

**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)

1. The titration curve of a weak acid is shown to the right. Its  $pK_a$  is:

- a) 2.0
- b) 5.0
- c) 7.0 -infection point in curve, also pH at 1/2 eq.**
- d) 14.0

2. When the pH of a solution is lower than the  $pK_a$  of an acid, then

- a) The concentration of [HA] exceeds [A-].**
- b) The concentration of [HA] is less than [A-]
- c) The concentration of [HA] is equal to [A-]
- d) The ratio of [HA] to [A-] cannot be determined.

3. When Edman degradation is used for peptide sequencing, it

- a) removes one residue from the carboxy-terminus.
- b) removes one residue from the amino-terminus.**
- c) removes the side-chain from the carboxy-terminus.
- d) removes the side-chain from the amino-terminus.

4. Which of the following are found in high abundance in *both* the core and on the surface of a protein?

- a) Positively charged amino acids.
  - b) Negatively charged amino acids
  - c) Polar amino acids.
  - d) Hydrophobic amino acids**
- The key word was *both* the core and the surface. When a protein folds, only 50% of the non-polar residues form the non-polar core. The remaining 50% remain solvent exposed. The core contains relatively few polar and usually no charged amino acids.

5. During the unfolding of a protein it requires about +5 kJ/mol to break a hydrogen bond. This implies:

- a) Hydrogen bonds are much weaker in proteins than in water
- b) Hydrogen bonds are much stronger in proteins than in water (+1 pt)**
- c) Hydrogen bonds are slightly weaker in proteins than in water.
- d) Hydrogen bonds are slightly stronger in proteins than in water.**

6. Antigens bind to which portions of an antibody?

- a) variable regions. **(+1 pt)**
- b)  $F_{ab}$  fragments. **(+1 pt)**
- c) only light chains.
- d) both a and b are correct.**

7. A protein contains two Tryptophan residues. The absorbance of a 1  $\mu$ M solution of the protein will be:

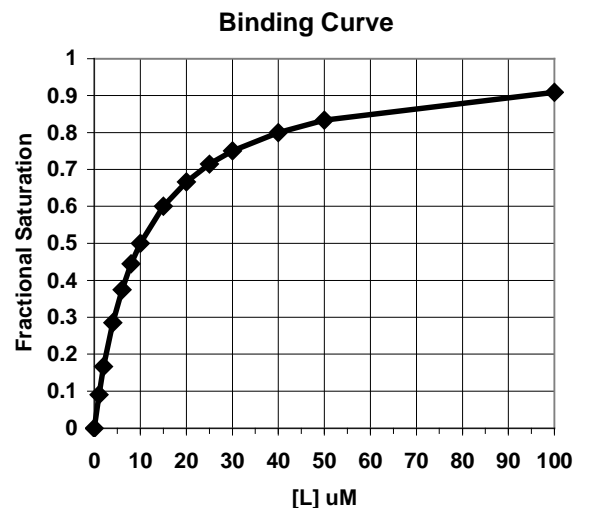
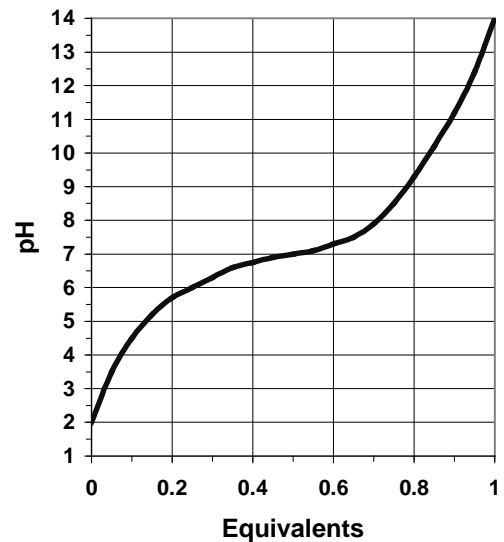
- a) 0.01  $\epsilon = 2 \times 5,000 = 10,000, A=c\epsilon l, l=1cm$**
- b) 0.02
- c) 0.05
- d) none of the above.

8. A ligand binding curve is shown on the right. The  $K_D$  for this ligand is:

- a) 1  $\mu$ M.
- b) 5  $\mu$ M.
- c) 10  $\mu$ M. [L] that gives  $Y=0.5$  is  $K_D$**
- d) none of the above.

9. The  $\Delta G^\circ$  for a protein unfolding is 0 kJ/mol. The fraction of folded proteins,  $f_N$ , is:

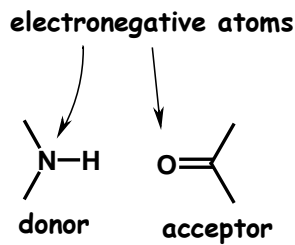
- a) 0.1
- b) 0.2
- c) 0.5  $\Delta G^\circ = 0$  means that the native and unfolded state are equal in energy  $\therefore f_n=f_u$ .**
- d) 0.9



**Part B:**

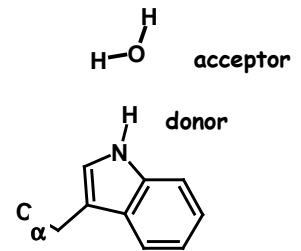
1. Some questions on hydrogen bonds (5 pts).

- 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).



The electronegative atom that is part of the donor group withdraws electron density from its attached proton, generating a partial positive charge on the proton. The electronegative acceptor (oxygen in this case) has a partial negative charge, making the donor-acceptor interaction favorable.

- 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)

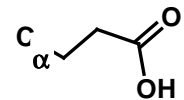
- a) Briefly define the  $pK_a$  (3 pts).

**This is the  $-\log$  of the equilibrium constant for deprotonation of an acid.**

**or**

**It is equal to the pH where 50% of the molecules are deprotonated.**

- 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)



**It is a weaker acid in this altered environment. Therefore, formation of the deprotonated species must be unfavorable. The deprotonated species is negatively charged, so the environment must be negatively charged.**

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

**It absorbs added acid or base, keeping the pH relatively constant.**

- 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

**Imidazole, since its  $pK_a$  is within one pH unit of the desired pH.**

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).

**Choices include Alanine, Valine, Leucine, Isoleucine, Phenylalanine. I've drawn Leucine.**

ii) Add *any* ionizable sidechain, with the exception of Glutamic acid, as the *amino-terminal* residue (1 pt).

**Choices include aspartic acid (pKa=4), Lysine (pKa=10), Arginine (pKa=10), Histidine (pKa=6). I've drawn Histidine.**

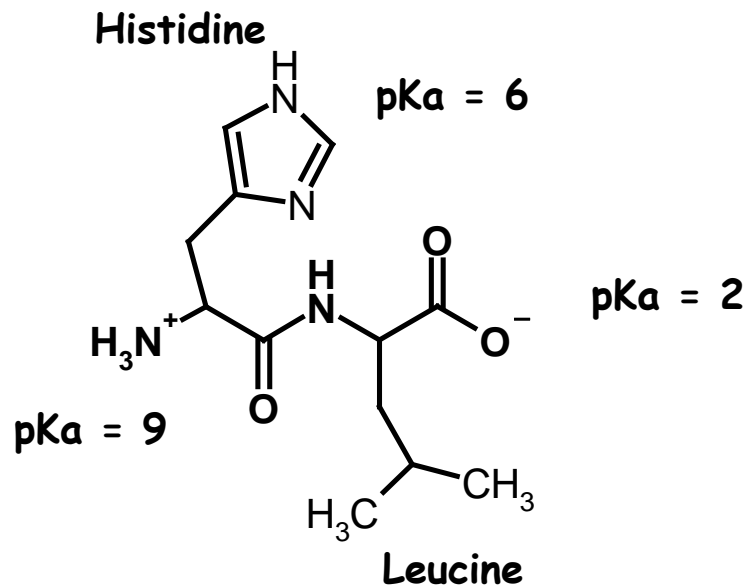
iii) Name your peptide (1 pt). **His-Leu**

iv) Label *all* ionizable groups with their approximate pK<sub>a</sub> values (1 pt). **See diagram**

v) Label the peptide bond and label an α-carbon (1 pt). **See diagram**

vi) Is the peptide bond free to rotate? What properties of the peptide bond support your answer (5 pts).

**No, because of partial double bond character (overlap of p<sub>z</sub> orbitals from the nitrogen and carbonyl carbon).**



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.

**Secondary structure is the structure of the mainchain atoms, tertiary structure is the structure of all of the atoms.**

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

**The quaternary structure describes the structure a protein with multiple chains, e.g. hemoglobin or immunoglobulin.,**

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

**Each point on the Ramachandran plot gives the phi and psi angle of a single residue - i.e. a concise description of its mainchain configuration. Therefore it gives the complete secondary structure of a protein, but does not provide any information about the tertiary structure.**

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.

**Example -  $\beta\alpha\beta$  - a two stranded  $\beta$ -sheet with an amphipathic  $\alpha$ -helix on top. It is stabilized by:**

**hydrogen bonds - between the strands and within the helix.**

**van der waals forces - between the helix and sheet**

**hydrophobic interaction - nonpolar residues will be buried at the interface between the sheet and helix.**

**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.

**Similarities;**

- Both only describe the mainchain atoms
- Both are stabilized by hydrogen bonding.

**Differences:**

- The helix is, well, a helix, and the sheet is formed of extended  $\beta$ -strands
- In the helix the sidechains point outward from the helical axis, in a  $\beta$ -sheet they point above and below the sheet.
- The hydrogen bonds in a helix are parallel to the helix axis, while they are perpendicular to the direction of the  $\beta$ -strand.

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?

**The kinetic on rate,  $k_{on}$  is usually defined by the number of collisions between the ligand and the protein, so it won't depend on any specific interaction between the two. Once the ligand is bound, the rate at which it comes off will depend on the number of interactions between the ligand and the protein. The more interactions, the slower the off-rate.**

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?

**The hydrophobic effect refers to the decrease in the entropy of water as it "coats" non-polar groups that are exposed to the solvent. When proteins unfold, they expose their non-polar groups to water, decreasing the entropy of the water. Since a decrease in entropy is unfavorable, this effect stabilizes the folded state.**

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.

**The configuration entropy is the entropy associated with the number of configurations a polymer chain can assume. It can be calculated from the number of configurations,  $W$ , using the following formula:**

$$S = R \ln W.$$

**For a protein,  $W=9/\text{residue}$  and an "n" residue protein will have  $9^n$  configurations.**

**Since the configurational entropy increased during unfolding, this stabilizes the unfolded state.**

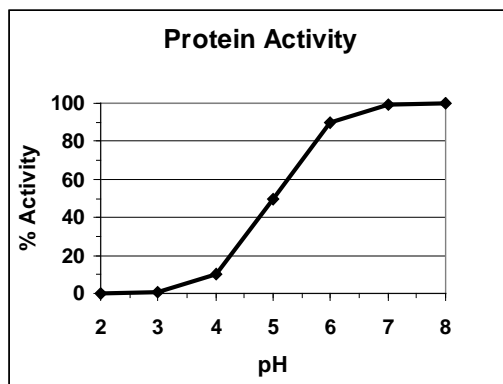
8. (10 pts) Please do **one** of the following three questions. Please indicate your choice when answering the question.

**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.

You need to calculate the fraction deprotonated, as this will be proportional to the amount of the active species. This is given by  $f_A = R/(1+R)$  where  $R=10^{pH-pK_a}$ . As an example calculation, at  $pH=6.0$ :

$$R=10^{6-5}=10, f_A = 10/11=0.90, \text{ or } 90\% \text{ active.}$$

The full curve is shown to the right. It was "sketched" by first plotting  $f_A = 0.5$  when the  $pH=pK_a$ . When the  $pH$  is one unit lower than the  $pH$ ,  $f_A$  drops to  $\sim 0.1$ , when the  $pH$  is one unit higher than the  $pK_a$  the  $f_A \sim 0.9$ , when the  $pH$  is two units higher than the  $pK_a$  the  $f_A \sim 0.99$ .



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

You need to calculate the ratio of  $[HA]$  and  $[A^-]$  to give the desired  $pH$ . This depends on the  $pK_a$  of the buffer that you picked.

Imidazole would be the best buffer, since its  $pK_a$  is closest to the desired  $pH$  of 7.0.

$$[HA] = [A_T] \frac{1}{1+R} \quad [A^-] = [A_T] \frac{R}{1+R} \quad R = 10^{pH-pK_a}$$

$R=10^{7-6} = 10$ .  $[A_T]=0.1$  moles, therefore:

$$[HA] = 0.1 \times (1/11) = 0.009 \text{ moles} \quad [A^-] = 0.1 \times (10/11) = 0.091 \text{ moles}$$

**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

a) Based on the above data, which amino acid(s) are recognized by chymotrypsin (2 pts)?

Two of the fragments end in Tryptophan (Trp), therefore chymotrypsin must cleave after this one. It also cleaves after Phe and Tyr - all large non-polar aromatics.

b) Based on the above data, give all possibilities for the sequence of the entire peptide (3 pts).

The first fragment given, Ala-Ala-Leu must be at the carboxy terminus, because it does not end in Trp, Phe, or Tyr. The order of the first two fragments is unknown, so there are two possibilities:

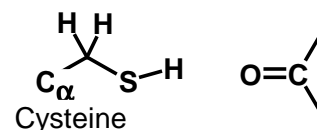
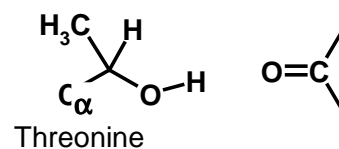
Ala-Lys-Trp-Ala-Met-Trp-Ala-Ala-Leu

Ala-Met-Trp-Ala-Lys-Trp-Ala-Ala-Leu

c) Briefly describe how you would use additional cleavage reagents to confirm which of these sequences correspond to the correct sequence (5 pts).

- If you cleaved with Trypsin, after Lysine, the left sequence would give the peptide Trp-Ala-Met while the right sequence would give Trp-Ala-Ala.
- If you cleaved with CNBr, after Methionine, the left sequence would give a peptide with sequence: Trp-Ala-Ala, while the right sequence would give a peptide Trp-Ala-Lys.
- Thus either cleavage reagent, Trypsin or CNBr, could be used to distinguish between the two possibilities.

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:

**The  $T_M$  is the temperature where the protein is 1/2 unfolded,  $f_U = 0.5$**

Threonine -  $T_M$ : ~333 K

Cysteine -  $T_M$ : ~307 K

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

**When the temperature is equal to  $T_M$ , there is an equal amount of folded and unfolded protein, this implies that the energy**

**difference between the two states is zero:**

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = 0 \text{ when } T = T_M. \text{ Therefore, } \Delta S^\circ = \Delta H^\circ / T_M$$

Threonine -  $\Delta S^\circ$ :

$$\Delta S^\circ = \frac{200 \text{ kJ/mol}}{333 \text{ deg}} \approx 600 \text{ J/mol} - \text{deg}$$

Cysteine -  $\Delta S^\circ$ :

$$\Delta S^\circ = \frac{190 \text{ kJ/mol}}{307 \text{ deg}} \approx 620 \text{ J/mol} - \text{deg}$$

- 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.]?

**The change in entropy suggests that there is also a difference in exposure of non-polar groups in the unfolded proteins - specifically with the Threonine containing protein, since  $\Delta S^\circ$  is lower, there must be more ordered water - therefore a larger non-polar sidechain becomes solvent exposed in the unfolded state.**

**A comparison of the two sidechains, Threonine versus cysteine, shows that the Threonine has a  $-CH_3$  group that is involved in van der Waals contacts in the folded state. When this residue is replaced by Cys, these contacts are missing, so the change in enthalpy includes contributions from both the change in hydrogen bond as well as van der Waals.**

