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Construction and optimisation of a computer model for a bacterial membrane*°

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The main steps in the construction of a computer model for a bacterial membrane are described. The membrane has been built of 72 lipid molecules, 54 of which being 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphatidylethanolamine (POPE) and 18 – 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphatidyl-*rac*-glycerol (POPG) molecules (thus in the proportion of 3:1). The membrane was hydrated with 1955 water molecules (approximately 27 water molecules per lipid). To neutralise the electronic charge (–e) on each POPG molecule, 18 sodium ions (Na^+) were added to the membrane close to the POPG phosphate groups. The atomic charges on the POPE and POPG headgroups were obtained from *ab initio* quantum mechanical restrained electrostatic potential fitting (RESP) (Bayly *et al.*, 1993, *J. Phys. Chem.* 97, 10269) using the GAMESS program at the 6-31G* level (Schmidt *et al.*, 1993, *J. Comput. Chem.* 14, 1347). The model constructed in this way provided an initial structure for subsequent molecular dynamics simulation studies intended to elucidate the atomic level interactions responsible for the structure and dynamics of the bacterial membrane.

Recent advances in the studies of biological processes promoted by biological membranes revealed their dependence on the lipid composition of the membrane. For example, the specificity of cationic peptides (class L antibiotics; Segrest *et al.*, 1990), which selectively

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphatidylcholine; POPE, 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphatidylethanolamine; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphatidyl-*rac*-glycerol; MD, molecular dynamics; RESP, restrained electrostatic potential fitting; OPLS, optimised potential for liquid simulations; TIP3P, three interacting points; RMSD, root mean square deviation; DHAD, dihedral angle deviation; MEMB-1, model bacterial membrane.

lyse bacterial cells but do not show any hemolytic activity, is postulated to arise from differences in the lipid composition between bacterial and animal cell membranes (Matsuzaki *et al.*, 1995). Unlike eukaryotic cell membranes, which are predominantly built of zwitterionic phosphatidylcholine (PC) lipids, prokaryotic membranes are rich in anionic phospholipids e.g., phosphatidylglycerol (PG) (van Klompenburg & de Kruiff, 1998). Moreover, eukaryotic cell membranes contain cholesterol which is not present in prokaryotic membranes. The role of zwitterionic phosphatidylethanolamine (PE), the most prevalent lipid in bacterial membranes (van Klompenburg & de Kruiff, 1998), remains still unclear. PE and PG aggregates form various phases in aqueous solutions. Transformations between phases may arise in response to surface pH, temperature, and concentration of monovalent and divalent cations (Watts & Marsh, 1981; Watts *et al.*, 1981; Marsh *et al.*, 1983). This indicates a great flexibility in packing and space organisation of the PE and PG lipids.

There is experimental evidence that relatively small changes in the chemical structure of lipid headgroups induce large differences in physical properties of the lipid assemblies (Boggs, 1987). In particular, PE bilayers are characterised by a smaller surface area per lipid compared with bilayers built of PCs (Hauser *et al.*, 1981). The higher density of PE packing arises from intermolecular hydrogen bonding between the phosphate and amino groups of adjacent PE molecules. Such interactions do not take place between the phosphate and choline groups of PCs. Recently the role of short distance interactions at the water/PC membrane interface has been studied by means of the molecular dynamics (MD) simulation method (Pasenkiewicz-Gierula *et al.*, 1997; 1999). These interactions were shown to contribute significantly to the membrane stability.

Mixed-phospholipid charged bilayer membranes can serve as a useful model for studying the structure and dynamic behaviour of bacterial membranes, but such studies have been performed much less frequently than those involving mono-phospholipid, neutral bilayers, which are models of eukaryotic membranes. In the present study, the construction of a computer model for a bacterial membrane is described. The model provides an initial structure for subsequent MD simulation. The number of MD simulation studies of charged membranes is rather small (López Cascales *et al.*, 1996; Charifson *et al.*, 1990). Thus we believe that our studies will provide an insight into the structural organisation and dynamic processes in the bacterial membrane at the molecular level.

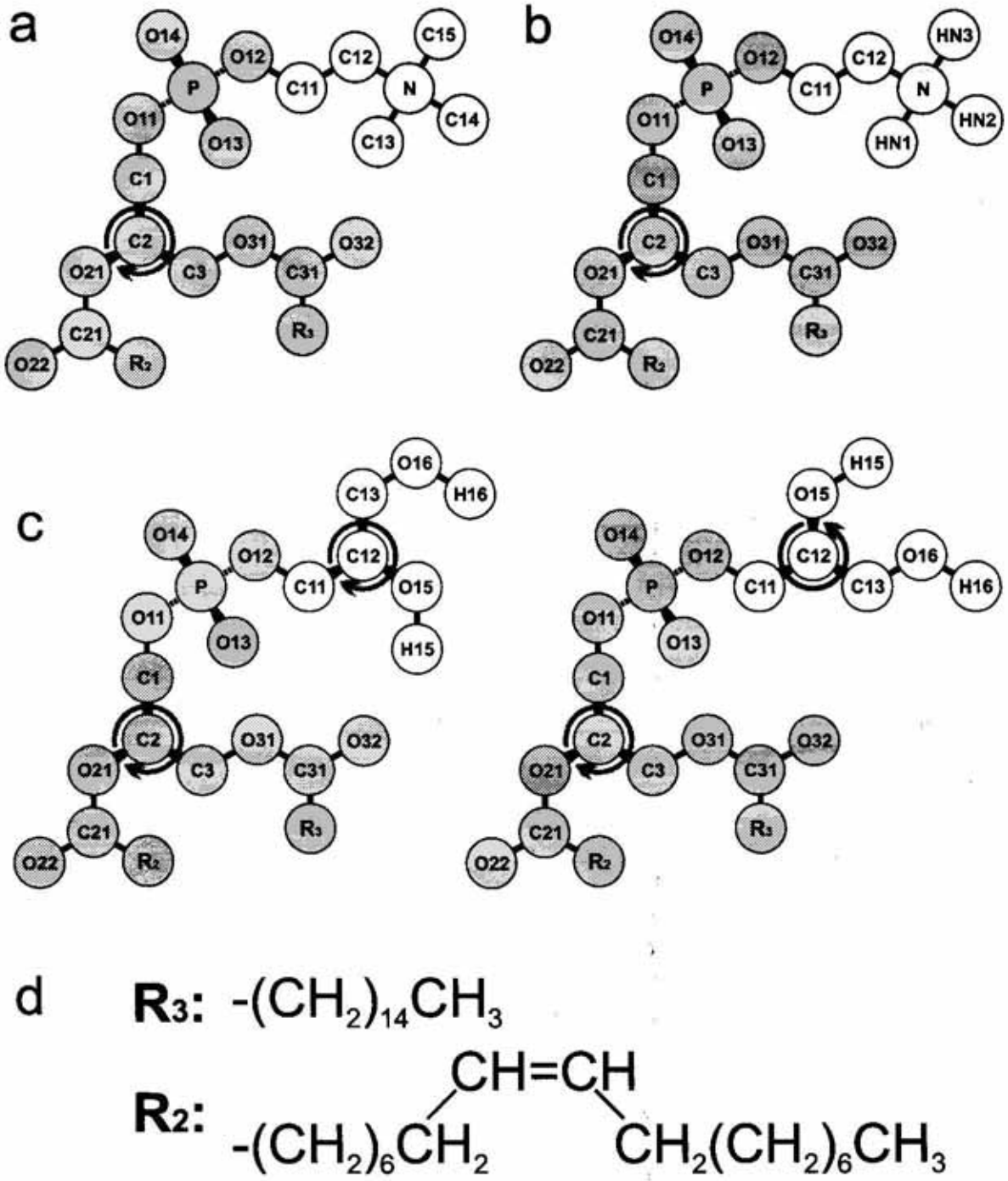
METHODS

Simulation system. The liquid-crystalline phase (L_α) is the biologically active phase of the lipid bilayer matrix of biomembranes. In this phase, the hydrocarbon chains of lipids are disordered and contain on average 3–4 *gauche* dihedral angles. Again, the surface area per lipid assumes a certain value characteristic of the bilayer lipid composition. So, building an initial configuration of a lipid bilayer for molecular modelling studies is not as straightforward as for proteins. In the present study, the initial configuration of the model bacterial membrane (MEMB-1) was based on a well equilibrated (after 6 ns of MD simulation) 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphatidylcholine (POPC) bilayer membrane (unpublished data). We believe that such a choice will reduce the thermal equilibration time of MEMB-1, which in our experience can last longer than 2 ns (Pasenkiewicz-Gierula *et al.*, 1997; 1999; Murzyn *et al.*, 1999).

The main lipid components of a bacterial membrane are 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphatidylethanolamine (POPE) and 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphatidyl-*rac*-glycerol (POPG) in the proportion 3:1

(van Klompenburg & de Kruiff, 1998) (Fig. 1), so that the POPC and model bacterial membranes have the same hydrocarbon chain core and differ only in the terminal groups of α-chains (Fig. 1). The PC choline moiety is replaced by ethanolamine in POPE, and by glycerol in POPG. Figure 1 shows the structure and numbering of atoms in POPC, POPE and POPG. Transformations of the POPC headgroups into headgroups of POPE and POPG were done with the use of an 'in-house' program, which operates on internal coordinates.

MEMB-1 contained 72 lipid molecules (54 POPEs and 18 POPGs). In physiological con-



d

$\mathbf{R_3}$: $-(CH_2)_{14}CH_3$

$\mathbf{R_2}$: $-(CH_2)_6CH_2-CH=CH-CH_2(CH_2)_6CH_3$

Figure 1. Molecular structure of phospholipids.

(a) POPC, (b) POPE, and (c) POPG headgroups with numbering of atoms according to Sundaralingham (1972). The absolute configurations of chiral centres are shown with arrows; (d) chemical structure of palmitoyl (R_2) and oleoyl (R_3) hydrocarbon chains. Atoms common to PE and PG are in grey.

ditions, POPE molecules are neutral whereas POPG are negatively charged (-e, the charge of an electron). In each leaflet of the bilayer, POPGs were uniformly distributed among POPEs to provide a reasonable spacing between negatively charged moieties. To neutralise the negative charge on the POPG phosphate group, a sodium ion (Na^+) was placed in its proximity. The total number of Na^+ in the membrane was 18. In Fig. 2a the initial positions of the PE and the PG molecules are shown for the upper leaflet of the membrane, together with the sodium ions. In the final step, 1955 water molecules were added, which gives about 27 water molecules per lipid. The equilibrium number of water molecules/lipid in a mixed-phospholipid bilayer is not known. However, both in pure PE and pure PG membranes, about 15 water molecules/lipid were found (Rand & Parsegian, 1989). This leads us to believe that about 27 water molecules/lipid in the PE-PG bilayer will effectively prevent undesirable electrostatic interactions between two charged surfaces of the membrane under periodic boundary conditions (López Cascales *et al.*, 1996). The total number of atoms in the system is 9645, 3780 of them being solute and 5865 solvent atoms.

Simulation parameters. For POPE, POPG and sodium ions, optimised potentials for liquid simulations (OPLS) parameters (Jorgensen & Tirado-Rives, 1988) were used. The procedure for supplementing the original OPLS base with the missing parameters for phospholipids was described by Pasenkiewicz-Gierula *et al.* (1999). For water, the three interacting points, 3-site model (TIP3P) (Jorgensen *et al.*, 1983) was used. To reduce computation time the united atom approximation was applied to the POPE and POPG molecules. The atomic charges on the PE and PG headgroups were obtained from *ab initio* quantum mechanical multiconformational restrained electrostatic potential fitting (RESP) (Bayly *et al.*, 1993) using the GAMESS program at the 6-31G* level (Schmidt *et al.*, 1993). The choice of headgroups conformations for atomic charge calculations as well as the derived charges are given in the Results section. The atomic charges on the hydrocarbon chains CH_2 groups of 0.006 e, terminal CH_3 groups of -0.084 e, and CH groups involved in the double bond of 0.008 e (Fig. 1d) were the same as those used for the POPC hydrocarbon chains (unpublished data).

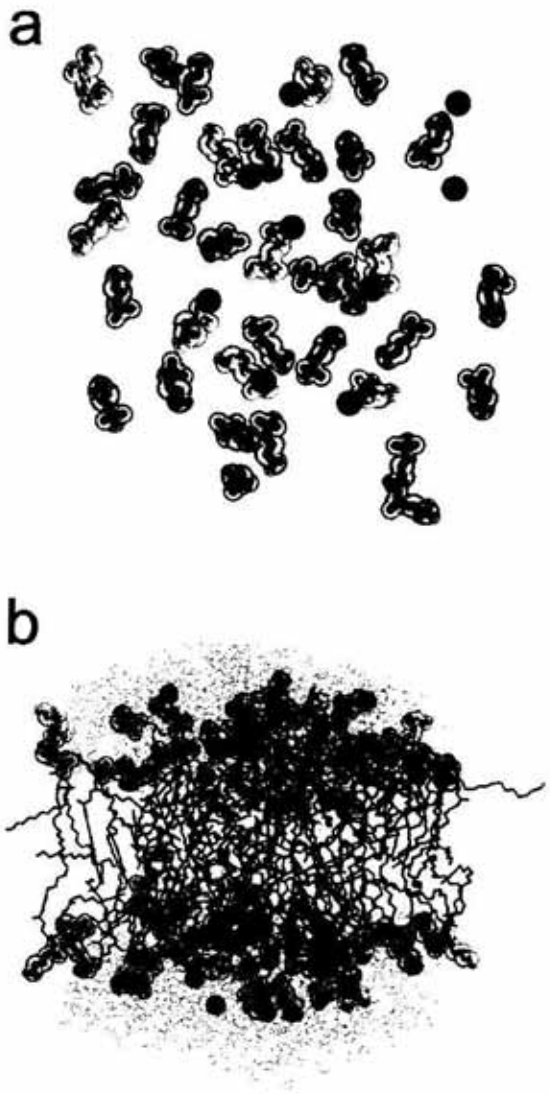


Figure 2. The optimised structure of MEMB-1.

(a) A top view of the membrane surface: the headgroups of POPG and POPE are in yellow and red, respectively, the sodium ions are in blue. (b) A side view of the hydrated membrane. The Figure has been drawn using MOLSCRIPT (Kraulis, 1991) and RASTER3D (Merritt & Bacon, 1997).

Simulation conditions. The MEMB-1 system was built, optimised and simulated with the AMBER 4.0 package (Perlman *et al.*, 1991). Three-dimensional periodic boundary conditions, using the usual minimum image convention, were used. The SHAKE algorithm (Ryckaert *et al.*, 1977) was used to preserve the bond lengths of the water molecule, and the time step was set at 2 fs (Egberts *et al.*, 1994). For nonbonded interactions, a residue-based cutoff was used with a cutoff distance of 12 Å. To reduce calculation time for nonbonded interactions, each lipid molecule (e.g., POPE and POPG) was divided into six residues, being chosen so as to make the total electrostatic charge on the residue close to zero, except for the headgroup of POPG for which the net charge was -e. The integrity of chemical groups in each residue was preserved. To eliminate the high energy conformations and configurations, the potential energy of the membrane system was minimised. After that, the dimensions of the simulation box were L_x = 44.8 Å, L_y = 50.8 Å and L_z = 63.3 Å, where x and y are the membrane in-plane axes, and z-axis is the membrane normal. The snapshot of the optimised MEMB-1 system is given in Fig. 2b.

Conformational analysis. To compare conformations of lipid molecules which were used in RESP fitting, the root mean square deviation (RMSD) and the dihedral angle deviation (DHAD) parameters were used. Both RMSD and DHAD express the measures of similarity between two structures. RMSD is calculated for Cartesian coordinates (Günter, 1998) while DHAD works on internal coordinates (Havel, 1990).

RESULTS AND DISCUSSION

Charge derivation

RESP charges were found to be highly reliable and conformation independent provided that during the multiconformational charge fitting a set of significantly different conformations had been used (Bayly *et al.*, 1993). To establish the partial charges on the PE and PG molecules (Fig. 1b and c), six distinct structures were used. The crystal structures for derivatives of PE (Hitchcock *et al.*, 1974) and PG (Pascher *et al.*, 1987) are known. In this paper they were denoted: PE-A for PE, and PG-A and PG-B for two conformations of PG found in the asymmetric unit cell. The remaining three conformations denoted: PE-X for PE, and PG-X and PG-Y for PG, were obtained by molecular mechanics optimisation in the AMBER 3.0 forcefield (Weiner *et al.*, 1984). The quantum mechanical calculations of the electrostatic potential were performed for all 6 conformations. In these calculations, lipid tails (β and γ chains, Fig. 1d) were substituted by the methyl groups (R_2 = R_3 = CH_3, Fig. 1b and c).

To obtain atomic charges on the phospholipid headgroups, the quantum mechanical potentials were fitted to the classical Coulombic energy function in a series of consecutive calculations performed with the RESP program (Bayly *et al.*, 1993). First, multiconformational RESP fitting was applied to three pairs of lipid conformers: PE-A/PE-X, PG-A/PG-B and PG-X/PG-Y. As a result, three sets of partial charges were obtained. They are given in the first three columns of Table 1. It was noticed that the partial charges on the glycerol backbone atoms varied among these three sets in a narrow range. Hence, in the following calculations, the number of conformations in multiconformational RESP fitting was extended to four. Fitting was performed separately in two groups of conformations. One group included both PE conformers (PE-A/PE-X) and a pair of PG crystal conformers (PG-A/PG-B). The other included both PE conformers (PE-A/PE-X) and a pair of PG optimised conformers (PG-X/PG-Y). In these calculations, a set of charge restraints was imposed. The restraints were established on the basis of a chemical equivalence between PE and PG molecules

Table 1. Point atomic RESP charges calculated at the 6-31G* basis set level.

The first column: charges on the PE molecule derived for two conformations PE-A and PE-X (see text) labelled here PE-AX; the second and third column: charges on the PG molecule derived for two crystal conformations (second) PG-A and PG-B (see text) labelled here PG-AB, and for two optimised conformations (third) PG-X and PG-Y (see text) labelled here PG-XY; the fourth and fifth column: charges on PE and PG molecules calculated with restraints (see text).

	Groups of conformations for RESP calculations				
Atom Name	PE-AX	PG-AB	PG-XY	PE-AX PG-XY set I	PE-AX PG-AB set II
PE, PG common atoms					
P	1.185	1.178	1.241	1.303	1.265
O11/O12	-0.450	-0.471	-0.445	-0.476	-0.469
O13/O14	-0.732	-0.771	-0.780	-0.789	-0.785
O21/O31	-0.409	-0.417	-0.410	-0.395	-0.387
O22/O32	-0.602	-0.617	-0.615	-0.610	-0.603
C21/C31	0.821	0.822	0.803	0.809	0.803
C1	0.235	0.223	0.167	0.245	0.247
C2	0.159	0.243	0.212	0.150	0.138
C3	0.270	0.191	0.254	0.253	0.228
PE specific atoms					
N	-0.324	-	-	-0.443	-0.500
HN1/HN2/HN3	0.298	-	-	0.327	0.339
C11	0.170	-	-	0.380	0.428
C12	0.247	-	-	0.145	0.151
PG specific atoms					
O15/O16	-	-0.580	-0.613	-0.701	-0.615
H15/H16	-	0.372	0.345	0.350	0.368
C11	-	0.243	0.083	-0.182	0.047
C12	-	0.182	0.361	0.702	0.363
C13	-	0.156	0.204	0.249	0.180
Std. Error of estimation	0.004	0.003	0.004	0.007	0.007

(Fig. 1b and c): the atoms common to both molecules i.e., the glycerol backbone, phosphate and acetyl groups atoms were forced to bear the same partial charges on both molecules. The partial charges on atoms specific to PE and PG, i.e., the ethanolamine and glycerol groups atoms, were not restricted. The results of calculations for the first group are given in the fourth column of Table 1 (set I), and for the second in the fifth column of Table 1 (set II). The approach described above reduces the influence of atomic charges on the rate of torsion transitions.

Conformational analysis

The reliability of partial charges derived for a given molecule by means of the multi-

conformational RESP method (Bayly *et al.*, 1993) depends on the diversity of conformations used. The diversity of both PE and PG conformations was compared in terms of RMSD and DHAD (see Methods). The results are summarised in Table 2. As can be seen, conformations of the pair of PG crystal structures (PG-A/PG-B) differ most while those of the pair of PG optimised structures (PG-X/PG-Y) are similar. Comparison of the partial charges obtained for set I, which includes the PG-A/PG-B pair, with those for set II, which includes the PG-X/PG-Y pair (Table 1), reveals that relatively small differences between PG-X and PG-Y give rise to an artificially high charge on C12. C12 in PG is well screened by surrounding groups (Fig. 1c) so that only really diverse conformations of the α-chain are able to reveal the proper charge. Eventually, for subsequent molecular dynamics simulations, atomic charges obtained for set II were used.

Table 2. The root mean square deviation (RMSD) and dihedral angle deviation (DHAD) parameters for PE and PG pairs of conformers (labelled as in Table 1).

Pairs of conformers	RMSD (Å)	DHAD (deg)
PE-AX	1.38	46.7
PG-AB	1.44	80.5
PG-XY	0.97	23.9

Determination of dipole moments

The dipole moments (μ) of the six initial conformations of PE and PG discussed above, were calculated for the set I and set II partial charges (Table 1). The dipole moments were not significantly affected by the choice of a charge set (not shown).

The average dipole moments for POPE and POPG in the optimised MEMB-1 system consisting of 54 POPE and 18 POPG molecules were then calculated. From the results given in Table 3 it can be seen that POPG has a distinctly larger μ than POPE. In POPG, the largest contribution to μ comes from the z-component, while in POPE the contributions to μ from μ_x, μ_y and μ_z are similar. The value of 22 ± 4 D obtained for the POPE dipole moment (Table 3) compares well with experimental estimates (Bowen & Lewis, 1983), whereas, the calculated value of 36 ± 5 D for the POPG dipole moment (Table 3) could not be verified due to the lack of experimental data. The average dipole moment of lipid molecules in the optimised MEMB-1 system might not be fully relevant as minimisation does not relax initial conformations of lipid headgroups. However, one should not expect significantly different values for the dipole moments calculated for 'relaxed' conformations of PE and PG because standard deviations of μ constitute less than 20% of the μ values (Table 3).

Table 3. The dipole moment (μ) and its components (μ_x, μ_y, μ_z) for POPE and POPG in MEMB-1.

x and y are the membrane in-plane axes and z-axis is the membrane normal.

Dipole moment (D)	POPE	POPG
μ	22 ± 4	36 ± 5
μ_x	9 ± 6	7 ± 5
μ_y	13 ± 8	8 ± 6
μ_z	10 ± 7	34 ± 5

CONCLUSIONS

The first goal of this work was to establish reliable partial charges for the PE and PG molecules required to construct a model bacterial membrane for structure optimisation and molecular dynamics simulations. By applying the RESP method (Bayly *et al.*, 1993) with restraints based on the structural similarities between PE and PG, a set of partial charges for each molecule was obtained. The second goal was to propose and test a general method for building membrane systems consisting of different types of lipids. The 3 ns MD simula-

tion performed so far indicates that the system constructed in the way described in this paper, is stable. It is hoped that MD simulation studies of the bacterial membrane will make it possible to elucidate the atomic level interactions responsible for the structure and dynamics of the membrane.

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