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Intrinsic Potential of Cell Membranes: Opposite Effects of Lipid Transmembrane Asymmetry and Asymmetric Salt Ion Distribution

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Using atomic-scale molecular dynamics simulations, we consider the intrinsic cell membrane potential that is found to originate from a subtle interplay between lipid transmembrane asymmetry and the asymmetric distribution of monovalent salt ions on the two sides of the cell membrane. It turns out that both the asymmetric distribution of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) lipids across a membrane and the asymmetric distribution of NaCl and KCl induce nonzero drops in the transmembrane potential. However, these potential drops are opposite in sign. As the PC leaflet faces a NaCl saline solution and the PE leaflet is exposed to KCl, the outcome is that the effects of asymmetric lipid and salt ion distributions essentially cancel one another almost completely. Overall, our study highlights the complex nature of the intrinsic potential of cell membranes under physiological conditions.

I. Introduction

The electrostatic properties of plasma membranes are crucial for numerous membrane-mediated biological phenomena such as the activation of voltage-gated membrane proteins, conductance of ionic channels, binding of therapeutic solutes to membranes, and trafficking across cell membranes.[1,2] It is well-established that plasma membranes of most living cells are characterized by a nonzero potential drop over the membrane, but the origin of this potential is not well understood. Although, in general, it is believed that the potential drop arises from a slight charge imbalance of salt ions across the plasma membrane,[3] there are strong experimental indications that such a picture is oversimplified.

This view becomes evident when considering biological membranes, which are multicomponent structures in a complex physiological environment. The membranes of living cells are known to be asymmetric with respect to the lipid compositions in the two opposite leaflets.[4,5] This transmembrane lipid asymmetry is vital for a variety of membrane properties and functions such as their mechanical stability[6] and programmed cell death.[7] Several experimental studies have demonstrated that the asymmetric distribution of lipid molecules across the membrane is able to give rise to a nonzero potential drop between the two membrane leaflets,[8−10] thereby contributing to the membrane potential. Recent atomic-scale computational studies of asymmetric lipid membranes have confirmed this observation.[11,12]

What about the effects of ions? Under physiological conditions, plasma membranes are always surrounded by a saline solution. Salt ions are known to interact with lipids, thereby affecting the structural, dynamic, and electrostatic properties of cell membranes.[13−15] What is more, the concentrations of salt ions (e.g., sodium and potassium ions) differ considerably for intracellular and extracellular fluids, giving rise to a pronounced asymmetry in ionic composition of electrolyte solutions facing the two sides of a plasma membrane.[3] It seems apparent that, if the ion concentrations on the two sides of a membrane are distinctly different, then the concentration gradient likely gives rise to a nonzero membrane potential. However, it turns out that an asymmetric ion distribution across a membrane is able to induce a nonzero transmembrane potential difference even in the absence of ion charge imbalance across the membrane.[16,17]

Thus, the intrinsic potential of cell membranes is complex in nature. In this article, we consider the possibility that the intrinsic membrane potential results from a subtle interplay between the transmembrane lipid asymmetry and the asymmetry in ionic composition of saline solutions on a membrane's intracellular and extracellular sides. To consider whether this view holds, we put forward an atomic-scale model of a lipid membrane that accounts for both types of asymmetries. Because phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the main representatives of zwitterionic lipids in the outer and inner leaflets of eukaryotic plasma membranes, respectively, we consider a model in which one of the two membrane leaflets is composed of PC lipids and is in contact with an aqueous solution with NaCl salt. The other monolayer is built from PE lipids and is hydrated by a saline solution with KCl salt; see Figure 1 (top).

Extensive atomic-scale molecular dynamics simulations of such a PC/PE membrane in saline solution show that the effect of monovalent salt ions is opposite to the effect of transmembrane lipid asymmetry: whereas one of these factors increases the membrane potential, the other aims to decrease it. Overall, we find for the present model that asymmetrically distributed ions profoundly reduce (or even cancel) a nonzero

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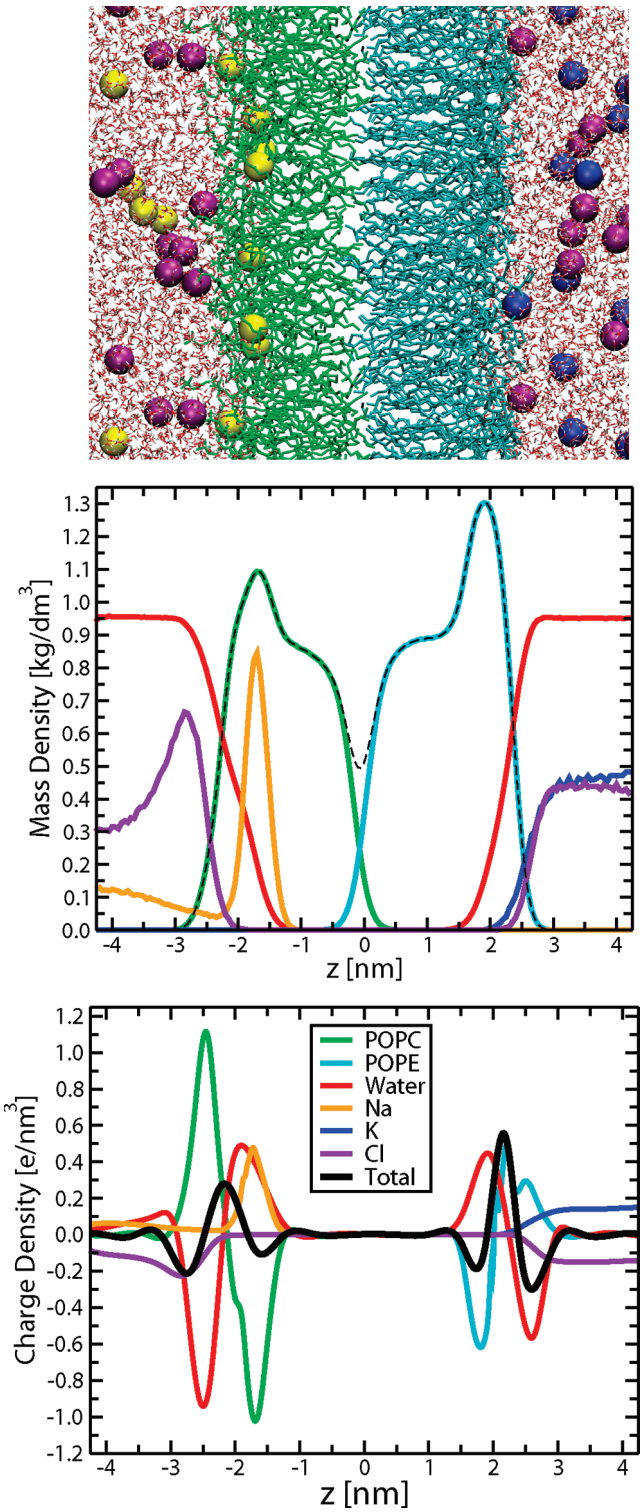

Figure 1. (Top) Snapshot of a POPC/POPE membrane with asymmetric distribution of salt ions. POPC lipids are shown in green, POPE lipids in cyan, water in red, sodium ions in yellow, potassium ions in blue, and chloride ions in purple. (Middle) Component-wise mass density profiles of a POPC/POPE membrane that separates water compartments with NaCl and KCl salts (profiles for ions were scaled by a factor of 50). Shown are the averages over two bilayers. (Bottom) Corresponding charge density profiles of the system.

transmembrane potential difference that is induced by an asymmetric distribution of lipid molecules across the membrane.

II. Materials and Methods

We performed atomic-scale molecular dynamics (MD) simulations of an asymmetric lipid membrane composed of zwitterionic palmitoyl oleoyl phosphatidylcholine (POPC) and palmitoyl oleoyl phosphatidylethanolamine (POPE) single-component monolayers. The initial structure of a PC/PE asymmetric membrane was taken from ref 11 and consisted of 51 POPC and 64 POPE lipids. The number of lipids in the two leaflets of a PC/PE membrane was adjusted such that the average area per lipid in each leaflet closely reproduced the average area per lipid in corresponding single-component bilayers.[11] For the purposes of this study, we employed a so-called double bilayer setup;[16−22] that is, we used a simulation box that contained two PC/PE membranes arranged in an antiparallel (PC/PE to PE/PC) fashion. Such a setup allows for independent control of the electrolyte compositions of the aqueous compartments on the two sides of a bilayer. Furthermore, it nullifies an overall dipole moment of a simulation box; we recall that an asymmetric PC/PE membrane is characterized by a nonzero transmembrane potential difference.[11]

Two PC/PE membranes were solvated with ∼10200 water molecules, amounting to ∼42500 atoms for the entire system. Sodium, potassium, and chloride ions were added to the double bilayer system in such a way that KCl was in direct contact with POPE leaflets and NaCl was in direct contact with POPC leaflets, thereby reproducing a situation that is typical for cell membranes [Figure 1 (top)]. The concentration of both KCl and NaCl was ∼0.2 M, close to the physiological values.

POPC and POPE lipids were described by the united-atom force field of Berger et al.[23] Water was modeled using the simple point charge (SPC) model.[24] For sodium, potassium, and chloride ions we employed the set of parameters supplied within the GROMACS force field.[25] The Lennard-Jones interactions were cut off at 1 nm. The electrostatic interactions were handled using the particle-mesh Ewald method (PME).[26] The simulations were performed in the *NpT* ensemble at the physiological temperature (T = 310 K) and at a pressure of 1 bar; the Berendsen scheme was used for controlling both temperature and pressure.[27] Periodic boundary conditions were applied in all three dimensions. The time step used was 2 fs. The simulations of an asymmetric PC/PE membrane with an asymmetric composition of saline solution were performed over a period of 100 ns. As a reference, a 100-ns MD run was performed for the same double bilayer system without salt. The GROMACS suite was used in all simulations.[25]

III. Results and Discussion

The asymmetry of the PC/PE membrane in NaCl/KCl saline solution is essentially two-fold and is clearly realized through the component-wise density profiles presented in Figure 1 (middle). First, there is an asymmetric distribution of lipids, and second, there is an asymmetric distribution of ions. Both asymmetries are particularly pronounced at the lipid/water interface. The results and the discussion below show that they both contribute significantly to the membrane potential.

First consider the distribution of lipids and its effects. We found that the lipid density profiles of the opposite monolayers are highly different. This is directly related to the different natures of PC and PE headgroups: POPE lipids are capable of forming both intra- and interlipid hydrogen bonds, whereas POPC lipids are not.[28] As a result, the POPE/water interface is narrower and more densely packed than its POPC counterpart; see Figure 1 (middle). Furthermore, as water permeates much more easily into the POPC leaflet, the hydration levels and

average orientations of the headgroup dipoles on the two sides of a membrane differ significantly.

As for the ion distributions and their effects, Figure 1 (middle) indicates that there is a pronounced difference in how monovalent ions interact with PC and PE leaflets. The POPC monolayer, being a model of the outer part of plasma membrane, is in direct contact with a water bath containing NaCl. Figure 1 (middle) shows that sodium ions interact strongly with POPC lipids: they permeate deep into the POPC leaflet and become bound to the PC carbonyl region. This finding is in full agreement with the results of several previous computational studies.[13,16,29,30] As a result of the binding of sodium, the PC/water interface becomes positively charged, promoting the confinement of Cl anions in the water phase near the lipid/water interface; this is demonstrated by the peak in the Cl ion density profile in Figure 1 (middle). Furthermore, one can observe a more vertical reorientation of headgroup dipoles of POPC as compared to a salt-free system. (The average angle between the PN vector and the outward bilayer normal decreases from 78° to 71°.) The spatial separation of Na and Cl ions induced by the POPC leaflet, coupled with PC headgroup reorientation, generate an additional dipole moment that increases the overall electrostatic potential across the POPC leaflet of the membrane (see below). This is in contrast to the POPE side of the membrane, which is adjacent to the aqueous solution with KCl: the PE/water interface, being stitched by inter-PE hydrogen bonds, is only weakly affected by potassium and chloride ions.[30] The headgroup orientation does not change compared to a salt-free system (as the corresponding angle between the PN vector and the bilayer normal is around 92° in both cases). Further, K and Cl ions do not become separated in the proximity of the PE surface of the membrane. Therefore, one can expect that KCl does not significantly affect the electrostatic properties of the POPE leaflet.[30]

All of the features of PC and PE leaflets seen through the mass density profiles are translated into the partial charge densities, which are crucial for the electrostatic properties of a membrane [see Figure 1 (bottom)]. To compensate for the positive charges of sodium ions bound to the PC/water interface and the negative charges of the confined chloride ions near the PC leaflet, POPC headgroups become considerably more vertically oriented compared to their POPE counterparts. POPC also exhibits a much higher lipid charge density peak (PC choline groups vs NH_3 groups of PE lipids) and a much deeper drop in the charge density corresponding to phosphate groups [Figure 1 (bottom)]. This implies that POPC lipids have a larger dipole moment across the membrane than POPE lipids. Furthermore, water molecules are more ordered on the POPC side of the membrane as a result of related ordering of Cl ions.

With charge densities at hand, one can employ the Poisson equation to compute the electrostatic potential across the membrane by twice integrating over the charge densities. In Figure 2 (top), we plot the total electrostatic potential for PC/PE membranes in NaCl/KCl and salt-free aqueous solutions. For the system without salt, the potential difference between the two lipid/water interfaces is found to be around 108 mV, which is in excellent agreement with the results of our previous study.[11] Note that this potential drop is negative on the POPE side, in agreement with the conditions in plasma membranes.

The central result of the present study regards the change in the total electrostatic potential due to the ions. We find that the asymmetrically distributed monovalent salt ions reduce the above potential of 108 to ~14 mV, i.e., the asymmetric ion

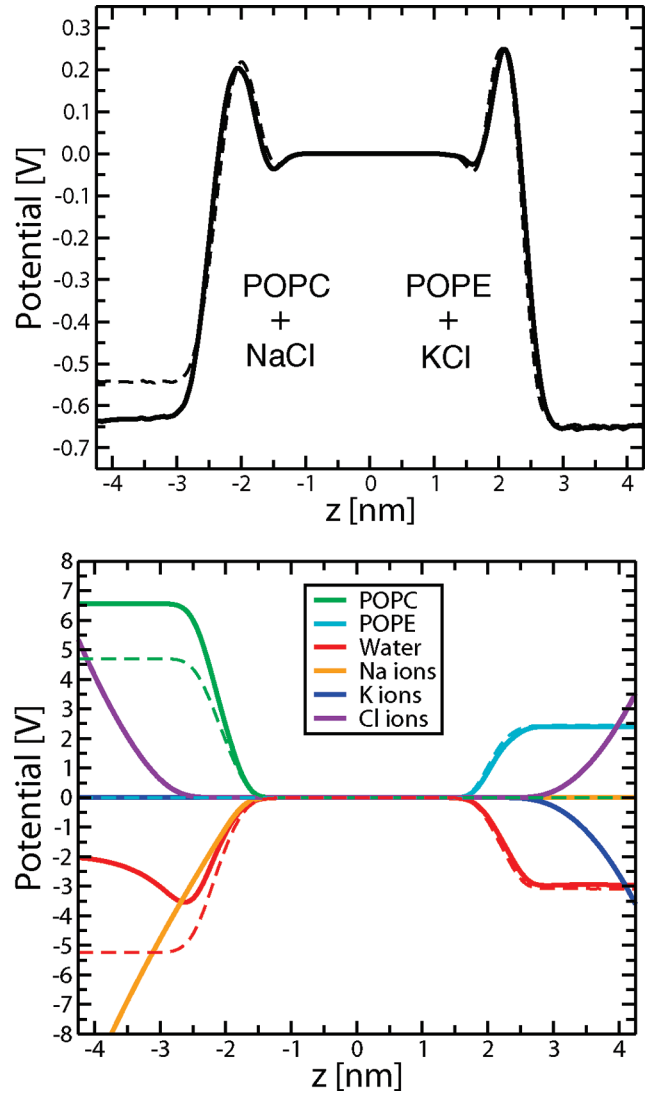

Figure 2. (Top) Electrostatic potential vs distance z from the center of mass (CM) of a POPC/POPE membrane. The potential is chosen to be zero at the CM of the membrane and averaged over two bilayers. The corresponding potential for a salt-free system is shown by a dashed line. (Bottom) Component-wise contributions to the electrostatic potential for POPC/POPE membranes in NaCl−KCl saline solution (solid lines) and in water without salt (dashed lines).

distribution practically cancels the nonzero potential drop across the asymmetric PC/PE membrane; see Figure 2 (top).

A detailed consideration of the component-wise contributions to the total electrostatic potential reveals the origin of this phenomenon [Figure 2 (bottom)]. Starting from the POPE monolayer, it is clear that KCl does not alter the potential on this side of the membrane. Meanwhile, on the POPC side, the effect of NaCl is two-fold. First, NaCl induces PC headgroups to become more vertically oriented, so that their (positive) contribution to the total electrostatic potential increases. This effect is enhanced by the salt-induced decrease in the (negative) contribution of water molecules; see Figure 2 (bottom). Second, sodium ions, being separated from chloride ions by ~1.5 nm [see Figure 1 (middle)] bring about a significant (negative) uncompensated contribution to the electrostatic potential. It turns out that the contribution coming from Na ions is somewhat larger than that arising from PC headgroup reorientation; it gives rise to an additional (negative) increment in the potential across the POPC leaflet, compensating the potential difference between the opposite sides of a PC/PE membrane.

The potential difference due to the asymmetric distribution of sodium and potassium ions, being positive on the POPE leaflet with respect to the POPC leaflet, is found to be around 108 − 14 = 94 mV. It is instructive to compare this value to previously reported results. It was first demonstrated by Gurtovenko[16] that one can observe a transmembrane potential of ~85 mV when a symmetric phosphatidylcholine membrane separates salt-free and saline (NaCl) water baths. This result was later confirmed in a computational study by Kandasamy and Larson,[21] where a potential of 90 mV was observed in a similar setup. Recently, Baker et al.[17] reported a transmembrane potential drop of 70 mV when a symmetric PC membrane was placed between two water compartments, one with NaCl and the other with KCl salt. This result is in line with refs 16 and 21: The effect of KCl salt on a PC membrane is highlighted by a slight increase in the electrostatic potential across a monolayer as compared to a salt-free solution. In particular, for the force field employed for ions in ref 17, it was shown[30] that the potassium-induced increase in membrane potential is around 12 mV. Therefore, substitution of a salt-free water bath considered in ref 16 with KCl saline solution should lead to a transmembrane potential drop of 85 − 12 = 73 mV, which almost coincides with the result of Baker et al.[17] In our case, KCl salt is in contact with POPE lipids, which are known to be quite insensitive to potassium ions.[30] Therefore, one can expect the potential difference to be around 85−90 mV, which is indeed the case. Thus, the value of the salt-induced potential difference reported in this article turns out to be very close to those observed in previous studies where symmetric membranes were considered, so that the potential drop due to the asymmetric salt distribution most likely does not correlate with the lipid composition of the membranes.

It is also very instructive to discuss the sensitivity of the results presented here to the force field employed for the salt ions. For the purposes of this study, we used ion force-field parameters developed by Straatsma and Berendsen.[31] These parameters are consistent with the SPC water model employed and incorporated in the Gromacs suite.[25] As an alternative, one can consider Charmm ion parameters,[32] especially for K ions, as it is generally believed that the Gromacs force field[31] exaggerates the size of a potassium ion. In a very recent computational study,[30] the above two sets of ion parameters were systematically compared, with emphasis on the effect of ions on lipid membranes. It was found that the effects of Na ions on PC bilayers are almost indistinguishable for the two force fields. Regarding the effect of K ions, it was shown that potassium ions do not affect PE bilayers regardless of which force field is used.[30] Therefore, we do not expect that the main findings of this work will be sensitive to the force field employed for ions.

Finally, we note that native plasma membranes of eukaryotic cells are considerably more complex than those discussed in this article. Among the main lipid components not included in our present work are anionic lipids such as phosphatidylserine (PS). These lipids are localized mostly in the inner membrane leaflet and definitely affect the electrostatic properties of plasma membranes. It has been recently demonstrated[12] that an asymmetric distribution of anionic lipids can lead to an additional nonzero potential drop of the same sign as that arising from the transmembrane lipid asymmetry considered here. To clarify the role of ion-induced and lipid-induced asymmetries in membrane potential in models of plasma membranes that are one step closer to native membranes, we are currently exploring membranes with such asymmetry with regard to phosphatidylserines.

IV. Summary

To summarize, in this article, we have presented the first computational study of a model lipid membrane that is characterized by lipid transmembrane asymmetry and an asymmetric distribution of monovalent salt ions on the two sides of a membrane. Both asymmetries are inherent features of biological membranes of most animal cells under physiological conditions. Through atom-scale molecular dynamics simulations, we have provided a detailed picture of how the above asymmetries can contribute to the intrinsic potential of cell membranes. It turns out that the effect of monovalent salt is opposite to the effect of transmembrane lipid asymmetry: the nonzero intrinsic potential of an asymmetric PC/PE membrane is found to be strongly reduced (and practically canceled) by putting its PC and PE leaflets into contact with NaCl and KCl saline solutions, respectively.

It will be particularly interesting to extend this work to more realistic membrane models whose lipid composition matches that of cellular membranes. Such studies will provide a great deal of insight to better understand the dependence of membrane potential on lipid composition that varies along the membrane plane from one membrane domain type to another. Coupling of different membrane domains with the associated membrane potential will also facilitate understanding the potential that proteins partitioned in a given domain are likely to feel. This is especially interesting, for example, in the case of voltage-gated channels, which play an important role in a number of cellular functions.[33]

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