Reply to "Comment on 'Thermodynamics of vesicle growth and instability' "

Duccio Fanelli
Dipartimento di Energetica, University of Florence and INFN, Via S. Marta 3, 50139 Florence, Italy

Alan J. McKane
Theoretical Physics, School of Physics and Astronomy, University of Manchester, Manchester M13 9PL, United Kingdom
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We respond to the Comment of Božič and Svetina [Phys. Rev. E **80**, 013401 (2009)] by first questioning the consistency of their approach. We then argue, on the basis of numerical estimates of relevant experimental quantities, that the vesicles may be assumed to be turgid as they grow in size, thus justifying our earlier methodology. Finally, we comment on the remaining issues where our approaches differ.

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Božič and Svetina (referred to as BS from now on) wrote a Comment [1] on our paper [2] (referred to as FM from now on) in which they criticize the approach we used to study the growth of vesicles through successive accretion of lipid molecules. In our paper we drew attention to two deficiencies in earlier work by BS [3,4]: (i) they used an incorrect dynamical equation, and (ii) the reflection coefficient, σ, was not used consistently. In their Comment [1] BS responded to point (ii)—the less important point—but made no comment about the more important point (i). Instead they criticize another aspect of our work, related to the whether the vesicle is flaccid or turgid. To respond to these criticisms we therefore first reiterate point (i) since we contend that the omission by BS of a term in this equation impacts on the discussion of the state of the vesicle. We then go on to discuss the flaccidity or turgidity of the vesicle. Finally, we address other secondary criticisms that BS make.

By far the majority of theoretical studies of vesicle shape and instability has used variational methods and so has assumed the vesicle to be a purely static entity with no dynamics [5]. Only a very few studies have used dynamics to investigate how a vesicle grows with time through successive accretion of lipid molecules increasing its surface area and subsequently increasing its volume through water (and solute) flowing in through its walls. In their 2004 paper [3], the starting point of BS is the following: "the vesicle volume (V) changes with time because of the net flow of water across the membrane and is given by

$$\frac{dV}{dt} = L_p A \Delta P, \tag{1}$$

where L_p is the hydraulic permeability and ΔP is the difference between the pressures outside and inside the vesicle." Here A is the surface area of the vesicle. On the other hand, FM uses the result (Eq. (4) of [2]):

$$\frac{dV}{dt} = L_p A\left[\Delta P - \frac{\partial E}{\partial V}\right]. \tag{2}$$

Clearly there is an extra term in the equation that FM use which comes from the bending energy, E, of the membrane. It was the omission of this term by BS that was our main criticism of their paper, yet they do not address this in any way in their Comment [1]. In fact, in their Comment [1] BS appear to retreat further from the dynamical analysis used in part in [3], and apart from restating Eq. (1) in the text, they mostly confine themselves to using results taken from studies using the variational method. For instance, their discussion makes extensive use of well-known relations (such as Eqs. (1) and (2) of their Comment [1]) which are found through minimizing the functional E (the membrane energy) under specific constraints.

The relationship between a static variational treatment and one based on the formalism of nonequilibrium thermodynamics [6] is not at all clear. FM use the latter formalism throughout their analysis, but BS appear to use it in the beginning of their analysis [as in Eq. (1), for instance], but then invoke results from variational studies and mix these together in a way which is inconsistent. For instance, they use the variational result $\Delta P = -\partial E/\partial V$, but it is not clear how to derive this result from the physical process of water flowing through a permeable membrane. It certainly is not consistent with their result [Eq. (1)] in equilibrium ($dV/dt=0$). In fact, the pressure difference that BS invoke arises as a Lagrange multiplier in the variational problem and can be explicitly calculated for the case of a spherical vesicle, giving a value different from zero. To reconcile these observations BS seem to suggest a rather artificial evolution of the vesicle, switching between a regime where the vesicle grows as a sphere, the pressure difference following from Eq. (1) to another where the variational constraint $\Delta P = -\partial E/\partial V$ is used so mixing results from two different formalisms.

By contrast, in the scheme used by FM, the dynamics of the vesicle is simply given by Eq. (2). As discussed below, the relevant regime is a stationary state where the pressure adjusts itself to balance the vesicle growth and the pressure term $\partial E/\partial V$. If the surface growth mechanism is turned off, then the system naturally relaxes toward an equilibrium state with $\Delta P = \partial E/\partial V$. Relaxation to such a state seems to us to be a minimal consistency requirement which the scheme of BS fails to meet. We can summarize the first part of this reply by reiterating the point that, while there have been a very large number of studies of this problem using a variational approach, the aim of FM was to investigate the problem in a self-consistent dynamical way. By contrast, BS in their Comment [1] and in their original papers use a strange amalgam of variational results and dynamics, which we believe are not consistent.

This discussion takes us to the central point of the Comment [1] by BS: that their analysis covers the possibility that the vesicle becomes flaccid, whereas that of FM does not. We absolutely agree that we do not consider the possibility that the vesicle becomes flaccid. That the vesicle is turgid is assumed throughout the analysis presented in [2], and we will now explain why this assumption is justified.

Let us begin by being clear as to what we mean by the terms flaccid and turgid. If the flow of water into the vesicle is sufficiently slow, then the membrane will become limp and lack any resilience. A consequence will be that various forces relating to the bending energy, lateral tension, etc. will not be engaged. Typically, a flaccid vesicle will be nonspherical, but vesicles with a larger L_p (allowing for a greater flow of water) will eventually become nearly spherical, and vesicles with a still larger L_p will reach a turgid state. These vesicles will then be tense and will respond to surface forces.

We now assume that the vesicle, with a given L_p, is almost a sphere but slightly flaccid. We will ask whether the vesicle quickly becomes turgid and then subsequently evolves as such, or whether it stays flaccid. The growth of the surface area by accretion is given by (Eq. (1) of [3] and Eq. (5) of [2])

$$\frac{dA}{dt} = \lambda A, \tag{3}$$

where $\lambda = \ln 2/T_d$ is the rate of growth of the area and T_d is the time taken for the membrane to double its area. Now if the vesicle is almost a sphere, $V \sim A^{3/2}$ and so $(dV/dt)/V = 3(dA/dt)/2A$. Furthermore, if the vesicle is flaccid, the pressure due to bending energy will not be engaged, as discussed above. Therefore, the change in the area due to the growth by water intake is

$$\frac{dA}{dt} = \frac{2L_p}{3}\frac{A^2}{V}\Delta P. \tag{4}$$

If the ratio, ζ, of Eq. (4) to Eq. (3) is less than one, the vesicle will remain flaccid. If $\zeta > 1$, then it will immediately become turgid, as water flows into it. Taking the ratio of the two equations gives

$$\zeta = \frac{2L_p}{3\lambda}\frac{A}{V}\Delta P = \frac{2L_p}{\lambda R}\Delta P, \tag{5}$$

where R is the radius of the vesicle. The hydraulic permeability, L_p, is usually given in terms of the water permeability coefficient (equal to $L_p \mathcal{R} T/\mathcal{V}$, where $\mathcal{R}$ is the gas constant, T is the temperature, and $\mathcal{V}$ is the molar volume of water). Taking, a value for the water permeability coefficient of 10^{-4} m s^{-1} (used by BS, page 569 of [3]) gives $L_p = 7.5 \times 10^{-13}$ m s^{-1} Pa^{-1}. Estimating the pressure is more difficult. There were early discussions of pressure differences in vesicles [7,8], but these were mainly concerned with matters of principle rather than obtaining realistic estimates. However, molecular-dynamics simulations have since become feasible, and a recent study [9] used values for ΔP of the order of 10 or 100 bar for small vesicles ($R = 10$ nm). We will use the values given in [9] and take $\Delta P = 10$ bar and $R = 10$ nm but revisit this question below. Finally, we take λ from the range suggested by BS: 8×10^{-6} s$^{-1} < \lambda < 1 \times 10^{-3}$ s^{-1} (page 569 of [3]). Using $\lambda = 1 \times 10^{-3}$ s^{-1}, the value most likely to favor a flaccid state, we find that

$$\zeta > 1.5 \times 10^4 \tag{6}$$

so that we are well into the turgid regime.

It could be argued that in the estimates used above, the vesicles were smaller than those typically studied. A more realistic size for vesicles is 100 nm [10], so we now give an alternative argument for turgidity of the vesicles that uses this value. We expect that if the vesicle begins in a flaccid state, it immediately becomes turgid because of the inflow of water due to the fact $\zeta \gg 1$. From then on we expect it to be in a stationary state where the flux of water into the vesicle exactly balances its growth due to surface accretion of lipids. So we ask: what is the pressure difference required for a turgid vesicle to remain a sphere while growing at a steady rate given by λ? This is given by Eq. (21) of [2]:

$$\Delta P = \frac{\lambda R}{2L_p} + \frac{\kappa C_0 R(C_0 R - 2)}{R^3}, \tag{7}$$

where the second term on the right-hand side is $\partial E/\partial V$, with κ being the bending modulus and C_0 being the spontaneous curvature. To estimate this term, we employ the values used by BS: κ of order 10^{-19} J (see also [11]) and $C_0 R$ of order 1, which gives for a vesicle of size 100 nm, a value of about 10^{-3} bar. The first term is of approximately the same order [which incidentally shows that $\partial E/\partial V$ cannot be ignored in Eq. (2)]. Therefore for pressure differences of order 10^{-3}–10^{-2} bar the system will settle down in the stationary state and grow in a turgid manner. As we have already discussed, the actual pressure differences are estimated to be much larger. Therefore when considering the instability of the vesicle (well after an initial transient flaccid state has disappeared), the correct equation to use is Eq. (7) or the generalization to deformed shapes such as the ellipsoid, as discussed in [2].

Finally, we come to the other points where BS and we differ. Point (ii) mentioned above has been discussed by BS in their Comment [1], where they agree that future consideration will have to be given to including the reflection coefficient in a more consistent way. The other outstanding point is that BS claim that the neglect of the lateral tension by FM is responsible for the observed discrepancies in the two approaches. There are several separate points here. First, BS again introduce the lateral tension through a static picture (they cite previous work where it is a Lagrange multiplier in the variational approach) and not through a systematic thermodynamic perspective, therefore the same criticism we have made above holds. Second, it is not correct to say, as BS do in their Comment [1], that "…the reason FM obtained this unrealistic prediction is the omission of the lateral tension…." We did not obtain an unrealistic prediction: we never considered the flaccid regime for reasons explained above, which is the regime BS are referring to; the figures in [2] only refer to a turgid vesicle. Third, our paper was designed to supply a framework within which to describe vesicle growth and instability, and in the conclusion we mention several aspects which have been omitted (e.g., thermal fluctuations, varying C_0, etc). We also say that there are "undoubtedly others," and this includes lateral tension. This and several other effects as well should be included in a full treatment. But we stress again that this has to be done systematically and in a controlled way if it is to be at all meaningful. In the case of the lateral tension this can be achieved within the framework that FM use by transforming it into an effective force which makes a special addition to the energy of the "PdV" type [8]. In a forthcoming paper we will extend the calculation reported in [2] to include other effects, among them the lateral tension.

In summary, the Comment by BS [1] does not respond to our criticism regarding the absence of a term involving the bending energy in the equation for the volume growth. It concerns itself with flaccid vesicles when there is little evidence that this is an important regime. Even electron micrographs indicate that the vesicles are turgid [10]. The precise mapping between quantities defined using the formalism of nonequilibrium thermodynamics and those used in variational calculations is not clear and needs to be elucidated before they can be used together in calculations of actual permeable membranes. What is clear is that this is an area where there is considerable scope for more comprehensive models and most of all for more experiments to guide the building of these models.

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