

Supplementary material for:
Shape of the membrane neck around a hole during plasma membrane
repair

1 Definitions and assumptions

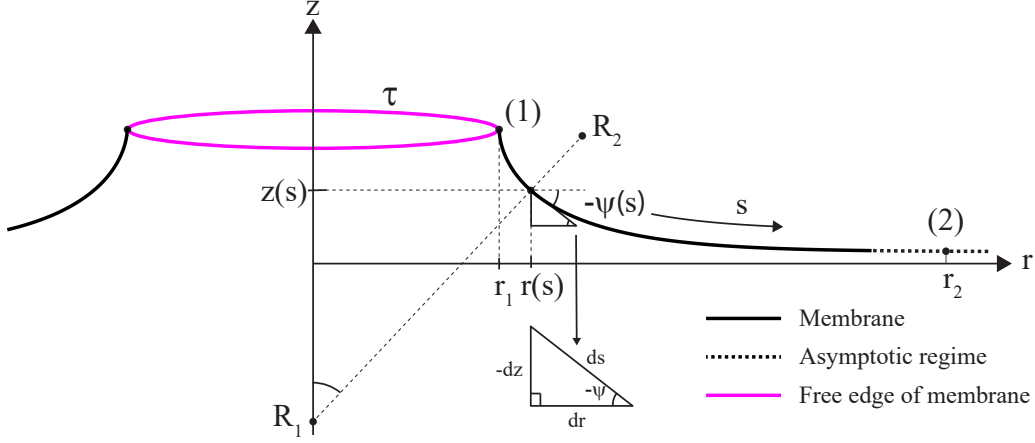


Figure S1: Geometry and definitions in a model for membrane curvature around a circular hole. The neck shape is given by coordinates $r(s)$, $z(s)$ and $\psi(s)$ which are functions of the arc length s .

We aim to model the membrane shape near a hole using the simplifying assumption of axial symmetry. Upon plasma membrane rupture and Ca^{2+} influx, binding of curvature-inducing proteins generates a spontaneous curvature (c_0) of the membrane. The combined effects of spontaneous curvature, membrane tension and line tension (τ) at the free edge results in a curved conformation of the membrane near the region of the hole described by coordinates $r(s)$, $z(s)$ and the angle $\psi(s)$ with s being the arc length. The equilibrium conformations of the membrane near the hole is determined through variational calculus of the total energy functional.

2 Energy of the neck conformation

The free energy of the membrane neck is the sum of the Helfrich energy plus a contribution from line tension at the free membrane edge:

$$G_{\text{neck}} = \int_A \left[\frac{k}{2} (2H - c_0)^2 + k_G K \right] dA + 2\pi\tau r_1 \quad (\text{S.1})$$

Here $2H$ is the total curvature and H is the mean curvature, c_0 is the spontaneous total curvature, K is the Gaussian curvature, k is the bending modulus, k_G is the Gaussian curvature modulus and $r_1 = r(s_1)$ is the radius at the hole edge. The area integration is over a sufficiently large area A away from the hole that state (2) can be considered to lie in the asymptotic regime. It is useful to parameterize the shape coordinates by the arc length s , defined to be increasing when traveling away from the membrane free edge. The system is subject to the following geometrical constraints on the coordinates: $r(s)$, $z(s)$ and

$\psi(s)$:

$$\frac{dz}{ds} = \dot{z} = \sin \psi \quad (\text{S.2})$$

$$\frac{dr}{ds} = \dot{r} = \cos \psi \quad (\text{S.3})$$

The total curvature $2H$ and the Gaussian curvature K are expressed in terms of the principal curvature radii R_1, R_2 as:

$$2H = \frac{1}{R_1} + \frac{1}{R_2} = \dot{\psi} + \frac{\sin \psi}{r} \quad (\text{S.4})$$

$$K = \frac{1}{R_1 R_2} = \dot{\psi} \frac{\sin \psi}{r} \quad (\text{S.5})$$

Using the area element $dA = 2\pi r ds$ and defining the energy functional S , equation (S.1) becomes:

$$\frac{G_{\text{neck}}}{2\pi} = S = \int_{s_1}^{s_2} \left[\frac{k}{2} \left(\dot{\psi} + \frac{\sin \psi}{r} - c_0 \right)^2 r + k_G \dot{\psi} \sin \psi \right] ds + \tau r_1 \quad (\text{S.6})$$

The Gaussian curvature term is integrated to yield:

$$S = \int_{s_1}^{s_2} \mathcal{L}_0 ds - k_G \cos \psi|_{s_1}^{s_2} + \tau r_1 \quad (\text{S.7})$$

where the 'Lagrangian' function (\mathcal{L}_0) has been defined as:

$$\mathcal{L}_0 = \frac{k}{2} \left(\dot{\psi} + \frac{\sin \psi}{r} - c_0 \right)^2 r \quad (\text{S.8})$$

To achieve mechanical equilibrium, the membrane is allowed to have a constant, non-zero tension (σ). The tension energy G_{tension} associated with an area change $A - A_0$, can be written:

$$G_{\text{tension}} = \sigma(A - A_0) = 2\pi \int_{s_1}^{s_2} \sigma r ds - \sigma A_0 \quad (\text{S.9})$$

The tension (σ) can be regarded as a Lagrange multiplier for the requirement of constant area. When applying variational calculus to the energy functional (below), the constant area term A_0 does not enter. According to equation (S.9) it means that the membrane tension only contributes with a term σr to the Lagrangian. The membrane is also subject to the geometrical constraints (S.2,S.3) which further generates two Lagrange multipliers: $t(s)$ and $v(s)$. In total, the full Lagrangian relevant for energy minimization becomes:

$$\mathcal{L} = \frac{k}{2} \left(\dot{\psi} + \frac{\sin \psi}{r} - c_0 \right)^2 r + t(\dot{r} - \cos \psi) + v(\dot{z} - \sin \psi) + \sigma r \quad (\text{S.10})$$

3 Energy minimization

The total energy functional is written as:

$$S = \int_{s_1}^{s_2} \mathcal{L} ds - k_G \cos \psi|_{s_1}^{s_2} + \tau r_1 \quad (\text{S.11})$$

The equilibrium conformation of the membrane is established using variational calculus with the requirement that $\delta S = 0$. The total variation δS is:

$$\begin{aligned} \delta S = & \int_{s_1}^{s_2} \left[\left(\frac{\partial \mathcal{L}}{\partial \psi} - \frac{d}{ds} \left(\frac{\partial \mathcal{L}}{\partial \dot{\psi}} \right) \right) \delta \psi + \left(\frac{\partial \mathcal{L}}{\partial r} - \frac{d}{ds} \left(\frac{\partial \mathcal{L}}{\partial \dot{r}} \right) \right) \delta r + \left(\frac{\partial \mathcal{L}}{\partial z} - \frac{d}{ds} \left(\frac{\partial \mathcal{L}}{\partial \dot{z}} \right) \right) \delta z \right] ds \\ & - \mathcal{H} \delta s|_{s_1}^{s_2} + \frac{\partial \mathcal{L}}{\partial \dot{\psi}} \delta \psi|_{s_1}^{s_2} + \frac{\partial \mathcal{L}}{\partial \dot{r}} \delta r|_{s_1}^{s_2} + \frac{\partial \mathcal{L}}{\partial \dot{z}} \delta z|_{s_1}^{s_2} + k_G \sin \psi \delta \psi|_{s_1}^{s_2} + \tau \delta r_1 \\ = & 0 \end{aligned} \quad (\text{S.12})$$

The three Euler-Lagrange terms in equation (S.12) must vanish separately at equilibrium, leading to the following differential equations:

$$\frac{\partial \mathcal{L}}{\partial \psi} - \frac{d}{ds} \left(\frac{\partial \mathcal{L}}{\partial \dot{\psi}} \right) = 0 \quad (\text{S.13})$$

\Downarrow

$$\ddot{\psi} = -\dot{\psi} \frac{\cos \psi}{r} + \frac{\sin \psi \cos \psi}{r^2} + t \frac{\sin \psi}{kr} - v \frac{\cos \psi}{kr} \quad (\text{S.14})$$

$$\frac{\partial \mathcal{L}}{\partial r} - \frac{d}{ds} \left(\frac{\partial \mathcal{L}}{\partial \dot{r}} \right) = 0 \quad (\text{S.15})$$

\Downarrow

$$\dot{t} = \frac{k}{2} \left[(\dot{\psi} - c_0)^2 - \frac{\sin^2 \psi}{r^2} \right] + \sigma \quad (\text{S.16})$$

$$\frac{\partial \mathcal{L}}{\partial z} - \frac{d}{ds} \left(\frac{\partial \mathcal{L}}{\partial \dot{z}} \right) = 0 \quad (\text{S.17})$$

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$$\dot{v} = 0 \quad (\text{S.18})$$

The Hamiltonian \mathcal{H} in equation (S.12) is:

$$\begin{aligned} \mathcal{H} &= -\mathcal{L} + \dot{\psi} \frac{\partial \mathcal{L}}{\partial \dot{\psi}} + \dot{r} \frac{\partial \mathcal{L}}{\partial \dot{r}} + \dot{z} \frac{\partial \mathcal{L}}{\partial \dot{z}} \\ &= \frac{kr}{2} \dot{\psi}^2 - \frac{kr}{2} \left(\frac{\sin \psi}{r} - c_0 \right)^2 + t \cos \psi - v \sin \psi - \sigma r = 0 \end{aligned} \quad (\text{S.19})$$

Equation (S.19) arises due to endpoint variations of s in the variational calculus of S . The relation $\mathcal{H}=0$ is e.g. useful for obtaining relations between the coordinates.

The boundary terms in equation (S.12) must be zero for all variations at the boundaries. Since we will use the exact asymptotic solution for the membrane shape far away from the hole at $s = s_2$ (see below), the variations at this boundary are zero: $\delta\psi_2 = 0$, $\delta r_2 = 0$, $\delta z_2 = 0$.

At the membrane hole $s = s_1$, the boundary terms in equation (S.12) reduce to the following equation:

$$- \left(\frac{\partial \mathcal{L}}{\partial \dot{\psi}} \Big|_{s_1} + k_G \sin \psi_1 \right) \delta\psi_1 + \left(\tau - \frac{\partial \mathcal{L}}{\partial \dot{r}} \Big|_{s_1} \right) \delta r_1 - \frac{\partial \mathcal{L}}{\partial \dot{z}} \Big|_{s_1} \delta z_1 = 0 \quad (\text{S.20})$$

The three terms in equation (S.20) must vanish separately, giving the following equations:

$$\dot{\psi}_1 = c_0 - \left(1 + \frac{k_G}{k} \right) \frac{\sin \psi_1}{r_1} \Leftrightarrow k_G = k \left[(c_0 - \dot{\psi}_1) \frac{r_1}{\sin \psi_1} - 1 \right] \quad (\text{S.21})$$

$$t(s_1) = t_1 = \tau \quad (\text{S.22})$$

$$v(s_1) = 0 \quad (\text{S.23})$$

Equation (S.21) provides the connection between the membrane conformation at the free edge and the Gaussian curvature modulus k_G . Equation (S.22) provides a stop criterium for terminating integration. Equation (S.23) and (S.18) combined gives $v(s)=0$ for all s . In summary, the following set of coupled

shape equations must be solved to obtain the membrane conformation via the functions: $r, \psi, \dot{\psi}, t, z$:

$$\dot{r} = \cos \psi \quad (\text{S.24})$$

$$\dot{\psi} = u \quad (\text{S.25})$$

$$\dot{u} = -u \frac{\cos \psi}{r} + \frac{\sin \psi \cos \psi}{r^2} + t \frac{\sin \psi}{kr} \quad (\text{S.26})$$

$$\dot{t} = \frac{k}{2} \left[(\dot{\psi} - c_0)^2 - \frac{\sin^2 \psi}{r^2} \right] + \sigma \quad (\text{S.27})$$

$$\dot{z} = \sin \psi \quad (\text{S.28})$$

$$\dot{A} = 2\pi r \quad (\text{S.29})$$

Here we have added equation (S.29) in order to determine the membrane area A . Additionally the following relationship between the variables exists:

$$\mathcal{H} = \frac{kr}{2} \dot{\psi}^2 - \frac{kr}{2} \left(\frac{\sin \psi}{r} - c_0 \right)^2 + t \cos \psi - \sigma r = 0 \quad (\text{S.30})$$

4 Asymptotic solution to shape equations

Numeric integration of the shape equations (S.24)-(S.29) requires a defined initial state. We take the initial state to be the point $s = s_2$ defined as being located far away from the hole. Luckily, an analytic solution to the shape equations in this asymptotic limit can be found. Below, we determine the asymptotic solution to the shape equations for large values of s . It is assumed that $\psi \rightarrow 0$ for $s \rightarrow \infty$. First, equation (S.26) is expanded to first order in ψ :

$$\ddot{\psi} \approx -\frac{\dot{\psi}}{r} + \frac{\psi}{r^2} + \frac{t\psi}{kr} \quad (\text{S.31})$$

Next, t is found to zero'th order in ψ from either equation (S.27) or (S.30):

$$t \approx \left(\frac{k}{2} c_0^2 + \sigma \right) r \quad (\text{S.32})$$

Upon insertion of equation (S.32) into (S.31), the following differential equation is found:

$$r^2 \ddot{\psi} + r \dot{\psi} - \psi \left[1 + \frac{r^2}{\lambda^2} \right] = 0 \quad (\text{S.33})$$

with $\frac{1}{\lambda^2} = \frac{c_0^2}{2} + \frac{\sigma}{k}$ and λ being a characteristic length scale in the asymptotic limit. Equation (S.33) is identified as the modified Bessel differential equation and the relevant solution is (using $ds = dr$):

$$\psi\left(\frac{r}{\lambda}\right) = C \cdot K_1\left(\frac{r}{\lambda}\right) \quad (\text{S.34})$$

-where C is a constant and K_1 is a modified Bessel function of the second kind and order one. By differentiation of the solution in equation (S.34), $\dot{\psi}$ can be found as $\dot{\psi}(p) = \frac{C}{\lambda} (-K_0(p) - \frac{K_1(p)}{p})$ with $p = \frac{r}{\lambda}$.

5 Shape equations with dimensionless variables

The equations defining the membrane shape can be reformulated using dimensionless variables. This eliminates the parameters k, c_0 from the shape equations and makes it simpler to map the possible membrane configurations when fewer input variables are needed. The following characteristic quantities emerge:

Length scale: $l_c = \frac{1}{c_0}$
Tension: $\sigma_c = kc_0^2$
Line tension: $\tau_c = kc_0$
Energy: $E_c = k$

Dimensionless versions of the previously defined variables, as denoted with $\tilde{\cdot}$ can now be used to reformulate the shape equations and the Hamiltonian:

$$\dot{\tilde{r}} = \cos \psi \quad (\text{S.35})$$

$$\dot{\tilde{\psi}} = \tilde{u} \quad (\text{S.36})$$

$$\dot{\tilde{u}} = -\tilde{u} \frac{\cos \psi}{\tilde{r}} + \frac{\sin \psi \cos \psi}{\tilde{r}^2} + \tilde{t} \frac{\sin \psi}{k\tilde{r}} \quad (\text{S.37})$$

$$\dot{\tilde{t}} = \frac{1}{2} \left[(\dot{\tilde{\psi}} - 1)^2 - \frac{\sin^2 \psi}{\tilde{r}^2} \right] + \tilde{\sigma} \quad (\text{S.38})$$

$$\dot{\tilde{z}} = \sin \psi \quad (\text{S.39})$$

$$\dot{\tilde{A}} = 2\pi\tilde{r} \quad (\text{S.40})$$

$$\tilde{\mathcal{H}} = \frac{\mathcal{H}}{kc_0} = \frac{\tilde{r}}{2} \dot{\tilde{\psi}}^2 - \frac{\tilde{r}}{2} \left(\frac{\sin \psi}{\tilde{r}} - 1 \right)^2 + \tilde{t} \cos \psi - \tilde{\sigma} \tilde{r} = 0 \quad (\text{S.41})$$

using the definitions: $\tilde{r} = rc_0$, $\tilde{z} = zc_0$, $\dot{\tilde{r}} = \frac{dr}{ds}$, $\dot{\tilde{\psi}} = \dot{\psi}c_0$, $\tilde{t} = tkc_0$, $\dot{\tilde{t}} = \dot{t}kc_0^2$, $\tilde{\sigma} = \sigma kc_0^2$, $\tau = \tau kc_0$, $\tilde{A} = Ac_0^2$. The analytic solution (S.34) in the asymptotic limit becomes:

$$\psi\left(\frac{\tilde{r}}{\tilde{\lambda}}\right) = C \cdot K_1\left(\frac{\tilde{r}}{\tilde{\lambda}}\right) \quad (\text{S.42})$$

with $\frac{1}{\tilde{\lambda}^2} = (\frac{1}{2} + \tilde{\sigma})$. Equation (S.21) and (S.22) for the Gaussian curvature becomes in dimensionless units:

$$\frac{k_G}{k} = \alpha = (1 - \dot{\tilde{\psi}}_1) \frac{\tilde{r}_1}{\sin \psi_1} - 1 \quad (\text{S.43})$$

$$\tilde{t}(\tilde{s}_1) = \tilde{t}_1 = \tilde{\tau} \quad (\text{S.44})$$

The Lagrangian of equation (S.10) becomes in dimensionless units:

$$\tilde{\mathcal{L}} = \frac{\mathcal{L}}{kc_0} = \frac{1}{2} \left(\dot{\tilde{\psi}} + \frac{\sin \psi}{\tilde{r}} - 1 \right)^2 \tilde{r} + \tilde{t}(\dot{\tilde{r}} - \cos \psi) + \tilde{\sigma} \tilde{r} \quad (\text{S.45})$$

and the energy functional corresponding to equation (S.11) becomes:

$$\tilde{S} = \frac{S}{k} = \int_{\tilde{s}_1}^{\tilde{s}_2} \tilde{\mathcal{L}} d\tilde{s} - \alpha \cos \psi|_{\tilde{s}_1}^{\tilde{s}_2} + \tilde{\tau} \tilde{r}_1 \quad (\text{S.46})$$

6 Procedure for solving shape equations

We solve the shape equations in dimensionless variables, since only $\tilde{\tau}$ and $\tilde{\sigma}$ needs to be specified in this case. Note that although we work in a constant area ensemble, the membrane area is not an input to the numerical solver, but emerges from the result. The following procedure is used to obtain solutions to the shape equations (S.35)-(S.39):

1. Values for the tension parameters $\tilde{\sigma}$, $\tilde{\tau}$ are chosen along with a value of $\alpha(\text{target}) = k_G/k$.
2. A point \tilde{r}_2 , ψ_2 in the asymptotic limit is chosen ($\psi_2 \approx 0$).

3. Values of \tilde{t}_2 and $\dot{\psi}_2$ are determined from the asymptotic solution, as described above. The \tilde{z} -scale has an arbitrary offset and we choose $\tilde{z}_2 = 0$.
4. The shape equations (S.35)-(S.39) are integrated using the `ode45` solver in Matlab[1] with \tilde{s} decreasing from 0 to $-\tilde{s}_{\text{final}}$. The value of \tilde{s}_{final} is chosen sufficiently large to ensure all physical solutions are captured.
5. All solutions fulfilling equation (S.44) are stored during integration.
6. The above steps (2-5) are repeated using a 'shooting' procedure: The ratio α is determined from equation (S.43) and ψ_2 is scanned to identify the usable solutions that fulfill: $\alpha = \alpha(\text{target})$.

Note that the radius \tilde{r}_2 in the asymptotic limit cannot be chosen freely, but is subject to a constraint: $\tilde{r}_2 > \tilde{\tau}\tilde{\lambda}^2$. This comes from the fact that $\tilde{t}_2 > \tilde{\tau}$, i.e. integration has to start outside the edge of the hole.

Shooting is performed by varying, on a log-scale, the input angle ψ_2 in the asymptotic limit over 8 orders which is sufficient to exhaust the solution space. All solutions fulfilling the boundary in equation (S.44) are stored during integration and from these the solutions also fulfilling equation (S.43) are identified. Solutions containing self-intersecting membrane conformations are filtered out as being unphysical.

A fixed ratio of the two elastic constants $\alpha(\text{target}) = k_G/k = -0.75$ is used throughout. This value is within the span of available experimental estimates[2] and also agrees well with simulations[3].

As a reference state for the membrane energy in the curved state we use a flat membrane with the same area as the neck and without a hole.

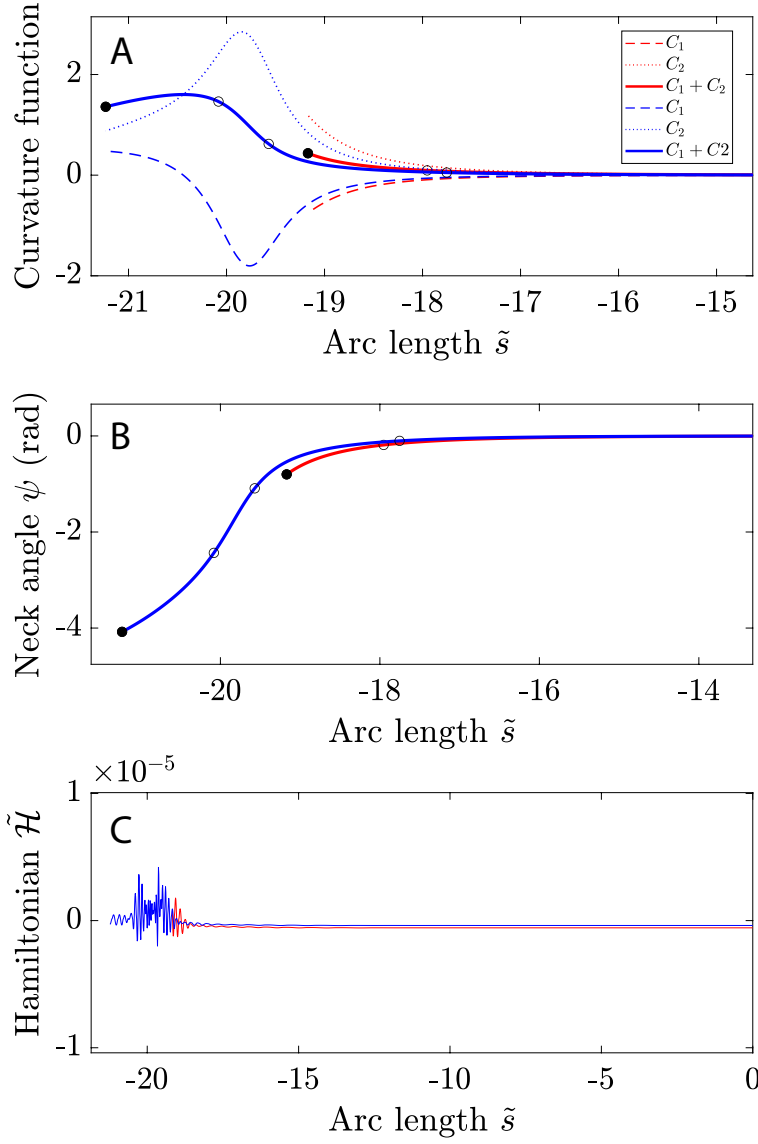


Figure S2: Plots of additional parameters related to the sample neck calculation using the line tension $\tilde{\tau}=1$ and membrane tension $\tilde{\sigma}=-0.1$. Red curves correspond to the weakly curved solution while blue correspond to the highly curved. Curvatures as function of the arc length \tilde{s} are shown in (A). Here $C_1 = 1/\tilde{R}_1$ and $C_2 = 1/\tilde{R}_2$ and $C_1 + C_2$ is the total curvature in dimensionless units. The neck angle ψ is shown as function of the arc length (B). Likewise, the Hamiltonian in equation (S.41) is shown as function of the arc length.

As a control and validation of the numeric solutions, the Hamiltonian function $\tilde{\mathcal{H}}$ of equation (S.41) is plotted against the arc length \tilde{s} in Figure S2C. It is found that $|\tilde{\mathcal{H}}| \ll 10^{-5}$ everywhere and confirming, to within the numerical precision, the prediction that $\tilde{\mathcal{H}}=0$, and validating the internal consistency of the shape equations (S.35-S.39). It is also found from figure S2A,S2B that the high curvature solution has a total curvature above one in the neck region and a neck angle $|\psi_1| > \pi$.

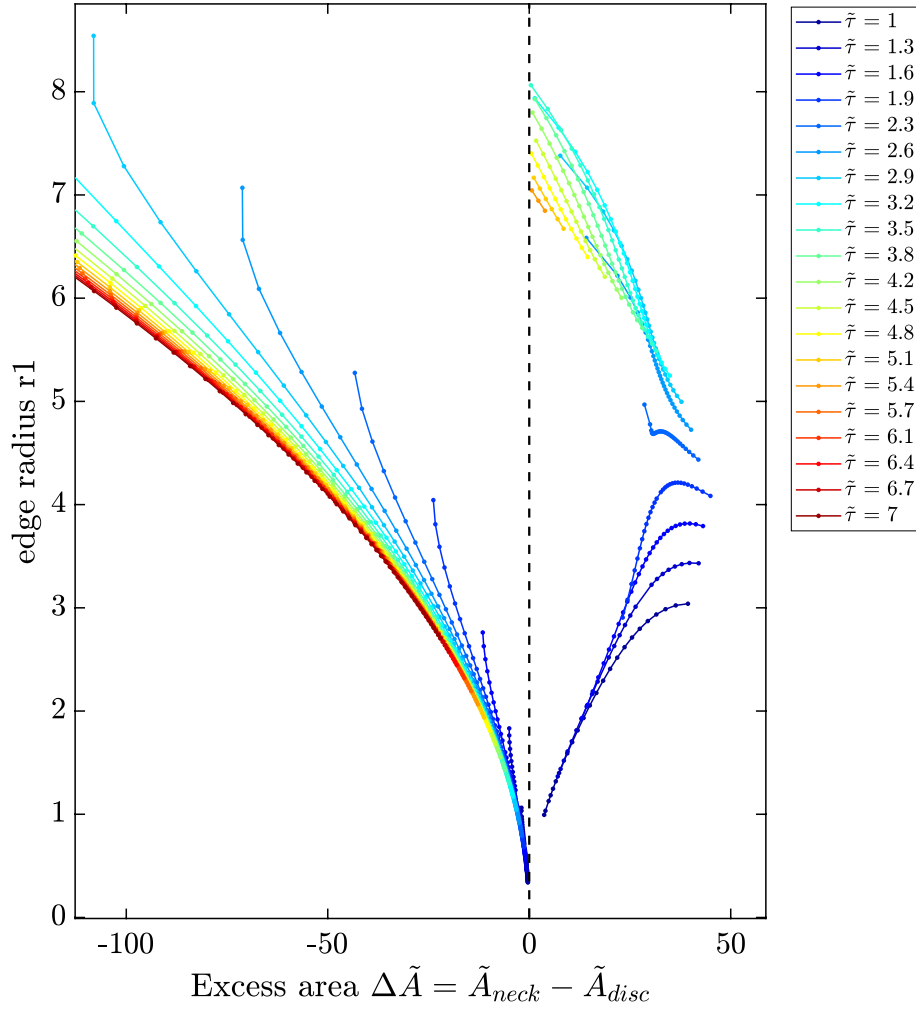


Figure S3: State diagram of the neck profiles in figure 5 in a representation of the radius r_1 of the free edge versus excess area. This figure can be compared with figure 5 which has a similar appearance, but shows the neck radius versus excess area.

References

- [1] J. R. Dormand, P. J. Prince, A family of embedded Runge-Kutta formulae, *Journal of computational and applied mathematics* 6 (1) (1980) 19–26.
- [2] D. Marsh, *Handbook of lipid bilayers*, CRC press, 2013.
- [3] M. Hu, J. J. Briguglio, M. Deserno, Determining the Gaussian curvature modulus of lipid membranes in simulations, *Biophysical journal* 102 (6) (2012) 1403–1410.