

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 headgroup 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. Cation interactions with lipid bilayers serving as a model for cellular membranes are thus widely studied with experimental [1–5] and theoretical methods [6]. General conclusion from experimental studies has been that multivalent ions and lithium have weak specific binding in phospholipid bilayers, while other monovalent ions do not essentially bind [1–3, 7]. The presence of anionic lipids, like PS or PG, increase the concentration close to the bilayer and thus the amount of bound ions, but do not affect the specific binding constant [7]. The binding details, like binding sites and stoichiometry are not yet fully resolved but interpretation of NMR and scattering experiments suggest that one Ca^{2+} interacts mainly with the choline groups [8–10] of two phospholipid molecules [11].

Classical molecular dynamics simulations have potential to solve the ion binding details in lipid bilayers and reveal its relevance to various biological problems [12, 13]. However, the available molecular dynamics simulation models have a strong tendency to overestimate cation binding on zwitterionic bilayers and none of them can reproduce the experimental data with the accuracy required for the interpretation of experiments [14]. The overestimated specific cation binding also makes zwitterionic bilayers effectively positively charged, which could potentially lead to significant artefacts in applications. Thus, there is a vast demand for classical molecular dynamics simulation model which correctly reproduces cation binding in lipid bilayers.

The lack of electronic polarizability from the classical MD simulation models is a potential source of artefact, which could lead to overbinding of cations. The issue has been considered highly relevant since the early days of lipid bilayer simulations and pioneering simulation studies scaled the partial charges to effectively include electronic polarizability [15, 16]. The explicit inclusion of electronic polarizability has been, however, turned to be practically complicated and implementations for lipids are rare [17].

In this work we show that the cation overbinding in classical MD simulations can be corrected by including electronic polarizability by using effective continuum correction (ECC) [18] for polar region of zwitterionic lipid molecules. This is essentially physically well justified version of partial charge scaling implemented in early days of lipid and surfactant simulations [15, 16]. The approach has been previously shown to improve bulk performance of ion models against neutron scattering data [19, 20]. The better bulk behaviour was not, however, sufficient to correct binding in lipid bilayers [14].

II. METHODS

A. Electronic continuum correction for lipid bilayers

According to the electronic continuum correction (ECC) [18], 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 [18]. Measurements of high frequency dielectric constant gives values of approximately $\epsilon_{el} \approx 2$ for almost any biomaterial [18]. Such a continuum can be easily included in standard MD simulation by a formal transformation of

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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 $f_q = 0.75$ [18?], which has been successfully used to improve the performance of ion force fields [19? , 20].

Here we apply the electronic continuum correction to lipid bilayers to accurately describe the lipid headgroup response to Na⁺ and Ca²⁺ concentrations [14]. We use the Lipid14 [21] force field parameters as a starting point, because they give the most realistic headgroup response with added cations and relatively realistic glycerol backbone and headgroup structures when compared with other state of the art lipid models [14, 22]. The partial atomic charges in Lipid14 were derived by fitting the electrostatic potential to its model quantum chemistry representation (RESP[?]) in vacuum. If the charges are obtained by using RESP in an implicit solvent, they vary the most for the polar moieties [23]. In order to implicitly capture solvent induced polarization in models with fixed charges, we shall use the average of partial charges from both environments (i.e. vacuum and solvent), so called implicitly polarized charges (IPolQ) [24]. By taking the charges of oxygen atoms in vacuum and implicit water solvent for POPC from [23], we represent the IPolQ charges in the electronic continuum correction of the Lipid14 model by increasing the scaling factor f_q to 0.8. [42]

Hydrocarbon chains in Lipid14 and other lipids models are highly optimized and give generally a good description for hydrophobic part of lipid bilayers in various conditions [25], in contrast to glycerol backbone and headgroup regions which require some improvement in all available lipid models [22]. To minimize the detuning of the highly optimized hydrocarbon parameters, we apply ECC only to the headgroup, glycerol backbone and carbonyl in acyl chains. These regions are also the most polar parts in lipids and are expected to most affect cation binding.

Mere scaling of partial charges in the modified region by the factor f_q reduces the area per molecule to ??2.find the value, which is significantly smaller than the experimental value ([]) and the original Lipid14 values ([])3.Add the values. The decrease of area was speculated 4.SAMULI: Did you analyze the effect of this to hydration or did we only speculate? JOE: I found a systematic decrease of water density in the heagroup region even if I kept APL constant. to arise from reduced hydration of headgroup due to the lower polarity of molecules with scaled charges. The hydration can be increased by decreasing the radius of atoms by reducing the σ parameters in Lennard-Jones potential for the selected atoms. This allows water molecules to approach closer to lipid atoms and have stronger electrostatic interactions with them. 5.SAMULI: This effect may have an official name. In that case we should mention it. Indeed, by reducing σ with a scaling factor $f_\sigma = 0.89$ we increased the area per molecule to a level close to experimental and original Lipid14 values.

B. Comparison to experimental data

Structures sampled by individual lipid molecules in simulations were compared to experimental data by using C-H bond order parameters [25]

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

where θ is the angle between C-H bond and membrane normal and average is taken over all sampled configurations.

Bilayer dimensions were compared to experiments by using the scattering form factor [25]

$$F(q) = \int_{-D/2}^{D/2} \left(\sum_{\alpha} f_{\alpha}(q_z) n_{\alpha}(z) - \rho_s \right) \exp(izq_z) dz, \quad (3)$$

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

Ion binding was compared between experiments and simulations by using lipid headgroup order parameters and "electrometer concept" introduced by Seelig et al. [14, 27]. The concept is based on the experimental observation that the order parameters of α and β carbons in lipid headgroup (see Fig. ?? 6. Figure with chemical structure and labeling to be added) are proportional to the amount of bound charge in lipid bilayer [27]. 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 [14, 25]. The observations are rationalized as a change of lipid headgroup dipole tilt to more vertical orientation with bound positive charge and *vice versa* for negative charge [27].

The used experimental data report order parameters as a function of equilibrium cation concentration in the bulk solvent [11, 28]. Such a condition is reached in simulations by adjusting the simulation box size to dimensions large enough that ion concentration 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. 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]

C. Simulation details

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 [29]. In order to test transferability of the newly developed ECC-lipids model, we also performed several additional simulations with water models

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

| model | APL (\AA^2) | Temperature [K] |
|---|------------------------|-----------------|
| Lipid14 [21] | 65.6 ± 0.5 | 303 |
| ECC-lipids | | |
| ($4.6 \cdot 5.1 \text{ nm}^2$), 72 lipids patch, OPC3 | 63.2 | 313 |
| (6.4 nm^2), 128 lipids patch, OPC3 | 64.2 | 313 |
| (6.4 nm^2), 128 lipids patch, SPC/E | 65.1 | 313 |
| (6.4 nm^2), 128 lipids patch, OPC | 64.4 | 313 |
| (6.4 nm^2), 128 lipids patch, TIP4p/2005 | 66.8 | 313 |
| experiment [40] | 64.3 | 303 |
| experiment | 67.3 | 323 |

OPC [30], SPC/E [31] and TIP4p/2005 [32] presented in Supporting Information (SI). We used ECC-ions model for ions [20, 33?]. Simulations with Lipid14 use ion model by Dang [34–36]. The code AmberTools [37] was then used to generate the Amber-type force field parameters. The parameters were converted to the Gromacs format by using acpype tool [38]. The partial charges were then manually modified to approximately correspond to their equivalent segments in Lipid14 [21]. 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.

III. RESULTS AND DISCUSSION

A. Lipid bilayers without ions

The scattering form factors, NMR order parameters and area per lipid calculated using the ECC-lipids model for POPC are compared to experiments and original Lipid14 results in Fig. 1 and in Table I. The structural quality is comparable to the state of art lipid models available in literature [25], thus we conclude that the ECC-lipids model reproduces the lipid bilayer structure in liquid disordered phase with similar accuracy to other available models.

Headgroup order parameter response to the bound charge was evaluated against experimental data measured with the cationic surfactant (dihexadecyldimethylammonium bromide, $\text{C}_{12}\text{Cl}_{16}^+\text{N}_2\text{C}_1\text{Br}^-$) [41]. 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 headgroup 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.

10.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 headgroup order parameter response to the bound cation concentration is in experiments approximately linear up to ~ 0.3 mole fraction of the cation [41]. The linear-

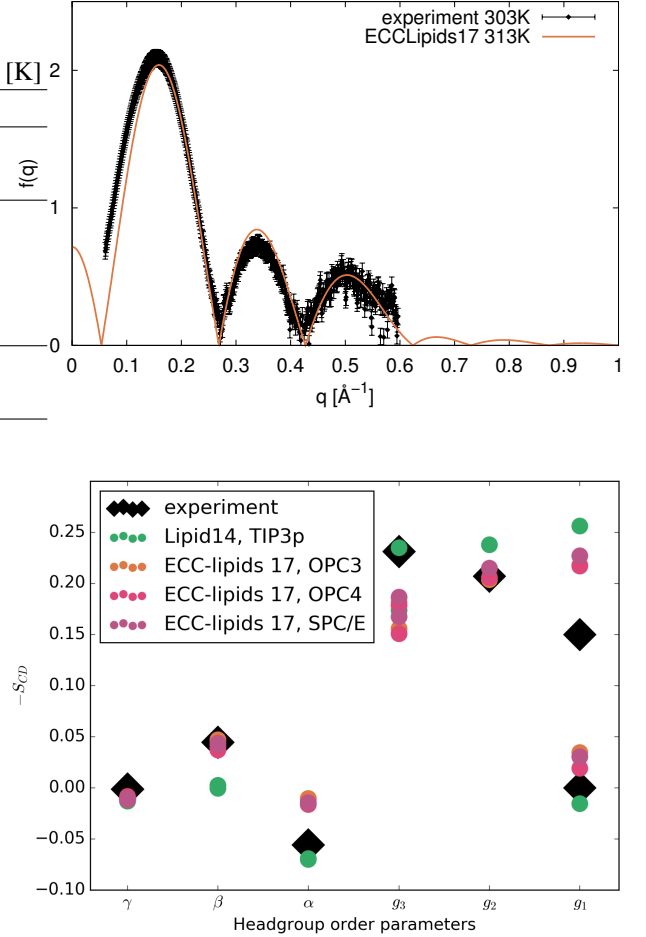


FIG. 1: X-ray scattering form factors from experiments [?] and simulations using Lipid14 [21] and ECC-lipids models. Headgroup and glycerol backbone order parameters from simulations with Lipid14 [21] and ECC-lipids models compared with experimental order parameters from [39].

8.Add acyl chain order parameters, POPC chemical structure

ity 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 **11.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 headgroup order parameter changes suggests that the observed overestimated response of all models to increasing CaCl_2 concentration in [14] can be explained at least in part by a high sensitivity of the headgroup order parameter response to the bound charge.

B. Cation binding to POPC membrane

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 overestimate the head group order parameter response to CaCl_2 concentration [14]. 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 [21] and Dang ions [34–36] show a pronounced peak in the position of the phosphate moieties of POPC. The use of a ECC-ion model [19, 20] 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). **12.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), the decrease in cation density of ECC-lipids demonstrates that ion binding to zwitterionic phospholipid membranes can be accurately described with classical MD simulation models that account for electronic polarizability.

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 headgroup order parameter experiments [11]. In addition to the ternary complexes, there also is a non-negligible probability of one Ca^{2+} cross-bridging three POPC molecules. Technical details of the analysis are in the SI.

14.Put details of the cation-binding stoichiometry analysis to SI. 15.Update the binding isotherm figure with new simulations 16.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. 17.SAMULI: I would also analyze how much there is contact between ions and different parts of the lipid (phosphate, carbonyl, etc.). 18.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.. 19.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 sim-

ulation models, where electronic polarization is effectively included by using electronic continuum correction. This is a significant improvement over other available lipid models, which all overestimate specific cation binding affinities [14]. The newly proposed model, which we denote as "ECC-lipids 17", reproduces the lipid bilayer structural details with similar accuracy as other state of the art lipid models. ECC-lipids 17 exhibit accurate headgroup order parameter response to bound cations, monovalent Na^+ and cationic surfactant dihexadecyldimethylammonium bromide, and divalent Ca^{2+} . Several water models (OPC3[29], OPC [30], SPC/E [31] and TIP4p/2005 [32]) 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 [11]. In this model 1 calcium binds to 2 POPC molecules, which together form a ternary complex.

The electronic continuum correction is applied here on Lipid14 POPC model [21], but we expect that the correction can be generalized also for other lipids and force fields.

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

Acknowledgments

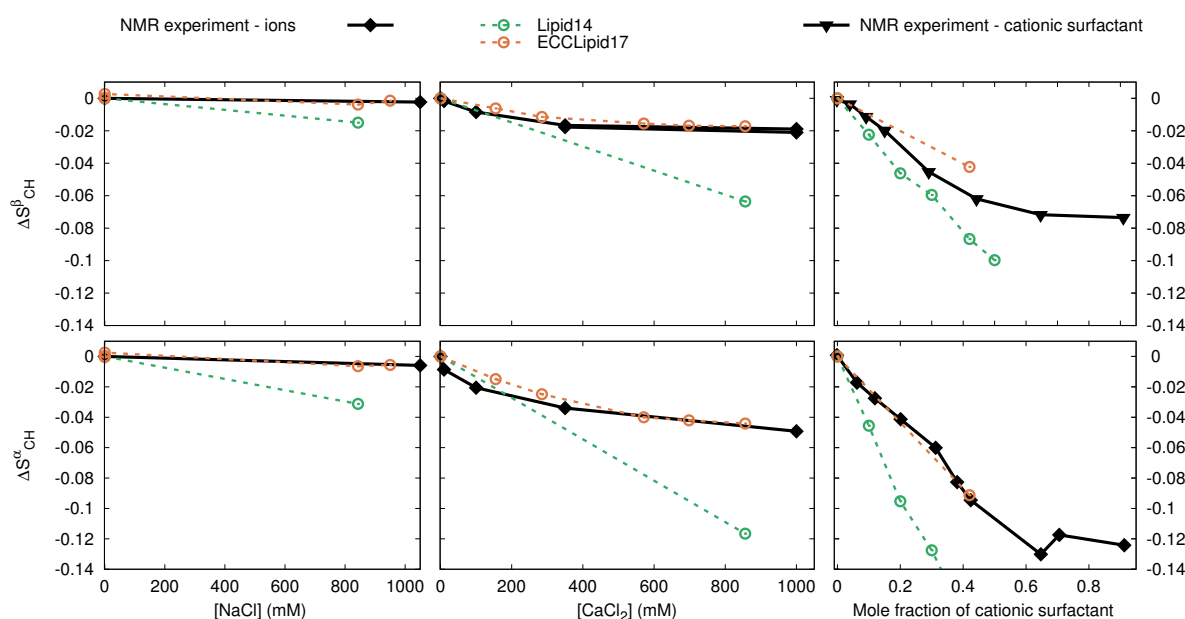


FIG. 2: Headgroup order parameter changes as a function of NaCl, CaCl_2 concentration and cationic surfactant (dihexadecyldimethylammonium bromide, $\text{C}_{12}\text{Cl}_{16}^+\text{N}_2\text{C}_1\text{Br}^-$) mole fraction from simulations and experiments (DPPC [28], POPC [11], surfactant [41]).

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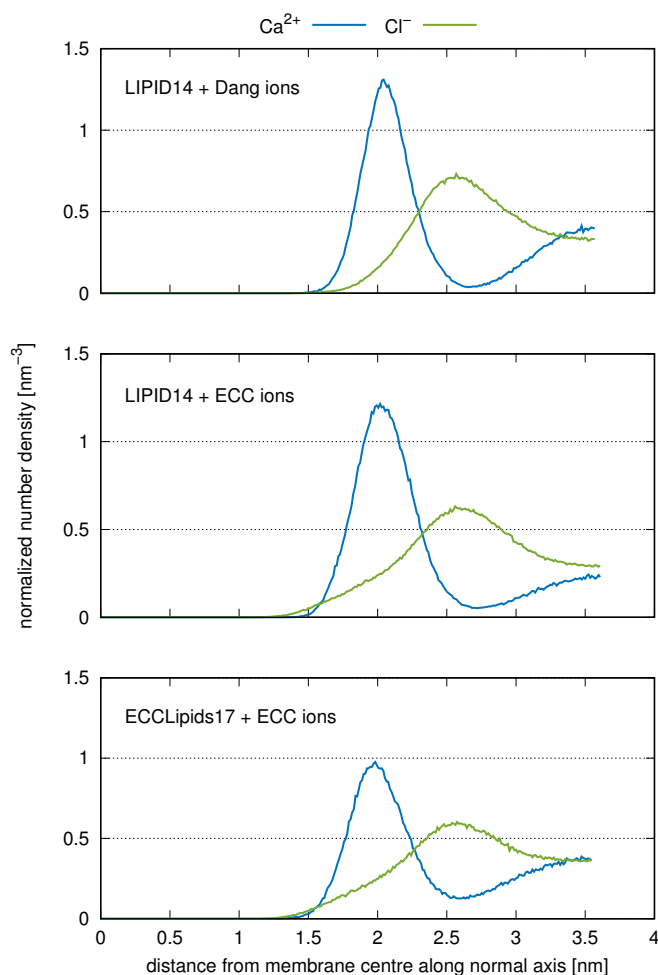


FIG. 3: Density profiles of Ca^{2+} and Cl^- for Lipid14 model with Dang ions and with ECC-ions and ECC-lipids model with ECC-ions.

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

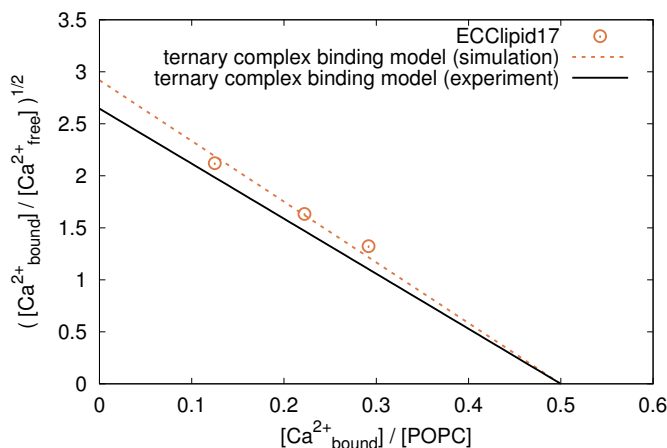


FIG. 4: Binding isotherm assuming stoichiometry of 2 POPC:1 Ca^{2+} as used in [11] fits our simulation data well.

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- [42] Depending on which QM method you arrive at values from 0.76 to 0.83, averages across atom types being around 0.78–0.80. Even though the methods are almost identical, authors of Lipid 14 find lower partial charges in vacuum than here – so I prefer the higher value. As the choice of charges is arbitrary anyway, I use 0.80 as an approximate round value. The use of 0.78–.79 might be more appropriate, though.

ToDo

P.

1. Abstract directly from Joe's conference abstracts. To be rewritten. 1
2. find the value 2
3. Add the values 2

| | | | |
|---|---|---|---|
| 4. SAMULI: Did you analyze the effect of this to hydration or did we only speculate? JOE: I found a systematic decrease of water density in the heagroup region even if I kept APL constant. | 2 | 14. Put details of the cation-binding stoichiometry analysis to SI. | 4 |
| 5. SAMULI: This effect may have an official name. In that case we should mention it. | 2 | 15. Update the binding isotherm figure with new simulations | 4 |
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| 9. put original references, not Slipids param. paper. . . | 3 | 18. 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. | 4 |
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| 8. Add acyl chain order parameters, POPC chemical structure | 3 | 13. Change the figure so that it contains a membrane background | 6 |
| 11. 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. | 3 | | |
| 12. Add OP-response of Lipid14+ECC-ions into the OP-changes plot in SI | 4 | | |