Published in final edited form as:

Interdiscip Sci. 2011 December ; 3(4): 272–282. doi:10.1007/s12539-011-0107-4.

Concentration Dependence of NaCl ion distributions around DPPC lipid bilayers

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Abstract

We study the coordination of excess NaCl to zwitterionic DPPC lipid bilayers using molecular dynamics simulations. We find that Na ions directly coordinate with the DPPC lipid carbonyl groups. As the number of excess ions increases, the number of coordinated ions increases, until it reaches a plateau at a ratio near 1 ion per every four lipids at 310 K, and 1 ion per every six lipids at 323 K. The area per lipid decreases as the number of excess ions is increased. For low number of ions per lipids (1:16 and 1:8), most Na ions are bound to the lipid carbonyls, while the Cl form an ionic cloud around the lipid choline groups. As a result of the Na binding, the lipid has an effective positive charge density. The residence time of Na ions bound to the lipid is longer than 40 ns, while Cl ions exchange faster than the nanoseconds timescale. We find that the bound Na ions replace ordered water around the carbonyls. The net linear charge density near the carbonyl groups stays positive, regardless of the presence of excess salt in the solution.

Keywords

ion binding; survival time; electrostatic potential; antimicrobial peptides; molecular dynamics simulations, hydration

I. INTRODUCTION

Biological membranes are composed of a complex mixture of lipids, ions, proteins and other biomolecules. The interaction of proteins with the cell membrane will depend on the local ionic environment, the nature of the protein, and the lipids. Of particular interest is the binding of antimicrobial peptides and cell penetrating peptides to lipids. Antimicrobial peptides have attracted much attention because of their potential use as new antibiotics [1, 39]. The antimicrobial peptide protegin-1 (PG-1) is an example of an, arginine rich, positively charged peptide. A balance of cationic and hydrophobic groups in PG-1 appears to be responsible for its ability to kill bacteria through membrane disruption [31]. Solid state NMR experiments have suggested that eight PG-1 chains form a stable toroidal pore when inserted into the lipid bilayer [32, 33]. When inserted into a bilayer, the Arg side chains interact with the phospholipid head groups and the hydrophobic regions interact with the carbon chains. The binding of the Arg side chains to the phospholipid headgroups affects the interaction of the lipids with ions, and the presence of ions may reduce the binding of peptides to lipids. Recently, based on molecular dynamics simulations, Herce proposed a mechanism for the translocation of cell a penetrating peptide, the HIV-1 tat-peptide, which is also an Arg rich peptide [14]. A similar mechanism has been observed for a polycationic peptide, (*Arg*)₉ [13]. In this model, the mechanism of insertion includes a phosphate group mediated insertion of Arg residues into a lipid bilayer. A feature of the pre-insertion stage is that Arg sidechains bind to the lipid phosphate and carbonyl groups— the region to which Na cations have been found to bind when excess salt is present. The response of a lipid bilayer

to ions will give insights into how these bilayers will respond to binding of polycationic peptides, like the ones described above.

Cations are known to bind to zwitterionic lipids, while anions behave like an ionic cloud, without specific binding [19, 21, 24, 34]. Computer simulations have become a useful tool to study lipid bilayers under various ionic and temperature conditions [20]. Computer simulations have shown that Na cations bind to lipids containing PC headgroups [4-8, 11, 12, 18, 22, 25, 26, 29, 36, 37]. The understanding of the coordination of Na to PC should provide insight about the balance between interactions leading to ion binding, lipid and ion solvation and lipid structural changes. Here we study a simple system composed of a zwitterionic lipid, DPPC, in aqueous NaCl excess salt. Biological membranes are composed of a complex mixture of charged and neutral lipids with different size head groups and carbon chains, and proteins, surrounded by an ionic environment. Therefore, our model system is an oversimplification of the cell membrane. The complexity of lipid bilayers and the long relaxation times of lipid molecules and bound ions require long simulations in the 200 nanosecond timescale. Although the results of these simulations depend on the choice of force fields and the treatment of long range electrostatic interactions, the findings regarding the binding of ions appear to be robust [7, 8].

Here we explore the behavior of a DPPC lipid as we increase the number of excess NaCl ion pairs from 0 to 280, with ion pairs to lipid ratios from 1:16 to over 2:1 and corresponding to ion concentration up to 1.63 molal excess NaCl. We show that the bilayer area per lipid decreases with increases of excess salt. The reduction of area per lipid and ion binding effects are saturated at higher excess content. We describe the effect of ion binding in the linear charge distribution and the electrostatic potential of the systems. In particular, we find that Na ion binding to the lipid carbonyl groups replaces ordered water molecules that provided a similar charge density near the carbonyl region. This interplay between ion binding and hydration may be relevant for understanding the binding of highly charged antimicrobial peptides to lipid bilayers.

II. METHODS

Molecular dynamics simulations were performed to study the interaction of excess NaCl ion pairs with a zwitterionic DPPC lipid bilayer. Excess NaCl ions were placed at random the water solvent in a periodically repeating box containing a pre-equilibrated lipid membrane (at 323 K) composed of 128 DPPC lipids and water molecules. We simulated systems containing 8, 16, 35, 70, 140, 210 and 280 excess NaCl ion pairs, corresponding to ion concentration of 0.045 to 1.63 molal excess NaCl, as well as a system without excess ions. All systems contained around 10k water molecules. The simulations were performed by using the GROMACS package [15] on a cluster of Opteron processors. The overall temperature of the water, lipids, and peptides was kept constant, coupling independently each group of molecules at 310 K and 323 K with a Nose-Hoover thermostat [16, 23]. The pressure was coupled to a Parrinello-Rahman barostat at 1 atm separately in every dimension. The temperature and pressure time constants of the coupling were 0.1 and 5 ps, respectively. The integration of the equations of motion was performed by using a leap frog algorithm with a time step of 2 fs. Periodic boundary conditions were implemented in all systems. A cut-off of 1 nm was implemented for the Lennard Jones and the direct space part of the Ewald sum for Coulomb interactions. The Fourier space part of the Ewald splitting was computed by using the particle-mesh Ewald method [9], with a grid size $64 \times 64 \times 112$. The box length for the system with no ions is $6.13 \text{ nm} \times 6.13 \text{ nm} \times 12.3 \text{ nm}$. We used the SPC/E [2], the lipid parameters were from Berger et al. [3], and the ions and peptide parameters were taken from the GROMOS force field. The ionic systems were simulated for periods exceeding 200 ns, each. One system (35 NaCl excess ions) showed a slow equilibration and

was simulated for 500 ns. All averages are calculated during the production stage. Table I summarizes the composition of the PC/NaCl/Water systems simulated.

We monitored the area per lipid and number of ions bound to the carbonyl oxygens of the DPPC lipid. We consider a Na ion to be bound to the lipid if the distance between the ion and the carbonyl oxygen atoms is within 0.32 nm. The radial distribution function show that most Na ions coordinated to carbonyl oxygens are within 0.25 nm. We consider Cl^- ions to be bound if they are within 0.65 nm from the nitrogen atom of the choline group. The distance of 0.65 nm was determined from the ion-atom radial distribution functions, shown in Figure 1. We calculated the survival times of ions bound to their respective partners in the lipid using the survival probability function described by Garcia and Stiller (Garcia and Stiller, 1993) [10]. Briefly, we monitored the residence time series and studied the distribution of residence times of the ions. We used a 10 ps window centered at a given time to filter the residence of each ion at that time. If an ion is bound the majority of the times within the window, the ion is considered to be bound at the center of the window. This window was moved along the trajectory and for every ion.

The lipid tail $C-D$ order parameters are calculated at two temperatures (310 K and 323 K) at various concentrations of excess ions to monitor the effect of salt in ordering the tails. At 310 K the system should be close to the experimental gel to liquid crystal transition, but we do not know the transition temperature for the force field and system size (128 lipids) used in our calculations. The lipid tail order parameters were calculated using the program *g_order* in Gromacs. Given that simulations do not represent the lipid chain hydrogen atoms explicitly, we assume that the hydrogens are positioned in their ideal tetrahedral configuration relative to the lipid chains. The order parameters were calculated for the Sn-1 and Sn-2 tails separately. We also calculated the parameters for the two leaflets separately. The difference in the S_{CD} order parameters for the Sn-1 tails in each leaflet are used as an indicator of errors in the calculations.

The electrostatic potential is calculated using the equation [6, 18]

$$\phi(z) - \phi(0) = -\frac{1}{\epsilon_0} \int_0^L dz' \left(\int_0^{z'} \rho(z'') dz'' + D_0 \right) \quad (1)$$

where

$$D(z) = \int_0^z \rho(z') dz' + D_0 \quad (2)$$

is the electric displacement field and

$$D_0 = -\frac{1}{L} \int_0^L dz \int_0^z \rho(z') dz' \quad (3)$$

is the displacement constant. L is the length of the simulation box in the direction perpendicular to the bilayer surface. Notice that including D_0 in the calculation is equivalent

to replacing $\rho(z) \rightarrow \rho(z) - Q_{tot}/L$, where $Q_{tot} = \int_0^L \rho(z) dz$. These equations are valid for systems under periodic boundary conditions, with a zero potential at infinity. These equations are integrated numerically. We imposed electroneutrality

$$Q_{tot} = \int_0^L \rho(z) dz = 0 \quad (4)$$

within the accuracy of the integration method used by redefining the density

$$\rho(z)_{num} = \rho(z) - Q_{tot}/L. \quad (5)$$

The value of $|Q_{tot}| < 10^{-4}e$. When calculating the contribution to the electrostatic potential coming from different components of the system, we group Na and Cl ions together such that the overall subsystem is electroneutral. Another option would be to subtract a uniform charge density from each component density profile, as is done in Ewald summations [17].

III. RESULTS AND DISCUSSION

First we studied the coordination of ions to DPPC. Figure 1 shows the radial distribution functions for Na to the DPPC carbonyl groups and for Cl to the Nitrogen atom in the choline group, for the system with 280 excess ions. The plot shows that Na ions are distributed with a sharp peak near 0.25 nm, and broader and smaller peaks at larger distances. Based on this result we use a distance of 0.32 nm to describe the first coordination shell around Na ions. The radial distribution function of Cl around the choline group, shown in Figure 1b, shows a peak centered near 0.5 nm, and the peak extends to 0.65 nm. We define a first coordination shell radius for Cl ions around the choline group to be 0.65 nm. The ions within these distances from the phospholipid carbonyl and the choline nitrogen atoms are considered bound in our subsequent analysis. For both Na and Cl ions we used a distance slightly larger distance than the first coordination shell to identify bound ions.

We monitored the steady state area per lipid and the number of bound Na and Cl ions for various systems. Figures 2a-b show the number of coordinated Na and the area per lipid for a system containing 70 excess ions, at 323 K. The number of coordinated Na ions and the area per lipid reach steady state values after 50 ns or more. Based on these results we selected the the first 100 ns of every calculation as the equilibration stage, except for the system with 35 excess ions (at 310 K), which we simulated for 500 ns, and use the first 250 ns as for equilibration. Details of the analysis are summarized in Table II. Figure 2c shows that many Na ions (~14) remain bound through the 150 ns production stage of the simulations, and many Na ions (~30) never bind to the lipid. In contrast, all Cl ions are bound to the choline nitrogen atom for an average of 25% of the configurations. Figure 2d shows the distribution of residence times for Na ions. This distribution is dominated by residence times of 1 ns. However, fewer events of 100 times longer residence times dominate the survival probability distribution. Figure 3 shows the survival times for Na and Cl ions for various systems. The survival times for Na ions follow a single exponential decay with decay times in excess of 40 ns, independent of ionic concentration. We consider the survival time to be a lower bound, since the simulations are not sufficiently long to get a better estimate. For Cl ions the survival time is below 0.1 ns. At higher concentration, there is a small number of ions that survive for a few ns. Our results suggest that Cl ions are acting as an ionic cloud around the lipid choline group. The number of Cl ions increase at high excess salt, in response to larger charge density that results due to the binding of Na ions to the lipid carbonyls.

Figure 4a shows that the area per lipid decreases as we increase the number of excess ions in the system, at two temperatures. We see that the area change is more pronounced at 310 K than at 323 K. Figure 4b shows that, as expected, the number of bound Na and Cl ions also increases with the number of excess ions in the solution. However, we observe a plateau in the number of bound ions when the number of ions exceeds half the number of DPPCs. At 310 K we get a plateau at ~32 ions, which gives one bound Na ion per four lipids. At 323 K the number of bound Na ions plateaus at ~23, which gives one ion per every 6 lipids. The number of bound Cl ions is slightly less than the number of bound Na at high excess salt.

The number of bound Na ions, N_b as a function of excess salt concentration, c_{ex} , can be described by the Langmuir binding isotherm,

$$N_b = \frac{NK_i c_{ex}}{1 + K_i c_{ex}}, \quad (6)$$

where K_i is the binding constant of the ions, and N is the number of sites where Na can bind. Fitting of this equation to the Na binding data at 310 K, shown in Figure 4b, gives $N = 40.8$ and $K_i = 4.94/M$. Our fitting gave a maximum of ~ 41 Na bound to 128 lipids, which is close to a ratio of [Ions:Lipid] = [1:3]. The fitting of the simulations at 323 K, gives $N = 32$ and $K_i \sim 7/M$. The calculated Na binding coefficients, K_b , are much larger than the value of $0.15 - 0.61/M$ calculated by Pandit [25] and the measured value of 0.15 ± 0.1 by Tatulian [34]. We will discuss this after we describe the ion distributions around the lipid headgroups.

The $|S_{CD}|$ in order parameters for the DPPC lipid bilayers our simulations at two temperatures are shown in Figure 5. At low excess salt (16 ion pairs) and 310 K the order parameters are slightly larger than for the same system at 323 K. For a system with 16 excess ion pairs (0.089m) the maximum value of S_{CD} are 0.244 ± 0.001 at 310 K and 0.226 ± 0.002 at 323 K. These values agree with calculated values reported by others in systems with no or low excess salt [11, 18, 35] and measurements [28, 30]. As the excess salt is increased we see a larger increase in the order parameter at 310 K. This increase shows a significant increase in the ordering of the lipid tails, and probably signals a trend of the system toward an ordered gel phase. However, our 250 ns simulations are not long enough to sample the phase transition. At 323 K we also observe an increase in the order parameters, although not as large as at 310 K. The general trend shown by the order parameters is that the lipid order increases with excess salt.

Figure 6 shows the charge density profiles and the contribution from different components of the system for systems containing 0, 16, 35, 70, and 140 excess ion pairs. Figure 6a shows the total charge density profile. This profile is complex, but some features are evident. (i) If we take the peaks of maximum charge density as a measure of the width of the bilayer, it is clear that the lipid system with no excess ions is narrower, consistent with a decrease in the area per lipid as the number of excess ions increases. (ii) The height of the positive charge density peaks near $z \sim 4.5$ and $z \sim 8.5$ nm do not change with excess salt. Interestingly, in absence of ions this charge density is contributed by the solvent. In presence of excess Na, this charge density is provided by bound Na ions. (iii) The height of negative density peaks ($z \sim 3, 5, 8$ and 10 nm) increase with excess salt.

The origin of these changes can be better described if we consider the contribution from the lipid, ions, and water to the total charge density, shown in Figures 6b-d. The contribution to the charge density from DPPC (Figure 6b) shows that the charge density peaks are narrower and larger in presence of excess salt. The density peaks magnitude increase with excess salt. The charge distribution coming from ions (Figure 6c) becomes larger and more sharply peaked as the number of excess ions is increased. We see that the number of Na ions increase near the phosphate and carbonyl groups. This increase is more distinct than the increase in size of the negative ion distribution near the lipids. This is consistent with the observation that Na ions bind the lipid even at low excess salt, while Cl ions bind at higher concentrations. The binding of Na provides a net surface charge density to the bilayer. Then the Cl ions, which do not bind deeply into the bilayer, act as a neutralizing ionic cloud around this charged surface. We find that there is a significant charge density contribution from the water molecules (Figure 6d). If we take the solvent contribution in the absence of excess ions as a reference, we see that there is a large decrease near $z \sim 5$ and 8 nm, where Na ions will bind in presence of excess salt. In absence of excess salt, water will adopt an

orientation that results in this increase in charge density. We also see an increase in charge density near $z \sim 3$ nm, where the Cl density increases with excess salt. The changes in charge density due to the solvent are as large as the total charge density, indicating that the solvent polarizes near the bilayer significantly.

The number density of Na and Cl ions, in Figures 6e-f, show a depression, relative to the average (bulk) value, in the number of Na ions near the lipid-water interface ($z \sim 4$ nm and $z \sim 9$ nm). We also observe an increase, relative to the bulk value, in the Cl ion density near the lipid-water interface. This is consistent with the expected distribution of ions near a positively charged surface. This positive charge density results from the binding of Na to the lipid carbonyls. Notice that these ions remain bound for most of the production stages of the simulations, while the Cl ions and other Na ions not directly coordinated to the carbonyls but close to the surface, exchange quickly and behave like an ionic cloud. These observations can help us understand the discrepancy between the measured binding coefficients of ions measured by Tatulian [34], and our computed values, which are much larger. The measured coefficients were obtained by analyzing the measured ζ potentials in terms of the Guy-Chapman theory. The ionic clouds follow the GC behavior, but not the ions bound to the carbonyls. Similar conclusions have been reached by Yi et al., [38] although for anionic lipids. Yi et al. [38] analyzed the Na binding to DOPG bilayers obtained utilizing simulations that yielded results for two terms— one arising from the bound Na ions and another from the diffuse ionic cloud— and found that the ionic cloud term followed the Guy-Chapman theory without any need for fitting. Their system is different than ours since they simulated an anionic lipid (DOPG) in presence of a large number of Na counterions (one per PG group), and excess salt. For a large number of Na counterions the binding of Na to the carbonyls will be saturated. Yi et al. found that these bound Na ions reduced the lipid surface charge density to 1/4 its value, and the ionic cloud distribution followed the GC description for this reduced surface charge density. In our simulations, if we separate the bound ions from the ionic cloud effect, we find that the lipid with bound Na ions has a net positive surface charge density, and as a consequence the Cl ionic cloud is enhanced at the surface, while the Na ionic cloud is reduced from its bulk value at the surface. The analysis for the DPPC is more complex since we do not saturate the Na binding capacity even with excess salt above 1 molal—when ions are expected to be strongly correlated and the GC theory fails.

Figure 7a shows the electrostatic potential as a function of the coordinate perpendicular to the bilayer plane, $\phi(z)$, calculated from the total linear charge density obtained for the system without excess salt. The electrostatic potential is defined by Eqn. 1. The displacement constant, D_0 , is 0.0046 V/nm. The electrostatic field at the center of the bilayer ($z \sim 6.4$ nm), $\phi \sim 0.585 \pm 0.025$ V, when taking the reference potential to be zero in the solvent region, far from the bilayer. Here the error is estimated from the difference in potential obtained from analyzing the simulation with and without invoking the symmetry of the system to $(x, y, z) \rightarrow (x, -y, -z)$. The asymmetry in the density distributions arise due to limited sampling (~ 250 ns). Figure 7b shows the resulting electric field, calculated with the symmetric charge distribution.

Figure 8 shows a decomposition of the electrostatic potential into contributions from different components of the system. In this figure we show the linear charge density profiles (Figure 8a) for a system containing 70 excess ion pairs, simulated at 323 K. These charge distributions are not symmetric. The features shown here are similar to what was shown in Figure 6. Figure 8b shows the contribution to the electrostatic potential from various components. The black curve shows the net potential, which is much smaller than most of the individual components, and of the same order of magnitude as the contribution from the solvent (red curve). In particular we find that the Na and Cl ions, together, have large

contributions to the potential. At large distance from the bilayer, these ion contributions cancel each other. The solvent contribution to the field extends from 2 to 10 nm, and affects the potential away from the bilayer. Part of this contribution is canceled by ions. Figure 8c shows a comparison of the electrostatic field, $\phi(z)$, for systems without excess ions and for a system with an excess of 70 ions. The net potential difference measured at the center of the bilayer is 0.075 V. To further explore the source of the potential differences we also calculated the potential contributions by system components for a system without excess ions. Interestingly, the solvent component is very similar to the ionic component in the system with excess salt. This result shows that in the absence of excess ions (and possibly in the absence of strong cationic binding, like for K) water is structured around the phospholipids and partially cancels the large electrostatic potential produced by the phospholipid charge distribution. We saw before that in absence of excess ions, water provides a positive charge density near the carbonyl groups and a negative charge density near the choline groups.

IV. CONCLUSIONS

Our results show that Na ions bind tightly, with long survival times, to the carbonyl groups. Cl ions bond to the choline groups, but as an ionic cloud. The residence time of Cl to choline groups is below the 10 ns timescale. We observed a saturation in the number of Na ions bound. At low excess salt (8 and 16 excess pairs) almost all Na ions are bound to the lipid carbonyls, while Cl ions do not coordinate strongly to choline groups. At high excess salt, the number of bound Na ions saturates to ~32 ions at 310 K and ~23 at 323 K, with a ratio of 1:4 Na:lipid at 310 K and 1:6 at 323 K. The effect of excess ions in the lipid bilayer is such that the area per lipid decreases with increasing excess ions. This effect is more distinct at 310 K where ions have been shown to increase the order of the lipid tails. The binding of ions to the carbonyl groups and the long residence times of these ions make an effective lipid-bound Na ion system that presents itself as a positively charged bilayer to the fast relaxing Cl ions and water molecules. At low excess salt, most positive ions are bound to the bilayer and the effective surface charge density of the bilayer increases with excess salt. At higher salt, the number of bound ions is saturated and the system behaves like a positively charged surface in presence of co-ions and an excess salt solution, which is lower than the bulk density. At 310 K, the positive surface charge density is $\sim 0.6 \text{ e/nm}^2$.

Our results agree with previous simulations on similar systems [5, 7, 8, 11, 18, 29]. The differences with Pandit et al. [27] can be easily explained by the slow relaxation time of the system toward steady state and the length of the simulations. We observed that the area per lipid and the number of bound Na ions converged in timescales exceeding 100 ns. We observed the Na ion binding and the reduction in surface area per lipid. We find that Na ions have a very strong affinity for DPPC carbonyl groups. This affinity has been studied by others in terms of their force field dependence [8]. Although the specifics of the Na binding are different for different models, all force fields show preferential binding of Na over K, and a variation in Na binding between 0.09 to 0.24 ions per lipid [8]. At 0.2 M excess salt and 323 K (the system with 35 excess ions) we observed that 18 ions bound to the lipid carbonyl groups (20 if we consider all oxygens in the lipid). This gives a binding of 0.16 Na ions per lipid and an area per lipid of 0.58 nm^2 in agreement with the values of 0.17 and 0.58 nm^2 reported by Cordomi [7]. Recently Cordomi et al reported a binding of 0.22 Na per lipid and an area of 0.54 nm^2 per lipid, which are larger than our values [8].

Water orientation also plays an important role in the balance between charged groups in the lipid. We found that in the absence of excess ions water is structured around the phospholipids and partially cancels the large electrostatic potential produced by the phospholipid charge distribution. The contribution of water to the electrostatic potential

opposes the effect of the lipid and is of almost equal magnitude (~ 10 V). This cancelation gives a net electrostatic potential at the center of the lipid of ~ 0.6 V. In absence of excess ions, water provides a positive charge density near the carbonyl groups and a negative charge density near the choline groups. In presence of excess NaCl, Na ions bind to the lipids and water does not provide the same effect as when there are no excess ions. In presence of excess salt, charge density from water molecules contributes a smaller potential (~ 0.5 V), while the potential resulting from the excess ions cancels most of the potential contribution resulting from the lipids charge density.

The interplay between water and ions should be important when considering the binding of positively charged peptides to DPPC. The binding of Na gives a net positive surface charge density to the lipid, and as a result positive peptides should not bind. Simulations of (Arg)₉ and the tat peptide have shown that Arg side chains prefer to bind to the phosphates and carbonyl groups in the lipid [13, 14]. In presence of excess salt, the Arg side chains may compete for the same binding sites as the Na, which may result in an attraction of these peptides to the lipid. The competition of charged peptides for Na binding sites merits further studies.

Acknowledgments

This work is being funded by NIH grant GM086801. JRR was supported by the NSF award DMR 0850934 REU site at Rensselaer. The authors acknowledge Kun Huang, Henry Herce, and Alan Chen for advise, comments and suggestions.

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FIG. 1.

a) Radial distribution function of Na ions around the lipid carbonyl oxygen atoms. (b) Radial distribution function of the Cl ions around the choline nitrogen atom. The distributions are normalized by the bulk density of ions. The distributions were calculated from configurations during the last 5 ns of the simulation for a system with 280 excess Na and Cl ions. These distributions suggest that the first coordination shell are around 0.3 nm for Na and 0.65 nm for Cl ions. The residence of ions within these first coordination shells is used to calculate the survival time of the ions.

FIG. 2.

(a) Number of bound Na ions to the DPPC lipid bilayer as a function of simulation time for a system with an excess of 70 ion pairs. b) Area per lipid as a function of simulation time. The number of bound ions and area per lipid reach a steady state after 50 ns of simulation. Averages shown in c-d are calculated over the last 150 ns of the simulation. c) Percentage of the time that each of the 70 Na (solid line) and Cl (dashed line) ions remained bound to the lipid bilayer. The occupancy times are sorted from largest to smaller. The occupancy time for Cl ions shorter, but all ions bind at some time during the simulation. d) Distribution of residence time of ions among the phosphates. The majority of the Na binding events last 1-10 ns. However, fewer events that last for 100s of ns dominate the survival distribution. The dashed line shows the product (not to scale) of the distribution of times by the time, $t * P(t)$, which is dominated by long residence times. The temperature is 323 K.

FIG. 3.

Survival function for ions coordinated to the DPPC lipid bilayer. The plot shows curves for Na and Cl ions at 16, 35, 70, and 140 excess ion pairs. The intercept at short time corresponds to the average number of ions bound to the bilayer. Na ions survive for times similar to half the production time in the simulations. We consider these times to be lower bounds, since longer simulations produce longer survival times. Cl ions exchange faster and have survival times below 10 ns. There are two characteristic survival times for Cl, one ~ 0.1 ns, corresponding to a cloud of ions surrounding the bilayer. Another time, $\sim 1 - 10$ ns, corresponding to tightly coordinated Cl ions, is present at higher salt content.

FIG. 4.

a) Area per lipid as a function of excess NaCl ions. We use the number of excess ions in the x-axis. b) Number of ions coordinated to the phosphate P atom (for Na) and the choline N4 atom (for Cl). Na ions are considered coordinated if they are within 0.32 nm of any DPPC carbonyl oxygen atom. Cl ions are considered coordinated if they are within 0.65 nm to any nitrogen atom of the choline group in DPPC.

FIG. 5. $C-D$ order parameters, S_{CD} , for DPPC lipid bilayers at various excess salt contributions, at (a) 310 K and (b) 323 K. At a temperature of 310 K there is a marked change in S_{CD} with increases in excess salt. At 323 K there is also an increased in chain order, although it less pronounced than for 310K. The scales in a-b are kept the same for comparison.

FIG. 6.

Linear charge density, $\rho(z)$, for systems containing 0 (red line), 16 (green line), 35 (blue line), 70 (magenta line) and 140 (cyan line) excess ion pairs. The figure shows the density profiles for (a) the total charge density, (b) the charge density due to DPPC lipids; (c) the charge density due to Na and Cl ions, (d) the charge density due to the water solvent, (e) the number density, $n(z)$, of Na ions, and (f) Cl ions. Notice that the number density of Na ions is depressed from the bulk value near $z=4$ and 9 nm, and the number density of Cl ions is enhanced near $z=3.5$ and 9.5 nm. These features are common of ions near a positively charged surface.

FIG. 7.

(a) Electrostatic potential for a lipid bilayer, without excess ions. The blue line is the potential with the calculated charge density. The green line shows the potential for the charge density for the bilayer rotated by 180 Deg. about the x- or y-axes (i.e., $(x, y, z) \rightarrow (x, -y, -z)$ transformation), and the black line shows the potential for a symmetrized charged density (average of the two other distributions). The difference in these profiles give an estimate of the errors in the simulations. Here the difference in potential at the center of the bilayer is 0.025 V. (b) Electric field along the z -direction (in V/nm).

FIG. 8.

(a) Contributions to the total linear charge density arising from the whole system (black), lipids (blue), solvent (red), Na (green) and Cl (magenta), for a lipid bilayer with 70 excess ion pairs. (b) Contributions to the electrostatic potential arising from the distribution of all atoms in the system (black), lipids (blue), solvent (red), Na (green) and Cl (magenta), for a lipid bilayer with 70 excess ion pairs. (c) Electrostatic potential for a lipid bilayer, with 70 excess ion pairs. The red line show the electrostatic potential for the system with no excess ions. The difference in potentials near the center of the bilayer (6.4 nm) is 0.07 V. (d) Contributions to the electrostatic potential in the system with no excess salt. The color code is the same as in (b). Notice that the electrostatic potential contribution from water in the system without excess salt is similar to that of ions in the system with excess salt.

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TABLE I

Composition of DPPC/water/NaCl systems simulated

DPPC	SPC/E	c_{ex} (molal)	Na^{+}	Cl^{-}	t_{sim} (ns)
128	10235	0	0	0	105
128	9980	.045	8	8	255
128	9980	0.089	16	16	255
128	9980	0.195	35	35	505
128	9980	0.390	70	70	255
128	9840	0.791	140	140	255
128	9700	1.23	210	210	205
128	9560	1.628	280	280	255

TABLE II

Ion Binding and Survival Time Statistics

Na ⁺	T (K)	t_p (ns)	Area/I (nm ²)	Bound Na	Bound Cl	t_{surv} (ns)
8	310	150	0.583	7.0	1.3	63
16	310	150	0.572	12.3	3.6	55
16	323	150	0.605	11.8	3.6	54
35	310	250	0.512	19.7	8.5	109
35	323	150	0.578	17.8	14.8	50
70	310	150	0.497	28.1	17.3	69
70	323	150	0.558	24.5	22.2	42
140	310	100	0.500	32.0	25.7	42
140	323	150	0.552	26.6	23.7	43
210	310	100	0.477	35.3	35.1	51
280	310	100	0.473	35.9	35.6	36
280	323	100	0.518	33.1	33.1	36