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Kirk's Exam 2 Review

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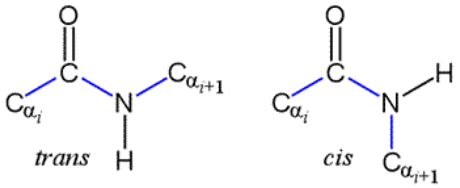
CHAPTER 6: PROTEIN FOLDING

1. Average packing density of 0.74 for protein
   - If the sum of the van der Waals forces of a protein's constituent amino acids is divided by the volume occupied by the protein, the packing density is 0.74 on average.

2. Definitions for orders of protein structure, including supersecondary and domains
   - Primary: sequence of amino acids and their linkage by peptide bonds to form polymer, including post-translational modifications and disulfide bonds
   - Secondary: regular recurring arrangements of polymer in pace, i.e, the helices formed by the polymer backbone
     a. Super secondary structure (motif): short-range associations of secondary structural elements, often through side chain interactions
     b. Domains: associations of lower order structure to form a 3-D unit.

3. Planar structure and cis/trans configuration of peptide bond unit; reason for and consequences of
   - The six atoms of the peptide bond group are always planar due to its double bond character. The resonance stabilization energy of this structure is ~88kJ/mol.
   - Due to this high energetic barrier, only two stable configurations are possible, cis and trans; however, the trans configuration is by far the most common.



4. Definition of ϕ and ψ angles; ϕ angle of pro is fixed
   Φ (phi) – represents the angle about the C_α-N bond
   Ψ (psi) – represents the angle about the C_α-C_o bond.
   Psi angle of proline is fixed at -60^o

5. What a Ramachandran plot shows; why glycine can be located anywhere on the plot
   - A Ramachandran plot is a plot of ψ vs. ϕ, and it shows the sterically reasonable values of those angles.
   - Glycine can be located anywhere on the plot because it does not have a side chain that would create steric crowding.
   - Upper left quadrant contains the β-sheets, lower left quadrant contains the α-helices.

6. Types of secondary structure: α, β, etc; their characteristics, e.g., pitch, handedness, foldedness and placement of side chains
   - Alpha Helix
     - 3.6 residues per turn; interchain H-bonds between the N-H and O=C every 5^{th} residue, i.e., every fourth peptide bond.
     - Stabilized by the H-bonds between the N-H and C=O groups of peptide bonds.
     - Called 3.6_{13} because a single turn involves 13 atoms

- As helix turns you come back to the exact same location every 5 turns, or 18 residues, which is also why it is also called 18_5
- Pitch = 3.6 residues x 0.15nm per residue = 0.54nm per turn
- Left handed, side chains extend outward from the core structure of the helix.

- Beta sheet
  - 2 fold helix, i.e., 2 residues per repeat
  - Extended zig-zag structure
  - Repeat length 0.650 (parallel) – 0.695 nm (antiparallel)
  - Interchain H-bonds b/t N-H and O=C of peptide bonds
  - Parallel or anti-parallel alignment.
  - Anti-parallel is energetically more favorable.
  - Parallel sheets distribute hydrophobic side chains on both sides of the sheet, whereas antiparallel sheets are usually arranged with all their hydrophobic residues on one side of the sheet.

7. Why α helix and β conformation predominate (i.e., because they represent minimum free energy states)
   - Because they represent minimum free energy states.

8. Why parallel β sheets are in the interior of proteins and are less stable than antiparallel sheets (viz., less favorable skewed H-bonds can't compete with H-bonding to water)
   - Parallel β-sheets are in the interior of proteins because they have hydrophobic residues sticking out on both sides. They are less stable because the H-bonds formed in parallel β-sheets are bent significantly

9. To recognize the pattern of hydrophobic residues alternating with hydrophilic residues in polypeptide strands in antiparallel sheets at the surface of proteins
   - Just a definition question, know that residues in polypeptide chains in anti-parallel sheets at the surface of proteins will have alternating hydrophilic and hydrophobic residues.

10. To recognize the pattern of Gly-X-Pro or Gly-Pro-X found in collagen strands
    - Another definition: just know that collagen has a Gly-X-Pro or Gly-Pro-X pattern, where X can be any amino acid but is frequently proline. Hydroxyproline can also usually be found in the place of proline.

11. Five types of supersecondary structure, including α helix coiled-coil (e.g. leucine zipper with heptad repeats having leu or similar amino acid at positions 1 and 4 of 7-residue repeats)
    - **Coiled coil**: bindle of alpha helices wound into a super helix. The coils interact through nonpolar residues at positions 1 and 4 of heptad repeats. The left-handed twist of the structure reduces the number of residues per turn to 3.5 so that the positions of the side chains repeat every 7 residues. So two right handed alpha helices coil together to make a left handed coil.
      - The leucine zipper is a classic example of a coiled coil, with heptad repeats having leucine or similar amino acid (isoleucine) at positions 1 and 4 of 7-residue repeats.
    - **Helix-turn-helix**: two alpha helices joined by a short peptide chain
    - Helix-loop-helix: alpha helixes joined by a peptide chain that loops

- **Beta Hairpin**: formed by two anti-parallel beta-strands connected by a hairpin peptide sequence that is often assisted by Proline, which allows it to make that turn back around to the opposite direction.
- Beta Meander: two or more beta anti-parallel beta strands connected by hairpin loops
- **Beta Greek Key**: consists of two sets of anti-parallel beta strands. 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-alpha-Beta**: two parallel beta strands are linked as 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.

12. Structures of β barrels, β sandwiches, and β propellers as bases of domains; what supersecondary structures they contain
    - **Beta barrels**: formed when a beta sheet is wrapped around to make a barrel, can be made up by hairpins, greek keys, or beta-alpha-beta supersecondary structures.
    - **Beta sandwich**: a flattened beta barrel
    - **Beta propeller**: is made up of anti-parallel beta sheets arranged kind of like a circle, forming a propeller. But the anti-parallel sheets can also be perpendicular to the central axis. I think the hairpin loops are what make this kind of domain?

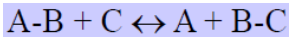
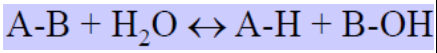
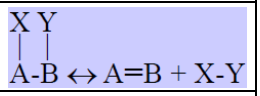
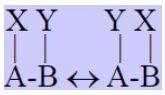
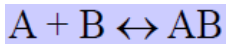
LOOK AT KIRK'S NOTES ON SUPERSECONDARY STRUCTURES!

13. Properties of IUPs (Intrinsically Unstructured Proteins)
    - Some proteins exist and function normally in a partially unfolded state
    - These IUPs do not possess uniform structural properties but are still essential for cellular function
    - These proteins are characterized by a nearly complete lack of structure and a high intramolecular flexibility
    - IUPs adopt well-defined structures in complexes with their target proteins
    - IUPs are characterized by an abundance of polar residues and a lack of hydrophobic residues

CHAPTER 13: ENZYME KINETICS

14. Names of the six E.C. classes of enzymes and be able to recognize their reactions
    - Oxidoreductase: oxidation-reduction in which oxygen or H is gained or lost
    - Transferase: transfer of functional group
    - Hydrolase: addition of water
    - Lyase: addition to double bond or reverse
    - Isomerase: rearrangement of atoms within a molecule
    - Ligase: formation of a bond with cleavage of ATP

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. 
3	Hydrolases	Add water across a bond, hydrolyzing it. 
4	Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds. 
5	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others. 
6	Ligases	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP. 

15. Difference between Michaelis and allosteric enzymes
    - Allosteric enzymes require effector molecules that bind to a site other than the active site in order for it to be activated or inhibited. Their curve is sigmoidal.
    - Michaelis enzymes do not have effector molecules that exert a positive or negative effect on enzyme activity. Michaelis enzymes have a hyperbolic curve.

16. Michaelis-Menton and Briggs-Haldane equations; be able to use to solve problems
    - Michaelis-Menton: assumes rapid equilibrium, where the ES complex is dissociated back into their free forms and product formation is rate limiting: k_1 and $k_{-1} >> k_2$.

      $$\mathbf{v_o} = \frac{V_{max}[S]}{K_S + [S]}$$
    - Briggs-Haldane: assumes steady-state. ES is formed as rapidly as it is lost: either by dissociation to regenerate more E and S, or by reaction to generate E and P.

      $$\mathbf{v_o} = \frac{V_{max}[S]}{K_m + [S]}$$

17. Understand meaning of K_S, K_m, k_{cat} (turnover number), and k_{cat}/K_m ratio
    - K_s: dissociation constant for rapid equilibrium
    - K_m: dissociation constant for steady-state equilibrium
    - k_{cat}: The turnover number, k_{cat}, is a measure of its maximal catalytic activity.
    - It is the number of substrate molecules converted to product per enzyme molecule per unit time when the enzyme is saturated with substrate: $[ES] = [E_T]$.
    - **$k_2 = k_{cat} = V_{max}/[E_T]$**, in most cases $k_2 = k_{cat}$
    - **k_{cat}/K_m:** measures the **catalytic efficiency** in $M^{-1}sec^{-1}$. Cannot exceed 10^9 $M^{-1}sec^{-1}$.

18. Know expected cellular [S] based on K_m and efficiency of perfect enzyme based on k_{cat}/K_m ratio
    - Inside the cell, [S] is seldom saturating, and is usually less than K_m.
    - Efficiency of a perfect enzyme is on the order of 10^9 $M^{-1}sec^{-1}$.

19. Kinetic basis for enzyme assay. How K_m is determined
    - See slides 16 and 17 in Chapter 13A Enzyme Kinetics.

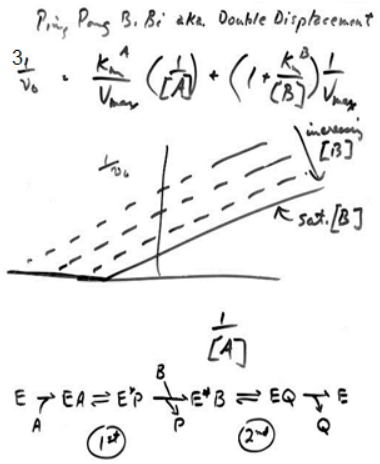
20. The three types of reversible enzyme inhibitors; their distinguishing effects on K_m and V_{max}; and their diagnostic Lineweaver-Burk plots
    - Competitive: K_m increased, V_{max} unchanged. Inhibitor competes for same active site as substrate
    - Uncompetitive: K_m and V_{max} decreased by the same proportion. Inhibitor binds to the ES complex, preventing the catalytic breakdown of substrate to product.
    - Noncompetitive: K_m unchanged, increased, or decreased, V_{max} unchanged. Inhibitor binds to both free enzymes and ES complex outside of the active site, modifying the structure of the enzyme so that substrate affinity is unchanged, reduced, or increased and product formation is prevented. See slide 11 on Enzyme Kinetics Cont. for diagnostic plots.

21. Formulas for K_m apparent for different types of inhibition, e.g. K_m app = $K_m(1+[I]/KI)$ for competitive inhibition; be able to solve for K_I or true K_m
    - Competitive: $\mathbf{K_{(m)} = K_m(1 + [I]/K_I)}$ $\mathbf{V_{(max)} = V_{max}}$
    - Uncompetitive: $\mathbf{K_{(m)} = K_m/(1 + [I]/K_I)}$; $\mathbf{V_{(max)} = V_{max}/(1 + [I]/K_I)}$
    - Pure Noncompetitive: $\mathbf{K_{(m)} = K_m}$: $\mathbf{V_{(max)}} = \frac{V_{max}}{1+\frac{[I]}{K_I}}$
    - Mixed Noncompetitive: $\mathbf{K_{(m)}} = \frac{K_m(1+\frac{[I]}{K_I})}{1+\frac{[I]}{\alpha K_I}}$; $\mathbf{V_{(max)}} = \frac{V_{max}}{1+\frac{[I]}{\alpha K_I}}$

LOOK AT KIRK'S AMAZING NOTES ON ENZYMES!

22. How sulfa drugs and viagra work and why ethanol is an antidote for methanol or anti-freeze poisoning
    - How Viagra works…
      - Increased cGMP levels result in relaxation of smooth muscle in walls of blood vessels.
      - Inhibiting cGMP hydrolysis by inhibition of phosphodieaterase in penile tissue boosts cGMP levels, increasing penile vascular muscle relaxation and improving erection.
    - How sulfa drugs work…
      Sulfa drugs such as sulfanilamide are structurally similar to substrates such as PABA, which are required in bacteria to make folic acid. The sulfa drug acts as a competitive inhibitor to PABA to block the synthesis of folic acid, and thus limits the growth of bacteria.
    - Why ethanol is an antidote for methanol or anti-freeze poisoning.
      - Methanol is oxidized to formaldehyde and then to formic acid, attacking the optic nerve, causing blindness and death.
      - Alcohol is detoxified in the liver by conversion to acetaldehyde (by alcohol dehydrogenase) and then acetaldehyde is oxidized to acetate by aldehyde dehydrogenase. Acetate can then be broken down by the body. Both enzymes require NAD^+.
      - Ethanol is a competitive inhibitor of methanol because alcohol dehydrogenase (ADH) has a higher affinity for ethanol than for methanol. This prevents accumulation of toxic metabolites (ex. formaldehyde) derived from methanol. Thus, methanol can be excreted from the body while ADH acts on ethanol.
      - Anti-freeze contains ethylene glycol, which converts to glycolic acid, glyoxylic acid and oxalic acid, which are highly toxic compounds. Ethanol acts as a competitive inhibitor to anti-freeze in the same way as it does for methanol.

23. Types of bisubstrate reactions and their diagnostic Lineweaver-Burk plots (Don't need to know the equations)
    - Single Displacement: both substrates bind first before products are formed.
      E + A + B → AEB → PEQ → E + P + Q
    - Random: either A or B may bind to the enzyme first, followed by the other substrate
      - Ordered: where A, designated the leading substrate, must bind to E first before B can be bound.
      - See pg. 403 in text for Single displacement graph (know how to draw and label!).
    - Double Displacement: two substrates bind and react separately in a ping pong manner.
      E binds to A to form AE, which converts to PE', then P is released, then B bind to E′ to form E'B, which converts to EQ, and Q is released with free E.
      - See bottom of pg. 406 in text for reaction mechanism, and top of pg. 407 for the plot.

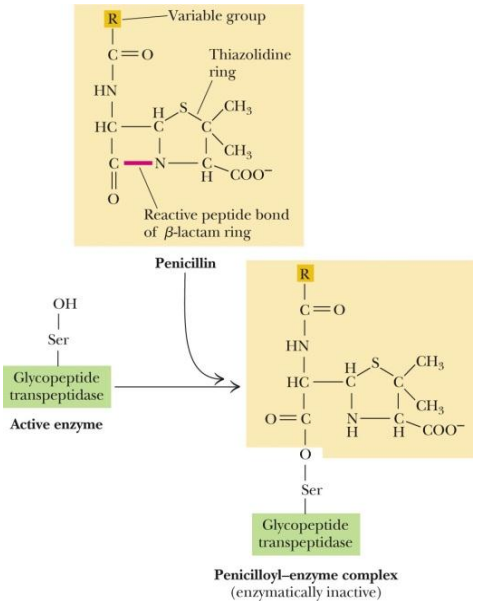


24. Other ways to diagnose type of bisubstrate reaction (i.e., ligand binding studies and exchange reactions)
    1. Kinetic analysis – e.g., LB plot to distinguish if mechanism is single or double displacement.
    2. Substrate binding assay to distinguish if substrate addition is random or ordered. Number of binding sites and K_s are determined by a Scatchard Plot.
    3. Exchange reactions – i.e., attempt reverse reaction in absence of one substrate to distinguish if mechanism is single or double displacement.
       See slide 23 for better details.

25. What a Scatchard plot shows
    - This is a plot of $[S]_{bound}/[S]_{free}$ vs. $[S]_{bound}$
    - It is used to determine the number of binding sites and K_s for a substrate or any other ligand.
    - Scratchard Plot formula: $[S]_b/[S]_f = (-1/K_S)[S]_b + (n[E]_T/K_S)$

26. How irreversible inhibitors work (they covalently bond to enzymes) and several examples: organophosphorus nerve gases and insecticides, penicillin (suicide substrates)
    - Irreversible inhibitors usually covalently modify an enzyme which then causes the inhibition to not be reversed. They work by altering the active site of their target which decreases the concentration of active enzyme.

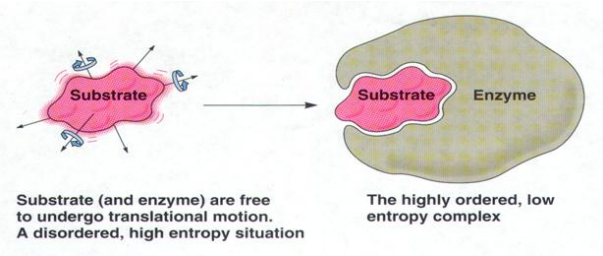
- Suicide substrates: has a very reactive group generated which then forms a covalent bond with a nearby functional group within the active site of the enzyme causing irreversible inhibition. They are a type of affinity label in which it becomes covalently linked to a functional group.
- Penicillin: exerts its effects by covalently reacting with an essential serine residue in the active site of glycopeptides transpeptidase. Penicillin consists of a thiazolidine rings fused to the beta-lactam ring. A reactive peptide bond in the beta lactam ring covalently attaches to the serine residue. The penicillinoyl-enzyme complex is catalytically inactive.
- Nerve gases and organophosphorus insecticides work by irreversibly inhibiting the breakdown of the neurotransmitter acetylcholine by acetylcholinesterase. The active site of acetylcholinesterase is blocked by phosphorylation of the catalytic serine residue.



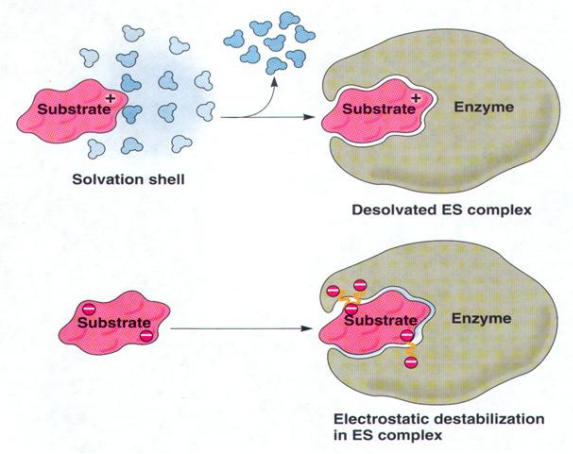
CHAPTER 14: ENZYME MECHANISMS

27. Enzymes catalyze reactions by lowering activation energy, not by changing ΔG for the reaction
    - Just a definition, but see section 14.2 on pg 420-421 for a nice explanation.
    - ΔG and K_{eq} do not change but forward rate of rxn increases, achieved by lowering TS $\Delta G^{\ddagger}$

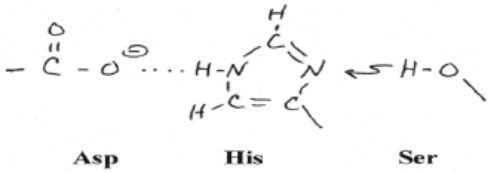
28. The seven reasons listed in lecture notes for catalysis by enzymes
    1. Stabilization of transition state intermediates: lowering 4 transition states in graph = faster
        a. Transition state analogs-tight binding than substrate, competitive inhibitor
           Examples –Tamiflu: transition state analog to viral neuraminidase
        b. Protease inhibitor: transition state analog to HIV protease
    2. Proximity and orientation of substrates
        a. Orientation effects in intramolecular reactions (steric effects)
    3. Entropy loss in ES formation: more ordered and thus it has a higher energy state



4. Destabilization of ES due to:
   - geometric strain, electronic strain, or desolvation of substrate = more energy

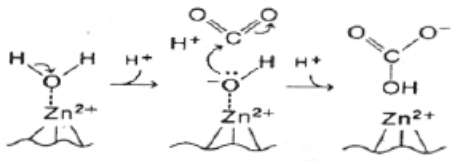


5. Formation of unstable covalent intermediates (side chains)
   - Side chain of reactive amino acid
   - Prosthetic groups, i.e., coenzymes: pyridoxol phosphate, thiamine pyrophosphate
6. General acid/base catalysis: proton donation or acceptance
   a. By amino acid side chains: acid and conjugate base of glu, asp, his



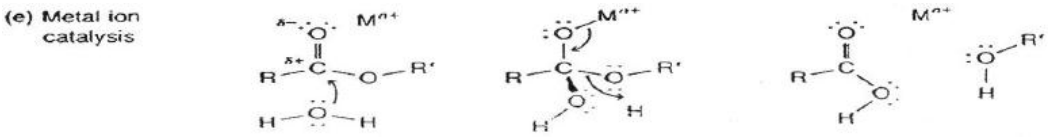
serine proteases

   b. Indirectly via metal ion catalysis, Zn^{2+} bound OH^-



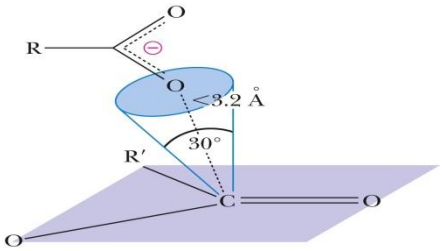
carbonic anhydrase

7. Metal ion catalysis: (Zn^{2+}, Mn^{2+})
   a. As above via general base catalysis
      - Neutralization of (-) charge in the substrate → approach of the nucleophile
      - Stabilization of leaving group (neutralization of charge)
      - Binds to negative groups or ones with lone pairs such as histidine, cysteine, aspartate, and glutamate)
   b. Via electronic strain due to ability to polarize bonds

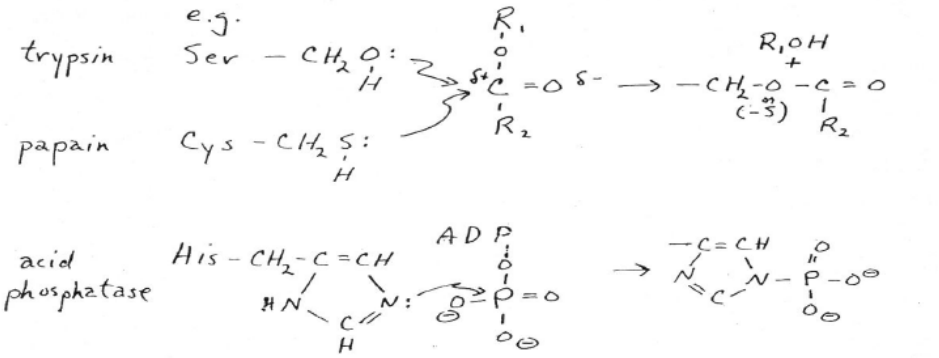


29. The properties of NACs (Near-Attack Conformations)
    - Near attacking conformations (NACs) are when catalytic groups are precisely positioned for their roles by the enzyme.

- Such preorganization selects substrate conformations in which the reacting atoms are in van der waals contact and at an angle resembling the bond to the formed transition state.
- NACs are precursors to reaction transition states.
- They are characterized as having reacting atoms with 3.2 Å and an approach angle of +/- 15 degrees of the bonding angle in the transition state - precursors to transition state.



30. The amino acids that can form unstable covalent intermediates during catalysis (ser, thr, tyr, cys, lys, his, asp, glu) and the types of unstable covalent bonds formed, including Schiff base formation with lys
    - Formation of unstable covalent intermediates occur with side chains of reactive amino acids (ser, thr, tyr, cys, lys, his, asp, glu) and can form the following unstable covalent bonds: trypsin, papain, acid phosphatase, or aldolase (lysine forming a Schiff base). RCH=NR'

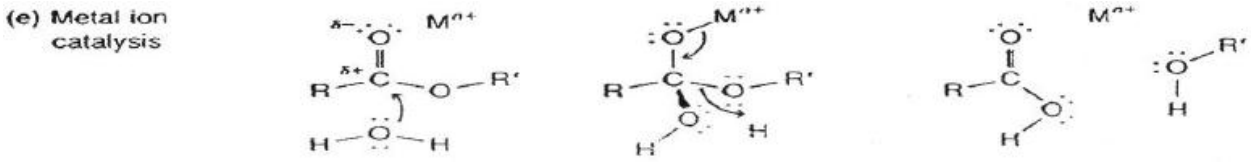


31. Characteristics of general acid and general base catalysis and the amino acids that can act as general acid and general base catalysts (asp, glu, his, cys, tyr, lys)
    - Specific acid base catalysis: in which reaction is accelerated by H^+ or OH^- diffusing around in the solution
    - General acid-base catalysis: in which H^+ or OH^- is created in the transition state by another molecule or group. By definition, general acid-base catalysis is catalysis in which a proton is transferred in the transition state.
    - Formation of hydrogen bond between proton donor and acceptor = rate limiting step

32. Ways pKa values can be shifted to permit catalysis (i.e. exam 1 info)
    - Please read pg. 433, "A Deeper Look", for a good example and explanation. The shifting of pKa values depend on the environment that the residue is in…. For example, the pKa of an acid increases if it is in a hydrophobic environment, whereas the pKa of a base decreases. Such changes allow for transferring of protons in catalysis.
    - $Lys^{2+} \rightarrow lys^{+} \rightarrow lys^{0} \rightarrow lys^{-}$ (by changing environment and its dielectric constant-hydrophobicity)
        - Facilitates transfer of proton in catalysis

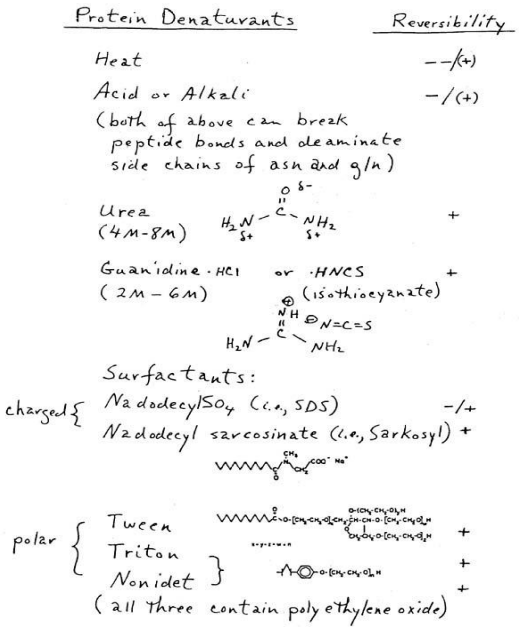
33. Properties of LBHBs (Low-Barrier H-Bonds) and role in catalysis
    - As distance of H bonds get closer, they become stronger and are almost like covalent bonds (<0.25 nm)
    - Stabilization energies can approach 60 kJ where the pKa values of the 2 EN atoms must be similar to form an LBHB
    - The energy released in forming an LBHB can help in catalysis

34. Characteristics of metal ion catalysis with Zn^{2+} as an example
    - Metal ion catalysis: occurs via general base catalysis or electronic strain due to ability to polarize bonds. One role for metals is to act as an electrophile catalyst, stabilizing the increased electron density or negative charge that develop during reactions
    - Metals such as Zn, Cu, Mo, Mg, or Fe bind to negative groups or ones with lone pairs such as histidine, cysteine, aspartate, and glutamate)
    - Zn^{2+} often is chelated with 3 side chains (e.g., His) and in the 4th position it can be chelated with H_2O to generate OH^- at pH 7 to serve as a nucleophile.



35. The catalytic amino acids and their roles in the serine (also applies to cysteine) and asp proteases
    - Serine proteases: consists of the triad: His, Asp, and Ser. Its mechanism follows a mixture of covalent and general acid-base catalysis
        - asp-102 functions to immobilize and orient His-57 via charge interaction
        - his-57 acts as a general acid and base
        - ser-195 forms a covalent bond with peptide to be cleaved
        - covalent bond formation turns trigonal C to tetrahedral C
        - the tetrahedral oxyanion intermediate is stabilized
    - Aspartic protease mechanism: all involved 2 asp residues at the active site. They work together as general acid base catalysts
        - asp proteases employ LBHB in their mechanism
    - Lysozymes: hydrolyzes polysaccharide chains and ruptures certain bacterial cells by breaking down cell wall
        - glu-35 – general acid
        - asp-52 – forms covalent intermediate

36. Types and examples of protein denaturants and how they work
    - Detergents, urea, high pH
        - Urea is a biological compound that handles the disposal of excess nitrogen in the body and acts as an agent in the denaturation of proteins. These denaturants unravel the tertiary structure of proteins by destabilizing internal, non-covalent bonds between atoms. One method involves direct interaction via hydrogen bonding to polarized areas of charge. Urea can also denature proteins indirectly.

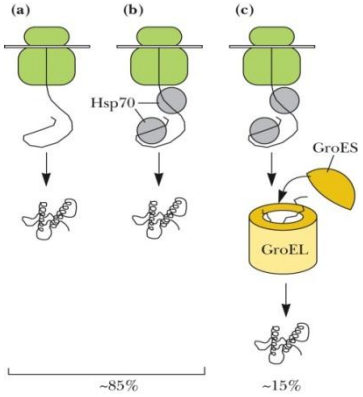


+ Often a protein can be renatured

-/+ Sometimes a protein can be renatured after mild treatment

37. T_m of a protein is the melting temperature, where 50% of the protein molecules are denatured
    - T_m represents a characteristic melting temperature, anything above this temperature causes the structure to unfold and aggregate thereby losing its function

38. Chaperones: catalysts for protein folding – Hsp70 and chaperonin (aka. Hsp60) – and how they work using ATP hydrolysis to drive conformational changes
    - Chaperones help some proteins fold. Many are heat shocked proteins (HSPs) where the principal ones are Hsp70, Hsp60, and Hsp90. Binding and hydrolysis of ATP by these three chaperones drives the folding process. They work by preventing intermolecular nonproductive interactions between hydrophobic sequences until productive folding interaction can occur.



39. RNA enzymes are called ribozymes, which are limited in reactions catalyzed due to presence of only –OH catalytic groups on the ribose

40. The peptidyl transferase of ribosomes is a ribozyme

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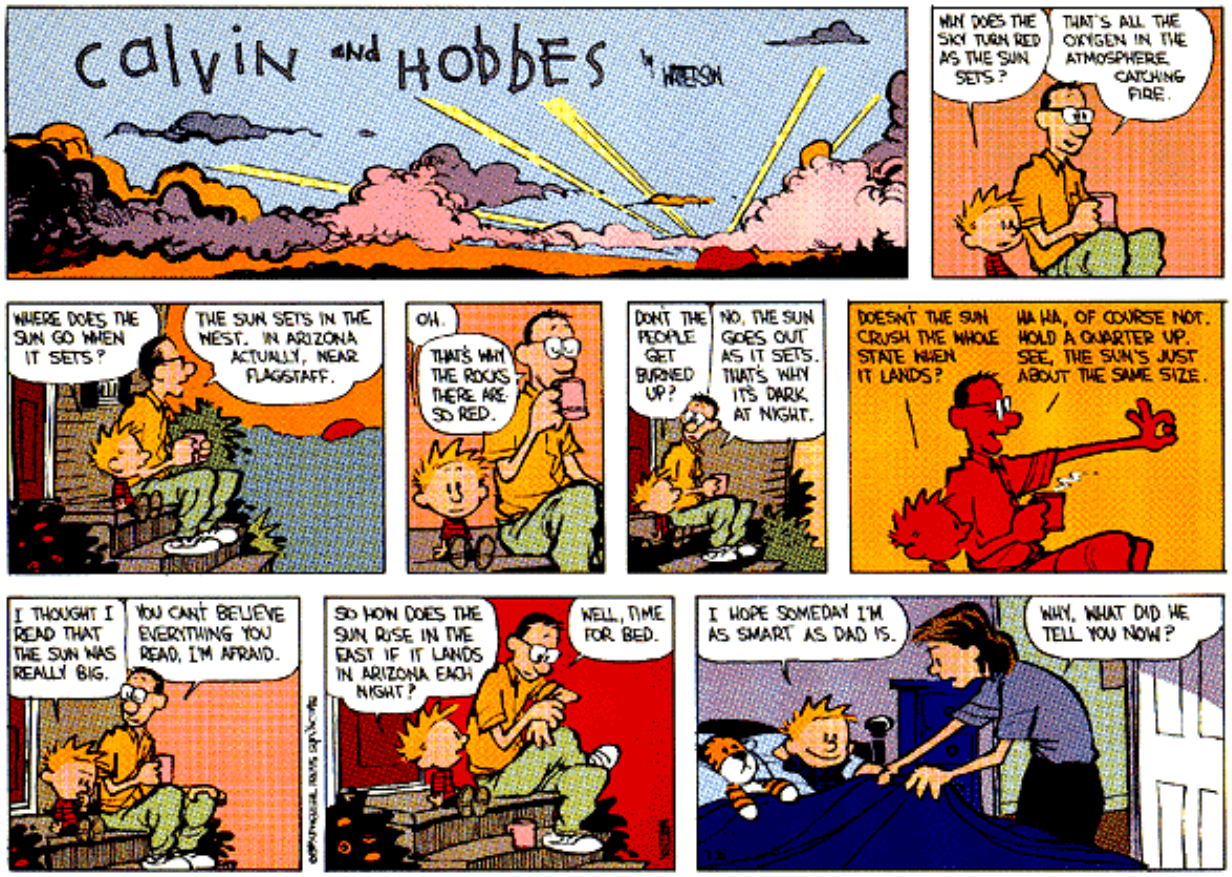

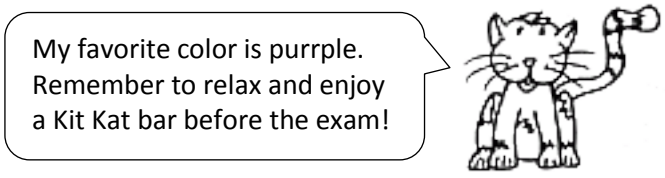

Problem Set #2: Due Friday 10/19 at 5:00PM in FO 3.602
Exam #2 Review: TBA

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