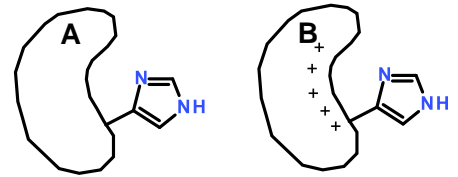


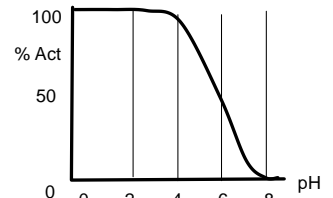
This exam consists of 6 pages and 11 questions with 2 bonus questions. **Total points are 100. Allot 1 min/2 points.** On questions with choices, all of your answers will be graded and the best scoring answer will be used. Please use the space provided, or the back of the preceding page.

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. $pH \gg pK_a$.** The “+” symbols on protein B refer to positive charges that are close to the histidine residue.

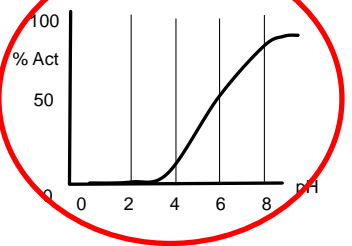


i) (4 pts) Assume that the pK_a of the imidazole group in protein A is 6. Will the pK_a of this imidazole group in protein B be higher or lower? **Briefly justify your answer.**

- The energy of the deprotonated His is not affected by the + charge on the protein since it is uncharged.
- The energy of the protonated His will be affected by the + charges. Since the charges are the same, the energy of the system will be raised, in other words the protonated form is unfavorable.
- Because the protonated form is unfavorable, the group will have an increased tendency to ionize, making it a stronger acid with a pK_a less than 6.



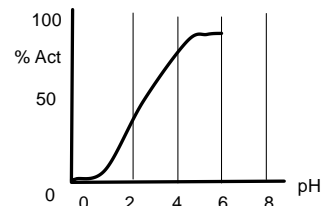
ii) (4 pts) Circle the curve on the right that correspond to the % activity of **protein A** as a function of pH, assuming the **deprotonated** form of the imidazole is the active form. **Briefly justify your answer.**



It is the middle diagram. There should be 50% activity when $pH = pK_a$ (1/2 the molecules are in the active form).

The activity should be zero at low pH since all the protein is in the inactive protonated state.

The activity should be 100% at high pH since all of the protein is in the active (deprotonated form)



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:

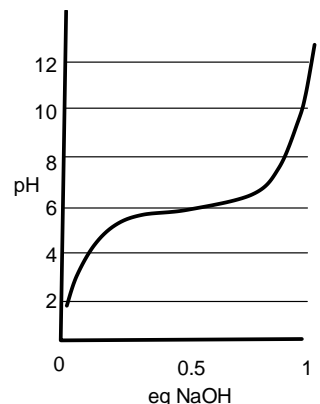
- a) Acetic acid ($pK_a = 5.0$), b) HEPES ($pK_a = 6.0$)

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

This is the titration curve for HEPES, since the pH at 0.5 equivalents is 6, the pK_a of HEPES.

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

HEPES since its pK_a is within one unit of the desired pH.



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 ($R = 10^{pH - pK_a}$ $f_A = R/(1+R)$ $f_{HA} = 1/(1+R)$)

moles of weak acid = $0.1 \text{ M} \times 1 \text{ L} = 0.1 \text{ moles}$ (Note: this is independent of the pH of the solution)

The desired $pH = pK_a$, so $f_A = 0.5$ and $f_{HA} = 0.5$

The equivalents of NaOH = $f_A = 0.5$. Moles of NaOH = $eq \times A_T \times V = 0.5 \times 0.1 \text{ M} \times 1 \text{ L} = 0.05 \text{ moles}$

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.

In the buffer region, any added OH is neutralized by protons released from the weak acid. Similarly, any added protons (from HCl), will protonated the conjugate base to form the protonated acid. In either case the added OH or H is removed and does not affect the pH.

4. (17 pts)

- 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).
- Give the sequence of the peptide (2 pts)
- Circle the mainchain atoms and indicate one **mainchain** hydrogen bond acceptor with an "A". (2 pts)

See diagram.

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

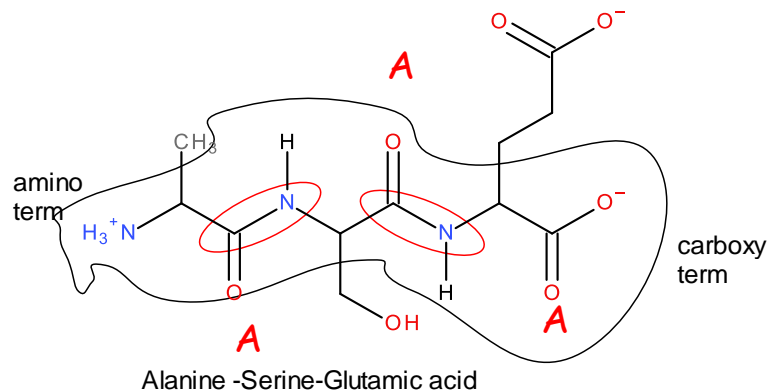
This is the peptide bond (red circle), it is planar because the nitrogen is sp² and trans to reduce van der Waals contacts.

- 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].

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



- at pH=2 -
 - amino term is +1 (fully protonated)
 - Glu charge = 0 (fully protonated)
 - C-term charge = -0.5 (1/2 protonated since pH = pKa)
- the charge is +1/2, so it has weak (unfavorable) interactions with the protein so it leaves quickly.



- at pH=7 -
 - amino term is +1 (fully protonated)
 - Glu charge = -1 (fully deprotonated)
 - C-term charge = -1 (fully deprotonated since pH = pKa)
- the charge is -1, so it has favorable interactions with the protein, leading to a lower off rate.

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).

The amino terminal sequence shows that there should be a CNBr (cleaves after Met) that starts with Glu, so the order of CNBr fragments is 1-3-2

Ala-Gly-Val-Met- Glu-Arg-Trp-Met- Val-Gln-Asp-Thr

This is consistent with the data for the trypsin fragments.

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

There is a only a single Trp residue:

$$A = 10^{-6} \times 5,050 \times 1 \text{ cm} = 0.00505$$

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

$$A = \log \frac{I_0}{I} = \epsilon [X] l$$

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.

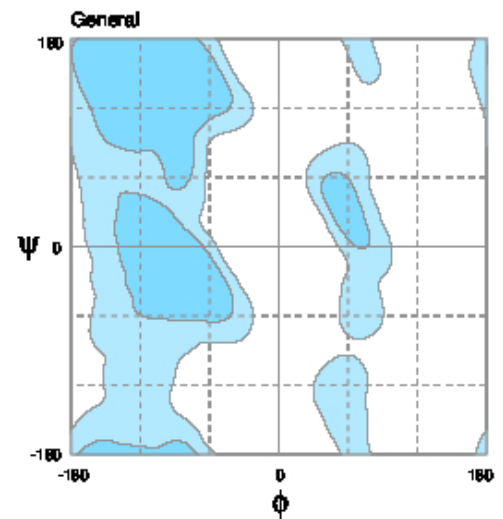
- Polar and charged residues are on the surface.
- The core is entirely non-polar and well packed
 - van der Waals interactions result in ideal packing
 - the hydrophobic effect causes the non-polar groups to be in the core, releasing ordered water when the protein folds.
- There can be non-polar residues on the surface.

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.

Structure should show the general chain direction, where the side-chains are, and the location of the sidechain.



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)

For beta structures, the points would be in the upper left quadrant.

For alpha structures, the points would be in the lower dark area to the left (psi=0 deg)

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

The shaded areas correspond to conformations with favorable van der Waals

The white areas correspond to conformations that give unfavorable van der Waals (due to side chain atoms coming too close together)

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)?

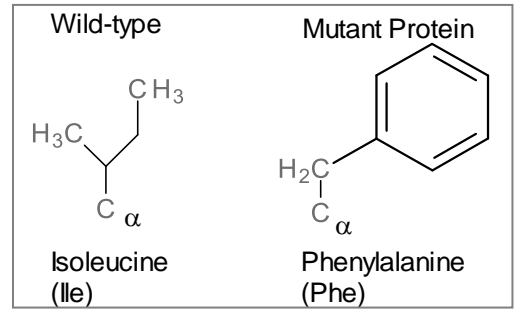
Glycine - there would be more shaded regions on the right side (due to the lack of a sidechain)

OR

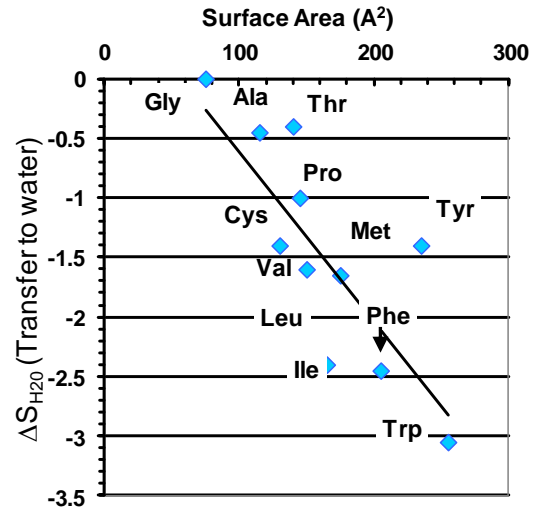
Proline - only the left side will be shaded (conformations on the right are not allowed because of the ring)

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°) for unfolding of the wild-type protein is +200 kJ/mol and for the mutant it is +180 kJ/mol.
- Briefly describe how you would have obtained the enthalpy from experimental measurements of $K_{eq}(=f_u/f_N)$ versus T (2 pts).
 - Why is the enthalpy positive (2 pts)?
 - Explain the difference in enthalpy between the two proteins (4 pts).



- Plot $\ln K$ versus $1/T$, slope = $-\Delta H/R$
- It requires energy to break the favorable van der Waals and hydrogen bonds, since the unfolded form has absorbed heat, the ΔH is positive.
- The larger Phe will disrupt the ideal packing in the core of the protein, reducing van der Waals between the other residues in the core.



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

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

- The overall entropy is positive because the increase in the entropy of the chain is larger than the decrease in the entropy of the water due to exposure of the non-polar residues.
 - The positive term is the conformational entropy that can be calculated using $S = R \ln W$, $W = 9^n$
 - The negative entropy term is due to the ordering of water and can be calculated using the above graph that indicates larger non-polar sidechains order more water. $S = \sum S_{\text{NONPolar}}$
- Both isoleucine and phenylalanine have the same entropy change when placed in water (-2.5 J/mol-K), therefore the entropy due to the hydrophobic effect will be the same. If we assume that the chain (conformational) entropy is the same for both proteins, then the observed entropy will be the same.

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?

Choice A:

Quaternary structure refers to the arrangement of multiple chains. Antibodies have four chains, two light and two heavy. Hemoglobin also has four chains, two alpha and two beta.

Choice B:

Should be a Y shaped diagram with two light and two heavy chains.

There should be three hypervariable loops projecting off of each chain at the ends of the Y

The antigen binds to the tips of the Y – to the hypervariable loops

The Fv fragment would contain the VL and VH domains.

Choice C:

A disulfide bond is a covalent bond between the sulfur atoms on two cysteine residues. It stabilizes proteins by reducing the entropy of the unfolded state, so the overall (favorable) increase in the conformational entropy is reduced by the disulfide bond.

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.*

b-strand, since it is alternating polar and non-polar. There is one non-polar residue that would be on the outside of the protein, marked with an (*)

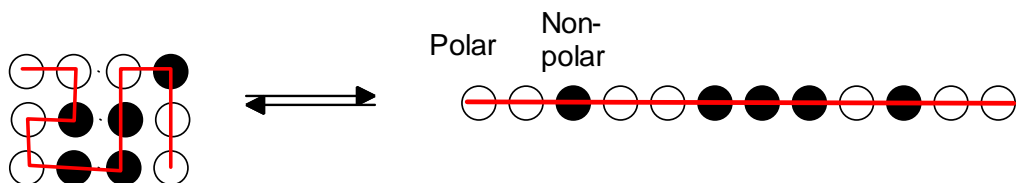


Partial Credit: If you said helix and stated that the non-polar residues have to be every 3 or 4, then +3 pts.

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

A chiral center is a (tetrahedral) carbon that forms bonds with four different groups. If a drug has a chiral center it has two enantiomers, which may have very different biological properties and it may be necessary to either synthesize a pure chiral compound or separate the enantiomers from a racemic mixture.

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.



Any structure that buries a similar number of non-polar groups is acceptable.

Bonus (1 pt): My blood type is type O, what other blood group can I receive blood from? Why? Since I am type O I have both anti-A and anti-B antibodies present. So I cannot receive blood from either A, B, or AB. I can only get blood from type O since there are no A or B antigens present on the red blood cells.