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Lipid Carbonyl Groups Terminate the Hydrogen Bond Network of Membrane-Bound Water

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Ⓢ Supporting Information

ABSTRACT: We present a combined experimental sum-frequency generation (SFG) spectroscopy and *ab initio* molecular dynamics simulations study to clarify the structure and orientation of water at zwitterionic phosphatidylcholine (PC) lipid and amine *N*-oxide (AO) surfactant monolayers. Simulated O−H stretch SFG spectra of water show good agreement with the experimental data. The SFG response at the PC interface exhibits positive peaks, whereas both negative and positive bands are present for the similar zwitterionic AO interface. The positive peaks at the water/ PC interface are attributed to water interacting with the lipid carbonyl groups, which act as efficient hydrogen bond acceptors. This allows the water hydrogen bond network to reach, with its (*up*-oriented) O−H groups, into the headgroup of the lipid, a mechanism not available for water underneath the AO surfactant. This highlights the role of the lipid carbonyl group in the interfacial water structure at the membrane interface, namely, stabilizing the water hydrogen bond network.
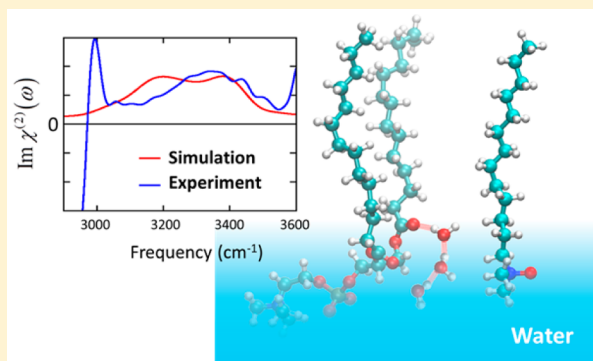
The cell membrane consists primarily of amphiphilic lipids that self-assemble into bilayers, which separate the inside from the outside of the cells. The membrane is essentially a hydrophobic core flanked by a pair of water/lipid interfaces.[1,2] Many proteins in eukaryotic organisms are embedded in different membranes and play an important role in transporting ions and molecules across the membrane. A key question concerns the nature of the water−lipid interactions that stabilize this unique platform and provide the driving force to generate the self-assembly of lipids into bilayers. Clearly, obtaining a molecular picture of the water−lipid contacts is essential for the fundamental understanding of cell membranes.[3] Despite the apparent importance of water−lipid interactions, microscopic insight into the water molecules near lipid layers such as their interaction sites as well as the molecular orientation and binding geometry of water molecules at the lipid headgroups have remained largely unresolved.[4,5]

Sum-frequency generation (SFG) spectroscopy has been demonstrated to be a powerful tool to probe the interfacial water structure[6−9] such as the water structure near the lipid or surfactant monolayers.[10−17] SFG can provide the vibrational spectrum associated with the O−H (O_W−H_W) stretch vibration of water at interfaces, where the water is not randomly oriented and the centro-symmetry is broken.[18,19] The phase-sensitive (PS) extension of the SFG technique has been applied to the water/vapor and water/surfactant interfaces,[6−14] probing *up*/

down-oriented water species at the interfaces. In particular, the water/negatively (positively) charged surfactant interface shows positive (negative) O_W−H_W stretch SFG peaks,[10−12] indicating that the negatively (positively) charged surfactant headgroups causes the O_W−H_W dipole moments to point *up* toward the surfactant layer (respectively *down* to the bulk water). Because zwitterionic lipids consist of both positive and negative charges, the O_W−H_W bond orientation near the zwitterionic lipid layers is more complicated.

Nagata and Mukamel computed the PS-SFG spectrum at the water/zwitterionic phosphatidylcholine (PC) interface using force field molecular dynamics (MD) simulation,[20] showing positive peaks at ∼3300, ∼3500, and 3600 cm^{-1}, attributed to the O_W−H_W groups interacting with the different moieties of the PC headgroup but all uniformly pointing *up* toward the PC lipid layer.[20] A similar molecular picture was further presented by Skinner and co-workers.[21,22] Experimentally, the signs of the simulated SFG peaks have been confirmed later by PS-SFG experiments.[11,14] However, a different microscopic picture of periodically repeated *up*- and *down*-oriented water structure was proposed.[14,23] As the isotopically diluted water/amine-*N*-oxide

(AO) surfactant interfaces exhibited both positive ~3200 cm^{-1} and negative ~3500 cm^{-1} SFG peaks, it was concluded, by extension, that both *up*- and *down*-oriented water molecules exist near the zwitterionic lipid and surfactant.[14] An essential question that therefore arises is whether the water molecules near the PC lipid layer orient periodically both *up* and *down* or whether they orient uniformly *up* toward the lipid layer.

Force field MD simulation has an inherent problem of arbitrary parameter settings, leading to potentially different interpretations and/or predictions of the SFG spectra of water.[24−26] Because *ab initio* MD (AIMD) simulation predicts both the molecular structure and vibrational signature from the electronic structure theory,[27] it provides a unique platform for understanding the SFG spectra of water near the zwitterionic lipid/surfactant monolayers. In this letter, we present experimental and AIMD-simulated SFG spectra[28] of the O_W−H_W stretch mode at the water/PC and water/AO interfaces. By establishing good agreement between simulation and experiment, we address the water orientation near the zwitterionic PC lipid and AO surfactant. Our results highlight the physical role of the lipid carbonyl group on the water orientation at the lipid interface.

For the experiment, the self-assembled monolayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) and *N,N*-dimethyldodecylamine *N*-oxide (DDAO) were produced onto isotopically diluted water (H$_2$O/HOD/D$_2$O = 1/8/16). The PS-SFG spectra were recorded under *ssp* (SFG, visible pulse probe, infrared pulse probe) polarization. Further information can be found in the Supporting Information. For the AIMD simulation, we ran 40 ps AIMD simulations for the water/DMPC and water/*N,N*-dimethyltetradecyl AO (DTAO) interfaces in the *NVT* ensemble at 330 K with the BLYP functional, after 10 ps AIMD runs for equilibration. The slightly elevated temperature is used to compensate for the over-structured water in the BLYP functional.[29] The water/DTAO (water/DMPC) interfaces consisted of 4 DTAO and 80 water molecules (4 DMPC and 170 water molecules). The TZV2P basis sets were used for the water molecules, while the DZVP basis sets were used for the DTAO and DMPC molecules.[30] Core electrons were described by norm-conserving GTH pseudopotentials.[31] We used the recently developed efficient algorithm to compute the SFG spectra via the surface-specific velocity−velocity autocorrelation function (ssVVAF).[28] The *xxz*-component of the SFG susceptibility was calculated, where the *z*-axis forms the surface normal and the *xy* plane is parallel to the interface. Note that the *ssp* SFG susceptibility can be connected to the *xxz* SFG susceptibility.[32] The details of AIMD simulation and SFG response calculations are given in the Supporting Information.

The simulated O_W−H_W stretch SFG responses ($R_{xxz}^{SFG}(\omega)$) are displayed in Figure 1 together with the experimental data obtained using isotopically diluted water. Both the simulation and experimental spectra show a broad 3100−3600 cm^{-1} positive band at the water/PC interface and a positive band below ~3300 cm^{-1} (very weak in experiment) and a negative band above ~3300 cm^{-1} at the water/AO interface. Below 3000 cm^{-1} the contribution from the C−H stretch mode of the lipid and surfactant governs the experimental SFG signal, which is not taken into account in our simulation. These results are in good agreement with previous experiments[13,14] and simulations.[20] Despite both having a zwitterionic headgroup, the SFG responses are remarkably different. The small discrepancy between experiment and simulation especially below 3200 cm^{-1}
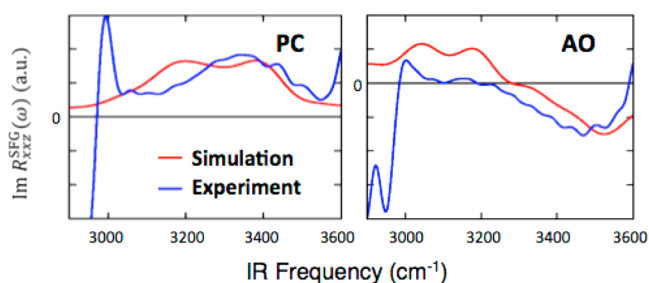

Figure 1. Imaginary parts of the SFG response of the water O−H stretch mode for the isotopically diluted water−PC lipid (left) and water−AO surfactant (right) interfaces.

could be caused by a small phase error, interference with the CH modes, or inter/intramolecular coupling (see Figure S2 in the Supporting Information).

To clarify the influence of the negatively and positively charged parts of the AO and PC headgroups on the O_W−H_W group orientations, we calculated the water radial distribution functions (RDFs) around the AO- and PC-headgroups. Here, the dangling phosphate oxygen (O_P) atom of the PC headgroup and the oxygen (O_N) atom of the AO headgroup were chosen as the representative atoms for the negatively charged parts, while the nitrogen (N) atom was chosen for the representative atom of the positively charged parts (see Figure 2a). Figure 2b displays the H_W...O_P, O_W...O_P, H_W...O_N, and O_W...O_N RDFs. The first peaks of the H_W···O_P and O_W···O_P RDFs for the water-PC interface, represented by the solid lines of Figure 2b, are located at 1.79 and 2.76 Å, respectively. The peak position difference of 0.97 Å is very close to the O_W−H_W bond distance of 0.99 Å, indicating that the O_W−H_W bond of water adjacent to the O_P atom is radially oriented away from the O_P atom. As is shown in the broken lines in Figure 2b, a similar tendency can be found near the O_N atom of the AO headgroup; the H_W···O_N (O_W···O_N) RDF exhibits the first peaks at 1.76 Å (2.75 Å), which is very close to that of the H_W···O_P (O_W···O_P) RDF. These results indicate that both the (partially) negatively charged O_P and O_N atoms strongly affect the O_W−H_W bond orientations. To study the range over which ordered structure of the water molecules may be present, we calculated the RDFs by excluding the first solvation shell, namely the water molecules within the 3.5 Å sphere centered at the O_P and O_N atoms. These are shown in Figure 2c. The difference in the peak positions of ~0.9 Å between the H_W···O_P and O_W···O_P RDFs and between the H_W···O_N and O_W···O_N RDFs indicates that the radially oriented water structure persists, albeit more weakly, even in the second hydration shell.

It is apparent that the negatively charged parts of the headgroups greatly affect the local water orientation. To investigate whether water orientation around the positively charged parts of the headgroups is equally strongly affected, we calculated the H_W−N and O_W−N RDFs, which are shown in Figure 2d. The H_W···N and O_W···N RDFs at the water/PC interface overlap, indicating that the O_W−H_W bonds are not preferentially oriented relative to the N atoms. In contrast, the RDFs for the AO headgroup exhibit sharp peaks. However, because the N atom is next to the O_N atom in the AO headgroup, the sharp peaks of the RDFs can be shown to originate from the H_W···O_N hydrogen bond rather than the H_W−N interactions. To confirm this, we also calculated the H_W−N and O_W−N RDFs by excluding the water molecules in the first hydration shell from the O_P or O_N atoms, which is
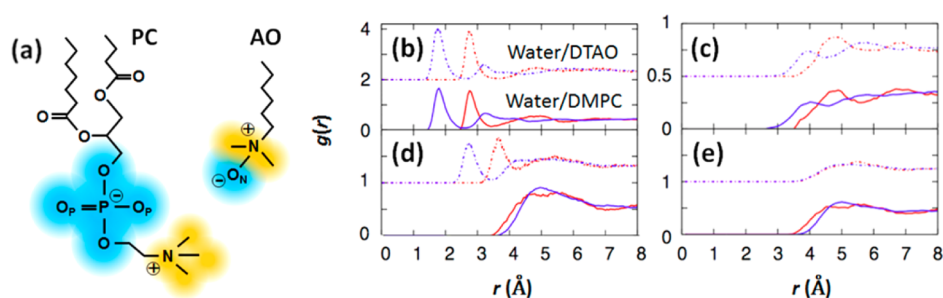
Figure 2. (a) Schematics of PC and AO headgroups. The negatively (positively) charged parts are marked in sky blue (orange). (b−e) The RDFs of the O_w (red) and H_w (blue) atoms calculated at the water/DMPC (solid) and water/DTAO (dotted) interfaces. The RDFs with respect to the (b) O_P and O_N atoms and (d) N atoms are plotted. The RDFs without the contribution from the first hydration shell, water molecules are plotted for the (c) O_P and O_N atoms and (e) N atoms.
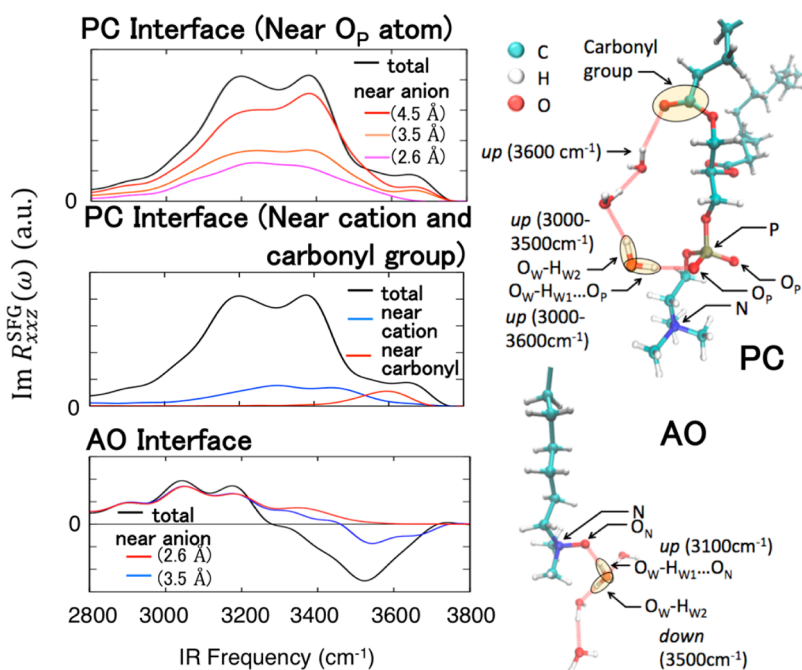

Figure 3. (Left) Selected $O_W−H_W$ chromophores' contributions to the SFG spectra. The SFG spectra in the top and bottom panels were computed using eq S6, while the spectra in the middle panel were computed using eqs S8 (blue) and S6 (red). The lengths in the parentheses denote the cutoff radii; "total" indicates the SFG spectra contributed by all the water molecules at the interface, which are the same as the spectra shown in Figure 1. (Right) Snapshots of the structures of the water molecules near the PC and AO headgroups.

shown in Figure 2e. This shows the $H_W{\cdots}N$ and $O_W{\cdots}N$ RDFs overlap, indicating that the orientations of the water molecules are not substantially influenced by the positively charged parts. This is because the positive charge on the N atom is very delocalized (see Supporting Information) and therefore not able to strongly influence the alignment of water. Further confirmation from the RDFs with respect to the hydrogen atoms of −CH$_3$ groups can be found in the Supporting Information. These observations explain why the PS-SFG response of water interfacing with zwitterionic lipids looks extremely similar to water interfacing with negatively charged lipids: in both cases, the SFG signal originates from water molecules interacting with the negatively charged part.

To extract the individual contributions of the positively and negatively charged parts of the PC and AO headgroups to the $O_W−H_W$ stretch SFG response functions, we computed $R_{xxz}^{SFG}(\omega)$ selectively including only the contribution from the water molecules in a sphere centered on a given atom. The simulation protocols are given in the Supporting Information.

First, we discuss the water/PC interface. The SFG spectra contributed by the $O_W−H_W$ stretch chromophores near the O_P atom are shown in the left top panel of Figure 3. The SFG response originating from the $O_W−H_W$ chromophores located in a 2.6 Å sphere around the O_P atom is dominated by the positive contribution of the $O_W−H_W$ stretch chromophores bound to the O_P atom ($O_W−H_{W1}{\cdots}O_P$ chromophore, see the right top panel of Figure 3) over a surprisingly wide frequency range from 3000 to 3600 cm^{-1}. When the other half $O_W−H_W$ chromophore of the water molecule with the $O_W−H_{W1}{\cdots}O_P$ chromophore ($O_W−H_{W2}$ chromophore, see the right top panel of Figure 3) is included in the SFG response by using the 3.5 Å cutoff radius, the spectrum becomes even more positive. This demonstrates that both the $O_W−H_{W1}{\cdots}O_P$ and $O_W−H_{W2}$ chromophores have the transition dipole moment pointing *up* to the lipid layer. The SFG response function is further enhanced with the cutoff radius of 4.5 Å, consistent with the conclusion above that the *up*-oriented $O_W−H_W$ groups persist even in the second hydration shell.

We subsequently explored the contribution of the O_W-H_W stretch chromophores near the positively charged part of the PC headgroups to the SFG responses, which are plotted in the left middle panel of Figure 3. This indicates that the O_W-H_W stretch chromophores near the N atom do not show any negative SFG features. Our AIMD simulation result clearly illustrates that the O_W-H_W transition dipole moments orient uniformly *up* to the lipid layer near both the negatively charged and positively charged parts of the PC headgroups, although the intensity is smaller for those around the positively charged parts, which is consistent with previous reports.[21]

The combined *up-* and *down*-oriented water conformation was proposed under the assumption that the zwitterionic AO and PC headgroups influence the interfacial water orientation in a similar way.[14] We thus surveyed the orientations of the water molecules at the water/AO interface by computing the SFG responses within the cutoff sphere centered at the O_N atom. These are shown in the left bottom panel of Figure 3. This indicates that the 3100 cm^{-1} positive peak is dominated by the O_W-H_W stretch chromophore bound to the O_N atom ($O_W-H_{W1}\cdots O_N$ chromophore; see the right bottom panel of Figure 3). Such a low frequency of the $O_W-H_{W1}\cdots O_N$ stretch mode has been indicated from IR spectra and AIMD simulation for aqueous trimethylamine-*N*-oxide solution.[33,34] In contrast, the other half O_W-H_W chromophore contained within the same water molecule (O_W-H_{W2} chromophore, see the right bottom panel of Figure 3) provides a negative ~3500 cm^{-1} feature. The angle profiles of the O−H groups near these interfaces are also given in Figure S4 of the Supporting Information. This indicates that the orientation of the O_W-H_{W2} transition dipole moment of the AO interface is very different from that of the water/PC interface.

The molecular mechanism of the different orientation of the O_W-H_{W2} groups can be attributed to the different conformations of the water molecules bound to the O_P and O_N atoms. In particular, in the case of the water/PC interface a larger fraction of water molecules aligns along the surface normal (*up*-oriented waters) in addition to those directly bound to the O_P and O_N atoms. Indeed the water molecules can interact with the carbonyl group, as is evident from both experiments[13,14] and MD simulations,[20,35] forming a continuous hydrogen bond network from the bulk up to the carbonyl group of the PC molecule (see the right top panel of Figure 3). Thus, the negatively charged part of the PC is fully solvated, allowing the O_W-H_{W2} transition dipole moment to point *up*. In contrast, the AO molecule does not contain the carbonyl group; thus, the water molecules tend to form the hydrogen bond network from the bulk to the AO headgroup, making the O_W-H_{W2} transition dipole moment point *down* to the bulk (see the right bottom panel of Figure 3). Our analysis demonstrates that the water molecules interacting with the carbonyl group are responsible for the *up*-oriented O_W-H_{W2} transition dipole moment at the water/PC interface.

Finally, we comment on the previous dipole potential measurements for the DPPC and dihexadecylphosphatidylcholine (DHPC) around lipid headgroups in water, where the large dipole moment difference is found between DPPC and DHPC and is attributed to the carbonyl group.[4] The current study indicates that the carbonyl group of DPPC can enhance the hydration of the lipid headgroup and the dipole moment of the water molecule has uniform *up*-orientations, whereas the DHPC lacking the carbonyl group should have less hydration than the DMPC. The different degrees of hydration can account for the net dipole moment of hydrated DPPC being much larger than that of DHPC.

In summary, experimental and ab initio SFG spectra reveal that the orientation of water for the more complex water/PC interface is more homogeneous than for the chemically simpler AO interface. Water at the PC interface exhibits only positive features, while at the AO interfaces it exhibits both a negative >3400 cm^{-1} feature and a positive <3400 cm^{-1} feature. The different orientation of the half O_W-H_W transition dipole moment can be attributed to the presence of the carbonyl group of the PC molecule. Because of this carbonyl group of PC molecules, the water molecules can form a continuous hydrogen bond network of *up*-oriented waters from the bulk to the carbonyl group, unlike the water/AO interface. This indicates that the carbonyl group of the lipid is very important for interfacial water orientation near the lipid layer.

■ ASSOCIATED CONTENT

ⓈSupporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.5b02141.

Detailed descriptions of the experimental setup and the simulation protocol (PDF)

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Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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