

Instructions: This exam is out of 100 points, you should allot 1 min/2 pts. Please use the space provided or the back of the previous page. On questions with more than one choice, all of your attempts will be graded and you will receive the grade for your best attempt.

1. (6 pts) Draw the amino acid glycine (sidechain = H), in the space to the right. Indicate any chiral centers and assume a pH =7.0. Indicate the approximate pK_a values for any ionizable groups on this amino acid.

2. (6 pts) Please do one of the following two questions. Please indicate your choice.

Choice A: Briefly explain why weak acids act as buffers at pH values near their pK_a.

Choice B: Briefly explain why pK_a values can be sensitive to environment, give an example.

3. (6 pts) A peptide contains a single histidine residue (pK_a=6), two tryptophan residues, and four phenylalanine residues. Please do **one** of the following choices.

Choice A: Which of the following pH values would be best for purifying this peptide using *positively* charge beads (circle your answer). Briefly justify your answer.

pH=2 pH=4 pH=6 pH=8 pH=10

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

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

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

Choice B: What would the absorbance be for a 1 μM solution of this protein at a wavelength of 280 nm and a 1 cm path length?

$$A = [X] \epsilon l$$

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

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

$$\epsilon_{280}^{Phenylalanine} = 220 M^{-1} cm^{-1}$$

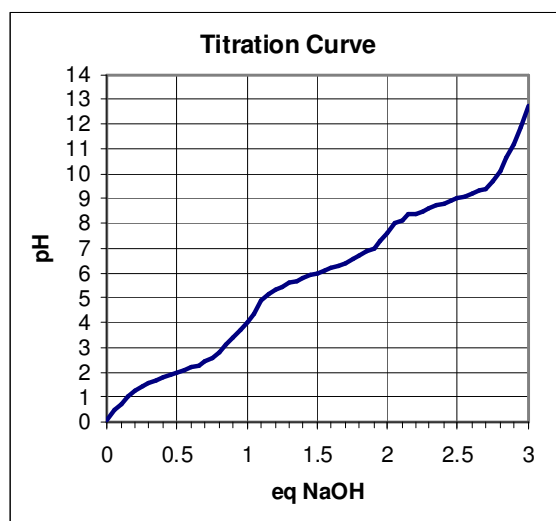
4. (12 pts) This question is based on the titration curve of a protein shown on the right. Only one sidechain is ionizable in this protein, and its pKa is 6.0. Please do **one** of the following two choices. Please do *all* parts within a choice.

Choice A: Assuming that the activity of this protein requires a deprotonated side chain:

- sketch a curve that would show the activity as a function of pH (8 pts).
- Briefly outline how you would actually calculate data points for such a curve (4 pts).

Choice B: The protein is being used as a buffer at pH 7.0. Assume that you are starting with a solution of *fully protonated protein*, please answer the following questions.

- (8 pts) Using graphical methods, i.e. the titration curve, estimate how many equivalents you would need to produce this buffer solution. *Justify your answer for full credit.*
- (4 pts) Show, with a numerical calculation, that your estimate in part i was close to the actual value.

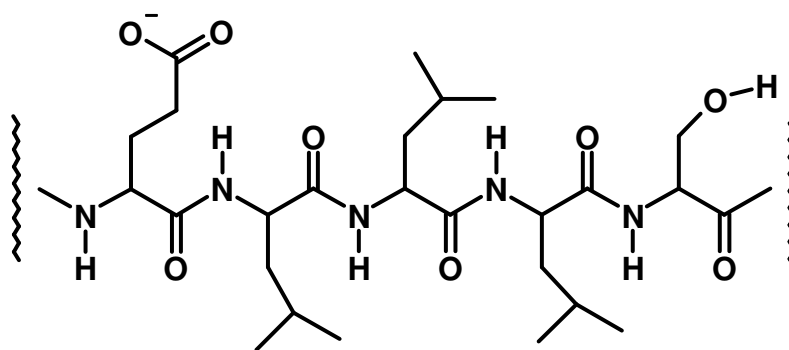


$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

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

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

5. (16 pts) The following diagram represents a short segment within a larger globular protein. This segment is found on the surface of the protein and contacts both the solvent (water) and participates in the core of the protein.



- i) Label *each* amino acid with its name, place the name above each residue. If you don't know a name(s), simply use A, B, etc. so that you can answer *part ii* (1 pt).
- ii) Write the primary structure of this segment below the diagram, using your names from *part i* (1 pt).
- iii) Circle the *sidechain* of an amino acid that would most likely be buried in the core of this protein (3 pts), *justify your answer in the space to the right*.
- iv) Put an "X" through **one** peptide bonds (1 pt).
- v) Label any **one** mainchain hydrogen bond acceptor with the letter "A" (1 pt).
- vi) Label any **one** sidechain hydrogen bond donor with "D-SC" (1 pt).
- vii) (8 pts) What is the most likely secondary structure of this segment? Justify your choice of secondary structure with reference to the above sequence (1 pt). If you cannot decide which secondary structure is appropriate, choose any one and proceed. Illustrate your answer with a sketch that captures the three-dimensional structure, the hydrogen bonding pattern, and location of sidechains for that secondary structure (7 pts).

6. (6 pts)

- i) List **two** key properties of the peptide bond.
- ii) Provide a molecular explanation for **one** of the two properties.

7. (16 pts) Please do **one** of the following choices:

Choice A: Describe how the *sidechain* of a *buried* amino acid contributes to the stability of the folded form of a protein. Your answer should discuss an interaction/force that is related to enthalpic changes (ΔH°) (8 pts) **AND** an interaction/force this is related to entropic changes (ΔS°) (8 pts)

Choice B: When a protein unfolds, there are changes in one enthalpic interaction involving *mainchain* atoms that favors the native state and changes in another entropic interaction that favors the unfolded state. State the nature/name of these interactions and provide a brief description of BOTH of them.

8. (4 pts) Please do one of the following choices.

Choice A: Briefly describe how you would obtain the enthalpy of either protein unfolding or ligand binding from experimental equilibrium constants.

Choice B: Distinguish quaternary structure from tertiary structure, give an example.

9. (6 pts) In the case of ligand binding, which of the two kinetic rate constants is more sensitive to interactions between the ligand and the protein – k_{ON} or k_{OFF} ? Briefly justify your answer.

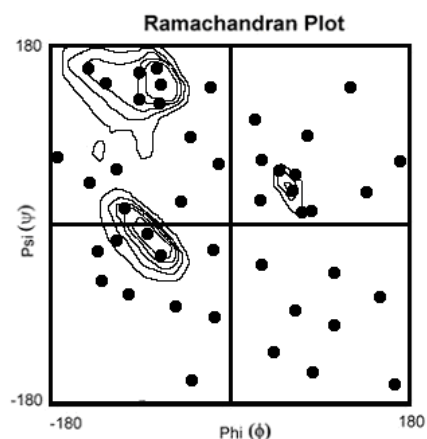
10. (8 pts) Please do **one** of the following two choices.

Choice A: The primary sequence of a 10 residue peptide is being determined using Edman degradation and cleavage. Note that only the sequence of the first **four** residues of a peptide are obtainable, regardless of its length. The following data were obtained:

- a) Sequencing of each peptide produced from Trypsin cleavage gave:
Ala-Cys-Met-Val Phe-Thr-Ser-Gly
- b) Sequencing of each peptide produced from Chymotrypsin cleavage gave:
Ala-Cys-Met-Val Thr-Ser-Gly-Met
- c) Sequencing of the intact peptide gave: Ala-Cys-Met-Val

Determine the peptide sequence; be sure to justify your answer.

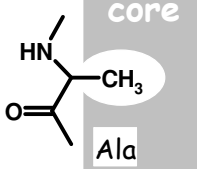
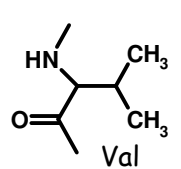
Choice B: The three dimensional structure of a new protein was recently determined. The Ramachandran plot on the right was generated from that structure. Given that this protein does not contain any glycine residues, do you believe that this structure is correct? Why or why not?



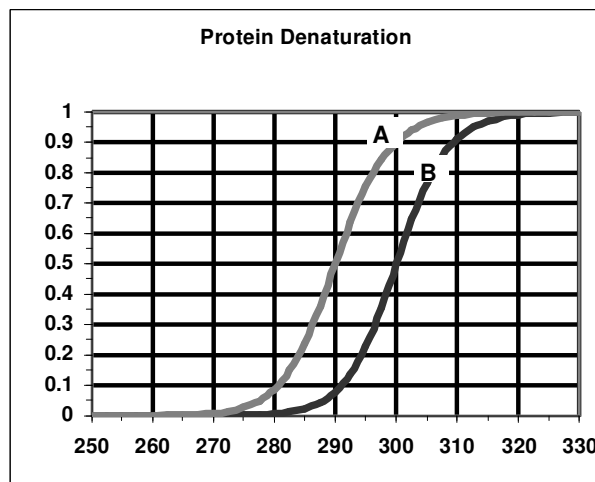
11. (14 pts) Please do **one** of the following questions. Please complete all parts of each choice.

Choice A:

The structure of a wild-type and mutant protein are shown on the right. The wild-type protein contains a buried alanine (Ala) residue while in the mutant protein this residue is replaced by a valine (Val). The thermal denaturation curves for both proteins are also given. The enthalpy and entropy of denaturation are also given.

 Wild-type Protein	 Valine substitution (mutant)
$\Delta H^\circ = +180,000 \text{ J/mol}$ $\Delta S^\circ = +600 \text{ J/mol-K}$	$\Delta H^\circ = +159,500 \text{ J/mol}$ $\Delta S^\circ = +550 \text{ J/mol-K}$

- Which denaturation curve, A or B, corresponds to the wild-type protein, justify your answer with a *quantitative* calculation (4 pts).
- Explain the difference in enthalpy for denaturation (180 kJ/mol versus 159.5 kJ/mol) for the two proteins (5 pts).
- Explain the difference in entropy for denaturation (600 J/mol-K versus 550 J/mol-K) for the two proteins (5 pts).



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

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

$$S = R \ln W$$

For the reaction: $N \rightleftharpoons U$:

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

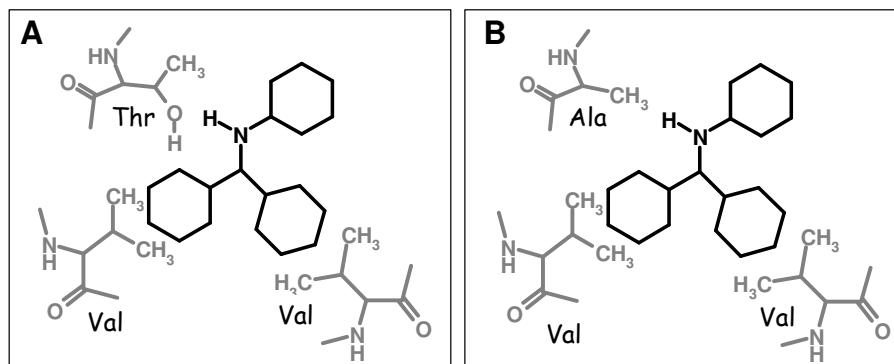
$$f_u = K_{eq}/(1+K_{eq})$$

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

Choice B: A 100 residue protein, which contains no glycine residues, unfolds with an enthalpy of +200 kJ/mol, and an entropy of +600 J/mol-K. Replacement of 10 residues with the amino acid glycine lowers the enthalpy change, ΔH° to +180 kJ/mol and increases the entropy change, ΔS° , to +800 J/mol-K. $RT = 2.494$ kJ/mol @ 300K.

- Provide one possible explanation for the reduction in enthalpy in the glycine containing protein (4 pts).
- Provide **two** possible explanations for the increase in entropy in the glycine containing protein (6 pts)
- Is the folded form of the mutant (glycine containing) protein more stable at 300 K? ($f_{\text{folded}} > 0.5$). (4pts).

Choice C: The following image shows the drug PCP bound to two different antibodies, A and B. The residues from the antibody are colored gray and the PCP is black. The binding sites differ in that a threonine (Thr) residue in A is replaced by an alanine (Ala) residue in B.



- Indicate on the diagram to the right the location of the binding site(s) of PCP (2 pts).
- Place a box around an antibody fragment that will still bind PCP and give the name of that fragment (2 pts).
- Which of the two antibodies, A or B, would be more likely to have a lower K_D , A or B? Why? (5 pts)
- Do you expect the two antibodies to differ mainly in their enthalpy (ΔH°) or entropy (ΔS°) of binding? Briefly justify your answer. (5 pts).

(Use the back of the preceding page to answer parts *iii* and *vi*).

