

Supporting Information for

Surface Potential of a Planar Charged Lipid-Water Interface – What Do Vibrating Plate Methods, Second Harmonic and Sum Frequency Measure?

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Experimental Procedures

Sample Preparation. 1,2-dipalmitoyl-3-trimethylammonium-propane (chloride salt) (DPTAP) and 1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt) (DPPG) were obtained from Avanti Polar Lipids, Inc. and used as received. The lipid powders were dissolved at a concentration of $4.3 \cdot 10^{-4}$ mol/l in a chloroform (Fischer Scientific, stabilized with amylene, >99%) and methanol (VWR Chemicals, 99.8%) mixture with a ratio of 9:1. The DPTAP and DPPG solutions were then mixed at different ratios. H₂O was de-ionized using a Millipore unit (resistivity ≥ 18.2 M Ω cm). To remove potential organic contamination, sodium chloride ($\geq 99.5\%$, Sigma-Aldrich) was baked at 650°C for around 8 hours and dissolved in H₂O directly after it had cooled down.

Sum Frequency Generation Experiments. SFG experiments were performed using a Ti:sapphire regenerative amplifier (Spitfire Ace, Spectra-Physics), generating 800 nm pulses with 40 fs pulse duration, a pulse energy of 5 mJ and a repetition rate of 1 kHz. Around 1.7 mJ of the laser output was used to pump an optical parametric amplifier with a collinear DFG stage (TOPAS-C, Spectra-Physics) in order to generate broad band IR pulses. Another part of the output was directed through a Fabry-Perot etalon (SLS Optics Ltd.) to achieve pulses of 15 cm⁻¹ FWHM. Both beams were subsequently focused and overlapped at the sample. The generated SFG pulse was collimated, focused onto a spectrograph (Acton SP 300i, Princeton Instruments) and detected with an electron-multiplied charge coupled device (Newton 970, Andor Instruments). SFG experiments were performed in three different polarization combinations, ssp (s-polarized SFG, s-polarized VIS, and p-polarized IR), ppp and pss. The angles of incidence were approximately 34° for the visible and 36° for the IR. The non-resonant signal of z-cut quartz was used to normalize the data after background subtraction.

The experimental procedure is explained in detail elsewhere (S1). Briefly, the SFG experiments were performed with a Teflon coated trough containing approximately 20 ml of H₂O with

10 mM of NaCl to maintain the ionic strength constant for the different lipid mixtures. The lipid mixtures were deposited on the water surface with a click syringe, yielding a constant area per molecule of 54 \AA^2 . The lipid film was left to equilibrate for 5 minutes before each measurement. During the measurement, the trough was rotated with a velocity of 0.05 m/s at the sample spot to prevent two subsequent laser pulses to hit at the same position. In this manner, local heating effects could be prevented (S2).

Phase-Resolved Sum Frequency Generation Experiments. The phase-resolved SFG measurements were performed on a setup comparable to the conventional SFG setup described above. However, in this setup, the IR pulse was generated in a non-collinear DFG stage. The generated narrow band visible pulse (VIS) in the phase-resolved setup had a width of 25 cm^{-1} . The IR and VIS pulses were overlapped at a gold surface in space and time to generate the local oscillator (LO). The reflected IR and VIS pulses, as well as the generated LO, were focused and overlapped on the sample at an angle of incidence of approximately 45° (IR) and 40° (VIS). A 1 mm thick fused silica plate was used to delay the LO as compared to the VIS and IR pulses. Phase-resolved SFG spectra were recorded in ssp polarization. The sample and the z-cut quartz reference were placed at the same height with the help of a height sensor. The tilt of the quartz was adjusted with a HeNe laser. A previously described method (S3) was used for data analysis. The spectra were inverse Fourier transformed, filtered and Fourier transformed back into the frequency domain. The interferogram of the sample was then normalized by the quartz reference interferogram. To account for the height change due to evaporation during the measurement, a D_2O spectrum was measured and used as a reference to apply a 40° phase correction to all spectra.

As a high lipid density was used, the phase-resolved SFG experiments were performed in a non-rotating trough (S2) filled with 5 ml H_2O containing 10 mM NaCl. DPTAP and DPPG solutions were added to achieve layers with a density of 54 \AA^2 per molecule.

Second Harmonic Generation (SHG) experiments. In the SHG reflection experiments a pulsed Yb:KGW (ytterbium-doped potassium gadolinium tungstate) laser system (Pharos, Light Conversion Ltd) was used, generating pulses with a wavelength of roughly 1028 nm, a pulse duration of roughly 210 fs, a repetition rate of 1 MHz and a pulse energy of 15 \mu J . In the current experiment, the pulse energy was reduced to 250 nJ. Measurements were performed with 45° -in/s-out, p-in/p-out, s-in/p-out, p-in/s-out and s-in/s-out polarization combinations. The incoming beam was set to an angle of 26° relative to the surface normal, the generated SHG pulse was collimated with a lens, focused onto a spectrograph (Minimate 2 Model 1681 B, SPEX GmbH) to selectively detect light at a wavelength of 514 nm and subsequently detected by a photomultiplier tube (R928, Hamamatsu).

For the SHG reflection experiments, the same trough and rotation speed was used as for the SFG experiments. Approximately 20 ml of H₂O containing 10 mM NaCl was filled into the trough. Subsequently, the lipid solution was drop-cast onto the surface. Before each measurement, the film was left to equilibrate for 3 minutes.

Surface potential measurements. The surface potential measurements were performed at Biolin Scientific in Helsinki with a surface potential sensor (SPOT) from KSV NIMA. For those measurements, a stainless steel counter electrode was placed in the solution underneath the vibrating plate electrode. The vibrating electrode was placed ~2 mm above the water surface. The surface pressure was measured with a Wilhelmy plate. The measurements were performed in a KSV NIMA Langmuir trough by compressing the lipid layers as well as in the round trough used for the SFG and SHG experiments by drop-casting the lipid solutions. For each experiment the trough was rinsed with ethanol, dried with a brush and rinsed with MilliQ water. MilliQ water with 10 mM NaCl was used as a subphase. The water surface was further cleaned by sucking away the top water layers with a pump. In the experiments with the Langmuir trough, the cleanliness was checked by closing the barriers and monitoring the surface pressure increase. The experiments were started if the increase in surface pressure was less than 0.3 mN/m. The surface pressure, as well as the surface potential, were set to zero before the addition of lipids to the surface. For the compression experiments, the film was left to equilibrate for 10 minutes before starting the compression with a rate of 15 mm/min.

Additional Experiments

The SHG experiment was performed at three different lipid coverages, namely, 150, 70 and 54 Å² per molecule. The results are shown in Figure S1. At low area per molecule, the signal of the DPPG:water interface is significantly smaller than that of a pure water surface. This stems from the destructive interference between the different contributions to the SHG signal discussed in the main text. It is thus consistent with our interpretation of the SHG results shown in Figure 1c.

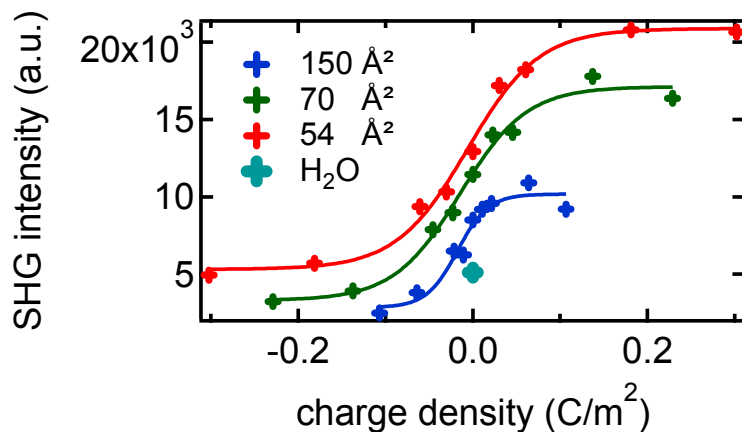


Figure S1: SHG intensities for the lipid-water interface with lipid coverages of 150 (blue), 70 (green) and 54 Å² (red) per molecule as a function of charge density. The signal of the water subphase without lipids on top is shown in light blue. All measurements were performed in 45°in/s-out polarization.

SFG Data Analysis

In order to quantify the signal variations for the different lipid mixtures, all intensity SFG spectra $I \propto |\chi^{(2)}E_1E_2 + \chi^{(3)}E_1E_2E_{DC}|^2 \propto |\chi_{eff}^{(2)}E_1E_2|^2$ shown in the main text are fitted with a Lorentzian model based on the following equation $\chi_{eff}^{(2)} = A_0e^{i\varphi} + \sum_n(A_n/(\omega_{IR} - \omega_n + i\Gamma_n))$. Where A_n , ω_n and Γ_n represent the area, frequency and half-width at half maximum of the resonances, while A_0 and φ are the area and phase of the non-resonant contribution. The fitting parameters shown in the Figures 1d, 2c, 3 and 4b are the areas A_n of the respective molecular vibrations. In case of the OH signals shown in Figure 1d and 2c it is the sum of the areas of the two water bands.

The SFG spectra shown in Figure 2 are fitted with eight resonant contributions and one non-resonant contribution. The resonant contributions are assigned to two O-H bands and six C-H bands. The width and frequency of the bands is kept constant throughout the fits for the different lipid mixtures containing an excess of DPTAP or DPPG; only the amplitudes vary. The O-H bands at around 3200 and 3490 cm⁻¹ are fitted with widths between 200 and 300 cm⁻¹. While the C-H bands positioned between 2740 and 2960 cm⁻¹ are fitted with widths between 19 and 60 cm⁻¹. The non-resonant phase was kept constant at 0.7 and 0.55 for mixtures containing an excess of DPTAP and DPPG respectively, while the non-resonant amplitude varied.

The SFG spectra obtained in the carbonyl region were fitted with two resonant contributions and one non-resonant contribution. The signal at around 1730 cm⁻¹ with a width of 25 cm⁻¹ is attributed to the carbonyl vibration. The non-resonant phase was kept constant at 2.3, while the non-resonant amplitude varied. The additional signal, necessary to fit the data, at 1816 cm⁻¹ had a width of 114 cm⁻¹.

Exemplary $\text{Im}\chi^{(2)}$ Spectra Obtained by Fitting the Intensity Spectra

Fitting the intensity spectra as described above yields a real and an imaginary part. The imaginary part of the resulting fits for three representative spectra are shown in Figure S2. As mentioned above the sum of the area A_n for the two water bands is shown in Figure 2c in the main text.

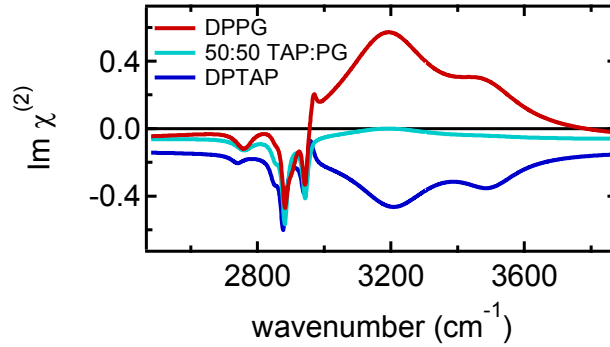


Figure S2: $\text{Im}\chi^{(2)}$ spectra obtained from fitting the intensity spectra of the pure DPTAP and DPPG monolayers as well as the 50:50 mixture in ssp polarization shown in Figure 2a in the main text.

Calculation of Dipole Potential

We have calculated the effect of the C=O dipoles of the lipid molecules on the interfacial potential using the following simple equation:

$$\Delta V = \frac{2\mu_n \cos\theta}{A\epsilon_0}$$

Here μ_n is the molecular dipole moment, A is the area occupied by one lipid molecule, ϵ_0 is the vacuum permittivity and ϑ is the angle of the dipole to the surface normal (S4). The factor 2 originates from having two CO groups per lipid molecule. The dipole moment of the carbonyl is reported to be around 1.8 D (S5) which corresponds to $5.9 \cdot 10^{-30}$ Cm. Our surface coverage corresponds to $54 \cdot 10^{-20}$ m² per lipid molecule and $\epsilon_0 = 8.85 \cdot 10^{-12}$ C/Vm. This calculation yields a contribution of the C=O dipole moment between 0 and 2.5 V upon changing the angle of the group from 0° to 90°. However, we would not expect the carbonyl moieties to be oriented along the surface normal but at a relative large angle with respect to the surface normal. Furthermore, this calculation does not take into account any screening effects from the solvent or dipole-dipole interactions (S6). Those two contributions would considerably decrease the

expected impact of the carbonyl groups on the surface potential. Taking these two effects into account, the calculated value is well within the range of previous dipole potential estimates (S7) and fits well to the range of potential values (200 – 1000 mV) we obtained experimentally. Upon changing the surface coverage to $78 \cdot 10^{-20} \text{ m}^2$, we obtain a potential range from 0 to 1.7 V, i.e. a decrease of roughly 30 % if one assumes no change in orientation. This is in good agreement with the 25 % reduction of the surface potential upon decreasing the amount of lipid molecules on the surface that we observe experimentally (Figure 1 in the main text).

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