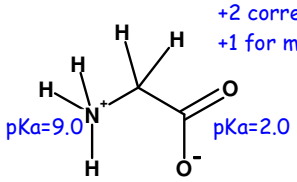
Biochemistry 03-232 Exam I – 2011 – KEY Name:_____

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.

+3 correct structure
+2 correct ionization state @ pH=7
+1 for mainchain pKa values



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 pKa values can be sensitive to environment, give an example.

Choice A:

When the pH is near the pKa there are appreciable quantities of HA and A-. (3 pts)

If base is added (NaOH) the weak acid deprotonates to neutralize the base, so the pH doesn't increase as much.

If acid is added (e.g. HCl) the weak acid protonates (A^- + H^+ to give HA) to neutralize the acid, so the pH doesn't increase much (3 pts, either example).

Choice B:

Since ionization changes the charge on the group, the presence of nearby charges can stabilize or destabilize either the protonated state or deprotonated state (4 pts)

For example, if a neutral group (COOH) ionizes it generates a negative charge (COO-). If there are other negative charges around, the deprotonated state will be more unfavorable than normal, and the acid will be less likely to deprotonate, it will become a weaker acid. (2 pts)

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.

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

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

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

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. 1pt 2pt

pH=2 pH=4 pH=6 pH=8 (pH=10)

The peptide needs to have a negative charge to bind to the bead (+ 2 pts).

The only negatively charged group is the mainchain carboxy, the amino terminus and the histidine are positively charged when protonated.

Therefore to generate a negative charge, both of these must be substantially deprotonated, which will occur at pH of 10. (+ 2 pts)

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?

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

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

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

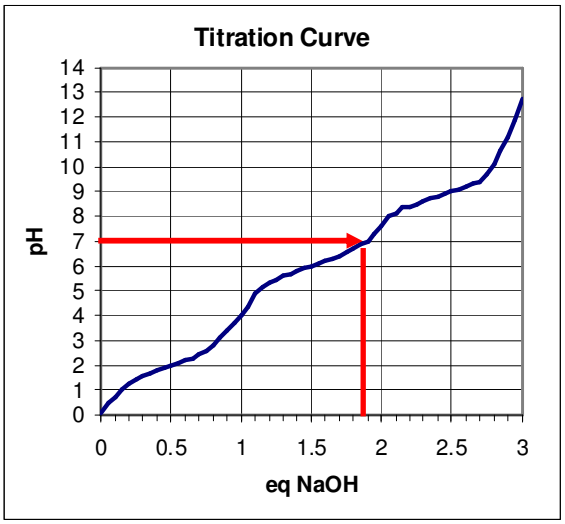
$$\varepsilon_{280}^{Phenylalanine} = 220 M^{-1}cm^{-1}$$

The extinction coefficient is the weighted sum for each group:

ε = 2x5000+4x220 = 10,880 (+4 pts)

A = 10,880 x 1 x 10^{-6} x 1 = 0.0108 (+2 pts)

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:

i) *sketch* a curve that would show the activity as a function of pH (8 pts).

ii) *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.

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

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

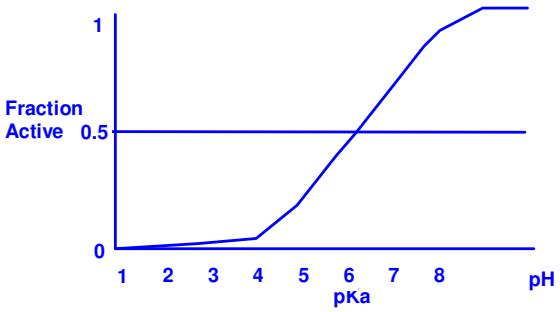
$$R = 10^{pH-pKa}$$

i) (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.*

ii) (4 pts) Show, with a numerical calculation, that your estimate in part i was close to the actual value.

Choice A:

i) Since the group must be deprotonated, the activity will be proportional to the fraction deprotonated. This will 0.09 at one pH unit below the pKa, 0.5 at the pKa, and 0.9 one pH unit above the pKa. The sketch is to the right (8 pts for correct plot, 5 pts if fraction protonated was plotted.) 1/2 way point should be at pH=6, the pKa.



ii) For any pH value, calculate R, then $f_{A^-}=1-f_{HA}=R/(1+R)$.

Choice B:

i) To reach a pH of 7.0 from the fully protonated form of the peptide you would need to add approximately 1.9 equivalents of base, as shown on the titration curve (8 pts).

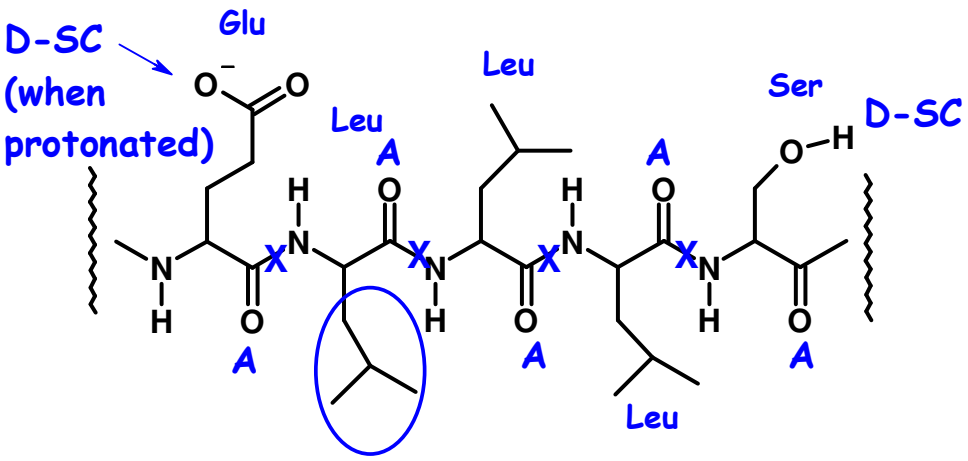
ii) To calculate the value, the desired pH=7, the pKa=6. (4 pts)

$R=10^{7-6}=10$, $f_{HA}=1/11=0.09$, $f_{A^-}=10/11=0.9$.

f_{A^-} is the number of equivalents of base to add from the 1st equivalence point to reach the desired pH, so the total number is 1+0.9.

Full credit if ii is the only answer provided (if correct of course).

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).

Glu-Leu-Leu-Leu-Ser (amino end first)

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*.

Non-polar groups comprise the core of proteins (2 pts for justification).

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).

Since this segment is on the surface one side must be polar and the other non-polar.

A sheet/strand would should alternating polar-nonpolar residues, so this is most likely a helix, which would show polar/non-polar every 3-4 residues. (1 pt)

Sketch should show (7 pts).

Helix – correct general shape.
Hydrogen bonds || to helix axis
Sidechains projecting out.

Sheet – correct extended shape
H-bonds perp to strand direction
Sidechains pointing up and down.

6. (6 pts)

i) List **two** key properties of the peptide bond.

ii) Provide a molecular explanation for **one** of the two properties.

Planer – due to partial double bond, or orbital overlap between p_z on carbon and nitrogen.
Trans – since this avoids an unfavorable vdw contact with the alpha hydrogens.

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^o) (8 pts) **AND** an interaction/force this is related to entropic changes (ΔS^o) (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.

Choice A:

Enthalpic – well packed core provides optimal van der Waals interactions.

Entropic – exposure of the buried non-polar group will order water molecules, decreasing the entropy of the system for the unfolded state, therefore stabilizing the folded state.

Choice B:

Enthalpic – the mainchain hydrogen bonds are more stable in secondary structure than to solvent, stabilizing the folded form.

Entropic – The unfolded form has a much higher entropy ($S=R\ln W$, $W=10^N$), making it more stable.

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.

Choice A: The slope of a plot of ln(Keq) versus 1/T gives the enthalphy $-\Delta H/R$.

Choice B; The quaternary structure describes the organization of multiple chains, the tertiary structure the complete three-dimensional structure of one chain.

Hb and immunoglobulins (antibodies) have four chains and therefore have a quaternary structure.

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.

The **off-rate**, since more interactions will make it less likely for the ligand to leave the binding site. (+6 pts)

The on-rate only depends on the frequency of collisions, which will be the same for all protein-ligand pairs.

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.

The amino terminal sequence is Ala-Cys-Met-Val from (c.

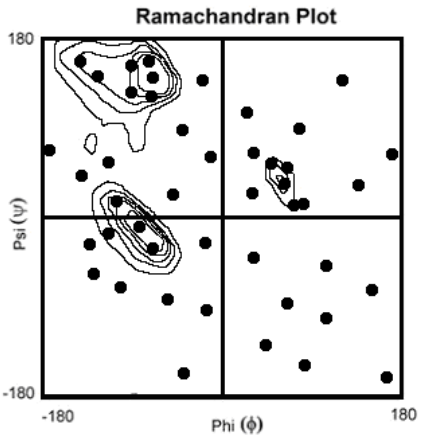
The second fragments overlap in sequence: Phe-Thr-Ser-Gly
Thr-Ser-Gly-Met
Giving Phe-Thr-Ser-Gly-Met

One residue is missing, so the possible choices are:

Ala-Cys-Met-Val-X-Phe-Thr-Ser-Gly-Met
Ala-Cys-Met-Val-Phe-Thr-Ser-Gly-Met-X

The second possibility is not consistent with the Trypsin data, X is either Lys or Arg. Final solution is:
Ala-Cys-Met-Val-X- Phe-Thr-Ser-Gly-Met
X is either Lys or Arg (not necessary to state)

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?

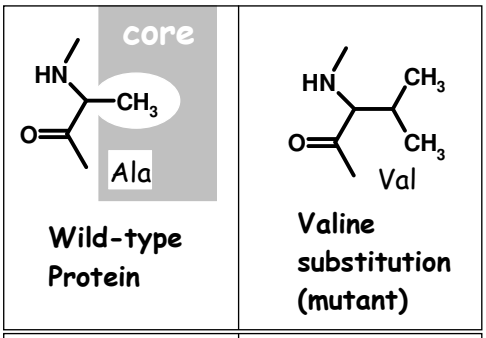


No, it is not correct. Non-glycine residues should fall within the contour lines. There are many residues with phi and psi angles that are in the high energy area.

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.

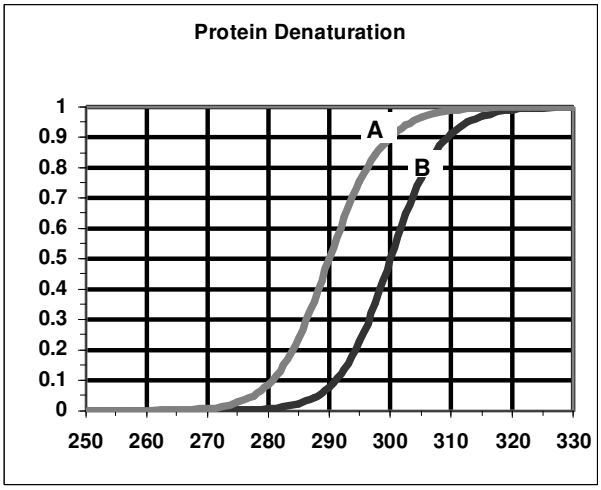


ΔH^o= +180,000 J/mol ΔS^o= + 600 J/mol-K	ΔH^o= + 159,500 J/mol ΔS^o= + 550 J/mol-k

i) Which denaturation curve, A or B, corresponds to the wild-type protein, justify your answer with a *quantitative* calculation (4 pts).

ii) Explain the difference in enthalpy for denaturation (180 kJ/mol versus 159.5 kJ/mol) for the two proteins (5 pts).

iii) Explain the difference in entropy for denaturation (600 J/mol-K versus 550 J/mol-K) for the two proteins (5 pts).



i) Calculating Tm = $\Delta H^o / \Delta S^o$. For the wild-type protein. Tm=300 K, corresponding to curve B. (+2 for correct answer, +2 for just).

ii) The decrease in enthalpy indicates the mutant has weaker vdw interactions (+2 pts).

This is due to the fact that the larger Valine residue doesn't fit when it replaces the Alanine, causing some sort of disruption in packing (+3 pts)

Alternatively, if you assumed the Val wasn't buried anymore, then the decrease in enthalpy is due to loss of direct vdw contacts with the Val.

iii) The difference in entropy is due to the hydrophobic effect (+3 pts).

The larger non-polar valine orders more water, decreasing the overall entropy change more than the alanine (+2 pts). Note that this explanation would not apply if the Val was exposed in both the folded and unfolded state.

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

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

$S = R\ln W$

For the reaction: N ⇔ 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^o to +180 kJ/mol and increases the entropy change, ΔS^o, to +800 J/mol-K. RT = 2.494 kJ/mol @ 300K.

i) Provide one possible explanation for the reduction in enthalpy in the glycine containing protein (4 pts).

ii) Provide **two** possible explanations for the increase in entropy in the glycine containing protein (6 pts)

iii) Is the folded form of the mutant (glycine containing) protein more stable at 300 K? ($f_{folded} > 0.5$). (4pts).

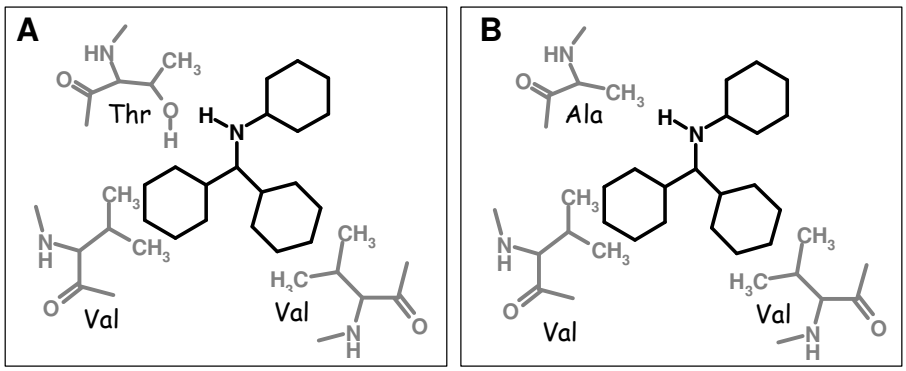
i) Must be vdw effects since glycine can still form H-bonds. The smaller sidechain on glycine has reduced vdw contacts with other core residues.

ii) The glycine isn't non-polar, so it won't order as many water molecules when the protein unfolds, so the overall entropy is larger since the hydrophobic effect does not reduce the change in conformational entropy (+5 pts)

Alternatively, glycine will show a larger increase in conformational entropy when the protein unfolds since glycine can assume more conformations of its mainchain atorms (+1 pt).

iii) Calculate Tm = 180000/800 = 225 K. Since the Tm is lower than 300K, more than ½ of the protein will be unfolded at 300.

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.



i) Indicate on the diagram to the right the location of the binding site(s) of PCP (2 pts).

ii) Place a box around an antibody fragment that will still bind PCP and give the name of that fragment (2 pts).(Either Fv or Fab OK)

iii) Which of the two antibodies, A or B, would be more likely to have a lower K_D, A or B? Why? (5 pts).

A shows more interactions with the PCP (H-bond from Thr, which is lost in B), therefore it will show a smaller k_{off} and a smaller K_D.

iv) Do you expect the two antibodies to differ mainly in their enthalpy (ΔH^o) or entropy (ΔS^o) of binding? Briefly justify your answer. (5 pts). Enthalpy, since the difference between A and B is loss of a hydrogen bond.