INTERACTIONS OF MOLECULAR IONS WITH MODEL PHOSPHOLIPID MEMBRANES

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Dedicated to Dr. Zdeněk Havlas on the occasion of his 60th birthday.

The affinities of a series of biologically relevant ions for a hydrated phospholipid membrane were investigated using molecular dynamics simulation. Interactions of molecular ions, such as guanidinium, tetramethylammonium, and thiocyanate with the bilayer were computationally characterized for the first time. Simulations reveal strong ion specificity. On one hand, ions like guanidinium and thiocyanate adsorb relatively strongly to the headgroup region of the membrane. On the other hand, potassium or chloride interact very weakly with the phospholipids and merely act as neutralizing counterions. Calculations also show that these ions affect differently biophysical properties of the membrane, such as lipid diffusion, headgroup hydration and tilt angle.

Keywords: Biophysics; Molecular dynamics; Phospholipids.

Cellular membranes represent a semipermeable barrier separating the cytosol from the extracellular environment. One of the most striking differences between the intra- and extracellular solutions is in the cationic content. The ionic strength of these solutions of about 150 mM is maintained dominantly by potassium at the inside and by sodium salts at the outside of the membrane[1]. Another strong ionic gradient across the membrane (exploited in signaling) is that of calcium, which is almost absent from the cytosol. This ionic imbalance is maintained at a considerable energy cost by an intricate system of proteins forming selective ion channels and pumps in the membrane[1].

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On top of ion channeling and active pumping through membrane proteins ions can interact directly with the lipid molecules, particularly with their polar or charged headgroups. It is not very well established whether these interactions could assist in selection process of ion channels and pumps. A necessary condition for being able to address this question is to quantitatively establish affinities of biologically relevant ions for the most common lipid molecules forming cellular membranes. For the simplest ions, i.e., alkali cations and halide anions this issue has been addressed in numerous computational and experimental studies during the last decade[2–16].

First molecular dynamics (MD) simulation studies focused on the interaction of alkali cations with model phospholipid membranes, formed from DOPC or POPC [2,3,6,13,14]. These calculations showed that smaller cations, such as Li^+ and Na^+, exhibited an appreciable affinity for the lipid headgroups, while larger cations like K^+ or Cs^+ preferred to stay in the aqueous solution. The small cations were attracted both to the carbonyl and phosphate moieties of the headgroup, with the relative strength of the two being to some extent force field dependent. Simulations employing nonpolarizable potentials did not show any strong affinity of halide anions for the phospholipid membrane. In contrast, experiments pointed more to an anionic rather than cationic effect on the membrane, particularly for large soft anions, such as iodide[9,15]. This situation was to a large extent reconciled by polarizable MD simulations, which showed that indeed iodide can penetrate all the way to the onset of the hydrophobic region of the bilayer[16]. Simulations also allowed to formulate the basic principles which govern the interactions of alkali halides with zwitterionic phospholipid membranes. The net effect was shown to be a result of interactions of ions with headgroup moieties, with counterions in the solutions and of interfacial and steric interactions[16]. Many recent computational findings concerning ion-membrane interactions are summarized in the review by Berkowitz et al.[17].

While alkali halide interactions with phospholipid membranes have been studied rather extensively, little is known about the behavior of more complex molecular ions at the membrane/solution interface. To the best of our knowledge, the only such ion investigated experimentally is thiocyanate (SCN^-), which exhibits strong affinity for the phospholipid bilayer[15], while calculations are lacking completely. Here, we extend our previous studies of alkali halides at membrane surfaces to four selected molecular ions. On the anionic side, we modeled the previously experimentally studied SCN^-. Thiocyanate ion is particularly interesting since it was recently shown to

play an important role as an antioxidant which protects lung cells[18,19]. On the cationic side we chose three important molecular ions. The first one is guanidinium (Gdm^+), which is a common protein denaturant[20]. It also represents the functional side chain group of arginine, which is known to penetrate efficiently into phospholipid membranes[21]. The second cation is tetramethylammonium (TMA^+) as the smallest representative of tetraalkylammonium ions, which are used as phase transfer catalyst due to their ability to cross from aqueous to less polar liquid phases, some of them also exhibiting antibacterial activity[22]. The third cation, taken also for comparison with TMA^+, is ammonium ion (NH_4^+). Using extensive MD simulations we characterize and quantify the interactions of these ions together with their alkali or halide counterions with a model phospholipid membrane composed of DOPC molecules.

COMPUTATIONAL METHODS

The ion-membrane interactions were studied by means of classical molecular dynamics (MD) simulations. Computation setup was consistent with our previous study of ion-membrane interactions[12]. Briefly, the simulated system was composed of 72 DOPC lipid molecules, 2627 molecules of water, and ions. The DOPC phospholipid bilayer was located in the center of a slab surrounded by a salt solution. Each system contained specific salt with the corresponding ions put into the aqueous phase. The following salt solutions were studied: KCl, NH_4Cl, $(CH_3)_4NCl$, $C(NH_2)_3Cl$, and KSCN. Ion concentration was set to ~1 mol/l (i.e., 50 anions and 50 cations). For the system containing KCl additional concentrations of 0.1, 0.5, 2, and 5 mol/l were considered.

Molecular dynamics simulations were carried out employing the computational package GROMACS version 4.0.3 [23]. We used the non-polarizable, all atom, force-filed for DOPC lipid molecules which was recently developed based on the general amber force field[12,24]. Force field parameters of ions are listed in Table I. Parameters for guanidinium cation by Camilloni et al. were employed[25]. Water molecules were described with the SPC/E model[26]. In the case of 1 M KCl solution, equilibration was performed for 20 ns. The properties of the membrane (area per lipid, density profiles, etc.) were converged after this time; the convergence of membrane properties in the tens of nanosecond timescale was recently demonstrated also in another study involving all-atom force field for membrane-potassium interactions[6]. The simulation was continued for 80 ns, and this part of the trajectory was used for further analysis. The initial configurations in simu-

lations for remaining ions were taken from the system containing 1 M KCl solution after 100 ns of simulation with all potassium cations removed and the other cations with a desired concentration added to the water phase. Then 20 ns of equilibration was performed, followed by 80 ns of the production run. Equation of motions were integrated with a step of 2 fs. The NPγT canonical ensemble was used with temperature equal to 310 K and pressure equal to 1 atm, both using the Berendsen algorithm[27]. Surface pressure of 22 dyn/cm was employed in the membrane plane.

TABLE I
Force field parameters of the ions

Ion	Charge, e	σ, nm	ε, kJ/mol
K^+	1.000	0.3048655	0.41840
Cl^-	–1.000	0.43200	0.41840
NH_4^+			
N	–0.70753	0.32500	0.71128
H	0.42690	0.106908	0.06569
$(CH_3)_4N^+$			
C	0.0160	0.339967	0.45773
N	0.6960	0.324999	0.71128
H	1.0080	0.195998	0.06569

RESULTS AND DISCUSSION

Interactions of Ions with the Membrane

In this section we discuss interactions of ions with the DOPC membrane in 1 M salt solutions as obtained from the MD simulations. Figure 1 depicts the electron density of lipid molecules in the systems containing 1 M solutions of all considered salts: KCl, NH_4Cl, $(CH_3)_4Cl$, $C(NH_2)_3Cl$, and KSCN. No major structural changes in the monolayer can be observed for these salts. This conclusion is supported by relatively small changes in the area per lipid and membrane thickness (Table II). With respect to the salt-free system, the DOPC in the presence of the considered salts does not undergo any significant lateral compression or expansion; the area per lipid is virtually not changed in the considered salt solutions. Similarly, based on mem-

brane thickness data, no sizable membrane compression or expansion along the membrane normal can be observed.

Interactions of ions with individual components of a lipid bilayer can be studied by means of partial density profiles. Figure 2 shows density profiles of ions, along with the density profiles of different membrane components and water. From the point of view of ion-membrane interactions, the polar groups at the membrane/water interface are of main interest. Therefore, we

TABLE II
Area per lipid and membrane thickness for DOPC membrane in water and 1 M salt solutions

Solution	Area per lipid, nm^2 (±0.1 nm^2)	Membrane thickness, nm (±0.1 nm)
Water[12]	0.72	3.6
KCl	0.69	3.7
NH_4Cl	0.71	3.6
$C(NH_2)_3Cl$	0.72	3.6
$(CH_3)_4NCl$	0.68	3.8
KSCN	0.70	3.8

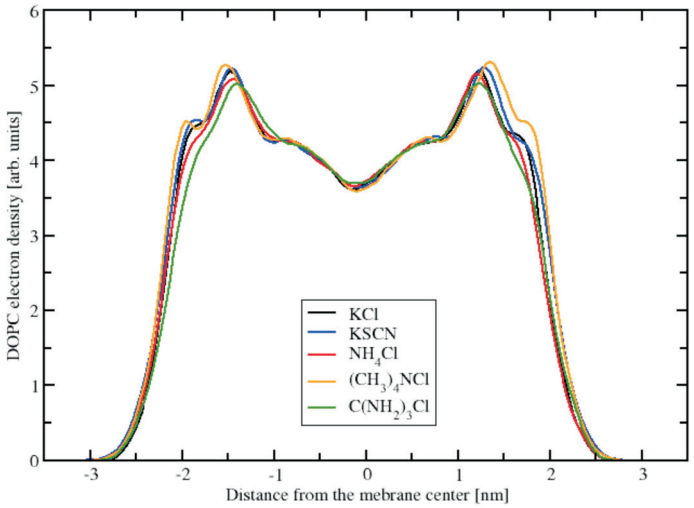

FIG. 1
Electron density profiles for DOPC lipid molecules in different 1 M salt solutions

consider also density profiles of phosphate, choline, and carbonyl groups of lipid molecules, which are the main polar components of DOPC lipid molecules. Additionally, in order to quantify ion-membrane interactions, we calculated the ion adsorption coefficients (A_i). These are defined as ratios of

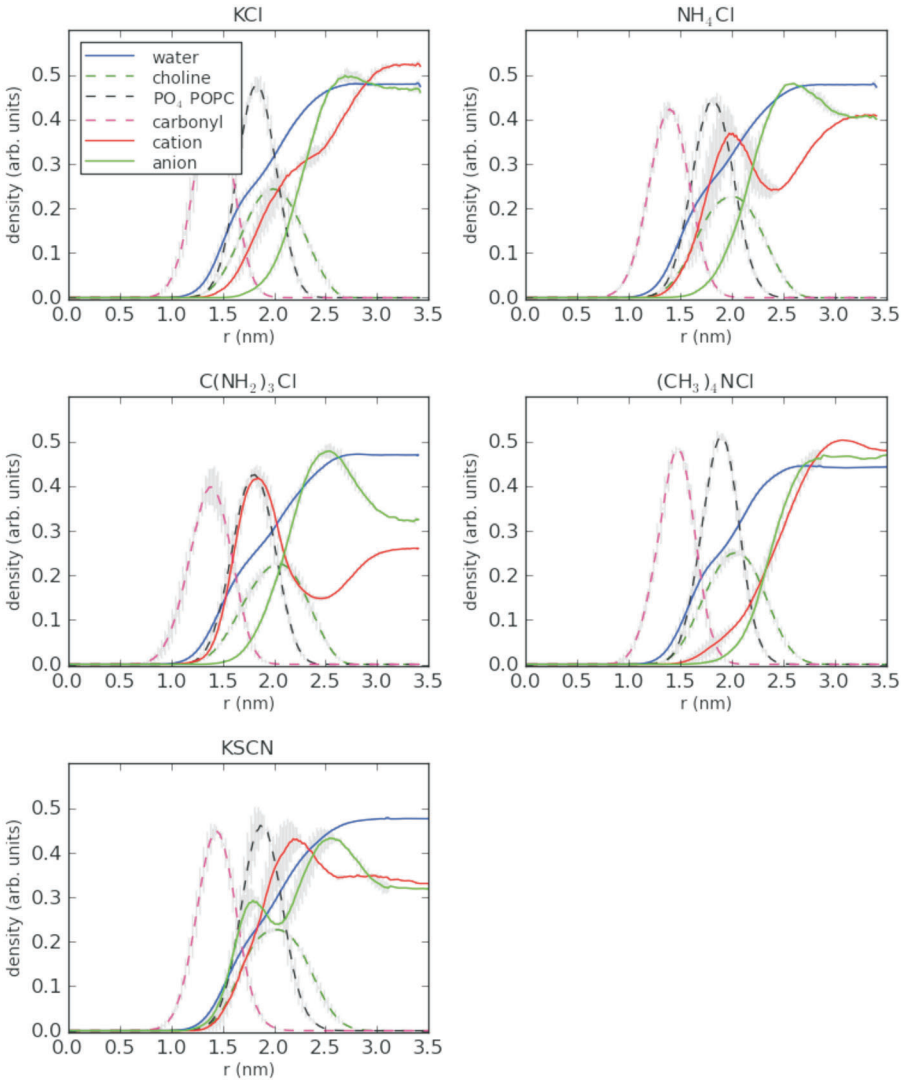

FIG. 2
Scaled number density profiles for ions, phosphate, choline and carbonyl oxygen atoms calculated for DOPC membrane in 1 M salt solutions

the averaged ion density in the region of the considered group of lipid atoms, weighted by the density of this group, to the density of the ion in the water phase. Effectively, these adsorption coefficients represent partition coefficients between the considered regions in the membrane and the water phase. In Table III adsorption coefficients for cations and anions in the region of both phosphate and carbonyl groups are presented. In 1 M KCl solution no significant adsorption of ions occurs (A_{PO4} = 0.29), although potassium is penetrating slightly closer to the membrane than chloride, predominantly towards phosphate groups of DOPC. Chloride anions are virtually absent in the headgroup region (A_{PO4} = 0.09). This is consistent with previous computational studies of membranes in KCl solutions which demonstrated that K^+, in contrast to both Li^+ and Na^+, is not exhibiting significant affinity towards a membrane[6,12].

In contrast to K^+, ammonium cation exhibits strong propensity towards the membrane, as visible in both density profiles and adsorption coefficients calculated for the NH_4Cl solution (A_{PO4} = 0.60). It occupies mainly the region of phosphate groups, however, penetration toward the region of carbonyl groups is also evident ($A_{C=O}$ = 0.11). Chloride anions are following K^+ counterions, which can be attributed to the charge neutralization effect, and is consistent with previous computational studies, employing nonpolarizable force fields, where no specific adsorption of Cl^- was observed[12]. The strongest propensity of cations towards the membrane is present in the guanidinium chloride solution. The density of Gdm^+ ion in the phosphate groups region is enhanced with respect to the water phase (A_{PO4} = 1.21).

TABLE III
Adsorption of ions in different regions of DOPC membrane in 1 M salt solutions. Adsorption coefficients are estimated with the error value of ±0.05

Salt (1 M)	Adsorption in phosphate volume		Adsorption in carbonyl volume	
	Cation	Anion	Cation	Anion
KCl	0.29	0.09	0.04	0.00
NH_4Cl	0.60	0.19	0.11	0.01
$C(NH_2)_3Cl$	1.21	0.28	0.34	0.02
$(CH_3)_4NCl$	0.13	0.06	0.01	0.00
KSCN	0.75	0.81	0.14	0.27

Additionally, the region of carbonyl groups is populated ($A_{C=O}$ = 0.34). The Cl^- density peaks outside of the membrane, close to the position where the Gdm^+ density is depleted; and it can be rationalized as the result of the charge neutralization effect.

None of the ions present in the tetramethylammonium chloride solution exhibits propensity towards the DOPC membrane. The values of adsorption coefficients for both TMA^+ and Cl^- are the lowest among the considered cations; accordingly, the density profiles of both ions decrease smoothly in the region of DOPC headgroups. A peak around r = 3.1 nm at the TMA^+ density can be attributed to a weak propensity of tetramethylammonium cation to the water/membrane interface. It should be noted, that the absence of TMA^+ adsorption in the headgroup region can be caused by a relatively high free-energy barrier for migration of the bulky TMA^+ ion from the water phase towards the phosphate and carbonyl groups in the membrane. It cannot be excluded that it was not possible to overcome this barrier at the timescale of our MD simulations. Umbrella sampling free energy calculations for such bulky cations will be performed in the future.

In the solution of potassium thiocyanate distinctive adsorption trends were observed. In the SCN^- density two maxima occur; the first one is located between the carbonyl and the phosphate region, and the second one appears outside of the membrane. The first maximum (r ~ 1.8 nm) originates from relatively strong adsorption of SCN^- in the headgroup region. This peak is followed by a maximum in the K^+ density (r ~ 2.2 nm) which occurs due to charge neutralization and interactions of potassium mainly in the phosphate region. At a larger distance from the membrane (r ~ 2.6) the second maximum in the SCN^- density occurs; accumulation of SCN^- in this region can be attributed to the neutralization of the charge of adsorbed potassium layer by thiocyanate anions. These observations are in accord with the recent vibrational sum frequency generation experimental study of DPPC and DMPE monolayers in the presence of SCN^- [28]. Two distinct populations of anion were observed there, and they were assigned to a weakly and strongly bound SCN^- at the water/lipid interface.

Influence of Salt Concentration on Potassium Cation Adsorption

We studied the influence of salt concentration on the extent of ion adsorption at the membrane. We simulated DOPC membrane in KCl solutions of 0.1, 0.5, 1, 2, and 5 mol/l concentration. Figure 3 depicts electron density profiles of lipids in each system. The corresponding values of area per lipid and membrane thickness are given in Table IV. No major structural changes

of the bilayer can be observed; main features of density profiles are constant. This is also evident from small changes in both the area per lipid and membrane thickness. At relatively high salt concentrations (2 and 5 mol/l) a small increase of the membrane thickness occurs; however, this effect is relatively low, in the range of the changes induced in the membrane by the presence of different salts at 1 mol/l concentration (see Table II).

TABLE IV
Area per lipid and membrane thickness for DOPC membrane in KCl solutions of different concentrations

KCl concentration mol/l	Area per lipid nm^2 (±0.1 nm^2)	Membrane thickness nm (±0.1 nm)
0.1	0.69	3.7
0.5	0.70	3.7
1	0.69	3.7
2	0.68	3.8
5	0.67	3.8

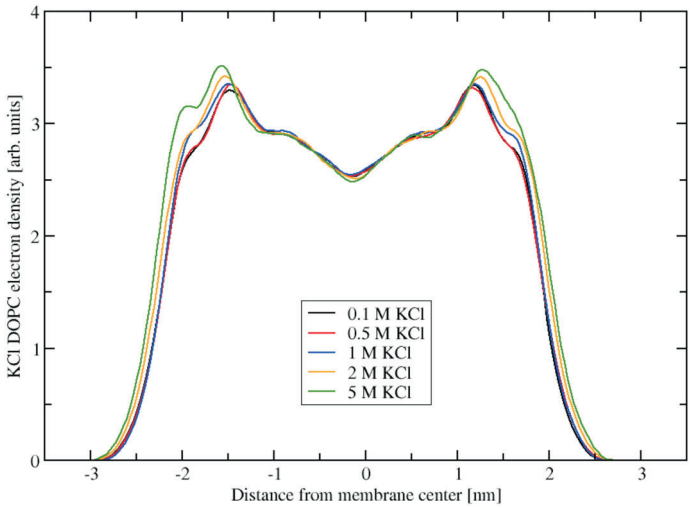

FIG. 3
Electron density profiles for DOPC lipid molecules in solutions of KCl of different concentrations

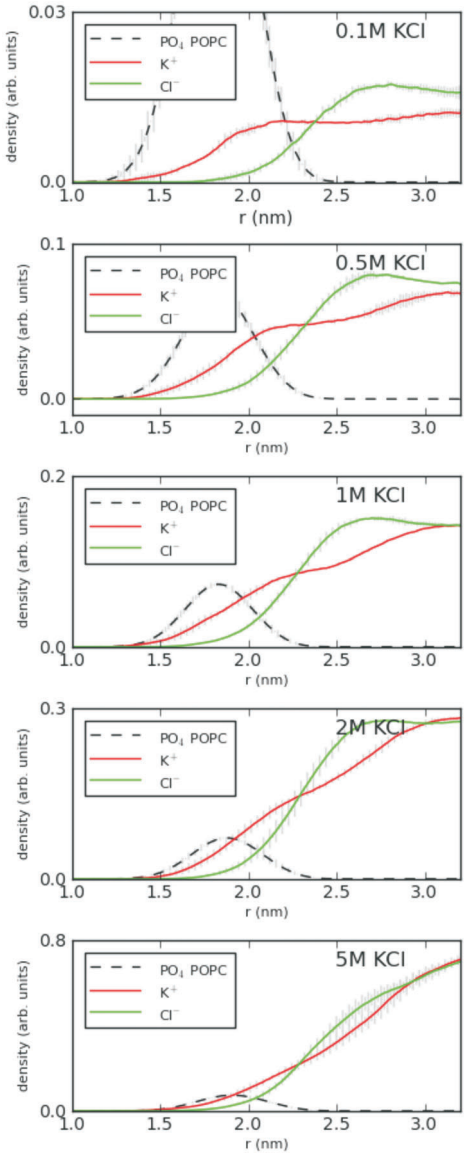

FIG. 4
Scaled number density profiles for ions and the phosphate group calculated for DOPC in KCl solutions of different concentrations. Note the different scale of the density axis and the corresponding decrease of the phosphate peak height. For presentation purposes, the densities were rescaled with respect to Fig. 2, however, a consistent rescaling was used for all concentrations

Partial density profiles of ions along with the density profiles of phosphate groups in each system are presented in Fig. 4. Penetration of K^+ into the phosphate region occurs at each concentration. As evident from adsorption coefficients presented in Table V, potassium adsorbs in the phosphate region; to a lesser extent it also penetrates towards carbonyl groups of DOPC. Similarly to the other chloride salts considered here, in KCl solutions in each concentration Cl^- forms a neutralizing layer outside of the membrane. Adsorption coefficients reveal an interesting trend of K^+ adsorption. Namely, the relative enhancement of the K^+ density at the membrane is decreasing with increasing concentration, for instance, A_{PO4} equals to 0.50 and 0.11 in 0.1 and 5 M salt solution, respectively.

Changes in the Membrane Properties upon Adsorption of Ions

Under physiological conditions phospholipid membranes act as semipermeable barriers. Thus any changes in hydration of a membrane may be crucial for its function, as they can influence membrane's permeability not only for water but also for other polar and non-polar molecules. We analyzed hydration of the lipid headgroups by means of the radial distribution function, $g(r)$, between phosphorus atoms of lipids and oxygen atoms of water molecules. Figure 5 depicts $g(r)$ calculated for systems with 1 mol/l concentration of different salts. Structural properties of phosphate–water pairs are similar for all salts, as the locations of maxima and minima of $g(r)$ curves do not change. However, the intensity of the first peak is salt-dependent. The decrease of this intensity indicates the decrease in the

TABLE V
Adsorption of ions in different regions of DOPC membrane in KCl salt solution. Adsorption coefficients are estimated with the error value of ±0.05

KCl conc. mol/l	Adsorption in phosphate volume		Adsorption in carbonyl volume	
	K^+	Cl^-	K^+	Cl^-
0.1	0.50	0.06	0.09	0.00
0.5	0.35	0.09	0.06	0.00
1	0.29	0.09	0.04	0.00
2	0.22	0.09	0.03	0.00

number of phosphate-water contacts, in other words it indicates dehydration of the phosphate region of the membrane. The considered salts can be ordered in the following way, with respect to their tendency to dehydrate the phosphate region:

$$KCl \sim KSCN < (CH_3)_4NCl < NH_4Cl < C(NH_2)_3Cl \, .$$

It can be observed that dehydration depends mainly on the extent of cation adsorption; with the guanidinium salt dehydrating the membrane most efficiently.

Dehydration of the phosphate groups and adsorption of ions in the corresponding region of a membrane have an influence on the conformation of lipid headrgoups. In Fig. 6 the distribution of P–N headgroup angles is depicted. A cationic effect cannot be observed as the angle histograms are similar for KCl, NH_4Cl, and $(CH_3)_4NCl$. Moreover, the most probable value for these salts resembles that for DOPC in water (~67°)[12]. However, for GdmCl a strong reorientation of DOPC headgroups is evident, with the most probable value shifted to ~50°. This corresponds to rising of the headgroups with respect to both the other salts solutions and water. Inter-

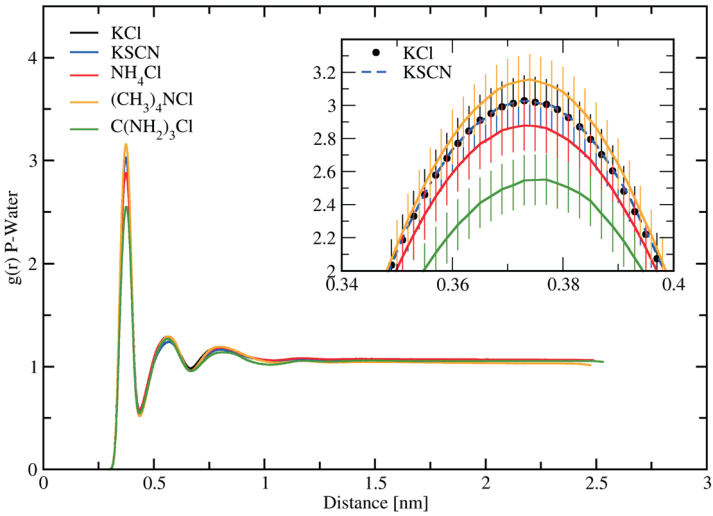

FIG. 5
Radial distribution function between phosphate atoms and water in different salt solutions. The inset shows the detailed picture of the first peak (for presentation purposes, the KCl peak is shown with points and the KSCN one with a dished line)

estingly, in the KSCN solution an increase of the most probable P–N angle can be observed (~80°) which demonstrates that in this system the DOPC headgroups are lowered with respect to both the other considered salts and water. The influence of both Gdm^+ and SCN^- on the P–N angle can be rationalized taking into account adsorption properties of both ions. Namely, the "bulky" Gdm^+ cations are strongly incorporated between the headgroups, thus they stiffen the membrane and cause the rise of DOPC headgroups. It is also likely that the repulsive Coulomb interaction between Gdm^+ and positively charged choline groups of lipid heads contribute to this effect. In the case of SCN^-, the population of ions incorporated between the headgroups is smaller compared to guanidinium ions; moreover, attractive interaction between the anions and choline groups may be responsible for the lowering of the headgroups. In the series of strongly adsorbed chlorides (KCl, NH_4Cl, and GdmCl) the influence of the cation size on the rising of DOPC headgroups can be observed. The rising of DOPC headgroups is stronger as the size of cation increases ($K^+ < NH_4^+ < Gdm^+$). This demonstrates that probably both Coulomb and steric interactions play the role in the rising of the headgroups. Another physiologically relevant property of phospholipid membranes which can be affected by ion adsorption is the

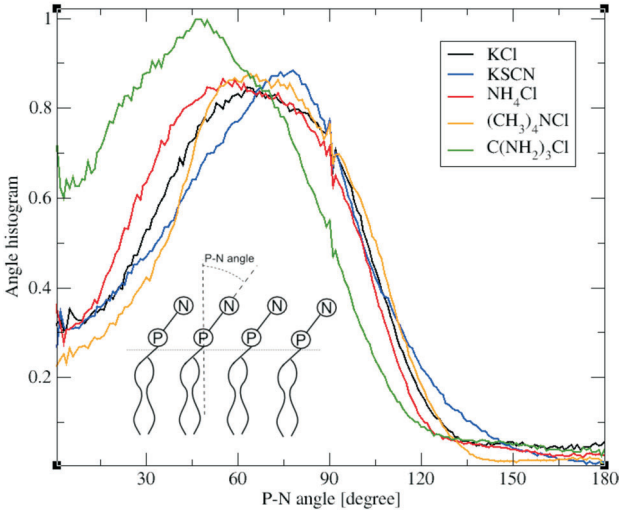

FIG. 6
Distributions of headgroup tiltangles defined as an angle between P–N vector, connecting phosphorus and nitrogen atom (see the inset) and the outward normal to the membrane. The most probable value of the angle is estimated with the error of is ±5°

lateral diffusion of lipid molecules. In Fig. 7 a lateral mean squared displacement of lipid molecules in DOPC membrane was calculated for different salts present in the system. The increase of the tendency for ion adsorption results in the decrease of the mean squared displacement of lipids, in other words, adsorption of ions in the considered salt solutions hinders lateral diffusion of lipids. Adsorption of both cations and anions influences the membrane with this respect, which is evident from the difference of the diffusion in the presence of KCl and KSCN. Tetramethylammonium chloride is causing the strongest hindering of lipid diffusion, which is probably a result of the tetramethylammonium cations accumulating right outside the membrane. In should be mentioned that the rate of lateral diffusion estimated using MD simulations is strongly dependent on the employed force field, moreover, the statistical errors in typical simulation setups are too large to accurately determine diffusion coefficients[16]. Regarding the strong influence of TMA^+ on the lateral diffusion a similar effect, i.e., hindering of the diffusion in the presence of weakly-bound ions, was observed. Clustering of lipids may also influence the lateral motion in the membrane; however, we have not observed significant clustering in the course of simulations.

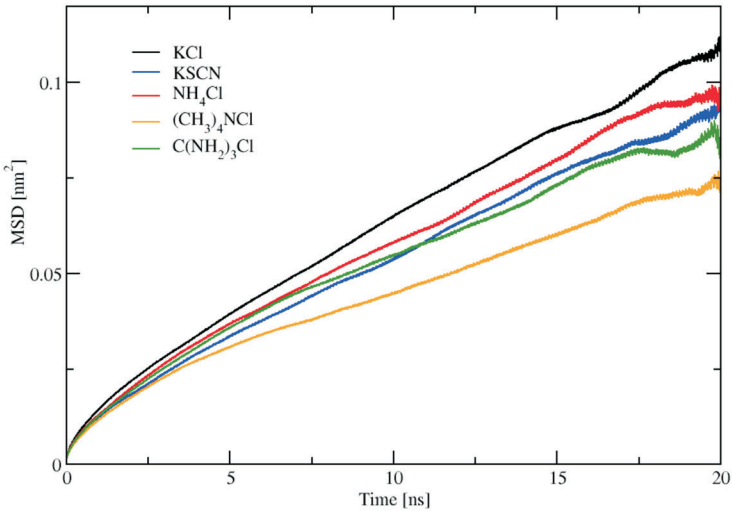

FIG. 7
Mean squared displacements of lipids in the membrane plane for different 1 M salt solutions

CONCLUSIONS

Using MD simulations we have analyzed interactions of biologically relevant ions with a model phospholipid membrane, extending previous computational studies to molecular ions. Cations interact predominantly in the regions of phosphate and carbonyl groups. The strongest adsorption, leading to surface enhancement, was observed in the case of guanidinium cations. Weaker adsorption was observed for ammonium cations. Interaction of K^+ was found to be dependent on the behavior of its counterion. Namely, in the KCl solution cation adsorption is relatively weak whereas in KSCN it is stronger, probably due to charge compensation effects in the presence of strongly-adsorbed SCN^-. In the case of TMA^+ it cannot be excluded that adsorption requires crossing of a high free-energy barrier which may originate due to steric interactions of bulky cations with the membrane, most likely with the choline groups which carry a partial positive charge. Free-energy calculations are needed to resolve this issue. It should be noted, that the partitioning of adsorbed cations between phosphate and carbonyl groups in MD simulations of phosphatidylcholine membranes is to some extent force field dependent. In the studies employing all-atom force fields, as the one used in the present work, adsorption of monovalent cations at phosphate groups is usually dominant whereas the united-atom force fields show predominant adsorption in the region of carbonyls[6]. We also investigated concentration effects for potassium chloride as a representative salt. No strong influence of concentration on the salt effect on the membrane was observed, while counterion neutralizing effects become important at higher concentrations.

Interactions of Cl^- anions are mostly governed by adsorption of countercations. Namely, no specific adsorption of Cl^- was observed; however, a neutralizing layer of Cl^- is formed in the outer region of the bilayer in those solutions for each significant adsorption of cations occurs. Chloride anions are unable to penetrate to the region of carbonyl groups; those of them which are in the contact with the membrane reside almost exclusively in the region of phosphates. SCN^- ions exhibit a relatively strong adsorption in the regions of both phosphate and carbonyl groups. The distinctive density profile of SCN^- originates from the fact that these ions both act as an adsorbent in the headgroup region and form a neutralizing layer outside the membrane. The behavior of SCN^- is in accord with recent experimental studies of phospholipid monolayers.

Although adsorption of the investigated ions does not significantly influence either the thickness or area per lipid of DOPC bilayer, several of

physiologically important membrane properties are affected. For instance, hydration in the headgroup region is diminished in the presence of strongly adsorbing ions. Orientation of headgroups is also changed; a strongly adsorbing cation (Gdm^+) induces rising of the PC headgroups, whereas strongly adsorbing anion (SCN^-) lowers the lipid headgroups. Lateral mobility of lipids is also affected; adsorption of both cations and anions is hindering diffusion of lipids in the bilayer. Interestingly, a relatively strong hindering of lateral diffusion was observed in the presence of the tetramethylammonium salt for which no specific adsorption occurs.

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