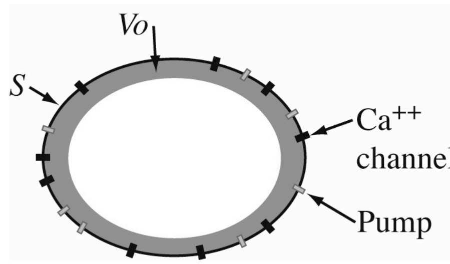
580.439/639 Midterm Exam, 2003

Closed book except for 1 sheet of paper. 1.5 hours. Do all problems

Problem 1

Consider the calcium concentration inside a cell. In a simplified system, there are two transport systems involved: voltage-gated ion channels that admit Ca^{++} to the cell and a pump that transports Ca^{++} out of the cell; of course, both transporters are bidirectional, but under normal conditions these are their predominant directions. The cell's surface area is *S* and the volume of cytoplasm from which Ca^{++} exchanges with the outside is *Vo* (shaded). The steady-state (resting) calcium concentrations are 1.5 mM outside the cell and 0.0015 mM inside the cell. For this problem, model the Ca^{++} movement through the ion channels using the GHK constant field equation:



$$I_{Ca} = P_{Ca}\, m V \frac{Ca_{in} e^{2FV/RT} - Ca_{out}}{e^{2FV/RT} - 1}$$

where I_{Ca} is the calcium current density through the membrane (units Amp/m^2), P_{Ca} is a constant (units mho·m/mole), *m* is a dimensionless HH gating variable and the remaining variables have their usual meanings; recall that z=2 for Ca^{++}. Model the pumping as a first-order interaction, as follows:

$$\mathrm{Ca_{in}} \underset{k_{in}}{\overset{k_{out}}{\rightleftharpoons}} \mathrm{Ca_{out}}$$

where the units of k_{in} and k_{out} are m/s. Of course, this transporter depends on other things, like the ATP concentration, but ignore those for now.

Part a) (11 points) Write a differential equation for the concentration Ca_{in} of calcium in the shaded volume, ignoring transport out of the volume into the rest of the cell (into the white region). That is, consider only the transport across the surface of the cell. Include both calcium current and pumping and express the equation in terms of I_{Ca} and whatever else you need. Assume that Ca_{out} is constant, fixed by regulatory mechanisms elsewhere. The left hand side should be something like "$Vo \cdot dCa_{in}/dt$ = ". This is mainly a problem in getting the units correct.

Part b) (6 points) Consider I_{Ca} at membrane potentials near the resting potential (-60 mV). Show that $I_{Ca} \approx$(const)$\cdot V$ at this potential. Give the value of the constant. This is mainly a problem in approximations.

Part c) (6 points) Rewrite the differential equation for Ca_{in} from Part a) using the approximation of Part b). At the resting potential, calcium must be in steady state. Write an equation that expresses this condition; the equation should specify Ca_{in} in terms of the other variables.

Part d) (8 points) If a small puff of Ca^{++} is injected into volume Vo, so that $Ca_{in}(0)=C_0$ is different from the equilibrium value discovered in Part c, solve for $Ca_{in}(t)$ for $t>0$. Assume for this part that V does not change from its resting value (reasonable if the calcium conductance is small compared to the other conductances in the system).

Problem 2

Porestickalate is an organic ion similar to TEA that blocks a certain potassium channel (Kxx), which has the same pore structure as the KcsA channel. Porestickalate works by sticking in the internal water-containing cavity of the channel. It is clearly way too big to fit through the selectivity filter.

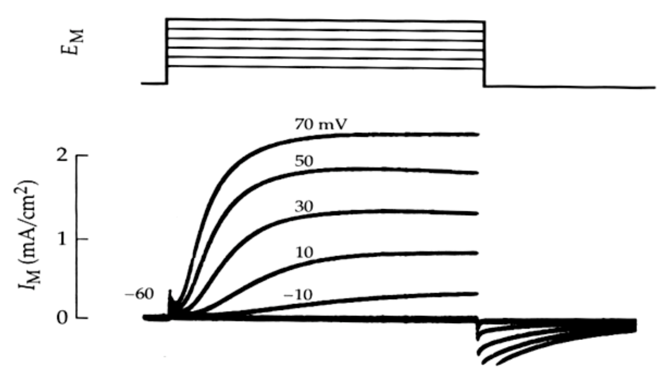
Part a) (6 points) The block only occurs if the channel's gate is open. Provide a hypothesis to explain this fact.

Part b) (6 points) Is porestickalate equally likely to block the channel if introduced in the internal and external solutions (i.e. inside the cell versus outside the cell)? Explain why.

Part c) (6 points) Is porestickalate likely to be a cation or an anion? Explain why you think so, based on the known structure of the KcsA channel.

Part d) (6 points) Assume that binding of porestickalate to the open channel is a single-step process like P + Kxx → P-Kxx. Draw a single-barrier model for this process and specify the minimum number of variables needed to account for 1) the kinetics of the binding, i.e. the rate constants; 2) the free energy difference between porestickalate in the channel and in solution; and 3) the effects of membrane potential on the porestickalate binding (independent of its effects on channel gating).

Part e) (11 points) The figure at right shows currents through the Kxx channel in a standard voltage clamp protocol, in the absence of porestickalate. Given what you know about porestickalate, draw the currents for the same voltage clamp protocol in the presence of porestickalate at a blocking concentration. Ignore the effects of membrane potential on the porestickalate ion for this part.



Part f) (8 points) In terms of your barrier model of part d), write an expression for the conditions at which 50% of the channels would be blocked in steady state by porstickalate. For this, assume that all channels are open and that porestickalate is placed on the appropriate side of the membrane. You should obtain a relationship between membrane potential V and the concentration of porestickalate in solution.

Problem 3

Part a) (6 points) Returning to the situation of problem 1, consider a more accurate model in which membrane potential also varies. Assume, to keep it simple, that the only voltage-gated channel in the membrane is the calcium channel, and model the remaining currents in the membrane as a fixed linear conductance G_M (units mho/m^2) with a reversal potential of E_M, equal to the rest potential. How many state variables are there in this system and what are they?

Part b) (8 points) Write an equation for the membrane potential of the cell of the form "$C \cdot dV/dt$ = . . .". In writing this equation, make whatever assumptions seem appropriate to reduce the number of state variables to two, which are Ca_{in} and V. Use the approximation for calcium current derived in Problem 1b and remember that charge can be carried through the membrane through both ion channels and the pumps. Assume that no external current is injected into the cell.

Part c) (12 points) Consider the system defined in terms of the two differential equations, one for Ca_{in} from Problem 1c and the second for V from problem 3b. Show that $V=E_M$ at the equilibrium point of this two-state-variable system. There is a hard, algebraically hairy, way to do this and a simpler way based on arguments about the nature of the equilibrium point. Either answer is acceptable.

Part d) (EXTRA CREDIT 8 points; don't do this until you are finished with all the above) Sketch a phase plane for this system (Ca_{in} vs V). You do not have many of the parameters needed to actually draw the nullclines. However, you should be able to figure out their general form and, taking into account that there is an equilibrium point at the resting potential E_M, you should be able to draw approximate curves. Show the nullclines and the equilibrium point(s), and show arrows to indicate the direction of trajectories away from the nullclines.