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Bioelectrochemistry 56 (2002) 141–146

Bioelectrochemistry

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Electric field effects in proteins in membranes

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Received 30 January 2002; accepted 4 February 2002

Abstract

Both the organization and function of protein nanostructures in membranes are related to the substructural properties of the lipid portion of the membrane. Potential differences that are established across the membrane and generate electric fields in these very thin portions are shown to modulate the organizational and functional properties of the protein modules. Many protein modules also have nonisotropic distributions of charged sites, including configurations in which there are regions containing predominantly positive fixed charges, juxtaposed with adjacent regions containing predominantly negative fixed charges. In these double fixed charge regions, very large electric fields can manifest in the ionic depletion layer at the junction of the two fixed charge regions. Consideration is also given to the manner in which the intense electric fields that are established in protein modules, such as proton ATPases, can modulate the chemical reactions that are associated with proton transport and dehydration reactions. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Membrane; Electric Field; Bilayer; Water dissociation; Molecular organisation; Fixed charges

1. Introduction

The overall thickness of cell membranes is typically around 6 nm. In living cells, electric potential differences are maintained across the membrane between the cytoplasmic compartment and the external medium. The membrane potential (difference) ranges from a modest 10 mV (for instance, in human erythrocytes) to more than 240 mV (for instance, in the giant algal cell of *Chara corallina*). Neurones typically have a ''resting'' membrane potential of around 60–80 mV and this is a value typical of many other cells (Fig. 1).

The average electric field strength in cell membranes is therefore of the order of 10^7 V/m. Furthermore, the electric field is not expected to be uniform and hence, the peak field strength is likely to be much greater than the average. Here, we will explore some of the mechanisms by which such intense electric fields may modulate the molecular organisation and functional properties of cell membranes.

2. Molecular organisation of membranes

Cell membranes, which separate the exterior environment from the cell interior, contain functional protein modules imbedded in a bimolecular lipid sheet or bilayer. The bilayer serves both as an electrical insulating and diffusion barrier as well as a supporting matrix for the protein modules [1]. Such membranes similarly delineate many of the internal organelles of cells.

The measured electrical conductance of a lipid bilayer membrane is of the order of 1 mS/m^2 [2–4]. This makes it an extremely good electrical insulator, despite the fact that the bilayer has pore ''defects''. The bilayer conductance is also orders of magnitude lower than measured conductances of cell membranes. The conduction properties of cell membranes therefore arise from embedded protein modules. Nonetheless, the insulating properties of the supporting lipid bilayer that derive from the hydrophobic properties of the lipid molecules, play a very important role in the organisation of these modules and feature strongly in the electrical characteristics of the cell membrane.

Many of the protein modules are composed of a number of subunits that span the membrane with transmembrane subunits connected by flexible strands that remain external to the membrane. The positioning and axial orientation of

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PII: S1567-5394(02)00061-0

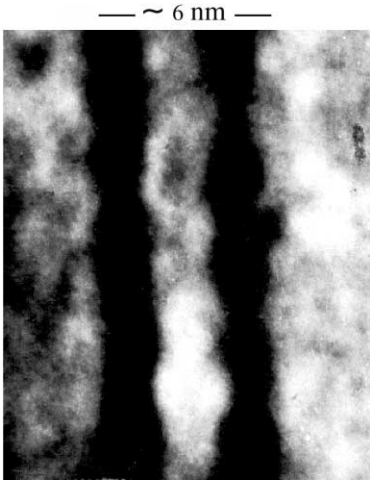

Fig. 1. An electron micrograph of the cell membrane (of *C. corallina*). The membrane is "fixed" by cross-linking using OsO_4 that also enhances the contrast. The two electron opaque regions in its OsO_4 stained structure corresponds to the polar, hydrophilic regions of the membrane, while the central electron-lucent layer corresponds to the nonpolar, hydrophobic region.

the fully functional modules result from a balance between opposing hydrophobic and hydrophilic forces, between the aqueous environment, the nonpolar lipid interior of the membrane and the polar and nonpolar portions of the subunits.

2.1. Protein aggregation

Membrane proteins have a central nonpolar region that approximately aligns with the central hydrophobic region of the lipid bilayer and polar regions that protrude into the aqueous environments. Any significant mismatch in, for instance, the dimensions of the nonpolar region of the protein and the hydrophobic region of the lipid bilayer will be associated with a substantial hydrophobic interaction energy. In principle, it is possible to evaluate the interaction energy for the many proteins for which the structure is now known. However, an estimate can be readily made from the total area of hydrophilic–hydrophobic interfaces and assuming a hydrophobic interaction similar in magnitude to that of an oil–water interface, which is ~ 50 mJ/m^2.

The outcome of a mismatch is generally to induce an aggregation of the protein subunits [5,6]. For example [7], consider the hypothetical protein subunits shown in Fig. 2.

In this example, the dimensions of the nonpolar region of the central portion of the protein (d_p) is fractionally larger than that of the nonpolar region of the lipid bilayer membrane (d_m). It is hypothesised that the subunits exist in the bilayer either separately or in an aggregation of six subunits. In the hexamer configuration, the flat faces of the subunits that interact with their neighbours have matching nonpolar regions. In the monomer configuration, the hydrophobic parts of these flat faces would be partially exposed to the aqueous environment; to an extend of ($h_d - h_m$). This gives rise to a difference in the interaction energies, E_h, for the two configurations. At equilibrium, the chemical potentials for the subunits in these two configurations must be equal. Thus,

$$\mu_{1,0} + kT\ln X_1 + E_h = \mu_{6,0} + \frac{kT\ln X_H}{6}$$

where X_1 and X_H are the mole fractions (concentrations) for the monomer and hexamer configurations, respectively, and $\mu_{1,0}$ and $\mu_{6,0}$ represent the respective standard chemical potentials for a subunit in these configurations and includes all the concentration independent components to the interaction energies. Designating X_6 as the mole fraction of subunits locked up as hexamers we have:

$$X_6 = 6X_H \text{ or } \mu_{1,0} + kT\ln X_1 + E_h = \mu_{6,0} + \frac{kT\ln(X_6/6)}{6}$$

E_h can be substantial, even for very small mismatches between the hydrophobic regions of the protein and bilayer. For the hypothetical protein shown in Fig. 2, the mismatch involves an "oil–water" area of $2r(d_p - d_m)$ per subunit, where r is the radius of the hexamer. For d_p=2.1 nm, d_m=2.0 nm and r=1 nm, $E_h \sim 10^{-18}$ mJ (or 0.062 eV or ~ 2.4 units of kT at room temperature).

Assuming $\mu_{1,0}=\mu_{6,0}$ and $X_6=10^5$ subunits per unit volume of membrane locked up in hexamers, the Boltzmann distribution function then yields a concentration ratio for the

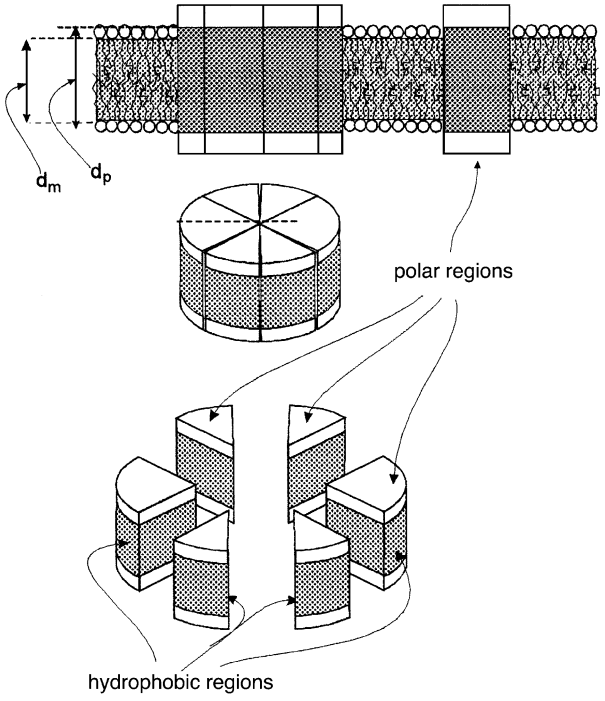

Fig. 2. A hypothetical protein module made up of six subunits, embedded in a lipid bilayer membrane. The subunits may either be dispersed as single subunits or may aggregate as hexamers. The statistical–mechanical equilibrium distribution of these configurations is determined by the chemical potentials of the monomer and hexamers (after Ref. [3]).

subunits (i.e. X_6/X_1) of $\sim 2.25\times10^5$, indicating that the mismatch, of only 0.1 nm, results in essentially all the subunits forming hexamers.

2.2. Electrostriction effects

Although electrostriction effects in solvent-free artificial bilayers are minimal [8], the extremely high electric field strengths in a cell membrane may give rise to electrostriction effects in proteins imbedded in the bilayer and this can have profound effects on the molecular organisation of the proteins in these membranes. This may be illustrated by considering the effect on the protein module and subunits shown in Fig. 3.

Since most of the electric field generated by the membrane potential V appears across the nonpolar part of the membrane, compression of the proteins due to electrostriction will also be largely confined to the nonpolar parts of the protein modules. Take the case where the proteins have a dielectric constant ε_p and an uncompressed thickness of the hydrophobic portion of d_0. When a field is applied, the electric stress is balanced by elastic restoring forces and assuming that the proteins are ideal elastic materials, this yields [9,10]:

$$\frac{\varepsilon_p\varepsilon_0 V^2}{2d^2} = Y\int_{d_0}^{d}\frac{dx}{x} = Y\ln\frac{d_0}{d}$$

where d is the thickness of the nonpolar portion of the modules or subunits in the presence of the field, Y is the elastic modulus of the subunits and ε_0 is the dielectric permittivity of free space.

2.3. Effect of membrane potential on protein aggregation

As the membrane potential changes, the electrostriction effects on embedded proteins may induce the type of mismatch discussed above that result in strong aggregation of protein subunits. Alternatively, the effect may result in nonpolar regions of the proteins becoming more closely matched dimensionally with those regions of the lipid bilayer. The latter would cause the subunits to disassociate. At very high membrane potentials, the electrostriction may be so severe that the nonpolar regions of the proteins become smaller than those regions of the bilayer and aggregation of the subunits would then again be induced.

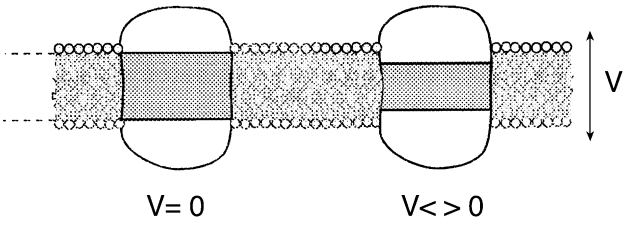

Fig. 3. Electrostriction of protein modules imbedded in a lipid bilayer membrane.

Thus, the large electric fields present in cell membranes and the large changes in those fields that are associated with membrane phenomena such as action potentials, electrogenic ion transport, etc., can potentially be a major determinant of protein organisation and function in membranes.

2.4. Fixed charges in proteins

The detailed structure of a number of functional membrane proteins are now available. As expected, these proteins contain fixed charges that arise from the ionization of basic and acidic amino acids comprising the protein. Such fixed charges modify the permeation of mobile ions into the modules that they form in a membrane. The resulting electro-chemical profiles and space charge effects can control electrical conduction through the modules and can give rise to highly nonlinear electrical characteristics.

The ion partitioning into a fixed charge system such as a membrane protein is not only affected by the fixed charges, but also by the Born (image) forces [11] that are determined by the dielectric properties of the protein and the external aqueous solution. The Born image energies are dependent on the ionic radius R, charge ez (valency z) as well as the dielectric constants of the solution (ε_1) and protein (ε_2) and is given by:

$$W_{\mathbf{B}} = \frac{z^2e^2}{8\pi\varepsilon_0 R}\left[\frac{1}{\varepsilon_2}-\frac{1}{\varepsilon_1}\right]$$

For a potassium ion, the Born energy for partitioning into a protein with a dielectric constant ε_2=10 is $\sim$0.1 eV. For comparison, the partitioning energy for such an ion into the interior of a lipid bilayer (ε_2=2.1) is $\sim$3 eV (or $\sim$120 units of kT at room temperature).

The effect of ion partitioning on the concentration profiles of mobile ions (hydrated cations and anions assumed to be of equal radius) permeating a protein module with no fixed charges is illustrated in Fig. 4a.

On the other hand, if the dielectric effects are ignored (ε_1=ε_2), the fixed charges give rise to the usual Donnan equilibrium which is dominated by the requirement for macroscopic neutrality. The concentration profiles in this instance are more like those shown in Fig. 4b for the case when the fixed charge concentration C_{x^-} is much larger than the ionic concentration of the solution C_o, or as in Fig. 4c for the case when $C_{x^-}C_o$. It should be noted that while in aqueous plant cells, the situation C_{x^-}C$_o$ is not uncommon for most isotonic solutions in mammalian tissue $C_{x^-}\sim C_o$.

When both dielectric and fixed charge effects occur, the profiles for the mobile ions are likely to be similar to those shown in Fig. 5. Here, the concentration of those ions with charge of the same sign as the fixed charges (co-ions) are depressed in the protein both by the Born energy and the Donnan effect, while the counter-ions reach values close (slightly higher than) to the fixed charge concentration. Note that this also establishes a large lattice potential

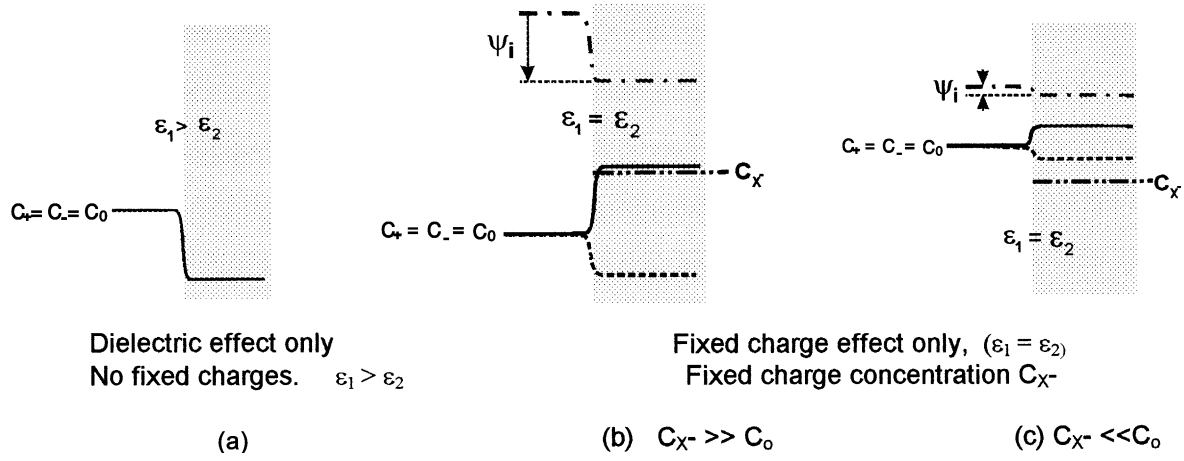

Fig. 4. Modules in contact with aqueous ionic solutions which permeate the system. (a) The effect of the dielectric alone. (b, c) The effect of fixed charges without dielectric effects; (b) the fixed charge concentration, C_{x^-}, is much greater than the ionic concentrations, C_o, in the external solution and (c) when the fixed charge concentration is similar or less than the concentration of ions in the external solution.

between the solution and protein matrix given by the Donnan potential:

$$\Psi_i = \frac{kT}{e} \ln \frac{C_{x^-}}{\gamma C_o} \qquad \text{where } \gamma = e^{-W_B/(kT)}$$

γ is the partition coefficient determined by the Born energy W_B.

2.5. Double fixed charge modules

An interesting case arises when a transmembrane protein module contains a negatively charged region in juxtaposition with a positive fixed charge region. The ionic profiles are then like those shown in Fig. 6.

At the junction of the positive and negative fixed charge regions, a layer develops which is largely depleted of both anions and cations. This is a layer with a large space charge associated with a large junction potential Ψ_j (the sum of the two solution-protein Donnan potentials, Ψ_o and Ψ_i). Most cells maintain sizeable "resting" potentials (V) which are normally negative on the cytoplasmic side with respect to the external solution. This membrane potential results in an additional bias appearing across the low conductance depletion layer. For the DFCM orientation depicted in Fig. 6, Gauss's law yields that the increased bias across the depletion layer will lead to an increase in the width of the central depletion layer where the space charge is almost equal to the fixed charge concentration.

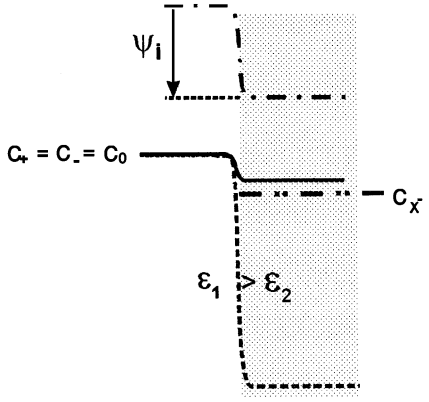

Fig. 5. Ion profiles in a fixed charge system in which differences in the dielectric constants also give rise to ion partitioning as a result of the Born image forces.

2.6. The chemiosmotic theory: vectorial chemistry

The ATPase is a protein module that can utilise the differences in the chemical potential of protons and the electrical potential difference (that is, the electrochemical potential difference for protons) across the membrane, to drive the synthesis of ATP from ADP and phosphate. This process requires the transport of protons from one side of the membrane to the other. In most systems at moderate pH, the concentration of protons, however, is extremely low and may limit the reaction kinetics. For instance, the total number of protons in the lumen of a mitochondrium at pH 6 is only 10. While the driving force for the ATP synthesis derives from the difference in the electrochemical potential of proteins across the inner membrane of the mitochondria, the kinetics may be limited by the number of protons locally available and their rate of production from the dissociation of water.

The structure of the ATPase modules is now becoming clear and it appears that the F_o portion of these modules contain DFCM regions [12]. The overall scheme is as shown in Fig. 7.

The depletion layer in the DFCM region in the F_o portion of the module, may play an important role in that this is a region where extremely large fields are generated [13]; exceeding 10^8 V/m.

The significance of this is that the dissociation of water is drastically altered in such electric fields.

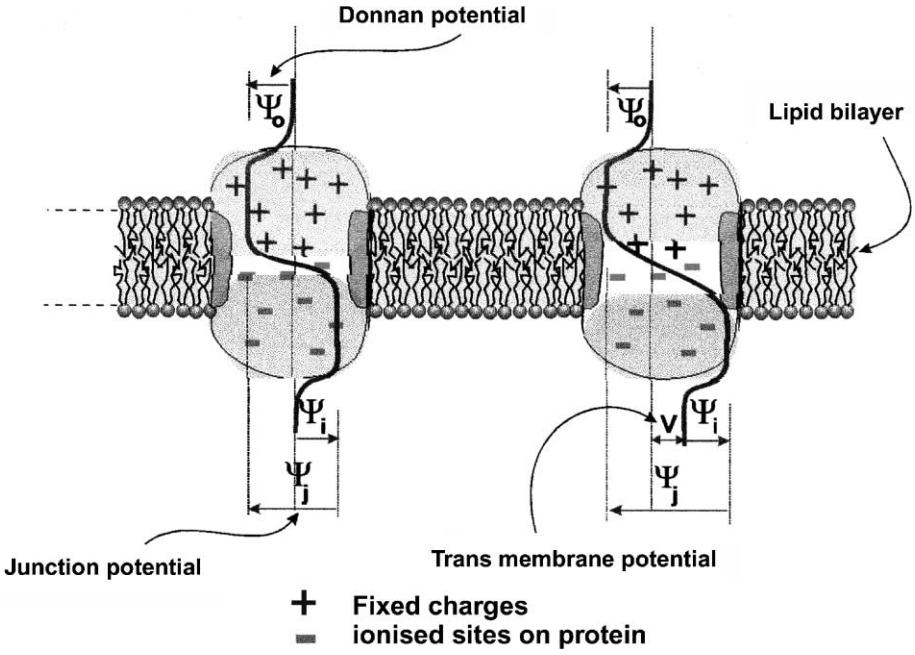

Fig. 6. Profiles of electric potential in double fixed charge modules (DFCM) in membranes. The fixed charges in each half of the lattice are opposite in sign and are neutralised by mobile counter ions that permeate the module. The co-ions in each lattice are depressed. At the junction of the two fixed charge regions, a layer forms, which is largely depleted of mobile ions and which will have a low conductance. A large junction potential will develop across this region as will an external potential when applied across the membrane. The additional potential will increase the width of the depletion layer for the orientation of the DFCM and potential shown in this diagram.

The conversion of ADP to ATP involves the following reaction:

$$\mathrm{ADP} + \mathrm{POH} + 2\mathrm{H}_0^+ \Leftrightarrow \mathrm{ATP} + \mathrm{H_2O} + 2\mathrm{H}_i^+$$

The equilibrium for this reaction is given by:

$$K_1 = \frac{[\mathrm{ATP}] \times [\mathrm{H_2O}] \times [\mathrm{H}_i^+]^2}{[\mathrm{ADP}] \times [\mathrm{POH}] \times [\mathrm{H}_0^+]^2}$$

The reaction is driven therefore by the relative chemical potentials for the various components in this reaction. Note that in this scheme, the protons on the external and internal sides of the membrane are treated as different species. The equilibrium concentrations of ATP and ADP will depend on the concentration of the water molecules. The water undergoes dissociation according to the scheme:

$$\mathrm{H_2O} \Leftrightarrow \mathrm{H}^+ + \mathrm{OH}^-$$

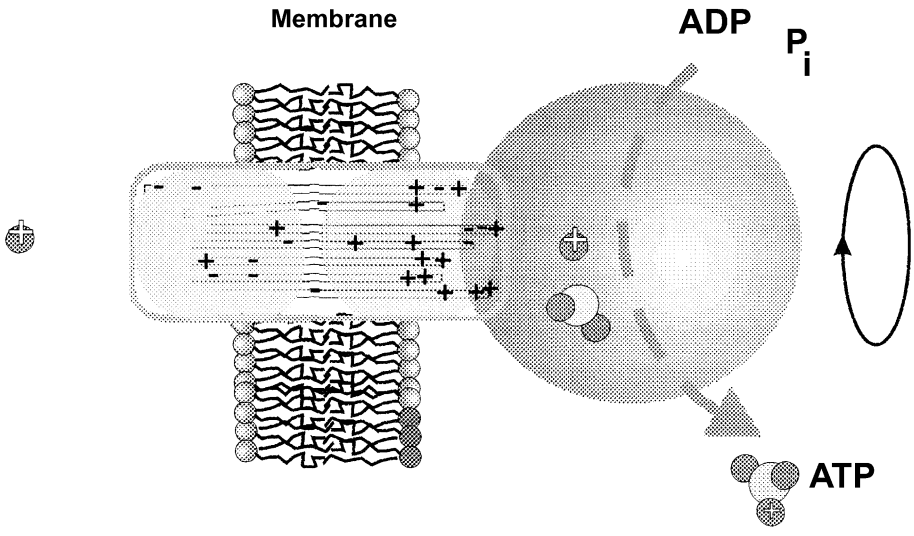

Fig. 7. A schematic drawing of the ATPase module. The F_o portion contains fixed charges that form a DFCM region. This will establish an intense electric field in the depletion layer in this region. Here, field-enhanced water dissociation might supply "dry" protons that drive the kinetics of the ATP synthesis.

with an equilibrium constant given by:

$$K = [H^+] \times [OH^-]$$

If we take the concentration of water molecules in aqueous phases as approximately constant ($\sim$55 molar), then for a neutral solution, $K=10^{-14}$ with $[H^+]=[OH^-]=10^{-7}$.

When the electric field is very intense, the equilibrium constant for the dissociation of water increases dramatically (Wien dissociation effect [14–16]). Indeed, greatly enhanced water dissociation has been observed in DFCM (bipolar) membranes when sufficiently large bias potentials were applied [17,18]. The current, then also rises very rapidly with increased bias and the current is then carried predominantly by protons and hydroxyl ions.

Water dissociation induced by intense electric fields in the F_o portion of the ATPase would have major consequences because it:

(i) generates larger numbers of protons for the reaction and
(ii) reduces the concentration of water molecules which will drive the synthesis reaction kinetics, which is in essence a dehydration reaction.

3. Conclusion

The presence of thin membranes in living cells, in conjunction with transmembrane potentials, create large internal electric fields that have an important role in membrane protein organisation and function. Furthermore, in membranes containing proteins with fixed charge regions, the fields create regions where additional field effects occur. These additional effects may be sufficiently intense to considerably alter the aqueous chemistry. It may be that biological evolution has exploited this to its advantage.

Acknowledgements

The authors gratefully acknowledge the support of the University of New South Wales and the Australian Research Grants Commission.

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