

- sides, *J. Am. Chem. Soc.* **114**, 9188 (1992).
11. There is no reason to believe that 5 cm is an upper limit on the radius of curvature because we can use  $\mu$ CP to pattern a flat surface.
  12. E. Kim, A. Kumar, G. M. Whitesides, *J. Electrochem. Soc.* **142**, 628 (1995).
  13. G. P. López, H. A. Biebuyck, G. M. Whitesides,

- Langmuir* **9**, 1513 (1993).
14. Supported in part by the Office of Naval Research, the Advanced Research Projects Agency, and the National Science Foundation (PHY-9312572). It also used MRSEC Shared Facilities supported by the NSF under award DMR-9400396. R.J.J. acknowledges a scholarship from NSERC and J.L.W. ac-

knowledges a postdoctoral fellowship from the NIH (1-F32 GM16511-01). R.J.J. and J.L.W. thank Y. Lu and S. Shepard for their assistance with instrumentation at the MRSEC. We thank F. Frankel for photography (Fig. 1D and Fig. 3A).

21 March 1995; accepted 15 May 1995

## Observation of Stable Shapes and Conformal Diffusion in Genus 2 Vesicles

Xavier Michalet\* and David Bensimon

The observed equilibrium shapes of phospholipid vesicles of topological genus 2 (shapes with two holes) are found to be in agreement with theoretical predictions on the basis of a minimization of the elastic curvature energy for fluid membranes under the constraints of constant area, volume, and area difference (between the inner and outer layers of the membrane). For some particular geometrical characteristics, the shapes of the vesicles change continuously and randomly on a slow time scale (tens of seconds) and thus exhibit conformal diffusion. This phenomenon is a reflection of the conformal degeneracy of the elastic curvature energy. Its observation sets a limit (three constraints) on the number of physical constraints relevant to the determination of the shapes of vesicles.

Phospholipid vesicles are closed fluctuating bags (less than a few micrometers in size) whose surfaces are made of phospholipid molecules organized in a membrane, a fluid bilayer structure a few nanometers thick. These vesicles, also known as liposomes and used as such in a number of applications (from cosmetics to pharmacology), are often studied as a simplified model of the cell membrane. They are easily formed from a sample of phospholipids dissolved in water and can be observed by phase-contrast microscopy (1). Understanding the shapes of these vesicles is a crucial test for the validity of the various physical models that describe fluid membranes. This has been an active experimental field since the mid-1970s when the first models were proposed as an explanation for the various shapes of red blood cells that had been observed (2). These models are all based on an elastic description of the fluid (shearable) membrane, its energy being

$$E = \frac{\kappa}{2} \iint \left( \frac{1}{R_1} + \frac{1}{R_2} \right)^2 dS \quad (1)$$

where  $\kappa$  is the elastic modulus,  $R_1$  and  $R_2$  are the local principal radii of curvature of the membrane (3), and  $S$  is the differential surface element.

The shape of the vesicle can be determined by minimizing its elastic curvature energy under various physical constraints, among which its area  $A$  and volume  $V$  are

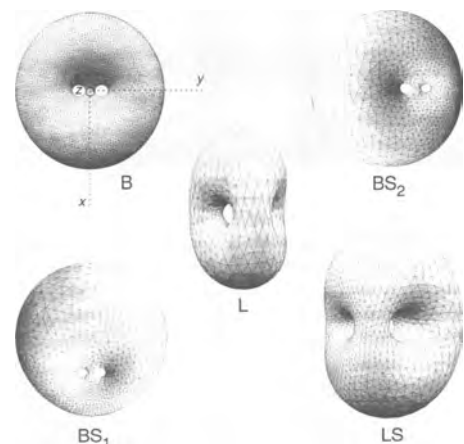
the most obvious. However, these are not sufficient to account for all the vesicle shapes that have been observed, for example, the variety of red blood cell morphologies. To account for those cases, other constraints have been introduced, such as a spontaneous curvature (which might result from a bilayer asymmetry) or a constraint on the area difference  $\Delta A$  between the inner and outer layers of the membrane [which could be constant as a result of a very low rate of lipid exchange (flip-flop) between the two layers], or a combination of both (4–6). Recently Jülicher, Seifert, and Lipowsky (JSL) pointed out that the number of relevant constraints could be determined to be three on the basis of the observation in vesicles of topological genus 2 or higher (that is, shapes

with two holes or more) of a new phenomenon, which they called conformal diffusion (7). Here, we report the observation of this phenomenon.

To understand the possible shapes of vesicles, it is helpful to first consider the invariance properties of their elastic curvature energy (Eq. 1). This energy is obviously invariant under translations and rotations. It is also invariant under dilations. Indeed, if we scale a vesicle (if it is spherical, its radius) by a factor  $\alpha$ , because its area increases as  $\alpha^2$ , its overall energy and shape remain unchanged. This invariance has an important consequence: The number of relevant geometrical characteristics is reduced by one, and adimensional parameters are defined as the reduced volume  $v$  and the reduced area difference  $\Delta a$  (8). Another more subtle property of the energy is its invariance with respect to sphere inversions. A sphere inversion is defined simply by choosing an inversion center  $O$  and “inverting” the distances (9). In contrast with the previous symmetries, the sphere inversions, although they may preserve the energy of a vesicle, may alter its shape. All of these transformations combined—translations, rotations, dilations, and inversions—form the group of three-dimensional conformal transformations, and, as just explained, the elastic curvature energy does not change as a result (that is, it is conformally invariant).

In particular, the state of minimal elastic curvature energy (the ground state) is conformally invariant. For vesicles of spherical

**Fig. 1.** Some of the absolute minimal shapes of topological genus 2 (shapes with two holes) for the elastic curvature energy (Eq. 1). These shapes can be obtained by special conformal transforms (SCTs) of the Lawson surface  $L$ ; SCTs are defined by  $\mathbf{I} \cdot \mathbf{B} \cdot \mathbf{I}$ , where  $\mathbf{B}$  indicates the translation of vector  $\mathbf{b}$ , and  $\mathbf{I}$  is the sphere inversion with its center at the origin of coordinates (7, 9). For the sake of commodity, we start from the “button” surface  $B$ . With  $\mathbf{b}$  parallel to the  $z$  axis,  $B[(v, \Delta a) = (0.68, 1.068)]$  can be continuously transformed into  $L[(v, \Delta a) = (0.67, 1.021)]$ , which has a threefold symmetry axis, and finally into a sphere with two infinitesimal handles (a “genus 2 sphere”)  $[(v, \Delta a) = (1, 1)]$ , going through surfaces of the  $LS$  kind shown here. With  $\mathbf{b}$  parallel to the  $x$  axis, it is possible to continuously reach a genus 2 sphere going through surfaces of the  $BS_1$  kind; the same is true for  $\mathbf{b}$  parallel to the  $y$  axis and surfaces of the  $BS_2$  kind. Shapes were calculated with the SURFACE EVOLVER program (17). This figure was inspired by a similar figure published in (7), which was based on a different numerical algorithm.



Laboratoire de Physique Statistique, Ecole Normale Supérieure, 24 rue Lhomond, 75231 Paris Cedex 5, France.

\*Present address: Laboratoire de Biophysique de l'ADN, Institut Pasteur, 25, 28 rue du Dr Roux, 75724 Paris Cedex 15, France.

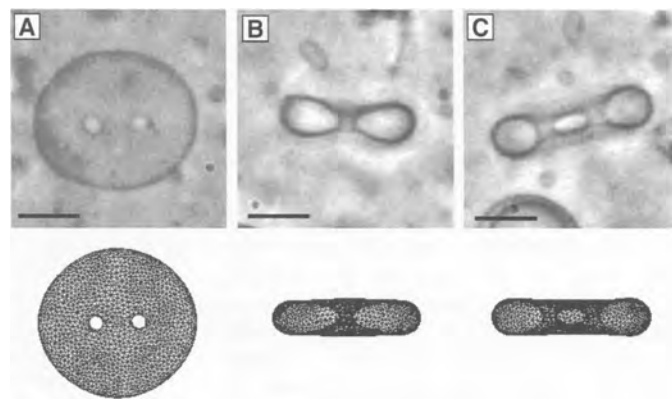
topology this ground state is the sphere, which remains a sphere under any three-dimensional conformal transformation. For the toroidal topology (surfaces of topological genus 1 or shapes with one hole) the Clifford torus, a particular axisymmetric doughnut, is an absolute minimal-energy shape (10). It is not unique. Upon inversion, it can be deformed continuously into a nonaxisymmetric, one-parameter family of minimal-energy tori known as the Dupin cyclides. The ground state of genus 1 shapes is thus degenerate. This degeneracy is, however, raised with the introduction of physical constraints, such as  $\nu$ , which selects one particular family member for each value  $0.711 < \nu < 1$  (11, 12).

For vesicles of topological genus 2, the ground state is the Lawson surface L (Fig. 1) and its conformal transforms (13). Because of the low symmetry of L, the minimal surfaces form in this case a three-parameter family  $\mathcal{W}$  (14). JSL pointed out that if two of these degrees of freedom are enough to satisfy the relevant constraints, then one degree of freedom remains to continuously deform the shape while preserving its elastic curvature energy and the constraints. This means, for example, that for certain values of  $\nu$  and  $\Delta a$  there exists a one-parameter family of vesicles with minimal energy. Because it costs them no energy, these vesicles are expected to randomly explore the various shapes of their family, a process called conformal diffusion. Because this process requires redistribution of fluid volumes, these vesicles will evolve on a long hydrodynamic time scale (tens of seconds), which can be differentiated from the fast (subsecond) thermal fluctuations of their membranes.

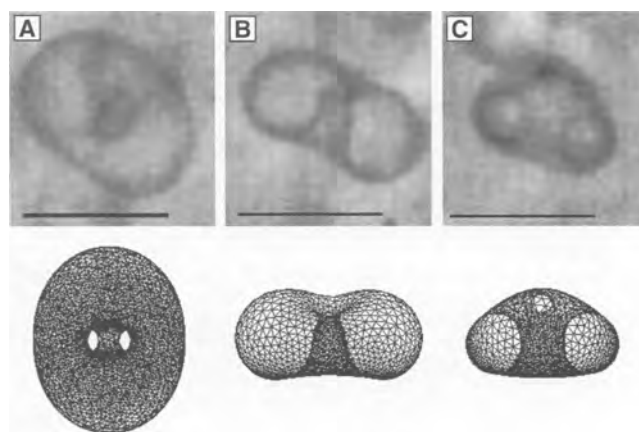
For geometrical constraints not reached by the degenerate ground state  $\mathcal{W}$ , a unique stable shape is expected (15). Our observations fully confirm these predictions. Figures 2, 3, and 4 present observations of vesicles of genus 2 with a unique stable shape. The “button”-like vesicle shown in Fig. 2 has been reported by our group (12). In Fig. 2 we compare its shape with a direct numerical minimization of the energy (Eq. 1) under the constraints  $\nu = 0.52$ ,  $\Delta a = 1.17$ . The good agreement between the observed and calculated shapes and the fact that the corresponding calculated elastic curvature energy is higher than the ground-state ( $\mathcal{W}$ ) energy support the arguments of JSL presented above. This agreement is further confirmed in Figs. 3 and 4, in which our observations are compared with a numerical minimization of Eq. 1 under appropriate constraints. Other stable shapes with different symmetries have also been observed, in agreement with the theoretical predictions of JSL.

Conformal diffusion is expected for vesicles whose geometrical parameters are

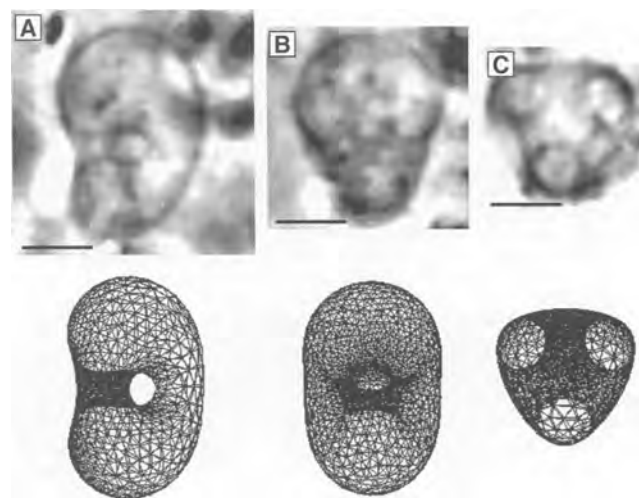
**Fig. 2.** Genus 2 button-like vesicle (18, 19): (A) top view, (B) side view, (C) front view. Scale bar, 10  $\mu\text{m}$ . For comparison, the numerically equilibrated surface that minimizes the elastic curvature energy  $E$  [with constraints  $(\nu, \Delta a) = (0.52, 1.17)$ ] is shown as a wire frame oriented in the corresponding directions with respect to the observer. These values do not belong to the  $\mathcal{W}$  domain calculated by JSL ( $E_{\text{calc}}/8\pi\kappa = 2.13 > E_{\mathcal{W}}/8\pi\kappa \approx 1.75$ ), which explains the stability of this vesicle. The symmetries of the vesicle are in agreement with the predictions of JSL for these  $(\nu, \Delta a)$  values.



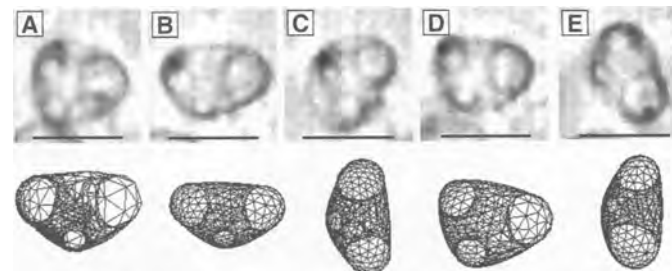
**Fig. 3.** Genus 2 vesicle: (A) top view, (B) side view, (C) front view. Scale bar, 10  $\mu\text{m}$ . In comparison, the numerical surface represents the SCT of the minimal button surface B of Fig. 1 with vector  $\mathbf{b} = (0, 0, 1.4/R_0)$ , that is, an intermediate surface between B and L, which lies on the boundary of the  $\mathcal{W}$  region (7) [ $(\nu, \Delta a) = (0.69, 1.029)$ ;  $E/8\pi\kappa \approx 1.75$ ;  $R_0 = \sqrt{A/4\pi}$ ].



**Fig. 4.** Genus 2 Lawson-like vesicle, with an approximate  $D_{3h}$  symmetry: (A) top view, (B) vertically tilted threefold symmetry axis, (C) fully tilted threefold symmetry axis. Scale bar, 10  $\mu\text{m}$ . We did not try to find its geometrical parameters. Corresponding views of the exact Lawson surface L of Fig. 1 are shown as an aid in visualizing the orientation of the vesicle with respect to the optical axis [ $(\nu, \Delta a) = (0.67, 1.021)$ ;  $E/8\pi\kappa \approx 1.75$ ].



**Fig. 5.** (A through E) Conformal deformation of a genus 2 vesicle (elapsed time between images,  $\sim 30$  s). Scale bar, 10  $\mu\text{m}$ . For comparison, numerical shapes belonging to the same one-parameter family of absolute minimal shapes are shown. They were obtained by a succession of SCTs that preserved  $(\nu, \Delta a)$ , as discussed in (7), starting from a  $(0.7/R_0, 0, 1.4/R_0)$  transformed button B [ $(\nu, \Delta a) = (0.67, 1.025)$ ;  $E/8\pi\kappa = 1.75$ ].





reached by the ground state  $\mathcal{W}$ . In Fig. 5 (top), successive images,  $\sim 30$  s apart, of the same vesicle indicate that it exhibits clear changes in shape. This sequence is compared with the numerically equilibrated surfaces within the  $\mathcal{W}$  family shown in Fig. 5 (bottom), all of which have the same geometrical parameters ( $v, \Delta a$ ) and of course the same minimal energy. The very long time scale of the observed deformation mode demonstrates that this is not a thermally excited bending mode, the time scale for which is typically less than a second (16). We conclude that this deformation is a zero-energy mode and thus is experimental evidence of conformal diffusion. We have generalized these observations to genus 3 vesicles, although the description of conformal diffusion for these vesicles is much more delicate.

## REFERENCES AND NOTES

- For a review, see R. Lipowsky, *Nature* **349**, 475 (1991).
  - P. B. Canham, *J. Theor. Biol.* **26**, 61 (1970); W. Helfrich, *Z. Naturforsch. Teil C Biochem. Biophys. Biol. Virol.* **28**, 693 (1973); E. A. Evans, *Biophys. J.* **14**, 923 (1974).
  - The value of the elastic modulus is  $\kappa \approx 10$  to  $20k_B T \approx 10^{-12}$  erg for most phospholipids (where  $k_B$  is the Boltzmann constant and  $T$  is temperature). It is very small but nonetheless many times greater than the thermal energy scale ( $k_B T/2$ ), which represents the energy attributed to each degree of freedom by thermal excitation. This explains why vesicles spontaneously fluctuate but do so within limits that enable the definition of stable shapes.
  - H. J. Deuling and W. Helfrich, *J. Phys. (Paris)* **37**, 1335 (1976).
  - L. Miao, B. Fourcade, M. Rao, M. Wortis, R. K. P. Zia, *Phys. Rev. A* **43**, 6843 (1991); U. Seifert, K. Berndt, R. Lipowsky, *ibid.* **44**, 1182 (1991); J. Käs and E. Sackmann, *Biophys. J.* **60**, 825 (1991); R. Podgornik, S. Svetina, B. Zeks, *J. Phys. II (Paris)* **3**, 631 (1993).
  - The two models, as well as a third that combines both of them, have the same sets of shapes as equilibrium solutions. They differ in the elastic curvature energy attributed to each surface and thus in their definition of the most stable shape for a given set of geometrical constraints. This has consequences in the prediction of shape changes, induced, for example, by temperature changes (5). The models also differ in their definition of the relevant physical characteristics of the vesicles; in one, the asymmetry of the membrane is described by the area difference constraint,
- $$\Delta A = D \int \left( \frac{1}{R_1} + \frac{1}{R_2} \right) dS \quad (2)$$
- where  $D$  is the bilayer thickness, and in the other by a spontaneous curvature, considered as a physicochemical characteristic.
- F. Jülicher, U. Seifert, R. Lipowsky, *Phys. Rev. Lett.* **71**, 452 (1993).
  - By a proper rescaling of the vesicle, the area constraint can be eliminated and the number of physical constraints relevant to the determination of its shape can thus be reduced by one. Hence, we may consider only rescaled constraints such as the reduced volume  $v = 6\sqrt{\pi}V/A^{3/2}$  and the reduced area difference  $\Delta a = \Delta A/4\sqrt{\pi D V A}$ , where  $V$  is the volume,  $A$  is the area of the surface,  $D$  is the thickness of the membrane, and  $\Delta A$  is the area difference between the layers of the membrane. With these definitions, for a spherical vesicle,  $v = 1$  and  $\Delta a = 1$ .
  - The image  $M'$  of any point  $M$  of the shape obtained by sphere inversion is defined by
- $$OM' = \frac{OM}{OM^2} \quad (3)$$
- where  $O$  is the inversion center.
- T. J. Willmore, *Total Curvature in Riemannian Geometry* (Horwood, New York, 1982).
  - Z.-C. Ou-Yang, *Phys. Rev. A* **41**, 4517 (1990); U. Seifert, *Phys. Rev. Lett.* **66**, 2406 (1991); Z.-C. Ou-Yang, *Phys. Rev. E* **47**, 747 (1993); B. Fourcade, *J. Phys. II (Paris)* **2**, 1705 (1992); F. Jülicher, U. Seifert, R. Lipowsky, *ibid.* **3**, 1681 (1993).
  - M. Mutz and D. Bensimon, *Phys. Rev. A* **43**, 4525 (1991); B. Fourcade, M. Mutz, D. Bensimon, *Phys. Rev. Lett.* **68**, 2251 (1992); X. Michalet and D. Bensimon, *J. Phys. II (Paris)* **5**, 263 (1995); X. Michalet, thesis (Université Paris VII, Denis Diderot, 1994).
  - L. Hsu, R. Kusner, J. Sullivan, *Exp. Math.* **1**, 191 (1992); R. Kusner, *Pac. J. Math.* **138**, 317 (1989); H. B. Lawson Jr., *Ann. Math.* **92**, 335 (1970).
  - The three parameters are related to the position of the inversion center, which is defined by three spatial coordinates. In the spherical case, because of the high symmetry of the sphere, all positions are equivalent. In the toroidal case, because of the symmetry axis, nonequivalent positions differ only in terms of their distance from the axis; the family of minimal shapes of genus 1 is thus a one-parameter family.
  - For equilibrium genus 2 surfaces that are not absolute minima of the elastic curvature energy  $E$ , there exist at least two orthogonal symmetry planes, which reduces the degeneracy of  $E$  [see (7, 14)].
  - J. F. Faucon, M. D. Mitov, P. Méléard, I. Bivas, P. Bothorel, *J. Phys. (Paris)* **50**, 2389 (1989); X. Michalet, B. Fourcade, D. Bensimon, *Phys. Rev. Lett.* **72**, 168 (1994).
  - SURFACE EVOLVER is a public domain program written by K. Brakke [*Exp. Math.* **1**, 141 (1992)].
  - The phospholipids used were mostly DMPC (dimyristoylphosphatidylcholine) and DC<sub>8</sub>PC [1,2-bis(10,12-tricosadinoyl)-sn-glycero-3-phosphatidylcholine] obtained in crystal powder form (Avanti Polar Lipids, Alabaster, AL). We prepared vesicles by hydration of a small amount of crystal, following a procedure described in (12).
  - We used a phase-contrast optical microscope (Nikon, Diaphot) coupled to a charge-coupled device camera (Sony). Pictures, which show a "cut" (that is, roughly the contour) of the vesicles in the focal plane, are recorded on video tape or further analyzed on a personal computer. The size of the vesicles is on the order of 10  $\mu$ m, and their topology is determined by comparison of their different contours (which can be successively observed on a time scale of some minutes because of the slow Brownian rotation of the vesicles) to numerical models of genus 2 surfaces calculated with the SURFACE EVOLVER program (17).
  - We thank F. Jülicher, U. Seifert, and R. Lipowsky for stimulating discussions, V. Croquette for help with the digital processing of the pictures, and K. Brakke for the use of SURFACE EVOLVER.

24 February 1995; accepted 26 May 1995

## Two-Dimensional Imaging of Potential Waves in Electrochemical Systems by Surface Plasmon Microscopy

Georg Flätgen, Katharina Krischer,\* Bruno Pettinger, Karl Doblhofer, Heinz Junkes, Gerhard Ertl

The potential dependence of resonance conditions for the excitation of surface plasmons was exploited to obtain two-dimensional images of the potential distribution of an electrode with high temporal resolution. This method allows the study of spatiotemporal patterns in electrochemical systems. Potential waves traveling across the electrode with a speed on the order of meters per second were observed in the bistable regime of an oscillatory electrochemical reaction. This velocity is close to that of excitation waves in nerve fibers and is far greater than the velocity of reaction-diffusion waves observed in other chemical systems.

All disciplines of science exhibit the phenomenon of spontaneous formation of spatiotemporal patterns from an originally homogeneous state (1). In chemical systems, spatial patterns may develop if an autocatalytic reaction is coupled to diffusion of the reacting species. These systems can be described by reaction-diffusion equations and have been intensively investigated (2). However, diffusion is not the only mechanism by which information can be passed on. For example, in nerve fibers, excitation states propagate as a result of electric transport phenomena. This is also true in electrochemical systems, where potential gradi-

ents are responsible for communication between different parts of the electrode. The coupling of the electrode kinetics to the bulk electrolyte, which is electroneutral, leads to phenomena that are qualitatively different from those expected in systems that can be described by reaction-diffusion equations (3). To date, the investigation of these patterns has been hindered by a lack of suitable experimental techniques. Here, we present images of two-dimensional (2D) potential waves in an electrochemical system. They were obtained with surface plasmon (SP) microscopy, a newly developed method for the study of rapid spatial variations at electrochemical interfaces.

In electrochemical systems, spatial patterns are composed of different voltage drops across the electrode-electrolyte interface

Fritz-Haber-Institut der Max-Planck-Gesellschaft, Faradayweg 4-6, D-14195 Berlin (Dahlem), Germany.

\*To whom correspondence should be addressed.