The following equations and constants may be useful:

T=300K and pH=7.0 unless otherwise stated.

To convert from C to K, add 273.

R=8.3 J/mol-K RT=2.5 kJ/mol @ 300K

Log2=0.3 ln10=2.3

ln9=2.20 ln2=0.69

$\Delta G^0 = -RT\ln K_{eq}$

$\Delta G = \Delta H - T\Delta S$

$S = R\ln W$

$\ln(a^n) = n \ln a$

For the reaction: N $\Leftrightarrow$ U:

$K_{eq} = [U]/[N]$

$f_u = K_{eq}/(1+K_{eq})$

$f_n = 1/(1+K_{eq})$

$pH=pK_A+\log([A^-]/[HA])$

$R=10^{(pH-pKa)}$

$[HA]=[A_T]/(1+R)$

$[A^-]=[A_T]R/(1+R)$

$R=[A^-]/[HA]$

Beer's law: $A=\varepsilon[X]l$

Tryptophan $\varepsilon_{280} = 5{,}000\ M^{-1}cm^{-1}$

Tyrosine $\varepsilon_{280} = 1{,}000\ M^{-1}cm^{-1}$

Amino Acid Names:

Alanine: Ala
Arginine: Arg
Asparagine: Asn
Aspartic Acid: Asp
Cystine: Cys
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
Glutamine: Gln
Glutamic Acid: Glu

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 ($CH_3CH_2CH_2CH_2OH$)
   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.
   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:
   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.
   c) the difference in the residual energy of reactants and products at equilibrium.
   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:
   a) extracellular proteins.
   b) dimeric proteins.
   c) intracellular proteins.
   d) multisubunit proteins.

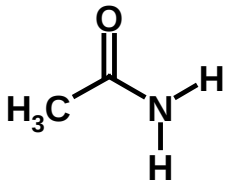
6. Which of the following are characteristics of the immunoglobulin (Ig) fold?
   a) It is found only in IgG molecules.
   b) It is composed of two anti-parallel β-strands folded into a globular domain.
   c) It is β-barrel like, composed of a three- and a four-stranded antiparallel β-sheet.
   d) It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



B2. (8 pts)

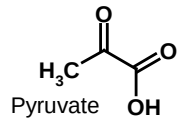
a) *Sketch* either an α-helix or a β-sheet in the box to the right (please indicate your selection). Indicate the location of the amino acid sidechains in your diagram. (4 pts)

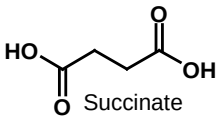
b) *Briefly* discuss the role of hydrogen bonds in the stabilization of an α-helix, β-sheet, or any other super-secondary structure. You may find it helpful to refer to your diagram (4 pts).

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.

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)

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)

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?

ii) why the two pK_A values of succinate differ?

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

B5. (12 pts)

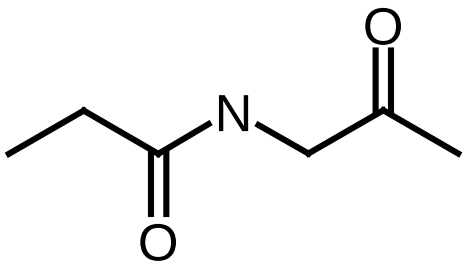
a) The following is a partial drawing of a dipeptide, in the extended chain conformation, *without* sidechain groups. In addition several atoms are missing. Please complete the drawing, **assuming a pH of 0.0**, by adding the following:

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).

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

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



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

i) a peptide bond.
ii) a bond that is freely rotatable.

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

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

Choice A:

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

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

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.

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.

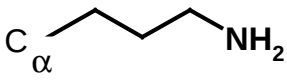
B7. Please do **one** of the following **two** questions (8 pts)

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).

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.



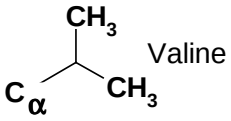
a) Why does poly-lysine bind to the surface at low pH but not at high? What fundamental force is involved? (4 pts)

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

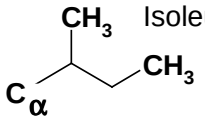
B8. (12 pts) Please do **one** of the following **two** questions:

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 **<u>or</u>** ΔS^o values differ between the two proteins. Be sure to indicate your choice (ΔH **<u>or</u>** ΔS) (4 pts).

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).

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

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

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)

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

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