580.439/639 Homework #2 Solutions

Problem 1

Part a) Using the principle of conservation of mass, the time rate of change of concentration in the i^{th} well is given by the difference between the flux into the well and the flux out of the well. Let C_i be the concentration at the i^{th} location in the membrane. Note that, because the wells are discrete locations, it makes sense to consider C_i to have units of moles/area, i.e. the x direction through the membrane is considered to be discrete and not continuous. Then

$$\frac{dC_i}{dt} = \left\{\text{flux into } i^{th} \text{ site}\right\} - \left\{\text{flux out of } i^{th} \text{ site}\right\} = J_{i-1} - J_i$$

In the steady state, $dC_i/dt = 0$ so that $J_{i-1} = J_i$ for all sites. This is a slight adaptation of the derivation given in class, differing in the definition of C_i (Note the difference in units in the two cases).

Part b) There are N flux equations, which can be written as N simultaneous linear equations in N as below.

$$\begin{aligned}
J &= \lambda(k_0 C_0 - k_{-1} C_1) \\
J &= \lambda(\qquad k_1 C_1 - k_{-2} C_2) \\
&\qquad\qquad \text{etc.} \\
&\qquad\qquad \text{etc.} \\
J &= \lambda(\qquad\qquad k_{N-1} C_{N-1} - k_{-N} C_N)
\end{aligned}$$

These can be solved simultaneously by successively multiplying them by the products of rate-constant ratios as

$$J = \lambda(k_0 C_0 - k_{-1} C_1)$$

$$\frac{k_{-1}}{k_1} J = \lambda\left(\qquad k_{-1} C_1 - \frac{k_{-1}}{k_1} k_{-2} C_2\right)$$

$$\frac{k_{-2}}{k_2}\frac{k_{-1}}{k_1} J = \lambda\left(\qquad \frac{k_{-1}}{k_1} k_{-2} C_2 - \frac{k_{-2}}{k_2}\frac{k_{-1}}{k_1} k_{-3} C_3\right)$$

etc.

$$\prod_{i=1}^{N-1} \frac{k_{-i}}{k_i} J = \lambda\left(\qquad k_{-(N-1)} \prod_{i=1}^{N-2} \frac{k_{-i}}{k_i} C_{N-1} - k_{-N} \prod_{i=1}^{N-1} \frac{k_{-i}}{k_i} C_N\right)$$

Summing the equations above gives

$$J\left[1+\sum_{j=1}^{N-1}\prod_{i=1}^{j}\frac{k_{-i}}{k_i}\right]=\lambda\left[k_0C_0-k_{-N}\prod_{i=1}^{N-1}\frac{k_{-i}}{k_i}\,C_N\right]$$

from which Eqn. (∗) of the problem set follows:

$$J=\lambda k_0\,\frac{C_0-C_N\dfrac{k_{-N}}{k_0}\displaystyle\prod_{i=1}^{N-1}\frac{k_{-i}}{k_i}}{1+\displaystyle\sum_{j=1}^{N-1}\prod_{i=1}^{j}\frac{k_{-i}}{k_i}} \tag{1}$$

Note that this equation is a completely general description of a barrier system that obeys independence. That is the particular barrier heights (G_B and G_W) assumed in the statement of this problem have not been used in the derivation so far, nor has the distribution of electrical potential.

Part c) The rate constants are given by the expressions discussed in class. In writing the rate constants, it is assumed that the electrical potential at the i^{th} barrier peak is $(i-0.5)\cdot\Delta V/N$ and that the electrical potential at the i^{th} potential well is $i\cdot\Delta V/N$. That is, we assume that the electrical potential varies linearly through the model,.

$$\begin{aligned}
&k_0=(\text{const})\,e^{-(G_B+zF\Delta V/2N)/RT} && \\
&k_i=(\text{const})\,e^{-(G_B-G_W+zF\Delta V/2N)/RT} && \text{for } i=1,\ldots N-1\\
&k_{-i}=(\text{const})\,e^{-(G_B-G_W-zF\Delta V/2N)/RT} && \text{for } i=1,\ldots N-1\\
&k_{-N}=(\text{const})\,e^{-(G_B-zF\Delta V/2N)/RT} &&
\end{aligned} \tag{2}$$

To evaluate Eqn. (1), we need ratios of the rate constants as follows:

$$\begin{aligned}
&\frac{k_{-N}}{k_0}=\frac{(\text{const})\,e^{-(G_B-zF\Delta V/2N)/RT}}{(\text{const})\,e^{-(G_B+zF\Delta V/2N)/RT}}=e^{zF\Delta V/NRT} &&\\
&\frac{k_{-i}}{k_i}=\frac{(\text{const})\,e^{-(G_B-G_W-zF\Delta V/2N)/RT}}{(\text{const})\,e^{-(G_B-G_W+zF\Delta V/2N)/RT}}=e^{zF\Delta V/NRT} && \text{for } i=1,\ldots N-1
\end{aligned} \tag{3}$$

Part d) Now substituting the expressions in Eqns. (2) and (3) into Eqn. (1) gives

$$J=\lambda\,(\text{const})\,e^{-G_B/RT}\;e^{-zF\Delta V/2NRT}\;\frac{C_0-C_N\,e^{zF\Delta V/NRT}\left(e^{zF\Delta V/NRT}\right)^{N-1}}{1+\displaystyle\sum_{j=1}^{N-1}\left(e^{zF\Delta V/NRT}\right)^{j}} \tag{4}$$

The sum in the denominator can be evaluated using the relationship

$$\sum_{n=0}^{N-1}x^n=\frac{1-x^N}{1-x}$$

by noting that

$$1 + \sum_{j=1}^{N-1} \left(e^{zF\Delta V/NRT}\right)^j = \sum_{j=0}^{N-1} \left(e^{zF\Delta V/NRT}\right)^j = \frac{1 - e^{zF\Delta V/RT}}{1 - e^{zF\Delta V/NRT}}$$

so that Eqn. (4) becomes

$$J = \lambda\,(\text{const})\, e^{-G_B/RT}\; e^{-zF\Delta V/2NRT} \left(1 - e^{zF\Delta V/NRT}\right) \frac{C_0 - C_N\, e^{zF\Delta V/RT}}{1 - e^{zF\Delta V/RT}} \tag{5}$$

Converting Eqn. (5) to current density by multiplying by -zF, reversing the sense of the positive flow as usual, gives

$$I = zF\lambda\,(\text{const})\, e^{-G_B/RT}\; e^{-zF\Delta V/2NRT} \left(e^{zF\Delta V/NRT} - 1\right) \frac{C_0 - C_N\, e^{zF\Delta V/RT}}{1 - e^{zF\Delta V/RT}} \tag{6}$$

where the minus sign has been used to reverse one of the exponential terms. Eqn. (6) is essentially the same as the GHK constant-field equation, except for the exponential terms in front of the ratio. If N is large enough that $zF\Delta V/NRT << 1$, then these terms can be approximated as

$$e^{-zF\Delta V/2NRT} \left(e^{zF\Delta V/NRT} - 1\right) = e^{zF\Delta V/2NRT} - e^{-zF\Delta V/2NRT}$$

$$\approx \left(1 + \frac{zF\Delta V}{2NRT}\right) - \left(1 - \frac{zF\Delta V}{2NRT}\right) = \frac{zF}{NRT}\,\Delta V$$

where use has been made of the approximation $\exp(1+\varepsilon) \approx 1 + \varepsilon$ for $\varepsilon << 1$. Now Eqn. (6) can be written as

$$I = (zF)^2\, \frac{\lambda\,(\text{const})\, e^{-G_B/RT}}{NRT}\, \Delta V\, \frac{C_0 - C_N\, e^{zF\Delta V/RT}}{1 - e^{zF\Delta V/RT}}$$

which is identical to the GHK constant-field equation with the mobility u defined as

$$u = \frac{\lambda^2\,(\text{const})\, e^{-G_B/RT}}{RT}$$

Use has been made of the fact that the thickness of the membrane, d, equals $N\lambda$. Note that the depth of the potential wells G_W does not enter into the result, as is always the case when independence is assumed, i.e. when the number of sites in the model is assumed to be large, so that the system is working far from saturation. In this situation, permeation is controlled by the energy peaks G_B only.

Problem 2

Part a) Rewrite Eqn. (*) from the problem set as follows:

$$J = \lambda k_0 \frac{C_0 - C_N f(\Delta V)}{g(\Delta V, \{G_i\})} \tag{1}$$

where the sums and products of rate constants from Eqn. (∗) of the problem set have been summarized into the functions *f*() and *g*(). f() depends on membrane potential only, as will be shown below. *g*() may also depend on barrier heights, that is on the set $\{G_i\}$. The unidirectional fluxes predicted by Eqn. (1) can be obtained by setting either C_0 or C_N to 0. That is, if there is no ion on side 0 (C_0=0), the the net flux equals the unidirectional flux from side N and vice-versa. Independence is necessary to this definition of unidirectional flux because we assume that the fluxes from the two sides add linearly in producing the net flux in Eqn. (1). To see that this is so, look back through the derivation of Eqn. (∗) and notice that nothing changes if C_0 is assumed to be 0 at the start.

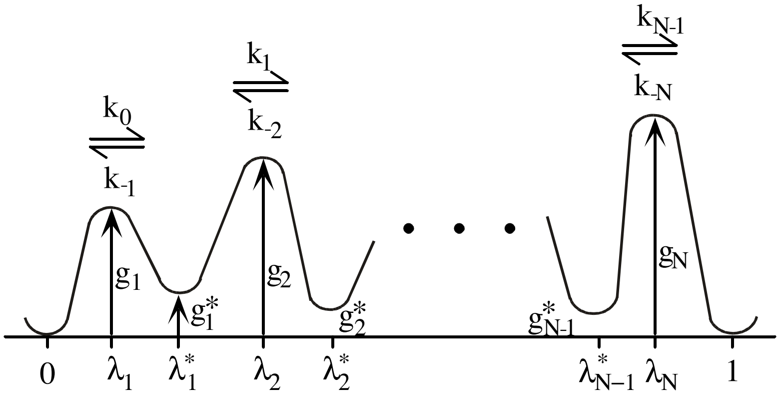
With this assumption, the unidirectional fluxes $J_{0\to N}$ and $J_{N\to 0}$ and the flux ratio are given by ($\Delta V = V_N - V_0$)

$$J_{0\to N} = \lambda k_0 C_0 \big/ g(\Delta V, \{G_i\}) \qquad \text{and} \qquad J_{N\to 0} = \lambda k_0 C_N f(\Delta V) \big/ g(\Delta V, \{G_i\})$$

$$\begin{matrix}\text{flux}\\ \text{ratio}\end{matrix} = \frac{J_{0\to N}}{J_{N\to 0}} = \frac{C_0}{C_N\, f(\Delta V)} \tag{2}$$

The minus sign that should appear in the equation for $J_{N\to 0}$ has been deleted because it is the magnitudes of the fluxes we care about.

To evaluate f(ΔV), consider the general barrier system sketched below. The notation here is the same as for Problem 1.



The barrier heights are expressed in normalized form, i.e. as $g_1 = G_1/RT$. The λ_i are fractions of the transmembrane potential that appear at each barrier, so that the membrane potential at barrier g_1 is $\lambda_1 v$, where $v = zF\Delta V/RT$. The components of Eqn. (∗) can now be written as follows.

$$\frac{k_{-i}}{k_i} = \frac{(const)e^{-[g_i+\lambda_i v - g_i * - \lambda_i * v]}}{(const)e^{-[g_{i+1}+\lambda_{i+1} v - g_i * - \lambda_i * v]}} = e^{g_{i+1}-g_i+(\lambda_{i+1}-\lambda_i)v}$$

$$\frac{k_{-N}}{k_0} = \frac{(const)e^{-[g_N+(\lambda_N-1)v]}}{(const)e^{-[g_1+\lambda_1 v]}} = e^{g_1-g_N+(\lambda_1-\lambda_N+1)v}$$

$$\frac{k_{-N}}{k_0}\prod_{i=1}^{N-1}\frac{k_{-i}}{k_i} = e^{g_1-g_N+(\lambda_1-\lambda_N+1)v}\exp\left[\sum_{i=1}^{N-1} g_{i+1}-g_i+(\lambda_{i+1}-\lambda_i)v\right] = e^{v} \tag{3}$$

With this result, the flux ratio Eqn. (2) now takes the Ussing form. Note that this derivation depends directly on the independence assumption because if independence is not true, then there will be terms involving concentrations in the denominator of Eqn. (∗) in the problem set. However, this assumption does not depend on any details of the barrier system or of the distribution of electrical potential through the membrane (i.e. no constant-field assumption).

Note that we could have argued that $f(\Delta V) = e^{v}$ because of the necessity that J=0 at equilibrium in Eqn. (∗). This is the way the argument is usually made.

If independence does not hold, then the unidirectional fluxes take the form of Eqn. 11 of problem 2 above

$$I_{1\to 2} = f(V)\frac{A_1}{g(V, A_1, A_2 = 0, \{G_i\})} \quad \text{and} \quad I_{2\to 1} = f(V)\frac{A_2 e^{zFV/RT}}{g(V, A_1 = 0, A_2, \{G_i\})}$$

where the denominator function $g(..)$ now depends on the concentrations and is different for the two unidirectional fluxes, giving a flux ratio that varies with concentration.

Part b) The Hodgkin-Huxley independence test involves the ratio of the currents obtained with two different sets of concentrations of an ion S in the bounding solutions. From Eqn. 1 above, with the independence assumption,

$$\frac{I'_S}{I_S} = \frac{S'_o - S'_i f(\Delta V)}{g(\Delta V, \{G_{S'}\})}\,\frac{g(\Delta V, \{G_S\})}{[S_o - S_i f(\Delta V)]} \tag{4}$$

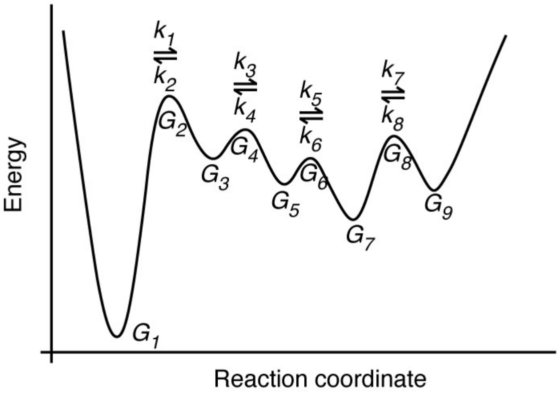
where *S* and *S'* are two different concentration gradients for the ion in question. Eqn. 4 can be simplified using the fact (Eqn. 3) that $f(\Delta V) = e^{v} = e^{zF\Delta V/RT}$ and the fact that the function $g(..)$ is the same for the two different ion concentrations, with the independence assumption. Then

$$\frac{I'_S}{I_S} = \frac{S'_o - S'_i f(\Delta V)}{S_o - S_i f(\Delta V)}$$

which is the HH independence test. Note that $g(\Delta V, \{G_{S'}\}) = g(\Delta V, \{G_S\})$ because the barriers are the same, regardless of ion concentration.

The ratio fails in the absence of independence because $g(..)$ is then a function of ion concentration.

Problem 3



The figure at right shows definitions of the rate constants for use in this problem.

Part a) Steady state means the fraction of channel in each state is fixed in time. As argued in class, that means the net transitions across each energy peak are zero, so that

$$J_{i\to i+1} = k_i x_i - k_{i+1}x_{i+2} = 0 \ ,$$

where $J_{i\to i+1}$ is the net flux from energy well *i* to well *i+1* and x_i is the fraction of the channel in the i^{th} well. Note that $x_1 + x_3 + x_5 + x_7 + x_9 = 1$. Writing a similar flux equation for each energy peak and applying the zero-flux condition gives the following ratios

$$\frac{x_1}{x_3} = \frac{k_2}{k_1} = \frac{const\ e^{-(G_2-G_3)/RT}}{const\ e^{-(G_2-G_1)/RT}} = e^{-(G_1-G_3)/RT} \gg 1 \ ,$$

and

$$\frac{x_3}{x_5} = \frac{k_4}{k_3} = e^{-(G_3-G_5)/RT} < 1, \quad \frac{x_5}{x_7} = \frac{k_6}{k_5} = e^{-(G_5-G_7)/RT} < 1, \quad \frac{x_7}{x_9} = \frac{k_8}{k_7} = e^{-(G_7-G_9)/RT} > 1 \ ,$$

where the relative sizes of the ratios follow from the energy differences in the diagram.

The fractions can be obtained by solving successively, using the fact that the x_i 's sum to 1:

$$\begin{aligned} 1 &= x_1 + x_3 + x_5 + x_7 + x_9 \\ &= \left[\frac{k_2}{k_1}\frac{k_4}{k_3}\frac{k_6}{k_5}\frac{k_8}{k_7} + \frac{k_4}{k_3}\frac{k_6}{k_5}\frac{k_8}{k_7} + \frac{k_6}{k_5}\frac{k_8}{k_7} + \frac{k_8}{k_7} + 1\right] x_9 \\ x_9 &= 1\Big/\left[\frac{k_2}{k_1}\frac{k_4}{k_3}\frac{k_6}{k_5}\frac{k_8}{k_7} + \frac{k_4}{k_3}\frac{k_6}{k_5}\frac{k_8}{k_7} + \frac{k_6}{k_5}\frac{k_8}{k_7} + \frac{k_8}{k_7} + 1\right] = 1/Z \end{aligned}$$

and

$$x_7 = \frac{k_8}{k_7}\Big/Z, \quad x_5 = \frac{k_6}{k_5}\frac{k_8}{k_7}\Big/Z, \quad x_3 = \frac{k_4}{k_3}\frac{k_6}{k_5}\frac{k_8}{k_7}\Big/Z, \quad x_1 = \frac{k_2}{k_1}\frac{k_4}{k_3}\frac{k_6}{k_5}\frac{k_8}{k_7}\Big/Z \ .$$

Note that these simplify when written in terms of energies

$$x_9 = 1/Z, \quad x_7 = e^{-(G_7-G_9)/RT}\big/Z, \quad x_5 = e^{-(G_5-G_9)/RT}\big/Z, \quad x_3 = e^{-(G_3-G_9)/RT}\big/Z, \quad x_1 = e^{-(G_1-G_9)/RT}\big/Z$$

$$(*)$$

$$Z = 1 + e^{-(G_7-G_9)/RT} + e^{-(G_5-G_9)/RT} + e^{-(G_3-G_9)/RT} + e^{-(G_1-G_9)/RT} \ .$$

For the activated state, the solution is the same except that G_1 is replaced by G_1' and G_9 is replaced by G_9'. Note that the relative sizes change so that $x_1/x_3<1$ and $x_7/x_9<1$.

The same result could be obtained by assuming that the conformations are in thermodynamic equilibrium so that

$$G_i + RT\ln x_i = G_j + RT\ln x_j \quad \text{so that} \quad x_i = x_j e^{-(G_i - G_j)/RT} \ ,$$

and so on.

Part b) From the relative sizes given above, most of the unactivated molecule is in state 1 which has a much lower energy level than the other states. After the molecule is activated, state 1 becomes the highest energy state and the molecule changes conformation so that most of it ends up in state 9'. The photon absorbtion thus drives a change of state of the molecule.

Part c) Using the rate constants above, the differential equations for the conformations can be written as

$$\begin{aligned}
\frac{dx_1}{dt} &= -k_1' x_1 + k_2 x_3 \\
\frac{dx_3}{dt} &= k_1' x_1 - (k_2 + k_3) x_3 + k_4 x_5 \\
\frac{dx_5}{dt} &= k_3 x_3 - (k_4 + k_5) x_5 + k_6 x_7 \\
\frac{dx_7}{dt} &= k_5 x_5 - (k_6 + k_7) x_7 + k_8' x_9 \\
\frac{dx_9}{dt} &= k_7 x_7 - k_8' x_9
\end{aligned} \quad .$$

The primes mark the rate constants that change with activation. Notice that these equations are singular because any one can be obtained by adding and subtracting the others; for example dx_9/dt is the sum of the other equations.. Using the fact that $x_1 + x_3 + x_5 + x_7 + x_9 = 1$ allows the last equation to be eliminated and the x_9 term in the d_7/dt equation to be written as follows:

$$\begin{aligned}
\frac{dx_1}{dt} &= -k_1' x_1 + k_2 x_3 \\
\frac{dx_3}{dt} &= k_1' x_1 - (k_2 + k_3) x_3 + k_4 x_5 \\
\frac{dx_5}{dt} &= k_3 x_3 - (k_4 + k_5) x_5 + k_6 x_7 \\
\frac{dx_7}{dt} &= -k_8' x_1 - k_8' x_3 + (k_5 - k_8') x_5 - (k_6 + k_7 + k_8') x_7 + k_8'
\end{aligned} \quad .$$

The problem didn't ask for exact initial conditions, but these are given in Eqn. (*).