20.320 Problem Set 6
October 23, 2009

General Instructions:

1. You are expected to state all your assumptions and provide step-by-step solutions to the numerical problems. Unless indicated otherwise, the computational problems may be solved using Python/MATLAB or hand-solved showing all calculations. Both the results of any calculations and the corresponding code must be printed and attached to the solutions. **For ease of grading (and in order to receive partial credit), your code must be well organized and thoroughly commented, with meaningful variable names.**

2. You will need to submit the solutions to each problem to a separate mail box, so please prepare your answers appropriately. Staple the pages for each question separately and make sure your name appears on each set of pages. (The problems will get sent to different graders, which should allow us to get the graded problem set back to you more quickly.)

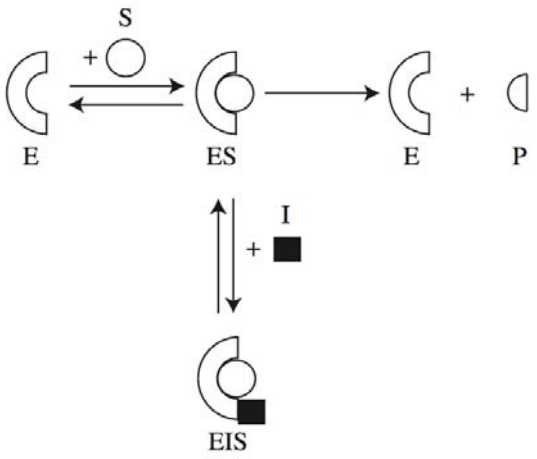
3. Submit your completed problem set to the marked box mounted on the wall of the fourth floor hallway between buildings 8 and 16.

4. This problem set is due at noon on Friday, October 30th. There will be no extensions of deadlines for any problem sets in 20.320. Late submissions will not be accepted.

5. Please review the information about acceptable forms of collaboration, which was provided on the first day of class and follow the guidelines carefully.

20.320 Problem Set 6
Question 1

Uncompetitive enzyme inhibitors bind to a site distant from the active site on the enzyme-substrate complex and allosterically inhibit catalysis. A schematic of this process is shown below (Figure 6.19 from Wittrup and Tidor).



A) Write a system of Ordinary Differential Equations to describe the dynamics of uncompetitive inhibition. Label the above schematic with the rate constants you use in your equations. You should have one differential equation for each species in the system.

B) Derive the Michaelis-Menten equation for reaction velocity in terms of $[S_0]$, [I], $[E_0]$, and the relevant rate and equilibrium constants. **Clearly state the assumptions you make in your derivation.**

C) Based on your answer to Part B), describe the effect of an uncompetitive inhibitor on the v_{max} and overall K_M of the reaction. What scaling factor(s) are applied to these terms?

D) Given K_I = 75 nM, K_M = 25 μM and $[S_0]$ = 5 mM, what concentration of inhibitor is needed to achieve IC_{50}?

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20.320 Problem Set 6
Question 2

In order to estimate the kinetics for a given enzyme-substrate reaction, an *in vitro* reaction is typically set up with a reporter for product formation. For instance*, in vitro* kinase reactions typically use ^{32}P, a radioactive isotope of phosphate in the γ-position of ATP, and then measure the amount of radioactivity incorporated into the product. Although most of these reactions are performed with high substrate:enzyme ratio, often it is difficult to obtain large amounts (or large concentration) of substrate.

Consider a single-substrate enzymatic reaction with no inhibition and the following parameters:

- Reaction volume: 100 µL
- Initial substrate concentration: 5 µM
- Enzyme concentration: 0.5 µM
- $k_1 = 3 \times 10^5$ L $mol^{-1}sec^{-1}$
- $k_{-1} = 5$ sec^{-1}
- $k_{cat} = 3$ sec^{-1}

A) Under the above conditions, calculate the characteristic time for this system to reach quasi-steady state.

B) What is the characteristic time to deplete substrate under these conditions?

C) Use MATLAB to compare the kinetics of product formation in this system with and without applying the Michaelis-Menten approximation.

   i. For simulating the reaction with no approximations, use `ode23s` to solve the representative system of differential equations with the appropriate initial conditions. Simulate the system under Michaelis-Menten conditions by simplifying your equations with the appropriate assumptions. Plot product formation over time for the first minute of the reaction on the same axes for both simulations.

   ii. Based on your plot and on the criteria discussed in class, evaluate the validity of the Michaelis-Menten approximation under these conditions. Discuss which assumptions hold and which do not. Why are your curves different?

   iii. Change an aspect of the original system (either a rate constant or an initial condition) such that the Michaelis-Menten approximation is valid for this time scale. On a new plot, overlay your two curves to show they are the same.

20.320 Problem Set 6
Question 3

Enzymes can typically catalyze reactions involving many different substrates, and can therefore be used to produce multiple products. Often these reactions have different K_M and k_{cat} values, which provides a degree of specificity. This problem will examine the effects of competition for enzyme binding on the enzyme's substrate specificity.

A) Provide a schematic diagram and write out the differential equations with the appropriate rate constants for two substrates reacting with the same enzyme to form two different products. Assume that the enzyme has one active site that can be occupied by a single substrate molecule at a time.

B) To estimate the temporal effects as well as the specificity effects, we will compare the level of product formation for each substrate at various times up 100 s, in the presence and absence of competition. Using an initial enzyme concentration of 50 μM and an initial substrate concentration of 175 μM for each substrate, graph the formation of product 1 assuming no product 2 is formed, product 2 assuming no product 1 is formed, and product 1 and product 2 assuming that the other can be formed on the same graph in MATLAB (you should have 4 lines total on the graph) for the time period of 0 to 100 seconds. Use the following rate constants:

Rate of association between Enzyme and Substrate 1:	5×10^{3} $M^{-1}s^{-1}$
Rate of dissociation of the Enzyme–Substrate 1 complex:	3×10^{1} s^{-1}
Rate of formation of Product 1 from Enzyme–Substrate 1 complex:	2×10^{1} s^{-1}
Rate of association between Enzyme and Substrate 2:	2×10^{6} $M^{-1}s^{-1}$
Rate of dissociation of the Enzyme–Substrate 2 complex:	2×10^{1} s^{-1}
Rate of formation of Product 2 from Enzyme–Substrate 2 complex:	2×10^{-1} s^{-1}

C) Compare the concentration of each product at a time of 20 seconds as the enzyme concentration increases from 1 to 100 μM, repeat for each substrate in the absence of competition, then repeat with both substrates together as in Part B).

D) Explain the shape of the shape of the curve of product 1 formation in Parts B) and C). What type of inhibition is the early part of the curve analogous to? How does the overall curve shape from this type of inhibition differ with the curves you produced and why?

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