

Accurate binding of calcium to phospholipid bilayers by effective inclusion of electronic polarization

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1. Abstract directly from Joe's conference abstracts. To be rewritten. Classical molecular dynamics simulations give detailed information about membrane structure and dynamics. However, there is still a room for improvements in current force fields. It is known from the literature, that the binding of ions, especially cations, to phospholipid membranes is overestimated in all classical models [1]. We suggest that the membrane-ion interactions can be corrected by including implicit electronic polarizability into the lipid models through the electronic continuum correction (ECC) [2], which was already applied to monovalent and divalent ions yielding models that feature correct ion pairing [3]. Using the electrometer concept [3, 4] and x-ray scattering form factors, our simulations point out that our hypothesis is correct and ECC is indeed a missing important contribution in current classical lipid models. Moreover, the solid physical principles behind ECC are found not to hamper other relevant properties of a phospholipid bilayer. The new lipid model, "ECC-lipids", shows accurate binding affinity to sodium and calcium cations and head group order parameter response to bound charge. We also provide for the first time a realistic stoichiometry of bound calcium cations to a POPC membrane, and their binding sites. This work will continue as an open collaboration project NMRlipids VI (<http://nmrlipids.blogspot.fi>).

I. INTRODUCTION

Cation interactions with cellular membranes play a key role in several biological processes, like in signal propagation in neurons and vesicle fusion. **2. JOE: list more introductory examples, where it has interesting effects.** Zwitterionic phosphocholine (PC) lipid bilayers have been widely used as model systems to understand molecular level details of specific cation interactions with cellular membranes by using experimental [1–5] and theoretical methods [6?–8]. **3. PAVEL: introduce previous theoretical work that discusses cation binding to POPC w.r.t. its specific moieties, e.g. Lukas' paper.** While relative binding affinity of different ions is agreed to follow Hoffmeister series, the molecular details of binding and binding energetics are not agreed in the literature [1–3, 9? , 10]. Non-invasive spectroscopic methods, like nuclear magnetic resonance (NMR), scattering and infrared spectroscopy, give accurate information about ion binding in lipid bilayers [3, 11–15]. Molecular level interpretation of the results, however, requires assumptions about binding models and is often not fully conclusive [?]. On the other hand, the accuracy of the state of the art atomistic resolution lipid and ion models have turned out insufficient for the detailed interpretation of cation binding details [10]. The overestimated specific cation binding observed in current lipid models [10] may lead to artificially positively charged membranes and significant artefacts in MD simulations (e.g. divalent cation-induced charge inversion of bacterial membranes [16]). **4. JOE: this may be a too daring statement.**

SAMULI: I think that the divalent cation induced charge inversion is not an artefact, but it might happen with too small concentrations. On the other hand, it is not a new observation either, see e.g. literature cited in NMRlipids IV project.)

In this work we show that the accuracy of ion binding, especially calcium, in zwitterionic PC lipid bilayer can be significantly improved for classical MD simulation models of zwitterionic lipids by including electronic polarizability in the polar region of lipid molecules using the electronic continuum correction (ECC) [17]. This approach has been previously shown to improve performance of ion models against neutron scattering data in concentrated solutions and ab-initio simulations [18? , 19]. The realistic structure of ions in a bulk solvent was not, however, sufficient to correct binding in lipid bilayers [10]. To fix it, we propose a classical fixed-charge MD simulation model of 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) lipid that accounts for electronic polarizability by using ECC. We will validate such a model by comparing its lipid bilayer structure to x-ray scattering data and its head group structure to NMR experiments. We will then quantify the accuracy of the head group order parameter response to the bound charge and compare it to the experimental NMR data of sodium and calcium binding in PC lipid bilayers. In addition, we will quantify relative binding affinities of different moieties in POPC to such cations. This will be discussed in terms of binding modes, stoichiometry and binding isotherms and their models used in experiments. The proposed POPC model, ECC-lipids, and the approach to obtain it is, hence, highly useful for future MD simulations with physiological salt conditions, and for effects where membrane electrostatics play a major role.

II. METHODS

A. Electronic continuum correction for lipid bilayers

The lack of electronic polarizability in the standard MD simulation force fields has been considered highly relevant is-

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sue since the early days of lipid bilayer simulations. In this work we circumvent the rather demanding explicit inclusion of electronic polarization effects [20] by implicitly including electronic polarizability in lipid bilayer simulations by using the electronic continuum correction (ECC) [17]. Technically, it is a similar approach to the phenomenological charge-scaling as applied in the early studies where a scaling factor one half was used [21, 22]. However, the concept of ECC is physically well justified and rigorously derived [17, 23, 24], but significantly more simple than the explicit inclusion of electronic polarizability [20] both for derivation and application.

According to ECC, electronic polarizability can be implicitly included in classical MD simulations by placing all particles into a homogeneous dielectric continuum with a dielectric constant ϵ_{el} , which is the electronic part of the dielectric constant of the media [17]. Measurements of high frequency dielectric constant gives values of approximately $\epsilon_{el} \approx 2$ for almost any biomaterial [17?]. Such a dielectric continuum can be easily included in standard MD simulation by a formal transformation of partial charges

$$Q^{ECC} = f_q \cdot Q \quad (1)$$

with a constant scaling factor $f_q = \epsilon_{el}^{-1/2}$ effectively representing the newly introduced electronic continuum. The value measured for water, $\epsilon_{el} = 1.78$, gives a scaling factor of $f_q = 0.75$ [17?], which has been successfully used to improve the performance of force field for ions [18? , 19].

While the scaling factor of $f_q = 0.75$ for ions in water is well justified and improves the model performance against experimental scattering data [18, 19, 25], it is not clear if the same factor should be used for partial charges in molecules, i.e, lipids in our case. Unlike the total charge of molecules, atomic partial charges within each molecule are not experimental observables. **5.Text should be clarified from here to the end of the paragraph** Although there have been proposed several schemes for the assignment of partial charges of molecules, methods targetting at fitting of electrostatic potentials are most popular among classical fixed charge force fields for biomolecules. [26] Currently the most commonly employed scheme is the RESP scheme [27, 28], which fits the electrostatic potential from quantum mechanical calculations. The resulting set of fixed partial atomic charges is hence the best fit to electrostatic potentials of an ensemble of conformations of a molecule. Such a physical meaning is especially well suited for the application of ECC, which targets at improving electrostatic interactions. Depending to the details, partial charge calculations may also partially include some of the solvent electronic polarizability effects. Thus, we expect that the correct ECC scaling factor, f_q , for molecular partial charges does not have to be strictly $f_q = \epsilon_{el}^{-1/2}$, but rather lies between this value (no electronic polarizability in the charge calculation) and 1 (full electronic polarizability already included in the charge calculation).

In this work, we empirically sample this parameter space $f_q \in [0.75, 1.0]$ to find an atomistic MD simulation force field parameters for lipids that accurately describe the lipid head

group response to varying concentrations of cations when compared to NMR data [14, 15, 29]. Such data can be used to accurately asses ion binding in PC bilayers, as discussed in Ref. 10 and in section II B. We chose Lipid14 [30] force field parameters as a starting point, because their response to bound ions was apparently most realistic against NMR data in recent work by NMRlipids project (see Fig. 5 in Ref. 10). Also glycerol backbone and head group structures in Lipid14 model were relatively realistic when compared with other state of the art lipid models [31]. The ECC correction was applied to Lipid14 parameters by scaling partial charges of the head group, glycerol backbone and carbonyl regions, which are the most polar parts in lipids and are expected to have the largest contribution to the cation binding. The hydrocarbon chain parameters are not modified, because they are already highly optimized and give generally a good description for hydrophobic part of lipid bilayers in various conditions in most lipids models, including Lipid14 [32]. In contrast, improvements in glycerol backbone and head group parameters are required in all available lipid models [31].

Exploring different scaling factor values, applied to head group, glycerol backbone and carbonyl charges of Lipid14 model, we found out that ion binding and related head group order parameter responses become weaker. The optimal behaviour of ion binding was observed with the scaling factor $f_q = 0.8$. Interestingly, this scaling factor is in line with the estimate given by “implicitly polarized charges” (IPolQ) [33] combined with RESP calculations in vacuum and implicit solvent reported in [34]. IPolQ charges are obtained as the average of partial charges given by RESP calculation [27] in vacuum and in a solvent. Applying the scaling factor of 0.75 to IPolQ charges calculated from the data in Ref. [34], gives similar partial charges to ones obtained by scaling Lipid14 charges with a factor 0.8.

While, the charge scaling improved the behaviour of lipid-ion interactions, it reduced the area per molecule of lipid bilayer without ions below experimental values. Simulations with Lipid14 parameters having partial charges of head group, glycerol backbone and carbonyls scaled with 0.8 gave the area per molecule value of $\approx 60 \text{ \AA}^2$, which is significantly smaller than the experimental value 64.3 \AA^2 ([6.missing REF for APL experiment) and the original Lipid14 value $(65.6 \pm 0.5) \text{ \AA}^2$ [30]. The decrease of area was found to arise from a lower hydration of the lipid head group region, which can be explained by the increased solvation free energy due to the lower polarity of molecules with scaled charges. The hydration can be increased by reducing the effective radius of atoms by changing the σ parameters in Lennard-Jones potential for the selected atoms similarly as done for free ions in solution [18, 19, 25]. This decreases the solvation free energy by allowing water molecules to approach closer to lipid atoms and have stonger electrostatic interactions with them. After reducing the σ parameters with a factor of $f_\sigma = 0.89$ for the same atoms for which charges were scaled, the area per molecule value was again in agreement with experimental value (see Table II).

B. Electrometer concept

Ion binding was compared between experiments and simulations by using lipid head group order parameters and "electrometer concept" [10, 36]. The concept is based on the experimental observation that the C-H bond order parameters of α and β carbons in lipid head group (see Fig. 1) are proportional to the amount of bound charge in a lipid bilayer [36]. This is rationalized as a change of lipid head group dipole tilt to more vertical orientation with bound positive charge and *vice versa* for negative charge [36]. More recent analysis in the NMRlipids project concluded that the order parameters decrease with bound positive charge and increase with bound negative charge when the signs are taken in account [10, 32]. The concept was also shown to be suitable for the comparison of ion binding affinity in lipid bilayers between simulations and experiments [10].

The order parameters for α and β segments in head group, as well as for all C-H bonds in lipid molecules, can be accurately measured by using ^2H NMR or ^{13}C NMR techniques. The order parameters can be calculated from MD simulations by using the definition

$$S_{\text{CH}} = \frac{3}{2} \langle \cos^2 \theta - 1 \rangle, \quad (2)$$

where θ is the angle between the bond and membrane normal and average is taken over all sampled configurations [32].

According to the electrometer concept, the change of the head group order parameters as a function of the amount of bound charge per lipid X^\pm can be written as [37]

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

where $S_{\text{CH}}^i(0)$ denote the order parameter in the absence of bound charge, i refers to either α or β carbon, m_i is an empirical constant depending on the valency and position of bound charge, and the value of the quadrupole coupling constant is $\chi \approx 167 \text{ kHz}$. Atomic absorption spectra and ^2H NMR data gave $m_\alpha = -20.5$ and $m_\beta = -10.0$ for Ca^{2+} binding to POPC bilayer (in the presence of 100 mM NaCl) [10, 15, 32]. Before using electrometer concept to analyze cation binding, we calibrate the response of head group order parameters to the bound charge in the simulation models. This is done by comparing the slope m_i in Eq. 3 between simulations and experiments of cationic surfactants in POPC bilayer [29]. Since essentially all surfactants locate in bilayer, the molar ratio directly gives the amount of bound charge per lipid X^\pm in these systems.

C. Ion concentrations and binding affinity

The salt concentrations are reported in two different ways in the used experimental data for the lipid head group order parameters. The data for DPPC is reported by using solution concentrations before solvating the lipids [14], while the data for POPC [15] is reported by using supernatant concentration

measured with atomic absorption spectroscopy after solvation of lipids. The former was used in comparison with experiments and calculated from simulations in previous work as $[\text{salt}] = N_c \times [\text{water}] / N_w$, where $[\text{water}] = 55.5 \text{ M}$ [10]. Here we use the latter, because the focus is on POPC bilayers. This is determined from the ion concentrations, which reach a constant value at the edges of large enough simulation box. The two concentrations differ somewhat for CaCl_2 systems, because some amount of ions bind in lipids. However, the small difference do not affect the conclusions in this or in previous work [10].

The ion concentrations with constant value at the simulation box edges are also used to quantify the ion binding affinities in different simulation models by using the relative surface excess of ions with respect to water Γ_i^w . This is defined as [38]

$$\Gamma_i^w = \Gamma_i - \Gamma_w \frac{C_i - C_i'}{C_w - C_w'} \quad (4)$$

$$\Gamma_a = \frac{n_a - C_a V - C_a' V'}{A}; \quad a = i, w, \quad (5)$$

where index i and w denote ions and water respectively, prime accent denotes the phase inside bilayer, whereas non-prime symbols account for bulk water phase, n is the total amount of water or ions, C denotes bulk concentrations, V is the volume of the respective phase separated by the Gibbs dividing plane, A is the area of the interface, and Γ_a is the surface excess of water or ions. As Γ_i^w does not depend on the definition of the interfacial region and the position of the Gibbs dividing plane [38], we consider the whole simulation box as an interface. Because bilayers have an interface in both leaflets, the total area of the interface in the simulation is given by the simulation box area multiplied by two, $2A_b$. The concentrations of water and ions inside the bilayer can be expected to vanish, thus $C_{i,w}' = 0$. This provides us a simplified relation for the relative surface excess of lipid bilayers in simulations

$$\Gamma_i^w = \frac{1}{2A_b} \left(n_i - n_w \frac{C_i}{C_w} \right), \quad (6)$$

where the bulk concentrations, C_w and C_i , are concentrations with constant value at the simulation box edges.

D. Validation of lipid bilayer structure against experiments

Lipid bilayer structure without ions was validated against NMR experiments by order parameters for C-H bonds and x-ray scattering experiments by using form factors. The former validates the structures sampled by individual lipid molecules in simulations with segmental resolution, while the latter validates the dimensions of the lipid bilayer (thickness and area per molecule) [32].

The order parameters were calculated from simulations for all C-H bonds in lipid molecules by using Eq. 2. Form factors

were calculated from equation

$$F(q) = \int_{-D/2}^{D/2} \left(\sum_{\alpha} f_{\alpha}(q_z) n_{\alpha}(z) - \rho_s \right) \exp(izq_z) dz, \quad (7)$$

where $f_{\alpha}(q_z)$ is the density of atomic scattering length, ρ_s is the density of solvent scattering length, $n_{\alpha}(z)$ is the number density of atom α and z is the distance from the membrane centre along its normal spanning the membrane with thickness D .

E. Simulation details

1. Simulations with aqueous ions

The simulated systems consisted of 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayer and an aqueous salt solutions of varying concentrations. In particular, the periodic orthorhombic simulation box contained 128 POPC molecules and approximately 50 water molecules per each lipid. Water molecules were described by OPC3 model [39] as it is currently the most accurate three site rigid water model. In order to test transferability of the newly developed ECC-lipids model, we also performed several additional simulations with water models OPC [40], SPC/E [41], TIP3p-FB and TIP4p-FB [42], and TIP4p/2005 [43] presented in Supporting Information (SI). We used ECC-ions model for ions [19, 25]. Simulations with Lipid14 use ion models by Dang [44–46], and by Åqvist [47]. Classical molecular dynamics simulations were performed using the GROMACS [48] simulation package (version 5.1.4). The simulation settings used in this work are summarized in Table I, and they are based on previously used settings in [10] available at [49].
7.As far as remember, I used there Langevin dynamics instead of thermostated MD, because this is done in Amber by default. If this is correct, the information in the table do not match with this sentence. Based on semi-extensive testing I made few years ago this do not change anything. Anyway, this should be reported consistently. Simulation trajectories and parameters are available at [?] 8.To be uploaded to Zenodo.

2. Simulations with cationic surfactants

Automated topology builder [56] was first used to create the structure of dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}^+N_2C_1Br^-$, molecule. AmberTools program [57] was then used to generate the Amber-type force field parameters. The parameters were converted to the Gromacs format by using acpype tool [58]. The partial charges were then manually modified to approximately correspond to their equivalent segments in Lipid14 [30]. The surfactants were randomly placed among the lipids to form bilayer structures with mole fractions 10%, 20%, 30%, 42% and 50% of surfactant in the POPC bilayer. All systems contained 50 POPC molecules per leaflet, 6340 TIP3P water molecules and 6, 14, 21, 35 or 50 surfactants per leaflet. The systems were simulated for 200 ns

TABLE I: Simulation parameters

simulation property	parameter
time-step	2 fs
equilibration time	100 ns
simulation time	200 ns
temperature	313 K
thermostat	v-rescale [50]
barostat	Parrinello-Rahman, semi-isotropic [51]
long-range electrostatics	PME [52]
cut-off scheme	Verlet [53]
Coulomb and VdW cut-off	1.0 nm
constraints	LINCS, only hydrogen atoms [54]
constraints for water	SETTLE [55]

9.This could be moved to SI. Only simulation lengths needs to be mentioned in the main paper.

TABLE II: Area per lipid (APL) from different models of POPC without ions

model	APL (\AA^2)	Temperature [K]
Lipid14 [30]	65.6 ± 0.5	303
ECC-lipids		
$(4.6 \cdot 5.1 \text{ nm}^2)$, 72 lipids patch, OPC3	63.2	313
$(6.4 \text{ nm})^2$, 128 lipids patch, OPC3	64.2	313
$(6.4 \text{ nm})^2$, 128 lipids patch, SPC/E	65.1	313
$(6.4 \text{ nm})^2$, 128 lipids patch, OPC	64.4	313
$(6.4 \text{ nm})^2$, 128 lipids patch, TIP4p/2005	66.8	313
experiment [60] 14.REF	64.3	303
experiment	67.3	323

15.SAMULI: I would put here Lipid14 in 303K, ECC-lipid in 303K and experiment in 303K. Rest in SI. The best experimental value would be the one analyzed from the form factor shown in previous figure, if available.

using Lipid14 model for POPC. First 20 ns were omitted from the analysis.

The same systems were also simulated with ECC-lipid model for POPC using the same setup. In these simulations the ECC correction was also applied to the cationic surfactant by scaling all charges with the same factor as for ECC-lipids, $f_q = 0.8$, and by using the atom types with reduced σ parameters from ECC-lipids.

III. RESULTS AND DISCUSSION

A. POPC membrane and its structure

In order to validate the newly developed model, ECC-lipids, we compared our simulation results without any ions to NMR order parameters measurements and x-ray scattering form factors (Fig. 1 and Table II). The tail order parameters being already highly optimized in the original Lipid14 model [30] are found to match the experimental values nicely.

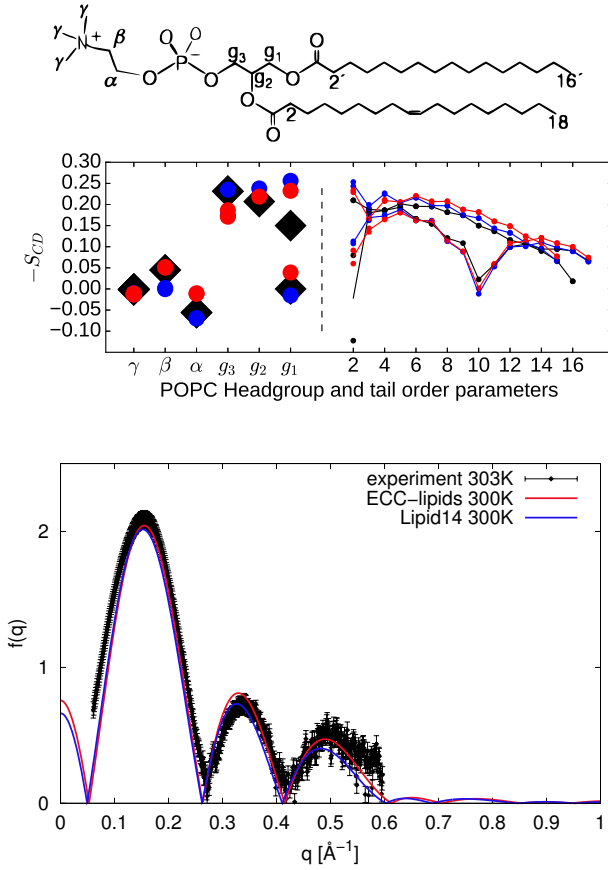


FIG. 1: X-ray scattering form factors from experiments [?] and simulations using Lipid14 [30] and ECC-lipids models. Order parameters of head group, glycerol backbone and sn-1 and sn-2 tails from simulations with Lipid14 [30] and ECC-lipids models compared with experimental order parameters from [59].

10. There seems to be a typo in experimental value for other order parameter for C2 carbon in sn-2 chain; it should be negative. The scale of the y-axis should be then zoomed when this is fixed.

11. I would put the acyl chain order parameters also for original Lipid14; JOE: Already done, check whether your figures are up to date. I usually push only *.eps.

12. x-axis scale in form factor figure would be better from 0 to 0.6. The experimental data is available for this range.

13. $S_{CD} \rightarrow S_{CH}$

The head group and glycerol backbone order parameters accuracy is comparable to the state of art lipid models available in literature [32]. The agreement between the x-ray scattering form factors and the areas per molecule from simulations and experiments confirm that the membrane structural properties are well captured. A structural comparison of ECC-lipids with Lipid14 can be found in SI along with results with other water models.

B. Response of POPC head group to bound charge

Before proceeding to the ion binding affinity studies, we quantify the response of headgroup order parameters to the

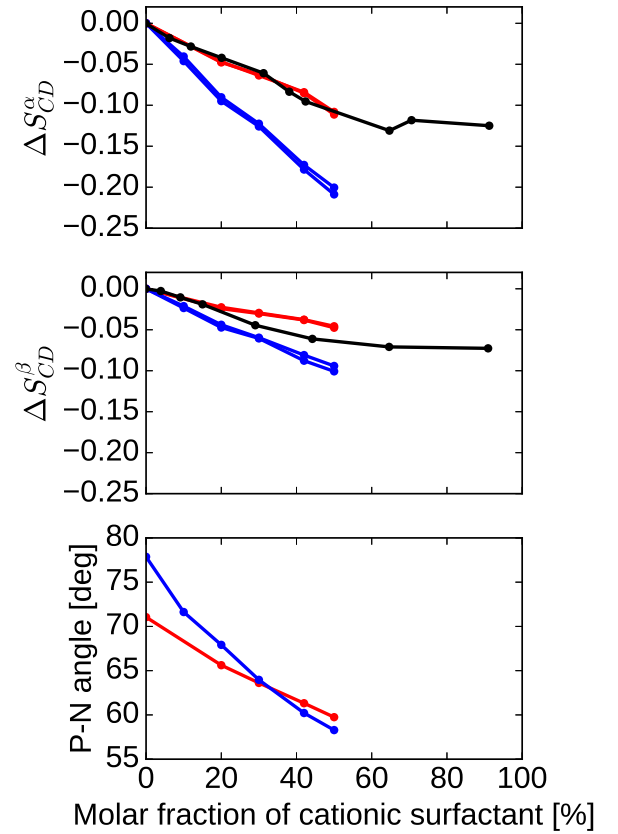


FIG. 2: Headgroup order parameter changes and P-N vector orientation as a function of cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}^+N_2C_1Br^-$) in PC bilayer from simulations and experiments [29].

16. Labels are missing.

17. Order should be the same as in other figures, i.e., β segment on top.

18. I would put x-axis from 0 to 51 and maybe zoom y-axis little bit as well.

amount of bound charge by using mixtures of monovalent cationic surfactants and POPC [29]. The amount of bound charge per PC in these systems is given by the molar fraction of cationic surfactants, because essentially all surfactants locate in the lipid bilayers. Experimental data for these systems can be used to validate the sensitivity of lipid headgroup order parameters to the amount of bound charge in simulations.

The headgroup order parameter changes with increasing amount of cationic surfactant dihexadecyldimethylammonium bromide ($C_{12}Cl_{16}^+N_2C_1Br^-$) is compared between experiments [29] and simulations in Fig. 2. Approximately linear decrease of the order parameters is observed in simulations and experiments with increasing amount of cationic surfactants up to mole fractions of $\sim 50\%$, as expected from Eq. 3. The slope is, however, too steep in Lipid14 model indicating that the head group order parameters are too sensitive to a bound charge. The ECC-lipids model gives a slope in very good agreement with experiments for the α segment, while the slope is slightly underestimated for the β segment.

19. SAMULI: We could calculate the slopes from simulations, but I am not sure if we would actually learn anything useful from this.

The headgroup P-N vector angle with respect to the membrane normal is also shown as a function of cationic surfactant mole fraction in Fig. 2. The headgroup orients more parallel to the membrane normal with increasing amount of bound cations, as expected [36]. The effect is more pronounced in Lipid14 than in ECC-lipids model, which is in line with the order parameter results and the reduced charge-dipole interactions in the ECC-lipid model. The response of α -order parameter to bound positive charge in ECC-lipid model is in good agreement with experiments. Therefore we use the data in Fig. 2 to suggest a linear relation between α -carbon order parameter and average P-N vector angle changes $\Delta\Theta_{P-N} = \Delta S_{CH}^\alpha$. This can be used to estimate the change in P-N vector angle from experiments measuring changes in α -carbon order parameter. **20.SAMULI: I think that we should establish the relation between order parameter and P-N vector angle changes by using ECC-lipids model. This would be useful for people measuring alpha order parameter changes from experiments.** For example, $\Delta S_{CH}^\alpha = 0.018$ measured due to the addition of $66.8 \mu\text{M}$ concentration of Mellittin in bulk water [61] gives $\Delta\Theta_{P-N} = 2.5^\circ$. **21.I looked this roughly from the figure, should be calculated from the actual relation if we decide to put this.**

C. Cation binding affinity in POPC

The binding affinity of aqueous cations in lipid bilayers can be measured by using the headgroup order parameters, because they decrease linearly with the bound positive charge [10, 36]. The headgroup order parameter responses to aqueous NaCl and CaCl₂ concentrations are shown in Fig. 3 from experiments (DPPC [14] and POPC [15]) and different simulation models for POPC.

Negligible changes of the headgroup order parameters are measured with submolar concentrations of NaCl due to the very low affinity of Na⁺ in PC bilayers [14]. While Na⁺ binding and the related headgroup order parameter changes were overestimated in almost all the available simulation models, the low affinity and negligible order parameter changes were reproduced by Lipid14 model when simulated with Åqvist ions [10]. However, the same combination of force field parameters overestimated the headgroup order parameter response to CaCl₂ concentration, which was the case also in all other models tested in Ref. 10. Using ion model by Dang et al. [44–46] or ECC-ions [19, 25?] with more realistic bulk behaviour did not improve the results for the CaCl₂ interactions with Lipid14 model, as seen in Figs. 3 and ?? (in SI), respectively. **22.Add OP-response of Lipid14+ECC-ions plot in SI.** The results support the conclusion of the previous work [10] that improvements also in lipid models are needed to correctly describe cation binding in PC bilayers.

Significant improvement can be achieved by using the ECC approach also for lipids. The headgroup order parameter changes as a function of CaCl₂ concentration from ECC-lipid model with ECC-ions show a good agreement with experiments in Fig. 3. As discussed in previous section, the model gives also a good agreement with experiments for the headgroup response to bound charge. Thus, the model can be used for more detailed analysis of the binding affinity.

The binding affinities are quantified by using the ion density profiles along membrane normal shown in Fig. 4. The density profiles show larger Ca²⁺ density peak in lipid headgroup region for Lipid14 model with Dang and ECC-ions than for the ECC-lipid model. The relative surface excess calculated from Eq. 6 gives $\Gamma_i^w = 0.07 \pm 0.01 \text{ nm}^{-2}$ for the ECC-lipid model, which is significantly smaller than $\Gamma_i^w = 0.13 \pm 0.01 \text{ nm}^{-2}$ for Lipid14 with Åqvist and $\Gamma_i^w = 0.3 \pm 0.03 \text{ nm}^{-2}$ with Dang ions. Rough estimates for the free energy difference between bound and unbound cation are given by

$$\Delta G = k_B T \log\left(\frac{p_o}{p_i}\right), \quad (8)$$

where p_o and p_i are estimated from the Ca²⁺ densities in bulk water and in the maximum density in bilayer, respectively. The density profiles in Fig. 4 give $\sim 0.8 k_B T$ for the free energy difference between bound and unbound Ca²⁺ ions in ECC-lipid model and $\sim 1.4 k_B T$ in Lipid14 with Åqvist.

23.SAMULI: Maybe we should discuss the repeat distances and area per molecules measured at [4, 5, 62]

Since the lipid headgroup order parameter responses to the amount of bound charge and to the aqueous ion concentrations are both in good agreement with experiments in the ECC-lipid model with ECC-ion parameters, we consider the Na⁺ and Ca²⁺ binding affinities to be realistic in this model. On the other hand, the Ca²⁺ binding affinity is overestimated by Lipid14 model when simulated with all the tested ion models. Similar conclusions were previously made based only on the headgroup order parameter data with aqueous cations [10]. However, the discrepancies with experiments in previous work could partly arise also from the inaccurate sensitivity of the headgroup to bound charge. Here we quantify this effect and conclude that the improvement due to ECC is partly, but not completely, caused by more realistic headgroup sensitivity to bound charge. This indicates that the issue should be carefully considered also when the electrometer concept is used to compare ion binding between experiments and simulations with other models.

D. Binding stoichiometry

The good agreement of ECC-lipids model with experiments encourages us to analyse the molecular binding details from MD simulations. Direct analysis of contacts between ions and lipids from simulations suggest that the most abundant POPC:Ca²⁺ complex has stoichiometry of 2 POPC:1 Ca²⁺. As shown in Fig. 5 this is in agreement with the ternary complex model suggested based on head group order parameter experiments [15]. In addition to the ternary complexes, there also is a non-negligible probability of one Ca²⁺ cross-bridging three POPC molecules. Technical details of the analysis are in the SI.

In addition, we estimated the relative binding affinities of several moieties in POPC: phosphate moiety, side chain 1 carbonyl group and side chain 2 carbonyl group. Using the probability isodensity contours (see Fig. 6), we estimate that the

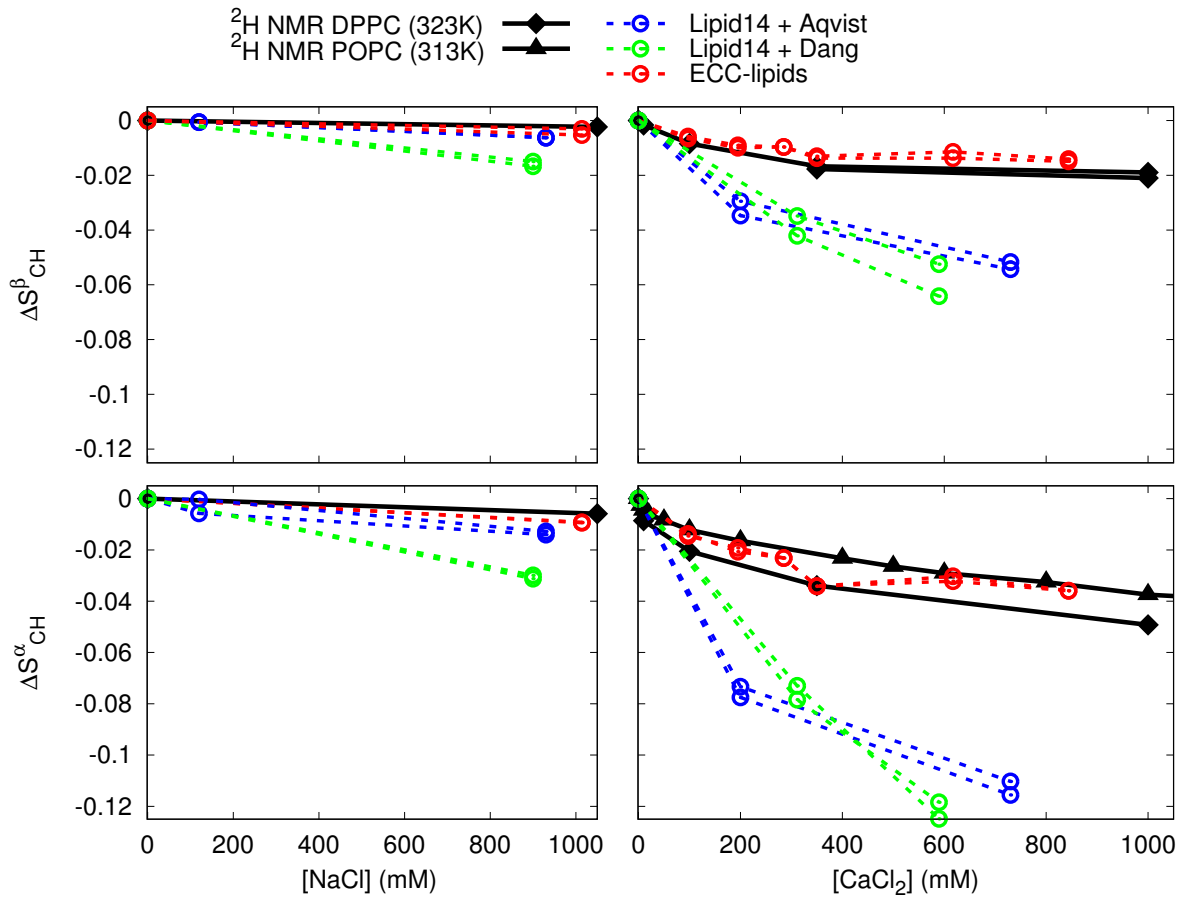


FIG. 3: Changes of head group order parameters of POPC bilayer as a function of NaCl and CaCl_2 concentrations are shown from simulations with different force fields together with experimental data (DPPC [14] and POPC [15]). Ion concentrations in bulk water are shown in x-axis. Values from simulations are calculated from the of cation number density C_{np} from the region at the simulation box edge with the constant ion concentration as $[\text{ion}] = C_{np}/0.602$. Simulation data with Lipid14 and Åqvist ion parameters is taken directly from Ref. [10].

largest contribution to the binding of Ca_2^+ to POPC membranes likely comes from the phosphate group. Although the isodensity plots are relatively easy to interpret, the contours shown in Fig. 6 cannot conclusively tell on the details of Ca_2^+ binding to any of the two carbonyl moieties.

27.JOE: Put details of the cation-binding stoichiometry analysis to SI.

28.JOE: Update the binding isotherm figure with new simulations

29.SAMULI: The same authors have also literature, where they say that ternary complex may not be the only option. I will recheck and come back to this. SAMULI: This is written in [63]: "Ca²⁺-binding to POPC bilayers over the whole concentration range can be best described in terms of formation of a ternary complex involving complexation of two lipids to one calcium ion (Altenbach and Seelig, 1984). The addition of a sodium competition term has not changed this conclusion. However, if Ca²⁺ concentrations up to 100 mM are considered, the data can be equally well explained by a 1:1 binding mechanism (cf. Figure 7). In contrast, the Ca²⁺ binding to POPC-POPG mixtures can be best described by assuming a 1:1 stoichiometry regardless of the range of Ca²⁺-concentrations." We might or might not want to discuss about this.

30.SAMULI: I would also analyze how much there is contact between ions and different parts of the lipid (phosphate, carbonyl, etc.). JOE: Pavel suggest using

volumetric map (e.g. in VMD) to visualise the amounts of Ca, Na and Cl bound to different moieties.

31.SAMULI: I think we should quantify this, i.e. how much there are these. Maybe also the other possible complexes? Maybe also the correlation between complexes and binding sites, if it is not too much work. JOE: This looks like a careful work for the next paper to me. I'd only add a relatively simple analysis of binding sites and probably the propensity of 1-2-3 membered clusters. SAMULI: I agree that we should not use too much time on this now..

32.Finalize stoichiometry analysis for Na^+ , Ca^{2+} , their interaction energies with the lipid membrane, etc, and finalize the discussion after these results.

IV. CONCLUSIONS

We show that the Na^+ and Ca^{2+} binding in phospholipid bilayers can be accurately described with classical MD simulation models, where electronic polarization is effectively included by using electronic continuum correction (ECC) [17]. This is a significant improvement over other available lipid models, which all overestimate specific cation binding affini-

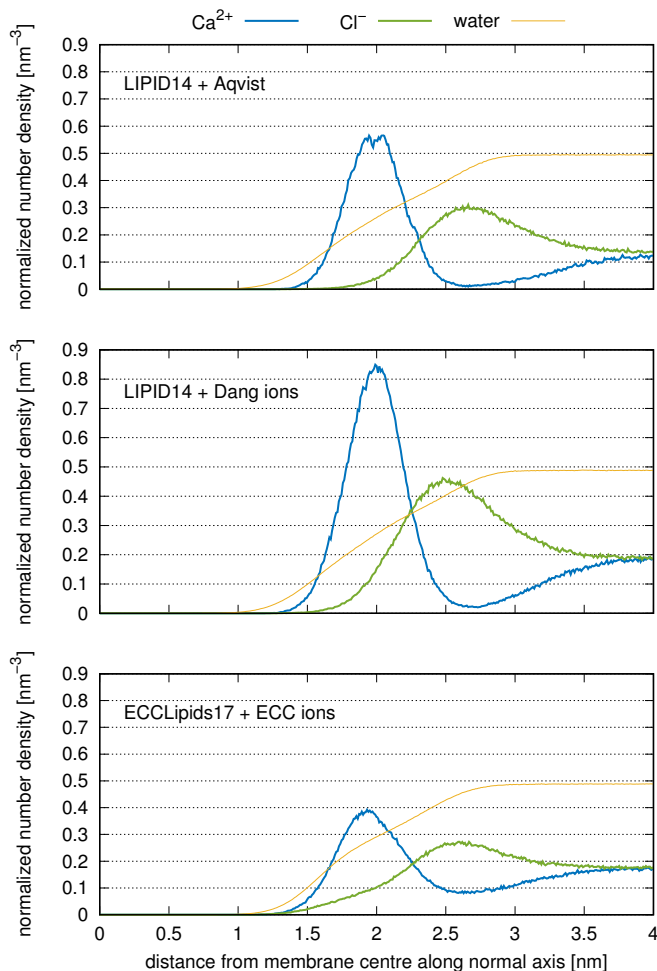


FIG. 4: Number density of Ca^{2+} and Cl^- as a function of membrane normal axis for different force fields. Data for Lipid14 with Åqvist ions are taken directly from Ref. 10. Densities of Cl^- and water are divided with 2 and 200, respectively, to visualize them with the same scale as Ca^{2+} . The molar concentration of the ions in water is 350 mM in all systems presented here.

24.PAVEL: draw phosphate position with its variance, add water density (scaled) and include the number of T-surface access.

25.JOE: Change the figure so that it contains a membrane background

26.The current data for Dang simulation seems to contain more ions than others.

ties [10]. The newly proposed model, which we denote as "ECC-lipids 17", exhibits accurate head group order parameter response to bound cations, monovalent Na^+ and cationic surfactant dihexadecyldimethylammonium bromide, and divalent Ca^{2+} also quantifying their binding affinities. Moreover, ECC-lipids 17 reproduce the lipid bilayer structural details with similar accuracy as other state of the art lipid models [10]. Several water models (OPC3[39], OPC [40], SPC/E [41] and TIP4p/2005 [43]) were used to exemplify the transferability of the parameters of the new ECC-lipids 17 force field.

Direct analysis of calcium binding details from MD simulations is in agreement with ternary complex model, which is suggested based on NMR data [15]. In this model 1 calcium

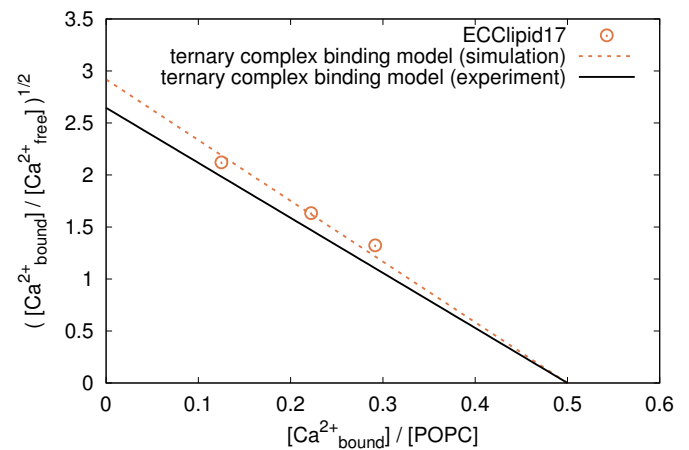


FIG. 5: Binding isotherm assuming stoichiometry of 2 POPC:1 Ca^{2+} as used in [15] fits our simulation data well.

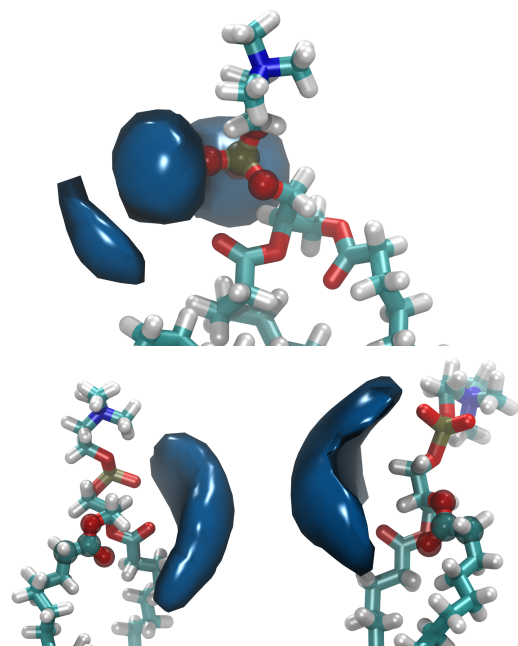


FIG. 6: Contours of probability isodensities of Ca_2^+ with respect to various moieties fixed in space (highlighted with transparent spheres): phosphate moiety, side chain 1 carbonyl group and side chain 2 carbonyl group. Shown contours suggest that the dominant contribution to Ca_2^+ binding likely comes from the phosphate group, but remain inconclusive on the details of the interactions with any of the two carbonyl groups.

33.JOE: I'll update this figure with some ensemble of configuration to support binding preference of Ca_2^+

binds to 2 POPC molecules, which together form a ternary complex. **34.**Continue summary using previous section once it is finished.

The electronic continuum correction is applied here on Lipid14 POPC model [30], but we expect that the correction can be generalized also for other lipids and force fields. The parameters can be used with existing standard nucleic acid and

protein force fields, e.g. AMBER-FB15 [64]. We suggest using state of the art water models like OPC3[39] or OPC [40], which yield higher accuracy than the traditional TIP3p water model [65].

This work can be reached as a repository containing all data at [zenodo.org:\dots\dots\dots](http://zenodo.org/dots/dots/dots) and as project NMRlipids VI in nmrlipids.blogspot.fi.

Acknowledgments

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ToDo

- | | P. |
|---|----|
| 1. Abstract directly from Joe's conference abstracts. To be rewritten. | 1 |
| 2. JOE: list more introductory examples, where it has interesting effects. | 1 |
| 3. PAVEL: introduce previous theoretical work that discusses cation binding to POPC w.r.t. its specific moieties, e.g. Lukas' paper | 1 |
| 4. JOE: this may be a too daring statement. SAMULI: I think that the divalent cation induced charge inversion is not an artefact, but it might happen with too small concentrations. On the other hand, it is not a new observation either, see e.g. literature cited in NMRlipids IV project. | 1 |
| 5. Text should be clarified from here to the end of the paragraph | 2 |
| 6. missing REF for APL experiment | 2 |
| 7. As far as remember, I used there Langevin dynamics instead of thermostated MD, because this is done in Amber by default. If this is correct, the information in the table do not match with this sentence. Based on semi-extensive testing I made few years ago this do not change anything. Anyway, this should be reported consistently. | 4 |
| 8. To be uploaded to Zenodo | 4 |
| 9. This could be moved to SI. Only simulation lengths needs to be mentioned in the main paper. | 4 |
| 14. put original references, not Slipids param. paper. | 4 |
| 15. SAMULI: I would put here Lipid14 in 303K, ECC-lipid in 303K and experiment in 303K. Rest in SI. The best experimental value would be the one analyzed from the form factor shown in previous figure, if available. | 4 |
| 10. There seems to a typo in experimental value for other order parameter for C2 carbon in sn-2 chain; it should be negative. The scale of the y-axis should be then zoomed when this is fixed. | 5 |
| 11. I would put the acyl chain order parameters also for original Lipid14; JOE: Already done, check whether your figures are up to date. I usually push only *.eps. | 5 |
| 12. x-axis scale in form factor figure would be better from 0 to 0.6. The experimental data is available for this range. | 5 |
| 13. $S_{CD} > S_{CH}$ | 5 |
| 16. Labels are missing. | 5 |
| 17. Order should be the same as in other figures, i.e. β segment on top. | 5 |
| 18. I would put x-axis from 0 to 51 and maybe zoom y-axis little bit as well. | 5 |
| 19. SAMULI: We could calculate the slopes from simulations, but I am not sure if we would actually learn anything useful from this. | 5 |

20. SAMULI: I think that we should establish the relation between order parameter and P-N vector angle changes by using ECC-lipids model. This would be useful for people measuring alpha order parameter changes from experiments. 6
21. I looked this roughly from the figure, should be calculated from the actual relation if we decide to put this. 6
22. Add OP-response of Lipid14+ECC-ions plot in SI. 6
23. SAMULI: Maybe we should discuss the repeat distances and area per molecules measured at [4, 5, 62] . 6
27. JOE: Put details of the cation-binding stoichiometry analysis to SI. 7
28. JOE: Update the binding isotherm figure with new simulations 7
29. SAMULI: The same authors have also literature, where they say that ternary complex may not be the only option. I will recheck and come back to this. SAMULI: This is written in [63]: "Ca²⁺ binding to POPC bilayers over the whole concentration range can be best described in terms of formation of a ternary complex involving complexation of two lipids to one calcium ion (Altenbach and Seelig, 1984). The addition of a sodium competition term has not changed this conclusion. However, if Ca²⁺ concentrations up to 100 mM are considered, the data can be equally well explained by a 1:1 binding mechanism (cf. Figure 7). In contrast, the Ca²⁺ binding to POPC-POPG mixtures can be best described by assuming a 1:1 stoichiometry regardless of the range of Ca²⁺ concentrations." We might or might not want to discuss about this. 7
30. SAMULI: I would also analyze how much there is contact between ions and different parts of the lipid (phosphate, carbonyl, etc.). JOE: Pavel suggest using volumetric map (e.g. in VMD) to visualise the amounts of Ca, Na and Cl bound to different moieties. 7
31. SAMULI: I think we should quantify this, i.e. how much there are these. Maybe also the other possible complexes? Maybe also the correlation between complexes and binding sites, if it is not too much work. JOE: This looks like a careful work for the next paper to me. I'd only add a relatively simple analysis of binding sites and probably the propensity of 1-2-3 membered clusters. SAMULI: I agree that we should not use too much time on this now. 7
32. Finalize stoichiometry analysis for Na⁺, Ca²⁺, their interaction energies with the lipid membrane, etc, and finalize the discussion after these results. 7
24. PAVEL: draw phosphate position with its variance, add water density (scaled) and include the number of T⁻ surface access. 8
25. JOE: Change the figure so that it contains a membrane background 8
26. The current data for Dang simulation seems to contain more ions than others. 8
33. Continue summary using previous section once it is finished. 8