NMRlipids III: Lipid-cholesterol interactions in atomistic resolution molecular dynamics simulations

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The quantitative quality of lipid-cholesterol interactions in atomistic resolution models will be determined against NMR and scattering data.

INTRODUCTION

1.We should emphasize the validation aspect of intermolecular interactions in binary lipid mixtures in the introduction.

Details of intermolecular interactions determine the phase behaviour and details lateral organization of lipid and bilayers containing cholesterol [1]. Formation of lateral heterogeneities [2], lipid rafts [3] and superlattices [4] in cellular membranes have been suggested to be driven by interactions between cholesterol and lipids. While detailed experimental information of these interactions is relatively sparse, atomistic resolution molecular dynamics simulations have been widely applied to give detailed explanations of lipid bilayer lateral organization and lipid-cholesterol interactions [5**?** , 6]. However, the simulations must reproduce the measured experimental details, such as NMR order parameters and scattering form factors, to be useful in intepretation of molecular details in lipid bilayer mixtures.

Simulations qualitatively reproduce the cholesterol condensation effect, but quantitative comparison to robust experimental data has not been typically done. This is partly due to the lack of availability of systematic experimental data sets for the comparison and partly due to the lack of protocols for validating intermolecular interactions in lipid bilayer simulations against experiments. Scattering experiments are more difficult to interpret for mixed lipid bilayers than for single component bilayers [7**?** –9], thus area per molecule values, one of the main quantity used to compare simulations to experiments, have not been available for lipid cholesterol mixtures. Systematic experimental data set for lipid C-H bond order parameters with different cholesterol concentrations in POPC bilayer has been published only relatively recently [10].

In this work we present the experimental scattering form factor data for POPC-cholesterol mixtures by systematically increasing the cholesterol concentration, scanning the same concentrations that were previously studied with ssNMR [10]. Our goal is to show that the combination of systematically measured C–H bond order parameter and scattering form factor data can be used to validate the quality of lipid–cholesterol intermolecular interactions in MD simulations. MD simulations can be also potentially used to give structural interpretation for form factors measured from mixed systems, which is a major challenge for current methods [7**?** –9]. The approach should be also applicable for mixed lipid bilayers with other than lipid–cholesterol mixtures.

Order parameters for C-H bond vectors in lipid bilayer systems, measured with ^{13}C or ^{2}H NMR techniques, give indirect information about the structural sampling of indidual molecules [11]. The observed increase of acyl chain order parameters with added cholesterol in simulations and experiments can be explained by increased trans conformations in acyl chains [10**?**], which is suggested to play critical role in the phase behaviour of PC-lipid–cholesterol mixtures [1]. Consequently, the correct cholesterol ordering effect is expected to be a necessary condition for a model used to understand lipid–cholesterol phase behaviour.

METHODS

X-ray scattering experiments

SAXS data on POPC multilamellar vesicles (MLVs) at various cholesterol concentrations has been measured. Data have been obtained at the EMBL BioSAXS beamline (Hamburg) using 20 keV photons, T = 27°C. Data were analyzed in terms of the SDP-GAP model described in Heftberger et al., J. Appl. Cryst. 2013 and Heftberger et al. Biophys. J. 2015. Data from MLVs are a convolute of structure factor (the crystalline lattice) and form factor. By fitting the scattered intensity data we obtain both contributions. Here we posted only form factors (ASCII format). For information on the quality of the fit we also give plots of the fitted intensity data. The electron density profile has been modelled in terms of the SDP model (see papers by Kucerka and coworkers), that is volume distribution functions are modelled by individual Gaussians or error

functions. Cholesterol is also accounted for by two Gaussians. This model has been proposed by Jianjun Pan (USF, Tampa, FL), but is to the best of our knowledge not published (see also PhD Thesis by Peter Heftberger). Additional figures show the volume distribution functions and the resulting electron density profiles.

Authors to consult and potentially include in publications using this data: Peter Heftberger (peter.heftberger@gmx.at), Georg Pabst (georg.pabst@uni-graz.at)

Molecular dynamics simulations

Molecular dynamics simulation data was collected using the open collaboration method [12]. Simulated systems are listed in Table I and the full simulation details are given in references or in supplementary material.

Quantitative quality measure for lipid bilayer structure

2.To be written once we have agreed on the used measure.

TABLE I: Simulated lipid bilayers containing cholesterol. The simulation file data sets marked with * include also part of the trajectory. [a] The number of lipid molecules [b] The number of cholesterol molecules [c] Cholesterol concentration (mol%) [d] The number of water molecules [e] Simulation temperature [f] The total simulation time [g] Time frames used in the analysis [h] Reference link for the downloadable simulation files [i] Reference for the full simulation details

Force field	lipid	[a]N_l	[b]N_{chol}	[c]C_{CHOL}	[d]N_w	[e]T (K)	[f]t_{sim}(ns)	[g]t_{anal} (ns)	[h]Files	[i]Details
Berger-POPC-07 [13] /Höltje-CHOL-13 [10, 16]	POPC	128	0	0%	7290	298	270	240	[14]*	[15]
	POPC	120	8	6%	7290	298	100	80	[17]*	[10]
	POPC	110	18	14%	8481	298	100	80	[18]*	[10]
	POPC	84	44	34%	6794	298	100	80	[19]*	[10]
	POPC	64	64	50%	10314	298	100	80	[20]*	[10]
	POPC	50	78	61%	5782	298	100	80	[21]*	[10]
CHARMM36[22, 23]	POPC	200	0	0%	9000	310	?	100	[24]*	SI
	POPC	200	22	10%	9000	310	?	100	[24]*	SI
	POPC	200	50	20%	9000	310	?	100	[24]*	SI
	POPC	200	86	30%	9000	310	?	100	[24]*	SI
	POPC	200	134	40%	15030	310	109	100	[25]*	SI
	POPC	200	200	50%	18000	310	109	100	[25]*	SI
Slipids[26–28]	POPC	200	0	0%	?	310	?	100	[29]*	SI
	POPC	512	0	0%	23943	310	170	100	[30]*	SI
	POPC	200	22	10%	?	310	?	100	[29]*	SI
	POPC	200	50	20%	?	310	?	100	[29]*	SI
	POPC	200	86	30%	?	310	?	100	[29]*	SI
	POPC	358	154	30%	21183	298	170	100	[31]*	SI
	POPC	200	134	40%	?	310	109	100	[32]*	SI
	POPC	200	200	50%	?	310	109	100	[32]*	SI
	POPC	256	256	50%	20334	298	170	100	[33]*	SI
MacRog[34]	POPC	128	0	0%	6400	310	400	200	[35]*	[12]
	POPC	114	14	11%	6400	310	400	200	[35]*	[12]
	POPC	72	56	44%	6400	310	400	200	[35]*	[12]
	POPC	64	64	50%	6400	310	400	200	[35]*	[12]
	POPC	56	72	56%	6400	310	400	200	[35]*	[12]

RESULTS AND DISCUSSION

Monitoring molecular interactions between POPC and cholesterol in lipid bilayer using NMR and MD simulations

Addition of cholesterol substantially increases the acyl chain C-H order parameters in PC lipid bilayers (Fig. 1), which can be explained by increased trans conformations in acyl chains [10**?**]. The absolute values of order parameters of sn-1 chain from ^{13}C NMR experiments [10] exhibit approximately linear increase with cholesterol up to equimolar mixture (Fig. S2), which is expected because phase separation is not observed for this mixture [10, 36].

Experimental acyl chain order parameters of pure POPC lipid bilayers are well reproduced by most force fields (Fig. 1), except for the C_2 carbon in *sn*-1 chain, which is typically the case in the state of the art lipid force fields (for review see [11]). However, the order parameters in the beginning of *sn*-1 chain are underestimated in the MACROG simulation and CHARMM36 simulations slightly overestimates the order parameters, also when used with other than Gromacs simulation packages (section S1 in the supplementary information). The absolute values of *sn*-1 acyl chain order parameters exhibit approximately linear increase upon addition of cholesterol also in simulations below equimolar mixture (Figs. S3-S7). However, the slope of the increase (Fig. 2) and order parameters in equimolar mixtures (Fig. 1) are overestimated by all force fields. The overestimation of order parameters observed in CHARMM without cholesterol and in all force fields with cholesterol is smaller than the contribution by undulations in large simulations (section S3 in the supplementary information).

4.Also experimental cholesterol order parameters are available. Maybe these should be calculated from simulations as well.

Lipid bilayer dimensions and density profiles as a function of cholesterol

Lipid bilayer dimensions can be experimentally accessed by measuring X-ray scattering form factor, which is related to the electron density along membrane normal via Fourier transformation [7–9, 11**?**]. The form factor can be translated to density profiles, area per lipids and bilayer thickness using SDP model or its combination with MD simulations [7**?** –9]. Sophisticated approaches for single component lipid bilayers are available [**?**], but multicomponent systems are more difficult to interpret [7**?** –9].

State of the art force fields give good agreement with experimental form factors for pure POPC lipid bilayers [**?**], which is the case also here, except for the Berger simulation (Fig. 3). Upon addition of cholesterol, the third minima in form factor systemically decreases from $\sim$0.42 to $\sim$0.32 This may be related to the reduced area per molecule upon addition of cholesterol (Fig. 4). The area per total amount of molecules decreas with cholesterol in all simulations, partly due to the smaller area covered by the cholesterol than lipids, but also because lipids ordered by cholesterol require less space. Due to the latter effect, the area per PC headgroup does not essentially increase up to the addition of $\sim$15 mol% of cholesterol.

Visual inspection of form factors (Fig. 3) and quantitative quality estimation (Fig. 5) suggests that the Berge/Holtje force field gives the best agreement with experiments with large cholesterol concentration. This is surprising because the same model overestimated acyl chain order parameter increase upon addition of cholesterol more than CHARMM36 or Slipids simulations (Figs. 1 and 2). The Berger/Holtje model also exhibits the most pronounced decrease in the area per molecule upon addition of cholesterol (Fig. **??**). On the other hand, the electron density profiles from Berger/Holtje simulations are closer to the result from the SDP model then other MD simulation models for both pure POPC bilayer and equimolar POPC/cholesterol mixture, especially in the middle of the bilayer where SDP model gives substantially lower density than MD simulations (Fig. 6). Therefore, the good quality of form factor Berger/Holtje model with large amounts of cholesterol may rather indicate the importance of this low electron density in the bilayer center, rather than better quality of lipid-cholesterol interactions in this model.

Presenting the quality of lipid bilayer MD simulation with binary mixtures

All the tested MD simulation models qualitatively reproduced experimentally observed condenced and ordering effects of cholesterol. However, the acyl chain ordering was overestimated and quality of form factors reduced with increasing amount of cholesterol. Although the biological importance of these details is unclear, overestimated ordering may promote formation of liquid ordered phases and inaccuate form factors complicate interpretation of scattering experiment with MD simulations. Therefore, MD simulation force fields correctly reproducing the order effects upon addition of the cholesterol would be invaluable.

To facilitate the comparison of the quality of complex lipid bilayers, we introduce the quantitative quality measures which measure the quality of intermolecular interactions in binary lipid bilayers from different perspectives. The simple difference between experimental and simulated C-H bond order parameters give a very detailed picture about the quality of molecular conformations in all positions of lipid bilayer (Fig. 7). However, complexity of such presentation increases with increasing amount of data and it may be hard to present in practical format readable for human or machine. Using root mean square deviation across regions in the molecule simplifies the presentation, but compromizes the spatial resolution. Here, we divide the lipid into headgroup, glycerol backbone, sn-1 and sn-2 chains to present quality of binary lipid bilayer in more convenient format (Fig. 7). **9.Discussion to be finished once we have the fitness function defined.**

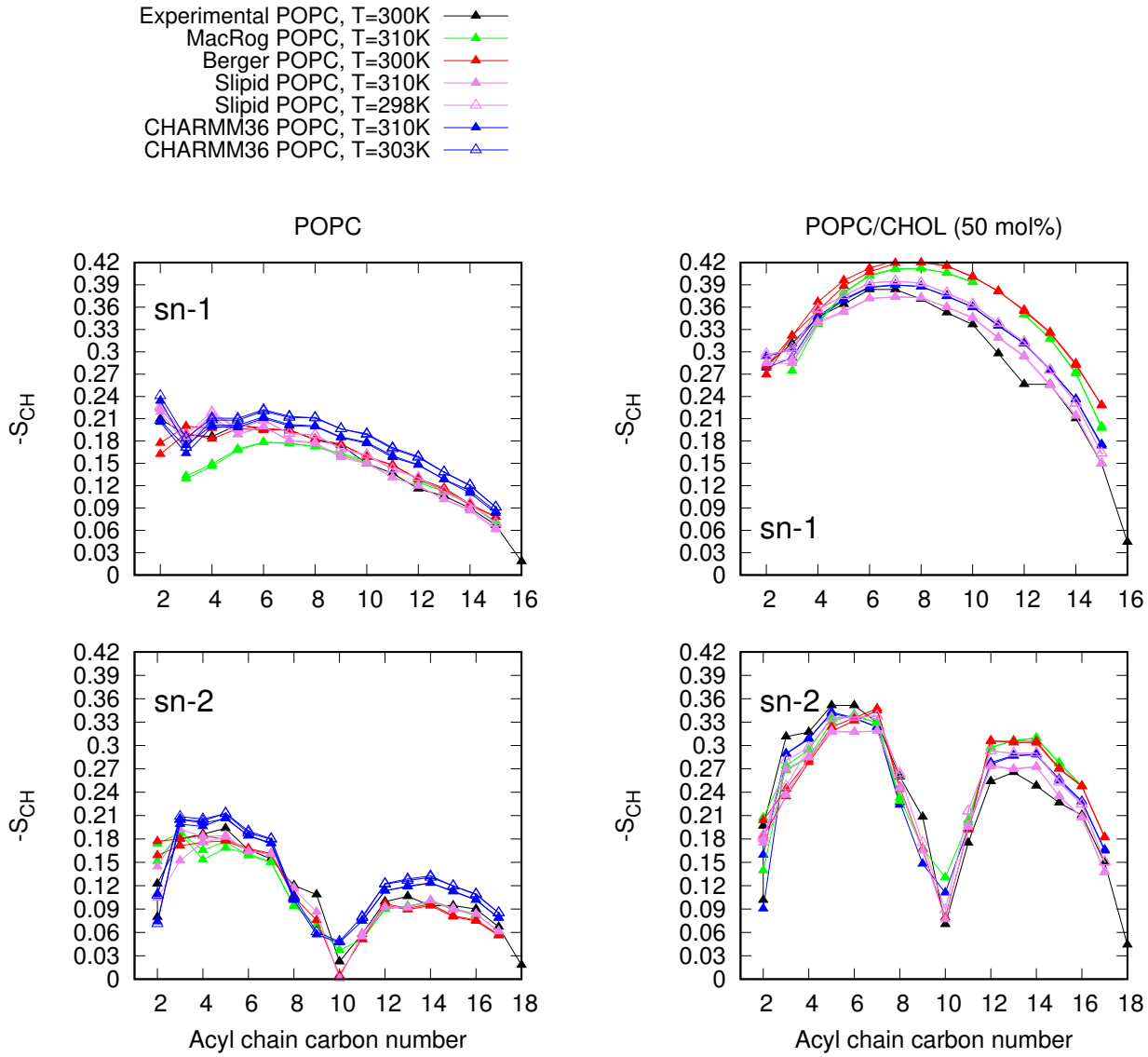

FIG. 1: Order parameters from simulations and experiments for acyl chains of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC).

3.Lipid14 results?

CONCLUSIONS

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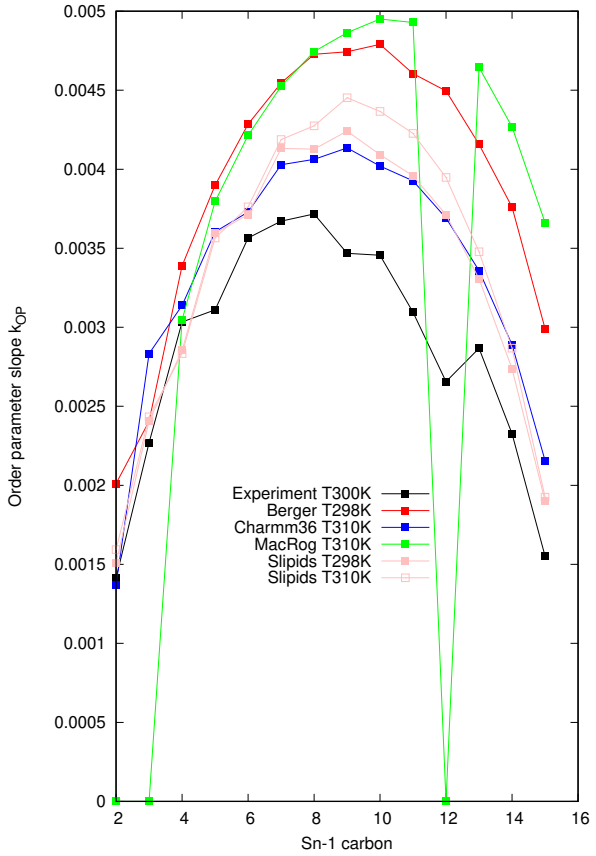

FIG. 2: Slopes of order parameters as a function of cholesterol. Determined by fitting equation $S_{\mathrm{CH}}(C_{\mathrm{chol}}) = k_{\mathrm{OP}} C_{\mathrm{chol}} + S_{\mathrm{CH}}(0)$ to the data in figures S2-S7.

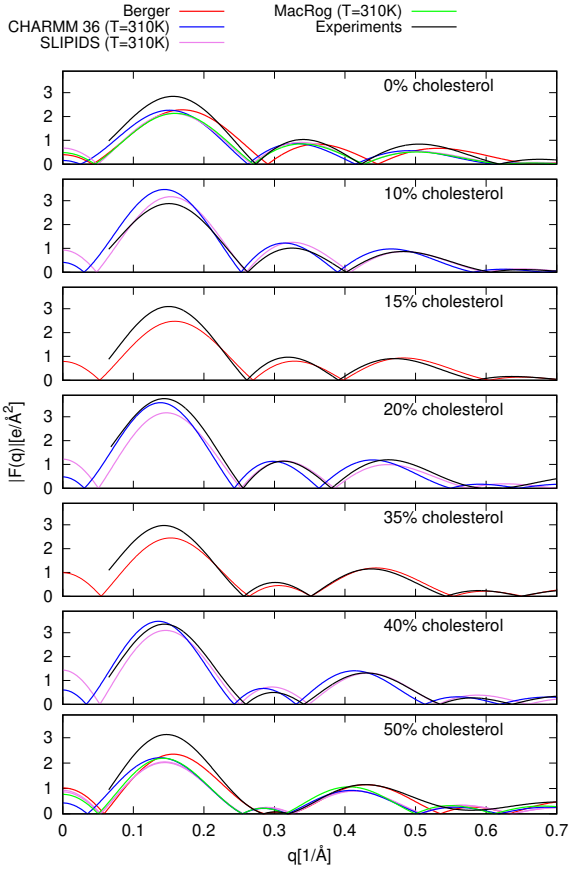

FIG. 3: Form factors from simulations and experiments.

5.Details about form factor calculation code is discussed in issues https://github.com/NMRLipids/MATCH/issues/56 and https://github.com/NMRLipids/MATCH/issues/50. Once the code is finalized, we should recalculate and check the form factors. CURRENTLY, 10%-40% cholesterol concetrations are calculated with a code which gives possibly incorrect heigths for the maxima.

6.The y-axis scale cannot be explicitly measured in experiments. Therefore, the y-axis of the experimental form factor is typically scaled to match with simulations. This currently not done, because we have several simulations which give inequal form factors, and therefore it is not clear against which simulation results we should scale the experimental results. I have created a issue for this discussion: https://github.com/NMRLipids/NmrLipidsCholXray/issues/18

7.Not all experimental and simulation data is here (maybe?).

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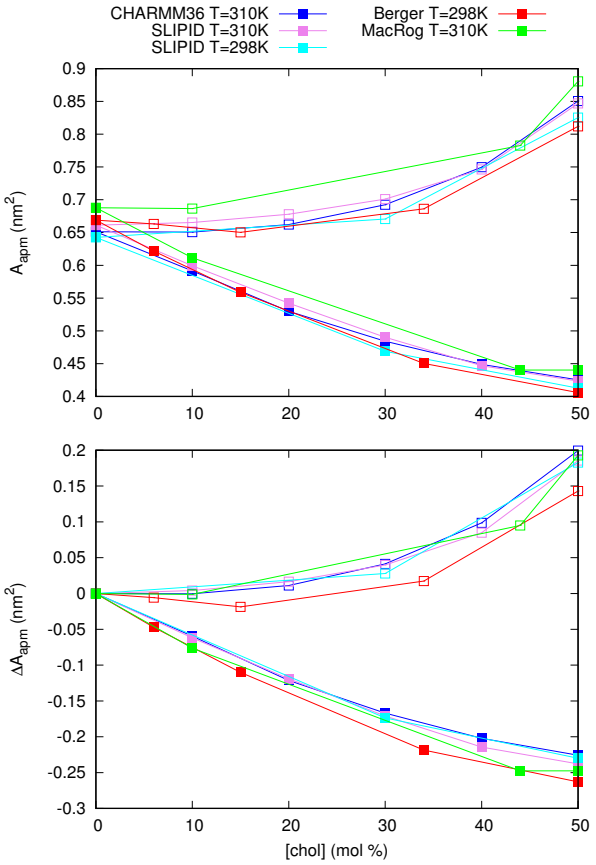

FIG. 4: Area per molecules calculated from different simulation models as a function of cholesterol concentration. The solid symbols are area per total amount of molecules (chol+PC) and the empty symbols area per PC headgroups. Top figure shows absolute values and bottom figure shows changes respect to pure lipid system.

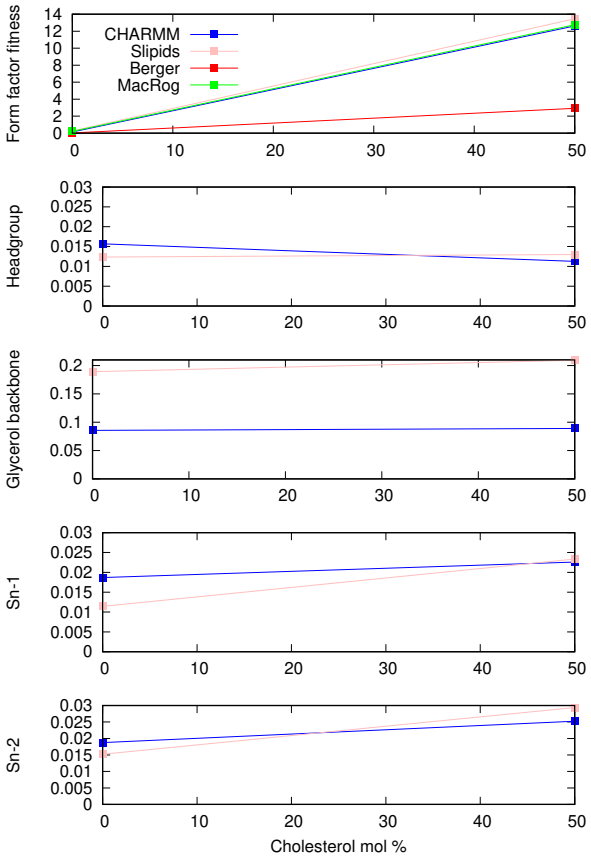

FIG. 5: Fitness of form factors between simulations and experiments.

8.Figure to be done properly when the fitness code is finished. This is discussed in issues https://github.com/NMRLipids/MATCH/issues/65, https://github.com/NMRLipids/MATCH/issues/64, https://github.com/NMRLipids/MATCH/issues/56, and https://github.com/NMRLipids/MATCH/issues/50

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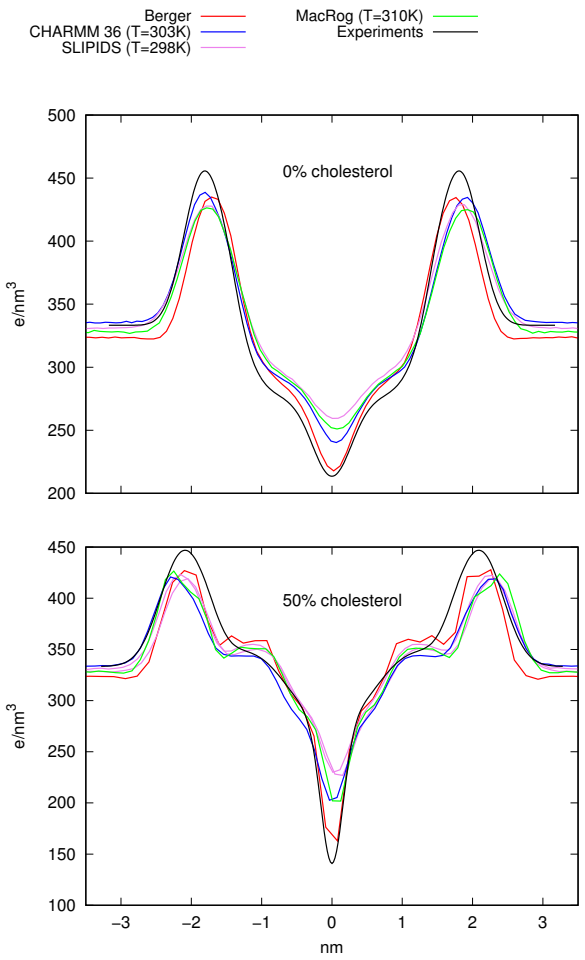

FIG. 6: Electron density profiles from MD simulations and SDP model.

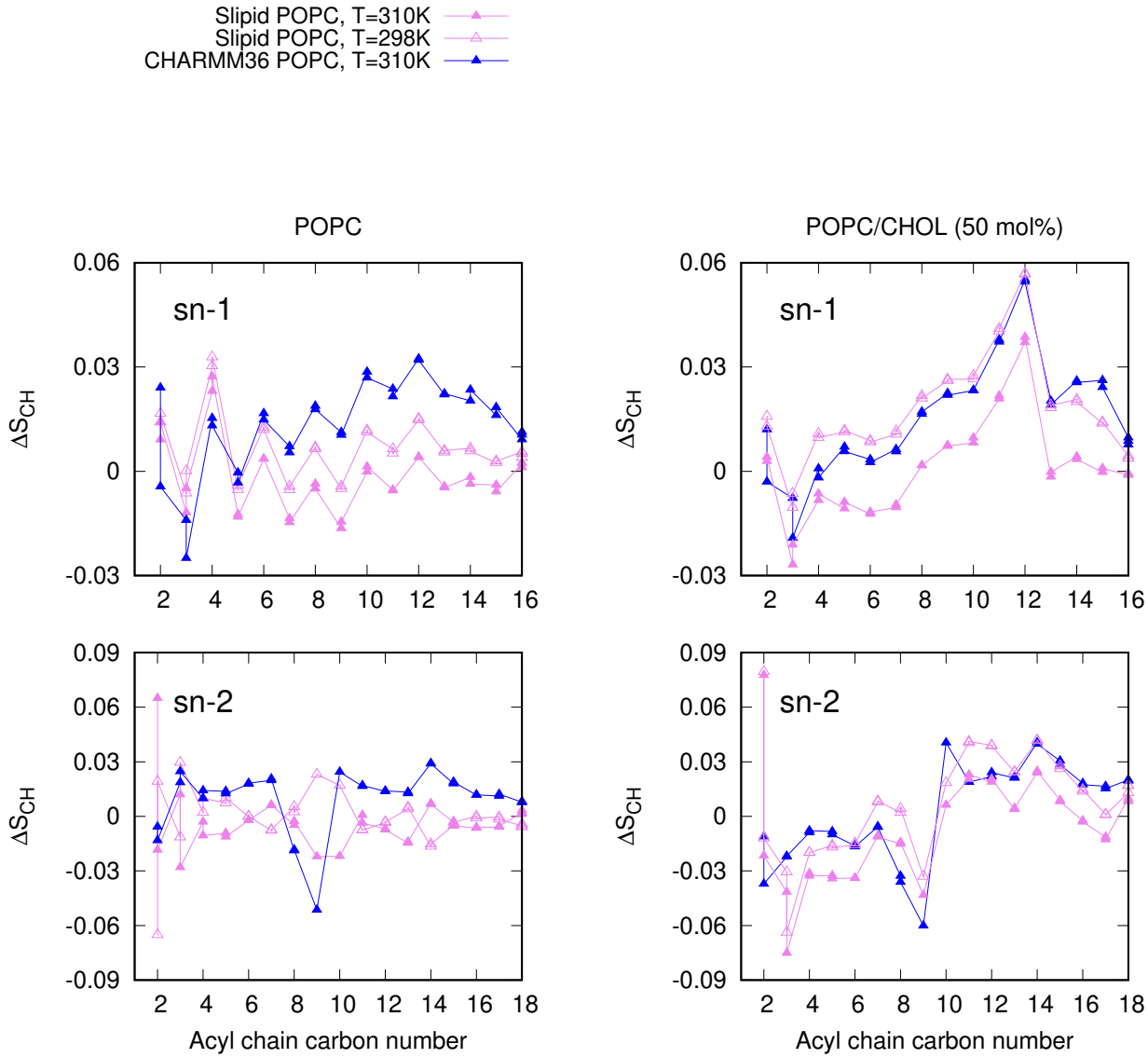

FIG. 7: Difference between order parameters from simulations and experiments for acyl chains of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC).

10.Figure to be done properly when the fitness code is finished. This is discussed in issues https://github.com/NMRLipids/MATCH/issues/65, https://github.com/NMRLipids/MATCH/issues/64, https://github.com/NMRLipids/MATCH/issues/56, and https://github.com/NMRLipids/MATCH/issues/50