

Acyl chain order parameter profiles in phospholipid bilayers: computation from molecular dynamics simulations and comparison with ^2H NMR experiments

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Abstract Order parameters from deuterium NMR are often used to validate or calibrate molecular dynamics simulations. This paper gives a short overview of the literature in which experimental order parameters from ^2H NMR are compared to those calculated from MD simulations. The different ways in which order parameters from experiment are used to calibrate and validate simulations are reviewed. In the second part of this review, a case study of cholesterol in a DMPC bilayer is presented. It is concluded that the agreement between experimental data and simulation is favorable in the hydrophobic region of the membrane, for both the phospholipids and cholesterol. In the interfacial region the agreement is less satisfactory, probably because of the high polarity of this region which makes the correct computation of the electrostatics more complex.

Keywords Deuterium · Molecular dynamics simulation · MD · Solid state NMR · Cholesterol · DMPC

Introduction

In the latest decades, research on membranes and membrane proteins has been growing steadily (Marsh 2003a; Marsh and Páli 2004; Tieleman et al. 2001). Lipid bilayers in the liquid crystalline phase, also known as the L_α or liquid disordered (L_d) phase, are generally considered to be relevant for models of biological membranes. The liquid ordered (L_o) phase is described as an intermediate state between the L_α and the L_β (gel) phase, usually associated with bilayers containing cholesterol. For a review on the properties of a fluid lipid bilayer see Bloom et al. (1991), for lipid-cholesterol interactions see Ohvo-Rekilä et al. (2002). An extensive review of experimentally determined structural data of fully hydrated bilayers for many different lipids is given in a paper by Nagle and Tristram-Nagle (2000). Lipids can influence the structure and function of membrane proteins and in turn membrane proteins have an influence on the lipid dynamics (Jensen and Mouritsen 2004; Lee 2004, 2005; Marsh 2003b). Therefore, it is important to be able to characterize the structure and dynamics of a lipid bilayer.

First of all, one should realize that there is no such thing as an atomic level structure of a bilayer in the liquid crystalline phase. The appropriate description of a bilayer is given by broad statistical distribution functions (Petrache et al. 1999). In addition to experiment, such distributions can be evaluated by molecular dynamics (MD) simulations. Simulations can also be used to provide additional information on an atomic level, in a resolution that cannot be obtained experimentally. However, simulations may suffer from uncertainties in the forcefield and relatively short simulation times. Since simulations result from a modeling of the actual interactions and forces in a bilayer, they should be validated by comparing to experimental data

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(Tieleman et al. 1997; Nagle and Tristram-Nagle 2000). Unfortunately, this is not as easy as it seems. Simulations and experiments have different timescales, and data obtained by these methods are fundamentally different (Benz et al. 2005). Also, there is a significant amount of uncertainty in some experimental data (Nagle and Tristram-Nagle 2000).

The properties of a lipid bilayer are commonly described using the area per lipid, bilayer thickness and acyl chain order parameters. These properties are closely related, and a considerable amount of research has been dedicated to find the equations that relate them to each other (Petrache et al. 1999, 2000; Leekumjorn and Sum 2006; Nagle and Tristram-Nagle 2000).

Lipid acyl chain order parameters are obtained easily from deuterium NMR quadrupole splittings. They can be directly measured by NMR (Seelig and Niederberger 1974), are model free (Petrache et al. 2000), and reliable (reproducible). Deuterium NMR determination of acyl chain order parameter profiles has been reported for multi- and unilamellar vesicles (Renault et al. 2006; Oldfield et al. 1971), bicelles (Aussenac et al. 2003) and lipid bilayers oriented at 90° with respect to the magnetic field (Marsan et al. 1999), on either uniformly or specifically labelled lipids. Oriented bilayers provide the best resolution and direct access to quadrupole splittings, while vesicles are easier to manipulate and can also provide quadrupole splittings at 90° with a good resolution, especially when a dePakeing algorithm (McCabe and Wassall 1997; Lafleur et al. 1989) is used.

This mini-review will focus on the comparison between experimental order parameters from deuterium NMR and those calculated from molecular dynamics simulations. It is not in the scope of this mini review to cover all the aspects of MD simulations of bilayers. Reviews on this subject can be found elsewhere (Tieleman et al. 1997; Scott 2002; Pasenkiewicz-Gierula et al. 2000). However, some remarks about the choice of MD parameters will be made. We will also review the use of order parameters for calculation of the area per lipid, a property that is often used to check the equilibration of a simulation. In the final part of this minireview, a case study of lipid–cholesterol interactions that was carried out in our lab will be presented.

Order parameters of the lipid chains

Lipids in a fluid bilayer are highly dynamic. Many movements on different timescales take place: rotation around chemical bonds and *trans/gauche* isomerisations (picoseconds), rotation (axial diffusion) around the lipid axis (nanoseconds), wobbling (nanoseconds), lateral diffusion (microseconds), flip-flop across the bilayer (milliseconds–

seconds) and undulatory movements of the membrane (milliseconds–seconds). Most of these movements influence the order parameters of the acyl chains. It is of vital importance to compare the timescale of these movements to the timescale of the experimental method (about 10 μs for ²H NMR). The characteristic timescale for ²H NMR is generally considered to be long enough to sample the dynamic processes in the membrane that influence the order parameters.

Nowadays, most molecular dynamics simulations are done with small systems (several hundreds of lipids), for relatively short times (typically ranging from tens to hundreds of nanoseconds). Although these times allow for some lipid movement, the bilayer size of these systems is too small to adequately sample undulations and the time is too short for lipid lateral diffusion and slower processes to take place. Some longer simulations (Wohlert and Edholm 2006), and simulations of bilayer undulations in bigger systems (Hofsäß et al. 2003; Marrink and Mark 2001; Lindahl and Edholm 2000) have been reported to investigate these undulations and/or obtain information on lipid lateral diffusion.

Lipid order parameters are a measure for the orientational mobility of the C–D bond and are defined as

$$S = \left\langle \frac{3 \cos^2 \Theta - 1}{2} \right\rangle \quad (1)$$

where Θ is the (time dependent) angle between the C–D bond vector and a reference axis. The angular brackets denote a time and ensemble average.

Order parameters do not only depend on the (dis)order of the system, but also on orientation. For example, an order parameter of zero can be either an unordered (isotropic) system or a perfectly ordered system oriented at the magic angle of 54.7° with respect to the magnetic field. A value of –0.5 indicates a perfectly ordered acyl chain in all-*trans* conformation, rapidly rotating around the bilayer normal. A complete description of lipid order parameters is given by Seelig (1977). In general, order decreases from the interface region to the bilayer center.

Calculating lipid order parameters from solid-state deuterium NMR spectra

It was first shown by Oldfield et al. (1971) that deuterium resonance can be used to study the chain mobility in lipid systems. A detailed explanation of the physics involved is given in a review article by Seelig (1977). The order parameters of the C–D bond can be calculated directly from experimentally determined quadrupolar splittings $\Delta\nu_Q$ (Eq. 2a). A_Q is the quadrupolar coupling constant $A_Q = e^2qQ/h$, equal to 168 kHz for a C–D bond in lipid

acyl chains (Burnett and Muller 1971). Order parameters reflect the average orientation of internuclear C–D vectors with respect to the direction of the external magnetic field. The averaging occurs as a consequence of molecular rotations about different axes. In bilayers in a fluid phase, the lipids are rapidly rotating around the bilayer normal, which is thus taken as the reference axis. If the movements are independent, the general expression for order parameters can be factored into a product of individual contributions (Fig. 1, Eq. 2b–h). These individual contributions describe the orientation of the bilayer normal with respect to the magnetic field (S_{bilayer}), the fluctuations of the bilayer normal (S_{coll}), reorientations of the molecular director with respect to the bilayer normal (S_{mol}), and reorientations of the C–D vector with respect to the molecular director (S_{intra}). Note that a different usage of the symbol S_{mol} can be found in the literature, where it is used to describe the

order parameter of the vector perpendicular to the CD_2 plane (Seelig and Niederberger 1974; Stockton et al. 1976).

$$\Delta\nu_Q = \frac{3}{2}A_Q S \tag{2a}$$

$$S = S_{\text{bilayer}} \cdot S_{\text{CD}} \tag{2b}$$

$$S_{\text{bilayer}} = \left\langle \frac{3 \cos^2 \theta - 1}{2} \right\rangle \tag{2c}$$

$$S_{\text{CD}} = \left\langle \frac{3 \cos^2 \alpha - 1}{2} \right\rangle \tag{2d}$$

$$S_{\text{CD}} = S_{\text{coll}} \cdot S_{\text{mol}} \cdot S_{\text{intra}} \tag{2e}$$

$$S_{\text{coll}} = \left\langle \frac{3 \cos^2 \varphi - 1}{2} \right\rangle \tag{2f}$$

$$S_{\text{mol}} = \left\langle \frac{3 \cos^2 \beta - 1}{2} \right\rangle \tag{2g}$$

$$S_{\text{intra}} = \left\langle \frac{3 \cos^2 \gamma - 1}{2} \right\rangle \tag{2h}$$

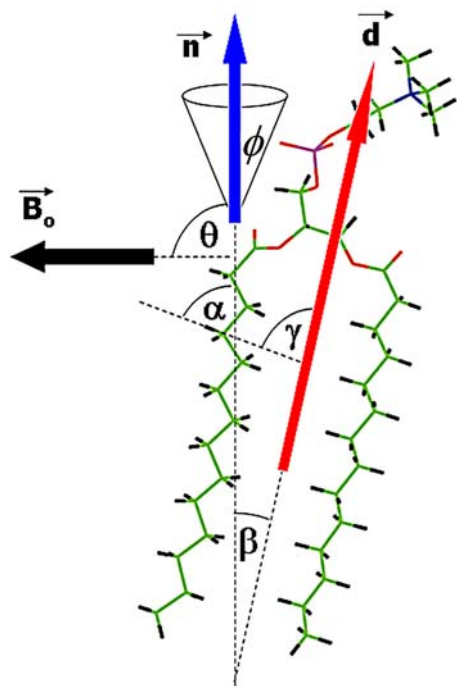


Fig. 1 Visual representation of different contributions to the observed order parameter S (Eq. 2a). Fast rotations of lipids about the vector normal to the bilayer permit to separate the contribution to the order parameter due to this movement from the contribution due to the overall positioning of the oriented bilayer with respect to the external magnetic field (Eq. 2b). The bilayer normal, symbolized by the vector \mathbf{n} , is oriented at an angle θ to the external magnetic field \mathbf{B}_0 (Eq. 2c). The observed CD vector is tilted at an angle α with respect to the bilayer normal (Eq. 2d). If the rotations of lipids about different axes are independent, the order parameter may be represented as the product of all individual contributions (Eq. 2e). The bilayer normal is subject to fluctuations, shown here as wobbling of the vector \mathbf{n} within a cone, characterized by an angle ϕ (Eq. 2f). The molecular director \mathbf{d} is at an angle β to the bilayer normal (Eq. 2g). The observed CD vector is at an angle γ with respect to the molecular director (Eq. 2h)

Calculating order parameters from MD simulations

From simulations, when averaging by fast axial rotation is assumed, the order parameter S_{CD} is calculated according to Eq. 1. If a united atom forcefield is used (without hydrogen or deuterium atoms), the C–D bond vector needs to be reconstructed. To do so, the $C_{(i-1)} - C_{(i+1)}$ vector is usually taken to be the z -axis. The x - and y -axis are defined perpendicular to the z -axis and to each other, with the y -axis in the $C_{(i-1)} - C_{(i)} - C_{(i+1)}$ plane. Using this definition, $S = (2/3)S_{xx} + (1/3)S_{yy}$ and can be compared directly to $^2\text{H-NMR}$ data (Egberts et al. 1994). For lipids rotating rapidly around the z -axis, $S_{xx} = S_{yy}$ and $S_{xx} + S_{yy} + S_{zz} = 0$ (Patra et al. 2006), hence the equation becomes $S = -S_{zz}/2$. Details on this so called recursion relation can be found in Douliez et al. (1998) and references therein. It is important to realize that this calculation only holds when the deuterons on a methylene are equivalent. This could be a problem in short (ps–ns) or small scale simulations where time and ensemble averaging are insufficient. One should also note that it is not valid around unsaturated bonds due to their different geometry.

It might be more interesting to compare $C_n - C_{(n-1)}$ order parameters (S_{CC}), calculated from experimentally measured S_{CD} to simulated ones, because S_{CC} provides more information on the chain conformations (Smondyrev and Berkowitz 1999b).

Calculation of bilayer properties from order parameters

An extensive review of experimentally determined structural data of fully hydrated bilayers for many different lipids is given in a paper by Nagle and Tristram-Nagle (2000).

Area per lipid, bilayer thickness, elasticity

Order parameters can be determined accurately and reproducibly by ^2H NMR. There is little disagreement between their values measured at different times, in different laboratories. However, there still is considerable uncertainty in the area per lipid calculated from experimentally determined order parameters (Nagle and Tristram-Nagle 2000). Attempts have been made to improve this (Petrache et al. 2000), but it is concluded that NMR order parameters cannot (yet) quantitate the bilayer structure (Petrache et al. 1999). Nevertheless, the area per lipid in the tail region and the methylenic travel distance can be estimated with reasonable accuracy (Petrache et al. 1999; Falck et al. 2004). It has been suggested that due to a difference in timescales, order parameters measured by NMR might not be the same as the ones calculated from MD simulations (Petrache et al. 1999).

Based on ^2H NMR measurements of different lipids, at different temperatures, with and without cholesterol, Laflaur et al. (1990) showed that localized changes at specific positions along the normal of the bilayer do not lead to localized changes in order parameters, but affect the entire curve. The length of the hydrocarbon chain and the hydrophobic thickness can be calculated from the average order parameter S_{CD} (Bloom et al. 1991; Smondyrev and Berkowitz 1999b; Douliez et al. 1995 and references therein). In a paper by Henriksen et al. (2006) about the effect of sterols on POPC membranes, the first moment of the acyl chain order parameters is correlated with membrane rigidity and area expansion modulus. The elastic properties (compressibility) are also related to the order parameter, as was hypothesized by Bloom et al. (1991) and confirmed by Henriksen et al. (2006).

Differences between the sn-1 and sn-2 lipid chains

A correlation of the orientation of a lipid chain with that of its nearest neighbours has been demonstrated by Takaoka et al. (2000), which is a good example for a property that is available from MD simulations, but has never been measured experimentally. Based on their simulations, de Vries et al. (2005) suggest a lateral correlation length of 3–5 nm for hydrated liquid crystalline DPPC. In a 2 ns simulation by Shinoda et al. (1997), it was shown that the sn-1 and sn-2

chain of DPPC are parallel to each other at 353 K. Given that the lipid headgroup is aligned (almost) parallel to the bilayer surface, the sn-2 chain has to be bent. It was indeed found that the gauche-ratio of the sn-2 C2–C3 bond was 30%. However, this value is still too low to explain why the chains are parallel. Another effect contributing to the bending of the sn-2 chain is the fact that it was found to favour one specific stable conformation, while the conformations of the sn-1 chains were equally distributed over two stable states. In the same paper (Shinoda et al. 1997), the authors use NMR relaxation data to show that the disordered conformation of the tail carbons is due to movement, and not orientation. The calculated order parameter profile reproduces the shape of the profile from deuterium NMR, but the calculated profile is shifted to higher values. The observation of qualitative, but not quantitative agreement has been reported often: the simulated order parameters are usually lower than the experimental ones.

A study on the differences between the sn-1 and sn-2 chain by Smondyrev and Berkowitz (1999b) reveals that the sn-1 tail in DPPC is more ordered in the beginning of the chain, while the sn-2 chain is more ordered at its end. They also noted a stronger “odd–even” effect in the sn-2 chains, which is likely to be a result of the chain’s tilt.

To explain the unusual quadrupolar splitting of C2 in the sn-2 chain as was reported by Seelig and Seelig (1974), a histogram of instantaneous order parameters rather than an average over time is presented in a paper by Takaoka et al. (2000). In the histogram, a second peak was observed for $\beta\text{-C2}$, which possibly explains the experimentally observed splitting.

Parameters affecting molecular dynamics simulations

The importance of carefully choosing the parameters for an MD simulation has been stressed in many papers on MD methodology (Anézo et al. 2003; Tieleman et al. 1997; Merz 1997; Feller 2000). A review on MD methodology by Norberg and Nilsson (2003) describes the current state of MD simulations.

There has been a lively discussion on the ensemble that should be chosen and on the correct treatment of electrostatic interactions. Nowadays, most authors agree upon the use of particle mesh Ewald (PME) (Essmann et al. 1995) to treat the electrostatics (Patra et al. 2003, 2004) because the area per lipid and the other properties of bilayers are very sensitive to the electrostatic cut-off distance (Anézo et al. 2003). However, simulations by Zaraiskaya and Jeffrey (2005) show no significant difference between PME and a twin-range cut-off model. The authors suggest two possible reasons for this apparent contradiction: they simulate a larger system (1,024 lipids), for a relatively short time

(4 ns). It should be mentioned that other lattice-sum methods exist, but they seem to be used less frequently than PME. Therefore, and because using a cut-off distance for electrostatic interactions is generally considered “bad” this review will focus on PME simulations, even though it has been demonstrated that PME can introduce artificial periodicity (Tobias 2001 and references therein). Note that most force-fields have been developed before it was common practice to employ PME. Next-generation force fields can be expected to address this issue.

As for the ensemble, a constant number of molecules, constant pressure and constant temperature (NPT) are most often used. In these simulations, the size of the simulation box is allowed to fluctuate in the xy -plane and z -direction separately (semi-isotropic), or all three dimensions are allowed to fluctuate independently (anisotropic). For simulations of systems that are large enough both choices are correct (Anézo et al. 2003). Ensembles with constant area per lipid (NPAT) or constant surface tension (NP γ T) have been used as well. Although NPAT simulations tend to agree better with experimental data (Castro-Román et al. 2006), they require as input an accurate value for the area per lipid from experiment, which is often a problem (Nagle and Tristram-Nagle 2000). Note that some force-fields require NPAT simulations, as simulations carried out with these force-fields are known to yield unrealistic values for the area per lipid (Jensen and Mouritsen 2004; Jensen et al. 2004). In NP γ T simulations, the area per lipid is sensitive to the surface tension γ (Feller and Pastor 1999; Marrink and Mark 2001).

Constant volume simulations (NVT, NVE) often lead to artefacts (Tieleman et al. 1997). However, it was shown by Suits et al. (2005) that after an equilibration of the density by an NPAT simulation, constant volume simulations can be carried out, although the authors note that the confidence in their method rests on both the confidence in the experimental data used to calibrate the simulation and the strength of the relation between order and surface area. An investigation of the effect of system size under constant volume conditions was carried out by de Vries et al. (2005), showing that 16 lipids per leaflet already reproduce many experimental properties after 3 ns, including the order parameters.

Several different forcefields (and adaptations thereof) exist. Obviously, the forcefield affects the properties of the simulated system. An analysis of different force fields for different applications is given in a review by Norberg and Nilsson (2003).

Setting up the system

In a paper by Suits et al. (2005), the average NMR order parameter was used to set up the simulation parameters.

The authors were able to reproduce the experimental order parameters for all atoms along the acyl chain. This seems to contradict the conclusion by Sternin et al. (2006), who recommend using the shape of the order parameter profile.

Another approach was taken by Feller et al. (1997): four MD simulations (NPAT) with a different area per lipid were carried out, and the order parameters and electron densities were compared to experimental data. The simulation with the order parameters and electron density that matched the experiment was considered to have the best value for the area per lipid. This led to a value of 62.9 Å, close to the value of 64 Å from Nagle and Tristram-Nagle (2000). Because Feller et al. tested for values of 59.3, 62.9, 65.5 and 68.1 Å, this can be considered a good agreement between simulation and experiment.

A simulation by Takaoka et al. (2000) was started from a crystal structure of DMPC. To pass the activation energy barrier of the phase transition to the L_α phase, the authors raised the temperature from 310 to 510 K for only 20 ps, and then slowly lowered it to 310 K, leading to an average lipid chain tilt angle close to the experimental value of 0°. After additional equilibration at 360 K, the ^2H NMR order parameters from Seelig and Seelig (1974) were successfully reproduced after 2.6 ns. Various other properties needed different times to equilibrate. The only way the authors could reproduce the experimental data while using PME was by choosing a constant surface-tension ensemble and an all-atom model.

In this work we have largely neglected the effect of undulations on calculated order parameters from the simulations, as in most simulations the membrane patches only allow minor undulations due to their limited size. Recent simulations (Marrink and Mark 2001; Lindahl and Edholm 2000) have shown that significantly larger patch sizes are required to model undulations accurately, and that undulations take a long time to develop. It is unlikely, however, that the suppressed undulations due to this approximation explain the reported differences between experimental and simulated order parameters, as these differences mainly correspond to simulation order parameters being lower than the experimental ones.

Comparison of simulations and experiments

Table 1 presents a selection from the literature where NMR data and MD simulations are compared.

Mixed lipids and asymmetric bilayers

Bilayers constituted of different lipids have different properties. In cells, membranes are composed of a complex

Table 1 Selection of publications where order parameters from molecular dynamics simulations are compared to experimental order parameters from ^2H NMR

Lipid(s)	Use of order parameter	Reference
DMPC	Comparison of DMPC (sim) to DPPC (exp), histogram of instantaneous order parameters	Takaoka et al. (2000)
DMPC	Decomposition of S_{CD} into S_{coll} , S_{intra} and S_{mol}	Aussenac et al. (2003)
DMPC	Effect of cholesterol and ergosterol	Czub and Baginski (2006)
DMPC	Effect of SDS	Bandyopadhyay et al. (2001)
DMPC	Influence of hydration and temperature	Högberg and Lyubartsev (2006)
DMPC	Setting up the initial bilayer configuration	Tang et al. (1999)
DPhPC	Comparison of simulated and experimental average order parameter	Husslein et al. (1998)
DPPC	Brownian dynamics, different field potentials	Pastor et al. (1991)
DPPC	Comparison of NMR relaxation data to simulated order parameters	Wohlert and Edholm (2006)
DPPC	Comparison of simulation and experiment	Egberts et al. (1994)
DPPC	Comparison of simulation and experiment	Tieleman et al. (1997)
DPPC	Differences between sn-1 and sn-2 chain	Smondirev and Berkowitz (1999b)
DPPC	Effect of cholesterol	Hofsäß et al. (2003)
DPPC	Effect of DMSO	Sum and de Pablo (2003)
DPPC	Effect of DMSO	Smondirev and Berkowitz (1999a)
DPPC	Effect of force-field parameters	Chandrasekhar et al. (2003)
DPPC	Effect of halothane	Koubi et al. (2000)
DPPC	Influence of different (constant) surface areas	Feller et al. (1997)
DPPC	Influence of system size and simulation time	de Vries et al. (2005)
DPPC	Temperature and disaccharide concentration dependence	Sum et al. (2003)
DPPC	Trans/gauche ratio and order parameters	Shinoda et al. (1997)
DPPC	Validation of forcefield	Berger et al. (1997)
DPPC	Validation of forcefield	Smondirev and Berkowitz (1999c)
DPPC, DDPS	Effect of asymmetry, order parameters for each bilayer leaflet separately	López Cascales et al. (2006)
DPPC, DPPE	Influence of different DPPC/DPPE ratios	Leekumjorn and Sum (2006)
DPPC, POPC, PLPC, PAPC	Effect of the number of double bonds	Hyvönen and Kovanen (2005)
DPPG, GalCer	Different GalCer/DPPG ratios	Zaraiskaya and Jeffrey (2005)
POPC	Analysis of lipid conformation and mobility	Heller et al. (1993)
POPC	Effect of NaCl	Böckmann et al. (2003)
SOPE	Simulation set up using POPC experimental order parameters	Suits et al. (2005)

Note that the description (in the second column) does not necessarily correspond to the main subject of the article, but rather gives a description of the use of order parameters in the cited publication

mixture of lipids and proteins, unevenly distributed over the two monolayers.

In a simulation study on a mixed DPPC/DPPE bilayer (Leekumjorn and Sum 2006), the DPPC order parameters agree with NMR data, but there is a poor agreement for DPPE, suggesting that a refinement of the DPPE forcefield is needed.

A simulation by López Cascales et al. (2006) of an asymmetric DPPC/DPPS bilayer shows a good agreement with experimental data for both the area per lipid and order parameters. They find that the properties of both leaflets are different and that the DPPS that is present in only one of the leaflets does not influence the properties of the other (DPPC only) leaflet.

The effect of ions

In biological systems all sorts of different ions interact with the membrane. These interactions influence many processes, such as membrane fusion and the binding of anti-microbial peptides (Kandasamy and Larson 2006a). In MD simulations, ions are usually added to neutralize a system in order to prevent inaccurate calculation of the electrostatic interactions. But due to the relatively small amount of water molecules in these simulations, this quickly leads to high salt concentrations. For a recent review of simulation studies on the effect of ions and water on a lipid bilayer, see Berkowitz et al. (2006).

An MD simulation of a POPC bilayer by Böckmann et al. (2003), shows that Na^+ ions interact with the lipid carbonyl oxygens, leading to a decrease in the area per lipid, a thickening of the bilayer and an increase of the order parameters. The addition of Na^+ and Cl^- ions decreased the self-diffusion of the lipids. The self-diffusion agreed well with experimental data, suggesting that it takes place on a timescale below the simulation time (100 ns). Other simulations of POPC (Kandasamy and Larson 2006a) and DPPC (Pandit et al. 2003) confirm the increase in order parameters with increasing salt concentration. Kandasamy and Larson find that the Na^+ ions bind to the glycerol ester group, deep in the interface region and they compare these findings to results from Sachs et al. (2004), who do not find this deep binding. This difference could be due to the shorter simulation time (50 and 5 ns, respectively) or the different simulation conditions (forcefield, ensemble). Indeed, it has been shown that the binding of ions, especially divalent ions, is a slow process that takes on the order of a hundred nanoseconds to equilibrate (Böckmann et al. 2003).

On POPS lipids, Na^+ ions have a similar effect. Ions are found to bind to the carbonyl oxygen deep in the interface region, resulting in a small increase in the order parameters of the first atoms in the acyl chain (Mukhopadhyay et al. 2004). Contrary to what one might expect, the area per lipid is unaffected. This is an important finding, because it shows that the area per lipid and the order parameter are not always straightforwardly related. It might—at least partly—explain the errors in the calculation of area per lipid from order parameters.

Hydration, the effect of water on bilayer simulations

Most MD simulations are carried out on fully hydrated bilayers. But since some experiments have been carried out at low hydration, simulations that investigate the effect of water on the properties of a bilayer are also interesting.

Molecular dynamics simulations of a DMPC bilayer show an increase in order parameters upon dehydration. This ordering effect of dehydration is confirmed by NMR data, although the increase is smaller in the simulations (Högberg and Lyubartsev 2006). Two recent reviews explain in detail the effect of (de)hydration on model membranes (Berkowitz et al. 2006; Milhaud 2004).

Small molecules

A comprehensive review article on the effect of small molecules has been published (Lee et al. 2005). A few examples are given below.

Sucrose and trehalose decrease the mean square displacement of the lipid phosphorus atoms without affecting

the DPPC area per headgroup and the order parameters (Sum et al. 2003).

In a paper by Sum and de Pablo (2003) the effect of DMSO on a DPPC bilayer is studied by simulating a DPPC bilayer with DMSO from 0 to 100 mol%. DMSO appeared to accumulate below and above the headgroup, while it was excluded from the headgroup region itself. It was shown that low concentrations of DMSO have a large effect on the area per lipid, while the effect on the order parameter of the tails is very small, demonstrating (again) the complexity of the often assumed relation between order parameter and area per lipid.

A study of the effect of ethanol and methanol on lipid bilayers was carried out by Patra et al. (2006). They find a small increase in order upon adding 1 mol% of alcohol. The ordering increased close to the glycerol group, or below it, for methanol and ethanol, respectively, in agreement with the mass density of these molecules. These results seemed to contradict those from Feller et al. (2002), which was explained by the fact that alcohols have two modes of action: a local effect proportional to the alcohol concentration and a global effect as a consequence of the changes in surface tension. A more detailed analysis showed that the lipid order parameter decreased for the molecules bound to ethanol, but increased for the unbound lipids.

A mole fraction of 6.6% SDS does not significantly increase the ordering of DMPC lipids, while the order of the SDS tails is increased significantly, in agreement with experimental data (Bandyopadhyay et al. 2001).

Peptides and proteins

Among the many reviews on MD simulations of membrane proteins in lipid bilayers, two recent examples are those of Ash et al. (2004) and Tieleman (2006).

A simulation by Kandasamy and Larson (2006a) shows that the antimicrobial peptide magainin leads to disordering of POPC lipids in its vicinity. The binding of the peptide is stronger at lower concentrations of salt. The authors also note that their 50 ns simulation is not sufficient to equilibrate the position of the peptide in the bilayer.

In a simulation of a DPPC bilayer by Appelt et al. (2005), ^2H NMR order parameters slightly increase in the presence of cyclo(RRWRF) peptide. The authors compare this effect to the effect of Na^+ ions described by Böckmann et al. (2003). An NMR study by Vogel et al. (2005) reveals that a peptide derived from N-ras protein drastically lowers DMPC order parameters. The DMPC packing however, is only slightly affected. A publication of a ^2H NMR study by Dave et al. (2004) suggests no changes in order parameters when 6 mol% phospholamban binds to a POPC bilayer.

Peptides with a positive and negative hydrophobic mismatch influence the lipid order parameters differently, as demonstrated in a simulation of “KALP” model peptides (Kandasamy and Larson 2006b).

These examples illustrate the complexity of peptide–lipid interactions. Different peptides and proteins may have different (or even opposite) effects on the order of lipid acyl chains.

Cholesterol and other sterols

Cholesterol increases the order in L_α phase bilayers. The amount of additional ordering induced by cholesterol differs, depending on the type of lipids. This effect has been investigated by deuterium NMR and MD simulations, where MD simulations are used to provide atomic detail on the cholesterol–lipid interactions. Reviews of interactions of cholesterol with phospholipids in membranes are given by Ohvo-Rekilä et al. (2002) and Mouritsen and Zuckermann (2004).

A paper by Henriksen et al. (2006) describes the correlation between the first moment of the ^2H -NMR spectra of a POPC bilayer and the membrane bending rigidity and area expansion modulus. They note that this correlation is the same for lanosterol, ergosterol and cholesterol, and independent of sterol concentration, even though these molecules have different effects on membrane thickness and sterol–lipid packing.

According to MD simulations, cholesterol increases the ordering in both the sn-1 and the sn-2 chain of DMPC, while in POPC it affects only the fragment of the sn-2 chain above the double bond (Róg and Pasenkiewicz-Gierula 2006; Chiu et al. 2001). These simulations also show that the α and β face of cholesterol have a preferential interaction with saturated and unsaturated chains, respectively.

It is beyond the scope of this publication to review all the work that has been carried out on lipid–cholesterol and protein–cholesterol interactions. Instead we present a study that was carried out in our lab, where experimental data and MD simulations were used to investigate the order parameters of cholesterol and lipid molecules in a DMPC bilayer.

Lipid–cholesterol interactions: a case study

Order parameter values have been obtained from measurements, performed on DMPC molecules with deuterated sn-2 chains. Also, for completeness, experimental results from other sources (Aussenac et al. 2003) have been included in the analysis. Cholesterol molecules have been selectively deuterated as shown in Fig. 2. High resolution

^2H spectra obtained with oriented bilayers and proton decoupling (Fig. 3) yielded low error bars (<2%) on the values of the experimental order parameters.

The simulation of molecular dynamics has been performed on a periodic 2:1 DMPC/cholesterol system, composed of 256 DMPC molecules, 128 cholesterol molecules and 7,310 H_2O molecules. The size of the system was $9.6 \text{ nm} \times 9.6 \text{ nm} \times 6.9 \text{ nm}$. The system was equilibrated until no changes in the energy were observed. For comparison, a simulation of pure DMPC was carried out as well. This system contained 128 DMPC molecules and 1920 water molecules. For both systems, a 200 ns trajectory has been generated with the GROMACS software package (Van der Spoel et al. 2005; Lindahl et al. 2001), using the Berger lipid force field (Berger et al. 1997). The center-of-mass motion was removed after each iteration. The Berendsen coupling (Berendsen et al. 1984) was used to keep the system at constant temperature (303 K) while the semi-isotropic Berendsen coupling kept the pressure at 1 bar (Berendsen et al. 1984). The electrostatic interactions were calculated using the PME algorithm (Essmann et al. 1995; Darden et al. 1993). The LINCS algorithm (Hess et al. 1997) was used to constrain bond lengths, allowing a time step of 2 fs. SPC was chosen as water model (Berendsen et al. 1981).

In previous simulations two types of DMPC molecules with respect to the dihedral angle θ_1 (based on atoms C1, C2, C3 and O in glycerol), referred to as type A and B, have been observed using InsightII and Discover 2000 software. The distribution of these angles, as reported in Aussenac et al. (2003) shows two maxima, the major one at 60° (gauche-, type A) and one at 180° (trans, type B). In our recent simulations, using GROMACS, we saw one broad peak centered at ca. 20° , which corresponds to neither of the two aforementioned types. The major difference between the two approaches is the choice of the force field for the simulations.

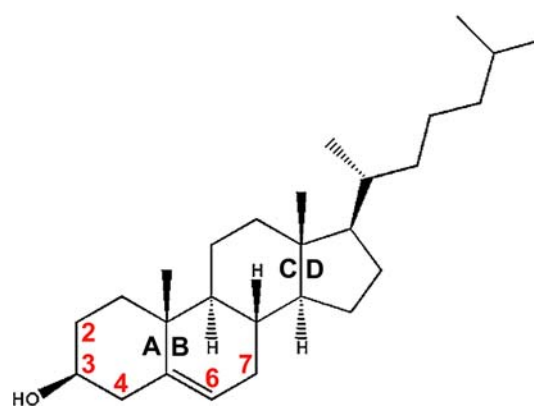


Fig. 2 Numbering of atoms labeled with deuterium (rings A and B)

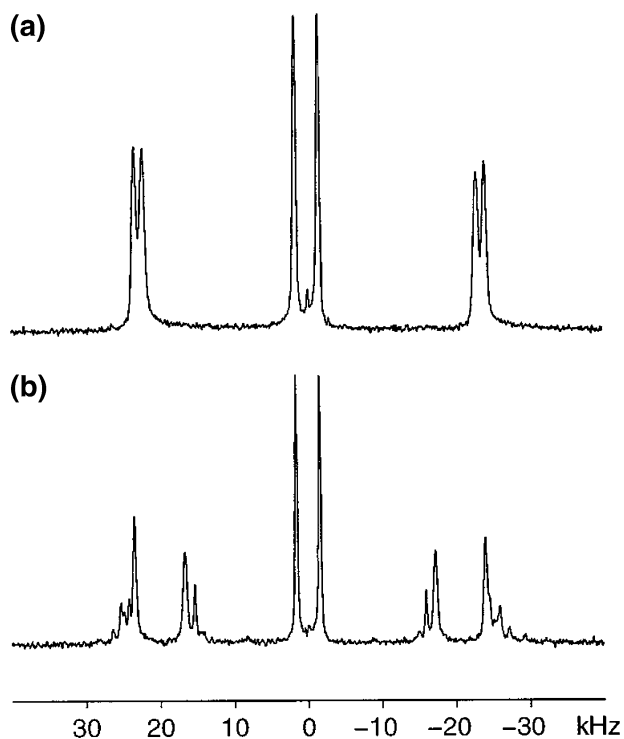


Fig. 3 ^2H NMR spectra of cholesterol labeled with deuterium (Marsan et al. 1999). Label at atom: (a) 6, 7a, 7e, (b) 2a, 2e, 3, 4a, 4e, 6

The order parameters were calculated from the MD trajectories. Since the bilayers in the NMR experiment were oriented perpendicular to the magnetic field, $S_{\text{bilayer}} = -1/2$. The fluctuations of the bilayer normal were found to be negligible in the simulations, since the value of S_{coll} is 0.9990 for DMPC, and increases to 0.9995 upon addition of 30% of cholesterol, which translate (using Eq. 2f) to fluctuations of 1.48° and 1.05° respectively. S_{mol} (the orientation of the molecular director with respect to the bilayer normal) is 0.55 for pure DMPC and 0.90 for DMPC in the mixture, while the corresponding value for cholesterol molecules is 0.88. These values are in agreement with those found in the literature (Marsan et al. 1999). Figure 4 shows the order parameter $-S_{\text{CD}}$, calculated for the sn-2 chain of pure DMPC as well as those measured experimentally. The agreement between experiment (filled symbols) and simulation (open symbols) is best at the end of the chain and acceptable for most carbon positions in the middle of it. However, for position 2 there is no agreement. The calculated values of the order parameter for deuterons proR and proS are similar, while the experimental values are noticeably different. Similar conclusions can be drawn from Fig. 5, showing order parameters for the 2:1 DMPC/cholesterol mixture. The agreement for most of the carbon positions is excellent. However, the carbon position 2 causes similar problems, as the predicted proS and proR

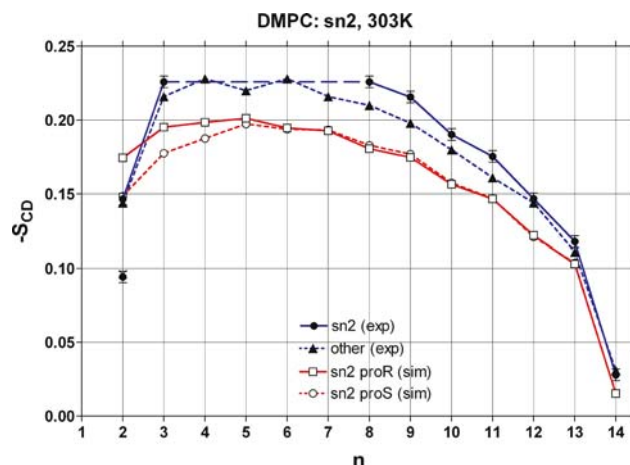


Fig. 4 Order parameter $-S_{\text{CD}}$ for the sn-2 chain of pure DMPC at 303 K as a function of the carbon atom index

values are nearly identical, and the experimental ones are very different. The apparent plateau in the experimental data is due to lack of resolution and specific assignments.

The comparison of order parameters calculated from the trajectories with the available experimental data for the choline and glycerol shows differences up to one order of magnitude higher than the width of experimental error bars. However, a conversion of these differences into the average inclination of the C^2H vector with respect to the bilayer normal yields tolerable differences, below 10° .

Figure 6 shows order parameters for cholesterol in the mixture. Again, the agreement is poor in the sense that the differences far exceed the limits of experimental uncertainties. However, the resulting differences in the inclination are small, as the maximal difference in orientation of individual vectors amounts to $4\text{--}5^\circ$.

A straightforward conversion of the observed differences between calculated and measured average vector orientations into quantitative corrections concerning the studied molecules is not feasible, since average molecular structures have no physical meaning. With this in mind, we may attempt to bring the calculated results in agreement with the experimental ones by calculating corrections to the orientations determined from NMR order parameters. This means (1) lifting the H2pro-S on sn-2 by $\sim 4^\circ$, (2) turning glycerol by $\sim 10^\circ$ about the $g_2\text{--}g_1$ axis and (3) lifting the entire choline unit by $\sim 6^\circ$. For cholesterol in the 2:1 mixture, two corrections should be introduced: (1) rotation of the director of the molecule by $\sim 4^\circ$ in the direction of C2 and (2) tilting the molecule by $\sim 4^\circ$ towards the protruding methyl groups.

Another interesting question is that of determining the average area per lipid (APL) (Greenwood et al. 2006; Edholm and Nagle 2005). Molecular dynamics simulations have shown that the size of the simulation box remained

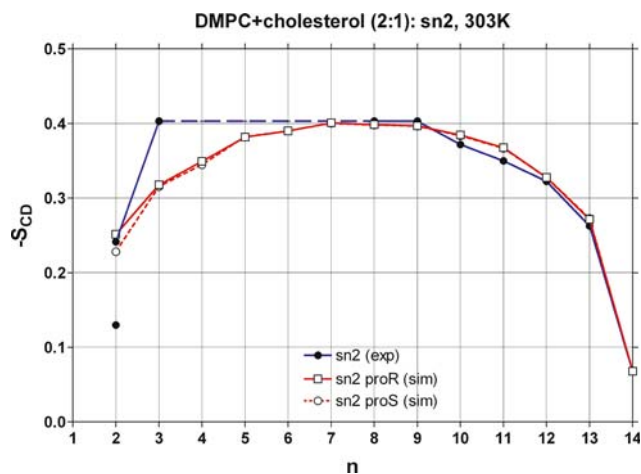


Fig. 5 Order parameter $-S_{CD}$ for DMPC in the DMPC-cholesterol 2:1 mixture at 303 K as a function of the carbon atom index

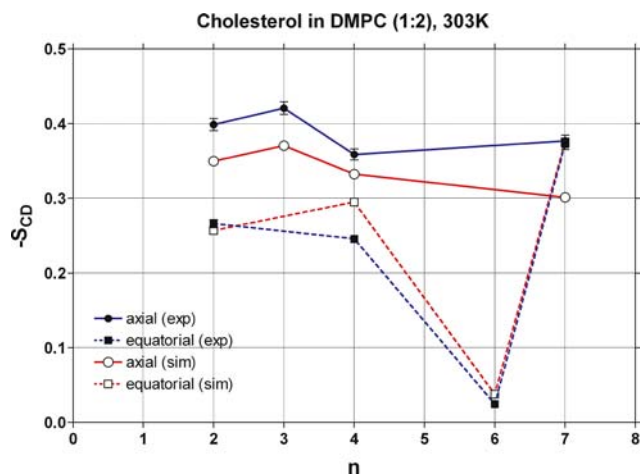


Fig. 6 Order parameters $-S_{CD}$ for cholesterol in the DMPC-cholesterol 2:1 mixture as a function of the carbon atom index

practically constant after adding cholesterol molecules (APL = 63.13 Å for DMPC and 63.02 Å in the mixture). This finding is in agreement with experiments by Chapman et al. (1969), where it was shown that cholesterol has little effect on condensed monolayers.

NMR order parameters have been used as basis for calculations of the APL (Petrache et al. 2000), with limited success. Depending on the theoretical approach used, the results give widely differing numbers for the APL, often far from experimental values. Clearly, a different approach and a better theory are desirable.

An important conclusion, inferred from the MD simulations, is that no clustering of cholesterol molecules occurs within the time span of the trajectory. The analysis of intermolecular contacts leads to the conclusion that there is on average one transient hydrogen bond formed between

the OH-group of cholesterol and the lipids, predominantly with the fatty-acyl chain esters, with the average distance between oxygen atoms (O3 of cholesterol and O2 of sn-1) of 2.6 Å (Fig. 7). In Soubias et al. (2004) and Hémin and Chipot (2006) the hydrogen bonding patterns between cholesterol and its partners in lipid bilayers are discussed in detail, taking into account different rotameric states of cholesterol and bonding partners (acyl esters, phosphate groups, water).

Experimental results from neutron scattering data show that the hydrophobic thickness of the DMPC bilayer is 30.1 Å and that it increases to 34.6 Å upon addition of cholesterol (Léonard et al. 2001). Depending on the choice of reference atoms in defining the bilayer thickness, our calculations for DMPC give 27.3 Å for the distance between C2 (sn-1) atoms and 34.7 Å for the P-P distance. In case of the mixture we obtain the estimates of 34.1 and 42.2 Å.

In order to deduce the position of cholesterol in the bilayer, the neutron scattering studies used deuterated cholesterol and determined the distance between peaks in the corresponding density profiles. These peaks reflect the distance between the centers of gravity of the deuterated cholesterol fragments (rings A and B). The experiment yields 32.7 Å for the 2:1 mixture. Our calculations give the estimate of 29.3 Å between C4 atoms and 33.1 Å for O3 atoms. The latter value is in better agreement with the experiment, but does not correspond to the center of

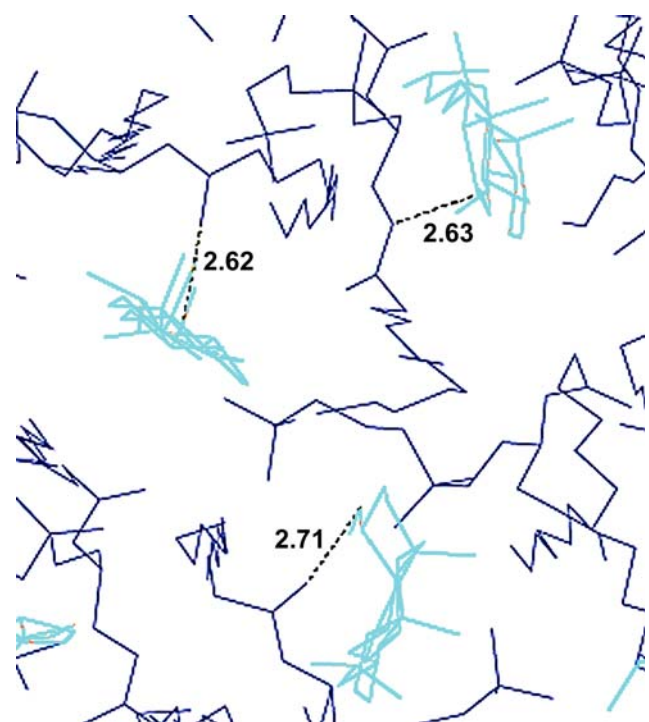


Fig. 7 Cholesterol molecules form hydrogen bonds with neighboring lipids ($d_{O-O} = 2.6$ Å)

gravity of the deuterated rings. The C4 atoms give a much better estimate of the center of gravity.

Conclusions

The inner, hydrophobic region of the membrane is rather well represented in simulations. Upon approaching the interfacial region, more and more discrepancies with experiment can be observed. The differences may originate from the parametrization of the unified-atom force field used in our studies. It will be interesting to see how the next-generation of all-atom lipid force fields performs in this respect. These force fields, that are currently being developed, use PME during calculations and are calibrated on and validated against state-of-the-art experimental data. In the region of the worst fit, high polarity is predominant and fluctuating charges would have to be taken into account to improve the overall agreement. It is therefore possible that perfect agreement can only be expected after including polarizability explicitly in the employed force fields (Palmo et al. 2003; Swart and van Duijnen 2006; Patel and Brooks 2004; Anisimov et al. 2005).

The differences between the calculated and experimental order parameters indicate that the orientations of molecules are described with an accuracy reaching $\pm 10^\circ$ and often better. However, these differences are statistically significant because they far exceed the limits of the experimental uncertainties, including possible errors arising from the interpretation of the NMR data.

The question is whether these differences are important for biological function. In many cases, the achieved level of accuracy is probably sufficient. However, there may be specific cases, such as interaction between a transmembrane protein and a ligand in a binding site located in the proximity of lipidic polar heads, where the reported differences may be critical. Hence, future development including continuous cross-validation between simulation and experiment will be required to address these issues.

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