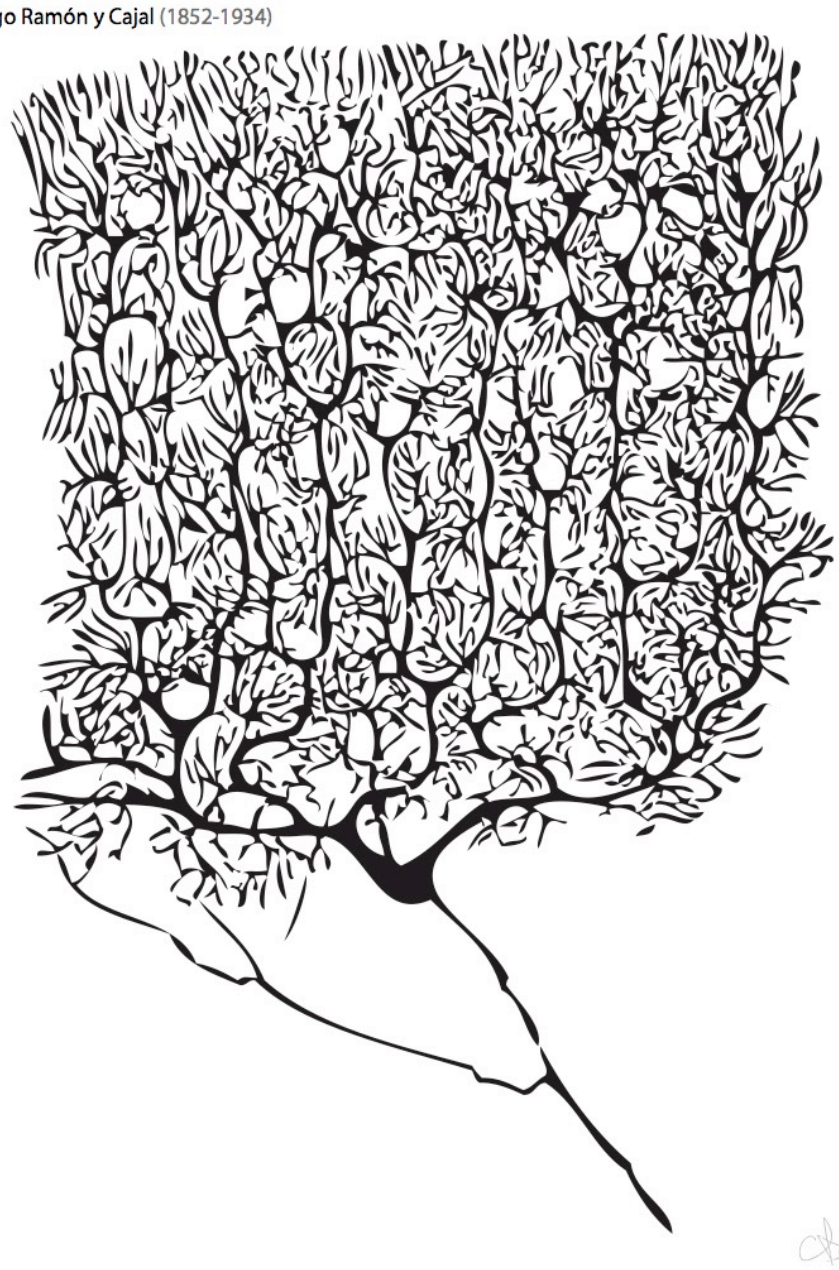
Cellular Electrodynamics



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York University
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BPHS 4080 Lecture 5

Reference/Acknowledgement:
- TF Weiss (Cellular Biophysics)
- D Freeman

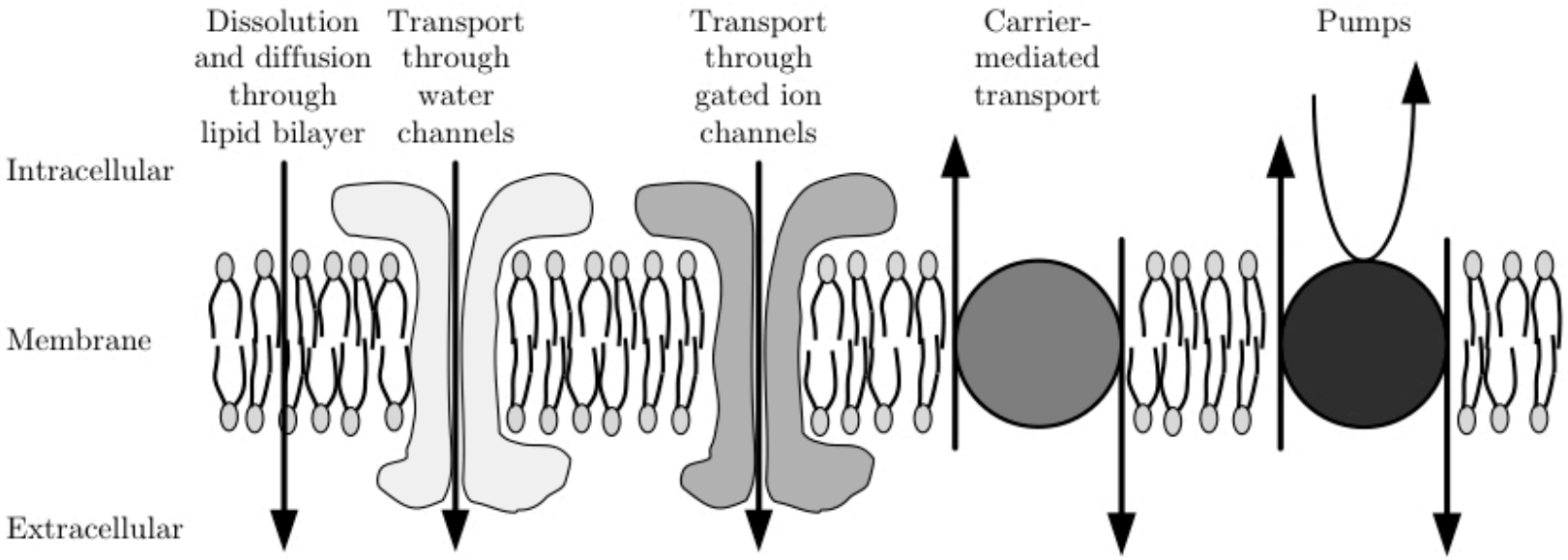

Dissolution and diffusion through lipid bilayer
Transport through water channels
Transport through gated ion channels
Carrier-mediated transport
Pumps
Intracellular
Membrane
Extracellular

Figure 2.19

Passive Transport: More than diffusion?

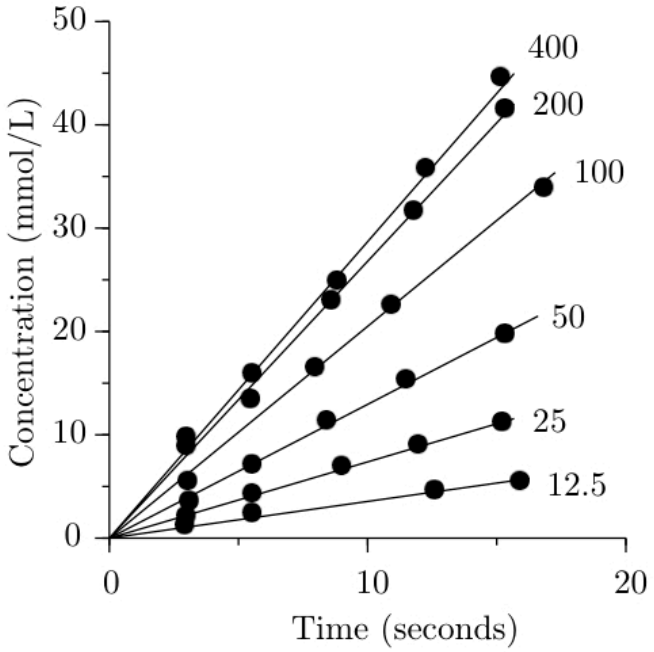


Figure 6.1

- Saturation occurs

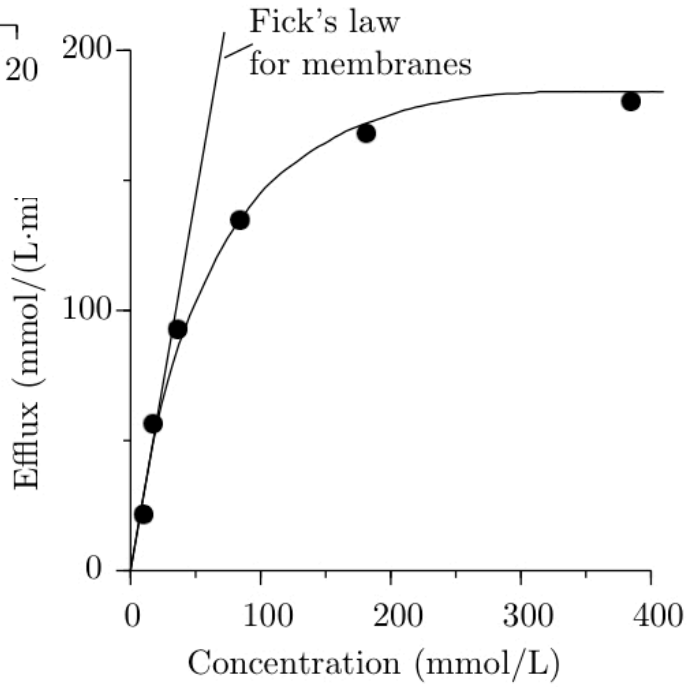


Figure 6.2

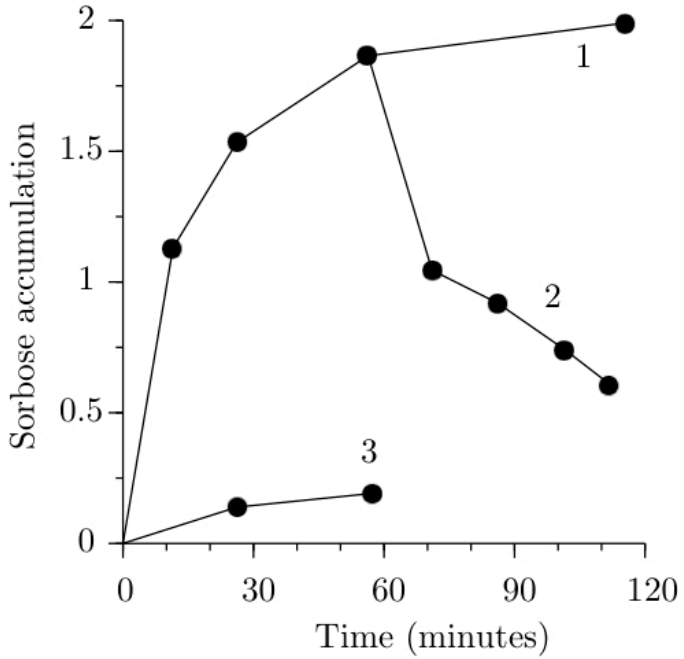


Figure 6.3

- Adding in other sugars affects things in a selective way

Passive Transport: More than diffusion?

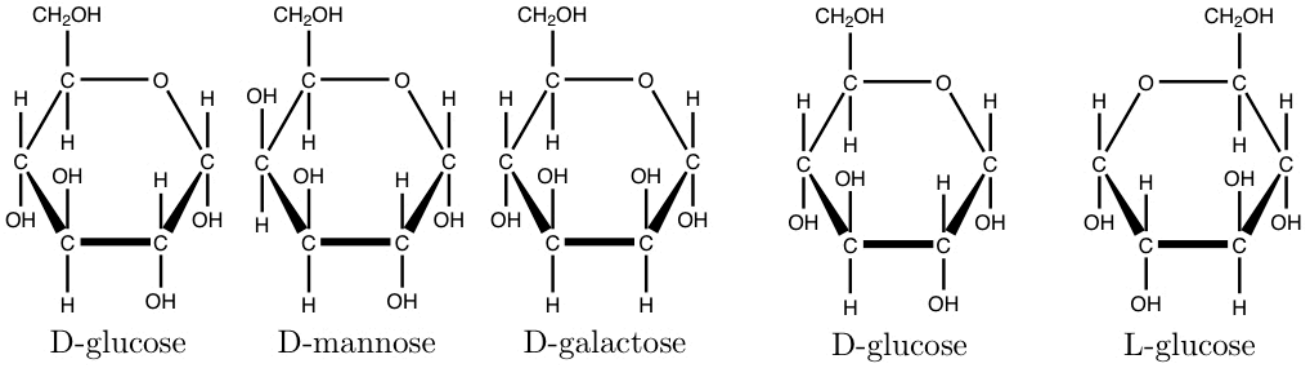


Figure 1.7

Figure 1.8

→ Structure of different solutes can have a big effect

Notion of a “carrier”

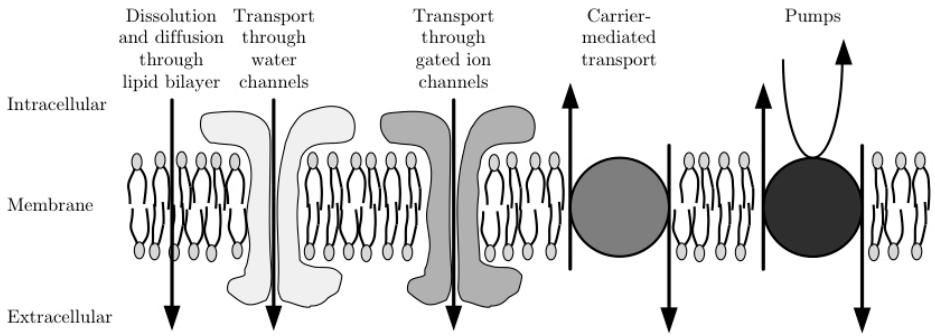
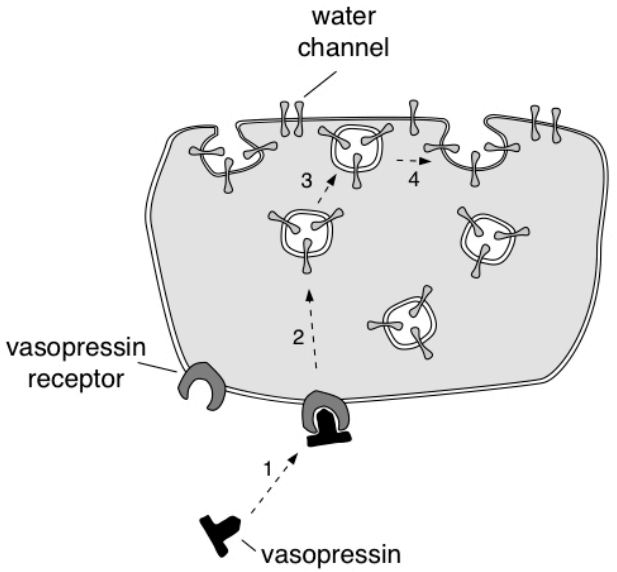


Figure 2.19



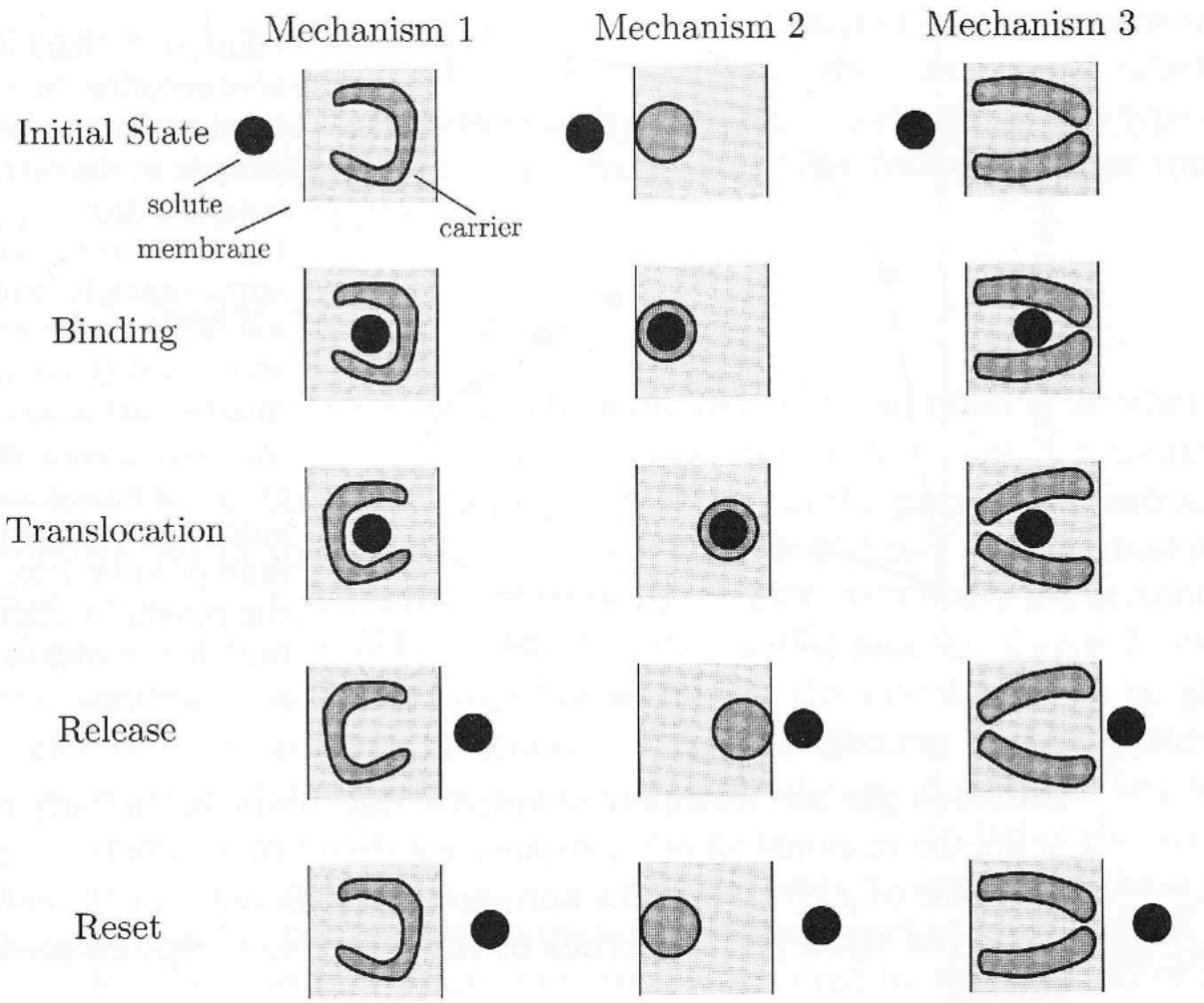
Carrier-Mediated Transport: glucose transporter as example

Distinguishing characteristics of glucose transport:

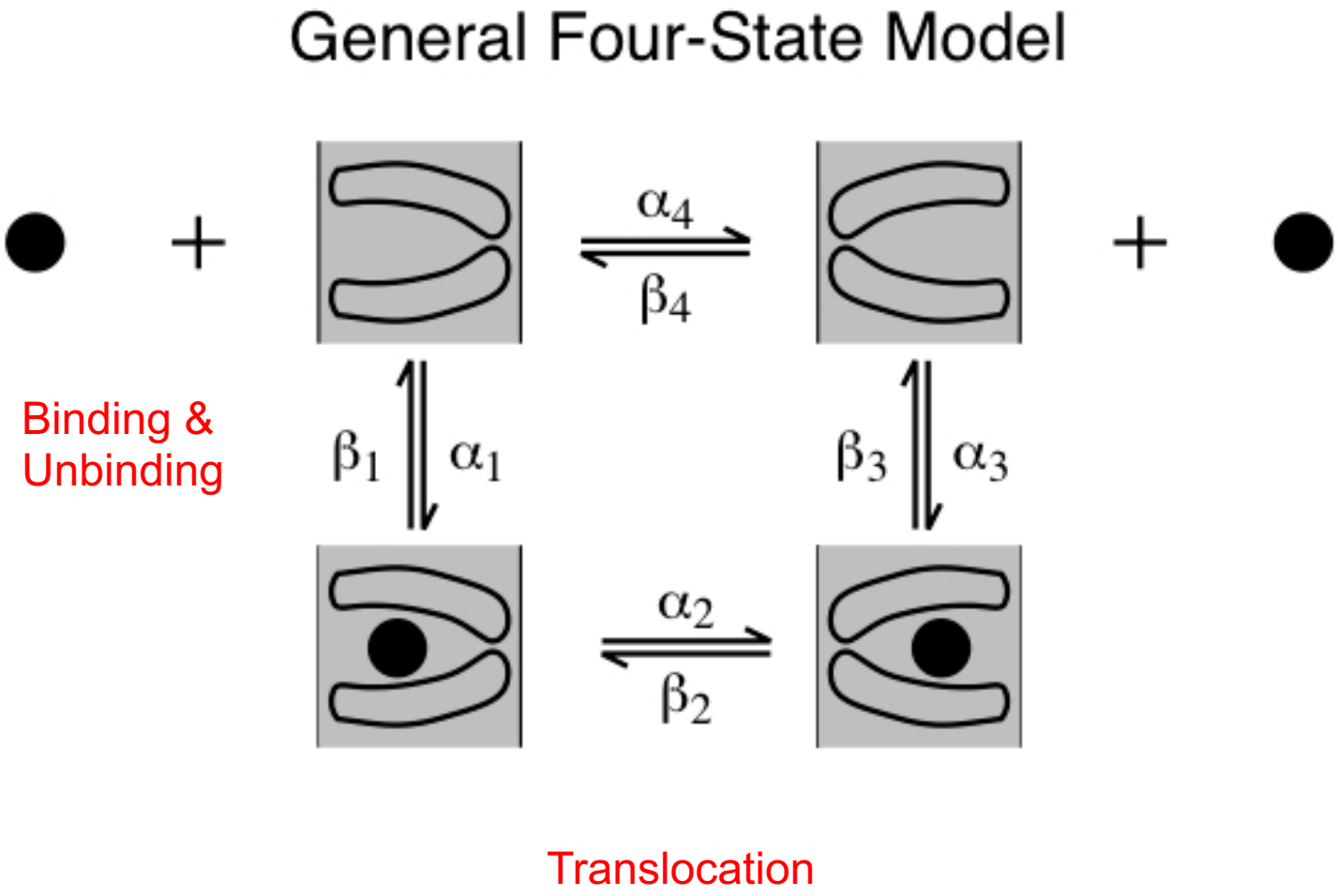
- facilitated -- i.e., faster than dissolve and diffuse
- structure specific -- different rates for even closely related sugars
- passive -- given a single solute, flow is down concentration gradient
- transport saturates -- solute-solute interactions
- transport can be inhibited -- solute-other interactions
- pharmacology (cytochalasin B)
- hormonal control (insulin)

similar to water channels (Hg, vasopressin)

Possible ‘Carrier’ Mechanisms



General Four-State Carrier Model



Chemical Kinetics (v1)

First-order, reversible reaction

$$R \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} P$$

$$\frac{dc_R(t)}{dt} = \beta c_P(t) - \alpha c_R(t) \quad \text{AND} \quad \frac{dc_P(t)}{dt} = \alpha c_R(t) - \beta c_P(t)$$

Equilibrium:

$$\frac{dc_R(t)}{dt} = \frac{dc_P(t)}{dt} = 0 \quad \rightarrow \quad \beta c_P(\infty) = \alpha c_R(\infty)$$

$$\frac{c_P(\infty)}{c_R(\infty)} = \frac{\alpha}{\beta} = K_a \quad \left(\begin{array}{c}\text{association, equilibrium, affinity,}\\ \text{stability, binding, formation constant}\end{array}\right)$$

Kinetics: assume total amount of reactant and product is conserved

$$c_R(t) + c_P(t) = C$$

$$\frac{dc_R(t)}{dt} = \beta\Big(C - c_R(t)\Big) - \alpha c_R(t)$$

$$\frac{dc_R(t)}{dt} + (\alpha + \beta)c_R(t) = \beta C$$

First-order, reversible reaction

$$R \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} P$$

First-order linear differential equation with constant coefficients

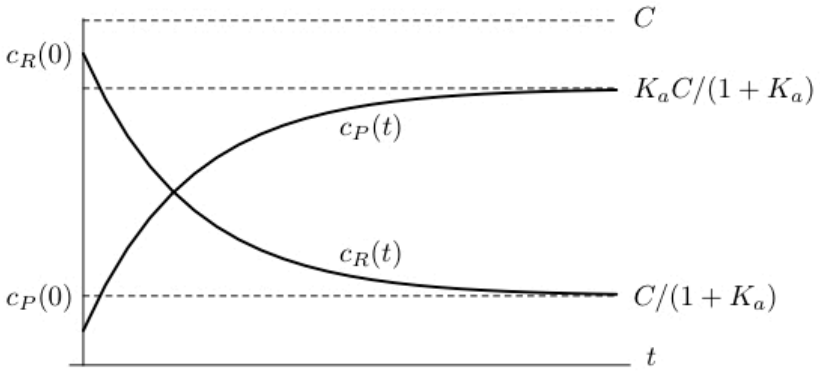
$$c_R(t) = c_R(\infty) - \Big(c_R(\infty) - c_R(0)\Big)\, e^{-t/\tau}, \text{ for } t > 0$$

$$c_R(\infty) = \frac{\beta}{\alpha+\beta} C = \frac{1}{1+K_a} C \quad \text{AND} \quad \tau = \frac{1}{\alpha+\beta}$$

First-order, reversible reaction

$$R \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} P$$

$$c_P(t) = C - c_R(t)$$



$$\tau = \tfrac{1}{\alpha+\beta}$$

Chemical Kinetics (v2)

Second-order reversible (binding) reaction

$$S + E \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} ES$$

$$\begin{aligned} \frac{dc_{ES}(t)}{dt} &= \alpha c_S(t) c_E(t) - \beta c_{ES}(t), \\ \frac{dc_S(t)}{dt} = \frac{dc_E(t)}{dt} &= \beta c_{ES}(t) - \alpha c_S(t) c_E(t), \end{aligned}$$

→ Law of mass action

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.

$$C_{ET} = c_E(\infty) + c_{ES}(\infty)$$

$$C_{ET} = \frac{K c_{ES}(\infty)}{c_S(\infty)} + c_{ES}(\infty) = \left(\frac{K}{c_S(\infty)} + 1 \right) c_{ES}(\infty)$$

$$c_{ES}(\infty) = \left(\frac{c_S(\infty)}{K + c_S(\infty)} \right) C_{ET}$$

→ Michaelis-Menten kinetics

Chemical Kinetics (v2)

Second-order reversible (binding) reaction

$$S + E \underset{\beta}{\overset{\alpha}{\rightleftharpoons}} ES$$

$$C_{ET} = c_E(\infty) + c_{ES}(\infty)$$

Second-order reversible (binding) reaction

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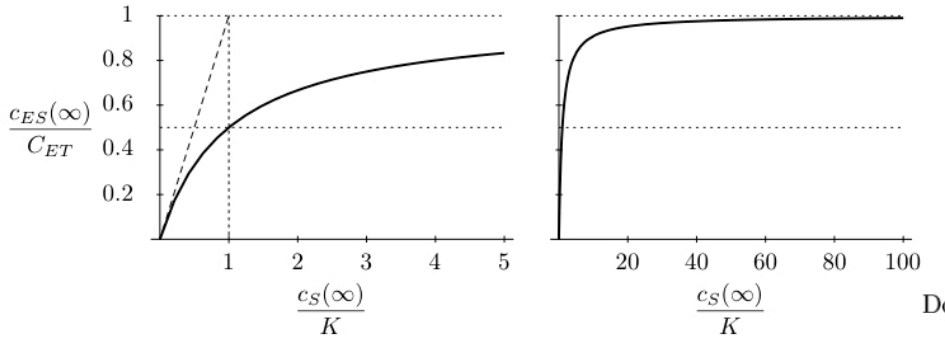
$$C_{ET} = \frac{K c_{ES}(\infty)}{c_S(\infty)} + c_{ES}(\infty) = \left(\frac{K}{c_S(\infty)} + 1\right) c_{ES}(\infty)$$

$$c_{ES}(\infty) = \left(\frac{c_S(\infty)}{K + c_S(\infty)}\right) C_{ET}$$

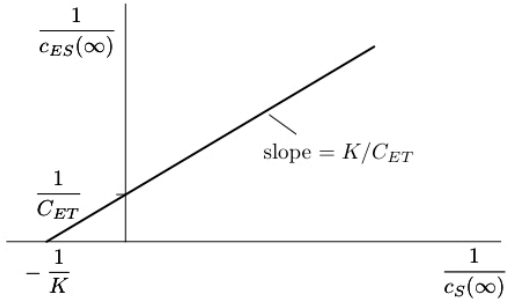
Rectangular hyperbola: Michaelis-Menten Relation



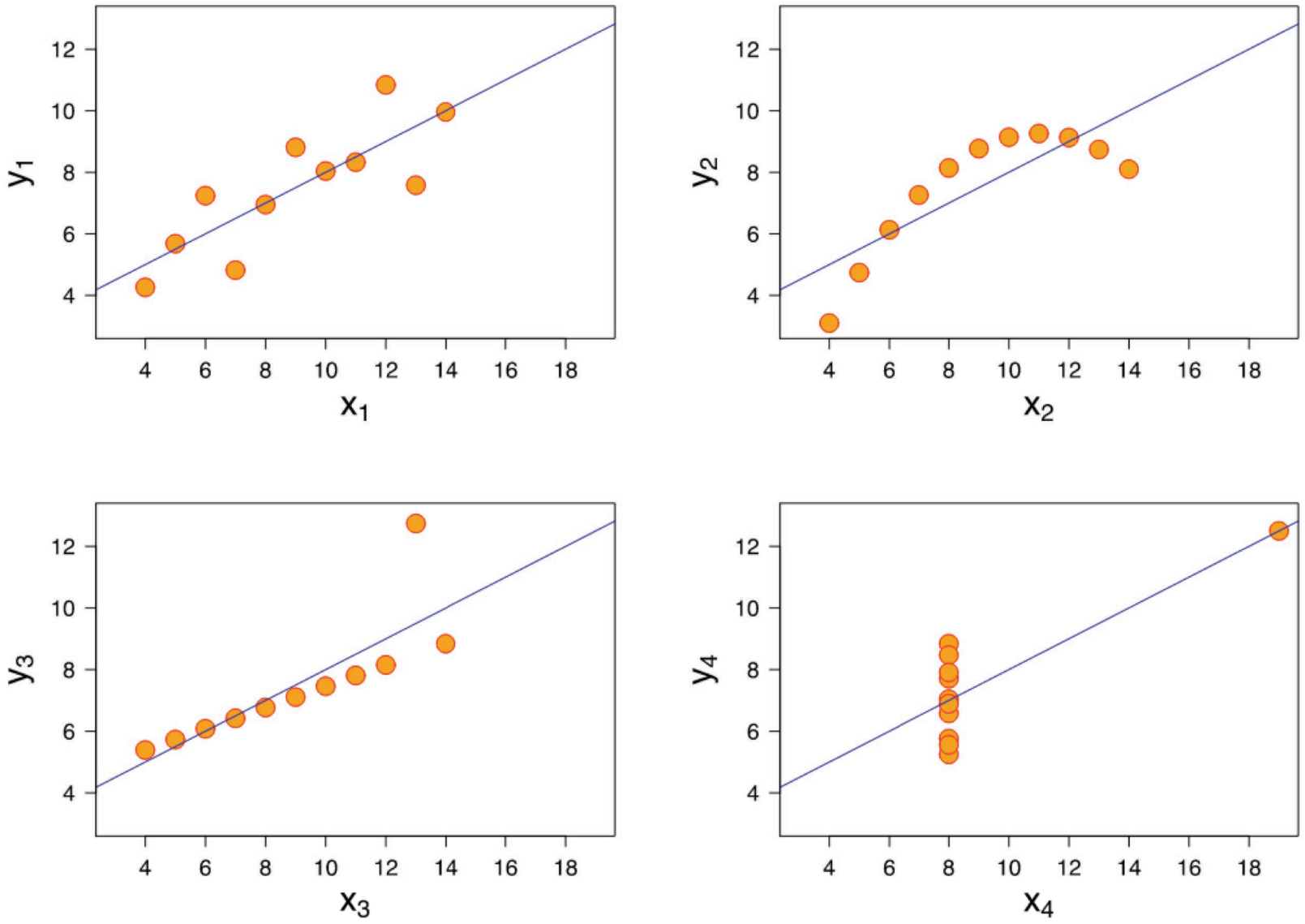
Doubly-reciprocal coordinates: Lineweaver-Burk plot

$$\frac{1}{c_{ES}(\infty)} = \left(1 + \frac{K}{c_S(\infty)}\right)\frac{1}{C_{ET}} = \left(\frac{K}{C_{ET}}\right)\frac{1}{c_S(\infty)} + \frac{1}{C_{ET}}$$

→ Linear way to plot nonlinear relationship!

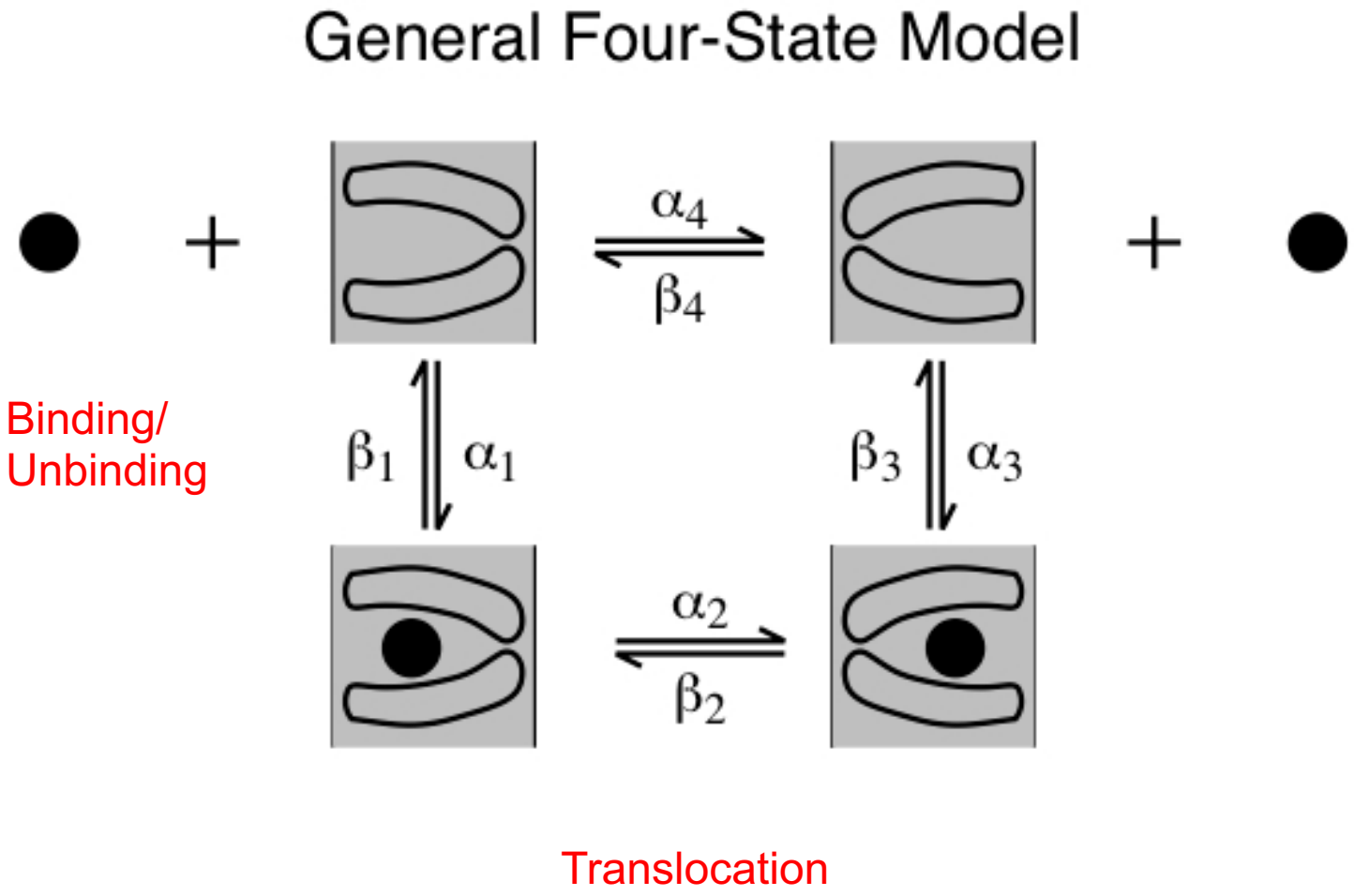


Aside: Anscombe's Quartet

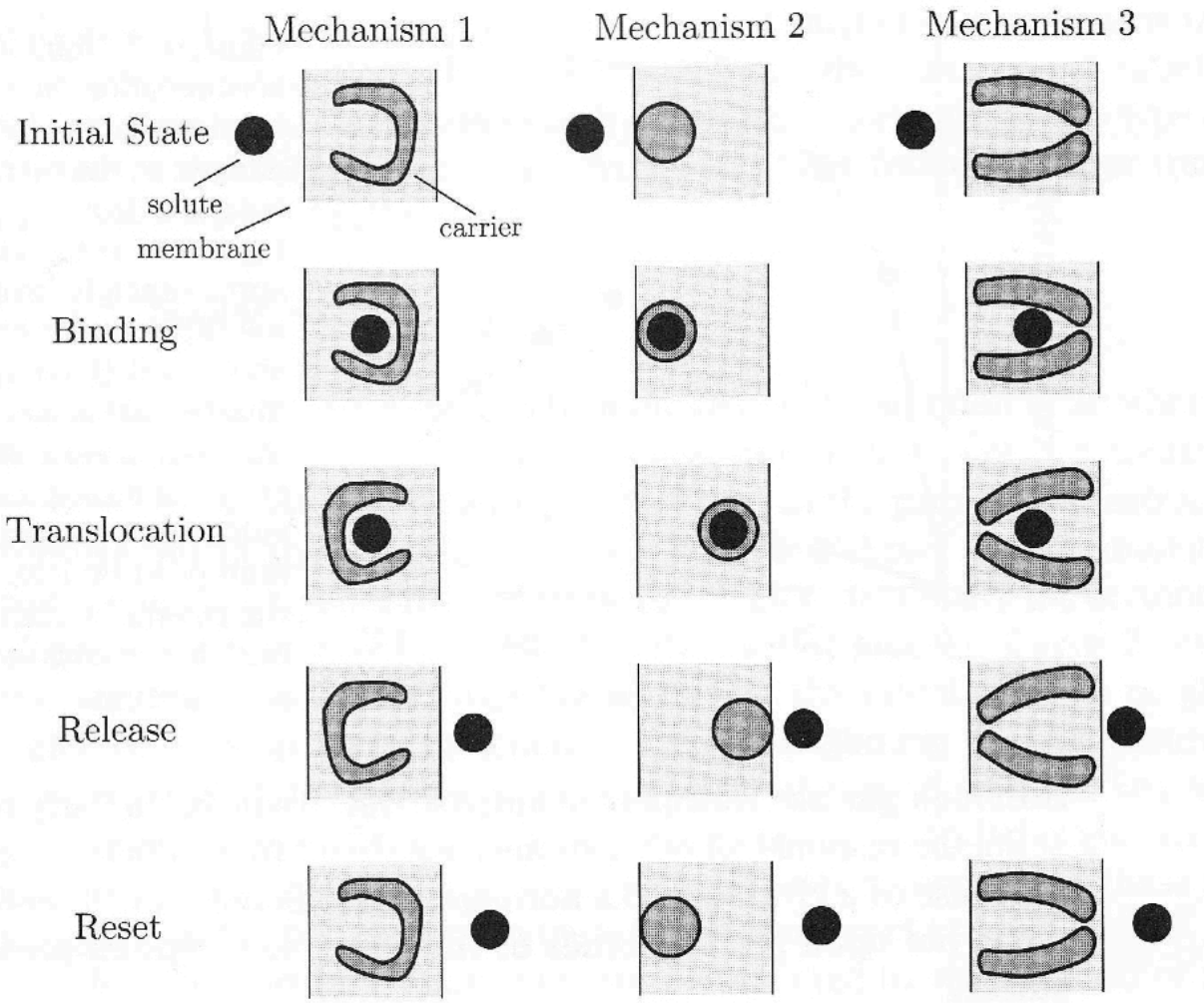


Anscombe (1973) *Graphs in Statistical Analysis*

General Four-State Carrier Model

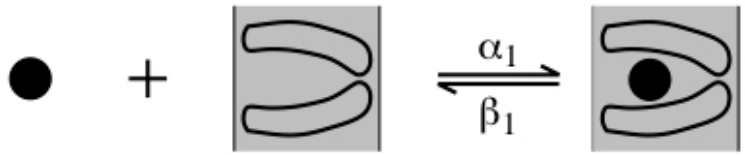


Possible ‘Carrier’ Mechanisms



Chemical Kinetics & 'Carriers'

Binding

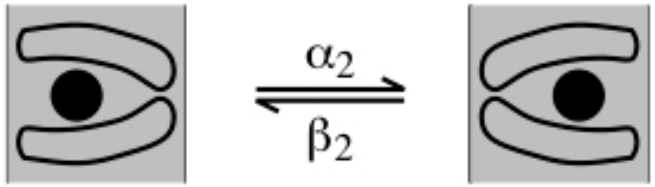


$$S^i + E^i \underset{\beta_1}{\overset{\alpha_1}{\rightleftharpoons}} ES^i$$

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

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

Translocation



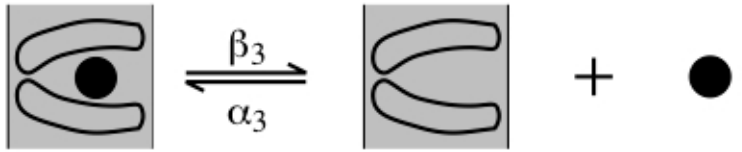
$$ES^i \underset{\beta_2}{\overset{\alpha_2}{\rightleftharpoons}} ES^o$$

$$\frac{dC^o_{ES}}{dt} = \alpha_2 C^i_{ES} - \beta_2 C^o_{ES}$$

$$\frac{dC^i_{ES}}{dt} = \beta_2 C^o_{ES} - \alpha_2 C^i_{ES}$$

Chemical Kinetics & 'Carriers'

Unbinding

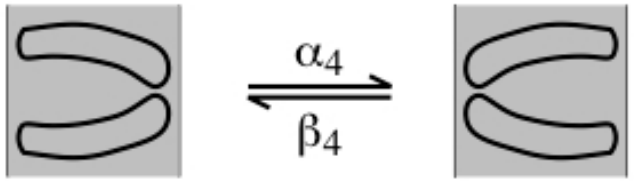


$$ES^o \underset{\alpha_3}{\overset{\beta_3}{\rightleftharpoons}} E^o + S^o$$

$$\frac{dC^o_{ES}}{dt} = \alpha_3 C^o_S C^o_E - \beta_3 C^o_{ES}$$

$$\frac{dC^o_S}{dt} = \frac{dC^o_E}{dt} = \beta_3 C^o_{ES} - \alpha_3 C^o_S C^o_E$$

Translocation



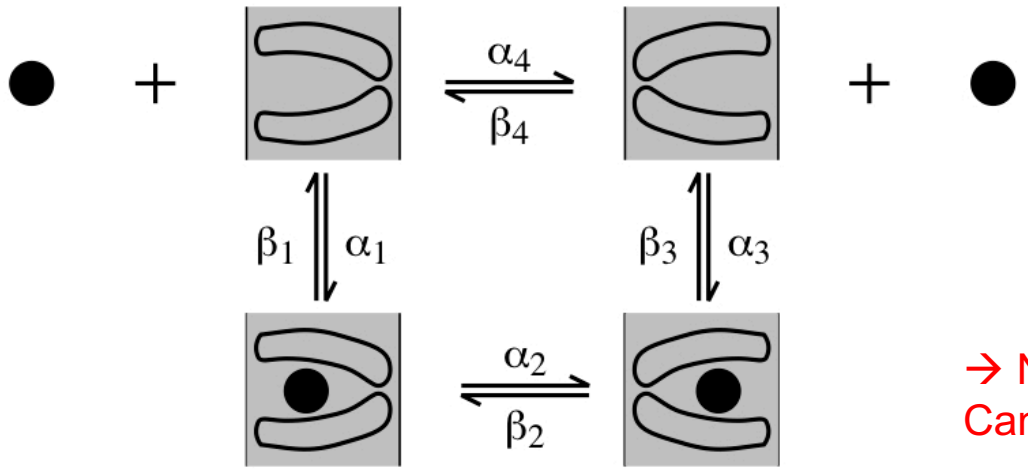
$$E^i \underset{\beta_4}{\overset{\alpha_4}{\rightleftharpoons}} E^o$$

$$\frac{dC^o_E}{dt} = \alpha_4 C^i_E - \beta_4 C^o_E$$

$$\frac{dC^i_E}{dt} = \beta_4 C^o_E - \alpha_4 C^i_E$$

Chemical Kinetics & 'Carriers'

General Four-State Model



→ Numerous free parameters.
Can we simplify?

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

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

$$\frac{dC^o_{ES}}{dt} = \alpha_3 C^o_S C^o_E - \beta_3 C^o_{ES}$$

$$\frac{dC^o_S}{dt} = \frac{dC^o_E}{dt} = \beta_3 C^o_{ES} - \alpha_3 C^o_S C^o_E$$

$$\frac{dC^o_{ES}}{dt} = \alpha_2 C^i_{ES} - \beta_2 C^o_{ES}$$

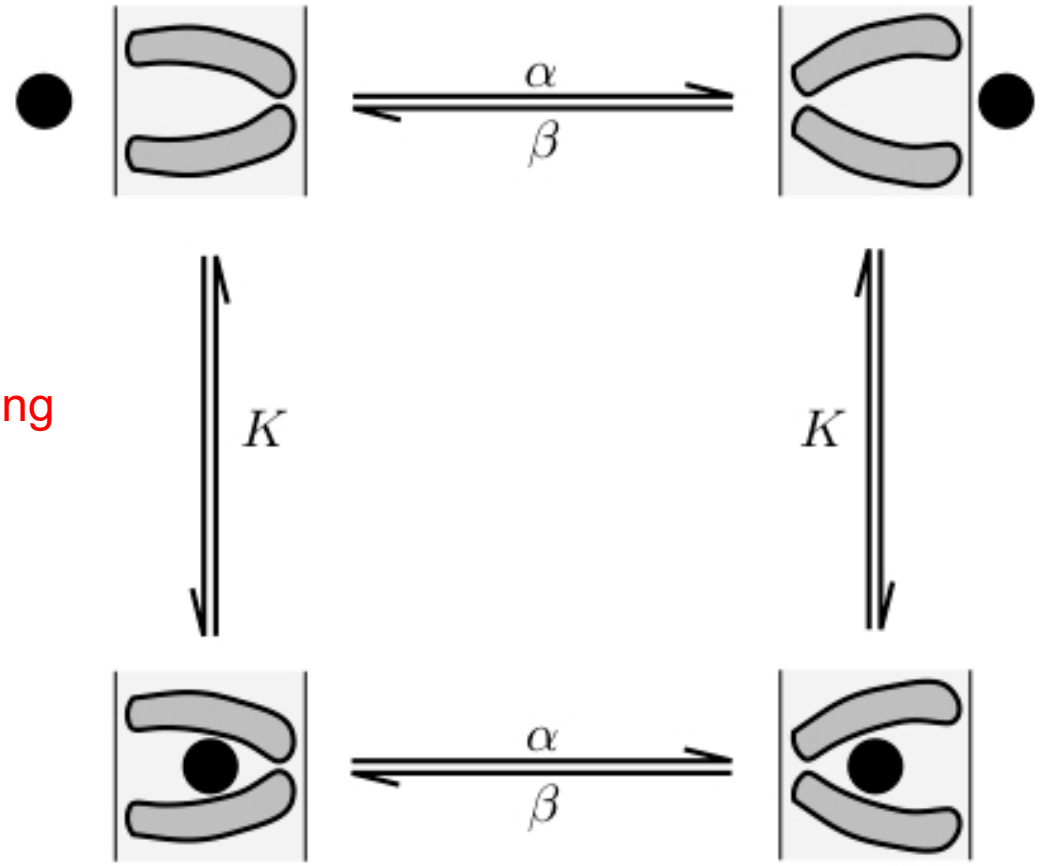
$$\frac{dC^i_{ES}}{dt} = \beta_2 C^o_{ES} - \alpha_2 C^i_{ES}$$

$$\frac{dC^o_E}{dt} = \alpha_4 C^i_E - \beta_4 C^o_E$$

$$\frac{dC^i_E}{dt} = \beta_4 C^o_E - \alpha_4 C^i_E$$

Simple, Symmetric Four-State Model

Translocation



Binding/Unbinding
(at equilibrium)

Figure 6.20

Assumption: Steady-state
(i.e., carrier densities are independent of time)

Simple, Symmetric Four-State Model

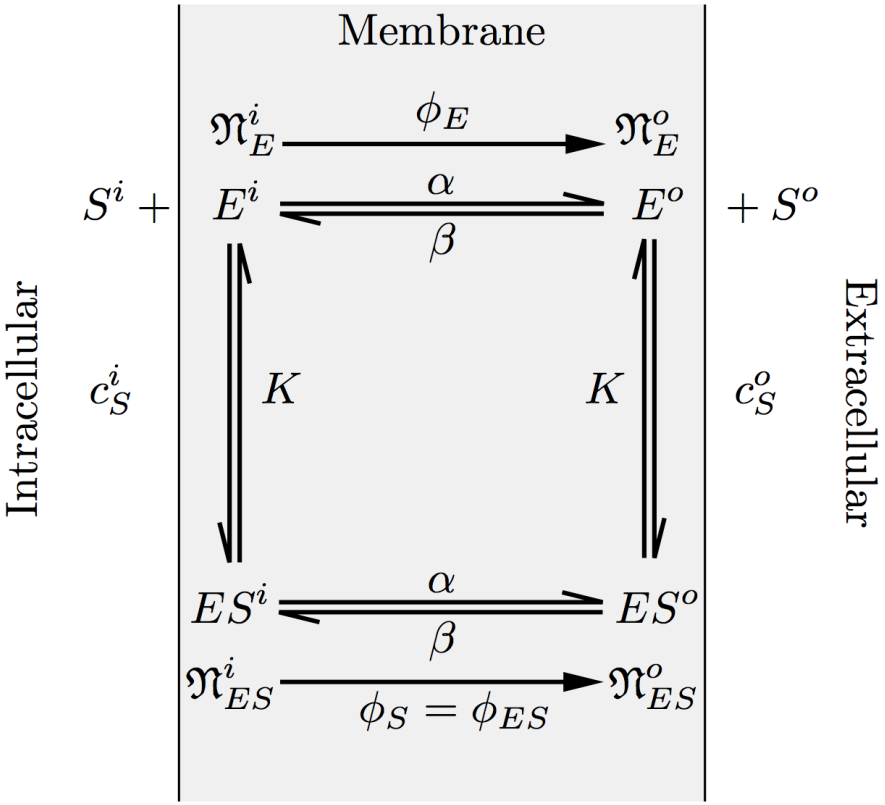


Figure 6.21

1. Conservation of enzyme:

$$\mathfrak{N}_E^i + \mathfrak{N}_E^o + \mathfrak{N}_{ES}^i + \mathfrak{N}_{ES}^o = \mathfrak{N}_{ET}$$

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

$$K = \frac{c_S^i \mathfrak{N}_E^i}{\mathfrak{N}_{ES}^i} = \frac{c_S^o \mathfrak{N}_E^o}{\mathfrak{N}_{ES}^o}$$

3. Translocation characterized by fluxes:

$$\phi_{ES} = \alpha \mathfrak{N}_{ES}^i - \beta \mathfrak{N}_{ES}^o$$

$$\phi_E = \alpha \mathfrak{N}_E^i - \beta \mathfrak{N}_E^o$$

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

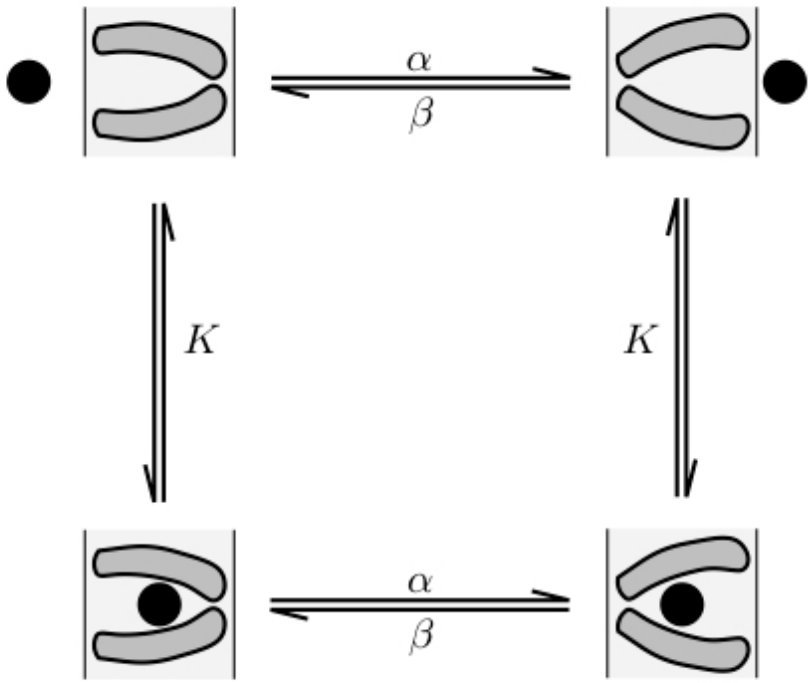


Figure 6.20

$$\mathfrak{N}_E^i + \mathfrak{N}_E^o + \mathfrak{N}_{ES}^i + \mathfrak{N}_{ES}^o = \mathfrak{N}_{ET}$$

$$K = \frac{c_S^i \mathfrak{N}_E^i}{\mathfrak{N}_{ES}^i} = \frac{c_S^o \mathfrak{N}^o}{\mathfrak{N}_{ES}^o}$$

$$\phi_{ES} = \alpha \mathfrak{N}_{ES}^i - \beta \mathfrak{N}_{ES}^o$$

$$\phi_E = \alpha \mathfrak{N}_E^i - \beta \mathfrak{N}^o$$

$$\phi_E + \phi_{ES} = 0$$

Combining equations...

$$\mathfrak{N}_{ES}^i = \left(\beta \frac{}{\alpha + \beta} \right) \left(\frac{c_S^i}{c_S^i + K} \right) \mathfrak{N}_{ET}$$

$$\mathfrak{N}_E^i = \left(\frac{\beta}{\alpha + \beta} \right) \left(\frac{K}{c_S^i + K} \right) \mathfrak{N}_{ET}$$

$$\mathfrak{N}_{ES}^o = \left(\frac{\alpha}{\alpha + \beta} \right) \left(\frac{c_S^o}{c_S^o + K} \right) \mathfrak{N}_{ET}$$

$$\mathfrak{N}_E^o = \left(\frac{\alpha}{\alpha + \beta} \right) \left(\frac{K}{c_S^o + K} \right) \mathfrak{N}_{ET}$$

Solving for the solute flux yields:

$$\phi_S = \left(\frac{\alpha\beta}{\alpha + \beta} \right) \mathfrak{N}_{ET} \left(\frac{c_S^i}{c_S^i + K} - \frac{c_S^o}{c_S^o + K} \right)$$

$$K = \frac{c_S^i \mathfrak{N}_E^i}{\mathfrak{N}_{ES}^i} = \frac{c_S^o \mathfrak{N}_E^o}{\mathfrak{N}_{ES}^o}$$

$$\phi_S = \left(\frac{\alpha\beta}{\alpha+\beta}\right) \mathfrak{N}_{ET} \left(\frac{c_S^i}{c_S^i + K} - \frac{c_S^o}{c_S^o + K}\right)$$

Figure 6.22

→ Steady-state

(i.e., carrier densities are independent of time)

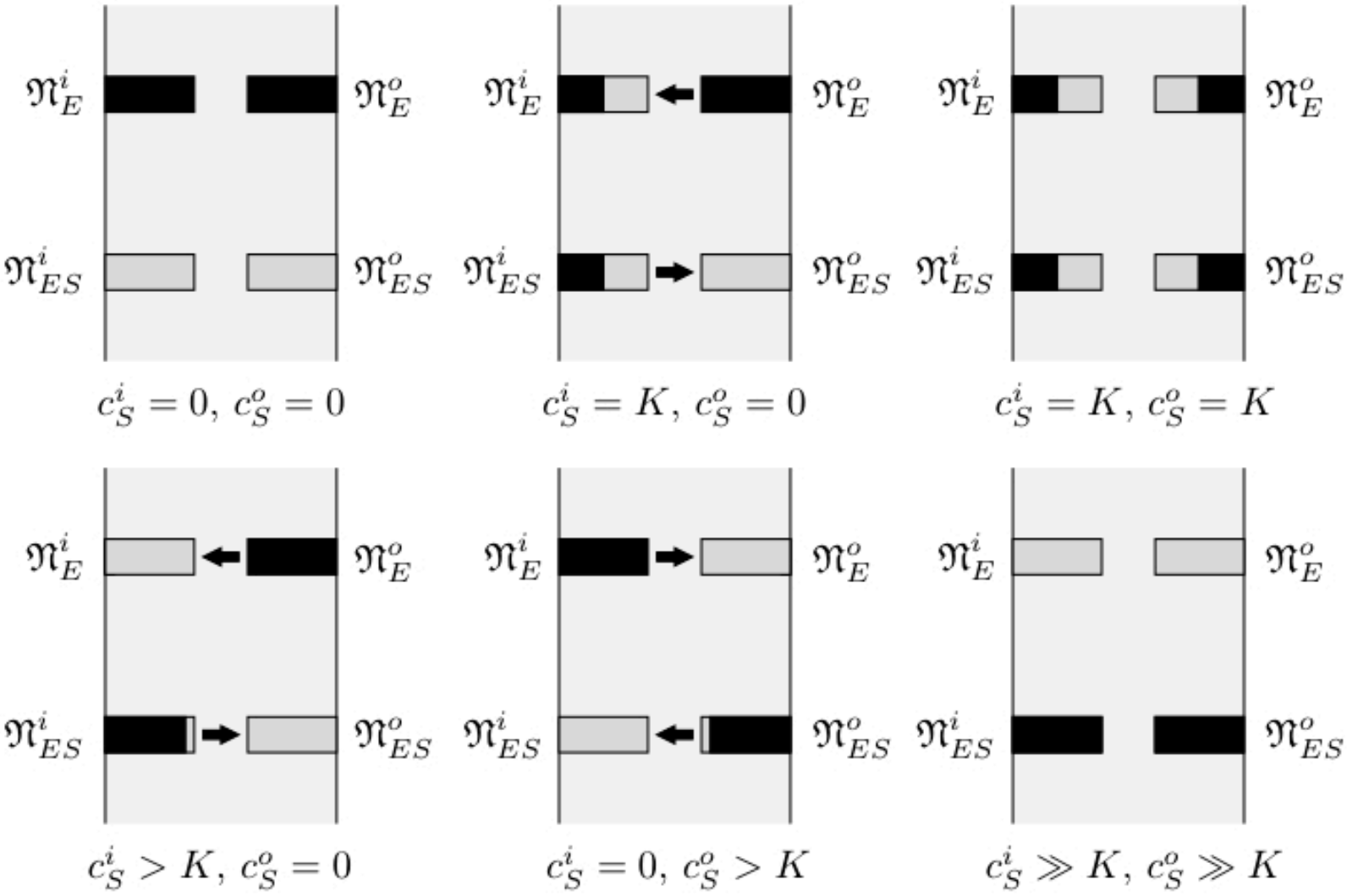


Figure 6.23

$$K = \frac{c_S^i \mathfrak{N}_E^i}{\mathfrak{N}_{ES}^i} = \frac{c_S^o \mathfrak{N}^o}{\mathfrak{N}_{ES}^o} \qquad \phi_S = \left(\frac{\alpha\beta}{\alpha+\beta}\right)\mathfrak{N}_{ET}\left(\frac{c_S^i}{c_S^i+K} - \frac{c_S^o}{c_S^o+K}\right)$$

Practice problems

6.8 Consider the simple, symmetric, four-state carrier shown in Figure 6.21. For each of the following conditions, find $\mathfrak{N}_E^i$, $\mathfrak{N}_E^o$, $\mathfrak{N}_{ES}^i$, $\mathfrak{N}_{ES}^o$, and ϕ_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 α/β. Give a physical interpretation of the results.

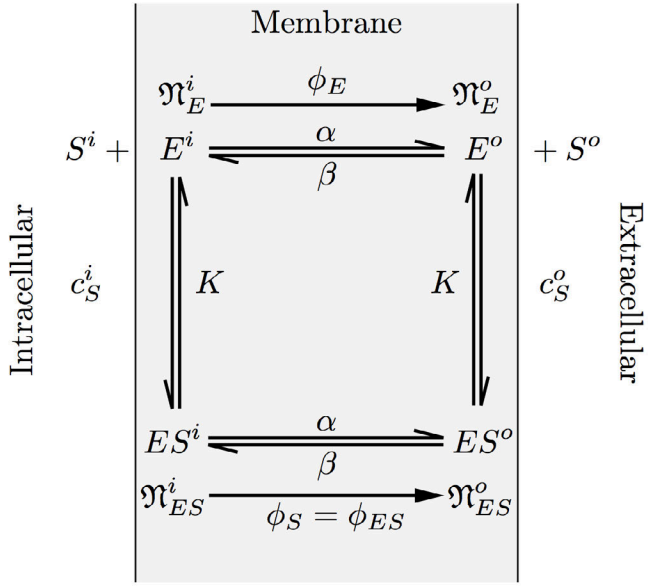


Figure 6.21

Practice problems

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$$\mathfrak{N}_{ES}^i = \left(\frac{\beta}{\alpha+\beta}\right)\left(\frac{c_S^i}{c_S^i+K}\right)\mathfrak{N}_{ET}$$

$$\mathfrak{N}_E^i = \left(\frac{\beta}{\alpha+\beta}\right)\left(\frac{K}{c_S^i+K}\right)\mathfrak{N}_{ET}$$

$$\mathfrak{N}_{ES}^o = \left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{c_S^o}{c_S^o+K}\right)\mathfrak{N}_{ET}$$

$$\mathfrak{N}_E^o = \left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{K}{c_S^o+K}\right)\mathfrak{N}_{ET}$$

$$\phi_S = \left(\frac{\alpha\beta}{\alpha+\beta}\right)\mathfrak{N}_{ET}\left(\frac{c_S^i}{c_S^i+K} - \frac{c_S^o}{c_S^o+K}\right)$$

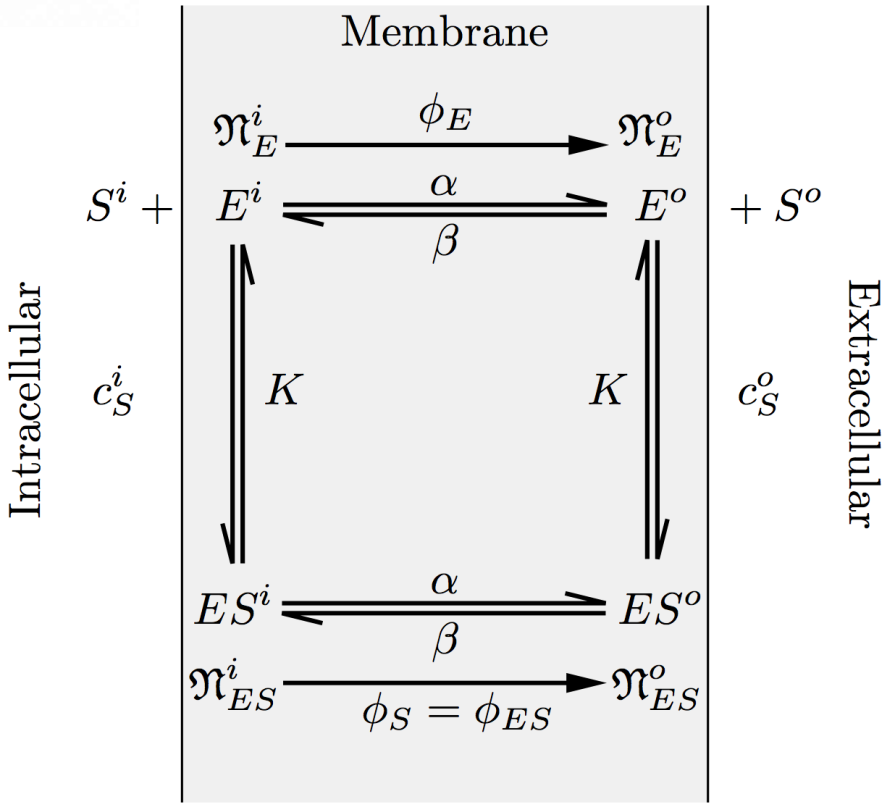

Figure 6.21

Exercise 6.8

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

$$\mathfrak{N}^i_{ES} = \frac{c^i_S}{c^i_S + K}\mathfrak{N}_{ET} \text{ and } \mathfrak{N}^i_E = \frac{K}{c^i_S + K}\mathfrak{N}_{ET}.$$

Since the enzyme cannot translocate, the flux of solute ϕ_S is also zero.

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

$$\mathfrak{N}^o_{ES} = \frac{c^o_S}{c^o_S + K}\mathfrak{N}_{ET} \text{ and } \mathfrak{N}^o_E = \frac{K}{c^o_S + K}\mathfrak{N}_{ET}.$$

Since the enzyme cannot translocate, the flux of solute ϕ_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 $\mathfrak{N}^i_E$ and $\mathfrak{N}^o_E$ will be zero. The bound densities will partition by the forward and reverse translocation rate constants, so that

$$\mathfrak{N}^i_{ES} = \frac{\beta}{\alpha + \beta}\mathfrak{N}_{ET} \text{ and } \mathfrak{N}^o_{ES} = \frac{\alpha}{\alpha + \beta}\mathfrak{N}_{ET}.$$

Since the solute cannot unbind, there will be no transport, ϕ_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) $\mathfrak{N}_{ES}^i = \mathfrak{N}_{ES}^o = 0$. However, from Equations 6.56 and 6.58 (Weiss, 1996a) it follows that

$$\mathfrak{N}_E^i = \frac{\beta}{\alpha+\beta}\mathfrak{N}_{ET} = \frac{1}{(\alpha/\beta)+1}\mathfrak{N}_{ET},$$

$$\mathfrak{N}_E^o = \frac{\alpha}{\alpha+\beta}\mathfrak{N}_{ET} = \frac{(\alpha/\beta)}{(\alpha/\beta)+1}\mathfrak{N}_{ET}.$$

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 α/β is increased, more of the carrier is found in the outside configuration, whereas as α/β is decreased, more of the carrier is found in the inside configuration

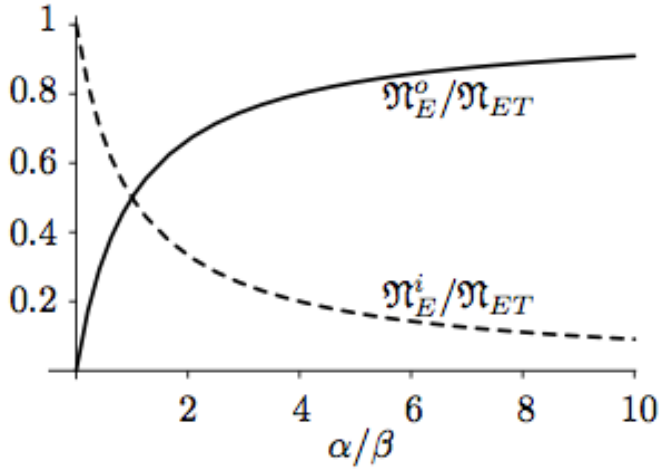

Figure 6.2: Density of carrier for a case when the solute concentration is zero on both sides of the membrane (Exercise 6.9).