Influence of *cis* double-bond parametrization on lipid membrane properties: How seemingly insignificant details in force-field change even qualitative trends

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(Received 14 April 2008; accepted 7 August 2008; published online 10 September 2008)

We have employed atomistic molecular dynamics simulations to investigate the effect of double-bond parametrization on lipid membrane properties. As models, we use one-component membranes composed of glycerol-based phosphatidylcholines (PCs) with monounsaturated acyl chains, and we complement these studies by additional PC/cholesterol simulations. We compare differences between double-bond parametrizations by varying the position of the double bond systematically along the lipid hydrocarbon chains. The results give rise for concern: They indicate that the double-bond description may change not only the quantitative but also the qualitative nature of membrane behavior. In particular, we find that the double-bond description which accounts for skew states in the vicinity of a double bond predicts a maximum in membrane disorder, when the double bond resides at the middle of an acyl chain, in agreement with experiments. The more commonly used description which does not accommodate skew states, however, predicts membrane disorder to decrease monotonically as the double bond is shifted from the glycerol backbone to the end of an acyl chain. The results highlight the importance of properly describing double bonds especially in many-component membranes, where the interplay of different molecule types is difficult to predict on intuitive grounds. © *2008 American Institute of Physics*.
[DOI: 10.1063/1.2976443]

INTRODUCTION

Cell membranes are fascinating examples of soft interfaces involved in a wide range of biological functions.[1] The functions are largely due to membrane proteins embedded in membranes, yet the activation and therefore the functionality of membrane proteins are in part controlled or even governed by cell membranes.

Membranes affect the functionality of membrane proteins by various means. On the one hand, proteins such as rhodopsin have been shown to favor interactions with specific lipids such as those having polyunsaturated fatty acids: Experimental studies of membrane-protein structures have indicated that polyunsaturated lipids may be an integral part of the protein structure and affect it through specific lipid-protein interactions.[2,3] On the other hand, there are also proteins such as mechanosensitive channels whose activation can be governed by the elastic properties of membranes.[4,5] In this case, instead of specific lipid-protein interactions, the interplay between a protein and a membrane arises from more generic membrane properties such as the lateral pressure profile[6,7] exerted by the membrane on the protein. Interestingly, in the context of polyunsaturated lipids, it has recently been shown that the lateral pressure profile of membranes comprised of polyunsaturated lipids is distinctly different from the pressure profile found in saturated membranes,[8] the differences being the largest in the hydrophobic membrane core where the double bonds of lipid hydrocarbon chains are located. Not only the structure but also the dynamics of polyunsaturated lipid membranes in the absence of proteins is distinctly different from their saturated counterparts.[8] From another perspective, the role of unsaturation is believed to be fundamental in the raft hypothesis. Differential interaction of cholesterol (Chol) with saturated and unsaturated membrane lipids is the basis for the formation of liquid-ordered and liquid-disordered phases that underpin raft formation.[9] As a consequence, it is clear that the double bonds in unsaturated lipid hydrocarbon chains have a major role to play in numerous membrane functions.

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Besides experiments, atomistic molecular dynamics (MD) simulations have become a more and more appealing technique to elucidate the complexity of cell membranes. Recently, they have been employed with success to a wide variety of membrane systems ranging from single-component bilayers to lipid rafts and further to complex membrane-protein complexes.[10,11] Yet, there is a reason to stress that there are also significant limitations associated with simulation models that one has to account for before simulations are being conducted.[12] The core of the issue is the reliability of MD simulations that crucially depends on the interaction potential (force field), which determines the properties of the system being considered. The quality of the force field is indeed the decisive factor, since seemingly tiny details can cause rather drastic changes in membrane properties. For example, it has been shown that the use of truncation instead of Ewald type summation schemes for electrostatic interactions may give rise to artificial ordering in the membrane plane, thus changing the phase behavior.[13,14] Also, the partial charge distribution in the polar head groups of lipids plays an important role in membrane packing, and may drive the membrane system to an incorrect phase,[15] again changing the phase behavior. In the same spirit, the presence of small charge at the hydrocarbon chain can lead to changes in phase behavior.[16]

In this work, we show through atomistic MD simulations that the description of a double bond in a lipid hydrocarbon chain is an equally subtle matter. At present, some of the available force fields for lipids parametrize the *cis* double bond as a simple improper torsion (which dictates the planarity of the double-bond region) together with small corrections for the adjacent dihedrals (see, for example, Ref. 17). Recently, by means of *ab initio* calculations of small molecules such as *cis*-2-hexen, Bachar *et al.*[18] developed another set of force-field parameters to describe the double-bond region, accounting for skew states in the vicinity of the double bond. Though it is likely that different descriptions of the double bond give rise to somewhat different membrane properties, in particular, in the hydrophobic region of a membrane,[8,19] the actual relevance of the issue has remained unclear since results of careful and systematic studies of this topic have not been available.

Here, we unravel this issue. We employ atomistic MD simulations to consider the effect of the double-bond description in one-component membranes composed of glycerol-based phosphatidylcholines (PCs) with monounsaturated acyl chains. For completeness, we complement these studies with additional PC/chol simulations. To be as systematic as possible, we compare different schemes for the double-bond treatment by varying the position of the double bond along the lipid hydrocarbon chains. The results give rise for concern: They indicate that the double-bond description can change not only the quantitative but also the qualitative nature of membrane behavior. Being more specific, we find that the description which accounts for skew states shows a maximum in the area per lipid when the double bond resides at the middle of an acyl chain, in agreement with experimental data on the main transition temperature T_m.[20,21] The description which does not accommodate skew states, however, predicts a monotonically decreasing area per lipid as the double bond is shifted from the glycerol backbone to the end of an acyl chain. Further data for other structural properties are consistent with this view.

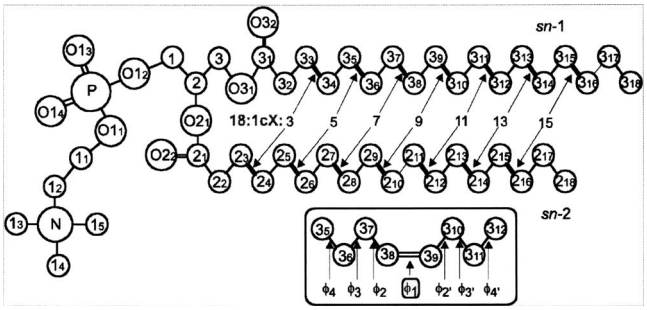

FIG. 1. Molecular structure of the PC molecules used in our simulations, including numbering of atoms. The unsaturated bonds in the studied species are marked and numbered in both chains. In the inset, the dihedral angles (ϕ_i) affected by the double-bond parameterization are shown for $sn-1$ chain of 18:1c9 (DOPC) moiety. The chemical symbol for carbon atoms, C, is omitted.

The results highlight the importance of describing double bonds in a realistic manner. If the double-bond description is too simplistic, artifacts may arise if the role of double bonds is important for the quantity being explored. In the present case, we find that the force field that does not account for skew states in the vicinity of a double bond yields results that are not only quantitatively but also qualitatively incorrect with respect to experimental findings. The implications of these findings in a more general context are briefly discussed in the end of this article.

MODELS AND COMPUTATIONAL DETAILS

Atomic-scale MD simulations of 14 different membrane systems have been carried out for each double-bond description. The first seven (pure) bilayers were composed of a total of 128 PC molecules, and the remaining seven (mixed) systems contained 32 chol molecules in addition to the 128 PCs. All the bilayers were hydrated with 3900 water molecules. The PC lipids had two stearyl 18 carbon chains (*sn*-1 and *sn*-2), both being equally *cis* monounsaturated. The position of the double bond was varied systematically and symmetrically along the chain as follows (see Fig. 1): between atoms $C3_3$–$C3_4$ (18:1c3), $C3_5$–$C3_6$ (18:1c5), $C3_7$–$C3_8$ (18:1c7), $C3_9$–$C3_{10}$ (18:1c9, DOPC), $C3_{11}$–$C3_{12}$ (18:1c11), $C3_{13}$–$C3_{14}$ (18:1c13), and $C3_{15}$–$C3_{16}$ (18:1c15) in the *sn*-1 chain, and atoms $C2_3$–$C2_4$ (18:1c3), $C2_5$–$C2_6$ (18:1c5), $C2_7$–$C2_8$ (18:1c7), $C2_9$–$C2_{10}$ (18:1c9, DOPC), $C2_{11}$–$C2_{12}$ (18:1c11), $C2_{13}$–$C2_{14}$ (18:1c13), and $C2_{15}$–$C2_{16}$ (18:1c15) in the *sn*-2 chain. In the rest of this paper, we refer to these bilayers by the index corresponding to the position of the double bond (the first carbon atom index in Fig. 1). Figure 1 shows the structure and the numbering of atoms as well as the positions of the double bonds in the unsaturated species. Full details of initial configurations are given in our previous papers.[22,23]

The simulations were performed using the GROMACS software package.[24,25] We used the standard united-atom force-field parameters that have been extensively tested and

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verified for saturated dipalmitoylphosphatidylcholine (DPPC) molecules (see e.g., Refs. 13 and 26 and references therein). The partial charges were taken from the underlying model description.[27] The simple point charge (SPC) model[28] was used for water. For chol, we used the description of Holtje *et al.*[29] The SETTLE algorithm[30] was used to preserve the bond lengths in water molecules, whereas the lipid bond lengths were constrained with the LINCS algorithm.[31] A single 1.0 nm cutoff distance was used for the Lennard–Jones interactions.[13] The long-range electrostatic interactions were handled using the particle-mesh Ewald method[32] with a real space cutoff of 1.0 nm, β spline interpolation (of order 6) and a direct sum tolerance of 10^{-5}. Periodic boundary conditions with the usual minimum image convention were used in all three directions, and the time step was set to 2 fs.

The simulations were carried out in the NpT (constant particle number, pressure, and temperature) ensemble at $P=1$ atm. The main body of studies were carried out and $T=338$ K. The temperature was chosen to be high enough to allow us to model the fluid phase: the temperature has to be above the main phase transition temperature of DSPC (328 K) that is the highest among the studied lipid species.[33] Additional shorter simulations were conducted for DOPC (18:1c9) at 303 K to facilitate more detailed comparisons with experiments. The temperature and pressure were controlled by using the weak coupling method[34] with the relaxation times set to 0.6 and 1.0 ps, respectively. The temperatures of the solute and solvent were controlled independently, and the pressure coupling was applied separately in the bilayer plane (xy) and the perpendicular direction (z). The simulation protocol applied here has been successfully applied in a number of previous MD simulations.[10,19,22,23,35]

Each of the simulations at 338 K was run for a total of 100 ns for the pure systems and 150 ns for the mixed bilayers. The first 20 ns were considered for equilibration and the remaining parts of the trajectories were used for analysis. The simulations at 303 K were simulated over a period of 60 ns, using the last 40 ns for analysis. To test the two different double-bond parametrizations side by side, we used both parametrizations for each of the different bilayers as listed above. For each such a pair, we used identical initial configurations such that the only difference was in the description of the double bond. That allows us to monitor how the systems evolve due to different parametrizations and how the differences are reflected in physical observables in pure and mixed bilayers. As an idea, this is analogous to the use of shadow Hamiltonians in the analysis of numerical integration methods.[36]

In the first set of simulations, we used the double-bond description by Bachar *et al.*[18] which explicitly describes the skew states of saturated bonds next to a double bond. Given that an accurate description is of particular importance in the case of polyunsaturated lipids,[3,8,19] Bachar *et al.* derived their parameters from *ab initio* calculations.

The other set of simulations was done using the standard double-bond parameters of the GROMOS87 force field,[17,37] which does not account for the skew states.[18,19] That is the parameter set which is among the most common ones used in MD simulations of lipids.

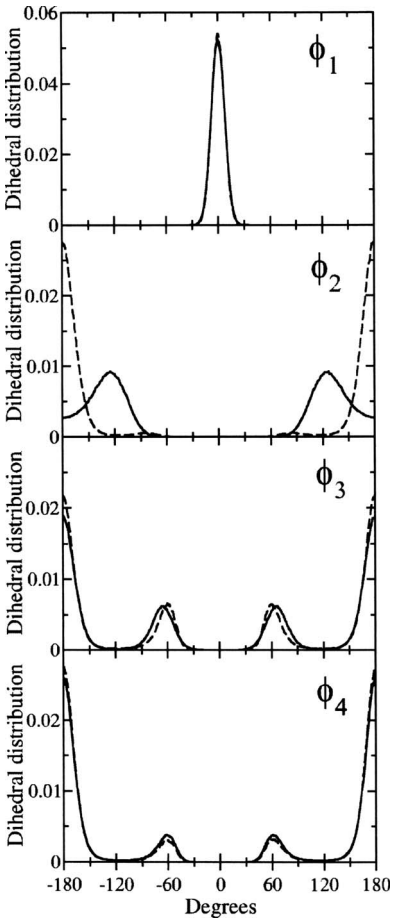

FIG. 2. Dihedral angle distributions for the dihedrals ϕ_1 (double bond), ϕ_2, ϕ_3, and ϕ_4 shown for the 18:1c9 moiety (DOPC). The solid lines correspond to the force field by Bachar *et al.* (Ref. 18) that accommodates skew states, whereas the dashed curves result from the force-field parametrization (Refs. 17 and 31) without them (see text for details).

To analyze the differences between the two parametrizations, we consider the dihedral angles ϕ_1, ϕ_2, ϕ_3, and ϕ_4 close to the double bond (in Fig. 1, these dihedrals have been marked for DOPC). Figure 2 depicts the normalized probability distributions of dihedral angles for both force fields for the case of a pure DOPC bilayer (other moieties in pure and mixed bilayers yield similar behavior). When the two double-bond descriptions are compared to each other, the dihedral next to the double bond (ϕ_2) is significantly modified, ϕ_3 displays only a rather small change, whereas ϕ_1 and ϕ_4 are mostly unaltered. The GROMOS87 force field[17,37] that ignores skew states keeps the ϕ_2 dihedral in a rather fixed *trans* configuration (180°), whereas the parameters of Bachar *et al.*[18] (which do account for the skew states) allow a more flexible and dynamic configuration between the two maxima at −120° and 120°. Therefore, PCs whose parametrization accounts for the skew states give rise to a less packed configuration and more extended torsions.

In previous studies, we have also used the OPLS (Optimized parameters for liquid simulations) united-atom force field[38] which uses an alternative description of double bonds. That parametrization leads to a broad distribution of ϕ_2 angles between 60° and −60° (range of angles equivalent to skew states) with a maximum at 180°.[39,40] In this paper, we

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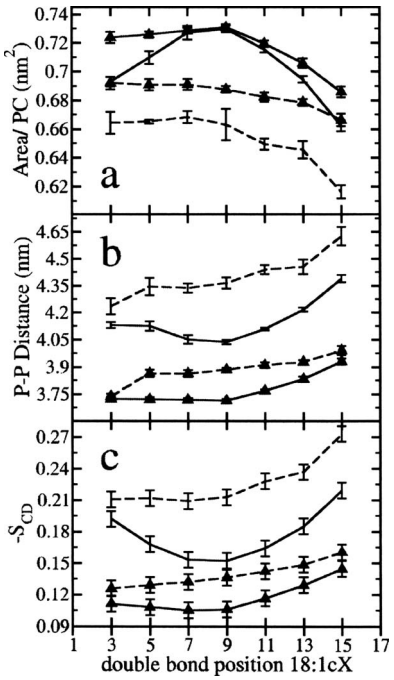

FIG. 3. (a) The area per PC, (b) the membrane thickness, and (c) the average values of the $-S_{CD}$ order parameter shown as a function of double-bond position. The solid lines correspond to the force-field parametrization by Bachar *et al.* (Ref. 18), while the dashed curves stand for the parametrization which does not account for skew states. Results are shown for both pure PC bilayers (triangles) and PC/chol mixtures (lines without symbols).

did not test this parametrization due to rather limited usage of this force field in current membrane simulations.

Finally, let us briefly discuss a technical matter related to the double-bond parametrization of Bachar *et al.*[18] The force field for the double-bond region in Ref. 18 was developed for a diunsaturated hydrocarbon region where two double bonds were separated by an individual single bond. In this work, however, the acyl chains are monounsaturated. To confirm that the description of Ref. 18 can be exploited in our model, we performed *ab initio* calculations using GAUSSIAN03 with two basis sets (B3LYP/6-31G(d) and B3LYP/6-31G**) for *cis*-4-octene, which includes a single double bond. For this molecule, we focused on the dihedral potential next to the double bond since that potential is the most sensitive one. Both basis sets yielded similar potentials whose qualitative nature was in line with the results reported in Ref. 18. The minor quantitative difference in the barrier height results from the different test molecules used in Ref. 18 and the present study; nonetheless they indeed yield identical angle distribution profiles.

RESULTS AND DISCUSSION

Of the many membrane properties, the average area per lipid in the membrane plane is the most commonly used quantity used to characterize packing as well as structure and dynamics of lipid bilayers. Here, we compute the average area per PC by dividing the total area of the simulation box by 64, the number of PC molecules in a single leaflet. Figure 3(a) shows that the area per molecule is significantly higher for the systems where the parametrization accounts for skew states.[18] This finding is in agreement with the form of the dihedral angle ϕ_2.

It is worthwhile to note the two important features from Fig. 3(a). First, the change in the area per molecule extends up to 0.06 nm^2, which is surprisingly large considering that this change can attributed to a single dihedral angle. Such a strong effect was also noticed by the developers of the force field,[18] although in their case only one lipid type was being explored. Second, changes are not systematic but rather system dependent, resulting in a different qualitative behavior with respect to the location of the double bond. The force field by Bachar *et al.* yields a maximum in the area per molecule for the 18:1c9 PC bilayer,[22] in agreement with experiments (see below). The presence of chol in the PC/chol system increases this maximum further.[23] Meanwhile, the force field that does not account for skew states brings forth an area per molecule that increases monotonically as the double-bond position is translated from the end of an acyl chain toward the head group, and this behavior is found in both pure PC and mixed PC/chol bilayers. The largest differences between the two force-field descriptions are found for PC/chol systems, when the acyl chains of PCs have a double bond at the center part of the chain. This is due to the fact that the ϕ_2 dihedral is in these cases interacting with the most rigid part of the chol ring system and also with one of the off-plane methyl groups.[41]

The nonmonotonic behavior in the area per lipid observed particularly in the mixed PC/chol bilayers has been proposed to explain the natural preference to synthesize lipids with double bonds located at the middle of the stearyl acyl chains.[23] This effect is only obtained when the force field accommodates skew states.

Above, we pointed out the agreement between our results and the experiments reported in Refs. 20 and 21. Being more specific, the experiments have shown that the main phase transition temperature for lipids with monounsaturated acyl chains is the lowest when the double bond resides at the middle of a chain. We in turn have found that the area per lipid is the largest when the double bond is located in the chain's center. To confirm the coupling between the two related findings, Table I summarizes the experimental data for a number of one-component lipid systems, showing the inverse correlation between the average area per lipid and the main phase transition temperature. The data shown in Table I concentrates on the results reported by Petrache, Kucerka, and Nagle *et al.* to minimize possible systematic effects due to the measurements and analysis. Clearly, the lower the T_m, the larger the average area per lipid, in agreement with the view used above.

Further comparison with experiments is less straightforward to conduct, since Refs. 20 and 21 are the only ones dealing with effects due to varying location of a double bond in monounsaturated acyl chains. Consequently, the below comparisons between simulations and experiments focus mainly on DOPC due to the available experimental data.

Additional comparison with experimental data shows good agreement with the simulations that exploit the force field with skew states: x-ray data for fully hydrated DOPC

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TABLE I. The inverse relation between the main phase transition temperatures T_m (from Ref. 56) and the average area per lipid for a number of different lipid species and temperatures.

Lipid	Temperature (K)	Area/lipid (Å^2)	T_m (K)	Reference
DLPC	338	71.2	272	51
DMPC	338	68.5	296	51
DPPC	338	67.1	314	51
DSPC	338	66.0	328	51
DLPC	323	67.1	272	51
DMPC	323	65.4	296	51
DPPC	323	63.3	314	51
DPPC	323	64.4	314	52
DOPC	303	72.4	253	53
DOPC	303	72.4	253	54
POPC	303	68.3	271	53
DLPC	303	62.6	272	51
DLPC	303	63.2	272	55
DMPC	303	60.0	296	51
DMPC	303	60.6	296	55

bilayers have resulted in values of 0.721–0.725 nm^2 for the area per lipid,[42–44] while the force field of Ref. 18 yields 0.731 nm^2. Meanwhile, the force field that does not account for skew states leads to an area of 0.688 nm^2 per DOPC molecule. The comparison here is suggestive, however, since the experiments were conducted at 303 K while the simulations have been carried out at 338 K. Below, we compare the simulation data to experiments more concretely through the S_{CD} profiles and x-ray form factors.

To gain more insights into the transverse structure, we computed the thickness of the bilayers by considering the average distance between the phosphorus atoms in the opposite leaflets (P–P distance). Figure 3(b) shows the resulting membrane thicknesses as a function of the double-bond position. We find that the results express similar features as the area per lipid discussed above. The force field by Bachar *et al.* yields a nonmonotonic behavior with a minimum in the 18:1c9 bilayers. This behavior is enhanced again in the presence of chol. The other force field that does not accommodate skew states, on the other hand, predicts membrane thickness to increase monotonically as the double bond is shifted from the glycerol group region to the end of an acyl chain. Changes in membrane thickness due to the skew state are also rather pronounced, and especially in the PC/chol systems the change can be as large as 0.4 nm. Thickness changes of this size have been shown to alter the functionality of some membrane proteins.[45]

Comparison to experimental data for DOPC membranes displays best agreement with the results obtained through the force field that incorporates skew states: the membrane thickness determined from electron density profiles[42–44,46] varies between 3.67 and 3.71 nm, while the force field with skew states results in a value of 3.71 nm, and the other one yielding a thickness of 3.89 nm.

The property that can accurately be measured from NMR experiments, providing information of lipid chain order, is the deuterium order parameter (S_{CD}) profile along the chain.[47] S_{CD} is defined as

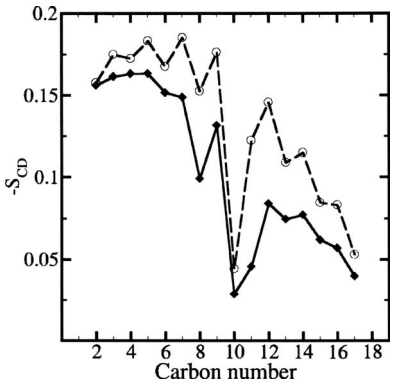

FIG. 4. The S_{CD} order parameter profile for the *sn*−2 chain of DOPC (18:1c9) with the two different double-bond parametrizations studied in this work. Carbon numbers follow the notation in Fig. 1. The solid lines correspond to the force field by Bachar *et al.* (Ref. 18) that accommodates skew states, whereas the dashed curves result from the force-field parametrization without them.

$$S_{CD} = \left\langle \tfrac{3}{2}(\cos^2\theta) - \tfrac{1}{2} \right\rangle,$$

where θ is the angle between the C–D bond and the bilayer normal, and the angular brackets denote averaging over time and over all C–D bonds. Since we employed the united-atom model, the deuterium positions were constructed from the neighboring carbon assuming ideal geometries. The S_{CD} mean values (averages over segments 4–18 with the exception of the segments containing the double bond) for the *sn*-2 PC acyl chains are plotted as a function of the double-bond position in Fig. 3(c). For the force field by Bachar *et al.*, we find a minimum in the S_{CD} curves when the double bond resides at the middle of an acyl chain, in agreement with the above discussed behavior for the area per lipid. The minimum is particularly evident in the PC/chol system where the interplay between the double bond and the rigid steroid structure enhances the effect. The force field that does not account for the skew states is again qualitatively different from the above picture. Instead of yielding a minimum, the S_{CD} profile increases monotonically as the double bond is translated towards the end of an acyl chain.

As an additional criterion to judge the relative merits of the two force-field descriptions for the double-bond region, consider Fig. 4. It shows the S_{CD} order parameter profile for DOPC (18:1c9), where the double bond resides at the middle of the acyl chains. The fact that the two double-bond parametrizations yield different quantitative results is no news, since the average areas are also different. What is more striking is the different qualitative behaviors close to the double bond, for carbon 9–11. For comparison, Warschawski and Devaux[48] have reported the NMR data for lipid chain ordering in fluid DOPC membranes at 310 K. They found that the chain order parameter had a major minimum at carbon position 10, followed by a minor increase for carbon 11. This behavior is consistent with the simulations where skew states have been taken into account. In the other force field without skew states, the S_{CD} profile for carbon 10–12 is substantially different, however. Nonetheless, a full comparison with experiments is difficult to make due to the different temperatures used in experiments (310 K) and simulations (338 K).

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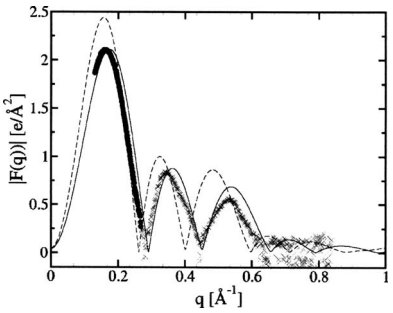

FIG. 5. Absolute form factors as a function of the wave number q for the simulated pure DOPC bilayers at 303 K. The two cases shown by the solid and dashed lines correspond to the force-field parametrization by Bachar *et al.* (Ref. 18) and the parametrization without the skew state, respectively. The solid circles and crosses correspond, respectively, to the experimental ULV and ORI data for DOPC at 303 K (for more details, see Ref. 50).

The comparison is rendered even more difficult due to the fact that the order parameter values reported in Ref. 48 are based on $^{1}H-^{13}C$ dipolar couplings, for which reason the order parameters found in Ref. 48 are smaller than those measured through the more common ^{2}H NMR (see discussion in Ref. 48), which in turn is consistent with our simulation data. Despite this difficulty regarding quantitative comparison between experiments and simulations, the qualitative nature of the order parameter data for carbon 10–12 is in favor of the force field that accounts for the skew state.

Considering the difficulties to rigorously compare simulation data with experiments, let us finally use the scattering form factor, $F(q)$, to confirm that the skew state makes a difference in the DOPC force field. The form factor computed from simulations can be compared to experimental "model-free" measurements. First, the relative electron density profile $\rho_r(z)$ is computed by subtracting the electron density profile of bulk water from that of the total system. The scattering form factors are then calculated as[49]

$$|F(q)| = \sqrt{\left(\int_{-L/2}^{L/2} \rho_r(z)\cos(qz)dz\right)^2 + \left(\int_{-L/2}^{L/2} \rho_r(z)\sin(qz)dz\right)^2},$$

where L is the length of the simulation cell in the z direction. In Fig. 5, the form factors are plotted for the simulated pure DOPC membranes using the two force fields at 303 K. The data extracted from the experimental form factor in Ref. 50 are also plotted for comparison. The results indicate a better agreement between the experimental scattering form factor[50] and the simulation using a force field that accounts for the skew state. The performance of the DOPC force field where the skew state is not included is substantially weaker.

In summary, comparison of DOPC simulations with experiments allows us to conclude that the force field including the skew state performs substantially better than the force field that does not take it into account. The results shown in Fig. 2 bring about the main reason why this is the case; the skew state is a crucial part of the parametrization of a double bond. The main results of this work are, though, the data shown in Fig. 3.

CONCLUDING REMARKS

Overall, force-field parametrization is a complex issue since there is no such a thing as a unique force field for any given system. It has been found that certain interactions are particularly important in the sense that seemingly minor changes in their parametrization may have a pronounced influence on system properties. In soft and biological matter, the interactions that are usually considered to express this concern are electrostatic interactions and hydrogen bonds, since they are the strongest interactions with respect to thermal energy. A number of lipid membrane studies have shown that these concerns are justified.[13–15] Meanwhile, as van der Waals interactions in the hydrophobic core of a membrane are considerably weaker than interactions based on electrostatics, one usually assumes that the parametrization of hydrocarbon chains is a matter of less concern.

Here, we have questioned this view by considering the treatment of double bonds in atomistic MD simulations of unsaturated lipid membranes. Not surprisingly, we have found that changes in the double-bond parametrization lead to quantitative differences in membrane properties. However, what is considerably more alarming is the fact that the different double-bond descriptions lead to results that are *qualitatively* different.

The membrane systems dealt with in the present study have been comprised of PC lipids with monounsaturated acyl chains. However, instead of letting the double bond to reside in a fixed position, we have varied its location systematically along the acyl chain. The force field that accounts for skew states in the vicinity of a double bond predicts that the membrane is most disordered when the double bond resides at the center of an acyl chain. This is manifested as a maximum in the area per lipid and a minimum in the S_{CD} order parameter profile, when the double bond is at the center of a chain. This behavior is consistent with experimental findings,[20,21] which have shown the main phase transition temperature of single-component lipid bilayers to have a minimum under similar conditions. Meanwhile, if the force field does not accommodate skew states next to the double bond, similar nonmonotonic behavior (and a minimum or maximum) in area per lipid and S_{CD} is not observed.

In more complex systems that include chol, the trends discussed above are even more pronounced, suggesting that the proper description of double bonds becomes increasingly important in many-component membranes, where the interplay of different molecule types is difficult to predict on intuitive grounds. Nonetheless, while the results presented in this work give rise to some concern, we wish to emphasize that not all membrane properties are particularly sensitive to the details of double-bond description. For example, if one is only interested in processes taking place at the membrane-water interface, the double-bond treatment is likely not highly relevant as long as the area per lipid and hence membrane packing properties are in order. One should also note that numerous differences between chol interactions with saturated and unsaturated lipids were reproduced even with the older parametrizations which do not take the skew states into account. However, as the relevance of double bonds is

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usually not known beforehand, why not avoiding possible artifacts by using descriptions that are consistent with experiments?

ACKNOWLEDGMENTS

We thank John F. Nagle and his group for experimental data and for allowing us to reproduce it in Fig. 5. T. R. has been supported by the Marie Curie Intra-European Fellowship 024612-Glychol. This work was carried out under the HPC-EUROPA project (RII3-CT-2003-506079), with the support of the European Community—Research Infrastructure Action under the FP6 "Structuring the European Research Area" Programme. Computational resources were provided by: the Barcelona Supercomputing Center (www.bsc.es), The Finnish IT Centre for Science (CSC), and the SharcNet grid computing facility (www.sharcnet.ca). We wish to thank the Academy of Finland, the Emil Aaltonen Foundation, and the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support. Partial financial support was provided by SEID through the project FIS200603525 and by DURSI through the project 2005-SGR000653.

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