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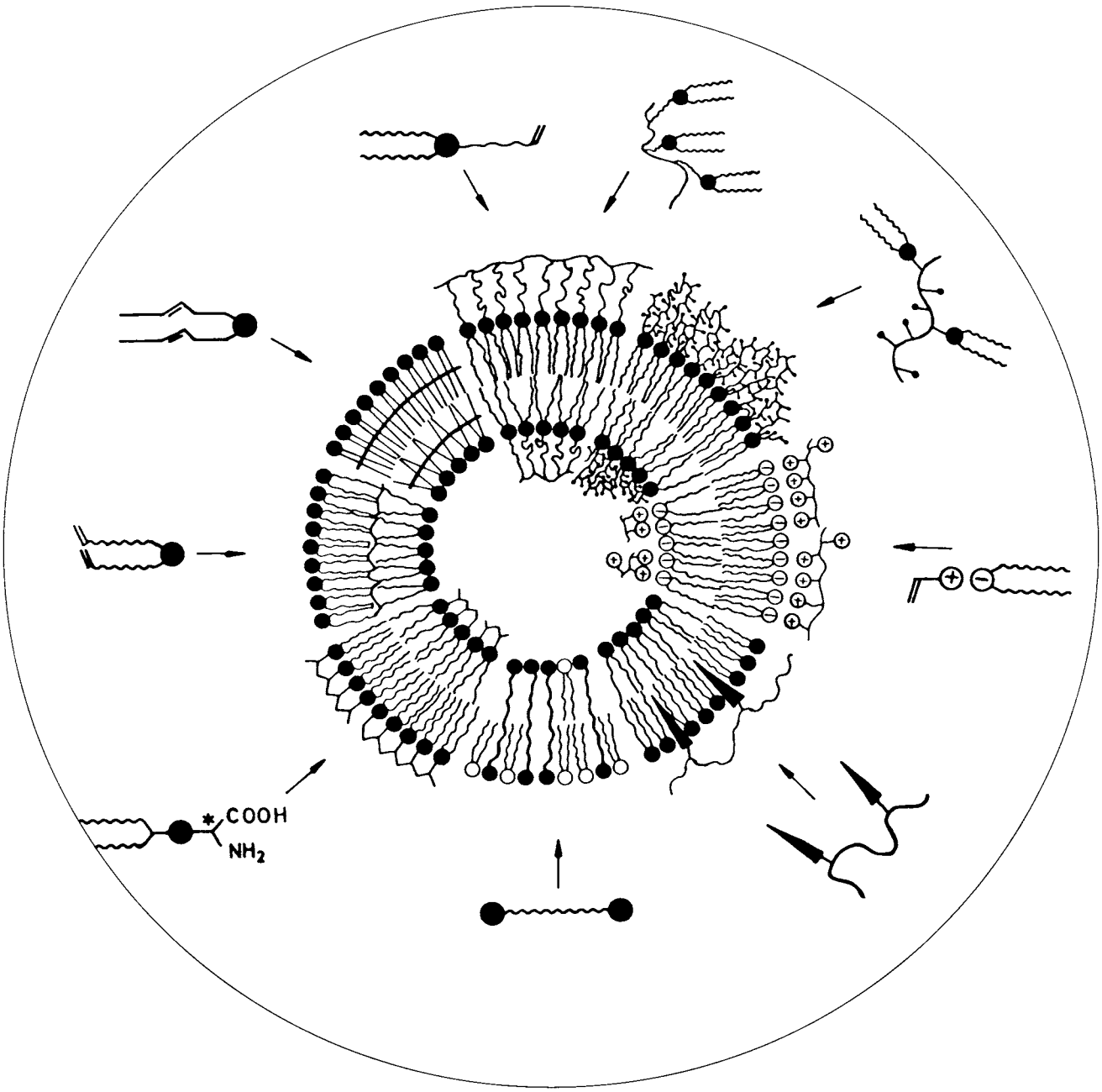

Helmut Ringsdorf, Bernhard Schlarb, and Joachim Venzmer

Molecular Architecture and Function of Polymeric Oriented Systems: Models for the Study of Organization, Surface Recognition, and Dynamics of Biomembranes

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Molecular Architecture and Function of Polymeric Oriented Systems: Models for the Study of Organization, Surface Recognition, and Dynamics of Biomembranes**

By Helmut Ringsdorf,* Bernhard Schlarb, and Joachim Venzmer

The Part and the Whole. The principle of self-organization for the creation of functional units is not an invention of modern natural sciences. It was already a basic idea of the ancient philosophies in Asia and Europe: only the mutuality of the parts creates the whole and its ability to function. Translated into the language of chemistry this means: the self-organization of molecules leads to supramolecular systems and is responsible for their functions. Thermotropic and lyotropic liquid crystals are such functional units, formed by self-organization. As highly oriented systems, they exhibit new properties. The importance of lyotropic liquid crystals for the life sciences has been known for a long time. They are a prerequisite for the development of life and the ability of cells to function. In materials science this concept of *function through organization* led to the development of new liquid-crystalline materials. From the point of view of macromolecular chemistry, this review tries to combine these two different fields and especially hopes to stimulate their interaction and joint treatment. To exemplify this, the molecular architecture of polymeric organized systems will be discussed. Polymeric liquid crystals combine the ability to undergo spontaneous self-organization—typical of liquid-crystalline phases—with the polymer-specific property of stabilizing these ordered states. As new materials, polymeric liquid crystals have already been investigated intensively. As model systems for biomembranes as well as for the simulation of biomembrane processes, they so far have been little discussed. The intention of this review article is to show that polymer science is able to contribute to the simulation of cellular processes such as the stabilization of biomembranes, specific surface recognition, or even the "uncorking" of cells. Polymer science, having an old tradition as an interdisciplinary field, can no longer restrict itself to common plastics. Attempts to reach new horizons have already begun. The borderland between liquid crystals and cells will certainly play an important role. Basic requirements to work in this frontier area between organic chemistry, membrane biology, life science, and materials science will be the delight in scientific adventures as well as the courage to go ahead. The most important prerequisite will be the willingness to cooperate with disciplines which so far have not really accepted each other. From this point of view, this review does not aim at giving defined answers. It wants instead to encourage the scientific venture: too often we cling to painfully acquired knowledge, fearing adventures.

To the question of what he was up to at the moment, Mr. K. answered: "I'm hard at work preparing my next error"
Bertolt Brecht[1]

1. Introduction: Polymer Science Today—Ancient Roots, Young Branches?[2]

1.1. Tradition and Innovation in Science

Two decisive aspects in the field of science are tradition and innovation. Tradition is the basis, for it is the cumulation of wisdom in the body of knowledge. To know what a subject is all about and to control it creates self-confidence, thus paving the way for innovations. Innovation is the adventure, since with the challenge comes the risk of calling into question (or even losing) one's own scientific identity, gained through tradition.

Persisting in tradition without innovation, however, soon leads to tiresome routine, to the science of yesterday: the longing for new adventures withers and dies.[3] On the other hand, pure innovation harbors the danger of superficiality. The sum of knowledge is immense and growing! Tradition and solid, successful work are honored and admired. Nevertheless, science can only be justified by a challenge, and demands the willingness to give up long-held classical or traditional views[4] in the attempt to discover new horizons.

1.2. One Example to Introduce the Topic: Order versus Chaos

Only a few years ago, it was still difficult to bring the evolution of life in line with the basic laws of physics. Ac-

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[**] The preceding page shows Figure 24.

cording to the principles of classical thermodynamics, disorder in the world was supposed to increase constantly. All controlled functional processes should therefore end one fine day; all order should dissipate. During the big bang, when the world evolved from chaos, there was no order at all. So how could organized structures, living organisms, develop from this start?[5]

It was a stormy development in the field of nonequilibrium phenomena that helped to resolve this dilemma. During the last few decades, the term self-organization has been synonymous with theories developed in various disciplines that deal with the further development, dynamics, and differentiation of systems. On the borderland between chemistry and biology, these include, in particular, concepts for the development of highly complicated organic molecules and the role they play in the evolution of biological information in a prebiotic world.[6b] Even the somewhat new overlapping discipline of synergetics,[6] with its concepts of ordered, self-organized, and collective behavior, had its share in this process.

The fascinating phenomena of self-organization, which can be observed in physical, chemical, and biological systems (either near to or far from thermodynamical equilibrium), are characterized by great variety and complexity. A large number of molecules organize spontaneously, eventually exhibiting well-organized behavior on the macroscopic scale. The variety of organized states ranges from relatively simple spatial or chronological forms of organization all the way to the complicated interaction between order and function in biological systems. Over the last few years, the phenomenon of self-organization has developed into a subject of interdisciplinary research with overlapping topics.[5,6]

1.3. From Polymeric Liquid Crystals to Functional Biomembrane Models?

Right from its very beginnings, polymer science was a field on the borderland between chemistry and physics, i.e., between two classical disciplines. Thus, scientists in this field had to dare to make innovations. It was no less a man than *Hermann Staudinger* who experienced this. It is highly instructive to observe how at the height of his creativity in the field of organic chemistry, he left his traditional field of work to enter the frontier between chemistry and physics in order to tackle the problems of macromolecular chemistry.[7] In the meantime, macromolecular chemistry, too, has become a classical discipline, a mature science, with all the advantages and handicaps of maturity. Harvest is plentiful, the results are abundant—but one has to ask: where is the future, where are the adventures?

Modern materials science once developed out of the interaction between metallurgy, ceramics, and macromolecular technology, and in the jubilee issue of *Angewandte Chemie* in honor of the 100th anniversary of *Hermann Staudinger*'s birth, the old "Geheimrat" *Hermann Mark* asked the question of what direction polymer science was about to take:[2] Is it going in the direction of life science? Is it possible to reduce polymer science, cell biology, and medicine to a common denominator? So from that point of view, polymer science finds itself not only on the borderland between chemistry and physics, but also between materials science and life science (Fig. 1).

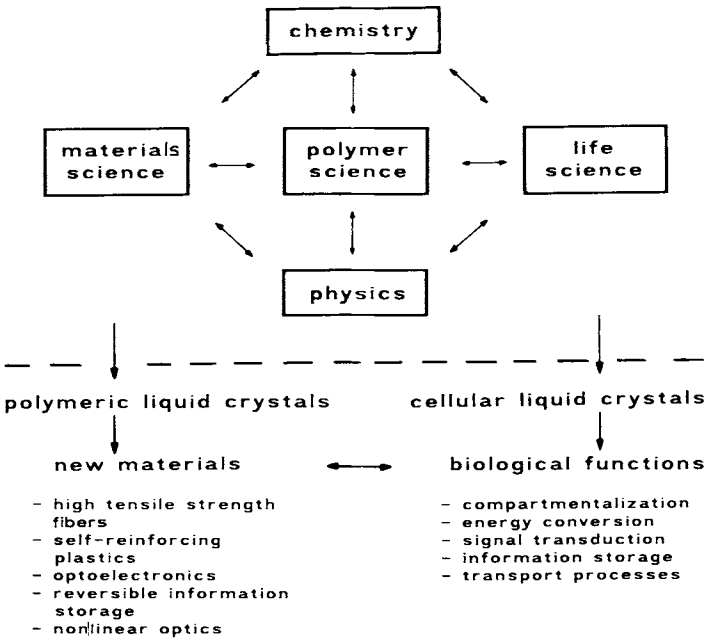


Fig. 1. Polymer science as an interdisciplinary field of science.

Taking self-organization and the molecular architecture of macromolecular systems as examples, this article aims to show that these fields of science can no longer be dealt with separately. In this review, the biological macromolecules of the cell such as polysaccharides or nucleic acids are not discussed as such. Molecular biology today is able to understand the central role which these polymers play in life science. But it has to be pointed out that their functions are based in all cases on the combination of molecular mobility and high order. This is achieved by their incorporation into membranes, by self-organization in solution, or by orientation at cell surfaces. These are combinations of properties which are typical of liquid-crystalline behavior. Thus, liquid crystals are chosen as *one* principal example of self-organizing systems, which are important for materials science as well as for life science (Fig. 1).

It was not by chance that, about 50 years ago, *J. D. Bernal* called the cell itself a natural liquid crystal, based on the knowledge of that time.[8] Liquid crystals really feature a behavior which is considered to be typical of a living cell: they react to a large number of stimulations such as light, sound, mechanical pressure, heat, and electric and magnetic fields as well as to changes in the chemical environment. The significance of cellular liquid crystals has been accepted for quite a long time. The significance of low-molecular-weight and of polymeric liquid crystals is on its way to becoming widely accepted. However, although the basic principles of self-organization are the same for both cellular and technical liquid crystals, only on rare occasions have they been looked at together.[9]

But what about the molecular architecture of the building blocks of self-organizing systems such as synthetic and biological liquid crystals and what about the structure of the resulting highly oriented supramolecular systems?

How does the molecular architecture relate to the functions of these materials? A hint as to possible answers to this problem will be given in an overview in Section 2. The goal is to stimulate interest in the overlapping fields of synthetic and biological liquid crystals in the broadest sense (cf. Fig. 2). In Section 3, thermotropic and lyotropic mesogens will briefly be defined and exemplified. Section 4 will show that scientists today are learning, step by step, what Mother Nature has long since known, namely to stabilize ultrathin, organized membranes and to functionalize them. Sections 5 and 6 will then concentrate on the connection between structure, function, and organization in biomembrane models. Even though the subject is still in its infancy, it is fascinating to observe how chemistry, physics, and membrane biology have to cooperate in order to mimic surface reactions (Section 5) and dynamic processes of biomembranes (Section 6).

Endocytosis and exocytosis, patching and capping—foreign words to chemists? Corkscrews for corked lipo somes?—a funny, maybe even nonscientific question? They originate from biology and are used on the borderland of polymer science between liquid crystals and the cell, an area of research which is only just beginning. The willingness to closely cooperate with adjacent disciplines, which hitherto have often been ignorant of one another,[10] will be a basic requirement to go ahead in this field. From that point of view, this article can neither be a perfect summary nor a presentation of detailed research results. To give definite answers is out of the question. It is simply meant to encourage innovations and scientific ventures: much too often we cling to our own knowledge out of the fear of new adventures.[11]

2. Self-Organization and Formation of Supramolecular Systems

Order and mobility are two basic principles of Mother Nature. On a molecular level two extreme cases can be found: the perfect order of crystals, in which the molecules exhibit almost no mobility at all, and the disordered state in gases and liquids, which exhibit randomness of highly mobile molecules. Both principles, order and mobility, are combined in liquid-crystalline phases. Although there are many possibilities for self-organization, the molecular basis is almost always simple: form-anisotropic or amphiphilic molecules make up the simplest building blocks. These already suffice—as shown in Figure 2—to construct a broad range of substances able to form supramolecular systems,[12] from thermotropic and lyotropic liquid crystals and the manifold micellar systems up to the highly ordered membranes in liposomes and cells.

In materials science, the significance of liquid crystals and micellar systems has long been known; it is based on exactly that combination of order and mobility in form-anisotropic molecules, leading to anisotropic (i.e., direction-dependent) properties of their materials and solutions.

From the life sciences we know that no life would be possible without the lipids' self-organization into bilayers within the cell membrane. In this case, too, order and mobility are related to the structure of functional units formed by lipids and proteins. In all cases, the functions of such supramolecular systems, formed by aggregation, are based on their organization.

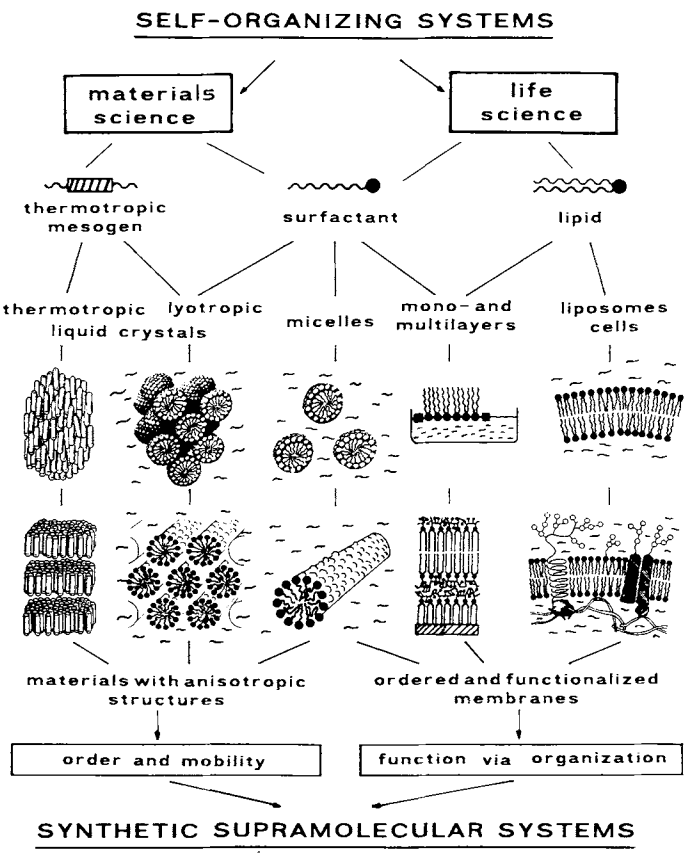


Fig. 2. Self-organization and supramolecular systems [12] in materials science and life science. The supramolecular structures range from simple nematic liquid crystals to complex biomembranes.

The self-organization and the construction of supramolecular systems is an interdisciplinary area which cannot be understood without the cooperation of different fields of science: chemistry alone does not fulfill that task nor does physics or biology. Over the last few years it has been possible to observe an increase in the number of studies which reveal the connection between synthetic and cellular liquid crystals and in which a mutual understanding opens up new perspectives. Macromolecular chemistry may play an important role in this context. On the one hand, macromolecules can stabilize organized systems without hampering their mobility. On the other hand, it is well known in macromolecular chemistry that creative curiosity for new compounds leads to new properties due to novel structures.

3. Molecular Architecture of Synthetic Self-Organizing Structures—or How to Attract Molecules to Form Supramolecular Systems

3.1. Structure and Properties of Thermotropic Polymeric Liquid Crystals

Numerous organic compounds with a rigid, rodlike molecular geometry (mesogens) do not directly go from the

crystalline into the isotropic state when they melt. Instead, they form intermediate liquid-crystalline phases (Fig. 3),[13] known as mesophases. These mesophases combine the order of the crystal with the mobility of the isotropic liquid. The parallel orientation of the longitudinal molecular axes is common to all mesophases (long-range orientational order). Two major classes can be distinguished: nematic (molecular centers distributed isotropically) and smectic (molecular centers organized in layers) phases. The individual smectic phases are further subdivided[14] into phases with or without tilt of the mesogens in the direction of the normal to the smectic layer. The molecules can either be in a disordered state within the respective layers or show different states of order. On the one hand, the driving forces

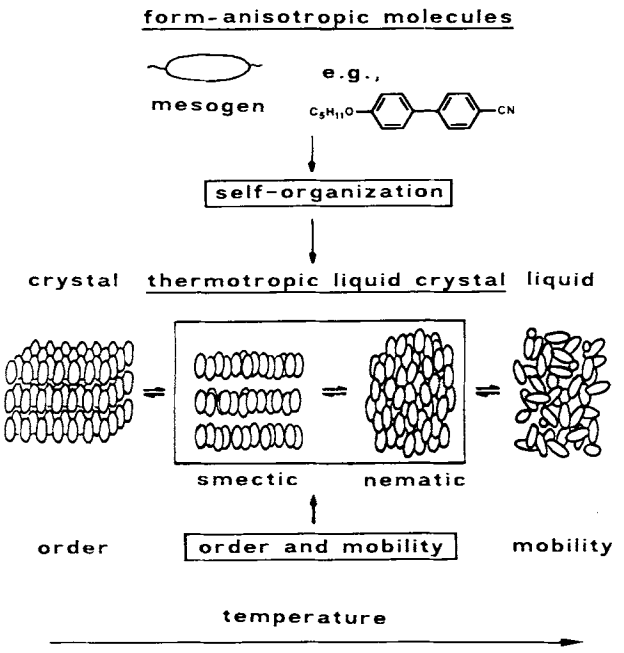


Fig. 3. Structure and phase behavior of thermotropic liquid crystals.

for the formation of mesophases are the anisotropic dispersion interactions[15] caused by the form-anisotropy of the mesogens. On the other hand, there are the steric repulsion forces[16] which are also orientation dependent.

The mesophases of rod-shaped compounds had been known for almost 100 years[17] when, in 1977, the discotic liquid-crystalline phases of disc-shaped molecules were found by *Chandrasekhar* et al.[18]. One can distinguish between a nematic discotic phase and several columnar discotic phases.[19] In the latter, molecular discs form stacks which, in turn, are organized in a two-dimensional lattice (cf. Fig. 5). These investigations reopened the game of molecular design of liquid-crystalline systems. So, additional phases were found: tubular,[20] pyramidic,[21] phasmidic,[22] and sanidic[23] phases. However, it still remains uncertain whether this is only a funny nomenclature or whether these are really new phases.

The development in the field of polymeric liquid crystals[24] started with polymers whose stiff main chain as a whole functions as the mesogen. Such polymers form liquid-crystalline phases either in solution[25] or in the melt.[26] At present, they are used as high-tensile-strength fibers (e.g., Kevlar®) or as thermoplastically processable, self-reinforcing plastics (e.g., Xydar®, Vectra®, Ultrax®).

Parallel to these industrial developments, numerous studies have been carried out in the area of basic research during the last ten years. They have dealt with the incorporation of rod-shaped mesogenic groups, known from low-molecular-weight liquid crystals, into polymers. This incorporation is either carried out by connecting the mesogenic groups via flexible spacers,[27] thus forming semiflexible liquid-crystalline (LC) main-chain polymers[28] (type B, Fig. 4) or, as in the case of the LC side-group polymers, by fixing the mesogens via flexible spacers to the polymer backbone[29] (type A, Fig. 4). Both types of liquid-crystalline polymers were, and still are, intensively investigated. Their structure–property relationships are discussed in numerous monographs[30] and reviews.[31]

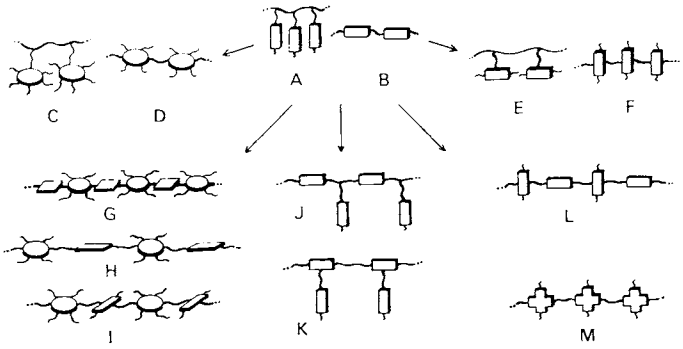


Fig. 4. Molecular architecture of polymeric liquid crystals: A) side-group polymers; B) main-chain polymers; C) discotic side-group polymers [32]; D) discotic main-chain polymers [33]; E) side-group polymers with laterally fixed mesogens [34, 35]; F) main-chain polymers with laterally linked mesogens [35]; G, H, I) combined disc-rod polymers, i.e., combinations of disc- and rod-shaped mesogens [23, 36, 37]; J, K) combined main-chain/side-group polymers [38]; L) polymers with laterally and terminally linked mesogenic groups; M) polymers with cross-shaped mesogenic groups [35].

From these two "classical" types (A and B), variations of the macromolecular architecture of liquid-crystalline polymers have recently been carried out. They are illustrated schematically in Figure 4.

On the one hand, it is possible to realize discotic phases, as observed in the case of low-molecular-weight liquid crystals, by using side-group[32] (type C) and main-chain[33] (type D) polymers. On the other hand, it is, of course, also possible to incorporate rod-shaped mesogens in different ways than in the two classical types A and B: examples are side-group polymers[34,35] (type E) as well as main-chain polymers[35] (type F) with laterally fixed mesogens. These strange-looking architectures do not prevent the formation of liquid-crystalline phases. As far as polymers of type E are concerned, the limited rotation of the mesogenic groups around their longitudinal axes leads to the formation of biaxial nematic phases.[34a-c] These phases have not been observed yet with low-molecular-weight liquid crystals. The third group of structural variations makes use of a well-known principle in polymer chemistry, which is rarely used in the field of liquid-crystalline materials: the com-

binations of different structural elements and different building principles to design one macromolecule. This includes the different combinations of rod- and disc-shaped mesogens[23, 36, 37] (types G, H, and I) and the combination of the structural principles of the two classical types of liquid-crystalline polymers (types A and B) in the combined main-chain/side-group polymers[38] (types J and K). Furthermore, the polymers with cross-shaped mesogens[35] (type M) should be mentioned, as well as the idea of combining laterally and terminally connected mesogenic groups (type L), which has not yet been realized.

Here only two examples are given to illustrate the structure-property relationships of such new polymeric liquid crystals. Figure 5 shows a discotic main-chain polymer[33a, b] of type D. This one features a columnar phase which can be mechanically oriented and frozen below the glass transition temperature. It is noteworthy that this polymer forms a highly ordered D_{ho} phase. Thus, the polymer chains do not disturb the packing of the discs.

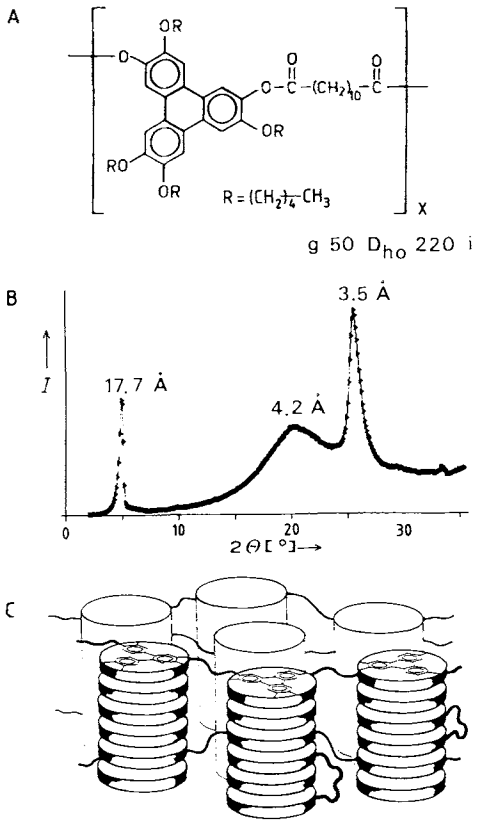


Fig. 5. A) Chemical structure and phase behavior of a discotic main-chain polymer with triphenylene moieties as core of the discogen [33a] (g = *g*lassy; D_{ho} = *d*iscotic *h*exagonal *o*rdered; i = *i*sotropic). B) X-ray scattering diagram (λ = 1.54 Å) of the polymer (I = scattering intensity, θ = scattering angle) [33b]. The scattering maxima are given by the Bragg spacings and can be interpreted in analogy to the values found for low-molecular-weight triphenylene derivatives [19b] as follows: 17.7 Å corresponds to the columnar distance in the hexagonal lattice; 4.2 Å corresponds to the nonoriented liquid-like arrangement of the alkyl chains; 3.5 Å corresponds to the constant distance of the triphenylene cores in the column. C) Structural model for the D_{ho} phase of the discotic main-chain polymer based on results from X-ray measurements of mechanically oriented samples [33b]. The columns are perpendicular to the stretching direction.

In Figure 6, a combined main-chain/side-group liquid-crystalline polyester of type J (A) is compared to one of type K (B). Depending on the point of attachment of the mesogenic side group, either mostly smectic (A) or nematic (B) mesophases are observed.

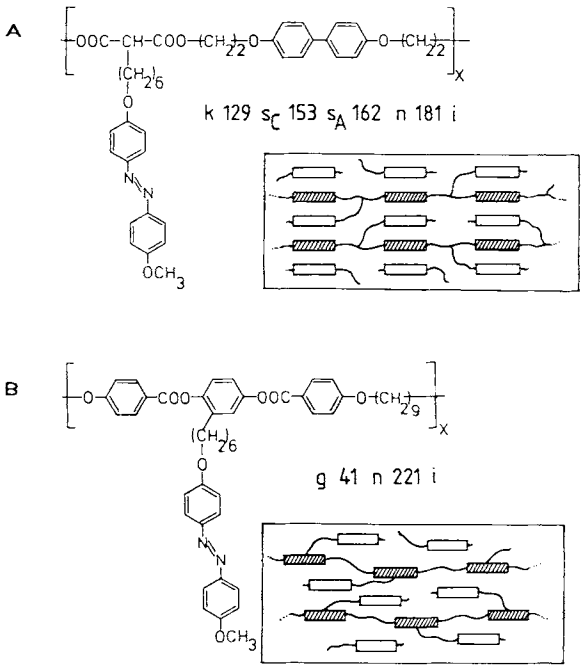


Fig. 6. Chemical structure, phase behavior, and structural models of the mesophase of combined main-chain/side-group liquid-crystal polymers. A) The attachment of the mesogenic groups to the flexible spacer of the polymalonate main chain [38a] favors smectic mesophases (s). B) The attachment of the mesogenic side groups directly to the polyester main-chain mesogens [38b] leads to broad nematic phases (n).

3.2. Structural Variations in Micellar and Lyotropic Liquid-Crystalline Systems

Contrary to thermotropic ones, lyotropic liquid-crystalline systems always consist of at least two components. One of them is a solvent, which plays a crucial role in determining order and mobility. The state of lyotropic liquid crystallinity is not primarily achieved by a change in temperature, but, as shown in Figure 7, by a change in solvent content.[39]

With decreasing solvent content, molecules that are isotropically dispersed at lower concentrations only form aggregates. These aggregates organize into nematic phases or into the more ordered lamellar (comparable to the thermotropic smectic phases), columnar, and cubic mesophases. Nematic phases differ from the isotropic solution by the long-range orientational order of the aggregates only, lamellar and columnar phases additionally by a long-range positional order of the aggregate centers in one or two dimensions; cubic phases merely show a long-range positional order in three dimensions. On further decreasing the solvent content, solvent-free crystals are formed—often via solvent-containing crystals. As typical of amphiphiles, lyotropic mesogens are composed of soluble (solvatophilic) and insoluble (solvatophobic) moieties within one molecule. Fatty acid salts and long-chain alkyl ammonium salts are representatives of this type of structure. In aqueous solution, they aggregate—if their concentration lies above

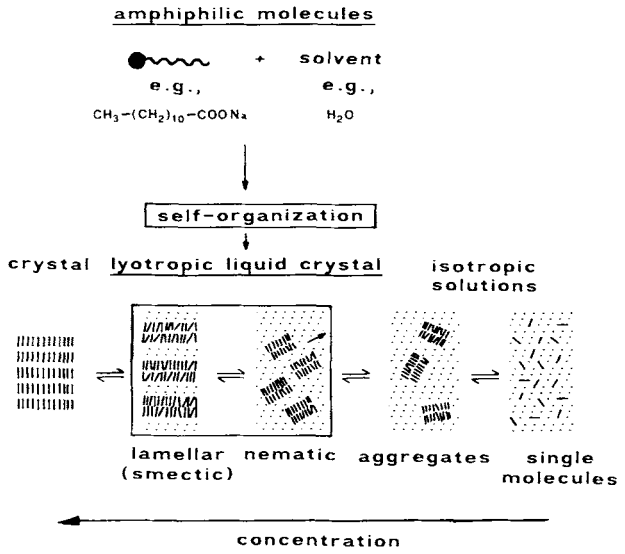


Fig. 7. Structure and phase behavior of lyotropic liquid crystals.

the critical micelle concentration (CMC)—to micelles, which may be spherical, cylindrical, or plate-shaped (Fig. 8).

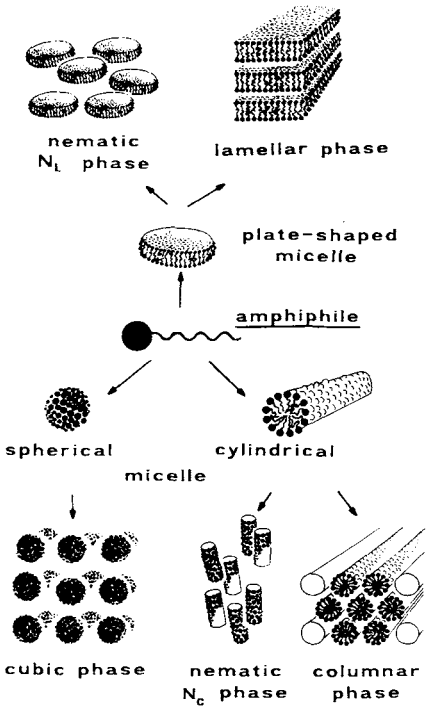


Fig. 8. Aggregation of amphiphiles to form micelles of various shapes and the formation of lyotropic mesophases from these micelles.

For spherical micelles, intermicellar interactions lead to cubic mesophases; for cylindrical micelles, to nematic N_C as well as to columnar mesophases. Plate-shaped micelles can form an additional type of nematic phase (N_L) and further a lamellar mesophase.[40] Thus, the different types of lyotropic mesophases are not directly determined by the structure of the single molecule (mesogen)—as is the case of thermotropic systems—but by the structure of the micelle[41] or the aggregate. The structure of the micelle, however, depends on several factors, such as temperature, concentration, and the structure of the amphiphile itself. The latter refers, in particular, to the hydrophobic-hydrophilic balance between the head group and the alkyl chain as well as to the geometry of the amphiphile.[42] Most of the amphiphiles form micelles of different shapes and thus often several types of lyotropic mesophases.

It is mainly this complexity of the aggregation behavior that has stimulated the syntheses of various types of amphiphilic compounds. So as to get to specific supramolecular systems, one now tries to influence the shape, size, and stability of the aggregates through molecular architecture of low-molecular-weight and polymeric amphiphiles. Two different approaches have been taken so far (Fig. 9).

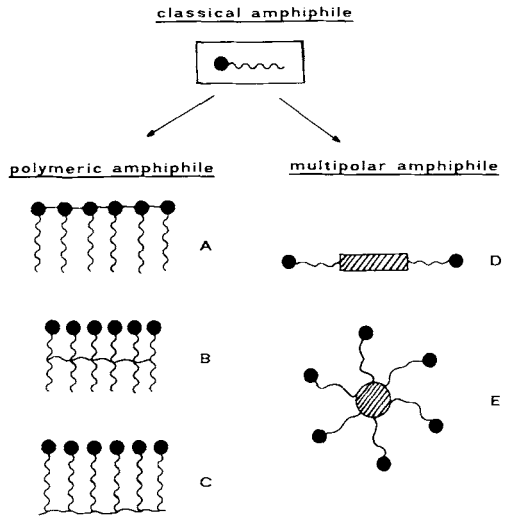


Fig. 9. Molecular architecture of polymeric (A, B, C) and multipolar amphiphiles (D, E).

The first approach starts from polymeric amphiphiles. Although they have long been known, the relationship between their structure and mesophase behavior has hardly been investigated. As depicted in Figure 9, amphiphiles can be synthesized with the polymerizable unit in the head group, in the hydrophobic chain, or attached to its end. Their polymerization leads to polymers of type A, B, or C, with the mobility of the amphiphiles, which are now fixed by the polymer, being extremely different from that of the monomeric compounds. As a consequence, aggregation and mesophase behavior may also change. In view of the few examples known,[43] hardly any changes have been observed as to the type of mesophase—not so as to the concentration and temperature ranges which were largely extended through polymerization. This is demonstrated by the amphiphilic polymer of type C shown in Figure 10.[43a]

Monomer **1** and the respective polymer only form lamellar phases in water. The monomer exhibits lyotropic liquid-crystalline behavior within a concentration range of 65–88 wt%, whereas the polymer exhibits such behavior in the range of 37–100 wt%. The maximum clearing tempera-

ture of the lyotropic mesophase of the monomer is 87 °C, that of the lyotropic mesophase of the polymer, however, 185 °C.

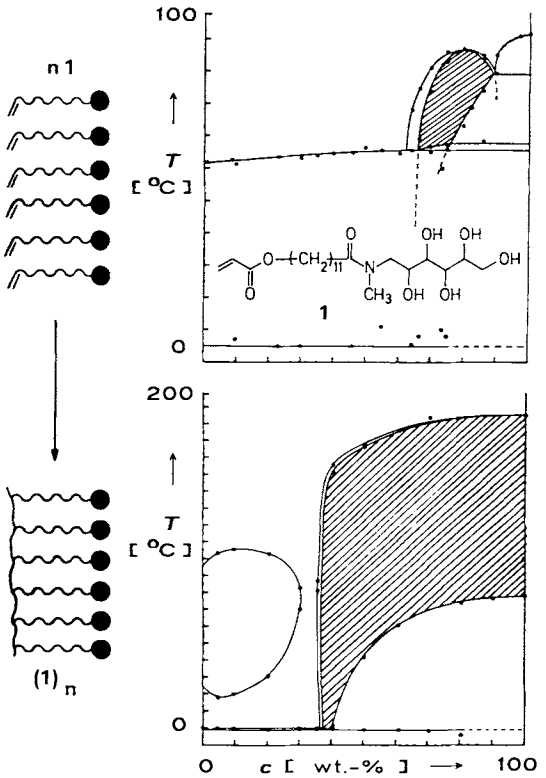


Fig. 10. Extension of the concentration and temperature ranges of the lyotropic mesophases (hatched) of **1** due to polymerization [43a].

The second approach to achieve a specific type of mesophase starts from amphiphiles of novel structure. These new structures combine the molecular design of thermotropic and lyotropic mesogens (see Section 3.3), i.e., the form-anisotropy of thermotropic liquid crystals (rod- and disc-shaped) with the amphiphilic character of lyotropic liquid crystals (see Fig. 9, types D and E). Some examples are shown in Figure 11. If, for instance, the alkyl chains of rod-shaped thermotropic liquid crystals are replaced by hydrophilic chains,[43i,k,44] rod-shaped bipolar amphiphiles result (e.g., **2** and **3**). In water, compound **2** forms only platelike aggregates, which arrange into a lamellar or smectic phase in a concentration range of 53–84 wt% at temperatures between 8.7 °C and 33.5 °C.[43i] As for the rod-shaped amphiphiles **2** and **3**, disc-shaped multipolar amphiphiles (e.g., **4**–**7**) are obtained by introducing hydrophilic chains into discotic molecules such as benzene or triphenylene derivatives. The amidation of the triphenylene-hexayl hexaester **8** with amino alcohols to form hexaamides,[47] e.g., **7** (Fig. 12), provides a simple reaction that involves this transformation of a thermotropic discotic liquid crystal to a lyotropic discotic liquid crystal. In this case, the structural similarity between the thermotropic discotic mesogen and the new lyotropic discotic mesogen is responsible for the same type of mesophase, namely, the columnar one. The lyotropic mesophase is formed by cylindrical aggregates present in the isotropic solution.

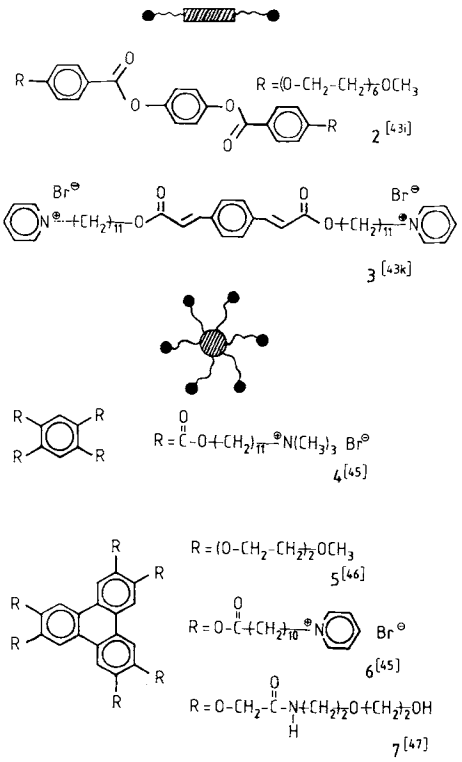


Fig. 11. Examples of multipolar amphiphiles (types D and E from Fig. 9).

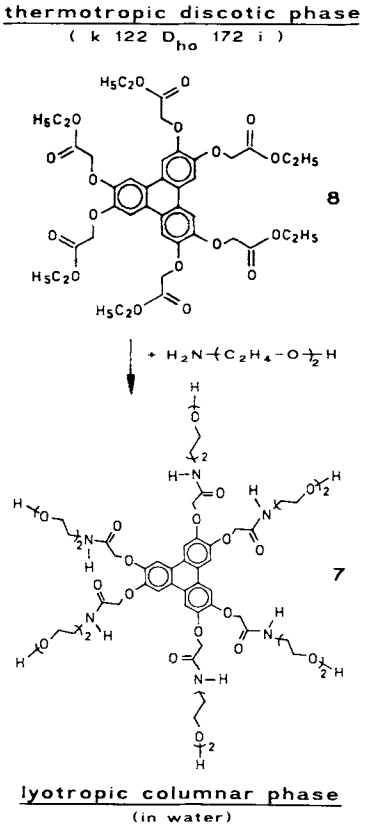


Fig. 12. Transformation of a thermotropic discotic mesogen to a multipolar amphiphile [47] (k = crystalline). Distances between the columns in the thermotropic phase (cf. Fig. 5C) at 140 °C, 19.2 Å; in the aqueous lyotropic phase (cf. Fig. 8) at 20 °C and 60 wt%, 28 Å.

3.3. Amphotropic Systems—All-round Compounds

In lyotropic mesophases, the combination of order and mobility can be achieved by using a solvent. The formation of thermotropic liquid-crystalline phases is based on the temperature-induced mobility of form-anisotropic molecules in the melt.

Amphotropic molecules (Greek: ampho = both, in two ways; trepein = align, direct) are compounds which can be organized according to at least two different principles owing to their structural properties. Figure 13 illustrates this using an example from the field of liquid crystals.

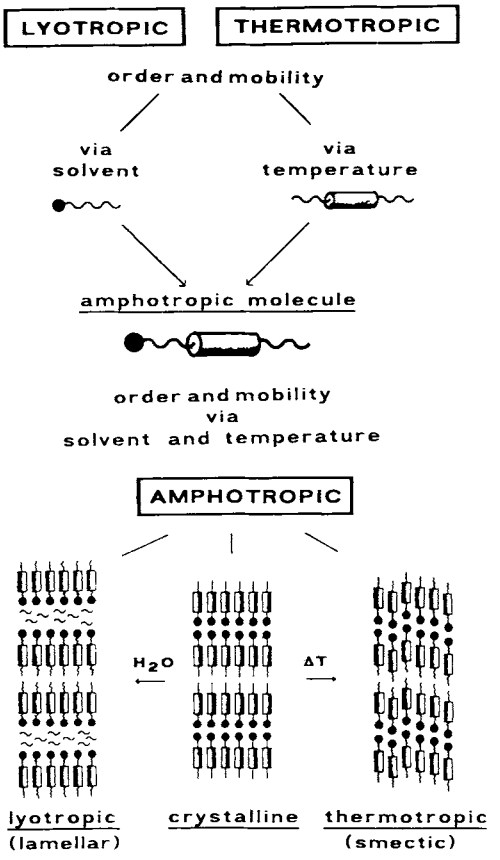


Fig. 13. Schematic representation of an amphotropic system.

Although the term amphotropic liquid crystal has not been used so far, there are many examples of amphotropic behavior:[39b, 43a, 48] some phospholipids,[48a] alkylated monosaccharides,[48b, c] and amphiphilic metal salts[39b, 48d] not only form lyotropic but also thermotropic mesophases. As regards their molecular structure, these molecules are normal amphiphiles. However, they reveal amphotropic behavior.

The range of amphotropic compounds can be considerably increased by the additional incorporation of typical thermotropic liquid-crystal building blocks (rod- or disc-shaped) into amphiphiles. *Kunitake* et al. have investigated a large number of amphiphilic substances which contain rod-shaped mesogens in the hydrophobic chains (e.g., biphenyl, azobenzene, diphenylazomethine moieties).[48e] Apart from various ordered structures in the aqueous phase, thermotropic liquid-crystalline behavior was observed for some of these compounds. Moreover, some polymers also show amphotropic properties.[43a, 48f, g, h] For example, it is possible to organize modified hydroxycellulose derivatives[48f, g] and various polyisocyanates,[48h] not only lyotropically, but also thermotropically.

As for amphotropic molecules, promising perspectives turn up in the field of Langmuir-Blodgett (LB) multilayers.[49, 50] A large number of possible technical applications of these ordered layer systems are nowadays a focal point of discussion. They could be used, for example, as electron beam resists[51] and photoresists,[52] in nonlinear optics (NLO),[53] in the field of reversible information storage,[54] and in the development of bio- and immunosensors.[55, 82]

LB multilayers show—owing to the stepwise transfer mechanism—a well-defined layer structure in the vertical direction. However, within each individual layer, the amphiphiles form two-dimensional lattices with a distinct domain structure (Fig. 14).

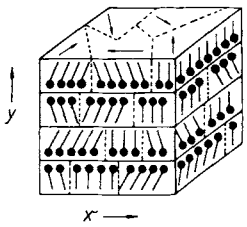
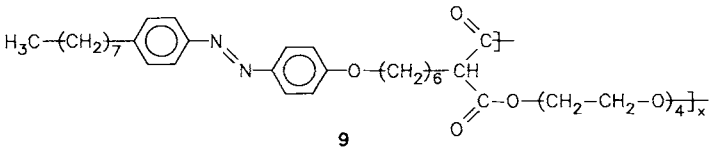


Fig. 14. Structure of LB multilayers: perfectly ordered layers in vertical direction y; irregular domain structure in horizontal direction x.

The crystalline packing of the molecules in the individual layers and the related severe limitation of the molecular mobility have so far not allowed a subsequent, specific manipulation of the molecular order. It has not yet been possible to obtain homogeneous, domain-free (i.e., uniformly oriented) lipid layers. Indeed, it is possible to induce a slight mobility of the molecules and thus a minute change in orientation by heating the multilayers within a certain temperature range. The melting of the layers, however, usually leads to the irreversible collapse of order.[56] On the one hand, amphotropic molecules are amphiphiles, i.e., capable of building up LB multilayers. On the other hand, they are thermotropic liquid crystals owing to the incorporation of mesogenic building blocks. Thus, amphotropic molecules could be used as an approach to introduce mobility within the individual layers, necessary for the subsequent manipulation process. The orientation of the molecules in the individual layers could then, for example, be carried out by means of electric or magnetic fields (Fig. 15).

Mesogen-containing amphiphiles have already been investigated in multilayers.[57] The example discussed here demonstrates that it is indeed possible to introduce thermal mobility in multilayers by using amphotropic molecules. The polymeric malonate **9** contains azobenzene



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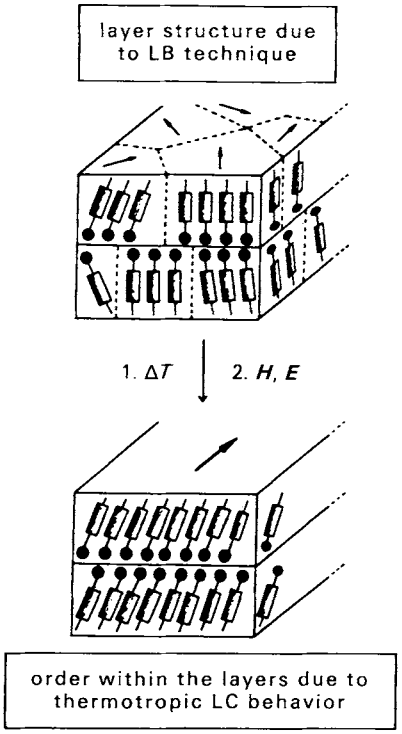


Fig. 15. LB multilayers made from amphotropic molecules: orientation within the layers by electric (*E*) or magnetic (*H*) fields in the mobile LC phase.

moieties as mesogens and an ethylene glycol main chain as the hydrophilic part.[58] In bulk, the polyester **9** is liquid crystalline (g - 275 55i). At the gas/water interface it forms a solid-analogous monolayer, which can be transferred onto solid substrates by means of the LB technique. The organization of the layers has been demonstrated by using small angle X-ray scattering (Fig. 16).

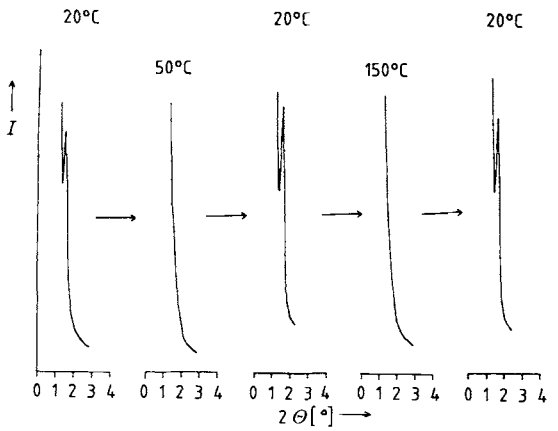
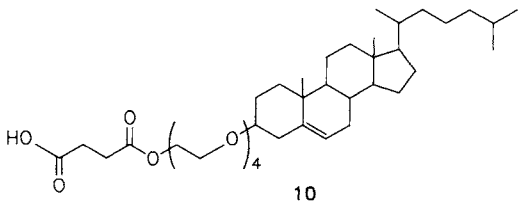


Fig. 16. Temperature-dependent small angle X-ray scattering of 30 layers of the polymer **9** on a polyethylene-therephthalate support: layer structure reappears at 20°C after melting above 50°C.

If the temperature is increased to about 50°C, the scattering reflex in the X-ray diagram disappears. The multilayer melts and the highly ordered structure is lost. It is decisive, however, that the capacity to reorientate is maintained: after cooling to 20°C, the scattering reflex reappears with its original intensity. Even after heating to 150°C, the multilayer structure reassembles at room temperature. The incorporation of mesogenic building blocks into the amphiphilic system and their fixation to the polymer seems to prevent a direct isotropic melting. Although the layer correlation is lost, the parallel alignment of the polymer-fixed mesogenic amphiphiles is maintained (cf. Fig. 13).

The field of molecular engineering of thermotropic and lyotropic liquid crystals is broad. The search for compounds capable of self-organization and formation of specific supramolecular structures will lead to numerous new classes of substances. The first examples have already been described.[48d, 59]

One compound that is capable of self-organization in manifold ways—and thus amphotropic—is the cholesterol derivative **10**. Its different possibilities of orientation are described in Figure 17.[60]



Owing to its form-anisotropy, this compound forms a thermotropic mesophase of the type "bilayer smectic S_A" between −28°C and 113°C (g −28 S_A 113 i). If it is slowly cooled, the substance solidifies at −28°C in the form of an anisotropic glass which does not crystallize even after a long period of annealing.

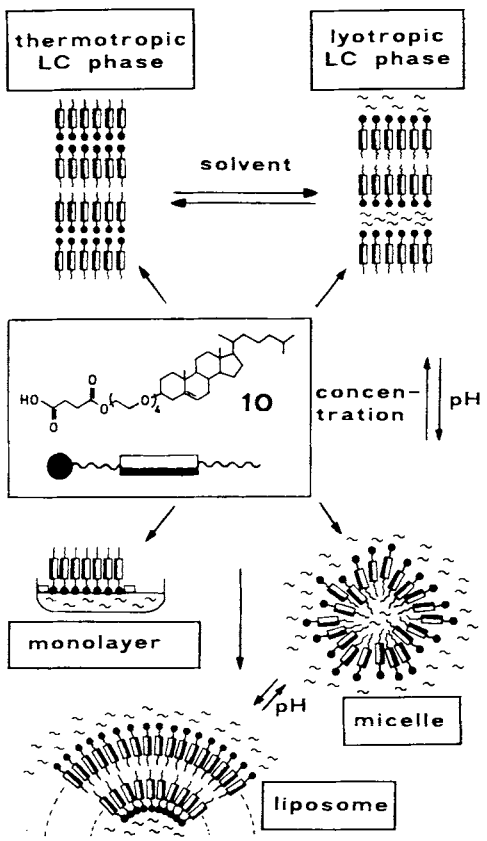


Fig. 17. Example of an amphotropic system: various ways of self-organization of the cholesterol derivative **10** [60].

Since the cholesterol derivative **10** is also an amphiphile, it can form numerous supramolecular structures in aqueous media: lyotropic LC phases, liposomes, micelles, and ordered monolayers. A lyotropic lamellar phase can be obtained by swelling in water or even in absolute diethylene glycol. In both media, spherically closed lipid bilayers (liposomes) develop spontaneously from the lamellar phase after further dilution. The formation of liposomes was demonstrated by means of entrapment of a water-soluble fluorescent marker as well as by phase contrast and electron microscopy. In addition, pH variations lead to the reversible formation of micelles. With a value of 1.6×10^{-3} mol L^{-1} (pH = 9.3), its CMC is in the range of normal surfactants. Drying of the aqueous aggregates leads back to the thermotropic liquid crystal.

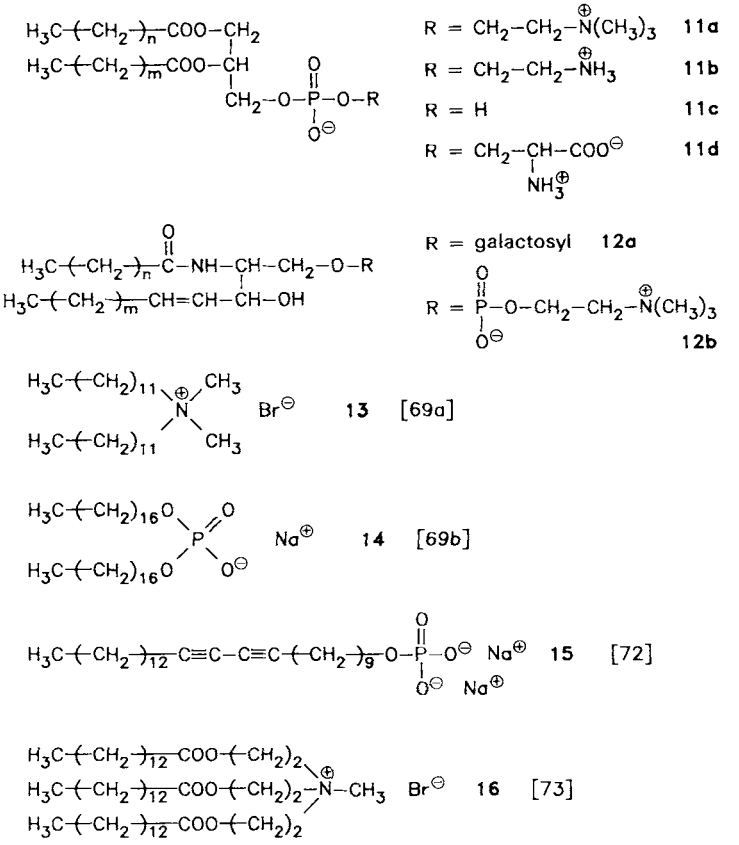
As vesicle-forming molecules, ethylene oxide cholesterol derivatives[60,61] possess an atypical structure, since the hydrophobic portion of bilayer-forming lipids usually consists of fatty acid chains. The molecular architecture of these "normal" lipids will be dealt with in greater detail in the next section.

3.4. Lipids as Bilayer-Forming Amphiphiles

Whereas the micelle-forming amphiphiles discussed in Section 3.2 still show a rather high solubility in water (CMC about 10^{-3} mol L^{-1}), it is a lot lower for membrane-forming lipids (CMC about 10^{-8} mol L^{-1}). Lipid molecules, i.e., double-chain amphiphiles, are the basic building blocks of all biological membranes.[62,63] Their self-organization in water is the result of the hydrophobic effect,[64] as is the case with surfactants, and depends also on the relative proportion of hydrophobicity and hydrophilicity of the lipid, as well as on its geometry.[42]

The phase diagram of a mixture of water with a typical lecithin lipid such as dipalmitoylphosphatidylcholine (DPPC) features the lipid bilayer as the basic structure over a very broad temperature and concentration range.[65] Depending on the water content, homogeneous, smectic phases of parallel lipid bilayers (lyotropic phases) and heterogeneous dispersions of multilamellar or single-walled liposomes can be observed. For low water content and high temperature, other lyotropic liquid-crystalline phases exist, such as the hexagonal, the cubic, and the ribbon phase.

In accordance with the importance of lipids for the formation of biomembranes, Nature is equipped with a wide range of possibilities in order to vary the molecular architecture of its membrane-forming amphiphiles (Scheme 1). Most of the natural lipids are zwitterionic (e.g., lecithin **11a** or ethanolamine **11b**) or negatively charged (e.g., phosphatidic acid **11c** or phosphatidylserine **11d**). There are also uncharged lipid structures found in Nature (e.g., glycolipids[66] such as **12a**). Not only the head-group structure but also the hydrophobic region is varied to a large extent. Depending on whether rigid or fluid membranes are needed, either saturated or unsaturated fatty acid chains are incorporated, respectively. Hydrogen bonds between the head groups, as, for example, in the case of sphingolipids (e.g., **12**), lead to an additional stabilization of the membrane.[67]



Scheme 1. Examples of natural and synthetic bilayer-forming amphiphiles.

Many of the lipids occurring in Nature have been synthesized.[68] In 1977, *Kunitake* et al. described fully synthetic amphiphiles having simple structures, which, just like natural lipids, are capable of forming bilayer membranes. Typical examples are the quaternary ammonium salts (e.g., **13**) and dialkyl phosphates (e.g., **14**) with two long chains.[69] The idea that even such simple amphiphilic structures can form bilayer membranes soon resulted in a large arsenal of artificial membrane-forming lipids.[70] These also include chiral compounds,[71] as they usually occur in Nature. Apart from the double-chain "classical" lipids discussed so far, synthetic bilayer-forming amphiphiles with only one (e.g., **15**[72]) or three (e.g., **16**[73]) hydrophobic alkyl chains also exist. Furthermore, there are membrane-spanning lipids; some of these bipolar amphiphiles will be presented in Section 4.7.

4. Stabilization and Surface Variation of Model Membranes

4.1. The Architecture of Biomembranes[74,75]

A decisive step in the development of the early forms of life was the self-organization of amphiphiles to form plasma membranes. It is unknown which principles led to the formation of macromolecules from purines, pyrimidines, or amino acids, but their self-replication would not have been possible without the protective shell of the lipid membrane. The cell membrane plays an important role in all essential biological phenomena. These include prob-

lems related to biological transport, energy metabolism, cell division, and macromolecular synthesis. The cell surface, in particular, is of significant importance as regards many aspects of cell biology, immunology, and cell-cell interaction as well as cell differentiation and the transformation of normal cells into malignant ones.

The biomembrane can be divided into three layers that are connected with one another: glycocalix, protein-lipid bilayer, and cytoskeleton (Fig. 18). The middle, protein-containing lipid bilayer is the component which indeed separates the cell into compartments.[76] Furthermore, it functions as an active filter and participates in motion and transport processes.

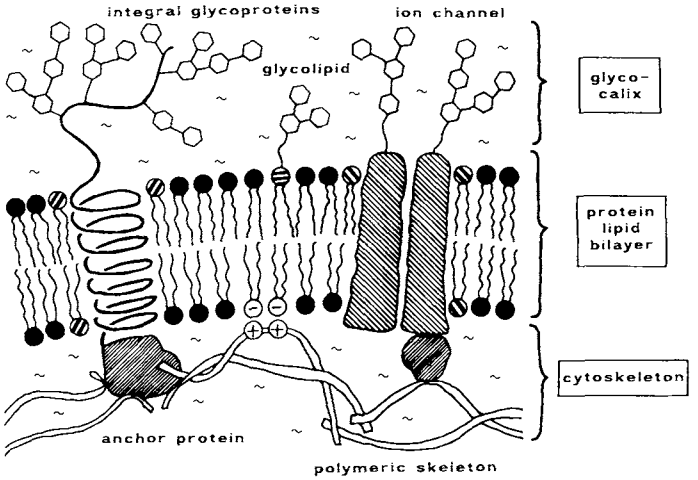


Fig. 18. Schematic representation of a biomembrane. The three layers are the glycocalix, the protein-lipid bilayer, and the cytoskeleton.

Outside, the phospholipid membrane is coated by a carbohydrate-rich layer, the glycocalix. It mainly consists of the oligosaccharide head groups of the glycoproteins and glycolipids, which are usually incorporated into the membrane. The glycocalix determines the surface recognition of cells: the oligosaccharide side chains, for example, are antigenic determinants in reactions of the immune system (see Section 5). In the case of plant cells, the glycocalix usually occurs as a solid, closed cell wall, in which the sugar groups (polysaccharides) additionally act as a skeleton (see Section 4.8.1). In the case of bacteria, the polysaccharide chains are cross-linked by oligopeptide chains. Thus, they form an extremely stable murein coat which represents one single macromolecule.

In animal cells, the stabilization of the cell membrane is mainly achieved by the cytoskeleton, which is linked to the inner side of the central lipid bilayer (see Section 4.8.2). From the point of view of polymer science, the cytoskeleton, in particular, as a polymeric network, represents an ideal basis for simulation experiments: reversible cross-linking, fixing of networks to membranes, dynamical processes in gels—these are all processes the cell constantly carries out in manifold ways.

To get a deeper insight into the structure-function relationships of biomembranes, a large number of bioorganic investigations are necessary. This is therefore an attractive and broad field of activity for synthetic chemists. However, natural membranes show a high complexity. Thus, in order to study the properties of individual membrane components, it is necessary to concentrate, at first, on simple model membranes having a defined chemical composition.

4.2. Model Membranes as Supramolecular Systems

In order to study the structure-property relationships of individual membrane components (lipids or proteins) at a supramolecular level, one has to rely on model membranes. On the one hand, they can provide information about the physical properties of membranes that only consist of lipids (pure or in mixture). On the other hand, in reconstitution experiments, membrane proteins can be incorporated into a physically and chemically defined surrounding. Then, the activity of the individual membrane proteins can be studied. Figure 19 gives a survey of the commonly used model membrane systems.

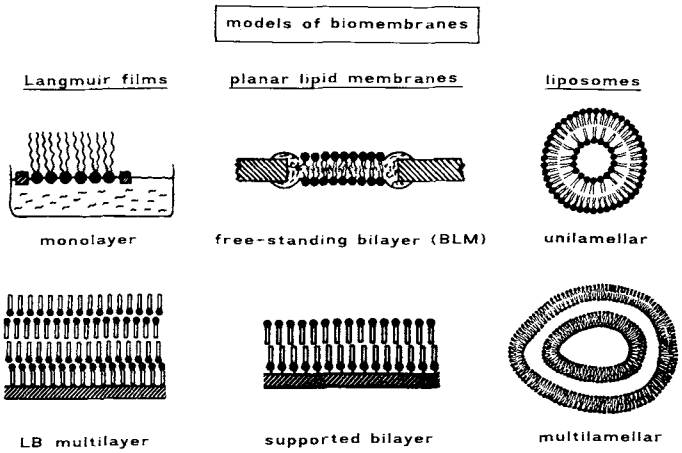


Fig. 19. Commonly used model membranes.

The spreading of a lipid at a gas/water interface leads to the formation of monolayers.[49c, 77] Formally, they represent only one-half of the lipid bilayer of a cell membrane. Thus, the monomolecular films—compared with other model membranes—seem to be the least similar to biomembranes. Nevertheless, investigations of monolayers supply precise information about the molecular packing and orientation of amphiphiles which cannot be gained from other model membranes. With the help of a film balance, monomolecular films can be characterized in the form of surface pressure-area diagrams (isotherms). This is a very sensitive method which responds to the slightest change in the structure of lipids. By means of isotherms, it is possible to gain information not only as to the orientation behavior of amphiphiles, their mobility in membranes, but also as regards the interactions with substances dissolved in the subphase (e.g., proteins; see Sections 5.2 and 5.3). Furthermore, by using a fluorescence microscope, phase-separation processes within the monolayer can be visualized.[78]

Highly ordered, ultrathin Langmuir-Blodgett (LB) films are obtained by transferring monolayers onto solid substrates.[49, 50, 79] As model membranes, multilayers are less

interesting. They seem to be technically more important as functional ultrathin films (see Section 3.3).

Planar lipid bilayers resemble much more the structure of biological membranes. They can be prepared either as free-standing black lipid membranes or as supported planar membranes. Black lipid membranes (BLMs)[80] are lipid bilayers which span a hole in a separating wall between two aqueous compartments. That is why they are ideal to carry out electrical measurements from which conclusions can be drawn as regards membrane capacity, thickness, and resistance. Thus, they are often used to study transport processes (e.g., of ions) through lipid membranes.[81] Supported planar membranes permit the use of measuring methods that cannot be applied to other model membranes.[82] If, for instance, such a planar membrane is fixed on a quartz substrate, one can detect fluorescence probes by means of TIRF spectroscopy (TIRF: total internal reflection fluorescence) only when they are close to the surface of the lipid bilayer. Therefore, such supported membranes are especially suitable for the investigation of surface recognition reactions.[82, 83, 163b]

Liposomes (vesicles)[84] are spherically closed lipid bilayers, which, in analogy to the cell membrane, enclose an aqueous compartment. Vesicles can be prepared by numerous methods,[84h, 85] which lead to the formation of completely different vesicle systems. These differ in diameter (between 20 nm and 100 μm) as well as in the number of bilayers. The sonication of lipid suspensions in water, for example, leads to small unilamellar vesicles (SUV, 20–100 nm). The swelling of lipid films, however, leads to uni- or multilamellar vesicles of the size of a cell. Liposomes are suitable for a large number of biophysical and biochemical investigations: e.g., for the measurement of membrane permeability,[84a] for the reconstitution of active membrane proteins,[86] and for the study of surface recognition reactions[84d, e] or dynamic membrane processes.[155] Furthermore, the possible use of liposomes as drug carriers is also being discussed.[87, 144a]

The five following sections (Sections 4.3 to 4.8) mainly deal with liposomes as model membranes. Apart from their surface variations, strategies for the preparation of polymerized liposomes are also discussed. On the one hand, these attempts should lead to more stable model membranes; on the other hand, they should help to simulate the stabilizing elements of the biomembrane.

4.3. Surface Variation of Model Membranes

In addition to direct variations of the membrane components, chemical surface variations of the cell membrane also play an important role in Nature: the generation of asymmetrical membranes or variations of the glycocalix are important examples. In model membranes, the molecular architecture of lipids can subsequently be varied within the supramolecular structure by means of chemical reactions. Such reactions in ordered systems are of interest because the highly organized lipid matrix represents a medium to control them. Differences in the course of these reactions, compared with the ones in isotropic solution, are caused by the orientation or the local concentration of the molecules involved.[88] Such reactions can be regarded not only as models for the transformation of lipids within biomembranes, but also, more generally, as models of enzymatic catalysis.[89] If vesicles are used as model membranes, a separation into different reaction sites[70e] (entrapped volume, hydrophobic interior of the membrane, membrane surface, aqueous outer volume), i.e., a spatial division of reaction partners, has to be considered. Thus, vesicles have been used, for example, to study artificial photosynthetic processes.[90] Moreover, the formation of spherically closed lipid bilayers by using reactive lipid molecules allows reactions to be carried out with water-soluble agents located only at the outer membrane surface. Thus, vesicle membranes with an asymmetrical distribution of head groups can be prepared.[70c, 90a] In the following examples, some reactions taking place at the surface of ordered membranes are illustrated.

The transformation of a negatively charged membrane surface to a positively charged one, under mild conditions, has been investigated in monolayers.[91] The maleic acid monoamide **17** with a carboxylic acid head group was used as an anionic reactive lipid. Under acidic conditions, the *cis* carboxyl group of **17** catalyzes the intramolecular hydrolysis of the amide bond.[92] In the course of this process, the dioctadecylammonium ion is formed. In order to observe the cleavage process, **17** was spread onto an acidic subphase. The isotherms of the acid, as well as that of the ammonium salt formed by the cleavage of the amide bond, are shown in Figure 20.

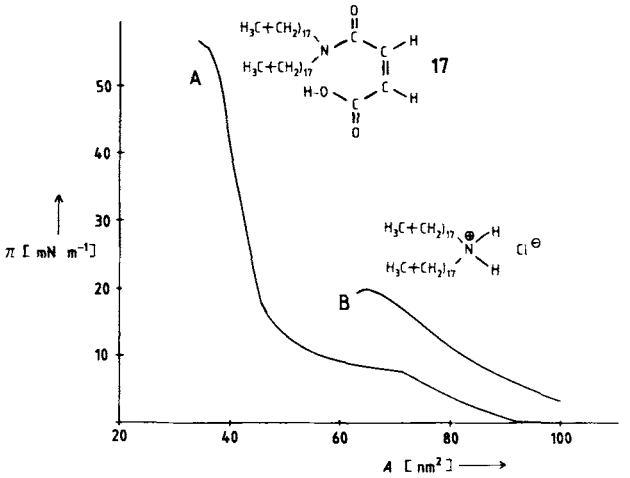


Fig. 20. Variation of the head-group charge in a monolayer [91]. A) Isotherm of the maleic acid amide **17** ($T = 31$ °, pH of the subphase = 2). B) Isotherm of a monolayer from the amide **17** after head-group cleavage with the formation of the corresonding ammonium salt. The cleavage was achieved by leaving the film for 14 h at 0 mN m^{-1} and 31 °C on the acidic subphase (pH = 2). π = surface pressure, A = area per molecule.

Apart from a liquid-analogous phase, **17** also features a solid-analogous phase (curve A). In contrast, the isotherm of the product, the ammonium salt, shows only a liquid-analogous film (curve B). This behavior is in good agreement with the isotherm of a reference sample of dioctadecylamine. The formation of this amine in the monolayer was also demonstrated by means of thin layer chromatography and by mass spectrometry. The intramolecular

cleavage of the head group of this compound depends on its structure; it does not take place in the case of the analogous succinic acid monoamide of dioctadecylamine.

The variation of membrane properties can also be achieved photochemically;[93,94] some examples are summarized in Figure 21. The alteration of the membrane per-

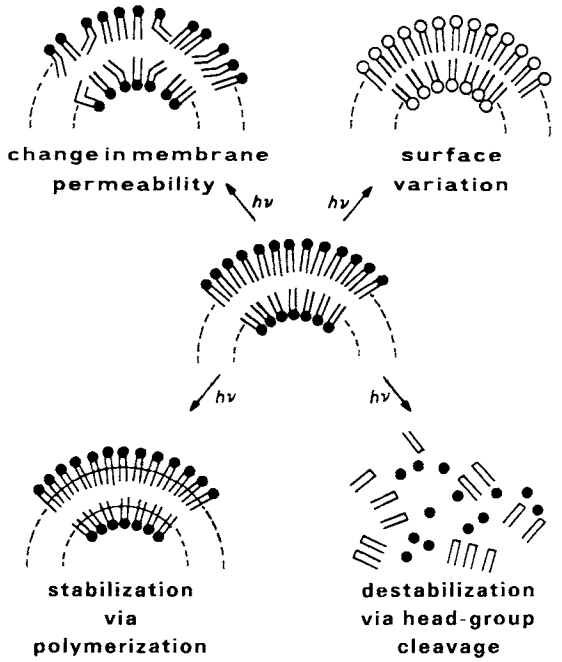
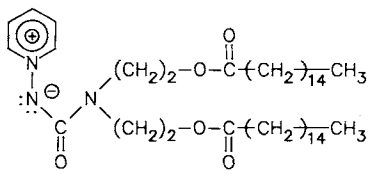


Fig. 21. Possibilities for the photochemical variation of membrane properties.

meability by means of the photochemical *cis-trans* isomerization of amphiphilic dyes has already been described.[94] The stabilization of model membranes via photochemically initiated polymerization reactions will be described in Section 4.5.1.

The photochemical variation and destabilization of the surface of monolayers and liposomes has been investigated by using lipids containing a zwitterionic photoreactive *N*-(1-pyridinio)amidate head group[95] (e.g., **18**).



18

Upon UV irradiation, these ylides can undergo ring expansion to give the isomeric 1,2-diazepines, or their head group can be cleaved, thus forming pyridine and an intermediary nitrene.[96] In liposomes composed of **18**, the photoreaction leads to the formation of 1,2-diazepine in high yield (Fig. 22).

Although the hydrophilicity of the diazepine formed in the head group is rather low, the vesicles neither aggregate nor precipitate during the photoreaction. However, these diazepine liposomes are metastable and can therefore be destroyed by mechanical stress such as shearing forces. A

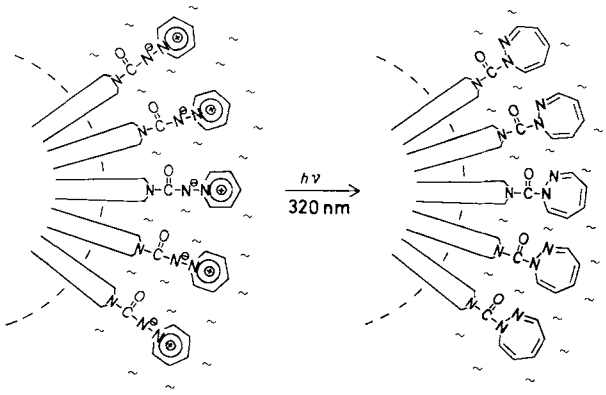
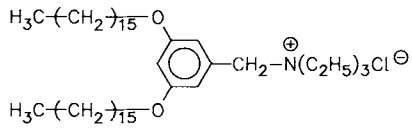


Fig. 22. Photochemical reaction of *N*-(1-pyridinio)amidate head groups forming diazepines in liposomal membranes: reduction of the head-group hydrophilicity [95].

subsequent reformation of vesicles from the isolated photoproduct is not possible. Thus, vesicle membranes composed of molecules that are not able to form such supramolecular systems by themselves can be generated by means of photochemical variation within the supramolecular system.

In a different photoreaction, lipid **19** is destroyed upon photolysis of its quaternary benzylammonium head group.[97] UV irradiation of **19** in monolayers and lipo-



19

somes mainly leads to the formation of the corresponding toluene derivative.[93a] The ordered membranes then collapse and form crystals (Fig. 23).

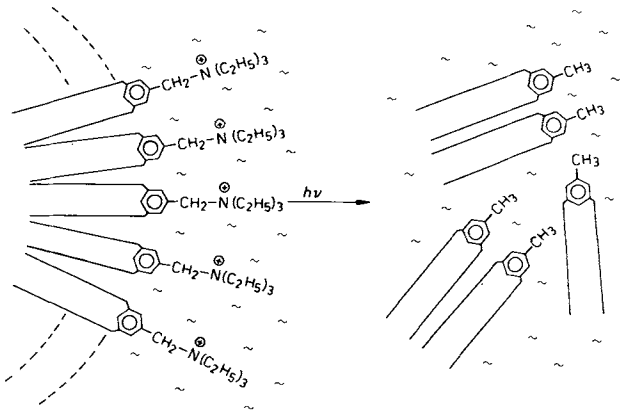


Fig. 23. Photochemical destruction of liposomes made from benzylammonium lipids [93a].

This process has also been studied in giant liposomes by means of phase contrast microscopy.[93a] Upon extended irradiation (about 40 s) the formation of crystallites is observed. A short irradiation (about 2 s), which does not result in a quantitative conversion of the reactive head groups, leads to the detachment of small liposomes from the larger ones. This process will again be dealt with briefly in Section 6.2 (Fig. 61).

4.4. Strategies for the Stabilization of Model Membranes

In the last few years, numerous methods to stabilize model membranes have been developed, mainly by using polymeric systems. A survey of the possible molecular architectures of stabilized liposomes is given in Figure 24.

The polymerization of lipids before or after their orientation in model membranes has been studied most intensively (see Section 4.5). As an alternative, the polycondensation of lipid molecules has also been described (see Section 4.6). Apart from such covalent binding reactions of lipid molecules, other possibilities to stabilize lipid bilayers are based on the noncovalent binding of polymers to the membrane surface (see Section 4.8). One method to achieve stabilization without using polymers involves the incorporation of membrane-spanning lipids into the bilayer (see Section 4.7).

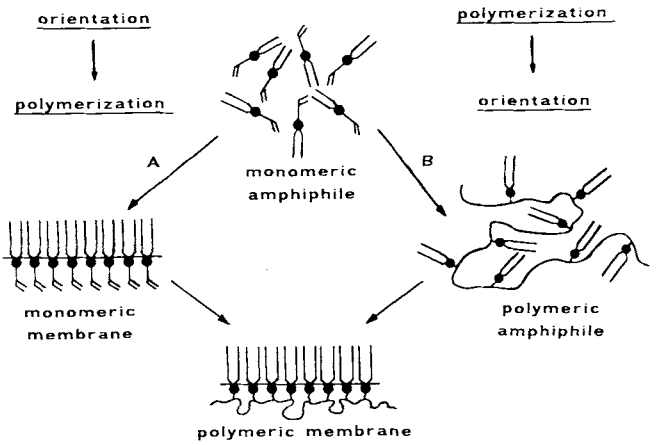


Fig. 25. Strategies for the preparation of polymeric model membranes. A) Orientation of the monomers in model membranes with subsequent polymerization. B) Polymerization of the monomers in isotropic solution and subsequent orientation of the polymeric amphiphiles.

4.5. Ordered Membranes from Monomeric and Prepolymerized Amphiphiles

Two approaches, schematically shown in Figure 25, have been used to prepare ordered polymeric model membranes. On the one hand, polymerizable amphiphiles can be used to build up monomeric model membranes, which can then be converted into polymeric ones (approach A). On the other hand, the use of oligomeric amphiphiles or the incorporation of spacer groups into the polymeric amphiphile to decouple the disordered polymer chain from the ordered membrane allows the formation of membranes from prepolymerized amphiphiles (approach B).[98]

4.5.1. Polymerizable Lipids

The polymerization behavior of amphiphiles with polymerizable units was first studied in monomolecular films.[99] In 1979, the formation of polymerized vesicles by using a lipid containing butadiyne units was mentioned for the first time.[100] In the meantime, many polymerizable groups have been incorporated into various lipid structures (see Scheme 2) to prepare polymerized liposomes.

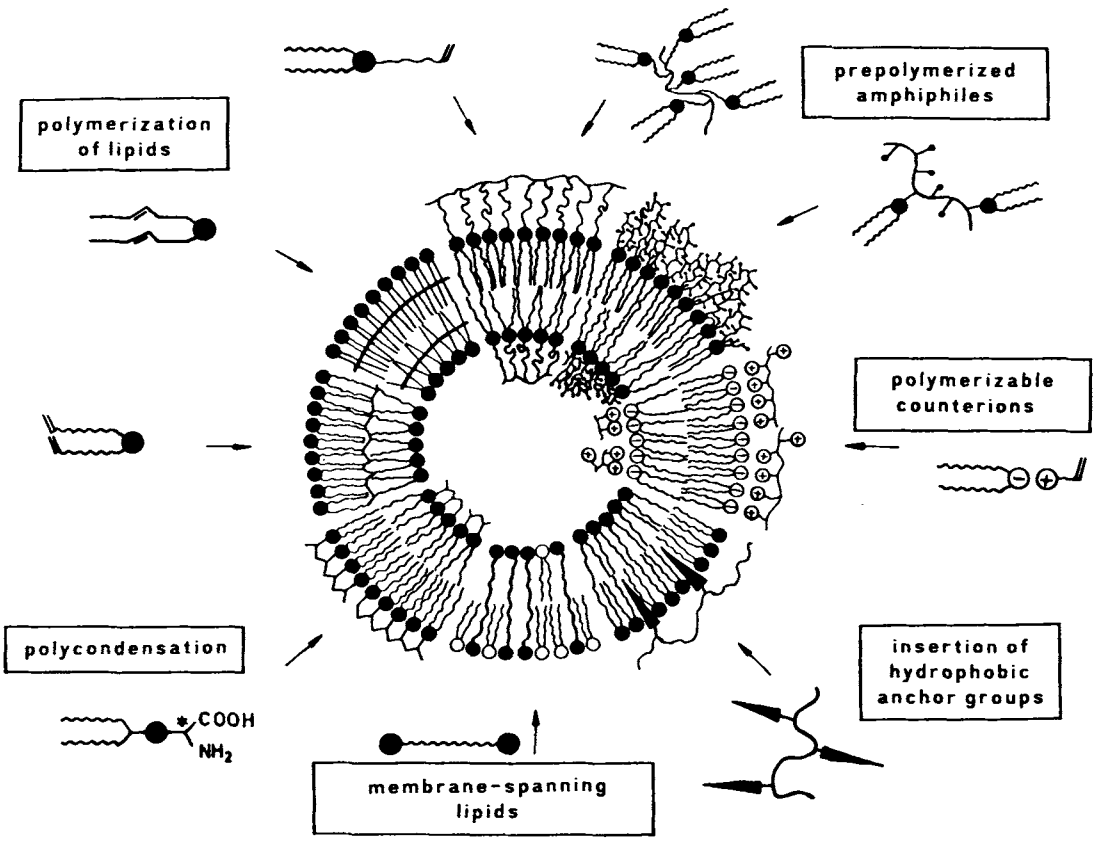
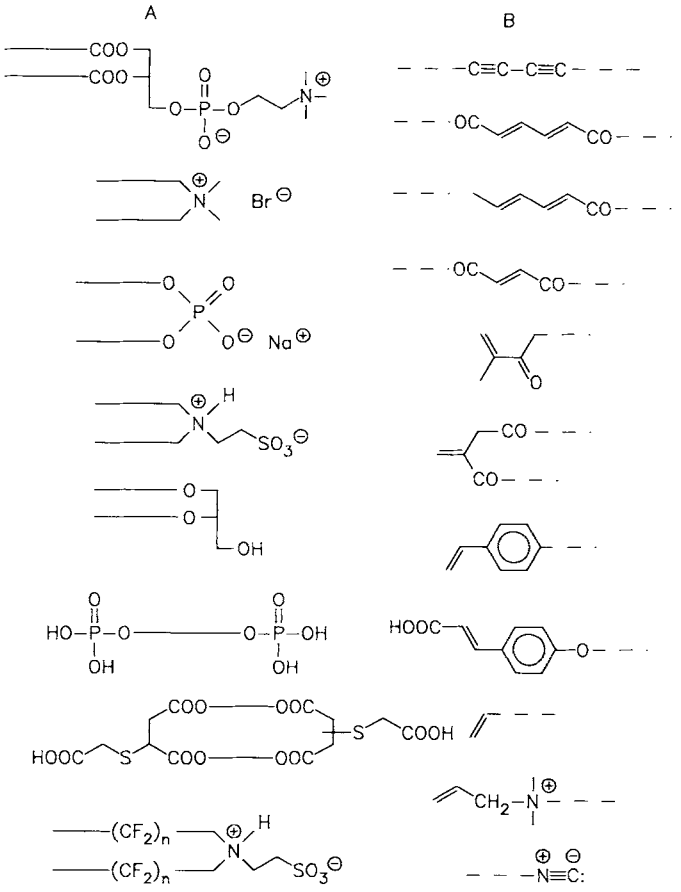


Fig. 24. Strategies for the stabilization of lipid bilayer membranes.

Scheme 3 shows only a few characteristic examples from the large number of polymerizable lipids.



Scheme 2. Building blocks of polymerizable lipids: A) lipid structures; B) polymerizable groups.

The game of structural variations of polymerizable amphiphiles (lipids, surfactants) and their use in monolayers, liposomes, and multilayers have enriched the monomeric and polymeric landscape and given rise to new hopes. These compounds and their perspectives of application will not be discussed further, since quite a few reviews on that topic already exist.[114]

4.5.2. *Model Membranes from Prepolymerized Amphiphiles*

A more recent concept for the preparation of polymeric membranes is based on the use of amphiphiles which have already been polymerized. At first sight, it seems surprising that this approach was taken much later than that via monomers. The problem with prepolymerized amphiphiles is the two-dimensional orientation of a three-dimensional polymer coil: An individual, motile molecule can easily be incorporated into a membrane. For a polymer-fixed molecule, however, its incorporation as a cooperative process is more complicated. Two different pathways can be followed to organize the membrane-forming alkyl chains of a polymer into ordered mono- or bilayers, despite the unordered structure of the polymer chain. On the one hand, polymers with low degrees of polymerization can be used. On the other hand, the incorporation of flexible spacers allows the decoupling of the different motion and organization processes taking place between the alkyl chains and the polymer main chain. Nature commonly uses the spacer concept, especially when functional groups with high mobility have to be kept close together. Already used for pharmacologically active polymers,[115] a similar concept has also been applied to the synthesis of liquid-crystalline polymers.[28,29] As regards polymeric amphiphiles, there exist three different possibilities as to the incorporation of a spacer group (Fig. 26).[116]

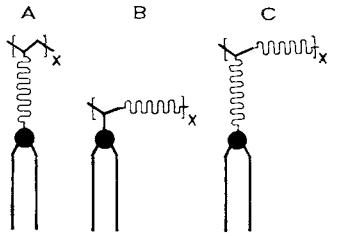


Fig. 26. Three possible ways to introduce hydrophilic spacer groups into polymeric amphiphiles [116a]: A) side-group spacer; B) main-chain spacer; C) main-chain and side-group spacer.

Homopolymers with side-group spacers (A) have been synthesized from lipids containing a hydrophilic spacer group between the membrane-forming amphiphilic part and the polymerizable unit. By means of copolymerization of simple lipids with hydrophilic comonomers, amphiphilic copolymers with main-chain spacers (B) can be prepared. The combination of both spacer types (C) can be realized by the copolymerization of spacer-containing lipids with hydrophilic comonomers.[116] The formation of polymeric model membranes by using such amphiphilic spacer-containing copolymers is illustrated in Figure 27.

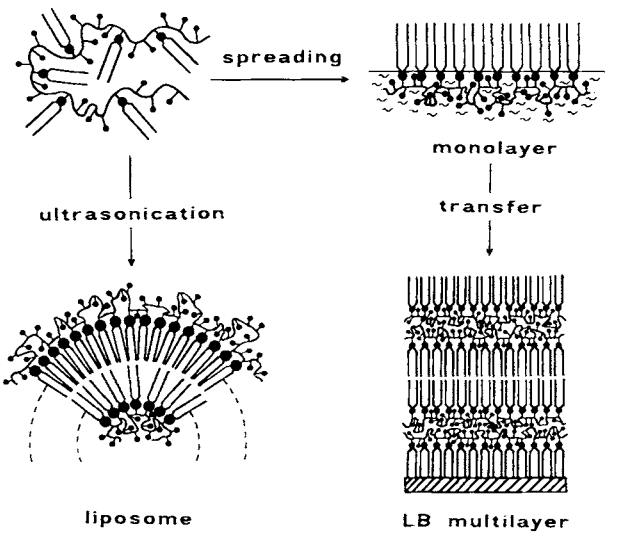
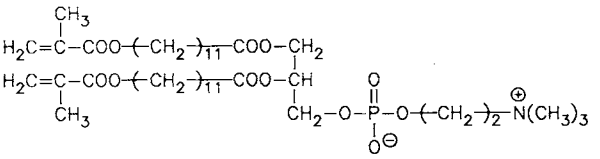
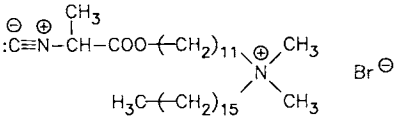


Fig. 27. Preparation of polymeric model membranes from copolymers containing hydrophilic comonomers.

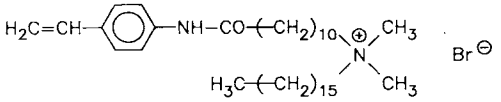
The preparation of vesicles from prepolymerized lipids has so far only been described for homopolymers with



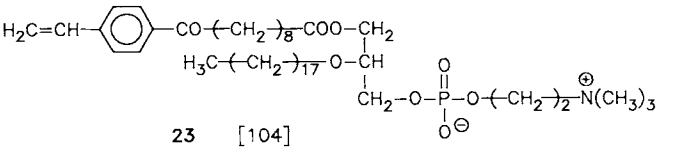
20 [101]



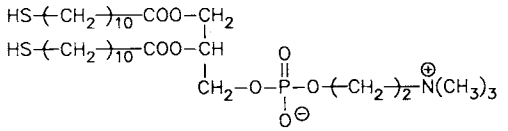
21 [102]



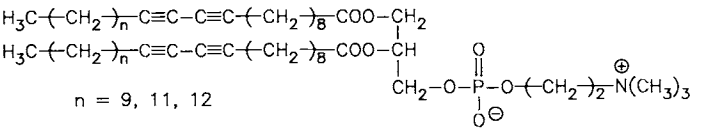
22 [103]



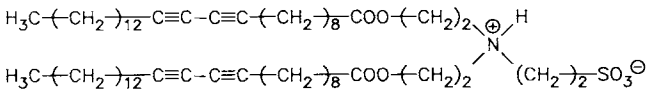
23 [104]



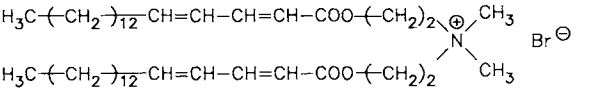
24 [105]



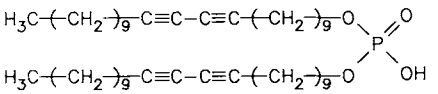
25 [72,106]



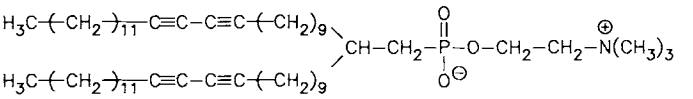
26 [106a,107]



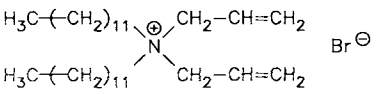
27 [72,108]



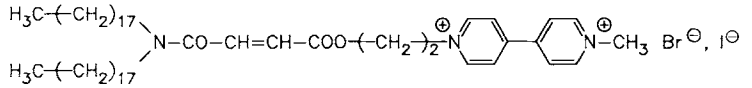
28 [106e, f]



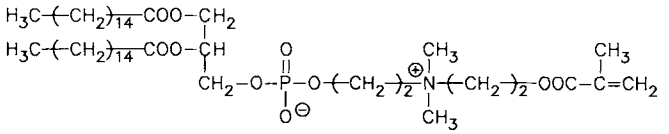
29 [109]



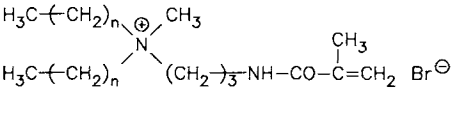
30 [110]



31 [111]



32 [112]



n = 15, 17

33 [113]

Scheme 3. Typical examples of liposome-forming polymerizable lipids.

side-group spacers (type A, Fig. 26).[98] In addition, the self-organization of a polyionene as a vesicle membrane has also been discussed.[98a]

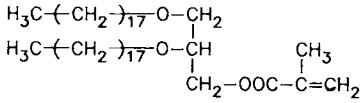
Fendler et al. described the copolymerization of maleic acid derivatives (e.g., **31**) with isotropically dissolved acrylonitrile as comonomer.[90a, 111] This kind of copolymerization also leads to the formation of polymers with main-chain spacers.

It is possible to prepare and investigate highly ordered stable monolayers by spreading the prepolymerized lipids (Fig. 27). They can also be used for the preparation of polymeric LB films via transfer onto solid substrates according to the LB technique.[116–119]

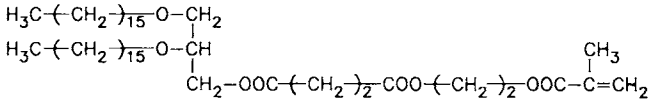
Scheme 4 shows some of the monomers from which amphiphilic homo- and copolymers with main-chain, side-group, or combined spacers have been synthesized. A fur-

ther interesting system consists of alternating copolymers made from maleic anhydride (e.g., with long-chain vinyl ethers or olefins), which have been investigated, in particular, by *Hodge* et al.[119]

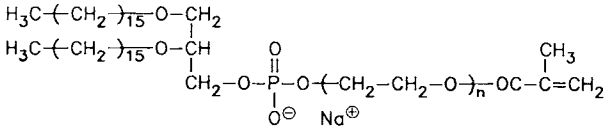
A



34 [116a]



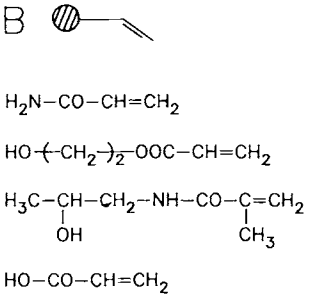
35 [116a]



n = 1: **36a** [116a]

n = 4: **36b** [98b]

B



Scheme 4. A) Polymerizable lipids; B) hydrophilic comonomers for the synthesis of spacer-containing amphiphilic copolymers.

The necessity of introducing a decoupling spacer becomes obvious when looking at the isotherms of the monomeric and polymerized spacer-free lipid **34** (Fig. 28). The

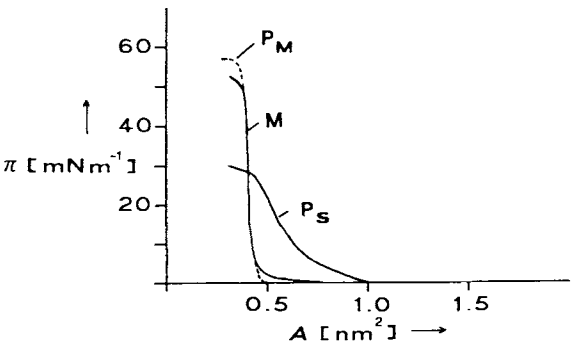
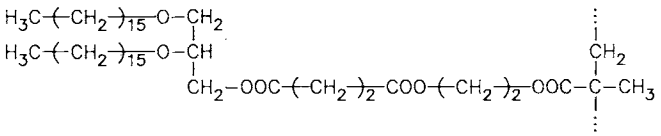


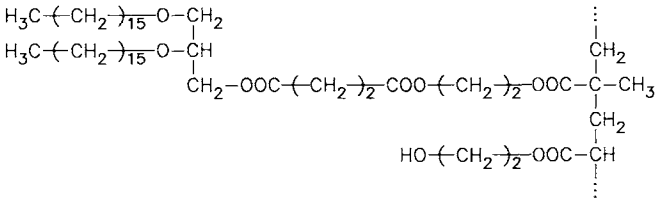
Fig. 28. Comparison of the isotherms of the spacer-free monomeric lipid **34** with its polymers [116a]. M) Monomeric lipid forms a highly ordered monolayer with a solid-analogous phase. P_M) Polymerization in the ordered monolayer leads to a slightly increased stability of the solid-analogous phase. P_S) Upon spreading of the polymer after polymerization in isotropic solution, only a liquid-analogous phase is formed. π = surface pressure, A = area per molecule.

monomeric lipid **34** forms a stable film with solid-analogous packing (curve M). After polymerization in the ordered monolayer, the isotherm exhibits a similar behavior, showing a slightly increased stability of the film (curve P_M). If, however, this spacer-free lipid is polymerized in isotropic solution and then spread, it is no longer capable of forming a highly ordered solid-analogous film (curve P_S). Here, an important role should be played by the molecular weight as well as the tacticity:[118c, 120] The isotherm of a lipid polymerized in isotropic solution should show a similar behavior to that of a monomeric lipid when a small degree of polymerization and a high fraction of syndiotactic units are used.

The isotherms in Figure 29 show that it is possible to maintain mobility and orientability of the alkyl side chains of a copolymer with a main-chain spacer. Whereas the monomeric lipid **35** with a short side-group spacer exhibits a liquid- and a solid-analogous phase (Fig. 29A), the corresponding polymer **37**, prepolymerized in solution, can



37



38

only form a solid-analogous phase (Fig. 29B). After the polymerization of the lipid in the head-group region, the mobility of the alkyl chains is extremely hindered. However, it is recovered by incorporating hydrophilic comonomer units. Indeed, the introduction of an equimolar amount of hydroxyethylacrylate as hydrophilic comonomer (**38**) already suffices to give rise to a liquid-analogous phase (Fig. 29C).

In ordered multilayers, the polymerization of amphiphiles[121] usually leads to the formation of defects.[52, 122] An elegant way to avoid defects induced by polymerization is to prepare LB films from prepolymerized amphiphiles. In Figure 30, the example of copolymer **39** illustrates in how far the content of hydrophilic comonomer influences the structure of LB multilayers. By means of small angle X-ray scattering, it has been shown that the spacings between the individual layers increase with increasing comonomer content.[116a] Furthermore, an increased portion of hydroxyethylacrylate allows a higher layer correlation.

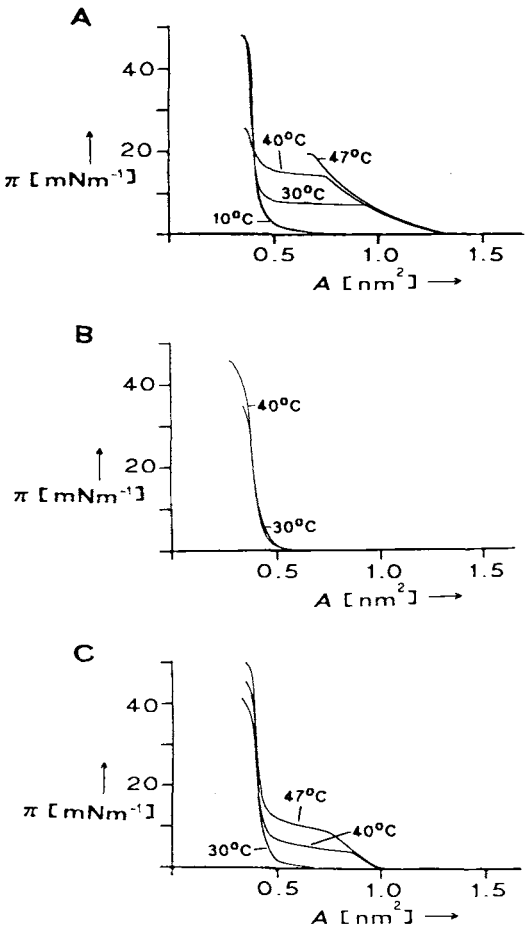


Fig. 29. Isotherms of the monomeric methacrylate **35** and its homo- and copolymers [116a]. A) The monomer **35** with a short side-group spacer forms temperature-dependent liquid- and solid-analogous phases. B) The homopolymeric methacrylate **37** ($=(\mathbf{35})_n$) is only capable of forming solid-analogous phases. C) The statistical 1:1 copolymer **38** from lipid **35** with hydroxyethylacrylate as main-chain spacer forms temperature-dependent liquid- and solid-analogous phases. π = surface pressure, A = area per molecule.

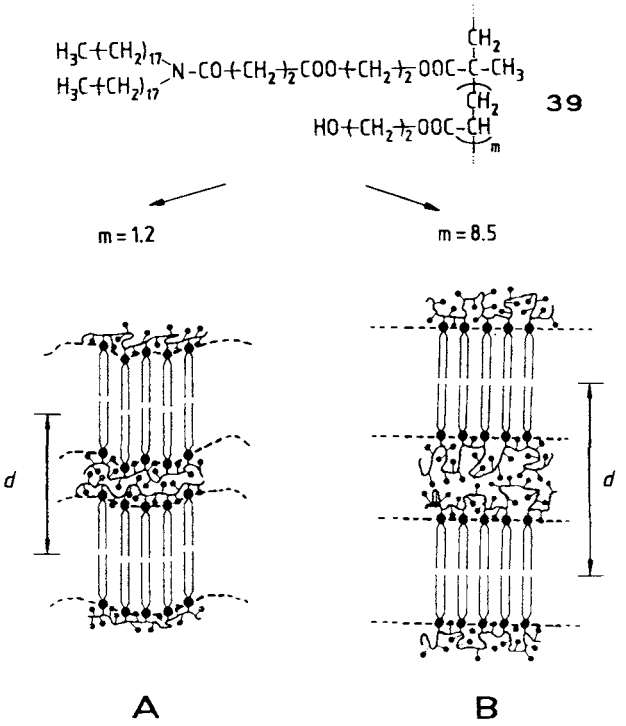


Fig. 30. Multilayers from the amphiphilic copolymer **39**. Schematic representation of the layer thickness d and the layer correlation depending on comonomer contents [116a]. A) Hydroxyethylacrylate content $m = 1.2$ (short hydrophilic main-chain spacer); small distance between layers, poor layer correlation. B) Hydroxyethylacrylate content $m = 8.5$ (long hydrophilic main-chain spacer); larger distance between layers, good layer correlation.

4.6. Polypeptide Liposomes—Bilayers from Polycondensable Lipids

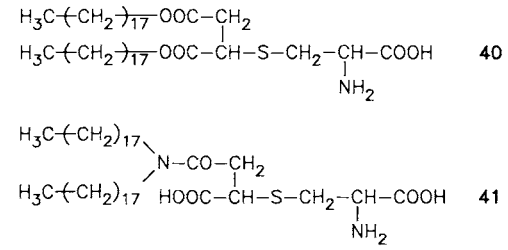
Considering the interest placed in liposomes as drug carrier systems,[87,114a] the idea of building up stabilized but biologically degradable systems looks exciting. The first attempts to prepare biodegradable polymerized liposomes have been described by *Regen* et al. They have used a reversibly polymerizable dimercaptolecithin[105] (cf. lipid **24**, Scheme 3). In this context, polypeptide liposomes seem to be promising.

Up to now, only a few papers exist on polycondensation reactions in ordered systems. The first one was published as early as 1948 by *Katchalsky* et al. It describes the condensation of octadecyl or hexadecyl esters of glycine and alanine in LB multilayers.[123] By simply allowing the films to stand, the corresponding polypeptides could be obtained within the films via aminolysis of the esters. In an isotropic solution, no reaction takes place under comparable mild conditions. Thus, the increased reactivity of the amphiphiles in the multilayers is presumably due to their orientation. *Fukuda* et al. later resumed these investigations.[124] Other attempts to achieve a polycondensation in ordered systems are the cross-linking of long-chain urea derivatives with formaldehyde[125] and of glycolipids with epichlorohydrin or divinylsulfone.[126]

The aminolysis of long-chain amino acid esters has also been tried in vesicle membranes. However, the transformation of free amino groups to amide groups leads to a decrease in the hydrophilicity of the lipid head groups to such an extent that the liposomes precipitate.[127] If long-chain Leuchs anhydrides are used for the polycondensation in vesicle membranes, neither educts nor products are capable of forming vesicles. Thus, it is necessary to use mixed membranes with lipids such as the phosphatidylcholine DPPC.[128]

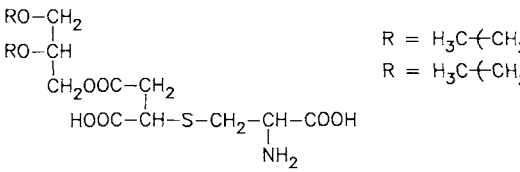
To prepare stable polypeptide liposomes, amino dicarboxylic acid lipids have been introduced by *Neumann* et al.[129] Some examples of polycondensable amino acid and amino dicarboxylic acid lipids are the compounds **40** and **41**–**45**. Liposomes prepared by using the lipids **41**–**45** remain in solution after polycondensation, owing to the additional hydrophilic carboxyl group (Fig. 31).

The lipids **40**–**45** were synthesized by the addition of cysteine or homocysteine to maleic acid derivatives. In water, they form vesicles upon ultrasonication. Furthermore, giant vesicles can be obtained from the glycerine ether derivatives **42**–**45** by means of swelling. The polycondensation of liposomes was achieved by sonicating the lipids **41**–**45** in the presence of a water-soluble carbodiimide at pH = 6.5. The formation of amide bonds, under these conditions, was confirmed by FTIR spectroscopy. Vapor-pressure osmometry reveals that oligopeptides with a mean de-



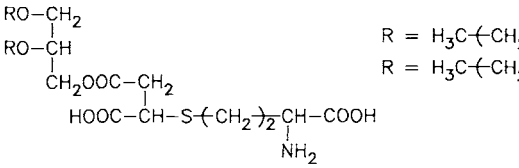
40

41



42

43



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45

gree of polymerization of 4 are formed by the polycondensation reaction.

In order to characterize the permeability of the monomeric and oligomeric amino acid vesicles, the release of entrapped markers was measured.[129b] The twofold negatively charged fluorescent dye 6-carboxyfluorescein (6-CF) as well as [^{3}H]glucose were used as markers. The monomeric liposomes feature a remarkably small permeability towards these two markers. By using the relatively bulky, charged 6-CF, for example, less than 0.1% release is measured within 30 h at room temperature. The permeability is remarkably higher after polycondensation; however, it is still rather low: after 30 h, these vesicles lose about 1.5% of 6-CF. The diffusion through the membrane is much faster for the uncharged marker compared with the charged 6-CF. The release behavior of monomeric and polycondensed vesicles from lipid **43** is exemplified in Figure 32.

As a comparison, the release kinetics of distearoylphosphatidylcholine (DSPC) vesicles is also given. This lipid was chosen since, just like the monomeric and condensed amino acid lipids, it is in the gel state at room temperature.

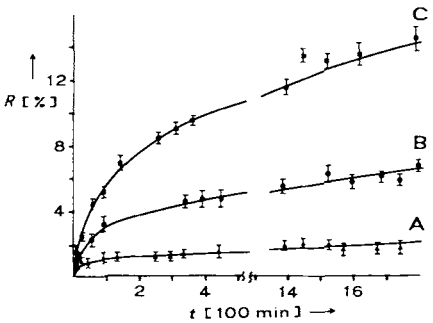


Fig. 32. Release of [^{3}H]glucose from amino acid liposomes (24 °C) [129b]. A) Liposomes made from the monomeric amino acid lipid **43**. B) Peptide liposomes made from the lipid **43**. C) Liposomes made from DSPC.

Figure 32 shows that monomeric amino acid liposomes exhibit a low permeability even when [^{3}H]glucose is used as a marker; their release rate is the lowest found for monomeric vesicles. In this case, too, the permeability is increased by the polycondensation; i.e., the packing density of the lipids is impaired by the oligopeptide formation. Nevertheless, the release rates of polycondensed liposomes are clearly below those of DSPC vesicles.

By means of sonication, isolated oligopeptides can also be used to prepare liposomes, analogously to the use of prepolymerized amphiphiles (see Section 4.5.2). The peptides of the lipids **42**–**45**, together with cholesterol, are even capable of forming giant liposomes.

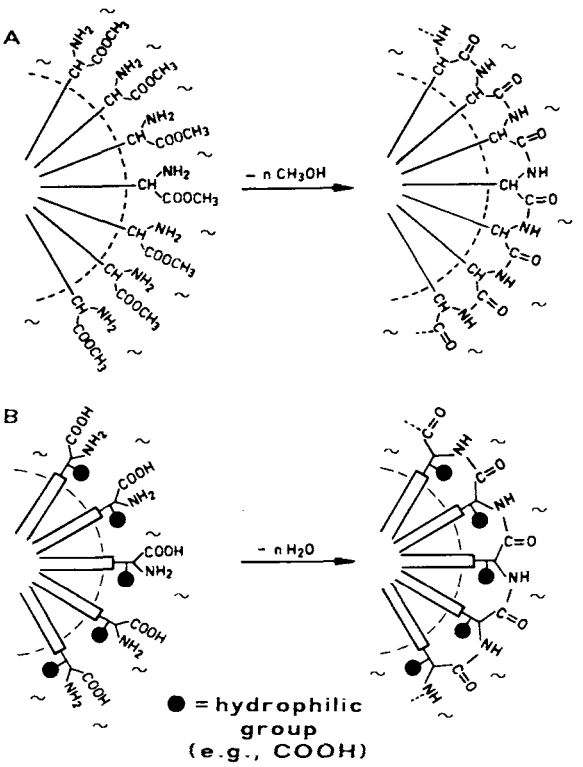
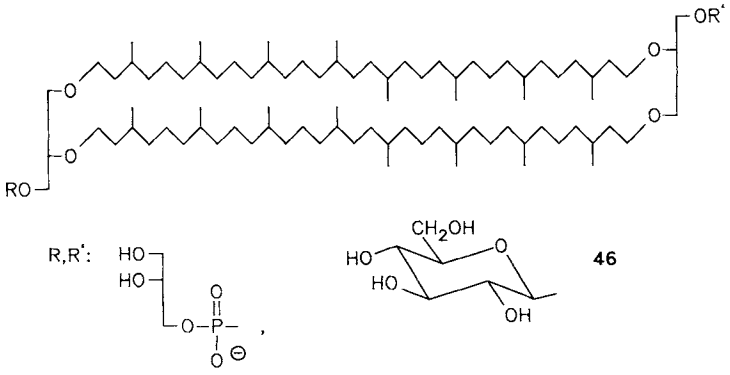


Fig. 31. Formation of peptide liposomes. A) Aminolysis of amino acid esters: reduction of the head-group hydrophilicity leads to the precipitation of liposomes [127]. B) Polycondensation of aminodicarboxylic acids: owing to the additional hydrophilic head group (COOH), such peptide liposomes remain in solution [129].

4.7. Stabilization by Means of Membrane-Spanning, Polymerizable Lipids

In archebacteria Nature offers an interesting example of membranes with high stability. They survive under extreme conditions and are therefore able to fill ecological niches. Thermophilic archebacteria thrive at temperatures up to 110 °C, acidophilic bacteria at pH values around 1, and halophilic ones even in saturated sodium chloride solution. Owing to their adaption to unusual habitats, the membrane components of these microorganisms also exhibit unusual structures.[130] The most important membrane lipid fraction is composed of membrane-spanning bipolar lipids (e.g., **46**). They consist of two isoprenoid diphytanyl (C_{40}) chains which are connected via acid-stable ether linkages to form macrocyclic glycerol tetraethers.

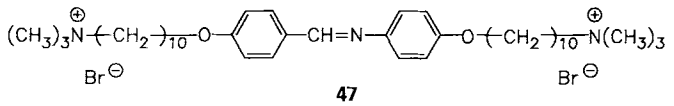


46

An asymmetrical structure of the bacterial membrane can be achieved with macrocycles carrying two different head groups. Commonly occurring polar head groups are phosphatidylglycerol and mono- or oligosaccharides. Some representatives of these archebacteria do not even possess a cell wall. Thus, the cell membrane is the only barrier towards the aggressive external environment.

Synthetic analogues of the membrane-spanning lipids of archebacteria have been prepared (e.g., **47**–**52**).

Okahata and *Kunitake* succeeded in preparing monolayer vesicle membranes by using a single-chain bipolar ammonium salt like **47**.[44] The term "bola amphiphile" was used by *Fuhrhop* et al. to describe numerous vesicle-forming bipolar, double-chain (macrocyclic) lipids (e.g., **48**).[131]



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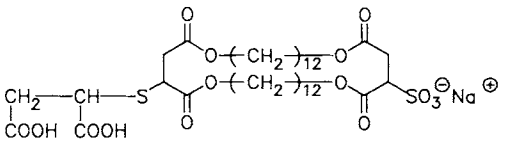

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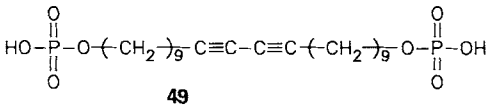

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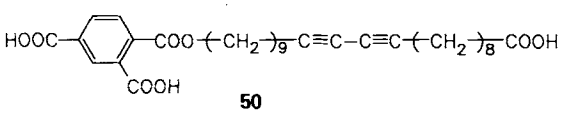

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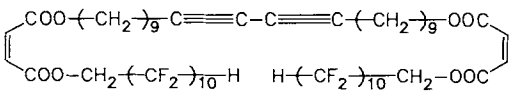

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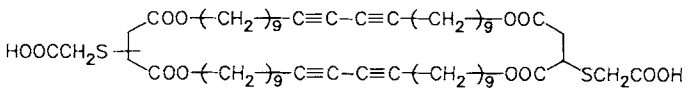

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Polymerizable bipolar amphiphiles containing butadiyne units, such as **49**–**52**, were introduced by *Bader* et al.[132] In the presence of cholesterol, the single-chain bipolar amphiphile **49** forms vesicle membranes which can be polymerized by UV irradiation at 0 °C.[132a] By using the macrocyclic dicarboxylic acid **52**, small unilamellar vesicles as well as giant liposomes can be obtained. They are in the liquid state and exhibit no phase transition to the solid-analogous state down to a temperature of 4 °C. Since the topochemical polymerization of butadiynes takes place only in the solid-analogous phase,[164] it is not possible to polymerize vesicles of the macrocycle **52**.

Asymmetrical vesicle membranes can be obtained by using the bipolar amphiphiles **48** and **50**, bearing head groups of different size, since the smaller and bigger head groups will preferably point inwards and outwards, respectively, in small liposomes of asymmetrical amphiphiles (Fig. 33). This concept was first used by *Fuhrhop* et al.[131b]

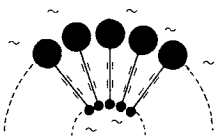


Fig. 33. Asymmetrical membranes of small, unilamellar vesicles made from membrane-spanning butadiyne lipids with head groups of different sizes [132b].

From **50**, vesicles can be prepared by sonication in the presence of 20–30 mol% of an amine (*N*-methylmorpholine, cyclohexylamine). The packing of **50** meets the requirements for a topochemical polymerization. Upon UV irradiation at 0 °C, the clear, colorless vesicle solution of **50** can be polymerized, forming a deep blue solution.[132b] The bulky trimellitic acid head group is located preferentially at the outside of the vesicle. This was proven by NMR experiments using the line-broadening effect of externally added $Mn^{2\oplus}$ ions on the signals of the aromatic protons.

Although liposomes made from a single lipid species are useful in studying basic membrane properties, those made from mixtures of structurally different lipids are a closer match to the complexity of natural systems. One can expect that mixed membranes prepared with membrane-spanning amphiphiles and conventional lipids should show a higher stability, as is schematically pointed out in Section 4.4 (Fig. 24). This stabilizing effect corresponds to that of archebacterial membranes and can also be compared to that of integral proteins. Indeed, electron microscopy reveals that mixed liposomes, made from the membrane-spanning macrocyclic amphiphile **52** and dimyristoylphosphatidylcholine (DMPC) (1:1), have homogeneously mixed membranes.[132b] Differences as to head groups or chain lengths do not result in phase separation (no DMPC domains can be seen by means of their ripple structures; cf. Section 4.8.1, Fig. 39, and Section 6.3.2, Fig.

64). Moreover, the thermal stability of these vesicles is increased by the membrane-spanning component. This has been demonstrated by temperature-dependent measurements of their permeability using 6-CF as an entrapped fluorescent marker.[132b]

4.8. "Liposomes in a Net"—Stabilization of Model Membranes by Polymeric Skeletons

Whereas model membranes can be stabilized by polymerization or polycondensation, i.e., by connecting the lipid molecules covalently, Nature makes use of completely different strategies to stabilize its membranes. Polymeric frames that are attached to the biomembrane provide it with flexibility and stability. Depending on the type of cell, these supporting elements differ considerably. Usually, they are attached to only one side of the membrane. Typical representatives are the cytoskeleton of erythrocytes and the cell walls of plant cells and bacteria.

Can model membranes be stabilized analogously to biomembranes by using polymeric frames?

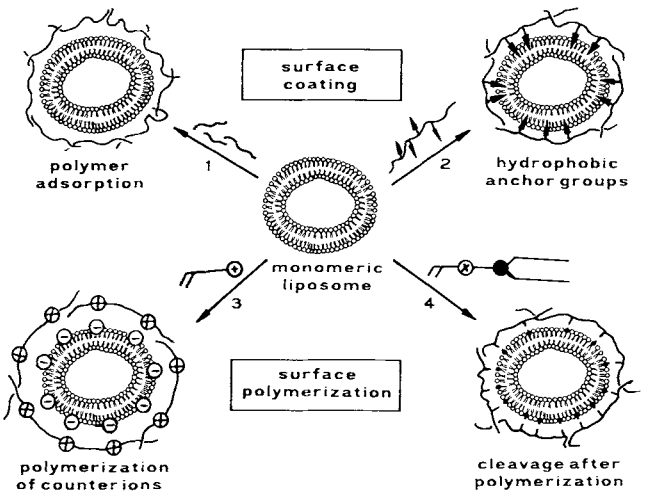


Fig. 34. Possible ways to prepare "liposomes in a net."

The four ways to bind polymers to liposomal membranes, shown in Figure 34, which are also common in Nature, are summarized as follows:

1. Water-soluble polymers can be adsorbed to vesicle membranes by means of hydrophilic or ionic interactions. Examples are the stabilization of lecithin vesicles by the adsorption of carboxymethyl chitin[133] as well as the binding of polylysine to negatively charged vesicle surfaces.[134] *Tirrell* et al. investigated the interaction between synthetic polyelectrolytes and liposomal membranes.[135]
2. Water-soluble polymers can be hydrophobized by modification with fatty acids or cholesterol. By inserting the hydrophobic anchor groups into membranes, these micellar, water-soluble, polymeric amphiphiles can be attached to membrane surfaces. *Sunamoto* et al. made use of this method in order to stabilize liposomes by coating them with hydrophobized polysaccharides.[136] These "artificial cell walls" will be dealt with in greater detail in Section 4.8.1.
3. In order to fix polyelectrolytes to membrane surfaces, charged monomers can be linked to the membrane-forming lipids via ionic forces. The subsequent "matrix polymerization" then proceeds at the membrane surface.[137] The introduction of polymerizable counterions only at the inside of vesicles leads to the formation of polymeric networks attached to the inner membrane surface. Such "artificial cytoskeletons" will be discussed in Section 4.8.2.
4. Liposomes might also be prepared from polymerizable lipids which carry a cleavable spacer (X) between the lipid part and the polymerizable group. The covalent linkage between the liposomal membrane and the enveloping polymer net can be split by the subsequent cleavage of that spacer. Such systems have not been realized yet.

4.8.1. Artificial Cell Walls or Wigs for Liposomes

To simulate the cell wall of plants, water-soluble polymers can be anchored in membranes via hydrophobic residues (Fig. 35). The driving force for the insertion of the anchor groups is the gain in free energy resulting from the transition from a dynamic, micellar system with partially solvent-exposed alkyl chains to a system in which the hydrophobic residues lie in the hydrophobic interior of the membrane.

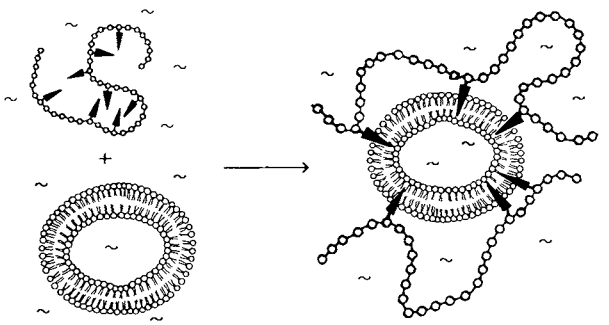


Fig. 35. Schematic drawing of the interaction of a liposomal membrane with a micellar polymer with hydrophobic anchor groups.

Examples of such interactions between polymers and membranes have been known for some time: The adsorption of synthetic polymers with hydrophobic anchor groups to cell membranes, for example, was described as early as 1964.[138] The first experiments with *O*-stearoyl derivatives of the polysaccharide dextran followed soon after.[139] *Wolf* et al. used similar polymers to study the lateral diffusion of polymers incorporated or attached to BLMs and cells.[140] In 1984 *Sunamoto* et al. introduced polysaccharide-coated liposomes as carrier systems for drugs.[136, 141] These are small, uni- or multilamellar vesicles, coated with palmitoyl- or cholesteryl-derivatized polysaccharides (pullulan, amylopectin). These asymmetrically coated liposomes feature a decreased permeability for 6-CF and an increased stability towards enzymatic de-

gradation by phospholipase D. *Kobayashi* et al. described mixed liposomes made from DPPC and synthetic *O*-octadecyl dextran.[142]

The coating of liposomes with polymers is of interest not only as regards the increase in stability, but also as regards surface variation. For example, it was demonstrated that a polysaccharide coating of liposomes leads to an altered distribution in the body.[136, 141, 143] Recently, even fragments of monoclonal antibodies were attached to a polysaccharide in addition to the hydrophobic anchor groups.[144] In vitro, the resulting "immunoliposomes" are bound to specific cells to a higher extent than the corresponding antibody-free liposomes.

Hydrophobized polysaccharides interact also with BLMs. Indeed, *Möllerfeld* et al. observed a dramatic increase in the mechanical stability of BLMs made from glycerol monoleate.[145] All other membrane properties, such as the transport of the lipophilic ion dipicrylamine, however, are influenced only slightly by this method of stabilization.

So far, attempts to simulate an artificial cell wall have only been made as regards the BLM and small vesicle model systems. However, for a direct observation of the insertion process of the anchor groups and its consequent effects on the membrane (e.g., morphological changes in the system), one has to use giant liposomes[146] as model membranes. These are form-flexible and can be observed with a light microscope. For these measurements, a temperature-controlled flow chamber[148] has been constructed, in which giant liposomes (ca. 20 μm in diameter) are prepared directly by swelling a lipid film. By using a syringe, the medium can be exchanged or additional components can be injected (Fig. 36).

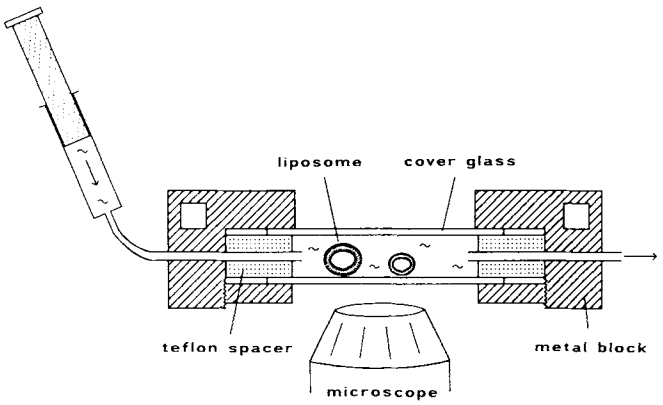


Fig. 36. Construction of the flow chamber for the observation of giant liposomes (schematic cross sectional view). The volume is 200 μL; the metal block is temperature-controlled. With the help of a syringe the medium can be gradually exchanged. Observations are carried out by using an inverse microscope (phase contrast and epifluorescence).

A series of amphiphilic polymers consisting of *O*-palmitoyl derivatives of the natural, linear polysaccharide dextran have been synthesized[60] (Fig. 37).

When a solution of one of those amphiphilic polymers is added to a preparation of giant liposomes, above a critical concentration, a significant morphological change can be observed: tubelike liposomes become constricted. In the course of this process structures resembling strings of pearls develop along the original longitudinal axis of the liposome (Fig. 38). These morphological changes are due to the insertion of the hydrophobic anchor groups into the bilayer. On the one hand, the unmodified dextran, without hydrophobic residues, shows no effect. On the other hand, a clearly membrane-associated fluorescence can be observed when a hydrophobized and fluorescence-labeled dextran is used. The critical concentration, above which these "strings of pearls" are formed, decreases with increasing degree of substitution and increasing molecular weight of the dextran derivative.[147] The formation of structures resembling strings of pearls is shown in Figure 38 together with a schematic representation of the insertion process.

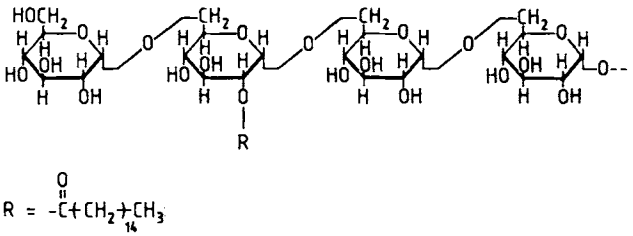


Fig. 37. *O*-Palmitoyldextrans [60] with mean molecular weights ranging from 43000 to 160000 and degrees of substitution between 3 and 6 mol% were used for the studies of giant liposomes described in the text. These dextrans have 15 to 40 anchor groups per polymer chain.

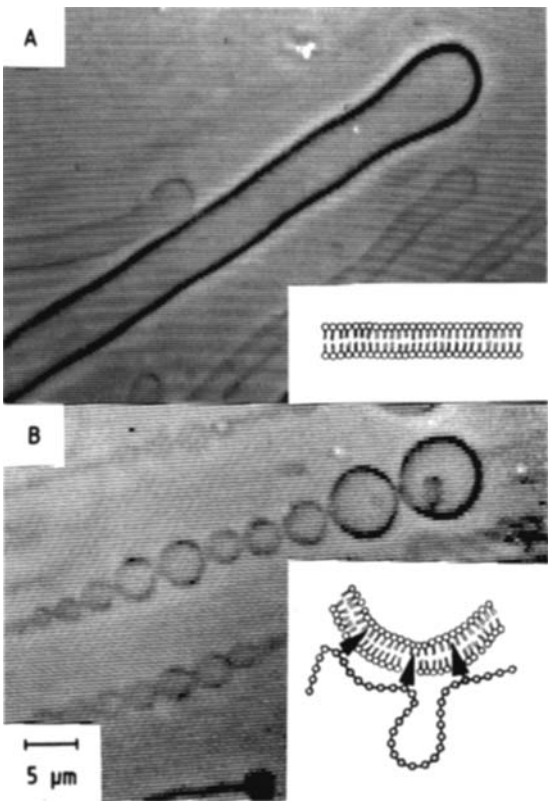


Fig. 38. Morphological change of a tubelike liposome owing to the interaction with a hydrophobized dextran (phase contrast micrograph and schematic explanation) [60, 147]. A) Before injection of the micellar polymer. B) After the injection.

According to these observations, the insertion of the anchor groups only in the outer half of the membrane induces a curvature (see Section 6.2) which leads to the formation of the "string of pearls." This is obviously a case of a polymer effect: Unsubstituted dextrans do not induce this process, whereas it occurs with octyl glucoside only at concentrations which are five orders of magnitude higher. This polymer effect is based on the fact that the locally high concentration of the anchor groups leads to a cooperative insertion into small membrane regions.

A similar kind of constriction process plays an important role in Nature in receptor-mediated endocytosis. In this case, the attachment of the protein clathrin to the inside of the cell membrane leads to the formation of "coated pits" and to the consequent detachment of "coated vesicles." They have an important transport function in the uptake of LDL (low-density lipoproteins).[149] These types of biomembrane processes will be discussed in detail in Section 6.2.

By means of freeze fracture electron microscopy,[150] it is possible to directly demonstrate the insertion of the hydrophobic anchor groups into the outer membrane of DMPC liposomes.[60, 147] By using this method, surface structures of membranes can be observed with a transmission electron microscope. Upon annealing between the pretransition and the main transition, typical ordered structures develop in liposomes from natural lecithins.[151] Owing to their appearance, they are called ripple phases. A liposomal membrane made from pure DMPC exhibits homogeneous ripple spacings of 12 ± 2 nm. Small amounts of impurities in the membrane already lead to a significant disturbance of these ripple structures.[152] Figure 39 explicitly illustrates the insertion of the hydrophobic anchor groups by means of the interaction between a hydrophobized polyvinylpyrrolidone derivative and DMPC liposomes.[60] The outer surface of the liposome shows a disturbed and widened ripple structure. However, the inner membrane surfaces of the multilamellar vesicle, which are made visible by the fracture, exhibit the perfect, original ripple structure of pure DMPC. This demonstrates that the added amphiphilic polymers, as schematically shown in Figure 35, only interact with the outer liposome surface and cannot penetrate through the membrane. A further attempt to insert amphiphilic compounds into liposomal membranes is described in Section 5.4.

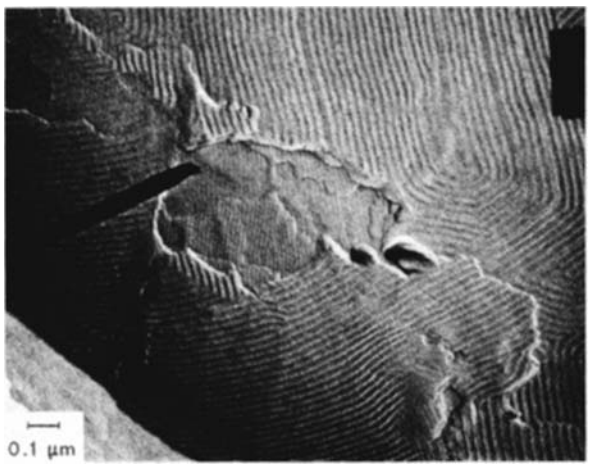
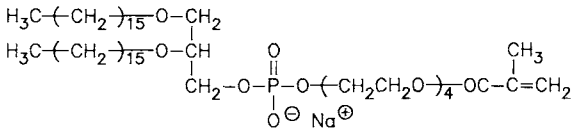


Fig. 39. Freeze fracture electron micrograph [204] of a multilamellar DMPC liposome after the interaction with a hydrophobized polyvinylpyrrolidone derivative; widened and disturbed ripple structure in the outer surface; undisturbed ripple structure of pure DMPC in the inner membrane layers [60].

4.8.2. Cytoskeleton Models or the Stabilization from Inside

In animal cells, the highly structured interior is spanned by a three-dimensional protein network, namely, the cytoskeleton. Among other functions, it serves as a static skeleton of the cell, thus maintaining its shape and stabilizing the plasma membrane. A typical example for the stabilizing function of the cytoskeleton is the spectrin network of erythrocytes.[153] Here, the combination of stability and flexibility is achieved by a two-dimensional protein network which is coupled to the lipid/protein bilayer (cf. Fig. 18). The protein network consists of filamentous spectrin molecules. Tetramers of this protein are cross-linked by another protein, actin. Some of these actin molecules are fixed to the cytoplasmic end of an integral membrane protein (Band III) by an anchor protein, known as ankyrin. A similar coupling of the cytoskeleton probably exists to the membrane-spanning protein glycophorin.[153a] Furthermore, it is assumed that the spectrin molecules are bound to negatively charged lipids in the inner-membrane leaflet by electrostatic forces.[154]

Because of the high complexity of the interaction between plasma membrane and cytoskeleton, it is hardly possible to understand the mechanical and structural properties of erythrocyte membranes without parallel model investigations. A simple model of an erythrocyte membrane was prepared from a mixture of DMPC and the polymerizable lipid **36b**.[154, 155] Here, the polymerizable methacrylate unit is kept in the aqueous phase by the hydrophilic tetraethylene oxide spacer[98b] (see Section 4.5.2).



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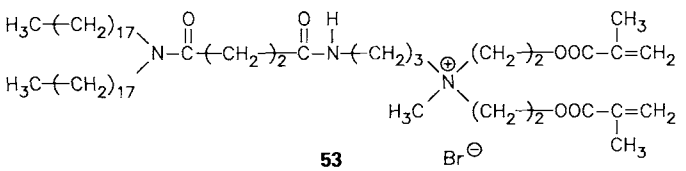

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The polymerization of such mixed liposomes leads to the formation of linear macromolecules at both membrane surfaces. By using a mixture of **36b** and lipid **53**, which contains two polymerizable units in the head-group region, cross-linked macrolipids are obtained. This leads to the formation of polymeric networks at both bilayer leaflets (Fig. 40).

An even better approach to simulate the cytoskeleton of the cell is a liposome, in which polymeric networks are

only inside the vesicles. It is possible either to attach two-dimensional networks to the inner membrane surface or to fill the interior of vesicles with three-dimensional cross-linked polymeric gels. Such three-dimensional networks,

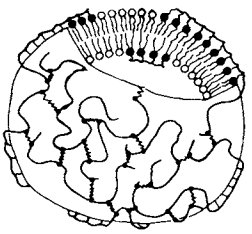


Fig. 40. Schematic drawing of a mixed liposome composed of polymerized lipids which are embedded in the monomeric lipid matrix. Additionally, the polymer chains are connected with one another forming a polymer network [155].

which span the complete interior of liposomes, are readily accessible by the polymerization of liposome-encapsulated hydrophilic monomers. By using this method, cross-linked polyacrylamide gels in the aqueous interior of liposomes have been prepared.[156] After destroying the lipid bilayer by addition of a surfactant, the size of the gel particles, as determined by light scattering and electron microscopy, remains nearly unchanged.

Two-dimensional polymeric networks that are attached only to the inner or the outer surface of the vesicle membrane via electrostatic interactions can be prepared by the exchange of the counterions at the outside of SUVs.[157] This is shown schematically in Figure 41.

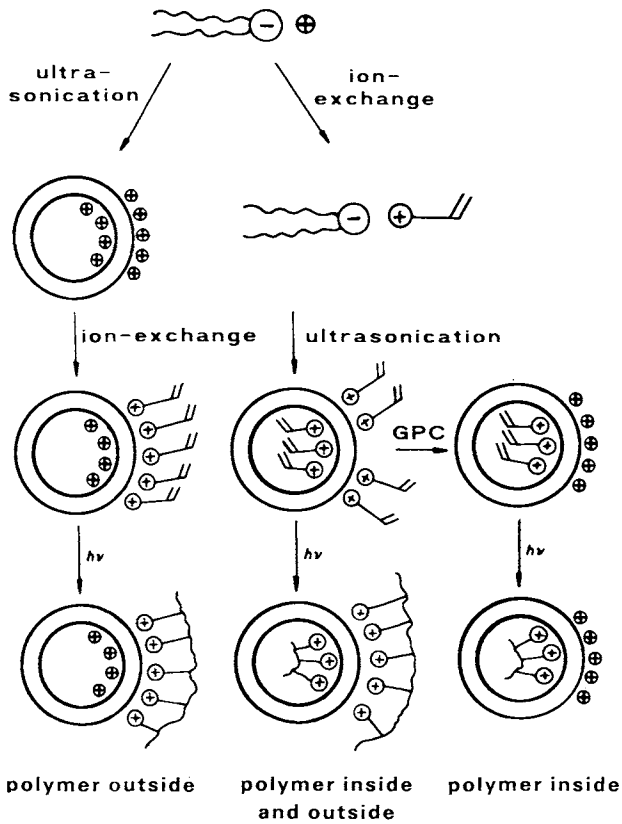
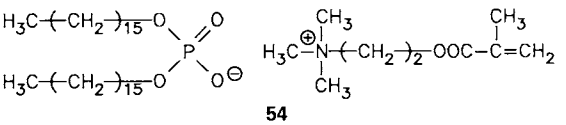


Fig. 41. Preparation of vesicle membranes with asymmetrically or symmetrically bound polyelectrolytes from lipids with polymerizable counterions [157]. GPC = gel permeation chromatography.

To realize this concept, the salts **54**–**57**, prepared from anionic lipids and polymerizable counterions, were used.[157] In the case of **54** and **56**, choline methacrylate is



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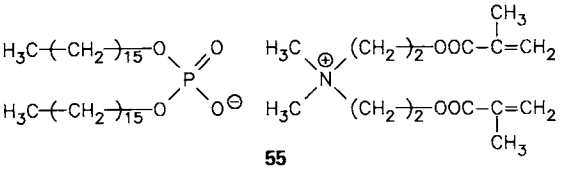

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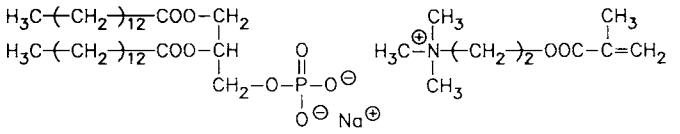

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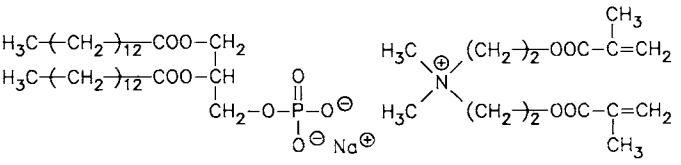

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bound as a counterion to negatively charged lipids (dihexadecyl phosphate or the anion of dimyristoylphosphatidic acid (DMPA)). This leads to linear polymer chains. Additionally, the salts **55** and **57**, with a counterion bearing two polymerizable groups, were used to prepare polymeric networks. The different routes to prepare vesicles with ionically linked polymers made with these lipids, as shown in Figure 41, are discussed briefly below.

Polymerizable counterions are introduced to the outer surface of unilamellar vesicles by exchanging $Na^{\oplus}$ against alkylammonium ions containing a methacrylate moiety. This process can be monitored by UV spectroscopy. Upon irradiation with UV light, the counterions can be polymerized at the outer membrane surface. In order to create macromolecules at the inner membrane surface, vesicles with polymerizable counterions on both sides of the lipid bilayer have to first be prepared. The polymerizable counterions at the outer membrane surface can then be replaced by $Na^{\oplus}$ by means of gel permeation chromatography (GPC). The polymerization of this vesicle system leads, in the case of lipid **55**, to a highly cross-linked polymeric network which is only attached to the inside of the vesicle membrane.

The phase transition temperature of the lipid bilayers strongly depends on the sort of counterion bound to the vesicle. Here, DMPA vesicles with a symmetrical distribution of the counterions are given as an example. Liposomes made from **56** with polymerizable counterions exhibit a phase transition temperature which is decreased to 31 °C, compared with liposomes made from the corresponding disodium salt (50 °C). By polymerization of the

cations of **56** at both membrane surfaces, the phase transition temperature is increased again.[157] Analogous results were obtained for dihexadecyl phosphate vesicles.[158] Figure 42 shows a model for the strong influence of the monomeric or the polymeric counterions on the phase transition.

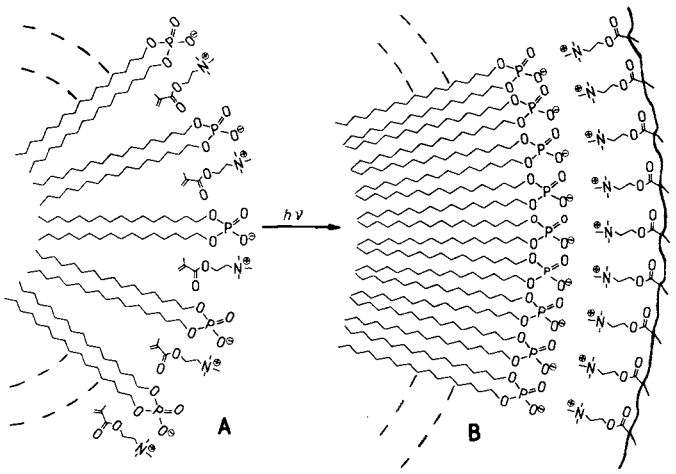


Fig. 42. Schematic drawing of the influence of choline methacrylate counterions on the packing of lipids in the membrane [158]. A) The monomeric counterions are partially inserted in the membrane, thus disturbing the packing of the lipids. B) The polymer is not inserted in the membrane, thus allowing a denser packing of the lipid molecules.

The monomeric choline methacrylate counterion thus leads to a fluidization of the membrane, owing to its partial insertion into the lipid bilayer. Since the phase transition temperature is shifted to higher values after polymerization, it can be assumed that the resulting polymer is no longer embedded in the membrane, thus allowing a denser packing of the lipid molecules. This model is also supported by results from permeability measurements.[158] Furthermore, comparative investigations of the stability of symmetrical vesicles of lipid **54** towards organic solvents were carried out using monomeric and polymeric counterions. Turbidity measurements reveal that the polymeric "liposomes in a net," in contrast with the unpolymerized counterparts, are even stable in 60% methanol.

A further step in simulating the cytoskeleton of erythrocytes is the additional fixation of an ionically fixed polymeric network to the inner membrane surface via covalently bound hydrophobic anchor groups. Such an "artificial cytoskeleton" was built up with vesicles consisting of a mixture of the lipids **53** and **54**[158] (Fig. 43). First, the polymerizable counterions are removed only from the outer vesicle surface. Then, the choline methacrylate counterions, bound to the inner membrane leaflet, are copolymerized with the cross-linking lipid **53** by means of UV irradiation. Thus, a two-dimensional polyelectrolyte network is formed at the inner-membrane surface. In addition to its electrostatic bonds, this network is fixed to the lipid bilayer via covalently linked hydrophobic anchor groups.

Comparative release measurements carried out on monomeric and polymerized vesicles using [^{3}H]glucose revealed that such an "artificial cytoskeleton" does not change the membrane permeability. Thus, it is the lipid bilayer, and not the polymeric skeleton, which indeed acts as the permeability barrier.[158] Hence, these polymerized vesicles formally behave like erythrocytes: the polymer controls their physical stability as well as their shape, whereas the lipid bilayer controls their permeability.

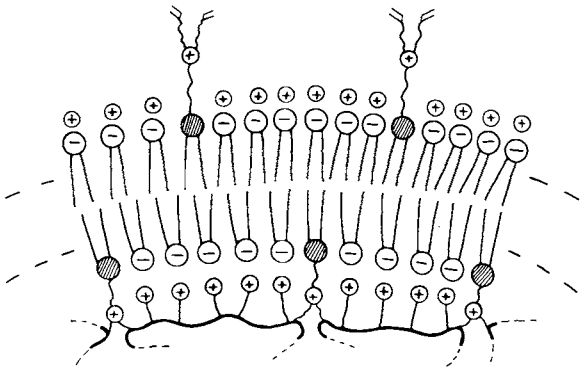


Fig. 43. "Artificial cytoskeleton" at the inner surface of vesicle membranes, prepared according to the following procedure [158]: (1) preparation of liposomes from 90 mol% of **54** (polymerizable counterions) with 10 mol% of **53** as a cross-linker (covalently bound methacrylate groups); (2) removal of the polymerizable counterions from the outer surface of the vesicle membrane via GPC; (3) copolymerization of the ionically or covalently bound methacrylates at the inner surface of the vesicle membrane.

5. The Use of Model Membranes to Study Biological Recognition Processes

The complex molecular processes in biological systems can only proceed along ordered paths if they are selectively controlled. Thus, the development of multicellular organisms can hardly be imagined without the capability of intercellular communication. The decisive step in such an information exchange is the binding of a signal molecule to a specific receptor which is usually bound to the membrane. Analogous recognition reactions are of significant importance for the immune system of vertebrates, too. The various responses of the immune system recognize and eliminate invading organisms as well as toxic substances produced by them. Because immune reactions are destructive, it is essential that they be made only in response to molecules which are foreign to the host. Without this distinction between foreign and autologous molecules, fatal autoimmune reactions would result. Therefore, biologists, biochemists, and biophysicists place great interest in the structure and the function of biological membranes as well as their interaction with signal molecules. In the course of these investigations, simple model membranes have also been used during the last few years.[82,159] In addition, the surface recognition of liposomes by target cells is of decisive importance for specific drug delivery.[87]

What kind of substances play a role in the manifold recognition reactions in Nature? The most important membrane components for surface recognition are those of the glycocalix, the glycoproteins and the glycolipids.[66] In the cell membrane, they are responsible for vital functions and properties such as cell recognition, antigenicity, histocom-

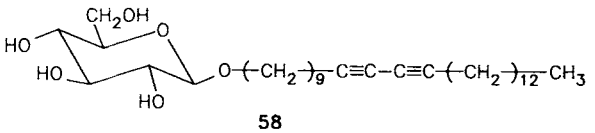
patibility, and lectin affinity. Sections 5.1, 5.3, and 5.4 will deal with the recognition of sugar structures in model membranes by means of lectins and antibodies. Moreover, the most well known and the best-investigated regulated process is the replication and transcription of DNA as well as the translation of RNA.[160] In this case, the recognition reaction is the base-coupling of nucleic acids. Model experiments in which these reactions are applied will be discussed in Section 5.2.

5.1. Reversible Sugar–Lectin Interaction with Polymerized Liposomes

Sugar structures attached to membranes can be recognized by lectins. These proteins, originally only found in plants, possess specific binding sites for certain sugars.[161] A well-studied representative is concanavalin A (Con A) which can bind α-glucopyranosides and α-mannopyranosides. It is a tetrameric protein where each subunit carries one binding site. The saturation of these binding sites with the low-molecular-weight sugars does not lead to aggregation of Con A. If, however, several of those sugar units are components of a supramolecular system such as a cell, agglutination and precipitation occurs after lectin addition. This process is reversible since the addition of a large amount of one of those pyranosides leads to the dissolution of the precipitate, thus reforming the original cell dispersion.[162]

Two principles can be used to carry out such biological recognition reactions with polymerized membrane systems. On the one hand, natural lipids with recognizable structures can be incorporated into polymeric membranes. On the other hand, polymerizable lipids with a recognizable head group can be synthesized.

Several polymerizable glycolipids with different sugar head groups were prepared a few years ago.[163] These carry a butadiyne unit as the polymerizable group in the hydrophobic chain.



58

The interaction between the glucose derivative **58** and Con A has been investigated in monolayers[163] and liposomes.[114a,b] Liposomes of **58** can be polymerized by UV irradiation, exhibiting a color change from colorless via blue to red, which is typical for butadiyne derivatives.[72,164] The experiment with Con A and sugar-bearing polymerized liposomes is schematically shown in Figure 44.

After adding Con A to a deep red solution of polymerized liposomes prepared from **58**, agglutination occurs within a few seconds: a red precipitate is formed, the supernatant becoming colorless. If, however, α-methylmannopyranoside, which binds much stronger to Con A, is then added, the precipitate is redispersed, thus leading to the original clear red solution of the liposomes. So the result is a competitive replacement of the liposomal-bound sugar groups by the soluble sugar.

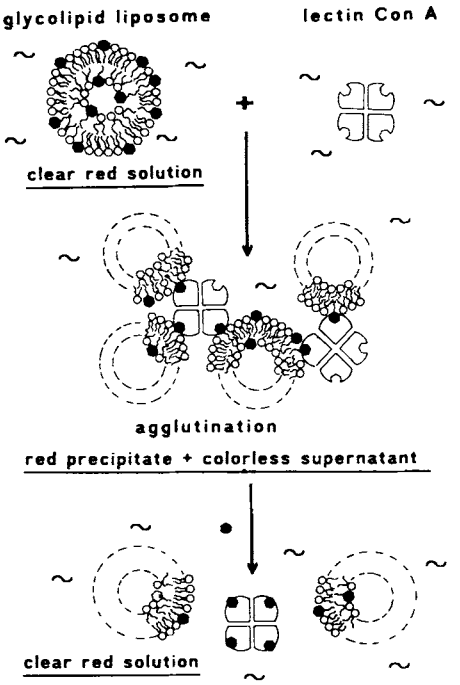


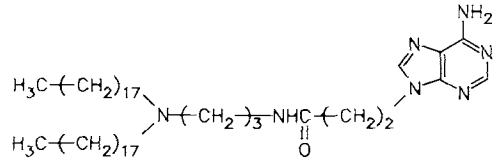
Fig. 44. Schematic representation of the reversible interaction of concanavalin A with polymerized glycolipid liposomes made from **58** [114b]. ● = sugar.

From simple qualitative experiments of this kind, one can proceed to investigations using synthetic and biologically relevant sugar structures[165] or natural glycolipids. Such model investigations gain increasing significance, for example, as regards the simulation of immunological phenomena and their medical application (affinity chemotherapy[166]).

5.2. Nucleolipid–Nucleobase Interaction in Monolayers

Another recognition reaction that occurs in Nature is the base-pairing in DNA. To learn more about the base–base interaction and the relation between structure and function, a large number of polynucleotide analogues have been synthesized.[167] The most common models are linear, uncharged polymers with nucleobases as side groups. With these polymers, specific interactions have been observed.[167,168] In none of these investigations were the nucleobases in an oriented state. In order to use the specific base-pairing in surface recognition reactions, nucleolipid monolayers and their interaction with nucleobases, dissolved in the subphase, were also recently investigated.[169] *Kornberg* et al. used such ordered nucleolipid monolayers to show that certain proteins, such as the B1 subunit of ribonucleotide reductase, are specifically bound in the form of a two-dimensional crystal.[170]

Compounds **59**–**62** are examples of new, uncharged nucleolipids, which form ordered structures like monolayers and liposomes.[169b,171] Despite their consistently big and only weakly hydrophilic head group, these nucleolipids



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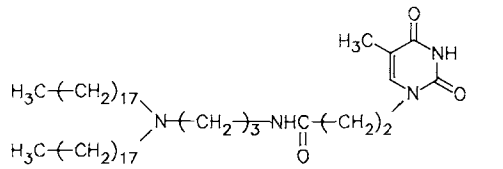

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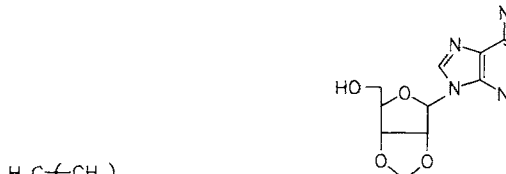

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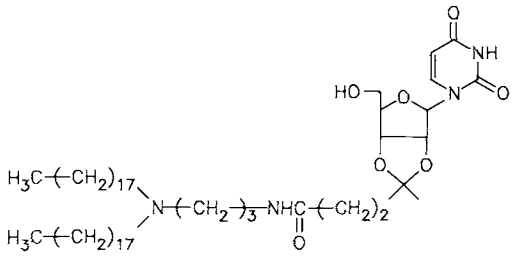


62

form stable, solid-analogous monolayers, with the nucleobase head groups dipping into the subphase. Their isotherms should be affected by the interaction of these nucleolipids with nucleobases as substratum in the aqueous phase. Figure 45 exhibits the surface-pressure behavior of the uridine lipid **62** to exemplify the influence of a monomeric nucleobase dissolved in the subphase.

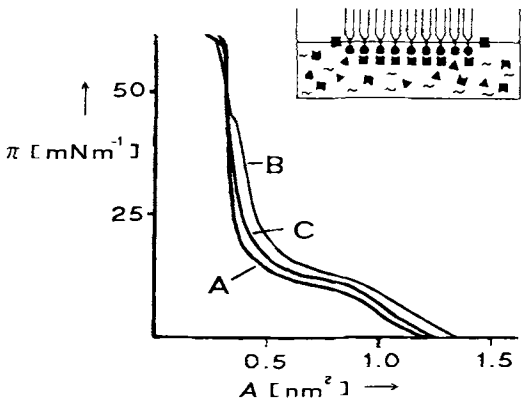


Fig. 45. Isotherms of the uridine lipid **62** on different subphases (20°C) [169b]: A) water; B) 0.01 M adenine (complementary) (■); C) 0.01 M thymine (noncomplementary) (▲). π = surface pressure, A = area per molecule.

The characteristic phase behavior of the nucleolipid **62** (curve A) is basically not changed by the dissolved nucleobases. Liquid-analogous as well as solid-analogous phases are observed, and the molecular area is slightly increased after addition of the bases. The influence of adenine (curve B), which is complementary to the nucleolipid, is slightly stronger than that of thymine (curve C).

The interaction between a nucleolipid monolayer and polymeric nucleotide bases is illustrated by means of the adenosine lipid **61** (Fig. 46). The two polynucleotides re-

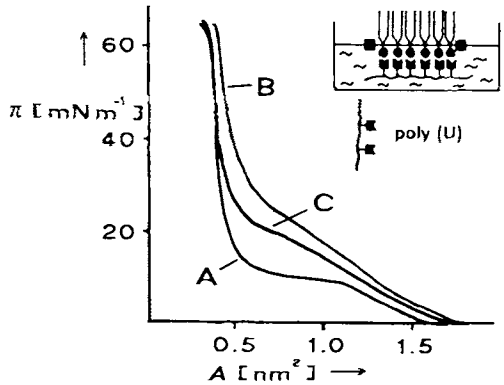


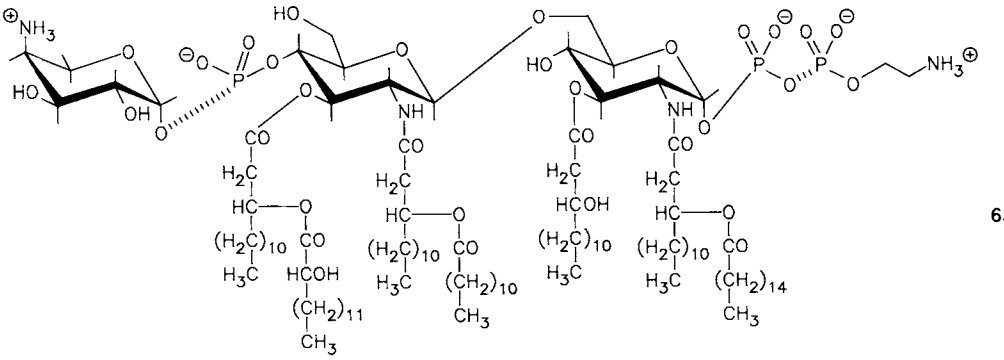
Fig. 46. Isotherms of the adenosine lipid **61** [169b] (subphase: PBS (= phosphate-buffered saline) buffer, pH 7.3, 1.5×10^{-3} M): A) in the absence of a polymeric nucleobase; B) 1.5×10^{-5} mol base L^{-1} poly(U) (complementary); C) 1.5×10^{-5} mol base L^{-1} poly(A) (noncomplementary). π = surface pressure, A = area per molecule.

veal a noticeably different influence on the isotherms of **61** if the surface pressure exceeds 15 mN m^{-1}. As regards the noncomplementary polyadenine nucleotide poly(A), it is already expelled from the head-group region at a surface pressure of 40–50 mN m^{-1}. Thus it loses all influence on the solid-analogous packing of the monolayer (curve C). In contrast, the poly(U) base, which is complementary to the lipid head group, exhibits a considerable interaction with the monolayer (curve B) even at high surface pressures.

In the case of liposomes made with nucleolipids and the phosphatidylcholine DPPC (3 : 1), no clear interaction with monomeric nucleobases can be demonstrated. Nevertheless, a significant interaction between liposomes and polymeric nucleobases was observed. However, the hypochromicity effects (decrease of the extinction coefficient after base-coupling) display no significant difference between specific and nonspecific interaction.[169b] This may be due to the fact that the stronger ionic interaction (see Section 4.8) of the polynucleotides with DPPC is superimposed on the weaker base-base interaction.

5.3. Model Investigations of the Interaction between Bacteria and the Complement System

In humoral immune reactions, the outer membrane of gram-negative bacteria is damaged by the complement system. The resulting hydrophilic pores lead to the death of the bacterium. The complement cascade (C) consists of at least 14 serum proteins which are activated in a specific order.[172] The classical pathway of C-activation is initiated by the adsorption of the first component C1 to the surface of the bacterium. This involves the binding of the C1 subcomponent C1q to the lipid A (**63**) of the outer membrane.[173] This first step of the complement cascade can



63

also be investigated by using model membrane systems. Indeed, **63**, the lipid anchor of the lipopolysaccharide (LPS), has already been incorporated into model membranes.[174] In cooperation with the group of *Loos*, the interaction between the complement component C1q and **63** was studied in monolayers and liposomes.[175] Lipid A (**63**) consists of seven hydrophobic alkyl chains of different lengths and one hydrophilic head group made up of glucosamine units as well as phosphate and ammonium groups.[176] C1q is built up of six collagen-like triple-stranded helices which are linked together at one end (Fig. 47). The free ends carry globular head groups that function as recognition sites. The C1q molecule has a molecular weight of 410000 and consists of 18 polypeptide chains.[177]

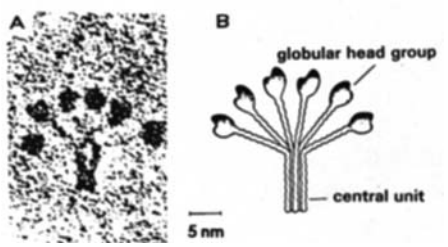


Fig. 47. A) Electron micrograph; B) schematic representation of the complement component C1q [177].

Figure 48 shows the isotherms of the purified oligomer-like **63** at different temperatures. The monolayers exhibit liquid- and solid-analogous phases and have a remarkable stability up to a temperature of 37 °C. The molecular area of about 1.3 nm^2 per molecule in the solid-analogous phase is within the range expected for a lipid with seven alkyl chains.

In order to investigate the interaction between **63** and C1q, the lipid A monolayer was kept under constant pressure while C1q was injected into the subphase. After the injection of C1q, one can clearly see a strong increase in molecular area if the monolayer is kept at a pressure of 2 mN m^{-1}, i.e., in the liquid-analogous state (Fig. 49). If, however, a higher surface pressure (20 mN m^{-1}) is applied, no change in the area per molecule is observed. In the solid-analogous state, the lipid A molecules are evidently so densely packed that the C1q head groups cannot penetrate the monolayer. For a recognition reaction to take place, not only does the specificity of the recognizable

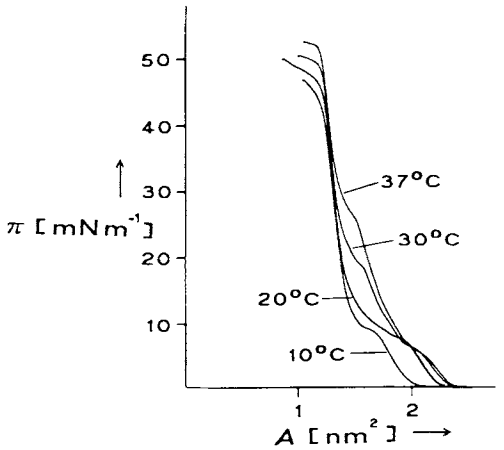


Fig. 48. Isotherms of **63** at 10, 20, 30, 37 °C (subphase: water). π = surface pressure, A = area per molecule.

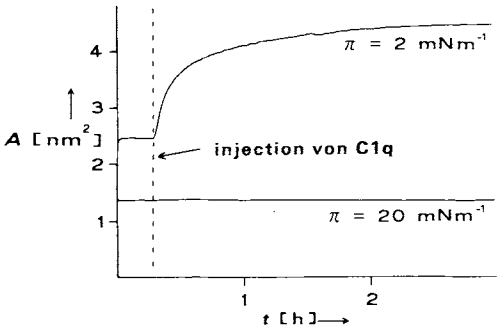


Fig. 49. Interaction of the protein C1q (1 μg mL^{-1}) with lipid A monolayers (20 °C): Injection of C1q (----) into the subphase at a constant pressure of 20 mN m^{-1} (solid-analogous phase) and 2 mN m^{-1} (liquid-analogous phase); strong interaction (expansion of the monolayer) only in the fluid state. A = area per molecule.

groups seem to be important, but also the existence of a liquid-analogous phase, common in biological membranes.[178]

The binding of C1q to lipid A monolayers was confirmed by investigations with a fluorescein-labeled anti-C1q antibody. These experiments were carried out with a film balance which was combined with a fluorescence microscope[180] (Fig. 50). Mixing diagrams of **63** and DPPC exhibit a complete miscibility of both components. However, these experiments do not give evidence as to whether the phase separation, which can be seen in Figure 50 (see also Section 6.1), already occurs owing to the adsorption

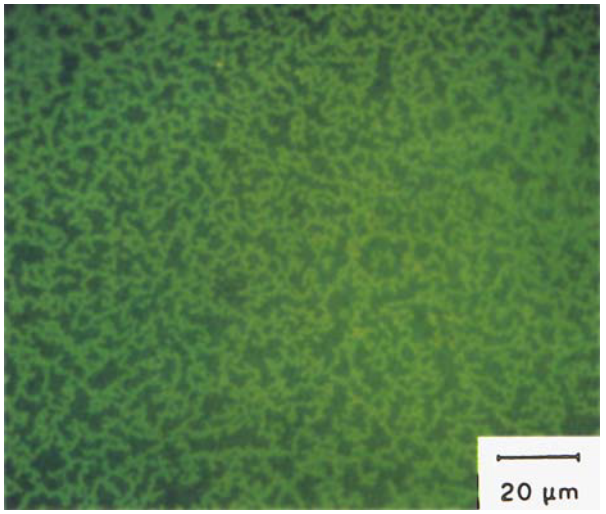


Fig. 50. Epifluorescence micrograph to show the binding of C1q to a monolayer formed from lipid A and DPPC (1:4) (cf. Fig. 51). Injection of a fluorescein-labeled antibody against C1q (anti-C1q FITC IgG [179], FITC = fluorescein isothiocyanate) into the subphase of the monolayer with bound C1q, resulted in the appearance of a fluorescence due to binding of the FITC antibody to the membrane-fixed C1q-lipid A complex.

of C1q to the lipid A monolayer, or if it is caused later by the C1q-antibody interaction.

As is known from other studies, C1q binds to **63** via its globular head-group structures. Therefore, the simplified model shown in Figure 51 can be postulated to explain the monolayer results described above.

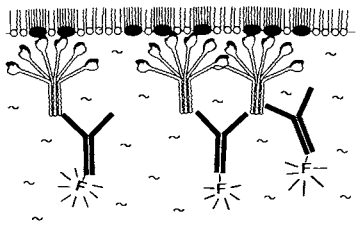


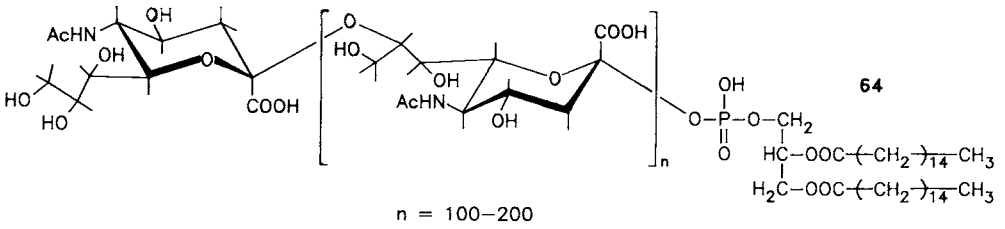
Fig. 51. Schematic representation of the adsorption of C1q to a monolayer formed from lipid A and DPPC and the binding of the fluorescein-labeled antibody. F stands for fluorescein isothiocyanate.

By making use of giant liposomes, it was also possible to demonstrate the interaction between C1q and **63** using FITC-labeled antibodies (for this method see Section 5.4). Here, too, it was found that, after the addition of C1q, only those liposomes containing **63** are made visible in the fluorescence microscope by the antibody.

5.4. Induction of Membrane Processes by Recognition Reactions at the Surface of Vesicles

A further example of a recognition reaction at the membrane surface is the binding of a specific antibody to its corresponding antigen at the membrane. Together with the group of *Bitter-Suermann*,[181] the polysaccharide K1 (**64**) from *E. coli* was used as a model system to investigate such surface reactions.[60]

K1 consists of α-(2-8)-linked sialic acid units[182] which are connected to a lipid residue. It belongs to the group of acidic capsular polysaccharides of the outer bacterial membrane. They are supposed to play a role in the serum and phagocytosis resistance of gram-negative bacteria.[181a] To prove the incorporation of **64** into model membranes, giant liposomes of the phosphatidylcholine DMPC were used. A micellar solution of **64** is injected into the chamber, shown in Figure 36, containing such a liposome preparation (Fig. 52A). After the insertion of the lipid anchors into the liposomal membrane (Fig. 52B, see also Section 4.8.1), the fluorescence-labeled anti-K1 antibody[181b] is added. The recognition between antigen and antibody (Fig. 52C) can be shown by means of fluorescence microscopy:



the originally diffuse fluorescence of the dissolved antibody becomes concentrated on the liposome surface.

The comparison of a phase-contrast micrograph (Fig. 53A) with the corresponding epifluorescence one (Fig.

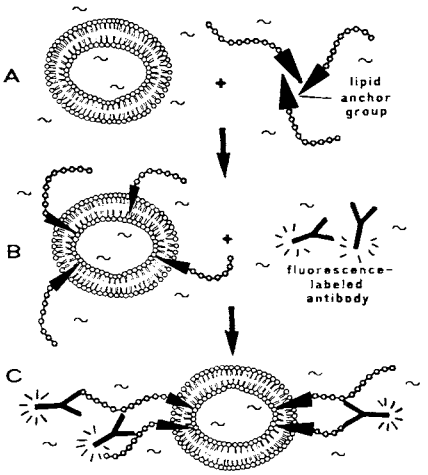


Fig. 52. Schematic representation of the experiment to prove the insertion of the capsular polysaccharide K1 (**64**) into liposomal membranes by using immunofluorescence [60]. A) Addition of a micellar solution of K1 to a liposome preparation. B) Insertion of the lipid anchor groups into the lipid bilayers; addition of the fluorescein-labeled anti-K1 antibody. C) Binding of the antibody and induction of a phase separation due to cross-linking of the K1 molecules (patching).

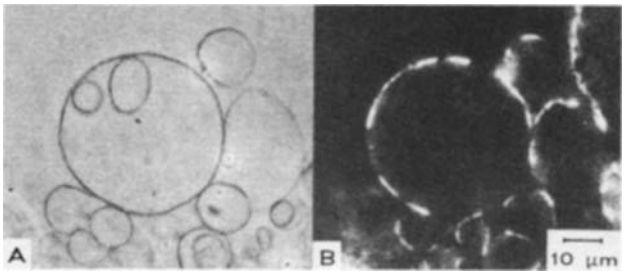


Fig. 53. Proof for the incorporation of **64** into DMPC liposomes [60]. A) Phase-contrast micrograph; B) epifluorescence micrograph of the same liposome (patching according to schematic drawing in Fig. 52C).

53B) clearly shows that the fluorescence of the antibody is associated with the membrane; i.e., **64** is detected at the membrane surface. Furthermore, it becomes evident that the fluorescence, dependent on time and concentration, is no longer distributed homogeneously in the membrane: patching[183] can be observed. It is caused by the reaction between the multivalent antigen, which is capable of lateral diffusion within the membrane, and the bivalent antibody, thus leading to a phase separation. This process might be compared to a precipitation reaction in two dimensions. Therefore, this experiment is not only an example of a recognition reaction between antigen and antibody; the final result, the clustering of one membrane component, also represents a simple membrane process (see Section 6.1).

6. Simulation of the Dynamics of Biomembranes

The dynamics of biomembranes (shape fluctuations, membrane instabilities), which is responsible for motion and transport processes, is fascinating and of great biological importance. It is a challenging task to explain cellular processes by phenomenological physical properties of the plasma membrane.[155a] The plasma membrane with its manifold functions is much more than just a passive permeability barrier which encloses cytosol from the surrounding medium. On the one hand, membrane transport proteins or ion carriers provide for a "local," ordered exchange of substances in both directions. On the other hand, dynamic processes are responsible for the important "bulky" transport through the membrane. Examples of these uptake and release processes of the cell are illustrated in Figure 54.

Cells ingest macromolecules and particles by a process called endocytosis: the substances are progressively enclosed by a small portion of the plasma membrane, which first invaginates and then pinches off to form an intracellular vesicle containing the ingested material.[185] Analogous mechanisms exist for the release of substances from the cell: as regards exocytosis, the intracellular vesicles, containing the substance to be released, fuse with the plasma membrane and open to the extracellular space. In the case of budding, bulges of the membrane to the outside are detached, whereby the substances given off remain enclosed by a lipid bilayer. This process plays a role when, for example, a virus leaves its host cell.

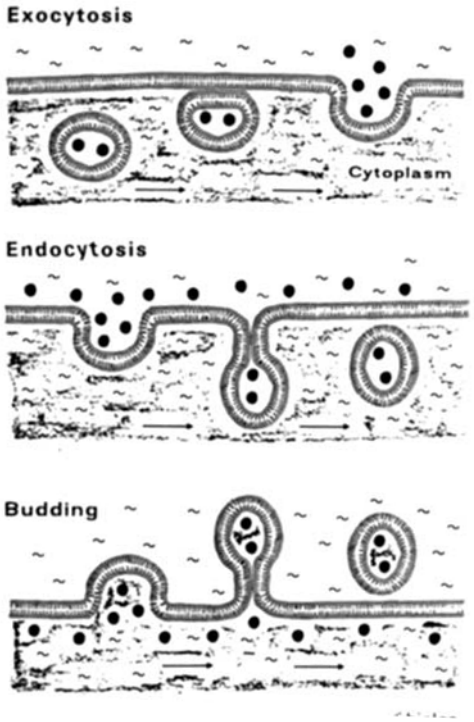


Fig. 54. Schematic drawing of three important transport processes of biological membranes [184]: exocytosis, endocytosis, and budding.

All these events require dynamic processes within the membrane, including the possibility of forming local domains in the membrane (patching and capping). In membrane biology, the mechanism of such dynamic cell processes is habitually described as extremely complex, usually involving proteins and being energy-consuming. Phase-separation phenomena, however, can be triggered off in lipid bilayers depending on counterions or temperature.[186] Simplified model membranes thus help to answer the question of what minimum requirements are necessary in a given system for a certain process to take place at all. The following sections illustrate how dynamic processes can be induced in purely synthetic, polymeric model systems in such a way that they correspond, at least formally, to biomembrane processes.

6.1. Patching and Capping in Polymerized Mixed Liposomes

Patching and capping are simple dynamic processes of the biomembrane caused by lateral diffusion. They are triggered off at the cell surface by antigen–antibody interactions and can be observed directly with a light microscope by using a fluorescence-labeled antibody.[183, 187] This is schematically shown in Figure 55. The fluorescence-labeled antibodies bind to the antigens at the cell surface (Fig. 55A) and a diffuse fluorescence, which is homogeneously distributed over the whole cell membrane, can be observed. Since the antibody molecules are bivalent, they are able to cross-link with the antigens. This soon leads to a phase separation within the membrane and thus to the fluorescent patches (Fig. 55B). An example of such a patching process in a vesicle membrane has already been described in Section 5.4. In the cell, the individual

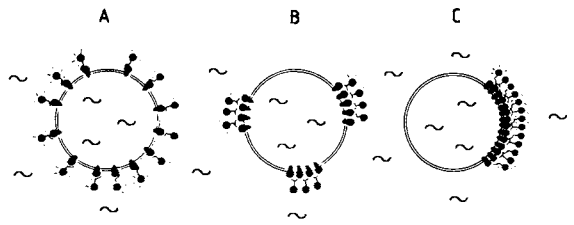


Fig. 55. Schematic drawing of the aggregation of membrane receptors induced by the binding of bifunctional fluorescence-labeled antibodies. A) Diffuse fluorescence of the homogeneously bound antibody. B) Clustering of the receptors forming fluorescent domains (patching). C) Aggregation of the domains forming a single fluorescent area (capping).

domains move, within a few minutes, to one certain area of the membrane, thus forming a cap (Fig. 55C). Whereas patching only requires lateral diffusion, it is assumed that the subsequent aggregation to one single cap is an energy-promoted process.[183, 187]

In mixed membranes of unsaturated and natural lipids, a phase separation can be induced via polymerization. This domain formation resembles the immunologically important processes of patching and capping.[188] Such experiments have been carried out with mixed giant liposomes made from the polymerizable lipid **27** (Scheme 3) and DMPC (1:1). Their membrane dynamics are made visible by the addition of a small amount of a fluorescent lipid. The liposomes composed of the monomeric components reveal complete miscibility of both lipids and the incorporated fluorescent dye, as can be seen by the diffuse fluorescence of the giant liposomes. After photopolymerization of **27** at 40°C (diffuse fluorescence still observable, Fig. 56A) and cooling down of these partially polymerized liposomes below the phase transition of the polymeric lipid, isolated areas of intense fluorescence occur (Fig. 56B).

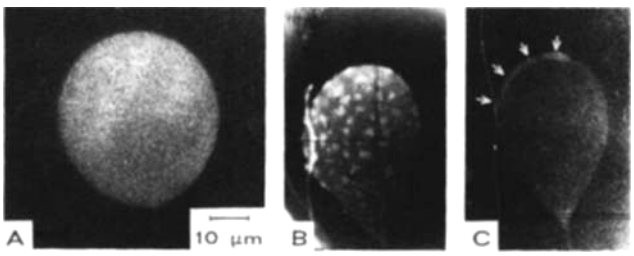


Fig. 56. Simulation of patching and capping in polymerized giant liposomes; epifluorescence micrographs of a giant liposome made from a mixture of the butadiene derivative **27** and DMPC (1:1) (0.1 mol% fluorescent lipid as marker) [188a]. A) Homogeneous fluorescence in the polymerized liposome at 40°C (above the phase transition of the polymer). B) Phase separation forming fluorescent domains in the same liposome immediately after lowering the temperature to 28°C (below the phase transition of the polymer). C) Formation of a single fluorescent cap in the same liposome after a few minutes.

This is caused by the immiscibility between the polymeric lipid, which is in the crystalline state, and the other membrane components, which are still in the fluid state. After a few minutes, the visible patches "flow" together, thus forming one single membrane cap (Fig. 56C). The time scale and appearance of these processes are completely analogous to the patching and capping observed on cell surfaces during their interaction with bivalent antibodies. These natural biomembrane processes can thus be mimicked with striking similarity using polymeric model systems. The initiating mechanisms are, however, completely different. Nevertheless, such experiments may contribute to the understanding of the biological phenomenon. It is generally assumed that the process of capping is actively driven, thus requiring ATP. However, the results reported here provide the first experimental evidence that such a cap formation can take place passively without energy consumption.

6.2. Morphological Changes of Giant Liposomes

The transport mechanisms of endocytosis and budding involve a local bulge either to the inside or to the outside of the membrane with the subsequent detachment of a small vesicle (cf. Fig. 54). The first step of this two-stage process is a local reversible change in the spontaneous curvature of the membrane. In contrast, the second step, the detachment of the vesicle, is irreversible. Figure 57 schematically illustrates how this process is triggered off in Nature by taking erythrocytes as an example. Here a local change in the spontaneous curvature of the membrane occurs, probably due to a lateral contraction of the cytoskeleton.[154]

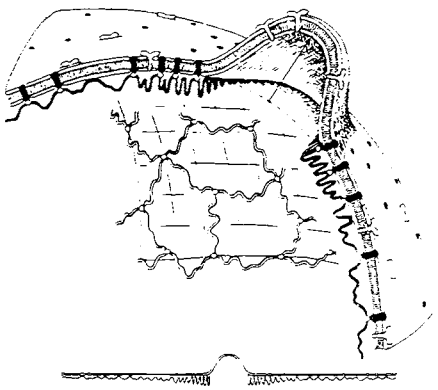


Fig. 57. Proposed pathway of triggering of endocytosis in an erythrocyte; by a local lateral contraction of the cytoskeleton, the removal of the network is expected to lead to a local change in the spontaneous curvature [154].

Protrusions caused by lateral phase separation can also occur in synthetic model systems. Figure 58A shows a giant liposome composed of a mixture of DMPC and the polymerizable spacer lipid **36b** (Scheme 4). Upon UV polymerization under a light microscope, clearly visible protrusions (Fig. 58B) develop out of the initially smooth surface. They continue to grow even after the UV light has been turned off, and their number increases with increasing amount of polymerizable lipid. This leads to the conclusion that a phase separation between the formed polymeric lipid and DMPC takes place, as shown schematically in Figure 58C.

Fairly well known examples of reversible transitions between different cell morphologies are the shape changes of erythrocytes. These can be achieved by different environmental changes such as changes in pH, temperature, and ionic strength or by the addition of drugs.[189] In the last

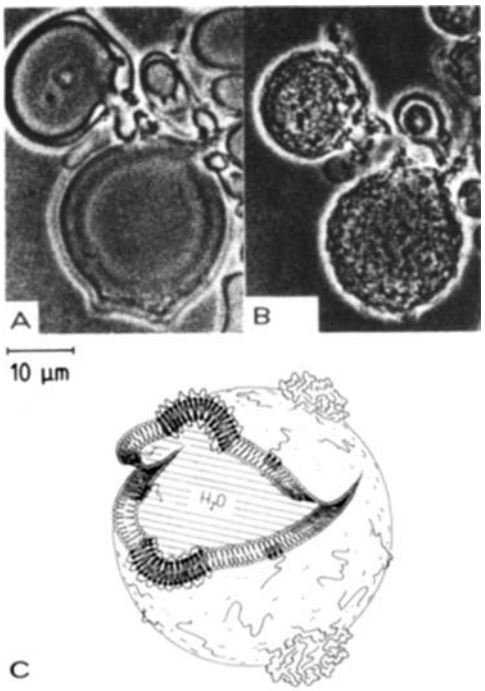


Fig. 58. Polymerization-induced protrusions in a giant liposome made from DMPC and **36b** (1:1) [154]. A) Phase-contrast micrograph of monomeric giant liposomes. B) After UV polymerization (100 s). C) Schematic representation of the microstructure of the bilayer after polymerization; lateral phase separation leads to domains of the polymeric lipid exhibiting a higher spontaneous curvature than the monomeric lipid matrix.

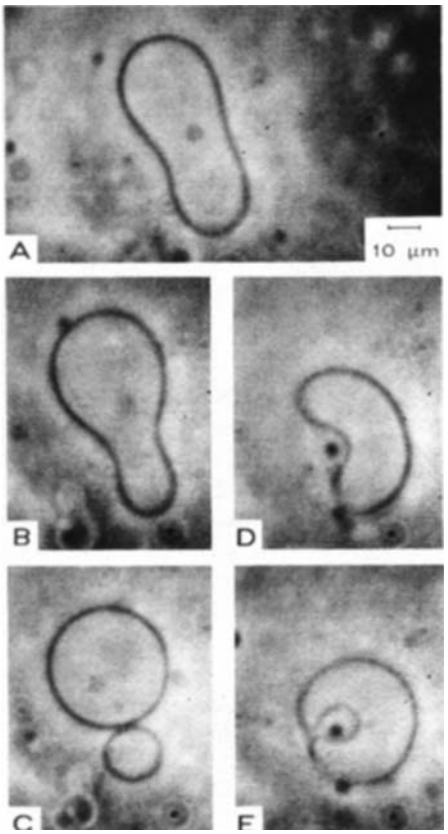


Fig. 59. Phase-contrast micrographs of temperature-induced, reversible morphology changes of a giant liposome made from the ammonium lipid **27** [155b]. A–B–C: Constriction of a discoid liposome at increasing temperature, reminiscent of exocytosis (cf. Fig. 54). A–D–E: Invagination in the same discoid liposome at decreasing temperature, reminiscent of endocytosis (cf. Fig. 54). A) 42°C; B) 42.5°C; C) 43°C; D) 41.5°C; E) 41°C.

few years, a series of theoretical studies have been published in order to explain the stability of the different cell shapes observed.[190] In this context, the model of *Svetina* and *Zeks* must be mentioned in particular.[190d] This model explains the stability of the different cell morphologies without any assumption as to the structure of the cell membrane. It is based on the idea that both leaflets of the lipid bilayer can expand and contract independently of one another. If, however, the changes in area of the two bilayer halves differ, the result is a change in the spontaneous curvature and thus a morphological change of the cell.

This concept has been confirmed by the observation of giant unilamellar liposomes.[155b] Figure 59 shows a series of reversible morphological changes of a giant vesicle made from the ammonium lipid **27**. These processes are temperature dependent and occur near the phase transition temperature (42°C). At a temperature of 42°C, the vesicle has a discoid shape (A). When the temperature is raised to 43°C, the vesicle constricts (C) via a bulge (B) to the outside. If, however, the temperature is lowered to 41°C, the opposite, i.e., a bulge to the inside, can be observed (D, E). Formally, these processes resemble budding and endocytosis and are completely reversible. By using the model of *Svetina* and *Zeks*, they can be explained in terms of a difference in the electric surface potential between the inner and outer membrane halves.[155b]

An additional example of an invagination involving a giant vesicle made from DMPC and the spacer lipid **36b** (4:1) is shown in Figure 60. In this case, however, the process is not triggered off by changing the temperature,

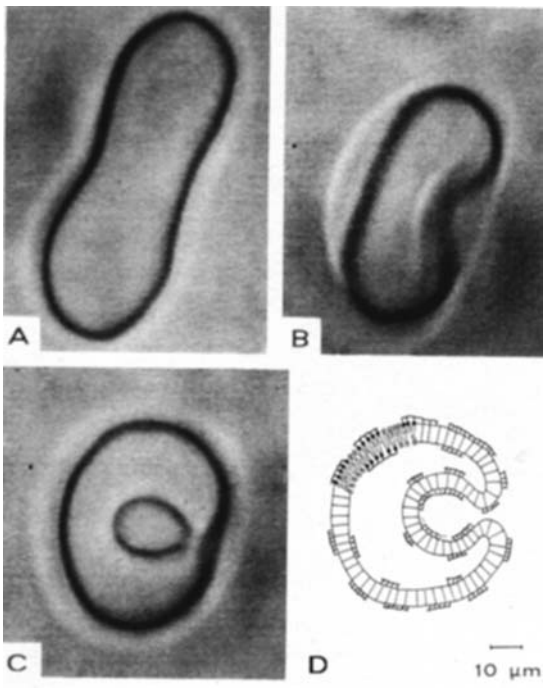


Fig. 60. Endocytosis-like shape change of a giant liposome made from DMPC and **36b** (4:1) caused by UV polymerization [155a]. A) Monomeric liposome at 29°C. B, C) Transformation occurring after 2 min of UV irradiation. D) Possible explanation for the shape transition in terms of a larger reduction of the area per lipid molecule in the outer monolayer compared with the inner leaflet. This might be due to an asymmetric distribution of **36b** which is probably preferentially distributed in the outer leaflet because of its large head group.

but by UV polymerization of one membrane component. This experiment does not lead to the protrusions of phase-separated polymer (cf. Fig. 58), because the portion of the polymerizable lipid is only 20 mol%. The membrane process observed is a bulge of the membrane to the inside, analogous to endocytosis. This can also be explained by the model of *Svetina* and *Zeks*: It can be assumed that the concentration of the spacer lipid **36b** is slightly higher in the outer membrane half than in the inner one, owing to its large head group. The polymerization going hand in hand with a decrease in area per molecule then leads to a reduction in area of the outer leaflet of the membrane, thus causing the invagination.[155a]

The experiments described show that even slight changes in the membrane or its environment may cause the morphological changes which are important for transport processes in biological systems. The second step of these processes, the irreversible detachment of smaller vesicles, however, requires more than only a change in curvature. In Section 4.3, a photoreactive system has already been presented which allows the realization of this process in model membranes. Upon short-term irradiation of interconnected, giant vesicles of the benzylammonium lipid **19**

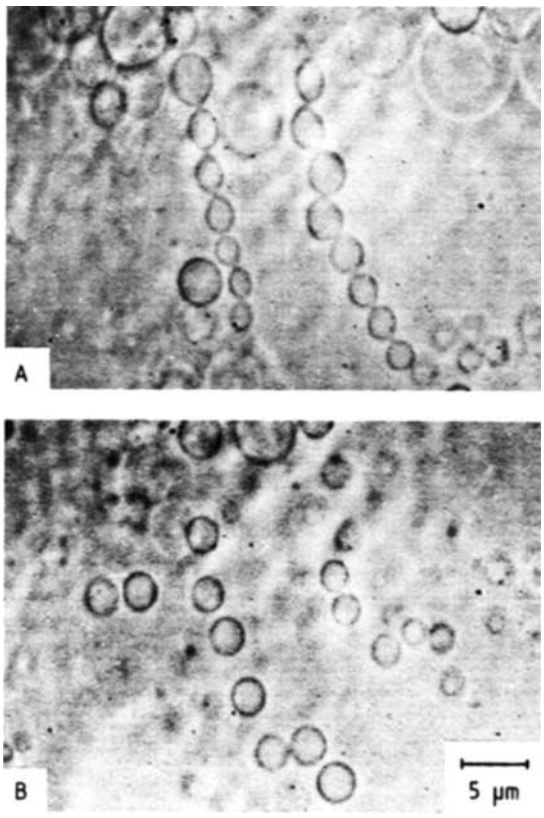


Fig. 61. Photochemically induced separation of interconnected giant liposomes made from the benzylammonium lipid **19** (cf. Fig. 23) [155b, 191]: A) before irradiation; B) after 2 s of UV irradiation.

(Fig. 61A), which resemble strings of pearls, the individual vesicles undergo separation via a photoinduced phase-separation process (Fig. 61B).[155b, 191]

6.3. Selective Opening of Phase-Separated Liposomes

6.3.1. Death of a Tumor Cell—Is Simulation Possible?

The great variety of biomembrane processes and the interaction of molecular and cellular events is reflected in a rather fascinating way in the death of a tumor cell that cannot escape from the immune system (Fig. 62).[192] The scanning electron micrograph shows the attack of an activated macrophage on a tumor cell (Fig. 62A).

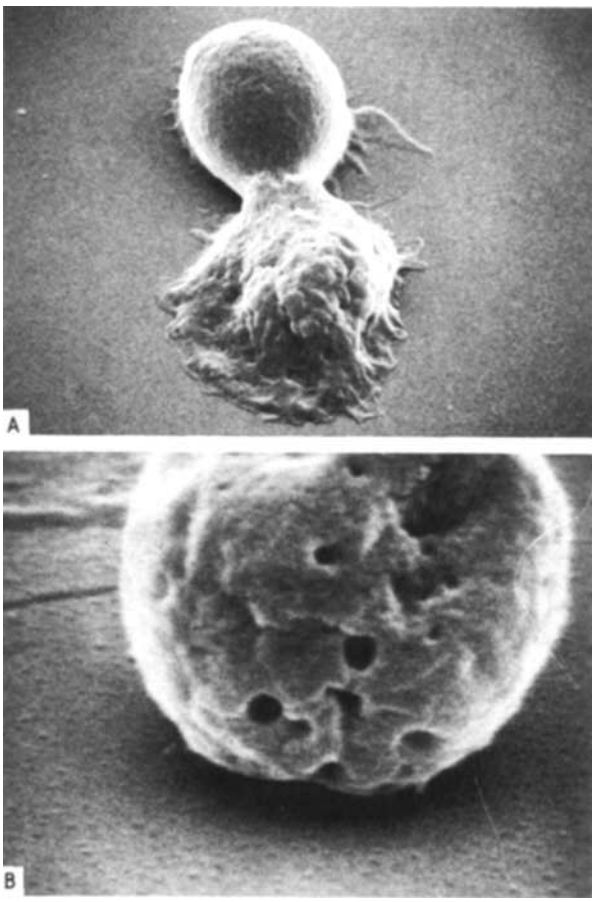


Fig. 62. Scanning electron micrograph of a tumor cell attacked by an activated macrophage [192]. A) Close contact between tumor cell (upper part) and activated macrophage (lower part). B) Holes in the membrane of the tumor cell after the attack.

On a molecular level, via antigen-antibody interaction, the macrophage recognizes the target cell and establishes a close membrane contact. Then, on a cellular level, a patching of the antigen-antibody complexes presumably takes place. In the subsequent membrane processes, which are not yet completely understood, the defense cell is able to drill holes in the originally stable membrane of the tumor cell. As a result, the cytoplasmic interior leaks out, which causes the death of the cell. What remains is a ghost cell (Fig. 62B).

Can this process be simulated? And if so, what is needed to do so? On the one hand, it is surprising that the lipid-protein bilayer does not collapse despite the visible holes. This gives evidence for its stability, the simulation of which has been discussed several times in this article. On

the other hand, the question of how these holes are created comes up. Conceivably, this occurs by means of a local process in the phase-separated membrane. One possibility could be that the macrophage tears open the tumor cell's membrane by means of a local endocytosis process of its own membrane. Another possibility is that the opening process is triggered by the macrophage and is accomplished by the cytoskeleton of the tumor cell. A further possible approach would be the lysis of membrane domains by enzymatic cleavage of the phospholipids, forming water-soluble lysophospholipids. The real course of this process is unknown.

The following sections describe the formal simulation of this process using the simple model of phase-separated polymerized liposomes.

6.3.2. *Formation of Domains in Vesicle Membranes*

Phase-separated liposomes can be used to simulate the hole formation process of the cell. In order to avoid a complete destruction of these liposomes by "uncorking," it is important that they are composed of a polymerized lipid matrix surrounding the labile domains of cleavable or nonpolymerizable lipids (Fig. 63).

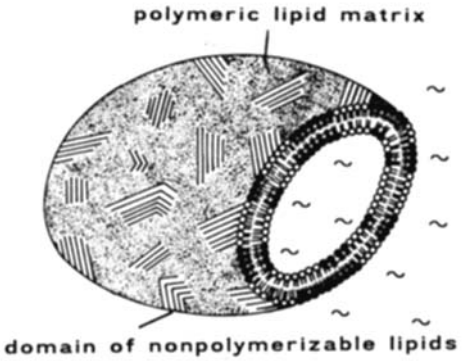
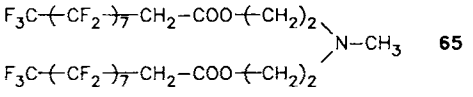


Fig. 63. Schematic drawing of a phase-separated liposome; the polymerized lipids form the stable matrix, surrounding the domains of nonpolymerizable lipids.

The induction of such a phase separation via polymerization has already been described in Section 6.1. Alternatively, the formation of domains can be predetermined by choosing suitable, incompatible lipid mixtures. Examples are mixtures of hydrocarbon and fluorocarbon lipids.[193] Mixed liposomes made from DMPC and the fluorocarbon amine **65** feature two phase transitions, e.g., in differential



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scanning calorimetry (DSC) measurements. This observation clearly demonstrates that these two lipids are immiscible since these phase transition temperatures are identical with those of the corresponding pure lipids.[193a] Freeze fracture electron micrographs reveal that, indeed, phase-separated liposomes with domains of the individual lipids are formed from the lipid mixture instead of two different liposome populations. Thus, domain formation in mixed membranes prepared from DMPC and **65** (95:5) can be made directly visible (Fig. 64) by means of the ripple structure typical of lecithin liposomes (see Section 4.8.1).

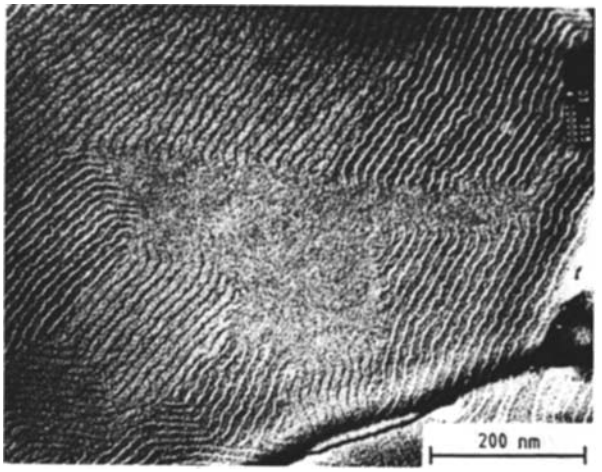
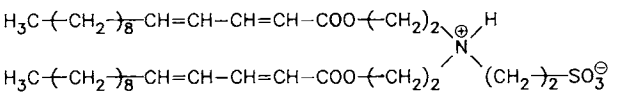


Fig. 64. Freeze fracture electron micrograph of a phase-separated liposomal membrane (95 mol% DMPC and 5 mol% fluorinated lipid **65**). The ripple structure shows the parts of the membrane composed of DMPC, surrounding a domain of the fluorinated lipid (smooth surface) [193a, 204].

To obtain a stable polymeric matrix needed for uncorking experiments of mixed liposomes, the polymerizable lipids **66** and **67** with hydrocarbon and fluorocarbon chains, respectively, have been used.



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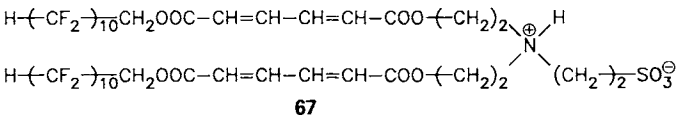

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Small unilamellar liposomes can be prepared from both lipids by means of sonication. The tightness of the polymeric matrix of vesicles formed by lipid **66** was demonstrated by time-dependent release measurements using eosin as a fluorescent marker. Figure 65 shows a comparison of the eosin release of monomeric and polymeric vesicles

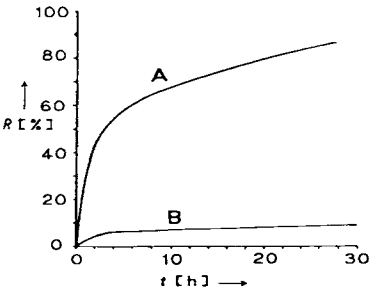


Fig. 65. Release R of eosin from vesicles (SUV) made from **66** [200]: A) monomeric; B) polymerized (5 mol% azobisisobutyronitrile, 60°C, 12 h).

made from the lipid **66**. The monomeric vesicles release half of the dye within 5 h, whereas the polymeric ones have lost only about 10% of their contents after 30 h.

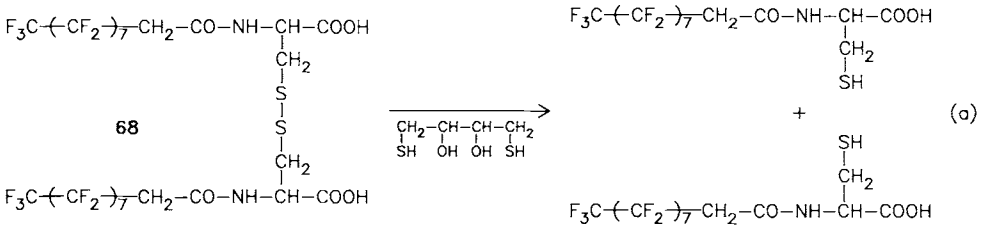
6.3.3. *Corkscrews for Corked Liposomes*[194]

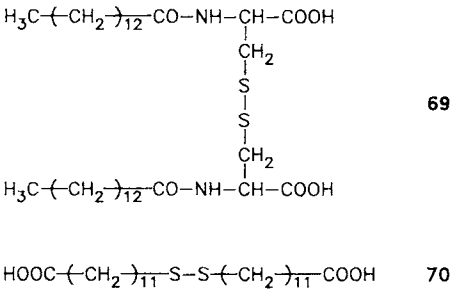
Numerous works proposing various mechanisms to destabilize membranes of liposomes have already been published. These include photochemical[94] as well as temperature-induced[195] increases in permeability or the use of pH-sensitive liposomes.[196] These investigations have exclusively been carried out with liposomes made from nonpolymerizable lipids.

In order to simulate the attack on a tumor cell by a macrophage, as shown in Figure 62, it is important not to destroy the whole liposome by membrane-destabilizing processes. Only labile domains within a stable lipid matrix should be selectively dissolved.[197] Figure 66 shows different processes that result in selective opening of such stabilized, phase-separated liposomes.

The polymeric components of these mixed liposomes form the stabilizing, shape-maintaining matrix. In order to destroy the labile domains, at least two methods are conceivable: dissolving or cleavage of the lipids. On the one hand, it is possible to dissolve the lipids of those domains with surfactants (detergents), thus forming mixed micelles, or by using organic solvents (e.g., acetone or ethanol). On the other hand, lipid cleavage through enzymatic or chemical reactions can be carried out. The enzymatic hydrolysis of phospholipids, forming lysophospholipids, by means of phospholipase A_2 (cf. Fig. 75) is the method Nature uses to synthesize and decompose membranes.[198] Several years ago, *Büschl* et al. already succeeded in transferring these reactions to polymeric model systems.[199]

The simulation of such lipid cleavage reactions by means of chemical processes offers a great number of synthetic ways to locally open the labile lipid domains in polymerized liposomes. Two principles are shown in Figure 66: head-group cleavage of double-chain lipids or chain cleavage of membrane-spanning lipids, each of them forming two surfactant molecules. To realize this concept by chemical reactions, a series of double-chain (**68**, **69**) or membrane-spanning (**70**) lipids with a disulfide group as





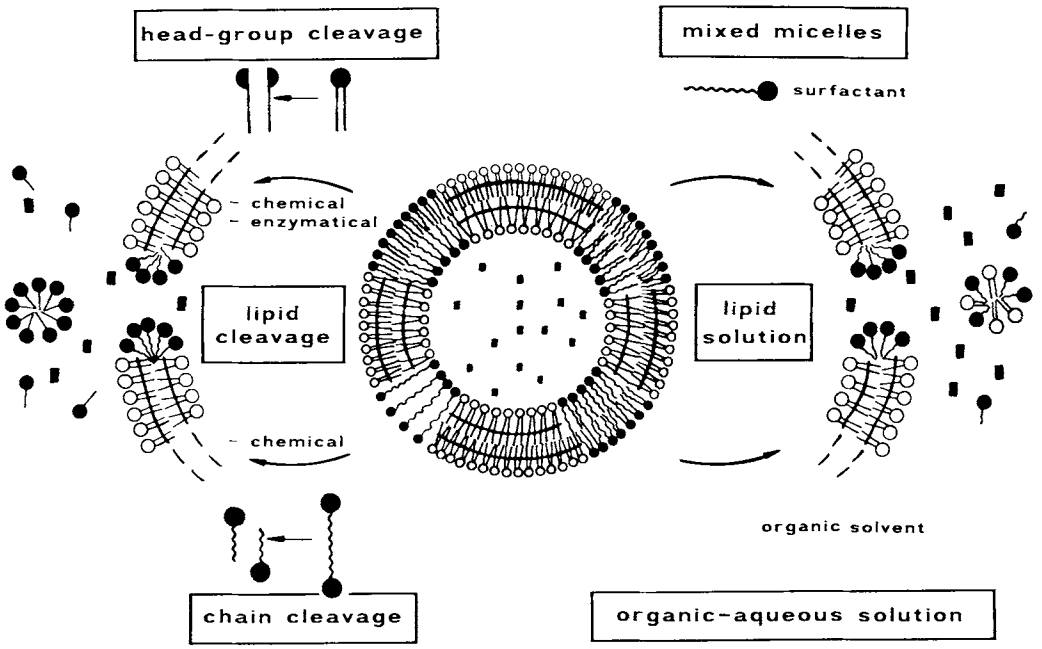


Fig. 66. Methods to uncork phase-separated liposomes composed of a stable polymeric matrix with labile domains.

cleavable unit have been synthesized.[200] The reductive cleavage of the water-insoluble disulfides—for example, with dithiothreitol (DTT)—leads to the formation of the corresponding partially water-soluble, single-chain thiols [Eq. (a)].

The cystine lipids **68** and **69** are capable of forming liposomes by sonication. The membrane-destruction process was followed by fluorescence spectroscopy using liposomes (made from **68**) containing eosin (Fig. 67). The

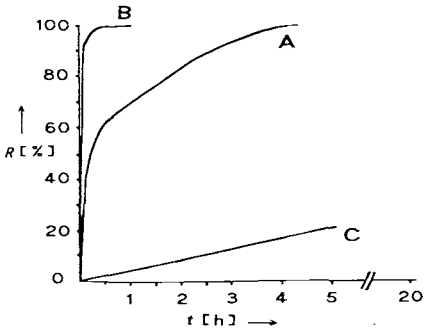


Fig. 67. Release R of eosin from vesicles (SUV) made from the fluorinated cystine derivative **68** at pH = 9 [200]. A) Cleavage of the disulfide bond by using 0.1 M Na_2SO_3. B) Cleavage by using dithiothreitol (DTT) (2 mg mL^{-1}). C) In the absence of a cleaving reagent.

cleavage process of the disulfide bonds within the cystine-lipid liposomes is much faster with DTT compared with sodium sulfite. Thus, DTT was used as a corkscrew[201] for all further opening reactions.

6.3.4. Chemical and Enzymatic Opening of Polymerized Mixed Liposomes

The combination of polymerizable taurine lipids (**66** and **67**) with cork-forming amphiphiles (**68**–**70**) results in stable polymeric liposomes with cleavable "cork" domains, as discussed in Section 6.3.3. Evidence for the opening of the labile domains in mixed liposomes was provided by electron microscopy as well as by the release of entrapped eosin.[202] Figure 68 schematically illustrates the opening process.

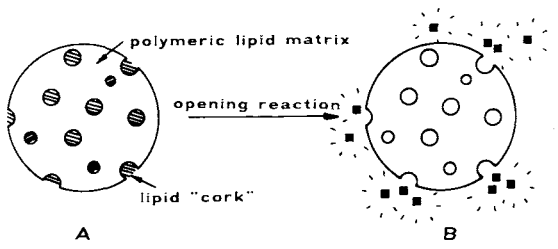


Fig. 68. Proof of the hole formation in polymerized vesicles containing labile domains. A) No fluorescence of the entrapped marker owing to self-quenching. B) Fluorescence of the released and therefore diluted marker after the hole formation in the membrane.

Head-group cleavage of lipids: In order to confirm the effect of phase separation on the release behavior of entrapped markers, liposomes with a homogeneous distribution of the cork component (i.e., not phase-separated) were studied as a comparison. Therefore, mixed liposomes from the polymerizable lipid **66** and the cystine lipid **69** (9 : 1), which form a homogeneous mixture, were used (Fig. 69).

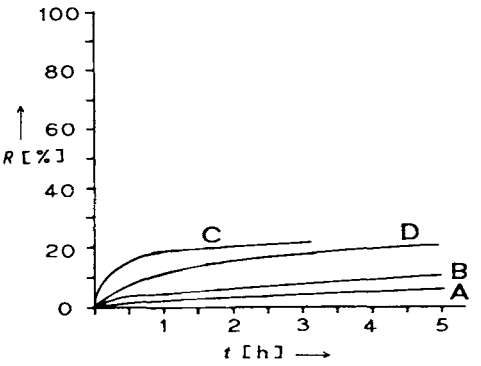


Fig. 69. Release R of eosin from domain-free vesicles (SUV) (90 mol% **66**, 10 mol% **69**, pH = 9) [200]. A) Polymerized vesicles in the absence of a cleaving reagent. B) Monomeric vesicles in the absence of a cleaving reagent. C) Polymerized vesicles after addition of DTT (1 mg mL^{-1}). D) Monomeric vesicles after addition of DTT (1 mg mL^{-1}).

In the absence of DTT as a cleavage reagent for the disulfide group of **69**, the release rates of the monomeric (curve B) and the polymerized liposomes (curve A) differ only very little. After the addition of DTT, the cleavage of the lipids does not lead to the formation of holes, because the cleavable lipids are distributed homogeneously within the membrane. The compatible lysolipids, formed during that process, cannot be pushed out of the membrane. Thus, after a short period of time, the release rates (curves C and D) are comparable to those measured without DTT (curves A and B).

A dramatic change in release behavior occurs if phase-separated liposomes from the same polymerizable lipid **66** and the domain-forming "fluorocarbon cork" **68** (9 : 1) are used (Fig. 70).

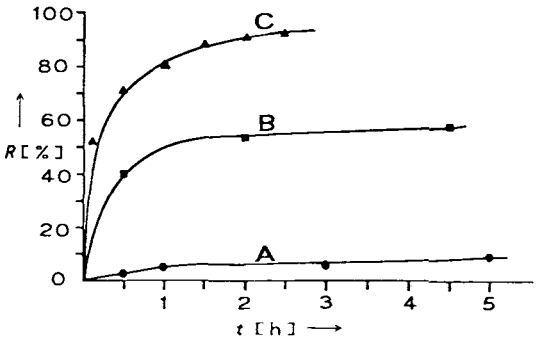


Fig. 70. Release R of eosin from phase-separated, domain-containing vesicles (SUV) (90 mol% **66**, 10 mol% **68**, pH = 9) [200]. A) Polymerized vesicles in the absence of cleaving reagent. B) Monomeric vesicles after addition of DTT (1 mg mL^{-1}). C) Polymerized vesicles after addition of DTT (1 mg mL^{-1}).

In this case, the domains of **68** are rapidly destroyed by DTT during the disulfide cleavage in unpolymerized liposomes. However, owing to the lateral diffusion of the monomeric lipids, the membrane defects are healed rather quickly. After cleavage of the whole cork component (about 1 h), the release rate (curve B) decreases to that

without DTT (curve A). After addition of DTT to the polymerized liposomes, the stiff polymeric matrix is uncorked and eosin is rapidly and almost completely released (curve C).

Is the hole formation in these experiments really similar to that which occurs in a cell (cf. Fig. 62)? It was possible to confirm this using two electron microscopy techniques. Figure 71 shows a scanning electron micrograph of a phase-separated polymeric liposome made from the taurine lipid **66** and the cleavable cystine lipid **68** before and after the destruction of the labile domains. As can be seen,

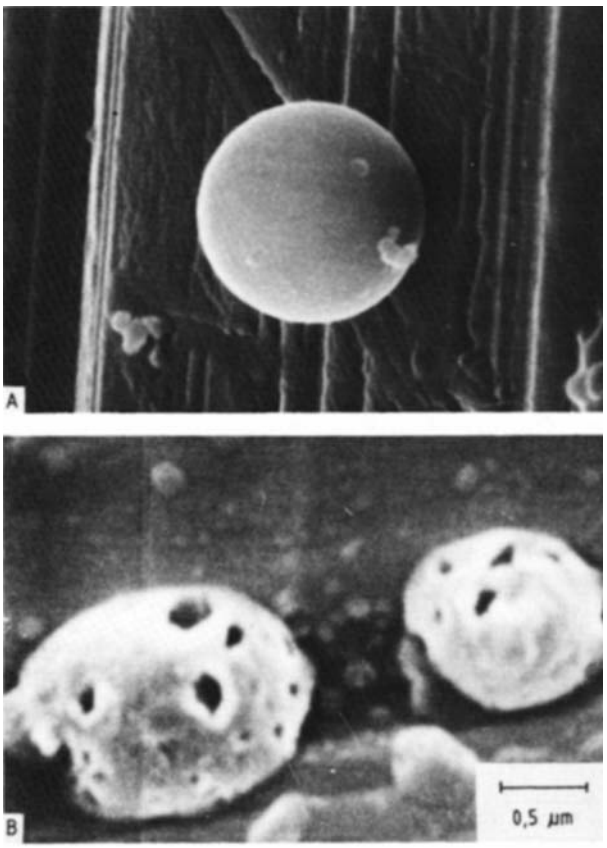


Fig. 71. Scanning electron micrograph of phase-separated, polymerized liposomes (90 mol% **66**, 10 mol% **68**) [200, 203]. A) Liposome before the cleavage reaction. B) Perforated membrane after the cleavage reaction (uncorked liposome!).

the lipid bilayers do not collapse. The function of the cytoskeleton of the cell, i.e., the stabilization of the membrane, is thus fulfilled by the polymeric matrix of the liposomes. The pore formation observed is not an artifact of the preparation technique for the scanning electron micrograph. This was proven by means of freeze fracture electron microscopy (Fig. 72). Figure 72A shows the smooth, unstructured surface of the polymeric mixed liposome before it is uncorked. In Figure 72B the membrane again features clearly visible holes after the cleavage has been carried out. The different diameters of the holes in the uncorked liposomes (Figs. 71B and 72B) are due to varying domain sizes, caused by different annealing times of the mixed liposomes before their polymerization.

Chain cleavage of lipids: All experiments discussed so far have been carried out with the cystine derivative **68** as the lipid cork. An analogous behavior has been observed with

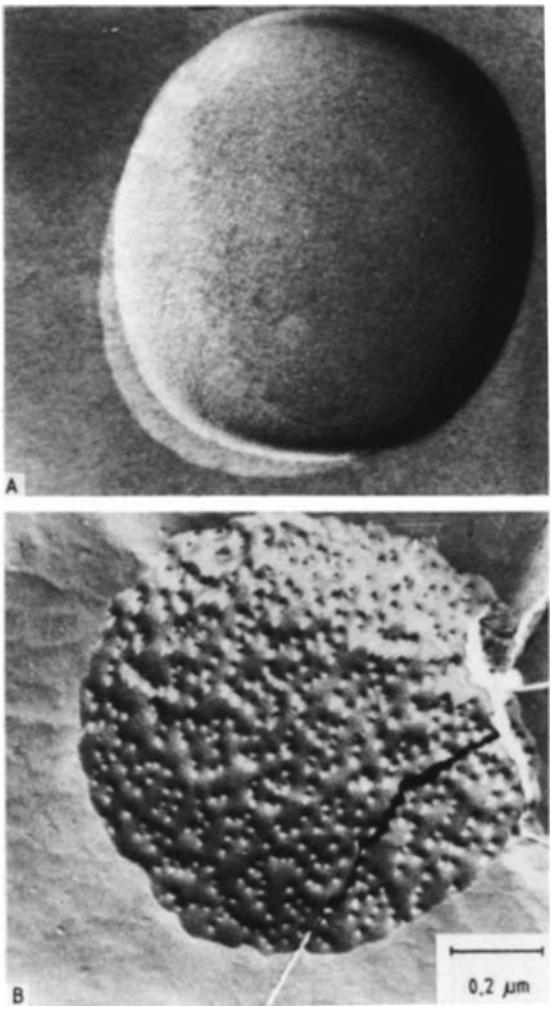


Fig. 72. Freeze fracture electron micrograph of phase-separated, polymerized liposomes [200, 204] (see caption to Fig. 71).

the bipolar disulfide amphiphile **70**. The release of eosin from phase-separated liposomes containing this cleavable lipid and the fluorinated muconic acid derivative **67** is shown in Figure 73.

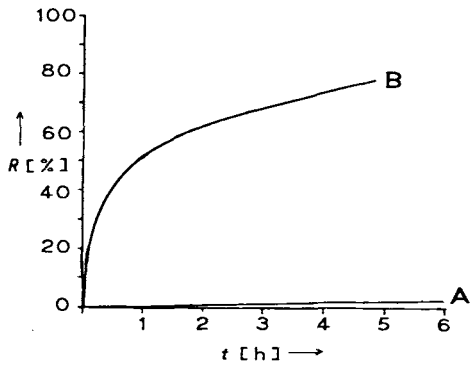


Fig. 73. Release R of eosin from phase-separated, domain-containing vesicles (SUV) (90 mol% muconic acid derivative **67**, 10 mol% disulfide **70**, pH = 9) [200]. A) Polymerized vesicles in the absence of a cleaving reagent. B) Polymerized vesicles after addition of DTT (1 mg mL^{-1}).

Polymerized vesicles with 10 mol% of the supposedly membrane-spanning cork **70** again exhibit only minor eosin release before cleavage (curve A). In this case, also, the

addition of DTT leads to a rapid release of the fluorescent marker, even though the rate is slower than that observed in the cleavage experiments with the cystine lipid **68**. This is quite understandable since the cleavage reagent does not attack the head groups; it first needs to diffuse into the membrane. However, no difference can be seen by electron microscopy between the double-chain and membrane-spanning cleavable lipid systems (Fig. 74).

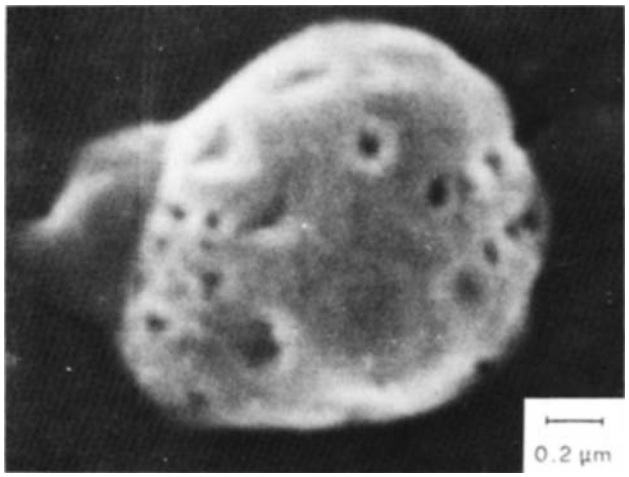


Fig. 74. Scanning electron micrograph of phase-separated, domain-containing liposomes (90 mol% **67**, 10 mol% **70**); hole formation after addition of DTT [200, 203].

Dissolving the cork without cleavage: As already illustrated schematically (Fig. 66), apart from a cleavage reaction, even the simple dissolving of the labile domains out of a polymeric matrix can lead to the formation of pores in a vesicle membrane.[200] This concept is based on the well-known fact that polymerized vesicles exhibit a higher stability towards surfactants or solvents than their monomeric analogues.[72,205]

To carry out an opening reaction by using solvents, mixed membranes made from the cationic polymerizable ammonium lipid **27** with the fluorinated amine **65** (9 : 1) were prepared. The dissolving of the domains formed by the fluorocarbon lipid after the addition of acetone to the solution of polymerized vesicles (1 : 1) was demonstrated by scanning electron microscopy. Here, too, holes are formed in the vesicle. In the course of this process, the polymerized lipid matrix again maintains its shape. In the meantime, *Tsuchida* et al. have also reported similar experiments.[206]

Enzymatic uncorking:[207] The opening reactions discussed so far proceed under nonphysiological conditions and with a relatively high concentration of cleavage reagent. The biochemical opening, in contrast, uses phospholipases and allows the process to take place under physiological conditions. Phospholipase A_2 specifically catalyzes the hydrolysis of the ester bond at the C-2 position of L-α-glycerophospholipids (Fig. 75).

This enzymatic reaction can be transferred to model membranes composed of polymerized and natural lipids. This was demonstrated first by using monolayers.[199] To investigate this cleavage reaction in liposomes,[208] mixed

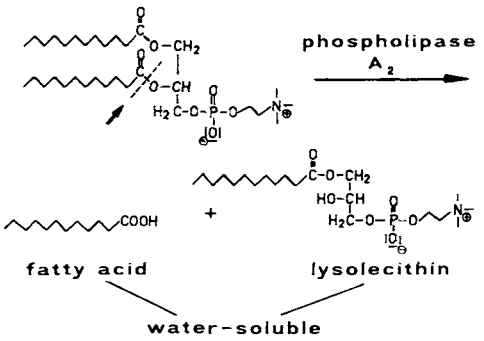
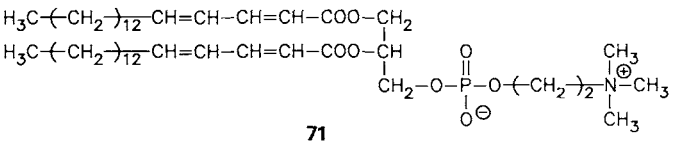


Fig. 75. Enzymatic cleavage of the C-2 ester bond of a lecithin by phospholipase A_2; the products (fatty acid and lysolecithin) are water-soluble surfactants.

membranes of the polymerizable lecithin **71** and DPPC (1 : 1) were used. Here, DPPC acts as the cork, cleavable by



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enzymes. The liposomes formed from these two lipids do not show domains since they are homogeneously miscible. Polymerization, however, induces a phase separation. It has to be pointed out that the highly specific phospholipase A_2 attacks the ester group of the dienoyl lecithin neither in the monomeric nor in the polymeric form. Indeed, liposomes prepared only from the dienoyl lecithin **71** remain tight and do not release eosin after addition of the enzyme. Thus, the release of 6-CF from mixed liposomes (Fig. 76) is clearly due only to the cleavage of the saturated lecithin (DPPC).

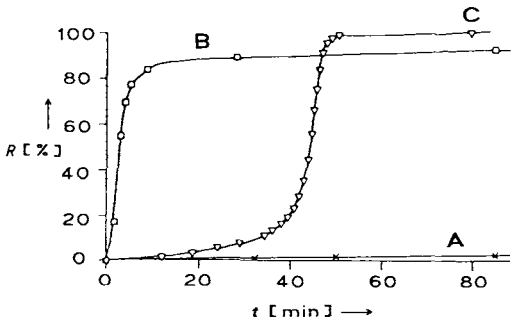

Fig. 76. Release R of 6-CF from monomeric and polymerized mixed vesicles (SUV) (50 mol% **71**, 50 mol% DPPC) [208]. A) Monomeric and polymerized vesicles in the absence of the enzyme. B) Polymerized vesicles after addition of phospholipase A_2 (30 µg mL^{-1}). C) Monomeric vesicles after addition of phospholipase A_2 (30 µg mL^{-1}).

In the absence of the enzyme, the release rate of 6-CF for the monomeric as well as the polymeric liposomes remains below 1% (curve A) during the experiment. After the addition of phospholipase A_2, a dramatic change in the release behavior occurs. The polymerized, phase-separated vesicles very rapidly lose the entrapped marker: already after 10 min, 90% of the vesicle contents are released

(curve B). In contrast, the analogous monomeric system exhibits an "incubation time" of about 40 min. In this case, too, a fast and almost complete release of the marker subsequently takes place within a few minutes (curve C). How can these different release rates be explained?

At first the 6-CF marker is only slowly released from the homogeneously mixed membrane of the monomeric liposomes. This is due to the fact that the lysolipids formed dissolve in the membrane, thus disturbing it only slowly. Since the ratio of cleavable to noncleavable lipid is 1 : 1, the membrane reaches such a high content of lysolipids and fatty acids after a certain incubation time (40 min) that the liposomes spontaneously decay, forming mixed micelles. The difference between the monomeric and the polymerized mixed system can be explained by the polymerization-induced phase separation. Experiments with monolayers of analogous systems also revealed that phospholipase A_2 attacks phase-separated membranes much faster than the corresponding homogeneous mixtures.[199] Due to phase separation, the lysolipid- and fatty-acid-rich domains are rapidly dissolved. However, the formation of a stable polymeric matrix may be impossible in these systems owing to the high contents (50 mol%) of DPPC. Thus, the polymerized liposomal membrane, too, is completely destabilized during the cleavage reaction with the formation of mixed micelles.

These concepts are supported by release measurements with mixed liposomes of the same composition but with different extents of polymerization (conversion).[208] As can be seen in Figure 77, the incubation time for the spontaneous destabilization depends on the amount of polymer in the membrane. Due to the polymerization-induced phase separation, the portion of cleavable lipid within the monomeric areas increases with increasing polymerization. Therefore, the time needed to reach the critical concentration of water-soluble products in the monomeric domains, i.e., the incubation time, depends on the extent of polymerization.

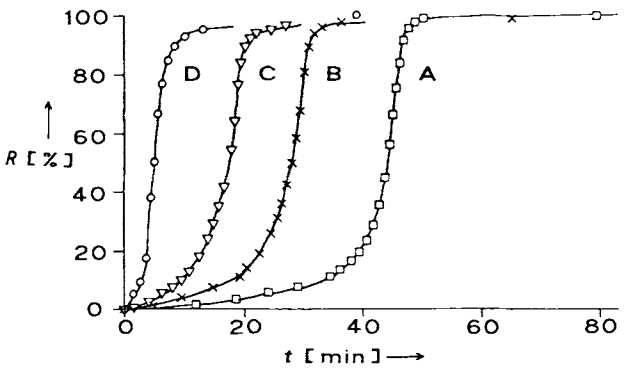

Fig. 77. Enzymatic release of 6-CF from partially polymerized vesicles (SUV) (50 mol% **71**, 50 mol% DPPC, 30 μg mL^{-1} phospholipase A_2) [208]. Dependence of the release kinetics on the extent of polymerization: A) 0%; B) 15%; C) 45%; D) 85%.

Synthetic corkscrews for cells? The experiments discussed so far show that it is indeed possible to formally simulate the biological process of local cell membrane opening by using phase-separated, partially polymerized liposomes. However, one is still miles away from the natural process. Figure 78 leads back to biological processes and does not merely show an additional polymerized liposome before and after it has been uncorked.

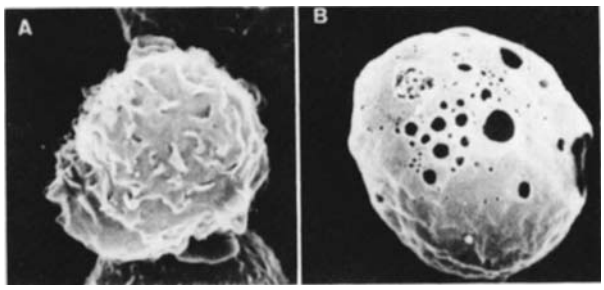

Fig. 78. Scanning electron micrograph of the hole formation in leukemia cells [209e]. A) Untreated leukemia cell. B) Leukemia cell after treatment with *O*-methyllysolecithin.

This scanning electron micrograph shows leukemia cells of a cell culture before (A) and after (B) treatment with special surfactants, namely, methyl ether derivatives of lysophospholipids which are, at present, also considered as anti-tumor agents.[209,68a] The result of the uncorking process of the cell by the synthetic lysolipid can clearly be seen. In whatever form the natural process of uncorking cells by activated macrophages (cf. Fig. 62) may proceed, it can at least be simulated in cells by using surfactants.

7. Outlook or Where to Go?

Many parts of this review are certainly incomplete. This is due partly to a lack of sufficient insight, partly to insufficient data. The reader should therefore not look at this contribution as a crystal-clear definition of facts and problems, but rather as an impulse for possible developments. This review represents an attempt, using the concept of self-organization, to jointly discuss the molecular architecture of supramolecular systems such as liquid crystals, micelles, liposomes, and biomembranes as a problem of mutual interest.[210]

The attempt to review different areas, which have traditionally been separate, is always a risky enterprise. It is impossible without generalizations and one thus, and rightly so, has to accept the eventuality of becoming superficial.[211]

On the other hand, *Georg Christoph Lichtenberg*, already 200 years ago, stated clearly: "He who understands nothing but chemistry doesn't even understand chemistry." Originally discussed in a more philosophical context, it nowadays (in times of rapidly increasing specialization) certainly holds true even for the natural sciences alone. Additionally, one has to accept that, owing to modern methods of measurements and sophisticated computer techniques, it is possible to measure to high precision and to evaluate more and more exactly: to measure and to verify is intensively taught and studied, but, too often, intuitive thinking is neglected.[212] Interdisciplinary research, however, is one way to stimulate intuition. In a refreshingly encouraging contribution, which recently appeared

in this journal, *Roald Hoffmann* describes "How Chemistry and Physics Meet in the Solid State."[211a] He stresses intuition as a strength of chemists. This frontier area of solid-state chemistry and physics can also only be tackled jointly and, like the formation and investigation of functional supramolecular systems, represents a fascinating chapter of modern materials science.

The fascination evoked in human beings by biological processes is old. The fascination of simulating these processes and transferring them into technical dimensions has just begun. The field is open and modern chemistry can offer essential contributions to it. Where to go? At present, materials science certainly can profit more from life science than vice versa. A technical analogue for biological membranes mimicking the specificity of their recognition and translocation processes has not yet been found—despite the countless varieties of synthetic polymer membranes already known. There have been many attempts to mimic the natural prototype and a few examples have been mentioned in this contribution. The breakthroughs are still missing: the knowledge is available, but innovation is a long time coming. Especially with respect to the next generation of technical membranes—switchable, functional, asymmetric, and suitable for specific and selective transport—the chemist should not leave the field of oriented supramolecular systems to the physicist and biologist alone. The physicists tend to like "too simplified" models, while biologists tend to reject all approaches which are not in accord with the "too complicated" prototypes of Mother Nature. Thus organic chemistry and macromolecular chemistry can play the important role of a mediator. One hint that this might work is given in the contribution by *J.-M. Lehn* in this issue.[12d] Molecular recognition processes for organic and inorganic compounds are a first step towards simulating highly specific recognition reactions common in biology.[12c,213] It will also be exciting to see what possibilities will be opened by the synthesis of glycolipids and glycoproteins representing tumor-specific antigens. They might be a bridge between organic chemistry and immunology.[214]

The art of synthesizing exceedingly complex molecules and characterizing them perfectly has reached dizzying heights and opens fascinating opportunities in the area of molecular engineering. But what is it good for? This art certainly will remarkably increase our chemical knowledge—but is this enough? It is not unlikely that, independent of the enormously increasing number of new synthetic compounds, the number of true innovations or innovative compounds has constantly decreased during the last few decades. In his aggressively but optimistically written contribution on "Chemical Needs and Possibilities at the End of the Century,"[215] *C. Frejacques* points out the importance of chemical systems based on self-organization. He stresses that, above and beyond the chemistry of covalent bonds, a chemistry of supramolecular systems is now emerging.

This review on the molecular architecture and function of polymeric oriented systems intends to show that such chemical systems, like their natural prototypes, may lead to functional units which can contribute to the understanding of biological processes and to the development of new materials. Both tradition and innovation are needed for that. On the borderland between the different disciplines of science, adventures are waiting. Neither uncritical optimism nor obstructive pessimism are justified. The excitement and the courage to set out for new frontiers and the willingness for close cooperation are basic prerequisites for this adventure of science. All the knowledge is available, we only have to learn to use it.

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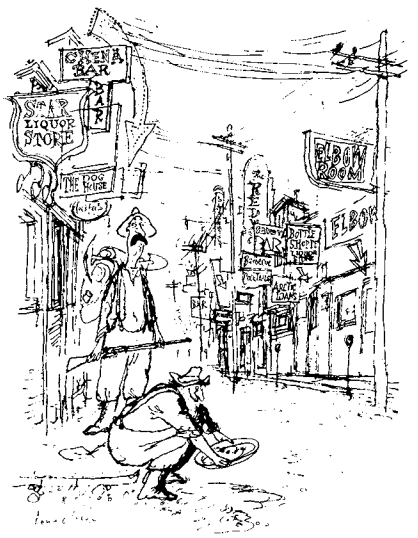
Translated by *Eva Grunwald* and Dr. *Christian Salesse*, Mainz

[1] B. Brecht: *Geschichten*, Bibliothek Suhrkamp, Frankfurt am Main 1962, p. 167. In this essay and under the heading "Hardship of the best," Mr. K. answers this question. But he certainly was a scientist! Don't you think so?

[2] H. Mark, *Angew. Chem. 93* (1981) 309; *Angew. Chem. Int. Ed. Engl. 20* (1981) 303.

[3] a) *Ronald Searle*, the scalpel-sharp British cartoonist, illustrates that even for the old gold diggers it was an absurd adventure to pan for gold by the light of the silvery neon in the main street of downtown Klondike (see [3b], p. 25):



Already in 1788 (!) *Georg Christoph Lichtenberg*, the great and critical philosopher and physicist from Göttingen, was very clear and outspoken in this respect when he characterized one of his colleagues: "... he was still hanging at his university like a wonderful chandelier without a tiny candle lit for years" (G. Ch. Lichtenberg, "*Sudelbücher*"). b) R. Searle: *From Frozen North to Filthy Lucre*, Viking Press, New York 1964.

[4] *Erwin Chargaff*, biochemist and critical essayist, depicts this as follows: "In science today smaller and smaller rooms are furnished more and more luxuriously and completely." (E. Chargaff: *Bemerkungen*, Klett-Cotta, Stuttgart 1981, p. 33).

[5] A. Dress, H. Hendrichs, G. Küppers: *Selbstorganisation - Die Entstehung von Ordnung in Natur und Gesellschaft*, Piper, München 1986.

[6] a) H. Haken: *Erfolgsgeheimnisse der Natur - Synergetik: Die Lehre vom Zusammenwirken*, Deutsche Verlags-Anstalt, Stuttgart 1986; b) B.-O. Küppers (Ed.): *Ordnung aus dem Chaos. Prinzipien der Selbstorganisation und Evolution des Lebens*, Piper, München 1987.

[7] From the standpoint of the history of science, it is most interesting to see how, very often, out of controversies between different disciplines, new research areas develop. The famous and historical controversy in the twenties between the then well-established colloid science (*W. Ostwald*) and the rising macromolecular chemistry (*H. Staudinger*) is an

instructive example for that: weak interactions in the formation of aggregates (colloids) as opposed to covalently linked small molecules (macromolecules). These competitive concepts have long since inevitably fused, as perfectly demonstrated in the supramolecular systems of biological membranes. However, it is also interesting to note that—at least in Germany—colloid science did not recover from this controversy for quite a while. For at least two or three decades colloid chemistry nearly disappeared from German universities. It regained its reputation only via its industrial importance. a) H. Morawetz, *Angew. Chem. 99* (1987) 95; *Angew. Chem. Int. Ed. Engl. 26* (1987) 93; b) *Polymers. The Origins and Growth of a Science.* Wiley, Chichester 1985; c) E. Jostkleigrewe (Ed.): *Makromolekulare Chemie - Das Werk Hermann Staudingers in seiner heutigen Bedeutung.* Schnell und Steiner, München 1987.

[8] a) J. D. Barnal, *Trans. Faraday Soc. 29* (1933) 1022; b) *The Physical Basis of Life.* Routledge and Kegan, London 1951.

[9] a) G. H. Brown, J. J. Wolken: *Liquid Crystals and Biological Structures,* Academic Press, New York 1979; b) D. M. Small, *J. Colloid Interface Sci. 58* (1977) 581; c) D. Chapman in F. D. Saeva (Ed.): *Liquid Crystals: The Fourth State of Matter,* Dekker, New York 1979, p. 305; d) Y. Bouligand in L. Liebert (Ed.): *Liquid Crystals (Solid State Phys. Suppl. 14* (1978) 259).

[10] As far as the often discussed and adjured willingness of scientists to cooperate is concerned, one has to be skeptical. Again one can use the point of view of *Ronald Searle* to describe the situation: People tend to cling to the old ways; a homesteader's cabin is still his castle (see [3b], p. 14 and [4]):

[11] The courage to set out and the willingness to turn around and search for prey from a different perspective are prerequisites for the adventure of science (see [3b], p. 21):

[12] Supermolecules ("Übermolekeln") were already defined by *K. L. Wolf* in 1937 to describe associations of molecules, the function of which was based on their organization: a) K. L. Wolf, F. Frahm, H. Harms, *Z. Phys. Chem. Abt. B 36* (1937) 17; b) K. L. Wolf, R. Wolff, *Angew. Chem. 61* (1949) 191. The conception of "Supramolecular Chemistry" has been realized by *Jean-Marie Lehn* and his group during the last few years: c) J.-M. Lehn, *Science (Washington) 227* (1985) 849; d) *Angew. Chem. 100* (1988) 91; *Angew. Chem. Int. Ed. Engl. 27* (1988) 89. In this contribution the term *supramolecular systems* is not only used for small molecular complexes of single molecules, such as substrate-receptor complexes. It is also applied to large molecular aggregates (high aggregation numbers), the function of which is again based on their organization. The ideal system to exemplify this definition is the biomembrane.

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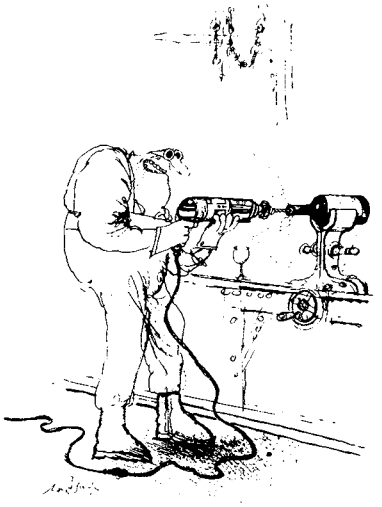

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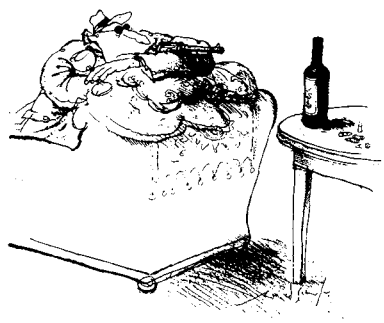
[200] T. Folda, *Dissertation*, Universität Mainz 1984.
[201] A pretty rapid method to uncork a bottle is the so-called "Sicilian opening" also known in chess and from time to time applied in science too (see [194b]):



[202] For the use of fluorescent dyes to measure the permeability of liposomal membranes see: a) J. N. Weinstein, S. Yoshikami, P. Henkart, R. Blumenthal, W. A. Hagins, *Science (Washington) 195* (1977) 489; b) J. N. Weinstein, E. Ralston, L. D. Leserman, R. D. Klausner, P. Dragsten, P. Henkart, R. Blumenthal in [84h], Vol. III, p. 183.

[203] The sanning electron micrographs were taken at Case Western Reserve University, Cleveland, Ohio, USA (Prof. *J. B. Lando*).

[204] The freeze fracture electron micrographs were taken by *C. Fahn* at the Physics Department of the Technische Universität München (Prof. *E. Sackmann*).

[205] a) W. F. Reed, L. R. Guterman, *J. Radiat. Curing 1986*, 18; b) R. L. Juliano, M. J. Hsu, S. L. Regen, M. Singh, *Biochim. Biophys. Acta 770* (1984) 109.

[206] H. Ohno, S. Takeoka, E. Tsuchida, *Polym. Bull. 14* (1985) 487.

[207] Enzymatic processes are common in the wine business. To uncork bottles, however, physical methods are usually preferred. As far as elegance is concerned, the Swiss variant can hardly be surpassed (see [194b]):

[208] R. Büschl, *Dissertation*, Universität Mainz 1984.

[209] a) P. G. Munder, M. Modolell, W. Bausert, H. F. Oettgen, O. Westphal, *Prog. Cancer Res. Ther. 16* (1981) 411; b) R. Andreesen, A. Schulz, U. Costabel, P. G. Munder, *Immunobiology 163* (1982) 335; c) M. Modolell, R. Andreesen, W. Pahlke, U. Brugger, P. G. Munder, *Cancer Res. 39* (1979) 4681; d) M. H. Runge, R. Andreesen, A. Pfleiderer, P. G. Munder, *JNCI J. Natl. Cancer Inst. 64* (1980) 1301; e) R. Andreesen, M. Modolell, G. H. F. Oepke, P. G. Munder, *Exp. Hematol. (NY) 11* (1983) 564.

[210] "It is just like someone dragging a stone and claiming: I'm building a Gothic cathedral."

[211] a) R. Hoffmann, *Angew. Chem. 99* (1987) 871; *Angew. Chem. Int. Ed. Engl. 26* (1987) 846; b) *Roald Hoffmann*—certainly one of the last ones to be reproached for superficiality—defines the problem of generalization saying: "Typologies and generalizations often point not so much to reality as to the weakness of the mind that proposes them." This has to be accepted!

[212] *Georg Christoph Lichtenberg* in his *"Aphorisms"*: "The wit is the finder and the intelligence the observer". "It is really necessary to seriously investigate why most inventions have to be made by chance. The main reason might be the fact that people tend to accept the views of their teachers and their environment. It thus might be beneficial to develop instructions how to systematically deviate from rules." *Aphorismen*, Inseltaschenbuch Suhrkamp, Frankfurt am Main, 1979, p. 202 and 204.

[213] a) F. Vögtle, H.-G. Löhr, J. Franke, D. Worsch, *Angew. Chem. 97* (1985) 721; *Angew. Chem. Int. Ed. Engl. 24* (1985) 727; b) F. Vögtle, E. Weber (Eds.): *Host Guest Complex Chemistry I–III, Top. Curr. Chem. 98* (1981), *101* (1982), *121* (1984); c) F. Vögtle, E. Weber (Eds.): *Biomimetic and Bioorganic Chemistry I–III, Top. Curr. Chem. 128* (1985), *132* (1986), *136* (1986); d) D. J. Cram, *Angew. Chem. 98* (1986) 1041; *Angew. Chem. Int. Ed. Engl. 25* (1986) 1039.

[214] See [165] and references cited therein.

[215] C. Frejacques, *Chem. Ind. (London) 1985*, 780.

[216] Thanks to all the coworkers for their diligent and devoted ...!

However, one really should remember that the Chinese already knew 2000 years ago: you learn a lot from your teachers, you learn more from your friends but you learn most from your students. (Picture taken from [3b], p. 101.)