This exam consists of 8 pages. There are a total of 100 points. Allot 1 minute for every 2 points.

Part A: Please circle the best answer (2 pts/question, 14 pts total)

1. In the titration of the amino acid glycine, an inflection point occurs:
   a) when there is no net charge on the amino acid.
   b) when one molar equivalent of base has been added to the fully protonated form.
   c) when 1.5 molar equivalent of base has been added to the fully protonated form.
   d) at neutral pH.

2. Which of the following is *most* correct about the core of a globular, folded protein?
   a) Extensive hydrogen bonding occurs between the side chains of polar residues.
   b) Hydrogen bonding between main chain C=O and side chain NH_2 groups is maximized.
   c) Extensive van der Waals interactions occur between predominantly non-polar residues.
   d) There are nine possible configurations per residue due to rotation about ∏ and ∏ angles.

3. Which of the following terms opposes protein folding?
   a) The enthalpy change due to the breaking of noncovalent bonds.
   b) The enthalpy change due to the breaking of covalent bonds.
   c) The configurational entropy change.
   d) The entropy change of the solvent.

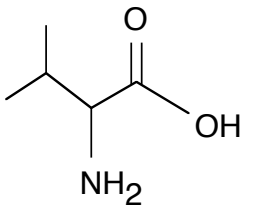
4. The peptide bond:
   a) has partial double bond character and is almost always in the cis configuration.
   b) is planar and rigid and almost always in the trans configuration.
   c) has three possible configurations due to rotation about the N-C bond.
   d) is hydrolyzed spontaneously in water.

5. Which of the following are features shared by both an □-helix and a □-sheet?
   a) Identical ∏ (phi) and ∏ (psi) torsional angles.
   b) Hydrogen bonds between electronegative backbone atoms.
   c) Hydrogen bonds that are parallel to the main chain.
   d) □-branched side chains are uncommon.

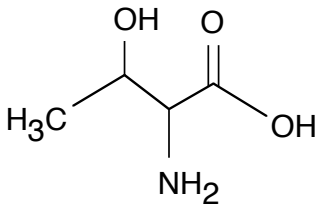
6. Amino acids with □-branched side chains are *best* accommodated in:
   a) an □-helix.
   b) a □-sheet.
   c) a □-turn.
   d) a coiled coil □-helical structure.

7. Which of the following is the "driving force" for protein folding?
   a) The restriction of ∏ (phi) and ∏ (psi) torsional angles.
   b) The hydrophobic effect.
   c) The change in configurational entropy.
   d) The formation of □-helices and □-sheets.

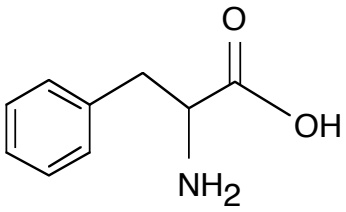
B1. (9 pts) The structure of three amino acids is shown below:



Val



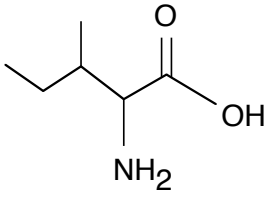
Thr



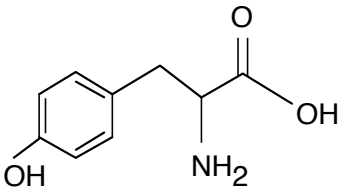
Phe

For *each* of these amino acids:

a) Identify the amino acid. Then show, by the removal, addition, or replacement of a small group, such as CH_3, OH, etc. (not the entire side-chain), how you could convert your chosen amino acid to another amino acid that is chemically most similar to the starting amino acid. For example: Alanine ($R=CH_3$) can be converted to Glycine (R=H) by the replacement of the methyl group with a hydrogen. Include the name of both the original and resulting amino acid. Either redraw the modified amino acid below or indicate your changes on the diagram above. **(3 pts, 1 pt each)**

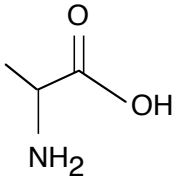


Val changed to **Ile** by addition of CH_3 group

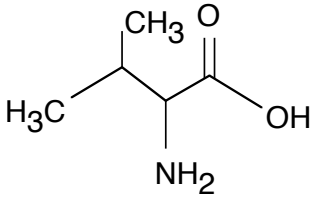


Phe changed to **Tyr** by addition of OH group

OR

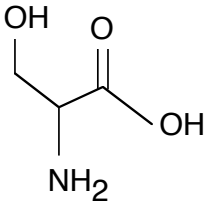


Phe changed to **Ala** by removal of phenyl group



Thr changed to **Val** by replacing OH group with CH_3 group

OR *(either of these is correct)*



Thr changed to **Ser** by removal of CH_3 group

Other accepted changes: **Val to Ser** by removal of a methyl group and addition of a hydroxyl group; **Phe to Ala** by removal of phenyl ring.

b) Did your change increase, decrease, or not affect the solubility of the amino acid in water? Briefly justify your answer. **(3 pts, 1 pt each)**

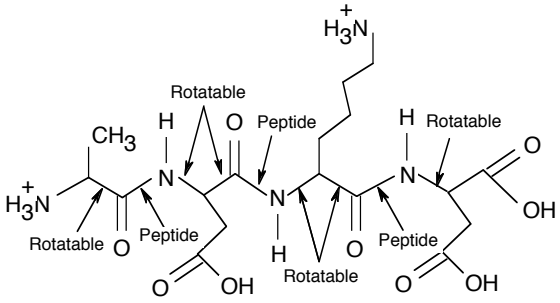
Val to Ile: Solubility is **decreased** by **addition of a nonpolar** methyl group.
Phe to Tyr: Solubility is **increased** by **addition of a polar** hydroxyl group.
Phe to Ala: Solubility is **increased by removal of a bulky aromatic ring**.
Thr to Val: Solubility is **decreased** by **removal of a polar** hydroxyl group and addition of a nonpolar methyl group. *OR*
Thr to Ser: Solubility is **increased** by removal of a **nonpolar** methyl group.
Credit was given if it was just stated whether the solubility increased or decreased.

c) Did your change increase, decrease, or not affect the ability of the amino acid to form a hydrogen bond to water? Briefly justify your answer. **(3 pts, 1 pt each)**

Val to Ile: H-bond capability is **unchanged**.
Phe to Tyr: H-bond capability is increased. The added **OH group can now donate an H bond**. *OR*
Phe to Ala: H-bond capability is **unchanged**.
Thr to Val: H-bond capability is **unchanged**. *OR*
Thr to Ser: H-bond capability is increased. The added **OH group can now donate an H bond**.

B2. (18 pts) The structure of the fully protonated form of a tetrapeptide is shown:

a) Indicate the location of a peptide bond and a freely rotatable bond on the diagram. (4 pts) **One of each was sufficient.**



b) List the sequence of the peptide. (2 pt) **Ala-Asp-Lys-Asp**

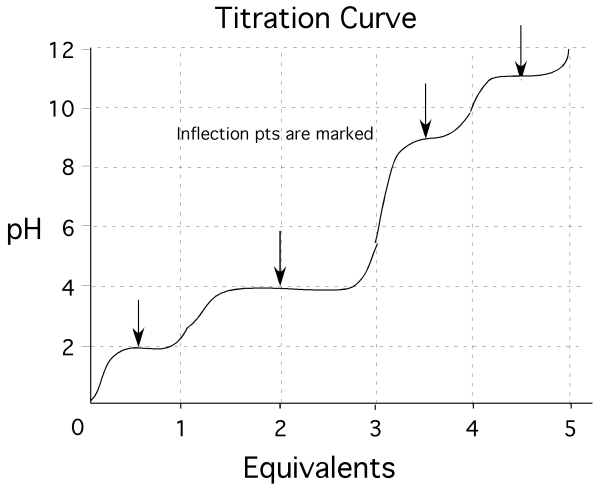
c) List each side chain with an ionizable functional group by name and indicate its approximate pKa. (2 pts)
Asp pKa ~4 (two of these)
Lys pKa ~11 (accept pKa's 9-12)
[Credit was given here even if the amino acid was misidentified in part b and the pKa of Glu instead of Asp was listed as ~4].

d) *Sketch* the pH titration curve of the tetrapeptide. Be sure to label the axis of your graph, provide the appropriate scale and numbers, and indicate the inflection points. You may assume that the pKa of the amino and carboxy terminus are 9 and 2, respectively. (6 pts)

General appearance of graph correct with 4 inflection points (2 pts)

Inflection points at flattest part of each curve at pH 2 (or 0.5 eq), 4 (or 2 eq), 9 (or 3.5 eq), 11 (or 4.5 eq) (2 pts)

Equivalents scale to 5 with 2 equivalents required to titrate the two Asp residues (2 pts)



e) At what pH will the net charge on this peptide be 0? (4 pts) **The net charge will be 0 when half the Asp residues are deprotonated at pH 4. (4 pts)**

B3. (6 pts)

a) *Sketch* an α-helix. You need not draw the individual atoms. However, indicate the direction of hydrogen bonds as well as the general location of the amino acid side chains in your diagram. Indicate the number of residues per turn of the helix and also state the

rule for H-bonding (ie, the __ group of residue n donates/accepts an H-bond to/from the __ group of residue n+_).

1.5 pts if the structure was helical.
Side chains pointed outward (1.5 pts)
There are ~3.6 (3.4 and 3.5 ok) residues per turn of the helix. (1.5 pts)
The carbonyl oxygen (C=O) of residue n accepts an H-bond from the amide proton (N-H) of residue n+4. (1.5 pts) [Full credit if it was just stated that there is an H-bond between residues n and n+4].

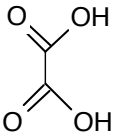
B4. (6 pts) Entropy plays an important role in defining the stability of the folded state of globular proteins. List, and then *briefly* discuss, the molecular nature of the entropic terms that affect protein folding. You should clearly state whether the term stabilizes or destabilizes the folded state. You are welcome to use an equation(s) as part of your answer.

The **configurational entropy** of a polypeptide or protein (S=RlnW) changes dramatically upon protein unfolding. **This is due to a large increase in freedom of rotation** about the phi and psi angles in the polypeptide backbone. The **configurational entropy change favors protein unfolding**. **(3 pts)**

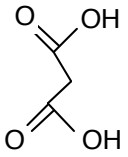
The second entropic term comes from the **hydrophobic effect. When a protein folds, nonpolar residues become buried in the core of the protein. This increases the entropy of the water molecules previously ordered around the nonpolar residues.** The hydrophobic effect **favors the folded state. (3 pts)**

B5. (15 pts)
You would like to make a 0.1 M buffer for an experiment at pH=6.0. You have the following two organic acids to choose from:

Acid	pKa1	pKa2
Oxalic acid	1.23	4.19
Malonic acid	2.83	5.69



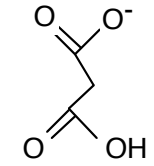
Oxalic acid



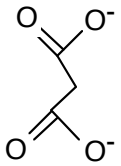
Malonic acid

a) Which of these two compounds would you choose and why? **(1 pt)**
Malonic acid would be the better choice because one of its pKa's is within one unit of the desired pH.

b) Draw the conjugate acid and base pair in your pH 6.0 buffer, indicating which is the acid and which is the base. **(2 pts)**



conjugate acid



conjugate base

c) You have only the fully protonated form of the acid in hand and a 1 M solution of NaOH. How would you make a 1 liter solution of your buffer? The amount of acid, as well as the amount of NaOH used should be given in moles. Please show all calculations. (6 pts)

This question is right out of problem set 1.

First, **the amount of acid needed is just 0.1 M x 1 L = 0.1 mole**. **(1 pt)**
Second, you need to titrate 0.1 mole of the malonic acid to titrate past the first pKa of 2.8. You need 0.1 mole of base to do this.
Then, you need to calculate what fraction of the 0.1 mole of malonic acid has to have its second ionizable group deprotonated to give a pH of 6.0. (See part b for the relevant acid/base pair). Use the H-H equation for this. $[A^-]/[HA]=R=10^{(pH-pKa)}$.
$R=10^{(6-5.69)}=2$. Thus $R=[A^-]/[HA]=2$. Since $At=A^-+HA$, and $HA=0.5A^-$,
$f_{A^-}=A^-/At=A^-/(A^-+0.5A^-)=A^-/1.5A^-=$**0.66. Thus 0.66*0.1 mole, or 0.066 mole of malonic acid has to have its second ionizable group deprotonated. Adding the original 0.1 mole to titrate the first ionizable group gives 0.166 mole of base required to get malonic acid to pH 6.0. (5 additional pts)**

[Only 2 additional pts were given if it was stated that 0.066 mole of base is required.

3 additional pts were given if it was stated that it takes 1 + less than 1 molar equivalent of base to titrate the acid to the desired pH.]

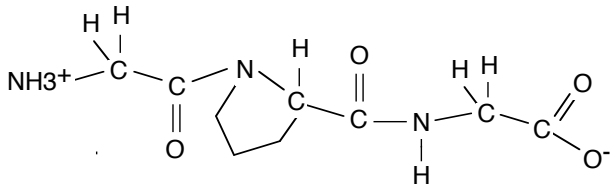
d) *Briefly* explain why each of the above acids has two different pKa values. **(2 pts)**
After deprotonation of the first carboxylic acid, the ionization of the second carboxylic acid is less favored because of the resulting charge repulsion between the two COO^- groups. [2 pts if it was stated that ionization of one group changes the environment of the second group].

e) *Briefly* explain why the pH of your buffer will not substantially decrease when protons (H^+) are released during a biological experiment carried out in your buffer. **(2 pts)**
The pH will not substantially decrease because the H^+ released during the experiement will be absorbed by the conjugate base of the buffer. It was not sufficient to just say that there is a buffer.

f) During the course of the biological experiment, protons (H^+) are released from one of the substrate organic compounds into the solvent. What would be the consequence of having forgotten to include the buffer if 10 μmole ($1x10^{-5}$ mole) of protons (H^+) were released? [Hint: what would the resulting pH be in the absence of buffering?] **(2 pts)**
In the absence of buffering, the **pH would be 5 (-log[H^+]). [1 pt if it was stated that the pH will drop].**

B6. (8 pts) The amino acids Proline and Glycine occur frequently in □-turns but only very infrequently in □-helices.
□-helices.

a) Draw the structure of the tripeptide with the following sequence: Gly-Pro-Gly. **(2 pts)**



b) State a reason why Pro residues are not commonly found in □-helices. **(2 pts)**
1) Because the Pro side chain is bonded to the amide nitrogen of the polypeptide backbone, a polypeptide containing Pro lacks an amide proton

(N-H) for H-bonding. This would leave a corresponding C=O group without an H-bonding partner.

OR

2) **Because the Pro side chain is bonded to the amide nitrogen of the polypeptide backbone, it is too strained** to conform to the ϕ torsion angles typically found in an α-helix.

[2 pts were given if it was simply stated that the conformation of Pro strains the backbone].

c) State a reason why Gly residues are not commonly found in α-helices. (2 pts)

Because Gly does not have a side chain, it can adopt a much wider range of ϕ and ψ torsional angles. [2 pts were given if it was stated that Gly lacks a side chain and thus doesn't contribute to helical stability because it doesn't form favorable interactions].

d) State one way that α-helices and β-turns differ with respect to hydrogen bonding. **(2 pts)**

1. **H-bonding within the polypeptide backbone is maximized in an α-helix, whereas β-turns typically have backbone (N-H and C=O) groups that are not H-bonded to other backbone (N-H and C=O) groups.**

OR

2. **H-bonds in an α-helix form between residue n and n+4, whereas an H-bond typically forms between residue n and n+3 in a β-turn. Give 2 pts if it is stated that H-bonds form between n and n+2 in a β-turn.**

OR

3. **H-bonds in an α-helix run parallel to the helix whereas H-bonds in a β-turn connecting two helices run perpendicular to the helices.**

B7. (8 pts) There are four levels of protein structure.

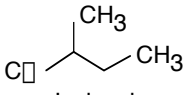
a) Using the immunoglobulins as an example, briefly discuss the major features of the four levels of protein structure, beginning with the primary structure. (6 pts)

Primary Structure: **Simply the amino acid sequence (2 pts)**
Secondary Structure: **Predominantly β-sheets, no α-helices (2 pts)**
Tertiary Structure (includes Supersecondary Structure or Domains): **Immunoglobulins are made up of repeating elements of supersecondary structure called a β-sandwich or Ig fold.** There are 4 of these in each heavy chain and two in each light chain, making a total of 12. (2 pts)
Quaternary Structure: **2 heavy chains and 2 light chains (2 pts)**
[For each answer, 2 pts were given if there is a general description of the type of structure but 1 additional bonus pt at each level of structure if the precise type of structure in immunoglobulins is identified].

b) How many antigen binding sites per molecule are there in an intact immunoglobulin? How is this number affected upon cleavage with papain? (2 pts)

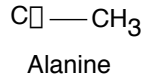
An intact Ig has 2 antigen binding sites. (1 pt) Cleavage with papain releases **two Fab fragments, each with 1 antigen binding site per molecule. (1 pt)**

B8. (16 pts) An altered version of T4 lysozyme, with a single amino acid substitution of Ile to Ala at position 27 (I27A) has been generated in the lab. In the wild type protein, Ile 27, in the middle of 3 anti-parallel strands, is buried in the hydrophobic core of the protein. The enthalpy and



entropy of unfolding (reaction direction $N \rightleftharpoons U$) were measured for both proteins and the values obtained are shown below:

	ΔH	ΔS
Isoleucine side-chain (wt)	116 kJ/mole	360 J/mol-K
Alanine side-chain (I27A)	98 kJ/mol	312 J/mol-K



Alanine

a) Provide an explanation for why the ΔH values differ between the two proteins. (4 pts)
The Ala mutant has a lower ΔH of unfolding because being smaller it forms fewer van der Waals interactions in the core; thus fewer bonds need to be broken for unfolding; less heat input is required. [2 pts if it is stated that the Ala mutant has fewer bonds; 4 pts if van der Waals interactions are mentioned].

b) Provide an explanation for why the ΔS values differ between the two proteins. (4 pts)
Ala has a smaller hydrophobic surface and orders less water around it. Thus there is less driving force for removing the Ala residue from water into the core of the protein. [2 pts if it is stated that Ala is less hydrophobic].

c) What is the melting temperature (T_M) of each protein? (2 pts)
$T = T_M$ when $\Delta G = 0$; $T_M = \Delta H/\Delta S$
For wt, T_M = 116,000 J/m / 360 J/mol-K = **322 K**.
For mutant, T_M = 98,000 J/m / 312 J/mol-K = **314 K**.

d) What fraction of the mutant protein is unfolded at the T_M of the wild type protein? (6 pts)

At 322 K, the T_M of the wt protein, the fraction of the mutant protein unfolded is as follows:
$\Delta G = \Delta H - T\Delta S$; ΔG = 98,000 J/mol – 322K (312 J/mol-K) = 98,000 – 100,464 = -2464 J/mol.

Since $\Delta G = -RT \ln K_{eq}$ = -2464 J/mol, K_{eq} can be calculated as follows:
$K_{eq} = e^{-\Delta G/RT} = e^{0.92} = 2.5$
$F_U = K_{eq}/1+K_{eq} = 2.5/3.5 = 0.71$. **Thus 0.71 or 71% of the mutant protein is unfolded at the T_M of the wt protein.**

[Many students misread the question as asking the fraction of unfolded mutant protein at the Tm of the mutant protein. In this case, 3 pts were given for answering that 0.5 of the mutant protein is unfolded].