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

(Dated: June 12, 2017)

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).

[1] Catte, A., Girych, M., Javanainen, M., Melcr J., Miettinen, M. S., Oganesyan, V. S. and Ollila H. S., PCCP 18(47) 32560-32569 (2016) [2] Leontyev, I. V., and Stuchebrukhov, A. A., JCTC 6(5) 14981508 (2010) [3] Kohagen, M., Mason, P. E., and Jungwirth, P., J. Phys. Chem. B 120(8) 145460 (2015) [4] Seelig, J., MacDonald, P. M., and Scherer, P. G., Biochemistry 26(24) 75357541 (1987)

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.

To date, there is no lipid model that can describe such a behaviour, the electrometer response and cation binding, quantitatively well [4]

Almost all lipid bilayer simulations reported in the literature are done with force fields that represent interactions between atoms with pairwise additive empirical potential and exclude the electronic polarizability [**?** **?**]. While the issue has been considered higly relevant since the early days of lipid bilayer simulations, practical solutions have turned out to be complicated [**?**]. Here we propose that electronic polarizability can be effectively included in lipid bilayer simulations by using the electronic contimuun correction [1].

II. METHODS

A. Electronic continuum correction for lipid bilayers

According to the electronic continuum correction (ECC) the electronic polarizability can be effectively included in MD simulations by scaling the partial charges with a constant factor $f_q = \epsilon_{el}^{-1/2}$, where ϵ_{el} is the electronic part of the dielec-

*samuli.ollila@helsinki.fi

tric constant of media [1]. Measurements of high frequency dielectric constant gives values of approximately $\epsilon_{el} \approx 2$ for almost any biomaterial [1**?**]. The value measured for water, $\epsilon_{el} = 1.78$, gives a scaling factor of $f_q = 0.75$ [1**?**], which has been successfully used to improve the performance of ion force fields [2**?** , 3].

Here we apply the same approach to lipid bilayers to accurately describe the lipid headgroup response to Na+ and Ca2+ concentrations [4]. We use the Lipid14 [5] 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 [4, 6]. The partial atomic charges in Lipid14 were derived by fitting the electrostatic potential to its model quantum chemistry representation in vacuum. **4.I do not fully understand the end of the paragraph** If such charges are obtained in an implicit solvent, they vary the most for the polar moieties [**?**]. In order to represent this difference in polarity in a model with fixed charges, we shall use the average charges from both environments (IpolQ charges) [**?**]. By taking the charges of oxygen atoms in vacuum and implicit water solvent for POPC from [7], we represent the IpolQ charges in the electronic continuum correction of the Lipid14 model by increasing the scaling factor for charges to $f_q = 0.8$. The newly presented ECC-POPC model is based on the POPC model from

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 [8], in contrast to glycerol backbone and headgroup regions which require some improvement in all available lipid models [6]. To minimize the intercalation with the highly optimized hydrocarbon parameters, we decided to apply ECC correction only to the headgroup, glycerol backbone and carbonyl in acyl chains. These regions are also most polar parts in lipids and are expected to most affect on cation binding.

The scaling of these partial charges by a factor of 0.8 reduce the area per molecule to ??, which is significantly smaller than the experimental and original Lipid 14 values. The decrease of area was speculated **5.Did you analyze the effect of this to hydration or did we only speculate?** 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 σ term in Lennart-Jones potential. This allows water molecules approach closer to lipid atoms, where electrostatic interactions are stronger and, thus, increases hydration level. **6.This effect may have an official name. In that case we should mention it.** **7.Is there some justification for the below sentence?** In line with the electronic continnum correction, the scaling factor for sigma parameters, f_σ, has to lay between f_q and 1. Scaling factor value of ?? for σ was found to reproduce area per molecule values close to experiments and original Lipid14 model when charges were scaled with 0.8.

B. Comparison to experimental data

Structures sampled by individual lipid molecules were compared to experimental data by using C-H bond order parameters [8]

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

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 [8] **8.Add equation**.

Ion binding was compared between experiments and simulations by using lipid headgroup order parameters and "electrometer concept" introduced by Seelig et al. [4, 9]. The concept is based on the experimental observation that the order parameters of α and β carbons in lipid headgroup (see Fig. **??**) are proportional to the amount of bound charge in lipid bilayer [9]. 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 [4, 8]. 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 [9].

9.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. SAMULI: I thought about this a little bit. My current conclusion is that we can take the number concentration from simulations with large box having a clear bulk concentration region and calculate the bulk concentration in mol/l unit like this

$$C_{eq}[mol/l] = \frac{C_{plateau}[mM]}{0.602} \tag{2}$$

C. Simulation details

10.To be written

III. RESULTS AND DISCUSSION

A. Lipid bilayers without ions

The structural quality of new ECC lipid model is evaluated against scattering form factors and NMR order parameters in Fig. 1 The optimal value for f_σ was found by representing the overall membrane structure well by matching scattering form factors to experimental data [10–12]. This ensures that the presented model describes correctly the membrane strucutre, adopts a liquid disordered phase, and provides area per lipid agreeing with experiments (see Fig 1 and Table I).

Order parameter changes from experiments [9] and simulations with Lipid14 model and with ECC-lipids model as a function of bound charge are shown in Fig. 2. Approximately linear decrease of headgroup order parameters

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 [**?**]16.REF	62.7	293
experiment	64.3	303
experiment	67.3	323
experiment	68.1	333
experiment POPE	56.6	303

is observed both in simulations and experiments with the cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$). The use of a cationic surfactant has the benefit that it is 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 simulation. 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 [4] 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 VALsim11.). 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}. **12.Add acyl chain order parameters, POPC chemical structure 13.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 **14.check that this the case for the used values** are shown in Table I. **15.finalize figure (NMR headgr. OPs + SAXS, continue in the discussion.**

B. Cation binding in POPC bilayer

Ion density profiles between different simulation models are compared in Fig. 3. Density profiles from simulations with original Lipid14 and Dang ions [20–22] 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 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 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 ECC-lipid model simulated with ECC

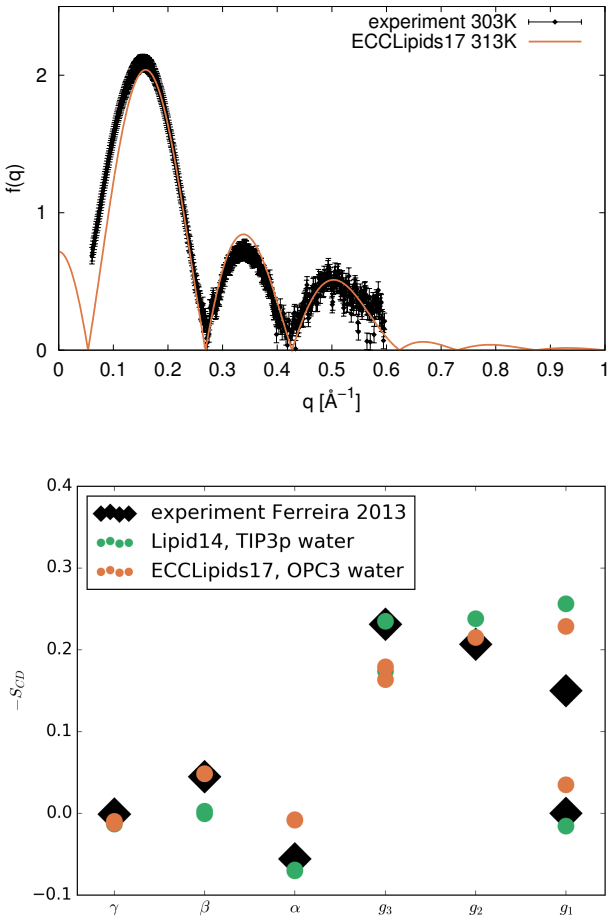

FIG. 1: X-ray scattering form factors from experiments [**?**] and simulations using Lipid14 and ECCLipids17 models. Headgroup and glycerol backbone order parameters from simulations with Lipid14 [5] and EECLipid17 models compared with experimental order parameters from [13].

model of $CaCl_2$. This is a significant improvement over previous models available for lipid–ion interactions studies [4] and indicates that the new model can be used for detailed studies of lipid-ion interactions.

One possible example is to adress the discourse on the stoichiometry of Ca^{2+} binding to POPC [**?**] **18.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 assumes 2 POPC per 1 Ca^{2+}. **19.Analyze stoichiometry for Na^{+}, Ca^{2+}, their interaction energies with the lipid membrane,**

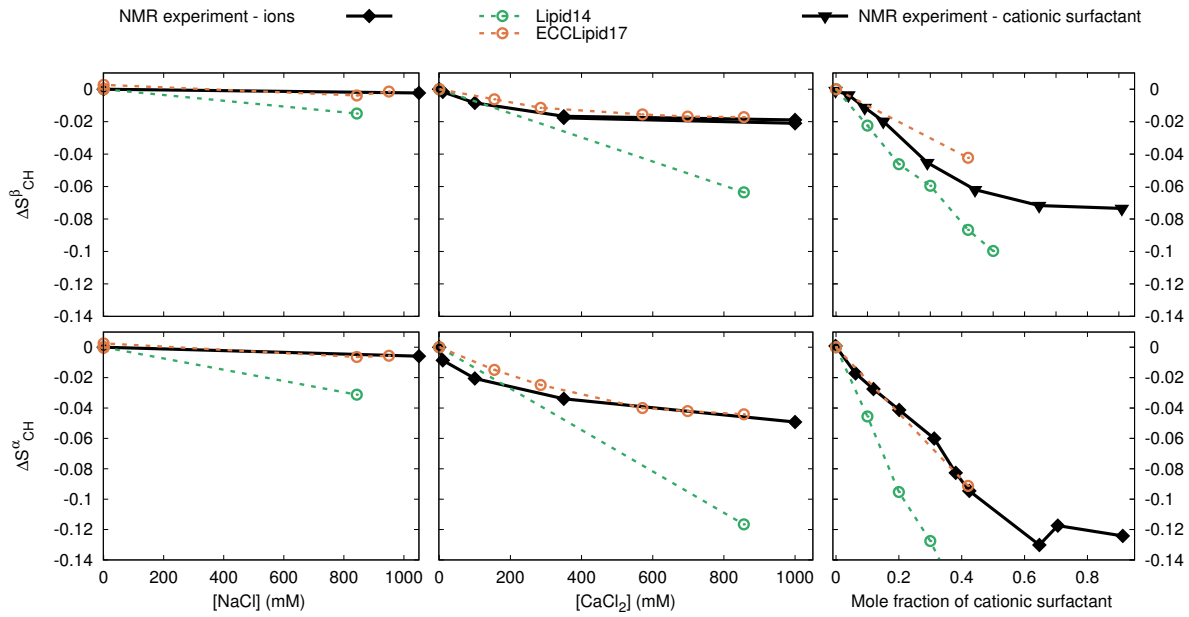

FIG. 2: Headgroup order parameter changes as a function of NaCl, $CaCl_2$ concentration and cationic surfactant (dihexadecyldimethylammonium bromide, $C_{12}Cl_{16}{}^{+}N2C_1Br^{-}$) from simulations and experiments (DPPC [14], POPC [15], surfactant [16]). Simulations with Lipid14 and qvist ion model from [4, 17–19].

17.Add Lipid14-Aquist data.

etc, and finalize the discussion after these results.

It is also suggested that the addition of NaCl to the solution of $CaCl_2$ enhances the hedgroup order parameter response compared to the solution with only $CaCl_2$. [**?**] **20.Simulate this effect and discuss it further**

21.The difference between DPPC and POPC – simulate and compare with experiment.

Discussion:

22.It might be worth acknowledging each experimental finding in [?] and observations in [?].

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 **23.REFs**. On the other hand, it might be the best if we used a model that doesn't have the dielectric constant from nuclei 78, but rather 44 – TIP4p2005 is the closest. [**? ?**]

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.

IV. CONCLUSION

We present models of POPC and DPPC lipids that for the first time exhibit accurate headgroup order parameter response to cation binding. The models are derived from Lipid14 model [5] by applying the electronic continuum correction. The models were optimized to represent correct membrane structure, and they were validated with the use of electrometer concept [9, 23**?**]. Compared to the phenomenological observations of calcium:POPC stoichiometry in the experimental work [**?**], our simulations reveal the same stoichiometry of 2 POPC molecules per 1 calcium but through direct observation. This tells us on the subtle effects of calcium in phospholipid membranes possibly leading to a better understanding of their physical properties (elasticity etc.). **24.Improve this concluding-**

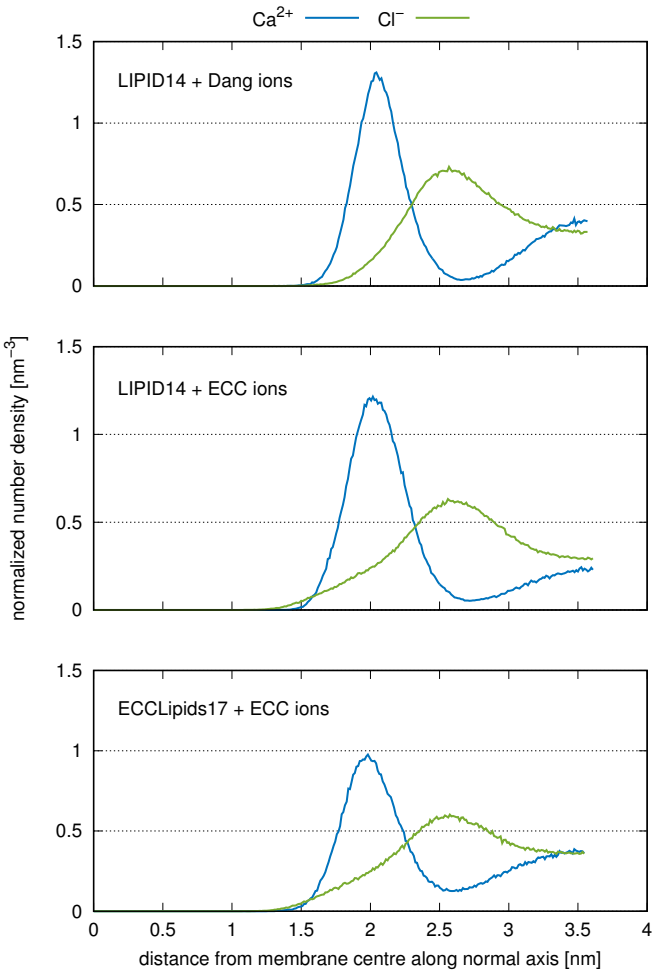

FIG. 3: Density profiles of Ca^{2+} and Cl^- for Lipid14 model with Aquist parameters and with ECC ions and ECCLipids17 with ECC ions.

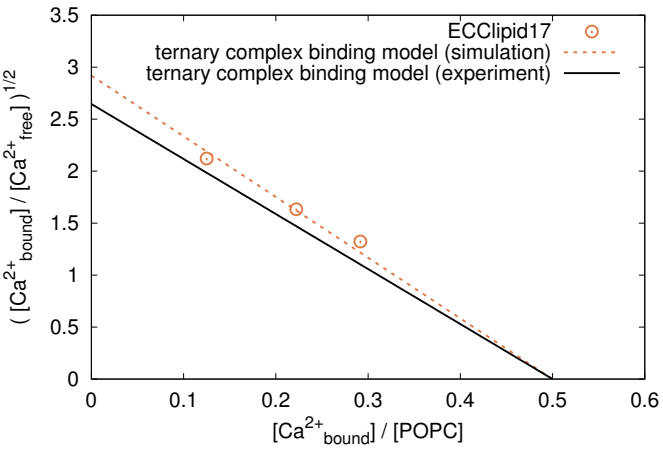

FIG. 4: Binding isotherm assuming stoichiometry of 2 POPC:1 Ca^{2+} as used in [**?**] fits the simulation data nicely.

discussion with actual insights.

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

Acknowledgments

SUPPLEMENTARY INFORMATION

[1] I. Leontyev and A. Stuchebrukhov, Phys. Chem. Chem. Phys. **13**, 2613 (2011).

[2] M. Kohagen, P. E. Mason, and P. Jungwirth, J. Phys. Chem. B **118**, 7902 (2014).

[3] M. Kohagen, P. E. Mason, and P. Jungwirth, J. Phys. Chem. B **120**, 1454 (2016).

[4] A. Catte, M. Girych, M. Javanainen, C. Loison, J. Melcr, M. S. Miettinen, L. Monticelli, J. Maatta, V. S. Oganesyan, O. H. S. Ollila, et al., Phys. Chem. Chem. Phys. **18** (2016).

[5] C. J. Dickson, B. D. Madej, . A. Skjevik, R. M. Betz, K. Teigen, I. R. Gould, and R. C. Walker, J. Chem. Theory Comput. **10**, 865 (2014).

[6] A. Botan, F. Favela-Rosales, P. F. J. Fuchs, M. Javanainen, M. Kanduč, W. Kulig, A. Lamberg, C. Loison, A. Lyubartsev, M. S. Miettinen, et al., J. Phys. Chem. B **119**, 15075 (2015).

[7] A. Maciejewski, M. Pasenkiewicz-Gierula, O. Cramariuc, I. Vattulainen, and T. Rog, J. Phys. Chem. B **118**, 4571 (2014).

[8] O. S. Ollila and G. Pabst, *Atomistic resolution structure and dynamics of lipid bilayers in simulations and experiments* (2016), in Press, URL `http://dx.doi.org/10.1016/j.bbamem.2016.01.019`.

[9] J. Seelig, P. M. MacDonald, and P. G. Scherer, Biochemistry **26**, 7535 (1987).

[10] H. I. Petrache, S. Tristram-Nagle, D. Harries, N. Kucerka, J. F. Nagle, and V. A. Parsegian, J. Lipid Res. **47**, 302 (2006).

[11] N. Kucerka, J. F. Nagle, J. N. Sachs, S. E. Feller, J. Pencer, A. Jackson, and J. Katsaras, Biophys. J. **95**, 2356 (2008), ISSN 0006-3495, URL `http://www.sciencedirect.com/science/article/B94RW-4VB4SVM-S/2/7ede236c4e83d16a6fe57cc3b1894349`.

[12] G. Pabst, N. Kucerka, M.-P. Nieh, M. Rheinstdter, and J. Katsaras, Chem Phys Lipids **163**, 460 (2010), ISSN 0009-3084, URL `http://www.sciencedirect.com/science/article/B6T2N-4YRHCWP-1/2/539e61bf10683661bd62b21d109fcb9a`.

[13] T. M. Ferreira, F. Coreta-Gomes, O. H. S. Ollila, M. J. Moreno, W. L. C. Vaz, and D. Topgaard, Phys. Chem. Chem. Phys. **15**, 1976 (2013).

[14] H. Akutsu and J. Seelig, Biochemistry **20**, 7366 (1981).

[15] C. Altenbach and J. Seelig, Biochemistry **23**, 3913 (1984).

[16] P. G. Scherer and J. Seelig, Biochemistry **28**, 7720 (1989).

[17] M. Girych and O. H. S. Ollila, *Popc_amber_lipid14_verlet* (2015), URL `http://dx.doi.org/10.5281/zenodo.30898`.

[18] M. Girych and O. H. S. Ollila, *Popc_amber_lipid14_cacl2_035mol* (2015), URL `http://dx.doi.org/10.5281/zenodo.34415`.

[19] M. Girych and O. H. S. Ollila, *Popc_amber_lipid14_cacl2_1mol* (2015), URL `http://dx.doi.org/10.5281/zenodo.35074`.

[20] D. E. Smith and L. X. Dang, J. Chem. Phys **100** (1994).

[21] T.-M. Chang and L. X. Dang, J. Phys. Chem. B **103**, 4714 (1999), ISSN 1520-6106, URL `http://dx.doi.org/10.1021/jp982079o`.

[22] L. X. Dang, G. K. Schenter, V.-A. Glezakou, and J. L. Fulton, J. Phys. Chem. B **110**, 23644 (2006), ISSN 1520-6106, URL `http://dx.doi.org/10.1021/jp064661f`.

[23] C. Altenbach and J. Seelig, Biochim. Biophys. Acta **818**, 410 (1985).

ToDo