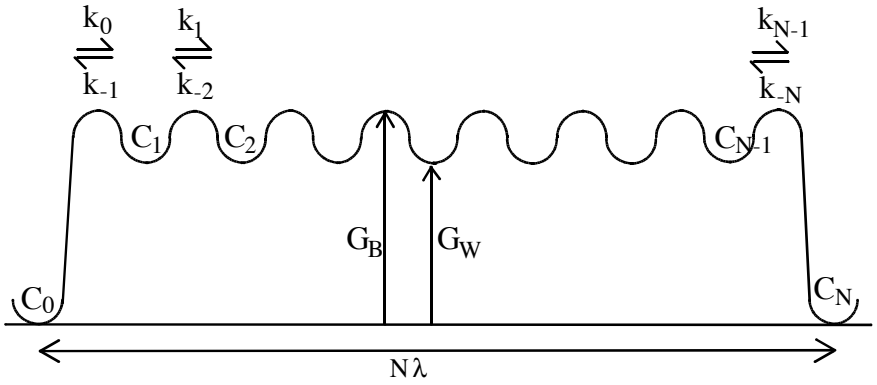
580.439/639 Homework #2

Due 9/22/14

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

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

c) Write expressions for k_i and k_{-i} from the parameters of the barrier model. Include the membrane potential in these expressions and assume constant field. 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 $\varepsilon << 1$ may be useful.)

Problem 2 (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:

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

$J_{A\to B}$ 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. (∗)?). How would the flux ration in a non-independent system deviate from Eqn. (**)?

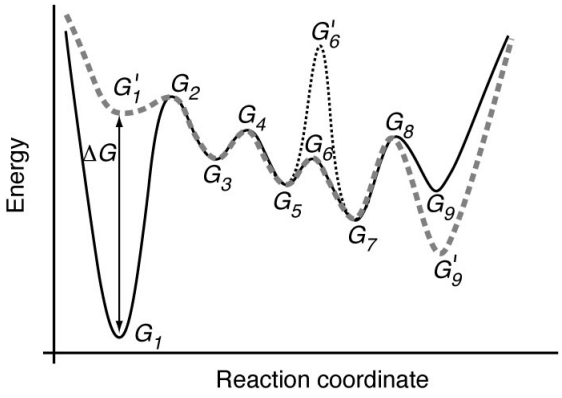
b) Derive the Hodgkin/Huxley test for independence from Eqn. (∗). This test is based on the currents that are measured with two different concentration gradients of an ion across a membrane. If current I_S is measured with concentrations S_i and S_o on the two sides of a membrane and current I'_S is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

Problem 3

Consider the diagram at right which shows the energy landscape of a receptor molecule, like rhodopsin, in the absence of activating energy (solid black line) and after activation by absorbing a photon (dashed gray line). This is a simplified version of the steps in visual transduction (based on Deupi and Kobilka *Physiology* 25:293, 2010). The diagram shows the relative energy of various conformations of the receptor molecule. This is different from the assumption made in analyzing ion channels in which the reaction coordinate was the position of an ion within the channel molecule, whose conformation was assumed fixed. In this case, the reaction coordinate corresponds to various conformations of the molecule itself and there is no mobile ion involved. The molecule can move among the various states indicated on the energy diagram according to kinetics like those used to model ion movements. For simplicity, we ignore movements of charges within the molecule as it changes conformations.



When the molecule absorbs a photon, its energy diagram changes (assumed instantaneously) from the solid line to the dashed line, with a change in the energy level of the G_1 state to G_1' and the G_9 state to G_9'. The other energy levels do not change. (Ignore the dotted lines and the G_6' state for parts a) through c) below.)

Part a) Assuming that the system is in steady state, compute the fraction of receptor molecules in each of the various conformations (G_1, G_3, G_5, G_7, and G_9) of the unactivated molecule. Repeat this calculation for the activated molecule (for states G_1', G_3, G_5, G_7, and G_9'), again assuming a steady state. (The answer is long-winded, so for the repeat, just state how the equations change for the activated state without rewriting the whole thing.) It may be useful to define rate constants for transitions along the reaction coordinate, which will be needed later.

Part b) Based on the calculations of part a), explain qualitatively what happens to the conformation of the molecule when it absorbs a photon. The energy difference ΔG is large compared to RT.

Part c) Write differential equations to model the flux of molecules through the energy barrier system immediately after absorbing a photon. Assume a fixed total number of molecules and assume that at time 0 (when the photon is absorbed), all the molecules are in the G_1' state. This will require defining appropriate rate constants (just define them, don't write out their values in terms of the energy levels). The differential equations will describe the time derivatives of the state variables x_1, x_3, x_5, x_7, and x_9, which are the fraction of molecules in each of the corresponding states. Writing this in the naïve and straightforward way gives a system like $d\vec{x}/dt = \mathbf{M}\vec{x}$, with a singular 5x5 matrix **M**. Rewrite the system in a similar form with a 4x4 matrix that is not (obviously) singular.