Enyzme kinetics: from Michaelis-Menten to Thermodynamics.[1]

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[1] Readings: Chapter 2 Sengupta book; Any introduction to enzyme kinetics. Though the correspondence between thermodynamics and kinetics emphasized here is not discussed much in anyplace.

> Instead of traditional lecture, here we will work through a series of problems in the form of a worksheet to try to understand chemical kinetics – and in particular the Michaelis-Menten equation which describes enzymes from *both the kinetic and thermodynamic points of view*. This will be essential since as we discussed at the beginning of the last lecture a central feature of living systems is that they use kinetics to control when and where free-energy transduction occurs.

Living systems are really defined by the enzymes they possess almost as much as their DNA. Enzymes are the main actors in metabolism - the chemicial process through which living systems extract energy from their environment. This ability to control kinetics in order to transduce Free Energy is what allows living cells to reliably process information, extract energy, and as we will see in the next lecture reduce errors beyond what can be done in equilibrium. Here, we will discuss the simplest model of enzyme kinetics: the Michaelis-Menten kinetics. It will also introduce us to the basics of Gibbs free energy and thermodynamics. Using what we learn here, next lecture we will explore the idea of Kinetic Proofreading.

INSTRUCTIONS: Instead of a traditional lecture, this is structured as a worksheet to be done in small groups. Please form groups of 3 people. For most problems, there are two answer slots (individual and group). For the individual answer slot, please first make an honest attempt at solving the problem by yourself. After you are done/or stuck, compare/discuss your answer with the group and put answer in group slot. If you are satisfied as a group move on to the next question.

NEXT CLASS: We will do a Journal Club style discussion of the original Hopfield Kinetic Proof Reading paper [2]

[2] Hopfield. Kinetic Proofreading: A New Mechanism for Reducing Errors in Biosynthetic Processes Requiring High Specificity. PNAS 71 4135 (1974). Available at `http://www.pnas.org/content/pnas/71/10/4135.full.pdf`

What is an enzyme?

An enzyme is a protein molecule that affects the kinetics of the reaction but not the free energy. Look at the following two figures (Fig. 1) from Chapter 10 of Phil Nelson's book

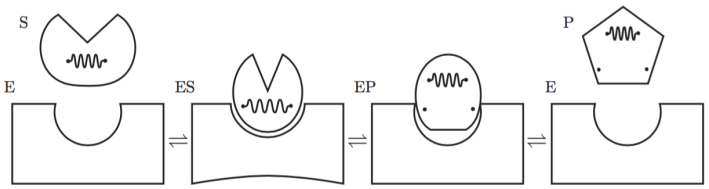

Figure 10.16: (Schematic.) Conceptual model of enzyme activity. (a) The enzyme E has a binding site with a shape and distribution of charges, hydrophobicity, and H-bonding sites approximately matching those presented by the substrate S. (b) To match perfectly, however, S (or both E and S) must deform. (Other, more dramatic conformational changes in the enzyme are possible too.) One bond in the substrate (shown as a spring in S) stretches close to its breaking point. (c) From the ES state, then, a thermal fluctuation can readily break the stretched bond, giving rise to the EP complex. A new bond can now form (upper spring), stabilizing the product P. (d) The P state is not a perfect fit to the binding site either, so it readily unbinds, returning E to its original state.

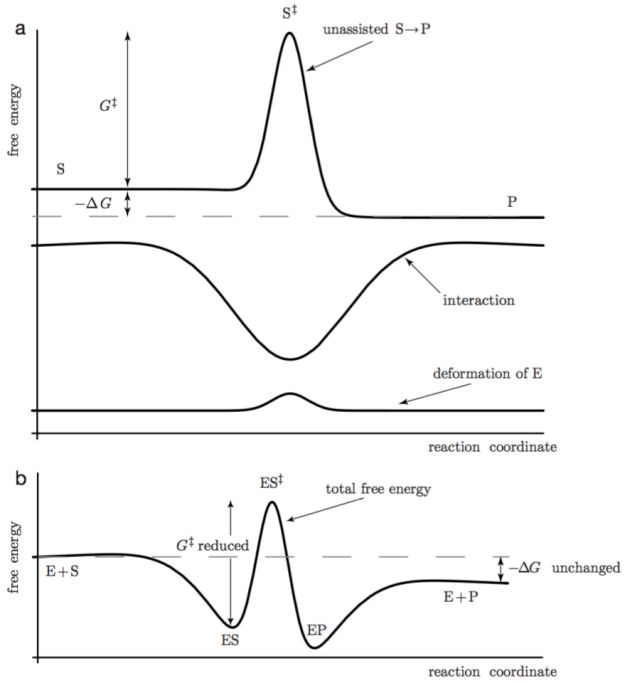

Figure 10.17: (Sketch graphs.) (a) Imagined free energy landscapes corresponding to the story line in Figure 10.16. *Top curve:* The substrate S can spontaneously convert to P only by surmounting a large activation barrier $G^{\ddagger}$, the free energy of the transition state $S^{\ddagger}$ relative to S. *Middle curve:* the interaction free energy between substrate and product includes a large binding free energy (dip), as well as the entropic cost of aligning the substrate properly relative to the enzyme (slight bumps on either side of the dip). *Lower curve:* The binding free energy may be partly offset by a deformation of the enzyme upon binding, but still the net effect of the enzyme is to reduce the barrier $G^{\ddagger}$. All three curves have been shifted by arbitrary constants in order to show them on a single set of axes. (b) Imagined net free energy landscape obtained by summing the three curves in (a). The enzyme has reduced $G^{\ddagger}$, but it cannot change ΔG.

Figure 1: Figures from Chapter 10 on Nelson's Biological Physics explaining how enzymes functions

- Discuss Figure 1 and explain in your own words the various features in the Free Energy landscape.
  Group Answer:

- What is the probability of reaching the active state $S^{\ddagger}$ and $ES^{\ddagger}$ if the enzyme is operating at temperature T?
  Group Answer:

A simple thermodynamics model of a two-state system

In this section, we will study a simple statistical model of a two-state system. This is a system that can be in one of two states A and B. In the context of enzymes, this can serve as an elementary statistical mechanics of an enzyme that can be bound or unbound with a substrate. Let us denote the free energies of the the two states by G_a and G_b and the difference between these free energies as $\Delta G = G_b - G_a$.

- Using the Boltzmann formula, show that the probability that the system is in state a is just give by

$$P_a = \frac{1}{1 + e^{-\beta \Delta G}} \tag{1}$$

  Individual Answer:

  Group Answer:

Now consider an $n + 1$ state system where the system can be in one on $+1$ state $j = 0, 1, \ldots n$. Consider the case where the free energy of the j-th state is $G_j = jG_0$ (in other words the free energy of each state increases by a constant factor G_0.).

- Write down the probability of the system to be in state j in terms of β and G_0. Write down approximate expressions valid when $G_0 \gg 1$. How can we understand this expression intuitively?
  Individual Answer:

Group Answer:

Reversible Reaction Kinetics

Now consider the reversible reaction where A and B combine to make a complex AB but AB can also dissociate back into A and B.

$$\mathrm{A} + \mathrm{B} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{AB}, \tag{2}$$

with k_+ and k_- the rate constants for the forward and backward reactions, respectively. From the law of mass action, this is described by the kinetic equation

$$\frac{d[\mathrm{C}]}{dt} = k_+[\mathrm{A}][\mathrm{B}] - k_-[\mathrm{AB}] \tag{3}$$

Denote the initial concentration of [A] by $[A_0]$ and define the equilibrium constant $K_{eq} = k_-/k_+$.

- Show that:

$$\frac{[\mathrm{AB}]}{A_0} = \frac{[\mathrm{B}]}{[\mathrm{B}] + K_{eq}} \quad \text{or} \quad [\mathrm{AB}] = \frac{A_0[\mathrm{B}]}{[\mathrm{B}] + K_{eq}}. \tag{4}$$

  and sketch a plot of the probability that A is found in the complex AB as a function of the concentration $[B]$.
  Individual Answer:

  Group Answer:

- Notice that we can view each molecule of A as being a two-state system with the two states corresponding to free and abound A

and AB. Combine this with the problem in the last section to show that the free energy difference between $[AB]$ and $[A]$ is

$$\Delta G = \log \frac{[B]}{K_{eq}}. \tag{5}$$

Individual Answer:

Group Answer:

- Discuss the logarithmic dependence on the concentration of $[B]$ in light of the chemical potential of the ideal gas. How do the kinetic and thermodynamic pictures relate?
  Group Answer:

Michaelis-Menten kinetics

A special class of catalytic reactions of great importance in biology are enzymes acting on their substrates.

$$\mathrm{E} + \mathrm{S} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{ES} \overset{k_{\mathrm{cat}}}{\rightarrow} \mathrm{E} + \mathrm{P}. \tag{6}$$

The enzyme E captures the substrate S and makes a complex ES reversibly. Occasionally the complex gives rise to the product P, a modification of the substrate, and releases the enzyme for further action. We will derive a kinetic equation for this.

- This derivation makes the following crucial assumptions: (i) Product formation is irreversible (Strictly speaking this thermodynamically impossible) (ii) the concentration of product can be neglected compared to that of the substrate (i.e $[\mathrm{P}] << [\mathrm{S}]$) and (iii) the

amount of the intermediate ES is approximately at steady-state (the quasi-equilibrium condition). Discuss why these assumptions are reasonable.
Group Answer:

- If we define the total amount of enzyme at $[E_0]$, show that under these assumptions

$$\frac{[\mathrm{ES}]}{[E_0]} = \frac{1}{1 + \frac{k_- + k_{\mathrm{cat}}}{k_+[\mathrm{S}]}} = \frac{1}{1 + \frac{K_M}{S}} \tag{7}$$

Individual Answer:

Group Answer:

- What is the $\Delta G^{\ddagger}$ for this enzyme?
Individual Answer:

Group Answer:

Under the assumptions outlined above, we can think of the rate of product formation as

$$\frac{d[\mathrm{P}]}{dt} = \frac{k_{\mathrm{cat}}[\mathrm{E}]_{\mathrm{total}}[\mathrm{S}]}{[\mathrm{S}] + K_M}, \tag{8}$$

with $K_M = (k_- + k_{\text{cat}})/k$ the Michaelis-Menten constant. This is the famous Michaelis-Mentin equation.

Understanding the equation

We now discuss how this equation is used to measure enzymatic properties [3]. Much ink has been spent on the conditions under which this standard derivation holds, or on how to derive it better. Instead of delving into these details, we will think of this formula as a phenomenological description, capturing the intuitive expectations in the limits of small or of large amount of substrates. For a small amount of substrates, the bottleneck is the enzyme and the substrate finding each other. Hence the rate is proportional to the product of $[\text{E}]_{\text{total}}$ and $[\text{S}]$. In the other limit, there is so much substrate that almost all the enzymes are in the complex ES. Hence the rate is just $k_{\text{cat}}[\text{E}]_{\text{total}}$. The Michelis constant, K_M can be thought of, operationally, as the substrate concentration where the rate is half of the maximum value.

[3] This section is literally copied directly from Chapter 2 of Sengupta.

For a fixed amount of enzyme, $[\text{E}]_{\text{total}}$, the velocity, $v([\text{S}]) = d[\text{P}]/dt$, satisfies

$$\frac{1}{v([\text{S}])} = \frac{1}{v_{\text{max}}} + \frac{K_M}{v_{\text{max}}}\frac{1}{[\text{S}]}. \tag{9}$$

with $v_{\text{max}} = k_{\text{cat}}[\text{E}]_{\text{total}}$. Thus, the plotting inverse of velocity against inverse of substrate concentration is expected to produce a straight line. This plot, known as Lineweaver-Burk plot, is often used in enzyme kinetics. The slope and the intercept of the straight line fit to the data can be used to extract parameters like v_{max} and K_M.

The tables 1 and 2 show that the values for the parameters K_M and k_{cat} vary widely from molecule to molecule. Note that when the concentration of the substrate is low compared to K_M, the product formation rate is reworked paragraph below to separate efficiency from collisions more explicitly

$$\frac{k_{\text{cat}}}{K_M}[\text{E}]_{\text{total}}[\text{S}] = k_+[\text{E}]_{\text{total}}[\text{S}] \times \left(\frac{k_{\text{cat}}}{k_{\text{cat}} + k_-}\right). \tag{10}$$

- Why don't I need a table for k_+? Can you derive a formula for the on-rate as a function of substrate concentration from first principles? (Hint: use an analogy with electrostatics to solve the Diffusion equation)
  Group Answer:

This equation has a simple interpretation as the rate of collisions between enzyme and substrate, $k_+[\mathrm{E}]_{\mathrm{total}}[\mathrm{S}]$, times the probability that a collision gives rise to a product. Thus, we see that the rate of the reaction is bounded above by the rate at which the substrate collides with the reactive pocket of the enzyme. The chance of collision, on the other hand, is limited by the diffusion rate (we will return to this in a few weeks). The highest observed values (k_{cat}/K_M) turns out to be in the range $10^8 - 10^9 \mathrm{s}^{-1}\mathrm{M}^{-1}$. These numbers are the same order of magnitude expected from diffusion limited kinetics. These enzymes are believed to have achieved kinetic perfection, in the sense that every encounter with a substrate is highly likely to lead to the product. We will return to diffusion-limited rates in the next chapter (also see exercise below).

Enzyme	Substrate	$K_M(\mu\mathrm{M})$
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-N-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400S
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

Table 1: K_M values for some enzymes and substrates (based on **Biochemistry by Berg, Tymoczko and Stryer** ****)

Enzyme	$k_{\mathrm{cat}}(\mathrm{s}^{-1})$
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Table 2: k_{cat} values for some enzymes (based on **Biochemistry by Berg, Tymoczko and Stryer** ****)

Homework

This exercise introduces the Monod-Wyman-Changeux (MWC) model of allosteric interactions. The MWC model was first proposed to explain the sigmoidal response of hemoglobin to oxygen and has since become one of the canonical models allostery in biochemistry and biophysics [4]. The main idea of the model is that an enzyme or protein can exist in multiple, interconvertible conformations with the probability that the enzyme is in a given confirmation determined by thermal equilibrium. The presence of ligands biases the enzyme towards one of these confirmations by shifting the relative free energies of the underlying protein confirmations. In this exercise, we will derive the main results of the MWC model from simple thermodynamic and statistical mechanical arguments.

[4] We will return to an interesting application of the MWC model to describe quantitative data on bacterial chemotaxis.

- Consider a protein with a single conformational state that can bind a ligand [L] from the environment. In thermal equilibrium, show that the free energy difference, ΔG, between the bound and unbound state is given by

$$\Delta G = -\log \frac{[\mathrm{L}]}{K_D}, \tag{11}$$

  with $K_D = k_-/k_+$, k_+ the ligand binding rate, and k_- is the ligand unbinding rate. K_D is called the binding affinity of the protein

- Now consider a protein that can exist in two states, an active state A, and an inactive state I. In the absence of ligand, the free energy of the active state is ϵ_A and the inactive state is ϵ_I. Furthermore, denote the binding affinity of the protein in the active state by K_D^A and the binding affinity in the inactive state K_D^I. Calculate the probability that the protein is in the active state. Show that in the limit where ligand binding strongly favors the active state $K_D^I \gg [\mathrm{L}] \gg K_D^A$, this expression reduces to a form similar to the Michaelis-Menten equation. Briefly discuss the meaning of K_M and the relationship to the Michaelis Metin equation.

- Generalize the calculation above to the case when the protein is composed on 2 independent, identical subunits each of which can bind ligand. For this case, there are 8 total possible states: the protein can be active or inactive with 0,1, or 2 ligands bound to the protein. Show that when $K_D^I \gg [\mathrm{L}] \gg K_D^A$, your expression reduces to a form similar to the Hill equation with a Hill coefficient of 2 [5].

[5] See Sengupta Chapter 2/Wikipedia if you do not know what the Hill equation is.