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Model-free thermodynamics of fluid vesicles

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Motivated by a long-standing debate concerning the nature and interrelations of surface-tension variables in fluid membranes, we reformulate the thermodynamics of a membrane vesicle as a generic two-dimensional finite system enclosing a three-dimensional volume. The formulation is shown to require two tension variables, conjugate to the intensive constraints of area per molecule and volume-to-area ratio. We obtain the relation between these two variables in various scenarios, as well as their correspondence to other definitions of tension variables for membranes. Several controversies related to membrane tension are thereby resolved on a model-free thermodynamic level. The thermodynamic formulation may be useful also for treating large-scale properties of vesicles that are insensitive to the membrane's detailed statistical mechanics and interactions.

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I. INTRODUCTION

Membrane vesicles are flexible fluid envelopes, made of a bilayer of amphiphilic molecules in aqueous solution [1,2]. Since Helfrich's work almost three decades ago [3], these prevalent structures have been one of the most extensively studied systems in soft-matter physics, exhibiting rich statistical-mechanical [4] and dynamic [5] behaviors. It may seem odd, therefore, to go back now and reformulate their equilibrium thermodynamics. Nevertheless, this is the goal of the current work. The reason is a long and ongoing debate concerning various definitions of the surface tension(s) of fluid membranes, their physical meanings, and interrelations [6–20]. Such issues as the exact number of independent tension variables and their possible vanishing [6,8,12,19], or even sign reversal [16–18], have remained controversial. The debate has been conducted at the statistical-mechanical level, emphasizing the role of thermal fluctuations and employing various analytical and simulation techniques. While certain issues are inherently statistical mechanical, it seems plausible that at least some of the other fundamental issues mentioned above should be settled at the lower, more general level of thermodynamics (i.e., without specifying a Hamiltonian).

Two key properties of fluid membranes make their thermodynamic characterization special and confusing. The first is that, while being extensive in two dimensions, the membrane is embedded in three dimensions. Consequently, it was suggested early on that the thermodynamics of open membranes required two spatial constraints rather than one [6] (the membrane area A and its projection onto a reference planar frame A_p) entailing two conjugate variables [8]. The variable conjugate to A, Π, is a surface pressure, analogous to ordinary thermodynamic pressure. The one related to A_p, the frame tension τ, is the force per unit length exerted at the edges of the frame, in the plane of reference, to maintain a given A_p.

The second property of bilayer membranes which sets them apart from ordinary fluids is the fact that they have a preferred area per molecule [12,21]. This boils down to the hydrophobic effect, which opposes the exposure of the inner core of the bilayer to the outer solvent [21]. As a result, the membrane possesses in-plane elasticity; unlike an ordinary fluid it can sustain not only a global compressive stress but also a global dilative one.

The discussion of membrane thermodynamics is further obscured when the in-plane elasticity is suppressed by taking the limit of membrane incompressibility. This commonly used assumption simplifies calculations and allows one to theoretically fix the total area of the membrane. Yet, at the same time, it makes essentially independent thermodynamic variables dependent: the area A and number of membrane molecules N become linearly related, and so do the conjugate surface pressure and chemical potential [8,12].

To these complications, which are special to fluid membranes, one should add the common pitfalls in thermodynamic analyses: the necessity to precisely define which variables are kept fixed when another variable is varied, and the fact that two variables, which turn out to be equal under certain considerations, are not necessarily identical.

To circumvent some of the foregoing complications we consider a closed envelope, a vesicle, rather than an open membrane. We expect (and will demonstrate below) that, once the thermodynamic behavior of a vesicle is clarified, the consequences for an open membrane should be readily inferred. In the case of a closed vesicle the three-dimensional constraint is an ordinary volume constraint on the enclosed system, rather than a projected-area constraint on the surface. Thus, we explicitly consider the contents of the envelope as a thermodynamic system, for which the properties of the membrane become surface effects. As a result, the starting point of the formulation resembles that of other studies of finite-size systems such as droplets or domains. It contains only well known, unambiguous thermodynamic variables, such as volume and surface area. The special properties of the membrane subsequently emerge without further assumptions or an externally imposed reference frame. In addition, membrane incompressibility is nowhere assumed.

The current work should be distinguished from other thermodynamic formulations for membranes, which were presented in the past [22,23]. The aim of those theories was to use a *local* thermodynamic description, based on Gibbs' theory of interfaces [22] or molecular considerations [23], to construct a coarse-grained, local free energy of a nonfluctuating mesoscopic element of the membrane. Subsequently, the resulting free energy per unit area could be used, like the Helfrich Hamiltonian [3], as a starting point for statistical-mechanical or other calculations on a larger scale. By contrast, we directly address the *global*, large-scale thermodynamics of a membrane vesicle.

In Sec. II we formulate the thermodynamics of the vesicle and present the resulting tension variables. In Sec. III we examine the consequences of the formulation in various experimental and theoretical scenarios, and the resulting relations between the two tension variables themselves, and between them and other previously defined variables. While the formulation is for a closed vesicle, we examine the analogous results for open membranes as well. Section IV summarizes the findings, relates them with earlier results, and discusses their implications.

II. THERMODYNAMIC FORMULATION

Consider a three-dimensional system of volume V, containing Q particles [24], and being in thermal contact with a bath of temperature T. In the thermodynamic limit the Helmholtz free energy of the system $F_3(T,V,Q)$ is extensive in Q while keeping the volume per particle fixed

$$F_3(T,V,Q) = Qf_3(T,v), \quad v \equiv V/Q. \tag{1}$$

If we double the number of particles, and at the same time also double the volume, the free energy F_3 will double. Conjugate to T is the entropy per particle, $s_3 \equiv -(\partial f_3/\partial T)_v$. Conjugate to v is the internal pressure

$$p_{\text{in}} \equiv -\left(\frac{\partial f_3}{\partial v}\right)_T. \tag{2}$$

The volume is enclosed by a two-dimensional envelope of surface area A and N particles [25]. As in studies of other finite-size systems (e.g., droplets, bubbles, domains), we restrict the analysis to the leading correction introduced by the surface to the thermodynamic limit $F = F_3$ of Eq. (1). This leading correction arises from contributions to the free energy which are extensive in the surface size N. Since the envelope is closed in three dimensions, its Helmholtz free energy generally depends on both A and V, $F_2 = F_2(T,A,V,N)$. For example, the envelope's entropy (which is extensive in N and should be included, therefore, in F_2) will decrease if V is increased while keeping A fixed. The dependence of F_2 on A and V implies that the thermodynamic description of the envelope requires *two* intensive variables apart from temperature. We choose these variables to be the area per particle $a \equiv A/N$ and the dimensionless volume-to-area ratio $\alpha \equiv 6\sqrt{\pi}V/A^{3/2}$ (defined such that it has the maximum value of unity for a perfectly spherical envelope). For F_2 to be extensive in N, one should fix *both* a and α,

$$\begin{aligned} F_2(T,A,V,N) &= Nf_2(T,a,\alpha), \\ a \equiv A/N, &\quad \alpha \equiv 6\sqrt{\pi}V/A^{3/2}. \end{aligned} \tag{3}$$

This is a key point which is worth dwelling upon. Imagine, for example, that we double both the number of particles making the envelope and its area, but do not change the enclosed volume. The surface free energy arising from in-plane short-range interactions and in-plane entropy will double. Yet, evidently, the resulting object is not merely a rescaled version of the original one. It is less spherical and bound to have more out-of-plane fluctuation entropy per molecule, leading to more than double the surface free energy. If we double N and A, and at the same time also increase V by a factor of $2^{3/2}$, the envelope will be properly rescaled. The in-plane as well as the out-of-plane contributions per molecule will remain unchanged, and the surface free energy F_2 will double. We stress that Eq. (3) contains only contributions which are extensive in the envelope's size.

The appearance of the thermodynamic variable α reflects a three-dimensional constraint imposed on the envelope. Here the constraint is simply that the envelope be closed and contain a volume V. One may impose other three-dimensional constraints, such as a given projected area A_p onto a reference surface. Such a thermodynamic constraint is not independent of the volume constraint; it may replace it but not be added to it. For example, if the area A of the envelope and the volume V that it encloses are known, then, evidently, the area of the sphere of volume V (taken as a reference surface) $A_p = (4\pi)^{1/3}(3V)^{2/3}$, and its deviation from A, are known as well. It is clear, however, that the foregoing considerations of A and V are more transparent and independent of any reference frame.

The flexibility of the thermodynamic formulation allows for adding other constraints. For example, one may suggest that F_2 should depend on additional characteristics of membrane shape apart from A and V, such as the (average) curvature. Yet, one should examine whether such additional constraints are beneficial or experimentally relevant. It is hard to conceive a practical scenario where the shape of a membrane is controlled to such an extent. We prefer, therefore, to remain with the standard constraints of volume and area as considered for any finite-size system. In statistical-mechanical terms the membrane will sample all those additional shape characteristics which are consistent with the global constraints of A and V (with appropriate Boltzmann weights).

Returning to Eq. (3), we write the entropy per particle of the envelope as $s_2 \equiv -(\partial f_2/\partial T)_{a,\alpha}$. The dependence of f_2 on two intensive variables other than temperature a and α implies that there are necessarily *two independent conjugate variables*

$$\Pi(T,a,\alpha) \equiv -\left(\frac{\partial f_2}{\partial a}\right)_{T,\alpha}, \tag{4}$$

$$\gamma(T,a,\alpha) \equiv \frac{3\alpha}{2a}\left(\frac{\partial f_2}{\partial \alpha}\right)_{T,a}. \tag{5}$$

The prefactor in the definition of γ has been introduced to make the following presentation clearer. Both variables have surface-tension dimensions. The first, Π, is the surface pressure (the two-dimensional analogue of p_{in}) describing the response of the envelope's molecules to in-plane strain (i.e., changes in the area per molecule without a change in the volume-to-area ratio). At the optimum area per molecule Π vanishes. The second variable γ, referred to hereafter as the Laplace tension, is related to the response of the envelope to out-of-plane strain (i.e., changes in the enclosed volume without a change in the area per molecule).

We can further define three surface moduli

$$K_{aa} = \left(\frac{\partial^2 f_2}{\partial a^2}\right)_{T,\alpha} = -\left(\frac{\partial \Pi}{\partial a}\right)_{T,\alpha},$$
$$K_{\alpha\alpha} = \left(\frac{\partial^2 f_2}{\partial \alpha^2}\right)_{T,a} = \frac{2a}{3\alpha}\left[\left(\frac{\partial \gamma}{\partial \alpha}\right)_{T,a} - \frac{\gamma}{\alpha}\right], \quad (6)$$
$$K_{a\alpha} = \frac{\partial^2 f_2}{\partial \alpha \partial a} = -\left(\frac{\partial \Pi}{\partial \alpha}\right)_{T,a} = \frac{2a}{3\alpha}\left[\left(\frac{\partial \gamma}{\partial a}\right)_{T,\alpha} + \frac{\gamma}{a}\right],$$

of which only the first is usually considered (aK_{aa} is the membrane's compression modulus). The last equality in Eq. (6) is a Maxwell relation between the two variables Π and γ. In addition, the convexity of f_2 demands that $K_{aa}K_{\alpha\alpha} > K_{a\alpha}^2$.

Now consider the combined system, with total Helmholtz free energy $F(T,V,Q,A,N)$. The fundamental differential relation for F is

$$dF = -SdT - pdV + \mu_3 dQ - \sigma dA + \mu_2 dN, \quad (7)$$

where S is the total entropy, p the total pressure exerted by the environment, σ the total surface pressure in the envelope, and μ_3 and μ_2 the chemical potentials, respectively, of the particles enclosed inside the vesicle and those making the envelope. In what follows we assume for simplicity that the particles in the volume interact with those in the envelope via hard-core repulsion only (i.e., they are merely enclosed by the envelope). In this case the total Helmholtz free energy is simply given by the sum of the volume and surface contributions

$$F = F_3 + F_2 = Qf_3(T,v) + Nf_2(T,a,\alpha). \quad (8)$$

Note, however, that there is an implicit coupling between the two terms via their mutual dependence on the volume. Taking the differential of Eq. (8) and equating it with Eq. (7), we identify

$$S = Qs_3 + Ns_2, \quad (9)$$
$$p = p_{\rm in} - \frac{2}{\alpha}\left(\frac{Na}{4\pi}\right)^{-1/2}\gamma, \quad (10)$$
$$\mu_3 = f_3 + p_{\rm in}v, \quad (11)$$
$$\sigma = \Pi + \gamma, \quad (12)$$
$$\mu_2 = f_2 + \Pi a. \quad (13)$$

Thus, the total surface pressure σ, the variable conjugate to A in the total free energy, is manifestly broken into two distinct contributions, Π and γ [Eq. (12)]. We underline the different roles played by each of these two tension-like variables, defined in Eqs. (4) and (5). The similarity between Π and ordinary thermodynamic pressure is reflected in its relation to the chemical potential of the envelope's molecules, Eq. (13), in which γ is absent. The Gibbs free energy of the envelope alone, whose sole extensive variable is N, is $G_2 = \mu_2 N = F_2 + \Pi A$ (*not* $F_2 + \sigma A$). By contrast, the mechanical nature of γ is reflected in its contribution to the pressure that the vesicle exerts on the external environment, Eq. (10), in which Π does not appear. We may define for the vesicle an effective thermodynamic radius of curvature, as

$$R_{\rm c} \equiv \alpha\left(\frac{Na}{4\pi}\right)^{1/2} = \frac{3V}{A}, \quad (14)$$

which for a perfectly spherical envelope coincides with the sphere's radius. With this definition Eq. (10) becomes Laplace's law, $p_{\rm in} - p = 2\gamma/R_{\rm c}$.

III. EXPERIMENTAL AND THEORETICAL SCENARIOS

As in any thermodynamic analysis, we can apply the formulation given above to various scenarios, in which certain variables are controlled while others are free to relax. These scenarios correspond to different statistical ensembles. In the following sections we describe several scenarios of particular relevance to experimental conditions or to the debate over membrane tension.

A. Volume or pressure constraint

Let us begin with a scenario in which we prescribe the volume enclosed by the envelope, as well as the temperature and the number of particles in the volume and in the envelope [i.e., we fix (T,V,Q,N)]. In practice, the volume may be considered fixed if we assume the inner solvent to be incompressible and neglect the permeation of solvent into and out of the vesicle. (This is valid, for example, over a sufficiently short time.) Previous studies considered an additional constraint of either a fixed area A or a fixed total surface pressure σ. We argue that this is unnecessary. Indeed, it is impractical to dictate the area of the membrane. If we do not impose an external surface pressure ($\sigma = 0$; the case of finite σ is discussed in the next section), the area will relax under the given constraints of (T,V,Q,N) [26]. The Helmholtz free energy is then minimized with respect to A, leading to

$$\Pi + \gamma = 0. \quad (15)$$

After the minimization, F remains dependent only on (T,V,Q,N). The force balance expressed by Eq. (15) has the following intuitive interpretation. The volume constraint acts as if an external surface pressure were applied to the envelope, $\Pi_{\rm ex} = -\gamma$ [27]. In response, the particles of the envelope develop an internal surface pressure $\Pi = \Pi_{\rm ex}$. The membrane's response to out-of-plane strains must be restoring, $\gamma \geqslant 0$. Hence, in the absence of an actual external surface pressure, the in-plane balance is between two negative pressures (i.e., the volume constraint acts to increase the area, whereas the internal pressure acts to decrease it).

Note that Eq. (15) is not an identity but an equation of state, relating the envelope's intensive variables, T, a, and α; Π and γ are in general independent variables, which become related in this scenario due to area relaxation. In addition, using Eqs. (2), (4), (5), and (15), together with the known dependence of α on V and A, one can calculate for a given model the unknown variables, such as p, a, Π, and γ, as functions of the constraints (T,V,Q,N) [28].

Now consider a different case, where the pressure of the external environment is controlled, while the volume can vary [i.e., we fix (T,p,Q,N)]. This scenario is quite similar to the preceding one; we discuss it separately because it is the most relevant for actual equilibrium vesicles, where the solvent has sufficient time to permeate in and out of the vesicle. In this case we should minimize the Gibbs free energy $G \equiv F + pV$ with respect to V and A, leading again to Eqs. (10) and (15). After the minimization G remains dependent only on (T,p,Q,N). We note that, although we dictate p, we do not directly fix $p_{\rm in}$ because of the surface effect represented by the Laplace tension γ in Eq. (10).

If there is neither a volume constraint nor a pressure one (i.e., $p = p_{\rm in}$), we have a tensionless membrane $\Pi = \gamma = 0$ [8].

B. Contact with a reservoir of amphiphilic molecules

Let us examine a scenario in which the set of constraints is (T,p,Q,μ_2). We explicitly consider this case for two reasons. First, it becomes the practically relevant scenario at sufficiently long times, when the vesicle exchanges amphiphilic molecules with the surrounding solution. Second, it highlights the crucial role played by the three-dimensional embedding of the membrane. The latter issue is recognized once we notice that the set (T,p,Q,μ_2) does not include any constraint that is extensive in membrane size. On the one hand, such a thermodynamic formulation would normally be underdefined and, hence, invalid. In the absence of any constraint on the envelope's size, the vesicle can grow indefinitely by drawing more amphiphilic molecules from the reservoir. On the other hand, it is clear that this scenario can be realized in experiments and simulations. This apparent contradiction is resolved below.

The free energy to minimize in this case is $\tilde{G} \equiv F + pV - \mu_2 N$. The minimization of $\tilde{G}$ with respect to V, A, and N recovers Eqs. (10), (13), and (15), which we should be able to solve for V, A, and N, given the constraints (T,p,Q,μ_2). Let us explicitly rewrite the equations in terms of these unknowns

$$
\begin{aligned}
0 &= f_2\left(T,\frac{A}{N},\frac{V}{A^{3/2}}\right) + \frac{A}{N}\Pi\left(T,\frac{A}{N},\frac{V}{A^{3/2}}\right) - \mu_2,\\
0 &= \Pi\left(T,\frac{A}{N},\frac{V}{A^{3/2}}\right) + \gamma\left(T,\frac{A}{N},\frac{V}{A^{3/2}}\right),\\
0 &= p_{\rm in}\left(T,\frac{V}{Q}\right) - \frac{2A}{3V}\gamma\left(T,\frac{A}{N},\frac{V}{A^{3/2}}\right) - p.
\end{aligned}
$$

The first two equations, describing in-plane equilibrium, are invariant to the scaling $(N \to \lambda N, A \to \lambda A, V \to \lambda^{3/2} V)$ by an arbitrary scale factor λ. It is the third equation (Laplace's law), accounting for the out-of-plane force balance, which violates this scaling and prevents the set of equations from being underdefined.

C. Surface pressure constraint

Just as it is impractical to impose a fixed area A on a vesicle, it is unclear how one could, in practice, apply to the vesicle a fixed in-plane force per unit length σ [29]. Nonetheless, in statistical-mechanical studies of membranes such an external surface-pressure term σA is frequently added to the Hamiltonian as a means to control the mean area. In the current discussion there is no need to introduce such a term and, moreover, we shall see that it leads to unnecessary complications; we consider it merely to relate the emergent variables Π and γ with the commonly used σ. Within our thermodynamic formulation, such a modification adds a term σA to the thermodynamic internal energy and free energy [30]. Consequently, the minimization of the free energy with respect to A now yields Eq. (12), $\sigma = \Pi + \gamma$, as the equation of state instead of Eq. (15). One can again interpret this result as if the particles of the envelope responded through an internal surface pressure Π to an external surface pressure, which consists of σ and the pressure $-\gamma$ due to the volume constraint, $\Pi = \Pi_{\rm ex} = \sigma - \gamma$. Thus, just as prescribing p in Sec. III A did not fix $p_{\rm in}$ because of the existence of γ, introducing the external surface pressure σ does not directly fix the internal one Π for a similar reason.

D. Projected area and frame tension

For open membranes a constraint on the membrane's projected area A_p onto a certain reference plane, is widely used. In the case of a vesicle the choice of the reference surface itself is nontrivial and may depend on the specific scenario [18]. We emphasize again that such a projection constraint is unnecessary in the current analysis since, as noted in Sec. II, constraints on A_p and V are mutually dependent. We may introduce a projection constraint *instead* of the volume constraint (on V or p) to clarify the relations between the current formulation and previous ones. Possible choices of a reference are the mean surface about which the vesicle fluctuates [18,31], the spherical shell that has the same area as the vesicle [10], or the spherical shell that holds the same volume as the vesicle [10,18,32]. We choose the last option as the reference surface, whose area is

$$A_p = \alpha^{2/3} A. \tag{16}$$

We may also define the appropriate intensive variable $a_{\rm p} \equiv A_p/N$. Thus, fixing the projected area corresponds to fixing neither a nor α separately, but rather the product $\alpha^{2/3} a$.

Related to the projected area is the frame tension τ. We have not used the term "conjugate" intentionally because the variable conjugate to A_p would be the partial derivative of the membrane's free energy with respect to A_p while keeping T, N, *and* A fixed,

$$
\begin{aligned}
\tau_1 \equiv \left(\frac{\partial F_2}{\partial A_p}\right)_{T,A,N} &= \left(\frac{\partial f_2}{\partial a_{\rm p}}\right)_{T,a}\\
&= \left(\frac{\partial f_2}{\partial \alpha}\right)_{T,a}\left(\frac{\partial \alpha}{\partial a_{\rm p}}\right)_{a} = \alpha^{-2/3}\gamma.
\end{aligned}
\tag{17}
$$

However, the frame tension as measured or controlled in experiments and simulations is related to the reversible work required to change A_p while keeping only T and N fixed [13], as it is impractical to fix A. This corresponds to differentiating f_2 with respect to $a_{\rm p}$ while keeping only T fixed; both a and α are allowed to vary while satisfying Eq. (16) and the equation of state $a = a(T,\alpha)$. (We have been assuming here the absence of an external surface pressure; if a nonzero σ is considered, σ should be kept constant as well.) This procedure yields

$$
\begin{aligned}
\tau \equiv \left(\frac{\partial f_2}{\partial a_{\rm p}}\right)_{T} &= \left(\frac{\partial f_2}{\partial \alpha}\right)_{T}\left(\frac{\partial \alpha}{\partial a_{\rm p}}\right)_{T}\\
&= \left[\left(\frac{\partial f_2}{\partial \alpha}\right)_{T,a} + \left(\frac{\partial f_2}{\partial a}\right)_{T,\alpha}\left(\frac{\partial a}{\partial \alpha}\right)_{T}\right]\\
&\quad\times\left[\frac{2}{3}\alpha^{-1/3} a + \alpha^{2/3}\left(\frac{\partial a}{\partial \alpha}\right)_{T}\right]^{-1}\\
&= \alpha^{-2/3}\frac{\gamma - \epsilon\Pi}{1+\epsilon}, \quad \epsilon \equiv \frac{3\alpha}{2a}\left(\frac{\partial a}{\partial \alpha}\right)_{T}.
\end{aligned}
\tag{18}
$$

The general expression for τ, Eq. (18), contains a parameter ϵ, which requires knowledge of the equation of state $a(T,\alpha)$. This parameter vanishes for an incompressible membrane having fixed a. It also cancels out in Eq. (18) if we use the equation of state (15) (i.e., if we consider $\sigma = 0$). In these two cases, therefore, we get the simple relation

$$\sigma = 0 \text{ or incompressible membrane:}$$
$$\tau = \tau_1 = \alpha^{-2/3}\gamma = (A/A_p)\gamma \geqslant \gamma. \tag{19}$$

Another simple limit is when the vesicle approaches a perfect spherical shape (or the open membrane approaches a flat state), where $\alpha \to 1$ and $\epsilon \to \infty$. Then we have $\tau = -\Pi$ (i.e., the frame tension provides the entire pressure required to balance the internal surface pressure). In the absence of an external surface pressure $\sigma = 0$, this gives the expected convergence for $\alpha = 1$ of the frame and normal-response (Laplace) tensions $\tau = \gamma$.

If a nonzero σ is considered, however, Eqs. (12) and (18) yield a different relation between the frame tension and the other tension variables

$$\tau = \alpha^{-2/3}\left(\gamma - \frac{\epsilon}{1+\epsilon}\sigma\right), \tag{20}$$

which requires model-dependent knowledge of ϵ. The parameter ϵ can also be represented in terms of the surface moduli defined in Eq. (6). Using the equation of state (15) or (12) to write $d(\Pi + \gamma) = 0$ and extracting from it the derivative $(\partial a/\partial\alpha)_T$, we obtain

$$\epsilon = \frac{3}{2}\frac{(3/2)\alpha^2 K_{\alpha\alpha} - a\alpha K_{a\alpha} + a\gamma}{a^2 K_{aa} - (3/2)a\alpha K_{a\alpha} + a\gamma}. \tag{21}$$

To assess the typical values of ϵ we make the following estimates. We neglect the coupling modulus $K_{a\alpha}$; for a vesicle approaching a spherical shape $K_{\alpha\alpha} \sim k_\mathrm{B}T/(1-\alpha)^2$ [33], where $k_\mathrm{B}T$ is the thermal energy; γa does not exceed a few $k_\mathrm{B}T$ before the membrane ruptures; $K_{aa}a^2$ is of order $10^2\, k_\mathrm{B}T$. Thus, for vesicles which are not extremely swollen $\epsilon \ll 1$, and the difference between Eq. (19) for $\sigma = 0$ and Eq. (20) for $\sigma \neq 0$ is small. For strongly swollen vesicles ($1 - \alpha < 10^{-2}$), however, ϵ becomes large. In this case we get $\tau \to \gamma - \sigma$. Such a finite difference between the frame tension and the Laplace tension as the envelope and its reference surface coincide is hard to physically justify; it is clearly related to the artificial inclusion of an external $\sigma \neq 0$.

IV. DISCUSSION

The requirement that a membrane vesicle, as complicated as its statistical mechanics may be, must comply with the thermodynamics of finite-size systems has yielded a surprising amount of information. This information is in line with certain earlier findings and disagrees with others.

The thermodynamics of a fluid vesicle requires two distinct tension variables, defined here as the surface pressure Π and the Laplace tension γ [Eqs. (4) and (5)]. We did not assume their existence but obtained them as a result of the standard geometrical constraints on the area and volume of the vesicle. When the area per molecule is allowed to relax (the relevant scenario in actual systems and molecular dynamics simulations) the total surface stress is zero, $\sigma = \Pi + \gamma = 0$, and the two tension variables become related. Unlike early suggestions [6], this does *not* imply that the membrane is tensionless, but just reflects the balance between the surface stress arising from a three-dimensional constraint and an equal internal surface pressure. As already recognized in Ref. [8], a state of vanishing tension, in the sense that the membrane does not experience a normal restoring force due to tension, is where $\gamma = 0$. Then, upon area relaxation, we also have $\Pi = 0$ (i.e., the area per molecule relaxes to its optimum value). This is achieved in a vesicle, for instance, when its volume is allowed to relax in conditions of zero pressure difference $p_\mathrm{in} = p$ [Eq. (10)]. A similar state is achieved in an open membrane (unless a $\sigma \neq 0$ is introduced, or a nonoptimum area per molecule is imposed), when its projected area is allowed to relax under conditions of zero frame tension $\tau = 0$ [Eq. (19)].

Another tension coefficient that is frequently used in the literature is the fluctuation tension, or "q^2-coefficient," r. It is extracted from the membrane's normal fluctuation spectrum $\langle u(\mathbf{q})u(-\mathbf{q})\rangle = k_\mathrm{B}T/[rq^2 + O(q^4)]$, where $u(\mathbf{q})$ is the Fourier-transformed normal displacement of the membrane as a function of wave vector $\mathbf{q}$. Evidently, this definition of tension is purely statistical mechanical and cannot be directly examined here. It is clear, nonetheless, that r is the tension related to the membrane's response to normal strain. We thus suggest to identify it as $r = \gamma$. Indeed, micropipette-aspiration experiments have shown that the measured r is consistent with the imposed vesicle's Laplace tension [34]. For open membranes it was analytically argued [8,9,12,13,19,20] and numerically demonstrated [13,20,35] that $r = \tau$, the frame tension, but other works disagree [14,16,36,37]. Our analysis confirms the former results. We have found that, when there is no external surface pressure, $\sigma = 0$, or when the membrane's compressibility can be neglected, the Laplace tension γ is proportional to, but slightly smaller than, the frame tension τ, $\gamma = \alpha^{2/3}\tau = (A_p/A)\tau$. This is in line with the reports of $r = \tau$ since the difference $(\tau - \gamma)u(\mathbf{q})u(-\mathbf{q})$ is only fourth order in the normal displacement. It suffices to redefine the Fourier transform using the membrane's material coordinates rather than its projected ones to get a slightly modified spectrum with a q^2 coefficient $r' = (A_p/A)r = (A_p/A)\tau = \gamma$. A recent numerical study suggests, in fact, that the fluctuation tension is equal to $(A_p/A)\tau$ rather than τ [19]. As the vesicle becomes spherical (or an open membrane gets planar) the frame tension coincides with the Laplace tension, as expected.

The term σA, added to the energy in many theories and simulations, is the origin of a lot of confusion. Thermodynamically, prescribing the variable σ corresponds to applying an external surface pressure [30], similar to the pV term introduced when a three-dimensional system is in contact with a pressure bath. We are used to the fact that, for three-dimensional systems, we must prescribe either the volume or the external pressure; if we do not, there will be nothing to counterbalance the system's internal pressure. One initially expects that for a membrane we would similarly have to prescribe either A or σ. (The latter is more widely used since studying fluctuations at fixed A is theoretically difficult [10,32].) However, in an actual vesicle nothing fixes the area, and there is no in-plane barrier that can apply a fixed external surface pressure [29]. Unlike the three-dimensional case, such an external pressure is not necessary to balance the internal pressure Π of the two-dimensional fluid because the volume constraint (i.e., the embedding of the membrane in three dimensions), be it the actual volume, a pressure difference, a projected area, or a frame tension, provides such a balancing pressure, equal to $-\gamma$. Even if we tune the volume constraint down to zero (e.g., by imposing $p_{\text{in}} = p$ or $\tau = 0$), the membrane can support a state of zero internal surface pressure by attaining its optimum area per molecule, without the need for an external pressure. Thus, the area is unconstrained, leading to $\Pi + \gamma = 0$ [28]; equivalently, we may say that the external surface pressure vanishes, $\sigma = 0$. In this restricted sense, the thermodynamics of area changes is similar to the thermodynamics of photons, whose number is unconstrained or, equivalently, whose chemical potential vanishes.

Hence, the ensembles of fixed (T,V,Q,N) or (T,p,Q,N) with $\sigma = 0$ (Sec. III A) are the ones that we wish to advocate as the most physically relevant and the least confusing. The corresponding ensembles for open membranes are (T,A_p,N) or (T,τ,N), again with $\sigma = 0$. (At sufficiently long times the constraint on N should be replaced by one on μ_2; see Sec. III B.) The distinction between ensembles is not merely technical or semantic, mainly because after removing the constraint on A or σ the ensembles mentioned above contain one less constraint than the ones usually studied. In addition, even ensembles having the same number of constraints may not be equivalent for the finite systems under consideration, which contain both volume and surface free-energy contributions [19,38].

If one insists on including a nonzero σ in the theory or simulation, then area relaxation will lead to $\sigma = \Pi + \gamma$, implying that $\sigma \neq \gamma = r$ in general. The inequalities $\sigma \neq r$ and $\sigma \neq \tau$ were observed in simulations [16,19,20]. We point out that the numerically measured difference $\sigma - \gamma = \sigma - r$ provides an indirect measurement of the internal surface pressure Π in such nonzero-σ computations. The inclusion of $\sigma \neq 0$ entails two additional problems which, as we have been trying to show, are superfluous. (a) Even when the membrane is tensionless, $\gamma = 0$, there is a residual surface stress, $\Pi = \sigma$, causing the area per molecule not to relax to its optimum value. (b) The frame tension τ is not proportional to $\gamma = r$ [Eq. (20)]. Although the deviation should be usually small, when the vesicle is strongly swollen (or an open membrane is nearly flat), we get $\tau \to \gamma - \sigma$, which is physically questionable for an envelope arbitrarily close to its projection.

We end by commenting on the possible signs of the various tension variables [16–18]. The Laplace tension, which characterizes the membrane's normal restoring force, must be nonnegative for the membrane to be stable, $\gamma \geqslant 0$. This is reflected also in our identification $\gamma = r$ since a negative r will cause unstable large-wavelength fluctuations. If we let the area relax without an external surface pressure $\sigma = 0$ (the conditions which, as we have argued, are the valid ones for actual vesicles) then the condition $\gamma \geqslant 0$ entails a nonpositive (compressive) internal pressure Eq. (15) $\Pi \leqslant 0$ and a nonnegative frame tension Eq. (19) $\tau \geqslant 0$. However, if a $\sigma \neq 0$ is imposed, Π can be also positive (dilative) Eq. (12) and τ can be also negative Eq. (20).

The simple thermodynamic framework, laid out above, may be practically useful in cases where the detailed statistical mechanics and interactions of a membranal system are not crucial. For example, a similar formulation has recently been used to unravel a universality in the osmotic swelling of vesicles, arising from the mere competition of volume and surface effects [39]. There are issues which obviously cannot be dealt with using thermodynamics alone. Important examples are the fluctuation spectra, renormalization of membrane parameters by fluctuations [6–9,40], and the equivalence (or lack of it) of different statistical ensembles [19,38]. Nonetheless, within its limitations, the remarkable strength of classical thermodynamics lies in its simplicity, broad applicability, and rigor [41]. We hope that the formulation and conclusions presented here will be useful as guidelines for future studies.

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[24] For simplicity we consider a single type of molecule, but the formulation can be readily extended to a mixture of several species.

[25] No assumption concerning the smoothness of the envelope is necessary, so long as one can calculate its area (e.g., by considering the particles as nodes and triangulating).

[26] The constraints are assumed here to *allow* area relaxation. If V is too large, Q is too large, or N is too small, such that the required area per molecule relative to its optimum value involves a strain of more than a few percent, the vesicle will rupture.

[27] This is a bit reminiscent of the recent realization that an intrinsic metric of a solid elastic sheet, upon embedding in three dimensions, may act as a constraint which spatially confines and stresses the sheet. E. Efrati, E. Sharon, and R. Kupferman, J. Mech. Phys. Solids **57**, 762 (2009).

[28] The fact that the area constraint can be relaxed with $\sigma = 0$ implies that we could, in principle, start from a surface free energy F_2 which did not depend on A, assuming A *a priori* to be the relaxed area as a function of (T,V,Q,N). Such a formulation, though equivalent to the one presented here (where we introduce an area constraint and then relax it), would be much less transparent.

[29] It might be possible to control the in-plane surface pressure of vesicles by adhesion to a substrate at zero contact angle.

[30] In the statistical mechanics of membranes one distinguishes between intrinsic tension (a coefficient that multiplies the area at the "microscopic" level of the Hamiltonian) and renormalized tension (the proportionality coefficient of A in the free energy). Evidently, this distinction lies beyond the scope of the current thermodynamic discussion.

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