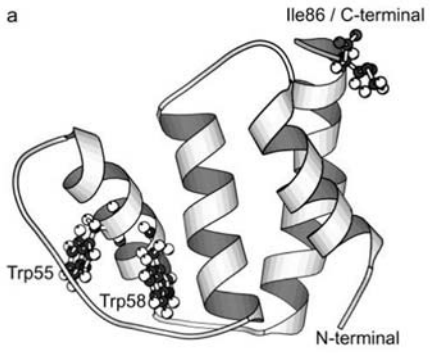
"Kinetic; Simbiology (II)"

The purpose of this lab is to compare two- and three-state models of protein folding kinetics using the steady-state approximation. This exercise is based on the following paper:

"Teilum, K., Maki, K., Kragelund, B. B., Poulsen, F. M., & Roder, H. (2002). Early kinetic intermediate in the folding of acyl-CoA binding protein detected by fluorescence labeling and ultrarapid mixing. Proceedings of the National Academy of Sciences of the United States of America, 99(15), 9807–9812. doi:10.1073/pnas.152321499"



In this work, the folding kinetics of an 86-residue four helix-bundle protein called ACBP (acyl-CoA binding protein) was measured using time-resolved fluorescence spectroscopy (monitoring UV tryptophan fluorescence at 280 nm), after fast dilution from chemical denaturant (guanidinium hydrochloride, GuHCl) in a capillary mixer. The authors find that their data can be fit to the following kinetic model:

$$U \underset{k_{IU}}{\overset{k_{UI}}{\rightleftharpoons}} I \underset{k_{NI}}{\overset{k_{IN}}{\rightleftharpoons}} N$$

where U is a highly fluorescent unfolded state, I is a partially folded intermediate, and N is the folded state.

Procedure:

Part A— Use Simbiology to simulate this kinetic model:

Use SimBiology to construct a model of the three-state folding kinetics, using the following kinetic rate parameters determined by the authors at 299 K:

$k_{UI} = 11000\ s^{-1}$ $\quad\rightleftharpoons\quad$ $k_{IU} = 7000\ s^{-1}$

$k_{IN} = 650\ s^{-1}$ $\quad\rightleftharpoons\quad$ $k_{NI} = 0.07\ s^{-1}$

Using initial concentrations of [U] = 10.0, [I] = 0.0, and [N] = 10.0, visualize the concentrations of each species over time. (Set stop time = 0.045 s for your simulation). Answer questions #1 and #2.

Part B— Modeling the two-state mechanism using steady-state approximation:
Let's now apply the steady-state approximation to this three-state model. This approximation is a simplification saying that the concentration of the intermediate [I] is constant throughout the reaction. Since the formation of the intermediate is fast compared to the slower, rate-limiting step of folding to the native state N, the steady-state approximation is still fairly accurate. To find the steady-state approximation, we go through the following steps:

1. Write down an expression for $d[I]/dt$:

$$d[I]/dt = -k_{IU}[I] - k_{IN}[I] + k_{UI}[U] + k_{NI}[I]$$

2. Solve $d[I]/dt = 0$ (i.e. steady-state conditions) to get [I] as a function of [U] and [N] :

$$\begin{aligned} 0 &= -k_{IU}[I] - k_{IN}[I] + k_{UI}[U] + k_{NI}[N] \\ [I](k_{IU} + k_{IN}) &= k_{UI}[U] + k_{NI}[N] \\ [I] &= (k_{UI}[U] + k_{NI}[N])/(k_{IU} + k_{IN}) \end{aligned}$$

3. Next, we plug this expression for [I] into the rate laws for [U] and [N]:

$$\begin{aligned} d[U]/dt &= -k_{UI}[U] + k_{IU}[I] \\ &= -k_{UI}[U] + k_{IU}\,(k_{UI}[U] + k_{NI}[N])/(k_{IU} + k_{IN}) \end{aligned}$$

$$\begin{aligned} d[N]/dt &= -k_{NI}[N] + k_{IN}[I] \\ &= -k_{NI}[N] + k_{IN}\,(k_{UI}[U] + k_{NI}[N])/(k_{IU} + k_{IN}) \end{aligned}$$

4. Finally, to get rates for a two-state U ⇌ N model of folding, consider that at equilibrium, $d[U]/dt = d[N]/dt = 0$. Set the two expressions for $d[U]/dt$ and $d[N]/dt$ above to be equal to each other, and rearrange the equation to get it in the following form:

$$k_{forward}\,[U] = k_{backward}[N]$$

Answer questions #3, #4 and #5.

Name: **TUID:**

Section:

Part A:

Q1. Using the results of your SimBiology model, estimate the populations (concentration) of U and N at equilibrium.

Q2. Estimate the equilibrium constant and free energy of folding.

$$K_{eq} = \frac{[N]_{eq}}{[U]_{eq}} \qquad \Delta G^{\circ} = -RTLn(K_{eq}) \qquad R = 8.314\ \frac{j}{mol\ K}, T = 299\ K$$

Part B: Q3. What are your computed values for $k_{forward}$ and $k_{backward}$? (You need to derive the following equations using step 4 in part B. Then, calculate the values of $k_{forward}$ and $k_{backward}$).

$$k_{forward} = k_{IN}\left(\frac{k_{UI}}{k_{IU} + k_{IN}}\right)$$

$$k_{backward} = k_{NI}\left(\frac{k_{IU}}{k_{IU} + k_{IN}}\right)$$

Q4. Estimate from $k_{forward}$ and $k_{backward}$ the equilibrium constant and free energy of folding.

$$K_{eq} = \frac{k_{forward}}{k_{backward}}$$

Q5. Compare your values of free energy of folding calculated in Q2 (Simbiology simulation) and Q4 (steady-state approximation)?