Lecture 2
These notes provide a more detailed description of the quantitative representation of enzyme and receptor action.

Mathematical Representation of Enzymatic Activity

Enzymes are a class of proteins that catalyze chemical reactions, enabling the formation and breaking of covalent bonds. Enzymes are present throughout the body: in the cytoplasm, embedded within the cell membrane, in specific cellular organelles, and in the bloodstream.

All enzymes have two major characteristics: 1) they specifically bind the ligands, called substrates, that they are able to covalently modify; and 2) they catalyze the formation and breaking of covalent bonds by stabilizing intermediate states the substrates need to go through when they are converted to a product. The minimal set of reactions describing enzyme-catalyzed conversion of a substrate to a product is commonly given by:

$$[E]+[S] \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} [ES] \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} [E]+[P]$$

The ES complex is made up of three distinct states, ES the enzyme substrate complex; ES*, the enzyme with the activated substrate in the transition state; and EP the enzyme product complex. This gives rise to a more detailed reaction sequence.

$$[E]+[S] \rightleftarrows [ES] \rightleftarrows [ES^*] \rightleftarrows [EP] \rightleftarrows [E]+[P]$$

Substrates share a characteristic with receptor agonist. Both induce conformational changes in the protein they bind that allow the protein to express its functional capability. In the case of receptors it is communication with its partner in the signaling pathway. In the case of the enzyme it is conversion of substrate to product. The important and obvious difference from the perspective of the ligand is that agonists are released from the receptor without chemical modification while enzymes convert substrates to products that are different chemical entities. Nevertheless the specificity with which enzymes recognize their substrates has been exploited to design molecules that bind the enzyme specifically but are not converted to products, analogous to competitive inhibitors of receptors. Such enzyme inhibitors are the major class of drugs used to treat a broad range of diseases from hypertension (high blood pressure) to HIV infection. Understanding the therapeutic function of these enzyme inhibitors also requires computational analyses.

Ordinary Differential Equations Based Models are useful for understanding important characteristics of enzymes

To understand the properties and capabilities of an enzyme it is useful to determine two characteristics: 1) Substrate affinity, defining how well the enzyme binds its substrate; and 2) maximal velocity, defining how quickly bound substrate can be converted into product. These enzyme characteristics are typically obtained by deriving the Michaelis-Menton equation as shown below

The simplified scheme listed above implies the following complete set of ODEs:

$$\frac{d[E]}{dt} = -k_1[E][S] - k_{-2}[E][P] + k_{-1}[ES] + k_2[ES]$$

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]$$

$$\frac{d[P]}{dt} = -k_{-2}[E][P] + k_2[ES]$$

$$\frac{d[ES]}{dt} = -k_{-1}[ES] - k_2[ES] + k_1[E][S] + k_{-2}[E][P]$$

Through simplifications and some algebra, we can derive the Michaelis-Menten equation that expresses the initial velocity of the reaction as a function of the substrate concentration. First, since we are interested only in the initial rate of change, we can assume that we are examining the reaction when there is no product. If [P]=0, then several terms above drop out and the set of equations becomes:

$$\frac{d[E]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_2[ES]$$

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]$$

$$\frac{d[P]}{dt} = k_2[ES]$$

$$\frac{d[ES]}{dt} = -k_{-1}[ES] - k_2[ES] + k_1[E][S]$$

Next, we consider the fact that in many reactions, the abundance of substrate is much greater than the abundance of enzyme. Mathematically if [S] >> [ES] then we can assume d[S]/dt = 0 and the equations simplify to:

$$\frac{d[E]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_2[ES]$$

$$\frac{d[P]}{dt} = k_2[ES]$$

$$\frac{d[ES]}{dt} = -k_{-1}[ES] - k_2[ES] + k_1[E][S]$$

Next we notice that d[ES]/dt = -d[E]/dt. This is equivalent to saying $[E]_{TOTAL}$ = [E] + [ES]. This is not an assumption, but rather simple conservation of mass. This also follows from the definition of a catalyst, which is neither produced nor consumed in the reaction it catalyzes. This reduces the system from three equations to two. Moreover, we can substitute $[E]_{TOTAL}$-[ES] wherever we encounter free enzyme [E]:

$$\frac{d[P]}{dt} = k_2[ES]$$

$$\frac{d[ES]}{dt} = -k_{-1}[ES] - k_2[ES] + k_1([E]_{TOTAL} - [ES])[S]$$

Finally, we make the steady-state assumption, which is that enzyme substrate complex concentration is constant. If this is true, we can assume that d[ES]/dt = 0. Setting the left hand side of the second equation to zero allow us to solve for the steady-state value of [ES] as follows:

$$0 = -k_{-1}[ES] - k_2[ES] + k_1([E]_{TOTAL} - [ES])[S]$$

$$k_{-1}[ES] + k_2[ES] + k_1[ES][S] = k_1[E]_{TOTAL}[S]$$

$$[ES] = \frac{[E]_{TOTAL}[S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

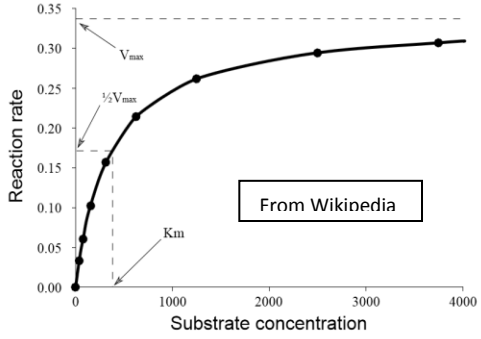
Finally, remember that d[P]/dt = k_2[ES]; that is the initial reaction velocity is equal to k_2 times the quantity above expression: $V_0 = \frac{k_2[E]_{TOTAL}[S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$

If we define $V_{max} = k_2[E]_{TOTAL}$, and $K_M = (k_{-1} + k_2)/k_1$, we obtain the famous Michaelis-Menten equation:

$$V_0 = \frac{V_{\max}[S]}{K_M + [S]}$$

The relationships between substrate concentrations and velocity are typically displayed graphically From these graphs and equations the following characteristics of an enzymes can be calculated

V_{max} - this represents the maximal velocity of product formation an enzyme is capable of achieving when all enzyme molecules are occupied by a substrate molecule. This value is proportional to the total concentration of enzyme and therefore increases when more enzyme is present.



K_M – As derived above this represents the concentration of the substrate at which an enzyme velocity equal to one half of V_{max}. The K_M is an approximate measure of the affinity of a substrate for the enzyme.

Another informative characteristic of enzymes is K_{cat}

K_{cat} - This number represents the intrinsic capability of the enzyme to convert substrate to product per unit time. Typically the value is given as molecules of product formed per molecule of enzyme per second, so this value is also called the turnover number. This is a widely variable number in the range of 1 to 1000

<u>Competitive inhibitors of enzyme action</u>

Competitive inhibitors bind to the enzyme and block the binding of the substrates. In addition, inhibitors themselves do not undergo any chemical changes due to the activity of the enzyme. If they are reversible inhibitors they are bound and released as the same chemical entity. Analogous to the equations presented earlier for ligand-receptor binding in the presence of a competitive inhibitor, the overall reaction scheme when the enzyme is exposed to both substrate and inhibitors is given by:

$$[EI] + [S] \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} [E] + [S] + [I] \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} [ES] + [I] \xrightarrow{k_{cat}} [E] + [P] + [I]$$

For such a system we can obtain an equation for the initial velocity of the reaction at a given combination of substrate and inhibitor:

$$V_0 = \frac{V_{\max}[S]}{[S] + K_M + \frac{K_M}{K_I}[I]}$$

For competitive inhibitors that are used as drugs it is often useful obtain the K_i value. This value represents the concentration of the inhibitor to obtain 50% inhibition of the maximal activity of the enzyme. Experimentally, to obtain K_i enzyme activity is measured at different substrate concentrations in the presence of varying concentrations of the inhibitors. Typical plots obtained from an experiment such as this are shown in Figure 12.4

ODE models of multiple coupled enzyme systems.

The above sections described how to quantitatively describe the function of a single receptor or enzyme and how these activities might be modified by inhibiting drugs. Within cells, however, the output of one biochemical reaction frequently either serves as the input (ligand or substrate) to another reaction, or is responsible for activating or inhibiting another biochemical reaction. Moreover, intracellular concentrations of important signaling molecules are influenced by reactions that both increase and decrease their activities, and these processes may be differentially regulated. Thus, to understand signal flow the simultaneous activities of multiple related enzymes need to be taken into account. Consider the case of the intracellular messenger cAMP that activates protein kinase A. The canonical description of this pathway is that information flows from a receptor, to adenylyl cyclase, to cAMP, and then to protein kinase A. Activation of adenylyl cyclase increases intracellular [cAMP], and this leads to an increase in the activity of the kinase. To understand information flow through this pathway in a cell, however, one must also consider the fact that cAMP is degraded by the enzyme phosphodiesterase . To determine cAMP as a function of time, therefore, we must quantitatively compare the rate of synthesis by adenylyl cyclase and the rate of degradation by phosphodiesterase. The relevant differential equation therefore has both a positive and a negative term:

$$\frac{d[cAMP]}{dt} = J_{produtions} - J_{\deg radation}$$

$$\frac{d[cAMP]}{dt} = k_{AC}[AC]_{active} - \frac{V_{\max,PDE}[cAMP]}{[cAMP] + K_{m,PDE}}$$

Integrating this equation from the start of reaction (0) to time t computes the balance between the amount of cAMP synthesized versus the amount of cAMP degraded. An important point to note is that an increase in cAMP can result from an increase in adenylyl cyclase activity or from a *decrease* in phosphodiesterase activity. Thus, although we are interested in understanding the linear flow of signal from receptor to adenylyl cyclase to protein kinase A, we need to "look" sideways at the degradation of cAMP to determine how much signal passes from adenylyl cyclase to protein kinase A. This can only be done by quantitatively comparing the rates of synthesis and degradation of cAMP. This quantitative comparison requires the solution of a differential equation that accounts for both processes, and this solution can then be used to predict the level of activation of protein kinase A.

Allosteric Regulation of Enzymes can be studied by ODE models

Many proteins, including enzymes and receptors, have multiple binding sites whereby activity (binding or catalysis) at one site affects activity at the other. This effect is called allostery. Binding of oxygen to hemoglobin was among the earliest observed examples of allostery. Monod, Changeux and Jacob used the term allosteric to describe the effects of regulators of enzyme activity that bore no structural resemblance to the substrate of the enzyme. Enzymes that are subject to allosteric regulation do not follow Michaelis-Menton kinetics. Rather they the display sigmoidal velocity curves. The simplest case where binding of substrate to one site enhances the activity of the second site results in a response that looks switch-like, a sharp transition from low to high activity states

The original discovery of the regulation of bound oxygen on the subsequent binding of oxygen or carbon monoxide by Hill led him to develop the following mathematical formulation

$$[LR] = \frac{R_{TOT}[L]^n}{K_D^n + [L]^n}$$

Originally Hill thought that *n* (eventually called the Hill coefficient) represented the number of oxygen binding sites per molecule of hemoglobin. However this is not strictly true: *n* represents not only the number of binding sites but also the level of interaction between the sites. For enzymatic reactions the Hill equation can be written as follows

$$\frac{\upsilon}{V} = \frac{[S]^n}{K' + [S]^n}$$

Where *v* velocity, and *V* is maximal velocity and S is substrate concentration and K' is a composite coefficient representing substrate affinity and interactions at multiple sites.

The value of the Hill coefficient is a widely used number to described interaction between binding or enzymatic sites. Hill-coefficients of 1 represent independent interacting sites. Hill coefficients of greater than 1 indicate positive cooperativity wherein binding or enzymatic activity at one sites increases the function of the second sites. Typically, systems with Hill coefficients greater than 1.5 are considered to exhibit strong positive cooperativity. Hill coefficients less than one are considered to show negative cooperativity, wherein binding or activity at one site decreases or inhibits binding or activity at the second sites. Receptors that have multiple non-interacting binding sites display $n_H < 1$ for ligand binding.

ODE models can be used to study many properties of receptor –ligand interactions

The idea of receptors as physical entities arose from the studies of P. Erhlich in Germany and J. N. Langley in England in the late nineteen early twentieth century. Langley, while studying neuro-muscular transmission, coined the term "receptive substance" to explain the opposing actions of the toxin curare and nicotine on muscle cells. Erhlich, studying agents that could be used for chemotherapy, developed the concept of selectivity that eventually gave rise to understanding of the specificity of reactions. Erhlich's studies on selectivity of therapeutic agents are summed by his statement "*corpora non agunt nisi fixate*" (agents cannot act unless they are bound). Soon thereafter, this line of reasoning was

formulated in quantitative terms by A. J. Clark . The interaction between the ligand and receptor is governed by the law of mass action

$$[L]+[R] \underset{k_b}{\overset{k_f}{\rightleftarrows}} [LR]$$

This scheme implies the following set of coupled ordinary differential equations (ODEs):

$$\frac{d[L]}{dt} = -k_f[L][R] + k_b[LR]$$
$$\frac{d[R]}{dt} = -k_f[L][R] + k_b[LR]$$
$$\frac{d[LR]}{dt} = k_f[L][R] - k_b[LR]$$

The rate of formation of the LR complex is an indication of the rate initiation of information flow into the cell when the ligand L is an agonist. Computations that measure the rate of LR formation are very useful in understanding the how physiological responses are initiated. The ratio of the k_b and k_f give the dissociation constant K_D which is the most common measure of affinity of a ligand for its receptor. The affinity constant defines, for a given [L], the fraction of receptors that will be occupied at steady state.

When radiolabeled ligands were developed as receptor probes, the most common studies focused on the determining the affinity of a ligand for the receptor. In these experiments the fractional receptor occupancy by the ligand was studied at varying ligand concentrations and a typical hyperbolic relationship that is governed by the law of mass action can be seen. There are several ways to plot and analyze this relationship using both linear and semi-logarithmic plots. From both types of plots we can determine the K_D of the ligand of interest.

From nonlinear plots of bound receptor versus free ligand, it is not always easy to deduce important quantities such as the dissociation constant K_D, and linear transformations of the relevant equations are frequently employed to illustrate important principles. A commonly used linear transformation is the relationship of the ratio of bound to free receptors (B/F) on the ordinate plotted versus bound receptors on the abscissa . This is called the Scatchard plot. This is a very useful plot as the both the slope and the intercept of the line on the abscissa gives the value of the total number of receptors in the preparation that is being measured. When the curve deviates from a straight line its shape is also informative as it indicates whether receptors interact with one another and the type of interaction between receptors. Thus ODE models of ligand receptor interactions allow us to determine many useful characteristics of both the ligands and the receptors.

Computational Receptor models can be used to understand the action of drugs

Many drugs act by blocking signaling pathways that are over-activated or otherwise dysregulated in the disease state. Small molecules that inhibit the action of endogenous agonists for G protein coupled receptors are used as therapeutic agents to treat a broad range of diseases. The key feature of an antagonist to block the action an endogenous agent is its ability to occupy the required number of binding sites on the target in the presence of normal to elevated levels of endogenous agonists. This competitive relationship is the critical criterion in determining if an antagonist drug is effective or not. The relationship is best understood by determining the relative affinities of the agonist and antagonist for the

receptor. A typical approach to determine the binding affinity are competition studies where varying concentrations of agonist are used in the presence of different fixed concentrations of the antagonist. These dose-response curves can be used to determine the affinity of the antagonist. For this consider the reactions

$$[IR]+[L] \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} [L]+[R]+[I] \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} [LR]+[I]$$

where [L] is the receptor's ligand (agonist), and [I] is an inhibitor (antagonist) that binds to the same site. In this case the receptor can exist in one of three forms, unbound ([R]), bound to ligand ([LR]), or bound to inhibitor ([IR]), i.e. $[R]_{TOTAL} = [R] + [LR] + [IR]$.
This scheme has the following steady-state solution for ligand-receptor complex as a function of free ligand:

$$[LR] = \frac{[R]_{TOTAL}[L]}{[L] + K_D + \frac{K_D}{K_I}[I]}$$

This equation is nearly identical to the equation for ligand-receptor complex in the absence of inhibitor except for the presence of the term $I*K_D/K_I$ in the denominator. Adding a term to the denominator has identical quantitative effects to an increase in K_D: in other words the presence of the competitive inhibitor essentially decreases the affinity of the receptor for the ligand, and the decrease in affinity can be computed as the apparent K_D:

$$K_{D,apparent} = K_D\left(1 + \frac{[I]}{K_I}\right)$$

The plot -log [Antagonist] vs. ratio of log ([Agonist Ratio]-1) was first devised by H.O. Schild and is called the Schild plot These type of experiments and computation are commonly used to determine which of a set of similar drugs is likely to be more effective. These types of computation are commonly used in the drug discovery process.