BIOL/CHEM 3361 Biochemistry I
Fall 2011

Due: Fri., Oct. 14 at 5:00 pm in FO 3.602
(*No late Problem Sets will be accepted.)*
(You may turn them in early, at lecture on Wed. 10/12, at a workshop, or at FO 3.602, to void last minute emergencies.)

PROBLEM SET 2

For full credit, **all steps** to the solutions of the following problems must be shown. You may work together on the problems, but you may not copy or plagiarize. Your answers must show your own math steps and be in your own words.

1. The nonapeptide leu-val-leu-trp-glu-lys-leu-his-arg was isolated from a partial trypsin-digest of a leucine zipper protein. Assume the pK_as for the amino acid side chains are as given in Table 4.1 in the Garrett and Grisham text and that the pK_as of any N- and C-termini are 4.5 and 9.5, respectively.
   a. If this nonapeptide is hydrolyzed by V8 protease, what products will result?
   b. If these products are separated by reverse-phase chromatography at pH 5.5, in what order will the products emerge from the column? Explain Why.
   c. Are the chances high, medium, or low that this tryptic peptide originated from the leucine zipper part of the protein? Explain.

2. Assume you completely digest the nonapeptide in question 1 with trypsin.
   a. What would be the digestion products?
   b. If you choose to separate these products using CM-chromatography, calculate the specific pH range you should use to bind both peptides to the column prior to elution? Assume a pK_a of 4.5 for the chromatography resin; other pK_as as in question 1.
   c. Which peptide will elute first from the column? Explain why.

3. Cell membranes are comprised of a 3.0 nm-thick lipid bilayer plus embedded proteins. To create a channel through the membrane, proteins can have several α helices bundled side-by-side and inserted across the membrane with the helix axis perpendicular to the plane of the membrane. Alternatively several β strands can form a β barrel that spans the membrane, with the β strands running essentially perpendicularly to the plane of the membrane.
   a. Given a 90 kd protein with a membrane-spanning domain containing 6 bundled α-helices, what percent of its mass would reside in the membrane-spanning portion?
   b. What would the percent be if a 12-stranded β-meander barrel were used instead to create the channel?

4. For a Michaelis enzyme, $k_1 = 2.0 \times 10^9\ M^{-1}\ s^{-1}$, $k_{-1} = 5.0 \times 10^4\ s^{-1}$, and $k_2 = 4.0 \times 10^2\ s^{-1}$.
   a. Calculate K_S and K_m for this reaction.
   b. Does substrate binding approach equilibrium or only achieve steady state, i.e. does the enzyme follow rapid equilibrium or only steady state kinetics? Explain.

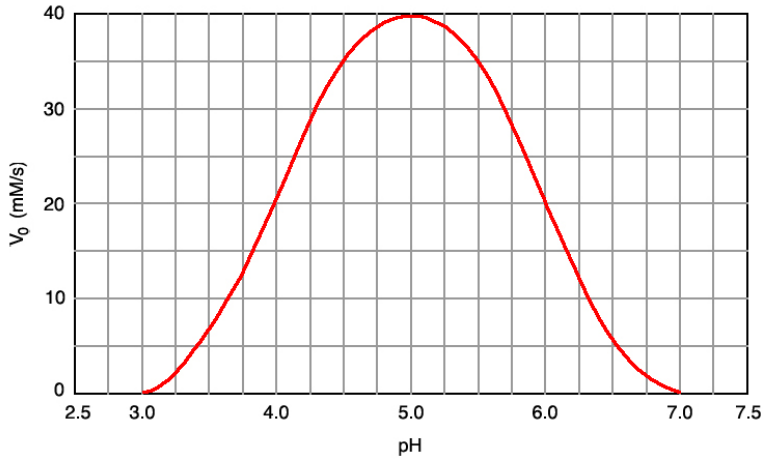
5. For a Michaelis-Menten enzyme, by what factor must the concentration of substrate be increased (i.e., how many fold) to go from an initial velocity that is 25% of V_{max} to 75% of V_{max}?

6. Ibuprofen [α-methyl-4-(isobutyl)phenylacetic acid; M_r 206] reversibly inhibits cyclo-oxygenase (COX-2), a Michaelis enzyme that converts arachidonate to prostaglandin G_2 (PPG_2) thereby preventing the fever, inflammation and pain induced by prostaglandins. The following table shows a kinetic analysis of COX-2 in the absence and presence of 10 mg/ml Ibuprofen.
   a. Make a fully labeled Lineweaver-Burk plot of the data (either manually or with a curve fitting program) and use it to determine V_{max}, and K_m for COX-2 and the apparent V_{max}, and K_m values in the presence of the Ibuprofen.
   b. What kind of inhibitor is Ibuprofen (viz., competitive, noncompetitive, uncompetitive)? Explain the basis of your assignment.
   c. What is the K_I for Ibuprofen with COX-2?

[Arachidonate] mM	v_o, mM/min w/o Ibuprofen	v_o, mM/min w/ Ibuprofen
0.5	23.5	16.67
1.0	32.2	25.25
1.5	36.9	30.49
2.5	41.8	37.04
3.5	44.0	38.91

7. a. COX-2 enzyme is a homodimer of M_r 140,000 with two active sites, and has a turnover number of 5.0×10^7 min^{-1} under the assay conditions used to generate the data in the above table. How many grams of COX-2 per ml were present in the above assays?
   b. Calculate the efficiency value of this enzyme. Would you rate it a perfect enzyme? Explain.

8. Below is a graph showing the relationship between the pH and the rate of substrate hydrolysis by an enzyme Q.

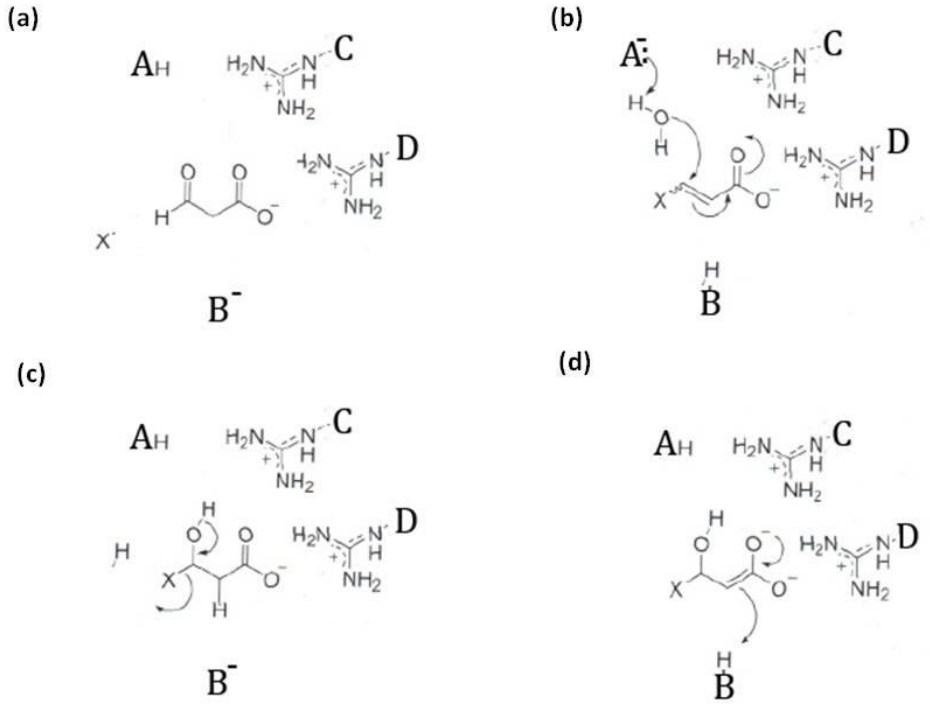


   a. Two catalytically important residues must be charged for the optimal enzyme reaction. Based on the pKa values of amino acid side chains from Table 4.1, which two residues are likely involved in the enzyme reaction?
   b. The enzyme Q hydrolyzes polysaccharides $(A\text{-}B)_x$, or $(A\text{-}A)_x$. Below is a table of rate constants of the enzyme Q for several oligosaccharides.

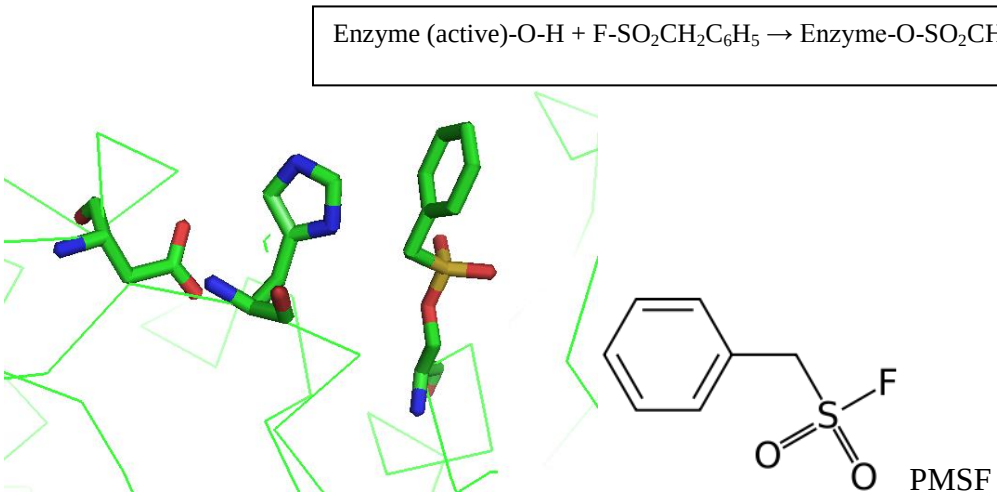
Oligosaccharide	Rate Const., kcat
$(A\text{-}B)_6$	0.5
$(A\text{-}B)_4$	0.5
$(A\text{-}B)_2$	10^{-5}
A_8	0.3
A_7	0.3
A_6	0.3
A_5	0.025
A_4	10^{-7}
A_3	10^{-8}

What would be the minimal length for the substrate? Some of the shorter substrates have inhibitory effect for the enzyme Q. What would be the reason?

9. The following drawings (a)-(d), which are not in order, show 4 stages of proposed reaction mechanisms of a dehalogenase. X in the drawing represents a halogen atom.
   a. Draw a theoretical energy diagram for the reaction (i.e., plot of free energy vs. stage of the reaction; don't worry about absolute G values) and put the letters A-D in the correct order next to the points on the diagram at which they would exist.
   b. Name a catalytic mechanism shown in stage (b) and describe its specific roles in the catalysis.
   c. Under what EC type would this enzyme be classified?
   d. Suggest three amino acids that could function as B residue in stage (d). Describe their specific role in the catalysis.



10. The following figure shows a protease covalently modified by PMSF (structure and reaction shown). Three key active site residues including the covalently modified residue are shown. Color key: green-C, red-O, blue-N, orange-S



a. To which group of enzymes does this protein belong judging by the key active site residues?

b. Sketch the step in a feasible reaction mechanism for the covalent modification. Use a similar enzyme in your textbook as an analogy.