

Part A:

- 1: C The most common property is hydrogen bond donation/acceptance (1 pt given for a or b)
- 2: D Any pK_a will be a buffer region since there is an inflection point at that pH. (1 pt given for b)
- 3: C Glu is acidic and Ile is nonpolar.
- 4: C
- 5: D is the best answer, 1 pt was given for *b* as neighboring could imply neighbors across the sheet.
- 6: B Only the secondary, tertiary and quaternary structure will be destroyed by unfolding.
- 7: B If the temperature is below the T_m , then less than half of the protein must be denatured (1 pt for d)
- 8: B Hydrophobic interactions cause a change of entropy of the water.

Part B:**B1**

i) 3 pts for correct backbone structure. 1.5 points for each amino acid. Grading should have been liberal.

- Acceptable polar: Asn, Gln, Ser, Thr, Cys. **Gly** was often given, but this is a poor choice compared to the others, Glycine would be weakly polar at best.
- Acceptable non-polar: Tyr, Trp, Phe, Ile, Leu, Val, Ala.

ii) Hydrogen bonding to sidechain: 2 pts for a suitable diagram with reasonable bond vectors/angles, 1/2 pt for donor/acceptor, 1/2 pt for distance.

iii) Self explanatory.

B2:

- i) Hydrogen bonds: || to helical axis in α -helix, perpendicular to strand direction in β -sheet
- ii) Side chains: Point on the *outside* of the α -helix, point up and down in the β -sheet
- iii) Length: 12 residues is about three turns of a helix - 15 Å. To estimate the length of a β -sheet, it is 3 bonds /amino acid (N-Ca-C-peptide), or about 4.0 Å/residue = ~20Å.

β -sheet: 1/2 pt removed if no directionality was given, -1/2 if drawn with no apparent explanations.

B3:

i) Both Tyrosine and Tryptophan absorb most strongly at 280 nm (1pt)

ii) The 'official' answer is 4.0, but if you said 3 or 5, you should have gotten a point. Please see me if you did not. (1 pt).

iii) The extinction coefficient of the protein is calculated from the composition:

$$\epsilon = 4(5,050) + 4(1,400) = 25,800 \text{ M}^{-1}\text{cm}^{-1}$$

(-1/2 pt if you forgot the units, -1/2 if you thought Asp absorbed)

iv) The correct formula to use is $A = \epsilon[C]l$. Since you were not explicitly given l , you should assume that it is 1 cm. Therefore the concentration is just:

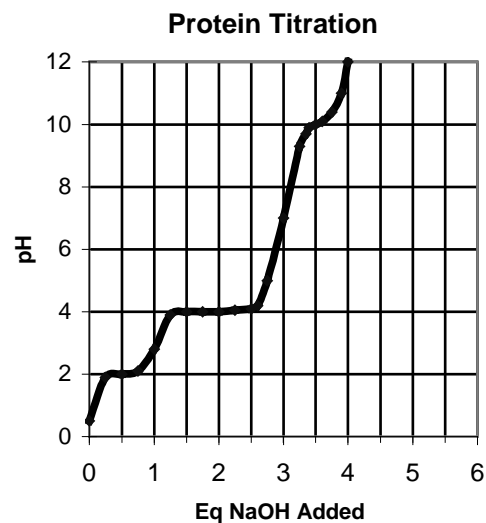
$$[C] = A/(\epsilon l) = 1.0 / 25,800 = 3.88 \times 10^{-5} \text{ M.}$$

v) There are a total of **four** titratable groups, the carboxy terminus ($pK_a=2.0$) the two sidechains on the Asp residues ($pK_a=4.0$) and the amino terminus ($pK_a=10.0$). You were told to ignore Tyrosine ionization, but if included it would add four additional titratable groups with a pK_a of 10). Tryptophan does **NOT ionize** over any pH range that you could generate with NaOH; its pK_a would be about 30, similar to an alcohol (-1 pt).

The correct titration curve is shown to the right. You should have labeled the numerical axis from 0 to 4 (1 pt), and include inflection points at the proper locations (3 pts).

0.5 equivalent at pH 2.0, 1.5 equivalents at pH 4.0,
2.5 equivalents at pH 4.0, 3.5 equivalents at pH 10.0,

and carried the titration to the final equivalence point of four equivalents (1 pt)



- vi) There are four titratable groups on this protein, the amino and carboxy termini and the two Asp side chains (again ignoring Tyrosine). Therefore you will need four moles of NaOH for *each* mole of protein because you are removing a total of four protons. Therefore you would need to have added :

$$4 \times 3.88 \times 10^{-5} \text{ moles} = 1.55 \times 10^{-4} \text{ moles of NaOH.}$$

There is no need to use the Henderson-Hasselbach equation here, since you are removing *all* of the protons from the protein, not stopping at a defined pH.

B4:

Van der Waals:

- Responsible for a well packed central core of globular proteins.
- Enthalpic
- You needed to say something along the following lines: Attractive force between all atoms, caused by an induced dipole as the electron clouds of atoms approach each other.

Hydrophobic

- Cause non-polar groups to be in the core of the protein.
- Entropic
- You needed to say something along the following lines: When a non-polar group is exposed to water it orders the water molecules around it in an ice like shell, decreasing the entropy of the water. When the non-polar group is brought into the non-polar core, this water is released, increasing the entropy of the system.

B5:

- a) In a β -sheet the hydrogen bonds on the edge of the sheet have to be to water. In contrast the β -barrel will allow those hydrogen bonds to be formed between strands, lowering their energy by 1-2 kJ/mol per h-bond. In addition you could have said that the β -barrel allows the formation of a hydrophobic core. (3 pts for description, 3 pts for correct discussion of stability)
- b) There were many possibilities here. In general β -strands will be held together with hydrogen bonds. Any other interactions, such as α -helix pairing (i.e. $\alpha\alpha$ structure) or $\beta\alpha\beta$ could involve any of the following between the secondary structural elements: sidechain-sidechain hydrogen bonds, van der waals, hydrophobic effect, electrostatics (list sound familiar now)? 3pts for correct drawing, 3 pts for energetics.
- c) If the protonated form of the amino acid is stabilized then it becomes a weaker acid, with a higher pKa. If the deprotonated form of the amino acid is stabilized then it becomes a stronger acids, with a lower pKa. Here are some specific examples:
- Histidine is positively charged when protonated, therefore it will be a stronger acid in a positively charged environment (stabilizing the deprotonated or neutral form). In a negatively charged environment, there will be a favorable electrostatic interaction between the charged His and its environment, causing it to become a weaker acid because it would prefer to keep its proton.
 - With Aspartic acid or Glutamic acid the protonated form is uncharged. Therefore the deprotonated state would be favored with a positively charged environment making it a stronger acid. In contrast a negatively charged environment would tend to make the sidechain keep its proton, becoming a weaker acid.

B6:

- i) A Y-shaped molecule was sufficient to answer the question. You needed to identify the two chains and indicate 'most' of the disulfide bonds between the heavy and light chains as well as between the two heavy chains.
- ii) Cysteine.
- iii) The bind to toxins (2 pts) with high specificity (1 pt), preventing the toxin from interacting with anything else.

B7: There were two ways to answer this question.

Choice a)

A) Assuming that there was *insufficient* information:

- i) Although you can tell which of the three fragments come first, the order of the latter two is unknown.
- ii) If you digested with Trypsin (cleaving after the Lysine) or with Met (cleaving after the Met) you would generate overlapping fragments that could then be used to assemble the sequence. You needed to supply some information about how this would help determine the sequence in order to get credit, it was insufficient to simply state a cleavage reagent.
- iii) Trp, Tyr, Phe

B) Correctly assuming that there is sufficient information.

- i) Two sequences are possible, depending on the order of the last two fragments:

Possibility a: Ala-Gly-Leu-Phe-**Lys-Cys-Ser**-Arg-Cys-Glu-Met-Tyr

Possibility b: Ala-Gly-Leu-Phe-Arg-Cys-Glu-Met-Tyr-**Lys-Cys-Ser**

Cleavage of *possibility a* with chymotrypsin will **not** generate the observed fragments, therefore *possibility b* is the correct sequence.

- ii) It was not necessary to answer this for full credit.
- iii) Trp, Tyr, Phe

Choice b)

Since the pH of the buffer is equal to the pKa of the acid, the ratio of [HA] to [A⁻] is 1:1. This is from the $\text{pH} = \text{pKa} + \log(A^-/\text{HA})$, $0 = \log(A^-/\text{HA})$, $1 = A^-/\text{HA}$. Therefore the solution would have to have a concentration of [HA]=0.025 M and a concentration of [A⁻] =0.025 such that the total concentration of buffer is 0.05.

Since you cannot purchase the acid [HA] in pure form, the only way to make this buffer is to begin with 0.05 moles of the basic form [A⁻] and add 0.025 moles of HCl, which will convert exactly 0.025 moles of [A⁻] to [HA] giving the desired ratio. You lost 1 pt if you neglected to do this last step.

C1:

$$i) \Delta S^\circ = S_U - S_N$$

$$S = R \ln W$$

$$S_N = R \ln 1 = 0$$

$$S_U = R \ln 9^{60} = (8.3)(60) \ln 9 = 8.3(60)(2.2) = +1095.6 \text{ j/mol-deg}$$

You are assuming that there are two rotatable bonds (N-C α , C α -C) that can have three different conformations. These conformations are independent of each other, therefore giving 9 possibilities/residue.

- ii) The contribution of the above entropy to the free energy difference between the native and the unfolded state is: $\Delta G^\circ = -T\Delta S^\circ = -(300)(1095.6) = -328.7 \text{ kJ/mol}$. Note that the negative sign tells you that the unfolded state is more stable based solely on the entropy of the disordered chain.

Since the folded form is 10 kJ/mol *more* stable than the unfolded form at 300 K, the contribution of van der waals, h-bonding, and the hydrophobic effect to the free energy of the native state is 338.7 kJ/mol. As for the sign of this term:

N \rightarrow U $\Delta G^\circ = +338.7 (G_U - G_N)$, the unfolded state would be higher in energy.

U \rightarrow N $\Delta G^\circ = -338.7 (G_N - G_U)$, the native state is lower in energy because of vdw, hbond, and the hydrophobic effect.

- iii) This introduces a third rotational bond in the peptide backbone, therefore the entropy of the unfolded state increases considerably:

$$S = R \ln W = R \ln (3 \times 3 \times 3)^{60} = R \ln (27)^{60}$$

$$(8.3)(60)(3.3) = 1641 \text{ j/mol-deg.}$$

The free energy associated with this entropy is, at 300k, +492 kJ/mol, far exceeds the stabilizing energy of 338 kJ/mol from van der waals, hydrogen bonds, and hydrophobic effects, therefore the protein unfolds.

C2:

i) At T_M , $\Delta G^0=0$. Therefore $\Delta H^0=T_M\Delta S^0$, or $T_M = (300,000 \text{ j/mol})/(1000 \text{ j/mol-deg}) = 300 \text{ K}$.

ii) If $T_M=292$, and $\Delta S^0=1000 \text{ j/mol-deg}$, then $\Delta H^0 = 292 \text{ deg} * 1000 \text{ j/mol-deg} = 292 \text{ kJ/mol}$.

iii) The temperature is the melting temperature of the *wild-type* protein, or 300 K. You lost a point if you took the temperature to be 292K (which made the following calculations quite easy since $K_{EQ}=1$).

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = 292 - (300)(1000) = -8 \text{ kJ/mol (2 pts)}$$

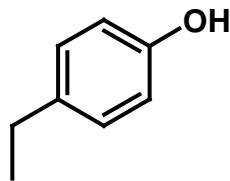
$$\Delta G^0 = -RT \ln K_{eq}$$

$$K_{EQ} = e^{(-\Delta G/RT)}$$

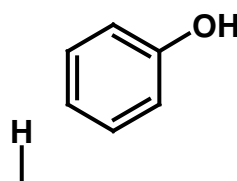
$$K_{EQ} = e^{8/2.5} = e^{3.2} = 24.5 \text{ (1 pt)}$$

$$F_U = 24.5/(1+24.5) = 0.96 \text{ or } 96\%. \text{ Most of the mutant protein is unfolded at 300K. (1 pt)}$$

iv) Since the phenol only affects the melting temperature of the mutant protein, and not the wild-type, it cannot have a *general* effect on protein stability here, but rather an effect that is due to the specific interaction between phenol and the mutant protein. Since the mutant has replaced a Tyrosine with a glycine, it has potentially lost van der waals interactions and hydrogen bond interactions between the original Tyr sidechain and the rest of the protein, accounting for the 8 kJ/mol difference in enthalpy. However, if phenol binds to the Glycine mutant in the cavity that was created with the Tyrosine sidechain was removed, these interactions can be restored, raising the melting temperature.



Wild-Type



Mutant (Gly) with bound Phenol