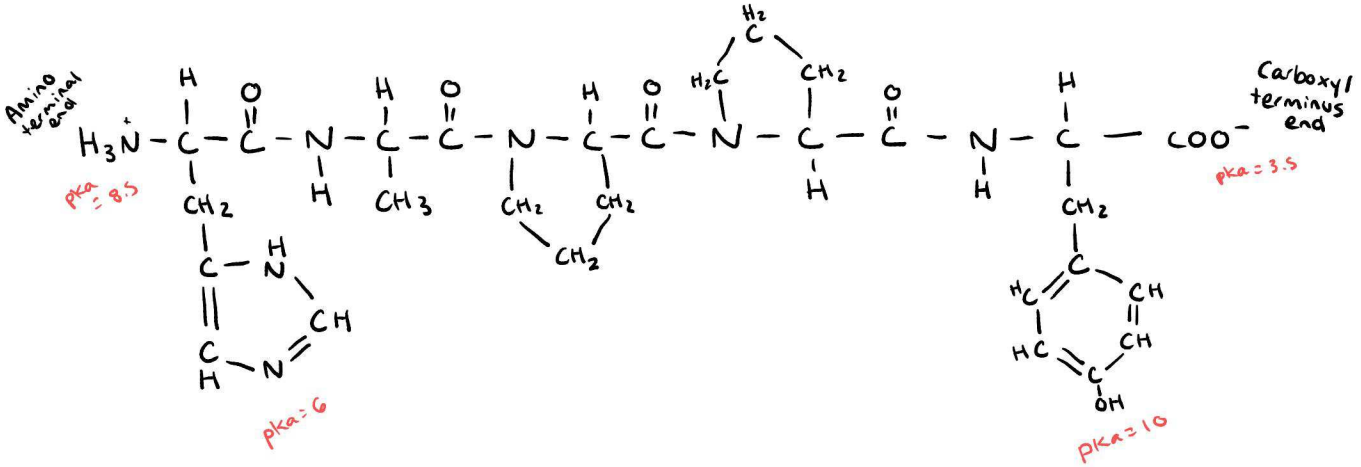
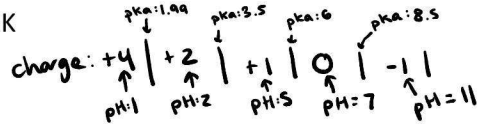
1. (21 pts) For the questions on this page use the peptide: **H – A – P – P – Y**

A. (8 pts) Hand draw (computer generated images will not be counted) the peptide at physiological pH (pH = 7.4):



B. (3 pts) Calculate the pI of the peptide. SHOW ALL YOUR WORK

$$pI = \frac{8.5 + 6}{2} = \boxed{7.25}$$



At pH=5, the net charge is +1, for pH=7 it's 0, and for pH=8 it's -1, the relevant pka's between these pH's are 6 (His) and 8.5 (NH_3^+ terminal).

C. (10 points) To do an experiment with this peptide you need to make two buffers. One where the peptide has a net **negative** charge (**Buffer A**) and one where the peptide has a net **positive** charge (**Buffer B**). The pH of both buffers has to be within 2 pH units of the pI, but you want the largest net charge you can have along with having the best buffer possible.

pH ~ 8

pH ~ 5

You have concentrated solutions of the following compounds available to you in the lab:
Acetic Acid (pKa = 4.8); Acetate; H_3PO_4 (pKa = 2); $H_2PO_4^-$ (pKa = 7.2); HPO_4^{2-} (pKa = 12); PO_4^{3-}; carbonic Acid (pKa = 6.4); bicarbonate (pKa = 10.3); carbonate; and powders of each amino acids.

Briefly describe how you would make each buffer:

must be > 7.25 and close to 9.25

7.2 ± 1 = buffer region

Buffer A: What is the intended pH of this buffer: 9.25

Protocol to make the buffer: (2 sentences Max)

I would create a solution with 1 mol alanine powder, whose free amino end has a pKa of 9.5. Then add slightly under 1.5 equivalents of NaOH until the pH is 9.25.

Buffer B: What is the intended pH of this buffer: 5.25

Protocol to make the buffer: (2 sentences Max)

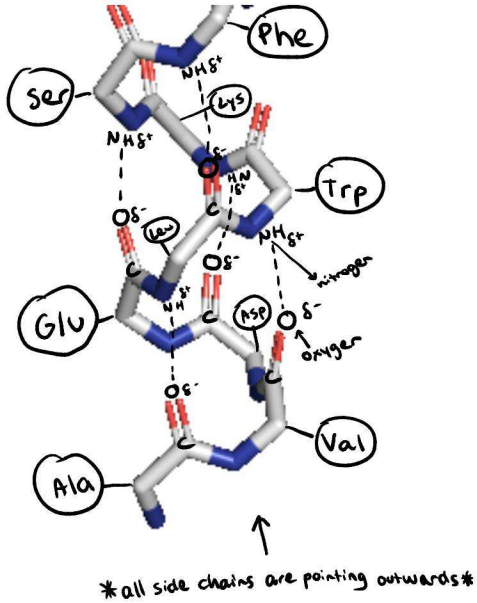
I would create a solution with a 1:1 ratio of acetic acid (pKa = 4.8) to acetate. Then I'd add a little bit more than 0.5 equivalents of NaOH to bring the pH to 5.25.

[redacted]

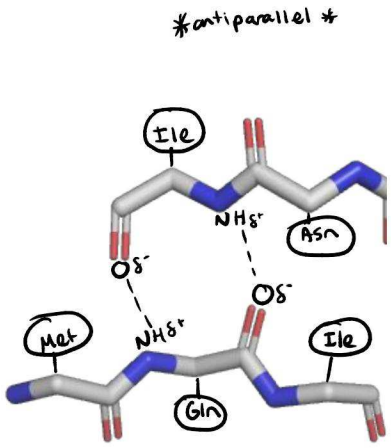
2. (10 points) Below are two portions of peptide backbone displaying their secondary structure.

- Draw in the hydrogen bonds that stabilize these structures. Include the atoms involved and label any charges (full or partial) that are involved.
- The sequence of each structure is shown. Indicate the orientation of the side chains using a circle and the 3 letter code for the amino acid.

A – V – D – E – L – W – K – S – F



Top: N – I; Bottom: M – Q – I



3. (10 pts) A). (4 pts) Explain how the structure of a biochemical macromolecule leads to its function.
(2 sentences Max)

The structure of a macromolecule determines the type of interactions it can have within itself or with other molecules. Bond type, such as alpha and beta linkages, branched/linear bond distribution, and the polar/nonpolar charge distributions all influence what the molecule can do in terms of energy storage, transport, and structural integrity.

B). (6 pts) Compare and contrast two carbohydrates as an example of your explanation.
(3 sentences Max)

For example, cellulose and starch's differences in structure are the reasons for these carbohydrates' differences in structure. Cellulose's beta glycosidic linkages and linear bond distribution add a lot of strength to the molecule, making it very difficult to break. On the other hand, starch's alpha glycosidic linkages and branched distribution allow it to be broken down easily and used more efficiently for storing energy, as well as for fast mobilization of glucose.

[redacted]

4. (12 points) You have been studying the metabolism of compound X that binds cooperatively to protein A. You have just identified another protein (B), that binds compound X with the same affinity but non-cooperatively.

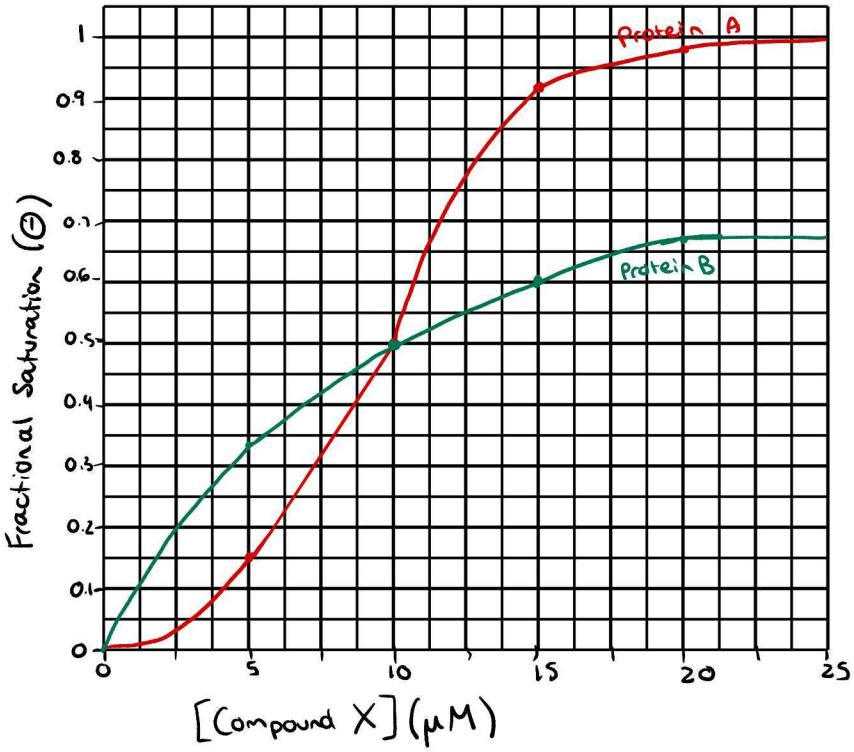
A). (8 points) The table below contains the data for compound X binding to protein A. Fill in the values for the binding of X to protein B. **Graph the two binding curves**.

$\Theta = \frac{[X]}{K_D + [X]} \rightarrow 0.5 = \frac{10}{K_D + 10} \rightarrow K_D = \frac{10}{.5} - 10 \rightarrow K_D = 10$

[X]	Θ for Protein A (μM)	Θ for Protein B (μM)
5	0.15	0.33
10	0.5	0.5
15	0.92	0.6
20	0.98	0.67

$\Theta = \frac{5}{10+5} \rightarrow \frac{1}{3} = 0.33$ $\Theta = \frac{15}{10+15} = \frac{3}{5} = 0.6$

$\Theta = \frac{10}{10+10} \rightarrow \frac{1}{2} = 0.5$ $\Theta = \frac{20}{10+20} = \frac{2}{3} = 0.67$



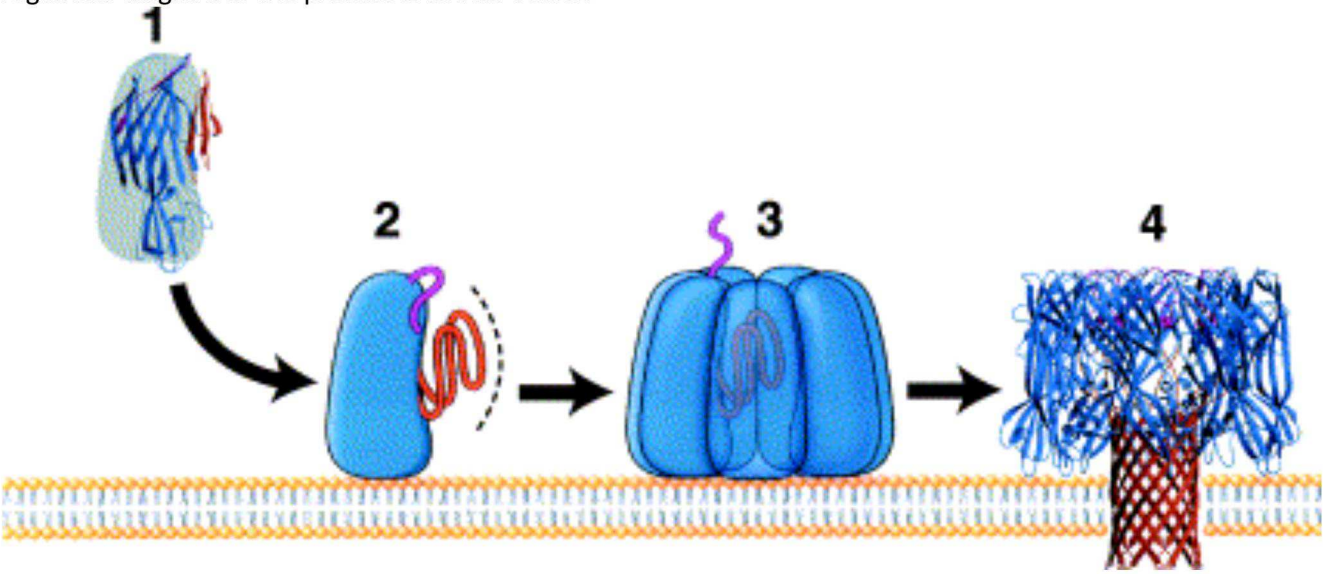
B). (4 points) The average physiological concentration of compound X is 12 μM. Which protein can respond more to slight changes in the concentration of compound X? Briefly explain your reasoning.
(2 sentences Max)

Protein A would respond more to slight changes in compound X's concentration because Protein A exhibits cooperative binding and, as a result, a steeper slope in its graph. Cooperative binding entails that the binding of compound X would cause a conformational change in Protein A that would increase its affinity for compound X, therefore, a change in the amount of compound X available for binding would affect Protein A's binding affinity and elicit a greater response than what would be seen with Protein B.

[redacted]

5. (20 pts) Protein A is a member of the bacterial pore-forming toxins family of proteins. They fold as a water soluble monomer (1), then assemble into a heptameric (7 subunit) complex (3), part of which is a transmembrane beta barrel (4). The beta barrel creates a hole in the membrane which disrupts the selective permeability of the membranes and eventually leads to cell lysis.

A general diagram of the process is shown below:



A). (2 pts) In part 3 what type of interactions most likely holds the subunits together?

Hydrophobic (ID-ID) interactions most likely hold the subunits together.

B). (2 pts) In part 3 what type of interactions most likely attaches the complex to the membrane?

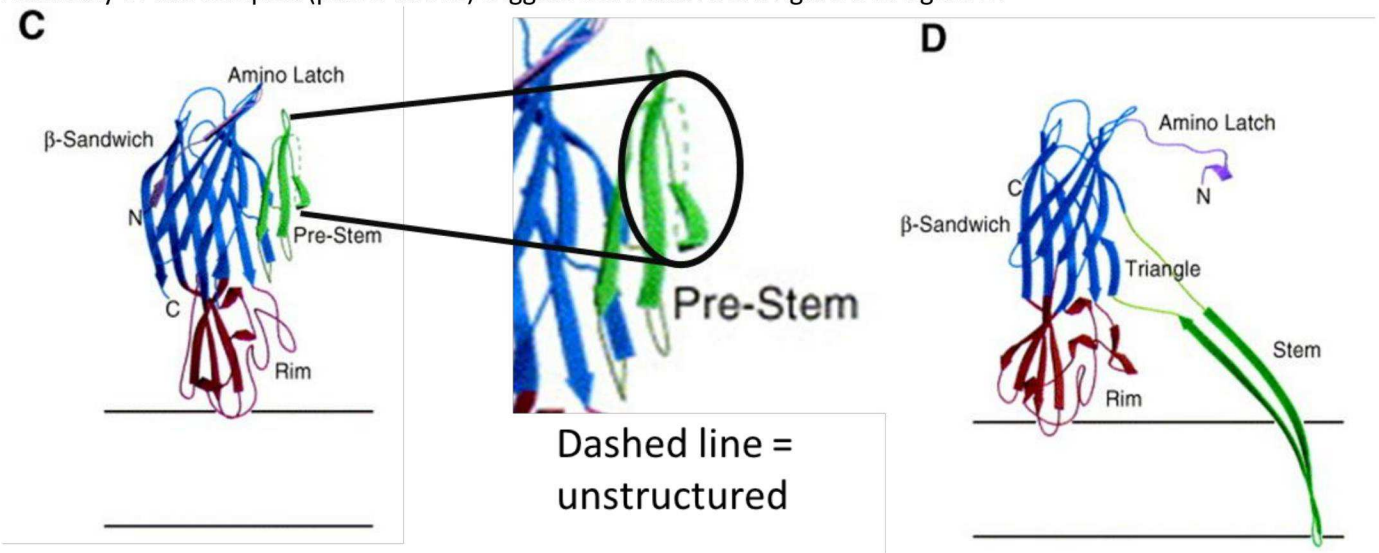
Dipole - Dipole (hydrogen bonds) interactions most likely attach the complex to the membrane.

C). (4 pts) Propose an arrangement of amino acid characteristics that you would expect to find in the transmembrane portion of this protein. Briefly explain your reasoning.
(2 sentences Max)

I propose for the amino acids characteristics to be arranged in an alternating amphipathic polar - nonpolar fashion with the protein's exterior being nonpolar and it's interior being polar so as to mimic the cellular membrane's phospholipid bilayer. Doing so would allow the protein to insert itself into the cellular membrane since the nonpolar exterior could interact with the nonpolar tails of the membrane's phospholipids, and the protein's polar interior would allow for molecules, such as water, to pass through the protein and into the cell.

[redacted]

Question 5 continued:
Below are structures of the monomer in the water soluble form (**C**) and in the membrane spanning form (**D**):
Assembly of the complex (part 3 above) triggers the switch from figure C to figure D.



In figure C the dashed line in the green pre-stem structure indicates a part of the sequence that is unfolded.
D). Transferring an **<u>unfolded peptide bond</u>** from an aqueous environment into a membrane has a ΔG=5.0 kJ/mol.

i). (4 pts) What might be the main reason that this is thermodynamically unfavorable? **(1 sentence Max)**

Since this would involve breaking bonds that are stronger than the ones being formed (dipole-dipole to dipole-induced dipole), there would be a positive change in enthalpy, which is thermodynamically unfavorable.

ii). (4 pts) Since we know that this process occurs, propose a mechanism to mitigate the unfavorability of inserting an unfolded peptide bond into a membrane. **(1 sentence Max)**

To mitigate the unfavorability, I'd propose coupling the process with the formation of the quaternary structure.

E). (4 pts) You have been tasked with finding a treatment for exposure to this toxin. You discover in the literature that assembly of the complex and the beta barrel occurs cooperatively once a large enough concentration of monomer is present on the surface of susceptible cells. Using this information propose a way to inhibit the cell lysis caused by these proteins. Briefly explain your reasoning. **(2 sentences Max)**

To prevent the resulting cell lysis I would introduce a negative, heterotrophic allosteric effector to the system. Since such effectors lower the protein's binding affinity, the toxin will, as a result, remain in the low-activity T state, therefore inhibiting the assembly of the complex and beta barrel and ultimately preventing cell lysis.