This exam consists of 8 pages. There are a total of 90 points, allot 1 minute/2 points

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

1. Which of the following alcohols would be *least* soluble in water?
   a) methanol (CH_3OH)
   b) ethanol (CH_3CH_2OH)
   c) butanol ($CH_3CH_2CH_2CH_2OH$)
   +2 d) octanol ($CH_3[CH_2]_6CH_2OH$)

2. In the titration of a diprotic weak acid that has two *identical* pK_a values, an inflection point occurs:
   a) at the beginning of the titration.
   b) when two equivalents of base have been added.
   +2 c) when the pH equals the pK_a.
   d) when one-half equivalent of base has been added.

3. Which of the following is most correct:
   +2 a) Charged amino acids are never buried in the interior of a protein.
   b) All hydrophobic amino acids are buried when a protein folds.
   c) Tyrosine is only found in the interior of proteins.
   d) Glycine is rarely found in proteins because it is too destabilizing.

4. The standard Gibb's energy, ΔG^o, is
   a) the residual energy present in the reactants at equilibrium.
   b) the residual energy present in the products at equilibrium.
   +1 c) the difference in the residual energy of reactants and products at equilibrium.
   +2 d) The energy required to convert one mole of reactants to one mole of products.

5. Disulfide bonds most often stabilize the native structure of:
   +2 a) extracellular proteins.
   b) dimeric proteins.
   c) intracellular proteins.
   +1 d) multisubunit proteins.

6. Which of the following are characteristics of the immunoglobulin (Ig) fold?
   a) It is found only in IgG molecules.
   +1 b) It is composed of two anti-parallel β-strands folded into a globular domain.
   +2 c) It is β-barrel like, composed of a three- and a four-stranded antiparallel β-sheet.
   d) It is found six times in the IgG light chain.

7. The fact that the core of most globular proteins is tightly packed is due to:
   a) covalent bonding.
   b) hydrogen bonding.
   c) electrostatic effects.
   +2 d) van der Waals forces.

A: _____/14

B1:_____/ 5

B2:_____/ 8

B3:_____/ 8

B4:_____/15

B5:_____/12

B6:_____/ 8

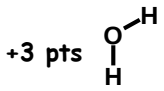
B7:_____/ 8

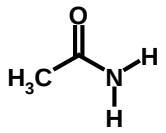
B8:_____/12

Tot:_____/90

B1. (5 pts)

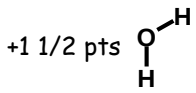
a) Draw a water molecule *donating* a hydrogen bond to the functional group shown on the right (3 pts). Your drawing should reflect the important features of the hydrogen bond. List **one** of these features in the space below (2 pts).





- Hydrogen bond are linear
- Hydrogen bonds involve an H-X donor and Y acceptor where X and Y are electronegative
- The distance between X and Y is about 2.8 A

(any one of these give +2 pts).



B2. (8 pts)

a) *Sketch* either an α-helix or a β-sheet in the box to the right (please indicate your selection). Indicate the location of the amino acid sidechains in your diagram. (4 pts)

Helix:

1. Overall shape as a helix (+1)
2. Mainchain hydrogen bonds parallel to helical axis (+2)
3. Sidechains project outward (+1)

Sheet:

1. Correct shape, multiple strands, parallel or antiparallel (+1)
2. Mainchain hydrogen bonds perpendicular to strand directions (+2).
3. Sidechains point above and below sheet (+1)

b) *Briefly* discuss the role of hydrogen bonds in the stabilization of an α-helix, β-sheet, or any other super-secondary structure. You may find it helpful to refer to your diagram (4 pts).

- Mainchain hydrogen bonds, with the N-H group as the donor and the O=C group as the acceptor stabilize secondary and super-secondary structures. (3 pts)

- All possible mainchain hydrogen bonds are made in these structures (1 pt)

B3. (8 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 form of the protein. You are welcome to use an equation as part of your answer.

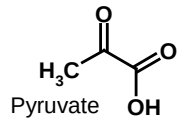
- Configurational entropy: Relates to the number of conformations the polypeptide chain can assume. Calculated as S=R ln W, where W is the number of conformations. Greatly favors the unfolded state (6 or 2 pts).

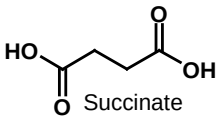
- Hydrophobic effect: Relates to the disorder of the water. In the unfolded state non-polar groups are exposed, ordering water and thus reducing the entropy of the water. Therefore, this stabilizes the folded state because the water that is released during folding from the non-polar groups is an increase in entropy (6 or 2 pts).

If a complete description of one of the above was given, then 6 points. If both are given then 8 points.

B4. (15 pts)

You wish to make a 0.1 M buffer for an experiment at pH=5.0. You have the following two organic acids to choose from (their structures are shown on the right):





- Pyruvic acid, $pK_A = 2.50$
- Succinic acid: 1^{st} $pK_A = 5.0$, 2^{nd} $pK_A = 7.0$

a) Which of these two compounds would you choose? Why? (3 pts)

Succinic acid, since one of its pKa values (the first one) is within one pH unit of the desired pH.

b) Assume that you only have both the fully protonated acid in hand, and a 1M 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 you plan to use can be given in moles. Please show all calculations. (8 pts)

We can ignore the second ionization of the succinate, since we are using the first pKa, or ionization, for the buffer.

Calculate $R = 10^{(pH-pKa)} = 10^{(5-5)} = 10^0 = 1$ (+2 pts)

$f_{HA} = 1/(1+R) = 0.5$ $f_{A^-}=R/(1+R) = 0.5$

$[HA]=f_{HA} \times 0.1\ M = 0.05\ M$ (+2 pts) $[A^-]=f_{A^-} \times 0.1\ M = 0.05\ M$ (+2 pts)

Alternatively, if you stated the pH = pK_a, therefore the concentration of each species ([HA], [A^-]) is the same, and therefore $f_{HA}=f_{A^-}= 0.5$, you should get +6 points.

So, you would begin with 0.1 moles of HA, and then add 0.05 moles of NaOH to convert 0.05 moles of HA to A^-. (2 pts).

c) Please do **one** of the following **two** choices (4 pts)

Choice A: Explain **one** of the following:

i) why the pK_A of pyruvate is less then both pK_A values for succinate?

The α-carbon of pyruvate has an electronegative atom attached to it, stabilizing the ionized form of the carboxylate, making it a stronger acid.

ii) why the two pK_A values of succinate differ?

The two carboxylic acid groups are close in space. So if you deprotonate one, generating a negative charge, then it becomes more difficult to deprotonate the second, making it a weaker acid for the second ionization.

Choice B: *Briefly* explain why your solution will be resistant to pH changes.

In the buffer region, added acid will be absorbed as A^- is converted to HA (+2 pts). Similarly, added base (OH^-) will be absorbed as the HA form of the buffer is converted to A^- (+2 pts).

Simply stating that the weak acid absorbed or released protons was worth +3 pts.

B5. (12 pts)

a) The following is a partial drawing of a dipeptide, in the extended chain conformation, *without* sidechain groups. In addition several atoms are missing. Please complete the drawing, **assuming a pH of 0.0**, by adding the following:

i) a glycine (sidechain = H) residue to the carboxy-terminal end of the peptide (2 pts).

ii) missing atoms *and* charges to the diagram. Please indicate the pKa values of the ionizable groups (2 pts).

Since the pH is 2 pH units lower than both pKa values, both are fully protonated (+1 pts for correct ionization state, +1 for pKa values).

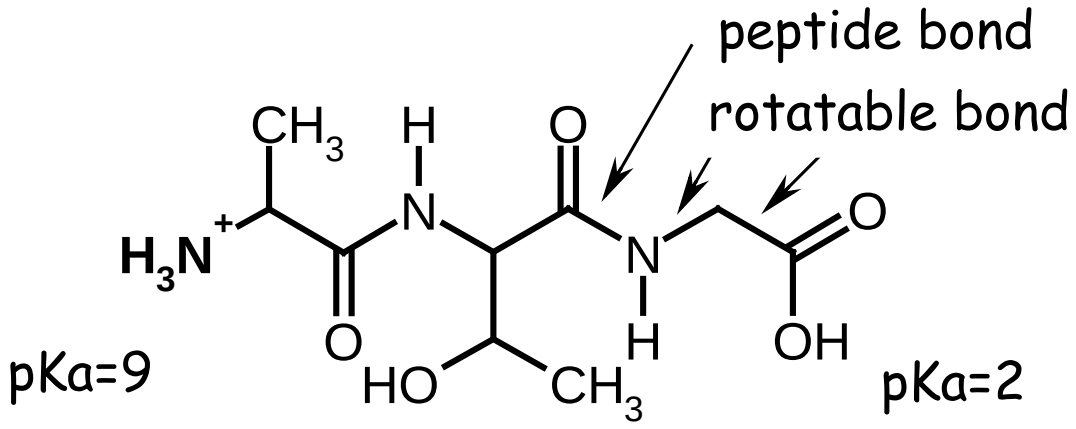
iii) the sidechain of any non-polar residue, with the exception of valine or isoleucine, to the first residue of the peptide (2 pts).

Ala, Leu, Phe, Tyr, Trp, Met are the best answers.

iv) any *polar*, but uncharged, sidechain to the second residue of the peptide (2 pts).

Asn, Gln, Ser, Thr, are the best answers Tyr and Cys are ok..

In parts iii and iv, 3/4 credit should be given if the functional groups are correct, even if it is not a common amino acid sidechain.



b) On your diagram, please indicate the following (2 pts).

i) a peptide bond. (+1 pt)
1/2 point, if all four atoms are identified (O-C-N-H)

ii) a bond that is freely rotatable. (+1 pt)

c) Give the name of this peptide (2 pt).

I added Alanine as my non-polar and Threonine as my polar, therefore the sequence is:

Ala-Thr-Gly

(+1 pt if Gly was the ending amino acid, but the other two are not close, based on the structures drawn above)

B6. Please do **one** of the following **three** questions (8 pts)

Choice A:

a) Why is the peptide bond planer? (4 pts)

Partial double bond character.

b) Which form of the peptide bond is more stable, the *trans* form or the *cis* form? Why? (4 pts)

trans (+2), because it reduces steric clashes (unfavourable van der Waals interactions) (+2)

Choice B:

A protein contains 4 tyrosine residues, 5 histidine residues, and 1 tryptophan residue. What is the absorption of a 10 μM solution of this protein at λ=280 nm, assuming a path length of 1 cm. The extinction coefficients (molar absorption coefficients) can be found on the face page.

Calculate the extinction coefficient from the composition:

$\varepsilon = 4 \times 1{,}000 + 1 \times 5{,}000 = 9{,}000\ M^{-1}\ cm^{-1}$. The histidine does not absorb (+4 pts).

$A = c\ \varepsilon\ l = 10 \times 10^{-6}\ M \times 9{,}000\ M^{-1}\ cm^{-1} \times 1\ cm$

$= 0.09$ (unitless)

(3.5 pts for correct numerical answer, 1/2 pt for correct use of units)

Choice C:

Using immunoglobulins, or other suitable examples, briefly discuss the major *features* of the four levels of protein structure, beginning with the primary structure.

(+1 1/2 points for correct name and description, additional +2 for *two* reasonable examples).

Primary: the amino acid sequence.

Secondary or super-secondary: Configuration of mainchain atoms, e.g. helices and sheets. (Ig fold)

Tertiary: Three dimensional structure of all (mainchain and sidechain atoms) in the protein. Any globular protein, e.g. protein G, myoglobin.

Quaternary: Three dimensional structure of multiple chains (e.g. immunoglobulins have four chains).

B7. Please do **one** of the following **two** questions (8 pts)

Choice A: A 10 residue peptide was treated *separately* with cyanogen bromide, trypsin, or chymotrypsin. After the cleavage reaction the peptide fragments were purified and subject to four rounds of Edman amino-terminal sequencing. The treatment and the peptide sequences are shown in the table below:

Cyanogen bromide treatment:	Met,	Ala-Thr-Ser-Phe
Trypsin treatment:	Met-Ala-Thr-Ser,	Gly-Asp-Trp
Chymotrypsin treatment:	Met-Ala-Thr-Ser,	Leu-Lys-Gly-Asp

a) Reconstruct the original sequence from these data (4 pts). Briefly indicate your approach (2 pt). Show that you answer is consistent with the original data (2 pts).

+2 for strategy (need not be this detailed)

Since the first trypsin fragment is the same as the first chymotrypsin fragment, the amino terminus of the peptide is Met-Ala-Thr-Ser.

Since treatment with Cyanogen bromide, which cleaves after Met, gives Met, plus Ala-Thr-Ser-Phe, and that the first three amino acids of this peptide match the last three of the trypsin and chymotrypsin frament (underlined) we can extend the sequence by one residue, Phe, to give: Met-Ala-Thr-Ser-Phe

Phe is the cleavage site for Chymotrypsin, so we expect the chymotryptic fragment to follow the Phe residue, giving:

Met-Ala-Thr-Ser-Phe-Leu-Lys-Gly-Asp

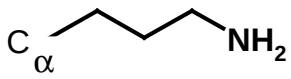
This sequence has a lysine, which is recognized by trypsin, so the Gly-Asp-Trp fragment must be the carboxy-terminus, giving the complete sequence:

Met-Ala-Thr-Ser-Phe-Leu-Lys-Gly-Asp-Trp (+4 for correct answer)

+2 for showing consistency with original data, this does not have to be a separate section of the answer. In otherwords, +4 points if the student somehow indicates how they have solved the problem.

- Cleavage with Cyanogen bromide gives : Met, Ala-Thr-Ser-Phe-Leu-Lys-Gly-Asp-Trp
- Cleavage with Trypsin gives: Met-Ala-Thr-Ser-Phe-Leu-Lys, Gly-Asp-Trp
- Cleavage with Chymotryspin gives: Met-Ala-Thr-Ser-Phe, Leu-Lys-Gly-Asp-Trp

Choice B: Poly-lysine is a long polypeptide that contains only lysine residue, the sidechain of lysine is shown on the right. Poly-lysine strongly adheres to negatively charged surfaces at low pH values (e.g. pH < 6). However, its adherence properties become progressively *weaker* as the pH is raised.



a) Why does poly-lysine bind to the surface at low pH but not at high? What fundamental force is involved? (4 pts)

When protonated, the sidechain is postively charged, opposite charges attract, so the peptide sticks by electrostatic forces (+2 pts). As the pH is raised, the lysine will become deprotonated, and there will no longer be an electrostatic interaction.

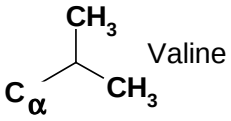
b) At what pH will the adhesive force between this peptide and the surface be reduced to approximately 50%? Why? (4 pts).

When the pH = pKa of the sidechain (approximately 9). At this point it is half protonated and half deprotonated, therefore the adhesive force should be reduced by 50%.

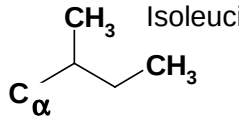
B8. (12 pts) Please do **one** of the following **two** questions:

Choice A:

A valine residue that is buried in the core of a protein is changed to an isoleucine residue. The sidechains of these two residues are shown to the right.



Valine



Isoleucine

The enthalpy and entropy of unfolding (reaction direction N→U) were measured for both the wild-type and mutant proteins and these values are shown in the following table:

	ΔH^o	ΔS^o
Valine sidechain (wild-type)	200 kJ/mol	505 J/mol-deg
Isoleucine sidechain (mutant)	190 kJ/mol	500 J/mol-deg

a) Provide an explanation for why the ΔH^o **or** ΔS^o values differ between the two proteins. Be sure to indicate your choice (ΔH **or** ΔS) (4 pts).

$\Delta H^{0:}$: The enthalpy has decreased, indicating that it takes less energy to break the non-covalent bonds in the isoleucine containing protein. Since both sidechains are non-polar, the only interaction that can be affected is van der Waals. Therefore the isoleucine protein must be less well packed in the core of the protein, i.e. the larger sidechain has disrupted an otherwise well packed core (+2 for van der waals, +2 for reasonable description)

ΔS^o: The entropy has decreased for the mutant. If you assume that the configurational entropy change will be the same when either protein unfolds, then the entropy difference must be due to a different hydrophobic effect. The more non-polar isoleucine will order more water in the unfolded state, therefore the ΔS will be less. (+2 for hydrophobic effect, +2 for reasonable description)

b) Briefly explain how the ΔH^o values would be obtained from experimental data. A well-labeled sketch can be used for your answer (2 pt).

Plot *ln* K_{EQ} versus 1/T, slope of the line is $-\Delta H/R$.

c) What is the T_M for the wild-type protein? Please show you work (2 pt).

At T_N $\Delta G^o=0$, therefore $\Delta H/\Delta S = T_M$

T_M = 200,000/505 = 396 K.

d) How much of the mutant protein is unfolded at the T_M of the wild-type protein? Please show your work (4 pts).

+3 pts for setting up the problem, additional +1 for getting the correct numerical answer.

ΔG^o = 190,000 - 396 × 500 = -8,000 J/mol [this tells you the unfolded form is preferred]

$K_{EQ} = e^{[-\Delta G/RT]} = e^{[8000/(8.3 \times 396)]}$ = 11.4 [this also tells you the unfolded form is preferred]

$F_U = K_{EQ}/(1+K_{EQ})$ = 0.91 [finally, this clearly tells you the unfolded form is preferred]

A number of students used the T_N for the wild-type protein. If they did the calculations correctly only deduct 1/2 point. If they simply stated that $T=T_M$ and therefore $f_{HA}=0.5$, deduct 1 1/2 points.

Choice B:

A five stranded β-sheet protein can inter-convert between a planer sheet and a β-barrel without unfolding. This conversion does *not* change the number of non-polar buried residues and therefore the hydrophobic effect is of no importance in this problem.

a) Assuming that each strand is 10 residues long, estimate the relative stability (i.e. ΔG^o) of the two structures. Please state your assumptions (6 pts)

Assuming that the only difference between the two structures is the hydrogen bonding, then the planer sheet will have two edges that hydrogen bond to water, while the β-barrel is closed with all hydrogen bonds between the mainchain atoms (+4 pts).

The strands are 10 long, in a β-strand each edge will have 10 hydrogen bonds to water (+1 pt)

Therefore, the enthalpy change in going from sheet to barrel is -20 kJ/mol, assuming each hydrogen bond between the mainchain atoms is 2 kJ/mol more stable than that to water. Since they are more stable in the barrel, the sign of the enthalpy change is negative, for this direction of the reaction (+1 pt).

b) Does the ΔG^o depend on temperature? Why or why not? (3 pt)

No, since hydrogen bonding is purely enthalpic (+2 pts), $\Delta S^o = 0$ for this reaction. Since $\Delta G^o = \Delta H - T\Delta S$, there is no effect of temperature on the free energy (but there is on the equilibrium constant, see part c) (+1 pt).

c) Calculate the fraction of the protein in the β-barrel form at 300K (3 pts)

+2 pts for setting up the problem, +1 for numerical answer.

ΔG^o = - 20 kJ/mol [this tells you the barrel form is preferred]

$K_{EQ} = e^{[-\Delta G/RT]} = e^{[20000/(8.3 \times 300)]} = 3078$ [this also tells you the barrel form is preferred]

$F_{Barrel} = K_{EQ}/(1+K_{EQ}) = 0.999$ [finally, this clearly tells you the barrel form is preferred]

Some students may have set this up as a protein unfolding problem. If they did, just deduct 2 points, and then grade the question based on what the student thought the equilibrium was..