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$_2$O, 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):

![H-D exchange diagram]

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:

\[
\begin{align*}
&k_1 \\
&P_{\text{H}} \rightleftharpoons P^*_{\text{H}} \\
&k_{-1} \\
&P_{\text{H}} + \text{OH}^- + D_2O \rightarrow P^*_{\text{D}} + \text{OH}^- + H_2O
\end{align*}
\]

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$_2$O 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$_2$O is high enough that it is effectively zero-order, so none of your equations should contain [D$_2$O]. (4 points)

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

\[
\frac{d[P^*D]}{dt} = k_{ex}[PH], \text{ where } k_{ex} = \frac{k_2k_1[OH^-]}{k_{-1} + k_2[OH^-]}
\]

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 \( k_2[OH^-] \ll k_1 \)? 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 \( k_2[OH^-] \gg k_1 \)? 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 \( A \rightarrow P \):

\[
A \xrightleftharpoons[k_1]{k_2} B \xrightarrow[k_3]{k_4} P
\]

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 \( K_1 = \frac{k_1}{k_{-1}} \), \( K_2 = \frac{k_2}{k_{-2}} \), and \( K_3 = \frac{k_3}{k_{-3}} \). \( k_4 \) 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)

\( \text{Hint #1: } \) 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.

\( \text{Hint #2: } \) 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)