

Dynamics of water at membrane surfaces: Effect of headgroup structure

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Atomistic molecular dynamics simulations of fully hydrated 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE), and 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG) bilayers in the liquid-crystalline state were carried out to investigate the effect of different lipid headgroups on the dynamics of water at the bilayer surface in short 80 ps time scales. Results obtained in these studies show that the hydrogen bonding amine group of POPE and the glycerol group of POPG slow water motion more than the equivalent choline group of POPC. Therefore, it is surprising that the effect of a POPC bilayer surface on water dynamics is similar to that of POPE and POPG bilayers. That result is due to a much higher number of water molecules interacting with the choline group of POPC than hydrogen-bonded molecules interacting with amine or glycerol groups of POPE and POPG. © 2006 American Vacuum Society. [DOI: 10.1116/1.2354573]

I. INTRODUCTION

Water is a key element in determining and controlling a variety of structural and functional properties of biological membranes.¹ For example, the very formation of membrane bilayers depends on water. Water also regulates and mediates membrane-membrane and membrane-protein interactions. Due to this, water-membrane interactions have been a subject of intense research. Milhaud² and Pratt and Pohorille³ provide recent reviews.

It is firmly established that biological macromolecules and assemblies modify the properties of the neighboring water molecules.⁴ Similarly, any hydrophilic surface, such as a membrane or a micelle, affects properties such as rotational, translational, and vibrational motions of water.^{5,6} For instance, the dynamics of water molecules is slowed down in the hydration layer around peptides and proteins,^{7–11} DNA,¹² and sugars.¹³ For proteins, it has been demonstrated that the dynamics of hydration water is sensitive to the secondary structure of the protein—an observation having potential biological implications.⁸ Studies of phosphatidylcholine (PC) bilayers at low and full hydration suggest that translational

motion of water near the membrane surface is restricted.^{14,15} The self-diffusion coefficient of water in the first hydration layer has been reported to be as much as five times smaller than in bulk.¹⁶

The presence of an interface also induces other changes: density of water in the interfacial region is increased^{17,18} and the freezing point of the interbilayer water is depressed^{19–22}. The network of hydrogen bonds in the interfacial region is perturbed, the probability of water molecules hydrogen bonding with neighboring molecules is increased, but due to a decrease in the number of neighbors, the total number of hydrogen bonds actually decreases.²³ In the interfacial region, water is also hydrogen bonded with lipid headgroups; the lifetime of such bonding is five to eight times longer than that of water-water hydrogen bonds.²⁴ Water dipoles also become ordered in the interfacial region up to 1 nm away from the membrane surface.²⁵

Water dynamics at the membrane surface have been a subject of few molecular dynamics (MD) simulation studies. In previous MD simulation studies on water dynamics near 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) membrane surface, it was shown that translational and rotational motions of water in 100 ps time scale near the membrane surface are restricted.²⁶ The effect was the strongest for water molecules that were hydrogen bonded to the phosphate and carbonyl oxygen atoms, as well as those clathrating choline

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groups of POPC. Both the translational and rotational motions of water that was hydrogen bonded to carbonyl oxygen were slower than the motions of those bonded to phosphate oxygen. It was also observed that water clathrating the POPC choline group was less affected than phosphate and carbonyl hydrogen bonded water. Furthermore, translational diffusion of all membrane water was faster along the membrane plane than along the membrane normal.

In another study, Åman *et al.* showed that bonded water in the interfacial region may be described by two regions characterized by different structures and dynamics.²⁷ It was shown that the slow component of the reorientational correlation function of bonded water molecule is due to the exchange between free and bonded water. This component was not observed in long (i.e., nanosecond) time scales. Bhide and Berkowitz performed comparative studies of water dynamics on neutral phosphatidylcholines and negatively charged phosphatidylserines (PS).²⁸ Sega *et al.* analyzed diffusion of intralamellar water in a ganglioside bilayer, i.e., in a confined geometry, and clearly demonstrated the importance of boundary effects.²⁹

In this article, the authors' previous studies were extended to include bilayers composed of two lipids—neutral phosphatidylethanolamine (PE) and anionic phosphatidylglycerol (PG). PE and PG are typical lipids of bacterial membrane, while PC and PS are characteristic for animal cell membranes.^{30,31} Here, we focus on PEs, PGs, and PCs. Our studies show, somewhat unexpectedly, that the three different headgroups (PE, PG, and PC) modify water dynamics in the interfacial region in a similar way, despite the fact that the strongly hydrogen bonding amine group of PE and the glycerol group of PG slow down bonded water much more than the choline group of PC. The weaker effect in the case of PC is compensated by the much higher number of water molecules bonded in the clathrate-like structure around this group (about 11) rather than hydrogen bonded with amine and glycerol groups (one to two molecules).

II. METHOD

A. Simulation systems

MD simulations of three different lipid bilayers, each composed of 128 lipids, were performed. The first bilayer consisted of POPC molecules, the second of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE) molecules, and the third of 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG) molecules. All three bilayers were hydrated with about 3600 water molecules. Since POPG is anionic (carrying a unit charge), 128 sodium (Na^+) counterions were added to preserve charge neutrality in the POPG system. The simulations were performed using the Groningen Machine for Chemical Simulation (GROMACS) software package.³² Figure 1 shows the structure and numbering of atoms and torsion angles in POPC, POPE, and POPG molecules. A water box with 1000 water molecules (no lipids) was used as a reference system.

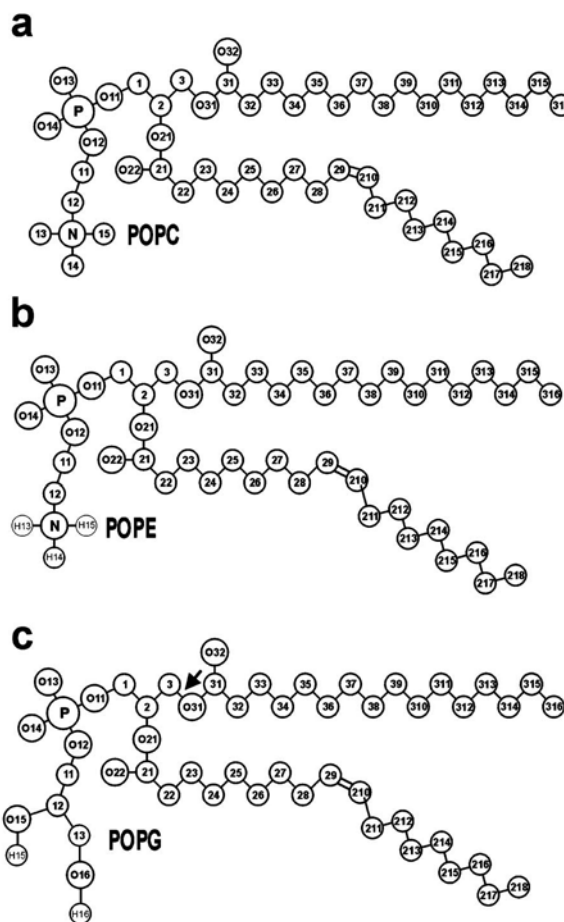


FIG. 1. Molecular structures of POPC (a), POPE (b), and POPG (c) with numbering of atoms.

B. Simulation parameters

The parameters for bonded and nonbonded interactions for POPC and POPE molecules were taken from a study of a pure dipalmitoylphosphatidylcholine (DPPC) bilayer.³³ The partial charges are from the underlying model description.³⁴ Validation of the force field is presented in Ref. 34 (as well as in numerous other articles citing it). For POPG, the same parameter set was used, except for partial charges for the glycerol group which were taken from 1,2-propanediol parameterized with GROMACS forcefield.³⁵ The POPG parameters and equilibrated bilayer structures are available on-line.³⁶ Validation of the POPG model is shown in Ref. 36. For water, the Simple Point Charge (SPC) model was used as it is consistent with the current lipid parameterization.³⁷

C. Simulation conditions

Three-dimensional periodic boundary conditions were applied to the system. The usual minimum image convention was used for short-range non-bonded interactions. The LINear Constraint Solver (LINCS) algorithm was used to preserve the lengths of the bonds between heavy atoms and hydrogen.³⁸ The time step was set to 2 fs. The simulations were carried out at a constant pressure of 1 bar and a con-

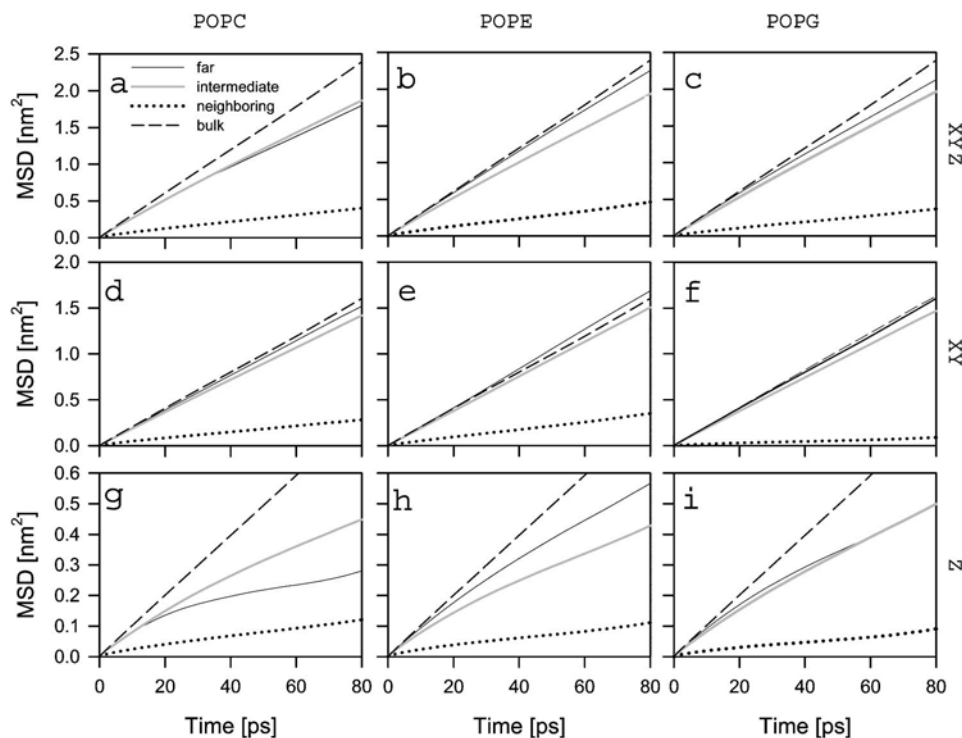


FIG. 2. MSD of the neighboring water (dotted line), intermediate water (gray line), far water (solid line), and bulk water (dashed line) in POPC [(a), (d), (g)], POPE [(b), (e), (h)], and in POPG [(c), (f), (i)]. In the membrane [(a), (b), (c)], in the membrane plane [(d), (e), (f)], and along the bilayer normal [(g), (h), (i)].

stant temperature of 310 K. Both simulations were controlled using the Berendsen method.³⁹ The temperatures of the solute and solvent (water and ions) were coupled independently to the thermostat. The relaxation times for temperatures and pressure were set at 0.1 and 1.0 ps, respectively, and the semi-isotropic barostat was used. Lennard–Jones interactions were cut off at 1.0 nm. For electrostatic interactions, the particle-mesh Ewald method with real space cutoff of 1 nm, β -spline interpolation of order 5, and direct sum tolerance of 10^{-6} was used.⁴⁰ Electrostatic interactions within 1.0 nm were calculated at each time step, while interactions beyond this range were determined every 10 steps.

The systems were equilibrated before the simulation runs. That took 30 ns for POPC, 50 ns for POPE, and 95 ns for POPG. Equilibrium was monitored in the conventional way by monitoring the area per lipid. After equilibration, the simulations were continued for 1 ns while storing data at very short intervals (details later). Those fragments are analyzed in this article to capture the details of interfacial water with high sampling resolution in equilibrium. This follows the standard simulation protocol that has been successfully applied before. The full details are provided in our earlier papers.^{36,41–43}

D. Data analysis

To calculate the mean-square displacement and the reorientational-autocorrelation function, the last 1 ns of each trajectory was sampled at every 50 fs. This 1 ns was further divided into five 200 ps fragments, the results presented be-

low are averaged over these 200 ps fragments. To calculate the velocity-autocorrelation functions and the angular velocity-autocorrelation functions, 100 ps fragments of each trajectory were sampled at every 2 fs. The data analysis was performed with the Molecular Modeling Tool Kit library and the nMoldyn program.^{44,45}

The translational diffusion coefficients were fitted to the linear part of the mean-square displacement curves obtained for each fragment of the trajectory. After fitting, the coefficients were averaged and the standard deviation was calculated to obtain an error estimate.

To study the effect of membrane surface on the dynamics of water, the water molecules were classified into six groups, following the convention used in our previous studies.²⁶ The first group consisted of water molecules that were not further than 4 Å from any membrane atom. We call these water molecules “neighboring water.” The number of water molecules in this group was approximately 1000. During the analyzed trajectories, fragments of all of the neighboring water molecules were located in the interface region of the bilayer. We did not observe any water in the hydrocarbon core of the membrane. The second group consisted of water molecules within a layer between 4 and 12 Å from any membrane atom (called “intermediate water”) and consisted typically of 1000–1800 water molecules. The third group was made up of water molecules that were not closer than 7 Å from any membrane atom (“far water”). That group had about 500 water molecules in the POPC bilayer and about 1000 in the other bilayers.

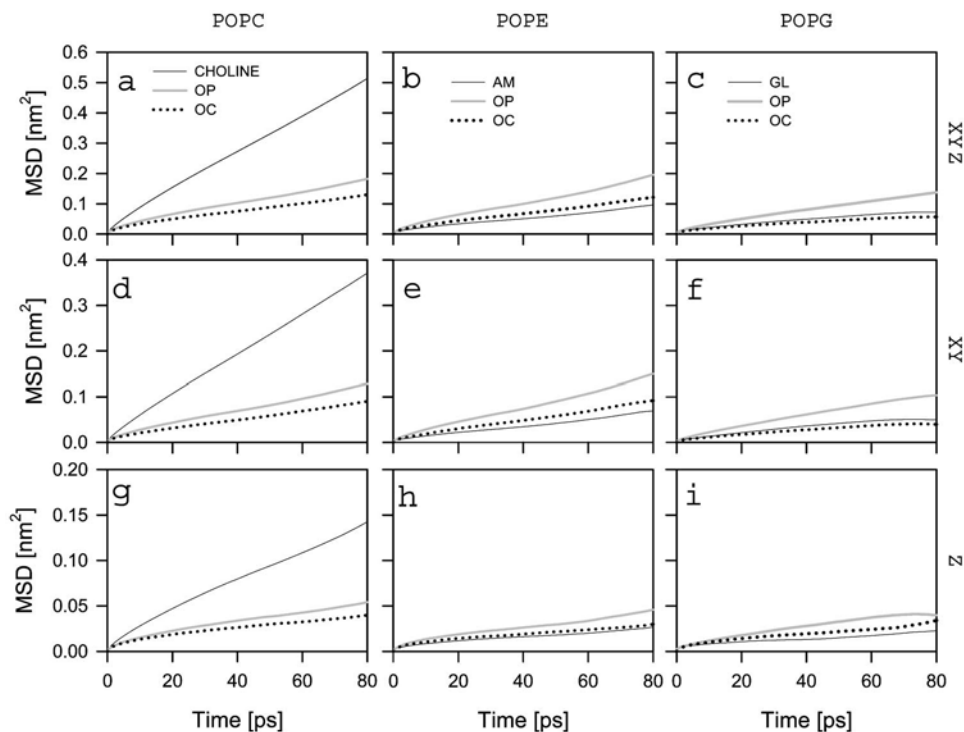


FIG. 3. MSD of the OC water (dotted line), OP water (gray line), choline/AM/GL water (solid line), in POPC [(a), (d), (g)], POPE [(b), (e), (h)], and POPG [(c), (f), (i)]. In the membrane [(a), (b), (c)], in the membrane plane [(d), (e), (f)], and along the bilayer normal [(g), (h), (i)].

Using the earlier criteria, it is possible that a water molecule belonged to both intermediate and far water. The overlap was about 30%. It is also worth noticing that the thickness of the water shell is lower in the POPC bilayer due to the much larger area per lipid. This results in a smaller size of the far water group and a larger overlap between this group and the intermediate water. The next three groups consisted, respectively, of water molecules that were hydrogen bonded to phosphate oxygens (“OP water”), carbonyl oxygens (“OC water”), the amine group of POPE (“AM water”), the glycerol group of POPG (“GL water”), and those clathrating choline groups of POPC (“choline water”). A hydrogen bond between a hydrogen donor ($D-H$) and a hydrogen acceptor (A) is judged to be formed when the $D\cdots A$ distance (r) is ≤ 3.25 Å and the angle θ between the $D\cdots A$ vector and the $D-H$ bond (the $A\cdots D-H$ angle) is $\leq 35^\circ$. The distance 3.25 Å is the position of the first minimum in the radial distribution function (RDF) of the water oxygen atoms (OWs) relative to an oxygen atom of a PC.⁴⁶ A water molecule clathrating a choline group is defined when a water molecule’s oxygen atom is within 4.75 Å from a $N-CH_3$ group. The distance 4.75 Å is the position of the first minimum of the RDF of the OWs relative to a $N-CH_3$ group.⁴⁶ Other groups consisted of a smaller number of molecules: AM, GL, and OC water of 50–100 molecules; OP of 150 molecules; and choline of 800 water molecules. Overlap between these groups is possible due to the possibility of simultaneous hydrogen bonding of a water molecule to two or more oxygens and since hydrogen bonding does not exclude participation in clathrate around a choline group.

A water molecule was determined to belong to one of the above groups if it fulfilled the given criteria for at least 70% of the analysed time (200 ps; selection was performed independently for each trajectory fragment). The criterion of 70% was used because of the dynamics of hydrogen bonding; during a hydrogen bond’s lifetime, short bonding and non-bonding intervals are observed. Thus, using stronger criterion would lead to an unnaturally small group not reflecting actual bonding.⁴⁶

III. RESULTS

A. Translational motion

Figures 2 and 3 show the mean-square displacement (MSD) curves of water molecules belonging to selected groups. The diffusion coefficients were obtained by fitting to the linear part of MSD curves between 20 and 80 ps (Table I).

In Fig. 2, MSD the curves of far, intermediate and neighboring water are shown in three dimensions [Figs. 2(a)–2(c)], in the membrane plane [Fig. 2(d)–2(f)], and along the bilayer normal [Figs. 2(g)–2(i)] for the different bilayers. As observed in experiments,¹⁴ as well as in previous MD simulation studies,^{26–28} translational diffusion of neighboring water is significantly slower than that of far water. This is observed both along the membrane plane and along the bilayer normal. Similar effects are observed for all three bilayers. However, the reduction in translational diffusion seems to be greater in the case of the PE and PG than in the case of the PC bilayer. The influence of membrane surface is also

TABLE I. Self-diffusion coefficients in three dimensions (D_{\parallel}), in the membrane plane (D_{\perp}), and along the membrane normal (D_{\parallel}) obtained from fitting to the MSD curves of water molecules belonging to the selected groups. Units: ($\text{cm}^2/\text{s} \cdot 10^{-8}$).

SYSTEM	Group	D_{\parallel}	D_{\perp}	D_{\parallel}
Water box		0.50 ± 0.02	0.50 ± 0.02	0.50 ± 0.02
POPC	Far	0.36 ± 0.02	0.47 ± 0.03	0.12 ± 0.02
	Intermediate	0.36 ± 0.02	0.44 ± 0.02	0.24 ± 0.01
	Neighboring	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
	Choline	0.10 ± 0.01	0.11 ± 0.01	0.08 ± 0.01
	OP	0.035 ± 0.005	0.035 ± 0.005	0.030 ± 0.005
	OC	0.02 ± 0.005	0.025 ± 0.005	0.017 ± 0.005
POPE	Far	0.47 ± 0.02	0.53 ± 0.02	0.33 ± 0.03
	Intermediate	0.40 ± 0.02	0.47 ± 0.02	0.24 ± 0.02
	Neighboring	0.09 ± 0.01	0.11 ± 0.02	0.06 ± 0.01
	AM	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
	OP	0.04 ± 0.01	0.05 ± 0.01	0.02 ± 0.01
	OC	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
POPG	Far	0.44 ± 0.02	0.51 ± 0.02	0.27 ± 0.02
	Intermediate	0.40 ± 0.03	0.46 ± 0.02	0.28 ± 0.02
	Neighboring	0.08 ± 0.01	0.08 ± 0.02	0.06 ± 0.01
	GL	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
	OP	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
	OC	0.008 ± 0.002	0.009 ± 0.003	0.006 ± 0.002

^aCalculated along two dimensions (X, Y).

^bCalculated along one dimension (Z).

apparent for intermediate water for POPC and POPE bilayers—the effects in these bilayers are the opposite. In the case of the POPC bilayer, the diffusion of intermediate water along the bilayer normal is faster than of far water, while in the POPE bilayer it is slower. The unexpected behavior of far water in the case of the POPC bilayer may be a result of a slightly too small thickness of the water layer. One should keep in mind that the area of the POPC membrane is larger than that of the POPE and POPG bilayers. In the POPG bilayer, we do not observe differences in the rate of translational diffusion between intermediate and far water. The translational diffusion of far water is slightly slower than that of bulk water. That is due to the reduction of translational diffusion along the membrane normal. In contrast, diffusion in the membrane plane is equal to the diffusion of bulk water within error bars.

In Fig. 3, the MSD curves of OC, OP, CHOLINE, AM, and GL water are shown in three dimensions [Figs. 3(a)–3(c)], in the membrane plane [Figs. 3(d)–3(f)], and along the bilayer normal [Figs. 3(g)–3(i)]. As can be seen, the choline group has the weakest effect on water translational diffusion. In all bilayers, OC water is more affected than OP water. Amine and glycerol groups have modified water translational diffusion to the same degree as carbonyl groups.

Table I summarizes the results for translational diffusion. Anisotropy in translational diffusion is apparent: it is slower along the membrane normal than in the membrane plane. Diffusion in bulk water is isotropic, as expected. Our results

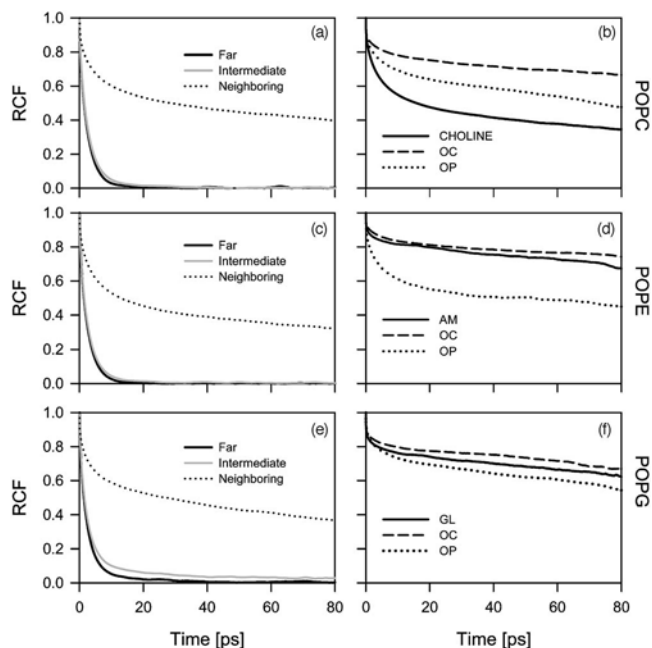


FIG. 4. RCF of the neighboring water (dotted line), intermediate water (gray line), and far water (black line) [(a), (c), (e)]; choline/AM/GL water (solid line), OP water (dotted line), and OC water (dashed line) [(b), (d), (f)]. In POPC [(a), (b)], POPE [(c), (d)], and POPG [(e), (f)] bilayers.

support the analysis of Segal *et al.* and Liu *et al.* but we could not determine whether the in-plane and out-of-plane dynamics are completely decoupled.^{29,47}

B. Rotational motion

In Figs. 4 and 5, reorientational correlation function (RCF) curves of water molecules belonging to the selected groups in POPC, POPE, and POPG bilayers are shown. RCF was calculated according to the algorithm given in Ref. 45 for a coefficient set of 1, 0, 0. This set of coefficients represents rotational motions measured by optical spectroscopy.⁴⁸ The RCF curves could not be satisfactorily fitted to a single or a sum of two exponentials and, thus, the results are only qualitative.

In Fig. 4, comparisons between selected water groups in the POPC [Figs. 4(a) and 4(b)], POPE [Figs. 4(c) and 4(d)], and POPG [Figs. 4(e) and 4(f)] bilayers are shown. In all three bilayers, we observed that rotation is strongly affected by the vicinity of the membrane surface. A small effect is also observed for intermediated water in the case of the POPG bilayer. This result is in agreement with the observation that in POPG bilayers, water dipoles remain ordered for a long distance away from the interface.³⁶ The choline group had the weakest effect on water rotation, while the carbonyl groups had the strongest. The amine and glycerol groups had a stronger effect than the phosphate groups.

The RCF curves of water molecules belonging to selected groups are compared in Fig. 5. We do not observe differences between near and intermediate water for the POPC and POPE bilayers, while for the POPG bilayer rotation is

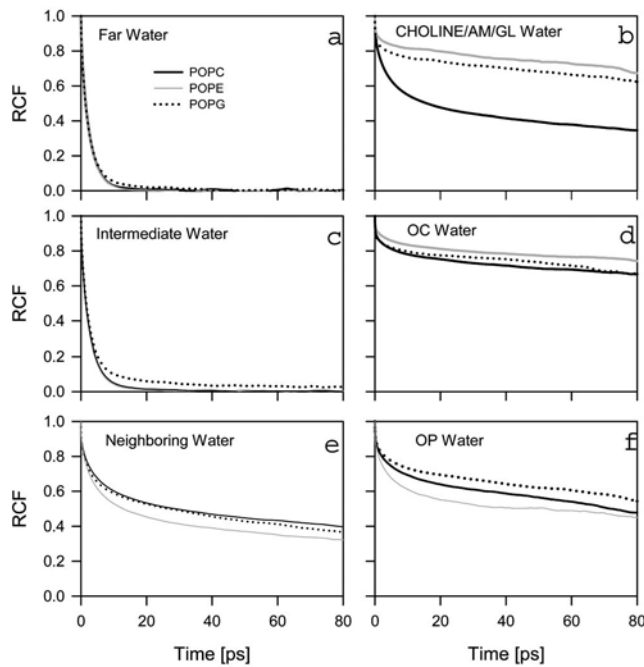


FIG. 5. RCF of the far water (a); choline/AM/GL water (b); intermediate water (c); OC water (d); neighboring water (e); and OP water (f) in the POPC (solid line), POPE (gray line), and POPG (dotted line) bilayers.

slightly affected. For neighboring water, rotation is similar in the POPC and POPG bilayers, while in the POPE bilayer it is less affected.

C. Velocity-autocorrelation functions

Figure 6 shows the velocity-autocorrelation function (VACF) of water molecules belonging to the selected groups in POPC, POPE, and POPG bilayers. In Fig. 6(a), the VACFs for neighboring, intermediate, and far water in the POPC bilayer are shown. As can be seen, intermediate and far water are indistinguishable from each other. They are also indistinguishable from bulk water (data not shown). Similar results were obtained for the POPE and POPG bilayers. Thus, in further analysis, we concentrate only on neighboring water.

D. Angular velocity-autocorrelation functions

Figure 7 shows the angular velocity-autocorrelation function (AVACF) of water molecules belonging to the selected groups in POPC, POPE, and POPG bilayers. In Fig. 7(a), each AVACF for neighboring, intermediate, and far water in the POPC bilayer is shown. As can be seen, intermediate and far water are almost indistinguishable from each other, and also from bulk water (data not shown). Similar results were obtained for POPE and POPG bilayer. Thus in further analysis, we concentrate only on neighboring water. Comparison of the AVACF of neighboring water in POPC bilayer in three dimensions, along the bilayer normal, and in the bilayer plane is shown in Fig. 7(b). The correlations in angular motions persist longer along the bilayer normal than in the bilayer plane. Similar results were obtained for POPE and POPG bilayers. Comparisons of water bound to the various

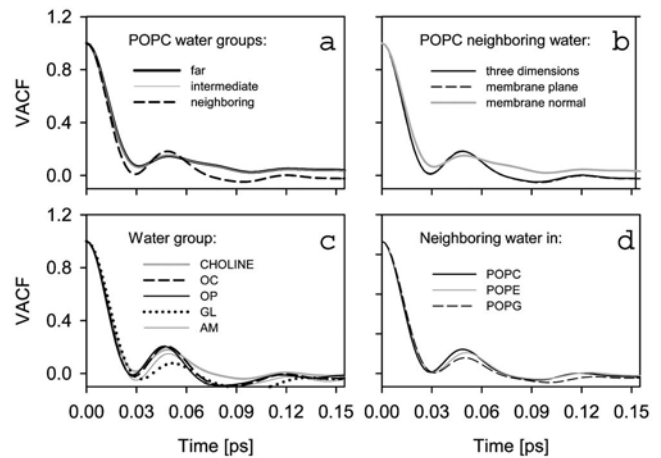


FIG. 6. VACF of neighboring (dashed line), intermediate (gray line), and far (solid line) water in the POPC bilayer (a); neighboring water in three dimensions (solid line), in the membrane plane (dashed line) and along the bilayer normal (gray line) in the POPC bilayer (b); OC (dashed line), OP (thick line), and CHOLINE (gray line) water in the POPC bilayer; AM (thin line) and GL water in the POPG bilayer (dotted line) (c); neighboring water in the POPC (solid line), POPE (gray line) and POPG (dashed line) bilayers (d).

polar groups of lipids show similar behavior of AVACF [Fig. 7(c)]. The results are very similar in all three bilayer systems, as are the results for near water in different bilayers [Fig. 7(d)].

IV. DISCUSSION

In this article, we have analyzed interface effects of three different lipid bilayers—POPC, POPE, and POPG—on water dynamics. Using a simple criterion, we selected three groups of water, neighboring water, intermediate water, and far wa-

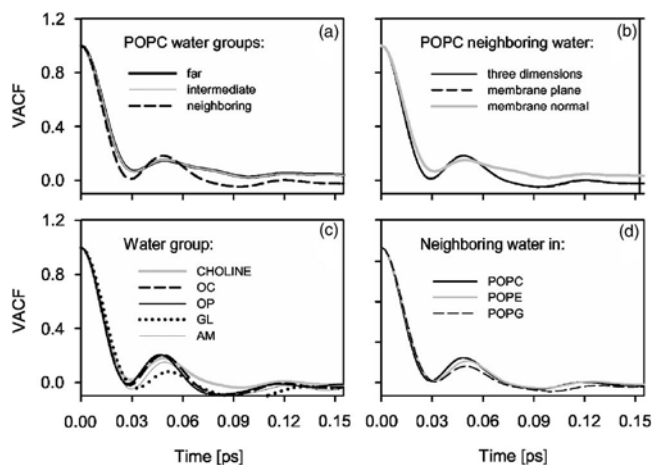


FIG. 7. AVACF of neighboring (dashed line), intermediate (gray line), and far (solid line) water in the POPC bilayer (a); neighboring water in three dimensions (solid line), in the membrane plane (dotted line), and along the bilayer normal (gray line) in the POPC bilayer (b); OC (dashed line), OP (thick line), CHOLINE (gray line) water in the POPC bilayer; AM (thin line) and GL water in the POPG bilayer (dotted line) (c); neighboring water in the POPC (solid line), POPE (gray line), and POPG (dashed line) bilayers (d).

ter (see Sec. II). In agreement with our previous studies, we observed a strong effect on neighboring water dynamics in all three bilayers. Interestingly, we did not observe any pronounced effects due to the head group structure on the translational diffusion of neighboring water (Table I) and only a small effect on the rotational motion (in the POPE bilayer, neighboring water rotation is less restricted than in POPC and POPG bilayers).

The earlier results are surprising when we compare how the lipid groups interact with water molecules. The choline group of POPC affects water rotation less effectively than the equivalent amine group in POPE and the glycerol group in POPG molecules. Thus we should expect stronger effects of POPE and POPG headgroups on water dynamics, compared to POPC. The reason for these ambiguous results is the number of water molecules involved in interactions with different group. The choline group binds about 11 water molecules in the clathrate, while the amine and glycerol groups bind only one to two water molecules. As a result, almost all neighboring water molecules in the POPC bilayer interact with the lipid headgroups (30% are hydrogen bonded and 80% belong to the clathrate). In contrast, in the case of the POPE and POPG bilayers, most of the neighboring water molecules do not interact directly with the lipid headgroups (35%–40% are hydrogen bonded). In the case of the POPG bilayer, a presence of counterions strongly adsorbed at the bilayer interface³⁶ slows down water dynamics in the interfacial region, compared to the POPE bilayers.

Bhide and Berkowitz used an alternative definition for water molecules on the basis of the water-density profile corrected for the membrane roughness.²⁸ Bhide and Berkowitz selected three regions of water: region 1, water in carbonyl groups region; region 2, water in phosphatidylcholine or phosphatidylserine group region; and region 3, far water. They observed that the translational motions in region 1 were slowed down by a factor of 100 and in region 2 by a factor of 6 in the case of a DPPC bilayer. Results obtained for region 2 correspond to choline water (slowed down by a factor of 3.6) and OP water (slowed down by a factor of 10) in our case. These groups are equivalent to region 2 of Bhide and Berkowitz. The slowing down observed for region 1 was greater than for our OC group (slowed down by a factor of 25), likely because our definition potentially includes water molecules from region 2 of and does not include water molecules not hydrogen bonded with PC but buried in the membrane core. These water molecules can obviously affect the results significantly. Another alternative approach to the selection of water groups was presented by Åman and co-workers.²⁷ They based their selection on the profile of the water order parameter along the bilayer normal. Despite different selection procedures, both studies show qualitatively similar effects on the translational motion of water.

In our previous study, we performed an analysis of water dynamics in POPC bilayers using the same water-group definition and simulation conditions, but different force fields for water (TIP3P water model⁴⁹) and lipids (OPLS forcefield⁵⁰).²⁶ Using the current parameter set (which is

commonly used by other research groups as well), translational diffusion slowed down much more than in the previous study. This is a consequence of the stronger effects of the phosphate, carbonyl, and choline groups, as well as the higher number of water molecules in clathrate around the choline group (11 water molecules in the present parameterization and six in the previous one per PC molecule). Both effects seem to result from higher partial charges in the choline methyl groups (for partial charges, see Refs. 34 and 51) and stronger van der Waals interactions between water and lipid atoms (for this point see Ref. 52). These stronger interactions also influence water dynamics in the intermediate and far water groups, which are slower than in bulk water. The difference between bulk and far water was less pronounced in the previous parameterization.

To conclude, the hydrogen bonding POPE amine group and the glycerol group of POPG slow down water motion more than the equivalent POPC choline group does. Despite the different structures and hydrogen bonding properties, their *overall* effect turned out to be similar. The reason for this is the much higher number of water molecules interacting with the choline group than is H-bonded with the glycerol or amine groups of POPG or POPE, respectively.

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