NMRlipids IV: Headgroup & glycerol backbone structures, and cation binding in bilayers with PE, PG and PS 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. Simulations for bilayers containing PG and PS with low salt conditions are ran with parameters from CHARMM GUI and compared to experiments. Some order parameters from simulations seems to be off from experimental values. Simulation data from other models would be highly useful to see if some of the existing models would reproduce the experimental order parameters and to analyze different conformations predicted by different models respect to experiments.

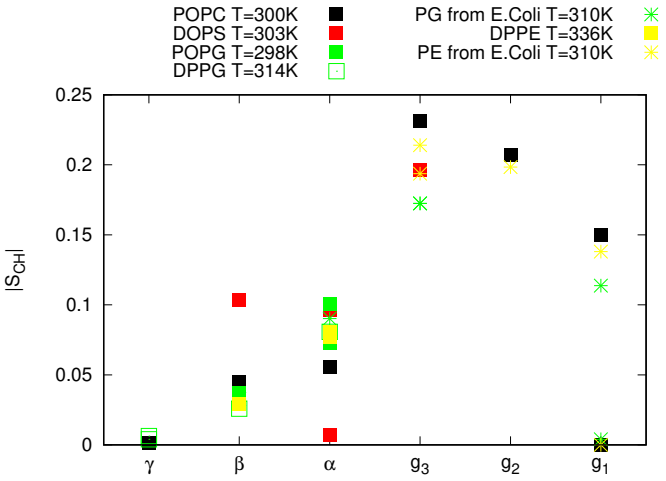

FIG. 1: Absolute values of order parameters for headgroup and glycerol backbone with different headgroups from experiments. POPC values are from [3], DOPS from [4] contains 0.1M of NaCl, POPG from [5] contains 10nM PIPES, DPPG from [6] contains 10mM PIPES and 100mM NaCl, DPPE from [7], E.coliPE and E.coliPG are from [8].

EXPERIMENTAL GLYCEROL BACKBONE AND HEADGROUP ORDER PARAMETERS FOR PE, PG AND PS LIPIDS

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).

GLYCEROL BACKBONE AND HEADGROUP ORDER PARAMETERS FOR PE, PG AND PS LIPIDS IN SIMULATIONS

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).

PE headgroup

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

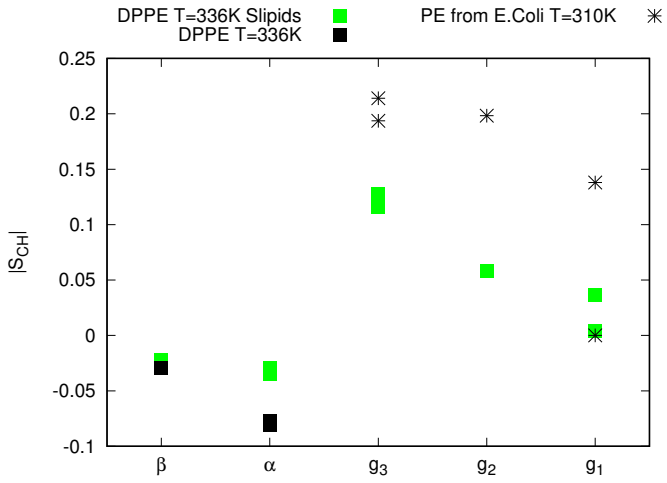

FIG. 2: Order parameters for DPPE headgroup and glycerol backbone from simulations with Slipids [**?**] and experiments (DPPE from [7] and E.coliPE from [8]). Absolute values are shown, because signs are not known experimentally.

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

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.

PS headgroup

The headgroup order parameters of DOPS and POPS bilayers from different experiments and simulations are shown in Figs. 3. None of the tested models gives satisfactory agreement with experiments for order parameters in headgroup α and β carbons.

Glycerol backbone order parameters seems similar in all models, except in Slipids. Even thought glycerol backbone order parameter values are not yet experimentally available for PS lipids, the comparison with the results for PC lipids suggest that Slipid model do not correctly capture the glycerol backbone structure [1]. The glycerol backbone structures between PC and PS lipids simulated with CHARMM36 are compared with the structures simulated with CHARMM36 in Fig. 4. The differences in sampled conformation leading to the order parameter differences are clearly visible in the figure.

PG headgroup

Comparison between experiments and simulations for PG lipids is shown in Fig. 5. 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.

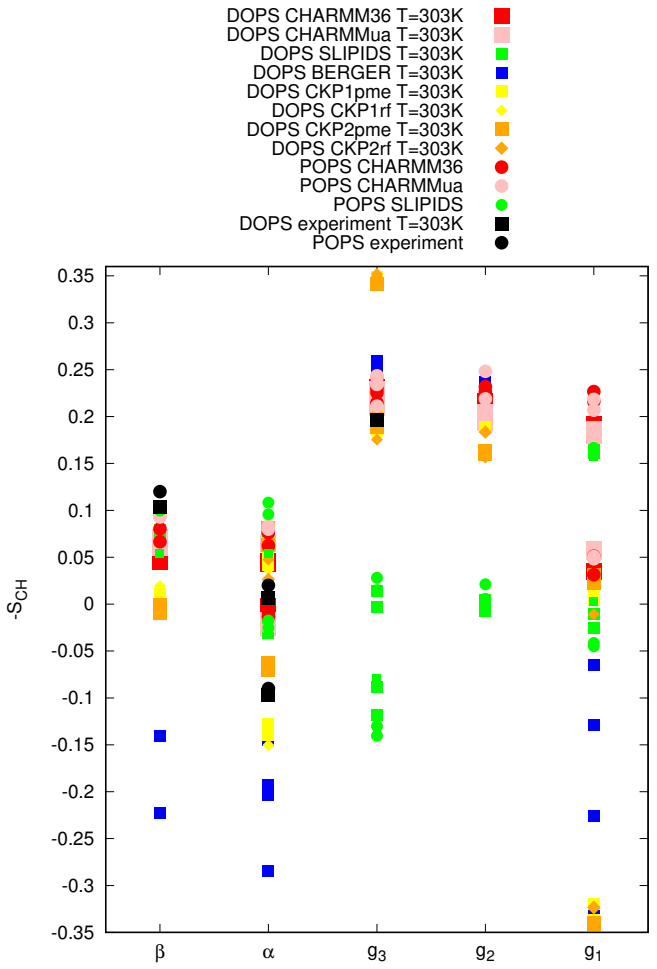

FIG. 3: Order parameters for DOPS headgroup and glycerol backbone from simulations with different models and experiments without $CaCl_2$ Experimental data from [4] contains 0.1M of NaCl. Signs are taken from experiments for POPS described in Supplementary Information.

3.Check and report all the counterions. 4.Glycerol backbone order parameters should be available from the spectra measured by Tiago Ferreira.

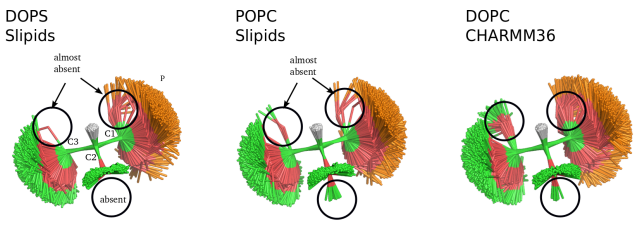

FIG. 4: Snapshots overlayed from different simulations for glycerol backbone region by Pavel Buslaev.

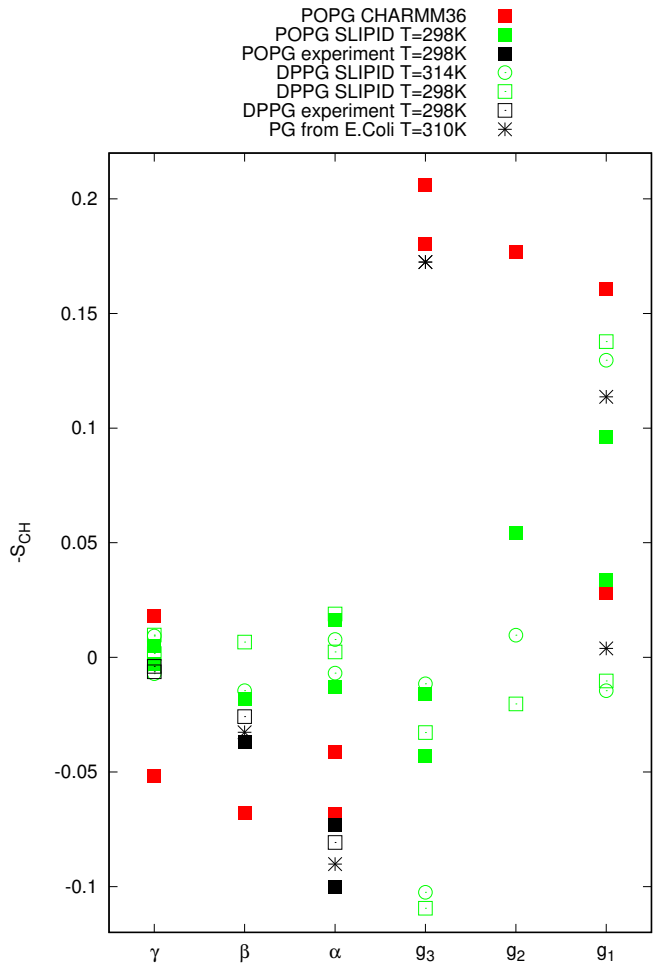

FIG. 5: Order parameters for PG headgroup and glycerol backbone from simulations and experiments without $CaCl_2$ (POPG from [5] contains 10mM of PIPES, DPPG from [6] contains 10mM PIPES and 100mM CaCl, E.Coli PG results from [8]). Signs are not known for experimental data. They are determined to give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

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

6.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.

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

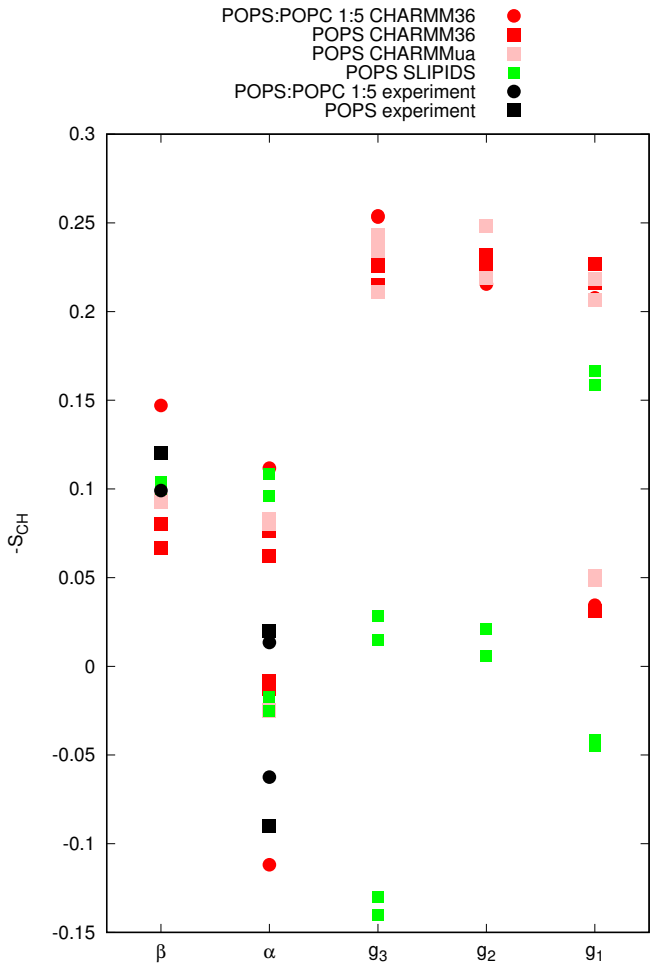

FIG. 6: Headgroup and glycerol backbone order parameters for systems with POPS from simulations with different models and experiments [12]. Signs are taken from experiments described in Supplementary Information.

8.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.

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

LIPID BILAYERS WITH MIXED COMPOSITIONS

PC/PS mixtures

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, 13]. This molecular electrometer concept can be used also for bilayers containing PC lipids mixed with charged lipids [5, 12, 14]. This is demonstrated in Figs 7, 8 and 9, 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 [13] (see Fig. 8). 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. 9). At $CaCl_2$ concentrations ($\sim$ 50-300mM) where order parameters reach the values for pure PC, the Ca2+ binding presumably

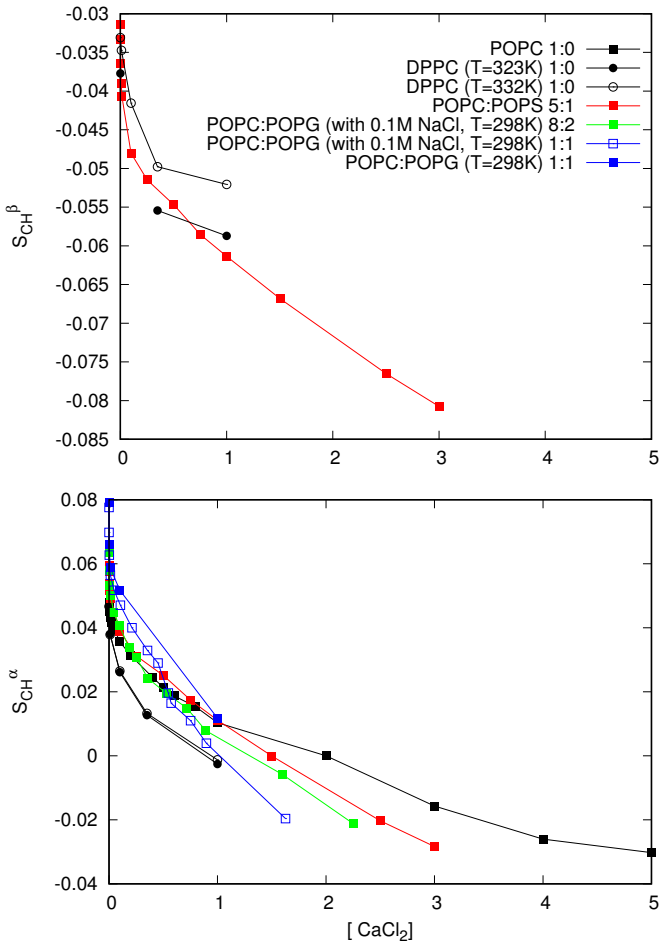

FIG. 7: PC headgroup order parameters as a function of CaCl concentration from experiments containing charged lipids. Pure DPPC data from [15], pure POPC data from [16], POPC:POPS mixture data from [12], POPC:POPG mixture data with 0.1M NaCl from [14] and POPC:POPG mixture data without NaCl from [5].

10.Check the NaCl concentrations in the samples.

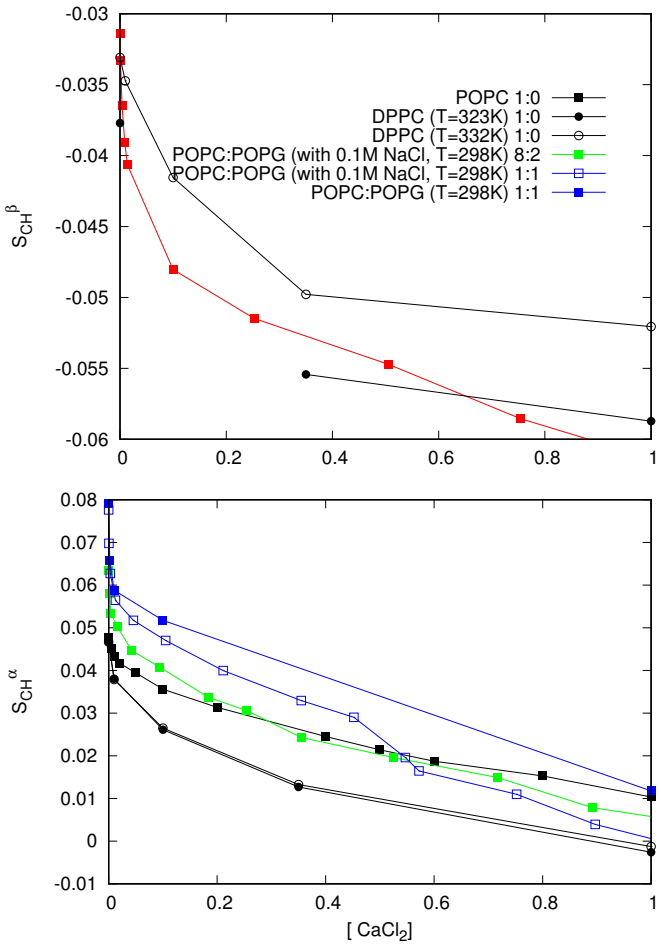

FIG. 8: Figure 7 zoomed to smaller concentrations.

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."*

Also the experimental order parameters for PS and PG headgroups as a function of $CaCl_2$ concentration are shown in Fig. 10. **11.These should be compared to simulations for potential structural interpretation of the changes.**

CA2+ BINDING IN BILAYERS WITH NEGATIVELY CHARGED PG AND PS LIPIDS IN SIMULATIONS

Comparison of Ca2+ binding in PG between CHARMM36 simulations and experiments [5] is shown in Fig. 11. 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 $\Delta S_\beta = 0.43 \Delta S_\alpha$ [15]. Anyway, the data presented in NMRlipids II project and in Fig.

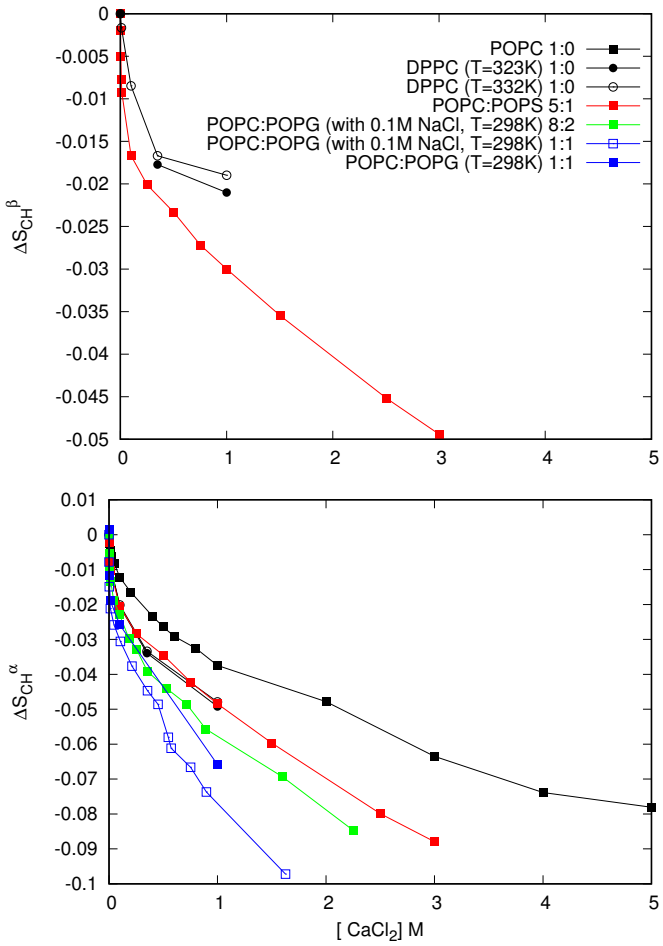

FIG. 9: The change of PC headgroup order parameters in the presence of different amount of negatively charged lipids respect to the values without added $CaCl_2$. The original data is the same as in Fig. 7.

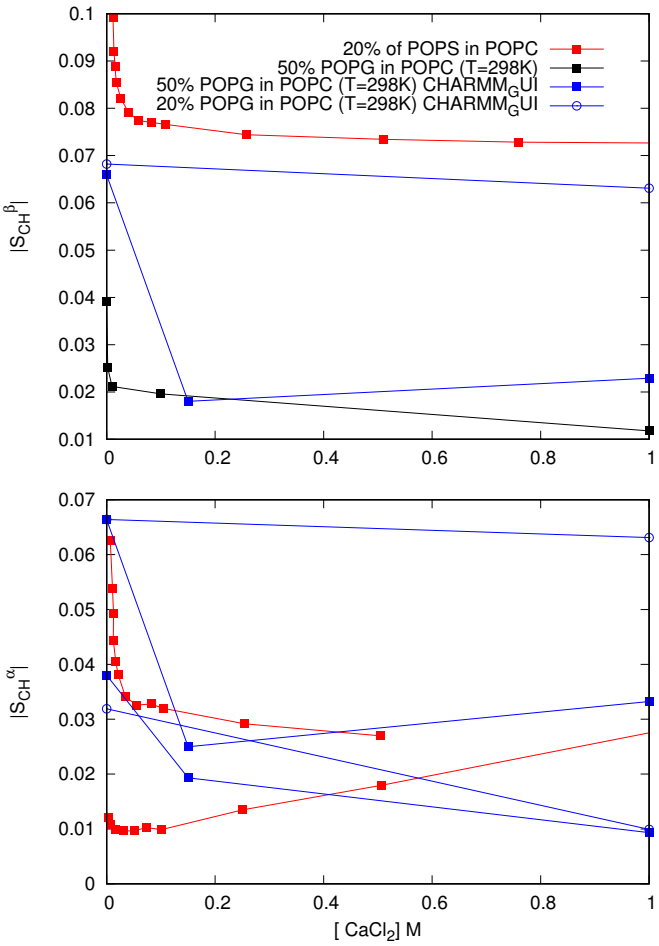

FIG. 10: PG and PS order parameters a function $CaCl_2$ concentration taken from [5] and [12], respectively.

12.Get the small concentration data from the inserts

11 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 **13.Should we check this against cationic surfactant experiments [17]?**.

Also dependence of β-carbon of PG on $CaCl_2$ concentration is compared with experiments [5] in Fig. 10. 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.

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

CONCLUSIONS

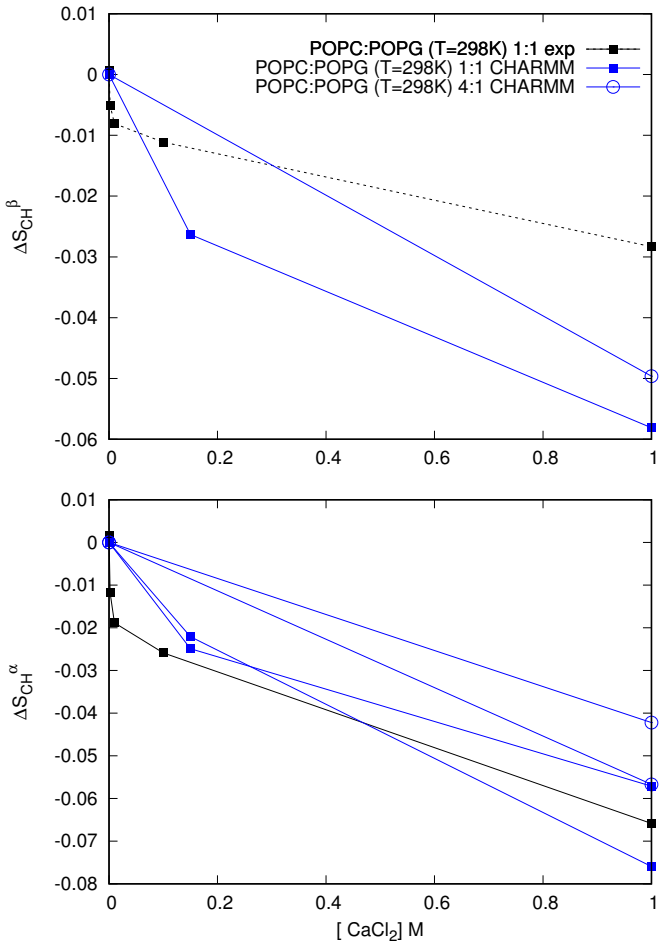

FIG. 11: PG order parameters as a function $CaCl_2$ concentration from experiments [5] and CHARMM36 simulations. Note that beta order parameter is calculated from empirical relation $\Delta S_\beta = 0.43\Delta S_\alpha$ [15], not actually measured.

SUPPLEMENTARY INFORMATION

Simulated systems

Measurements of order parameter sign

Fig. 12 summarizes the experimental results on the order parameter sign measurement for POPS sample. The experimental protocol is the same used in Ref. 27. In (a) you see the headgroup region of the INEPT spectrum where alpha and beta are identified. In (b) you have the R-PDLF slices for alpha and beta where you see one single splitting for beta (which gives an order parameter equal to 0.12), and for alpha a superposition of a large splitting (order parameter equal to 0.09) and a very small splitting which cannot be calculated. On the bottom you have the S-DROSS slices of these two carbons. The grey lines show a random collection of slices from noise such that it gets clear what is significant. The S-DROSS slice for

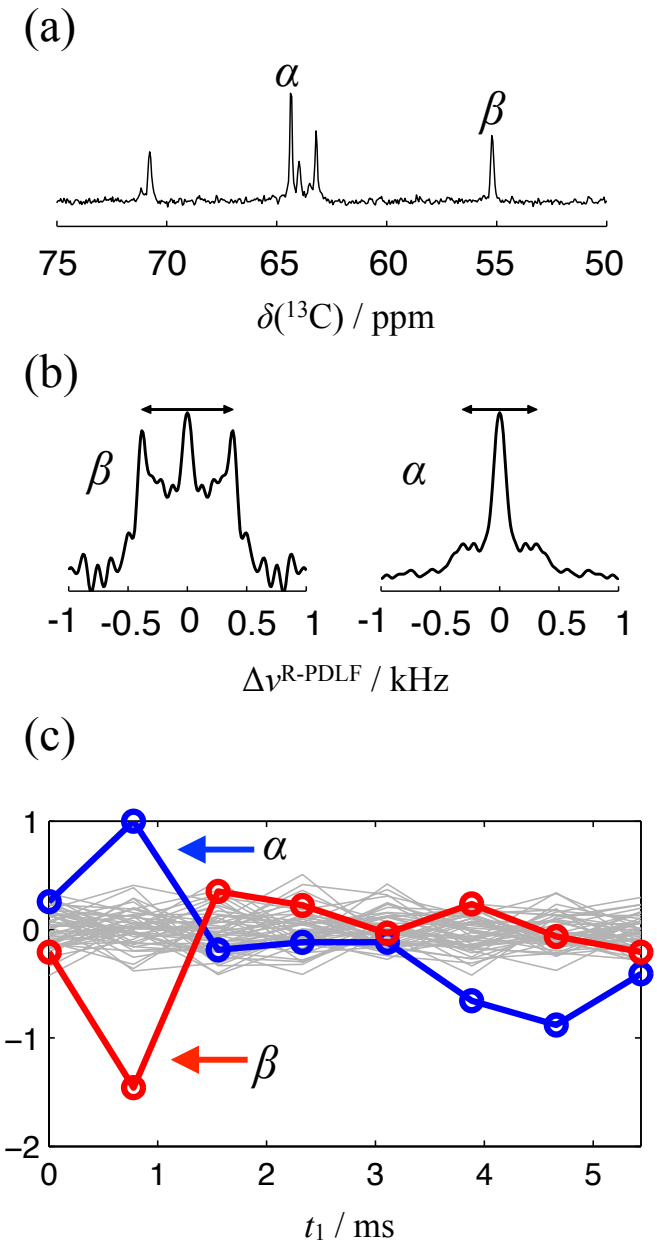

FIG. 12: Experimental results for sign measurement for POPS sample

beta clearly shows that the order parameter is negative. The slice for alpha shows that the higher order parameter is positive and suggests that the smaller order parameter is negative (from the deviation towards negative values in the longer t1 times).

The results updated with SIMPSON simulations for the SDROSS profiles are shown in Fig. 13. The value for the smaller alpha order parameter is taken from Fig 3 in Ref. 28, because resolution in 13C NMR experiments was nor high enough to determine numerical value for this. The plots in Fig. 13 (c) show the following. The error bars and points are

the experimental SDROSS data. The thick lines are SIMPSON simulations. The simulations were done by using the order parameter for beta equal to -0.12 and for alpha one order parameter equal to 0.09 and the other equal to -0.02 (black) or 0.02 (grey). Since the black lines agree with experimental data, we conlude that the order parameters for β carbon are -0.12 and for α order parameters are 0.09 and -0.02.

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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 [18]	0	0	288	9386	0	336	200	100	[19]
DOPS/Na^+	CHARMM36 [**?**] **15.**	0	0	128	4480	0	303	500	100	[**?**] **16.**
DOPS/Na^+	CHARMM36ua [**?**] **17.**	0	0	128	4480	0	303	500	100	[**?**] **18.**
DOPS/Na^+	Slipids [20]	0	0	128	4480	0	303	500	100	[**?**] **19.**
DOPS/Na^+	Slipids [20]	0	0	288	11232	0	303	200	100	[21]
DOPS/Na^+	Berger [22]	0	0	128	4480	0	303	500	100	[**?**] **20.**
DOPS/Na^+	GROMOS-CKP [**?**] **21.**	0	0	128	4480	0	303	500	100	[**?**] **22.**
POPS/Na^+	CHARMM36 [**?**] **23.**	0	0	128	4480	0	298	500	100	[**?**] **24.**
POPS/Na^+	CHARMM36ua [**?**] **25.**	0	0	128	4480	0	298	500	100	[**?**] **26.**
POPS/Na^+	Slipids [20]	0	0	128	4480	0	298	500	100	[**?**] **27.**
POPC:POPS (5:1)/Na^+	CHARMM36 [23**?**] **28.**	0	0	?	?	0	?	?	?	[**?**] **29.**
POPG/Na^+	CHARMM36 [**?**] **30.**	0	0	?	?	0	?	?	?	[**?**] **31.**
POPG/Na^+	Slipids [20]	0	0	288	10664	0	298	250	100	[24]
DPPG/Na^+	Slipids [20]	0	0	288	11232	0	314	200	100	[25]
DPPG/Na^+	Slipids [20]	0	0	288	11232	0	298	400	100	[26]

[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

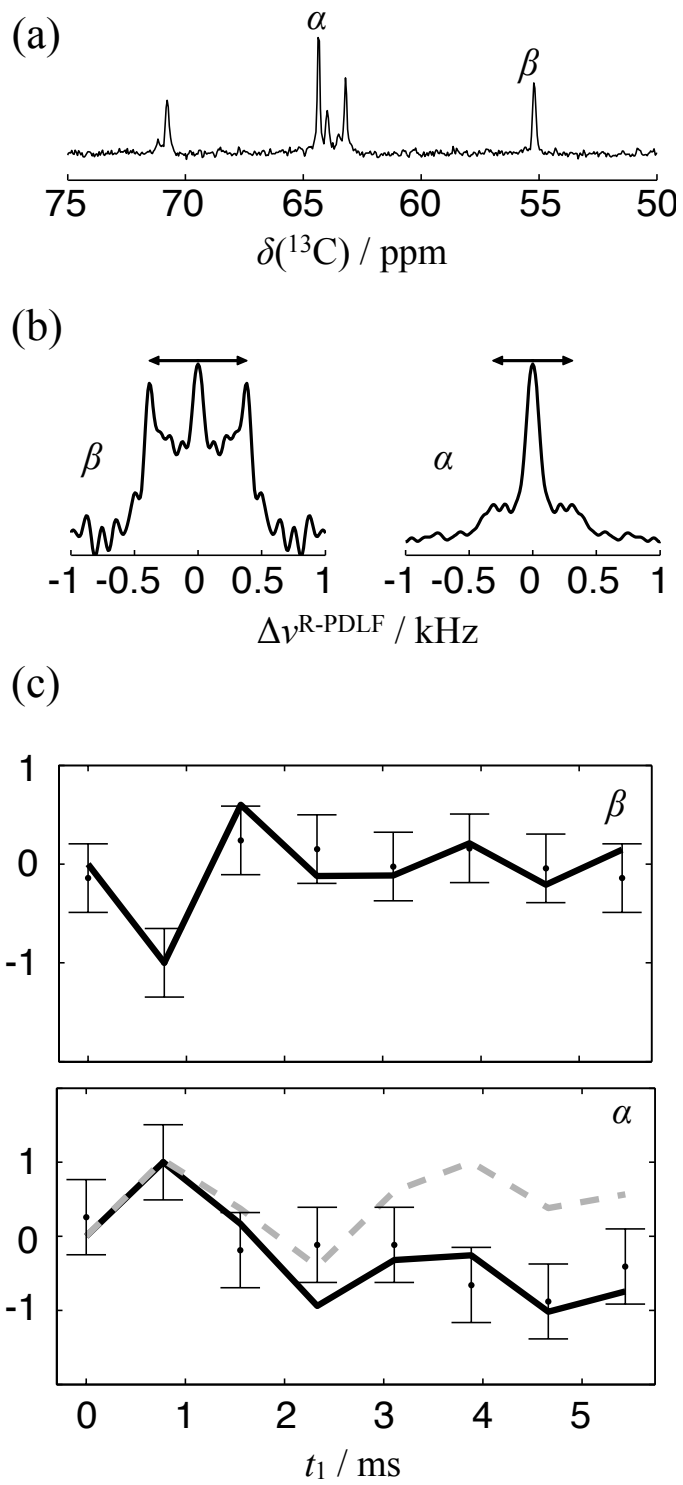

FIG. 13: Experimental results for sign measurement for POPS sample