Origin of gradients in lipid density and surface tension between connected lipid droplet and bilayer: supplementary text

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1   Theoretical description: basic assumptions

The phospholipid (PL) bilayer (b), as well as the monolayer at the "external" droplet interface (me) and the monolayer at the "internal" droplet interface (mi), are described as soft and homogeneous fluid interfaces joining at a contact line, Figure 1B and C. The free energy of this three interfaces system is,

$$F = F_{me} + F_{mi} + F_b + \tau L \ , \tag{1}$$

where F_b, F_{me} and F_{mi} are the free energy of the three interfaces, the last term is the energy cost associated to the contact line with L the length of the contact line and τ its line tension. The oil phase in the droplet and aqueous phase around the interfaces are supposed incompressible; their bulk contributions to the free energy are constant (independent of the droplet shape and PL distribution between the interfaces) and skipped in equation (1).

    This description is valid for droplets with a radius R much larger than monolayer elastic length $\lambda = \sqrt{\kappa_m/\gamma_m} \sim 1 - 10$ nm, where κ_m is the bending rigidity of a monolayer at the droplet interface and γ_m its surface tension. In this large droplet regime, the bending elasticity of the interfaces and the long-range interactions between the monolayers are negligible. In fact, elasticity and interfaces interaction control the
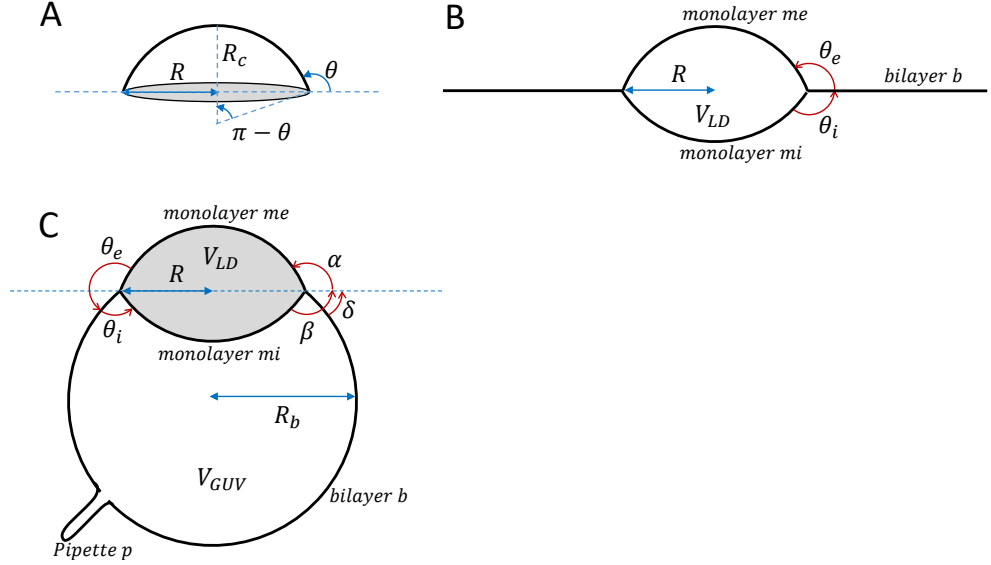
Figure 1: A) Geometry of a spherical cap of curvature radius R_c, angle $\pi - \theta$. B) Scheme of a LD connected to a flat bilayer. C) Scheme of a DEV: a LD connected to spherical bilayer.

shape of the interfaces at the nanometer scale at the vicinity of the contact line, in a region of width λ reminiscent to the "transition" region in wetting phenomena. Elasticity and interfaces interaction ensure in particular the smooth transition between monolayers and bilayer [1, 2]. In a large scale description, $R \gg \lambda$, the transition region is considered to be infinitesimal and the energy contribution of this region corresponds to the line energy (the last term) in equation (1). The line tension is due mainly to the elastic energy associated to the monolayers deformation near the contact line and scales as $\tau \sim \sqrt{\kappa_m \gamma_m}$ [1] and is thus of the order of 10 pN.

For a droplet embedded in a large membrane under very tension, this effective line tension has been shown to be able to destabilize the droplet leading to its spontaneous budding. However, in this paper focusing on large droplets in situations where they are stable, embedded in their host membrane, the line tension is irrelevant since its influence to the shape is negligible as compare to that of the surface tensions (see discussion section 3). So in the following and in the main text, we make the approximation $\tau = 0$.

2 Chemical potentials and surface tensions definitions

We denote A_b, A_{me} and A_{mi} the area of the three interfaces; N_{me} and N_{mi} the number of phosholipids in each monolayer at the LD interface; N_{be} and N_{bi}, the numbers of phospholipids in the external and internal leaflets of the bilayer. The surface densities of phosholipids in the LD monolayers and in the bilayer leaflets are denoted $\rho_{me} = N_{me}/A_{me}$, $\rho_{mi} = N_{mi}/A_{mi}$, $\rho_{be} = N_{be}/A_b$ and $\rho_{bi} = N_{bi}/A_b$.

The chemical potentials of the external and internal droplet monolayers, and of the internal and external leaflets of the bilayer are defined by,

$$\mu_{me} = \left.\frac{\partial F_{me}}{\partial N_{me}}\right|_{A_{me}} , \quad \mu_{mi} = \left.\frac{\partial F_{mi}}{\partial N_{mi}}\right|_{A_{mi}} , \quad \mu_{be} = \left.\frac{\partial F_b}{\partial N_{be}}\right|_{A_b, N_{bi}} , \quad \mu_{bi} = \left.\frac{\partial F_b}{\partial N_{bi}}\right|_{A_b, N_{be}} . \tag{2}$$

The surface tensions of the droplet interfaces and of the bilayer are defined by,

$$\gamma_{me} = \left.\frac{\partial F_{me}}{\partial A_{me}}\right|_{N_{me}} , \quad \gamma_{mi} = \left.\frac{\partial F_{mi}}{\partial A_{mi}}\right|_{N_{mi}} , \quad \gamma_b = \left.\frac{\partial F_b}{\partial A_b}\right|_{N_{be}, N_{bi}} . \tag{3}$$

The interfaces are homogeneous and soft (no rigidity), by extensivity, the interfaces free energy can be written,

$$F_{me}(A_{me}, N_{me}) = A_{me} f_m(\rho_{me}) , \quad F_{mi}(A_{mi}, N_{mi}) = A_{mi} f_m(\rho_{mi}) , \quad F_b(A_b, N_{be}, N_{bi}) = A_b f_b(\rho_{be}, \rho_{bi}) ,$$

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where f_m and f_b are the free energy per surface unit of a LD interface and of a PL bilayer. The chemical potentials and surface tensions can hence be written in term of f_m and f_b as,

$$\mu_{me} = \mu_m(\rho_{me}) \ \ \text{and} \ \ \mu_{mi} = \mu_m(\rho_{mi}) \ , \ \ \text{where} \ \ \mu_m(\rho) = \partial_\rho f_m(\rho) \ , \tag{4}$$

$$\gamma_{me} = \gamma_m(\rho_{me}) \ \ \text{and} \ \ \gamma_{mi} = \gamma_m(\rho_{mi}) \ , \ \ \text{where} \ \ \gamma_m(\rho) = f_m(\rho) - \rho\partial_\rho f_m(\rho) \tag{5}$$

$$\mu_{be} = \mu_b(\rho_{be}, \rho_{bi}) \ \ \text{and} \ \ \mu_{bi} = \mu_b(\rho_{bi}, \rho_{be}) \ , \ \ \text{where} \ \ \mu_b(\rho, \rho') = \partial_\rho f_b(\rho, \rho') \ , \tag{6}$$

$$\gamma_b(\rho_{be}, \rho_{bi}) = f_b(\rho_{be}, \rho_{bi}) - \rho_{be}\mu_{be} - \rho_{bi}\mu_{bi} \ , \tag{7}$$

where $\mu_b(\rho, \rho')$ is the chemical potential of the bilayer leaflet of density ρ, so that the chemical potential of the other leaflet is $\mu_b(\rho', \rho)$ since $\partial_{\rho'} f_b(\rho, \rho') = \partial_\rho f_b(\rho', \rho)$. Chemical potentials and surface tensions are related by Gibbs-Duhem relations,

$$d\gamma_m = -\rho_m d\mu_m \ \ \text{and} \ \ d\gamma_b = -\rho_{be} d\mu_{be} - \rho_{bi} d\mu_{bi} \ , \tag{8}$$

which can be derived straightforwardly from equations (4, 5, 6, 7).

For later convinience, we define the stretching modulus of a LD interface and of the bilayer,

$$K_{me} = A_{me}\frac{\partial\gamma_{me}}{\partial A_{me}} \ , \ \ K_{mi} = A_{mi}\frac{\partial\gamma_{mi}}{\partial A_{mi}} \ , \ \ K_b = A_b\frac{\partial\gamma_b}{\partial A_b} \ . \tag{9}$$

They can be written as,

$$K_{me} = K_m(\rho_{me}) \ \ \text{and} \ \ K_{mi} = K_m(\rho_{mi}) \ , \ \ \text{where} \ \ K_m(\rho) = -\rho\partial_\rho\gamma_m(\rho) \tag{10}$$

$$K_b(\rho_{be}, \rho_{bi}) = K_{be} + K_{bi} \ , \ \ \text{where} \ \ K_{be} = -\rho_{be}\frac{\partial\gamma_b}{\partial\rho_{be}}\bigg|_{\rho_{bi}} \ \ \text{and} \ \ K_{bi} = -\rho_{bi}\frac{\partial\gamma_b}{\partial\rho_{bi}}\bigg|_{\rho_{bi}} \ , \tag{11}$$

3 Thermodynamic equilibrium conditions

At equilibrium, the area and shape of the three interfaces (LD interfaces and bilayer), as well as their numbers of phospholipids, minimize the free energy $F = F_b + F_{me} + F_{mi}$ under the constrains of the conservation of the total number of phospholipids in each membrane leaflet (bilayer leaflet + contiguous LD monolayer), and the conservation of the volumes delimited by the interfaces. The minimization leads to well-known equilibrium conditions. The morphology of the interfaces obey the Laplace law,

$$P_{LD} - P_e = 2\gamma_{me}H_{me} \ , \ \ P_{LD} - P_i = 2\gamma_{mi}H_{mi} \ , \ \ P_i - P_e = 2\gamma_b H_b \ , \tag{12}$$

where P_e, P_i, P_{LD} are the pressure of the fluid on the external and internal side of the membrane, and the droplet pressure ; H_{me}, H_{mi}, H_b are the mean curvature of the external monolayer, internal monolayer and bilayer. At the contact line, the balance of the forces, and the balance of the phosholipid fluxes between the LD monolayers and the bilayer, impose the equilibrium relations (neglecting the line tension of the droplet),

$$\vec{\gamma}_{me} + \vec{\gamma}_{mi} + \vec{\gamma}_b = 0 \ , \tag{13}$$

$$\mu_{me} = \mu_{be} \ , \tag{14}$$

$$\mu_{mi} = \mu_{bi} \ . \tag{15}$$

The equilibrium state of the system is determined by these conditions supplemented by external constrains, which depend on the practical situation considered. Different cases are discussed in sections 4 and 5.

Remarques:

- The mechanical equilibrium conditions (12, 13) can also be recovered starting from a more complete description of the system, taking into accounts the elasticity of the LD interfaces and bilayer, and taking the limit of a large droplet $R \gg \sqrt{\kappa_m/\gamma_m}$ where κ_m is the bending rigidity of the monolayer a the droplet interface, see ref. [1].

- Including the line tension, the force balance at the droplet rim reads $\vec{\gamma}_{me} + \vec{\gamma}_{mi} + \vec{\gamma}_b - (\tau/R)\,\vec{e}_r$ where $\vec{e}_r$ is the radial unit vector in the plan contact line. For a droplet of radius $R = 1$ μm and a line tension $\tau = 10$ pN, the line tension is totally irrelevant unless the bilayer tension is lower than 10^{-5} N/m. At such very low bilayer surface tension, for a droplet in a large membrane as compare to the droplet size, the embedded droplet may become unstable and buds ouward the membrane. In other situations (larger bilayer tension, larger droplet, finite size membrane that fordids droplet budding), on which we focus in this paper, where the droplet is stable in the membrane, the line tension has a negligible effect since τ/R is always much lower than the droplet surface tensions $\gamma_{me,mi}$.

4 Equilibrium state of a droplet in a flat bilayer under tension

This situation is depicted figure 1B. The applied tension on the bilayer, γ_{ext}, the oil volume V_{LD} and the number of phospholipids in each membrane leaflet $N_{me} + N_{be} = N_e$ and $N_i = N_{mi} + N_{bi}$ are fixed. The LD shape is axisymmetric and can then be characterized by three variables: the contact angles θ_e, θ_i and the contact line radius R. The two monolayers have a spherical cap shape; the mean curvature of the interfaces are $H_{me} = \sin\theta_e/R$, $H_{mi} = \sin\theta_i/R$, $H_b = 0$. We denote

$$A_c(R,\theta) = 2\pi R^2 \frac{1+\cos\theta}{\sin^2\theta} \quad \text{and} \quad V_c(R,\theta) = \frac{\pi R^3}{3} \frac{2-\cos^3\theta + 3\cos\theta}{\sin^3\theta} \ , \tag{16}$$

the area and volume of a spherical cap with base radius R and polar angle $\pi - \theta$, figure 1A. The volume of the droplet and area of the monolayers expressed in terms of θ_e, θ_i and R, are, $V_{LD} = V_c(R,\theta_e) + V_c(R,\theta_i)$ and, $A_{me} = A_c(R,\theta_e)$, $A_{mi} = A_c(R,\theta_i)$.

4.1 General case

There are eight independent variables free to adjust and fully characterizing the thermodynamic state of the system: ρ_{me}, ρ_{mi}, ρ_{be}, ρ_{bi}, θ_e, θ_i, R and A_b. Their equilibrium value satisfies the equilibrium conditions,

$$\mu_m(\rho_{me}) = \mu_b(\rho_{be}, \rho_{bi}) \quad \text{and} \quad \mu_m(\rho_{mi}) = \mu_b(\rho_{bi}, \rho_{be}) \ , \tag{17}$$

$$\gamma_m(\rho_{me})\cos\theta_e + \gamma_m(\rho_{me})\cos\theta_i + \gamma_b(\rho_{be}, \rho_{bi}) = 0 \quad \text{and} \quad \gamma_m(\rho_{me})\sin\theta_e = \gamma_m(\rho_{mi})\sin\theta_i \ , \tag{18}$$

where the second line is the mechanical equilibrium condition (14) an axisymmetrical system, and the constrains,

$$\gamma_b(\rho_{be}, \rho_{bi}) = \gamma_{\text{ext}} \ , \tag{19}$$

$$V_c(R,\theta_e) + V_c(R,\theta_i) = V_{LD} \ , \tag{20}$$

$$\rho_{me}A_c(R,\theta_e) + \rho_{be}A_b = N_e \quad \text{and} \quad \rho_{mi}A_c(R,\theta_i) + \rho_{bi}A_b = N_i \ . \tag{21}$$

In general, morphology of the droplet (characterized by R and $\theta_{e/i}$) and PL distribution between the interfaces are coupled though the constrains PL conservation (21). The values of the densities and angles depend upon the oil volume V_{LD} and the number of PL in each leaflet of the membrane N_e, N_i. However, there are two important situations discussed in the following where morphology and PL distribution are thus disentangled: (i) symmetrical membrane and (ii) infinite bilayer.

4.2 Flat symmetrical membrane

For a symmetrical membrane with identical leaflets, $\rho_{me} = \rho_{mi} = \rho_m$ and $\rho_{be} = \rho_{bi} = \rho_b$, the PL densities are directly imposed by the bilayer tension and chemical potential balance (14),

$$\mu_m(\rho_m) = \mu_b(\rho_b) \ , \tag{22}$$

$$\gamma_b(\rho_b) = \gamma_{\text{ext}} \ . \tag{23}$$

Hence, the densities are independent of the morphology and size of the droplet, and independent of the total number of PL molecules in the membrane. The surface tensions of the monolayers are equal, $\gamma_{me} = \gamma_{mi} = \gamma_m(\rho_m)$ and then the mechanical equilibrium (14) gives,

$$\theta_e = \theta_i = \theta \quad \text{and} \quad 2\gamma_m(\rho_m)\cos\theta + \gamma_b(\rho_b) = 0 \ , \tag{24}$$

which determine the contact angle. The contact line radius is determined by the oil volume, $V_c(R,\theta) = V_{LD}/2$, and the area of the bilayer by the total number of PL per leaflet $N_e = N_i = \rho_m A_c(R,\theta) + \rho_b A_b$.

4.3 Flat infinite membrane

In the case of an asymmetrical membrane whose area is much larger than the area of the droplet interfaces, $A_{me/mi} \ll A_b$, the density of PL in each leaflet of the bilayer is independent of the presence of the droplet, $\rho_{be} = N_e/A_b$ and $\rho_{bi} = N_i/A_b$ (according to (21)). Under the condition of imposed bilayer tension, the PL densities in the bilayer leaflets are given by,

$$\rho_{be}/\rho_{bi} = N_e/N_i \ , \tag{25}$$

and (19). The density of PL in the monolayers are then given by (17). As for a symmetrical membrane, the densities are independent of the droplet morphology and size.

The two contact angles are determined by the mechanical equilibrium condition (18) and the contact line radius by the oil volume (20).

5 Equilibrium state of a droplet in a spherical vesicle (DEV system)

The geometry of a droplet embedded in the membrane of a spherical vesicle (DEV system) is axisymmetric and depicted Figure 1C. The three interfaces (monolayers and bilayer) are spherical caps. The morphology can be characterized by the contact line radius R and the three angles α, β, δ. The mean curvature of the interfaces is then $H_{me} = \sin\alpha/R$, $H_{mi} = \sin\beta/R$, $H_b = \sin\delta/R$. The contact angles between the monolayers and the bilayer at the contact line are,

$$\theta_e = \alpha + \delta \quad \text{and} \quad \theta_i = \beta - \delta \ . \tag{26}$$

5.1 Free DEV

For a free DEV, the fixed quantities are the oil volume V_{LD}, the inner volume in the vesicle V_{GUV} and the number of PL in each leaflet, N_e and N_i. There are eight free variables in this system, ρ_{me}, ρ_{mi}, ρ_{be}, ρ_{bi}, θ_e, θ_i, R and δ. These free variables satisfy the four equilibrium relations (17, 18) as well as four constrains, fixed oil volume, fixed vesicle volume and fixed PL number in each leaflet, which read

$$V_c(R,\alpha) + V_c(R,\beta) = V_{LD} \quad \text{and} \quad V_c(R,\delta) - V_c(R,\beta) = V_{GUV}, \tag{27}$$

$$\rho_{me}A_c(R,\alpha) + \rho_{be}A_c(R,\delta) = N_e \quad \text{and} \quad \rho_{mi}A_c(R,\beta) + \rho_{bi}A_c(R,\delta) = N_i \ , \tag{28}$$

where the angle α and β are related to the contact angles by equation (26) and the functions V_c and A_c are given by equations (16).

5.2 DEV under fixed tension

Experimentally, the bilayer tension of the DEV can be controlled by pipette aspiration. For this purpose, a part of the vesicle bilayer is sucked in a thin pipette. In this system, there is an additional free variable as compared to the free DEV, the area A_p of the bilayer in the pipette, and an additional constrain, the imposed bilayer tension γ_{ext}. The nine free variables obey the four equilibrium relations (17, 18), the condition of fixed bilayer tension (19), the conditions of oil and vesicle volumes conservation given by (27) since the volume of the vesicle sucked in the pipette is neglible, and the conservation of PL in each leaflet,

$$\rho_{me}A_c(R,\theta_e - \delta) + \rho_{be}\left(A_c(R,\delta) + A_p\right) = N_e \quad \text{and} \quad \rho_{mi}A_c(R,\theta_i + \delta) + \rho_{bi}\left(A_c(R,\delta) + A_p\right) = N_i \ , \tag{29}$$

5.3 DEV with tensionless bilayer

When the surface tension of the bilayer vanishes, the mechanical equilibrim condition (13) imposes $\vec{\gamma}_{me} = -\vec{\gamma}_{mi}$ and thus,

$$\gamma_{me} = \gamma_{mi} \quad \text{and} \quad \theta_i = \theta_e - \pi \ ,$$

the droplet is spherical. The equality of the monolayer tension $\gamma_m(\rho_{me}) = \gamma_m(\rho_{mi})$ implies the equality of the monolayer density and then, following equation (14), the equality of the bilayer leaflet density,

$$\rho_{me} = \rho_{mi} = \rho_m \quad \text{and} \quad \rho_{be} = \rho_{bi} = \rho_b \ ,$$

where ρ_m and ρ_b obey $\gamma_b(\rho_b) = 0$ and $\mu_b(\rho_b) = \mu_m(\rho_m)$. In the case of a tensionless bilayer, as in the cases of symmetrical membrane and infinite bilayer, the PL distribution is independent of the LD and vesicle size.

6 Relations between surface tension variations

We investigate here the change of the LD surface tensions triggered by an infinitesimal change of the applied tension to the bilayer $d\gamma_{\text{ext}} = d\gamma_b$, while the oil volume, number of PL in each leaflet (and vesicle inner volume in the case of a DEV) remain constant.

In the general situation of an oil droplet embedded in a finite size membrane with asymmetric leaflet, such as in the DEV system, the morphology of the oil droplet and the distribution of PL in the different bilayer leaflets and monolayers are entangled. Deriving the variations of LD surface tensions, requires to differentiate the full set of coupled equations satisfied by the free variables and solve the resulting linear system. Though there is no technical difficulty, it leads to very complicated, and therefore useless, expressions.

However, in the particular cases considered section 4.2 and 4.3, the distribution of PL, and thus the values of the surface tensions, are independent of the droplet morphology and size. The surface tensions of the bilayer and monolayer are coupled only through the condition of chemical potential equilibrium between adjacent LD monolayer and bilayer leaflet (14, 15), allowing to derive simple relations between the infinitesimal changes of surface tensions of the three interfaces. We also consider the case of an initially tensionless bilayer where, though the change of morphology and PL distribution are linked, simple relations can be obtained.

6.1 Surface tension coupling due to chemical potential equilibrium

A change of the bilayer tension is associated to a change of the density of its leaflets. This last induces a migration of the PL toward (or from) the monolayers at the droplet interface to re-establish the equilibrium of the chemical potentials (17). This redistribution modifies the monolayers densities and thereby the droplet interfaces tensions. A general relation between the surface tension variations due to this mechanism can be derived, by differentiating equations (14, 15), $d\mu_{be} = d\mu_{me}$ and $d\mu_{bi} = d\mu_{mi}$ and using the Gibbs-Duhem relations (8),

$$d\gamma_b = \frac{\rho_{be}}{\rho_{me}}d\gamma_{me} + \frac{\rho_{bi}}{\rho_{mi}}d\gamma_{mi} \ . \tag{30}$$

6.2 Symmetric membrane

In the situation considered section 4.2, PL distribution, and thus surface tensions, are only coupled by the condition of chemical potential balance (23, 22), there is no feedback from the system morphology. Then, there is an unequivocal relation between bilayer and monolayer tension. The variation of the bilayer and monolayer chemical potentials are $d\mu_m = d\gamma_m/\rho_m = d\mu_b = d\gamma_b/\rho_b$, and thus,

$$d\gamma_m = \frac{\rho_m}{2\rho_b}d\gamma_b \ .$$

6.3 Flat infinite bilayer

In the situation considered section 4.3, where the bilayer area is much larger than that of the droplet, equation (25) implies that the relative change of the bilayer leaflet densities are equal and given by,

$$\frac{d\rho_{be}}{\rho_{be}} = \frac{d\rho_{bi}}{\rho_{bi}} = -\frac{d\gamma_b}{K_b} \ , \tag{31}$$

where the second equality follows from equation (11). The variation of the chemical potentials of contiguous bilayer leaflet and monolayer must be equal, according to (14, 15), and are given by,

$$d\mu_{me} = d\mu_{be} = \frac{1}{\rho_{be}}\frac{K_{be}}{K_b}d\gamma_b \ \text{ and } \ d\mu_{mi} = d\mu_{bi} = \frac{1}{\rho_{bi}}\frac{K_{bi}}{K_b}d\gamma_b \ . \tag{32}$$

To demonstrate this formula, one should use,

$$d\mu_{be} = \frac{\partial\mu_{be}}{\partial\rho_{be}}d\rho_{be} + \frac{\partial\mu_{be}}{\partial\rho_{bi}}d\rho_{bi} = \left(\rho_{be}\frac{\partial\mu_{be}}{\partial\rho_{be}} + \rho_{bi}\frac{\partial\mu_{be}}{\partial\rho_{bi}}\right)\frac{d\rho_{be}}{\rho_{be}} = \left(\rho_{be}\frac{\partial\mu_{be}}{\partial\rho_{be}} + \rho_{bi}\frac{\partial\mu_{bi}}{\partial\rho_{be}}\right)\frac{d\rho_{be}}{\rho_{be}} = \frac{\partial\gamma_b}{\partial\rho_{be}}\frac{d\rho_{be}}{\rho_{be}} \ ,$$

where we have used successively equation (31), the equality $\partial_{\rho_{be}}\mu_{bi} = \partial_{\rho_{bi}}\mu_{be}$ and equation (8). (The second equality in eq. 32 is obtained in analogous manner by exchanging the subscript e and i.) Inserting (32) in the Gibbs-Duhem relation (8) directly gives,

$$d\gamma_{me} = \beta_e\frac{\rho_{me}}{\rho_{be}}d\gamma_b \ \text{ and } \ d\gamma_{mi} = \beta_i\frac{\rho_{mi}}{\rho_{bi}}d\gamma_b \ , \tag{33}$$

with,

$$\beta_e = \frac{K_{be}}{K_b} \ \text{ and } \ \beta_i = 1 - \beta_e = \frac{K_{bi}}{K_b}$$

where K_{be} and K_{be} are defined in equation (11).

6.4 Tensionless bilayer

In the case $\gamma_b = 0$, as shown section 4.3, $\theta_i = \theta_e - \pi$ (spherical droplet), $\gamma_{me} = \gamma_{mi}$, $\rho_{me} = \rho_{mi} = \rho_m$ and $\rho_{bi} = \rho_{be} = \rho_b$. Differentiating equations (18) gives,

$$\cos\theta_e d\gamma_{me} + \cos\theta_i d\gamma_{mi} + d\gamma_b - \gamma_{me}\sin\theta_e d\theta_e - \gamma_{mi}\sin\theta_i d\theta_i \ ,$$
$$\sin\theta_e d\gamma_{me} - \sin\theta_i d\gamma_{mi} + \gamma_{me}\cos\theta_e d\theta_e - \gamma_{me}\cos\theta_i d\theta_i.$$

Using $\gamma_{me} = \gamma_{mi}$, $\cos\theta_i = -\cos\theta_e$ and $\sin\theta_i = \sin\theta_e$, one deduce,

$$d\gamma_{me} - d\gamma_{mi} = -\cos\theta_e d\gamma_b \tag{34}$$

On the other hand, equation (30) in the case of a tension less membrane gives,

$$d\gamma_{me} + d\gamma_{mi} = \frac{\rho_m}{\rho_b} \ .$$

Combining the last two equations gives simple relations between the variations of the surface tensions,

$$d\gamma_{me} = \frac{1}{2}\left(\frac{\rho_m}{\rho_b} - \cos\theta_e\right)d\gamma_b \ \ \text{and} \ \ d\gamma_{mi} = \frac{1}{2}\left(\frac{\rho_m}{\rho_b} + \cos\theta_e\right)d\gamma_b \ . \tag{35}$$

According to these results, if a droplet bud outward ($\theta_e < \pi/2$) in a tensionless vesicle, an increase of the bilayer tension will lead to a larger increase of the external monolayer tension than the internal one $d\gamma_{me} > d\gamma_{mi}$ (and conversely if the droplet bud inward).

7 Theoretical description of the DEV system

7.1 Variables and equations

We provide here the complete set of equations that is used and solved in order to compare the theory to the experimental data obtained in the DEV system. As explained above, the morphology and thermodynamics state of the DEV under fixed tension is characterized by nine variables (see above), ρ_{me}, ρ_{mi}, ρ_{be}, ρ_{bi}, θ_e, θ_i, R, δ, A_p, free to adjust and sufficient to express any other thermodynamic quantity. They obey nine equations,

$$\mu_m(\rho_{me}) = \mu_b(\rho_{be}, \rho_{bi}) \ \ , \ \ \mu_m(\rho_{mi}) = \mu_b(\rho_{bi}, \rho_{be}) \ ,$$
$$\gamma_b(\rho_{be}, \rho_{bi}) = \gamma_{\text{ext}} \ \ , \ \ \gamma_m(\rho_{me})\cos\theta_e + \gamma_m(\rho_{me})\cos\theta_i + \gamma_b(\rho_{be}, \rho_{bi}) = 0 \ \ , \ \ \gamma_m(\rho_{me})\sin\theta_e = \gamma_m(\rho_{mi})\sin\theta_i \ ,$$
$$V_c(R, \theta_e - \delta) + V_c(R, \theta_i + \delta) = V_{LD} \ \ , \ \ V_c(R, \delta) - V_c(R, \theta_i + \delta) = V_{GUV},$$
$$\rho_{me}A_c(R, \theta_e - \delta) + \rho_{be}\left(A_c(R, \delta) + A_p\right) = N_e \ \ , \ \ \rho_{mi}A_c(R, \theta_i + \delta) + \rho_{bi}\left(A_c(R, \delta) + A_p\right) = N_i \ ,$$

with,

$$A_c(R, \theta) = 2\pi R^2 \frac{1 + \cos\theta}{\sin^2\theta} \ \ \text{and} \ \ V_c(R, \theta) = \frac{\pi R^3}{3}\frac{2 - \cos^3\theta + 3\cos\theta}{\sin^3\theta} \ .$$

The surface tensions and chemical potentials are related to the PL densities through the equations of state presented in the main text,

$$\gamma_m(\rho) = \gamma_{o/w} - \pi(\rho) \ \ , \ \ \gamma_b(\rho, \rho') = \gamma_{\text{phob}} - \pi(\rho) - \pi(\rho') \ , \tag{36}$$

$$\mu_m(\rho) = \int \frac{1}{\rho}\frac{d\pi}{d\rho}d\rho + \epsilon_{\text{mo}} \ \ , \ \ \mu_b(\rho, \rho') = \mu_m(\rho) + \epsilon_{\text{mm}} - \epsilon_{\text{mo}} \ , \tag{37}$$

with the lateral pressure, and its contribution to the chemical potentials, given van der Waals equations of state,

$$\pi(\rho) = \frac{k_B T \rho}{1 - a\rho} - u\rho^2 \ \ , \ \ \int \frac{1}{\rho}\frac{d\pi}{d\rho}d\rho = -k_B T \ln\left((a\rho)^{-1} - 1\right) + \frac{k_B T}{1 - a\rho} - 2u\rho \ .$$

Given the parameters $\gamma_{o/w}$, γ_{phob}, $\epsilon_{\text{mo}} - \epsilon_{\text{mm}}$, a, u, V_{LD}, V_{GUV}, N_e, N_i and γ_{ext}, the state of the DEV is computed by solving numerically the set of equations written above (with an original code written in C using the GSL library). One can compute the equilibrium states of a DEV (and in particular the surface tensions of the droplet) for different applied tension γ_{ext} and compare with the experimental measurement done with the double pipette method (see main text).

7.2 Determination of the parameters

The parameter γ_{ext} is the control parameter, it is fixed by pipette aspiration and its value is known.

The parameters $\gamma_{o/w}$, γ_{phob}, $\epsilon_{\text{mo}} - \epsilon_{\text{mm}}$, a, u dependent on the PL and oil used to make the DEVs. They should be the same for all the DEVs of a sample. At the opposite, the geometrical parameters V_{LD}, V_{GUV}, N_e, N_i are different for each individual DEV and are deduced by image analysis for a given DEV (see below).

The parameters $\gamma_{o/w}$ (bare oil/water interfacial tension), a (excluded area) and u (lateral PL-PL interaction strength) are obtained by fitting the equation of state $\gamma_m(\rho)$ to the tension - area data obtained with the pending drop experiment. The parameters γ_{phob} and $\epsilon_{\text{mo}} - \epsilon_{\text{mm}}$ are adjusted by comparing the theoretical output of the DEV model of sect. 7.1 to the γ_{me} versus γ_b data of measured for a selected DEV.

The macroscopic parameters V_{LD}, V_{GUV}, N_e, N_i are deduced from the simple morphology of the DEV at very low bilayer tension $\gamma_b \simeq 0$. It is assumed that these parameters remain constant during the experiment, in particular when the bilayer tension is varied. At vanishing tension, the droplet is spherical. Image analysis allow the measurement of the LD radius $R_{LD,0}$, the GUV curvature radius $R_{b,0}$ and the angle $\theta_{e,0}$. We can deduce the angles $\theta_{i,0} = \pi - \theta_{e,0}$ and

$$\delta_0 = \frac{(R_{LD,0}/R_{b,0}) \sin \theta_{e,0}}{(1 - (R_{LD,0}/R_{b,0}) \cos \theta_{e,0})} \ ,$$

and then, the radius of the contact line $R_0 = R_{b,0} \sin \delta_0$. The oil and vesicle volumes are then

$$V_{LD} = \frac{4\pi R_{LD,0}^3}{3} \ \ \text{and} \ \ V_{GUV} = V_c(R_0, \delta_0) - V_c(R_0, \theta_{i,0} + \delta_0) \ .$$

The numbers of PL are given by,

$$N_e = \rho_m^0 A_c(R_0, \theta_{e,0} - \delta_0) + \rho_b^0 A_c(R_0, \delta_0) \ \ \text{and} \ \ N_i = \rho_m^0 A_c(R_0, \theta_{i,0} + \delta_0) + \rho_b^0 A_c(R_0, \delta_0) \ ,$$

where ρ_m^0 and ρ_b^0 are the monolayer and bilayer leaflet density at vanishing bilayer tension. (At zero bilayer tension, $\rho_{me} = \rho_{mi} = \rho_m$ and $\rho_{be} = \rho_{bi} = \rho_b$, see section 5.3.) The value of ρ_b^0 and ρ_m^0 are obtained in term of the microscopic parameters $\gamma_{o/w}$, γ_{phob}, $\varepsilon_{\text{om}} - \varepsilon_{\text{mm}}$, a, u from the equations $\gamma_b(\rho_b^0) = 0$ and $\mu_b(\rho_b^0) = \mu_m(\rho_m^0)$.

8 Approximate expressions for the density and tension difference between connected bilayer and monolayer

Using the balance of chemical potentials (14) and the equations of state (37), the density ρ_b and ρ_m of contiguous bilayer leaflet and LD monolayer obey,

$$\mu_m(\rho_m) - \mu_m(\rho_b) = \varepsilon_{\text{mm}} - \varepsilon_{\text{mo}} \ .$$

Assuming a small difference of density, $-\mu_m'(\rho_b)(\rho_b - \rho_m) = \varepsilon_{\text{mm}} - \varepsilon_{\text{mo}}$ and using the Gibbs-Duhem relation (8), $\mu_m'(\rho) = -\gamma_m'(\rho)/\rho = K_m(\rho)/\rho^2$ (the prime denote the derivative of the function), one obtains an approximate expression of the relative difference of density between the LD interface and the bilayer,

$$\frac{\rho_b - \rho_m}{\rho_b} \simeq \frac{(\varepsilon_{\text{om}} - \varepsilon_{\text{mm}})\rho_b}{K_m(\rho_b)} \ .$$

At zero bilayer tension, the equilibrium conditions (13) impose $\gamma_{me} = \gamma_{mi} = \gamma_m^0$, and thus $\rho_{me} = \rho_{mi} = \rho_m^0$. It follows, from the balance of the chemical potentials (14, 15), that the density in the two bilayer leaflets are also equal. According to the equation of state (36), this density obeys $\pi(\rho_b^0) = \gamma_{\text{phob}}$. The monolayer tension is given by the equation of state (37), $\gamma_m^0 = \gamma_{o/w} - \pi(\rho_m^0)$. Under the assumption of a small relative difference between the monolayer and bilayer density, $\gamma_m^0 = \gamma_{o/w} - \pi(\rho_b^0) - \pi'(\rho_b^0)(\rho_m^0 - \rho_b^0)$. According to the equations of state (36) and the Gibbs-Duhem relations (8), $\pi'(\rho) = -\gamma_m'(\rho) = -\rho\mu_m'(\rho)$. Then, according to the equation of state (37), $\pi'(\rho) = -\rho(\varepsilon_{\text{mm}} - \varepsilon_{\text{om}})$. Finally, the surface tension of a monolayer connected to a tensionless bilayer is,

$$\gamma_m^0 \simeq \gamma_{o/w} - \gamma_{\text{phob}} + (\varepsilon_{\text{mo}} - \varepsilon_{\text{mm}})\rho_b^0 \ .$$

References

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