

# Electronic Supplementary Information for The buckling-condensation mechanism driving gas vesicle collapse

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## 1 Prior Consideration of Condensation

In a comprehensive review of gas vesicles [1], Walsby considered the condensation mechanism for possible liquid occupation of the vesicle:

$$p_V/p_{sat} = (e) \exp\left(\frac{2\sigma_{lv}V_L}{r_c T}\right) \quad (1)$$

This equation can be obtained by solving the Young-Laplace equation for  $p_L$  and substituting into Eqn. (4) in the main text. The factor  $e$  written by Walsby appears to be

$$e = \exp\left(\frac{V_L}{T}(p_V - p_{sat})\right). \quad (2)$$

From eqns. 1 and 2, Walsby argued that condensation should occur inside the vesicle when the interior vapor pressure exceeds the saturation pressure (eg,  $p_V > p_{sat}$ ), for a fixed radius  $r_c$  of the liquid nucleus.

Walsby then applies the same equation to analyze the relationship between the vapor pressure inside the vesicle and the vesicle radius  $R$ :

$$p_V/p_{sat} = (e) \exp\left(\frac{-2\sigma_{lv}V_L}{RT}\right) \quad (3)$$

where the negative sign arises from the concave curvature of the vesicle shell with respect to the vapor. From Eqn. 3, Walsby claims that the vapor pressure inside the vesicle must be less than the saturation pressure (eg,  $p_V < p_{sat}$ ) for a finite, constant  $R$ .

From eqns. 1 and 3, Walsby concludes that presence of liquid condensate within the vesicle would cause the interior vapor pressure to be simultaneously greater and smaller than the equal pressure of the liquid nucleus and the ambient exterior liquid. Condensation inside the vesicle therefore triggers a paradox. However, this argument (eqn. 3) assumes the vesicle membrane is equivalent to a liquid-vapor interface with the same effective surface tension,

But approximating the structure of the vesicle as a microbubble proves to be a poor model. For a gas vesicle, mechanical equilibrium exists between the interior vapor and the inside of the protein shell, as well as between the outside of the protein shell and the exterior liquid. Internal stresses of the protein shell account for mechanical mismatch between the interior vapor pressure and the exterior liquid pressure. Thus, GVs are rigid and do not show significant changes in size under varying external pressure (their species dependent linear compressibility is around  $0.0155 \text{ MPa}^{-1}$ ) [1].

Given that the vesicle radius stays approximately constant as the exterior pressure increases, the interior vapor pressure  $p_V$  likewise does not change according to eqn. 3 since there is no dependence of  $p_V$  on the external liquid pressure. This would only hold if the vesicle membrane remained impermeable to vapor, which Walsby disproved [1].

In fact, vapor can enter GVs through pores in the membrane [1]. Thus there exists mechanical equilibrium across the two phase interface between the exterior liquid and the vapor within the pore; this interface within each pore is concave with respect to the interior vapor due to surface tension balancing the pressure difference between the two phases. The interface curvature is not convex as 3 implies by assuming the cylindrical vesicle wall acts as a large liquid-vapor interface. The paradox is therefore resolved, and condensation proves to be a viable mechanism by which liquid water can occupy the vesicle interior and remove its echogenicity.

## 2 Impact of New Experimental Measurements of GV Width

To begin this discussion, it is important to note that there are many open questions surrounding gas vesicles, but even very basic concepts, such as their geometric size are still being evaluated. Recently, Dutka et al. showed that the reported diameter of GVs can vary widely, depending on the experimental technique used to measure their width [2]. Specifically, they demonstrated that measuring the width of GVs using negative stain electron microscopy (ns-EM) on intact vesicles resulted in an over prediction of the mean diameter, when compared to the gold-standard technique, cryo-EM. Unfortunately, cryo-EM is a technique with higher access barriers, due to the cost of equipment, time, and sample quantities required. Luckily, they were able to confirm that ns-EM performed on flat collapsed GVs can be used to indirectly calculate an accurate diameter which agrees well with the cryo-EM results using the simple equation  $D = 2w/\pi$ , where  $w$  the width measured from ns-EM on a flat collapsed vesicle (as opposed to an intact vesicle). This equation allows for some previously published but overpredicted GV diameters [3] to be corrected and utilized in our work, while other previously reported data on collapsed GV widths [4] are confirmed to be accurate. It also demonstrates the challenges associated with studying the fundamental mechanics of these structures, due to the abundance

of knowledge gaps.

### 3 Condensation Dynamics

The large density difference between vapor and liquid water may potentially pose a barrier to the diffusion of vapor into the vesicle during condensation. The key flux of interest is then the evaporation rate of liquid water in the pores of the vesicle membrane. Assuming the liquid water in the pores is constantly replenished from the bulk exterior phase, such that the liquid-vapor meniscus is static, we can use statistical rate theory to find an expression for the evaporation flux  $J$  [5].

$$J = 2 \frac{p_V}{\sqrt{2\pi m k T}} \sinh\left(\frac{\Delta S}{k}\right) \quad (4)$$

where  $k$  is the Boltzmann constant,  $m$  is the mass of a water molecule, and  $\Delta S$  is the change in entropy associated with the transfer of one water molecule from the liquid to the vapor phase. The total mass of vapor entering the vesicle per unit time can be obtained from:

$$M = J N r_p^2 \pi \quad (5)$$

where  $N$  is the number of pores in the membrane and  $r_p^2 \pi$  gives the area of each pore. Walsby estimates  $N$  to be around 21000 and the  $r_p$  as approximately 0.63 nm. If we assume the vapor pressure in the vesicle is 90% of the equilibrium value  $p_V$  due to the non-equilibrium formation or growth of the liquid nucleus, the minimum evaporation flux for external pressures between 1.3 to 13 bar (the relevant range for vesicle collapse) is  $J = 0.80 \frac{kg}{m^2 s}$  [5]. The mass of vapor per unit time entering the vesicle through evaporation is approximately  $M = 6.59 \times 10^{-14} \frac{kg}{s}$ .

Given the mass of liquid  $m_l$  needed to form a liquid nucleus of critical size and the total mass of vapor  $m_v$  in the vesicle, the time  $t_f$  necessary to fill the vesicle with sufficient mass of water for the condensate nucleus to form is:

$$t_f = \frac{m_l - m_v}{M}$$

The maximum filling time  $t_f$  for the range of vesicle sizes considered is 1.9 milliseconds.

### References

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