

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).

i) 2 for the carboxyl and 9 for the amino, based on the pH at the $\frac{1}{2}$ equivalent points (0.5) and (1.5) where the pH=pKa.

ii) In these regions the weak acid is deprotonated and the released proton will neutralize the added base. Since the base is reacting with a proton from the weak acid, and not a proton in solution (which would increase the pH), the pH changes only a little.

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

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.

Choice A:

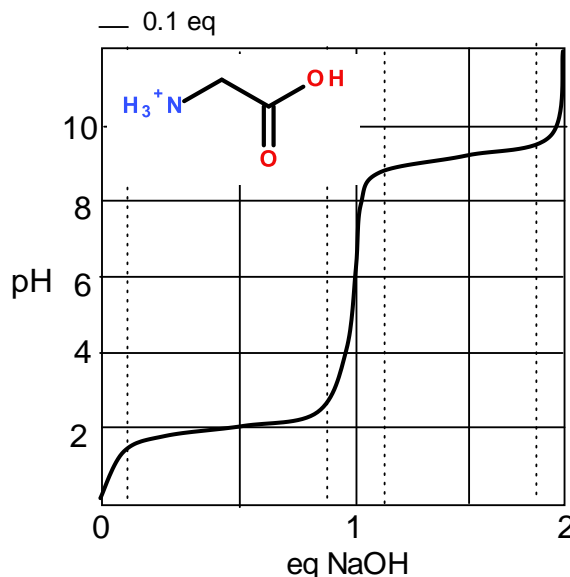
- Calculate the **difference** in fraction protonated at the two pH values:
- pH = 3.0, $R = 10^{pH-pK_a} = 10^{3-2} = 10^1 = 10$, $f_{HA} = 1/(1+10) \sim 0.1$
- pH = 2.0, $R = 10^{pH-pK_a} = 10^{2-2} = 10^0 = 1$, $f_{HA} = 1/(2) = 0.5$
- A total of 0.4 equivalents would be added, moles = $0.4 \times A_T \times V = 0.4 \times 0.1 \times 0.5 = 0.02$ moles

Choice B:

- moles of glycine = $A_T \times V = 0.1 \text{ M} \times 0.5 \text{ L} = 0.05$ moles
- Determine f_{HA} at pH 9.0, using 2nd pKa value. Since pH = pKa, $f_{HA}=f_A=0.5$.
- Since you are starting with H_2A , you need to add one full equivalent to deprotonated the carboxylate, then 0.5 equivalents to partially deprotonated the amine, total equivalents = 1.5
- moles NaOH = $eq \times A_T \times V = 1.5 \times 0.1 \times 0.5 = 0.075$ moles

Choice C:

- The production of acid will cause the pH to drop.
- Solution A, at a pH 1.5 is below the pKa so it is closer to the start of the buffer region than solution B. Solution B, which is at pH 2.5 is to the right of the pKa, so it can absorb more protons before the edge of the buffer region is reached. Therefore buffer B will be able to absorb more protons than A.



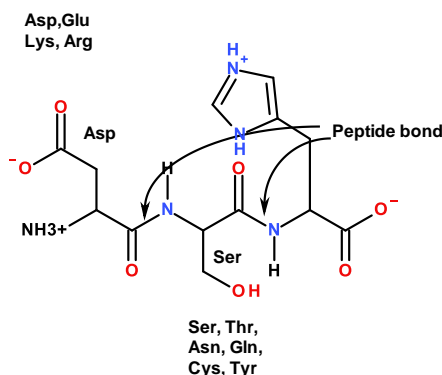
3. (11 pts) Draw the chemical structure of a tri-peptide (e.g three amino acids), adding to the histidine residue shown below (the histidine is shown with its sidechain in its protonated form). Your first amino acid should be charged and the second one should be polar (but not charged), assume a pH=6.0 (5 pts 3 pts for correct mainchain, 2 points for sidechain – full credit if there are only minor errors e.g. if they say Asp but drew Glu, it is OK.). 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) **Asp - Ser - His** (The order does not matter, as long as the structure is correct and agrees with the sequence.

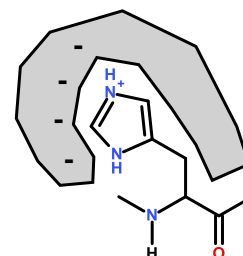
ii) a peptide bond (1 pt) **See diagram**

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

+1 (amino) - 1 (sidechain, Asp) + 0.5 (His sidechain) - 1 (carboxyl) = -0.5 (this answer will depend on what they choose as their charged residue/ **Asp & Glu = -0.5, Lys & Arg = +1.5**)



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.



- The negative charge stabilizes the protonated form of His, due to the unlike charges.
- Therefore the histidine would like to remain protonated, making it a weaker acid.
- Weaker acids have higher pK_a values.

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).

Ala Gly Met Phe Arg Ser Trp Met Ala Ala Trp
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.

The 1st chymo fragment indicates a Phe follows the first three residues. This phe is contained in the 3rd CNBr fragment. The Met at position 8 is found in the 3rd Chymo fragment. The colored arrows indicate the predicted fragments, which agree with the experimental ones.

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). **$A = [X]\epsilon l$**

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

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

The protein contains two tryptophan residues and zero tyrosine residues, so the extinction coefficient is $10,000 \text{ M}^{-1}\text{cm}^{-1}$. The concentration is $A/\epsilon l = 1/10,000 = 10^{-4} \text{ M}$.

Points on page: _____

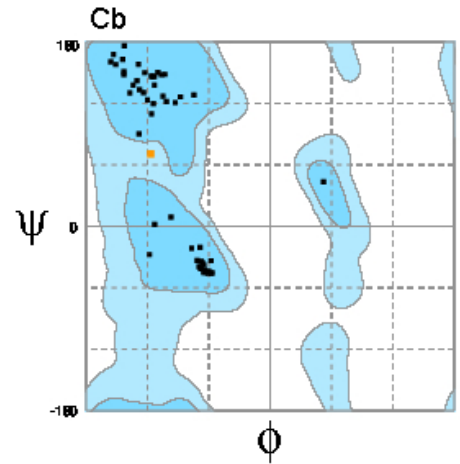
7. (7 pts) The Ramachandran plot is shown on the right.

- 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)?

i) The phi and psi rotational angles of one residue in the protein.

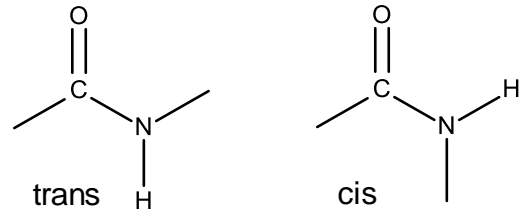
ii) Regions of low energy due to favorable vdw.

iii) No. The shaded regions on this plot are for residues with C_{β} carbons. glycine, which lacks a beta-carbon would have a larger area that would be low energy. Proline, which cannot rotate around the N-Ca bond would have a smaller shaded area.



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)?



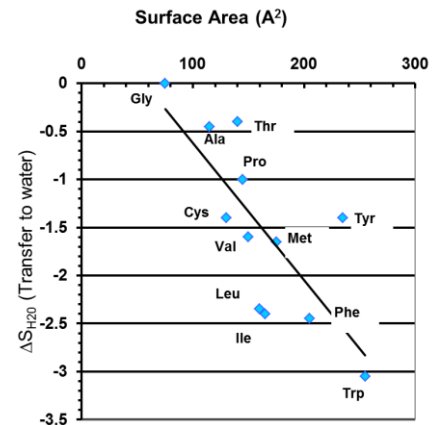
i) Cis and trans. In cis the N-H is on the same side as the C=O, in trans it is on the opposite side.

ii) The trans is more stable. In the cis form there is unfavorable overlap of the alpha protons (+1 pt) between adjacent residues, leading to van der Waals repulsion (+3 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 $S = R \ln W$.

Choice A: The entropy of the chain stabilizes the unfolded form, it can be approximated as $S = R \ln W$, where $W = 9^N$, N = number of residues. 9 conformations/residue are assumed for the unfolded form, 3 for the mainchain and 3 for the sidechain.

Choice B: The entropy of the water stabilizes the folded form due to the increase in entropy of the water that occurs when non-polar residues are buried. This can be estimated from the entropy of transfer of the free amino acids from a non-polar solvent to water, which are indicated on the graph.



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!).

Choice A: The disulfide bonds reduce the entropy of the unfolded state because they crosslink the chain, reducing the number (W) of conformations it can take. This destabilizes the unfolded state, thus making the folded state more stable.

Choice B: Ethanol is less polar than water, so it would interfere with the hydrophobic effect, causing a smaller decrease in the entropy during unfolding. The -OH group on the ethanol may also disrupt hydrogen bonds.

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

i) 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.

ii) Describe **all** of the forces that stabilize the structure that you drew.

i) Drawing should reflect the overall geometry.

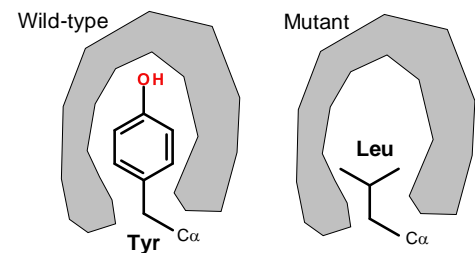
Helix should have the sidechains point outwards, 3.6 residues/turn, ~5 Å/turn, hbonds || to helix axis.

Strand should have sidechains alternating up and down, ~3 Å/residue, Hbonds perp. to strand direction.

Stands that face the core will have alternating polar-non-polar residues. Helices that face the core will have non-polar residues every 3rd or 4th residue.

ii) Stabilized by mainchain H-bonds and vdw. The b-a-b structure may also be stabilized by hydrophobic effect between the sheet and helix. **3 pts**

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*.



i) Please do one of the following two choices:

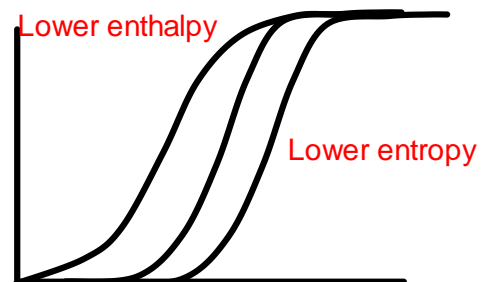
Choice A: How will the enthalpy of unfolding change for the mutant protein? Will it be higher or lower than the wild-type protein? Justify your answer (5 pts).

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).

Choice A: Enthalpy - the smaller Leu will have weaker vdw interactions with the core, so the ΔH will be smaller for the mutant. The -OH group on tyrosine is also likely involved in a hydrogen bond, this hydrogen bond is not possible with leucine, causing an even smaller enthalpy

Choice B: Entropy - Leu is more non-polar than tyrosine (see graph on Q9). This will mean the overall entropy for the mutant will be smaller since there will be a larger reduction of the conformational (chain) entropy by the hydrophobic effect. This will stabilize the mutant.

ii) 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).



- If you did choice A, the protein will be less stable with a lower T_M since the enthalpy is lower. It will be slightly less cooperative as well.

- If you did choice B, the protein will be more stable, because the entropy change is smaller for the mutant protein.

- Since the change (reduction) in enthalpy lowers T_M , but the change in entropy raises T_M , you cannot predict what will happen unless quantitative values are available.

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

1. Calculate $K_{eq} = f_u/(1-f_u)$ at a number of temperatures
2. Plot $\ln K_{eq}$ versus $1/T$, slope is $-\Delta H^\circ/R$, so $\Delta H^\circ = -\text{slope} \times R$

1. Determine T_m , where $f_u=0.5$, at this temp: $\Delta S^\circ = \Delta H^\circ/T_m$.

- 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.

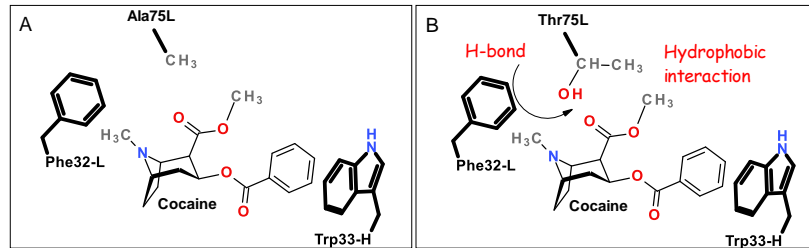


The isopropanol binds in the cavity that was formed due to the size difference between tyrosine and leucine, "repairing" the vdw energy that was lost. The isopropanol can also participate in a hydrogen bond. Both of these would increase the enthalpy, stabilizing the protein. This effect is conceptually very similar to using the increase in T_m as a method to detect the binding of potential drugs to enzyme.

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 μM

B: 1 μM



- 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).

B has the higher affinity due to the lower K_D . This is due to the **hydrogen bond** between Thr75L and the bound cocaine and the **hydrophobic interaction** between the methyl group on threonine and the methyl on cocaine.

- 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).

The off-rate will be slower for B, since this depends on the number of interactions and there are more interactions between B and the bound ligand.

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?

Choice A: A Y shaped with two light chains and two heavy chains. The light chains pair with one end of the heavy chains, forming the variable region responsible for antibody binding.

Choice B: A Fab fragment contains a light chain and the 1st half of the heavy chain, the antigen binds in the location, at the variable regions. An Fv is just the two variable regions, V_L and V_H . The antigen binds at the same location as the Fab fragment.

Choice C: They are smaller and can penetrate into tissues more readily.

- 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). Any fold that buries most of the non-polar residues is fine.

