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 headgroup 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 IV (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 phopholipid bilayers, while other monovalent ions do not essentially bind [1–3, 7]. The presence of anoinic 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 Ca2+ 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.

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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 higly 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 physicalle well justified version of partial charge scaling implemented in early days of lipid and surfctant simulations [15, 16]. The approach has been previously shown in to improve bulk performace 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

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 $f_q = 0.75$ [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. vaccum 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. [53]

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 stonger 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]

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

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]

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

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.

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 conceration reaches a clear plateau in the bulk solvent. The concentrations in the units of $\mathrm{mol/l}$ were then determined as

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

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: Simulation parameters

simulation property	parameter
time-step	2 fs
equilibration time	100 ns
simulation time	200 ns
temperature	313 K
thermostat	v-rescale [36]
barostat	Parrinello-Rahman, semi-isotropic [37]
long-range electrostatics	PME [38]
cut-off scheme	Verlet [39]
Coulomb and VdW cut-off	1.0 nm
constraints	LINCS, only Hydrogen atoms [40]
constraints for water	SETTLE [41]

OPC [30], SPC/E [31] and TIP4p/2005 [32] presented in Supporting Information (SI). We used ECC-ions model for ions. [20, 33**?**] Classical molecular dynamics simulations were performed using the GROMACS [34] simulation package (version 5.1.4). The simulation settings used in this work are are summarized in Table I, and they are based on previously used settings in [14] available at [35]. Simulation trajectories and parameters are available at [**?**] **8.To be uploaded to Zenodo**.

1. *Simulations with cationic surfactants*

Automated topology builder [42] was first used to create the structure of dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$, molecule. The code AmberTools [43] was then used to generate the Amber-type force field parameters. The parameters were converted to the Gromacs format by using acpype tool [44]. 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 lipids calculated from the ECC corrected lipid model for POPC are compared to experiments and original Lipid14 results in Fig. 1 and in Table II. The structural quality is comparable to the state of art lipid models available in literature [25], thus we conclude that the ECC corrected lipid model reproduces the lipid bilayer structure in liquid disordered phase with similar accuracy than other available models **9.Discussion to be finished when we have all the results in the figure**. **12.finalize figure (NMR headgr. OPs + SAXS, continue in the discussion.**

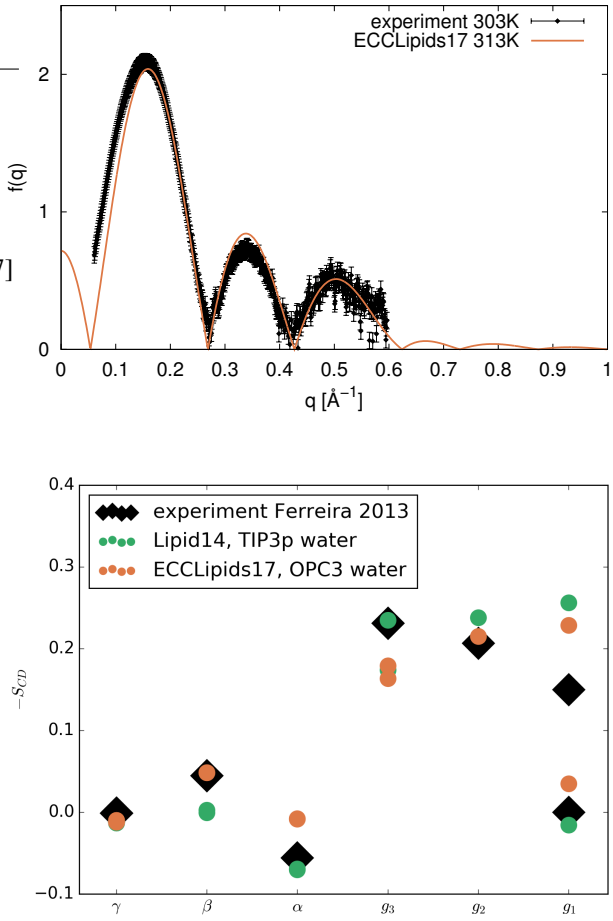

FIG. 1: X-ray scattering form factors from experiments [**?**] and simulations using Lipid14 and ECCLipids17 models. Headgroup and glycerol backbone order parameters from simulations with Lipid14 [21] and EECLipid17 models compared with experimental order parameters from [45].

10.Add acyl chain order parameters, POPC chemical structure 11.Should we add the results from original lipid14?

TABLE II: Area per lipid from different models for POPC without ions

model	A (^{2})	Temperature [K]
Lipid14 (literature)	65.6± 0.5	303
Lipid14ecc0.80+sigma0.875		313
GMX small patch	64.9	
GMX 4xbig patch	65.5	
oMM small patch	63.65	
oMM 4xbig patch	63.7	
experiment [46]**13.REF**	62.7	293
experiment	64.3	303
experiment	67.3	323
experiment	68.1	333
experiment POPE	56.6	303

Headgroup order parameter response to bound charge was evaluated against experimental data measured with cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$) [47]. The exact amount of bound charge in the membrane is known these systems, because practically all cationic surfactant molecules are embedded in lipid bilayer due to their amphiphilic nature. Thus, the headgroup order parameter changes as a function of mole fraction of cationic surfactants in Fig. 2 gives also the order parameter changes as a function of bound cations. **14.SAMULI: I think that we should make a separate figures for this one and ion concentrations. In the current figure it would be difficult to show this with a reasonable scale. Currently Lipid14 results are cut out, thus the scale should be increased. This would, however, make the changes with CaCl too small. The change of headgroup tilt could be incorporated in the same figure with order parameter response to cationic surfactant.** The headgroup order parameter response to bound cation concentration is approximately linear up to ∼0.3 mole fraction in experiments [47]. The linearity is also observed in simulations with original Lipid14 and with ECC correction. Quantitative comparison, however, reveals that the response is overestimated in original Lipid14 for both segments, while the ECC corrected model gives a good agreement for the change of α segment order parameter, but slightly underestimates the β segment order parameter change. The overestimation of order parameter changes with original Lipid14 model suggests that the overestimated response with $CaCl_2$ concentration in [14] can be partly explained by the high sensitivity of the headgroup order parameter response to bound charge.

Secondly, the S^{α}/S^{β} ratio of the response is found to be slightly larger than in experiments (VALexp vs VALsim **15.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.**). This property is kept also by the newly derived model, ECC-lipid, and, hence, it shows a perfect agreement in the response of the headgroup order parameter S^{α} but underestimates slightly the response of S^{β}.

B. Cation binding in POPC bilayer

Headgroup order parameter response to increasing $CaCl_2$ concentration from experiments, original Lipid14 model and ECC corrected model are shown in Fig. 2. The order parameter response is significantly overestimated in original Lipid14 model, while results from ECC corrected model are in good agreement with experiments. This is a significant improvement over previously available models, which always overstimate the order parameter response to $CaCl_2$ concentration [14]. The good agreement with experiments indicate that the binding details of Ca2+ are realistic in the ECC corrected model and it can be thus used to study lipid-ion interaction details.

Ion density profiles between different simulation models are compared in Fig. 3. Density profiles from simulations with original Lipid14 and Dang ions [50–52] show a pronounced peak in the position of the phosphate moieties of POPC. The use of a ECC-ion model [19, 20] along with original Lipid14 does not significantly change it **17.SAMULI: If we show density profile for this, we should show also the order parameter changes.** The new ECC-lipid model with scaled ions exhibits on the other hand smaller density in this region suggesting overall weaker binding of cations (Fig. 2). This demonstrates that cation binding in zwitterionic phospholipid bilayer can be accurately described with classical MD simulation model with effective included of electronic polarizability.

Good agreement of ECC corrected 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 most common complex are ones with stoichiometry of 2 POPC:1 Ca^{2+}. **20.SAMULI: Details of this analysis have to be added.** As shown in Fig. 4 this is in agreement with the ternary complex model suggested based on headgroup order parameter experiments [11]. **21.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. 22.SAMULI: I would also analyze how much there is contact between ions and different parts of the lipid (phosphase, carbonyl, etc.).** In addition to the ternary complexes, there also is a non-negligable probability of one Ca^{2+} cross-bridging three POPC molecules **23.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 cites, if it is not too much work..**

24.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 Ca2+ binding in phospholipid bilayers can be accurately described with MD simulation models, where electronic polarization is effectively included by using ECC correction. This is a significant advantage to the other available lipid models, which all overestimate specific cation binding affinities. The proposed model reproduces the lipid bilayer structural details with similar accuracy as the other state of the art lipid models. The 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.

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.

This will be a foundation stone of a new open-collaboration project NPRlipid 6 in nmrlipids.blogspot.fi....

Acknowledgments

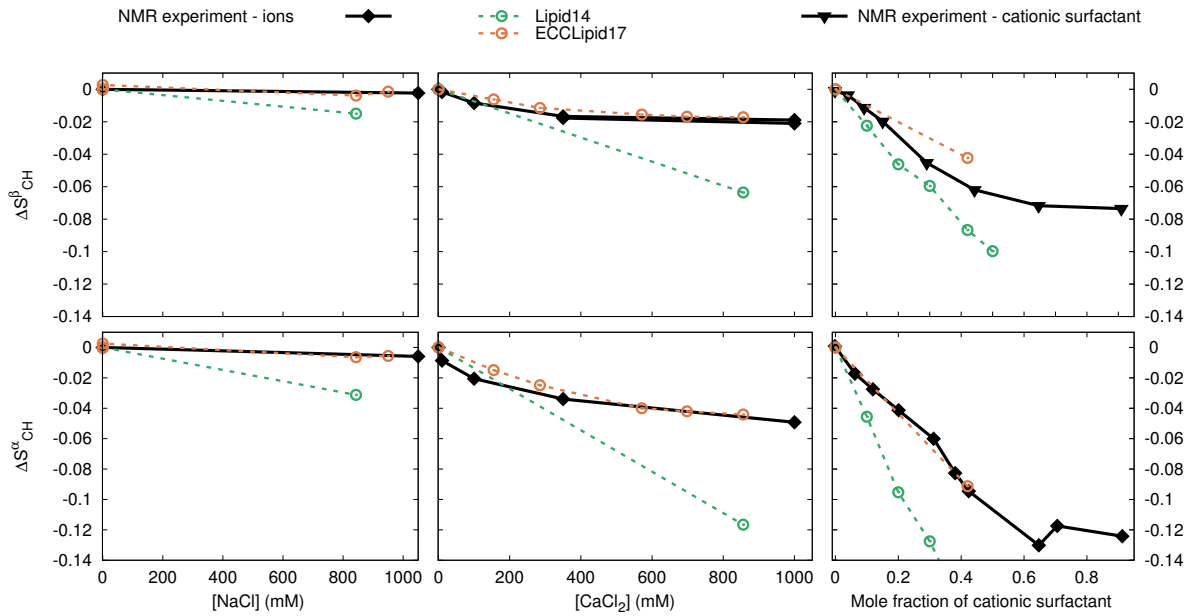

FIG. 2: Headgroup order parameter changes as a function of NaCl, $CaCl_2$ concentration and cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$) from simulations and experiments (DPPC [28], POPC [11], surfactant [47]). Simulations with Lipid14 and qvist ion model from [14, 35, 48, 49].

16.Add Lipid14-Aquist data. Lipid14/qvist data to be added from https://github.com/NMRLipids/lipid_ionINTERACTION/blob/master/Data/POPC/CaCl/LIPID14/LIPID14caclCONSchange.dat. Joe: I'm not sure what is meant – I think Aquist data are actually plotted there. I recently changed it to L14+Dang ions (both data and plot). I think just one ion model is sufficient (and ECC-ions are based on Dang model). Samuli: I think that we could add also the Aqvist data. This is in the NMRlipids II publication so it might make easier to follow for people who have red it publication.

SUPPLEMENTARY INFORMATION

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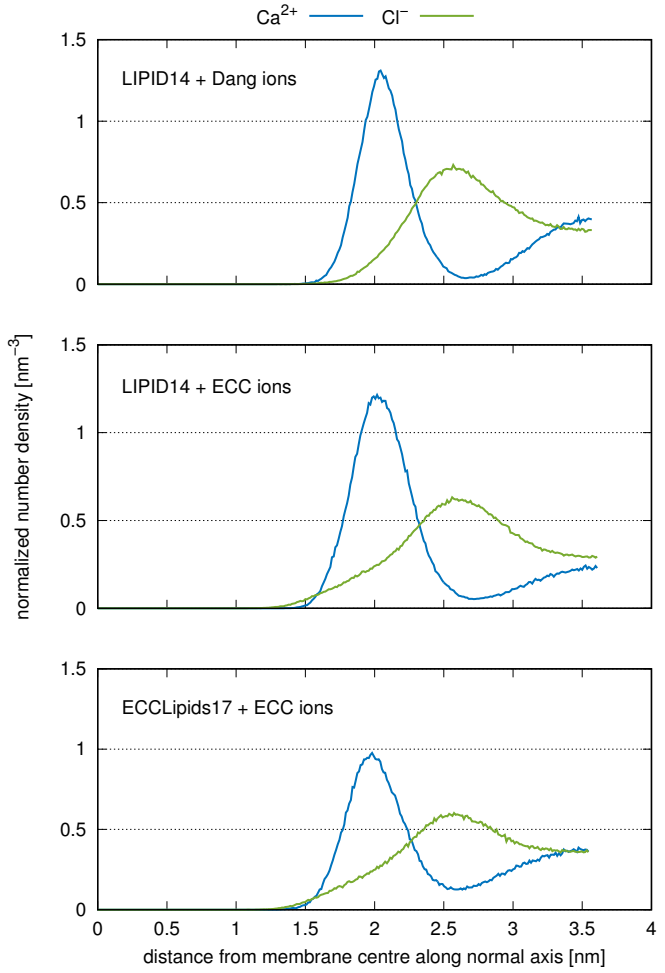

FIG. 3: Density profiles of Ca^{2+} and Cl^- for Lipid14 model with Aquist parameters and with ECC ions and ECCLipids17 with ECC ions.

18.SAMULI: We should add the location of bilayer here somehow. In NMRlipids II the location of phosphate was shown with green vertical line. 19.SAMULI: I would add Aqvist data from NMRlipids II in here as well.

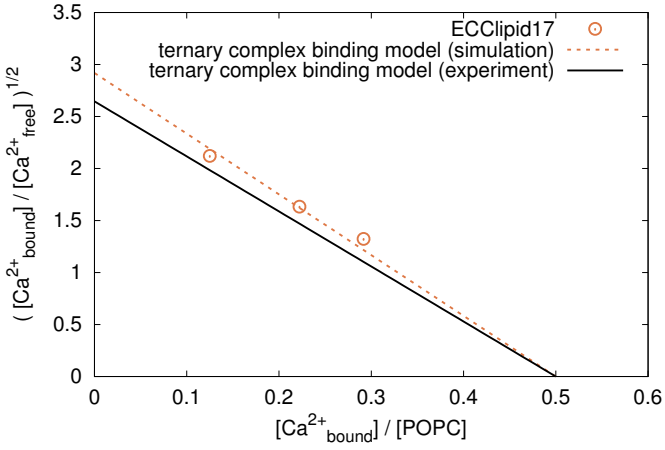

FIG. 4: Binding isotherm assuming stoichiometry of 2 POPC:1 Ca^{2+} as used in [11] fits the simulation data nicely.

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[53] 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