Response of the hydrophilic part of lipid membranes to changing conditions – a critical comparison of simulations to experiments

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We compare the order parameters predicted for the hydrocarbon segments in lipid bilayer headgroup region by the Berger molecular dynamics simulation model to those measured by Nuclear Magnetic Resonance (NMR) experiments. We first show results for a fully hydrated POPC bilayer, and then focus on changes of the order parameters as a function of hydration level, NaCl and CaCl$_2$ concentrations, and cholesterol content. The experimental headgroup order parameters are never reproduced. This indicates that under all of these conditions the used model is unable to correctly reproduce the headgroup structure. Consequently, many of the conclusions drawn over the years from this model might be erroneous. This manuscript has not been submitted to any journal, instead its contents are discussed at nmrlipids.blogspot.fi.

I. INTRODUCTION

Lamellar lipid bilayer structures have been widely studied with various experimental and theoretical techniques [1–6]. A typical motivation for these studies has been the use of the lipid bilayer as a simple model for a cell membrane. Due to this great interest in these systems, a large effort has been put to model them quantitatively at the atomistic level [2–5]. Naturally, this is a huge challenge, and to extract useful information from the classical atomistic models one has to know how close to the reality the molecular details are under different conditions. The most straightforward assessment of these models can be done by comparing to scattering and NMR data [2,3]. These comparisons have shown that several simulation models reproduce with relatively good accuracy many structural (area per molecule, electron densities, acyl tail order parameters) and dynamical (acyl tail dynamics, diffusion) properties [2,4]. However, the structure and dynamics of the lipid headgroup part, that is the glycerol and choline parts, are described with variable success. For example, the united atom Berger force field has been recently shown to reproduce incorrect structures and too slow dynamics for these parts [7,8]. Regarding the structure, all atom force fields CHARMM and GAFF seems to perform better [9,10].

Importantly, there are several open questions where atomistic level behaviour of glycerol and choline regions are of interest. Related to the model cell membranes, for example the issue of “hydration repulsion” that has been suggested to originate from hindered headgroup movements instead of water mediated effects [11–13]: also interaction of ions with the headgroup region is of great interest due to its importance in various physiological processes [14]; there is also the cholesterol condensation effect that has been suggested to arise from the “umbrella effect” mediated by lipid headgroups [15]. Atomistic level simulations have been used to study all these topics [16–31].

Order parameters for hydrocarbon C-H vectors, defined as $S_{CH} = \frac{1}{2}(\cos^2 \theta - 1)$, where the average is ensemble average and $\theta$ is the angle between the C-H bond and membrane normal, give very detailed information concerning the structure of lipid molecules. The order parameters can be measured with various NMR techniques and with very high accuracy and reproducibility, furthermore the values are available for a large amount of different lipids [32]. The drawback is that several different distributions of $\theta$ can result in the same order parameter. Thus the sampled structures cannot be uniquely determined from the order parameters. Crucially however, any suggested structure must reproduce the measured order parameters, otherwise it can not be correct. These properties of the $S_{CH}$ order parameter makes it an ideal parameter to compare with molecular dynamics simulations: The quality of the simulation is tested and, if the simulation is correct, the interpretation of the experiment is done simultaneously.

The behaviour of the NMR order parameters for lipid headgroups under varying experimental conditions is relatively well known. For example, the addition of positively charged surfactants leads to an increase of the order parameter for $\beta$ carbon and decrease for $\alpha$ carbon (for labeling, see Fig. 1), and vice versa for negatively charged surfactant [33]. Based on this result, it was postulated that the increase of the $\beta$ carbon order parameter together with decrease of the $\alpha$ carbon order parameter arises from the headgroup dipole orienting more parallel with the membrane normal, and vice versa [33]. The order parameter measurements as a function of hydration agree well with this idea: the $\beta$ carbon order parameter decreases and $\alpha$ increases by dehydration, as would be expected if the headgroup orients more perpendicular to the membrane normal [34,35]. Furthermore, the addition of multivalent cations has a similar effect as cationic surfactants: They increase the $\beta$ and decrease the $\alpha$ carbon order parameters [36]. This is logical, as multivalent cations are expected to penetrate close to the phosphate group [14], thus residing where the charges of cationic surfactants embedded into a lipid bilayer would reside.

In this manuscript we compare the experimentally measured order parameters for glycerol and choline regions with the predictions from the widely used Berger model [37] as a function of hydration level, ion concentration (NaCl,CaCl$_2$) and cholesterol content. It has already been shown that the Berger
model cannot reproduce the experimental order parameters for a fully hydrated POPC bilayer and that its behaviour as a function of cholesterol content is incorrect [7]. Here we show that also the qualitative behaviour as a function of hydration and ion concentration is not correctly described.

II. METHODS

A. Simulated systems

For the POPC bilayer a standard simulation setup with 128 lipid molecules and 7290 water molecules was used [7, 38]. The order parameters were calculated from the last 80 ns of the trajectory totalling 100 ns. For low hydration, the number of water molecules was decreased to 896, corresponding to 7 water molecules per lipid, and the order parameters were calculated from the last 50 ns of a trajectory totalling 60 ns. For simulations with NaCl, the POPC bilayer was solvated with 7202 water molecules, 44 Na\(^+\) and Cl\(^-\) ions, corresponding roughly to [NaCl] \(\approx\) 200 mM. For simulations with CaCl\(_2\), the POPC bilayer was solvated with 7157 water molecules, 44 Ca\(^{2+}\) and 88 Cl\(^-\) ions, corresponding roughly to [CaCl\(_2\)] \(\approx\) 200 mM. The order parameters were calculated from the last 50 ns of a trajectory totalling 110 ns. The results for the POPC/cholesterol systems were taken directly from Ref. [7].

B. Simulation details

All simulations were done with Gromacs 4 simulation package [39]. The Berger force field was used for the POPC [37], with the dihedral potential next to the double bond taken from [40]. GROMOS parameters in ffgmxnb.itp were used for ions and parameters from Höltje [41] for cholesterol. More details for POPC/cholesterol simulations can be found from Ref. [7]. The rest of the simulation details are provided in the *.mdp–files available at [42].

III. RESULTS AND DISCUSSION

A. POPC bilayer under full hydration

The glycerol and choline group order parameters for pure POPC bilayer under full hydration are shown in Fig. 2 These results from simulations and experiments have been discussed previously [7]. Note, however that the larger order parameter for \(\beta\) carbon was missing in Ref. [7], which led to a conclusion that the simulated order parameter for that carbon was in a good agreement with experiments. Fig. 2 shows that the simulations produced different order parameters for different hydrogens attached to the same carbon for \(\beta\), \(\alpha\) and \(g_3\) segments, but in experiments the same values are measured. This means that in simulations these segments sample significantly different conformations than in the experimental system [43]. Most likely the motions in simulations are more restricted, as was also seen in NMR measurements of lipid segment dynamics [8]. For the \(g_1\), separate order parameters for different hydrogens are seen in both, the experiments and simulations, but in simulations the values are significantly higher than in experiments. Also for \(g_1\) and \(g_2\) groups the dynamics is slower in simulations than in experiments [8].

These results indicate that in the used model the structure of the lipid molecules close to the interface between water and hydrophobic region of a bilayer is not correct. However, from this data it is still difficult to conclude if this discrepancy is large enough to affect the qualitative conclusions made using

![FIG. 1: Chemical structure of POPC.](image)

![FIG. 2: Order parameteres from simulations and experiments for glycerol and choline groups of POPC. Experimental values taken from [7].](image)
this model. In the sections below, we show that in addition to this “initial structure”, also its structural response to changes in conditions is incorrect.

B. Change of hydration level

It is known from calorimetric and surface force experiments that there is a strong repulsion between two bilayers at distances below 1 nm [11, 13]. Originally it was suggested that this repulsion arises from removing the water molecules between bilayers, thus it was called the “hydration repulsion”. However, later it has been shown that the origin of the force is entropic and it has been suggested that it actually arises from the hindered movement of lipid molecules. For a review see Refs. [11, 13]. Molecular scale behaviour of lipid bilayers as a function of dehydration has been studied by both NMR [34, 35, 44] and classical atomistic molecular dynamics simulations [16–20], but the comparison between simulations and NMR measurements is typically not conducted.

Fig. 3 shows the behaviour of experimental and simulated order parameters as a function of hydration level. The experimental values are for DMPG at 314 K, taken from Ref. [44], and they are in good agreement with the NMR measurements for POPC at 296 K [34] and for DOPC at 303 K [35]. Thus, the headgroup behaviour as a function of hydration is comparable between these systems. The simulation model and protocol are almost identical to those of Schneck et al. [20] (SPC water model is used in here, while SPC/E was used by Schneck et al.).

From Fig. 3 we clearly see that the used simulation model is not able to reproduce the effects of dehydration on the molecular scale structure of the lipids. For example, order parameters for the α carbon are increasing, and g3 are decreasing in the simulations, against the experimental observations. Thus, we conclude that the Berger lipid model is not suitable to study the molecular level effects of dehydration or the molecular origin of the hydration repulsion.

C. Change of ion concentrations

Ion membrane interactions have been widely studied with various experimental techniques since the 1970s [14]. It has been observed that most monovalent ions have very mild effects on phase transition temperature, electrophoretic mobility, infrared spectra, area per molecule, bending rigidity and deuterium order parameters of lipids [14, 36, 45–47]. The exception in these studies was lithium, for which larger effects were observed [14]. Multivalent ions, on the other hand, typically have a larger effect on the above quantities [14, 36, 45–47].

In contrast, in atomistic simulations both, Na+ and Ca2+ ions have been observed to bind lipid bilayers and change their properties significantly [21, 23]. Support for these results has been found from calorimetry, AFM experiments and diffusion measurements with probes [22, 28, 48–50]. The conclusions from these experiments, however, often contradict with the conclusions from other calorimetric experiments and spectroscopy [14, 36, 45].

Especially the Na+ binding has been widely discussed in recent molecular dynamics simulation literature. It seems that all force fields predict binding, but the strength of binding depends on the force field [24, 27]. Generally the CHARMM based force fields predict less Na+ binding and changes in bilayer properties than the GROMOS based force fields [26, 51]. Comparisons have been done between simulations and electrophoresis, calorimetric and diffusion experiments [22, 28, 52, 53]. However, direct comparison of atomistic simulations to these measurements is nontrivial, because the measured changes can arise from many different origins. For example, the electrophoretic mobility depends on the exact location of the shear plane, the changes in water properties in the presence of ions might affect the results, and in diffusion experiments the ions might directly affect the probe diffusion without actually interfering with the bilayer.

The most straightforward comparison between atomistic simulations and experiments can be done by comparing with the NMR order parameters. In this manuscript we concentrate only on Na+ and Ca2+ since those are the most discussed ions
in the simulation literature. In NMR measurements it was observed that the lipid order parameters were unaffected by the addition of NaCl into a lipid bilayer system, whereas a systematic change was observed by the addition of CaCl$_2$ [36]. Fig. 4 shows the results from our simulations of a lipid bilayer system with NaCl and CaCl$_2$ compared with the NMR experiments for a DPPC bilayer at 323 K by Akutsu and Seelig [36] in Fig. 4. Essentially similar results have been measured also for a POPC bilayer at 298 K [54], thus the results are comparable.

The order parameters calculated from simulations were clearly affected by the presence of both NaCl and CaCl$_2$ (Fig. 4). This is not surprising since both Na$^+$ and Ca$^{2+}$ ions penetrate into the headgroup region and affect the molecular structure of the lipid model, as discussed previously [21–23]. The behaviour of the lipid bilayer predicted by the simulations with increased ion concentration is clearly not in agreement with the NMR experiments: Most order parameter values changed too much and typically to the wrong direction. The result indicates that this model is not suitable for ion–membrane interactions studies. As the all–atom models like GAFF and CHARMM reproduce better order parameters for pure phospholipid headgroups, and as the ion partitioning is less in the CHARMM model [9, 10, 26, 51], these models might perform better. However, this remains to be tested.

D. Change of cholesterol content

Cholesterol is often present in cell membranes, where it has been suggested to be an important player in domain formation [55, 56]. For this reason, phospholipid–cholesterol mixtures are widely studied experimentally and theoretically. It has been found that cholesterol orders lipid acyl tails, decreases area of bilayer (condensing effect), and induces phase separation in model membranes [55]. However, the molecular origins of these effects are not completely understood [55, 56]. For example, different views about the role of lipid headgroups has been presented [15, 56]: In the “umbrella model” cholesterol molecules would cause the tilting and decrease of dynamics in the headgroups as they shield cholesterol from water [15]; in the “superlattice model” cholesterol would act as a spacer for the headgroups and increase their entropy and dynamics [56]. NMR order parameter measurements, however, indicate that the headgroup structure and dynamics are not affected even by very large cholesterol concentrations, which would suggest that the headgroup behaviour is uncoupled from the rest of the molecule [7, 8, 57, 58]. This inter-
FIG. 5: The effect of cholesterol content on glycerol and choline order parameters in experiments [7] and simulations [7].

petition is also in agreement with recent field-cycling NMR experiments [5].

A careful comparison between NMR data and molecular dynamics simulations for POPC–cholesterol mixture has been recently published [7]. Fig. 5 shows the results obtained by Ferreira et al. [7] for the headgroup region in Fig. 5. Note again that both order parameters for the β carbon (one of which was missing in the Ref. [7]) are shown here. We see that experimentally cholesterol induces only very slight changes for the choline and glycerol order parameters, largest change being observed in the g_3 carbon. In simulations, significantly larger cholesterol–induced effects are seen for order parameters of the α, g_2 and g_1 segments. In summary, as already pointed out in Ref. [7], no conclusions about the lipid headgroup behaviour as a function of cholesterol can be made based on the used model.

E. Other systems than POPC

In addition to the above systems, order parameters for hydrocarbon segments in the headgroup have been reported from experiments and simulations at least for lidocaine/PC [60] and glycolipid/PC mixtures [61]. The issue is not paid much attention in these publications, but it seems that also here the simulation model has difficulties in reproducing the measured order parameters for the headgroup region. In general, the comparison between simulations and NMR for the headgroup region is not usually done, even though the experimental data would be available. The available NMR data for different systems has been recently reviewed [32].

IV. CONCLUSIONS

We have demonstrated that the Berger force field for phospholipids is not able to correctly reproduce the experimental order parameters for glycerol and choline parts for fully hydrated bilayer, dehydrated bilayer, bilayer interacting with ions, or bilayer with cholesterol. Our results thus indicate that some conclusions made using the Berger force field would have to be reconsidered.

For example, as the structural changes due to dehydration are not correct in the Berger model, also the contribution from changes in the headgroup energy to hydration repulsion analyzed in Refs. [18, 20] might not be correct.

Similar significant structural changes in the headgroup region are seen upon the addition of sodium and calcium ions in the Berger model. This contradicts with the experiments where sodium does not change the headgroup structure and the changes observed for calcium are different from those seen in simulations. These results indicate that the changes in the headgroup structure due to a permeating charge are not correct in the Berger model and, in particular, that sodium ions penetrating the headgroup region is erroneous.

Finally, the POPC headgroup structure is changing as function of cholesterol content when the Berger model is used for lipids and Höltje model for cholesterol. In experiments only the order parameter of the g_3 segment and the glycerol group dynamics are changing; The choline structure and dynamics stays intact [8]. Thus the structural changes of the lipid glycerol and headgroup regions due to the interaction with cholesterol, as analyzed in Refs. [30, 31] might be overestimated.

Recent NMR results indicate that the glycerol and choline parts are too rigid in this model [8]. It is possible that other atomistic models would correctly reproduce the bilayer behaviour under the conditions studied here, however this remains to be tested.

This work will be further progressed and discussed through the blog: nmrlipids.blogspot.fi. Everyone is invited to join the discussion and make contributions through the blog. The manuscript will be eventually submitted to an appropriate scientific journal. Everyone who has contributed to the work through the blog will be offered coauthorship. For more details see: nmrlipids.blogspot.fi.

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