Chemistry and Biochemistry 153A, Spring 2010

Exam 2

Instructions:

- You will have 1 hour 45 minutes to complete the exam.
- You may use a pencil (recommended) or blue or black ink pen to write your answers. Other color inks will not be graded. Your choice of writing utensil will not affect your ability to request a regrade.
- Only answers on the answer sheet, in the indicated space, will be graded; writing anywhere else will be ignored. Be sure to write your name on the answer sheet.
- Do not write in the score boxes on your answer sheet; you will be docked points if you do.
- For answers with a word or sentence limit, words beyond this limit will not be read or graded.
- For short- or multi-answer questions, including irrelevant or wrong information or selections in your answer will cause you to lose points.
- Write legibly. If the grader cannot read your answer, you won't get credit.
- Items you may have on your desk:
  - non-programmable scientific calculator, *without its case or cover*
  - writing utensil(s)
  - student ID

  ALL other items must be placed into a bag, which must be zipped up or closed and pushed *completely* under your chair.
- No hats, hoods, earphones, or cellphones are allowed.
- If you continue to write after 'time' is called, your exam will be taken and docked 10 points.
- **Questions are printed on both sides, as is the answer sheet. Be sure you've answered all of the questions!**

Approximate pK_a values of ionizable groups of amino acids and peptides
(side chains listed unless otherwise noted):

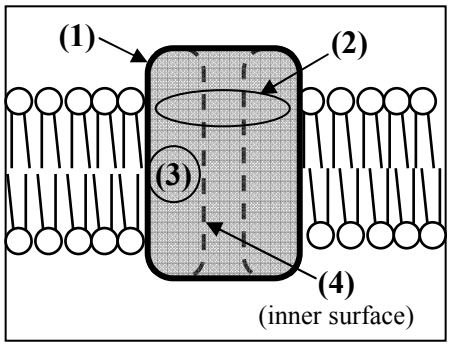
Aspartate:	4	Serine:	13
Glutamate:	4	Threonine:	13
Histidine:	6	α-carboxyl of free amino acid:	2
Cysteine:	8.5	α-carboxyl at C-terminus of peptide:	3
Tyrosine:	10.5	α-amino of free amino acid:	9.5
Lysine:	10.5	α-amino at N-terminus of peptide:	8
Arginine:	12.5		

1. (5) Membrane fluidity varies with changing (choose all that apply):
    a. Temperature
    b. pH
    c. Cholesterol content
    d. Sphingolipid content
    e. Glycolipid content
    f. Fatty acyl length
    g. Fatty acyl saturation

2. (3) Order the following compounds from most to least hydrophilic:
    a. Cholesterol
    b. A cholesteryl ester
    c. A bile acid

3. (4) Given the following diagram of a pore-forming integral membrane protein (shown in grey), match each amino acid to the *surface* location most likely to contain it.

    a. Leu
    b. Pro
    c. Tyr
    d. Asn



4. (3) Briefly explain why an antiparallel β-barrel, like those seen in some integral membrane proteins, must be composed of an even number of β-strands. In your answer, include an explanation of why an odd number of strands would not work. (50 words or fewer.)

5. (2) True or false? Cholesterol can easily diffuse *within* each leaflet of the plasma membrane, and therefore it is evenly distributed within a given leaflet.

6. (2) True or false? In deriving the Michaelis-Menten equation, substrate concentration is assumed to be constant becaue the substrate is at steady state.

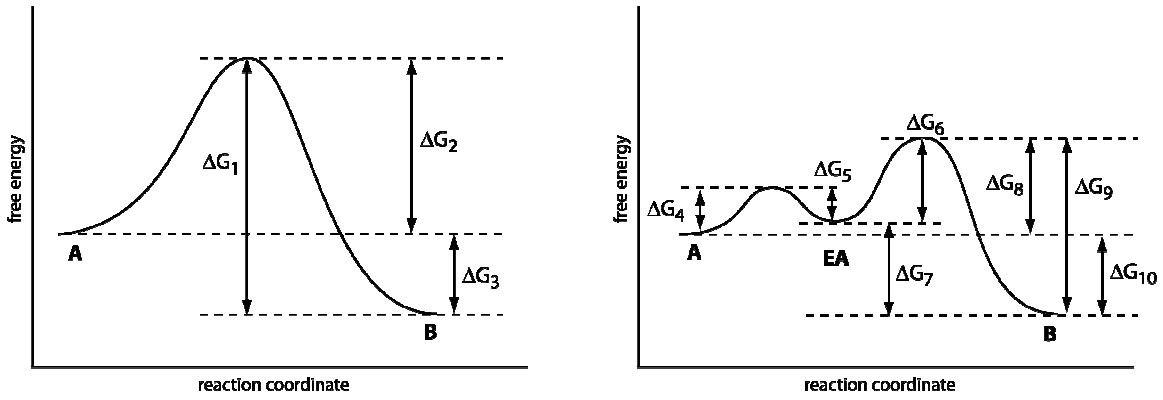
7. (2) True or false? In the sequential model of cooperativity, binding of a ligand can lead to a reduced affinity for subsequent ligand binding.

8. (2) True or false? Enzymes that can experience uncompetitive inhibition likely follow an ordered, sequential mechanism.

9. (12) The P_{50} for oxygen binding to myoglobin is 2.8 torr. Binding experiments with an altered myoglobin show that it is 90% saturated at an oxygen partial pressure of 44 torr.
    a. Calculate the P_{50} for oxygen binding to the altered myoglobin. Show your work.
    b. Draw the binding curves for normal (N) and altered (A) myoglobin. Label the axes with names, units, and number values, and indicate which curve is which.
    c. Does the altered myoglobin have higher or lower oxygen-binding affinity than normal myoglobin?
    d. Additional experiments show that the normal and altered myoglobins bind oxygen *equally quickly*. Briefly explain how this is possible (in 35 words or fewer).

10. (5) For each reaction, name the *class and subclass* (if applicable) of the enzyme that would catalyze that reaction:
    a. $ATP + H_2O \rightarrow ADP + P_i$
    b. $A + B + ATP \rightarrow C + ADP + P_i$
    c. $A \rightarrow B$

11. (5) Given the following free energy diagrams:



    a. Which ΔG determines the rate of the enzyme-catalyzed reaction?
    b. Write an expression (using ΔG's) for the *reduction* in activation energy provided by the enzyme.
    c. What is the molecularity of this reaction?

12. (5) a. Briefly define the catalytic mechanism known as 'proximity and orientation effects' (45 words or fewer).
    b. True or False? Proximity and orientation effects are a feature of all enzyme catalyzed reactions.

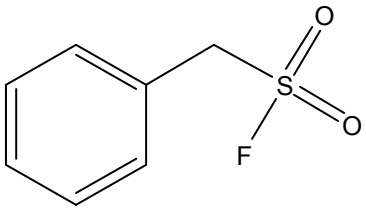
13. (26) Consider the enzyme carbonic anyhdrase:
    a. Where in the blood is carbonic anhydrase located?
    b. Write a balanced equation for the reaction that is catalyzed by carbonic anhydrase.
    c. What *class* of enzyme is carbonic anhydrase?
    d. Explain how the action of carbonic anhydrase helps hemoglobin release oxygen as it passes through the capillaries of the peripheral tissues. (60 words or fewer.)

    Carbonic anhydrase acting on bicarbonate has a k_{cat} of 4×10^5 s^{-1} and a catalytic efficiency of 1.5×10^7 $M^{-1}s^{-1}$.
    e. Briefly define k_{cat} in 20 words or fewer.
    f. Calculate K_m and V_{max} for the reaction of bicarbonate carried out by 1 μM carbonic anhydrase at body temperature (37°C). Show your work.
    g. Draw a double-reciprocal (Lineweaver-Burk) plot for this reaction (from part f). Label axes with names, units, and number values.
    h. To the graph from part f, add a dashed line for the reaction carried out with double the concentration of carbonic anhydrase.
    i. Carbonic anhydrase acting on carbon dioxide has a k_{cat} of 10^6 s^{-1} and a catalytic efficiency of 8.3×10^7 $M^{-1}s^{-1}$. Which molecule, bicarbonate or carbon dioxide, binds with higher affinity to carbonic anhydrase? (Assume binding/unbinding is much faster than catalysis.) Show your reasoning.
    j. True or False? Carbonic anhydrase is catalytically perfect.

14. (4) a. What is a 'retaining glycosidase?' (Explain in 20 words or fewer.)
    b. SN_2 reactions, by definition, lead to an inversion of substituent groups at the reacting carbon. How, then, is it possible for the retaining glycosidases to act via an SN_2 mechanism? Briefly explain in 15 words or fewer.

15. (4) Why is the proposed SN_2 mechanism for lysozyme action more widely accepted than the SN_1 mechanism? Choose all that apply:
    a. A lactone analog binds the enzyme with high affinity.
    b. A covalent intermediate was trapped and its structure determined.
    c. The D-site sugar is forced to adopt a half-chair conformation.
    d. Most retaining glycosidases act via an SN_2 mechanism.
    e. An oxonium ion is very unstable.
    f. NAG_4 as a substrate is cleaved only slowly.

16. (10) PMSF (phenylmethylsulfonyl fluoride) is an inactivator of serine proteases. It is commonly used in the production of protein for experimental study; it prevents cellular proteases from digesting the protein of interest. The structure of PMSF is shown:



a. Starting with the provided framework of a serine protease active site, show how PMSF is bound once it has inactivated the enzyme. In your drawing, also complete the missing portions of the enzyme, and show all important interactions in the active site. Draw any H-bonds as dotted lines.

b. PMSF is a (choose all that apply):
   A. Transition state analog
   B. Substrate analog
   C. Product analog
   D. Competitive inhibitor
   E. Uncompeititive inhibitor
   F. Mixed inhibitor

c. True or false? Lowering the pH of the solution would alter binding by PMSF.

17. (6) In studying the mechanism of serine proteases, scientists A and B introduce mutations into the enzymes and monitor the resulting enzyme activity. One mutation changes the serine of the catalytic triad to cysteine.

a. Scientist A thinks this mutation will *increase* the activity of the enzyme. Briefly explain the reasoning behind scientist A's hypothesis (25 words or fewer).

b. Scientist B thinks this mutation will *reduce* the activity of the enzyme. Briefly explain the reasoning behind scientist B's hypothesis (25 words or fewer).