Supplementary Material (ESI) for PCCP
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# Nanoscale Chemical Imaging of Segregated Lipid Domains using Tip-Enhanced Raman Spectroscopy

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## Reference Raman Spectra of DPPC and DOPC Powders

Figure S1 shows the Raman spectra of DPPC and DOPC powders. Spectral differences can be found in the fingerprint region (600-1800 cm$^{-1}$) as well as in the C-H stretching region (2800-3100 cm$^{-1}$). The two *cis* double bonds in the oleoyl chains of DOPC show up as a sharp Raman band at 1654 cm$^{-1}$ representing the C=C stretching vibration. This band is absent in the spectrum of DPPC, which has two saturated acyl chains. The three distinct peaks at 1066 cm$^{-1}$, 1102 cm$^{-1}$ and 1128 cm$^{-1}$ in the DPPC spectrum represent the carbon backbone vibrations, which appear as one broad signal in the DOPC spectrum.

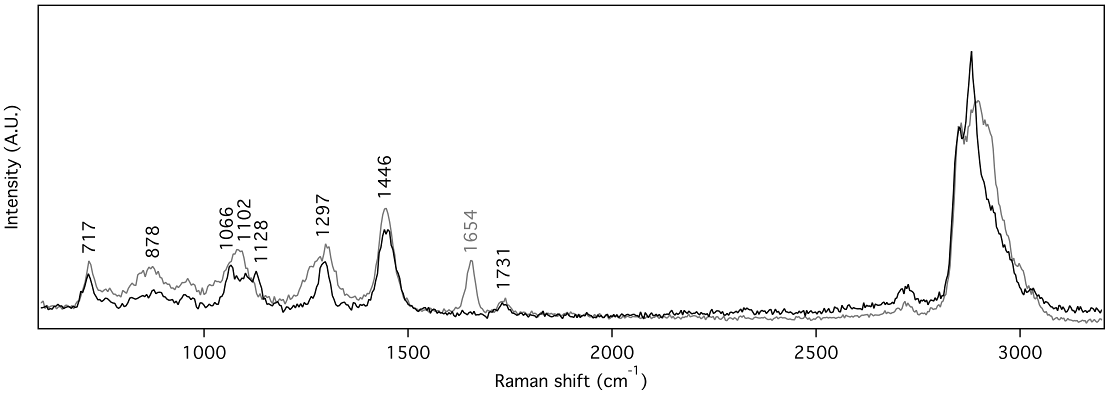

**Fig. S1** Extended version of Figure 2a. Raman spectra of DPPC (black) and DOPC (gray) over the full spectral range.

## Preparation of Lipid Monolayers by Langmuir-Blodgett technique

Figure S2 shows the surface pressure – area isotherms for a pure DPPC and a pure *d62*-DPPC monolayer at the air-water interface. The step observed for both isotherms is caused by a phase transition from the liquid-expanded to the liquid-condensed phase.[1] The isotherm for the *d62*-DPPC monolayer is shifted to lower packing densities at any given surface pressure and the phase transition occurs at a higher surface pressure compared to the DPPC monolayer. Thus, the deuteration of the two acyl chains of DPPC has a significant influence on the molecular interaction within the lipid monolayer. We take this into account by using a higher surface pressure for transfer to the substrate when substituting DPPC by *d62*-DPPC in the mixed monolayer to ensure clear phase separation.

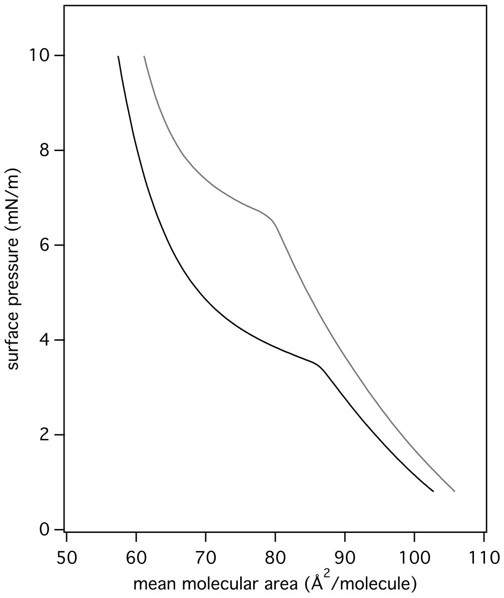

**Fig. S2** Surface pressure – area isotherm for a pure monolayer of DPPC (black) and *d62*-DPPC (gray) at room temperature. The observed plateau present in both curves is caused by a phase transition occurring at the corresponding surface pressures.

Figure S3 shows the surface pressure – area isotherm for the *d62*-DPPC/DOPC mixture (molar ratio of 1:1) up to a surface pressure of 20 mN/m used for transfer to the substrate. The mean molecular area achieved at this pressure is 71 Å$^2$/molecule (corresponding value on the x-axis).

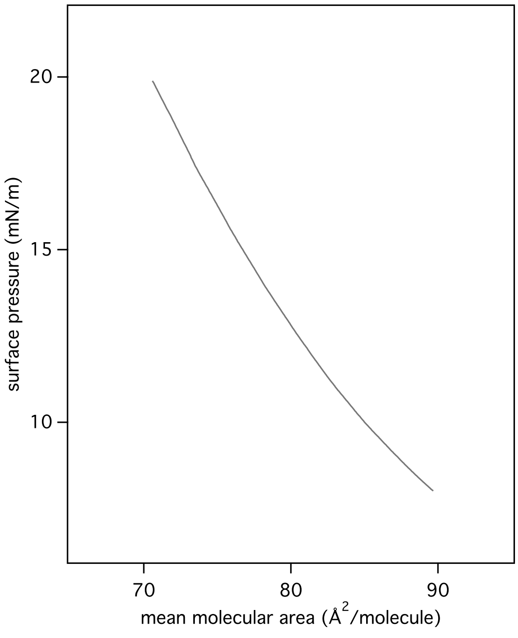

**Fig. S3** Surface pressure – area isotherm for *d62*-DPPC/DOPC (molar ratio of 1:1) at room temperature. Only the last compression before transfer to the substrate is shown.

### TERS Map on the Mixed *d62*-DPPC/DOPC Monolayer

Figure S3 shows three different stages during processing of the collected data from the 128 x 128 raster scan. The scan direction was from bottom left to top right. In the first image, the brightness represents the background corrected intensity of the Raman band at 2100 cm$^{-1}$ extracted from the full spectrum associated with each pixel. Doing the same for the Raman band at 2900 cm$^{-1}$, yields the second image. If we combine these two data sets by taking the ratio of the two peaks, we end up with the third image. It can be noted that this procedure leads to a significant improvement of the image contrast. This is because overall variations of the signal enhancement (e.g. due to differences in tip – sample distance) are eliminated. For example the sudden drop of the overall intensity seen in the middle of image (a) and (b) is successfully removed.

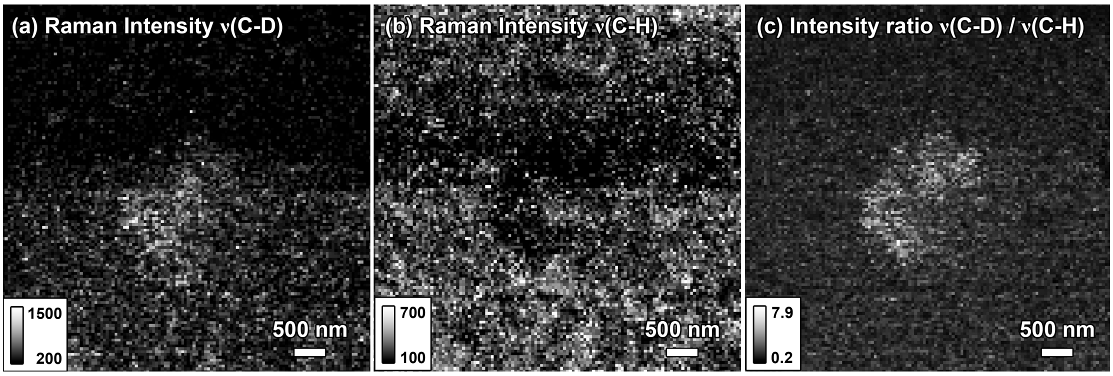

**Fig. S3** Intensity maps for the Raman band at 2100 cm$^{-1}$ (a), the Raman band at 2900 cm$^{-1}$ (b) and the ratio of both (c). Intensities in (a) and (b) are given in counts. The images were generated and processed using the NOVA software (NT-MDT, Russia).

Figure S4 shows the constant current mode STM image acquired during the 128 x 128 pixel TERS raster scan shown in Figures 3d and S3. The condensed phase domain is visible as a darker area in the middle of the image, which is due to a lower tip position to maintain the tunnel current. This could be due to the higher molecular density of the condensed phase domain leading to a higher insulation. An exact value for the step size in z direction cannot be determined because of the low signal-to-noise ratio. However, the difference in tip-sample distance does not affect the spectral information used for the TERS image, because each pixel represents an intensity ratio of two Raman bands and is therefore not affected by changes in the overall enhancement.

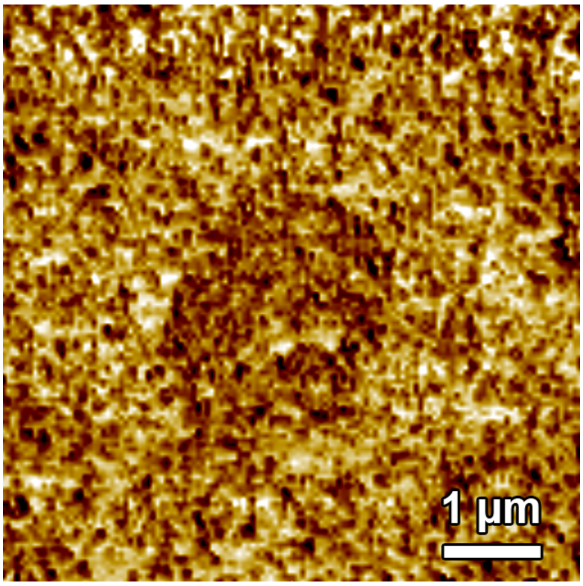

**Fig. S4** Constant current mode STM image measured during the 128 x 128 pixel TERS raster scan. A bias voltage of 200 mV was applied between silver tip and gold surface and the tunnel current was set to 0.1 nA. The dark region (lower tip position) in the middle of the image matches the condensed phase domain observed in the TERS raster scan.

## References

1 S. L. Duncan and R. G. Larson, *Biophys. J.*, 2008, **94**, 2965.