Optimization of Hydrocarbon Chain Interaction Parameters: Application to the Simulation of Fluid Phase Lipid Bilayers

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We have carried out molecular dynamics simulations of *n*-hexane, *n*-decane, *n*-pentadecane, and 5-decene in order to find an optimal set of parameters for nonbonded interactions between atoms on different molecules. This optimization is necessary because previous parameter sets optimized by fitting to a single *n*-alkane do not work well when applied to the simulation of a liquid alkane with a chain length different from the fitted value. For the simulation of fluid phase lipid bilayers in which the hydrocarbon environment is different at different depths in the bilayer interior, it is essential to have a robust parameter set for reliable simulations. We have found such a set, and in this paper we describe the optimization procedure and give the results.

In recent years the number of simulations of hydrated, fluid phase, lipid bilayers has grown rapidly.[1−10] All of the simulations employ molecular dynamics (MD) and/or Monte Carlo (MC) methods to explore the configuration space of a simulation cell typically consisting of ∼100 lipids and a few thousand waters. Most simulations are currently done under the constraints of constant temperature and isotropic or anisotropic constant pressure. All of the simulations agree reasonably well with some of the experimental data, such as order parameters, but none of the simulations so far agree with *all* of the experimental data. Two quantities that tend to differ between the various simulations are the area per molecule and the dipole potential of the bilayer. These quantities are very sensitive to the parameters that are input to each simulation. (In a constant surface tension or constant pressure simulation, the area per molecule is a prediction of the simulation.)

The set of interaction parameters that must be input into a simulation consists of parameters for interactions between bonded atoms, parameters for interactions between nonbonded atoms on the same molecule, and parameters for interactions between nonbonded atoms on different molecules. While all of these interactions are important, probably the subset that is most important for the determination of such thermodynamic variables as density (or area per molecule) and dipolar potential is the set of parameters for interactions between nonbonded atoms on different molecules. This set consists of three parameters for each atom type; the partial electrical charge, and the 6-12 parameters in a typical 1-6-12 interaction function:

$$V(i) = \sum_{j \neq i} \left[\frac{q_i q_j}{r_{ij}} + 4\sqrt{\epsilon_i \epsilon_j} \left(\left(\frac{\sqrt{\sigma_i \sigma_j}}{r_{ij}} \right)^{12} - \left(\frac{\sqrt{\sigma_i \sigma_j}}{r_{ij}} \right)^{6} \right) \right] \quad (1)$$

where q_i, σ_i, and ϵ_i represent the partial charge, 6-12 radius, and 6-12 strength, respectively, for atom type *i*. For a lipid bilayer simulation one must assign values to these parameters for all different atom types in the simulation.

If the lipid bilayer to be simulated is in its fluid phase a potential simplification in computing is to simplify the lipid molecules by using "united atoms", i.e. atoms in which all methyl and methylene groups are represented by single centers of 6-12 interaction, and no explicit hydrogen atoms are introduced. For closely packed, ordered, chains this approximation will lead to erroneous results.[11] For fluid phases, where intermolecular densities are lower and directional interactions are subject to averaging over fast time scales, the inclusion of explicit hydrogens is less critical. It *is* critical in these systems to simulate as large a system as possible for as long as possible in order to achieve the best possible conformational sampling. In order to do this we do make several assumptions that maximize the speed of the computations without unduly compromising accuracy in ways that are significant for this type of calculation, including the use of united atom models, interaction range cutoffs, and weak pressure coupling (to be discussed below).

Generally, values for interaction parameters are selected after a set of simulations on simpler systems are carried out. Parameters that accurately reproduce experimental data in simulations of simpler systems are the best choices for lipid bilayer simulations. For saturated lipid hydrocarbon chains, the clear choice for such reference systems is the liquid alkanes. Early work by Jorgensen and co-workers used a comprehensive set of Monte Carlo simulations to establish a set of optimal parameters for liquid hydrocarbons.[12] More recently, Berger et al.[1] have suggested that 6-12 parameters for interactions between methyls and methylenes on different molecules should be chosen on the basis of simulations of longer chain hydrocarbons if they are to be used in lipid bilayer simulations. They found that earlier parameter sets based on simulations of liquid hexane did not accurately reproduce the density or the heat of vaporization of liquid pentadecane. They have revised their simulation parameters on the basis of fitting the density and heat of vaporization of liquid pentadecane.[1] In all cases to date,

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simulations for the purpose of testing parameter sets have been carried out only on short chain *n*-alkanes (i.e., up to hexane), or only on long chain *n*-alkanes, i.e., pentadecane.

For the selection and testing of simulation parameters, experimental data are available for a variety of thermodynamic properties of liquid hydrocarbons over a wide range of chain length. In particular, the specific molecular volume of liquid alkanes shows a simple linear dependence on the chain length.[13] Therefore, it is desirable to find an optimal parameter set for simulations that will successfully reproduce liquid alkane thermodynamical properties over a wide range of chain lengths. Such a parameter set could then be used with increased confidence for simulation of lipid membranes.

In this paper we describe simulations of three different alkanes, hexane, decane, and pentadecane, and one alkene, 5-decene. We have determined a set of 6-12 parameters for nonbonded interactions between methyls and methylenes on different molecules for which MD simulations yield densities and heats of vaporization for the *n*-alkanes that are in good agreement with experiment for $n = 6$, 10, and 15. In addition, we have used these parameters in 5-decene simulations to find appropriate parameters for the double-bonded carbons in these chains. This is important for the simulation of lipids with unsaturated chains.

Method

We carried out all MD simulations using the GROMOS[14] simulation package, with alterations in the interaction function parameter (ifp) files for the different values of the 6-12 parameters for methyls and methylenes. In all cases the time step size was 2 fs. Constant temperature and pressure were maintained using the weak coupling method[14] with the coupling constant to the pressure reservoir set at 0.5 ps and the coupling constant to the temperature reservoir set at 0.4 ps. It has been pointed out[15] that the weak coupling algorithm does not correspond to any known thermodynamic ensemble. This makes the algorithm unreliable for the calculation of fluctuation-based quantities such as heat capacity or compressibility. However, it is a simple and efficient algorithm so we have used it in these simulations because we are interested only in first-order thermodynamic variables, namely density and heat of vaporization. Additionally, we use a simple coupling algorithm because we aim to use the same procedure to carry out fluid phase lipid bilayer simulations for large numbers of lipid and water molecules.

For all simulations the following general procedure was followed. First, the molecules were placed on a lattice with the density near the experimental value. Next, the initial configuration was energy minimized to remove high-energy contacts (this was mainly necessary for the pentadecane simulation). Then, a simulation was run for 40 ps at constant volume and at the constant temperature of 500 K (NVT) with velocity reassignments every 2 ps. This run was followed by a 40 ps NVT run at 300 K, again with velocity reassignment every 2 ps. Finally, a NPT run at 300 K and 1 atm pressure was carried out. The *x*, *y*, and *z* components of the internal virial were calculated separately. This allowed the simulation cell size to change in shape as well as in size. The length of the final run varied from 300 ps, in runs that used parameter sets from Egberts et al.[16] or as altered by Berger et al.,[1] to between 800 ps and 1 ns for runs testing new parameters. Simulations were carried out for hexane, decane, 5-decene, and pentadecane. Hexane simulations contained 751 molecules, while simulations for decane, 5-decene, and pentadecane all contained 216 molecules. 5-Decene simulations were carried out using the optimized methyl and methylene parameters from the pentadecane, decane, and hexane simulations while varying 6-12 parameters for the double-bonded carbons to fit the experimental density.

In all simulations a spherical cutoff of 1.5 nm was used for the nonbonded interactions. To determine the appropriate van der Waals cutoff distance, constant pressure simulations of hexane were done with cutoffs of 10, 15, and 20 Å. The simulations were not stable with a 10 Å cutoff, showing a steadily rising specific volume even at a temperature well below the boiling point of liquid hexane. The simulations at 15 and 20 Å cutoff were stable, and asymptoted to densities very close to each other. From these simulations we judged that the van der Waals forces could be cut off at 15 Å without serious compromise in accuracy for the purposes of these calculations.

United atom models were used (that is, no explicit hydrogens were included in the simulations), and all atomic charges were set to zero. In particular, because united atom approximation is used, no explicit charges are associated with the carbon−carbon double bond. The partial charges associated with the C=C bond are small,[17] and for common lipids containing an oleate chain the double bond is located at the 9 position fairly far from the lipid−water interface. For these reasons, and because we are interested in the simulation of disordered, fluid phase lipid membranes, the neglect of partial charges is an approximation that will speed up simulations without sacrificing thermodynamic accuracy. For computing torsion angle motions around saturated bonds, third neighbor 6-12 interactions were replaced with the dihedral potential function due to Ryckaert and Bellmans.[18] The gas phase trans/gauche ratio will not be sensitive to the changes we are making in the van der Waals parameters, and the liquid phase trans/gauche ratio will only be sensitive insofar as the density is changed. Density changes are in the range of 1−5% for the range of LJ parameters we have used for trials. For computing torsion angle motions around the unsaturated bond in 5-decene, the parameters of Feller et al.[17] were used to essentially fix the unsaturated bond in the cis conformation.

Molar heats of vaporization were calculated by the same method utilized by Berger et al.:[1]

$$\Delta H_{vap} = E_{gas} - E_{liquid} + RT \qquad (2)$$

where E_{gas} and E_{liquid} are the nonbonded potential energies per mole of gas and liquid phases, respectively, T is the absolute temperature, and R is the gas constant. E_{liquid} is also corrected for the finite cutoff range for 6-12 interactions using the method described by Berger et al.[1] The corrections ranged from −0.63 kJ/mol (hexane) to −1.84 kJ/mol (pentadecane). The above expression for the heat of vaporization assumes the molar volume of the gas phase is very large compared to that of the liquid phase and that the ideal gas equation of state holds for the gas phase. E_{gas} is obtained from 300 ps MD simulations of single molecules in a large box.[1] In all cases, $T = 300$ K, which is in reality below the boiling point of all systems simulated. At the boiling point one might expect that the contribution to ΔH_v from the liquid potential energy would be less but that this would be compensated to some degree by the increased RT contribution.

Results

Figure 1 shows a plot of the mean volume per molecule versus number of carbons for liquid alkanes. On this figure we plot the experimental densities (diamonds),[19] densities cal-

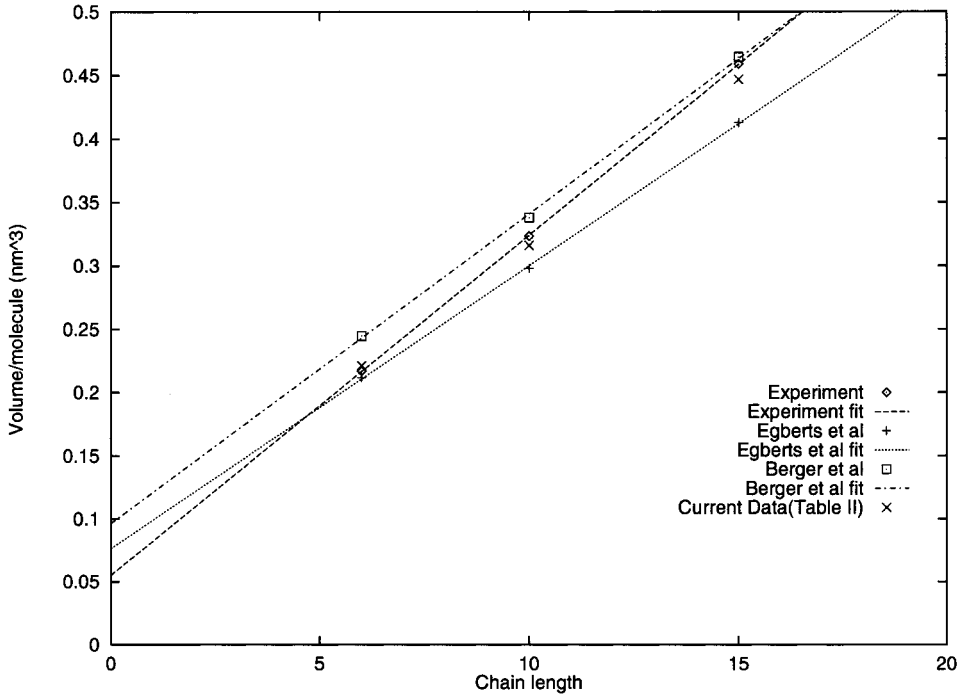

Figure 1. Plot of volume per molecule versus carbon number including experimental data points,[19] volumes calculated from simulations described in this paper, volumes calculated using parameters from Egberts et al.,[16] and volumes calculated using parameters from Berger et al.[1]

culated from the Egberts[16] parameter set (crosses), and densities calculated from the revised parameter set of Berger et al. (boxes)[1] from 300 ps NPT MD runs at 300 K and 1 atm. For all three data sets (experiment, Egberts, and Berger) the points lie on a straight line. However, the three lines are different in both slope and intercept. The line between points calculated with the Egberts[16] parameter set lies closest to the experimental data for hexane, as should be expected since the 6-12 parameters in this set were adjusted to give a good fit to the hexane density. But these parameters produce molecular volumes that are substantially smaller than experiment for decane and pentadecane. The line between points calculated with the Berger et al.[1] parameter set lie closest to the experimental data for pentadecane, as should be expected since the 6-12 parameters in this set were adjusted to give a good fit to the pentadecane molecular volume. But these parameters produce molecular volumes that are substantially larger than experiment for hexane and decane.

From Figure 1 it is clear that the molecular volume of the *n*-alkanes, plotted versus carbon number *n*, must obey an equation of the form

$$V_{\mathrm{mol}} = an + b \tag{3}$$

The slope *a* of this line is the volume per methylene group, and the intercept *b* is related to the volume per methyl by substituting $n = 2$ in the above equation:

$$V_{\mathrm{methyl}} = (2a + b)/2 \tag{4}$$

For the experimental points, the slope from a least squares fit is 0.0269 nm^3, and the intercept is 0.0553 nm^3, yielding volumes $V_{\mathrm{CH_2}} = 26.9$ Å^3, and $V_{\mathrm{CH_3}} = 54.5$ Å^3. For comparison, Nagle and Wilkinson have measured $V_{\mathrm{CH_2}} = 27.6$ Å^3 for fluid phase DPPC.[20] It is reasonable to expect the volume per CH_2 to be slightly smaller for liquid alkanes compared to DPPC, because the chains in DPPC are constrained by attachment to the polar groups. From least square fits to the GROMOS volume points we find $V_{\mathrm{CH_2-Egberts}} = 22.3$ Å^3. From least squares fits to the data points from the Berger et al.[1] parameters, $V_{\mathrm{CH_2-Berger}} = 24.5$ Å^3.

TABLE 1: Comparison of 6-12 Parameters

	CH_2		CH_3	
	σ (nm)	ϵ (kJ/m)	σ (nm)	ϵ (kJ/m)
this paper	0.400	0.380	0.351	0.570
Egberts et al.[16]	0.375	0.430	0.375	0.625
Berger et al.[1]	0.396	0.380	0.396	0.570
5-decene CH	0.375	0.380		

We conclude from Figure 1 that both of the previously used parameter sets yield volumes per methylene that are too small, because the slopes of both lines are less than that of the line through the experimental points. Similarly, both of the previously used parameter sets yield volumes per methyl that are too large compared to the experimental value.

Our attempts to find 6-12 parameters for methyls and for methylenes were guided by the above conclusions. For an interaction function of the 6-12 form between atoms on different molecules, we assume that, to lowest order, the molecular volume is primarily influenced by the values of the σ parameters, while the heat of vaporization is primarily determined by the ϵ parameters. Therefore, our approach was to do trial simulations of ~10 ps in length with $\sigma_{\mathrm{CH_2}}$ enlarged in comparison to previously used values, and $\sigma_{\mathrm{CH_3}}$ reduced in comparison to previously used values. The values of $\epsilon_{\mathrm{CH_2}}$ and $\epsilon_{\mathrm{CH_3}}$ were set at the values used by Berger et al.[1] as they agreed well with the experimental heat of vaporization of pentadecane. When we settled on values for $\sigma_{\mathrm{CH_2}}$ and $\sigma_{\mathrm{CH_3}}$, which appeared promising, longer simulations of 300−500 ps were run.

Table 1 gives the values for the 6-12 parameters that produce the best fit to the molecular volumes and to the heats of vaporization for the three *n*-alkanes and for 5-decene. It is interesting to note that it is only possible to simultaneously fit molecular volumes for all three *n*-alkanes if $\sigma_{\mathrm{CH_3}}$ is substantially smaller than $\sigma_{\mathrm{CH_2}}$. While this result seems counterintuitive, the same trend has been noted earlier by Jorgensen et al.[12] The value

TABLE 2: Calculated and Experimental Molar Volumes and Heats of Vaporization

system	calc V (nm^3)	expt V	calc ΔH_v (kJ/m)	expt ΔH_v[19]
hexane	0.2212	0.2170	23.3	31.9
decane	0.3163	0.3236	41.7	45.6
pentadecane	0.4470	0.4590	63.6	61.2
5-decene	0.3055	0.3128	42.0	42.8

TABLE 3: Calculated and Experimental Average Number of Trans and Gauche Bonds per Chain[a]

n-alkane	g_+	g_-	$g_+ + g_-$	trans	t/g ratio
hexane	0.416	0.439	0.855	2.15	2.51
decane	0.844	1.03	1.87	5.13	2.75
pentadecane	1.60	1.63	3.23	8.77	2.72
tridecane (expt)[21]			3.5	6.5	1.86

[a] The sum of columns 3 and 4 is the total number of dihedrals per chain.

of σ_{CH} is close to that used by Feller et al.[17] (0.375 is our result, while Feller et al. used $\sigma_{CH} = 0.372$ in simulations of dioleoylphosphatidylcholine). Our value of ϵ_{CH} is larger than that of Feller et al.[17] An important difference between the simulations of Feller et al.[17] is that they include all hydrogen atoms. For this reason we chose a larger value of ϵ_{CH}, namely the same value as ϵ_{CH_2}, and then varied σ_{CH} to fit experiment. We also point out that there is no fundamental reason that the 1-6-12 potential function should be appropriate for individual subunits in polymer chains. It is used almost universally for lack of a better alternative, but the simple physical interpretations of σ and ϵ may be compromised in the case of chain molecules.

Table 2 compares molecular volumes and heats of vaporization calculated using our new parameters with experiment. The agreement for molecular volumes is between 1.7 and 1.9% for the *n*-alkanes and 2.3% for 5-decene. In the case of 5-decene the fit is less accurate because, after fixing 6-12 parameters for methyls and methylenes, the molecular volume is less sensitive to variation of 6-12 parameters for the two ethene carbons. In general, heats of vaporization calculated from the simulations agreed very well with experimental values. The estimated error in molecular volume calculations is ± 0.004 nm^3, and the estimated fluctuation in heat of vaporization calculations is ± 0.6 kJ/m.

As a check that the new parameter set, along with the Ryckaert−Bellmans dihedral potential, produces the correct conformational disordering in the liquid alkanes, we have calculated the concentrations of gauche and trans bonds for the three *n*-alkanes. Table 3 summarizes the data and compares with experimental data for tridecane.[21] This table reports the average number of trans, g_+, and g_- bonds per chain. The sum of columns 3 and 4 is the total number of dihedrals per chain (number of bonds − 2), and column 5 is the ratio of column 4 divided by column 3. The experimental data of ref 21 on their face suggest that our computed trans/gauche ratio is a bit too large. However, we note firstly that assignment of Raman bands to specific vibrational modes in liquid alkanes and in lipid bilayers is a difficult task, and in some cases alternative assignments are possible.[22] Secondly, we note that the data of Holler and Fallis[21] would also suggest that the trans/gauche ratio for saturated chain linear alkanes is lower in bulk solution than in membranes, where measured trans/gauche ratios are approximately 3:1.[23] We find essentially the same trans/gauche ratio (approximately 3:1) in simulations of both bulk liquid and fluid phase membranes, using the Ryckaert−Bellmans dihedral

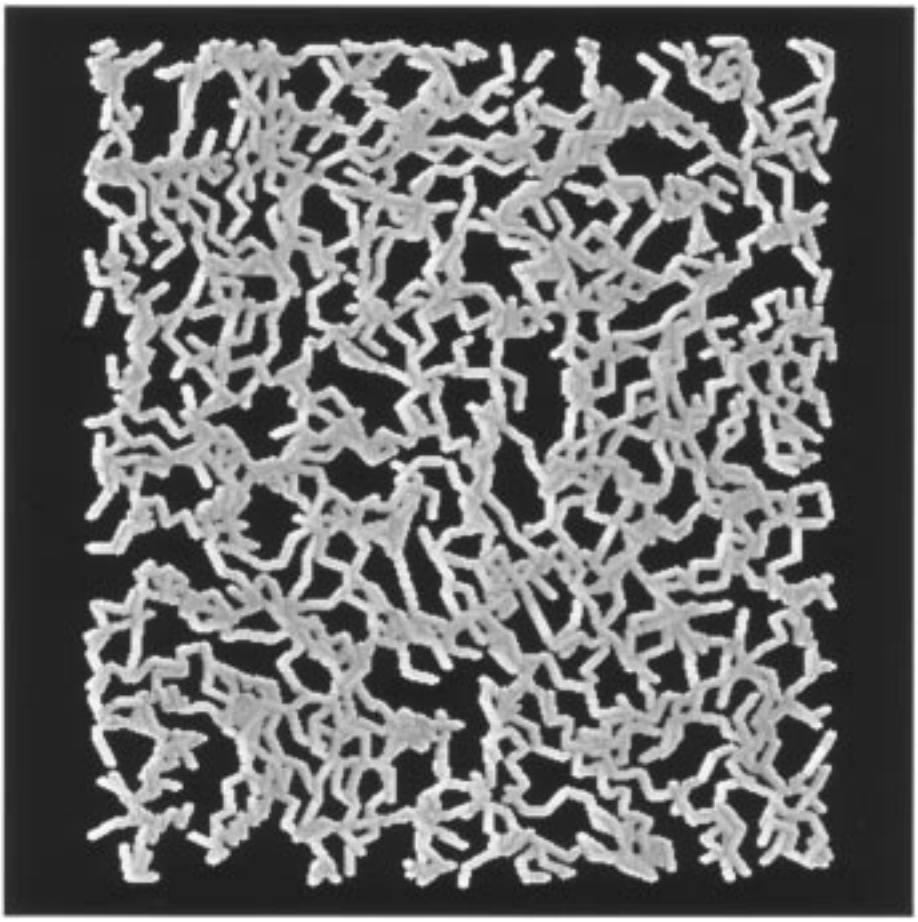

Figure 2. Snapshot of liquid *n*-pentadecane, shown as a stick model.

potential. In both cases this is close to the gas phase trans/gauche ratio for the same potential. Thus it is not clear to us why the trans/gauche ratio in hydrocarbon chains, averaged over entire chains, should be substantially different in bulk liquid alkanes as compared to fluid phase membranes, as is suggested by comparing[21] to the experimental determinations of the trans/gauche ratio in membrane environments.

Figure 2 is a snapshot of the pentadecane system at the end of the 300 ps MD simulation.

Discussion

Through a set of simulations of three representative *n*-alkanes using $n = 6$, 10, and 15, we have obtained 6-12 parameters that agree reasonably well with experimental volumes per molecule and heat of vaporization. These parameters should now be well suited for application to acyl chains in fluid phase lipid bilayer simulations. We have tested this conclusion by using the new parameters in a simulation of dipalmitoylphosphatidylcholine (DPPC), which we continued from the end of a recently completed run that used combined MD and configurational bias Monte Carlo (CBMC) methods.[24] This simulation was 150 ps in length, using the NγT ensemble.[2] The simulation was run at an elevated temperature of 342 K for comparison with experimental data. The order parameter profile averaged over the last 50 ps of this run is consistent with experimental data.[25,26] The average area per molecule is 60.5 ± 1.5 Å^2, consistent with the experimental value of 62.9 ± 1.3 Å^2.[27] A longer run combining Monte Carlo and MD as in earlier work[24] would likely result in a larger area per molecule due to the increased value of σ_{CH_2} in our new parameter set.

Recently, Daura et al.[28] have revised CH_2 and CH_3 united atom nonbonded parameters in the GROMOS96 force field by fitting to simulation results for a number of cyclic and short-chain alkanes from methane through butane. They obtain for methylene $\sigma_{CH_2} = 3.920$ Å and $\epsilon_{CH_2} = 0.489$ kJ/m and for methyl $\sigma_{CH_3} = 3.875$ Å and $\epsilon_{CH_2} = 0.732$ kJ/m. Since they used short chain alkanes in their optimization procedure, we tested their parameter set in a simulation of pentadecane at 300 K with a 1.5 nm cutoff radius. We obtained a molecular volume of 0.43 nm^3, smaller than the experimental value and smaller than the value predicted by our parameters in Table 2. This is not surprising, since their value for σ_{CH_3} is relatively large and their value of σ_{CH_2} is relatively small, both of which would lead to calculated molecular volumes that are too small for longer chain alkanes.

We believe it is important that future simulations of lipid membranes make use of a robust parameter set such as ours, which gives reasonable fits to a wide range of experimental data for liquid hydrocarbons of chain length that varies from $n \sim 6$ to lengths similar to the longest phospholipid hydrocarbon chain ($\sim$14−18). This is important because the ratio of methylenes to methyls is different for different *n*-alkanes. In a lipid bilayer this ratio *effectively* changes also as one moves from the head group region to the bilayer center. Near the head groups the hydrocarbon region consists mainly of methylenes, corresponding to the internal environment of very long chain liquid alkanes. Near the center of the bilayer there is a higher fraction of methyls, corresponding to the internal environment of very short chain liquid alkanes. The force field parameter presented in this paper is the first one that produces appropriate densities for the full range of methylene to methyl ratios seen in the various regions of a fluid phase lipid bilayer membrane.

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