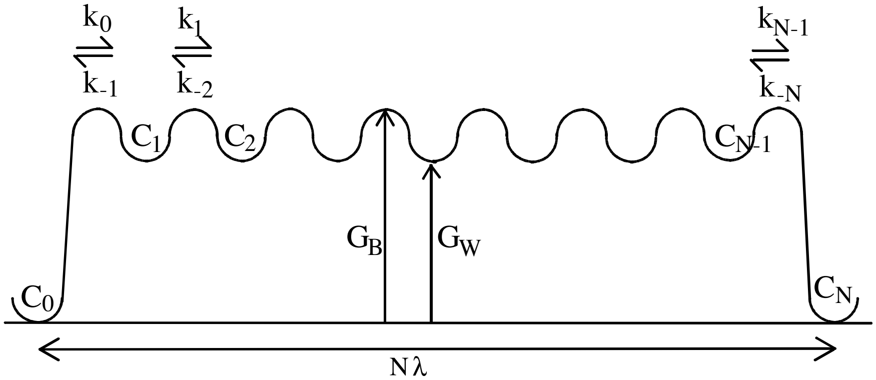
580.439/639 Homework #2

Due 9/23/09

Problem 1

Flux as described by the Nernst-Planck equation can be shown to be equivalent to flux through the barrier system diagrammed below, where it is assumed that the diffusion barrier (membrane) consists of a large number of identical small barriers.



There are N barriers, each separated from its neighbors by a distance λ, so that the thickness of the membrane is $N\lambda$. The barriers have height G_B and separate energy wells at G_W. The solutions on either side of the membrane are represented by sites 0 and N (C_0 and C_N), with 0 energy. The C_i are the concentrations of ion in each potential well. Added to the energy diagram above is a trans-membrane potential difference $\Delta V = V_N - V_0$, which is not shown; the membrane potential difference is assumed to obey the constant-field assumption, i.e. $V(x) = \Delta V x/(N\lambda)$ within the membrane.

a) Let the flux over each barrier be J_i, i=1 . . . N. Argue that the steady-state assumption implies that

$$J = J_1 = J_2 = \cdots = J_N$$

b) Assume that the net flux over each barrier is given by the following equation, which is similar to the barrier model used in class except that the distance λ between potential wells is pulled out of the (const) term in the equations for the rate constants k_i.

$$J_i = \lambda k_{i-1} C_{i-1} - \lambda k_{-i} C_i$$

Assume also that flux obeys the independence principle; that is, the concentrations are well below saturation so that the number of potential wells does not have be considered in the analysis (or equivalently, the number of potential wells is much larger than the number of occupied potential wells). Write a flux equation for each barrier and solve them simultaneously to show that

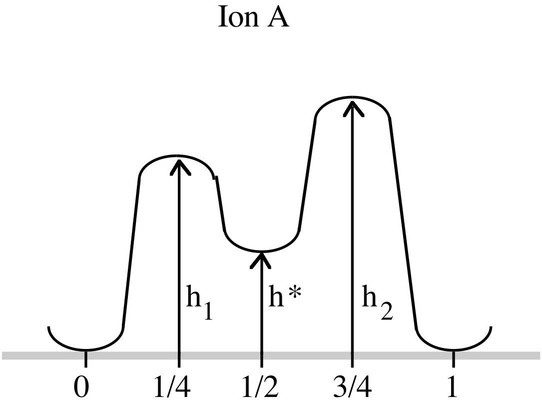
$$J = \lambda k_0 \frac{C_0 - C_N \dfrac{k_{-N}}{k_0} \displaystyle\prod_{i=1}^{N-1} \frac{k_{-i}}{k_i}}{1 + \displaystyle\sum_{j=1}^{N-1} \prod_{i=1}^{j} \frac{k_{-i}}{k_i}} \qquad (*)$$

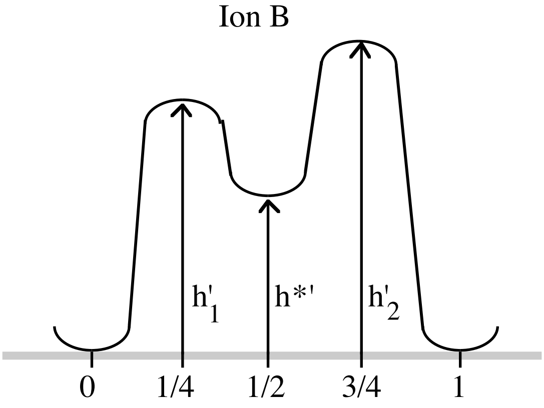
c) Write expressions for k_i and k_{-i} from the parameters of the barrier model. Include the membrane potential in these expressions. Actually, all you really care about are the terms appearing in Eqn. (∗): k_0, k_{-N}/k_0, and k_{-i}/k_i.

d) Using the rate constants from c), show that, if N is large, then Eqn. (∗) reduces to a form equivalent to the Goldman-Hodgkin-Katz constant-field equation derived from the Nernst-Planck equation in Hille and discussed in class. Give an explicit equation for mobility u in terms of the parameters of the model above. (Hint: the approximation $\exp(\varepsilon) \approx (1+\varepsilon)$ for $\varepsilon \ll 1$ may be useful.)

Problem 2

Consider a channel which is adequately modeled by the two-barrier, single binding site model considered in class and on pp. 478-483 of Hille. This model can be used to explain how the relative permeability of the channel for two ions A and B can change depending on the concentrations of the ions in test solutions. Energy barriers for the two ions are drawn below.





Energies are given in normalized form, i.e. if G_1 is the height in Joules of a barrier, then the barrier is represented in the diagram as $h_1 = G_1/RT$. Distances through the membrane are given in fractional form. Do not assume independence for this problem, unless it is appropriate for the specific conditions under consideration.

Consider first a permeability ratio experiment, in which a membrane containing only this channel is put into a test situation in which there are concentrations A_1 and B_1 on one side of the membrane and concentrations A_2 and B_2 on the other side. If ions A and B are the only permeant ions in the system, then the membrane potential is usually assumed to be given by the GHK equation:

$$\Delta V = \frac{RT}{F} \ln \frac{P_A A_1 + P_B B_1}{P_A A_2 + P_B B_2} \qquad (1)$$

Eqn. (1) can be solved for P_A/P_B, which provides an estimate of the permeability ratio of the two ions, given a measured value of ΔV and the known concentrations.

a) Eqn. (1) applies to the channel model above only under special circumstances. Assume that the following two constraints hold:

   1) The difference in barrier heights between the ions are fixed at Δh, i.e.

   $$h_1' = h_1 + \Delta h \quad \text{and} \quad h_2' = h_2 + \Delta h$$

   Note that no assumption is made about h^* and $h^{*\prime}$.

   2) The ionic concentrations are low, so that the channel is well away from saturation.

   Show that with these two constraints, the membrane potential is given by Eqn. (1). Start from the equations for flux (or current) through this sort of channel that were derived in class, as simplified for the case of low concentrations, and apply the same steady-state assumptions usually used to derive equations like Eqn. (1) (e.g.. no net charge transfer through the membrane and $z_A = z_B = +1$).

b) Give an expression for P_A/P_B in terms of the parameters of the channel model for the situation considered in a). The ratio should not be voltage-dependent.

c) Show that if constraint 1) is eliminated, i.e. the differences in barrier heights between the ions are different for the two barriers, then the membrane potential ΔV is described by an equation like Eqn. (1) , except that now the permeabilities are voltage-dependent (Retain constraint 2 for this part.)

   What happens to the voltage dependence if one barrier is dominant, e.g. $h_1 > h_2$ and $h_1' > h_2'$? (i.e. how serious a limitation is constraint 1?)

Now consider a different way of measuring permeability, based on current ratios. In the simplest case, each ion is individually tested by placing the channel between solutions containing only ion A or only ion B on side 1 with no permeant ion on side 2 (e.g. $B_1 = A_2 = B_2 = 0$ or $A_1 = A_2 = B_2 = 0$). The currents I_A and I_B are measured in the two separate tests solutions (with the same membrane potential ΔV) and the permeability ratio is defined as I_A/I_B.

d) Show that the permeability ratio I_A/I_B is the same as that defined in a) above (P_A/P_B), if constraints 1) and 2) hold.

e) Finally, consider the case where constraint 2) is eliminated and the concentrations of A and B are in the saturation range. Give expressions for saturation currents I_{Amax} and I_{Bmax} and show that the permeability ratio I_{Amax}/I_{Bmax} differs from the ratios defined at lower concentrations. Note that it is not even necessary for the permeabilities P_i and I_i to be monotonically related for a series of ions! (constraint 1 should turn out to be irrelevant to this part).

Problem 3 (Independence)

In the first problem, an equation was derived for flux through a channel system in which independence holds, meaning that the concentration of ion in the system is low enough that we can ignore the fact that there is a finite amount of channel present. It should be evident that Eqn. (∗) above applies generally to any barrier system in which independence holds. That is, the specific simple barrier structure assumed for Problem 1 does not affect the derivation of parts a) and b) of either of the previous two problems. Begin with Eqn. (∗) for this problem.

a) The Ussing flux ratio is a condition for independence of ion fluxes. It is usually written as follows:

$$\frac{J_{A\to B}}{J_{B\to A}} = \frac{C_A}{C_B} e^{zF(V_A - V_B)/RT} \tag{**}$$

$J_{A\to B}$ and $J_{B\to A}$ are unidirectional fluxes from side A to B or vice versa and C_A, C_B, V_A, and V_B are the concentrations and electrical potentials on the two sides of the membrane. If Eqn. (**) holds for a flux, then it is consistent with independence (see the discussion on pp. 358-360 of Hille). Derive the Ussing flux ratio equation from Eqn. (*). In doing this show that the result is not affected by the specifics of the barrier system assumed. (Hint: what are the unidirectional fluxes predicted by Eqn. (∗)?).

b) Derive the Hodgkin/Huxley test for independence from Eqn. (∗). This test is based on the currents that are measured with two different concentration gradients of an ion across a membrane. If current I_S is measured with concentrations S_i and S_o on the two sides of a membrane and current I'_S is measured with concentrations S'_i and S'_o, then the ion is transported independently if

$$\frac{I'_S}{I_S} = \frac{S'_i - S'_o e^{-z_S F\Delta V/RT}}{S_i - S_o e^{-z_S F\Delta V/RT}}$$

You will have to make additional assumptions. (See Hille, p. 472-476).