

Research paper

Role of phosphatidylglycerols in the stability of bacterial membranes

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Abstract

An extensive 100-ns molecular dynamics simulation of lipid bilayer composed of mixture of phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) was performed to elucidate the role of PGs to the stability of bacterial membranes. In addition, a control simulation of pure PE over 150 ns was performed. We observed that PGs decrease both the PE headgroup protrusions into the water phase, and the PE headgroup motion along bilayer normal. The above effects are caused by stronger inter-lipid interactions in the mixed bilayer: the number of hydrogen bonds created by PEs is 34% higher in the mixed than in the pure bilayer. Another contribution is due to the numerous ion-mediated inter-lipid links, which strongly enhance interface stability. That provides a plausible mechanism for preventing lipid desorption from the membrane, for example, under the influence of an organic solvent. A more compact and less dynamic interface structure also decreases membrane permeability. That provides a possible mechanism for stabilizing, e.g., bacterial membranes.

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1. Introduction

In addition to the much studied phosphatidylcholines (PCs), phosphatidylethanolamines (PEs) and phosphatidylglycerols (PGs) are among the most common lipids in nature. PEs,

like PCs, are neutral and zwitterionic under physiological conditions [1]. PEs are present in both eukaryotic and prokaryotic membranes, participating in a multitude of tasks such as determining fusion, vesiculation and curvature of bilayers [2–5]. PEs also influence permeation [6], and even processes such as cell division [7]. Unlike PEs and PCs, PGs are anionic carrying a unit negative charge. PGs are primarily present in higher plants [8], and they are one of the major constituents of bacterial membranes where the usual amount is around 25% [9]. In *Staphylococcus aureus*, the PG fraction may be up to 80% of lipids, whereas in *Escherichia coli* PEs are dominant with a fraction of about 80% of the lipids [9]. The PG concentration in eukaryotic membranes is low, but they are present, e.g., in mitochondria. In red blood cells they constitute about 2% of phospholipids [10].

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; POPC, palmitoyloleoylphosphatidylcholine; POPE, palmitoyloleoylphosphatidylethanolamine; POPG, palmitoyloleoylphosphatidylglycerol; MD, molecular dynamics.

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Due to the presence of an ammonium group in PE and hydroxyl groups in the PG molecule, both lipids are capable in participating in hydrogen bonding as both hydrogen donors and acceptors, while PCs can only function as hydrogen acceptors. PE–PE hydrogen bonds have been observed both experimentally [11,12] and in molecular dynamics (MD) simulations [13–16]. The presence of PG–PG hydrogen bonds in PG mono- and bilayers is not so obvious; their presence has been suggested from infrared studies [17,18]. Limited formation of such bonds has been demonstrated in MD simulations as well [19,20].

Since direct electrostatic interactions between charged species are rather strong, PGs, as well as other charged lipids, function both as membrane stabilizers and destabilizers, and are believed to play an important role in controlling membrane peptide/protein interactions [21]. The importance of electrostatic interactions between PGs is stressed by the fact that main phase transition temperature as well as phase behavior strongly depend on ionic strength [22,23].

Furthermore, there is strong evidence that bacteria are able to adjust their relative concentrations of PEs and PGs when subjected to toxic organic solvents [24,25]. Such an alteration in headgroup composition seems to be a means for changing membrane permeability, and hence preserving stability. Molecular mechanisms responsible for these changes are not known, however. Studies using model membranes and PGs have revealed complex effects on vesicle morphology, phase behavior, and mixing properties of PE–PG mixtures [26, 27].

Unlike for PCs, the number of computational studies involving PEs and PGs has been more limited. Properties of e.g., SOPE and POPE [28,29], DOPE [30], DPPE [15,16], DLPE [14] and POPG bilayers [20,31] have been studied, but the number is very limited compared to the abundant studies of PCs, and no systematic studies exist to our knowledge. POPE–POPG mixture, the topic of this paper, has recently been used to model bacterial membranes [32–35]. The current study goes beyond the earlier ones [32,33] as the time scales here are almost two orders of magnitude longer providing the access to the important hydrogen bonding and charge-mediated interactions. An excellent review of MD simulations of charged lipid species and the effect of ions on lipid bilayer properties is given by Berkowitz et al. [36].

In this paper, we present results from large-scale MD simulations of a mixed POPE–POPG bilayer in proportion 3:1. This proportion was chosen as it models bacterial membranes [32,33]. Our main findings can be summarized as follows: based on our measurements, we propose that the stability of bacterial membranes significantly increases due to the presence of PGs. PGs also decrease protrusions of PE molecules and reduce their motion along the bilayer normal. These changes occur due to strong ion-mediated electrostatic interactions, and an increase in inter-lipid hydrogen bonds. Consequences to domain formation in bacterial membranes [37] and antimicrobial peptides [38] will be discussed at the end of this paper.

The rest of this paper is organized as follows: in Section 2 we describe the simulation method, protocol and parameters.

In Section 3 we present the results, and we finish with a discussion and conclusions in Section 4.

2. Method

We studied lipid bilayers consisting of 96 POPEs, 32 POPGs, and 3623 water molecules. Since the POPG lipids are anionic and carry a unit charge, 32 Na⁺ counterions were added for charge neutrality. A pure POPE bilayer made of 128 POPEs and 3655 water molecules was studied as a reference system; the structures of the POPE and POPG molecules are shown in Fig. 1. The lipid force-field parameters were taken from the widely used and validated united atom force-field of Berger et al. [39]. They are based on the GRO-MOS force-field bonding parameters for lipid headgroups, with Ryckaert–Bellemans potential functions [40, 41] for hydrocarbon chains. For the Lennard–Jones interactions, the OPLS (Optimized Parameters for Liquid Simulations) parameters were used [42]. For the hydrocarbon chains, the Lennard–Jones parameters optimized for long hydrocarbon were used [39]. For POPE, we used the description by Tieleman et al. [43] (available at <http://moose.bio.ucalgary.ca/>), and for POPG we used the same description (based on the above parameterization) as in our previous study of a pure POPG bilayer [20] (available at <http://www.softsimu.org/downloads.shtml>). The SPC (Simple Point Charge) model [44] was used for water as it is consistent with the above force-field, and the standard GROMACS force-field parameters were used for sodium ions.

The simulations were performed in the NpT (constant particle number, pressure and temperature) ensemble using the open source GROMACS package [45]. All the Lennard–Jones interactions were cut off at 1.0 nm, and the particle-mesh Ewald (PME) method [46] was used for electrostatics. Lipid bonds were constrained using the LINCS algorithm [47], while SETTLE [48] was used for water. The list of non-bonded pairs was updated every 10 steps. The time step was set to 2 fs and temperature was kept constant at 310 K using the weak coupling method [49] with a coupling time constant of 0.1 ps. Temperature of 310 K was selected since it is physiological temperature and thus of biological interest. The selected lipids are in the fluid phase in that temperature. Following standard practice, lipids and solvent (water and ions) were separately coupled to a heat bath. The Berendsen barostat [49] with a coupling constant of 1.0 ps was employed (1 bar), and semi-isotropic pressure coupling was used. The above parameters and the simulation protocol have been widely used, validated and tested by us and other groups, see e.g., Refs. [50–54] and references therein.

As the initial structure for the mixed POPE–POPG bilayer, we used a pre-equilibrated palmitoylcholine phosphatidylcholine (POPC) bilayer [50] where the choline moieties of POPC lipids were replaced with ammonium and glycerol groups. The resulting POPE–POPG (POPE) bilayer system was simulated for 100 (150) ns; the time range from 30 to 100 ns (30 to 150 ns) was used in data analysis. Equilibration was monitored by using the area per lipid, potential energy and

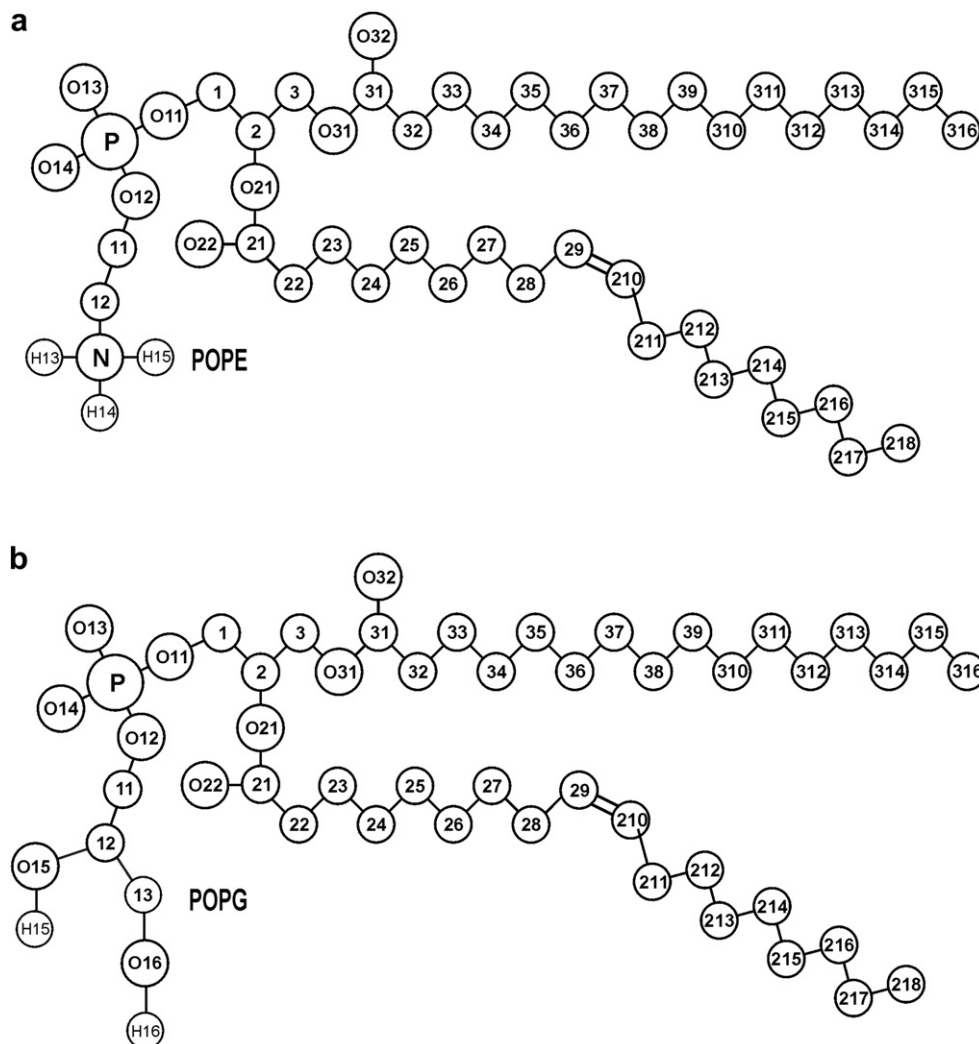


Fig. 1. Chemical structures of (a) POPE and (b) POPG molecules.

temperature which had settled to their equilibrium values after 20 ns. Snapshots of the POPE–POPG system are shown in Fig. 2.

3. Results

3.1. Characteristics of the membrane systems

We start with a discussion of the system dimensions. In the pure POPE system the area per lipid was determined to be $0.514 \pm 0.006 \text{ nm}^2$. It compares reasonably well with experiments which give about 0.56 nm^2 at $T = 303 \text{ K}$ [55] and the simulations of Mukhopadhyay et al. [56] which give 0.49 nm^2 at $T = 298 \text{ K}$. Unlike for the commonly used dipalmitoylphosphatidylcholine (DPPC), there is only a very limited amount of data available for POPE. Mukhopadhyay et al. also discuss possible problems in comparing simulations of POPE with experiments. For the POPE–POPG mixture we measured $0.510 \pm 0.007 \text{ nm}^2$ and in our previous study of the pure POPG system we found $0.530 \pm 0.006 \text{ nm}^2$ [20]. To our

knowledge, there is no experimental data that can be compared to the mixed system.

We also measure the deuterium order parameter S_{cd} (Fig. 3) [57]:

$$S_{\text{cd}} = \left\langle \frac{3}{2}(\cos^2 \theta) - \frac{1}{2} \right\rangle$$

where θ is an instantaneous angle between the n th segmental vector, i.e., (C_{n-1}, C_{n+1}) vector linking $n-1$ and $n+1$ carbon atoms in the acyl chain and the bilayer normal; $\langle \dots \rangle$ denotes both the ensemble and the time averages. As compared to the pure POPE bilayer, the sn-1 chain of the POPG in the mixed bilayer shows reduced order at the beginning of the chain. The PG sn-1 chain is, however, more ordered than in the case of a pure POPG bilayer.

3.2. Head group orientation and rotation

The orientations of the POPE P–N dipoles are influenced by the Na^+ ions, which bind to the POPE carbonyl oxygen

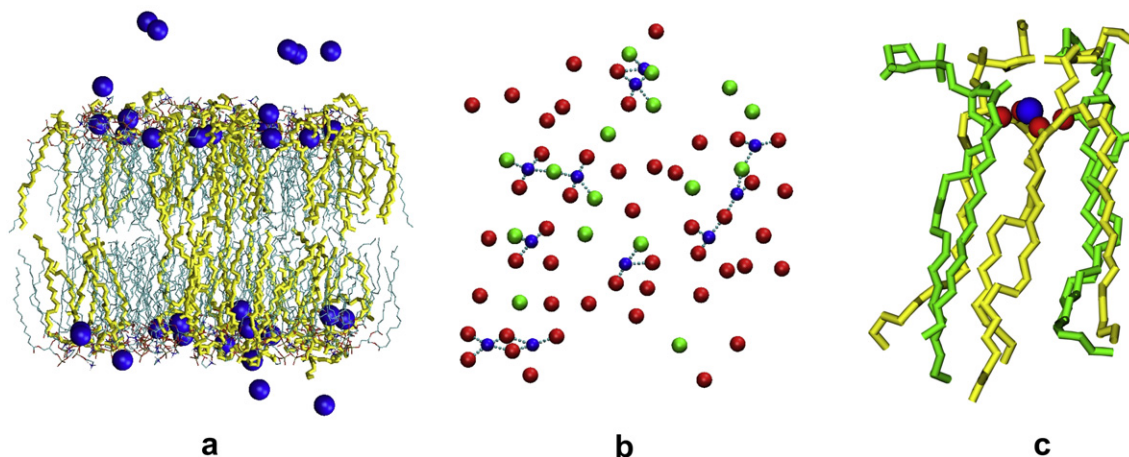


Fig. 2. Snapshots of (a) the structure of the POPE–POPG bilayer (water not shown for clarity), and (b) configuration of the upper leaflet after 100 ns (each lipid is represented by the average position of its two carbonyl oxygen atoms. Color scheme: red, POPE; green, POPG; blue, ions; and yellow, lipid-ion 'bonds'). (c) Structure of four lipids bonding to a Na^+ ion.

O22 in the POPE–POPG system, see Fig. 1 for the numbering of atoms. To study this, POPEs were divided into two categories: those bound to a Na^+ ion and those not. We define a POPE– Na^+ pair bound if the lifetime of the 'bond' exceeds

half of the whole trajectory. We found that about 1/3 of POPEs were bound during the simulation run. The average angle between the bilayer normal and the P–N vector was $87^\circ \pm 4^\circ$ for bonded and $96^\circ \pm 2^\circ$ for non-bonded PEs. Hence, binding to an ion reorients the P–N dipole toward the water phase. The orientation of the P–N dipoles in the pure POPE system was found to be $92^\circ \pm 1^\circ$. That is, within the error bars, the same as the average value in the POPE–POPG system ($93^\circ \pm 3^\circ$). That may be the result of a balance between ion bonding, hydration, and inter-lipid hydrogen (H) bonds. Consistently with previous studies, the effect of Na^+ ions on the POPG head group orientation was found to be negligible [20].

To examine the effect of ion bonding on headgroup rotation, we calculated the reorientational autocorrelation function (RAF) of the P–N vector for the first Legendre polynomial of the ion-bonded and non-bonded POPE headgroups both in the mixed POPE–POPG and the pure POPE bilayers (Fig. 4). As can be seen in Fig. 4, the rotation of PE headgroups is slower in the mixed bilayer including both bonded and non-bonded PEs. However, the dynamics of the bonded headgroups is more influenced than those of non-bonded PEs. We can conclude that head group rotation is influenced both by ion bonding and H-bonds (see Section 3.4)

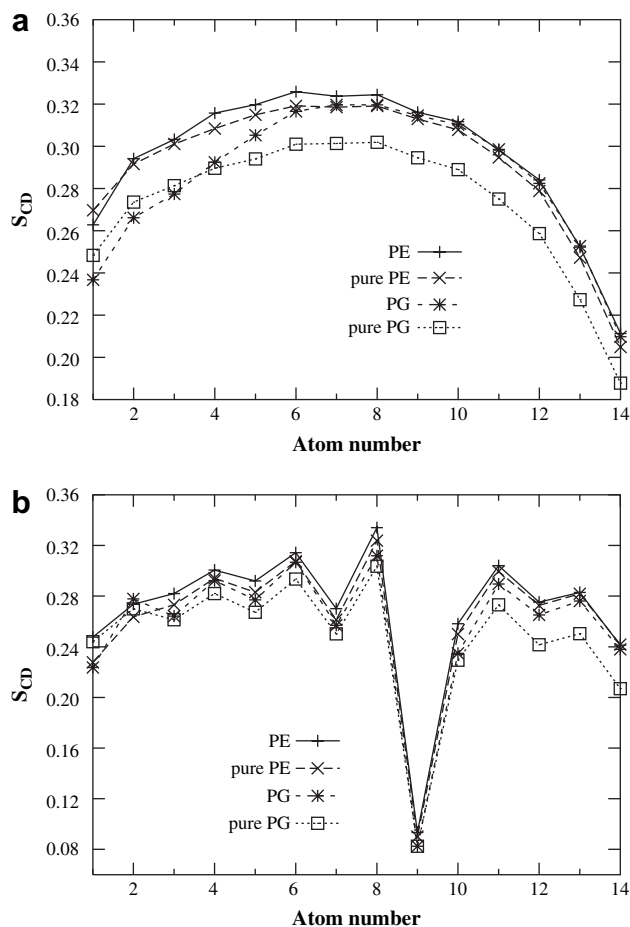


Fig. 3. Deuterium order parameters S_{CD} of sn-1 (a) and sn-2 (b) chains of POPE and POPG in pure POPE, pure POPG, and POPE/POPG bilayers.

3.3. Interactions with ions

In the POPE–POPG system, the Na^+ ions interact actively with the carbonyl oxygen atoms, especially with the POPG ones. To characterize those interactions, the radial distribution functions (RDFs) of Na^+ ions relative to the various lipid oxygen atoms and water oxygens are shown in Fig. 5. The coordination numbers around a Na^+ ion (that is, the average number of atoms within the first hydration shell of an ion, see [20]) were found to be 3.28, 1.10, 0.80, 0.06, 0.01, 0.11, and 0.03 for water oxygen atoms (errors are less than 0.03), POPE carbonyls (O22, O32), POPG carbonyls (O22, O32), POPE phosphodiester oxygen atoms (O13, O14), POPG

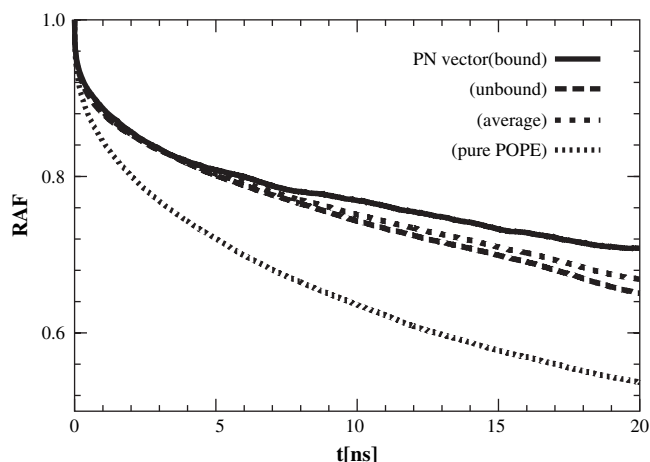


Fig. 4. Reorientational autocorrelation function of the first Legendre polynomial for P–N vector in the POPE molecules in the POPE and POPE–POPG bilayers.

phosphodiester oxygen atoms (O13, O14), O15 (POPG), and O16 (POPG), respectively. The sodium ions prefer to interact with the POPG carbonyl oxygen atoms since each POPE carbonyl oxygen atom binds only 0.37 sodium ions, while each

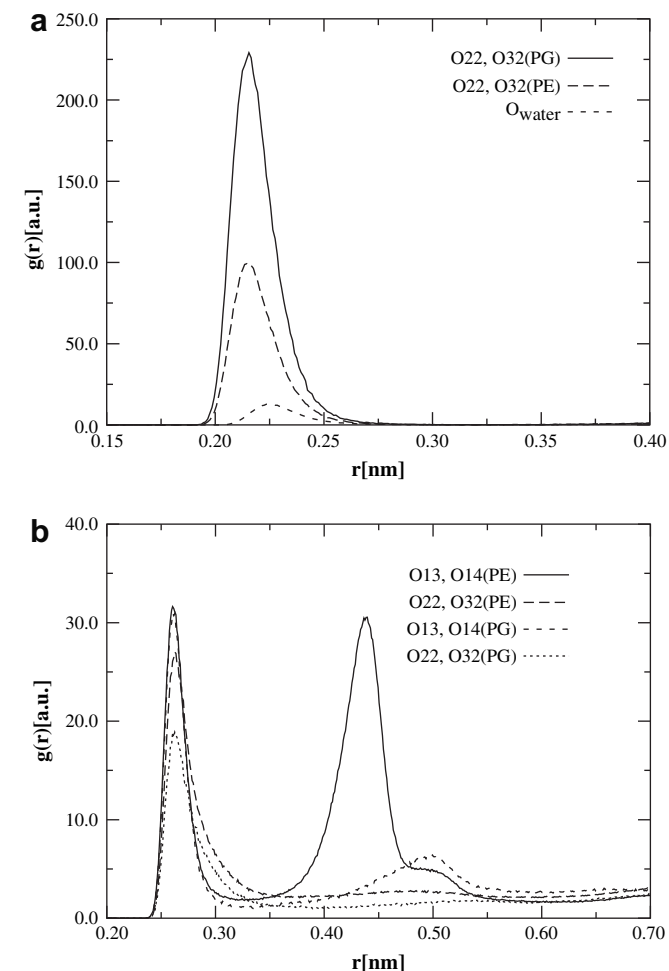


Fig. 5. Radial distribution functions (RDF) of phosphodiester and carbonyl oxygen atoms of POPE and POPG with Na⁺ ions (a); and with POPE nitrogens (bottom) in the POPE–POPG system (b). See Fig. 1 for numbering of atoms.

POPG binds 0.80. In addition, the two carbonyl oxygen atoms of both POPE and POPG show different occurrence around Na⁺ ions. The coordination numbers of O22 and O32 of POPE around a Na⁺ ion were found to be 0.70 and 0.39, respectively. For POPG, the relative coordination numbers are 0.58 and 0.22.

Ions can link two lipids together by forming ‘bridges’. We define an ion bridge to exist when a carbonyl oxygen atom (O22, O32) is within the first hydration shell (0.33 nm [20]) of a Na⁺ ion. We chose this definition, since bonding between Na⁺ ions and carbonyl oxygen atoms is more stable and frequent in comparison to interactions between the Na⁺ ions and other lipid oxygen atoms. It is also possible to consider all lipid oxygen atoms as binding sites available for ion bridges [36]. We found 60 ± 6 ion–lipid pairs, which lead to the formation of 13 ± 1 ion–lipid clusters. An ion–lipid cluster is defined to be a connected network of lipids, which are bound together by ion bridges. Distribution of the cluster sizes is shown in Fig. 6a. A snapshot of a cluster (viewed from the water side) is shown in Fig. 2b. As compared with POPEs, there are more POPGs which bind to two Na⁺ ions. Majority of Na⁺ ions were found to interact with three or four lipids. To illustrate this, the fractions of ions bonded to

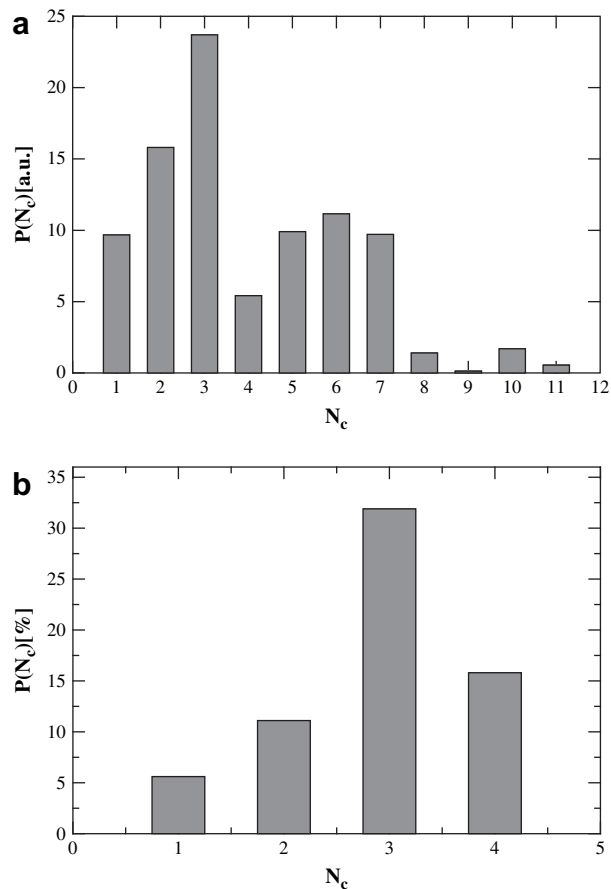


Fig. 6. (a) Distributions of cluster sizes containing at least one Na⁺ ion in the POPE–POPG system. (b) Fractions of Na⁺ ions bonding to different numbers of lipids.

different number of ions are shown in Fig. 6b. A snapshot of four lipids bonded to one Na^+ is shown in Fig. 2c.

Concluding, the introduction of PGs and related counterions to a PE bilayer gives rise to strong bonding and formation of stable ion–lipid clusters, this will be discussed in detail below.

3.4. Lipid–lipid and lipid–water hydrogen bonding

To determine whether a H-bond exists, we used the common definition: the distance r between the hydrogen and an acceptor has to be $r \leq 0.25$ nm, and the donor–hydrogen acceptor angle must be $\alpha \leq 30$ [58]. As for lipid–lipid H-bonds, a POPE participates, on average, in 1.27 H-bonds in the mixed bilayer (0.79 with other PEs and 0.48 with PGs; H-bonds with PGs include 0.40 bonds where the H donor is the PE ammonium group, and 0.08 where the H donor is the PG O16–H16 group) and only in 0.95 H-bonds in the pure POPE bilayer (errors are less than 0.02). A POPG, in turn, participates in 1.55 H-bonds (1.44 with PEs and 0.11 with PGs; H-bonds with PEs include 1.20 bonds where the H donor is a PE and 0.24 where the H donor is PG), and only in 0.36 H-bonds in a pure POPG bilayer [20]. Detailed numbers of H-bonds between the different groups of POPE and POPG molecules are given in Table 1.

Lipids also H-bond with water molecules. The POPE carbonyl oxygen atoms (O22 and O32) H-bond to 1.35 water

Table 1
Average number of intermolecular and intramolecular hydrogen bonds in POPE–POPG and POPE bilayers

H donor	H acceptor	Intermolecular	Intramolecular
<i>POPE–POPG bilayer</i>			
N (PE)	O12 (PE)	–	1.00 ± 0.00
N (PE)	O13, O14 (PE)	0.49 ± 0.04	–
N (PE)	O22 (PE)	0.24 ± 0.03	0.13 ± 0.03
N (PE)	O32 (PE)	0.06 ± 0.02	0.03 ± 0.01
N (PE)	O13, O14 (PG)	0.16 ± 0.03	–
N (PE)	O15 (PE)	0.10 ± 0.02	–
N (PE)	O16 (PE)	0.03 ± 0.02	–
N (PE)	O22 (PE)	0.08 ± 0.01	–
N (PE)	O32 (PE)	0.03 ± 0.01	–
O15 (PG)	O12 (PG)	–	1.00 ± 0.00
O16 (PG)	O11 (PG)	–	0.02 ± 0.02
O16 (PG)	O12 (PG)	–	–
O16 (PG)	O13, O14 (PG)	0.01 ± 0.01	–
O16 (PG)	O15 (PG)	0.01 ± 0.01	0.04 ± 0.03
O16 (PG)	O16 (PG)	0.01 ± 0.01	–
O16 (PG)	O21 (PG)	–	0.03 ± 0.01
O16 (PG)	O22 (PG)	0.06 ± 0.03	0.01 ± 0.01
O16 (PG)	O31 (PG)	–	–
O16 (PG)	O32 (PG)	0.02 ± 0.02	0.01 ± 0.01
O16 (PG)	O13, O14 (PE)	0.03 ± 0.03	–
O16 (PG)	O22 (PE)	0.16 ± 0.05	–
O16 (PG)	O32 (PE)	0.05 ± 0.04	–
<i>POPE bilayer</i>			
N (PE)	O13, O14 (PE)	0.63 ± 0.05	–
N (PE)	O22 (PE)	0.26 ± 0.03	0.18 ± 0.03
N (PE)	O32 (PE)	0.07 ± 0.02	0.01 ± 0.01

The errors are standard error estimates.

molecules in the mixed bilayer, and to 1.78 in the pure POPE bilayer. Thus, the observed dehydration is likely due to bonding. A POPG binds to 0.97 water molecules in the carbonyl region in the mixed bilayer and to 0.63 in a pure POPG bilayer [20]. Other lipid oxygen atoms were found to bind to a similar number of water molecules both in mixed and pure bilayers.

Concluding, the introduction of PGs and related counterions to a PE bilayer leads to an increase in intermolecular H-bonding (34% more H-bonds per PE) and to a decrease in hydration in the carbonyl region of the bilayer. This may also have other consequences as the area per lipid of a mixed PC–POPG bilayer has been shown to depend on hydration (together with the mixing ratio) [59].

3.5. Water ordering and electrostatic potential

Ordering of water molecules in the vicinity of the water/membrane interface can be characterized by the time averaged projection of the water dipole unit vector onto the interfacial normal [20]. The results are shown in Fig. 7a, which shows

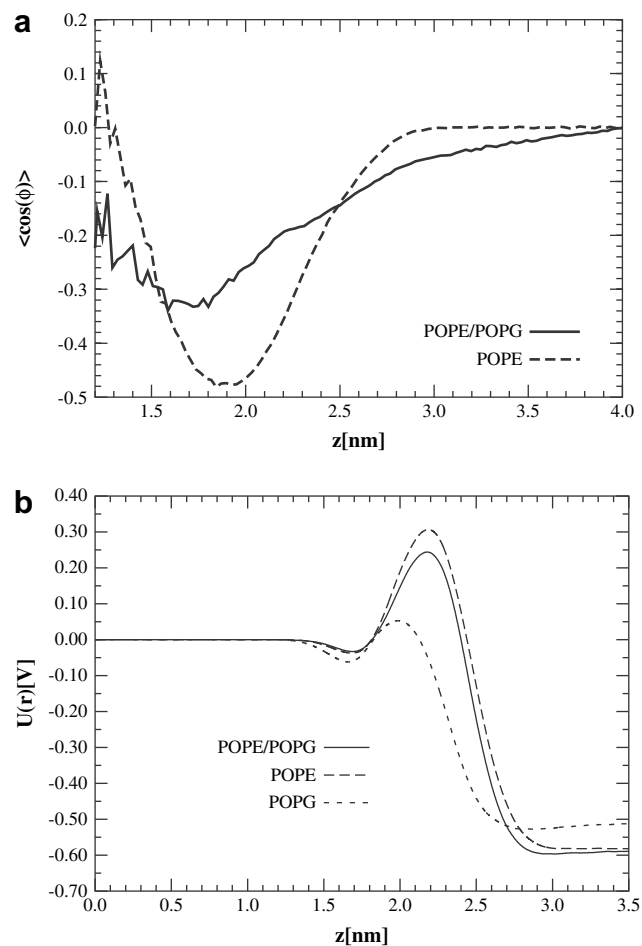


Fig. 7. Profiles across the bilayer of (a) water orientation represented by the average cosine of the angle between the water dipoles and the bilayer normal; (b) electrostatic potential for pure POPE, pure POPG, and POPE–POPG bilayers.

that the water molecules are less ordered in the carbonyl oxygen region of the mixed POPE–POPG than in the pure POPE bilayer.

To a large extent, the electrostatic potential determines the permeability of ionic solutes through the lipid bilayer. Hence, to calculate the electrostatic potential across the bilayer, the average charge density profile was first computed, the bilayer center being assigned to $z=0$ for each simulation frame. Then, the electrostatic potential was determined by integrating the charge density twice with the initial condition $V(z=0)=0$ [50,51]. Fig. 7b shows the electrostatic potential profiles of pure POPE, pure POPG, and POPE/POPG systems. The potential differences between the interior of bilayer and the water phase are, respectively, -0.58 , -0.51 , and -0.59 mV.

3.6. Protrusions characterized by POPE glycerol backbone

Protrusions, i.e., extensions of a lipid or lipids outward to the water phase are an important characteristic of membranes as they tell about the hydrophobic thickness and its fluctuations, but they are relatively difficult to characterize experimentally as well as in a simulation. Protrusions may be due to single lipid or they may be collective [60,61]. Here, we focus on the former. Here, we describe protrusions by measuring the partial distributions of the nitrogen atoms in the z -direction and, as a second characteristic, we study the positional autocorrelation functions in the z -direction using the local neighborhood of the lipid as a reference. These measures characterize the amount and lifetime of fluctuations in the z -direction. Ideally, free energy [60] should be used but it was not possible here. The distribution of the nitrogen atoms in the two systems is shown in Fig. 8. It has a slightly narrower distribution in the mixed bilayer as compared with the pure POPE bilayer. This suggests that the protrusions in the mixed bilayer are suppressed as compared with the pure bilayer. To examine that better, we calculated the autocorrelation functions for the positions of the glycerol groups along the bilayer normal with

respect to their neighborhood, see Fig. 9. The neighborhood was defined as molecules with their centers of mass at a distance <1 nm. The z -position was then calculated with respect to the average z -position of the neighborhood. Fig. 9 indicates a slower motion in the direction of the bilayer normal in the mixed bilayer.

4. Discussion

We have studied the properties of POPE–POPG bilayers to elucidate the role of charged PG lipids on the stability of bacterial membranes. Key results of this study are shown in Figs. 8 and 9. The distribution of nitrogens is sharper in the mixed bilayer than in the pure POPE system (Fig. 8); that indicates a more compact bilayer interfacial region and less lipid protrusions into the water phase. Decrease in protrusions is associated with slower dynamics in the interface as shown in Figs. 4 and 9. Both phenomena contribute to increased membrane stability by preventing lipid desorption from the membrane, for example, under the influence of an organic solvent, thus preventing the membrane from disintegrating. A more compact and less dynamic structure of the interface should also decrease membrane permeability. To our knowledge such a mechanism of stabilizing bacterial membranes by PG has not been previously proposed.

Our data suggest that the atomic level mechanism responsible for the decrease of protrusions and the dynamics of head-groups is related to the strong bonding between PEs and PGs. This is realized through H-bonds and ion bridges. As for H-bonds, the PE ammonium group preferentially H-bonds with the PG phosphodiester oxygen atoms. Due to this, the total number of H-bonds created by the ammonium groups is increased by about 30% in comparison with the pure POPE bilayer. Additionally, PEs are H-bonded with POPGs via the PG hydroxyl groups. Similar H-bonding and water mediated bonding (water bridges) between PEs and PGs have been shown in a previous study of a POPE–POPG bilayer [33].

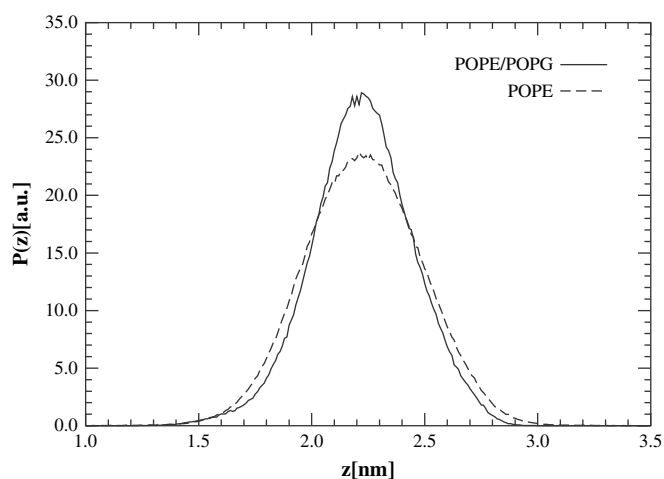


Fig. 8. Partial densities of the nitrogen atoms (bilayer is centered at 0 nm).

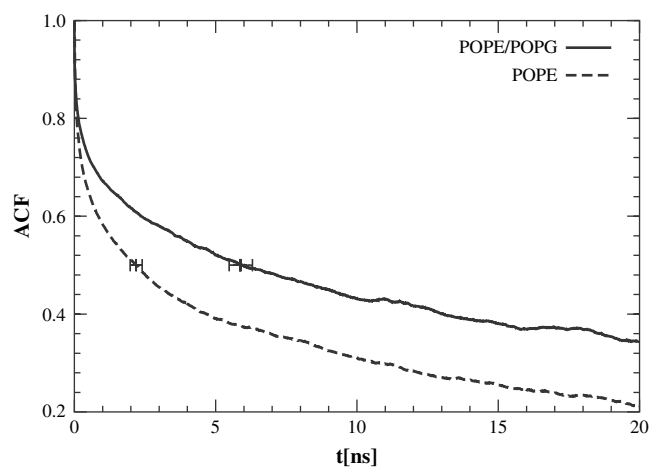


Fig. 9. Autocorrelation functions of the vertical positions of the glycerol group for the pure POPE and mixed POPE–POPG bilayers.

The second mechanism responsible for the decrease of protrusions is due to ion binding and the formation of ion bridges between PEs and PGs. Fig. 2a shows that most of the ions are bonded in the interface. That causes local dehydration as water bridges are replaced by much stronger ion links. The lifetime of ion links is much longer than that of water bridges – many ions were bonded for more than half of the simulation time, some of them even for the whole simulation (70 ns). For comparison, the lifetime of water bridges is of the order of 100–200 ps [62].

Let us finish by discussing some aspects related to the wider importance of our results. In eukaryotic membranes the issue of domains, in particular rafts [63], has been one of the most discussed topics. In bacterial membranes, domains have received much less attention although they do exist [37]. In mammalian cells, cholesterol, saturated PCs and sphingomyelin are the main components in rafts, but in the latter, domains have been mostly associated with PE and cardiolipin, which is negatively charged. PEs have been suggested to be the key component [37] due to their small head group size and H-bonding capabilities. Our results show in detail that PEs bond strongly with PGs, and hence it is very reasonable to expect that to be the case with cardiolipin as well – it seems also plausible to have domains in PE–PG dominated systems, although we are not aware of such experiments. As discussed above, the total number of H-bonds by the PE ammonium groups significantly increases when PG is added in a pure PE bilayer. As also discussed above, ion bridges mediate further PE–PG interactions at the water–membrane interface. Thus, our studies show the detailed microscopic mechanisms, which may be responsible for domain formation in bacterial membranes. Simulating systems so large that one would be able to see the formation and dissociation of such domains is currently beyond any computational capabilities.

Finally, lipid charge, in particular that of PGs, together with variation of the hydrophobic chain length, has been shown [38] to be an important factor in the disruption of lipid membranes when exposed to certain antimicrobial peptides. One possibility, although cannot be verified here, is that the penetration of the peptide breaks the H-bond and ion bonding network and that may trigger destabilization of the membrane, e.g., due to like-charge interactions. Whether or not that is the case will be left as a topic for further studies.

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References

- [1] T.M. Devlin, Textbook of Biochemistry with Clinical Corrections, fifth ed. Wiley-Liss, New York, 2002.
- [2] R. Birner, M. Bürgermeister, R. Schneider, G. Daum, Roles of phosphatidylethanolamine and of its several biosynthetic pathways in *Saccharomyces cerevisiae*, Mol. Biol. Cell 12 (2001) 997–1007.
- [3] K.A. Dill, D. Stigter, Lateral interactions among phosphatidylcholine and phosphatidylethanolamine head groups in phospholipid monolayers and bilayers, Biochemistry 27 (1988) 3446–3453.
- [4] H. Nikaido, Molecular basis of bacterial outer membrane permeability revisited, Microbiol. Mol. Biol. Rev. 67 (2003) 593–656.
- [5] E.E. Williams, J.A. Cooper, W. Stillwell, L.J. Jenki, The curvature and cholesterol content of phospholipids bilayers alter the transbilayer distribution of specific molecular species of phosphatidylethanolamine, Mol. Membr. Biol. 17 (2000) 157–164.
- [6] K. Iwamoto, S. Kobayashi, R. Fukuda, M. Umeda, T. Kobayashi, A. Ohta, Local exposure of phosphatidylethanolamine on the yeast plasma membrane is implicated in cell polarity, Gen. Cells 9 (2004) 891–903.
- [7] K. Emoto, M. Umeda, An essential role for a membrane lipid in cytokinesis: regulation of contractile ring disassembly by redistribution of phosphatidylethanolamine, J. Cell Biol. 149 (2000) 1215–1224.
- [8] J.F. Mead, R.B. Alfin-Slater, D.R. Howton, G. Popják, Lipids – Chemistry, Biochemistry and Nutrition, Plenum Press, New York, 1986.
- [9] W. Dowhan, Molecular basis for membrane phospholipids diversity: why are there so many lipids? Ann. Rev. Biochem. 66 (1996) 199–232.
- [10] S. Uran, A. Larsen, P.B. Jacobsen, T. Skotland, Analysis of phospholipid species in human blood using normalphase liquid chromatography coupled with electrospray ionization ion-trap tandem mass spectrometry, J. Chrom. B 758 (2001) 265–275.
- [11] J.M. Boggs, Lipid intermolecular hydrogen bonding: influence on structural organization and membrane function, Biochim. Biophys. Acta 906 (1987) 353–404.
- [12] W. Hubner, A. Blume, Interactions at the lipid–water interface, Chem. Phys. Lipids 96 (1998) 99–123.
- [13] D.A. Pink, S. McNeil, B. Quinn, M.J. Zuckermann, A model of hydrogen bond formation in phosphatidylethanolamine bilayers, Biochim. Biophys. Acta 1368 (1998) 289–305.
- [14] K.V. Damodaran, K.M. Merz, A comparison of DMPC- and DLPE-based lipid bilayers, Biophys. J. 66 (1994) 1076–1087.
- [15] S. Leekumjorn, A.K. Sum, Molecular simulation study of structural and dynamic properties of mixed DPPC/DPPE bilayers, Biophys. J. 90 (2006) 3951–3965.
- [16] S. Leekumjorn, A.K. Sum, Molecular investigation of the interactions of trehalose with lipid bilayers of DPPC, DPPE and their mixture, Mol. Sim. 32 (2006) 219–230.
- [17] A. Dicko, H. Bourque, M. Pezolet, Study by infrared spectroscopy of the conformation of dipalmitoylphosphatidylglycerol monolayers at the air–water interface and transferred on solid substrates, Chem. Phys. Lipids 96 (1998) 125–139.
- [18] Y.P. Zhang, R.N.A.H. Lewis, R.N. McElhaney, Calorimetric and spectroscopic studies of the thermotropic phase behavior of the n-saturated 1,2-diacylphosphatidylglycerols, Biophys. J. 72 (1997) 779–793.
- [19] Y.N. Kaznessis, S.T. Kim, R.G. Larson, Simulations of zwitterionic and anionic phospholipid monolayers, Biophys. J. 82 (2002) 1731–1742.
- [20] W. Zhao, T. Róg, A.A. Gurtovenko, I. Vattulainen, M. Karttunen, Atomic-scale structure and electrostatics of anionic POPG lipid bilayers with Na⁺ counterions, Biophys. J. 92 (2007) 1114–1124.
- [21] A. Tari, L. Huang, Structure and function relationship of phosphatidylglycerol in the stabilization of phosphatidylethanolamine bilayer, Biochemistry 28 (1989) 7708–7712.
- [22] C.H. Huang, S.S. Li, Calorimetric and molecular mechanics studies of the thermotropic phase behavior of membrane phospholipids, Biochim. Biophys. Acta 1422 (1999) 273–307.
- [23] M.T. Lamy-Freund, K.A. Riske, The peculiar thermo-structural behavior of the anionic lipid DMPG, Chem. Phys. Lipids 122 (2003) 19–32.

- [24] S. Isken, J.A.M. de Bont, Bacteria tolerant to organic solvents, *Extremophiles* 2 (1998) 229–238.
- [25] F.J. Weber, J.A.M. de Bont, Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes, *Biochim. Biophys. Acta* 1286 (1996) 225–245.
- [26] B. Pozo-Navas, K. Lohner, G. Deutsch, E. Sevcsik, G. Pabst, Composition dependence of vesicle morphology and mixing properties in a bacterial model membrane system, *Biochim. Biophys. Acta* 1716 (2005) 40–48.
- [27] B. Pozo-Navas, V.A. Raghunathan, J. Katsaras, M. Rappolt, K. Lohner, G. Pabst, Discontinuous unbinding of lipid multibilayers, *Phys. Rev. Lett.* 91 (2003) 028101.
- [28] M.C. Pitman, F. Suits, K. Gawrisch, S.E. Feller, Molecular dynamics investigation of dynamical properties of phosphatidylethanolamine lipid bilayers, *J. Chem. Phys.* 122 (2005) 244715.
- [29] F. Suits, M.C. Pitman, S.E. Feller, Molecular dynamics investigation of the structural properties of phosphatidylethanolamine lipid bilayers, *J. Chem. Phys.* 122 (2005) 244714.
- [30] A. de Vries, A.E. Mark, S.J. Marrink, The binary mixing behavior of phospholipids in a bilayer: a molecular dynamics study, *J. Phys. Chem. B* 108 (2004) 2454–2463.
- [31] D.E. Elmore, Molecular dynamics simulation of a phosphatidylglycerol membrane, *FEBS Lett.* 580 (2006) 144–148.
- [32] M. Pasenkiewicz-Gierula, K. Murzyn, T. Róg, C. Zzaplewski, Molecular dynamics simulation studies of lipid bilayer systems, *Acta Biochim. Pol.* 47 (2000) 601–611.
- [33] K. Murzyn, T. Róg, M. Pasenkiewicz-Gierula, Phosphatidylethanolamine–phosphatidylglycerol bilayer as a model of the inner bacterial membrane, *Biophys. J.* 88 (2005) 1091–1103.
- [34] T. Róg, K. Murzyn, M. Pasenkiewicz-Gierula, Molecular dynamics simulation studies of charged and uncharged lipid bilayers: a treatment of electrostatic interactions, *Acta Biochim. Pol.* 50 (2003) 789–798.
- [35] K. Murzyn, T. Róg, M. Pasenkiewicz-Gierula, Interactions of magainin-2 amide with membrane lipids, *Lect. Notes Comput. Sci.* 3037 (2004) 325–331.
- [36] M.L. Berkowitz, D.L. Bostick, S. Pandit, Aqueous solutions next to phospholipid membrane surfaces: insights from simulations, *Chem. Rev.* 106 (2006) 1527–1539.
- [37] K. Matsumoto, J. Kusaka, A. Nishibori, H. Hara, Lipid domains in bacterial membranes, *Mol. Microbiol.* 61 (2006) 1110–1117.
- [38] E. Sevcsik, G. Pabst, A. Jilek, K. Lohner, How lipids influence the mode of action of membrane-active peptides, *Biochim. Biophys. Acta* 1768 (2007) 2586–2595.
- [39] O. Berger, O. Edholm, F. Jahnic, Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature, *Biophys. J.* 72 (1997) 2002–2013.
- [40] J.P. Ryckaert, A. Bellemans, Molecular dynamics of liquid *n*-butane near its boiling point, *Chem. Phys. Lett.* 30 (1975) 123–125.
- [41] J.P. Ryckaert, A. Bellemans, Molecular dynamics of liquid alkanes, *Faraday Discuss. Chem. Soc.* 66 (1978) 95–106.
- [42] W.L. Jorgensen, J. Tirado-Rives, The OPLS potential functions for proteins: energy minimization for crystals of cyclic peptides and crambin, *J. Am. Chem. Soc.* 110 (1988) 1657–1666.
- [43] D.P. Tieleman, H.J.C. Berendsen, A molecular dynamics study of the pores formed by *Escherichia coli* OmpF porin in a fully hydrated palmitoyl-oleoylphosphatidylcholine bilayer, *Biophys. J.* 74 (1998) 2786–2801.
- [44] H.J.C. Berendsen, J.P.M. Postma, W.F. van Gunsteren, J. Hermans, Interaction models for water in relation to protein hydration, in: B. Pullman (Ed.), *Intermolecular Forces*, Reidel, Dordrecht, 1981, pp. 331–342.
- [45] E. Lindahl, B. Hess, D. van der Spoel, GROMACS 3.0: a package for molecular simulation and trajectory analysis, *J. Mol. Model.* 7 (2001) 306–317.
- [46] U. Essman, L. Perela, M.L. Berkowitz, H.L.T. Darden, L.G. Pedersen, A smooth particle mesh Ewald method, *J. Chem. Phys.* 103 (1995) 8577–8592.
- [47] B. Hess, H. Bekker, H.J.C. Berendsen, J.G.E.M. Fraaije, LINCS: a linear constraint solver for molecular simulations, *J. Comput. Chem.* 18 (1997) 1463–1472.
- [48] S. Miyamoto, P.A. Kollman, SETTLE: an analytical version of the SHAKE and RATTLE algorithms for rigid water models, *J. Comput. Chem.* 13 (1992) 952–962.
- [49] H.J.C. Berendsen, J.P.M. Postma, W.F. van Gunsteren, A. DiNola, J.R. Haak, Molecular dynamics with coupling to an external bath, *J. Chem. Phys.* 81 (1984) 3684–3690.
- [50] M. Patra, E. Salonen, E. Terama, I. Vattulainen, R. Faller, B.W. Lee, J. Holopainen, M. Karttunen, Under the influence of alcohol: the effect of ethanol and methanol on lipid bilayers, *Biophys. J.* 90 (2006) 1121–1135.
- [51] M. Patra, M. Karttunen, M.T. Hyvönen, E. Falck, P. Lindqvist, I. Vattulainen, Molecular dynamics simulations of lipid bilayers: major artifacts due to truncating electrostatic interactions, *Biophys. J.* 84 (2003) 3636–3645.
- [52] E. Falck, M. Patra, M. Karttunen, M.T. Hyvönen, I. Vattulainen, Lessons of slicing membranes: Interplay of packing, free area, and lateral diffusion in phospholipid/cholesterol bilayers, *Biophys. J.* 87 (2004) 1076–1091.
- [53] M. Patra, M. Karttunen, M.T. Hyvönen, E. Falck, I. Vattulainen, Lipid bilayers driven to a wrong lane in molecular dynamics simulations by truncation of long-range electrostatic interactions, *J. Phys. Chem. B* 108 (2004) 4485–4494.
- [54] S. Vainio, M. Jansen, M. Koivusalo, T. Róg, M. Karttunen, I. Vattulainen, E. Ikonen, Desmosterol cannot replace cholesterol in lipid rafts, *J. Biol. Chem.* 281 (2006) 1121–1135.
- [55] R.P. Rand, N. Fuller, V.A. Parsegian, D.C. Rau, Variation in hydration forces between neutral phospholipid bilayers: evidence for hydration attraction, *Biochemistry* 27 (1988) 7711–7722.
- [56] H.J. Mukhopadhyay, P.D. Vogel, P.D. Tieleman, Distribution of pentachlorophenol in phospholipid bilayers: a molecular dynamics study, *Biophys. J.* 86 (2004) 337–345.
- [57] J.H. Davis, The description of membrane lipid conformation, order and dynamics by ²H-NMR, *Biochim. Biophys. Acta* 737 (1983) 117–171.
- [58] K. Murzyn, W. Zhao, M. Karttunen, M. Kurdziek, T. Róg, The dynamics of water at the membrane surface – effect of the headgroup structure, *Biointerphases* 1 (2006) 98–105.
- [59] T. Wiedmann, A. Salmon, V. Wong, Phase behavior of mixtures of DPPC and POPG, *Biochim. Biophys. Acta* 1167 (1993) 14–20.
- [60] R. Lipowsky, S. Grotehans, Hydration vs. protrusion forces between lipid bilayers, *Europhys. Lett.* 23 (1993) 599–604.
- [61] J.P. Douliez, A. Leonard, E.J. Dufourc, Conformational order of DMPC sn-1 versus sn-2 chains and membrane thickness: an approach to molecular protrusion by solid state ²H NMR and neutron diffraction, *J. Phys. Chem. B* 100 (1996) 18450–18457.
- [62] M. Pasenkiewicz-Gierula, T. Róg, K. Kitamura, A. Kusumi, Cholesterol effects on the phosphatidylcholine bilayer polar region: a molecular simulation study, *Biophys. J.* 78 (2000) 1376–1389.
- [63] K. Simons, E. Ikonen, Functional rafts in cell membranes, *Nature* 387 (1997) 569–572.