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.

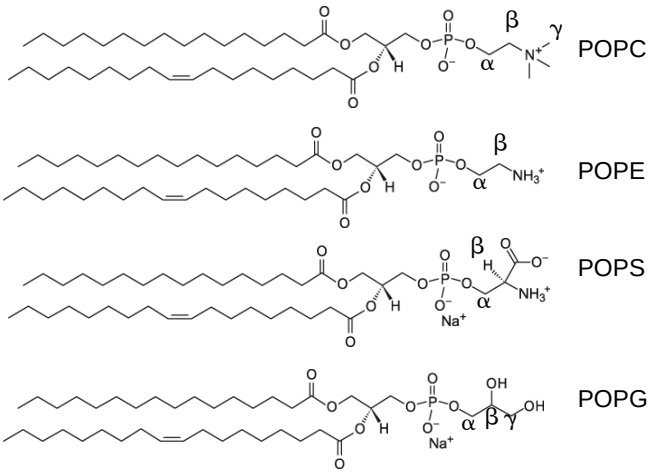

FIG. 1: Chemical structures and labels for the headgroup carbons.

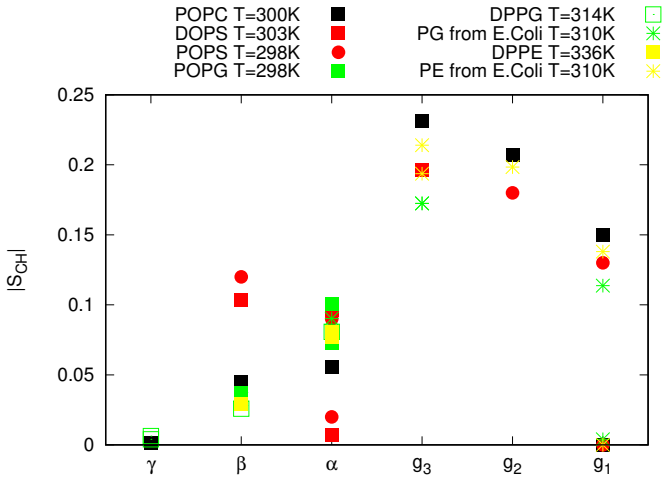

FIG. 2: Absolute values of order parameters for headgroup and glycerol backbone with different headgroups from experiments. POPC values are from [3], DOPS from [4] contains 0.1M of NaCl, POPG from [5] contains 10nM PIPES, DPPG from [6] contains 10mM PIPES and 100mM NaCl, DPPE from [7], E.coliPE and E.coliPG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

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. 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).

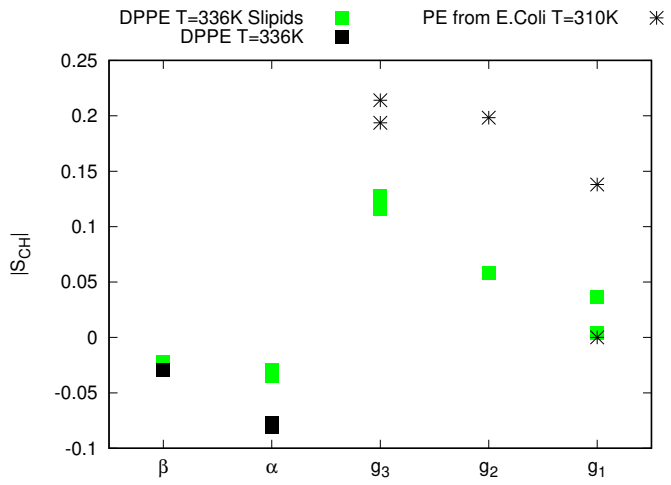

FIG. 3: Order parameters for DPPE headgroup and glycerol backbone from simulations with Slipids [**?**] and experiments (DPPE from [7] and E.coliPE from [8]). Absolute values are shown, because signs are not known experimentally.

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

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. 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 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. 4. 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. 6. 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. 7. 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 BILAYERS WITH MIXED COMPOSITIONS

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. 9 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. 9. 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 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, 15] are shown in Fig. 10. The effect

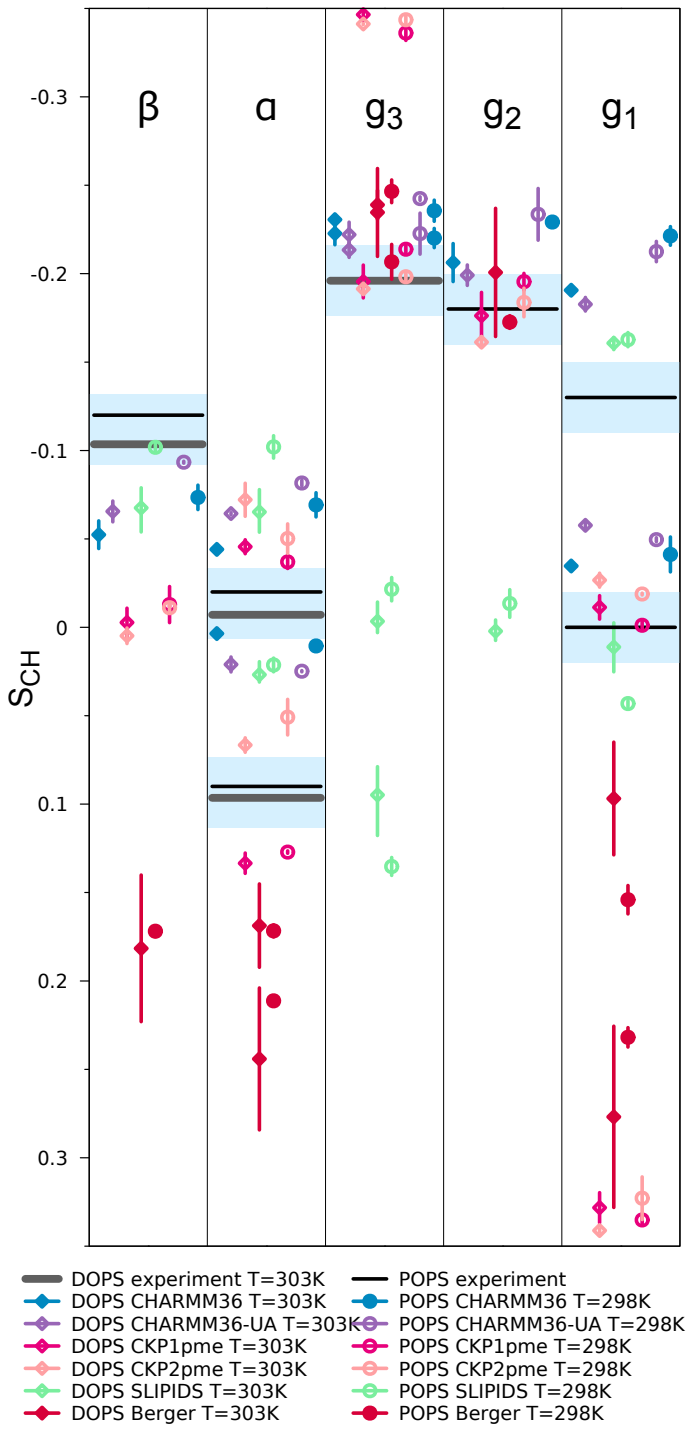

FIG. 4: Order parameters for PS headgroup and glycerol backbone from simulations with different models and experiments without $CaCl_2$ Experimental data from [4] contains 0.1M of NaCl. Signs are taken from experiments for POPS described in Supplementary Information. The vertical bars shown for the computational values are not error bars, but demonstrate that for these systems we had at least two data sets; the ends of the bars mark the extreme values from the sets, and the dot marks their measurement-time-weighted average.

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

	β	α	g_3	g_2	g_1	Σ
CHARMM 36	M	M F	M	M	**M** F	8
CHARMM 36-UA	M	M	M	M	**M** F	8
GROMOS-CKP1	**M**	M **F**	**M F**		**M F**	14
GROMOS-CKP2	**M**	M F	**M F**		**M F**	14
Slipid	M	**M**	**M F**	**M**	M **F**	14
Berger	**M**	**M F**	M F	M	**M** F	15

FIG. 5: Rough subjective ranking of force fields based on Figure 4. Here M indicates a magnitude problem, F a forking problem; letter size increases with problem severity. Color scheme: within experimental error (dark green), almost within experimental error (light green), clear deviation from experiments (light red), and major deviation from experiments (dark red). The Σ-column shows the total deviation of the force field, when individual carbons are given weights of 0 (matches experiment), 1, 2, and 4 (major deviation). For full details of the assessment, see Supplementary Information.

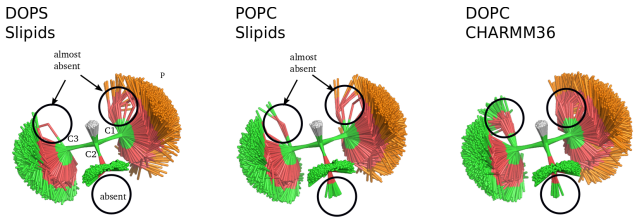

FIG. 6: Snapshots overlayed from different simulations for glycerol backbone region by Pavel Buslaev.

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.

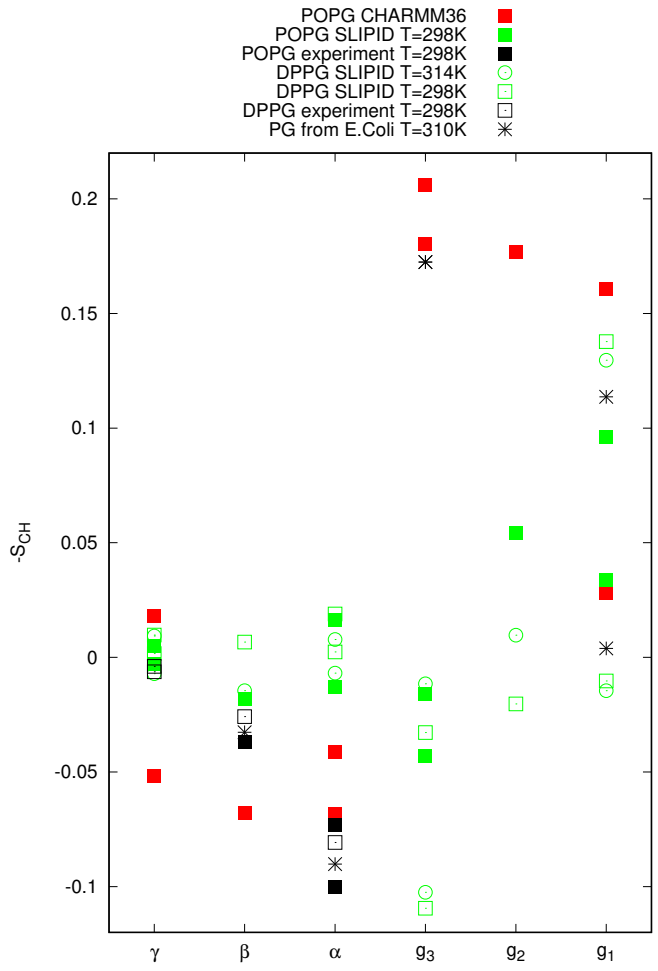

FIG. 7: Order parameters for PG headgroup and glycerol backbone from simulations and experiments without $CaCl_2$ (POPG from [5] contains 10mM of PIPES, DPPG from [6] contains 10mM PIPES and 100mM CaCl, E.Coli PG results from [8]). Signs are not known for experimental data. They are determined to give 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.

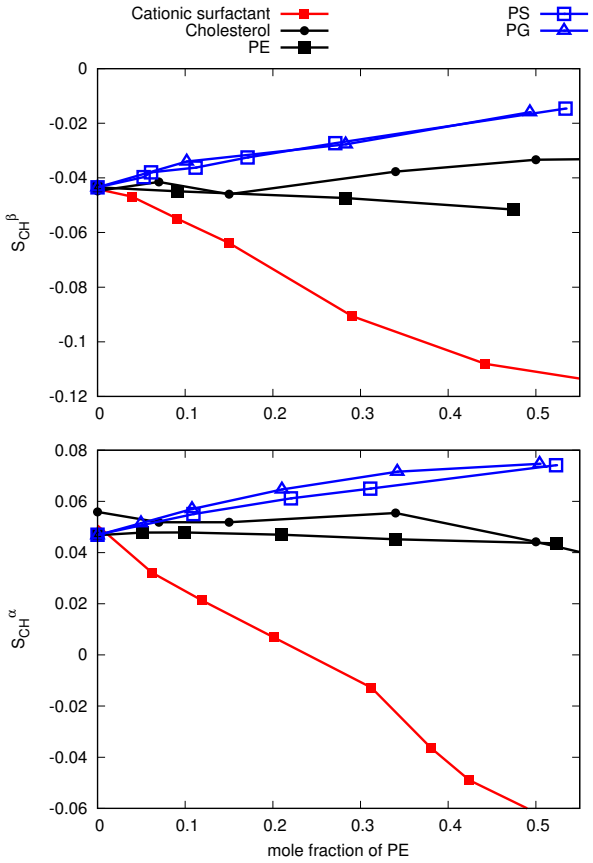

FIG. 8: PC headgroup order parameters from experiments of mixtures with PE, PS, PG and cholesterol [3, 9, 13]. Signs are determined as discussed in [1, 14].

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, 16]. This molecular electrometer concept can be used also for bilayers containing PC lipids mixed with charged lipids [5, 15, 17]. This is demonstrated in Figs 11, 12 and 13, 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 [16] (see Fig. 12). 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. 13). 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*

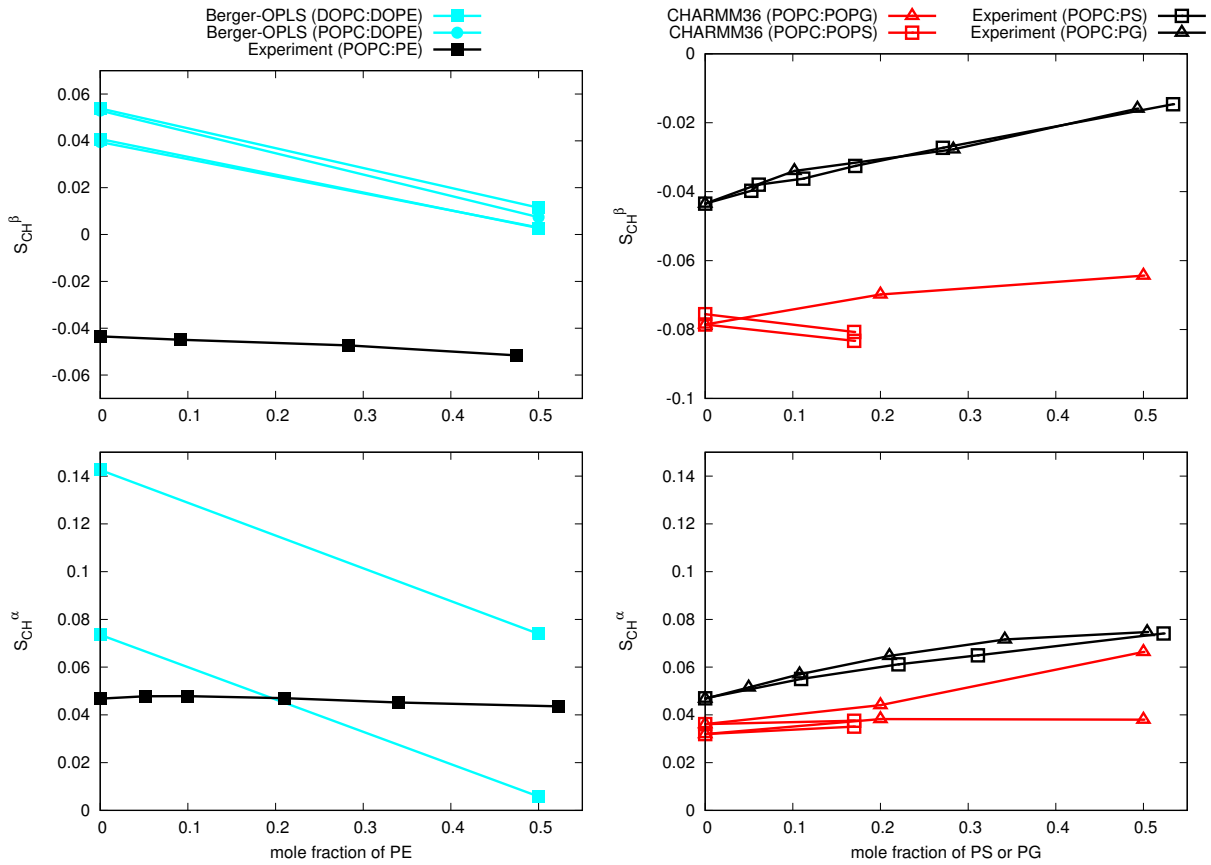

FIG. 9: PC headgroup order parameters from mixtures with PE, PS and PG lipids with various mole fractions from different simulation models and experiments [9]. Signs are determined as discussed in [1, 14].

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

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. 14. **12.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. 15. 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. 15 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 **14.Should we check this against cationic surfactant experiments [13]?**.

Also dependence of β-carbon of PG on $CaCl_2$ concentration is compared with experiments [5] in Fig. 14. 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.

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

CONCLUSIONS

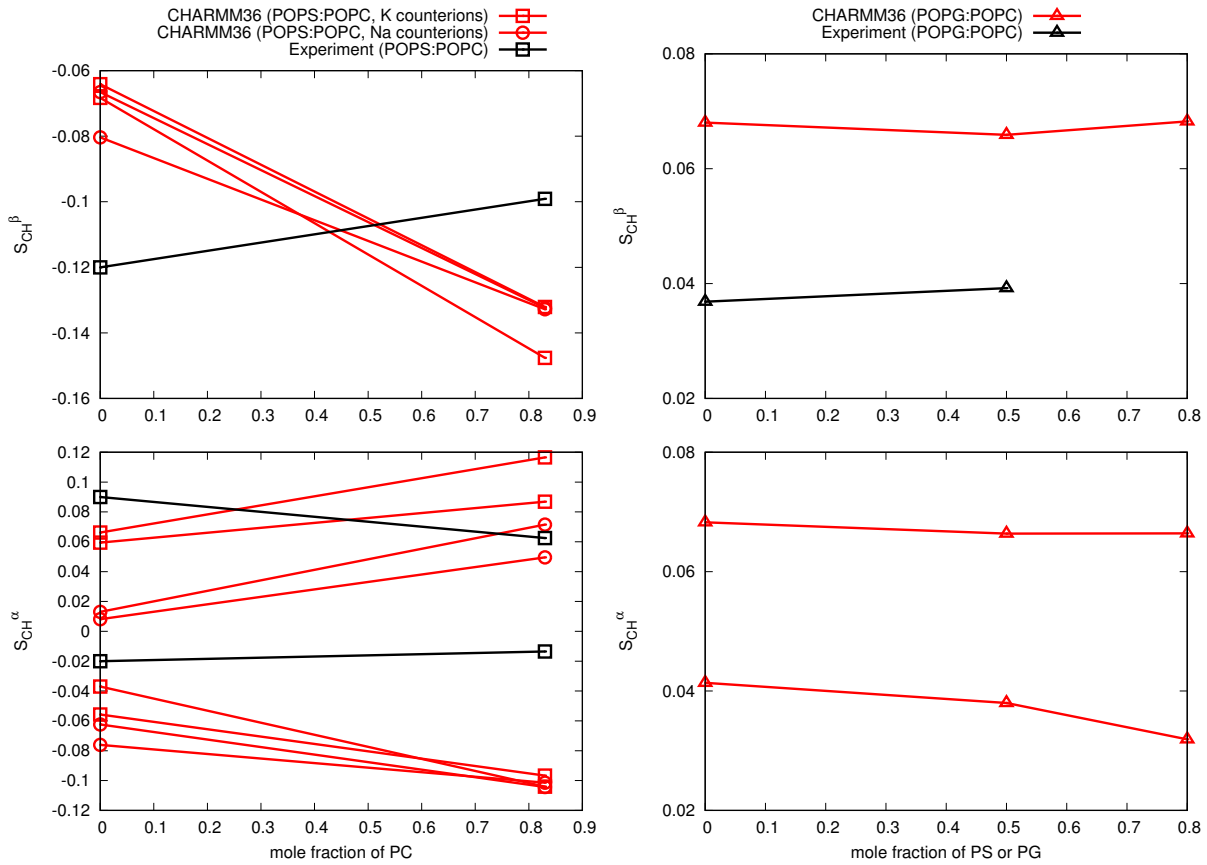

FIG. 10: PS and PG lipid headgroup order parameters from mixtures with PC lipids with various mole fractions from different simulation models and experiments [5, 15]. Signs are not yet known experimentally for PG, thus the signs give by simulations are used. Signs for PS are measures as described in SI.

9.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.

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

SUPPLEMENTARY INFORMATION

Simulated systems

Measurements of order parameter sign

Fig. 16 summarizes the experimental results on the order parameter sign measurement for POPS sample. The experimental protocol is the same used in Ref. 29. 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 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. 17. The value for the smaller alpha order parameter is taken from Fig 3 in Ref. 30, because resolution in 13C NMR experiments was nor high enough to determine numerical value for this. The plots in Fig. 17 (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.

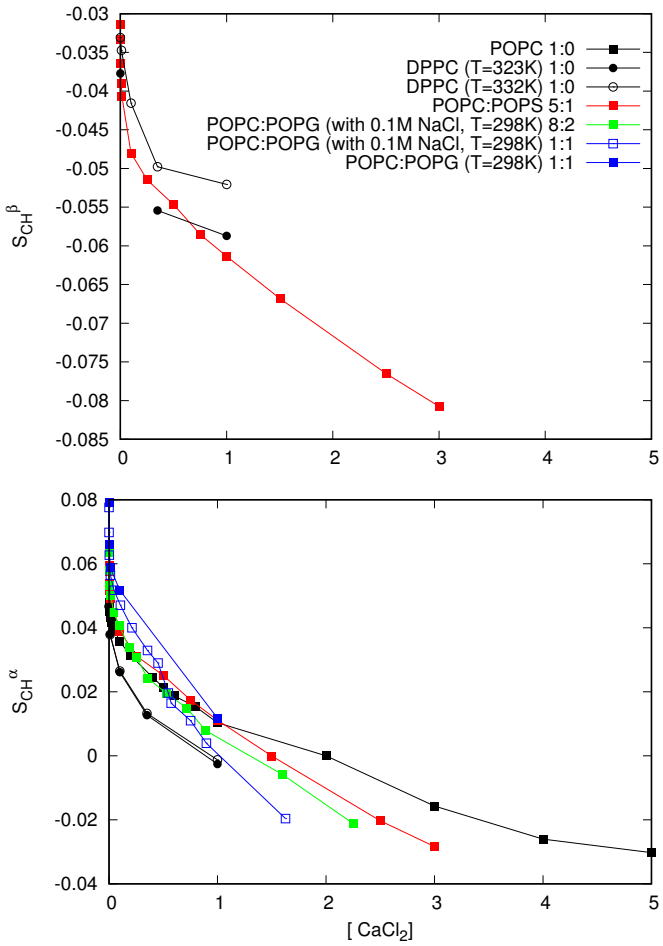

FIG. 11: PC headgroup order parameters as a function of CaCl concentration from experiments containing charged lipids. Pure DPPC data from [18], pure POPC data from [19], POPC:POPS mixture data from [15], POPC:POPG mixture data with 0.1M NaCl from [17] and POPC:POPG mixture data without NaCl from [5].

11.Check the NaCl concentrations in the samples.

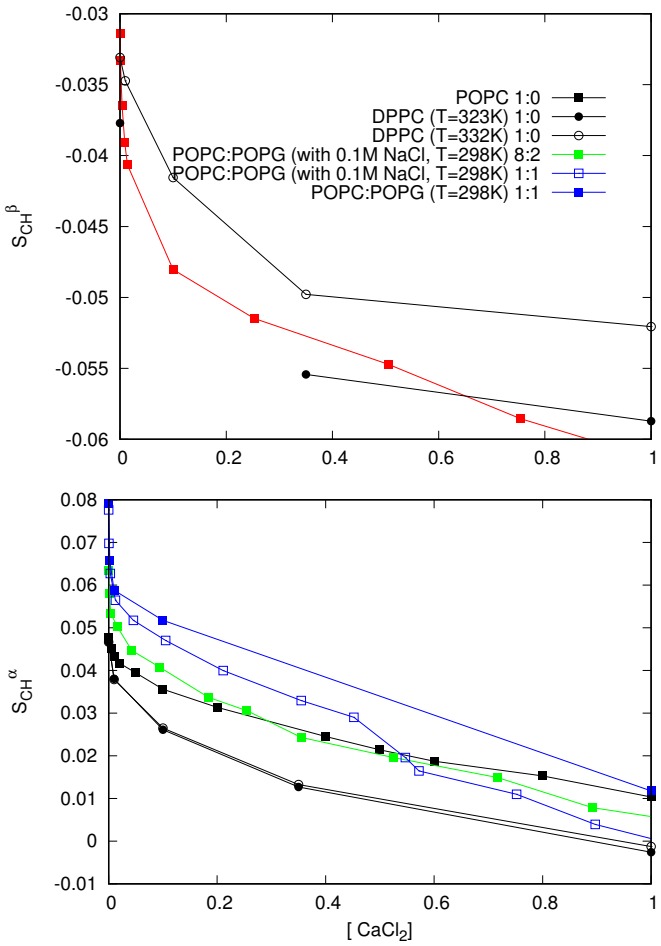

FIG. 12: Figure 11 zoomed to smaller concentrations.

Dihedrals

Details of the rough subjective force field ranking (Fig. 5)

The assessment was based fully on the Fig. 4. First, for each carbon (the columns in Fig. 4) in each force field (the rows), we looked separately at deviations in magnitude and forking.

Magnitude deviations, i.e., how close to the experimentally obtained C–H order parameters (OPs) the force-field-produced OPs were. For each carbon, the following 5-step scale was used:

0 ( ): More than half of all the calculated OPs (that is, of all different hydrogens in all different lipids) were within the *subjective sweet spots* (SSP, blue-shaded areas in Fig. 4).

1 (ᴍ): All the calculated OPs were < 0.03 units away from the SSP.

2 (M): All the calculated OPs were < 0.05 units away from the SSP.

3 (M): All the calculated OPs were < 0.10 units away from the SSP.

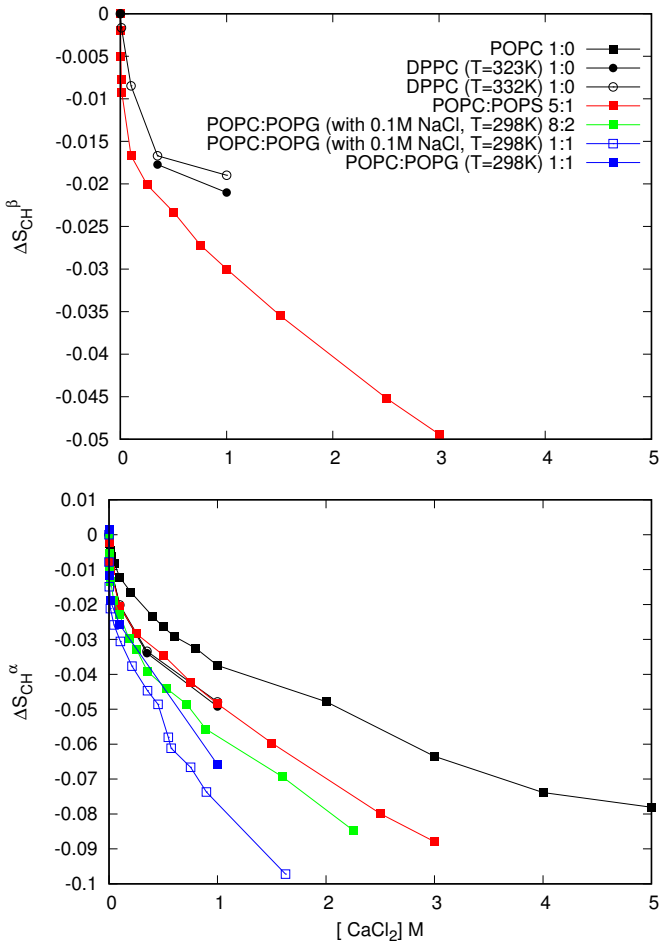

FIG. 13: The change of PC headgroup order parameters in the presence of different amount of negatively charged lipids respect to the values without added $CaCl_2$. The original data is the same as in Fig. 11.

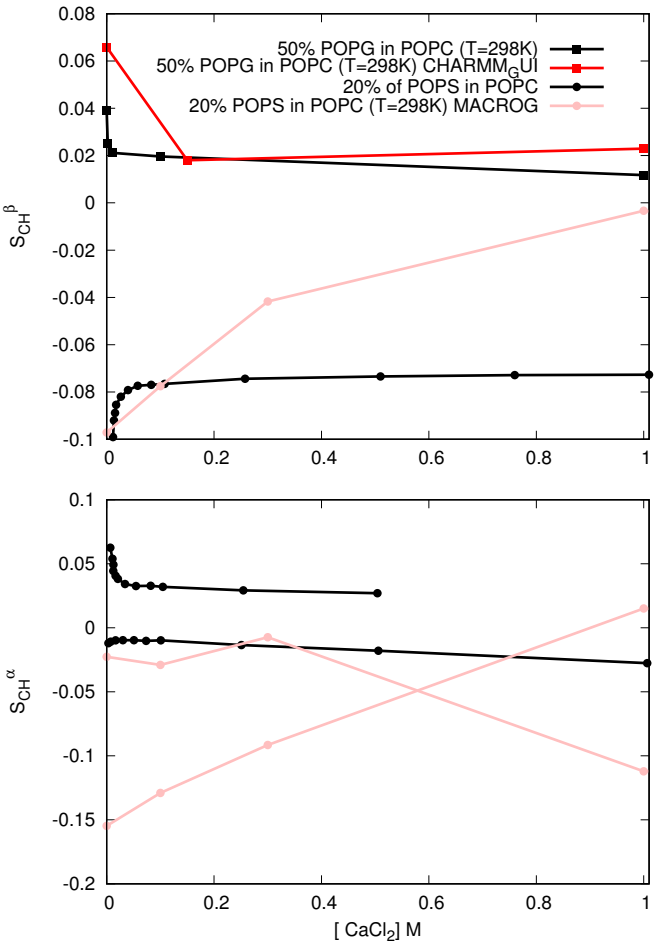

FIG. 14: PG and PS order parameters a function $CaCl_2$ concentration taken from [5] and [15], respectively.

13.Get the small concentration data from the inserts

4 (M): Some of the calculated OPs were > 0.10 units away from the SSP.

Forking deviations, i.e., how well the difference in order parameters of two hydrogens attached to a given carbon matched that obtained experimentally. Note that this is not relevant for β and g_2, which have only one hydrogen. For the α carbon, for which a considerable forking of 0.105 is experimentally seen, the following 5-step scale was used:

0 ( ): The distance D between the dots (that mark the measurement-time-weighted averages in Fig. 4) was $0.08 < D < 0.13$ units for all the calculated OPs (that is, for all different lipids).

1 (F): $(0.06 < D < 0.08)$ OR $(0.13 < D < 0.15)$.

2 (F): $(0.04 < D < 0.06)$ OR $(0.15 < D < 0.17)$.

3 (F): $(0.02 < D < 0.04)$ OR $(0.17 < D < 0.19)$.

4 (F): $(D < 0.02)$ OR $(0.19 < D)$.

For the g_3 carbon, for which no forking is indicated by experiments, the following 5-step scale was used:

0 ( ): $D < 0.02$.

1 (F): $0.02 < D < 0.04$.

2 (F): $0.04 < D < 0.06$.

3 (F): $0.06 < D < 0.08$.

4 (F): $0.08 < D$.

For the g_1 carbon, for which a considerable forking of 0.13 is experimentally seen, the following 5-step scale was used:

0 ( ): $0.11 < D < 0.15$.

1 (F): $(0.09 < D < 0.11)$ OR $(0.15 < D < 0.17)$.

2 (F): $(0.07 < D < 0.09)$ OR $(0.17 < D < 0.19)$.

3 (F): $(0.05 < D < 0.07)$ OR $(0.19 < D < 0.21)$.

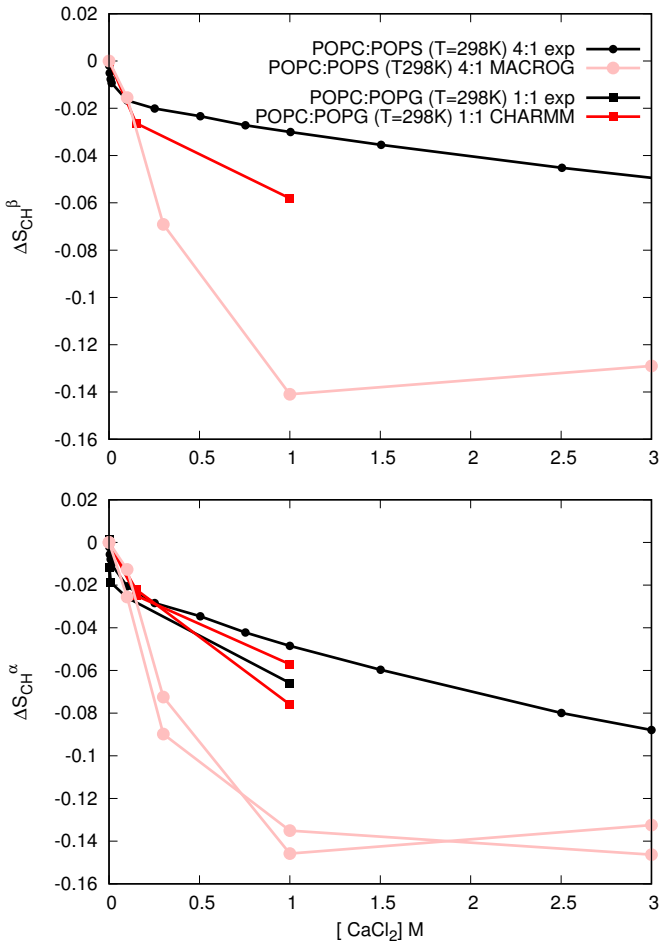

FIG. 15: PG order parameters as a function $CaCl_2$ concentration from experiments [5] and CHARMM36 simulations. Note that beta order parameter is calculated from empirical relation $\Delta S_\beta = 0.43\Delta S_\alpha$ [18], not actually measured.

4 (F): $(D < 0.05)$ OR $(0.21 < D)$.

Based on these assessments of magnitude and forking deviations, each carbon was then assigned to one of the following groups: "within experimental error" (magnitude and forking deviations both on step 0 of the scales described above), "almost within experimental error" (sum of the magnitude and forking deviation steps 1 or 2), "clear deviation from experiments" (sum of magnitude and forking deviation steps from 3 to 5), and "major deviation from experiments" (sum of magnitude and forking deviation steps from 6 to 8). These groups are indicated by colors in Fig. 4. (Note that for β and g_2, for which there can be no forking, the corresponding group assigment limits were: 0, 1, 2, and 3.)

Finally, the total ability of the force field to describe the headgroup and glycerol structure was estimated. To this end, the groups were given the following weights: 0 (within experimental error), 1 (almost within experimental error), 2 (clear deviation from experiments), 4 (major deviation from experiments), and the weights of the five carbons were summed up. The sum, given in the Σ-column of Fig. 4, was then used to (roughly and subjectively, as should be clear from the above description) rank the force fields.

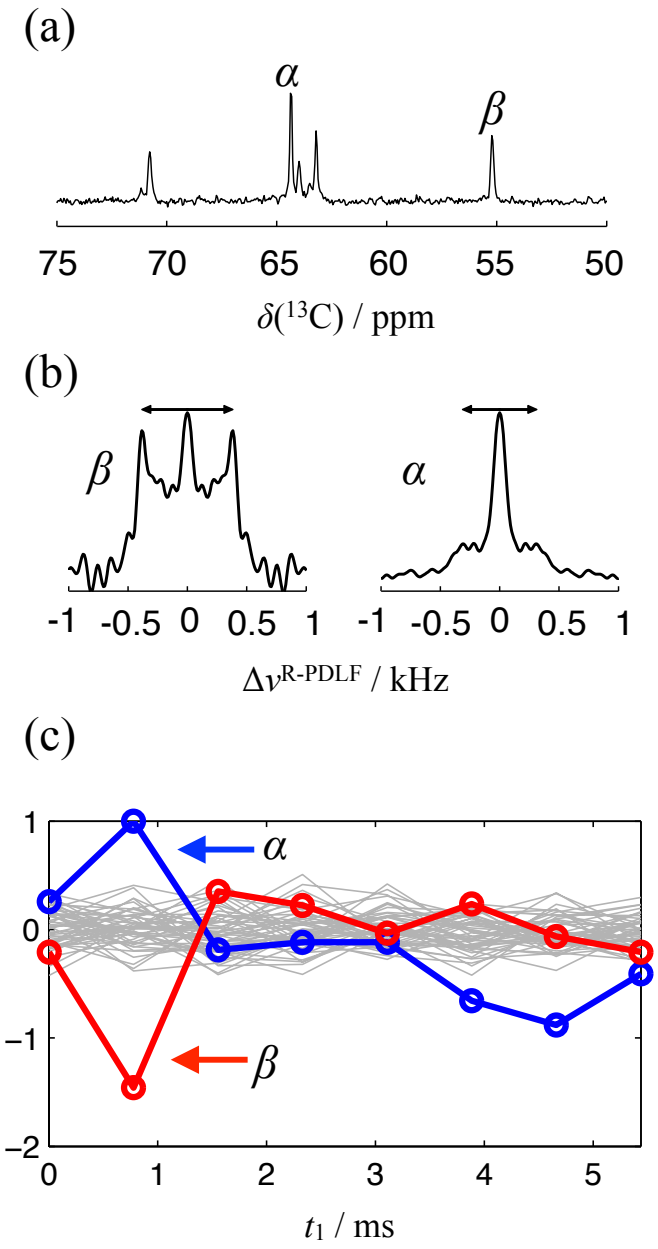

FIG. 16: Experimental results for sign measurement for POPS sample

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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 [20]	0	0	288	9386	0	336	200	100	[21]
DOPS/Na^+	CHARMM36 [**?**] **16.**	0	0	128	4480	0	303	500	100	[**?**] **17.**
DOPS/Na^+	CHARMM36ua [**?**] **18.**	0	0	128	4480	0	303	500	100	[**?**] **19.**
DOPS/Na^+	Slipids [22]	0	0	128	4480	0	303	500	100	[**?**] **20.**
DOPS/Na^+	Slipids [22]	0	0	288	11232	0	303	200	100	[23]
DOPS/Na^+	Berger [24]	0	0	128	4480	0	303	500	100	[**?**] **21.**
DOPS/Na^+	GROMOS-CKP [**?**] **22.**	0	0	128	4480	0	303	500	100	[**?**] **23.**
POPS/Na^+	CHARMM36 [**?**] **24.**	0	0	128	4480	0	298	500	100	[**?**] **25.**
POPS/Na^+	CHARMM36ua [**?**] **26.**	0	0	128	4480	0	298	500	100	[**?**] **27.**
POPS/Na^+	Slipids [22]	0	0	128	4480	0	298	500	100	[**?**] **28.**
POPC:POPS (5:1)/Na^+	CHARMM36 [25**?**] **29.**	0	0	?	?	0	?	?	?	[**?**] **30.**
POPG/Na^+	CHARMM36 [**?**] **31.**	0	0	?	?	0	?	?	?	[**?**] **32.**
POPG/Na^+	Slipids [22]	0	0	288	10664	0	298	250	100	[26]
DPPG/Na^+	Slipids [22]	0	0	288	11232	0	314	200	100	[27]
DPPG/Na^+	Slipids [22]	0	0	288	11232	0	298	400	100	[28]

[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

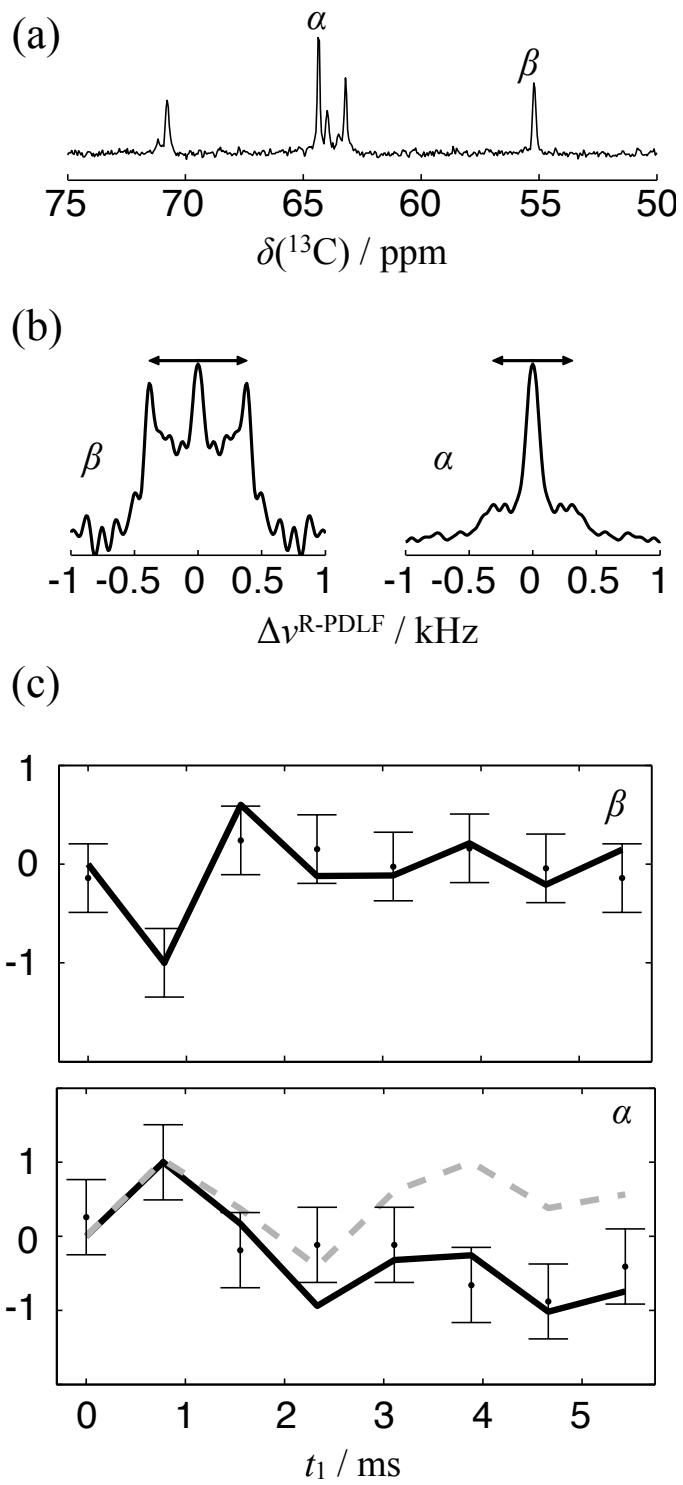

FIG. 17: Experimental results for sign measurement for POPS sample

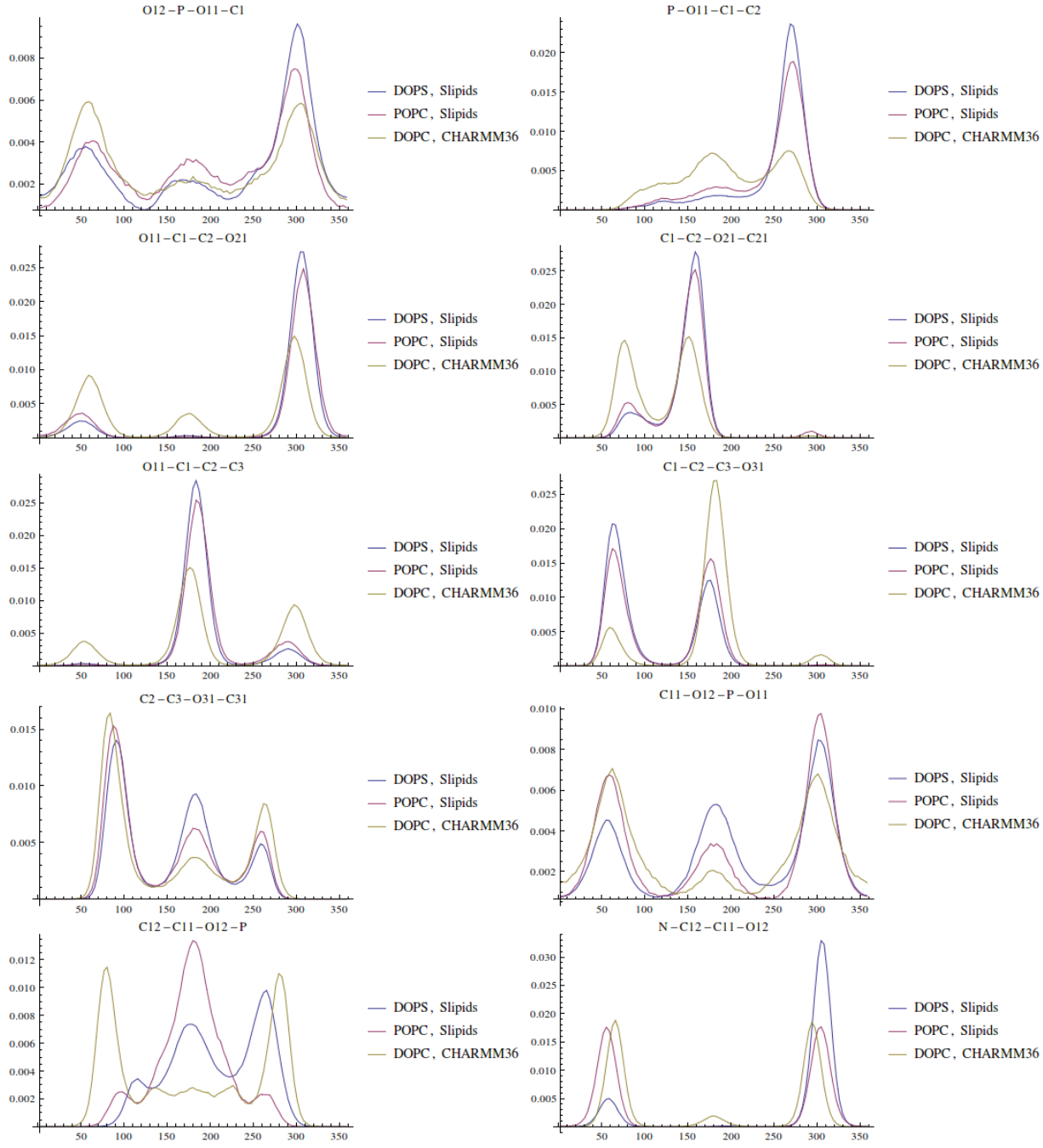

FIG. 18: Experimental results for sign measurement for POPS sample