

**Biochemistry I - Exam Face Page**

- This exam consists of 9 pages, including this one.
- There are a total of 90 points - or approximately 2 points /minute of exam time.
- 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:

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

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

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

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

**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:**

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

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

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

**Ligand Binding:**

For [M]+[L] ↔ [ML]

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

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

1: \_\_\_\_\_ / 9

2: \_\_\_\_\_ / 6

3: \_\_\_\_\_ / 12

4: \_\_\_\_\_ / 8

5: \_\_\_\_\_ / 12

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

7: \_\_\_\_\_ / 11

8: \_\_\_\_\_ / 14

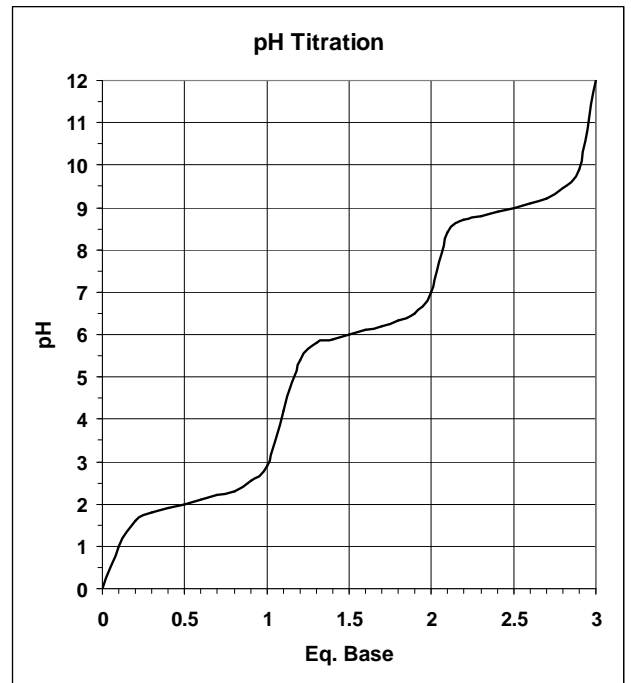
9: \_\_\_\_\_ / 12

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

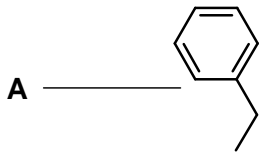
1. (9 pts) A titration curve of an amino acid is shown on the right. Please answer the following questions.

i) What are the  $pK_a$  values associated with this amino acid? Briefly explain how you got these values (3 pt).

ii) Mark **one** pH range on this curve where this amino acid would serve as a buffer. Briefly justify your answer (3 pts).



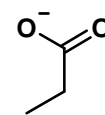
iii) Select from the choices of *sidechains* below, the amino acid that would most likely produce the above titration curve. Write the approximate  $pK_a$  for its sidechain next to its structure on the diagram below. Give the name of this amino acid (3 pts).



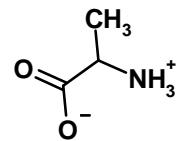
2. (6 pts) The  $pK_a$  of the carboxyl group in the *sidechain* of aspartic acid (Asp) is 4.0. In alanine (Ala), the  $pK_a$  of the *mainchain* carboxyl is 2.0. Please answer either of the following **two** choices. Circle your choice.

**Choice A:** When the Asp sidechain in a protein is close to an amino group, its  $pK_a$  usually decreases, why?

**Choice B:** When the Asp sidechain in a protein is close to another carboxylate group, its  $pK_a$  usually increases, why?



Aspartate sidechain



Alanine

3. (12 pts) Select **one** of the following four interactions or effects. *Briefly* describe the molecular nature of your choice, state whether it is related to the entropy or enthalpy of the system, and then describe its role in the stabilization (or destabilization) of typical globular proteins. Rank the importance of your choice in stabilizing/destabilizing the folded form of the protein relative to the other three choices.

Sample answer: Electrostatic effects involve attraction and repulsion of charged groups in proteins (e.g. Asp and Lys). This is largely an enthalpic ( $\Delta H$ ) effect. It has very little influence on stabilizing either the folded or unfolded form of the protein, all other effects are more important.

- i) the hydrophobic effect
- ii) hydrogen bonds

- iii) van der Waals forces
- iv) Conformational entropy

4. (8 pts) Describe how an  $\alpha$ -helix is similar to a  $\beta$ -sheet (or  $\beta$ -hairpin). How do they differ? (A well labeled diagram is an acceptable answer).

5. (12 pts) Draw, in the space below, *any* dipeptide that contains two *different* residues (Yes, you can use the various amino acid structures found in this exam). In your drawing, the peptide bond should be *trans* (4 pts). On your drawing indicate:
- The peptide bond (1 pt).
  - The bonds whose torsional angles are defined by  $\phi$  and  $\psi$  (order not important) (2 pt).
  - The name of your dipeptide (1 pts).

iv) Please answer any **one** of the following three choices (4 pts). Please circle your choice.

**Choice A:** Which bond(s) in the dipeptide is/are planer and not free to rotate. Why is this so?

**Choice B:** Why are most peptide bonds *trans*?

**Choice C:** Briefly describe the Ramachandran plot and the origin (or source) of the low energy conformations that are contained within the plot.

6. (6 pts) Please do either of the following **four** choices. Please circle your choice:

**Choice A:** Briefly describe the term “quaternary structure”. Provide an example of one.

**Choice B:** What are super-secondary structures? Give an example.

**Choice C:** Distinguish between the secondary and tertiary structure of a protein.

**Choice D:** Describe the overall structure of an antibody molecule. Indicate where antigen binds on this molecule and the location of the Fab fragment.

7. (11 pts) A peptide that is 12 residues in length was subject to Edman degradation. Due to the small amount of the original peptide, only the first 5 residues could be unambiguously identified. The sequence obtained from these data is:

Gly-Ser-Arg-Phe-Phe

The original peptide was then treated with the protease chymotrypsin, and the peptides or free amino acids that were produced from this cleavage reaction were sequenced to give the following data:

Gly-Ser-Arg-Phe, Phe(free amino acid), and a peptide with the sequence: Ser-Thr-Lys-Leu-Met. One additional amino acid was also released by chymotrypsin digestion.

i) Give as much of the original sequence as possible in the space below. The first five residues are already listed for you. *Briefly* justify your answer (5 pts).

Gly – Ser – Arg – Phe – Phe – \_\_\_\_\_ – \_\_\_\_\_ – \_\_\_\_\_ – \_\_\_\_\_ – \_\_\_\_\_ – \_\_\_\_\_ – \_\_\_\_\_  
 1    2    3    4    5    6    7    8    9    10    11    12

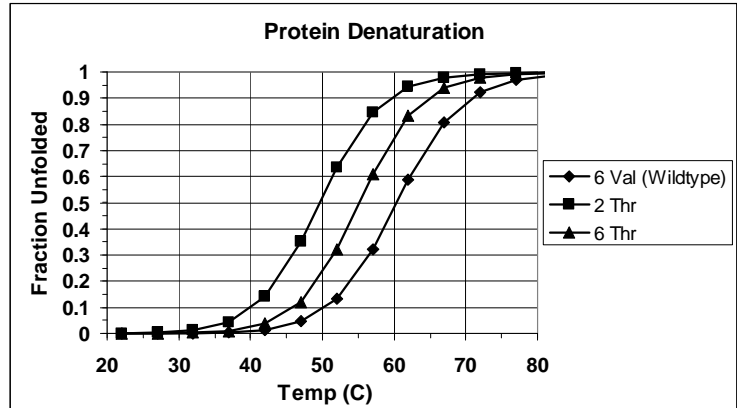
iv) If you wanted to complete the sequence using another cleavage reagent, which would you use and why? (1 pts)

v) A 10 μM (1 μM = 10<sup>-6</sup> M) solution of the peptide has an absorbance of 0.1 (assume path length of 1 cm). Based on this information, can you determine the 11<sup>th</sup> and 12<sup>th</sup> amino acids? How? [Note: Ignore the UV absorption of Phe in this problem, it's small anyway.] (5 pts)

$A = \epsilon[X]l$ $\epsilon_{TRP} = 5,000 M^{-1}cm^{-1}$ $\epsilon_{TYR} = 1,500 M^{-1}cm^{-1}$
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8. (14 pts) Please select **one** of the following three choices. Circle your choice.

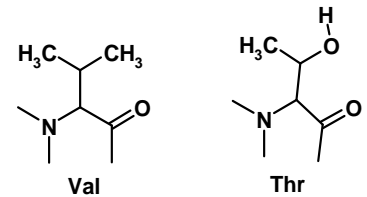
**Choice A:** The core of a globular protein consists entirely of six valine residues. The melting curve for this protein, as well as a number of variants is shown to the right. The thermodynamic parameters for these proteins are shown in the table below.



Protein	$\Delta H^{\circ}_{(N \rightarrow U)}$	$\Delta S^{\circ}_{(N \rightarrow U)}$
Native protein (6 Val)	+200 kJ/mol	+600 J/mol-deg
Variant 1 (2 Thr+4 Val)	+200 kJ/mol	+620 J/mol-deg
Variant 2 (6 Thr)	+210 kJ/mol	+640 J/mol-deg

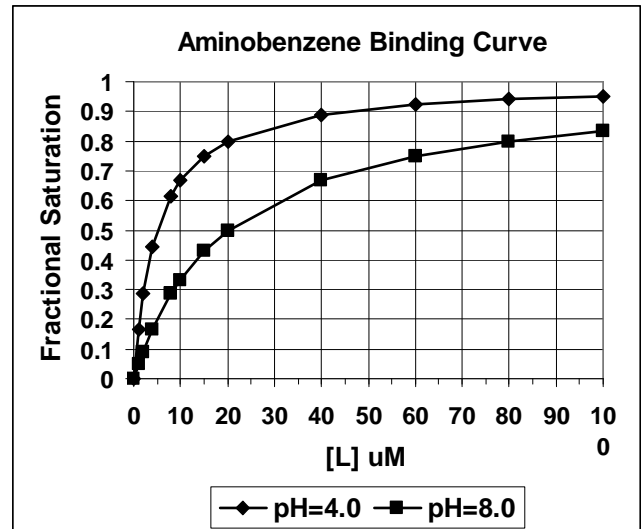
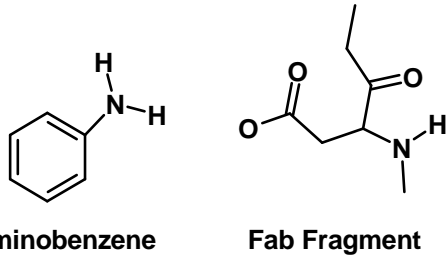
i) Briefly describe how you would obtain the enthalpies from these denaturation curves (2 pts).

ii) Replacement of two of the valine residues with the amino acid threonine causes the protein to become much less stable than the original. Briefly explain which interactions have been affected by this replacement and explain why the protein is less stable. You should include the above changes in the thermodynamic parameters in your discussion. (**Note:** Pay close attention to the structure of the sidechains of valine and threonine when formulating your answer.) (6 pts)



iii) Surprisingly, replacement of all six of the valine residues with threonine increases the stability of the protein somewhat, giving the middle denaturation curve in the above graph. Interpret this result using the thermodynamic parameters given in the table. (6 pts)

**Q8 Choice B:** The binding of aminobenzene to a Fab fragment was measured using equilibrium dialysis at two different pH values. The binding curves for each pH value are shown to the right. The structure of aminobenzene interacting with one of the amino acid residues (aspartic acid) from the antibody is shown below.



i) Determine the  $K_D$  for binding at each pH value. Enter your answer in the table to the right. Briefly justify your approach. (5 pts)

pH	$K_D$
4.0	
8.0	

ii) At what pH value is the binding the strongest? Justify your answer by reference to the  $K_D$  values. (5 pts)

iii) Briefly explain the effect of pH on the binding affinity. Your answer should clearly indicate how the interaction between the aminobenzene and the Fab fragment changes as a function of pH and how this affects the measured  $K_D$ . You can assume that the  $pK_a$  values of the aminobenzene and the sidechain of the amino acid from the Fab fragment are both 4.0 (4 pts).

**Q8 – Choice C:** A 60 residue globular protein unfolds with an enthalpy of +200 kJ/mol with a melting temperature of 333 K. Please answer the following questions:

$$\begin{aligned}\Delta G^{\circ} &= \Delta H^{\circ} - T\Delta S^{\circ} \\ &= -RT \ln K_{EQ} \\ R &= 8.3J / mol - K \\ S &= R \ln W\end{aligned}$$

i) Determine the overall *entropy* of unfolding (3 pts).

ii) Estimate the change in *entropy* due to conformational changes when this protein unfolds (4 pts).

iii) Based on your calculated value for *part ii*, what is the contribution of the solvent entropy to the overall protein stability? (3 pts).

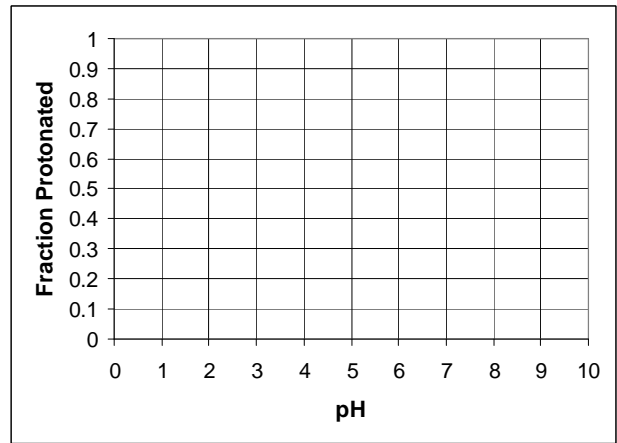
iv) Calculate how much of this protein would be unfolded at 343 K (4 pts).



9. (12 pts) Please do **one** of the following four choices. Please indicate your choice. The following equations may be useful, but a graphical solution is also acceptable.

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

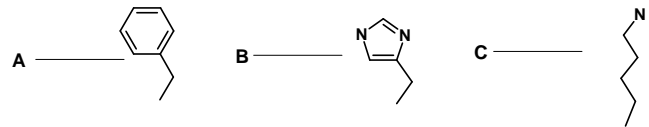
$$f_{HA} = \frac{1}{(1 + R)} \quad f_{A^-} = \frac{R}{1 + R}$$



**Choice A (Buffer construction):** You wish to make a buffer solution at pH 3.0 using phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) as the starting material. How much NaOH do you have to add to 1L of solution to make a 1 molar solution of this buffer? You may assume that the pK<sub>a</sub> values of phosphate are 2, 7, and 12. (Hint: Assume phosphate is a monoprotic buffer with a pK<sub>a</sub>=2).

**Choice B (pH adjustment):** A 1 M buffer solution of phosphate at pH = 3.0 is being used to buffer a reaction. The reaction that you are buffering absorbs protons, causing the pH to increase to 5.0. How much HCl do you have to add to restore the pH to 3.0? You may assume that the pK<sub>a</sub> values of phosphate are 2, 7, and 12. (Hint: Assume phosphate is a monoprotic buffer with a pK<sub>a</sub>=2).

**Choice C (Charge calculation):** What is the net charge at pH 9 for the amino acid that you selected for your answer in problem 1? Please show your work. (The structures are repeated here for your convenience.)



**Choice D (Enzyme activity & pH):** An enzyme has an Aspartic acid residue that is important for function. The enzyme activity at pH=4.0 is ~10%, at pH=5.0 it is 50%, and at pH=6.0 it is ~90%. What is the pK<sub>a</sub> of this group? Which form is active (protonated or deprotonated)? Justify your answers to both of these questions.