Amplitude Hierarchy of Vesicle Shapes

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Abstract. Shapes of fluid lipid vesicles are governed by the bending elasticity of their membrane as described by the Area-Difference-Elasticity (ADE) model. These shapes can be quantified using a suitable modal representation of the vesicle contour. Prolate vesicles are characterized by a hierarchy in their shape amplitudes. Experimentally, we find an ordering of the amplitudes with mode number both in large (100 nm) as well as giant (10 μ m) unilamellar vesicles. Mean shapes are found only within the small energetically stable region of the prolate phase. Our study demonstrates that bending energy concepts may be quantitatively used on cellular length scales ranging from the size of organelles to the plasma membrane.

Key words: Vesicle shapes, Bending energy, Spontaneous curvature.

The morphology of fluid lipid vesicles is governed by the bending energy of their membrane [1, 2]. These vesicles form a bewildering variety of shapes [3, 4, 5] including prolates and oblates [6], pears and stomatocytes [7, 8], tubes and strings of beads [9, 10]. Even more exotic shapes like starfish [11] or self-adhering structures [12] can be understood within the framework of bending elasticity (and mutual contact potentials) of membranes. Bending elasticity of membranes is relevant for biological functions involving morphological transformations [13]. For instance, the vesicular transport of proteins between various organelles [14] involves budding and fission [10] of small satellite vesicles from their membranes of origin and final fusion with the target membranes. Usually these vesicles are covered with a protein coat [15].

Recently, a comprehensive theory of vesicle shapes, the Area-Difference-Elasticity (ADE) model, which includes the effects of bilayer elasticity, has been developed [16, 17]. Briefly, the shape of a vesicle is determined by minimizing the bending energy of its bilayer with respect to constraints on the area A and volume V of the membrane capsule. The energy scale is set by the bending modulus of the membrane κ . The vesicle size, which is characterized by the area equivalent radius $R_A = (A/4\pi)^{1/2}$, determines the length scale. Finally, the relevant shape parameters are the reduced volume $v = 3V/4\pi R_A^3$ and the effective spontaneous curvature $\bar{c}_0 = \bar{C}_0 R_A$, where $\bar{C}_0$ includes contributions from both spontaneous curvature and differential area between the monolayers. There are numerous quan-

Figure 1. Typical examples of extracted vesicle contours of A) phase-contrast video images and B) Cryo-TEM pictures. The algorithm used is detailed in [23]. Whereas analysis of TEM pictures is restricted to single snapshots only, video image analysis proceeds on a sequence of frames in real-time [24].

titative measurements of the bending modulus [18, 19, 20]. In contrast, vesicle shapes were so far classified experimentally mainly with respect to their symmetry [7, 21, 22]. A detailed analysis of vesicle shapes and a measurement of the effective spontaneous curvature was attempted only recently [23, 24]. In this short communication, we present an investigation of prolate shapes over more than two orders of magnitude in vesicle size. We monitor the shape details of giant unilamellar vesicles (GUVs) and large uni-lamellar vesicles (LUVs). The ADE model of vesicle shapes describes the morphology of 2-dimensional membrane sheets. Corrections to this theory due to the finite thickness of the bilayer D are smaller than the dominant contributions by a factor $(D/R_A)^2$ [17]. Thus, the ADE model is expected to be valid up to rather small curvature radii on the order of a few times the bilayer thickness. Indeed, we find that LUVs with a mean diameter of 100 nm quantitatively show the same scale-invariant shape characteristics to be detailed below than GUVs with diameters of a few 10 μm .

Shape contours were obtained by image analysis from maximal cross-sections of prolate vesicles. Whereas data on giant vesicles from video phase contrast microscopy are abundantly available in our laboratory [23, 24], measurements for large vesicles were done on original TEM pictures taken from [22]. In Figure 1, representative examples of extracted contours of the two cases are shown. These 2-dimensional curves may be written in terms of an arclength s which starts at one pole ($s = 0$) of the prolate vesicle under investigation and ends at the other

($s = s^*$). We take the direction of the polar axis to be $\hat{y}$ and the perpendicular direction to be $\hat{x}$. A convenient representation is

$$\psi(s) = \pi \frac{s}{s^*} + \sum_{n=1}^{\infty} a_n \sin(n\pi \frac{s}{s^*}), \quad (1)$$

where $\psi(s)$ is the angle between $\hat{y}$ and the outward-pointing normal to the curve. The overall length scale is set by s^* . The first term on the right describes a semicircular arc, i.e., a spherical vesicle shape. The coefficients $\{a_n\}$ parameterize deviations from the sphere. For shapes like prolates, which exhibit equatorial symmetry, the odd- n coefficients vanish. Thus, prolate shapes are characterized by the coefficients $a_{2n}(v, \bar{c}_0)$ alone. A spherical shape corresponds to $v = 1$. It is therefore clear that the coefficients a_{2n} will all be small when v is near unity. From minimization of the bending energy in this representation, it follows that these non-vanishing coefficients have the structure of a well-defined hierarchy [17, 23],

$$\begin{aligned} a_2 &= A_2 (1-v)^{\frac{1}{2}} + B_2(\bar{c}_0) (1-v) + O((1-v)^{\frac{3}{2}}), \\ a_4 &= B_4(\bar{c}_0) (1-v) + O((1-v)^{\frac{3}{2}}), \\ a_6 &= O((1-v)^{\frac{3}{2}}), \end{aligned} \quad (2)$$

where $A_2 = (135/64)^{1/2} \simeq 1.45$ and the coefficients B_2 and B_4 are linear functions of $\bar{c}_0$ with coefficients of order unity. It is a consequence of this structure that for v near unity $a_2 \gg a_4 \gg a_6 \dots$, a hierarchy which reflects the fact that modes of higher n correspond to shorter wavelengths and cost more bending energy. The leading amplitude, a_2 , is independent of $\bar{c}_0$ at lowest order, because this is the only contribution of order $(1-v)^{\frac{1}{2}}$ and is, therefore, entirely determined by the constraint on the reduced volume v . Only in next-higher order does the shape become dependent on $\bar{c}_0$. Prolate vesicles become unstable at a minimal, respectively, maximal value of the spontaneous curvature corresponding to the spinodals of the prolate phase. This leads to just a small band of allowed a_4 -amplitudes near zero. Indeed, we find this behavior for the mean amplitude of the fourth mode both with small and giant vesicles. As a side remark, we note that it was crucial for the development of our technique to measure spontaneous curvature to identify the hierarchical ordering of the shape amplitudes [23]. It is evident from Equation 2 that the amplitude a_4 is most appropriate for a determination of $\bar{c}_0$, whereas the reduced volume v may be obtained from the amplitude a_2 . In practice, Equation 2 is not used. The mapping $(\langle a_2 \rangle, \langle a_4 \rangle) \rightarrow (v, \bar{c}_0)$ is done numerically. It turns out that the hierarchical ordering persists even for shapes which deviate substantially from a sphere.

We performed statistical analysis of several tens-of-thousands of experimental contours of a large number of *single*, thermally fluctuating GUVs. Experimental shapes imaged by video phase-contrast microscopy were compared with high precision to theory. Generally, one finds that all defect-free prolate vesicles are located exclusively within the theoretical-allowed narrow a_4 -band near zero [23, 24]. The

mean amplitudes $\langle a_6 \rangle$ are zero within experimental error. Thus, all observable amplitudes obey the expected hierarchy.

For LUVs imaged by Cryo-TEM, we have to rely on an average of only a few shape contours obtained from *different* vesicles at comparable reduced volumes. We have collected contour data of 15 prolate LUVs with a range of leading amplitudes $\langle a_2 \rangle \in [0.3, 0.5]$ and corresponding reduced volumes $\langle v \rangle \in [0.88, 0.96]$. This leads to an allowed range of amplitudes $a_4 \in [-0.02, 0.02]$. Indeed, we find $\langle a_4 \rangle = 0.008 \pm 0.04/\sqrt{15} = 0.008 \pm 0.01$. Thus, although we can extract only coarse-grained information out of the static Cryo-TEM pictures, we conclude that the mean shapes of LUVs fulfil the amplitude hierarchy as well and fall into the expected a_4 -band within experimental error. We emphasize, that the limits on the amplitudes are quite restrictive. Theoretical (unstable) vesicle shapes with a_4 values, even of an order of magnitude outside the allowed region, still look visibly like smooth prolate vesicles.

To summarize, we have demonstrated that a particular feature of prolate vesicle shapes holds true over three orders of magnitude of vesicle size. This shows that bending energy concepts may be quantitatively used from the small cellular length scale of organelles up to the size of the morphological structures of the plasma membrane, e.g., exo- and edocytotic vesicles.

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