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Lipid Transmembrane Asymmetry and Intrinsic Membrane Potential: Two Sides of the Same Coin

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The intrinsic membrane potential affects or even governs a variety of biological phenomena associated with cell membranes, such as the binding of charged proteins and drugs to membranes, insertion and orientation of integral membrane proteins, conductance of channels, and membrane transport processes overall.[1] Nonetheless, despite its major biological relevance, the origin of this potential is not well understood. It is generally accepted that the potential difference arises from the salt ion imbalance across the plasma membrane as the concentrations of, for example, proton, sodium, and potassium ions on the extracellular and intracellular sides of the cell are considerably different.[2] Recent atomic-scale computer simulations have provided support for this point of view.[3−5]

However, in this work, we show that this intriguing issue may be considerably more complex: we show that a nonzero transmembrane potential can readily be observed in phospholipid membranes in the *absence* of ions. More specifically, we provide compelling evidence that a *nonzero* electrostatic potential between two membrane surfaces is an intrinsic property of lipid membranes that are characterized by an *asymmetric lipid distribution across the membrane*. This is shown here to give rise to an electrostatic transmembrane potential difference of about 100 mV, which is of the same order of magnitude as the potential difference (about 50−100 mV) in native plasma membranes.[2,6]

The asymmetry in transmembrane distribution of lipids is an inherent feature of membranes of most animal cells.[7] It plays an important role in various membrane properties and phenomena such as membrane mechanical stability[8] and programmed cell death.[9] It is well-known that, in plasma membranes of eukaryotic cells, cholinephospholipids such as phosphatidylcholine (PC) and sphingomyelin are located mostly in the extracellular leaflet, whereas aminophospholipids such as phosphatidylethanolamine (PE) and phosphatidylserine are predominant lipids in the intracellular leaflet.[10]

In this communication, we use this insight to consider an asymmetric lipid membrane through atomic-scale molecular dynamics simulations. The asymmetric lipid membrane is comprised of palmitoyl-oleoyl-PC (POPC) and palmitoyl-oleoyl-PE (POPE) single-component monolayers, which therefore mimic the outer and inner leaflets of plasma membranes, respectively (see Figure 1).

The simulations over a period of 100 ns were performed at the physiological temperature ($T = 310$ K) using a united-atom forcefield of Berger et al.[11] The periodic boundary conditions were applied in all three dimensions. Additional studies were conducted with the use of the slab geometry,[12] which were found to yield results consistent with those reported here.

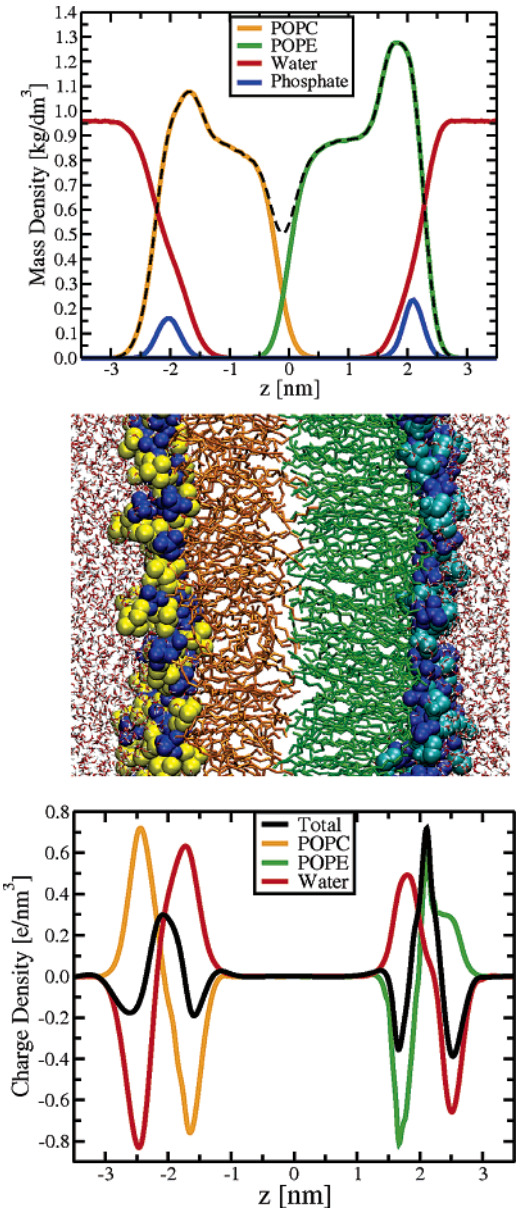

Figure 1. (Top) Mass density profiles of an asymmetric POPC/POPE membrane. (Middle) A snapshot of a POPC/POPE membrane with phosphate groups (blue), choline groups (yellow), amine groups (cyan), POPC acyl chains (orange), POPE chains (green), and water (red). (Bottom) Charge density profiles of a POPC/POPE membrane.

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The asymmetric membrane was formed by adjoining POPC and POPE monolayers extracted from corresponding 100 ns long simulations of conventional single-component POPC and POPE bilayers. Since lipids in a POPE bilayer are more densely packed than in a POPC bilayer, the number of lipids in the two leaflets of an asymmetric membrane was adjusted such that the average area per lipid in each leaflet reproduced closely the average area per lipid in corresponding single-component bilayers. This led us to an asymmetric membrane with 51 lipids in the POPC leaflet and 64 lipids in the POPE leaflet. All of the simulations were performed using the GROMACS suite.[13]

The major difference between POPC and POPE lipids lies in the nature of their head groups. In contrast to POPC, POPE has a primary amine that makes it capable for the formation of intra- and intermolecular hydrogen bonds to lipid phosphate groups.[14] As a result, the water−lipid interface on the POPE side of an asymmetric membrane is more densely packed and is considerably narrower than on the POPC side, as seen from the peak heights of lipid density profiles shown in Figure 1 (top). Water permeates into the membrane on the POPC side to a significantly larger extent compared to the POPE side (see Figure 1 (top)), reflecting the well-known difference in hydration level of PC and PE bilayers.[14]

Strong hydrogen bonding between PE head groups renders the average orientation of PN vectors (from phosphorus to nitrogen in the head group) on the two sides of the membrane different: the average angle between the PN vector and the outward bilayer normal is found to be 78 and 91° for POPC and POPE leaflets, respectively. This, in particular, means that there is a pronounced difference in lipids' dipole moment per unit area in the two membrane leaflets.

The partial charge densities of an asymmetric membrane, being crucial for its electrostatic properties, also differ considerably in the two leaflets. On the POPC side water molecules readily permeate into the membrane−water interface and reorient themselves to partly compensate for charges of phosphate and choline lipid groups, leading to a smooth profile for the total charge density with a rather low peak (see Figure 1 (bottom)). In contrast, the permeation of water into the POPE leaflet is considerably hindered by the densely packed PE head groups, so that water molecules fail to compensate for the charges of lipid head groups to a significant extent. This leads to a sharp high peak in the total charge density profile in the POPE leaflet and, correspondingly, to a pronounced asymmetry in charge distribution across the POPC/POPE membrane (see Figure 1 (bottom)).

The asymmetry in charge density distribution is translated into the electrostatic potential across the membrane, computed from the Poisson equation by twice integrating over charge densities: the resulting electrostatic potential of the POPC/POPE membrane turns out to be asymmetric with respect to membrane center; the value of the potential difference between the two surfaces of the membrane is found to be *nonzero* and equal to 104 ± 25 mV (see Figure 2). Importantly, this potential is found in the *absence* of salt ions and is therefore not related to the transmembrane potential which arises from transmembrane concentration differences of ionic substances: the observed potential originates here from a difference in the dipole moments of the two leaflets of the asymmetric zwitterionic POPC/POPE membrane. These findings are in good agreement with reported experimental data on dipole potential measurements in asymmetric membranes formed from bacterial PE and 1,3 Diolein, two lipids with different polar (neutral) head groups.[15]

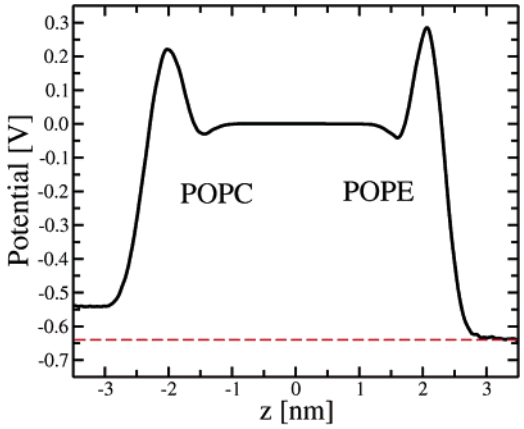

Figure 2. Electrostatic potential versus distance *z* from the center of mass (CM) of a POPC/POPE membrane. The potential is here chosen to be zero at the CM of the membrane.

If we consider the POPC monolayer of the asymmetric membrane as mimicking the outer leaflet of a cell membrane, we find the intrinsic potential of the membrane in the inside of the cell to be negative with respect to the outside. Thus, the observed intrinsic potential due to lipid asymmetry has the same sign as the transmembrane potential in plasma membranes.[2] Furthermore, the magnitude of the intrinsic potential turns out to be of the same order of magnitude as the potential in plasma membranes.[2,6] Overall, despite the simplified nature of the membrane model considered, it is however possible or even likely that transmembrane lipid asymmetry typical of living cells can contribute to some extent to the potential of cell membranes.

To summarize, in this communication, we have provided an atomic-scale picture of how an asymmetric distribution of zwitterionic lipids between the two membrane leaflets can give rise to a *nonzero* intrinsic potential difference between the two sides of the lipid membrane. Our findings strongly support the idea that the transmembrane lipid asymmetry and the intrinsic membrane potential are coupled.[15] From now on, it would be of major interest to understand how the intrinsic potential due to lipid asymmetry is possibly related to the various asymmetry-associated cellular processes.[9]

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