# Two-state folding process

The rate data and thermodynamic data for the unfolding of the protein racemase is given below. Determine the rate constants for folding and unfolding. The reaction is a two-state process shown below:

$$F \leftarrow\rightarrow U$$

| Temperature (K) | Equilibrium Constant | Observed Unfolding Rate |
|---|---|---|
| 310 | 1.2 | $1.3 \times 10^3$ s$^{-1}$ |
| 340 | 5.6 | $3.2 \times 10^4$ s$^{-1}$ |

A. Calculate the rate constant for folding and unfolding at each temperature.
B. Calculate the activation energy for the folding and unfolding processes.

# Two-state folding process

A. Calculate the rate constant for folding and unfolding at each temperature.

| Temperature (K) | Equilibrium Constant | Observed Unfolding Rate |
|---|---|---|
| 310 | 1.2 | $1.3 \times 10^{3}\ s^{-1}$ |
| 340 | 5.6 | $3.2 \times 10^{4}\ s^{-1}$ |

Solution: Solve two equations and two unknowns to obtain the intrinsic rate constants.

$$K = \frac{k_u}{k_f} \quad ; \quad k_{obs} = k_u + k_f$$

$$k_u = k_{obs} - k_f$$

$$Kk_f = k_{obs} - k_f$$

$$(K+1)k_f = k_{obs}$$

$$k_f = \frac{k_{obs}}{K+1} = \frac{1300\ s^{-1}}{2.2} = 590\ s^{-1}\ \ at\ 310\ K$$

# Two-state folding process

A. Calculate the rate constant for folding and unfolding at each temperature.

| Temperature (K) | Equilibrium Constant | Observed Unfolding Rate |
|---|---|---|
| 310 | 1.2 | $1.3 \times 10^3\ s^{-1}$ |
| 340 | 5.6 | $3.2 \times 10^4\ s^{-1}$ |

$$k_f = \frac{k_{obs}}{K+1} = \frac{1300\ s^{-1}}{2.2} = 590\ s^{-1}\ \ at\ 310\ K$$

$$k_u = k_{obs} - k_f = 1300 - 590 = 710\ s^{-1}\ at\ 310\ K$$

$$k_f = \frac{k_{obs}}{K+1} = \frac{3.2\ x\ 10^4\ s^{-1}}{6.6} = 4800\ s^{-1}\ \ at\ 340\ K$$

$$k_u = k_{obs} - k_f = 32000 - 4800 = 27200\ s^{-1}\ at\ 340\ K$$

# Two-state folding process

B. Calculate the activation energy for the folding and unfolding processes.

Solution: Using the rate constants calculated in part A use the Arrhenius equations for each pair to obtain the activation energy.

$$E_a = \frac{-R \ln \frac{k_2}{k_1}}{\left(\frac{1}{T_2} - \frac{1}{T_1}\right)}$$

For folding we have

$$E_{a,f} = \frac{-R \ln \frac{5400}{590}}{\left(\frac{1}{340} - \frac{1}{310}\right)} = 64.6\ kJ/mol$$

# Two-state folding process

B. Calculate the activation energy for the folding and unfolding processes.

Solution: Using the rate constants calculated in part A use the Arrhenius equations for each pair to obtain the activation energy.

$$E_a = \frac{-R \ln \frac{k_2}{k_1}}{\left(\frac{1}{T_2} - \frac{1}{T_1}\right)}$$

And for unfolding we have

$$E_{a,u} = \frac{-R \ln \frac{27200}{710}}{\left(\frac{1}{340} - \frac{1}{310}\right)} = 106.4\ kJ/mol$$