Física Biológica

Transporte a través de la membrana.

1. *Reproduciendo cálculos de la teórica.* Calcule el flujo estacionario de partículas de una dada especie que pasan de un lado a otro de una membrana de espesor d suponiendo que ese flujo ocurre solo en la dirección perpendicular a la membrana a través de un poro de área transversal, A, y que es suficientemente pequeño como para que las concentraciones a un lado y al otro de la membrana (c_i y c_e, respectivamente) permanezcan invariantes. Haga el cálculo en estos dos casos:

   (a) Las partículas no tienen carga eléctrica o el campo eléctrico en el poro es nulo.

   (b) Las partículas tienen carga ze y hay un campo eléctrico dentro del poro de la forma $E\hat{x}$ con E uniforme y constante.

   En ambos casos calcule el número de partículas que atraviesan el poro por unidad de tiempo como función de c_i y c_e y, en el segundo caso, determine la corriente eléctrica. Para este segundo caso, suponiendo que $c_i = 1mM$, $c_e = 100\mu M$, que el potencial de membrana es $V = 50mV$, que el espesor de la membrana es $d = 5nm$, que el poro tiene un área, $A = 100nm^2$, y que el sistema se encuentra a temperatura ambiente ($T = 300^0$K), calcule la corriente en pA (pico Ampere).

2. *Equilibrio de Gibbs-Donnan; ejercicio tomado de "Mathematical Physiology" de Keener y Sneyd.* Suponga un volumen, V, cerrado subdividido en dos volúmenes, V_i y V_e, separados por una membrana que permite el paso de dos tipos de iones "pequeños", unos de carga $z\,e$ y los otros de carga $-ze$ (con e la carga del electrón en valor absoluto y $z > 0$) pero que impide el paso de moléculas grandes (proteínas) de carga z_X que quedan confinadas al volumen, V_i. Suponga que las concentraciones son espacialmente uniformes dentro de cada volumen, que hay neutralidad eléctrica dentro de cada volumen y que el número total de iones de cada especie en V (N_{tot} y N'_{tot}) es constante. Llamando $[S_i]$, $[S_e]$, $[S'_i]$ y $[S'_e]$ a las concentraciones de los iones pequeños a un lado y otro de la membrana y $[X]$ a la concentración de proteínas en V_i, calcule todas las concentraciones como función del tamaño de los volúmenes, las valencias (z y $-z_X$), la concentración de proteínas, $[X]$, y el número total de iones pequeños de un tipo, N_{tot}, en el equilibrio (es decir, cuando no hay flujo neto de ninguna especie a través de la membrana; para esto último es necesario que haya un potencial de membrana, V, que debe calcular en función de los parámetros antes mencionado). Repita el cálculo haciéndolo en función de N'_{tot} en lugar de N_{tot} y determine cómo deberían relacionarse N'_{tot} y N_{tot} entre sí para que las respuestas obtenidas en ambos casos coincidan.

3. *Ejercicio tomado de "Mathematical Physiology" de Keener y Sneyd.* En las páginas 64-65 del libro "Mathematical Physiology" de Keener y Sneyd que se adjuntan al final de la guía se introduce un modelo para el transportador de glucosa sencillo. En dicho modelo se supone que cada conformación del transportador es igualmente probable y que la afinidad del sitio de unión de la glucosa no se ve

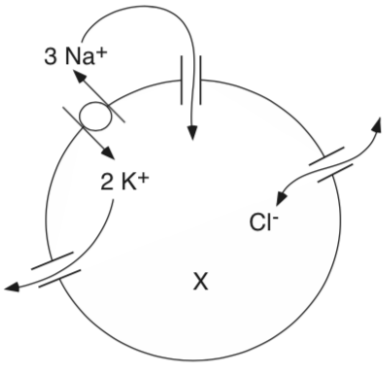
afectado por los cambios conformacionales. Construya un modelo más detallado en el que se relajen estos supuestos y calcule el flujo a través de la membrana. Diga qué unidades tiene el flujo calculado (acá no estoy pidiendo unidades específicas como *cm* o *s*, sino unidades de qué, *e.g.*, volumen/tiempo o lo que corresponda) e interprete qué significa. Escriba la condicón que debería satisfacerse en el nuevo modelo para que se anule dicho flujo.

4. *Ejercicio tomado de "Mathematical Physiology" de Keener y Sneyd.* En las páginas 69-70 del libro "Mathematical Physiology" de Keener y Sneyd que se adjuntan al final de la guía se introduce un modelo para el intercambiador de Na^+-Ca^{2+}. Re-escriba las ecuaciones dinámicas suponiendo que los procesos de pegado y despegado del Na^+ y el Ca^{2+} al intercambiador ocurren en una escala de tiempo mucho más rápida que la de los procesos de intercambio entre el interior y el exterior de la célula. *Ayuda:*, suponga que se alcanza rápidamente el equilibrio dado por $k_1 c_i x_1 = k_{-1} n_i^3 x_2$ y $k_3 n_e^3 y_2 = k_{-3} c_e y_1$. Luego introduzca las nuevas variables $X = X_1 + X_2$ e $Y = Y_1 + Y_2$ y obtenga las ecuaciones para X e Y.

5. *Repaso: i)* Considere un capacitor plano-paralelo de área A cuyas placas están separadas por una distancia d. ¿Cuál es su capacidad? ¿Cómo se vincula la carga en cada placa conductora con la diferencia de potencial entre placas? *ii)* Considere un capacitor de capacidad C conectado a un cable por el que circula una corriente I. Diga cómo varía la carga, Q, en el capacitor como función de I. Discuta la relación entre el signo de I y el de dQ/dt.

6. Considere una célula cuya membrana actúa como un capacitor de capacidad C. Suponga que hay un canal con conductancia $g(V)$ en la membrana tal que la corriente que lo atraviesa puede escribirse como $I = g(V)(V - V_0)$, donde V es el potencial de membrana. Escriba una ecuación de evolución para V (es decir, la ecuación $dV/dt = ...$).

7. *Regulación del volumen celular, descripción sencilla tomada del libro "Mathematical Physiology" de Keener y Sneyd.* Considere el modelo simplificado de la figura que tiene en cuenta 4 especies: los iones simples Na^+, K^+ y Cl^- y la especie, X, que representa moléculas grandes (proteínas) de carga negativa. Las moléculas de X no atraviesan la membrana. Los iones simples sí lo hacen a través de canales específicos para cada uno de ellos. El modelo incluye también una bomba que, a una tasa por unidad de tiempo, p, extrae del medio intracelular 3 iones Na^+ por cada 2 iones K^+ que ingresa a dicho medio. Suponga que la corriente de cada uno de estos iones a través de sus canales está dada por una función lineal, $g_i(V - V_{eq,i})$, del potencial de membrana, V, con una conductividad, g_i, constante.



(a) Escriba las ecuaciones que rigen la variación de la concentración intracelular (en número de moles por unidad de volumen) de los 3 iones como función de V, p, la temperatura, T, las concentraciones extracelulares, las conductividades, g_{Na}, g_K, g_{Cl}, y las constantes que haga falta.

(b) Suponiendo que la presión hidrostática vale lo mismo a un lado y a otro de la membrana, escriba la ecuación que rige la variación del volumen, w, de la célula debida a la presión osmótica como función de las cantidades ya mencionadas y la resistencia de la membrana, r, al flujo de agua a través de ella ($rQ = \Delta P$, donde Q es el caudal en unidades de volumen a la menos 1 y P, la presión).

(c) Escriba la ecuación que rige la variación del potencial de membrana despreciando el efecto de la bomba de Na^+-K^+.

(d) Suponiendo que las concentraciones extracelulares permanecen constantes y que hay electroneutralidad tanto dentro como fuera de la célula, encuentre las condiciones que deben satisfacerse en el equilibrio.

(e) Definiendo el volumen, $\mu \equiv w[Cl^-]_e/[X]$, y la tasa, $P = peF/(RTg_{Na})$, adimensionales donde $e = 1.610^{-19} Coulomb$ es la carga del electrón, y tomando $\gamma = g_{Na}/g_K = 0.11$, $z_X = -1$, $[Na^+]_e = 437mM$, $[K^+]_e = 20mM$, $RT/F = 25.8mV$, grafique μ, el potencial de membrana y los potenciales de equilibrio del $Na^]$ y el K^+ commo función de P (es decir, reproduzca las figuras 2.15 y 2.16 del libro "Mathematical Physiology" que se incluyen en lo que sigue).

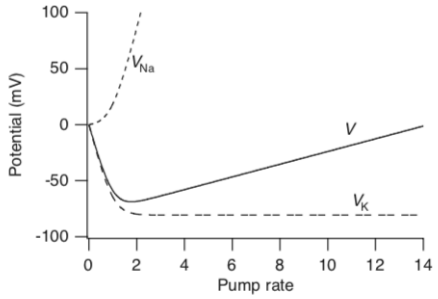


Figure 2.16 Membrane potential, Na^+ equilibrium potential, and K^+ equilibrium potential as functions of the pump rate.

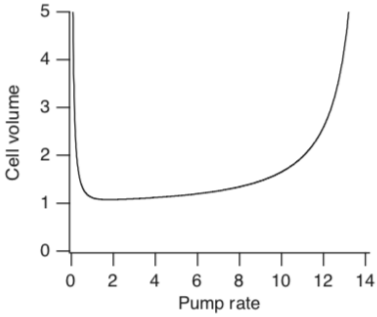


Figure 2.15 Cell volume as a function of the pump rate.

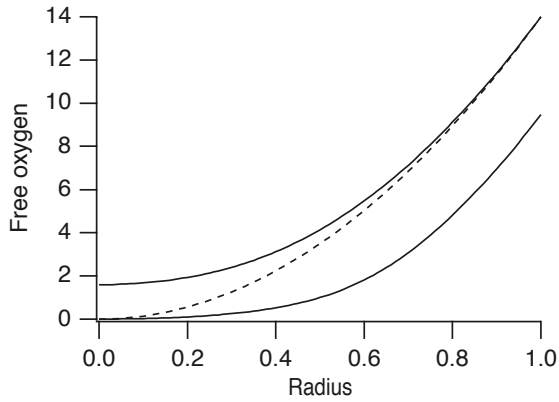


Figure 2.5 Free oxygen σ as a function of radius y. Solid curves show oxygen concentration in the presence of myoglobin ($\rho = 5$), the lower of the two having the critical external oxygen concentration. The dashed curve shows the oxygen concentration without facilitation at the critical external concentration level.

previous section. Carrier-mediated transport is the means by which some sugars cross the cell membrane to provide an energy source for the cell. For example, glucose, the most important of the sugars, combines with a carrier protein at the outer boundary of the membrane, and by means of a conformational change is released from the inner boundary of the membrane.

There are three types of carrier-mediated transport. Carrier proteins that transport a single solute from one side of the membrane to the other are called *uniports*. Other proteins function as coupled transporters by which the simultaneous transport of two solute molecules is accomplished, either in the same direction (called a *symport*) or in the opposite direction (called an *antiport*).

2.4.1 Glucose Transport

Although the details are not certain, the transport of glucose across the lipid bilayer of the cell membrane is thought to occur when the carrier molecule alternately exposes the solute binding site first on one side and then on the other side of the membrane. It is considered highly unlikely that the carrier molecule actually diffuses back and forth through the membrane.

We can model the process of glucose transport as follows: We suppose that the population of enzymatic carrier proteins C has two conformational states, C_i and C_e, with its glucose binding site exposed on the cell interior (subscript i) or exterior (subscript e) of the membrane, respectively. The glucose substrate on the interior S_i can bind with C_i and the glucose substrate on the exterior can bind with enzyme C_e to form the complex P_i or P_e, respectively. Finally, a conformational change transforms P_i into P_e and vice versa. These statements are summarized in Fig. 2.6.

The equations describing this model are

$$\frac{dp_i}{dt} = kp_e - kp_i + k_+ s_i c_i - k_- p_i, \tag{2.49}$$

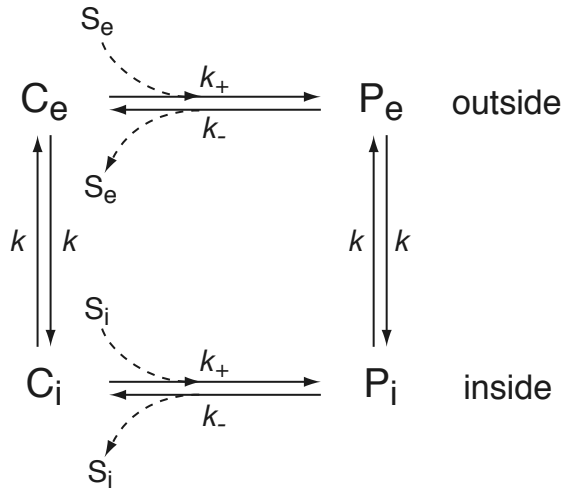


Figure 2.6 Schematic diagram of the glucose transporter described by (2.49)–(2.52).

$$\frac{dp_e}{dt} = kp_i - kp_e + k_+ s_e c_e - k_- p_e, \tag{2.50}$$

$$\frac{dc_i}{dt} = kc_e - kc_i + k_- p_i - k_+ s_i c_i, \tag{2.51}$$

$$\frac{dc_e}{dt} = kc_i - kc_e + k_- p_e - k_+ s_e c_e. \tag{2.52}$$

where $s_i = [\mathrm{S}_i]$, $p_i = [\mathrm{P}_i]$, etc. Since the total amount of receptor is conserved, we have $p_i + p_e + c_i + c_e = C_0$, where C_0 is a constant (the total transporter concentration). Hence there are only three independent equations, not four. The flux, J, is

$$J = k_- p_i - k_+ s_i c_i = k_+ s_e c_e - k_- p_e, \tag{2.53}$$

where we have defined a flux from outside to inside to be positive.

We find the steady-state flux by setting all derivatives to zero and solving the resulting algebraic system. It follows that

$$J = \frac{1}{2} K k C_0 \frac{s_e - s_i}{(s_i + K + K_d)(s_e + K + K_d) - K_d^2}, \tag{2.54}$$

where $K = k_-/k_+$ and $K_d = k/k_+$. Since k is the rate at which conformational change takes place, it acts like a diffusion coefficient in that it reflects the effect of random thermal activity at the molecular level.

The nondimensional flux is

$$j = \frac{\sigma_e - \sigma_i}{(\sigma_i + 1 + \kappa)(\sigma_e + 1 + \kappa) - \kappa^2}, \tag{2.55}$$

where $\sigma_i = s_i/K, \sigma_e = s_e/K, \kappa = K_d/K$. A plot of this nondimensional flux is shown in Fig. 2.7, plotted as a function of extracellular glucose σ_e, with fixed intracellular glucose and fixed κ. We can see that the rate of transport is limited by saturation of the enzyme kinetics (this saturation is observed experimentally) and thermal conformational

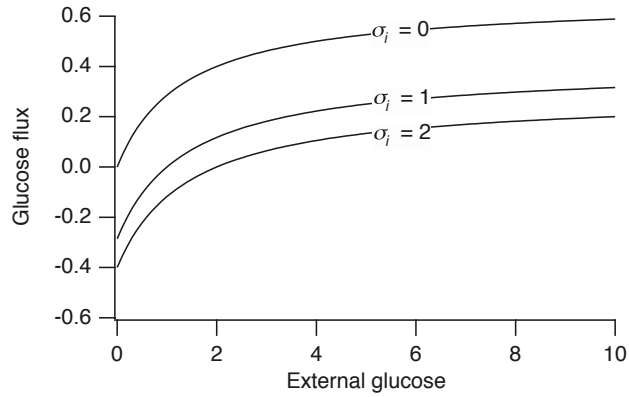


Figure 2.7 Plot of the (nondimensional) flux of glucose as a function of extracellular glucose, for three fixed intracellular glucose concentrations (σ_i), with $\kappa = K_d/K = 0.5$.

change is crucial to the transport process, as transport J drops to zero if $K_d = 0$. The binding affinity of the carrier protein for glucose (k_+), and hence the flux of glucose, is controlled by insulin.

It is important to recognize that the above expression for J is for the steady-state flux only. If the system is not at steady state, then the flux from the outside to the transporter, $J_{\text{on}} = k_+ s_e c_e - k_- p_e$, need not be the same as the flux off the transporter to the inside, $J_{\text{off}} = k_- p_i - k_+ s_i c_i$. Obviously, in this case the differential equations must be solved to obtain J_{on} and J_{off}.

It should be noted that there are two ways that the model of Fig. 2.6 can be understood. First, as we did here, we can let each variable represent the concentration of transporters in each of the four possible states. In this case, the conservation relationship is $s_i + p_i + s_e + p_e = C_0$. If each of the variables is scaled by C_0, the conservation relationship becomes $s_i + p_i + s_e + p_e = 1$, and each variable is then the fraction of the population in each state.

However, there is another way to interpret this second conservation relationship. If $s_i + p_i + s_e + p_e = 1$ we can interpret the model as referring to the behavior of a single exchanger, in which case the variables are probabilities of being in a given state, and the exchanger is modeled as a Markov process (see the Appendix to this chapter).

Markov models such as that shown in Fig. 2.6 can often be simplified by assuming that some of the transitions are much faster than others. The technique of reduction using a fast time scale is used in many places throughout this book; indeed, it is used in Chapter 1, in the equilibrium and quasi-steady-state approximations of enzyme kinetics; even though the technique is described in Chapter 1, it is sufficiently important that it warrants repeating.

The procedure can be simply illustrated with this model of the glucose transporter. Suppose that the binding and release of glucose is much faster than the change in conformation, i.e., that the transitions between C_e and P_e, and between C_i and P_i, are

For an antiport, the subscripts on one of the substances must be exchanged, to give

$$J = \frac{1}{2} K_d K k_+ C_0 \frac{s_e^m t_i^n - s_i^m t_e^n}{(s_i^m t_e^n + K + K_d)(s_e^m t_i^n + K + K_d) - K_d^2}. \tag{2.63}$$

The effectiveness of this type of exchanger is determined by the coefficients m and n. For this antiport, flux is positive (S flows inward and T flows outward) if

$$\left(\frac{s_e}{s_i}\right)^m > \left(\frac{t_e}{t_i}\right)^n. \tag{2.64}$$

For example, for the Na^+–Ca^{2+} exchanger (discussed in more detail in the next section) which exchanges three Na^+ ions for one Ca^{2+} ion, a ratio of extracellular to intracellular Na^+ of about 8 can be used to effectively pump Ca^{2+} out of a cell even when the ratio of extracellular to intracellular Ca^{2+} is 500.

2.4.3 Sodium–Calcium Exchange

For the glucose transporter described above, membrane flux is driven by a concentration difference of glucose across the membrane, and if glucose concentrations equilibrate, the transmembrane flux becomes zero. However, because it relies on two concentration differences, an antiport transporter such as the Na^+–Ca^{2+} exchanger can act as a pump. Although this transporter is a passive pump (because it consumes no chemical energy directly), it is often described as a secondarily active pump; it uses the Na^+ gradient to pump Ca^{2+} out of the cell against its concentration gradient, but energy is required to establish and maintain the Na^+ gradient. Na^+–Ca^{2+} exchange is an important mechanism for Ca^{2+} removal in a number of cell types, particularly cardiac ventricular cells, in which much of the Ca^{2+} that enters the cell during an action potential is removed from the cell by the Na^+–Ca^{2+} exchanger (Chapter 12). It has therefore been studied extensively, and a number of highly detailed models have been constructed (Hilgemann, 2004; Kang and Hilgemann, 2004). Here we describe a simple model of this important transporter.

In our model (see Fig. 2.9), E_i is the exchanger protein in the conformation for which the binding sites are exposed to the interior of the cell, and E_e is the conformation for which the binding sites are exposed to the exterior. Starting at state X_1 in the top left of the figure, the exchanger can bind Ca^{2+} inside the cell, simultaneously releasing three Na^+ ions to the interior. A change of conformation to E_e then allows the exchanger to release the Ca^{2+} to the outside and bind three external Na^+. A return to the E_i conformation completes the cycle. Of course, it is a crude approximation to assume that one Ca^{2+} and three Na^+ ions bind or unbind the exchanger simultaneously.

It is now straightforward to calculate the steady flux for this model. As with the previous transporter models, we first solve for the steady-state values of x_1, x_2, y_1, and y_2, the fraction of exchangers in the state X_1, X_2, Y_1, and Y_2, respectively. There are four equations: three differential equations for exchanger states and one conservation

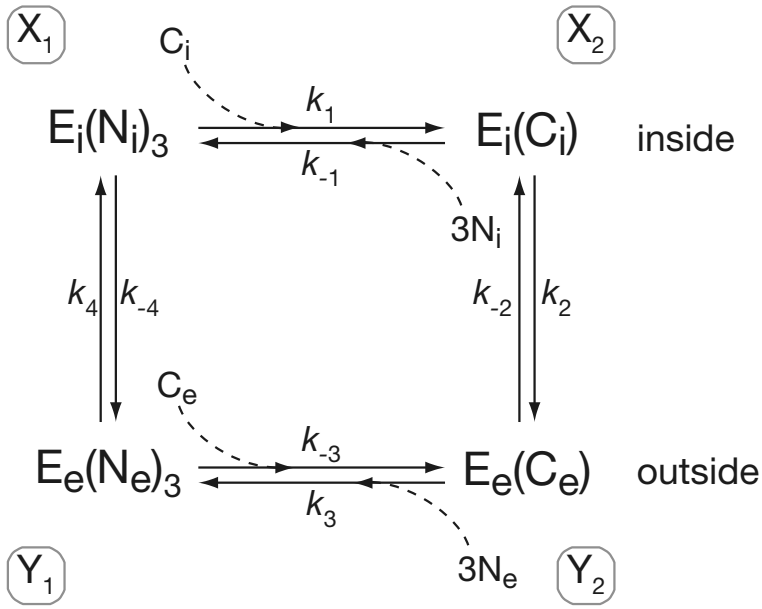


Figure 2.9 Schematic diagram of a simple model of the Na^+–Ca^{2+} exchanger.

equation. These are

$$\frac{dx_1}{dt} = k_{-1}n_i^3 x_2 + k_4 y_1 - (k_1 c_i + k_{-4})x_1, \tag{2.65}$$

$$\frac{dx_2}{dt} = k_{-2}y_2 + k_1 c_i x_1 - (k_2 + k_{-1}n_i^3)x_2, \tag{2.66}$$

$$\frac{dy_1}{dt} = k_{-4}x_1 + k_3 n_e^3 y_2 - (k_4 + k_{-3}c_e)y_1, \tag{2.67}$$

$$1 = x_1 + x_2 + y_1 + y_2. \tag{2.68}$$

Here c and n denote, respectively, Ca^{2+} and Na^+ concentration, and the subscripts e and i represent external and internal concentrations.

Using a symbolic package such as Maple, the steady-state solution of these equations is easily calculated. The flux, J, is found to be

$$J = k_4 y_1 - k_{-4}x_1 = \frac{k_1 k_2 k_3 k_4 (c_i n_e^3 - K_1 K_2 K_3 K_4 c_e n_i^3)}{16 \text{ positive terms}}, \tag{2.69}$$

where, as usual, $K_i = k_{-i}/k_i$.

Notice that the units of the flux J here (1/time) are different from those in the previous examples (concentration/time), because here the variables x_i and y_i are fractions of exchangers in a particular state (or probabilities) rather than concentrations of exchangers in a particular state. Hence, the flux in this model is a turnover rate, i.e., the

number of times the exchanger goes around the cycle per unit time. This can easily be converted to a concentration per time if the concentration of the exchangers is known.

An Electrogenic Exchanger

An important difference between the Na^+–Ca^{2+} exchange process and the transport processes discussed previously is that Na^+ and Ca^{2+} are ions. Since each cycle of the Na^+–Ca^{2+} exchanger transports two positive charges out and three positive charges in, it generates an electric current. Such exchangers are said to be *electrogenic*.

As is discussed in Section 2.6, all cells have an electrical potential difference across their membranes. Clearly, additional work is necessary for the exchanger to move electric current against a potential difference. To take this into account, consider a ligand, L, with a charge z, and suppose that there is a process that moves L from the cell interior with potential V_i to the cell exterior with potential V_e, i.e.,

$$L_i \rightarrow L_e. \tag{2.70}$$

The change in chemical potential (cf. Section 1.2) for this reaction is

$$\begin{aligned}\Delta G &= G^0_{L_e} + RT\ln([L_e]) + zFV_e - G^0_{L_i} - RT\ln([L_i]) - zFV_i \\ &= RT\ln\left(\frac{[L_e]}{[L_i]}\right) - zFV,\end{aligned} \tag{2.71}$$

where $V = V_i - V_e$ is the transmembrane potential. (The standard convention is to define the potential difference across the membrane as the internal potential minus the external potential, as discussed further in Section 2.6.1.) The standard free energy for L is the same on both sides of the membrane, so $G^0_{L_e} = G^0_{L_i}$. At equilibrium, $\Delta G = 0$, so that

$$K = \frac{[L_i]_{eq}}{[L_e]_{eq}} = \exp\left(\frac{-zFV}{RT}\right), \tag{2.72}$$

where K is the equilibrium constant for the reaction.

For the Na^+–Ca^{2+} exchanger, the overall reaction begins with three Na^+ outside the cell and one Ca^{2+} inside the cell, and ends with three Na^+ inside the cell and one Ca^{2+} outside. We can write this as

$$3Na^+_e + Ca^{2+}_i \longrightarrow 3Na^+_i + Ca^{2+}_e. \tag{2.73}$$

The change in chemical potential for this reaction is

$$\Delta G = RT\ln\left(\frac{n_i^3 c_e}{n_e^3 c_i}\right) + FV. \tag{2.74}$$

At equilibrium we must have $\Delta G = 0$, in which case

$$\frac{n^3_{i,eq} c_{e,eq}}{n^3_{e,eq} c_{i,eq}} = \exp\left(-\frac{FV}{RT}\right). \tag{2.75}$$