NMRlipids IV: Headgroup & glycerol backbone structures, and cation binding in bilayers with PE and PG lipids

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Abstract

INTRODUCTION

PE and PG lipids are most common lipids in bacteria [1]. Zwitterionic PE is the second most abundant glycerophospholipid in eukaryotic cells and has been related to the diseases [2–4]. Anionic PG lipids are less abundant, but is also proposed to be fundamental for terrestrial life [5]. PE and PG affect membrane protein functionality [6] and bind to various proteins [7]. PE headgroup is also prone for negative membrane curvature and causes membrane fusion [3, 8]. Therefore, the PE and PG headgroup structures play probably essential roles in many biological processes.

Structural details of lipid headgroups are mainly studied using NMR experiments, which suggest that the glycerol backbone structures are largely similar irrespectively of the headroup [9], glycerol backbone and headgroup structure and behaviour are similar in model membranes and in bacteria [9–11], and the headgroup structures are similar in PC, PE and PG lipids, while headgroup is more rigid in PS lipids [12, 13]. Some attempts to resolve conformational ensembles from NMR for PC and PE lipids have been made, but lesser extend for PG or PS lipids [14–16]. Classical molecular dynamics simulations could potentially give such ensembles and therefore enable the detailed studies of lipid headgroup behaviour in complex biomolecular systems, but current force fields are not accurate enough to reproduce the correct conformational ensembles for PC and PS headgroups [17, 18]. Several MD simulations of PE and PG lipids have been published especially in the context of modeling inner membrane of Gram-negative bacteria [19–31] **1.There may be some relevant publication missing from here**, but evaluation of glycerol backbone and headgroup structures against experiments is rare [25].

Besides the structure, also ion binding may regulate biophysical activity of especially negatively charged lipid headgroups [11]. Monovalent cation (except Lithium) binding to zwitterionic PC and anionic PS headgroups is very weak, while multivalent ion binding is stronger but still weak [18, 32–35]. The ion binding affinity data for PE is more scarce [36], but large differences to PC would be surprising. Negatively charged lipids are suggested to bear same cation binding constants than zwitterionic lipids, but the amount of bound ions to negatively charged membranes would still be larger because the concentration of cations in the vicinity of membranes would be higher [11]. On the other hand, anionic PS lipids are proposed chelate with calcium ions [37–39]. In simulations, the cation binding affinity to PC and PS membranes is typically overestimated [18, 35], which can be improved by applying the ECC to the partial charges of the force fields [40, 41].

Here, we use open collaboration and order parameters of glycerol backbone and headgroup to evaluate the accuracy of PE and PG heagroup structures, and the cation binding affinity to anionic membranes containing PG lipids in the current MD simulation force fields. The force field giving the best description for glycerol backbone and headgroup structures of PC, PS, PG and PE headgroups (CHARMM36) reproduces the essential differences in order parameters between these headgroups, and therefore enables the analysis of structural differences between the headgroups.

METHODS

Experimental C–H bond order parameters

The headgroup and glycerol backbone C–H bond order parameter magnitudes and signs of POPE and POPG were determined by measuring the chemical-shift resolved dipolar splittings with a R-type Proton Detected Local Field (R-PDLF) experiment [42] and S-DROSS experiments [43] using natural abundance ^{13}C solid state NMR spectroscopy as described previously [44, 45]. POPE and POPG powder were purchased from Avanti polar lipids. The NMR experiments were identical to our previous work [18]. **2.Is this enough and correct, or should we repeat some methods from the NMRlipidsIVps paper?** The POPE experiments were recorded at 310 K and POPG experiments at 298 K, where the bilayers are in the liquid disordered phase [46].

Absolute values of the headgroup and glycerol backbone order parameters from PE and PG lipids are measured previously using ^{2}H NMR [9, 12, 133, 134]. Because also the order parameter signs bear essential information about the lipid structures [17, 135], we measured the magnitudes and signs of POPE and POPG C–H bond headgroup and glycerol backbone order parameter in liquid phase using the 2D-RPDLF and S-DROSS experiments, as described previously [18, 44, 45]. For POPE, the glycerol backbone and α-carbon peaks in INEPT spectra were assigned based on previously measured POPC spectra [44] and the β-carbon peak was assigned based on ^{13}C chemical shift table for amines available at `https://www.chem.wisc.edu/areas/reich/nmr/c13-data/cdata.htm` (Fig. S3). For POPG, the glycerol backbone peaks in INEPT spectra were assigned based on previously measured POPC spectra [44], while α and γ-carbon peaks **3.How were these assigned?** (Fig. S4). The numerical value of the β-carbon order parameter could not be determined, because its peak overlapped with the g_2 peak from glycerol backbone in POPG. However, the order parameter of β-carbon is expected to be clearly smaller than for g_2 based on previous ^{2}H NMR measurements [9, 12, 134]. Therefore, the beginning of the S-DROSS curve gives the sign for g_2 order parameter and end for β (Fig. S4 (E)). This is confirmed with SIMPSON calculations using negative value for g_2 and positive value for β order parameter (Fig. S5). **4.Details to be checked by Tiago.**

Molecular dynamics simulations

Molecular dynamics simulation data were collected using the Open Collaboration method [17], with the NMRlipids Project blog (`nmrlipids.blogspot.fi`) and GitHub repository (`github.com/NMRlipids/NMRlipidsIVotherHGs`) as the communication platforms. The simulated systems of pure PE and PG bilayers without additional ions are listed in Tables S1 and S2, and lipid mixtures with additional ions in Table S4. Further simulation details are given in the SI, and the simulation data are indexed in a searchable database available at `www.nmrlipids.fi`, and in the NMRlipids/MATCH repository (`github.com/NMRlipids/MATCH`).

The C–H bond order parameters were calculated directly from the carbon and hydrogen positions using the definition

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

where θ is the angle between the C–H bond and the membrane normal (taken to align with z, with bilayer periodicity in the xy-plane). Angular brackets denote average over all sampled configurations. The order parameters were first calculated averaging over time separately for each lipid in the system. The average and the standard error of the mean were then calculated over different lipids. Python programs that use the MDAnalysis library [47, 48] used for all atom simulations is available in Ref. 49 (`scripts/calcOrderParameters.py`). For united atom simulations, the trajectories with hydrogens having ideal geometry were constructed first using either buildH program [50] or (`scratch/opAAUA_prod.py`) in Ref. 49, and the order parameters were then calculated from these trajectories. This approach has been tested against trajectories with explicit hydrogens and the deviations in order parameters are small [50, 51].

5.BuildH program is now cited with a direct link to the GitHub repo. I think that a release to Zenodo would be nice in the final publication.

6.Maybe we should also shortly discuss here about the reasons for slight dependence of order parameter values on the method used to reconstruct hydrogens?

The ion number density profiles were calculated using the `gmx density` tool of the Gromacs sofware package [52].

Analysis of molecular dynamics simulation data

The big data set of MD simulations was analysed in the NMRlipids databank manner. Unique naming convention for lipid atoms in each force field was defined using the mapping files and analysis for all simulations indexed in NMRlipids databank manner were performed using python codes.

Analysis of lipid conformations bound to proteins

Dihedral angles of all available conformations in the PDB databank were calculated using the API access to the databank.

RESULTS AND DISCUSSION

Conformational ensembles of different lipid headgroups in bulk bilayer

The conformational ensembles of different lipid headgroups and their order parameters have been discussed previously [**?**], but these studies have been inconclusive because order parameter signs have been unknown and tools to interpret the conformational ensembles have not been available. Our experimental order parameters witht the information of the sign from different lipid headgroups combined with the literature data are shown in Fig. 1. The glycerol backbone order parameters are similar for all the lipids, although they move slightly toward positive values (closer to zero) in the order PC $<$ PE $<$ PS $<$ PG. While headgroup order parameters of PC and PE lipids are rather similar, PG and PS lipids exhibit distinct values compared with other lipids. In PS lipids, the α-carbon order parameter exhibits significant forking and the β-carbon order parameter is more negative than others. The α-carbon order parameter of PG is similar to PE and PG, while the positive value of β-carbon is distinct from the other lipids. Notably, this difference was not observed in previous ^{2}H NMR experiments, because absolute value of β-carbon order parameter is similar in PG, PE and PC lipids and the order parameter signs were not measured [9, 12, 134].

In conclusion, the order parameter experiments suggest that the glycerol backbone conformations in all lipids and the headgroup conformations in PC and PE lipids are relatively similar, while PS and PG headgropus exhibit distinct conformational sampling. The details of sampled conformation are difficult to deduce from order parameters only, but the distinct headgroup order parameters of PS lipids are previously related to the more rigid structure of the headgroup [13, 18, 136].

Headgroup and glycerol backbone order parameters of PE and PG lipids show wide variation between different force fields and none of the force fields reproduce all values within experimental error bars. (Figs. S1 and S2), as observed previously also for PC and PS lipids [17, 18]. The poor performance of headgroup order parameters in Berger model can be probably explained by ring like structures seen in Fig. 6 in Ref. 137, which is a typical feature for Berger based lipid force fields containing explicit hydrogen atoms in the head group [24, 25, 138]. The poor performance of glycerol backbone of Slipids simulations is systemically observed also for other lipids in previous studies [17, 18]. **8.Should we comment more the relative quality of different force fields and/or make the subjective force field ranking figures? https://github.com/NMRLipids/NMRlipidsIVPEandPG/issues/8**

Without further discussion about poorly performing force fields, we focus on more detailed analysis of CHARMM36 simulations, which captures the essential differences between PC, PS, PG and PE headroup order parameters (Fig. 5) with the exception of β-carbon order parameter of PC which is too negative when compared with PS or PE order parameter, or with experiments [17]. Characteristic dihedral conformations in PS headgroup are asymmetric conformations prefer-

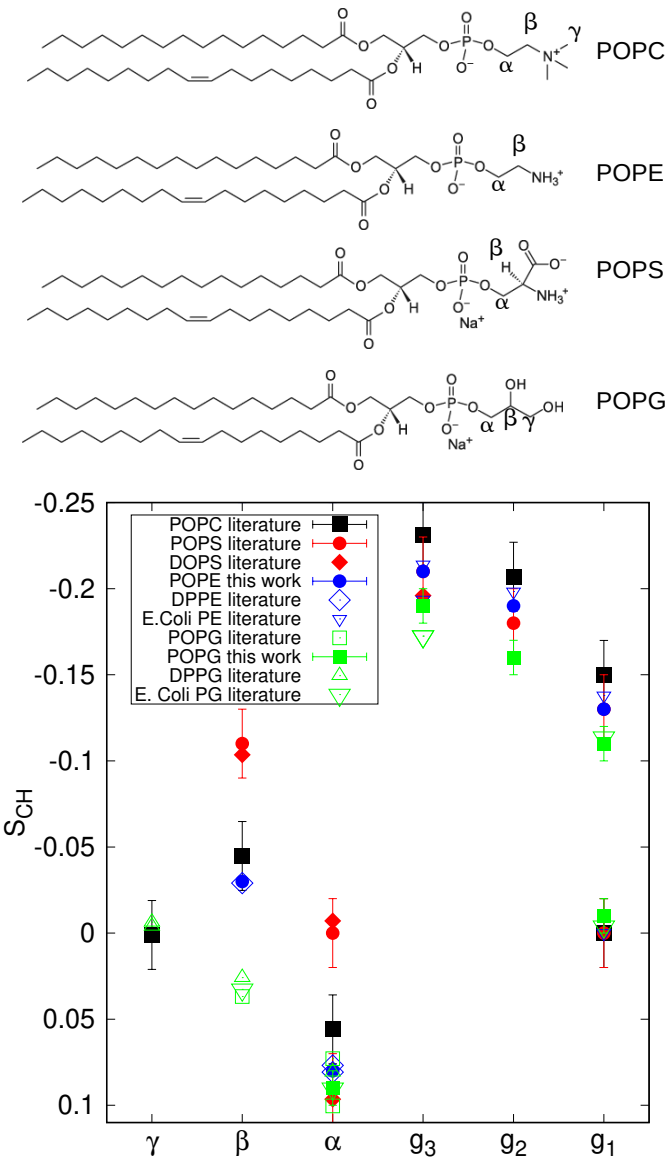

FIG. 1: (top) Chemical structure of different lipids. (bottom) Headgroup and glycerol backbone order parameters from different experiments in lamellar liquid disordered phase. The values and signs for POPE (310 K) and POPG (298 K) measured in this work, and for POPS (298 K) [18] and POPC (300 K) [44, 45] previously using ^{13}C NMR. The literature values for DOPS with 0.1M of NaCl (303 K) [136], POPG with 10nM PIPES (298 K) [134], DPPG with 10mM PIPES and 100mM NaCl (314 K) [12], DPPE (341 K) [133], E.coliPE and E.coliPG (310 K) [9] are measured using ^{2}H NMR. The signs from ^{13}C NMR are used also for the literature values.

7.The bottom figure could be clarified as Fig. 2 in the NMRlipids IVps paper.

ring gauche 270° conformations in N-C_β-C_α-O_α and C_β-C_α-O_α-P dihedrals. In PG headgroup, the O_β-C_β-C_α-O_α dihedral (corresponding N-C_β-C_α-O_α dihedral in other lipids) is mostly in trans conformation, and C_β-C_α-O_α-P has asymmetric tendency to be in gauche 60° conformation. Main difference between PC and PE is the lower probability of trans state

in C_β-C_α-O_α-P PC dihedral, which could be a potential reason for the too negative β headgroup order parameter in PC.

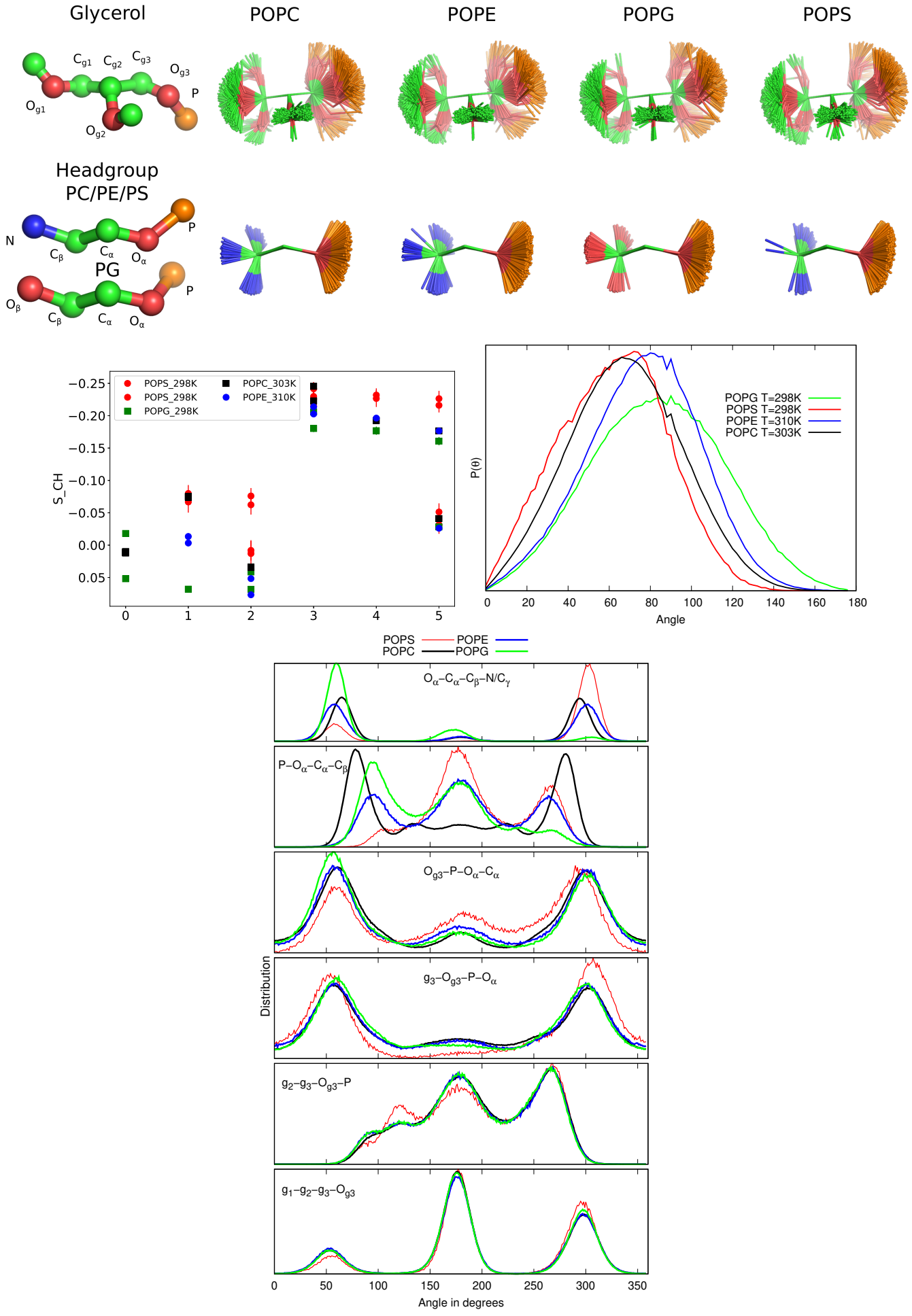

FIG. 2: Overlayed snapshots and dihedral angle distributions from CHARMM36 simulations of different lipids which give the best agreement with experiments.

9.The differences observed in dihedral distributions are not visible in the snapshot figures.

10.More detailed discussion of this figure is in https://github.com/NMRLipids/NMRlipidsIVPEandPG/issues/9

Lipid conformational ensembles in lipid bilayers with bound ions

To evaluate the calcium binding in simulations of lipid bilayers containing PG lipids, we calculated the changes in headgroup order parameters of POPC:POPG (1:1) and (4:1) mixtures upon addition of $CaCl_2$, and compared these with the available experimental data [134, 139]. The headgroup order parameters of PC lipids can be used to measure the ion binding affinity to lipid bilayers because their magnitude is linearly proportional to the amount of bound charge in bilayer [35, 140]. This molecular electrometer concept can be used also for bilayers containing PC lipids mixed with charged lipids [18, 34, 134, 139]. The headgroup order parameters can be used to evaluate MD simulations against experimental data, because they can be directly calculated from MD simulations [35].

The decrease of POPC headgroup order parameters in mixtures with POPG lipids with increasing $CaCl_2$ concentration is overestimated in Slipids and Lipid17 simulations (Figs. 3 and S8) indicating too strong binding affinity of calcium into the bilayers as previously observed for pure PC lipid bilayers and mixtures with PS lipids [18, 35]. **11.CHARMM results to be mentioned once we have the new simulations.** The calcium binding affinity to lipid bilayers with PC and PS lipids was recently improved by applying the electronic continuum correction (ECC) to Amber Lipid14/17 force fields [40**?**]. In this approach, the electronic polarizability is implicitly included in the classical force fields by scaling the charges with constant factors [145]. Here, we make a ECC-POPG force field by applying the scaling factors originally used for POPS also to POPG, i.e., we multiply charges and Lennard-Jones σs of headgroup, glycerol backbone, and carbonyl regions with f_q=0.75 and f_σ=0.89, respectively [**?**]. ECC-POPG model gives a weaker calcium binding affinity (Fig.4) and better agreement with the experimental PC headgroup order parameter changes (Fig. 3) for POPC:POPG mixtures than the original Lipid17 model **12.to be finished when we have all the data**, indicating that the ECC improves the simulation predictions of calcium binding affinity as previously observed for PC and PS lipids [40**?**].

Experimental data for the β-carbon order parameter of POPG shows a rapid decrease with increasing $CaCl_2$ concentrations up to 10 mM and more modest decrease with larger concentrations (Fig. 3) [134]. This behaviour is similar to that of β-carbon order parameters of POPC, but essentially different than observed for POPS, where β-carbon order parameters increases with addition of calcium [18]. Experimentally measured changes of PG α-carbon order parameters upon addition of calcium are not available. Lipid17 and Slipids force fields correctly capture the PG β-carbon order parameter response to $CaCl_2$ even thought the binding affinity was too large based on the comparison of PC headgroup order parameter changes with experiments. While applying ECC to Lipid17 improved the PC headgroup order parameter response and binding affinity, the response of PG β-carbon order parameter to calcium is too weak in this model. The response of PG α-carbon order parameters to $CaCl_2$ differs between force fields, but experimental data to evaluate these predictions is not available.

13.To be finished once we have the new CHARMM simulations and conformational changes of PG analyzed.

14.We still need more data to finish the discussion. More detailed discussion is in https://github.com/NMRLipids/NMRlipidsIVPEandPG/issues/12

Protein bound lipid conformations

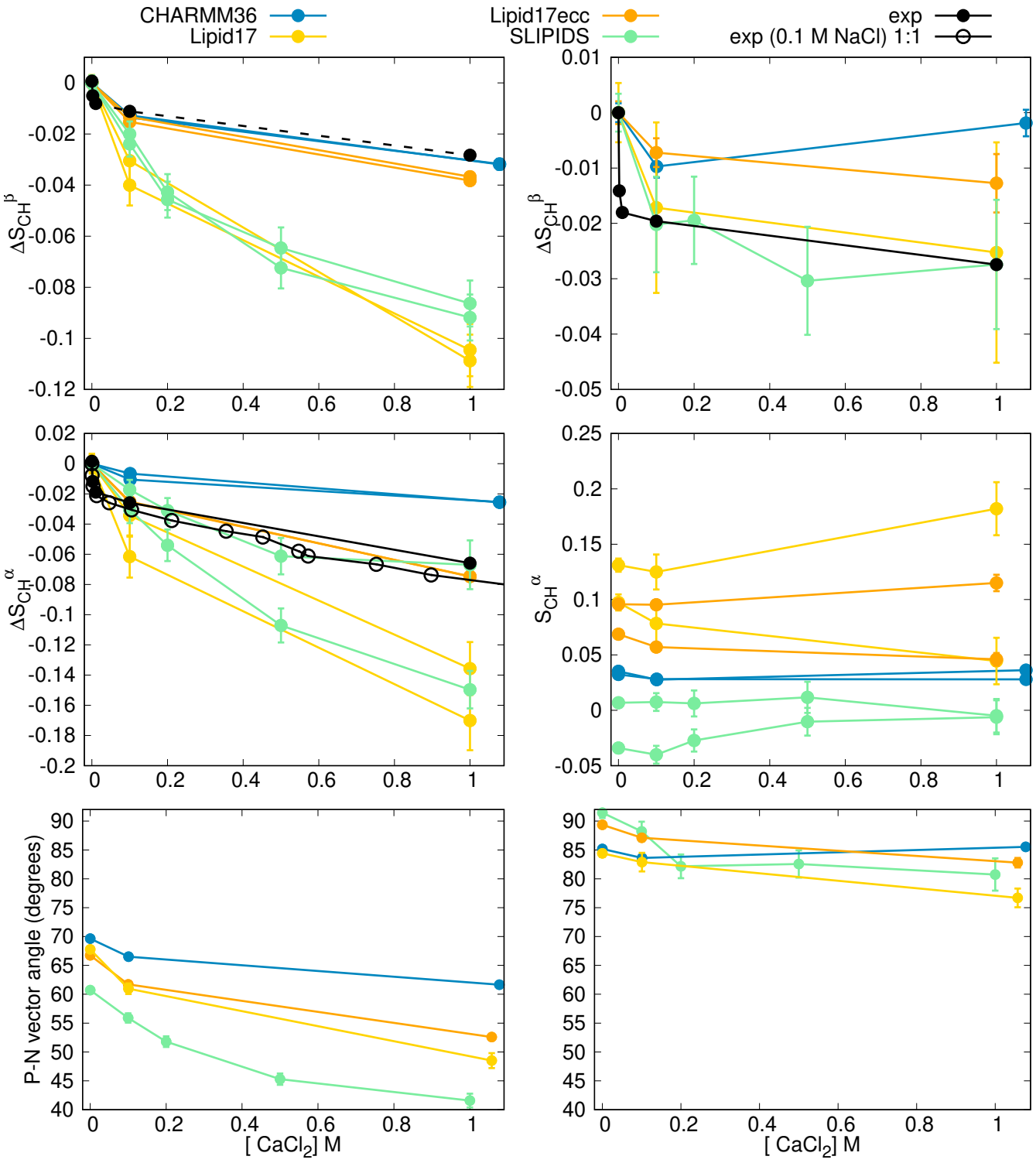

FIG. 3: Modulation of headgroup order parameters of POPC (*left*) and POPG (*right*) in POPC:POPG (1:1) mixture upon addition of $CaCl_2$ in 298 K temperature from experiments [134, 139] and simulations. The β-carbon order parameter of POPC (dashed line on top left) is not directly measured but calculated from empirical relation $\Delta S_\beta = 0.43\Delta S_\alpha$ [144]. The changes with respect to the systems without $CaCl_2$ are shown for other data than for the α-carbon of POPG for which experimental order parameter is not available.

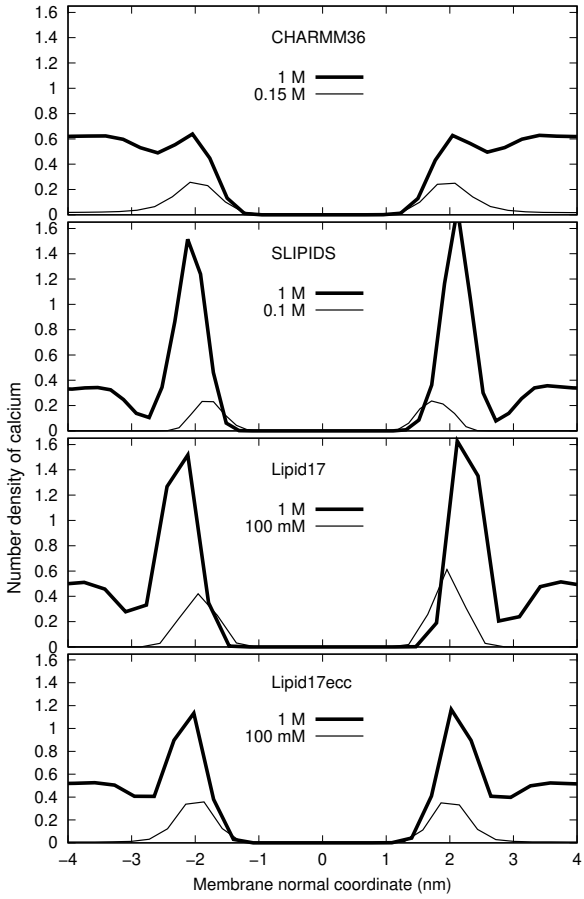

FIG. 4: Calcium ion density profiles along membrane normal from simulations of POPC:POPG (1:1) mixtures with different force fields.

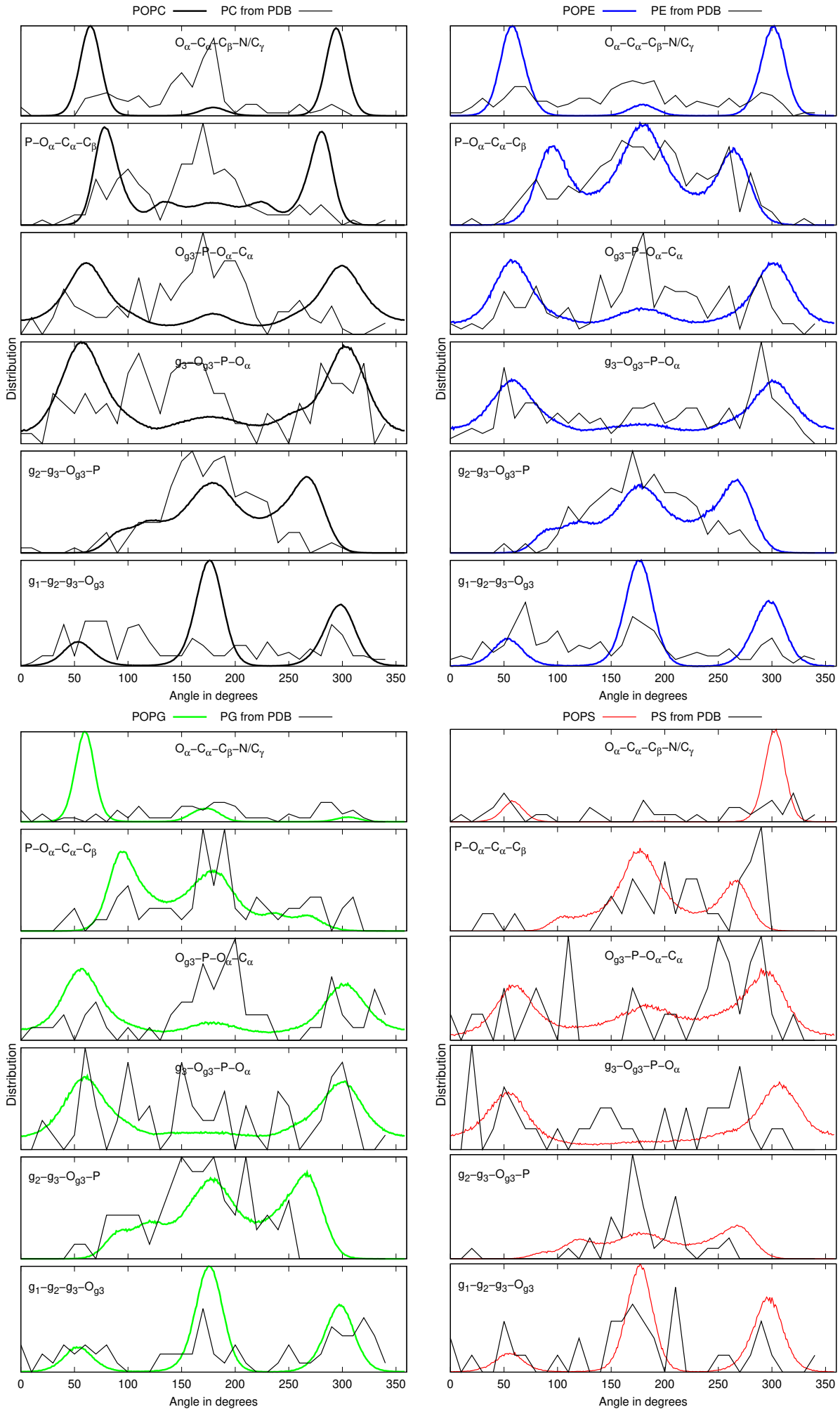

FIG. 5: Dihedral distributions from simulations and lipid structures in PDB.

CONCLUSIONS

AP is grateful to the Centro de Supercomputacin de Galicia (CESGA) for use of the Finis Terrae computer

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