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

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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. 2. 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).

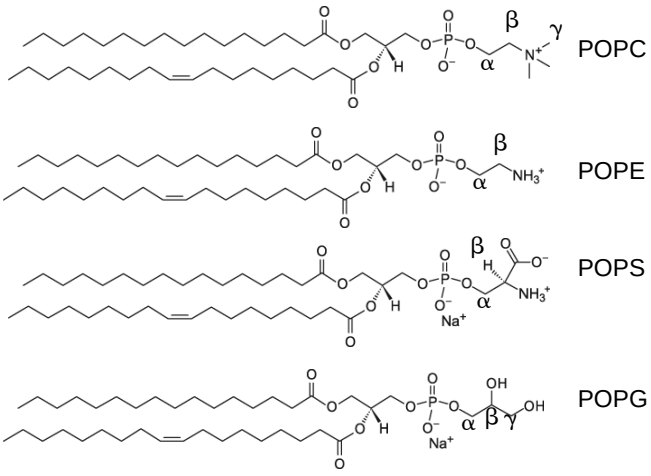

FIG. 1: Chemical structures and labels for the headgroup carbons.

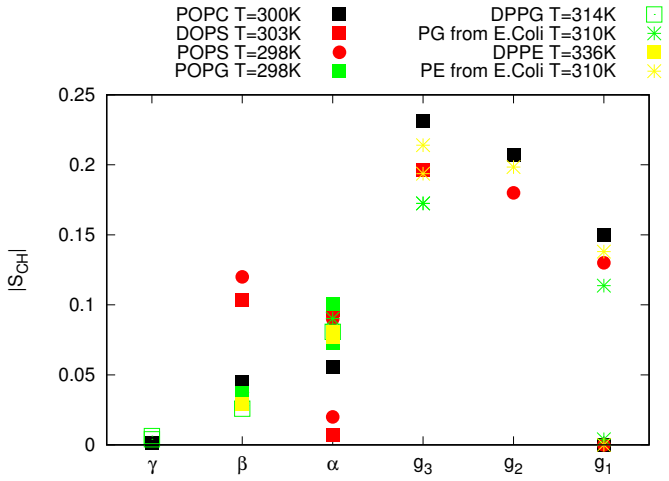

FIG. 2: Absolute values of order parameters for headgroup and glycerol backbone with different headgroups from experiments. POPC values are from [3], DOPS from [4] contains 0.1M of NaCl, POPG from [5] contains 10nM PIPES, DPPG from [6] contains 10mM PIPES and 100mM NaCl, DPPE from [7], E.coliPE and E.coliPG are from [8].

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).

RESULTS AND DISCUSSION

PE headgroup and glycerol backbone

Order parameters from Slipids simulations and experiments for DPPE are shown in Fig. 3. 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

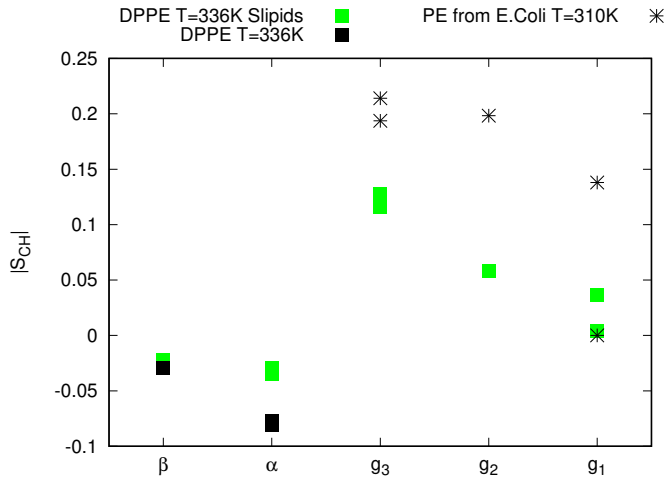

FIG. 3: Order parameters for DPPE headgroup and glycerol backbone from simulations with Slipids [**?**] and experiments (DPPE from [7] and E.coliPE from [8]). Absolute values are shown, because signs are not known experimentally.

2.Experimental signs of the order parameters would be highly useful.

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.

PG headgroup and glycerol backbone

Comparison between experiments and simulations for PG lipids is shown in Fig. 4. 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.

Effect of PE, PS and PG on PC headgroup

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 [12] in contrast to experiments [9]. On the other hand, the increase of the

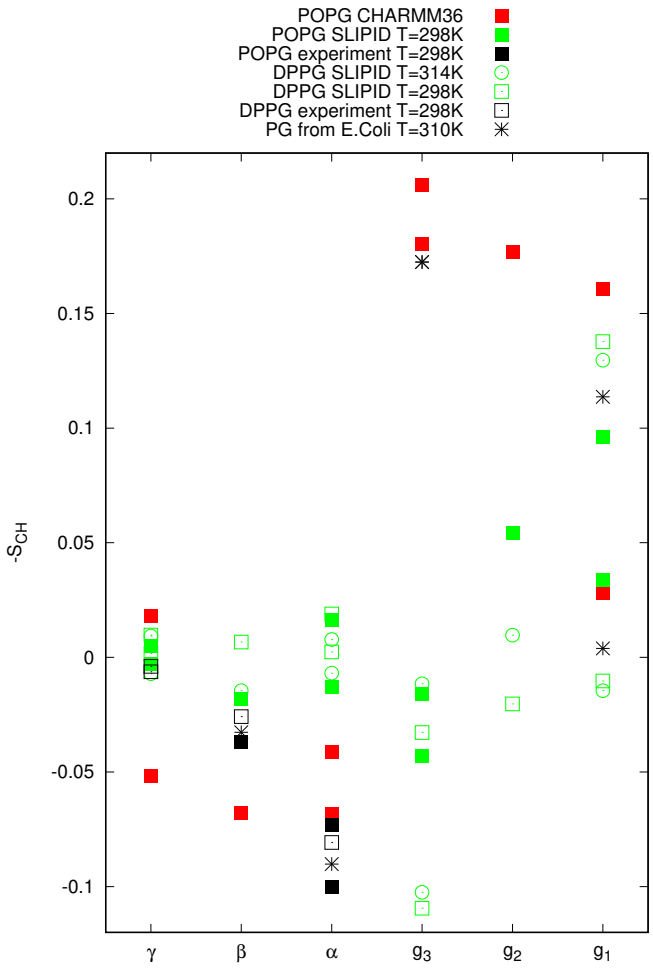

FIG. 4: Order parameters for PG headgroup and glycerol backbone from simulations and experiments without $CaCl_2$ (POPG from [5] contains 10mM of PIPES, DPPG from [6] contains 10mM PIPES and 100mM CaCl, E.Coli PG results from [8]). Signs are not known for experimental data. They are determined to give best agreement with simulations. This is not reliable and should be corrected when experimental data becomes available.

3.More simulation data for lipids with different headgroups to be collected

4.CHARMM GUI simulation contains only counter ions as potassium. All experiments here contain some amount of sodium salt. The best ion concentrations for comparison should be figured out.

5.Experimental signs of the order parameters would highly useful.

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

Effect of PC on PS and PG headgroups

The headgroup order parameters for PS and PG lipid mixtures with PC having different mole fractions from simulations and experiments [5, 14] are shown in Fig. 6. The effect of increasing amount of PC to PS headgroup seems to quali-

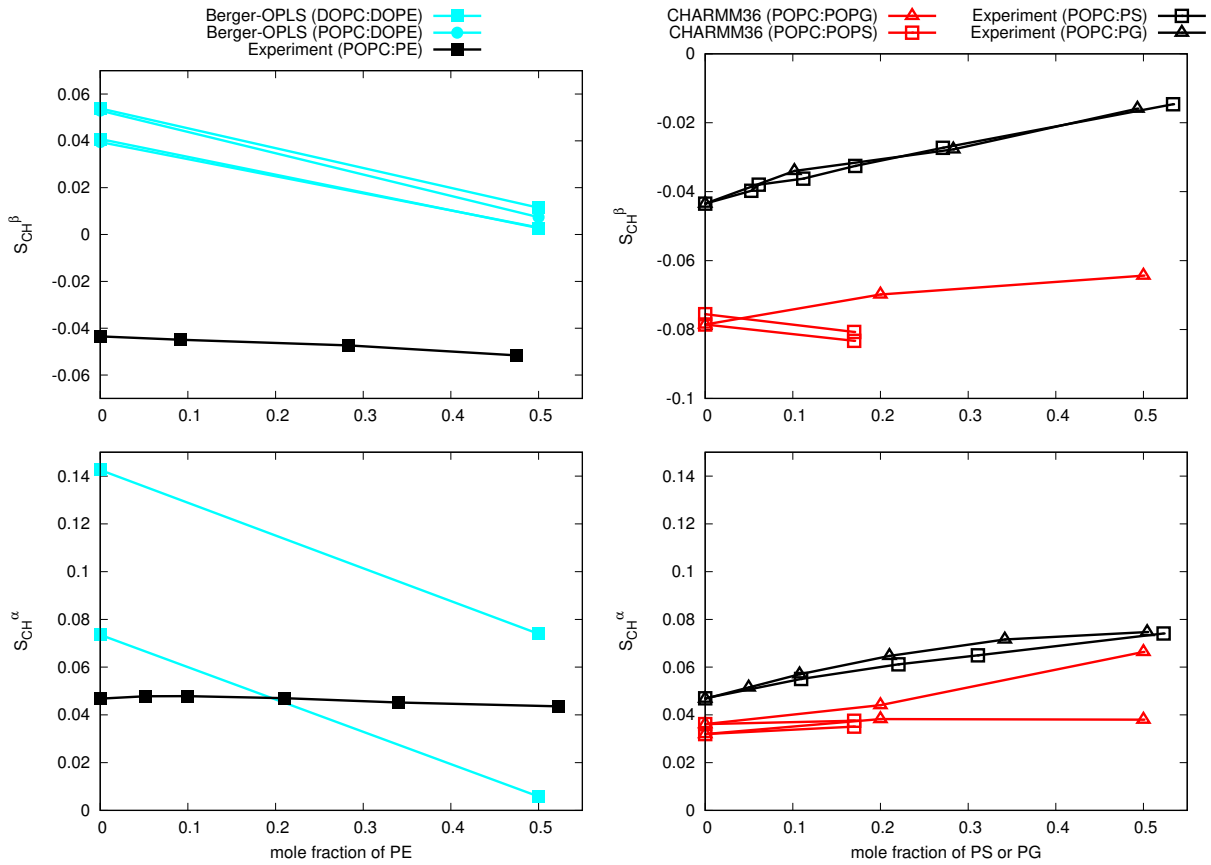

FIG. 5: PC headgroup order parameters from mixtures with PE, PS and PG lipids with various mole fractions from different simulation models and experiments [9]. Signs are determined as discussed in [1, 13].

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

tatively 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.

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, 15]. This molecular electrometer concept can be used also for bilayers containing PC lipids mixed with charged lipids [5, 14, 16]. 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 [15] (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 in-*

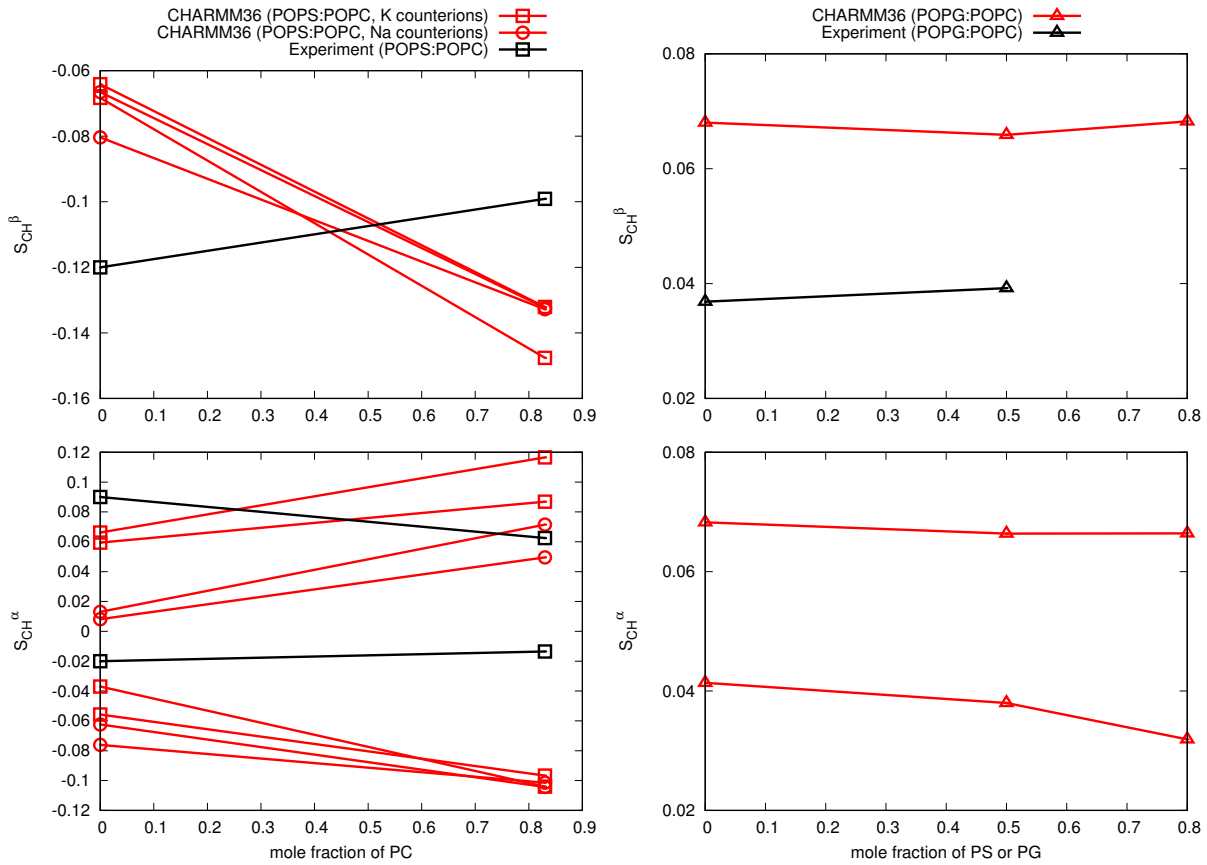

FIG. 6: PS and PG lipid headgroup order parameters from mixtures with PC lipids with various mole fractions from different simulation models and experiments [5, 14]. Signs are not yet known experimentally for PG, thus the signs give by simulations are used. Signs for PS are measures as described in SI.

7.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.

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

creased 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. 7. 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 $\Delta S_\beta = 0.43\Delta S_\alpha$ [17]. Anyway, the data presented in NMRlipids II project and in Fig. 7 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 **9.The response of CHARMM36 to cationic surfactant against experiments [18] 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. 8. 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.

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

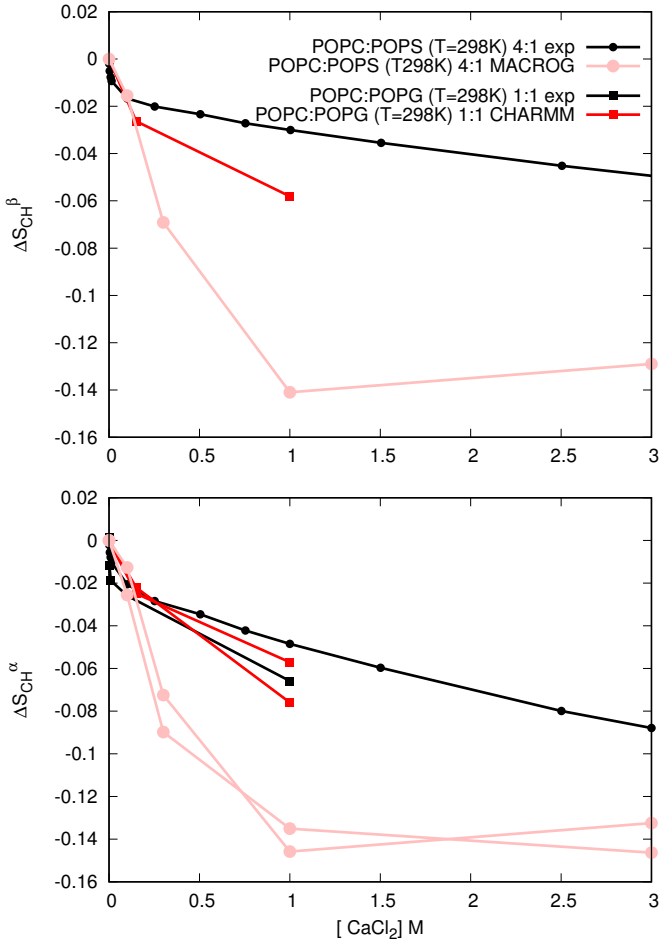

FIG. 7: PG order parameters as a function $CaCl_2$ concentration from experiments [5] and CHARMM36 simulations. Note that beta order parameter is calculated from empirical relation $\Delta S_\beta = 0.43\Delta S_\alpha$ [17], not actually measured.

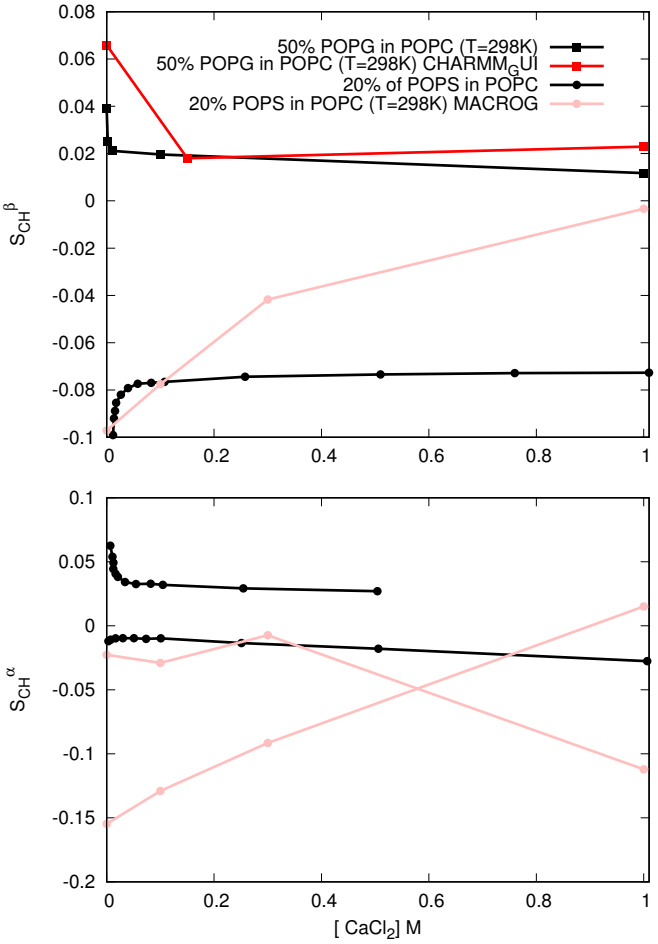

FIG. 8: PG and PS order parameters a function $CaCl_2$ concentration taken from [5] and [14], respectively.

12.Get the small concentration data from the inserts

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. 8. **11.These should be compared to simulations for potential structural interpretation of the changes.**

CONCLUSIONS

SUPPLEMENTARY INFORMATION

Simulated systems

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ToDo

TABLE I: List of MD simulations. The salt concentrations calculated as [salt]=$N_c \times$[water] / N_w, where [water] = 55.5 M.

lipid/counter-ions	force field for lipids / ions	NaCl (mM)	$CaCl_2$ (mM)	[a]N_l	[b]N_w	[c]N_c	[d]T (K)	[e]t_{sim}(ns)	[f]t_{anal} (ns)	[g]files
DPPE	Slipids [19]	0	0	288	9386	0	336	200	100	[20]
DOPS/Na^+	CHARMM36 [**?**] **13.**	0	0	128	4480	0	303	500	100	[**?**] **14.**
DOPS/Na^+	CHARMM36ua [**?**] **15.**	0	0	128	4480	0	303	500	100	[**?**] **16.**
DOPS/Na^+	Slipids [21]	0	0	128	4480	0	303	500	100	[**?**] **17.**
DOPS/Na^+	Slipids [21]	0	0	288	11232	0	303	200	100	[22]
DOPS/Na^+	Berger [23]	0	0	128	4480	0	303	500	100	[**?**] **18.**
DOPS/Na^+	GROMOS-CKP [**?**] **19.**	0	0	128	4480	0	303	500	100	[**?**] **20.**
POPS/Na^+	CHARMM36 [**?**] **21.**	0	0	128	4480	0	298	500	100	[**?**] **22.**
POPS/Na^+	CHARMM36ua [**?**] **23.**	0	0	128	4480	0	298	500	100	[**?**] **24.**
POPS/Na^+	Slipids [21]	0	0	128	4480	0	298	500	100	[**?**] **25.**
POPC:POPS (5:1)/Na^+	CHARMM36 [24**?**] **26.**	0	0	?	?	0	?	?	?	[**?**] **27.**
POPG/Na^+	CHARMM36 [**?**] **28.**	0	0	?	?	0	?	?	?	[**?**] **29.**
POPG/Na^+	Slipids [21]	0	0	288	10664	0	298	250	100	[25]
DPPG/Na^+	Slipids [21]	0	0	288	11232	0	314	200	100	[26]
DPPG/Na^+	Slipids [21]	0	0	288	11232	0	298	400	100	[27]

[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