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 phopholipid 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 stochiometry 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 moiteties, 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-invansive spectroscopic methods, like nucelar magnetic resosnance (NMR), scattering and infrared scpectroscopy, 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 articifially positively charged memranes and significant artefacts in MD simualtions (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 performace 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 structue 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 higly 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 polariization 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 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

$$Q^{ECC} = f_q \cdot Q \tag{1}$$

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. 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 striclty $f_q = \epsilon_{el}^{-1/2}$, 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 Na^+ and Ca^{2+} concentrations when compared with NMR data [14, 15]. These 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 [29] 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 [30]. The ECC correction was applied to Lipid14 parameters 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 [31]. In contrast, improvements in glycerol backbone and headgroup parameters are required in all available lipid models [30].

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 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) [32] combined with RESP calculations in vacuum and implicit solvent reported in [33]. 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. [33], 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 headgroup, glycerol backbone and carbonyls scaled with 0.8 gave the area per molecule value of ≈60 $Å^2$, which is significantly smaller than the experimental value 64.3 $Å^2$ ([]**5.missing REF for APL experiment**) and the original Lipid14 value (65.6 ± 0.5) $Å^2$ [29]. 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 the σ parameters in Lennard-Jones potential for the selected atoms similarly as done for free ions in solution [18, 19, 25]. 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 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. 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, 35]. The concept is based on the experimental observation that the order parameters of α and β carbons in lipid head group (see Fig. **??** 6.Figure with chemical structure and labeling to be added) are proportional to the amount of bound charge in lipid bilayer [35]. 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, 31]. 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 [35]. Order parameters for α and β segments in headgroup, as well as for all C-H bonds in lipid molecules, can be accurately measured by using H^2 NMR or ^{13}C NMR techniques. The experimental order parameters can be compared with the the ones calculated from MD simulations by using the definition

$$S_{\mathrm{CH}} = \frac{3}{2}\langle \cos^2\theta - 1\rangle, \tag{2}$$

where θ is the angle between the bond and membrane normal and average is taken over all sampled configurations [31].

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 [36]

$$\Delta S^i_{\mathrm{CH}} = S^i_{\mathrm{CH}}(X^{\pm}) - S^i_{\mathrm{CH}}(0) = \frac{4m_i}{3\chi}X^{\pm}, \tag{3}$$

where $S^i_{\mathrm{CH}}(0)$ 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 $\chi \approx 167$ 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, 31]. Recent work published by the NMRlipids project showed that the concept works qualitatively also in simulations and can be used to compare ion binding affinitity between simulations and experiments [10].

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 relative molar concentration in water [salt]=$N_c\times$[water]$/N_w$, where [water] = 55.5 M, which was accurate enough for the conclusions in that study [10]. Since we are now targeting a model with quantitatively accurate binding affinity, we rather use a definition that corresponds more closely to the concentrations reported in experiments. By using larger simulation box dimensions that allow the determination of bulk ion concentration, the equilibrium concentration corresponding to the experimental data in the units of $\mathrm{mol/l}$ can be then determined from the simulations as

$$C_{eq} = \frac{C_{bulk}}{0.602}, \tag{4}$$

where the bulk concentration, C_{bulk}, is the number density in the units of nm^{-3} in the bulk solvent far from the membrane interface. 7.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 ion binding affinities between different models were compared by calculating the relative surface excess of ions with respect to water, Γ^w_i, as [37]

$$\Gamma^w_i = \Gamma_i - \Gamma_w \frac{C_i - C'_i}{C_w - C'_w} \tag{5}$$

$$\Gamma_a = \frac{n_a - C_a V - C'_a V'}{A}\ ;\ a = i, w\,, \tag{6}$$

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 divinding plane, A is the area of the interface, and Γ_a is the surface excess of water or ions. As Γ^w_i does not depend on the definition of the interfacial region and the position of the Gibbs dividing plane [37], we consider the whole simulation box as an interface. Note that phospholipid membranes are bilayers, and hence the interface occurs in the simulation box twice providing a surface area $2A$. 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

$$\Gamma^w_i = \frac{1}{2A}\left(n_i - n_w\frac{C_i}{C_w}\right), \tag{7}$$

where the bulk concentrations, C_w and C_i, can be determined from the simulation as C_{bulk} in Equation 4.

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) [31].

Order parameters were calculated from simulations for all C-H bonds by using Eq. 2 and form factors as

$$F(q) = \int_{-D/2}^{D/2}\left(\sum_\alpha f_\alpha(q_z)n_\alpha(z) - \rho_s\right)\exp(izq_z)\mathrm{d}z, \tag{8}$$

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

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 [49]
barostat	Parrinello-Rahman, semi-isotropic [50]
long-range electrostatics	PME [51]
cut-off scheme	Verlet [52]
Coulomb and VdW cut-off	1.0 nm
constraints	LINCS, only hydrogen atoms [53]
constraints for water	SETTLE [54]

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

centre along its normal spanning the membrane with thickness D.

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. In particular, the periodic orthorhombic simulation box conatined 128 POPC molecules and approximately 50 water molecules per each lipid. Water molecules were described by OPC3 model [38] 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 [39], SPC/E [40], TIP3p-FB and TIP4p-FB [41], and TIP4p/2005 [42] presented in Supporting Information (SI). We used ECC-ions model for ions [19, 25**?**]. Simulations with Lipid14 use ion models by Dang [43–45], and by Åqvist [46]. Classical molecular dynamics simulations were performed using the GROMACS [47] 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 [48]. Simulation trajectories and parameters are available at [**?**]

8.To be uploaded to Zenodo.

2. *Simulations with cationic surfactants*

Automated topology builder [55] was first used to create the structure of dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$, molecule. AmberTools program [56] was then used to generate the Amber-type force field parameters. The parameters were converted to the Gromacs format by using acpype tool [57]. The partial charges were then manually modified to approximately correspond to their equivalent segments in Lipid14 [29]. 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. These systems were first simulated for 200 ns using Lipid14 model. First 20 ns was omitted from the analysis. Simulations with ECC-lipids used the same simulation setup. We also applied electronic continuum correction to the cationic surfactant by scaling all its 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.

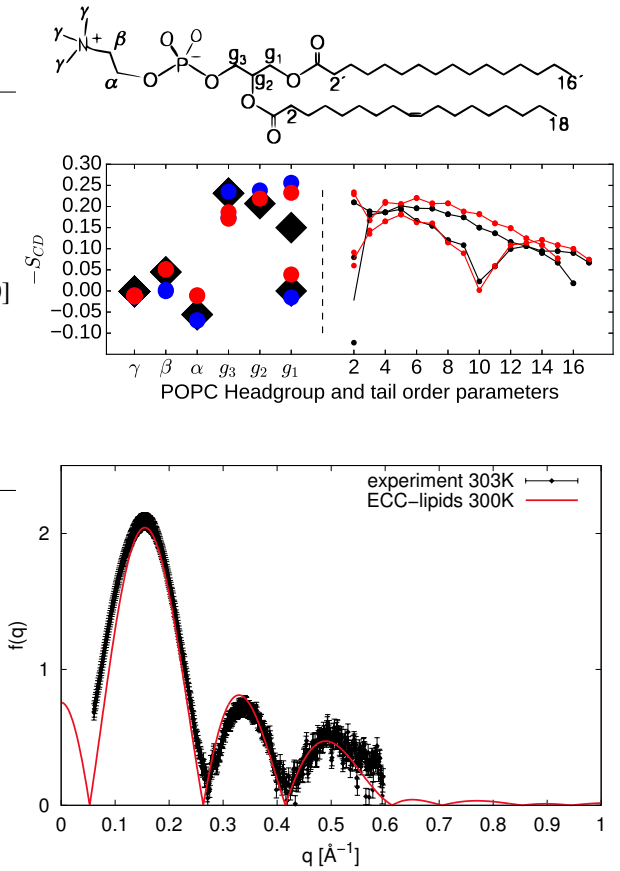

FIG. 1: X-ray scattering form factors from experiments [**?**] and simulations using Lipid14 [29] and ECC-lipids models. Order parameters of head group, glycerol backbone and sn-1 and sn-2 tails from simulations with Lipid14 [29] and ECC-lipids models compared with experimental order parameters from [58].

10.We should run a simulation for the system withtout 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.

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

model	APL (Å^2)	Temperature [K
Lipid14 [29]	65.6± 0.5	303
ECC-lipids		
$(4.6 \cdot 5.1\,\mathrm{nm}^2)$, 72 lipids patch, OPC3	63.2	313
$(6.4\ \mathrm{nm})^2$, 128 lipids patch, OPC3	64.2	313
$(6.4\ \mathrm{nm})^2$, 128 lipids patch, SPC/E	65.1	313
$(6.4\ \mathrm{nm})^2$, 128 lipids patch, OPC	64.4	313
$(6.4\ \mathrm{nm})^2$, 128 lipids patch, TIP4p/2005	66.8	313
experiment [59]11.REF	64.3	303
experiment	67.3	323

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 [29] 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 [31]. 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 headgroup to bound charge

REWRITE: The headgroup response to bound charge 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 cationinc surfactants and PC lipids [60].

The amount of the bound charge is known excatly in these systems, because essentially all surfactants with a unit charge locate in the lipid bilayers.

Changes of headgroup order parameters as a function of added cationic surfactant dihexadecyldimethylammonium bromide ($C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$) from experiments [60] and simulations are shown in Fig. 2. As expected from Eq. 3, the head group order parameter response to the bound cation concentration is approximately linear at least up to 30% mole fraction in experiments and in both simulation models, original Lipid14 and ECC-lipids. 15.We might want to calculate the slopes from simulations.

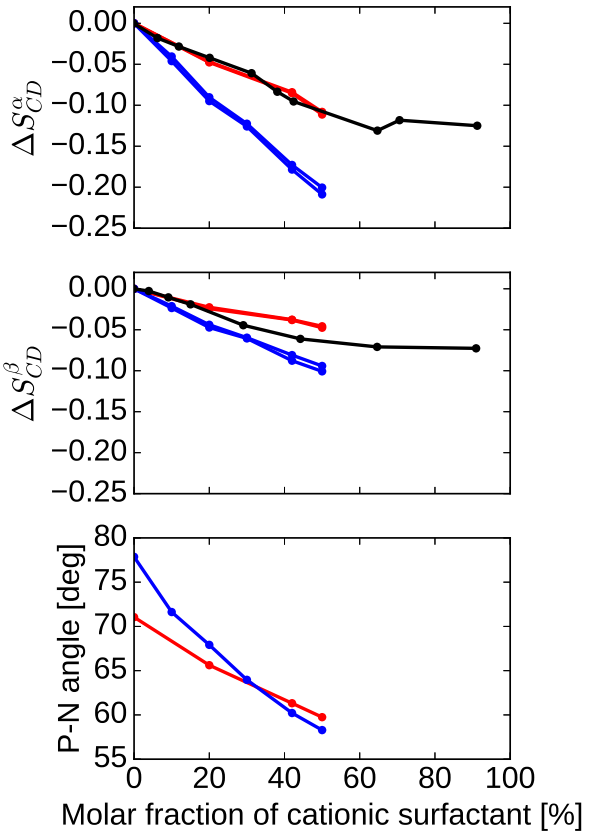

FIG. 2: Headgroup order parameter changes and P-N vector orientation as a function of cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$) in PC bilayer from simulations and experiments [60].

12.Labels are missing.

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

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

The slope of the head group order parameters response is, however, too steep in Lipid14 model indicating that its head group response is too sensitive to a bound charge. This has to be taken into account when interpreting the head group order parameter response to $CaCl_2$ concentration in Lipid14 model. 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.

Also the angle of the headgroup P-N vector with respect to membrane normal is shown in Fig. 2 as a function of bound cations. As expected [35], the headgroup orients more parallel with the membrane normal with an increasing amount of bound cations. The effect is stronger in Lipid14 model than in ECC-lipids model, which is in line with the reduced charge–dipole interactions in the ECC framework leading to the lower head group sensitivity to bound charges in ECC-lipids model.

16.It might be a good idea to establish a relation between order parameter change and P-N vector angle by using ECC-lipids model.

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 II B and in previous literature [10, 35]. The headgroup order parameter response of PC lipids to NaCl and $CaCl_2$ concentrations from experiments (DPPC [14] and POPC [15]) and different simulation models are shown in Fig. 3. The simulation results of Lipid14 with Åqvist ions [46] (from Ref. 10) are shown together with the results from Lipid14 simulation with ions by Dang [43–45] and from ECC-lipids simulations with ECC-ions.

As already discussed in a recent work by NMRlipids project [10], the headgroup order parameters are almost unaffected by addition of NaCl in Lipid14 simulations with Åqvist ion model due to a very weak binding of Na^+ in PC bilayer. This is in agremeent with experimental data, in contrast to almost all the other lipid models, which significantly overestimate Na^+ binding in PC bilayers [10]. Despite the good performance with NaCl, the response of headgroup order parameters to $CaCl_2$ concentration are significantly overestimated in Lipid14 simulations with Åqvist ion model, see Fig. 3 and Ref. 10.

Wide range of data presented in previous work [10] suggested that $CaCl_2$ binding behaviour cannot be corrected by only improving ion models. This was not, however, tested specifically for Lipid14 model, so we ran also Lipid14 simulations with ions by Dang [43–45] and ECC-ions [19, 25**?**], which have more realistic bulk behaviour than Åqvist model [46]. In line with previous work, the headgroup order parameters response to $CaCl_2$ concentration was overestimated also with these ion models, as seen in Figs. 3 and **??** (in SI), respectively **18.Add OP-response of Lipid14+Dang in Fig. 3 and OP-response of Lipid14+ECC-ions plot in SI.**

Applying ECC correction also to lipids, introduced in section II A, brings the order parameter response to $CaCl_2$ concentration to a good agreement with experiments, as shown in Fig. 3. This is a significant improvement over previously introduced models, which all overestimate the head group order parameter response to $CaCl_2$ concentration [10]. The good agreement with experiments indicates that the ECC corrected lipid model has a sufficient accuracy for realistic studies of details of ions binding to lipid bilayers.

Ion density profiles along membrane normal from different simulations are shown in Fig. 4. **19.This paragraph is to be finalized when we have the surface excess analysis done by using Eq. 7.** 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 [29] and Dang ions [43–45] show a pronounced peak in the position of the phosphate moieties of POPC. The use of ECC-ion model [18**? ?** , 19] or the ion model by Åqvist [46] 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^{2+} complex has stoichiometry of 2 POPC:1 Ca^{2+}. 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-negligable probability of one Ca^{2+} 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 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.

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 [61]: "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:l stoichiometry regardless of the range of Ca2+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 (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.

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 betweem 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^+, 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 affinities [10]. The newly proposed model, which we denote as

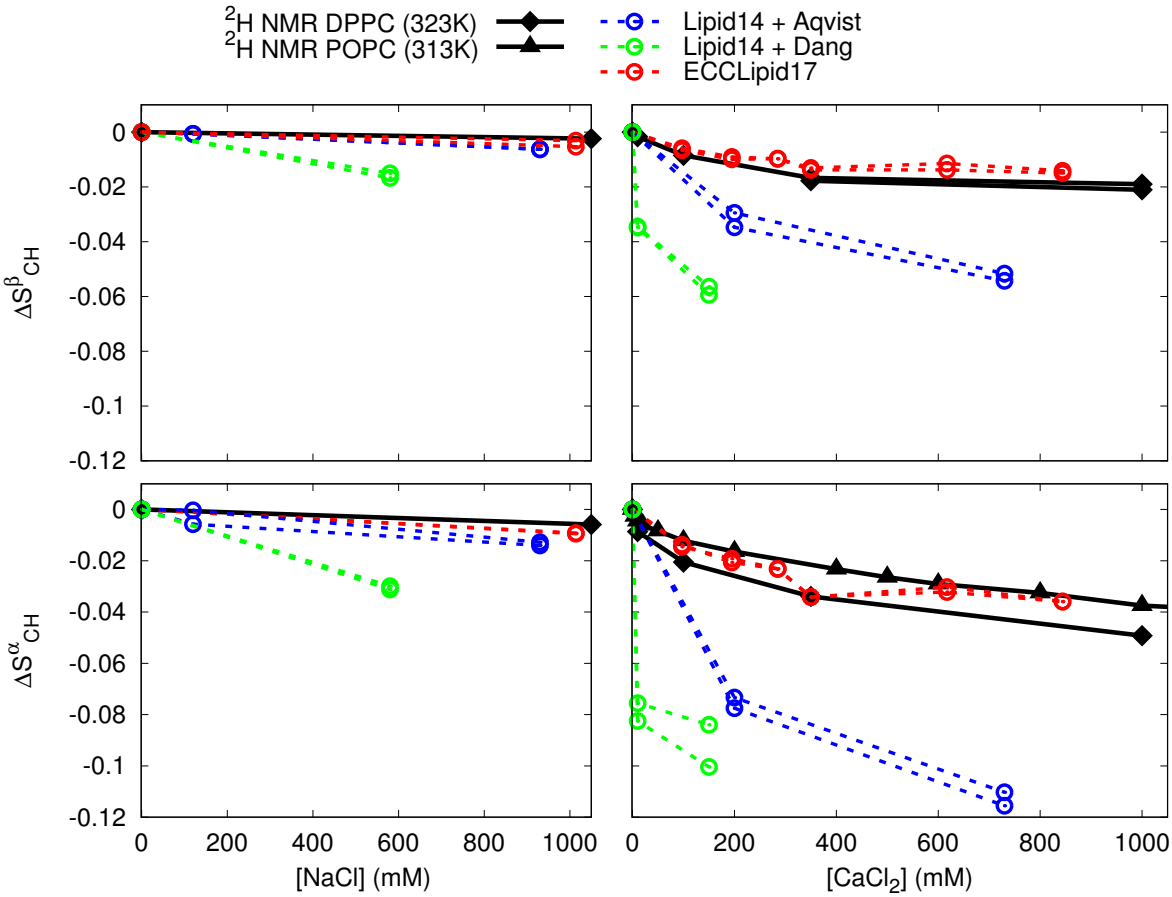

FIG. 3: Headgroup order parameter changes as a function of NaCl, $CaCl_2$ concentration from simulations with different force fields and experiments (DPPC [14] and POPC [15]). Simulations with Lipid14 parameters and Åqvist ions are directly from Ref. [10], except that the concentration are determined with Eq. 4.

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

"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[38], OPC [39], SPC/E [40] and TIP4p/2005 [42]) 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 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 [29], 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 [62]. We suggest using state of the art water models like OPC3[38] or OPC [39], which yield higher accuracy than the traditional TIP3p water model [63].

This work can be reached as a repository containing all data at `zenodo.org:\dots\dots\dots` and as project NMRlipids VI in `nmrlipids.blogspot.fi`.

Acknowledgments

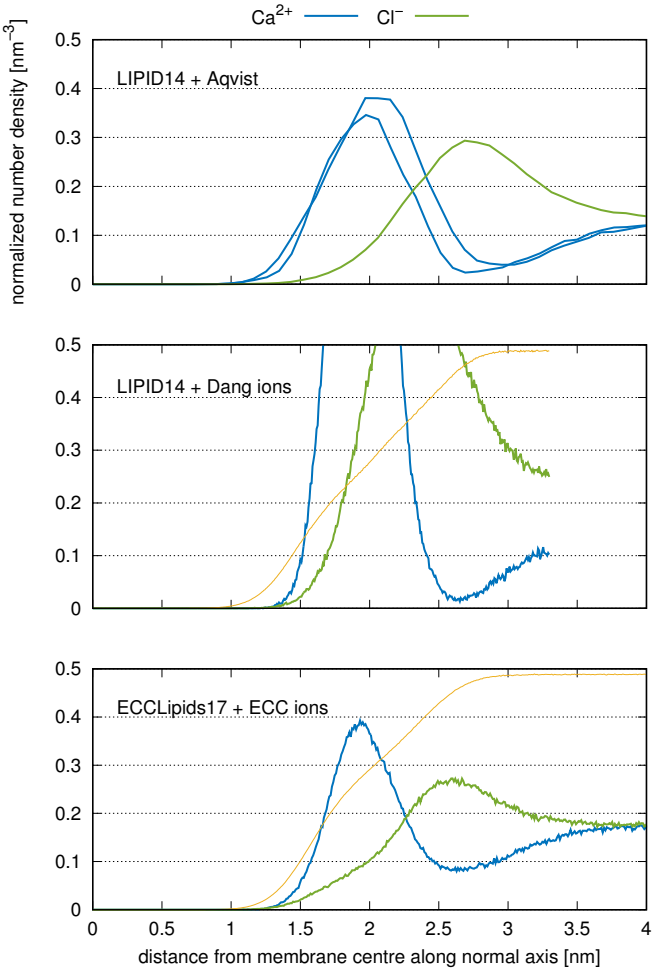

FIG. 4: Number density of Ca^{2+} and Cl^- as a function of membrane normal axis for different force fields. Data for Lipid14 with Åqvist ions are taken directly from Ref. 10. Relative surface excess for the respective simulations are: Lipid14 with Åqvist ions $\Gamma_i^w = XXnm^{-3}$; Lipid14 with Dang ions $\Gamma_i^w = 0.3nm^{-3}$; ECC-lipids with ECC-ions $\Gamma_i^w = 0.07nm^{-3}$. Densities of Cl^- and water are divided with 2 and 200, respectively, to visalize them with the same scale as Ca^{2+}. The molar concentration of the ions in water is 350 mM in all systems presented here.

20.Calculate the surface excess for L14+Aqvist sim. 21.PAVEL: draw phosphate position with its variance, add water density (scaled) and include the number ofΓ-surface access.

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

23.Resuls with Lipid14+Dang to be added once done – running with larger box.

SUPPLEMENTARY INFORMATION

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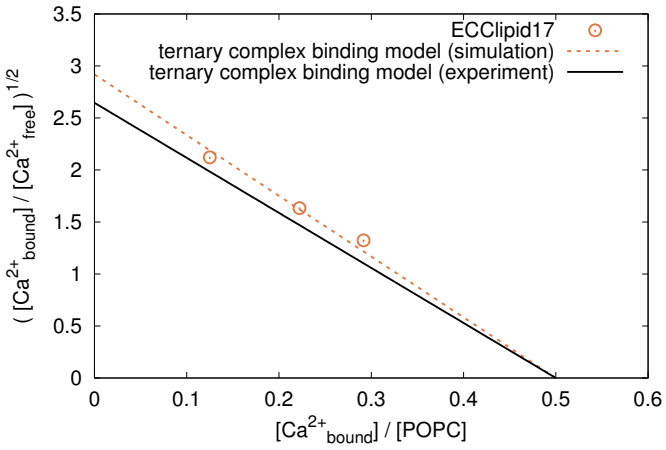

FIG. 5: Binding isotherm assuming stoichiometry of 2 POPC:1 Ca^{2+} as used in [15] fits our simulation data well .

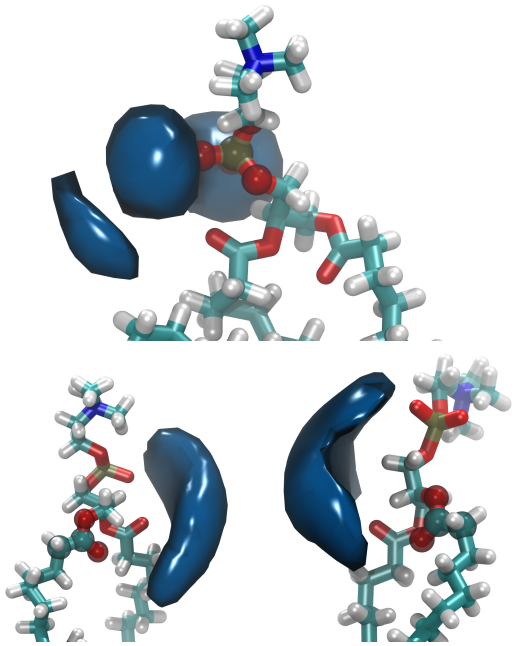

FIG. 6: Contours of probability isodensities of $Ca_2{}^+$ with respect to various moieties fixed in space (highlighted with transparent spheres): phosphate moiety, side chain 1 carbonyl group and side chain 2 carbonyl group. Shown contours suggest that the dominant contribution to $Ca_2{}^+$ binding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

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