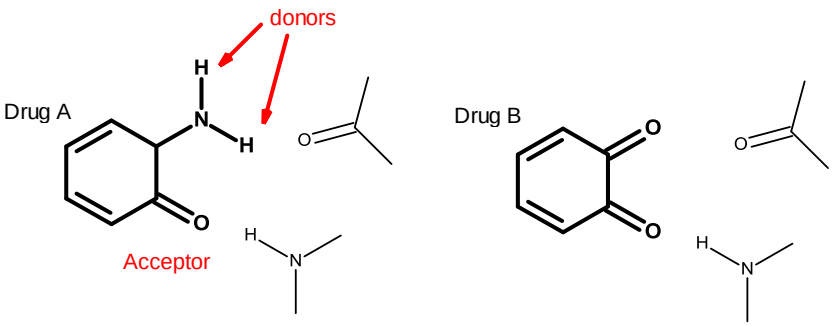
Instructions: This exam consists of 100 pts, 14 questions, 5 pages. On questions with choices all of your attempts will be graded and you will receive the best grade. **Allot 1 min/2 points.**

1. (6 pts) The following shows two drug that are bound to a protein. The structure of the drug is in bold.

i) label **either** a hydrogen bond donor or an acceptor on **drug A**. (4 pts)

ii) Which of the two drugs would have a lower K_D ($=k_{off}/k_{on}$)? Drug A or drug B? Briefly justify your answer by reference to k_{ON} and k_{OFF} (2 pt).



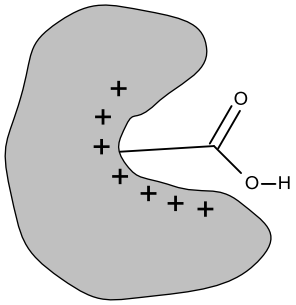
Drug A has two hydrogen bonds with the protein, but drug B only has one. Therefore drug A will have the slower off-rate and lower K_D.

2. (2 pts) Why are most salts soluble in water, even though the ions may interact less favorably with the water molecules than in the crystal.

The increase in entropy or disorder of the dissolved ions is very favorable.

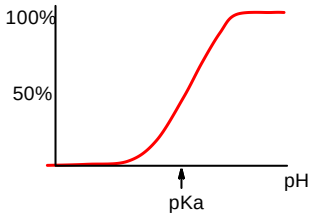
3. (8 pts) The normal pKa for a carboxylic acid side chain in an amino acid is 4.0. You are measuring the pKa of the same group, but incorporated into a protein. This residue is located in a positively charged pocket (see sketch on the right).

i) Do you expect the pKa to be higher or lower than 4.0 for this residue in the protein? *Briefly justify your answer* (4 pts).



- There is no charge on the protonated species, so the energy of the HA state is not affected.
- When deprotonated, there will be a favorable interaction with the neg charge on the carboxylate and the pos. charges on the protein, so the deprotonated state will be stabilized.
- This will result in a stronger acid, with a **lower** pKa.

ii) Assume that the **de**protonated species is the active form of this protein, sketch the activity versus pH on the plot to the right (4 pts).



The activity is proportional to the fraction deprotonated, so it will be 10% at one pH unit below pKa, 50% at the pKa, and 90% one pH unit above the pKa. Curve should look like the one on the right, Most important landmark is 50% active when the pH = pKa.

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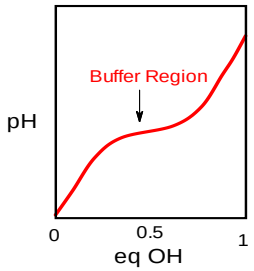
4. (16 pts) You wish to make a solution that is buffered against pH changes. The desired pH of the solution is 4.0. The volume is 0.5L and the total concentration of the weak acid is 0.1 M The following monoprotic buffers are available: pyruvate (pKa=2.0), acetate (pKa=5), Tris (pKa=8).

$R=10^{pH-pKa}$, $f_{HA} = 1/(1+R)$, $f_{A^-} = R/(1+R)$

i) Which of these would you select and why (2 pt)?

Acetate, since its pKa is (just) within one pH unit of the desired pH.

ii) Why does the weak acid reduce (buffer) pH changes when a strong acid or base is added to the solution, i.e. explain how a buffer works. A sketch of a titration curve may be useful to illustrate your answer. Feel free to use the diagram on the right (6 pts).



- In the buffer region there are significant amounts of HA and A.
- If a strong base is added to the solution, the HA will dissociate and neutralize the base.
- If a strong acid is added, the A will become protonated and neutralize the acid.

iv) Please do **one** of the following two choices (8 pts).

Choice A: Describe how you would make the buffer – i.e. give moles of HA & H, and the number of moles of NaOH or HCl that would be required, depending on the method you selected to make your buffer.

Choice B: Although the starting pH of your solution was 4.0, it has shifted to 5.0 due to the production of base by the reaction. How many moles of HCl do you add to return the pH to 4.0?

Choice A: $R=10^{4-5}=10^{-1}=0.1$

Fraction protonated = 1/(1+0.1) = 0.9

Fraction deprotonated = 0.1

Method A: mix 0.9 x 0.5 L x 0.1 M = 0.045 moles of HA

mix 0.1 x 0.5 L x 0.1 M = 0.005 moles of A (note these sum to .05 = .5Lx.1M)

Method B: Start with 0.05 moles of HA, add 0.1 x 0.5 L x 0.1 M = 0.005 moles of NaOH

Method C: Start with 0.05 moles of NaA, add 0.9 x 0.5 L x 0.1 M = 0.045 moles of HCl.

Choice B:

Since the ending pH = pKa, the fraction protonated is 0.5

At pH =4 (starting pH) the fraction protonated was 0.9 (see above)

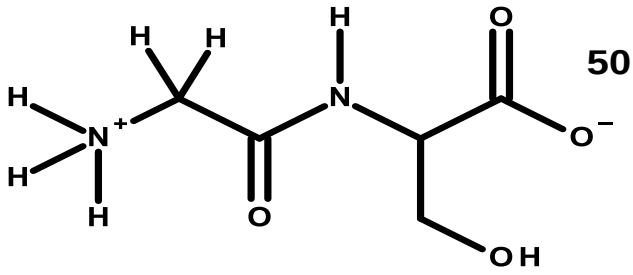
Number of equivalents of HCl to add is 0.4 ($=\Delta f_{HA}$) Moles = 0.4 x 0.5L x 0.1M = 0.02 moles

5. (10 pts) Draw the chemical structure of a dipeptide that contains any two amino acids that are **different**. *Do not use alanine or threonine.* Assume that the pH of the solution is 2.0 and indicate the correct protonation state of any ionizable groups. You can assume the relevant pKa values are 2.0 and 9.0 (8 pts).

Grading: 6 points for correct mainchain, 1 point for correct ionization state, ½ pt for each correct sidechain.

i) Indicate the sequence of your peptide (1 pt). Example is Glycine-Serine (key point is they start at the amino terminus)

ii) *Estimate* the charge on your peptide, at pH=2.0 (1 pts). +1 for the amino, -1/2 for the carboxylate = +1/2 (**Full credit if the charge is consistent with their drawing**).



50 % deprotonated

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6. (6 pts) Please do **one** of the following choices:
Choice A: Is there free rotation about the peptide bond? Why or why not?
Choice B: The atoms that participate in the peptide bond, O=C-N-H, all lie in a plane, why?

Choice A: No, because of overlap between the pz orbitals on the carbon and nitrogen, makes the peptide bond behave like a double bond.

Choice B: Both the carbon and nitrogen are sp2 hybridized to maximize overlap between their pz orbitals.

7. (10 pts) The sequence of an 11 residue peptide is determined by Edman sequencing of fragments that are produced after cleavage by CNBr and Chymotrypsin. You should assume that it is possible to only obtain the first five (5) residues of any peptide during the sequencing reaction.

CNBr Fragments:	Ala-Gly-Met	Ala-Ala-Leu	Phe-Arg-Ser-Trp-Met
Chymotrypsin fragments:	Ala-Gly-Met-Phe	Arg-Ser-Trp	Met-Ala-Ala-Leu

Reconstruct the original sequence of the peptide, write your answer here:

Ala – Gly – Met – Phe

Phe – Arg – Ser – Trp – Met

Met-Ala-Ala-Leu

1 2 3 4 5 6 7 8 9 10 11

Justify your approach to receive partial credit in case your answer is wrong.

8. (3 pts) Circle the interactions that stabilize secondary structure. Put a box around those which stabilize tertiary structure. ~~Strike out~~ any that have no effect on either secondary or tertiary structure.

a) van der Waals

b) Hydrogen bonds

~~c) Electrostatic interactions~~

d) Hydrophobic effect

+1 for striking out c), +1 for circling a) and b). They may have omitted the boxes around a) and b) -1/2.

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9. (8 pts)

i) Sketch **one** regular secondary structure and indicated the location of the sidechains and Hydrogen bonds (6 pts).

ii) If the secondary structure that you drew faced the core of the protein, what type of side chains would point into the core and how often would you find them in the sequence? (2 pts)

Helix:

- Sidechains point out
- H-bonds parallel to axis
- Every 3rd or 4th residue would be non-polar

Sheet

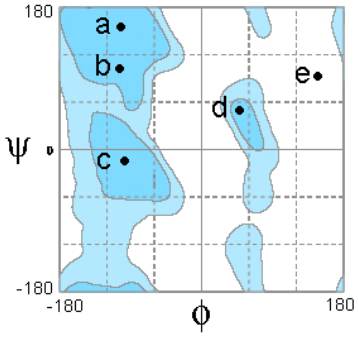
- Sidechains point up or down, alternating
- H-bonds perpendicular to strand direction
- Every second residue would be non-polar.

10. (1 pt) How many residues are in one turn of an α-helix? **3.6 (can be off a decimal or so)**

11. (8 pts) The following is a Ramachandran plot. The values for five residues (a-e) have been plotted. Please answer the following questions.

i) What do the contour lines/shaded areas represent? (6 pts)

ii) Which of the five points most likely corresponds to a glycine residue? Briefly justify your answer. (2 pts)



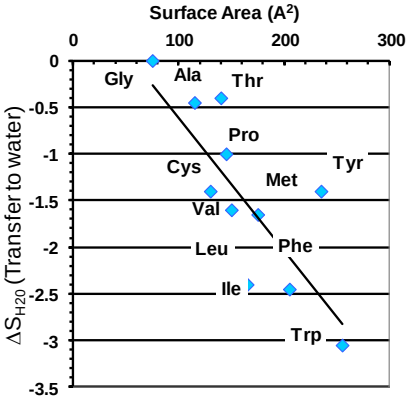
i) Shaded areas represent regions of favorable van der Waals. White regions are unfavorable.

ii) **e** – it is in an unfavorable region for residues with beta carbons, but since glycine lacks a beta carbon, the unfavorable van der Waals would not exist.

12. (11 pts) In the equation: $\Delta S^0 = \Delta S^0_{CHAIN} + \Delta S^0_{SOLVENT}$

i) What does the term $\Delta S^0_{SOLVENT}$, represent with respect to protein unfolding? Your answer should include a brief description at the molecular level (8 pts).

ii) *Briefly* describe how you could calculate, or estimate, **either** of the two terms, ΔS^0_{CHAIN} **or** $\Delta S^0_{SOLVENT}$. The chart on the right may be helpful, as might this equation: S=R ln W (3 pts).



i) $\Delta S^0_{SOLVENT}$ is the hydrophobic effect. It is negative for protein unfolding. At the molecular level, it is the ordering of water molecules around non-polar groups, decreasing their entropy.

ii)

$\Delta S^0_{CHAIN} = S_U - S_F = R \ln 9^N - R \ln 1$. 9^N = the number of different conformations of the unfolded chain.

$\Delta S^0_{SOLVENT}$: This can be estimated using the transfer free entropies on the chart, for example, Tryptophan would contribute -3 J/mol-K.

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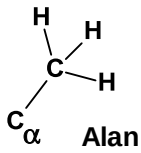
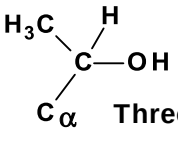
13. (6 pts) An alanine residue is found in the core of a folded globular protein (wild-type protein). It is replaced by a threonine residue (mutant protein). The amount of unfolded protein for the mutant (threonine) is measured as a function of temperature and plotted on the right. A van't Hoff plot from this data is also on the right. The slope of the line is indicated on the plot.



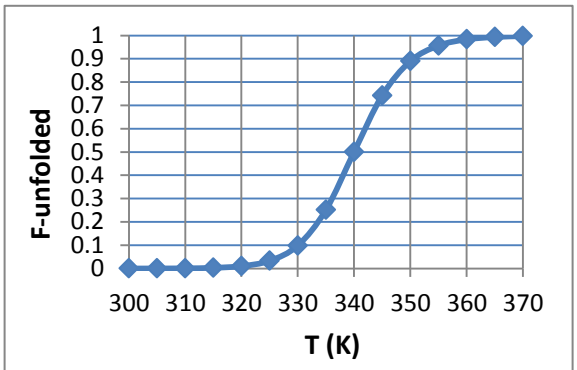
Alanine



Threonine

$\Delta G^o = \Delta H^o - T\Delta S^o$
$\ln K_{EQ} = -(\Delta H^0/R)(1/T) + (\Delta S/R)$
R=8.31 J/mol-K

The ΔH^o for unfolding of the wild-type protein is +200 kJ/mol. The entropy for unfolding, ΔS^0, is +600 J/mol-K for ***both*** proteins.



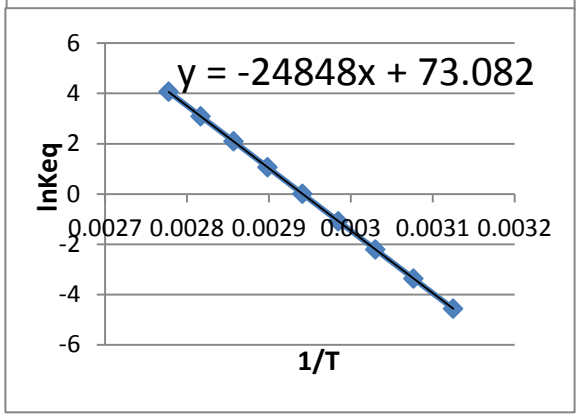
Please do **both** parts *i* and *ii*, you have a choice for part *ii*.

i) Determine the enthalpy for unfolding of the mutant (threonine) containing protein. You can either use the van't Hoff plot, or use T_M = 340K (3 pts).

ii) Please do **<u>one</u>** of the following choices (3 pts):

Choice A: Explain the difference in enthalpy between the wild-type and the mutant protein in terms of interactions that have either stabilized or destabilized the mutant protein.

Choice B: Why is the entropy the same for both proteins? The graph on the previous page may be useful?



i) van't Hoff: Slope = -24848.

ΔH^o = 8.31 x 24848 = 206 kJ/mol.

T_M ; 0 = $\Delta H^o - T_M \Delta S^o$.

$\Delta H^o = T_M \Delta S^o$ = 340 x 600 = 204 kJ/mol (these are slightly different since the Tm ~340K)

ii)

Choice A: It requires more heat to unfold the threonine containing protein. Additional van der Waals interactions are a possibility; however Thr is larger than Ala, so **disruption** of van der Waals is more likely, which would lower ΔH^o. Thr has an H-bonding group, so perhaps it is making an H-bond in the core that wasn't present with the Alanine, providing an additional ~4 kJ/mol of stability **(2 points if they say van der Waals, 3 for H-bonds)**

Choice B: Both Alanine and threonine show the same change in the entropy of water when exposed in the unfolded state (plot on previous page), so ΔS^o_{H2O} will be the same.

14. (5 pts) Please do **<u>one</u>** of the following choices – *use the back of the previous page if you need more space.*

Choice A: Describe, or draw, either an intact antibody, Fab fragment, or F_V fragment. Your answer should describe the separate chains that make up the protein and you should also indicate where the antigen binds and the location of the hypervariable loops.

Choice B: Why do disulfide bonds stabilize the folded structure of antibodies?

Choice C: A protein contains 2 tryptophan (Trp) residues and one tyrosine (Tyr) residue. Write an expression that would allow you to calculate the extinction coefficient for this protein, given that you know ε_{Trp} and ε_{Tyr}.

$A = [X] l \varepsilon$

Choice A: Ig = 2 light + 2 heavy/Fab = 1 Light + V_H-C_{H1}/Fv = V_L + V_H.. Should show 3 hypervariable loops associated with each variable domain, antigen contacts hypervariable loops.

Choice B: The crosslink in the chain reduces the entropy of the unfolded state, making it less stable, therefore the folded form is more stable.

Choice C: $\varepsilon = 2 \times \varepsilon_{TRP} + 1 \times \varepsilon_{TYR}$

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