

# Hydrated Phospholipid Surfaces Probed by Ultrafast 2D Spectroscopy of Phosphate Vibrations

Rene Costard, Ismael A. Heisler, Thomas Elsaesser

Max-Born-Institut f. Nichtlineare Optik und Kurzzeitspektroskopie, D-12489 Berlin, Germany

Author e-mail address: elsasser@mbi-berlin.de

**Abstract:** Phosphate stretching vibrations probe interfacial dynamics in hydrated phospholipids. Two-dimensional spectra in the 1000-1300  $\text{cm}^{-1}$  range reveal structural fluctuations on a 300 fs time scale while water-phosphate hydrogen bonds persist for longer than 10 ps.

**OCIS codes:** (320.7130); (300.6530); (300.6340).

The interaction of phospholipids with water plays a key role for the structure and function of biological membranes. Interfacial water molecules hydrate the charged phospholipid headgroups at the membrane's surface where the interplay of long-range Coulomb polarization forces and local hydrogen bonds determines the molecular geometries [1]. While the time-averaged equilibrium structures of hydrated phospholipids have been studied in substantial detail, insight into their dynamics on the time scale of vibrational motions and structural fluctuations, processes relevant for biological function, is limited. Femtosecond infrared spectroscopy has mainly addressed water stretching vibrations of hydrated membranes [2] and reverse micelles, an approach inherently connected with a spatial averaging over all water environments, i.e., not allowing for a selective observation of interfacial water. Here, we introduce the phosphate stretching vibrations of phospholipid head groups as novel specific interfacial probes to study ultrafast dynamics of the phospholipid-water interface in a wide range of hydration levels. Applying two-dimensional (2D) photon-echo spectroscopy in the frequency range from 1000 to 1300  $\text{cm}^{-1}$ , we map subpicosecond structural fluctuations of the hydrated membrane surface and show that water-phosphate hydrogen bonds persist for longer than 10 ps.

As a model system, reverse dioleoylphosphatidylcholine (DOPC) micelles containing a nanopool of water molecules (Fig. 1a) were prepared in benzene solution for different hydration levels  $w_0$ , where  $w_0$  represents the ratio of the water to DOPC concentration [3,4]. The infrared absorption spectra of reverse micelles at different  $w_0$  display the asymmetric and symmetric  $(\text{PO}_2)^-$  stretch absorption around  $\nu_{\text{AS}}=1250 \text{ cm}^{-1}$  and  $\nu_{\text{S}}=1095 \text{ cm}^{-1}$  (Figure 1b), both showing a red-shift with increasing water content. Femtosecond 2D photon-echo spectroscopy was implemented for the first time in the 1000 to 1300  $\text{cm}^{-1}$  range by generating two phase-locked pulse pairs with the help of diffractive optics and using heterodyne detection based on frequency-domain interferometry [5]. A 2D spectrum recorded with a  $w_0=16$  (fully hydrated) sample at a population time  $T=300 \text{ fs}$  is presented in Fig. 1(c). The 2D lineshapes of  $\nu_{\text{AS}}$  at an excitation frequency  $\nu_1=1235 \text{ cm}^{-1}$  and of  $\nu_{\text{S}}$  at  $\nu_1=1090 \text{ cm}^{-1}$  consist of a positive component (yellow red) due the  $\nu=0$  to 1 transition of the oscillator and a negative component (blue) due the  $\nu=1$  to 2 transition. All contours are elongated along the diagonal  $\nu_1=\nu_3$ , indicating a substantial inhomogeneous broadening that reflects the structural heterogeneity of the phospholipid-water interface. The other 2D peaks originate from other fingerprint vibrations in this range as discussed in Ref. [5]. In Fig. 1(d), we show 2D spectra of the symmetric  $(\text{PO}_2)^-$  stretch mode  $\nu_{\text{S}}$  which has a 1-1.5 ps population lifetime, for hydration levels  $w_0=1$  and 16 and up to population times  $T=4000 \text{ fs}$ . Even for full hydration ( $w_0=16$ ), the 2D lineshapes are preserved over this time range, as is also evident from the unchanged slopes of the center lines shown in blue. This fact demonstrates an essentially constant inhomogeneous broadening and, thus, a practically static disorder of the hydrated phospholipid.

For a detailed quantitative analysis, the measured 2D spectra were compared to lineshape calculations based on a Kubo formalism for the frequency-time correlation function (tcf). We find excellent agreement with the full data set for a tcf consisting of an initial 300 fs decay, necessary in order to account for the antidiagonal widths of the 2D envelopes, and a slow second component for which we estimate a lower limit of its decay time of 10 ps in order to account for the 'static' inhomogeneous broadening. The fast decay present also at low hydration reflects structural fluctuations of the phospholipid geometry and – at high  $w_0$  – restricted motions of water molecules. The slow contribution shows that frequency jumps from the breaking and reformation of water-phosphate hydrogen bonds are absent on a 10 ps time scale, suggesting a comparably rigid hydration pattern of the phosphate groups. This finding which is in line with theoretical molecular dynamics simulations of hydrated phospholipid membranes, also suggests a minor influence of structure fluctuations in the inner water nanopool on the hydration of the interface. An extension of such work to other systems, e.g., phosphate hydration in DNA, will be discussed.

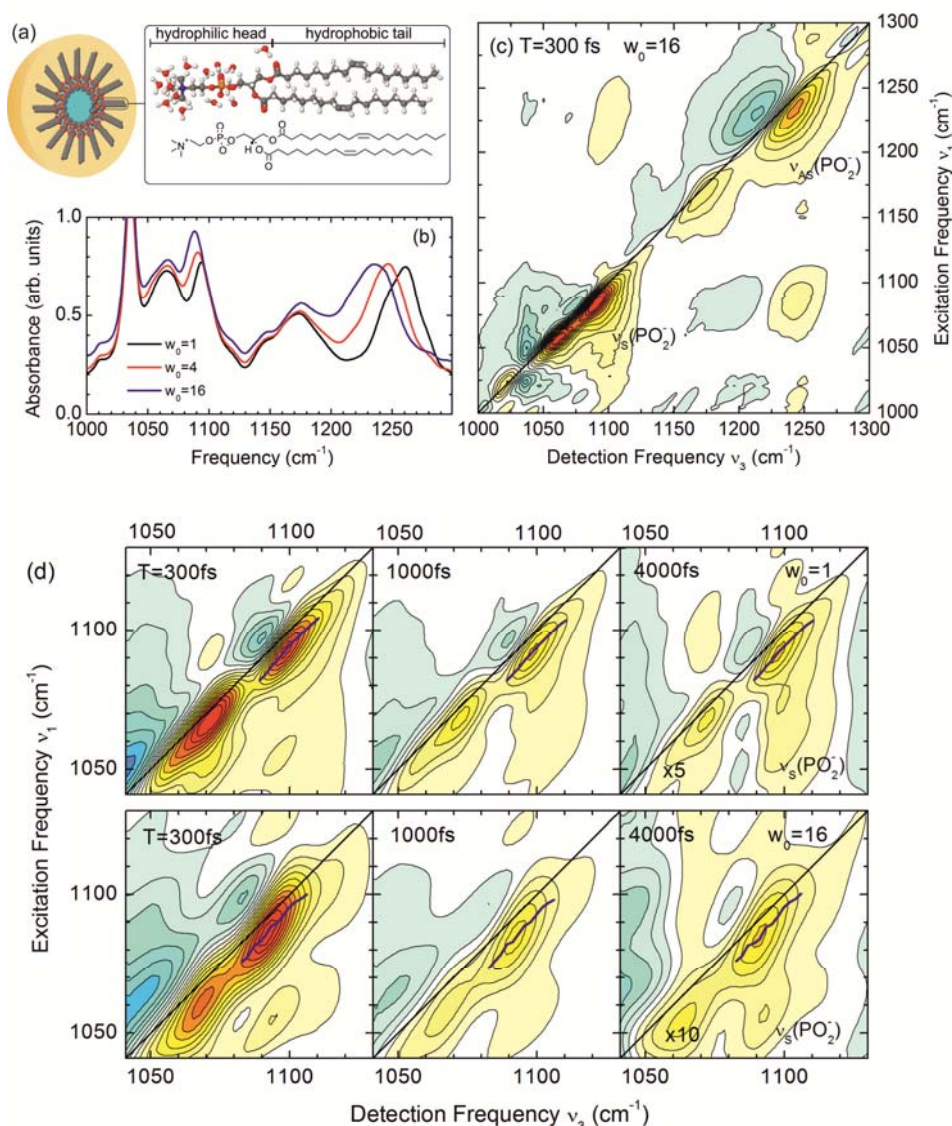


Fig. 1 (a) Chemical structure of the phospholipid DOPC and schematic of a DOPC reverse micelle containing a water nanopool. (b) Infrared absorption spectra of DOPC reverse micelles for hydration levels of  $w_0=1$ , 4, and 16. Both the asymmetric ( $\text{PO}_2^-$ ) stretch vibration  $\nu_{as}$  around  $1250\text{ cm}^{-1}$  and the symmetric ( $\text{PO}_2^-$ ) stretch vibration  $\nu_s$  around  $1095\text{ cm}^{-1}$  undergo a redshift with increasing hydration level. (c) Absorptive 2D infrared spectrum of DOPC reverse micelles at  $w_0=16$  and a population time  $T=300$  fs. Yellow-red contours are positive signal, blue contours are negative signals. The phosphate stretch contributions are marked. (d) Series of 2D spectra of the symmetric ( $\text{PO}_2^-$ ) stretch vibration  $\nu_s$  at  $w_0=1$  (top row) and  $w_0=16$  (bottom row). There is a minor reshaping with population time  $T$ , as also evident from the center lines shown in blue.

## References

- [1] L. Saiz and M. L. Klein, "Structural properties of a highly polyunsaturated lipid bilayer from molecular dynamics simulations", *Biophys. J.* **81**, 204–218 (2001).
- [2] W. Zhao, D. E. Moilanen, E. E. Fenn, and M. D. Fayer, "Water at the surfaces of aligned phospholipid multibilayer model membranes probed with ultrafast vibrational spectroscopy", *J. Am. Chem. Soc.* **130**, 13927–13937 (2009).
- [3] N. E. Levinger, R. Costard, E. T. J. Nibbering, and T. Elsaesser, "Ultrafast energy migration pathways in self-assembled phospholipids interacting with confined water", *J. Phys. Chem. A* **115**, 11952–11959 (2011).
- [4] R. Costard, C. Greve, I. A. Heisler, and T. Elsaesser, "Ultrafast energy redistribution in local hydration shells of phospholipids: a two-dimensional infrared study", *J. Phys. Chem. Lett.* **3**, 3646–3651 (2012).
- [5] R. Costard, I. A. Heisler, and T. Elsaesser, "Structural dynamics of hydrated phospholipid surfaces probed by ultrafast 2D spectroscopy of phosphate vibrations", *J. Phys. Chem. Lett.* **5**, 506–511 (2014).