ENTROPY IN BIOLOGY

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Lecture 5: Control of Cell Volume

Overview of the model
Osmotic flow & electroneutrality

Ionic fluxes
Homework (involves the Einstein relation)

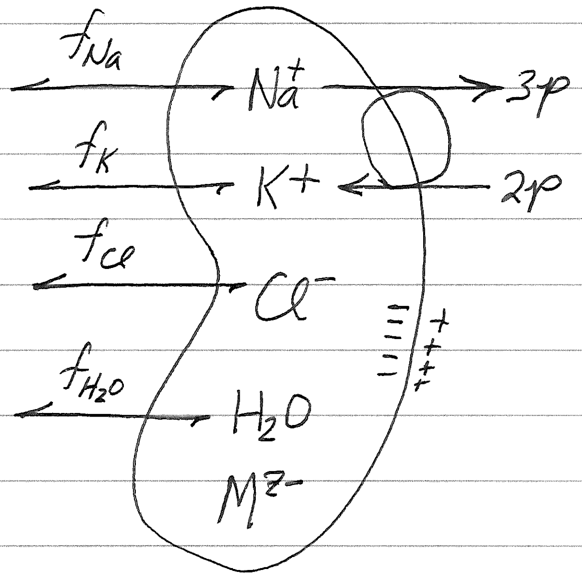
Space-charge layers
    The cell membrane as a capacitor
    Ionic content of the space-charge layers

Dynamical equations of the model

Dynamics on the slow manifold

Steady state: Homework

Control of Cell Volume



Biological cells contain macromolecules that carry a net negative charge. The macromolecules themselves are too few to produce a substantial osmotic pressure, but their counterions are much more numerous and cause a significant osmotic problem for the cell. Plant cells and bacteria cope with this problem by building a thick cell wall, but animal cells are bounded only by a lipid bilayer membrane that is not capable of withstanding any substantial pressure difference.

Animal cells cope with their osmotic problem by pumping ions. This is done by Na^+/K^+ exchange pumps located in the cell membrane. Every turn of the pump exports three Na^+ ions and imports two K^+ ions. The energy source for this, as for many cellular processes, is the hydrolysis of ATP.

Let V be the cell volume, and let A be the area of the cell membrane.
We regard A as constant, since the deformations of lipid bilayer membranes are known to be area-preserving to a good approximation. This restricts V to the interval $(0, V_{max})$, where V_{max} is the volume of a sphere of area A:

(1) $$V_{max} = \frac{4}{3}\pi\left(\frac{A}{4\pi}\right)^{3/2}$$

When V exceeds V_{max}, the cell membrane breaks, so the goal of cell volume control is to keep V below V_{max}.

Since the cell can freely adjust its shape to accomodate changes in volume as long as the volume is below Vmax, we may assume that there is no hydrostatic pressure difference across the cell membrane. In this situation, the flow of water (expressed as volume per unit time, per unit area of cell membrane, positive outward) is given by

$$f_{H_2O} = \gamma_{H_2O}\, kT \Big([Na^+]^{ext} + [K^+]^{ext} + [Cl^-]^{ext} - [Na^+]^{int} - [K^+]^{int} - [Cl^-]^{int} \Big) \tag{2}$$

In this equation, γ_{H_2O} is the hydraulic permeability of the membrane, k is Boltzmann's constant, T is the absolute temperature, $[\ ]$ denotes concentration (number of ions per unit volume), "ext" and "int" denote the external and internal solutions, and we are using the dilute-solution form of the osmotic pressure law, in which the osmotic pressure of a solute species is kTc, where c is its concentration.

Note that in the above formula for f_{H_2O}, we neglect the direct osmotic effect of the macromolecules that are trapped inside the cell. This is because that direct osmotic effect is overwhelmed by the much larger effect of the counterions

In equation (2) the external concentrations are regarded as given, and we are assuming for now that the only external solutes are Na^+, K^+, and Cl^-. Since the external solution has to be electrically neutral, the given concentrations must satisfy the constraint that

(3) $$[Na^+]^{ext} + [K^+]^{ext} = [Cl^-]^{ext}$$

The equation of electroneutrality inside the cell must take into account the charges on the macromolecules. Let the total amount of this charge be denoted $-Q_M$, with Q_M positive, and assume that this charge is uniformly distributed over the volume of the cell. Then interior electroneutrality gives the equation

(4) $$q\,[Na^+]^{int} + q\,[K^+]^{int} = q\,[Cl^-]^{int} + \frac{Q_M}{V}$$

where q is the elementary charge, i.e., the charge on a proton. Dividing by q, we get

(5) $$[Na^+]^{int} + [K^+]^{int} = [Cl^-]^{int} + \frac{Q_M}{qV}$$

As we shall see later, equations (3) & (5) are both violated in thin boundary layers known as space-charge layers adjacent to the cell membrane. Thus, the concentrations that appear in these equations are the concentrations in the bulk solutions, outside of the space-charge layers.

Given the above remark about the space-charge layers, it may seem strange that we use the bulk concentrations rather than the concentrations immediately adjacent to the membrane in the formula for f_{H_2O}, equation (2). Indeed, a similar issue will arise later when we derive formulae for the ionic fluxes. There, too, we shall assume that the fluxes are determined by the bulk concentrations, and also by the electrostatic potential of the internal bulk solution with respect to the external bulk solution. Thus, the transmembrane fluxes of our model should be thought of as running from bulk solution to bulk solution across an entity consisting of the membrane together with its space-charge layers.

Ionic fluxes

Here we derive expressions for the ionic fluxes f_{Na}, f_K, f_{Cl}.

Consider an ionic species in which each ion carries a charge zq. Let the internal and external concentrations of this ionic species be denoted c^{int} and c^{ext}. Let ϕ be the electrical potential of the internal solution with respect to that of the external solution. We need to determine the function

$$(6) \qquad f(c^{int}, c^{ext}, \phi)$$

which gives the net number of ions per unit time per unit area of membrane that move from the internal solution to the external solution.

We make three assumptions that together determine the form of the flux function f:

$$f(c_1, c_2, \phi) = c_1 f_1(\phi) - c_2 f_2(\phi) \tag{7}$$

$$f\left(c e^{-\frac{qz\phi}{kT}}, c, \phi\right) = 0 \tag{8}$$

$$f(c, c, \phi) = \gamma c \phi q z \tag{9}$$

Equation (7) separates the net flux into an outward and an inward flux, and each of these is assumed to be governed by mass action. The basic assumption here is that individual ions move across the membrane independently of each other.

Equation (8) is required by thermodynamics, provided that the only driving force acting on the ionic species in question is the electrical potential difference ϕ.

Equation (9) is essentially Ohm's law for the special case in which the concentrations are equal on the two sides of the membrane. The fact that c appears linearly on the right-hand side of (9) is then required by (7). The constant γ will be further interpreted below.

We are not claiming here that ion transport across cell membranes always satisfies the hypotheses (7-9). There are many different transport mechanisms, each with its own flux law. These assumptions do, however, imply a formula for ionic flux that is often observed in practice, and we shall use that formula here.

Substituting (7) into (8) & (9), and dividing by c in each case, we get two equations in two unknowns for $f_1(\phi)$ and $f_2(\phi)$:

$$f_1(\phi)e^{-\frac{qz\phi}{kT}} - f_2(\phi) = 0 \tag{10}$$

$$f_1(\phi) - f_2(\phi) = \gamma \phi q z \tag{11}$$

These equations are solved by

(12) $$f_1(\phi) = \frac{qz\gamma\phi}{1 - e^{-\frac{qz\phi}{kT}}}$$

(13) $$f_2(\phi) = \frac{qz\gamma\phi\, e^{-\frac{qz\phi}{kT}}}{1 - e^{-\frac{qz\phi}{kT}}}$$

$$= \frac{qz\gamma\phi}{e^{\frac{qz\phi}{kT}} - 1}$$

Note that $f_2(-\phi) = f_1(\phi)$. Substituting (12) & (13) into (7) with $c_1 = c^{int}$ and $c_2 = c^{ext}$, we get

(14) $$f(c^{int}, c^{ext}, \phi) = \frac{qz\gamma\phi\left(c^{int} - c^{ext} e^{-\frac{qz\phi}{kT}}\right)}{1 - e^{-\frac{qz\phi}{kT}}}$$

When $\phi = 0$, L'Hospital's rule gives

(15) $$f(c^{int}, c^{ext}, 0) = \gamma kT \left(c^{int} - c^{ext}\right)$$

This suggests an interpretation for the coefficient γ. Suppose ion transport takes place through cylindrical channels of length l_m, where l_m is the thickness of the cell membrane. Let α be the fraction of the membrane area that is taken up by the pores of these channels. When $\phi = 0$ we then expect a flux given by

(16) $$f(c^{int}, c^{ext}, 0) = \alpha D \frac{c^{int} - c^{ext}}{l_m}$$

where D is the diffusion coefficient of the ion in the channel. Comparison of (15) & (16) shows that

(17) $$\gamma = \frac{\alpha}{l_m} \frac{D}{kT}$$

Indeed, one can derive the entire flux law (14), including the interpretation of γ, by assuming that ion transport occurs by drift and diffusion through cylindrical channels in a constant electric field. The steady-state equations for the concentration $c(x)$ of the ionic species in its channel are then as follows:

(18) $$\frac{dF}{dx}=0 \quad , \qquad F=-D\frac{dc}{dx}+\mu\frac{qz\phi}{l_m}c(x)$$

on the interval $0<x<l_m$, with boundary conditions

(19) $$c(0)=c^{int} \quad , \qquad c(l_m)=c^{ext}$$

In these equations F is the flux (number of ions per unit time traversing the channel divided by the cross-sectional area of the channel). The two terms in the formula for F represent diffusion and drift. In the drift term, ϕ/l_m is the constant electric field, so $qz\phi/l_m$ is the force on each ion. The constant μ is called the mobility, and $\mu qz\phi/l_m$ is the drift velocity. Multiplying the drift velocity by the local concentration $c(x)$, we get the contribution of drift to the flux, F.

Homework

- Solve equations (18-19) for $c(x)$, and evaluate F.
- Impose the condition that $F = 0$ when

(20) $$c^{int} = c^{ext} e^{-\frac{qz\phi}{RT}}$$

and use this to express the mobility μ in terms of the diffusion coefficient D. This is called the Einstein relation. It will be discussed further, below.

- Compare your formula for F to the equation for f that was derived above. The result should be

(21) $$f = \alpha F$$

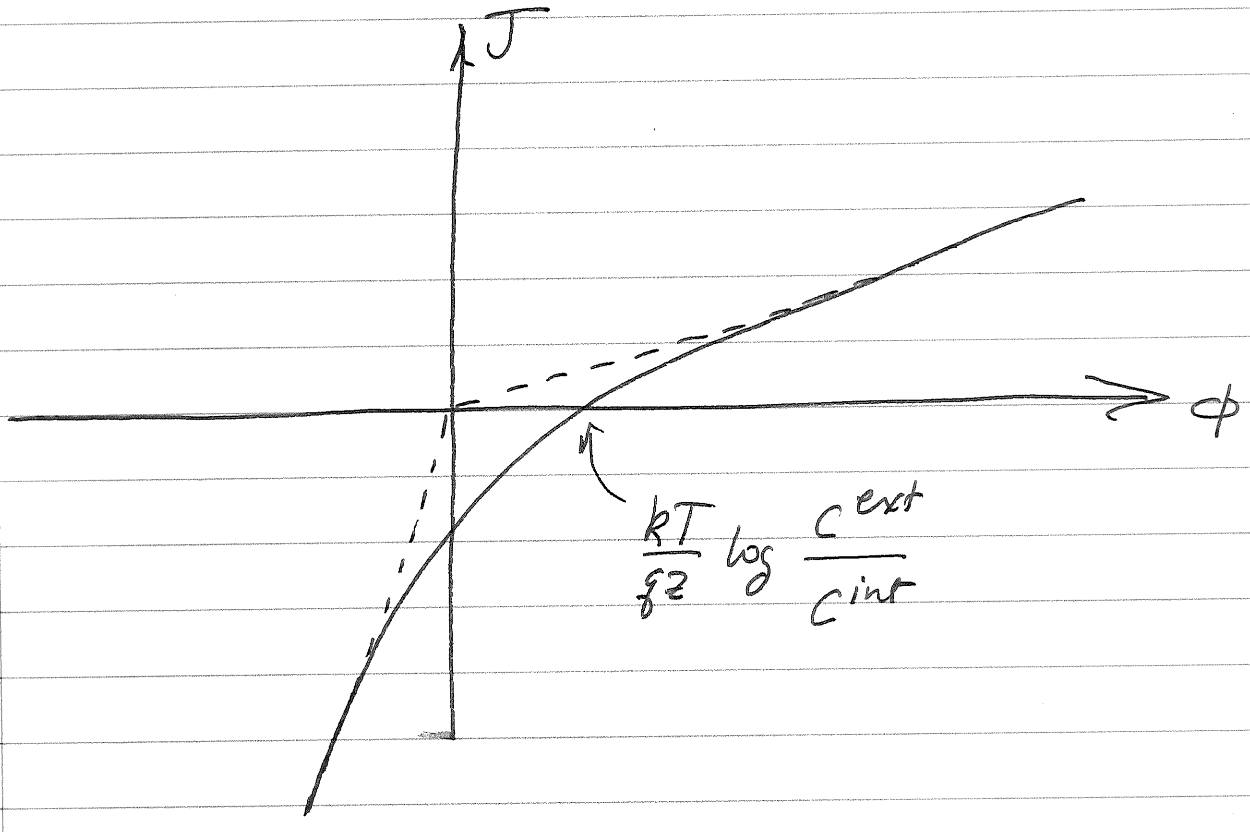
since F is per unit area of channel, but f is per unit area of membrane.

The Einstein relation, derived in the above homework, reveals a deep connection between drift and diffusion. Although it was derived by considering thermodynamic equilibrium and detailed balance, it is applicable to non-equilibrium conditions as well. To establish this, we make the assumption that D and μ are only functions of the temperature, T. At any given temperature, then, D and μ are constant, and the Einstein relation is a relationship between two constants. Such a relationship obviously remains valid if conditions change (at constant temperature) in such a way that the system is no longer at equilibrium.

The flux law (14) with γ given by (17) is known as the Goldman-Hodgkin-Katz (GHK) formula, and also as the constant-field equation. The traditional way to derive it is via equations (18-19) as in the above homework. The derivation based on the hypotheses (7-9) requires only a few general principles and may therefore explain why the flux law (14) is so widely observed.

To convert f to current density J we must multiply by qz. From (14) & (17) we then get

(22) $$J = \frac{\alpha}{l_m} \frac{D}{kT} (qz)^2 \phi \frac{c^{int} - c^{ext} e^{-\frac{qz\phi}{kT}}}{1 - e^{-\frac{qz\phi}{kT}}}$$



Note that $J=0$ when

$$\phi = \frac{kT}{qz}\log\frac{c^{ext}}{c^{int}} \tag{23}$$

This value of ϕ is called the equilibrium potential for the ion species that is under consideration.

Also, as $\phi \to \pm\infty$

$$J \sim \frac{\alpha}{l_m}\frac{D}{kT}(qz)^2\phi \left\{ \begin{array}{ll} c^{int} & \text{as } \phi \to +\infty \\ c^{ext} & \text{as } \phi \to -\infty \end{array} \right\}. \tag{24}$$

This asymptotic result has a nice interpretation in terms of the behavior of $c(x)$ within the channel (see above homework) as $\phi \to \pm\infty$.

Space-charge layers

Although the bulk solutions in the interior and exterior of the cell are electrically neutral, there is a potential difference across the cell membrane, and this requires space-charge layers adjacent to the cell membrane. These space-charge layers are very **thin**: the charge density decays exponentially to zero with distance away from the face of the membrane, and the space constant of this exponential decay, called the Debye length, is less than the thickness of the membrane itself.

The thinness of the space-charge layers has two important consequences. One of these is that we can think of the space-charge layers as if they were in thermodynamic equilibrium with the adjacent bulk solutions at all times. The other consequence is that we can ignore membrane curvature and use a one-dimensional theory to analyze the structure and ionic content of the space-charge layers.

Thus, we use the one-dimensional Poisson-Boltzmann equation to model the space-charge layers. Let x be the spatial coordinate running orthogonal to the faces of the membrane, with the interior face of the membrane at $x=0$ and the exterior face at $x=l_m$. The interior of the cell is represented in this model by the domain $(-\infty,0)$ and the exterior of the cell by the domain (l_m,∞). Of course, the bulk solutions are not really infinitely far away from the faces of the membrane but they may as well be infinitely far away from the point of view of the space-charge layers. This is yet another consequence of the thinness of these layers in comparison to other cell dimensions.

On the interior domain $(-\infty,0)$, we assume that there is a uniform background charge density

$$\rho_b(x) = \frac{-Q_M}{V}, \quad x<0 \tag{25}$$

There is no background charge in the membrane itself or in the external domain.

The electrical potential $\phi(x)$ therefore satisfies the following differential equations:

(26) $$-\varepsilon \frac{d^2\phi}{dx^2} = \frac{-Q_M}{V} + \sum_{i=1}^{3} q z_i c_i(x) , \quad x<0$$

(27) $$-\varepsilon_m \frac{d^2\phi}{dx^2} = 0 , \quad 0<x<l_m$$

(28) $$-\varepsilon \frac{d^2\phi}{dx^2} = \sum_{i=1}^{3} q z_i c_i(x) , \quad l_m < x$$

with boundary conditions

(29) $$\phi(-\infty) = \phi^{int} , \quad \phi(\infty) = \phi^{ext} = 0$$

(30) $$\phi(0^-) = \phi(0^+) , \quad \phi(l_m^-) = \phi(l_m^+)$$

(31) $$\varepsilon \frac{d\phi}{dx}(0^-) = \varepsilon_m \frac{d\phi}{dx}(0^+) , \quad \varepsilon_m \frac{d\phi}{dx}(l_m^-) = \varepsilon \frac{d\phi}{dx}(l_m^+)$$

In these equations, ε is the dielectric constant of water, which we assume is the dielectric constant of the ionic solution, both inside and outside of the cell, and ε_m is the dielectric constant of the cell membrane. Of course,

ε_m has no effect in equation (27), but the ratio $\varepsilon_m/\varepsilon$ plays an important role in the the boundary condition (31), so it is worthwhile to mention that $\varepsilon_m/\varepsilon$ is small, with the numerical value of this ratio being about $2/80 = 0.025$.

In equations (26) & (28), $i=1$ denotes Na^+, $i=2$ denotes K^+, and $i=3$ denotes Cl^-, so $z_1 = z_2 = +1$, and $z_3 = -1$.

For $c_i(x)$ in equations (26) & (28), we use the *linearized* Boltzmann formulae

(32) $$c_i(x) = c_i(\pm\infty)\left(1 - \frac{q z_i(\phi(x) - \phi(\pm\infty))}{kT}\right),$$

in which "$\pm\infty$" denotes $-\infty$ when $x < 0$ and $+\infty$ when $x > l_m$.

In equation (32), the values of the concentrations at $\pm\infty$ are those of the bulk solutions internal ($c_i(-\infty)$) and external ($c_i(+\infty)$) to the cell:

(33) $c_1(-\infty) = [Na^+]^{int}, \quad c_1(+\infty) = [Na^+]^{ext}$

(34) $c_2(-\infty) = [K^+]^{int}, \quad c_2(+\infty) = [K^+]^{ext}$

(35) $c_3(-\infty) = [Cl^-]^{int}, \quad c_3(+\infty) = [Cl^-]^{ext}$

Recall that the bulk concentrations satisfy the electroneutrality conditions, equations (3) & (5). In the internal case, the electroneutrality condition includes not only the mobile ions but also the immobile background charge.

Thus, when we substitute (32) into (26) & (28) and take into account the electroneutrality conditions at $x = \pm\infty$, we are left with the following:

(36) $$\frac{d^2\phi}{dx^2} = \frac{q^2 c^{int}}{kT\varepsilon}\left(\phi - \phi^{int}\right), \quad x < 0$$

(37) $$\frac{d^2\phi}{dx^2} = \frac{q^2 c^{ext}}{kT\varepsilon}\phi, \quad l_m < x$$

where

WARNING: This notation was **previously** used to refer to any one species. Now it refers to the sum.

$$c^{int} = [Na^+]^{int} + [K^+]^{int} + [Cl^-]^{int} \tag{38}$$

$$c^{ext} = [Na^+]^{ext} + [K^+]^{ext} + [Cl^-]^{ext} \tag{39}$$

Note in particular that positive and negative ions both contribute positively to c^{int} and c^{ext}, and also that only the mobile ions contribute. (We are only considering monovalent ions here, but more generally each species contributes in proportion to z^2.)

The only solutions of (36) & (37) that remain bounded as $x \to -\infty$ in the case of (36) and as $x \to +\infty$ in the case of (37) are

$$\phi(x) = \phi^{int} + \left(\phi(0) - \phi^{int}\right) \exp\left(\sqrt{\frac{q^2 c^{int}}{kT\varepsilon}}\, x\right), \qquad x < 0, \tag{40}$$

and

$$\phi(x) = \phi(l_m) \exp\left(-\sqrt{\frac{q^2 c^{ext}}{kT\varepsilon}}\,(x - l_m)\right), \qquad l_m < x. \tag{41}$$

The values of $\phi(0)$ and $\phi(l_m)$ remain to be determined. To do so, we first evaluate

(42) $$\phi'(0^-) = \left(\phi(0) - \phi^{int}\right)\sqrt{\frac{q^2 c^{int}}{kT\varepsilon}}$$

(43) $$\phi'(l_m^+) = -\phi(l_m)\sqrt{\frac{q^2 c^{ext}}{kT\varepsilon}}$$

Because of equation (27), the potential profile in the interior of the membrane is linear, and therefore

(44) $$\phi'(0^+) = \phi'(l_m^-) = \frac{\phi(l_m) - \phi(0)}{l_m}$$

Substituting (42-44) into the boundary conditions (31), we get two equations in the two unknowns $\phi(0)$ and $\phi(l_m)$, namely

(45) $$\varepsilon\left(\phi(0) - \phi^{int}\right)\sqrt{\frac{q^2 c^{int}}{kT\varepsilon}}$$

$$= \varepsilon_m \frac{\phi(l_m) - \phi(0)}{l_m} = -\varepsilon\,\phi(l_m)\sqrt{\frac{q^2 c^{ext}}{kT\varepsilon}}$$

Note that we have implicitly used the boundary conditions (30), since we have not made any distinction between $\phi(0^+)$ and $\phi(0^-)$ or between $\phi(l_m^+)$ and $\phi(l_m^-)$.

For a parallel plate capacitor with dielectric material between the plates, the capacitance per unit area is given by the dielectric constant divided by the thickness of the dielectric material (i.e., by the distance between the plates). Thus the capacitance per unit area of the cell membrane is given by

(46) $$C_m = \frac{\varepsilon_m}{l_m}$$

and by analogy with this it is natural to define

(47) $$C_{int} = \varepsilon\sqrt{\frac{\delta^2 c^{int}}{kT\varepsilon}} = \frac{\varepsilon}{l_D^{int}}$$

(48) $$C_{ext} = \varepsilon\sqrt{\frac{\delta^2 c^{ext}}{kT\varepsilon}} = \frac{\varepsilon}{l_D^{ext}}$$

in which l_D^{int} and l_D^{ext} are the Debye lengths interior and exterior to the membrane, respectively. The pair of equations (45) then becomes

(49)
$$C_{int}\left(\phi(0)-\phi^{int}\right)=C_m\left(\phi(l_m)-\phi(0)\right)$$
$$=-C_{ext}\,\phi(l_m)$$

Solving for $\phi(0)$ and $\phi(l_m)$, and expressing the results in terms of the reciprocals of the capacitances per unit area, we get

(50)
$$\phi(0)=\frac{C_m^{-1}+C_{ext}^{-1}}{C_{int}^{-1}+C_m^{-1}+C_{ext}^{-1}}\,\phi^{int}$$

(51)
$$\phi(l_m)=\frac{C_{ext}^{-1}}{C_{int}^{-1}+C_m^{-1}+C_{ext}^{-1}}\,\phi^{int}$$

This completes the determination of $\phi(x)$.

The charge per unit area in the space-charge layer on the inner surface of the cell membrane is given by

(52) $$\sigma^{int} = \int_{-\infty}^{0} -\varepsilon \frac{d^2\phi}{dx^2}\, dx = -\varepsilon \frac{d\phi}{dx}(0^-)$$

$$= \varepsilon\left(\phi^{int} - \phi(0)\right)\sqrt{\frac{z^2 c^{int}}{kT\varepsilon}}$$

$$= C_{int}\, \frac{C_{int}^{-1}}{C_{int}^{-1} + C_m^{-1} + C_{ext}^{-1}}\, \phi^{int}$$

$$= \frac{\phi^{int}}{C_{int}^{-1} + C_m^{-1} + C_{ext}^{-1}}$$

Similarly, the charge per unit area in the space-charge layer on the outer surface of the cell membrane is given by

(53) $$\sigma^{ext} = \int_{l_m}^{\infty} -\varepsilon \frac{d^2\phi}{dx^2}\, dx = \varepsilon \frac{d\phi}{dx}(l_m)$$

$$= -\varepsilon\, \phi(l_m) \sqrt{\frac{q^2 c^{ext}}{kT\varepsilon}}$$

$$= -C_{ext} \frac{C_{ext}^{-1}}{C_{int}^{-1} + C_m^{-1} + C_{ext}^{-1}}\, \phi^{int}$$

$$= -\frac{\phi^{int}}{C_{int}^{-1} + C_m^{-1} + C_{ext}^{-1}} = -\sigma^{int}$$

Thus, the membrane is a capacitor, with capacitance per unit area given by

(54) $$\overline{C}_m = \frac{1}{C_{int}^{-1} + C_m^{-1} + C_{ext}^{-1}}$$

Note that the right-hand side of (54) is the recipe for combining the capacitances of capacitors connected in series. If one of the capacitors is much smaller than the others, it dominates the series combination. In our case, C_m is much smaller than C_{int} and C_{ext}, since $\varepsilon_m << \varepsilon$

and also since l_D^{int} and l_D^{ext} are smaller than l_m, see equations (46-48).

Thus, from now on we shall make the approximation that

$$\overline{C_m} = C_m \tag{55}$$

The capacitance per unit area of a lipid bilayer membrane is about 10^{-6} coulomb/(volt · cm^2). This is equal to 1 in a system of units sometimes used in electrophysiology, in which the unit of time is the millesecond, the unit of voltage is the millivolt, and the unit of current is the microamp (so the unit of charge is the microamp · millesecond, which is equal to 1 nanocoulomb).

Note that the approximation (55) is consistent with our use of the linearized Poisson-Boltzmann equation, since (55) implies that most of the voltage difference $\phi^{int} - \phi^{ext}$ appears across the membrane itself, with only small voltage difference occurring across the two ionic solutions, see equations (50-51).

Next, we need to determine the ionic content of the space-charge layer on the inner surface of the cell membrane. This is needed to do proper bookkeeping of the total number of ions of each species inside of the cell.

To find the excess number of ions of each species associated with the space-charge layer on the inner surface of the cell membrane, per unit area of membrane, we evaluate

(56) $$\int_{-\infty}^{0} \left(c_i(x) - c_i(-\infty) \right) dx$$

From equations (32) & (40), and then using (50), (47), and (52), this integral is equal to

(57) $$c_i(-\infty)\,\frac{qz_i}{kT}\,\frac{\phi^{int}-\phi(0)}{\sqrt{\frac{q^2c^{int}}{kT\varepsilon}}}$$

$$= c_i(-\infty)\,\frac{qz_i}{kT}\,\frac{1}{\sqrt{\frac{q^2c^{int}}{kT\varepsilon}}}\,\frac{C_{int}^{-1}\,\phi^{int}}{C_{int}^{-1}+C_m^{-1}+C_{ext}^{-1}}$$

$$= c_i(-\infty)\,\frac{qz_i}{kT}\,\frac{\sigma^{int}}{\varepsilon\left(\frac{q^2c^{int}}{kT\varepsilon}\right)}$$

$$= \frac{c_i(-\infty)z_i}{c^{int}}\quad\frac{\sigma^{int}}{q}$$

Note the factor z_i in the foregoing. In a positive space-charge layer ($\sigma^{int}>0$), there is an excess of positive ions and a deficit of negative ions, and in a negative space-charge layer ($\sigma^{int} < 0$) there is a deficit of positive ions and an excess of negative ions.

We can put the above result in a slightly nicer form by making the definition

(58) $$\sigma_i^{int} = q z_i \int_{-\infty}^{0} \left(c_i(x) - c_i(-\infty) \right) dx$$

$$= \frac{c_i(-\infty)\, z_i^2}{c^{int}} \, \sigma^{int}$$

$$= \frac{c_i(-\infty)}{\sum_{j=1}^{3} c_j(-\infty)} \, \sigma^{int}$$

since $z_i^2 = 1$ for all of the ions that we are considering. Thus, each of the mobile ions makes a contribution to the charge density of the space charge layer in proportion to its concentration in the adjacent bulk solution, and regardless of its charge. In the case of ions of the opposite sign to that of the overall space charge layer, this contribution comes about through a deficit of the concentration of that ion in comparison to its concentration in the adjacent bulk solution.

Although we are only considering monovalent ions here, we remark that the generalization of (58) is

$$\sigma_i^{int} = \frac{c_i(-\infty)\, z_i^2}{\sum_{j=1}^{n} c_j(-\infty)\, z_j^2}\, \sigma^{int} \tag{59}$$

Making use of the above results, we can relate the numbers of mobile ions of each species within the cell to their interior bulk concentrations in the following way

$$N_{Na^+} = V[Na^+]^{int} + A\left(\frac{[Na^+]}{[Na^+]+[K^+]+[Cl^-]}\right)^{int} \frac{C_m \phi^{int}}{q} \tag{60}$$

$$N_{K^+} = V[K^+]^{int} + A\left(\frac{[K^+]}{[Na^+]+[K^+]+[Cl^-]}\right)^{int} \frac{C_m \phi^{int}}{q} \tag{61}$$

$$N_{Cl^-} = V[Cl^-]^{int} + A\left(\frac{[Cl^-]}{[Na^+]+[K^+]+[Cl^-]}\right)^{int} \frac{C_m \phi^{int}}{-q} \tag{62}$$

If we multiply both sides by q in equations (60) & (61), and by $-q$ in equation (62), add the results, and then make use of bulk interior electroneutrality, equation (5), we get

(63) $$q N_{Na^+} + q N_{K^+} - q N_{Cl^-} - Q_M = A C_m \phi^{int}$$

The left hand side of this equation is the total charge within the cell. On the right-hand side, AC_m is the total capacitance of the cell membrane.

For future reference, it will be useful to solve equations (60-62) for the concentrations. To clean up the notation, let $c^{int} = \left([Na^+]+[K^+]+[Cl^-]\right)^{int}$, as before, and also let

(64) $$N = \frac{A C_m \phi^{int}}{q}$$

Then

$$[Na^+]^{int} = \frac{N_{Na^+}}{V + \frac{N}{c^{int}}} \tag{65}$$

$$[K^+]^{int} = \frac{N_{K^+}}{V + \frac{N}{c^{int}}} \tag{66}$$

$$[Cl^-]^{int} = \frac{N_{Cl^-}}{V - \frac{N}{c^{int}}} \tag{67}$$

Adding these equations, we get c^{int} on the left-hand side, and hence an equation for c^{int} that can be written as follows

$$1 = \frac{N^+}{Vc^{int} + N} + \frac{N^-}{Vc^{int} - N} \tag{68}$$

where

$$N^+ = N_{Na^+} + N_{K^+} , \quad N^- = N_{Cl^-} \tag{69}$$

From (68), we get a quadratic equation

(70) $$(Vc^{int})^2 - (N^+ + N^-)(Vc^{int}) + (N^+ - N^- - N)N = 0$$

and this is solved by

(71) $$c^{int} = \frac{N^+ + N^-}{2V}\left(1 + \sqrt{1 - 4\frac{(N^+ - N^- - N)N}{(N^+ + N^-)^2}}\right)$$

Here, we have chosen the root that gives $c^{int} = \frac{N^+ + N^-}{V}$ when $N = 0$, i.e., when there is no space-charge layer.

From equations (63) & (64), we have

(72) $$N^+ - N^- - N = \frac{Q_M}{q}$$

and therefore the above formula for c^{int} can be rewritten as

$$(23) \quad c^{int} = \frac{N_{Na^+} + N_{K^+} + N_{Cl^-}}{2V} \left(1 + \sqrt{1 - 4\frac{Q_M A C_m \phi^{int}}{\zeta^2 (N_{Na^+} + N_{K^+} + N_{Cl^-})^2}} \right)$$

With c^{int} known, we can substitute it back into (65-67) to get equations for the internal bulk concentrations of the mobile ions.

Dynamics

We can now formulate a system of ODE to describe the cell. The state variables are the numbers of mobile ions of each species in the cell, and the cell volume:

(74) $$N_{Na^+}, \ N_{K^+}, \ N_{Cl^-}, \ V$$

From these variables, we can evaluate the potential ϕ^{int} from equation (63), and then the concentrations

(75) $$[Na^+]^{int}, \ [K^+]^{int}, \ [Cl^-]^{int}$$

from equations (73) and (65-67).

Next, we can evaluate the fluxes:

(76) $$f_{Na^+} = \gamma_{Na^+}\, q\phi^{int} \frac{[Na^+]^{int} - [Na^+]^{ext} e^{-\frac{q\phi^{int}}{kT}}}{1 - e^{-\frac{q\phi^{int}}{kT}}}$$

(77) $$f_{K^+} = \gamma_{K^+}\, q\phi^{int} \frac{[K^+]^{int} - [K^+]^{ext} e^{-\frac{q\phi^{int}}{kT}}}{1 - e^{-\frac{q\phi^{int}}{kT}}}$$

(78) $$f_{Cl^-} = \gamma_{Cl^-}(-q\phi^{int}) \frac{[Cl^-]^{int} - [Cl^-]^{ext} e^{+\frac{q\phi^{int}}{kT}}}{1 - e^{+\frac{q\phi^{int}}{kT}}}$$

(79) $$f_{H_2O} = \gamma_{H_2O}\, kT\Big([Na^+]^{ext} + [K^+]^{ext} + [Cl^-]^{ext}$$
$$- [Na^+]^{int} - [K^+]^{int} - [Cl^-]^{int}\Big)$$

With these fluxes defined, we have:

(80) $$\frac{dN_{Na^+}}{dt} + A\left(f_{Na^+} + 3p\right) = 0$$

(81) $$\frac{dN_{K^+}}{dt} + A\left(f_{K^+} - 2p\right) = 0$$

(82) $$\frac{dN_{Cl^-}}{dt} + A f_{Cl^-} = 0$$

(83) $$\frac{dV}{dt} + A f_{H_2O} = 0$$

In these equations, A and p are positive constants.

An important remark is that the above formulation automatically preserves the electroneutrality of the interior bulk solution. This is not a consequence of the dynamics, however, but rather of the algebra by which the interior concentrations are evaluated from the numbers of ions of the mobile species within the cell. To confirm this, recall the steps by which equation (63) was derived from (60-62). There, electroneutrality of the interior bulk solution was used to derive (63). Reversing the steps, we see that electroneutrality of the interior bulk solution is indeed implied by (60-62) together with (63).

In any code that implements the above dynamics, one should build in a check that bulk electroneutrality of the interior ionic solution is indeed satisfied and moreover that the computed values of $[Na^+]^{int}$, $[K^+]^{int}$, $[Cl^-]^{int}$, and ϕ^{int} satisfy equations (60-62).

Dynamics on the slow manifold

There are two very different time scales in the dynamical system described above. One of these is the electrical time scale associated with the membrane capacitance. It is on the order of milliseconds and is the scale on which neuronal signalling operates. The other is the substantially slower time scale on which changes in volume and concentration occur.

If the electrical time scale is not of interest, it can be removed from the problem by setting $C_m = 0$. In that case, there are no space-charge layers and the whole cell is electrically neutral at all times. The relationship between the internal concentrations and the numbers of ions of the different species within the cell simplifies to

(84) $$[Na^+] = \frac{N_{Na^+}}{V}, \quad [K^+] = \frac{N_{K^+}}{V}, \quad [Cl^-] = \frac{N_{Cl^-}}{V}$$

Since the cell must remain electrically neutral at all times, there cannot be any net electrical current flowing across the cell membrane, and this condition now becomes the way in which the electrical potential within the cell (relative to that of the external ionic solution) is determined:

(85) $$0 = q\left(f_{Na^+} + 3p + f_{K^+} - 2p - f_{Cl^-}\right)$$

$$= pq +$$

$$\frac{q^2\phi^{int}}{1-e^{-\frac{q\phi^{int}}{kT}}}\Bigg(\left(\gamma_{Na^+}[Na^+]^{int} + \gamma_{K^+}[K^+]^{int} + \gamma_{Cl^-}[Cl^-]^{ext}\right)$$

$$-e^{-\frac{q\phi^{int}}{kT}}\left(\gamma_{Na^+}[Na^+]^{ext} + \gamma_{K^+}[K^+]^{ext} + \gamma_{Cl^-}[Cl^-]^{int}\right)\Bigg)$$

Here we have used equations (76-78), with f_{Cl^-} rewritten in such a way that it has the same denominator as f_{Na^+} and f_{K^+}.

Aside form the pump current, pg, the right hand side of (85) has the form of a single Goldman-Hodgkin-Katz current density, with an effective γ equal to

(86) $$\gamma_{Na^+} + \gamma_{K^+} + \gamma_{Cl^-}$$

and with effective internal and external concentrations equal to

(87) $$\frac{\gamma_{Na^+}[Na^+]^{int} + \gamma_{K^+}[K^+]^{int} + \gamma_{Cl^-}[Cl^-]^{ext}}{\gamma_{Na^+} + \gamma_{K^+} + \gamma_{Cl^-}}$$

and

(88) $$\frac{\gamma_{Na^+}[Na^+]^{ext} + \gamma_{K^+}[K^+]^{ext} + \gamma_{Cl^-}[Cl^-]^{int}}{\gamma_{Na^+} + \gamma_{K^+} + \gamma_{Cl^-}}$$

Note in particular that $[Cl^-]^{ext}$ appears with the internal concentrations of Na^+ and K^+, and that $[Cl^-]^{int}$ appears with the external concentrations of Na^+ and K^+ in these expressions.

The beautiful fact that the GHK formula replicates itself in this way is only true because all of the ions that we are considering have the same magnitude of their charge.

The right-hand side of (85) is a strictly increasing function of ϕ^{int}, and its range is the whole real line, since it approaches $+\infty$ as $\phi^{int} \to +\infty$, and approaches $-\infty$ as $\phi^{int} \to -\infty$. Thus, equation (85) does indeed define ϕ^{int} as a function of the other variables. In a numerical scheme, the value of ϕ^{int} can be found, for example, by Newton's method, with an initial guess given by the value that was found at the previous time step.

Note that nature has been somewhat unkind to us here by employing a pump ratio of 3:2 instead of 1:1. A non-electrogenic pump that imports one K^+ ion for each Na^+ ion that it exports can also control cell volume and has a pump current of zero. With a 1:1 pump, the term pq does not appear on the right-hand side of (85), and then we get an explicit formula for ϕ^{int}:

$$(89)\quad \phi^{int} = \frac{kT}{q} \log \frac{\gamma_{Na^+}[Na^+]^{ext} + \gamma_{K^+}[K^+]^{ext} + \gamma_{Cl^-}[Cl^-]^{int}}{\gamma_{Na^+}[Na^+]^{int} + \gamma_{K^+}[K^+]^{int} + \gamma_{Cl^-}[Cl^-]^{ext}}$$

In summary, if we are not interested in the electrical time scale, we can set $C_m = 0$ and again solve equations (80-83), but with the simpler relations (84) between the numbers of ions in the cell and their concentrations, and with ϕ^{int} defined by the solution of equation (85), or in the particular case of a 1:1 pump by the explicit formula (89).

In this version of the dynamical system (80-83) we are enforcing intracellular electroneutrality only by requiring the rate of change of the total charge in the cell to be zero. Thus, we have to be careful to choose initial conditions in which the cell is electrically neutral, and even then we should monitor the total charge on the cell to ensure that numerical error does not cause the cell to drift away from electroneutrality, since there is no mechanism in the dynamics to correct for this kind of error.

Steady state

The steady-state equations of the model cell are obtained from (80-83) by setting all of the time derivatives equal to zero. The membrane area A then cancels out, and we are left with

(90) $$f_{Na^+} + 3p = 0$$

(91) $$f_{K^+} - 2p = 0$$

(92) $$f_{Cl^-} = 0$$

(93) $$f_{H_2O} = 0$$

The fluxes that appear in these equations are given by (76-79). These are 4 equations in the 5 unknowns

(94) $$[Na^+]^{int}, [K^+]^{int}, [Cl^-]^{int}, \phi^{int}, V$$

and the fifth equation is that of intracellular electroneutrality, which is equation (5).

We start by thinking of the equations of osmotic equilibrium and electroneutrality as a pair of equations in the unknowns

(95) $$\left([Na^+]+[K^+]\right)^{int}, \quad [Cl^-]^{int}$$

This pair of equations is

(96) $$\left([Na^+]+[K^+]\right)^{int}+\left([Cl^-]\right)^{int}=\left([Na^+]+[K^+]\right)^{ext}+[Cl^-]^{ext}$$
$$=2[Cl^-]^{ext}$$

(97) $$\left([Na^+]+[K^+]\right)^{int}-\left([Cl^-]\right)^{int}=\frac{Q_M}{qV}$$

and the solution is

(98) $$\left([Na^+]+[K^+]\right)^{int}=[Cl^-]^{ext}+\frac{Q_M}{2qV}$$

(99) $$[Cl^-]^{int}=[Cl^-]^{ext}-\frac{Q_M}{2qV}$$

Since Cl^- is not pumped, it is in thermodynamic equilibrium when the cell as a whole is in a steady state. This implies that

(100) $$[Cl^-]^{int} = [Cl^-]^{ext} e^{+\frac{q\phi^{int}}{kT}}$$

as we can see from (78) together with $f_{Cl^-}=0$.

Combining equations (99) & (100), we get a relationship between the cell volume and the intracellular potential

(101) $$e^{\frac{q\phi^{int}}{kT}} = 1 - \frac{Q_M}{qV\,2[Cl^-]^{ext}}$$

This equation shows that

(102) $$\phi^{int} < 0$$

and it also implies a restriction on V:

(103) $$V > \frac{Q_M}{q\,2[Cl^-]^{ext}}$$

Equation (103) gives an important limitation to what the control of cell volume can achieve. If the right-hand side of (103) is greater than Vmax as given by equation (1), then the cell is certain to swell until it becomes spherical and bursts, regardless of its pump rate p.

Let

(104) $$R = \frac{Q_M}{q V 2 [Cl^-]^{ext}}$$

Then (101) becomes

(105) $$e^{\frac{q \phi^{int}}{kT}} = 1 - R$$

and

(106) $$R \in (0, 1)$$

If we can solve for R, then we will have determined V.

Equations (90-91) with the passive fluxes defined by (76-77) can be rewritten as follows

(107) $$q\phi^{int}\,\frac{[Na^+]^{int}\,e^{\frac{q\phi^{int}}{kT}} - [Na^+]^{ext}}{e^{\frac{q\phi^{int}}{kT}} - 1} = -\frac{3p}{\gamma_{Na^+}}$$

(108) $$q\phi^{int}\,\frac{[K^+]^{int}\,e^{\frac{q\phi^{int}}{kT}} - [K^+]^{ext}}{e^{\frac{q\phi^{int}}{kT}} - 1} = +\frac{2p}{\gamma_{K^+}}$$

Adding these equations, rearranging the result, and recalling that $[Na^+]^{ext} + [K^+]^{ext} = [Cl^-]^{ext}$, we get the following

(109) $$\left([Na^+]^{int} + [K^+]^{int}\right) e^{\frac{q\phi^{int}}{kT}}$$

$$= [Cl^-]^{ext}$$

$$-p\left(\frac{3}{\gamma_{Na^+}} - \frac{2}{\gamma_{K^+}}\right)\left(e^{\frac{q\phi^{int}}{kT}} - 1\right)\left(\frac{kT}{q\phi^{int}}\right)\frac{1}{kT}$$

Dividing by $[Cl^-]^{ext}$ and rewriting the result in terms of R, we get

(110) $$\frac{[Na^+]^{int}+[K^+]^{int}}{[Cl^-]^{ext}}(1-R)$$

$$= 1 - \hat{p}\,\frac{(-R)}{\log(1-R)}$$

where

(111) $$\hat{p} = \frac{p}{kT[Cl^-]^{ext}}\left(\frac{3}{\gamma_{Na^+}}-\frac{2}{\gamma_{K^+}}\right)$$

From equation (98), we also have

(112) $$\frac{[Na^+]^{int}+[K^+]^{int}}{[Cl^-]^{ext}} = 1+R$$

So equation (110) becomes

(113) $$1-R^2 = 1 - \hat{p}\,\frac{R}{\log\left(\frac{1}{1-R}\right)}$$

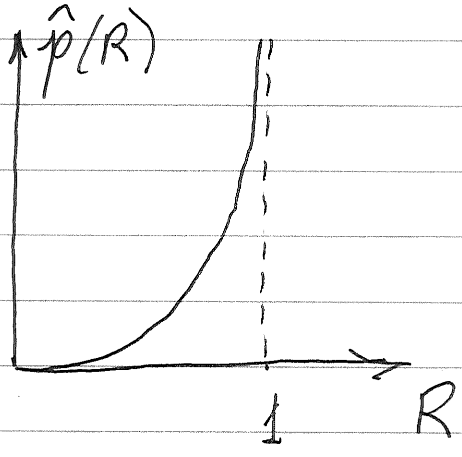
Therefore, thinking of $\hat{p}$ as a function of R defined on $(0,1)$, we have

(114) $$\hat{p}(R) = R\log\left(\frac{1}{1-R}\right)$$

Note that

(115) $$\hat{p}'(R) = \frac{R}{1-R} + \log\left(\frac{1}{1-R}\right)$$

This is positive for $R \in (0,1)$ and equal to zero for $R=0$. Thus $\hat{p}$ is strictly increasing for $R\in(0,1)$, and therefore invertible.



We can tell where the cell operates on the above curve merely by measuring its resting potential. Setting

(116) $$\frac{kT}{q} = 25 \text{ millivolts}, \quad \phi^{int} = -75 \text{ millivolts}$$

we see that equation (105) becomes

(117) $$e^{-3} = 1 - R$$

Then

(118) $$R \approx 1$$

and from (114)

(119) $$\begin{aligned}\hat{p}(R) &= \left(1 - e^{-3}\right)\log\left(e^{3}\right)\\ &= 3\left(1 - e^{-3}\right) \approx 3\end{aligned}$$

By setting $R = 1$, we get the following *approximate* equations for the steady state of the cell:

$$V = \frac{Q_M}{q\,2[Cl^-]^{ext}} \tag{120}$$

$$[Na^+]^{int} + [K^+]^{int} = 2[Cl^-]^{ext} \tag{121}$$

$$[Cl^-]^{int} = 0 \tag{122}$$

Equations (121-122) imply osmotic equilibrium, since the total concentration of mobile ions within the cell matches the external concentration of solute.

Also, equations (120-122) imply internal electroneutrality, since the number of negative elementary charges per unit volume attached to intracellular macromolecules is equal to $Q_M/(qV) = 2[Cl^-]^{ext}$, and this is the same as the concentration of positive counterions in the interior of the cell (and there are no negative counterions in the limit under consideration here).

Note that in the limit under consideration here, in which the pump rate is so large that $R \approx 1$, the cell becomes a perfect osmometer. This means that the cell volume is inversely proportional to the concentration of solute in the external solution. Remarkably, in this limit, the cell volume becomes independent of all other parameters except for the number of negative charges attached to macromolecules within the cell. Thus, as the cell grows by synthesizing additional macromolecules, the cell volume automatically keeps pace.

Homework

Part 1: Although the above discussion suggests that R can be made arbitrarily close to 1, there is actually an upper limit $R_{max} < 1$ which comes from the requirement that $[Na^+]^{int} \geq 0$. Derive a formula for R_{max} in terms of $[Na^+]^{ext}/([Na^+]^{ext} + [K^+]^{ext})$ and $\gamma_{Na^+}/\gamma_{K^+}$. Use the following realistic values to evaluate R_{max}: $\gamma_{Na^+}/\gamma_{K^+} = 0.01$

$[Na^+]^{ext} = 140\,mM$, $[K^+]^{ext} = 4\,mM$

Part 2: Plot the following as functions of $\hat{p}$

$$\frac{V}{\left(\frac{Q_M/\xi}{2[Cl^-]^{ext}}\right)}, \quad \frac{\phi^{int}}{\left(\frac{kT}{\xi}\right)}, \quad \frac{[Na^+]^{int}+[K^+]^{int}}{[Cl^-]^{ext}}$$

$$\frac{[Cl^-]^{int}}{[Cl^-]^{ext}}, \quad \frac{[Na^+]^{int}}{[Cl^-]^{ext}}, \quad \frac{[K^+]^{int}}{[Cl^-]^{ext}}$$

Remarks:

The easy way to make these plots is to use R as a parameter

For the last two plots, use the data mentioned on Part 1 of this homework.