Budding and fission of a multiphase vesicle

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Received 21 April 2006 and Received in final form 26 July 2006 / Published online: 7 September 2006 – ª EDP Sciences / Società Italiana di Fisica / Springer-Verlag 2006

Abstract. We present a model of bi-phasic vesicles in the limit of large surface tension. In this regime, the vesicle is completely stretched and well described by two spherical caps with a fold, which concentrates the membrane stress. The conservation laws and geometric constraints restrict the space of possible shapes to a pair of solutions labeled by a parameter $\sigma$ given by line tension/pressure. For a given value of $\sigma$, the two solutions differ by the length of the interface between domains. For a critical value, $\sigma_c$, the two vesicle shapes become identical and no connected solution exists above this critical value. This model sheds new light on two proposed mechanisms (osmotic shocks and molecule absorption) to explain the budding and the fission in recent experiments.

PACS. 87.16.Dg Membranes, bilayers, and vesicles – 87.10.+e General theory and mathematical aspects – 68.03.Cd Surface tension and related phenomena

Introduction

The cell membrane is a bilayer made out of a mixture of lipid species. The membrane can be either the outer boundary of the cell or an interface separating different compartments inside the cell. This soft structure has many biological properties and also realizes intracellular traffic: a membrane vesicle buds from one compartment, travels through the cytosol and fuses with another compartment. Despite the fluidity of the lipid bilayer, the cellular membrane presents a lateral inhomogeneity due to the formation of dynamical microdomains, called rafts [1]. These microdomains have been shown to be rich in cholesterol and sphingolipid [2]. In vivo, the rafts have not been directly observed but their size has been estimated to be between 20 and 700 nm [3]. A central question in membrane biology and biophysics is to understand how this spatial organization is used by the cell, in particular to favor interactions with proteins. Due to their size and specific composition, it has been argued that rafts play a role in protein docking, signaling, intracellular traffic [4] or virus budding [3].

Recently, a model system of Giant Unilamellar Vesicles (GUV) including sphingomyelin-cholesterol domains was developed [5]. These domains, which are supposed to reproduce the raft composition, are the result of a phase separation of the lipid species [6]. They are more structured than the surrounding classical liquid bilayer but remain in a liquid state. For this reason, they are called “liquid-ordered” domains whereas the classical membrane is called “liquid-disordered” domain. A large number of studies have focused on the thermodynamics of the liquid-ordered phases, in particular the effect of temperature or composition on domain formation [6,7]. Multi-phase vesicles are elegant and efficient tools to study the mechanical properties of microdomains. They can be used to understand how rafts bud and make daughter vesicles for intracellular traffic, but also how detergent addition can isolate rafts from the cell membrane. Recent experiments have shown that liquid-ordered domains can be separated from the initial vesicle by using tubular deformations [8], osmotic shocks [9,10] or absorption of external molecules like proteins or detergents [11,12]. Here, we develop a macroscopic theory for the last two situations. Our model describes the budding preceding the fission where the liquid-ordered domain lifts up from the liquid-disordered vesicle.

Budding and fission have already attracted many theoretical works for vesicles made by a mixture of lipids, segregated into two phases or not [13–19]. The models vary depending on the physical interactions involved but they are all based on the minimization of the bilayer energy. For a vesicle with one phase, the budding is induced by molecules whose shape is different from the main lipids of the membrane: if the molecules prefer a curved bilayer, they will favor the formation of a bud. These models rest on a continuous approach and are well adapted to describe the absorption of external molecules [20,21] or a mixture of lipids [13,22]. Even when the concentration of molecules is strongly inhomogeneous, this approach does not describe a true phase separation. For a bi-phasic vesicle, a line tension exists between the two phases, trying to reduce the interface length, and favoring budding [23]. Previous studies have mainly focused on two cases: a constant
surface with a volume free to adjust \([24,15]\) and a constant surface with a fixed volume \([15,25]\). In both cases, the shape of the vesicle is controlled by the geometric constraints and by the invagination length \(\zeta\), the ratio between the bending rigidity and the line tension. The main results of these numerical studies where elasticity plays the dominant role is the preferred budding and ejection of large domains as the line tension increases.

Here we focus our attention on multi-phase stretched vesicles, a situation often encountered in experiments, and not considered in the previous treatments \([15]\). In this case, osmotic-pressure effects dominate and stretch the bilayer. The volume can change but not at negligible cost. The vesicles can then be described by two spherical caps with an elastic junction. Our model is also suitable for a vesicle with a fixed volume, as long as the two-spherical-cap description remains valid. The variational procedure with surface constraints allows to find two solutions for any ratio \(\sigma = \text{line tension/pressure} \) less than a critical value \(\sigma_c\). The stable solution is the one observed experimentally. An osmotic shock increases the control parameter \(\sigma\) and induces a budding, which may lead the system to a complete fission if \(\sigma\) reaches the critical value. The case of detergents is slightly different since it requires an additive energy for molecular absorption in the membrane. As a large number of different detergents can be used to achieve the budding \([12]\), we restrict on a common characteristic: the conical shape. Therefore, when detergent molecules are added in the membrane, they locally deform the bilayer. According to Leibler’s model \([20]\), the deformation due to these molecules is taken into account via an elastic contribution simultaneously proportional to the average curvature and the molecule concentration. Stretched bilayers favor homogeneous concentration of molecules except at the interface between domains. We show that chemical inhomogeneities contribute to an effective line tension, not necessarily positive. Depending on the concentration, this effective line tension can induce a budding and a possible fission into two independent vesicles.

This paper is organized as follows. The next section is devoted to a detailed description of the model and defines the energies involved in the physics of the vesicle, such as the stretching of the bilayer, the bending of the vesicle or the absorption of molecules. Then, we explain how we can describe analytically the shape of stretched multi-domains vesicles below a critical parameter. Above this critical parameter, we predict a topological bifurcation. This explains the fission induced by a change of osmotic pressure. The last section extends this treatment to the absorption of molecules.

Membrane description

Inhomogeneous lipid bilayer

We consider an inhomogeneous vesicle made of two lipid phases: a “liquid-ordered” phase \(l_o\) and a “liquid-disordered” one \(l_d\). Both phases are in the liquid state but the \(l_o\) domain is more structured than the \(l_d\) phase for the following reasons: there exist specific interactions between molecules \([26]\) and/or a possible optimization of biphilic space packing \([27]\). Steady morphologies and their out-of-plane deformations are commonly described by the Canham and Helfrich’s model with energy for each phase \(i\) given by

\[
F_{ni}^i = \int_S \left[ 2k_iH^2 + \kappa^i_GK + \Sigma_i \right] dS; \quad (1)
\]

\(H\) and \(K\) are, respectively, the mean and Gaussian curvature. The elastic bending rigidity \(\kappa_i\) and Gaussian rigidity \(\kappa^i_G\) are expected to be higher in the \(l_o\) phase. Typical values can be found for example in \([28]\): \(\kappa_{l_o} \approx 20k_bT\) and \(\kappa_{l_d} \approx 80k_bT\). Values of Gaussian moduli are notoriously more difficult to measure but a recent study mentions values of order \(\kappa^i_G = -0.83\kappa_i\) \([29]\). Although \(F_{ni}^i\) is a surface integral, the Gaussian contribution to the energy is indeed a contour integral calculated at the interface between the two domains, according to the Gauss-Bonnet theorem. The most general Canham and Helfrich’s model also includes a spontaneous curvature, allowing a difference between the two leaflets. This difference can induce a difference of area between the two leaflets, which is not described by the spontaneous curvature \([30,31]\) but can induce a budding and a possible fission. In general, for multi-phases vesicles, the lipid bilayer is symmetric, with no spontaneous curvature \([32]\). Introducing the spontaneous curvature does not change the results of this paper so, for simplicity, we assume a symmetric bilayer. A difference of area between the two leaflets induces a lateral constraint; as the lifetime of the stretched vesicle we are considering here is larger than the flip-flops rate of the lipids (minutes compared to seconds), we assume that these constraints are relaxed. Then, we do not consider explicitly the difference of area here. Moreover, the description of molecule absorption treated in this paper can be extended to the case of a difference of area. The last contribution in equation (1) is related to the possible extension of the membrane. For a stretched vesicle, this contribution is large compared to the elastic energy. Moreover, stretching the surface of the vesicle for more than 3\% forms pores in the bilayer \([28]\), not observed in the fission experiments. Therefore, the membrane surface can be considered as constant. This is taken into account by introducing the Lagrange multiplier \(\Sigma_i\).

The total energy of the two-domain vesicle includes the energy (Eq. (1)) of each phase plus two coupling terms. First, a sharp interface of vanishing thickness exists between the \(l_o\) and \(l_d\) phases. Any increase of its length requires an energy proportional to a line tension \(\sigma\). Second, the vesicle membrane is lightly permeable to the water but not to the ions or large molecules present in the surrounding water. This induces an osmotic pressure \(P\). The energy of the coupling terms is

\[
F_c = \sigma \int_C dl - \int_V PdV. \quad (2)
\]

The osmotic pressure normally varies with the enclosed volume. For a tense membrane, it is possible to assume
the osmotic pressure to be constant (see App. A), with an unvixed volume. Using a variable osmotic pressure will complicate the calculations without modifying the main results of this paper. All the physical parameters involved in the description of the membrane are functions of the chemical composition. Therefore, as a deformation of the vesicle may induce a shift in the chemical equilibrium between the two domains, these parameters could also change with the deformation. For clarity, we do not take into account this effect.

Proteins or detergent-membrane interactions

External molecules such as proteins or detergents can be absorbed in both phases but with different efficiencies. We choose a Landau’s approach with an optimal homogeneous concentration \( \phi_{eq} \). Departure from this value or inhomogeneity of concentration \( \phi \) has a cost in energy, quadratic to leading order and measured by two positive constants in each phase: \( \alpha_i \) and \( \beta_i \). If the proteins are soluble or not in the surrounding medium, we can either set the number of these molecules in each phase or set the chemical potential \( \mu_i \) of the phase \( i \). We choose to fix the chemical potential \( \mu_i \), but this has no real consequence on the results since it only modifies the definition of \( \mu_i \). Therefore the free chemical energy of absorption for each phase is

\[
F_p^i = \int_{S_i} \left( \frac{\alpha_i}{2} (\phi - \phi_{eq})^2 + \frac{\beta_i}{2} (\nabla \phi)^2 + \mu_i \phi \right) dS + \int_{S_i} \Lambda_i \phi dS. \tag{3}
\]

The last integral in equation (3) represents the local distortion of the membrane induced by the absorbed molecules [20,33]. It is proportional to the mean curvature of the membrane with a weight depending on the local concentration \( \phi \), as suggested by S. Leibler [20], \( \Lambda_i \) being a coupling constant.

The energy (Eq. (3)) is valid only for passive molecules (no chemical activity), their physical characteristics being included in the parameters \( \mu_i, \alpha_i, \beta_i \), and \( \Lambda_i \). Values of these parameters can be derived from molecular dynamic simulations or estimated by simple physical arguments. Typical values of the interaction energy between two molecules soluble in the surrounding water is \( kT \) (it is more for insoluble molecules). Hence the chemical potential of detergents is about \( nkT \) with \( n \) the number of molecules inside the bilayer. For a flat membrane of surface \( S \), the optimal concentration \( \phi_{eq} \) is related to the parameter \( \alpha \) by \( \mu = \alpha \phi_{eq} = \alpha \phi_{eq}/S \). Therefore, \( \alpha \approx SKT \).

In our case, we set \( \alpha \approx R^2kT \). The size \( l_c \) of a step in concentration is given by the relation \( \beta = \alpha l_c^2 \). Then, \( \beta \approx l_c^2R^2kT \), the chemical length being typically 10nm.

The estimate of the coupling constant \( \Lambda \) is more difficult. Many studies have focused on the microscopic deformations of the curvature by molecules (see for example [34–37]) but, to our knowledge, there is no obvious link between the microscopic and macroscopic descriptions, except for one molecule [38]. We propose the following argument for a naive estimate of \( \Lambda \). Its dimension is an energy multiplied by a length. The typical energy associated with the deformation of the bilayer is \( k \). Each molecule imposes a conical deformation to the bilayer (\( \varphi \) being the angle of the cone). For a conical molecule, this angle is related to its shape: for a molecule with a chain in the surrounding water, the angle is a function of the free displacements of the chain [33]. The induced curvature increases with \( \varphi \). Hence, the parameter \( \Lambda \) can be estimated as \( \Lambda \approx \pm k \rho R_m \) with \( R_m \) a typical length. For a conical molecule, \( R_m \) is the lateral size of the molecule and for a molecule with a mobile hydrophilic chain, \( R_m \) is the radius of gyration of the chain.

The sign of the coupling constant is given directly by the deformation of the bilayer. This sign depends on the definition of the mean curvature. We choose the convention that a spherical vesicle has a positive mean curvature. Then, positive values of \( \Lambda \) imply that the molecules tend to decrease the local vesicle radius. For example, this is obtained for conical molecules absorbed on the external leaflet, when the molecule hydrophilic part is larger than the hydrophobic one. We restrict to the case where the adsorption takes place on one side only. When the two layers are concerned, our theoretical framework can be easily extended by the introduction of two concentration fields.

Finally, the total free energy of the system is given by

\[
F_{TOT} = F_m^d + F_m^o + F_p + F_p + F_c. \tag{4}
\]

The standard variational procedure to find extrema of this energy produces the so-called Euler-Lagrange equations.

Euler-Lagrange equations

Minimization of the free energy \( F_{TOT} \) gives the stable static solutions for the membrane. Looking for axisymmetric shapes, we choose the cylindrical coordinates and we parameterize the surface by the arclength \( s \). The vesicle shape is given by \( r(s) \) and \( \psi(s) \) (see Fig. 1). The Euler-Lagrange equations associated with \( F_{TOT} \) are [39]:

\[
\psi'' = \frac{\sin(\psi) \cos(\psi)}{r^2} - \frac{\psi'}{r} \cos(\psi) - \frac{Pr}{2k_t} \cos(\psi) + \frac{\gamma}{K_t} \sin(\psi) + \frac{\Lambda_i}{K_i} \phi, \tag{5a}
\]

\[
\gamma' = \frac{K_t}{2} \psi'^2 - \frac{K_t}{2} \sin(\psi)^2 + \frac{\Lambda_i}{K_i} \phi, \tag{5b}
\]

\[
\phi'' = -\phi \frac{\cos(\psi)}{r} - \frac{\Lambda_i}{\beta_i} \left( \frac{\sin(\psi)}{r} + \psi' \right) + \frac{\alpha_i}{\beta_i} \phi + \frac{\mu_i}{\beta_i}, \tag{5c}
\]

\[
r' = \cos(\psi). \tag{5d}
\]

with \( \gamma \) a local Lagrange multiplier used to impose the geometric constraint between \( r' \) and \( \cos(\psi) \) in the minimization process.
To simplify the notations, we introduce the following parameters:

$$\tilde{\Sigma}_i = \Sigma_i + \alpha_i / 2 \phi_{eq}, \quad \tilde{\mu}_i = \mu_i - \alpha_i \phi_{eq}.$$  

Assuming (\(\phi = 0\)) gives the classical equations of an axisymmetric vesicle [15,39]. These equations have to be solved with the correct boundary conditions at the border between the two domains, these conditions being the results of the variational procedure. Assuming continuity of the radius \(r\), the angle \(\psi\) and the molecule concentration \(\phi\), one finds

\[
\begin{align*}
\kappa_1 \psi'(s_J - \epsilon) r(s_J) + \left( \kappa_1 + \kappa_G \right) \sin(\psi(s_J)) & \quad - A_1 \phi(s_J) r(s_J) - A_2 \psi'(s_J + \epsilon) r(s_J) \\
- \left( \kappa_2 + \kappa_G \right) \sin(\psi(s_J)) + A_2 \phi(s_J) r(s_J) & = 0, \\
\gamma(s_J - \epsilon) - \gamma(s_J + \epsilon) + \sigma & = 0, \\
\beta_1 \phi'(s_J - \epsilon) - \beta_2 \phi'(s_J + \epsilon) & = 0,
\end{align*}
\]

where \(s_J\) is the arclength at the junction, label 1 denotes the phase for \(s \leq s_J\) and label 2 the phase for \(s \geq s_J\).

Since the equations of the shape and the boundary conditions are highly non-linear, there is no exact solution for arbitrary values of the physical parameters. However, further analytical progress can be obtained in the limit of large pressure (stretched membrane). This treatment only requires simple analytical algebra and allows to explain experimental features such as the complete budding of the ordered phase as it has been observed by different groups using either osmotic shocks [9], proteins [11] or detergent molecules [12].

### Analytical treatment of the membrane shape

We first consider a membrane without addition of extra molecules. An obvious solution of the Euler-Lagrange equations can be easily found if we discard the elasticity. Our purpose is then to use this simple solution as zeroth order and correct it for small but not vanishing values of the bending rigidity by using boundary layer analysis. We consider also the inclusion of passive molecules and show that at least for weak coupling between curvature and concentration, a similar strategy applies.

The constraint on the total area \(S_{TOT}\) allows to define a length scale \(R_0\) by \(4\pi R_0^2 = S_{TOT}\) and an energy scale with the osmotic pressure: \(PR_0^2\). Hereafter, we will use dimensionless parameters. For example, we have:

\[
\tilde{s} = \frac{s}{R_0}, \quad \tilde{R} = \frac{R}{R_0}, \quad \tilde{\kappa} = \frac{\kappa}{PR_0^2} \quad \text{or} \quad \tilde{\sigma} = \frac{\sigma}{PR_0^2}.
\]

### The exact zero-order model: the capillary solution

After electro-formation, GUV vesicles are usually stretched and the osmotic pressure dominates the elastic energy. The two-spherical caps, solution of the Euler-Lagrange equations with \(\kappa_i = 0\), are defined by a set of four geometrical parameters: the radii of the two caps \(R_1\), \(R_2\) (or \(\tilde{R}_1\), \(\tilde{R}_2\)) and the two angles at the boundary \(\theta_1\) and \(\theta_2\) (see Fig. 2). The Euler-Lagrange equations (Eqs. (5)) give the values of the two Lagrange multipliers \(\tilde{\Sigma}_i\) and \(\gamma_i\), without direct information on the vesicle shape:

\[
\begin{align*}
2 \tilde{\Sigma}_i & = \tilde{R}_i, \\
\gamma_i(s) & = \frac{\tilde{R}_i^2}{2} \sin \psi \cos \psi,
\end{align*}
\]

where \(\kappa_1\) and \(\kappa_2\) are the bending rigidity of the two domains, these conditions being the results of the variational procedure. Assuming continuity of the radius \(r\), the angle \(\psi\) and the molecule concentration \(\phi\), one finds

\[
\begin{align*}
\kappa_1 \psi'(s_J - \epsilon) r(s_J) + \left( \kappa_1 + \kappa_G \right) \sin(\psi(s_J)) & \quad - A_1 \phi(s_J) r(s_J) - A_2 \psi'(s_J + \epsilon) r(s_J) \\
- \left( \kappa_2 + \kappa_G \right) \sin(\psi(s_J)) + A_2 \phi(s_J) r(s_J) & = 0, \\
\gamma(s_J - \epsilon) - \gamma(s_J + \epsilon) + \sigma & = 0, \\
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- \left( \kappa_2 + \kappa_G \right) \sin(\psi(s_J)) + A_2 \phi(s_J) r(s_J) & = 0, \\
\gamma(s_J - \epsilon) - \gamma(s_J + \epsilon) + \sigma & = 0, \\
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\]
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experimentally, it is easier to change the reduced line tension,
reduced line tension ~
produced in blue here and the L4 in red. Scale bar, 5 μm. Reproduced
from [9] with the authorizations of the authors and editor.

Constraints on the fraction f occupied by the domain 1:
\[ \begin{align*}
R_1 \sin \theta_1 &= R_2 \sin \theta_2, \\
\frac{1}{2} R_1^2 \sin \theta_1 \cos \theta_1 &= \frac{1}{2} R_2^2 \sin \theta_2 \cos \theta_2 - 2\sigma, \\
0.5 R_1^2 (1 - \cos \theta_1) &= f, \\
0.5 R_2^2 (1 + \cos \theta_2) &= 1 - f.
\end{align*} \]

Two parameters control the shape of the vesicle: the re-
duced line tension \( \sigma = \sigma / R_2^2 \) and the fraction \( f \). Experiment-
ally, it is easier to change the reduced line tension, by an osmotic shock for example. We will see that also addition of molecules may modify \( \sigma \). Notice that the angle \( \psi \) is not continuous at the interface. This is possible if elasticity is ignored.

This approach assumes a stretched vesicle, with a
changing volume and a constant osmotic pressure. For a
floppy vesicle, at fixed volume, we can apply an analogous
analysis providing that both domains remain nearly spher-
ical which is seldom the case [13]. In this case, numerical
approaches are more suited.

The set of equations (Eqs. (8)) has either two solu-
tions or no solution depending on the value of \( \sigma \) at fixed
\( f \). As an example, consider the vesicles prepared by electros- swelling [40], which makes usually tense membranes but without control on the final osmotic pressure. Refer-
ing to Figure 1b in Baumgart et al.’s work [9], reproduced
here in Figure 3, we calculate \( R_0 = 10.7 \, \mu m \), \( f = 9.5\% \) and
\( \sigma = 0.18 \), from estimated values of \( R_1 \), \( R_2 \), \( \theta_1 \) and \( \theta_2 \). Us-
ing the value of the line tension measured by Baumgart et al. [40] \( (\sigma = 7 \cdot 10^{-13} N) \), we get \( P = 3 \cdot 10^{-2} \, \text{Pa} \). The re-
duced bending rigidity is then \( \kappa \approx 0.01 \) which shows that elasticity can be neglected in a first step.

Solving equations (8) for the experimental values of \( \sigma \) and \( f \) gives two possible solutions: \( R_1 = 0.5 \), \( R_2 = 0.98 \), \( \theta_1 = 1.34 \) and \( \theta_2 = 0.514 \), the measured values (Fig. 4(a)), but also \( R_1 = 0.37 \), \( R_2 = 0.97 \), \( \theta_1 = 1.34 \) and \( \theta_2 = 0.514 \)
for the second solution (Fig. 4(b)). In order to explain
why the first solution is preferred in the experiment, we
calculate the dimensionless energy reduced to \( F_{TOT} =
- V/R_0^3 + 2 \pi \sigma R_1 \sin \theta_1 \). It gives, respectively, \((-3.64)\) compared to \((-3.59)\), which is consistent with experimental observations since only stable vesicles (with the lowest en-
ergy) can be observed.

A systematic study of the pair of solutions for arbitrary
values of \( \sigma \) is straightforward and the results are
presented in Figure 4. Figure 4b) is a classical bifurcation
diagram when a pair of solutions exists but only one is
stable. In this problem, \( \sigma \) is the control parameter and
the interface radius \( \tilde{r}_1 \) is the order parameter. These two
solutions differ geometrically, the unstable solution pre-
senting a smaller neck at the junction compared to the
stable one (obvious if \( \sigma = 0 \) (see Fig. 5b)). As \( \sigma \) increases,
the two solutions become geometrically closer up to a
finite value of \( \sigma_c \). Above the critical value (\( \sigma > \sigma_c \)), there
is no connected solution to the Euler-Lagrange equations
but a couple of disjoining spheres remains a plausible
solution. This critical value \( \sigma_c \) is determined by equation (8b)
which gives the equilibrium of the forces in the radial di-
rection (axis \( r \)). The term in \( \sigma \) is due to the line tension and
its effect pinches the vesicle. The two other terms (in
\( R_1^2 \) and \( R_2^2 \)) are related to the stretching of the domains
and are bounded. The critical value \( \sigma_c \) corresponds to
the maximal force on the vesicle. For higher line tensions
(or smaller pressures), it is no longer possible to compen-
sate the stretching by the line tension and the system splits
into two independent vesicles.

The transition is a dynamical process and has similarities
with the breaking of a soap film between two rings
(the catenoid instability) [41]. Nevertheless, complete
fission requires a microscopic reorganization, such as hemi-
fission [42] which occurs at small scales and is out of reach.
Fig. 5. Dimensionless energies $\tilde{F}_{\text{TOT}}$ (a) and interface radius $\tilde{r}_J$ (b) of the solutions of the E.-L. equations (Eqs. (8)) versus the control parameter $\tilde{\sigma} = \sigma/PR_i^2$. The calculation has been done for a fraction of liquid-ordered phase of 9.5%. In both figures, the solid line corresponds to the stable solution, experimentally observable, the dashed line to the unstable solution and the dotted line to $\tilde{\sigma} = \tilde{\sigma}_c$, the critical value of the control parameter. For $\tilde{\sigma} \geq \tilde{\sigma}_c$, there is no longer a solution. Four pictures of vesicles showing the shape transformation with the dimensionless line tension have been added.

of the present treatment. A detailed analysis may be found in [43] for example. It is why we cannot predict if the two daughter vesicles remain connected by a small filament of lipids or prefer to separate.

The shape of the vesicle is also controlled by the fraction $f$ of the domain 1. Variations of the critical parameter $\tilde{\sigma}_c$ with the fraction $f$ leads to a phase diagram for the connected vesicle (see Fig. 6). The diagram is symmetric with respect to $f = 1/2$ since in the absence of elasticity the two domains are equivalent. Small domains are less stable than large ones for a stretched vesicle. A physical explanation can be found by considering flat domains on a flat surface. If the radius of the domain is $r$, the pinching energy (due to the line tension) is approximatively $\sigma r$ and the resistance energy (due to the pressure) is approximatively $Pr^3$. The balance of both energies gives $\sigma/P \approx r^2$.

![Fig. 6. Values of the critical control parameter $\tilde{\sigma}_c$ versus the fraction $f$ of the upper domain (label 1). The reduced areas of the domains are given by $A_1 = f$ and $A_2 = (1 - f)$. The dashed line shows the fraction 0.5.](image)

Therefore, it is harder to destabilize a large domain than a small one. Theoretical studies on floppy vesicles with fixed volume show that large domains are ejected before the small ones [15], contrary to the case studied here which concerns stretched vesicles at constant osmotic pressure. Controlling the osmotic pressure for weakly stretched vesicles could lead to a better understanding of all intermediate cases between these two limits.

Localization of the elastic deformation

We study now the robustness of the model when elasticity is taken into account. Comparing the bending energy (Eq. (1)) to the osmotic-pressure energy (Eq. (2)), one finds that elastic effects can be neglected if $\kappa_i \ll PR_i^3$ in each phase. However, the zero-order model gives a slope discontinuity at the interface between the two domains, responsible for a singularity in the curvature. As soon as the bending modulus is not exactly zero, this discontinuity produces an infinite elastic energy density, localized near the junction, in contradiction with the weakness of elasticity. We are faced with a classical boundary layer model, as found for example in the crumpling of an elastic plate [44] or the folding of an elastic shell [45]. For small but not zero $\kappa_i$ values, the elasticity of the membrane prevents the discontinuities near the junction by smoothing out the vesicle profile locally (see Fig. 2) on a characteristic distance of order the elastic length $l_e$ in each phase:

$$l_e = \sqrt{\frac{\kappa_i}{PR_i}}.$$

Experimental determinations of the osmotic pressure give values higher than $10^{-2}$Pa [9,40,46]. Using typical values for giant vesicles ($\kappa \approx 10^{-19}$J, $R \approx 10 \mu$m), we get $l_e \leq 1 \mu$m, which is small compared to $R_i \approx 10 \mu$m. We can model our system as two spherical caps slightly distorted.
at the junction on a distance of order \( l_c \): far away from the fold, the spherical solution (denoted by \( S \)) is a good approximation but it has to be corrected by a boundary layer (denoted by \( B \)) of size \( \tilde{l} = l_c/R \) near the interface. We decompose \( \psi, r \) and \( \gamma \) into

\[
\psi = \psi_S + \psi_B(\tilde{l}), \quad r = r_S + \tilde{l}_c r_B(\tilde{l}), \quad \gamma = \gamma_S + \tilde{l}_c \gamma_B(\tilde{l}) \tag{9}
\]

with \( \psi_S = \theta_1 \) or \( \psi_S = \theta_2 \) and the new arclength parameter \( \tilde{l} = (\tilde{s} - \tilde{s}_j)/l_c \). The quantities \( \psi_B, r_B \) and \( \gamma_B \) must vanish far away from the junction.

The leading order of equation (5) gives

\[
\hat{\psi}_B = \sin \psi_B. \tag{10}
\]

This is the pendulum equation, which has explicit analytical solutions (see App. B). The shape in the boundary layer does not depend on the Gaussian modulus \( \kappa_G \). This modulus appears in the boundary condition equation (6a) and has the same order of magnitude as the bending modulus \( \kappa \). In the general case, its contribution can be important on the membrane shape \([8, 40]\) but, for a stretched vesicle, it disappears (see Eq. (B.2) of App. B). Taking into account the possible spontaneous curvature of the membrane does not modify the pendulum equation.

From equations (4) and (10), we derive the elastic energy in each phase. This energy is localized at the junction and is proportional to the interface length, in the same way as a line tension:

\[
\sigma_{\text{cusp}} = \sqrt{\kappa_l P R_i} \left\{ 2 \left[ 1 - \cos \left( \frac{\psi_j - \theta_i}{2} \right) \right] + \sin \theta_i \left[ \sin \theta_i - \sin \left( \frac{\psi_j + \theta_i}{2} \right) \right] \right\}. \tag{11}
\]

The angle \( \psi_j \) is the angle \( \psi \) at the interface (see Eq. (B.3) of App. B). It is fixed by the boundary conditions (Eq. (6a)) and depends on the ratio between bending rigidities (see App. B). Using Baumgart et al.'s work \([40]\), we determine \( \kappa_R/\kappa_d \approx 4 \), in agreement with \textit{ab initio} numerical simulations which give \( \kappa_R/\kappa_d = 5.0 \pm 1.56 \) \([40]\).

The effective line tension, including the elastic contributions, modifies our control parameter

\[
\sigma = \frac{(\sigma + \sigma_{\text{cusp}})}{PR_i^2}. \tag{12}
\]

The bifurcation diagram for a given fraction is the same as previously and, in particular, the value of the critical parameter \( \sigma_c \) is the same with and without elasticity. The elastic contribution \( \sigma_{\text{cusp}} \) is a non-trivial function of the physical constants \( (P, \kappa_R \text{ and } \kappa_d) \) and geometrical constants like the angles \( \theta \). Note that the effective line tension \( \sigma_{\text{cusp}} \) is not necessarily positive, contrary to \( \sigma \) but it remains small for weak membrane elasticity. If the elastic contribution \( \sigma_{\text{cusp}} \) is positive, it slightly favors the budding of the domains.

A variation of the control parameter \( \sigma \) modifies the angles \( \theta_1, \theta_2 \) and \( \psi_j \) and then the elastic line tension \( \sigma_{\text{cusp}} \). Figure 7 shows the values of the reduced line tension of the fold \( \tilde{\sigma}_{\text{cusp}} = \sigma_{\text{cusp}}/PR_i^2 \) versus the reduced total line tension \( \tilde{\sigma} \). The solid line is \( \tilde{\sigma}_{\text{cusp}} \) for the low-energy solution. The dashed line is \( \tilde{\sigma}_{\text{cusp}} \) for the high-energy solution. The line tension of the fold must be smaller than the total line tension \( \sigma + \sigma_{\text{cusp}} \) since the line tension \( \sigma \) due to the interface is positive. Therefore, some shapes are no longer physically allowed for the unstable solution.
The existence of this instability does not mean that the rrippled shape, more complex than a spherical cap [39,11].

characterizes each phase individually which may exhibit a cap model remains a solution of the Euler-Lagrange equations. However, in a previous paper we have shown that the two-cap solutions may be unstable either above a critical homogeneous concentration given by $¹$ for very strong coupling $l_i$ and $l_j$ domains (respectively, label 2 and 1) do not have the same effect since the elastic moduli are not equal. Contrary to a true line tension, this effective line tension can be negative for $f$ near 0.5.

Figure 8 shows the energies of the vesicle versus the control parameter $σ$ for the allowed solutions.

Elastic line tension is in general smaller than the physical line tension $σ$ (see Fig. 8). However, it may have a noticeable effect on the shape of the vesicle when the control parameter approaches the critical value $σ_c$: the interface length changes abruptly with the control parameter $σ$ in this region. The elasticity also breaks the symmetry $f$ versus $(1−f)$ in the diagram 9. Figure 9 shows the elastic line tension versus the fraction $f$ of the domain 1. Note that the elastic line tension is negative for large domains, meaning that the elasticity resists to the pinching.

In the following, we treat the absorption of external molecules with the same approach.

**Budding by molecule insertion**

When specific molecules such as conical-shaped molecules or proteins (PLA$_2$) are introduced in the outer bilayer, they succeed to eject a domain which is transformed into an independent vesicle (see Fig. 10). This instability looks like the observation made with a variation of the osmotic pressure and suggests a similar treatment.

When the molecules are distributed uniformly, the two-cap model remains a solution of the Euler-Lagrange equations. However, in a previous paper we have shown that the two-cap solutions may be unstable either above a critical homogeneous concentration given by $φ_{eq} = PR_i^2/L_i$ or for very strong coupling $A_i^2/κ_α α_i ≫ 1$ [39]. This instability characterizes each phase individually which may exhibit a rippled shape, more complex than a spherical cap [39,11]. The existence of this instability does not mean that the ejection process is inhibited but it cannot be treated by a simple analytical treatment. Therefore, we consider the opposite limit with stable spherical caps. This implies that the concentration far away from the interface is homogeneous and smaller than $φ_{eq}$, the ratio between the stretching effect (the osmotic pressure) and the coupling constant $λ_i$. For concentrations higher than $φ_{eq}$, the coupling effect due to the molecules is stronger than the vesicle tension. Our choice is justified by the fact that at each step of the fission the domains remain spherical (see Fig. 10).

Equations (5) connect the concentration of molecules to the chemical potential ($μ_i$) and modify the area Lagrange multiplier $Σ_i$:

\[
μ_i = \frac{2A_i}{R_i} - α_i(φ_i - φ_{eq}),
\]

\[
2Σ_i R_i^2 = PR_i^2 - 2λ_i φ_i R_i + α(φ_i^2 - φ_{eq}^2)R_i^2,
\]

\[
γ_i = \frac{PR_i^2}{2} \sin \psi \cos \psi.
\]

In the following, we extend the analytical treatment to the absorption of molecules and we give an interpretation of the experimental observations.

**Fold description**

The interface is the place where strong chemical gradients of molecule distribution are found with typical lengthscale given by

\[
l_c = \sqrt{\frac{κ_α}{α_i}},
\]

![Image](https://example.com/image.png)
which must be compared to the vesicle lengthscale $R_v$. Typical values of the chemical length $l_c$ are 10 nm and the concentration gradients are localized at the fold ($l_c \ll R_v$). We can use a boundary layer method, in the same spirit as the treatment of the elasticity.

For distances larger than $l_c$, the concentration of molecules is constant and reaches the value $\phi_i$ that we choose as unit in each phase: so $\phi_i = \phi_i/\phi_i$. In this description, the concentration does not have to be constant in the boundary layer, which is the main difference with the spontaneous curvature approach. Far away from the interface, the spherical solution (denoted by $S$) remains a solution but a boundary layer (denoted by $B$) is necessary in the close vicinity of the interface. We use again the decomposition for the case of the elastic fold (Eq. (9)), with $\tilde{\phi} = 1 + \tilde{\phi}_B$. Three dimensionless parameters describe the fold: the chemical length $\bar{l}_c$, the ratio $\bar{\lambda}_c$ between the curvature due to the molecules and the stretching of the vesicle and the ratio $\bar{\lambda}_e$ between the curvature due to the molecules and the cost of absorption:

$$\bar{l}_c = \sqrt{\frac{\beta}{\alpha R_1^2 l_c}}, \quad \bar{\lambda}_c = \frac{A_0 \bar{\phi}}{PR_1^2} \text{ and } \bar{\lambda}_e = \frac{A_1}{\alpha \bar{\phi}_R l_c}.$$  \hspace{1cm} (13)

Remember that the stability of each spherical cap requires $\bar{\lambda}_c \leq 1$ and $\bar{\lambda}_e \bar{\lambda}_c \leq 1$ [39]. Expanding the shape equations (Eqs. (5)) to leading order gives

$$\psi_B'' = \sin \psi_B + \bar{\lambda}_c \bar{\phi}_B'$$ \hspace{1cm} (14a)

$$\bar{l}_c^2 \bar{\phi}_B'' = \bar{\phi}_B - \bar{\lambda}_c \psi_B'.$$ \hspace{1cm} (14b)

The fold energy is given by the leading orders of equation (4) and is proportional to $r_j$, the interface length. Therefore, it defines a new effective line tension $\sigma_{cusp}$. This line tension is partly due to the difference of concentration of absorbed molecules between the two domains but it is also due to the coupling of the molecules with the curvature. Therefore, this contribution can be positive or negative, depending on the chemical species.

The two-spherical-cap zeroth-order solution remains valid and the uniform insertion of molecules in the vesicle modifies only the Lagrange multipliers which have no direct physical content, despite the modification of the energy level of the system. Therefore, we conclude that the bifurcation diagram remains unchanged with the same threshold value found previously although the numerical values of the energy are modified. For $\bar{\sigma}_c \leq \bar{\sigma}_c$, two ideal solutions still exist, the stable one being observed experimentally. Only gradients which appear at the interface modify the cusp shapes and we need to evaluate them if they are responsible for a change in the line tension value.

The equations (Eqs. (14)) have no explicit solution but some interesting limits can be considered. We focus here on three independent limits: $\bar{\lambda}_c \ll 1$, $\bar{l}_c \ll 1$ and $\bar{l}_c \ll 1$, the last one corresponding to the experimental case.

**First case ($\bar{\lambda}_c \ll 1$):** the elastic coupling regime. It modiﬁes the shape of the fold near the fold: the chemical length $\bar{l}_c$ and the stretching of the vesicle are inserted from the outer monolayer.

This limit decouples equation (14a) at zero order, giving exactly the same solution as the case without molecule. Equation (14b) allows to calculate the molecule distribution but as the terms in $\bar{\lambda}_c$ can be neglected in the energy, the effect of the molecules is negligible. The elastic line tension is not modiﬁed by molecule addition.

**Second case ($\bar{\lambda}_c \ll 1$):** the weak chemical coupling length. This limit decouples equation (14b), leading to $\bar{\phi}_B = \bar{\phi}_B \exp (\pm l_c^2 / l_c)$ with $\bar{\phi}_B$, the molecule excess at the interface, given by the boundary conditions (Eqs. (6)). In physical units and taking into account both sides of the fold, we get for the increase of the line tension:

$$\delta \sigma_{cusp} = \frac{\sqrt{\alpha_1 \beta_1 \beta_2}}{2(\sqrt{\alpha_1} + \sqrt{\beta_2})} (\bar{\phi}_2 - \bar{\phi}_1)^2.$$  \hspace{1cm} (15)

The molecule absorption increases the effective line tension, which may induce the fission. This effect is only due to chemical gradients near the interface. It increases with the number of molecules added to the system.

**Third case ($\bar{l}_c \ll 1$):** the case of a small chemical length. The molecule concentration has two very different length-scales: $\bar{l}_c$ and $l_c$. The chemical length $\bar{l}_c$ contributes to the junction between the two domains and can be treated as a boundary layer. However, the associated energy is proportional to $l_c$ and is then negligible. For size larger than $\bar{l}_c$, equation (14) becomes

$$\bar{\phi}_B = \bar{\lambda}_c \psi_B,'$$ \hspace{1cm} (16a)

$$(1 - \bar{\lambda}_e \bar{\lambda}_c) \psi_B'' = \sin \psi_B.$$ \hspace{1cm} (16b)

The effective line tension is then given by

$$\sigma_{cusp} = \frac{A_1 \bar{\phi}_1 (\theta_1 - \psi_{cusp}) + A_2 \bar{\phi}_2 (\psi_{cusp} - \theta_2)}{1 - \bar{\lambda}_e \bar{\lambda}_c \sigma_{el}},$$ \hspace{1cm} (17)

with $\sigma_{el}$ the elastic line tension calculated without molecules. The multiplicative factor $\sqrt{1 - \bar{\lambda}_e \bar{\lambda}_c}$ is independent of the molecule concentration but decreases the contribution of the elastic fold when absorbed molecules are present. Remember that in our framework $\bar{\lambda}_e \lambda_c$ is always smaller than 1 since the cost associated with the concentration gradients $\beta$ is neglected [39]. The two first terms are proportional to the concentration of added molecules. They are positive when conical molecules are introduced in the outer monolayer of the membrane (positive $A$), which is the case found in the experiments. However, if the molecules are added in the inner monolayer, it becomes negative and budding and pinching are inhibited. In conclusion, the net effect of molecule insertion is a decrease of the line tension at low concentration, then a net increase as the concentration increases if the molecules are inserted from the outer monolayer.

**Budding and fission processes**

The absorption of molecules does not change the zeroth-order shape equations of the stretched vesicle in the weak-coupling regime. It modifies the shape of the fold near...
the interface giving a new contribution to the effective line tension. If the absorption takes place in the external leaflet, it contributes to an increase of the line tension. This increase puts the system closer to the bifurcation point controlled by the parameter \( \sigma = (\sigma + \sigma_{\text{cusp}})/P \) and induces a budding of the smaller phase: as \( \sigma \) increases, the neck radius decreases (see Fig. 5b) and the small domain seems to lift up. If the concentration is high enough so that \( \sigma > \sigma_c \), the budding is automatically followed by a fission process, making two separated vesicles, one for each phase. If the concentration is not high enough, the lifting will stop before the change of topology. In the meantime, it is possible that the vesicle looses some of its molecules and recovers its initial configuration. This reversibility is impossible when fission is complete for two reasons: first, the system relaxes the Gaussian elastic energy and two daughter vesicles may be energetically favored; second due to thermal fluctuations, the vesicles move away from each other and the coalescence process is unlikely. The fact that the fission occurs proves that the time scale for fission is much smaller than the possible rearrangement of molecules between the leaflets.

Figure 10 reproduces experimental results from Staneva et al. [11], showing fission of a liquid-ordered domain induced by Phospholipase A\(_2\) protein addition. The vesicle is obtained by electro-formation (the electrode is visible on the left of the pictures). It is made with a 45 : 45 : 10 mol/mol mixture of phosphatidylcholine (PC), sphingomyelin (SM) and cholesterol (Chol). The vesicles show one liquid-ordered domain made visible by fluorescence microscopy (not reproduced here): a small fraction (10\%) of the PC is replaced by an analogous fluorescent lipid which is excluded from the liquid phase. So \( l_o \) appears as a dark disk. The proteins are injected in the neighborhood of the vesicle by a micropipette (visible on the right of the first picture). Phospholipase A\(_2\) transforms the PC lipids into LysoPC, a conical molecule, by cutting one of the two hydrophobic tails. Fission occurs about 10 seconds after protein injection.

Similar fission processes have been observed when detergents like LysoPC, Triton X100 or Brij 98 are added in significant quantities near a similar vesicle [12]. However, in this case, the fission is not always complete: the daughter vesicles may remain connected by a small lipid filament, as also observed in [18]. This is not in contradiction with our model since the fission process requires to split the lipid bilayers at the molecular level [42]. The fission process prefers small domains, as predicted by our model. If the concentration in detergents is not high enough or if the Phospholipase A\(_2\) is not activated, the liquid-ordered domains bud without complete fission. It is also possible to observe a relaxation of the vesicle, which recovers its initial shape.

**Conclusion**

Liquid-ordered domains are supposed to be implicated in the cellular processes, such as penetration of virus or active transport. Various recent experiments on model systems have shown that liquid-ordered domains can be ejected specifically from a tense vesicle, either by an osmotic shock or by the absorption of molecules. Extrapolating these results to real cells necessitates the determination of the important physical effects for the fission.

Our approach here consists in using the tension of the vesicle as a ground for a common interpretation of fission by osmotic shock or molecule absorption. Tense vesicles have a very simple shape: a spherical cap for each domain, connected by a sharp cusp. Theoretically, a simple analytical description of the vesicle shape can be found, controlled by two parameters only: the fraction of the liquid-ordered domain and the reduced line tension \( \sigma/P \). Elastic effects, as described through Helfrich’s energy, are taken into account. They show themselves in the cusp near the interface and only contribute as a correction to the line tension. For a given pair of control parameters (the fraction and line tension), two connected solutions exist: one has the lowest energy and can be observed experimentally, the other being not observable.

Experimentally, one can modify the reduced line tension by acting on the osmotic pressure: a decrease of the osmotic pressure will increase the reduced line tension. For vanishing line tension, the observable solution is nearly spherical. As the line tension increases, the liquid-ordered domain buds, until a critical value of the reduced line tension. At this value, stable and unstable solutions are identical and, above, no connected solution exists. This explains the experimental fission [9]. A controlled change of the osmotic pressure will allow to test this model: budding remains reversible until a critical value.

The relative size of the domain is also studied. It mainly modifies the critical value of the line tension for the fission: small domains are easier to eject than large ones. This prediction is opposite to the other limiting case of a floppy vesicle, controlled by its elasticity and line tension [23]. Clearly, a more complete study, both theoretical and experimental, of the interplay between tension and elasticity on fission is needed to understand the mechanical properties of liquid-ordered domains. This approach relies on the control of the osmotic pressure in the model system, which is still at an early age for multi-domains vesicles. However, it is likely that in vivo systems are controlled by a mixing of lateral tension and elasticity.

The simple description of tense vesicles allows to model the fission by the absorption of molecules. The experiments at the origin of this work used mainly detergents [12] but also proteins like PLA\(_2\) [11]. This model assumes that the absorbed molecules force the bilayer to curve due to their conical shape or to an entropic pressure. However, the effects of external molecules on a lipid bilayer are generally complex: detergents can solubilize the bilayer, proteins are chemically active and can transform the lipids of the bilayer. A more complete analysis has to account for the specificities of each molecule. Nevertheless, our simple approach reproduces the different features of the observed budding.

Coupling between curvature and concentration of external molecules on a tense membrane can lead to two
different kinds of instabilities. The strong coupling concerns mostly the stability of the spherical shape: the presence of molecules is strong enough to overcome the tension of the bilayer and the domain gets a rippled shape [39]. The other instability is the budding and fission, which can be observed for spherical but also rippled domains. Here, we restrict on weak coupling and the domains remain spherical allowing an analytical treatment. The impurities only modify the vesicle shape in the cusp. Their effect can be described by an effective line tension. The budding and fission processes are then explained by the same first-order transition than for a change of osmotic pressure. In particular, the small domains are ejected at first, as observed experimentally [12].

The variation of the line tension with the concentration is calculated explicitly. For conical detergents, an increase of concentration gives an increase of the line tension (with a budding) if the molecules are absorbed on the external leaflet of the bilayer, as done experimentally but can give a decrease if the molecules are absorbed on the internal leaflet. However, more quantitative results require a quantitative determination of the coupling constant. If the microscopic details of the coupling between external molecules and the bilayer have been studied, the transition to a mesoscopic theory has not be done yet. Quantitative values of the coupling constants could help to determine the importance of the curvature forcing in the cellular fission.

We would like to thank M. Angelova, P. Bassereau, T. Baumgart and G. Staneva for various discussions on experimental aspects and A. Goriely for critical reading.

Appendix A. Osmotic pressure

Ions or large molecules cannot cross the lipid bilayer. The number of ions or big molecules enclosed in the vesicle is fixed, as long as no pore is created. However, the bilayer remains permeable to the water molecules, meaning that the enclose volume can change with an energy cost.

When the interactions between the ions or the big molecules are assumed negligible, it is possible to describe such molecules as a perfect gas. The energy associated with the osmotic pressure is [14]

\[
F_V(V) = R g T \left[ n \ln \frac{V}{V_0} - c_0 (V - V_0) \right], 
\]

where \(c_0\) is the external concentration of these osmotically active molecules, \(n\) the number of these molecules confined within the vesicle and \(V\) the volume of the vesicle. The volume \(V_0 = n/c_0\) is the volume for which the osmotic pressure vanishes.

When the enclosed volume \(V\) is close to the volume \(V_0\), we can assume \(V/V_0 - 1 \ll 1\) and the energy (Eq. (A.1)) is approximatively

\[
F_V(V) \approx R g T c_0 V_0 \left( \frac{V}{V_0} - 1 \right)^2 .
\]

Typical values for the concentration is \(c_0 \approx 10^{-4}\) mol/L, for the enclosed volume \(V \approx 10^{-15}\) m\(^3\), which give an energy of about \(10^{-13}\) J, huge compared to the bending energy of the vesicle \(\kappa\). Therefore, the bending energy is not enough to modify the enclosed volume for a floppy vesicle. It is the classical assumption of the studies of equilibrium shapes in the case of fluctuating vesicles.

If the enclosed volume \(V\) is large compared to the volume \(V_0\), the energy (Eq. (A.1)) becomes

\[
F_V(V) \approx -R g T c_0 V .
\]

We can then assume that the bilayer is stretched by a constant osmotic pressure

\[
\Pi = R g T c_0 \approx 10^{4} \text{Pa},
\]

which is of the order of experimental values [46]. Note that the energy associated with the osmotic pressure is large compared to the bending energy. This also means that the bending energy is not strong enough to change the enclosed volume of a tense vesicle.

Appendix B. Analytical treatment without molecules

We present here the analytical treatment for the boundary layer in the case of a stretched vesicle without molecules. The shape of the vesicle near the interface is given by the pendulum equation (Eq. (10)):

\[
\ddot{\psi}_B = \sin(\psi_B).
\]

Remember that we use the arclength parameter \(\tilde{t} = (s - s_j)/l_c\), which is the dimensionless arclength from the interface. We multiply the pendulum equation by \(\dot{\psi}_B\) and integrate:

\[
\frac{1}{2} \dot{\psi}_B^2 = -\cos \psi_B + 1 .
\]

The constant 1 appears because the angle \(\psi_B\) and its derivatives must vanish far away from the junction. The angle \(\dot{\psi}_B\) is then given by the differential equation:

\[
\dot{\psi}_B = \pm 2 \sin \left( \frac{\psi_B}{2} \right) ,
\]

which can be integrated into

\[
\tan \frac{\psi_B}{4} = \left( \tan \frac{\psi_0}{4} \right) \exp(\pm \tilde{t}).
\]

The condition of vanishing angle far away from the junction implies that the sign plus is for the domain 1 (\(\tilde{t} \leq 0\)) and the sign minus is for the domain 2 (\(\tilde{t} \geq 0\)). The angle \(\psi_0\) is the contribution of \(\psi_B\) to the angle at the interface \(\psi_j = \theta_1 + \psi_0\).

An expression of the angle \(\psi_0\) is given by the boundary condition (Eq. (6a)). As usual for the boundary layers, we
kept only the terms of higher degree in $1/\bar{l}$. With the dimensionless arclength $\bar{l}$, the boundary condition becomes

$$\sqrt{\kappa_1 R_1} \psi_B'(-\epsilon) = \sqrt{\kappa_2 R_2} \psi_B'(+\epsilon).$$

(B.2)

Using the differential equation (B.1) and the relation at the interface $\psi_B(0) = \psi_0 = \psi_J - \theta_i$, the expression becomes

$$\sqrt{\kappa_1 R_1} \sin \left( \frac{\psi_J - \theta_1}{2} \right) + \sqrt{\kappa_2 R_2} \sin \left( \frac{\psi_J - \theta_2}{2} \right) = 0.$$

(B.3)

The angles $\theta_1$, $\theta_2$ and $\psi_J$, as the radii $R_1$ and $R_2$, can be measured experimentally [40] and can be used to determine the ratio $\kappa_1/\kappa_2$.

References

10. G. Staneva, personal communication.