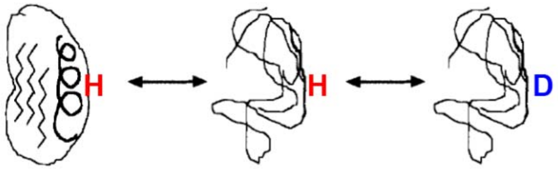
Biophysical Chemistry – CH 4403 01
Assignment 8 (60 points)

Due Friday, November 7 at 4:30 pm

1. Tinoco Chapter 9, question #2 (10 points)

2. Tinoco Chapter 9, question #24. (5 points)

3. Under equilibrium conditions, proteins are constantly folding and refolding. The backbone amide proton is labile, and therefore if a protein is placed in a solution of D_2O, individual backbone H_N atoms will exchange to become deuterium over time. This process is called H-D exchange, and it is a common technique used to study protein structure. Schematically, it is illustrated below (adapted from Englander, S. W., *et al.* (1997) *Protein Sci.* **6**: 1101.):



Analytically, this effect can be measured using mass spectrometry or NMR spectroscopy. The following mechanism is used to describe the process of H-D exchange:

$$PH \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} P^*H$$

$$P^*H + OH^- + D_2O \xrightarrow{k_2} P^*D + OH^- + H_2O$$

In the model shown above, the protein unfolds (P to P^*), exposing the amide proton. Then, the base (OH^-) catalyzes exchange between the proton and the deuteron. Since D_2O is in excess, it's extremely unlikely that a proton will exchange back, so the second step in the mechanism is unidirectional.

a. Write differential equations to describe how [PH], [P^*H], and [P^*D] change with time. The concentration of D_2O is high enough that it is effectively zero-order, so none of your equations should contain [D_2O]. (4 points)

b. Under steady-state conditions ($\frac{d[P^*H]}{dt} = 0$), the initial rate of exchange is given by the following equation:

$$\frac{d[P^*D]}{dt} = k_{ex}[PH], \text{ where } k_{ex} = \frac{k_2 k_1 [OH^-]}{k_{-1} + k_2[OH^-]}$$

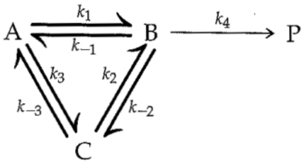
Show that this equation is true. (*Hint:* Set $\frac{d[P^*H]}{dt}$ to zero and solve for [P^*H].) (5 points)

c. How does your expression for k_{ex} in (b) simplify in the limit that $k_2[OH^-] << k_{-1}$? When this is true, exchange is said to be in the EX2 regime. (1 point)

d. How does your expression for k_{ex} in (b) simplify in the limit that $k_2[OH^-] >> k_{-1}$? When this is true, exchange is said to be in the EX1 regime. (1 point)

e. Experimentally, it is possible to measure k_{ex} and [OH$^-$]. Devise a way to linearize the expression in for k_{ex} in part (b) so that, by measuring k_{ex} at several different values of [OH$^-$], you could obtain k_1 (the rate of unfolding) from a linear fit. (*Hint:* Consider the expression for k_{ex}^{-1}.) (4 points)

4. Consider the following proposed mechanism for the reaction A → P:



a. Write the differential equation for the rate of formation of B. (4 points)

b. The formation of B from A and C is fast to equilibrium, with equilibrium constants $K_1 = k_1/k_{-1}$, $K_2 = k_2/k_{-2}$, and $K_3 = k_3/k_{-3}$. k_4 is much smaller than all the other k's. Write a differential equation for the formation of P in terms of the concentration of A, equilibrium constants, and k_4. (3 points)

c. Write an expression (containing no derivatives) for the concentration of P as a function of time. At zero time, $[A] = A_0$, and $[B]$, $[C]$, and $[P]$ are all zero. (8 points)

Hint #1: Since you are given a starting value for A_0, use conservation of mass to relate the amount of product formed (P) to the initial concentration of A_0 and the time-dependent concentrations of A, B, and C. Then, use the fast equilibrium approximation to eliminate the concentrations of $[B]$ and $[C]$ from your expression above. This will allow you to determine the concentration of A after fast equilibrium has been established, but before any P has been formed.

Hint #2: Differentiate the conservation of mass expression with respect to time to relate $\frac{dP}{dt}$ and $\frac{dA}{dt}$. This expression, combined with part (b) and the knowledge that A, B, and C are in fast equilibrium, can be used to derive a simple first-order differential equation for [A] that you can solve. Finally, once you know $[A]$, the concentration of P can be calculated from your conservation of mass equation. (8 points)

5. Tinoco Chapter 9, question #37. For part (f), show that the initial slope of your curve is equivalent to the answer you stated in part (e) when the initial concentration of B is zero. You may find it helpful to remember that, when *t* is close to zero, $e^{-kt} \approx 1 - kt$. (15 points)