On the surface tension of fluctuating quasi-spherical vesicles

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Abstract. We calculate the stress tensor for a quasi-spherical vesicle and we thermally average it in order to obtain the actual, mechanical, surface tension \( \tau \) of the vesicle. Both closed and poked vesicles are considered. We recover our results for \( \tau \) by differentiating the free-energy with respect to the proper projected area. We show that \( \tau \) may become negative well before the transition to oblate shapes and that it may reach quite large negative values in the case of small vesicles. This implies that spherical vesicles may have an inner pressure lower than the outer one.

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1 Introduction

Biological membranes are nanometer-thick sheets composed essentially of a bilayer of phospholipids [1]. Although very thin, they form a barrier for ions and larger molecules. They are thus essential for cells, forming a protection from the external media and preventing diffusion. Moreover, they are fluid and very flexible, exhibiting a strong resistance to area change but no shear resistance. As a consequence, they present large thermal fluctuations [2].

Bilayers composed of one single type of phospholipid are usually used to study the physical properties of membranes. For this purpose, unilamellar vesicles, consisting of one closed bilayer, are widely used in experiments. Different techniques allow to obtain these vesicles, whose size range from a few tens of nanometers to a hundred of micrometers (giant vesicles) [3, 4, 5]. They appear in several fluctuating shapes, depending on the enclosed volume \( V \), on the total area \( A \) and on the area difference between the monolayers [6, 7].

For liquid interfaces, the surface tension is a constant depending only on the nature of the phases in contact. The situation for membranes is quite different. Membranes consist of a fixed number of insoluble lipids possessing an equilibrium density. Mechanically, the tension depends on the lipid density but also on the curvature of the membrane [8, 9, 10]. Nonetheless, for fluid membranes, the concept of surface tension is threefold: (i) Experimentally, one measures a tension \( \tau \) from the quadratic dependence in the wavenumber of the fluctuation spectrum [2]. (ii) Theoretically, one can introduce in the Hamiltonian a Lagrange multiplier \( \sigma \) multiplying the total membrane area. In principle \( \tau \) can be deduced from \( \sigma \) by renormalization procedures [11, 12]. (More accurately, one may fix the total membrane area by a constraint [13] or, even better, keep the lipid density as a parameter [8].) (iii) Experimentally, one measures another tension, \( \tau \), through the pressure difference across the membrane by means of the Laplace relation [14, 15]. In principle, this is the actual (macroscopic) tension, since it corresponds to the average force that is exerted tangentially to the average membrane’s surface. In principle, \( \tau \) can be calculated by differentiating the total free-energy with respect to the projected area [12], but this is a tricky matter [16]. The confusion associated with these different tensions inspired many articles [11, 12, 16, 17, 18, 19, 20, 21].

Recently, a new method was introduced to calculate \( \tau \), based on thermally averaging the stress tensor of the membrane [16]. It has the advantage to be directly related to the very definition of \( \tau \). This method was applied to planar membranes and it was shown that the expression of \( \tau \) obtained in this way coincides with that obtained from carefully differentiating the total free-energy [16].

The aim of this paper is to calculate the surface tension \( \tau \) of quasi-spherical vesicles by using the same method as in [16], i.e., by averaging the stress tensor. There are several interesting questions that we shall address:

1. What is the difference between \( \tau \) for a vesicle and \( \tau \) for a planar membrane? Is there a characteristic radius over which they coincide?
2. How does the volume constraint influence the expression for \( \tau \)?
and fixed volume $V$.

We consider a quasi-spherical vesicle with fixed area $A$ and fixed volume $V$. Its shape is parametrized in spherical coordinates by (see Fig. 1)

$$ r = R [1 + u(\theta, \phi)] e_R, $$

where $u \ll 1$. A convenient choice for the reference sphere is $R = (\frac{4V}{\pi})^{1/3}$, corresponding to the sphere of volume $V$. The volume and area constraints read:

$$ V = \frac{1}{3} R^3 \int_0^\pi d\theta \int_0^{2\pi} d\phi \ [1 + u(\theta, \phi)]^3 \sin \theta, $$

$$ A = R^2 \int_0^\pi d\theta \int_0^{2\pi} d\phi (1 + u) \times \sqrt{(1 + u)^2 \sin^2 \theta + u_\theta^2 + u_\phi^2 \sin^2 \theta}. $$

Here, and in the following, $u_i \equiv \partial u/\partial i$, $u_{ij} \equiv \partial^2 u/\partial i \partial j$, where $i, j \in \{\theta, \phi\}$. Latin indices will denote either $\theta$ or $\phi$, not $r$.

There are three variants of the free-energy describing the elasticity of a fluid vesicle: i) the “spontaneous curvature”, or Helfrich model [22], ii) the “bilayer couple” model [23,24], and iii) the “area-difference elasticity” model [25,26], the latter being the most accurate [7]. As shown by Seifert [13], the three models are all equivalent in the quasi-spherical limit to the minimal Helfrich model, i.e., to the spontaneous curvature model with vanishing spontaneous curvature [22]. We therefore adopt the latter, which corresponds to the free-energy, or effective Hamiltonian: $H = 2\kappa \int dS C^2$, supplemented by the area and volume constraints. Here the integral runs over the vesicle’s surface, $C$ is the local mean curvature of the membrane and $\kappa$ is the bending rigidity constant. While the volume constraint is quite easy to implement, it is difficult to handle the surface constraint exactly [13]. We shall therefore use the traditional approach, in which the area constraint is approximatively taken into account by means of a Lagrange multiplier $\sigma$ playing the role of an effective surface tension. As discussed in Ref. [13], the latter approach gives correct results in the small excess area limit (in which we shall place ourselves).

Thus, the effective Hamiltonian we shall use is

$$ H = \int dS \left(2\kappa C^2 + \sigma\right), $$

together with the volume constraint (2). Explicitly, in terms of $u(\theta, \phi)$, the Hamiltonian reads

$$ H = \int d\theta \ d\phi \ h(u, \{u_i\}, \{u_{ij}\}), $$

with [28,29]:

$$ h = (2\kappa + R^2 \sigma) \sin \theta \\
+ 2 \sin \theta \left[R^2 \sigma u - \kappa \left(u_{\phi\phi} \csc^2 \theta + u_{\theta\theta} \cot \theta + u_{\theta\phi}\right)\right] \\
+ \frac{1}{2} \sin \theta \left[2R^2 \sigma u^2 + (2\kappa + R^2 \sigma)(u_\theta^2 + u_\phi^2 \csc^2 \theta)\right] \\
+ \kappa \left(u_{\theta\theta} \cot \theta + u_{\phi\phi} \csc^2 \theta\right)^2 + \kappa u_{\theta\phi}(u_{\theta\theta} + 4u) \\
+ 2\kappa (u_{\theta\theta} + 2u) \left(u_{\theta\theta} \cot \theta + u_{\phi\phi} \csc^2 \theta\right) + O(u^3). $$

2.2 Stress tensor components

Let us consider an infinitesimal cut at constant longitude ($\phi$ constant) separating a region 1 from a region 2 (see Fig. 1). The normal to the projection of this cut onto the reference sphere is $m = e_R$. By definition, the projected stress tensor $\Sigma$ in spherical geometry relates linearly the
force \(dF\) that region 1 exerts on region 2 to the angular length \(ds = d\theta\) of the projection of the cut onto the reference sphere:

\[
dF = \mathbf{\Sigma} \cdot \mathbf{ds} = (\Sigma_{\theta\theta} e_\theta + \Sigma_{\theta\phi} e_\phi + \Sigma_{\phi\phi} e_\phi) d\theta.
\] (7)

Likewise, for a cut at constant latitude (\(\theta\) constant), with \(m = e_\theta\) and \(d\phi = d\phi\), we have

\[
dF = \mathbf{\Sigma} \cdot \mathbf{ds} = (\Sigma_{\theta\theta} e_\theta + \Sigma_{\phi\phi} e_\phi + \Sigma_{\theta\phi} e_\phi) d\phi.
\] (8)

For an oblique cut, \(dF\) is obtained by decomposing \(m\) along \(e_\theta\) and \(e_\phi\).

The projected stress tensor associated with the Hamiltonian of the minimal Helfrich model has been calculated in planar geometry [10] and in cylindrical geometry [27] (see also Ref. [9] for the original covariant formulation). The derivation in spherical geometry is lengthier but it follows the same route, which we sketch now. We consider a patch of membrane delimited by a closed curve, corresponding to a domain \(\Omega\) on the reference sphere enclosed by \(\partial\Omega\). The membrane within the patch is assumed to be deformed, at equilibrium, by means of a distribution of surface and boundary forces (and a distribution of boundary torques). To each point of the patch, we impose an arbitrary displacement \(\delta a = \delta a_r e_r + \delta a_\theta e_\theta + \delta a_\phi e_\phi\) that keeps constant along the boundary the orientation of the membrane’s normal \(\mathbf{n}\) (in this way the torques will produce no work). On the one hand, the boundary energy variation reads (after integration by parts):

\[
\delta H = \int_{\partial\Omega} ds m_1 \left[ h \delta i + \left( \frac{\partial h}{\partial u_i} - \frac{\partial \phi}{\partial u_i} \delta i + \frac{\partial \phi}{\partial u_i} \delta u_j \right) \right],
\] (9)

where \(ds\) is the arc-length in the \((\theta, \phi)\) space, \(h\) is given by eq. [13], \(m\) is the normal to \(\partial\Omega\), and \(\delta i \in \{\delta \theta, \delta \phi\}\) corresponds to the variation of \(\partial\Omega\). On the other hand, the work of the force exerted through the boundary reads

\[
\delta H = \int_{\partial\Omega} ds \delta a \cdot \mathbf{\Sigma} \cdot \mathbf{m}.
\] (10)

By identifying eqs. [13] and [14], one can obtain \(\Sigma\). Details of this calculation, as well as all the components of the stress tensor are given in appendix A. In particular, we obtain

\[
\Sigma_{\theta\theta} = \frac{1}{2R} \left\{ R^2 \sin \theta (2 + 2u + u^2 \csc^2 \theta - u^2) + \kappa \left[ u^2 \csc^2 \theta - u^2 \sin \theta 
+ 2u \left( u_{\phi\phi} \csc \theta + u_{\theta\theta} \sin \theta \right) \right] 
+ \kappa \csc \theta \left[ 2u^2 - u^2 \cos^2 \theta - 2u_{\phi\phi} (1 + u_\theta \cot \theta) \right] 
+ 4\kappa u \csc \theta \left( u_{\phi\phi} - u_{\theta\theta} \csc^2 \theta \right) 
+ 2u \left( u_\theta \left( \sin \theta + u_\theta \cot \theta \right) + (2u - 1) u_\theta \cos \theta \right) \right\} 
+ \mathcal{O}(u^3).
\] (11)

### 2.3 Thermal averages and fluctuation spectrum

In order to calculate the vesicle’s mechanical tension \(\tau\), we need to determine the thermal average \(\langle \Sigma_{\theta\theta} \rangle\). To this aim, we perform the standard decomposition of \(u(\theta, \phi)\) in spherical harmonics [13,28,29]:

\[
u(\theta, \phi) = \frac{u_{0,0}}{\sqrt{4\pi}} + \sum_{\omega} u_{l,m} Y_{l,m}(\theta, \phi),
\] (12)

with \(u_{l,-m} = (-1)^m u_{l,m}\) and

\[
\sum_{\omega} = \sum_{l=2}^{L} \sum_{m=-l},
\] (13)

\(L\) being a high wavevector cutoff (see below). Note that the modes \(l = 1\), which correspond to simple translations, are discarded.

In terms of \(u_{l,m}\) and up to order \(u^2\), eq. (2) takes the form:

\[
V = R^3 \left[ \frac{4\pi}{3} \left( 1 + \frac{u_{0,0}}{\sqrt{4\pi}} \right) ^3 + \sum_{\omega} |u_{l,m}|^2 \right].
\] (14)

The volume constraint \(V = \frac{4}{3}\pi R^3\) (recall the definition of \(R\)) implies therefore [13]:

\[
u_{0,0} = -\frac{1}{\sqrt{4\pi}} \sum_{\omega} |u_{l,m}|^2.
\] (15)

With the help of the relation:

\[
\cot \theta \frac{\partial Y_{l,m}}{\partial \theta} + \csc^2 \theta \frac{\partial^2 Y_{l,m}}{\partial \phi^2} = -\frac{\partial^2 Y_{l,m}}{\partial \theta^2} - l (l + 1) Y_{l,m},
\] (16)

and using eq. (15), one obtains then [13]:

\[
H = 4\pi R^2 \sigma + \frac{1}{2} \sum_{\omega} \tilde{H}_{l} |u_{l,m}|^2 + \mathcal{O}(u^3),
\] (17)

where

\[
\tilde{H}_l = \kappa (l - 1) (l + 2) (l^2 + l + \sigma).
\] (18)

Here,

\[
\sigma = \frac{\sigma}{\kappa / R^2}
\] (19)

is the reduced tension. Note that we have discarded in \(H\) a constant energy term, \(8\pi \kappa\).

We emphasize that negative values of \(\sigma\) are allowed [13]. Indeed, the minimum of the Hamiltonian [17] corresponds for \(\sigma > -6\) to a perfectly spherical vesicle \((u_{l,m} = 0, \forall l \geq 2)\). The mean-field transition to an oblate shape occurs thus at \(\sigma = -6\) (non harmonic terms being then needed to stabilize the system).

Standard statistical mechanics yields then \(\langle u_{l,m} \rangle = 0, \forall l \neq 0\), and

\[
\langle u_{l,m} u_{l',m'} \rangle = (-1)^m \frac{k_B T}{H_l} \delta_{l,l'} \delta_{m,-m'},
\] (20)

where \(k_B T \equiv 1/\beta\) is the temperature in energy units.
We may now calculate the fluctuation amplitudes. Using eq. (20) and the addition theorem for spherical harmonics:

\[
\sum_{m=-l}^{l} Y_{l}^{m}(\theta, \phi) Y_{l}^{m}(\theta, \phi)^{*} = \frac{2l + 1}{4\pi},
\]

we obtain

\[
\langle u \rangle = \frac{\langle u_{0,0} \rangle}{\sqrt{4\pi}} = -\frac{1}{4\pi} \sum_{\omega} \langle |u_{\omega,\omega}|^2 \rangle
\]

\[
= \frac{k_{B} T}{4\pi} \sum_{l=2}^{L} \frac{2l + 1}{H_l},
\]

\[
\langle u^2 \rangle = \sum_{\omega} \sum_{\omega'} Y_{l}^{m}(\theta, \phi) Y_{l}^{m'}(\theta, \phi) \langle u_{\omega,\omega'} u_{\omega',\omega} \rangle
\]

\[
= \sum_{\omega} \frac{k_{B} T}{H_l} Y_{l}^{m}(\theta, \phi) Y_{l}^{m}(\theta, \phi)^{*} = \frac{k_{B} T}{4\pi} \sum_{l=2}^{L} \frac{2l + 1}{H_l} = -\langle u \rangle, \tag{23}\]

\[
\langle u_{2} \rangle = \sin^{2} \theta \frac{k_{B} T}{4\pi} \sum_{l=2}^{L} \frac{l(l+1)(2l+1)}{2H_l}, \tag{24}\]

\[
\langle u_{4} \rangle = \frac{k_{B} T}{4\pi} \sum_{l=2}^{L} \frac{l(l+1)(2l+1)}{2H_l}, \tag{25}\]

The correlations of the other derivatives of \( u \) are given in appendix B. Note the interesting relation \( \langle u \rangle = -\langle u^2 \rangle \) (valid at second order in \( u \)), showing how the temperature-dependent fluctuations affect the mean-shape.

### 2.4 Cutoff

The large wavenumber cutoff \( L \) should be related with the smallest wavevector allowed, \( \Lambda \approx a^{-1} \), where \( a \) is a length comparable to the membrane thickness (i.e., \( \pi/\Lambda \) of the order of a few times \( a \)). With spherical harmonics, however, this is not easy to implement. The requirement that we should recover the planar limit for large values of \( R \) will guide us.

For a square patch of fluctuating membrane with reference area \( A_{p} \) and periodic boundary conditions, the wavevectors are quantified according to \( q = 2\pi \sqrt{A_{p}} (n_{x}, n_{y}) \), where \( n_{x} \) and \( n_{y} \) are integers and \( |q| < A \). The number of modes is then \( \pi \Lambda^2 / (2\pi \sqrt{A_{p}})^2 \) and the number of modes per unit area is

\[
\frac{N_{\text{modes}}}{A_{p}} = \frac{\Lambda^2}{4\pi}. \tag{26}\]

For the vesicle, we have

\[
\frac{N_{\text{modes}}}{A_{p}} = \frac{1}{4\pi R^2} \sum_{l=2}^{L} (2l + 1) = \frac{(L - 1)(L + 3)}{4\pi R^2}. \tag{27}\]

Asking that the number of degree of freedom per unit area (per lipid, in some sense) be the same in both case, we require these two quantities to be equal. Hence, we get

\[
(L - 1)(L + 3) = \Lambda^2 R^2, \tag{28}\]

which gives \( L = \lfloor \sqrt{\frac{4 + R^2}{A}} - 1 \rfloor \) (\( \lfloor x \rfloor \) is the integer part of \( x \)). In the limit \( R \gg \Lambda^{-1} \), this gives simply \( L \approx aR \).

### 2.5 Validity of the Gaussian approximation

Since our calculations are limited to \( O(u^2) \), we should check, in principle, that higher order terms are negligible. In practice this is not feasible. To check the smallness of \( u \) (which is especially critical in the case \( \sigma \leq 0 \)) we shall require:

\[
\langle u^2 \rangle = \frac{k_{B} T}{4\pi \kappa} \sum_{l=2}^{L} \frac{2l + 1}{(l-1)(l+2)(l^2 + l + \bar{\sigma})} \leq U_{\text{max}}^2. \tag{29}\]

To be definite, in the following we shall take

\[
U_{\text{max}} = 5 \%. \tag{30}\]

Note that the presence of the factor \((l^2 + l + \bar{\sigma})\) in the denominator of eq. (29), together with the condition \( l \geq 2 \), implies \( \bar{\sigma} \in [0, \infty) \), as already discussed.

Solving condition (29) for the typical values \( A^{-1} \approx 5 \text{nm} \), \( \kappa = 25 k_{B} T \), and taking \( U_{\text{max}} = 0.05 \), we find

\[
\bar{\sigma} \geq \bar{\sigma}_{\text{min}} \approx -4, \tag{31}\]

almost independently of \( R \). It follows that negative tensions \( \sigma \) are in fact within the validity range of our Gaussian approximation.

An interesting control parameter is the excess area, defined by

\[
\alpha = \frac{(A) - A_{p}}{A_{p}} = \frac{k_{B} T}{8\pi \kappa} \sum_{l=2}^{L} \frac{2l + 1}{l^2 + l + \bar{\sigma}}. \tag{32}\]

Our validity condition (31) yields \( \alpha \leq \alpha_{\text{max}} \), with \( \alpha_{\text{max}} \) shown in fig. 2. One can see that \( \alpha_{\text{max}} \approx c_{1} + c_{2} \ln R \), where \( c_{1} \) and \( c_{2} \) are constants. Indeed, the sum in eq. (32) is dominated by the modes \( l = 2 \) and \( l = 3 \), the rest being well approximated for \( \bar{\sigma} = O(1) \) by an integral proportional to \( \ln R \).

### 3 Actual mechanical surface tension

Our aim is to calculate the mechanical tension \( \tau \) of the vesicle. Imagine replacing the fluctuating vesicle by a shell coinciding with its average shape. The actual mechanical tension \( \tau \) is the average force per unit length that is exerted tangentially to the shell’s surface. Because of the spherical symmetry, we may evaluate it at any point in any direction. Let us consider at \( (\theta, \phi) \) a cut with \( \theta \) constant of extension \( d\phi \). The component along \( e_{\theta} \) of the force...
exchanged through the cut is on average $\langle \Sigma_{\theta \theta} \rangle$. The length of the cut is on average $(R(1 + u) \sin \theta \, d\phi)$. Hence,

$$\tau = \frac{\langle \Sigma_{\theta \theta} \rangle}{R \sin \theta (1 + \langle u \rangle)}.$$  \hfill (33)

Since $\langle u \rangle = -\langle u^2 \rangle$, because of the volume constraint, and since $\Sigma_{\theta \theta} = \sigma R \sin \theta + O(u)$, we obtain equivalently

$$\tau = \frac{1}{R \sin \theta} \langle \Sigma_{\theta \theta} \rangle + \sigma \langle u^2 \rangle + O(u^3).$$  \hfill (34)

Using eq. (11) and the results obtained in sec. 2.3 and appendix 1, we obtain

$$\tau = \sigma - \frac{k_B T \kappa}{8\pi R^2} \frac{\int_{l=2}^{L} \left( (l-1)(l+1)(l+2)(2l+1) \right)}{l^2 + l + \sigma} \tilde{H}_l$$  \hfill (35)

$$= \sigma - \frac{k_B T \kappa}{8\pi R^2} \sum_{l=2}^{L} \frac{l(l+1)(2l+1)}{l^2 + l + \sigma}.$$  \hfill (36)

As expected this result is independent of point $(\theta, \phi)$ where the calculation was made.

We show in fig. 3 the behavior of $\tau$ as a function of the excess area $\alpha$, obtained numerically from eqs. (35) and (36). This presentation is the most physical, because $\tau$ and $\alpha$ are both measurable, while $\sigma$ is not. There are several salient points:

1. The results for $\tau$ deviate from the flat limit ($R \to \infty$) essentially for $R \leq 1 \mu m$.
2. Negative and quite large values of $\tau$ are indeed accessible.
3. There exists a well-defined excess area corresponding to $\tau = 0$. Since, as we shall show, the curves in fig. 4 are similar without the volume constraint, this value corresponds to the spontaneous excess area taken up by a poked vesicle (vanishing Laplace pressure).
4. There is a plateau at large values of $\alpha$, which probably corresponds to the actual transition to oblate shapes.

It occurs for $\tilde{\tau} \equiv \tau R^2/\kappa < -6$, i.e., below the mean-field threshold (see the discussion after eq. (19)). The high symmetry phase (spherical vesicle) is thus stabilized by its entropic fluctuations, as one might have expected.

We have also calculated the normal and orthogonal components of the tension. They vanish: $\langle \Sigma_{\theta \phi} \rangle = 0$ and $\langle \Sigma_{\phi \phi} \rangle = 0$. While the latter result is obvious on symmetry grounds, the former one is interesting, implying that the shell mentioned above can indeed be considered as a purely tense surface. (This would probably not hold for a vesicle with a non-spherical average shape.) As a consequence, the Laplace law can be used without curvature corrections for a fluctuating quasi-spherical vesicle—i.e., one uses $\tau$ instead of $\sigma$. Indeed, this could be expected from renormalisation arguments, since the Laplace law is exact (despite the curvature energy) for a perfectly spherical membrane.

It is interesting to examine $\tau$ in the limit of large vesicles. In this case, the sum in eq. (36) may be replaced by an integral:

$$\tau - \sigma \approx \frac{k_B T \kappa}{8\pi R^2} \int_{l=2}^{R} dl \frac{l(l+1)(2l+1)}{l^2 + l + \sigma} \frac{A}{R}.$$  \hfill (37)

$$\approx \frac{k_B T A^2}{8\pi} \left[ 1 - \frac{\sigma}{\kappa A^2} \ln \left( 1 + \frac{\kappa A^2}{\sigma} \right) \right] + O(\frac{A}{R}).$$  \hfill (38)
The dominant term in eq. (38) correctly matches the expression of \( \tau - \sigma \) for flat membranes. In this case, the relation between \( \alpha \) and \( \sigma \) is analytical, yielding

\[
\tau_{\text{flat}}(\alpha) = \frac{\kappa A^2}{\epsilon^{8\pi \beta \kappa}} \frac{k_B T A^2}{8 \pi},
\]

(39)

which is shown in fig. 3.

Finally, we show in fig. 4 the excess area corresponding to a vanishing lateral tension \( (\tau = 0) \). Its value is very much radius dependent for \( R \leq 1 \mu m \), but one recovers for \( R \geq 2 \mu m \) the flat membrane limit [16]:

\[
\alpha_0 = \frac{\ln (8\pi \beta \kappa)}{8\pi \beta \kappa},
\]

(40)

4 Derivation using the free-energy

For a flat membrane, one may also obtain \( \tau \) by differentiating the free-energy with respect to the projected area \( A_p \) [12,20,21,16], but there are two pitfalls [16]. One must: i) take the thermodynamic limit \( (A_p \to \infty) \) only after the differentiation, and ii) introduce a cutoff to the order that the total number of modes remains constant during the differentiation, as discussed in [21].

Let us investigate the free-energy method in the case of quasi-spherical vesicles. The free-energy, \( F \), is given by

\[
F = -\frac{1}{\beta} \ln \int \mathcal{D}[r] e^{-\beta H},
\]

(41)

the integral running over all the configurations of the vesicle. At the Gaussian level, \( H \) is given in terms of spherical harmonics by eq. [17], and since \( r = R e_r + R u(\theta, \phi) e_r \), we may write (in agreement with Ref. [13]):

\[
\mathcal{D}[r] = \prod_{l=2}^{L} \left( \prod_{m=0}^{l} R d r_{l,m} \right) \left( \prod_{m=1}^{l} R d u_{l,m} \right),
\]

(42)

where the superscripts \( \mathcal{R} \) and \( \mathcal{I} \) signify real part and imaginary part, respectively. This measure corresponds to the so-called normal gauge, which is known to be correct for small fluctuations [13]. We note that the radius \( R \) of the reference sphere appears explicitly and that for each value of \( l \), only half of the allowed values of \( m \) have to be considered, as \( r \) is real.

Performing the Gaussian integrals yields

\[
F = 4\pi R^2 \sigma + k_B T \sum_{l=2}^{L} \frac{2l + 1}{2} \ln \left( \beta H_l/R^2 \right).
\]

(43)

In order to obtain \( \tau \), we must differentiate \( F \) with respect to the vesicle’s “projected area” \( A_p \). Which one, however? The area \( 4\pi R^2 \) of the reference sphere (i.e., the sphere having the same volume as the vesicle’s), or the area of the vesicle’s average shape, defined as \( A_m = 4\pi (R(1 + w))^2 \)? It will turn out that the former choice is the correct one. In a sense, this is natural because it corresponds to our parametrization. However, it is not that obvious, because the definition of \( \tau \) in eq. (39) involves the area of the average vesicle’s shape.

Let us thus pick \( A_p = 4\pi R^2 \). It is worth noticing that \( H_l \) depends on \( A_p \) only through \( \bar{\sigma} = \sigma R^2/\kappa \), yielding

\[
\frac{\partial \bar{H}_l}{\partial R^2} = (l - 1)(l + 2) \sigma.
\]

(44)

With the choice:

\[
\tau = -\frac{k_B T}{8\pi R^2} \sum_{l=2}^{L} \frac{l(l + 1)(2l + 1)}{l^2 + l + \bar{\sigma}},
\]

(45)

we obtain

\[
\tau = \sigma - \frac{k_B T}{8\pi R^2} \sum_{l=2}^{L} \frac{l(l + 1)(2l + 1)}{l^2 + l + \bar{\sigma}},
\]

(46)

which is identical to the result obtained from the stress tensor approach, eq. (46). How about the pitfalls mentioned above? First, we didn’t take the thermodynamic limit before differentiating. (Actually, this would not be problematic, since the quantification of the modes does not involve the size of the system, like it is the case for planar membranes.) Second, we kept \( L \) (hence the number of modes) constant during the differentiation, in agreement with the fact that \( L = [\sqrt{4 + R^2 \Lambda^2} - 1] \) is constant for a mathematically infinitesimal change of \( R \).

We may obtain a more intrinsic expression for \( \tau \). With \( \langle A \rangle = A_p(1 + \alpha) \), where \( \alpha \) is given by eq. (32), and \( N_{\text{modes}} = \sum_{l=2}^{L} (2l + 1) \), we may rewrite \( \tau \) as

\[
\tau A_p = \langle A \rangle \sigma - \frac{k_B T}{2} N_{\text{modes}}.
\]

(47)

The quickest way to this result is to keep separate, when differentiating with respect to \( R^2 \), the two terms coming from \( \ln(\bar{H}_l) \) and \( \ln(1/R^2) \) in eq. (43). The interpretation of this equation is not straightforward, because \( k_B T/2 \) is the internal energy per mode (not the free-energy per mode). Note that the same form for \( \tau \) is also valid in the planar case, as shown in [21] (with further justifications in [15]).
In addition, we have checked that differentiating $F'$ with respect to $A_m = 4\pi[R(1 + (u))^2]$, which differs from $4\pi R^2$ by terms of $\mathcal{O}(u^2)$, gives a wrong result (in the sense that it differs from the result obtained by the stress tensor method).

### 4.1 Poked vesicle

In the case where the volume is unconstrained, e.g., a vesicle poked by a micropipette or by specific proteins, the natural choice for the reference sphere (of radius $R$) is the average vesicle’s shape. By definition, then, $u_{0,0} = 0$, which implies $A_m = 4\pi R^2$. In this case, the Hamiltonian in the Gaussian approximation becomes:

$$H' = 4\pi R^2\sigma + \frac{1}{2} \sum_{\omega} \hat{H}_{\text{H}} |u_{\omega,\omega}|^2,$$

where $\hat{H}_{\text{H}} = \hat{H} + 4\kappa \sigma$. Equation (45) gives $\tau$ by the stress tensor method, is valid whatever the form of $\hat{H}_{\text{H}}$. Hence, we need just replace $\hat{H}_{\text{H}}$ by $\hat{H} + 4\kappa \sigma$, which yields:

$$\tau' = \sigma - \frac{k_BT}{8\pi R^2} \sum_{l=2}^{\infty} \frac{l(l+1)(2l+1) + \kappa}{(l-l)(l+2)}.$$

(To avoid confusion, we denote here by $\tau'$ the mechanical tension in the case of poked vesicles.) Although eqs. (49) and (50) differ mathematically, it turns out that their difference is numerically irrelevant. Indeed, the extra term $4\sigma/(l-1)(l+2)$ in the denominator of eq. (49) is only important for small $l$’s while the sum is dominated by large $l$’s.

The free-energy, is given by the same expression as eq. (49) with $\hat{H}_{\text{H}}$ replaced by $\hat{H}'$:

$$F' = 4\pi R^2\sigma + k_BT \sum_{l=1}^{\infty} \frac{2l+1}{2} \ln \left( \beta \hat{H}'_{\text{H}}/R^2 \right).$$

It turns out, again, that $\tau' = \partial F'/\partial(4\pi R^2)$ exactly. This result is satisfying, but at the same time it shows how slippery the free-energy approach can be: differentiating with respect to the area of the average vesicle is correct in the case of poked vesicles but not in the case of closed vesicles. The stress tensor method is thus a much safer method.

Our expression for $\tau'$ differs from that obtained in Ref. [20], where the authors considered also a spherical membrane without volume constraint. In particular, the mechanical tension obtained in that reference cannot take negative values. We believe that the discrepancy between the two results comes from the omission in Ref. [20] of the factors $R$ within the measure. Indeed the factor $1/R^2$ in the logarithm of our eq. (50) is absent in the corresponding expression (A.9) of Ref. [20].

### 5 Summary and discussion

In this paper we have applied the stress-tensor method [16] to the calculation of the surface tension $\tau$ of quasi-spherical vesicles, investigating both closed vesicles (volume constraint) and poked vesicles (no volume constraint). In both cases we have shown that the more standard method based on differentiating the free energy with respect to the projected area gives the same results, provided it is performed in the right way.

In order to describe the elasticity of the vesicle we have used the simplest Helfrich model, since all its variants (ADE, BC, etc.) are equivalent in the quasi-spherical limit [13]. We have made two approximations: i) we have replaced the membrane area constraint by a Lagrange multiplier approach with effective surface tension $\sigma$; ii) we have kept all calculations at the Gaussian level. The former approximation is justified for small excess areas (see sec. 4C of Ref. [13]). To control the validity of the latter approximation, we have restricted our investigations to relative height variations of the membrane with respect to the reference sphere of less than 5%.

We have calculated the projected stress tensor in spherical geometry. This tool should be useful in other contexts. We have thermally averaged it in order to calculate the actual surface tension $\tau$ acting tangentially along the average membrane surface. Its expression in terms of $R$ (vesicle radius), $\sigma$, $\kappa$, $k_BT$, $A$ (cutoff) depends on whether the vesicle is closed or poked. However, numerically the difference is practically indistinguishable. The flat membrane limit holds when $R \geq 1 \mu$m. We have re-obtained the same expressions for $\tau$ by the free-energy approach. This confirms that the so-called naive measure that we have used is valid in the Gaussian approximation (see, e.g., Ref. [13]). We have discussed whether the free-energy should be differentiated with respect to the area of the sphere that has the same volume as the vesicle or with respect to the sphere corresponding to the average vesicle’s shape. We have shown that the correct choice depends on whether the vesicle is closed or poked, and therefore that the stress tensor approach is the safest one.

It was shown in Ref. [20] that $\sigma$ (the Lagrange multiplier) may become negative. One of our motivation was to check whether the actual mechanical tension $\tau$ could become negative or not. This would imply that vesicles could sustain inner pressures lower than the outer pressure (which is impossible for liquid drops). Our analysis shows that $\tau$ may indeed become negative within the validity range of our Gaussian analysis. For instance, small vesicles with $R = 50 \mathrm{nm}$ can reach negatives tensions of $-10^{-4} \mathrm{N/m}$ with an excess area still less than 1% (see fig. [3]). The so-called “giant vesicles”, with $R \geq 1 \mu$m, can sustain negatives tensions $\approx -k_BT A^2/8\pi$ which may be of order $-10^{-6} \mathrm{N/m}$ or $-10^{-5} \mathrm{N/m}$ depending on the uncertainty on the value of the cutoff. The possibility to sustain negative tensions, or negative pressure differences, might be investigated: i) by controlling the outer osmotic pressure, in the case of small vesicles, or ii) by poking a giant vesicle through by a micropipette to which it would adhere and gently decreasing its inner pressure.
An interesting feature, which might be tested experimentally by controlling the pressure outside the vesicle, is the plateau of fig. 3. One sees that when $\tau$ reaches a critical value $\tau_c < 0$, the excess area rises dramatically. As discussed above, this behavior (still within the validity range of our Gaussian approximation) probably corresponds to the transition to oblate shapes. For small vesicles we find that it corresponds roughly to $\tau_c R^2/\kappa \approx -5$ while for giant vesicles it is given by $\tau_c \approx -k_B T A^2/(8\pi)$. Hence, applying the Laplace pressure formula $\Delta P = 2\sigma R$, we find that the critical pressure difference yielding the shape transition is $\Delta P_c \approx \text{sup}[10\kappa/R^3, k_B T A^2/(4\pi R)]$.

Finally, a possible way to measure the difference between $\tau$ and $\sigma$, suggested to us by L. Limozin [33], would be to proceed experiments on adhering vesicles similar to those of Raëdler et al. [31]. In these experiments, the tension is deduced both from the fluctuation spectrum of the adhering part and from the contact angle $\theta_{\text{ad}}$ via the Young-Dupré relation, the adhesion energy being estimated by controlling the pressure outside the vesicle. In order to compare eqs. (9) and (10), one needs to obtain $\delta u_{\phi}$

$$\delta u_{\phi} = \frac{1}{1 + u} \left\{ \delta \phi \left[ (1 + u)^2 - 2u_{\phi}^2 - (1 + u) u_{\theta \phi} \right] \right. \right.$$  \( + \left. \frac{\delta \phi}{\sin \theta} \left[ (1 + u) u_{\phi} \cos \theta - (1 + u) u_{\theta \phi} \sin \theta \right] + \delta u_{\theta} \right\}, \quad (54)

$$\delta \phi = \frac{1}{1 + u} \left\{ \delta \theta \left[ (1 + u) u_{\phi} \cot \theta + 2u_{\phi} u_{\theta} \right] - (1 + u) u_{\theta \phi} \right\} + \delta \phi \left[ (1 + u)^2 \sin^2 \theta + 2u_{\phi}^2 \right] - (1 + u) \left( u_{\phi} \cos \theta \sin \theta + u_{\phi \theta} \right) + \delta u_{\phi} \right\}. \quad (55)

These equations, combined with eqs. (51) and (53), allow us to write $\delta u_{\theta}$ and $\delta u_{\phi}$ in terms of $\delta a$. Up to order $u^2$, we obtain

$$\delta \theta = \frac{\delta a_{\phi}}{R} (1 - u + u^2), \quad (56)$$

$$\delta \phi = \frac{\delta a_{\theta}}{R \sin \theta} (1 - u + u^2), \quad (57)$$

$$\delta u_{\theta} = \frac{1}{R} \left\{ \delta a_{\theta} - (1 - u) \left( \delta a_{\phi} \csc \theta + \delta a_{\phi} u_{\theta} \right) \right\}, \quad (58)$$

$$\delta u_{\phi} = \frac{1}{R} \left\{ \delta a_{\phi} \left[ (1 - u) \cot \theta + u_{\theta} \right] \right. \right.$$  \( + \left. \delta a_{\phi} \csc \theta u_{\theta} \right\} - (1 - u) \left( u_{\phi} \cos \theta \sin \theta + u_{\phi \theta} \right) \right\}. \quad (59)

These equations are to be inserted into eq. (9). Note that it is necessary to expand $h$ at $O(u^3)$ in order to obtain $\partial h/\partial u_{ij}$ and $\partial h/\partial u_{ij}$ consistently at $O(u^2)$ in eq. (9). This means adding

$$h_3 = -\kappa \sin \theta \left\{ 4 \csc^2 \theta u_{\phi} u_{\theta} u_{\phi \theta} + 2u_{\phi}^2 u_{\theta} \right.$$  \( - 2u_{\phi}^2 \csc^2 \theta \left( u_{\phi \theta}^2 \csc^2 \theta - u_{\phi \theta} \cot \theta \right) \right.$$  \( + \left. u \left[ u_{\theta \theta}^2 + u_{\theta}^2 \left( 2 + \cot^2 \theta \right) + 2u_{\phi}^2 \csc^2 \theta u_{\phi \theta}^2 \csc^2 \theta \right] + 2u_{\phi} u_{\theta \phi} \csc^2 \theta \cot \theta + 2u_{\phi} u_{\phi \theta} \csc^2 \theta \csc^2 \theta \right.$$  \( + \left. 2u^2 \left( u_{\phi \theta}^2 + u_{\phi \theta} \cot \theta + u_{\phi \theta} \csc^2 \theta \right) \right\}, \quad (61)

to eq. (9) before calculating the derivatives. Comparing eqs. (9) and (10), we obtain finally

Let $\delta n = \vec{n} - \delta \theta \sin \theta \delta \phi$, $\delta \phi$, $\delta \theta$, $\delta u_{\theta}$, and $\delta u_{\phi}$ in terms of $\delta a$.

A1 Planar limit

In order to check the expressions (62) and (64), we consider the limit $R \to \infty$, which should yield the stress tensor for a
flat membrane. We consider a general point $(\theta, \phi)$ on the sphere and we define a local system of Cartesian coordinates $(x, y, z)$ such that $dx = R \sin \theta \, d\theta$, $dy = R \sin \theta \, d\phi$, and $dz = dr$. We want to determine the stress tensor $\Sigma'$ in these new coordinates. The component of the elementary force $df_\alpha$ along a general direction $\alpha \in \{x, y, z\}$ exerted through a cut perpendicular to $x$ reads $df_\alpha = \Sigma'_{\alpha x} \, dl = \Sigma_{\alpha \theta} \, d\phi$, with the correspondence $dl = R \sin \theta \, d\phi$. Thus we have $\Sigma'_{\alpha x} = \Sigma_{\alpha \theta} / (R \sin \theta)$. Likewise, $\Sigma'_{\alpha y} = \Sigma_{\alpha \phi} / R$ since in this case $dl = R \, d\theta$. In the limit of large $R$, the plane tangent to the sphere at the point $(\phi, \theta)$ becomes the reference plane of the membrane, and the height of the membrane over this plane is $h(x, y) = R \mu \theta(\phi)$. Hence, $u_\theta = R u_\phi = h_x$, $u_\phi = R \sin \theta \, u_y = \sin \theta \, h_y$, $u_{\phi \theta} = R^2 u_{\phi x} = R h_{x x}$, $u_{\phi \phi} = R^2 \sin^2 \theta \, u_{yy} = R \sin^2 \theta \, h_{yy}$, etc. Keeping the terms that are dominant in the limit $R \to \infty$, we obtain

$$\Sigma_{\alpha \theta} = \frac{1}{2R} \left\{ R^2 \sigma \sin \theta \left( 2 + 2u_\alpha + u_{\phi \phi} \cos^2 \theta - u_{\theta \theta} \sin \theta + 2u_{\phi \theta} \left( u_{\phi \phi} \cos \theta + u_{\theta \theta} \sin \theta \right) \right) + \kappa \left[ u_{\phi \phi} \cos \theta - u_{\theta \theta} \sin \theta + 2u_{\phi \theta} \left( u_{\phi \phi} \cos \theta + u_{\theta \theta} \sin \theta \right) \right] \right\},$$

$$\Sigma_{\phi \phi} = \frac{1}{2R} \left\{ R^2 \sigma \sin \theta \left( 2 + 2u_\alpha + u_{\phi \phi} \cos^2 \theta - u_{\theta \theta} \sin \theta + 2u_{\phi \theta} \left( u_{\phi \phi} \cos \theta + u_{\theta \theta} \sin \theta \right) \right) + \kappa \left[ u_{\phi \phi} \cos \theta - u_{\theta \theta} \sin \theta + 2u_{\phi \theta} \left( u_{\phi \phi} \cos \theta + u_{\theta \theta} \sin \theta \right) \right] \right\},$$

$$\Sigma_{\phi \theta} = \frac{1}{2R} \left\{ R^2 \sigma \sin \theta \left( 2 + 2u_\alpha + u_{\phi \phi} \cos^2 \theta - u_{\theta \theta} \sin \theta + 2u_{\phi \theta} \left( u_{\phi \phi} \cos \theta + u_{\theta \theta} \sin \theta \right) \right) + \kappa \left[ u_{\phi \phi} \cos \theta - u_{\theta \theta} \sin \theta + 2u_{\phi \theta} \left( u_{\phi \phi} \cos \theta + u_{\theta \theta} \sin \theta \right) \right] \right\},$$

where $\sigma = \kappa h_x \partial_x \nabla^2 h$, $\sigma_{xy} = -\sigma h_x h_y - \kappa h_x h_y V^2 h + \kappa h_y \partial_y \nabla^2 h$, $\sigma_{xz} = \kappa h_x \partial_z \nabla^2 h$, $\sigma_{yy} = \kappa h_y \partial_y \nabla^2 h$, $\sigma_{yz} = -\sigma h_x h_y - \kappa h_x h_y V^2 h + \kappa h_y \partial_z \nabla^2 h$, $\sigma_{xy} = -\sigma h_x h_y - \kappa h_x h_y V^2 h + \kappa h_y \partial_y \nabla^2 h$, $\sigma_{yz} = \kappa h_y \partial_z \nabla^2 h$.

B Correlation functions and relations among them

There are five fundamental correlation functions, which we have calculated explicitly:

$$\langle u_{\theta \theta} \rangle = -\langle u_{\phi \phi} \rangle,$$

$$\langle u_{\phi \theta} \rangle = -\langle u_{\phi \phi} \rangle.$$

which holds also in the presence of derivatives with respect to $\theta$, one deduces the following rule: when averaging a product of two terms one may pass a derivative with respect to $\phi$ from one term to the other one while multiplying by $-1$. Hence,

$$\langle u_{\phi \phi} \rangle = 0,$$

$$\langle u_{\theta \theta} \rangle = 0,$$

$$\langle u_{\phi \theta} \rangle = 0.$$
Starting from the addition theorem for spherical harmonics, one may show by suitable differentiations that the sum
\[ \sum_{m=-l}^{l} \frac{\partial^k Y_l^m(\theta, \phi)}{\partial \theta^k} \frac{\partial^{p-l} Y_l^m(\theta, \phi)^*}{\partial \theta^{p-l}} = 0 \]
vanishes for \( k + p \) odd, implying:
\[ \langle u \ u_{\theta \phi} \rangle = 0, \quad \langle u \ u_{\theta \theta} \rangle = 0. \]

Note that this does not hold when derivations with respect to \( \phi \) are also involved.

Starting again from the addition theorem, one may show that
\[ \sum_{m=-l}^{l} \frac{\partial^{k+1} Y_l^m(\theta, \phi)}{\partial \theta^{k+1}} \frac{\partial^{p-1} Y_l^m(\theta, \phi)^*}{\partial \theta^{p-1}} = \sum_{m=-l}^{l} \frac{\partial^k Y_l^m(\theta, \phi)}{\partial \theta^k} \frac{\partial^{p-l} Y_l^m(\theta, \phi)^*}{\partial \theta^{p-l}}. \]

It follows that, when averaging a product of two terms one may pass a derivative with respect to \( \theta \) from one term to the other one while multiplying by \(-1\). This holds only, however, in the absence of derivatives with respect to \( \phi \).

As a consequence,
\[ \langle u_{\theta} \ u_{\phi} \rangle = -\langle u \ u_{\theta \phi} \rangle, \quad \langle u_{\theta} \ u_{\theta \theta} \rangle = -\langle u_{\theta \theta} \ u_{\theta \theta} \rangle. \]

Finally, one may also use the fact that \( \Delta Y_l^m(\theta, \phi) = 0 \), where \( \Delta \) is the laplacian in spherical coordinates, to obtain
\[ \langle u_{\theta} \ u_{\theta \theta} \rangle = \cot \theta \langle u \ u_{\theta \theta} \rangle - \csc^2 \theta \langle u_{\theta} \ u_{\phi \phi} \rangle. \]

Since \( \langle u_{\theta} \ u_{\theta \phi} \rangle \) vanishes, on obtains
\[ \langle u_{\theta} \ u_{\phi \phi} \rangle = -\sin \theta \cos \theta \langle u_{\phi \phi} \rangle. \]

In conclusion, eqs. (81), (88), together with eqs. (86), (88), and eq. (87) give all the correlation functions of the derivatives of \( u \).

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\[ \langle u_{\theta}^2 \rangle = \sum_{\omega,\omega'} \partial_{\theta} Y_{l m}^m (\theta, \phi) \partial_{\theta} Y_{l m}^{m'} (\theta, \phi) \langle u_{lm} u_{l'm'} \rangle = \sum_{\omega} \frac{k_B T}{H_1} \partial_{\theta} Y_{l m}^{m} \partial_{\theta} (Y_{l m}^{m})^* = \sum_{\omega} \frac{k_B T}{H_1} m^2 |Y_{l m}^{m}|^2, \]
\[ = \sin^2 \theta \frac{k_B T}{4\pi} \sum_{l=2}^L \frac{l(l+1)(2l+1)}{2H_1}, \quad (84) \]

\[ \langle u_{\phi \phi}^2 \rangle = \sum_{\omega} \frac{k_B T}{H_1} m^4 |Y_{l m}^{m}|^2 = \sin^2 \theta \frac{k_B T}{4\pi} \sum_{l=2}^L \frac{l(l+1)(2l+1)}{8H_1} \left[ 4 + 3 \left( -2 + l + l'^2 \right) \sin^2 \theta \right], \quad (85) \]

\[ \langle u_{\theta}^2 \rangle = \sum_{\omega} \frac{k_B T}{H_1} \partial_{\theta} Y_{l m}^m \partial_{\theta} (Y_{l m}^{m})^* = \frac{k_B T}{4\pi} \sum_{l=2}^L \frac{l(l+1)(2l+1)}{2H_1}, \quad (86) \]

\[ \langle u_{\theta}^2 \rangle = \sum_{\omega} \frac{k_B T}{H_1} \partial_{\theta} Y_{l m}^m \partial_{\theta} (Y_{l m}^{m})^* = \frac{k_B T}{4\pi} \sum_{l=2}^L \frac{l(l+1)(2l+1)}{8H_1} \left( -2 + 3l + 3l'^2 \right), \quad (87) \]

\[ \langle u_{\theta \phi \phi} \rangle = -\sum_{\omega} \frac{k_B T}{H_1} m^2 \partial_{\theta} Y_{l m}^m \partial_{\theta} (Y_{l m}^{m})^* = -\frac{k_B T}{4\pi} \sum_{l=2}^L \frac{l(l+1)(2l+1)}{2H_1} \left[ 1 + \frac{(l+3)(l-2)}{4} \sin^2 \theta \right]. \quad (88) \]