

This exam consists of 5 pages and 12 questions. Total points are 100. Allot 1 min/2 points. On questions with choices, all of your answers will be graded and the best scoring answer will be used. Use the space provided.

1. (2 pts) Which of the following does **NOT** play a predominate role in the energetic of protein folding (circle best answer)?
 - a) van der Waals forces
 - b) hydrogen bonds
 - c) electrostatic forces
 - d) hydrophobic effect
2. (4 pts) Briefly discuss why all of the following compounds are soluble in water and then indicate which of the three would be the most soluble in water. Justify your answer.
 - a) methanol (CH_3OH)
 - b) ethanol ($\text{CH}_3\text{CH}_2\text{OH}$)
 - c) propanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$)

All three would form hydrogen bonds with water via the alcohol group ($-\text{OH}$) (2 pts)

Methanol would be the most soluble since it has the smallest non-polar group (1 pt), which would decrease the entropy of the water by the smallest amount (1 pt).

3. (3 pts) Briefly relate **one** of the following two properties of water to its molecular structure.

Choice A: density of ice.

Choice B: heat capacity of liquid water.

Choice A:

Water can both donate and accept hydrogen bonds due to its electronegative oxygen (2 pts)

The oxygen in water is sp^3 hybridized and therefore tetrahedral. When frozen this creates a structure with open channels, reducing the density of ice relative to liquid water (1 pt)

Choice B:

Water can both donate and accept hydrogen bonds due to its electronegative oxygen (2 pts)

The hydrogen bonds are present in liquid, allowing water to absorb heat without a large increase in temperature (1 pt)

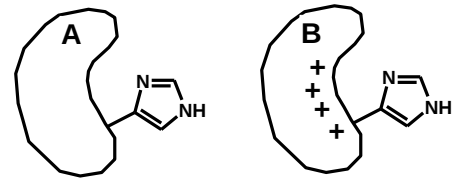
4. (6 pts) The peptide bond is described as “planer and trans”. Briefly describe why the peptide bond has these characteristics.

Planer - the four atoms lie in a plane because the nitrogen is sp^2 hybridized giving it a planer geometry (2 pts)

It is sp^2 hybridized because this allows the nitrogen to share its p_z orbital with the pi bond between the carbon and oxygen (1 pt)

Trans - this geometry prevents unfavorable van der Waals contacts that occur in the cis conformation (3 pts)

5. (9 pts) Two different proteins (A and B) contain an imidazole group as part of one of their amino acids. The structure of these two proteins is shown on the right. The "+" symbols refer to positive charges. [Hint: The imidazole is shown in the deprotonated state.]



- i) (1 pt) Which of the following amino acid has an imidazole as part of its sidechain (circle correct answer):

Glutamine Asparagine **Histidine** Lysine Arginine.

- ii) (4 pts) Assume that the pK_a of the imidazole group in protein A is 6, predict the pK_a of this group in protein B. Briefly justify your answer.

When the histidine becomes protonated it will become positively charged (1 pt)

The positive charge on the **protonated** histidine will be energetically unfavorable due to the positive charges on the protein (2 pts)

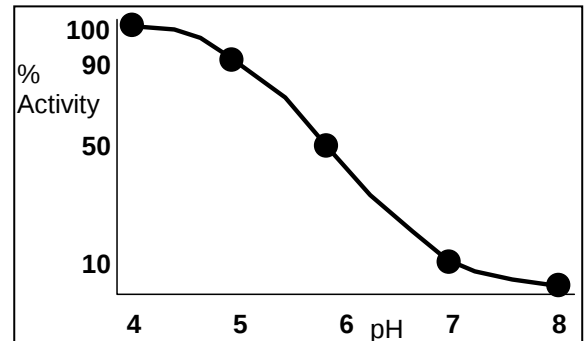
Therefore the deprotonated state will be preferred, making this a stronger acid with a lower pK_a . (1 pt)

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

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

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

- iii) (4 pts) Sketch, in the box on the right, the % activity of protein A as a function of pH, assuming the protonated form of the imidazole is the active form. The pH range should be from 4 to 8. Be sure to label the axes.



+2 pts for correct "shape" of curve

+2 pts for correct positioning of curve ($pK_a = 50\%$)

(-1 if you plotted the curve for the deprotonated state being more active.)

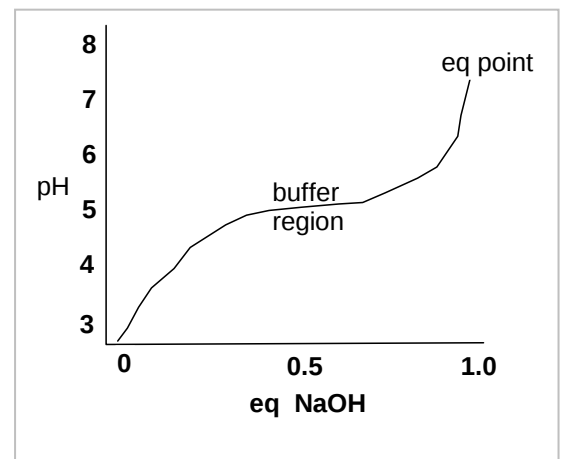
6. (12 pts) You wish to make a buffer solution at $pH = 4$. The reaction that you are buffering produces acid, i.e. it will lower the pH. Your choices of weak acids for the buffer are:

- Acetic acid ($pK_a = 5.0$),
- Pyruvic acid ($pK_a = 2.0$)
- Tris ($pK_a = 8.0$)

- a) (2 pts) Which buffer would you choose and why?

Acetic acid, it is the only acid with a pK_a close to the desired pH (2 pts)

Although pyruvic acid seems like a better choice due to its more acidic pK_a , it would have no buffer capacity at pH 4 (1 pt if pyruvate was selected).

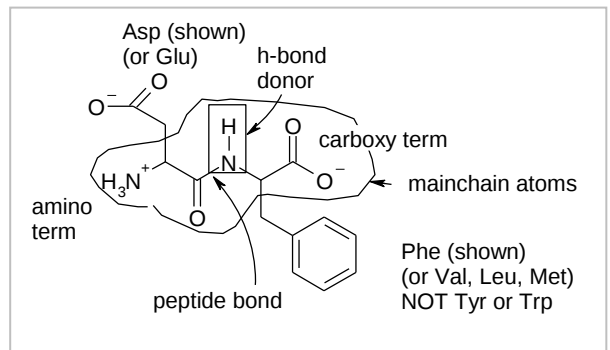


- b) (4 pts) Sketch the titration curve for the buffer of your choice in the space on the right. Be sure to label both axes and indicate the major features of this curve. **Monoprotic acid (one pK_a).**

- c) (6 pts) Assuming that you are beginning with the fully protonated form of the buffer (HA), calculate how many **equivalents** of NaOH would you need to add to the solution of protonated weak acid. Show your work on the back of pg 1, write your answer here:

~ 0.1 eq of base are required since the desired pH is one unit below the pK_a (4 pts). A more accurate value is obtained using the formula: $R = 10^{4-5} = 10^{-1} = 0.1$. $f_{A^-} = .1/1.1 = 0.09$ (+2 pts)

7. (16 pts) Draw the chemical structure of a dipeptide in the box on the right (4 pts). Your first amino acid can be any acidic residue and your second amino acid should be **completely** non-polar, but do not use isoleucine (Ile) or alanine (Ala).



- i) label the amino- and carboxy-terminus of your peptide and give the names of the two amino acids (2 pts)

- ii) Indicate the peptide bond (1 pt)

- iii) circle the mainchain atoms (1 pt)

- iv) indicate one hydrogen bond donating group on the mainchain atoms with the square box (2 pts)

- v) estimate the net charge on your peptide, **assuming that the pH is the same as the pK_a value of your acidic residue**. For partial credit, show your work on the back of the previous page, write your answer in the space to the right (4 pts):

$$q_{total} = \sum (f_{HA} \cdot q_{HA} + f_{A-} \cdot q_{A-})$$

The charges on the end of the peptide cancel each other. The acidic side chain will be 50% protonated, so the **average charge is -0.5 e. (4 pts)**

- vi) Do you expect your peptide to bind to the protein shown on the right? Justify your answer (2 pts)

Yes, the negative charge on the peptide will interact favorably with the positive charges on the protein (2 pts)



8. (10 pts) You are trying to sequence a **12 residue** peptide using Edman degradation. The following peptide sequences were obtained after cleavage of the initial peptide with the indicated cleavage reagents. You can assume that it was possible to sequence the first five residues of each peptide. The peptide begins with the sequence: Ala-Gly-Val-Met-Glu

CNBr fragments: CNBr 1: Ala-Gly-Val-Met CNBr 2: Val-Gln-Asp-Thr CNBr 3: Glu-Arg-Trp-Met

Trypsin Digest: P1 : Ala-Gly-Val-Met-Glu P2: Trp-Met-Val-Gln-Asp

- i) (6 pts) Determine the sequence of the original peptide. Instead of writing out the sequence, just give the correct order of the CNBr fragments, e.g. 1-2-3. Justify your approach on the back of the previous page.

1 - 3 - 2. (4 pts for correct answer)

CNBr 1 is the first fragment since its sequence agrees with the amino-terminal sequence of the peptide. The third CNBr fragment has an Arginine, which is a cleavage site for trypsin, so one of the Trypsin fragments should begin with Trp-Met. P2 does, showing that CNBr3 must come before CNBr2. **(2 pts for some sort of justification)**

- ii) (4 pts) What is the absorbance at 280 nm for a 1 uM (10⁻⁶ M) solution of this peptide?

Amino acid	ϵ
Trp	5,050 M ⁻¹ cm ⁻¹
Tyr	1,440 M ⁻¹ cm ⁻¹
Phe	220 M ⁻¹ cm ⁻¹

The peptide contains only a single Trp residue, therefore its extinction coefficient will be the same as Trp **(3 pts)**

$$A = \log \frac{I_0}{I} = \epsilon [X] l$$

$$A = 5,050 \times 10^{-6} \times 1 = 5.05 \times 10^{-3} \quad \text{(1 pt)}$$

9. (10 pts) Please do **one** of the following three choices.

Choice A: Briefly describe the molecular basis of the hydrophobic effect and indicate its role in the stability of folded proteins.

Choice B: Briefly describe conformational entropy and indicate its role in the stability of folded proteins.

Choice C: Briefly describe the role of van der Waals forces in the formation/stability of secondary **and** tertiary structures of proteins.

Choice A:

The hydrophobic effect relates to a change in the entropy of the water due to the presence of non-polar groups (3 pts)

The non-polar groups order the water, reducing its entropy - which is unfavorable. (3 pts)

When proteins unfold they expose their buried non-polar groups. This makes unfolding unfavorable (or folding favorable) since this decreases the entropy of the system. (4 pts)

Choice B:

Conformational entropy describes the disorder of the polypeptide chain (2 pts)

The entropy increases with disorder, according to $S = R \ln W$, where W is the number of different conformations (2 pts)

Since unfolded proteins can assume a large number of conformations there is a large increase in the entropy when they unfold - this stabilizes the unfolded state. (6 pts)

Choice C:

Van der Waals forces can be attractive or repulsive if the atoms get too close (4 pts)

The repulsive nature reduces the number of conformations in secondary structures to 3. (3 pts)

The attractive force is responsible for the well packed core in the tertiary structure of proteins (3 pts)

10. (16 pts) Three Ramachandran plots are shown on the right, labeled A, B, C.

i) (2 pts) What do the regions enclosed by contour lines represent?

Regions of low energy due to favorable van der Waals interactions.

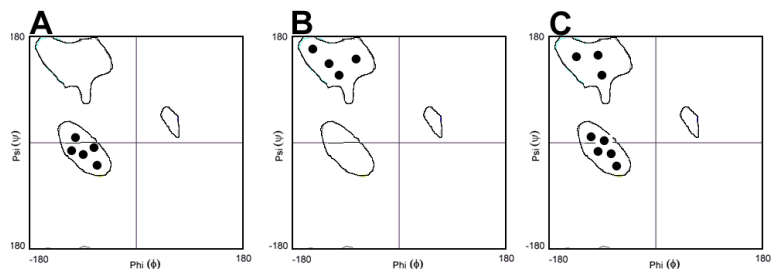
ii) (3 pts) Indicate which plot corresponds to which secondary/super-secondary structure (circle correct answer)

A	B	C	helical peptide
A	B	C	β -sheet
A	B	C	$\beta\alpha\beta$

iii) (6 pts) Pick any **one** of the above three secondary/super-secondary structures and briefly describe its structure (a sketch is an acceptable answer). Your sketch should indicate the location of hydrogen bonds and sidechain atoms.

iv) (3 pts) For the same structure that you picked in iii), describe the **main** energetic feature that is important for its stability.

H-bonds acceptable for all three. $\beta\alpha\beta$ unit - hydrophobic & vdw for helix-sheet interaction. (3 pts)



Helical drawing: (2 pts each)

- Right handed
- H-bonds || to helix axis
- Sidechains point out

Sheet

- Peptide strands running in same direction
- H bonds perp to strand direction
- Sidechains alternate up and down

$\beta\alpha\beta$

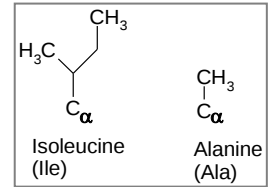
Combination of above structures

11. (6 pts) Please do **one** of the following four choices:

Choice A: A protein consists of the following sequence of polar (open circles) and non-polar (closed circles) amino acids. Indicate which part of the protein will likely be helical and which part sheet? Briefly justify your answer.



Choice B: An isoleucine residue is buried in the core of a globular protein. In a mutant protein the isoleucine is replaced by an alanine residue. Briefly discuss how this replacement will affect **either** the enthalpy **or** the entropy associated with unfolding of the protein.



Choice C: A protein unfolds with an enthalpy of +200 kJ/mol and an entropy of 600 J/mol-K. How much of this protein is unfolded at 330K?

Choice D: What is a disulfide bond and why does it stabilize proteins which contain them?

Choice A:

First $\frac{1}{2}$ will be a helix because the non-polar residues are every 4th residue. Second $\frac{1}{2}$ sheet because the non-polar residues alternate.

Choice B:

ΔH° : The smaller Ala will have reduced vdw interactions, therefore the enthalpy will decrease - less energy will be required to denature the protein.

ΔS° : The smaller Ala will order less water in the unfolded state. Therefore the net change in entropy will increase because there will be less cancellation of the conformational entropy by the hydrophobic effect.

Choice C:

Calculate the standard energy: $\Delta G^\circ = 200,000 - (330)600 = + 2,000 \text{ J/mol}$

Calculate the equilibrium constant: $K_{EQ} = e^{-\Delta G/RT} = e^{-2000/8.3 \times 330} = 0.48$

Calculate the fraction unfolded = $K_{EQ}/(1 + K_{EQ}) = 0.48/1.48 = 0.325$

Choice D:

A disulfide bond is a covalent bond between the sulfur atoms on the sidechain of cysteine.

It stabilizes folded proteins because it decreases the entropy of the unfolded state by reducing the number of conformations.

12. (6 pts) Please do **one** of the following two choices.

Choice A: Using immunoglobulins (antibodies) as an example, briefly describe:

- i) quaternary structure
- ii) protein domains.

Choice B: Draw a "cartoon" diagram of an antibody and indicate on your diagram the following:

- i) the location of the hypervariable loops.
- ii) an Fab fragment
- iii) Where the antigen binds.

Choice A:

Quaternary structure is a description of the number of different polypeptide chains. Immunoglobulins have 2 light and 2 heavy chains.

Protein domains are independently folding units of a longer polypeptide. Immunoglobulins consist of Ig folds or Ig domains. The light chain contains 2 such domains.

Choice B:

Y-shaped diagram (showing both H & L chains) with the hypervariable loops at the ends of the top of the Y. An Fab fragment is one of the upper arms. The antigen binds to the region with the hypervariable loops