

Indira Chandrasekhar · Mika Kastenholz
Roberto D. Lins · Chris Oostenbrink
Lukas D. Schuler · D. Peter Tieleman
Wilfred F. van Gunsteren

A consistent potential energy parameter set for lipids: dipalmitoylphosphatidylcholine as a benchmark of the GROMOS96 45A3 force field

Received: 22 July 2002 / Accepted: 11 November 2002 / Published online: 21 January 2003
© EBSA 2003

Abstract The performance of the GROMOS96 parameter set 45A3 developed for aliphatic alkanes is tested on a bilayer of dipalmitoylphosphatidylcholine (DPPC) in water in the liquid-crystalline L_α phase. Variants of the force-field parameter set as well as different sets of simulation conditions or simulation parameter sets are evaluated. In the case of the force-field parameters, the van der Waals constants for the non-bonded interaction of the ester carbonyl carbon and the partial charges and charge group definition of the phosphatidylcholine head group are examined. On the methodological side, different cut-off distances for the non-bonded interactions, use of a reaction-field force due to long-range electrostatic interactions, the frequency of removal of the centre of mass motion and the strength of the coupling of the pressure of the system to the pressure bath are tested. The area per lipid, as a measure of structure, the order parameters of the chain carbons, as a measure of membrane fluidity, and the translational diffusion of the lipids in the plane of the bilayer are calculated and compared with experimental values. An optimal set of simulation parameters for which the GROMOS96 parameter set 45A3 yields a head group area, chain order parameters and a lateral diffusion coefficient in accordance with the experimental data is listed.

Keywords Molecular dynamics simulation · Lipids · Potential energy parameters · GROMOS

Introduction

Molecular dynamics (MD) simulation is a powerful means of evaluating interactions between molecules at the atomic level and interpreting the dynamic behaviour of a biomolecular system. The data obtained from the simulation depend critically on the force field used to calculate the potential energy of the inter-particle interactions and on the simulation parameters. The potential-energy or force-field parameters used in simulating biomolecular systems are generally obtained by a process of refinement against experimental or *ab initio* quantum-mechanical data (see, for example, van Gunsteren and Berendsen 1990). The GROMOS force field has developed (Hermans et al. 1984; van Gunsteren and Berendsen 1987; Egberts et al. 1994; van Gunsteren et al. 1996; Daura et al. 1998; van Gunsteren et al. 1998; Schuler and van Gunsteren 2000; Schuler et al. 2001) alongside the growth of computational techniques that allow larger systems to be simulated for longer times, allowing us to address increasingly more subtle and complex problems. The latest effort at parametrization was prompted by the observation that although the established force-field parameter set, 43A2, was extremely successful for a variety of biomolecular systems, it yielded incorrect densities when simulating simple aliphatic chains that contained more than six CH_2 groups. This feature of the force field did not pose a problem for proteins, sugars or nucleotides as these molecules do not contain long aliphatic carbon chains. However, in order to obtain a force-field parameter set that is also applicable to lipids, the aliphatic alkane parameters were refined in an exhaustive effort, resulting in a new GROMOS96 force-field parameter set termed 45A3 (Schuler et al. 2001). In the current paper the 45A3 parameter set is tested on lipids using bilayers of the model lipid dipalmitoylphosphatidylcholine (DPPC). The structural as well as the dynamic efficacy of this parameter set is established by examining the head

I. Chandrasekhar (✉) · M. Kastenholz · R.D. Lins
C. Oostenbrink · L.D. Schuler · W.F. van Gunsteren
Laboratory of Physical Chemistry,
Swiss Federal Institute of Technology Zürich,
ETH-Hoenggerberg, 8093, Zurich, Switzerland
E-mail: indira@igc.phys.chem.ethz.ch
Fax: +41-1-6321039

D.P. Tieleman
Department of Biological Sciences,
University of Calgary,
2500 University Drive NW, Calgary,
Alberta T2N 1N4, Canada

group area per lipid, the diffusion of the glycerol backbone in the plane of the lipid bilayer and the order parameters of the aliphatic chains. An optimal set of simulation parameters for membranes that is compatible with the GROMOS96 force-field parameters is derived, stressing the interdependence of different simulation parameters and the need for a consistent approach in order to reproduce observable data. The relevance of the current force field is that, unlike many other force fields for lipid simulation, it is consistent with the parameters used for simulating other biomolecules such as peptides and nucleotides, allowing for easy application to simulations of the types of complex membrane systems discussed in the following references (Tieleman et al. 1997;

Feller 2000; Forrest and Sansom 2000; Tieleman et al. 2002).

Potential-energy or force-field parameters

The semi-empirical potential-energy terms used in classical molecular mechanics and MD calculations have been extensively described (for example, Ramachandran and Sasisekharan 1969; van Gunsteren et al. 1996). We make use of the standard GROMOS96 interaction function (van Gunsteren et al. 1996, 1998; Scott et al. 1999) and the aliphatic alkane parameters from the 45A3 parameter set (Schuler et al. 2001). The covalent interaction parameters (Fig. 1, Tables 1, 2, 3, 4) and the partial charges and charge group definitions (Fig. 2) of the glycerophosphatidyl head group and the van der Waals parameters for the aliphatic alkanes and the ester carbon (Table 5) are detailed below. The bond, bond-angle and torsional parameters for the ester group have been discussed elsewhere (Chandrasekhar and van Gunsteren 2002). In the present simulations the

Fig. 1a, b The molecular topology of the DPPC molecule. **a** Atom names taken from Chandrasekhar and Sasisekharan (1989), covalent bond type codes and bond-angle type codes from the GROMOS96 force field (van Gunsteren et al. 1996). Names: *X*; numbering: ⁿ*X*; bond type codes: *thin*; bond-angle type codes: *italics*. **b** Atom types and van der Waals integer atom codes from the GROMOS96 force field. Atom types: *X*; numbering: ⁿ*X*; integer atom codes: *X*^{IAC}

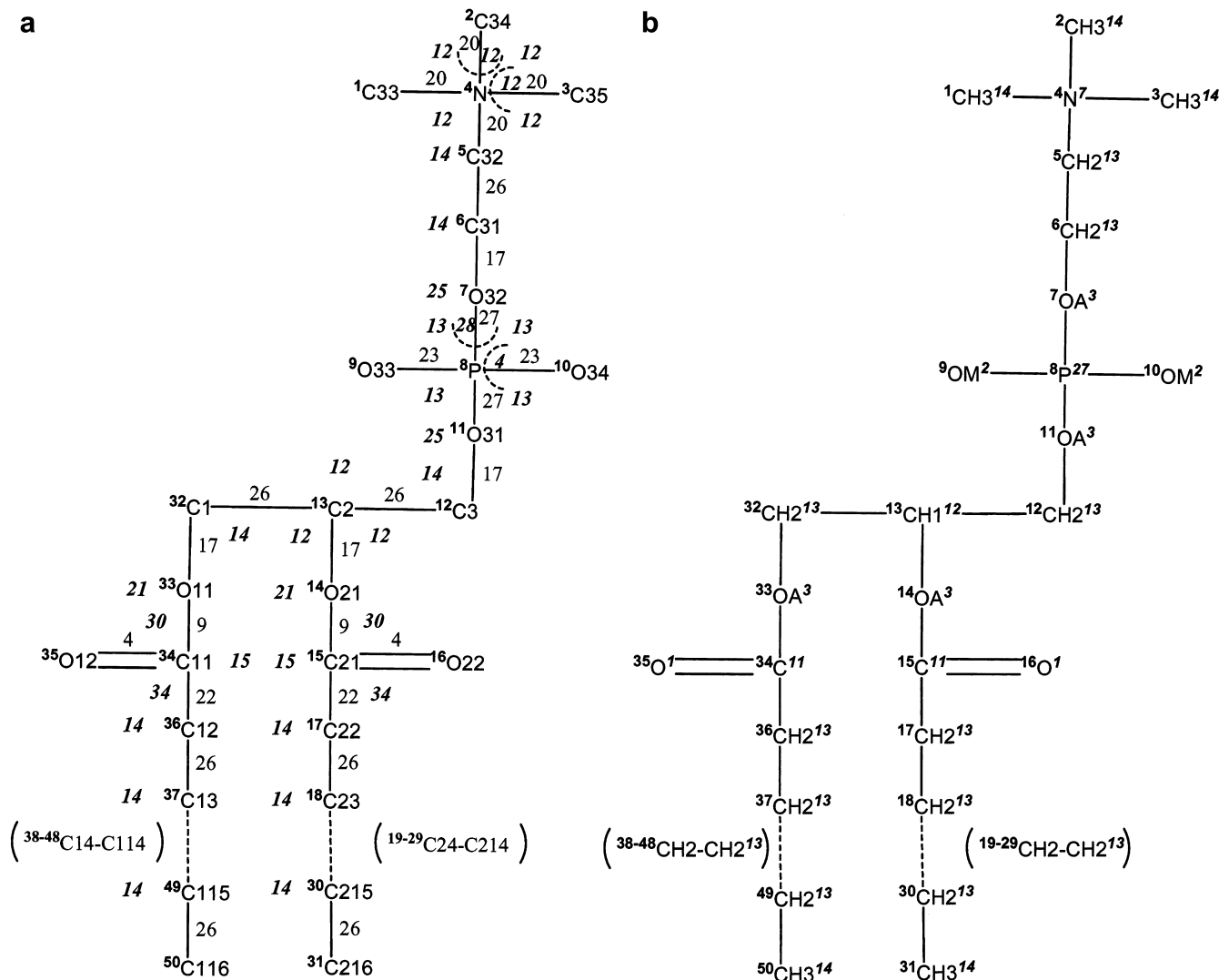


Table 1 The force-field parameters for the bond terms in DPPC (taken from van Gunsteren et al. 1996)

Bond type code	Relevant bond in terms of atom types ^a	Force constant, K_{b_n} (10^7 kJ mol ⁻¹ nm ⁻⁴)	Ideal bond length, b_{0_n} (nm)
4	C=O	16.60	0.123
9	C-OA	11.80	0.133
17	CH _n -OA	8.18	0.143
22	C-CH _n	7.64	0.148
23	P-OM	8.60	0.148
26	CH _n -CH _n	7.15	0.153
27	P-OA	4.84	0.161

^a The appended letter *n* is equal to 1, 2 or 3 and identifies whether the atom is a GROMOS96 united atom type CH1, CH2 or CH3 (Fig. 1b)

Table 2 The force-field parameters for the bond angle terms in DPPC (taken from van Gunsteren et al. 1996)

Bond-angle type code	Relevant bond angle in terms of atom types ^a	Force constant, K_{θ_n} (kJ mol ⁻¹)	Ideal bond angle, θ_{0_n} (degree)
4	OA-P-OA	420	103.0
12	CH _n -N, CH _n -CH _n	520	109.5
13	OM-P-OA	450	109.6
14	CH _n -CH _n -CH _n , OA	530	111.0
15	OA-C-CH _n	545	113.0
21	CH _n -OA-C	635	117.0
25	CH _n -OA-P	530	120.0
28	OM-P-OM	780	120.0
30	O=C-OA	700	122.0
34	O=C-CH _n	750	125.0

^a The appended letter *n* is equal to 1, 2 or 3 and identifies whether the atom is a GROMOS96 united atom type CH1, CH2 or CH3 (Fig. 1b)

parameters for the CH_n-OA bond are represented by the GROMOS96 bond type code 17 with a bond length of 0.143 nm (Table 1) rather than bond type code 20 as used previously (Chandrasekhar and van Gunsteren 2002). The effect on the area per lipid when tested over 300 ps of simulation is not significant (data not shown). The water molecules are modelled using the SPC model (Berendsen et al. 1981).

Alkane van der Waals parameters

In the parameter set 45A3 the van der Waals parameters for the aliphatic united atoms, CH₁, CH₂ and CH₃, have been refined. Further, two new atom types, type CH2r

for CH₂ groups in cyclic structures and type CH0 for tetrahedral sp³ carbons bound to four non-hydrogen atoms, have been introduced. The parameters relevant to DPPC are listed in Table 5. Variants of the 45A3 parameter set that were tested on the DPPC bilayer differ in the van der Waals parameters for the planar ester carbon, indicated by C, which is known to have a significant effect on the cross-sectional area per lipid (Schuler 2000; Chandrasekhar and van Gunsteren 2001, 2002). Initial tests of the simulation parameters (discussed below) make use of the parameter set 45A3-45, where the number after the hyphen refers to the type number of the atom from the GROMOS96 set whose parameters are used to represent the planar ester carbon. Thus, in 45A3-45, instead of the standard GROMOS96 van der Waals parameter for sp² carbons in planar groups (type number 11), the type CH0 parameters for the tetrahedral sp³ atom (type number 45) are used to represent the planar ester carbons. Other variants include 45A3-45×11 and 45A3-45×12. Here, the geometric mean of the bare tetrahedral carbon CH0 (type number 45) and, respectively, the planar carbon type C (type number 11) and the united tetrahedral carbon type CH1 (type number 12) are used. The van der Waals parameters used for the planar ester carbon C are listed in Table 5.

Partial charges and charge group definitions

Two sets of partial charges and three different charge group definitions for the phosphatidylcholine (PC) head group have been evaluated for their effect on the area per lipid in the plane of the bilayer. Shown in Fig. 2 are (a) the charges from GROMOS96 with the head group divided into three charge groups, a charge group convention consistent with GROMOS96 (van Gunsteren et al. 1996), (b) the partial charges due to Chiu et al. (1995) that are significantly larger than the GROMOS96 charges, with the charge group convention followed during the equilibration of the system (Tieleman and Berendsen 1996) where the entire head group is treated as one charge group, and (c) the partial charges due to Chiu et al. (1995) with a third charge group definition consistent with the GROMOS96 standards, where the head group is divided into two charge groups. Note that option (b) requires the use of larger cut-off radii for calculation of the non-bonded interactions than is consistent with GROMOS96 parameter sets (Daura et al. 1998; Schuler et al. 2001).

Table 3 The force-field parameters for the improper dihedral angle terms in DPPC (taken from van Gunsteren et al. 1996)

Improper dihedral-angle type code	Relevant improper dihedral angle in terms of atom names	Force constant, K_{ξ_n} (kJ mol ⁻¹ degree ⁻²)	Ideal improper dihedral angle, ξ_n (degree)
1 (planar group)	Cn1-On1-Cn2-On2	0.0510	0
2 (tetrahedral centre)	C2-C1-C3-O21	0.102	35.26439

^a The appended letter *n* refers to the sn chain number and is either 1 or 2 (Fig. 1a)

Table 4 The force-field parameter terms and assignment of torsional dihedral angles in DPPC (taken from van Gunsteren et al. 1996)

Torsional angle type code	Relevant torsional angle in terms of atom names ^{a,b}	Force constant, $K_{\varphi_n}^K \varphi_n$ (kJ mol ⁻¹)	Phase shift, $\cos\delta_n$	Multiplicity, m_n
14	C33-N-C32-C31	3.77	+1	3
17	N-C32-C31-O32	5.86	+1	3
14	C32-C31-O32-P	3.77	+1	3
11	C31-O32-P-O31	1.05	+1	3
9	C31-O32-P-O31	3.14	+1	2
11	O32-P-O31-C3	1.05	+1	3
9	O32-P-O31-C3	3.14	+1	2
14	P-O31-C3-C2	3.77	+1	3
17	O31-C3-C2-C1	5.92	+1	3
14	C3-C2-O21-C21	3.77	+1	3
17	C3-C2-C1-O11	5.92	+1	3
14	C2-C1-O11-C11	3.77	+1	3
3	$Cn-On1-Cn1-Cn2$	16.7	-1	2
20	$On1-Cn1-Cn2-Cn3$	1.0	+1	6
17	$Cnm-Cn(m+1)-Cn(m+2)-Cn(m+3)$	5.92	+1	3

^a The appended letter n refers to the sn chain number and is either 1 or 2 (Fig. 1a)

^b The appended letter m refers to the sequence number of the methylene or methyl carbon along the chain and varies between 1 and 13 for DPPC (Fig. 1a)

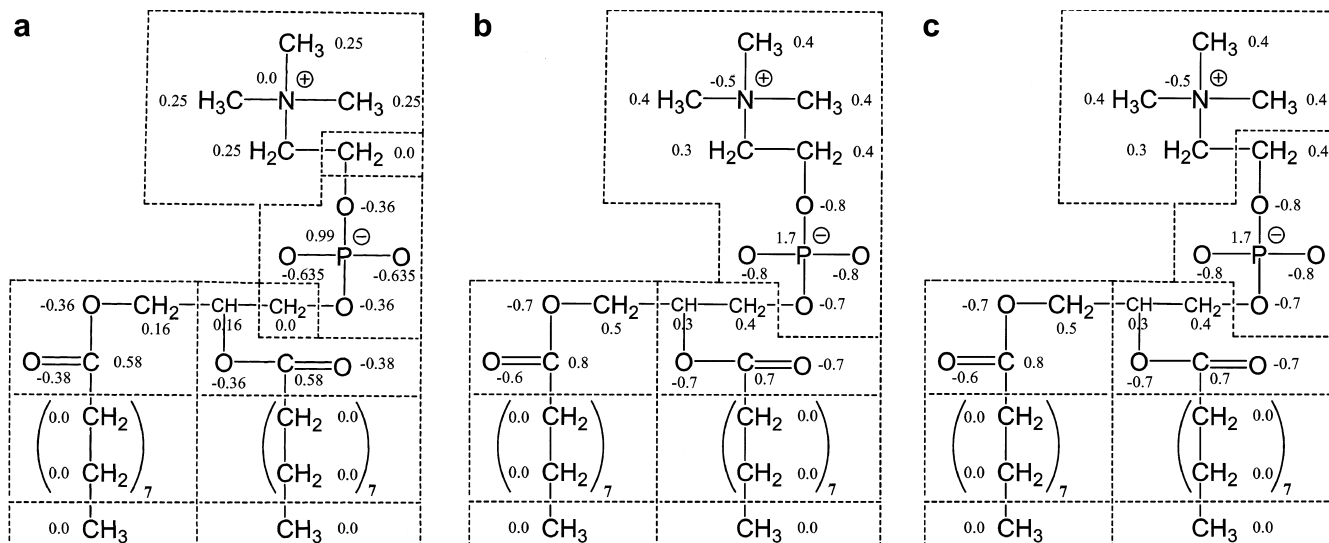
Methods

Simulation parameters

An equilibrated and well-characterized DPPC bilayer in the liquid-crystalline L_α phase, consisting of two layers of 64 lipids each, separated in the z -direction by a layer of 3655 water molecules, was used as a test system (Tieleman and Berendsen 1996). The system was first minimized using the force-field parameter set 45A3-45 (Table 5) and simulation parameter set A (Table 6) with the steepest descent minimization method in GROMOS96. This was followed by 3 ns of MD simulation using the simulation parameters indicated in column A of Table 6, which resulted in a shrinking

of the bilayer in the lateral directions from 0.65 nm², which is within the experimental range for the L_α phase, to 0.49 nm², which is well below it (Nagle and Tristram-Nagle 2000). Consequently, a series of different simulation parameter sets, B to G, listed in Table 6, were tested using short simulations of up to 3 ns. The test conditions involve either the charges and charge group definitions of the DPPC head groups (Fig. 2) or the simulation procedure and parameters (Table 6). The latter include the two radii used in the triple-range method to evaluate interactions used in GROMOS96 (van Gunsteren et al. 1996), the permittivity value of the reaction-field approximation to the third range, the strength of the coupling to the pressure bath (Berendsen et al. 1984) and the frequency of removal of the centre of mass motion. The standard conditions used in GROMOS96 were used as one point of reference (column A, Table 6), while the conditions used in the initial set-up of the DPPC bilayer chosen for simulation by Tieleman and Berendsen (1996) (column E, Table 6) were used as the other.

Fig. 2a–c The partial charges (in units of the electron charge) and different charge group definitions (*dashed lines*) for the glycerol ester and phosphatidylcholine (PC) groups. **a** GROMOS96 charges with the PC head group split into three charge groups. **b** Charges due to Chiu et al. (1995), where the entire PC group is treated as one charge group. **c** Charges due to Chiu et al. (1995), with the PC head group split into two charge groups



Simulation conditions

Initial velocities were taken from a Maxwellian distribution at 325 K, 10 K above the gel to liquid-crystalline transition temper-

Table 5 The van der Waals parameters for the aliphatic alkane carbons in the united atom parameter set 45A3 and the parameters used to represent the planar ester carbon^a

Atom type	Type number or integer atom code	ϵ (kJ mol ⁻¹)	σ (nm)
CH3	14	0.8672	0.3748
CH2	13	0.4105	0.4070
CH1	12	0.09489	0.5019
CH0	45	0.006995	0.6639
C	11	0.4059	0.3361
C	45	0.006995	0.6639
C	45×11 ^b	0.05328	0.4724
C	45×12 ^c	0.02577	0.5773

^a The van der Waals interaction is defined as $4\epsilon[(\sigma/r)^{12} - (\sigma/r)^6]$. The parameters of the first five rows are from parameter set 45A3 (taken from Schuler et al. 2001), the first four representing the aliphatic alkane carbons and the fifth the planar ester carbon. The parameters of the last three rows are variants of the planar ester carbon parameters

^b The geometric mean of type number 45 and type number 11

^c The geometric mean of type number 45 and type number 12

ature for DPPC (Small 1986). The temperature of the lipids and the water molecules were separately coupled to a temperature bath with coupling time 0.1 ps (Berendsen et al. 1984). Rectangular periodic boundary conditions were applied. In all cases, anisotropic pressure coupling was used (Berendsen et al. 1984). The isothermal compressibility, κ_T , was 0.46×10^{-3} (kJ mol⁻¹ nm⁻³)⁻¹. Bond lengths were constrained to ideal values using the SHAKE algorithm (Ryckaert et al. 1977) with a geometric tolerance of 10^{-4} . The time step for the leap-frog integration scheme was set to 2 fs.

In the standard GROMOS96 set-up, the non-bonded interactions are treated using a triple-range cut-off scheme (van Gunsteren and Berendsen 1990; Daura et al. 1998). The force-field parameters have been developed with a short-range cut-off radius of 0.8 nm, within which interactions are determined every time step with the long-range interactions updated every five time steps with a cut-off radius of 1.4 nm. Beyond this long-range cut-off radius, a reaction-field approximation with different values for the permittivity ϵ_{RF} is applied (Tironi et al. 1995).

Analysis

Evaluation of chain order parameters

The deuterium order parameters for acyl chains in lipids may be evaluated in simulation from the long-time average of the second-order Legendre polynomial that expresses the reorientation correlation function of the C-D vector (for example, Pastor and Venable 1993). Thus the order parameter tensor may be defined as

$S_{ij} = 1/2 \langle 3 \cos \theta_i \cos \theta_j - 1 \rangle$, where θ_i is the angle between the i th molecular axis and the bilayer normal (in this case the z -axis) (Egberts and Berendsen 1988). The average is taken over time and over the 128 DPPC molecules. The molecular axes for the n th carbon atom, C_n , along the chain, is defined as follows: z -axis: from C_{n-1} to C_{n+1} ; y -axis: perpendicular to the molecular z -axis and in the plane through C_{n-1} , C_n and C_{n+1} , pointing from the line connecting the C_{n-1} and C_{n+1} atoms towards the C_n atom; x -axis: perpendicular to the molecular y - and z -axes according to a right-handed coordinate system. The molecular axes must be defined independently of the bond lengths and bond angles. If not, variation of the geometry of the chain results in increased mobility (Schuler 2000) due to intramolecular fluctuations.

Diffusion coefficients

The lateral self-diffusion coefficient of the DPPC molecules, D , is calculated from the Einstein relation, valid at long times t : $2NtD = \langle |r(t) - r(0)|^2 \rangle$, where the right-hand side of the equation refers to the mean square displacement of the chosen atom [the glycerol atom C2 (Fig. 1a)], and N is the number of translational degrees of freedom. As we are concerned solely with lateral displacement in the plane of the bilayer (in our case the x - y plane), $N=2$. Note that τ is the initial time and $r(t)$ refers to the position of the chosen atom at time t . The average is taken over time τ , and over 128 DPPC molecules.

Note that in the calculation of the order parameters and diffusion coefficients we have confined ourselves to short time scales of the order of 300 ps. It is generally believed that although lipid systems are known to undergo long-time relaxations (Pastor et al. 2002), the time scale of reorganization and re-ordering is relatively short. This supposition is supported, in our case, by the fact that the system responds quickly to variation in simulation parameters, as well as to changes in the force field parameters (see Figs. 3 and 4 below). While we are aware that order of magnitude increments have been achieved both in system size and simulation time length (Lindahl and Edholm 2000), we see that for the assessment and evaluation of parameters a standard system size of an 8×8 bilayer of DPPC and the relatively short time length of 300 ps are revealing.

Results and discussion

Partial charges and simulation conditions

The simulated area per lipid for each of the models listed in Table 6 is shown in Fig. 3. The GROMOS96 charges (Fig. 2c) result in a reduction in the area per lipid (models A to D) below the lowest reported experimental value of 0.57 nm² (Nagle and Tristram-Nagle 2000),

Table 6 Different simulation set-ups for the DPPC bilayer^a

	A	B	C	D	E	F	G
Charges and charge groups for glycerophosphatidylcholine							
Charges	G	G	G	G	C	C	C
Number of charge groups	3	1	1	1	1	1	2
Simulation parameters							
Cut-off radii	0.8/1.4	0.8/1.4	1.0/2.0	1.0/2.0	1.0/2.0	1.0/2.0	0.8/1.4
Reaction field, ϵ_{RF}	54	54	54	1	1	54	54
pressure coupling, τ_p	0.5	0.5	0.5	0.5	1.0	1.0	0.5
COM motion removal	50,000	50,000	50,000	500	1	1	50,000

^aGROMOS96 charges (Fig. 2a) are indicated by G and the charges from Chiu et al. (1995) (Figs. 2b and 2c) by C. The number of charge groups into which the phosphatidylcholine head group is split is listed. Cut-off radii are given in nm, the pressure coupling constant in ps, the frequency of removal of centre of mass motion in steps of 2 fs

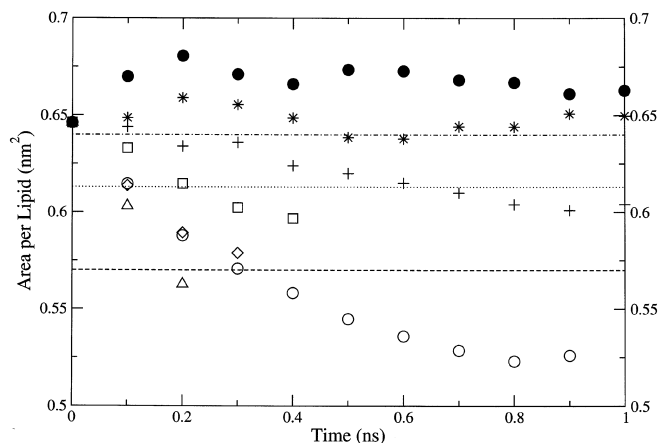


Fig. 3 The area per DPPC molecule as a function of time for the simulation parameter sets A to G listed in Table 6. A: open circles; B: squares; C: diamonds; D: triangles; E: plus signs; F: stars; G: filled circles. The force-field parameter set 45A3-45 (Table 5) is used. The horizontal dashed line indicates the lower limit of the range of experimental values (Nagle and Tristram-Nagle 2000), the horizontal dotted line the area per PC lipid extrapolated to infinite chain length (Petrache et al. 2000) and the horizontal dot-dash line is the best estimate of the area per DPPC molecule in the liquid-crystalline phase (Nagle and Tristram-Nagle 2000)

while the larger partial charges (Fig. 2c) maintain a larger head group area per lipid (models E to G). This is in contrast to simulations of non-polar lipids, where the use of the larger partial charges at the ester group (the charges ascribed to the sn-1 chain in Fig. 2c were used in the case of triglycerides) has little effect on the area per molecule (Chandrasekhar and van Gunsteren 2001). The inclusion of a reaction-field force tends to enlarge the area per lipid, as seen by comparing simulations C (diamonds) and D (triangles) or simulations F (stars) and E (plus signs) in Fig. 3. Extending the cut-off radius for non-bonded interactions lowers the area per lipid, as is observed when comparing simulations C (diamonds) and B (squares) or simulations F (stars) and G (filled circles). The use of larger charge groups implies fewer non-bonded interactions for a given cut-off radius, which results in an increase of the area per lipid as illustrated by comparing simulations B (squares) and A (circles) of Fig. 3. Hence the definition of a larger charge group would require a larger cut-off radius for the non-bonded interactions, increasing the computational expense. The simulation parameter set G (Table 6) shows that the larger charges and the smaller charge group definition (Fig. 2c) are consistent with the triple-range cut-off radii of 0.8/1.4 nm (Fig. 3). The charge group convention shown in Fig. 2c, where the larger partial charges are used to maintain head group separation, along with the standard GROMOS96 simulation parameters, namely the use of the reaction-field forces with a permittivity of 54.0, a pressure coupling parameter of 0.5 ps, a frequency of centre of mass removal of once per 50,000 steps and, as discussed above, cut-off radii of 0.8/1.4 nm for the triple-range cut-offs, is used in the continuing investigations.

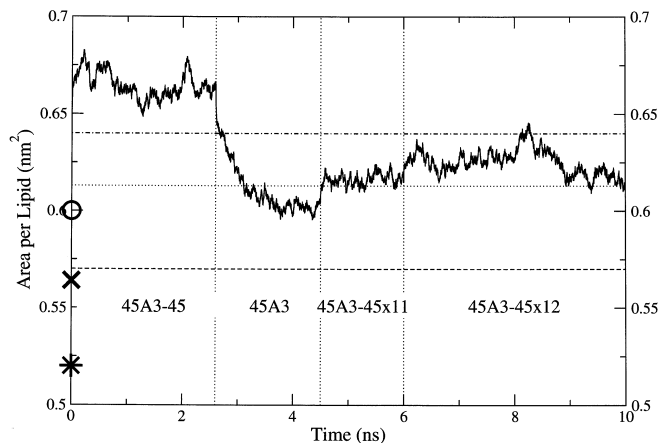


Fig. 4 The area per DPPC molecule as a function of time for variants of the force-field parameter set 45A3. The nomenclature for the parameter sets is given in Table 5. The simulation parameter set G (Table 6) is used. Partial charges are due to Chiu et al. (1995) (Fig. 2c) unless otherwise indicated. The van der Waals radii of the planar ester carbons are changed at the time points indicated by the vertical dotted lines. The mean area per lipid averaged over 300 ps of stable trajectory for the simulations carried out using the force-field parameter set 45A3, not including the phosphodiester torsional terms under simulation conditions G (Table 6), is shown on the ordinate as a bold circle; the value for the 43A2 parameter set under the conditions of simulation indicated by column G of Table 6 as a bold cross, and that using the GROMOS96 partial charges (Fig. 2a) under simulation conditions A (Table 6) and the 45A3 parameter set supplemented with torsional energy parameters obtained by fitting (not shown in Fig. 6) the molecular mechanics profiles of the model fragments discussed in the text and the caption to Fig. 6 to the ab initio profile (Fig. 6, thick lines with circles), as a bold asterisk. The horizontal dashed line indicates the lower limit of the range of experimental values (Nagle and Tristram-Nagle 2000), the horizontal dotted line the area per PC lipid extrapolated to infinite chain length (Petrache et al. 2000) and the horizontal dot-dash line is the best estimate of the area per DPPC molecule in the liquid-crystalline phase (Nagle and Tristram-Nagle 2000)

Planar carbon parameters

The effect of varying the planar carbon van der Waals parameters on the area per lipid, the calculated chain order parameters and the extent of lateral diffusion is shown in Figs. 4 and 5 and Table 7, respectively. The area per lipid is acutely sensitive to the change in size of the ester carbon (Fig. 4), as has also been observed in the case of triglycerides in the gel phase (Chandrasekhar and van Gunsteren 2001, 2002), though it must be noted that all variants of the force-field parameter set 45A3 result in an area per lipid within an acceptable range of the idealized experimental value for DPPC of 0.64 nm^2 (Nagle and Tristram-Nagle 2000) and the value of $0.613 \pm 0.08 \text{ nm}^2$ for phosphatidylcholine lipids extrapolated from experiment to infinite chain length (Petrache et al. 2000). However, the chain mobility or, in other words, the chain order parameter, while it responds to variations in the ester carbon size, is relatively insensitive to them (Fig. 5). This has been seen in the case of the non-polar triglycerides as well, where *gauche-trans* isomerization was found to show no significant differences

Fig. 5 The S_{CD} order parameter as function of chain carbon number for the (a) sn-1 and (b) sn-2 chains of DPPC. Experimental values for the sn-2 chain (Douliez et al. 1995): stars; 43A2: squares; 45A3: circles; 45A3 combined with the Ryckaert-Bellemans potential: small filled circles; 45A3-45: triangles; 45A3-45x11: small filled diamonds; 45A3-45x12: diamonds. The simulation parameter set G (Table 6) is used. The S_{CD} values are averaged over 128 DPPC molecules and over 300 ps of the stable trajectories

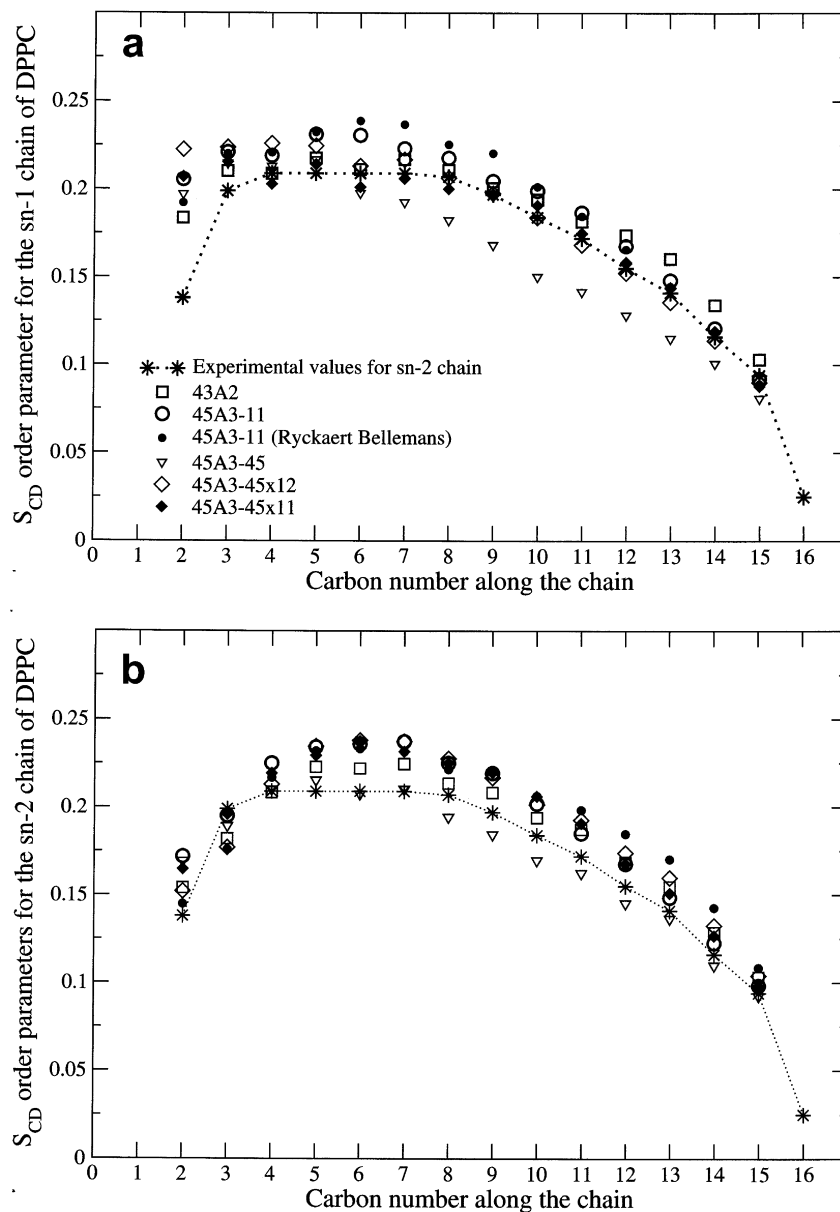


Table 7 The lateral self-diffusion coefficient for the DPPC molecules simulated using the force-field parameter set 43A2 and variants of parameter set 45A3^a

Parameter set	Diffusion coefficient ($10^{-7} \text{ cm}^2 \text{ s}^{-1}$)
43A2	4.1
45A3	1.8
45A3-45	3.2
45A3-45x11	4.5
45A3-45x12	1.9

^aThe codes for the parameter sets are given in Table 5. Simulation parameter set G (Table 6) was used. The values were evaluated as averages over 128 DPPC molecules from 300 ps of stable trajectory. Experimental values are of the order of magnitude 10^{-8} to $10^{-7} \text{ cm}^2 \text{ s}^{-1}$ (Blume 1993; Lipowsky and Sackman 1995; Kusba et al. 2002)

between models simulated using different variants of the force-field parameter set 45A3 (Chandrasekhar and van Gunsteren 2002). As expected, the parameter set

45A3-45, with a large ester carbon radius of 0.66 nm, results in the most mobile chains (Fig. 5), whereas the parameter sets 45A3, 45A3-45x11 and 45A3-45x12 with intermediate radii have similar S_{CD} values that cluster around the experimental figures (Seelig and Niederberger 1974; Douliez et al. 1995). The Ryckaert-Bellemans torsional potential (Ryckaert and Bellemans 1975) was also tested for comparison, in conjunction with the 45A3 parameter set. It shows a small reduction in mobility relative to the GROMOS96 torsional form (van Gunsteren et al. 1996). Although the force-field parameter set 43A2 is notoriously inadequate in reproducing the density of long-chain liquid alkanes (Schuler et al. 2001), and the area per lipid in lamellar non-polar triglycerides in the gel phase (Chandrasekhar and van Gunsteren 2001, 2002) and in DPPC bilayers in the liquid-crystalline phase (Fig. 4), it reproduces the experimental chain order parameters for DPPC. This may

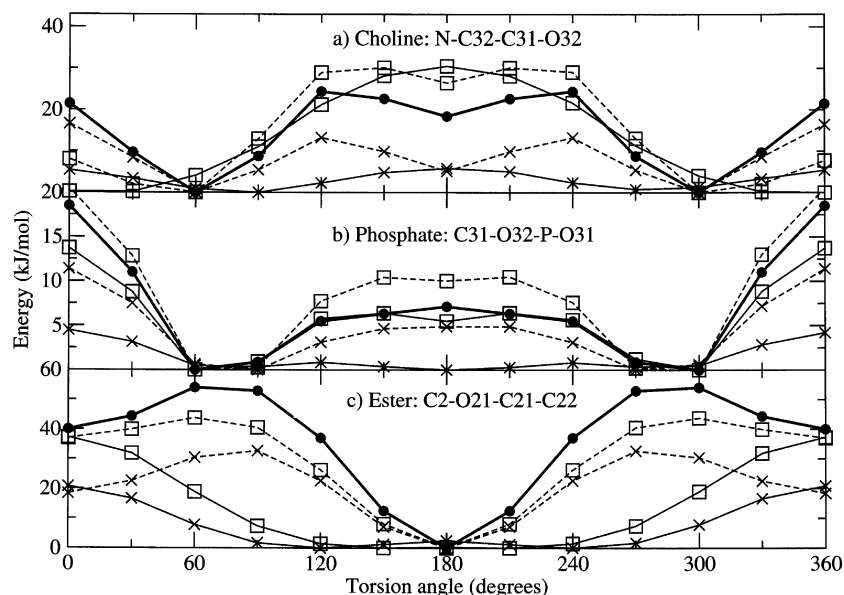


Fig. 6 Energy as a function of dihedral angle (dihedral angle specified below) for (a) the choline fragment of the PC head group, (b) the dimethyl phosphate (DMP) and (c) the glycerol ester fragment. The energies obtained from MP2/6-31-G** ab initio calculations are shown by *thick solid lines with circles*, the *thin solid lines* indicate the GROMOS96 molecular-mechanics energy calculated excluding the torsional dihedral energy term, and the *dashed lines* indicate the GROMOS96 molecular-mechanics energy including the torsional dihedral energy term in the GROMOS96 parameter set 45A3. The *squares* imply the use of the larger charges due to Chiu et al. (1995) (Fig. 2c) and the *crosses* the smaller GROMOS96 charges (Fig. 2a). The relevant molecular fragments using the nomenclature indicated in Fig. 1a and the dihedral angle that is varied in steps of 30° are (a) for the choline molecule: C33-(C34/C35)-N-C32-C31-O32-H, with the torsion angle of interest being N-C32-C31-O32; the dihedral angles about the bonds N-C32 and C31-O32 are allowed to relax; (b) for DMP: C31-O32-P(O33/O34)-O31-C3, where the terminal carbons are treated as methyl groups, with the relevant dihedral angle being C31-O32-P-O31; the symmetrical dihedral angle O32-P-O31-C3 is kept at the ideal *trans* value; and (c) for the glycerol-ester fragment: C2-O21-C21(O22)-C22 with energy as a function of the ester dihedral angle C2-O21-C21-C22 being evaluated

be due to the fact that although the lateral molecular separation is considerably reduced in the simulation using parameter set 43A2 (Fig. 4), it is maintained above the gel phase value (Nagle and Tristram-Nagle 2000) by the use of the larger partial charges described in Fig. 2c (simulation conditions G in Table 6). In all cases, the order parameters of the C2 atom of both the sn-1 (atom C12, Fig. 1a) and the sn-2 (atom C22, Fig. 1a) chains do not reproduce experimental values. This is generally the case in shorter MD simulations of lipid bilayers (e.g. Tieleman and Berendsen 1996), probably reflecting the short time length of the simulations relative to the slow relaxation times that contribute to NMR spin-lattice relaxation (for a discussion, see Pastor and Venable 1993; Pastor et al. 2002).

Although the test simulations are not of sufficient length to generate good statistics, the values of the diffusion coefficients for the 45A3 parameters, in combi-

nation with the larger charges shown in Fig. 2c evaluated over 300 ps of stable trajectory, are of the right order of magnitude compared to experiment (Blume 1993; Lipowsky and Sackman 1995; Kusba et al. 2002) (Table 7).

The glycerophosphatidylcholine group

To understand the ability of the larger partial charges due to Chiu et al. (1995) (Fig. 2c) to maintain the simulated area per lipid within the experimental range under GROMOS96 simulation conditions [Fig. 3, simulation parameter set G (closed circles)], we examine the different fragments of the glycerophosphatidylcholine head group individually, first the choline group, second the phosphodiester moiety and third the glycerol ester group. For each fragment, we generate an ab initio quantum-mechanical torsional profile of a suitable model molecule (individual models discussed below) with the Gaussian 98 program (Frisch et al. 1998) using second-order Moller-Plesset perturbation theory (MP2) and the 6-31G** basis set, as a function of the corresponding dihedral angle at 30° intervals (thick lines with circles, Fig. 6). The corresponding molecular-mechanics energy profiles (Fig. 6) are obtained by minimizing the model molecules using the conjugate gradient minimizer in GROMOS96 (van Gunsteren et al. 1996), while restraining the relevant torsional dihedral angles at the indicated values, for the two sets of partial charges shown in Figs. 2a and 2c. The calculations are carried out using the simulation conditions used in simulation parameter set G (Table 6).

The torsional profiles for the N-C31-C32-O32 torsion in the choline group [C33(C34/C35)-N-C32-C31-O32-H] (see Fig. 1a for nomenclature) are shown in Fig. 6a. The molecular-mechanics energy profiles that do not include a torsional contribution (only bond, bond-angle, im-

proper dihedral-angle and the non-bonded van der Waals and electrostatic energy contributions) indicated by the solid lines in Fig. 6a reveal that the larger partial charges (squared solid line) shown in Fig. 2c overestimate the energy of the *trans* conformer but do not contribute to the *cis* energy barrier. Even after inclusion of the GROMOS96 torsional dihedral term listed in Table 4, the *cis* energy value continues to be lower than 10 kJ mol⁻¹, significantly lower than the 24 kJ mol⁻¹ value of the ab initio energy profile (Fig. 6a, squared dashed lines). The use of the smaller GROMOS96 charges (Fig. 2a) underestimates the *gauche-gauche* barrier and the *trans* energy value, although the *cis* barrier is fairly well reproduced (Fig. 6a, crossed dashed lines).

A model system for the phosphodiester group is dimethyl phosphate (DMP) [C31-O32-P(O33/O34)-O31-C3] (see Fig. 1a for nomenclature), where a methyl group is attached to each of the ester oxygens of the phosphodiester group. The energy profile of DMP as a function of the torsion angles around the two P-O single bonds is a complex one, with minimum energy structures at the *gauche-gauche* (75°, 75°), *trans-gauche* (-170°, 74°) and *trans-trans* (156°, 156°) conformations (Florian et al. 1998; Alber et al. 1999; Leulliot et al. 1999; Banavali and MacKerell 2001; and this work, data not shown). To simplify the problem, we generate an ab initio quantum-mechanical torsional profile of DMP, keeping one P-O torsion fixed at the ideal *trans* conformation of 180° and varying the other in steps of 30° (Fig. 6b). The molecular-mechanics energy profiles indicate that the larger partial charges (Fig. 2c) match the ab initio profile even without the inclusion of the torsional contribution to the energy (Fig. 6b, squared thin solid lines). The mean area per lipid averaged over 300 ps simulation under simulation conditions G (Table 6) in the absence of the GROMOS96 phosphodiester torsions (Table 4) is shown in Fig. 4 as a circle, showing that the removal of the torsional term in this case has little effect on the area per head group. The inclusion of the torsional energy term for the phosphodiester bond from the parameter set 45A3 does not sufficiently raise either the *cis* or *trans* barriers in the case of the GROMOS96 (Fig. 2a) charges (Fig. 6b, crossed dashed lines) and overestimates them when the larger partial charges (Fig. 2c) are used (Fig. 6b, squared dashed lines).

Finally, the energy profiles for the ester torsion *Cn-On1-Cn1-Cn2*, where *n* = 1 or 2 refers to the sn chain number (see Fig. 1a for nomenclature), show that inclusion of the GROMOS96 two-fold torsional term, while it does neither sufficiently raise the *cis-trans* barrier nor reproduce the *cis-trans* energy difference in the case of the GROMOS96 (Fig. 2a) charges (Fig. 6c, crossed dashed line), results in a *trans-cis* barrier of over 30 kJ mol⁻¹, suggesting that the *trans* to *cis* transition will be rare. Indeed, in the simulations of lamellar triglycerides in the gel phase using the 45A3 parameter set, no transitions of the ester torsion have been observed (Chandrasekhar and van Gunsteren 2002). The use of the

larger partial charges (Fig. 2c) results in a *cis-trans* energy difference that is closer to the current ab initio value (Fig. 6c, squared dashed line), although it has been suggested that this energy difference would in fact be lowered in the presence of solvent (Wiberg and Wong 1993).

The energy profiles about the individual dihedral angles of the polar head group clearly reveal that significant differences result from the use of different partial charges. However, the cause for the ability of the larger charges to maintain a larger area per lipid is not immediately evident. Attention is drawn to significant differences in the torsional energy profiles generated using different partial charges. The use of the larger partial charges (Fig. 2c) underestimates the *cis* barrier for the choline torsion (Fig. 6a, squared dashed line), allowing for mobility of the large choline moiety between the *gauche* conformations even while overestimating the *trans* barrier. The use of the dihedral torsional terms in conjunction with the larger partial charges (Fig. 2c) overestimates the *trans* barrier for the phosphodiester torsion and holds the phosphate group more rigid. Further, the difference in the magnitude of the negative charges at the ester oxygens has an effect on the area per lipid of the DPPC bilayer (Chandrasekhar and van Gunsteren manuscript in preparation). Analysis of lipid crystal structures shows that lipid phosphodiester torsions sample not only the two *gauche* but also the *trans* conformations (Hauser et al. 1981), unlike nucleotides and nucleic acids that experience considerable conformational restriction due to the presence of the sugar moieties as well as to helical constraints (Gupta et al. 1982). This emphasizes the importance of proper sampling of the allowed conformations. The torsional dihedral force constants of the dihedral angles discussed above are fitted to reproduce the ab initio profiles in conjunction with the smaller GROMOS96 partial charges (Fig. 2a). The area per lipid at the end of 500 ps of simulation using the GROMOS96 charges (Fig. 2a), simulation parameter set A (Table 6) and the 45A3 force-field parameter set supplemented by the fitted torsional dihedral terms for the dihedral angles discussed above, is shown in Fig. 4 (asterisk). It has a value of 0.51 nm², well below the lowest experimental value determined. Reproducing the ab initio torsional profiles alone is not sufficient to maintain the simulated area per lipid close to the experimental value, indicating that a correct representation of the intramolecular interactions alone does not maintain the lipid bilayer in the correct phase. This deterred more detailed analysis of the differences in the conformational behaviour of the lipids resulting from the differences in the torsional energy profiles generated using the different sets of partial charges.

Conclusion

The well-known challenge of parameterizing lipids for molecular dynamics simulation has been discussed in detail elsewhere (Egberts and Berendsen 1988; Stouch

et al. 1991; Chandrasekhar 1992; Schlenkrich et al. 1996; Berger et al. 1997; Feller et al. 1997; Tieleman et al. 1997; Tobias et al. 1997; Chiu et al. 1999; Smondyrev and Berkowitz 1999; Feller and MacKerell 2000; Schuler 2000; Chandrasekhar and van Gunsteren 2002). The need for an improved GROMOS96 parameter set for lamellar and micellar lipid systems has been well recognized (Chiu et al. 1999; Schuler 2000; Schuler et al. 2001). Our intention has been to develop a parameter set where not only the potential energy parameters but also the simulation parameters are transferable to a wide range of biomolecular systems that include not only lipids but also peptides and nucleotides. The advantage of a consistent parameter set is of particular importance when simulating complex membrane systems containing peptides and proteins (Tieleman et al. 1997; Feller 2000; Forrest and Sansom 2000; Tieleman et al. 2002). In this paper we establish the efficacy of the newly refined GROMOS96 aliphatic alkane parameter set 45A3 for lipids in combination with the partial charges for the glycerophosphatidylcholine head group as proposed by Chiu et al. (1995) on both structural and dynamic grounds using a DPPC model bilayer in the L_α phase and present an optimal set of simulation conditions that is compatible with the GROMOS96 force field. Initial tests indicate that the current force field, like other lipid force fields (Berger et al. 1997; Feller and MacKerell 2000; Forrest and Sansom 2000; Tieleman et al. 2002), is robust as regards transferability to lipids with different chain lengths (dimyristoylphosphatidylcholine), chain unsaturation (palmitoyloleoylphosphatidylcholine) and head group composition (dipalmitoylphosphatidylethanolamine) (data not shown). Further investigations to evaluate the effect of the use of lateral pressure, the interactions between the polar head-group and water, and the use of infinite lattice sum methods to treat long-range electrostatic interactions such as particle mesh or Ewald methods, are in progress.

Acknowledgements D.P.T. is a Scholar of the Alberta Heritage Foundation for Medical Research. Financial support by the National Center of Competence in Research (NCCR) Structural Biology of the Swiss National Science Foundation is gratefully acknowledged.

References

- Alber F, Folkers G, Carloni P (1999) Dimethyl phosphate: stereoelectronic versus environmental effects. *J Phys Chem B* 103:6121–6126
- Banavali NK, MacKerell AD Jr (2001) Reevaluation of stereoelectronic contributions to the conformational properties of the phosphodiester and N3'-phosphoramidate moieties of nucleic acids. *J Am Chem Soc* 123:6747–6755
- Berendsen HJC, Postma JPM, van Gunsteren WF, Hermans J (1981) Interaction models for water in relation to protein hydration. In: Pullman B (ed) *Intermolecular forces*. Reidel, Dordrecht, pp 331–342
- Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR (1984) Molecular dynamics with coupling to an external bath. *J Chem Phys* 81:3684–3690
- Berger O, Edholm O, Jahnig F (1997) Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature. *Biophys J* 72:2002–2013
- Blume A (1993) Dynamic properties. In: Cevc G (ed) *Phospholipids handbook*. Dekker, New York, pp 455–511
- Chandrasekhar I (1992) Parameter development for molecular dynamics simulation of lipids. In: Gaber BP, Easwaran KKK (eds) *Biomembrane structure and function state of the art*. Adenine Press, Albany, NY, pp 353–363
- Chandrasekhar I, Sasisekharan V (1989) The nomenclature and conformational analysis of lipids and lipid analogues. *Mol Cell Biochem* 91:173–182
- Chandrasekhar I, van Gunsteren WF (2001) Sensitivity of molecular dynamics simulations of lipids to the size of the ester carbon. *Curr Sci* 81:1325–1327
- Chandrasekhar I, van Gunsteren WF (2002) A comparison of the potential energy parameters of aliphatic alkanes: molecular dynamics simulations of triacylglycerols in the alpha phase. *Eur Biophys J* 31:89–101
- Chiu SW, Clark M, Subramaniam S, Scott HL, Jakobsson E (1995) Incorporation of surface tension into molecular dynamics simulation of an interface: a fluid phase lipid bilayer membrane. *Biophys J* 69:1230–1245
- Chiu SW, Clark M, Jakobsson E, Subramaniam S, Scott HL (1999) Optimisation of hydrocarbon chain interaction parameters: application to the simulation of fluid phase bilayers. *J Phys Chem B* 103:6323–6327
- Daura X, Mark AE, van Gunsteren WF (1998) Parametrization of aliphatic CH_n united atoms of the GROMOS96 force field. *J Comput Chem* 19:535–547
- Douliez J, Leonard A, Dufour EJ (1995) Restatement of order parameters in biomembranes: calculation of C-C bond order parameters from C-D quadrupolar splittings. *Biophys J* 68:1727–1739
- Egberts E, Berendsen HJC (1988) Molecular dynamics simulation of a smectic liquid crystal with atomic detail. *J Chem Phys* 89:3718–3732
- Egberts E, Marrink S-J, Berendsen HJC (1994) Molecular dynamics simulation of a phospholipid membrane. *Eur Biophys J* 22:423–436
- Feller SE (2000) Molecular dynamics simulations of lipid bilayers. *Curr Opin Colloid Interface Sci* 5:217–223
- Feller SE, MacKerell D Jr (2000) An improved empirical potential energy function for molecular simulations of phospholipids. *J Phys Chem B* 104:7510–7515
- Feller SE, Yin D, Pastor RW, MacKerell D Jr (1997) Molecular dynamics simulation of unsaturated lipid bilayers at low hydration: parametrization and comparison with diffraction studies. *Biophys J* 73:2269–2279
- Florian J, Strajbl M, Warshel A (1998) Conformational flexibility of phosphate, phosphonate, and phosphorothioate methyl esters in aqueous solution. *J Am Chem Soc* 120:7959–7966
- Forrest LR, Sansom MS (2000) Membrane simulations: bigger and better? *Curr Opin Struct Biol* 10:174–181
- Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Zakrzewski VG, Montgomery JA Jr, Straatmann RE, Burant JC, Dapprich S, Millam JM, Daniels AD, Kudin KN, Strain MC, Farkas O, Tomasi J, Barone V, Cossi M, Cammi R, Mennucci B, Pommeli C, Adamo C, Clifford S, Ochterski J, Petersson GA, Ayala PY, Cui Q, Morokuma K, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ciolowski J, Ortiz JV, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Gomperts R, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Gonzalez C, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Andres JL, Gonzalez C, Head-Gordon M, Replogle ES, Pople JA (1998) Gaussian 98, revision A, 6th edn. Gaussian, Pittsburgh
- Gupta G, Rao SN, Sasisekharan V (1982) Conformational flexibility of DNA: an extension of the stereochemical guidelines. *FEBS Lett* 150:424–428

- Hauser H, Pascher I, Pearson RH, Sundell S (1981) Preferred conformation and molecular packing of phosphatidylethanolamine and phosphatidylcholine. *Biochim Biophys Acta* 650:21–51
- Hermans J, Berendsen HJC, van Gunsteren WF, Postma JPM (1984) A consistent empirical potential for water-protein interactions. *Biopolymers* 23:1513–1518
- Kusba J, Li L, Gryczynski I, Piszczek G, Johnson M, Lakowicz JR (2002) Lateral diffusion coefficients in membranes measured by resonance energy transfer and a new algorithm for diffusion in two dimensions. *Biophys J* 82:1358–1372
- Leulliot N, Ghomi M, Scalamani G, Bertier G (1999) Ground state properties of the nucleic acid constituents studied by density functional calculations. I. Conformational features of ribose, dimethyl phosphate, uridine, cytidine, 5'-methyl phosphate-uridine, and 3'-methyl phosphate-uridine. *J Phys Chem A* 103:8716–8724
- Lindahl E, Edholm O (2000) Mesoscopic undulations and thickness fluctuations in lipid bilayers from molecular dynamics simulations. *Biophys J* 2000:426–433
- Lipowsky R, Sackman E (eds) (1995) Structure and dynamics of membranes: from cells to vesicles. *Handbook of biological physics*, vol 1. Elsevier, Amsterdam
- Nagle JF, Tristram-Nagle S (2000) Structure of lipid bilayers. *Biochim Biophys Acta* 1469:159–195
- Pastor RW, Venable RM (1993) Molecular and stochastic dynamics simulation of lipid membranes. In: van Gunsteren WF, Weiner PK, Wilkinson AJ (eds) *Computer simulation of biomolecular systems: theoretical and experimental applications*, vol 2. Escom, Leiden, pp 443–463
- Schlenkrich M, Brickmann J, MacKerell AD Jr, Karplus M (1996) An empirical potential energy function for phospholipids: criteria for parameter optimization and applications. In: Merz K, Roux B (eds) *Biological membranes: a molecular perspective from computation and experiment*. Birkhauser, Boston, pp 31–81
- Schuler LD (2000) Molecular dynamics simulation of aggregates of lipids: development of force-field parameters and application to membranes and micelles. PhD thesis, ETH-Zürich, Zürich, Switzerland
- Schuler LD, van Gunsteren WF (2000) On the choice of dihedral angle potential energy functions for *n*-alkanes. *Mol Simulation* 25:301–319
- Schuler LD, Daura X, van Gunsteren WF (2001) An improved GROMOS96 force field for aliphatic hydrocarbons in the condensed phase. *J Comput Chem* 22:1205–1218
- Scott WRP, Hünenberger PH, Tironi IG, Mark AE, Billeter SR, Fennen J, Torda AE, Huber T, Krüger P, van Gunsteren WF (1999) The GROMOS biomolecular simulation program package. *J Phys Chem A* 103:3596–3607
- Seelig J, Niederberger W (1974) Deuterium labelled lipids as structural probes in liquid crystalline bilayers. *J Am Chem Soc* 96:2069–2072
- Small DM (1986) The physical chemistry of lipids from alkanes to phospholipids. In: Hanahan D (ed) *Handbook of lipid research*, vol 4. Plenum, New York
- Smondyrev AM, Berkowitz ML (1999) United atom force field for phospholipid membranes: constant pressure molecular dynamics simulation of dipalmitoyl-phosphatidylcholine/water system. *J Comput Chem* 20:531–545
- Stouch TR, Ward KB, Altieri A, Hagler AT (1991) Simulation of lipid crystals: characterisation of potential energy functions and parameters for lecithin molecules. *J Comput Chem* 12:1033–1046
- Tieleman DP, Berendsen HJC (1996) Molecular dynamics of a fully hydrated dipalmitoylphosphatidylcholine bilayer with different macroscopic boundary conditions and parameters. *J Chem Phys* 105:4871–4880
- Tieleman DP, Marrink SJ, Berendsen HJC (1997) A computer perspective of membranes: molecular dynamics studies of lipid bilayer systems. *Biochim Biophys Acta* 1331:235–270
- Tieleman DP, Hess B, Sansom MSP (2002) Analysis and evaluation of channel models: simulations of alamethicin. *Biophys J* 83: (in press)
- Tironi IG, Sperb R, Smith PE, van Gunsteren WF (1995) A generalised reaction field method for molecular dynamics simulations. *J Chem Phys* 102:5451–5459
- Tobias DJ, Tu K, Klein ML (1997) Atomic scale molecular dynamics simulations of lipid membranes. *Curr Opin Colloid Interface Sci* 2:115–126
- van Gunsteren WF, Berendsen HJC (1987) *Groningen molecular simulation (GROMOS) library manual*. Biomos, Groningen, The Netherlands
- van Gunsteren WF, Berendsen HJC (1990) *Computer simulation of molecular dynamics: methodology, applications and perspectives in chemistry*. *Angew Chem Int Ed Engl* 29:992–1023
- van Gunsteren WF, Billeter SR, Eising AA, Hünenberger PH, Krüger P, Mark AE, Scott WRP, Tironi IG (1996) *Biomolecular simulation: the GROMOS96 manual and user guide*. Hochschulverlag an der ETH Zürich/Biomos, Zürich/Groningen
- van Gunsteren WF, Daura X, Mark AE (1998) The GROMOS force field. In: Schleyer PvR (ed) *Encyclopedia of computational chemistry*, vol 2. Wiley, Chichester, pp 1211–1216
- Wiberg KB, Wong MW (1993) Solvent effects. 4. Effect of solvent on the *E/Z* energy difference for methyl formate and methyl acetate. *J Am Chem Soc* 115:1078–1084