Supporting information

Membrane elastic energy

We assume that the membrane elastic energy is minimized at zero curvature. The membrane elastic energy is then expressed by

$$E_{bound} = \kappa_{bound} H_{bound}{}^2 A_{bound} \quad (4)$$

$$E_{swell} = \kappa_{swell} H_{swell}{}^2 A_{swell} \quad (5)$$

where κ_i and A_i denote the bending rigidity and area, respectively, of a state *i*, and H_i represents the mean curvature of the membrane of the state *i*. We also assume energy storage around the hole, which is expressed by

$$E_h = \frac{\lambda(H_{bound} + H_{bound,\max})}{2\pi(r_p - r_{swell})} \quad (6)$$

The volume of the buffer solution contained in a vesicle is approximately expressed as

$$V_{swell} = \pi r_{swell}{}^2 \times 2\pi r_p = 2\pi^2 \left(\frac{1}{2H_{swell}}\right)^2 r_p \quad (a1)$$

Parameters r_{swell} and r_p are shown in Figure 7a. Thus, E_{swell} may be rewritten as

$$E_{swell} = \kappa_{swell} \frac{\pi^2 r_p}{2V_{swell}} A_{swell} \quad (a2)$$

H_{bound} and r_p are dependent on each other by a geometric constraint:

$$r_p = \left(\frac{A_{bound}}{\pi}\right)^{1/2} \left(1 - \frac{A_{bound}}{4\pi} H_{bound}{}^2\right)^{1/2} \quad (a3)$$

Here we use

$$\sin\theta = \frac{r_p}{r_{bound}}, \quad A_{bound} = 2\pi r_{bound}{}^2 (1 - \cos\theta)$$

The first equation is an approximation. Using eq. a3, E_{swell} and E_h are expressed by

$$E_{swell} = \kappa_{swell} \frac{\pi^{3/2}}{2} \frac{A_{swell} A_{bound}{}^{1/2}}{V_{swell}} \left(1 - \frac{A_{bound}}{4\pi} H_{bound}{}^2\right)^{1/2} \quad (a4)$$

$$E_h^{-1} = 2\pi\left(\lambda(H_{bound} + H_{bound,\max})\right)^{-1} r_p \left\{1 - \left(\frac{V_{swell}}{2\pi^2}\right)^{1/2}\left(\frac{A_{bound}}{\pi}\right)^{-3/4}\left(1 - H_{bound}^{*}{}^{2}\right)^{-3/4}\right\} \quad (a5)$$

Here we introduce

$$H_{bound}^{*}{}^{2} = \frac{A_{bound}H_{bound}^{2}}{4\pi}, \quad p_A^{*} = \frac{A_{swell}A_{bound}^{1/2}}{V_{swell}} \quad (a6)$$

to obtain

$$\begin{aligned} E^{*} &= \frac{E_{bound} + E_{swell} + E_h}{2\pi\kappa_{swwell}} \\ &= 2\kappa^{*}H_{bound}^{*}{}^{2} + \frac{\sqrt{\pi}}{4}p_A^{*}\left(1 - H_{bound}^{*}{}^{2}\right)^{1/2} \\ &\quad + \frac{\lambda^{*}}{2\pi}\frac{\left(H_{bound,\max}^{*} + H_{bound}^{*}\right)}{\left(1 - H_{bound}^{*}{}^{2}\right)^{1/2}}\frac{1}{1 - \frac{1}{\pi^{1/4}}\frac{1}{(2r_A^{*}p_A^{*})^{1/2}}\left(1 - H_{bound}^{*}{}^{2}\right)^{-3/4}} \end{aligned} \quad (a7)$$

where $E^{*} = (E_{bound} + E_{swell} + E_h) / (2\pi\kappa_{swell})$, and dimensionless bending rigidity is defined as $\kappa^{*} = \kappa_{bound} / \kappa_{swell}$.

V_{swell} given by eq. a1 and is conserved. Eqs. a1 and a3 yield

$$r_{swell} = \frac{1}{2H_{swell}} = \frac{V_{swell}^{1/2}}{\sqrt{2}\,\pi}\left(\frac{A_{bound}}{\pi}\right)^{-1/4}\left(1 - \frac{A_{bound}}{4\pi}H_{bound}^{2}\right)^{-1/4} \quad (a8)$$

Using eqs. a3 and a8, we obtain

$$A_{swell} = 2\pi\, r_{swell}\, 2\pi\, r_p = 2\sqrt{2}\pi V_{swell}^{1/2}\left(\frac{A_{bound}}{\pi}\right)^{1/4}\left(1 - \frac{A_{bound}}{4\pi}H_{bound}^{2}\right)^{1/4} \quad (a9)$$

Eqs. a6 and a9 give

$$p_A^{*} = 8\sqrt{\pi^3}\, r_A^{*}\left(1 - H_{bound}^{*}{}^{2}\right)^{1/2} \quad (a10)$$

where r_A^{*} is defined as A_{bound} / A_{swell}.

The substitution of eq. a10 for p_A^{*} in eq. a7 gives

$$E^{*} = 2\kappa^{*}H_{bound}^{*}{}^{2} + 2\pi^{2}\, r_A^{*}\left(1 - H_{bound}^{*}{}^{2}\right) + \frac{\lambda^{*}}{2\pi}\frac{H_{bound,\max}^{*} + H_{bound}^{*}}{\left(1 - H_{bound}^{*}{}^{2}\right)^{1/2}}\frac{1}{1 - \frac{1}{4\pi\, r_A^{*}}\frac{1}{1 - H_{bound}^{*}{}^{2}}} \quad (a11)$$

which generates eq. 7 in the main text.

The total area of the membrane (A_{total}) is conserved. The equation $A_{total} = A_{swell} + 2A_{bound}$, eq. a9 and $r_A^* = A_{bound} / A_{swell}$ yield eqs. 9c and 9d in the main text. Here we used $\alpha_{swell}^* = 1 - 2\alpha_{bound}^*$, that is derived from $A_{swel\ l} = A_{total} - 2A_{bound}$.