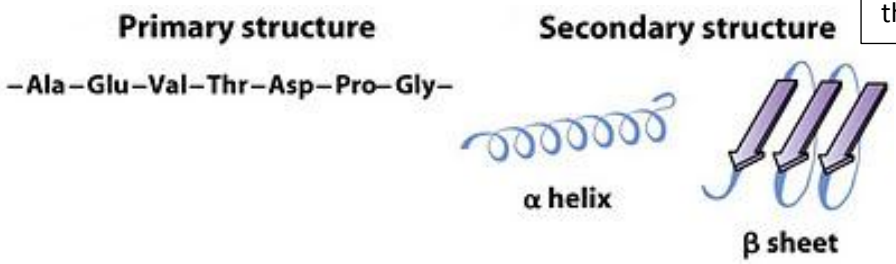
Contact email:
knh093020@utdallas.edu

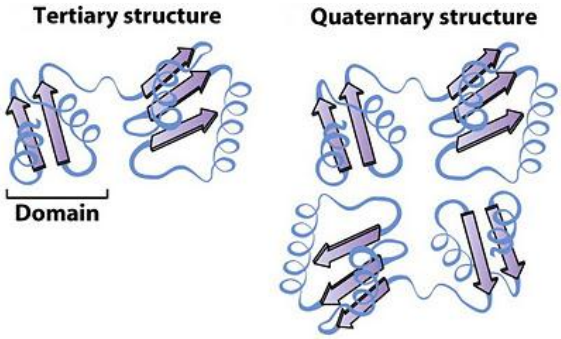
Kirk's Notes on Supersecondary Structures

Check which version (bottom-right corner) you have to make sure you have the most updated notes!



The primary structure refers to the sequence of amino acid residues in the polypeptide chain written left-to-right from the N-terminus to the C-terminus.

Secondary structures are ordered structures formed by internal hydrogen bonding between amino acid residues. The common secondary structures are the α helix, the β strand, and various loops and turns. The β sheet is often counted as secondary structure although, strictly speaking, it is a motif (see below).



http://sandwalk.blogspot.com/2008/03/levels-of-protein-structure.html

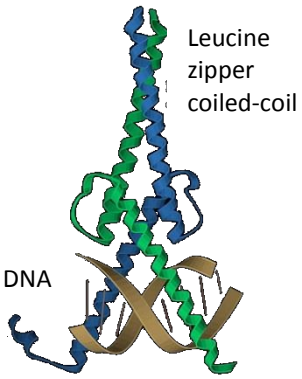
Loops and turns connect α helices and β strands. The most common types cause a change in direction of the polypeptide chain allowing it to fold back on itself to create a more compact structure.

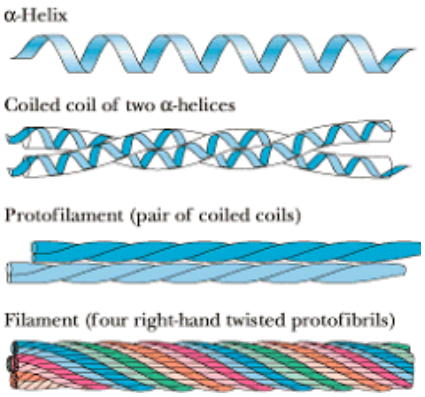
Loops are not well defined. They generally have hydrophilic residues and they are found on the surface of the protein. Loops that have only 4 or 5 amino acid residues are called turns when they have internal hydrogen bonds.

Protein domains consist of structural motifs. A structural motif is a supersecondary structure.

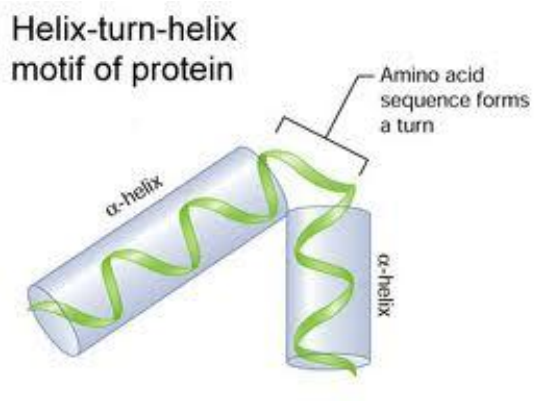
There are many different types of supersecondary structures but the five types as covered in chapter 6 are: α helix coiled coil, helix-turn-helix, β hairpin, β greek key, and beta-alpha-beta.

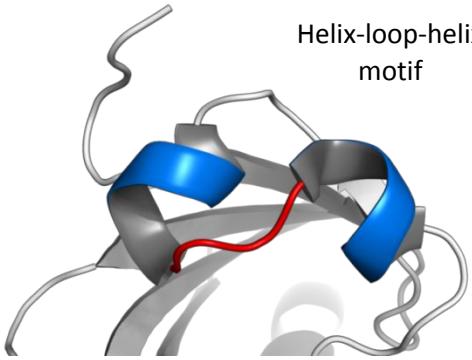
Alpha helix coiled coil are made of a bundle (2-7) alpha helices wrapped together like the strands of a rope. Coiled coils usually contain a repeated pattern of nonpolar residues at positions 1 and 4 in a heptad (7) repeat. Leucine zippers are an example of a coiled coil structure.





A helix-turn-helix is composed of two alpha helices connected by a short strand of amino acids. A helix-loop-helix is composed of two alpha helices joined by a loop. Loops (or turns) are elements of secondary structure in proteins where the polypeptide chain reverses its overall direction.





Helix-loop-helix
motif

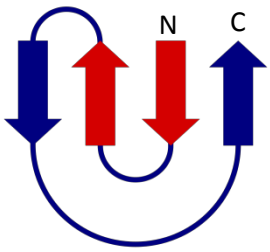
Beta hairpins (also called β-turns) are made of only two beta strands. Each strand contains antiparallel beta sheets and are used to change peptide direction with a hairpin loop. The hairpin loop is often assisted by proline, which allows it to make that turn back around in the opposite direction. Beta meanders are similar to beta hairpins but contain 2 or more consecutive antiparallel β-strands linked together by hairpin loops. Beta barrels and beta propellers are their common domain forms. Beta sandwich is a flattened beta barrel.

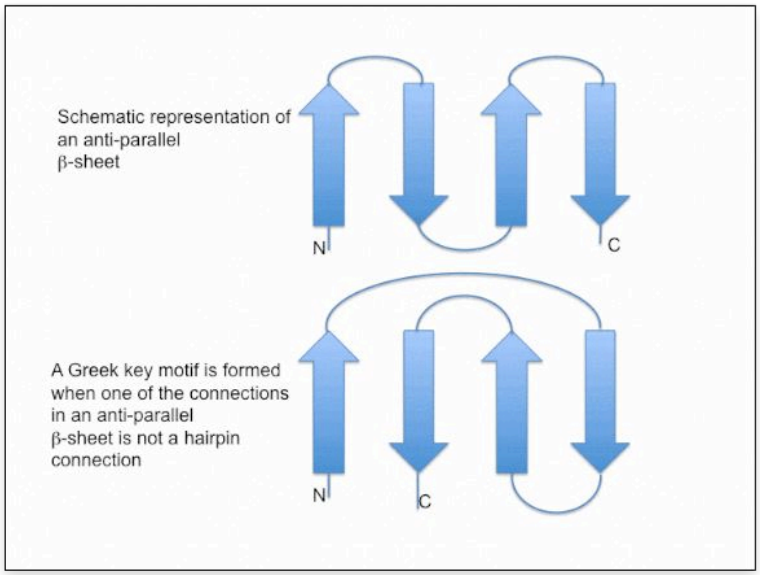
Beta
Hairpin

Beta
Meander

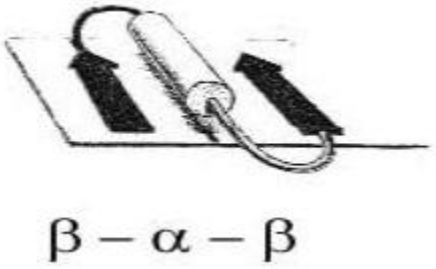
Beta greek key motif comprise of four adjacent antiparallel beta strands and their linking loops. The first three anti-parallel strands are connected by hairpin loops, while the fourth strand is connected to the first by a longer loop. Beta barrels are one of their domain forms. Beta sandwich is a flattened beta barrel.

Beta
Greek Key





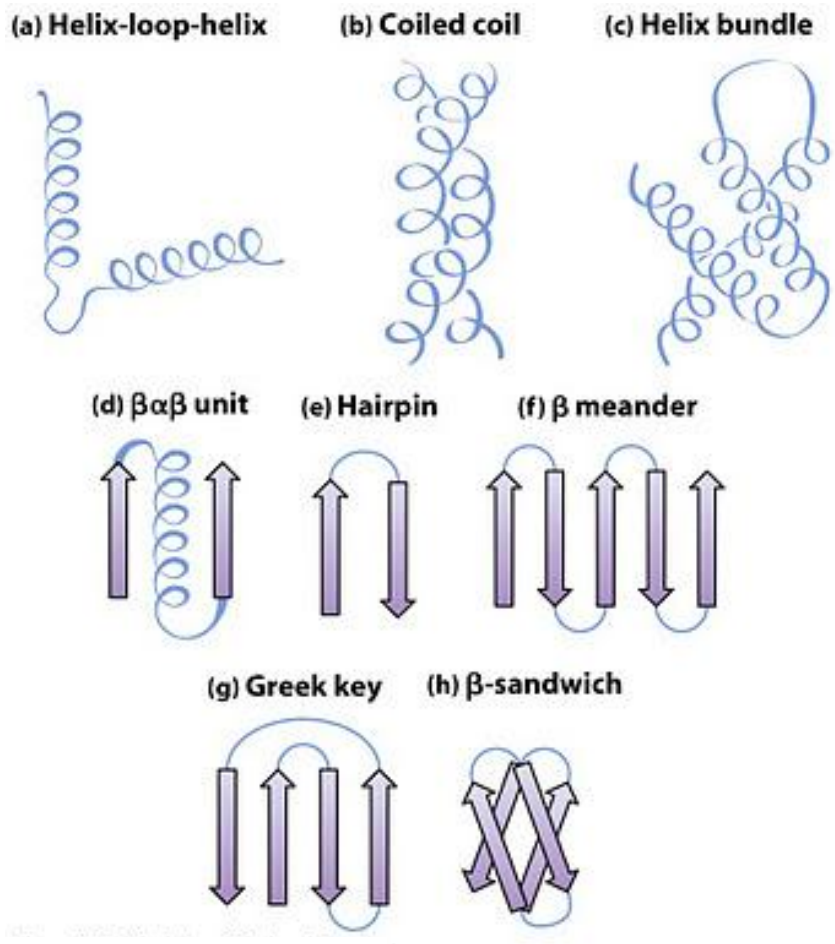
Beta-alpha-beta is an important supersecondary motif with two parallel beta strands linked by a cross-over alpha helix. This could either be a right-handed or left-handed crossover, but the right-handed crossover is more favorable. Beta barrels are their most common domain form. Beta sandwich is a flattened beta barrel.



Beta-alpha-beta

$\beta - \alpha - \beta$

Overview



Problem Set #2: Due Friday 10/19 at 5:00PM in FO 3.602
Exam #2 Review: TBA

Exam #2: Monday 10/22 at 10:00AM (Lee) in normal classroom
Tuesday 10/23 at 1:00PM (Marsh) in normal classroom

Contact email:
knh093020@utdallas.edu

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

E = enzyme, S = substrate, ES = enzyme-substrate complex, P = product

Assuming that [S] >> $[E]_o$, E binds to S to form ES complex with a 2nd order rate constant of k_1. ES can either (1) dissociate back into E and S with a 1st order rate constant of k_{-1} or it can (2) form E and P with a 1st order rate constant of k_2. In rapid equilibrium, we assume $k_{-1} >> k_2$ and that the ES complex falls apart much more quickly than S is converted to P. The rate limiting step is k_2 (also called k_{cat}). In steady-state equilibrium, we do NOT assume $k_{-1} >> k_2$ and that the ES complex converts S to P either more or less quickly than the ES complex falling apart back into E + S.

Definitions:

K_m: Michaelis constant with units in Molarity. It is the substrate concentration at which the initial velocity is half of its V_{max}. The lower the K_m, the higher affinity of the enzyme for the substrate and greater rate of reaction. It is important to note that K_m in the general equation does not equal K_S.

V_{max}: represents the maximum rate achieved by the system, at maximum (saturating) substrate concentrations

k_{cat}: catalytic rate constant (also called the turnover number) with units in s^{-1}; measure of how many bound substrate molecules turnover or form products in 1 second. In most cases, $k_2 = k_{cat}$ so that $v_0 = k_{cat}[ES]$. ES converts to form E and P with a 1st order rate constant of k_2.

k_{cat}/k_m: measures enzyme efficiency. If [S] << K_m then $v_o = k_{cat}/k_m\ [E]_t[S]$ and k_{cat}/k_m is a 2nd order rate constant with units of $M^{-1}s^{-1}$. A perfect enzyme has an efficiency of 10^8-10^9 and is only limited by diffusion.

Rapid Equilibrium (Michaelis-Menten):

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

Rapid equilibrium means $k_2 = k_{cat}$ = rate limiting

k_1 & $k_{-1} >> k_2$

$[E][S]k_1 = [ES](k_{-1})$

$$\frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} = K_S$$

K_S = substrate dissociation constant

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

V_{max} = $k_2[E]_{total}$; K_S = $\frac{k_{-1}}{k_1}$

If $k_{-1} >> k_2$, $k_S = K_m$ (follows rapid equilibrium assumption)

Steady-State Equilibrium (Briggs & Haldane):

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

Steady-state equilibrium means ES formation = rate of its breakdown

k_2 has to be relatively large enough to consider steady-state eq.

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

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

K_m = Michaelis constant

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

V_{max}

K_m

If $k_{-1} >> k_2$, $K_m = K_S$ (follows rapid equilibrium assumption)

Basis for enzyme assay:

$$v_o = \frac{k_2[E]_{total}[S]}{K_m+[S]}$$

V_{max}

$V_{max} = k_2[E]_{total}$ and K_m depends on k_1, k_{-1}, and k_2

As the concentration of substrate approaches infinity ($S >> K_m$), initial velocity reaches maximal velocity and the reaction is dependent only on E ($v_o = V_{max} = k_2[E]_o$). If the concentration of substrate is decreased drastically ($S << K_m$), then the reaction is bimolecular and dependent on both S and E ($v_o = V_{max}[S]/K_m = k_2[E]_oS/ K_m$).

$$v_o = \frac{k_2[E]_{total}[S]}{K_M+[S]} \rightarrow v_o = \frac{v_{max}[S]}{K_M+[S]} \rightarrow \frac{v_o}{v_{max}} = \frac{[S]}{K_M+[S]}$$

Enzyme Kinetics – Reversible Inhibition
Inhibitor: Any ligand, natural or synthetic, that decreases the velocity of an enzyme-catalyzed reaction
Effect: A change in K_m and/or V_{max} (apparent K_m (written as $K_{(m)}$ or $K_{m(app)}$)means presence of an inhibitor)

Types of Inhibitors:
<u>Competitive</u> – K_m ↑, V_{max} ↔
A direct competition exists between substrate and inhibitor for binding to the free enzyme. Thus, a higher level of substrate is required to obtain ½V_{max}. In most cases, the inhibitor binds in the active site.

<u>Uncompetitive</u> – K_m and V_{max} ↓ by same proportion
Inhibitor binds to the ES complex preventing the catalytic breakdown of substrate to the product. This results in a lower V_{max}. Since the substrate cannot compete with the inhibitor for binding (i.e., it is uncompetitive), the K_m, being [S] at ½V_{max}, is lowered proportionally.

<u>Noncompetitive</u> – K_m ↔ or ↑ or ↓ V_{max} ↓ proportionally to inhibitor concentration
Inhibitor binds to both free enzymes and ES complex outside the active site, modifying the structure of the enzyme so that substrate affinity is unchanged, reduced, or increased and product formation is prevented.
Two types of noncompetitive inhibition occur:
Pure (aka classic) – K_m ↔. This holds when α = 1.
Mixed - $K_{(m)}$ ↑. This holds when α > 1. Higher affinity for free enzyme.
$K_{(m)}$ ↓. This holds when α < 1. Higher affinity for ES complex.

K_I: dissociation constant for enzyme inhibitor complex. Effects best observed in Lineweaver-Burk plots.

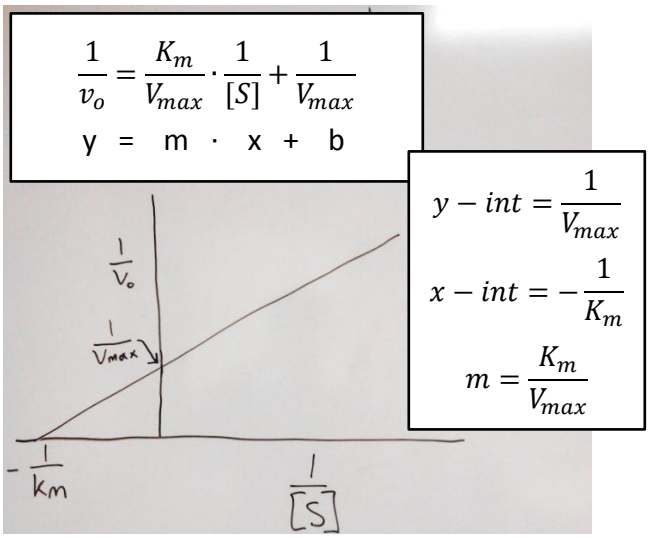
How can V_{max} and K_m be determined from experimental data?

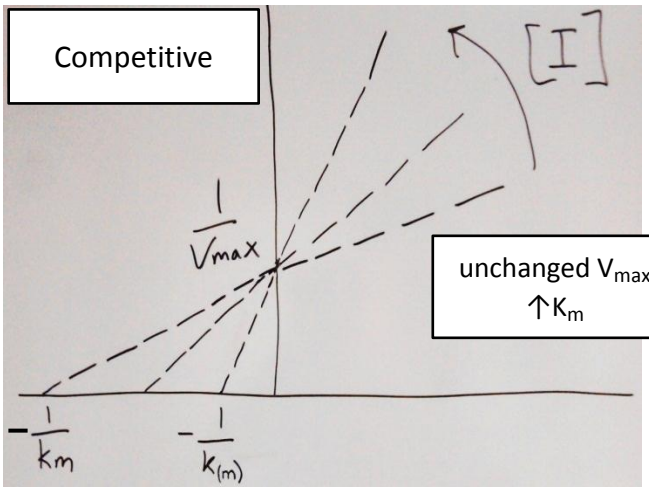
- nonlinear hyperbolic fit – plots v_o vs. [S]
  - $v_o = k_1$[reactant]

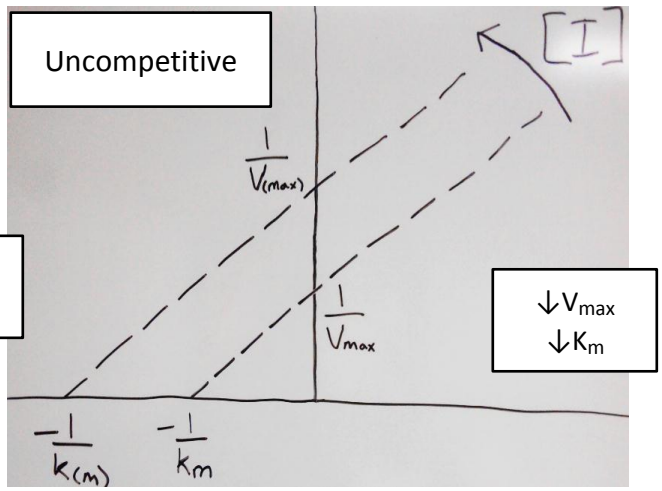
- double reciprocal plot (Lineweaver-Burk plot)
  - rearrange Michaelis-Menten equation
  - $\frac{1}{v_o} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$

- Scatchard plot – plots $[S]_{bound}$ / $[S]_{free}$ vs. $[S]_{bound}$
  - used to determine no. of binding sites and K_S of a substrate or any other ligand
  - $[S]_{bound} = \frac{[S]_{free}}{K_S}[E]_{free}$

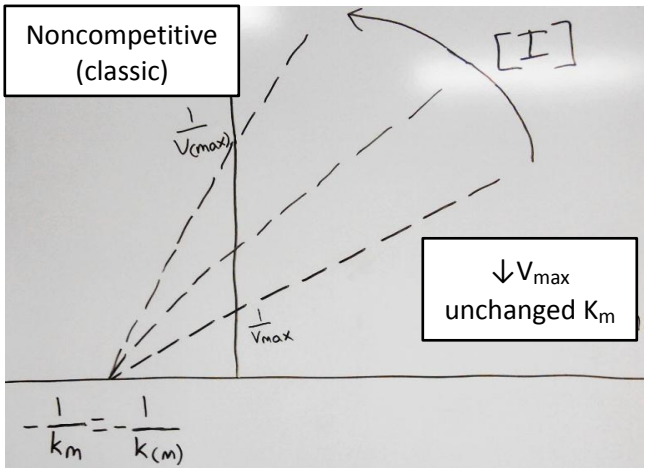
<u>Lineweaver-Burk Plot</u>:







Noncompetitive (classic)



Which type of inhibitor (competitive, uncompetitive, or noncompetitive)?

Can be determined from the trendlines on the graph and from the calculated V_{max}, K_m, $V_{(max)}$, and $K_{(m)}$.

Does V_{max} increase, decrease, or unchanged?

V_{max} → ____ $V_{(max)}$

Does K_m increase, decrease, or unchanged?

K_m → ____ $K_{(m)}$

Graph not shown for noncompetitive (mixed)

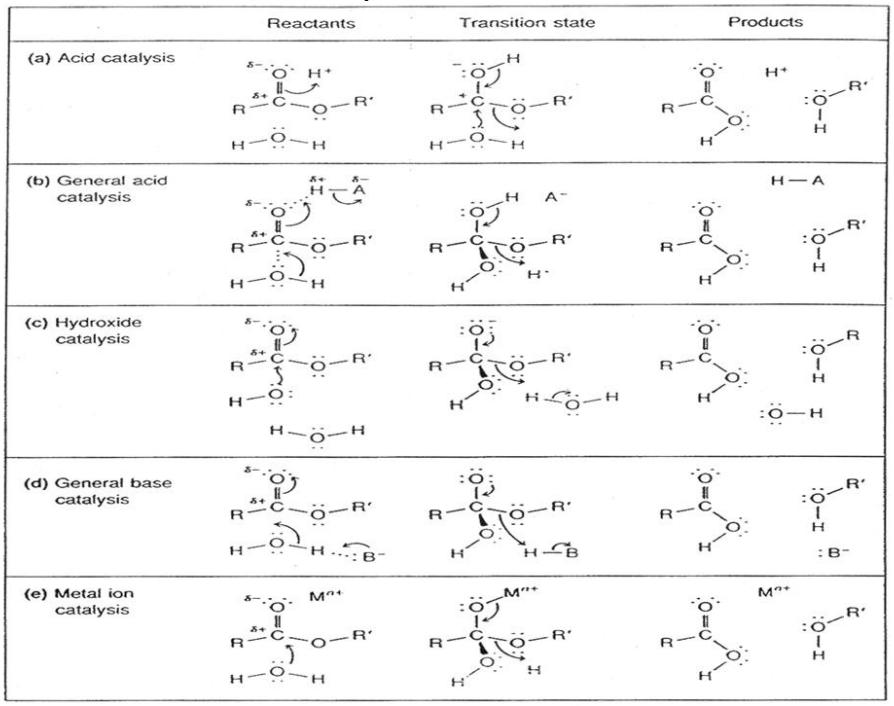
↓V_{max}

↑K_m if α > 1 ↓K_m if α < 1

TABLE 13.6 The Effect of Various Types of Inhibitors on Apparent K_m and Apparent V_{max}

Inhibition Type	Apparent K_m	Apparent V_{max}
None	K_m	V_{max}
Competitive	$K_m(1 + [I]/K_I)$	V_{max}
Noncompetitive	K_m	$V_{max}/(1 + [I]/K_I)$
Mixed	$K_m(1 + [I]/K_I)/(1 + [I]/\alpha K_I)$	$V_{max}/(1 + [I]/\alpha K_I)$
Uncompetitive	$K_m/(1 + [I]/K_I)$	$V_{max}/(1 + [I]/K_I)$

Catalytic Mechanisms

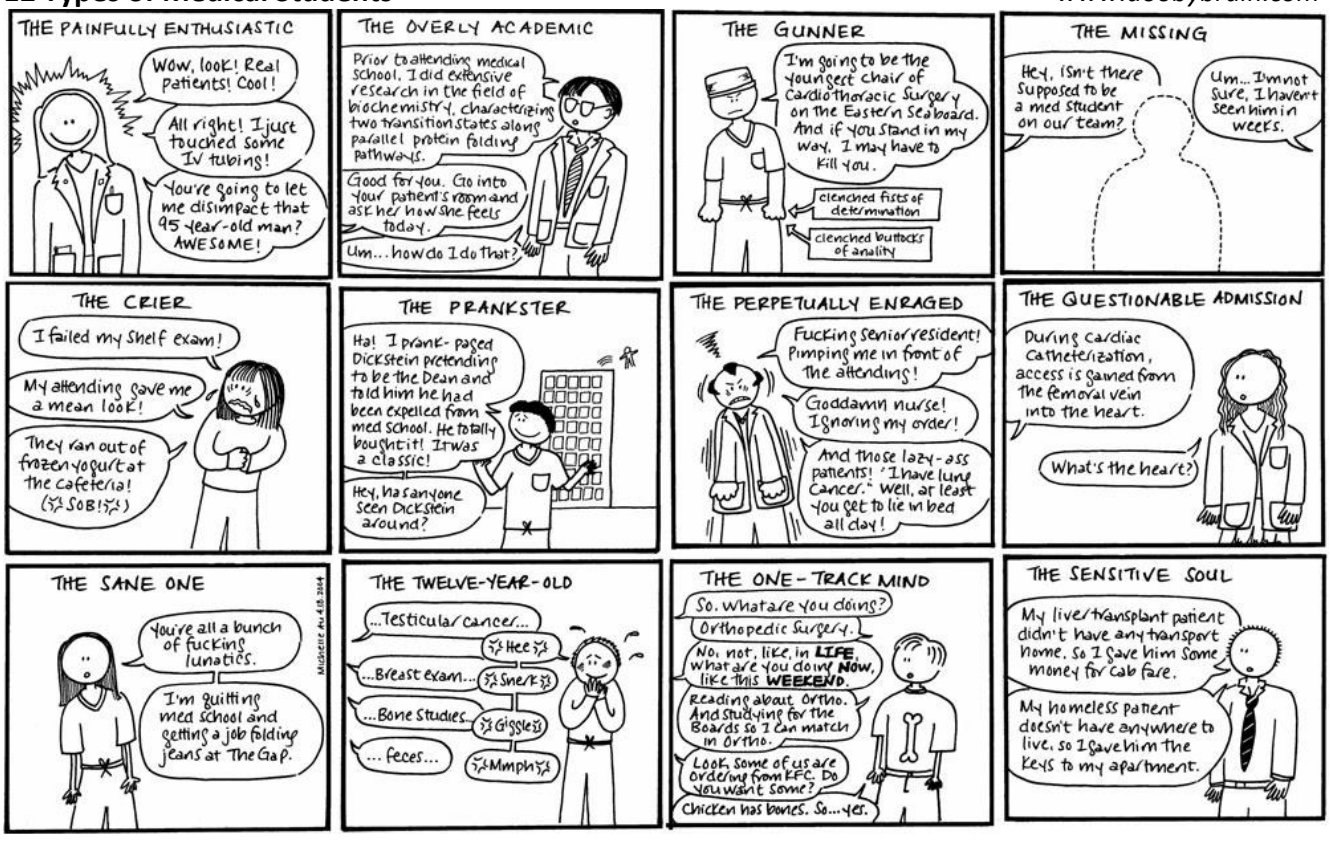


4

Kirk's Amazing Notes for Enzymes

Number	Classification	Biochemical Properties
1	Oxidoreductases	Act on many chemical groupings to add or remove hydrogen atoms. $A^- + B \leftrightarrow A + B^-$
2	Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules. $A\text{-}B + C \leftrightarrow A + B\text{-}C$
3	Hydrolases	Add water across a bond, hydrolyzing it. $A\text{-}B + H_2O \leftrightarrow A\text{-}H + B\text{-}OH$
4	Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds. $\begin{matrix} X & Y \\ \vert & \vert \\ A\text{-}B & \end{matrix} \leftrightarrow A{=}B + X\text{-}Y$
5	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others. $\begin{matrix} X & Y \\ \vert & \vert \\ A\text{-}B & \end{matrix} \leftrightarrow \begin{matrix} Y & X \\ \vert & \vert \\ A\text{-}B & \end{matrix}$
6	Ligases	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP. $A + B \leftrightarrow AB$

12 Types of Medical Students *www.doobybrain.com*



Problem Set #2: Due Friday 10/19 at 5:00PM in FO 3.602
Exam #2 Review: TBA
Exam #2: Monday 10/22 at 10:00AM (Lee) in normal classroom
Tuesday 10/23 at 1:00PM (Marsh) in normal classroom

Kirk's Amino Acids Chart

Note: an amino acid may be considered to belong to more than one category. For charge state, consider physiological condition (~pH 7). The range of pKa for α-COOH (1.7-2.6) and for α-NH_3^+ (8.8 to 10.8).

Name	Letter Codes	Chemical Structure of Side Chain R	Hydrophobic	Polar	Charged	Aromatic	Side Chain pKa
Alanine	Ala A	—CH_3	Y	N	N	N	
Arginine	Arg R	—$(CH_2)_3NH$-$C(NH)NH_2$	Y	Y/N	Y(+)	N	12.5
Asparagine	Asn N	—CH_2CONH_2	N	Y/N	N	N	
Aspartic acid	Asp D	—CH_2COOH	N	Y/N	Y(-)	N	3.9
Cysteine	Cys C	—CH_2SH	Y/N	Y/N	N	N	8.3
Glutamine	Gln Q	—$CH_2CH_2CONH_2$	N	Y/N	N	N	
Glutamic acid	Glu E	—CH_2CH_2COOH	N	Y/N	Y(-)	N	4.3
Glycine	Gly G	—H	Y/N	Y/N**	N	N	
Histidine	His H	—CH_2-$C_3H_3N_2$	Y	Y/N	Y(+)	Y	6.0
Isoleucine	Ile I	—$CH(CH_3)CH_2CH_3$	Y	N	N	N	
Leucine	Leu L	—$CH_2CH(CH_3)_2$	Y	N	N	N	
Lysine	Lys K	—$(CH_2)_4NH_2$	Y	Y/N	Y(+)	N	10.5
Methionine	Met M	—$CH_2CH_2SCH_3$	Y	N	N	N	
Phenylalanine	Phe F	—$CH_2C_6H_5$	Y	N	N	Y	
Proline	Pro P	—$CH_2CH_2CH_2$-	Y	N	N	N	
Serine	Ser S	—CH_2OH	Y/N	Y/N	N	N	13
Threonine	Thr T	—$CH(OH)CH_3$	Y	Y/N	N	N	13
Tryptophan	Trp W	—$CH_2C_8H_6N$	Y	Y/N*	N	Y	
Tyrosine	Tyr Y	—CH_2-C_6H_4OH	Y	Y/N*	N	Y	10.1
Valine	Val V	—$CH(CH_3)_2$	Y	N	N	N	

AVLIPMF have entirely nonpolar R groups. The rest are amphiphilic (varying amounts of polar and nonpolar parts).
*Y and W have the smallest polar portion.
**Glycine with only H as its R group can be accommodated in both polar and nonpolar environments. As such, it is found both inside and on the surface of proteins. Its polar peptide linkages are the major determinant of its property.

20 Standard Amino Acids