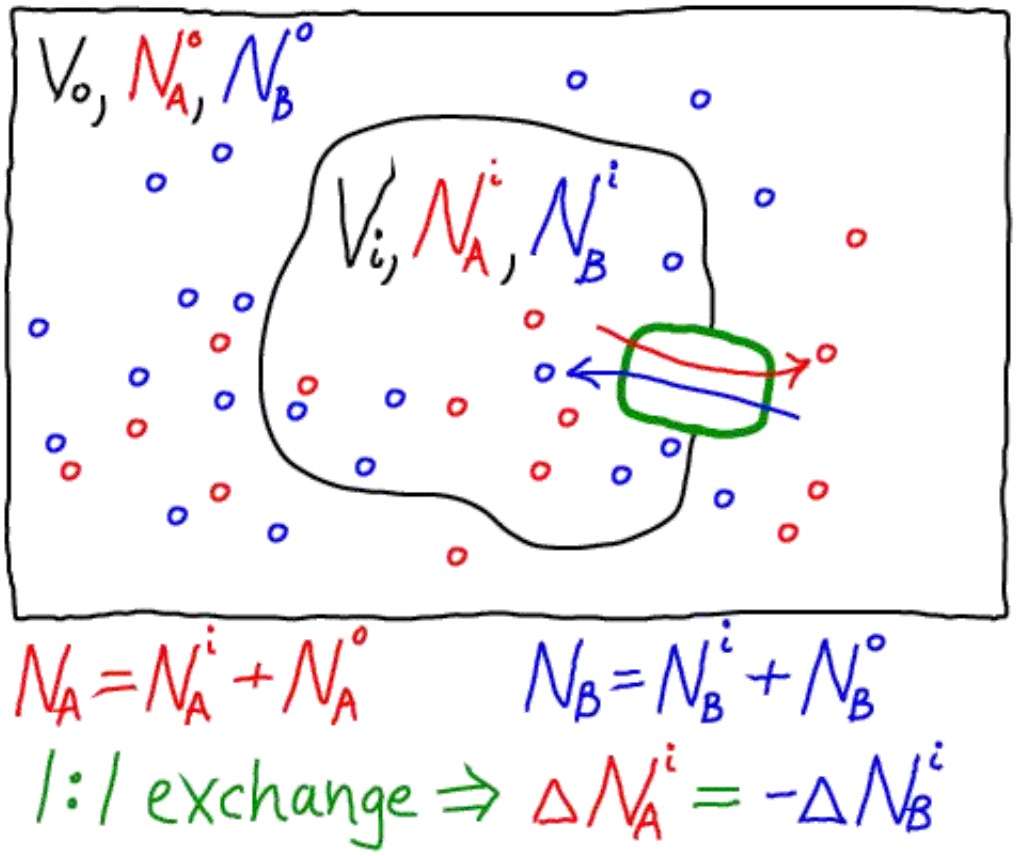
$$\frac{[A]_{out}^{eq}}{[A]_{in}^{eq}} = \frac{[B]_{out}^{eq}}{[B]_{in}^{eq}} \tag{4}$$

As we saw in the simulations, equilibrium is reached when the outside-to-inside concentration ratios match for A and B. In fact, because our transporter is a passive element, this result is independent of the rates chosen -- even if the rates are not symmetric (but be careful that the set of rates in a cycle has to be physically consistent).

Note that, perhaps surprisingly, the equilibrium point will depend on the initial condition. This is true because our simple antiporter model does not allow "slippage" (where only an A or a B is transported in some cycles). If there were some mechanism for A and/or B to switch compartments independently of the other, then the equilibrium point would not only have equal concentration ratios, but the ratios would be 1 -- equal inside and outside concentrations. A more advanced discussion of the antiporter cycle describes further possibilities and the relation to a symporter cycle.

Thermodynamic analysis

In contrast to the kinetic analysis above, which includes a description of specific states of the antiporter, we can perform a thermodynamic analysis of the same system. Using an ideal gas description, in analogy to what was done for binding and for understanding free energy storage in a gradient, arguably leads to a simpler description than we encountered using kinetics. The thermodynamic analysis will directly lead to the equilibrium condition (4).



The thermodynamic model consistent with the mass-action picture above consists of ideal gases for each of the components: A molecules inside (i) and out (o), as well as B molecules inside and out. The combined free energy F for the whole system is the sum of the free energies for each of these ideal gases.

$$\begin{aligned} F =& F^{\text{idl}}(N_A^i, V_i) + F^{\text{idl}}(N_B^i, V_i) \\ &+ F^{\text{idl}}(N_A^o, V_o) + F^{\text{idl}}(N_B^o, V_o), \end{aligned} \tag{5}$$

where we have omitted the temperaure dependence that will not be pertinent here. The total free energy is the simple sum of the component free energies because there are no (energetic) interactions between the components. In an ideal gas, by definition, there are no interactions among molecules.

To obtain concrete results, we must specify one specific state of our system - something like an initial condition. After all, the 1:1 exchange of A and B means that not every possible set of populations ($N_A^i, N_A^o, N_B^i, N_B^o$) is possible. For simplicity, we shall assume that our reference state has all A molecules inside ($N_A^i = N_A, N_A^o = 0$) and all B molecules outside ($N_B^o = N_B, N_B^i = 0$).

Further, because we are considering a 1:1 antiporter (where one A always exchanges for one B), there is really only one variable that can change. To see this, we can express all the species numbers in terms of N_A^o, the number of A molecules outside, and the *constant* species totals N_A and N_B:

- because an A molecule must be either inside or out, we have $N_A^i = N_A - N_A^o$;
- because of the 1:1 exchange, $N_B^i = N_A^o$; and
- because a B molecule must be either inside or out, $N_B^o = N_B - N_B^i = N_B - N_A^o$.

Using these relations and noting that $F^{\mathrm{idl}}(N, V, T) = Nk_BT \ln[N/(V/\lambda^3)]$, we can now re-write the total free energy from (5) as

$$\begin{aligned}\frac{F}{k_BT} =& \left(N_A - N_A^o\right) \ln\left(\frac{N_A - N_A^o}{V_i/\lambda_a^3}\right) \\ &+ N_A^o \ln\left(\frac{N_A^o}{V_o/\lambda_a^3}\right) + N_A^o \ln\left(\frac{N_A^o}{V_i/\lambda_b^3}\right) \\ &+ \left(N_B - N_A^o\right) \ln\left(\frac{N_B - N_A^o}{V_o/\lambda_b^3}\right)\end{aligned} \tag{6}$$

We find the most probable state (which is equilibrium in the large N limit) by setting to zero the derivative of F with respect to N_A^o. After some algebra, the result is

$$\frac{N_A^o/V_o}{N_A^i/V_i} = \frac{N_B^o/V_o}{N_B^i/V_i}, \tag{7}$$

which is equivalent to (4) because N_X/V is the concentration of species X.

Importantly, *the thermodynamic analysis does not require knowledge of the system's substates, but only the 1:1 stoichiometry*.

More on antiporters

The stoichiometry of transport has very interesting effects: see the discussion of 2:1 exchange. Also, all the analysis above assumed perfect 1:1 exchange. In reality slippage could be important, and is a critial general phenomenon in complex molecular machines.

References:

- B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).
- T.L. Hill, "Free Energy Transduction and Biochemical Cycle Kinetics," (Dover, 2005). Absolutely **the** book on cycles. Describes the effects of including additional states and transitions.

Exercises:

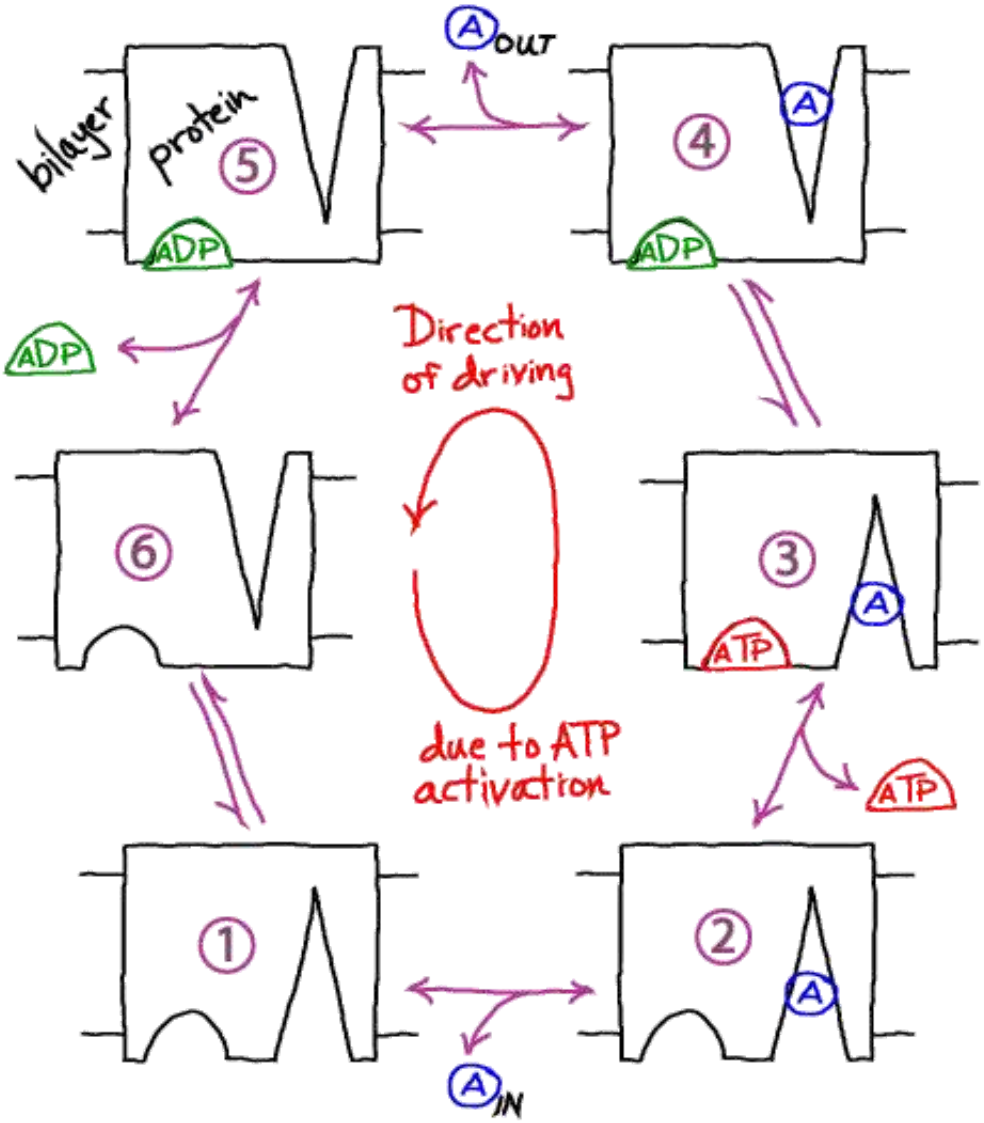
1. Derive (4).
    1. Derive (4) using arbitrary rates $\{k_{12}, k_{21}, k_{23}, \dots\}$. In doing so, you will want to be sure your cycle is "thermodynamically consistent" with equilibrium principles. In other words, you will need to determine a constraint, which can be done by assuming the system starts at the state of minimum global free energy in which each species has equal concentrations on both sides.
2. Derive (7).

ATP-driven Transporters

Active transport driven by ATP hydrolysis

Free energy stored in the activated carrier ATP can be used to drive the transport of an ion or small molecule against a gradient - i.e., transport from a region of low to high concentration.

- Membrane-embedded transporter proteins can function as machines because binding and catalytic events are coupled to conformational changes.
- The cycle below is a simplified, schematic model of a transporter whose ATP hydrolysis step is coupled to "eversion" - transition between inward-facing and outward-facing conformations.
- The alpha helices in membrane proteins achieve eversion in roughly the same manner as you might re-adjust the orientations of a group of irregular pens and pencils held in your hand.



Basics of the Cycle

- This cycle will pump molecule A from inside to outside a cell or compartment in typical conditions (relatively high ATP concentration).
- Like all cycles, this one can be reversed with sufficiently large [A] outside and sufficiently small [ATP]. Thus, free energy stored in the gradient of A can be used to synthesize ATP: see below.
- As in the case of gradient-driven transport, the pump itself is actually a passive element or catalyst for the cycle. The free energy is fully supplied by ATP.
- Note that the model as shown is a simplified version that omits some possible states and connections among states - e.g., ATP cannot bind the empty receptor in our model.
    - Additional states and connections could reduce the efficiency of pumping. See discussion in Hill's book.
    - The presence of additional/states connections would have to be verified on a system-by-system basis.

Understanding how the cycle is driven

- A simple but powerful qualitative analysis starts by considering the condition of equilibrium. Equilibrium describes a (hypothetical) very large set or ensemble of identical systems of which - on average - an equal number are executing the forward and reverse of every process. Thus, for example, between steps 2 and 3, if there are N_{23} systems in which ATP binds every second, there is an equal number ($N_{32} = N_{23}$) for which ATP unbinds.
- In the perfectly balanced state of equilibrium, there will be equal numbers of systems performing full clockwise and full counter-clockwise cycles per unit time. Thus, although individual cycles may pump A or synthesize ATP, there is no net pumping or synthesis/hydrolysis of ATP.
- Because the balance of equilibrium is so perfect, however, it can be disturbed at almost any point in the cycle. Most obviously, adding an excess of ATP will cause more 2-to-3 transitions, which will cause 3-to-4 transitions and so on, leading to counter-clockwise cycling. It is the binding process that provides directionality. *Binding events can be considered the "handles" used to drive a cycle in a given direction.*
- Importantly, the cycle can also be driven by imbalancing the detailed equilibrium at *any* point in the cycle:
    - Excess A added inside will also drive the cycle counter-clockwise.
    - Excess A added outside will drive the cycle *clockwise*.
    - Excess ADP will also drive the cycle clockwise.

A quantitative model: Simulation and analysis

- We will employ a chemical-kinetics model, which is formulated solely in terms of state populations and rate constants for transitions among states, assumed to obey mass-action behavior.
- To keep the model simple, we will make the following assumptions:

- We assume ATP hydrolysis to ADP is a unimolecular event -- i.e., no phosphate (Pi) is released. This does not affect the key conclusions.
- k_{on} will be the on-rate (see Notation) for both A and nucleotide binding -- for transitions from state 1 to 2, from 2 to 3, from 6 to 5, and from 5 to 4.
- k_{off} will be the off-rate for all unbinding of A or nucleotide (ATP, ADP) -- reversals of the transitions noted above.
- k_{conf} will be the rate for conformational transitions in both directions in the apo transporter (no A or nucleotide) -- transitions between states 1 and 6.
- The coupled process of conformational change and ATP conversion to ADP will be governed by the rates k_{td} and k_{dt} for the processes 3 to 4 and 4 to 3, respectively.
- We will assume that the outside and inside volumes are the same -- so that the numbers of A, ATP, and ADP molecules tell us the concentrations in a simple way.

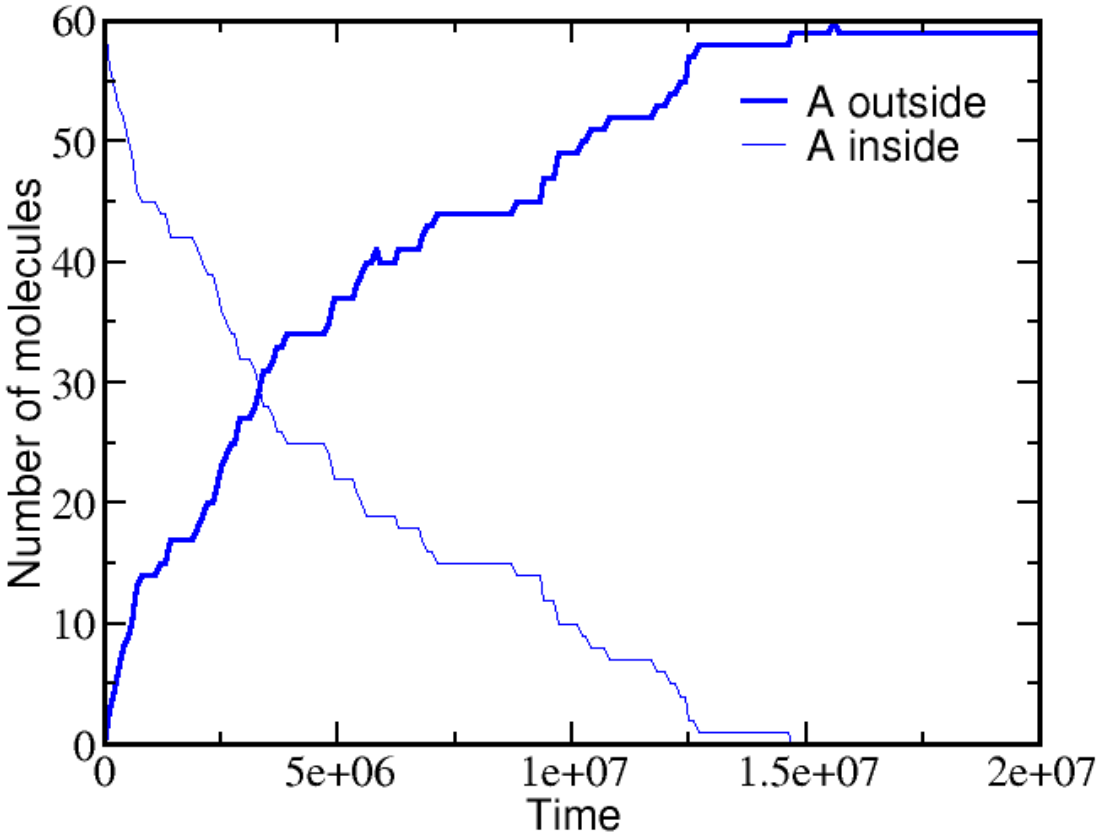
- The model is now fully specified. For example, the differential equation governing the population of state 1 (denoted [1]) is gievn by

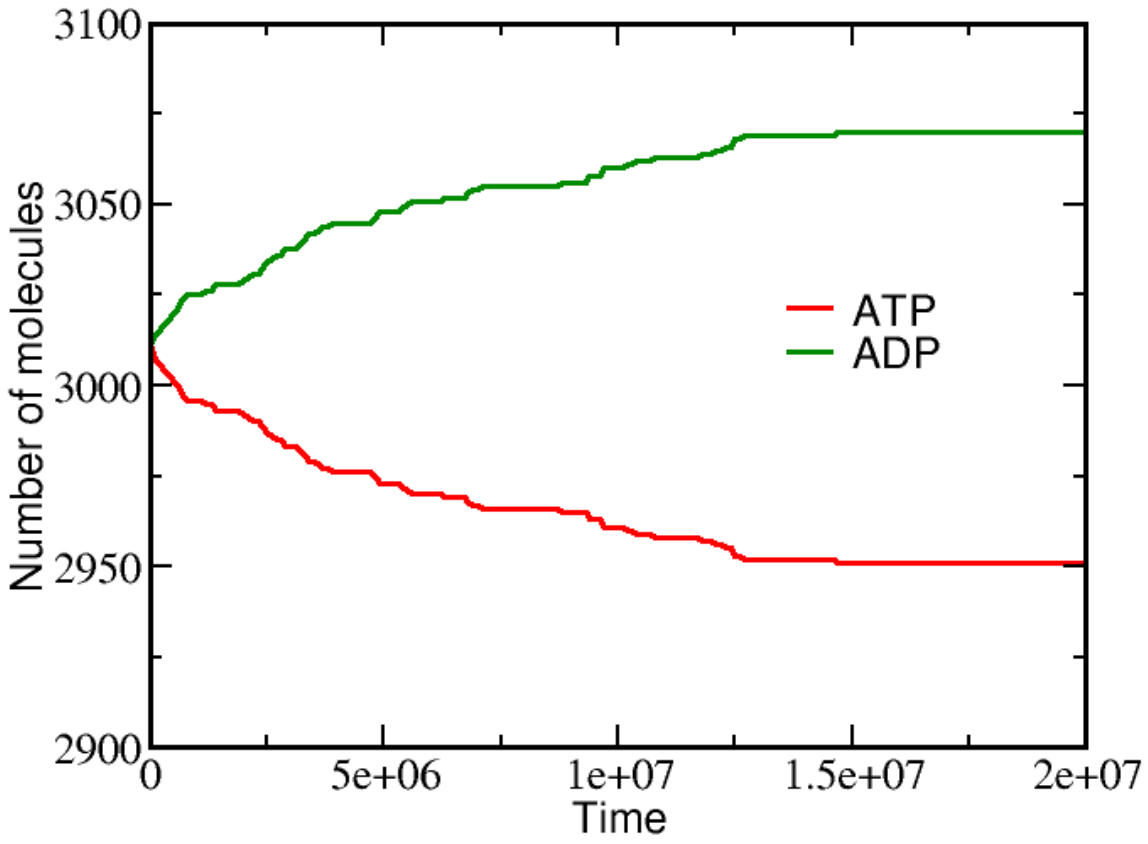
$$\frac{d[1]}{dt} = -[1][\text{A}]_{\text{in}}\, k_{\text{on}} - [1]\, k_{\text{conf}} + [2]\, k_{\text{off}} + [6]\, k_{\text{conf}}$$

- In words, the equation means that the population of the unbound state 1 decreases due to binding of A with rate k_{on} and due to conformational transitions to state 6 with rate k_{conf}; it increases due to unbinding from state 2 with rate k_{off} and due to conformational transitions from state 6 with rate k_{conf}.

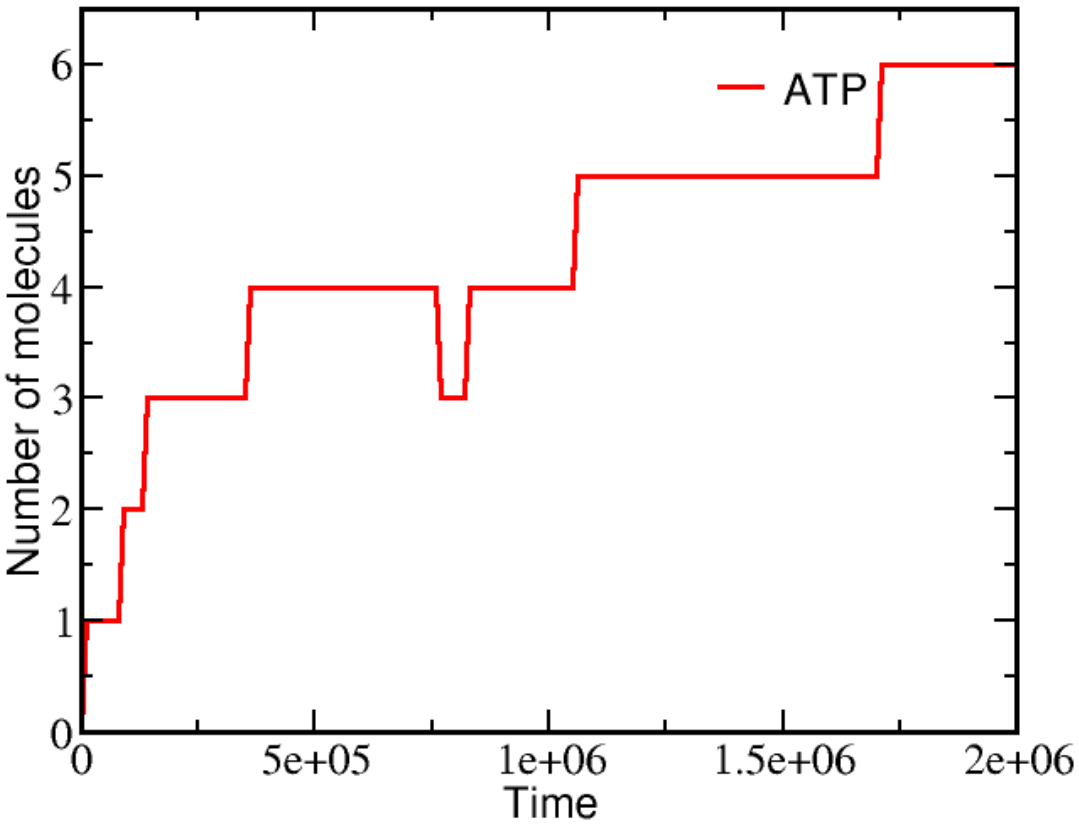
Simulating the model

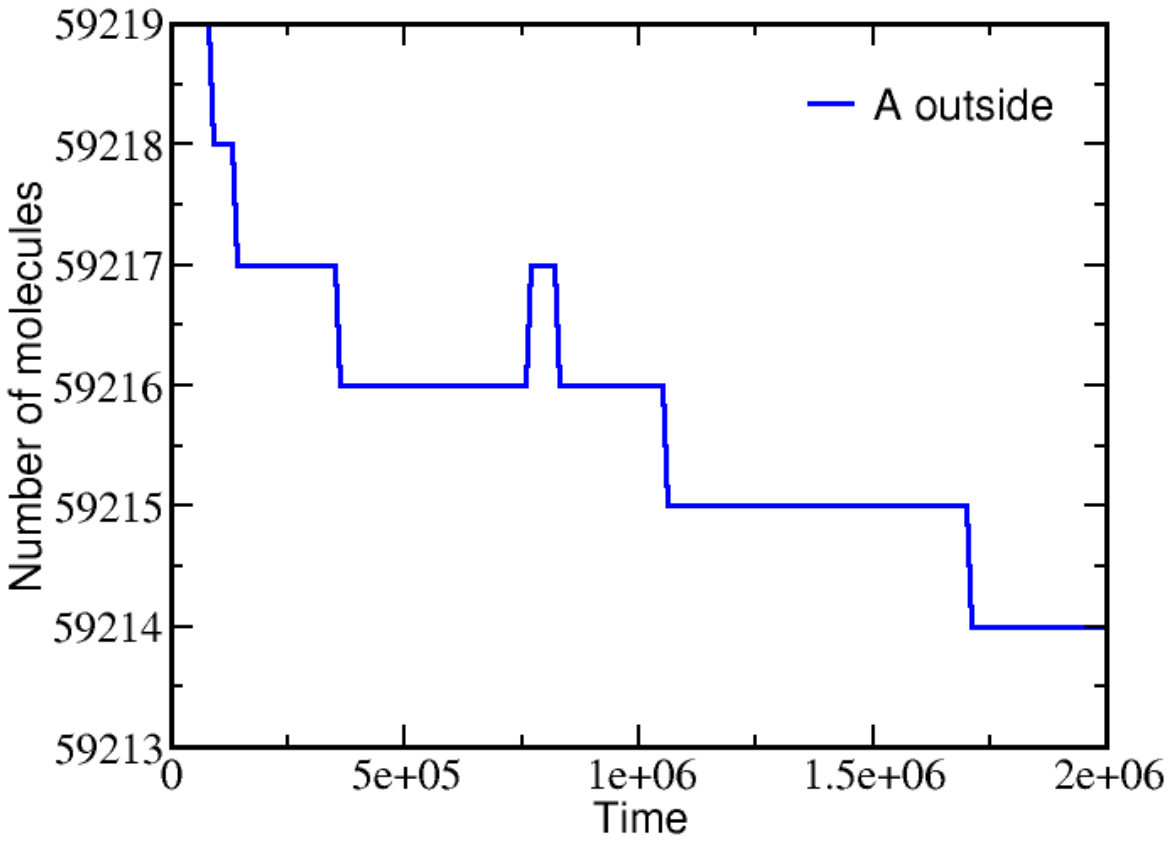
If we start a simulation with all A molecules on the inside and equal numbers of ATP and ADP (which is far from equilibrium), the system equilibrates to a state with essentially all the A molecules pumped outside. A molecules are pumped because the initial condition of equal ATP and ADP concentrations is far from equilibrium: ADP is greatly favored and the system moves toward increased ADP concentration even at the (lesser) cost of pushing A beyond its apparent equilibrium of equal inside and outside concentrations. The data below show the behavior due to a single transporter.


60
50
40
30
20
10
0
Number of molecules
0
5e+06
1e+07
1.5e+07
2e+07
Time
A outside
A inside



On the other hand, if we start from a very different non-equilibrium condition where *no* ATP is present and all the A is outside, then ATP will be sythesized (provided there is a sufficiently large amount of A present). The data below show the system evolution when multiple transporters are present.


ATP
Number of molecules
0
1
2
3
4
5
6
0
5e+05
1e+06
1.5e+06
2e+06
Time



These simulations were performed using BioNetGen, a rule-based platform for kinetic modeling. The brief source code for the model (a .bngl file) can be downloaded by right-clicking here.

Analyzing the model

Although molecular bio-machines tend to operate out of equilibrium, an equilibrium analysis is the simplest and clearest reference point from which to understand their behavior. In equilibrium, every process (i.e., arrows in the model) will be in balance with its reverse process. In terms of the simple mass-action kinetics we are employing (see above for rates), this means

$$[1]^{eq}\,[A]_{in}^{eq}\,k_{on} = [2]^{eq}\,k_{off} \quad (1)$$

$$[2]^{eq}\,[ATP]^{eq}\,k_{on} = [3]^{eq}\,k_{off} \quad (2)$$

$$[3]^{\mathrm{eq}}\, k_{\mathrm{td}} = [4]^{\mathrm{eq}}\, k_{\mathrm{dt}} \tag{3}$$

and so on.

Solving the full set of equations (by going around the cycle and eliminating one of the numbered-state concentrations at a time) yields the result

$$\frac{[\mathrm{A}]^{\mathrm{eq}}_{\mathrm{out}}}{[\mathrm{A}]^{\mathrm{eq}}_{\mathrm{in}}} = \frac{[\mathrm{ATP}]^{\mathrm{eq}}}{[\mathrm{ADP}]^{\mathrm{eq}}}\,\frac{k_{\mathrm{td}}}{k_{\mathrm{dt}}} \tag{4}$$

Despite its simple appearance, this is actually a *constrained* equilibrium, as we will see below.

We need to carefully determine the ratio $k_{\mathrm{td}}/k_{\mathrm{dt}}$. We can do this by considering *any* equilibrium situation because the ratio is not a property of the various rates involved, but rather of the overall "stoichiometry" of the machine (i.e., of the fact that one ATP is required to pump one A molecule). We can imagine that our transporter is present in a membrane where there is also a simple channel that allows A to flow in or out without impediment. In this case, A must equilibrate to equal inside and outside concentrations. But also, ATP and ADP will be unconstrained and hence will relax to their "natural" equilibrium -- i.e., the equilibrium they would reach in solution if they were not coupled to any other process. This equilibrium is governed by the ratio of solution ("sol") rates:

$$\frac{[\mathrm{ATP}]^{\mathrm{eq}}}{[\mathrm{ADP}]^{\mathrm{eq}}} = \frac{k^{\mathrm{sol}}_{\mathrm{dt}}}{k^{\mathrm{sol}}_{\mathrm{td}}} \equiv K^{\mathrm{eq}} \tag{5}$$

which is the same as the equilibrium constant by definition.

To finish our analysis, we substitute (5) into the full-system equilibrium condition (4) for the special case $[\mathrm{A}]^{\mathrm{eq}}_{\mathrm{out}}/[\mathrm{A}]^{\mathrm{eq}}_{\mathrm{in}} = 1$, finding

$$\frac{[\mathrm{A}]^{\mathrm{eq}}_{\mathrm{out}}}{[\mathrm{A}]^{\mathrm{eq}}_{\mathrm{in}}} = 1 = \frac{k^{\mathrm{sol}}_{\mathrm{dt}}}{k^{\mathrm{sol}}_{\mathrm{td}}}\,\frac{k_{\mathrm{td}}}{k_{\mathrm{dt}}} \tag{6}$$

We thus conclude

$$\frac{k_{td}}{k_{dt}} = \frac{k_{td}^{sol}}{k_{dt}^{sol}}, \tag{7}$$

which is a result for the *particular choices* we made for rate constants. Had we chosen different rates for the various processes, a different relation would have been found in terms of the various rates. Note that (7) does *not* say that the rates in and out of solution are the same: it only says their *ratio* is the same.

A Constrained Equilibrium

Let us re-write the equilibrium condition (4) using the constraint (7):

$$\frac{[A]_{out}^{eq}}{[A]_{in}^{eq}} = \frac{[ATP]^{eq}}{[ADP]^{eq}} \frac{k_{td}^{sol}}{k_{dt}^{sol}} \tag{8}$$

This is actually a *constrained* equilibrium, assuming that the only way A is transported -- and the only way ATP and ADP interconvert -- is through our transporter. Specifically, the concentrations of A generally will not reach their "natural" equilibrium (equal inside and outside concentrations) for arbitrary initial conditions of ATP and ADP. This point is illustrated in the simulation examples above. The equilibrium is constrained by the fact that one ATP is converted to ADP for each A pumped from inside to outside (or the reverse).

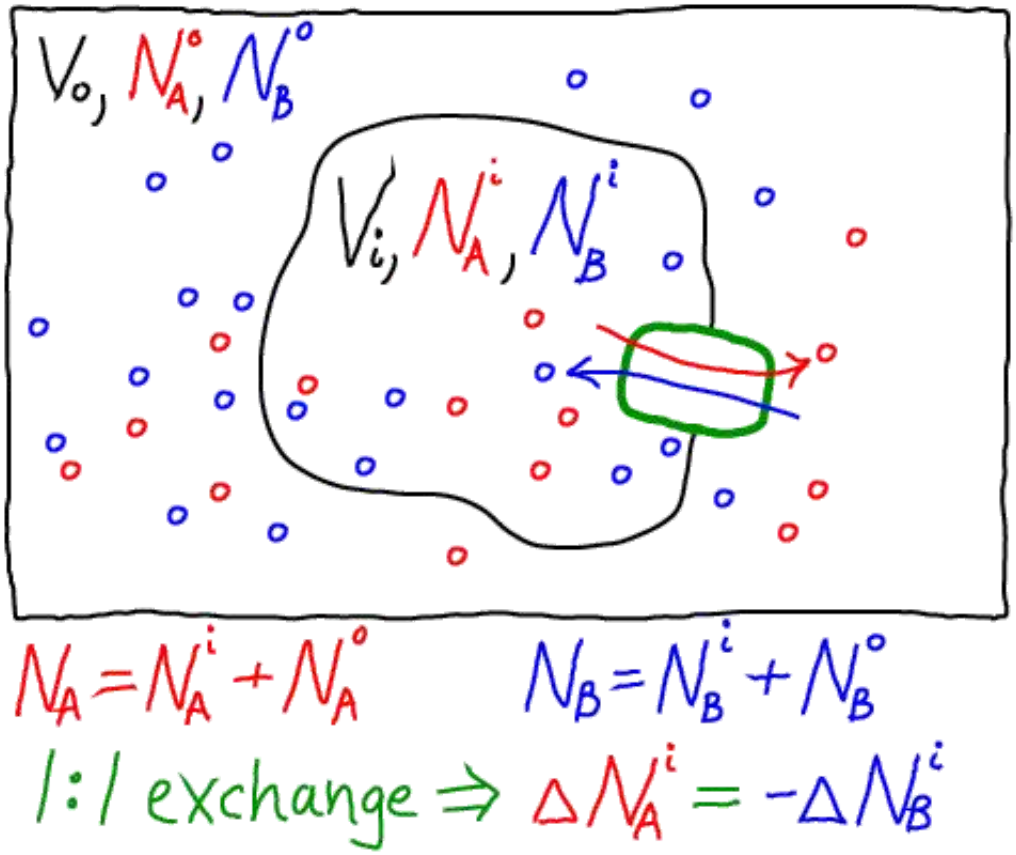
Furthermore, as you can show in an **excercise**, the constrained equilibrium (8) does *not* depend on our simple rate choices. The same result holds for any rate choices in our cycle which allow detailed balance to be satisfied in equilibrium.

Another **exercise**: Write an equation for the equilibrium values of all species based on an arbitrary initial condition *using a single variable.* Ignore molecules that might remain in the transporter. [Hint: Consider the consequences for all species of a single A molecule transported by this model.]

References:

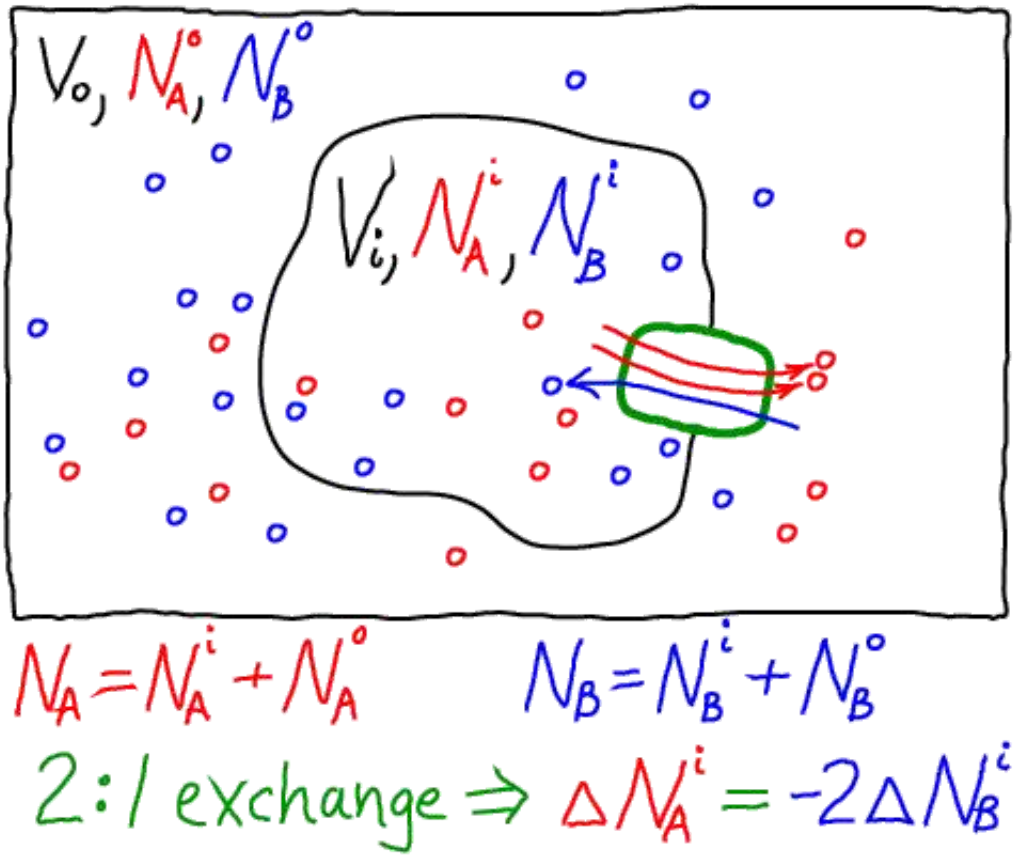
- B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).
- T.L. Hill, "Free Energy Transduction and Biochemical Cycle Kinetics," (Dover, 2005). Absolutely **the** book on cycles. Describes the effects of including additional states and transitions.
- C. Hallam and R. Whittam, "The Role of Sodium Ions in ATP Formation by the Sodium Pump," Proc. R. Soc. Lond. B (1977) 198:109-128, doi: 10.1098/rspb.1977.0088

Stoichiometric Effects in Transport



Although our main discussion of exchange focused on a simple 1:1 antiporter (above), other stoichiometries occur. That is, instead of exchanging precisely one A molecule for a B molecule many exchangers use other stoichiometries. One example is the sodium-calcium exchanger NCX, which is important for pumping calcium out of neurons after an action potential.

Thermodynamic analysis of a 2:1 antiporter



We can readily construct a thermodynamic model consistent with our usual a href="javascript:changeTo('transportStoich','massAction')">mass-action picture. As for the 1:1 antiporter, the model consists of a href="javascript:changeTo('transportStoich','idealGas')">ideal gases for each of the components: A molecules inside (i) and out (o), as well as B molecules inside and out. The combined free energy F for the whole system is the sum of the free energies for each of these ideal gases.

$$F = F^{\mathrm{idl}}(N_A^i, V_i) + F^{\mathrm{idl}}(N_B^i, V_i) + F^{\mathrm{idl}}(N_A^o, V_o) + F^{\mathrm{idl}}(N_B^o, V_o), \quad (1)$$

where we have omitted the temperaure dependence that will not be pertinent here. The total free energy is the simple sum of the component free energies because there are no (energetic) interactions between the components. In an ideal gas, by definition, there are no interactions among molecules.

Again following our treatment of the 1:1 antiporter, we must specify one specific state of our system - something like an initial condition. The 2:1 exchange of A and B means that not every possible set of populations ($N_A^i, N_A^o, N_B^i, N_B^o$) is possible. For simplicity, we shall assume that our reference state has all A molecules inside ($N_A^i = N_A, N_A^o = 0$) and all B molecules outside ($N_B^o = N_B, N_B^i = 0$).

Because we are considering a 2:1 antiporter (where two A always exchanges for one B), there is really only one

variable that can change. To see this, we can express all the species numbers in terms of N_B^i, the number of A molecules outside, and the *constant* species totals N_A and N_B:

- because a B molecule must be either inside or out, we have $N_B^o = N_B - N_B^i$;
- because of the 2:1 exchange, $N_A^o = 2N_B^i$; and
- because an A molecule must be either inside or out, $N_A^i = N_A - N_A^o = N_B - 2N_B^i$.

Using these relations and noting that $F^{\mathrm{idl}}(N, V, T) = Nk_BT \ln[N/(V/\lambda^3)]$, we can now re-write the total free energy from (1) as

$$\begin{aligned}\frac{F}{k_BT} =& \left(N_A - 2N_B^i\right) \ln\left(\frac{N_A - 2N_B^i}{V_i/\lambda_a^3}\right) \\ &+ 2N_B^i \ln\left(\frac{2N_B^i}{V_o/\lambda_a^3}\right) + N_B^i \ln\left(\frac{N_B^i}{V_i/\lambda_b^3}\right) \\ &+ \left(N_B - N_B^i\right) \ln\left(\frac{N_B - N_B^i}{V_o/\lambda_b^3}\right)\end{aligned} \tag{2}$$

We find the most probable state (which is equilibrium in the large N limit) by setting to zero the derivative of F with respect to N_B^i. After some algebra, the result is

$$\left(\frac{N_A^o/V_o}{N_A^i/V_i}\right)^2 = \frac{N_B^o/V_o}{N_B^i/V_i}. \tag{3}$$

This result is very different from what we saw in the case of the 1:1 antiporter, where there was no 2nd power present. In the 2:1 antiporter at equilibrium, the concentration ratio for A will be "magnified" in B. Such an antiporter can therefore pump a target molecule (B) more effectively than a 1:1 antiporter.

The ability for a 2:1 antiporter to pump more thoroughly should not be taken to mean that the antiporter itself does work. No work is done. Rather, the antiporter acts as a catalyst for bringing the whole system to its minimum free energy - among the accessible states. The only states which are accessible are the initial state

and those that differ from it by 2:1 exchange, and the antiporter will tend toward the state with lowest free energy (as in our analysis above). That being said, we should recognize that (i) real antiporters may be subject to slippage and hence have a less well-defined stoichiometry; and (ii) in a cell, there will be other transport mechanisms (e.g., other pumps) and hence a wide variety of concentrations will be accessible.

Importantly, our thermodynamic analysis did not require knowledge of the system's substates, but only the 2:1 stoichiometry.

Exercises:

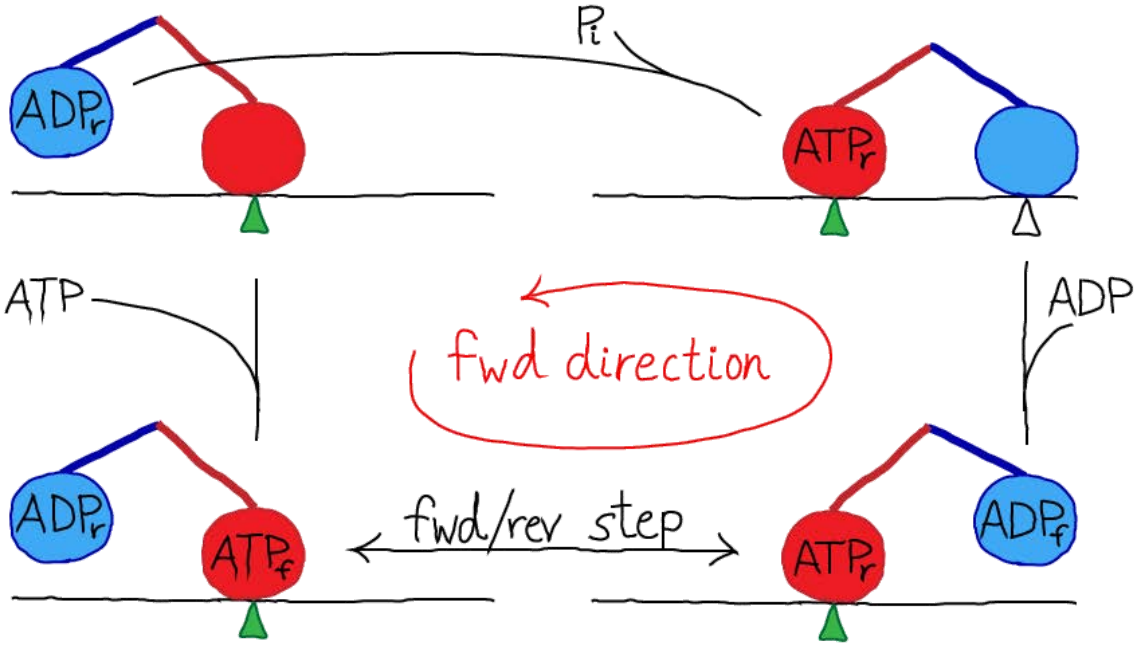
1. Derive (3).

Molecular Motors

Kinesin: A Prototypical Molecular Motor

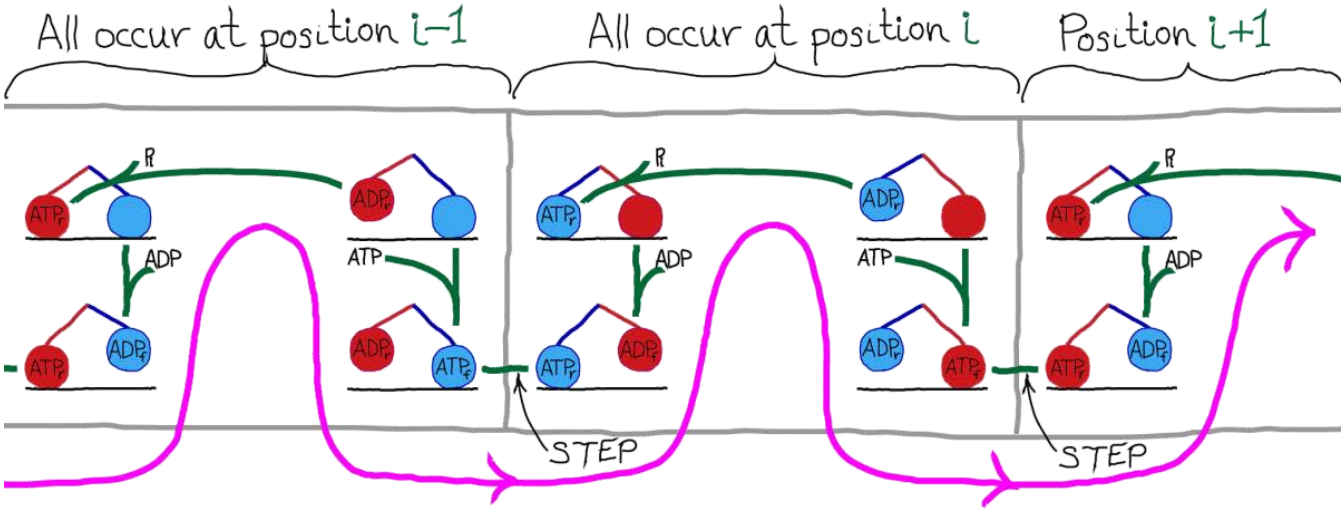
Molecular motors generate motion by coupling conformational changes to ATP hydrolysis. In doing so, motors use the free energy stored in ATP (and ATP itself stores free energy because it is maintained out of equilibrium as an activated carrier).

Kinesin is a two-headed motor, like many others (dynein, and most myosins). Each motor head is capable of hydrolyzing ATP, which results in a conformational change. The heads are also coupled to one another via "neck linker" domains. A simplified version of kinesin's cycle is shown below.

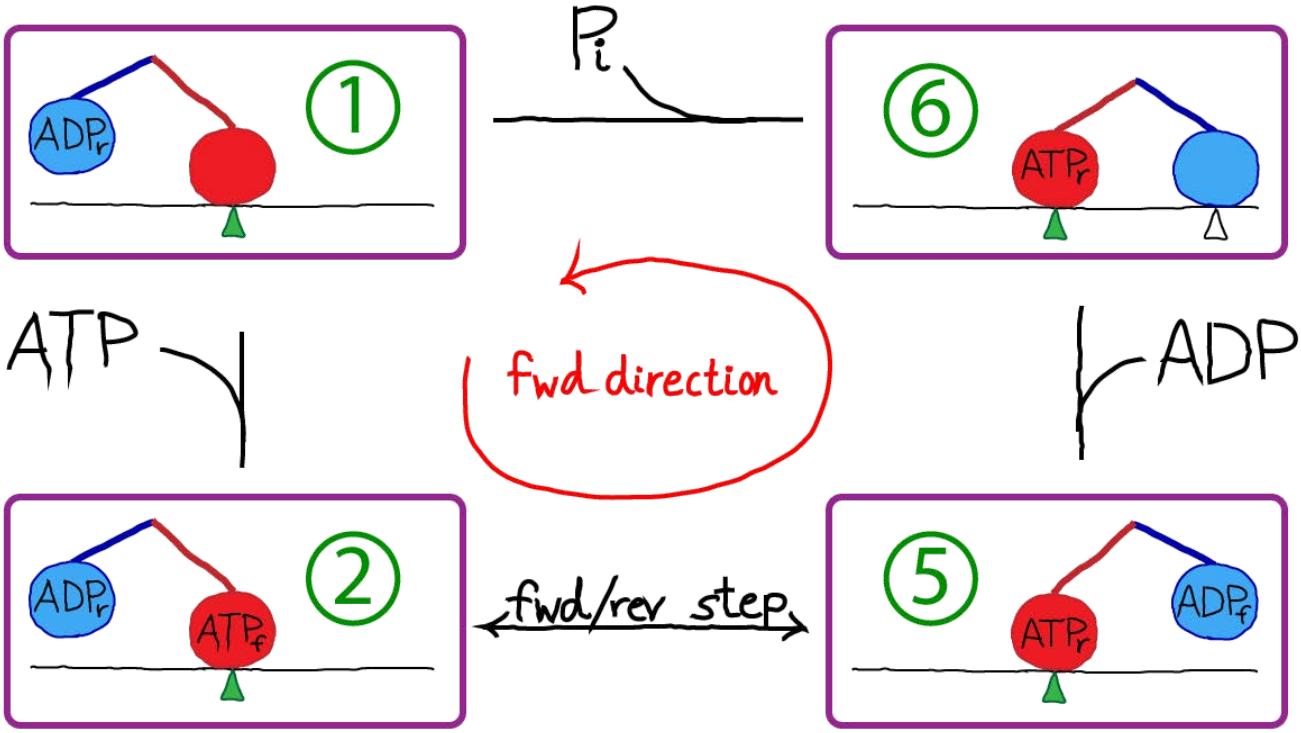


In the figure, the green triangle represents a fixed location along the microtubule, which is shown as a simple horizontal line. The colored ovals are the two heads, and the colored lines are the neck linkers. The chemical state is indicated in the oval, with a subscript noting whether a head is forward (f) or in the rear (r). The top-most transition, which is simply unbinding of the rear head, is somewhat confusing in the four-state diagram because the identity of the forward/rear heads has switched: note the green triangle and see figure below.

Kinesin moves along microtubulue filaments in the cell and plays a key role in mitosis. The steps are 8nm in length and discrete based on the polymeric structure of the microtubule. Hence we can label the positions as (..., i-2, i-1, i, i+1, i+2, ...). The cycle of chemical and mechanical steps is probably best visualized in the context of a microtubule. The pink line below shows the order of steps in purely forward motion.



A simple quantitative model for kinesin motion



To turn our original cartoon into a concrete model, we simply need to assign rate constants. We will assign rates derived by Liepelt and Lipowski ("LL" - see References) on the basis of experiments. Notice that the state numbers (1, 2, 5, 6) are not consecutive: this is simply for consistency with LL; our states are a subset of theirs, although this does not lead to significant deviations from the original model's behavior for the cases examined here.

The first thing to do with a kinetic model is write out the basic mass-action equations. For brevity, we'll note the first two here,

$$\frac{d[1]}{dt} = [6]\,k_{61} + [2]\,k_{21} - [1]\,[\mathrm{ATP}]\,k_{12} - [1]\,[\mathrm{Pi}]\,k_{16} \quad (1)$$

$$\frac{d[2]}{dt} = [1]\,[\mathrm{ATP}]\,k_{12} + [5]\,k_{52} - [2]\,k_{21} - [2]\,k_{25} \quad (2)$$

where $[\mathrm{X}]$ is the concnetration of state X and k_{ij} is the rate constant from state i to j. Remember, you can always consult the Notation page.

Equilibrium analysis of the cycle

It is necessary to perform an equilibrium analysis of the cycle for two reasons: (i) to ensure the rates permit detailed balance to hold in equilibrium, and (ii) to ensure that the rates are consistent with the thermodynamic balance holding between ATP and its hyrolysis product, ADP.

We apply the condition of detailed balance (equal and opposite flows) to all legs of the cycle. For the 1-2 and 2-5 steps, we have

$$[\mathrm{ATP}]^{\mathrm{eq}}\,[1]^{\mathrm{eq}}\,k_{12} = [2]^{\mathrm{eq}}\,k_{21} \quad (3)$$

$$[2]^{\mathrm{eq}}\,k_{25} = [5]^{\mathrm{eq}}\,k_{52} \quad (4)$$

$$[5]^{\mathrm{eq}}\,k_{56} = [\mathrm{ADP}]^{\mathrm{eq}}\,[6]^{\mathrm{eq}}\,k_{65} \quad (5)$$

$$[6]^{\mathrm{eq}}\,k_{61} = [\mathrm{Pi}]^{\mathrm{eq}}\,[1]^{\mathrm{eq}}\,k_{16} \quad (6)$$

To be clear, we are *not* assuming our system is always in equilibrium. Rather, we are establishing that our system should be capable of reaching equilibrium consisent with physical principles.

Although each of the (3) - (6) indidvidually represents a detailed-balance condition for a particular process, together they yield a single constraint on the rate constants. To see this, solve for $[2]^{\mathrm{eq}}$ in (4) and substitute the result in (3); then continue this process and successively eliminate *all* the state concentrations. You will be left with a relation between the rate constants and the concentrations of ATP, ADP, and Pi:

$$\frac{k_{12}\,k_{25}\,k_{56}\,k_{61}}{k_{21}\,k_{52}\,k_{65}\,k_{16}} = \frac{[\mathrm{ADP}]^{\mathrm{eq}}\,[\mathrm{Pi}]^{\mathrm{eq}}}{[\mathrm{ATP}]^{\mathrm{eq}}} \equiv K_d \qquad (7)$$

where we have noted that the ratio of concentrations on the right is nothing other than the equilibrium constant for ATP, its effective "dissociation" constant. The relation (7) implies that once 7 of the rate constants are known, the 8th is determined by thermodynnamic considerations.

Following LL, we set $K_d = 4.9 \times 10^5$ M, which is a large value reflecting that, in solution, ATP hydrolysis is strongly favored over synthesis - ADP will predominate over ATP unless ATP is synthsesized. In a cell, of course, ATP is constantly being synthesized to maintain a non-equilibrium condition in which ATP stores free energy available for use by many processes.

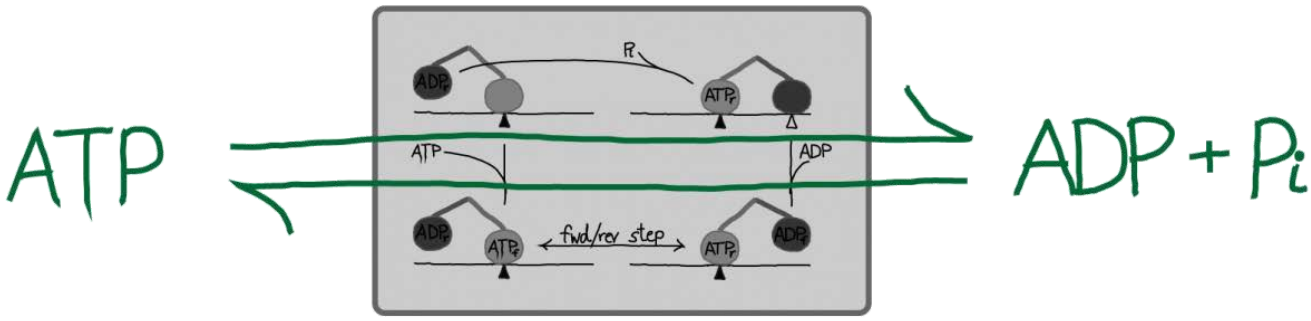
A Subtlety. As shown in the diagram with the "cycle" superimposed on a microtubule, the states really are specific to the position (i, i+1, ...). Nevertheless, the equilibrium analysis we just performed ignoring the position is correct. To see this, consider an ensemble of single kinesin molecules, each walking on a separate *circular* microtubule. In such a case, it is clear that the average population of each chemical/configurational state is independent of the positional state i - e.g., the population of state 6 is the same at every position. Hence, the equilibrium properties can be ascertained by assuming we have a simple cycle: the correct populations are obtained, aside from a trivial factor accounting for the number of positions, which is the same for each state.

The equilibrium constant and directionality.

When the concentrations of ATP, ADP and Pi are at equilibrium values, which satisfy (7), the system will not be driven. That is, on average, the number of forward and reverse steps per unit time will be the same. In typical cellular conditions, however, the ratio of concentrations will greatly favor ATP - and hence forward steps will predominate. That is, we expect

$$\frac{[\mathrm{ADP}]\,[\mathrm{Pi}]}{[\mathrm{ATP}]} \ll \frac{[\mathrm{ADP}]^{\mathrm{eq}}\,[\mathrm{Pi}]^{\mathrm{eq}}}{[\mathrm{ATP}]^{\mathrm{eq}}} \equiv K_d \qquad (8)$$

See the discussion of simulation data, below, for some examples. Although reverse stepping is possible in principle, and in the present model, note that realistic concentrations in fact make reversals highly unlikely - see exercises.



An alternative point of view is that kinesin may be seen merely as a catalyst for ATP hydrolysis (and synthesis, under suitable conditions), as sketched above. In this picture, the stepping of kinesin can be considered an unnecessary consequence of catalyzed event: see also the "thought experiment" proposed for ATP in its role as an activated carrier. The basic view of ATP is correct although to be fully accurate, one would have to account for work done against viscous forces in typical steps - see below.

The issue of work. In the present model, for simplicity, there is no "load" or force on our motor - and so mechanical forward/backward steps perform no work. This is unphysical because a viscous/frictional force will always oppose the direction of motion. Nevertheless, the zero-load case is of interest for building understanding of motor systems, and compared to experimental situations, the viscous force may be considerably smaller than forces which are applied on such motors.

Model parameters

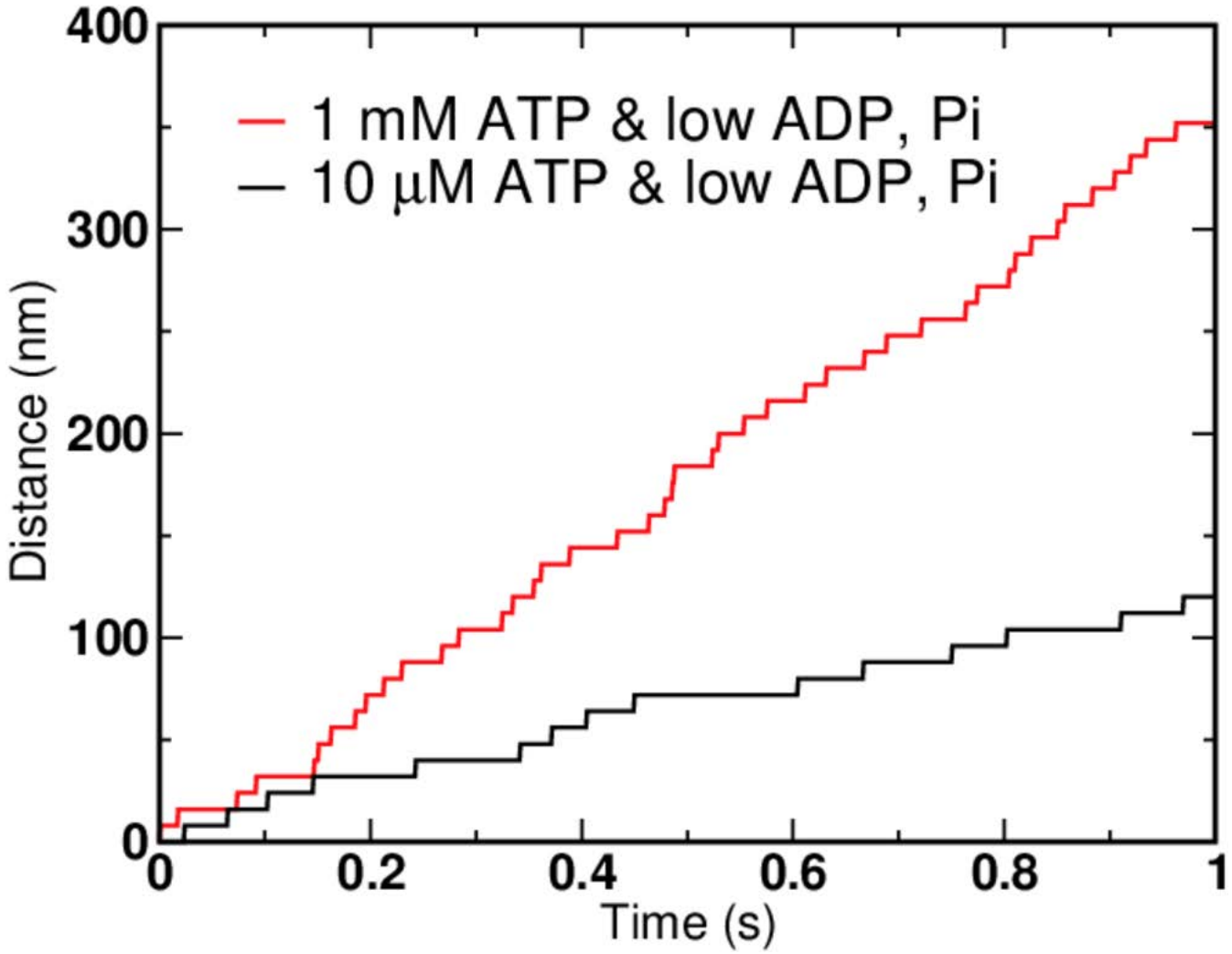
Specific rate constants are given in the table below, which are derived from the LL paper.

Process	Symbol	Value
ATP binding	k_{12}	2×10^6 / (M s)
ATP unbinding	k_{21}	100 / s
Forward step	k_{25}	3×10^5 / s
Reverse step	k_{52}	0.24 / s
ADP unbinding	k_{56}	100 / s
ADP binding	k_{65}	2×10^4 / (M s)
Hydrolysis and Pi unbinding	k_{61}	0.74 / s
Pi binding and synthesis	k_{16}	2×10^4 / (M s)

Note that k_{61} was calculated from other rate constants and K_d using (7).

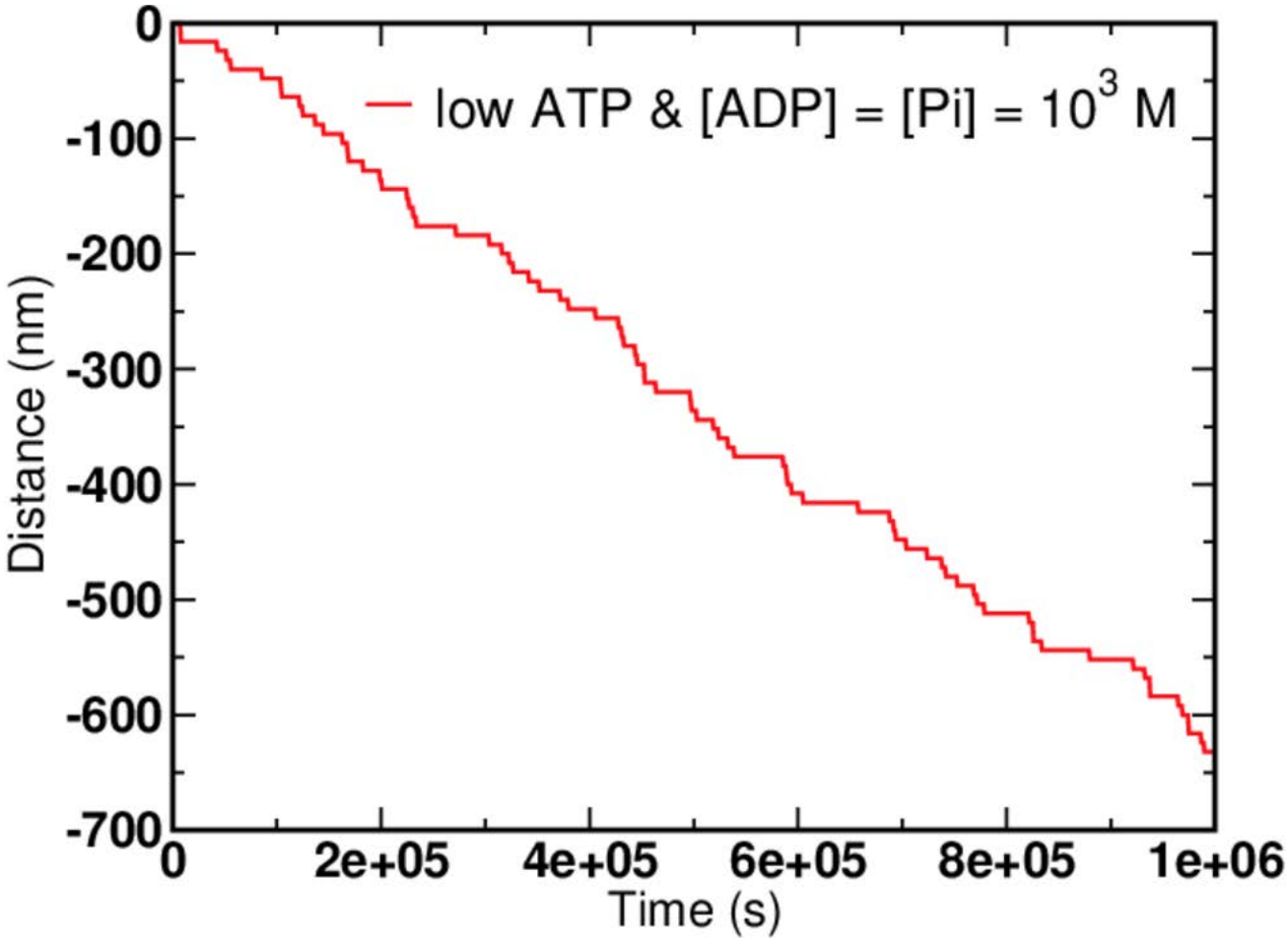
Simulating the model

We can simulate the simple kinetic model using BioNetGen, a rule-based platform for kinetic modeling. If we assign each mechanical step (the 2-to-5 transition) the experimentally determined value of 8 nm, we can graph the simulation results for different concentrations of ATP, ADP and Pi.



Not surprisingly, higher ATP concentration leads to faster motion. But there are limits on how fast the motor can move - see the exercises for hints. In the graph above, the ATP concentration increased 100-fold, but the speed of the motor clearly did not.

Although it is safe to say that a given kinesin motor (there are many kinds) evolved to move in a single direction, it is of physical interest to push our model to extremes. In particular, given that all molecular processes are reversible, can we get our motor to run in reverse? You should recognize that forward steps are expected whenever the nucleotide concentrations are shifted toward larger ATP - i.e., when the actual concentrations differ from their equilibrium values and excess ATP is present as in (8). By the same argument, *reverse steps* are expected when there is an excess of ADP and/or Pi - when the opposite of (8) holds.

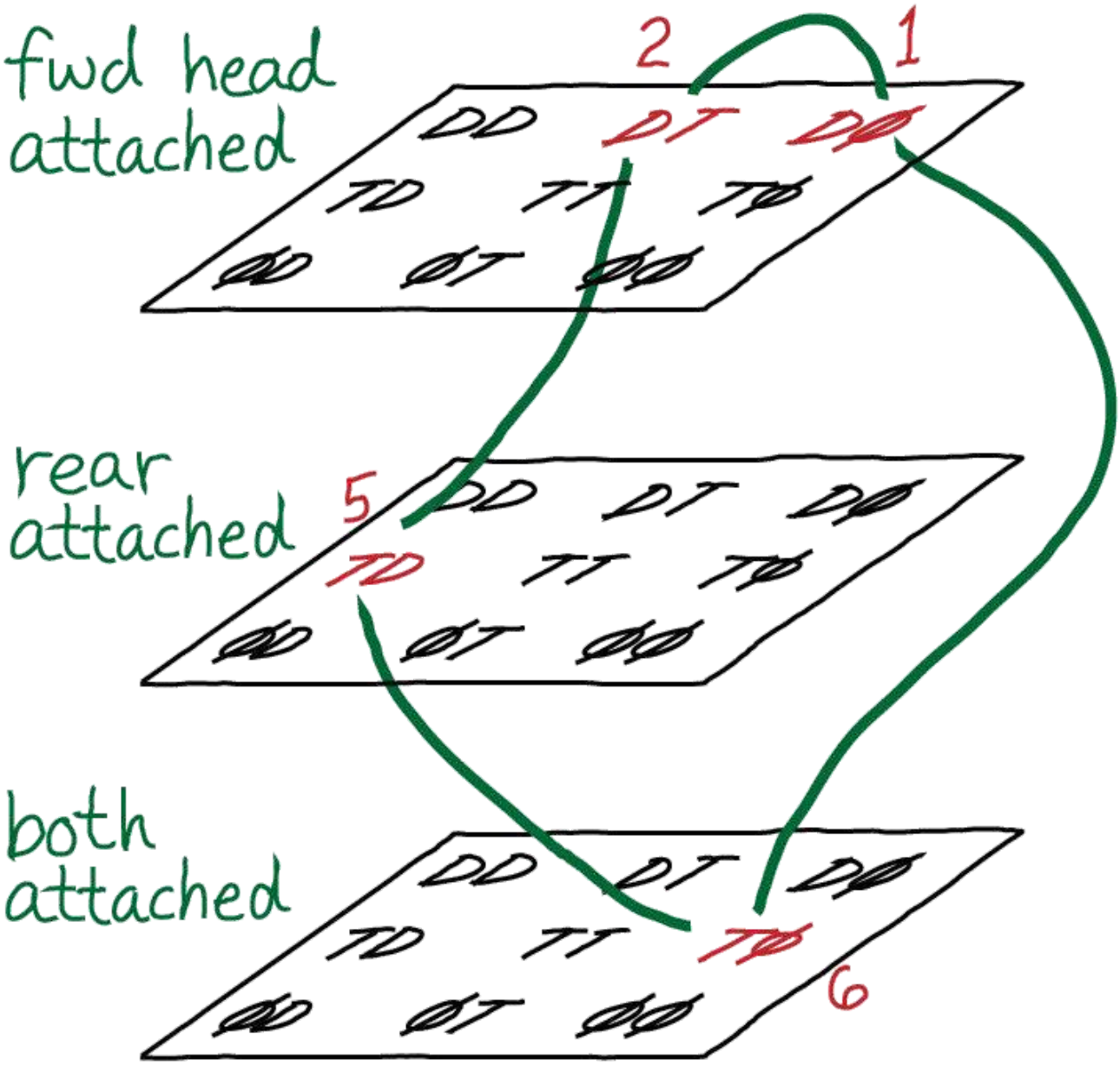


Note the timescale and nucleotide concentrations compared to previous data with forward stepping. Such concentrations are unphysical as can be seen from the exercises.

These simulations were performed using BioNetGen, a rule-based platform for kinetic modeling. The source code for the model (a .bngl file) can be downloaded by right-clicking here.

Making a more complete model

Our discussion has avoided two issues important to detailed consideration of motor proteins: the effect of a significant opposing force which may be present if a cargo is attached to the motor, and a more complete view of the potential state space. Qualitatively, it is clear that an opposing force or "load" will decrease the speed of the motor at given nucleotide concentrations; for further details, the reader is referred to the LL paper and references therein.



Using a conceptual framework similar to what was presented in our advanced discussion of cycle logic, we can also consider a more complete state space for the kinesin motor. In the figure above, D, T, and ϕ represent possible states of an indivdual motor head: ADP-bound, ATP-bound, and empty, respectively. A pair of symbols represents the rear/forward combination - e.g., "DT" means the rear head is ADP-bound and the forward head is ATP-bound - using the convention that right is forward.

The space shown here may seem large and complicated, but really we have omitted a number of possibilities: that both heads could become detached, the position on the filament, additional configurational states, and states with both ADP and Pi bound.

References:

- General references
  - B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).

- T.L. Hill, "Free Energy Transduction and Biochemical Cycle Kinetics," (Dover, 2005).
- J. Howard, "Mechanics of Motor Proteins and the Cytoskeleton," (Sinauer, 2001).

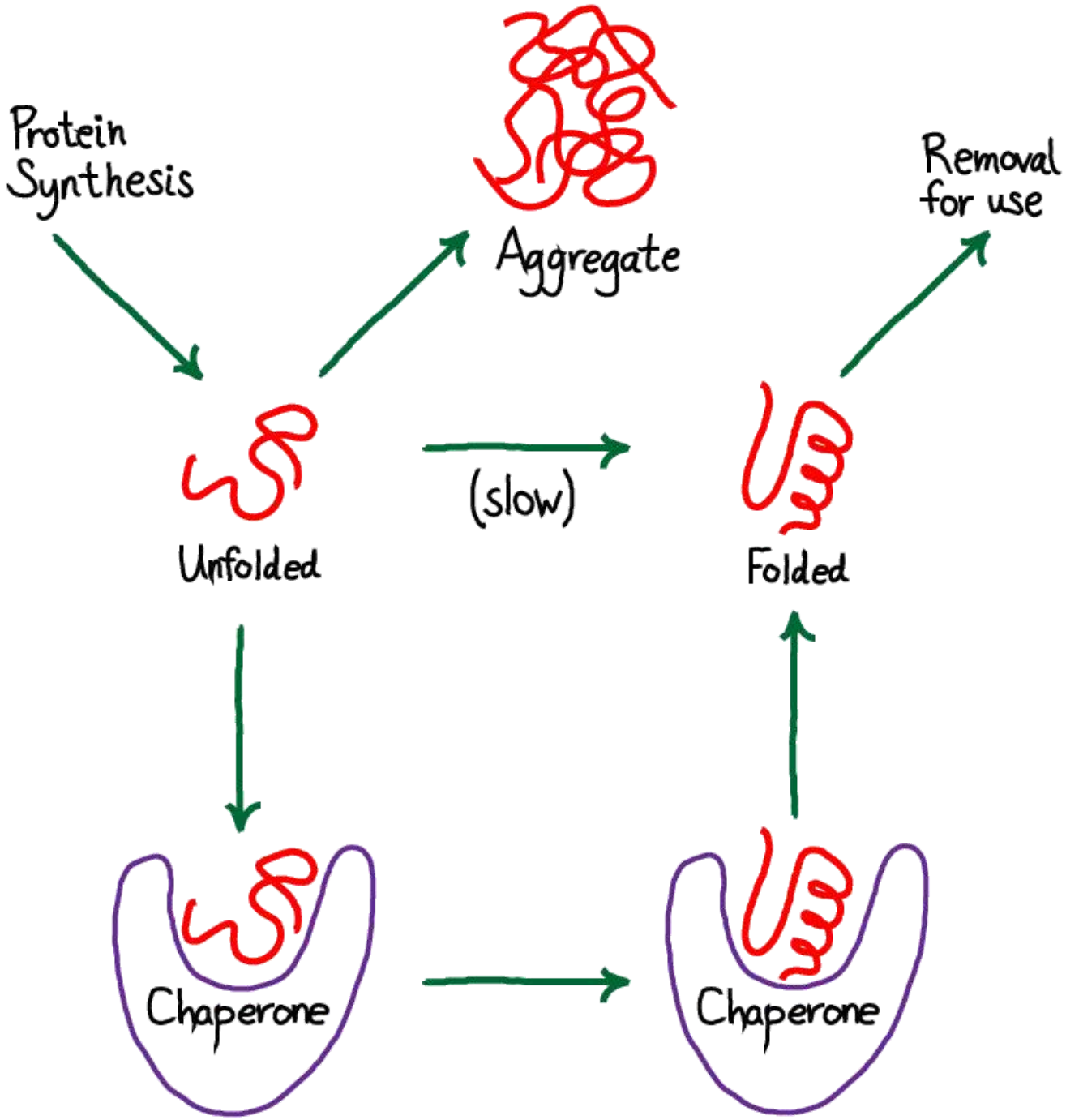
- The specific kinesin model used is based on the following paper:
  - S. Liepelt and R. Lipowsky, "Kinesin's Network of Chemomechanical Motor Cycles," Phys. Rev. Lett. 98, 258102 (2007).

- Experimental studies of kinesin's chemomechanical cycle - only a tiny selection of a vast literature:
  - N. Carter and R. Cross, "Mechanics of the kinesin step," Nature 435, 308-312 (2005).
  - N. Guydosh and S. Block, "Backsteps induced by nucleotide analogs suggest the front head of kinesin is gated by strain," PNAS 103 (21) 8054-8059 (2006).
  - D. Hackney, "The tethered motor domain of a kinesin-microtubule complex catalyzes reversible synthesis of bound ATP," PNAS 102:18338-18343 (2005).
  - W. Schief, R. Clark, A. Crevenna, and J. Howard, "Inhibition of kinesin motility by ADP and phosphate supports a hand-over-hand mechanism," PNAS 101:1183-1188 (2004).

Exercises:

1. Derive (7).
2. In a mass-action model, the overall rate of ATP binding and hydrolysis will increase in proportion to the the ATP concentration without limit. In reality, a maximum saturating value is expected for the binding step: Why? [Hint - review the mass-action page.]
3. Even if the world truly obeyed mass-action principles, there would still be a limit on the speed with which the motor could move given the present model. Discuss this issue by examining rate constants and limiting cases.
4. Use a geometric argument based on the size of an ADP molecule and the molecular spacing at $[\mathrm{ADP}] = 10^3$ M to show that concentration is unphysical. Explain why the mass-action picture breaks down.

Chaperone-aided Protein Folding



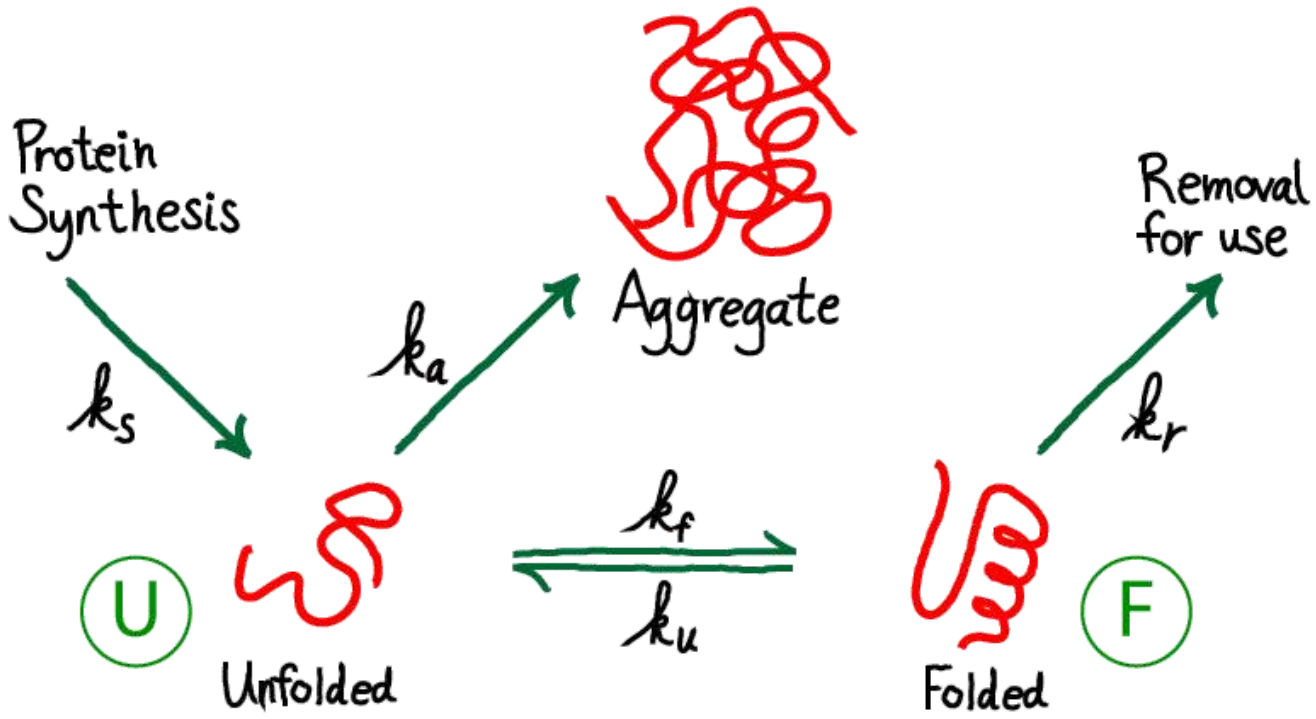
Surprisingly, unfolded proteins are toxic to the cell because of their potential to form large, difficult-to-degrade aggregates consisting of many proteins. Machinery for safely "catalyzing" protein folding is therefore an essential part of cell functioning. *Chaperones* are a class of proteins and protein complexes that enable successful protein folding. We will see that to be maximally effective, chaperones must use free energy, such as from hydrolysis of the activated carrier ATP.

Our discussion, as usual, will focus on the essential biophysics rather than on more detailed models of specific systems. The simpler discussion enables understanding of the key driving forces and mechanisms, which in

turn can provide building blocks for more sophisticated modeling.

A very valuable preliminary model

We can gain a surprising amount of insight from studying a very simple model of folding and aggregation *without* chaperones. The model is instructive on its own, but also establishes a key reference point for models chaperones.



From the figure above, you should see immediately that this is a driven system. Driving occurs because unfolded protein is being synthesized (at a rate k_s) and folded protein is removed (at a rate k_r) for trafficking to other parts of the cell where the proteins will be used. Unfolded proteins are also assumed to aggregate irreversibly at rate k_a. We are not concerned here with the source of energy for this driving, but it is critical to appreciate that free energy is being expended in the process. The spontaneous flow or driving indicates that indeed free energy is being expended. The system is not in equilibrium.

The need for chaperones implies that the rate of folding - at least for some proteins - is small compared to other rates, especially that for aggregaton. We will also assume that, once folded, proteins are reasonably stable so that the unfolding rate is even smaller than the folding rate. Hence, our picture is that $k_u < k_f$ and both are smaller than other rate constants in the model. This picture applies to the subset of proteins which are not fast folders.

Our goals are to determine the amount of protein which ends up aggegated compared to what is folded, and to understand how this ratio depends on the parameters of our simple model. Thus, we want to calculate the ratio

$$\frac{\text{Aggregation}}{\text{Folding}} = \frac{[\text{U}]\, k_a}{k_r}, \tag{1}$$

where the populations of the unfolded and folded states have been denoted by [U]and [F]. This ratio of fluxes or overall rates (as opposed to rate constants alone) derives from basic mass action principles.

Given the input and removal of molecules from the system, it is natural to analyze the system in a steady state, which conveniently is the simplest analysis. (Note that subjecting a system to a steady-state analysis is not a claim that the system in question will always exhibit steady behavior. Rather, the steady state is a convenient and informative condition to examine.) We will therefore formulate our analysis in terms of steady-state concentrations: $[\text{X}]^{\text{SS}}$ for species X.

Our mathematical task is simplified by the observation that the ratio (1) does not require the absolute values of the concentrations, but only their ratio. This ratio is determined using the continuity of flow from the unfolded to folded to the "removed" state (upper right in figure above). That is, the *net* flow from U to F must match the flow that is removed:

$$[\text{U}]^{\text{SS}}\, k_f - [\text{F}]^{\text{SS}}\, k_u = [\text{F}]^{\text{SS}}\, k_r, \tag{2}$$

which in turn implies the ratio

$$\frac{[\text{U}]^{\text{SS}}}{[\text{F}]^{\text{SS}}} = \frac{k_u + k_r}{k_f} \tag{3}$$

We can now simply substitute (3) into (1) (which applies in steady or non-steady conditions) to obtain the aggregation ratio in steady state:

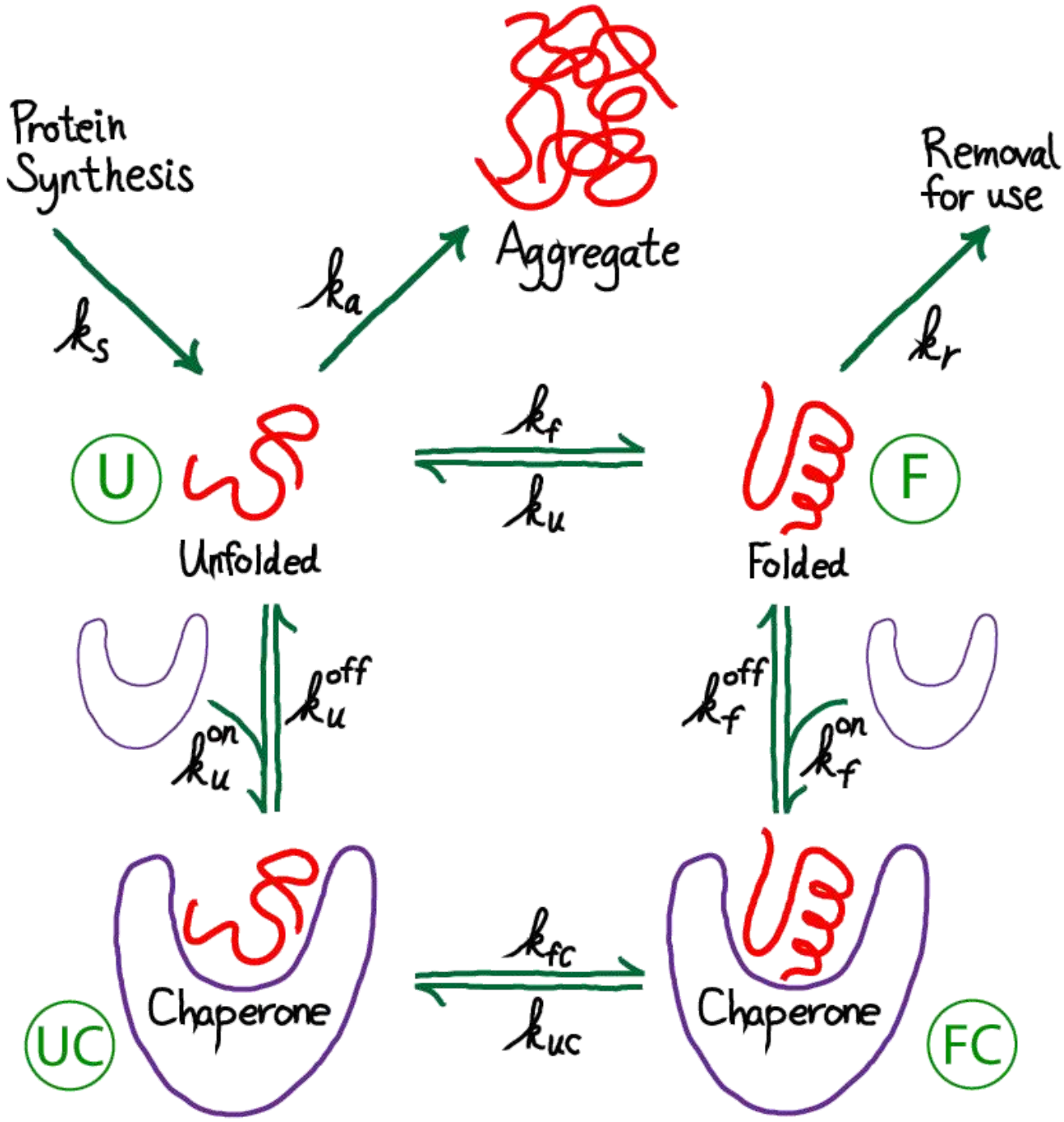
$$\text{Steady State: } \frac{\text{Aggregation}}{\text{Folding}} = \frac{k_a}{k_r}\frac{k_u}{k_f} + \frac{k_a}{k_f}. \tag{4}$$

The result depends only on rate constants and not on the absolute concentrations, which makes it straightforward to interpret.

To solidify our understanding of this almost-but-not-quite trivial model, we can rewrite (4) as $(k_a/k_f)\,[(k_u/k_r)+1]$. For proteins that are slow to fold spontaneously, we expect that the aggregation rate k_a is much larger than the folding rate k_f; this is, after all, why chaperones are needed in the first place. Our re-write of the ratio shows that aggregation is indeed expected to be significant in our simple analysis without the presence of chaperones: even though the first term in the square brackets may be small due to slow unfolding (i.e., protein stability), it must be positive and hence the whole ratio must exceed k_a/k_f, which is large. In the limit that unfolding is much slower than removal ($k_u \ll k_r$), the ratio approaches $k_a/k_f \gg 1$ reflecting the fractional outflows from unfolded state. So we've done a little math to quantify our intuition that some kind of chaperone mechanism is needed when folding is slow, and equally importantly, set the stage for more realistic models.

It is worth noting that the ratio of unfolded protein in *steady state* given in (3) generally will be far from the equilibrium value. The balance condition which must hold in equilibrium would dictate a ratio of k_u/k_f, which differs significantly from (3) given our assumption that k_u is small compared to other rates. Thus, perhaps ironically, the driving in this case shifts the populations toward the dangerous unfolded state, though this would appear to be intrinsic to the directionality of the system - proteins start out unfolded!

The simplest chaperone model - no ATP



Although this model is more complicated than our previous one, it has the distinct advantage of actually including chaperones! Note that the chaperones are purely "passive" in the model as shown - they store no free energy and do not use ATP. The chaperones will act simply as catalysts. However, because we are considering a driven non-equilibrium condition, the chaperones' presence can alter the aggregation ratio.

To give away the punchline first, note that our new model adds to the prior model only by adding an additional pathway between the unfolded and folded state. Other processes are not altered. Hence, the net result of the model will be modified, "effective" rate constants that will replace k_f and k_u in our analyses above. All we need to do is set up the math to figure out what happens.

Before getting into detailed analysis of the model, we immediately see that it contains a cycle (U-F-FC-UC),

and therefore the rates must satisfy a constraint, as holds for all cycles. In other words, among the eight rate constants in the cycle, only seven can be considered as adjustable parameters due to the cycle constraint

$$\frac{k_f\, k_{\mathrm{F}}^{\mathrm{on}}\, k_{uc}\, k_{\mathrm{U}}^{\mathrm{off}}}{k_u\, k_{\mathrm{F}}^{\mathrm{off}}\, k_{fc}\, k_{\mathrm{U}}^{\mathrm{on}}} = 1. \tag{5}$$

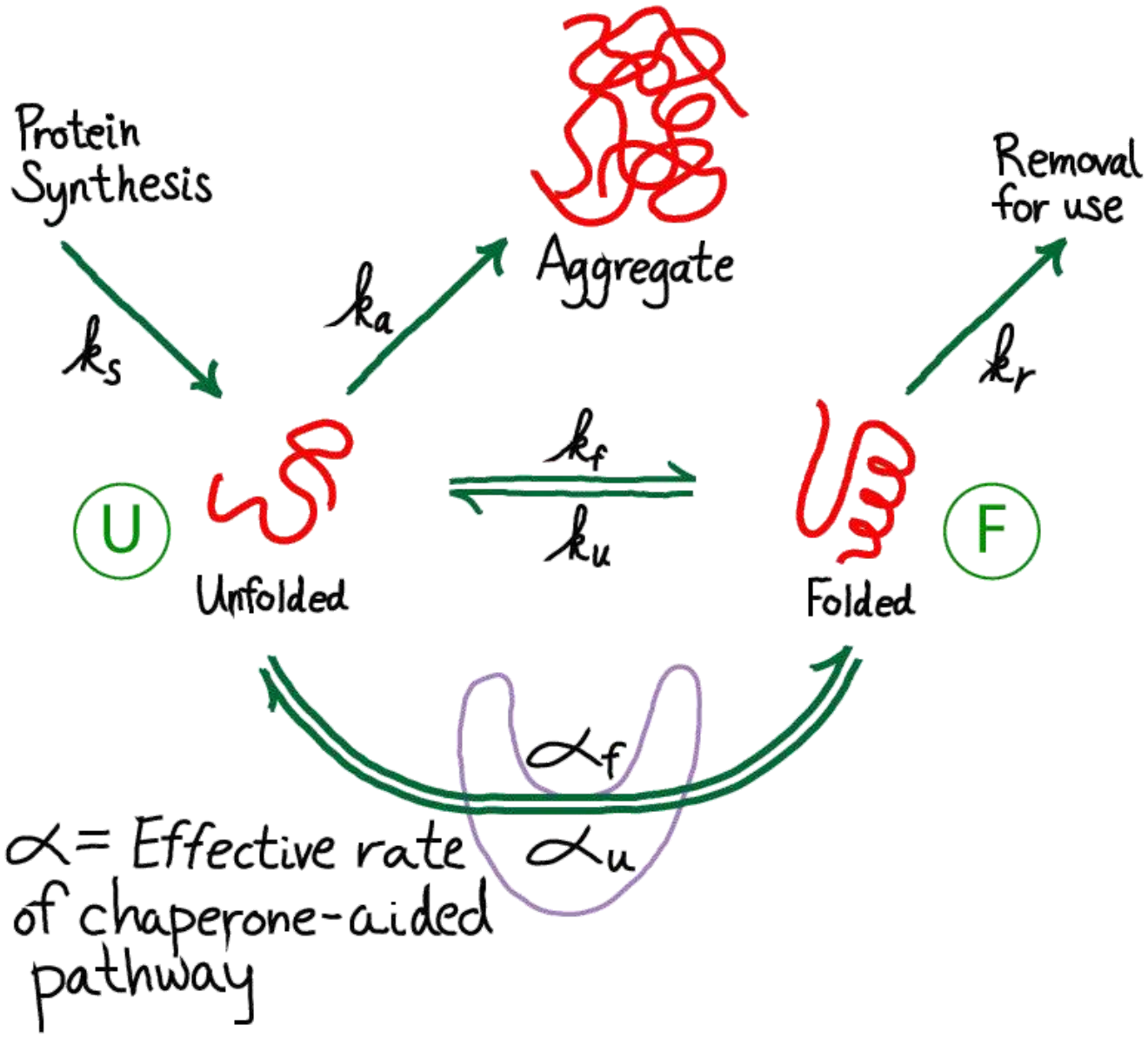
To extract biophysical information for this model, we will solve for its steady state. The algebra is somewhat complicated, although straightforward, and we just sketch it here. (Derivations of some results are given as exercises, with hints.) Fortunately, the basic idea is simple. We use the fact that the net flow through the chaperone pathway will be constant in a steady state - i.e., the flow from stat U to UC will match that from UC to FC and from FC to F. Our standard mass action machinery enables us to write down the corresponding equations easily:

$$[\mathrm{U}][\mathrm{C}]\, k_{\mathrm{U}}^{\mathrm{on}} - [\mathrm{UC}]\, k_{\mathrm{U}}^{\mathrm{off}} = [\mathrm{UC}]\, k_{fc} - [\mathrm{FC}]\, k_{uc} \tag{6}$$

$$[\mathrm{UC}]\, k_{fc} - [\mathrm{FC}]\, k_{uc} = [\mathrm{FC}]\, k_{\mathrm{F}}^{\mathrm{off}} - [\mathrm{F}][\mathrm{C}]\, k_{\mathrm{F}}^{\mathrm{on}}, \tag{7}$$

where we have omitted the "SS" (steady state) superscripts to keep the equations cleaner.

Using a strategy described in the Exercises, we can solve these equations for the effective rate constants, α_f and α_u , along the chaperone path.



These are

$$\alpha_f = \frac{[\mathrm{C}]\, k_{\mathrm{U}}^{\mathrm{on}}\, k_{fc}\, k_{\mathrm{F}}^{\mathrm{off}}}{k_{fc}\, k_{\mathrm{F}}^{\mathrm{off}} + k_{\mathrm{U}}^{\mathrm{off}}\, k_{\mathrm{F}}^{\mathrm{off}} + k_{\mathrm{U}}^{\mathrm{off}}\, k_{uc}} \tag{8}$$

$$\alpha_u = \frac{[\mathrm{C}]\, k_{\mathrm{F}}^{\mathrm{on}}\, k_{uc}\, k_{\mathrm{U}}^{\mathrm{off}}}{k_{fc}\, k_{\mathrm{F}}^{\mathrm{off}} + k_{\mathrm{U}}^{\mathrm{off}}\, k_{\mathrm{F}}^{\mathrm{off}} + k_{\mathrm{U}}^{\mathrm{off}}\, k_{uc}} \tag{9}$$

The graphic above demonstrates that the presence of chaperones in the model, which initially appeared a great complication, can be included as a parallel pathway with the effective first-order rate constants α_f and α_u. That is, the probability of folding (transitioning from state U to F) per unit time is $[\mathrm{U}]\,(k_f + \alpha_f)$ and for unfolding is $[\mathrm{F}](k_u + \alpha_u)$. To put it another way, the overall rate constants, accounting for both paths between U and F, are:

$$k_f^{\mathrm{tot}} = k_f + \alpha_f \tag{10}$$

$$k_u^{\mathrm{tot}} = k_u + \alpha_u \tag{11}$$

Biophyscial discussion of passive chaperone effects

Our goal is to determine how the presence of passive chaperones (which do not use ATP or another energy source) can affect the aggregration ratio (1). In the presence of the chaperone pathway, (4) must be modified to account for both processes:

$$\text{Steady State: } \frac{\text{Aggregation}}{\text{Folding}} = \frac{k_a}{k_r}\frac{k_u^{\mathrm{tot}}}{k_f^{\mathrm{tot}}} + \frac{k_a}{k_f^{\mathrm{tot}}}. \tag{12}$$

Let's examine the aggregation ratio term by term. We'll focus first on the factor $k_u^{\mathrm{tot}}/k_f^{\mathrm{tot}}$ and compare it to the trivial case given in (4). In fact, this factor is unchanged, as we can see by examining the ratio

$$\frac{\alpha_u}{\alpha_f} = \frac{k_{\mathrm{F}}^{\mathrm{on}}\, k_{uc}\, k_{\mathrm{U}}^{\mathrm{off}}}{k_{\mathrm{U}}^{\mathrm{on}}\, k_{fc}\, k_{\mathrm{F}}^{\mathrm{off}}} = \frac{k_u}{k_f}, \tag{13}$$

where the last equality derives from the cycle constraint (5). This ratio of effective rates does not change, and hence the first term in (12) is the same as the corresponding term in the chaperone-free case, (4).

The second term in (12) clearly can differ from the chaperone-free case. In the limit of large chaperone concentration $[\mathrm{C}]$, the term can become very small (within our mass-action picture; in reality, there is a strict limit to the concentration of a large protein or complex). So the second term can get small, but the first term remains as it was in the absence of chaperones.

The bottom line is that the presence of chaperones can indeed decrease the aggregation ratio, hence increasing folding, down to a limit. Namely, in our mass-action picture,

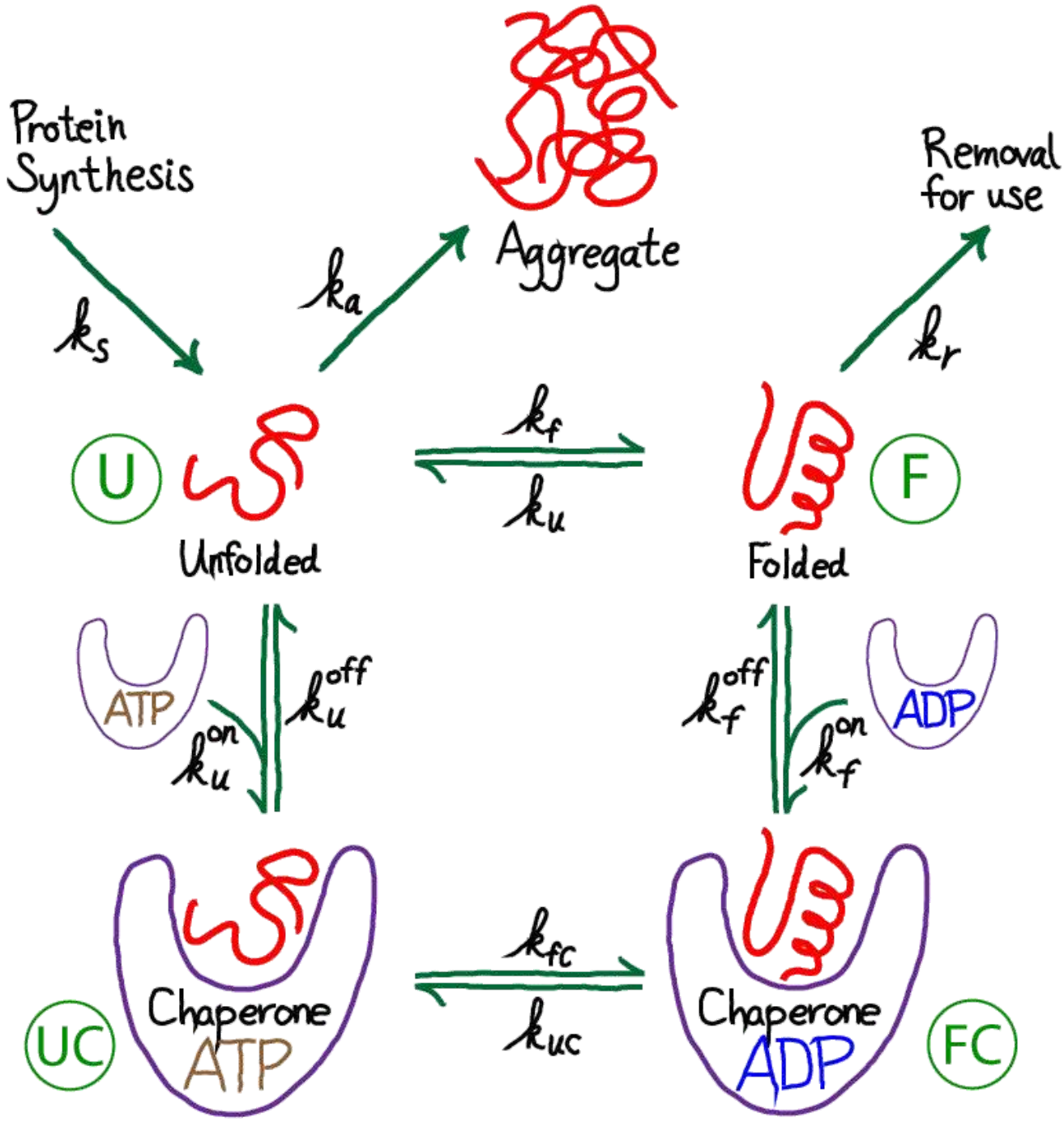
$$\text{Passive Chaperones: } \frac{\text{Aggregation}}{\text{Folding}} > \frac{k_a}{k_r}\frac{k_u}{k_f}, \tag{14}$$

where the "tot" superscripts are omitted because $k_u^{\text{tot}}/k_f^{\text{tot}} = k_u/k_f$ in the case of passive chaperones. We can see that for proteins with a strong tendency to aggregate (large k_a) and/or modest stability (k_u significant compared to k_f), significant aggregation could still occur.

The only way to improve on (14) within our current chaperone cycle is to somehow drive the chaperone function.

ATP-driven chaperone-aided folding

Let's now consider chaperones that use ATP based on the schematic below, which is not meant to indicate specfics as to when ATP hydrolysis occurs.



ATP-driven chaperones can achieve a higher level of successful folding compared to the passive case. Such chaperones convert the free energy stored in the cell's non-equilibrium concentration of ATP (relative to ADP) into greater folding "fidelity" - i.e., more folding, less aggregation. This exchange bears qualitative similarities to the cell's exchange of free energy for greater fidelity in translation.

The basic mechanism for the increased folding with ATP driving is easy to see within our simple kinetic modeling. As we showed in the previous section, without driving, the ratio $k_u^{\rm tot}/k_f^{\rm tot}$ that appears in (12) cannot change. This is because, in essence, the passive chaperone acts simply as a catalyst. The ATP-driven chaperone, by contrast, can modify the ratio. The distinction between the two underscores the differences in cycle structure, as discussed in the cycle logic section: the distinguishability between ATP- and ADP-bound

chaperones provides a "handle" to drive the cycle in one direction, whereas passive chaperones (no ATP or ADP) act to drive the cylce in both directions equally.

The effect of ATP-driving can be seen in the effective rate constants, α given in (8) and (9). Instead of $[\mathrm{C}]$ in α_f , we will have $[\mathrm{C}\cdot\mathrm{ATP}]$ and in α_u we will have $[\mathrm{C}\cdot\mathrm{ADP}]$. In turn, these will modify k_f^{tot} and k_u^{tot} in (10) and (11), and lead to a significantly modified aggregation ratio (12). In particular, the first term in (12) can be decreased well below the passive-case minimum given in (14) - and we expect significantly more folding.

To see this more explicitly, we can revisit the first term in (12). Recall that the solution folding and unfolding rates, k_u and k_f are presumed small compare to other rates (necessitating chaperone use in the first place). Hence we have

$$\frac{k_u^{\mathrm{tot}}}{k_f^{\mathrm{tot}}} = \frac{k_u + \alpha_u}{k_f + \alpha_f} \simeq \frac{\alpha_u}{\alpha_f} = \frac{k_u\,[\mathrm{C}\cdot\mathrm{ADP}]}{k_f\,[\mathrm{C}\cdot\mathrm{ATP}]}, \tag{15}$$

where we used the constraint (5). The fraction (15) can be much less than k_u/k_f because we expect that any protein evolved to use ATP will bind much more strongly to ATP than to ADP. That is, we expect $[\mathrm{C}\cdot\mathrm{ATP}] \gg [\mathrm{C}\cdot\mathrm{ADP}]$. Recall from the section on ATP that the concentrations of the two nucleotides are about the same.

Summing Up

To avoid aggregation, chaperone systems encourage folding in two ways. The first way is simply to catalyze folding without using free energy, but this is a weak effect that we have seen is severely limited. More importantly, the use of free energy stored in ATP allows the system to be driven toward greater folding. In terms of "cycle logic", ATP-bound chaperones provide a handle with which the system can be driven uni-directionally - which wouldn't be possible if ATP did not bind or did not get hydrolyzed to ADP.

We have not touched on quite interesting questions regarding details of how free energy from ATP is used - e.g., whether chaperones perform mechanical work to aid folding or simply prevent aggregation (see work by Lorimer and by Horwich). Our simple analysis suggests that such mechanistic details may be less important than general process of transducing free energy for the end result of more folded protein.

Arguably, the driven process of chaperone-aided folding echoes the driven or "kinetic" proofreading which occurs in protein translation.

References:

- General reference
  - B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).

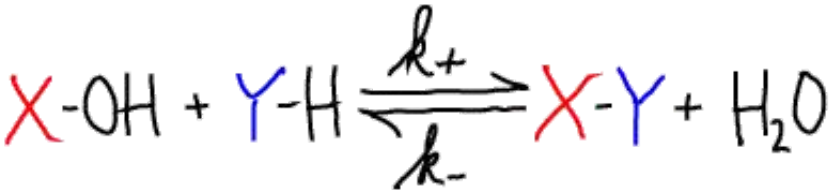
- The following are biophysical studies and perspectives on chaperones, which can help you get started in the large body of literature:
  - D. Thirumalai, G. H. Lorimer, "Chaperonin-mediated protein folding," Annu Rev Biophys Biomol Struct 30:245-269 (2001).
  - Arthur L. Horwich, Adrian C. Apetri, Wayne A. Fenton, "The GroEL/GroES cis cavity as a passive anti-aggregation device," FEBS Letters 583:2654-2662 (2009).
  - Nicholas C. Corsepius and George H. Lorimer, "Measuring how much work the chaperone GroEL can do," PNAS 110:E2451-E2459 (2013).

Exercises:

1. Derive (5).
2. Derive Eqs. (8) and (9) in several stages. (a) First use (6) to solve for $[\mathrm{UC}]$ in terms of other variables. (b) Substitute this result into (7) and solve for $[\mathrm{FC}]$ in terms of $[\mathrm{U}]$, $[\mathrm{F}]$ and $[\mathrm{C}]$. (c) Use the result for $[\mathrm{FC}]$ in your expression for $[\mathrm{UC}]$. (d) Solve for the *net* flow from state U to UC: the left-hand side of (6). The coefficients of $[\mathrm{U}]$ and $[\mathrm{F}]$ are the effective rate constants α_f and α_u.

Principles of Synthesis

Biosynthesis is driven by free energy



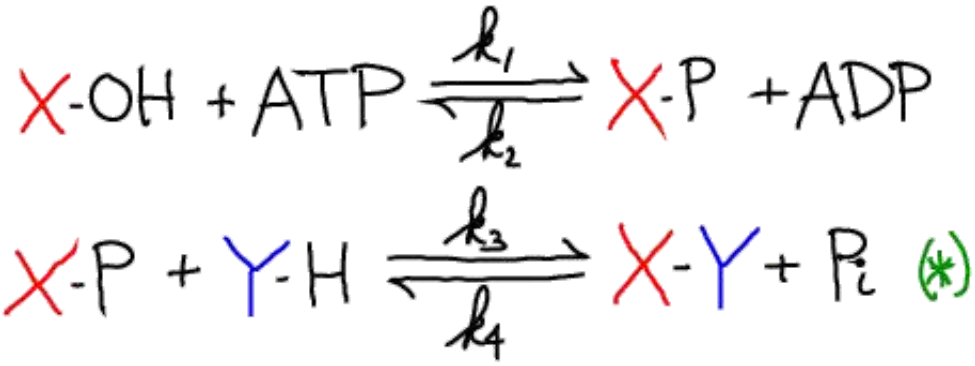
Chemical syntheses in the cell, such as the formation of X-Y above, typically require an input of free energy. Free energy could be supplied directly if there were an over-abundance of reactants (compared to equilibrium) and/or a dearth of products. More commonly, there is a complex sequence of reactions required for synthesis, and free energy drives these intermediate reactions. Below, we see how the hydrolysis of ATP can be coupled to a series of two synthesis reactions. Other carriers could also supply the free energy to drive synthetic reactions.

Note that if a synthetic reaction were favorable (did not require free energy), it would only be necessary for the cell to supply a catalyst.

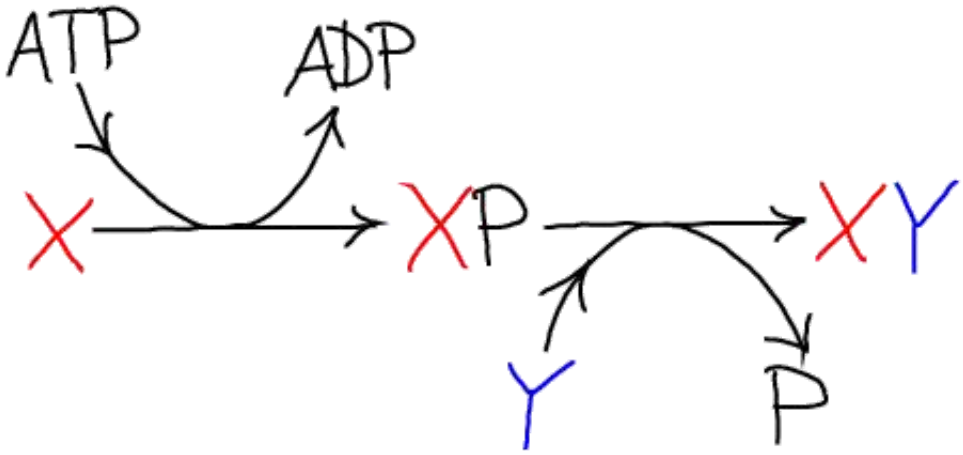
Reaction coupling, driven by ATP

An example of the mechanism here is the synthesis of glutamine from glutamic acid, where X-OH = glutmatic acid, Y-H = NH_3. Below, -P = -O-PO_3.

The synthesis of X-Y can be coupled to ATP hydrolysis by the following two reactions



The net result of the two reactions is the synthesis of X-Y *and* the hydrolysis of ATP. Graphically the process looks like this



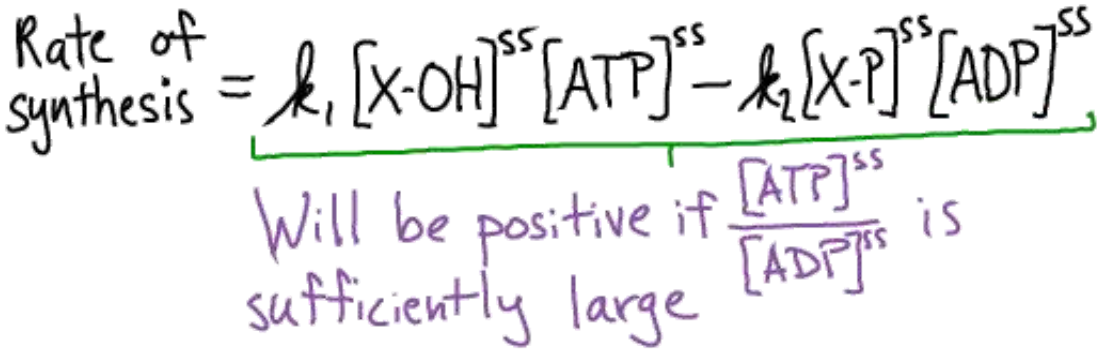
Steady-state analysis of the coupled reactions

Will the two-step synthesis above really occur under typical cellular conditions, even if the direct synthesis ($X + Y \rightarrow XY$) is unfavorable? We can answer this question using a steady-state analysis of the equations above in a mass action picture. Specifically, we can calculate the rate of formation of XY (and Pi) in the second of the two reactions, which is marked with a green (*). We use a steady-state condition on the intermediate XP:

$$0 = \frac{d[X\text{-}P]}{dt} = k_1[X\text{-}OH][ATP] - k_2[X\text{-}P][ADP] - \{k_3[X\text{-}P][Y\text{-}H] - k_4[X\text{-}Y][P_i]\}$$

Precisely net flow to right in (*) = rate of synthesis

Because the last two terms are precisely the net rate of XY and Pi synthesis - based on Eq (*) - and the four terms together sum to zero in a steady state, we can rewrite the synthesis rate to see the dependence on ATP and ATP:



So long as the ratio $[ATP]/[ADP]$ is sufficiently large, the rate of XY synthesis will be positive. And because ATP is an activated carrier that ratio *is* large.

Bottom line: XY is synthesized at a positive rate if ATP is sufficiently activated.

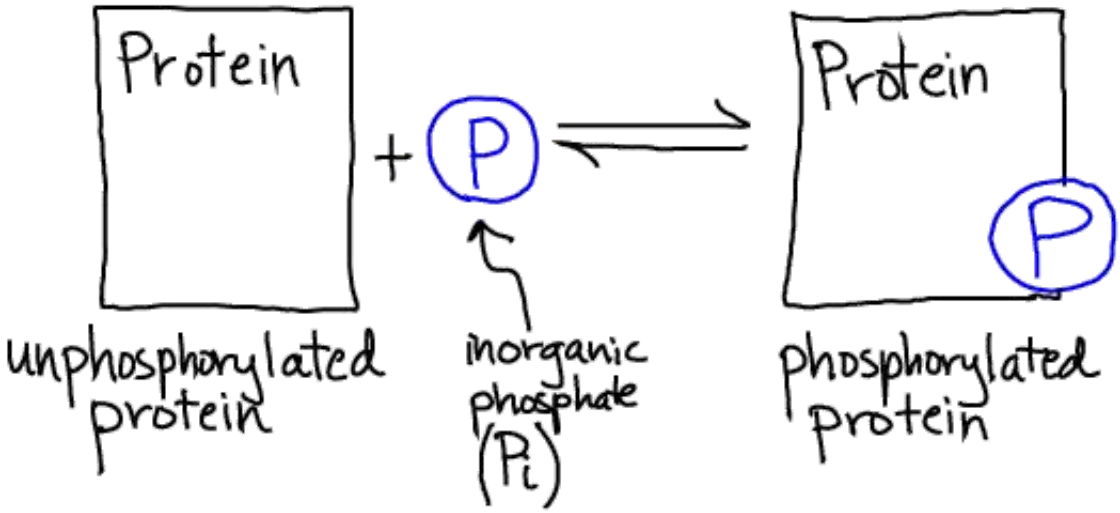
References:

- B. Alberts et al., "Molecular Biology of the Cell", Garland Science (many editions available). See the 2nd chapter's discussion of ATP-driven synthesis.
- J. M. Berg et al., "Biochemistry", W. H. Freeman. The 2002 edition is online for free.
- Any biochemistry or cell biology book will discuss synthesis.

Phosphorylation, Methylation, etc

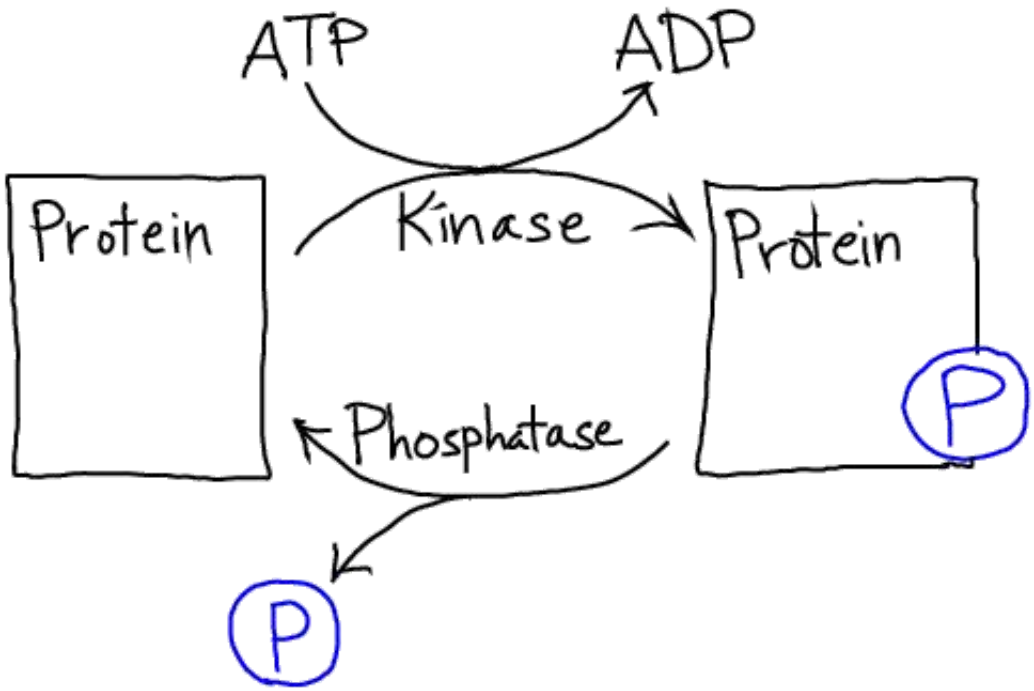
The Mysteries of Phosphorylation

Phosophorylation is the covalent attachment of a phosphate group (Pi or P) to a protein, which typically affects the protein's behavior during a signaling sequence. For example, phosphorylation can "activate" or turn on the activity of the target protein.



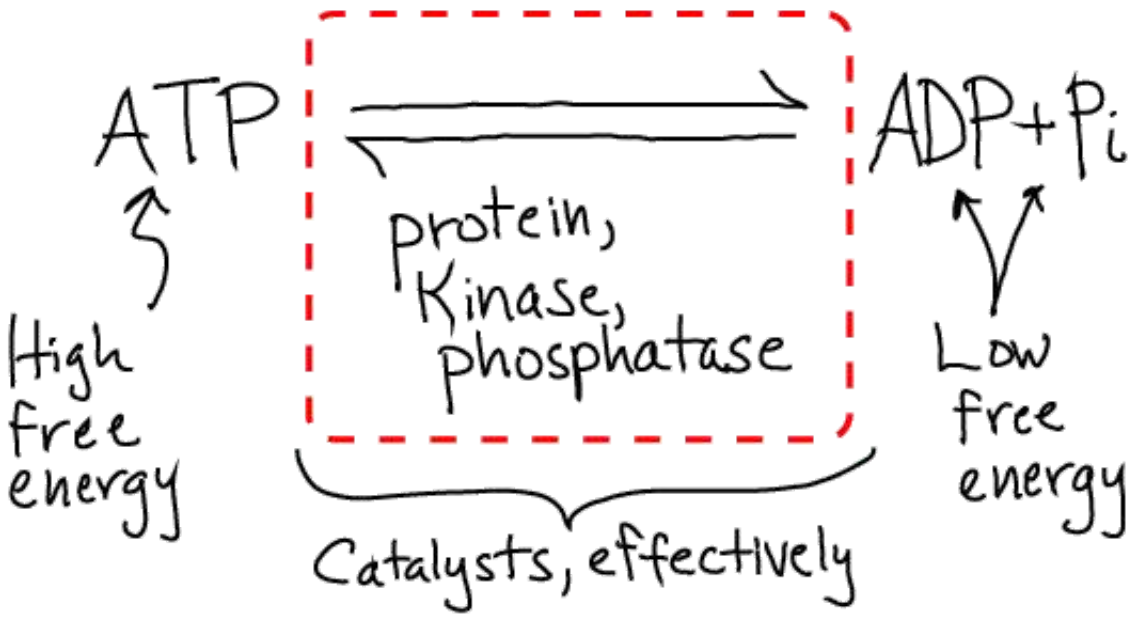
Our interest is not in the details of phosphorylation chemistry, but rather in the role of free energy in controling the directionality of signaling. Most generally, how is reversibility in signaling (e.g., phosphorylation) controlled? After all, every molecular process is reversible, but the cell requires its processes to be sequenced correctly. Why doesn't the enzyme, called a kinase, which places phosphate groups on proteins also catalyze the reverse reaction? And a related question: how can a single enzyme control which side of the phosphorylation reaction is favored?

The answers are very interesting. In a cell, the process of phosphorylation is driven by the free energy stored in ATP in its role as an activated carrier. That is, the same source of free energy that can drive "uphill" chemical synthesis and even locomtion via molecular motors is used to "power" signaling in the sense of ensuring that processes happen in the right order. This is much easier to see in the more accurate cartoon below.



The specific strategy evolved by cells involves two separate reactions which both are free energetically favorable. The kinase catalyzes hydrolysis and attaches the phosphate group, while the kinase catalyzes cleavage of the phosphate group from the protein in a separate reaction. The reactions don't occur in reverse (typically) because the products and reactants are far out of equilibrium, as is always the case when free energy is stored. The cell regulates the duration of the signal - how long the phosphate stays on the protein - by controling the whether or not the phosphatase is present and active. Note that the cell has many types of kinases and phosphatases, each of which is specific to (de)phosphorylating one or a small set of proteins. The activity of these key enzymes in turn may be regulated by other kinase/phosphatase pairs!

From a bottom-line chemistry perspective, the net reaction that occurs is the usual hydrolysis of ATP to ADP and Pi. However, the full reaction is catalyzed by a *set* of proteins instead of just a single enzyme.



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Signalling

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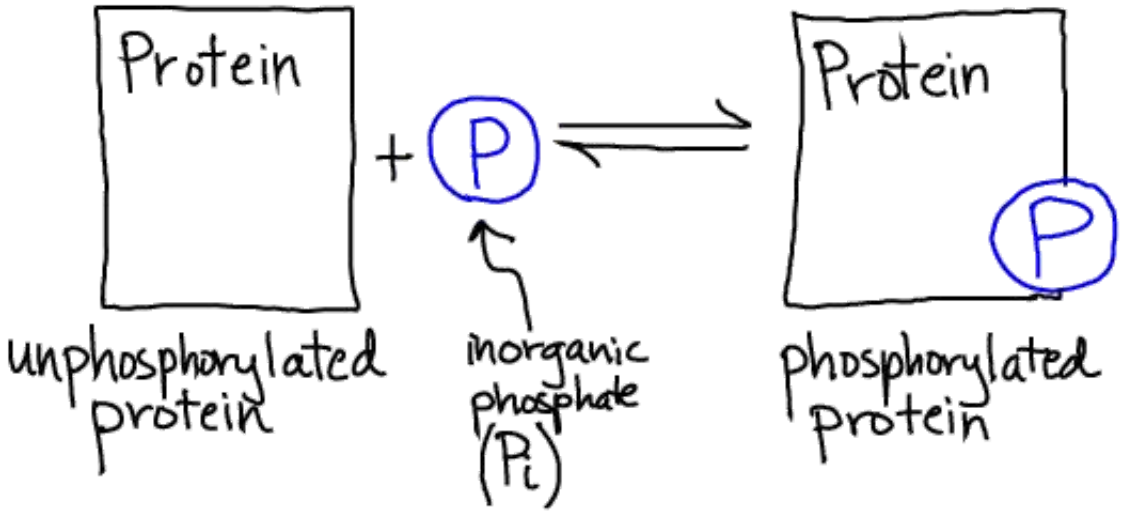

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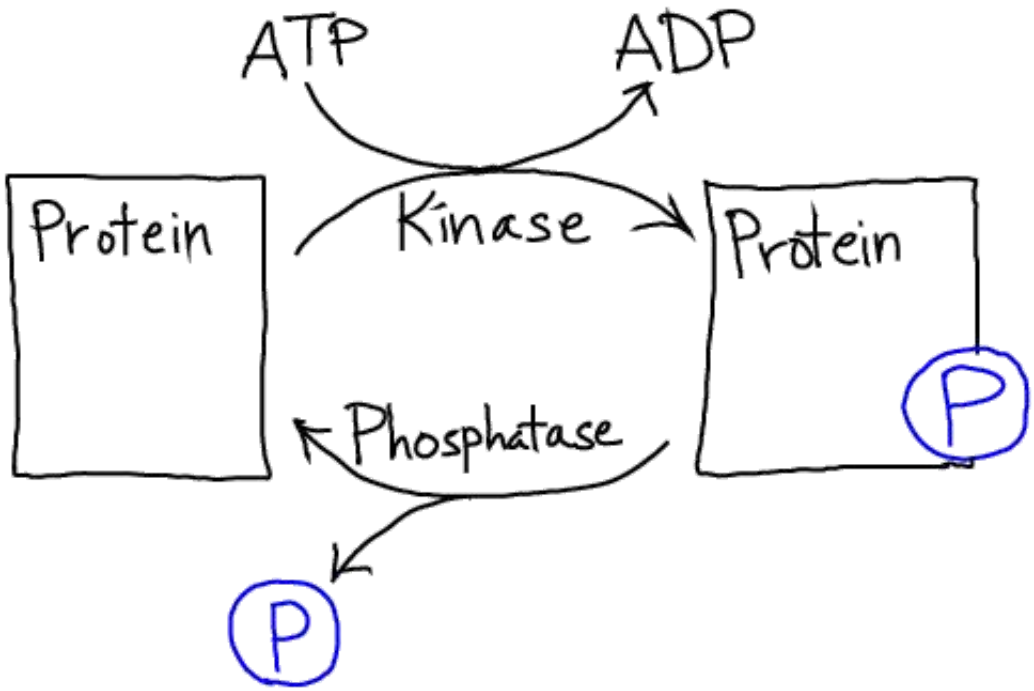

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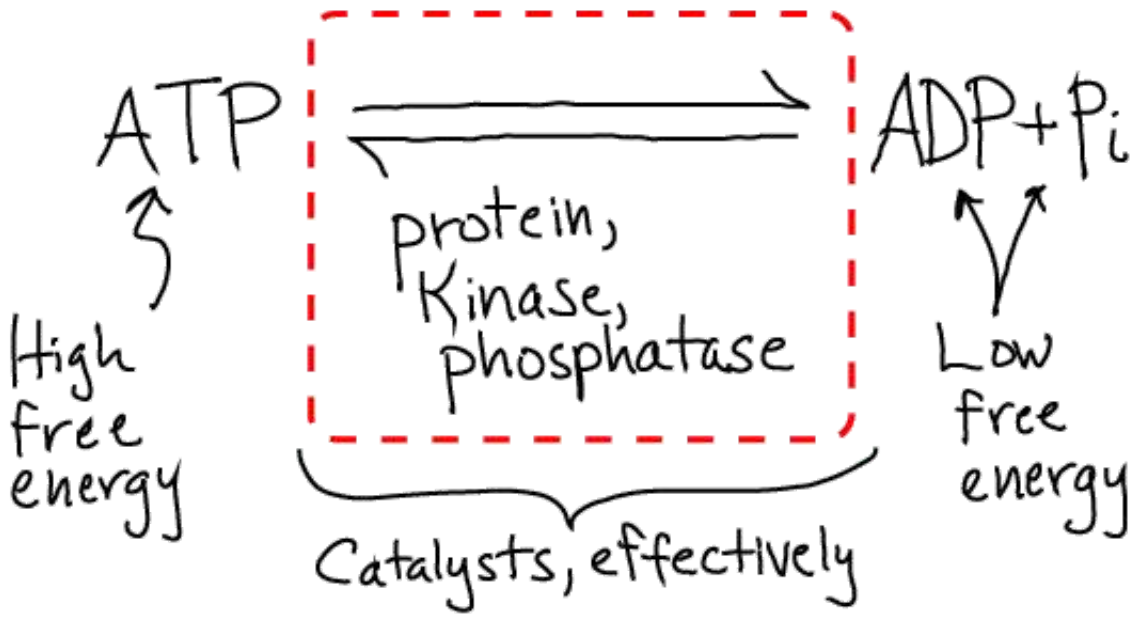

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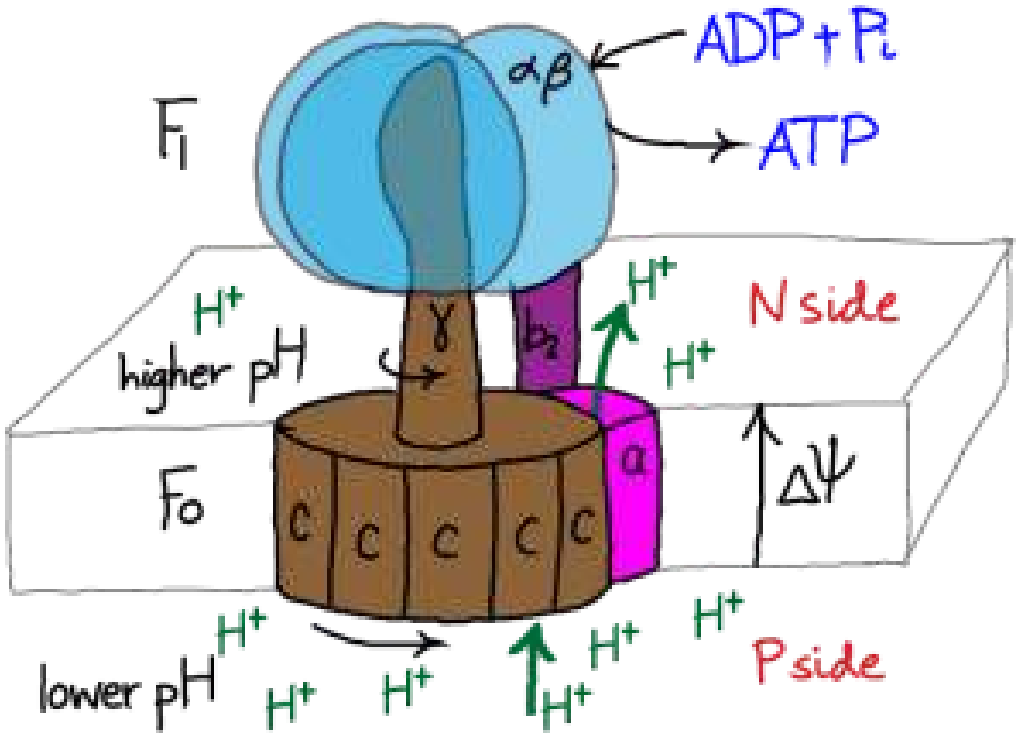
Synthesis of ATP by ATP synthase

Making the Miracle Molecule: ATP Synthesis by the ATP Synthase

ATP is the most important energized molecule in the cell. ATP is an activated carrier that stores free energy because it is maintained out of equilibrium with its hydrolysis products, ADP and Pi. There is a strong tendency for ATP to become hydrolyzed (split up) into ADP and Pi, and any process that couples to this reaction can be "powered" by ATP - even if that process would be unfavorable on its own. Examples include transport of other molecules across a membrane against the natural gradient and the action of motor proteins.



ATP is synthesized by a machine that may be even more remarkable, the ATP synthase (also called F-ATPase or FoF1-ATPase). As shown in the figure, the ATP synthase is an intricate rotary machine consisting of ~ 20 proteins. The machine is driven by a difference in proton electrochemical potential across the bilayer (grey outline), which consists of both a pH difference (difference in proton concentrations) and an electrostatic potential - i.e., voltage - difference $\Delta\psi$. The proton-coupled free energy difference is sometimes called the "proton motive force" or pmf. The pmf drives proton transport (green arrows) from the positive P-side to the negative N-side of the membrane, which in turn causes rotation of the c-ring (brown) of the Fo subunit which is attached to the γ stalk (brown). Rotation of the asymmetric γ stalk within the three catalytic $\alpha\beta$ domains (light blue) of the F1 subunit suppplies energy sufficient for the synthesis of ATP - more precisely, for the phosophorylation of ADP.

In overview, an electrochemical gradient for protons is transduced into mechanical motion (rotation) which in turn creates sufficient conformational energy (elastic and perhaps electrostatic) to enable the highly unfavorable chemical reaction snythesizing ATP. This is fairly incredible.

Thermodynamic analysis of ATP synthesis

We can understand the driving forces through which the ATP synthase works *without* detailed knowledge or assumptions regarding structural details of the machine. For example, although mechanical/conformational free energy is transiently stored during the synthesis cycle, that is part of the inner workings of the machine that does not affect the overall thermodynamics. This is because all molecular machines are "passive devices" that reset to the exact same state after a cycle - in essence, machines simply are catalysts for complicated processes that may involve both chemical reactions and transport. The machines themselves do not supply energy but rather use free energy stored in other sources, such as activated carriers like ATP or concentration gradients.

The ATP synthase always generates three ATP molecules in a complete 360-degree rotational cycle because of the three catalyic $\alpha\beta$ domains, but the number of protons n transported (which depends on the number of c-subunits - see figure above) varies from 8 to 15, depending on the organism and/or organelle. The overall "reaction" catalyzed by the ATP synthase (omitting water) is therefore

$$3\,\mathrm{ADP} + 3\,\mathrm{Pi} + n\,\mathrm{H}^+(\text{"P side"}) \rightleftharpoons 3\,\mathrm{ATP} + n\,\mathrm{H}^+(\text{"N side"}), \quad (1)$$

where "P" denotes the positive side of the membrane where the proton chemical potential is higher, and "N" is the "negative" side of lower chemical potential.

The overall free energy change per ATP synthesized is therefore the difference bewteen the change in chemical potential μ for $n/3$ protons and the free energy cost for phopshorylating ADP:

$$\Delta G_{\mathrm{tot}} = (n/3)\,\Delta\mu(\mathrm{H}^+) - \Delta G(\mathrm{ADP} \rightarrow \mathrm{ATP}). \quad (2)$$

We know that the overall ΔG_{tot} must be negative, because the process does occur. The negative $\Delta\mu$ term will outweigh the positive ΔG for phosphorylating ADP.

Although the individual terms cannot really be known exactly, we can approximate them reasonably using our usual ideal gas (ideal solution) framework. We will build on the detailed discussion found in the chemical potential section, where we explored the free energy of ATP *hydrolysis*, $\Delta G(\mathrm{ATP} \rightarrow \mathrm{ADP})$, which is simply the negative of the ΔG value we want. We therefore have

$$\Delta G(\mathrm{ADP} \rightarrow \mathrm{ATP}) \simeq -RT \ln \left(\frac{[\mathrm{ADP}][\mathrm{Pi}]/\,[\mathrm{ATP}]}{[\mathrm{ADP}]^{\mathrm{eq}}[\mathrm{Pi}]^{\mathrm{eq}}/\,[\mathrm{ATP}]^{\mathrm{eq}}} \right), \quad (3)$$

where $[\mathrm{X}]$ denotes the cellular concentration of molecule X and $[\mathrm{X}]^{\mathrm{eq}}$ is the equilibrium concentration. ATP is an "activated carrier" of free energy because its concentration is maintained far from equilibrium ... by the action of the ATP synthase. The state of activation for ATP is equivalent to the condition that $\Delta G(\mathrm{ADP} \rightarrow \mathrm{ATP}) > 0$.

When equilibrium and typical cellular values for the concentrations are substituted into (3), one finds that $\Delta G(\mathrm{ADP} \rightarrow \mathrm{ATP}) \simeq 12$ kcal/mol, which is about 20 times the thermal energy (RT or $k_B T$ significant amount of energy!

For ATP synthesis to proceed, we know the total ΔG_{tot} in (2) must be negative, so the change in proton chemical potential must be large and negative:

$$\Delta\mu(\mathrm{H}^+) < (3/n)\,(-12\,\mathrm{kcal/mol}) \quad (4)$$

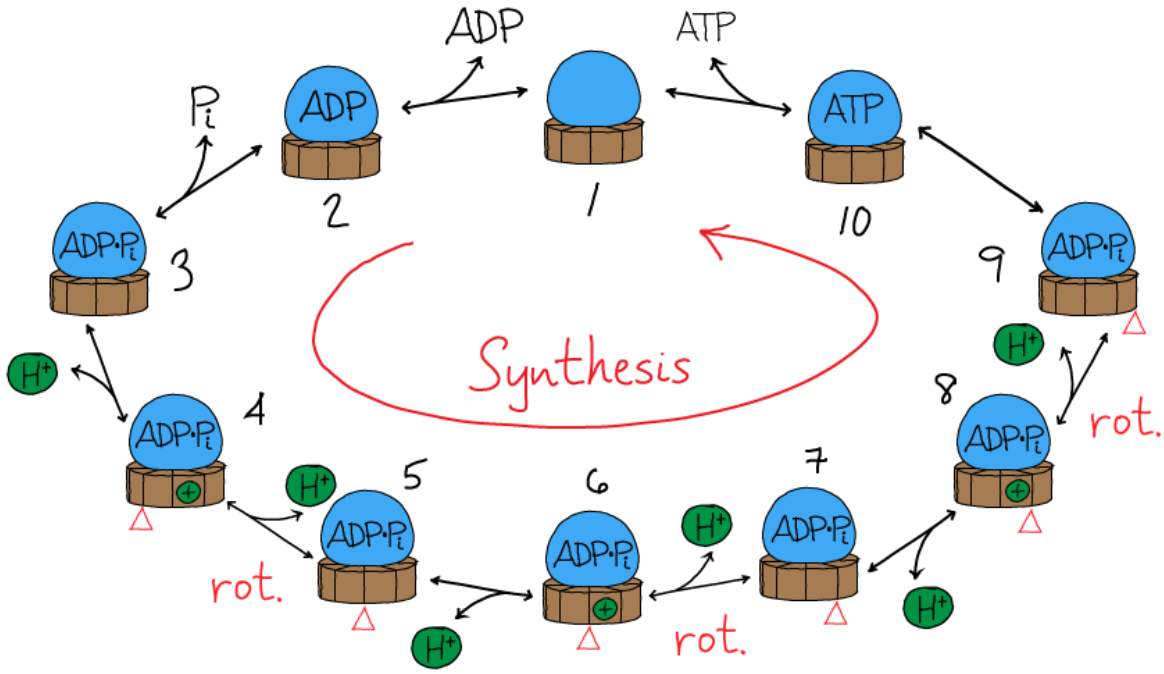
where $n = 8$ for the mammalian ATP synthase. As noted above, there are two driving forces acting on the protons: (i) the electrostatic potential difference across the membrane $\Delta\psi$, which simply creates an electric field that exerts a force on the proton as you learned in high school physics, and (ii) the pH difference ΔpH across the membrane which creates a diffusive force that tends to equalize concentrations because pH is simply a measure of concentration. A more complete discussion of membrane electrostatics is available.

We can quantify the preceding description of proton driving forces, the pmf, again within ideal solution theory, via

$$\Delta\mu(\mathrm{H}^+) = -F\Delta\psi + 2.3RT\Delta\mathrm{pH}, \quad (5)$$

where F is Faraday's constant which simply converts the potential difference $\Delta\psi$ into energy units for a single proton charge. To interpret the overall effect of these terms, we must know the conventions adopted for the "Δ" terms: for pH, it is P-side pH value minus the N-side pH making ΔpH negative; for ψ it is the same directionality, so the N-side voltage is subtracted from the P-side's, making $\Delta\psi$ positive. The net result that both terms of $\Delta\mu(\mathrm{H}^+)$ are negative by convention. Physically, the absolute value of $\Delta\mu(\mathrm{H}^+)$ is the free energy available per proton transported down the electro-chemical gradient. Note that sometimes the electro-chemical potential difference is assigned the symbol $\Delta\tilde{\mu}$ to remind us that there is an electric field present.

A simple kinetic model for ATP synthesis



We can get a more concrete understanding for how the synthase will behave by building a simple mass action model which incorporates some of the key features of its behavior. To allow us to build a model based on a relatively small number of equations, we will essentially model the synthesis of a single ATP - in effect, we will model 120 out of the full 360 degrees of the rotary cycle. And given that restriction, we will want the number of protons n to be divisible by 3. We will choose $n = 9$ which is close to the $n = 8$ value for mammalian ATP synthases.

The model will attempt to mimic the rotary mechanism in the following sequence of steps, as shown in the figure. Starting from state 1, ADP and Pi will bind the synthase, leading to state 3. A proton will then bind from the P side of the membrane, leading to state 4, followed by proton unbinding to the N side and state 5. (Rotation is implicitly included in the transitions $3 \rightarrow 4$ and $4 \rightarrow 5$, though for concreteness the figure shows it occuring with the $4 \rightarrow 5$ transition.) Proton binding, unbinding and rotation repeat two more times, leading to state 9. The transition to state 10 is the catalytic step yielding ATP, and then ATP unbinding occurs leaving the machine back in state 1. All the steps will be reversible as is true for the cycle for any molecular machine.

The mass-action equations governing the model's kinetics are straightforward to write down. Using the notation that $[i]$ is the population of the machine in state i, the first equation is:

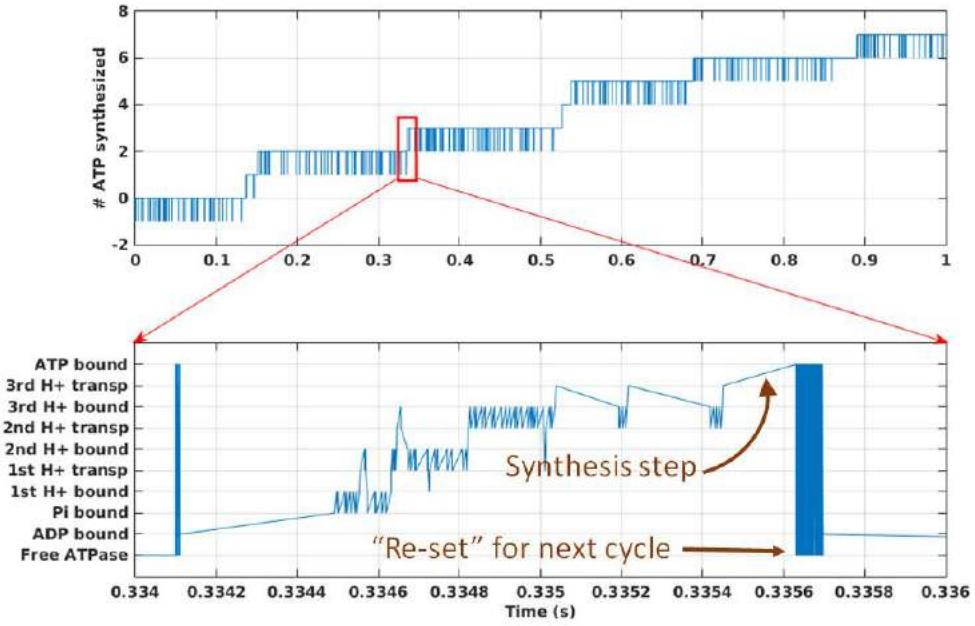
$$\frac{d[1]}{dt} = -\left([1][\mathrm{ADP}]\, k_{12} + [1][\mathrm{ATP}]\, k_{1,10}\right) + [2]\, k_{21} + [10]\, k_{10,1}; \quad (6)$$

where k_{12} and $k_{1,10}$ are on-rates, while k_{21} and $k_{10,1}$ are off-rates. The other equations take similar forms. For example,

$$\frac{d[9]}{dt} = -\left([9][\mathrm{H}^+(\mathrm{N})]\,k_{98} + [9]\,k_{9,10}\right) + [8]\,k_{89} + [10]\,k_{10,9}\,; \quad (7)$$

where $[\mathrm{H}^+(\mathrm{N})]$ is the proton concentration on the N side, k_{98} is an on-rate, k_{89} is an off-rate, while $k_{9,10}$ and $k_{10,9}$ are the catalytic rates for synthesis and hydrolysis, respectively.

To fully specify the model, we need all the rate constants. Some are available from the literature and others can be estimated, which is a fairly technical process that will not be discussed here. (The parameters used in the numerical data shown below are given in the model file also available below.) However, it is worth noting that not all the rate constants can take arbitrary parameters because of the usual constraint that occurs with any cycle.



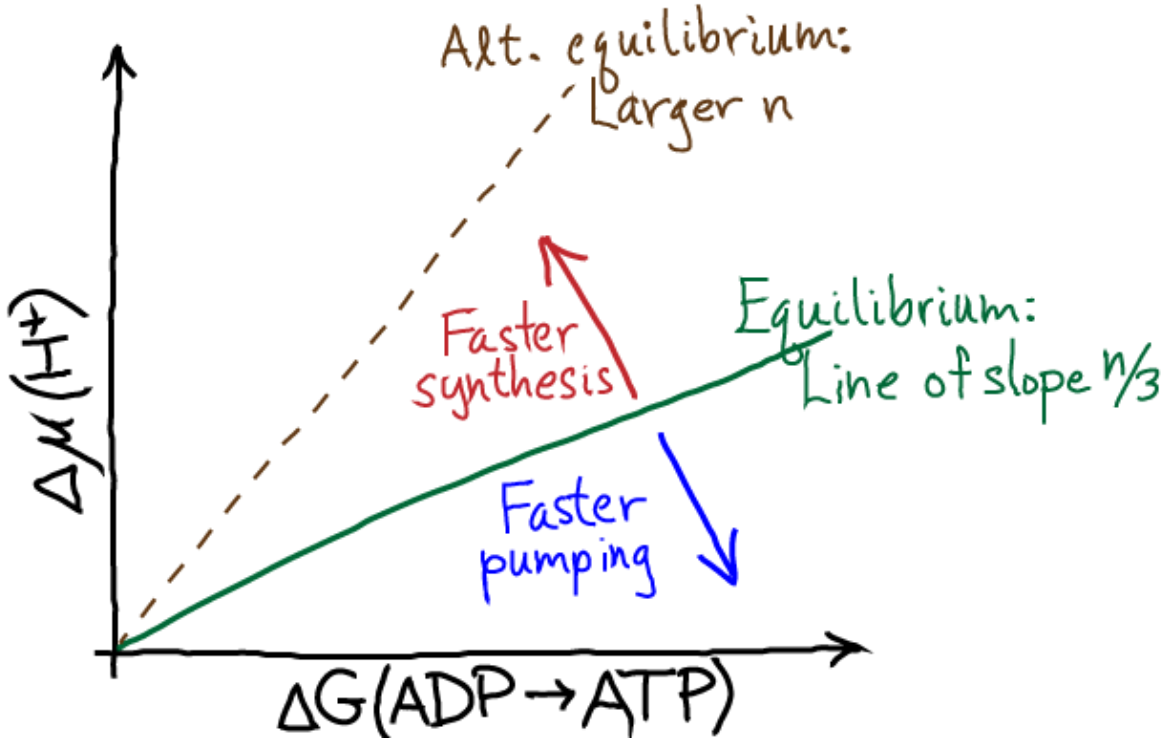
The figure shows stochastic simulation of our rotary model for a (molecular) time of 1 sec. Synthesis proceeds at a rate of about ~10 ATP/s, but note that there are frequent stochastic reversals. Such reversals, which occur at the level of overall ATP synthesis as driven by "microscopic" reversals among the 10 states we have set up, are expected to be an intrinsic part of the functioning of many molecular machines. On aggregate, however, with thousands of synthases per mitochondrion, there will be a very steady amount of synthesis at fixed driving conditions. The source code for the model (a .bngl file) can be downloaded here. The simulation was performed using BioNetGen, a rule-based platform for kinetic modeling.

Speed vs. efficiency and Synthesis vs. pumping

We can get a very useful overall understanding of the relation between the thermodynamics (driving free energy) and the kinetics of ATP synthesis by taking a closer look at Eq. (2). The equilibrium condition is when $\Delta G_{\mathrm{tot}} = 0$, so the driving free energy for the protons exactly balances the free energy necessary to phosphorylate ("synthesize") ATP:

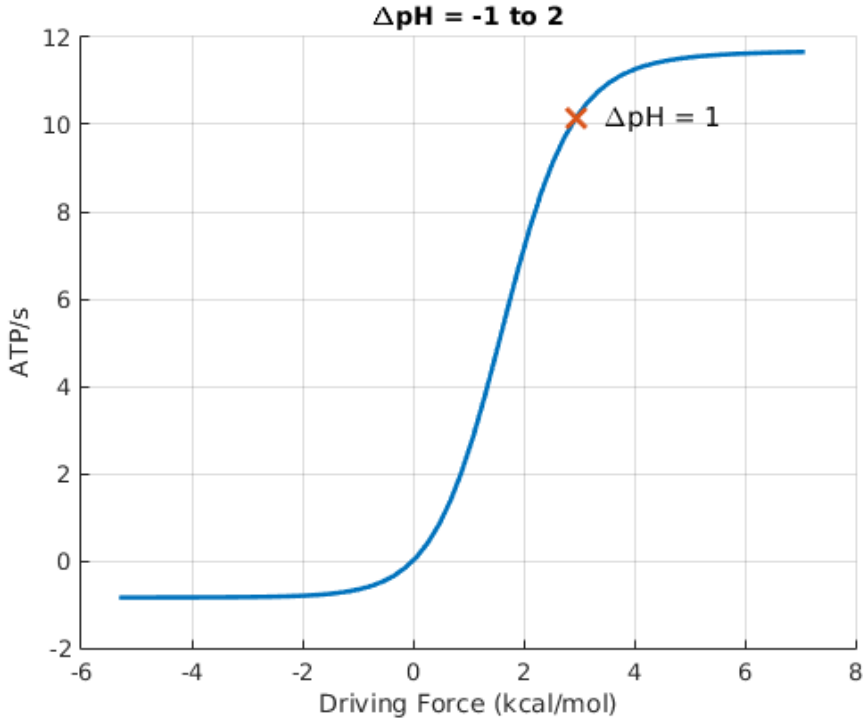
$$\text{Equilibrium: } (n/3)\,\Delta\mu(\mathrm{H}^+) = \Delta G(\mathrm{ADP} \to \mathrm{ATP}). \quad (8)$$

In a plot of the chemical potential vs. the synthesis free energy, this corresponds to a straight line of slope $n/3$ as shown in the figure.



At equilibrium, there is zero net synthesis - which follows immediately from the balanced-flow definition of equilibrium. That is, while an occasional ADP might get phosphorylated by a synthase, that will be balanced out by an equal number of hydrolysis events. The further the system gets from equilibrium, the stronger the driving. With stronger driving we expect faster synthesis (in the region above the equilibrium line). There will be, however, a maximum speed at which the machine can operate, so ultimately the synthesis rate will level off even with very significant driving.

To visualize the relationship between thermodynamic driving and the ATP synthesis rate more quantiatively, it is useful to define the driving free energy as the negative of the total free energy chage, $\Delta G_{\mathrm{drive}} = -\Delta G_{\mathrm{tot}}$. By convention, $\Delta G_{\mathrm{drive}}$ is positive under synthesis conditions and negative for reverse operation of the synthase. In the figure below, the point marked with a red X represents the conditions used to generate the stochastic trajectories shown above.



As with any molecular machine, the synthase can be driven in reverse, in which case it will act as a proton pump driven by ATP hydrolysis - as has been shown experimentally many times. The stronger the driving, the faster the pumping until the performance plateaus due to rate-limiting chemical and conformational steps. This behavior is seen in the figure for negative driving potential.

On the question of efficiency, Eq. (2) tells us that for any finite amount of driving, there must be some free energy "spilled" ($\Delta G_{\rm tot}$ is dissipated as heat) when synthesis proceeds at a finite rate. Hence, the efficiency - measured as the fraction of the proton free energy converted to ATP chemical free energy - is always less than 100%. The lower the driving, the higher the efficiency - at the price of reducing the speed of synthesis. The cell must optimize the tradeoff between speed and efficiency for its own purposes ... and note that some heat generation is not entirely wasteful for many organisms that need to maintain body temeperature.

Rotary ATPases have evolved many different stoichiometries (n values) enabling them to function under different conditions, presumably optimized for different organisms or organelles. A given set of thermodynamics conditions can be used either for ATP hydrolysis-driven proton pumping or pmf-driven ATP synthesis, depending on the stoichiometry. See the dashed line in the $\Delta\mu$ vs. ΔG figure.

Acknowledgements

Many thanks to Ramu Anandakrishnan and Zining Zhang for helpful discussions as we all learned about the rotary ATPases. Ramu Anandakrishnan prepared the data-based figures.

References:

A basic discussion of the ATP synthase can be found in any biochemistry or cell biology book. More detailed treatments are given in bioenergetics texts. For example, see

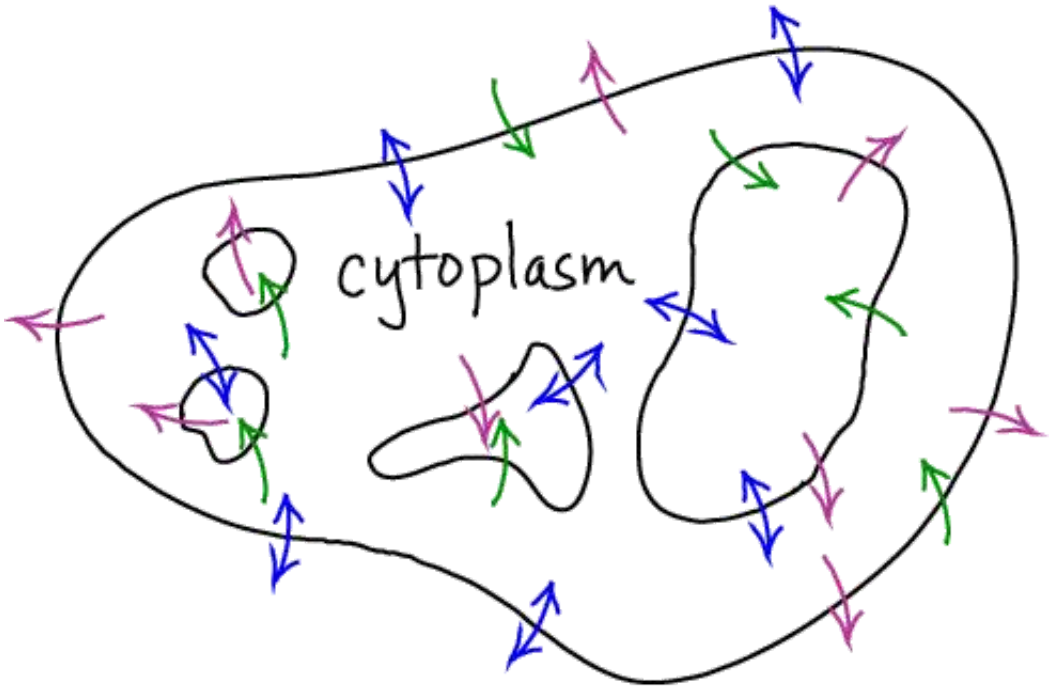
- D. G. Nicholls et al., "Bioenergetics", Academic Press.
- T. P. Silverstein, "An exploration of how the thermodynamic efficiency of bioenergetic membrane systems varies with c-subunit stoichiometry of F1F0 ATP synthases," J Bioenerg Biomembr (2014) 46:229-241.
- J. E. Walker, "The ATP synthase: the understood, the uncertain and the unknown," Biochem. Soc. Trans. (2013) 41:1-16.

Exercises:

1. Using the definition of pH, show that the ΔpH term in Eq. (5) corresponds precisely to the free energy change for a simple concentration difference as in our chemical potential discussion.
2. Write down the mass-action equations governing states 3, 4, and 5 in the model for rotary synthesis.
3. Write down the cycle constraint for the model in terms of rate constants, and then group the rate constants to see the dependence of the constraint equation on various equilibrium constants.
4. Sketch the expected behavior of a stochastic simulation of a single ATP synthase under equilibrium conditions. Also sketch the average behavior - i.e., the average ATP synthesized per second considering many synthases.

Transport

Transport in the Cell



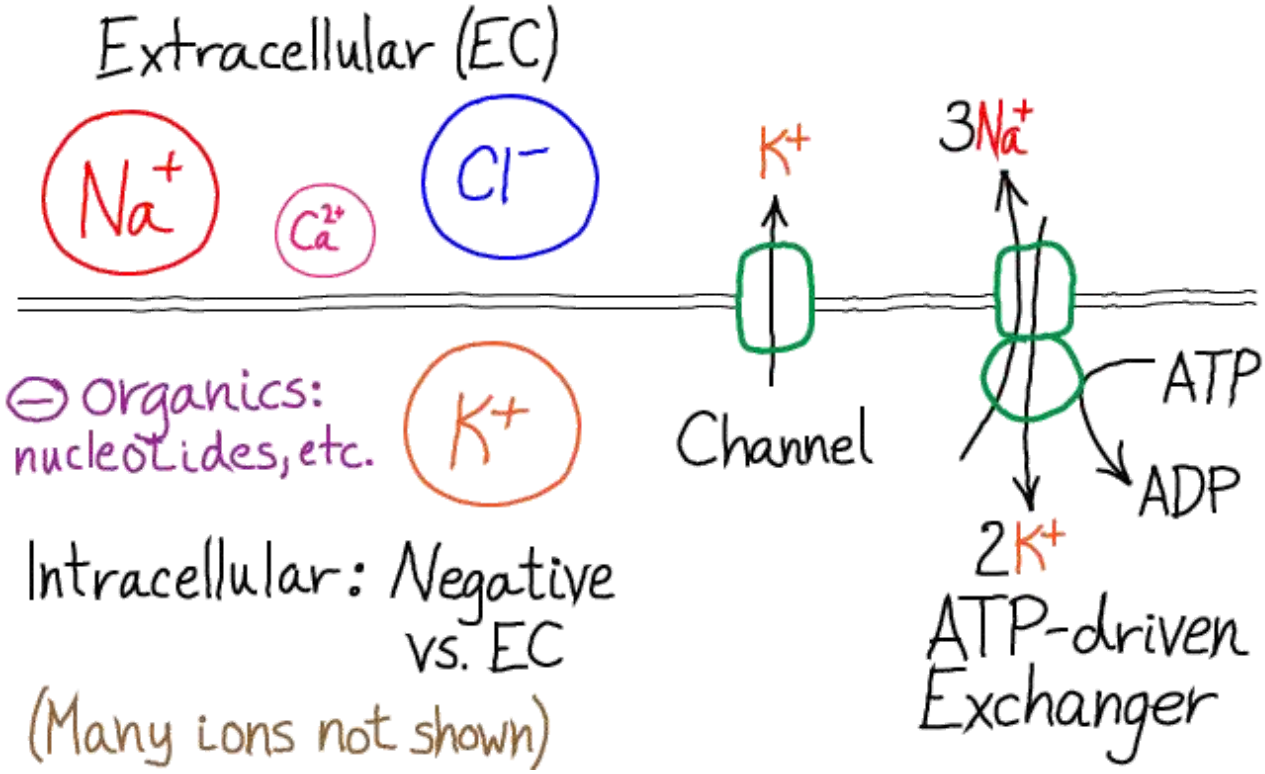
Basics

- **Transport** refers to the cell transporting molecules and ions across membranes - cellular or organelle membranes.
  - Transport is critical to the cell, as it must obtain nutrients, sort molecules, and export waste.
  - Transport can be active or passive.

- Passive transport occurs when molecules flow from a region of high to low concentration. Such flow requires no free energy, but the cell may use free energy to regulate the flow, by turning it on or off as needed.
  - Example: The action potential in a neuron is gated passive transport.
  - See the discussion of ion concentration gradients to learn the basics of electrostatic influences in passive transport.
  - **Active transport** uses free energy to pump a molecule against its concentration gradient (i.e., from low to high concentration). This can occur by two generic mechanisms depending on the type of free energy expended to do the pumping:
    1. ATP-driven transport, or
    2. Transport driven by the gradient of another molecule or ion.

The Membrane, Ions, and "Electrostatic Health"

Ions and the Membrane: An Introduction



Besides its role in separating the cell from the rest of the world and organelles from the cytoplasm, the membrane and its integral proteins are critical in controlling the "electrical health" of the cell. This involves keeping ions of a given type primarily on one side of the membrane - see above - until their flow to the other side is needed, as in the action potential of nerve cells or calcium signaling. And once the physiological need for such flow ends, ions must be pumped back to the "resting" state (which is *not* an equilibrium state).

The figure above sketches key ion species - sodium (Na^+), chloride (Cl^-), potassium (K^+), and calcium (Ca^{++}) - shown on the side of the membrane where the concentration of the given ion is highest. The sizes of the circled ions crudely represent the relative concentrations between species. The charged organic molecules include, DNA, RNA, and ATP, which have negative phosphate groups. The figure also shows a potassium 'leak channel' and the Na-K pump, two of the transmembrane transporters involved in electrical behavior.

Basics: 'Activated ions' and passive ions

Although the overall behavior of ions is complex, because all ions interact with one another based on the laws of electrostatics, a basic picture emerges by dividing ions into two classes. As background, note that the extracellular and intracellular concentrations of a given species will tend to adjust to the (Donnan) equilibrium point based on the potential difference across the membrane, which is embodied in the Nernst equation and

results from *all* charged species present.

- **Activated ions** are those with concentrations *not* dictated by the Donnan equilibirum/Nernst equation - i.e., nonequilibrium concentrations. In precise analogy with activated carriers, such ions store free energy and can be used to power other processes, such as transport or can rapidly flow across the membrane as part of a physicological process such the action potential of nerve cells or calcium signaling. In most cells, sodium (Na^{+}) and calcium (Ca^{++}) are activated. (Note that 'activated ions' is not standard terminology, but seems very descriptive.)
- **Passive ions** are those whose intra/extra-cellular ion concentrations are essentially consistent with the Donnan equilibirum/Nernst equation.

Importantly, just because there is a substantial ion concentration gradient across the membrane - which is true for all ions - does *not* mean the species is out of equilibrium. Rather, because the cell maintains a potential differene across the membrane in its resting state, the Donnan equilibrium concentrations do *not* match across the membrane. The cell has evolved to keep only a subset of ions 'activated'.

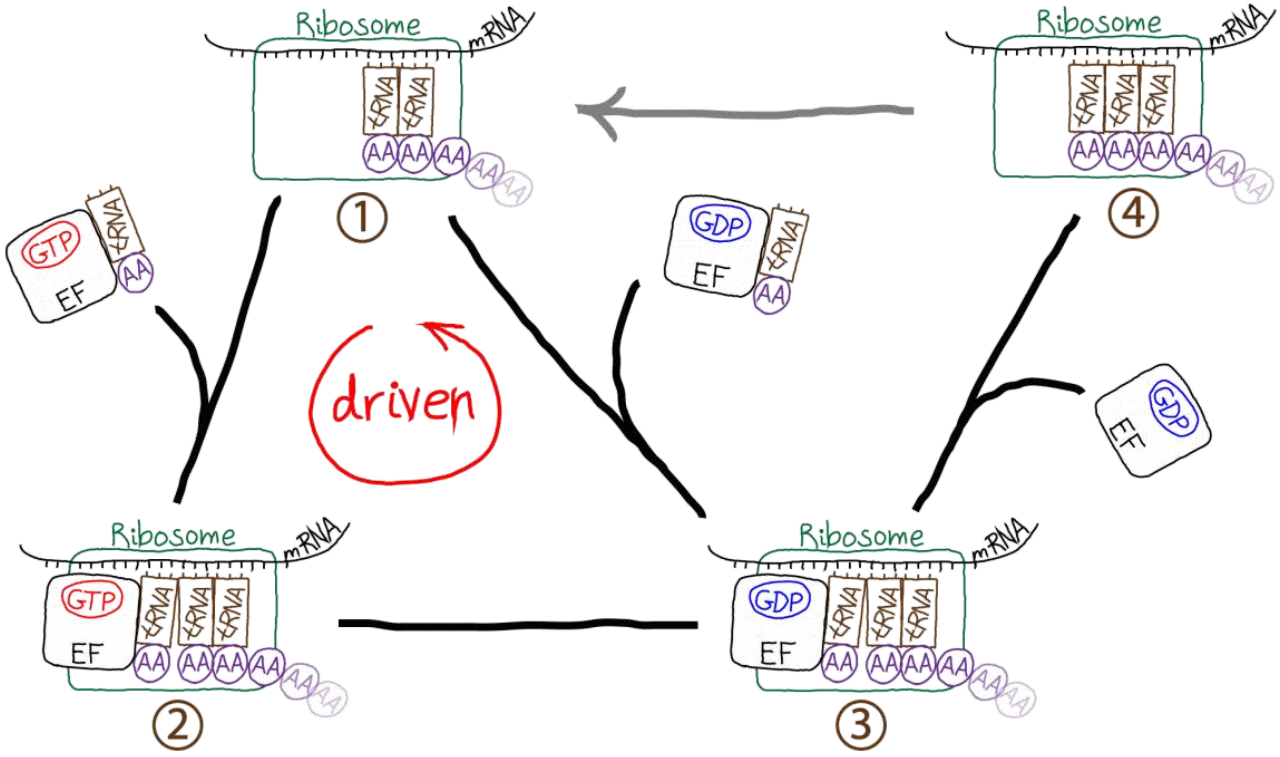
References:

- R. Phillips et al., "Physical Biology of the Cell," (Garland Science, 2009).
- B. Alberts et al., "Molecular Biology of the Cell", Garland Science (many editions available).

Active ("Kinetic") Proofreading

One of the most remarkable tasks accomplished by the cell is the exchange of energy for information, as originally hypothesized in separate works by Hopfield and Ninio. That is, the cell performs processes like transcription and translation with greater fidelity than would be possible if it only relied on binding affinity to attach the correct base or amino acid. The cell achieves this using "proofreading" processes that are driven by the expenditure of free energy. These processes conventionally are described as *kinetic proofreading* though driven or active proofreading would be terminology more consistent with discussions presented in these pages - e.g., active vs. passive transport.

We will study proofreading which occurs in the translation process - in the synthesis of protein based on an mRNA sequence. The source of free energy turns out to be the activated carrier GTP rather than ATP.

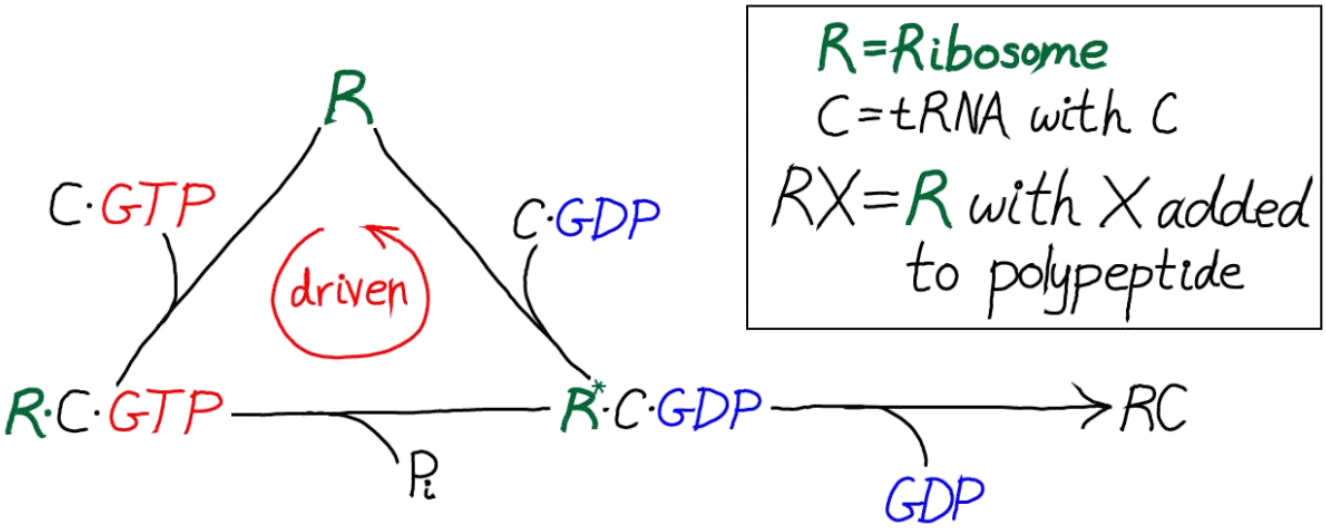


The figure schematically demonstrates the translation process occurring in a cell. The ribosome is the molecular machine responsible for translation. Starting in the upper left (state 1), the ribosome links a tRNA molecule bound with an amino acid (AA) to a complex including the preceding two tRNA molecules and the growing polypeptide chain. The incoming tRNA/AA molecules are complexed to an EF protein which is also bound to GTP. GTP hydrolysis drives the cycle in a counter-clockwise direction, adding a step (state 2) to the process which seems to be extraneous. In fact, the extra step is what makes proofreading possible by permitting a second opportunity for unbinding of tRNA/AA from the ribosome - which ultimately aids discrimination between correct and incorrect amino acids. From state 3, the polypeptide chain is extended by the formation of a covalent bond to the new AA; EF/GDP unbinds and the system yields state 4 which cannot receive a new tRNA/AA because the binding site is occupied. In a driven multi-step process not shown

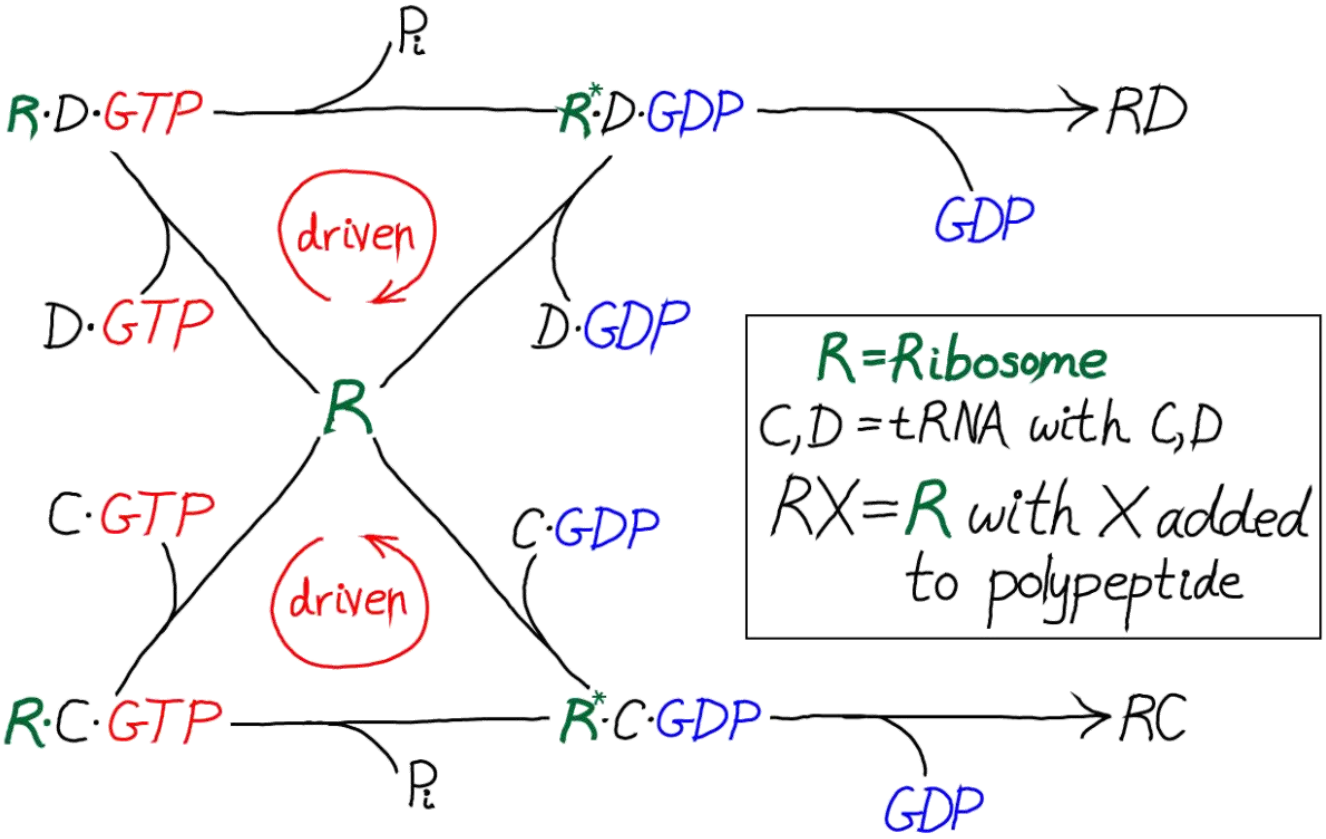
(grey arrow) the ribosome also translates the peptide chain and three tRNA molecules to the right, ejecting the rightmost tRNA which was added earliest.

A model for driven proofreading in translation

This subtle process is best studied with a specific model. We can construct a tractable model for driven/kinetic proofreading by slightly simplifying the process sketched above.

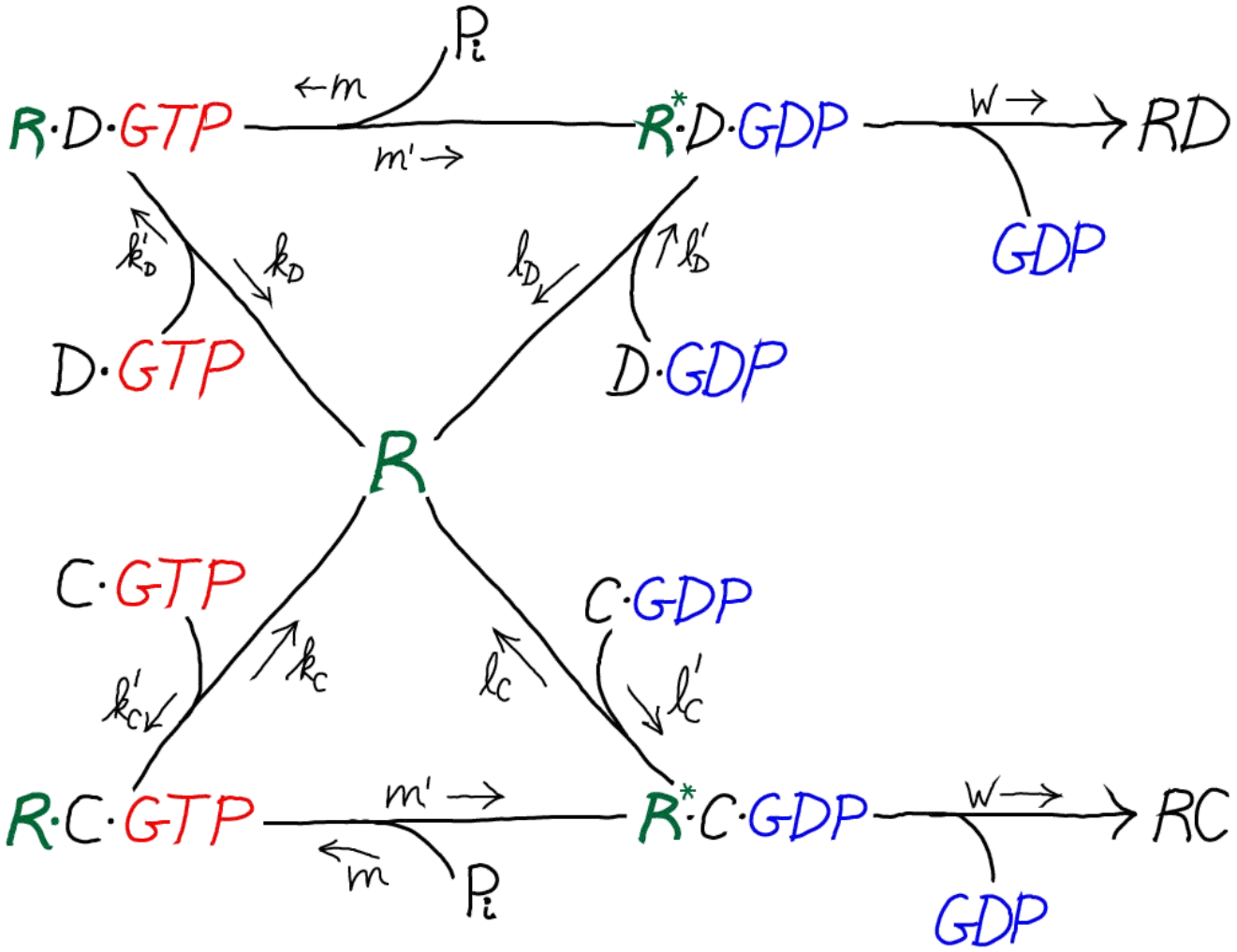


In this kinetic scheme, we have omitted the EF protein but retained GTP, which is critical as the activated carrier providing the driving force for proofreading. We have also further simplified the final step of covalent addition of the amino acid. The symbol "C" should be thought of as the correct amino acid, in contrast to the wrong amino acid "D" shown in the full model below.



Only by including the dual cycles with both C and D can we assess the discriminatory power of the model. That is, we will not to know the relative likelihoods for C and D to be incorporated into the polypeptide chain. And proofreading *is* discrimination.

Model specifics



The model is specified according to the rates in the diagram above, where the notation follows Hopfield's paper. It is a standard mass action model: letters besides k are used for the rate constants of different processes to avoid excessive sub- and super-scripts. The model has the following features and assumptions, again following Hopfield:

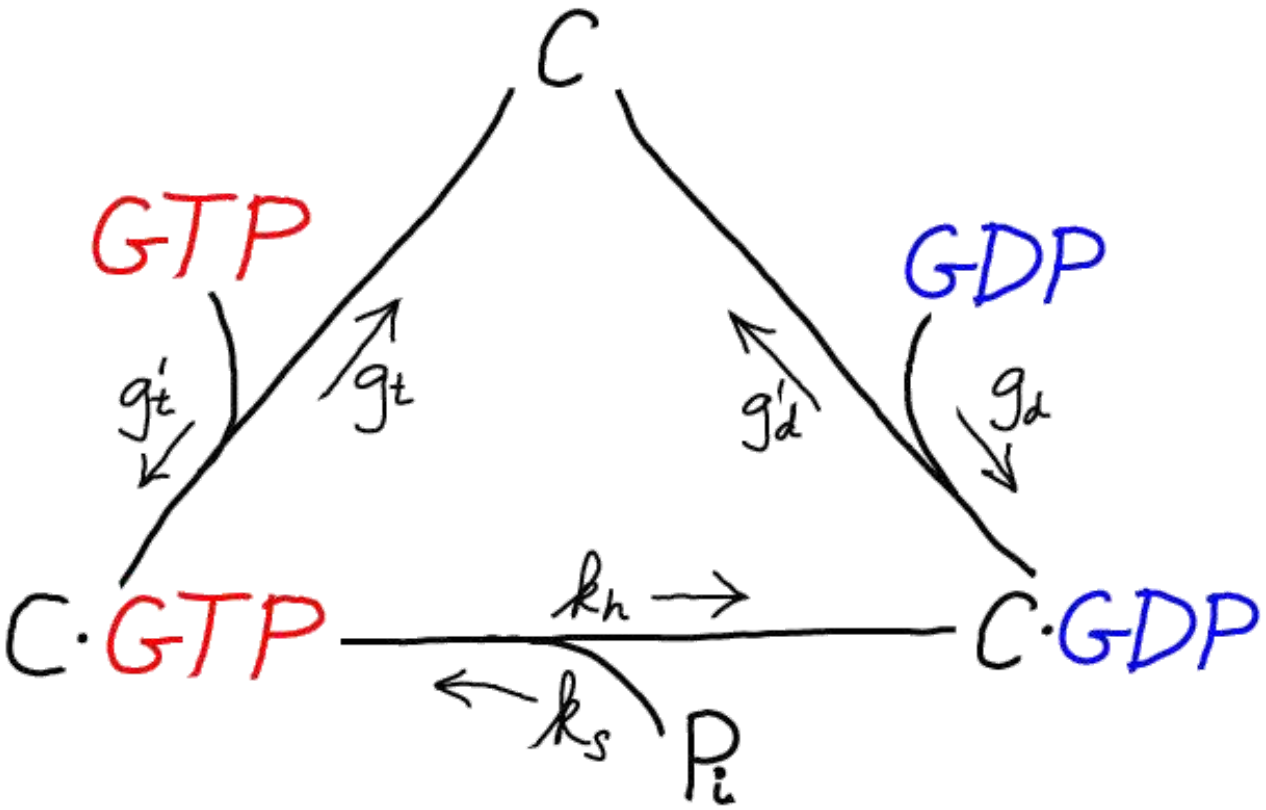
- The amino acid complexes for C and D are assumed to be equally available in solution, so $[\mathrm{C}\cdot\mathrm{GTP}] = [\mathrm{D}\cdot\mathrm{GTP}]$.
- The rates m, m' and w are identical for both the C and D processes.
- On-rates are also assumed to be the same for both processes, so that $k'_C = k'_D$ and $l'_C = l'_D$.
- Discrimination is assumed to occur via unbinding processes, with the more favorable C being slower to unbind compared to D. Specifically, off-rates are chosen to differ by a factor of $f_0 < 1$ so that $l_C = f_0\, l_D$ and $k_C = f_0\, k_D$.
- $\mathrm{C}\cdot\mathrm{GTP}$ binding is assumed to be more favorable than $\mathrm{C}\cdot\mathrm{GDP}$ binding, which can be realized via $l'_C < k'_C$, and similarly for D.
- The transition from RX to R* X (with X = C or D) is assumed to be much slower than the R* X unbinding processes: $m' \ll k_C, k_D$
- The rate w for incorporating an amino acid onto the polypeptide chain (which involves multiple steps) is

assumed to be much slower than all other rates.

You should recognize that, as with any cycle, there is a constraint which ensures thermodynamic consistency, so that only five of six rates are independent. This will be discussed further below.

An auxiliary cycle for activating the tRNA complexes

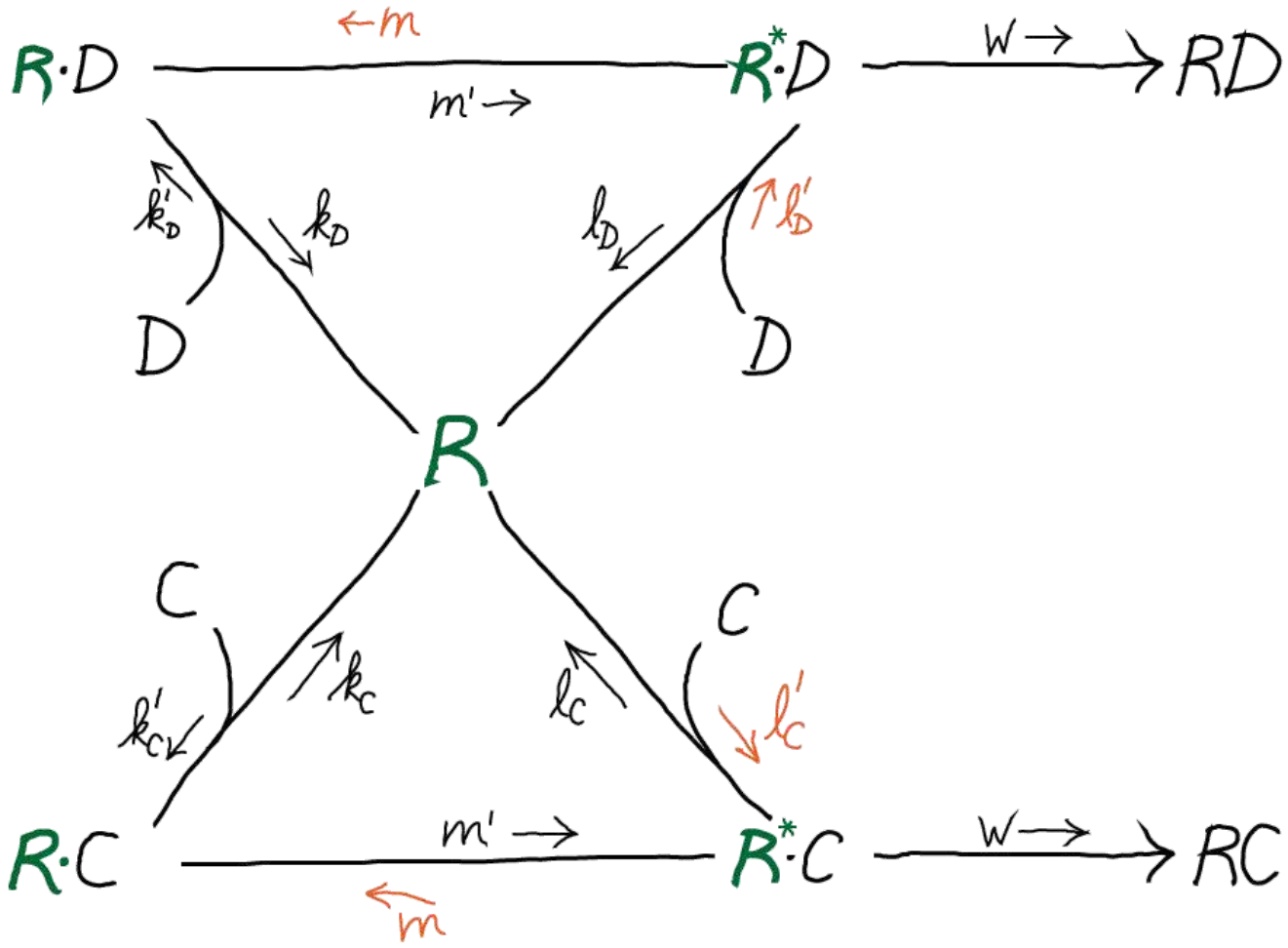
Although not essential for understanding the basics of driven proofreading, there is an additional cycle that governs the activation of the tRNA complexes: the unbinding of GDP and the binding of GTP to C or D. This activation cycle is interesting because it brings in the "raw" free energy associated with GTP hydrolysis/activation.



The C/GTP cycle is driven by the cell's continual synthesis of GTP. GTP is an activated carrier and its cellular concentration far exceeds its equilibrium value relative to the hydrolysis products GDP and Pi. An analogous cycle, with identical rate constants, is assumed for D/GTP binding and hydrolysis.

"Back-of-the-envelope" analysis

With only a few algebraic steps, and without including the auxiliary C/GTP cycle, we can demonstrate the basics of driven proofreading. In fact, for a quick analysis, it is easiest to exclude GTP and including driving only implicitly as will described. Thus we consider a somewhat simplified cycle.



The rates shown in orange will later be used to model driving.

Translation without driving, roughly

First, let's consider the cycle "as is" with all rates/processes included. Because the rate w is so slow (see above) and there is no driving, the cycles can be considered to be in equilibrium. In other words, all the processes happen so fast compared to the w process that their relative populations are essentially equal to the equilibrium values. In this model, the equal availability of the two amino acid complexes amounts to assuming $[\mathrm{C}] = [\mathrm{D}]$.

Our goal is to calculate the discrimination ratio of correct (C) incorporation by the w process to incorrect (D) incorporation in a steady state:

$$\frac{[\mathrm{RC}]}{[\mathrm{RD}]} = \frac{[\mathrm{R^*{\cdot}C}]\,w}{[\mathrm{R^*{\cdot}D}]\,w} = \frac{[\mathrm{R^*{\cdot}C}]}{[\mathrm{R^*{\cdot}D}]} \tag{1}$$

In the scenario just described, it is straightforward to solve for $[\mathrm{R^*{\cdot}X}]$ using detailed balance along the right leg of each cycle. For the C cycle, we have $[\mathrm{R}][\mathrm{C}]\,l'_C = [\mathrm{R^*{\cdot}C}]\,l_C$ and similiar for D. Substituting into (1) and

using assumptions stated above, we find

$$\frac{[\mathrm{RC}]}{[\mathrm{RD}]} = \frac{l'_C\, l_D}{l_C\, l'_D} = \frac{l_D}{l_C} = \frac{1}{f_0} \tag{2}$$

In other words, in this *un*driven scenario, discrimination occurs according the ratio of unbinding rates, which in turn is just the ratio of equilibrium dissociation constants because the on-rates are the same: see the discussion of binding.

Driven translation, roughly

To obtain a quick estimate of the effects of GTP-driving on the translation process, we will assume simply that the reverse processes shown in orange above are eliminated. That is, we will simply set the rate constants $m, l'_C, l'_D = 0$. You should recognize that this leads to an unphysical model (because detailed balance cannot be satisfied), but our goal is to construct a crude model of the effect of driving - without including the complications of the driving components. Don't worry - we will show later that the full model yields the same basic behavior as the simple version.

Procedurally, we will pursue a steady state analysis. We cannot use a quasi-equilibriium analysis, as we did for the undriven case, because we have explicitly put a net flow into the cycles by setting rate constants to zero.

In steady state, the net flow into every state balances the net flow out. For the $\mathrm{R^*{\cdot}C}$ state, we have

$$[\mathrm{R{\cdot}C}]\, m' = [\mathrm{R^*{\cdot}C}]\, (l_C + w) \simeq [\mathrm{R^*{\cdot}C}]\, l_C \tag{3}$$

where we used our assumption of very small w. The steady-state condition for the $\mathrm{R{\cdot}C}$ state is

$$[\mathrm{R}][\mathrm{C}]\, k'_C = [\mathrm{R{\cdot}C}]\, \left(k_C + m'\right) \simeq [\mathrm{R{\cdot}C}]\, k_C \tag{4}$$

where we again used one of our original assumptions that $m' \ll k_C$. Exactly analogous results are obtained for the D process.

We can combine the C and D steady state results to obtain the overall discrimination ratio. We use the preceding steady-state equations to solve for $[\mathrm{R^*{\cdot}C}]$ in terms of $[\mathrm{R}][\mathrm{C}]$, and similarly for D. From these steps we can obtain

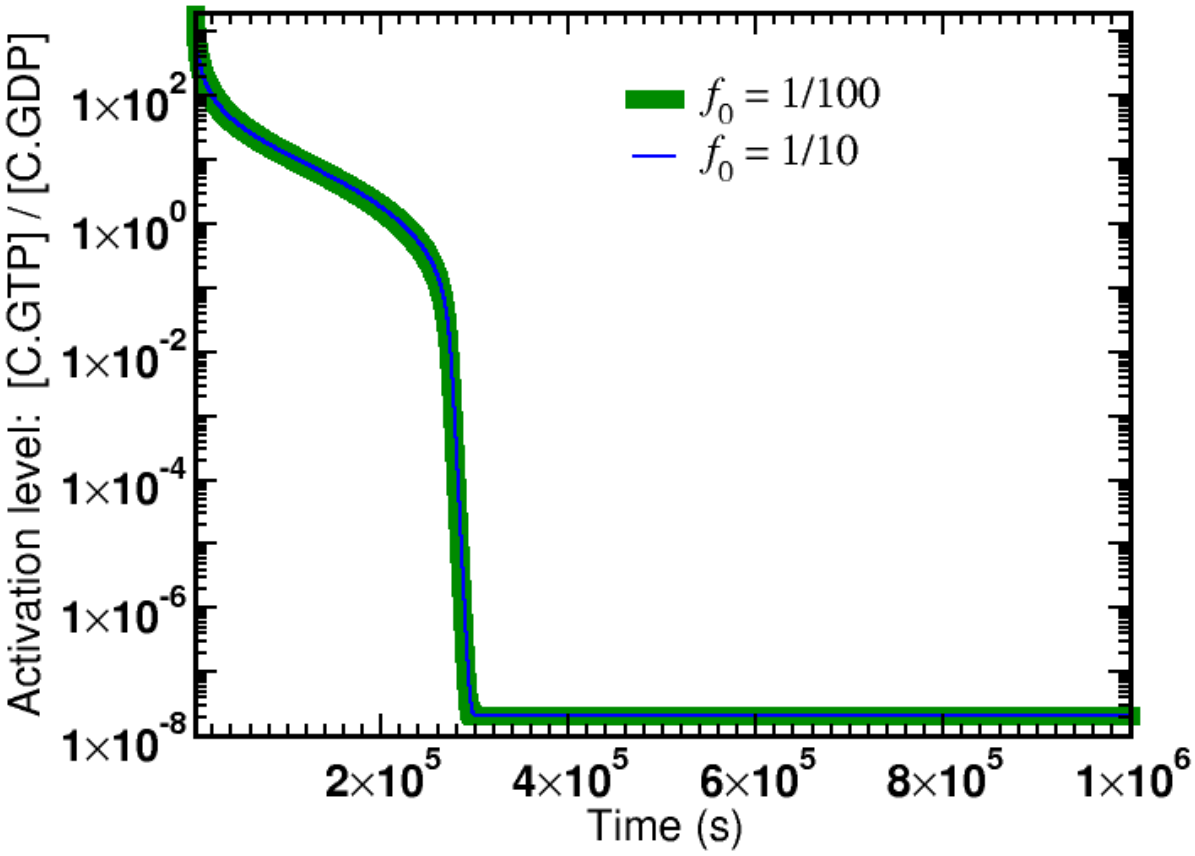
$$\frac{[\mathrm{RC}]}{[\mathrm{RD}]} = \frac{[\mathrm{R^*{\cdot}C}]}{[\mathrm{R^*{\cdot}D}]} \simeq \frac{k'_C\, l_D\, k_D}{l_C\, k_C\, k'_D} = \frac{1}{{f_0}^2} \tag{5}$$

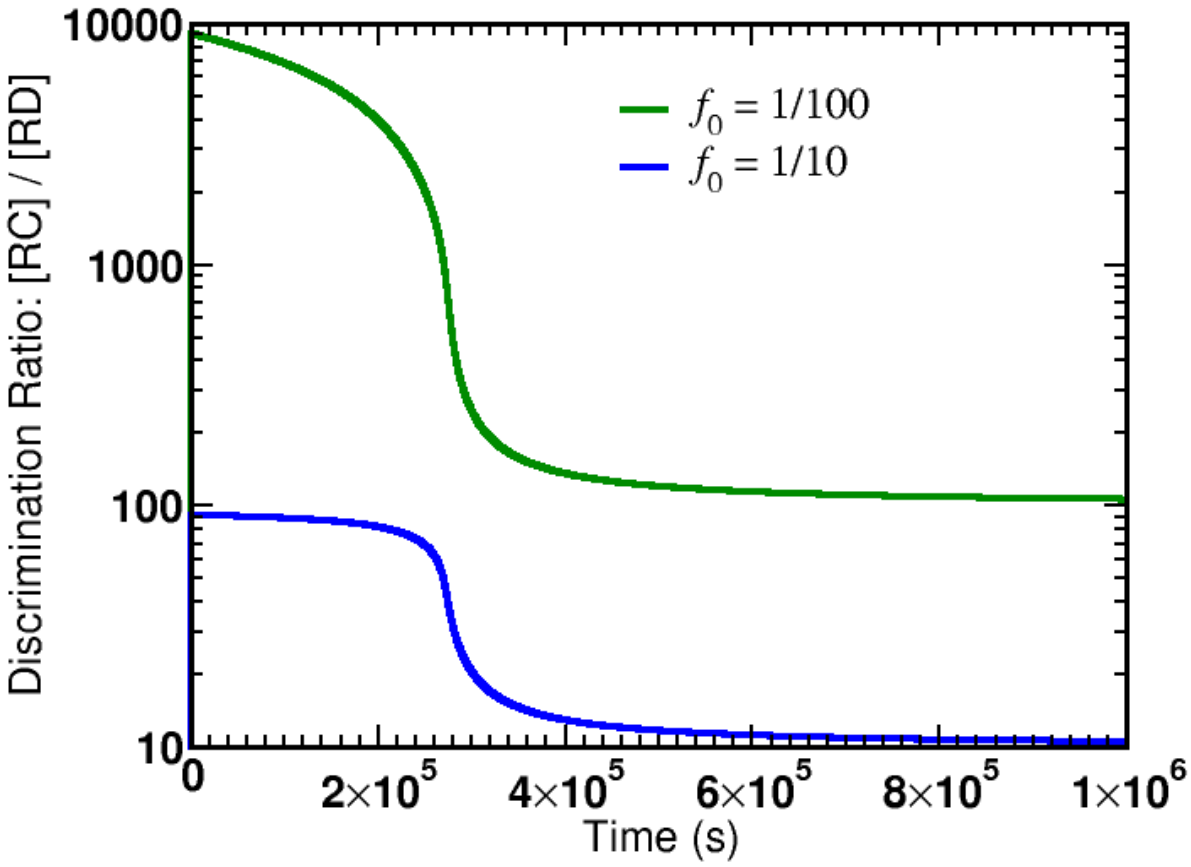
where we used the original assumption $k'_C = k'_D$.

The driven discrimination ratio (5) is dramatically improved compared to the undriven case (2), when we recall that $f_0 < 1$. In a cellular context, f_0 can be as small as $1/100$ (see book by Alon) making driven proofreading 100 times better! Although we derived (5) using apparently unphysical assumptions, Hopfield has shown the same result is obtained from a full steady-state analysis. Our numerical data on the full model, below, are also consistent with the result.

Simulation of the full driven proofreading model

Simulation data is presented from the model specified below. Two cases were examined: afffinity ratios of $f_0 = 1/10, 1/100$. In both cases, simulations started from an initial condition where GTP (and hence $\mathrm{C{\cdot}GTP}$) was highly activated; simulations were run long enough so that $\mathrm{C{\cdot}GTP}$ became de-activated (via sufficient hydrolysis to GDP) and, accordingly, the proofreading became less driven.


Activation level: [C.GTP] / [C.GDP]
1×10^2
1×10^0
1×10^-2
1×10^-4
1×10^-6
1×10^-8
2×10^5
4×10^5
6×10^5
8×10^5
1×10^6
Time (s)
f0 = 1/100
f0 = 1/10



The first graph shows the time course of the de-activation of $\mathrm{C}\cdot\mathrm{GTP}$, which is identical for both affinity ratios. The system is started in a highly activated non-equilibrium state and relaxes toward equilibrium.

There are two key lessons from the figures plotting the discrimination ratio $[\mathrm{RC}]/[\mathrm{RD}]$: (i) The degree of discrimination decreases over time as GTP becomes de-activated, which is what we would expect. (ii) The discrimination ratio goes from a nearly maximal initial value $\sim 1/f_0{}^2$ as predicted by our simple analysis to the equilibrium value of $\sim 1/f_0$. Although it is not shown in the graphs, more than one GTP hydrolysis occurs per successful addition of amino acid in both cases.

The simulation data shown corresponds to an initial condition relaxing toward equilibrium, but it certainly is possible to run a non-equilibrium steady-state simulation in which GTP stays activated and the proofreading remains driven/active. This could be achieved by mimicking the cell's continual synthesis of GTP from GDP and Pi in a one-way reaction.

Rate constants for the model are specified below. Initial conditions were $[\mathrm{GTP}] = 10^{-3}$ M, $[\mathrm{GDP}] = [\mathrm{Pi}] = 10^{-6}$ M, $[\mathrm{C}] = [\mathrm{D}] = 10^{-4}$ M, with 1,000 ribosomes (R). Simulations were performed using BioNetGen, a rule-based platform for kinetic modeling. The source code for the model (a .bngl file) can be downloaded by right-clicking here.

Process	Symbol	Value
Reference on-rate	k_{on}	10^8 / (M s)
Reference off-rate	k_{off}	100 / s
Affinity ratio	f_0	Adjustable parameter
C·GTP binding	k'_C	k_{on}
C·GTP unbinding	k_C	k_{off}
C·GDP binding	l'_C	$0.01\, k_{\text{on}}$
C·GDP unbinding	l_C	k_{off}
GTP hydrolysis (Rib.)	m'	$0.1\, k_{\text{off}}$
GTP synthesis (Rib.)	m	cycle constraint (below)
Amino acid attachment	w	$0.001\, k_{\text{off}}$
D·GTP binding	k'_D	k_{on}
D·GTP unbinding	k_D	k_{off} / f_0
D·GDP binding	l'_D	$0.01\, k_{\text{on}}$
D·GDP unbinding	l_D	k_{off} / f_0
GTP binding	g'_t	k_{on}
GTP unbinding	g_t	k_{off}
GDP binding	g'_d	g'_t
GDP unbinding	g_d	$10\, k_{\text{off}}$
GTP hydrolysis (aux.)	k_h	$10^{-8}\, k_{\text{off}}$
GTP synthesis (aux.)	k_s	cycle constraint (below)

Cycle constraints

We first analyze the main cycle for ribosome (R) binding to $\text{C}\cdot\text{GTP}$. As with any cycle, we apply detailed

balance to each process and eliminate unwanted concentration terms to obtain

$$\frac{l_C\, k'_C\, m'}{l'_C\, k_C\, m} = \frac{[\mathrm{C}\cdot\mathrm{GDP}][\mathrm{Pi}]}{[\mathrm{C}\cdot\mathrm{GTP}]} \tag{6}$$

The right-hand side has the form of an equilibrium (dissociation) constant but it's not one we can look up in a biochemistry book. Instead we can express that constant in terms of the GTP constant by using the auxiliary cycle.

We therefore turn to the auxiliary cycle of C + GTP binding. From the detailed balance conditions, the constraint one finds is

$$\frac{g_d\, k_h\, g'_t}{g'_d\, k_s\, g_t} = \frac{[\mathrm{GDP}][\mathrm{Pi}]}{[\mathrm{GTP}]} = K_{\mathrm{GTP}} \tag{7}$$

where the right-hand side is the GTP hydrolysis equilibrium constant. However, we can also look directly at the detailed balance condition for GTP hydrolysis to find

$$\frac{k_h}{k_s} = \frac{[\mathrm{C}\cdot\mathrm{GDP}][\mathrm{Pi}]}{[\mathrm{C}\cdot\mathrm{GTP}]} \tag{8}$$

To get the relations into a useful form, we solve for the left-hand side of (8), namely k_h/k_s, from (7) and subtitute the result back into (8) to obtain

$$\frac{[\mathrm{C}\cdot\mathrm{GDP}][\mathrm{Pi}]}{[\mathrm{C}\cdot\mathrm{GTP}]} = K_{\mathrm{GTP}}\frac{g'_d\, g_t}{g_d\, g'_t} \tag{9}$$

This the result we want because it expresses the $\mathrm{C}\cdot\mathrm{GTP}$ equilibrium constant in terms of a known equilibrium constant and rates from the model. Finally, we re-write (6) using (9) as

$$\frac{l_C\, k'_C\, m'}{l'_C\, k_C\, m} = K_{\mathrm{GTP}} \frac{g'_d\, g_t}{g_d\, g'_t} \tag{10}$$

Equations (7) and (10) are the constraints used to define missing model parameters, along with $K_{\mathrm{GTP}} = 5 \times 10^5$ M. The constraint for the R + $\mathrm{D \cdot GTP}$ cycle is analogous to (10).

References:

- B. Alberts et al., "Molecular Biology of the Cell," Garland Science (many editions available).
- J. J. Hopfield, "Kinetic proofreading: A new mechanism for reducing errors in biosynthetic processes requiring high specificity," Proc. Nat. Acad. Sci. 71:4135-4139 (1974).
- J. Ninio, "Kinetic amplification of enzyme discrimination," Biochimie 57:587-595 (1975).
- U. Alon, *An introduction to systems biology*, CRC Press (2006).

Exercises:

1. Derive the constraint (6) among the rates for the R, RC, R* C cycle.
2. Derive the constraint (7) for the C/GTP "auxiliary" cycle. Show that the constraint from the previous cycle can be expressed in terms of rate constants from the C/GTP cycle.
3. Derive the "back of the envelope" driven discrimination ratio (5).

About

Physical Lens on the Cell attempts to offer a new perspective on physical cell biology education, both in terms of content and form.The goal is to provide interdisciplinary material, now a staple of modern science, in a modern way. Lens is not a traditional linear textbook in internet guise but a cross-linked tool intended for learners from a variety of backgrounds.

Content

Modern cellular and molecular biophysics, like so many other fields, brings together a range of disciplines starting with traditional cell biology, but including the physical and quantitative sciences. Physical Lens on the Cell uses a physical approach to organize cellular phenomena in a manner that is much more concise than the usual cell biology text of 1,000+ pages. This site makes no attempt at comprehensiveness, and thus cannot replace the traditional textbook, but rather emphasizes deeper understanding of physical driving forces that necessarily underlie the myriad cellular phenomena whose diversity often seems bewildering.

The site will most obviously be useful for physical or quantitative scientists seeking to understand cell biology without getting through the 1,000-page textbook. The basic physical driving forces are explained along with their manifestation in a surprising array of contexts - synthesis of molecules, transport of molecules to organize the cell and maintain its health, locomotion along the internal road system of filaments, and even information processing (error correction). The physics, which is largely non-equilibrium thermodynamics and statistical mechanics, is fascinating in itself but presented in a formulation that should be readily accessible to undergraduates - based on mass-action kinetics and ideal-gas thermodynamics.

Biologists also have much to gain from the site. It should deepen and quantify their understanding of the physical driving forces, the free energies, which underlie all cellular processes. After all, if there is no driving force, a process won't happen. The goal is to understand the commonalities linking the incredible diversity of cellular phenomena. Yes, there are equations, but these are restricted to the relatively simple algebra and calculus that are common in biochemistry books. Familiarity with chemical kinetics will give the biochemist or biologist an advantage over the physical scientist.

Platform

Because Physical Lens on the Cell covers interdisciplinary material, unlike a conventional textbook, it cannot make assumptions about the reader's level of knowledge. In fact, the notion of a knowledge "level" is not really appropriate. For example, many very able physicists may have minimal familiarity with non-equilibrium phenomena, whereas such ideas (if not the formalism) may be intuitive to biochemists. The author of a traditional book would be left to puzzle over appropriate content for a "Chapter 1."

Physical Lens on the Cell therefore does not have traditional chapters, though it does have an organization. Most importantly, the site is fully hyperlinked so the reader can readily bring up content from both internal

and external links. Thus, one can click through to just the right information. The site is organized hierarchically, with the most fundamental concepts presented "first" and progressing to cell biology phenomena. But it may be most interesting to start reading about a more biological topic and clicking through as needed to get the physical background.

Two-Panel Reading

Science is not easy and learning science rarely is linear. The two-panel layout (click on the splitscreen icon from any content page) allows ready cross-referencing of any and all material in the website. It is even possible to view two copies of the same page to allow comparison of figures or equations and the accompanying text that otherwise would cause the original item to scroll off the screen.

About the PDF Version

To enable off-line reading of the site's content, a pdf version is available. Readers of the pdf should be aware of two points: (i) the pdf represents a static "snapshot" taken of the site and may not include all updated material; and (ii) the pdf presents the material as it is ordered in the menus, with the result that a number of pages are duplicated. Duplications arise because a given page (e.g., on activated carriers) may be listed, appropriately, under more than one category (e.g., Physical/Molecular Processes and Energy Economy). Duplication seemed preferable to excluding the material from a section where it is highly relevant.

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