Chemistry and Biochemistry 153A
Spring 2011

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 separate 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 colored answer sheet. Be sure you've answered all of the questions!**

1. (5) True or False?
    a. (1) Enzymes can change the rate of a reaction.
    b. (1) Enzymes can change the direction of a reaction.
    c. (1) Protein-ligand binding usually involves covalent bonds
    d. (1) A protein could have differing affinities for two ligands, but bind them at the same rate.
    e. (1) In divergent evolution, two proteins share a common ancestor.

2. (6) For each enzyme listed below, name the enzyme *class* to which it belongs:
    a. (1) Citrate synthase
    b. (1) Phosphoglycerate mutase
    c. (1) Cytochrome oxidase
    d. (1) Phosphoprotein phosphatase
    e. (1) Hexokinase
    f. (1) Chymotrypsin

3. (8) Consider the composition and properties of membrane microdomains or rafts.
    a. (4) Which of the following membrane-associated molecules would you be *more likely* to find *within* rafts than outside of rafts? Choose all that apply:
        A. Cholesterol
        B. Sphingolipids
        C. Phosphatidylethanolamine
        D. GPI-linked proteins
        E. Palmitoylated proteins
        F. Glycosylated proteins
        G. Integral membrane proteins
        H. Prenylated proteins
        I. Lipid-linked proteins
    b. (2) What do the molecules you selected have in common (other than their presence in rafts)? Briefly explain in 10 words or fewer.
    c. (2) Use comparative adjectives (ex: 'faster') to complete the following sentence describing the *physical* properties of rafts: The clustering of these molecules makes raft regions _____ and _____ than non-raft regions of the membrane.

4. (2) Complete the following sentence, being as specific as possible: A protein with part of its polypeptide chain inserted into the membrane is called a(n) _____ protein.

5. (6) Below are various ionic species and their concentrations both inside and outside the cell. Refer to these values for the questions below.

Ion	Intracellular concentration (mM)	Extracellular concentration (mM)
Na^+	5-15	145
K^+	140	5
Mg^{2+}	0.5	1
Ca^{2+}	10^{-4}	1-2
Cl^-	5-15	110

   a. (2) List the ion(s) that could be used to drive *efficient* import of a neutral compound into the cell as *symport*.

   b. (2) List the ion(s) which could be used to drive *efficient* import of a neutral compound into the cell as *antiport*.

   c. (2) The types of transport described in questions a and b above would both be classified as:
      A. Primary active transport
      B. Secondary active transport
      C. Facilitated diffusion
      D. Passive transport

6. (4) You are sequencing a protein sample via Edman degradation, and after the first round of degradation, you identify several different amino acid derivatives. Which of the following are reasonable explanations for this result? Choose all that apply:
   a. The N-terminus of the protein is modified.
   b. The sample is impure and contains multiple different proteins.
   c. The protein is denatured.
   d. The protein is composed of several subunits.
   e. The protein has begun to degrade.
   f. The protein is glycosylated.

7. (3) For each of the following techniques, state what experimental parameter of the molecule is measured and used to evaluate differences between molecules:
   a. (1) Mass spectrometry
   b. (1) Circular dichroism
   c. (1) FTIR

8. (13) The hemoglobin tetramer is in equilibrium with its alpha-beta dimer form:

tetramer dimer

$$\alpha_2\beta_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} 2\,\alpha\beta$$

This equilibrium is described by the dissociation constant $K_{4,2}$. For oxyhemoglobin, $K_{4,2}$ is ~5 μM. For deoxyhemoglobin, $K_{4,2}$ is ~1 nM. In the presence of organic phosphates, such as 2,3-BPG, $K_{4,2}$ for *oxy*hemoglobin is lowered by ~2 orders of magnitude.

a. (2) Write two different expressions for $K_{4,2}$ based on the equilibrium reaction shown above.

b. (2) Do *oxygenated* or *deoxygenated* αβ dimers have greater affinity for one-another?

c. (5) The concentration of tetrameric hemoglobin in arterial red blood cells is ~5 mM. What *percent* of hemoglobin exists as dimers in arterial blood? Show your reasoning.

d. (4) Briefly explain how the presence of 2,3-BPG lowers the $K_{4,2}$ of oxyhemoglobin (20 words or fewer).

9. (15) Shown below are several O_2 binding curves. The curve in bold (#3) represents O_2 binding by hemoglobin of a normal person. For each of the following hemoglobin mutations, choose the curve below that best represents O_2 binding for that hemoglobin.

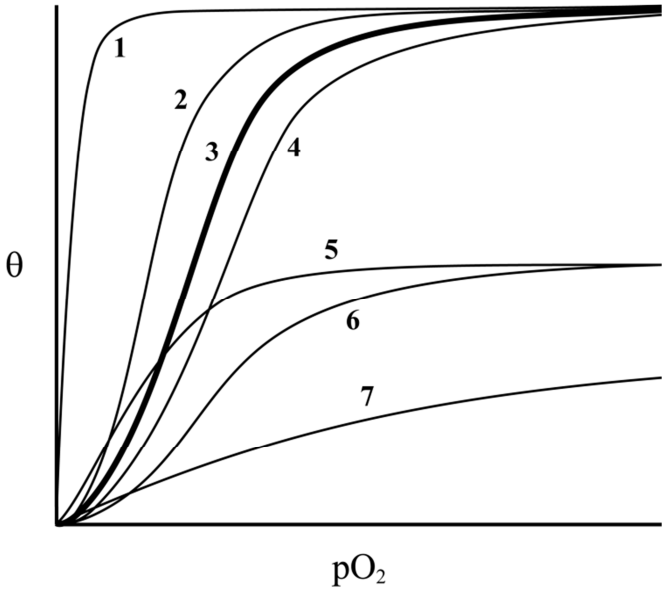
a. (3) A mutation that allows hemoglobin to only adopt the R-state.

b. (3) His58→Tyr, which promotes methemoglobin formation (heme with Fe^{3+}).

c. (3) Asn102→Thr, which disrupts a hydrogen bond that stabilizes the R-state

d. (3) A mutation that disrupts chloride binding

e. (3) A mutation resulting in hemolytic anemia, that is, breaking of red blood cells.



10. (6) Briefly state 3 reasons why the S_N2 mechanism for lysozyme is more likely to be correct than the S_N1 mechanism (8 words or fewer per reason).

11. (4) What is the role of the specificity pocket of the serine proteases? Choose all that apply:
    a. To allow the enzyme to bind a particular substrate
    b. To help the enzyme preferentially bind the tetrahedral intermediate
    c. To position the substrate in the correct orientation with respect to the catalytic triad.
    d. To stabilize the transition state
    e. To react with the substrate

12. (4) What is the role of the oxyanion hole of the serine proteases? Choose all that apply:
    a. To allow the enzyme to bind a particular substrate
    b. To help the enzyme preferentially bind the tetrahedral intermediate
    c. To position the substrate in the correct orientation with respect to the catalytic triad.
    d. To stabilize the transition state
    e. To react with the substrate

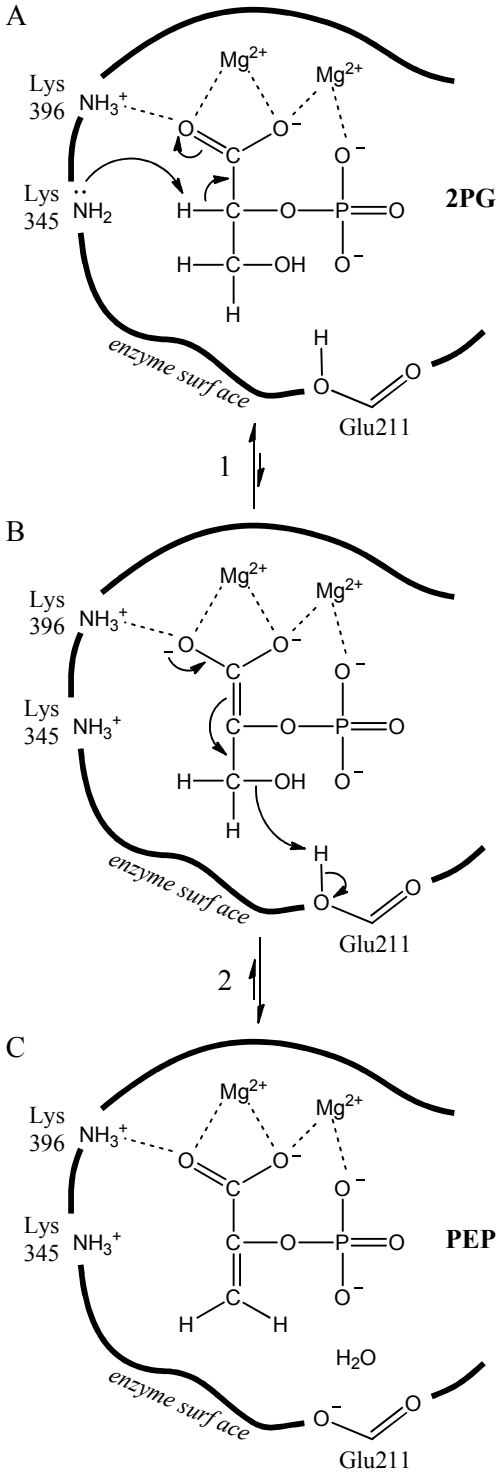
13. (2) Given the following reactions, write an expression (using concentrations and rate constants) for C at steady state.

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B + C \qquad \text{and} \qquad C \underset{k_4}{\overset{k_3}{\rightleftharpoons}} D$$

14. (3) In the derivation of the Michaelis Menten equation, why do we assume $[S] >> [E_T]$? Briefly explain in 10 words or fewer.

15. (4) Which of the following descriptions of k_{cat} are correct? Choose all that apply:
    a. k_{cat} is dependent on enzyme concentration
    b. k_{cat} is a measure of catalytic power
    c. k_{cat} is a measure of catalytic efficiency
    d. k_{cat} is a measure of enzyme-substrate affinity
    e. k_{cat} reflects saturating levels of substrate
    f. k_{cat} reflects the slowest catalytic step (or steps) of a reaction
    g. k_{cat} is a constant for an enzyme, independent of substrate

16. (15) Enolase catalyzes the conversion of 2-phosphoglycerate (2PG) to phosphoenolpyruvate (PEP), an important step in the breakdown of sugars. The mechanism of enolase is shown below:



a. (8) Consider the enzyme's functional groups that are shown in this mechanism (Lys396, Lys345, Glu211, and Mg^{2+} ions). For each, list the catalytic mechanism(s) in which it participates. If, in the figure, the group is not shown to participate in any catalytic mechanisms, write 'none shown.' (You may not need all the lines.)

b. (2) What class of enzyme is enolase?

c. (2) Enolase normally functions at neutral pH. Given that, what is unusual about the starting state of the enzyme (as depicted in panel A)? Briefly explain in 10 words or fewer.

d. (3) Provide a hypothesis about features of the enzyme's active site that could give rise to this unusual starting state (from part c). Briefly explain in 20 words or fewer.