Biophysics I (BPHS 4080)

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Chemical Kinetics (v2)

Second-order reversible (binding) reaction

\[ S + E \xrightarrow{\alpha} ES \]
\[ \frac{dc_{ES}(t)}{dt} = \alpha c_s(t)c_E(t) - \beta c_{ES}(t), \]
\[ \frac{dc_s(t)}{dt} = \frac{dc_E(t)}{dt} = \beta c_{ES}(t) - \alpha c_s(t)c_E(t), \]

Equilibrium:
\[ \frac{dc_{ES}(t)}{dt} = \frac{dc_s(t)}{dt} = \frac{dc_E(t)}{dt} = 0 \]
\[ \alpha c_s(\infty)c_E(\infty) - \beta c_{ES}(\infty) = 0 \]
\[ \frac{c_{ES}(\infty)}{c_s(\infty)c_E(\infty)} = \frac{\alpha}{\beta} = K_a \quad \text{(association constant)} \]
\[ \frac{1}{K_a} = \frac{c_s(\infty)c_E(\infty)}{c_{ES}(\infty)} = K \quad \text{(dissociation constant)} \]

Assume enzyme conserved: \( c_E(t) + c_{ES}(t) = C_{ET} \)
How does \( c_{ES} \) depend on \( c_s \)? Eliminate \( c_E \).

\[ C_{ET} = c_E(\infty) + c_{ES}(\infty) \]
\[ C_{ET} = \frac{Kc_{ES}(\infty)}{c_s(\infty)} + c_{ES}(\infty) = \left( \frac{K}{c_s(\infty)} + 1 \right) c_{ES}(\infty) \]
\[ c_{ES}(\infty) = \left( \frac{c_s(\infty)}{K + c_s(\infty)} \right) C_{ET} \]

→ Law of mass action

→ Michaelis-Menten kinetics
Possible ‘Carrier’ Mechanisms

- **Initial State**
  - Mechanism 1: [Diagram]
  - Mechanism 2: [Diagram]
  - Mechanism 3: [Diagram]

- **Binding**
  - Mechanism 1: [Diagram]
  - Mechanism 2: [Diagram]
  - Mechanism 3: [Diagram]

- **Translocation**
  - Mechanism 1: [Diagram]
  - Mechanism 2: [Diagram]
  - Mechanism 3: [Diagram]

- **Release**
  - Mechanism 1: [Diagram]
  - Mechanism 2: [Diagram]
  - Mechanism 3: [Diagram]

- **Reset**
  - Mechanism 1: [Diagram]
  - Mechanism 2: [Diagram]
  - Mechanism 3: [Diagram]
General Four-State Carrier Model

Translocation

Binding & Unbinding
Chemical Kinetics & ‘Carriers’

**Binding**

\[ S^i + E^i \xleftrightarrow{\alpha_1/\beta_1} ES^i \]

\[
\frac{dC^i_{ES}}{dt} = \alpha_1 C^i_S C^i_E - \beta_1 C^i_{ES} \\
\frac{dC^i_S}{dt} = \frac{dC^i_E}{dt} = \beta_1 C^i_{ES} - \alpha_1 C^i_S C^i_E
\]

**Translocation**

\[ ES^i \xleftrightarrow{\alpha_2/\beta_2} ES^o \]

\[
\frac{dC^o_{ES}}{dt} = \alpha_2 C^i_{ES} - \beta_2 C^o_{ES} \\
\frac{dC^i_{ES}}{dt} = \beta_2 C^o_{ES} - \alpha_2 C^i_{ES}
\]
Chemical Kinetics & ‘Carriers’

Unbinding

\[ E \equiv \frac{\beta_3}{\alpha_3} \quad \text{ES}^o \quad \xleftarrow{\beta_3} \quad E^o \quad + \quad S^o \]

\[ \frac{dC_{ES}^o}{dt} = \alpha_3 C_S^o C_E^o - \beta_3 C_{ES}^o \]

\[ \frac{dC_S^o}{dt} = \frac{dC_E^o}{dt} = \beta_3 C_{ES}^o - \alpha_3 C_S^o C_E^o \]

Translocation

\[ E^i \quad \xrightarrow{\beta_4} \quad \alpha_4 \quad E^o \]

\[ \frac{dC_E^o}{dt} = \alpha_4 C_E^i - \beta_4 C_E^o \]

\[ \frac{dC_E^i}{dt} = \beta_4 C_E^o - \alpha_4 C_E^i \]
Numerous free parameters. Can we simplify?

\[
\begin{align*}
\frac{dC_{ES}^i}{dt} &= \alpha_1 C_S^i C_E^i - \beta_1 C_{ES}^i \\
\frac{dC_S^i}{dt} &= \frac{dC_E^i}{dt} = \beta_1 C_{ES}^i - \alpha_1 C_S^i C_E^i \\
\frac{dC_{ES}^o}{dt} &= \alpha_2 C_{ES}^o - \beta_2 C_{ES}^o \\
\frac{dC_E^o}{dt} &= \frac{dC_S^o}{dt} = \beta_3 C_{ES}^o - \alpha_3 C_S^o C_E^o \\
\frac{dC_{ES}^i}{dt} &= \beta_2 C_{ES}^o - \alpha_2 C_{ES}^i \\
\frac{dC_E^i}{dt} &= \beta_4 C_E^o - \alpha_4 C_E^i \\
\end{align*}
\]
Simple, Symmetric Four-State Model

Assumption: Steady-state
(i.e., carrier densities are independent of time)
Simple, Symmetric Four-State Model

1. Conservation of enzyme:

\[ \mathcal{N}_E^i + \mathcal{N}_E^o + \mathcal{N}_{ES}^i + \mathcal{N}_{ES}^o = \mathcal{N}_{ET} \]

2. Binding is fast (always in steady state):

\[ K = \frac{c_S^i \mathcal{N}_E^i}{\mathcal{N}_{ES}^i} = \frac{c_S^o \mathcal{N}_E^o}{\mathcal{N}_{ES}^o} \]

3. Translocation characterized by fluxes:

\[ \phi_{ES} = \alpha \mathcal{N}_{ES}^i - \beta \mathcal{N}_{ES}^o \]
\[ \phi_E = \alpha \mathcal{N}_E^i - \beta \mathcal{N}_E^o \]

4. Net flux of enzyme is zero:

\[ \phi_E + \phi_{ES} = 0 \]

→ Steady-state
(i.e., carrier densities are independent of time)
Simple, Symmetric Four-State Model

\[ \eta^i_E + \eta^o_E + \eta^i_{ES} + \eta^o_{ES} = \eta_{ET} \]

\[ K = \frac{c^i_S \eta^i_E}{\eta^i_{ES}} = \frac{c^o_S \eta^o_E}{\eta^o_{ES}} \]

\[ \phi_{ES} = \alpha \eta^i_{ES} - \beta \eta^o_{ES} \]

\[ \phi_E = \alpha \eta^i_E - \beta \eta^o_E \]

\[ \phi_E + \phi_{ES} = 0 \]

Combining equations...

\[ \eta^i_{ES} = \left( \frac{\beta}{\alpha + \beta} \right) \left( \frac{c^i_S}{c^i_S + K} \right) \eta_{ET} \]

\[ \eta^i_E = \left( \frac{\beta}{\alpha + \beta} \right) \left( \frac{K}{c^i_S + K} \right) \eta_{ET} \]

\[ \eta^o_{ES} = \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{c^o_S}{c^o_S + K} \right) \eta_{ET} \]

\[ \eta^o_E = \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{K}{c^o_S + K} \right) \eta_{ET} \]

Solving for the solute flux yields:

\[ \phi_S = \left( \frac{\alpha \beta}{\alpha + \beta} \right) \eta_{ET} \left( \frac{c^i_S}{c^i_S + K} - \frac{c^o_S}{c^o_S + K} \right) \]
\[ K = \frac{c_s^i \mathcal{N}_E^i}{\mathcal{N}_{ES}^i} = \frac{c_s^o \mathcal{N}_E^o}{\mathcal{N}_{ES}^o} \]

\[ \phi_s = \left( \frac{\alpha \beta}{\alpha + \beta} \right) \mathcal{N}_{ET} \left( \frac{c_s^i}{c_s^i + K} - \frac{c_s^o}{c_s^o + K} \right) \]
Steady-state (i.e., carrier densities are independent of time)

\[ \phi_s = \left( \frac{\alpha \beta}{\alpha + \beta} \right) n_{ET} \left( \frac{c^i_s}{c^i_s + K} - \frac{c^o_s}{c^o_s + K} \right) \]

\[ K = \frac{c^i_s n^i_E}{n^i_{ES}} = \frac{c^o_s n^o_E}{n^o_{ES}} \]
Practice problems

6.8 Consider the simple, symmetric, four-state carrier shown in Figure 6.21. For each of the following conditions, find $n_E^i$, $n_E^o$, $n_{ES}^i$, $n_{ES}^o$, and $\phi_S$. Explain the physical significance of each of your answers.

a. $\alpha = 0$.
b. $\beta = 0$.
c. $K = 0$.

6.9 For the simple, symmetric, four-state carrier shown in Figure 6.21, let $c_S^i = c_S^o = 0$. Sketch the carrier density in each of its four states as a function of $\alpha/\beta$. Give a physical interpretation of the results.
Practice problems

6.8 Consider the simple, symmetric, four-state carrier shown in Figure 6.21. For each of the following conditions, find \( \nu_i^E, \nu_o^E, \nu_{iES}, \nu_{oES}, \) and \( \phi_S \). Explain the physical significance of each of your answers.

a. \( \alpha = 0 \).
b. \( \beta = 0 \).
c. \( K = 0 \).

6.9 For the simple, symmetric, four-state carrier shown in Figure 6.21, let \( c_s^i = c_s^o = 0 \). Sketch the carrier density in each of its four states as a function of \( \alpha/\beta \). Give a physical interpretation of the results.

\[
\begin{align*}
\nu_{iES}^i &= \left( \frac{\beta}{\alpha + \beta} \right) \left( \frac{c_s^i}{c_s^i + K} \right) \nu_{ET}^i \\
\nu_i^E &= \left( \frac{\beta}{\alpha + \beta} \right) \left( \frac{K}{c_s^i + K} \right) \nu_{ET}^E \\
\nu_{oES}^o &= \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{c_s^o}{c_s^o + K} \right) \nu_{ET}^o \\
\nu_o^E &= \left( \frac{\alpha}{\alpha + \beta} \right) \left( \frac{K}{c_s^o + K} \right) \nu_{ET}^o \\
\phi_S &= \left( \frac{\alpha \beta}{\alpha + \beta} \right) \frac{c_s^i}{c_s^i + K} - \frac{c_s^o}{c_s^o + K}
\end{align*}
\]

Figure 6.21
Exercise 6.8

a. Since $\alpha$ is zero, none of the enzyme can translocate to face the extracellular solution. Therefore the densities of outward facing enzymes $n_{ES}^o$ and $n_E^o$ are zero. The inward facing densities partition in proportion to the intracellular concentration of solute and the dissociation constant for the binding reaction. Therefore,

$$n_{ES}^i = \frac{c_S^i}{c_S^i + K} n_{ET} \quad \text{and} \quad n_E^i = \frac{K}{c_S^i + K} n_{ET}.$$ 

Since the enzyme cannot translocate, the flux of solute $\phi_S$ is also zero.

b. The case $\beta = 0$ is similar to the case $\alpha = 0$ except that the enzyme cannot face the intracellular solution. Therefore the densities of inward facing enzymes $n_{ES}^i$ and $n_E^i$ are zero. The outward facing densities partition in proportion to the extracellular concentration of solute and the dissociation constant for the binding reaction. Therefore,

$$n_{ES}^o = \frac{c_S^o}{c_S^o + K} n_{ET} \quad \text{and} \quad n_E^o = \frac{K}{c_S^o + K} n_{ET}.$$ 

Since the enzyme cannot translocate, the flux of solute $\phi_S$ is also zero.

c. If $K = 0$, the enzyme cannot dissociate. Therefore, if there is any extracellular or intracellular solute, it will bind to the enzyme and never unbind. Therefore the unbound densities $n_E^i$ and $n_E^o$ will be zero. The bound densities will partition by the forward and reverse translocation rate constants, so that

$$n_{ES}^i = \frac{\beta}{\alpha + \beta} n_{ET} \quad \text{and} \quad n_{ES}^o = \frac{\alpha}{\alpha + \beta} n_{ET}.$$ 

Since the solute cannot unbind, there will be no transport, $\phi_S$ will be zero.
Exercise 6.9 For $c_S^i = c_S^o = 0$ there is no carrier bound to enzyme. Therefore, on this basis and by inspection of Equations 6.55 and 6.57 (Weiss, 1996a) $\mathcal{N}^i_{ES} = \mathcal{N}^o_{ES} = 0$. However, from Equations 6.56 and 6.58 (Weiss, 1996a) it follows that

$$\mathcal{N}^i_E = \frac{\beta}{\alpha + \beta} \mathcal{N}_{ET} = \frac{1}{(\alpha/\beta) + 1} \mathcal{N}_{ET},$$

$$\mathcal{N}^o_E = \frac{\alpha}{\alpha + \beta} \mathcal{N}_{ET} = \frac{(\alpha/\beta)}{(\alpha/\beta) + 1} \mathcal{N}_{ET}.$$

These relations are plotted in Figure 6.2. If $\alpha/\beta = 1$ then half the carrier is in the inside configuration and the other half is in the outside configuration. As $\alpha/\beta$ is increased, more of the carrier is found in the outside configuration, whereas as $\alpha/\beta$ is decreased, more of the carrier is found in the inside configuration.

Figure 6.2: Density of carrier for a case when the solute concentration is zero on both sides of the membrane (Exercise 6.9).