HTML ABSTRACT + LINKS

Asymmetry of lipid bilayers induced by monovalent salt: Atomistic molecular-dynamics study

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Interactions between salt ions and lipid components of biological membranes are essential for the structure, stability, and functions of the membranes. The specific ionic composition of aqueous buffers inside and outside of the cell is known to differ considerably. To model such a situation we perform atomistic molecular-dynamics (MD) simulations of a single-component phosphatidylcholine lipid bilayer which separates two aqueous reservoirs with and without NaCl salt. To implement the difference in electrolyte composition near two membrane sides, a double bilayer setup (i.e., two bilayers in a simulation box) is employed. It turns out that monovalent salt, being in contact with one leaflet only, induces a pronounced *asymmetry* in the structural, electrostatic, and dynamical properties of bilayer leaflets after 50 ns of MD simulations. Binding of sodium ions to the carbonyl region of the leaflet which is in contact with salt results in the formation of "Na-lipids" complexes and, correspondingly, reduces mobility of lipids of this leaflet. In turn, attractive interactions of chloride ions (mainly located in the aqueous phase close to the water-lipid interface) with choline lipid groups lead to a substantial (more vertical) reorientation of postphatidylcholine headgroups of the leaflet adjoined to salt. The difference in headgroup orientation on two sides of a bilayer, being coupled with salt-induced reorientation of water dipoles, leads to a notable asymmetry in the charge-density profiles and electrostatic potentials of bilayer constitutes of the two leaflets. Although the overall charge density of the bilayer is found to be almost insensitive to the presence of salt, a slight asymmetry in the charge distribution between the two bilayer leaflets results in a *nonzero* potential difference of about 85 mV between the two water phases. Thus, a transmembrane potential of the order of the membrane potential in a cell can arise *without* ionic charge imbalance between two aqueous compartments. © *2005 American Institute of Physics*. [DOI: 10.1063/1.1942489]

I. INTRODUCTION

Biological membranes represent complex, self-assembled structures which consist mainly of lipids and proteins. Membrane lipids, being organized into a bilayer, serve as a basic matrix for other constitutes of biomembranes.[1] The lipids in biomembranes are very diverse and can differ in headgroups and in acyl chain length and saturation. The most abundant lipid components in biomembranes are zwitterionic phospholipids; some charged (normally anionic) lipids are also present. Furthermore, biomembranes are known to be asymmetric in lipid composition of their leaflets.[1]

To get information about the structural properties and functions of lipid components of such complex structures as biomembranes, one often studies simplified, model lipid bilayers. In the simplest case, the lipid bilayers are formed by a single lipid component; however, studying lipid mixtures is also feasible. Along with a wide range of experimental methods, molecular-dynamics (MD) computer simulations become nowadays a standard tool for studying biomolecular systems. As far as lipid membranes are concerned, the MD simulations provide a deep insight into the atomistic details of their structure and dynamics and in many cases are able to complement experimental data. By far most MD computer simulations of bilayer systems have dealt with phosphatidylcholine (PC) lipids.[2–5] Considerably less attention has been paid to other phospholipid major components of living cells such as phosphatidylethanolamine[6–8] (PE) and sphingomyelin (SM).[9–12] All these lipids are zwitterionic (neutral). Recently MD studies of charged membranes (built from anionic[13–15] as well as from cationic lipids[16]) have also been performed. Furthermore, simulating many-component mixed lipid bilayers[16–21] seems to become one of the general directions for future MD studies.

Since physiological conditions always imply salt in aqueous solution, the problem of interactions of lipid bilayers with salt ions is of fundamental interest. Experimental studies show that salt can affect the structural and electrostatic properties of bilayers built from zwitterionic phospholipids, this influence is sensitive to a particular type of ions and their valency.[22–27] Also, salt ions can have impact on membrane fusion[28] and on transport across membranes.

Only very recently first MD computer simulations of PC lipid bilayers in aqueous solution with salt have been carried

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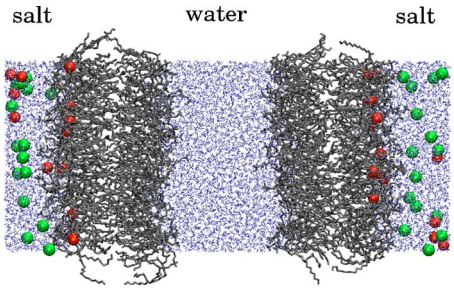

FIG. 1. (Color online). Snapshot of the simulation system: two lipid bilayers, the salt-free water reservoir between the bilayers, and the "outer" water reservoir with NaCl salt (salt ions are shown by van der Waals (vdW) spheres). Shown is the final structure after 50 ns of MD simulations.

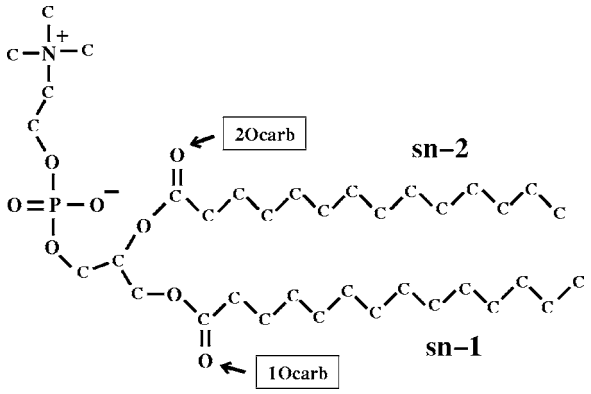

FIG. 2. Chemical structure of a zwitterionic dimyristoylphosphatidylcholine (DMPC) lipid considered in this work.

out.[29,30] The major problem here is that binding of salt ions to a zwitterionic lipid membrane is a very slow process, it requires very long simulation times until the equilibrium state is reached. For instance, the equilibration time needed for a PC lipid bilayer with monovalent salt is found to be about 20 ns (Ref. 29) (and more than 90 ns for divalent salt[30]). It was shown that salt alters both the structure and the dynamics of PC lipid bilayers: It decreases the area of a bilayer, enhances the ordering of acyl chains, and slows down the lipid self-diffusion.[29,30]

In this work we focus on another aspect of interactions between lipid membranes and salt. It is related to the very well-known function of biomembranes to serve as a barrier between the cell interior and extracellular fluid. The composition of electrolyte solution inside and outside of the cell is quite different, and, therefore, the influence of salt on the outer and inner leaflets of biological membranes can differ considerably. We also recall that the composition of biological membranes themselves is asymmetric with respect to their inner and outer leaflets, and this makes the situation very complicated.

As a first step towards an understanding of how the asymmetry in composition of electrolyte buffers near two leaflets of lipid membranes affects their structural and dynamical properties, one can start with studying one-component lipid bilayers which separate compartments with different salt concentrations. Experimentally, such a situation can be realized for planar bilayer membranes which allow adjustment of electrolyte conditions on each side of the bilayer.[1]

To address this problem, we employ atomistic MD simulations of a phosphatidylcholine lipid bilayer in the liquid-crystalline phase, two leaflets of the bilayer being kept under different salt concentrations. To simplify the situation even more, we assume salt-free aqueous solution from one side of the lipid membrane, while another side is in contact with monovalent salt. A standard setup of MD simulations of lipid bilayers (a bilayer in a box of water) cannot be used for this purpose because of periodic boundary conditions: Salt inserted to one side of the bilayer will eventually diffuse to another side within a nanosecond time span. To overcome the periodicity problem one needs to consider *two* bilayers in a simulation box instead of *one*, see Fig. 1. In our case lipid bilayers in Fig. 1 separate two compartments: The interior ("intracellular fluid") is salt-free aqueous solution and the exterior ("extracellular fluid") is aqueous solution with salt (note that periodic boundary conditions are applied in all three dimensions). In other words, here we explicitly model the lipid lamellar phase and have independent control over electrolyte conditions on the "inner" and "outer" leaflets of the bilayers. Such a double bilayer setup was used very recently for modeling the transmembrane potential gradient across a phosphatidylcholine bilayer.[31] A similar setup was also employed in recent MD simulations of ion transport through a hydrophobic pore.[32]

Molecular-dynamics simulations of the double bilayer system, see Fig. 1, demonstrate that keeping leaflets of an initially symmetrical, one-component phospholipid bilayer under different electrolyte conditions leads to establishing a pronounced asymmetry of the bilayer after 50 ns of MD simulations. This asymmetry of bilayer's leaflets can be revealed through their structural, electrostatic, and also dynamic properties. As a reference, we also simulated single bilayer systems (with and without salt). A comparison between the properties of leaflets of the resulting asymmetrical lipid bilayer and those of symmetric bilayers (extracted from conventional single bilayer simulations) is discussed.

II. MODEL AND SIMULATION DETAILS

Zwitterionic dimyristoylphosphatidylcholine (DMPC) lipid was used for the bilayer simulations presented here. The DMPC lipid within a united atom representation consists of 46 interaction sites, see Fig. 2. Force-field parameters for the DMPC lipids were taken from the united atom force field of Berger *et al.*[33] This force field was previously validated[34,35] and was shown to reproduce experimentally observed values of the area and of the volume per lipid.[36] The parameters for this force field are available on-line at http://moose.bio.ucalgary.ca/Downloads/files/lipid.itp. Water was modeled using the simple point charge (SPC) water model.[37] For sodium and chloride ions we used the default set of parameters supplied within the Gromacs force field,[38,39] while being aware of the effects of different models for NaCl.[40]

We performed atomistic MD simulations of three different, fully hydrated bilayer systems, their initial configurations were prepared as follows. We started with the equilibrated DMPC bilayer structure taken from Ref. 16 (the pure

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TABLE I. Summary of MD simulations of lipid bilayer systems

System	Atoms	Lipids	Water	Na ions	Cl ions	$\langle A \rangle$ (nm^2)	Thickness (nm)
2B_salt	42 238	256	10 134[a]	30	30	0.633±0.005	3.45±0.03
1B_water	21 179	128	5097	0	0	0.661±0.010	3.35±0.05
1B_salt	21 059	128	5037	30	30	0.609±0.008	3.54±0.04

[a]This includes 5097 water molecules in the "inner" salt-free compartment and 5037 water molecules along with 30 Na^+ and 30 Cl^- ions in the "outer" electrolyte bath, see Fig. 1.

DMPC bilayer after 20 ns of simulations). This bilayer structure consists of 128 DMPC lipids and 3655 water molecules and is available on-line at http://www.softsimu.org/downloads.shtml. Because a binding of water molecules by salt ions can decrease the hydration level of lipids, we increased the number of water molecules by a factor of 1.4. The resulting DMPC bilayer, preequilibrated for 1 ns, was then used as our initial configuration for simulations of a single bilayer in salt-free aqueous solution (referred to as 1B_water system). For salt simulations of a single bilayer, randomly chosen water molecules of the 1B_water system were replaced by 30 sodium and 30 chloride ions, corresponding to a NaCl concentration of about 300 mM (referred to as 1B_salt system). The number of salt ions was chosen to stay not far away from physiological conditions and, at the same time, to provide reasonable statistics. Finally, a double bilayer system was formed by replicating the salt-free bilayer system (1B_water) along the direction of the bilayer normal. After preequilibrating for 1 ns, 30 Na and 30 Cl ions were added in the outer water compartment (see Fig. 1) in the same fashion as it was done for the 1B_salt system. The resulting system (referred to as 2B_salt system) consists of two DMPC bilayers which separate the inner water bath (water content is the same as that of the 1B_water system) and the outer water reservoir with salt (salt concentration is the same as that of the 1B_salt system), see Fig. 1. Note that since periodic boundary conditions were applied in all three dimensions, the words inner and outer are used (here and below) just for the sake of convenience. Details related to all simulated systems are summarized in Table I.

The Lennard-Jones interactions were cut off at 1 nm without a shift or switch function. Since truncations of long-range electrostatic interactions are known to lead to artifacts in MD simulations of phospholipid bilayers,[41–44] the electrostatic interactions were handled using the particle-mesh Ewald (PME) method.[45] The simulations were performed in the NpT ensemble. The temperature was kept constant using a Berendsen thermostat[46] with a coupling time constant of 0.1 ps. Lipid molecules and water along with salt ions were separately coupled to a thermostat. All the simulations were done at a temperature of 323 K, such that the bilayers are in the liquid-crystalline phase (the main transition temperature of a DMPC bilayer is 297 K).[47] Pressure was controlled by a Berendsen barostat[46] with a coupling time constant of 1.0 ps.

All bond lengths of the lipid molecules were constrained using the LINCS algorithm,[48] whereas the SETTLE algorithm[49] was used for water molecules. The time step of 2 fs was used. All simulations were performed using the GROMACS package.[38,39] All three bilayer systems were simulated for 50 ns each, only last 20 ns of the trajectories were used for analysis (see below). Each simulation was run on a PC with a single 3,2-Ghz Pentium 4 processor. For our biggest 2B_salt system of ~42 000 atoms each nanosecond required about 55 h of computation time.

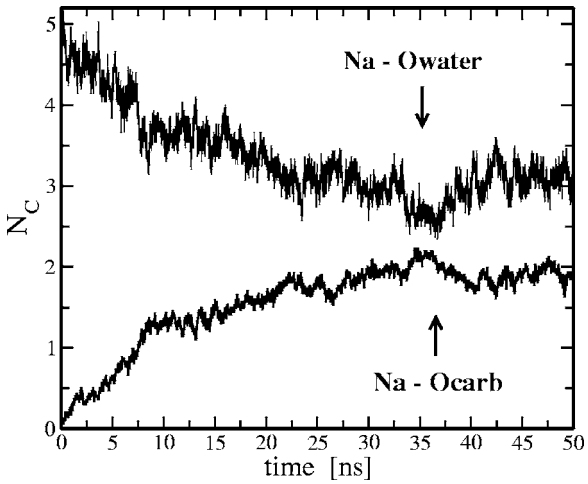

FIG. 3. Time evolution of coordination numbers N_C of sodium ions with lipid carbonyl oxygens and with water oxygens for the 2B_salt system.

III. RESULTS AND DISCUSSION

A. Equilibration, area per lipid, and bilayer thickness

As was emphasized in the Introduction, the system equilibration is of main concern in simulating zwitterionic lipid bilayers with salt. In Refs. 29 and 30 the authors demonstrated that the slowest processes in such systems are associated with the binding of cations to lipid carbonyl oxygens. Therefore, here we use the coordination numbers of sodium ions with some principal oxygens in the system to monitor the system equilibration.

In Fig. 3 we present the time evolution of coordination numbers $N_C(t)$ of sodium ions with lipid carbonyl oxygens (oxygens denoted as 1Ocarb and 2Ocarb in Fig. 2) and with water oxygens for leaflets of the 2B_salt system, which are in contact with salt. Coordination numbers were calculated by counting the total numbers of oxygens in question within the first hydration shell of Na; the shell radius was extracted from corresponding radial distribution functions and was found to equal 0.31 nm. As it is seen in Fig. 3, in the course of simulations sodium ions bind to lipid carbonyl oxygens; at the same time they loose water molecules from their first hydration shell. This process requires about 25 ns. The very similar picture was also found for the single lipid bilayer with salt (1B_salt) in agreement with previous studies.[29,30] Thus, for each simulated system only last 20 ns (out of 50 ns) are used for subsequent analysis.

One of the fundamental structural characteristics of lipid bilayers is the average area per lipid, $\langle A \rangle$, which can be measured rather accurately through experiments on model lipid membranes.[50] From computational point of view, it is crucial for validating the model. For the salt-free DMPC bilayer (1B_water) the average area per lipid was found to have a

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value of $\langle A \rangle = 0.661 \pm 0.010$ nm^2, see Table I. As far as experimental data on the area per lipid are concerned, for the fully hydrated DMPC bilayer at 323 K values of 0.629, 0.654, and 0.703 nm^2 have been reported, see Refs. 51–53, respectively. Therefore, the model used in our study is able to reproduce rather well the experimentally observed values of $\langle A \rangle$ for salt-free DMPC bilayers.

Adding monovalent salt to a lipid bilayer (1B_salt system) leads to the compression of the bilayer (by about 8%, see Table I) because of the complexation of sodium ions with lipid molecules, this finding being in very good agreement with previously reported computational results.[29] Now, in the case when salt is added to one leaflet only (2B_salt system), the observed bilayer compression is roughly *twice* smaller, see Table I. This is something what one can expect because sodium ions bind to lipids of the outer leaflet only. Similar to previous studies,[29,54] the bilayer compression is accompanied by the enhanced ordering of acyl lipid chains in a leaflet which is in contact with salt.

We note that the inner (salt-free) leaflet of the 2B_salt bilayer system also gets compressed since the inner and the outer leaflets are two parts of the same bilayer. This leads, in turn, to the fact that nonpolar hydrocarbon chains of lipids in the salt-free leaflet of the 2B_salt system are more ordered as compared to those in the leaflets of the salt-free single bilayer (1B_water system). This effect is, however, a consequence of an artificial coupling between the two leaflets in bilayer simulations and should be considered as an artifact arising from the simulation setup.

The effect of salt on the bilayer thickness is also of some interest. The thickness is measured as the average P–P distance between phosphorous atoms of the two monolayers. For the fully hydrated DMPC bilayer in salt-free solution (1B_water system) the P–P distance was found to be 3.35 ± 0.05 nm. NaCl salt, being added to a single bilayer (1B_salt system), increases the bilayer thickness by 0.19 nm, see Table I, in full agreement with the findings of Ref. 29, where this increase was attributed to the electrostatic repulsion between two monolayers which became positively charged after the binding of sodium ions.[29] When NaCl salt is added to one bilayer leaflet only (2B_salt system), the bilayer thickness is found to be intermediate between those of single bilayers with and without salt. We also measured the average thickness of each leaflet independently (with respect to the bilayer center) and found essentially the same increase (by about 0.05 nm) in the thickness for both inner (salt-free) and outer (in contact with salt) leaflets. Since for our 2B_salt system sodium ions bind to the outer leaflet only, the increase in the bilayer thickness cannot be explained by the above-mentioned electrostatic repulsion between two positively charged monolayers.[29] Therefore, the observed changes in the membrane thickness are presumably due to the decrease in the area per lipid coupled with the enhanced ordering of acyl lipid chains, i.e., result from the lipid-sodium complexation.

B. Ion binding

To quantify locations of salt ions in the 2B_salt bilayer, we calculated the number density profiles for different constituents of the system. In Fig. 4 we plot the number densities (reduced for clarity's sake by the maximal values of the densities of corresponding constituents) for the 2B_salt bilayer (salt is located on the left side from the bilayer center, $z=0$). First of all, one sees a clear signature of the sodium-lipid complexation: Sodium ions penetrate deep into the carbonyl region of the outer (in contact with salt) leaflet, the corresponding peak of the sodium number density almost exactly coincides with those of lipid carbonyl oxygens, 1Ocarb and 2Ocarb. It is in great contrast with chloride ions which are mainly located in bulk water in some 0.5 nm from the water-lipid interphase, see Fig. 4. Furthermore, it is seen that the outer and the inner leaflets are asymmetrical. The average distance between phosphorous and nitrogen atoms of the outer (in contact with salt) leaflet is clearly larger than that of the inner salt-free leaflet; this is a sign of the salt-induced reorientation of phosphatidylcholine headgroups (see Sec. III C). Also, in the inner, salt-free leaflet the lipid carbonyl oxygens (1Ocarb) of *sn*-1 acyl chains are located deeper than those of *sn*-2 chains (2Ocarb); this is also the reason why sodium ions, being added to a bilayer system, bind mostly to the *sn*-2 carbonyl oxygens, which are easier to access (data not shown). In contrast, the number density peaks for carbonyl oxygens of both acyl chains are almost indistinguishable in the outer leaflet which is contact with salt, see Fig. 4.

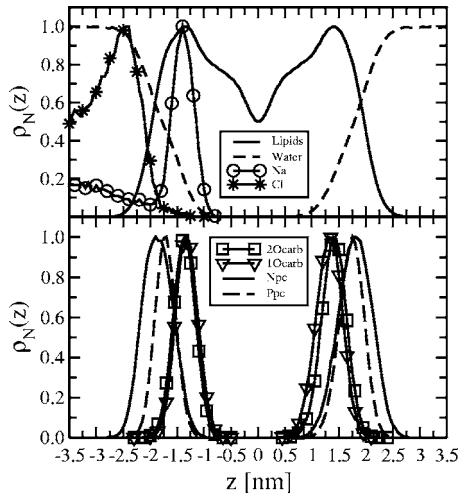

FIG. 4. Scaled number densities $\rho_N(z)$ for the 2B_salt system. (Top): Number density profiles for lipids, salt ions, and water. (Bottom): Number density profiles for nitrogen and phosphorous atoms of lipid headgroups and for lipid carbonyl oxygens. The case $z=0$ corresponds to the center of the bilayer; all the number densities are averaged over two bilayers.

Now we turn to the detailed analysis of the Na-lipid complexation. The temporal behavior of the coordination number of sodium ions with lipid carbonyl oxygen atoms in Fig. 3 was computed by means of the averaging over all Na ions in the system. To obtain the actual average number of lipids bound to a sodium ion one needs to make the averaging only over sodium ions which have lipid carbonyl oxygens in the first coordination shell. This gives us 2.89 ± 0.17 lipids for the outer leaflet of the 2B_salt system (last 20 ns of the trajectory are used for the averaging). For a single bilayer system (1B_salt) we found the very close value of the aver-

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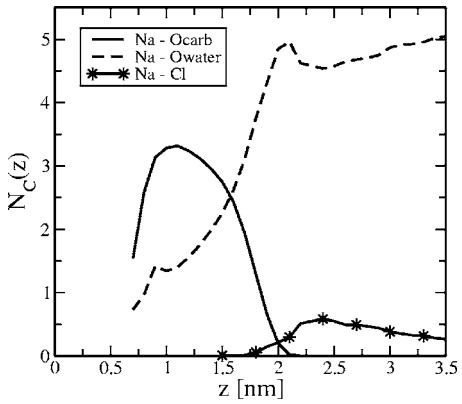

FIG. 5. Coordination numbers $N_C(z)$ of sodium ions with lipid carbonyl oxygens, with water oxygens, and with chloride ions as a function of distance z from the bilayer center (2B_salt system).

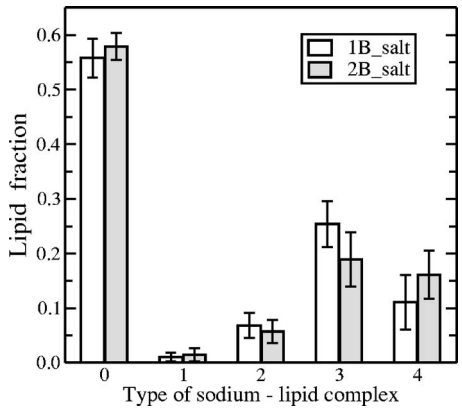

FIG. 6. Details of Na-lipid complexation. Shown are the average fractions of lipid molecules involved in sodium-lipid complexes of various types: "0" means that lipids are not bound to sodium ions, "1" corresponds to a sodium ion bound to a single lipid, "2" means that a sodium ion forms a complex with two lipids, etc. For comparison, results for the double bilayer (gray) as well as for the single bilayer with salt (white) are shown.

age coordination number of Na ions with lipid carbonyl oxygen atoms, namely, 2.85 ± 0.18. This means that in both cases a sodium ion binds on average to *three* lipid molecules. This is in agreement with the results of Ref. 29 where the binding of a sodium ion to three PC lipids was also observed. Another recent computational study[54] revealed that only two dipalmitoylphosphatidylcholine (DPPC) lipid molecules are bound (on average) to each Na ion. We note, however, that the time scales of the simulations in Ref. 54 were rather short: The total simulation time was 10 ns and the last 5 ns were used for analysis. Repeating our measurements for the 1B_salt system with the use of the same time scales as those in Ref. 54 gives the average coordination number Na-Ocarb equal to 2.28, which is considerably smaller than $\langle N_C \rangle$ measured after the equilibration has been established (i.e, after 30 ns).

To illustrate how coordination numbers of sodium ions vary within the bilayer system, in Fig. 5 we plot various sodium N_C with lipid carbonyl oxygens, with water oxygens, and also with Cl ions as a function of distance z from the bilayer center for the outer leaflet of the 2B_salt bilayer. It is seen that when a sodium ion goes through a water-lipid interface ($z \sim 2$ nm), it starts to loose its coordinated water. At the same time, the coordination of Na with lipid carbonyl oxygens goes up and approaches its maximal value at around 1.2 nm from the center of the bilayer, see Fig. 5. Note that the maximal values of the coordination number Na-Ocarb is equal to 3.32, i.e., exceeds the corresponding average value $\langle N_C \rangle \simeq 2.89$. For the single bilayer system (1B_salt) we found similar results. As for chloride ions, they have no influence on the binding of Na ions inside of the bilayer because they are coordinated with Na mainly in bulk water, rather far away from the water-lipid interface, see Figs. 4 and 5.

Of particular interest is more detailed analysis of the sodium-lipid complexation. Since a sodium ion binds on average to three lipid molecules, one can expect that the sodium-lipid complexes are formed by three lipids, see also Ref. 29. This is, however, a simplified, "averaged" picture. In Fig. 6 we plot fractions of lipids involved in Na-lipid complexes of various types. For a conventional single bilayer with NaCl (1B_salt system) we found that about 56% of all lipids are not bound to sodium ions (sodium-lipid complex of type "0"). This is in good agreement with the results of MD simulations of PC bilayers in aqueous solution with similar NaCl concentration.[29] The majority of lipids bound to sodium ions in the 1B_salt system is indeed organized into complexes consisting of three lipids (lipid fraction 0.25). However, there are also a substantial number of lipids which are involved in complexes of other types, these complexes are formed by two and by four lipids with fractions equal to 0.07 and 0.11, respectively. This result is not very unexpected and does not contradict with the fact that the average coordination number of sodium ions with lipid carbonyl oxygens is about 3: Lipids organized into complexes of two and of four lipids, being presented in the bilayer in ratio 1:2, indeed lead to the situation when a sodium ion binds (*on average*) to three lipids.

It turns out that the distribution of lipids over various types of Na-lipid complexes differs for the system with an asymmetric setup of electrolyte solution (2B_salt system), see Fig. 6. First, the fraction of nonbound lipids, 0.58, in its outer leaflet (which is contact with salt) is slightly larger than that for the 1B_salt system. Second, the main contribution to the sodium-lipid complexation is provided by complexes of three lipids as well as of four lipids with lipid fractions given by 0.19 and 0.16, respectively. Thus, despite of approximately the same average coordination number Na-Ocarb, the organization of lipids into complexes is found to be notably different for the 2B_salt and for the 1B_salt systems. This can be associated with the difference in their area per lipid, see Table I, and in the packing of lipids: The outer leaflet of the 2B_salt system is less compressed as compared to that of the single bilayer with salt (1B_salt), and its lipids have more freedom for rearrangements.

C. Orientation of lipid headgroups

Headgroups of DMPC lipids are zwitterionic and possess a dipole moment along the $P-N$ vector, see Fig. 2. The orientation of the headgroups with respect to the outward bilayer normal is of particular interest since it contributes to the electrostatic potential across a lipid monolayer. For inner

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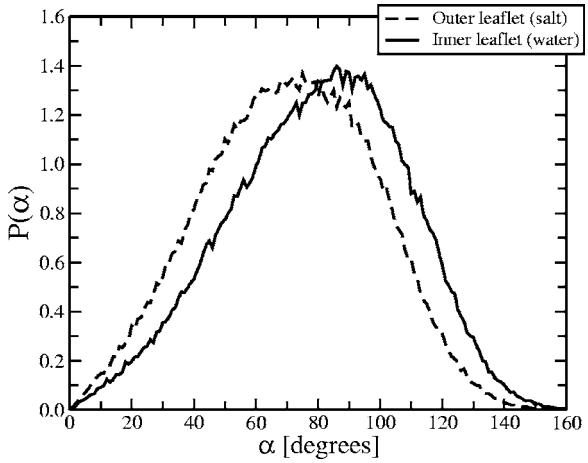

FIG. 7. Distribution function $P(\alpha)$ of the angle α between the $P-N$ vector of DMPC headgroups and the outward bilayer normal. Shown are results for the inner (solid line) and for the outer (dashed line) leaflets of the 2B_salt system. $P(\alpha)$ is given in arbitrary units.

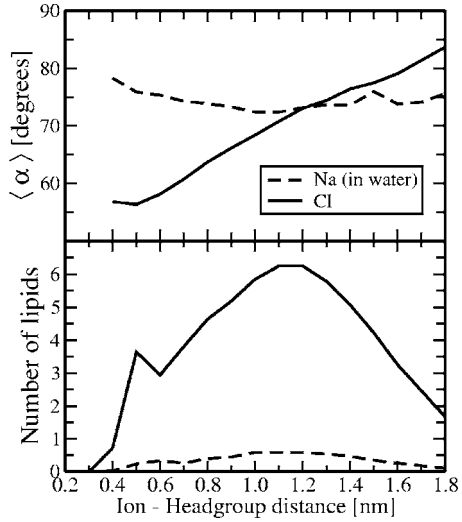

FIG. 8. (Top): The headgroup tilt angle α averaged over lipid headgroups which have salt ions within a certain distance as a function of this distance for the outer leaflet of the 2B_salt bilayer. The "headgroup-ion" distance is defined as (Na^+-P_{pc}) and (Cl^--N_{pc}) distances for sodium and chloride ions, respectively. (Bottom): The corresponding average number of lipids having salt ions within a certain distance from their headgroups. Shown are results for "free" sodium ions in bulk water (dashed lines) and for chloride ions (solid lines).

and outer leaflets of the 2B_salt bilayer, Fig. 7 shows the distribution function $P(\alpha)$ for the angle α between the $P-N$ vector and the outward bilayer normal. One sees a pronounced asymmetry in the PC headgroup orientation induced by monovalent salt: The maximum of the angle distribution $P(\alpha)$ for the outer leaflet (which is contact with salt) is shifted to smaller angles as compared to that for the inner, salt-free monolayer, see Fig. 7. The average values of the angle α are found to be $(79.8^\circ \pm 1.2^\circ)$ and $(70.7^\circ \pm 1.3^\circ)$ for the inner and the outer leaflets, respectively. Thus, monovalent salt results in a considerable reorientation of the PC headgroups.

Interestingly, this headgroup reorientation turns out to be rather robust to the change in the area per lipid. For the 1B_salt bilayer (which has the area per lipid smaller than that of the 2B_salt bilayer, see Table I) one has $\langle\alpha\rangle=(70.6^\circ \pm 1.4^\circ)$; this coincides with $\langle\alpha\rangle$ for the outer leaflets of the double bilayer system. In turn, for the salt-free single bilayer (1B_water) we found $\langle\alpha\rangle=(79.2^\circ \pm 1.3^\circ)$. Note that this value is in agreement with the experimental data[55,56] and with MD simulations of phosphatidylcholine lipid bilayers under salt-free conditions.[57]

The observed reorientation of the zwitterionic PC headgroups under the influence of salt agrees well with the computational results of Refs. 29 and 30, where it was demonstrated that both monovalent and divalent salt made lipid headgroups more vertically oriented. We note that such a re-orientation was not observed in Ref. 54; this, however, may be again attributed to rather short time scales (see also Sec. III B). Another MD study of palmitoyloleoylphosphatidylcholine (POPC) lipid bilayers[58] reported large local changes in headgroup tilt induced by salt ions; the average value of the tilt was found to be almost unchanged, though. A direct comparison of that work with our study is, however, rather difficult because another force field (all-atom CHARMM force field) and another ensemble (NAP_NT ensemble implying constant surface area) were used.[58] Furthermore, the simulation time was again very short (5 ns).

Now we turn to a more detailed analysis of how salt ions affect the orientation of the PC headgroups in the outer leaflet of the 2B_salt bilayer. Because of the strong binding of sodium ions to the carbonyl region of the outer leaflet, "free" Na ions in bulk water should be distinguished from those bound to the membrane. As far as sodium ions in *bulk water* are concerned, they are found to have a rather weak effect on the headgroup tilt as it is seen in Fig. 8 (top), where we plot the average headgroup tilt $\langle\alpha\rangle$ as a function of a headgroup-ion distance in the outer leaflet (more specifically, Na^+-P_{pc} and Cl^--N_{pc} distances for sodium and chloride ions, respectively). When a sodium ion from bulk water approaches a headgroup (at distances smaller than 1 nm), it causes a slight increase in the angle between the headgroup dipole vector and the outward bilayer normal (see also Ref. 58). It has to be noted, however, that the number of lipid headgroups which have such sodium ions within a certain distance is small and their contribution to the overall headgroup orientation is, therefore, not very significant, see Fig. 8 (bottom). The latter is, to some extent, a consequence of salt concentration considered: On average, about 10 sodium ions (out of 15) are found to bind to lipids of the outer leaflet. Therefore, there are only a few sodium ions in bulk water.

Surprisingly, sodium ions *bound* to carbonyl lipid oxygens do not have a strong influence on the headgroup tilt either. In Fig. 9 we plot the average angle between the $P-N$ vector of PC headgroups and the outward bilayer normal for various types of lipid-sodium ion complexes in the outer leaflet. It is seen that the formation of the lipid complexes does not lead to a notable reorientation of the corresponding lipid headgroups as compared to free lipids (lipid complex of type 0 in Figs. 9 and 6).

In contrast, the effect of chloride ions on the headgroup tilt turns out to be significant. As discussed in Sec. III B, chloride ions do not penetrate deep into the membrane and

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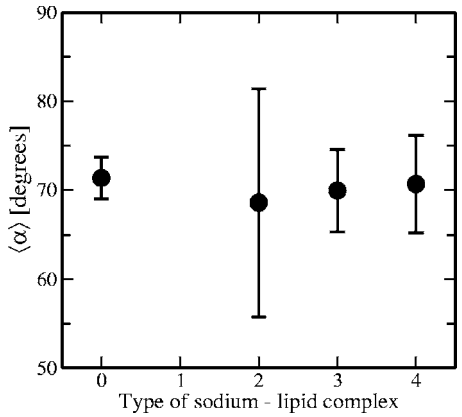

FIG. 9. The average angle $\langle\alpha\rangle$ between the $P-N$ vector of PC headgroups and the outward bilayer normal for lipids involved in various types of lipid-sodium ion complexes in the outer monolayer of the 2B_salt system (see Fig. 6 for a detailed description of the types of the lipid complexes). Results for the case when a sodium ion binds to a single lipid (lipid complex of type "1") are not included because its contribution is negligible and the corresponding data are very noisy.

are mainly located in bulk water, see Fig. 4. Therefore, the average number of lipids which have Cl ions within a certain distance from their headgroups is considerable, see Fig. 8 (bottom). Remarkably, a chloride ion approaching a nitrogen of a PC headgroup results in a systematic decrease in the average angle $\langle\alpha\rangle$ between the headgroup $P-N$ vector and the outward monolayer normal as seen in Fig. 8 (top). Our finding is in agreement with the computational results of Ref. 58.

To illustrate the influence of Cl ions in another way, we classified all lipid headgroups of the outer leaflet as follows: Lipids which do not have chloride ions within 1 nm from their headgroup nitrogens and lipids which have 1 or 2 chloride ions within the same distance from Npc. It turns out that if a Cl ion approaches a headgroup nitrogen closer than 1 nm, the headgroup tilt angle $\langle\alpha\rangle$ decreases by about 12°, see Fig. 10. This pronounced effect seems to be caused by

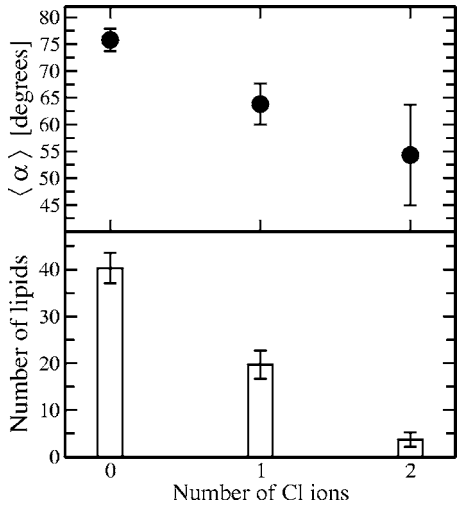

FIG. 10. (Top): The average angle $\langle\alpha\rangle$ between the $P-N$ vector and the outward bilayer normal for lipids which have 0, 1, and 2 cloride ions within 1 nm of their headgroup nitrogens. (Bottom): The corresponding average numbers of such lipids. Shown are results for the outer leaflet of the 2B_salt system.

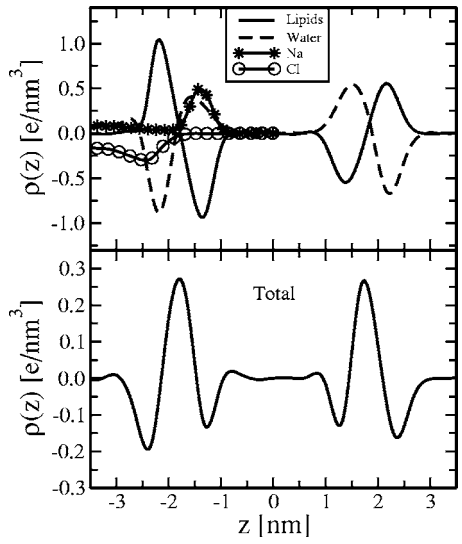

FIG. 11. (Top): Charge densities $\rho(z)$ across the 2B_salt bilayer due to lipids (solid line), water (dashed line), sodium ions (stars), and chloride ions (open circles). (Bottom): The total charge density across the 2B_salt bilayer. The case $z=0$ corresponds to the center of the bilayer; all the charge densities are averaged over two bilayers.

electrostatic attraction between Cl^- and N_{pc}.

To conclude, we note that all the results found for the orientation of the PC headgroups in the outer leaflet of the 2B_salt system also hold for a single lipid bilayer in aqueous solution with salt (1B_salt system).

D. Electrostatic potential and orientation of water molecules

The asymmetry induced by monovalent salt is clearly seen by inspecting the partial charge densities due to different constitutes of a bilayer system, see Fig. 11 (top). The charge densities were calculated in the same fashion as the number density profiles in Fig. 4; furthermore, due to the noise, the data were smoothed by means of spline approximation.[59] To compensate negative charges of chloride ions close to the water-lipid interface the charge-density peak of choline lipid groups in the outer leaflet becomes considerably higher than that for the salt-free inner leaflet. In turn, the depth of the charge-density minimum related to negatively charged phosphate groups increases to compensate positive charges of sodium ions penetrated deep into the outer leaflet. All in all, salt ions lead to a notable difference in the charge-density profiles of phosphatidylcholine lipids on two sides of the 2B_salt membrane.

As it is seen in Fig. 11 (bottom), the total charge density of the 2B_salt bilayer turns out to be nearly symmetrical, though. This means that the above-mentioned considerable changes in the charge distributions of zwitterionic lipids result in almost full compensation of the effects induced by salt ions and by reoriented water molecules. However, some subtle differences still exist because of a small charge imbalance in the lipid-water interface of the outer leaflet.

We also computed the electrostatic potential $V(z)$ across the bilayer by twice integrating the charge density, see Fig. 12. The electrostatic potential was taken to be zero at the center of the bilayer $(z=0)$ and was averaged over two bi-

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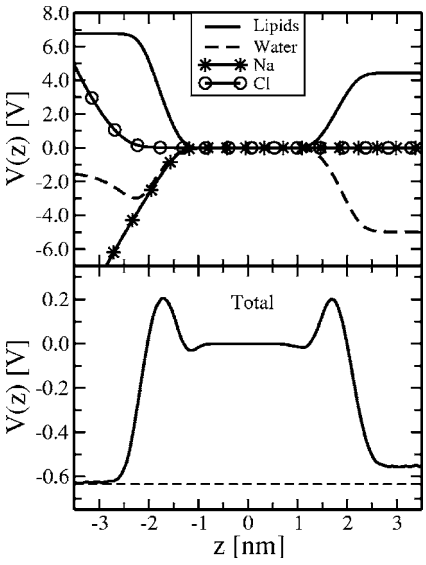

FIG. 12. (Top): Componentwise contributions to the electrostatic potential $V(z)$ across the 2B_salt bilayer due to lipids (solid line), water (dashed line), Na (stars), and Cl (open circles) ions. (Bottom): The total electrostatic potential $V(z)$ across the 2B_salt bilayer. The electrostatic potential is taken to be zero at the center of the bilayer ($z=0$) and is averaged over two bilayers.

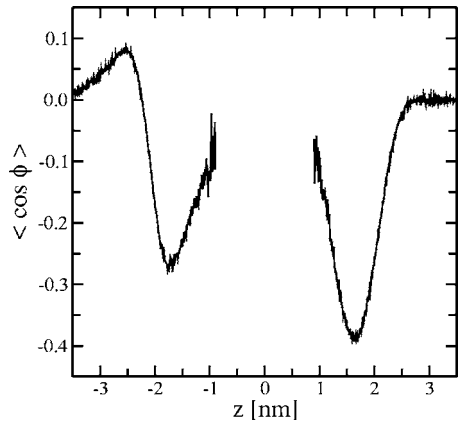

FIG. 13. Average orientation of water molecules with respect to the outward bilayer normal for the 2B_salt system, see text for details. The case $z=0$ corresponds to the center of the bilayer; salt is located at $z<0$. Since the number of water molecules inside the bilayer is very small, data for $|z|<1$ are noisy and not shown.

layers of the 2B_salt system. Again, the componentwise contributions to the $V(z)$, see Fig. 12 (top), considerably differ for the inner and for the outer leaflets. Salt ions induce the potential increase due to lipid headgroups and the potential drop due to reoriented water molecules when compared with the salt-free inner leaflet. The same was also found for single bilayer systems upon adding salt (1B_water and 1B_salt systems). These findings agree with the results of Ref. 29 (note the difference in the definition of zero of the potential). Similar to the charge-density profiles, the *total* electrostatic potential $V(z)$ across the 2B_salt lipid bilayer turns out to be not very sensitive to salt, see Fig. 12 (bottom), in agreement with recent experimental[60] and computational studies.[29,54]

For the salt-free inner leaflet of the 2B_salt bilayer (as well as for a single DMPC bilayer without salt, 1B_water) we found that the overall difference in the potential between the bilayer center and the water phase is about 550 mV, see Fig. 12 (bottom), which is in agreement with previous computational studies.[16,57,61,62] Experimental data[60,63–66] for phospholipid membranes scatter from 200 to 600 mV. For a single bilayer system (1B_salt) the presence of 0.3 M of NaCl is found to increase the total electrostatic potential of a *monolayer* up to 645 mV (data not shown). This effect was also observed in previous MD simulation studies;[29,54] it seems to be slightly dependent on salt concentration.[29] Similar potential increase (up to 635 mV) is found for the outer 2B_salt leaflet which is in contact with salt, see Fig. 12 (bottom) at $z<0$.

The increase in the total electrostatic potential of the outer monolayer with respect to the salt-free monolayer results in a *nonzero* potential difference between two phases, see Fig. 12 (bottom). The origin of the nonzero transmembrane potential is a net dipole moment induced on the 2B_salt lipid bilayer due to a slight asymmetry of the charge distribution between the two leaflets, see Fig. 11 (bottom). Remarkably, this transmembrane potential ΔV is found to be 85 ± 10 mV, i.e., turns out to be close to typical values of membrane potentials in a cell. Therefore, as we demonstrated, a nonzero transmembrane potential can arise in the *absence* of any ionic charge imbalance between two water compartments (compare with Ref. 31).

In addition, we computed the average orientation of water molecules on both sides of the 2B_salt bilayer, see Fig. 13. Bulk water in the salt-free compartment ($z>0$), being far away from the water-lipid interface, does not have a preferred orientation, i.e., $\langle\cos\phi\rangle=0$ at $z>2.5$ nm, where ϕ is the angle between the water dipole vector and the outward bilayer normal. Close to the interface, water molecules get oriented in such a way that their dipoles point toward the bilayer center, i.e., $\langle\cos\phi\rangle<0$. This ordering is normally attributed to the hydrogen bonding between oxygens of phosphatidyl lipid groups and water hydrogens.[42,67]

In the outer leaflet ($z<0$) the presence of salt changes the orientation of water molecules close to the water-lipid interface as compared to the salt-free inner leaflet, see Fig. 13: At distances larger than 2.25 nm from the center of the 2B_salt bilayer one sees that the average direction of the water dipoles gets inverted, i.e., $\langle\cos\phi\rangle$ becomes positive, see also Ref. 58. Thus, there is an evident difference in the orientation of water molecules on both sides of the 2B_salt bilayer, which is directly related to an asymmetric setup in electrolyte composition of the outer and the inner compartments, see Fig. 1.

E. Lateral diffusion

To conclude, it is worthwhile to discuss dynamical properties of lipids of a bilayer with the asymmetric electrolyte composition. To quantify the motions of lipids in the bilayer plane, we calculated the mean-square displacements $\langle[r(t)]^2\rangle$ of the center-of-mass (C.M.) positions of lipids in the inner and in the outer monolayers of the 2B_salt bilayer, see Fig. 14. To improve accuracy, we divided the overall production run of 20 ns into two pieces; for every piece of 10 ns we computed $\langle[r(t)]^2\rangle$ up to $t=5$ ns. Furthermore, the effects of

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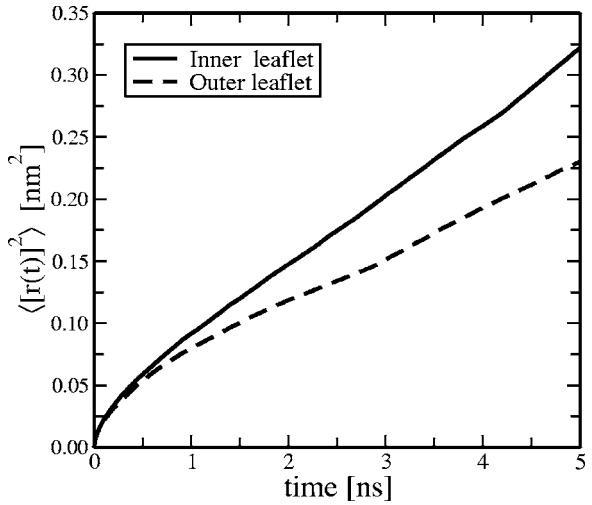

FIG. 14. Mean-square displacements $\langle [r(t)]^2 \rangle$ of DMPC lipid molecules of the salt-free inner leaflet (solid line) and of the outer leaflet which is in contact with NaCl salt (dashed line).

the motion of C.M. of two monolayers relative to each other as well as barostat effects were excluded. In practice, we computed the mean-square displacements following the standard approach described elsewhere.[44] Figure 14 clearly shows that the mobility of lipids in the inner, salt-free leaflet is higher than that for lipids in the outer leaflet which is in contact with salt. Remarkably, this is despite of the fact that both leaflets have the same area.

The lateral diffusion of lipid molecules is normally characterized by the lateral diffusion coefficient D_L defined from

$$D_L = \lim_{t \to \infty} \frac{1}{4t} \langle [r(t)]^2 \rangle, \tag{1}$$

i.e., from the slope of the mean-square displacements $\langle [r(t)]^2 \rangle$ of C.M. of lipids in the bilayer plane. For the lateral diffusion coefficient we found $D_L = (14.2 \pm 4.2) \times 10^{-8}$ cm^2/s for the inner leaflet and $D_L = (9.3 \pm 1.4) \times 10^{-8}$ cm^2/s for the outer leaflet. Remarkably, the value of D_L for the salt-free DMPC leaflet is within the range of experimentally observed values.[68–71] Thus, adding salt to the outer leaflet decreases the lateral lipid self-diffusion and results in a notable difference in the lateral mobility of lipids in two monolayers of the membrane.

Our finding agrees with the results of Ref. 29, where similar decrease in the lipid self-diffusion under the influence of NaCl was found both experimentally and through MD simulations. The decrease was attributed to the formation of tight lipid-sodium ion complexes with reduced mobility.[29] In our case the influence of such complexes on the lipid mobility is even more pronounced since the outer and the inner leaflets, being two sides of the same membrane, have the same area. We also estimated the lateral diffusion coefficients for single bilayers (1B_water and 1B_salt systems). The results are consistent with those for the double bilayer case, namely, salt is found to decrease the lipid self-diffusion. A quantitative comparison of D_L for the double and single bilayer systems is, however, difficult because our production run is not long enough (20 ns) to provide sufficient statistical accuracy in determining the lateral diffusion coefficients (compare with Refs. 29, 43, and 44).

IV. CONCLUSIONS

Electrostatic interactions between lipid membranes and salt ions have attracted substantial interest since they are essential for the structure and stability of the membranes and are presumed to affect many biologically relevant processes such as membrane fusion and insertion of proteins into membranes. The main goal of this work is to model a situation which occurs in a biological cell: The specific electrolyte composition inside of the cell differs greatly from that of the surrounding fluid. For this purpose we perform extensive 50-ns MD simulations of a single-component DMPC lipid bilayer which separates two aqueous compartments with different salt concentrations: The salt-free "inner" compartment and the "outer" compartment containing 0.3 M of NaCl salt.

Our atomistic MD study demonstrates that monovalent salt, being in contact with only one leaflet of an initially symmetrical membrane, induces a pronounced *asymmetry* in the structural, electrostatic, and dynamic properties of the membrane leaflets. Sodium ions penetrate deep into the membrane up to the carbonyl region of the outer (in contact with salt) leaflet, bind to its lipid molecules, and result in the formation of "Na-lipids" complexes (preferably consisting of three and of four lipids). Chloride ions do not penetrate that deep and are located in the aqueous phase close to the water-lipid interface; their attractive interactions with choline groups of lipids of the outer leaflet seem to be responsible for a substantial (more vertical) reorientation of the PC lipid headgroups with respect to the outward bilayer normal (by about 9°) as compared to the inner, salt-free leaflet. Furthermore, the lateral mobility of lipids of the outer (in contact with salt) leaflet is reduced by the above-mentioned formation of massive lipid complexes with sodium ions. This leads to a considerable difference in diffusion coefficients of lipids of the two leaflets of the bilayer.

The salt-induced reorientation of lipid headgroup dipoles, being coupled with a reorientation of water dipoles, leads to a noticeable difference in the charge-density profiles (as well as in the electrostatic potentials) of membrane constitutes on different sides of the bilayer. The overall charge density of the bilayer turns out to be almost insensitive to the presence of salt. However, there is a slight asymmetry in the charge distribution between the two bilayer leaflets; the asymmetry induces a nonzero dipole moment on the bilayer. This leads, in turn, to a *nonzero* potential difference ΔV between the two aqueous phases. Remarkably, this transmembrane potential $\Delta V = 85 \pm 10$ mV is of the same order as the membrane potential in a cell. Thus, a nonzero transmembrane potential can arise *without* ionic charge imbalance between two aqueous compartments.

All in all, the atomistic molecular-dynamics study of a PC lipid membrane separating electrolyte buffers of different ionic compositions allows us to shed more light on a fundamental problem of the influence of salt ions on lipid components of biological membranes. To implement such a study we needed to use a double bilayer setup implying two lipid

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bilayers in a simulation box. This approach, being computationally rather expensive, offers new perspectives in studying model lipid membrane systems. In addition to the recently reported modeling of the transmembrane potential gradient due to ionic charge imbalance,[31] one can think of studying the hydration effects in the lipid lamellar phase and of modeling "true" asymmetrical lipid membranes which possess an asymmetry both in the lipid composition of leaflets and in the ionic composition of electrolyte buffers on the two sides of membranes. These problems are addressed in our ongoing studies.

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[1] R. B. Gennis, *Biomembranes: Molecular Structure and Function* (Springer-Verlag, New York, 1989).
[2] D. P. Tieleman, S. J. Marrink, and H. J. C. Berendsen, Biochim. Biophys. Acta **1331**, 235 (1997).
[3] S. E. Feller, Curr. Opin. Colloid Interface Sci. **5**, 217 (2000).
[4] L. Saiz and M. L. Klein, Acc. Chem. Res. **35**, 482 (2002).
[5] H. L. Scott, Curr. Opin. Struct. Biol. **12**, 495 (2002).
[6] K. V. Damodaran, K. M. Merz, and B. P. Gaber, Biochemistry **31**, 7656 (1992).
[7] F. Zhou and K. Schulten, J. Chem. Phys. **99**, 2194 (1995).
[8] D. P. Tieleman and H. J. C. Berendsen, Biophys. J. **74**, 2786 (1998).
[9] S. W. Chiu, S. Vasudevan, E. Jacobsson, R. J. Mashl, and H. L. Scott, Biophys. J. **85**, 3624 (2003).
[10] E. Mombelli, R. Morris, W. Taylor, and F. Fraternali, Biophys. J. **84**, 1507 (2003).
[11] M. T. Hyvönen and P. T. Kovanen, J. Phys. Chem. B **107**, 9102 (2003).
[12] P. Niemelä, M. T. Hyvönen, and I. Vattulainen, Biophys. J. **87**, 2976 (2004).
[13] J. J. L. Cascales, J. G. de la Torre, S. J. Marrink, and H. J. C. Berendsen, J. Chem. Phys. **104**, 2713 (1996).
[14] S. A. Pandit and M. L. Berkowitz, Biophys. J. **82**, 1818 (2002).
[15] P. Mukhopadhyay, L. Monticelli, and D. P. Tieleman, Biophys. J. **86**, 1601 (2004).
[16] A. A. Gurtovenko, M. Patra, M. Karttunen, and I. Vattulainen, Biophys. J. **86**, 3461 (2004).
[17] S. J. Marrink, E. Lindahl, O. Edholm, and A. E. Mark, J. Am. Chem. Soc. **123**, 8638 (2001).
[18] S. A. Pandit, D. Bostick, and M. L. Berkowitz, Biophys. J. **85**, 3120 (2003).
[19] A. H. de Vries, A. E. Mark, and S. J. Marrink, J. Phys. Chem. B **108**, 2454 (2004).
[20] S. A. Pandit, E. Jakobsson, and H. L. Scott, Biophys. J. **87**, 3312 (2004).
[21] K. Murzyn, T. Róg, and M. Pasenkiewicz-Gierula, Biophys. J. **88**, 1091 (2005).
[22] S. A. Tatulian, Eur. J. Biochem. **170**, 413 (1987).
[23] B. A. Gunningham, E. Gelerinter, and L. J. Lis, Chem. Phys. Lipids **46**, 205 (1988).
[24] M. Roux and M. Bloom, Biochemistry **29**, 7077 (1990).
[25] R. J. Clarke and C. Lüpfert, Biophys. J. **76**, 2614 (1999).
[26] M. Rappolt, G. Pabst, H. Amenitsch, and P. Laggner, Colloids Surf., A **183**, 171 (2001).
[27] H. Binder and O. Zschörnig, Chem. Phys. Lipids **115**, 39 (2002).
[28] S. Ohki and K. Arnold, Colloids Surf., B **18**, 83 (2000).
[29] R. A. Böckmann, A. Hac, T. Heimburg, and H. Grubmüller, Biophys. J. **85**, 1647 (2003).
[30] R. A. Böckmann and H. Grubmüller, Angew. Chem., Int. Ed. **43**, 1021 (2004).
[31] J. N. Sachs, P. S. Crozier, and T. B. Woolf, J. Chem. Phys. **121**, 10847 (2004).
[32] J. Dzubiella, R. J. Allen, and J.-P. Hansen, J. Chem. Phys. **120**, 5001 (2004).
[33] O. Berger, O. Edholm, and F. Jahnig, Biophys. J. **72**, 2002 (1997).
[34] D. P. Tieleman and H. J. C. Berendsen, J. Chem. Phys. **105**, 4871 (1996).
[35] E. Lindahl and O. Edholm, Biophys. J. **79**, 426 (2000).
[36] J. F. Nagle and M. C. Wiener, Biochim. Biophys. Acta **942**, 1 (1988).
[37] H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, and J. Hermans, in *Intermolecular Forces*, edited by B. Pullman (Reidel, Dordrecht, 1981), pp. 331–342.
[38] H. J. C. Berendsen, D. van der Spoel, and R. van Drunen, Comput. Phys. Commun. **91**, 43 (1995).
[39] E. Lindahl, B. Hess, and D. van der Spoel, J. Mol. Model. **7**, 306 (2001).
[40] M. Patra and M. Karttunen, J. Comput. Chem. **25**, 678 (2004).
[41] D. J. Tobias, Curr. Opin. Struct. Biol. **11**, 253 (2001).
[42] M. Patra, M. Karttunen, M. T. Hyvönen, E. Falck, P. Lindqvist, and I. Vattulainen, Biophys. J. **84**, 3636 (2003).
[43] C. Anézo, A. H. de Vries, H. D. Höltje, D. P. Tieleman, and S. J. Marrink, J. Phys. Chem. B **107**, 9424 (2003).
[44] M. Patra, M. Karttunen, M. T. Hyvönen, E. Falck, and I. Vattulainen, J. Phys. Chem. B **108**, 4485 (2004).
[45] T. Darden, D. York, and L. Pedersen, J. Chem. Phys. **98**, 10089 (1993).
[46] H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, A. DiNola, and J. R. Haak, J. Chem. Phys. **81**, 3684 (1984).
[47] G. Cevc and D. Marsh, *Phospholipid Bilayers: Physical Principles and Models* (Wiley, New York, 1987).
[48] B. Hess, H. Bekker, H. J. C. Berendsen, and J. G. E. M. Fraaije, J. Comput. Chem. **18**, 1463 (1997).
[49] S. Miyamoto and P. A. Kollman, J. Comput. Chem. **13**, 952 (1992).
[50] J. F. Nagle and S. Tristram-Nagle, Biochim. Biophys. Acta **1469**, 159 (2000).
[51] J. F. Nagle, R. Zhang, S. Tristram-Nagle, W. Sun, H. I. Petrache, and R. M. Suter, Biophys. J. **70**, 1419 (1996).
[52] H. I. Petrache, S. W. Dodd, and M. F. Brown, Biophys. J. **79**, 3172 (2000).
[53] S. C. Costigan, P. J. Booth, and R. H. Templer, Biochim. Biophys. Acta **1468**, 41 (2000).
[54] S. A. Pandit, D. Bostick, and M. L. Berkowitz, Biophys. J. **84**, 3743 (2003).
[55] H. Hauser, I. Pascher, R. H. Pearson, and S. Sundell, Biochim. Biophys. Acta **650**, 21 (1981).
[56] P. G. Scherer and J. Seelig, Biochemistry **28**, 7720 (1989).
[57] A. M. Smondyrev and M. L. Berkowitz, J. Chem. Phys. **111**, 9864 (1999).
[58] J. N. Sachs, H. Nanda, H. I. Petrache, and T. B. Woolf, Biophys. J. **86**, 3772 (2004).
[59] B. J. Thijsse, M. A. Hollanders, and J. Hendrikse, Comput. Phys. **12**, 393 (1998).
[60] R. J. Clarke, Adv. Colloid Interface Sci. **89**, 263 (2001).
[61] S. W. Chiu, M. Clark, V. Balaji, S. Subramaniam, H. L. Scott, and E. Jacobsson, Biophys. J. **69**, 1230 (1995).
[62] R. R. Gabdoulline, G. Vanderkooi, and C. Zheng, J. Phys. Chem. **100**, 15942 (1996).
[63] S. B. Hladky and D. A. Haydon, Biochim. Biophys. Acta **318**, 464 (1973).
[64] A. D. Pickard and R. Benz, J. Membr. Biol. **44**, 353 (1978).
[65] R. F. Flewelling and W. L. Hubbel, Biophys. J. **49**, 541 (1986).
[66] K. Gawrisch, D. Ruston, J. Zimmerberg, V. A. Parsegian, R. P. Rand, and N. Fuller, Biophys. J. **61**, 1213 (1992).
[67] M. T. Hyvönen, T. T. Rantala, and M. Ala-Korpela, Biophys. J. **73**, 2907 (1997).
[68] W. L. C. Vaz, R. M. Clegg, and D. Hallman, Biochemistry **24**, 781 (1985).
[69] P. F. F. Almeida, W. L. C. Vaz, and T. E. Thompson, Biochemistry **31**, 6739 (1992).
[70] A. Filippov, G. Orädd, and G. Lindblom, Biophys. J. **84**, 3079 (2003).
[71] A. Filippov, G. Orädd, and G. Lindblom, Langmuir **19**, 6397 (2003).

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