

# 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 fully understood [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 effectively 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 included in classical MD simulations by placing all particles into a homogeneous dielectric continuum with a dielectric constant  $\epsilon_{el}$ , 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 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 ions, atomic partial charges in classical MD simulations are not experimental observables. Instead they are based on theoretical calculations and the derived values depend, for example, on the conformational sampling of the molecules and description of solvent in the calculations [? ? ]. Depending to the details, partial charge calculations may partially include some of the solvent electronic polarizability effects. Thus, we expect that the correct ECC scaling factor,  $f_q$ , for molecular partial charges would lie between  $\epsilon_{el}^{-1/2}$  (no electronic polarizability in charge calculation) and 1 (full electronic polarizability in charge calculation).

In this work, we empirically sample this parameter space to find a atomistic MD simulation force field parameters for lipids that accurately describe the lipid head group response to  $\text{Na}^+$  and  $\text{Ca}^{2+}$  concentrations when compared with NMR data [14, 15]. This data can be used to accurately asses ion binding in PC bilayers, as discussed in Ref, 10 and in section II B. We chose the Lipid14 [26] 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 [27]. The ECC correction was applied to Lipid14 pa-

rameters by scaling partial charges of the headgroup, 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 [28]. In contrast, improvements in glycerol backbone and headgroup parameters are required in all available lipid models [27].

Exploring different scaling factor values, applied to headgroup, glycerol backbone and carbonyl charges of Lipid14 model, we found out that ion binding and related headgroup order parameter response became weaker with the scaling of partial charges. 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) [29] combined with RESP calculations in vacuum and implicit solvent reported in [30]. IPolQ charges are obtained as the average of partial charges given by RESP calculation [? ] in vacuum and in a solvent. Applying the scaling factor of 0.75 to IPolQ charges calculated from the data in Ref. [30], gives similar partial charges to ones obtained by scaling Lipid14 charges with a factor 0.8. **5.SAMULI: I am still not sure if this is correct and/or makes sense.**

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 headgroup, 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  $64.3 \text{ \AA}^2$  ([6.missing REF for APL experiment) and the original Lipid14 value  $(65.6 \pm 0.5) \text{ \AA}^2$  [26]. The decrease of area was found to arise from a lower hydration of the lipid head group region, which can be explained by the decreased 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  $\sigma$  parameters in Lennard-Jones potential for the selected atoms similarly as done for free ions in solution [18? , 19]. This increases solvation free energy by allowing water molecules to approach closer to lipid atoms and have stonger electrostatic interactions with them. After reducing the  $\sigma$  parameters of the same atoms for which charges were scaled with a factor of  $f_\sigma = 0.89$ , the area per molecule value was again in agreement with experimental value (see Table II).

## B. Comparison of ion binding affinity to experiments by using the electrometer concept

Ion binding was compared between experiments and simulations by using lipid head group order parameters and “electrometer concept” introduced by Seelig et al. [10, 32]. The concept is based on the experimental observation that the order parameters of  $\alpha$  and  $\beta$  carbons in lipid head group (see Fig. ?? **7.Figure with chemical structure and labeling to be added**) are proportional to the amount of bound charge in lipid bilayer

[32]. More recent analysis included also the order parameter signs and concluded that the order parameters decrease with bound positive charge and increase with bound negative charge [10, 28]. The observations are rationalized as a change of lipid head group dipole tilt to more vertical orientation with bound positive charge and *vice versa* for negative charge [32]. Order parameters for all C-H bonds in lipid molecules can be accurately measured by using  $H^2$  NMR or  $^{13}C$  NMR techniques. The same order parameters can be calculated from MD simulations directly from the definition

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

where  $\theta$  is the angle between the bond and membrane normal and average is taken over all sampled configurations [28].

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 [33]

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

where  $S_{CH}^i(0)$  denote the order parameter in the absence of bound charge,  $i$  refers to either  $\alpha$  or  $\beta$  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  $\chi \approx 167$  kHz. Atomic absorption spectra and  $^2H$  NMR data, combined with the information about order parameter signs 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, 28].

Recent work published by the NMRlipids project showed that the concept works qualitatively also in simulations and can be used to compare ion binding affinity between simulations and experiments [10]. However, only systems with lipid bilayers and aqueous cations were used in that study. The headgroup response to bound charge then depends on both, the binding affinity and the response of the headgroup on bound charge. Thus, the headgroup response to bound charge has to be correctly described in the model if the concept is used for quantitative studies of binding affinity. Hence, we first quantify the response of head group order parameters to the bound charge by using experimental data measured from mixtures of cationic surfactants and PC lipids [34]. The amount of the bound charge is known exactly in these systems, because essentially all surfactants with unit charge locate in lipid bilayers. Thus, the headgroup order parameter response to bound charge can be first quantified against experimental data before proceeding to binding affinity studies.

The experimental data used to quantify cation binding reports order parameters as a function of equilibrium cation concentration in the bulk solvent [14, 15]. Recent work by NMRlipids project estimated this concentration by using the molar concentration in water  $[salt] = N_c \times [water] / N_w$ , where  $[water] = 55.5$  M,

**8.SAMULI: I call here concentration we used to call "nominal" as "molar concentration in water". I think that the "nominal" is not a good term for this. We need to decide how we will call this.**

which was accurate enough for the conclusions in that study

[10]. Since we are now targeting a model with quantitatively accurate binding affinity, we used larger simulation box dimensions that allow the determination of bulk ion concentration. Equilibrium concentration corresponding the experimental data in the units of mol/l can be then determined from the ion concentration plateau in the bulk solvent at the boundaries of the simulation box as

$$C_{eq} = \frac{C_{plateau}}{0.602}, \quad (4)$$

where plateau concentration is the number density in the units of  $nm^{-3}$ . **9.SAMULI: Once we have to final results, we can probably say that the repeat distance is not far from the experimentally measured distance [4, 5]**

The binding affinities between different models were compared by calculating the relative surface excess between water and ions  $\Gamma_w^i$  [35]

$$\Gamma_w^i = \Gamma_w^x - \Gamma_i^x \frac{c_w - c'_w}{c_i - c'_i}, \quad (5)$$

where  $c_w$  and  $c_i$  are concentrations in bulk water,  $c'_w$  and  $c'_i$  are concentrations inside bilayer, and  $\Gamma_w^x$  and  $\Gamma_i^x$  are surface excess of water and ions, respectively. Here consider the whole simulation box as a interface region, thus the surface excesses are  $\Gamma_w^x = n_w/A$  and  $\Gamma_i^x = n_i/A$ , where  $A$  is the area of the simulation box, and  $n_w$  and  $n_i$  are total number of water and ions in simulation box, respectively. The concentrations of water and ions inside bilayer can be expected to vanish, thus  $c'_w = c'_i = 0$ . Then we get the relative surface excess for lipid bilayer simulations

$$\Gamma_w^i = \frac{n_w}{A} - \frac{n_i}{A} \frac{c_w}{c_i}, \quad (6)$$

where concentrations in bulk,  $c_w$  and  $c_i$ , can be determined from the simulation box boundary.

### C. Comparison of lipid bilayer structure to 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) [28]. Form factor was calculated from simulations as

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

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

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 [45]
barostat	Parrinello-Rahman, semi-isotropic [46]
long-range electrostatics	PME [47]
cut-off scheme	Verlet [48]
Coulomb and VdW cut-off	1.0 nm
constraints	LINCS, only hydrogen atoms [49]
constraints for water	SETTLE [50]

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

model	APL ( $\text{\AA}^2$ )	Temperature [K]
Lipid14 [26]	$65.6 \pm 0.5$	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 [55] <b>13.REF</b>	64.3	303
experiment	67.3	323

### D. 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. Water molecules were described by OPC3 model [36]. In order to test transferability of the newly developed ECC-lipids model, we also performed several additional simulations with water models OPC [37], SPC/E [38] and TIP4p/2005 [39] presented in Supporting Information (SI). We used ECC-ions model for ions [19, 25? ]. Simulations with Lipid14 use ion model by Dang [40–42]. Classical molecular dynamics simulations were performed using the GROMACS [43] 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 [44]. Simulation trajectories and parameters are available at [? ] **10.To be uploaded to Zenodo.**

#### 2. Simulations with cationic surfactants

Automated topology builder [51] was first used to create the structure of dihexadecyldimethylammonium bromide,  $\text{C}_{12}\text{Cl}_{16}^+\text{N}_2\text{C}_1\text{Br}^-$ , molecule. AmberTools program [52] was then used to generate the Amber-type force field parameters. The parameters were converted to the Gromacs format by using acpype tool [53]. The partial charges were then manually modified to approximately correspond to their equivalent segments in Lipid14 [26]. 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. These systems were ran with original Lipid14 POPC model and with ECC-corrected model. **11.Describe the changes in surfactant model used with ECC-corrected 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 [26] are found to match the experimental values nicely. The head group and glycerol backbone order parameters accuracy is comparable to the state of art lipid models available in literature [28]. 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 headgroup on bound charge

Headgroup order parameter response to the amount of bound charge can be quantified by using ionic surfactants with a known valency [34]. All surfactants locate in lipid bilayers due to their amphiphilic nature, thus the exact amount of bound charge in the membrane is known in these systems. The headgroup order parameter changes as a function of bound charges can be then explicitly determined from such systems.

Changes of headgroup order parameter as a function of added cationic surfactant dihexadecyldimethylammonium bromide ( $\text{C}_{12}\text{Cl}_{16}^+\text{N}_2\text{C}_1\text{Br}^-$ ) from experiments [34] and simulations are shown in Fig. 2). The head group order parameter response to the bound cation concentration is approximately linear at least up to  $\sim 0.3$  mole fraction in experiments and in both simulation models, original Lipid14 and ECClipids **17.Yet to be shown..**

The slope is, however, too steep in Lipid14 model indicating that the headgroup order parameters are too sensitive for bound charge in this model. This has to be taken into account when interpreting the headgroup order parameter response

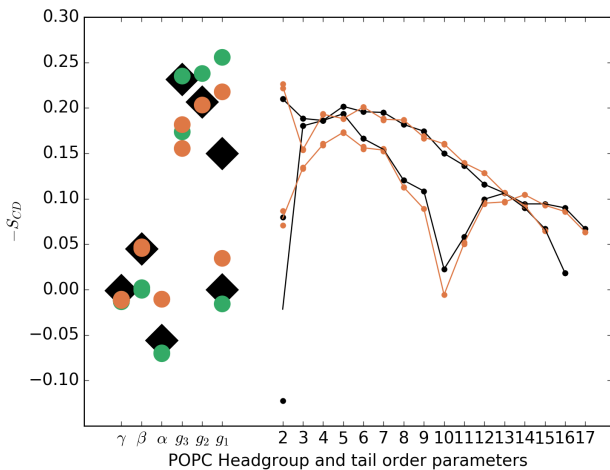
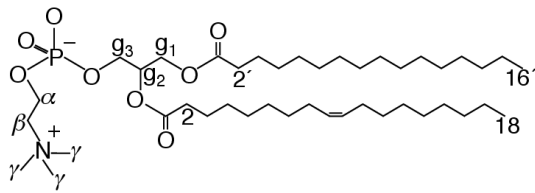
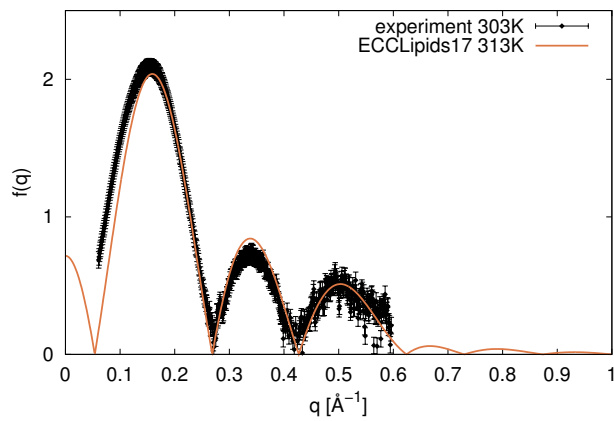


FIG. 1: X-ray scattering form factors from experiments [?] and simulations using Lipid14 [26] and ECC-lipids models. Order parameters of head group, glycerol backbone and sn-1 and sn-2 tails from simulations with Lipid14 [26] and ECC-lipids models compared with experimental order parameters from [54].

12. We should run a simulation for the system without ions with the same (or close) temperature as the experimental data. The NMR data is at 300K so that would be good. I think that this would be also close enough for the scattering data at 303K.

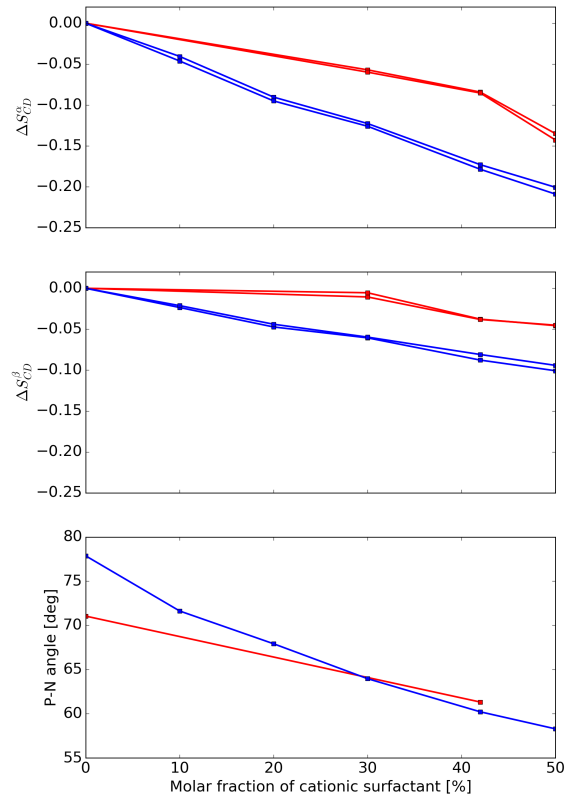


FIG. 2: Headgroup order parameter changes and P-N vector orientation as a function of cationic surfactant (dihexadecyldimethylammonium bromide,  $C_{12}Cl_{16}^+N_2C_1Br^-$ ) in PC bilayer from simulations and experiments [34].

14. Labels are missing.

15. Experimental data points are missing.

16. Order should be the same as in other figures, i.e.,  $\beta$  segment on top.

to  $CaCl_2$  concentration in Lipid14 model. The ECC-lipids model gives a slope in very good agreement with experiments for the  $\alpha$  segment, while the slope is slightly underestimated for the  $\beta$  segment.

The headgroup P-N vector angle is shown in Fig. ?? as function of bound cations. As expected [32], the headgroup order parameter decrease due to bound cations is related to the orientation of headgroup more parallel to membrane normal. The headgroup orientational effect is stronger in Lipid14 parameters than in ECClipid parameters, which is in line with smaller dipole moment and order parameter changes in ECClipid model.



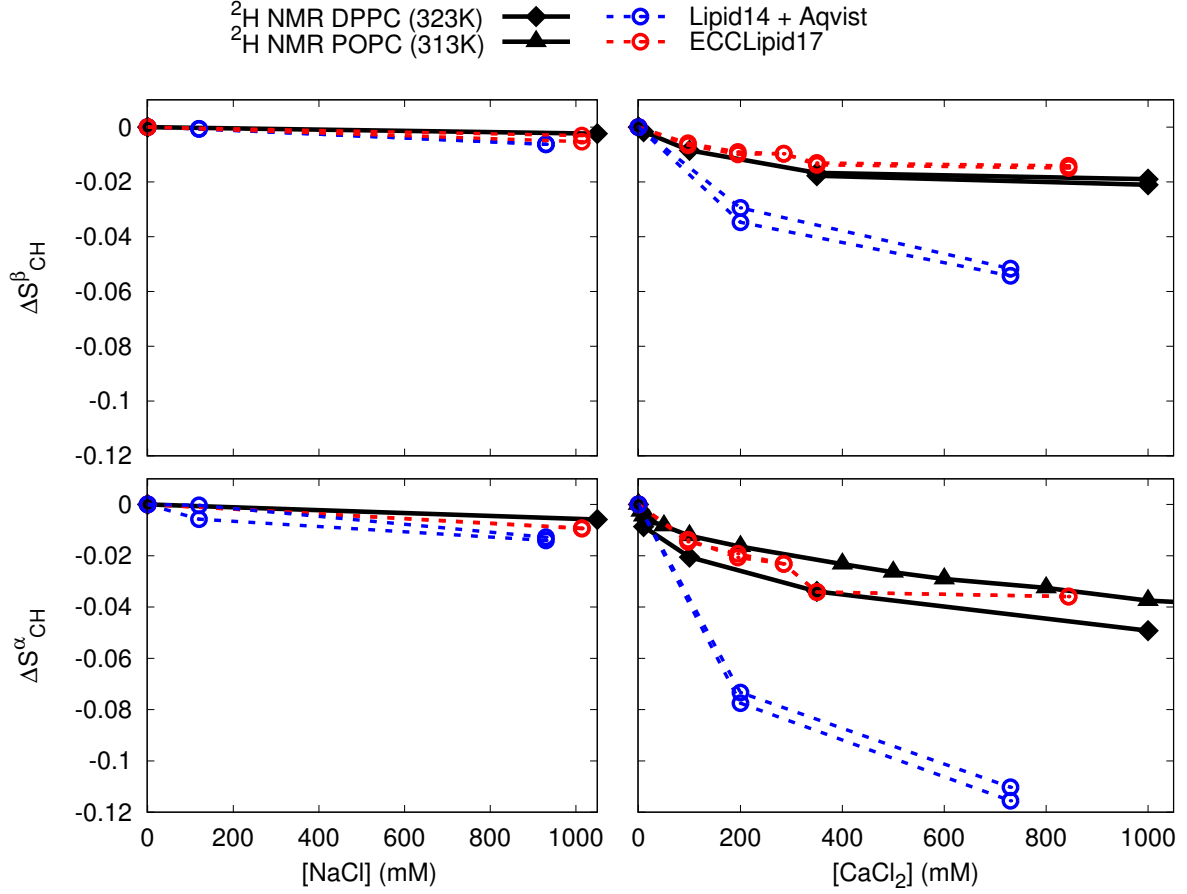


FIG. 3: Headgroup order parameter changes as a function of NaCl,  $\text{CaCl}_2$  concentration from simulations with different force fields and experiments (DPPC [14] and POPC [15]). Simulations with Lipid14 parameters and Aqvist ions are directly from Ref. [10], except that the concentration are determined with Eq. 4.

18.Simulation results with original Slipids and Dang ions to be added when we have the results.

### C. Cation binding affinity in POPC

The headgroup order parameter response to aqueous ionic concentration can be used to measure ion binding affinity in experiments and simulations, as discussed in section ?? and in previous literature [10, 32]. The headgroup order parameter response of PC lipids to NaCl and  $\text{CaCl}_2$  concentrations from experiments (DPPC [14] and POPC [15]) and different simulation models are shown in Fig. 3. The simulation results of Lipid14 with Aqvist ions [?] taken directly from Ref. 10 are shown together with the results from Lipid14 simulation with Dang ions [?] and from ECClipid simulations with ECC-ions.

As already discussed in the recent work by NMRlipids project [10], the headgroup order parameters are almost unaffected by addition of NaCl in Lipid14 simulations with Aqvist ion model due a very weak binding of  $\text{Na}^+$  in PC bilayer. This is in agreement with experimental data, in contrast to almost all the other lipid models, which significantly overestimate  $\text{Na}^+$  binding in PC bilayers [10]. Despite of good perfor-

mance with NaCl, the response of headgroup order parameters to  $\text{CaCl}_2$  concentration are significantly overestimated in Lipid14 simulations with Aqvist ion model, see Fig. 3 and 10.

Wide range of data presented in previous work [10] suggested that  $\text{CaCl}_2$  binding behaviour cannot be corrected by only improving ion models. This was not, however, tested for Lipid14 model, so we ran also Lipid14 simulations with Dang [40–42] and ECC corrected [?] ions having more realistic bulk behaviour as Aqvist model [?]. In line with previous work, the headgroup order parameters response to  $\text{CaCl}_2$  concentration was overestimated also with these ion models, as seen in Figs. 3 and ?? (in SI), respectively 19.Add OP-response of Lipid14+Dang in Fig. 3 and OP-response of Lipid14+ECC-ions plot in SI.

Applying ECC correction also for lipids, introduced in section ??, brings the order parameter response to  $\text{CaCl}_2$  concentration to a good agreement with experiments, as shown in Fig. 3. This is a significant improvement over all models introduced previously which in general overestimate the head group order parameter response to  $\text{CaCl}_2$  concentration [10]. The good agreement with experiments indicates that the ECC

corrected lipid model has a sufficient accuracy for realistic studies of ion binding details on lipid bilayers.

Ion density profiles along membrane normal coordinate from different simulations are shown in Fig. 4. **20.** This paragraph is to be finalized when we have the surface excess analysis done by using Eq. 6. This is directly related to the amount of surface excess charge as shown along with ion density profiles in Fig. 4. The density profiles from simulations with original Lipid14 [26] and Dang ions [40–42] show a pronounced peak in the position of the phosphate moieties of POPC. The use of a ECC-ion model [18? ? , 19] or the standard Amber ion model by Åqvist [56] along with Lipid14 does not significantly change it (Headgroup order parameter responses for these models in SI). The new ECC-lipids model with ECC-ions [18? ? , 19] exhibits on the other hand smaller density in this region suggesting overall weaker binding of cations (Fig. 3). It is then likely that the models that overestimate head group order parameter response [10] also overestimate the amount of bound cations.

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:Ca<sup>2+</sup> complex has stoichiometry of 2 POPC:1 Ca<sup>2+</sup>. 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 Ca<sup>2+</sup> cross-bridging three POPC molecules. Technical details of the analysis are in the SI.

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

**25.JOE:** Update the binding isotherm figure with new simulations

**26.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 [57]: "Ca<sup>2+</sup>-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 Ca<sup>2+</sup> 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 Ca<sup>2+</sup> binding to POPC-POPG mixtures can be best described by assuming a 1:1 stoichiometry regardless of the range of Ca<sup>2+</sup>-concentrations." We might or might not want to discuss about this.

**27.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.

**28.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..

**29.Finalize stoichiometry analysis for Na<sup>+</sup>, Ca<sup>2+</sup>, their interaction energies with the lipid membrane, etc, and finalize the discussion after these results.**

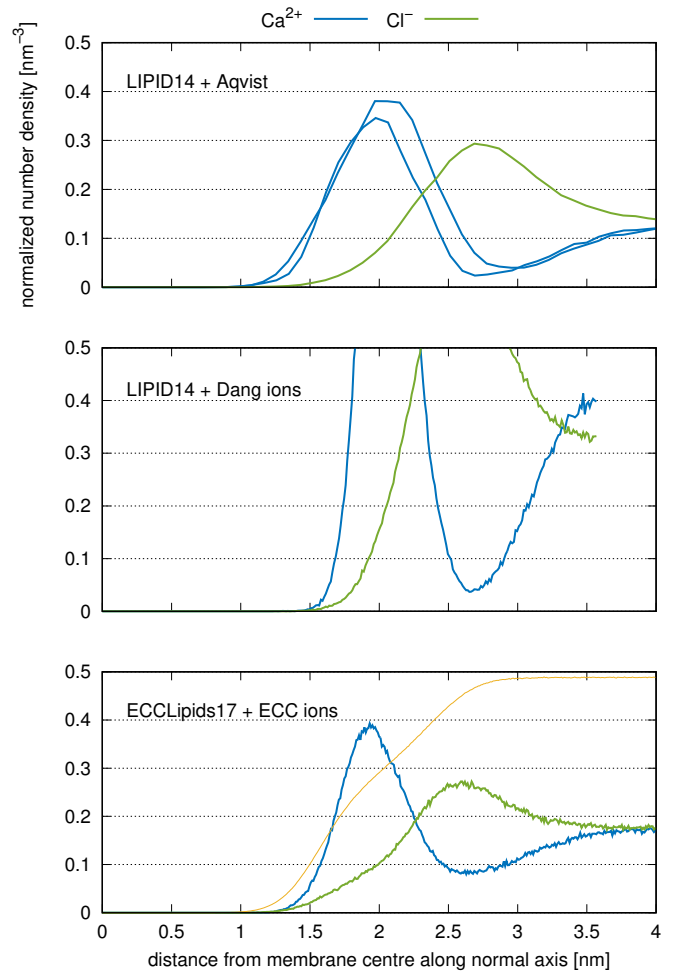


FIG. 4: Number density of Ca<sup>2+</sup> and Cl<sup>-</sup> as a function of membrane normal axis for different force fields. Data for Lipid14 with Aqvist ions are taken directly from Ref. 10. Densities of Cl<sup>-</sup> and water are divided with 2 and 200, respectively, to visualize them with the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

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

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

**23.**Results with Lipid14+Dang to be added once done.

#### IV. CONCLUSIONS

We show that the Na<sup>+</sup> and Ca<sup>2+</sup> 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 affinities [10]. The newly proposed model, which we denote as "ECC-lipids 17", exhibits accurate head group order parameter response to bound cations, monovalent Na<sup>+</sup> and cationic surfactant dihexadecyldimethylammonium bromide, and divalent Ca<sup>2+</sup> also quantifying their binding affinities. Moreover, ECC-lipids 17 reproduce the lipid bilayer structural de-

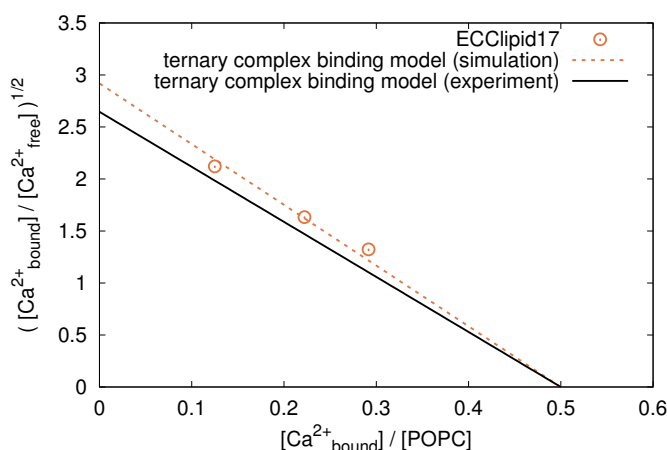


FIG. 5: Binding isotherm assuming stoichiometry of 2 POPC:1  $\text{Ca}^{2+}$  as used in [15] fits our simulation data well.

tails with similar accuracy as other state of the art lipid models [10]. Several water models (OPC3[36], OPC [37], SPC/E [38] and TIP4p/2005 [39]) were used to exemplify the trans-

ferability 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 binds to 2 POPC molecules, which together form a ternary complex. **30.Continue summary using previous section once it is finished.**

The electronic continuum correction is applied here on Lipid14 POPC model [26], 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 [58]. We suggest using state of the art water models like OPC3[36] or OPC [37], which yield higher accuracy than the traditional TIP3p water model [59].

This work will serve as a foundation stone of a new open-collaboration project NMRlipids VI in nmrlipids.blogspot.fi.

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### SUPPLEMENTARY INFORMATION

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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. SAMULI: I am still not sure if this is correct and/or makes sense. . . . . 2
6. missing REF for APL experiment . . . . . 2
7. Figure with chemical structure and labeling to be added 2
8. SAMULI: I call here concentration we used to call "nominal" as "molar concentration in water". I think that the "nominal" is not a good term for this. We need to decide how we will call this. . . . . 3
9. SAMULI: Once we have to final results, we can probably say that the repeat distance is not far from the experimentally measured distance [4, 5] . . . . . 3
10. To be uploaded to Zenodo . . . . . 4
11. Describe the changes in surfactant model used with ECC-corrected lipids. . . . . 4
13. put original references, not Slipids param. paper. . 4
17. Yet to be shown. . . . . 4
12. We should run a simulation for the system without ions with the same (or close) temperature as the experimental data. The NMR data is at 300K so that would be good. I think that this would be also close enough for the scattering data at 303K. . . . . 5
14. Labels are missing. . . . . 5

15. Experimental data points are missing. . . . .	5	27. SAMULI: I would also analyze how much there is contact between ions and different parts of the lipid (phosphase, 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
16. Order should be the same as in other figures, i.e, $\beta$ segment on top. . . . .	5	28. 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
18. Simulation results with original Slipids and Dang ions to be added when we have the results. . . . .	6	29. Finalize stoichiometry analysis for $\text{Na}^+$ , $\text{Ca}^{2+}$ , their interaction energies with the lipid membrane, etc, and finalize the discussion after these results. . . . .	7
19. Add OP-response of Lipid14+Dang in Fig. 3 and OP-response of Lipid14+ECC-ions plot in SI . . . . .	6	21. PAVEL: draw phosphate position with its variance, add water density (scaled) and include the number of $\Gamma$ -surface access. . . . .	7
20. This paragraph is to be finalized when we have the surface excess analysis done by using Eq. 6. . . . .	7	22. JOE: Change the figure so that it contains a membrane background . . . . .	7
24. JOE: Put details of the cation-binding stoichiometry analysis to SI. . . . .	7	23. Results with Lipid14+Dang to be added once done. . . . .	7
25. JOE: Update the binding isotherm figure with new simulations . . . . .	7	30. Continue summary using previous section once it is finished. . . . .	8
26. 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 [57]: "Ca <sup>2+</sup> 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 Ca <sup>2+</sup> 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 Ca <sup>2+</sup> binding to POPC-POPG mixtures can be best described by assuming a 1:1 stoichiometry regardless of the range of Ca <sup>2+</sup> -concentrations." We might or might not want to discuss about this. . . . .	7		