Accurate binding of calcium to phospholipid bilayers by effective inclusion of electronic polarization

Josef Melcr, Hector Martinez-Seara Monne, and Pavel Jungwirth
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague 6, Czech Republic

O. H. Samuli Ollila*
Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague 6, Czech Republic and Institute of Biotechnology, University of Helsinki

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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, "ECClipids", 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).

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I. INTRODUCTION

motivation, significance of membranes, phospholipids and simulation.

assumptions (so that we have structure of the paper like in a mathematical proof) – MD simulation is a good tool for studying molecules. classical MD models can describe lipids accurately,

MD is ... and it serves ... it is useful for ... (describe throuhg references)

Lipid membranes, especially phospholipid membranes; their significance for life, sciences, society, pharma ...

Current force fields – pros and cons, at a good shape in many aspects, agree on various properties. – write basic ideas from the lipid-FF whitepaper I did recently.

lipid force fields fail in description of membrane-cation interaction – could be answered by ECC? Cations were shown to generally overbind in PC lipid bilayers in NMRlipids II project. Here we propose that the cation overbinding can be corrected by implicitly inducing electronic polarizability in lipid headgroups by scaling the partial charges – i.e. MDEC/ECC [1]**1.Bulid BibTex references database.** paradigm.

MDEC – or – ECC **2.choose one**We have to decide whether we will refer to the method as a correction (ECC) or as a MD simulation paradigm (MDEC) – and choose on of these labels. – Joe: use ECC as we apply MDEC rather as a correction to current state not as a new paradigm for FF development. MD in electronic continuum as in [1] or electronic continuum correction as in [**?**]

Describe ECC: good physical concept for treating part of the polarizability (electronic) in a simple mean-field way.

Succesful application: Works for cations [2**?** , 3]**3.REF** – motivation for its application to zwitterionic lipids like POPC.

Hypothesis: ECC helps in describing even zwitterionic molecules like POPC, will be demonstrated through headgroup order parameter response to cationic molecules.

II. METHODS

ECC and solvation ΔG.(from [1] **4.**: hydration ΔG_{hyd} can provide good structure and energetics, but will fail in good interactions – already proved for cations) In addition, ΔG_{hyd} – the usual target for parametrization of small molecules in classical force fields – is not the right target in the MDEC/ECC paradigm – part of $\Delta G = \Delta G_{nuc} + \Delta G_{el}$ is already included in the polarization of electrons, and only the remining part of ΔG, ΔG_{nuc}, belongs to the polarization of the nuclei. Hence it is non trivial, how large should be the scaling factor f_σ – it should lay between 1 and f_q, the limits for the original interaction energy and its limit when we neglect the ΔG_{el} term in ΔG_{hyd}.

How do I apply ECC on Lipid14 POPC and why such choice (i.e. f_σ, f_q and definition of the scaled region). Region definition: headgroup requires optimization, tails appear already accurate enough. Choice of f_q: value 0.8 reflects the fact that the charges in Lipid14 were derived in vacuum, whereas they should rather be the average of vacuum and sol-

*samuli.ollila@helsinki.fi

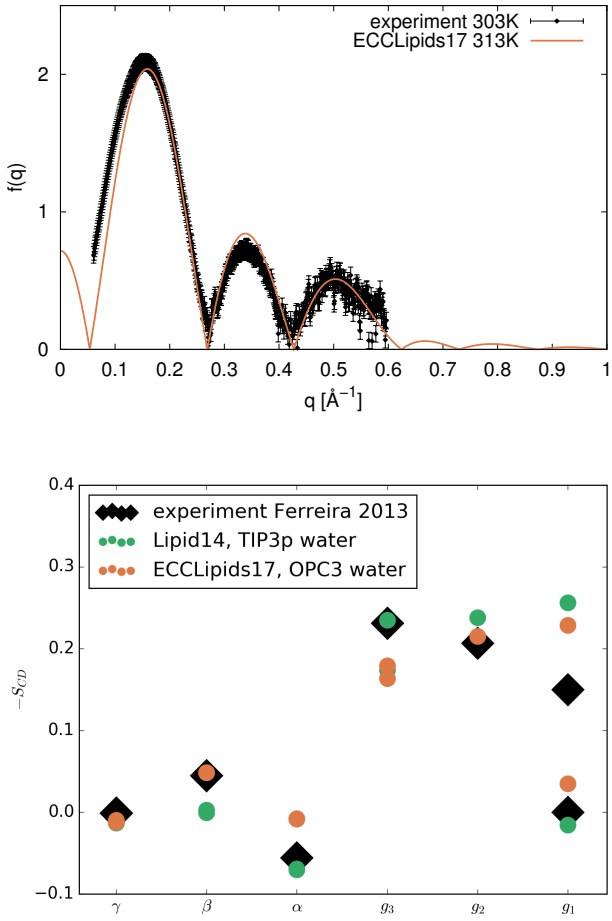

FIG. 1: X-ray scattering form factors from experiments [**?**] and simulations using Lipid14 and ECCLipids17 models. Headgroup and glycerol backbone order parameters from simulations with Lipid14 [7] and EECLipid17 models compared with experimental order parameters from [8].

vated charges (so called IPolQ charges [**?**], both charge-sets can be taken from [4]).

why did I choose Lipid 14 – good ratio of α/β response [5] but not perfect.

Detailed and robust structural information of lipid bilayers can be reached with C-H bond order parameter from NMR experiments, which tell on the lipid structure sampled by each individual molecule. On the other hand, form factors from scattering experiments validate overall bilayer properties, lateral density (area per molecule) and thickness [6]. The structural quality of new ECC lipid model is evaluated against scattering form factors and NMR order parameters in Fig. 1 **5.Add acyl chain order parameters, POPC chemical structure 6.Why original Lipid14 model seems to deviate from experiments, in contrast to the original publication? Different simulation setup used?** Area per molecules extracted from MD simulations and SPD model fitted to scattering data **7.check that this the case for the used values** are shown in Table I **8.finalize figure (NMR headgr. OPs + SAXS, continue in the discussion.**

Ion binding in lipid bilayers can be accurately measured

TABLE I: 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 [**?**]**9.REF**	62.7	293
experiment	64.3	303
experiment	67.3	323
experiment	68.1	333
experiment POPE	56.6	303

and compared between experiments and simulations by using lipid headgroup order parameters and "electrometer concept" introduced by Seelig et al. [5, 9]. This is based on the experimental observations that order parameters for α and β carbons are proportional to the amount of bound charge in lipid bilayer, which was rationalized as a charge induced tilt of the headgroup dipole [9]. Later analysis including signs of the order parameters showed that the order parameters are actually decreasing with bound positive charge and *vice versa* for negative charge [5, 6].

III. RESULTS AND DISCUSSION

Order parameter changes as a function of bound charge are shown in Fig. 2. Approximately linear decrease of headgroup order parameters is observed both in simulations and experiments with the cationic surfactant. The use of a cationic surfactant has the benefit that it not a subject of partitioning between water and lipid phase. This means that we know the exact amount of bound charge in the membrane both in experiment and simulaiton. Quantitative comparison reveals that in the original Lipid14 model the response is overestimated for both order parameters, α and β. This suggests that the observed overestimated response in [5] is at least in part due to the high sensitivity of the headgroup order parameter response, not only due to overestimated binding of cations. Secondly, the S^α/S^β ratio of the response is found to be slightly larger than in experiments (VALexp vs VALsim**10.**). This property is kept also by the newly derived model, ECClipid, and, hence, it shows a perfect agreement in the response of the order parameter S^α but underestimates slightly the response of $/S^\beta$.

11.ongoing,Actual concentration of cations in simulation has yet to be estimated. If it varies too much from the nominal concentration, I may need to tweak the scaling factors, f_q or only f_σ, to accomodate it. However, it is very unlikely, response to the surfactant DHMDMAB is OK. Big patches with loads of solvent are running at the moment to guide me on the possible finite-size errors and this conc-error.

Ion density profiles between different simulation models are compared in Fig. 3. Density profiles from simulations

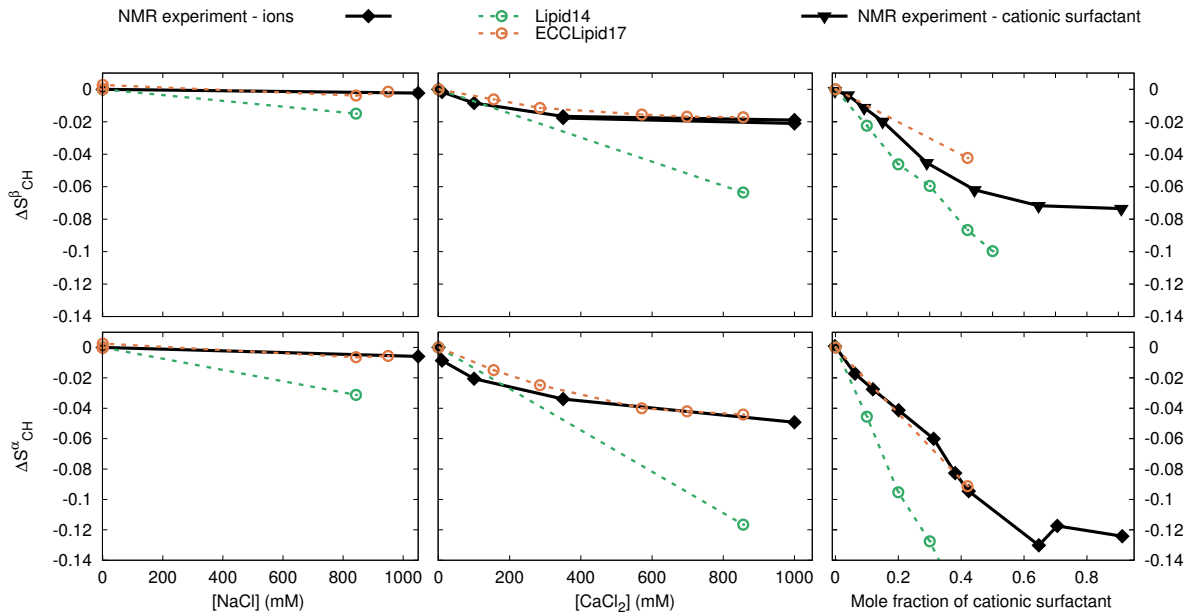

FIG. 2: Headgroup order parameter changes as a function of NaCl, $CaCl_2$ concentration and cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$) from simulations and experiments (DPPC [10], POPC [11], surfactant [12]). Simulations with Lipid14 and qvist ion model from [5, 13–15].

12.Add Lipid14-Aquist data.

with original Lipid14 and Dang ions [16–18] show a pronounced peak in the position of the phosphate moieties of POPC. The use of a ECC-ion model [2, 3] along with original Lipid14 does not significantly change it. The new ECClipid model with scaled ions exhibits on the other hand smaller density in this region suggesting overall weaker binding of cations (Fig. 2). This demonstrates how importnat it is to account for electronic polarizability also in molecules with zero total charge. Alltogether, the response of headgroup order parameters as a function of $CaCl_2$ concentration is in agreement with experiments in the new ECClipid model simulated with ECC model of $CaCl_2$. This is a significant improvement over previous models available for lipid–ion interactions studies [5] and indicates that the new model can be used for detailed studies of lipid-ion interactions.

For example, there is a debate in the literature on the stoichiometry of Ca^{2+} binding to POPC [**?**] **13.REFs**. In line with the early experimental finding [**?**], we find that our data fit well a ternary complex model, which assumes 2 POPC molecules per 1 Ca^{2+}. In contrast to this experiment, however, we possess also a complete atomistic detail of this phenomenon. Hence we can support this empirical finding with a direct observation of transient complexes of Ca^{2+} and POPC. The simulation suggests that the stoichiometry 2 POPC:1 Ca^{2+} is the most common complex. In addition, there also is a non-negligable probability of one Ca^{2+} cross-bridging three POPC molecules. This cannot be observed in experiments without atomistic detail as it makes only a small perturabation to the binding isotherm that assume 2 POPC per 1 Ca^{2+}. **14.Analyze stoichiometry for Na^{+}, Ca^{2+}, their interaction energies with the lipid membrane, etc, and finalize the discussion after these results.**

Discussion:

other lipids: charged lipids? – ongoing research in our lab. I'm currently working on POPE with Aniket for curved (and flat) membranes.

Samuli: From the point of view of this paper, the most relevant other lipid to study would be DPPC to see if we can reproduce the difference between POPC and DPPC in experiments.

Joe: In addition, there are more experimental OP data on DPPC. However, it is not necessary and we can leave it to the community project.

Role of water model: we use OPC3 (current best), it would be worth giving an estimate how results change when we use say SPCE or even TIP3p at least in the SI (so that the reader knows what errors to expect comming from these sub-optimal models). In addition, there is protein force field Amber15-FB, which uses water close to OPC3, TIP3pFB **15.REFs**.

questionable charge distribution from RESP – a leeway for further tweaks of the FF. It is not obvious that RESP charges provide the best description, especially due to its non-uniqe solution. Shall we solve RESP fitting with the constraint of full charges and then scale down, or shall we rather solve the fitting with a scaled total charge target?

acheivable accuracy of the MD engines themselves (mainly Hector's worry) is another limiting factor in fine tuning parameters – solid physical ground helps.

application of the correction to other lipid models: The rule looks general, however, it depends on how accurate the original model was. From preliminary simulations with POPE, it

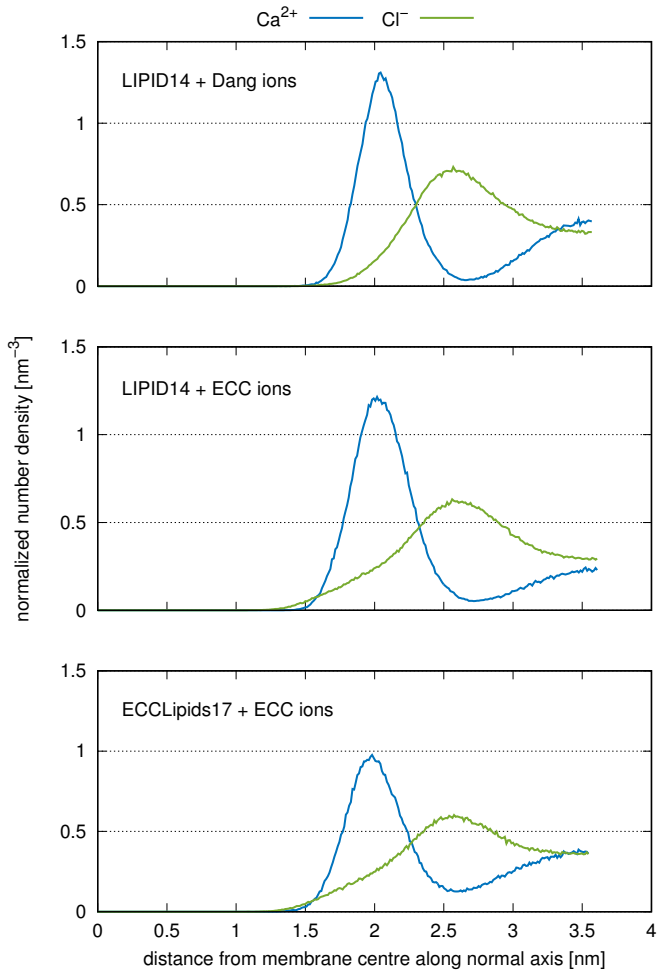

FIG. 3: Density profiles of Ca^{2+} and Cl^- for Lipid14 model with Aquist parameters and with ECC ions and ECCLipids17 with ECC ions.

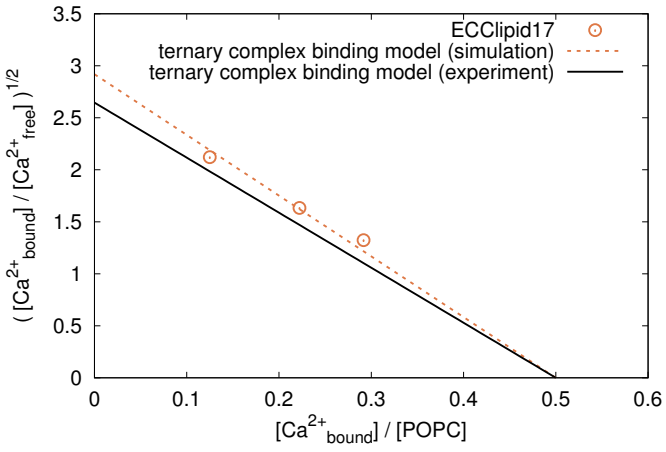

FIG. 4: Binding isotherm assuming stoichiometry of 2 POPC:1 Ca^{2+} as used in [**?**] fits the simulation data nicely.

looks that the rule works for Lipid14 FF, at least for zwitterionic headgroups.

Conclusion:

Reiterate what we did...

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

Acknowledgments

SUPPLEMENTARY INFORMATION

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ToDo

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