

Part A: Multiple Choice

1. c: is planar and usually found in a trans conformation. 1 pt was given for b)
2. a: Buffers are considered to be effective within one pH unit of their pKa.
3. a: Only the complete secondary structure is known. The locations of the side-chain atoms do not involve the phi and psi angles.
4. d: Is the best answer since 2 kJ/mol is a small amount of energy. 1 pt was given for b)
5. c: This is the correct definition of ΔG° .
6. b: This is the best answer, since occasionally charged residues can be found in the interior. 2 pts were given for a).
7. a: Conformational entropy decreases during folding, clearly unfavorable. The remaining three answers are generally favorable for protein folding.
8. a: Variable regions is the better answer since regions outside the hypervariable loops can also be involved in binding. However, full credit was given for c).

Part B: Short Answer

B1: i) The alpha carbon is the carbon directly attached to the amide nitrogen.

ii) Replacement of the γ carbon with an OH yields Ser. Addition of an OH to the β carbon yields Thr. These are the best answers. Full credit was given for Tyr, although this was not the simplest choice. Asn and Gln were given 2 pts.

iii) 2 points was given for drawing a water molecule in the correct location, 1 point was given for the donor/acceptor label.

B2: i) Six (6) equivalents of base are required to fully titrate the protein. Since the carboxy-terminus accounts for the pKa=2 and the amino terminus accounts for the pKa=10, the remaining inflection points must arise from the sidechains. The pKa of these groups is 4.0. Therefore Asp is the correct amino acid. The pKa of His(6) and Lys(11) are not appropriate.

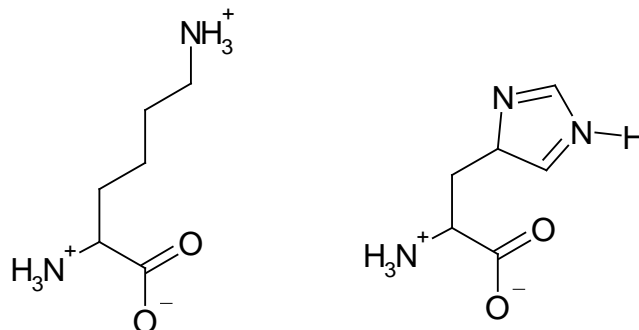
ii) Since two equivalents are required for the carboxy and amino terminus, there must be four (4) Asp residues in the protein ($6-2=4$, most days anyhow)

iii) The only “tricky” part of this question arises from the fact that there are FOUR ionizable side chains. The following table was the best answer, but qualitative descriptions were also accepted. You must have indicated that the pI is the pH where the net charge is zero.

| pH | Charge on COOH, pKa=2.0 | Charge on amino terminus (NH ₂). pKa=10 | Charge on all <u>four</u> Asp sidechains | Net Charge |
|----|-------------------------|---|--|------------|
| 0 | 0 | +1 | 0 | +1 |
| 2 | -0.5 | +1 | 0 | +0.5 |
| 4 | -1.0 | +1 | -2 (4 x -0.5) | -2.0 |

Therefore, the **pI is between 2 and 4.**

B3: i) The pKa values of Lys (left) is about 10, that for Histidine (right) is about 6.0 2 pts were given for the correct ionization state of the amino and carboxy groups, 1 pt for the correct pKa, and 2 points for the correct charge/protonation on the sidechain.



ii) This should have looked very similar to the extended chain in the notes. 2 pts were given for the overall structure; you had to give some indication of the right geometry for an extended chain. 2 pts were given for a trans peptide bond.

iii) Lys-His or His-Lys were accepted.

B4a: There are two major entropic changes:

1. A reduction in conformational entropy as the phi and psi angles go from 3x3 possible conformations/residue to 1 in the folded protein.
2. A increase in the entropy of the water (hydrophobic effect) as water that is bound to exposed non-polar groups is released as the non-polar groups form the hydrophobic core.

If you provided sufficient detail for one of these you got 8 points. You needed to provide both to get full points.

B4b: An environment that stabilizes the protonated form will raise the pKa while an environment that stabilizes the deprotonated form will lower the pKa. You should have been given 4 points for the general concept and 2 more points if you discussed how both protonated and deprotonated states were stabilized. Four additional points were given for a correct example: e.g. The pKa of glutamic acid will increase if it is in a negatively charged environment and it will decrease if it is in a positively charged environment. In this case a favorable electrostatic (unlike charges) lowers the pKa.

B4c: Four of the 'forces' are the same: Hydrophobic, van der Waals, H-bonding and electrostatic interactions. Conformational entropy is much more important in protein folding than ligand binding.

B5:

| | |
|--|---|
| Primary Sequence: linear sequence of amino acids | e.g. hypervariable regions of heavy and light chains |
| Secondary Structure: conformation of backbone (α -helix, β -sheet) | e.g. β -sheets/ β -strands in immunoglobulin fold |
| Tertiary Structure: three dimensional arrangement of mainchain and sidechain | Immunoglobulin fold |
| Quaternary: Structure/arrangement of multiple chains | Y-shaped molecule. Heavy and light chains held together by disulfide bonds. |

B6:

- i) The secondary structure is β -strand.
- ii) Surface, since there are polar residues on one side of the strand and non-polar residues on the other side of the strand.
- iii) The third residue is Histidine, any reasonable diagram showing a water hydrogen bonded to the amide proton or the C=O was fine.
- iv) $\beta\beta$, $\alpha\beta\alpha$, β -meander, greek key, β -barrel.
- v)

A,B: A hydrophobic amino acid

G: A ψ angle (F was given 1/4 pt)

D: The carboxy-terminus

A: An amino acid that strongly absorbs UV light

E: A peptide bond

F: A ϕ angle (G was given 1/4 pt)

C: The amino terminus.

C1:

i) Use acetic acid since its pKa is closest to the desired pH of the buffer.

$$\text{pH} = \text{pKa} + \log [A^-]/[\text{HA}]$$

$$4 = 5 + \log R$$

$$-1 = \log R$$

$$R = 0.1$$

$$[\text{HA}] = A_T (1/(1+R)) = 0.1 (1/(1+0.1)) = 0.09 \text{ moles}$$

$$[A^-] = A_T (R/(1+R)) = 0.1 (0.1/1+0.1) = .01 \text{ moles}$$

ii) Calculate the extinction coefficient for the entire protein:

$$\epsilon = 2 \times 5000 + 1000 = 11,000 \text{ M}^{-1}\text{cm}^{-1}$$

$$C = A/\epsilon l = 2.0/11,000 = 1.8 \times 10^{-4} \text{ moles/l} = 182 \mu\text{M} = 0.18 \text{ mM}$$

iii) At pH 6.0, since this is one pH unit below the pKa of the side chain 90% of the sidechain will be protonated. Therefore 10% will be deprotonated. Consequently 10% of the enzyme is active.

iv) There are two possibilities from the Trypsin digest:

a) Ala-Gly-Met-Thr-Lys-X₁-X₂-X₃

or

b) X₁-X₂-X₃-Ala-Gly-Met-Thr-Lys

where X₁-X₂-X₃ is unknown

X₁-X₂-X₃ must be either Leu-Leu-X₃, or X₁-Leu-Leu since neither sequence above has a cleavage site for chymotrypsin (cleaves after Phe, Tyr, Trp). Either X₁ or X₃ is Phe, Tyr, Trp. Regardless, the second choice (b) above can't produce a 5 residue Trypsin fragment because X₃ is either Phe, Tyr, Trp, or Leu. Therefore choice (a) is the only possibility. Therefore the answer is:

Ala-Gly-Met-Thr-Lys-X₁-Leu-Leu, with X₁ being either Phe, Tyr, or Trp.

C2: i) There are three ways to approach this problem:

a) Calculate $T_M = \Delta H^\circ / \Delta S^\circ = (360 \times 10^3 \text{ J/mol}) / 1000 \text{ J/mol-K} = 360 \text{ K}$. At T_m the protein is only 1/2 denatured. Since the T_m is equal to the highest allowed temperature, the protein will not be 99% denatured at 360K.

b) Calculate K_{eq} at 360 and then F_u :

$$\Delta G^\circ = -RT \ln K_{eq} = \Delta H^\circ - T\Delta S^\circ = 360 \text{ kJ/mol} - 360 (1000 \text{ J/mol-K}) = 0$$

Therefore K_{eq} must be 1 ($\ln 1 = 0$), therefore $F_u = K_{eq} / (1 + K_{eq}) = 1/2$. Therefore it is only unfolded by 50% at 360 K.

c) Determine the temperature at which it is 99% unfolded:

$$F_u = 0.99, F_n = .01 \rightarrow K_{eq} = F_u / F_n = 99.$$

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

ΔH° , ΔS° and K_{eq} are known, so you can solve for T, giving the temperature where 99% is unfolded.

$$T = \frac{\Delta H^\circ}{(\Delta S^\circ - R \ln K_{eq})} = \frac{360,000}{1000 - 8.3 \ln 99} = 374 \text{ K}$$

This is the temperature at which 99% is denatured. This is 14 degrees above the high limit. Therefore it can't be done which simply raising the temperature.

ii) You could try changing the pH (higher or lower) or adding a safe organic solvent, such as ethanol!