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Accelerating All-Atom MD Simulations of Lipids Using a Modified Virtual-Sites Technique

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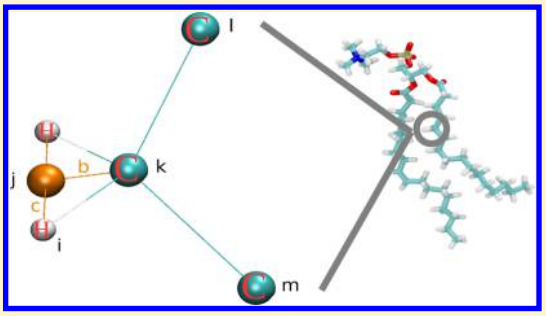
Supporting Information

ABSTRACT: We present two new implementations of the virtual sites technique which completely suppresses the degrees of freedom of the hydrogen atoms in a lipid bilayer allowing for an increased time step of 5 fs in all-atom simulations of the CHARMM36 force field. One of our approaches uses the derivation of the virtual sites used in GROMACS while the other uses a new definition of the virtual sites of the CH2 groups. Our methods is tested on a DPPC (no unsaturated chain), a POPC (one unsaturated chain), and a DOPC (two unsaturated chains) lipid bilayers. We calculate various physical properties of the membrane of our simulations with and without virtual sites and explain the differences and similarity observed. The best agreements are obtained for the GROMACS original virtual sites on the DOPC bilayer where we get an area per lipid of 67.3 ± 0.3 Å^2 without virtual sites and 67.6 ± 0.3 Å^2 with virtual sites. In conclusion the virtual-sites technique on lipid membranes is a powerful simulation tool, but it should be used with care. The procedure can be applied to other force fields and lipids in a straightforward manner.



1. INTRODUCTION

We report a method allowing an increase, by more than twofold, of the time step of all-atom molecular dynamics (MD) simulations of membrane and protein−membrane systems that employ the CHARMM force field,[1] by extending the virtual-site[2,3] framework to lipids represented by the CHARMM C36 force field.[4] Our strategy is applicable to other force fields as well.

Biological molecules are inherently hydrogen-rich. The high-frequency vibrations of hydrogen-containing bonds and angles limits the time-step in all-atom simulations to 1 fs. Constraining bonds using SHAKE[5] and both bonds and angles using LINCS[6,7] increases the time step to 2 fs, beyond which both constraining algorithms often become unstable. To improve performance, one strategy is to use a united-atom force field, where nonpolar hydrogen atoms are united onto the attached heavy atom.[8,9] However, the time step used is still constrained to 2 fs, and one loses information about nonpolar hydrogen atoms, which may be desired in many cases. An alternate strategy is to eliminate these fast degrees of motion completely by treating each hydrogen atom as a massless dummy atom, the so call virtual-site procedure.[2,3] The hydrogen masses are delegated to the closest heavy atom, and their coordinates are recalculated after each time step from the three nearest heavy atoms using geometric considerations. The strategy has been successfully used with a time step of up to 5 fs for simulating fully hydrated protein with the CHARMM27 force field.[3]

Protein−lipid systems are typically simulated using the CHARMM,[4] AMBER,[10] OPLS,[11] or one of the GROMOS force fields. The choice of force field while simulating protein−lipid complexes depends on considerations of speed and accuracy, the problem of interest, limitations of the simulation engine, and even historical reasons. Without going into detailed arguments about force-field choice, we will simply state that besides AMBER, CHARMM is an all-atom force field with a fully self-consistent, sufficiently accurate parameter set for all biological macromolecules: proteins, lipids, carbohydrates, and nucleic acids.[1,4,12] Furthermore, parameters for organic molecules and drugs can be now be automatically acquired using the newly developed CHARMM general force field,[13] much like what has been possible with the Antechamber module of AMBER.[14]

Unlike the Berger force-field for lipids, in which nonpolar hydrogen atoms in the lipid tails are united into the connected carbon, the CHARMM lipid force field employs explicit hydrogens, which more than doubles the number of interaction sites representing each lipid, ultimately resulting in significant performance loss. United-atom parameter sets of the CHARMM lipids force field were developed to address this issue,[15,16] but it will ultimately become tedious to do this for each new generation of lipid force fields. A better solution is to extend the virtual-sites algorithm to the hydrogen atoms to the lipids. In the existing implementation of virtual sites[2,3] in the simulation engine GROMACS,[17−20] virtual sites can be applied to proteins and additionally to lipids with only minor modification. The current implementation of virtual sites within GROMACS does not work with CHARMM lipids for

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a variety of reasons. First, some of the default parameters for the position of the CH3 groups are values from OPLS, and not CHARMM. Second, a few parameters are missing from the topology in order to handle the specific structure found in the lipid headgroups. We then propose two approaches to derive the virtual-sites parameters: one based on the geometrical properties of an equilibrated CHARMM36 bilayer at the temperature of interest and the other relying on the default procedure originally implemented in GROMACS for calculation of the protein virtual sites albeit with newly tuned parameters for CHARMM. We will compare both approaches and show that the latter gives better agreement with the simulation without virtual sites at the temperature and pressure of interest.

To test the accuracy of our virtual-sites implementation for CHARMM lipids, we calculate typical properties of three fully hydrated lipid bilayers consisting of 1,2-dipalmitoyl-phosphatidylcholine (DPPC), 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), and 1,2-dioleoyl-phosphatidylcholine (DOPC) lipids.

2. COMPUTATIONAL METHODS

2.1. Virtual-Site Parameters Derivation. In this section we detail the two methods we used in order to use virtual sites with the lipids. One relies on the same automatic calculation as implemented in GROMACS for proteins and is referred to as "original virtual sites". The other uses a different virtual-sites construction for the CH2 group of the lipid tails and focuses on reproducing the average properties of the individual lipids as extracted from simulations without virtual sites. This second method is named "fitted virtual sites".

In the phosphatidylcholine (PC) lipids the hydrogen atoms can be divided in four groups: (i) the hydrogens from the CH3 groups in the choline headgroup and the chain ends, (ii) the hydrogens from the CH2 groups of the saturated carbon atoms, (iii) the hydrogens bound to the unsaturated carbon atoms in the carbon chains, and (iv) the CH group of the glycerol backbone.

2.1.1. Original Virtual Sites. In this paper we treat the CH3 groups as in the original virtual-site publications:[2,3] the whole group (the carbon atom and the three hydrogens) is replaced by two particles having each half the mass of the CH3 group. The distances between these masses and the atom to which the CH3 group is bound are constrained. These distances are calculated such that the group formed by the two masses has the same center of mass and moment of inertia as the CH3 group. For POPC there are two such sets of parameters as the CH3 groups can be bound either to a carbon (tails) or to a nitrogen (headgroup). Therefore, we introduce a new particle type named MCH3N for the headgroup. The dummy masses for the POPC tails conserve the original MCH3 denomination as there is no difference in the bond length between a CTL2 carbon atom of the CH2 groups of the lipid tails and a CTL3 carbon atom of the CH3 group of the lipids and CT2 and CT3 in proteins in the CHARMM36 force field. The actual values for the constraints are recalculated for the CHARMM36 force field because the default values used by GROMACS are for the OPLS force field. We show the calculated values in Table 1. All other virtual sites are treated as per the original publications.[2,3] We subsequently modified the topology so that the pdb 2gmx program of the GROMACS package can be used directly with one of the -vsite options on a PC lipid to obtain its topology with virtual sites. See the Supporting Information for details on which topology files have been modified.

Table 1. Parameters for the Virtual Sites of the CH3/NH3 Groups Updated for CHARMM36[a]

CHARMM atom types		constrain length (nm)
	new parameters	
MCH3N	NTL	0.182707
MCH3N	MCH3N	0.091314
MCH3	CTL2	0.185772
	updated parameters	
MNH3	CT1	0.174907
MNH3	CT2	0.174907
MNH3	CT3	0.174907
MNH3	MNH3	0.082619
MCH3	CT1	0.186636
MCH3	CT2	0.185772
MCH3	CT3	0.185945
MCH3	MCH3	0.093582
MCH3S	S	0.211217
MCH3S	MCH3S	0.092844

[a]The parameters marked as "new" were not present in the topology and had to be added. The parameters marked as "updated" were already present but their values have been changed.

2.1.2. Fitted Virtual Sites. The "3out" construction usually used for the CH2 groups[2] is made from construction vectors that are not normalized to avoid the division operations required and to increase performance. However, for lipids, this means that the actual location of the hydrogens depends on the nearby dihedral angle whose value fluctuates during the simulation. Therefore, we change the way the virtual sites for the CH2 groups are calculated. To define the new virtual sites for the hydrogens we need the position of the atoms bound to the main carbon atom of the CH2 group, atoms l and m in Figure 1. We use new intermediate constructing particles with no mass nor interaction named VCXX where XX stands for the carbon atom number to which the hydrogens are bound, atom j

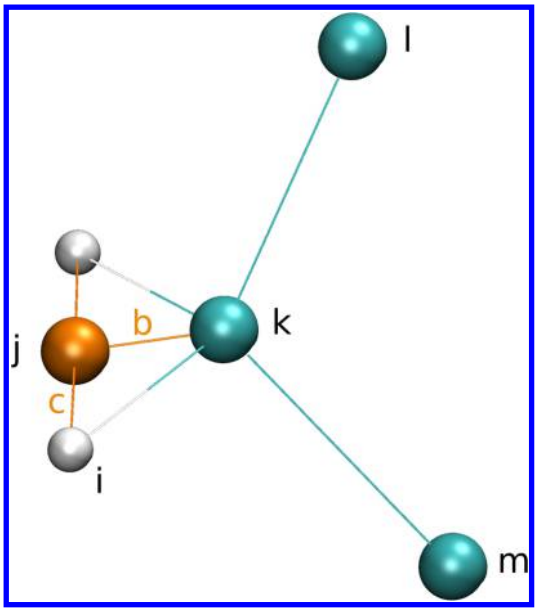

Figure 1. New virtual site used for the CH2 groups. Atoms k, l, and m are the atoms from which the virtual sites are constructed. We first construct atom j using the positions of atoms k, l, and m. We then construct the virtual sites for the hydrogens (atom i in the Figure) using the position of atoms j, k, l, and m

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in Figure 1. These new virtual-site particles are placed such that they are the normal projection of the hydrogen atoms on the plane spanned by the atoms k, l, and m. They are constructed using the 3fd virtual site construction of GROMACS. Subsequently, the positions of the hydrogen atoms are calculated using the 4fdn virtual-site construction of GROMACS. The main difference from the default method used for the CH2 groups in the original virtual sites is that the vectors used here are normalized. Our method is somewhat slower due to the division needed for the normalization. However, it allows greater control over the position of the hydrogens. The other virtual sites for CH and CH3 groups of the lipids are calculated as per the original virtual-sites procedure. However, the actual values of all the parameters are calculated from the average angles and bond lengths extracted from the simulations without virtual sites. These are different from the values used in the original virtual-sites construction which use the values from the topology, ignoring the Urey–Bradley terms and other interactions.

2.2. Simulations. We report three sets of simulations each of which are described in the following. All systems contain a 128 lipid bilayer and 5688 water molecules. We use three phosphatidylcholine lipid types in our simulations: DPPC which has two saturated chains, POPC with one saturated and one unsaturated chain, and DOPC with two unsaturated chains. The lipids and waters are independently coupled by the heat bath at 303 K for the POPC and DOPC system and at 323.15 K for DPPC. A semi-isotropic pressure coupling at 1 bar with zero average tension is used. All membranes were equilibrated for at least 30 ns using the Velocity rescale thermostat and Berendsen pressure coupling.[21] The production runs use the Nose–Hover thermostat[22,23] and the Parrinello–Rahman barostat.[24]A Lennard-Jones potential switch function is applied from 8 to 12 Å, and electrostatic interactions are treated using particle mesh Ewald (PME)[25] with a real space cutoff of 12 Å. The CHARMM TIPS3P water model with explicit Lennard-Jones interactions on the hydrogens is used.[1] For the simulations without virtual sites we use a 2 fs time step with the covalent bonds involving hydrogen atoms constrained with the LINCS algorithm.[6,7] For the virtual-sites simulations we use the same options except that all bonds are constrained, as required by the virtual-sites procedure, and the time step is increased to 5 fs. We used the CHARMM36 force field[4] as implemented in GROMACS (4.6.3).[3,17,26]

We have run two simulations of 200 ns with different initial velocities for each of the DPPC, POPC, and DOPC lipid bilayers without virtual sites and with the fitted and original virtual sites. This gives a total of 3.6 μs of simulation.

3. RESULTS

To validate our new set of parameters we calculate the area per lipid, chain order parameters, P–N vector orientation, the diffusion constant, the autocorrelation of the vector connecting the first carbon atom of each chain, and the hydration of the headgroups with and without virtual sites for all three membranes. The area per lipid, the C–C correlation time, the average P–N angle, and the diffusion coefficient are shown in Table 2. The figures for the P–N vector distribution, the deuterium order parameters, the mean square displacement (msd), and the C–C autocorrelation are show in Figures 2, 3, and 4 for our DPPC, POPC, and DOPC membranes, respectively. The time evolution of the area per lipid and the hydration figures can be found in the Supporting Information.

Table 2. Calculated Values as Average and Standard Error over All Sets of Simulations without Virtual Sites, with the Fitted Virtual Sites and with the Original Virtual Sites[a]

lipids	type	APL (Å^2)	C–C (ns)	P–N (deg)	D (10^{-8} cm^2/s)
DPPC	no vs	59.0 ± 0.3	2.2	108.8 ± 0.2	11.8 ± 0.1
	fitted vs	49 (gel)	9.1	107.0 ± 0.8	3.4 ± 0.1
	ori vs	61.3 ± 0.3	2.2	108.6 ± 0.2	15.3 ± 0.6
POPC	no vs	63.1 ± 0.3	3.3	111.0 ± 0.3	7.8 ± 0.3
	fitted vs	61.0 ± 0.3	4.5	111.0 ± 0.4	8.9 ± 0.2
	ori vs	64.6 ± 0.3	4.1	110.6 ± 0.3	7.8 ± 0.3
DOPC	no vs	67.3 ± 0.3	2.9	111.1 ± 0.2	8.7 ± 0.3
	fitted vs	65.6 ± 0.3	3.8	111.0 ± 0.3	7.9 ± 0.2
	ori vs	67.6 ± 0.3	3.6	110.7 ± 0.3	7.5 ± 0.3

[a]The diffusion coefficient is calculated from a linear fit to the msd between 5 and 25 ns.

Overall the original virtual sites gives results closer to the simulations without virtual sites than the fitted virtual sites. Also the parameters of the virtual-sites simulations get closer to the ones without virtual sites as we increase the number of unsaturated chains in the lipids. For DPPC the lipids of the fitted virtual site goes into a gel like structure after 30 ns of simulation, and this is reflected in all the calculated properties. We will postpone an explanation for this behavior to the next section and we will ignore this case in the following discussion. In all the other simulations the virtual-sites lipids are in a liquid crystalline phase. The fitted virtual sites have a lower area per lipid for all the lipids studied while the original virtual sites have a greater one. The area per lipid obtained from our simulations without virtual sites is lower than the one reported in the original CHARMM36 publication[4] due to the use of a potential switch instead of a force switch for the Lennard-Jones interactions. However, this should not modify the behavior of the virtual sites when compared to the simulations without virtual sites using the same cutoff scheme. The higher/lower area per lipid translates into a lower/higher order parameter for the lipid tails, and the unsaturated chains with virtual sites have a closer order parameter to the one without virtual sites. The P–N vector orientation as well as the hydration (see the Supporting Information) show only minor differences suggesting that the virtual-sites procedure does not affect the properties of the lipid head groups. The virtual sites increase the C–C correlation time while the diffusion coefficient can be increased or decreased by the procedure.

4. DISCUSSION

Let us discuss in more details the difference between the fitted and the original virtual sites. In Figure 5 we show the angle distribution of one of the CH2 angles in the lipid tails for all our simulations with the POPC molecules. In this figure we can see that the fitted virtual sites conserves the average structure of the CH2 group but completely suppresses the fluctuations. The fitted virtual sites then gives a better average structure of the lipids, for example, see the P–N angle distribution in Figures 3 and 4. It will also always give a lower area per lipid, and this will lead to severe artifacts, as seen for DPPC in Figure 2. The original virtual sites has a distribution which is broader than the one for the fitted virtual sites, and its average is different from the simulation without virtual sites. This is because of the use of non-normalized vectors in the construction of those virtual sites which makes the actual position of the hydrogens dependent

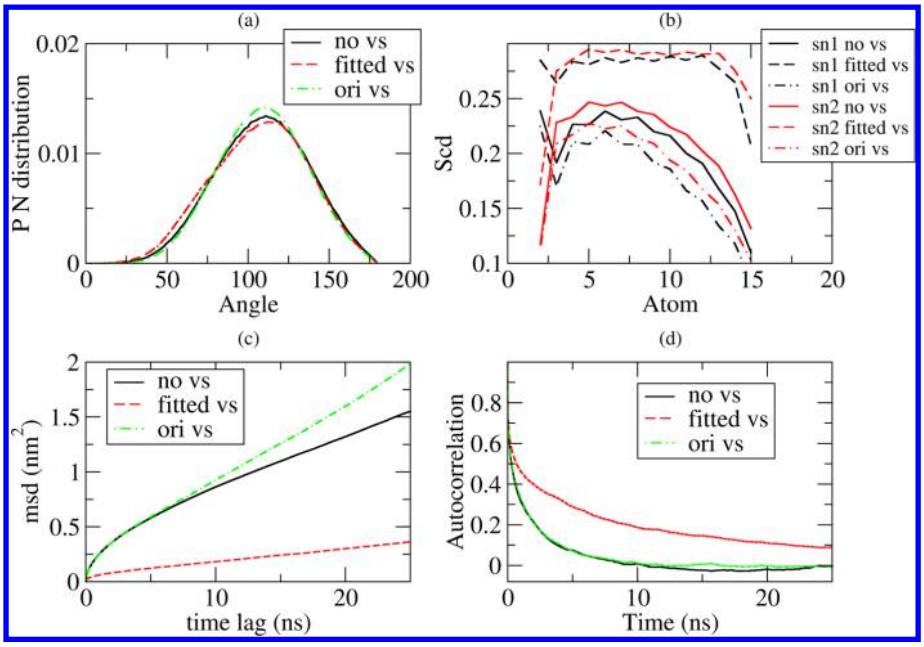

Figure 2. Calculated properties for the DPPC membranes. (a) Distribution of the P−N vector angle with the z axis. (b) Deuterium order parameters of the lipid tails. (c) Mean square displacement of the lipids center of mass. (d) Autocorrelation of the angle between the x axis and the vector connecting the first two carbon atoms of the lipid chains (atom name C21 and C31). In all figures the continuous line is for the simulation without virtual sites while the dashed and dot-dashed lines are for the fitted and original virtual sites, respectively. Note that the lipids in the simulation with the fitted virtual sites are in a gel phase

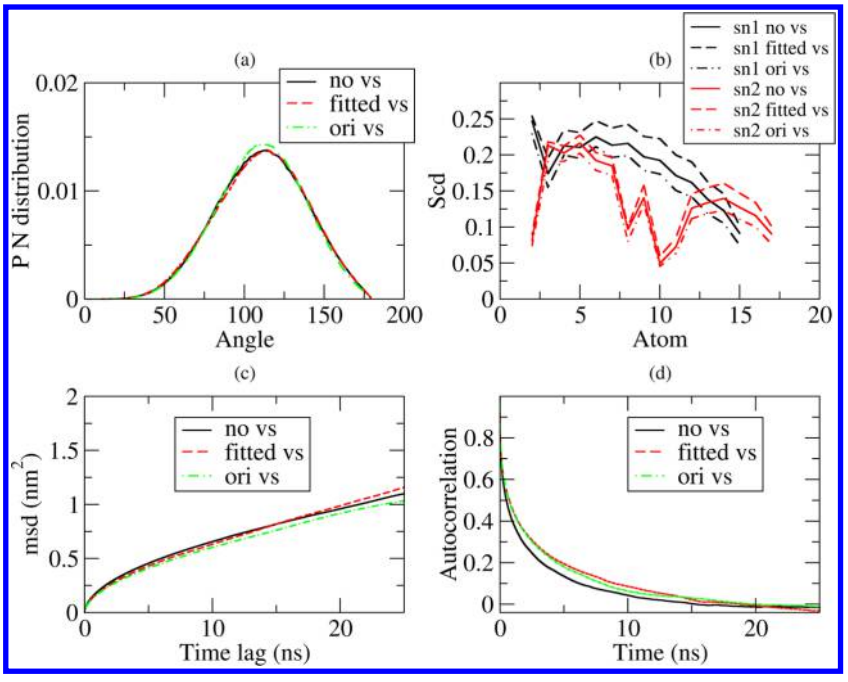

Figure 3. Calculated properties for the POPC membranes. The properties shown are the same as in Figure 2.

on the position of the neighboring heavy atoms. Note that this makes the average value of the position of the hydrogen atoms difficult to predict because they depend on the position of the nearby atoms which themselves depend on the position of the hydrogen atoms. As an example, the positions of the H5R and H5S atoms are technically calculated to give a value for the CH2 angle equal to the one given in the topology, 109°, but end up being 102°. Interestingly, this procedure accidentally conserves some kind of fluctuations of the CH2 group. These fluctuations, together with the altered structure of the lipids, give an area per lipid which is higher for all the lipids studied here. Even though the lowering of the fluctuation observed in Figure 5 should decrease the area per lipid, the change in the average structure in the original virtual sites overcompensates for this change. In particular, these changes avoid the collapse of the lipids observed for the fitted virtual sites for DPPC.

One could try to find optimal bond lengths and angles that would fit the area per lipid and other properties of the lipids, but this would be akin to develop a new parameter set which was not the aim of our approach.

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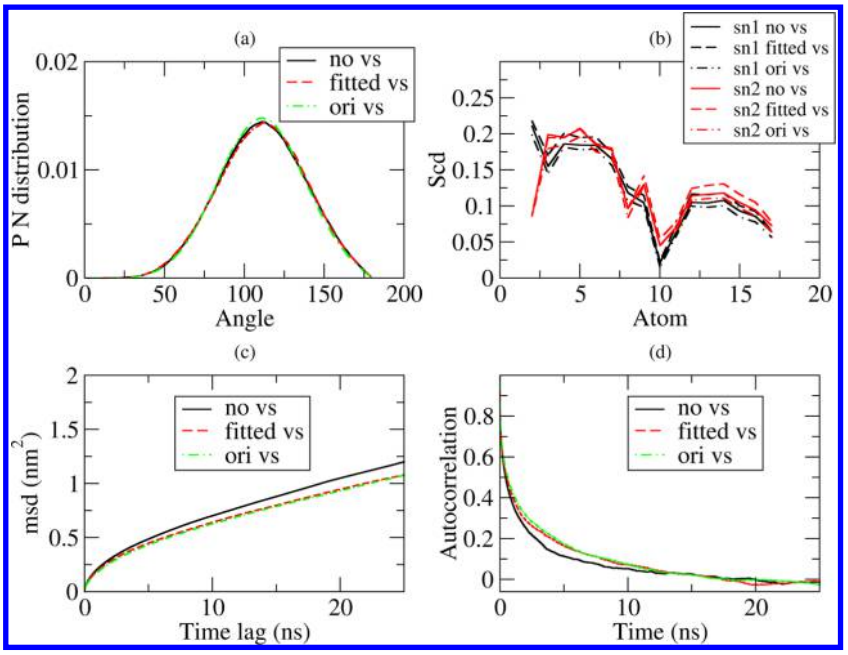

Figure 4. Calculated properties for the DOPC membranes. The properties shown are the same as in Figure 2.

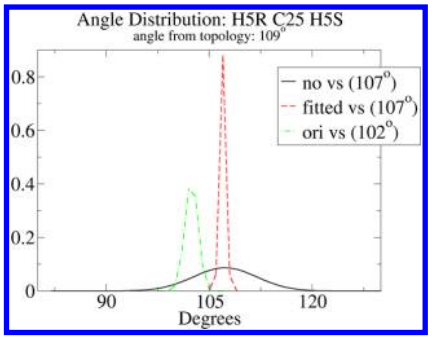

Figure 5. Distribution of the angle of the fifth CH2 group of the first chain for all POPC lipids in all our simulations. Note that the fitted virtual sites and the simulation without virtual sites have the same average angle while the original virtual sites have a broader distribution than the fitted virtual sites.

The time-step used in the simulation and, hence, the performance increase will depend on the fastest degrees of freedom after the removal of the hydrogens degrees of freedom. In the original virtual-sites paper,[2] where the virtual sites were only applied to proteins, a time-step up to 7 fs was used. However, in our simulations, with the CHARMM36 force field, a time-step above 5 fs leads to unstable systems.[3] The water model used is also important as it can limit the time-step below 5 fs independently of the lipids and proteins in the system. The CHARMM36 force field uses the TIPS3P water model (slightly modified TIP3P model, with Lennard-Jones interactions on the hydrogens) where the water is modeled as a completely stiff molecule, and therefore it is a good candidate for the application of the virtual sites. The actual performance increase will typically be slightly lower than a factor 2.5;, for our simulation we typically get a factor ∼2.48, and this is because of the additional steps required to calculate the virtual-sites positions.

As with all MD approaches, a specific set of parameters should only be used in the conditions it was derived for. In the case of the CHARMM force field used for biomembrane simulation, one should not try to obtain meaningful results with pressure and temperature far from the biological range. The same applies to our procedure, but by freezing the hydrogen atoms of the lipid tails we introduce another limitation, namely, the environment of the lipids should not change drastically during the simulation. For example, the virtual sites procedure might be unfit to describe spontaneous self-assembly of lipids in a water phase. Indeed, as the environment of the hydrogens of the lipid tails changes from water to being in contact with other lipid tails, a rearrangement of the hydrogen atoms can be expected. Such a rearrangement is not possible with virtual-sites hydrogens. This is one of the reasons why we developed the fitted virtual-sites procedure: a different set of parameters can be derived for different simulation conditions (temperature, pressure, etc.). Even though the original virtual sites works better in the conditions we have discussed here it could perform worse in others, for example, for a system originally in a gel phase without virtual sites.

Finally, we can say that the membrane properties are well reproduced by the original virtual sites, in particular for DOPC (with two unsaturated chains). The trend being that the higher degrees of unsaturation the better for the properties of the virtual sites. Given that the properties of the headgroup are mostly unaffected by the procedure, one could use the virtual sites to study the membrane interactions with interfacial proteins. It could also be used to accelerate the simulation of transmembrane proteins, but care should be taken to be sure that the change in order parameter does not affect the overall structure of the protein. From the topology we provide one can calculate the virtual sites for any PC lipid. However, the parameters for a new lipid should be thoroughly tested before being used. This is particularly true if the temperature of interest is close to a phase transition of the lipid.

5. CONCLUSION

We implement a method to improve performance of all-atom lipid simulations by a factor of 2.5. We extend the virtual-sites procedure to DPPC, POPC, and DOPC lipids, thus permitting

a time step of 5 fs. Some membrane static properties such as the orientation of the headgroup, the water hydration, and, in some cases the area per lipid, are similar to the simulations with and without virtual sites. The virtual-sites technique will influence the properties of the membrane and lipids and one must decide if the sacrifice in accuracy is reasonable for the properties of the system of interest. The procedure is compatible with the previously implemented virtual-sites method for proteins, thus allowing virtual-sites simulations of protein lipid complexes.

Currently the method has been implemented for the CHARMM36 force field and is applicable to other lipids, proteins, and, possibly, force fields. We will extend the range of available parameters in future works.

■ ASSOCIATED CONTENT

Supporting Information

Area per lipid and hydration graphs, details on the calculation of the diffusion coefficient, and the autocorrelation time as well as more information on the topology construction. This material is available free of charge via the Internet at http://pubs.acs.org/. The topology files can be found at http://memphys.dk/node/71.

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Notes

The authors declare no competing financial interest.

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