

1. A new fluorescent molecule called "fluoromol" is being studied by a startup company. The molecule is to be used as a label inside cells. The patent application states that the fluorescence quantum yield of the molecule is 0.98. A scientist measures the experimental lifetime for the fluorescence as one nanosecond. Please determine the fluorescence rate constant ( $k_f$ ) and the internal conversion (or non-radiative decay) rate constant ( $k_{IC}$ ).

Solution:  $\tau_{obs} = 1 \text{ ns} = 10^{-9} \text{ seconds}$

$k_{obs} = 1/\tau_{obs} = 10^9 \text{ s}^{-1}$ .  $\Phi_f = 0.98$ .

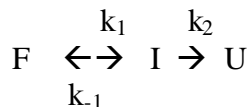
Two equations:  $k_{obs} = k_f + k_{IC}$ ,  $\Phi_f = k_f / (k_f + k_{IC}) = k_f / k_{obs}$

Solve for unknowns:  $k_f = \Phi_f k_{obs} = 0.98(10^9 \text{ s}^{-1}) = 9.8 \times 10^8 \text{ s}^{-1}$

$k_{IC} = k_{obs} - k_f = 10^9 \text{ s}^{-1} - 9.8 \times 10^8 \text{ s}^{-1} = 2.0 \times 10^7 \text{ s}^{-1}$

$k_f =$  \_\_\_\_\_  $k_{IC} =$  \_\_\_\_\_

2. A protein is observed to fold with an intermediate state called I. The experimental rate scheme is:



Fill in the following blanks with the correct rate expressions:

$d[F]/dt =$  \_\_\_\_\_  $-k_1[F] + k_{-1}[I]$  \_\_\_\_\_.

$d[I]/dt =$  \_\_\_\_\_  $k_1[F] - (k_{-1} + k_2)[I]$  \_\_\_\_\_.

$d[U]/dt =$  \_\_\_\_\_  $k_2[I]$  \_\_\_\_\_.

In this scheme the rate constant  $k_2 \gg k_1$  and  $k_2 \sim k_{-1}$ . Write an expression for the rate of unfolding ( $d[U]/dt$ ) in terms of only the concentration of the folded molecule [F].

Solution: Apply the steady-state approximation

$k_1[F] - (k_{-1} + k_2)[I] = 0$

$[I] = k_1[F] / (k_{-1} + k_2)$

Substitute this into:  $d[U]/dt = k_2[I]$

$d[U]/dt =$  \_\_\_\_\_  $k_1 k_2 / (k_{-1} + k_2) [F]$ .

3. Double helical DNA from a 20-mer is formed with an observed rate constant of  $1.1 \times 10^5 \text{ s}^{-1}$  and free energy of  $-5.733 \text{ kJ/mol}$ . The equilibrium is  $2 \text{ ssDNA} \leftrightarrow \text{dsDNA}$ .
- a. Calculate the equilibrium constant at 300 K.

$$K = \exp\{-\Delta G^{\circ}/RT\} = \exp\{-(-5733 \text{ J/mol})/(8.31 \text{ J/mol}\cdot\text{K})(300 \text{ K})\}$$

$$= \exp\{2.299\} = 9.964 \sim 10$$

$$K = \underline{\underline{10}}$$

b. Calculate the folding rate constant.

Two equations:  $k_{\text{obs}} = k_{\text{h}} + k_{\text{d}}$ ,  $K = k_{\text{h}}/k_{\text{d}}$

Solve:  $k_{\text{d}} = k_{\text{h}}/K$ ,  $k_{\text{obs}} = k_{\text{h}} + k_{\text{h}}/K$ ,  $k_{\text{obs}} = k_{\text{h}}(1 + 1/K)$ ,  $k_{\text{h}} = k_{\text{obs}}/(1 + 1/K)$

Substitute:  $k_{\text{h}} = k_{\text{obs}}/(1 + 1/K) = 1.1 \times 10^5 \text{ s}^{-1}/1.1 = 10^5 \text{ s}^{-1}$

$$k_{\text{h}} = \underline{\underline{10^5}} \text{ s}^{-1}$$