580.439/639 Homework #3 Solutions

Problem 1

Part a) If the membrane is depolarized, the number of open gates increases in the HH K$^+$ channel; in the model, this means that the gating charges move to the outer membrane surface. With this definition of gating charge movement, a positive gating charge z_G is required to favor the open states as membrane potential increases (depolarizes).

Part b) Using the rate theory formulation developed in class, the unidirectional rates of transfer over the barrier are given by (k is the constant associated with rate theory models):

$$\text{rate of opening} \;=\; \text{fraction closed} \;\cdot\; k\,e^{-\left(\Delta G' - \lambda z_G FV\right)/RT} \tag{1.1}$$

$$\text{rate of closing} \;=\; \text{fraction open} \;\cdot\; k\,e^{-\left(\Delta G + (1-\lambda) z_G FV\right)/RT} \tag{1.2}$$

and

$$\frac{dn}{dt} = \text{ rate of opening } - \text{ rate of closing}$$

Substituting Eqns. 1.1 and 1.2 and noting that the fraction closed is (1-n) and the fraction open is n gives

$$\frac{dn}{dt} = (1-n)\,k\,e^{-\left(\Delta G' - \lambda z_G FV\right)/RT} - n\,k\,e^{-\left(\Delta G + (1-\lambda) z_G FV\right)/RT}$$

Collecting terms in n:

$$\frac{dn}{dt} = -k\,e^{-\Delta G'/RT}\,e^{\lambda z_G FV/RT}\left[1 + e^{\left(\Delta G' - \Delta G - z_G FV\right)/RT}\right]n + k\,e^{-\Delta G'/RT}\,e^{\lambda z_G FV/RT} \tag{1.3}$$

To simplify, define the following dimensionless variables:

$$g = \Delta G / RT \qquad g' = \Delta G' / RT \qquad v_h = \left(g' - g\right)/z_G \qquad \alpha = k e^{-\Delta G'/RT} \qquad v = \frac{FV}{RT}$$

Then Eqn. 1.3 can be written in terms of simplified variables as:

$$\frac{dn}{dt} = -\alpha e^{\lambda z_G v}\left[1 + e^{-z_G (v - v_h)}\right]n + \alpha\,e^{\lambda z_G v} \tag{1.4}$$

which is the form given in the problem assignment.

Part c) The HH differential equation for n in terms of $n_\infty(V)$ and $\tau_n(V)$ is

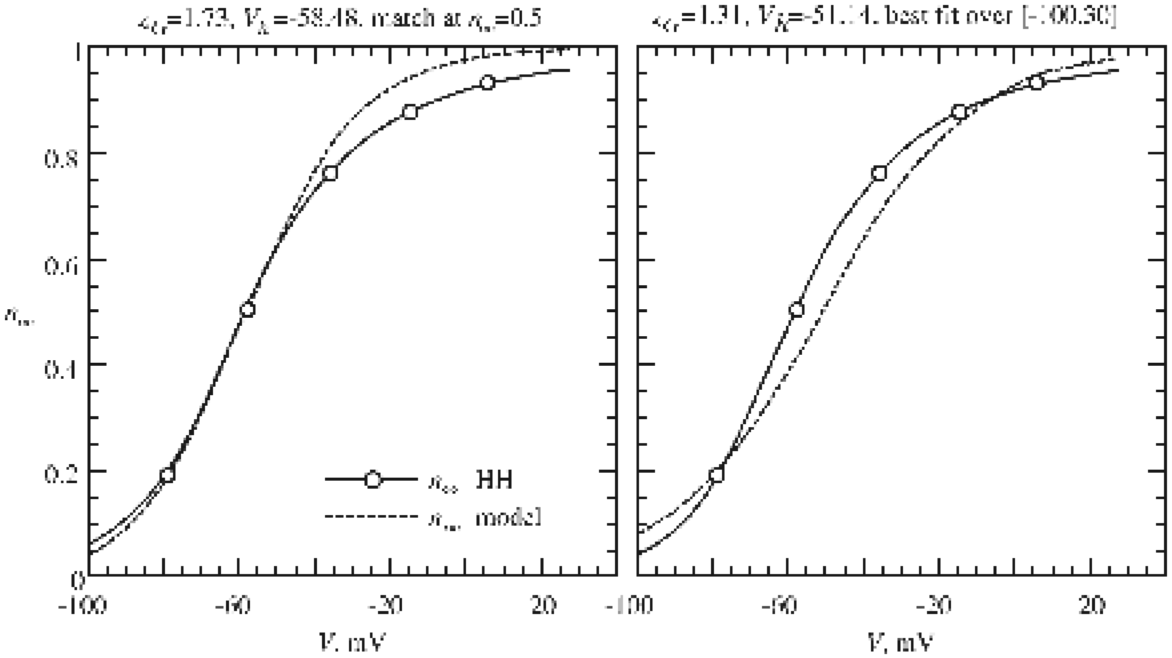
$$\frac{dn}{dt} = -\frac{n}{\tau_n} + \frac{n_\infty}{\tau_n} \tag{1.5}$$

Comparison of Eqns. 1.4 and 1.5 yields the following expressions for $n_\infty(V)$ and $\tau_n(V)$:

$$n_\infty(v) = \frac{1}{1+e^{-z_G(v-v_h)}} \qquad \tau_\mathrm{n}(v) = \frac{1}{\alpha e^{\lambda z_G v}\left[1+e^{-z_G(v-v_h)}\right]} \tag{1.6}$$

Part d) In order to fit Eqns. 1.6 to the HH model, the values of four parameters must be specified: z_G, v_h, α, and λ. Some trial and error will show that Eqns. 1.6 are similar to, but not identical to the HH equations. That is, using the HH equations in the problem set gives expressions for n_∞ and τ_n which are not the same as Eqn. 1.6; Eqns. 1.6 are an approximation of the HH model and can give a close, but not perfect fit. Therefore there is not one correct answer to this problem.

Because n_∞ involves only two parameters, it is easiest to start by estimating z_G and v_h from the n_∞ equation and then estimate α and λ from the τ_n equation. Two examples of fits of $n_\infty(V)$ from Eqn. 1.6 (labeled "n_∞ model") and n_∞(V) from the HH model (labeled " n_∞HH") are shown below.



The fit in the left graph was done by selecting z_G and $\mathrm{V_h} = v_h{\cdot}26$ mV to match the functions and their slopes at the $n_\infty = 0.5$ point. The fit at right was done by linearizing Eqn. 1.6 as

$$e^{-z_G(v-v_h)} = \frac{1-n_\infty}{n_\infty} \quad \text{or} \quad v = v_h - \frac{1}{z_G}\ln\left[\frac{1-n_\infty}{n_\infty}\right] \tag{1.7}$$

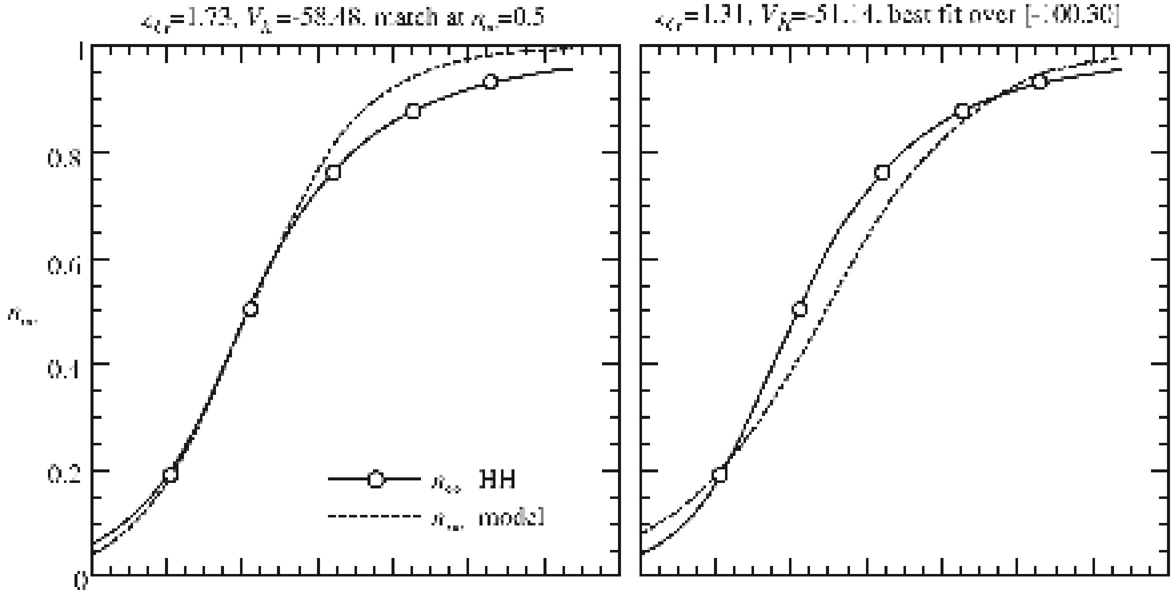
Eqn. 1.7 predicts that a plot of v versus $\ln[(1-n_\infty)/n_\infty]$ should be a straight line. n_∞ can be computed from the HH equations in the problem set and plotted in this form and a straight line fitted to the result by least squares. The slope of the straight line is then an estimate of $1/z_G$ and the intercept is an estimate of v_h. When the fit is done over the voltage range [-100, 30] mV, the result is the fit in the right hand graph above.

Neither fit is perfect; the gating charge ranges from 1.3-1.7 and $V_h = v_h RT/F$ ranges from -51 to -59 mV. V_h is a measure of $\Delta G' - \Delta G$, that is of the energy difference between the open and closed state, measured as the "equilibrium potential" for the two states. The fact that V_h is substantially negative (with $z_G > 0$) means that the open state is strongly preferred to the closed state at 0 membrane potential (as drawn in the problem set). For a discussion of gating charge z_G, see Hille, p. 54-57 or Johnston and Wu, pp. 161-169.

In order to fit the remaining parameters, α and λ, a similar procedure can be used. One way to approach this problem is to note that

$$\frac{n_\infty}{\tau_n} = \alpha e^{\lambda z_G v} \quad \text{so that} \quad \ln\left[\frac{n_\infty}{\tau_n}\right] = \ln \alpha + \lambda z_G v$$

and a plot of $\ln[n_\infty/\tau_n]$ versus v should be a straight line with slope λz_G and intercept lnα. Once again, least squares can be used to estimate the slope and intercept and α and λ computed from the parameters of the fit. When this is done, the results are shown below. The graph at left is based on a fit near $n_\infty = 0.5$, over the voltage range [-80, -40] mV. The values of z_G and V_h obtained above at $n_\infty = 0.5$ are used here. The graph at right is based on a fit over the wide voltage range used above [-100, 30] mV. In either case, the peak of the energy barrier is at (1-λ) ≈ 0.25-0.3 of the way through the membrane. Unlike the other parameters, the value of α varies widely with the fit strategy.



The rate theory model is only a crude fit to the HH equations (and to the HH data), but the fit could probably be improved by adding more barriers. Hodgkin and Huxley took a different approach by adding "fudge factor" terms to the rate-theory model in order to make the result a more accurate reflection of their data.

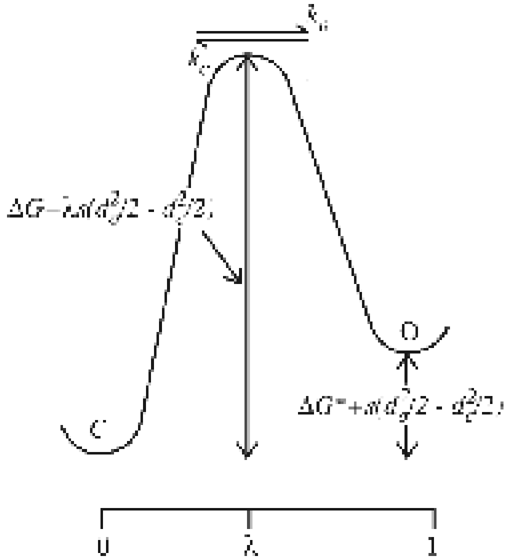
Part e) The value of 4.8 given by Hille (Fig. 13, p. 42) and Johnston and Wu (Fig. 6.20, p. 163) is for the potassium *conductance*, which is proportional to n^4, whereas we are considering the motion of only one gating particle in this problem, i.e. *n*. Hille's value should be about 4 times ours, which is only approximately so.

Problem 2

The energy diagram at right is the basis for the analysis. There is a difference ΔG^* between the closed and open states of the channel, without the contribution of the spring; $\Delta G^*>0$, so that the channel tends to be in the closed state. The spring's effect on the channel can be modeled by considering the energy transferred into or out of the spring when the channel closes or opens. Suppose the length of the spring with the channel closed is d_C and the spring's length with the channel open is d_O. Both d_C and d_O are functions of x, the separation between the cilia. Then the change in potential energy ΔG_S of the spring when the channel opens is

$$\Delta G_S = \int_{d_c}^{d_o} s\,\xi\,d\xi = s\left(\frac{d_o^2}{2} - \frac{d_c^2}{2}\right)$$

where *s* is the spring constant. This amount of energy is added to the open state of the channel in the diagram above, because when the channel opens, this amount of energy must be added to the system. In fact, $\Delta G_S < 0$ because $d_O < d_C$, and the attachment of the spring to the channel favors the open state of the channel; this makes sense in that the spring tends to pull the channel into the open state. We assume that a fraction λ of the spring's energy is transferred when the channel is passing over the energy maximum. In fact, λ does not affect the final answer to part a).



The rate constants k_O and k_C are given by

$$\begin{aligned} k_c &= (\text{const})\, e^{-(\Delta G-\Delta G^*-(1-\lambda)\Delta G_S)/RT} \\ k_o &= (\text{const})\, e^{-(\Delta G+\lambda\Delta G_S)/RT} \end{aligned} \tag{2.1}$$

The rate of channel opening is given by

$$\frac{dO}{dt} = -k_c O + k_o C = -k_c O + k_o(T-O) = k_o T - (k_o + k_c)O \tag{2.2}$$

where *T* is the total amount of channel, $T = C + O$. In the steady state, $dO/dt = 0$ and

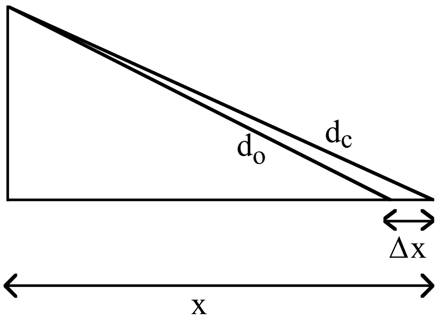
$$O = T\frac{k_o}{k_o + k_c} \tag{2.3}$$

so that

$$\text{prob. open} = \frac{O}{T} = \frac{(\text{const})\,\mathrm{e}^{-(\Delta\mathrm{G}+\lambda\Delta\mathrm{G}_\mathrm{S})/RT}}{(\text{const})\,\mathrm{e}^{-(\Delta\mathrm{G}+\lambda\Delta\mathrm{G}_\mathrm{S})/RT} + (\text{const})\,\mathrm{e}^{-(\Delta\mathrm{G}-\Delta\mathrm{G}^{*}-(1-\lambda)\Delta\mathrm{G}_\mathrm{S})/RT}}$$

with some algebra

$$\frac{O}{T} = \frac{1}{1+\mathrm{e}^{(\Delta\mathrm{G}^{*}+\Delta\mathrm{G}_\mathrm{S})/RT}} = \frac{1}{1+\mathrm{e}^{(\Delta\mathrm{G}^{*}+s(d_o^2/2-d_c^2/2))/RT}}$$



All that remains is to specify the dependence of ΔG_S on x, the spacing of the cilia. To do this fully requires various assumptions about the geometry of the system, and quite complex equations can be derived. Consider the simple case sketched at right: x is the spacing of the cilia, y is the vertical distance from the tip of one cilium to the tip of the next, and Δx is the change in position of the tip of the spring when the channel opens. In real hair cells, the distance traveled by the spring when the channel opens is small, i.e. $(d_O-d_C) << d_O, d_C$. In this case, the spring lengths d_O and d_C can be computed as

$$\begin{aligned}
d_c &= \sqrt{x^2+y^2} \\
d_o &= \sqrt{(x-\Delta x)^2+y^2} \\
&= \sqrt{x^2+y^2-2x\Delta x+\Delta x^2} \\
&= \sqrt{x^2+y^2}\sqrt{1-\frac{2x\Delta x-\Delta x^2}{x^2+y^2}} \\
&\approx \sqrt{x^2+y^2}\left(1-\frac{x\,\Delta x}{x^2+y^2}\right) = d_c\left(1-\frac{x\,\Delta x}{d_c^2}\right) = d_c - \frac{x\,\Delta x}{d_c}
\end{aligned}$$

where the assumption $\Delta x << x$ has been used to make the approximation of d_O on the last line. With these assumptions, $(d_O-d_C) \approx -x\cdot\Delta x/d_C$ which is negative because $d_O < d_C$. Furthermore, $d_O \approx d_C = d_C(x)$, the function of the spread of the cilia given on the first line above. With this assumption

$$s\left(d_o^2/2-d_c^2/2\right) == \frac{s}{2}\left(d_o+d_c\right)\left(d_o-d_c\right) \approx -s\,d_c\,x\,\Delta x\,/\,d_c = -s\,x\,\Delta x$$

so that

$$\frac{O}{T} = \frac{1}{1+e^{(\Delta G^{*}-s\,x\,\Delta x)/RT}}$$

As the cilia are spread apart, x increases, the exponential in the denominator decreases, and O/T increases, opening channels which depolarize the cell. This is thought the be the means by which mechanical transduction occurs in hair cells.

Part b) The rate of opening is given by Eqn. (2.2). At time 0^-, i.e. just before the cilia are moved apart, the system is in steady state with $O(0^-)$ open channels. At this time, $dO/dt = 0$, so Eqn. 2.3 applies, and

$$O(0^-) = T\frac{k_o(x_0)}{k_o(x_0)+k_c(x_0)} \tag{2.3a}$$

The rate constants in the equation above have their values at separation x_0. When the cilia are moved apart to $x=x_1$, the values of k_o and k_c will change and the system will no longer be in steady state. Now

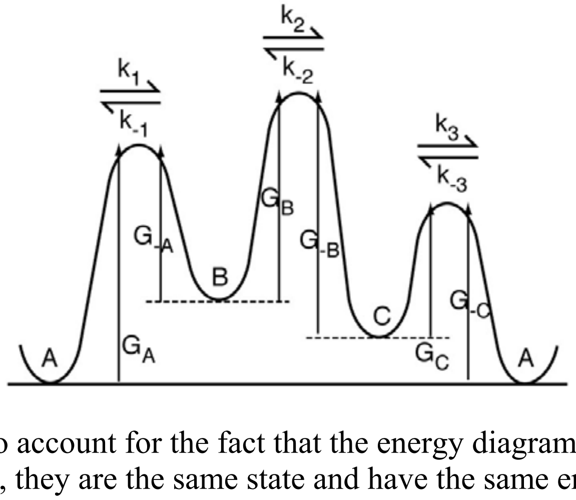
$$\frac{dO}{dt} = k_o(x_1 T - \left[k_o(x_1)+k_c(x_1)\right]O$$

Substituting the steady-state value for O at time 0^- from Eqn. 2.3a gives the initial rate of change of open channels

$$\begin{aligned}\left.\frac{dO}{dt}\right|_{t=0^+} &= k_o(x_1)T - \left[k_o(x_1)+k_c(x_1)\right]T\frac{k_o(x_0)}{k_o(x_0)+k_c(x_0)}\\ &= \frac{k_o(x_1)k_c(x_0)-k_o(x_0)k_c(x_1)}{k_o(x_0)+k_c(x_0)}T\end{aligned}$$

Problem 3

Part a) The barrier system is shown below.



Two A states are shown, to account for the fact that the energy diagram is cyclical, like the reaction system; of course, they are the same state and have the same energy level. Writing out the rate constants in terms of barrier energies:

$$\begin{aligned}k_1 k_2 k_3 &= (const)e^{-G_A/RT}\,e^{-G_B/RT}\,e^{-G_C/RT}\\ k_{-1}k_{-2}k_{-3} &= (const)e^{-G_{-A}/RT}\,e^{-G_{-B}/RT}\,e^{-G_{-C}/RT}\end{aligned} \tag{5}$$

taking the ratio $k_1k_2k_3 / k_{-1}k_{-2}k_{-3}$ gives

$$\frac{k_1 k_2 k_3}{k_{-1} k_{-2} k_{-3}} = e^{-\left[G_A - G_{-A} + G_B - G_{-B} + G_C - G_{-C}\right]/RT} \qquad (6)$$

The sum of barrier energies in the exponential of Eqn. 6 is zero, since it begins at the ground state energy of the *A* state and ends up at the same level. Thus, the cyclical nature of the energy diagram forces $k_1k_2k_3 \,/\, k_{-1}k_{-2}k_{-3}$ to be 1.

Part b) The fluxes are given by the three equations below. J_i is the flux leaving the i^{th} state.

$$\begin{aligned} J_A &= k_1 A - k_{-1} B \\ J_B &= k_2 B - k_{-2} C \\ J_C &= k_3 C - k_{-3} A \end{aligned} \qquad (7)$$

If the fluxes are zero,

$$\begin{aligned} 0 &= k_1 A - k_{-1} B & \Rightarrow \quad B &= \frac{k_1}{k_{-1}} A \\ 0 &= k_2 B - k_{-2} C & \Rightarrow \quad C &= \frac{k_2}{k_{-2}} B \\ 0 &= k_3 C - k_{-3} A & \Rightarrow \quad A &= \frac{k_3}{k_{-3}} C \end{aligned} \qquad (8)$$

Now, substituting the right-hand equations in Eqn. 8 into one another gives

$$A = \frac{k_3}{k_{-3}} C = \frac{k_3}{k_{-3}} \frac{k_2}{k_{-2}} B = \frac{k_3}{k_{-3}} \frac{k_2}{k_{-2}} \frac{k_1}{k_{-1}} A \qquad (10)$$

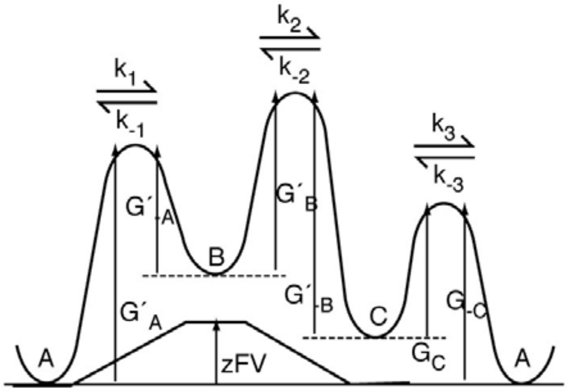
Because *A* must equal *A*, it follows from Eqn. 9 that $k_1k_2k_3 \,/\, k_{-1}k_{-2}k_{-3}=1$.

Part c) Using the definitions of fluxes in Eqn. 7, suppose that $J=J_A=J_B=J_C>0$. Recall that, in a steady state, the fluxes in a system like this one must all be equal in order that the concentrations be constant in time. Then, from Eqn. 7,

$$\begin{aligned} &J = k_1 A - k_{-1} B > 0 && \Rightarrow \quad A > \frac{k_{-1}}{k_1} B \\ &J = k_2 B - k_{-2} C > 0 && \Rightarrow \quad B > \frac{k_{-2}}{k_2} C \quad \Rightarrow \quad A > \frac{k_{-1}}{k_1}\frac{k_{-2}}{k_2} C \\ &J = k_3 C - k_{-3} A > 0 && \Rightarrow \quad C > \frac{k_{-3}}{k_3} A \quad \Rightarrow \quad A > \frac{k_{-1}}{k_1}\frac{k_{-2}}{k_2}\frac{k_{-3}}{k_3} A \end{aligned} \qquad (11)$$

The bottom rightmost equation implies that, in a steady state, $A>A$, which cannot be true, as long as *A*, *B*, and *C* are non-zero. Thus a steady state with non-zero net flux cannot exist in a system like this one.

Part d) Now the rate constants are modified by the membrane potential, so that the barrier diagram is changed by elevating the B state and the barriers leading to it.



The modified barrier heights include potential as

$$G'_A = G_A + \lambda_A zFV \qquad G'_{-A} = G_{-A} + (\lambda_A - 1)zFV$$
$$G'_B = G_B + (\lambda_B - 1)zFV \qquad G'_{-B} = G_{-B} + \lambda_B zFV$$

It is evident that the argument of part a) still holds and microscopic reversibility is still true. This would not be so if the charge *z* varied among the states, as in the transporter model considered in class.