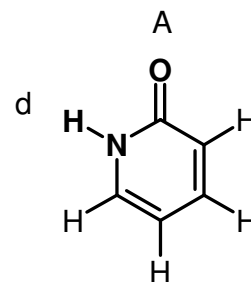


This exam consists of 100 points in 14 questions on 6 pages. Use the space provided.

1. (5 pts) Identify potential hydrogen bond donors *and* acceptors on the molecule shown to the right. Label them with "d" or "a", respectively (2 pts). Briefly explain why a group is a hydrogen bond donor (3 pts).



The oxygen can accept a hydrogen bond.

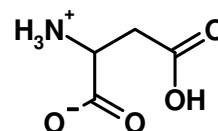
The NH group will donate a hydrogen bond.

A hydrogen bond donor has the general structure X-H, where X is electronegative. The higher electronegativity of X produces a partial positive charge on the proton. The partial positive charge on the proton forms a favorable electrostatic interaction with the electronegative acceptor.

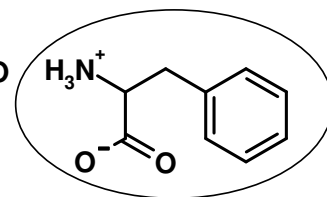
2. (2 pts) Two amino acids are shown on the right.

i) Name both amino acids (full name or 3 letter) (1 pt).

ii) Circle the amino acid that you would most likely find in the core of a folded protein (1 pt).

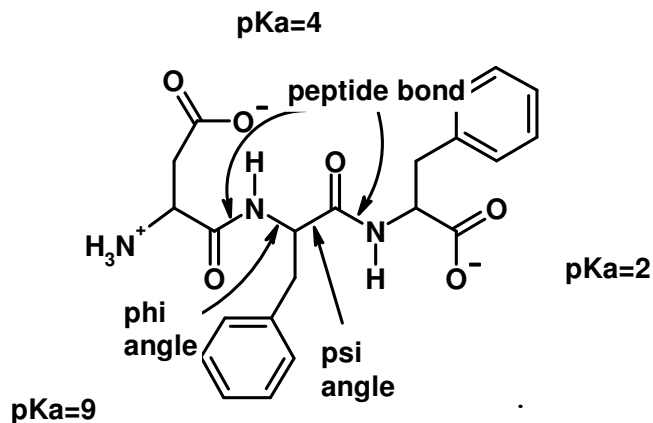


Aspartic Acid
(Asp)



Phenylalanine
(Phe)

3. (14 pts) Draw a tripeptide using the first of the above amino acids (question 2) once, and the second twice. The geometry of the peptide bonds should be drawn in the most prevalent form, i.e. *trans* (8 pts).



i) Name your peptide, using the names you used in question 2. (1 pt) **Asp-Phe-Phe**

ii) Indicate the amino and carboxy terminus (2 pts). *See diagram.*

iii) Label the peptide bond and the *phi* and *psi* bonds for the second residue (2 pts). *See diagram, any peptide bond was acceptable.*

iv) Indicate the pKa values of *all* ionizable groups (1 pt). *See diagram*

4. (4 pts) Briefly explain why the peptide bond is planer **OR** explain why it is trans.

Planer: The peptide bond is planer because the nitrogen and carbon are sp^2 hybridized because this enhances the interaction between the p_z orbitals of these two atoms. Stating partial double bond character was accepted, but this only explains why rotations about the peptide bond are unfavorable.

Trans: In this geometry there is minimal steric clash between the atoms. In the cis form, atoms will bump into each other.

5. (3 pts) A solution of the peptide from question 3 has a UV absorbance of 1.0 at $\lambda=280$ nm. Assuming a path length, l , of 1 cm, what is its concentration?

The units of the extinction coefficient are $M^{-1}cm^{-1}$. (omitted in the chart on the right).

$A = c \epsilon l$	
$\epsilon_{280}^{\text{Tryptophan}}$	= 5,000
$\epsilon_{280}^{\text{Tyrosine}}$	= 1,000
$\epsilon_{280}^{\text{Phenylalanine}}$	= 100

The peptide has two Phenylalanine groups, so the extinction coefficient for the entire peptide is $200 M^{-1}cm^{-1}$. The concentration is then:

$$C = A/\epsilon l = 1/(200 \times 1) = 0.005 M, \text{ or } 5 \text{ mM.}$$

[Full credit if the extinction coefficient used was consistent with your naming of the aromatic residue in Q2]

6. (9 pts) Please do **ONE** of the following two questions. Clearly indicate your choice.

Choice A: Explain why solutions of weak acids act as buffers in the range of $pH = pK_a \pm 1$.

- In this region there is appreciable amounts of HA and A^- (3 pts).
- Added base will remove a proton from HA, forming A^- , leaving the actual hydrogen ion concentration essentially constant (3 pts).
- Added acid will protonate A^- , forming HA, without increasing the hydrogen ion concentration (3 pts).

Choice B: The pK_a of a side chain amino group on a lysine residue in a protein is 7.0. What is the usual pK_a for a group of this type? Provide a possible reason for this shift in pK_a .

The usual pK_a for this group is 9.

This indicates that the amino group is a stronger acid. Therefore, the environment must be either stabilizing the deprotonated form or destabilizing the protonated form. Either could occur because what matters is the relative energy difference between the two states.

The protonated form has a positive charge. So one possibility is that it is nearby other positive charges, this would make protonation less favorable, making it a stronger acid.

7. (12 pts) Please do **ONE** of the following four questions. Indicate your choice and show your work.

Choice A (pH effects on activity): A group in a protein must be *deprotonated* to be active. The pKa of this group is 5.0. What is the % activity of this protein at a pH of 6.0?

The fraction deprotonated is $1 - f_{HA}$.

Calculating f_{HA} :

$$R = 10^{6-5} = 10^1 = 10$$

$$f_{HA} = 1/(1+10) = 1/11 = 0.09$$

Therefore $f_{A^-} = 0.91$. The protein is 91 % active.

Choice B (Buffer problem): The pKa of a weak acid is 5.0. Describe how you would make 1 L of a 1.0 M buffer at pH=6.0 using this acid.

The fraction protonated is 0.09 and the fraction deprotonated is 0.91 (see part A).

Therefore mixing 0.09 moles of HA and 0.91 of the sodium salt (A^-) will generate the buffer.

Alternatively: Start with 1 mol of HA and add 0.91 moles of NaOH.

Alternatively: Start with 1 mol of A^- and add 0.09 moles of HCl.

Choice C (Charge determination): Determine the isoelectric pH (pI) of the peptide from Q3.

- This tripeptide has only three ionizable groups and will behave as if it were just Aspartic acid. The pI is the average of two of the pKas. You don't know which two to average, so your choices are either $(2+4)/2 = 3$ or $(4+9)/2 = 7.5$.
- At pH =7.5 both carboxylates will be deprotonated and the amino group will be protonated. The net charge at this pH is $-2+1 = -1$.
- At pH=3.0 the carboxy terminus will be 10% protonated and the sidechain carboxylate will be 90% protonated. The sum of these two will be a charge of -1, which cancels the +1 charge on the amino group. Therefore the pI=3.

Choice D (pH adjustment): How many *equivalents* of base do you have to add to a solution of a weak acid (pKa = 5) to change the pH from 5.0 to 6.0?

- At the starting pH, the fraction protonated is 0.5 since the pH=pKa.
- At the ending pH, the fraction protonated is 0.09 (see choice A).
- Therefore 0.41 equivalents of base will have to be added.

8. (10 pts)

i) *Briefly* describe the hydrophobic effect.

ii) Does it result in a change of entropy or enthalpy (circle the correct choice)?

iii) What is the role of the hydrophobic effect in defining the native structure of globular proteins?

i) The ordering of water when non-polar groups are exposed to the water.

ii) The **entropy** of the water decreases.

iii) The hydrophobic effect forces results in a non-polar core.

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

$$f_{HA} = \frac{1}{1 + R}$$

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

$$\log 10 = 1, \log 0.1 = -1$$

9. (5 pts) Please do **ONE** of the following three choices. Clearly indicate your choice.

Choice A: In calculating the conformational entropy of a protein, the conformational entropy of the unfolded state is given as $S = R \ln 9^n$, where n is the number of residues. Briefly explain the origin of 9^n in this expression and why this formula should not be applied to proteins with a large number of glycine residues.

- Each residue has 9 different conformations, 3 possibilities for the mainchain and 3 for the sidechain.
- Since the conformation of each residue is independent, the conformation of the entire chain is $9 \times 9 \times 9 \dots 9$, or 9^n .
- Glycine has many more mainchain conformations because it lacks a sidechain, therefore 9 conformations/residues is not a good approximation.

Choice B: A classmate has recently determined the structure of a typical protein and shows you the Ramachandran plot for their protein. The points for each amino acid are found all over the plot. What should you tell your classmate about the likelihood that they have a correct structure?

Their structure is wrong. The points for non-glycine residues should fall in three distinct regions of the Ramachandran plot. Corresponding to β -strand, or α -helix.

Choice C: Briefly compare and contrast tertiary structure and quaternary structure. Give an example of a protein with a quaternary structure and discuss why the quaternary structure is essential for function (a drawing with some text is a suitable answer).

Tertiary structure is the 3D structure of one chain.

Quaternary structure is the arrangement of multiple chains.

Antibodies have a quaternary structure (2 light chains, 2 heavy chains). The antigen binds to both the light and heavy chain.

10. (10 pts) Sketch *either* an α -helix *or* a two stranded parallel β -sheet. Indicate the locations & direction of the hydrogen bonds and the sidechains.

α -helix:

Needed to draw something that looks like a helix (2 pts)

H-bonds || to helix axis (4 pts)

Sidechains project out from helix (4 pts)

β -sheet.

Two || strands running in same direction (N $\rightarrow$ C) (2 pts)

Hydrogen bonds perpendicular to strands (4 pts).

Sidechains alternate up and down (4 pts).

11. (1 pt) Name *one* super-secondary structure. **β -barrel or β - α - β unit.**

12. (10 pts) A 14 residue peptide of unknown sequence was divided into two samples. The first sample was treated with Trypsin and the second with chymotrypsin. The resultant peptides in each sample were separated and sequenced and their *complete* sequences are given below. What is the sequence of the original peptide?

You need not list each residue in your answer, the order of the chymotrypsin fragments will suffice, i.e., C1-C2 or C2-C1. **Justification** of your answer is required for full credit. *Write the justification on the back of the preceding page.*

Sequence of the four fragments from Trypsin:

T1: Phe-Arg

T2: Ala-Asp-Arg

T3: Gly-Met-Ser-Lys

T4: Leu-Ser-Ala-Ala-Gly

Sequence of the two fragments from Chymotrypsin cleavage:

C1: Arg-Leu-Ser-Ala-Ala-Gly

C2: Ala-Asp-Arg-Gly-Met-Ser-Lys-Phe

The two possibilities are C1-C2 or C2-C1

C2-C1 is correct: Ala-Asp-Arg-Gly-Met-Ser-Lys-Phe-Arg-Leu-Ser-Ala-Ala-Gly.

Digestion of this peptide with trypsin produces the following fragments:

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

which is consistent with the four peptides from the trypsin digestion.

Amino Acid Names:

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

13. (12 pts) Please do **ONE** of the following two questions (Choice B is on the following page).

Choice A: A protein has a ΔH° of +200 kJ/mol and a ΔS° of +600 J/mol-K.

- Sketch the denaturation curve of the protein, clearly marking T_M (6 pts).
- Briefly describe how the enthalpy would be obtained from your curve sketched in part i (3 pts).
- Determine how much of this protein is *unfolded* at 310K (3 pts).

i) The T_M is the temperature at which the native and denatured states are equal in energy, i.e. ΔG° is zero. Therefore $T_M = \Delta H^\circ / \Delta S^\circ$ or 333 K. Your curve should begin at $f_{\text{unfolded}}=0$ at low temperatures, rise sharply and pass through $T=333$ K when $f_{\text{unfolded}} = 0.5$, and then level off at $f_{\text{unfolded}}=1$ at high temperatures.

ii) Obtain K_{EQ} at a number of temperatures from $f_{\text{unfolded}}/(1 - f_{\text{unfolded}})$. Plot $\ln K_{EQ}$ versus $1/T$, slope is $-\Delta H^\circ/R$.

iii) At 310K, $\Delta G^\circ = 200 - (310) \times (0.6) = +14$ kJ/mol.

$K_{EQ} = e^{-\Delta G/RT} = e^{-14,000/(8.31 \times 310)} = e^{-5.43} = 0.0043$.

$f_U = K_{eq}/(1+K_{eq}) = .0043/1.0043 \approx 0.004$

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

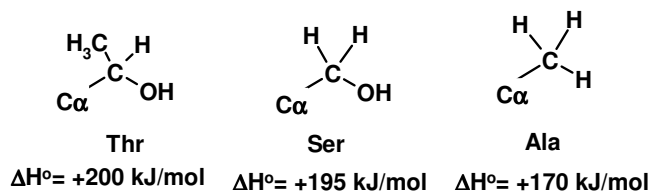
$$\Delta G^\circ = -RT \ln K_{EQ}$$

$$f_{\text{Native}} = \frac{1}{1 + K_{EQ}}$$

$$(N \rightarrow U)$$

$$R = 8.31 \text{ J/mol} - K$$

Q13- Choice B: A Threonine (Thr) residue *buried* in the core of a protein is replaced by Serine (Ser) or Alanine (Ala). The ΔH° values for unfolding of each protein are +200 kJ/mole, +195 kJ/mol, and +170 kJ/mol, respectively. The sidechains of all three amino acids are shown to the right.



i) Explain the change in enthalpy for the Threonine to Serine replacement (9 pts).

Serine differs from Threonine by loss of a methyl group. Therefore the decrease in enthalpy is due to a reduction in the van der Waals interactions in the Serine containing protein. Potential hydrogen bonding interactions between the Threonine and some other group would be preserved in the serine containing protein.

ii) Explain the change in enthalpy for the Serine to Alanine replacement (3 pts).

- The -OH group is missing in the Alanine residue, so the loss of a hydrogen bond is likely involved (+2 pts).
- The large decrease in enthalpy (25 kJ/mol) is due to the fact that the other group that formed a hydrogen bond to the Threonine (or serine) is without a donor/acceptor in the Alanine containing protein (+ 1 pt).

One way to visualize the effect of the Alanine mutation is consider 190 kJ/mol of the ΔH in the serine protein to account for all enthalpic effects **except** for the hydrogen bond to the serine.

In the unfolded state, the group that forms a hydrogen bond to serine is hydrogen bonded to water.

In the folded state, the hydrogen bond to water has been broken, at a cost of +20 kJ/mol and replaced by a hydrogen bond in the interior of the protein, releasing 25 kJ/mol. Therefore the net change in going from unfolded to folded is -5 kJ/mol. In the reverse direction, this becomes +5 kJ/mol. This adds to 190 kJ/mol to give 195 kJ.

For the alanine protein, the group that did form a hydrogen bond to serine still forms one to water in the unfolded state. A total of +20 kJ/mol has to be provided to break this bond, as above. However, it is not reformed in the folded state, so there no heat released. In the reverse direction (native to unfolded) the ΔH is -20 kJ/mol. Add this to the +190 and you get +170 kJ/mol.

14. (3 pts) Define K_D in terms of kinetic on- and off-rates for a protein-ligand interaction, and then *briefly* explain why ligands that bind tightly have small K_D values.

$$K_D = \frac{k_{off}}{k_{on}}$$
 Ligands that bind with many interactions show small off-rates, and therefore small K_D values.

1 .	_____ /	5
2 .	_____ /	2
3 .	_____ /	14
4 .	_____ /	4
5 .	_____ /	3
6 .	_____ /	9
7 .	_____ /	12
8 .	_____ /	10
9 .	_____ /	5
10 .	_____ /	10
11 .	_____ /	1
12 .	_____ /	10
13 .	_____ /	12
14 .	_____ /	3
TOT	_____ /	100