

Supporting Information

Dangling OD Confined in a Langmuir Monolayer

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Experimental

Materials. Acyl chain perdeuterated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (*d62*-DPPC) with a purity of 99% was purchased from Avanti Polar Lipids (Alabaster, AL). Chain perdeuterated palmitic acid (*d31*-PA) with a purity of 98% and D₂O with a purity of 99% were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Chloroform (spectrophotometric grade, 99.8%) was obtained from Sigma-Aldrich (St. Louis, MO). Stock solutions of *d62*-DPPC and *d31*-PA, both with a concentration of 1 mM, were made in chloroform.

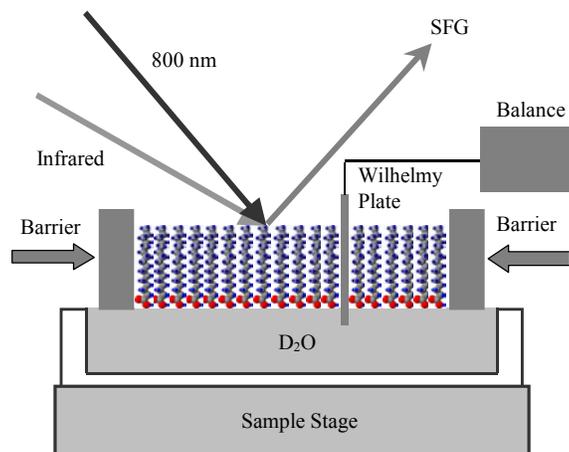
Method. In the study, VSFG spectroscopy, broad bandwidth, was employed. Detailed optical set up of the broad bandwidth VSFG can be found elsewhere.^{1,2} Briefly, the laser system consists of two 1-kHz repetition rate regenerative amplifiers, both of which are seeded by sub-50 fs 800 nm pulses from a Ti:Sapphire oscillator and pumped by a 527 nm beam from an all solid-state Nd:YLF laser. The two regenerative amplifiers provide 85 fs pulses at 800 nm (22 nm bandwidth) and 2 ps pulses at 800 nm (15 cm⁻¹ bandwidth). The fs broad bandwidth pulses were then used to generate broad bandwidth infrared light via an optical parametric amplifier (OPA). The wavelength tuning ability of the OPA is achieved by adjusting the phase-matching angles of BBO and AgGaS₂ crystals. In this work, the output energy of each 800 nm ps pulse was 400 μJ and the IR energy was 4 μJ per pulse. The infrared beam is broad enough to cover the dangling OD stretching region (2900 cm⁻¹-2600 cm⁻¹). Therefore, using the broad bandwidth system, a surface vibrational spectrum can be obtained without wavelength scanning. The 800 nm ps beam and the spectrally broad infrared beam are overlapped at the surface spatially and temporally. The generated VSFG signal containing spectral information from the surface was detected using a monochromator-CCD detection system.

The VSFG spectrum is polarization dependent. In this study, VSFG spectra obtained with the polarization combination of ssp (s-SFG; s-800nm; p-infrared) were presented. VSFG spectra from the air/D₂O/monolayer with the polarization combinations of sps (s-SFG; p-800nm; s-infrared) and ppp (p-SFG; p-800nm; p-infrared) were also obtained. However, the signal from the dangling OD was too weak to be discerned.

The VSFG spectrum was normalized against a nonresonant SFG spectrum from a GaAs crystal to remove the spectral distortion caused by the energy profile of the infrared pulse. To calibrate the SFG peak positions, a nonresonant SFG spectrum from the GaAs crystal surface was obtained with a polystyrene film covering the OPA infrared output port. The resulting SFG spectrum containing polystyrene infrared absorption bands was used for the calibration. The calibration accuracy is better than 1 cm⁻¹. The spectral resolution of the VSFG system was characterized with an approach proposed by Ishibashi and Onishi³ and was found to be comparable to 8 cm⁻¹.²

To carry out the measurements on a Langmuir monolayer, a Langmuir trough (KSV minitrough, KSV, Finland) was coupled to the VSFG sample stage, as shown in Scheme S1. The rectangular trough (176.5 mm × 85 mm) is made of Teflon and was thermostated by circulating water in channels underneath the trough. The use of two barriers provides symmetric film compression. The surface pressure – mean molecular area relationship (i.e. the compression isotherm) was continuously monitored during film compression by the Wilhelmy plate method. The Wilhelmy plate is made of filter paper. Before spreading lipids, the subphase surface (D₂O) was swept by the barriers to ensure that no significant surface pressure increase occurred. A known amount of the lipid solution was spread on the D₂O surface in a drop-wise manner with a Hamilton syringe, and 10 minutes was allowed to elapse for complete solvent evaporation before starting the compression. The barrier moving speed was 5 mm/min. The typical experimental routine is as follows. D₂O was placed in the trough and a VSFG spectrum of the neat D₂O surface was taken. Then a known amount of the lipid (such as *d62*-DPPC) chloroform solution was spread onto the D₂O surface between the two barriers to form a Langmuir monolayer. Then

a series of VSFG spectra under different surface coverages was obtained by varying the area between the two barriers. In this study, surface pressure is used to denote surface coverage for simplicity. Table S1 shows the surface pressure, phase, and its corresponding surface coverage. Monolayer phase assignment is based on previous literature.⁴



Scheme S1. A Langmuir trough coupled to VSFG sample stage.

Table S1. Surface pressure, phase, and surface coverage for *d62*-DPPC monolayer (297 K) and *d31*-PA monolayer (299 K).

	Surface pressure (mN/m)	Surface coverage (Å ² /molecule)
<i>d62</i> -DPPC	0.5 (LE)	101
	1 (LE)	98
	3 (LE)	89
	8 (LE)	72
	42 (C)	50
<i>d31</i> -PA	1 (LE)	44
	3 (LE)	39
	25 (C)	25

*LE: liquid-expanded phase; C: condensed phase.

VSFG spectral normalization

The VSFG spectrum was normalized against a nonresonant SFG spectrum from a GaAs crystal to remove the spectral distortion caused by the energy profile of the infrared pulse. A common problem associated with the normalization process is that the baseline on the left and right sides of the normalized VSFG spectrum become noisy after normalization, as denoted by the dashed arrow in Figure S1. To overcome this problem, a modified normalization was adopted in our study. In the modified method, a baseline-offset GaAs curve is used for normalization. As shown in Figure S1, in the baseline region denoted by the two arrows, the S/N level of the VSFG spectrum normalized with the offset GaAs curve is significantly improved as compared with the VSFG spectrum normalized with the original GaAs curve. As also shown in Figure S1, within our spectral region (i.e. $2550\text{ cm}^{-1} - 2900\text{ cm}^{-1}$) the two normalization methods give identical VSFG spectra. One obvious advantage of the modified normalization process is for the spectral curve-fitting. Those noisy spikes caused by the conventional normalization method could allow the curve-fitting program to falsely identify these regions as component peaks. This modified normalization method has been previously used by Geiger and coworkers in their VSFG studies.⁵

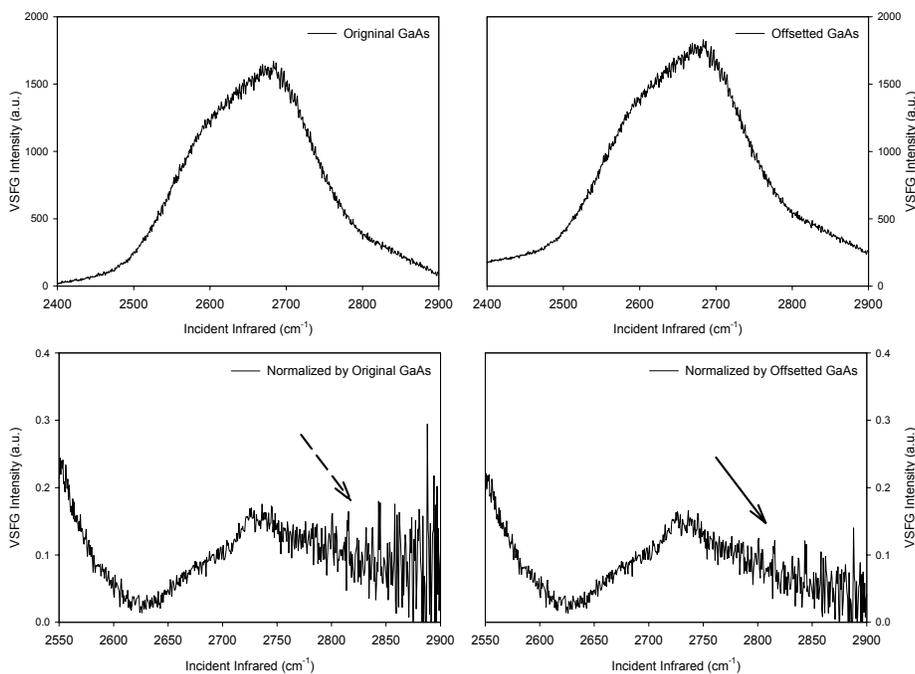


Figure S1. Comparison of the VSFG spectra obtained through two different normalization methods: normalized against the original GaAs curve versus normalized against the offset GaAs curve.

VSFG spectral curve-fitting

The spectral curve-fitting serves two purposes in this study. First, it helped to obtain more accurate peak positions than that obtained through a direct reading from the experimental VSFG spectrum. For example, the dangling OD peak of neat D₂O is determined to be at ~2740 cm⁻¹ through a direct reading from the measured spectrum. Yet, the fit shows the peak to be at 2735 cm⁻¹. Second, curve-fitting was used to deconvolve the overlapping component peaks of the perturbed dangling OD band.

A VSFG spectrum is a functional relationship between I_{SFG} and ω_{IR} . Curve-fitting gives values for $\chi_{NR}^{(2)}$, A_v , ω_v , and Γ_v . In the multi-component curve-fitting, each set of A_v , ω_v , and Γ_v defines each individual component peak. The commercial software IGOR, after adding additional home-written codes, was used in the curve-fitting. Lorentzian line-shapes were utilized.

Figure S2 shows the curve-fit of the VSFG spectrum of the air/D₂O surface in the dangling OD stretching region. The peak at 2735 cm⁻¹ is the fitted dangling OD component. The grey curve is part of the fitted hydrogen-bonded component, which is frequently called the “liquidlike” component.⁶ Figure S3 shows the curve-fits of the VSFG spectra of the air/D₂O/*d*62-DPPC monolayer surfaces in the dangling OD stretching region under different surface coverages. The fitting results reveal that upon increasing the surface pressure, the 2720 cm⁻¹ component peak (red peak) decreases while the 2700 cm⁻¹ component (blue peak) increases. A similar trend with respect to the 2720 cm⁻¹ and 2660 cm⁻¹ component peaks is observed from the curve-fits of the VSFG spectra of the air/D₂O/*d*31-PA monolayer surfaces in Figure S4.

In the fitting of the VSFG spectra in Figures S2, S3, and S4, the phases of the dangling OD and the “liquidlike” OD are set to be the same. This phase relationship is based on the recent phase-sensitive VSFG study of water by Shen and coworkers.⁶

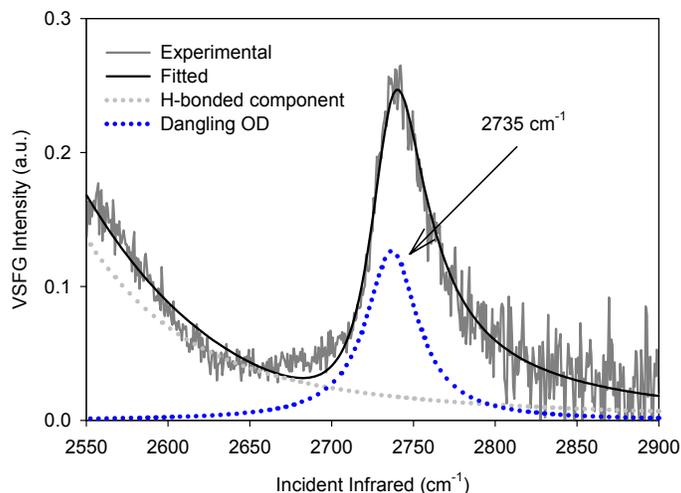


Figure S2. Curve-fit of the VSFG spectrum of the air/D₂O surface in the dangling OD stretching region at 297 K.

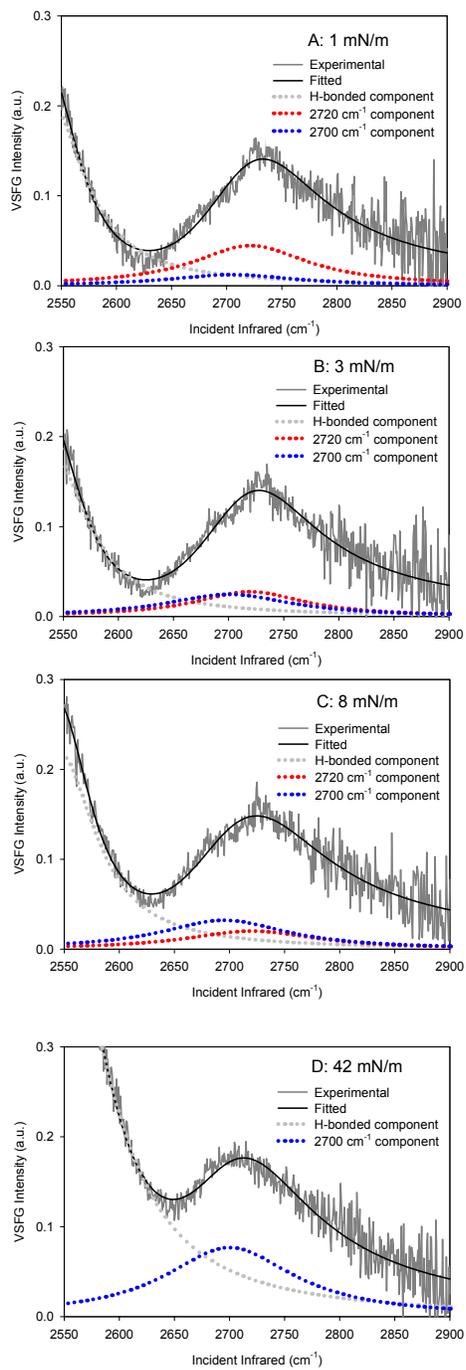


Figure S3. Curve-fits of the VSGF spectra of the air/ $\text{D}_2\text{O}/d62\text{-DPPC}$ monolayer surfaces in the dangling OD stretching region under different surface coverages at 297 K.

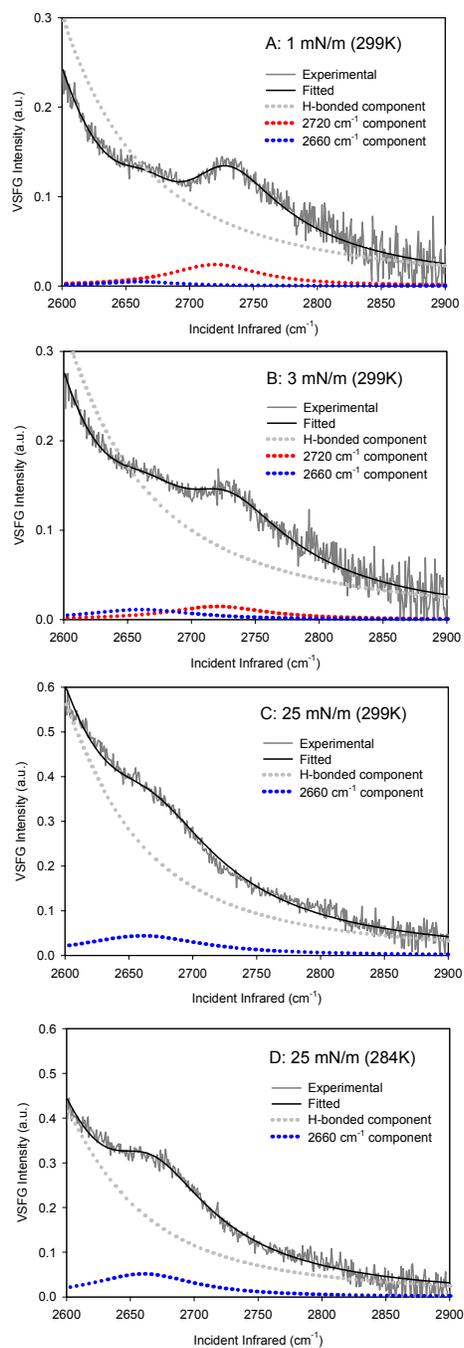


Figure S4. Curve-fits of the VSF spectra of the air/ $\text{D}_2\text{O}/d31$ -PA monolayer surfaces in the dangling OD stretching region under different surface coverages at 299 K and 284 K.

Surface number density discussion

A previous VSFG study showed that 25% of surface water molecules possess dangling OH bonds; after adding methanol to water, the dangling OH is completely removed once the methanol surface number density reaches $2.5 \times 10^{14}/\text{cm}^2$.⁷ For this study, when the surface is fully covered by DPPC, the mean area per DPPC molecule is 50 \AA^2 . This corresponds to a $2 \times 10^{14}/\text{cm}^2$ DPPC surface number density. Since DPPC has two C=O groups and one phosphate group that can form hydrogen bonds with the dangling OH (or dangling OD) groups, it is reasonable to assume that one DPPC molecule is at least comparable to three methanol molecules (3 hydrogen bonding sites). The surface density of DPPC is tripled. Therefore, there are adequate numbers of DPPC molecules on the surface to consume the dangling ODs. Yet, in our study the dangling OD was found to persist even when the D₂O surface was fully covered by DPPC molecules.

References

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