03-131 Genes, Drugs, and Diseases Problem set 4 September 27, 2015

1. (6 pts, 10 min) Shown to the right is one of the substrates for reverse transcriptase and two drugs, compound I and compound II, that are currently used to inhibit the enzyme.

i) Briefly describe the role of this enzyme in the HIV lifecycle and indicate why it would be a particularly good drug target.

ii) Based on the structure of the drugs, which drug is most likely to be an allosteric inhibitor and which is most likely to be a competitive inhibitor. Be sure to justify your answer.

2. (5 pts, 10 min) Both compound I and compound II can inhibit HIV reverse transcriptase. Which will be the better inhibitor if the substrate concentrations are high in the cell? Briefly justify your answer.

3. (5 pts, 5 min) Draw dAMP and label the 5’ and 3’ carbons. How does dATP differ from dAMP?

4. (4 pts, 2 min) Write the corresponding RNA sequence for the following DNA sequence. ATGCGTGACTAG. Please label the 5’ end.

5. (4 pts, 2 min) Write the duplex DNA representation of this DNA sequence. ATGCGTGACTAG. Please label the 5’ end.

6. (9 pts, 15 min) The diagram to the right shows three different basepairs, T-T, A-T (normal basepair) and A-G.

i) Identify hydrogen bonds **between** each pair of bases. How many hydrogen bonds do you see for each basepair?

ii) What is the relative size of each basepair, what is the distance between the riboses (e.g. large, medium, or small).

iii) When DNA is replicated by polymerases, the correct pairing of bases is A with T; other pairings, e.g. T-T, A-G are incorrect and would lead to mutations. Based on your answer to part i) and part ii), what other criteria do polymerases use to insert the correct base, besides numbers of hydrogen bonds?

**Jmol Questions – You will need to view two Jmol pages for this problem set**

7. Visit the first Jmol page (Jmol-A) and answer the following questions.

i) Is the label “A” at the 5’ or 3’ end of the molecule? Why?

ii) Is this DNA or RNA, justify your answer.

8. (16 pts, 20 min) The second Jmol page (Jmol-B) 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 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 a *mutant* HIV protease. Reciprocal plots are provided that give the activity of the wild-type protein without and with the cyclohexane drug. A separate plot is given for the mutant protein, without drug, with the cyclohexane drug, and with three other drugs. Please answer the following questions.

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

ii) Which residue is altered in the mutant HIV protease? What has the valine at position 82 been changed to? (1 pt)

iii) Does this residue contribute mostly to catalysis or specificity of substrate binding? Justify your answer. (2 pts)

iv) Explain, with reference to the change in KI (which you can obtain from the slopes of the reciprocal plots) and the structure of the enzyme-inhibitor complex for the wild-type and mutant enzymes, why the affinity to the original cyclohexane drug has been affected by this mutation (3 pts). **In calculating KI values, assume an inhibitor concentration of 1 nM.**

v) Which of the three drugs would be the *worst* inhibitor of the mutant protease? Justify your answer with reference to the reciprocal plot, 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 reciprocal plot, 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).

Structures of Drugs:

J-mol page instructions:

* The “Wild-type+CycloHex” button will load the “wild-type” or non-mutant enzyme with the original drug bound.
* 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 you 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 the drug 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.