

## Structure and Orientation of Water at Charged Lipid Monolayer/Water Interfaces Probed by Heterodyne-Detected Vibrational Sum Frequency Generation Spectroscopy

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**Abstract:** Cell membrane/water interfaces provide a unique environment for many biochemical reactions, and associated interfacial water is an integral part of such reactions. A molecular level understanding of the structure and orientation of water at lipid/water interfaces is required to realize the complex chemistry at biointerfaces. Here we report the heterodyne-detected vibrational sum frequency generation (HD-VSFG) studies of lipid monolayer/water interfaces. At charged lipid/water interfaces, the orientation of interfacial water is governed by the net charge on the lipid headgroup; at an anionic lipid/water interface, water is in the hydrogen-up orientation, and at the cationic lipid/water interface, water is in the hydrogen-down orientation. At the cationic and anionic lipid/water interfaces, interfacial water has comparable hydrogen bond strength, and it is analogous to the bulk water.

Phospholipid bilayers, the main constituent of cell membranes, are important structural and functional components in biological systems. The membrane/water interface provides a unique environment for many biochemical reactions, and the associated interfacial water is an integral part of such reactions. Water in this restrictive environment behaves differently, which affects many biochemical reactions.<sup>1–6</sup> Therefore, a molecular level elucidation of the structure and orientation of water at lipid/water interfaces is essentially important to understand the adsorption and desorption of various biomolecules, ions, and drugs at the biological interfaces.

In spite of its importance, we are yet to have a unanimous understanding of the water structure even for very simple lipid/water interfaces.<sup>7–11</sup> For instance, Sovago et al. concluded that the water takes the “hydrogen-down” (H-down) orientation at negatively charged and neutral (zwitterionic) lipid/water interfaces on the basis of the maximum entropy phase retrieval analysis (MEM) of homodyne VSFG spectra.<sup>12</sup> They claimed that the water structure at the charged lipid interface is different from that at simple charged surfactant/water interfaces and that the orientation of water at the lipid/water interfaces is not governed by electrostatics arising from the charge of the headgroup. In contrast, simulation studies suggested the “hydrogen-up” (H-up) orientation of interfacial water, even for a neutral (zwitterionic) lipid/water interface.<sup>13,14</sup> Indeed, the water structure at the interfaces is one of the most central subjects in the field of interfacial chemistry.<sup>15</sup>

In this communication, we report a phase-sensitive heterodyne-detected vibrational sum frequency generation (HD-VSFG) study of charged lipid/water interfaces to provide conclusive evidence about the structure and orientation of water at the lipid/water interface. Unlike the conventional (homodyne-detected) VSFG technique that measures the absolute square of  $\chi^{(2)}$  ( $\chi^{(2)}$  is the second-order nonlinear susceptibility), heterodyne measurements<sup>15–18</sup>

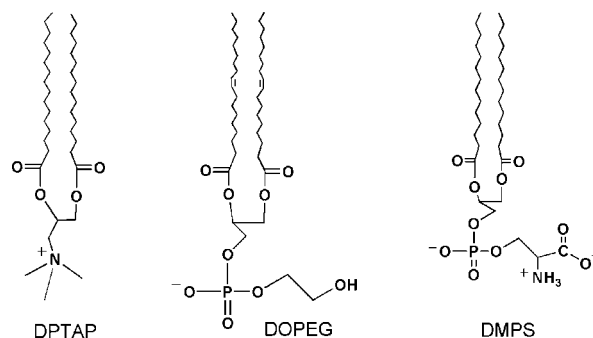


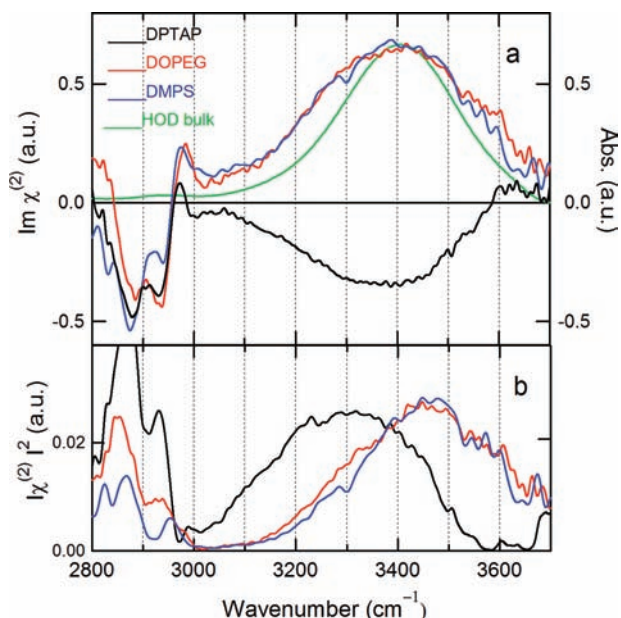
Figure 1. Chemical structures of the lipids studied.

separately provide the imaginary (Im) and real (Re) parts of  $\chi^{(2)}$  spectra with two important advantages. First, the sign of the  $\text{Im}\chi^{(2)}$  spectrum directly manifests the absolute orientation of molecules at the interface. Second, the  $\text{Im}\chi^{(2)}$  spectra provide the spectra of interfacial species free from spectral deformation by  $\text{Re}\chi^{(2)}$  and, hence, allow straightforward interpretation by comparison with bulk absorption spectra which correspond to  $\text{Im}\chi^{(1)}$ .

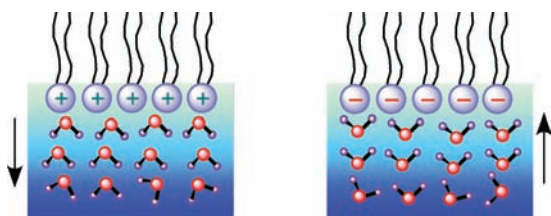
To investigate the structure and orientation of water at the lipid/water interface, we used three different model lipids with varying tail and head groups (Figure 1). The lipids are 1,2-dipalmitoyl-3-trimethylammonium propane (DPTAP), 1,2-dioleoyl-*sn*-glycero-3-phosphoethyleneglycol (DOPEG), and 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine (DMPS), respectively. We used isotopically diluted water ( $\text{H}_2\text{O}/\text{HOD}/\text{D}_2\text{O} = 1/8/16$ ) to remove the band split by intra- and/or intermolecular coupling.<sup>10</sup> Thus, HOD is the predominant species that gives the signal in the OH stretching region.

Figure 2a shows  $\text{Im}\chi^{(2)}$  spectra of DPTAP, DOPEG, and DMPS/water interfaces, in the CH and OH stretching regions, measured with the *ssp* polarization combination (*s*- for sum frequency, *s*- for visible, and *p*- for IR). In the CH stretching region, the negative bands with peaks at  $\sim 2878$  and  $\sim 2940$   $\text{cm}^{-1}$  are attributed to the methyl symmetric stretching ( $\text{CH}_{3,\text{SS}}$ ) and methyl Fermi resonance ( $\text{CH}_{3,\text{FR}}$ ), respectively, while the positive bands with the maximum at  $\sim 2972$   $\text{cm}^{-1}$  are due to the methyl antisymmetric stretching ( $\text{CH}_{3,\text{AS}}$ ).<sup>9</sup> The negative sign of  $\text{CH}_{3,\text{SS}}$  and  $\text{CH}_{3,\text{FR}}$  bands and the positive sign of  $\text{CH}_{3,\text{AS}}$  indicate that the terminal methyl CHs are oriented toward the air, for all the lipids studied.<sup>17</sup>

In the OH stretching region, the  $\text{Im}\chi^{(2)}$  spectrum has the positive sign for the negatively charged DOPEG and DMPS interfaces and has the negative sign for the positively charged DPTAP/water interface. The positive  $\text{Im}\chi^{(2)}$  band corresponds to the H-up, and the negative  $\text{Im}\chi^{(2)}$  band to the H-down orientation of interfacial water molecules.<sup>17</sup> Thus at the negatively charged lipid/water interface, the net orientation of interfacial water is H-up, whereas, at the positively charged lipid/water interface, the water is in the H-down orientation (Figure 3). In a recent homodyne



**Figure 2.** (a)  $\text{Im}\chi^{(2)}$  spectra of DPTAP (black), DOPEG (red), and DMPS (blue)/water interfaces measured with *ssp* polarization and surface pressure  $\approx 30 \pm 3$  mN/m. Isotopically diluted water ( $\text{H}_2\text{O}/\text{HOD}/\text{D}_2\text{O} = 1/8/16$ ) was used. Absorption spectrum of bulk HOD (green) is shown for comparison. (b)  $|\chi^{(2)}|^2$  spectra of the lipid/water interfaces calculated from the corresponding  $\text{Im}\chi^{(2)}$  and  $\text{Re}\chi^{(2)}$  spectra. Spectra were normalized for comparison.



**Figure 3.** Schematic representation of the net orientation of water molecules near the cationic and anionic lipid monolayer/water interfaces. Arrows indicate the direction of electric field generated by the charge on the head groups of the lipids.

VSFG study, it was claimed that the water molecules at lipid interface were essentially different from those observed for the charged surfactant/water interfaces and have an average orientation opposite to that predicted by electrostatics arising from the charge of the headgroup.<sup>12</sup> However, the present HD-VSFG measurements clearly showed that the orientation of interfacial water at the charged lipid interfaces is in harmony with the case of the charged surfactant/water interfaces.<sup>17</sup> Moreover, as shown in Figure 2a, the  $\text{Im}\chi^{(2)}$  spectra of DOPEG and DMPS interfaces have the same sign and very similar spectral shapes in the OH stretching region. This indicates that the structure and orientation of water around the head groups of DMPS and DOPEG are quite similar to each other, although these two lipids have significantly different chemical structures (see Figure 1). The  $\text{Im}\chi^{(2)}$  spectra did not change noticeably with the presence of 5 mM phosphate buffer at  $\text{pH} = 7.2$  (spectra not shown). Therefore, it can be concluded that, for the charged lipids, it is the net charge that dictates the net orientation and structure of interfacial water, at least, at a low ionic strength. The interfacial water monitored by VSFG is predominantly the water aligned below the charged headgroup in the electric double layer.

To address the hydrogen-bonding strength and structure at the charged lipid/water interfaces, we compared the  $\text{Im}\chi^{(2)}$  spectra of the lipid/water interfaces with the IR spectrum of bulk HOD (Figure 2a). This comparison revealed three important aspects. (1) The  $\text{Im}\chi^{(2)}$  spectra of the charged lipid/water interfaces do not exhibit any distinct feature assignable to the “ice-like” structure.<sup>8,9</sup> The  $\text{Im}\chi^{(2)}$  spectra show only a single band feature

(not a double peaked one) as in the case of the bulk liquid water. Thus the charged lipid/water interfaces have a continuum of water structures with varying hydrogen-bond strength.<sup>10,16</sup> (2) The width of the OH stretch band is larger than that of the bulk HOD spectrum. This indicates that the lipid/water interface provides a more inhomogeneous environment than the bulk. (3) The maxima of the OH stretch bands in the  $\text{Im}\chi^{(2)}$  spectra of different lipid/water interfaces are very close to each other and are similar to the maximum in the bulk HOD spectrum ( $\sim 3400$   $\text{cm}^{-1}$ ). This observation is in sharp contrast to the apparent peak shifts seen in the  $|\chi^{(2)}|^2$  spectra that were calculated from  $\text{Im}\chi^{(2)}$  and  $\text{Re}\chi^{(2)}$  (Figure 2b). The OH stretch band in the  $|\chi^{(2)}|^2$  spectrum of the cationic DPTAP/water interface appears substantially red-shifted (maximum  $\approx 3300$   $\text{cm}^{-1}$ ) compared to that at the anionic lipid/water interfaces (maxima  $\approx 3450$   $\text{cm}^{-1}$ ). This apparent shift in the  $|\chi^{(2)}|^2$  spectra may mislead some to think that the water hydrogen bonding strength at the cationic lipid/water interface is stronger than that at the anionic lipid/water interface. Indeed, such arguments have been made based on the homodyne-VSFG spectra.<sup>11</sup> The present HD-VSFG measurements clearly show that the  $|\chi^{(2)}|^2$  spectra at the charged lipid/water interfaces are heavily affected by  $\text{Re}\chi^{(2)}$  and that they cannot directly be compared with each other (see Supporting Information). The high similarity of the OH stretch bands in the  $\text{Im}\chi^{(2)}$  spectra of cationic and anionic lipid/water interfaces reveals that the water molecules at these oppositely charged interfaces have comparable hydrogen-bond strength, and it is analogous to that of bulk water.

In conclusion, for the charged lipid/water interface, interfacial water is in the H-up orientation at the anionic lipid/water interfaces and in the H-down orientation at the cationic lipid/water interfaces. The net orientation of interfacial water is governed by the net charge on the lipid headgroup. This water orientation of the charged lipid/water interface is essentially the same as that at a simpler charged surfactant/water interface. At cationic and anionic lipid/water interfaces, the interfacial water has comparable hydrogen bond strength and it is analogous to that of bulk water on average.

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**Supporting Information Available:** Description of materials and experimental methods. The imaginary, real, and absolute square of  $\chi^{(2)}$  spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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