Problem 7 - Due Monday March 27

1. (16 pts, 25 min). You work for a drug company and you are trying to determine which two drugs would be better to use in clinical trials to inhibit HIV reverse transcriptase. The structure of the normal substrate and the two drugs are shown to the right.

i) What is the role of this enzyme in the HIV lifecycle and indicate why it would be a particularly good drug target (2 pts).

ii) What type of inhibitors do you think compound A and B are? Briefly justify your answer (2 pts).

iii) Use the dry lab associated with this problem to obtain initial velocity data as a function of varying substrate +/- a fixed concentration of inhibitors. The dry lab will return two datasets, one labeled A and one labeled B. Generate a double reciprocal plot and submit that plot with your problem set (2 pts).

iv) For each of the datasets, determine the type of inhibition (i.e. competitive or mixed) and the appropriate inhibition constants ($K_I$ or $K'_I$ and $K''_I$) from the steady-state data. Note that there is some experimental error in the data, so the y-intercept on the double reciprocal plot for the competitive inhibitor may not be exactly the same as the value obtained in the absence of the inhibitor (7 pts).

v) Can you determine from these data which inhibitor will make the best drug? If not, what additional information beyond the binding affinities will be required? Many factors come into play in making a successful drug besides how well it may bind to its target enzyme in a laboratory setting. Think about where drugs have to function and what might be unknown in that environment. [Hint: A plot of $v$ versus $[S]$ in the absence and the presence of each inhibitor may give you an idea.] (3 pts)

2. (18 pts, 30 min) The Jmol page associated with this problem set shows wild-type and a mutant HIV protease in complex with a number of different HIV drugs. One of these drugs is the same as the one presented in class. This particular drug contains a cyclohexane ring at one end and it binds to the wild-type enzyme with high affinity.

Three different drugs, with alteration in the cyclohexane ring, have been developed for the purpose of inactivating the mutant HIV protease (see bottom of page).

The double reciprocal plots for the wild-type and mutant enzyme without and with the cyclohexane inhibitor are shown. Double reciprocal plots for the mutant enzyme with the three drugs are also shown below. Linear fits to these curves are shown in the above table. The linear fit for both the wild-type and mutant, in the absence of any inhibitor is $y=0.5x+0.1$.

i) What feature of the HIV life-cycle leads to a high level of mutations in the HIV genetic material (2 pts)?

ii) Based on the Jmol page, which residue is altered in the mutant HIV protease? How has it been changed? (1 pt)
iii) Does this residue contribute mostly to catalysis or specificity? Justify your answer. (2 pts)

iv) Determine the $K_i$ values from the double reciprocal plots. Assume $[I]=10$ nM (2 pts).

v) Explain, with reference to the change in the $K_i$ values and the structure of the two enzymes, why the affinity to the original cyclohexane drug has been affected by this mutation (e.g. compare lines 1 and 2 in the above table as well as the interaction between the protein and the drug.) (3 pts)

vi) Which of the three drugs would be the worst inhibitor of the mutant protease? Justify your answer with reference to the $K_i$ values as well as the interaction between the drug and the mutant enzyme. A simple sketch of the interaction between the drug and the inhibitor would be useful (4 pts).

vi) Which of the three drugs would be the best inhibitor of the mutant protease? Justify your answer with reference to the $K_i$ values as well as the interaction between the drug and the mutant enzyme. A simple sketch of the interaction between the drug and the enzyme would be useful (4 pts).

J-mol page instructions:
- The "unmodified" button will load the "wild-type" or non-mutant enzyme.
- The "simple view" button will show the backbone of the protein, the sidechain of key residues, and the bound drug.
- The check boxes will add surfaces to the indicated features, to orient yourself with respect to the molecule.
- The "Mut+cyclohexane" button will load the mutant HIV protease and the original, non-modified drug. This drug has a cyclohexane group that contacts the enzyme and is the same as "drug B" from lecture.
- The buttons labeled "Drug1", "Drug2", etc. will load the structure of the mutant HIV protease with a different drug bound in the active site.

3. (8 pts, 10 min) You will need to do an internet search to answer this question, please cite your sources.

i) Fosamprenavir is the pro-drug form of amprenavir. What is a pro-drug and why is it advantageous to administer Fosamprenavir over amprenavir (3 pts).

ii) The inhibitor discussed in class is not used in clinical treatment of HIV due to its relatively low potency. Saquinavir is one of the commonly used drugs to inhibit HIV. Although both drugs appear to be different, they share a common feature that is required of all competitive inhibitors. What is that feature and where is it found in the structure of both inhibitors (5 pts).

4. (10 pts, 30 min) You are given an equal mixture of all the proteins listed in the table below. The size of the protein, its solubility in ammonium sulfate, and the number of charged residues are presented in this table. Using the on-line protein purification simulator, develop a purification scheme that will result in the purification of one of the proteins in their native, non-denatured, form. The protein that you need to purify depends on your last name. Enter the value in the ‘Key’ column on the first page of the simulator, where it asks for your “first letter last name.” The correct target protein should be assigned to you.
In developing your purification scheme you may use ammonium sulfate precipitation, gel filtration, anion exchange, and cation exchange chromatography.

After completing the purification of your protein, you must **click on the submit button** to send your work to the server. Feel free to make as many practice runs as you like, but only hit submit when you are satisfied with your purification scheme and want to submit it for grading.

*Ammonium sulfate concentration at which 50% of the protein will precipitate. A concentration that is 0.5 M below and above this concentration will precipitate 25% and 75% of the protein, respectively. A concentration that is 1.0 M below or 1.0 M above will precipitate 0% and 100% of the protein, respectively.*

A link to the purification simulator can be found on the Jmol page for this problem set (& Blackboard).

**Starting amount and Yield:** The initial number of enzymatic units of each protein is 100, therefore your % yield = number of final units.

**How to evaluate purity?** The specific activity of each protein can be found on the “Handbook” tab. Due to round off error, your final specific activity may be ~5% lower, this will be considered as a pure protein. The specific activities of the pure protein are also listed in the above table.

**Instructions:**
1. Enter your Andrew id and the “Key” corresponding to the first letter of your last name.
2. Select the purification scheme from the drop-down menu. If you select anything else but gel filtration, you will also need to define the pH of the separation (ion exchange) or the amount of ammonium sulfate (M).
3. After the separation occurs, you need to find out where your enzyme is. In the case of column chromatography, click on the tubes that you think will have your enzyme. In the case of ammonium sulfate, the two fractions will be the precipitate or the supernatant. After you have selected the fractions that you want to assay, click on the “Do Enzyme Assay” button. The simulator will return the total amount of protein, the activity of your enzyme, and the specific activity of each fraction. In the above example, tubes 27, 35, 38, 39, and 40 were assayed for enzyme activity. Tube 27 showed a high level of activity, indicating that the first peak off of the column corresponds to the target protein.
4. Once you have determined where your enzyme is, you want to tell the program which fractions to pool to carry forward to the next step. Select those fractions by clicking on them, you must to this, even if you had selected the fractions for enzyme assays. Once you have selected the fractions you want, click on the “Collect Samples” button, this will pool all of your samples into the beaker. You can either do another separation step or if your protein is pure, click on the “Submit Assignment” button. **In this example, I would have selected tube 25, 26, 27, 28, and 29.**