Disentangling Sum-Frequency Generation Spectra of the Water Bending Mode at Charged Aqueous Interfaces

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ABSTRACT: The origin of the sum-frequency generation (SFG) signal of the water bending mode has been controversially debated in the past decade. Unveiling the origin of the signal is essential, because different assignments lead to different views on the molecular structure of interfacial water. Here, we combine collinear heterodyne-detected SFG spectroscopy at the water-charged lipid interfaces with systematic variation of the salt concentration. The results show that the bending mode response is of a dipolar, rather than a quadrupolar, nature and allows us to disentangle the response of water in the Stern and the diffuse layers. While the diffuse layer response is identical for the oppositely charged surfaces, the Stern layer responses reflect interfacial hydrogen bonding. Our findings thus corroborate that the water bending mode signal is a suitable probe for the structure of interfacial water.

I. INTRODUCTION

The bending mode of H$_2$O has a characteristic frequency around 1550–1700 cm$^{-1}$. This mode has been probed using vibrational spectroscopies, because it reports on the local structure of the hydrogen-bond network in water; when water is strongly (weakly) hydrogen-bonded, the frequency of the bending mode is blue-shifted (red-shifted). Probing the bending mode of water has several advantages over probing the H–O–H stretch mode. Whereas the O–H stretch mode of water cannot be spectrally distinguished from other molecules containing OH-groups, the H–O–H water bending mode is specific to water. Also, the vibrational coupling between bending modes has a limited impact on its spectral response, in sharp contrast to the O–H stretch mode. Furthermore, understanding the bending mode is essential to unveil the vibrational energy transfer from the O–H stretch mode of water and the amide mode of proteins to the local heat. The bending mode is believed to be an essential intermediate step to receive excess vibrational energy and release it to the local heat.

The H–O–H bending mode of specifically interfacial water molecules has been probed with sum-frequency generation (SFG) spectroscopy. Although SFG spectroscopy is surface-specific, the precise origin of the SFG signal has been highly debated. So far, three distinct contributions have been proposed, from interfacial dipoles, bulk quadrupoles, and interfacial quadrupoles. The dipole contribution refers to the first-order term of the second-order susceptibility, and a number of research groups have analyzed and interpreted the experimental and simulated SFG data of the bending mode based on the dipole mechanism. The bulk quadrupole mechanism was proposed by Tahara, Morita, and co-workers in 2016, in which the first-order dipole term is masked by a higher-order term. More recently, in 2020, a new set of the bending mode SFG spectra demonstrated the frequency shift of the bending mode due to the interaction of water with lipids/surfactants. Because the frequency shift cannot be accounted for via the bulk quadrupole mechanism, Tahara and co-workers proposed that the bending mode SFG signal is generated by the higher-order term arising from the interface (interfacial quadrupole mechanism).

Clarifying this apparent contradiction by unveiling the origin of the SFG signal is important, because the different assignments of the origin of the signal lead to different interpretations of the bending mode of water—and thereby of the structure of interfacial water. If the $\chi^{(3)}$ signal arises from the dipole mechanism, it provides information on the molecular orientation of the interfacial water molecules. If the signal arises through the interfacial quadrupole mechanism, one cannot obtain orientational information.

Currently, the bulk quadrupole mechanism is not supported by any experimental data. The remaining two mechanisms,
dipole mechanism and interfacial quadrupole mechanism, can be identified from the sign of the H–O–H bending mode ($\chi^{(2)}$) at the charged interfaces. If $\chi^{(2)}$ is governed by the dipole mechanism, the sign of the Im($\chi^{(2)}$) signal changes with the sign of the surface charge. If $\chi^{(2)}$ is governed by the interfacial quadrupole mechanism, the sign of the Im($\chi^{(2)}$) signal is positive, irrespective of the sign of the surface charge.

Extracting the $\chi^{(2)}$ contribution at the charged interfaces is, however, not straightforward because the water signal at these interfaces arises not only from the oriented water molecules in the Stern layer ($\chi^{(2)}$ term) which is invariant to the solution’s salt concentration, but also from those oriented along the interfacial electric field in the diffuse layer ($\chi^{(3)}$ term) (Figure 1a,b). This interfacial field and the magnitude of the $\chi^{(3)}$ contribution has been examined by varying the bulk electrolyte concentration. However, the $\chi^{(3)}$ contribution is controversial: Reference 26 indicated a substantial $\chi^{(3)}$ contribution, leading to the flipping of the sign of the Im($\chi^{(2)}$) peak due to the change of the negatively and positively charged interfaces (dipole mechanism), while ref 28 showed that the $\chi^{(3)}$ contribution is negligible, leading to the positive Im($\chi^{(2)}$) peak irrespective of negatively or positively charged interfaces (interfacial quadrupole mechanism).

Here, using collinear heterodyne (HD)-SFG, we measure the H–O–H bending mode of water at the positively charged lipid (1,2-dipalmitoyl-3-trimethylammonium propane, DPTAP) and negatively charged lipid (1,2-dipalmitoyl-3-glycero-3-phospho-glycerol, DPPG) interfaces. We unambiguously establish that the $\chi^{(3)}$ contribution is non-negligible and determine its spectrum. The careful extraction of the Im($\chi^{(2)}$) signal, by varying the electrolyte concentration, reveals that the sign of the Im($\chi^{(2)}$) signal is opposite at the water–DPTAP and water–DPPG interfaces. We highlight the importance of the homogeneous sampling of the water–lipid interface, which could be achieved by rotating the sample in the collinear HD-SFG setup.

II. METHODS

II.A. Sample Preparation. We dissolved DPPG (sodium salt) and DPTAP (chloride salt) purchased from Avanti Polar Lipids in a mixture of 90% chloroform (Fischer Scientific, stabilized with amylene, >99%) and 10% methanol (VWR Chemicals, 99.8%) at a concentration of 4.3 $\times$ 10^{-4} mol/L. Sodium chloride (Sigma-Aldrich, >99.5%) was baked in an oven for 8 h at 650 °C. We used D2O (>99.9%), which was purchased from Sigma-Aldrich. H2O was obtained from a Milli-Q machine (resistance >18.2 MΩ cm). We prepared the sodium chloride solutions with their concentrations of 1 M and 0.1 mM. We chose the concentrations of 0.1 mM and 1 M to see the spectral deformations in both imaginary and real parts due to the complex $\chi^{(2)}$ term, as is seen in what follows.

The 20 mL sodium chloride solutions were poured into a Teflon trough with an 8.0 cm diameter. We then deposited ~50 μL DPTAP and DPPG solutions onto the H2O and D2O solutions using a click syringe. The surface pressure of the DPTAP and DPPG monolayers was measured with a commercial surface tension meter (Kibron, Inc., Helsinki, Finland) and was determined to be ~44 ± 3 mN/m and 19 ± 3 mN/m, respectively. The surface area per lipid was estimated to be ~44 Å² and ~52 Å² for DPTAP and DPPG, respectively. The prepared samples were equilibrated for at least 40 min. For both HD-SFG and HD-SHG measurements, the trough was rotated to avoid the lipid monolayer distortion due to heat accumulation. The speed of the sample at the laser irradiation spot was ~1.0 cm/s.

In this study, we used the charged lipids of DPPG and DPTAP with the C=O groups. The C=O stretch mode contributions interfere with the H–O–H bending mode of water, which may potentially complicate the interpretation on the SFG spectra. The other choices which have been...
is challenging because the height of the sample polarization combination. The details of the HD-SFG setup and

The measurements were performed with to the rotation of the sample, causing phase modulations. The

uncover the χ the ionic strength with the salt concentration, allowing us to

produce sum-frequency signal serving as local oscillator (LO). These beams were then collinearly passed through an 8 mm pulse energy, 1 kHz repetition rate). The visible and IR beams

χprohibit the χeff contribution, unlike SDS and CTAB.

II.B. HD-SFG Measurements. The HD-SFG measurements were performed on a collinear beam geometry using a Ti:Sapphire regenerative amplifier (Spitfire Ace, Spectra-Physics, centered at 800 nm, ∼40 fs pulse duration, 5 mJ pulse energy, 1 kHz repetition rate). The visible and IR beams were first focused into a 20 μm-thick y-cut quartz plate to generate the LO signal, and fused silica plates for phase modulation, the fundamental beam was

focused onto the sample surface. All the measurements were

performed with s-in/p-out polarization combinations. The incident angle of the incoming beam was set to 45° relative to the surface normal. The generated second harmonic generation (SHG) signal was dispersed in a spectrograph and detected by an EMCCD camera.

III. RESULTS AND DISCUSSION

III.A. Evidence of Non-negligible χ\text{bend} Contribution. Figures 1c,f display the complex SFG susceptibility (χ(2)) at the D2O–DPTAP and H2O–DPTAP interfaces with two different salt concentrations. First, we focus on the Im(χ(2)) spectra at the D2O–DPTAP interface. For D2O, the bending mode is shifted to ∼1200 cm\(^{-1}\), outside the studied frequency window, so that these measurements serve as a reference. For all the salt concentrations, the spectra commonly show a large positive peak at ∼1720 cm\(^{-1}\) and a relatively small negative peak at ∼1740 cm\(^{-1}\). These peaks are attributed to the C—O stretch mode.\(^{51}\) A striking change of the spectra with increasing salt concentration is the elevation of the baseline (frequency-independent nonresonant contribution). We then turn our

commonly used for generating the charged surfaces are the surfactants without the C—O groups, such as sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB).\(^{24,44}\) However, SDS and CTAB have critical micellar concentrations of ∼0.1 mM to 1 mM, much higher than DPPG and DPTAP. In fact, for stable SFG measurements, researchers have used 1 mM–10 mM concentrations of SDS and CTAB.\(^{34–46}\) Such high SDS and CTAB bulk concentrations prohibit fine control of charge screening by sodium chloride to tune the χ(2) contribution, which requires concentrations down to 0.1 mM. The bulk concentrations of the DPPG and DPTAP samples were ∼1 μM, much smaller than the 0.1 mM salt concentration. For DPPG and DPTAP, one can control the ionic strength with the salt concentration, allowing us to uncover the χ(2) contribution, unlike SDS and CTAB.

II.C. HD-SHG Measurements. A pulsed Yb:KGW (ytterbium-doped potassium gadolinium tungstate) laser system (Pharos, Light Conversion Ltd.) was used, generating pulses with a wavelength of ∼1030 nm, a pulse duration of roughly 210 fs, a repetition rate of 1 MHz, and a pulse energy of 15 μJ. The pulse energy was reduced to 300 nJ. After passing through y-cut quartz to generate the LO signal, and fused silica plates for phase modulation, the fundamental beam was focused onto the sample surface. All the measurements were performed with s-in/p-out polarization combinations. The incident angle of the incoming beam was set to 45° relative to the surface normal. The generated second harmonic generation (SHG) signal was dispersed in a spectrograph and detected by an EMCCD camera.

Figure 2. (a–d) HD-SFG spectra at the H2O–DPPG and D2O–DPPG interfaces with two different NaCl concentrations. The blue and red data points indicate H2O–DPPG and D2O–DPPG data, respectively. The solids lines represent the fits.
focus to the $\text{Im}(\chi^{(2)}_{\text{eff}}(\omega))$ spectra of the H$_2$O–DPTAP samples. Here, the H–O–H bending mode appears as a 1650 cm$^{-1}$ peak feature.\cite{26,28} Upon increasing the salt concentration from 0.1 mM to 1 M, the 1650 cm$^{-1}$ peak varies substantially.

Subsequently, we measured the $\chi^{(2)}_{\text{eff}}$ spectra of the H$_2$O- and D$_2$O-negatively charged DPPG samples. The spectra are shown in Figure 2. The D$_2$O–DPPG samples also show the positive 1720 cm$^{-1}$ and negative 1740 cm$^{-1}$ C–=O stretch features, while the H$_2$O–DPPG samples possess the ∼1650 cm$^{-1}$ H–O–H bending mode contribution, in addition to the C–=O stretch features. Again, upon changing the salt concentration, the 1650 cm$^{-1}$ peak varies.

The SFG response of the H$_2$O and D$_2$O samples in the measured frequency range can be approximated by

$$\chi^{(2)}_{\text{eff},H_2O}(\omega) = \chi^{(2),\text{NR}}_{H_2O}(\omega) + \chi^{(2),R}_{\text{C}=O,H_2O}(\omega) + \chi^{(2),R}_{\text{bend}}(\omega)$$

$$\chi^{(2)}_{\text{eff},D_2O}(\omega) = \chi^{(2),\text{NR}}_{D_2O}(\omega) + \chi^{(2),R}_{\text{C}=O,D_2O}(\omega)$$

respectively, where $\chi^{(2),\text{NR}}$ represents the nonresonant contribution, $\chi^{(2),R}_{\text{C}=O}(\omega)$ denotes the resonant contribution from the C–=O stretch mode, $\zeta$, $\Phi$, $\Psi$, and $\Delta k_z$ denote ion concentration, the surface potential, the inverse of the Debye length, and the mismatch of the wave-vectors along the surface normal in the reflected SFG configuration, respectively.\cite{26,28} By assuming that $\chi^{(2),R}_{H_2O}(\omega) = \chi^{(2),R}_{D_2O}(\omega)$ and $\chi^{(2),R}_{\text{C}=O,H_2O}(\omega) = \chi^{(2),R}_{\text{C}=O,D_2O}(\omega)$, that is, the nuclear quantum effects (NQEs) are negligible, one can get the H–O–H bending mode contribution

$$\chi^{(2),R}_{\text{bend}}(\omega) = \chi^{(3),R}_{\text{bend}}(\omega)\Phi(\zeta)\frac{k(\zeta)}{k(\zeta) - i\Delta k_z}$$

by subtracting $\chi^{(2)}_{\text{eff},H_2O}(\omega)$ from $\chi^{(2)}_{\text{eff},D_2O}(\omega)$. Here, we assumed negligible NQEs on the spectral shape, in analogy with previous work.\cite{28} We will discuss the validity of this assumption in the following.

Figure 3 panels a and b show the subtracted spectra ($\Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega,c)) = \text{Im}(\chi^{(2)}_{\text{eff},H_2O}(\omega,c)) - \text{Im}(\chi^{(2)}_{\text{eff},D_2O}(\omega,c))$) of the DPTAP and DPPG samples, respectively. The $\Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega,c))$ response in the 1580–1630 cm$^{-1}$ frequency region decreases for the DPTAP sample, when the salt concentration increases from $c = 0.1$ mM to 1 M. On the other hand, the $\Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega,c))$ response in the 1580–1630 cm$^{-1}$ frequency region increases for the DPPG sample. The changes of the $\Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega,c))$ spectra with varying salt concentration signify the non-negligible $\Delta \text{Im}(\chi^{(2)}_{\text{bend}}(\omega,c))$ contribution.

We further calculated the spectra $\Delta \Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega)) = \Delta \text{Im}(\chi^{(2)}_{\text{eff},H_2O}(\omega,c = 0.1 \text{ mM})) - \Delta \text{Im}(\chi^{(2)}_{\text{eff},H_2O}(\omega,c = 1 \text{ M}))$, which reflect the $\Delta \Delta \chi^{(3),R}_{\text{bend}}(\omega)\Phi(\zeta)\frac{k(\zeta)}{k(\zeta) - i\Delta k_z}$ contribution (again under the assumption of negligible NQEs). The data are shown in Figure 3c,d for the DPTAP and DPPG samples, respectively. The $\Delta \Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega))$ spectra showed a positive 1580–1670 cm$^{-1}$ contribution for the DPTAP sample and a negative contribution for the DPPG sample. The $\Delta \Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega))$ contribution in the $\omega < 1650$ cm$^{-1}$ region is more apparent than that in the $\omega > 1650$ cm$^{-1}$ region, where 1650 cm$^{-1}$ is a typical H–O–H bending mode frequency. The prominent

![Figure 3](https://doi.org/10.1021/acs.jpcb.1c03258)

**Figure 3.** (a,b) The $\Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega,c))$ spectra obtained through the subtraction of D$_2$O data from the H$_2$O data of the (a) DPTAP and (b) DPPG samples at two different salt concentrations. The spectra are offset by 0.01 for clarity. (c,d) The $\Delta \Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega))$ spectra for the (c) DPTAP and (d) DPPG samples obtained through the subtraction of $\Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega,c = 1 \text{ M}))$ spectrum from $\Delta \text{Im}(\chi^{(2)}_{\text{eff}}(\omega,c = 0.1 \text{ M}))$ spectrum. The features appearing in the region shaded in light blue result from the residual C–=O contributions. The dotted lines indicate the residual nonresonant contribution inferred from the fits.
The positive and negative $\Delta \Delta \text{Im}(\chi^{(3)}(\omega))$ contributions for the DPTAP and DPPG samples indicate that the $\Delta \Delta \text{Im}(\chi^{(3)}(\omega))$ signal is governed by the

$$\text{Im}\left(\chi_{\text{bend}}^{(3)}(\omega)\Phi(c)\frac{k(c)}{k(c) - i\Delta k_z}\right)$$

term. Since the sign of the

$$\text{Im}\left(\chi_{\text{bend}}^{(3)}(\omega)\Phi(c)\frac{k(c)}{k(c) - i\Delta k_z}\right)$$

varies with the sign of the surface charge due to the surface potential of $\Phi(c)$, the flipping of the sign for $\Delta \Delta \text{Im}(\chi^{(3)}(\omega))$ for the positively charged DPTAP and negatively charged DPPG samples provides direct evidence for the $\chi_{\text{bend}}^{(3)}(\omega)$ contribution.

The current finding is at odds with that in ref 28 in which the H–O–H bending mode contribution is unchanged upon the addition of the salt. The reason for such a discrepancy may be attributable to the lipid monolayer formation. A lipid monolayer is easily displaced from the laser spot as a result of the heat accumulation due to continued laser irradiation.43 Because we used the rotating trough, such heat accumulation can be avoided.

This hypothesis can be confirmed by investigating the SFG signature of the C=O stretch mode at the H2O–DPTAP and D2O–DPTAP interfaces. First, the ratio of the C=O stretch peak amplitude vs the H–O–H bending mode amplitude in the Im$\chi^{(3)}_{\text{eff}}$ spectrum at the H2O–DPTAP interface is much larger in this work than that reported in ref 28. This implies that the coverage of the DPTAP is higher in this work than in ref 28. Furthermore, the C=O peak frequency is ~1730 cm$^{-1}$ in the intensity $|\chi_{\text{eff}}^{(3)}|$ spectra at the D2O–DPTAP interface in refs 51 and 26, as well as our measurement (see Supporting Information), while the C=O peak is located at ~1740 cm$^{-1}$ in ref 28. Because the lower surface coverage of DPTAP results in the blue-shift of the C=O stretch peak,54 the 1740 cm$^{-1}$ C=O peak observed in ref 28 indicates that the coverage of the DPTAP is likely strongly reduced in the probed region. With decreasing surface coverage of DPTAP, the surface charge decreases, lowering the impact of the $\chi_{\text{bend}}^{(3)}(\omega)$ contribution on the SFG spectra. Note that very recently, Bakker and co-workers also pointed out that too small a surface charge leads to negligibly small dipolar contribution of the bending mode in ref 44.

III.B. Determination of $\chi_{\text{bend}}^{(3)}$ and $\chi_{\text{bend}}^{(3)}$ Spectra. The above result of the significant $\chi_{\text{bend}}^{(3)}(\omega)$ contribution manifests that the $\chi_{\text{bend}}^{(3)}(\omega)$ and $\chi_{\text{bend}}^{(3)}(\omega)$ contributions are entangled in the measured $\chi_{\text{eff}}^{(3)}$ spectra. Thus, disentangling the $\chi_{\text{bend}}^{(3)}(\omega)$ contribution from the $\chi_{\text{bend}}^{(3)}(\omega)$ contribution requires fitting of the spectra. Here, before carrying out the fitting, we verify the assumption that the NQE is negligible between the H2O and D2O samples. In fact, the different nonresonant background between the H2O and D2O samples can be seen in the nonzero ~1800 cm$^{-1}$ region of $\Delta \Delta \text{Im}(\chi^{(3)}_{\text{eff}}(\omega,c))$ of the DPTAP and DPPG samples as well as the SDS data in ref 28. Because the nonresonant contribution critically affects the inferred amplitude of the H–O–H bending mode signal, we checked whether the NQE s differentiate the nonresonant background of the H2O and D2O samples at the water–DPTAP interface by using HD-SHG spectroscopy. The amplitudes and phases obtained in the HD-SHG measurements are plotted in Figure 4 panels a and b, respectively, for DPTAP. The amplitude of the nonresonant contribution is very similar for the H2O and D2O samples, while the phase differs significantly, particularly for the 10 mM salt concentration. Currently, we are not sure how the NQEs affect the nonresonant contributions. Simulation techniques, including the NQEs,52 may be able to clarify the origin of the difference between the H2O and D2O samples.

On the basis of the knowledge that the nonresonant contributions of the H2O sample $\chi_{\text{H2O}}^{(3)}$ and the D2O sample $\chi_{\text{D2O}}^{(3)}$ can be different, we extracted the $\chi_{\text{bend}}^{(3)}(\omega)$ and $\chi_{\text{bend}}^{(3)}(\omega)$ contributions from the SFG spectra at the DPTAP and DPPG interfaces. For fitting the H–O–H bending mode contribution, we used a Voigt profile:53,54

$$\chi_{\text{bend}}^{(3)}(\omega) = \frac{1}{\sqrt{2\pi}\Gamma_{\text{bend},\text{inh}}} \int_{0}^{\infty} \frac{A_{\text{bend}}}{\omega_{\text{bend}} - \omega' + i\Gamma_{\text{bend},\text{hom}}} \exp\left(-\frac{(\omega - \omega')^2}{2\Gamma_{\text{bend},\text{inh}}^2}\right) d\omega'$$

(3)}
Figure 5. (a,b) \(\text{Im}(\chi^{(2)}(\omega))\), \(\text{Im}(\chi^{(3)}(\omega))\) and \(\text{Im}(\chi^{(3)}(\omega))\Phi(c)\) spectra obtained from the fit for (a) water-DPTAP and (b) water-DPPG interfaces, respectively. The \(\text{Im}(\chi^{(2)}(\omega))\) and \(\text{Im}(\chi^{(3)}(\omega))\) contributions indicate the phase mismatching term, the effective diffuse layer contribution, respectively. The \(\text{Im}(\chi^{(3)}(\omega))\Phi(c)\) contribution includes the phase mismatching term, showing the effective diffuse layer contribution in the \(\text{Im}(\chi^{(3)}(\omega))\) spectra.

where \(A_{\text{bend}}\) \(\omega_{\text{bend}}\) and \(\Gamma_{\text{bend,inh}}\) denote, respectively, the amplitude, characteristic frequency, and line width associated with homogeneous broadening, and \(\Gamma_{\text{bend,inh}}\) accounts for inhomogeneous broadening. For the fit of the \(\text{C}==\text{O}\) stretch modes, we used the two Lorentzian functions corresponding to the positive and negative features.51

We performed the global fitting for all 16 spectra (\(\text{H}_2\text{O}/\text{D}_2\text{O} \times \text{two different salt concentration } \times \text{imaginary/real parts } \times \text{DPTAP/DPPG}\)). Here, \(\chi^{(2)}(\omega)\) was kept fixed across all eight \(\text{H}_2\text{O}\) spectra, because \(\chi^{(2)}(\omega)\) reflects the bulk water properties and should thus be independent of the lipid species. The four \(\text{H}_2\text{O}==\text{DPTAP}\) spectra and the four \(\text{H}_2\text{O}==\text{DPPG}\) spectra each had one fixed \(\chi^{(2)}(\omega)\), as the Stern layer contribution is largely independent of the ionic strength and thus is insensitive to the salt concentration. Furthermore, the parameters for the \(\text{C}==\text{O}\) stretch modes were identical between \(\text{H}_2\text{O}\) and \(\text{D}_2\text{O}\) samples. The global fitting provides the robust estimation of the \(\chi^{(2)}(\omega)\) and \(\chi^{(3)}(\omega)\) contributions. The details of the fitting functions and obtained parameters can be found in the Supporting Information.

The obtained fits are plotted in the solid lines of Figures 1(c–f) and 2, while the \(\chi^{(2)}(\omega)\) and \(\chi^{(3)}(\omega)\) spectra obtained from the fit are shown in Figure 5. The inferred \(\text{Im}(\chi^{(2)}(\omega))\) and \(\text{Im}(\chi^{(3)}(\omega))\Phi(c)\) spectra are negative and positive for the DPTAP samples, while these are positive and negative for the DPPG samples. The mechanism of the opposite sign of the \(\chi^{(2)}(\omega)\) and \(\chi^{(3)}(\omega)\) contributions was previously explained using \textit{ab initio} calculations.26 The term causes a line shape modulation of \(\text{Im}(\chi^{(3)}(\omega))\Phi(c)\) spectra in the low concentration regime (red dotted lines in Figure 5), giving rise to the low frequency contribution in the frequency region of less than \(\sim 1650\ \text{cm}^{-1}\), as discussed above. We would like to stress that the opposite signs of the \(\text{Im}(\chi^{(2)}(\omega))\) peak at the positively charged DPTAP and the negatively charged DPPG interfaces reveal that the \(\text{H}==\text{O}==\text{H}\) bending mode SFG feature arises from the dipole rather than from the quadrupole contribution.

The peak frequencies of the \(\chi^{(2)}(\omega)\) contribution at the \(\text{H}_2\text{O}==\text{DPTAP}\) and \(\text{H}_2\text{O}==\text{DPPG}\) interfaces were \(1672 \pm 10\) and \(1652 \pm 2\ \text{cm}^{-1}\), respectively. Since a higher bending mode frequency indicates a stronger hydrogen bond,1 the peak frequencies indicate that a water molecule in the vicinity of the DPTAP interface has stronger hydrogen bonding than those at the DPPG interface. This trend is consistent with the O–H stretch data of the DPTAP and DPPG interface; the HD-SFG spectra show that the \(\text{H}_2\text{O}==\text{DPTAP}\) interface (center-of-mass frequency of \(3360\ \text{cm}^{-1}\)) shows a slightly lower frequency than the \(\text{H}_2\text{O}==\text{DPPG}\) interface (3390 cm\(^{-1}\)).55 The slightly higher frequency of the \(\text{H}_2\text{O}\) molecules near the \(\text{PO}_4^{3-}\) part of the phospholipid can be rationalized by previous simulation data.56

This qualitative agreement between interfacial water stretch and bend frequencies substantiates the conclusion that the \(\chi^{(3)}(\omega)\) response originates from the interfacial dipole. As such, the \(\chi^{(3)}(\omega)\) peak contains information on the hydrogen bond structure of the interfacial water molecules.

The \(\chi^{(2)}(\omega)\) contribution has a peak frequency of \(1650\ \text{cm}^{-1}\) and a full-width at half-maximum (fwhm) of \(\sim 60\ \text{cm}^{-1}\). Since the \(\chi^{(2)}(\omega)\) contribution reflects the bulk properties, the peak frequency and fwhm of the \(\chi^{(2)}(\omega)\) spectra can be compared with the IR and Raman spectra of the water bending mode. Indeed, these values are very comparable to the 1644 cm\(^{-1}\) peak frequency and \(\sim 70\ \text{cm}^{-1}\) fwhm of the IR spectrum of the water bending.57

IV. CONCLUSIONS

We performed the HD-SFG measurement of the \(\text{H}==\text{O}==\text{H}\) bending mode of water at the water-positively charged DPTAP and water-negatively charged DPPG interfaces. Our data show that the \(\chi^{(2)}(\omega)\) contributions are not negligible at the charged interface. The sign of the \(\text{Im}(\chi^{(2)}(\omega))\) spectrum at the water–DPTAP interface is negative, whereas the sign of the \(\text{Im}(\chi^{(2)}(\omega))\) spectrum at the water–DPPG interface is positive. The change of the peak sign indicates that the bending mode signal arises from the dipole mechanism. Furthermore, we discussed the obtained frequency for the \(\text{Im}(\chi^{(2)}(\omega))\). The sensitivity of the peak frequency at different interfaces indicates that the bending mode of the interfacial water molecules can be a reporter for the hydrogen bonding structure at the interfaces.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.1c03258.
Further details for the experimental methods; discussion on frequency variations of the $\text{C}=\text{O}$ stretch mode between different research groups; fitting procedures; comparison of HD-SHG and HD-SFG data; phase-accuracy of HD-SFG measurements (PDF)

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**Notes**

The authors declare no competing financial interest.

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