ENZYMES

1. Enzyme Nomenclature—Enzymes Always end in -ase
   a. Some are named for the substrate attacked or product made (ATPase makes ATP)
   b. Some have generic names (trypsin, pepsin, papain)
   c. Most are named for the chemical reactions catalyzed
      1) Hydrolytic enzymes result in cleavage of a covalent bond by the addition of the atoms of water. For example, esterases break ester bonds, lipases hydrolyze lipids, phosphatases remove phosphate groups, pyrophosphatases cleave pyrophosphate into two phosphates, nucleases hydrolyze nucleic acids, and proteases or peptidases hydrolyze peptide bonds.
      2) Oxidation/reduction enzymes are called dehydrogenases or oxidases. In biological terms, oxidation usually involves removal of hydrogen ions, while reduction involves the addition of hydrogen ions
      3) Transferases transfer single carbons, acyl groups, amino groups, phosphate groups, lipid groups, or sugar groups from one reactant to another.
      4) Decarboxylases catalyze the removal of carboxyl groups.
      5) Ligases form covalent bonds between two reactants and lyases break covalent bonds.
      6) Isomerases catalyze the interconversion of various isomers. Examples include racemases, cis/trans isomerases, and epimerases.
      7) Dehydrases catalyze the elimination of water, resulting in unsaturations.

2. Enzyme active sites are lined with the side chains of amino acids
   a. The active sites bind the substrates and cofactors of the reaction.
   b. They contain amino acid residues that directly participate in the making and breaking of bonds.
   c. The active site is a relatively small area compared to the entire enzyme.
   d. The active site is a 3-dimensional cleft or crevice which usually excludes water unless it is a substrate of the reaction catalyzed.
   e. Substrate binding specificity depends on the precise spatial arrangement of the amino acids in the active site.
      Consider the "Lock and Key Model" as an example.

3. Enzymes have unique characteristics compared to other proteins.
   a. They have an extremely high specificity for their substrates.
   b. A very limited number of substrates will bind to a certain active site.
   c. They catalyze a single reaction, or in rare cases, two coupled reactions.
   d. They are very tightly regulated. Many enzymes which are part of "pathways" are controlled by "feedback loops." Examples of enzyme regulation are allosteric inhibition or stimulation, regulatory proteins such as calmodulin, covalent modification such as phosphorylation, proteolytic activation, and the simple availability of the enzyme itself (due to protein turnover: the synthesis and degradation of the enzyme in the cells).
   e. Enzymes transform energy. That is, the energy of reactants is converted with high efficiency into a new form (the products)
      1) Enzymes are catalysts only and cannot alter equilibria
         \[ DG < 0 \] (negative), reaction is spontaneous
         \[ DG = 0 \] the reaction is at equilibrium, no net change takes place
         \[ DG > 0 \] (positive), an input of free energy is needed to drive the reaction
      2) Enzymes accelerate the attainment of equilibria
      3) Enzymes accelerate reactions by stabilizing transition states
4. The Michaelis-Menten Relationship is a useful model for describing enzymatic catalysis. (See the handout on the Michaelis-Menten Relationship for the theory and derivation of the model.)

a. The $V_{\text{max}}$ and $K_m$ can be determined for an enzyme by observing the rate of catalysis at varied substrate concentrations. The inverse of the Michaelis-Menten equation, called the Lineweaver-Burk equation, can be plotted to give a straight line.

\[
\text{Michaelis-Menten equation: } V = \frac{V_{\text{max}} [S]}{K_m + [S]} \\
\text{Lineweaver-Burk equation: } \frac{1}{V} = \frac{K_m}{V_{\text{max}}[S]} + \frac{1}{V_{\text{max}}} \\
\text{slope } = \frac{K_m}{V_{\text{max}}[S]} \\
\text{y-intercept } = \frac{1}{V_{\text{max}}}
\]

Hence, for any enzyme, we can monitor the amount of product produced (the velocity) as we vary the concentration of substrate available to the enzyme (substrate concentration). We can plot the values obtained, draw the best straight line through the points, and calculate the $K_m$ and $V_{\text{max}}$ values.

b. $K_m$ is a significant value in describing an enzymatic reaction.
   1) $K_m$ is the concentration at which half the active sites of the enzyme are filled, or in other words, the concentration of substrate where the enzyme is functioning at half of its maximum possible velocity.
   2) Since, in the derivation of the Michaelis-Menten equation, $K_m$ is defined as $(k_2 + k_3)/k_1$, and since in most cases with enzymatic reactions $k_2 \gg k_3$, then we can assume $K_m = k_2/k_1$, and therefore $K_m$ is actually the dissociation constant for the enzyme/substrate complex. In other words, $K_m$ is descriptive of the binding of substrate to the enzyme active site. The LOWER the value of $K_m$, the MORE affinity the substrate has for the enzyme.

c. $V_{\text{max}}$ is also significant in describing an enzymatic reaction.
   1) By definition, $V_{\text{max}}$ is the maximum rate of the catalyzed reaction (when all of the enzyme active sites are completely filled with substrate).
   2) If the total number of active sites $[ET]$ is known, then the turnover rate for the enzyme (defined in the model as $k_3$) can be calculated from the relationship $V_{\text{max}} = k_3[ET]$.

5. The inhibition of enzymes can be described using the Michaelis-Menten model.

a. Enzyme inhibition may be irreversible, where the inhibitor dissociates very slowly from target enzyme, if at all. This involves very tight binding, often including the formation of covalent bonds. (In this case, there is no equilibrium established between the binding and release of inhibitor.)

b. Enzyme inhibition may be reversible, where the enzyme-inhibitor complex rapidly dissociates. This type of inhibition can be studied using the Michaelis-Menten relationship, especially in terms of how the inhibitor affects $K_m$ and $V_{\text{max}}$.
   1) In competitive inhibition, the inhibitor diminishes the rate of catalysis by reducing the number of substrate molecules which are bound to enzyme molecules, therefore affecting $K_m$ but not $V_{\text{max}}$. (The competitive inhibitor competes with the substrate for the enzyme active site; increasing the substrate concentration can overcome the inhibition.)
   2) In noncompetitive inhibition, the inhibitor actually decreases the turnover by the enzyme without reducing the number of substrate molecules bound to enzyme molecules, therefore affecting $V_{\text{max}}$, but not $K_m$. (A noncompetitive inhibitor binds to a completely different site on the enzyme than does the substrate, therefore inhibitor binding has no effect on substrate binding, and vice versa.)
   3) In uncompetitive inhibition, the inhibitor binds only the enzyme-substrate complex, therefore having what appears to be equivalent effects on both $K_m$ and $V_{\text{max}}$.

4) By studying the Lineweaver-Burk plots of uninhibited versus inhibited reactions for a given enzyme, the change in the plot of the straight line in the presence of the inhibitor can be used to determine which of the above types of inhibitors is present.
6. Enzymes can be controlled by allosteric effectors
   a. Cooperativity involves the influence of the binding of one ligand to an enzyme on the binding of a second ligand to the enzyme.
      1) In positive cooperativity, the binding of one ligand (substrate) affects the binding of the identical ligand (more substrate), to a second active site on a second subunit of the enzyme. Since both ligands involved are identical (they are both substrate), this can be termed a "homotropic effect."
      2) In negative cooperativity, the binding of one substrate molecule to one subunit decreases the affinity of the other subunits for substrate molecules.
   b. Allosteric inhibitors or stimulators of enzymes are ligands which bind to "allosteric sites" on the enzyme, which are separate and distinct sites from the active site. The binding of the these allosteric effectors causes a conformational shift which changes the structure of the active site.
      1) Binding of an allosteric inhibitor to an enzyme causes a conformational shift which decreases enzyme affinity for substrate (less substrate binds to enzyme).
      2) Binding of an allosteric stimulator to an enzyme causes a conformational shift in the enzyme which increases the enzyme affinity for substrate (more substrate binds enzyme).
      3) These allosteric effects are "heterotropic effects," where the binding of one ligand (the inhibitor or stimulator) affects the binding of a different ligand (the substrate).