Supplementary material

Coexistence of Lipid Phases in Multilayer Phospholipid Films Probed by Raman Mapping

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Calibration procedure

To estimate the composition of a sample from the Raman spectra we used the intensities of the spectral components obtained from the spectral decomposition. In our study, the spectra were decomposed into 4 components corresponding to DOPC, cholesterol, and the two conformational states of DPPC (Fig. 1 of the main text). The reference spectra of phospholipids were normalized by the integral intensity of CN mode at 718 cm^{-1}. The cholesterol spectrum was scaled by maximum intensity.

The intensity proportions between different components ($I_{DOPC}/I_{DPPCd62}/I_{Chol}$) were estimated from Raman spectra of the samples with the known average content ($C_{DOPC}/C_{DPPCd62}/C_{Chol}$). In linear assumptions, the intensity ratios are proportional to the molar fractions:

$$\frac{c_{DOPC}}{c_{DPPCd62}} = \alpha_{DOPC} \times \frac{I_{DOPC}}{I_{DPPCd62}}, \quad (1)$$

$$\frac{c_{Chol}}{c_{DPPCd62}} = \alpha_{Chol} \times \frac{I_{Chol}}{I_{DPPCd62}}, \quad (2)$$

where α_{DOPC} and α_{Chol} are the coefficients determined by the difference in Raman scattering cross-sections. The coefficients were obtained by fitting of the intensity ratio ($I_{DOPC}/I_{DPPCd62}/I_{Chol}$) to the known molar fractions $C_{DOPC}/C_{DPPCd62}/C_{Chol}$ = $\alpha_{DOPC}\times I_{DOPC}$ / $I_{DPPCd62}$ / $\alpha_{Chol}\times I_{Chol}$. Figure 1S shows the correspondence between concentrations evaluated from Raman and nominal $C_{DOPC}/C_{DPPCd62}/C_{Chol}$ values. Due to the sample heterogeneity, related to the phase coexistence and, in some samples, to the possible deviations of the real concentrations from the nominal ones, Raman data differs from the nominal data (Fig. S1). The minimal deviations were obtained at α_{DOPC} = 0.905 (the SD of measured data points is 8.4 mol. %) and α_{Chol} = 2.025 (SD = 5.9 mol. %). The SD for DPPC-d62 molar fraction was 9.6 mol. %. Since our reference Raman spectra for DOPC and DPPC-d62 were scaled by the intensity of stretching CN mode, it is expected for α_{DOPC} to be close to 1, which agrees with the calibration results.

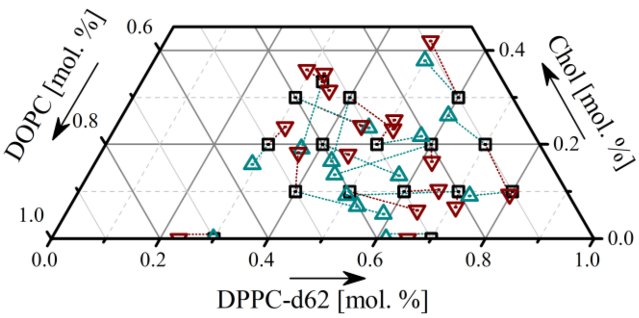

Figure S1. Fitting of the nominal sample compositions to the average compositions known by Raman data. The squares represent the nominal content of multilayer films, the triangles represent the concentrations evaluated from Raman spectra by fitting the coefficients α_{DOPC}, α_{Chol}. The top triangles (dark cyan) correspond to the concentrations of the dry films, the bottom triangles (vine) correspond to the concentrations of the hydrated films.

Phase coexistence in films at intermediate degree of hydration

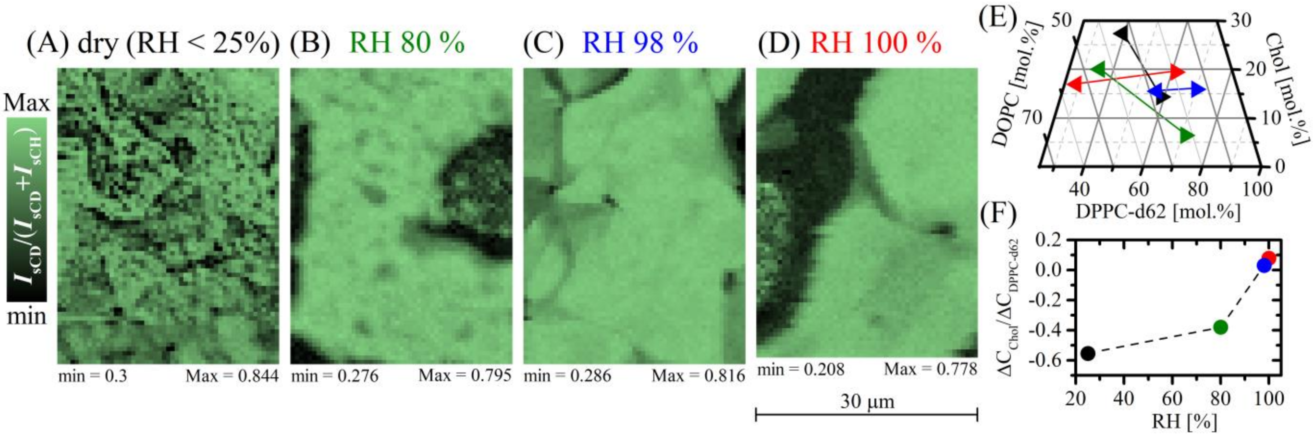

Figure S2. Raman maps and phase coexistence diagram for 30/50/20 films with different relative humidity. Black color was used to denote data corresponding to dry film with relative humidity (HR) below 25 %, green color denotes film with RH = 80 %, blue corresponds to RH = 98 %, and the data obtained from hydrated films (RH = 100%) are marked with red. Raman maps of DOPC/DPPC-d62/Chol films: (A) dry, (B) RH = 80 %, (C) RH = 98 %, (D) RH = 100 %. (E) Phase coexistane diagram. (F) Dependence of the tie-line slope ($\Delta C_{Chol}/\Delta C_{DPPC-d62}$) on film hydration.

To provide a better comprehension of the effect of hydration on phase coexistence, we carried out measurements of DOPC/DPPC-d62/Chol films with the same 30/50/20 content at intermediate relative humidities. Samples were kept at a given humidity for a day. Intermediate relative humidity (RH) was achieved using mixtures with different proportions of water and glycerol.

In general, films with intermediate hydration show intermediate characteristics between dry and fully hydrated films. Raman map of dry film (Fig. S2A) demonstrates higher heterogeneity in comparison to Raman map of a film with higher hydration (Figs S2B,C,D). This indicates that diffusion at 80% RH is high enough for the domains to coalesce within 24 h. Since coalescence depends on the diffusion rate and the time interval, the observed maps should depend on the exposure time under certain humidity conditions. Our results are consistent with the expected increase in diffusion rate with increasing RH. Using the same data, we investigated changes in the chemical content of coexisting domains (Fig. S2E). To quantify this change, we calculated the slope between cholesterol and DPPC-d62 concentrations (ΔCChol/ΔCDPPC-d62). The slope monotonically increases from low to high RH (Fig. S2F). The tie-line orientation between the dry sample and film at 80 % RH is less than the difference between 80 % and 98 %.