

Please answer all questions on a separate sheet of paper.

### I. Short essay

1. Describe the theory and practice of the polymerase chain reaction (PCR). What is the role of the primer? Why type of enzyme(s) is(are) used? What are applications of PCR?
2. Describe the methods and applications of chemiluminescence and fluorescence. What is the quantum yield for chemiluminescence? How does the quantum yield for fluorescence compare to that of chemiluminescence? Give an example of each phenomenon and describe how it works? What are applications of these methods in cell biology?

### II. Numerical problems

1. You are provided a van't Hoff plot and thermodynamic data for villin headpiece.
  - a. calculate the molar enthalpy of unfolding.

| ln Keq | 1/T                   |
|--------|-----------------------|
| 2.30   | $2.72 \times 10^{-3}$ |
| -2.9   | $3.30 \times 10^{-3}$ |

Assuming a two state model and using the two data points given determine the enthalpy of unfolding for the villin headpiece ( $R = 8.31 \text{ J/mol}\cdot\text{K}$ ).

Solution: the enthalpy of unfolding is provided by the slope =  $-\Delta H^\circ/R$ .

$$\text{Slope} = (-2.9 - 2.3)/(3.3 - 2.72) \times 1000 = -8655$$

$$\Delta H^\circ = R(-\text{Slope}) = 8.31(8655) = 74.5 \text{ kJ/mol}$$

- b. Again assuming a two-state model calculate the equilibrium constant and the unfolding and folding rate constants given the following data.

| T (°C) | $\Delta G^\circ$ (kJ/mol) | $k_{\text{obs}}$ ( $\text{s}^{-1}$ ) |
|--------|---------------------------|--------------------------------------|
| 30     | 6.325                     | $2.4 \times 10^5$                    |
| 90     | -7.175                    | $8.9 \times 10^5$                    |

Solution: Calculate the equilibrium constants

$$T_1 = 30 \text{ }^\circ\text{C} : K_1 = \exp\{-\Delta G_1^\circ/RT\} = \exp\{-6325/8.31/303\} = 0.0811$$

$$T_2 = 90 \text{ }^\circ\text{C} : K_2 = \exp\{-\Delta G_2^\circ/RT\} = \exp\{7175/8.31/303\} = 17.28$$

Using the fact that  $K = k_u/k_f$  and  $k_{\text{obs}} = k_u + k_f$  we have

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

$$K = k_u/(k_{\text{obs}} - k_u)$$

$$(k_{\text{obs}} - k_u)K = k_u$$

$$Kk_{\text{obs}} = k_u + k_u K = k_u(1 + K)$$

$$k_u = k_{\text{obs}}K/(1 + K) \text{ and } k_f = k_{\text{obs}}/(1 + K)$$

Therefore:

At  $T_1 = 30^\circ\text{C}$  :

$$k_u = (2.4 \times 10^5 \text{ s}^{-1})0.0811/1.0811 = 18000 \text{ s}^{-1}$$

$$k_f = (2.4 \times 10^5 \text{ s}^{-1})/1.0811 = 2.22 \times 10^5 \text{ s}^{-1}$$

$T_2 = 90^\circ\text{C}$ :

$$k_u = (8.9 \times 10^5 \text{ s}^{-1})17.28/18.28 = 8.43 \times 10^5 \text{ s}^{-1}$$

$$k_f = (8.9 \times 10^5 \text{ s}^{-1})/18.28 = 48700 \text{ s}^{-1}$$

c. Using the values of the rate constants for unfolding at two temperatures calculate the activation energy for the unfolding process using the Arrhenius equation.

Solution: The Arrhenius equation can be expressed as follows

$$\ln(k_2/k_1) = -E_a/R(1/T_2 - 1/T_1)$$

which is a form that is entirely analogous to the van't Hoff equation used in part a.

Thus,

$$E_a = -R \ln(k_2/k_1) / (1/T_2 - 1/T_1)$$

For the unfolding reaction

$$E_a = -8.31 \ln(8.43 \times 10^5 \text{ s}^{-1}/18000 \text{ s}^{-1}) / (1/363 - 1/303) = -58.5 \text{ kJ/mol}$$

For the folding reaction

$$E_a = -8.31 \ln(48700/2.22 \times 10^5 \text{ s}^{-1}) / (1/363 - 1/303) = 23.1 \text{ kJ/mol}$$

2. An enzyme that catalyzes the reaction  $S \rightarrow P$  was analyzed at several initial  $[S]$ . The measured values of the initial rate  $V_0$  are:

| $[S]$ (M)             | $V_0$ (mmol/liter/min) |
|-----------------------|------------------------|
| $10^{-2}$             | 75                     |
| $10^{-3}$             | 74.9                   |
| $10^{-4}$             | 60.0                   |
| $7.5 \times 10^{-5}$  | 56.25                  |
| $6.25 \times 10^{-6}$ | 15.0                   |

a. Determine  $K_M$  and  $V_{\text{max}}$  for the enzyme

Solution:  $V_{\text{max}}$  is given by the entry in the table with the highest  $[S]$ .

Therefore,  $V_{\text{max}} = 75 \text{ mmol/liter/min}$ .

Given this value we can determine  $K_M$  using the data at some lower value of  $[S]$

$$V_0 = V_{\text{max}}[S]/K_M + [S]$$

Solving for  $K_M$  we obtain

$$V_0(K_M + [S]) = V_{\text{max}}[S]$$

$$V_0 K_M = (V_{\text{max}} - V_0)[S]$$

$$K_M = (V_{\text{max}} - V_0)/V_0[S]$$

Using the lowest concentration for  $[S]$

$$K_M = (75 - 15)/15[6.25 \times 10^{-6}] = 2.5 \times 10^{-5} \text{ M}$$

b. What would  $V_0$  be if the initial  $[S] = 2.5 \times 10^{-5} \text{ M}$ ?

Solution: Since  $[S] = K_M$  for this value this corresponds to  $V_{1/2} = 1/2V_{\max}$ .  
 $V_{1/2} = 37.5$  mmol/liter/min.

- c. What would be the  $V_0$  if the initial  $[S] = 10^{-4}$  M and the enzyme concentration were doubled?

Solution: recall that  $V_{\max} = k_b[E_0]$  so that if the enzyme concentration is doubled the maximum rate is also doubled. Therefore,  $V_{\max} = 150$  mmol/liter/min under these conditions.

$$V_0 = V_{\max}[S]/K_M + [S] = 150(10^{-4})/(2.5 \times 10^{-5} + 10^{-4}) = 120 \text{ mmol/liter/min}$$