Supplementary material

Deciphering the orientation of lipid molecules by principal component analysis of Raman mapping data

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In the main part of the article we demonstrated Raman based orientation analysis for dry DMPC and hydrated DPPC samples. One may ask whether the observed differences do result from different samples hydration or from different materials. To ensure that these differences between PCA data for dry and hydrated samples are not related to different types of phospholipids, we also carried out Raman mapping and provided PCA analysis for a dry DPPC sample and a hydrated DMPC vesicle.

Planar dry DPPC sample

The planar DPPC sample was obtained in a similar way as described for the preparation of planar DMPC structures in materials and methods in the main text. Changes were caused by lower solubility in isopropanol for DPPC than for DMPC. In this regard, the concentration of DPPC in the solution was two times lower (25 mg/ml), the solution was heated to 55 °C. The differences observed between planar samples (see Figs. 1b and S1a) are associated with the different material properties of these two phospholipids.

As seen from Fig. S1, spatial distributions of PCs are similar to those that were obtained for the DMPC sample (Fig. 2). Same similarity can be found for PCs spectral representations (see Fig S1f and Fig 2e).

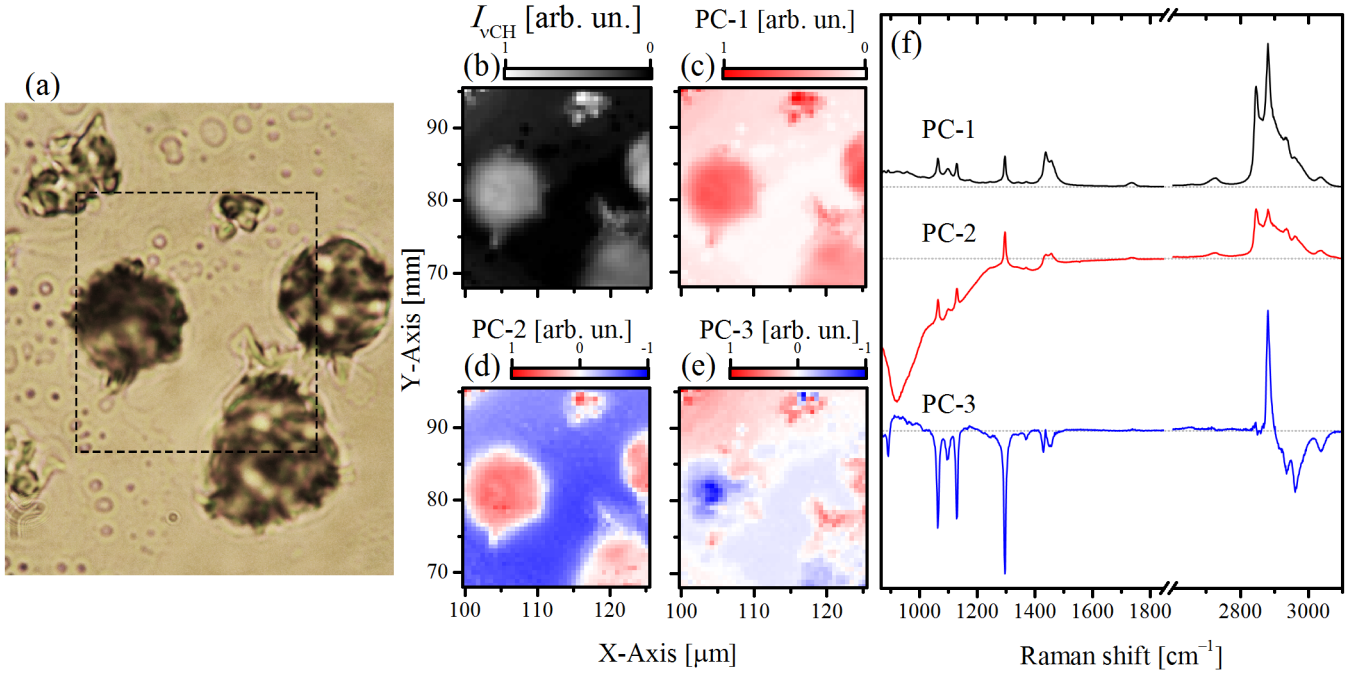

Fig. S1. Raman PCA analysis of the planar dry DPPC sample. (a) Bright-field microscopy photo; (b) mapping of the integral intensity of CH stretching vibrations; (c) mapping of the PC-1; (d) mapping of the PC-2; (e) mapping of the PC-3; (e) spectral representations of the first three PCs. Spectra are vertically shifted for clarity.

Hydrated DMPC sample

DMPC vesicles were prepared using the same protocol as for DPPC vesicles. Unlike DPPC, which main transition temperature is 42 °C, DMPC undergoes this transition at 25 °C. To study DMPC vesicles in the gel phase, the sample was cooled to 22 °C in optical cryostage (THMS350V, Linkam). Cryostage induced some sample displacements; however, in Fig S2d it can be seen that PC-3 distribution is similar to the distribution shown in Fig. 4a for vertical polarization of incident radiation. Spectral representations in Fig. S2e also look identical to those shown in Fig. 4b.

Therefore, we can conclude that PCA analysis works in the same way for DPPC and DMPC phospholipids in dry and hydrated states.

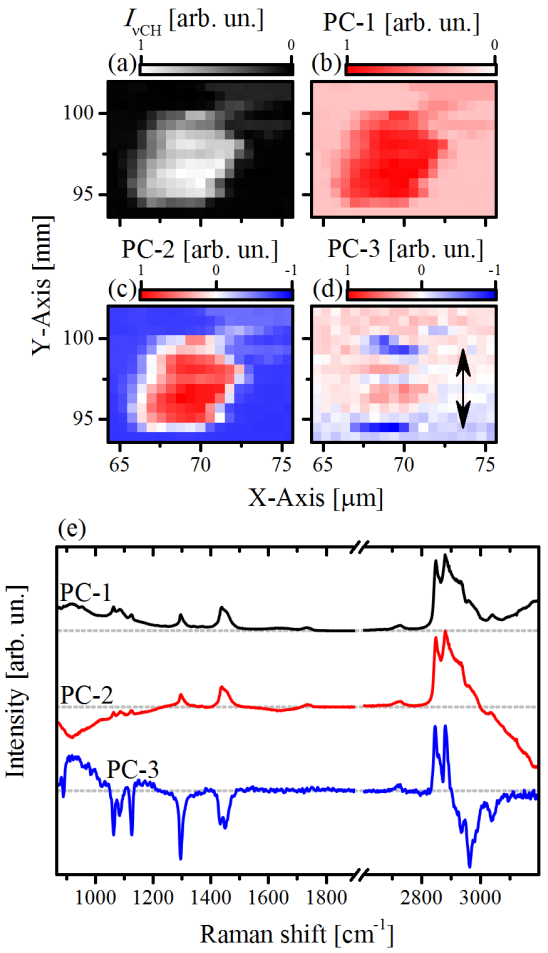

Fig. S2. Raman mapping of DMPC vesicle (at T=+22°C): (a) Raman mapping of the integral intensity of CH stretching vibrations; (b) mapping of the PC-1; (c) mapping of the PC-2; (d) mapping of the PC-3, arrow indicate polarization of incident radiation, each map is individually scaled from 0 to max magnitude; (e) the first three components of PCA. Spectra are vertically shifted for clarity.