

Supporting Information

**Femtosecond Hydrogen Bond Dynamics of Bulk-like and Bound Water at Positively and Negatively Charged Lipid Interfaces Revealed by 2D HD-VSFG Spectroscopy**

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## 2D HD-VSFG Spectroscopy:

In the 2D HD-VSFG setup used in this study, the local oscillator (LO) SFG was generated in the transmission configuration prior to the SFG from the sample, as has been described before.<sup>[1]</sup> In brief, visible ( $\omega_1$ ) light (center wavelength: 795 nm, bandwidth: 24  $\text{cm}^{-1}$ , pulse width: 500 fs) and infrared ( $\omega_2$ ) light (center wavelength: 3400  $\text{cm}^{-1}$ , bandwidth: 250  $\text{cm}^{-1}$ , pulse width: 100 fs) were focused onto a 10- $\mu\text{m}$  thick  $y$ -cut quartz crystal (Crystal Base Co., Ltd.) to generate the LO SFG ( $\omega_1+\omega_2$ ). The LO pulse passed through a 2-mm thick silica plate which delayed the LO pulse with respect to the  $\omega_1$  and  $\omega_2$  pulses by 3300 fs. The  $\omega_1$ ,  $\omega_2$ , and LO pulses were refocused onto the lipid/water interface by a concave mirror ( $R=150$  mm) where a tunable infrared  $\omega_{\text{pump}}$  pulse (bandwidth: 150  $\text{cm}^{-1}$ , pulse width: 200 fs) was also focused for the vibrational excitation. The  $\omega_{\text{pump}}$  pulse was generated by the difference frequency generation between frequency-doubled idler from a parametric amplifier and the 795 nm light using a potassium titanyl phosphate (KTP, 3 mm thick) crystal. The frequency of the  $\omega_{\text{pump}}$  pulse was tuned by rotating the KTP crystal. The  $\omega_1+\omega_2$  pulse generated at the water surface and the LO pulse were collinearly introduced into a polychromator and detected by a multichannel detector. The height of the sample interface was kept constant with the accuracy of 1  $\mu\text{m}$  by monitoring through a displacement sensor (Keyence, SI-F10). The  $\omega_1+\omega_2$ ,  $\omega_1$ ,  $\omega_2$ , and  $\omega_{\text{pump}}$  pulses were S-, S-, P-, and P-polarized, respectively. The incident angles of the  $\omega_1$ ,  $\omega_2$ , and  $\omega_{\text{pump}}$  beams were 37°, 42°, and 23°, respectively. The average pulse energies of the incident  $\omega_1$ ,  $\omega_2$ , and  $\omega_{\text{pump}}$  beams at the sample surface were 10, 13, and 10  $\mu\text{J}$ , respectively. The time resolution of the measurements was estimated by measuring the third-order nonlinear signal of  $\omega_1+\omega_2+\omega_{\text{pump}}$  generated at the lipid/water interfaces with changing the  $\omega_{\text{pump}}$  delay, and it was found to be  $\sim 200$  fs for all the  $\omega_{\text{pump}}$  frequencies.

The steady-state  $\chi^{(2)}$  spectra of the lipid/water interfaces were measured without the  $\omega_{\text{pump}}$  pulse. The steady-state spectra were normalized by the spectrum of the air/D<sub>2</sub>O interface.<sup>[2]</sup> For time-resolved measurements, the  $\omega_{\text{pump}}$  pulse excited a certain portion of the broad OH stretch band of water, and the subsequent  $\omega_1$  and  $\omega_2$  pulses generated  $\omega_1+\omega_2$  pulse at the sample interface after a certain delay time which provided the  $\Delta\text{Im}\chi^{(2)}$  for that delay time. The  $\Delta\text{Im}\chi^{(2)}$  spectra were measured for six different  $\omega_{\text{pump}}$  frequencies centered at 3100, 3200, 3300, 3400, 3500, 3550  $\text{cm}^{-1}$ , and they were interpolated and combined to obtain 2D HD-VSFG spectra at a particular delay time.

**Materials:**

The lipids DPTAP and DPPG were purchased as lyophilized powders from Avanti Polar Lipids. Ultrapure water (resistivity: 18.2 M $\Omega$  cm) was used for all measurements as H<sub>2</sub>O, and D<sub>2</sub>O (NMR grade, 99.9%) was purchased from Wako. Chloroform (GC grade, 99.7%) was purchased from Kanto Chemical Co. and was used as obtained. A Langmuir monolayer of the lipid at the water surface was prepared by dissolving a few milligrams of lipid in chloroform and spreading the solution on the water surface in a Petri dish (diameter: 3 cm). The surface pressure was monitored with a commercial surface tensiometer (Kibron, Inc.) throughout the experiment. The surface pressure of the DPTAP and DPPG aqueous interfaces were maintained at 30 $\pm$ 5 mN m<sup>-1</sup> which corresponds to the liquid condensed phase of the lipids. Isotopically diluted water was prepared by mixing H<sub>2</sub>O and D<sub>2</sub>O in 1:4 molar ratio (H<sub>2</sub>O:HOD:D<sub>2</sub>O = 1:8:16). Thus, HOD is a predominant species that gives the signal in the OH stretch region. All the measurements were performed at 296 K.

**References:**

- [1] K.-i. Inoue, S. Nihonyanagi, P. C. Singh, S. Yamaguchi, T. Tahara, *J. Chem. Phys.* **2015**, *142*, 212431.
- [2] a) S. Nihonyanagi, R. Kusaka, K.-i. Inoue, A. Adhikari, S. Yamaguchi, T. Tahara, *J. Chem. Phys.* **2015**, *143*, 124707; b) S. Yamaguchi, *J. Chem. Phys.* **2015**, *143*, 034202.