This exam consists of 6 pages and 11 questions with 1 bonus question. **Total points are 100. Allot 1 min/2 points**. On questions with choices, all of your answers will be graded and the best scoring answer will be used. Please use the space provided, or the back of the preceding page.

1. (6 pts) Please do both parts:

   i) Draw two "hydrogen bonded" water molecules. Indicate on your diagram a donor and an acceptor.

   ii) What is the key property of the atoms that result in the formation of a hydrogen bond?
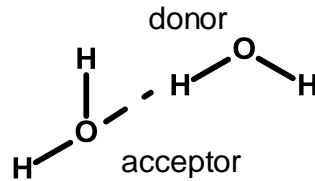
ii) the oxygen is electronegative, which gives the hydrogen a partial positive charge and the oxygen a partial negative charge.

2. (7 pts) Two different proteins (A and B) both contain an imidazole group as part of one of their amino acids (histidine). The structure of these two proteins is shown on the right. The imidazole group is shown in the deprotonated state in both proteins. The "-" symbols on protein B refer to negative charges that are close to the histidine residue. Please do parts i and ii.
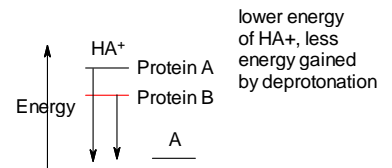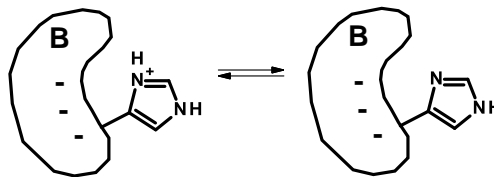
   i) (3 pts) Assume that the pKa of the imidazole group in protein A is 6. Will the pKa of this imidazole group in protein B be higher or lower? Briefly justify your answer.

The imidazole is positively charged when protonated.

Therefore the protonated state would be stabilized due to favorable charge-charge interactions. The imidazole would therefore become a **weaker acid with a higher pKa**

   ii) (4 pts) Circle the curve on the right that correspond to the % activity of protein A as a function of pH, assuming the **deprotonated** form of the imidazole is the active form. **Briefly justify your answer**.

The activity will be proportional to the fraction deprotonated. Therefore there should be little activity at low pH since the group will be protonated – this excludes the top graph.

Since the pKa=6, 50% will be deprotonated at pH=6, so the graph should pass through 50% at that point, so the middle graph is correct.
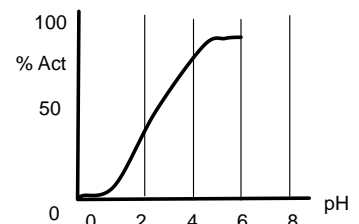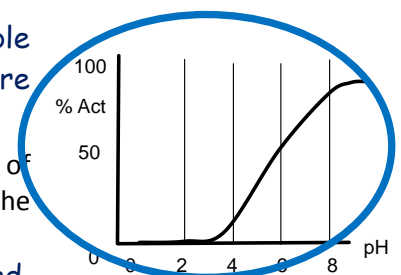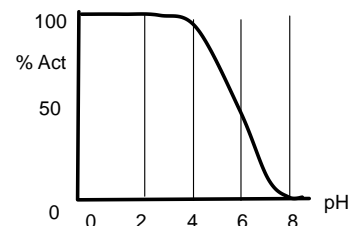
3. (10 pts) You wish to make 1L of a 0.1 M ($=[A_T]$) buffer solution at pH = 6. Your choices of weak acids for the buffer are the following. Please do all parts of the question.
  a) Acetic acid (pK_a=5.0),
  b) Hepes (pK_a = 6.0)
  c) Tris (pK_a =8.0)

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

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

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

i) (2 pts) Which buffer would you choose and why?

Either acetic acid or hepes could be used, since both buffer regions include pH=6. The buffer region is pKa +/- 1 unit.

Hepes would be the best choice since the pH=pKa and this buffer could absorb both acid or base. The acetic acid solution would only be good at absorbing acid, the addition of any base would move the pH out of the buffer region for acetic acid.

ii) (2 pt) Complete the x-axis of the titration curve shown on the right by providing a scale. Which compound, a), b) or c), corresponds to this titration curve. Justify your answer.

The scale goes from 0 to 1 since this is a monoprotic acid (single inflection point).

The infection point, at 0.5 equivalents, is equal to the pKa. In this graph it is 6, so the answer is **b – hepes**
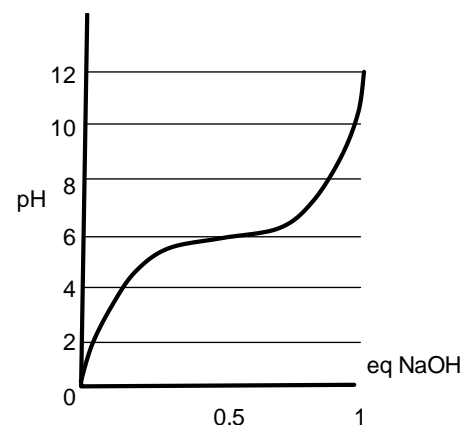
iii) (6 pts) Assuming that you are beginning with the fully protonated form of the buffer (HA), calculate how many **moles** of NaOH would you need to add to the solution of protonated weak acid.

Since the pH=pKa, the number of equivalent of base you would need to add is 0.5 equivlants. The moles are: eq base x $[A_T]$ x Vol = 0.5 x 0.1 mole/L x 1L = 0.05 moles of NaOH

4. (6 pts) The peptide bond is described as "planer and trans". Please do all of the following.
  i) Briefly describe why the peptide bond has these characteristics (2 pts).
  ii) Circle the trans form of the peptide bond shown on the right (**2 pt**).
  iii) Why is the trans form generally more stable than the cis form (2 pts)?
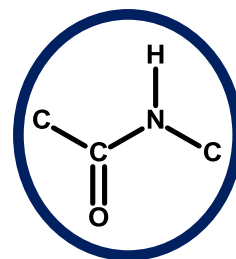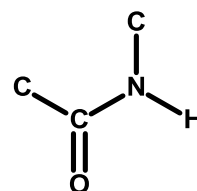
Planer – the four atoms lie in a plane because the nitrogen and carbon are both sp2 hybridized giving it a planer geometry (**1 pts**)

The nitrogen is sp2 hybridized because this allows the nitrogen to share its p_z orbital with the pi bond between the carbon and oxygen (**1 pt**)

Trans – this geometry prevents unfavorable van der Waals contacts that occur in the cis conformation (**2 pts**)

5. (12 pts) Part of the structure of a dipeptide is shown on the right. Please do all of the following.

i) Complete the diagram, as follows (3 pts):

- pH=7.0 *The amino terminus should be protonated, the carboxy deprotonated.*
- One amino acid should be **_without_** a chiral center. *Glycine is the only choice here.*
- The second amino acid can be any amino acid whose sidechain has **_both_** polar and non-polar character, but is **not** charged at pH=7.
- *Best choices are threonine, tyrosine, or tryptophan. Asparagine, glutamine, serine also accepted, although these do not have significant non-polar character.*
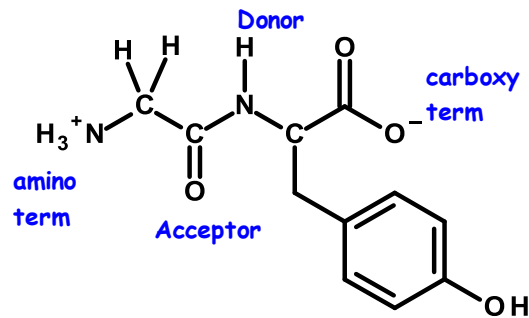
ii) Label the amino-and carboxy-terminus of your peptide (2 pts) *See diagram.*

iii) Give the sequence (primary structure) of the peptide (2 pts) *See diagram.*

iv) *Estimate* the net charge on your peptide at pH=7. For partial credit (if needed), briefly justify your answer/show your work on the back of the previous page. Write your final answer in the space to the right (4 pts).

$$q_{total} = \Sigma(f_{HA} \cdot q_{HA} + f_{A-} \cdot q_{A-})$$

Since the sidechains are not ionized, you only need to worry about the ends. The net charge is zero, the positive charge on the amino terminus is canceled by the negative charge of the carboxylate group.

v) Indicate one hydrogen bond donor and one acceptor on your dipeptide (1 pt). *See diagram*

6. (11 pts) You are trying to sequence a **12 residue** peptide using Edman degradation. The following peptide sequences were obtained after cleavage of the initial peptide with the indicated cleavage reagents. You can assume that it was possible to sequence the first five residues of each peptide. The peptide begins with the sequence: Ala-Gly-Val. Please do both parts of this question.

CNBr fragments: CNBr1: Ala-Gly-Val-Met   CNBr2: Val-Gln-Asp-Thr   CNBr3: Glu-Arg-Trp-Met

Trypsin Digest: T1: Ala-Gly-Val-Met-Glu   T2: Trp-Met-Val-Gln-Asp

i) (7 pts) Determine the sequence of the original peptide. Instead of writing out the sequence, you can just give the correct order of the CNBr fragments, e.g. 1-2-3. **For full credit, you must briefly justify your approach on the back of the previous page for full credit.**

Since the amino terminal sequence is Ala-Gly-Val, there are only two possible choices:

`Ala-Gly-Val-Met-Val-Gln-Asp-Thr-Glu-Arg-Trp-Met    (1 – 2 – 3), or`

`Ala-Gly-Val-Met-Glu-Arg-Trp-Met-Val-Gln-Asp-Thr    (1 – 3 – 2).`
`T2:                      Trp-Met-Val-Gln-Asp`

The sequence of the T2 fragment confirms the second choice, 1 – 3 - 2

ii) (4 pts) What is the absorbance at 280 nm for a 1 uM (10^{-6} M) solution of this peptide? Assume a path length (l) of 1 cm.

There is a single Trp, so the extinction coefficient of the peptide is equal to that of Trp. The Absorption is just: $[C]l\varepsilon = 10^{-6}$ *M × 1 cm × 5,500 M^{-1}cm^{-1} = 0.055*

Amino acid	ε
Trp	5,500 M^{-1}cm^{-1}
Tyr	1,490 M^{-1}cm^{-1}

$$A = \log\frac{I_O}{I} = \varepsilon[X]l$$

7. (10 pts) Briefly describe the overall structure of folded globular proteins, in particular where you would find polar, non-polar, and charged side chains.

- Polar and charged residues on **all** the outside (4 pts)
- Non-polar residues in the core **and** on the outside (4 pts)
- Well packed core. (2 pts)

There is no requirement for any secondary structure, but most globular proteins have considerable secondary structure because this is an efficient way to generate hydrogen bonds.

8. (12 pts) Please do **_two_** of the following choices:

   Choice A: Briefly describe the molecular basis of the hydrophobic effect and indicate its role in the stability of folded proteins.

   Choice B: Briefly describe conformational entropy and indicate its role in the structure of folded proteins.

   Choice C: Briefly describe van der Waals effects and indicate their role in the structure of folded proteins.

Choice A:

The hydrophobic effect relates to a change in the entropy of the water due to the presence of non-polar groups. When proteins unfold they expose their buried non-polar groups. This makes unfolding unfavorable (or folding favorable) since this decreases the entropy of the system. (6 pts)

Choice B:

Conformational entropy describes the disorder of the polypeptide chain. Since unfolded proteins can assume a large number of conformations there is a large increase in the entropy when they unfold – this stabilizes the unfolded state. (6 pts)
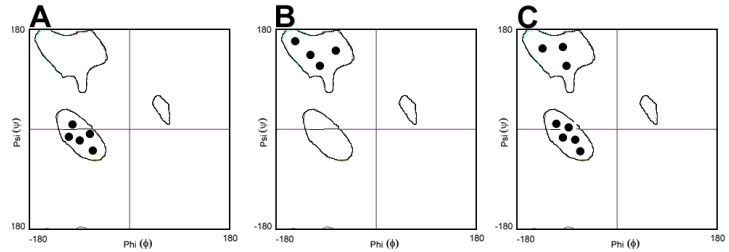
Choice C:

Van der Waals forces can be attractive or repulsive if the atoms get too close.

The attractive force is responsible for the well packed core in the tertiary structure of proteins (6 pts)

9. (14 pts) Three Ramachandran plots are shown on the right, labeled A, B, C.). Please do all parts of this question.

i) (2 pts) Indicate which plot corresponds to which secondary/super-secondary structure (circle correct answer)

(A)	B	C	helical peptide
A	(B)	C	β-sheet
A	B	(C)	βαβ
A	(B)	C	β-barrel

ii) (2 pts) What do the regions enclosed by the contour lines represent? Why are most of the points found inside these lines?

Regions of low energy due to favorable van der Waals interactions. The area outside the contour lines represent repulsive van der Waals interactions, largely involving the C_β atom (i.e. the sidechains). See bonus question below related to glycine (2 pts).

iii) (6 pts) Pick any **one** of the above four secondary/super-secondary structures and briefly describe its structure (a sketch is an acceptable answer). Your sketch should indicate the location of hydrogen bonds and sidechain atoms and some information on its geometric properties (e.g. length of one residue).

Helical drawing
- Right handed
- H-bonds || to helix axis
- Sidechains point out
- 3.6 residues/turn or 1.5 Å/amino acid or 5.5 Å/turn

Sheet
- Peptide strands running in same direction
- H bonds perp. to strand direction
- Sidechains alternate up and down
- 3 Å/amino acid.

βαβ
Combination of above structures, with the alpha helix on top of the two stranded sheet.

β barrel
β-sheet wrapped into a barrel shape structure. Necessary to indicate where the strands are.

iv) (4 pts) For the same structure that you picked in iii), describe the **main (i.e. discuss only one)** energetic feature that is important for its stability.

H-bonds would be acceptable for all four. βαβ unit – hydrophobic & vdw for the interaction between the helix and the two-stranded sheet. **Hydrogen bonds between the helix and sheet would not be common.**

Bonus (2 pts). Where could you find glycine residues on the Ramachandran plot? Why?

The unfavorable regions are due to repulsive van der Waals interactions with the beta carbon. Since glycine has no beta carbon it could be found almost anywhere on the plot. **Note**: glycine has phi and psi angles and can be found in either helices or sheets. It does destabilize then however because of its higher conformational entropy.

10. (6 pts) An isoleucine residue is buried in the core of a globular protein. Two different mutant proteins are studied, where the isoleucine has been replaced by Ala or Phe.

Discuss the relative stability of **_one_** these two mutant proteins with respect to the stability of the wild-type. Do you expect the mutant that you have selected to more, or less stable? Your answer should discuss **_either_** enthalpic **or** entropic terms that affect stability.
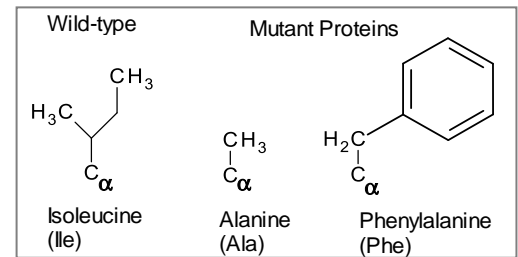
Alanine:

Smaller sidechain would have reduced van der Waals interactions with the other residues in the core, reducing stability.

The hydrophobic effect would be smaller as well, since less water would be ordered during unfolding, this would also reduce stability, because the overall entropy change is positive and larger ($\Delta G = \Delta H - T\Delta S$, note minus sign).

Phenyalanine:

Larger side chain would also disrupt van der Waals since the ring would not pack as well into the pocket where the smaller Ile sidechain is located.

The larger non-polar sidechain would increase stability since more water would be ordered in the unfolded protein.

Overall the protein is likely to be less stable, i.e. the van der Waals disruption would outweigh the entropy difference, but it would be difficult to predict.

The entropy diagram on the right was not required, but it helps you think about the competing entropy factors in protein unfolding (N→U).
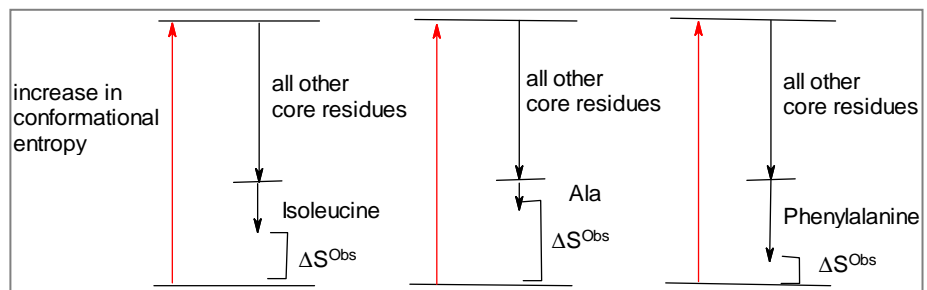
11. (6 pts) Please do **one** of the following choices. Do all parts within a choice.

Choice A: Using immunoglobulins (antibodies) as an example, briefly describe:

    i) quaternary structure

    ii) protein domains/motifs.

Quaternary structure is a description of the number of different polypeptide chains. Immunoglobulins have 2 light and 2 heavy chains.

Protein domains are independently folding units of a longer polypeptide. Immunoglobulins consist of Ig folds or Ig domains. The light chain contains 2 such domains.

Choice B: Draw a "cartoon" diagram of an antibody and indicate on your diagram the following:

    i) The location of the hypervariable loops.

    ii) Where the antigen binds.

    iii) Label the chains, and domains within each chain.

Y-shaped diagram (showing both H & L chains) with the hypervariable loops at the ends of the top of the Y. An Fab fragment is one of the upper arms. The antigen binds to the region with the hypervariable loops.

Choice C: What is a disulfide bond and why does it stabilize proteins which contain them?

A disulfide bond is a covalent bond between the sulfur atoms on the sidechain of cysteine.

It stabilizes folded proteins because it decreases the entropy of the unfolded state by reducing the number of conformations.