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Supplemental Information

An Asymmetry in Monolayer Tension Regulates Lipid Droplet Budding Direction

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A Tension Asymmetry Between Monolayers Regulates the Budding Direction of Reconstituted Lipid Droplets

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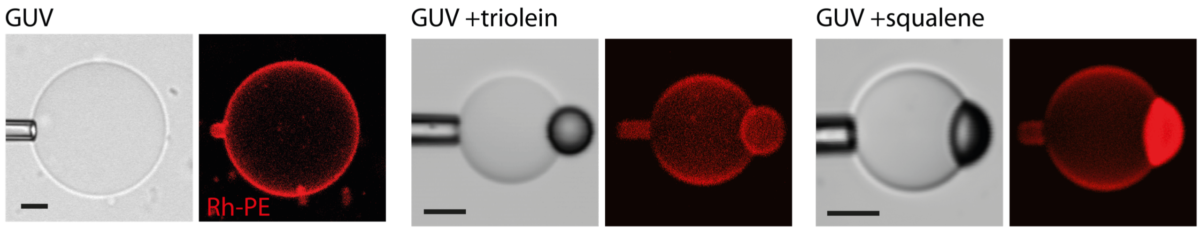
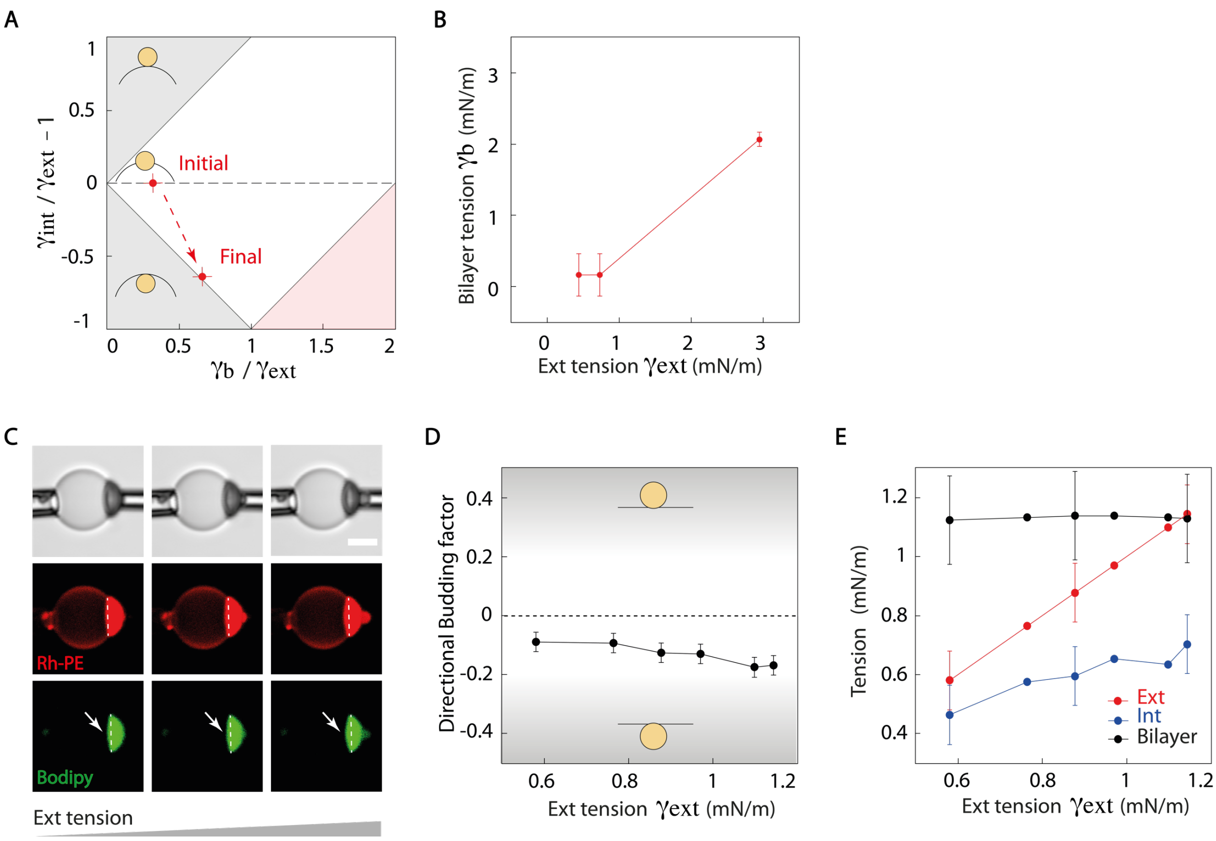
Supporting Material

Figures S1



Supplementary Figure S1 | Illustration of micro-aspiration measurement of the bilayer surface tension of PC GUV, PC GUV embedded with a triolein LD and PC GUV embedded with squalene LD. Scale bar is 10 µm.

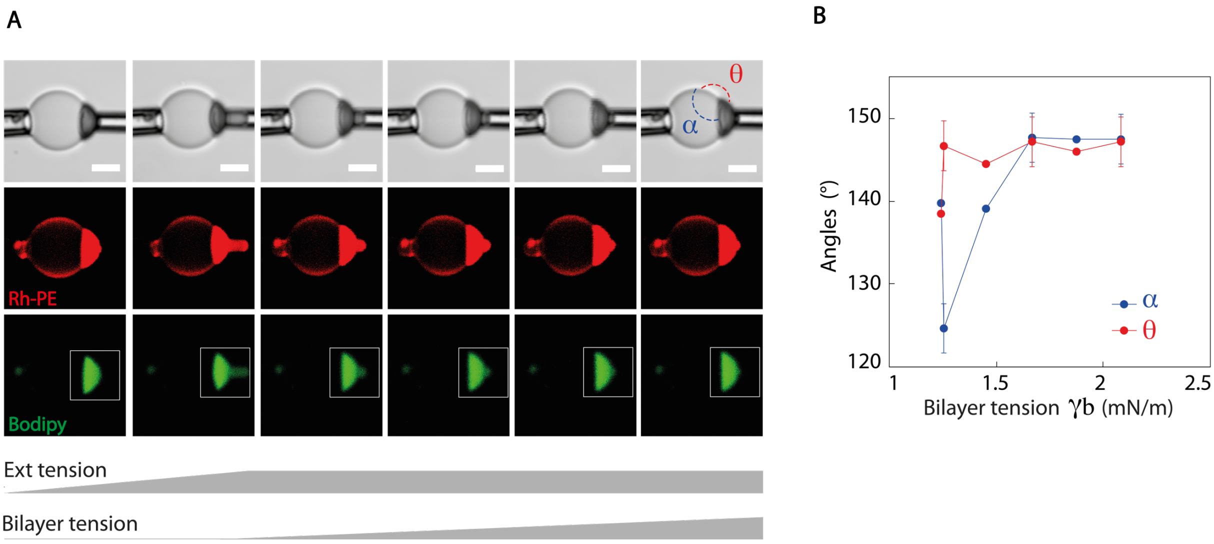
Figures S2



Supplementary Figure S2 | A) Initial and final states of the LD shape from the Fig. 4A, indicated on the phase diagram of Fig.3A. **B**) Bilayer tension corresponding to the experiment shown in Fig. 4A, calculated using the technique describe in supplementary text 2 **C)** Squalene LD embedded in a GUV.

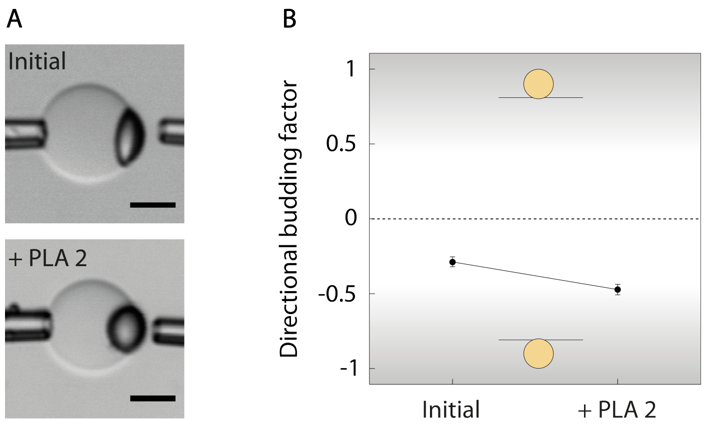
External monolayer tension in increased with right micropipette and bilayer tension is kept constant with the left micropipette. The LD tends to internalized (arrow). Scale bar is 10 µm. **D)** Corresponding Directional budding factor as a function of the external tension. **E)** Evolution of the three tensions as the external tension is increased. Bilayer tension is kept constant.

Figures S3



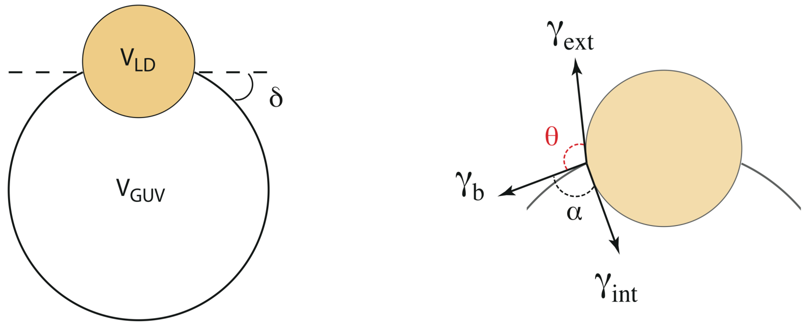
Supplementary Figure S3 | A) Full frame view of the experiment shown in (Fig. 5A). Scale bar is 10 µm **B)** Corresponding angles alpha and theta plotted as a function of the bilayer tension.

Figures S4



Supplementary Figure S4 | A) PLA2 enzyme is added to the external medium of a PC/PA (70/30) GUV embedding a triolein LD. (PA: dioleoylphosphatidic acid**)** PA was added to increase the bilayer tension and obtain droplets that were not readily budded, which often occurred with pure PC. The evolution of the droplet is observed over time. Scale bar is 10 µm. **B)** Decreasing directional budding factor showing a asymetrization of the LD position.

Supplementary text 1 | Determination of the shape and position of an LD in a GUV bilayer.



Considering that the lipid droplet's shape is driven by the equilibrium of the three surface tensions (bilayer γ_b, external monolayer γ_{ext} and internal monolayer γ_{int}) (Eq. 1), can be projected on the bilayer axis and lead to (Eq. 2).

$$\overrightarrow{\gamma_b} + \overrightarrow{\gamma_{int}} + \overrightarrow{\gamma_{ext}} = \vec{0} \qquad (1)$$

$$\gamma_b = -\gamma_{int}\cos(\alpha) - \gamma_{ext}\cos(\theta)$$
$$\gamma_{ext}\ \sin(\theta) = \gamma_{int}\ \sin(\alpha) \qquad (2)$$

Moreover, the conserved volume of the LD and the GUV gives two other equations (Eq. 3,4):

$$V_{LD} = \frac{4\,\Pi Ro^3}{3}\left(\frac{2-\cos(\theta-\delta)^3+3\cos(\theta-\delta)}{\sin(\theta-\delta)^3} + \frac{2-\cos(\alpha-\delta)^3+3\cos(\alpha-\delta)}{\sin(\alpha-\delta)^3}\right)$$
(3)

$$V_{GUV} = \frac{4\,\Pi Ro^3}{3}\left(\frac{2-\cos(\delta)^3+3\cos(\delta)}{\sin(\delta)^3} - \frac{2-\cos(\alpha-\delta)^3+3\cos(\alpha-\delta)}{\sin(\alpha-\delta)^3}\right)$$
(4)

In order to determine the shape of the droplet, we only need three parameters: alpha, theta and delta, as a function of the tensions γ_b, γ_{int} and γ_{ext}.

Rearranging these equations leads to a set of 3 equations (Eq. 5,6,7) that can be solved numerically. The following equations (5, 6) Gives alpha and theta:

$$\cos(\theta) = \frac{\gamma_{int}{}^2 - \gamma_{ext}{}^2 - \gamma_b{}^2}{2\,\gamma_b\,\gamma_{ext}} \qquad (5)$$

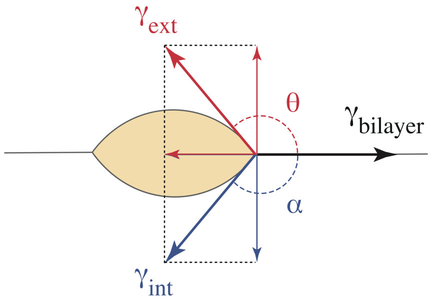
$$\cos(\alpha) = \frac{\gamma_{ext}{}^2 - \gamma_{int}{}^2 - \gamma_b{}^2}{2\,\gamma_b\,\gamma_{int}} \qquad (6)$$

The last parameter delta is determined numerically, keeping the volumes constant (Eq. 7):

$$\frac{V_{LD}}{V_{GUV}} = \frac{\dfrac{2-\cos(\theta-\delta)^3+3\cos(\theta-\delta)}{\sin(\theta-\delta)^3} + \dfrac{2-\cos(\alpha-\delta)^3+3\cos(\alpha-\delta)}{\sin(\alpha-\delta)^3}}{\dfrac{2-\cos(\delta)^3+3\cos(\delta)}{\sin(\delta)^3} - \dfrac{2-\cos(\alpha-\delta)^3+3\cos(\alpha-\delta)}{\sin(\alpha-\delta)^3}}$$
(7)

The profiles of the embedded LDs displayed in (Fig. 4B) are obtained using this mathematical model.

Supplementary text 2 | Determination of surface tensions.



Given that the LD shape is driven by surface tension, the following equation describes the link between the bilayer tension γ_b, the external tension γ_{ext}, and the internal tension γ_{int}:

$$\gamma_b = -\gamma_{int}\cos(\alpha) - \gamma_{ext}\cos(\theta) \quad (1)$$

$$\gamma_{ext}\sin(\theta) = \gamma_{int}\sin(\alpha) \quad (2)$$

Measuring one of the tensions and the two angles (θ and α) allows us to determine the two other tensions using the two former equations. For example, knowing alpha, theta, and the external tension enable the determination of the bilayer tension by $\gamma_b = -(\gamma_{ext}\mathrm{cotan}(\alpha)\ \sin(\theta) + \cos(\theta))$, and γ_{int} through equation (2).