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Molecular electrometer and binding of cations to phospholipid bilayers[†]

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Despite the vast amount of experimental and theoretical studies on the binding affinity of cations — especially the biologically relevant Na^+ and Ca^{2+} — for phospholipid bilayers, there is no consensus in the literature. Here we show that by interpreting changes in the choline headgroup order parameters according to the 'molecular electrometer' concept [Seelig *et al.*, *Biochemistry*, 1987, **26**, 7535], one can directly compare the ion binding affinities between simulations and experiments. Our findings strongly support the view that in contrast to Ca^{2+} and other multivalent ions, Na^+ and other monovalent ions (except Li^+) do not specifically bind to phosphatidylcholine lipid bilayers at sub-molar concentrations. However, the Na^+ binding affinity was overestimated by several molecular dynamics simulation models, resulting in artificially positively charged bilayers and exaggerated structural effects in the lipid headgroups. While qualitatively correct headgroup order parameter response was observed with Ca^{2+} binding in all the tested models, no model had sufficient quantitative accuracy to interpret the Ca^{2+} :lipid stoichiometry or the induced atomistic resolution structural changes. All scientific contributions to this open collaboration work were made publicly, using `nmrlipids.blogspot.fi` as the main communication platform.

1 Introduction

Due to its high physiological importance — nerve cell signalling being the prime example — interaction of cations with phospholipid membranes has been widely studied via theory, simulations, and experiments. The relative ion binding affinities are generally agreed to follow the Hofmeister series^{1–9}, however, consen-

sus on the quantitative affinities is currently lacking. Until 1990, the consensus (documented in two extensive reviews^{2,3}) was that while multivalent cations interact significantly with phospholipid bilayers, for monovalent cations (with the exception of Li^+) the interactions are weak. This conclusion has since been strengthened by further studies showing that bilayer properties remain unaltered upon the addition of sub-molar concentrations of monovalent salt^{4,10,11}. Since 2000, however, another view has emerged, suggesting much stronger interactions between phospholipids and monovalent cations, and strong Na^+ binding in particular^{6–9,12–18}.

The pre-2000 view has the experimental support that (in contrast to the significant effects caused by any multivalent cations) sub-molar concentrations of NaCl have a negligible effect on phospholipid infrared spectra⁴, area per molecule¹⁰, dipole potential¹⁹, lateral diffusion¹¹, and choline head group order parameters²⁰; in addition, the water sorption isotherm of a NaCl–phospholipid system is highly similar to that of a pure NaCl solution — indicating that the ion–lipid interaction is very weak⁴.

The post-2000 'strong binding' view rests on experimental and above all ~~simulation~~–simulation findings. At sub-molar NaCl concentrations, the rotational and translational dynamics of membrane-embedded fluorescent probes ~~decreased~~decreased^{7,9,12}, and atomic force microscopy (AFM) ex-

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periments ~~show~~showed changes in bilayer hardness^{14–18}; in atomistic molecular dynamics (MD) simulations, phospholipid bilayers consistently ~~bind~~bound Na⁺, although the binding strength ~~depends~~depended on the model used^{12,13,21–26}.

Some observables have been interpreted in favour of both views. For example, as the effect of monovalent ions (except Li⁺) on the phase transition temperature is tiny (compared to the effect of multivalent ions), it was initially interpreted as an indication that only multivalent ions and Li⁺ specifically bind to phospholipid bilayers²; however, such a small effect in calorimetric measurements was later interpreted to indicate that also Na⁺ binds^{8,12}. Similarly, the lack of significant positive electrophoretic mobility of phosphatidylcholine (PC) vesicles in the presence of NaCl (again in contrast to multivalent ions and Li⁺) suggested weak binding of Na⁺^{1,8,14,15,27}; however, these data ~~have also been~~were also explained by a countering effect of the Cl[−] ions^{22,28}. ~~To Furthermore, to~~ reduce the area per lipid in scattering experiments, molar concentrations of NaCl ~~are~~were required¹⁰, ~~which indicates~~indicating weak ion–lipid interaction; in MD simulations, however, already orders of magnitude lower concentrations ~~result~~resulted in Na⁺ binding and a clear reduction of area per lipid^{12,23}. Finally, ~~in noninvasive NMR experiments,~~ lipid lateral diffusion ~~is~~was unaltered by NaCl ~~in noninvasive NMR experiments~~¹¹; however, ~~it is reduced in simulations as it was reduced~~ upon Na⁺ binding, ~~which supports interpreting in simulations,~~ the reduced lateral diffusion of fluorescent probes^{7,9,12} ~~as favouring~~has been interpreted to support the post-2000 'strong binding' view.

In this paper, we set out to solve the apparent contradictions between the pre-2000 and post-2000 views. To this end, we employ the 'molecular electrometer' concept, according to which the changes in the C–H order parameters of the α and β carbons in the phospholipid head group (see Fig. 1) can be used to measure the ion affinity ~~to for a~~ PC lipid bilayer^{20,29–31}. ~~As~~^{20,29–32}. ~~As the~~ order parameters can be accurately measured in experiments and directly compared to simulations³³, ~~employing~~³³, ~~applying~~ the molecular electrometer as a function of cation concentration allows the comparison of binding affinity between simulations and experiments. In addition to demonstrating the usefulness of this general concept, we show that the response of the α and β order parameters to penetrating cations is qualitatively correct in MD simulations, but that in several models the affinity of Na⁺ for PC bilayers is grossly overestimated. Moreover, we show that the accuracy of lipid–Ca²⁺ interactions in current models is not enough for atomistic resolution interpretation of NMR experiments.

This work ~~has been~~was done as an Open Collaboration at nmrlipids.blogspot.fi; all the related files³⁴ and almost all the simulation data (<https://zenodo.org/collection/user-nmrlipids>) are openly available.

2 Results and Discussion

2.1 Background: Molecular electrometer in experiments

The ~~molecular electrometer concept is based on basis for the~~ molecular electrometer is the experimental observation that bind-



Fig. 1 Chemical structure of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), and the definition of γ , β , α , g_1 , g_2 and g_3 segments.

ing of any charged objects (e.g.—ions, peptides, anesthetics, amphiphiles) on a PC bilayer interface ~~induces~~induced systematic changes in the choline ~~β - and α segment and β segment~~ C–H order parameters^{20,29–31,35–40}. ~~Thus~~^{20,29–32,35–40}. Being systematic, these changes ~~can be used to determine~~could be employed for determining the binding affinities of the charged objects. ~~The in question.~~ Originally the molecular electrometer was ~~originally~~ devised for cations^{20,29}^{20,29,32}, but further experimental quantification with various positively and negatively charged molecules showed that the choline order parameters S_{CH}^{α} and S_{CH}^{β} in general vary linearly with small amount of bound charge per lipid^{29–31,35–40}. ~~The Let now~~ $S_{CH}^i(0)$, where i refers to either α or β , denote the order parameter in the absence of bound charge; the empirically observed linear relation ~~can~~can then be written as⁴¹

$$\Delta S_{CH}^i = S_{CH}^i(X^{\pm}) = S_{CH}^i(0) + \frac{4m_i}{3\chi} X^{\pm} \quad (1)$$

where $S_{CH}^i(0)$ is the order parameter in the absence of bound charges. Here X^{\pm} is the amount of bound charge per lipid, m_i is an empirical constant depending on the valency and position of bound charge, X^{\pm} is the amount of the bound charge per lipid, i refers to either α or β , and the value of the quadrupole coupling constant is $\chi \approx 167$ kHz. ~~The change in order parameters with respect to a bilayer without bound charges then becomes~~

$$\Delta S_{CH}^i = S_{CH}^i(X^{\pm}) - S_{CH}^i(0) = \frac{4m_i}{3\chi} X^{\pm}.$$

For Ca²⁺ binding to POPC bilayer (in the presence of 100 mM NaCl), combination of atomic absorption spectra and ²H NMR experiments gave $m_{\alpha} = -20.5$ and $m_{\beta} = -10.0$ ²⁹. kHz.

The absolute values of order parameters increase for ~~With~~ bound positive charge, the absolute value of the β and decrease for segment order parameter increases and the α segment with bound positive charge order parameter decreases (and vice versa

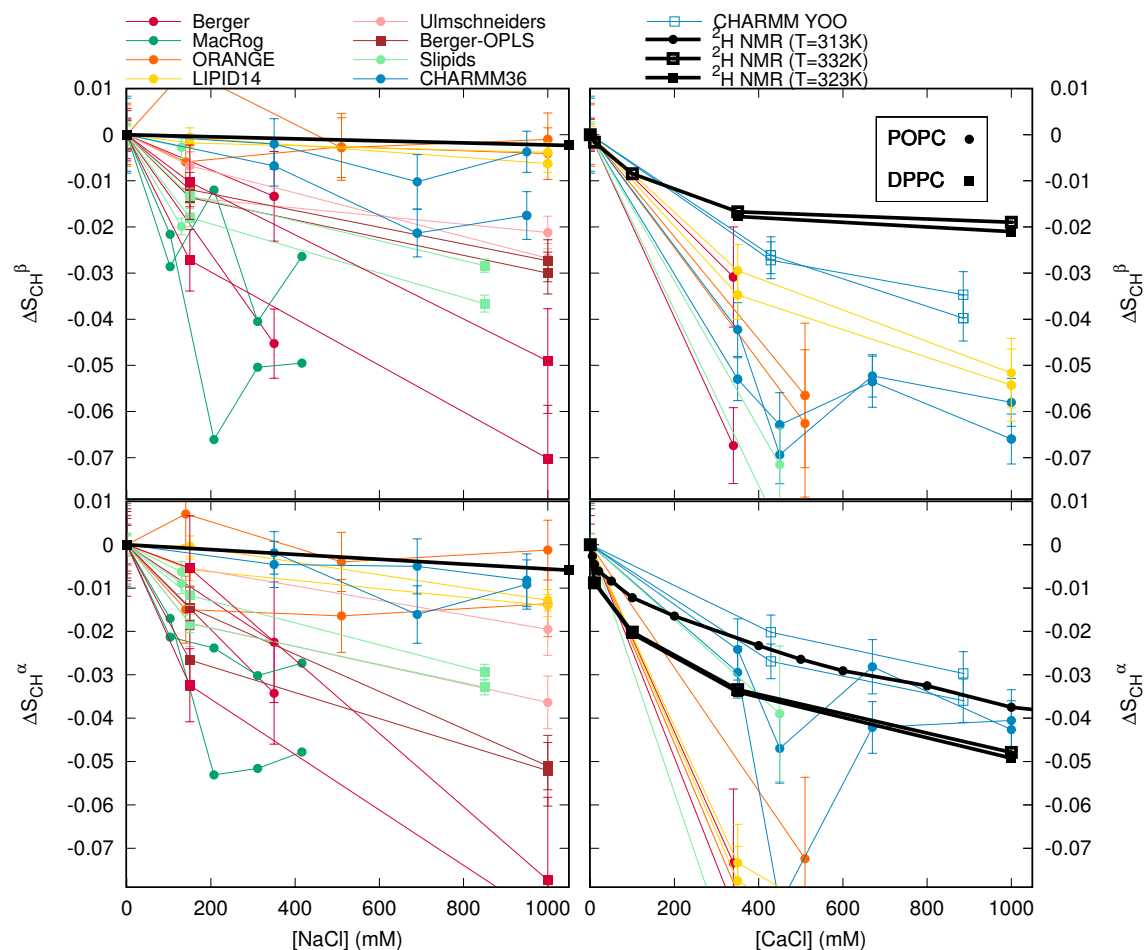


Fig. 2 The order-parameter changes for Changes in the PC lipid headgroup β (top row) and α segments as a function of (bottom) segment order parameters in response to NaCl (left column) and/or CaCl_2 (right column) salt solution concentration, from increase. Comparison between simulations (Table 1) and experiments ²⁰ (DPPCs from Ref. ²⁰, POPC with CaCl_2 from Ref. ²⁹). The signs of the experimental order-parameters values, taken from experiments without ions ^{42–44}, can be assumed to be unchanged with at these salt concentrations represented here ^{29,33}. It should be noted We stress that none of the models used here reproduces the order parameters without salt within experimental error for pure PC bilayer without ions, indicating structural inaccuracies with of varying severity in all models of them ⁴⁵. Note that the relatively large decrease drop in CHARMM36 with at 450 mM CaCl_2 arise arose from more equilibrated binding affinity due to a very long simulation timestep, see ESI†.

for negative charge ^{20,29–31,35,40} ^{20,29–32,35,40}. However, as the β -carbon order parameter is negative while α -carbon order parameter is positive $S_{\text{CH}}^{\beta}(0) < 0$ while $S_{\text{CH}}^{\alpha}(0) > 0$ ^{42–44}, we can conclude that both $\Delta S_{\text{CH}}^{\beta}$ and $\Delta S_{\text{CH}}^{\alpha}$ in fact decrease with bound positive charge (and increase with bound negative charge). Consequently, values of m_i are negative for bound positive charges; for Ca^{2+} binding to POPC bilayer (in the presence of 100 mM NaCl), combination of atomic absorption spectra and ^2H NMR experiments gave $m_{\alpha} = -20.5$ and vice versa. This $m_{\beta} = -10.0$ ²⁹. This decrease can be rationalised by electrostatically induced changes in tilting of the choline P–N dipole tilt ^{30,31,46}, which is ^{30,31,46} also seen in simulations ^{23,24,47,48}. This — and is in line with order parameter decrease the order parameter increase related to the P–N vector tilting more parallel to the membrane plane seen with decreasing hydration levels ⁴⁵.

The quantification of $\Delta S_{\text{CH}}^{\beta}$ and Quantification of $\Delta S_{\text{CH}}^{\alpha}$ with different cations have revealed that $\Delta S_{\text{CH}}^{\beta}/\Delta S_{\text{CH}}^{\alpha} \approx 0.5$ and $\Delta S_{\text{CH}}^{\beta}$ for a wide range of different cations (aqueous

cations, cationic peptides, cationic anesthetics) has revealed that $\Delta S_{\text{CH}}^{\beta}/\Delta S_{\text{CH}}^{\alpha} \approx 0.5$ ^{38,40}. More specifically, the relation $\Delta S_{\text{CH}}^{\beta} = 0.43\Delta S_{\text{CH}}^{\alpha}$ was found for a DPPC bilayer with to hold for DPPC bilayers at various CaCl_2 concentrations ²⁰.

2.2 Molecular electrometer concept in MD simulations

The headgroup order parameter changes as a function of ion concentration in solution from ^2H NMR experiments are shown black curves in Fig. 2 show how the headgroup order parameters for DPPC and POPC bilayers ^{20,29} change in ^2H NMR experiments as a function of salt solution concentration ^{20,29}: Only minor changes in order parameters are seen as a function of NaCl in solution, while [NaCl], but the effect of $[\text{CaCl}_2]$ is an order of magnitude larger. Thus, according to the molecular electrometer concept, the 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 ^{20,29}.

Figure 2 also reports order parameter changes calculated from

MD simulations of DPPC and POPC lipid bilayers as a function of NaCl or CaCl₂ initial concentrations in solution (for details of the simulated systems see [Tables 1](#), [?? Table 1](#) and [ESI[†]](#)). Note that [although](#) none of these MD models [reproduced](#) [reproduces](#) within experimental uncertainty the order parameters for a pure PC bilayer without ions ([Figure Fig. 2](#) in [Ref. 45](#)), [indicating which indicates](#) structural inaccuracies of varying severity in all models⁴⁵. [However,](#) [all the models qualitatively reproduce](#) the experimentally observed headgroup order parameter increase with dehydration [was qualitatively reproduced by all the models](#)⁴⁵, and similarly here [. Similarly here](#) ([Fig. 2](#)) the presence of cations [leads led](#) to the decrease of S_{CH}^{β} and S_{CH}^{α} ([Fig. 2](#)) and S_{CH}^{β} , in qualitative agreement with experiments. The changes [were](#), however, overestimated by most models. [According to the electrometer concept this,](#) [which according to the molecular electrometer](#) indicates overbinding of cations in most MD [simulation models simulations](#).

While [electrometer concept the molecular electrometer](#) is well established in experiments (see [previous section Sec. 2.1 above](#)), it is not *a priori* clear that it works in simulations. The overestimated order parameter decrease could, in principle, arise [also from the oversensitivity of choline headgroups on cation binding from an exaggerated response of the choline headgroups to the binding cations](#), instead of overbinding. [Here we analyse](#) [Therefore, to evaluate the usability of the molecular electrometer in MD simulations, we analysed](#) the relation between cation binding and choline order parameter decrease in simulations [in order to evaluate the usability of the electrometer concept in MD simulations](#).

According to the molecular electrometer [concept,](#) [the](#) order parameter changes are linearly proportional to the amount of bound cations [in bilayer](#) (Eq. (2.1)). Figure 3 shows [the changes in order parameter as a function of bound charge this proportionality](#) in MD simulations (see [ESI[†]](#) for the definition of bound ions); in keeping with the molecular electrometer, [a](#) roughly linear correlation between bound [cation](#) charge and order parameter change [is was](#) found in all [the eight](#) models. Note that quantitative comparison of the proportionality constants (i.e. slopes in Fig. 3) between different models and experimental slopes ($m_{\alpha} = -20.5$ and $m_{\beta} = -10.0$ for Ca²⁺ binding in DPPC bilayer in the presence of 100mM NaCl [in Eq. ??](#)²⁹) is not straightforward since the simulation slopes depend on the definition used for bound ions (see [ESI[†]](#)).

[The We note that the quantitative](#) comparison of order parameter changes in response to bound charge [is should be](#) more straightforward for systems with charged amphiphiles fully associated in [the](#) bilayer, as the amount of bound charge is then explicitly known in both simulations and experiments. [Such comparison between previously published simulation data⁴⁹ and experiments^{31,50} In such a comparison between experiments^{31,50} and previously published Berger-model-based simulations⁴⁹ we](#) could not rule out overestimation of order parameter response to bound cations (i.e., slopes m_{β} and slopes m_{α}) [in a Berger-based model \(and \$m_{\beta}\$ \), see ESI[†]](#). This might, in principle, explain the overestimated order parameter response

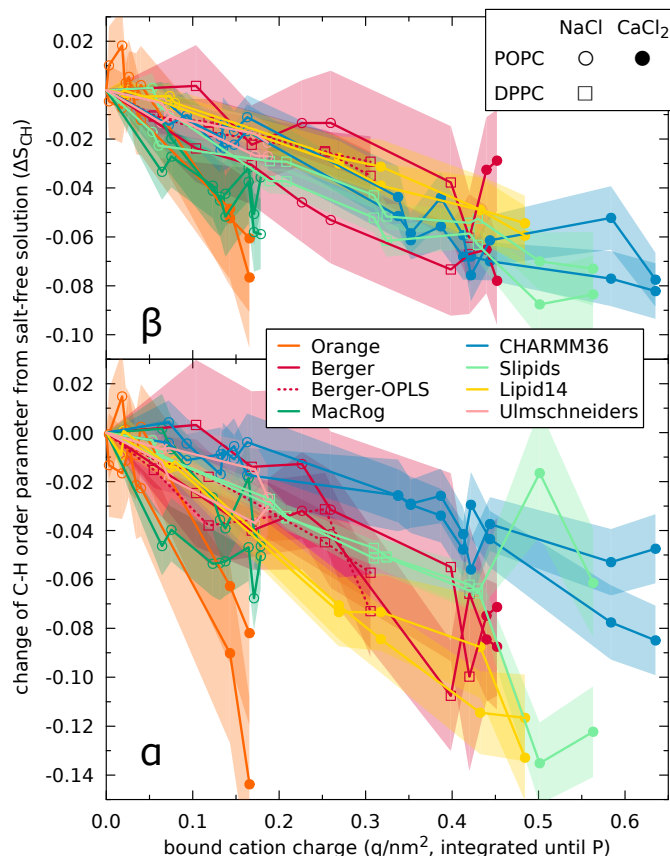


Fig. 3 Change of order parameters (from salt-free solution) of the β and α segments, $\Delta S_{\text{CH}}^{\beta}$ and $\Delta S_{\text{CH}}^{\alpha}$, shown as a function of bound cation charge. Eight MD simulation models compared; the two lines per model denote to the two hydrogens per carbon. The order parameters as well as the bound charge calculated separately for each leaflet; cations residing between the bilayer centre and the density maximum of Phosphorus considered bound; error bars (shaded) show standard error of mean over lipids.

of [the](#) Berger model to CaCl₂, but not to NaCl (see discussion in [ESI[†]](#)). Since simulation data with charged amphiphiles [from other models is not available,](#) [the are not available for other models,](#) an extended comparison with different models is left for further studies.

Figure 3 shows that the decrease [on order parameter clearly correlates of order parameters clearly correlated](#) with the amount of bound cations [also](#) in simulations. This is also evident from Fig. 4, which shows the Na⁺ density profiles of the MD models ordered according to the order parameter change ([reported](#) in Fig. 2) from the smallest (top) to the largest (bottom). The [general trend in the figure is that the](#) Na⁺ density peaks are larger for models with larger changes in order parameters, in line with the observed correlation between cation binding and order parameter decrease in Fig. 3. [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 in Fig. 2, from the smallest \(top panel\) to the largest \(bottom panel\). The lipid densities are scaled by 100 \(united atom\) or 200 \(all atom model\) to improve](#)

readability-

Figure 5 compares the relation between $\Delta S_{\text{CH}}^{\beta}$ and $\Delta S_{\text{CH}}^{\alpha}$ in experiments²⁰ and ~~different simulation in MD~~ models. Only Lipid14 ~~gives~~ $\Delta S_{\text{CH}}^{\beta}/\Delta S_{\text{CH}}^{\alpha}$ ratio in agreement with the experimental ratio. ~~In all the other models, all other models underestimated the α segment order parameter decrease with bound cations is underestimated with respect to the β order parameter segment decrease.~~

In conclusion, ~~the a~~ clear correlation between bound cations and order parameter decrease ~~is observed in all the tested was observed for all~~ simulation models. Consequently, the ~~electrometer concept molecular electrometer~~ can be used to compare the cation binding affinity between experiments and simulations. However, we ~~find that the quantitative found that quantitatively the~~ response of α and β segment order parameters to bound cations in simulations ~~do did~~ not generally agree with the experiments. ~~The, e.g., the $\Delta S_{\text{CH}}^{\beta}/\Delta S_{\text{CH}}^{\alpha}$ ratio agrees agreed~~ with experiments only in ~~the~~ Lipid14 model (Fig. 5). Thus, the observed overestimation of the order parameter changes with ~~cation concentrations may salt concentrations could~~, in principle, arise from overbinding of ~~ions or from too sensitive cations or from an oversensitive~~ lipid headgroup response ~~on bound cation to the bound cations~~ (see also discussion in ESI[†]). A careful analysis with current lipid models is performed in the next section.

2.3 Cation binding in different simulation models

The order parameter changes (Fig. 2) and density distributions (Fig. 4) demonstrate significantly different Na^+ binding affinities in different simulation models. The best agreement with experiments (lowest $\Delta S_{\text{CH}}^{\alpha}$ and $\Delta S_{\text{CH}}^{\beta}$) is observed for those models (Orange, CHARMM36, and Lipid14; see Fig. 2) that also predict the lowest Na^+ densities in the membrane proximity (Fig. 4). In all the other tested models, the choline order parameter responses to NaCl are clearly overestimated (Fig. 2), and the strength of the overestimation is clearly linked to the strength of the Na^+ binding affinity (compare Figs. 2 and 4); this leads us to conclude that sodium binding affinity is overestimated in all these models.

In the best three models, the order parameter changes with NaCl are small (< 0.02), so with the achieved statistical accuracy we cannot conclude which of the three has the most realistic Na^+ binding affinity, especially at physiological NaCl concentrations ($\sim 150\text{mM}$) relevant for most applications. The overestimated binding in the other models raise questions on the quality of the predictions from these models when NaCl is present. Especially interactions between charged molecules and lipid bilayer might be significantly affected by the strong Na^+ binding, as it makes the bilayer effectively positively charged.

Significant Ca^{2+} binding affinity to a phosphatidylcholine bilayer at sub-molar concentrations is agreed in the literature^{2,3,20,29}, however, several details are yet under discussion. Simulations suggest that Ca^{2+} bind to lipid carbonyl oxygens with coordination number of 4.2¹³, while interpretation of NMR and scattering experiments suggest that one Ca^{2+} interacts mainly with choline groups^{106–108} of two phospholipid molecules²⁹.

~~Simulation~~ A simulation model correctly reproducing the order

parameter changes would resolve the discussion by giving atomistic resolution interpretation for the experiments.

As a function of CaCl_2 concentration, all but one (CHARMM36 with recent ion model by Yoo et al.⁷⁶), model overestimate the order parameter decrease (Fig. 2). According to the molecular electrometer, this indicates overestimated Ca^{2+} binding. This is the most likely scenario for the models where changes in both order parameters were overestimated, however, in the case of CaCl_2 we cannot exclude the possibility that the headgroup response is oversensitive to bound cations (see ESI[†]). In CHARMM36 with ion model by Yoo et al.⁷⁶, ΔS_{CH} is overestimated for β but underestimated for α , in line with Fig. 5 where $\Delta S_{\text{CH}}^{\beta}/\Delta S_{\text{CH}}^{\alpha}$ ratio in CHARMM36 is larger than in experiments. Since we do not know if $\Delta S_{\text{CH}}^{\beta}$ or $\Delta S_{\text{CH}}^{\alpha}$ is more realistic in CHARMM36, we cannot conclude if Ca^{2+} binding is too strong or weak in this simulation model. This could be resolved by comparing CHARMM36 model to the experimental data with known amount of bound charge (e.g., experiments with amphiphilic cations^{31,50}), however, such simulation data are not currently available.

The ion density distributions with CaCl_2 in Fig. 6 show significant Ca^{2+} binding in all models, however, some differences occur in details. The Berger model predicts deeper penetration depth (density maxima close to $\pm 1.8\text{ nm}$) compared to other models (density maxima close to $\pm 2\text{ nm}$). The latter value is probably more realistic since ^1H NMR and neutron scattering data indicate that Ca^{2+} interacts mainly with the choline group^{2,106–108}. In CHARMM36, almost all Ca^{2+} ions present in simulation bind in bilayer indicating strongest binding affinity among the tested models. The difference is not as clear in Fig. 2 because α carbon order parameters are the least sensitive to bound charge in CHARMM36 (Fig. 3).

The origin of inaccuracies in lipid-ion interactions and binding affinities in different models is far from clear. Potential candidates could be, for example, discrepancies in the ion models^{109–111}, incomplete treatment of electronic polarizability¹¹², or inaccuracies in the lipid headgroup description⁴⁵. Cordomi et al.²⁴ showed that the Na^+ binding affinity decreases when ion radius increases in the model, however, also the models with the largest radius show significant binding in DPPC bilayer simulated with OPLS-AA force field¹¹³. In our results, the Slipids model gives essentially similar binding affinity with ion parameters from Refs. 92 and 90,91. Further, the compensation of missing electronic polarizability by scaling ion charge^{112,114} reduced Na^+ binding in Berger, BergerOPLS and Slipids models, but not enough to be in agreement with experiments (ESI[†]). The charge-scaled Ca^{2+} model¹¹⁵ slightly reduced binding in CHARMM36, but did not have significant influence on binding in Slipids (ESI[†]). Significant reduction of Ca^{2+} binding was observed with ion model by Yoo et al.⁷⁶, however, the CHARMM36 lipid model must be further analysed to fully interpret the results.

On the other hand, also the lipid models may have significant influence on ion binding behaviour. For example, the same ion model and non-bonded parameters are used in the Orange and BergerOPLS⁶⁰ simulations, but while Na^+ ion binding affinity appears realistic in the Orange model, it is significantly overestimated in the BergerOPLS (Fig. 4). However, realistic Na^+ bind-

Table 1 List of MD simulations performed in this work. The ion salt concentrations are calculated as $[\text{ionsalt}] = (N_{\text{ion}} \times c \times [\text{water}]) / N_w$, where $[\text{water}] = 55.5 \text{ M}$. These correspond the concentrations reported in the experiments by Akutsu et al.²⁰. The lipid force fields are named as in our previous work⁴⁵.

Force field (lipid, ion)	force field for lipids / ions	lipid	salt	[ionsalt] mM- (mM)	^a N _l	^b
Berger-POPC-07 ⁵¹ / -		POPC	no	0	128	7
Berger-POPC-07 ⁵¹ / ffgm ⁵³		POPG	NaCl	340 (NaCl)	128	7
Berger-POPC-07 ⁵¹ / ffgm ⁵³		POPG	340 (CaCl ₂)	128-7157-340	0	44-71
Berger-DPPC-97 ⁵⁶ / -		DPPC	no	0	72	2
Berger-DPPC-97 ⁵⁶ / ffgm ⁵³		DPPG-150 (NaCl)	72 NaCl	2880-150	8	
Berger-DPPC-97 ⁵⁶ / ffgm ⁵³		DPPG		1000 (NaCl)	72	2
BergerOPLS-DPPC-06 ⁶⁰ / -		DPPC	no	0	72	2
BergerOPLS-DPPC-06 ⁶⁰ / OPLS ⁶²		DPPG-150 (NaCl)	72 NaCl	2880-150	8	
BergerOPLS-DPPC-06 ⁶⁰ / OPLS ⁶²		DPPG		1000 (NaCl)	72	2
CHARMM36 ⁶⁵ / -		POPC	no	0	72-128	2242-52
CHARMM36 ⁶⁵ / -				0	303-72	22
CHARMM36 ⁶⁵ / CHARMM36 ⁶⁸		POPG	NaCl	350 (NaCl)	72	2
CHARMM36 ⁶⁵ / CHARMM36 ⁶⁸		POPG-690 (NaCl)	72	2085-690	26	
CHARMM36 ⁶⁵ / CHARMM36 ⁶⁸		POPG		950 (NaCl)	72	2
CHARMM36 ⁶⁵ / CHARMM36		POPG	350 (CaCl ₂)	350	128	6
CHARMM36 ⁶⁵ / CHARMM36		POPG		450 (CaCl ₂)	200	9
CHARMM36 ⁶⁵ / CHARMM36		POPG		670 (CaCl ₂)	128	6
CHARMM36 ⁶⁵ / CHARMM36		POPG	1000 (CaCl ₂)	128-1000	6400	
CHARMM36 ⁶⁵ / -		DPPC	no	0	128	80
CHARMM36 ⁶⁵ / Yoo ⁷⁶		DPPG	430 (CaCl ₂)	128-430	7760	0-77
CHARMM36 ⁶⁵ / Yoo ⁷⁶		DPPG	886 (CaCl ₂)	128-890	7520	0-75
MacRog ⁷⁷ / -		POPC	no	0	288-128	14400-64
MacRog ⁷⁷ / -				0	310-288	144
MacRog ⁷⁷ / OPLS ⁶²		POPG	NaCl	100 (NaCl)	288	14
MacRog ⁷⁷ / OPLS ⁶²		POPG		210 (NaCl)	288	14
MacRog ⁷⁷ / OPLS ⁶²		POPG		310 (NaCl)	288	14
MacRog ⁷⁷ / OPLS ⁶²		POPG		420 (NaCl)	288	14
Orange / OPLS ⁶² / -		POPC	no	0	72	2
Orange / OPLS ⁶²		POPG	NaCl	140 (NaCl)	72	2
Orange / OPLS ⁶²		POPG		510 (NaCl)	72	2
Orange / OPLS ⁶²		POPG		1000 (NaCl)	72	2
Orange / OPLS		POPG	510 (CaCl ₂)	72-510	2802	0-28
Slipids ^{86, 87} / -		DPPG-POPC	no	0	128	3840-0-51
Slipids ⁸⁶ / AMBER ^{90,91} / AMBER ⁹²		DPPG	150 (NaCl) NaCl	600-130	18000-200	49-90
Slipids ⁸⁶ / AMBER ^{90,91} DPPG ⁸⁷ / AMBER ⁶²		850 (NaCl)	128 CaCl ₂	3726-450	57	
Slipids ⁸⁷ / Slipids ⁸⁶ / -		POPG-DPPC	no	0	128	5120-38
Slipids ⁸⁶ / AMBER ^{90,91}			NaCl	150	-89-600	180
Slipids ⁸⁷ / AMBER ⁹² / AMBER ^{90,91}		POPG	130 (NaCl)	850	128	37
Slipids ⁸⁶ / AMBER ^{90,91}		21	0	21-1750	310	36
Slipids ⁸⁷ / AMBER ⁶² POPC ⁸⁶ / AMBER ^{90,91}		450 (CaCl ₂)	200	9000-2570	0	73-35
Lipid14 ⁹⁶ / AMBER ⁶² / -		POPC	no	0	128	5
Lipid14 ⁹⁶ / AMBER ⁶²		POPG-150 (NaCl)	128 NaCl	5120-150	12	
Lipid14 ⁹⁶ / AMBER ⁶²		POPG-1000 (NaCl)	128	5120-1000	77	
Lipid14 ⁹⁶ / AMBER ⁶²		POPG	350 (CaCl ₂)	128-350	6400	0-64
Lipid14 ⁹⁶ / AMBER ⁶²		POPG	1000 (CaCl ₂)	128-1000	6400	
Ulmschneiders ¹⁰² / OPLS ⁶² / -		POPC	no	0	128	5
Ulmschneiders ¹⁰² / OPLS ⁶²		POPG-150 (NaCl)	128 NaCl	5120-150	12	
Ulmschneiders ¹⁰² / OPLS ⁶²		POPG-1000 (NaCl)	128	5120-1000	77	

^a The number of lipid molecules

^b The number of water molecules

^c The number of Na⁺ molecules, ^d N_{Ca}, ^e N_{Cl} cations

^f Simulation temperature

^g The total simulation time

^h Time frames used in the for analysis

ⁱ Reference for simulation files

^j Journal Name, [year], [vol.], 1-11

^k The number of lipid molecules

^l The number of water molecules

^m The number of Na⁺ molecules

ing does not directly relate to realistic Ca^{2+} binding (see Orange, Lipid14 and CHARMM36 in Fig. 2) or realistic choline order parameter response to bound charge (see Orange and CHARMM36 in Fig. 5). It should be also noted that the low binding affinity of Na^+ in CHARMM36 model is due to the additional repulsion added between sodium ions and lipid oxygens (NBFIX)⁶⁸ (ESI^+). Altogether, our results indicate that probably both, lipid and ion force field parameters, need improvement to correctly predict the cation binding affinity, and the associated structural changes.

3 Conclusions

As suggested by the molecular electrometer concept^{20,29–31, 20,29–32}, the decrease in order parameters of α and β carbons in the PC head group of lipids bilayers is related to cation binding in all tested simulation models (Fig. 3), despite of known inaccuracies in the actual atomistic resolution structures⁴⁵. Hence the molecular electrometer concept allows a direct comparison of Na^+ binding affinity between simulations and noninvasive NMR experiments. The comparison reveals that most models overestimate 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 pre-2000 view that at submolar concentrations, in contrast to Ca^{2+} and other multivalent ions^{1–4,10,11,19,20,27,29}, Na^+ and other monovalent ions (except Li^+) do not specifically bind to phospholipid bilayers. Concerning the interpretation of existing experimental data, our work supports Cevc's view² that the observed small shift in phase transition temperature is not indicative of Na^+ binding. Further, our findings are in line with the noninvasive NMR spectroscopy work of Filippov et al.¹¹ that proved the results of Refs. 7,9,12 to be explainable by direct interactions between Na^+ ions and fluorescent probes. Finally, as spectroscopic methods are in general more sensitive to atomistic details in fluid-like environment than AFM, our work indirectly suggests that the ion binding reported from AFM experiments on fluid-like lipid bilayer systems^{14–18} might be confounded with other physical features of the system. Concerning contradictions in MD simulation results, we reinterpret strong Na^+ binding as an artefact of several simulation models, e.g., the Berger model used in Refs. 12,13.

The artificial specific Na^+ binding in simulations may lead to doubtful results, since it effectively leads to positively charged phosphatidylcholine (PC) lipid bilayers even at physiological NaCl concentration. Such a PC bilayer has distinctly different interactions with charged objects compared to a (more realistic) model without specific Na^+ binding. Furthermore, the overestimation of Na^+ binding affinity may extend also to other positively charged objects, say, membrane protein segments. This would affect lipid–protein interactions and could explain, for example, contradicting results on electrostatic interactions between charged protein segments and lipid bilayer^{116,117}. In conclusion, more careful studies and model development on lipid bilayer–charged object interactions are called for to make molecular dynamics simulations directly usable in a physiologically relevant electrolytic environ-

ment.

This work has been done as a fully open collaboration, using nmrlipids.blogspot.fi as the communication platform. All the scientific contributions have been communicated publicly through this blog or GitHub repository³⁴. All the related content and data is available at Ref. 34.

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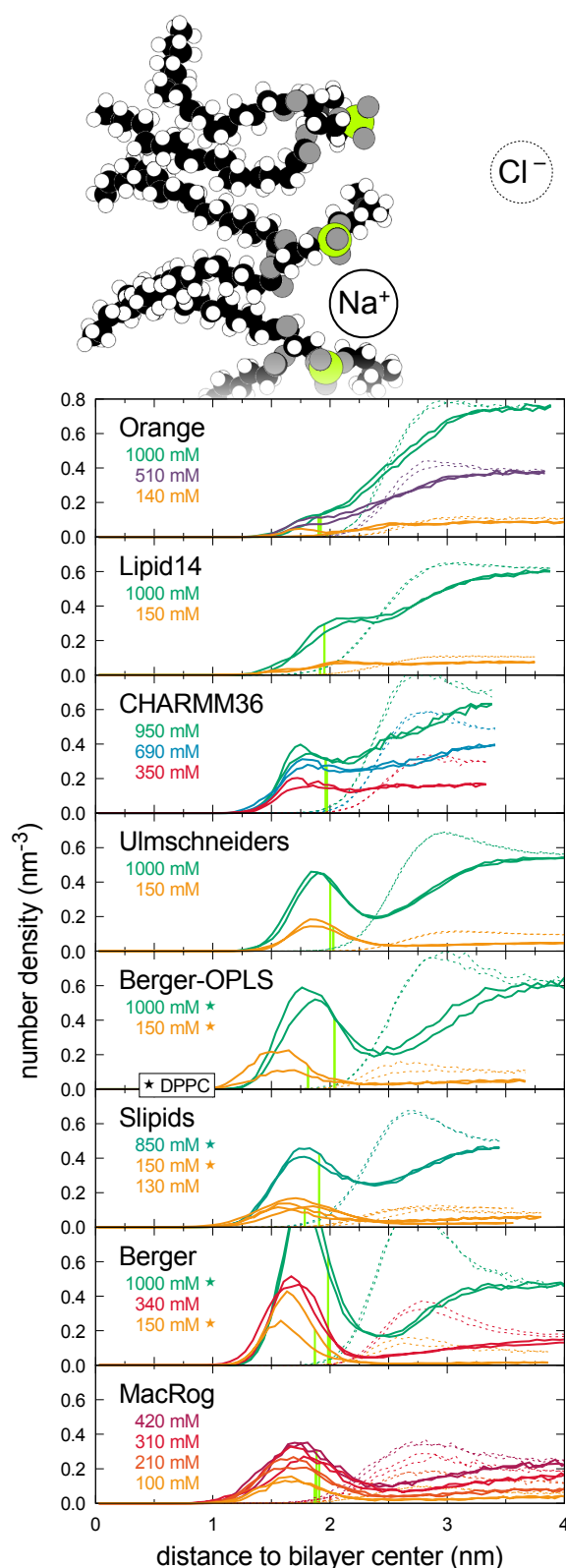


Fig. 4 Na^+ (solid line) and Cl^- (dashed) distributions along the lipid bilayer normal from MD simulations at several NaCl concentrations. The eight MD models are ordered according to their strength of order parameter change in response to NaCl (Fig. 2) from the weakest (top panel) to the strongest (bottom). The light green vertical lines indicate the locations of the Phosphorus maxima, used to define bound cations in Fig. 3.

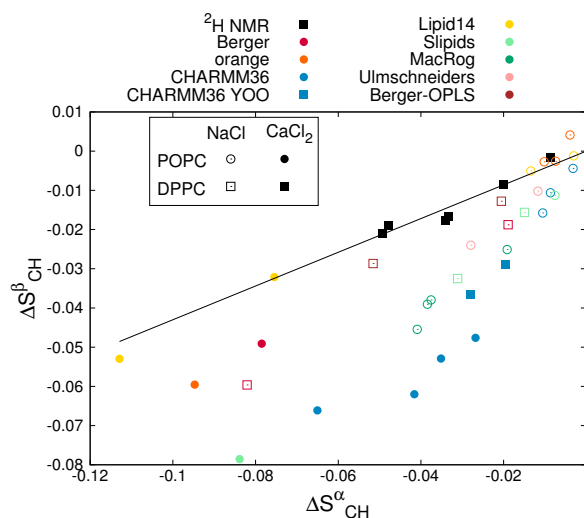


Fig. 5 Relation between $\Delta S_{\text{CH}}^{\beta}$ and $\Delta S_{\text{CH}}^{\alpha}$ from experiments²⁰ and different simulation models. Solid line is $\Delta S_{\text{CH}}^{\beta} = 0.43\Delta S_{\text{CH}}^{\alpha}$ determined for DPPC bilayer from ^2H NMR experiment with various CaCl_2 concentrations²⁰.

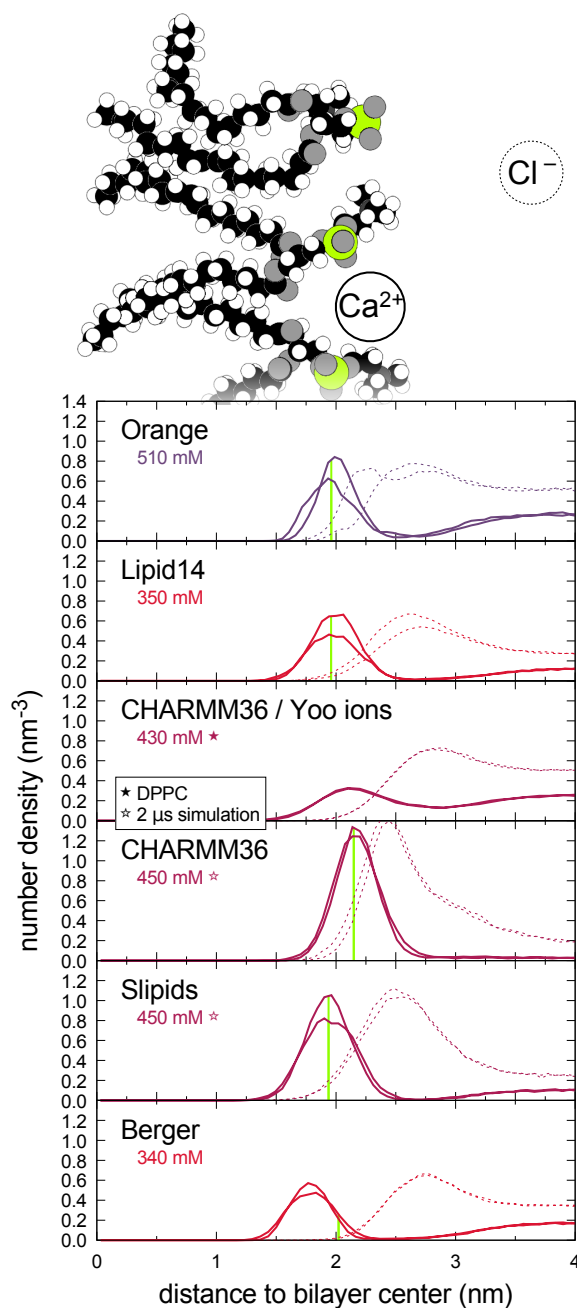


Fig. 6 Atom-number density profiles along the membrane normal coordinate z for lipids, Ca^{2+} (solid line) and Cl^- ions (dashed) distributions along the lipid bilayer normal from MD simulations with different force fields. The profiles for clarity, only with smallest available one CaCl_2 concentration are per MD model is shown; see ESI† for clarity. Figure a plot including all the available concentrations is shown in ESI†. The lipid densities are scaled with 100 (united atom) or 200 (all atom model) to make them visible with light green vertical lines indicate the locations of the Phosphorus maxima, used y-axis scale. The Cl^- density is scaled with 2 to equalise charge density of ions define bound cations in Fig. 3.