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Nanometre-scale structure of fluid lipid membranes

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Abstract. The lipid-bilayer component of biological membranes is a two-dimensional liquid under physiological conditions. Computer-simulation calculations based on statistical mechanical models predict this liquid to exhibit structure in the form of fluctuating lipid domains in the nanometre range. Indirect and direct experimental evidence for the small-scale structure is provided by fluorescence energy-transfer techniques and atomic-force microscopy, respectively.

1. Introduction

The conventional textbook picture of the lipid-bilayer component of biological membranes is that of a fairly structureless fluid which serves as an appropriate solvent for the membrane proteins. Recent evidence suggests that this picture is far from correct [1]. Not only does the lipid bilayer display a highly structured transverse profile; it also displays distinct static and dynamic structural organization on a small scale, e.g. in the form of differentiated lipid domains [2–4].

From the point of view of liquid physics, it is obvious that a fluid lipid bilayer, due to the various molecular interactions, must be structured at some scale and that density and compositional fluctuations persist and are characterized by non-zero correlation lengths. The crucial questions are what are the characteristic length scales, what controls the scales, how can they be detected, and what functional purpose may the small-scale microstructure serve in a biological membrane?

In the present paper, which reviews some previously published results as well as adding new data, these questions are addressed by a combined theoretical and experimental study of a series of model-membrane systems consisting of simple phospholipid bilayers. The focus is on the lateral-bilayer structure that arises as a consequence of the main phase transition in pure phospholipid bilayers. The main phase transition, which is of first order, takes the bilayer from a two-dimensional crystalline solid with lipid acyl chains of high conformational order to a two-dimensional fluid (liquid) in which the acyl chains of the lipid molecules have a high degree of conformational disorder [5]. This transition underlies the rich phase behaviour found in bilayers with more than one molecular species.

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2. Nano-scale structure from computer simulations

Computer-simulation studies of a class of simple lattice-gas-type models of the main phase transition have revealed that this transition, although of first order, is accompanied by strong lateral density fluctuations [6, 7]. In the fluid lipid phase these fluctuations correspond to dynamic heterogeneous structures which can be pictured as clusters of ordered lipid molecules in a fluid matrix of disordered lipid molecules, as illustrated in figure 1(a). The average cluster size, or the correlation length, increases towards the phase transition and the value of the correlation length is the larger the shorter the acyl chains of the lipid molecules are. Hence the bilayer can be tuned towards a critical point, as the chain length is decreased [8]. The macroscopic manifestations of these fluctuations are anomalies in the response functions and the elastic moduli of the membrane [6].

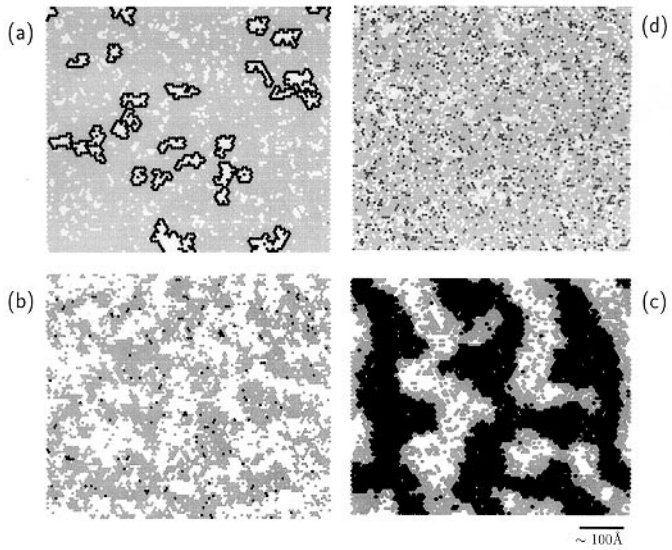

Figure 1. Snapshots of simulated lateral configurations of lipid bilayers containing 10 000 acyl chains. (a) Density fluctuations in the fluid phase of $DC_{16}PC$, with the interfaces of the lipid domains highlighted. (b) Compositional fluctuations in the fluid phase of a 1:1 binary mixture of $DC_{12}PC$–$DC_{20}PC$. (c) Non-equilibrium solid–fluid phase separation in a binary mixture of $DC_{14}PC$–$DC_{18}PC$. (d) Compositional fluctuations in a ternary lipid 22:12:66 mixture of $DC_{14}PC$–$DC_{18}PC$–$DC_{22}PC$, where the minority, intermediate lipid species is seen to accumulate at the lipid domains of the two other species.

For a binary lipid mixture of lipid species with different acyl-chain lengths† the consequence of the underlying phase equilibria is that, even in thermodynamic one-phase regions, a dynamic small-scale structure develops in the form of compositional fluctuations or dynamic micro-phase separation, as illustrated in figure 1(b). The length scale of these fluctuations can be varied by changing the temperature, composition, as well as the chain-length difference between the two lipid species [9]. An example of the correlation function for a fluid binary mixture is shown in figure 2(a). The structure of binary fluid mixtures can be further modified by introducing a small amount of a third lipid component which can mediate the boundaries between the micro-phase-separated regions, as illustrated in figure 1(d). This third component can conveniently be chosen as a lipid molecule with an acyl-chain length in

† Abbreviation used is DC_nPC: di-acyl phosphatidylcholine with n carbon atoms in each saturated acyl chain.

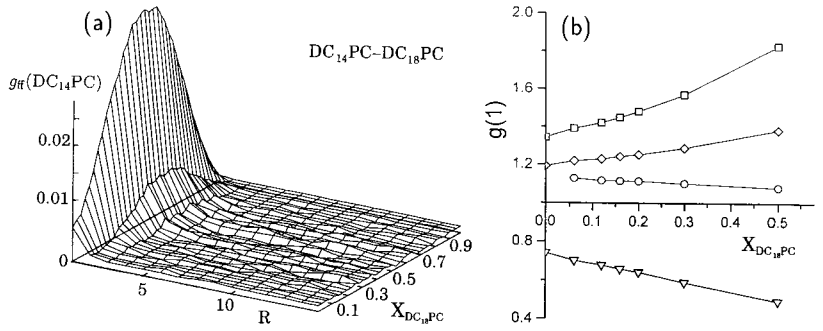

Figure 2. Correlation functions of fluid lipid mixtures. (a) Chain–chain pair correlation function for $DC_{14}PC$ lipids in $DC_{14}PC$–$DC_{18}PC$ for varying composition and acyl-chain separation, R. (b) Nearest-neighbour chain–chain correlation function in 1:1 $DC_{14}PC$–$DC_{22}PC$ mixtures with varying contents of a third species $DC_{18}PC$. □: 14–14; ◇: 18–18; ○: 22–22; ▽: 14–22.

between the ones of the majority species [20]. Hence, this third component acts as a surfactant. A nearest-neighbour correlation function characterizing this special ternary fluid mixture is shown in figure 2(b).

An example of the non-equilibrium small-scale structure that evolves in binary lipid mixtures in a fluid–solid coexistence region is illustrated in figure 1(c) for two lipid species of different acyl-chain length. The lipid species with the higher melting point solidifies and a non-equilibrium spinodal decomposition pattern emerges, where the domain interfaces are wetted by molecules of the low-melting-point component which takes on a more conformationally ordered state in order to mediate the chain-length mismatch at the domain boundaries [10].

3. Nano-scale structure from fluorescence spectroscopy

It is possible to design fluorescent analogues of the lipid molecules, that lead to the phase equilibria described above, and to introduce them in small amounts in order to probe the possible micro-environments of lipid bilayers as predicted by the simulations in figure 1. A special experimental assay involves a set of two fluorescent probes, each designed to have differential affinity for a specific lipid phase, e.g. a solid or a fluid lipid phase [11]. The probes will then tend to localize in their preferred regions of the membrane. In the case where the two probes are respectively a donor and an acceptor for fluorescence, transfer of fluorescence energy, which occurs when the probes collide, will be suppressed if the two probes localize in each of their compartments of the membrane. Formation of lipid domains as in figure 1(a) will then imply that the donor fluorescence intensity will increase.

In figure 3 we show the measured fluorescence intensity of the donor for three different lipid-bilayer systems incorporated with a donor–acceptor pair. A dramatic peak shows up at the phase transition temperature for each of the three systems. The peak is the stronger, the shorter the acyl chain of the lipid molecules. This behaviour is strong indirect evidence of lipid-domain formation in the transition region. It furthermore shows that the domain formation becomes systematically more pronounced as the lipid acyl-chain length is decreased. Similar evidence for lipid-domain formation and lateral bilayer heterogeneity has been reported for other membrane systems using fluorescent probes [12].

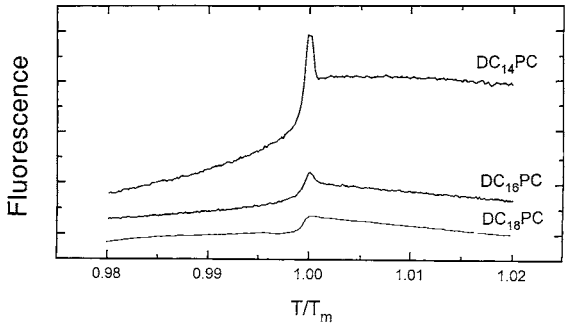

Figure 3. Fluorescence intensity of the donor molecule of a donor–acceptor pair as a function of temperature around the respective phase transition temperature T_m for three different lipid bilayers.

4. Nano-scale structure from atomic-force microscopy

Scanning-probe techniques like atomic-force microscopy (AFM) have opened up the possibility of a direct investigation of the lateral structure of membrane systems in the nanometre range [13]. In particular, it is possible to image soft lipid layers arranged on an appropriate solid support.

Lipid monolayers on air–water interfaces display phase equilibria which are thermodynamically equivalent to those of bilayers in water. Such monolayers can conveniently be transferred onto solid supports by Langmuir–Blodgett techniques which facilitate subsequent imaging by AFM. In figure 4(a) we show an AFM image of a one-component lipid monolayer in the fluid state near the phase transition (the liquid-condensed–liquid-expanded transition) and in figure 4(b) we show an image of a binary monolayer mixture. Both images demonstrate the presence of a small-scale structure, corresponding to density and compositional fluctuations, respectively. The patterns imaged by this technique are assumed to be the patterns present in the monolayer film before transfer, which leads to fixing of the film. These patterns resemble those discussed above for lipid bilayers. Furthermore, the domain sizes and the domain morphology

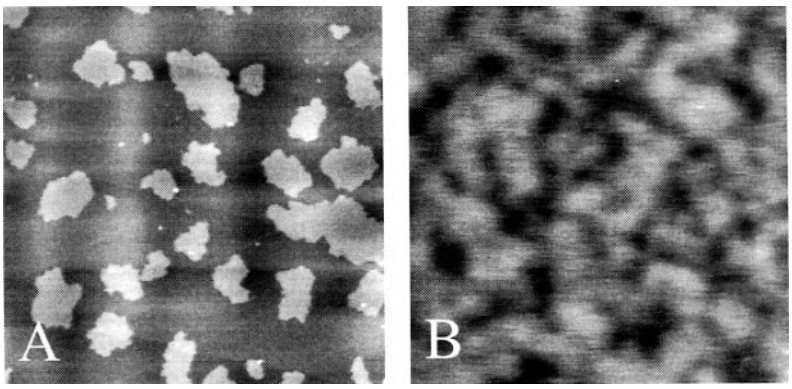

Figure 4. Lateral structure of transferred lipid monolayers imaged by atomic-force microscopy. The different grey tones reflect height differences. (a) A lipid monolayer of $DC_{14}PC$ in the fluid phase near the critical point. The image size is 5×5 μm. The maximum height difference is about 5 Å. (b) A lipid monolayer of a binary mixture of $DC_{14}PC$–$DC_{18}PC$ near the coexistence region. The image size is 250×250 nm. The maximum height difference is about 2 Å.

can be systematically varied by changing temperature, lateral pressure, and composition as expected according to the analogy with lipid bilayers [21]. Hence we take the finding of nanometre-scale structure in the Langmuir–Blodgett lipid films in figure 4 as direct evidence for lipid-domain formation in corresponding lipid bilayers.

Due to the softness of lipid bilayers in the fluid phase it is very difficult to resolve by AFM techniques lateral fluid structure of lipid bilayers in water. However, domains in the solid phases of binary lipid bilayers have recently been observed by AFM in supported aqueous lipid bilayers [14]. In the same systems, grazing-incidence neutron-reflectivity studies have furthermore provided evidence for domain formation in cases involving fluid phases [14].

5. Nano-scale structure and membrane function

It has been surmised that the presence of nano-scale structure in lipid bilayers would influence the functional properties of membranes. It is now well established that the passive permeability of lipid bilayers correlates with lipid-domain formation [15]. Active functions including binding of proteins and enzyme action have also been suggested to be controlled by the small-scale structure [16].

A particularly clear and striking example is that of phospholipase A_2 activity [17]. This enzyme hydrolyses phospholipid molecules organized in lipid layers. In figure 5 we show results for the enzyme activity as a function of temperature for lipid bilayers for the same three different lipid species as in figure 3. It is observed that the activity for each lipid is at its maximum at the respective phase transition temperature. Furthermore, the width of the peak is larger for the shorter lipid chain reflecting a close correlation with the intensity of the density fluctuations. These observations suggest that the activity of phospholipase A_2 is controlled by the small-scale structure of the lipid membrane. Other examples of active enzymes and proteins associated with membranes have also been reported as being sensitive to the physical state of the lipid bilayer [16]. It has furthermore been shown that the organization of trans-membrane proteins, to the extent that these have special affinity for certain lipid domains, can be controlled by the small-scale lipid structure [18].

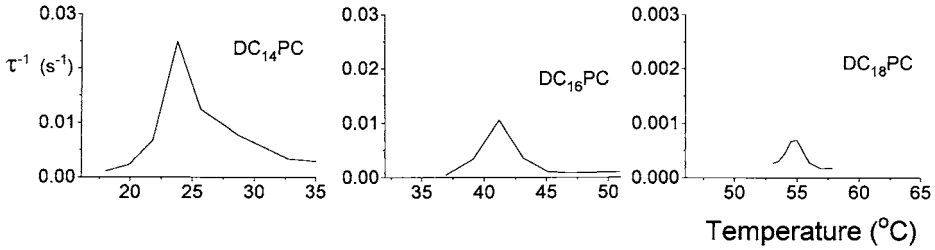

Figure 5. Activity of phospholipase A_2 (measured by the so-called inverse lag time, τ^{-1} [17]) as a function of temperature for three different lipid bilayers. In each panel, the peak occurs at the transition temperature of the lipid bilayer in question.

6. Conclusions

The fundamental physical principles of the lateral molecular organization of biological membranes were considered from the point of view of the lipid-bilayer membrane, its structure, dynamics, and cooperative phenomena. Results obtained from fluorescence spectroscopy,

atomic-force microscopy, as well as computer-simulation calculations, were interpreted as evidence for small-scale lipid-domain formation. It is suggested that the structural organization in the nanometre range is of importance for the functioning of biological membranes, including lateral protein organization [18] and the activity of membrane-bound enzymes [17]. Hence, small-scale lipid structure may serve as a vehicle for compartmentalizing biological membranes [19].

Acknowledgments

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