A model for membrane potential and intracellular ion distribution

A.K. Khitrin[1], K.A. Khitrin[2], and M.A. Model[3*]

[1]Department of Chemistry and Biochemistry, Kent State University, Kent, OH 44242, USA

[2]Department of Physics, Kent State University, Kent, OH 44242, USA

[3]Department of Biological Sciences, Kent State University, Kent, OH 44242, USA,

tel. 330-672-0774, mmodel@kent.edu

*Corresponding author

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Abstract

Most cells carry a negative electric charge. It produces a potential difference across the membrane, which regulates voltage-sensitive ion transport and ATP synthesis in mitochondria. The negative charge comes partly from an excess of negative ions in the cell interior (Donnan potential) and partly from ionized groups on the membrane (surface potential). In this work we propose some important modifications to the existing theory of membrane potential. First, we calculate the concentration profile of intracellular positive ions and derive a simple equation to assess the submembrane depletion of positive ions that gives rise to the Donnan potential. The extent of depletion varies with potential, which may provide a regulatory mechanism for ion pumps and channels. Next we consider the surface component of the potential and note that the standard Gouy-Chapman theory has been developed for planar membranes, whereas real cell membranes have a closed geometry. In this case, charges on the membrane surface are not expected to generate fields extending into the cell interior. This fact calls for reinterpretation of some theoretical points as well as experimental data. In particular, the experimentally demonstrated electrostatic attraction between cationic proteins and the negative membrane must now be explained without invoking intracellular fields, and we suggest a new mechanism that can account for this interaction.

INTRODUCTION

Most healthy cells and mitochondria are characterized by a negative resting potential. The process mainly responsible for its generation is intracellular accumulation of K^+ (by the Na^+,K^+ pump) combined with high permeability for K^+; this allows some ions to exit the cell down the concentration gradient, leaving behind an unbalanced negative charge. This is the classic Donnan mechanism, which is considered in detail in physiology textbooks (6, 48). Additionally, the stoichiometry of the Na^+,K^+ pump is such that bringing two K^+ ions into the cell is coupled with extrusion of three ions of Na^+. Direct electrogenic contribution of the pump to the membrane potential is usually small (47, 45) but can be significant or even dominant in some cell types (2, 18, 25). The large (150-200 mV) negative potential of mitochondria is generated exclusively by pumping out protons by the respiratory chain (35).

Regardless of the exact mechanism of potential generation, a deficit of positive ions is created inside a cell or an organelle. In a typical animal cell, the total concentration of charges is on the order of 300 mM. Negative charges are mostly associated with proteins and nucleic acids; the contribution of Cl^- can range from 4 to 60 mM, depending on the cell origin (7). Positive charges are mostly represented by freely diffusible K^+ and, sometimes, by Na^+; the ion composition of mitochondrial matrix is similar to that of the cytosol (1, 44). It is estimated that an excess of negative charges by about 3 μM should be sufficient to create a 90 mV difference across the membrane (6).

The other contribution to the total potential comes from fixed charges on the membrane. These charges are associated with anionic phospholipids that are present both on the inner and outer sides of the membrane (9). Surface charges are efficiently screened by the ions present in the aqueous media but can produce local fields extending about a Debye distance (on the order of a nanometer) into the aqueous phase. Quantitatively, this field is described by the Gouy-Chapman theory (17, 31); more detailed models include dipolar fields from zwitterionic lipids and explicit polarization of water molecules (7). Applications of the surface potential theory to biological membranes have been extensive (23, 30, 31, 34, 36, 45, 50); in particular, the field originating from surface charges has been implicated in binding of positively charged cytoplasmic proteins to the inner leaflet of the plasma membrane (19, 37).

In the present paper we propose some modifications to the existing theory of membrane potential. We note that whether the cell potential has a Donnan or a surface origin, the entire charge that generates the transmembrane potential difference is localized to a nanometer-deep strip under the membrane (in the case of Donnan potential, this layer has a reduced concentration of positive ions). This effect has been recognized before (26), but here we present a simple theoretical model to describe the concentration profile of intracellular charges. Next, we reexamine the origin of the surface potential. The standard treatment of surface charges is based on the assumption that the entire membrane is a plane sheet. In reality, biological membranes have a closed geometry, and we show that this fact is expected to have important consequences for electric phenomena: surface charges, on the average, are not supposed to generate internal fields. This calls for reassessment of a large body of experimental data; in particular, the new model must be reconciled with the evidence of electrostatic attraction between positively charged cytoplasmic proteins and the negative membrane. We thus suggest an alternative general mechanism of protein-membrane binding based on minimization of electric energy of the system.

THEORY AND DISCUSSION

1. Concentration profiles of intracellular charges. We assume for now that negative charges (e.g., anionic proteins) are fixed and uniformly distributed throughout the bulk of the cell. Monovalent positive charges, on the other hand, are mobile and present at a slightly lower net concentration. As with any conductor, an unbalanced charge should be confined to a thin surface layer (which, in our case, is the space immediately under the membrane), so that the potential deep inside the cell would be constant. The concentration profile for positive ions can be found from the balance between the diffusion flux and the flux created by electric field (**Fig. 1**):

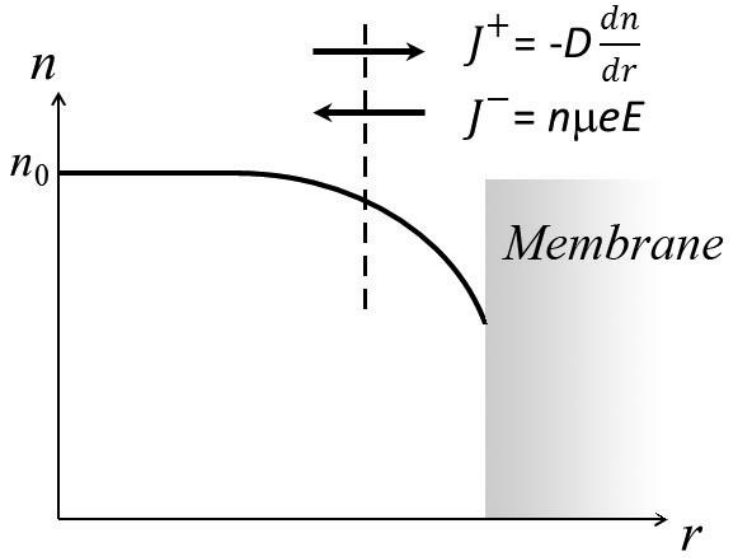

Figure 1. The balance between diffusion and electromotive fluxes in a sub-membrane layer. Membrane is shown by the shaded area and the ion concentration profile by the solid line. Nonuniform distribution of ions creates two opposite fluxes, electrostatic and diffusional; the balance between these fluxes at any point (indicated by the dashed line) determines the equilibrium concentration profile.

$$-D\,(\mathrm{d}n/\mathrm{d}r) + n\mu eE = 0 \qquad (1)$$

Here D is the diffusion coefficient, n is the concentration of positive ions (number per m^3), r is the distance along a normal to the membrane, μ is the mobility, e is the electron charge, and E is the electric field created by nonuniform distribution of charges. If we assume that a cell has a spherical or a flat shape, the electric field E can be calculated by applying the Gauss theorem:

$$E(\mathrm{r}) = \sigma/(\varepsilon\varepsilon_0) = (\varepsilon\varepsilon_0)^{-1} e \int_{-\infty}^{r} (n - n_0)\,\mathrm{d}r', \qquad (2)$$

where $\sigma(Cm^{-2})$ is the charge density per membrane area, ε and ε_0 are the relative and absolute dielectric permittivity, and n_0 is the concentration of negative ions. Only the total charge on the left of the dashed line in **Fig. 1** contributes to the electric field at the position of the line. By combining Eq. 1, Eq. 2, and the Einstein-Smoluchowski relation: $D = \mu kT$ (where k is the Boltzmann constant and T is the absolute temperature), one obtains the equation

$$dn/dr = \{e^2/(\varepsilon\varepsilon_0 kT)\} n \int_{-\infty}^{r} (n - n_0)\, dr'. \quad (3)$$

By introducing the relative concentration $c = n/n_0$ and the characteristic Debye-Hückel length

$$\lambda = \{\varepsilon\varepsilon_0 kT/(e^2 n_0)\}^{1/2}, \quad (4)$$

Eq. 3 can be rewritten as

$$dc/dr = \lambda^{-2}\, c \int_{-\infty}^{r} (c - 1)\, dr'. \quad (5)$$

For T = 300K, ε = 60, and n_0 corresponding to 0.1M, the Debye-Hückel length is about 1 nm. Finally, in terms of the dimensionless coordinate $x = r/\lambda$, Eq. 5 assumes the form

$$dc/dx = c \int_{-\infty}^{x} (c - 1)\, dx'. \quad (6)$$

This integral-differential equation describes the concentration profile resulting from the balance between the diffusion flux and the flux created by electric field. Because direct numerical solution of Eq. 6 is computationally inefficient, it was solved by converting it to a second-order differential equation:

$$d^2(\ln c)/dx^2 = c - 1. \quad (7)$$

The soliton-like solution describes a transition from c = 1 (at $x \rightarrow -\infty$) to c = 0 at large x (**Fig. 2a**). The solution can be shifted by an arbitrary constant along the x-axis; this shift represents one of the integration constants. In **Fig. 2a**, zero value of x was arbitrarily chosen at a point where c = 0.5. For practical calculations, the following empirical function was found to give an accurate approximation to the solution:

$$c(x) = 0.5\, tanh\, (0.0021236 - 0.632263\, x - 0.0642222\, x^2 - 0.0108275\, x^3) + 0.5 \quad (8)$$

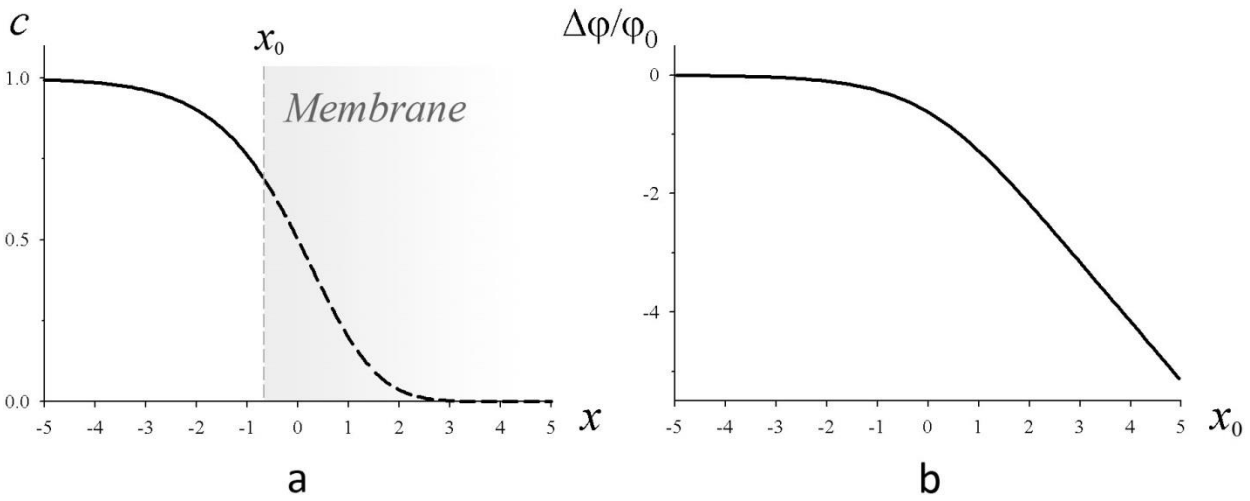

Figure 2. (a) Solution of Eq. 6 for the concentration profile. The membrane location at different x_0 results in different membrane potentials. (b) The membrane potential as a function of x_0.

To find the position of the membrane boundary x_0 we relate our solution to the cell potential Δφ. In doing so we assume that most of the potential drop Δφ occurs within the membrane; the rationale for this will be given later in the text. In this case we can estimate Δφ as the product of $E(r_0)$ found from Eq. (2) and the membrane thickness l. Relative dielectric permittivity ε of the electrolyte should now be replaced by that of the membrane ε_m.

$$\Delta\varphi = l\,(\varepsilon_m\varepsilon_0)^{-1} e \int_{-\infty}^{r0}(n - n_0)\,dr. \qquad (9)$$

After conversion to dimensionless units, as in Eq. 6, we have

$$\Delta\varphi = \varphi_0 \int_{-\infty}^{x0}(c - 1)\,dx, \qquad \varphi_0 = \gamma\,(\varepsilon/\varepsilon_m)\,(kT/e), \qquad (10)$$

where $\gamma = l/\lambda$ is the dimensionless thickness of the membrane, x_0 is the position of the membrane boundary and c is the solution of Eq. 6 (**Fig. 2a**). Estimates of the parameters in Eq. 9 give kT/e = 26 mV at T = 300K, and $\varphi_0 \approx 1$ V for $\gamma = 3$ and $\varepsilon/\varepsilon_m = 15$. **Fig. 2b** shows the potential inside the cell in units of φ_0. Once x_0 is determined from the data in **Fig. 2b** for a given Δφ, the concentration of positive ions at the membrane boundary c_m can be found from **Fig. 2a**. The dependence of c_m on Δφ is shown in **Fig. 3**. Note that $c_m(\Delta\varphi)$ describes the concentration at the inner membrane surface for any monovalent positive ion or their sum. Concentration profile $c(x)$ can also be viewed as an equilibrium Boltzmann distribution in a self-consistent potential. Therefore, if a divalent ion is present at a concentration small enough not to affect the potential, its relative concentration c_2 can be estimated as $c_2 = c^2$. The concentration of a divalent positive ion at the membrane boundary is shown in **Fig. 3** by a dashed line.

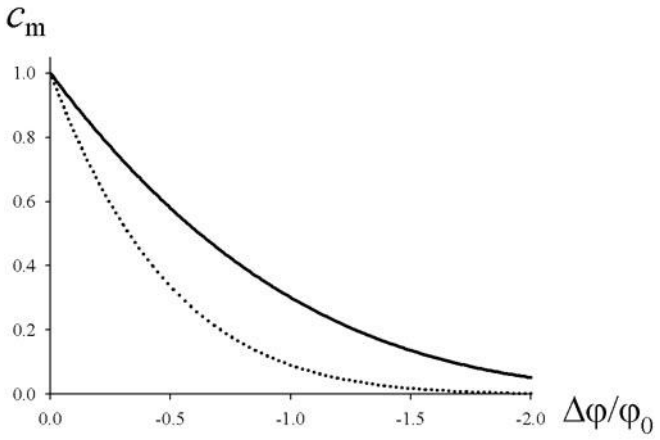

Figure 3. Concentration of monovalent (solid) and divalent (dashed line) ions at the membrane as a function of the cell potential.

The described model predicts a relatively small degree of depletion. Its magnitude can be estimated at ≈ 5% for a typical cell membrane potential of -60 mV and ≈ 15% for a three times larger mitochondrial potential. The effect for divalent cations, such as calcium, will be approximately twice as large (for small depletions). It needs to be recognized though that our model does not take into account the discreet nature of fixed charges. Indeed, discreet negative charges are likely to sequester a fraction of mobile positive ions, thus reducing their effective concentration. As a result, a greater relative amount of sub-membrane depletion will be needed to achieve the same potential. Quantitative assessment of this effect would require detailed knowledge of the distribution of negative charges, including dipolar effects (7), but, qualitatively, one can say that calculations based on the continuous model are likely to underestimate the effect. Since the extent of depletion is potential-dependent, it would be tempting to speculate that it might provide a stabilizing feedback, especially for mitochondria, where large changes in the potential are observed during cell growth, differentiation, motility, cancerous transformation, calcium signaling, excitotoxicity and apoptosis (12, 43).

2. Qualitative features of the potential profile. The total potential difference between the cell interior and the outside solution (the one that can be measured with electrodes) comprises three components: the voltage drop inside the membrane $\Delta\varphi_m$ and across the two regions on both sides of the membrane, $\Delta\varphi_{in}$ and $\Delta\varphi_{out}$ (**Fig. 4**). First, consider the potential created by fixed membrane charges (**Fig. 4a**). In the literature, the inner potential difference $\Delta\varphi_{in}$ is often shown positive (dashed line in **Fig. 4a**), as resulting from negative charges on the inner side of the membrane. Such conclusion is derived from the Gouy-Chapman model for a flat charged sheet immersed in a neutral electrolyte. Since the electric field is negligible at distances larger than Debye length, it must have been assumed that closing the membrane would not affect the result. We believe, however, that this is not so. Consider a spherical shell carrying uniformly distributed surface charges but without any electrolyte inside. According to the Gauss theorem, the electric field inside is zero. Now add an electrically neutral electrolyte. From the requirement of minimum energy of the system it follows that the electrolyte must remain unpolarized. Indeed, suppose that a spherically symmetric perturbation of the charge density inside the shell has developed. It will not

change the electrostatic energy density in the membrane and outside the cell since that depends only on the total charge inside the cell. However, the region with non-zero electric field will increase the electrostatic energy within the cell. Additionally, a non-uniform distribution of ions will increase the free energy associated with mixing. For these reasons we conclude that membrane-bound charges cannot polarize intracellular electrolyte and the potential profile would be more accurately represented by the solid line in **Fig. 4a**.

The above reasoning does not apply to a flat membrane, in which case it is possible to show that formation of double electric layers on both sides of the membrane actually reduces energy. The ultimate reason for the difference is that, for a closed geometry, the condition of electrolyte neutrality (charge conservation) becomes more restrictive.

Because the spherical membrane is positioned outside its inner surface but inside its outer surface, only inner charges will create the potential within the membrane. This again is different from the standard treatment, which assumes that the surface-related part of $\Delta\varphi_m$ is determined by the difference between surface charge densities inside and outside. The reasoning based on the Gauss theorem compels us to conclude that outside charges play no role in the generation of $\Delta\varphi_m$.

This, however, brings up an interesting question. It is known that certain treatments can eliminate the membrane potential; ionophores, metabolic inhibitors or the opening of the mitochondrial permeability transition pore dissipate the potential across the inner mitochondrial membrane (12, 35, 38, 42), and high extracellular potassium does the same to the plasma membrane (12, 28, 39). This fact presents no difficulty within the standard model because only the difference in surface charges would be responsible for any residual potential; thus, if the charge densities on both surfaces are approximately equal, then complete depolarization is possible. But for a spherical cell, dissipation of the Donnan potential is expected to leave the contribution from interior surface charges intact (the above-mentioned treatments are not expected to change the surface potential (16)). One possible explanation to this fact is that the actual inner surface charge density is smaller than is commonly thought. The cytosol is an extremely crowded space (41), and the dissociation constants of phosphate groups of lipids facing the cell interior may be different from those in a dilute buffer.

So far, we have only been considering the potential created by surface charges. A complete description of the membrane potential must include the effects of unbalanced negative charges in the bulk of the cell. As shown earlier, these uncompensated bulk charges are expected to be limited to a thin sub-membrane layer and to produce a potential that is shown qualitatively in **Fig. 4b**. The depletion-related $\Delta\varphi_{in}$ is at least an order of magnitude smaller than $\Delta\varphi_m$ for the following two reasons. First, submembrane electric fields extend only over the distance of about the Debye length, which is smaller than the 3-5 nm width of the membrane (14, 45). Second, the relative dielectric permittivity of the aqueous solution ε is much larger than that of the membrane $\varepsilon_m \approx 2$-5 (14, 31, 45), making the electric field inside the membrane correspondingly stronger (see also (26)). These considerations apply only to the inner potential. The outer potential $\Delta\varphi_{out}$ is reported to reach 15-30 mV on the plasma membrane (10, 30).

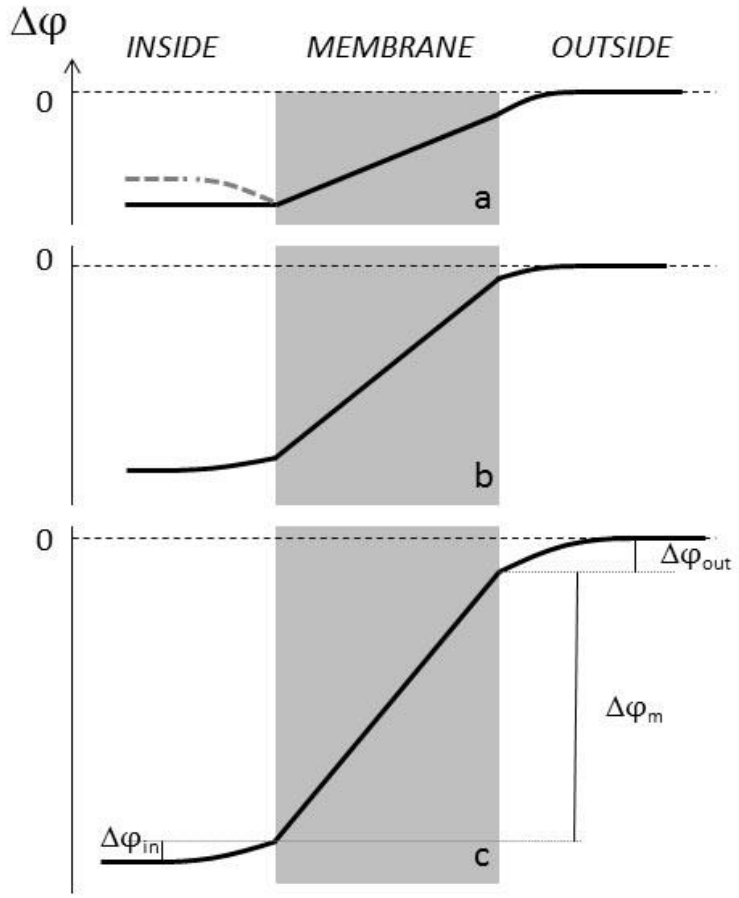

Figure 4. Potential profiles created by (a) immobile negative charges attached to the membrane surfaces; the dashed line shows the profile according to the open membrane model (b) sub-membrane depletion of positive mobile ions, and (c) the sum of the contributions in (a) and (b). See text for additional explanations.

3. Hypothesis for the mechanism of protein-membrane interactions. We have argued that the membrane surface creates no electric field in the cell interior. At the same time, there is substantial experimental evidence in favor of nonspecific electrostatic attraction between acidic phospholipids and positively charged intracellular proteins (19, 29, 49). This apparent paradox needs to be addressed. First, we note that the statement about the surface potential being zero inside requires some refinement: it applies only to average fields. For spherical or flat cells, zero average would also imply zero local fields, but deviations would be expected within small membrane protrusions or invaginations. Likewise, nonuniform distribution of charges within the membrane (15, 24) may give rise to local internal fields. Such effects, for example, may play a role in highly convoluted mitochondrial crystae. Future work may help evaluate the magnitude of this effect.

In addition to that, we hypothesize that there might be yet another, and presumably more universal, mechanism of electrostatic interaction between the membrane and intracellular charges. Consider a membrane with electric field E_m directed as shown in **Fig. 5**. The energy density associated with this field is proportional to E^2. When a large multiply charged molecule or a small particle is

located in a conducting liquid far from the membrane, its electric field E_{p1} decays at the Debye distance. However, if the molecule closely approaches the membrane, its electric field begins to penetrate the membrane without decay. Now a much stronger field E_{p2}, directed opposite to E_m, emerges within the membrane. The total field in the membrane becomes reduced, and so does the electrostatic energy of the system (**Fig. 5**). Therefore, close placement of a large positively charged molecule on the membrane would be thermodynamically favorable.

A simple estimate of the binding energy ΔG can be made by assuming that: (1) the protein is large compared to the membrane thickness l; (2) by neglecting the energy of the Debye layer; (3) by neglecting the entropic contribution from the ionic layer surrounding the protein:

$$\Delta G = (Sl/2\varepsilon_m\varepsilon_0)\,(\sigma_p^2 + 2\sigma_p\sigma_m), \qquad (11)$$

where S is the protein/membrane contact area, σ_m is the surface density of membrane charges, and σ_p is the surface density of protein charges. One can see that, for a given σ_m, the strongest binding (minimum ΔG) is experienced by particles with $\sigma_p = -\sigma_m$, but for low membrane potentials $|\sigma_m| < 0.5|\sigma_p|$ repulsion is expected.

This mechanism may operate either on planar or spherical membranes. An interesting situation might arise for a flat membrane when the potential is generated by positive charges adsorbed on the right side of the membrane (in the configuration depicted in **Fig. 5**). The expected attraction of positive molecules from the opposite side of the membrane would be equivalent to attraction of like charges! Needless to say, this hypothesis needs to be tested experimentally.

The other line of evidence for electrostatic interactions between phospholipids and positively charge molecules comes from the work on artificial lipid vesicles (3-5, 13, 21, 22, 27, 32, 33, 46, 51) or plane lipid layers (11, 20, 22, 40). However, flat membranes or the outer surface of vesicles represent a different electrical environment, and there is no contradiction between those results and our hypothesis.

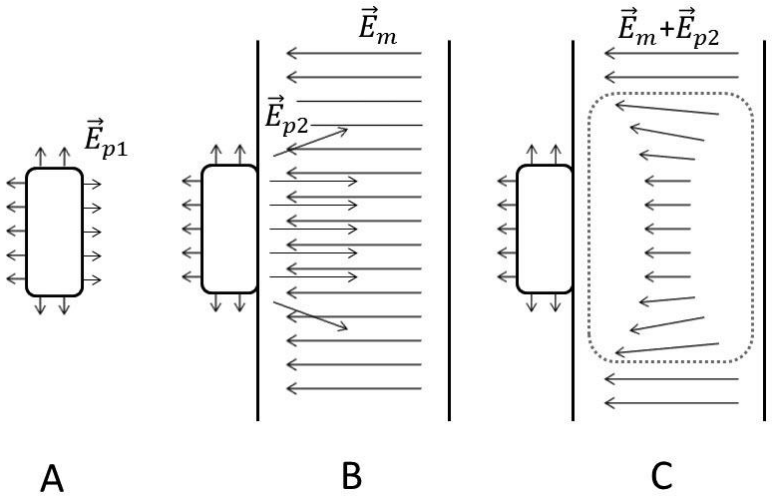

Figure 5. The proposed mechanism of binding of a positively charged large molecule or particle to the interior of the membrane surface. (A). When the particle is in a conducting liquid, the electric field created by ionized groups extends only a short distance into the liquid. (B). When the particle comes in apposition with the membrane, its field penetrates into the membrane in the direction opposite to the field already present in the membrane. (C) Superposition of the field from the particle with the field in the membrane results in a decrease in the total field in the area indicated by the dotted line. The energy density within that area decreases to create an energetically favorable condition for particle binding.

4. Conclusions. Near-membrane depletion of positive ions could in principle be verified by using appropriate membrane-linked ion probes. The loss of a negative potential would be expected to raise the subcellular concentration of positive ions and stimulate their efflux, which, indeed, is a common cellular response to depolarization (35). Of course, cells have others ways to regulate ion traffic, so the biological significance of the hypothesized depletion cannot be claimed at this point. Nevertheless, as a little-appreciated consequence of the Donnan potential, this effect may be interesting at least from the theoretical perspective.

With regard to the surface potential theory, we have shown that simple coulombic interactions between cytoplasmic proteins and the inner membrane are incompatible with the model of a spherical, uniform and continuously charged membrane. Since real membranes are not such, additional theoretical work is necessary to evaluate the importance of deviations from symmetry and uniformity; one might find, for example, that electrostatic interactions should be limited to certain areas of the membrane. The alternative (or complementary) mechanism of attraction between an electrically polarized membrane and charges immersed in a conducting liquid should be testable on model systems.

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