The molecular electrometer and binding of cations to phospholipid bilayers

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Despite the vast amount of experimental and theoretical studies, the binding affinity of cations, especially the biologically relevant Na^+ and Ca^{2+} ions, into a phosholipid bilayer is not agreed on in the literature. Here we show that the ion binding affinity can be directly compared between simulations and experiments by using the choline headgroup order parameters according to the 'molecular electrometer' concept. Our results strongly support the traditional view that Na^+ ions and other monovalent ions (bar Li^+) do not specifically bind to phosphatidylcholine lipid bilayers with mM concentrations, in contrast to Ca^{2+} and other multivalent ions. Especially the Na^+ binding affinity is overestimated by several molecular dynamics simulation models, leading to an artificially positively charged lipid bilayer. Qualitatively correct headgroup order parameter response is observed with Ca^{2+} binding in all the tested models, however, none of the tested models has sufficient quantitative accuracy to interpret the Ca^{2+}:lipid stoichiometry or the induced atomistic resolution structural changes.

This work has been, and continues to be, progressed and discussed through the blog `nmrlipids.blogspot.fi`*, through which everyone is invited to join the discussion and make contributions. The manuscript will be eventually submitted to an appropriate scientific journal. Everyone who has contributed to the work through the blog will be offered coauthorship. For more details see* `nmrlipids.blogspot.fi`.

I. INTRODUCTION

The interaction of cations with phospholipid membranes is important in many physiological processes, nerve cell signalling being the prime example. For this reason such interactions have been widely studied via experiments, simulations, and theory. While it is generally agreed that the relative binding affinity of different ions follows the Hofmeister series [1–9], no consensus emerges from the literature on the quantitative binding affinities of different ions to phospholipid bilayers. Two extensive reviews covering work done prior to 1990 [2, 3] concluded that monovalent cations interact only weakly with phospholipid bilayers (with the exception of Li^+), while for multivalent ions the interactions are significant. This conclusion has been supported by further studies showing that bilayer properties remain unaltered upon addition of millimolar concentrations of monovalent salt [4, 10, 11]. On the other hand, the weakness of interactions between phospholipids and monovalent ions has been questioned in several experimental and molecular dynamics simulation studies [6–9, 12–18] suggesting stronger binding especially for Na^+ ions.

More specifically, millimolar concentrations of NaCl have a negligible effect on the choline head group order parameters [19], area per molecule [10], dipole potential [20], and lipid lateral diffusion [11]; in contrast, these properties are significantly affected by the presence of $CaCl_2$ or other multivalent ions. In addition, water sorption isotherms are very similar for POPC/NaCl system and NaCl in pure water — indicating only weak interaction between ion and lipids [4]. Further, only minor changes in POPC infrared spectra are observed in the presence of NaCl, while changes are significant in the presence of Ca^{2+} and other multivalent ions, also confirming that interaction between Na^+ and lipids are weak [4]. In contrast, the decrease in rotational and translational dynamics of fluorescent probes in lipid bilayers with mM NaCl concentrations suggests significant Na^+ binding [7, 9, 12]. However, the reduced lateral diffusion is not observed in non-invasive NMR experiments, suggesting that fluorescence results arise from Na^+ interactions with probes rather than with lipids [11]. Also the interpretation of calorimetric measurements has been controversial. The effect of monovalent ions (bar Li^+) on phase transition temperature is small, compared to the effect of multivalent ions; this was initially interpreted by Cevc as an indication that only multivalent ions and Li^+ specifically bind to phosholipid bilayers [2]; however, more recently such small effect was interpreted as an indication that also Na^+ binds to lipid membranes [8, 12]. In electrophoresis measurements on phosphatidylcholine vesicles, NaCl increases the (initially negative) zeta potential to about zero; however, positive zeta potentials can be generally reached only with multivalent ions or Li^+ [1, 8, 14, 15, 21]. The lack of significant positive electrophoretic mobility in the presence of NaCl suggests weak binding of Na^+; however, the same data can also be explained by the effect of Cl^- ions [22, 23]. Finally, changes in bilayer hardness and area per lipid measured with Atomistic Force Microscopy (AFM) were inter-

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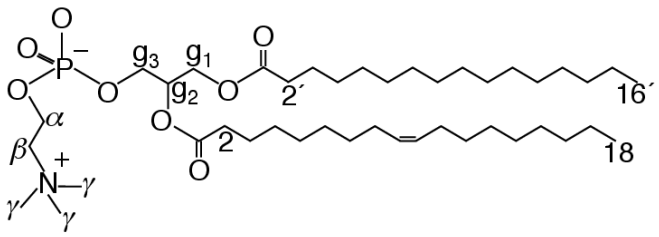

FIG. 1: Chemical structure of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC).

preted as Na^+ binding to phospholipids [14–18].

Atomistic molecular dynamics (MD) simulations are a powerful tool to interpret experimental data in terms of atomic-level interactions. In atomistic MD simulations, the majority of commonly used models predicts binding of Na^+ ions to phoshatidylcholine lipid bilayers, but the strength of binding depends on the specific model used [12, 13, 22, 24–27]. Some simulation studies confirmed a reduction in lipid lateral diffusion due to Na^+ binding, in agreement with fluorescent probe measurements [7, 9, 12] but in contrast with NMR experiments [11]. Other simulations showed a reduction in area per lipid in the presence of NaCl, in agreement with AFM experiments [14–18]; however, the reduction in area was observed at excessively low Na^+ concentrations, compared to observations from scattering experiments [10]. Predictions of electrophoretic mobility in the presence of NaCl yielded positive values, higher than in experiments; however, this could be explained by the behaviour of Cl^- ions [22, 23].

In the present work we set out to solve the apparent contradictions by directly comparing the headgroup hydrocarbon segment α and β (see Fig. 1) order parameters between simulations and experiments as a function of cation concentration. According to the 'molecular electrometer' concept, changes in order parameters of the α and β carbons in the phospholipid head group can be used to measure the ion affinity to the phophatidylcholine (PC) lipid bilayer [19, 28–30]. Order parameters can be accurately measured in experiments and straightforwardly compared to simulations [31], therefore the molecular electrometer allows the comparison of binding affinity between simulations and experiments. We show that the response of order parameters to penetrating cations is qualitatively correct in simulations, but the affinity of PC bilayers for Na^+ ions is significantly overestimated in several MD simulation models. Moreover, we show that the accuracy of tested models does not allow for an interpretation of lipid–Ca^{2+} interactions with atomistic resolution.

II. RESULTS AND DISCUSSION

A. Molecular electrometer concept in experiments

According to the molecular electrometer concept the binding of charged objects on PC bilayer interface induce systematic changes in choline β and α segment order parameters. Thus, the changes of these order parameters can be used to determine binding affinities of the charged objects. The concept is originally based on experimentally observed changes in choline order parameters with bound cations [19, 28], see Fig. 2 for data as a function of ion concentration in solution.

Further quantification with various positively and negatively charged molecules showed that choline order parameters vary linearly with small amount of bound charge per lipid [28–30, 36–41]. The relation between bound charge per lipid $X^{\pm}$ and choline order parameters can be then written as [**?**]

$$S_{\mathrm{CH}}^{(i)}(X^{\pm}) = S_{\mathrm{CH}}^{(i)}(0) + \frac{4}{3}\chi^{-1} m_i X^{\pm}, \qquad (1)$$

where the quadrupole coupling constant χ is approximately equal to 167 kHz, $S_{\mathrm{CH}}^{(i)}(0)$ is order parameter without bound charges, m_i is constant depending on the valency and position of bound charge, and i refers to either α or β. Combination of atomic absorption spectra and ^{2}H NMR experiments gave $m_\alpha = -20.5$ and $m_\beta = -10.0$ for Ca^{2+} binding in POPC bilayer [28].

In original experiments the absolute values of order parameters increased for β and decreased for α segment with bound positive charge and *vice versa* for negative charge [19, 28–30, 36, 41]. However, more recent experiments showed that β carbon order parameter is negative while α carbon order parameter is positive [32–34]. Thus, we can conclude that β and α segment order parameters decrease with bound positive charges and increase with bound negative charge. Consequently, values of m_i are negative for bound positive charges and *vice versa*. This can be rationalized by electrostatically induced changes in choline P-N dipole tilt [29, 30, 42]. This is in line with order parameter decrease related to the P-N vector tilting more parallel to membrane plane seen with decreased hydration levels [35].

The headgroup order parameter changes as a function of ion concentration in solution from H^2 NMR experiments are shown in Fig. 2 for DPPC and POPC bilayers [19, 28]. In contrast to the response as a function of bound charge in Eq. 1, the changes in Fig. 2 are not linear. This can be explained by electrostatic repulsion between already bound Calcium ions and ions in solution [28]. Experimental data in Fig. 2 shows only minor changes in order parameter as a function of NaCl is solution, while the effect of $CaCl_2$ is an order of magnitude larger. Thus, according to the molecular electrometer concept, monovalent Na^+ ions have negligible affinity for PC lipid bilayers at concentrations up to 1 M, while binding of Ca^{2+} ions at the same concentration is significant [19, 28]. This conclusion is in agreement with several other experimental studies [2–4, 10, 11].

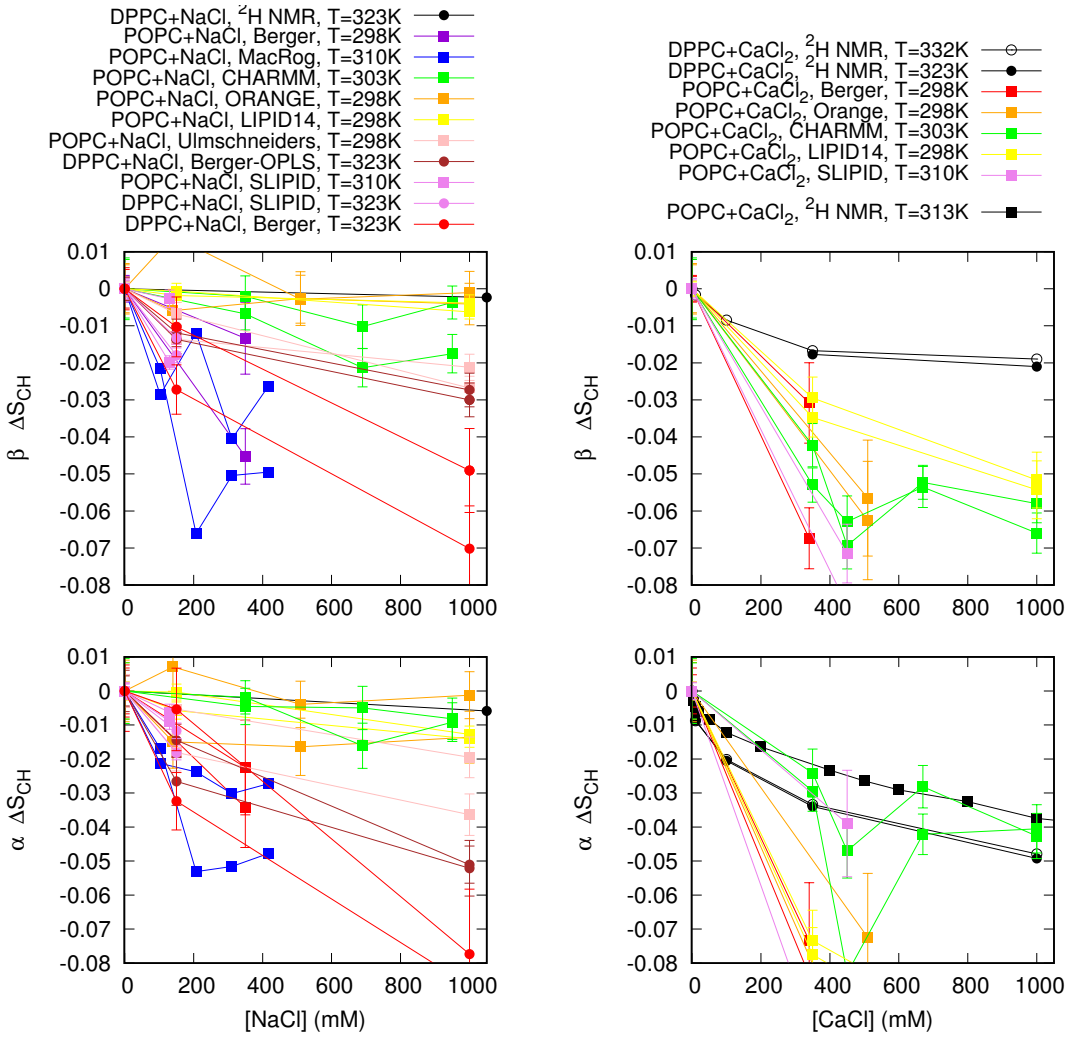

FIG. 2: The order parameter changes for β and α segments as a function of NaCl (left column) and $CaCl_2$ (right column) concentration, from simulations and experiments [19] (POPC with $CaCl_2$ from [28]). The signs of the experimental order parameters, taken from experiments without ions [32–34], can be assumed to be unchanged with concentrations represented here [28, 31]. It should be noted that none of the models used here reproduces the order parameters within experimental error for pure PC bilayer without ions, indicating structural inaccuracies with varying severity in all models [35]

1.We should figure out how to present and discuss the CHARMM results with shorter simulation times.

B. Molecular electrometer concept in MD simulations

Figure 2 also reports order parameter changes calculated from MD simulations of DPPC and POPC lipid bilayers as a function of NaCl or $CaCl_2$ concentrations in solution. Details of the simulated systems are reported in Table I and in Supplementary Information. It should be noted that none of the models used here reproduced the order parameters within experimental uncertainty for pure PC bilayer without ions in our previous study (Figure 2 in Ref. [35]), indicating structural inaccuracies with varying severity for all models [35]. On the other hand, the experimentally observed headgroup order parameter increase with dehydration was qualitatively reproduced by all the tested models [35]. Accordingly, the presense of cations in simulations leads to the decrease of choline order parameters in Fig. 2, which is in qualitative agreement with experiments. However, the changes are overestimated in most simulation results in Fig 2.

According to the electrometer concept the order parameter changes are proportional to the amount of bound cations in bilayer, see section II A. The order parameter changes as a function of bound charge from simulations are shown in Fig. 3. Roughly linear correlation between bound charge and order parameter change is found in all simulation models, which is in line with experiments [28]. However, there are some differences in the proportionality constans (i.e. slopes in Fig. 3) between different models; especially MacRog and Orange models give relatively steep slopes and CHARMM gives gentle slope for α carbon. The quantitative comparison to experimental slopes ($m_\alpha = -20.5$ and $m_\beta = -10.0$ for Ca^{2+} binding in POPC bilayer in Eq. 1 [28]) is not straightforward since the results from simulations depends on the definition of

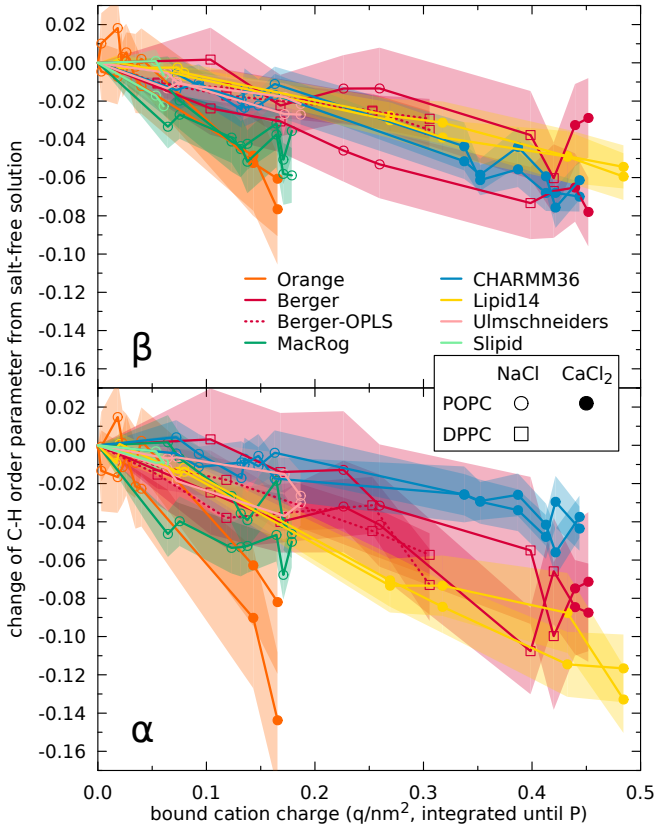

FIG. 3: Changes of order parameters.

2.Results from long CHARMM and Slipids simulations to be added.

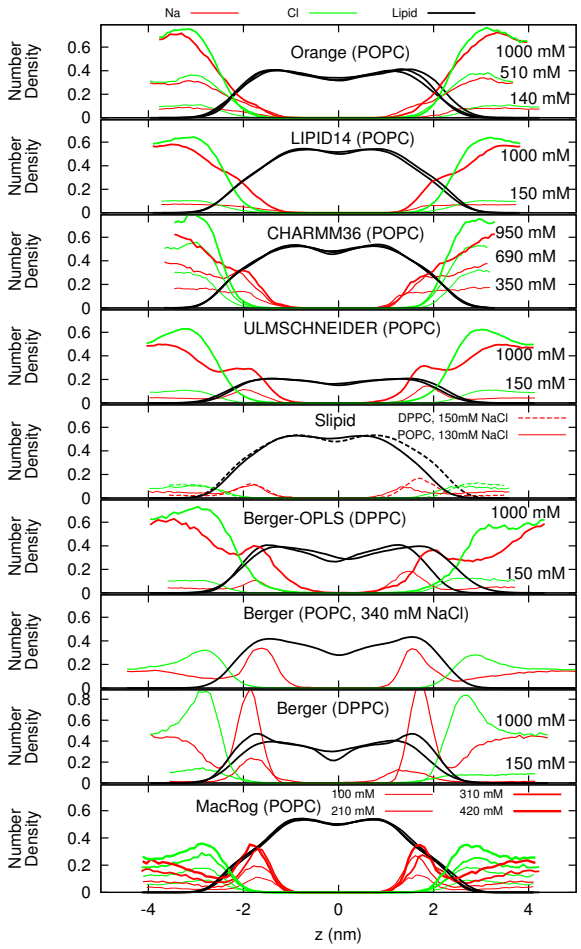

FIG. 4: Atom number density profiles along the membrane normal for lipids, Na^+, and Cl^- ions from simulations with different force fields and different NaCl concentrations. The force fields are ordered according to the order parameter changes reported Fig. 2, from the smallest (top panel) to the larges (bottom panel). The lipid densities are scaled by 100 (united atom) or 200 (all atom model) to improve readability. Figure discussed in https://github.com/NMRLipids/lipid_ionINTERACTION/issues/4.

bound ions. Thus the more detailed analysis is left for further studies.

Figure 3 shows that order parameter decrease clearly correlates with amount of bound cations also in simulations. This is also evident from Fig. 4 showing Na^+ density profiles from the different simulation models. In the figure, simulation models are ordered according to the order parameter changes (reported Fig. 2), from the smallest to the largest. The Na^+ density peaks are larger for models with larger changes in order parameters, in line with observed correlation between cation binding and order parameter decrease in Fig. 3.

In conclusion, the observed clear correlation between bound cations and order parameter decrease support the validity of the molecular electrometer in simulations. Thus, the choline order parameter changes can be used to directly compare the cation binding affinity between simulations and experiments.

C. Cation binding in different simulation models

The order parameter changes and density distributions in Figs. 2 and 4 with added NaCl show significantly different Na^+ binding affinities for different simulation models. The best agreement with experiments (i.e., lowest order parameter changes) is observed for the Orange, CHARMM36 and Lipid14 models (see Figs. 2). These also predict the lowest Na^+ densities in the proximity of the membrane (see Fig. 4). Since the order parameter changes with NaCl in these three models are small (less than 0.02), we cannot conclude with the achieved accuracy which of these three models has the most realistic Na^+ binding affinity, especially with physiological NaCl concentrations ($\sim$ 150mM) which is relevant for most applications.

On the other hand, the choline order parameter changes with NaCl are clearly overestimated in all other tested models (see Fig. 2). This can be explained by unrealistically strong Na^+ binding affinity to the bilayer in these models (see Fig. 4). Such observation raises questions on the quality of the predictions from these models when NaCl is present.

3.It has been suggested that we should add references here. The problem is that

TABLE I: List of simulations performed in this work. The ion concentrations are calculated as [ion]=($N_{ion} \times$[water])/N_w, where [water]=55.5M. These correspond the concentrations reported in the experiments by Akutsu et al. [19]. The lipid force fields are named as in our previous work [35].

Force field (lipid, ion)	lipid	[Ion] mM	[a]N_l	[b]N_w	[c]N_{Na}	[d]N_{Ca}	[e]N_{Cl}	[f]T (K)	[g]t_{sim}(ns)	[h]t_{anal} (ns)	Files
Berger-POPC-07[43]	POPC	0	128	7290	0	0	0	298	270	240	[44]
Berger-POPC-07[43], ffgmx[45]	POPC	340 (NaCl)	128	7202	44	0	44	298	110	50	[46]
Berger-POPC-07[43], ffgmx[45]	POPC	340 ($CaCl_2$)	128	7157	0	44	88	298	108	58	[47]
Berger-DPPC-97[48]	DPPC	0	72	2880	0	0	0	323	60	50	[49]
Berger-DPPC-97[48], ffgmx[45]	DPPC	150 (NaCl)	72	2880	8	0	8	323	120	60	[50]
Berger-DPPC-97[48], ffgmx[45]	DPPC	1000 (NaCl)	72	2778	51	0	51	323	120	60	[51]
BergerOPLS-DPPC-06[52]	DPPC	0	72	2880	0	0	0	323	120	60	[53]
BergerOPLS-DPPC-06[52], OPLS[54]	DPPC	150 (NaCl)	72	2880	8	0	8	323	120	60	[55]
BergerOPLS-DPPC-06[52], OPLS[54]	DPPC	1000 (NaCl)	72	2778	51	0	51	323	120	60	[56]
CHARMM36[57]	POPC	0	72	2242	0	0	0	303	30	20	[58]
CHARMM36[57], CHARMM36[59]	POPC	350 (NaCl)	72	2085	13	0	13	303	80	60	[60]
CHARMM36[57], CHARMM36[59]	POPC	690 (NaCl)	72	2085	26	0	26	303	73	60	[61]
CHARMM36[57], CHARMM36[59]	POPC	950 (NaCl)	72	2168	37	0	37	303	80	60	[62]
CHARMM36[57], CHARMM36	POPC	350 ($CaCl_2$)	128	6400	0	35	70	303	200	100	[63]
CHARMM36[57], CHARMM36	POPC	450 ($CaCl_2$)	200	9000	0	73	146	310	2000	100	[64]
CHARMM36[57], CHARMM36	POPC	670 ($CaCl_2$)	128	6400	0	67	134	303	200	120	[65]
CHARMM36[57], CHARMM36	POPC	1000 ($CaCl_2$)	128	6400	0	100	200	303	200	100	[66]
MacRog[67]	POPC	0	288	14400	0	0	0	310	90	40	[68]
MacRog[67], OPLS[54]	POPC	100 (NaCl)	288	14554	27	0	27	310	90	50	[69]
MacRog[67], OPLS[54]	POPC	210 (NaCl)	288	14500	54	0	54	310	90	50	[69]
MacRog[67], OPLS[54]	POPC	310 (NaCl)	288	14446	81	0	81	310	90	50	[69]
MacRog[67], OPLS[54]	POPC	420 (NaCl)	288	14392	108	0	108	310	90	50	[69]
Orange, OPLS[54]	POPC	0	72	2880	0	0	0	298	60	50	[70]
Orange, OPLS[54]	POPC	140 (NaCl)	72	2866	7	0	7	298	120	60	[71]
Orange, OPLS[54]	POPC	510 (NaCl)	72	2802	26	0	26	298	120	100	[72]
Orange, OPLS[54]	POPC	1000 (NaCl)	72	2780	50	0	50	298	120	80	[73]
Orange, OPLS	POPC	510 ($CaCl_2$)	72	2802	0	26	52	298	120	60	[74]
Slipid[75]	DPPC	0	128	3840	0	0	0	323	150	100	[76]
Slipid[75], AMBER[77, 78]	DPPC	150 (NaCl)	600	18000	49	0	49	323	100	40	-
Slipid[79]	POPC	0	128	5120	0	0	0	303	200	150	[80]
Slipid[79], AMBER[81]	POPC	130 (NaCl)	200	9000	21	0	21	310	105	100	[82]
Slipid[79], AMBER[54]	POPC	450 (CaCl)	200	9000	0	73	146	310	2000	100	[83]
Lipid14 [84], AMBER[54]	POPC	0	128	5120	0	0	0	298	205	200	[85]
Lipid14 [84], AMBER[54]	POPC	150 (NaCl)	128	5120	12	0	12	298	205	200	[86]
Lipid14 [84], AMBER[54]	POPC	1000 (NaCl)	128	5120	77	0	77	298	205	200	[87]
Lipid14 [84], AMBER[54]	POPC	350 ($CaCl_2$)	128	6400	0	35	70	298	200	100	[88]
Lipid14 [84], AMBER[54]	POPC	1000 ($CaCl_2$)	128	6400	0	100	200	298	200	100	[89]
Ulmschneiders [90], OPLS[54]	POPC	0	128	5120	0	0	0	298.15	205	200	[91]
Ulmschneiders [90], OPLS[54]	POPC	150 (NaCl)	128	5120	12	0	12	298.15	205	200	[92]
Ulmschneiders [90], OPLS[54]	POPC	1000 (NaCl)	128	5120	77	0	77	298.15	205	200	[93]

[a] The number of lipid molecules
[b] The number of water molecules
[c] The number of Na^+ molecules
[d] The number of Ca^{2+} molecules
[e] The number of Cl molecules
[f] Simulation temperature
[g] The total simulation time
[h] Time frames used in the analysis

there are a lot of them and it is difficult to choose which ones to pick. Any opinions? Especially interactions between charged molecules and lipid bilayer are significantly affected since strong Na^+ binding makes bilayer effectively positively charged.

All the tested models overestimate the order parameter decrease as a function of $CaCl_2$ concentration as seen in Fig. 2. According to the electrometer concept this indicates overestimated Ca^{2+} binding. On the other hand, the result could be also explained by the oversensitivity of the choline structure on bound Ca^{2+}, i.e. too strong slopes in Fig. 3. We cannot fully exclude this possibility since the quantitative comparison with experimental slope is not possible with current data as discussed in section II B. In this case the bindiging affinitity could be correct but the overestimated choline structural response would induce too large order parameter changes. The latter explanation may be relevant especially in the case of Orange model which gives steeper slope in Fig. 3 than other models.

The ion density distributions with $CaCl_2$ in Fig. 5 show significant Ca^{2+} binding in all models, however, some difference occur between different models. The Berger model predicts deeper penetration depth (density maxima close to ± 1.8 nm) compared to other models (density maxima close to ± 2 nm). The latter value is probably more realistic since ^{1}H NMR and neutron scattering data indicates that Ca^{2+} interacts mainly with the choline group [2, 100–102]. In CHARMM36 model almost all Ca^{2+} ions present in simulation bind in bilayer indicating strongest binding affinity among the tested models. On the other hand, CHARMM36 α carbon order parameters are least sensitive to bound charge (Fig. 3), thus the difference is not as clear in Fig. 2.

In contrast to Na^+, Ca^{2+} binding and related order parameter decrease is seen in experiments [2, 3, 19, 28] and in all tested simulation models, see Figs. 2 and 5. While the significant Ca^{2+} binding affinity to a phosphatidylcholine bilayer at mM concentrations is agreed in the literature, the estimations for lipid/Ca^{2+} stoichiometry vary between 17 and 0.24 [13, 21, 28]. The smallest number (0.24) indicating that one Ca^{2+} ion binds roughly four lipid molecules originates from simulation with Berger model [13]. 4.There is something wrong in these stoichiometry numbers. The direct comparison of order parameters between different simulation models and experiments in Fig. 2 shows that Ca^{2+} binding induced changes are overestimated in all tested models. In contrast to Na^+, clear correlation between Ca^{2+} binding affinity and order parameter changes is not found, thus the overestimation of order parameter change may arise, e.g. from overestimated binding, incorrect headgroup response to penetrating divalent cation or penetration depth. The ion model having scaled charges [99] used with CHARMM36 did not improve the results, see Supplementary Information.

The overestimated Na^+ binding may originate, e.g., from incorrect choline structure [35], lack of polarizability [94], other discrepancies in the ion models [95–97] or from combination of these and other issues. Interestingly, the same ion model and non–bonded parameters are used in the Orange and BergerOPLS [52] simulations, but while Na^+ ion binding affinity is realistic in the Orange model, it is seriously overestimated in the BergerOPLS (Fig. 4). This shows that the binding affinity significantly depends on the used lipid parameters. On the other hand, Na^+ binding with Berger, BergerOPLS and Slipid models is reduced but not yet in agreement with experiments when charges on the ions are reduced (to compensate for the lack of electronic polarizability [94, 98], see Supplementary Information). Further, the Slipid model gives similar binding affinity with two different ion parameters. Altogether, these results indicate that at least lipid models need improvement to correctly predict the Na^+ binding affinity.

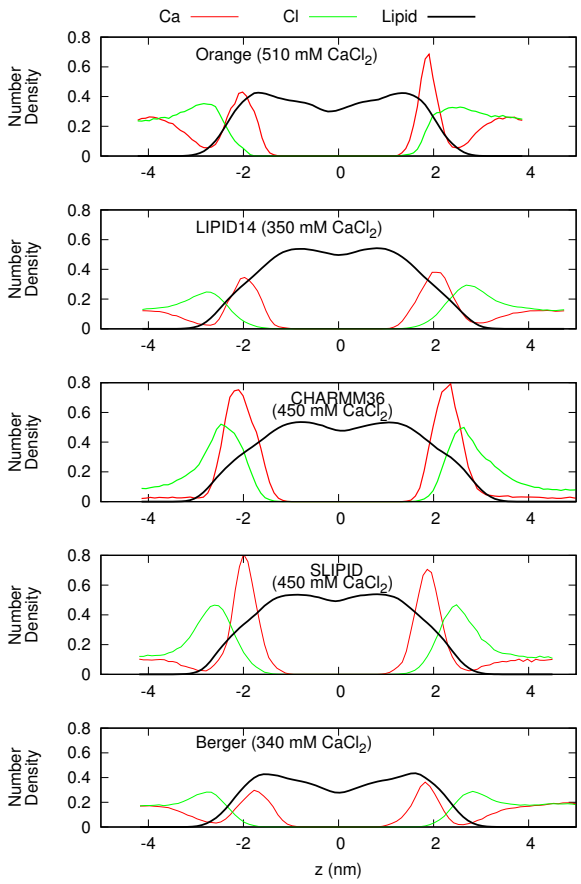

FIG. 5: Atom number density profiles along the membrane normal coordinate z for lipids, Ca^{2+} and Cl^- ions from simulations with different force fields. The profiles only with smallest available $CaCl_2$ concentration are shown for clarity. Figure including all the available concetrations is shown in the Supplementary Information. The lipid densities are scaled with 100 (united atom) or 200 (all atom model) to make them visible with the used y-axis scale. Figure discussed in https://github.com/NMRLipids/lipid_ionINTERACTION/issues/4.

III. CONCLUSIONS

As suggested by the molecular electrometer concept [19, 28–30], the decrease in order parameters of α and β carbons in the PC head group of lipids bilayers is related to cation bind-

ing in all tested simulation models, despite of inaccuracies in actual atomistic resolution structures [35]. The concept allows direct comparison of Na^+ binding affinity between simulations and NMR experiments by using changes in the head group order parameter. The comparison reveals that most models overestimate the Na^+ binding; only Orange, Lipid14, and CHARMM36 predict realistic binding affinity. None of the tested models has the required accuracy to interpret the Ca^{2+}/lipid stoichiometry or induced structural changes with atomistic resolution.

In general, our results support the traditional (pre-2000) view that Na^+ and other monovalent ions (bar Li^+) do not specifically bind to the phospholipid bilayer at mM concentrations, in contrast to Ca^{2+} and other multivalent ions [1, 4, 10, 11, 19–21, 28]. The contradicting results from previous molecular dynamics simulations [12, 13], fluorescent probe dynamics [7, 9, 12], calorimetry [8, 12] and AFM [14–18], suggesting stronger Na^+ binding, can be explained by simulation artefacts, direct interactions between Na^+ and fluorescent probes [11], alternative interpretations of the significance of small phase transition temperature shifts [2], and insufficient resolution of AFM.

The artificial specific Na^+ binding in simulations may lead to doubtful results, since it effectively leads to positively charged phoshatidylcholine (PC) lipid bilayers even at physiological NaCl concentration. Such PC bilayer has distinctly different interactions with charged objects compared to the more realistic model without specific Na^+ binding. Furthermore, the overestimation of Na^+ binding affinity may extend also to other positively charged objects, e.g. membrane protein segments. This would affect lipid protein interactions and could explain contradicting results on electrostatic interactions between charged protein segments and lipid bilayer [104, 105]. In conclusion, more careful studies and model development on lipid bilayer–charged object interactions are needed to make molecular dynamics simulations directly usable in physiologically relevant electrostatic environment.

This work has been, and will be, progressed and discussed through the blog `nmrlipids.blogspot.fi`, through which everyone is invited to join the discussion and make contributions. The manuscript will be eventually submitted to an appropriate scientific journal. Everyone who has contributed to the work through the blog will be offered coauthorship. For more details see `nmrlipids.blogspot.fi`.

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SUPPLEMENTARY INFORMATION

Appendix A: Effect of ion model and polarization

It has been suggested that the missing electronic polarizability can be compensated by scaling the ion charge in simulations [94]. To test if this would improve the Na^+ ion binding behaviour, we ran simulations with Berger-DPPC-97, BergerOPLS-DPPC-06 and Slipids with scaled Na^+ and Cl^- ions. For Berger-DPPC-97 and BergerOPLS-DPPC-06 models the ion charge in systems listed in Table I was simply scaled with 0.7 and the related files are available at [106–109]). For simulations with Slipids the ion model by Kohagen et al. was used [98] and the related files are available at [110]. The simulation parameters were identical to those employed in the simulation of POPC with 130 mM NaCl (see Methods). The order parameter changes and Na^+-binding affinity are decreased by the charge scaling but yet overestimated with respect to the experiments as seen from Figs. 6 and 7. Thus the overestimated binding affinity cannot be fixed by only scaling charges.

5.This should be rewritten due to the new data We also tested the effect of charge scaling in the case of $CaCl_2$ with CHARMM36 model. The ion model by Kohagen et al. [99] was used and the related files are available at [111]. Figures 6 and 8 show that the scaling does not improve the $CaCl_2$ binding behaviour respect to the experiments. The same scaled model with Slipid in the main text also overestimated the $CaCl_2$ effect. However, the effect of scaling cannot be analyzed in this case since Slipid simulation was no ran with non-scaled $CaCl_2$ model.

Appendix B: Density distributions with different $CaCl_2$ concentrations

The density distributions with all simulated $CaCl_2$ concentrations are shown in Fig. 8.

Appendix C: methods

1. Simulated systems

All simulations are ran with a standard setup for planar lipid bilayer in zero tension with periodic boundary conditions with Gromacs software package (version numbers 4.5-X-5.0.X).

2. Analysis

The order parameters were calculated from simulation trajectories directly applying the equation $S_{\mathrm{CH}} = \langle \frac{3}{2}\cos^2\theta - \frac{1}{2} \rangle$, where θ is the angle between a given C–H bond and the bilayer normal and average is taken over all lipids and time frames. For united atom models, the positions of hydrogen atoms were calculated for each molecule in each frame *a posteriori* by using the *protonate* tool in Gromacs 4.0.2 [112]. The statistical

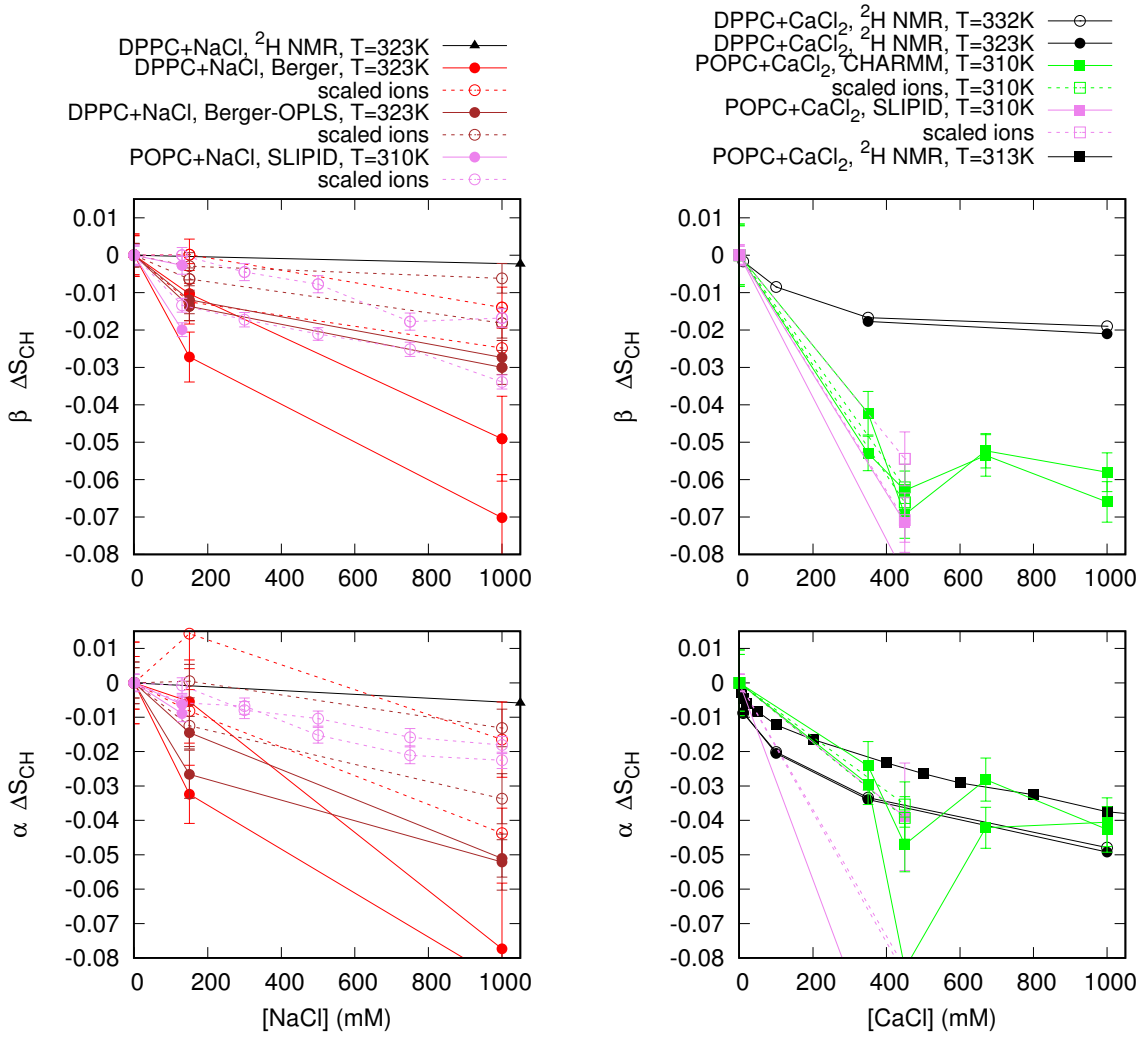

FIG. 6: Order parameter changes in simulations using ion models with scaled charge.

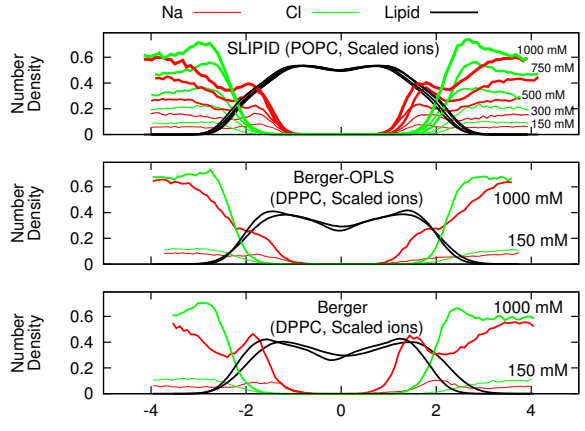

FIG. 7: Atom number density profiles along membrane normal coordinate z for lipids, Na^+ and Cl^- ions from simulations using ion models with scaled charges. The lipid densities are scaled with 100 (united atom) or 200 (all atom model) to make them visible with the used y-axis scale.

error in the order parameter was estimated by calculating the average value separately for each lipid molecule, and then the average and standard error of the mean over the ensemble of lipids (as done also in previous work [35]). All the scripts used in analysis and the resulting data are available in the GitHub repository [113]

3. Simulation details

a. Berger

POPC The simulation without ions is the same as in [114] and the files are available at [44]. The starting structures for simulations with ions is made by replacing water molecules with appropriate amount of ions (see Table I). The Berger force field was used for the POPC [115], with the dihedral potential next to the double bond taken from [116]. The ion parameters from ffmgx [45] were used. Timestep of 2 fs was used with leap-frog integrator. Covalent bond lengths were

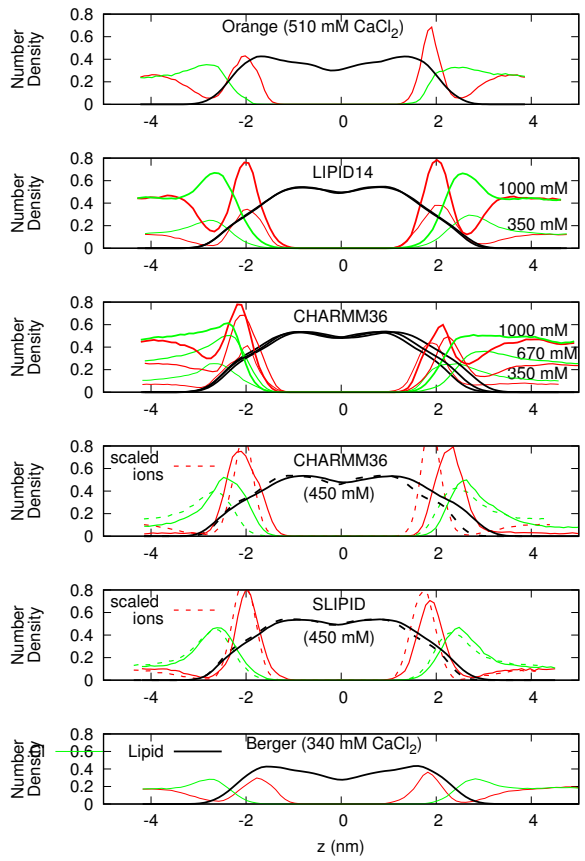

FIG. 8: Number density profiles for lipids, Ca^{2+} and Cl^{-} ions from simulations with different force fields and different $CaCl_2$ concentrations. The lipid densities are scaled with 100 (united atom) or 200 (all atom model) to make them visible with the used y-axis scale. Figure discussed in https://github.com/NMRLipids/lipid_ionINTERACTION/issues/4

constrained with LINCS algorithm [117, 118]. Coordinates were written every 10 ps. PME [119, 120] with real space cut-off 1.0 nm was used for electrostatics. Plain cut-off was used for the Lennard-Jones interactions with a 1.0 nm cut-off. The neighbour list was updated every 5th step with cut-off 1.0 nm. Temperature was coupled separately for lipids, water and ions to 298 K with the velocity-rescale method [121] with coupling constant 0.1 ps^{-1}. Pressure was semi-isotropically coupled to the athmospheric pressure with the Parrinello-Rahman barostat [122].

DPPC The simulation without ions is the same as in [35] and the files are available at [49]. The initial configuration contained 72 DPPC lipids and 2880 SPC water molecules. The standard Berger DPPC force field was used [115] (simulations indicated as Berger-DPPC-97 in Table I). The electrostatics were handled with PME [119, 120], with real-space Coulomb cut-off set at 1.0 nm. Lennard-Jones potentials were cut off 1.0 nm. The neighborlist for all non-bonded interactions was updated every 10 steps. Temperature was set to 323K with the velocity-rescale method [121] using a coupling constant of 0.1 ps^{-1}. Semi-isotropic pressure coupling at 1 ATM was handled with the Parrinello-Rahman barostat [122] with 1 ps coupling constant. The time step was 4 fs, and coordinates were written every 10 ps. The total simulation time was 120 ns (without pre-equilibration) and last 60 ns was used in the order parameter analysis.

For simulations with added salt, the appropriate number of SPC water molecules were randomly replaced with ions. Ions were described by the ffgmx parameters [45]. In simulations with scaled charges, charge-scaling was applied by scaling the ion charges by a factor 0.7. Conditions in the ion simulations were as with the pure DPPC described above. The duration of the simulations was 120 ns (without pre-equilibration) and last 60 ns was used in the order parameter analysis.

All the simulation files for pure DPPC simulations can be found at [49] and for the simulations with ions at [50, 51] and with scaled ions at [106, 107].

b. *BergerOPLS*

For simulations without ions, the initial configuration contains 72 DPPC lipids and 2880 SPC water molecules. For simulations with added salt, the appropriate amount of SPC water molecules were randomly replaced with ions. The number of ions is reported in Table I. For the lipids, we used the same version of Berger force field as in previous simulations, described in [115]; for the ions, we used the qvist parameters [54] (commonly used within the OPLS-AA force field). Issues related to the compatibility between Berger and OPLS-AA force fields are described in ref. [52]. A set of simulations was carried out using reduced electrostatic charges on the ions; in this case, a charge of 0.7 e was used on the ions, as described in refs. [94, 98]. Except for the ion force field, all simulation parameters (for non-bonded interactions, integration time step, thermostat, etc.) were identical to the parameters used in the Berger DPPC simulations described above.

All simulation files can be found at [53] for pure DPPC simulations, at [55, 56] for simulations with ions, and at [108, 109] for simulations with ions with scaled charges.

c. *CHARMM36*

POPC with NaCl The simulation without ions is taken directly from [35, 58]. The starting structures for simulations with NaCl were made by replacing randomly located water molecules of the structure of pure POPC simulation with appropriate amount of ions. The force field for lipid were the same as in [35, 58]. The ion parameters with NBFIX by Venable et al. [59] were used. Simulations were ran with Gromacs 4.5.5 software [123]. Timestep of 2 fs was used with leap-frog integrator. Covalent bonds with hydrogens were constrained with LINCS algorithm [117, 118]. Coordinates were written every 5 ps. PME with real space cut-off 1.4 nm was used for electrostatics. Lennard-Jones interactions were switched to zero between 0.8 nm and 1.2 nm. The neighbour list was updated every 5th step with cut-off 1.4 nm. Temperature was

coupled separately for lipids and solution to 303 K with the velocity-rescale method [121] with coupling constant 0.2 ps. Pressure was semi-isotropically coupled to the athmospheric pressure with the Berendsen method [124].

POPC with $CaCl_2$ The starting structures with varying amounts of $CaCl_2$ ions were constructed using the CHARMM-GUI Membrane Builder (http://www.charmm-gui.org/) online tool [125]. All runs were performed with Gromacs 5.0.3 software package [126] and CHARMM36 additive force field parameters for lipids [57] and ions were obtained from CHARMM-GUI input files. Standard CHARMM-GUI mdp options were used. Particularly, h-bond lengths were constrained with LINCS [117, 118]. The temperatures of the lipids and the solvent were separately coupled to the Nose-Hoover [127, 128] thermostat with a target temperature of 303 K and a relaxation time constant of 1.0 ps. Semi-isotropical pressure coupling to 1 bar was obtained with the Parrinello-Rahman barostat [122] with a time constant of 5 ps. Equations of motion were integrated with the Verlet algorithm [129] using a timestep of 2 fs. Long-range electrostatic interactions were calculated using the PME [119, 120] method with a fourth order smoothing spline. A real space cut-off of 1.2 nm was employed with grid spacing of 0.12 nm in the reciprocal space. Lennard-Jones interactions were smoothly switched to zero between 1.0 nm and 1.2 nm. Verlet cutoff-scheme [129] were used with the long-range neighbor list updated every 20 steps. Coordinates were written every 10 ps. After energy minimization and an equilibration run of 0.5 ns, 200ns simulations were ran and the last 100ns of each simulation was employed for the analysis.

d. MacRog

The simulation parameters are identical to those employed in our earlier study [35] for the full hydration and dehydration simulations. The initial structures with varying amounts of NaCl were constructed from an extensively hydrated bilayer by replacing water molecules with ions using the Gromacs tool genion [130]. Even at the highest considered salt concentration, the amount of water molecules per lipid after this replacement process was still greater than 50.

e. Orange

The systems contained 72 POPC lipids and 2880 SPC water molecules, and an appropriate amount of ions as indicated in Table I.

For the lipids, we used an unpublished force field coined Orange force field. Briefly, this includes most bonded interactions from Berger lipids [115], except for dihedrals which were derived via *ab initio* calculations on small model compounds. As in Berger lipids, Lennard-Jones parameters are from OPLS. ;; (A) Jorgensen et al, JACS (1984), 106, 6638-6646; ;; (B) Jorgensen, JPC (1986), 90, 1276-1284; ;; (C) Jorgensen et al, JPC (1986), 90, 2174-2182; ;; (D) Jorgensen et al, JACS (1988), 110, 1657-1666; ;; (E) Briggs et al, JPC (1991), 95, 3315-3322. Partial charges were derived on the basis of *ab initio* calculations. In simulations with ions, the qvist parameters were used [54]. The electrostatics were handled with PME [119, 120], with real-space Coulomb cut-off set at 1.8 nm. Lennard-Jones potentials were cut off at 1.8 nm. The neighborlists for the calculation of non-bonded forces were updated every 5 steps.

Temperature was set to 298K with the velocity-rescale thermostat [121] using a coupling constant of 0.1 ps^{-1}, and the pressure was set to 1 bar using the Berendsen weak coupling algorithm [124] (compressibility of 4.5*$10^{-}5$ $bar^{-}1$, time constant of 1 ps), coupling separately the x-y dimension and the z dimension to obtain a tensionless system. A time step of 2 fs was used for the integration (with the leap-frog algorithm), coordinates were written every 100 ps, and the total simulation time was 60 ns.

All the simulation files for pure lipid simulations are found at [**?**] and for the simulations with ions at [**?**].

f. Slipids

DPPC The simulation without ions from [35], available at [76] was used. For the simulations with ions, the starting DPPC lipid bilayer, which was built with the online CHARMM-GUI [125] (http://www.charmm-gui.org/), contained 600 lipids, 30 water molecules/lipid, Na^{+} and Cl^{-} ions (150 mM NaCl). The TIP3P water model was used to solvate the system and ion parameters by Roux [77, 78] were used. the GROMACS software package version 4.5.5 [123] and the Stockholm lipids (Slipids) force field parameters for phospholipids were used. After energy minimization and a short equilibration run of 50 ps (time step 1 fs), 100 ns production runs were performed using a time step of 2 fs with leap-frog integrator. All covalent bonds were constrained with the LINCS [117, 118] algorithm. Coordinates were written every 100 ps. PME [119, 120] with real space cut-off at 1.0 nm was used for Coulomb interactions. Lennard-Jones interactions were switched to zero between 1.0 nm and 1.4 nm. The neighbour lists were updated every 10^{th} step with a cut-off of 1.6 nm. Temperature was coupled separately for upper and bottom leaflets of the lipid bilayer, and for water to one of the temperatures reported above with the Nosé-Hoover thermostat [127, 128] using a time constant of 0.5 ps. Pressure was semi-isotropically coupled to the atmospheric pressure with the Parrinello-Rahman [122] barostat using a time constant of 10 ps.

POPC The simulation without ions from [35], available at [80] was used. Additionally, a POPC bilayer consisting of 200 lipids, hydrated with 45 water molecules per lipid, was simulated in the presence of 130 mM NaCl. **6.Details from the simulation with CaCl** The Slipids model [75, 79] was employed for lipids, the tip3p model [131] for water, and the ion parameters by Smith and Dang [81] for NaCl. The system was first equilibrated for 5 ns with a time step of 1 fs after which a 100 ns production run was performed using a time step of 2 fs. Trajectories were written every 100 ps. The system was kept in a tensionless state at 1 bar using a semi-isotropical Parrinello–

Rahman barostat [122] with a time constant of 1 ps. The temperature was maintained at 310 K with the velocity rescaling thermostat [121]. The time constant was set to 0.5 ps for both lipids and solvent (water and ions) which were coupled separately. Non-bonded interactions were calculated within a neighbor list with a radius of 1 nm and an update interval of 10 steps. The Lennard-Jones interactions were cut-off at 1 nm, whereas PME [119, 120] was employed for long-range electrostatics. Dispersion correction was applied to both energy and pressure. All bonds were constrained with the LINCS [117, 118]. algorithm.

g. *Lipid14*

The starting structures with varying amounts of ions were constructed using the CHARMM-GUI Membrane Builder (http://www.charmm-gui.org/) online tool [125]. The GROMACS compatible force field parameters generated in [35] and available at [132] were used. The TIP3P water model [131] was used to solvate the system and Åqvist [54] parameters were used for ions. All runs were performed with Gromacs 5.0.3 software package [126] and LIPID14 force field parameters for POPC [84].

H-bond lengths were constrained with LINCS [117, 118]. The temperatures of the lipids and the solvent were separately coupled to the Nose-Hoover [127, 128] thermostat with a target temperature of 298.15 K and a relaxation time constant of 0.1 ps. Semi-isotropical pressure coupling to 1 bar was obtained with the Parrinello-Rahman barostat [122] with a time constant of 2 ps. Equations of motion were integrated with the Verlet algorithm [129] using a timestep of 2 fs. Long-range electrostatic interactions were calculated using the PME [119, 120] method with a fourth order smoothing spline. A real space cut-off of 1.0 nm was employed with grid spacing of 0.12 nm in the reciprocal space. Lennard-Jones potentials were cut-off at 1 nm, with a dispersion correction applied to both energy and pressure. Verlet cutoff-scheme [129] were used with the long-range neighbor list updated every 20 steps. Coordinates were written every 10 ps.

After energy minimization and an equilibration run of 5 ns, 200ns production runs were performed and analysed. In case of the CaCl2 systems only the last 100ns of each simulation was employed for the analysis.

h. *Ulmschneiders*

The starting structures with varying amounts of ions were constructed using the CHARMM-GUI Membrane Builder (`http://www.charmm-gui.org`) online tool [125]. The force field parameters were obtained from Lipidbook [133]. The TIP3P water model [131] was used to solvate the system. Additionally, the simulations of ion-free bilayer were repeated with both Verlet and Group cutoff-schemes [91]. There was no significant difference in headgroup or glycerol backbone order parameters between these cutoff-schemes. All runs were performed with Gromacs 5.0.3 software package [126]. The glycerol backbone order parameters without iones were not the same as reported in the previous study [35]. The origin of discrepancy was located to the different initial structures which was taken from CHARMM-GUI in this work and from Lipidbook in the previous work. Since the order parameters with the initial structure from CHARMM-GUI are closer to the experimental values, the results indicate that the structure available from Lipidbook is stuck to a state with incorrect glycerol backbone struccuture, for more discussion see `https://github.com/NMRLipids/lipid_ionINTERACTION/issues/8`.

All-bond lengths were constrained with LINCS [117, 118]. The temperatures of the lipids and the solvent were separately coupled to the Nose-Hoover [127, 128] thermostat with a target temperature of 298.15 K and a relaxation time constant of 0.1 ps. Semi-isotropical pressure coupling to 1 bar was obtained with the Parrinello-Rahman barostat [122] with a time constant of 2 ps. Equations of motion were integrated with the Verlet algorithm [129] using a timestep of 2 fs. Long-range electrostatic interactions were calculated using the PME [119, 120] method with a fourth order smoothing spline. A real space cut-off of 1.0 nm was employed with grid spacing of 0.12 nm in the reciprocal space. Lennard-Jones potentials were cut-off at 1 nm, with a dispersion correction applied to both energy and pressure. Verlet cutoff-scheme [129] were used with the long-range neighbor list updated every 20 steps. Coordinates were written every 10 ps. After energy minimization and an equilibration run of 5 ns, 200ns simulations were ran and the last 100ns of each simulation was employed for the analysis.

Appendix D: Author Contributions

Andrea Catte
Mykhailo Girych ran and analyzed several simulations. Discussed the project actively with OHSO.
Matti Javanainen provided data with several lipid and ion models. Discussed the project actively with OHSO. Supervised the work of JT.
Markus S. Miettinen
Luca Monticelli
Jukka Määttä
Vasily S. Oganesyan
O. H. Samuli Ollila co-designed the project with MSM and managed the work. Ran and analyzed several simulations. Wrote the manuscript.
Joona Tynkkynen

TODO

P.

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