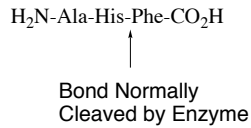
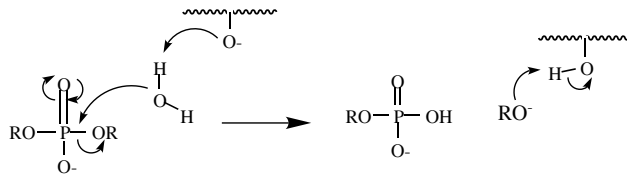


## Homework Three, Enzymes 4170

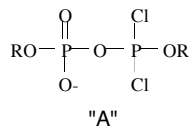
1. Consider the following (imaginary) example. An endogenous (naturally occurring) peptide called intelligensin is shown below. Intelligensin is responsible for learning and storage of memories in mammals. Some animals, such as Golden Retrievers, seem to express high levels of an enzyme that degrades intelligensin. This enzyme is called intelligensinase, it is a protease enzyme that degrades the peptide as shown below. Design an affinity-labeling agent for intelligensinase.



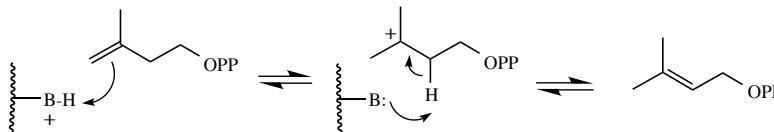
- Design a peptidomimetic reversible inhibitor for the protease described above (intelligensinase).
- What is the expected biological effect of the inhibitor described above? Will it make my dog smarter or (even) less intelligent?
- Enzymes called phosphodiesterases catalyze the hydrolysis of phosphodiesters as shown below.



Offer a mechanism that explains why "A" is a mechanism-based inactivator of phosphodiesterases. Show exact how the enzyme is covalently modified. (Hint One: enzyme inactivators are usually electrophiles... what is the electrophile generated here? Hint Two: remember the reactivity of acid chlorides that we saw in lecture:  $\text{RCOCl}$ .)

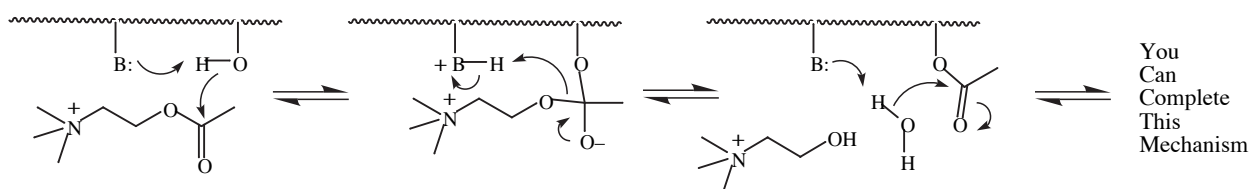


- When taking a reversible enzyme inhibitor, why is it necessary to take frequent doses?
- Design a transition state analog inhibitor for isopentenyl phosphate isomerase, whose mechanism is shown below.



7. Use a reaction coordinate diagram (plot of  $\Delta G$  vs rxn coordinate) to graphically depict two modes of enzymatic catalysis: transition state stabilization and substrate destabilization by strain and distortion.

8. Acetylcholinesterase is the enzyme responsible for deactivating (destroying) the neurotransmitter acetylcholine. The mechanism of this enzyme is shown below.



(a) using principles developed in this course, design a reversible inhibitor of this enzyme

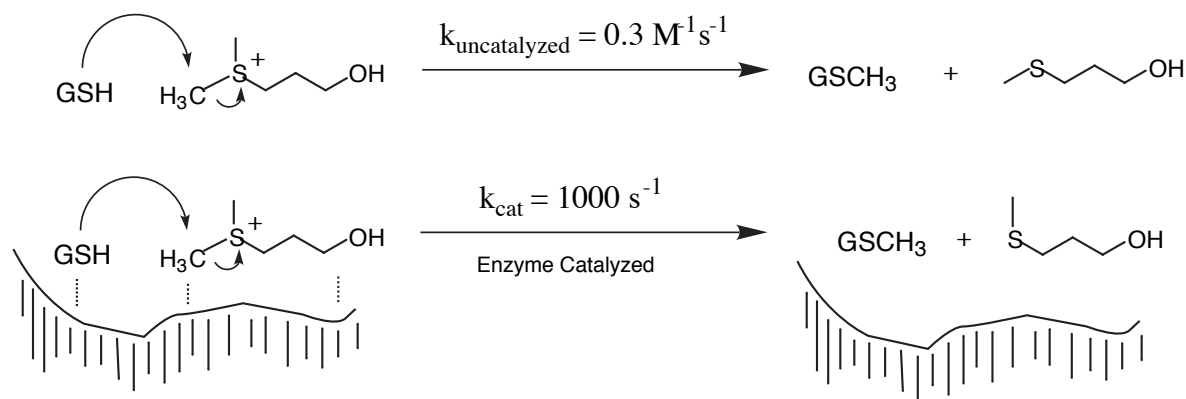
(b) design an affinity labelling agent for this enzyme (not the one I showed in class)

9. The  $K_i$  of drug **A** for its target enzyme is 6 nM. The  $K_i$  of drug **B** for its target enzyme is 10  $\mu$ M.

(a) based upon the data that you are given, which drug would you predict to be more potent?

(b) Let's assume that these drugs diffuse completely and randomly through all the fluid space of the human body. Let's also assume that there are 50 liters of fluid in a human (as we have calculated before). How much drug **A** will you need to administer to achieve 50% inhibition of the target enzyme? How much drug **B**? ASSUME THAT AN "AVERAGE" DRUG HAS A MW = 250 gm/mol. You may need to look at the handouts on enzyme kinetics that are posted on the course website.

10. One way that enzymes catalyze chemical reactions is by lowering the entropy of activation. In lecture, and in your textbook, this has been called "catalysis by proximity". A high "local concentration" of the reaction partners is created by holding the two molecules together in close proximity. This effect is quantitatively assessed using a term called "effective molarity". Consider the reactions of glutathione (GSH) with the sulfonium ion shown below and calculate the effective molarity of glutathione in the enzyme-catalyzed reaction.



Again, look at the handouts on enzyme kinetics posted on the course website...

11. For the system:



The reciprocal of an expanded form of the Michaelis-Menten equation is often written as follows:

$$\frac{1}{v} = \frac{k_{-1} + k_3 + k_1[S]}{k_1 k_3 [E_T][S]}$$

where: S is the substrate,  $E_T$  is the total enzyme, and v is defined as:

$$v = \frac{dP}{dt} = -\frac{dS}{dt}$$

a. Define the terms  $K_m$  and  $V_{max}$  using the parameters that are shown above.

b. Rewrite the Michaelis-Menten equation using the terms  $K_m$  and  $V_{max}$ .

c. Draw a plot and annotate it so that it shows how you can obtain the values of  $K_m$  and  $V_{max}$  from a series of measurements of the initial reaction velocities  $v_o$  at a set of initial  $[S_o]$ , in other words  $v_o [S_o]$  pairs.

12. Look at the handout regarding the kinetics of enzyme inhibition (posted on the website). Under the following conditions, calculate the enzyme velocity ( $v_0$ ) in terms of  $V_{\max}$ .

$$\text{enzyme } K_M = 1 \times 10^{-3} \text{ M}$$

$$[S] = 1 \times 10^{-3} \text{ M (1 mM)}$$

$$\text{drug } K_i = 1 \times 10^{-6} \text{ M}$$

$$[\text{Drug}] = 1 \times 10^{-6} \text{ M (1 } \mu\text{M)}$$

13. Enzymes (or any catalyst) accelerate reaction rates by decreasing the free energy of activation for the reaction in question. (a) show this on a reaction-coordinate energy diagram. (b) If an enzyme is able to decrease the energy of the transition state by, say, 5 kcal/mol *how much would this increase the reaction rate at physiological temperature?* For this question, you may need to remember that the Arrhenius equation provides a way to relate energy of activation (E) to reaction rates. In this equation, the parameter "A" can be taken as a constant (whose value you do not need to worry about).

$$\text{Arrhenius Eqn: } k = Ae^{(-E/RT)}$$

For additional problems related to enzyme-targeted drugs, work problems 1-15 on pages 218-221 and problems 1-14 on pages 303-307, in Silverman's text.