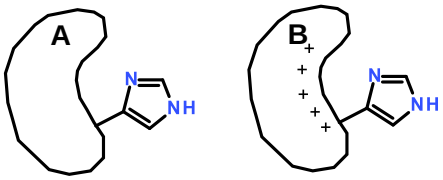
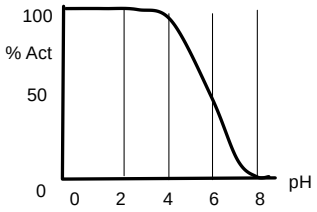
This exam consists of 6 pages and 11 questions with 2 bonus questions. **Total points are 100. Allot 1 min/2 points**. On questions with choices, all of your answers will be graded and the best scoring answer will be used. Please use the space provided, or the back of the preceding page.

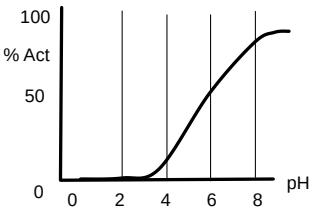
1. (8 pts) Two different proteins (A and B) both contain an imidazole group as part of one of their amino acids (histidine). The structure of these two proteins is shown on the right. **The imidazole group is shown in the deprotonated state in both proteins, i.e. pH>>pKa.** The "+" symbols on protein B refer to positive charges that are close to the histidine residue.

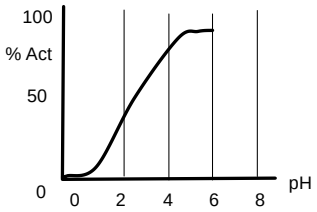


i) (4 pts) Assume that the pKa of the imidazole group in protein A is 6. Will the pK_a of this imidazole group in protein B be higher or lower? ***Briefly justify your answer.***



ii) (4 pts) Circle the curve on the right that correspond to the % activity of **protein A** as a function of pH, assuming the ***deprotonated*** form of the imidazole is the active form. ***Briefly justify your answer***.

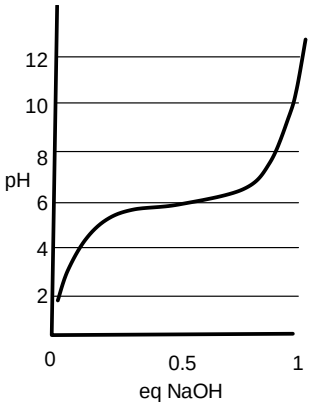




2. (9 pts) You wish to make 1L of a 0.1 M (A_T) buffer at pH = 6, using one of the following weak acids:

a) Acetic acid (pK_a=5.0), b) HEPES (pK_a = 6.0)

i) (2 pt) Which weak acid from the above list (a or b), corresponds to the titration curve on the right? Why?



ii) (1 pts) Which of the two weak acids would you choose to make this buffer and why?

iii) (6 pt) Describe how you would make this buffer assuming that you only had the fully protonated form of the acid. Give the total moles of weak acid required and the moles of NaOH required to adjust the pH ($R=10^{pH-pKa}$ $f_A = R/(1+R)$ $f_{HA} = 1/(1+R)$)

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3. (5 pts) Why do weak acids act as buffers? Briefly explain why the pH of the solution does not change very much in the buffer region when a strong base or acid is added to the solution.

4. (17 pts)

i) Draw any tripeptide that you like (assuming that the pH @pH=7) as long as it contains one non-polar, one polar, and one acidic charged residue (e.g. Aspartic acid or glutamic acid)**. Do not** use histidine, phenylalanine, or isoleucine in your drawing (6 pts).

ii) Give the sequence of the peptide (2 pts)

iii) Circle the mainchain atoms and indicate one ***mainchain*** hydrogen bond acceptor with an "A". (2 pts)

iv) Indicate a bond in your structure that is planer, and usually *trans*. Briefly indicate why it has these properties (5 pts).

v) You measure the binding of your peptide to the protein shown on the right at low pH and at high pH and find the following k_{off} values (1000 s^{-1} at pH=2, 1 s^{-1} at pH=7). Explain why the off-rate is slower at pH=7 (2 pts). [Hint: *estimate* the charge on the peptide at pH 2 and 7].

$$q_{total} = \sum(f_{HA} \cdot q_{HA} + f_{A-} \cdot q_{A-})$$

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5. (10 pts) You are trying to sequence a **12 residue** peptide using Edman degradation. The following peptide sequences were obtained after cleavage of the initial peptide with the indicated cleavage reagents. You can assume that it was only possible to sequence the first five residues of each peptide. Sequencing of the intact peptide gives the sequence: Ala-Gly-Val-Met-Glu

CNBr fragments: CNBr 1: Ala-Gly-Val-Met CNBr 2: Val-Gln-Asp-Thr CNBr 3: Glu-Arg-Trp-Met

Trypsin Digest: T1: Ala-Gly-Val-Met-Glu T2: Trp-Met-Val-Gln-Asp

i) (7 pts) Determine the sequence of the original peptide. Instead of writing out the sequence, you can just give the correct order of the CNBr fragments, e.g. 1-2-3. Justify your approach if you think you might need partial credit (in case your answer is incorrect).

ii) (3 pts) What is the absorbance at 280 nm for a 1 uM (10^{-6} M) solution of this peptide (assume l=1cm)?

Amino acid	**ε**
Trp	5,050 $M^{-1}cm^{-1}$
Tyr	1,440 $M^{-1}cm^{-1}$

$$A = \log \frac{I_O}{I} = \varepsilon [X] l$$

6. (8 pts) Briefly describe the overall tertiary structure of folded globular proteins, in particular where you would find polar, non-polar, and charged side chains. Also describe the properties of the core of the protein and briefly indicate why it has those properties.

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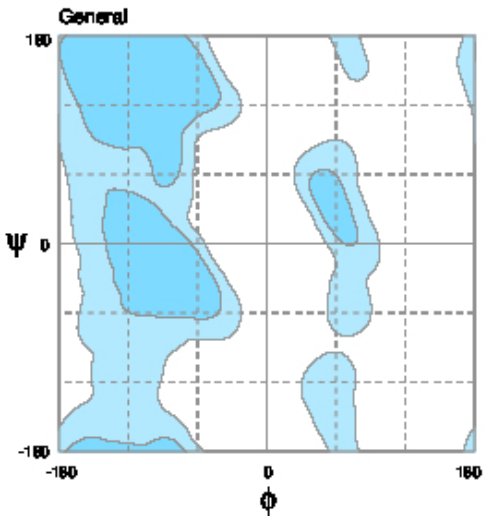
7. (14 pts) A blank Ramachandran plot for a non-glycine or non-proline residue is given on the right.



i) Provide a sketch of any **one** of the following structures (you need not draw all of the atoms, just indicate the approximate positions of C_α carbon, the location of the sidechains, and the location of hydrogen bonds (6 pts).

- α_R helix
- three stranded β sheet (antiparallel)
- β-barrel
- β-α-β super-secondary structure.
- Immunoglobulin domain.

ii) Indicate what the Ramachandran plot would look like for the protein that you sketched (i.e. where would the points be found on the plot?) (2 pts)

iii) Why are some regions of the plot white and some shaded (colored) (4 pts)?

iv) How would the shaded regions differ if the residue was a glycine or a proline. You only need to discuss **one** of the two (2 pts)?

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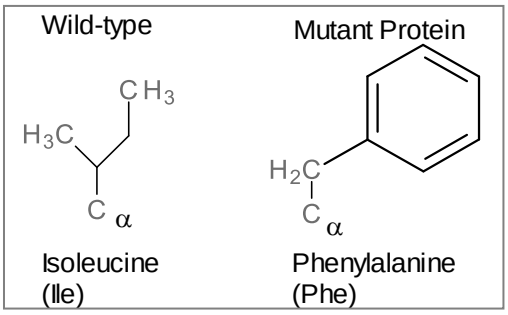
8. (16 pts) An isoleucine residue is buried in the core of a globular protein. A mutant protein is being studied, where the isoleucine has been replaced by Phe. **Please do all parts of this question.**

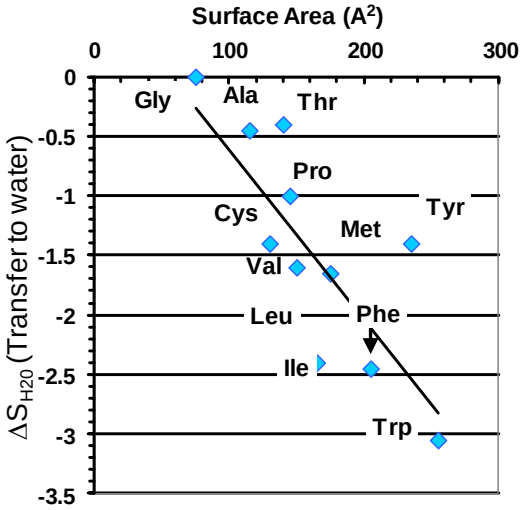


i) The enthalpy (ΔH^o) for unfolding of the wild-type protein is +200 kJ/mol and for the mutant it is +180 kJ/mol.

a) Briefly describe how you would have obtained the enthalpy from experimental measurements of $K_{eq}(=f_u/f_N)$ versus T (2 pts).

b) Why is the enthalpy positive (2 pts)?

c)Explain the difference in enthalpy between the two proteins (4 pts).



ii) The entropy (ΔS^o) for unfolding of both proteins is the same, +600 J/mol-K.

a) Explain why the entropy is positive, specifically what factors contribute to the overall entropy and what are their signs and relative sizes. ***Describe how you might estimate each term*** (6 pts).

b) Explain why the entropy change for both proteins is the same (2 pts)

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9. (6 pts) Please do **one** of the following choices.

Choice A: Define/describe quaternary structure, using immunoglobulins (antibodies) or hemoglobin as an example.

Choice B: Draw a "cartoon" diagram of an antibody and indicate on your diagram the following:

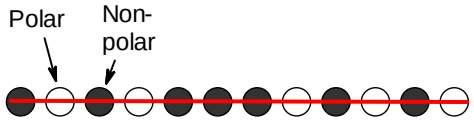
i) the location of the hypervariable loops.

ii) Where the antigen binds.

iii) The part that would be found in an Fv fragment.

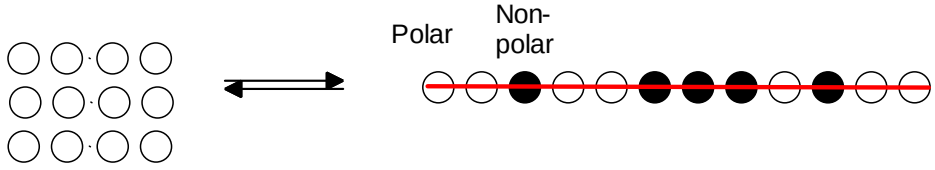
Choice C: What is a disulfide bond and why does it stabilize proteins which contain them?

10. (5 pts) What type of secondary structure would you most likely find the following sequence, assuming it is on the surface of a globular protein. *Justify your answer.*



11. (2 pts) What is a chiral center and why do pharmaceutical firms generally avoid developing drugs that have chiral centers?

Bonus (1 pts): Indicate the most stable fold of the following protein by connecting the circles with either vertical or horizontal lines between adjacent circles. The folded form is on the left, the unfolded on the right.



Bonus (1 pt): My blood type is type O, what other blood group can I receive blood from? Why?

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