BMB428 — Dr. Song Tan
Physical Chemistry with Biological Applications — Fall 2008

<u>Problem Set 24: Enzyme Kinetics - the Basics</u> — Due Dec 2, 2008

1. Consider the following set of elementary steps for an enzyme catalyzed reaction:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\bullet}S$$

$$E{\bullet}S \xrightarrow{k_2} E + P$$

   Using the same techniques that we applied to deriving rate laws for chemical reactions, derive an expression for d[P]/dt. Your answer should contain the term $\frac{k_{-1} + k_2}{k_1}$ which we will define as K_m, the Michaelis-Menten constant.

2. The equation that you derived in question 1 is not particularly useful since it (hopefully) has a term with the free enzyme concentration, [E]. Since enzyme concentrations are typically VERY low during steady state kinetics, it is difficult to determine what fraction of the enzyme has no substrate bound, E, and what fraction is in the E•S form. Fortunately, it is possible to eliminate the [E] term. Use the equation $[E]_0 = [E] + [E{\bullet}S]$ to derive a new equation for d[P]/dt. $[E]_0$ is the total (initial) enzyme concentration that is added to the reaction. It is usually easy to measure $[E]_0$ experimentally. You should substitute this new equation into the expression that you wrote for d[ES]/dt and then rewrite the equation for d[P]/dt.

3. Problem 10.5 (p. 398)
   An enzyme that has a K_M value of 3.9×10^{-5} M is studied at an initial substrate concentration of 0.035 M. After 1 min, it is found that 6.2 μM of product has been produced. Calculate the value of V_{max} and the amount of product formed after 4.5 min. Assume Michaelis-Menten kinetics apply.

4. The following data were obtained for an enzyme-catalyzed reaction following Michaelis-Menten kinetics as shown in question 1:

   k_1 and k_{-1} are very fast (much faster than k_2)
   $k_2 = 100\ s^{-1}$, $K_M = 1.0 \times 10^{-4}$ M at 280 K
   $k_2 = 200\ s^{-1}$, $K_M = 1.5 \times 10^{-4}$ M at 300 K

   (a) For [S] = 0.10 M and $[E]_0 = 1.0 \times 10^{-5}$ M, calculate the rate of formation of product at 280 K.
   (b) Calculate the activation energy for k_2 (use the Arrhenius equation).
   (c) What is the value of the equilibrium constant at 280 K for the formation of the enzyme-substrate complex ES from E and S?
   (d) What is the sign and magnitude of the standard thermodynamic enthalpy $\Delta H°$ for the formation of ES from E and S?