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.We need to select references for these**. 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-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 model 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 articfially positively charged memranes and significant artefacts in MD simualtions (e.g. Ca^{2+} induced charge inversion of bacterial membranes [16]).

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 [18**?** , 19] and ab-initio simulations [**?**]. 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 head group order parameter response to 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 classical MD simulation models has been considered higly relevant issue since the early days of lipid bilayer simulations. Some pioneering simulation studies scaled the partial charges of atoms by one half to effectively include polarizability [20, 21]. Also, approaches that explicitly include polarizability has been introduced, but this has turned out to be practically compli-

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cated [22]. In this work we effectively include 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 [20, 21]. In contrast to those studies, 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 [22] 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 continuum can be easily included in standard MD simulation by a formal transformation of partial charges

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

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 ion force fields [18**?**, 19].

The main goal of this work is to apply ECC correction to accurately describe the lipid head group response to Na^+ and Ca^{2+} concentrations in MD simulation models when compared with NMR data [10]. The Lipid14 [25] force field parameters were used as a starting point, because their response to bound ions was most realistic against NMR data (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 [26].

While the scaling factor of $f_q = 0.75$ for ions in water is well justified and shown to improve model performance against scattering data [18, 19, 27], it is not clear if the same factor should be used for lipid head groups. Unlike the total charge of cations and anions, atomic partial charges used in classical MD simulations are not experimental observables and can in principle adopt arbitrary values. Here we estimate the correct scaling factor for Lipid14 model by using so called "implicitly polarized charges" (IPolQ) [28], which are the average of partial charges given by restrained electrostatic potential fitting (RESP [**?**]) in vacuum and in solvent. Using the partial atomic charges obtained from RESP fitting in vacuum and implicit solvent reported in [29] and considering that the partial charges in original Lipid14 parametrization were derived using RESP [**?**] in vacuum, we conclude that the charges in Lipid14 model shall be scaled roughly by the factor $f_q = 0.8$.

Here we apply the ECC correction with the proposed scaling factor only to 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 modi-

fied, because they are already highly optimized in most lipids models including Lipid14 and give generally a good description for hydrophobic part of lipid bilayers in various conditions [30]. In contrast, all available lipid models require some improvements in glycerol backbone and head group regions [26]. The scaling of partial charges in the polar region was found to decrease area per molecule to 60 Å^2, which is significantly smaller than the experimental value 64.3 Å^2 ([]**4.missing REF for APL experiment**) and the original Lipid14 values (65.6 ± 0.5) Å^2 [25].. 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 solvation free energy and hydration is sensitive to Van der Waals interaction parameters, hence, they can be increased by reducing the radius of the selected atoms by changing the the σ parameters in Lennard-Jones potential. Here we reduce σ with a scaling factor of $f_\sigma = 0.89$ for the same atoms for which charges were scaled. This has lead to an increase of the area per molecule back close to the 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 α and β carbons in lipid head group (see Fig. **??** **5.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, 30]. 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 C-H bonds can be accurately measured for each C-H bond of lipids by using H^2 NMR or ^{13}C NMR techniques and calculated from MD simulations directly from definition

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

where θ is the angle between the bond and membrane normal and average is taken over all sampled configurations [30].

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]

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

where $S_{\mathrm{CH}}^i(0)$ 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 $\chi \approx 167\,\text{kHz}$. Combination of atomic absorption spectra, ^{2}H NMR experiments and 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, 30].

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, it also turned out that the sensitivity of the order parameters response to the bound charge has to be quantified more accurately for quantitative comparison of binding affinity. Hence, we first quantify the response of head group order parameters against experimental data from cationinc surfactants embedded in PC bilayer [34]. Essentially all surfactants locate in lipid bilayers in these experiments, thus the amount of the bound charge is known and can be plotted against order parameter change. Secondly we evaluate the response of the head group order parameters to varying salt concentrations in solution and estimate the amount of membrane bound charge.

The used experimental data report order parameters as a function of equilibrium cation concentration in the bulk solvent [14, 15]. Such a condition is reached in simulations by adjusting the simulation box size to dimensions large enough that ion conceration reaches a clear plateau in the bulk solvent. The concentrations in the units of mol/l were then determined as

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

where plateau concentration is the number density in the units of nm^{-3}. **6.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]**

C. Comparison of lipid bilayer structure to experimental data

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) [30]. Form factor was calculated from simulations as

$$F(q) = \int_{-D/2}^{D/2} \left(\sum_\alpha f_\alpha(q_z) n_\alpha(z) - \rho_s \right) \exp(izq_z)\mathrm{d}z, \quad (5)$$

where $f_\alpha(q_z)$ is the density of atomic scattering length, ρ_s is the density of solvent scattering lenght, $n_\alpha(z)$ 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.

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 [44]
barostat	Parrinello-Rahman, semi-isotropic [45]
long-range electrostatics	PME [46]
cut-off scheme	Verlet [47]
Coulomb and VdW cut-off	1.0 nm
constraints	LINCS, only hydrogen atoms [48]
constraints for water	SETTLE [49]

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 [35]. In order to test transferability of the newly developed ECC-lipids model, we also performed several additional simulations with water models OPC [36], SPC/E [37] and TIP4p/2005 [38] presented in Supporting Information (SI). We used ECC-ions model for ions [19, 27**?**]. Simulations with Lipid14 use ion model by Dang [39–41]. Classical molecular dynamics simulations were performed using the GROMACS [42] 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 [43]. Simulation trajectories and parameters are available at [**?**] **7.To be uploaded to Zenodo.**

2. Simulations with cationic surfactants

Automated topology builder [50] was first used to create the structure of dihexadecyldimethylammonium bromide, $\text{C}_{12}\text{Cl}_{16}{}^+\text{N2C}_1\text{Br}^-$, molecule. The code AmberTools [51] was then used to generate the Amber-type force field parameters. The parameters were converted to the Gromacs format by using acpype tool [52]. The partial charges were then manually modified to approximately correspond to their equivalent segments in Lipid14 [25]. 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. **8.Describe the changes in surfactant model used with ECC-corrected lipids.**
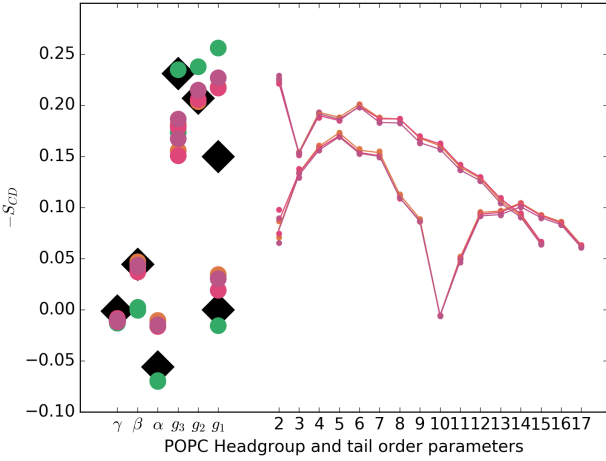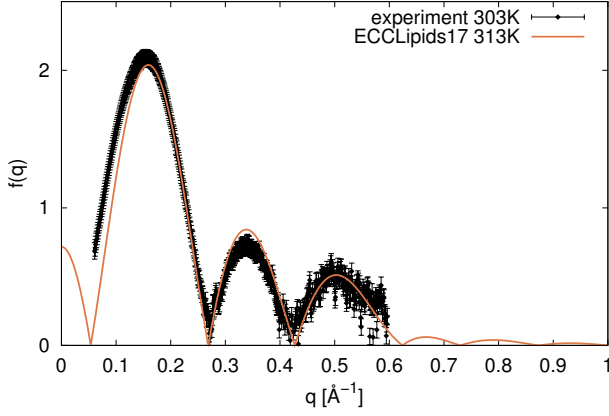
FIG. 1: X-ray scattering form factors from experiments [**?**] and simulations using Lipid14 [25] and ECC-lipids models. Order parameters of head group, glycerol backbone and sn-1 and sn-2 tails from simulations with Lipid14 [25] and ECC-lipids models compared with experimental order parameters from [53].

9.Add acyl chain order parameters, POPC chemical structure

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.

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 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 [25] 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 [30], The agreement between the x-ray scattering form factors and areas per molecule from simulations and experiments confirm that the membrane structural properties are

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

model	APL (Å^2)	Temperature [K]
Lipid14 [25]	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 [54]**11.REF**	64.3	303
experiment	67.3	323

well captured.

B.   POPC membrane and electrometer concept with cationic surfactant

Headgroup order parameter response to the bound charge was evaluated against experimental data measured with the cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^+N2C_1Br^-$) [34]. The exact amount of bound charge in the membrane is known in these systems, because practically all cationic surfactant molecules are embedded in the lipid bilayer due to their amphiphilic nature. Thus, the changes of head group order parameter as a function of mole fraction of cationic surfactants (Fig. 2) gives also the order parameter changes as a function of the amount of bound cations. **12.Split the OP-changes figure in two: cationic surfactant -and- binding of cations. The separate figure with the cationic surfactant shall be additionally accompanied with a plot of Head group tilt (PN-vector?) change/response.**

The head group order parameter response to the bound cation concentration is in experiments approximately linear up to ∼0.3 mole fraction of the cation [34]. The linearity is also observed in simulations with original Lipid14 and with newly derived ECC-lipids models. Quantitative comparison, however, reveals that the response is overestimated in Lipid14 for both segments, while the ECC-lipids model gives a good agreement for the change of α segment order parameter, and slightly underestimates the β segment order parameter change. Such a finding is not surprising, as the $\Delta S^\alpha / \Delta S^\beta$ ratio of the response of Lipid14 model is found to be slightly larger than in experiments (VALexp vs VALsim **13.Add values of** S^α / S^β **response for sim and experiment SAMULI: Maybe these could be put in the same figure with the order parameter response and P-N vector angle change with surfactants.**), and this property is also kept by the newly derived Lipid14-based model, ECC-lipids. The present comparison of head group order parameter changes suggests that the observed overestimated response of all models to increasing $CaCl_2$ concentration in [10] can be explained at least in part by a high sensitivity of the head group order parameter response to the bound charge.
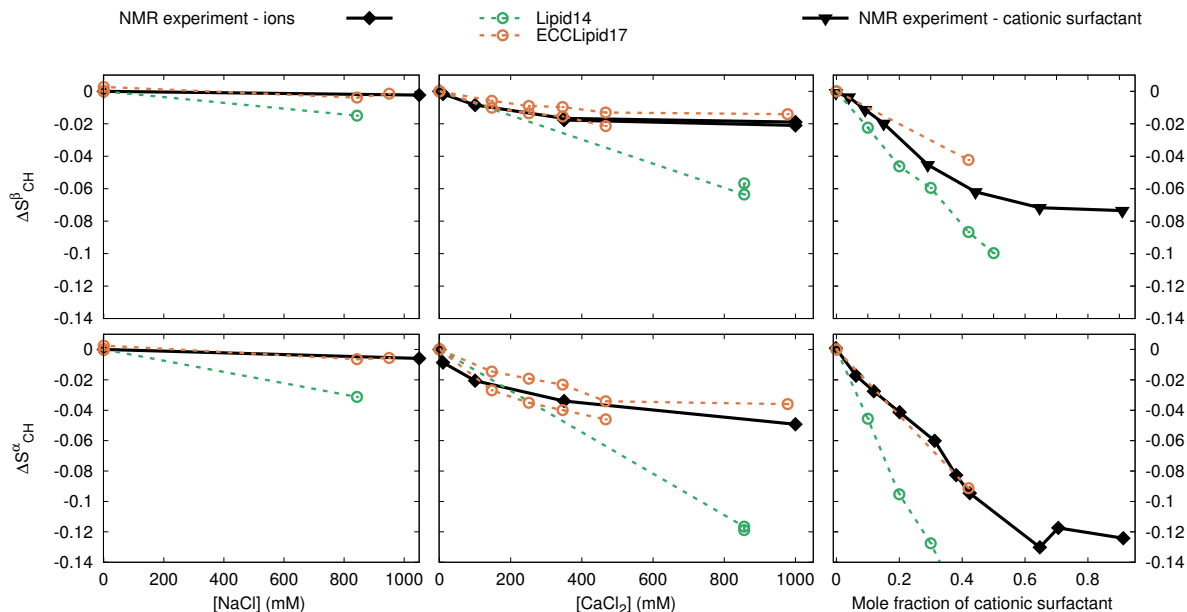
FIG. 2: Headgroup order parameter changes as a function of NaCl, CaCl$_2$ concentration and cationic surfactant (dihexadecyldimethylammonium bromide, C$_{12}$Cl$_{16}$$^+$N2C$_1Br^-$) mole fraction from simulations and experiments (DPPC [14], POPC [15], surfactant [34]).

C. POPC membrane and electrometer concept with cation binding

After achieving accurate head group order parameter response with cationic surfactant, we advanced to the response to solutions with varying concentrations of salts, namely NaCl and CaCl$_2$. Headgroup order parameter response to increasing CaCl$_2$ concentration from experiments, original Lipid14 model and ECC-lipids model are shown in Fig. 2. The order parameter response is significantly overestimated in original Lipid14 model, while results from ECC-lipids model are in a good agreement with the experiments. This is a significant improvement over current models, which in general overstimate the head group order parameter response to CaCl$_2$ concentration [10]. The good agreement with experiments indicate that the binding details of Ca^{2+} are realistic in the ECC-lipids model and, hence, it can be used to study lipid-ion interaction in detail.

Ion density profiles from simulations with different models are compared in Fig. 3. The density profiles from simulations with original Lipid14 [25] and Dang ions [39–41] 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 Åquist along with Lipid14 does not significantly change it (Headgroup order parameter responses for these models in SI). **17.Add OP-response of Lipid14+ECC-ions into the OP-changes plot in SI** The new ECC-lipids model with ECC-ions exhibits on the other hand smaller density in this region suggesting overall weaker binding of cations (Fig. 2). Together with the accurate head group order parameter response (Fig. 2), we can conclude that the present model, ECC-lipids, exhibits accurate ion binding affinity to zwitterionic phospho-

lipid membranes suggesting that the models that overestimate head group order parameter response [10] also overestimate the amount of bound cations.

Good agreement of ECC-lipids model with experiments encourages us to analyse the binding details from MD simulations. Direct analysis of contacts between ions and lipids from simulations suggest that the most abundant POPC:Ca^{2+} complex have stoichiometry of 2 POPC:1 Ca^{2+}. As shown in Fig. 4 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.

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

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

20.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 [55]: "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.

21.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.

22.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
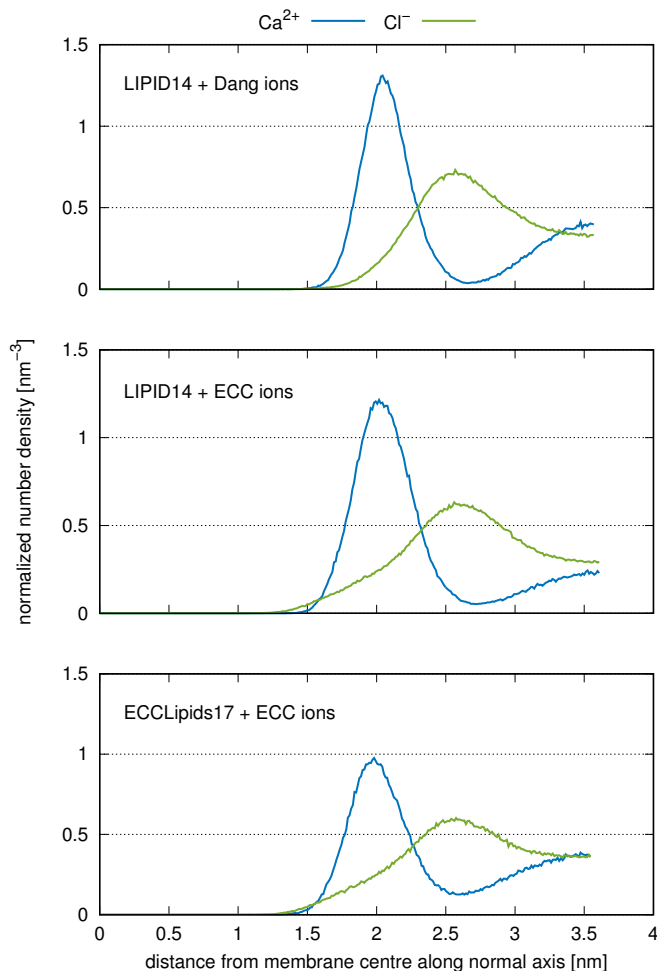
FIG. 3: Density profiles of Ca^{2+} and Cl^- for Lipid14 model with Dang ions and with ECC-ions and ECC-lipids model with ECC-ions.

14.Change the figure so that it contains a membrane background

15.What are the concentrations (calculated as in NMRlipids II) in this figure?

16.These should be also compared to the results with qvist ions, which are shown in NMRlipids II. NMRlipids II have results with 350mM and 1000mM. If we have the distributions with the same concentrations qvist results could be added also in this figure. If not, the comparison has to be made some other way.

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..

23.Finalize stoichiometry analysis for Na⁺, Ca²⁺, 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
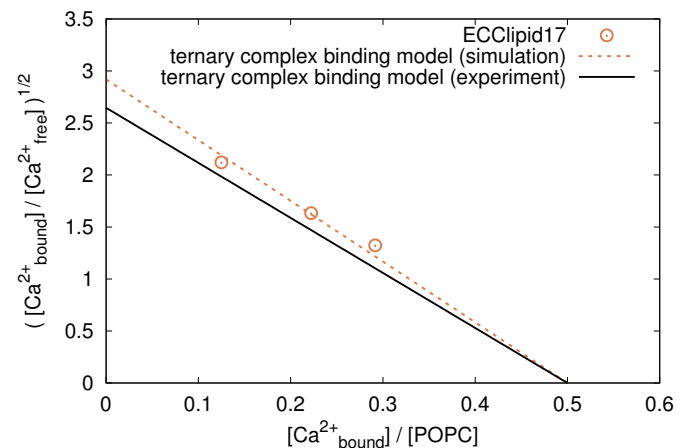
FIG. 4: Binding isotherm assuming stoichiometry of 2 POPC:1 Ca^{2+} as used in [15] fits our simulation data well .

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^+ 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[35], OPC [36], SPC/E [37] and TIP4p/2005 [38]) 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. **24.Continue summary using previous section once it is finished.**

The electronic continuum correction is applied here on Lipid14 POPC model [25], 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 [56].

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

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