

Comparing Simulations of Lipid Bilayers to Scattering Data: The GROMOS 43A1-S3 Force Field

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ABSTRACT.

Simulations of DOPC at $T=303$ K were performed using the united atom force field 43A1-S3 at six fixed projected areas, $A_P = 62, 64, 66, 68, 70$, and 72 \AA^2 as well as a tensionless simulation that produced an average $A_{NPT}=65.8 \text{ \AA}^2$. After a small undulation correction for the system size consisting of 288 lipids, results were compared to experimental data. The best, and excellent, fit to neutron scattering data occurs at an interpolated $A_N=66.6 \text{ \AA}^2$ and the best, but not as good, fit to the more extensive x-ray scattering data occurs at $A_X=68.7 \text{ \AA}^2$. The distance ΔD_{B-H} between the Gibbs dividing surface for water and the peak in the electron density profile agrees with scattering experiments. The calculated area compressibility $K_A=277\pm10 \text{ mN/m}$ is in excellent agreement with micromechanical experiment. The volume per lipid V_L is smaller than volume experiments which suggests a work-around that raises all the areas by about 1.5%. Although $A_X \neq A_N \neq A_{NPT}$, this force field obtains acceptable agreement with experiment for $A_L = 67.5 \text{ \AA}^2$ (68.5 \AA^2 in the work-around) which we suggest is a better DOPC result from 43A1-S3 simulations than its value from the tensionless NPT simulation. However, non-simulation modeling obtains better simultaneous fits to both kinds of scattering data which suggests that the force fields can still be improved.

Introduction

It is well-recognized that molecular dynamics simulations provide a level of quantitative detail unavailable to experiment. It is also well-recognized that this detail depends on the force fields used in the simulation. Accordingly, force field development and refinement for lipid bilayer simulations is ongoing.¹⁻¹³ An important test of force fields is comparing simulation results to experimental scattering data.^{1-4,8,14-22} The first goal of this paper is to show how to carry out a recently proposed refinement of these tests for lipid bilayers.²³ The main proposed refinement determines both the area A_x at which the simulation best agrees with the x-ray data and the area A_N at which it best agrees with the neutron data and asks how these areas agree with each other as well as with the area A_{NPT} for a tensionless simulation.

Our second goal is to critically test a particular united-atom force field 43A1-S3.¹ When it was developed, this force field was tested favorably against experimental x-ray, NMR and volumetric data for DLPC, DMPC, DPPC and DOPC lipid bilayers. It was not, however, compared to neutron scattering data which has become particularly important regarding the area per lipid molecule A_L .¹⁸ Also, x-ray scattering data have since been refined from many data sets and more accurate uncertainties provided. Furthermore, only tensionless (NPT) simulations were previously performed and insight can be gained from other values of A_L . We have restrained ourselves in this paper to the DOPC bilayer. This is partly because there are more extensive scattering data for DOPC than for other lipids and partly because DOPC has been a particularly hard case for other simulations.²³

Methods

MD Simulations

The initial starting configurations were obtained from a previously simulated fluid-phase bilayer system comprised of 288 Berger et al.²⁵ DOPC lipids and 9428 SPC waters (~32.7 waters/lipid corresponding to fully hydrated DOPC²⁴). The system was repurposed for the Chiu et al. lipid potentials and the SPC water was replaced with SPCE.¹ This system was then simulated for 100 ns under the isothermal-isobaric (NPT) ensemble with constant pressure and temperature (1 bar and 303 K respectively). Pressure coupling was applied using a semi-isotropic scheme, with the *xy*-periodic box dimensions coupled, and the *z*-dimension was allowed to vary freely. Figure 1 illustrates the A_P trajectory from the NPT system showing rapid convergence to an equilibrated $A_P = 65.8 \text{ \AA}^2$ that agrees with Chiu et al.¹ Initial configurations for constant area (NP_NAT ensemble) simulations were obtained from simulated frames at or near the desired projected area per lipid ($A_P = 62, 64, 66, 68, 70$ and 72 \AA^2) with the *xy*-box dimensions modified to achieve the specific A_P . These systems were then simulated for 50 ns at constant area where only the *z*-dimension of the periodic cell was allowed to fluctuate. The final 20 ns of each simulation were used for subsequent analysis.

All systems were simulated with the GROMACS 4 program using a leap-frog algorithm to integrate the equations of motion.²⁶⁻²⁹ Each system was run at 303 K using a 2 fs time step and recording coordinates every 5 ps. All run parameters were obtained from Chiu et al.¹ as follows. The Particle Mesh Ewald method³⁰ was used for long-range electrostatics with a direct space cut-off of 1.0 nm, Fourier spacing of 0.15 nm, and a sixth-order interpolation. A twin-range cutoff (1.0/1.6 nm) was applied for van der Waals interactions and the neighbor-pair list was updated

every 5 time steps. Lipid bonds were constrained using the LINCS algorithm.³¹ Water bonds were constrained using the SETTLE algorithm.³² A Nosé-Hoover thermostat³³ with a time constant of 0.5 ps was used to control the ensemble temperature, while a Parrinello-Rahman barostat³⁴ with a time constant of 1 ps was used to keep the pressure fixed.

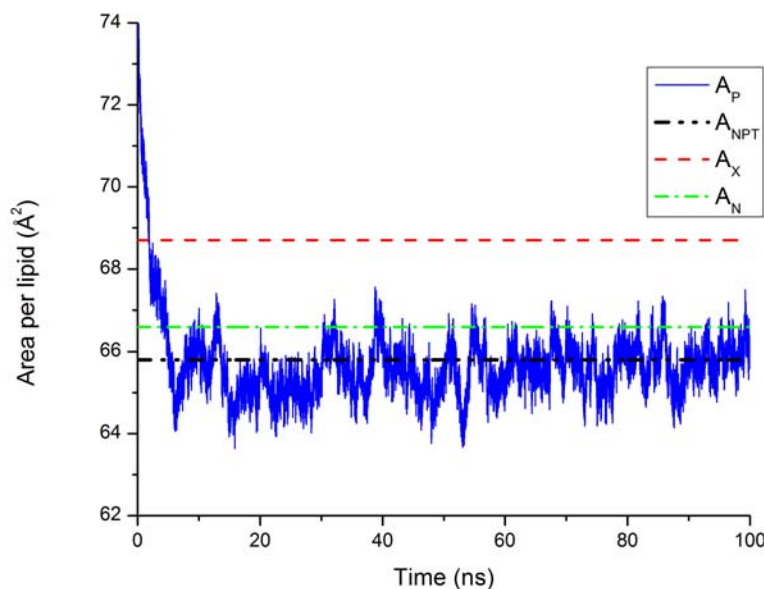


Figure 1. Time evolution of the projected area per lipid from the NPT simulation illustrating rapid convergence to an average $A_P = 65.8 \text{ Å}^2$. Lines for A_{NPT} (---), A_X (- -) and A_N (— · —) are provided for comparison.

Determination and Comparison of Structural Profiles

Number density profiles and local area per lipid (A_L) were determined using the MDAnalysis software package³⁵ and the surface referencing undulation correction method developed by Braun et al.³⁶ Number density profiles were calculated for each unique united atom type (54 in DOPC, 3 in SPCE) with a cut-off wave number of $q_0 = 1.0 \text{ [nm}^{-1}]$ to correct for bilayer undulations. These number density profiles were then used for input into the SIMtoEXP software program²⁰ that produces electron density profiles and their Fourier transforms, which are the X-ray form factors $F_x(q_z)$, and neutron scattering length profiles and their Fourier transform, which

are the neutron form factors $F_N(q_z)$. SIMtoEXP imports experimental form factor data and it provides the unknown experimental scaling factor by minimizing the χ^2 in fitting the data to each simulation. In addition to this straightforward procedure, an alternative procedure modified the simulated volumes of the water and the lipid by multiplying the original simulated water number density profiles by one factor to obtain the experimental density of water and by multiplying the lipid number density profiles by another factor to obtain the experimental lipid volume. The latter modification means that the simulated lipid area is also multiplied by the same factor.

Simulated volumes of water, lipid and its components were obtained using a SIMtoEXP app.³⁷ We first defined the components to be water, choline (Chol), phosphate (PO_4), glycerol (Gly), carbonyls (Carbs), chain methylenes (CH_2), methines (CH) and chain terminal methyls (CH_3). We also defined the components according to the SDP model³⁸ which combines the carbonyls and the glycerol and separates the phosphocholine into just the choline methyls and the rest. There were negligible differences in the volumes of the lipid V_L and the lipid headgroups V_H using the two different definitions of the components as shown in Tables S1 and S2.

Experimental Data and SDP Modeling

A composite experimental X-ray scattering data set for $F_X(q_z)$ was obtained as an average using seven sets of data from oriented stacks of bilayers and three sets of data from unilamellar vesicles.^{18,39-43} Neutron scattering $F_N(q_z)$ in D_2O were obtained from Kucerka et al.⁴³ Modeling from these data sets was done using the SDP analysis.¹⁸ This analysis requires estimates for various constraints such as the ratios of the volumes of the component groups in the headgroup and in the tails and for the widths of the headgroup methyl distribution, the double bond distributions, and the width of the Gibbs dividing surface for the total hydrocarbon core. As these quantities do not vary significantly with area in simulations, the simulated values provided

estimates¹⁸ and we have updated those estimates using the 43A1-S3 results. Lists of the constraints are given in Table S3. As the density of X-ray data points is greater than for neutron data, the neutron data were weighted more heavily in the fitting procedure so as to give roughly equal values for the average (i.e., reduced) χ^2 of x-ray and neutron data.

Results

Table 1 shows that the local area per lipid A_L is only about 0.1-0.2 \AA^2 larger than the projected area per lipid A_P of the simulation box. Although the number of lipids in the simulation is larger than most current simulations, the simulated system is still small enough to suppress most long wavelength undulations. The small, but systematic, decrease in the difference between A_L and A_P as area is increased is consistent with increased surface tension that further suppresses undulations. The volume of SPCE water in the simulations is 0.1 \AA^3 greater than experiment. Table I also shows that the volume V_L of DOPC gradually increases with increasing A_L , as would be expected because the hydrocarbon chains become more disordered. V_L also is consistently smaller than experiment. The ratio r of terminal methyl to methylene volume agrees well with the acceptable range of experimental values.⁴⁴⁻⁴⁶ Volumes of all components are compiled in Tables S1 and S2.

Property	Experiment	Simulations						
A_P		62	64	65.79	66	68	70	72
A_L	--	62.2	64.15	65.89	66.15	68.14	70.13	72.12
A_{LM}	--	63.1	65.0	66.8	67.0	69.0	70.9	72.8
V_W	30.0	30.1	30.1	30.1	30.1	30.1	30.1	30.1
V_L	1303	1283	1285	1286	1286	1288	1289	1289
V_H	319-331	329	330	331	329	330	330	331
V_C	972-984	954	955	955	957	958	959	958
r	1.8-2.1	2.0	2.01	2.03	1.96	1.94	1.96	1.98
ΔD_{H-C}	--	4.1	4.0	4.0	4.0	4.0	4.0	4.0
ΔD_{P-H}	--	0.9	0.7	0.6	0.7	0.7	0.6	0.5
ΔD_{B-H}	1.0-1.7	1.2	1.2	1	1.1	0.9	0.8	0.7

Table 1. A_P is the box area divided by half the number of lipids, A_L takes account the larger local area due to undulations and A_{LM} is modified as described in Methods. The volume subscripts are L for total lipid volume, C for hydrocarbon chains beginning at the second carbon and H for the headgroup volume consisting of the carbonyls, glycerol and phosphocholine. ΔD_{H-C} is the difference between half the head-head distance D_{HH} in the electron density profile and the Gibbs dividing surface D_C of the hydrocarbon region. ΔD_{P-H} and ΔD_{B-H} are the differences between the mean position of the phosphate (P) and the Gibbs dividing surface for the water (B), respectively, and $D_{HH}/2$. Units for Areas A, volumes V and distances D are in the appropriate powers of Å and the ratio r of terminal methyl to methylene volumes is dimensionless.

Figure 2 shows electron density profiles for the range of fixed A_P listed in Table I. As expected, the bilayer becomes systematically thinner as A_P is increased and this induces the reciprocal effect of spreading the X-ray form factors $F_X(q_z)$ to larger q_z as shown in Fig. 3. The most objective comparison with experimental data is with the experimental form factors.^{14,15,22}

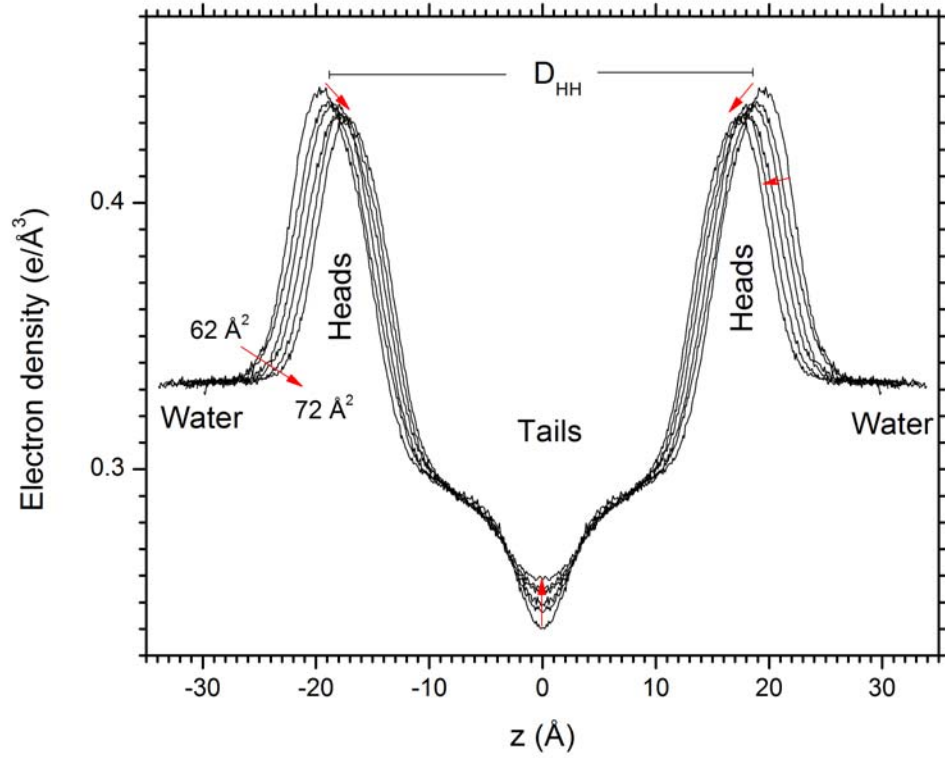


Figure 2. Simulated electron densities versus distance z from the center of the bilayer for the range of projected areas. The arrows point from the smallest $A_P=62 \text{ Å}^2$ to the largest $A_P=72 \text{ Å}^2$. D_{HH} defines the Head-Head bilayer thickness.

Figures 3 and 4 compare, respectively, the x-ray $F_x(q_z)$ and the neutron $F_N(q_z)$ scattering data. The simulations agree better with the experimental data for A near the middle of the simulated range, as is most clearly seen in Fig. 4. However, for visual clarity in Figs. 3 and 4, only one unknown scale factor for the experimental data could be chosen whereas the scale factors for best agreement with each simulation are slightly different. Figures 3 and 4 also show results for the SDP model.

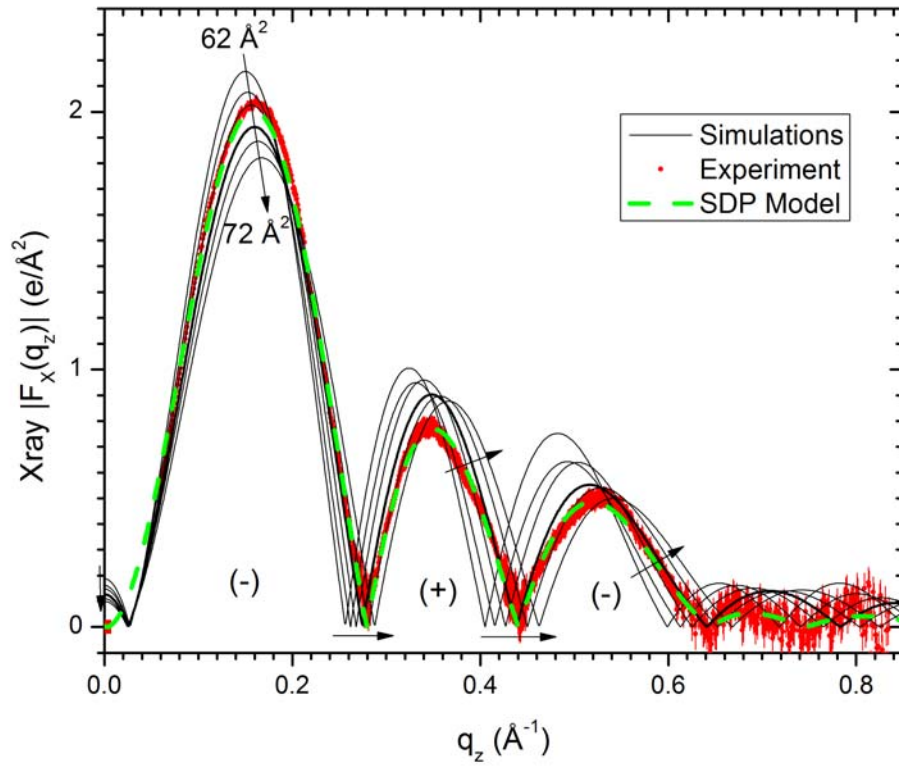


Figure 3. Simulated, experimental, and SDP model X-ray form factors $|F_x(q_z)|$ versus q_z in reciprocal space. The arrows indicate the progression from the smallest simulated projected area $A_P=62 \text{ \AA}^2$ to the largest $A_P=72 \text{ \AA}^2$ with the best fit to experiment shown in bold magenta. Scaled experimental data with uncertainties include the volumetric datum at $q=0$. Signs of $F_x(q_z)$ are indicated by (-) and (+). Negative values of experimental $|F_x(q_z)|$ propagate from experimental uncertainty when measured intensities are close to zero.

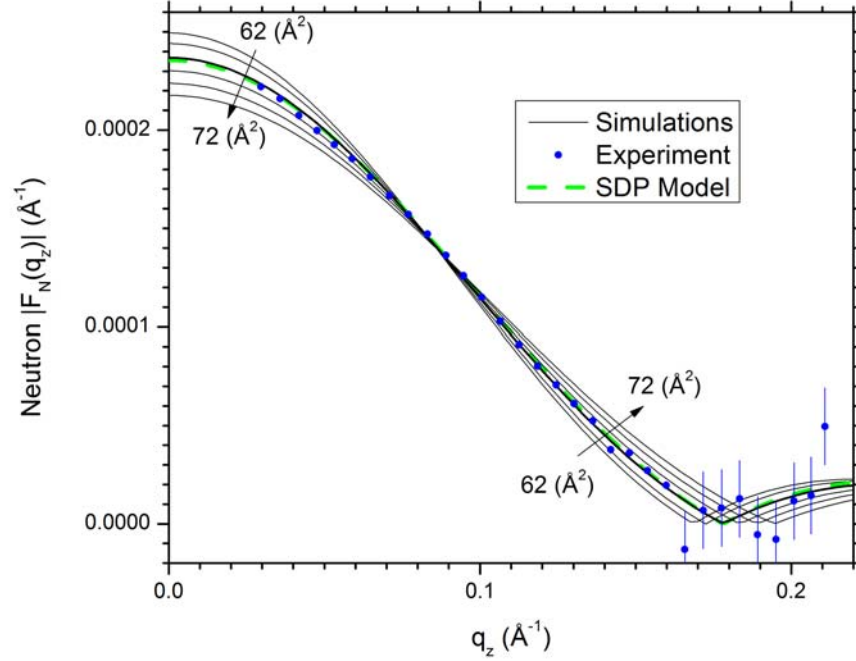


Figure 4. Simulated, experimental, and SDP model neutron form factors $F_N(q_z)$ versus q_z in reciprocal space. The arrows indicate the progression from the smallest simulated projected area, $A_P=62 \text{ \AA}^2$ to the largest $A_P=72 \text{ \AA}^2$ with the best fit to experiment shown in bold magenta. Estimated uncertainties for $q_z < 0.16 \text{ \AA}^{-1}$ are about the size of the data symbols.

The comparison of simulation with experiment is better quantified in Figure 5 which shows reduced χ^2 values for which the experimental scale factor was optimized separately for each simulated area. Quadratic interpolation to the minimal χ^2 gives $A_X = 68.7 \text{ \AA}^2$ for best agreement of the simulation with X-ray data and $A_N = 66.6 \text{ \AA}^2$ for best agreement with neutron data. Figure 5 also indicates the area $A_{NPT} = 65.9 \text{ \AA}^2$ from the tensionless NPT simulation.

There is a disagreement of the simulated $F_X(q_z)$ with the experimental point at $q_z=0$ in Fig. 3 that is related to the volumetric values in Table 1, and this leads to a possible modification in how the simulation is treated. There is a fundamental relation⁴⁷

$$A_L F_X(0) = 2(n_L - \rho_W V_L) , \quad (1)$$

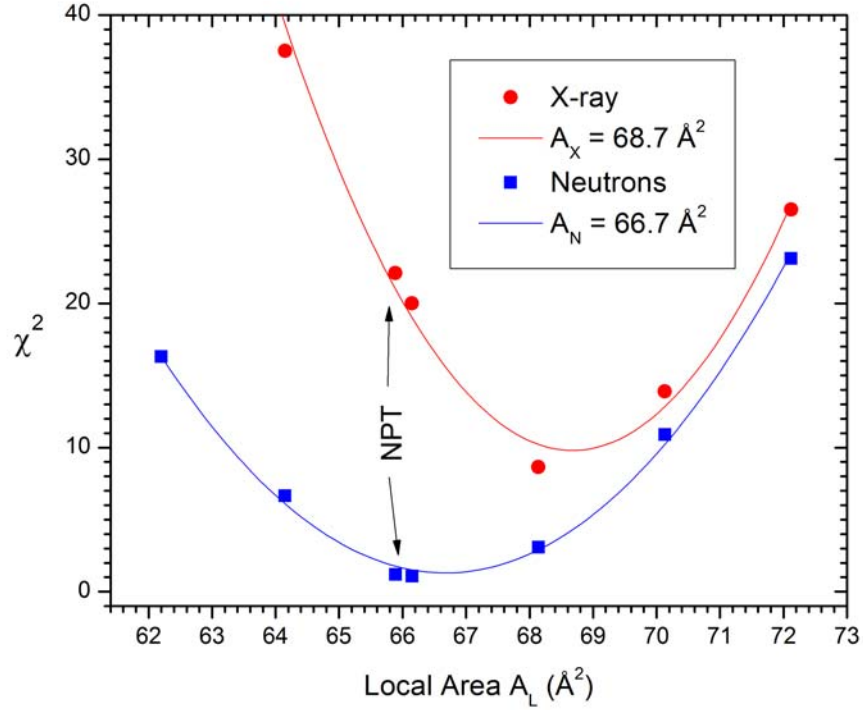


Figure 5. Symbols show values of χ^2 from fitting simulations with local areas A_L to X-ray and neutron data and lines show quadratic fits from which interpolated best areas $A_x = 68.7 \text{ \AA}^2$ and $A_N = 66.7 \text{ \AA}^2$ are obtained. NPT indicates the tensionless simulation and the other values were for the areas listed in Table 1.

where $n_L=434$ is the number of electrons in DOPC, V_L is the volume of DOPC given in Table 1 and $\rho_w = 0.333 \text{ e/\AA}^3$ is the electron density of water at 303 K. Equation (1) gives $F_x(0)=0$ independently of the value of A_L . The simulations give positive values of $F_x(0)$ because (a) ρ_w is slightly too small due to a slightly too large V_w shown in Table 1 and (b) more significantly, the simulated V_L is also too small. These are fairly minor flaws inherent in simulations and they invite a work-around to improve agreement with experiment, as follows. The number densities from the simulations may be multiplied by factors to give the experimental values of V_w and V_L . Although one could contemplate different factors for the headgroup components than for the chain components, we have here applied the same factor for all the lipid components. Because

the component distributions retain their same shape along the z -direction, there is no modification of volume along the z -direction, only in lateral directions, so lipid area should be multiplied by the same factor as the lipid volume, and all areas increase. Table 1 shows the values of the modified A_{LM} . Figure 6 shows the results of this modification compared to the χ^2 results repeated from Fig. 5. Furthermore, the minimal value of χ^2 improves substantially for the x-ray, but not the neutron, comparison. Moreover, this work-around increases the difference between A_X and A_N from 2.0 \AA^2 to 2.2 \AA^2 .

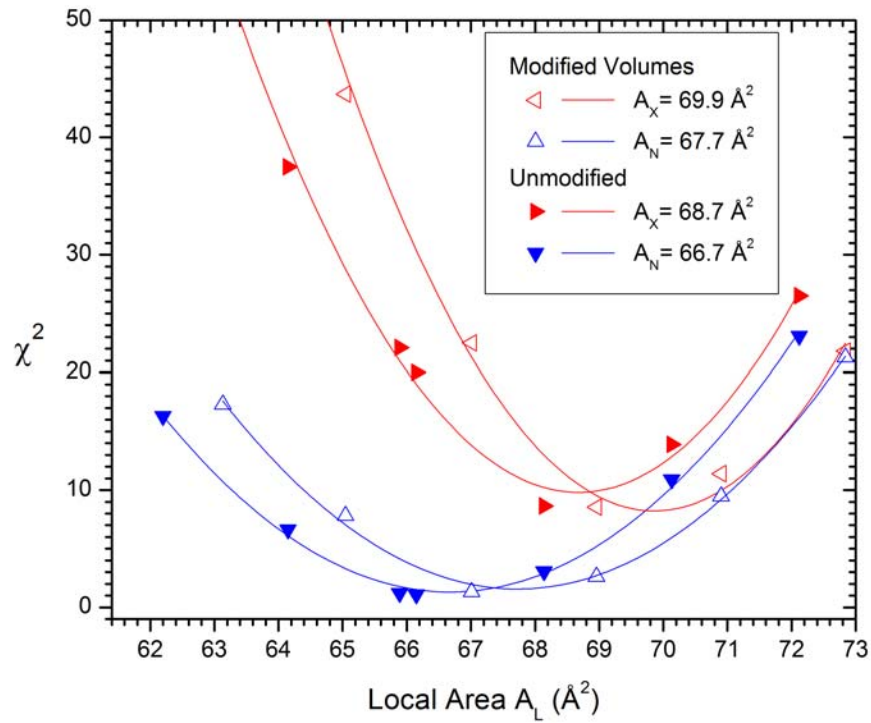


Figure 6. Same format as Fig. 5 with added values for the modified volume work-around described in the text.

Figures 3 and 4 also show results obtained from SDP modeling of the experimental data. These results agree much better with the experimental X-ray data and somewhat better with the neutron data than any of the simulations as shown quantitatively in Table 2.

Another property of general interest is the area compressibility modulus, defined as $K_A = A(\partial\gamma/\partial A)_T = (\partial\gamma/\partial(\ln A))_T$. The simulations provide a value of the surface tension γ for each simulated area A_L . The simulated numerical value, $K_A = 277 \pm 10$ dyn/cm, is obtained from the slope in the plot in Fig. 7.

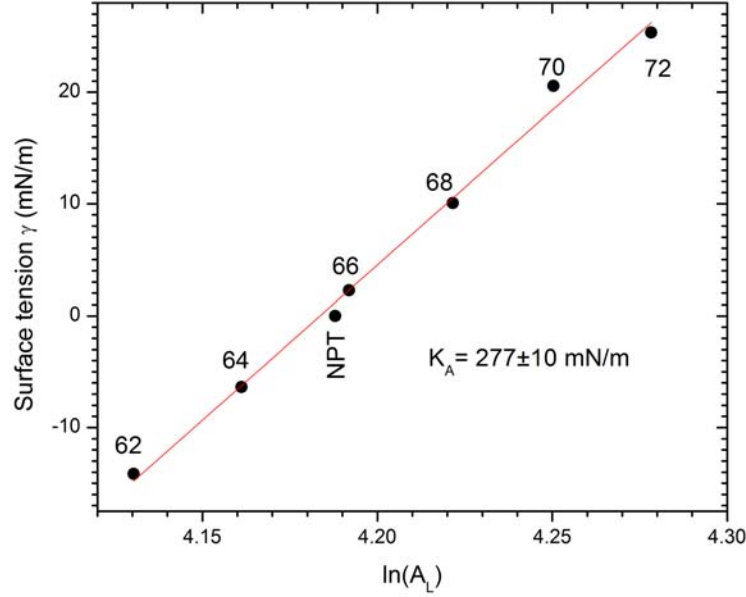


Figure 7. The slope of the simulated surface tension γ versus the logarithm of the local area A_L provides the area compressibility modulus K_A . The number next to each data point is the value of the projected area A_P in \AA^2 .

Discussion

The united atom force field 43A1-S3 obtains excellent agreement with neutron scattering data as shown qualitatively in Figure 4. Quantitatively, Table 2 shows that the reduced χ^2 of the fit of the data to the simulated $F_N(q_z)$ is close to 1 even for the NPT simulation (Fig. 5) and Table 2 gives its interpolated minimum as 1.31 at $A_N = 66.7 \text{ \AA}^2$. The χ^2 of the fit of the x-ray data to the simulated $F_X(q_z)$ is considerably poorer as shown in Fig. 5, with an interpolated minimal $\chi^2 =$

9.81 for $A_x=68.7 \text{ \AA}^2$. The large x-ray χ^2_x reflects that the simulation has smaller second and third lobes compared to the first lobe than the experimental data (Fig. 3); a very similar $F_x(q_z)$ was also reported by Chiu et al.¹

Property	Basic	Modified	SDP
A_{NPT}	65.9	66.7	67.6
$\chi^2_{\text{X+N}}$	11.7	13.3	1.17
A_{N}	66.7	67.7	N/A
χ^2_{N}	1.31	1.57	1.13
A_{X}	68.7	69.9	N/A
χ^2_{X}	9.81	8.22	1.21
V_{L}	1287	1303	1303
V_{C}	957	969	982
r	1.96	1.96	1.97

Table 2. The Basic column and Modified columns give simulated results for unmodified and modified simulations respectively where $\chi^2_{\text{X+N}}$ for NPT is half the sum of the neutron and x-ray χ^2 and χ^2_{N} and χ^2_{X} are interpolated minima, respectively, for neutron only and for x-ray only. The SDP column gives modeling results obtained from simultaneously fitting a model to both the experimental neutron and x-ray data. SDP fitting used the experimental volumes shown and ratios of other volumes consistent with the simulation as shown in Table S3. Units for all properties are in appropriate powers of \AA .

One reason for the poorer agreement with the x-ray data is that they extend to much larger q_z thereby including more structural detail and setting a greater challenge for simulations. Another reason is that the x-ray data are subject to systematic error as seen in the slightly distorted shapes of the higher lobes of $F(q_z)$, though this does not account for very much of the χ^2 . A final reason is that the simulation incorrectly obtains the $F(0)$ datum because the volume per lipid V_{L} is too small and the volume of water V_{w} is too large, as shown in Table I, with the consequence that $F_x(0)$ is too large according to Eq. (1). We have explored a work-around of these volumetric flaws that gives the χ^2 results in Fig. 6 with a summary of the salient results in

the Modified column in Table 2. The work-around increases the best areas A_X and A_N by about 1.5% and it improves the fit to the x-ray data (χ^2_x decreases), as expected, but the fit to the neutron data becomes poorer.

A simulation should give $A_X=A_N$; that is clearly not the case with or without the volumetric work-around. However, the difference between A_X and A_N is smaller for these 43A1-S3 force field simulations than for other simulations of DOPC.²³ A viable compromise value between the areas A_N and A_X obtained in this paper is an area per lipid $A_L = 67.5 \text{ \AA}^2$ which would increase to 68.5 \AA^2 for the volumetric work-around. Ideally, a simulation should also give $A_L=A_{NPT}$, but it has been argued that this is asking too much of the water potentials.²³ Instead, one should accept a non-zero value of the surface tension γ as another work-around for flaws in the water interfacial force fields.^{17,22}

It has been emphasized^{18,23} that the neutron $F_N(q_z)$ data are most strongly sensitive to the total (Luzzati) bilayer thickness D_B , so there is a best value of D_B for agreement with neutron data. The x-ray $F_X(q_z)$ data are most sensitive to the headgroup peaks in the electron density profile characterized by the Head-Head thickness D_{HH} and there is a best value of D_{HH} for agreement with the x-ray data. Therefore, for a simulation to have equal values of A_X and A_N , it has to obtain the best values of both D_{HH} and D_B at the same A_L . Of course, both thicknesses decrease as the area is increased as tabulated in Tables S1 and S2. The important quantity is therefore the difference $\Delta D_{B-H}=(D_B -D_{HH})/2$. Most importantly for a force field to obtain $A_X=A_N$ is that ΔD_{B-H} agree with experiment for the most relevant values of A_L and the 43A1-S3 force field succeeds according to this metric as shown in Table 1.

Other insights can be obtained from Table 1. The fact that the quantity $\Delta D_{H-C} = (D_{HH}-2D_C)/2$, where $2D_C$ is the hydrocarbon thickness, is constant suggests that the headgroup

conformation does not change with A_L . Then, the result that ΔD_{B-H} gradually decreases with increasing A_L is due to D_B decreasing more rapidly than D_{HH} which can be understood because water fills in more of the volume between the headgroups in the interfacial region, thereby bringing the Gibbs dividing surface for water closer to the hydrocarbon core. Table 1 also indicates that there is a non-zero distance ΔD_{P-H} between the average location of the phosphate and the peak in the electron density profile; this can be traced to the electron density of the carbonyl and glycerol groups being large enough to pull the peak of the total electron density from the phosphate significantly toward the center of the bilayer. Detailed locations of all the component groups and their volumes are provided in Tables S1 and S2.

A particularly noteworthy test of simulations is the ratio r of the volume of the terminal methyls on the hydrocarbon chains to the volume of the methylenes. One of the reasons that this paper has focused on the 43A1-S3 force field rather than the older, much utilized Berger et al. force field²⁵ is that the latter gives too large values of $r \sim 2.7$. Table I shows that the results for the 43A1-S3 force field agree well with the experimentally acceptable range. We note in passing that the Berger force field also provides excellent agreement with the x-ray and neutron experimental data at the same area $A_L=67.4 \text{ \AA}^2$.

An additional motivation for simulating many areas is to obtain the area compressibility modulus $K_A = 277 \text{ mN/m}$ as shown in Fig. 7. The value of K_A is insensitive to lipid type⁴⁸ so, as emphasized by Klauda et al.,⁵ K_A is a robust quantity for force field development. It may be noted that for DPPC, CHARMM36 gave a somewhat smaller $K_A=207\pm14 \text{ mN/m}$,^{4,5} CHARMM35b4 gave 240 mN/m ,⁴⁹ and the Berger et al. force fields gave a larger value of 348 mN/m .^{50,51} The latter value was obtained using the area fluctuations in an NPT simulation. We have also applied that method to our 43A1-S3 NPT simulation and obtain $K_A = 321 \text{ mN/m}$. Until

recently, the accepted experimental value for DOPC was $K_A = 265 \pm 18$ mN/m,⁴⁸ although a recent re-evaluation has suggested raising this to ~ 300 mN/m,⁵² so agreement of 43A1-S3 with the experimental K_A is excellent.

Determining the area A_L for DOPC has been especially challenging. For many years A_L was reported to be about 72 \AA^2 .^{24,39,53,54} However, simultaneous analysis of x-ray and neutron scattering data, called the SDP analysis, lowered A_L to 67.4 \AA^2 .³⁸ Since the experimental data have been updated, we have performed the SDP analysis again and now estimate $A_L = 67.6 \pm 0.5 \text{ \AA}^2$ (from Tables S4, S5 and column 5 of Table S3). The result $A_L = 67.5 \text{ \AA}^2$ that we suggest above to be the most appropriate unmodified result for the 43A1-S3 force field is in remarkably good agreement with the SDP results.

However, as is apparent from Figs. 3 and 4 and quantified in Table 2, SDP modeling fits the experimental data much better than the simulations; this suggests that the 43A1-S3 simulations can be improved. We have explored directions such improvements might take using the SDP program. The SIMtoEXP program²⁰ provides simulated values for the parameters that are used in the SDP model.¹⁸ Inserting these values into the SDP program essentially recovers the fits obtained by the SIMtoEXP program. The SDP program was then run while constraining a subset of these values to see whether better χ^2 can be obtained by allowing the other parameters to fit. Often, very good fits to the data can be obtained but the values of the parameters are completely unrealistic. For example, the distance between the Gibbs dividing surface for the hydrocarbon core (D_c) and the carbonyl/glycerol (CG) group becomes stereochemically too small, so this distance was constrained to its value 1.3 \AA obtained by the 43A1-S3 simulations and also by CHARMM simulations. We found that the fit was significantly improved when the widths of the headgroup distribution and the Gibbs dividing surface for the hydrocarbon core

were allowed to increase. The fit also improved when the volume of the CG group was allowed to decrease. Detailed numerical results are given in Tables S3-S5. Hopefully, these clues may suggest modifications in the force fields, especially regarding the CG moiety which is the lipid backbone.

Conclusions

Lipid force field development and subsequent experimental validation continues to be faced with the fundamental challenge of defining appropriate metrics for thorough comparison to experiment. Ideally, a force field simulated at zero surface tension would agree with both neutron and x-ray data. As this does not happen for other force fields,²³ we have devised a more refined test. By using a series of NPAT simulations, we compare the areas A_X and A_N at which the simulation best fits the x-ray and neutron data, respectively, and we suggest that $A_X=A_N$ is a primary criterion for testing a simulation. Then the comparison of the tensionless area A_{NPT} is a secondary criterion. This study has applied this refined test to the GROMOS 43A1-S3 united atom force field specifically for DOPC. Although agreement with neutron scattering data is excellent with A_N only 0.8 \AA^2 greater than A_{NPT} , agreement with the more challenging x-ray data is relatively poorer with A_X nearly 3 \AA^2 greater than A_{NPT} . Such detailed studies have not yet been performed for other force fields, but it appears that these results, while not perfect, make this force field quite competitive. Although our focus has been on validation with x-ray and neutron scattering experiments, we have also tested the 43A1-S3 force field against volumetric data, where it obtains excellent values for the relative methyl and methylene volumes, though it obtains somewhat small values for the overall hydrocarbon volume. Also, the 43A1-S3 force field agrees very well with the K_A mechanical micromanipulation datum. We suggest that the type of analysis in this paper be performed for other force fields and also for other lipids.

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Supporting Information.

A detailed description of component structural parameters for each simulation as well as SDP model fit results are provided in SI.

References

- (1) Chiu, S. W.; Pandit, S. A.; Scott, H. L.; Jakobsson, E. *J Phys Chem B* **2009**, *113*, 2748.
- (2) Poger, D.; Van Gunsteren, W. F.; Mark, A. E. *Journal of Computational Chemistry* **2010**, *31*, 1117.
- (3) Poger, D.; Mark, A. E. *Journal of Chemical Theory and Computation* **2010**, *6*, 325.
- (4) Klauda, J. B.; Venable, R. M.; Freites, J. A.; O'Connor, J. W.; Tobias, D. J.; Mondragon-Ramirez, C.; Vorobyov, I.; MacKerell, A. D., Jr.; Pastor, R. W. *J Phys Chem B* **2010**, *114*, 7830.
- (5) Klauda, J. B.; Venable, R. M.; MacKerell, A. D.; Pastor, R. W. In *Curr Top Membr*; Feller, S. E., Ed.; Elsevier Academic Press Inc: San Diego, 2008; Vol. 60, p 1.
- (6) Klauda, J. B.; Monje, V.; Kim, T.; Im, W. *J Phys Chem B* **2012**, *116*, 9424.
- (7) Lim, J. B.; Rogaski, B.; Klauda, J. B. *J Phys Chem B* **2012**, *116*, 203.
- (8) Jambeck, J. P. M.; Lyubartsev, A. P. *J Phys Chem B* **2012**, *116*, 3164.
- (9) Jambeck, J. P. M.; Lyubartsev, A. P. *Journal of Chemical Theory and Computation* **2012**, *8*, 2938.
- (10) Monticelli, L.; Kandasamy, S.K.; Periole, X.; Larson, R.G.; Tieleman, D.P.; Marrink, S.J. *Journal of Chemical Theory and Computation* **2008**, *4*, 819.
- (11) Marrink, S. J.; Risselada, H. J.; Yefimov, S.; Tieleman, D. P.; de Vries, A. H. *J Phys Chem B* **2007**, *111*, 7812.
- (12) Dickson, C. J.; Rosso, L.; Betz, R. M.; Walker, R. C.; Gould, I. R. *Soft Matter* **2012**, *8*, 9617.
- (13) Ulmschneider, J. P.; Ulmschneider, M. B. *Journal of Chemical Theory and Computation* **2009**, *5*, 1803.
- (14) Sachs, J. N.; Petrache, H. I.; Woolf, T. B. *Chemistry and Physics of Lipids* **2003**, *126*, 211.
- (15) Benz, R. W.; Castro-Roman, F.; Tobias, D. J.; White, S. H. *Biophysical Journal* **2005**, *88*, 805.
- (16) de Vries, A. H.; Chandrasekhar, I.; van Gunsteren, W. F.; Hunenberger, P. H. *J Phys Chem B* **2005**, *109*, 11643.
- (17) Kučerka, N.; Perlmutter, J. D.; Pan, J.; Tristram-Nagle, S.; Katsaras, J.; Sachs, J. N. *Biophysical Journal* **2008**, *95*, 2792.
- (18) Kučerka, N.; Nagle, J. F.; Sachs, J. N.; Feller, S. E.; Pencer, J.; Jackson, A.; Katsaras, J. *Biophysical Journal* **2008**, *95*, 2356.
- (19) Pan, J. J.; Tieleman, D. P.; Nagle, J. F.; Kucerka, N.; Tristram-Nagle, S. *Biochimica Et Biophysica Acta-Biomembranes* **2009**, *1788*, 1387.
- (20) Kučerka, N.; Katsaras, J.; Nagle, J. F. *J Membr Biol* **2010**, *235*, 43.
- (21) Braun, A. R.; Sachs, J. N. *Annual Reports in Computational Chemistry* **2011**, *7*, 125.
- (22) Klauda, J. B.; Kučerka, N.; Brooks, B. R.; Pastor, R. W.; Nagle, J. F. *Biophysical Journal* **2006**, *90*, 2796.
- (23) Nagle, J. F. *Faraday Discuss.* **2013**, *161*.
- (24) Nagle, J. F.; Tristram-Nagle, S. *Bba-Rev Biomembranes* **2000**, *1469*, 159.
- (25) Berger, O.; Edholm, O.; Jahnig, F. *Biophysical Journal* **1997**, *72*, 2002.
- (26) Hess, B.; Kutzner, C.; van der Spoel, D.; Lindahl, E. *Journal of Chemical Theory and Computation* **2008**, *4*, 435.
- (27) Van der Spoel, D.; Lindahl, E.; Hess, B.; Groenhof, G.; Mark, A. E.; Berendsen, H. J. C. *Journal of Computational Chemistry* **2005**, *26*, 1701.

- (28) Lindahl, E.; Hess, B.; Van Der Spoel, D. *J Mol Model* **2001**, 7, 306.
- (29) Berendsen, H. J. C.; Vanderspoel, D.; Vandrunen, R. *Comput Phys Commun* **1995**, 91, 43.
- (30) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. *Journal of Chemical Physics* **1995**, 103, 8577.
- (31) Hess, B. *Journal of Chemical Theory and Computation* **2007**, 116.
- (32) Miyamoto, S.; Kollman, P. A. *Journal of Computational Chemistry* **1992**, 13, 952.
- (33) Nose, S. *Molecular Physics: An International Journal at the Interface Between Chemistry and Physics* **1984**, 255.
- (34) Parrinello, M., and A. Rahman *J Appl Phys* **1981**, 7182.
- (35) Michaud-Agrawal, N.; Denning, E. J.; Woolf, T. B.; Beckstein, O. *Journal of Computational Chemistry* **2011**, 32, 2319.
- (36) Braun, A. R.; Brandt, E. G.; Edholm, O.; Nagle, J. F.; Sachs, J. N. *Biophysical Journal* **2011**, 100, 2112.
- (37) Petrache, H. I.; Feller, S. E.; Nagle, J. F. *Biophysical Journal* **1997**, 72, 2237.
- (38) Kucerka, N.; Nagle, J. F.; Sachs, J. N.; Feller, S. E.; Pencer, J.; Jackson, A.; Katsaras, J. *Biophysical Journal* **2008**, 95, 2356.
- (39) Pan, J.; Tristram-Nagle, S.; Kucerka, N.; Nagle, J. F. *Biophysical Journal* **2008**, 94, 117.
- (40) Pan, J. J.; Tristram-Nagle, S.; Nagle, J. F. *Phys Rev E* **2009**, 80, 021931 (1).
- (41) Liu, Y. F.; Nagle, J. F. *Phys Rev E* **2004**, 69, 040901.
- (42) Kucerka, N.; Tristram-Nagle, S.; Nagle, J. F. *J Membrane Biol* **2005**, 208, 193.
- (43) Katsaras, J.; Kucerka, N.; Gallova, J.; Uhrikova, D.; Balgavy, P.; Bulacu, M.; Marrink, S. J. *Biophysical Journal* **2009**, 97, 1926.
- (44) Nagle, J. F.; Wiener, M. C. *Biochimica Et Biophysica Acta* **1988**, 942, 1.
- (45) Wiener, M. C.; Suter, R. M.; Nagle, J. F. *Biophysical Journal* **1989**, 55, 315.
- (46) Wiener, M. C.; White, S. H. *Biophysical Journal* **1992**, 61, 428.
- (47) Nagle, J. F.; Wiener, M. C. *Biophysical Journal* **1989**, 55, 309.
- (48) Rawicz, W.; Olbrich, K. C.; McIntosh, T.; Needham, D.; Evans, E. *Biophysical Journal* **2000**, 79, 328.
- (49) Raghunathan, M.; Zubovski, Y.; Venable, R. M.; Pastor, R. W.; Nagle, J. F.; Tristram-Nagle, S. *J Phys Chem B* **2012**, 116, 3918.
- (50) Waheed, Q.; Edholm, O. *Biophysical Journal* **2009**, 97, 2754.
- (51) Brandt, E. G.; Braun, A. R.; Sachs, J. N.; Nagle, J. F.; Edholm, O. *Biophysical Journal* **2011**, 100, 2104.
- (52) Evans, E.; Rawicz, W.; Smith, B. A. *Faraday Discuss.* **2013**, 161.
- (53) Tristram-Nagle, S.; Petrache, H. I.; Nagle, J. F. *Biophysical Journal* **1998**, 75, 917.
- (54) Kučerka, N.; Tristram-Nagle, S.; Nagle, J. F. *J Membrane Biol* **2005**, 208, 193.