Bioenergetics:

Energy, Thermodynamics, and Enzymes

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

Two fundamental concepts govern energy as it relates to living organisms: the First Law of Thermodynamics states that total energy in a closed system is neither lost nor gained – it is only transformed. The Second Law of Thermodynamics states that entropy constantly increases in a closed system.

More specifically, the First Law states that energy can neither be created nor destroyed: it can only change form. Therefore, through any and all processes, the total energy of the universe or any other closed system is constant. In a simple thermodynamic system, this means that the energy is transformed either by the transfer of heat energy (i.e. heating and cooling of a substance) or by the production of mechanical work (i.e. movement). In biological and chemical terms, this idea can be extended to other forms of energy such as the chemical energy stored in the bonds between atoms of a molecule, or the light energy that can be absorbed by plant leaves.

Work, in this case, need not imply a complicated mechanism. In fact, there is work accomplished by each molecule in the simple expansion of a heated mass of gaseous molecules (as visualized by expansion of a heated balloon, for example). This is expressed mathematically as the Fundamental Thermodynamic Relation:

$$dE = T\,dS - p\,dV$$

in which E is internal energy of the system, T is temperature, S is entropy, p is pressure, and V is volume.

The Second Law dictates that entropy always seeks to increase over time. Entropy is simply a fancy word for chaos or disorder. The theoretical final or equilibrium state is one in which entropy is maximized, and there is no order to anything in the universe or closed system. Spontaneous processes, those that occur without external influence, are always processes that convert order to disorder. However, this does not preclude the imposition of order upon a system. Examining the standard mathematical form of the Second Law:

$$\Delta S_{system} + \Delta S_{surroundings} = \Delta S_{universe}, \text{ where } \Delta S_{universe} > 0$$

shows that entropy can decrease within a system as long as there is an increase of equal or greater magnitude in the entropy of the surroundings of the system.

Unlike the First Law which applies even to particles within a system, the Second Law is a statistical law – it applies generally to macroscopic systems. However, it does not preclude small-scale variations in the direction of entropy over time. In fact, the Fluctuation theorem (proposed in 1993 by Evans et al, and demonstrated by Wang et al in 2002) states that as the length of time or the system size increases, the probability of a negative change in entropy (i.e. going against the Second Law) decreases exponentially. So on very small time scales, there is a real probability that fluctuations of entropy against the Second Law can exist.

The phrase "in a closed system" is a key component of these laws, and it is with the idea encapsulated in that phrase that life can be possible. Let's think about a typical cell: in its lifetime, it builds countless complex molecules - huge proteins and nucleic acids formed from a mixture of small amino acids or nucleotides, respectively. On its surface,

The "universe" is a closed system by definition because there is nothing outside of it.

this example might seem to be a counterexample to the second law - clearly going from a mixture of various small molecules to a larger molecule with bonded and ordered components would seem to be a decrease in entropy (or an increase in order). How is this possible with respect to the second law? It is, because the second law applies only to closed systems. That is, a system that neither gains nor loses matter or energy.

A living cell is not a closed system: it has inputs and outputs. However, the second law is still useful if we recognize that the only way that it can be bypassed is through the input of energy. If a cell cannot take in food (input of matter and energy into the system) it dies, because the second law requires that everything eventually breaks down into more random/chaotic collections of smaller components. The order required to sustain life (think about all the different complex molecules that were mentioned in the previous chapter) is phenomenal. The same thing applies on the organismal level (fig. 1) - without an input of energy (in the form of food molecules for animals or in the form of light for plants), the organism will die and subsequently decompose.

Creating molecules from atoms costs energy because it takes a disordered collection of atoms and forces them, through chemical bonds, into ordered, non-random positions. There is likewise an energy cost to formation of macromolecules from smaller molecules. By imposing order in the system, there must be an associated input of energy. This happens at every level of the system: atoms to molecules, small molecules to macromolecules, groups of molecules to organelles, etc.

Where does that energy go? It ends up in the bonds that are holding the molecules or macromolecules in their ordered state. When such a bond is broken, and a molecule is turned back into a collection of atoms, energy is released. The energy in a chemical bond is thus *potential* energy - it is stored energy that, when released, has the ability to do work. This term, if you recall your high school physics, is usually learned along with *kinetic* energy, which is energy that is being used in the process of actually doing work (i.e. moving an object from one place to another). The classic example is the rock on the top of a hill: it has potential energy because it is elevated and could potentially come down. As it tumbles down, it has kinetic energy as it moves. Similarly in a cell, the potential energy in a chemical bond can be released and then used for processes such as putting smaller molecules together into larger molecules, or causing a molecular motor to spin or bend - actions that could lead to pumping of protons or the contraction of muscle cells, respectively.

Coming back to the second law, it essentially mandates that breaking down molecules releases energy and that making new molecules (going against the natural tendency towards disorder) requires energy. Every molecule has an intrinsic energy, and therefore

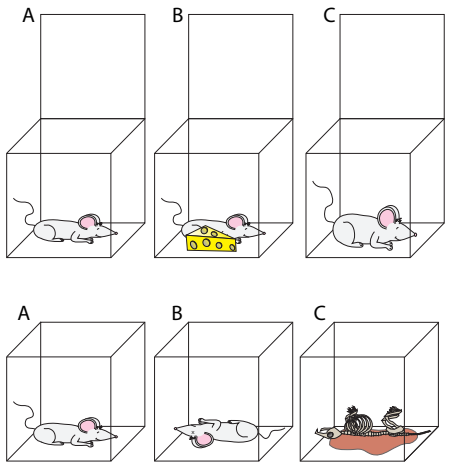

Figure 1. In the top panel, depicting an open system in which there are inputs for matter and energy (in the form of food), the box is open and air can be exchanged, food dropped in, etc, thus allowing the mouse to grow. However, in the bottom panel, depicting a closed system, the mouse does not have ready access any more oxygen than is in the box, nor does it have access to food. Without these inputs, the second law takes effect, and the mouse dies and decomposes into many smaller molecules.

As the polymerizing reaction reduces entropy, it requires energy generated (usually) by the breakdown of ATP into AMP and PPi, which is a reaction that increases entropy.

whenever a molecule is involved in a chemical reaction, there will be a change in the energy of the resulting molecule(s). Some of this change in the energy of the system will be usable to do work, and that energy is referred to as the free energy of the reaction. The remainder is given off as heat.

The Gibbs equation describes this relationship as $\Delta G=\Delta H-T\Delta S$. ΔG is the change in free energy, ΔH is the change in enthalpy (roughly equivalent to heat), T is the temperature at which the reaction takes place, and ΔS is the change in entropy. As a matter of convention, release of free energy is a negative number, while a requirement for input of energy is denoted with a positive number. Generally, a chemical reaction in which $\Delta G < 0$ is a spontaneous reaction (also called an *exergonic* reaction), while a chemical reaction in which $\Delta G > 0$ is not spontaneous (or *endergonic*). When $\Delta G = 0$, the system is in equilibrium. ΔG can also be expressed with respect to the concentration of products and reactants:

$$\Delta G = \Delta G^\circ + RT \ln ([P1] [P2] [P3] \ldots / [R1] [R2] [R3] \ldots)$$

Terms in square brackets denote concentrations, ΔG° is the standard free energy for the reaction (as carried out with 1M concentration of each reactant, at 298K and at 1 atm pressure), R is the gas constant (1.985 cal $K^{-1}mol^{-1}$), and T is the temperature in Kelvin. In a simpler system in which there are just two reactants and two products:

$$aA + bB \rightleftharpoons cC + dD$$

the equation for free energy change becomes

$$\Delta G = \Delta G^\circ + RT \ln \left(\frac{[C]^c [D]^d}{[A]^a [B]^b} \right)$$

This is important to us as cell biologists because although cells are not very well suited to regulating chemical reactions by varying the temperature or the pressure of the reaction conditions, they can relatively easily alter the concentrations of substrates and products. In fact, by doing so, it is even possible to drive a non-spontaneous reaction ($\Delta G > 0$) forward spontaneously ($\Delta G < 0$) either by increasing substrate concentration (possibly by transporting them into the cell) or by decreasing product concentration (either secreting them from the cell or by using them up as substrates for a different chemical reaction).

Changes in substrate or product concentration to drive a non-spontaneous reaction are an example of the more general idea of *coupling reactions* to drive an energetically unfavorable reactions forward. Endergonic reactions can be coupled to exergonic reactions as a series of reactions that ultimately is able to proceed forward. The only requirement is that the overall free energy change must be negative ($\Delta G < 0$). So, assuming standard conditions ($\Delta G = \Delta G^{\circ\prime}$), if we have a reaction with a free energy change of +5

kcal/mol, it is non-spontaneous. However, if we couple this reaction, to ATP hydrolysis for example, then both reactions will proceed because the standard free energy change of ATP hydrolysis to ADP and phosphate is an exergonic -7.3 kcal/mol. The sum of the two ΔG values is -2.3 kcal/mol, which means the coupled series of reactions is spontaneous.

In fact, ATP is the most common energy "currency" in cells precisely because the -7.3 kcal/mol free energy change from its hydrolysis is enough to be useful to drive many otherwise endergonic reactions by coupling, but it is less costly (energetically) to make than other compounds that could potentially release even more energy (e.g. phosphoenolpyruvate, PEP). Also, much of the -14.8 kcal/mol (ΔG°') from PEP hydrolysis would be wasted because relatively few endergonic reactions are so unfavorable as to need that much free energy.

Even when a reaction is energetically favorable (ΔG < 0), it may not occur without a little "push", chemically speaking. The "push" is something called *activation energy*, and it overcomes thermodynamic stability. Consider glucose, for instance. This simple sugar is the primary source of energy for all cells and the energy inherent within its bonds is released as it breaks down into carbon dioxide and water. Since this is large molecule being broken down into smaller ones, entropy is increased, thus energy is released from reaction, and it is technically a spontaneous reaction. However, if we consider a some glucose in a dish on the lab bench, it clearly is not going to spontaneously break down unless we add heat. Once we add sufficient heat energy, we can remove the energy source, but the sugar will continue to break down by oxidation (burn) to CO_2 and H_2O.

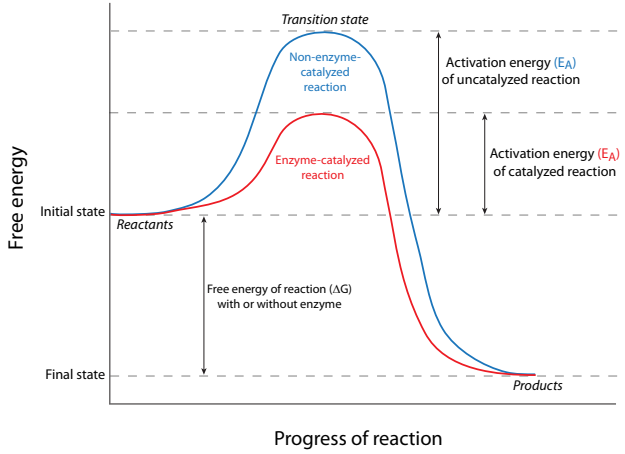

Figure 2. Catalysts lower the activation energy barrier to chemical reactions without altering the free energy change for that reaction.

Why is ATP different from other small phosphorylated compounds? How is it that the γ-phosphoanhydride bond (the most distal) of ATP can yield so much energy when hydrolysis of glycerol-3-phosphate produces under a third of the free energy? The most obvious is electrostatic repulsion. Though they are held together by the covalent bonds, there are many negative charges in a small space (each phosphate carries approximately 4 negative charges). Removing one of the phosphates significantly reduces the electrostatic repulsion. Keeping in mind that ΔG is calculated from the equilibrium of both reactants and products, we also see that the products of ATP hydrolysis, ATP and phosphate, are very stable due to resonance (both ADP and P_i have greater resonance stabilization) and stabilization by hydration. The greater stability of the products means a greater free energy change.

Put another way, the reactant(s) must be brought to an unstable energy state, known as the transtion state (as shown at the peak of the graphs in fig. 2). This energy requirement barrier to the occurrence of a spontaneous thermodynamically favored reaction is called the activation energy. In cells, the activation energy requirement means that most chemical reactions would occur too slowly/infrequently to allow for all the processes that keep cells alive because the required energy would probably come from the chance that two reactants slam into one another with sufficient energy, usually meaning they must be heated up. Again, cells are not generally able to turn on some microscopic bunsen burner to generate the activation energy needed, there must be another way. In fact, cells overcome the activation energy problem by using catalysts for their chemical reactions. Broadly defined, a *catalyst* is a chemical substance that increases the rate of a reaction, may transiently interact with the reactants, but is not permanently altered by them. The catalyst can be re-used because it is the same before the reaction starts, and after the reaction completes. From a thermodynamic standpoint, it lowers the activation energy of the reaction, but it does not change the ΔG. Thus it cannot make a non-spontaneous reaction proceed; it can only make an already spontaneous reaction occur more quickly or more often.

Enzymes

Biological catalysts are called enzymes, and the overwhelming majority of enzymes are proteins. The exceptions are a class of RNA molecules known as ribozymes, of which most act upon themselves (i.e. part of the RNA strand is a substrate for the ribozyme part of the strand). In this book (and most textbooks in this field), unless otherwise specified, the term enzyme refers to one made of protein. Enzymes confer extraordinary specificity to a chemical reaction: a reaction that might occur between a variety of potential substrates in an uncatalyzed situation may only be allowed between two specific substrates when catalyzed by an enzyme. Enzymes allow cells to run chemical reactions at rates from a million to even a trillion times faster than the same reactions would run under similar conditions without enzymes. In some cases, the enzymes allow reactions to proceed that would normally (i.e. sans enzyme) require more extreme temperature, pressure, or acidity/alkalinity. Finally, and perhaps most importantly for life, enzymes can be regulated. This is crucial for the cell, since it must be able to react to different situations, such as availability of energy, accumulation of toxic byproducts, the need to reproduce, etc. Not only can enzymes be modified either covalently or non-covalently to increase or decrease their activity, the cell can also regulate production of the enzymes, providing another level of control over particular cellular biochemical reactions.

Enzymes are the most diverse type of protein in a cell. They vary not only in size, but also in the number of independently manufactured subunits that must come together to form an active enzyme, or *holoenzyme*. Part of the reason for requiring so many different enzymes is that they are usually very specific for their substrate molecules, and that specificity is based upon a combination of shape and charge. The interactions between substrate and enzyme are often likened to a lock and key or pieces of a jigsaw puzzle. If the substrate fits the shape of the enzyme's active site (the part of the enzyme that carries out the actual catalytic reaction), and the charges interact (e.g. positively charged amino acids on the enzyme lining up with negative charges on the substrate), then there may be further stabilization of the interaction by Van der Waals and hydrogen bond interactions. In fact, formation of a stable Enzyme-Substrate (ES) intermediate is energetically analogous to the transition state (fig. 2) of reactions.

The specificity of enzymes is such that stereoisomers may not be recognized by some enzymes: for example, a protease (enzymes that chop up proteins into smaller pieces by hydrolyzing the peptide bonds between specific amino acids) such as trypsin can be stymied by the presence of a D-amino acid in place of the usual L-amino acid in a protein, even though it is a mirror image of the very same amino acid. This specificity means that enzymes are highly selective with respect to the reactions they catalyze, which means that specific reactions can be greatly enhanced without causing a general increase in many related chemical reactions. Another implication of the high specificity is that enzymes can (and often do) have high affinity for their substrates without the problem of binding non-substrate molecules (other than specific inhibitors - see below).

If most biochemical reactions would proceed extremely slowly, if at all, without catalysis, enzymes are needed to lower the activation energy needed for chemical reactions to support life. Exactly how does an enzyme lower the activation energy of a reaction? What exactly does "activation energy" mean in the context of a cell? To understand this, there are two principles to keep in mind: first, when we talk about chemical reactions, generally, we are concerned with populations of substrate, product, and enzyme molecules, not individuals; and second, the reactions are generally taking place between molecules dissolved in the aqueous cytoplasm of the cell.

Consider a reaction in which substrates A and B interact to form product C (fig. 3). If this reaction is not catalyzed, it depends on the happenstance that a molecule of A runs into a molecule of B in just the right orientation, and with the right amount of energy, to react and form the new molecule. We can conceptualize "activation energy" as the difficulty in getting A and B together perfectly so the reaction can proceed. How might an enzyme lower this activation energy? By making it easier for A and B to find each

Enzyme Classification

Enzymes have been catalogued and classified since the 1950's, during which time there was an explosion of enzyme discoveries and a need for a unified nomenclature and catalog. An International Commission on Enzymes was established (yes, of course I'm serious, why do you ask?) and thus started the Enzyme List. This list is now kept up-to-date online at http://www.chem.qmul.ac.uk/iubmb/enzyme.

All enzymes now have both recommended names for common usage, often reflecting historical naming, and a systematic name, which is highly specific. They also have a classification number based on their activity. The major classes of enzymes are (1) Oxidoreductases, which carry out oxidation-reduction reactions, (2) Transferases, which transfer functional groups, (3) Hydrolases, which carry out hydrolysis reactions, (4) Lyases, which eliminate groups to form double bonds, (5) Isomerases, which rearrange the bonds in a molecule but do not add or remove atoms, and (6) Ligases, which form bonds in reactions coupled to ATP hydrolysis.

As an example, DNA ligase (recommended name) catalyzes the formation of a phosphodiester bond between the 3' end of one DNA fragment and the 5' end of another. Its rather long and tedious systematic name is "poly(deoxyribonucleotide):poly(deoxyribonucleotide) ligase (AMP-forming)" and its classification number is 6.5.1.1. As a ligase, it is class 6; because it forms phosphoric ester bonds, it is subclass 5; the sub-subclass of 1 in this case is meaningless because it is the only sub-subclass of phosphoric-ester bond-forming ligases, but the final number designates the DNA ligase separately from other 6.5.1 enzymes such as RNA ligase, which is 6.5.1.3.

other with the right orientation and energy. So it could have binding sites for molecule A and molecule B, and once it has bound these two molecules, it changes its conformation, bringing A and B together under exactly the right conditions to react and form C. Once the reaction is complete, the product floats off because the enzyme has no affinity for it, and the enzyme returns to its initial shape, ready to bind more substrates.

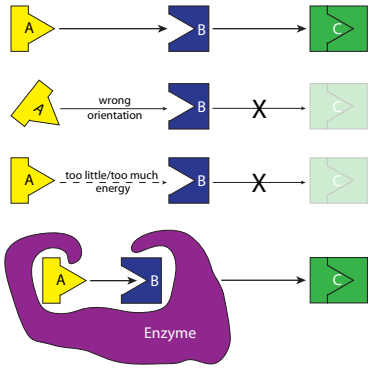

Figure 3. Enzymes can lower activation energy by binding substrates individually and bringing them together under optimal conditions to react.

Another example may be found with enzymes that break apart a molecule (figure 4). In order for a molecule to break apart, it may need to collide with another molecule with sufficient energy to break one or more of its covalent bonds. An enzyme that catalyzes

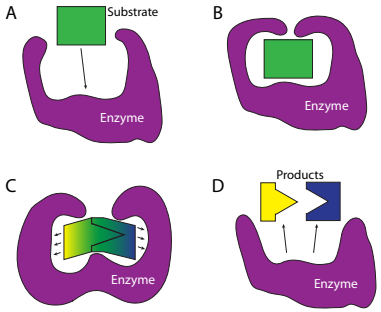

Figure 4. The enzyme (A) binds a specific substrate, leading to a conformational change (B) that stresses the molecule (C) until it breaks down into two smaller component molecules (D), which are released.

the breakdown reaction might bind to the molecule, and in binding it, undergoes a conformational shift that bends or twists the molecule in such a way that the bonds in the substrate molecule are weakened or broken. These two examples oversimplify the chemistry of enzyme activity into a mechanical idea, but the general relationship in how an enzyme lowers activation energy for a reaction is accurate.

Enzymes may also facilitate a chemical reaction by acting as a temporary holding site for an active group being transferred from one substrate to another. Alternatively, temporary formation of hydrogen bonds or even covalent bonds between the enzyme and substrate can alter chemical characteristics of the substrate to make it react more easily. An example of enzyme mechanisms on the molecular level is shown in Chapter 5: Figure 1.

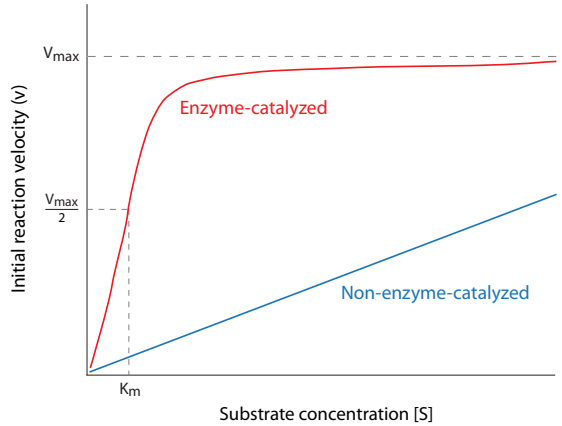

Figure 5. Saturation curve for a single-substrate enzyme-catalyzed reaction.

Enzyme Kinetics

Unlike uncatalyzed (but readily occurring) reactions, in which the rate of the reaction is dependent only on the concentration of the reactants, the rate of enzyme-catalyzed reactions is limited by the number of enzyme molecules available. This maximal rate of turnover from substrate to product is a function of the speed of the enzyme and the number of enzyme molecules. V_{max}, this theoretical maximal rate or reaction, is approached when there is such a high concentration of substrate molecules that not only is every available enzyme at a given time occupied, but as soon as an enzyme finishes converting substrate to product, it immediately binds a new substrate. Another term, K_m, is related to V_{max} in that K_m (the Michaelis constant) is the concentration of substrate at which half-maximal reaction rate ($V_{max}/2$)occurs. These two terms are related in the Michaelis-Menten equation, which describes the reaction rate *v* with respect to the substrate concentration [S].

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

Figure 6. Michaelis-Menten Equation

The Michaelis-Menten equation assumes a simple reaction of the form:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where E is an enzyme, S is the substrate, and P is the product. Note the formation of the intermediate enzyme-substrate complex, ES, which is a transition state (recall fig. 2) in which the substrate is unstable and associated with the enzyme. In fact, ES could as

The Michaelis-Menten equation was derived by Leonor Michaelis and his graduate student Maud Menten in 1913, based on work by Victor Henri, and is applicable only to simple enzyme kinetics in which there is only one substrate that is changed immediately to a product during the reaction without forming any intermediate compound, the enzyme in question shows no allostericity, and the reaction is unidirectional.

It should be noted that the reaction rate *v* is actually the initial reaction rate at a particular substrate concentration, and is sometimes denoted v_o. Naturally, as the reaction continues, the substrate concentration decreases, along with the reaction rate.

easily be considered EP, since this state is essentially the tipping point between the conversion from substrate to product. In this construction, the Michaelis constant, K_M, of an enzyme-catalyzed reaction is $(k_2 + k_3) / k_1$. That is the rate of ES dissociation over the rate of ES association. K_M, of course, varies not only depending on the enzyme, but also with respect to the identity of the substrate. Some enzymes can work with multiple substrates, and the K_M of that enzyme for the different substrates is usually different. Because the saturation curve in Fig. 5 can be difficult to work with, linearizations of the Michaelis-Menten equation were developed. The most common is the double reciprocal plot, better known as the Lineweaver-Burk plot. On this type of graphical representation of enzyme kinetics, the reciprocal of the substrate concentration is plotted against the reciprocal of the reaction velocity. This generates a line in which the x-intercept is then $-1/K_m$, the y-intercept is $1/V_{max}$, and the slope of the line is K_m/V_{max}.

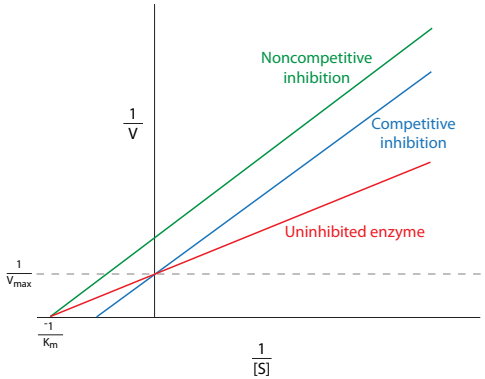

Figure 7. Lineweaver-Burk plot of enzyme kinetics and the effect of competitive and non-competitive inhibitors at constant concentrations.

Regulation of Enzyme Activity

Figure 7 (and 9) also illustrates the effects of two different types of inhibition on the different components of enzyme kinetics. Enzymes can be slowed down or even prevented from catalyzing reactions in many ways including preventing the substrate from entering the active site or preventing the enzyme from altering conformation to catalyze the reaction. The inhibitors that do this can do so either reversibly or irreversibly. The irreversible inhibitors are also called inactivators, and either bind to the enzyme with such high affinity as to be virtually irreversible, or they actually form covalent bonds with the enzyme. Reversible inhibitors are generally grouped into two basic types: competitive and non-competitive.

Obtaining V_{max} and K_m from a direct plot of v against [S] can be difficult because even at very high substrate concentrations, experimental data may still be significantly under the V_{max}. This leads to underestimation of the V_{max}.

The Lineweaver-Burk plot addresses this concern, but has some shortcomings of its own. Because it is easier to obtain data at high concentrations, most of the data points are near 0, and fewer data points are available further out (to the right of the graph). Because these are reciprocals, under these low [S] conditions, small errors in measured values of v turn into large errors in $1/v$, and therefore large errors in K_M and V_{max}. This is evident on examination of the Lineweaver-Burk equation:

$$\frac{1}{v} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Figure 8. Lineweaver-Burk Equation

Finasteride (trade names include Propecia and Proscar) is an irreversible inhibitor that binds very tightly to the enzyme 5-α-reductase, used in converting testosterone to dihydrotestosterone. It is used in the treatment of male pattern baldness, benign prostatic hyperplasia, and prostate cancer.

Aspirin is an example of an irreversible inhibitor that actually forms a covalent bond with the enzyme. The aspirin (acetylsalicylic acid) transfers its acetyl group onto a serine residue on cyclooxygenase-2 (COX-2). This stops the production of inflammation-producing prostaglandins and thromboxanes by COX-2.

Competitive inhibition is perhaps the simplest to understand. The inhibitor molecule competes directly with the substrate for the active site of an unbound enzyme. If an inhibitor binds to the active site, the substrate is unable to do so until the inhibitor has vacated the site. Thus, one could potentially overwhelm competitive inhibition with sufficiently larger concentrations of substrate so that the probablility that the enzyme bumps into a substrate to bind becomes exceeding large compared to the probablility of bumping into an inhibitor. Normal, uninhibited V_{max} is then achieved despite the presence of the competitive inhibitor, which has only affected the K_m, that is, the concentration of substrate needed to reach $V_{max}/2$. This is the kinetic signature of competitive inhibitors: with increasing inhibitor concentrations, K_M is increased but V_{max} is unaffected.

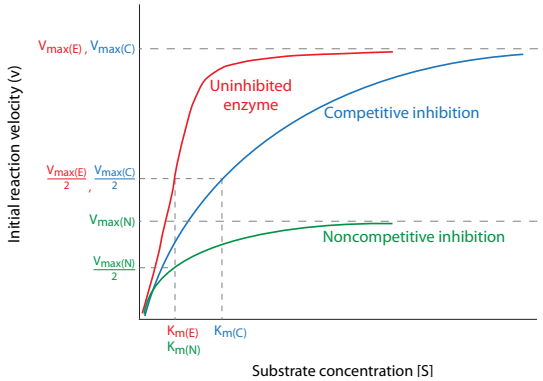

Figure 9. Saturation curves for enzyme-catalyzed reactions without inhibitor (red) with competitive inhibitor (blue) at a constant concentration, and with noncompetitive inhibitor (green) at a constant concentration.

Non-competitive inhibition involves inhibiting the enzyme by altering its ability to complete the catalyzed reaction through binding of the enzyme at a position that is not the active site. When the inhibitor binds to the enzyme, it causes a change, usually conformational, that may either prevent the enzyme from binding the substrate, or prevent the enzyme from acting upon a bound substrate. In either case, increasing the availability of substrate will not ultimately overcome the effect of the inhibitor. Thus, V_{max} is reduced because some proportion of the enzymes are no longer usable, but because the enzymes that are available have the same access to substrate as it would without inhibitor (that is, it is not in competition with an inhibitor), the K_m is not affected.

Non-competitive regulation is one example of *allosteric regulation* of enzymes. Allosteric interactions occur when the binding of a ligand (not necessarily a substrate) to a protein influences the binding of another ligand to the protein at a separate binding

Methotrexate is a competitive inhibitor of dihydrofolate reductase (DHFR), an enzyme that synthesizes tetrahydrofolate, which is a precursor for purine synthesis, and therefore for DNA and RNA. It has a very similar molecular structure for folic acid, the natural substrate of DHFR. Methotrexate is used as an anti-cancer drug because it affects rapidly reproducing cells (which need to make DNA sooner than other cells) more than non-cancerous cells.

Many of the enzymes in metabolic pathways (chapters 5 and 6) are regulated by a naturally occurring non-competitive inhibitor. One example is phosphofructokinase (PFK), which is involved in glycolysis, which produces ATP for the cell. However, if there are high enough levels of ATP in the cell that other cellular processes aren't using, the it can bind to phosphofructokinase outside of its active site (it binds fructose-6-phosphate), and turn it off. This blocks glycolysis and production of excess ATP when the cell does not need it. As ATP is used up, there is less available to inhibit PFK, and glycolysis starts back up.

There are two models for allosteric interactions. The symmetry model, also known as the concerted model, or MWC model (Monod, Wyman, and Changeux, 1965), proposes that the allosteric enzyme is an oligomer of several subunits, each of which are symmetrically related, and can be in either a "tensed" or "relaxed" state, but all of the subunits are in the same state and at equilibrium. When a ligand binds, it changes the state of the subunit(s) to which it binds, and to maintain equilibrium, that in turn causes the state of the other subunits to match, thus altering binding properties for a subsequent ligand. The sequential model, or the KNF model (Koshland, Nemethy, and Filmer, 1966), proposes

site. These kinds of interactions can be either positive (activating) or negative (inhibitory), and either homotropic (both ligands are identical) or heterotropic (ligands are different). Interestingly, sometimes the regulator ligand may actually be a product of the catalyzed reaction. In this kind of feedback mechanism, the progress of a reaction is self-regulating.

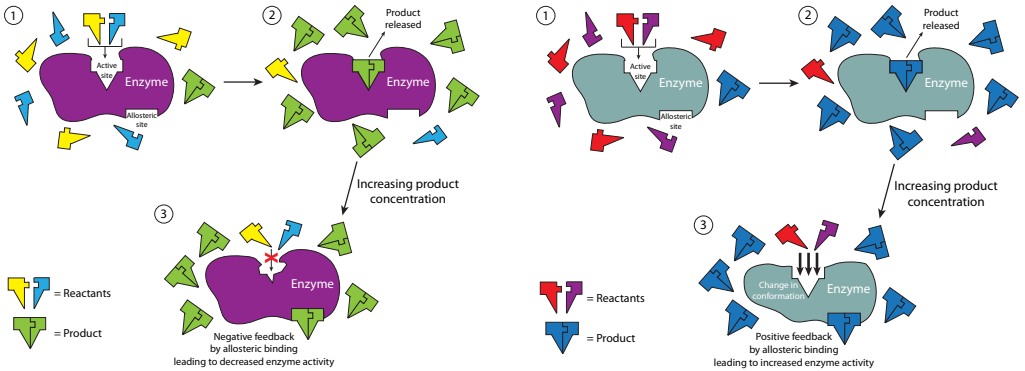

Figure 10. Feedback control loops. (Left) Negative feedback is a common biological mechanism for self-regulating processes. The product of the reaction, either by itself or inconjunction with other molecules, acts as an allosteric inhibitor of the enzyme. (Right) Positive feedback is less common because it can lead to rapid expansion of the scope of a reaction, and requires an external (relative to the enzymatic reaction) mechanism for slowing or stopping the reaction.

While important, especially pharmaceutically, the use of enzyme inhibitors is not the only way to regulate enzymes. There are numerous examples of one type of enzyme activating or inhibiting another. The most common general example are the protein kinases. These enzymes phosphorylate (transfer phosphate group to) other enzymes and thereby activate them. Kinases are generally fast and very specific, and this is an efficient method for activating large numbers of particular enzymes quickly. Conversely, protein phosphatases are enzymes (also quite fast, but much less specific than kinases) that remove the phosphate groups from phosphorylated proteins, thereby turning off those enzymes. Keep in mind that this is a generalization, and that not all phosphorylations are activating. In addition to enzymatic inhibition of enzymes, there is also inhibition by binding and sequestration of the substrates. In fact, the antibiotic vancomycin works just this way, binding to the substrate peptide for transpeptidase and preventing the enzyme from recognizing it. Transpeptidase normally helps stabilize the cell wall of certain bacteria by altering some of the proteins, and without its activity, the protection of the cell wall is compromised and the bacteria may be more easily killed.

something quite different: although it also supposes subunits in either tensed or relaxed states, it does not require all subunits to be connected in such a way as to mandate that all subunits be in the same state, and therefore conformational changes in one subunit need not be propagated to all. Instead, using an induced-fit model of ligand binding rather than the more rigid basic lock and key mechanism, it suggests that when a ligand binds to the enzyme, it induces a slight conformational change in the active site that increases its affinity for the ligand. The conformational change may slightly alter the conformation of other subunits of the enzyme, but may not constitute a state change between relaxed and tensed depending on how tightly the subunits are interacting. However, the change is enough to increase substrate affinity in adjacent subunits.

Most cell/molec courses stop the discussion of enzyme inhibitors at competitive vs non-competitive based on their kinetic profiles. However, it should be noted that if you take a biochemistry course, you may encounter the terms *uncompetitive* inhibitor and *mixed* inhibitor. These terms are defined not just by the enzyme kinetics, but the mechanism of interaction: *Uncompetitive inhibitors* bind only to the enzyme-substrate (ES) complex, and not to the enzyme before it has encountered substrate. This leads to decreased V_{max} and decreased K_m. *Mixed inhibition* means that the inhibitor can bind to either enzyme alone or the enzyme-substrate complex. Because the affinities of the inhibitor for the two forms of the enzyme are different, and because part of it depends on substrate concentration while the other kind of binding does not, generally, V_{max} decreases and K_m increases. Non-competitive inhibition is a special case of mixed inhibition in which the catalytic activity of the enzyme is diminished or abolished, but the ability to bind substrate is unaltered.

The activity of enzymes is greatly influenced by both pH and temperature, as expected from the discussion of protein structure in the previous chapter. Activity profiles of most enzymes shows a peak of activity that tails off on either side, whether it is pH or temperature. This is an innate characteristic of the enzyme. For example, pepsin, a digestive enzyme secreted into the stomach (pH 2) does not function when the pH > 5. On the other hand, another digestive enzyme, trypsin, which is secreted into the duodenum (proximal small intestine) where the pH is ~8, does not work in acidic environments. Changes in pH can change ionization of amino acid side chains that can thereby alter interaction with the substrate, or lead to changes in tertiary structure.

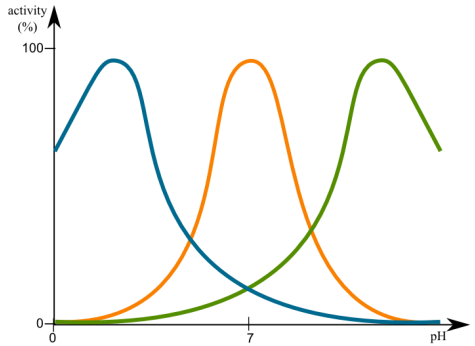

Figure 11. pH dependence of enzymatic activity. This graph depicts three hypothetical enzymes with acidic, neutral, and basic pH optima.

Similarly, at suboptimal temperatures, the likelihood of protein-substrate interaction is low but above the optimal temperature, the increased energy can lead to breaking of hydrogen bonds within the structure of the enzyme, resulting in changes that inactivate the catalytic ability of enzyme or prevent it from binding substrate with sufficient affinity. The temperature optimum of most enzymes is very close to its typical environment. Thus, a human enzyme would operate optimally around 37°C, while an enzyme from bacteria that live in deep-sea volcanic vents (e.g. Thermophilus aquaticus) might have temperature optima over 90°C. This is one of the reasons that refrigeration can slow down growth of microorganisms (which obviously have no ability to regulate their temperature), and why most microorganisms are killed (enzymes permanently denatured) when put into sustained high temperature environments. Interestingly, the DNA polymerase from the T. aquaticus bacteria, also commonly called Taq polymerase, is used in a rapid DNA-amplifying lab technique known as PCR (polymerase chain reaction, see Methods chapter) in which samples are repeatedly heated to high temperatures to separate DNA strands in preparation for making copies of them. DNA polymerases from most prokaryotic or eukaryotic species would be denatured and inactivated by the high heat, but Taq has evolved (with respect to its tertiary structure) for extraordinary structural stability even in heat extremes.

Well over half of the enzymes discovered so far do not act in the simplistic Michaelis-Menten one-substrate-one-product mechanism, but rather operate with two substrates and two products, usually with the transfer of an active group. These types of reactions are sometimes known as Bi Bi reactions. There are two major classes of these reactions: the *sequential reactions*, in which all substrates bind with the enzyme before the reaction proceeds, and the *ping pong reactions*, in which one or more products are created and released before all of the substrates have been bound. In fact, unlike sequential reactions, the two substrates do not interact with one another while bound to the enzyme.

Finally, many enzymes require a molecular partner that has no catalytic activity of its own, but like a catalyst, is not permanently altered by the chemical reaction. These molecules are *cofactors*. Some are simple: elemental, in fact, including metal ions such as Zn^{++} or Ca^{++}. Others are slightly more complex: small organic cofactors are called *coenzymes*, and accomplish the same thing, acting as a required partner to the enzyme in catalyzing a reaction. The interaction with the enzyme itself varies and may be only transient, as in NAD^{+}/NADH which are coenzymes used in redox reactions, or permanently bound to the enzyme by covalent bond like the heme group of hemoglobin. Often the function of the coenzyme is to provide an active group to facilitate the catalyzed reaction. Coenzyme A, in various metabolic pathways such as glycolysis or the tricarboxylic acid cycle, can be bound to a substrate to form a stable product that then acts as an intermediate. The Co-A is released from the molecule as it undergoes the next step in a series of reactions in the metabolic pathway (see Chapter 5).

From a human health standpoint, it is interesting to note that many coenzymes are vitamins, or derived from vitamins. These are the B vitamins biotin (B_7), cobalamin (B_{12}), folic acid (B_3), niacin/nicotinamide (B_9), pantothenic acid (B_5), pyridoxine (B_6), riboflavin (B_2), and thiamine (B_1). Vitamins are small organic compounds that are not synthesized by an organism and must therefore be ingested. They are generally needed only in small quanitities, but necessary nonetheless. Naturally, the vitamins we are familiar with are those required by humans. The specific roles of these vitamins and the consequences of not having enough of them are discussed later in this textbook, as the enzymes that they work with are introduced in detail.