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

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(Dated: April 17, 2019)

Primarily measured but also simulated NMR order parameters will be collected also for other than phophatidylcholine (these are discussed in NMRlipids I) headgroup. The information will be used to understand structural differences between different lipid molecules in bilayers.

INTRODUCTION

In NMRlipids I and II project we were looking for a MD model which would correctly reproduce headgroup and glycerol backbone structures and cation binding for PC lipid bilayers [1, 2]. Here we extend the same goal for other than PC lipids. Currently the focus is on PE, PG and PS bilayers and their mixtures with PC. Experimental data with different amounts of added salt is now collected and presented in this manuscript.

Absolute values of experimental order parameters for different lipid headgroups are collected in Fig. 1. Signs are measured only for PC as far as I know, thus only absolute values are used for now.

Based on superficial reading, the conclusions in the literature are roughly
1) glycerol backbone structures are largely similar irrespectively of the headroup [8],
2) glycerol backbone and headgroup structure and behaviour are similar in model membranes and in bacteria [8–10],
3) headgroup structures are similar in PC, PE and PG lipids, while headgroup is more rigid in PS lipids [6, 11].
Extensive discussion about structural details of PE, PG or PS headgroups do not exists (as far as I know), In contrast to PC lipids (see [1] and references therein).

Several simulations containing PE, PG and PS lipids have been published [**?**], **1.List should be completed** however, glycerol backbone and headgroup order parameters are not compared to the experiments (based on superficial reading of literature).

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 [**?**] and S-DROSS experiments [**?**] using natural abundance ^{13}C solid state NMR spectroscopy as described previously [3**? ?**]. **2.The rest of the details to be written. I am not sure how much we need to repeat the NMRlipidsIVps paper.**

MOLECULAR DYNAMICS SIMULATIONS

Molecular dynamics simulation data were collected using the Open Collaboration method [1], with the NMRlipids Project blog (`nmrlipids.blogspot.fi`) and GitHub repository (`github.com/NMRlipids/NMRlipidsIVotherHGs`) as the communication platforms. The simulated systems are listed in Table II (pure PE and PG bilayers without additional ions). 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 program (`scripts/calcOrderParameters.py`) that uses the MDAnalysis library [**?** **?**] is available in Ref. **?** . The ion number density profiles were calculated using the `gmx density` tool of the Gromacs sofware package [**?**].

RESULTS AND DISCUSSION

Headgroup and glycerol backbone order parameters of POPE and POPG from ^{13}C NMR

The glycerol backbone and α-carbon peaks in INEPT spectra of POPE were assigned based on previously measured POPC spectra (Fig. **??**) [3]. 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`. The order parameters for the glycerol backbone and headgroup C–H bonds were determined from 2D-RPDLF and S-DROSS experiments

TABLE I: List of single lipid type MD simulations without additional ions.

lipid/counter-ions	force field for lipids / ions	[a]N_l	[b]N_w	[c]T (K)	[d]t_{sim}(ns)	[e]t_{anal} (ns)	[f]files
DPPE	Slipids [19]	288	9386	336	200	100	[20]
DPPE	GROMOS-CKP [**?**]	128	?	342	2×500	2×400	[**?**] 3.
POPE	GROMOS 43A1-S3 [**?**]	128	?	313	2×200	2×100	[**?**] 4.
POPE	CHARMM36ua [**?**]	336	?	310	2×200	2×100	[**?**] 5.
POPE	CHARMM36 [**?**]	144	5760	310	500	400	[**?**]
POPE	Slipids [19**?**]	336	?	310	2×200	2×100	[**?**]
POPE	OPLS-UA vdW on H [**?**]	128	?	303	2×200	2×100	[**?**] 6.
POPE	OPLS-UA [**?**]	128	?	303	2×200	2×100	[**?**] 7.
POPE	Berger-based [**?**]	128	?	303	2×200	2×100	[**?**] 8.
POPE	Berger-based2 [**?**]	128	?	303	2×200	2×100	[**?**] 9.
POPE	GROMOS-CKP [**?**]	128	?	313	2×500	2×400	[**?**] 10.
DOPE	Berger-based [**?**]	128	?	271	2×200	2×100	[**?**] 11.
DOPE	Berger-based2 [**?**]	128	?	271	2×300	2×100	[**?**] 12.
DOPE	GROMOS-CKP [**?**]	128	?	271	2×500	2×400	[**?**] 13.
POPG/K^+	CHARMM36 [**?**] 14.	118	4110	298	100	100	[**?**]
POPG/Na^+	Slipids [21]	288	10664	298	250	100	[25]
DPPG/Na^+	Slipids [21]	288	11232	314	200	100	[26]
DPPG/Na^+	Slipids [21]	288	11232	298	400	100	[27]

[a]Number of lipid molecules with largest mole fraction
[b]Number of water molecules
[c]Simulation temperature
[d]Total simulation time
[e]Time used for analysis
[f]Reference for simulation files

(Fig. **??**), as described previously [**?**]. The POPE experiments were recorded at 310 K, where the bilayer is in liquid disordered phase [**?**]. **33.To be checked by Tiago.**

34.Figure and discussion about POPG experiments to be addded.

The headgroup and glycerol backbone order parameters of PE lipids are similar with different acyl chains and also close the values for POPC, althought PE gives systematically slightly more positive values (Fig. 1). These could be explained with slightly larger temperature in PE measurements, except for the α-carbon with the positive sign, for which the more positive value is farther away from zero. For PG lipids, the glycerol backbone order parameters are more positive than for other lipids. The headgroup α-carbon gives value close to PE, while the value of β-carbon is distinct from other lipid being only one which has positive sign, suggesting distinct conformation of PG lipids in this region. This was not observed in previous ^{2}H NMR study, where sign was not measured and β-carbon order parameter was apparently similar to the value for PE and PC results.

In conclusion, the results suggests that the glycerol backbone conformations in all lipids are relatively similar. Also, the headgroup conformations are similar for PC and PE lipids, while PS and PG are singnificantly different. For PS lipids, the differences are discussed previously [**?**].

PE headgroup and glycerol backbone

Order parameters from Slipids simulations and experiments for DPPE are shown in Fig. 2. Glycerol backbone order parameters in Slipids are off from experiments, as already observed previously for PC lipids [1]. Order parameter signs for PE are not experimentally measured yet. For headgroup the signs are set to give best agreement with simulations and for glycerol to be consistent with experimental signs for PC. Order parameter for β carbon shows apparent agreement with experiments. However, the sign of beta order parameter is positive, in contrast to PC where negative sign was measured. Thus, the the beta order parameter agrees with experiment with the assumption that its sign is opposite than for PC. This is yet to be confirmed by experiments. Order parameter for α carbon is too close to zero, even if the sign would be correct.

36.Berger results are not here yet, but we should mention the ring like structures pointed out by T. Piggot in the blog: The poor performance of headgroup order parameters in Berger model can be probably explained by ring like structures seen in Fig. 6 in Ref. 23. These ring like structures are a widespread feature of typical Berger based lipid force fields containing explicit hydrogen atoms in the head group [? ? ?].

TABLE II: List of MD simulations with lipid mixtures and additional ions.

lipid/counter-ions	force field for lipids / ions	NaCl (M)	$CaCl_2$ (M)	[a]N_l	[b]N_w	[c]N_c	[d]T (K)	[e]t_{sim}(ns)	[f]t_{anal} (ns)	[g]files
POPC	CHARMM36 [**?**]	0.11	0	500	25000	48	310	500	100	[**?**]
POPC:POPG (7:3)	CHARMM36 [**?**]	0.11	0	350	?	?	310	500	100	[**?**]
POPC:POPG (1:1)/K^+	CHARMM36 [**?**]	0	0	?	?	?	?	?	?	[**?**] 15.
POPC:POPG (1:1)/K^+	CHARMM36 [**?**]	0	0.15 16.	?	?	?	?	?	?	[**?**] 17.
POPC:POPG (1:1)/K^+	CHARMM36 [**?**]	0	1.0 18.	?	?	?	?	?	?	[**?**] 19.
POPC:POPG (4:1)/K^+	CHARMM36 [**?**]	0	0	?	?	?	?	?	?	[**?**] 20.
POPC:POPG (4:1)/K^+	CHARMM36 [**?**]	0	0.15 21.	?	?	?	?	?	?	[**?**] 22.
POPC:POPG (4:1)/K^+	CHARMM36 [**?**]	0	1.0 23.	?	?	?	?	?	?	[**?**] 24.
POPC	CHARMM36 [**?**]	0	0	256	8704	0	300	300	250	[**?**]
POPC:POPE (1:1)	CHARMM36 [**?**]	0	0	128	8704	0	300	300	250	[**?**]
POPC	Slipid [**?**]	0.11	0	500	25000	48	310	500	100	[**?**]
POPC:POPG (7:3)	Slipid [**?**]	?	0	?	?	?	310	500	100	[**?**] 25.
POPC	Berger [**?**] 26.	0	0	256	10240	0	300	300	200	[**?**]
POPC:POPE (1:1)	Berger [**?**] 27.	0	0	128	11008	0	300	300	200	[**?**]
POPC:DOPE (1:1)	Berger [**?**] 28.	0	0	128	10240	0	300	300	200	[**?**]
DOPC	Berger [**?**] 29.	0	0	256	11008	0	300	300	200	[**?**]
DOPC:DOPE (1:1)	Berger [**?**] 30.	0	0	128	11008	0	300	300	200	[**?**]
POPG	Slipids [**?**] 31.	0.11	0	500	25000	49	310	500	100	[**?**]
POPG	CHARMM36 [**?**]	0.11	0	500	25000	49	310	500	100	[**?**]
POPE	Slipids [**?**] 32.	0.11	0	500	25000	50	310	500	100	[**?**]
POPE	CHARMM36 [**?**]	0.11	0	500	25000	50	310	500	100	[**?**]

[a]Number of lipid molecules with largest mole fraction
[b]Number of water molecules
[c]Number of additional cations
[d]Simulation temperature
[e]Total simulation time
[f]Time used for analysis
[g]Reference for simulation files

PG headgroup and glycerol backbone

Comparison between experiments and simulations for PG lipids is shown in Fig. 3. The signs are not yet measured experimentally. They are set to give the best argeement with experiments. This would suggest that the β order parameter would be positive, in contrast to PC and PS headgroups, were negative signs were measured. Even thought the signs turned out to be correct, the tested models would not give a very good argeement with the experiments.

Lipid headgroup interactions in PC:PE and PC:PG mixtures

The headgroup order parameters increase with the addition of negatively charged PS and PG lipids, decrease when mixed with positively charged surfactants and are less affected by the addition of zwitterionic PE lipids or cholesterol. In addition to the results summarized in Fig. **??**, also mixtures of PC with negatively charged PI, CL, PA, and zwitterionic SM follow the eletrometer concept [9]. to mixtures of differently charged lipids are collected from different experiments in Fig. **??**. As shown in Fig. **??**, order parameters of PC headgroup behave in various lipid mixtures as expected from the electrometer concept [9, 12], i.e., order parameters increase when anionic lipids are mixed with PC and decrease with cationic surfactants. The changes with the addition of neutral lipids is significantly smaller.

The headgroup order parameters for PC lipids (POPC and DOPC) mixed with PE, PS and PG lipids are shown in Fig. 5 from different simulation model and experiments [9] with different mole fractions. As already discussed previosly, the PC lipid headgroup behaviour follows the electrometer concept in experiments when mixed with other lipids, i.e., the order parameters increase when mixed with negatively charged lipids (PS, PI, CL, PA and PG) remains almost unchaged when mixed with neutral lipids (PE and SM) [9]. This is not the case in simulation data shown in Fig. 5. The addition of DOPE into a POPC and DOPC bilayers significantly decreases the PC headgroup order parameters in simulations with OPLS compatible version of the Berger force field [15] in contrast to experiments [9]. On the other hand, the increase of the

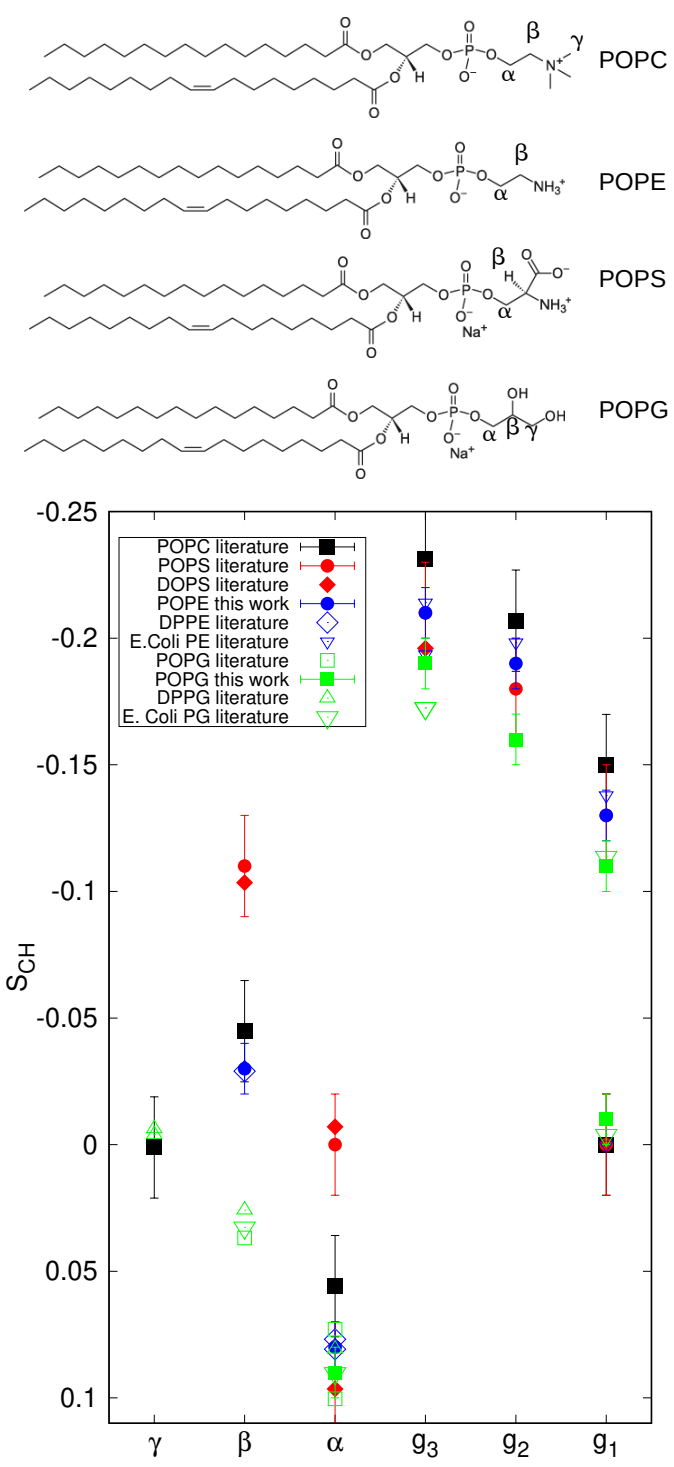

FIG. 1: (top) Chemical structure of different lipids (bottom) Headgroup and glycerol backbone order parameters measured from lipids with different headgroups in lamellar liquid disordered phase. The values and signs for POPE (310 K), POPG (298 K). POPS (298 K) [**?**] and POPC (300 K) [3**?**] are measured using ^{13}C NMR. The literature values for DOPS with 0.1M of NaCl (303 K) [4], POPG with 10nM PIPES (298 K) [5], DPPG with 10mM PIPES and 100mM NaCl (314 K) [6], DPPE (341 K) [7], E.coliPE and E.coliPG (310 K) [8] are measured using ^{2}H NMR. The signs from ^{13}C NMR are used also for the literature values.

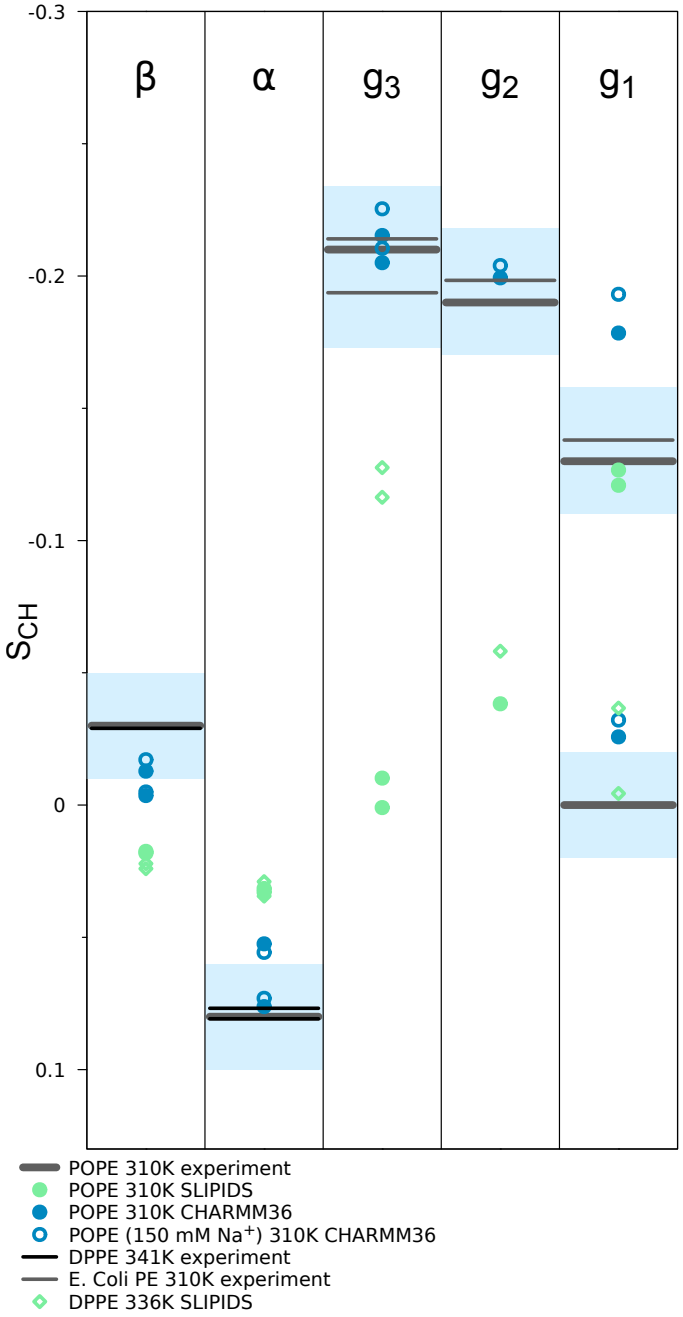

FIG. 2: Order parameters for DPPE headgroup and glycerol backbone from simulations with Slipids [**?**] and experiments (DPPE from [7] and E.coliPE from [8]). Absolute values are shown, because signs are not known experimentally.

35.Results from united atom simulations yet to be added

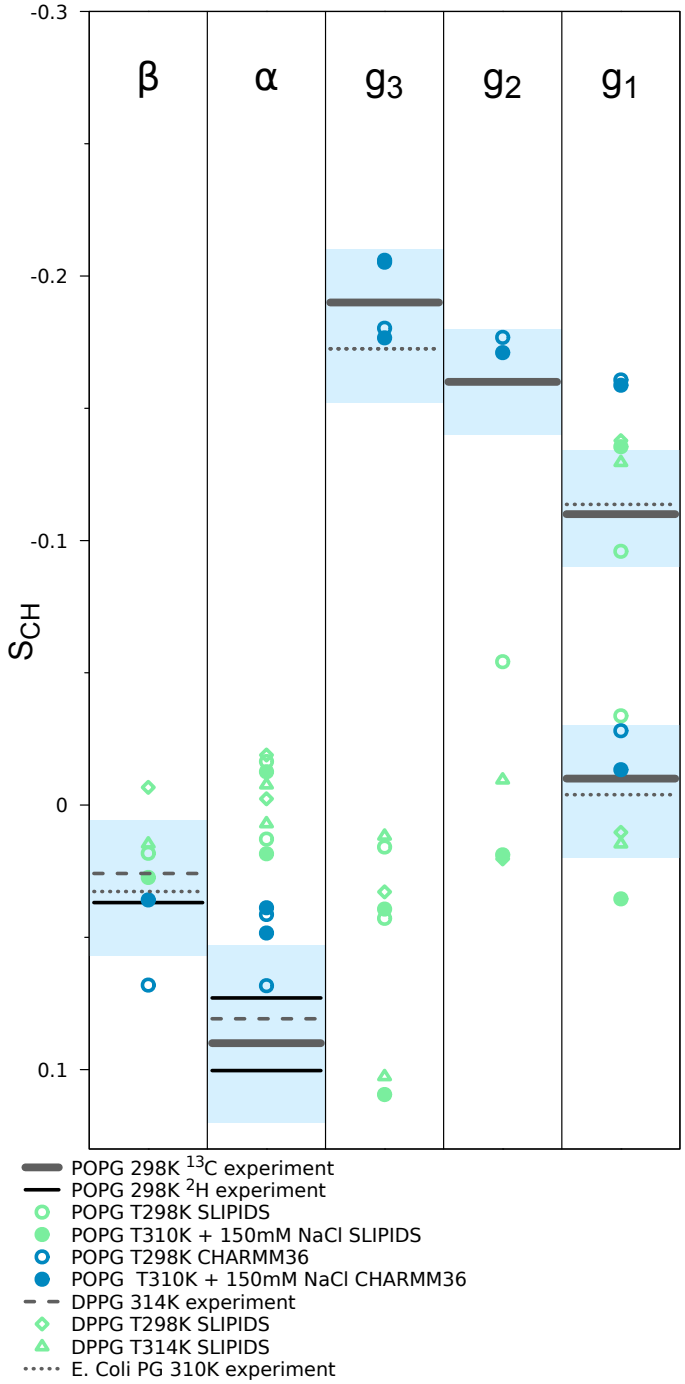

FIG. 3: Order parameters for PG headgroup and glycerol backbone from simulations and experiments without $CaCl_2$ (POPG from [5] contains 10mM of PIPES, DPPG from [6] contains 10mM PIPES and 100mM CaCl, E.Coli PG results from [8]). Signs are not known for experimental data. They are determined to give best agreement with simulations. This is not reliable and should be corrected when experimental data becomes available.

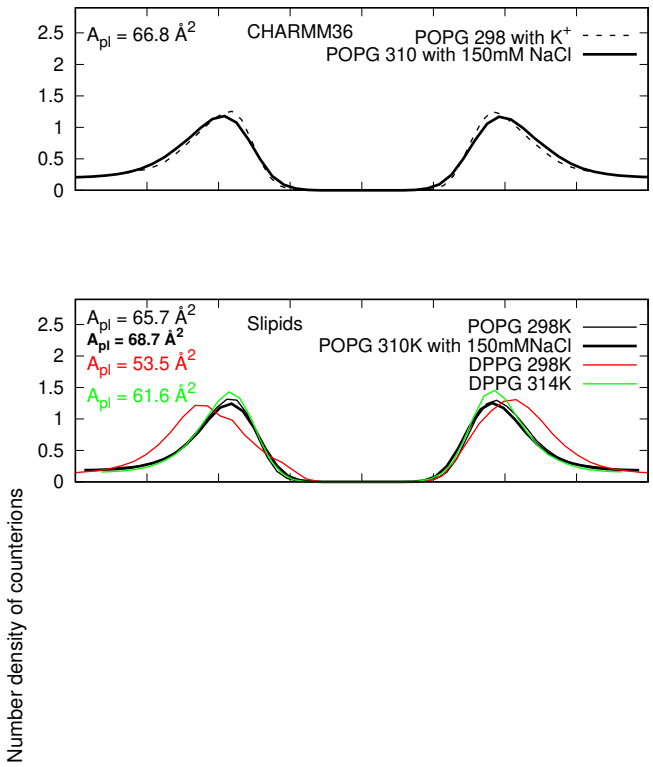

FIG. 4: Counterion densities from simulations with PG lipids.

PC headgroup order parameters in CHARMM36 simulations mixed with PS and PG lipids is significantly smaller than in experiments.

37.This is text by P. Fuchs, copied from the blog. Area results in nm^2, the error is ¡= 0.003 nm^2 - pure POPC CHARMM36: 0.624 Berger : 0.649 - POPC/POPE 50:50 CHARMM36 : POPC 0.609, POPE 0.557 Berger-hacked: POPC 0.637, POPE 0.632 —– One can see that CHARMM 36 predicts a drop in the area on going from pure POPC to POPC/POPE 50:50. This means that POPC pack tightly to POPE. In contrast, the values for Berger are not that changed. The POPE value predicted by CHARMM 36 (in the mixture POPC/POPE 50:50) is much

smaller than that predicted by Berger. **38.The experimental acyl chain order parameters for POPE [?] seem larger than reported for POPC [3], which supports the more condensed PE bilayer. In principle, this is beyond the scope of this work (lipid headgroups), but we can consider mentioning this.**

The headgroup order parameters for PS and PG lipid mixtures with PC having different mole fractions from simulations and experiments [5, 16] are shown in Fig. 6. The effect of increasing amount of PC to PS headgroup seems to qualitatively incorrect in CHARMM36 simulations. The β-carbon order parameter increases in experiment, but decreases in simulations with both tested counterions (Na+ and K+). Larger α-carbon order parameter decreases with the addition of PC in experiment, while the lower remains unchanged. In simulations the larger increases and the lower decreases. Interestingly, the α-carbon order parameters are closer to experiments in pure PS system with K+ counterions than with Na+. The changes in PG headgroup order parameters are minor in simulations, which is in line with the only available experiment for the β-carbon.

Ion binding to PE lipid bilayers

Ca^{2+} binding in bilayers with negatively charged PG and PS lipids

PC lipid headgroup order parameters can used to measure ion binding affinity, because their magnitude is linearly proportional to the amount of bound charge in bilayer [2, 12]. This molecular electrometer concept can be used also for bilayers containing PC lipids mixed with charged lipids [5, 16, 17]. This is demonstrated in Figs **??**, **??** and **??**, showing order parameters for PC headgroup α and β carbons as a function of $CaCl_2$ concentration in the presence of different amounts of negatively charged PS or PG lipids.

PC headgroup order parameters increase when negatively charged PS or PG are added to PC bilayer in the absense of added $CaCl_2$, as expected based on electrometer concept [12] (see Fig. **??**). Further, the order parameters decrease with the addition of $CaCl_2$ and the decrease is more pronounced for systems with more negatively charged lipids (see Fig. **??**). At $CaCl_2$ concentrations ($\sim$ 50-300mM) where order parameters reach the values for pure PC, the Ca2+ binding presumably fully cancels the charge from negative lipids and overcharging occurs above these concenterations. The interpretation of this data and some other results has been that [10]

> *"(i) Ca^{2+} binds to neutral lipids (phosphatidylcholine, phosphatidylethanolamine) and negatively charged lipids (phosphatidylglycerol) with approximately the same binding constant of K = 10-20 M^{-1};*
> *(ii) the free Ca^{2+} concentration at the membrane interface is distinctly enhanced if the membrane carries a negative surface charge, either due to protein or to lipid;*
> *(iii) increased inter-facial Ca^{2+} also means increased amounts of bound Ca^{2+} at neutral and charged lipids;*
> *(iv) the actual binding step can be described by a Langmuir adsorption isotherm with a 1 lipid:1 Ca^{2+} stoichiometry, provided the interfacial concentration C_M, is used to describe the chemical binding equilibrium."*

Comparison of Ca2+ binding in PG between CHARMM36 simulations and experiments [5] is shown in Fig. 8. The decrease of α order parameter is in agreement with experiments, while decerase of β order parameter is overestimated. The result is very similar to the results with PC in NMRlipids II publication [2]. It should be, however, noted that the β-order parameters are not actually measured for PG, but they are calculated from empirical relation $\Delta S_\beta = 0.43 \Delta S_\alpha$ [18]. Anyway, the data presented in NMRlipids II project and in Fig. 8 together suggest that Calcium binding is similarly overestimated by CHARMM36 model in pure POPC bilayers and mixtures with POPG. The good agreement of α carbon would be explained by too weak dependence of its order parameter of bound charge **42.The response of CHARMM36 to cationic surfactant against experiments [13] to be checked. I have already ran the simulations, analysis to be done.**.

Also dependence of β-carbon of PG on $CaCl_2$ concentration is compared with experiments [5] in Fig. **??**. Absolute value of the order parameter is too large without ions, but rapid decrease due to addition of $CaCl_2$ is observed in agreement with experiments for systems with 1:1 mixture of POPC and POPG. In addition, absolute value in systems with $CaCl_2$ is in agreement with experiments. However, system with 4:1 mixture of POPC and POPG behaves differently, but experimental data is not available for comparison for this mixture.

43.More simulation data for systems with negatively charged lipids and $CaCl_2$ to be collected

EFFECT OF CA2+ BINDING ON PG AND PE HEADGROUP

Also the experimental order parameters for PS and PG headgroups as a function of $CaCl_2$ concentration are shown in Fig. **??**.

CONCLUSIONS

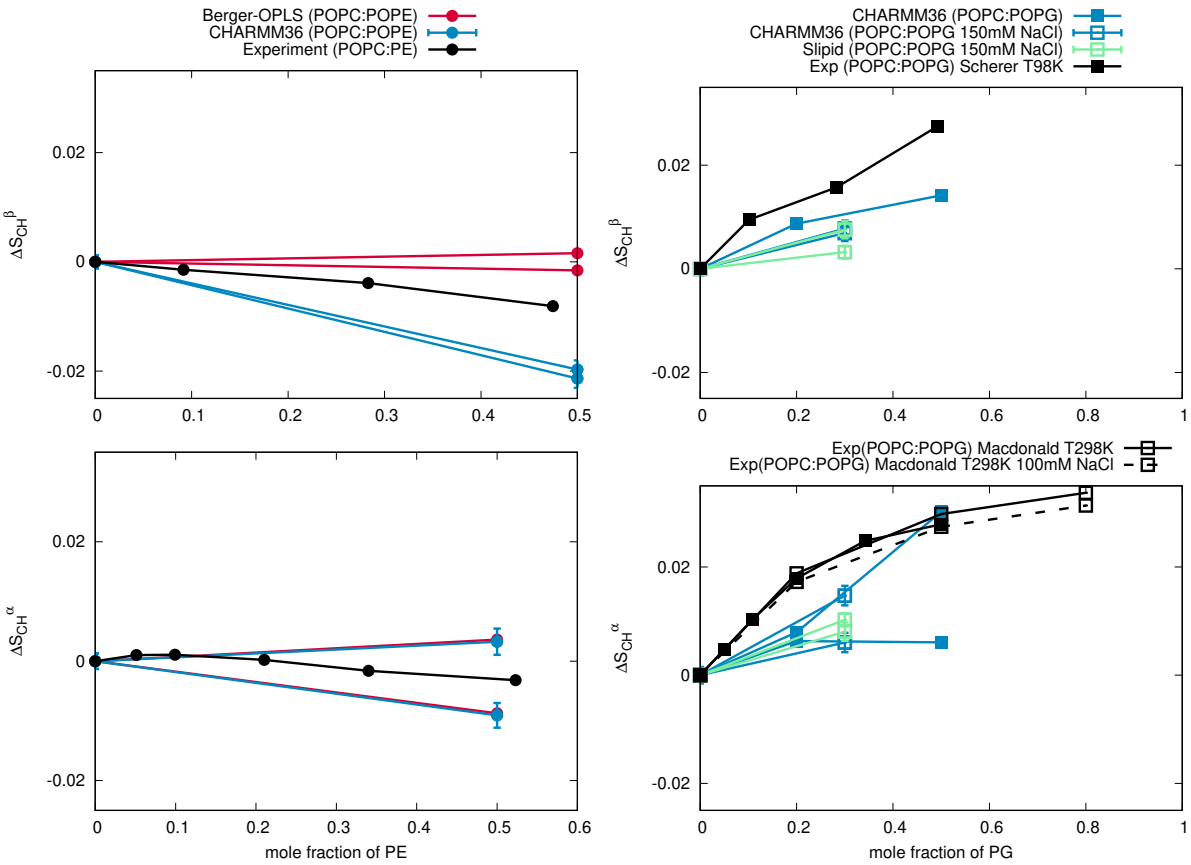

FIG. 5: PC headgroup order parameters from mixtures with PE, PS and PG lipids with various mole fractions from different simulation models and experiments [9]. Signs are determined as discussed in [1, 14].

39.Simulation of CHARMM36 at 298K should be maybe rerun with Gromacs 5.

SUPPLEMENTARY INFORMATION

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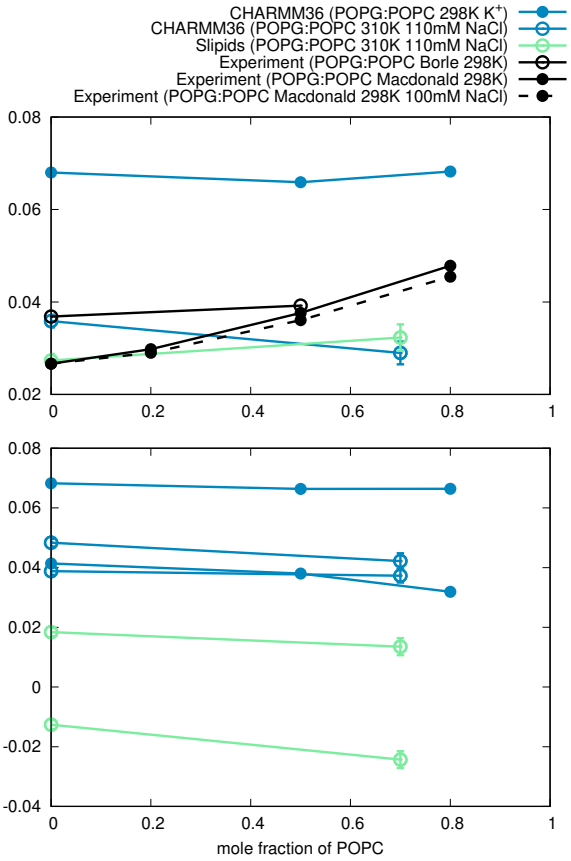

FIG. 6: PS and PG lipid headgroup order parameters from mixtures with PC lipids with various mole fractions from different simulation models and experiments [5, 16]. Signs are not yet known experimentally for PG, thus the signs give by simulations are used. Signs for PS are measures as described in SI.

40.Some simulations contain potassium as counterions, while some sodium. All experiments here contain some amount of sodium salt. The best ion concentrations for comparison should be figured out.

41.Why there is difference between CHARMM36 simulation results from POPS:POPC mixture and pure POPS? Discussion in https://github.com/NMRLipids/NMRlipidsIVotherHGs/issues/1

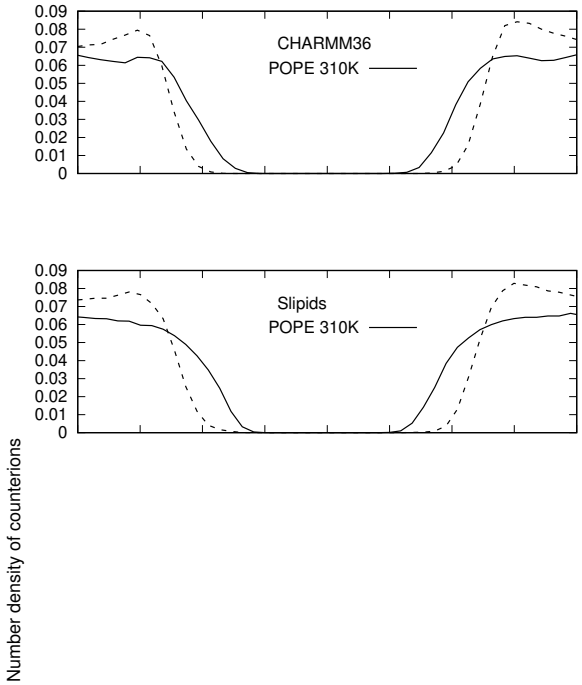

FIG. 7: Sodium (solid line) and choride ion density profiles along membrane normal from different simulations with PE lipids.

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ToDo

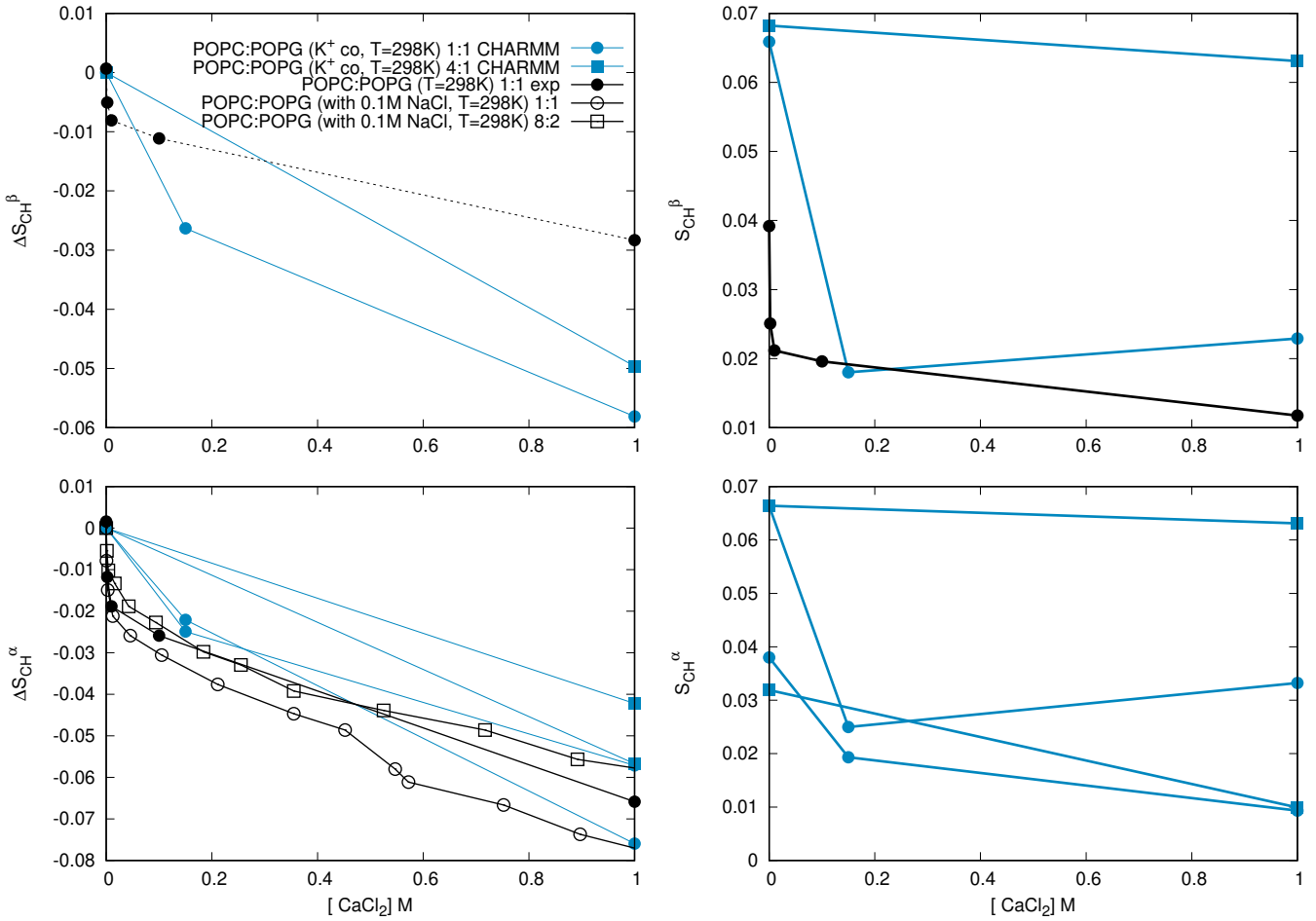

FIG. 8: PG order parameters as a function $CaCl_2$ concentration from experiments [5] and CHARMM36 simulations. Note that beta order parameter is calculated from empirical relation $\Delta S_\beta = 0.43 \Delta S_\alpha$ [18], not actually measured.

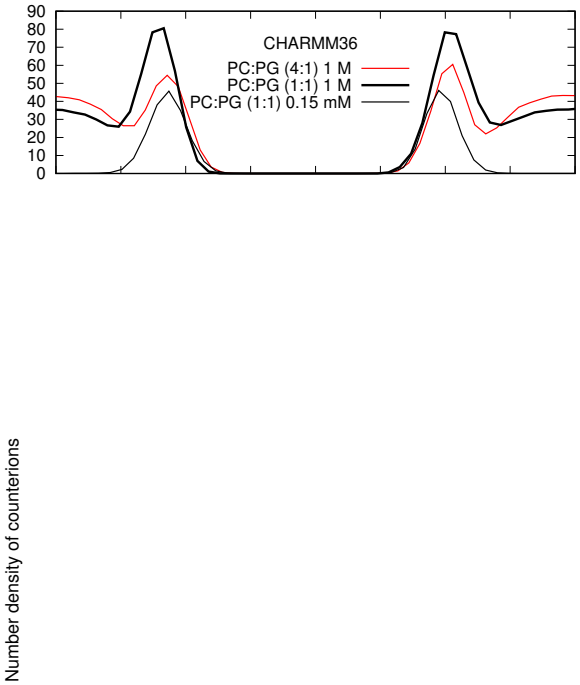

FIG. 9: Calcium ion density profiles along membrane normal from different simulations with PG lipids.