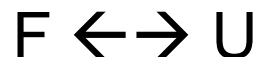


Two-state folding process

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:



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

- Calculate the rate constant for folding and unfolding at each temperature.
- 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	$1.3 \times 10^3 \text{ s}^{-1}$
340	5.6	$3.2 \times 10^4 \text{ s}^{-1}$

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

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

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

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

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

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

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	$1.3 \times 10^3 \text{ s}^{-1}$
340	5.6	$3.2 \times 10^4 \text{ s}^{-1}$

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

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

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

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

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

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

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

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