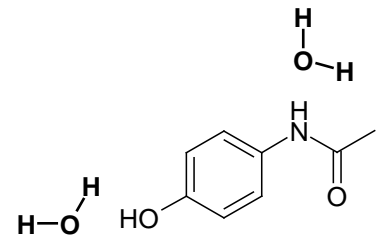


This exam consists of 5 pages and 15 questions. Total points are 100. Allot 1 min/2 points. On questions with choices, all of your answers will be graded and the best scoring answer will be used. Use the space provided, or the back of the previous page. The full name and three letter abbreviation of the amino acids is on the last page.

1. (4 pts) The following compound is a common drug (acetaminophen). Draw one water molecule accepting a hydrogen bond from this molecule. State the general rules for identifying donors and acceptors on any molecule.



The two possible accepting waters are shown (2 pts)

The general rule is that X-H is the H-bond donor, and Y is the H-bond acceptor, with X and Y both being electronegative (2 pts)

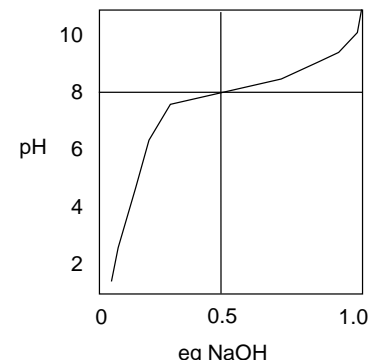
2. (6 pts) Entropy plays an important part in affecting the solubility of ions and non-polar compounds. Select one of these (ions or non-polar compounds) and briefly discuss the role of entropy in solubility.

Ions: The entropy of the ions increases considerably when the ion dissolves in water (the entropy of the crystalline salt is zero). An increase in entropy is favorable, facilitating high solubility.

Non-polar compounds: When these dissolve in water they reduce the entropy of the water by ordering the water molecules around them, this decrease in entropy is unfavorable, so non-polar groups have low solubility in water.

3. (8 pts) Sketch a titration curve of a **monoprotic** acid on the right, assuming the $pK_a=8$. What is the “buffer region” of a titration curve and explain why weak acids act as buffers.

Plot on the right should have the correct axis (2 pts), showing inflection point at 0.5 equivalents at pH 8.0 (2 pts).

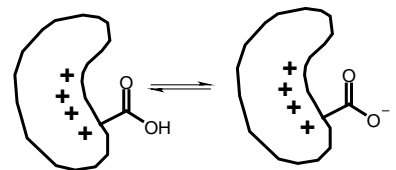


The buffer region is within one unit of the pK_a (from 7-9 in this example. And the pH is buffered because the weak acid is being deprotonated, neutralizing the added NaOH (4 pts).

4. (8 pts) Please do one of the following choices:

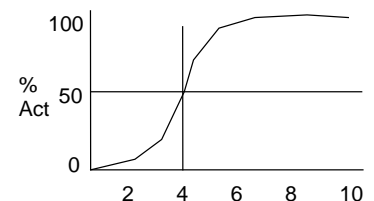
Choice A: A carboxylic acid residue is located in a positively charged pocket in a protein. The normal pK_a for this group, free in solution, is 4.0. Will the pK_a be higher or lower for the residue in the protein? Briefly justify your answer.

Choice B: A carboxylic acid group in a protein must be deprotonated for biological activity. Sketch the graph of activity versus pH for this protein, assuming the pK_a of the group is 4.0. Briefly justify your answer.



Choice A: There is a favorable electrostatic interaction with the deprotonated carboxylate and the positive charge on the protein. This will stabilize the deprotonated state, making it more likely for the acid to ionize, therefore it will be a stronger acid with a $pK_a < 4$.

Choice B: The activity will be proportional to the fraction deprotonated, with 50% activity when the $pH=pK_a$ (since $\frac{1}{2}$ of the molecules are deprotonated at that pH).



5. (12 pts) Please do one of the following two choices:

Choice A:

- i) Assuming that you have a monoprotic weak acid with a pKa of 8, calculate the number of *equivalents* of NaOH required to make a buffer solution at pH 9, assuming that you are beginning with the fully protonated form of the acid.
- ii) How would your answer to i) change if this were a diprotic acid, with the first pKa = 2 and the second pKa = 8?

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

$$f_{HA} = \frac{1}{1+R} \quad f_{A^-} = \frac{R}{1+R}$$

$$R = 10^{pH - pK_a}$$

Choice B:

You have a solution of a monoprotic weak acid at pH=9. How many *equivalents* of HCl do you need to add to bring the pH to 8.0? The pKa of the acid is 8. Briefly justify your answer.

Choice A:

- i) The pH is one unit above the pKa, so the f_{A^-} will be approximately 0.9. By calculation: $R = 10^1 = 10$, $f_{A^-} = 10/11 = 0.91$. You would have to add 0.91 equivalents of NaOH (8 pts, -1 if no calculation is shown)
- ii) You would have to add one additional equivalent to fully deprotonate the first group, so total equivalents is 1.91. (4 pts)

Choice B:

The number of equivalents is the difference in fraction protonated between the two pH values. (4 pts)

The f_{HA} at pH 9 is 0.09. $R = 10^{9-8} = 10^1$. $f_{HA} = 1/11 = 0.09$.

The fraction protonated at pH 8 is 0.5 since the pH = pKa, so a total of $0.5 - 0.09 = 0.41$ equivalents of HCl. (8 pts, -1 if no calculation is shown)

6. (15 pts)

- i) Draw the dipeptide Alanine(Ala)-Lysine(Lys) at pH=7.0, with the peptide bond in the trans conformation. The sidechain of Alanine is a methyl group (4 pts).

3 pts for correct chemical structure

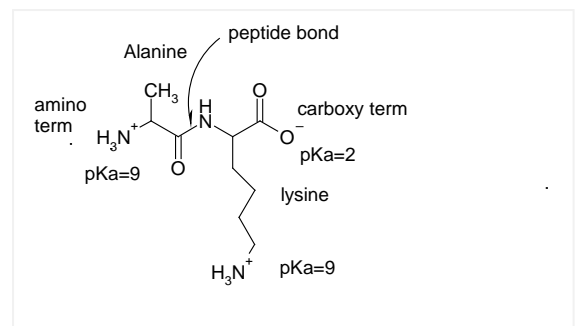
1 pt for correct charges at pH=7

- ii) Place the following labels on your drawing (3 pts, 1 pt each):

- amino terminus
- carboxy terminus
- peptide bond

- iii) What is the charge on this dipeptide, at pH=7. Briefly justify your answer with reference to individual pKa values (4 pts).

Since the pH is 2 units away from all pKa values, the two amino groups, with a pKa of 9 will be fully protonated with a charge of +2. The mainchain carbonyl will be fully deprotonated with a net charge of -1, overall charge is +1.



- iv) Could you use the absorption of UV light (280 nm) to measure the concentration of this peptide? Why or why not? (3 pts). [This was changed to 4 pts when grading, to bring the total for this question to 15 pts]

No, since there are no sidechains (Tyrosine, tryptophan) that absorb UV light at 280 nm.

7. (6 pts) The four atoms associated with the peptide bond are planar and usually found in the trans configuration.
- Briefly explain why it is planar and trans (5 pts).
 - The peptide bond assumes one conformation in the folded and unfolded state. If the peptide bond could assume more than one conformation in the unfolded state, but still one conformation in the folded state, how would it affect the stability of proteins (1 pt)?

i) It is planar because the nitrogen is sp^2 hybridized (planar) as is the carbon. This occurs because the remaining pz orbital of nitrogen can then delocalize over the entire peptide bond, forming a partial double bond. (Full credit for discussing hybridization and partial double bond, 3/5 if only partial double bond is mentioned).

ii) This would increase the conformational entropy of the unfolded state, making it more stable, thus the folded or native state would be less stable.

8. (8 pts) A 10 residue peptide was sequenced with Edman degradation. You should assume that only the first 5 residues can be obtained from any peptide, even though the peptide might be longer.

Cyanogen Bromide Cleavage (CNBr): Ala-Gly-Met & Ser-Thr-Lys-Trp-Ser
 Trypsin cleavage: Ala-Gly-Met-Ser-Thr & Trp-Ser-Leu-Leu
 Chymotrypsin cleavage: Ala-Gly-Met-Ser-Thr & Ser-Leu-Leu

Please write the correct sequence of the peptide below. Justification of your approach (on the back of the previous page) will be rewarded with partial credit if your answer is wrong. Otherwise there is no need to justify your answer.

1 Ala 2 Gly 3 Met 4 Ser 5 Thr 6 Lys 7 Trp 8 Ser 9 Leu 10 Leu

The peptide begins with Ala-Gly-Met-Ser-Thr since this fragment is common to one peptide that is produced by the different cleavage reagents.

The Met in this sequence suggest that there should be a CNBr fragment beginning with Ser-Thr. There is, allowing the sequence to extend three more residues:

Ala-Gly-Met-Ser-Thr
 Ser-Thr-Lys-Trp-Ser

The longer sequence has a Trp, which is a site for chymotrypsin, suggesting to look for a chymotrypsin fragment that begins with Ser, completing the sequence:

Ala-Gly-Met-Ser-Thr
 Ser-Thr-Lys-Trp-Ser
 Ser-Leu-Leu

9. (10 pts) There are only three stable secondary structures.

i) What are their names? β -strand or sheet, right handed α -helix, left handed α -helix

ii) Describe, or sketch, one of them, indicating the location of H-bonds and sidechains.

β -strand or sheet should show alternating pattern of sidechains (up-down) and H-bond perpendicular to strands.

α -helix should show helical structure, H-bonds parallel to helix axis, sidechains pointing out.

iii) Assuming that your secondary structure faced the core of the protein, what distribution of polar and non-polar amino acids along the polypeptide chain would you expect to observe?

β -strand - every second one would be non-polar.

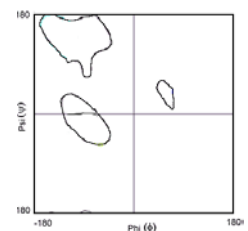
α -helix - every 3rd or 4th (3.6) would be non-polar.

iv) What is the role of van der Waals forces in stabilizing these structures, and what is the relationship between van der Waals and Ramachandran plots (a Ramachandran plot is shown if you would like to use it to illustrate your answer)?

For the values of phi and psi OUTSIDE of the contour regions, there is a repulsive van der Waals force because the beta carbons hit each other.

These unfavorable contacts reduce the number of phi and psi pairs per residue from 9 to 3, reducing the entropy of the unfolded state, thereby stabilizing the folded state.

For values of phi and psi INSIDE the contour regions, there are favorable van der waals contacts.



10. (5 pts) Please do one of the following two choices:

Choice A: Describe, or sketch, any super-secondary structure and briefly describe the forces that stabilize it.

Choice B: What are disulfide bonds, and how do they stabilize the folded form of proteins?

Choice A:

β -barrel - barrel like structure made of β -strands. Stabilized by H-bonds between the strands and van der Waals forces between the side chains

$\beta\alpha\beta$ - individual strands and helix stabilized by H-bonds. Residues between helix and sheet are non-polar stabilizing the structure by the hydrophobic effect (vdw stabilization acceptable)

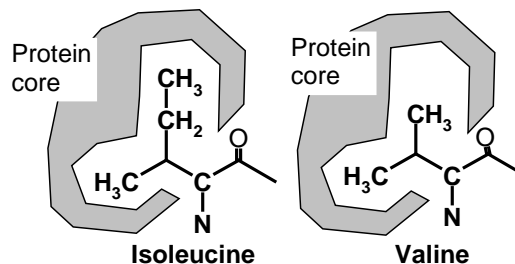
Ig Fold - two 3-4 stranded β -sheets that are stabilized by H-bonds within the sheet, a disulfide bond holds the two sheets together.

Choice B: A disulfide bond is a S-S bond between two Cysteine residues in a protein.

They reduce the conformational entropy of the unfolded state, thus making it less favorable, thus stabilizing the native (folded) form.

11. (1 pt) Typical globular proteins contain 100 % of their polar and charged residues on their surface (location).

12. (5 pts) The core of a protein contains an isoleucine residue in the wild-type enzyme. This is replaced by valine in a mutant. The thermodynamic parameter associated with unfolding of each of these proteins is provided below the diagram. The direction of the reaction is considered to be from the native state to the unfolded state (N \rightarrow U).



ΔH°	+200 kJ/mol	+190 kJ/mol
ΔS°	+600 J/mol-deg	+610 J/mol-K

Explain the difference in enthalpy (ΔH°) between the two proteins.

The enthalpy is reduced in the mutant protein, indicating either weaker vdw or H-bonds. Since neither the original residue, or the mutant have hydrogen bonding properties it must be vdw. The valine has one less methyl group than the Isoleucine, reducing the contact with the rest of the core of the protein, reducing the van der Waals interactions.

Bonus 2 pts: Explain the difference in entropy (ΔS°) between the two proteins.

The mutant (valine) has a smaller non-polar sidechain, therefore it will order less water in the unfolded state. Thus the observed entropy will be larger.

13. (6 pts) Draw the structure of an antibody and:

- Name the protein chain(s) and indicate the location of the hypervariable loops (2 pts)
- Indicate the location(s) of the bound antigen. (2 pts)
- Indicate either a Fab **or** an Fv fragment (2 pts)

Y-shaped structure with two heavy chains and two light chains

Hypervariable loops at the ends of each branch of the Y

Antigen binds at the end of each branch (to the hypervariable loops)

The Fab fragment is the entire arm of the Y,

The Fv fragment is just the top half of each arm (containing the variable segments of the heavy and light chains)

14. (2 pts) Briefly discuss one of the uses of antibodies.

Protein purification - antibody binds to protein of interest, allowing its purification.

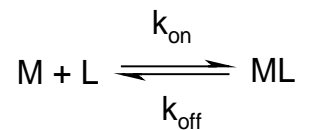
Cell labeling - antibody is fluorescent and binds to a cell component, indicating its location

Immune system - inactivates pathogens

Cancer treatment - antibody carries toxin to a cancer cell

Drug detoxification - antibody binds to the drug, or in some cases (cocaine) inactivates it.

15. (4 pts) In the case of ligand binding, the formation of the ligand-protein complex depends on the kinetic on-rate (k_{ON}) and the rate that the complex dissociates to free protein and ligand (kinetic off-rate, k_{OFF}). Which of these two parameters is most likely to differ when comparing different ligands binding to a protein? Why?



The off-rate since it depends on the number of interactions (which may differ).
The on-rate only depends on the number of collisions, which will be the same for both ligands.

Alanine:	Ala
Arginine:	Arg
Asparagine:	Asn
Aspartic Acid:	Asp
Cystine:	Cys
Glutamine:	Gln
Glutamic Acid:	Glu
Glycine:	Gly
Histidine:	His
Isoleucine:	Ile
Lysine:	Lys
Leucine:	Leu
Methionine:	Met
Phenylalanine:	Phe
Proline:	Pro
Serine:	Ser
Threonine:	Thr
Tryptophan:	Trp
Tyrosine:	Tyr
Valine:	Val