The morphology of lipid membranes

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Current research into the morphology of lipid membranes focuses on three areas: first, the transformations and fluctuations of the shape of freely suspended vesicles; second, the morphology of membranes that experience mutual interactions or external forces arising, for example, from a macroscopic surface or from optical tweezers; and third, the behavior of heterogeneous membranes with respect to bilayer asymmetry, intramembrane domains and anchored polymers. Our understanding of the morphology of lipid membranes has progressed substantially through the development of experimental techniques and theories.

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Introduction

To a large extent, the fascinating morphology of biological membranes is due to their fluidity, in other words, to the ability of the molecules within them to move laterally along the membranes. This property becomes apparent when studying lipid bilayers that contain only one or a few molecular components. Indeed, in their fluid state, even relatively simple lipid membranes exhibit a rather complex morphology, as will be reviewed below.

First, I will consider the morphology of freely suspended membranes. These membranes form closed vesicles which exhibit a variety of different shapes and shape transformations. In particular, toroidal vesicles have now become accessible using new preparation methods and have been observed to undergo a novel diffusion process, termed conformal diffusion. Next, I will discuss the morphology of interacting membranes: the adhesion of vesicles to a macroscopic interface, the mutual interaction of membranes within membrane bunches, and the interaction of membranes with optical tweezers. Finally, I will review our current understanding of the behavior of heterogeneous membranes. The heterogeneity can arise from an asymmetry in the bilayer composition, from the formation of intramembrane domains, and from flexible or stiff polymers that are anchored in the membrane.

This review covers experimental and theoretical work that has been performed during the past few years. I have also included some of the pioneering papers in which a certain approach has been used for the first time. In order to comply with the rules of this journal, I have annotated only those papers which were published since 1994. For a more complete discussion of the structure and dynamics of lipid membranes, the interested reader is referred to a recent handbook [1••].

Freely suspended membranes

There are several preparation methods by which one can produce relatively large vesicles (with a linear size of the order of 10 μm) which can then be studied by optical microscopy. In a freely suspended state, these vesicles can exhibit a large variety of different shapes and various shape transformations, as illustrated in Figure 1.

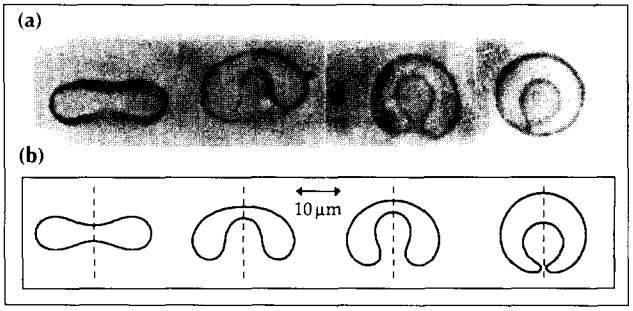

Fig. 1. Temperature-induced 'endocytosis'. **(a)** A unilamellar dimyristoyl phosphatidyl choline (DMPC) vesicle observed by phase contrast microscopy during a temperature increase of less than 1°C; **(b)** theoretical shapes of minimal bending energy with constraints on volume, area, and total mean curvature. The shape at far right represents a small spherical bud that is contained in the larger sphere; both spheres are connected by a small neck or 'worm-hole'.

Shape transformations of vesicles

It was realized about twenty years ago that the shape of vesicles composed of fluid membranes is governed by curvature and bending elasticity [2–4]. Even so, systematic attempts to change the vesicle shape via a

Abbreviations

DGDG—digalactosyl diglyceride; **DMPC**—dimyristoyl phosphatidyl choline; **PC**—phosphatidyl choline; **PE**—phosphatidyl ethanolamine; **PG**—phosphatidyl glycerol; **POPS**—palmitoyl oleyl phosphatidyl serine; **SOPC**—stearyl oleyl PC.

control parameter such as temperature and to compare experimental observations and theoretical calculations in a quantitative way have been made only quite recently. The example shown in Figure 1 represents one of the first attempts in this direction [5]. In the past couple of years, this work has been extended and refined in various ways. As a result, we now have a rather detailed theoretical model for lipid bilayers that have a homogeneous composition [6,7•]. For a guide to the literature on this subject, see [8•].

Shape fluctuations

Lipid membranes undergo thermally excited bending undulations which consist of fluctuations with a wide range of wavelengths from molecular dimensions up to the membrane size. Fluctuations with wavelengths which exceed about half a micrometer can be observed directly, under the light microscope. The intensity of these fluctuations depends on the temperature and the bending rigidity (the elastic modulus). Thus, these intensities can be used to estimate the elastic modulus; larger values indicate a more rigid membrane and smaller values a more flexible membrane. For dimyristoyl phosphatidyl choline (DMPC), the bending rigidities deduced in this way lie in the range $(1–2)\times10^{-19}$ J $\simeq(20–40)\,k_BT$ [9,10], whereas micropipette aspiration techniques gave the somewhat smaller estimate 0.6×10^{-19} J $\simeq 12\,k_BT$ [11]. For egg PC, the bending rigidities were found to be $(0.4–0.8)\times10^{-19}$ J [12,13].

Bending undulations with sufficiently long wavelengths are damped by the viscosity of the liquid surrounding the membrane [14]. Recently, Evans *et al.* [15] postulated another dissipation mechanism, one that arises from the friction between the two monolayers of the bilayer. The interplay between these two dissipation mechanisms leads to interesting cross-over phenomena in the relaxation times [16]. (These times are a function of the wavelength; the functional dependence [called a dispersion relation] depends on the dissipation mechanism. Thus, one has one dependence for small wavelengths, and another for large wavelengths.) The relaxation times are consistent with results from neutron spin echo experiments for a stack of bilayers [17].

Strong shape fluctuations pose several challenging theoretical problems, such as the constraint of constant area (which implies an effective lateral tension for the bending undulations [18,19,20•]) and the consistent prescription of how to sum over all shape configurations of the fluctuating membrane [21•]. The bending undulations also affect the size distribution of vesicles: until recently, it was generally believed that this distribution should have a broad maximum at intermediate sizes; in contrast, a recent calculation [22•] predicts that the peak of this distribution is at the minimal vesicle size.

The concept of a bending mode is no longer meaningful when its wavelength becomes of the order of the membrane thickness. On these small scales, the molecular structure of the lipid–water interface should be taken into account. This interface is roughened by the relative displacements of the lipid head groups, as has been observed in computer simulations [23,24] and in scattering experiments [25–27]. The resulting molecular roughness acts to reduce the bending rigidity [28•].

Ultraflexible membranes and branched vesicles

For bending rigidities that are large compared to k_BT, the bending undulations represent small perturbations of the average shape. On the other hand, if the bending rigidities were comparable to k_BT, these fluctuations would determine the shape as observed in Monte Carlo simulations [29,30•]: unless they were inflated by osmotic pressure (in which case they eventually became spherical), vesicles composed of such ultraflexible membranes were found to have the same geometric properties as branched polymers.

Toroidal vesicles

Even though the shapes shown in Figure 1 are quite different from the shape of a sphere, these shapes still have the same topology as a sphere. A different topology is obtained for toroidal vesicles with one or several 'handles'. Toroidal shapes have long been studied by mathematicians but the area and volume constraints as applicable to vesicles have been included only quite recently [31]. In addition, a new preparation technique has been introduced by which one can produce such vesicles from diacetylen PC [32].

One interesting aspect of toroidal vesicles with two or more handles is the appearance of a new diffusion process, termed conformal diffusion, which was first predicted theoretically [33]. In this process, the vesicle undergoes continuous shape changes which correspond to conformal transformations of the shape (see Fig. 2a–c). Very recently, such a process has also been observed experimentally (X Michalet, D Bensimon, personal communication). In another set of experiments, toroidal vesicles which consist of two concentric membranes connected by several necks have been observed to undergo strong positional fluctuations (Fig. 2d,e) [34••]. In addition, one expects strong fluctuations in the radius of such a neck [35].

Interacting membranes

When dissolved in water, lipid membranes form a large variety of structures in which the membranes experience mutual interactions: multilamellar vesicles, oriented bunches and stacks, 'myelin structures' consisting of multilamellar cylinders etc. In addition, these membranes can adhere to a macroscopic interface such as the container wall or the water–air interface. In the latter case, they experience an extended external force which depends on the chemical composition and structure of the interface. Finally, lipid membranes may interact

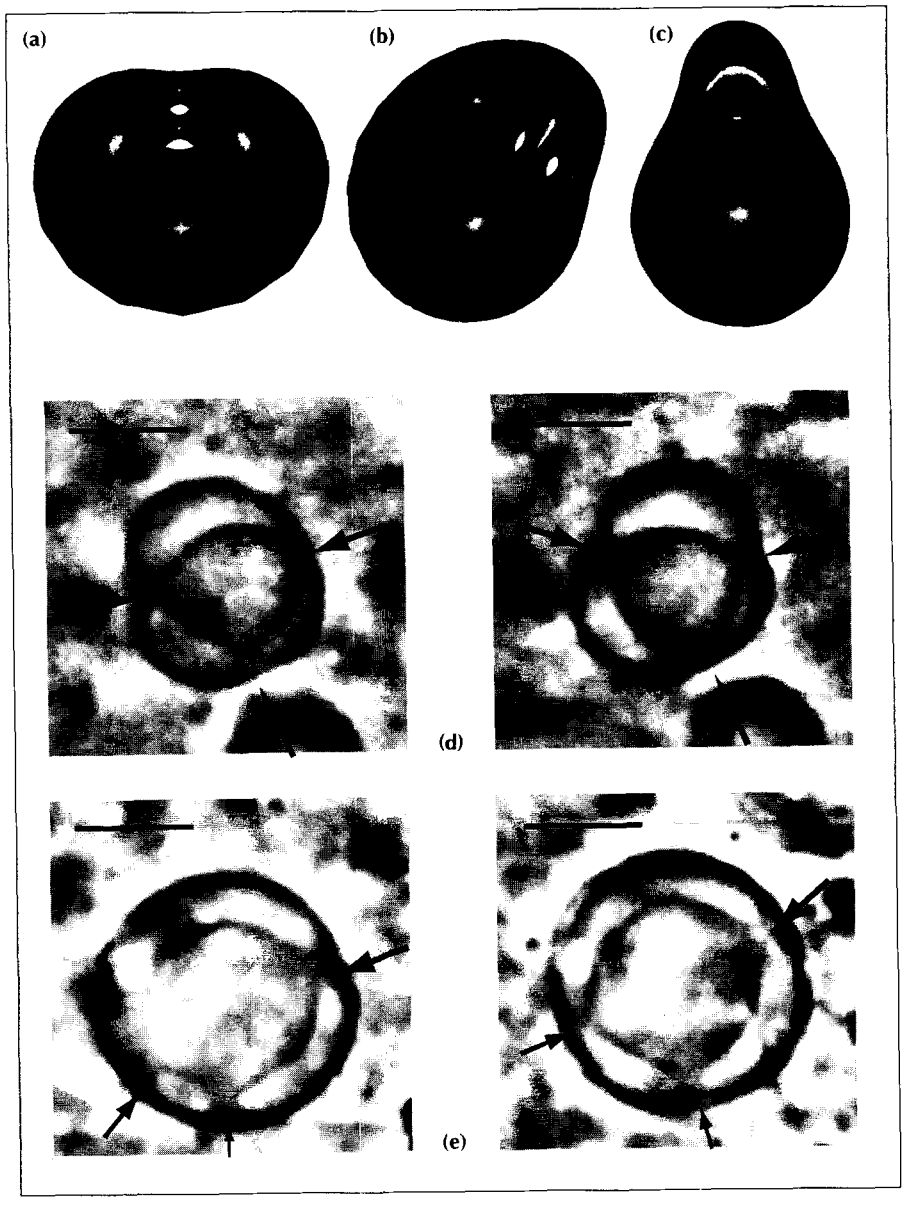

Fig. 2. Conformal diffusion of a toroidal vesicle with two handles: all shapes have the same bending energy, surface area, enclosed volume, and total mean curvature. The shapes in **(a)** and **(c)** possess one symmetry plane perpendicular to the plane of the figure. Toroidal vesicles consisting of two nearby concentric membranes connected by several necks (indicated with arrows). Rows **(d)** and **(e)** show two subsequent snapshots of one vesicle. The bars indicate 10 μm.

with localized forces arising, for example, from optical tweezers.

Adhesion of vesicles

A vesicle that is attracted towards another surface gains adhesion energy but has to increase its bending energy. This implies that the attractive surface potential must exceed a certain threshold value before adhesion sets in [36,37]. Two geometries have been experimentally explored: firstly, the mutual adhesion of two vesicles that are sucked into two micropipettes under the optical microscope [37,38] and secondly, one vesicle that is attracted towards a solid substrate and that is observed by reflection interference microscopy [39,40••]. In the latter case, the bound vesicle is usually stabilized by gravity, that is to say, it is filled with a solution that is denser than the surrounding liquid. Recent theoretical studies show that this stabilization has a substantial, deforming effect on the adhering shapes (M Kraus, U Seifert, R Lipowsky, unpublished data).

By reflection interference microscopy, one can also study the shape fluctuations within the contact region. This has been done recently using stearyl oleyl phosphatidyl choline (SOPC) vesicles [40••]. For relatively weak adhesion, the vesicle membrane is observed to undergo strong fluctuations, and one can measure both the mean separation between membrane and adhesive substrate and the roughness of the membrane. In this way, one may estimate the effective interaction between the membrane and the surface that is renormalized by the bending undulations.

Renormalization of repulsive interactions

Two surfaces which have an effectively repulsive interaction may be pushed together by an external pressure or constraint. In this case, the bending undulations induce a repulsive interaction which is long-ranged and behaves as $1/l^2$ for large surface separation l. This interaction was originally predicted by scaling arguments [41]. The widespread interpretation that this interaction reflects

the collisions or close contacts between the surfaces is misleading, because the probability for close contacts decays according to $1/l^3$ for large l (C Hiergeist, R Lipowsky, unpublished data) [42•].

Renormalization of attractive interactions

If the two surfaces experience a mutual attraction, the renormalization by bending undulations leads to an unbinding transition, as first predicted theoretically [43] and then observed experimentally using bunches of (glycolipid) digalactosyl diglyceride (DGDG) membranes [44]. For neutral surfaces, this transition is predicted to be continuous, but for charged surfaces, the direct interaction may exhibit a potential-energy barrier in addition to the attractive potential-energy well [45]. In the latter case, the fluctuating membrane 'tunnels' through sufficiently weak barriers but is trapped by sufficiently large ones. Thus, the unbinding transition is continuous for weak barriers and discontinuous for strong barriers [46•] (A Ammann, R Lipowsky, unpublished data).

Bunches and stacks of membranes

Bulk samples of a large number of oriented bilayers have been studied for a long time. In this case, the separation of the membranes can be controlled by the osmotic stress method, in which the membranes are pushed together by an osmotic pressure [47•]. One may also observe bunches and stacks that consist of a relatively small number of bilayers: one example is oriented bilayer stacks at the air–water interface [48]. The behavior of such systems has recently been studied theoretically by approximate mean-field theories [49] and by Monte Carlo simulations [50,51•]; analytical theory has also been developed for bundles of one-dimensional strings, which exhibit the same cooperative behavior as bunches of two-dimensional membranes [42•,52•].

Mutz and Helfrich [44] and Netz and I [50] found that a freely suspended bunch containing N identical membranes unbinds at a unique unbinding temperature which is independent of N. Furthermore, in the limit of large N, the transition is predicted to be rather abrupt and thus to resemble a discontinuous transition, even though it is still a continuous one [42•]. If the stack of N membranes is attracted towards a rigid surface, on the other hand, it can undergo N successive unbinding transitions in which one membrane after another peels off [50,52•]. Such peeling processes should always occur during the formation of vesicles from oriented samples and have been explicitly observed for many multilayer systems (see, for example, [53,54•]).

Tension-induced adhesion

Lateral tension suppresses the bending undulations and thus suppresses fluctuation-induced repulsion. Therefore, lateral tension can induce adhesion. This phenomenon has recently been studied with respect to the adhesion of vesicles [40••] (U Seifert, personal communication) and the adhesion of oriented membrane segments [51•]. Helfrich [55,56•] has argued that, for PC and for phosphatidyl ethanolamine (PE) bilayers, a detailed comparison between experiment and theory for this tension-induced adhesion process implies a hidden reservoir of membrane area. He has postulated that this area is stored in a membrane superstructure which consists of an array of saddle-shaped membrane segments. Additional evidence for such a superstructure has been obtained with electron microscopy [56•] but more experiments are certainly needed in order to clarify this issue.

Shape transformations induced by optical tweezers

Optical tweezers provide localized light traps and can be used to manipulate various objects in the micrometer size range. Recently, such tweezers have been directly applied to DMPC bilayers and were found to induce novel shape transformations (see Fig. 3) [57••]. For the cylindrical geometry shown in Figure 3a, the tweezers seem to induce a tension which transforms the cylinder into a chain of pearls (Fig. 3d), in analogy to the Plateau-Rayleigh instability of capillary tubes: the cylinder has a surface area (and, thus, a surface free energy) which exceeds the area (and the free energy) of the chain of pearls. The selected wavelength for inducing membrane instability has been determined in the framework of a hydrodynamic model [58•]. Dramatic effects on the membrane morphology can also be observed when these optical tweezers are applied to bunches of bound membranes (R Bar-Ziv, R Menes, E Moses, SA Safran, personal communication).

Heterogeneous membranes

Biomembranes contain a large number of different lipid and protein components which are organized in a rather complex manner. Firstly, the lipid–protein bilayer is asymmetric (i.e. the composition of the two monolayers is different) [59]; secondly, the lateral composition along the membrane is non-uniform, as both lipids and proteins are organized into intramembrane domains [60,61]; and thirdly, the membrane is decorated with macromolecules that stick out from the membrane and govern the interaction of the membrane with its surroundings. These aspects of membrane heterogeneity can be studied in different types of model membranes.

Flexibility of multicomponent membranes

Several attempts have been made to estimate the bending rigidity of a lipid bilayer that contains several components. For a membrane with two phospholipid components, the simplest approach is to use a lever rule for the inverse bending rigidities [62]. If the membrane contains a small number of molecules with a conical shape, the lateral diffusion of these molecules leads to a reduction of the bending rigidity [63], as has been estimated recently from refined theoretical models [64•] (RR Netz, P Pincus, personal communication).

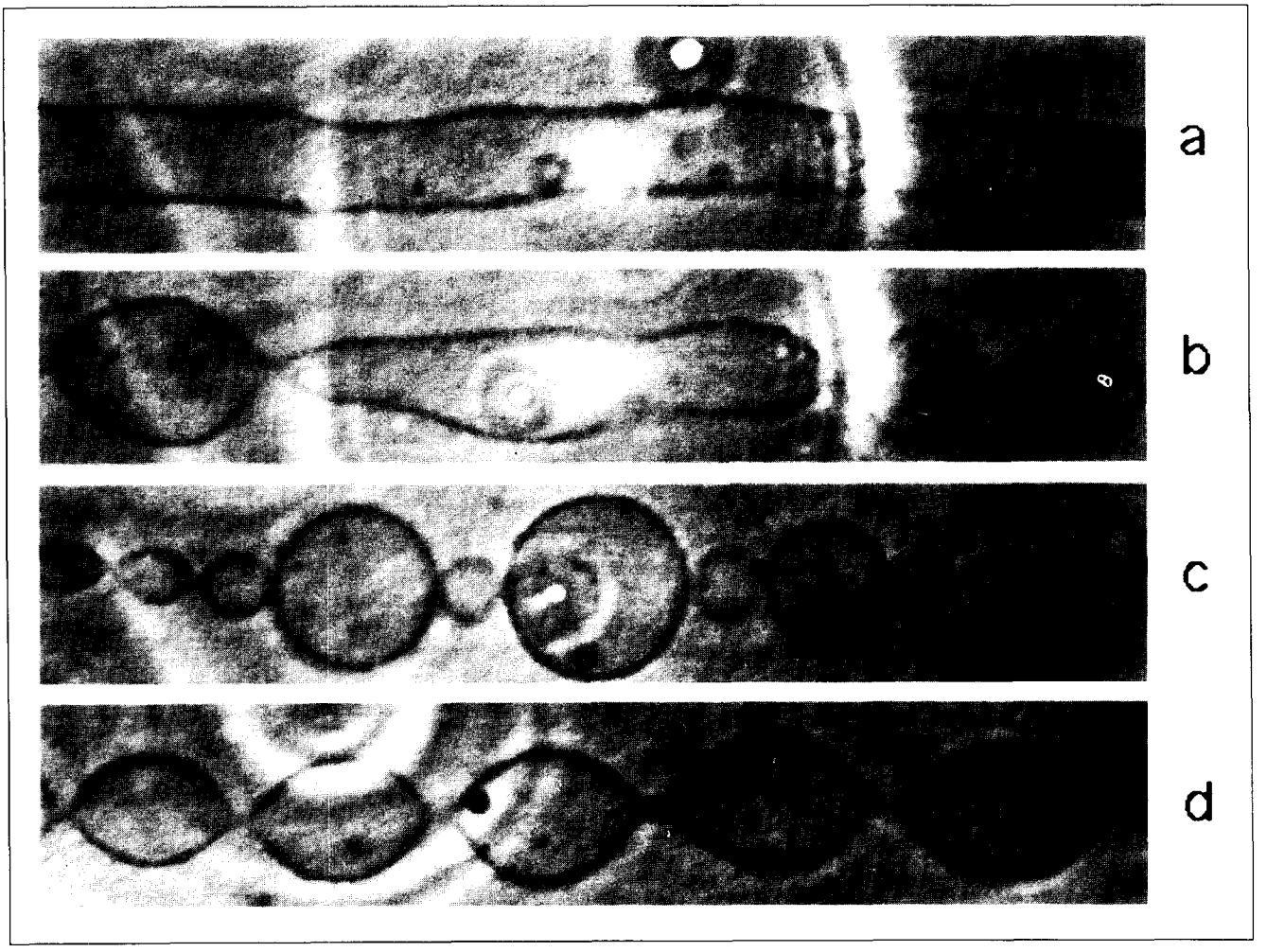

Fig. 3. Cylindrical tube of DMPC that is perturbed by optical tweezers undergoes a pearling instability. **(a)** Initial application of the tweezers at time $t = 0$; **(b)** $t = 9$ s; **(c)** the tweezers are turned off at $t = 30$ s; **(d)** periodic structure at a different position of the tube after $t = 223$ s. The length of the displayed tube segment is 81.5 μm. (Courtesy of E Moses).

On the experimental side, the bending rigidity of phospholipid/cholesterol mixtures has been studied in some detail: it was found to increase fourfold for DMPC bilayers with 30 mole percent cholesterol [10] and threefold for SOPC bilayers with (40–50) mole percent cholesterol [11,65].

Bilayer asymmetry and curvature

It has been known for a long time that small vesicles that are composed of two different lipids, such as PC and PE, exhibit a strong asymmetry in the composition of the two monolayers of the bilayer [66]. This compositional asymmetry reduces the mismatch, or frustration, between the spontaneous curvatures of the two monolayers as has been recently discussed for surfactant vesicles [67].

Bilayer asymmetry in large vesicles has been induced by two methods [68], (BLS Mui, HG Döbereiner, TD Madden, PR Cullis, personal communication): in the first method, in a lipid mixture containing the neutral lipid PC and a small amount of phosphatidyl glycerol (PG), the anionic PG was redistributed by means of a pH gradient; in the second method, lysolipid molecules, which have only one hydrocarbon chain, were added to the outer monolayer of the vesicle membrane. In both cases, the bilayer asymmetry led to pronounced shape transformations of the vesicles.

It has been found theoretically that a small asymmetry of the bilayer will have a rather strong effect on the shape transformations induced by a change in temperature [5]. In this way, a small asymmetry on molecular scales becomes amplified up to mesoscopic scales. This is one example of the sensitivity of lipid membranes to small perturbations in their molecular structure.

Curvature-induced segregation within membranes

In a multicomponent bilayer, surfactants or lipids with a conical shape will diffuse towards membrane regions with an appropriate curvature. Thus, the curvature of the membrane may induce phase segregation between the different molecules, as has recently been discussed theoretically [69]. Fischer [70•] proposed a similar mechanism in order to explain the dynamics of budding.

The lipids that are enriched in the curved membrane region could lower the bending ridigity of this region. This would increase the curvature and thus the concentration of lipids within this region (provided these lipids do not repel each other too strongly). Such positive feedback could lead to very flexible domains within the membrane and could explain the high degree of flexibility of vesicles composed of phospholipids and cholates which have recently been reported to penetrate the skin [71•].

Different mechanisms for domain formation

Mixtures of lipids exhibit two-phase coexistence regions, where several thermodynamic phases coexist [72•,73•]. In many cases, a fluid phase and a solid or gel phase coexist. In addition, some mixtures, such as dielaidoyl PC/dipalmitoyl PE [74], phospholipid/cholesterol [75,76,77•], and palmitoyl oleyl phosphatidyl serine (POPS)/didodecanoyl PC [78•], exhibit two-phase coexistence regions where both phases are fluid. If the bilayer is prepared in a coexistence region, it will undergo phase separation, which leads to the formation of intramembrane domains.

Integral proteins in biomembranes may be randomly distributed or may aggregate within the membrane. X-ray scattering, with the momentum transfer confined in the plane of the membrane, is a new experimental tool by which one may distinguish these different states [79]. From the theoretical point of view, the effective interaction between two integral proteins contains several contributions: short-range interactions, which are usually attractive, arising from the perturbation of the molecular structure of the lipid bilayer [80,81]; long-range repulsive interactions between conical molecules arising from the bending deformations of the bilayer [82,83]; and long-range attractive interactions induced by the thermally excited bending fluctuations [83].

Another mechanism for domain formation is provided by polymerizable lipids: lipid molecules that have two or even more reactive units can be polymerized within the bilayer membrane. As a result, one obtains 'fuzzy' domains consisting of a branched polymer which is completely embedded in the bilayer [84].

Ordered domains and curvature modulations

The domains that are produced by phase separation of lipid bilayers often form ordered patterns, as originally observed by freeze fracture and electron microscopy [85]. From the theoretical point of view, striped phases and hexagonal arrays of domains have been studied using essentially flat membranes under lateral tension [86,87,88•], and uniaxial striped domains have been studied using cylindrical vesicles [62,87]. If the domains and the membrane matrix have different elastic properties, the membrane will exhibit curvature modulations induced by the phase separation.

Domain-induced budding and fission

When subjected to low levels of lateral tension, a fluid domain can undergo a budding process, as shown in Figure 3 [89,90]. Inspection of this figure shows that the length of the domain boundary decreases during the budding process. Thus, this shape transformation is driven by the edge or line tension of this domain boundary. Simple estimates also show that the bud is likely to break off from the membrane matrix. Such budding and fission processes have been observed experimentally for vesicles composed of DMPC and cholesterol [91].

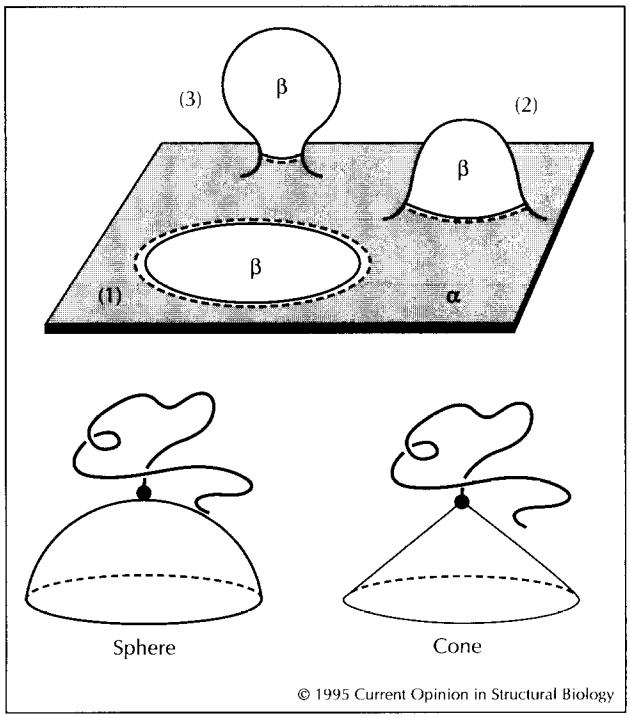

Fig. 4. Shape transformations of heterogeneous membranes. **(a)** An intramembrane domain (β) in a membrane matrix (α) undergoes a budding transformation as soon as its size has reached a certain threshold value; stages are indicated by (1), (2) and (3). **(b)** A membrane segment that is decorated with an anchored polymer (bold, wavy line) is bent by this polymer.

Membranes decorated by anchored polymers

A polymer can be attached to the bilayer membrane in several ways: by a lipid anchor, where one end of the polymer is covalently bound to the head group of the lipid molecule [92,93]; by hydrophobic sidegroups of the polymer which are inserted into the bilayer [94,95]; and by membrane-spanning hydrophobic domains of the polymer. The last method is the typical situation for membrane-bound proteins.

I have recently shown that such an anchored polymer tends to exert fluctuation forces on the bilayer membrane that bend the membrane away from the polymer (see Fig. 4) [96•]. This mechanism could explain some of the shape transformations of DMPC vesicles experimentally induced by changing the conformation of the anchored

polymers [94,95] (J Simon, M Kühner, H Ringsdorf, E Sackmann, personal communication).

Anchored rods and specific adhesion

Cell membranes adhere via specific cell adhesion molecules, which are relatively stiff rod-like molecules anchored in the bilayer membrane. In some cases, pairs of such molecules act as receptors and counter-receptors (or ligands) and form molecular bridges between the membranes [97,98,99•]. The binding energy of such molecular bonds can be larger than the energy required to uproot one of the receptors from the membrane; this effect has been observed with agglutinin-bonded red blood cells [100].

One receptor–ligand pair that has recently been studied in some detail is biotin and avidin or streptavidin [101•,102•]. In particular, it has been observed that dilauroyl PC vesicles that contain biotin with dipalmitoyl PE anchors start to aggregate if one adds streptavidin to the aqueous solution and to unbind again if one adds soluble biotin, which competes with the anchored biotin for binding streptavidin [102•]. During the aggregation process, the shape of the vesicles remains essentially unchanged, implying that the vesicles are bound by focal bonds consisting of one or a few clustered biotin–streptavidin–biotin bridges.

Conclusions

During the past couple of years, our understanding of the morphology of lipid membranes has improved substantially. On the one hand, new experimental techniques, such as interference reflection microscopy and optical tweezers, have been further developed that give rather detailed information on membrane behavior and allow us to manipulate the bilayer membranes in new ways. On the other hand, many qualitative ideas have been turned into systematic theories which make definite predictions about the cooperative behavior of these membranes. In many cases, theoretical considerations and experimental observations are in fair agreement.

The experimental and theoretical work has also shown, however, that the membrane behavior on mesoscopic scales is quite sensitive to small perturbations of the membrane structure on molecular scales. Such perturbations can arise, for example from chemical heterogeneities such as colipids with unsaturated hydrocarbon chains or from hydrophobic molecules embedded in the bilayer. In general, these additional molecules lead to local variations in the spontaneous curvature of the lipid membrane, increase the molecular roughness of the lipid water interfaces, and change the activation energies for structural defects such as pores.

In order to eliminate variables on the molecular level, the preparation procedures for lipid membranes should be further improved. In addition, more efforts should be devoted both to experimental work and to computer simulations in which one systematically studies the dependence of material parameters (such as the bending rigidity) on the chemical composition of the membranes.

Finally, another very promising avenue for future research involves lipid membranes coupled to polymer networks. One prominent example is the coupling between the cell membrane and the filaments of the cytoskeleton, which is believed to determine the mechanical properties of the cell such as cell shape, cell locomotion, and cell division. On the molecular level, a lot has been learned recently about the membrane–cytoskeleton interaction [103•]. What remains to be done is to build model systems in which the cooperative behavior arising from this interaction can be studied in a systematic way.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Lipowsky R, Sackmann E (Eds): **The Structure and dynamics of membranes.** Amsterdam: Elsevier Science; 1995. [Hoff A (Ed): Handbook of Biological Physics, **1**.]

This volume contains 19 review articles on the behavior of biomembranes and lipid bilayers. In addition to various chapters on membrane morphology, this volume covers lipid polymorphism, lipid monolayers, molecular modelling of lipid membranes, applications of liposomes, electroporation, electrofusion and membrane fusion induced by ions and polymers.

2. Canham PB: **The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell.** *J Theoret Biol* 1970, **26**:61–81.

3. Helfrich W: **Elastic properties of lipid bilayers: theory and possible experiments.** *Z Naturforsch* 1973, **28**:693–703.

4. Evans EA: **Bending resistance and chemically induced moments in membrane bilayers.** *Biophys J* 1974, **14**:923–931.

5. Berndl K, Käs J, Lipowsky R, Sackmann E, Seifert U: **Shape transformations of giant vesicles: extreme sensitivity to bilayer asymmetry.** *Europhys Lett* 1990, **13**:659–664.

6. Bozic B, Svetina S, Zeks B, Waugh RE: **Role of lamellar membrane structure in tether formation from bilayer vesicles.** *Biophys J* 1992, **61**:963–973.

7. • Miao L, Seifert U, Wortis M, Döbereiner H-G: **Budding transitions of fluid-bilayer vesicles: the effect of area-difference elasticity.** *Phys Rev E* 1994, **49**:5389–5407.

This paper describes our current theoretical understanding of the bending elasticity for homogeneous membranes.

8. • U Seifert and R Lipowsky: **Morphology of vesicles.** In *Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier Science; 1995:403–463.

This review covers recent theoretical and experimental work on the morphology of freely suspended and adhering vesicles.

9. Schneider MB, Jenkins JT, Webb WW: **Thermal fluctuations of large cylindrical phospholipid vesicles.** *Biophys J* 1984, **45**:891–899.

10. Duwe HP, Käs J, Sackmann E: **Bending elastic moduli of lipid bilayers: modulation by solutes.** *J Phys France* 1990, **51**:945–962.

11. Evans E, Rawicz W: **Entropy-driven tension and bending elasticity in condensed-fluid membranes.** *Phys Rev Lett* 1990, **64**:2094–2097.

12. Mutz M, Helfrich W: **Bending rigidities of some biological model membranes as obtained from the Fourier analysis of contour sections.** *J Phys France* 1990, **51**:991–1002.

13. Meleard P, Faucon JF, Mitov MD, Bothorel P: **Pulsed-light microscopy applied to the measurement of the bending elasticity of giant liposomes.** *Europhys Lett* 1992, **19**:267–271.

14. Brochard F, Lennon JF: **Frequency spectrum of the flicker phenomenon in erythrocytes.** *J Physique* 1975, **6**:1035–1047.

15. Evans E, Yeung A, Waugh R, Song J: **Dynamic coupling and nonlocal curvature elasticity in bilayer membranes.** In *The Structure and Conformation of Amphiphilic Membranes.* Edited by Lipowsky R, Richter D, Kremer K. Berlin: Springer; 1992:148–153. [*Springer Proceedings in Physics*, **66**.]

16. Seifert U, Langer SA: **Viscous modes of fluid bilayer membranes.** *Europhys Lett* 1993, **23**:71–76.

17. Pfeiffer W, König S, Legrand JF, Bayerl T, Richter D, Sackmann E: **Neutron spin echo study of membrane undulations in lipid multibilayers** *Europhys Lett* 1993, **23**:457–462.

18. Milner ST, Safran SA: **Dynamical fluctuations of droplet microemulsions and vesicles.** *Phys Rev A* 1987, **36**:4371–4379.

19. Bivas I, Bivolarski L, Mitov MD, Derzhanski A: **Correlations between the form fluctuations modes of flaccid quasispherical lipid vesicles and their role in the calculation of the curvature elastic modulus of the vesicle membrane.** *J Phys II* 1992, **12**:1423–1438.

20. • Seifert U: **The concept of effective tension for fluctuating vesicles.** *Z Physik B* 1995, **97**:299–309.
A systematic theory for the shape fluctuations of vesicles is developed corresponding to a low temperature or large rigidity expansion.

21. • Cai W, Lubensky TC, Nelson P, Powers T: **Measure factors, tension and correlations of fluid membranes.** *J Phys II France* 1994, **4**:931–949.
A detailed theoretical study about the pitfalls one encounters in the summation over all shape configurations.

22. • Morse DC, Milner ST: **Fluctuations and phase behavior of fluid membrane vesicles.** *Europhys Lett* 1994, **26**:565–570.
The fluctuation contribution to the free energy of a single vesicle is predicted to increase logarithmically with the vesicle size.

23. Egberts E, Berendsen HJC: **Molecular dynamics simulation of a smectic liquid crystal with atomic detail.** *J Chem Phys* 1988, **89**:3718–3732.

24. Marrink SJ, Berkowitz M, Berendsen HJC: **Molecular dynamics simulation of a membrane-water interface: the ordering of water and its relation to the hydration force.** *Langmuir* 1993, **9**:3122–3131.

25. König S, Pfeiffer W, Bayerl T, Richter D, Sackmann E: **Molecular dynamics of lipid bilayers studied by incoherent quasi-elastic neutron scattering** *J Phys II France* 1992, **2**:1589–1615.

26. Wiener MC, White SH: **Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of x-ray and neutron diffraction data.** *Biophys J* 1992, **61**:434–447.

27. McIntosh TJ, Simon SA: **Contributions of hydration and steric (entropic) pressures to the interactions between phosphatidylcholine bilayers: experiments with the subgel phase.** *Biochemistry* 1993, **32**:8374–8384.

28. • Lipowsky R, Grotehans S: **Renormalization of hydration forces by collective protrusion modes.** *Biophys Chem* 1994, **49**:27–37.
The molecular roughness of the lipid water interfaces bounding the bilayer is predicted to reduce the bending rigidity.

29. Kroll DM, Gompper G: **The conformation of fluid membranes.** *Science* 1992, **255**:968–971.

30. • Gompper G, Kroll DM: **Phase diagram and scaling behavior of fluid vesicles.** *Phys Rev E* 1995, **51**:514–525.
Scaling theories and computer simulations are combined in order to determine the phase diagram of ultraflexible vesicles.

31. Seifert U: **Vesicles of toroidal topology.** *Phys Rev Lett* 1991, **66**:2404–2407.

32. Fourcade B, Mutz M, Bensimon D: **Experimental and theoretical study of toroidal vesicles.** *Phys Rev Lett* 1992, **68**:2551–2554.

33. Jülicher F, Seifert U, Lipowsky R: **Conformal degeneracy and conformal diffusion of vesicles.** *Phys Rev Lett* 1993, **71**:452–455.

34. •• Michalet X, Bensimon D, Fourcade B: **Fluctuating vesicles of nonspherical topology.** *Phys Rev Lett* 1994, **72**:168–171.
Experimental observations and theoretical considerations for diacetylenic phosphatidyl choline vesicles consisting of two concentric membranes that are connected by many necks; these systems provide new and non-trivial checks for the theoretical modelling of lipid membranes.

35. Goos J, Gompper G: **Topological defects in lamellar phases: passages and their fluctuations.** *J Phys I France* 1993, **3**:1551–1567.

36. Seifert U, Lipowsky R: **Adhesion of vesicles.** *Phys Rev A* 1990, **42**:4768–4771.

37. Evans E: **Adhesion of surfactant-membrane covered droplets: special features and curvature elasticity effects.** *Colloids and Surfaces* 1990, **43**:327–347.

38. Evans EA: **Analysis of adhesion of large vesicles to surfaces.** *Biophys J* 1980, **31**:425–432.

39. Rädler J, Sackmann E: **Imaging optical thicknesses and separation distances of phospholipid vesicles at solid surfaces.** *J Phys II France* 1993, **3**:727–748.

40. •• Rädler JO, Feder TJ, Strey HH, Sackmann E: **Fluctuation analysis of tension-controlled undulation forces between giant vesicles and solid substrates.** *Phys Rev E* 1995, **51**:4526–4536.
Reflection interference microscopy is used to study the shape fluctuations in the contact zone of adhering vesicles; by this approach, several scales can be measured simultaneously, giving rather detailed information about the renormalized interaction.

41. Helfrich W: **Steric interaction of fluid membranes in multilayer systems.** *Z Naturforsch* 1978, **33a**:305–315.

42. • Lipowsky R: **From bunches of membranes to bundles of strings.** *Z Physik B* 1995, **97**:193–203.
Review about swollen bunches of membranes and their renormalized interactions. The cooperative behavior of these bunches is intimately related to the corresponding behavior of bundles of one-dimensional strings.

43. Lipowsky R, Leibler S: **Unbinding transitions of interacting membranes.** *Phys Rev Lett* 1986, **56**:2541–2544.

44. Mutz M, Helfrich W: **Unbinding transition of a biological model membrane.** *Phys Rev Lett* 1989, **62**:2881–2884.

45. Marra J, Israelachvili JN: **Direct measurements of forces between phosphatidylcholine and phosphatidyl-ethanolamine bilayers in aqueous electrolyte solutions.** *Biochemistry* 1985, **24**:4608–4618.

46. • Lipowsky R: **Discontinuous unbinding transitions of flexible membranes.** *J Phys II France* 1994, **4**:1755–1762.
Theoretical paper which clarifies the character of the unbinding transition for charged membranes.

47. • Parsegian VA, Rand RP: **Interaction in membrane assemblies.** In *The Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier; 1995:643–690. [Hoff A (Ed): Handbook of Biological Physics, **1**.]
Review about the mutual interactions of membranes as obtained by different experimental techniques for a variety of lipids.

48. Cevc G, Fenzl W, Sigl L: **Surface-induced X-ray reflection visualization of membrane orientation and fusion into multibilayers.** *Science* 1990, **249**:1161–1163.

49. Helfrich W: **Mean field theory of n-layer unbinding.** *J Phys II France* 1993, **3**:385–393.

50. Netz RR, Lipowsky R: **Unbinding of symmetric and asymmetric stacks of membranes.** *Phys Rev Lett* 1993, **71**:3596–3599.

51. • Netz RR, Lipowsky R: **Stacks of fluid membranes under pressure and tension.** *Europhys Lett* 1995, **29**:345–350.
Results of Monte Carlo simulations on bunches and stacks of membranes which are pushed together by an external pressure.

52. • Hiergeist C, Lässig M, Lipowsky R: **Bundles of interacting strings in two dimensions.** *Europhys Lett* 1994, **28**:103–108.
Analytical theory for bundles of interacting strings which behave in the same way as bunches of interacting membranes; compare with [42•].

53. Tsao Y, Evans DF, Rand RP, Parsegian VA: **Osmotic stress measurements fo DHDAA bilayers as a function of temperature and added salt.** *Langmuir* 1993, **9**:233–241.

54. • Hartung J, Helfrich W, Klösgen B: **Transformation of phosphatidylcholine multilayer systems in a large excess of water.** *Biophys Chem* 1994, **49**:77–81.
Experimental studies of bunches of PC bilayers by microscopy and X-ray diffraction indicating that the membranes peel off from the bunch.

55. Helfrich W:**Hats and saddles in liquid membranes.** *Liquid Crystals* 1989, **5**:1647–1658.

56. • Helfrich W:**Tension induced mutual adhesion and a conjectured superstructure of lipid membranes.** In*The Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier; 1995:691–721. [Hoff A (Ed): Handbook of Biological Physics, **1**.]
Review article which summarizes the evidence for a bilayer superstructure consisting of saddle-shaped membrane segments.

57. •• Bar-Ziv R, Moses E:**Instability and pearling states produced in tubular membranes by competition of curvature and tension.** *Phys Rev Lett* 1994, **73**:1392–1395.
Optical tweezers, when applied directly to tubes of lipid bilayers, are observed to induce novel shape transformations. This technique, which perturbs the membranes locally, represents a new and very promising experimental tool.

58. • Nelson P, Powers T, Seifert U: **Dynamic theory of pearling instability in cylindrical vesicles.** *Phys Rev Lett* 1995, **74**:3384–3387.
Hydrodynamic theory for the shape transformations of tubular vesicles as induced by optical tweezers.

59. Devaux PF: **Static and dynamic lipid asymmetry in cell membranes.** *Biochemistry* 1991, **30**:1163–1173.

60. Ededin M: **The super-ordered fluid: a domain model of cell membranes.** In *Biophysics of the cell surface.* Edited by Glaser R, Gingell D. Berlin: Springer-Verlag; 1990:51–59.

61. Glaser M: **Lipid domains in biological membranes.** *Curr Opin Struct Biol* 1993, **3**:475–481.

62. Markin VS: **Lateral organization of membranes and cell shapes.** *Biophys J* 1981, **36**:1–19.

63. Leibler S: **Curvature instability in membranes.** *J Phys (Paris)* 1986, **47**:507–516.

64. • Helfrich W, Kozlov MM: **Flexibility and roughness of mixed and partially polymerized bilayers in terms of the hat model and local bending frustration.** *J Phys II France* 1994, **4**:1427–1438.
A theory for the bending rigidity of multicomponent bilayers which is based on local curvature fluctuations of individual molecules.

65. Song J, Waugh RE: **Bending rigidity of SOPC membranes containing cholesterol.** *Biophys J* 1993, **64**:1967–1970.

66. Thompson TE, Huang C, Litman BJ: **Bilayers and biomembranes: compositional asymmetries induced by surface curvature.** In *The cell surface in development.* Edited by Moscona AA. New York: John Wiley; 1974:1–16.

67. Safran SA, Pincus PA, Andelman D, MacKintosh FC: **Stability and phase behavior of mixed surfactant vesicles.** *Phys Rev A* 1991, **43**:1071–1078.

68. Farge E, Devaux PF: **Shape changes of giant liposomes induced by an asymmetric transmembrane distribution of phospholipids.** *Biophys J* 1992, **61**:347–357.

69. Seifert U: **Curvature-induced lateral phase segregation in two-component vesicles.** *Phys Rev Lett* 1993, **70**:1335–1338.

70. • Fischer TM: **Mechanisms for determining the time scales in vesicle budding.** *Phys Rev E* 1994, **50**:4156–4166.
Theoretical estimates for the time scales which govern the different dynamic processes involved in the budding transformation of vesicles.

71. • Cevc G: **Material transport across permeability barriers by means of lipid vesicles.** In *The Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier; 1995:465–490. [Hoff A (Ed): Handbook of Biological Physics, **1**.]
Review on vesicles composed of phospholipids and cholates which seem to be ultraflexible and to represent possible delivery systems that penetrate directly through the skin.

72. • Sackmann E: **Physical basis of self-organization and function of membranes: physics of vesicles.** In *The Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier; 1995:213–304. [Hoff A (Ed): Handbook of Biological Physics, **1**.]
Review about the physical properties of lipid vesicles with a detailed discussion of multicomponent membranes.

73. • Almeida PFF, Vaz WLC: **Lateral diffusion in membranes.** In *The Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier; 1995:305–357. [Hoff A (Ed): Handbook of Biological Physics, **1**.]
Review about lateral diffusion in lipid bilayers which emphasizes the behavior in heterogeneous membranes.

74. Wu SH-W, McConnell HM: **Phase separations in phospholipid membranes.** *Biochemistry* 1975, **14**:847–854.

75. Almeida PFF, Vaz WLC, Thompson TE: **Lateral diffusion in the liquid phases of dimyristoylphosphatidyl-choline/cholesterol lipid bilayers: a free volume analysis.** *Biochemistry* 1992, **31**:6739–6747.

76. Thewalt JL, Bloom M: **Phosphatidylcholine: cholesterol phase diagrams.** *Biophys J* 1992, **63**:1176–1181.

77. • Bloom M, Mouritsen OG: **The evolution of membranes.** In *The Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier; 1995:65–95. [Hoff A (Ed): Handbook of Biological Physics, **1**.]
Review about the biological evolution of membranes and the special role played by cholesterol.

78. • Hinderliter AK, Huang J, Feigenson GW: **Detection of phase separation in fluid phosphatidylserine/phosphatidylcholine mixtures.** *Biophys J* 1994, **67**:1906–1911.
Experimental evidence for the coexistence of two fluid lamellar phases.

79. He K, Ludtke SJ, Wu Y, Huang HW: **X-ray scattering with momentum transfer in the plane of the membrane.** *Biophys J* 1993, **64**:157–162.

80. Marcelja S: **Lipid-mediated protein interaction in membranes.** *Biochim Biophys Acta* 1976, **455**:1–7.

81. Dan N, Pincus P, Safran SA: **Membrane-induced interactions between inclusions.** *Langmuir* 1993, **9**:2768–2771.

82. Gruler H: **Chemoelastic effects of membranes.** *Z Naturforsch* 1975, **30c**:608–614.

83. Goulian M, Bruinsma R, Pincus P: **Long-range forces in heterogeneous fluid membranes.** *Europhys Lett* 1993, **22**:145–150. [Published erratum appears in *Europhys Lett* 1993, **23**:155.]

84. Gaub H, Sackmann E, Büschl R, Ringsdorf H: **Lateral diffusion and phase separation in two-dimensional solutions of polymerized butadiene lipid in DMPC bilayers.** *Biophys J* 1984, **45**:725–731.

85. Gebhardt C, Gruler H, Sackmann E: **On domain structure and local curvature in lipid bilayers and biological membranes.** *Z Naturforsch* 1977, **32c**:581–596.

86. Leibler S, Andelman D: **Ordered and curved meso-structures in membranes and amphiphilic films.** *J Phys* 1987, **48**:2013–2018.

87. Kawakatsu T, Andelman D, Kawasaki K, Taniguchi K: **Phase transitions and shape of two component membranes and**

vesicles. I: Strong segregation limit. *J Phys II France* 1993, **3**:971–997.

88. • Harden JL, MacKintosh FC: **Shape transformations of domains in mixed-fluid films and bilayer membranes.** *Europhys Lett* 1994, **28**:495–500.
Theoretical study of essentially flat multicomponent membranes under lateral tension which exhibit striped and caplet phases.

89. Lipowsky R: **Domain-induced budding of fluid membranes.** *Biophys J* 1993, **64**:1133–1138.

90. Jülicher F, Lipowsky R: **Domain-induced budding of vesicles.** *Phys Rev Lett* 1993, **70**:2964–2967.

91. Döbereiner H-G, Käs J, Noppl D, Sprenger I, Sackmann E: **Budding and fission of vesicles.** *Biophys J* 1993, **65**:1396–1403.

92. Blume G, Cevc G: **Liposomes for a sustained drug release in vivo.** *Biochim Biophys Acta* 1990, **1029**:91–97.

93. Lasic DD, Martin FJ, Gabizon A, Huang SK, Papahadjopoulos D: **Sterically stabilized stealth liposomes: a hypothesis on the molecular origin of the extended circulation times.** *Biochim Biophys Acta* 1991, **1070**:187–192.

94. Decher G, Kuchinka E, Ringsdorf H, Venzmer J, Bitter-Suermann D, Weisgerber C: **Interaction of amphiphilic polymers with model membranes.** *Angew Makromol Chem* 1989, **166/167**:71–80.

95. Ringsdorf H, Simon J, Winnik FM: **Interactions of hydrophobically modified poly (N-isopropylacrylamides) with liposomes.** In *Colloid-polymer interactions.* Edited by Dubin PL, Tong P. Washington, DC: American Chemical Society; 1993:216–240. [Dubin PL, Tong P (Eds): ACS Symposium series, **532**.]

96. Lipowsky R: **Bending of membranes by anchored polymers.** *Europhys Lett* 1995, **30**:197–202.

97. Bell GI: **Models for the specific adhesion of cells to cells.** *Science* 1978, **200**:618–626.

98. Springer TA: **Adhesion receptors of the immune system.** *Nature* 1990, **346**:425–434.

99. • Bongrand P: **Adhesion of cells.** In *The Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier; 1995:755–803. [Hoff A (Ed): Handbook of Biological Physics, **1**.]
Review of the physical aspects of cell adhesion with a detailed description of several model systems.

100. Evans E, Berk D, Leung A: **Detachment of agglutinin-bonded red blood cells. I: Forces to rupture molecular-point attachments.** *Biophys J* 1991, **59**:838–848.

101. • Florin EL, Moy VT, Gaub HE: **Adhesion forces between individual ligand-receptor pairs.** *Science* 1994, **264**:415–417.
Atomic force microscopy is used to measure the strength of single molecular bonds between biotin and avidin.

102. • Chiruvolu S, Walker S, Israelachvili J, Schmitt FJ, Leckband D, Zasadzinski JA: **Higher order self-assembly of vesicles by site-specific binding.** *Science* 1994, **264**:1753–1756.
Aggregation of dilauroyl PC vesicles is induced by specific adhesion via lipid-anchored biotin as receptor and streptavidin as ligand.

103. • Janmey P: **Cell membranes and the cytoskeleton.** In *The Structure and Dynamics of Membranes.* Edited by Lipowsky R, Sackmann E. Amsterdam: Elsevier; 1995:805–849. [Hoff A (Ed): Handbook of Biological Physics, **1**.]
Review about the filaments and anchor proteins involved in the interaction of the cytoskeleton with the lipid/protein bilayer of cell membranes.

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