Direct measurement of the mechanical properties of lipid phases in supported bilayers

Laura Picas,* Felix Rico,* Simon Scheuring, *‡
* INSERM U1006, Institut Curie, 26 rue d'Ulm, Paris, France

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MATHERIAL AND METHODS

Sample preparation

SLBs were prepared following the method described (1). Briefly, large unilamellar vesicles (LUVs, diameter ~ 100 nm) were obtained by extrusion of multilamelar vesicles of DOPC : DPPC (1 : 1, mol : mol) in phosphate buffered saline (PBS). LUVs were deposited onto freshly cleaved mica disks mounted on a Teflon disk and incubated for 2 h at 60 °C. Bilayers were carefully rinsed with PBS buffer before imaging and were kept under aqueous environment.

Peak Force Quantitative Nano-Mechanics AFM measurements

AFM experiments were performed on a multimode-V microscope controlled by Nanoscope-V electronics and the Nanoscope 8 software (Bruker AXS Corporation, Santa Barbara, CA).

Images were acquired in PeakForce Quantitative Nano-Mechanics (PF-QNM) mode at different peak loading forces (100 – 550 pN). V-shaped Si_3N_4 cantilevers (MSNL, Bruker AXS Corporation, Santa Barbara, CA) with a nominal spring constant of 0.1 N/m and a nominal tip radius of 3 nm were used under liquid operation. Cantilever spring constant and sensitivity were calibrated before each experiment.

AFM image processing

Image and data processing was performed using commercial NanoScope Analysis Software (Bruker AXS Corporation, Santa Barbara, CA) and Gwyddion software, a modular free program for SPM data analysis (gwyddion.net).

Nanomechanical parameters of lipid phases

Average stiffness and deformation values obtained from different independent measurements at each of the studied loading forces are shown in supplementary table 1. Both mechanical parameters were acquired after typical roughness analysis of the images without application of plane fitting.

Determination of the Young's modulus of lipid bilayers on supported lipid vesicles.

Analogously to SLBs, unfused liposomes were topographically and mechanically mapped using PF-QNM AFM (Supplementary Fig. 2).

Force-distance curves acquired during PF-QNM imaging were analyzed assuming thin shell theory to determine the Young's modulus (E) of the liposome bilayer (3, 4). The slope in the contact region reflects the effective stiffness of the cantilever-liposome system (k_{eff}). The apparent stiffness of the liposome (k_{l}) was obtained assuming two springs loaded in series

$$k_l = \frac{k_c k_{eff}}{1 - k_{eff}}$$

where k_{c} is the spring constant of the cantilever.

For small deformations applied by a point load (4), the elastic force required to apply a deformation δ is given by

$$F = \frac{4}{\sqrt{3(1-\nu^2)}} \frac{Eh^2}{R_l} \delta = k_l \delta$$

being E and ν, the Young's modulus and Poisson ratio, respectively, R_l, the radius of the liposome (determined from AFM images to be $R_l = 129 \pm 14$ nm), h, the bilayer thickness, and δ, the deformation, which was calculated in terms of the point of contact (z_c) and deflection offset (d_0) as $\delta = z - z_c - (d - d_0)$. We assumed a Poisson ratio of 0.5 for an incompressible material. Using the average value of the slope obtained from curves at the center of the liposomes (12.3 ± 4.5 pN/nm), and the average thickness of the lipids in the gel and liquid phases, we obtained a Young's modulus of ~ 21 MPa, which lies between the values obtained on the liquid and gel phases. This result suggest that liposomes contained a mixture of DOPC and DPPC and confirm the measurements of E obtained on SLBs. Given the dimensions and geometry of liposomes, effects of the underlying mica substrate were not expected for the applied deformation (Supplementary Fig.2). Thus, the agreement in the

determination of E by both methods further confirms the reliability of our results presented in the main manuscript.

Determination of the elastic energetic cost of lipid mixing

The effect of having a mixture of lipids presenting hydrophobic mismatch leads to chain stretching that requires an energetic cost. Assuming volume conservation, the elastic energy of stretching a lipid molecule can be expressed by the difference in the actual thickness (h) and the preferred thickness (h_0) by

$$G_{stretch} = \frac{A_0}{h_0^2}\frac{K_A}{2}(h-h_0)^2$$

being A_0 the preferred lipid area (5). Using a typical value of A_0=0.73 nm^2 and A_0=0.55 nm^2 for DOPC and DPPC (5, 6), respectively together with the experimental values that we obtained for the bilayer thickness of DOPC and DPPC (4.1 ± 0.2 nm and 5.3 ± 0.4 nm, respectively) as the preferred and actual lipid thicknesses, we obtained an elastic stretching energy per molecule of 0.81 k_BT and 0.36 k_BT, respectively.

SUPPORTING FIGURES

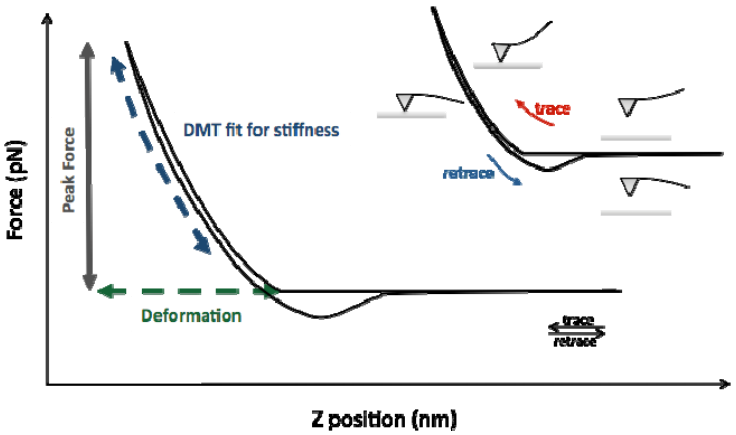

Fig S1. Scheme of a force distance curve used for the fitting of the different parameters that are obtained with the PF-QNM AFM imaging mode: Stiffness is obtained from fitting the contact region slope of the retracting curve using the Derjaguin-Muller-Toporov (DMT) model (2) and deformation is designated as the tip penetration at the peak force. The inset force curve picture displays the cantilever position and bending at each point of the approaching (trace) and retracting (retrace) curve.

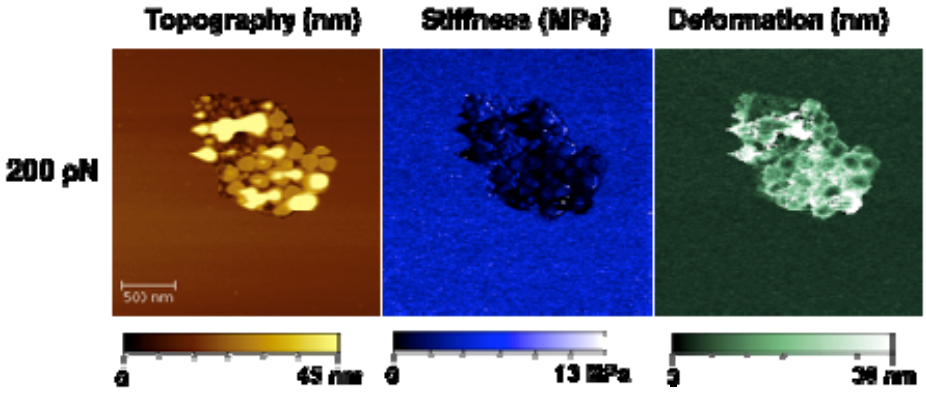

Figure S2. PF-QNM images of DOPC:DPPC (1:1) liposomes showing topography (nm), apparent stiffness (MPa), and deformation (nm) at a peak loading force of 200 pN. The false color scales are 20 nm (height; left), 13 MPa (apparent stiffness; middle) and 30 nm (deformation; right), respectively.

SUPPORTING TABLES

Table S1. Experimental values (Stiffness in MPa, and Deformation in nm) obtained from the nanomechanical measurement of the lipid phases at different loading forces.

	Stiffness (MPa)	
Loading force	**Liquid phase**	**Gel phase**
100 pN	5.3 ± 0.3	6.1 ± 0.4
200 pN	19.3 ± 2.6	28.1 ± 4.3
300 pN	40.3 ± 9.6	47.5 ± 6.5
400 pN	42.6 ± 5.9	55.2 ± 7.7
550 pN	49.0 ± 6.8	81.8 ± 13.1

	Deformation (nm)	
Loading force	**Liquid phase**	**Gel phase**
100 pN	1.2 ± 0.3	1.2 ± 0.4
200 pN	1.0 ± 0.3	1.2 ± 0.3
300 pN	1.4 ± 0.3	1.5 ± 0.3
400 pN	1.6 ± 0.3	2.0 ± 0.3
550 pN	2.4 ± 0.3	2.5 ± 0.2

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