

UNASSISTED AND ASSISTED ION TRANSPORT ACROSS MEMBRANES: INSIGHTS FROM COMPUTER SIMULATIONS

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Transport of ions is an ubiquitous cellular processes. It provides the cell with the capability to acquire nutrients, synthesize high-energy compounds and transduce signals from the environment. Principles of biological transport are exploited in a variety of medical, pharmaceutical and environmental applications of biomembrane technologies. The highly promising liposome technologies, which have wide applications ranging from targeted drug delivery to bioremediation and biosensors, can serve as examples.

In order to explain membrane-related cellular processes and to realize the full practical potential of biomembranes it is necessary to understand the mechanism of transport across membranes and the means to control its efficiency and selectivity. Since X-ray crystallography and Nuclear Magnetic Resonance, have only limited applicability to membrane systems, molecular-level computer simulations have an important role to play in this effort. Most of these simulations have been performed using the molecular dynamics method. Here, we recount some of the progress achieved by such simulations.

Unassisted ion transport

Most ions permeate membranes very poorly. The permeability coefficients of monovalent ions, such as Na⁺, K⁺ and Cl⁻, are in the range of 10⁻¹⁵ and 10⁻¹⁰ cm sec⁻¹ [1], approximately 8-10 orders of magnitude smaller than the permeability coefficients of small, neutral solutes. However, this does not mean that unassisted ion transport is biologically or technologically irrelevant. Such uncontrolled transport is usually unproductive, and sometimes harmful to cells, but may be useful in artificial, membrane-bound structures, such as liposomes.

Qualitatively, the inability of ions to penetrate the non-polar interior of the membrane is easy to understand. In the aqueous environment, ions strongly polarize the surrounding water so that dipoles of water molecules tend to align themselves with the electric field created by the ion. As the ion moves into the membrane, strong, favorable water-ion interactions are broken at a considerable free energy expense. In the language of classical, continuum electrostatics, this process can be considered as the transfer of charge from a high to a low dielectric medium.

The simplicity of this qualitative picture suggests that calculating the permeability coefficients for ions should be a relatively straightforward task.

The key step is to calculate the free energy of transferring the ion from bulk water into the middle of the membrane (ΔA_{wm}). ΔA_{wm} can be obtained from a continuum model, in which the membrane is represented as a lamella of width w and the dielectric constant ϵ_m , placed between two infinitely wide lamellae of a medium characterized by the dielectric constant of water [2]. If w , ϵ_m and the ionic radius are assigned ΔA_{wm} can be calculated from the analytical formula. For Na^+ , this yields $\Delta A_{wm} = 47 \text{ kcal mol}^{-1}$, which corresponds to a permeability coefficient of $10^{-29} \text{ cm sec}^{-1}$. This value is approximately 16 orders of magnitude lower than the measured permeability coefficient [3]. This large discrepancy cannot be simply explained by small inaccuracies in the ionic radius or the dielectric description of the membrane. Clearly, something is missing in the simple continuum model.

To explain the observed discrepancy it is necessary to abandon the continuum model and, instead, appeal to a molecular-level description of ion permeation. This was done using molecular dynamics simulations, in which a sodium ion was transferred across a membrane formed by molecules of glycerol 1-monooleate (GMO) arranged in a bilayer [3]. The simulations reveal a picture of ion permeation that is quite different from the picture implied by the continuum model. As the ion moves into the bilayer, it leaves behind a deep, local defect in the membrane. Water molecules penetrate the defect to remain near the ion. Similarly, the polar headgroups of neighboring GMO molecules "cave in" to be in contact with the ion. When Na^+ crosses the mid-plane of the bilayer the defect on the incoming side of the membrane relaxes and is replaced by a defect on the outgoing side.

The simulations clearly point out to the shortcomings of the standard continuum model. First, the width of the membrane cannot be assumed as locally constant. In fact, even in pure membranes, the width fluctuates forming capillary waves, but this effect is markedly larger in the presence of the ion. Second, even in the middle of the bilayer, the ion remains fairly well solvated by both water molecules and lipid headgroups. As a result, ΔA_{wm} is considerably reduced to 27 kcal mol^{-1} . This, in turn, yields the permeability coefficient of $10^{-15} \text{ cm sec}^{-1}$, in good agreement with experiment.

The results of computer simulations allow for making several general predictions regarding ion permeation across membranes. In particular, they suggest that permeability coefficients can be affected by the membrane width differently than predicted by the continuum models. As the width of the membrane increases the formation of thinning defects and the accompanied water penetration is progressively more difficult and, at some width, becomes impossible. Above this width the permeability coefficient should increase sharply. On the other hand, if the width decreases the free energy cost of forming defects should be low and the membrane should become fairly permeable to ions. These predictions have been confirmed by measuring

permeabilities to ions of bilayers made of a series of phospholipids, which differ only in the length of the hydrocarbon chain [4].

Although the picture of ion permeation, described above, appears to be quite general, there is one clear exception. Protons permeate through membranes approximately five orders of magnitude faster than other monovalent ions [5], which suggests that they are not transferred as hydronium ions but, instead, by some other mechanism. It has been proposed that this mechanism involves translocation of excess proton through a transient chain of properly aligned water molecules spanning the membrane [6]. This mechanism, which is available only to protons, is more efficient because it requires only small displacements of several protons between consecutive water molecules in the chain rather than a large translocation of a single ion.

Assisted ion transport

As we have mentioned, transport of ions across membranes requires assistance. Without assistance only a few sodium ions per day would permeate a typical liposome. This assistance is provided by either carrier molecules or transmembrane protein channels and pumps. In most instances, channel-forming proteins aggregate spontaneously to form a pore in the membrane surrounded by a bundle of α -helices or β -strands. Occasionally, a transmembrane protein has an intrinsic architecture that includes a pore. Gramicidin A is one such example. Although transmembrane proteins could be quite complex, the channel forming parts are usually remarkably simple. Moreover, they often retain their transport properties even if the rest of the protein is removed. In this respect, membrane proteins differ from water-soluble proteins, which are usually not amenable to similar simplifications.

Even though membrane proteins exhibit little structural variety around the pore, they can still achieve specificities that match or exceed those of much more diverse water-soluble proteins. How it can be done only through subtle manipulations of the amino acid sequence along the pore is the subject of this section. We selected gramicidin A, the M_2 protein from the human Influenza virus, aquaporins and the potassium channel as relevant examples. Symmetrically arranged dimers of gramicidin A form a very simple channel lined only by peptide backbone atoms. The channel transports water across the membrane with almost no free energy barrier. Computer simulations indicate that water molecules in the channel form a linear, hydrogen-bonded chain [7]. This may explain why the channel is capable of efficient proton transport [8]. The channel is also permeated by alkali metal ions such as Na^+ and K^+ .

The M_2 channel is an aggregate of four identical protein fragments, each folded into a helix. In response to an applied voltage, protons are conducted through space in the middle of the channel at remarkably high rates [9]. As in gramicidin A, it has been postulated that protons travel along the network of water molecules filling this space. However, in contrast to gramicidin, which is

not selective, the M_2 channel transports alkali ions rather poorly. This selectivity is achieved by forming a gate made of four histidine residues, one from each of the helices, which are sufficiently large to occlude the pore. Since the gate not only blocks transport of atomic ions but also interrupts the water network a question arises: how do protons get across? It has been proposed that this can be accomplished without opening the gate by capturing a proton on one side of a histidine residue and releasing another proton from the opposite side. Then, the channel returns to the initial state through tautomerization of the histidine [9]. Alternatively, histidines forming the gate can acquire additional protons and, due to repulsion between their positive charges, move away from one another, thus opening the gate just enough to facilitate penetration by water but not by monovalent ions. Molecular dynamics simulations of the channel with the histidine residues in different protonation states [10] reveal that all intermediate states of the tautomerization mechanism are structurally stable and the arrangement of water molecules in the channel is conducive to the proton transport. These results indicate that the tautomerization mechanism is possible. In contrast, if all histidines are protonated the channel loses its structural integrity, and one helix moves away from the remaining three. This indicates that the fully protonated state of the gate cannot be involved in proton transport. However, a possibility that the singly or doubly protonated gate can open sufficiently to allow for water penetration cannot be excluded.

While it might be inferred that increased rates of water transport must be associated with increased permeation of protons, this is not the case. Physiological proteins, called aquaporins, efficiently transport water but not protons. This is very fortunate because otherwise they could dissipate proton gradients across cell walls thus disrupting synthesis of ATP. Several structural features of aquaporins may be involved in ensuring their selectivity. In particular, it has been proposed that a chain of water molecules in the channel is disrupted by strong interactions between the conserved arginines lining the pore and a nearby water molecule [11]. Alkali ions are incapable of translocating through aquaporins at least in part because of a constriction of the pore diameter to about 3 Å over a span of one residue.

The KcsA K^+ channel provides another example of a four-helix bundle that achieves high efficiency and selectivity of ion transport across membranes. The recent determination of its three-dimensional structure [12] enabled electrostatic and molecular dynamics simulations of ion transport through this channel [13]. These simulations reveal that both the orientations of helices and the water filling the channel contribute to its selectivity towards positive, monovalent ions, excluding both divalent and negative ions. A short region in the channel located near the extracellular side acts as a selectivity filter for K^+ .

These examples show how computer simulations and modeling can contribute to our understanding of the mechanisms of action and selectivity of ion channels. In the future, this knowledge can be used to design novel channels

with desired properties. However, there are still several challenges that have to be met. In particular, both short and long ranged electrostatic interactions between ions and the rest of the system must be accurately accounted for and the lengths of simulations must be substantially increased. Also, more high-resolution structures of ion channels are needed. These issues are subjects of vigorous studies and considerable progress can be expected in the near future.

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