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

Details of intermolecular interactions determine the phase behaviour and details lateral organization of lipid and bilayers containing cholesterol [1]. Formation 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 details, like 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 quantitatve 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 also the experimental scattering form factor data for POPC-cholesterol mixtures by systematically increasing the cholesterol concentration. 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 MD simulations. MD simulations can be also potentially used to give structural interpretation for form factor 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.

RESULTS AND DISCUSSION

Structural sampling of molecules in bilayers with cholesterol

Order parameters for C-H bond vectors in lipid bilayer systems, measured with ^{13}C or ^{2}H NMR techniques, give indirect information about 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 phase behaviour in PC lipid-cholesterol mixtures [1]. Consequently, the correct cholesterol ordering effect is expected to be necessary condition for a model used to understand lipid-cholesterol phase behaviour.

Acyl chain order parameters for pure 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayer and mixture with 50 mol% of cholesterol from different simulations and experiments are shown in Fig. 1. Phase separation is not observed for this system [10, 12], thus the intermolecular interactions are expected to have maximum effect in equimolar mixture. Experimental acyl chain order parameters are typically well reproduced in state of the art lipid models for pure lipid bilayers (for review see [11]). This is also mainly observed in the data presented here, except that CHARMM36 slightly overestimates the order parameters and MACROG model underestimates order parameters in the beginning of sn-1 chain. In equimolar mixtures with cholesterol all models overestimate the order parameters. For Berger/Holtje and MACROG models the overestimation is significant, while less severe for CHARMM36 and Slipid models.

1.Is overestimation in CHARMM36 and Slipid significant or not?

2.Lipid14 results from recent article should be added in this figure

The order parameter changes as a function of cholesterol for each segment are shown in Fig. 2 (currently only sn-1).

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

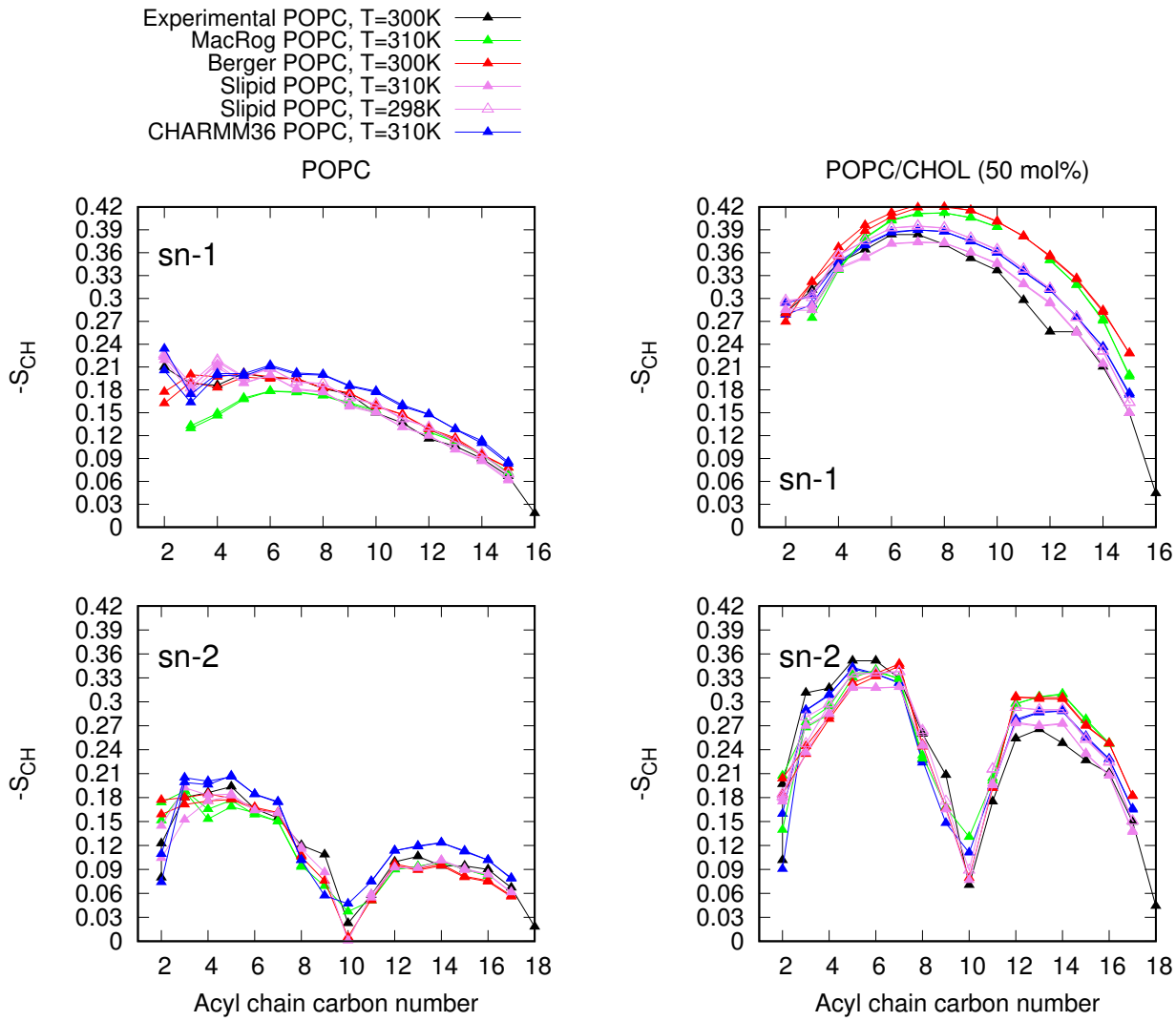

FIG. 1: Order parameters from simulations and experiments for acyl chains of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC).

Lipid bilayer dimensions and density profiles as a function of cholesterol

Cholesterol induced changes in lipid packing, thickness and density profiles are suggested to be relevant, for example, for membrane protein interactions and cellular membrane lateral organization [**?**]. Experimental and simulation studies show that the cholesterol induces the so called ”condensing effect”, i.e. acyl chain ordering increases the membrane thickness and reduces the area per molecule in lipid bilayers [**?**]. This is demonstrated from simulations in Fig. 3, showing the area per PC headgroups and per total number of molecules as a function of cholesterol concentration from different models. All models show reduction of area per total amount of molecules, 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 do not essentially increase up to the addition of $\sim$15 mol% of cholesterol.

5.Membrane thickness to be plotted as well.

Bilayer dimensions are indirectly experimentally accessible by scattering methods and have been widely used to validate lipid bilayer simulations [11**?**]. While sophisticated models are applied to extract area per molecule and thicknes for single component lipid bilayers, the results for multicomponent systems are more difficult to interpret [7**?** –9]. In such case the validation can be done by comparing directly measurable form factors between simulations and experiments. If simulation reproduces the form factor, it can be also used to interpret the experiments. The form factors from different simulation models with different cholesterol concentrations are compared to experimental data Fig. 4.

6.Discussion to be finished when the data is solid.

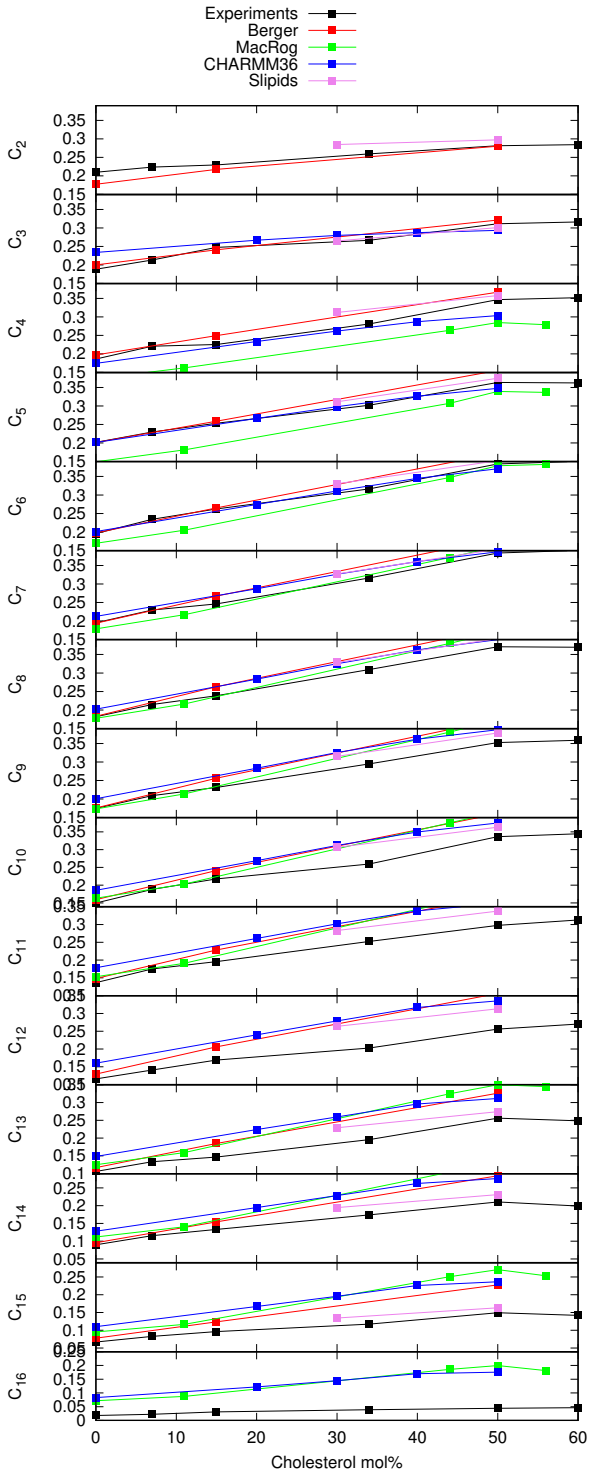

FIG. 2: Order parameter changes from simulations and experiments for each segment in sn-1 chain of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) as a function of cholesterol concentration.

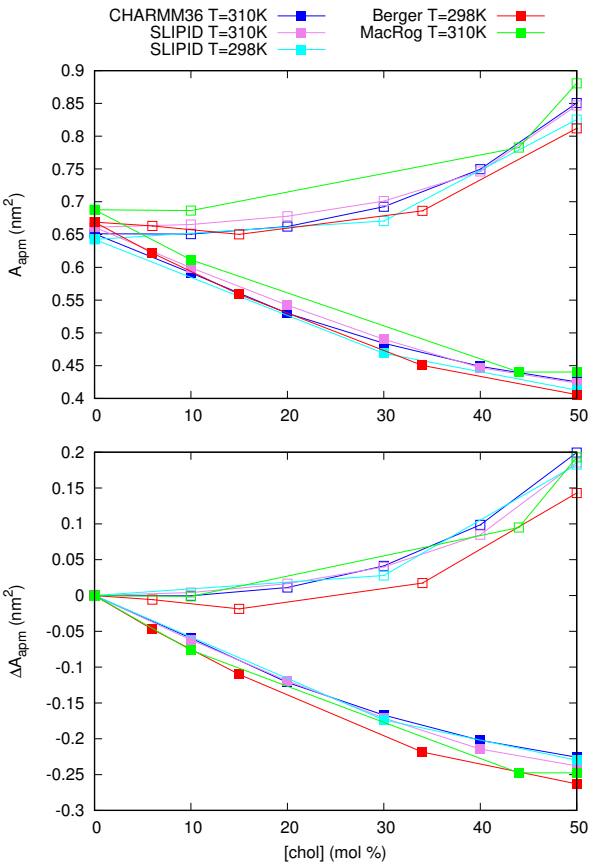

FIG. 3: Area per molecules calculated from different simulation models as a function of cholesterol concentration. Solid marks are area per total amount of molecules (chol+PC) and empty marks are area per PC headgroups. Top figure shows absolute values and bottom figure shows changes respect to pure lipid system.

CONCLUSIONS

Cholesterol ordering effect is overestimated in Berger/Holtje and MacRog models. Slight overestimation is observed also in CHARMM36 and Slipid models, but more careful analysis is required to conclude if this is significant or not.

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 = 27C. 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

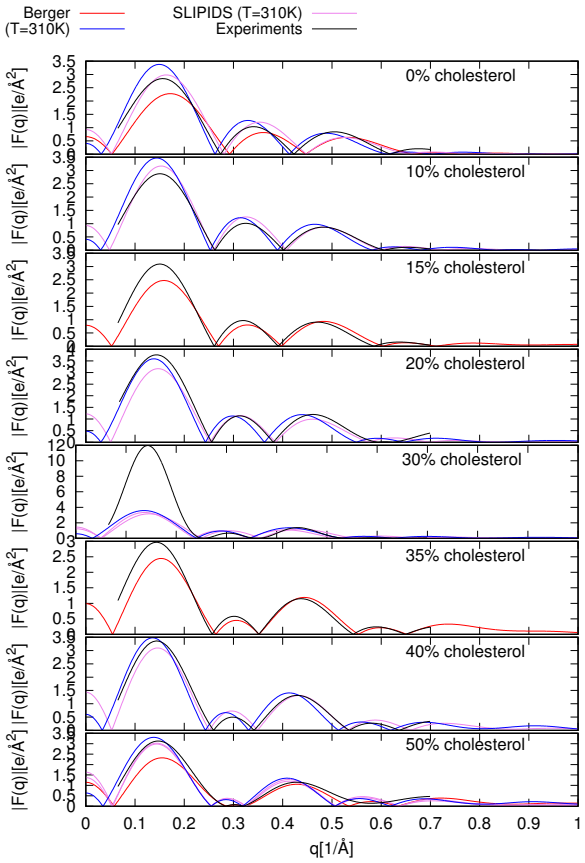

FIG. 4: Form factors from simulations and experiments.

7.Form factor calculation method should be double checked.

8.Experimental form factor amplitudes are not scaled to match with simulations, as done usually

9.Not all experimental and simulation data is here.

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

Simulated systems are listed in Table I and the full simulation details are given in references or in supplementary material.

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]	POPC	128	0	0%	7290	298	270	240	[14]*	[15]
/Höltje-CHOL-13 [10, 16]										
	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]*	[36]
	POPC	114	14	11%	6400	310	400	200	[35]*	[36]
	POPC	72	56	44%	6400	310	400	200	[35]*	[36]
	POPC	64	64	50%	6400	310	400	200	[35]*	[36]
	POPC	56	72	56%	6400	310	400	200	[35]*	[36]

SUPPLEMENTARY INFORMATION

CHARMM36 results from different simulation packages

The results from CHARMM36 model for lipid bilayers from different simulation packages have been reported to give different results in the literature [37, 38]. The results are mainly dependent on different Lennart-Jones cut–off settings, but all the details are not quite understood. In this work we use the results from Gromacs 5 with settings suggested to be optimal by Gromacs webpage. We also compared the results from Gromacs 5 with these settings to the results simulated with NAMD, OpenMM and literature values. Based on comparison shown in Fig. 5, we conclude that Gromacs 5 with settings suggested in webpage gives consistent results with the literature and other simulation packages. Thus these results are used in the main body of the paper. However, order parameters are slightly overestimated respected to the experiments also with these settings.

10.Should be extend this discussion based on discussion at https://github.com/NMRLipids/NmrLipidsCholXray/issues/4 ?

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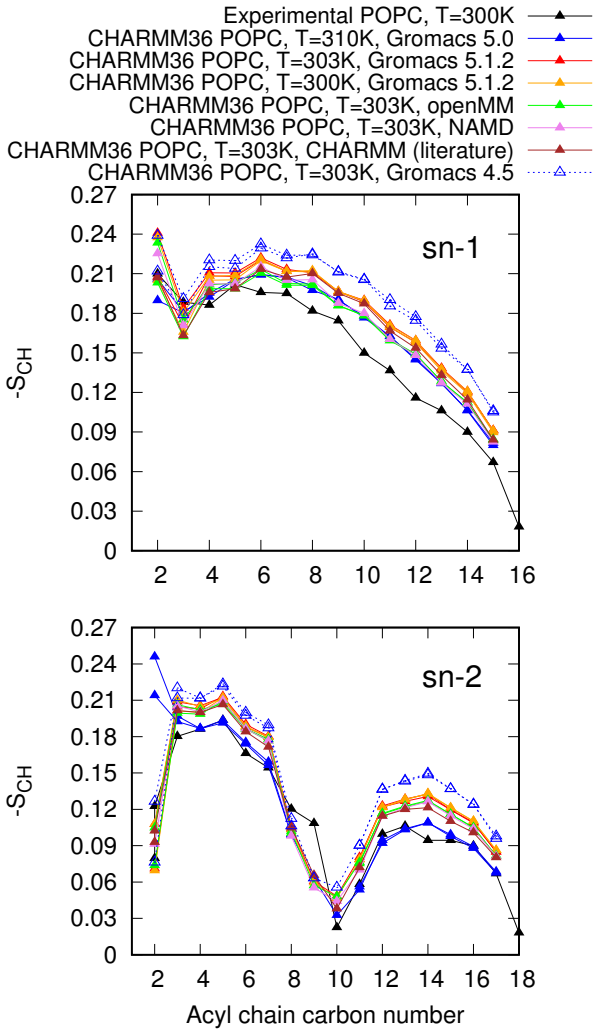

FIG. 5: Results for CHARMM36 model [22] from different simulation packages. Discussion going on at *https://github.com/NMRLipids/NmrLipidsCholXray/issues/4*.

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