Exam 2 Review Problems
DO NOT BRING TO EXAM

This packet contains problems from old exams, your book, supplemental materials, and even stuff from a TA from many years past. Use this as practice only. This is not, by any means, a definitive indication of what will be on your test.

Solutions will be provided during spring break. If you have questions, contact me via email.

There is one important note about this packet and the exam. You will **NOT** be allowed into the exam with this packet. If you are caught with this packet, copies of it or even retyped/recopied versions of it, there will be serious repercussions which may include your exam being voided.

The key is to be familiar with this material. Do not expect to see questions that mimic these problems. Professor Yaffe is working hard to make sure his material is original.

Good Luck!
-Ali

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Exam 1, 1999

1. Suppose theat you isolated an HIV strain from a patient's blood that contained a reverse transcriptase that proofread. All else being equal, would you consider this virus more or less of a danger, as compared to the strain that does not proofread? Explain briefly.

Exam 1, 1996

2. 2 part question

    1 Draw the chemical structure for the peptide with the sequence RCQAC (denoted in one letter abbreviations), in which the cysteins within the peptide are disulfide bonded to one another. What is the net charge of the peptide at pH 6? Explain briefly.

    2 You mix 0.1 micromoles fo the peptide with 0.8 micromoles of β-mercaptoethanol. Suppose that all of the peptide disulfide bonds are reduced as a consequence. Draw the structures of the two different forms of β-mercaptoethanol that will be present. After the reaction is complete, how many micromoles of each of these forms of β-mercaptoethanol will be present?

3. 2 part question

    1 How many possible ways are there to form 5 disulfide bonds within a protein that contains 10 cysteine residues?

    2 Suppose that you purify the completely reduced form of the protein, and then oxidize the protein. You determine that all of the oxidized protein molecules contain the same disulfide combination. What is the significance of your observations?

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Exam 1, 1995

4. You are given a peptide with a disulfide bond that has the following sequence:

Asp-Ala-Phe-Cys-Ile-Gly-Lys-Ser-Glu-Cys-Asp-Ser
|_____S-S_____|

1 Draw the structures of the PTH amino acid derivatives that you would expect to produce if you carry out two sequential Edman degradation reactions.

2 Now suppose that your sample was contaminated with trypsin, which cleaved the peptide. Draw the structures of the PTH amino acid derivatives that you would expect to result from the contaminated sample if you carry out two sequential Edman degradation reactions (ignore contributions from trypsin per se to the results of the Edman degradation). Explain briefly.

Exam 1, 1994

5. Consider the peptide: Ala-Glu-His-Lys. (Use table 3.1 from Stryer on page 50 [table 2.1 on page 23 in 4th edition].) Draw the structures of this peptide at pH 2 and a pH 12. Sketch a sodium hydroxide titration curve for this peptide, starting at pH 2 and ending at pH 12.

6. The amino acid sequence of insulin is given on page 53 of the 5th edition of Stryer and page 25 of the 4th edition.

1 Suppose that you performed an Edman degration analysis of bovine insulin. For the first two cycles of the analysis, what PTH derivatives would you expect to obtain?

2 Suppose that you had a protease that cleaved after Glu residues. How many fragments would you expect to obtain after exposing insulin to your protease, assuming that the reaction goes to completion? Give the amino acid sequence of the largest fragment.

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Exam 4, 1996

7. One can build an accurate model of the backbone of a protein given a complete set of phi and psi values for the protein, provided that one makes what critical assumptions? Explain briefly.

Exam 4, 1995

8. You get a protein with 5 disulfide bonds. You reduce the disulfide bonds with β-mercaptoethanol in the presence of urea and then dialyze the mixture versus water, ph 7. You then expose the protein solution to air (O_2) until all the cysteine residues are disulfide bonded. How many combinations of different disulfide bonds would you expect in the protein, if protein folding was a completely random process? How many combinations would you expect if protein folding was a process determined by the amino acid sequence only? Explain briefly.

PSET 1, 2001

9. Draw the derivative PTH-amino acid product and the resulting dipeptide from the initial Edman degradation of the following tripeptide: Arg-Lys-Lys

PSET 5, 2001

10. You have a peptide with the following sequence: HKEH. Plot a titration curve of this particular peptide. (A titration curve is a plot of a dependence of the pH of a solution on the amount of OH added.)

Exam 4, 1996

11. What are the significant conformational changes that occur when chymotrypsinogen is cleaved to yield π-chymotrypsin? Explain how these changes are thought to affect the enzymatic activity of the protein.

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Problem Set 11, 2001

12. The hypothetical enzyme studiase will convert the sugar anxietose to equal molar concentrations of two products, relievioxide and A. The $\Delta G^{0'}$ of this reaction is 1.5 kcal/mol. What metabolites are favored at equilibrium? Give the equilibrium concentration of each component of the reaction mixture if 2M anxietose were allowed to attain equilibrium in the presence of studiase. What would the equilibrium concentrations be in the absence of enzyme? Assume room temperature of 25°C.

    What additional information is needed to determine if the reaction occurs spontaneous? To determine the rate of reaction?

    Tell if the reaction occurs spontaneously under the following conditions:
    2M anxietose, 0.25M A, 0.5M relievioxide
    1M anxietose, 1M A, 0.5M relievioxide

13. Draw a free energy vs. reaction coordinate diagram for a typical substrate (S) to product (P) reaction in the absence of a catalyst. Label the transition state and the activation free energy.

    Suppose you have a protein that binds the product only (i.e. the protein does not bind the substrate or the transition state). With a dotted line on the same diagram, draw the corresponding free energy vs. reaction coordinate diagram for the same reaction in the presence of this protein. Label the binding free energy. Would you expect the initial rate of the reaction to be faster, slower, or the same in the presence of the protein? Explain.

Problem Set 12, 2001

14. Predict whether each of the following manipulations will increase or decrease the tendency of HbS to polymerize in vitro. Explain briefly.

    A) An increase in the partial pressure of oxygen

    B) Stripping the HbS molecule of BPG

    C) An increase in pH

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Exam 4 Review Questions

15. Determine whether each of the following statements regarding allostery is true or false. Justify your answer.

    A) A competitive inhibitor of an allosteric enzyme may actually increase the reaction rate when the inhibitor is at low concentrations relative to the substrate.

    B) The sequential model of allostery cannot explain negative cooperativity.

    C) At saturating amounts of an activator, an allosteric enzyme can exhibit Michaelis-Menten kinetics.

16. Why does the sequential model of allostery better explain negative cooperativity than the concerted model?

17. An anemic individual, whose blood has only half the normal Hb content, may appear to be in good health. However, a normal individual is incapacitated by exposure to sufficient carbon monoxide to occupy half his heme sites (CO binds Hb with 200 times greater affinity than does oxygen). Explain.

18. A mutant hemoglobin is isolated. In this mutant protein, the pK's for all ionizable groups are the same in the T and R states, except for one histidine residue that has a higher pK in the R state.

    A) Sketch a representation of the of the oxygen binding curve at pH 7.6 and pH 7.2 for the mutant hemoglobin.

    B) Why would a person with this mutant hemoglobin have medical problems?

19. Who would have a higher level of BPG: a person at low altitude or one at high altitude? Why?

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Random

20. G-proteins are involved in signal transduction in many types of cells in the body. In intestinal epithelial cells, a signal mediated by a G-protein regulates water transport in these cells through certain membrane proteins. The effects of certain drugs and toxins on the G-protein can cause an abnormality in which excess water exists from the blood into the intestinal tract through these cells. This water is then lost through diarrhea.

    A) The cholera toxin irreversibly modifies the Galpha-subunit of the G-protein so that it continuously stimulates adenylate cyclase. Cholera can lead to severe, potentially fatal dehydration if not treated properly.

        i) What funtion of the Galpha-subunit is altered by the toxin so that adenylate cyclase is continuously active?

        ii) What is the effect of the toxin on cAMP levels in the intestinal cells?

    B) A drug analogue of GTP (GTP-gammaS) has the property that once it is bound by the G-protein, GTP-gammaS cannot be hydrolyzed to GDP.

        i) If GTP-gammaS is given to an individual, what would its effect be on the intestinal epithelial cell?

        ii) Would the effects of GTP-gammaS be limited to intestinal epithelial cells?

From Your Book

21. (15-1) At what stages in the signaling pathway from epinephrine to cAMP does a significant amount of amplification occur?

22. (15-7) A mutated form of the α subunit of the heterotrimeric G protein has been identified; this form readily exchanges nucleotides even in the absence of an activated receptor. What effect would you expect this mutated α subunit to have on its signaling pathway?

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23. (15-10) Glucose is mobilized for ATP generation in muscle in response to epinephrine, which activates $G_{\alpha s}$. Cyclic AMP phosphodiesterase is an enzyme that converts cAMP into AMP. How would inhibitors of cAMP phosphodiesterase affect glucose mobilization in muscle?

24. (10-7) What is the effect of each of the following treatments on the oxygen affinity of hemoglobin A in vitro?

    (a) Increase in pH from 7.2 to 7.4
    (b) Increase in pCO_2 from 10 to 40 torr.
    (c) Increase in [2,3-BPG] from $2*10^{-4}$ to $8*10^{-4}$ M.
    (d) Dissociation of $\alpha_2\beta_2$

25. (10-11) Suppose that you have just examined a young boy with a bleeding disorder highly suggestive of classic hemophilia (factor VIII deficiency). Because of the late hour, the laboratory that carries out specialized coagulation assays is closed. However, you happen to have a sample of blood from a classic hemophiliac whom you admitted to the hospital an hour earlier. What is the simplest and most rapid test that you can perform to determine whether your present patient also is deficient in factor VIII activity?

26. (9-2) Contributing to your own demise. Consider the subtilisin (another serine protease) substrates A and B.

    A: Phe-Ala-Cln-Phe-X
    B: Phe-Ala-His-Phe-X

    These substrates are cleaved (between Phe and X) by native subtilisin at essentially the same rate. However, the His 64 - to - Ala mutant of subtilisin cleaves substrate B more than 1000-fold as rapidly as it cleaves substrate A. Propose an explanation.

27. (9-4) Adding a charge. In chymotrypsin, a mutant was constructed with Ser 189, which is in the bottom of the substrate specificity pocket, changed to Asp. What effect would you predict for this Ser 189 -> Asp 189 mutation?

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28. (8-4) Mode of inhibition. The kinetics of an enzyme are measured as a function of substrate concentration in the presence and in the absence of 2mM inhibitor (I).

[S] (μM)	Velocity (μmol / minute)	
	No inhibitor	Inhibitor
3	10.4	4.1
5	14.5	6.4
10	22.5	11.3
30	33.8	22.6
90	40.5	33.8

(a) What are the values of V_{max} and K_M in the absence of inhibitor? In its presence?
(b) What type of inhibition is it?
(c) What is the binding constant of this inhibitor?
(d) If [S] = 10 μM and [I] = 2mM, what fraction of the enzyme molecules have a bound substrate? A bound inhibitor?
(e) if [S] = 30μM, what fraction of the enzyme molecules have a bound substrate in the presence and in the absence of 2 mM inhibitor? Compare this ratio with the ratio of the reaction velocities under the same conditions.

29. (8-11) More Michaelis-Menton. For an enzyme that follows simple Michaelis-Menton kinetics, what is the value of V_{max} if V_0 is equal to 1μmol/minute at 1/10 K_m?

From Year 2002 Problem Sets:

30. What are the significant conformation changes that occur when chymotrypsinogen is cleaved to yield π-chymotrypsin? Explain how these changes are thought to affect the enzymatic activity of the protein. Would this transition occur if the cleaved product were incubated at pH 2? At pH 12? Why or why not?

31. What would happen if you replaced GTP with a nonhydrolyzable FTP analog Guanylylimido diphosphate in cAMP dependent receptor systems?

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32. Give the stabilizing bond types involved in each of the following stages of protein structure. Also provide a brief description (definition) of each stage.
    A. Primary Structure
    B. Alpha Helix
    C. Beta Sheet
    D. Tertiary Structure
    E. Quaternary Structure

More stuff from your book:

33. (3-4) An enzyme that catalyzes disultide-sulfhydryl exchange reactions, called PDI, has been isolated. PDI rapidly converts inactive scrambled ribonuclease into enzymatically active ribonuclease. In contrast, insulin is rapidly inactivated by PDI. What does this important observation imply about the relation between the amino acid sequence of insulin and its three-dimensional structure.

34. (3-5) A protease is an enzyme that catalyzes the hydrolysis of the peptide bonds of target proteins. How might a protease bind a target protein so that its main chain becomes fully extended in the vicinity of the vulnerable peptide bond?

35. (3-17G) You have a solution of HCl with a pH of 2.1. What is the concentration of HCl needed to make this solution?

36. (4-10G) A map of the electron density is necessary for the determination of the three-dimensional structure of a protein, but other information is also needed. Hydrogen atoms have one electron and cannot be visualized by x-ray analyses of proteins. Bearing this in mind, compare the structure of amino acids like valine, threonine and isoleucine and then describe what additional information would be needed along with an electron density map.

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37. (8-4) The kinetics of an enzyme are measured as a function of substrate concentration in the presence and absence of 2 mM inhibitor.

S (uM)	V (no I)	V (+I) (in umol/min)
3	10.4	4.1
5	14.5	6.4
10	22.5	11.3
30	33.8	22.6
90	40.5	33.8

(A) What are the values of V_{max} and K_M in the absence of I? In the presence?
(B) What type of inhibition is it?
(C) What is the binding constant of this inhibitor?
(D) if [S]=10 uM and [I]= 2 mM, what fraction of the enzyme molecules have a bound substrate? A bound inhibitor?
(E) if [S] = 30 uM, what fraction of the enzyme molecules have a bound substrate in the presence and the absence of 2 mM I? Compare this ratio with the ratio of the reaction velocities under the same conditions.

38. (8-3G) The enzyme hexokinase catalyzes the following reaction:
Glucose + ATP <–> glucose 6-P + ADP, $\triangle G$ = -4.0 kcal/mol

(A) Calculate the change in free energy $\triangle G'$ for this reaction under typical intracellular conditions using the following concentrations: glucose 55 mM; ATP 5 mM; ADP 1 mM; and glucose 6 P 0.1 mM. Assume the temperature is 25 °C.
(B) In the typical cell, is the reaction catalyzed by hexokinase close to equilibrium or far from equilibrium? Explain.

Thats all for now...I may send out some more over spring break.