Chem 162/262 (2004) Assignment #3:

Enzymes: Mechanisms & Inhibition

1. Most enzymes have high substrate specificity, that is, they act on only a single substrate. However, some enzymes catalyze reactions with a number of similar compounds; such enzymes are termed promiscuous. Kinetic studies with promiscuous enzymes can be used to gain some information about structure-activity relationships that help in designing inhibitors. The table below lists experimentally measured kinetic parameters for one such enzyme. The (*R*) and (*S*) designate absolute stereochemistry of the chirality center; the absence of label indicates that the compound is either achiral or a racemic mixture was used.

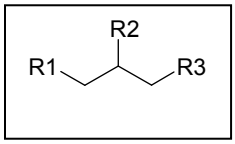

Table: Kinetic parameters for compounds with a general structure:

Label	R1	R2	R3	K_m	V_{max} (relative)	V/K_m (relative)
A	OH	OH	OH	5.1	100	19.6
B	OH	OH	SH	4.0	155	38.4
C	OH	OH	Cl	6.0	130	21.7
D	OH	OH	Br	6.1	109	17.9
E	OH	OH	NH2	180	104	0.6
F	OH	OH	H	0.06	105	1750
G	NH2	OH(*R*)	H	4.4	33	7.5
H	NH2	OH(*S*)	H	**500**	9	0.002

Questions:

1. The kinetic parameter V/K_m is a measure of enzyme's specificity toward the substrate. Identify two main structural factors that affect the specificity.
2. The three poorest substrates were compounds labeled H, E, and G. Which one would you pick as a lead compound for preparation of inhibitors via a bio-isosteric replacement strategy? Why?
3. Which atoms in your lead compound would you try to substitute in order to create a compound (inhibitor) that binds with high affinity but has lower turnover rate than the lead compound?

2. In their famous 1971 PNAS paper Michael Page and William Jencks proposed that enzymes may work as "entropy traps". Based on simple thermodynamic arguments they arrived to the conclusion that enzymes can accelerate bimolecular reactions by as much as 10^8.

a) Explain the "entropy trap" concept of Page and Jencks in your own words.
b) Many scientists now believe that while the basic idea behind the "entropy trap" concept remains valid, the 10^8 value largely overestimates rate acceleration that could be achieved by simply binding two reactants of the bimolecular reaction to the enzyme. Discuss reasons why the 10^8 estimate might be wrong.
c) Is the concept of "entropy trap" relevant to designing enzyme inhibitors as potential drugs? Discuss why.

3. Before the crystal structure of orotidine 5'-monophosphate decarboxylase (ODCase) was solved in 2000, three alternative mechanisms for the decarboxylation of OMP were considered likely. While the atomic-level analysis of this enzyme has helped to disprove some of these mechanisms, the molecular mechanism of catalysis by this highly proficient enzyme remains largely unsolved.

1. Draw (with appropriate chemical structures) the three alternative mechanisms that were considered prior to availability of the crystal structure. If you cannot find out from the literature what these three mechanisms were, you can come up with your own hypotheses.
2. Using molecular visualization software (SYBYL, PyMOL, any of your choice), prepare an image of the ODCase active site showing the bound nucleobase and the residues that are in direct contact with the nucleobase. You do not need to show the sugar nor 5'-phosphate moieties. Please do not use images that somebody else has made.
3. Discuss how the crystal structures of ODCase complexed with inhibitors disprove some of the mechanisms that you outlined earlier.

Project development.
You will be submitting a drug design proposal at the end of this course. As a second part of this project, you are expected to validate one (or few) targets pertaining to the disease that you want to work on. As part of your second assignment, present and critically discuss the evidence that validates your target(s). Make sure to read and reference the original research that provided validating data, and discuss limitations of such studies.

Some students may wish to work on a project where the target is unknown. For example, you may have heard or read about a surprisingly valuable side-effect of a currently existing drug and want to design a combinatorial library around this structure to find compounds that show stronger "side-effect". In this case, please describe what approaches you would take to identify the target of your drug.

Please see "Drug Design project tips" document for more information on how to approach writing the final project.