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Supplemental information

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Susanne Liese and Andreas Carlson

Supplemental Material: Membrane Shape Remodeling by Protein Crowding

Susanne Liese[1,2,*] and Andreas Carlson[1,*]

[1]Department of Mathematics, Mechanics Division, University of Oslo, N-0851, Oslo, Norway
[2]Current address: Max Planck Institute for the Physics of Complex Systems, D-01187 Dresden, Germany
[*]Email: sliese@pks.mpg.de, acarlson@math.uio.no

Membrane Shape - Analytic Approximation

For a spherical cap, where the entire crowded domain forms a single protrusion, the energy change going from a flat to a budding membrane, is written as:

$$\Delta E = 2\kappa A_{\mathrm{c}} \left[\left(\frac{1}{R_{\mathrm{s}}} - C_{\mathrm{ind}} \right)^2 - C_{\mathrm{ind}}^2 \right] + \sigma \left(A_{\mathrm{c}} - \pi R_{\mathrm{s}}^2 \sin^2 \alpha \right), \tag{S1}$$

with

$$A_{\mathrm{c}} = 2\pi R_{\mathrm{s}}^2 \left(1 - \cos \alpha \right). \tag{S2}$$

Inserting Eq. S2 into Eq. S1, the energy difference is expressed as

$$\Delta E = 2\kappa A_{\mathrm{c}} \left(\frac{1}{R_{\mathrm{s}}^2} - 2\frac{C_{\mathrm{ind}}}{R_{\mathrm{s}}} \right) + \sigma A_{\mathrm{c}} \frac{A_{\mathrm{c}}}{4\pi R_{\mathrm{s}}^2}, \tag{S3}$$

Minimizing Eq. S3 with respect to R_{s}, we find

$$R_{\mathrm{s}} = \frac{1 + \frac{\sigma A_{\mathrm{c}}}{8\pi\kappa}}{C_{\mathrm{ind}}}. \tag{S4}$$

The height of the spherical cap is written as:

$$\begin{aligned} L &= R_{\mathrm{s}} \left(1 - \cos \alpha \right), \\ &= \frac{A_{\mathrm{c}} C_{\mathrm{ind}}}{2\pi} \frac{1}{1 + \frac{\sigma A_{\mathrm{c}}}{8\pi\kappa}}. \end{aligned} \tag{S5}$$

Inserting Eq. S4 into Eq. S3 the energy of the minimal energy shape becomes:

$$\Delta E = -\frac{2\kappa A_{\mathrm{c}} C_{\mathrm{ind}}^2}{1 + \frac{\sigma A_{\mathrm{c}}}{8\pi\kappa}}. \tag{S6}$$

If the membrane shape is described by a cylinder with radius $1/(2C_{\mathrm{ind}})$, then the energy and the protrusion height are obtained directly as

$$\Delta E = -2\kappa A_{\mathrm{c}} C_{\mathrm{ind}}^2 + \sigma A_{\mathrm{c}} - \sigma\pi \frac{1}{(2C_{\mathrm{ind}})^2} \tag{S7}$$

and

$$L = \frac{A_{\mathrm{c}} C_{\mathrm{ind}}}{\pi}. \tag{S8}$$

Based on Eqs. S6, S7 the $C_{\rm ind}$-range for which a cylindrical shape is energetically favorable compared to a spherical cap shape, is given by

$$-2\kappa A_{\rm c} C_{\rm ind}^2 + \sigma A_{\rm c} - \sigma\pi \frac{1}{(2C_{\rm ind})^2} < -\frac{2\kappa A_{\rm c} C_{\rm ind}^2}{1+\frac{\sigma A_{\rm c}}{8\pi\kappa}}, \tag{S9}$$

which leads to

$$A_{\rm c} C_{\rm ind}^2 > \left(2\pi + \frac{\sigma A_{\rm c}}{4\kappa}\right) + \sqrt{\left(2\pi + \frac{\sigma A_{\rm c}}{4\kappa} - \frac{\pi}{4}\right)^2 - \frac{\pi^2}{16}}. \tag{S10}$$

Since $8\pi + \sigma A_{\rm c}/\kappa > \pi$, we approximate Eq. S10 as

$$A_{\rm c} C_{\rm ind}^2 > 2\left(2\pi + \frac{\sigma A_{\rm c}}{4\kappa}\right). \tag{S11}$$

The induced spontaneous curvature for which a cylindrical shape becomes energetically favorable is

$$C_{\rm ind} > \sqrt{\frac{4\pi + \frac{\sigma A_{\rm c}}{2\kappa}}{A_{\rm c}}}. \tag{S12}$$

To understand why the transition point between a flat and a tubular membrane shape depends on the size of the crowded domain, we examine in more detail the size dependence of the two energy contributions, bending energy and tension. We start with a completely flat membrane where the bending energy is given by $E_{\rm bend} = 2\kappa A_{\rm c} C_{\rm ind}^2$. Thus $E_{\rm bend}$ is extensive and proportional to $A_{\rm c}$. If we now remodel the membrane into a cylinder with radius $1/(2C_{\rm ind})$, the bending energy approximately vanishes. The membrane energy is dominated by the tension, where the energy difference between a flat and a cylindrical shape is written as $\sigma\left(A_{\rm c} - \frac{\pi}{4C_{\rm ind}^2}\right)$. Hence, the tension term is not simply proportional to $A_{\rm c}$. In a hypothetical scenario where the membrane is transformed into a cylinder with vanishing radius, the tension energy would be given by $\sigma A_{\rm c}$. The transition from a flat to a cylindrical shape, in this case given by the condition $2\kappa A_{\rm c} C_{\rm ind}^2 > \sigma A_{\rm c}$, would depend only on the ratio $\kappa C_{\rm ind}^2/\sigma$. However, a cylinder with vanishing radius is impeded by the divergent bending energy. In other words, while the bending energy of a flat membrane can be approximated as being extensive in $A_{\rm c}$, the tension term is not, since it contains an additional term proportional to $C_{\rm ind}^{-2}$. Consequently, the transition point between a flat and a cylindrical shape, determined by $2\kappa A_{\rm c} C_{\rm ind}^2 > \sigma\left(A_{\rm c} - \frac{\pi}{4C_{\rm ind}^2}\right)$, depends on both $\kappa C_{\rm ind}^2/\sigma$ and $A_{\rm c} C_{\rm ind}^2$.

Energy Functional

The energy functional (Eq. 1 in the main text) in the arc length parameterization reads

$$E = 2\pi \int_0^\infty dSR\left[\frac{\kappa}{2}\left(\frac{d\psi}{dS} + \frac{\sin\psi}{R}\right)^2 + \sigma\right] + 2\pi\int_0^\infty dS^* R^* p(\rho^*)\Theta(A_{\rm c} - A), \tag{S13}$$

with the Heaviside function Θ. The coordinates along the protein surface, which are shifted by $r_{\rm p}$ relative to the membrane surface, are indicated with an asterisk. If the protein radius $r_{\rm p}$ is small compared to the inverse of the membrane curvature, the coordinates along the protein surface (dashed line in Fig. 1c in the main text) are given by $R^* = R + r_{\rm p}\sin\psi$ and $Z^* = Z + r_{\rm p}\cos\psi$. An arc length element dS^* is then written as $dS^* = \sqrt{(dR^*)^2 + (dZ^*)^2} = dS\sqrt{(dR^*/dS)^2 + (dZ^*/dS)^2} = dS\left(1 + r_{\rm p}\frac{d\psi}{dS}\right)$ and the area element along the protein surface is given by $dA^* = dS^* R^* = dSR\left(1 + r_{\rm p}\frac{d\psi}{dS}\right)\left(1 + r_{\rm p}\frac{\sin\psi}{R}\right)$. The protein density ρ along the membrane surface is equivalent to $\rho = dN_{\rm p}/dA$, with $N_{\rm p}$ the number of proteins. Since the number of proteins is conserved, we write the protein density along the shifted surface as $\rho^* = dN_{\rm p}/dA^* = \rho\left[\left(1 + r_{\rm p}\frac{d\psi}{dS}\right)\left(1 + r_{\rm p}\frac{\sin\psi}{R}\right)\right]^{-1}$.

Next, we rewrite the lateral pressure p, by first expressing p in a virial expansion $p(\rho^*) = \sum_{i=1}^\infty k_{\rm B}T\nu_i(\rho^*)^i$ and then performing a Taylor expansion around $r_{\rm p}C_1 = r_{\rm p}C_2 = 0$, with $C_1 = d\psi/dS$ and $C_2 = \sin\psi/R$, up to second order in C_1

and C_2,

$$dS^*R^*p(\rho^*) = dSR\sum_{i=1}^{\infty} k_{\mathrm{B}}T\nu_i\rho^i\left(1+r_{\mathrm{p}}C_1\right)^{-i+1}\left(1+r_{\mathrm{p}}C_2\right)^{-i+1}, \tag{S14a}$$

$$\approx dSR\sum_{i=1}^{\infty} k_{\mathrm{B}}T\nu_i\rho^i\left[1+(1-i)r_{\mathrm{p}}(C_1+C_2)+\frac{i^2-i}{2}r_{\mathrm{p}}^2(C_1+C_2)^2+(1-i)r_{\mathrm{p}}^2C_1C_2\right], \tag{S14b}$$

$$\approx dSR\left[p(\rho)+\left(p(\rho)-\rho\frac{dp}{d\rho}\right)r_{\mathrm{p}}\left(C_1+C_2\right)+\frac{\rho^2}{2}\frac{d^2p}{d\rho^2}r_{\mathrm{p}}^2\left(C_1+C_2\right)^2+\left(p(\rho)-\rho\frac{dp}{d\rho}\right)r_{\mathrm{p}}^2C_1C_2\right]. \tag{S14c}$$

Inserting Eq. S14 into Eq. S13 and introducing the non-dimensional variables $c_1=\sqrt{A_{\mathrm{c}}}C_1$, $c_2=\sqrt{A_{\mathrm{c}}}C_2$, $\widetilde{p}=2pA_{\mathrm{c}}/\kappa\cdot\Theta(A_{\mathrm{c}}-A)$, and $\widetilde{\sigma}=\sigma A_{\mathrm{c}}/\kappa$, we can now write the total membrane energy, Eq. S13, as

$$\frac{E}{\pi\kappa}=\int_0^{\infty}dsr\left[(c_1+c_2)^2+2\widetilde{\sigma}+\widetilde{p}-\left(\rho\frac{d\widetilde{p}}{d\rho}-\widetilde{p}\right)\frac{r_{\mathrm{p}}}{\sqrt{A_{\mathrm{c}}}}(c_1+c_2)+\frac{\rho^2}{2}\frac{d^2\widetilde{p}}{d\rho^2}\frac{r_{\mathrm{p}}^2}{A_{\mathrm{c}}}(c_1+c_2)^2-\left(\rho\frac{d\widetilde{p}}{d\rho}-\widetilde{p}\right)\frac{r_{\mathrm{p}}^2}{A_{\mathrm{c}}}c_1c_2\right], \tag{S15}$$

where we also used the non-dimensional variables defined in the main text. For a fixed protein density the energy, Eq.S15, can be expressed, in terms of an induced spontaneous curvature C_{ind}, an effective increase of the bending rigidity $\Delta\kappa$, an effective Gaussian bending rigidity κ_{g}, and a constant γ as

$$\frac{E}{\pi\kappa}=\int_0^{\infty}dsr\left[\left(1+\frac{\Delta\kappa\Theta(A_{\mathrm{c}}-A)}{\kappa}\right)\left(c_1+c_2-2\sqrt{A_{\mathrm{c}}}C_{\mathrm{ind}}\Theta(A_{\mathrm{c}}-A)\right)^2+2\widetilde{\sigma}+\frac{\kappa_{\mathrm{g}}\Theta(A_{\mathrm{c}}-A)}{\kappa}c_1c_2\right]+\frac{\gamma A_{\mathrm{c}}}{\pi\kappa}, \tag{S16}$$

$$\text{with } C_{\mathrm{ind}}=\frac{\frac{r_{\mathrm{p}}}{2\kappa}\left(\rho\frac{dp}{d\rho}-p\right)}{1+\frac{\rho^2r_{\mathrm{p}}^2}{\kappa}\frac{d^2p}{d\rho^2}},\quad \Delta\kappa=\rho^2r_{\mathrm{p}}^2\frac{d^2p}{d\rho^2},\quad \kappa_{\mathrm{g}}=2r_{\mathrm{p}}^2\left(p-\rho\frac{dp}{d\rho}\right),\quad \gamma=2p(\rho)-4(\kappa+\Delta\kappa)C_{\mathrm{ind}}^2(\rho).$$

All four quantities C_{ind}, $\Delta\kappa$, κ_{g}, and γ are constant within the crowded domain. We note that γ has units of a surface tension, where γ combined the lateral pressure and a term that compensates the C_{ind}^2 term in the Helfrich energy. A crowding induced membrane tension was also described by Linden *et al.*, who discussed the interplay between the induced membrane tension and the opening and closing of protein channels [1]. The contribution from γ depends solely on the protein density ρ, but not on the membrane shape. Hence, $\gamma A_{\mathrm{c}}/\pi\kappa$ does not influence the energy minimizing shape since the size of the crowded domain, A_{c}, is fixed. In contrast, the overall membrane area A is not constrained, which means that $\widetilde{\sigma}$ cannot be neglected in the energy minimization.
If the protein size is much smaller than the size of the crowded domain, $r_{\mathrm{p}}\ll\sqrt{A_{\mathrm{c}}}$, we can omit all terms of order $r_{\mathrm{p}}^2/A_{\mathrm{c}}$ in Eq. S15, which simplifies the energy to

$$\frac{E}{\pi\kappa}=\int_0^{\infty}dsr\left[\left(c_1+c_2-2\sqrt{A_{\mathrm{c}}}C_{\mathrm{ind}}\Theta(A_{\mathrm{c}}-A)\right)^2+2\widetilde{\sigma}\right]+\frac{\gamma A_{\mathrm{c}}}{\pi\kappa}, \tag{S17}$$

$$\text{with } C_{\mathrm{ind}}=\frac{r_{\mathrm{p}}}{2\kappa}\left(\rho\frac{dp}{d\rho}-p\right)\text{ and }\gamma=2p(\rho)-4\kappa C_{\mathrm{ind}}^2(\rho).$$

We note that Eq. S17 is equivalent to Eq. 2 in the main text.

Shape Equations

In the previous section, we have shown that the membrane energy in non-dimensional variables is written as:

$$\frac{E}{\pi\kappa}=\int_0^{\infty}dsr\left[(\psi'+\frac{\sin\psi}{r}-2c_{\mathrm{ind}})^2+2\widetilde{\sigma}\right]+const., \tag{S18}$$

with $c_{\mathrm{ind}}=\sqrt{A_{\mathrm{c}}}C_{\mathrm{ind}}\Theta(A_{\mathrm{c}}-A)$. The derivative with respect to s is indicated by a prime, $d/ds=()'$. We use the Euler-Lagrange formalism to derive the shape equations that minimize Eq. S18. $\mathcal{L}$, a function similar to the Lagrangian in the Euler-Lagrange formalism, is given by

$$\mathcal{L}=r\left[(\psi'+\frac{\sin\psi}{r}-2c_{\mathrm{ind}})^2+2\widetilde{\sigma}\right]+\lambda_1(r'-\cos\psi)+\lambda_2 2\pi r\Theta(1-a), \tag{S19}$$

with the Lagrange multiplier function λ_1 enforcing the relation between r and ψ. The Lagrange multiplier λ_2 maintains a fixed area of the crowded domain.
Based on $\frac{d}{ds}\frac{\partial \mathcal{L}}{\partial \psi'} = \frac{\partial \mathcal{L}}{\partial \psi}$, we find

$$h' = \frac{\lambda_1}{4r}\sin\psi, \text{ with } h = \frac{1}{2}\left(\psi' + \frac{\sin\psi}{r}\right) - c_{\text{ind}}. \tag{S20}$$

And $\frac{d}{ds}\frac{\partial \mathcal{L}}{\partial r'} = \frac{\partial \mathcal{L}}{\partial r}$ leads to

$$\lambda_1' = (\psi' - 2c_{\text{ind}})^2 - \left(\frac{\sin\psi}{r}\right)^2 + 2\widetilde{\sigma} + 2\pi\lambda_2\Theta(1-a). \tag{S21}$$

We define $\mathcal{H}$ in analogy to a Hamiltonian, with

$$\mathcal{H} = -\mathcal{L} + \psi'\frac{\partial \mathcal{L}}{\partial \psi'} + r'\frac{\partial \mathcal{L}}{\partial r'} \tag{S22a}$$

$$= r\left((\psi')^2 - \left(\frac{\sin\psi}{r} - 2c_{\text{ind}}\right)^2\right) - 2\widetilde{\sigma}r + \lambda_1\cos\psi - \lambda_2 2\pi r\Theta(1-a). \tag{S22b}$$

We note that $\mathcal{H}$ is not an energy, but rather an auxiliary function that we use to derive the shape equations. The explicit and implicit dependence of $\mathcal{H}$ and $\mathcal{L}$ on the scaled arc length s are related as $d\mathcal{H}/ds = -\partial\mathcal{L}/\partial s$. Since $\mathcal{L}$ does not depend on s explicitly, $\mathcal{H}$ is constant. The upper integration boundary in Eq. S18 is not fixed. The functional variation of $\mathcal{L}$ then leads to $\mathcal{H} = 0$ [2, 3, 4, 5, 6]. Eq. S22 is now written as

$$2\widetilde{\sigma} + \lambda_2 2\pi\Theta(1-a) = (\psi')^2 - \left(\frac{\sin\psi}{r} - 2c_{\text{ind}}\right)^2 + \lambda_1\frac{\cos\psi}{r}. \tag{S23}$$

Inserting Eq. S23 into Eq. S21 we find

$$\lambda_1' = 2\left(\psi' + \frac{\sin\psi}{r} - 2c_{\text{ind}}\right)\left(\psi' - \frac{\sin\psi}{r}\right) + \frac{\lambda_1}{r}\cos\psi. \tag{S24}$$

Next, we define the auxiliary function $u := \lambda_1/(2r)$, so that

$$u' = \frac{\psi' + \frac{\sin\psi}{r} - 2c_{\text{ind}}}{r}\left(\psi' - \frac{\sin\psi}{r}\right) = \frac{4h}{r}\left(h - \frac{\sin\psi}{r} + c_{\text{ind}}\right). \tag{S25}$$

Eq. S20 and S25, together with the geometric relations between r, z, ψ, and a, lead to the shape equations (Eqs. 7 in the main text):

$$\frac{dr}{ds} = \cos\psi, \tag{S26a}$$

$$\frac{dz}{ds} = -\sin\psi, \tag{S26b}$$

$$\frac{d\psi}{ds} = 2h - \frac{\sin\psi}{r} + 2c_{\text{ind}}, \tag{S26c}$$

$$\frac{dh}{ds} = \frac{u}{2}\sin\psi, \tag{S26d}$$

$$\frac{du}{ds} = \frac{4h}{r}\left[h - \frac{\sin\psi}{r} + c_{\text{ind}}\right], \tag{S26e}$$

$$\frac{da}{ds} = 2\pi r. \tag{S26f}$$

Boundary Conditions and Numerical Implementation

The boundary conditions of the membrane shape at the center line (Eq. 8 in the main text) are given by $r = 0$, $a = 0$ and $\psi = 0$. At the outer boundary, in the protein-free region ($c_{\text{ind}} = 0$), the membrane transitions to a flat shape with

$\psi(s \to \infty) = 0$ and a vanishing mean curvature ($h(s \to \infty) = 0$). Hence, $\mathcal{H}$ as given in Eq. S22, at the outer boundary simplifies to

$$\mathcal{H}(s \to \infty) = 2r(u - \widetilde{\sigma}), \tag{S27}$$

which leads to the boundary condition for u, with $u(s \to \infty) = \widetilde{\sigma} = \sigma A_\mathrm{c}/\kappa$.

Since Eq. S26 has a singularity for $r = 0$, we have to shift the inner boundary from $s = 0$ to $s = \tau$ in the numerical calculations, where we set $\tau = 0.0001$. We denote the scaled mean curvature at the center line as $2c_0$. According to $\psi(\tau) = \int_0^\tau \psi' ds \approx \int_0^\tau c_0 ds$, we obtain the new boundary condition $\psi(\tau) \approx c_0\tau$. Analogously, from $r' = \cos\psi \approx 1 - \psi^2/2$ and $a' = 2\pi r$ we find the new boundary conditions $r(\tau) \approx \tau$ and $a(\tau) \approx \pi\tau^2$. In addition, we denote the value of u at $s = \tau$ as u_0. Evaluating $\mathcal{H}$ (Eq. S22) at $s = \tau$, we find the following relation between u_0 and the Langrange multiplier λ_2: $u_0 = \lambda_2\pi + \sigma + 2c_\mathrm{ind}^2 - 2c_0c_\mathrm{ind}$. In summary, we obtain the following boundary conditions for the numerical calculations:

$$r(\tau) = \tau, \tag{S28a}$$
$$z(s_\mathrm{end}) = 0, \tag{S28b}$$
$$\psi(\tau) = c_0\tau, \qquad \psi(s_\mathrm{end}) = 0, \tag{S28c}$$
$$h(\tau) = c_0 - c_\mathrm{ind}, \tag{S28d}$$
$$u(\tau) = u_0, \qquad u(s_\mathrm{end}) = \frac{\sigma A_\mathrm{c}}{\kappa}, \tag{S28e}$$
$$a(\tau) = \pi\tau^2, \tag{S28f}$$

with s_end the total arc length, which we set to values between $s_\mathrm{end} = 2.0$ and $s_\mathrm{end} = 6.0$, while ensuring that a change in s_end has no influence on the shape of the protein crowded domain.

In the numerical implementation, we set c_0 to a fixed value and vary u_0 such that the outer boundary condition $\psi(s_\mathrm{end}) = 0$ is satisfied. From $u(s_\mathrm{end}) = \sigma A_\mathrm{c}/\kappa$, we then obtain the scaled membrane tension that corresponds to c_0. Subsequently, from a systematic variation of c_0 and u_0 we determine the solution of the shape equation for any given value of the scaled membrane tension.

Transmembrane Pressure

Above, we derived the shape equations for a protrusion budding from an infinite flat membrane. We now turn to a protrusion budding from a spherical vesicle and discuss the limit where the two models are equivalent. In particular, the following derivation shows that the transmembrane pressure does not influence the shape equations in the limit of large vesicles.

The energy of a closed membrane shape reads:

$$E = 2\pi \int_0^{S_\mathrm{end}} dSR \left[\frac{\kappa}{2} \left(\frac{d\psi}{dS} + \frac{\sin\psi}{R} \right)^2 + \sigma - \Pi \frac{R}{2} \sin\psi \right] + 2\pi \int_0^{S_\mathrm{end}} dS^* R^* p(\rho^*) \Theta(A_\mathrm{c} - A), \tag{S29}$$

which is similar to Eq. S13, but we here consider the transmembrane pressure Π and the integration does not extend to infinity. The upper integration limit S_end (see Fig. S1) itself depends on the membrane shape. In dimensionless units the energy becomes:

$$\frac{E}{\pi\kappa} = \int_0^{s_\mathrm{end}} dsr \left[(\psi' + \frac{\sin\psi}{r} - 2c_\mathrm{ind})^2 + 2\widetilde{\sigma} - \widetilde{\Pi} r \sin\psi \right] + const., \tag{S30}$$

with $\widetilde{\Pi} = \Pi A_\mathrm{c}^{3/2}/\kappa$. The corresponding $\mathcal{L}$ and $\mathcal{H}$ then read:

$$\mathcal{L} = r \left[(\psi' + \frac{\sin\psi}{r} - 2c_\mathrm{ind})^2 + 2\widetilde{\sigma} - \widetilde{\Pi} r \sin\psi \right] + \lambda_1(r' - \cos\psi) + \lambda_2 2\pi r \Theta(1 - a), \tag{S31}$$

$$\mathcal{H} = -\mathcal{L} + \psi' \frac{\partial \mathcal{L}}{\partial \psi'} + r' \frac{\partial \mathcal{L}}{\partial r'} \tag{S32a}$$
$$= r \left((\psi')^2 - \left(\frac{\sin\psi}{r} - 2c_\mathrm{ind} \right)^2 \right) - 2\widetilde{\sigma} r + \widetilde{\Pi} r^2 \sin\psi + \lambda_1 \cos\psi - \lambda_2 2\pi r \Theta(1 - a). \tag{S32b}$$

Following the same arguments as in the derivation of Eq. S26, we find $\mathcal{H}(s) = 0$. Based on the Euler-Lagrange equations $\left(\frac{d}{ds}\frac{\partial L}{\partial \psi'} = \frac{\partial L}{\partial \psi}, \frac{d}{ds}\frac{\partial L}{\partial r'} = \frac{\partial L}{\partial r}\right)$ we find the following shape equations:

$$\frac{dr}{ds} = \cos\psi, \tag{S33a}$$

$$\frac{dz}{ds} = -\sin\psi, \tag{S33b}$$

$$\frac{d\psi}{ds} = 2h - \frac{\sin\psi}{r} + 2c_{\mathrm{ind}}, \tag{S33c}$$

$$\frac{dh}{ds} = \frac{u}{2}\sin\psi - \frac{\widetilde{\Pi}}{4} r\cos\psi, \tag{S33d}$$

$$\frac{du}{ds} = \frac{4h}{r}\left[h - \frac{\sin\psi}{r} + c_{\mathrm{ind}}\right] - \frac{\widetilde{\Pi}}{2}\sin\psi, \tag{S33e}$$

$$\frac{da}{ds} = 2\pi r. \tag{S33f}$$

Far away from the budding region, the membrane shape transitions into a spherical shape with radius R_{v} (Fig. S1), which implies $\psi' = \sin\psi/r = 1/r_{\mathrm{v}}$, with $r_{\mathrm{v}} = R_{\mathrm{v}} A_{\mathrm{c}}^{-1/2}$ the scaled curvature radius of the vesicle. Hence, the mean curvature h far away from the budding region is constant, which according to Eq. S33d means $u = \widetilde{\Pi}/2[\sin\psi/r]^{-1}\cos\psi$. Inserting these relations into Eq. S32, we find

$$\widetilde{\Pi} = \frac{2\widetilde{\sigma}}{r_{\mathrm{v}}}. \tag{S34}$$

If the protein crowded region is much smaller than the overall vesicle size ($r_{\mathrm{v}} \gg 1$), the scaled transmembrane pressure (Eq. S34) becomes negligible and Eqs. S33 are equivalent to Eqs. S26.

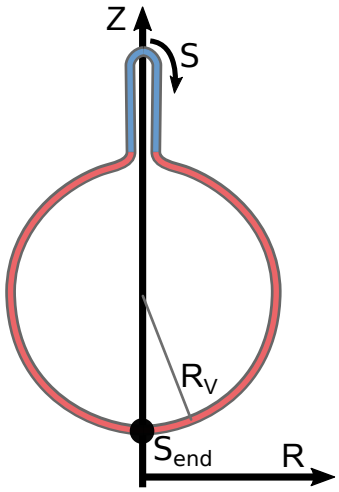

Figure S1: The protein crowded domain (blue) protrudes from an initially spherical vesicle (red). Far away from the crowded domain the membrane shape transitions into a spherical shape with a curvature radius R_{v}.

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