

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

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(Dated: August 23, 2017)

Primarily measured but also simulated NMR order parameters will be collected also for other than phosphatidylcholine (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.

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 headgroup [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).

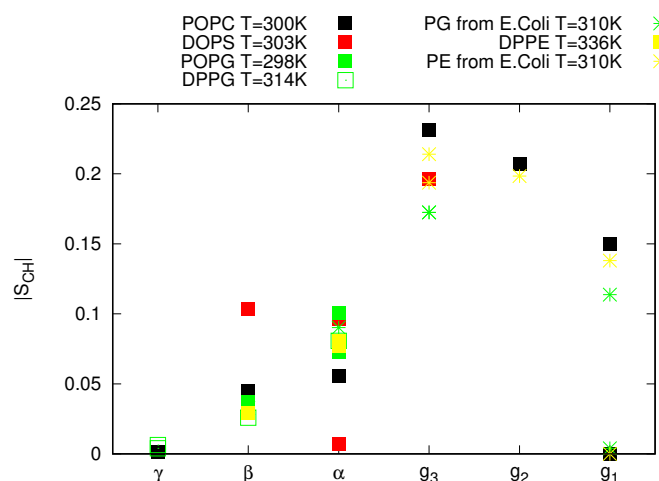


FIG. 1: Absolute values of order parameters for headgroup and glycerol backbone with different headgroups from experiments. POPC values are from [3], DOPS from [4] contains 0.1M of NaCl, POPG from [5] contains 10nM PIPES, DPPG from [6] contains 10mM PIPES and 100mM NaCl, DPPE from [7], E.coliPE and E.coliPG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

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

Absolute values of order parameters between Slipids simulations [?] and experiments for DPPE are shown in Fig. 2. Glycerol backbone order parameters in Slipids are off from experiments, as systematically observed in this and previous studies [?]. Alpha order parameter is too low, but beta 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

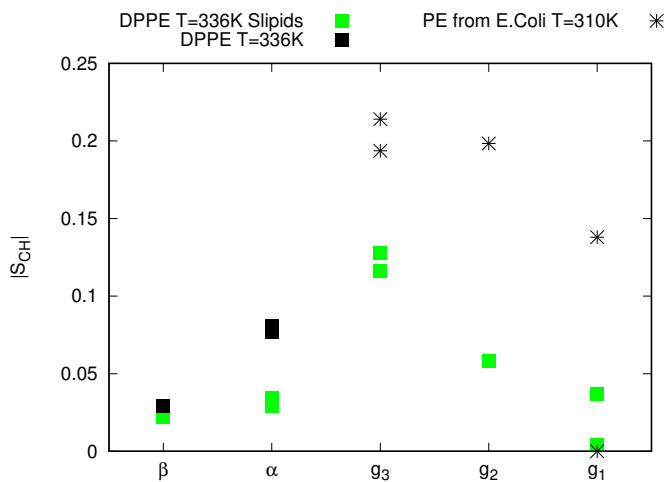


FIG. 2: Order parameters for DPPE headgroup and glycerol backbone from simulations with Slipids [?] and experiments (DPPE from [7] and E.coliPE from [8]). Absolute values are shown, because signs are not known experimentally.

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

opposite than for PC.

PS headgroup

Order parameters for bilayers with PS headgroups from different experiments and simulations are shown in Figs. 3 and 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 though 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. 5. The differences in sampled conformation leading to the order parameter differences are clearly visible in the figure.

PG headgroup

Comparison between experiments and simulations with CHARMM GUI parameters for PG lipids in Fig. 6 suggest that the model overestimates forking in γ and α order parameters, and magnitudes of β and g_1 order parameters.

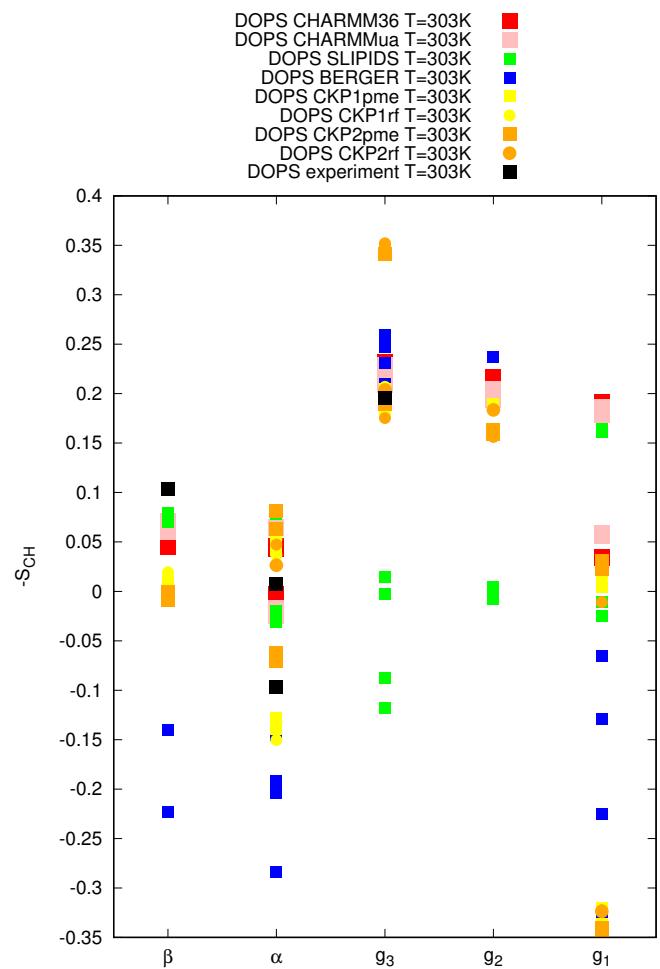


FIG. 3: Order parameters for DOPS headgroup and glycerol backbone from simulations with different models and experiments without CaCl_2 . Experimental data from [4] contains 0.1M of NaCl. Signs are taken from experiments for POPS described in Supplementary Information.

Ca^{2+} BINDING IN BILAYERS WITH NEGATIVELY CHARGED PG AND PS LIPIDS

PC lipid headgroup order parameters can be 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 absence 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

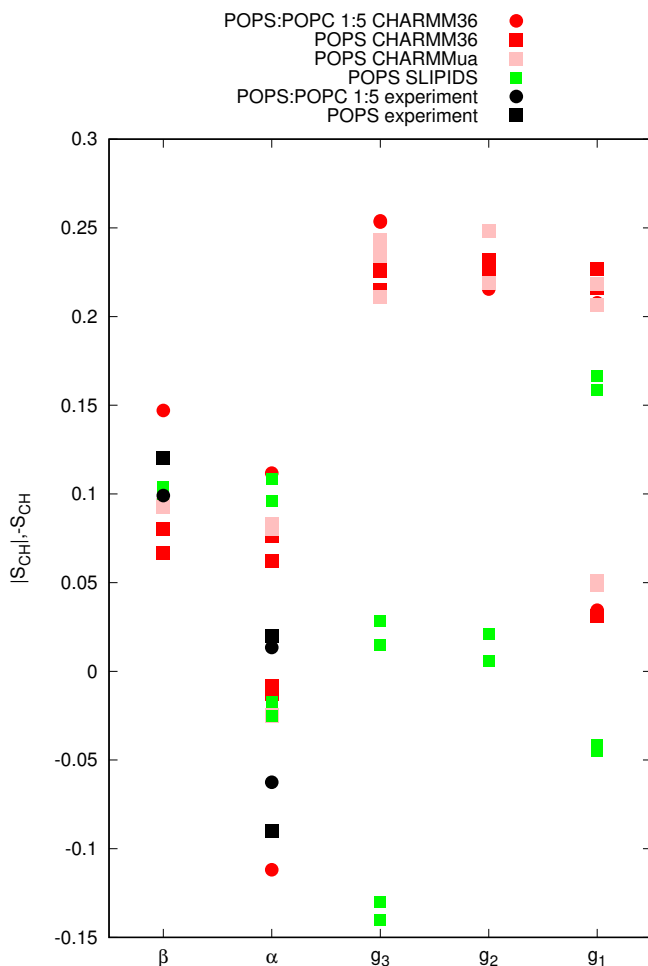


FIG. 4: Headgroup and glycerol backbone order parameters for systems with POPS from simulations with different models and experiments [12]. Signs are taken from experiments described in Supplementary Information.

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

4. 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.

5. Why there is difference between CHARMM36 simulation results from POPS:POPC mixture and pure POPS? Discussion in

<https://github.com/NMRLipids/NMRLipidsIVotherHGs/issues/1>

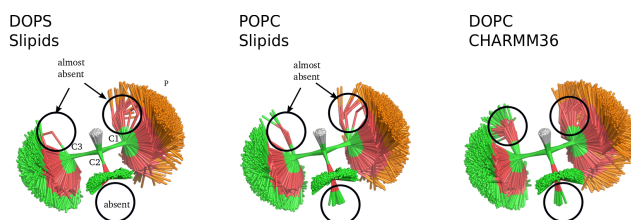


FIG. 5: Snapshots overlaid from different simulations for glycerol backbone region by Pavel Buslaev.

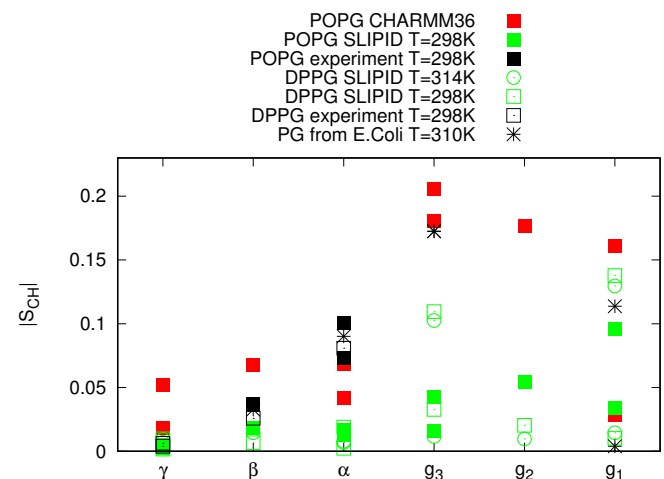


FIG. 6: Order parameters for PG headgroup and glycerol backbone from simulations with CHARMM GUI parameters and experiments without CaCl_2 (POPG from [5] contains 10mM of PIPES, DPPG from [6] contains 10mM PIPES and 100mM CaCl_2 , E.Coli PG results from [8]). Absolute values are shown, because signs are not known for experimental data. Glycerol backbone order parameters are negative in simulations, while α , β and smaller γ are positive.

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

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

CaCl_2 concentrations (~ 50 -300mM) where order parameters reach the values for pure PC, the Ca^{2+} binding presumably fully cancels the charge from negative lipids and overcharging occurs above these concentrations. 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. 10. These should be compared to simulations for potential structural interpretation of the changes.

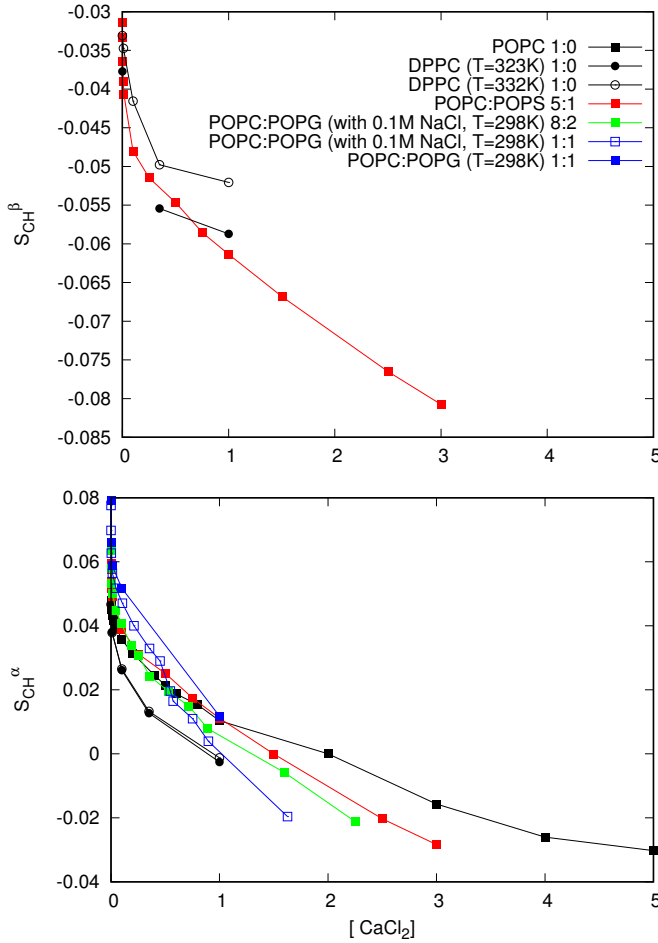


FIG. 7: PC headgroup order parameters as a function of CaCl_2 concentration from experiments containing charged lipids. Pure DPPC data from [15], pure POPC data from [16], POPC:POPS mixture data from [12], POPC:POPG mixture data with 0.1M NaCl from [14] and POPC:POPG mixture data without NaCl from [5].

9. Check the NaCl concentrations in the samples.

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

Comparison of Ca^{2+} 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 decrease 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. 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 12. Should we check this against cationic surfactant experiments

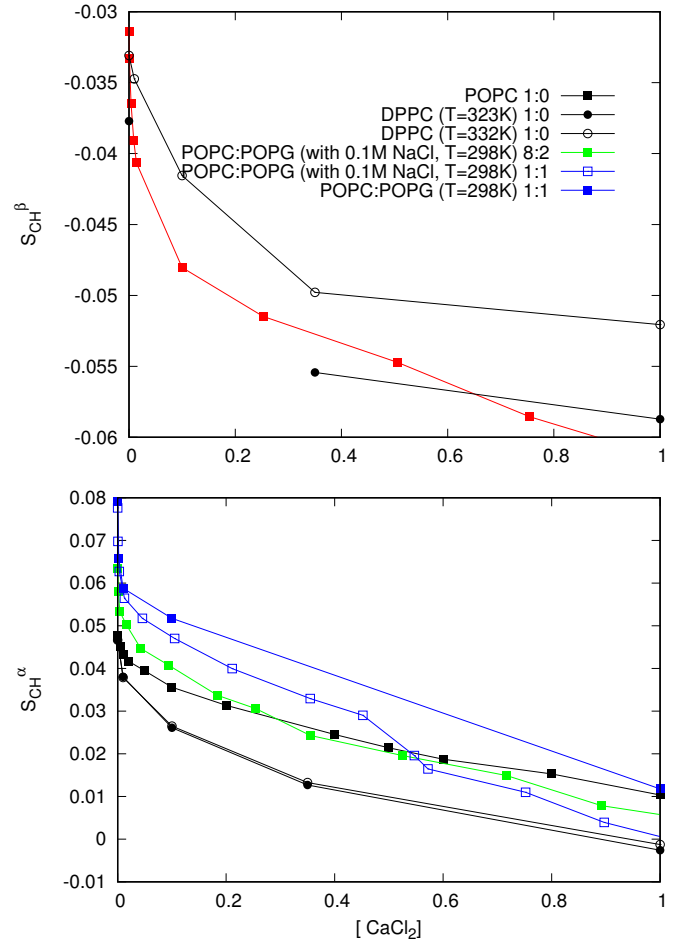


FIG. 8: Figure 7 zoomed to smaller concentrations.

[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.

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

CONCLUSIONS

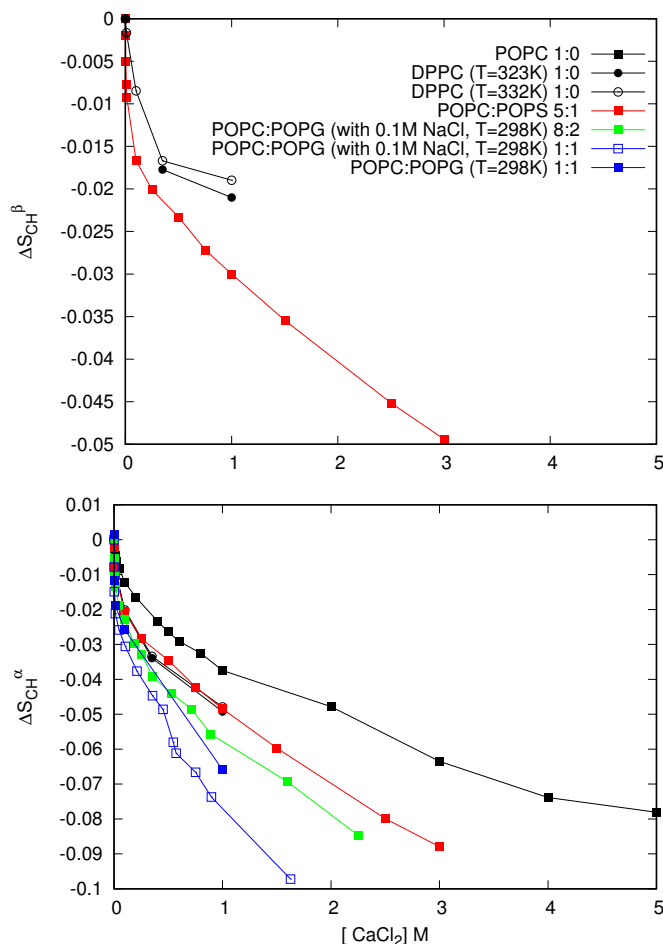


FIG. 9: The change of PC headgroup order parameters in the presence of different amount of negatively charged lipids respect to the values without added CaCl_2 . The original data is the same as in Fig. 7.

SUPPLEMENTARY INFORMATION

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. 18. 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 posi-

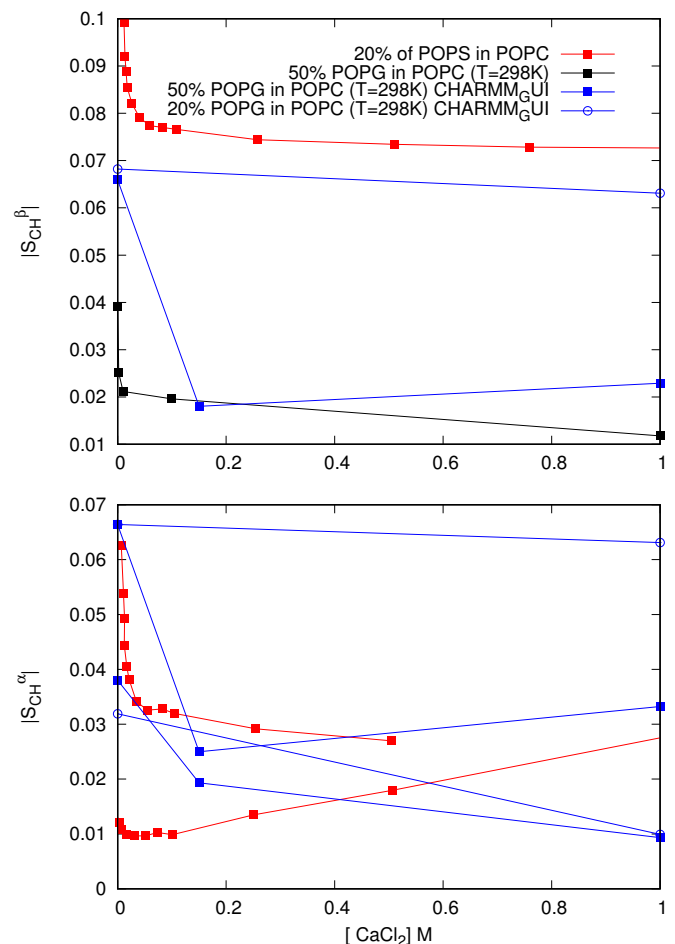


FIG. 10: PG and PS order parameters a function CaCl_2 concentration taken from [5] and [12], respectively.

11. Get the small concentration data from the inserts

tive 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. 19, because resolution in ^{13}C NMR experiments was not 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 conclude that the order parameters for β carbon are -0.12 and for α order parameters are 0.09 and -0.02.

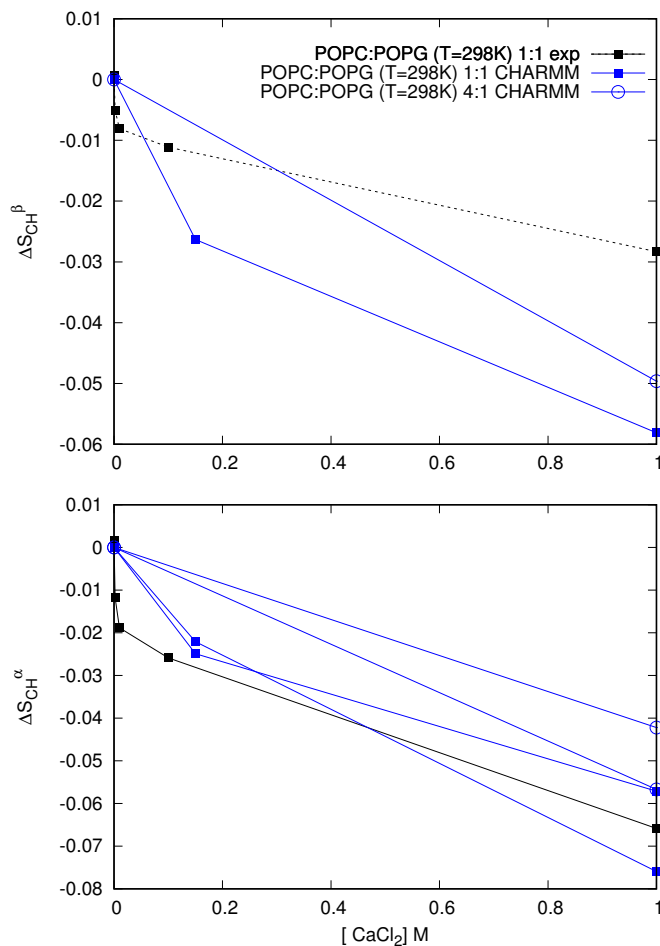


FIG. 11: PG order parameters as a function CaCl_2 concentration from experiments [5] and CHARMM36 simulations. Note that beta order parameter is calculated from empirical relation $\Delta S_\beta = 0.43\Delta S_\alpha$ [15], not actually measured.

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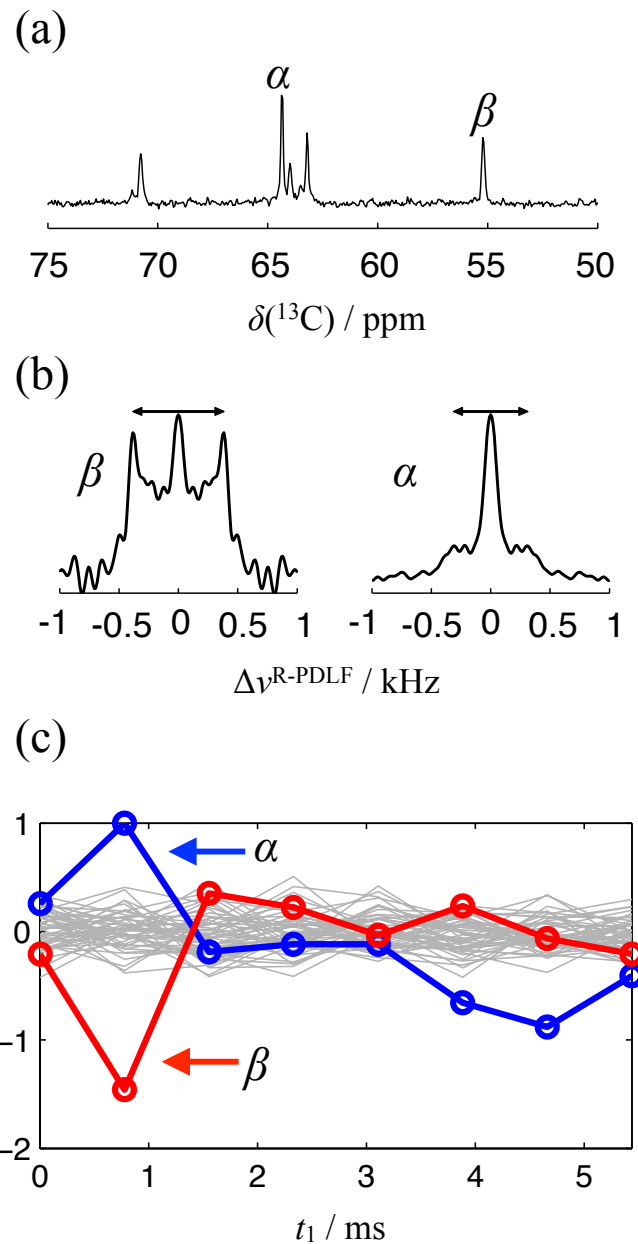


FIG. 12: Experimental results for sign measurement for POPS sample

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ToDo

1. List should be completed	P.	1		
2. Experimental signs of the order parameters would be highly useful.		2		
3. More simulation data for lipids with different head-groups to be collected		3	6. More simulation data for lipids with different head-groups to be collected	3
4. 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.		3	7. CHARMM GUI simulation contains only counterions as potassium. All experiments here contain some amount of sodium salt. The best ion concentrations for comparison should be figured out.	3
5. Why there is difference between CHARMM36 simulation results from POPS:POPC mixture and pure POPS? Discussion in https://github.com/NMRLipids/NMRLipidsIVotherHGs/issues/1		3	8. Experimental signs of the order parameters would highly useful.	3
			10. These should be compared to simulations for potential structural interpretation of the changes.	3
			9. Check the NaCl concentrations in the samples. . . .	4
			12. Should we check this against cationic surfactant experiments [17]?	4
			13. More simulation data for systems with negatively charged lipids and CaCl ₂ to be collected	4
			11. Get the small concentration data from the inserts . .	5

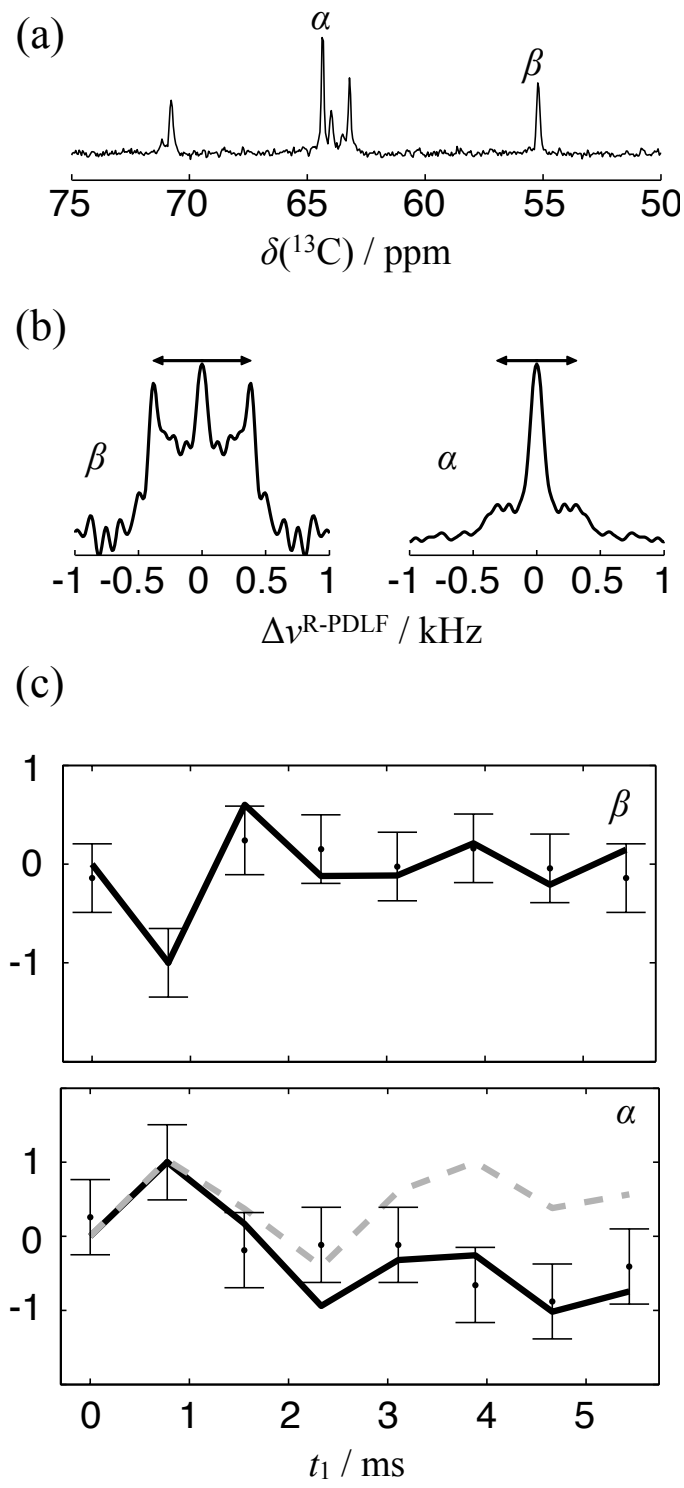


FIG. 13: Experimental results for sign measurement for POPS sample