Fluctuating Vesicles of Nonspherical Topology

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We report the observation of phospholipid vesicles of high topology, exhibiting strong thermal fluctuations. By deeply affecting the global shape of the vesicle, these differ from the usual local thermal undulations of the membrane. They can be described as positional fluctuations of necks linking two nearby concentric membranes. Using boundary layer methods we determine the shape and elastic energy of the necks and corroborate this analysis by a numerical solution of the minimization problem. This approach leads to a qualitatively correct description of our experiments.

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Artificial vesicles are closed membranes made of a phospholipidic bilayer. They have been extensively studied both theoretically and experimentally when their shape is a deformed sphere 

\[ \text{Surfaces of genus } g = 1 \text{ (and very recently of genus } 2) \text{ were described theoretically} \ [2,3] \text{ and observed experimentally in our laboratory} [2,4]. \text{ For higher genus } g \text{ surfaces } (g > 2), \text{ obtained by adding } g \text{ handles to a sphere, no exhaustive theoretical calculations are available} [5]. \text{ We report here the first observation of fluid vesicles with } g > 2, \text{ exhibiting strong thermal fluctuations. These are vesicles of small volume to surface ratio, which look like two nearby concentric membranes connected by tubular links (referred to as "necks" in the following): these necks wander around the surface, their relative distance varying by a factor of 2 in certain cases (see Fig.}

![Fig. 1. Two snapshots (a few seconds apart) of two fluctuating vesicles of genus 2 (a) and 4 (b) with reduced volume \( v \approx 0.4 \) [7]. The necks are indicated by arrows. Notice the strong (> 20%) fluctuations in the interneck distances. Bars indicate 10 \( \mu m \).](image)

Our vesicles are prepared using a phospholipid purchased from Avanti Polar Lipids [6], following a standard procedure described in [4]. The observations are made at a constant temperature, above the chain melting temperature \( T_m = 43 \degree C \), so that the membrane can be considered as fluid. Vesicles of various shapes and sizes (roughly 10 \( \mu m \)) are observed by phase contrast microscopy, which shows a cut of the membranes perpendicular to the focal plane. Pictures are digitized using a charged-coupled-device camera coupled to a contrast enhancing video acquisition board, and saved on a video tape recorder or on a computer.

Vesicles can be geometrically classified according to their volume \( (V) \) to surface \( (S) \) ratio, namely their dimensionless reduced volume: \( v = 6\sqrt{\pi V}/S^{3/2} \). Indeed, in our experiments, their volume and surface remain constant. The limiting cases are \( v = 1 \), which corresponds to the sphere (which cannot fluctuate because it is the single surface of its class), and \( v = 0 \), which corresponds to an empty vesicle.

We have already observed [4] that the shape of vesicles with high reduced volume presents only small thermal fluctuations at equilibrium, as may be expected from a simple model based on the curvature elastic energy first introduced by Canham, Helfrich, and Evans [8]:

\[
\mathcal{E} = \frac{\kappa}{2} \int \int H^2 dS + \bar{\kappa} \int \int K dS.
\]

where \( \kappa \approx (10-20)k_BT \) [9] and \( \bar{\kappa} \) are the elastic mean and Gaussian curvature moduli. \( H = c_1 + c_2 \) and \( K = c_1c_2 \) are the local mean and Gaussian curvatures (\( c_1, c_2 \) being the principal curvatures) [10]. The high value of \( \kappa/k_BT \) is consistent with the experimental observations for quasishperical vesicles (\( v \approx 1 \)) that the undulation modes of the membrane perturbed the overall shape by only a few percent [9]. This observation holds in fact for most vesicles of genus 0 or 1. For higher genus and small enough reduced volume \( v \), however, the examples of Fig. 1 show that strong fluctuations of the global shape are observed.
To understand this unusual behavior, we determine the shape of one neck, from which we deduce its energy and the energy of interaction between necks. For the sake of simplicity, we consider the shape of a neck of (mean) radius $r$ linking two asymptotically flat parallel surfaces, and in order to have a finite volume, take square parallel pieces of membrane of size $L \times L$ with periodic boundary conditions (PBC). The system is thus equivalent to a neck connecting two identical tori or to a periodic 2D lattice of necks, with an elementary square cell of size $L \times L$.

The problem now is to find a shape minimizing the elastic energy [Eq. (1)] with PBC on this cell. Minimization of Eq. (1) leads to the Euler-Lagrange equation:

$$
\Delta H + 2(H^2 - K)H = 0.
$$

(2)

In the Monge representation where the deviation of the membrane from the $(x, y)$ plane is given by $\xi(x, y)$, the curvature $H$ is [8]

$$
H = \frac{\xi_{xx}(1 + \xi_y^2) - 2\xi_{xy}\xi_x \xi_y + \xi_{yy}(1 + \xi_x^2)}{[1 + (\nabla \xi)^2]^{3/2}}.
$$

(3)

We solve Eq. (2) by boundary layer methods [11], distinguishing between two regions where Eqs. (2), (3) have simple asymptotic forms.

**Outer solution.** — Far from the neck ($a < r < L/2$), the derivatives of $\xi$ are small: $\xi_x, \xi_y = O(a/L)$. To leading order in $a/L$ we may neglect the nonlinear terms in (3) and set $H \equiv -\Delta \xi$. We have thus to solve the usual biharmonic equation describing small fluctuations of a flat membrane:

$$
\Delta H = -\Delta^2 \xi = 0.
$$

(4)

The solution of Eq. (4) describing a neck and satisfying the PBC $H(x \pm L, y \pm L) = H(x, y)$ can be obtained by solving the electrostatic (Poisson) problem given by integration of Eq. (4):

$$
-H \equiv \Delta \xi = 4\pi \sigma = -4\pi q/L^2,
$$

(5)

with a point charge $q = -a/2$ at the position of each neck, and PBC. The constant charge density (curvature) $\sigma = -q/L^2$ on the right hand side of Eq. (5) enforces the neutrality of the equivalent infinite periodic system of charges, i.e., it avoids the divergence of $\xi$ at infinity. The solution of Eq. (5) with PBC is $(z = x + iy)$

$$
\xi_0(z) = \text{Re} \left\{ a \ln \left( \frac{2L}{\pi a} \right) \prod_{m > 0} \left[ 1 + \frac{(\sin \pi z/L)}{(\sin \pi m)} \right]^2 \right\}
$$

$$
-\frac{\pi a}{L^2} \frac{\pi a}{L^2}.
$$

(6)

**Inner solution.** — Near the neck ($a \leq r \leq L/2$), the nonlinear terms in $H$ cannot be neglected anymore $\xi_x, \xi_y$ are $O(1)$. However, the minimal surface (MS) solution $H = 0$ clearly solves Eq. (2). It is the catenoid: $\xi_0(r) = a \cosh^{-1}(r/a)$.

Since the asymptotic form ($r \gg a$) of the catenoid diverges logarithmically [$\xi_0(r) \sim a \ln 2r/a$], this inner solution matches to leading order with the outer solution $\xi_0(x, y)$ which also diverges logarithmically when $r \ll L/2 [\xi_0(r) \sim a \ln 2r/a]$. Hence, if we consider only the solution around the $(m, n) = (0, 0)$ site, a uniform leading order approximation to the shape of the neck is [11]

$$
\xi_{\text{unif}}(r) = \xi_0(r) + \xi_0(r) - a \ln(2r/a),
$$

(7)

which can be easily generalized to the whole lattice.

Therefore, on the scale of $a$, the inner solution is a minimal surface. We checked this result by a direct numerical minimization's procedure (Powell's method), see Fig. 2, which also indicates that the outer solution has almost constant curvature as predicted by Eq. (5).

To leading order, the elastic energy of the neck comes from the bending of the outer region:

$$
\mathcal{E} = \frac{\kappa}{2} \iint H^2 \, dS = 2[2\pi^2 \kappa(a/L)^2].
$$

(8)

This result is corroborated by the numerical calculations (see Fig. 3). There are of course corrections to $\mathcal{E}$ of $O((a/L)^4)$, see inset in Fig. 3 coming from higher order corrections to the shape of the neck.

We point out that the stabilization of a finite size neck $a \neq 0$ in Eq. (8) is due to the reduced volume constraint. Indeed, the distance between two membranes connected by a neck of radius $a$ is of order $2a(L/2) \simeq 2a \ln(L/a)$. The reduced volume $v \simeq 3\sqrt{2}\pi a/L \ln(L/a)$ thus fixes the relative size of the neck, $a/L$, and its energy, $\mathcal{E}$ [12].

Up to now, we have dealt with a single neck per elementary cell of the lattice. We now consider $N$ such necks on each cell. Since to leading order the outer problem is linear [Eq. (4)], the outer solution for $N$ necks is a superposition of $N$ single-neck outer solutions. That is also true for the inner solution for $N$ necks (the catenoid), as long as their inner regions do not overlap (otherwise, our axisymmetric inner solution is no more valid). If $n$ necks ($N \geq n \geq 2$) begin to overlap, the inner problem for these necks consists in determining a surface subtended by $n$ disconnected contours, with an asymptotic logarithmic behavior matching the outer solution. A result due to Schoen [14] states that the only MS of that kind is the...
FIG. 3. Log-log plot of the elastic energy versus \( a/L \). Points are the numerical results \( (E_n) \). Straight line is the leading order theoretical prediction, \( E_l \). The inset represents the deviation from that prediction \( (E_n - E_l) \) which scales as \( (a/L)^4 \) as expected (straight line).

The catenoid \( (n = 1) \): the new inner solution is thus not a MS anymore \( (H \neq 0) \), implying that the elastic energy of the system increases. The overlap of the inner regions of nearby necks is thus energetically unfavorable, leading to an effective repulsion.

Therefore, to leading order, the necks behave as a gas of free particles with a hard core repulsion whose range is \( l_c \approx \sqrt{a}L \) (given by the matching region of the preceding outer and inner solutions). At long range, \( l_c \ll l \approx L \), higher order corrections to the shape of the necks might lead to repulsive (or attractive) interactions, but these are expected to be much smaller than the hard core repulsion (and also much smaller than \( k_B T [O(\kappa(a/l)^4)] \)).

Although we did not model true vesicles, it is interesting to compare these results to our observations. For a vesicle of area \( L \times L \) and \( N \) necks, the typical distance \( l \) between two neighboring necks is \( l \approx L/\sqrt{N} \). These necks will fluctuate freely if \( l \gg \sqrt{a}L \), i.e., if \( N \ll 3\sqrt{2}\pi/v \ln(L/a) \). For \( v = 0.4 \), we get \( N \ll 20 \), which is the case in Fig. 1. The simplest elastic theory for membranes is thus consistent with the experimentally observed strong thermal fluctuations of the vesicular shape. These fluctuations are due to a near degeneracy of the ground state of these high genus low \( v \) vesicles. They are different from the ones considered in Ref. [3], which result from an exact degeneracy of the ground state of genus 2, \( v \sim 1 \) vesicles, a consequence of the conformal invariance of the Hamiltonian (1).

Finally we emphasize the generality of our approach: We restricted ourselves to the case of two stacked membranes connected by \( N \) necks, which is adequate for Fig. 1. In fact we observed topologically more complicated vesicles, which can be described as many concentric nearby vesicles, connected by necks (see Fig. 4). Previous observations of such a topology had already been reported [16]. We can model these configurations by a periodic lattice (in the \( x, y \) directions) of \( M \) membranes connected by \( N \) necks. Let us consider for instance the simplest case \( M = 3 \), \( N = 2 \) (see Fig. 5). Far from the necks (outer solution), we solve Eq. (4) using the electrostatic analogy. The upper \( (u) \) and lower \( (l) \) parts are surfaces of almost constant mean curvature, as obtained previously. The middle \( (m) \) surface is obtained as the electrostatic potential of an infinite lattice, which elementary cell contains one +\( q \) and one -\( q \) charge. As a consequence, it is a surface of asymptotic zero curvature (a plane).

The inner solution for each neck is a catenoid if they are far enough one from another: in this case they do not interact, for the same reason as before. As they get closer, the elastic energy will remain constant if there exists a MS of genus 0 [17] which matches with the three preceding surfaces \( u, l, \) and \( m \). But the only MS of genus 0 are the plane and the catenoid [14]. Therefore the inner solution for two nearby necks is not a MS, and the elastic energy increases: we conclude that the necks repel.

FIG. 4. Fluctuating vesicle of very high genus; arrows indicate position of necks; bar indicates 10 \( \mu \)m.

FIG. 5. Doubly periodic stack of 3 membranes connected by 2 necks as obtained numerically with SURFACE EVOLVER [13] by imposing a volume constraint. One has to add a fourth fictitious planar membrane (not represented here) to define an inside and an outside. The area constraint comes from the imposed periodicity. \( a/L \) for each neck equals 0.04.
For the sake of simplicity, we considered up to now only simple configurations of membranes and necks, corresponding to genus-0 inner solutions, and we relied on a theorem of Schoen to prove their hard core repulsion. In fact there exist many MS with catenoidal ends sandwiching planar ones (e.g., the Costa surface and its derivatives) [18], with topological genus $g > 0$. They would correspond to more complicated configurations (with $M \geq 3$ and $N \geq 3$), but it is interesting to notice that they would then lead to the possible existence of bound states of $n$ necks linking $m$ membranes ($M \geq m \geq 3$, $N \geq n \geq 3$), i.e., nonfluctuating assembly of necks.

In conclusion, for sufficiently small density of necks and reduced volume, strong fluctuations in the position of the necks are observed, in agreement with our perturbative approach. In the limit where $M \to \infty$ and $N \to \infty$, one recovers the sponge phase. This approach might be an alternative to previous studies [19] which have dealt only with the triply periodic case.

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[7] Notice that since we only see a 2D section of the vesicle, the genus of the second vesicle ($g = 4$) has been estimated by setting a lower limit on the number of necks connecting the "inner" and "outer" membrane of the vesicle.
[10] The second term in (1) is a topological constant according to the Gauss-Bonnet theorem, therefore playing no role in the following discussion.
[12] From the analytical point of view, we look for a shape which minimizes the curvature energy given the periodic boundary conditions and the mean radius $a$ of the neck. In the numerical simulations, the condition on the neck radius is replaced by a volume constraint, which is taken care of by adding a pressure term to the free energy. Both procedures are equivalent.
[17] The genus of such unusual surfaces can be obtained as the number of handles one has to add to a punctured catenoid to get a conformally equivalent surface (see [18]).