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Cold Spring Harb Perspect Biol 2011;3:a004622 originally published online May 24, 2011

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Model Answers to Lipid Membrane Questions

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Ever since it was discovered that biological membranes have a core of a bimolecular sheet of lipid molecules, lipid bilayers have been a model laboratory for investigating physicochemical and functional properties of biological membranes. Experimental and theoretical models help the experimental scientist to plan experiments and interpret data. Theoretical models are the theoretical scientist's preferred toys to make contact between membrane theory and experiments. Most importantly, models serve to shape our intuition about which membrane questions are the more fundamental and relevant ones to pursue. Here we review some membrane models for lipid self-assembly, monolayers, bilayers, liposomes, and lipid–protein interactions and illustrate how such models can help answering questions in modern lipid cell biology.

“THEY ARE BAGS!”—A SHORT HISTORY OF MEMBRANE MODELS

It was a major discovery when Gorter and Grendel in 1925 observed that biological membranes were ultrathin, bimolecular sheets (Gorter and Grendel 1925), thereby suggesting lipid bilayers as simple models of biological membranes (Mouritsen and Andersen 1998). At that time lipid monolayers had already been studied in principle since the days of Benjamin Franklin, and more extensively by Ervin Langmuir and Katharine Blodgett using quantitative experimental techniques. A lipid monolayer at an air–water interface is an even simpler membrane model than a bilayer, basically being half a membrane. Proteins were added to the picture, first peripherally in the 1935 model because of Danielli and Dawson,

later in 1966 in Robertson's unit membrane model, and finally in 1972 the Singer–Nicolson fluid mosaic (Singer and Nicolson 1972) anticipated that membranes were composites of a lipid bilayer with integral and membrane-spanning proteins (for a list of references to the classical literature, see the recent review by Bagatolli et al. 2010).

The Singer–Nicolson model was a very successful model because it provided a simple framework within which to ask relevant membrane questions. Its simplicity was its strength, but also its drawback because many researchers, somewhat inadvertently, assumed that membranes were more fluid than mosaic (Engelman 2005). The parallel work on the physical chemistry and phase behavior of lipid bilayer systems during the period 1960–1980 (see, e.g., Shimshick and McConnell 1973) was unfortunately

Editor: Kai Simons

Additional Perspectives on The Biology of Lipids available at www.cshperspectives.org

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Cite this article as *Cold Spring Harb Perspect Biol* 2011;3:a004622

O.G. Mouritsen

only to a minor degree adopted by the biological community (Bergelson 1995). It was only with the introduction of the functional raft concept (Simons and Ikonen 1997; Kusumi and Suzuki 2005; Jacobson et al. 2007; van Meer et al. 2008; Pike 2009; Lingwood and Simons 2010) that the biological researchers became fully aware of the importance of lateral structure and lipid domains in membranes.

The fluid-mosaic model was already significantly refined by Israelachvili (1977) to account for lipid–protein interactions, membrane folding, and pore formation. Further insight into the fundamental mechanisms of membrane–protein interactions was furnished by the Marčelja model (Marčelja 1976) and the so-called mattress model (Mouritsen and Bloom 1984). The mattress model and the principle of triggering mechanisms proposed by Sackmann (Sackmann 1984) both anticipated the importance of differential structuring of membrane regions around integral membrane proteins. Sackmann also refined the model to incorporate the importance of extended cellular structures attached to the membrane, such as the glycocalyx and the cytoskeleton (Sackmann 1995).

A key discovery in biomembrane science is Alec Bangham’s discovery of liposomes (Bangham and Horne 1964) showing by electron microscopy that membranes form spontaneously from phospholipids dispersed in water as thin, closed bags of lipid bilayers. The importance for membrane modeling of the insight behind Bangham’s statement—“They are bags!” (A Bangham, pers. comm.)—cannot be overestimated because it pinpoints the importance of entropy as a driving force for membrane formation and stability.

Today researchers freely use all these fundamental ideas to help answering membrane questions using a range of membrane models of various complexities. In all cases lipids, lipid self-assembly, lipid bilayers, lipid domains, and lipid functionality have moved center stage (Mouritsen 2005; Heimburg 2007, 2009; Nag 2008). Lipidology and lipidomics have grown to become key sciences in cell biology.

It should be pointed out that the term “model” is used somewhat differently in different

communities of researchers. Physical scientists usually think of a useful model, whether it is an experimental model system or a theoretical model, as the simplest possible construction that captures the relevant properties of a given situation. The virtue of this kind of model is to have as little detail as possible. In contrast, life scientists usually insist on having as many details accounted for as possible in a model to be useful and realistic. We shall adopt the physical scientists’ ideal of a model in the present paper. It should also be clarified, that theoretical models come in many different flavors. Some models are of the theoretical variety involving a phenomenological construction of a free energy functional for the system formulated in terms of order parameter fields, such as density, composition, order, and curvature. Other models are statistical mechanical models operating with variables that account for some important, molecular degrees of freedom for the molecules. The most refined of such models are those building on the most accurate atomic-scale interatomic potentials available. Less refined models involve some coarse-grained variables that average over many atomic details. In most cases, the statistical mechanical models require computer simulation techniques, such as molecular dynamics, Monte Carlo, or dissipative particle dynamics simulations, for calculating the properties of the models (Merz and Roux 1996; Shillcock 2008; Marrink et al. 2009).

Biomembrane science has now reached a mature state in which it is possible to attach realistic problems and questions to membrane behavior using a combination of experiments, theoretical modeling, and large-scale computer simulation. The picture of membrane modeling advocated in the present paper is based on such a combined approach.

LIPID SELF-ASSEMBLY, CURVATURE, AND LIPID POLYMORPHISM

Because of their amphiphilic character, lipids self-assemble in water in a variety of different aggregates and extended structures, depending on lipid molecular structure and thermodynamic conditions. The polymorphism of lipids

and their phase behavior in water which owe so much to the pioneering work by Luzzati (1968) were fully anticipated in the context of biomembranes in a classical paper by Cullis and de Kruijff (1979) and later by Israelachvili introducing a phenomenological packing parameter (Israelachvili 1992). The effective shapes of the molecules were found to control the morphology of the structures being formed (cf. Fig. 1). Molecules of conical shape would stabilize phases with curvature, such as micelles, hexagonal phases, and cubic phases, whereas more cylindrical shapes would predominantly lead to lamellar bilayers (Seddon and Templer 1995).

It is important to note that even if thermodynamic conditions lead to a stable lamellar bilayer this bilayer may suffer from an in-built curvature stress because of a spontaneous curvature of the participating monolayers. This curvature stress can be released globally or even locally by external agents, solutes, and amphiphiles, as well as peptides and proteins

leading to destabilizing of the bilayer and/or formation of local defects such as invaginations, buds, or pores.

HALF A MEMBRANE: THE LIPID MONOLAYER

Lipid monolayers spread on air–water or oil–water interfaces (cf. Fig. 1B) are simple models of lipid membranes. The advantage of such models is that they can easily be studied and manipulated in a Langmuir trough in which thermodynamic relationships between surface tension and surface area can be measured (Dynarowicz-Latka et al. 2001). The in-plane and lateral structure of the monomolecular film, which can consist of lipids, proteins, and lipid–protein recombinants, can readily be investigated by various spectroscopies, such as fluorescence microscopy and Brewster-angle microscopy, by scattering techniques (X-ray and neutron), or by surface-sensitive scanning-probe

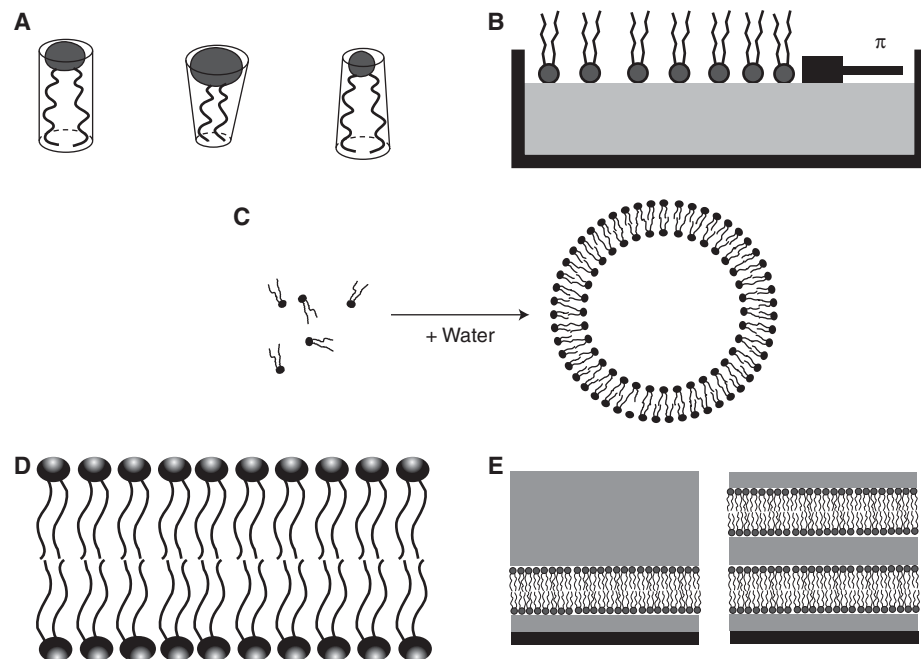


Figure 1. Lipid molecules and models of lipid membranes. (A) Lipid molecular shapes. (B) Lipid monolayer on an air–water interface. (C) Lipid self-assembly into a vesicle (unilamellar liposome). (D) Freestanding lipid bilayer. (E) Single and double lipid bilayers on solid supports.

O.G. Mouritsen

techniques, such as atomic-force microscopy, provided the monolayer is transferred to a solid support. The combined information obtained will hence cover scales ranging from the molecular scale, over the nanometer scale, to the micron and macroscopic length scales.

Importantly, lipid monolayers on air–water interfaces are good models to probe, in a well-defined setting, the interactions between the lipid compartment of cell membranes and various agents that are active on membranes, such as solutes, drugs, enzymes, and water-soluble and amphiphatic peptides.

An example of lateral monolayer lateral structure imaged by multiphoton excitation fluorescence microscopy using laurdan is shown in Figure 2A. The image illustrates the coexistence between liquid-expanded and liquid-condensed phases in a DPPC (dipalmitoylphosphatidylcholine) monolayer at an air–water interface (Brewer et al. 2010).

SUPPORTED LIPID BILAYERS AND MULTILAYERS

Freestanding lipid bilayers (cf. Fig. 1D) can be transferred to solid supports under full aqueous conditions (cf. Fig. 1E and Fig. 2B–F) using, for example, Langmuir–Blodgett deposition techniques, or self-assembled by using rehydration subsequent to spin-coating techniques, or by explosion of a vesicular dispersion onto an appropriate hydrophilic surface. Using proteoliposomes it is also possible to produce supported lipid bilayers with integral membrane proteins incorporated (cf. Fig. 2D). The correct incorporation and folding of large integral membrane proteins can be facilitated using solid supports with appropriate soft cushions in the form of grafted polymer layers or brushes (Tanaka and Sackmann 2005).

Proximal lipid bilayers on solid supports are significantly influenced by interactions with the substrate, and their thermodynamics and lateral organization and order is affected by the support (Jensen et al. 2007; Keller et al. 2005). Hence, caution should be exerted when comparing data for supported bilayers with corresponding data for free-standing bilayers. The

influence of the support can be significantly suppressed by adding a second, distal bilayer on top of the proximal one (cf. Fig. 2E) (e.g., using spin-coating techniques [Simonsen and Bagatolli 2004]).

The advantage of supported bilayers is that they can be manipulated and imaged in advanced fluorescence and atomic-force microscopes, hence revealing their lateral structure on scales from nanometers to microns (cf. Fig. 2B–E). Under suitable conditions, reconstituted proteins can also be visualized as shown in Figure 2D. In supported membranes with high protein contents (e.g., in natural membranes of the purple membranes of *Halobacterium halobium*), the crystalline arrays of bacteriorhodopsin reveal details of the protein arrangements (Oesterhelt et al. 2000; Butt 2009).

The top layer of double-supported lipid bilayers can help answer questions regarding lateral membrane structure on scales below the diffraction limit, and atomic-force microscopy can lead to valuable real-space information on membrane domains, phase separation, and molecular organization of model membranes (Leidy et al. 2002). As an example, the image in Figure 2C shows the small-scale ripple structure of the top layer of a DLDP/DPPC double-supported bilayer. In some cases dynamical phenomena can be studied—for example, the action of a phospholipase-assisted hydrolysis of a lipid bilayer as illustrated in Figure 2F (Simonsen 2008).

Studies of supported bilayers may eventually shed some light on one of the major outstanding and elusive questions in membrane biology, that of lipid diversity. It remains a puzzle why biological membranes consist of hundreds of different kinds of lipids. Part of the answer is likely to lie in the dynamics of the lateral structure of the lipid-bilayer component of the membranes.

CLOSED BILAYERS: VESICLES, LIPOSOMES, AND THE SUVs, THE LUVs, AND THE GUVs

Vesicles and unilamellar liposomes are possibly the simplest models of closed cell membranes (cf. Fig. 1C). They are freestanding in water,

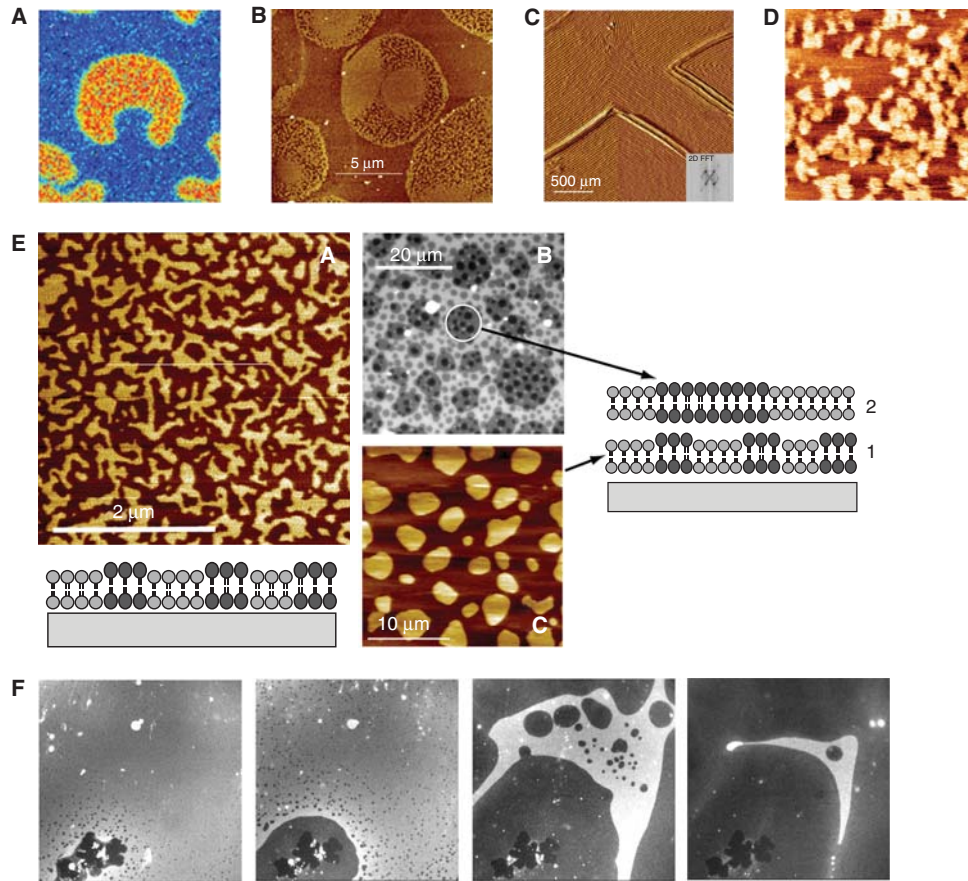


Figure 2. Lateral structure of lipid mono- and bilayers. (A) Multiphoton excitation fluorescence microscopy image, using laurdan, of the lateral structure of a DPPC monolayer in the liquid-extended liquid-condensed coexistence region at 8.8 mN/m and 21°C. (Adapted from Brewer et al. 2010; reprinted with permission from Elsevier © 2010). (B) Atomic-force microscopy image of the lateral structure of a single-supported bilayer of native pulmonary surfactant. (Adapted from Bernardino de la Serna et al. 2004.) (C) Atomic-force microscopy image of the lateral structure of the top bilayer of a double-supported bilayer system of DLPC/DPPC 1:1 at 20°C revealing the occurrence of ripple structures with a periodicity of 30 nm. (Courtesy of Dr. Uffe Bernchou Jensen.) (D) Lateral structure of a supported lipid bilayer reconstituted with individually discernable aquaporins. The image is 100 nm × 100 nm. (Courtesy of Dr. Danielle Keller.) (E) Atomic-force (E:A) and fluorescence microscopy (E:B and E:C) images of the lateral structure of single- and double-supported lipid bilayer of the raft mixture DOPC/DPPC/Chol 2:2:1 spin coated onto a mica surface. (Adapted from Jensen et al. 2007; reprinted courtesy of Dr. Adam Cohen Simonsen.) (F) Fluorescence microscopy images (120 μm × 120 μm) of the time evolution of the lateral structure of a single-supported POPC lipid bilayer being hydrolyzed by secretory phospholipase A₂. (Adapted from Bernchou Jensen and Simonsen 2005; reprinted with permission from Elsevier © 2005.)

they are single-walled, they are closed onto themselves, and they hence separate an aqueous compartment from the environment. As membrane models, the vesicles have the advantage that they can be formed in different sizes, SUVs (small unilamellar vesicles, <100 nm in

diameter), LUVs (large unilamellar vesicles, <1000 nm in diameter), and GUVs (giant unilamellar vesicles, >1000 nm in diameter). Hence, it is possible to study effects of membrane permeability barriers and curvature. Dispersions of vesicles give access to measurement

O.G. Mouritsen

of ensemble properties (e.g., thermodynamic and structural properties).

GUVs are particularly useful membrane models because they are large enough to be studied individually in a visual microscope (Bagatolli 2006, 2010). Moreover they can be manipulated by micromechanical devices and their mechanical properties can be assessed (e.g., by pipette aspiration techniques [Evans and Skalak 1980]). In recent years, GUVs have established themselves as a key model and versatile laboratory for answering a plethora of membrane questions, not least in relation to domain formation. It is noteworthy that special fluorescent lipid analogs such as laurdan and prodan can be used as simultaneous spectroscopic probes that not only reveal lateral structure but also aspects of the lipid chain order. This is of particular importance for questions regarding the order of domains related to membrane rafts (Dietrich et al. 2001; Fidorra et al. 2009).

Figure 3 shows some examples of the type of information that advanced fluorescence microscopy can reveal on lateral membrane structure in GUVs formed by simple and more complex lipid mixtures.

A quantitative analysis of images as those shown in Figure 3 can answer questions regarding phase diagrams and phase equilibria and domain size distributions, as well as domain shapes and their evolution in time (Baumgart et al. 2003). Moreover, modifications in the lateral organization can be studied under the influence of membrane-active agents, drugs, and enzymes.

FOUR CONSPICUOUS FEATURES OF LIPID MEMBRANES REVEALED BY MEMBRANE MODELS

Studies of membrane models as described above have provided critical answers regarding several fundamental questions pertaining to biomembranes. We shall briefly mention four of the more important lessons from such studies. The results are obtained by combining insight from using a range of experimental techniques, some of them mentioned above,

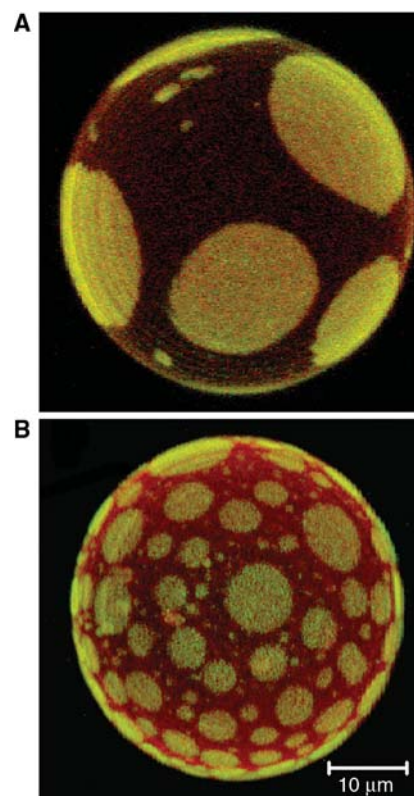


Figure 3. Lateral structure in giant unilamellar vesicles (GUVs) as visualized by fluorescence microscopy. (A) Mixture of DOPC/DPPC/Chol 2:2:1 at 20°C. (B) Native pulmonary surfactant mixtures. (B, Adapted from Bernardino de la Serna et al. 2004; reprinted with permission from *The Journal of Biological Chemistry* © 2004.)

in combination with theoretical modeling and computer simulation (Mouritsen 2005).

The Lateral Pressure Profile

The transverse structure of a lipid bilayer is possibly the most overlooked, but at the same time most obvious and fundamental property of a membrane (Cantor 1997a). Its transbilayer shape, as illustrated in Figure 4A, is highly non-trivial, revealing regions of expansive (positive) pressures and regions of large tensile (negative) pressures. The pressure profile owes its existence to the two opposed oil–water interfaces

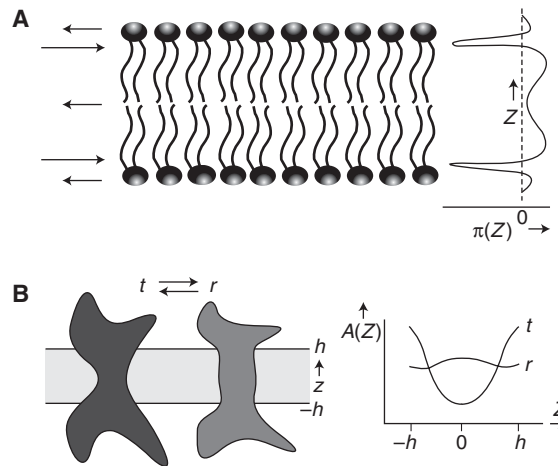


Figure 4. Transbilayer structure of lipid bilayers. (A) Schematic illustration of the lateral pressure profile, $\pi(z)$ of a lipid bilayer, revealing regions of expansive (positive) pressures and regions of large tensile (negative) pressures. (B) Illustration of how changes in the lateral pressure profile may shift the equilibrium between two conformational states (t, r) of an integral membrane protein. $A(z)$ describes how the protein cross-sectional area varies across the bilayer from $-h$ to h . (B, Adapted from Cantor 1997a; reprinted with permission from The American Chemical Society © 1997.)

sandwiching the hydrophobic core of the membrane interior.

The large variations in pressure, ranging from the equivalent of a hundred to a thousand atmospheres, are a direct consequence of the distribution of the oil–water interfacial tension over a bilayer thickness of ~ 5 nm.

The lateral pressure profile puts distinct constraints on the interaction between the membrane and integral proteins, and it is believed that protein and channel function may be regulated by the lipids via alterations in the pressure profile. In particular it has been surmised that certain solutes, ligands, and drugs exert part of their action on membrane proteins and receptors via changes in the pressure profile as illustrated schematically in Figure 4B. A prominent example is that of the molecular mechanism of general anesthesia, which is likely to involve modulation of the pressure profile, and then in turn the lipid–protein interactions, by the localization of the potent anesthetic agents in the interfacial regions of the membrane surface (Cantor 1997b).

The pressure profile, and its dependence on bilayer composition and thermodynamic conditions, has been carefully worked out by

theoretical models and computational techniques (Patra 2005; Sonne et al. 2005). Still no experimental technique has been devised to actually measure the profile, the reason being that no probe has been found that faithfully and without perturbing the system can measure pressure differences on the subnanometer scale.

Cholesterol and the Liquid-Ordered Phase

A single, special kind of lipid, cholesterol, has come to play a key role in the elucidation of membrane organization and domain formation (Mouritsen and Zuckermann 2004; Mouritsen 2010). Being the single most abundant type of lipid in all plasma membrane, typically in a molar concentration of $\sim 30\%$, a long-standing membrane question has been how cholesterol regulates membrane structure and function.

By the proposal of a new type of membrane phase, the liquid-ordered phase (Ipsen et al. 1987), that results as a compromise between cholesterol's schizophrenic affinity for fluid and solid lipid phases, cholesterol was shown to control membrane organization on different length scales. The proposal was based on a simple theoretical model of lipid bilayers and it was

O.G. Mouritsen



inspired by experimental NMR (nuclear magnetic resonance) and calorimetry data for the cholesterol-DPPC system (Vist and Davis 1990). The liquid-ordered phase was shown to be a fundamental physical and thermodynamic consequence of the symmetry of the binary lipid-cholesterol mixture, with two liquid phases of the same fluid symmetry but a different degree of acyl-chain order: the liquid-disordered (fluid) phase and the liquid-ordered phase. The resulting phase diagram is a manifestation of the basic facts that cholesterol has a preference for ordered acyl lipid chains (i.e., chain states characteristic of the solid-ordered [gel] phase), but at the same time it is more soluble in the fluid phase. This apparent conflict is resolved by inducing an intermediate phase—that is, a phase that is ordered from the point of view of the conformational structure of the lipid chains but is disordered from the point of view of the lateral positions of the molecules.

The proposal of the liquid-ordered phase from a simple membrane model was hence indirectly responsible for consolidating a sound physical basis for the functional membrane platforms that underlie the phenomenological raft hypothesis (Simons and Ikonen 1997).

Even if the liquid-ordered phase was proposed to exist in binary lipid-cholesterol systems, only little conclusive evidence is available as yet of the presence of a macroscopic liquid-ordered phase and macroscopic phase coexistence between liquid-ordered-liquid-disordered phases in binary lipid bilayers. Nevertheless, there is now a large body of experimental work that directly or indirectly has shown unequivocally the existence of smaller microscopic domains in model membranes, possibly of the size of tens of nanometers (Veatch et al. 2004; Pencer et al 2007; Davis et al. 2009).

Lateral Membrane Structure: Phases, Domains, and Rafts

Both theoretical and experimental studies of model membranes, monolayers as well as bilayers, very early showed that membranes are

structured laterally, first of all as a consequence of the many-bodyness and cooperative behavior of the system (Mouritsen and Jørgensen 1994; Mouritsen 2005). Phase equilibria can lead to large-scale phase separation, and local fluctuations in composition and density can lead to small-scale lipid domain formation. Inhomogeneities in the form of defects and coupling to cytoskeleton and external structures may eventually control the length scale of the domains. Examples of lateral organization in some simple model membranes were shown in Figures 2 and 3.

Subsequent to the proposal of the raft hypothesis, a large number of model studies have been performed on special lipid mixtures that are believed to be raft formers. The typical system is a ternary mixture including cholesterol together with a lipid with a high melting point (e.g., a saturated lipid or sphingolipid) and one with a low melting point (e.g., a mono-unsaturated or polyunsaturated lipid). Many ternary phase diagrams have been worked out (Veatch and Keller 2003; Jensen et al. 2007; Goñi et al. 2008), and paradoxically the identification of the liquid-ordered phase in coexistence with a fluid lipid phase has been much more easy in ternary systems compared to binary lipid-cholesterol systems (Bagatolli et al 2010; Mouritsen 2010).

Membrane Mechanics

Because of its very small thickness compared to its extension, a lipid bilayer may be modeled as a two-dimensional, flexible sheet imbedded in a three-dimensional space (Evans and Skalak 1980). Because membranes usually are prepared in a tensionless state and the surface tension hence is negligible, the energetics of the sheet is controlled by the mechanical modules (i.e., the bending modulus, κ , and the Gaussian curvature modulus, κ_G) and described by the Helfrich expression

$$dE_{\text{surface}} = \left[\frac{\kappa}{2} \left(\frac{1}{R_1} + \frac{1}{R_2} - \frac{2}{R_0} \right)^2 + \frac{\kappa_G}{R_1 R_2} \right] dA, \quad (1)$$

in which dE_{surface} is the energy differential associated with an area deformation dA . R_1 and R_2 are the principal radii of curvature of the sheet, and R_0 is a measure of the spontaneous curvature. Because the membrane is assumed to be fluid, there is no term in Equation (1) accounting for resistance to shearing. For closed bilayers like vesicles, the Gaussian term integrates to a topological constant when the total energy is evaluated by integrating Equation (1) over the entire surface of the vesicle. Hence, only the bending term and the bending modulus κ contribute to the mechanics in this approximation. Equation (1) would have to be modified if area differences between the two monolayers are significant (Miao et al. 1994).

The Helfrich Hamiltonian in Equation (1) has turned out to be a remarkably good model of membrane mechanics and it has been successful in accounting for the various shapes and shape transformations of lipid vesicles and even cells like the erythrocyte (Lim et al. 2002). The model has also proven to be a workhorse in the laboratory for determining mechanical parameters of lipid membranes, using micromechanical measurements such as vesicle fluctuation analysis, in which the undulations of fluctuating GUVs in the microscopy via Fourier analysis (Henriksen et al. 2004) can yield the bending modulus, even under physiological conditions (Méléard et al. 2009). In particular, this type of model approach has answered important questions regarding the effect of antimicrobial, amphiphatic peptides or sterols on membrane mechanics, thereby supplementing parallel model studies of the same systems using more conventional thermodynamic and spectroscopic investigations.

RECONSTITUTED MEMBRANES AND LIPID-PROTEIN INTERACTIONS

Modeling of biological membranes typically progresses in steps, starting with simple lipid bilayers with one or two lipid species, then proceeds to more complex lipid mixtures, including special lipids such as sterols, sphingolipids, and glycolipids. Another step may be using the full lipid extract from cell membranes.

This type of modeling focuses on the lipid properties and it has provided the foundation for interpretation of many experiments on real biological membranes and it has revealed to which extent lipids marshal membrane structure and function (Mouritsen 2005).

Parallel to this line of modeling is the formulation of models attempting to capture the essentials of protein-membrane interactions (e.g., integral membrane proteins). Most of the proposed models, however, fall short of describing real membranes in which half of the mass is proteins. Experimental models are typically lipid-protein recombinants in which purified membrane proteins are reconstituted into vesicles and liposomes of a well-defined lipid composition (de Kruiff 2004; Tamm 2005; Perez-Gil 2008).

Important membrane questions that the models should answer are related to, for example, lateral distribution and organization of the proteins in the plane of the membrane, the effect of lipid-protein interactions on lipid domains and rafts, the structure of the lipids in close proximity to the proteins, the possible impact of lipid properties on protein structure and hence function, and the possibly lipid-mediated interactions between proteins. These questions are all in some way or another interrelated.

To illustrate the potential of simple theoretical models for answering some of these systems we shall briefly describe how the concepts of hydrophobic matching, lateral pressure profile, and lipid propensity for curvature have proven to be useful.

Hydrophobic matching between the hydrophobic core of lipid bilayers and the hydrophobic stretch of integral membrane proteins has been considered by several authors (Sackmann 1984; Mouritsen and Bloom 1984; Jensen and Mouritsen 2004; Andersen and Koeppel 2007) as a key determinant of lipid protein interactions (cf. Fig. 5A). The idea is that lack of a perfect match carries an energy penalty that basically amounts to the elastic distortion of the lipid matrix around the protein. For a sufficient large value of this penalty, the protein may yield and undergo a conformational change, hence offering a mechanism for lipid-mediated

O.G. Mouritsen

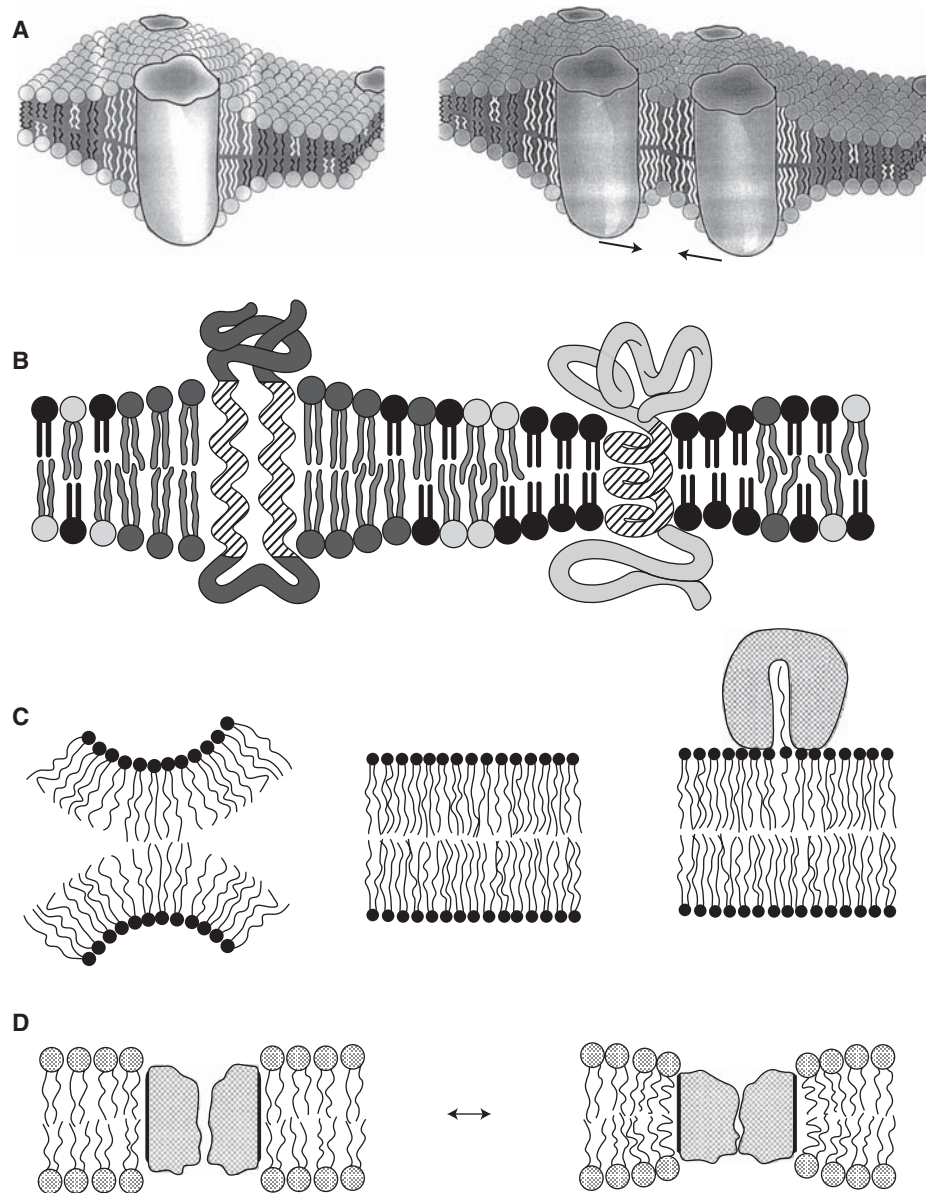


Figure 5. Lipid–protein interactions in membranes. (A) Schematic illustration of the principle of hydrophobic matching between lipid bilayers and integral membrane proteins. In the case of a mismatch, the deformation in the lipid matrix may induce an indirect, lipid-mediated attraction between the proteins. (B) Schematic illustration of a conformational change in an integral membrane protein induced by changes in the hydrophobic mismatch condition. (Data from Sackmann 1984.) (C) Release of the curvature stress in a lipid bilayer, composed of two lipid monolayers with spontaneous curvature, via the formation of the extended lipid chain conformation. One of the tails of the lipid molecule is captured in a hydrophobic pocket of a peripheral membrane protein. (Courtesy of Dr. P.K.J. Kinnunen.) (D) Curvature stress may induce conformational changes in a membrane channel and hence shift the equilibrium between an open and a closed state. (Courtesy of Dr. O.S. Andersen.)



effects on protein function, as illustrated in Figure 5B.

Another consequence of hydrophobic matching is the possibility of sorting, selection, or enrichment of certain lipids near the protein (Dumas et al. 1997, 1999). This is a purely physical effect and does not require special binding affinity of certain lipids for proteins. Obviously, specific interactions (e.g., by electrostatics) will add to this picture and may in some case overrule the matching.

For larger protein concentrations, the hydrophobic matching provides a mechanism for lipid-mediated protein–protein interactions, typically attractive as illustrated in Figure 5A, which will provide a driving force for protein aggregation and possibly crystallization in the plane of the membrane. The range of this protein–protein interaction will be controlled by the coherence length of the correlations between the lipids. These correlations can get rather long-ranged, in particular near special (critical) points in the system phase diagram. This effect is a version of the more general wetting phenomenon (Gil et al. 1997, 1998). Although a strictly equilibrium phenomenon, critical points in complex lipid mixtures (e.g., those containing cholesterol) may hence be interesting to consider in relation to membrane organization (Gil et al. 1997; Veatch et al. 2007; Honerkamp-Smith et al. 2008; Bagatolli et al. 2010). This is another example of the power of membrane models to provide intuition regarding important membrane questions.

The hydrophobic matching principle has been important in substantiating the concept of membrane rafts (Lingwood and Simons 2010) which are domains enriched in cholesterol and high-melting lipids and therefore generally thicker than the membrane matrix in which they reside. Matching then furnishes a mechanism for protein selection where those proteins that match the raft thickness the better (e.g., via appropriate acylation or prenylation) are recruited to the raft. Conversely, they can be released from the raft by appropriate enzymatic modification of the proteins. Hence, the concept of hydrophobic matching helps to establish a semiquantitative physical framework for signaling cascades.

Model Answers to Lipid Membrane Questions

It should be emphasized that in the present picture, lipid domains and rafts are dynamically maintained entities (Mayor and Rao 2004)—that is, they are by no means static but highly fluctuating and dynamic. They owe their existence to the correlations and many-bodyness of the of the entire membrane assembly.

The propensity of some lipids for inducing curvature stress and possibly nonlamellar phases provides, via the lateral pressure profile, provides another mechanism for lipid–protein interactions (Marsh 2007) as illustrated in Figure 4B. This mechanism is not necessarily independent from the hydrophobic matching mechanism. An illustration is given in Figure 5, C and D, in the case of release of curvature stress by binding a peripheral membrane protein and a shift between open and closed conformations of a membrane channel, respectively. Examples of systems that have been shown to operate according to these principles are the binding of cytochrome *c* to a membrane surface (Touminen et al. 2002) and the opening and closing of gramicidin A channels (Andersen and Koeppel 2007), respectively.

A CALL FOR NEW MEMBRANE MODELS

Virtually all existing theoretical models of membranes are based on equilibrium considerations. The same is true of most experimental models that typically are performed as test tube experiments. Many membrane systems studied in the laboratory are not considered in the full functional state and the experimental measurements usually pertain to some kind of equilibrium or near-equilibrium state. Obviously, real biological membranes are far from equilibrium or at best in a driven steady-state situation. In any case, the well-known principles from equilibrium thermodynamics do not apply. A typical example is a membrane with an ion pump that is driven by some kind of energy transduction mechanism (Sabra and Mouritsen 1998; Girard et al. 2005). Another example is the binding of ligands to receptors in which the binding is influenced by a force (e.g., the binding of collectins in the innate immune system to sugar groups on invading

O.G. Mouritsen

pathogens [Thormann et al. 2007]). A third example is the set of responsive membranes in the dermal barrier that is subject to a gradient in water chemical potential (Sparr and Wennerström 2001). A fourth example is the morphogenesis of the endoplasmic reticulum and the Golgi apparatus with membrane that owe their existence to nonequilibrium conditions of flow of energy and matter (Kühnle et al. 2010). It is interesting in this context to note that the plasma membrane can undergo phase separation when the cell dies (Lingwood and Simons 2010).

We are only about to begin facing questions as to how we by models study membrane structure in the functional state. For instance, how does bilayer transverse and lateral order become modified in an active membrane subject to transport, signaling, and enzymatic processes, and how do these processes in turn become controlled by the nonequilibrium state of the lipid matrix? Only few clues exist (Sabra and Mouritsen 1998; Høyrup et al. 2002; Girard et al. 2005; Turner et al. 2005; Lomholt 2006; Fan et al. 2010) and progress is hampered by the lack of appropriate, quantitative model systems that can be analyzed with the accuracy we are used to from equilibrium conditions.

Hence there is an urgent call for new models, experimental as well as theoretical, that can establish a framework for future work for answering pressing questions regarding membrane structure and function in nonequilibrium states.

MODEL MEMBRANES FOR HEALTH AND TECHNOLOGY

Membrane models have in many cases served as stepping stones in translating the insight from lipid membrane biophysics and biochemistry into technological applications, it be design of novel functional materials, nano-scale encapsulation technologies for foodstuff and drugs, sensors, and living technology.

As specific examples can be mentioned the design of two concrete new designs of liposome-based drug delivery systems for cancer therapy whose existence is to a large extent based on

extensive physical modeling. The first one is a thermosensitive liposome for delivering of anti-cancer drugs in which the essential triggering mechanism is a lipid bilayer phase transition that is tuned to take place a few degrees above physiological temperatures (Ponce et al. 2006). By heating tumors, in which the liposomes accumulate, from the outside the liposomal membranes go through their phase transition and become leaky. As a consequence, the encapsulated drug leaks out of the liposomes exactly where it is needed. The other example is a liposome that is made sensitive to the action of an endogenously up-regulated secretory phospholipase A₂ in cancer tissue. On accumulation in the tumor of intravenously administered liposomes with encapsulated anticancer drugs, the phospholipase opens the liposomal carrier and the drugs leak out (Jørgensen et al. 2002; Andreassen et al. 2005). This latter system can furthermore be expanded to include lipid prodrugs or double prodrugs that are turned into drugs by the action of the phospholipase, precisely at the target (Pedersen et al. 2010).

Both mentioned liposomal drug delivery systems have already been taken to clinical trials. They are good examples of how membrane models may provide answers to important problems in health and biotechnology.

ACKNOWLEDGMENTS

Illuminating discussion with members of MEMPHYS is gratefully acknowledged. MEMPHYS-Center for Biomembrane Physics is supported by the Danish National Research Foundation.

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O.G. Mouritsen

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