Supporting Information for

Reversible monolayer-bilayer transition in supported phospholipid LB films under the presence of water: morphological and nanomechanical behavior

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1. DPPC:Chol 1:1 Langmuir and Langmuir-Blodgett films

As reported before cholesterol (Chol.) induces the formation of a highly condensed phase in monolayers of 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC).[1] In addition, a cholesterol molar fraction of 0.5 in the DPPC:Chol mixtures is the highest reported before Chol. forms pure cholesterol domains in the mixture.[2] This is the reason why we have chosen a DPPC:Chol 1:1 proportion for the purpose of this work. The surface pressure of 35 mN·m^{-1} mimics the lateral pressure in biological membranes,[3] and this is the reason why the monolayers were transferred at this surface pressure.

To illustrate the main features of DPPC:Chol 1:1 Langmuir films Figure S1 shows the surface pressure, π, vs. the area per molecule, *A*, isotherm for DPPC, Chol, and DPPC:Chol 1:1 Langmuir films recorded at 20 ºC. In addition, the inset graph in Figure S1 shows the Young modulus or elastic modulus, K_s, of DPPC:Chol 1:1 vs. the surface pressure. K_s is defined as:[4]

$$K_s = -A \cdot \left(\frac{\partial \pi}{\partial A}\right)_T$$

The high value of the elastic modulus at 35 mN·m^{-1} is worth noting. From the isotherms in Figure S1 it can be concluded that the DPPC:Chol 1:1 mixture results in a highly compact monolayer exhibiting a negative excess area per molecule, A^E, (A^E = - 0.035 nm^2·molecule^{-1}) at 35 mN·m^{-1}. A^E is defined as $A^E = A_{DPPC:Chol} - (x_{DPPC} \cdot A_{DPPC} + x_{Chol} \cdot A_{Chol})$, where *A* represents the area per molecule and *x* the mole fraction. The high *Ks* value at 35 mN·m^{-1} accompanied by a compact film is indicative of a cholesterol-induced condensed (CC) phase, which has been comprehensively described recently by Miyoshi and Kato.[1]

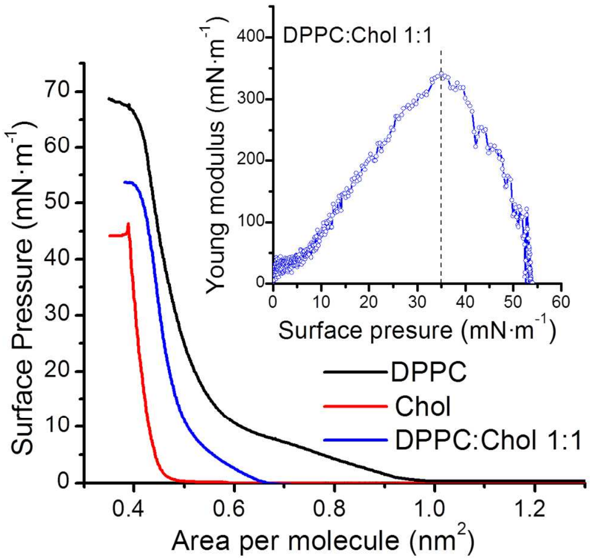

Figure S1. Surface pressure vs. area per molecule isotherms for the indicated compounds recorded onto pure water at 20 ºC. The inset graph shows the Young modulus vs. the surface pressure for a mixed DPPC:Chol 1:1 monolayer.

The monolayers of DPPC:Chol 1:1 transferred at a surface pressure of 35 mN·m^{-1} were studied by means of infrared spectroscopy. The experiment was carried out with a 2 cm^{-1} spectral resolution and 252 scans were accumulated in the region contained between 2800 and 3000 cm^{-1}. The presence of the C-H stretching band in 2850 cm^{-1} and 2920 cm^{-1} provides useful information relating to a well-ordered orientation of the phospholipid alkyl chains since the frequencies of methylene stretching vibration modes, $\nu_s(CH_2)$ and $\nu_a(CH_2)$ have been long used as a measure of the status of packing of the molecules in the monolayer.[5-8] When the alkyl chains are highly ordered (trans-zigzag conformation), the bands appear near 2918 and 2848 cm^{-1}, respectively. However, if the alkyl chains exhibit conformational disorder, the stretching vibration modes are shifted near 2927 and 2856 cm^{-1}, depending on the ratio of gauche conformations. The hydrocarbon tails in the DPPC:Chol 1:1 monolayers transferred at 35 mN·m^{-1} present bands at 2918 and 2848 cm^{-1} (Figure S2), which is indicative of a high degree of order in the alkyl chains with a trans-zigzag conformation.

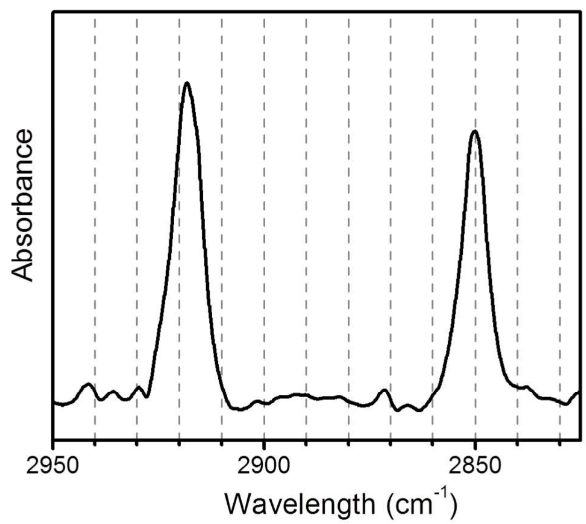

Figure S2. FTIR spectrum of a DPPC:Chol 1:1 monolayer transferred at a surface pressure of 35 mN·m^{-1}.

2. Bearing analysis of DPPC:Chol 1:1 LB films domain surface coverage

AFM imaging allows us to conclude that a variable surface coverage dependent on the immersion time has been achieved. Consequently, a bearing analysis of the recorded AFM images was carried out in order to estimate the percentage of the mica surface modified with DPPC:Chol domains. AFM images were reduced to binary images by thresholding with the depth value corresponding to the highest point of the peaks detected in the depth histograms. Thus, the bearing analysis tool allows obtaining the percentage of projected area occupied by features exhibiting depth values smaller than or equal to the thresholded one. The statistical analysis of coverage data registered over 5 x 5, 10 x 10, or 20 x 20 μm^2 areas from 5 different but equivalent AFM images rendered an average DPPC:Chol domain-coverage of 54 % for t = 20 min. (Figure S3), 49 % for t = 45 min. (Figure S4), and 47 % for t = 60 min. (Figure S5).

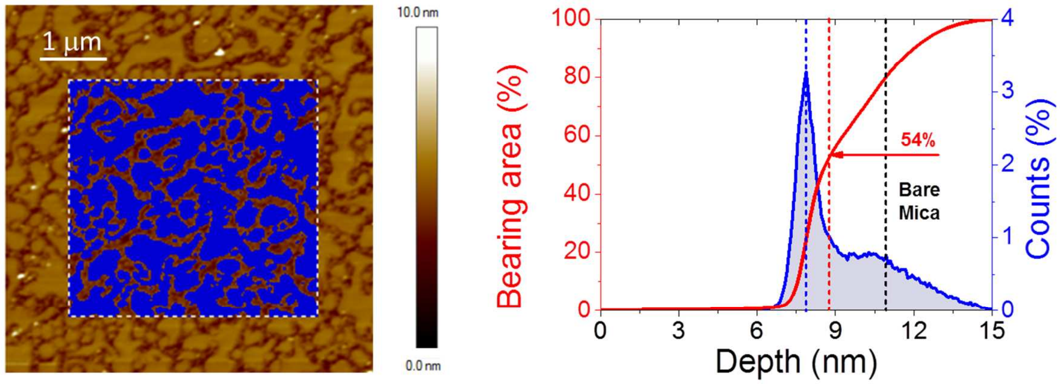

Figure S3. Left: 5.0 x 5.0 μm^2 AFM image of a monolayer LB film of DPPC:Chol 1:1 after immersion for 20 minutes in a 10 mM HEPES solution with the mask in blue unveiling molecule-free mica areas. Right: depth histogram showing the distribution of height data at different depths referred to a reference point, i.e. the highest pixel. The red line, i.e. bearing analysis, indicates the relative projected area covered at each depth value depicted as a blue mask in the topographic image corresponding to the white-dashed boxed area. The small peak in the histogram marked with a black-dashed vertical line is attributed to the unveiled bare mica substrate while the red arrow accounts for the selected height threshold corresponding to the average height of the measured bilayer domains.

Interestingly, and unlike those obtained for t = 20 min. and 60 min, the depth histogram registered for t = 45 min, depicted in Figure S4, exhibits two maxima, marked with blue-dashed vertical lines, corresponding to thicknesses of 4.9 and 5.4 nm for the as-obtained bilayer domains. These figures have been attributed to a different degree of packing for the time-dependent self-assembly of phospholipid alkyl chains in the arising upper leaflet layer. However, this slight height difference could also be tentatively related to the occurrence of less-coordinated phospholipid molecules located at the boundaries of the smaller bilayer islands which progressively incorporate to bigger domains. In any case, bearing analysis carried out at the afore-mentioned height threshold values, i.e. 4.9 and 5.4 nm, gave rise to surface coverages of ca. 24 and 25 %, respectively.

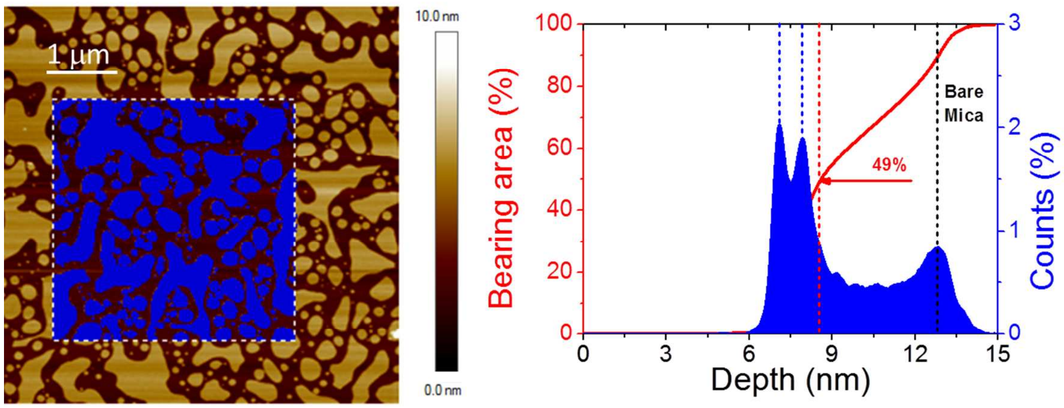

Figure S4. Left: 5.0 x 5.0 μm^2 AFM image of a monolayer LB film of DPPC:Chol 1:1 after immersion for 45 minutes in a 10 mM HEPES solution with the mask in blue unveiling molecule-free mica areas. Right: depth histogram showing the distribution of height data at different depths referred to a reference point, i.e. the highest pixel. The red line, i.e. bearing analysis, indicates the relative projected area covered at each depth value depicted as a blue mask in the topographic image corresponding to the white-dashed boxed area. The small peak in the histogram marked with a black-dashed vertical line is attributed to the unveiled bare mica substrate while the red arrow accounts for the selected height threshold corresponding to the average height of the measured bilayer domains.

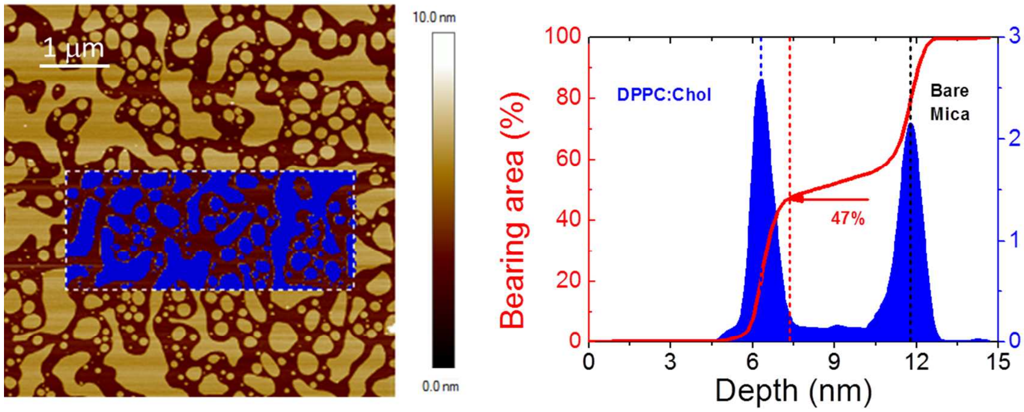

Figure S5. Left: 5.0 x 5.0 µm^2 AFM image of a monolayer LB film of DPPC:Chol 1:1 after immersion for 60 minutes in HEPES 10 mM with the mask in blue unveiling molecule-free mica areas. Right: depth histogram showing the distribution of height data at different depths referred to a reference point, i.e. the highest pixel. The red line, i.e. bearing analysis, indicates the relative projected area covered at each depth value depicted as a blue mask in the topographic image corresponding to the white-dashed boxed area. The small peak in the histogram marked with a black-dashed vertical line is attributed to the unveiled bare mica substrate while the red arrow accounts for the selected height threshold corresponding to the average height of the measured bilayer domains.

3. Restoration of DPPC:Chol monolayer after drying

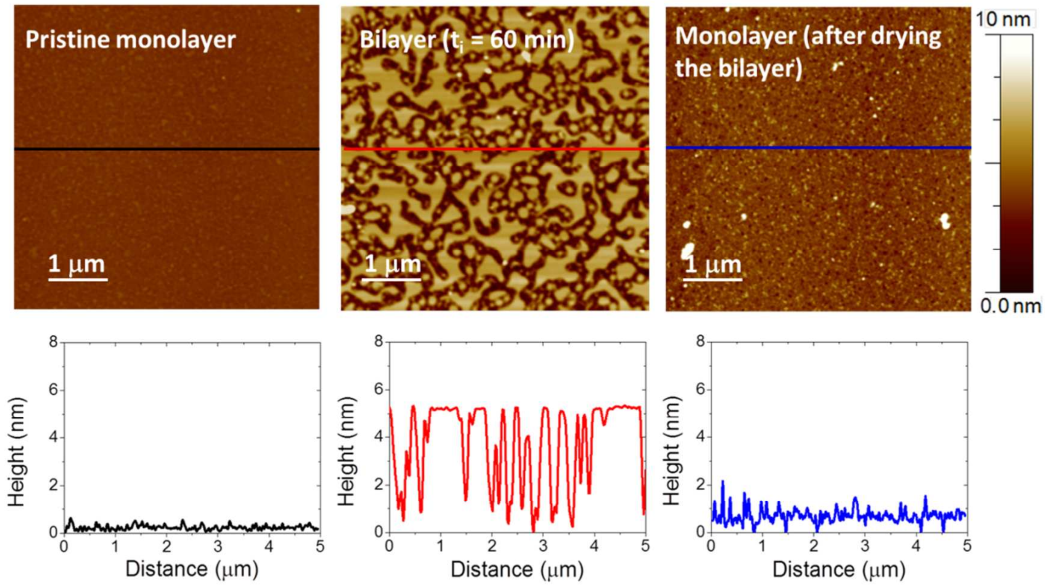

Figure S6. AFM topographic images corresponding to a pristine DPPC:Chol 1:1 monolayer LB film; bilayer formed after 60 minutes of immersion of the monolayer into a 10 mM HEPES solution; and regeneration of a monolayer after water was evaporated from the bilayer. Additionally, the corresponding cross-section profiles are included.

4. Measurement of the breakthrough force (F_b) for growing immersion times

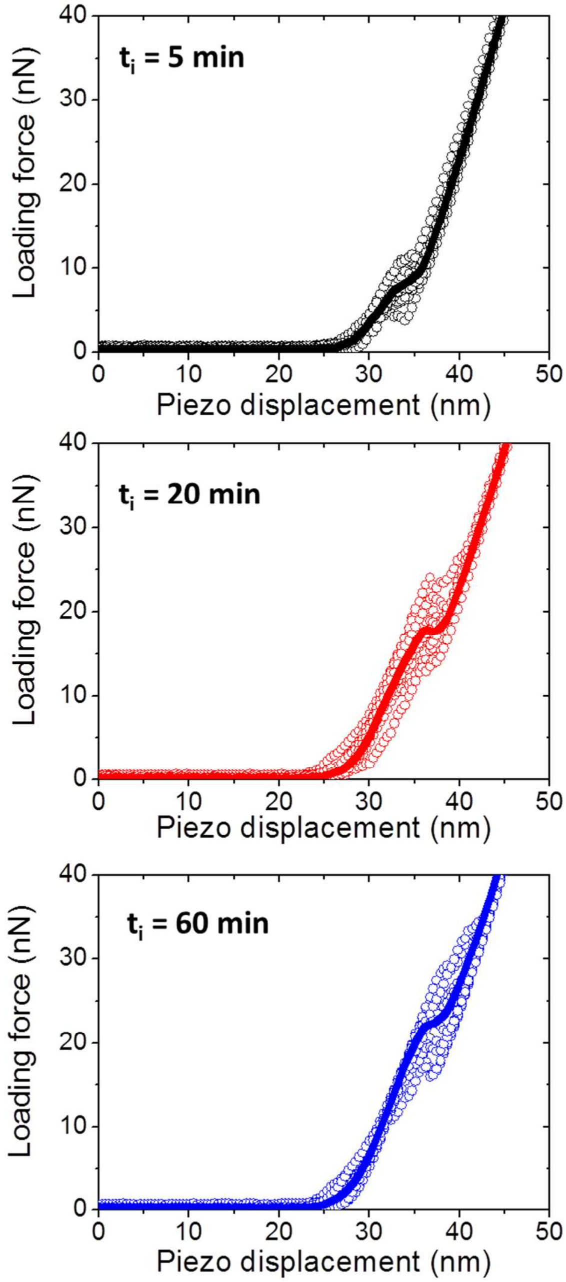

Figure S7. Representative loading force vs. piezoelectric displacement curves showing the breakthrough force value distribution for the indicated immersion times in a 10 mM HEPES solution. The corresponding average curves are depicted in solid line.

References

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