

Supplementary information for

Unraveling the Origin of the Apparent Charge of Zwitterionic Lipid Layers

Lisa B. Dreier^{1,2}, Amanuel Wolde-Kidan³, Douwe Jan Bonthuis⁴, Roland R. Netz³, Ellen H.G. Backus^{1,5}, Mischa Bonn^{1,*}

1. Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany
2. Graduate School Materials Science in Mainz, Staudingerweg 9, 55128 Mainz, Germany
3. Fachbereich Physik, Freie Universität Berlin, Arnimallee 14, 14195 Berlin, Germany
4. Institute of Theoretical and Computational Physics, Graz University of Technology, 8010 Graz, Austria
5. Department of Physical Chemistry, University of Vienna, Währinger Strasse 42, 1090 Vienna, Austria

* corresponding author

Experiments

The lipids DOPC and DOCPe were obtained from Avanti Polar Lipids and used as received. The lipids were dissolved in a 9:1 mixture of Chloroform (>99%, stabilized with amylene, Fischer Scientific) and Methanol (99.99%, Fischer Scientific) at a concentration of $4.3 \cdot 10^{-4}$ mol/l. To avoid oxidation of the unsaturated lipid molecules, DOPC and DOCPe were dissolved in a glovebox under nitrogen atmosphere. H₂O was deionized to a resistivity of 18.2 MΩ*m using a Millipore unit. D₂O (99.9%) was obtained from Euriso-Top and used as received.

The conventional SFG spectra were acquired on a setup using a Ti:sapphire regenerative amplifier (Spitfire Ace, Spectra-Physics), that generated 800 nm pulses with a 40 fs pulse duration, a pulse energy of 5 mJ and a repetition rate of 1 kHz. One part of the laser output was used to pump an optical parametric amplifier with a collinear DFG stage (TOPAS-C, Spectra-Physics) to generate the broad band IR pulses. Another part of the output was used to generate the 15 cm⁻¹ FWHM VIS pulses by directing it through a Fabry-Perot etalon (SLS Optics Ltd.). Subsequently, both beams were focused and overlapped in time and space at the sample. The SFG pulse that was generated at the sample was then collimated, focused onto a spectrograph (Acton SP 300i, Princeton Instruments) and detected with an electron-multiplied charge coupled device (Newton 970, Andor Instruments). All SFG spectra shown here were recorded in ssp polarization (s-polarized SFG, s-polarized VIS, and p-polarized IR). The angles of incidence were set to roughly 34° (VIS) and 36° (IR). After background subtraction, the spectra were normalized using the nonresonant signal of z-cut quartz.

The SFG experiments were performed in a Teflon coated trough. The DOCPe and DOPC solutions were deposited on the water surface using a click syringe. The conventional SFG experiments were performed at an area per molecule of 80 \AA^2 , corresponding to a surface pressure of roughly 22 mN/m and 42 mN/m for DOPC and DOCPe, respectively. The lipid layer was left to equilibrate for 3 minutes after the deposition. The trough was rotated with a velocity of 0.05 m/s during the measurement, to prevent two subsequent laser pulses to hit the sample at the same position. In this way, local heating effects could be avoided ¹. During the equilibration of the film and the measurements, the setup was flushed with nitrogen to prevent oxidation of the unsaturated alkyl chains of the lipid molecules.

The phase-resolved SFG experiments on the water-DOPC and water-DOCPe interface were performed on a setup using a Ti:sapphire regenerative amplifier (Spitfire Ace, Spectra-Physics, centered at 800 nm , 5 mJ pulse energy, roughly 40 fs pulse duration, 1 kHz repetition rate). The broadband IR pulse was generated in an optical parametric amplifier (Light Conversion TOPAS-C) with a non-collinear DFG stage. The narrow band VIS pulse, with a bandwidth of $\sim 25 \text{ cm}^{-1}$, was generated by directing the other part of the laser output through a Fabry-Perot etalon (SLS Optics Ltd.). The local oscillator (LO) was generated by overlapping the IR and VIS pulses in space and time in a $10 \text{ }\mu\text{m}$ thick y-cut quartz plate. Subsequently, all three pulses, the generated LO as well as the transmitted VIS and IR, were focused and overlapped on the sample surface at an angle of incidence of roughly 47° (VIS) and 62° (IR). A 2 mm thick fused silica plate was used to delay the LO as compared to the VIS and IR pulses. The data were analyzed using a previously described method ². Briefly, the spectra were inverse Fourier transformed, filtered and Fourier transformed back into the frequency domain. The as obtained interferogram of the sample was normalized by the z-cut quartz reference interferogram. During the experiment, we used a height sensor and a HeNe laser to make sure that the height and tilt of the sample surface and the z-cut quartz reference are the same.

The phase-resolved SFG experiments were performed at an area per molecule of $85 \text{ \AA}^2/\text{molecule}$. This corresponds to a surface pressure of roughly 15 mN/m for DOPC and 36 mN/m for DOCPe. Analogously to the conventional SFG experiments, the lipid monolayer was left to equilibrate for approximately 3 minutes before the experiment was started. During that time and the measurements, the setup was flushed with nitrogen to prevent oxidation of the unsaturated alkyl chains of the lipid molecules. D_2O spectra were acquired before each lipid measurement and used to apply a phase correction to the data, since D_2O does not have a vibrational resonance in the OH and CH stretch frequency window. A 45° and 50° phase-correction was applied to the shown DOCPe and DOPC spectra, respectively.

Simulations

Atomistically resolved, solvent explicit molecular dynamics simulations were performed using the GROMACS 2019.1 package³. Planar DOPC and DOCPe bilayers composed of $N_{lip} = 72$ lipid molecules (36 in each leaflet) were simulated at a hydration level of $n_w = N_{water} / N_{lip} = 30$ water molecules per lipid, which is well above the swelling limit for PC-lipids. Periodic boundary conditions were applied in all directions. Simulations were performed in the NpT-ensemble at $T = 300$ K and atmospheric pressure of $p = 1$ bar. The pressure was controlled by rescaling the box only perpendicular to the lipid interface. The lateral box dimensions were fixed in order to adjust the average area per lipid molecule to the experimental value of $A_{lip} = 85 \text{ \AA}^2$. The velocity re-scaling thermostat⁴ with a time constant of $\tau_T = 0.5$ ps was used in combination with the Berendsen barostat⁵, employing semi-isotropic pressure coupling with a time constant of $\tau_p = 1$ ps and a compressibility of $\kappa = 4.5 \cdot 10^{-5} \text{ bar}^{-1}$. A timestep of $\Delta t = 2$ fs was used together with a cutoff radius of $r_c = 0.9$ nm for electrostatics and Van der Waals interactions. Long ranged charge interactions were modeled using the particle-mesh-Ewald method⁶ and van der Waals interactions were described by Lennard Jones potentials shifted to zero at the cut-off. The SPC/E water model⁷ was used in combination with the Berger forcefield⁸ for the lipids. For the DOCPe lipid, the bonds, angles and dihedrals, as well as the Lennard-Jones parameters, were taken from the original DOPC Berger forcefield. Partial charges were also copied from the original forcefield and only adjusted in order to keep charge neutrality (see Fig. S1).

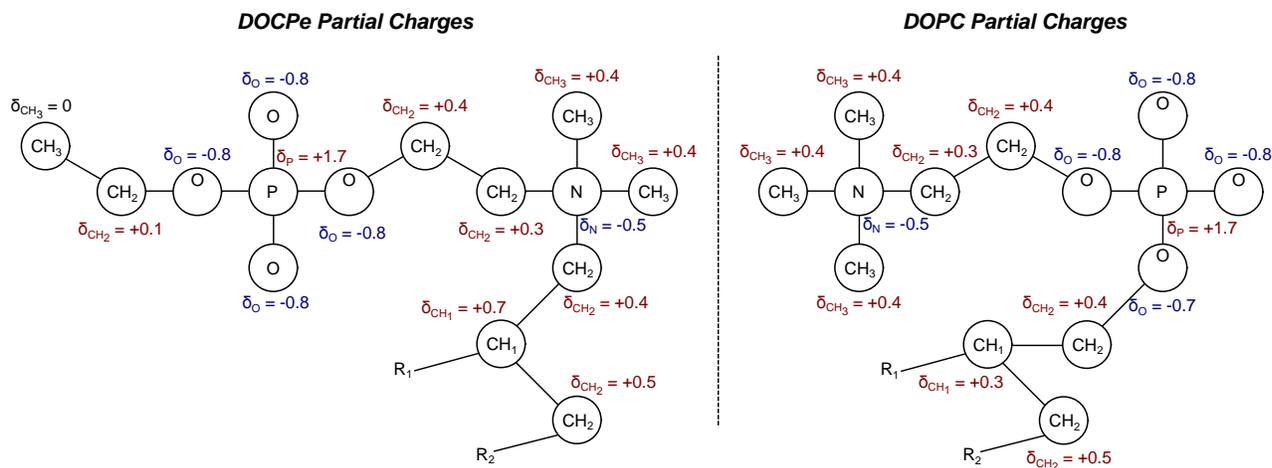


Figure S1: Partial charges used for the employed forcefields. In the case of the DOCPe forcefield, partial charges on two atoms, the oxygen of the phosphate group and the CH_1 of the lipid backbone were adjusted in order to keep charge neutrality. All other partial charges were copied from the original DOPC-lipid forcefield. R_1 and R_2 denote the oleic acid tails for which partial charges were not changed.

Observables were averaged over trajectories with a total runtime of 1 μ s. The electric dipole field of the water molecules was computed from the number density $n(z)$, the water dipole moment $p_0 = 2.35 \text{ D}^7$ and the dipole orientation $\cos\theta(z)$ as follows:

$$E_{dipole}(z) = - \frac{p_0 \cdot \cos\theta(z) \cdot n(z)}{\epsilon_0}$$

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