Chem 163, Lecture 8

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1 Two-state systems

Today we're going to talk about two-state systems. Almost any chemical reaction can be thought of in this way: you have reactants and products; or you have an ion inside the cell or outside the cell; or you have the nucleotide-bound state or the nucleotide-free state. Indeed everything we learned about molecular motors can be couched in the language of two-state systems. Here's how: If you watch a single molecular motor for some time long compared to the single step time, you can calculate the mean displacement and the variance. These quantities are the sums over all steps of the mean displacements and variances for a single step (central limit theorem!). You could get the same result by starting a large number of molecular motors all in some initial conformation, and then letting them all run for a short time t so that no motor takes more than one step. The mean displacement per motor, and the variance in this displacement, run for some short time t and summed over N motors, will be the same as for a single motor run for time Nt.

A molecular motor can be thought of as just a chemical reaction where the total amount of reactants that become products happens to be coupled to the total distance traveled, i.e. displacement is one of the reaction products, whose free energy depends on the force the motor is working against.

1.1 Modeling 2-state processes

You want to make a measurement on a system, and from this measurement to infer the rate constants. You then want to use this rate information to make a microscopic model of the system you're studying, namely to learn something about the shape of the potential energy surface and the motion on this surface. How do you do this?

One approach is to make a single-molecule measurement, for instance observing the folding and unfolding of a single RNA molecule under tension. Here's an example:

[SHOW EXAMPLES OF 2-STATE TRAJECTORIES:

- SCIENCE PAPER FROM LIPHARDT AND BUSTAMANTE ON "REVERSIBLE UNFOLDING OF SINGLE RNA MOLECULES BY MECHANICAL FORCE";

- FINZI AND GELLES SCIENCE PAPER ON TETHERED BEAD ASSAY;

- SINGLE-MOLECULE FRET EXPERIMENTS;

- SINGLE ION CHANNEL RECORDINGS: PATAPOUTIAN AND JULIUS NOBEL PRIZE PAPERS.]

How should we think about these two-state trajectories? label the two states A and B. We can assign the system a probability to be in either state at any time. The conditional probability of being in state A at time t, given that you were in state x at time t_0 is denoted $p(A, t|x, t_0)$. As a form of shorthand we will write this p_A, and similarly for p_B. Put these two probabilities on top of each other to form a vector. This vector evolves subject to the following equation:

$$\frac{d}{dt}\begin{pmatrix} p_A \\ p_B \end{pmatrix} = \begin{pmatrix} -\lambda & \mu \\ \lambda & -\mu \end{pmatrix}\begin{pmatrix} p_A \\ p_B. \end{pmatrix} \tag{1}$$

Here λ is the rate of going from A to B and μ is the rate of going from B to A. We also have the constraint that $p_A + p_B = 1$. There are various techniques for solving this equation. The most general is to write

$$\mathbf{V}(t) = e^{\mathbf{M}t}\mathbf{V}(0), \tag{2}$$

where $\mathbf{V}$ is the state-vector and $\mathbf{M}$ is the transition matrix. The exponential can be evaluated by diagonalizing $\mathbf{M}$. The nice thing about this approach is that it generalizes to systems with an arbitrary number of states.

We'll take a slightly different approach, and consider the time-evolution of the composite variable $X \equiv \lambda p_A - \mu p_b$. Then the matrix equation simplifies to:

$$\frac{dX}{dt} = -(\lambda + \mu)X, \tag{3}$$

with the trivial solution $X(t) = X(0)e^{-(\lambda+\mu)t}$. Solving for $p_A(t)$ and $p_B(t)$ yields:

$$\begin{aligned} p_A(t) &= \frac{\mu}{\lambda + \mu} + \exp\left[-(\lambda + \mu)t\right]\left(p_A(0) - \frac{\mu}{\lambda + \mu}\right) \\ p_B(t) &= \frac{\lambda}{\lambda + \mu} + \exp\left[-(\lambda + \mu)t\right]\left(p_B(0) - \frac{\lambda}{\lambda + \mu}\right). \end{aligned} \tag{4}$$

At steady state the exponentials decay and the equilibrium populations in A and B are:

$$\begin{aligned} p_A &= \frac{\mu}{\lambda + \mu} \\ p_B &= \frac{\lambda}{\lambda + \mu}. \end{aligned} \tag{5}$$

We see that the composite variable X is a measure of the deviation of the concentration from equilibrium.

In a real system, there are spontaneous fluctuations in the concentration because each molecule decides whether to react independently of all its neighbors. These spontaneous fluctuations relax as would fluctuations you put in by hand. In fact, you can write a Langevin equation for the concentration fluctuations, just as you would for the motion of a particle in a harmonic potential well:

$$\frac{dX}{dt} = -(\lambda + \mu)X + \xi(t), \tag{6}$$

where $\xi(t)$ is a generalized force, satisfying:

$$\langle \xi \rangle = 0 \tag{7}$$
$$\langle \xi(t)\xi(t+\tau)\rangle = 2(\mu+\lambda)\langle X^2\rangle\delta(\tau). \tag{8}$$

Solving this equation, just as we did for the case of a particle in a harmonic well gives:

$$\langle X(t)X(t+\tau)\rangle = \langle X^2\rangle \exp\left[-(\lambda+\mu)\tau\right]. \tag{9}$$

Thus the FDT rears its head again. In fact, the parallel between the chemical reaction system and the particle in a well is more apparent if you think about it from a thermodynamic perspective. The chemical potential of a species depends on its concentration. Near equilibrium, the *free energy* of each species depends quadratically on the deviation of its concentration from equilibrium. So we really do have an overdamped harmonic oscillator, just in an abstract chemical space.

How big are the fluctuations in concentration? This problem is mathematically the same as when we considered fluorescent molecules which had some probability p to be in a laser beam, and $q = (1-p)$ not to be in the laser beam. The statistics are governed by the binomial distribution. In our case $p = \lambda/(\lambda+\mu)$ and $q = \mu/(\lambda+\mu)$. For n molecules, the variance is just $\sigma^2 = npq$, or in our case:

$$\sigma_A^2 = \sigma_B^2 = n\frac{\mu\lambda}{(\mu+\lambda)^2}. \tag{10}$$

So now we have 3 ways to measure the kinetics: we could do a single-molecule experiment and calculate $p(A,t|x,t_0)$, and $p(B,t|x,t_0)$; we could disturb the system from equilibrium and watch its macroscopic relaxation, or we could look at the spontaneous fluctuations about equilibrium. All of these approaches yield the combined rate constant $\mu + \lambda$. Is there any experiment we could do to extract μ and λ separately?

Yes!! If we do the single-molecule experiment and look at the dwell-time histograms, we will see that dwell times are exponentially distributed, with decay constants λ for state A and μ for state B.

2 Effects of mechanical forces on rates and equilibria

So far we have been considering the motion of unperturbed biochemical systems. But these days many experiments involve pulling or pushing on a molecule, or otherwise interfering with its progress. Why do we do this, and how can we use it to learn about the nature of a biochemical step?

One of the most interesting aspects of biophysics is the strong coupling between mechanics and chemistry. Remember on the first day the video I showed of the developing C. elegans embryo, where the contraction of the actomyosin network determined the location of the head and the tail? Well throughout embryonic development much of the information that helps cells determine what to become and where to go is mechanical. Cells have a great many mechanisms for transducing mechanical signals into chemical ones. People keep learning about new ways in which cells sense the mechanical nature of their environment and react

accordingly. A trivial example of this is when you go to the gym and work out–the mechanical stresses on your muscles ultimately lead to biochemical cues to develop more muscle.

All of our senses can also be thought of as mechano-chemical transduction events. Touch is an obvious one, but hearing and temperature sensation fall in this category too. Vision is an opto-chemical process, but much of the same formalism applies. And taste and smell can be thought of as forms of sensing chemical potentials rather than mechanical potentials. For all of these reasons it is interesting to understand the general methods by which external forces may affect the balance in a chemical reaction.

When you were a small child, you probably learned about *Le Chatelier's* principle: that in a chemical reaction, if you create moles of gas, then an increase in pressure can favor the reactants. Creating moles of a gas is a way for a chemical reaction to do mechanical PdV work. So this is an example of a mechano-chemical coupling. Let's review where this principle came from, so we can see how to generalize it to other types of mechano-chemical couplings.

If you have a chemical reaction $aA + bB \rightleftharpoons cC + dD$ then the equilibrium constant is:

$$K_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b}. \tag{11}$$

If everything is in the gas phase, then you replace [A], [B], [C], [D] by the respective partial pressures. Now suppose you increase the total pressure, at constant temperature, by some amount x. Then the numerator is scaled by a factor of x^{c+d}, while the denominator is scaled by x^{a+b}. If $c+d > a+b$, then the only way to maintain the condition of chemical equilibrium is to shift the reaction toward the reactant side.

Ok, but where did the expression for the equilibrium constant come from? Why do the concentrations and stoichiometric coefficients appear in this odd way? This is a consequence of the Second Law of thermodynamics. The Second Law states that for any spontaneous process, the entropy of the Universe must increase. The entropy of the Universe comprises the entropy of our system, and the entropy of everything else (the "bath"). If we do a chemical process at constant volume and temperature, then the change of entropy of our system is just ΔS. The change in entropy of the bath is $\Delta S^{bath} = q_{rev}^{bath}/T$, where q_{rev}^{bath} is the heat transferred to the bath. But we have $q_{rev}^{bath} = -q_{rev}^{system} = -\Delta H_{rxn}$, where ΔH_{rxn} is the enthalpy change of the reaction (for a reaction at constant volume $\Delta H_{rxn} = \Delta U_{rxn}$, but if the reaction does PdV work, then we need to account for this work for the relation $q_{rev}^{system} = \Delta H_{rxn}$ to still hold).

So now we can express the seemingly too-deep-to-be-useful relation $\Delta S^{Universe} > 0$ in terms of system parameters only:

$$\Delta S_{rxn} - \Delta H_{rxn}/T > 0 \tag{12}$$

or equivalently

$$\Delta G_{rxn} < 0 \tag{13}$$

for a spontaneous process, where $\Delta G \equiv \Delta H - T\Delta S$. At thermal equilibrium, where $\Delta S^{Universe} = 0$, we have $\Delta G = 0$.

Now how does ΔG for a reaction change with concentrations or partial pressures? The ΔH term is all about the actual chemical reaction: the energies of the bonds broken or

formed. The effect of concentration is all in the entropy terms. Entropy is defined as $S \equiv k_B \ln \Omega$ where Ω is the number of microstates. For a gas, the number of microstates should be proportional to the accessible volume, so we have $S = S_0 + N k_B \ln V$, where N is the number of molecules (replace $N k_B$ by nR, where n is the number of moles, if you wish). The quantity S_0 contains all the entropy in internal molecular degrees of freedom (vibrations, rotations, etc.), as well as the entropy associated with the kinetic energy of the particles. For an ideal gas, $V \propto 1/P$, so for a reaction that changes the pressure of a gas, we have $\Delta S = -N k_B \ln P_f/P_i$. When we measure entropy relative to some standard state (1 Atm pressure, typically), it is customary to drop the P_i in the denominator, which is understood to be 1 Atm. Then we have $\Delta S = -N k_B \ln P$.

So for a reaction under non-standard conditions (concentrations and partial pressures deviating from 1 molar and 1 Atm, respectively), then we have:

$$\Delta G = \Delta G^0 + RT \ln Q \tag{14}$$

where Q is calculated by gathering all the logarithmic terms and putting the stoichiometric coefficients in the exponents. For our notional reaction above, this is:

$$Q = \frac{[C]^c [D]^d}{[A]^a [B]^b}. \tag{15}$$

The statement $\Delta G = 0$ then implies that:

$$\frac{[C]^c [D]^d}{[A]^a [B]^b} = e^{-\frac{\Delta G^0}{RT}}. \tag{16}$$

We call the term $e^{-\frac{\Delta G^0}{RT}}$ the equilibrium constant, K_{eq}. This is a really remarkable result, because we've found a relation between the concentrations and partial pressures for some arbitrary reaction conditions to the concentrations and partial pressures under a set of standard "reference" conditions.

The beauty of working with ΔG is that it seamlessly combines chemical, mechanical, and electrical contributions to the energy. For a reaction at constant pressure we have:

$$\Delta G = \Delta U^0 - T \Delta S^0 + RT \ln Q + P \Delta Vol + \sigma \Delta A + F \Delta x + \tau \Delta \theta + V \Delta q + ... \tag{17}$$

The terms $P \Delta Vol$, $\sigma \Delta A$, and $F \Delta x$ represent mechanical work in 3, 2, and 1 dimensions, respectively. The term $\tau \Delta \theta$ represents rotational work, and the term $V \Delta q$ represents the electrical work (moving a charge Δq through a potential drop V).

Explicitly, you can now see how all these different forces affect equilibrium constants:

$$K_{eq} = K_{eq}^{(0)} \exp \left[-(\sigma \Delta A + F \Delta x + \tau \Delta \theta + V \Delta q + ...)/k_B T \right]. \tag{18}$$

When you were a kid you learned about "Le Chatelier's" principle for pressure, but now you see it applies for all the other kinds of work that can couple to a chemical system.

2.1 Example: Nernst potential

Here's a simple example. Consider an ion translocating across a membrane. The sign convention for membrane voltage is to measure $\Delta V = V_{in} - V_{out}$. Since there is no chemical transformation, we have $\Delta G^0 = 0$. If we consider an ion flowing from outside the cell to inside the cell, the reaction quotient is $Q = \frac{[C_{in}]}{[C_{out}]}$. We then have

$$\Delta G = RT \ln Q + V \Delta q \tag{19}$$

where V is the membrane potential. Setting $\Delta G = 0$ and solving for V gives:

$$V = \frac{RT}{\Delta q} \ln \left(\frac{[C_{out}]}{[C_{in}]} \right). \tag{20}$$

This is the famous Nernst equation. The parameter RT/F has the numerical value of 26 mV at room temperature, where the Faraday constant, F, is the number of Coulombs in a mole. When logarithms are taken base 10, then the prefactor gets multiplied by $\ln 10 = 2.303$ and becomes 59 mV. The denominator, Δq is the charge on a mole of ions, which is ZF, where Z is the valence of the ion and F is Faraday's constant. If you wish, you can replace the prefactor by $k_B T/Ze$ where e is the charge of a proton.

2.2 Example: opening of voltage-gated ion channels

There are some ion channels which have a "voltage gate" as part of the molecular structure. This is a transmembrane helix with some charges associated with it, typically a few arginines. Many ion channels act as tetramers, in which case the charge is the sum of the charges of all the gates (assuming that the gates act in unison). In the presence of a transmembrane electric field, these charges can move, opening up an ion-conducting pore in the channel. If the channel opens at positive membrane voltages, then the probability of the channel being open depends on the membrane voltage, as follows. [MAKE DRAWING OF TWO STATES, LOWER STATE OF ENERGY ZERO (CLOSED STATE) AND OPEN STATE AT ENERGY V_0. AN INCREASE IN V LOWERS THE ENERGY OF THE OPEN STATE RELATIVE TO THE CLOSED STATE.]

$$\begin{aligned} P_{open} &= \frac{e^{q(V-V_{1/2})/k_B T}}{1 + e^{q(V-V_{1/2})/k_B T}} \\ &= \frac{1}{1 + e^{q(V_{1/2}-V)/k_B T}} \end{aligned} \tag{21}$$

Here $V_{1/2}$ is the voltage where the channel has a 50% probability of being open. If the conductance of a single open ion channel is g_1 and your patch pipette has n channels in it, then

$$g_n = n g_1 P_{open}. \tag{22}$$

[SKETCH g_n vs V for a NaV channel]. Example: voltage-gated Na and K channels have a probability of being open that increases e-fold per 4 mV, implying 6 charges move concertedly across the membrane ($k_B T/q = 6$ meV).

6

There will also be fluctuations in the number of open channels, because each channel will gate independently from the others. These fluctuations will have magnitude

$$\sigma_n^2 = n g_1^2 P_{open}(1 - P_{open}). \tag{23}$$

[SKETCH g_n and σ_n^2 vs V]

If you find the voltage where the channel is open half the time, then the product $P_{open}(1 - P_{open}) = 1/4$ and the fluctuations are maximized. The ratio of the fluctuations to the mean gives the unit conductance!

$$\frac{\sigma_n^2}{g_n} = \frac{1}{2} g_1. \tag{24}$$

The number of channels in the pipette is

$$n = \frac{(g_n)^2}{\sigma_n^2} \tag{25}$$

measured at the 50% activation point. People used fluctuation measurements like this to learn about the unit conductance of ion channels long before people invented techniques sensitive enough to record from a single channel. This estimate we just did is exactly the same as the math we did when we were considering FCS, though the physics is very different!

If you plot the fluctuations in channel conductance as a function of the mean conductance during a voltage sweep, you get an upside-down parabola.

2.3   Example: tension-sensitive ion channels, ligand-gated ion channels, temperature-sensitive ion channels

You can go through very similar calculations to look at the gating of the ion channels that sense membrane tension. Here you just replace $V\,dq$ by $\sigma\,dA$, where σ is the membrane tension and dA is the difference in membrane area between the closed and open states of the channel. Do you want dA to be big or small if you want a very sensitive mechanosensor?

You can also apply this argument to temperature sensation. [SEE CLAPHAM 2011 PNAS PAPER]. Vanilloid receptor channels change (e.g. the TrpV1 channel for whose discovery David Julius won the 2021 Nobel Prize in Physiology or Medicine) have an e-fold change in open probability over a 3 degree change in temperature. How do you get such a steep change? You need something which approaches a phase transition, as though a domain in the protein is melting. Let's see how that can happen. Combining:

$$\Delta G^0 = -RT \ln K_{eq} \tag{26}$$

with

$$\Delta G^0 = \Delta H^0 - T \Delta S^0 \tag{27}$$

gives

$$\ln K_{eq} = -\Delta H^0/RT + \Delta S^0/R. \tag{28}$$

From this you can see that the steepness with which K_{eq} varies with T depends on the enthalpy change, ΔH^0. For the TrpV1 channel, ΔH^0 is approximately 100 kCal/mole (equal

to 170 $k_B T$/molecule at room temperature), whereas the enthalpy change for most protein conformational transitions is typically 2-10 kCal/mole. This means that a relatively large portion of the receptor must be unfolding (approximately 50 amino acids).

The picture is actually more complicated than this because, for proteins, both ΔH^0 and ΔS^0 also depend on temperature. See the Clapham paper for an exploration of the consequences.

2.4 Example: 1-D freely-jointed chain

In a few weeks we'll see that the same math can be applied to stretching of a polymer, where you have segments that can either point left or right and you want to know the probability that a force causes a chain link to point to the left vs right.

2.5 Effect of forces on rate constants

The parameter ΔG is useful for determining equilibrium constants (the conditions under which a process stops), but also for estimating rates, since $\Delta G^\dagger$ shows up in the exponent in the rate constant. Even without a microscopic model of the rate constant, we can ask how do external forces affect the rates of forward and backward reactions.

The general notion behind calculating rates of processes is that we draw a potential energy surface connecting the two states. We assume that each state corresponds to a fairly deep minimum (deeper than $k_B T$). At the transition state we assume that the potential energy surface has a saddle point: it goes down if you head towards reactants or products, and it goes up if you head in any other direction.

For a molecule to cross between reactants and products, it has to get over the transition state. The number of molecules at the transition state is proportional to $e^{-\Delta G^\dagger / k_B T}$. Thus it is reasonable to assume that the forward rate of a reaction is:

$$k_{AB} = k_A^{(0)} e^{-\frac{G^\dagger - G_A}{k_B T}}, \tag{29}$$

where G is the free energy of the transition state, G_A is the free energy of the initial state A, and $k_A^{(0)}$ is some attempt frequency. Similarly the rate of the reverse reaction would be:

$$k_{BA} = k_B^{(0)} e^{-\frac{G^\dagger - G_B}{k_B T}}. \tag{30}$$

We can get the equilibrium constant from the rates as follows. The rate of change of population in A is:

$$\frac{d[A]}{dt} = -k_{AB}[A] + k_{BA}[B]. \tag{31}$$

At equilibrium, by definition $d[A]/dt = 0$, which implies that at equilibrium

$$
\begin{aligned}
K_{eq} &= \frac{k_{AB}}{k_{BA}} \\
&= \frac{k_A^0}{k_B^0} e^{-\frac{G_B - G_A}{k_B T}}
\end{aligned}
\tag{32}
$$

So in the equilibrium constant, all information about the height and shape of the barrier is lost.

But something interesting happens when we consider the kinetics. For concreteness, let's imagine that we're applying a force to the system, F, and states A and B are separated by a distance L. Then the whole potential energy surface gets tilted, just as we saw for the voltage-gated ion channel. The energy of the state B is increased relative to state A by an amount FL. The energy shift in the transition state depends on where it is along the reaction path. Let's call α the fractional position of the transition state (so $0 \leq \alpha \leq 1$). Then the energy shift of the transition state relative to state A is αLF and the energy shift relative to B is $-(1-\alpha)L$. So the rate constants become:

$$k_{AB} = k_{AB}^0 e^{-\frac{\alpha FL}{k_B T}}, \tag{33}$$

and

$$k_{BA} = k_{BA}^0 e^{+\frac{(1-\alpha)FL}{k_B T}}. \tag{34}$$

As before, the ratio of the rate constants gives the total equilibrium constant:

$$K_{eq} = K_{eq}^0 e^{-\frac{FL}{k_B T}}. \tag{35}$$

Recall from our general discussion of two-state kinematics, the relaxation rate constant for the whole system is the *sum* of the forward and backward rate constants, so

$$k_{Tot} = k_{AB}^0 e^{-\frac{\alpha FL}{k_B T}} + k_{BA}^0 e^{+\frac{(1-\alpha)FL}{k_B T}}. \tag{36}$$

This means that the rate goes up when F is either very positive or very negative. The system is slowest when it is balanced, i.e. when the forward and backward rate constants are equal and $[A] = [B]$.

3 Microscopic models of rate constants

Now we will talk about how to calculate forward and reverse rates of a reaction from an underlying model of the dynamics. The goal is to calculate the prefactors $k_A^{(0)}$ and $k_B^{(0)}$ which we've been waving our hands at up to this point.

Different models of rate processes are appropriate for different scenarios. The correct model depends on a number of factors, including:

- the number of degrees of freedom involved and how strongly they are coupled to their environment, i.e. whether their motion is overdamped or underdamped;

- whether quantum mechanics is important, i.e. whether electronic or vibrational splittings are comparable to the barrier height. For instance, if you consider electron-transfer reactions or photochemistry (as occurs in vision or photosynthesis), then quantum mechanics matters;

- how thermal energy compares to other energy scales. Different theories are needed depending on whether thermal energy is greater or less than the barrier height or the quantum level spacing.

- the shape of the potential energy landscape: whether it is symmetrical or asymmetrical between reactants and products or biased; and whether it is constant in time or time-varying.

Today we'll limit ourselves to overdamped motion, ignoring quantum mechanics, and assuming a large barrier height.

3.1 Eyring model

Before we tackle the Kramers theory, let's give a naïve picture, called the Eyring theory. In the Eyring theory, each molecule undergoes underdamped oscillations in its potential well, so the attempt frequency is just the natural oscillation frequency:

$$k^{(0)} = \frac{1}{2\pi}\sqrt{\frac{\alpha}{m}}, \tag{37}$$

where α is the spring constant. The idea is that in each cycle the total energy of the oscillation (and hence its amplitude) is randomized according to a Boltzmann distribution, so each cycle you get a new attempt to hop over the barrier. How this happens in an underdamped system (which can only interact weakly with its environment) is not specified in the model.

Let's poke at the Eyring model to see where it fails.

- If you're totally underdamped and you have enough energy to hop over the barrier from A to B, then you will keep going up the other side of B, swing back, and return back over the barrier back into A.

- This model also doesn't take into account any of the effects of damping or diffusion that are so critical in determining many rate processes. For instance, the Eyring model predicts that viscosity should play no role in determining the rate; but we know that in many cases viscosity is important.

- [DRAW TWO POTENTIAL ENERGY CURVES WITH THE SAME MINIMA AND BARRIER HEIGHTS, BUT ONE VERY BROAD BARRIER AND ONE NARROW BARRIER] Which of these will have a faster rate? Clearly the one with the broad barrier will have a slower rate. But the Eyring model doesn't pay any attention to the *shape* of the barrier

To see that the Eyring model does not apply to many biomolecular reactions, consider that the rates of most biomolecular processes do not change if you substitute in different isotopes, but change radically if you change the viscosity of the solution. The Eyring model gives an *upper limit* on the rate of any reaction.

3.2 Kramers Theory

To make a more accurate picture we have to delve into the details of the process. We'll start by considering a one-dimensional double-well. The analysis below closely follows that in Landauer and Swanson, "Frequency Factors in the Thermally Activated Process," *Phys.*

Rev. 121, 1668 (1961), posted on the website. There are a variety of different models for the potential energy surface which give slightly different expressions, though the qualitative behavior will be similar. Several of these alternative models (e.g. an 'N'-shaped well and a 'U'-shaped well are explored in Howard Ch. 5.

We will start out with all the particles in well A, and as they hop over the barrier into well B we pull them out and put them back in A. If the population of well A is P_A, and the number of particles crossing the barrier per unit time is j, then the forward rate constant for the reaction is:

$$k_{AB} = j/P_A. \tag{38}$$

We assume that the relaxation within well A is fast compared to the rate of escape of particles. So everywhere except possibly right near the transition state, the population distribution in A looks as though it is in equilibrium.

Our first trick is to write the total density as a small deviation on the equilibrium density. So for the near-equilibrium case we have:

$$\rho(x) = \beta(x)e^{-U/k_BT}. \tag{39}$$

This expression does not entail any loss of generality because $\beta(x)$ could be anything.

Now we will make some approximations for $\beta(x)$ that will allow us to solve the problem. Near the bottom of well A, β is pretty much constant. In well B $\beta(x) \approx 0$ (recall that we don't let particles accumulate in B. And it's a safe bet to assume that $\beta(x)$ goes monotonically from $\beta(A)$ to 0 as we cross the barrier. [INSERT DRAWING OF APPROXIMATE FORM OF $\beta(x)$.]

First let's get a relation between $\beta(A)$ and P_A. If we Taylor expand the potential near the minimum in well A as

$$U(x) = U_A + \frac{1}{2}\alpha_A x^2, \tag{40}$$

then the total population in well A is roughly given by:

$$\begin{aligned}
P_A &\approx \beta(A)e^{-U_A/k_BT} \int_{-\infty}^{\infty} e^{-\alpha_A x'^2/2k_BT} dx' \\
&= \beta(A)e^{-U_A/k_BT} \sqrt{\frac{2\pi k_BT}{\alpha_A}}.
\end{aligned} \tag{41}$$

Let's put this result aside for later.

Now let's figure out j, by looking at the flux across the midline. We start with the Smoluchowski formula in 1-D:

$$j = -\frac{\rho}{\gamma}\frac{dU}{dx} - D\frac{d\rho}{dx}. \tag{42}$$

Here U is the potential, D is the diffusion coefficient, and γ is the drag coefficient. This equation should look pretty familiar by now. The equilibrium density distribution ignoring leakage over the barrier is:

$$\rho_i = ce^{-U/k_BT}. \tag{43}$$

Plugging the *ansatz* $\rho(x) = \beta(x)e^{-U/k_BT}$ into the Smoluchowski equation yields:

$$j = -D\left(\frac{d\beta}{dx}\right)e^{-U/k_BT}. \tag{44}$$

11

Now we rearrange this equation, solving for $d\beta/dx$, and then we integrate dx. This gives:

$$\beta(x) = \beta_0 - \int \frac{j}{D} e^{U(x')/k_B T} dx'. \tag{45}$$

Where is this integral large? Well, the dominant contribution comes from where $U(x')$ is largest. Since $U(x')$ shows up in the exponential, let's expand the potential about the maximum:

$$U(x') = U_0 - \frac{1}{2}\alpha_0 x'^2 + \dots. \tag{46}$$

We want an expression for $\beta(A)$, and we can take advantage of the fact that we know $\beta(B) \approx 0$. So we have:

$$\beta(A) = -\int_B^A \frac{j}{D} e^{\left(U_0 - \frac{1}{2}\alpha_0 x'^2\right)/k_B T} dx'. \tag{47}$$

But now the right hand side is just a Gaussian integral, which we know and love. Evaluating the integral gives:

$$\beta(A) = \frac{j}{D} e^{U_0/k_B T} \sqrt{\frac{2\pi k_B T}{\alpha_0}}. \tag{48}$$

Rearranging this result yields

$$j = D\beta(A)\sqrt{\frac{\alpha_0}{2\pi k_B T}} e^{-U_0/k_B T}. \tag{49}$$

To get the rate we look at the ratio of the flux to the population:

$$
\begin{aligned}
k_{AB} &= \frac{j}{P_A} \\
&= D e^{-(U_0 - U_A)/k_B T} \frac{\sqrt{\alpha_0 \alpha_A}}{2\pi k_B T}.
\end{aligned}
\tag{50}
$$

As expected, the result is independent of our trial function $\beta(x)$.

We can rewrite this formula in a suggestive form which emphasizes the difference from the Eyring theory. Recall from the Stokes-Einstein formula $D/k_B T = 1/\gamma$. So we have:

$$k_{AB} = \frac{\sqrt{\alpha_A \alpha_0}}{2\pi\gamma} e^{-(U_0 - U_A)/k_B T}. \tag{51}$$

Note that for an oscillator of mass m subject to a restoring force α, the frequency of oscillation is

$$\omega = \sqrt{\frac{\alpha}{m}}. \tag{52}$$

This means that the forward rate is:

$$k_{AB} = \omega_0 \tau_r \frac{\omega_A}{2\pi} e^{-(U_0 - U_A)/k_B T}, \tag{53}$$

where $\tau_r = m/\gamma$ is the momentum relaxation time of the particle. This result differs from the Eyring result by a factor of $\omega_0 \tau_r$. We are considering motion that is highly overdamped,

so $\omega_0 \tau_r \ll 1$. Thus, not surprisingly, the Kramers rate is much slower than the Eyring rate. What is, perhaps, surprising is that the main difference has to do not with the shape of the potential at the minimum, but with the detailed shape of the potential barrier itself.

The Kramers model is pretty good, but it does slightly overestimate the rate. To see why, consider a particle that has just crossed over the maximum of the reaction coordinate. Very close to the maximum, the particle sees a locally flat potential. This means that the particle has some finite probability of crossing back over the maximum into the well from which it came. In other words, a particle perched exactly at the top of the well has a 50% probability of going down either side. A particle infinitesimally to the right of the maximum, which we just counted as a reaction, still has a roughly 50% probability of going down either side.

3.3 Multidimensional Kramers

We've left one very important fact out of our discussion. We considered a purely 1-D reaction coordinate, and lumped all the other degrees of freedom of the system into a "bath" whose only role is to provide viscous drag. In fact, life is not so simple.

Here's the problem. Complex molecules have a great many internal degrees of freedom. For instance, in a protein there are many bonds about which you can rotate. To undergo a transition from one state to another, you might have to adopt a particular conformation along many of your internal degrees of freedom. That is, you might have to squeeze through a conformational bottleneck. Doing so costs entropy. So far we've discussed the problem in purely energetic terms, as is appropriate for the 1-D case. In reality we should think about the *free energy*. How to factor that in?

[DRAW A 2-DIMENSIONAL POTENTIAL ENERGY SURFACE WITH TWO BASINS SEPARATED BY A SADDLE POINT]

We'll now repeat the derivation at an accelerated pace, considering motion in an arbitrary number of dimensions. The details are given in the paper by Landauer and Swanson, posted on the website.

The population of basin A is given by

$$P_A = \beta(A) \int \ldots \int dx_1 \ldots dx_n e^{-\frac{U(x_1,\ldots,x_n)}{k_B T}}. \tag{54}$$

Note that the integral on the r.h.s. is just the formula for the partition function! We'll return to that fact in a minute. Again we make a harmonic approximation for the potential about its minimum, so we get n Gaussian integrals. These are all the same, so we have

$$P_A = \beta(A) e^{-\frac{U_A}{k_B T}} \prod_{i=1}^{n} \sqrt{\frac{2\pi k_B T}{\alpha_i}}. \tag{55}$$

Now we evaluate the total flux across the saddle point (integrating over all the perpendicular degrees of freedom). There are only $n-1$ perpendicular degrees of freedom because one degree of freedom is the reaction coordinate which we have to handle specially. Again, we make a harmonic approximation for each of the degrees of freedom, so we get $n-1$ Gaussian integrals. Evaluating the integral gives:

$$j = D\beta(A) \sqrt{\frac{\alpha_0}{2\pi k_B T}} e^{-U_0/k_B T} \prod_{i=1}^{n-1} \sqrt{\frac{2\pi k_B T}{\kappa_i}}, \tag{56}$$

13

where the κ_i are the spring constants along all of the orthogonal degrees of freedom in the transition state. The rate constant becomes:

$$k_{AB} = \frac{1}{2\pi\gamma} \sqrt{\frac{\alpha_0 \prod_{i=1}^n \alpha_i}{\prod_{i=1}^{n-1} \kappa_i}} e^{-\frac{U_0 - U_A}{k_B T}}. \tag{57}$$

This is an explicit formula for the rate in terms of the spring constants along all the axes in the ground state and the transition state.

Now I'll show you two nice examples of application of Kramers' Theory:

[SHOW EXAMPLE FROM MCCANN ET AL, AND FROM MY PRL PAPER ON NANOPARTICLES IN ARBITRARY FORCE FIELDS]

3.4 Kramers and entropy

Let's discuss some subtleties associated with the Kramers picture.

[DRAW TWO STATES WITH ENERGIES U_A AND U_B]

We define the equilibrium constant for the reaction to be

$$K_{eq} = \frac{[B]}{[A]}, \tag{58}$$

and Boltzmann would tell us that this ratio is $K_{eq} = e^{-(U_B - U_A)/k_B T}$. Now let's connect these two states by a potential energy surface.

[DRAW A SURFACE IN WHICH STATE B IS IN A NARROW WELL AND STATE A IS IN A BROAD WELL]

But Kramers says $k_{AB} = \frac{\sqrt{\alpha_A \alpha_0}}{2\pi\gamma} e^{-(U_0 - U_A)/k_B T}$ and $k_{BA} = \frac{\sqrt{\alpha_B \alpha_0}}{2\pi\gamma} e^{-(U_0 - U_B)/k_B T}$, where α_i represents the absolute value of the curvature at position i. If we plug in the Kramers rates to the expression $K_{eq} = k_{AB}/k_{BA}$, we get:

$$K_{eq} = \sqrt{\frac{\alpha_A}{\alpha_B}} e^{-(U_B - U_A)/k_B T}. \tag{59}$$

This is different from the Boltzmann prediction. So what Gibbs?

The answer is that our two macrostates that we are calling A and B each correspond to an ensemble of microstates. Within each well, there is a large number of positions that the particle can occupy. Each of those states is occupied with a probability given by a Boltzmann factor, but the total probability to be in each well has to be summed over all of those microstates. If one well has more states available to it, the larger number of states can offset an energetic term that disfavors that well. Our next mission will be to figure out how to correct the Boltzmann distribution when there are multiple microstates (e.g. positions in the well) associated with each macrostate (e.g. well identity).

Let's start with the simple case, where we imagine that all the states within each well are degenerate. Clearly this is not the case for the parabolic double-well, but it will help us to build intuition. This would be the case for square-bottom wells. When there are degenerate states, the Boltzmann distribution is:

$$\frac{[B]}{[A]} = \frac{n_B}{n_A} e^{-(U_B - U_A)/k_B T}, \tag{60}$$

where n_B and n_A are the number of degenerate microstates within each well. Clearly it doesn't matter how finely we divide up space to count the microstates, because our discretization size will cancel in the ratio. For instance, if we had square-bottom wells of length l_B and l_A, respectively, then $n_B/n_A = l_B/l_A$.

For degenerate states, we define the entropy

$$S = k_B \ln(\Omega) \tag{61}$$

where Ω is the number of degenerate states. We can write

$$\frac{n_B}{n_A} = \exp[\ln(n_B) - \ln(n_A)] \tag{62}$$

$$= \exp[\frac{k_B T (\ln(n_B) - \ln(n_A))}{k_B T}] \tag{63}$$

$$= \exp[\frac{T \Delta S}{k_B T}]. \tag{64}$$

If we make this substitution into the expression for the equilibrium constant, we get

$$\frac{[B]}{[A]} = \exp[-\frac{U_B - U_A - T \Delta S}{k_B T}] \tag{65}$$

$$= \exp[-\frac{\Delta G}{k_B T}], \tag{66}$$

where we have introduced $\Delta G = \Delta U - T \Delta S$. So you see how the free energy emerges naturally just from our need to account for all the different microstates that correspond to each macrostate.

Now what do we do if the microstates are not degenerate? In this case, the entropy is defined as

$$S = -k_B \int_{-\infty}^{\infty} p(x) \ln(p(x)) dx. \tag{67}$$

Check that when all states are degenerate this reduces to the formula we gave above!

Let's define a helper function, called the partition function, defined as:

$$Z = \int_{-\infty}^{\infty} e^{-U(x)/k_B T} dx. \tag{68}$$

When each macrostate corresponds to multiple microstates, we no longer define the energy of the macrostate by the bottom of the well, but instead we define it by the expectation value of the energy in the well:

$$\langle U \rangle = \int_{-\infty}^{\infty} p(x) U(x) dx, \tag{69}$$

where $p(x)$ is governed by the Boltzmann distribution:

$$p(x) = \frac{1}{Z} \exp[-U(x)/k_B T]. \tag{70}$$

If we define the free energy as $G = \langle U \rangle - TS$, then we have

$$G = \int_{-\infty}^{\infty} p(x)U(x)dx + k_B T \int_{-\infty}^{\infty} p(x)\ln p(x)dx \tag{71}$$

$$= \int_{-\infty}^{\infty} p(x)[U(x) + k_B T \ln p(x)]dx \tag{72}$$

$$= \frac{1}{Z}\int_{-\infty}^{\infty} \exp[-U(x)/k_B T][U(x) + k_B T \ln\left(\frac{1}{Z}\exp[-U(x)/k_B T]\right)]dx \tag{73}$$

$$= \frac{1}{Z}\int_{-\infty}^{\infty} \exp[-U(x)/k_B T][U(x) - k_B T \ln Z - U(x)]dx \tag{74}$$

$$= -k_B T \ln Z. \tag{75}$$

Now if we have two states, A and B, then the ratio of the populations in the two states is just

$$\frac{[B]}{[A]} = \frac{\int_B \exp[-U(x)/k_B T]dx}{\int_A \exp[-U(x)/k_B T]dx} \tag{76}$$

$$= \frac{Z_B}{Z_A} \tag{77}$$

$$= \exp[-(G_B - G_A)/k_B T]. \tag{78}$$

Returning now to the Kramers formula, the quantities $\sqrt{k_B T/\alpha_A}$ and $\sqrt{k_B T/\alpha_B}$ are measures of the width of each of the wells, i.e. the horizontal distance you have to go to attain an energy of order $k_B T$. It is conventional to write the prefactors in Eq. 59 in a slightly different form:

$$K_{eq} = \exp\left[-\frac{(U_B - k_B T \ln \alpha_B^{-1/2}) - (U_A - k_B T \ln \alpha_A^{-1/2})}{k_B T}\right]. \tag{79}$$

The difference in entropy between the two wells is

$$\Delta S = k_B \ln \sqrt{\frac{\alpha_B}{\alpha_A}}, \tag{80}$$

and the difference in free energy is

$$\Delta G = (U_B - U_A) + \frac{1}{2}\ln\frac{\alpha_B}{\alpha_A}. \tag{81}$$

A more rigorous calculation of the entropy of a harmonic oscillator is in Howard Appendix 5.1 and gives the same result. Note that we were able to set $\Delta\langle U \rangle = U_B - U_A$ because the thermal energy just adds $k_B T/2$ to both states, which cancels when we take the difference! Equipartition again!

Returning to the original Kramers formula for a harmonic well, we can ask how to estimate the rate for some oddly shaped potential energy surface. The Kramers formula generalizes to the form

$$k_{AB} \approx \frac{D}{l_0 l_A}e^{-\frac{U_0 - U_A}{k_B T}} \tag{82}$$

where l_A and l_0 are the width of the initial state and the transition state, respectively. These widths correspond to the distance you have to go to attain an energy of approximately $k_B T$.

[DRAW TWO POTENTIAL ENERGY SURFACES, ONE WITH A SHARP MINIMUM AND MAXIMUM, AND ONE WITH BROAD MINIMA AND MAXIMA]

Which of these two surfaces would lead to a faster rate from A to B?

Models of this sort are the basis for our understanding of most molecular motors and motor proteins. We will return to many examples of this later in the course.

3.5   Comparison between Kramers and electronic transitions

In the Kramers model, we can think of a protein as gradually ambling up to the transition state, and then once it crosses the transition state it cruises downhill to the final state. Because of this, reaction rates are strongly dependent on viscosity and on mechanical forces that might assist or counteract this motion towards the transition state (more on mechanical forces later). This view contrasts sharply with the view of reactions you might have learned in other chemistry classes. For instance, in photochemical reactions we have something called the "Franck-Condon Principle" which tells us that the electrons move so much faster than the atoms that the atoms are essentially stationary during the fundamental reaction step, and that the atoms relax after the process is over.

An example of a scenario where the Kramers theory doesn't apply is in a molecule called Bacteriorhodopsin, that we're studying in my lab. Here the reaction is initiated by absorption of a photon, which occurs in a few hundred femtoseconds. This reaction causes a molecule of retinal to isomerize, which then drives a cascade of conformational changes that can take hundreds of milliseconds to complete. Here the initiation of the reaction occurs in an essentially rigid atomic framework, and all of the mechanical motion is associated with the aftermath. If we were to try to drive the photocycle *backwards*, then it would look like a Kramers process.

[GIVE TUTORIAL ON MICROBIAL RHODOPSINS]

Typically Kramers theory is good for reactions where a large conformational change *precedes* the reaction. In cases in which there is an electron-transfer or a photochemical component, then an Eyring picture is more appropriate.