

**Biochemistry I - Exam Face Page**

- This exam consists of 8 pages, including this one.
- There are a total of 90 points - or approximately 30 sec/point.
- You should be able to answer the question in the space provided, if not, please use the back of the adjacent page.
- Many questions have choices, if you answer more than one of the choices, your best answer will be counted.

The following equations and constants may be useful:

T=300K and pH=7.0 unless otherwise stated.

R=8.3 J/mol-K RT=2.5 kJ/mol @ 300K

Log2=0.3 ln10=2.3

T(K) = T(C) + 273.

**Thermodynamics:**

$$\Delta G^0 = -RT \ln K_{eq}$$

$$\Delta G = \Delta H - T\Delta S$$

$$S = R \ln W$$

$$\ln(a^n) = n \ln a$$

For the reaction: N ↔ U:

$$K_{EQ} = \frac{[U]}{[N]}$$

$$f_U = \frac{K_{EQ}}{1 + K_{EQ}}$$

$$f_N = \frac{1}{1 + K_{EQ}}$$

**Amino Acid Names:**

Alanine: Ala  
 Arginine: Arg  
 Asparagine: Asn  
 Aspartic Acid: Asp  
 Cystine: Cys  
 Glycine: Gly  
 Histidine: His  
 Isoleucine: Ile  
 Lysine: Lys  
 Leucine: Leu  
 Methionine; Met  
 Phenylalanine: Phe  
 Proline: Pro  
 Serine: Ser  
 Threonine: Thr  
 Tryptophan: Trp  
 Tyrosine: Tyr  
 Valine: Val  
 Glutamine: Gln  
 Glutamic Acid: Glu

**Acid-Base & Buffers**

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

$$R = 10^{pH - pK_a}$$

$$[HA] = [A_T] \frac{1}{1 + R}$$

$$[A^-] = [A_T] \frac{R}{1 + R}$$

**Beer's law:**

$$A = \epsilon [X] l$$

$$\epsilon_{\lambda=280} = 5,000 \text{ for Tryptophan (Trp)}$$

$$\epsilon_{\lambda=280} = 1,500 \text{ for Tyrosine (Tyr)}$$

**Ligand Binding:**

For [M]+[L] ↔ [ML]

$$K_{EQ} = \frac{k_{on}}{k_{off}}$$

$$Y = \frac{[ML]}{[M] + [ML]} = \frac{[L]}{K_D + [L]}$$

Part A: \_\_\_\_\_/18

1: \_\_\_\_\_/ 5

2: \_\_\_\_\_/12

3: \_\_\_\_\_/10

4: \_\_\_\_\_/ 5

5: \_\_\_\_\_/ 8

6: \_\_\_\_\_/ 4

7: \_\_\_\_\_/10

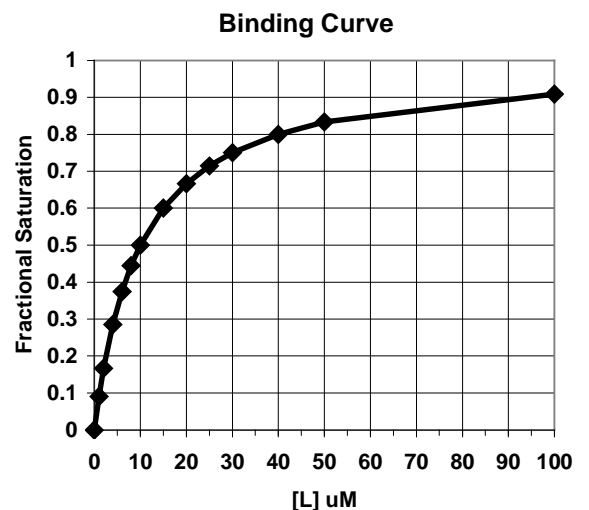
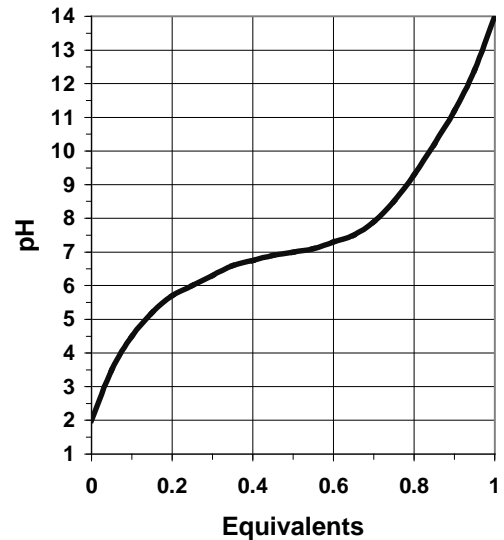
8: \_\_\_\_\_/10

9: \_\_\_\_\_/ 8

**TOTAL:** \_\_\_\_\_/90

**Part A: Multiple Choice:** Please circle the best answer (18 pts)

- The titration curve of a weak acid is shown to the right. Its  $pK_a$  is:
  - 2.0
  - 5.0
  - 7.0
  - 14.0
- When the pH of a solution is lower than the  $pK_a$  of an acid, then
  - The concentration of [HA] exceeds [A-].
  - The concentration of [HA] is less than [A-]
  - The concentration of [HA] is equal to [A-]
  - The ratio of [HA] to [A-] cannot be determined.
- When Edman degradation is used for peptide sequencing, it
  - removes one residue from the carboxy-terminus.
  - removes one residue from the amino-terminus.
  - removes the side-chain from the carboxy-terminus.
  - removes the side-chain from the amino-terminus.
- Which of the following are found in high abundance in *both* the core and on the surface of a protein?
  - Positively charged amino acids.
  - Negatively charged amino acids
  - Polar amino acids.
  - Hydrophobic amino acids.
- During the unfolding of a protein it requires about +5 kJ/mol to break a hydrogen bond. This implies:
  - Hydrogen bonds are much weaker in proteins than in water
  - Hydrogen bonds are much stronger in proteins than in water
  - Hydrogen bonds are slightly weaker in proteins than in water.
  - Hydrogen bonds are slightly stronger in proteins than in water.
- Antigens bind to which portions of an antibody?
  - variable regions.
  - $F_{ab}$  fragments.
  - only light chains.
  - both a and b are correct.
- A protein contains two Tryptophan residues. The absorbance of a 1  $\mu M$  solution of the protein will be:
  - 0.01
  - 0.02
  - 0.05
  - none of the above.
- A ligand binding curve is shown on the right. The  $K_D$  for this ligand is:
  - 1  $\mu M$ .
  - 5  $\mu M$ .
  - 10  $\mu M$ .
  - none of the above.
- The  $\Delta G^\circ$  for a protein unfolding is 0 kJ/mol. The fraction of folded proteins,  $f_N$ , is:
  - 0.1
  - 0.2
  - 0.5
  - 0.9

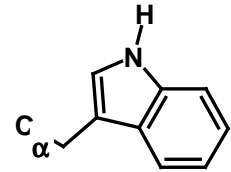


**Part B:**

1. Some questions on hydrogen bonds (5 pts).

- a) Briefly describe the characteristics of the atoms that compose a 'hydrogen-bond'. Feel free to answer this question by drawing and carefully labeling a chemical structure (3 pts).

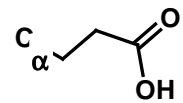
- b) The structure shown on the right is the sidechain of the amino acid tryptophan. Show how a water molecule would form a hydrogen bond with this amino acid. Be sure to label the donor and acceptor groups (2 pts).



2. Some questions on acid/base theory (12 pts)

- a) Briefly define the  $pK_a$  (3 pts).

- b) The sidechain of glutamic acid (shown to the right) has a  $pK_a$  of  $\sim 4.0$  in solution. A glutamic acid residue in a protein is found to have a  $pK_a$  of 6.0. Based on this change in  $pK_a$ , what can you say about the environment of the glutamic acid in the protein? Why has this environment affected the  $pK_a$ ? (5 pts)



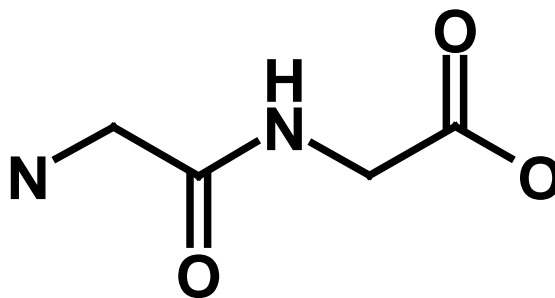
- c) Briefly describe the *most* important property of a buffer solution. (2 pts)

- d) Which of the compounds listed on the right would you select for use to buffer a solution at  $pH=7.0$ ? Why? (2 pt)

Compound	$pK_a$
Acetic Acid	4.0
Imidazole	6.0
Ethyl-amine	9.0

3. (10 pts) The partial structure of a dipeptide is shown below.

- i) Add *any* completely non-polar sidechain as the **carboxy-terminal** residue (1 pt).
- ii) Add *any* ionizable sidechain, with the exception of Glutamic acid, as the **amino-terminal** residue (1 pt).
- iii) Name your peptide (1 pt).
- iv) Label *all* ionizable groups with their approximate  $pK_a$  values (1 pt).
- v) Label the peptide bond and label an  $\alpha$ -carbon (1 pt).
- vi) Is the peptide bond free to rotate? What properties of the peptide bond support your answer (5 pts).



4. (5 pts) Please do **one** of the following three choices. Please indicate your choice when answering the question.

**Choice A:** Briefly distinguish between secondary and tertiary structure.

OR

**Choice B:** Define quaternary structure and give an example of a protein whose proper function requires an intact quaternary structure.

OR

**Choice C:** What is a Ramachandran plot? What, if anything, does it tell you about the secondary and tertiary structure of a protein?

5. (8 pts) Please do **one** of the following two choices. Please indicate your choice when answering the question.

**Choice A:** Sketch the structure of a super-secondary structural element and briefly discuss **two** molecular forces or interactions that stabilize this structural element.

OR

**Choice B:** How does an  $\alpha$ -helix differ from a  $\beta$ -sheet? How are they similar? Your answer should include a discussion of the overall structure, location of sidechains, and stabilizing force(s). You may answer this question with a carefully labeled drawing.

6. (4 pts) The equilibrium constant for the binding of different ligands to a protein largely depend on  $k_{off}$ , and not on  $k_{on}$ . Why?

7. (10 pts) Please answer **one** of the following two questions. Please indicate your choice when answering the question.

**Choice A:** What is the molecular nature of the "hydrophobic" effect? Is it enthalpic or entropic? In what way does it stabilize (or destabilize) the folded state of globular proteins?

OR

**Choice B:** What is configurational entropy? In what way does it stabilize (or destabilize) folded globular proteins? Briefly discuss how you could estimate this contribution to protein stability.

8. (10 pts) Please do **one** of the following three questions. Please indicate your choice when answering the question.

**Choice A:** A glutamic acid residue must be *deprotonated* for a protein to have biological function. The  $pK_a$  of this glutamic acid residue is 5.0. Draw a graph that shows the activity of this protein as a function of pH. Briefly justify your approach with an example calculation.

**Choice B:** Describe how you would make 1 L of a 0.1 molar buffer solution at  $pH = 7$  using one of the compounds shown to the right. Your answer should explicitly state the number of moles of the weak acid and its conjugate base that would be required to make the buffer; i.e. you do not have a strong acid or base available for pH adjustment. Please show your work.

Compound	pKa
Acetic Acid	4.0
Imidazole	6.0
Ethyl-amine	9.0

**Choice C:** A nine residue peptide was fragmented using chymotrypsin and three fragments were obtained. The complete sequences of these fragments are shown below:

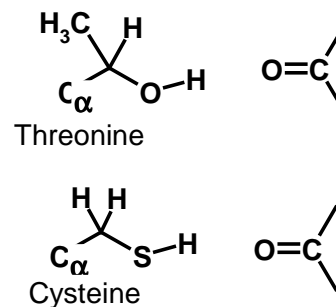
Ala-Ala-Leu

Ala-Lys-Trp

Ala-Met-Trp

- Based on the above data, which amino acid(s) are recognized by chymotrypsin (2 pts)?
- Based on the above data, give all possibilities for the sequence of the entire peptide (3 pts).
- Briefly describe how you would use additional cleavage reagents to confirm which of these sequences correspond to the correct sequence (5 pts).

9. (8 pts) You are interested in determining the contribution of -SH hydrogen bonds in protein stability. You select a protein that has a *buried* threonine residue that forms a hydrogen bond to a mainchain C=O group. You replace this threonine residue with a cysteine residue. The partial structure of the threonine and cysteine containing proteins are shown to the right.



The melting curve for both of these protein is shown below. The enthalpy for denaturation, or unfolding, of the threonine containing protein is +200 kJ/mol. The enthalpy for denaturation of the cysteine containing protein is +190 kJ/mol.

a) Use the melting curves to find the  $T_M$  for both proteins (2 pts). List your values here:

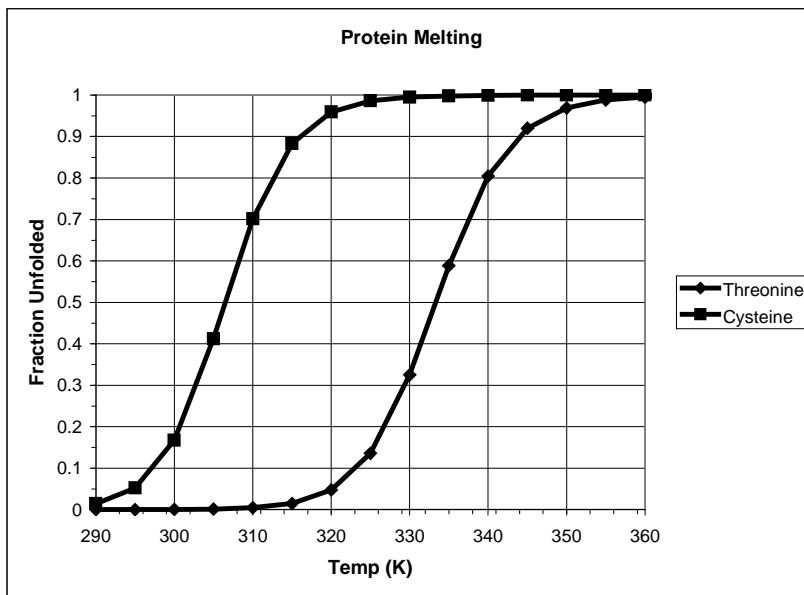
Threonine -  $T_M$  :

Cysteine -  $T_M$  :

b) Determine the  $\Delta S^\circ$  for denaturation for both proteins (2 pts). Show your work and list your values here:

Threonine -  $\Delta S^\circ$  :

Cysteine -  $\Delta S^\circ$  :



c) (4 pts) You prepare a scientific paper on these experiments, in which you claim that the energy of a SH hydrogen bond is 10 kJ/mol weaker than the energy of an OH hydrogen bond. This claim is rejected by the reviewers of your paper! Although your measurements are correct, what mistake have you made in the interpretation of your data [Hint: Do the  $\Delta S^\circ$  changes suggest other *enthalpic* interactions you've missed.]?