Dynamical description of vesicle growth and shape change

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We systematize and extend the description of vesicle growth and shape change using linear nonequilibrium thermodynamics. By restricting the study to shape changes from spheres to axisymmetric ellipsoids, we are able to give a consistent formulation which includes the lateral tension of the vesicle membrane. This allows us to generalize and correct a previous calculation. Our present calculations suggest that, for small growing vesicles, a prolate ellipsoidal shape should be favored over oblate ellipsoids, whereas for large growing vesicles oblates should be favored over prolates. The validity of this prediction is examined in the light of the various assumptions made in its derivation.

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I. INTRODUCTION

Vesicles are fascinating structures for several reasons [1]: they occur in a wide range of shapes and sizes, they are cell-like and are frequently used in the modeling of protocells, and they present a formidable task for the mathematical modeler. It is not hard to see why they are so difficult to describe theoretically. They are basically a closed membrane made up of a lipid bilayer, which allows water and solutes to permeate from the environment into the interior of the vesicle [1,2]. While they are frequently modeled as a two-dimensional surface, the finite thickness of the bilayer as well as its structure plays an important role in understanding their behavior [2]. In a pioneering work, Helfrich [3,4] used the analogy between the rodlike shape of nematic liquid crystals and the lipids forming the vesicle bilayer to write down an expression for the energy of the membrane. He envisaged the membrane as a curved surface and accounted for the fact that the membrane had structure through the introduction of a phenomenological constant C_0, the spontaneous curvature. Since then a variety of other models have been proposed, most of which start from the idea of a purely geometrical surface to represent the membrane, with additional structure introduced in various ways [2].

For most of the period since these models were proposed the main focus of their study has been to look for the shapes with the smallest energy under fixed conditions of constant volume V and constant surface area A. It was argued that this would give the expected shape of the vesicle at these values of V and A, and indeed a range of shapes emerged from this analysis [2]. However this is a purely static approach—there is no mechanism which postulates how the transitions between different shapes occurred or how long these changes take. We recently carried out a preliminary study of a dynamical process designed to describe the change in vesicle shape [5]. The change in surface area occurred because of the slow accretion of lipids onto the surface from the fluid surrounding the vesicle [6]. This resulted in a change in the volume of the vesicle due to the influx of water and solutes through the membrane. The process was assumed to be sufficiently slow that the formalism of linear nonequilibrium thermodynamics (LNET) could be used [7]. The expectation was to find stability conditions which determine the shape of the vesicle at various stages of its growth.

While we believe that modeling vesicles as surfaces with an energy of the Helfrich type, which change their shapes and sizes according to LNET, is a minimalist description which is capable of answering many interesting questions, it is still extremely mathematically complex. The surface may be of an arbitrary shape, and so requires the formalism of differential geometry for its study, the actual dynamics, as opposed to the statics, of mixtures of the rodlike lipids and the pointlike fluid molecules is very nontrivial, and the nonequilibrium thermodynamics of discontinuous structures such as vesicles has been little studied because of its difficulty. Some of these questions will be discussed in a forthcoming publication [8], where we will develop the formalism in a systematic fashion. However, in our view it is also valuable to proceed in stages, and build on what has gone before [5,9–12], and not to attempt to introduce all aspects of the problem simultaneously. This is the philosophy that we will follow in this paper; we will extend our previous calculation to include surface tension (frequently called lateral tension when discussing vesicles) in the context of axisymmetric ellipsoidal shapes, treating the area A as well as the volume V as thermodynamic variables. This will form the basis for the treatment of more general shapes in the future. We will also correct some of the analysis presented in [5].

II. FORMALISM

We shall in common with most other authors adopt the spontaneous curvature model of Helfrich [3] in which the energy of the membrane is given by

$$E_m = \frac{\kappa}{2}\oint_A (2H - C_0)^2 dA, \tag{1}$$

where H is the local mean curvature, dA is an element of the surface A, and κ is the bending rigidity. Since a purely static analysis of Eq. (1) shows that when a spherical surface becomes unstable it is replaced with an ellipsoid [13], we will restrict ourselves to shapes which are spheres or axisymmetric ellipsoids. These will be parametrized in Cartesian coordinates by

$$x = a \sin\theta \cos\varphi,$$

$$y = a \sin\theta \sin\varphi,$$

$$z = c \cos\theta, \tag{2}$$

where $0 \leq \varphi < 2\pi$, $0 \leq \theta \leq \pi$, and where a and c are constants. For a sphere $a=c \equiv r$, the radius. We have not included a term proportional to the Gaussian curvature in the expression for the energy since this is a constant for the kinds of surfaces that we are considering here [2,4].

The surface area, volume, and membrane energy (1) can all be found in closed form for an axisymmetric ellipsoid and are given explicitly in the appendix of [5] [there is however a typographical error in Eqs. (A9) of that paper: the minus sign in the factor $-2a^3/3c^4$ should be absent]. They are all functions of the two variables a and c which characterize the ellipsoid, with E_m additionally depending on the parameters κ and C_0. Since the volume takes the simple form $V = 4\pi a^2 c/3$, c may be explicitly eliminated in favor of a and V in the expressions for A and E_m. Subsequently $A = A(V,a)$ may be inverted to give $a=a(V,A)$. In this way we see that the energy of the membrane is a function of V and A only. Since in the Helfrich model the membrane is completely described by this energy, we deduce that the thermodynamic description of the membrane can be achieved using these two variables.

As previously indicated we use LNET to describe the time evolution of the vesicle. Throughout, we assume that the temperature T is fixed since we are not interested in changes in shape which come about because of a change in temperature. For simplicity, we assume that no solutes are present, although it is not too difficult to extend the treatment to include them.

The system is assumed to comprise two regions, the *interior* and *exterior*, that are separated by a third region, the *membrane*. This third region is assumed to be very thin, and our aim is to modify the thermodynamic description, so that the membrane can (following Helfrich) be simply regarded as a boundary, or geometrical surface, between the first two regions. In the formalism of LNET, each region is assumed to be in local equilibrium [7], with the thermodynamic relation $TdS=dE-dW$ holding in each region. Here, S is the entropy, E is the internal energy, and W is the work done on the system [14]. The three regions will be labeled i, e, and m, respectively.

The internal energy and work done have the following forms:

(i) The internal energies of the interior, E_i, and exterior, E_e, will be that of the fluid in these two regions, in our case water. Their sum is denoted by E_w. The internal energy of the membrane is given by Eq. (1).

(ii) Considering the interior and exterior regions individually, each will have work done on them of $-PdV$ if their volumes change, where P is the pressure in that region. Adding these gives the total contribution for work of this type to be $-P_i dV_i - P_e dV_e$. The membrane will be assumed to have negligible volume, and so gives no contribution. This also means that $dV_i = -dV_e \equiv dV$, and so the total work done on the system due to the interior increasing its volume by dV is $(P_e - P_i)dV$.

(iii) There will also be a work done if the area of the membrane increases by dA, equal to σdA, where σ is the surface tension [14]. This would exist even if the system consisted of two regions of different fluids, with no membrane separating them. The existence of a membrane with nontrivial structure separating the interior and exterior means that σ will have a more complex functional form which reflects this structure. For this reason we will follow the usual usage in this field and refer to it as the lateral tension.

Adding up the contributions from the three regions one finds

$$TdS = dE_w + dE_m - (\Delta P)dV - \sigma dA, \tag{3}$$

where now S is the total entropy of the system and $\Delta P \equiv (P_e - P_i)$ is the pressure difference between the exterior and interior. The membrane does not explicitly appear in the terms relating to work. We can also eliminate it from the internal energy by noting that, since E_m is a function of V and A,

$$dE_m = \left(\frac{\partial E_m}{\partial V}\right)_A dV + \left(\frac{\partial E_m}{\partial A}\right)_V dA. \tag{4}$$

This allows us to write the thermodynamic relation (3) for the system as

$$TdS = dE_w - (\Delta P)_{\text{eff}} dV - (\sigma_{\text{eff}})dA, \tag{5}$$

where

$$(\Delta P)_{\text{eff}} = \Delta P - \left(\frac{\partial E_m}{\partial V}\right)_A, \quad \sigma_{\text{eff}} = \sigma - \left(\frac{\partial E_m}{\partial A}\right)_V. \tag{6}$$

Equation (5) is the thermodynamic relation for two regions separated by a boundary with no material properties. The effect of the membrane simply changes the pressure difference and lateral tension from ΔP to $(\Delta P)_{\text{eff}}$ and from σ to σ_{eff}, respectively. Therefore, as long as we make these replacements, we may ignore the membrane from a thermodynamic point of view, and simply treat it as a boundary which separates the inside of the vesicle from the environment. In our previous analysis [5] we did not treat the area as an independent variable. This is clearly consistent for spherical vesicles, but not for those which have an axisymmetric ellipsoidal shape.

III. DYNAMICS

One of the central features of LNET is the relation between the forces X_a, which cause the state of the system to change, and the fluxes J_a, which are the result of these changes [7]. Within the formalism of LNET these are linearly related: $J_a = \Sigma_b L_{ab} X_b$, where the L_{ab} are constants, the Onsager coefficients. The forces and fluxes can be identified in a systematic way [8], but for the specific problem of interest to us here, where the relevant thermodynamic variables are simply V and A, we may proceed more directly. We will also restrict ourselves to just one of the fluxes: that due to

water flowing through the membrane into the interior of the vesicle. This will be denoted by J_w. The direct effect that causes this flux of water is the pressure difference between the exterior and interior regions [15,16], which incorporating the effect of the membrane is $(\Delta P)_{\text{eff}}$.

If we only took into account this direct effect, as we did in our previous treatment [5], we would write $J_w = L_p(\Delta P)_{\text{eff}}$, where L_p is the hydraulic conductivity of the membrane. However, there will also be an indirect effect [7] for which the driver will be σ_{eff}, and so

$$J_w = L_p(\Delta P)_{\text{eff}} + L_\sigma \sigma_{\text{eff}}, \tag{7}$$

where L_σ is a second Onsager coefficient. We shall justify the choices of forces and fluxes in more detail elsewhere [8], but we can give a simple microscopic argument showing how the flux proportional to the lateral tension comes about. For a positively curved membrane, the lipids are arranged in a funnel-like configuration which inhibits the flow of water across the membrane. Decreasing the curvature of a membrane at any point therefore permits a greater flow of water due to the lipids becoming increasingly parallel. In this way, for fixed shapes, an increase in the surface area causes a slight alignment of adjacent lipids, and hence permits a greater flow across the membrane. This resultant flow is proportional to the lateral tension, the measure of how the membrane energy changes with area.

We are now in a position to introduce the dynamics explicitly. The rate of increase in the volume of the vesicle will be given by $dV/dt = AJ_w$, and so from Eqs. (6) and (7) we have

$$\frac{dV}{dt} = A\left\{ L_p\left[\Delta P - \left(\frac{\partial E_m}{\partial V}\right)_A \right] + L_\sigma\left[\sigma - \left(\frac{\partial E_m}{\partial A}\right)_V \right] \right\}. \tag{8}$$

We also need to describe how the surface area of the vesicle grows due to the inclusion of lipids from the environment. The simplest assumption is that these attach themselves uniformly over the entire surface, so that the surface grows at a constant rate per unit area which we will denote by λ [9,5]:

$$\frac{dA}{dt} = \lambda A \Rightarrow A(t) = A(0)e^{\lambda t}. \tag{9}$$

Before we go on to investigate the dynamics for an ellipsoidal shape, let us briefly consider the result for a sphere. In this case V and A are not independent variables, and so the thermodynamic relation (5) should read

$$TdS = dE_w - (\Delta P)_{\text{eff}}^{\text{sphere}} dV, \tag{10}$$

where

$$\begin{aligned}(\Delta P)_{\text{eff}}^{\text{sphere}} dV &= (\Delta P)_{\text{eff}} dV + \sigma_{\text{eff}} dA \\ &= \Delta P dV + \sigma dA - \left(\frac{\partial E_m}{\partial A}\right)_V dA - \left(\frac{\partial E_m}{\partial V}\right)_A dV \\ &= \Delta P dV + \sigma dA - dE_m \\ &= \left[\Delta P - \left(\frac{dE_m}{dV}\right) + \sigma\left(\frac{dA}{dV}\right) \right] dV, \end{aligned} \tag{11}$$

where in the last line the derivatives are not partial derivatives since for a sphere $E_m = E_m(V)$ and $A = A(V)$. We may calculate these in a straightforward way. The mean curvature of a sphere of radius r is $1/r$, and so from Eq. (1)

$$E_m = 2\pi\kappa(C_0 r - 2)^2 \Rightarrow \frac{dE_m}{dV} = \frac{C_0\kappa}{r^2}(C_0 r - 2). \tag{12}$$

Using $dA/dV = 2/r$, we find from Eqs. (11) and (12) that

$$(\Delta P)_{\text{eff}}^{\text{sphere}} = \Delta P - \frac{C_0\kappa}{r^2}(C_0 r - 2) + \frac{2\sigma}{r}. \tag{13}$$

However, for a sphere $J_w = L_p(\Delta P)_{\text{eff}}$, and so in equilibrium when there is no flow of water, $J_w = 0$, Eq. (13) gives

$$\Delta P - \frac{C_0\kappa}{r^2}(C_0 r - 2) + \frac{2\sigma}{r} = 0. \tag{14}$$

This is a standard result from the studies of spherical vesicles in equilibrium [13], if we make allowance for the different sign conventions for the pressure difference and the lateral tension. In previous work [5,12], we did not include the lateral tension in our description, and so our equilibrium result did not include the final term in Eq. (14).

It should be emphasized that Eq. (14) is a consequence of asking that the vesicle is in static equilibrium, so that in particular no lipids are being added to the exterior surface, leading to no increase in the surface area A. A condition for dynamic equilibrium can also be obtained. This is a stationary state in which the vesicle remains turgid and grows like a sphere. In this case, recalling that the area grows according to Eq. (9), one gets $dV/dt = 2\pi\lambda r^3$. The spherical version of Eqs. (7) and (8), $dV/dt = AL_p(\Delta P)_{\text{eff}}^{\text{sphere}}$, therefore gives

$$\frac{\lambda r}{2L_p} = \Delta P - \frac{C_0\kappa}{r^2}(C_0 r - 2) + \frac{2\sigma}{r}, \tag{15}$$

a result that could not be derived from a purely static description. Equation (15) is Eq. (21) of [5], but with the lateral tension now taken into account.

The inclusion of the lateral tension is even more important for axisymmetric shapes of the kind we are considering here, because now A is an independent variable. Since we are concerned with questions of stability, we will only consider ellipsoids which differ in shape from the sphere very slightly. In this case the parameters a and c which describe the ellipsoid [see Eqs. (2)] may be expressed as

$$a = R(1 + a_1\epsilon), \quad c = R(1 + c_1\epsilon), \tag{16}$$

where ϵ is a small quantity and a_1 and c_1 are numbers which characterize the shape of the ellipsoid: if $a_1 > c_1$ it is oblate, and if $a_1 < c_1$ it is prolate. The quantity R reduces to the radius of the sphere as $\epsilon \to 0$, but care is required in its definition, as we will discuss in more detail below.

Using standard results [17] and Eq. (16), it is straightforward to calculate the surface area and volume of the ellipsoid for small ϵ. From the explicit forms given in the appendix of [5] it is found that

$$A = 4\pi R^2\left[1 + \tfrac{2}{3}(2a_1 + c_1)\epsilon + \mathcal{O}(\epsilon^2)\right],$$

$$V = \tfrac{4}{3}\pi R^3[1 + (2a_1 + c_1)\epsilon + \mathcal{O}(\epsilon^2)]. \tag{17}$$

However, as we will now show, it is not consistent to assume that R is independent of ϵ if we assume a growth law of form (9). To see this, we write the expression for A given in Eqs. (17) as $A=4\pi R^2\phi(\epsilon)$, where $\phi(\epsilon)$ is the expression in the square brackets. Then,

$$\lambda A = \frac{dA}{dt} = 8\pi R\phi\frac{dR}{dt} + 4\pi R^2\frac{d\phi}{dt} \Rightarrow \lambda = \frac{d}{dt}[\ln R^2\phi], \tag{18}$$

which implies that $R(t)=e^{\lambda t/2}[\phi(t)]^{-1/2}$, up to an overall multiplicative constant. Since ϕ is a function of ϵ, so is R. Therefore, for consistency, we cannot use R when carrying out a perturbative expansion in ϵ since it contains hidden ϵ dependence. Instead we should use the radial variable $r(t)=R(t)[\phi(t)]^{1/2}$, which is ϵ independent. This is equivalent to determining r through the condition $A=4\pi r^2$, for any axisymmetric ellipsoid. Clearly r is the radius of the sphere which has the same surface area as the ellipsoid.

The correct procedure to investigate the dynamics of vesicle growth perturbatively in ϵ is, therefore, to use closed-form expressions from [5] and Eq. (16), to determine results (17) to the required order, but then to set $A=4\pi r^2$. This can be inverted to find $R(t)=r(t)[\phi(t)]^{-1/2}$, allowing V and E_m to be found as functions of r and ϵ. To first order, this procedure gives

$$\phi(t) = 1 + \tfrac{2}{3}(2a_1 + c_1) + \mathcal{O}(\epsilon^2), \tag{19}$$

leading to $V=(4\pi r^3/3)[1+\mathcal{O}(\epsilon^2)]$. In fact, since $A=4\pi r^2$ exactly, the volume is directly related to the so-called reduced volume, defined by $v=6\sqrt{\pi}V/A^{3/2}$, by

$$V = \frac{4\pi r^3}{3}v. \tag{20}$$

Although V has no term of order ϵ when expressed in terms of the variable r, it does turn out to have a term of order ϵ^2:

$$v = 1 - \tfrac{4}{15}(a_1 - c_1)^2\epsilon^2 + \mathcal{O}(\epsilon^3). \tag{21}$$

As a check we note that $v<1$ for all cases except the sphere ($a_1=c_1$), as required. In our previous calculation [5] we used the variable R, rather than r, which gave incorrect results for the coefficients in the expansion in ϵ. Correcting these by eliminating R in favor of r makes previously cumbersome results look far more elegant. For example, the energy of the membrane becomes

$$E_m(r,\epsilon) = E^{(0)}(r) + \alpha_2 E^{(2)}(r)\epsilon^2 + \mathcal{O}(\epsilon^3), \tag{22}$$

where

$$E^{(0)}(r) = 2\pi\kappa(C_0 r - 2)^2, \quad E^{(2)}(r) = \frac{8\pi\kappa}{3}(6 - C_0 r), \tag{23}$$

and where

$$\alpha_2 = \tfrac{4}{15}(a_1 - c_1)^2. \tag{24}$$

A purely static analysis of the stability of an ellipsoidal vesicle would compare the energy of the membrane (22) to the energy of a spherical vesicle—the same equation, but with $\epsilon=0$ [13]. The conclusion would be that the ellipsoid is more stable if $E^{(2)}(r)<0$, i.e., if $C_0 r>6$. However, there is no dynamics in this picture at all. To achieve a more physical description of the time evolution of the vesicle we utilize Eq. (8). To do this we first need to evaluate $(\partial E_m/\partial V)_A$ and $(\partial E_m/\partial A)_V$, but we cannot proceed directly since we know $E_m=E_m(r,\epsilon)$ rather than $E_m=E_m(V,A)$. To circumvent this problem, suppose that we have inverted (in principle, not in practice) $V=V(r,\epsilon)$ to obtain $\epsilon=\epsilon(r,V)$. Then since $E_m=E_m(r,\epsilon(r,V))$,

$$\left(\frac{\partial E_m}{\partial V}\right)_A = \left(\frac{\partial E_m}{\partial V}\right)_r = \left(\frac{\partial E_m}{\partial \epsilon}\right)_r\left(\frac{\partial \epsilon}{\partial V}\right)_r,$$

$$\left(\frac{\partial E_m}{\partial A}\right)_V = \frac{1}{8\pi r}\left(\frac{\partial E_m}{\partial r}\right)_V = \frac{1}{8\pi r}\left\{\left(\frac{\partial E_m}{\partial r}\right)_\epsilon + \left(\frac{\partial E_m}{\partial \epsilon}\right)_r\left(\frac{\partial \epsilon}{\partial r}\right)_V\right\}. \tag{25}$$

So we need only to calculate $(\partial\epsilon/\partial V)_r$ and $(\partial\epsilon/\partial r)_V$. These may be found from Eq. (20) by noting that the reduced volume is a function only of ϵ. Then

$$v'(\epsilon)\left(\frac{\partial \epsilon}{\partial V}\right)_r = \frac{1}{4\pi r^3/3}, \quad v'(\epsilon)\left(\frac{\partial \epsilon}{\partial r}\right)_V = -\frac{3}{r}\frac{V}{4\pi r^3/3}, \tag{26}$$

where $v'(\epsilon)=dv/d\epsilon$. Substituting expressions (26) into Eqs. (25) gives

$$\left(\frac{\partial E_m}{\partial V}\right)_A = \frac{[v'(\epsilon)]^{-1}}{4\pi r^3/3}\left(\frac{\partial E_m}{\partial \epsilon}\right)_r,$$

$$\left(\frac{\partial E_m}{\partial A}\right)_V = \frac{1}{8\pi r}\left(\frac{\partial E_m}{\partial r}\right)_\epsilon - \frac{3V}{2A}\left(\frac{\partial E_m}{\partial V}\right)_A. \tag{27}$$

These two equations allow us to find $(\partial E_m/\partial V)_A$ and $(\partial E_m/\partial A)_V$ if we know E_m and V as functions of r and ϵ.

Using results (20)–(24) to second order in ϵ, the partial derivatives (27) are given by

$$\left(\frac{\partial E_m}{\partial V}\right)_A = -\left\{\frac{1}{4\pi r^3/3}\right\}E^{(2)}(r) + \mathcal{O}(\epsilon),$$

$$\left(\frac{\partial E_m}{\partial A}\right)_V = \frac{1}{8\pi r}\left[\frac{dE^{(0)}}{dr} + \frac{3}{r}E^{(2)}(r)\right] + \mathcal{O}(\epsilon). \tag{28}$$

It is important to note that while we have used the results for E_m and V correct to second order in ϵ, we have only been able to calculate the required derivatives to zeroth order in ϵ. The reason for this can be traced back to $v'(\epsilon)$ being of order ϵ and the differentiation of E_m with respect to ϵ also giving an expression of order ϵ. Together these reduce the powers of ϵ by 2 in the calculation of $(\partial E_m/\partial V)_A$. The result can however be used to check that we recover the previously derived form for the spherical vesicle in the limit $\epsilon\to 0$. Substituting

Eqs. (28) into Eq. (8), and using $dV/dt=2\pi\lambda r^3$, valid for a sphere, one finds

$$\frac{\lambda r}{2}=L_p\left\{\Delta P+\frac{2\kappa}{r^3}(6-C_0r)\right\}+L_\sigma\left\{\sigma-\frac{\kappa}{2r^2}[(C_0r)^2-4C_0r+12]\right\}. \quad (29)$$

This is identical to Eq. (15), already derived for the sphere provided that we make the identification $L_\sigma=2L_p/r$. It should be emphasized that this identification is only being made to obtain the correct result in the spherical limit, bearing in mind that L_σ is not defined in this case.

IV. STABILITY

To determine if and when the shape of the vesicle starts to deviate from that of a sphere, we calculate the time derivative of the reduced volume. This will be zero ($v=1$) when the vesicle remains spherical, but will start to decrease as soon as it adopts another shape. From Eq. (20),

$$\frac{dV}{dt}=4\pi r^2v\frac{dr}{dt}+\frac{4\pi r^3}{3}\frac{dv}{dt}. \quad (30)$$

The zeroth-order part of the first term on the right-hand side of Eq. (30) has already been included in the stationary condition (29). At first order in ϵ, the second term in this equation is the relevant one. To find the right-hand side of Eq. (8) to order ϵ, it is necessary to find $(\partial E_m/\partial V)_A$ to order ϵ^3. This can be carried out in the same way as described above for the calculation to order ϵ^2, although the intermediate steps are sufficiently complicated that we used MATHEMATICA [18]. Nevertheless, the final result is quite simple:

$$-2\alpha_2\epsilon\frac{d\epsilon}{dt}=\frac{4\kappa}{7r^4}(a_1-c_1)(5C_0r+6)[2L_p-rL_\sigma]\epsilon+\mathcal{O}(\epsilon^2), \quad (31)$$

or defining a critical radius r_c by $r_c=2L_p/L_\sigma$,

$$\alpha_2\frac{d\epsilon}{dt}=\frac{2\kappa L_\sigma}{7r^4}(a_1-c_1)(5C_0r+6)(r-r_c)+\mathcal{O}(\epsilon). \quad (32)$$

This stability condition differs from the one we had derived in our earlier study of this question [5], in that it predicts a linear growth away from the sphere, and not an exponential one. Since $\alpha_2>0$, if the right-hand side of Eq. is positive, the sphere is linearly unstable and if it is negative, it is linearly stable. We see that oblate ($a_1>c_1$) perturbations will destabilize a sphere if $r>r_c$, whereas prolate ($a_1<c_1$) perturbations will destabilize a sphere if $r<r_c$. The picture we have is that as the sphere grows, it is susceptible to fluctuations which give it a prolate ellipsoidal shape if it has a radius less than r_c, and it is susceptible to fluctuations which give it an oblate ellipsoidal shape if it has a radius greater than r_c. The actual value of r_c will be expected to vary over several orders of magnitude, reflecting the type of lipids making up the bilayer and so the values of L_p and L_σ.

How do these predictions compare with the currently available experimental results? According to our predictions, spherical shapes should be unstable in a purely dynamical setting where the membrane surface grows due to the successive inclusion of lipid molecules and where the volume then increases due to the inflow of water. Under static conditions, however, the Helfrich energy functional implies that spherical vesicles are stable below a critical radius. It can be argued that the reason why spherical shapes are often seen in experiments [1] is a reflection of the specific experimental setting adopted. If the dynamical mechanisms of the type considered here are essentially negligible, then static effects are expected to prevail and, consequently, spheres are the energetically favored configurations.

In [19], the process of vesicle formation was investigated in a rich and dynamic phospholipid mixture. Dynamical light scattering and transmission electron microscopy experiments were performed to resolve the vesicles' shape and quantify their associated sizes. The observed vesicles were relatively large (hydrodynamic radii $\simeq$200 nm or $>$500 nm) and corresponded to either oblate ellipsoids or triaxial ellipsoids. The fact that a significant fraction of the (giant) vesicles belonged to the oblate ellipsoids family is consistent with the prediction from our analysis.

Clearly, there are number of other effects (e.g., temperature fluctuations) which are implicated in the above-mentioned experiments and not included in our modeling efforts. We also need to consider more generic shapes, including triaxial ellipsoids. The probability that perturbations are axisymmetric is presumably small; nevertheless, oblate ellipsoids are observed in experiments where the dynamics plays a role and the vesicles are sufficiently large. This is, for instance, the case in [20,21], where initial spherical vesicles are experimentally shown to deform into oblate ellipsoids, rather then prolate ones, in the presence of osmotic driving pressures. This is a distinctive feature which is not captured by any static approaches to the problem of the morphology of vesicles and one that could perhaps be explained by a crucial interplay between shape and dynamics.

V. CONCLUSIONS

In this paper we have extended and corrected earlier work on the growth and stability of vesicles. The errors in our previous paper [5] were first an inappropriate choice of characteristic size for the ellipsoidal vesicle (R rather than r). This was a subtle point relating to the fact that assuming the radius to be independent of the degree of deformation ϵ was not compatible with the growth law we chose. The second error was one of omission: we did not include the lateral tension in our original analysis. Finally, there was a simple typographical error in Eqs. (A9) of [5]. While correcting the first point would not have changed the conclusions to any great extent, adding the surface tension does: the prediction is now that smaller vesicles should tend to be prolate and larger vesicles should tend to be oblate.

There are several caveats that we should make in regard to this prediction. Foremost among these is that the model we have adopted is very simple, and even though it is the one frequently used in the literature, we should keep this constantly in mind. It should be possible to extend these results

to the area-difference-elasticity [22] model, which is slightly more realistic. It would be interesting to see if the predictions are changed in any way. Another caveat is that we have assumed that the sphere becomes unstable to an ellipse defined by Eqs. (2). Although this is what is found from investigations using variational techniques [13], it would be more consistent not to assign a specific functional form to the shape of the membrane. This is currently being investigated; the analysis is considerably more complicated, and the results will be presented elsewhere [8]. Finally, LNET for this problem is very underdeveloped, and rather complex, with plenty of scope for pitfalls. This will also be more extensively discussed elsewhere [8].

Even given all these caveats, we still believe that the current work is a significant step forward in understanding vesicle growth and shape changes. Virtually all previous work was static and made much less contact with the physics of the problem than the dynamic approach adopted here. Work that did exist on the dynamics, including our own, has errors or inconsistencies which we hope that we have rectified. What is required most of all are more experiments in order to guide the theoretical development, ruling out and supporting the various theoretical approaches. We hope that the current work stimulates the carrying out of such experiments in the future.

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