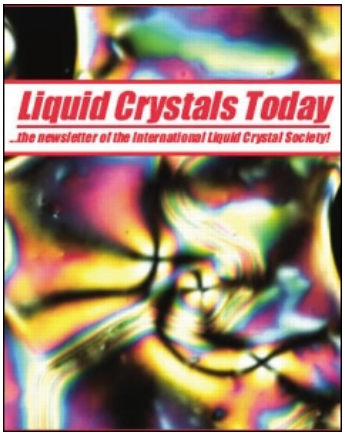

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Fluid bilayer vesicles Statistical physics of soft two-dimensional surfaces

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Fluid bilayer vesicles

Statistical physics of soft two-dimensional surfaces

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The combination of length-scales of several microns, time scales for dynamic fluctuations accessible to video frequency, and energy scales of a few k_BT all contribute to make vesicles a unique model system to study the statistical physics of soft surfaces in real space.

These vesicles are truly two-dimensional liquid-crystalline systems consisting of a single bilayer of phospholipid molecules. The lipid molecules owe their amphiphilic nature to the polar or charged headgroups and the two hydrophilic hydrocarbon chains. When introduced into an aqueous environment, these amphiphiles aggregate spontaneously into two mono-molecular layers held together by weak non-covalent forces due to the hydrophobic effect. As illustrated in figure 1, these membranes form large encapsulating 'bags' called vesicles, because open sheet-like configurations would involve a large energy along the hydrophobic edges. Even though the membrane is only a few nanometres thick, vesicles can reach macroscopic dimensions of up to 100 micrometres. Video microscopy studies have revealed an amazing variety of different shapes, among which shape transformations can be induced by changing parameters like the temperature or osmotic conditions. These membranes are so flexible that the thermal excitations of the membrane due to Brownian motion become visible in the microscope.

One of the original motivations to investigate vesicle shapes was the quest to understand the plethora of cellular configurations occurring in biology, in particular the shapes of red blood cells. The basic structural unit of both the plasma membrane of all cells and the membranes of cell organelles such as the golgi apparatus is a bilayer consisting of a mixture of different lipids and other molecules such as cholesterol. Proteins are incorporated into these membranes and

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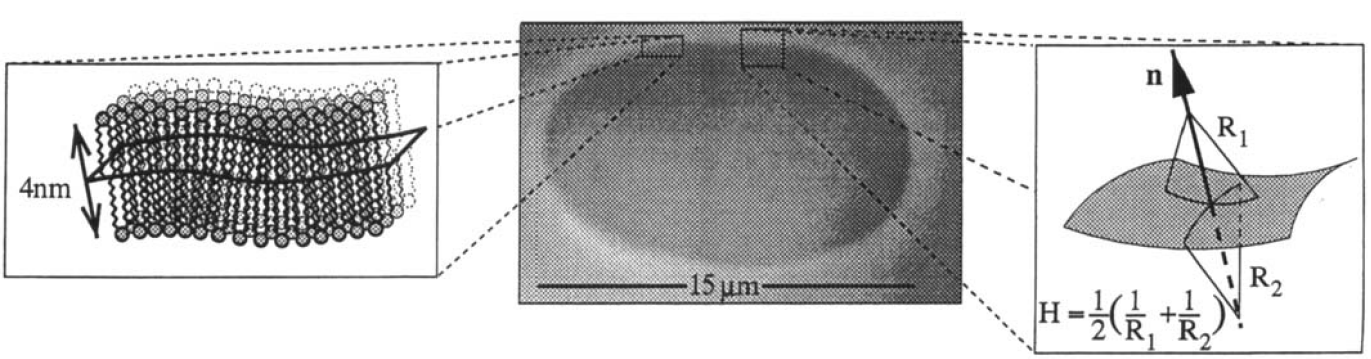

Figure 1. Video-microscopic image of a vesicle; sketches of the bilayer (left) and a surface with local mean curvature H (right).

very often attached polymeric networks endow them with additional structural stability.

Rather than studying the enormously complex systems of biomembranes, physicists searching for a well-defined model system have focused on the bilayer as just one element of this membrane. Complexity is further reduced by looking at a homogeneous one-component membrane. Even this apparently simple system has revealed a previously unexpected rich phenomenology which merits being studied in its own right. Thus, the study of fluid membranes has matured from its original motivation. The purpose of this article is to describe the appeal fluid membranes have acquired as one of the paradigmatic systems of soft matter research [1].

Curvature model

The basic theoretical model for fluid membranes and vesicles rests on a few physical properties of the bilayer membrane. The theoretical description on mesoscopic length-scales much larger than the bilayer thickness considers membranes as two-dimensional surfaces embedded in three-dimensional space. Because the bilayer is in its fluid (smectic A) state, there is no shear elasticity. Since it is energetically much cheaper to bend the membrane than to stretch or compress it, most shape transformations happen at constant area per lipid molecule. The number of lipid molecules in each monolayer is conserved because molecules neither flip between the layers nor dissolve into the

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aqueous solution on the typical experimental time scales of minutes. Even though membranes are permeable to water, the enclosed volume of a vesicle is typically a constrained variable, too. The reason lies in the presence of molecules to which the membrane is impermeable such as sugar molecules or large ions, that are either deliberately added to the aqueous solution, or inevitably present in low concentration due to impurities. In either case, any net transfer of water would generate an osmotic pressure that cannot be counter-balanced by the relatively weak forces arising from additional bending of the membrane.

Mathematically, the bending energy of such a bilayer membrane can be expressed by integrating the square of the mean curvature H over the closed vesicle surface, see figure 1. Therefore, the configurations of vesicles are fundamentally distinct from those of most interfaces since they are not determined by a surface tension but rather by bending elasticity. This fundamental difference is the reason for the great variety of non-spherical shapes of vesicles, in contrast to the characteristic spherical equilibrium shapes of simple liquids which are governed by isotropic surface tension.

Theoretically, vesicle shapes are thus determined as the minimum of the bending energy subject to the constraint of a fixed number of lipid molecules in each layer (which determines both the vesicle area and a preferred value of the total mean curvature) and a fixed enclosed volume. If either the bilayer or the liquid environments are asymmetric, a spontaneous curvature must be included. While the basic physical ideas of such a description were introduced in the early seventies by Canham, Helfrich and Evans [2], the systematic study of such a curvature model started less than ten years ago.

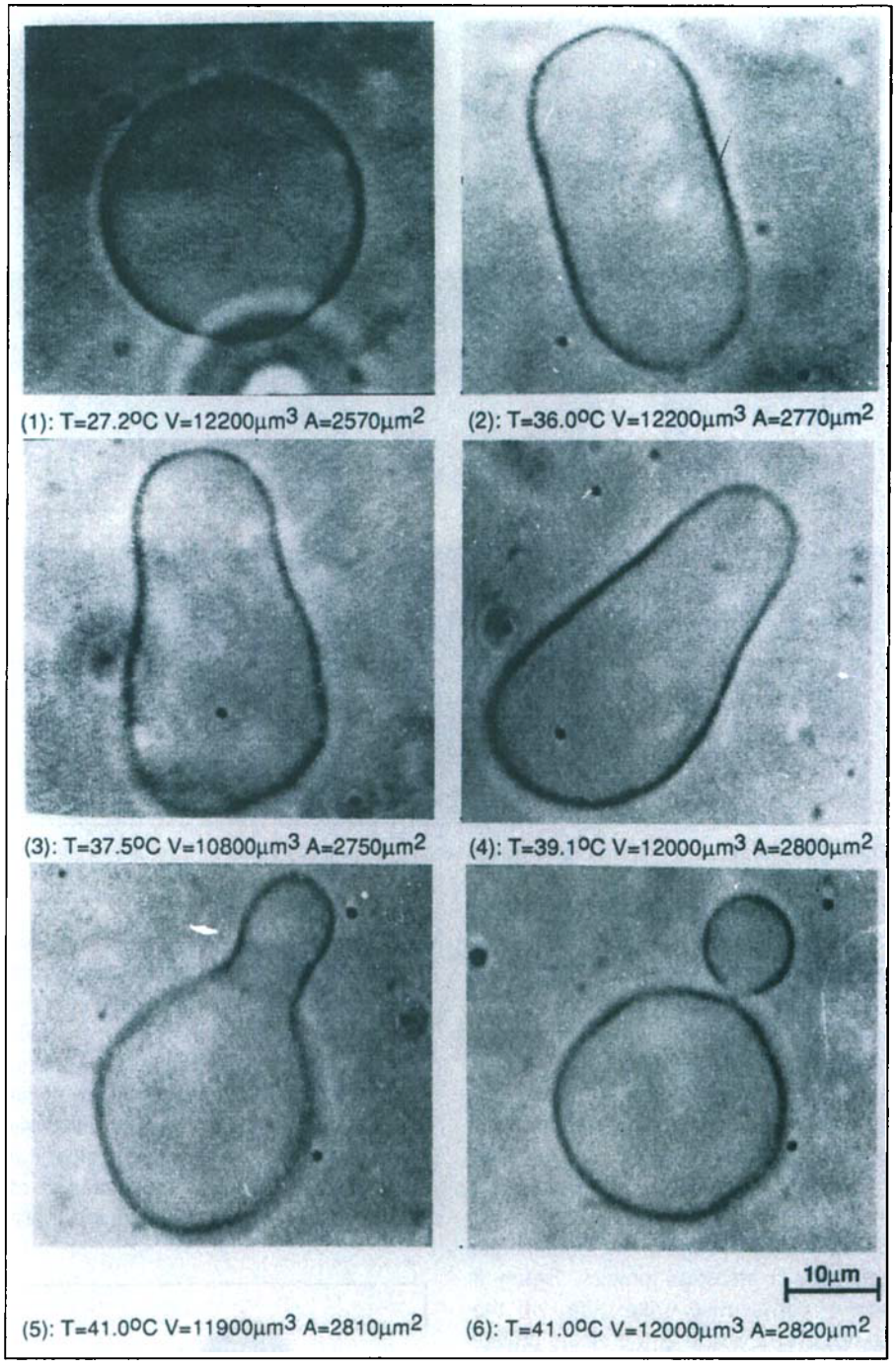

Figure 2. Temperature-induced budding transition (courtesy of J. Käs and E. Sackman).

Shapes and their transformations

The most prominent example of a shape transformation is the budding transition shown in figure 2, where the shape change of an initially spherical vesicle is recorded with video microscopy [3]. As the temperature increases, the sphere becomes a prolate ellipsoid. While this transformation may seem inevitable given that the thermal expansion of the area is much larger than that of the enclosed volume, the surprise is the occurrence of a pear-shape with broken up/down symmetry as the temperature is increased further. At even higher temperatures, the neck closes, resulting in two spherical compartments that sit on top of each other but remain connected by a narrow constriction. Despite the huge curvature in the neck region, the bending energy is still finite because the two principal curvatures have compensating signs. In all but the first and last shape, the video displays pronounced fluctuations which the still pictures, of course, cannot convey.

Based on the curvature model described above, we now understand this transformation. With increasing temperature, the ratio of volume to area becomes smaller. The theoretical studies predict that at some point shapes with a narrow neck become energetically favourable. In the language of phase diagrams, this transition is first order. Vesicles are finite systems and therefore metastability and

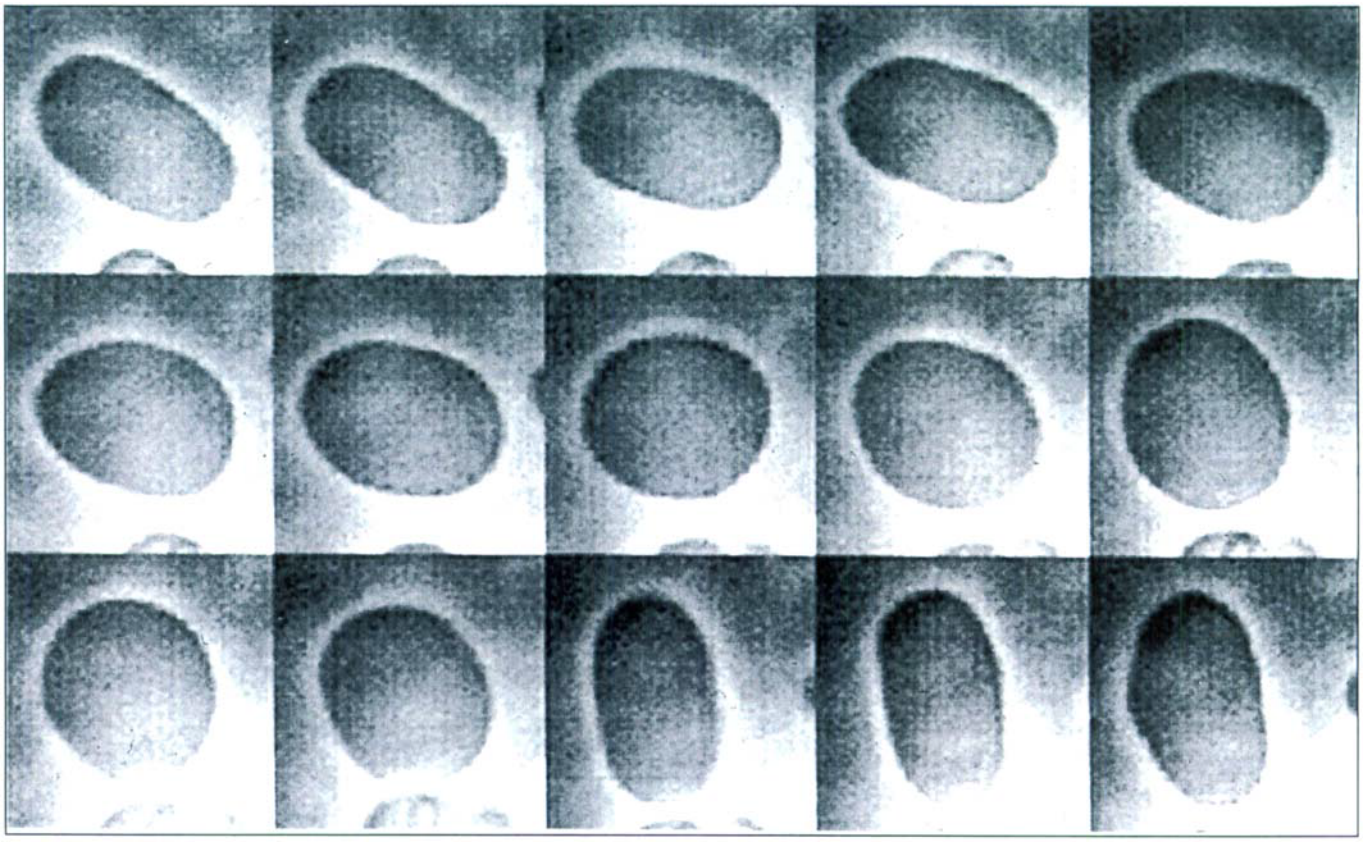

Figure 3. Consecutive snapshots of a vesicle exhibiting bistability between a prolate shape (with ellipsoidal contour) and an oblate shape (with circular contour).

hysteresis play a crucial role. Indeed, a careful analysis of the shape fluctuations around the prolate shape close to the spinodal, i.e. close to the point where the prolate loses its metastability, are in agreement with theoretical predictions based on a Landau-type argument for these fluctuations [4].

Another transition provides an intriguing macroscopic realization of bistability, which is one of the fundamental concepts in statistical physics. Figure 3 shows consecutive snap-shots of the contours of a vesicle at the oblate prolate transition. Since this transition is weakly first order with an activation energy of only a few k_BT, thermal fluctuations are strong enough to induce 'jumps' of the vesicles between the two minima. The vesicle at this transition is thus one of the rare examples in physics for which macroscopic bistability can be explored directly. Again, theoretical predictions based on the Kramers rate theory for diffusive motion in such a double well potential are in reasonable agreement with the experimental data [5].

Figure 4 shows a non-axisymmetric starfish vesicle computed by minimizing the bending energy on a dynamically triangulated surface and its experimentally observed counterpart [6]. It is surprising that this complex shape results from the minimization of even such a relatively simple energy as the curvature energy. The basic driving force for the formation of starfish shapes is a small volume to surface ratio and a slight surplus of molecules in the outer monolayer compared to those in the inner layer.

Even more fascinating from a mathematical perspective are vesicles of non-spherical topology. Usually, the topology of a given vesicle does not change because membranes have a fairly large rupture tension of about 3 erg cm^{-2} Still, vesicles with non-spherical topology

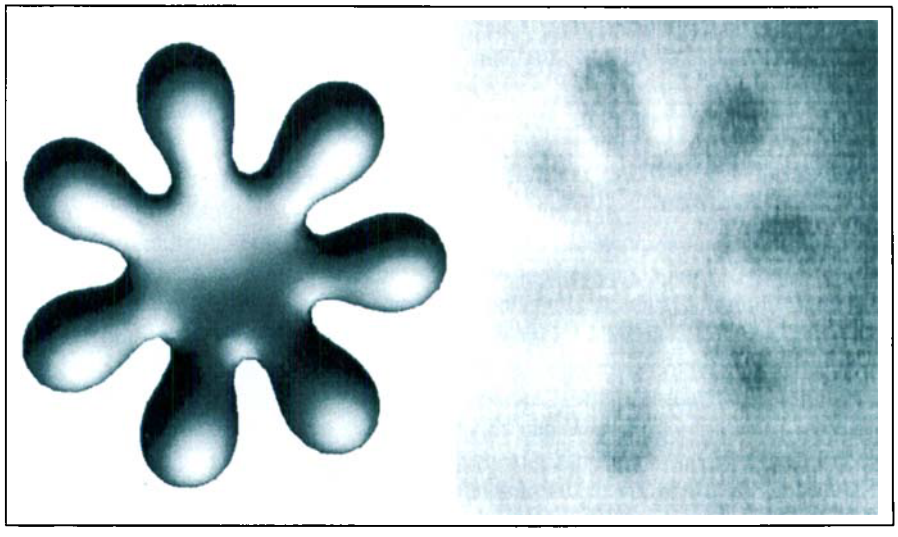

Figure 4. Seven-armed starfish.

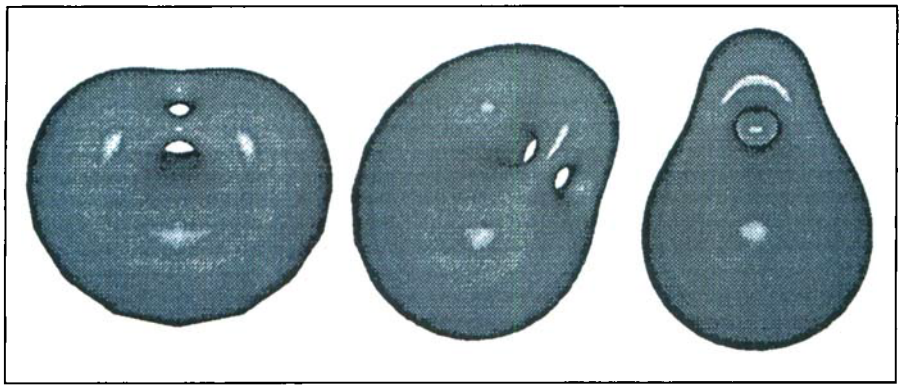

Figure 5. Vesicle with two handles along a conformal trajectory.

have been found in certain preparations. For shapes with at least two holes or handles, theory predicts that the shape of minimal energy is not unique but rather degenerate due to invariance of the curvature energy with respect to conformal transformations. Similar to rotational diffusion (which does not change the shape), this effect leads to a diffusion in shape space along a trajectory determined by a conformal transformation as shown in figure 5. It is quite satisfying that such a subtle theoretical prediction was verified experimentally soon after [7]. Besides the aesthetic appeal of rendering three-dimensional conformal transformations visible in the microscope, this experimental observation bears a fundamental significance. It confirms a certain variant of the curvature model, the area-difference–elasticity model, which pays special attention to the fact that the two leaflets of the bilayer do not exchange lipid molecules on short time scales [1].

In all of the studies discussed so far, the bending rigidity, which is the fundamental material parameter of the curvature energy, is of the order of 10–20 k_BT. This value is just in the range which makes the whole description meaningful. A much larger value would prevent the shapes from flickering, a much smaller value renders the concept of a mean shape with small fluctuations around it meaningless. In the latter regime, fluctuations are dominant and snapshots of the shape can exhibit large protrusions. The shape in this regime, as shown in figure 6, resemble branched polymer-like configurations [8]. It may be interesting to note that these configurations also arise in models studied within high energy physics. While homogeneous phospholipid membranes are too stiff to be a physical realization of these shapes, small amounts of impurities can soften the membrane substantially. Concomitant with a smaller bending rigidity is very often also a larger tendency to

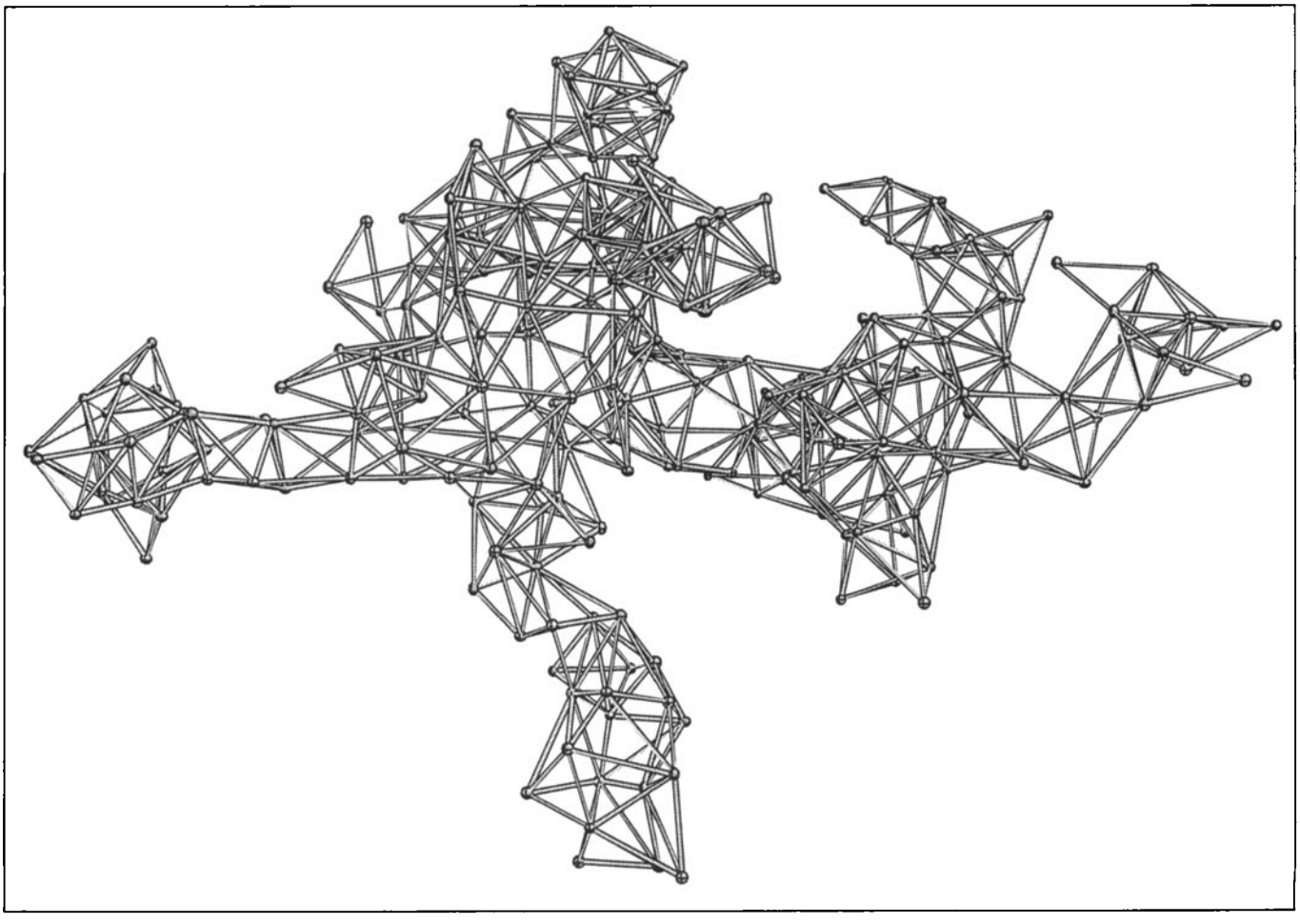

Figure 6. Vesicle in the branched polymer region (courtesy of G. Gompper).

change the topology, in which case the concept of a single vesicle with fixed area breaks down.

Non-equilibrium dynamics

The equilibrium aspects of isolated vesicle shapes and their fluctuations, as illustrated above with a few examples are well understood. To date, non-equilibrium aspects have been much less studied, not only because of their much greater challenge to theory, but also because of a lack of controlled experiments. Fortunately, new experimental developments have made well-defined non-equilibrium experiments possible.

The use of laser tweezers to excite membranes locally and study their global response has revealed a variety of unexpected phenomena. The best studied case so far is the so-called pearling instability of a cylindrical membrane shown in figure 7 [9]. The system is initially prepared as nearly straight, somewhat flaccid cylinders up to hundreds of microns long. Application of a localized laser spot on such a cylindrical vesicle produces a dramatic transformation to a stationary 'peristaltic' figure, i.e. a cylindrical shape whose radius at first varies roughly sinusoidally with distance from the trap. The shape transformation propagates outward from the laser trap, with a well-defined velocity. Longer excitation leads to a pearled state.

Theoretical modelling [10] exploits the phenomenological resemblance to the classical Plateau–Rayleigh instability of cylindrical fluid jets subject to surface tension. In the presence of bending rigidity, one needs a finite tension to initiate this static instability. The laser creates a local lateral tension by sucking lipid into the trap. This tension spreads rapidly along the cylinder since the membrane is nearly incompressible. After this very brief initial period, the cylinder is unstable towards long wavelength deformations whose growth law follows from the linearized hydrodynamics. The propagating character of the instability is captured by using the concept of marginal stability well known from front propagation in liquid-crystalline systems when a stable phase invades an unstable one in a controlled manner [11]. Theoretical predictions for both the front velocity and the wavelength of the pearling instability are in quantitative agreement with the experimental data. Less well explored and understood is the late stage dynamics of pearls moving along the tether, which could be caused by lateral gradients of tension.

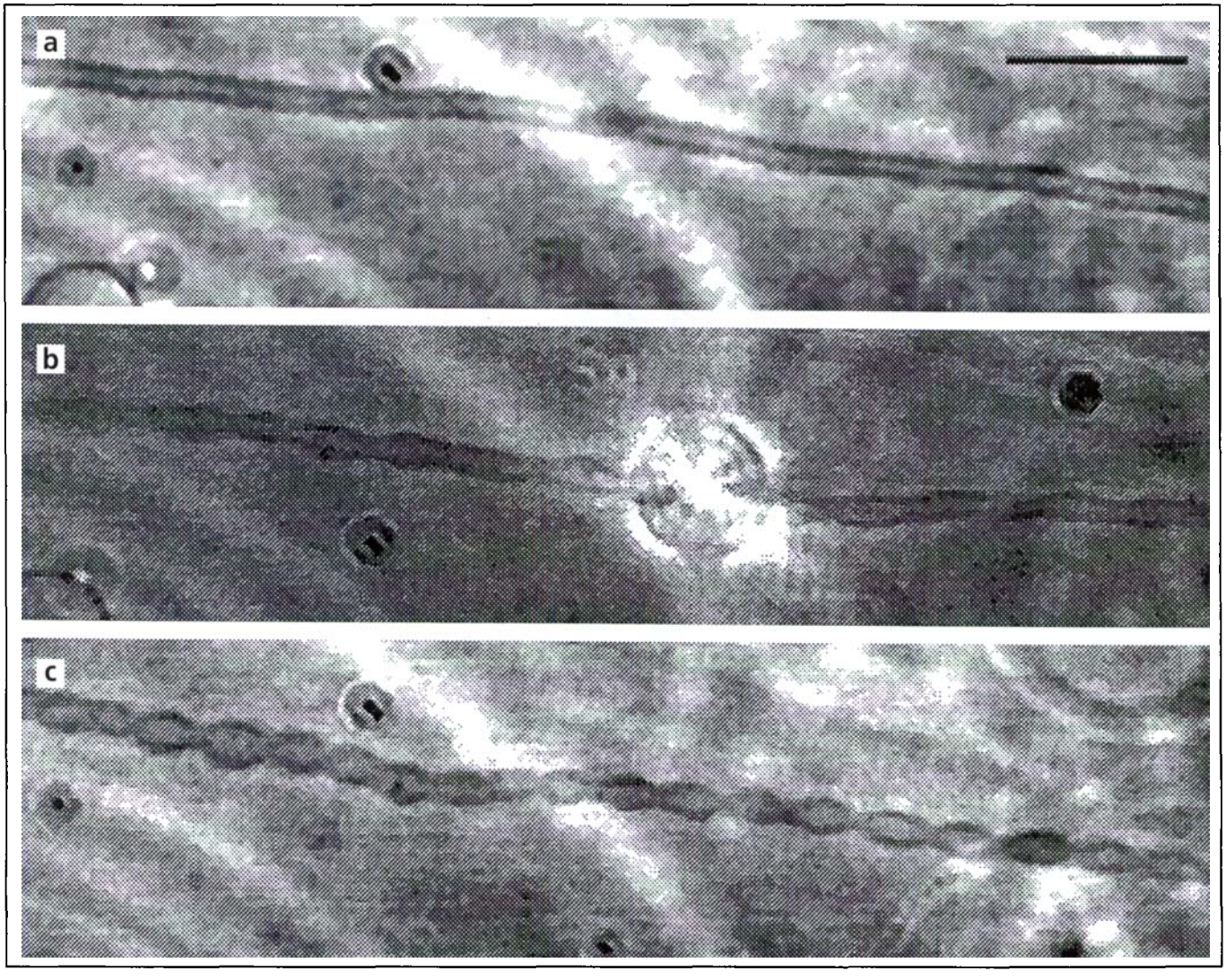

Figure 7. Pearling instability of a cylindrical vesicle (a) induced by the action of a laser (b) leading to a peristaltic mode (c) (courtesy of R. Bar-Ziv and E. Moses).

In other membrane geometries, applying the laser tweezers can cause quite different effects. A particularly spectacular phenomenon occurs if a laser is applied to an almost spherical vesicle in which a smaller vesicle is originally embedded [12]. At first, thermal fluctuations of the large vesicle cease because excess area is sucked into the trap. After shutting off the laser, these tense vesicles rupture and spontaneously expel vesicles caught in the interior. An intriguing explanation of this phenomenon is based on the assumption that the laser creates vesicles of sub-optical size. Osmotic pressure due to these vesicles is supposed to suck water between the two vesicles thereby pushing out the inner vesicle. While still somewhat speculative, this idea can be checked by looking for evidence of the small vesicles using scattering techniques.

Whereas the laser perturbs the membrane locally, hydrodynamic flow creates a global field to which a soft object like a vesicle can respond with a quite dramatic shape change. While a lot of studies have looked at the shape of liquid droplets in shear flow, similar work on vesicles is still scarce. Figure 8 shows the shape evolution of an initially oblate vesicle in modest shear flow [13]. The final state is a non-axisymmetric cigar-like stationary shape. Even though this shape is stationary, the fluid membrane is in perpetual tank-treading motion around this shape dragged along by the adjacent hydrodynamic flow through non-slip boundary conditions. This theoretical study leads to quantitative predictions for the inclination angle and the tank-treading frequency as a function of shear rate and volume to area ratio. A particularly intriguing result of the theory is that shear is a singular perturbation. Thus, even very small shear fields suffice to change a vesicle's shape dramatically. These predictions should now be tested in experiments.

Adhesion

For fundamental as well as practical reasons, studying the interactions between two vesicles or between a vesicle and a substrate are an important generalization of the statistical physics of single isolated vesicles described so far. Vesicles adhering to a substrate can be studied by reflection interference contrast microscopy [14]. This technique is sensitive enough to pick up both fluctuations of the bound part of the vesicle in the nanometre range and the shape of the contour where the membrane detaches from the substrate. The global shape of such a vesicle follows from the competition between bending energy and overall adhesion energy [1]. Several contributions enter the total adhesion energy. Van der Waals attraction and (unscreened) electrostatic interaction act on a scale of up to 10 to a 100 nanometres. Short-ranged interactions comprise hydration forces and protrusion forces on a molecular scale. Since membranes are a soft matter system, the confinement of fluctuations in the bound region leads to a substantial steric interaction that mimics a repulsive force [15]. A first step towards a systematic check of theoretical predictions by quantitative experiments has only recently become possible. Clearly, this technique has a lot of potential for elucidating the subtle interplay of entropy and energy that determine the interaction of membranes [16].

Prospects and applications

The fruitful interaction between theory and experiments over the course of the last ten years or so has led to a quantitative understanding of homogeneous fluid bilayer membranes. Based on such solid ground, systems more complex than the paradigmatic cases so far described can now be explored. This final section gives glimpses of further generalizations.

For a membrane consisting of two (or more) components the local composition is an additional degree of freedom that can couple non-trivially to the shape. If one of the lipids prefers a certain curvature, such a mixture will soften the rigidity of the membrane. Even if this effect does not lead to an instability, one of the components will aggregate in regions of high curvature. This effect is one of the simplest manifestations of a coupling between internal degrees of freedom (such as the composition) and external degrees of freedom (the shape) [1]. Likewise, polymers which are anchored in, or adsorbed to, the bilayer will have a similar effect [17].

Both vesicles that are softer due to several components and vesicles with attached polymers have interesting applications in cosmetics and pharmaceutics [18]. The flexibility of multi-component vesicles allows them, for example, to penetrate the intact skin thus delivering ingredients to subcutaneous regions and even to the circulatory system. For steric reasons, added polymers can prevent the sticking of vesicles to capillaries or blood components thus enhancing the lifetime of these drug carriers in the body.

For these biomedical purposes, a detailed understanding of the adhesive properties of membranes and their interactions is mandatory to ensure proper targeting. In addition to the unspecific adhesion discussed above, an important factor in biological adhesion is the formation of focal contacts between membranes due to receptor–ligand pairs that act as reversible lock–key molecules. The physics of such reversible bonds can be studied experimentally, if these molecules are integrated into membranes supported on a polymer-decorated substrate [19]. Ultimately, these experiments can help the development of more efficient biosensors. Along a similar route with technological potential the adhesion of membranes on laterally structured substrates has been used to investigate the coupling between nerve cells and semiconductors in order to obtain insight into signalling [20].

A technological application of studies on the non-equilibrium dynamics of vesicles as, for example, in shear flow, lies in the rheology of vesicular suspensions. Open problems concern the shape changes of vesicles in other flow geometries like capillary flow or elongational flow as well as the effective hydrodynamic interaction between several vesicles. Understanding these effects will be mandatory for bridging the gap between the mesoscopic characteristics of the membrane dynamics and the macroscopic rheological behaviour of such a suspension.

From the physicist's perspective, it will be gratifying to see how applying the time-honoured concepts of statistical physics illuminate the way to a better understanding of these more complex systems. Surely, the beauty and phenomenological complexity that such a simple system as the bilayer membrane in aqueous solution has revealed will resurface in even more guises in future studies. How productively the knowledge acquired on the physics of this paradigmatic soft matter system can ultimately be transformed into a better understanding of cell biology or technological advances, only time will tell.

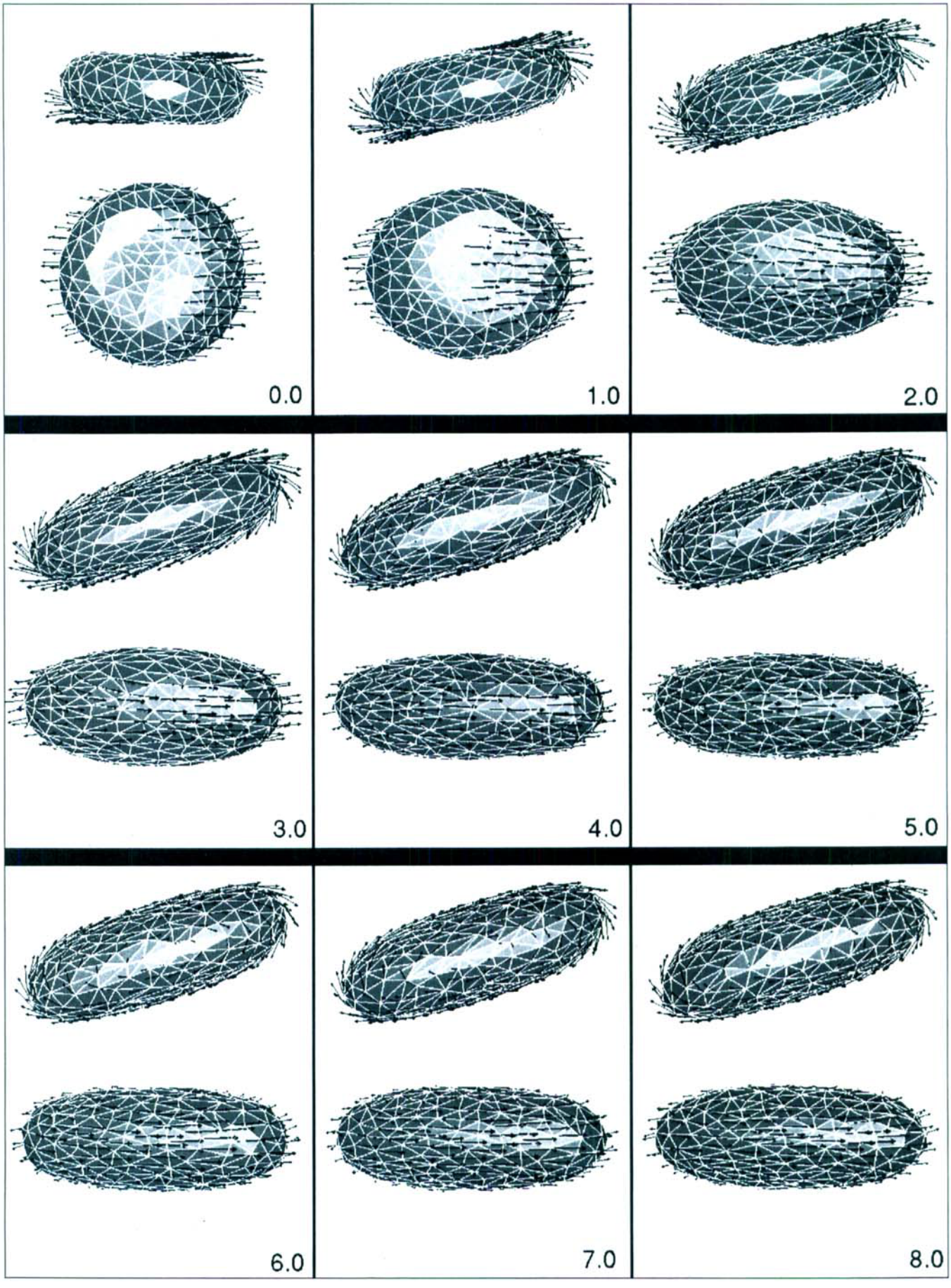

Figure 8. Shape transformation of an oblate vesicle in shear flow. The arrows give the local velocity of the membrane. The stationary state is reached after 8 s.

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