Part A: (14 points total: 2 points each, Circle the *best* answer)

1. Which of the following group can serve as an *effective* donor of hydrogen bonds.
   - **a) N-H**
   - b) C-H
   - **c) S-H (1/2 point should have been given for this answer.)**
   - d) C=O

2. A tri-protic weak acid can act as an *effective* buffer
   - a) at all pH values.
   - **b) when the pH is approximately equal to any of its pK_a values**
   - c) half-way between pK_{a1} and pK_{a2}.
   - d) at the middle pK_a only.

3. Which of the following is most correct:
   - a) Polar amino acids are never buried in the interior of a protein.
   - **b) Polar amino acids are seldom buried in the interior of a protein.**
   - c) All hydrophobic amino acids are buried when a protein folds.
   - d) Tyrosine is only found in the interior of proteins.

4. Formation of a hexa (6)-peptide from individual amino acids would release how many water molecules?
   - a) Three.
   - b) Four.
   - **c) Five. When an amino acid is added to a peptide chain, a water is released. In this case five are added to the first.**
   - d) Six.

5. Which of the following has *no* effect on the energetics of protein folding?
   - a) Conformational Entropy.
   - b) Hydrophobic Interactions.
   - **c) Covalent bonds. These are not changed during unfolding.**
   - d) Hydrogen Bonds.

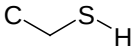
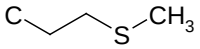
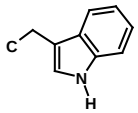
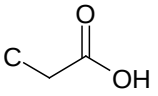
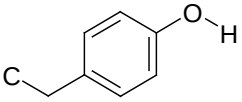
6. The free energy change due to *unfolding* of a protein is positive, therefore
   - a) the temperature is equal to T_M.
   - b) the enthlapy of the reaction is zero.
   - c) the temperature is above T_M.
   - **d) the temperature is below T_M. Since ΔG^o is positive, the unfolded state is higher in energy and therefore less favourable. Therefore $T<T_M$ since $f_U<0.5$.**

7. Which of the following regions of antibodies bind antigens?
   - a) Disulfide bonds.
   - b) Constant regions.
   - **c) F_v fragments.**
   - d) F_c fragments.

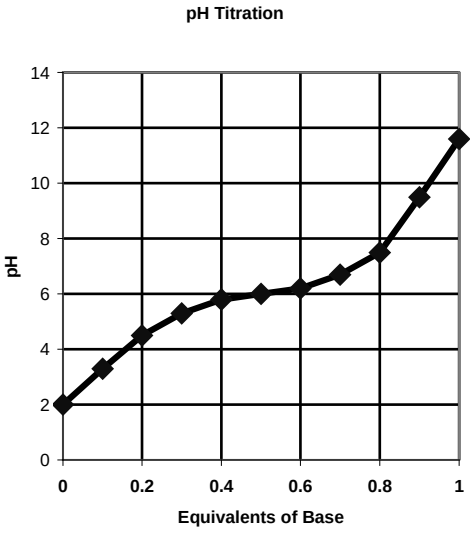
Part B: Short Answer

B1: (6 pts)

Correctly match **three** of the six descriptions to the amino acid side-chain (only the side chain of the amino acid is shown). Place the correct letter next to the description. In cases where there is more than one correct choice, only one is required for full credit. Item 4 has been done for you as an example.

1. Absorbs UV light	**__d,f_**	a. 
2. Forms disulfide bonds in proteins.	**__a_**	b. 
3. Side chain pK_a =4.0. Residue also found in the active site of HIV protease.	**__e__**	c. 
4. Amino acid that is not chiral.	**__C_**	d. 
5. Has both polar and non-polar character.	**__d,f__**	e. 
6. CNBr cleaves after this residue.	**__b__**	f. 

B2: (6 pts) A titration curve of a mono-protic acid is provided on the right.



i) Label the x-axis and y-axis, including the units (2 pts.)

See graph. (1 pt for each axis)

iii) Briefly explain how the pK_a of the acid is obtained from this experiment. What is the pK_a for this particular acid (3 pts).

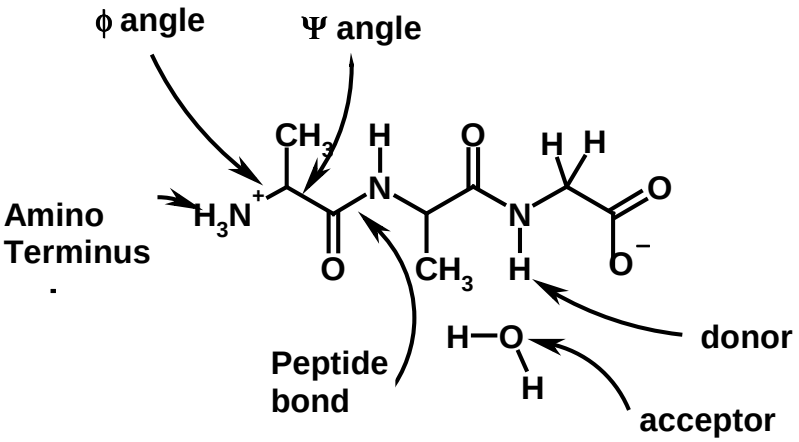
At the inflection point the pH=pKa, this also occurs when the number of equivalents =0.5 (1.5 pts)

In this case the pKa is 6.0 (1.5pts)

iv) Which amino acid has a side chain with the same pK_a (1 pt)?

Histidine (1 pt)

B3: (12 pts) The partial structure of a dipeptide is shown below.



i) Convert this to a tripeptide by adding a glycine residue to either end. [The sidechain of Gly is a proton](3 pts**).**

See diagram below, 2.5 pts were given for the correct structure, 1/2 point for getting the charge correct.

ii) Give the sequence of the peptide (e.g. Tyr-Trp-Phe) (1 pt).

Ala-Ala-Gly for the example given below. If you added Gly to the other end, it would be Gly-Ala-Ala

iii) Indicate the location of:

- The *first* peptide bond (1 pt).
- The *first* ϕ torsional angle or bond (1 pt).
- The *first* ψ. torsional angle or bond (1 pt).
- The amino terminus (1 pt).

See diagram below

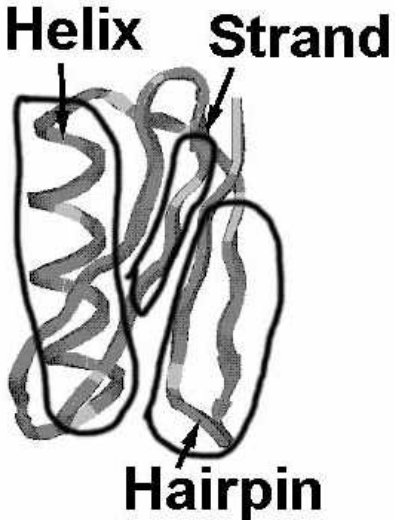
iv) Which of the above three bonds is always considered to be planer? Why is it planer? (2 pts)

The peptide bond (1pt) since it has partial double bond character (1pt).

v) Draw a water molecule forming a hydrogen bond to one of the amide groups in this peptide. Label the donor and acceptor in the diagram (2 pts)

See diagram - 1 pt for the water molecule, 1/2 pt each for donor and acceptor.

B4: (8 pts) An image of protein G is shown to the right.



i) An arrow has been drawn to the right of the figure. What is the *approximate* length of this arrow in angstroms. Briefly justify your answer (1 pt).

This helix has four complete turns (1/2pt) At 5.4 Å /turn this would be about 22Å(1/2 pt)

ii) Circle, or otherwise clearly indicate, on the structure to the right, **two** of the following three structural features (2 pts).

- α-helix
- β-strand
- β-hairpin

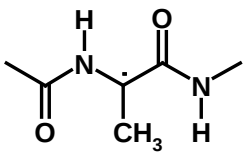
Be sure to indicate which of the three you have selected.

See diagram to right

B4: - Continued:

iii) Discuss the major energetic term that *stabilizes* any one of the above structural features. A simple chemical diagram of the interaction can be used in your answer. (5 pts)

Main-chain (1/2 pt) hydrogen bonds (3 1/2 pts) that involve the following interaction shown to the right (1 pt).



B5: (6 pts) Explain why the core of a folded protein consists mainly of non-polar residues. Your answer should include a discussion about changes in thermodynamic

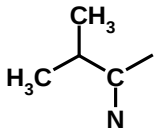
parameters as well as provide some information at the molecular scale, i.e. simply stating "It is lowest in free energy" is not sufficient.

Exposed hydrophobic residues will order water, decreasing the entriopy (ΔS) of the system, which is unfavourable.

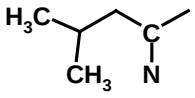
When the non-polar groups are buried, the ordered waters are released, which increases the entropy, which is favourable.

If you just discussed van der Waals interactions leading to tight packing, you received 3 pts.

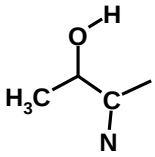
B6: (8 pts) The **core** of a protein contains a Valine residue in the wild-type enzyme. This is replaced by Leucine in one mutant and Threonine in another. The thermodynamic parameter associated with unfolding of each of these proteins is provided below the amino acid side-chain. The direction of the reaction is considered to be from the Native state to the Unfolded state (N→U).



Valine



Leucine



Threonine

ΔH^o	+200 kJ/mol	+190 kJ/mol	+205 kJ/mol-deg
ΔS^o	+600 J/mol-deg	+595 J/mol-K	+605 J/mol-deg

i) (5 pts) Select **ONE** of the mutants, either the Leucine or Threonine substitution, and provide an explanation for the change in **enthalpy (ΔH^o)**. Your answer should include a description of the molecular interactions in the folded form of the protein. You can supplement your answer with a simple sketch.

Leu: The enthalpy is lower, therefore fewer interactions need to be broken during unfolding (2 pts)

The larger leucine sidechain must not quite fit in the core as well as the valine, disrupting van der Waals interactions (3 pts)

Thr: The enthalpy is higher, therefore, more interactions have to be broken during unfolding (2 pts)

The OH group on the Threonine can form a hydrogen bond to either a mainchain or sidechain atom in the core of the protein. This hydrogen bond cannot be to water, otherwise there would be no change in the energetics because the same hydrogen bond would exist in both the folded and unfolded states (3 pts)

ii) Only do **one** of the following **three** parts (3 pts)

a) What is the T_M for the wild-type protein? Briefly justify your approach.

At T_M ΔG^o is zero. Therefore: $\Delta H^o = T_M \Delta S^o$ and T_M can be obtained from the ratio of the two: T_M=200,000/600 = 333 K. Note that it is necessary to change the kJ to J for the enthalpy.

b) Briefly explain how the enthalpy would be obtained from a protein melting (denaturation) curve.

Obtain $K_{EQ}=(f_u/f_N)$ at two temperatures. Plot $\ln K_{EQ}$ versus 1/T. Slope is $-\Delta H^o/R$

c) Briefly explain how you would obtain the fraction of wild-type molecules that are unfolded at **any** temperature.

Calculate $\Delta G^o=\Delta H^o+T\Delta S^o$ at the desired temperature. Obtain K_{EQ} from $\Delta G^o=-RT\ln K_{EQ}$. $f_U=K_{EQ}/(1+K_{EQ})$

C1: Do one of the following two questions (6 pts).

i) A protein that is 20 amino acid residues in length folds into a stable structure. Assume that the protein forms **all but one** hydrogen bond when it folds and that the unsatisfied H-bond is not accessible to water. Calculate the enthalpy of unfolding. State whatever assumptions you make regarding the energetics of hydrogen bond formation.

Consider this to be a two step reaction. First, break all of the hydrogen bonds to water, then reform all but one during the refolding.

In the first step ΔH^0 = 20 x 20 kJ/mol = + 400 kJ/mol. The sign is positive since we had to put heat into the system (increase the enthalpy) to break the bonds.

In the second step, 19 of these reform, releasing 25 kJ/mol. 25 kJ/mol are released because the hydrogen bond in secondary structure is 5 kJ/mol more stable than to water.

ΔH^o = -19 x 25 kJ/mol = - 475 kJ/mol.

The net change in enthalpy is the sum of the two:

ΔH^o=-475+400 = -75 kJ/mol.

The above is for folding, since the question asked for unfolding, we invert the sign:

ΔH^o = +75 kJ/mol.

ii) A 20 residue protein can fold into either an α-helix or a β-strand (i.e. both species can be found in a solution of the folded protein at the same time). Calculate the entropy for the transition from the unfolded to the folded states. If you do not have a calculator, simply write out the relevant equations.

Calculate the entropy of the folded and unfolded states, and then take the difference:

$S_U = R \ln 9^{20}$ (2 pts)

$S_F = R \ln 2$ There are <u>two</u> possible folded states since the helix and strand structures coexist (4 pts)

$\Delta S^o = S_F - S_U = R(\ln 2 - 20 \ln 9)$

C2: (8 pts) Do one of the following two questions:

i) The primary sequence of a 10 residue peptide is being determined using Edman degradation and proteolytic cleavage. Only the sequence of the first four residues of a peptide are obtainable, regardless of its length. The following data were obtained:

a). Sequencing of the intact peptide gave the first four amino acids: Ala-Cys-Met-Val

b) Sequencing of each peptide produced from Trypsin cleavage gave:

Ala-Cys-Met-Val Phe-Thr-Ser-Gly

c) Sequencing of each peptide produced from Chymotrypsin cleavage gave:

Ala-Cys-Met-Val Thr-Ser-Gly-Met

Determine as much of the peptide sequence as possible and give the most probable sequence for the missing residue(s).

The first four amino acids are Ala-Cys-Met-Val, since these were obtained from the sequence of the intact peptide. The second Trypsin peptide contains a site for chymotrypsin, so it is possible to overlap the 2nd Trypsin fragment with the second Chymotrypsin fragment: Phe-Thr-Ser-Gly-Met. At this point, there is only one amino acid missing - either Lys or Arg since Trypsin cleaves after either of these two:

Ala-Cys-Met-Val-[Lys/Arg]-Phe-Thr-Ser-Gly-Met

ii) A protein contains one Trp (ε_{280} = 5000 $M^{-1}cm^{-1}$) residue and five Tyrosine residues (ε_{280} = 1000 $M^{-1}cm^{-1}$). A solution of this protein has an absorbance of 0.5 for a 1cm path length. What is the concentration of the protein in solution?

First, calculate the molar extinction coefficient for the entire protein by summing all of the absorbing groups: ε_{280} = 5000 x1 + 1000 x 5 = 10,000 $M^{-1}cm^{-1}$

The concentration is obtained from Beer's law: $A=C\varepsilon l$: $C=A/\varepsilon l$ = 0.5/10,000 x 1 = 5 x 10^{-5} M.

C3: (10 pts) Do one of the following two questions:

i) You want to make 1 L of a 0.5 M buffer solution with a pH = 5.0. The reaction that you are trying to control the pH of generates protons.

Your choices of acids are acetic acid (pK_a = 4.0) or imidazole (pK_a = 6.0).

a) Explain which buffer compound you would use and why. If you are uncertain of what to choose, just pick one and move on, either choice will be graded in the following sections (2 pts).

You would select acetic acid because at pH 5.0 it is ionized to a greater extent than imidazole. Consequently it can absorb more of the protons that are generated from the reaction.

b) Determine the correct ratio of the acidic and conjugate base form of the buffer.(5 pts).

For acetic acid: pK_a=4.0, $R=10^{pH-pKa} = 10^{5-4}=10$.

$[HA]=0.5\ (1/(1+10))=0.045$.

$[A^-]=0.5\ (10/(1+10))=0.455$

For imidazole: pKa=6.0, $R=10^{pH-pKa}=10^{5-6} = 0.1$

$[HA]=0.5\ (1/1.1)=.455$

$[A^-]=0.5(.1/1.1)=.045$

c) Explain how you would make the buffer, assuming you only have the fully protonated form of the acid in your laboratory.(3 pts).

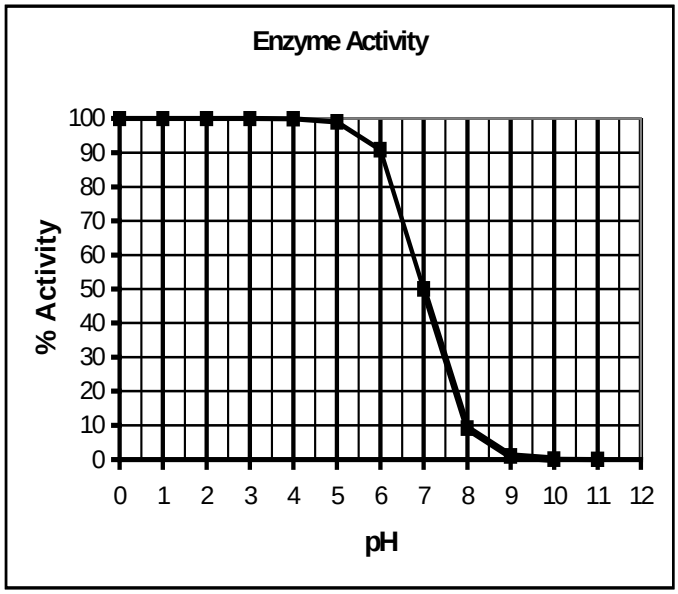
You would begin with 0.5 moles of the acidic form [HA] and add sufficient NaOH to create the desired amount of $[A^-]$.

Acetic acid: Add 0.455 moles of base.

Imidazole : Add 0.045 moles of base.

ii) An enzyme has a single lysine residue in its active site. In order for the enzyme to be fully active, this lysine side-chain must be positively charged (protonated). You can assume that the pK_a of the lysine side chain is 7.0.

a) Draw, using the graph to the right, the pH dependence of the enzyme activity. Justify your graph, preferably with a sample calculation. (6 pts)



The amount of active enzyme is proportional to the fraction in the [HA] form, this is just:

$\frac{[HA]}{[A_T]} = \frac{1}{1+R}$ **R is calculated from $R=10^{pH-pKa}= 10^{pH-7.0}$**

Some sample calculations:

pH	R	Activity
6.0	$R=10^{6-7} = 10^{-1} = 0.1$	1/1.1 = 90%
7.0	$R = 10^{7-7} = 10^0 = 1$	1/2 = 50%
8.0	$R = 10^{8-7} = 10^1 = 10$	1/11=9%

b) This pK_a is significantly lower than the normal pK_a for a lysine residue. Suggest how this might occur. Illustrate your answer with an appropriate diagram of the lysine residue in the active site of this enzyme (4 pts). **The pKa of the lysine sidechain is approximately 9.0. This particular lysine is a stronger acid, therefore either the protonated form is destabilized such that it wants to loose its proton, or the deprotonated form is stabilized. If this lysine was found in a positively charged environment, deprotonation would be favorable to reduce the electrostatic energy. The lysine could also be found in a non-polar environment, which would also favor the deprotonated or uncharged form.**