

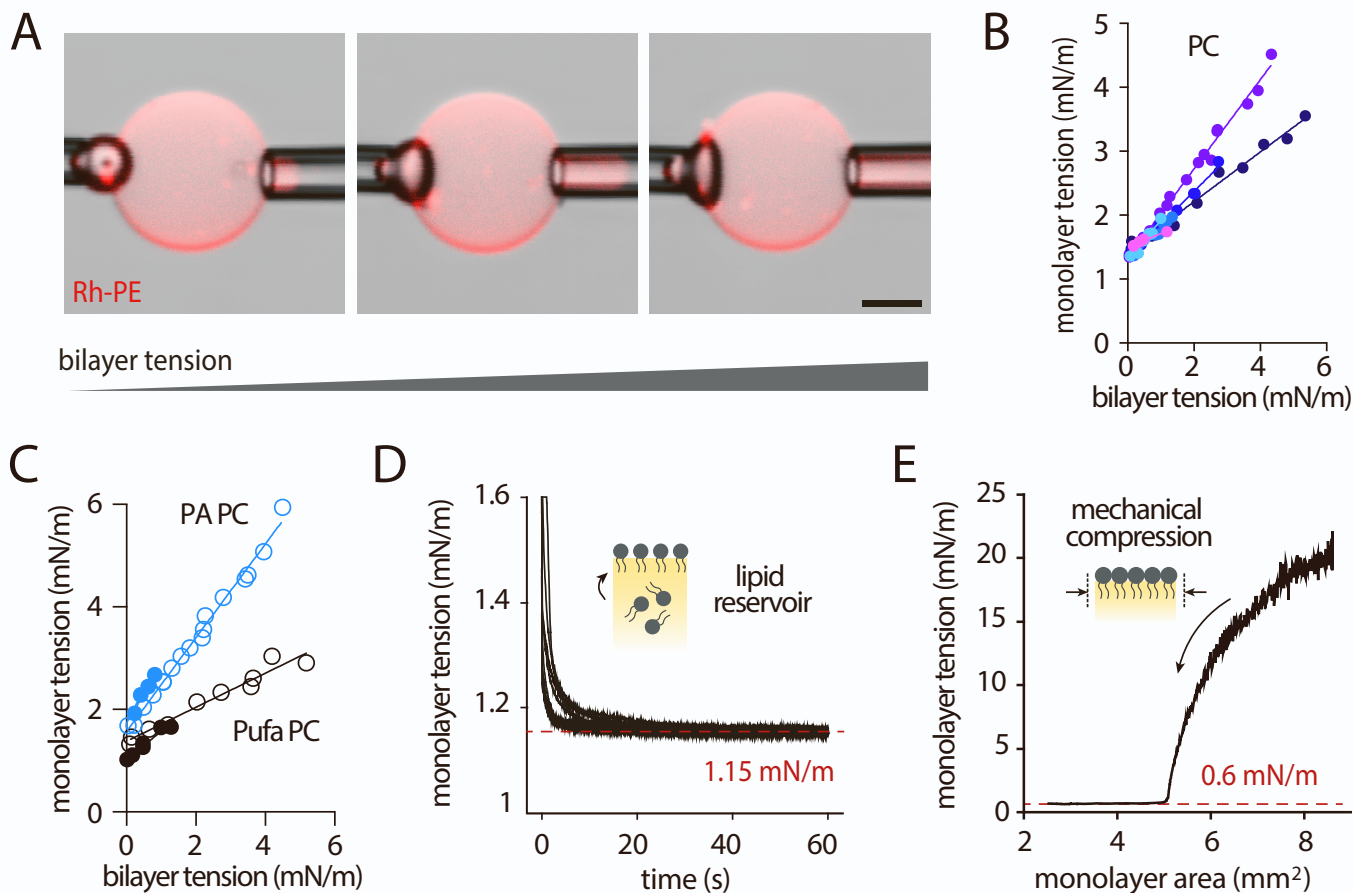
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**Supplemental information**

**Origin of gradients in lipid density and surface tension between connected lipid droplet and bilayer**

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## Supplemental Figures



### Supplementary figure 1, related to figure 2:

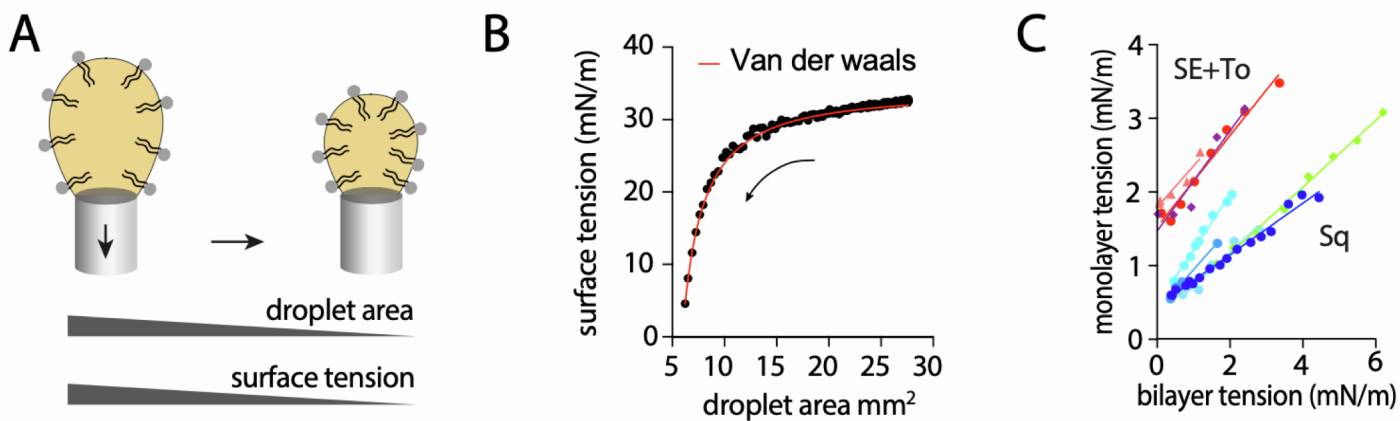
**A)** Confocal micrograph showing brightfield images of a triolein-DOPC DEV merge with the fluorescence image of the phospholipids (rhodamine-DOPE) while increasing its bilayer surface tension with the right micropipette and simultaneously measuring its monolayer surface tension using the left micropipette. Related to figure 2C. The scale bar is 10  $\mu\text{m}$ .

**B)** Monolayer-bilayer surface tension diagrams of triolein-DOPC DEVs having droplets with various asymmetric positions relative to the bilayer. Note that independently of the position of the droplet, the monolayer-bilayer surface tension curves have a near-linear variation.

**C)** Monolayer-bilayer surface tension diagrams of triolein-DEVs made with different phospholipid compositions: DOPA-DOPC (1-2) and Pufa-DOPC (1-2). The DEVs have droplets presenting various asymmetric positions relative to the bilayer. Note that independently of the position of the droplet and the phospholipid composition, the monolayer-bilayer surface tension curves have a near-linear variation.

**D)** Determination of the surface tension of a DOPC monolayer at a triolein- interface in contact with a phospholipid reservoir in the triolein phase. A triolein droplet containing a large amount of DOPC (0.5% w/w) is formed at the tip of a J-needle. Surface tension is recorded over time as the lipids relocate at the oil-buffer interface. The equilibrium surface tension is designated as the tension of a monolayer in contact with a large lipid reservoir. Several independent experiments are represented.

**E)** Determination of the surface tension of a DOPC monolayer at triolein-buffer interface when mechanically compressed. The surface tension of a DOPC-covered triolein droplet was recorded as the droplet surface area was reduced. The lower tension reached at the plateau regime is designated as the tension of a monolayer mechanically compressed.



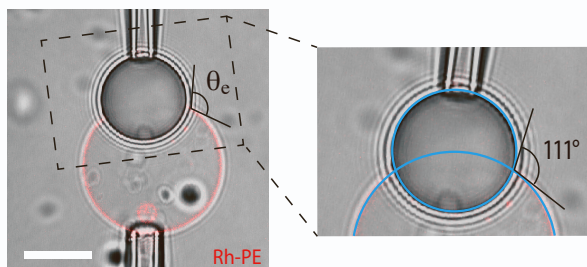
**Supplementary figure 2 related to figure 3:**

**A)** DOPC monolayer covering a triolein oil droplet was characterized by using a droplet tensiometer method. The phospholipid monolayer is compressed by reducing the droplet surface area while surface tension is recorded.

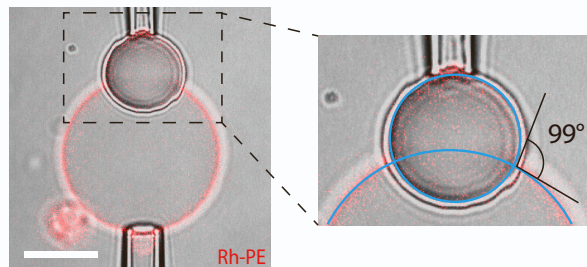
**B)** Resulting surface tension-droplet area isotherm fitted using van der Waals model, allowing to determine phospholipid monolayer characteristics (see material and methods).

**C)** Monolayer-bilayer surface tension diagrams of DOPC-DEVs of different oil compositions: sterol ester-triolein (1:3) (upper data) and squalene (lower data). The DEVs have droplets presenting various asymmetric positions relatively to the bilayer. Note that independently of the position of the droplet and the oil composition, the monolayer-bilayer surface tension curves have a linear variation.

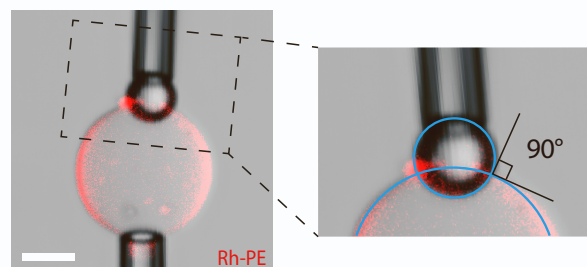
A



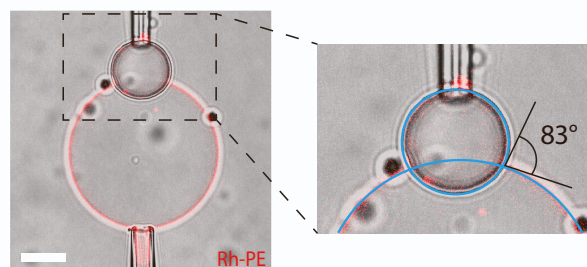
B



C



D



### Supplementary figure 3 related to figure 4:

Merge of brightfield and fluorescent micrographs of the DEVs used in Figure 4A at low bilayer tension. The phospholipids are reported by the red fluorescence signal of Rh-DOPE. The  $\theta_e$  angle reports the droplet asymmetry: if  $\theta_e > 90^\circ$ , the droplet is budding (A, B), if  $\theta_e = 90^\circ$  the droplet is centered (C), and if  $\theta_e < 90^\circ$ , the droplet is budding inward (D). Blue circles are the auscultating circles used to determine the contact angles (see material and methods). Scale bars are  $10\mu\text{m}$ .