Biophysical Chemistry – CH 4404 01
Assignment 8

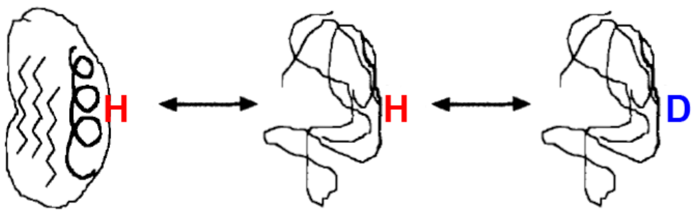
Due Friday, November 9 at 5pm

Please complete the answers to this assignment on a separate page (or pages), showing your work and sources (if you referred elsewhere for constants, enthalpies, etc.).

1. Tinoco Chapter 7, question #2 (10 points)

2. Tinoco Chapter 7, question #25. (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. Tinoco Chapter 7, question #15. *Hint:* For part (c), since you are given a starting value for A_0, use conservation of mass to relate the amount of product formed to the total concentrations of A, B, and C. Then, differentiate with respect to time to relate $\frac{dP}{dt}$ to $\frac{dA}{dt}$, $\frac{dB}{dt}$, and $\frac{dC}{dt}$. 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 differential equation for [A] that you can integrate and solve. (15 points)

5. Tinoco Chapter 7, question #29. Be sure to write the elementary steps you used in deriving your mechanism in part (a). *Hint*: Think about what would happen if B could be formed irreversibly by two different pathways, depending on whether A was protonated. (10 points)