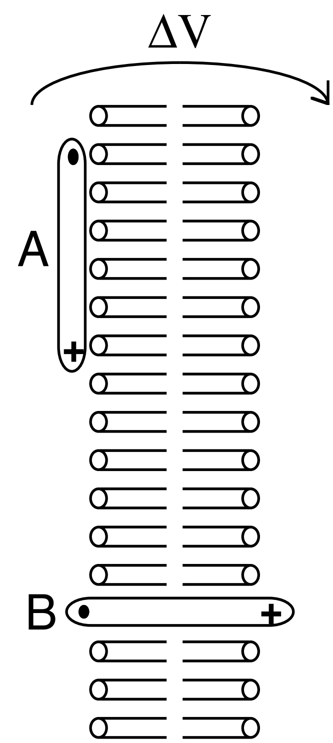
580.439/639 Homework #1

Due Sept. 14, 2009

Problem 1



One way in which membrane biophysics has been studied is to use artificial lipid bilayers; these are membranes composed of bilayers of pure lipid into which various channel-forming molecules can be inserted in order to study their properties. One such channel-forming molecule is alamethecin. As diagrammed at right, alamethecin is a rod-shaped molecule with a net positive charge on one end. It apparently exists in two states A and B. In state A it is absorbed to the surface of the lipid bilayer and in state B it is inserted through the lipid. Five or six molecules in state B can associate to form a channel which conducts ionic current through the membrane. Because the electrical potential at the position of the positive charge changes with the state of the molecule, the membrane potential ΔV affects the fraction of alamethecin that is in the B state.

a) Compute the equilibrium distribution of alamethecin between states A and B. Assume that the chemical potential of alamethecin is given by

$$\mu_{al} = \mu_{al}^{0} + RT\ln C_{al} + z_{al}FV$$

where the concentration C_{al} is the density of molecules in either state A or B per unit area of membrane. For this calculation, assume that the constant term μ_{al}^{0} varies with the state of the molecule, i.e. that $\mu_{al}^{0A} \neq \mu_{al}^{0B}$, due to interactions of the molecule with the lipid. Show that, at equilibrium,

$$\frac{C_{al}^{B}}{C_{al}^{A}} = K_{AB} = \exp\left[\frac{\mu_{al}^{0A} - \mu_{al}^{0B} - F\Delta V}{RT}\right]$$

b) Assume, for simplicity, that two molecules in state B can aggregate to form a channel with simple kinetics as

$$\mathrm{B} + \mathrm{B} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \text{channel}$$

compute the equilibrium distribution of the three species A, B, and channel in terms of the equilibrium constants K_{AB} and K_2, where K_{AB} is defined above and K_2 is k_2/k_{-2}. The total concentration of alamethecin in the membrane is Q. The results for the three concentrations should be

$$A = \frac{1+K_{AB}}{4K_2K_{AB}^2}\left[\sqrt{1+\frac{8K_2K_{AB}^2Q}{\left(1+K_{AB}\right)^2}}-1\right]$$

$$B = K_{AB}A$$

$$\text{channel } = K_2(K_{AB}A)^2$$

c) Consider two limiting cases, when $K_{AB} >> 1$ and when $K_{AB} << 1$; these cases correspond to a situation in which $\mu_{al}^{0A} - \mu_{al}^{0B}$ is positive (the interactions between alamethecin and the lipid stabilize form B in the membrane) or to a situation in which $\mu_{al}^{0A} - \mu_{al}^{0B}$ is negative (the interactions repel form B from the membrane). In these cases, the complicated expression for the concentration of A above can be simplified by approximations, if it is also assumed that $K_2Q << 1$. Carry out these simplifications and show that in one case, the membrane conductance through the alamethecin channel should be voltage dependent whereas in the other case, it is constant, i.e. linear. Explain why voltage dependence occurs in one case but not the other. (A useful approximation here is that $\sqrt{1+\varepsilon} \approx 1+\varepsilon/2$ for $\varepsilon<<1$.)

Problem 2

The Ca^{++} concentration inside neurons is maintained at a low level by active transport mechanisms. Consider one of these, the Na^+-Ca^{++} exchanger. This molecule moves 3 Na^+ ions into the cell and 1 Ca^{++} ion out of the cell in a coupled fashion. That is, the exchanger moves the Na^+ and Ca^{++} ions simultaneously. ATP and other cellular energy-sources are not directly involved; rather, energy for moving the Ca^{++} comes from the potential energy gradient of Na^+. Suppose the concentrations of Na^+ and Ca^{++} inside and outside the cell are as given in the table at right and the membrane potential of the cell is -60 mV. What is the lowest possible Ca^{++} concentration inside the cell that could be achieved by this mechanism? (Hint: at what calcium concentration would the exchanger be at equilibrium?)

	inside	outside
Na^+	10 mM	150 mM
Ca^{++}	??	2 mM

Problem 3

The ion concentrations in two extracellular fluid spaces of the mammalian cochlea, the endolymphatic and perilymphatic spaces, are shown below. There is an endolymphatic potential of +90 mV between these two spaces, endolymph positive w.r.t. perilymph (which is at the normal 0 mV extracellular reference potential).

Ion	perilymph	endolymph	
Na^+	145 mM	2 mM	
K^+	5	157	
Ca^{++}	1	0.02	
Cl^-	120	132	
$HCO3^-$	20	31	
urea	5	5	(urea is uncharged)

a) Tell which ions are at equilibrium between these spaces and which ions must be actively transported. For the actively transported ions, tell which direction the ions must be transported (out of or into the endolymph).

b) A Na-2Cl-K transporter moves one Na^+, one K^+, and 2 Cl^- ions through a membrane in the same direction on each transport step. Usually, it uses the energy in the sodium electrochemical potential gradient to transport K^+ and Cl^- against their electrochemical gradients (into a cell, for example). Given the ion concentration gradients described in part a), is there enough energy in the Na^+ gradient to allow this transporter to move potassium and chloride into the endolymph?

c) Suppose this transporter only moved 1 Cl^- each time (i.e. each transport step moved 1 Na^+, 1K^+, and 1 Cl^-, all in the same direction). Is your answer different? Why?

Problem 4

The Nernst-Planck equation can be integrated across a membrane to yield the following equation for the current-voltage relationship of an ion:

$$I_K = z_k F u_k RT \frac{C_k^2 e^{z_k F \Delta V/RT} - C_k^1}{\int_0^d e^{z_k FV/RT} dx} \qquad (*)$$

(shown in class, see also Hille, p345). I_k is the current carried by the k^{th} ion through the membrane of thickness d. The ion's concentrations in the bounding solutions are C_k^1 and C_k^2; V is the electrical potential profile in the membrane and ΔV is the transmembrane potential. z_k, u_k, F, R, and T have their usual meanings. The only assumption that is made is that the system is in steady state; i.e. the constant-field assumption has not been made.

a) Suppose that the only ions that can permeate the membrane are two monovalent cations (e.g. K^+ and Na^+). All other ions are impermeant. Argue that, in the steady state, the following equation must hold:

$$I_K + I_{Na} = 0 \qquad (**)$$

(that is, explain what parameters of the system are held steady if the equation above holds and what parameters are not held steady)

b) By substituting the current equations (*) for I_{Na} and I_K into the steady state equation, derive the following expression for membrane potential:

$$\Delta V = \frac{RT}{F} \ln \frac{u_k K_1 + u_{Na} Na_1}{u_k K_2 + u_{Na} Na_2} \qquad (***)$$

where $K_1 = C_K^1$, etc. In doing this, DO NOT ASSUME A CONSTANT FIELD IN THE MEMBRANE AND SHOW THAT THE INTEGRAL IN THE DENOMINATOR OF THE CURRENT EQUATION (*) DOES NOT NEED TO BE EVALUATED FOR THIS SPECIAL CASE.

c) Argue from Eqn. (∗∗∗) that the relative permeability P_A / P_B of two monovalent cations A^+ and B^+ can be determined from the steady-state transmembrane potential that is set up across a membrane to which only these two ions are permeable.

d) Suppose that there is an active transport enzyme (say Na^+-K^+ ATPase) in the membrane, as there is in a real cell. Assume that the enzyme transports r Na^+ ions in one direction through the membrane each time one K^+ ion is transported in the other direction (for Na^+-K^+ ATPase, r=1.5). Assume that the only ions with significant permeability through the membrane are Na^+ and K^+ as before. With these assumptions, both the active and passive transport of Na^+ and K^+ are part of the problem. Write a new steady-state condition (different from (∗∗)) which guarantees both a steady-state membrane potential and steady-state concentrations of Na^+ and K^+ on both sides of the membrane. Derive the membrane potential equation (∗∗∗) for this situation (Hint use the fact that r is a fixed ratio).