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

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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-

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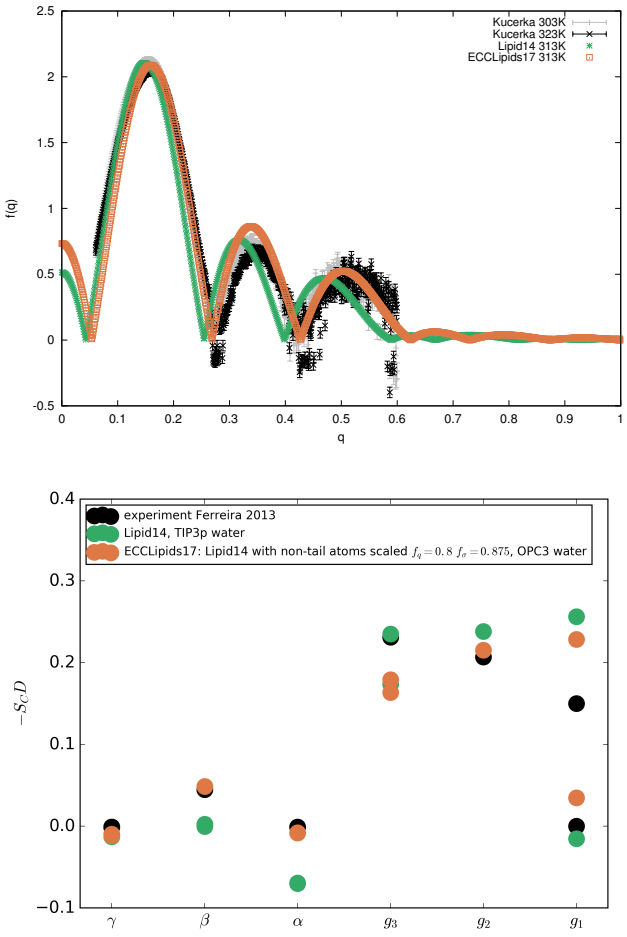

FIG. 1: X-ray scattering form factors from experiments [**?**] and simulations using Lipid14 and ECCLipids17 models. Headgroup and glycerol backbone order parameters from simulations with Lipid14 [7] and EECLipid17 models compared with experimental order parameters from [8].

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].

III. RESULTS AND DISCUSSION

Detailed and robust structural information of lipid bilayers can be reached with C-H bond order parameter from NMR and form factors from scattering experiments. The former can be used to validate structures sampled by individual molecules and latter to validate overall bilayer properties, lateral density (area per molecule) and thickness [6]. The structural quality of new ECC lipid model against scattering form factors and NMR order parameters is evaluated in Fig. 1 **5.Add acyl chain order parameters, POPC chemical structure and fix experimental alpha order parameter value 6.Why original Lipid14 models seems to deviate from experiments, in contrast to the original publication?** Area per molecules extracted from MD simulations and SPD model fitted to scattering data

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

7.check that this the case for the used values are shown in Table I **8.finalize figure and the discussion**

Ion binding in lipid bilayers can be accurately measured 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 carge induced tilt of 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].

Order parameter changes as a function of bound charge are shown in Fig. 2. Approximately linear decrease of headgroup order parameters is observed in simulations and experiments with cationic surfactant. Quantitative comparison reveals that in original Lipid14 model the proportionality constant is overestimated, thus the headgroup is too sensitive to the bound cations. However, the new ECC lipid model shows better agrement, althoght β responce is slightly underestimated.

What affects the headgroup OP response?: The response of the headgroup order parameters is improved both through diminished affinity towards the cations and through lower headgroup response to the bound charge (from simulations with DHMDMAB surfactant). The responses of headgroup OPs are shown in Fig. 2.

10.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 with original Lipid14 and qvist ions and new ECC lipid model with scaled ions are not much different, thus the overestimated order parameter decrease is explained by the overestimated headgroup response on bound charge in original lipid model (see Fig. 2). Introducing scaling in only on ion models increase the ion binding which is also seen in order parameter

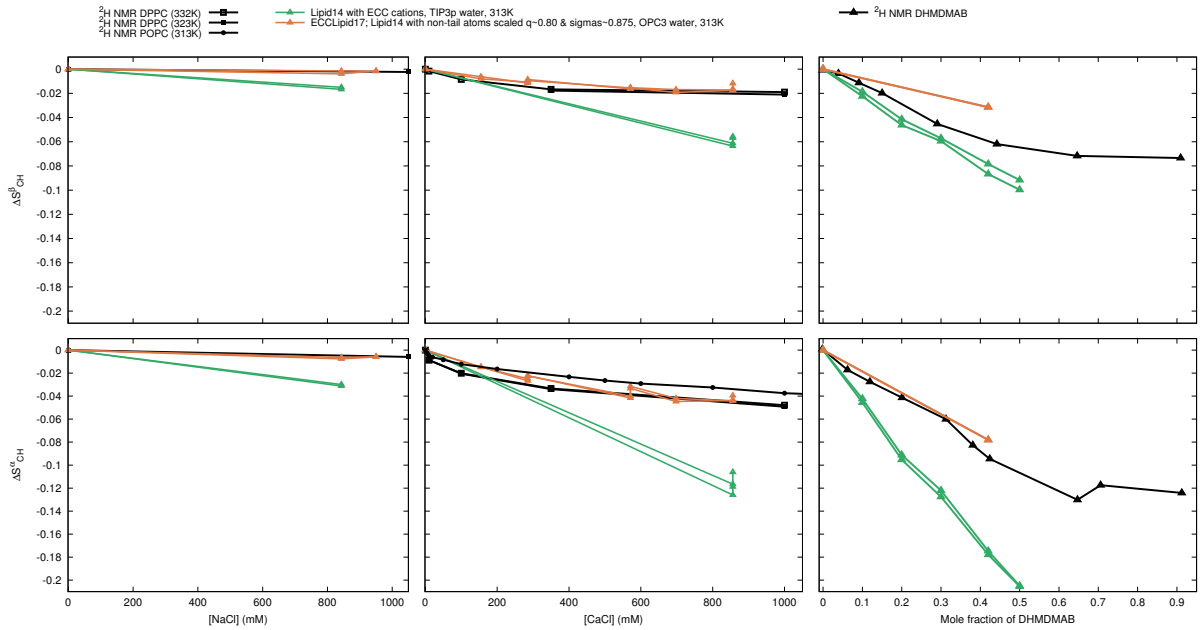

FIG. 2: Headgroup order parameter changes as a function of NaCl, $CaCl_2$ concentration and cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$) from simulations and experiments (DPPC [10], POPC [11], surfactant [12]). Simulations with Lipid14 and qvist ion model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

11.Lipid14/qvist data to be added from https://github.com/NMRLipids/lipid_ionINTERACTION/blob/master/Data/POPC/CaCl/LIPID14/LIPID14caclCONSchange.dat

changes in Fig. 2 **12.Final check to be done when the data from qvist model is there**. The increased binding can be probably explained by the changes in the size of the ion due to scaling **13.more detailed explanation?**. Using ECC approach also in lipid headgroups decreases the ion binding and also brings headgroup response on bound charge close to experiments in Fig. 2. Alltogether, the response of headgroup order parameters as a function of $CaCl_2$ concentration is in agreement with experiments in new ECC lipid model simulated with scaled $CaCl_2$ model. This is a significant improvement on previous modela available for lipid–ion interactions studies [5] and indicates that the new model can be used for detailed studies of lipid-ion interactions.

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 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

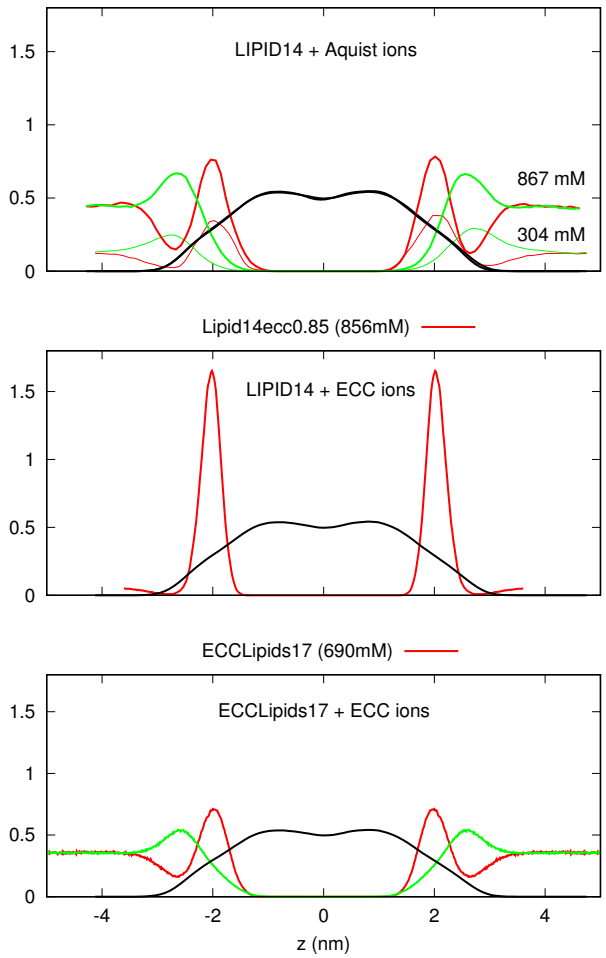

FIG. 3: Density profiles of Ca^{2+} and Cl^{-} for Lipid14 model with Aquist parameters and with ECC ions and ECCLipids17 with ECC ions.

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