This exam contains **100 pts** in **12 questions** on **5 pages**. Please use the space provided. **Allot 1 min/2pts**.

1. (8 pts) A titration curve for a dipeptide is shown to the right. In this dipeptide the termini have been chemically modified and can no longer ionize; only the sidechain groups can ionize.

i) (1 pt) The pK_a value for the first ionization is 3.8, what is the pK_a for the second ionization?

pK$_{a2}$ = 6.2
(inflection point at 1.5 equivalents).
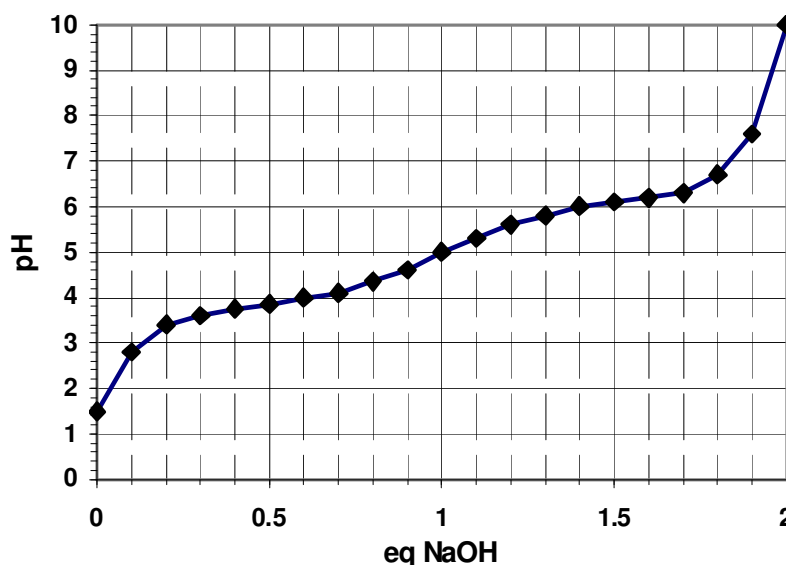
ii) (2 pts) What ionizable amino acids are contained in this peptide? [Hint, read choice B of the next question.]
Write your answers here →

Aspartic acid or Glutamic Acid	Histidine

iii) (5 pts) *Briefly* explain why the pH doesn't change very much around pH 4 or pH 6, as base is added.

In these regions the added base extracts a proton from the weak acid, not the hydronium ions (H⁺) in the water. Since the hydronium ion concentration does doesn't change much, the pH doesn't since pH = -log H⁺.

2. (12 pts) Please do **_one_** of the following two choices. [Hint: The equations to the right are not really required to do this problem.]

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$
$$f_{HA} = \frac{1}{1+R}$$
$$R = 10^{pH-pKa}$$

Choice A: Describe how you would make 2 liters of a 0.1 M buffer solution at pH 4.0 using the dipeptide discussed in the previous question. Assume that the only source of the weak acid is the fully protonated form, H_2A (i.e. you are starting at the left of the titration curve. Remember that the termini are modified and do not ionize. Show your work.

Choice B: Determine the charge at pH 3.8 for the peptide discussed in the previous question. You may assume that the more acidic residue has a neutral charge when protonated, and the other a positive charge when protonated. Remember that the termini are modified and do not ionize.

Choice A:
To reach a pH of 4.0 you would need to add 0.6 equivalents of NaOH, based on the titration curve. If you calculated this number: R = 10$^{4-3.8}$ = 1.584. f$_{AH}$ = 1/(1 + 1.584) = 0.387, f$_{A-}$ = 0.61.

The amount of NaOH required is 0.6 x 0.1 M (eq x [A$_T$]) = 0.06 moles/L of NaOH. Since 2L is required, 0.12 moles of NaOH would be required.

Choice B:
The group with a pKa = 3.8 is 1/2 ionized at that pH. Therefore its charge is -1/2:
q$_1$=f$_{HA}$ q$_{HA}$ + f$_{A-}$ q$_{A-}$ = 0.5 x (0) + 0.5 x (-1)

The group with a pKa =6.2 will essential completely protonated at this pH, so its charge is +1 (1 pt extra credit if you actually calculated its ionization state.
The overall charge is +1/2.

3. (8 pts) The diagram to the right shows a compound bound to a protein. The grey area represents the protein.
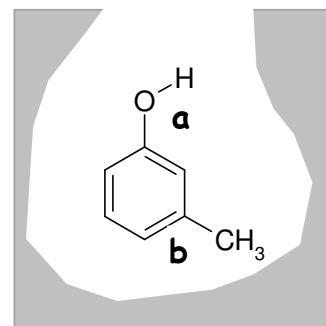
i) (4 pts) Based on the structure of the bound compound, what forces are likely to be responsible for its binding to the protein? Clearly label the functional groups on the compound (e.g. "a", "b", etc.) and *briefly* discuss how they could interact with the protein in the space below:

a) The -OH group donate a hydrogen bond to the protein as well as accept a hydrogen bond from the protein (+3 pts)

b) The non-polar atoms (methyl and benzyl) would likely be adjacent to other non-polar atoms, providing stability by van der Waals as well as the hydrophobic interaction (+1 for either van der waals or hydrophobic interactions.)

ii) (4 pts) If one of the interactions that you identified in *part i* was removed, how would the dissociation constant (K_D) change? Would it increase, decrease, or stay the same? Why?

The K_D would get larger as there are fewer interactions (4 pts). Largely due to an increase in the off-rate ($K_D = k_{off}/k_{on}$) .

4. (7 pts) Please do ***one*** of the following two choices.

Choice A: A 10 residue peptide is being sequenced. The partial amino terminal sequence is: Ala-Gly-Met-Phe The peptide is treated with a cleavage reagent and the resultant peptides are separated and their individual sequences are:

   Peptide A: Phe-Leu-Lys-Met   Peptide B: Asp-Ala-Met   Peptide C: Ala-Gly-Met

i) (5 pts) Determine the sequence of the peptide and write the sequence in the space below. Briefly describe how you arrived at your answer.

      Ala-Gly-Met-Phe-Leu-Lys-Met-Asp-Ala-Met
        1   2   3   4   5   6   7   8   9 10

Peptide C is the amino-terminal peptide, since its sequence is the same as the partial amino terminal sequence. This leaves peptide A or B to follow. Peptide A begins with Phe, so it must follow, leaving peptide B to be at the end:

Ala-Gly-Met-Phe
          Phe-Leu-Lys-Met
                  Asp-Ala-Met
Ala-Gly-Met-Phe-Leu-Lys-Met-Asp-Ala-Met

ii) (2 pts) Circle the name of the reagent that was used to cleave the peptide: ***Trypsin   Chymotrypsin   CNBr***

Choice B: A solution of a protein with 5 tyrosine residues and one tryptophan residue has an absorbance of 0.5 at 280 nm.

i) What is the concentration of the protein in solution (4 pts)?
ii) What assumption did you make to solve this problem (3 pts)?

$$A = C\varepsilon l$$
$$\varepsilon_{280}^{Tryptophan} = 5,000 M^{-1} cm^{-1}$$
$$\varepsilon_{280}^{Tyrosine} = 1,000 M^{-1} cm^{-1}$$

i) The overall extinction coefficient = 5 × 1000 + 5000 = 10,000.
     C = 0.5/(10,000 × 1) = 0.00005 M = 60 uM.

ii) You assumed that the individual amino acids in the protein absorb light independently, as if they were free amino acids. Alternative, you assumed that the maximum wavelength for absorption was 280 nm. Since the path length was given during the exam, this was not a valid assumption.

5. (8 pts) Sketch one regular secondary structure in the space to the right. Your drawing should provide:

i) A representation of the mainchain shape. (2 pts)	Helical like structure.	At least one extended linear strand.
ii) An indication of the location of hydrogen bonds (2 pts)	H-bonds || to helix axis	H-bonds perpendicular to the direction of the strand
iii) An indication of the location of the sidechains (2 pts)	Projecting outward from cylinder	Sidechains alternating up and down.
iv) Name (2 pts)	α -helix	β-sheet/strand

6. (12 pts)
  i) Draw a dipeptide using any two different amino acids, *except for* phenylalanine and valine (6 pts)
  ii) Provide the sequence for your peptide (e.g. Phe-Val) (2 pts)
  iii) Identify the peptide bond (2 pts)
  iv) Identify **all** bonds corresponding to the phi (φ) and psi (ψ) torsional angles (2 pts)
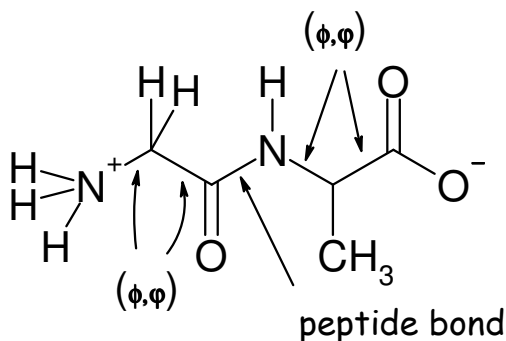
Above example is Gly-Ala

7. (8 pts) Please do **_one_** of the following two choices:
  Choice A: Describe/draw the most common conformation of the peptide bond and explain why this conformation is low in energy (You may refer to the above drawing, question 7.)
  Choice B: Briefly describe a Ramachandran plot and explain why there are three regions of low energy for most amino acids.

Choice A: trans and planer, as drawn above. Trans since it minimizes unfavorable van der waals. Planer due to orbital overlap (or partial double bond)

Choice B: A two dimensional plot of phi and psi angles for each residue (A sketch is fine).
  Residues with sidechains can only assume three low energy values due to unfavorable van der Waals contacts between sidechain atoms.

8. (8 pts) Please answer **_one_** of the following three choices. Be sure to indicate your choice.

Choice A: Briefly describe the major thermodynamic factor that *destabilizes* the native (folded) state of a protein. Use an equation if appropriate.

Choice B: Explain what thermodynamic factor(s) are responsible for the fact that most proteins have well packed cores.

Choice C: The energy to break a hydrogen bond is approximately 20 kJ/mol, yet hydrogen bonds have a relatively minor role in stabilizing folded proteins, on the order of 1-5 kJ/mol. Why is this so?

Choice A: When the protein unfolds the chain will have many different conformations, W, giving rise to a large and favorable entropy for the unfolded state. (S = R ln W).

Choice B: The tight packing optimizes van der Waals interactions. Hydrophobic interactions dictate what types of residues are found in the core, not the packing.

Choice C: The hydrogen bonding group in the protein is hydrogen bonded to water in the unfolded state. Although breaking this during the folding process requires 20 kJ/mol, it is reformed in secondary structure releasing 21-25 kJ/mol, so the net difference is 1-5 kJ/mol.

9. (8 pts) Describe the hydrophobic effect in molecular terms and explain why it stabilizes the tertiary structure of proteins but not isolated secondary structures.

The hydrophobic effect is the reduction in the entropy of water around exposed non-polar groups (+6 pts).

The solvent exposure of non-polar sidechains does not change when a helix or a sheet form, so there is no change in the entropy of the solvent (+1 pt)

When a protein folds into a tertiary structure, a number of non-polar groups become buried, changing the exposure of non-polar groups (+1 pt)

10. (3 pts) A simple drawing that represents the structure of an entire antibody molecule is shown on the right. Indicate on this diagram:
i) The location of the variable regions.
ii) The location of the antigen binding site.
iii) The region of the antibody that would be found in an F_{AB} fragment.

i) and ii) are shown on the right, iii) on the left. Since the molecule is symmetric, there are two Fab, two variable region segments, and two antigen binding sites.
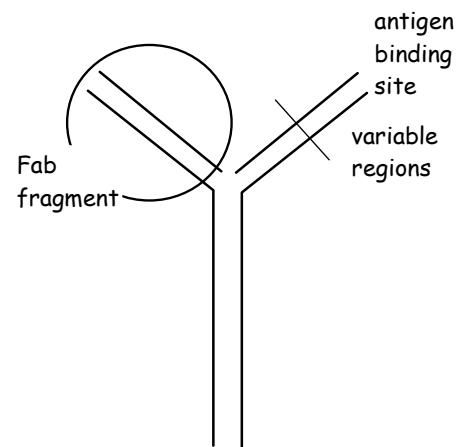
11. (2 pts) Provide one example of the use of antibodies**.**
Killing pathogens, drug detoxification, cancer treatment.

12. (16 pts) The thermodynamic stability of four proteins is compared, the wild-type sequence, two proteins with single amino acid replacements, and one protein with both replacements. The locations of these changes are with the core of the protein. The enthalpy and entropy of denaturation are given below and the structures of the sidechains of phenylalanine (Phe) and valine (Val) are shown on the right.

Phe   Val
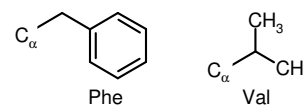
	Wild-type	**Phe57 → Val**	**Val98 → Phe**	**Phe57 → Val Val98 → Phe**
ΔH^o	+198 kJ/mol	+190 kJ/mol	+170 kJ/mol	+198 kJ/mol
ΔS^o	+600 J/mol-deg	+610 J/mol-deg	+590 j/mol-deg	+600 J/mol-deg

i) (4 pts) Determine how much of the wild-type protein is folded at 330 K. Please show your work.

Calculate ΔG^o first: $\Delta G^o = \Delta H^o - T\Delta S^o$ = 198,000 - (330)(600) = 0.

Since the energy difference between the two states (native and unfolded) is zero, they must be equally populated, one-half of the protein is folded, one-half is unfolded.

$$\Delta G = \Delta H - T\Delta S$$
$$\Delta G^o = -RT \ln K_{EQ}$$
$$f_{Native} = \frac{1}{1 + K_{EQ}}$$
$$(N \rightarrow U)$$
$$R = 8.31 \, j/mol - \mathbf{deg}$$

ii) (4 pts) Sketch, in the space to the right, the denaturation curve for the wild-type protein. Be sure to accurately represent the midpoint of the curve.

The mainpoints are:
- At 330 K (T_M) the fraction unfolded is 0.5
- the protein is completely folded at $T<T_M$.
- The protein is completely unfolded at $T>T_M$.
- The shape of the curve. A pH titration or ligand binding curve was **not** the correct curve to draw.
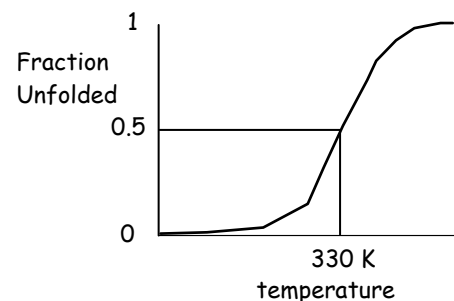
iii) (2 pts) *True   or (False)*

The enthalpy, ΔH^o, is obtained from the slope of the denaturation curve, at T_M. Circle the correct answer above. The correct plot is $\ln K_{EQ}$ versus 1/T, with a slope of -ΔH^o/R [No explanation was required.]

iv) (6 pts) Complete **one** of the following choices. Use the back of the preceding page if you need more space.

Choice A: Based on the changes in enthalpy (ΔH^o), what can you say about the relative location of residues 57 and 98 within the core of the protein? Are the next to each other, or far apart? Briefly justify your answer.

They must be close in space (4 pts).

When either one is altered, there is a decrease in the enthalpy, suggesting a disruption in van der Waals.

When both are changed (interchanged) the enthalpy is back to normal. You might imagine a structure as shown on the right:

Wild-type Protein   Two mutant protein
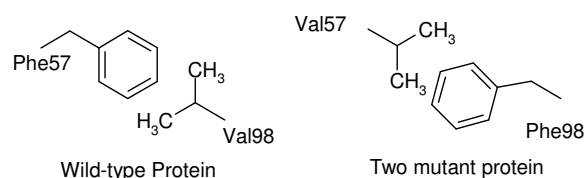
Choice B: Explain the difference in entropy (ΔS^o) between the wild-type protein and the Phe57→Val mutant, i.e. why is the overall entropy change larger for the mutant protein.

The overall entropy change is higher because the non-polar surface area of Val is smaller than Phe (3 pts), so less water will be ordered when it unfolds (2 pts), thus the negative entropy change of the solvent does not reduce the positive increase in conformational entropy as much. An alternative explanation, that Val would occupy more states in the denatured state, because was also accepted as long as the difference in W for Val versus Phe was clearly argued. Phe would actually have more states in the denatured protein because it has two rotatable bonds in the side chain, while Val has only one.

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