Supporting Information

Membrane Nanotubes Increase the Robustness of Giant Vesicles

Tripta Bhatia[1], Jaime Agudo-Canalejo[2,3], Rumiana Dimova[1] and Reinhard Lipowsky[1*]
[1] *Theory and Bio-Systems, Max Planck Institute of Colloids and Interfaces, Science Park Golm, 14424 Potsdam, Germany*
[2] *Rudolf Peierls Centre for Theoretical Physics, University of Oxford, Oxford OX1 3NP, UK*
[3] *Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802, USA* April 5, 2018

In this supporting information, we derive the equations (2) - (4) of the main text. The derivation of the mechanical tension in necklace-like tubes is supplemented by Figures S1 and S2.

Spherical and Cylindrical Membrane Segments

Consider a vesicle membrane with bending ridigity κ and spontaneous curvature m. The membrane is exposed to a certain pressure difference $P_{\rm in} - P_{\rm ex}$ between the pressures within the interior and exterior aqueous solutions and experiences the mechanical tension Σ. If a membrane segment has a shape with constant mean curvature and constant Gaussian curvature, the theory of curvature elasticity implies a shape equation that is polynomial in these curvatures. Examples for such shapes are spheres and cylinders. [1, 2] For a spherical membrane segment with mean curvature $M_{\rm sp}$, the polynomial shape equation can be written in the form [2]

$$P_{\rm in} - P_{\rm ex} = 2\hat{\Sigma} M_{\rm sp} - 4\kappa m M_{\rm sp}^2 \tag{S1}$$

with the total membrane tension

$$\hat{\Sigma} \equiv \Sigma + \sigma = \Sigma + 2\kappa m^2 \tag{S2}$$

which consists of the mechanical tension Σ and the spontaneous tension σ. For a cylindrical tube with mean curvature $M_{\rm cy}$, one obtains two shape equations that have the form

$$P_{\rm in} - P_{\rm ex} = 2\hat{\Sigma} M_{\rm cy} - 4\kappa\, M_{\rm cy}^3 \tag{S3}$$

and

$$P_{\rm in} - P_{\rm ex} = 4\hat{\Sigma} M_{\rm cy} - 16\kappa m M_{\rm cy}^2 + 8\kappa M_{\rm cy}^3 \,. \tag{S4}$$

Note that the total membrane tension $\hat{\Sigma}$, which includes the spontaneous tension $\sigma = 2\kappa m^2$, enters all three shape equations (S1), (S3), and (S4).

Mechanical Tension of Cylindrical Nanotubes

Next, consider a cylindrical nanotube that protrudes from a spherical mother vesicle with mean curvature $M_{\rm mv}$. Mechanical equilibrium between the tube and the mother vesicle imply the three shape equations (S3), (S4), and (S1) with $M_{\rm sp} = M_{\rm mv}$, from which one can eliminate the pressure difference $P_{\rm in} - P_{\rm ex}$ and the mechanical tension Σ. As a result, one obtains the mean curvature

$$M_{\rm cy} \approx m - \tfrac{1}{4} M_{\rm mv} \quad \text{for small} \quad M_{\rm mv}/|m| \,. \tag{S5}$$

When we eliminate the pressure difference by a combination of (S3) and (S4), we can express the mechanical tension in terms of the mean curvature $M_{\rm cy}$. Inserting the expression for $M_{\rm cy}$ as given by (S5), we obtain the mechanical tension [2]

$$\Sigma \approx \kappa\, m\, M_{\rm mv} = \frac{M_{\rm mv}}{2m}\sigma \approx -\frac{R_{\rm cy}}{R_{\rm mv}}\sigma \quad \text{for small} \quad R_{\rm cy}/R_{\rm mv} \tag{S6}$$

with the spontaneous tension $\sigma = 2\kappa m^2$. Because the radius $R_{\rm mv} = 1/M_{\rm mv}$ of the mother vesicle is much larger than the tube radius $R_{\rm cy} = 1/(2|M_{\rm cy}|) \approx 1/(2|m|)$, the absolute value of the mechanical tension, $|\Sigma|$, is much smaller than the spontaneous tension σ.

Mechanical Tension of Necklace-Like Nanotubes

Giant vesicles also form another type of nanotubes as provided by necklace-like tubes that consist of small spheres connected by closed membrane necks. [2, 3] The shape equation for each small sphere is given by (S1) with $M_{\rm sp} = M_{\rm ss}$ while the spherical mother vesicle satisfies the same equation (S1) with $M_{\rm sp} = M_{\rm mv}$. Eliminating the pressure difference from these two equations, we obtain the mechanical tension

$$\Sigma = 2\kappa m(M_{\rm ss} + M_{\rm mv}) - 2\kappa m^2\,. \tag{S7}$$

Particularly interesting shapes are provided by the limit shapes $L^{[N]}$ that consist of a spherical mother vesicle and necklace-like in-tubes with N small spheres which can form for negative spontaneous curvature $m < 0$. [3] Each small sphere of these in-tubes has the mean curvature $M_{\rm ss} = m$, which implies that the in-tubes have zero bending energy. The necks connecting two small spheres then satisfy the neck closure condition $M_{\rm ss} + M_{\rm ss} = 2m$ and the mechanical tension of the limit shapes $L^{[N]}$ is equal to

$$\Sigma = 2\kappa m(m + M_{\rm mv}) - 2\kappa m^2 = \frac{1}{mR_{\rm mv}}\sigma = -\frac{R_{\rm ss}}{R_{\rm mv}}\sigma \tag{S8}$$

as follows from the expression (S7) with $M_{\rm ss} = m$. Because the radius $R_{\rm mv}$ of the mother vesicle is again much larger than the radius $R_{\rm ss} = 1/|m|$ of the small spheres, the absolute value $|\Sigma|$ of the mechanical tension in (S8) is again much smaller than the spontaneous tension σ.

For a given membrane area, the limit shapes $L^{[N]}$ represent the equilibrium shapes of the tubulated vesicle for certain vesicle volumes or, equivalently, for certain values $A_{\rm nt}^{[N]} = N4\pi/m^2$ of the membrane area $A_{\rm nt}$ stored in the tubes. [3] Furthermore, each shape $L^{[N]}$ belongs to a whole branch of shapes, as illustrated by the four branches in Fig. S1. This figure displays the bending energy landscape $E_{\rm nt}$ for a necklace-like tube that grows as we reduce the volume of a GUV, the size of which is much larger than the width of the nanotubes. The deflation process decreases the membrane area $A_{\rm mv}$ of the mother vesicle and increases the area $A_{\rm nt}$ stored in the tube, for fixed total area $A = A_{\rm mv} + A_{\rm nt}$. The bending energy of the tubulated GUV is equal to $E_{\rm mv} + E_{\rm nt}$ where the bending energy $E_{\rm mv}$ of the mother vesicle is a monotonically decreasing function of $A_{\rm nt}$. Examples for the morphologies of the necklace-like tubes along several branches of the energy landscape are displayed in Fig. S2.

Inspection of the energy landscape in Fig. S1 reveals that the equilibrium shapes with the lowest bending energy $E_{\rm nt}$ are provided by short segments of the $[N]$-branches as obtained by slight deflation and slight inflation of the limit shapes $L^{[N]}$. Slight deflation of $L^{[N]}$ reduces the vesicle volume and increases the area $A_{\rm nt}$ of the necklace-like tubes until we reach the intersection point of the $[N]$-branch with the $[N+1]$-branch at tube area $A_{\rm nt} =$

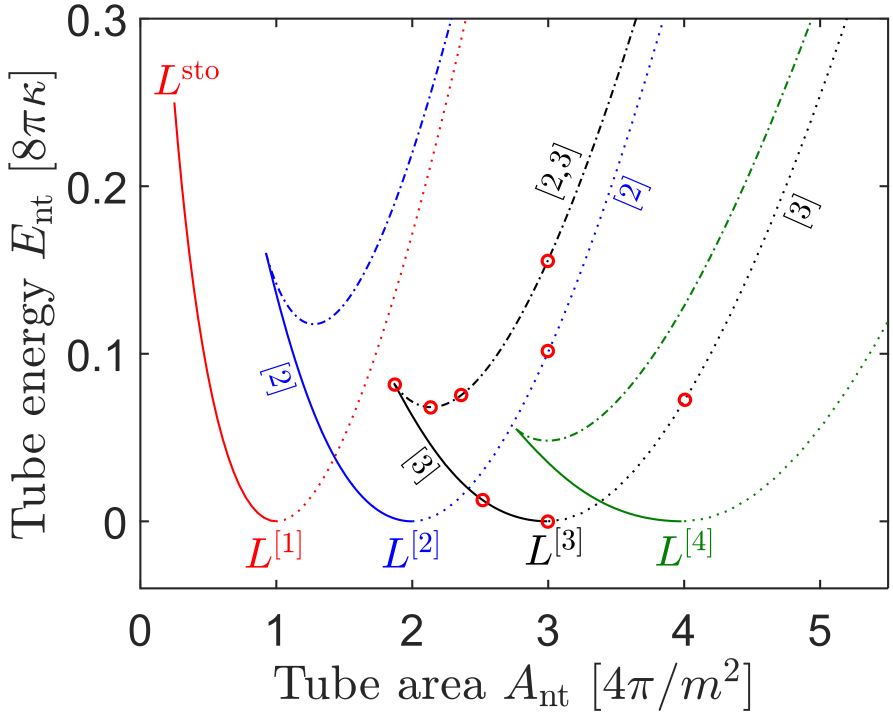

Figure S1: Energy landscape $E_{\rm nt}$ of a necklace-like nanotube protruding into a GUV as a function of membrane area $A_{\rm nt}$ stored in the tube. In accordance with the experiments, the size of the GUV is taken to be much larger than the width of the nanotubes. The energy landscape is built up from a discrete set of $[N]$-branches with $N \geq 1$. The different branches are distinguished by different colors. Each $[N]$-branch attains its energy minimum for the limit shape $L^{[N]}$ which consists of N small spheres with radius $R_{\rm ss} = 1/|m|$ and area $4\pi/m^2$. When we deflate the limit shape $L^{[N]}$, i.e., when we reduce the vesicle volume for fixed membrane area, we move towards larger values of the tube area $A_{\rm nt}$ along the dotted lines which represent necklace-like tubes with N small spheres of radius $R_{\rm ss} > 1/|m|$ and $N-1$ closed necks. When $R_{\rm ss}$ reaches the limiting value $R_{\rm ss} = 3/|m|$, the small spheres undergo a sphere-prolate bifurcation (outside of the figure). When we inflate the limit shape $L^{[N]}$, we move towards smaller values of $A_{\rm nt}$ along the full lines that represent necklace-like tubes with N bellies and $N-1$ open necks. The dash-dotted lines represent unstable necklace-like tubes corresponding to transition states $[N, N+1]$ between the (meta)stable $[N]$ and $[N+1]$ states. The red circles mark the nanotube morphologies displayed in Fig. S2.

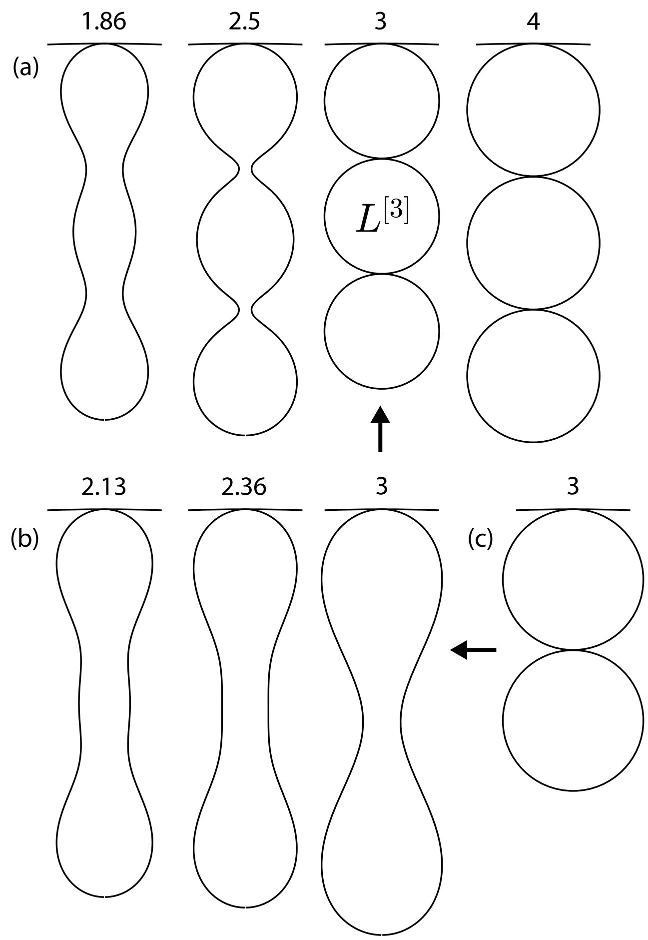

Figure S2: Morphologies of necklace-like nanotubes corresponding to the red circles in Fig. S1. The number at the top of each tube represents the tube area $A_{\rm nt}$ in units of $4\pi/m^2$: (a) Four shapes along the (meta)stable [3]-branch. The shape with $A_{\rm nt} = 1.86$ represents the bifurcation point between the [3]-branch and the unstable $[2, 3]$-branch of transition states. The shape with $A_{\rm nt} = 3$ is the limit shape $L^{[3]}$; (b) Three shapes along the $[2, 3]$-branch. The shape with $A_{\rm nt} = 2.13$ is located at the energy minimum of the $[2, 3]$-branch, the shape with $A_{\rm nt} = 2.36$ separates transition states with three from those with two bellies; and (c) Metastable shape of the [2]-branch that decays into the limit shape $L^{[3]}$ via the rightmost transition state in (b) with $A_{\rm nt} = 3$ (two arrows).

$(N + \epsilon_N)4\pi/m^2$ with a dimensionless coefficient ϵ_N that satisfies $0 < \epsilon_N < 1$. We now increase the tube area by

$$A_{\text{nt}} - A_{\text{nt}}(L^{[N]}) \equiv \delta_N \frac{4\pi}{m^2} \quad \text{with } 0 \leq \delta_N \leq \epsilon_N \tag{S9}$$

which leads to the small sphere curvature

$$M_{\text{ss}} = \frac{m}{\sqrt{1 + \delta_N/N}} \approx m\left(1 - \frac{\delta_N}{2N}\right) \quad \text{for large N.} \tag{S10}$$

The number N of small spheres is directly related to the length L_{nt} of the necklace-like nanotube via $L_{\text{nt}} = 2N/|M_{\text{ss}}|$ which implies

$$M_{\text{ss}} \approx m + \frac{\delta_N}{L_{\text{nt}}} \quad \text{for large } L_{\text{nt}} \gg R_{\text{ss}}, \tag{S11}$$

i.e., for a tube length L_{nt} that is large compared to the radius R_{ss} of the small spheres, with $0 < \delta_N < 1$. Using the general expression (S7) for the mechanical tension Σ of necklace-like tubes, we obtain

$$\Sigma \approx 2\kappa m \left(M_{\text{mv}} + \frac{\delta_N}{L_{\text{nt}}}\right) = \left(\frac{1}{mR_{\text{mv}}} + \frac{\delta_N}{mL_{\text{nt}}}\right)\sigma \tag{S12}$$

Therefore, the absolute value $|\Sigma|$ of a necklace-like tube is much smaller than the spontaneous tension σ if both the mother vesicle radius R_{mv} and the tube length L_{nt} are much larger than the small sphere radius $R_{\text{ss}} \approx 1/|m|$. Both conditions were fulfilled during the initial aspiration of all tubulated GUVs that were used to obtain the data in Fig. 5 of the main text. For these GUVs, the mechanical tension Σ can be neglected compared to the spontaneous tension σ for both cylindrical and necklace-like nanotubes as summarized by equation (2) of the main text.

Pressure balance during micropipette aspiration

When a GUV is aspirated by a micropipette, the vesicle membrane separates the aqueous solution into three distinct compartments: the interior solution with pressure P_{in}, the exterior solution with pressure P_{ex}, and the solution within the micropipette with pressure P_{pip}. Aspiration by a micropipette with radius R_{pip} leads to a membrane tongue with a spherical end cap that has the mean curvature $M_{\text{to}} \leq 1/R_{\text{pip}}$ which increases initially from the value $M_{\text{to}} = 1/R_{\text{ve}}$, the mean curvature of the nonaspirated mother vesicle, up to $M_{\text{to}} = 1/R_{\text{pip}}$ and then remains constant during further aspiration. Thus, it is useful to distinguish initial aspiration with $1/R_{\text{ve}} < M_{\text{to}} < 1/R_{\text{pip}}$ from prolonged aspiration with $M_{\text{to}} = 1/R_{\text{pip}}$.

The mean curvature $M_{\text{mv}} = 1/R_{\text{mv}}$ of the spherical mother vesicle satisfies the shape equation

$$P_{\text{in}} - P_{\text{ex}} = 2\hat{\Sigma}M_{\text{mv}} - 4\kappa m M_{\text{mv}}^2\,. \tag{S13}$$

as in (S1) with $M_{\text{sp}} = M_{\text{mv}}$. Likewise, the spherical end cap of the tongue is described by the shape equation

$$P_{\text{in}} - P_{\text{pip}} = 2\hat{\Sigma}M_{\text{to}} - 4\kappa m M_{\text{to}}^2 \tag{S14}$$

as in (S1) with $M_{\text{sp}} = M_{\text{to}}$ and the exterior pressure P_{ex} replaced by the pipette pressure P_{pip}. Substracting (S13) from (S14) and using the decomposition $\hat{\Sigma} = \Sigma + \sigma$ of the total membrane tension as in (S2), we obtain the relationship

$$P_{\text{ex}} - P_{\text{pip}} = [M_{\text{to}} - M_{\text{mv}}]\,[2\Sigma + 2\sigma - 4\kappa m(M_{\text{to}} + M_{\text{mv}})] \equiv P_{\text{el}} \tag{S15}$$

between the suction pressure $P_{\text{ex}} - P_{\text{pip}}$ and the elastic counter pressure P_{el}. With $M_{\text{to}} = 1/R_{\text{to}}$ and $M_{\text{mv}} = 1/R_{\text{mv}}$, the two equalities in (S15) become identical with equations (3) and (4) in the main text.

References

[1] Ou-Yang, Z.-C. & Helfrich, W. Bending Energy of Vesicle Membranes: General Expressions for the First, Second and Third Variation of the Shape Energy and Applications to Spheres and Cylinders. *Phys. Rev. A* **39**, 5280–5288 (1989).

[2] Lipowsky, R. Spontaneous Tubulation of Membranes and Vesicles Reveals Membrane Tension Generated by Spontaneous Curvature. *Faraday Discuss.* **161**, 305–331 (2013).

[3] Liu, Y., Agudo-Canalejo, J., Grafmüller, A., Dimova, R. & Lipowsky, R. Patterns of Flexible Nanotubes Formed by Liquid-Ordered and Liquid-Disordered Membranes. *ACS Nano* **10**, 463–474 (2016).