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Controlled shaping of lipid vesicles in a microfluidic diffusion chamber

M. Mally, B. Božič, S. Vrhovec Hartman, U. Klančnik, M. Mur, S. Svetina and J. Derganc

ESI: The stationary shapes of vesicles under gravity

1 Determination of the equilibrium vesicle shape

Under the influence of gravity the vesicles in the microfluidic chip sink to the bottom of the channels as they contain denser sucrose solution compared to the surrounding glucose solution. Depending on their volume relative to the membrane area and the difference between the areas of their membrane leaflets, their shape deviates from a shape of a sphere to a lesser or greater degree when resting on the substrate. The corresponding parameters that influence the vesicle shape, i.e. the relative volume (v) and the difference between the equilibrium surface areas of the membrane leaflets in relative units (Δa_0), are defined by the expressions

$$v = \frac{3V}{4\pi R_0^3}\,, \tag{SI1}$$

$$\Delta a_0 = \frac{\Delta A_0}{8\pi R_0 h}\,, \tag{SI2}$$

where V is the volume of the vesicle, R_0 is the radius of the sphere that has the same area as the vesicle (A), ΔA_0 is the difference between the equilibrium surface areas of the membrane leaflets, and h is the distance between the neutral surfaces of the outer and the inner leaflet.

The shape of a lipid vesicle is determined by the minimum of its mechanical energy. We analyze the shape of the lipid vesicle under the assumption that the vesicle volume and its area do not change. Therefore, the elastic energy of a closed bilayer is the sum of only two terms: (a) the bending energy term [1]

$$W_b = \frac{1}{2}k_c \int (C_1 + C_2 - C_0)^2 dA\,, \tag{SI3}$$

where k_c is the bending modulus, C_1 and C_2 are the principal curvatures, and C_0 is the spontaneous curvature, and (b) the area difference elasticity (relative expansivity) term [2]

$$W_{\mathrm{ADE}} = \frac{k_r}{2Ah^2}(\Delta A - \Delta A_0)^2\,, \tag{SI4}$$

where k_r is the non-local bending modulus and ΔA is the difference between the areas of the outer and the inner leaflets, which is equal to $h\int(C_1 + C_2)dA$.

In addition, in the case of the difference in solution densities ($\Delta\rho$) also the gravity has to be taken into consideration to describe the conformation of the vesicle that lies on the substrate. The corresponding gravitational potential energy can be conveniently expressed as [3]

$$W_g = \Delta\rho g \int Z dV \,, \tag{SI5}$$

where g is the gravitational field and Z is the vertical distance between the substrate and the point in the vesicle interior.

The minima of the vesicle mechanical energy correspond to stationary shapes. The shape of a flaccid vesicle is therefore obtained from the minimum of $W_b + W_{\rm ADE} + W_g$. The constraints in volume and area can be incorporated in the energy minimization by introducing the Lagrange multipliers (μ and λ). Thus the shape equation for the vesicle is obtained by minimizing the functional

$$\mathcal{G} = W_b + W_{\rm ADE} + W_g - \mu V - \lambda A \,. \tag{SI6}$$

The procedure for obtaining the vesicle shape from Eq. (SI6) is outlined in the following in some detail.

It is convenient to minimize the functional $\mathcal{G}$ separately with respect to ΔA, and for a given ΔA with respect to the vesicle shape [4, 5]. At equilibrium the derivative of the functional $\mathcal{G}$ with respect to the relative area difference equals zero

$$\left.\frac{d(W_b + W_{\rm ADE} + W_g)}{d\Delta A}\right|_{eq} = 0 \,. \tag{SI7}$$

Then the variation of the functional $\mathcal{G}$ with respect to the vesicle shape is performed at equilibrium. The variation of the relative expansivity term is proportional to the variation of the relative area difference, $\delta W_{\rm ADE} = (dW_{\rm ADE}/d\Delta A)\delta\Delta A$, since the relative expansivity term ($W_{\rm ADE}$) depends only on the relative area difference (ΔA). In order to perform the shape variation it is convenient to define a new parameter ν that represents the derivative of the relative expansivity energy with respect to ΔA:

$$\nu = -\frac{dW_{\rm ADE}}{d\Delta A} = -\frac{k_r}{Ah^2}(\Delta A - \Delta A_0) \,. \tag{SI8}$$

Using Eq. (SI8) the variation of the relative expansivity term ($\delta W_{\rm ADE}$) reads $-\nu\delta\Delta A$.

An axisymmetrical surface can be parametrized by the coordinates $R(S)$ and $Z(S)$ [6], where R is the distance between the symmetry axis and a certain point on the contour, and S is the arclength along the contour. The inclination of the contour is defined through the equation $\tan\psi = dZ/dR$, and the coordinates R and Z depend on the angle ψ through the equations $\dot{R} = \cos\psi$ and $\dot{Z} = \sin\psi$, where the overdots denote the derivatives with respect to S. In this parametrization the principal curvatures along the parallels and the meridians, and the mean curvature $[(C_1 + C_2)/2]$ can be expressed as $\sin\psi/R$ and $\dot{\psi}$, and $(\sin\psi/R + \dot{\psi})/2$. Accordingly, the parameters of the vesicle are given

by: $V = \pi \int_0^{S^*} R^2 \sin\psi dS$, which is the volume of the vesicle, $A = 2\pi \int_0^{S^*} R dS$, the area of the vesicle, and $\Delta A = 2\pi h \int_0^{S^*} R(\sin\psi/R + \dot{\psi}) dS$, the area difference, where S^* is the total length of the contour.

The angle ψ and coordinates R, Z are taken as three independent variables. The interrelation between them can be kept constant by introducing new Lagrange multipliers $\gamma(S)$ and $f(S)$, which represent the shear force in the radial and axial directions [7, 8]. The variation of the functional G can then be expressed for an axisymmetrical vesicle as

$$\delta\mathcal{G} = \delta \int_0^{S^*} \mathcal{L} dS \,, \tag{SI9}$$

where $\mathcal{L}$ is the Lagrange function:

$$\begin{aligned} \mathcal{L} &= \pi k_c R \left(\frac{\sin\psi}{R} + \dot{\psi} - C_0 \right)^2 - 2\pi\nu h(\sin\psi + \dot{\psi} R) + \pi\Delta\rho g R^2 Z \sin\psi \\ &\quad -\pi\mu R^2 \sin\psi - 2\pi\lambda R + \gamma(\dot{R} - \cos\psi) + f(\dot{Z} - \sin\psi) \,. \end{aligned} \tag{SI10}$$

The variation of the functional [Eq. (SI9)] with respect to all independent variables along the contour has to vanish ($\delta\mathcal{G} = 0$) [6], which leads to the system of differential equations

$$\ddot{\psi} = \frac{\sin\psi\cos\psi}{R^2} - \frac{\dot{\psi}\cos\psi}{R} + \frac{\Delta\rho g R Z \cos\psi}{2k_c} - \frac{\mu R \cos\psi}{2k_c} + \frac{\gamma \sin\psi}{2\pi k_c R} - \frac{f\cos\psi}{2\pi k_c R} \,, \tag{SI11}$$

$$\dot{\gamma} = \pi k_c \left(\left(\dot{\psi} - C_0\right)^2 - \frac{\sin^2\psi}{R^2} \right) - 2\pi\nu h\dot{\psi} + 2\pi\Delta\rho g R Z \sin\psi - 2\pi\mu R \sin\psi - 2\pi\lambda \,, \tag{SI12}$$

$$\dot{f} = -\pi\Delta\rho g R^2 \sin\psi \,. \tag{SI13}$$

The system of equations [Eqs. (SI11)-(SI13)] is solved by the shooting method. The integration is stopped at $\psi = \pi/2$. In the procedure for obtaining the vesicle shape the values of μ, λ, ν, the axial length, f and R at the bottom, and the membrane curvature at the top are found to fulfill the conditions of the chosen V, A, to fulfill the equilibrium condition [Eq. (SI8)], and to fulfill the conditions of the continuity of Z, γ, f and the meridian curvature at the equator.

The vesicle is treated as composed of units and can be described by the sum of the corresponding sections [9]. These sections have to possess the same Lagrange multipliers. The main vesicle body has a discoid shape (Fig. SI1). If we neglect the influence of gravity on the protrusion, its shape can be described by the string of spheres. In this case ($\dot{\psi} = 0$) the corresponding radii that fulfill the differential equations are

$$R_s = \frac{-\lambda + k_c C_0^2/2 \pm \sqrt{(\lambda - k_c C_0^2/2)^2 - 2\mu(\nu h + k_c C_0)}}{\mu} \,. \tag{SI14}$$

For the vesicle's equilibrium shape, influenced by gravity, the size of the small beads in the protrusion can be obtained by taking the minus sign before the root in Eq. (SI14). Therefore, in the procedure for obtaining the vesicle shape with a bead-like protrusion, at

chosen number of spherical beads also the contributions of volume, surface area and area difference of the protrusion to the volume, surface area and area difference of the vesicle have to be taken into consideration. With this theory, the shapes, characterized by a certain number of beads (n), with corresponding energies, can be calculated. At given equilibrium difference between the areas of the membrane leaflets, the conformation that has the lowest mechanical energy among the conformations with different n, corresponds to the stationary shape. At the border between two adjacent regions of stationary shapes the mechanical energy is continuous.

The shapes can also be determined approximately using a dome model as described in Sec. 2. Some characteristic shapes are shown in Fig. SI1. In experiments we frequently observe that the size of the first bead in the protrusion formation is fixed and therefore we can assume that the first neck closes up and the material transport is stopped. We hence

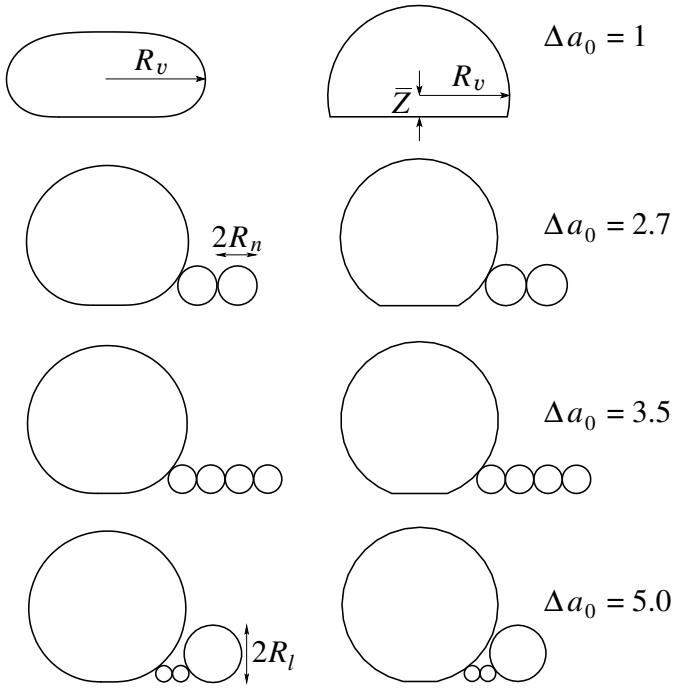

Figure SI1: The comparison of the shapes obtained by solving the differential equations (left column) and the shapes in the approximation of a dome (right column). The shapes at the bottom represent the vesicles where the first-formed bead (with the radius denoted by R_l) is the largest bead in the string. The radius of the main vesicle body at the equator, the distance of the equatorial plane from the substrate, and the radii of the small beads are marked by R_v, $\bar{Z}$, and R_n, respectively. The equilibrium differences between the areas of the membrane leaflets in dimensionless units for each row ($\Delta a_0 = \Delta A_0/8\pi h R_0$) are given in the panel. The other parameters, i.e. the relative volume (v), the vesicle size ($R_0 = \sqrt{A/4\pi}$), the difference in solution densities ($\Delta\rho$), the bending constant (k_c), and the non-local and local bending constants ratio (k_r/k_c), are taken to be 0.85, 8 μm, 18.5 kg/m^3, 10^{-19} J, and 3, respectively [10].

deduct the surface area and the volume of this first bead from the vesicle surface area and volume, and calculate the shape of the rest of the vesicle by minimizing the corresponding mechanical energy.

2 Predictions of the dome model

In a first approximation the shape of the main vesicle body resembles a dome of a geometrical sphere with a bottom cut off. If a protrusion is formed, its shape can be approximated with a string of spherical beads [9]. The dimensions of the vesicle with the protrusion, i.e. the radius of the main vesicle body (R_v), the distance of the equatorial plane from the substrate ($\bar{Z}$), the radius of the small beads (R_n) and their number (n), are determined in the minimization process considering the geometrical relations for the vesicle volume (V), its membrane area (A), and the difference between the surface areas of the membrane leaflets (ΔA) [11].

In Fig. SI1 some representative shapes for the vesicles are shown. It is evident that the difference between the shapes obtained by the approximation of a dome and the shapes obtained by solving the differential equations (Sec. 1) decreases with increasing difference between the surface areas of the membrane leaflets.

3 Finding the spontaneous curvature of the membrane with intercalated LPS

Intercalation of LPS molecules into the outer membrane leaflet changes the spontaneous curvature of the membrane (C_0). The expression for C_0 can be obtained from the expressions for the spontaneous curvatures of the outer and the inner membrane leaflet (C_{out} and C_{in}) [11]

$$C_0 = \frac{1}{2}(C_{\mathrm{out}} + C_{\mathrm{in}})\,. \tag{SI15}$$

We can write the outer spontaneous curvature as

$$C_{\mathrm{out}} = \frac{N_{\mathrm{POPC,out}} A_{\mathrm{POPC}} C_{\mathrm{POPC}} + N_{\mathrm{LPS}} A_{\mathrm{LPS}} C_{\mathrm{LPS}}}{N_{\mathrm{POPC,out}} A_{\mathrm{POPC}} + N_{\mathrm{LPS}} A_{\mathrm{LPS}}} \tag{SI16}$$

with N_i the number, A_i the surface area, and C_i the intrinsic curvature of either POPC or intercalating LPS molecules, and the curvature of the inner leaflet accordingly as

$$C_{\mathrm{in}} = -\frac{N_{\mathrm{POPC,in}} A_{\mathrm{POPC}} C_{\mathrm{POPC}}}{N_{\mathrm{POPC,in}} A_{\mathrm{POPC}}}\,, \tag{SI17}$$

where the minus sign accounts for the opposite orientation of the POPC molecules compared to that in the outer leaflet. Since LPS molecules are relatively large and their hydrophobic part not significantly curved, the preferred curvature of a LPS molecule can be assumed negligible when compared to the preferred curvature of POPC molecule ($C_{\mathrm{POPC}} \approx -0.022/\mathrm{nm}$

[12]), and the spontaneous membrane curvature is obtained in the form

$$C_0 = -\frac{N_{\mathrm{LPS}} A_{\mathrm{LPS}}}{2A} C_{\mathrm{POPC}} \tag{SI18}$$

where A is the membrane surface area.

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Video Captions

Movie 1: Reversible shape alterations between the equilibrium shapes of a vesicle at very low LPS concentrations (0.185 – 0.25 μg/ml): a vesicle with one outer bead (at 0.25 μg/ml), a vesicle without protrusions (0.22 μg/ml), and a vesicle with an inner bead (0.185 μg/ml).

Movie 2: Reversible transformation of the membrane shape at high LPS concentrations (5 μg/ml): formation of a string of beads after the addition of LPS to the microfluidic chip reservoir, and the shortening of the string of beads when the LPS molecules diffuse out of the microfluidic chamber.