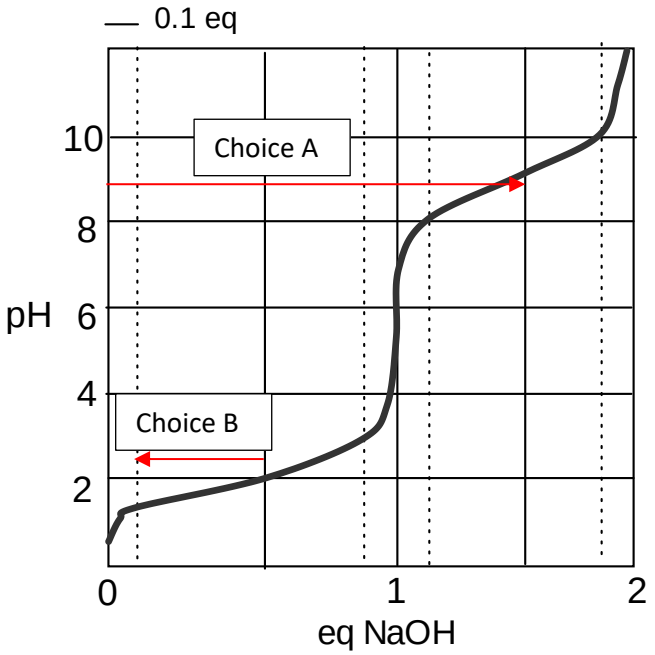
Instructions: this exam consists of 14 questions on 5 pages, for a total of 100 points. On questions with choices, all of your attempts will be graded and you will be awarded the highest grade. Please use the space provided or the back of the preceding page. Allot 1 min per 2 points.

1. (6 pts) A sketch of 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.

i) What are the pKa values for glycine? *Briefly Justify your answer*. (1 pt)

The pKa values are 2 and 9, because this is the pH when 0.5 and 1.5 equivalents have been added, generating a ratio of HA:A of 1:1, indicating that the pH = pKa.

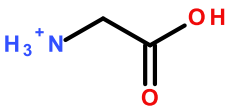


ii) Indicate a buffer region and explain why the pH changes very little in the buffer regions (5 pts).

In the buffer region the weak acid is deprotonating, and the proton that is released will neutralize the added base. Thus, the hydronium ion (pH=-log [H^+]) concentration doesn't change very much.

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

Choice A: 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, see right). Describe how you would make the buffer. Include in your answer the following:



i) the total number of moles of glycine you would need for this buffer solution.

ii) the total number of moles of NaOH you would need to add to the glycine solution to give a pH = 9.

Choice B: You have 0.5L of a 0.1M solution of glycine at pH 2.0. What is the buffer capacity of this buffer, i.e. how many moles of HCl could be neutralized by the buffer before the pH becomes outside the buffer region? *Justify your answer.*

Choice A:

i) the total moles of glycine are 0.1 moles/L x 0.5L = 0.05 moles.

ii) pH 9 is the pKa for the second ionization, at that pH, fHA = fA=0.5. Therefore you would have to add 1.5 eq of NaOH to go from the left side of the titration curve to pH=9.

Moles NaOH = eqNaOH x At = 1.5 x 0.05 = 0.075 moles

Choice B:

pH 2 is the pKa, so the starting pH would be in the middle of the buffer region.

The edge of the buffer region is +/- 1 pH unit from the pKa. It would take 0.4 eq of HCl to change the pH from 2 to 1.

Moles HCl = eqHCl x At = 0.4 x 0.05 = 0.02 moles

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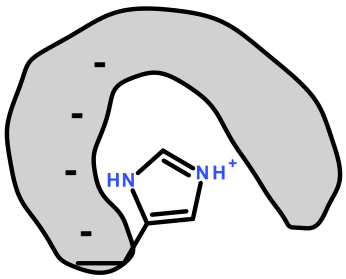
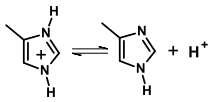
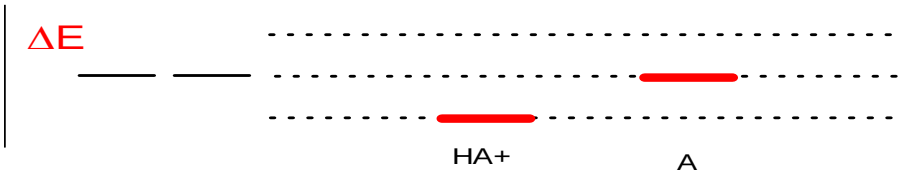
3. (6 pts) The diagram on the right shows a protein with a His residue near other ***negatively*** charged groups in the protein. You measure the pK_a of the histidine residue and discover that its pK_a is 7 instead of the usual 6.



i) Explain why the pK_a of the histidine has increased. The following diagram may be helpful (5 pts).





- The HA form of His has a positive charge, so its energy would be lowered in a negatively charged environment.
- The A form of His has no charge, so its energy will be unchanged.
- The relative energy of the HA form is now lower, so it is more stable – i.e. a weaker acid with a higher pKa.

ii) Proteins contain too many ionizable groups to measure pKa values by standard titration (measuring pH versus eq. NaOH). What technique can be used to measure pK_a values of individual sidechains in proteins (1 pt)?

NMR (using the effect of protonation on chemical shift)

4. (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

To receive partial credit, justify your answer on the back of the previous page.

Ala-Gly-Met-Phe (1st chymotrypsin)

Phe-Arg-Ser-Trp-Met (3rd CNBr)

Met-Ala-Ala-Trp (3rd chymotrypsin)

5. (4 pts) Briefly describe how could you determine the sequence of the above protein using mass spectrometry.

- The peptide would be fragmented into ions, a series of b ions are formed by breaking the peptide bond
- The b ions are the mass of the residues in the peptide +1 (to account for H2N on the amino terminus
- The mass of the smallest b-ion gives the first residue.
- The mass of the next smallest b-ion = the first two residues, so taking the difference, b2-b1 , gives the mass of the second residue.

6. (4 pts) A solution of the protein from the previous question (Q4) gives an absorbance of 1.0. What is the concentration of that protein in solution (l=1 cm). **A= [X]εl**

ε_{Trp}= 5500 $M^{-1}cm^{-1}$
ε_{Tyr}= 1500 $M^{-1}cm^{-1}$

There are 2 Trp residues and no tyr, so ε = 2 x 5,500 = 11,000 M-1cm-1.

[X]= 1/11,000 = 9 x 10-5 M = 90 uM

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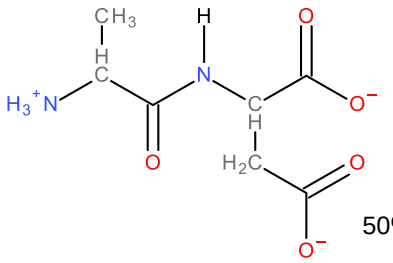
7. (12 pts)

i) Draw the chemical structure of Ala-Asp, assuming a pH=4.0 (7 pts). Justify the protonation state of ionizable groups (3 pts).

+5 pts for correct mainchain atoms, +2 pts for correct sidechains

- The amino group is fully protonated (pH 4 << pKa 9).
- The mainchain carboxyl group is fully deprotonated (pH 4 >> pKa 2)
- The sidechain carboxy group is 50% deprotonated & 50% protonated since pH = pKa



50% protonated

ii) The sequence of a segment of a protein is Ala-Asp-Val-Ser. Given that Ala and Val are non-polar and Asp and Ser are not, what is the more likely secondary structure for this sequence if it were part of a larger protein. Briefly justify your answer (2 pts).

b-strand, the non-polar residues alternate and would point towards the non-polar core of the protein.

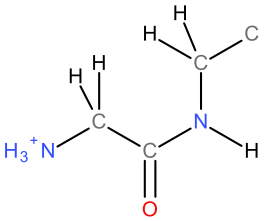
8. (8 pts) The peptide bond exists in two conformations, one of which is more stable than the other.

i) What are these two conformations? Draw or describe them (4 pt).

ii) Why is one conformation more stable than the other? What favorable or unfavorable interactions are responsible (4 pts)?

The two possible conformations are trans or cis. Trans is shown above, cis is shown on the right.

The cis is less stable because of unfavorable van der Waals between the protons on the two alpha carbons.



9. (8 pts) The Ramachandran plot for residues with C_β atoms is shown on the top right.

i) What does each dot on this plot represent? (1 pt)

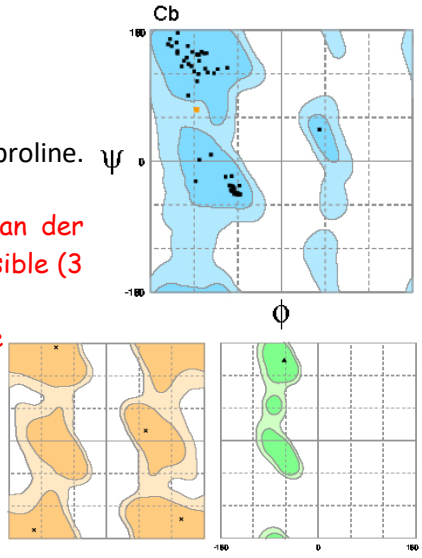
one residue in the protein.

ii) What do the colored/shaded regions represent? (3 pts)

regions that are low in energy because of favorable van der Waals

iii) The Ramachandran plots on the bottom right correspond to glycine and proline. Briefly explain why the shaded areas differ from the upper plot (4 pts).

- Glycine lacks a beta carbon so there are fewer unfavorable van der Waals contacts, more combinations of phi and psi angles are possible (3 pts)
- Proline sidechain is in a ring, and to rotate to phi angles on the right side would require the ring to break (1 pt)



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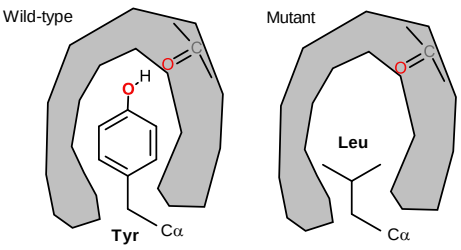
10. (8 pts) A tyrosine residue that is buried in the core of a globular protein is replaced by leucine. Tyrosine is the wild-type residue and leucine is the mutation. The sidechain of tyrosine and leucine are shown on the right, the gray shape represents the other residues in the core.

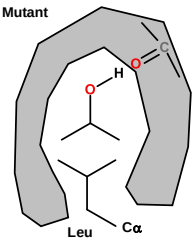


i) How will the enthalpy of unfolding change for the mutant protein? Will it be higher or lower than the wild-type protein? Justify your answer (6 pts).

The enthalpy will be smaller for the mutant due to:

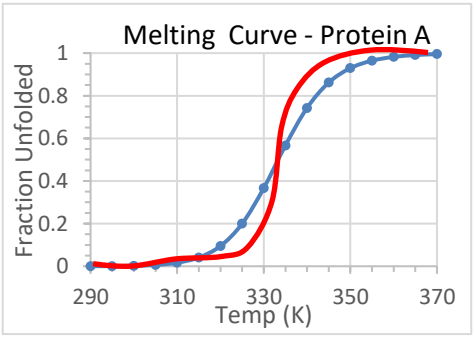
1. loss of H-bond between the Tyr and the O=C.
2. reduced van der Waals because the Leu sidechain is smaller.

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



- The isopropanol can fill the cavity and restore the interactions that were lost when the Tyr was replaced by Leu.

11. (6 pts) Two globular proteins differ in size by a factor of two. Protein A is 50 residues and protein B is 100 residues. The thermal denaturation curve for protein A is shown on the right.



i) Sketch the denaturation curve you would expect to observe for protein B. Justify your answer with reference to differences in entropy and enthalpy between the two proteins.

$\Delta G^o = \Delta H^o - T\,\Delta S^o = -RT\ln K_{EQ}$ $\qquad ln K_{eq} = -\frac{\Delta H^o}{R}\left[\frac{1}{T}\right] + \frac{\Delta S^o}{R}$

- Protein B is larger so it will have a larger enthalpy and entropy than A.
- The melting temperature depends the ratio of the two, so it will probably not change very much.
- The steepness of the transition depends on enthalpy, so the melting curve will be steeper.

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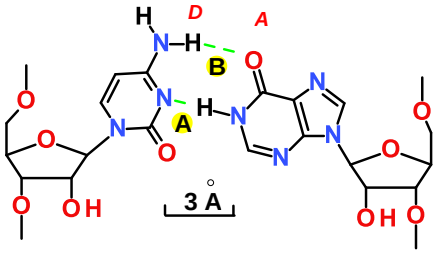
12. (8 pts) The structure of a basepair in a tRNA molecule is shown on the right. There are two hydrogen bonds connecting the bases, A and B.



i) Which of these two hydrogen bonds do you expect to be stronger? Justify your answer (6 pts).

Overall "A" will be stronger. Although "B" involves donation to a more electronegative atom "A" more ideal because:

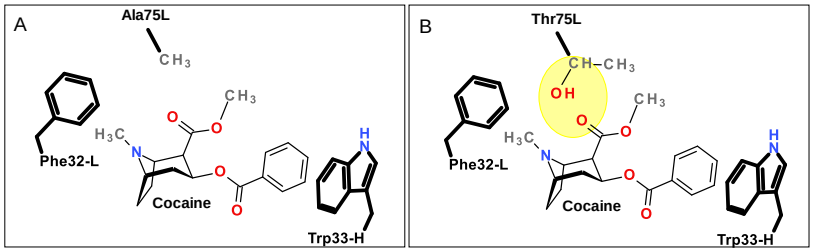
- It is the optimal distance of 3 A between the two electronegative atoms, in B the atoms are further apart.
- All three atoms line up, with an angle of 180 degrees, this is more favorable than the bend H-bond in B.

(4 pts for discussing electronegativity, 1 pt for distance effects, 1 pt for angular effects).

ii) Identify the donor with a "D" and the acceptor with an "A" for hydrogen bond B (2 pt). See diagram

13. (8 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 uM B: 1 uM



i) Which antibody has the higher affinity for cocaine? Justify your answer with reference to the K_D values. Also discuss how the interaction between the antibodies and cocaine affects the observed K_D (6 pts).

- B since it has the lower K_D
- This is due to the hydrogen bond between Thr75 and cocaine. This is absent in A. All other interactions are the same.

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 there are more interactions

14. (6 pts) Please do **one** of the following (use the space to the right to answer the question):

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

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

Choice A: Y-shaped molecule, 2 light chains, 2 heavy chains. Antigen bonds at tips of Y.
Choice B: Fab = entire light chain + 1st half of heavy chain. The antigen binds to the end that has the variable sequences.
Fv= just the variable segments of the light and heavy chains.

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