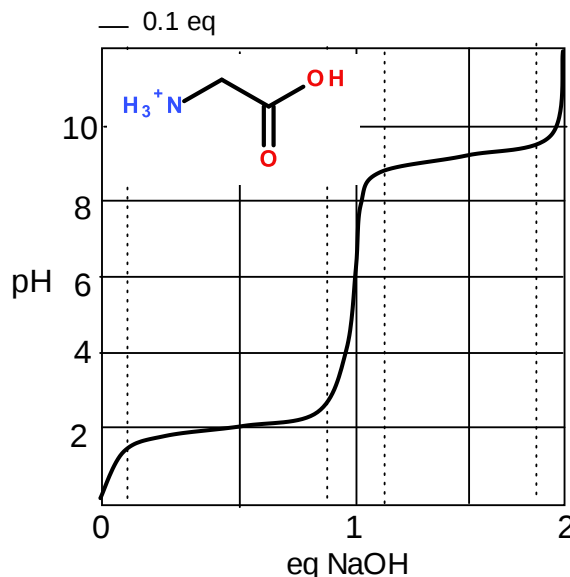


Instructions: this exam consists of 14 questions on 5 pages, for a total of 100 points. On questions with choices, all of your attempts will be graded and you will be awarded the highest grade. Please use the space provided or the back of the preceding page.

1. (6 pts) The titration curve for a diprotic acid, glycine, is shown on the right. The vertical dotted lines are 0.1 eq from the nearest solid line.

- What are the pKa values for glycine? *Justify your answer.* (1 pt)
- Explain why the pH changes very little in the buffer regions (5 pts).



2. (8 pts) This question is based on the titration curve shown in the previous question. Please do one of the following choices. $f_{HA} = 1/(1+R)$, $f_A = R/(1+R)$, $R = 10^{(pH-pKa)}$

Choice A: A 0.1 M solution of glycine (volume = 0.5 L) is at pH = 3.0. How many moles of acid (HCl) would you add to adjust the pH to 2.0? Please show your calculations.

Choice B: You want to make a 0.1 M buffer using glycine with a pH = 9.0, total volume of 0.5 L. You only have the fully protonated glycine available (e.g. H_2A). Describe how you would make the buffer. Include in your answer the following:

- the total number of moles of glycine you would need for this buffer solution.
- the total number of moles of NaOH you would need to add to the glycine solution to give a pH = 9.

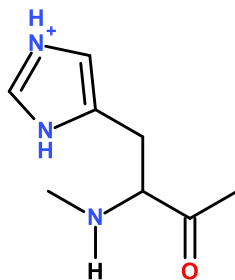
Choice C: You wish to use glycine as a buffer for two separate reactions, one at pH 1.5 (solution A) and the other at pH 2.5 (solution B). Assuming the concentration of glycine is the same in both solutions, and that the reaction will generate acid, which solution has the ability to absorb more protons? Justify your answer.

3. (11 pts) Draw the chemical structure of a tri-peptide (e.g three amino acids), adding to the histidine residue shown below (the histidine is shown with its sidechain in its protonated form). Your first amino acid should be charged and the second one should be polar (but not charged), assume a pH=6.0 (5 pts). You **cannot** use histidine, alanine, threonine, tyrosine, tryptophan, or leucine for your choices. Please indicate the following on your diagram.

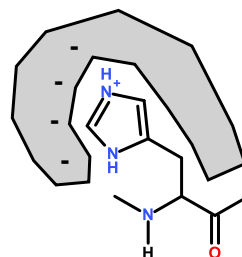
i) the sequence of your peptide (1 pt)

ii) a peptide bond (1 pt)

iii) the overall net charge on your peptide at pH=6.0, and justify your answer (4 pts).



4. (6 pts) Assume that the peptide you drew in the previous question binds to another protein, in a negatively charged binding pocket. You measure the pK_a of the histidine residue when bound to the protein and discover that its pK_a is 7 instead of the usual 6. Explain why the pK_a of the bound histidine has increased.



5. (8 pts) The sequence of an 11 residue peptide is determined by Edman sequencing of fragments that are produced after cleavage by CNBr or Chymotrypsin. You should assume that it is possible to only obtain the first five (5) residues of any peptide during the sequencing reaction.

CNBr Fragments: Ala-Gly-Met

Ala-Ala-Trp

Phe-Arg-Ser-Trp-Met

Chymotrypsin fragments: Ala-Gly-Met-Phe

Arg-Ser-Trp

Met-Ala-Ala-Trp

Reconstruct the original sequence of the peptide, write your answer here (the first three are done for you).

<u>Ala</u>	<u>Gly</u>	<u>Met</u>	_____	_____	_____	_____	_____	_____	_____	_____	_____
1	2	3	4	5	6	7	8	9	10	11	

Justify your approach on the back of the previous page to receive partial credit in case your answer is wrong.

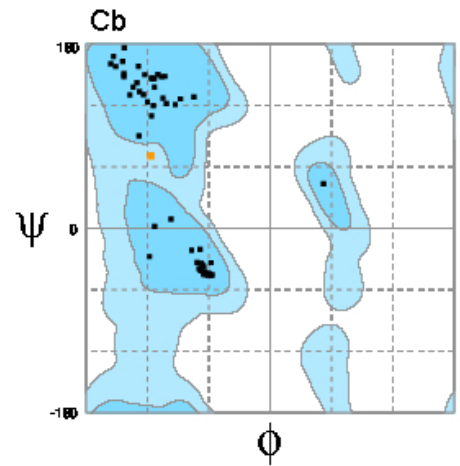
6. (5 pts) A solution of the protein from the previous question gives an absorbance of 1.0. What is the concentration of that protein in solution ($l=1$ cm). $A = [X]\epsilon l$

$$\epsilon_{\text{Trp}} = 5000 \text{ M}^{-1}\text{cm}^{-1}$$

$$\epsilon_{\text{Tyr}} = 1000 \text{ M}^{-1}\text{cm}^{-1}$$

7. (7 pts) The Ramachandran plot is shown on the right.

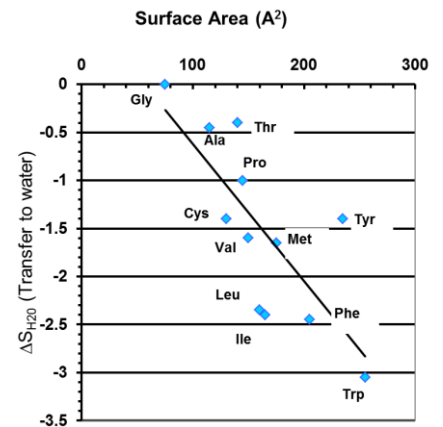
- What does each dot on this plot represent? (1 pt)
- What do the colored/shaded regions represent? (4 pts)
- Are the colored/shaded regions the same for all 20 of the amino acids? Justify your answer (2 pts)?



8. (6 pts) The peptide bond can exist in only two conformations, one of which is more stable than the other.

- What are these two conformations? Draw or describe them (2 pt).
- Why is one conformation more stable than the other? What favorable or unfavorable interactions are responsible (4 pts)?

9. (8 pts) Two entropy terms are important for protein unfolding. One of these stabilizes the folded form while the other stabilizes the unfolded form. Select **ONE** of these two terms and describe it. Also indicate how could you estimate that term, given the size of the protein and the number of non-polar residues. The plot on the right may be useful, also $S = R \ln W$.



10. (4 pts) Please do one of the following choices:

Choice A: Briefly explain why disulfide bonds stabilize folded proteins.

Choice B: Briefly explain why ethanol will destabilize folded proteins, unfolding them (an unconventional way to cook an egg!).

11. (8 pts) Pick either one secondary structure **or** super-secondary structure and:

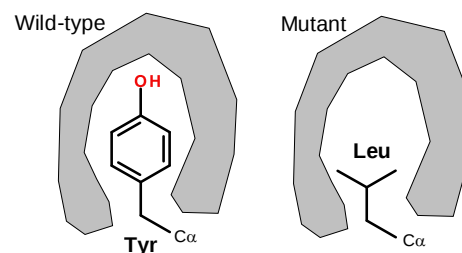
- Sketch (or describe) its structure, indicating both the mainchain and the sidechains (you need not draw all of the atoms), important geometric parameters, and if appropriate the distribution of polar and non-polar residues.
- Describe **all** of the forces that stabilize the structure that you drew.

12. (12 pts) A tyrosine residue that is buried in the core of a globular protein is replaced by leucine. Tyrosine is the wild-type residue and leucine is the mutation. The sidechain of tyrosine and leucine are shown on the right, the gray shape represents the other residues in the core. Please do all parts. Note that you have a choice in *part i*.

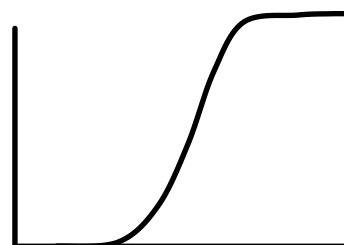
- Please do one of the following two choices:

Choice A: How will the enthalpy of unfolding change for the mutant protein? Will it be higher or lower than the wild-type protein? Justify your answer (5 pts).

Choice B: How will the entropy of unfolding change for the mutant protein? Will it be higher or lower than the wild-type protein? Justify your answer (5 pts).



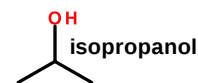
- The melting curve for the **wild-type** protein is shown on the diagram on the right. Label the axis and then sketch the melting curve that you would expect to see for the mutant protein. Briefly explain why you drew the curve that you did, *with reference to your answer to part i* (5 pts).



iii) Briefly describe how you would experimentally determine **either** ΔH° **or** ΔS° for unfolding from the above melting curve (2 pts). $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$, $\Delta G^\circ = -RT \ln K_{eq}$

iv) (**Bonus 2 pts**). The stability of the mutant protein increases in the presence of isopropanol.

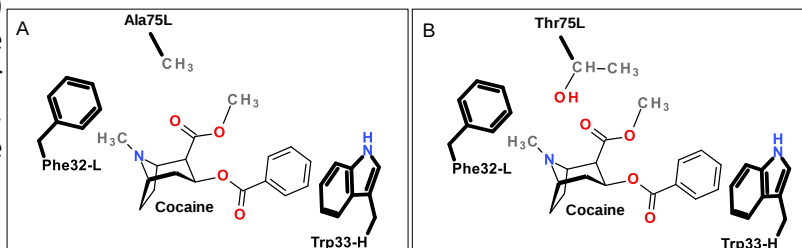
Why? Use the back of the previous page for your answer.



13. (6 pts) Two different antibodies (A and B) are being tested as drugs to treat a cocaine overdose. The antibody-cocaine complex for both of these are shown on the right. Residues from the antibody are in bold. The K_D values for each antibody are:

A: 10 μ M

B: 1 μ M



i) Which antibody has the higher affinity for cocaine? Justify your answer with reference to the K_D values and the interaction between the antibody and the cocaine (4 pts).

ii) What rate constant is most likely to be different when comparing antibody A to antibody B, the kinetic on-rate (k_{ON}), or the kinetic off-rate (k_{OFF})? Why? (2 pts).

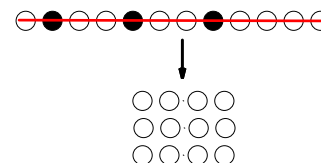
14. (5 pts) Please do **one** of the following choices:

Choice A: Describe/draw the overall quaternary structure of an antibody, including an indication of where the antigen binds.

Choice B: Describe/draw **either** an Fv or an Fab fragment. Indicate where the antigen binds.

Choice C: Why might an Fv or Fab fragment be more effective in antibody based cancer treatments?

Bonus (2 pts): Indicate the lowest energy fold for the following protein sequence. Show the location of non-polar residues and the tracing of the chain (solid = non-polar, open=polar).



Points on page: _____