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

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(Dated: June 5, 2017)

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.

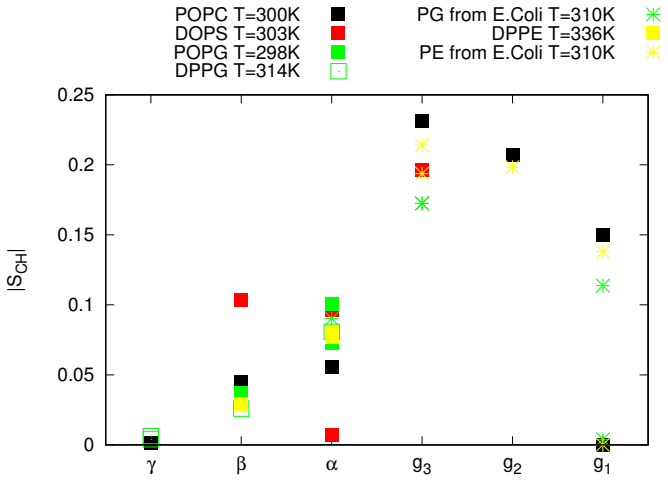

FIG. 1: Absolute values of order parameters for headgroup and glycerol backbone with different headgroups from experiments. POPC values are from [3], DOPS from [4] contains 0.1M of NaCl, POPG from [5] contains 10nM PIPES, DPPG from [6] contains 10mM PIPES and 100mM NaCl, DPPE from [7], E.coliPE and E.coliPG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

Absolute values of order parameters between Slipids simulations [**?**] and experiments for DPPE are shown in Fig. 2. Glycerol bakcbone 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 opposite than for PC.

Comparison between experiments and simulations with CHARMM GUI parameters for PS lipids in Fig. 3 suggest that the model overestimates magnitudes in β and g_3 order

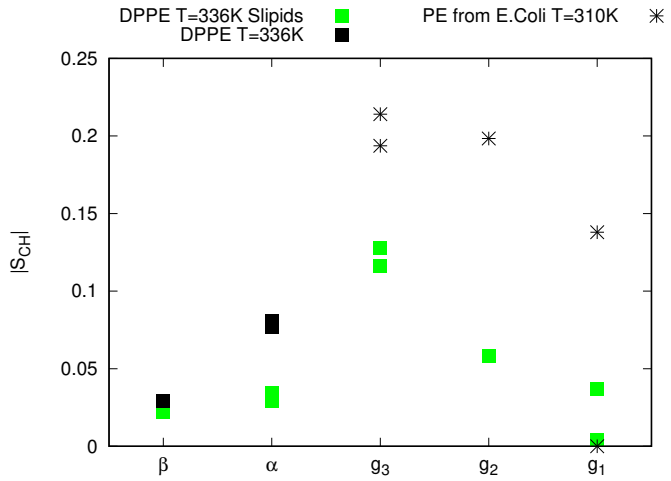

FIG. 2: Order parameters for DPPE headgroup and glycerol backbone from simulations with Slipids [**?**] and experiments (DPPE from [7] and E.coliPE from [8]). Absolute values are shown, because signs are not known experimentally.

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

parameters and forking in α.

Comparison between experiments and simulations with CHARMM GUI parameters for PG lipids in Fig. 4 suggest that the model overestimates forking in γ and α order parameters, and magnitudes of β and g_1 order parameters.

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 5, 6 and 7, 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. 6). 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. 7). 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;*

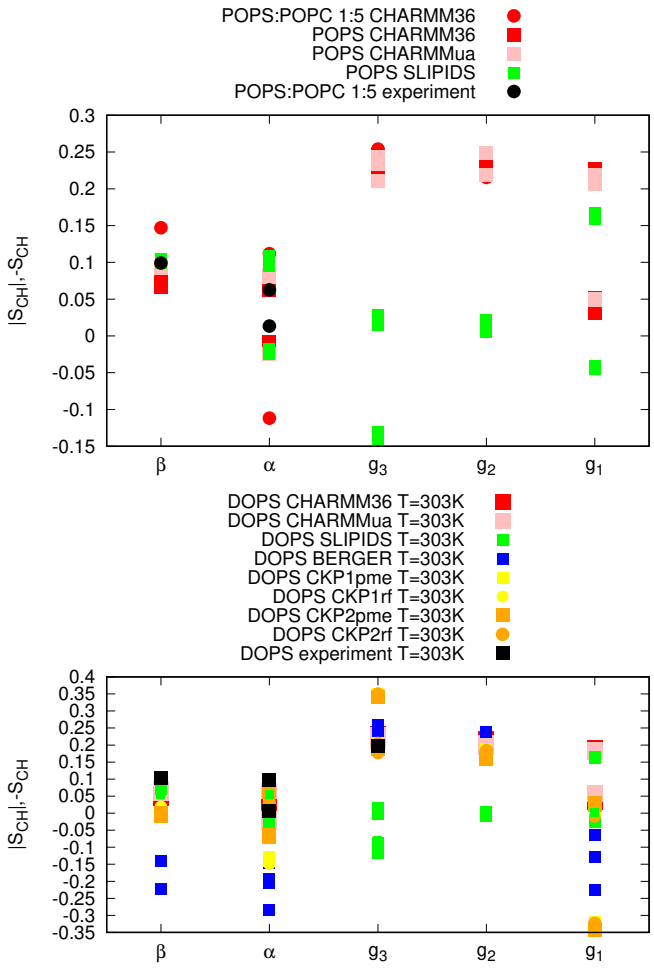

FIG. 3: Order parameters for POPS (top) and DOPS (bottom) headgroup and glycerol backbone from simulations with different models and experiments without $CaCl_2$ (DOPS from [4] contains 0.1M of NaCl, POPC:POPS mixture from [12]). Absolute values are shown for experimental data, because signs are not known. Simulations values are -S_{CH}

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.Experimental signs of the order parameters would highly useful.

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

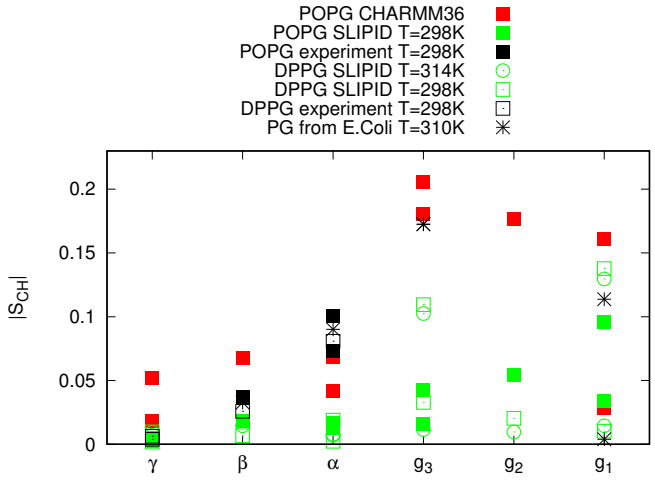

FIG. 4: Order parameters for PG headgroup and glycerol backbone from simulations with CHARMM GUI parameters and experiments without $CaCl_2$ (POPG from [5] contains 10mM of PIPES, DPPG from [6] contains 10mM PIPES and 100mM CaCl, E.Coli PG results from [8]). Absolute values are shown, because signs are not known for experimental data. Glycerol backbone order parameters are negative in simulations, while $alpha$, β and smaller γ are positive.

7.More simulation data for lipids with different headgroups to be collected 8.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. 9.Experimental signs of the order parameters would highly useful.

> *(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. 8. **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

13.Simulation data for systems with negatively charged lipids and $CaCl_2$ to be collected

CONCLUSIONS

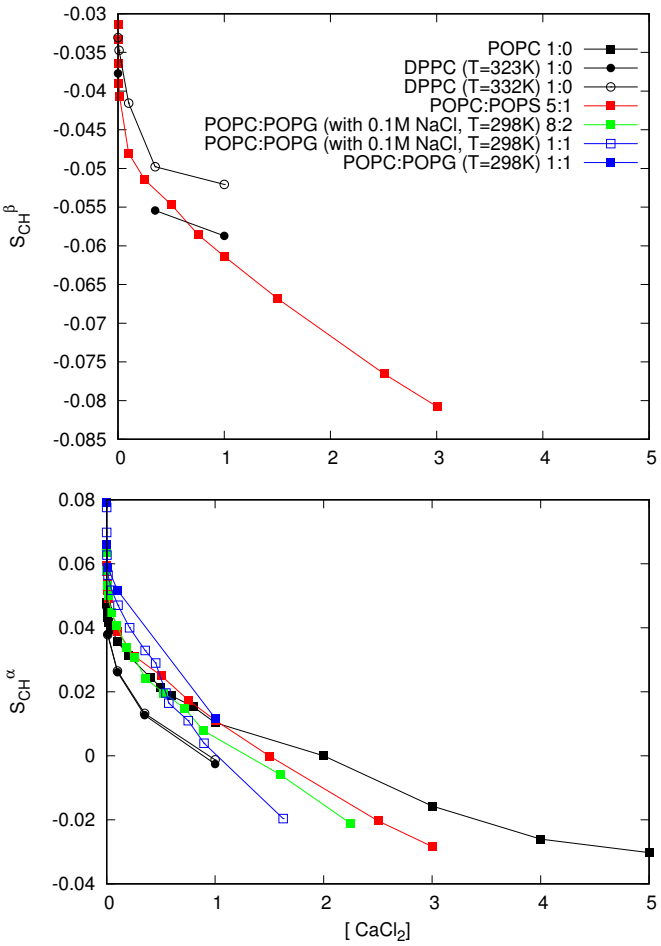

FIG. 5: PC headgroup order parameters as a function of CaCl concentration from experiments containing charged lipids. Pure DPPC data from [15], pure POPC data from [16], POPC:POPS mixture data from [12], POPC:POPG mixture data with 0.1M NaCl from [14] and POPC:POPG mixture data without NaCl from [5].

10.Check the NaCl concentrations in the samples.

SUPPLEMENTARY INFORMATION

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[1] A. Botan, F. Favela-Rosales, P. F. J. Fuchs, M. Javanainen, M. Kanduč, W. Kulig, A. Lamberg, C. Loison, A. Lyubartsev, M. S. Miettinen, et al., J. Phys. Chem. B **119**, 15075 (2015).
[2] A. Catte, M. Girych, M. Javanainen, C. Loison, J. Melcr, M. S. Miettinen, L. Monticelli, J. Maatta, V. S. Oganesyan, O. H. S. Ollila, et al., Phys. Chem. Chem. Phys. **18**, 32560 (2016).
[3] T. M. Ferreira, F. Coreta-Gomes, O. H. S. Ollila, M. J. Moreno, W. L. C. Vaz, and D. Topgaard, Phys. Chem. Chem. Phys. **15**, 1976 (2013).
[4] J. L. Browning and J. Seelig, Biochemistry **19**, 1262 (1980).
[5] F. Borle and J. Seelig, Chemistry and Physics of Lipids **36**, 263 (1985).

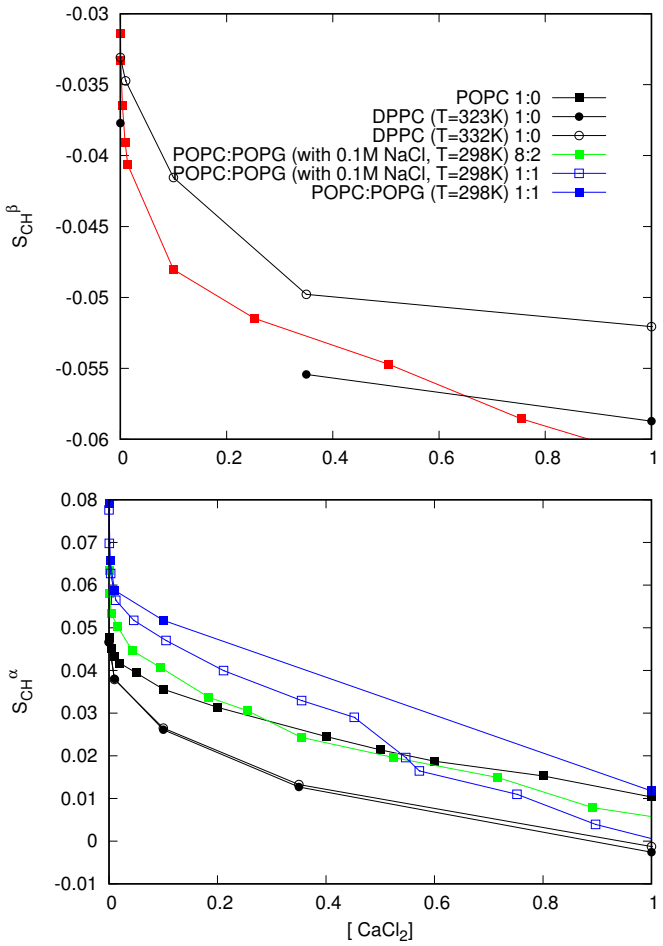

FIG. 6: Figure 5 zoomed to smaller concentrations.

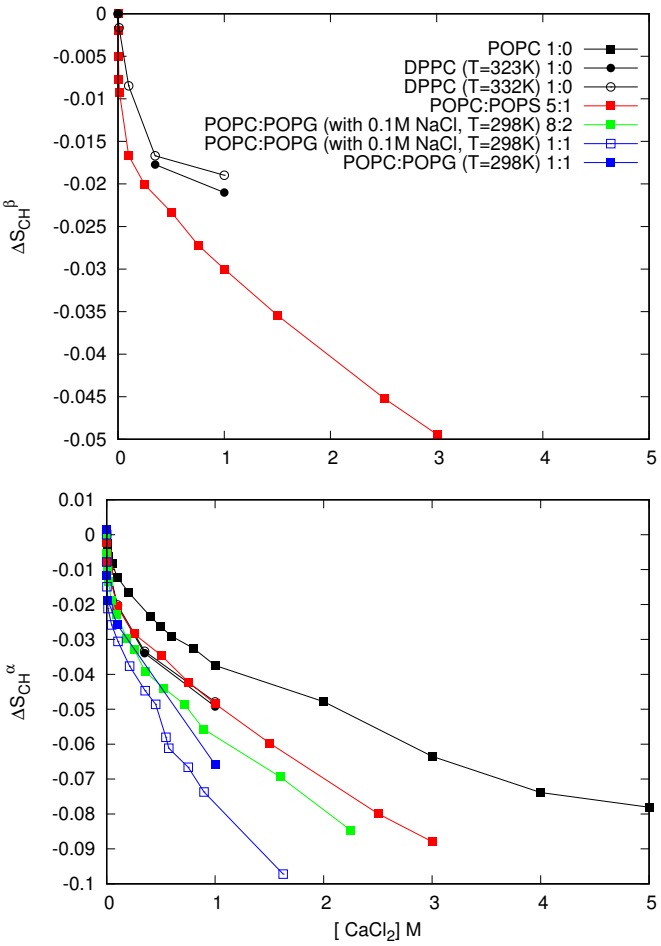

FIG. 7: The change of PC headgroup order parameters in the presence of different amount of negatively charged lipids respect to the values without added $CaCl_2$. The original data is the same as in Fig. 5.

[6] R. Wohlgemuth, N. Waespe-Sarcevic, and J. Seelig, Biochemistry **19**, 3315 (1980).

[7] J. Seelig and H. U. Gally, Biochemistry **15**, 5199 (1976).

[8] H. U. Gally, G. Pluschke, P. Overath, and J. Seelig, Biochemistry **20**, 1826 (1981).

[9] P. Scherer and J. Seelig, EMBO J. **6** (1987).

[10] J. Seelig, Cell Biology International Reports **14**, 353 (1990), ISSN 0309-1651, URL `http://www.sciencedirect.com/science/article/pii/030916519091204H`.

[11] G. Büldt and R. Wohlgemuth, The Journal of Membrane Biology **58**, 81 (1981), ISSN 1432-1424, URL `http://dx.doi.org/10.1007/BF01870972`.

[12] M. Roux and M. Bloom, Biochemistry **29**, 7077 (1990).

[13] J. Seelig, P. M. MacDonald, and P. G. Scherer, Biochemistry **26**, 7535 (1987).

[14] P. M. Macdonald and J. Seelig, Biochemistry **26**, 1231 (1987).

[15] H. Akutsu and J. Seelig, Biochemistry **20**, 7366 (1981).

[16] C. Altenbach and J. Seelig, Biochemistry **23**, 3913 (1984).

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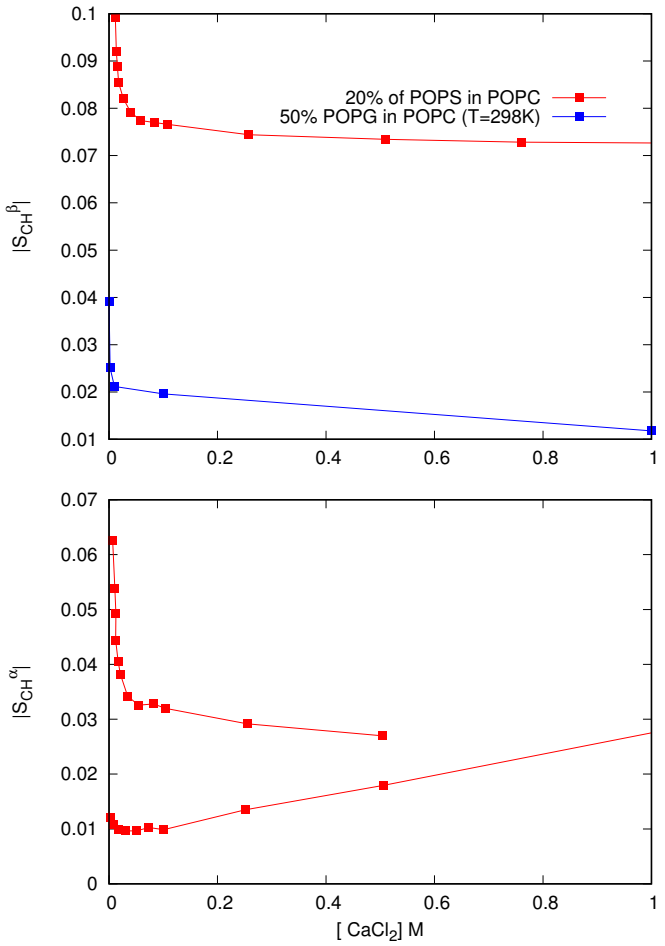

FIG. 8: PG and PS order parameters a function $CaCl_2$ concentration taken from [5] and [12], respectively.

12.Get the small concentration data from the inserts

8. 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. 3

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13. Simulation data for systems with negatively charged lipids and $CaCl_2$ to be collected 3

10. Check the NaCl concentrations in the samples. . . 3

12. Get the small concentration data from the inserts . 5