Membranes Display Nano-scale Heterogeneity - Beating the Randomness of the Fluid Lipid Bilayer[1]

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Abstract

Based on theoretical and experimental evidence obtained from model studies it is argued that lipid bilayer membranes display lateral heterogeneity in the nano-meter range. It is pointed out that this small-scale structure is important for various membrane functions.

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Introduction[2]

The conventional text-book picture of the fluid lipid-bilayer component of biological membranes as a fairly structure-less and disordered 'fluid mosaic' solvent needs modification (Mouritsen and Jørgensen, 1997). The lipid bilayer displays distinct static and dynamic structural organization on a small scale in the nano-meter range (1-100 nm), e.g. in terms of differentiated lipid domains. This scale is in between the molecular scale, accessible by most spectroscopic techniques as well as molecular dynamics calculations, and the micrometer scale which is accessible by scattering and various microscopy techniques. The lateral heterogeneity is an inescapable physical consequence of the fact that the lipid bilayer is a many-particle system, a structured fluid, controlled by cooperative molecular interactions. The question is not whether small-scale lipid domains and lateral heterogeneity is present in membranes, but rather on which time- and length-scales these phenomena occur. Indirect and some direct experimental and theoretical evidence is now becoming available in favor of small-scale lipid-domain formation. Furthermore, evidence is accumulating that small-scale lipid bilayer structure is of importance for the functioning of biological membranes, including trans-membrane permeation, activity of membrane-bound enzymes and receptors, as well as morphological changes at the cell surface. The small-scale structure of membranes may be a way

[1] An extended version of this contribution can be found in Mouritsen and Jørgensen (1997).
[2] Abbreviation: DC_nPC: di-acyl phosphatidylcholine with n carbon atoms in each acyl chain.

of beating the randomness of the fluid bilayer, creating specific differentiated regions or reaction compartments which can steer and possibly trigger the biochemical reactions of the membrane.

Small-Scale structure vs. Large-Scale Structure

Many cell membranes are known to be differentiated into lipid domains on the micrometer scale (Edidin, 1997; Sheetz, 1993; Tocanne, 1992; Bergelson et al., 1995) which seems to be important for gene expression (Norris and Madsen, 1995), for binding and activation of certain enzymes, e.g. protein kinase C (Glaser et al., 1996; Yang and Glaser, 1996), as well as membrane biogenesis and cell division (Welby et al., 1996). Domains of this size, which are visible in fluorescence microscopy, have long lifetimes and may be the result of equilibrium phase separation processes or coupling of the membrane proteins to the cytoskeleton (Kusumi and Yashushi, 1996; Felsenfeld at al., 1996). Indirect evidence exists of domain formation on a smaller, but less well-defined scale, in erythrocyte (More et al., 1996; 1997) and mitochondrial (Richelli et al., 1995) membranes.

Theoretical Evidence for Nano-scale Structure

It can been argued on general theoretical grounds, that lipid domains as well as differentiated regions near proteins should exist also on a much smaller scale, in the nano-meter range, due to the basic physical fact that the fluid lipid bilayer, in the capacity of being a many-particle system consisting of $10^9 - 10^{10}$ molecules, should behave as a structured liquid with a morphology and a lateral organization that reflect the underlying phase equilibria of the membrane (Mouritsen and Jørgensen, 1995; Mouritsen and Kinnunen, 1996). Membrane organization and lipid domains in the nano-meter range are much more difficult to investigate experimentally, and to date mostly indirect evidence of their existence is available. In this situation, computer-simulation calculations have proved useful to investigate, within simple model-membrane systems, under which conditions small-scale membrane structure arises, how it can be characterized, and how it can be modulated, e.g. by solutes, sterols, and integral membrane proteins. Examples of small-scale structure predicted by Monte Carlo computer-simulation calculations are shown in Fig. 1. It is not possible from these calculations to determine the lifetime of the domains.

The examples in Fig. 1 illustrate how order and compartments can arise from cooperative many-particle phenomena such as density fluctuations (Fig. 1a), compositional fluctuations (Fig. 1b), and phase separation processes (Fig. 1c).

The mutual relationship between nano-scale lipid bilayer heterogeneity as well as protein organization has also been studied by computer-simulation techniques (Mouritsen at al., 1996). It is found, that the proteins so to speak 'pick up' the small-scale structure which leads to the formation of differentiated regions around the protein of a structure and composition that is different from that of the bulk, cf. Fig. 1d. This type of cooperative lipid-protein interaction is important for protein organization in membranes, in particular two-dimensional crystallization, cf. Fig. 1e (Gil et al., 1997). Even more strikingly, special compartments can be formed if the integral proteins exhibit activity that drives the membrane assembly into a non-equilibrium steady state, cf. Fig. 1f (Sabra and Mouritsen, 1998).

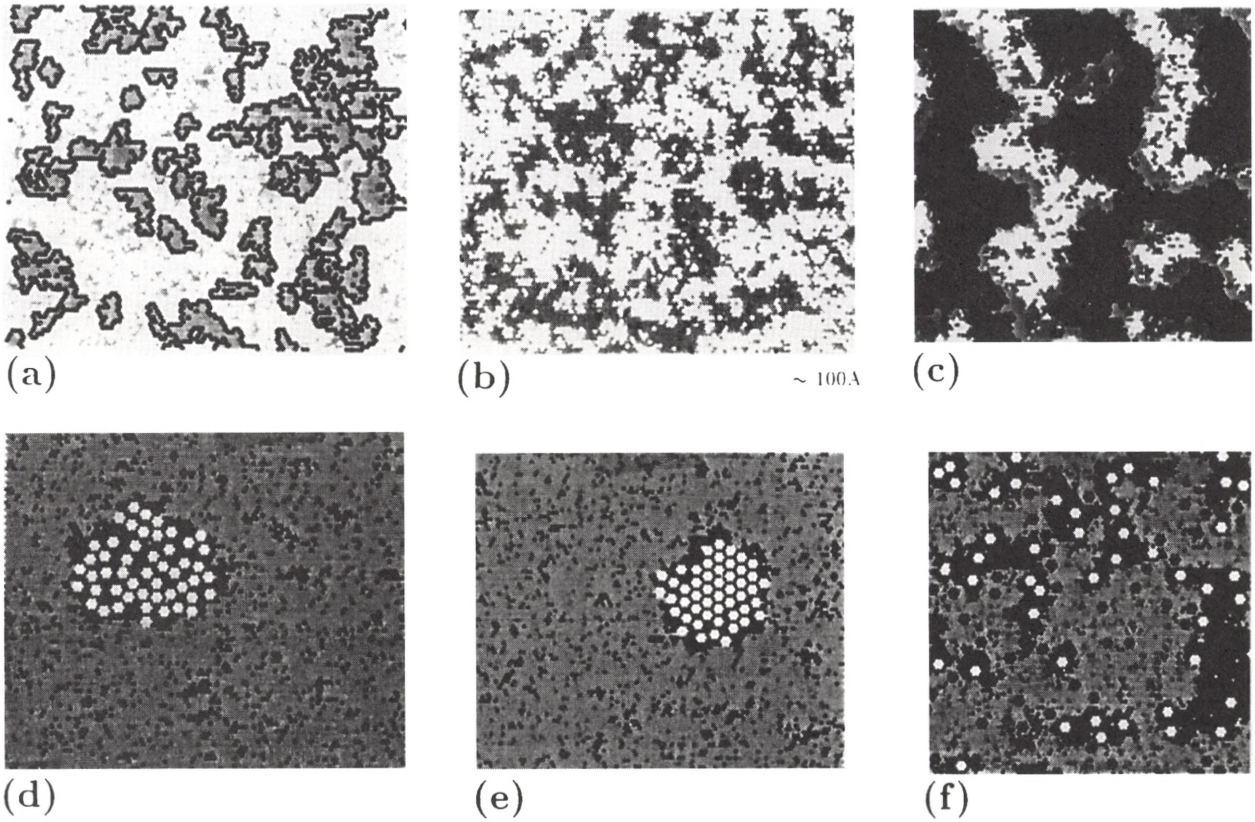

Figure 1. Computer simulation of lipid-bilayer nano-scale lateral organization. **(a):** Density fluctuations in fluid $DC_{14}PC$ bilayers. **(b):** Compositional fluctuations in fluid bilayer mixtures of $DC_{12}PC$ and $DC_{20}PC$. **(c):** Gel-fluid phase separation pattern in mixtures of $DC_{12}PC$ and $DC_{18}PC$. **(d):** A protein-rich domain formed as a capillary condensate in lipid bilayer mixtures of $DC_{14}PC$ and $DC_{18}PC$. **(e):** Two-dimensional protein crystallization induced by lipid phase structure in binary lipid bilayers. **(f):** Steady-state compartmentalization of lipid bilayer mixtures of $DC_{14}PC$ and $DC_{18}PC$ in the presence of active proteins.

Experimental Evidence for Nano-Scale Structure

In one-component lipid bilayers, fluorescence energy transfer techniques (Pedersen et al., 1996; Loura et al., 1996) and eximer-to-monomer fluorescence emission spectroscopy (Lehtonen et al., 1996) have been used to provide indirect evidence for lipid-domain formation, cf. Fig. 1a. These techniques are adequate to probe lateral structure in the range of about 10 nm. Evidence for lipid micro-domains consisting of less than fifty lipid molecules in the gel phase of lipid binary mixtures of phospholipids with different acyl-chain lengths comes from infrared spectroscopy (Mendelsohn et al., 1995). Although some evidence of fluid-fluid immiscibility has been reported for binary lipid mixtures with different polar head groups, phosphatidylserine and PC (Hinderliter et al., 1994), nano-scale lipid structure formation in the fluid phase of binaries, cf. Fig. 1b, has yet to be demonstrated experimentally.

In a series of experiments, T.E. Thompson and collaborators (Piknová et al., 1996; Schram et al., 1996) have over the last several years, by

different spectroscopic techniques, provided compelling evidence in favor of small-scale gel-phase domain formation and compartmentalization of binary mixtures of PC lipids with different acyl-chain lengths in the gel-fluid phase coexistence region, cf. Fig. 1c. The results have provided information on the diffusional characteristics of the probes which have been interpreted in terms of static percolation structures and domains of fractal structure. The sizes of the domains range up to several hundred molecules, depending on the degree of ideality of the mixture, the composition, and the temperature. It is difficult to reconcile the observation of static domains of finite size in the gel-fluid coexistence region of binary lipid mixtures with equilibrium thermodynamics and it is possible that these domains are consequences of slow non-equilibrium rearrangements (Jørgensen and Mouritsen, 1995; Jørgensen et al., 1996) or coupling to the bilayer curvature.

The binding of peripheral proteins, such as cytochrome c (Kinnunen et al., 1994) has been shown to be controlled by the formation of lipid domains of charged lipids leading to a local surface charge density that is larger that the average surface charge and hence locally promote binding. The binding, in turn, of the charged proteins leads to a stabilization of the small-scale lipid domain structure (Heimburg and Biltonen, 1996).

A number of investigations have been conducted on lipid bilayers reconstituted with integral membrane proteins in order to investigate the effect on lipid structure due to a hydrophobic mismatch between the lipid-bilayer thickness and the trans-membrane hydrophobic membrane domain of the protein (Mouritsen and Bloom, 1993; Mouritsen, 1998). A mismatch is theoretically predicted to not only influence the phase equilibria of the membrane but also to lead to a special lipid profile at the lipid-protein interface. In the case of lipid bilayers with more than one lipid species, this could lead to a physical lipid selectivity and sorting mechanism at the lipid-protein interface and the creation of a special local lipid structure that may be markedly different from the bulk, both with respect to molecular composition and lipid acyl-chain conformational order.

Studies of bacteriorhodopsin in binary lipid mixtures of $DC_{14}PC$-$DC_{18}PC$ lipids have indicated that the presence of the protein has a significant influence on the percolation properties and the topology of the domains in the gel-fluid phase coexistence region (Piknová et al., 1997; Schram and Thompson, 1997). It has been suggested, based on a combined fluorescence energy transfer experiment and Monte Carlo simulation on bacteriorhodopsin reconstituted into an even more non-ideal mixture of PC lipids, $DC_{12}PC$-$DC_{18}PC$, that the hydrophobic matching principle acts as to sort the two lipid species at the lipid-bacteriorhodopsin interface, such that there is an enrichment of that lipid species, which under the thermodynamic conditions given, most easily adapts to hydrophobic matching (Dumas et al., 1997). These results are clear manifestations of small-scale, nano-meter-range structural organization of lipid-protein assemblies.

Relationship Between Nano-Scale Membrane Structure and Function

The function of certain lipases active on lipids in aggregated form seems to be controlled by the nano-scale structure of the lipid bilayer. In the case of phospholipase A_2 it has been demonstrated that the activity, measured via the lag time for the onset of rapid hydrolysis, has a systematic dependence on temperature and acyl-chain length of the PC substrate which correlates closely with the degree of heterogeneity, i.e. small-scale bilayer structure (cf. Fig. 1a) (Hønger et al., 1996). The ac-

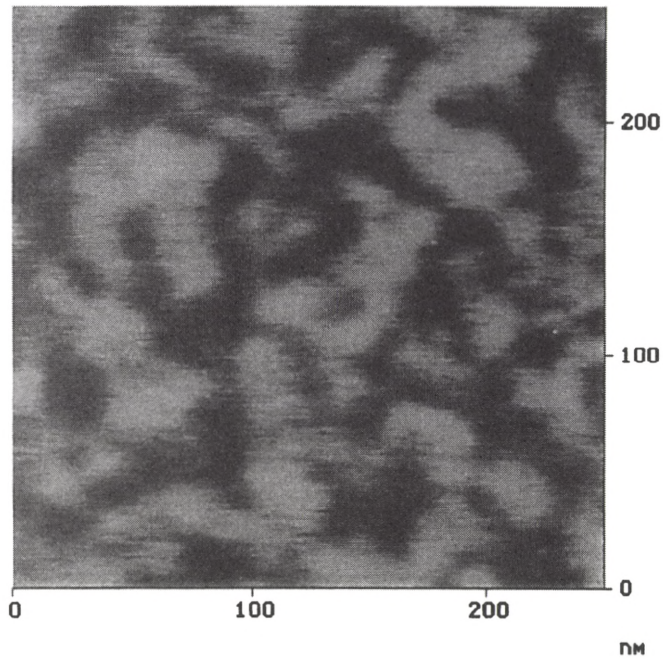

Figure 2. Image of a lipid monolayer 1:1 mixture of $DC_{14}PC$ and $DC_{18}PC$ on a solid support obtained by atomic force microscopy (L. Kildemark, T. Bjørnholm, and O. G. Mouritsen, unpublished).

tivity of phospholipase C has also been correlated with the small-scale lipid structure in terms of defects or lipid domains (Basanez et al., 1996).

Protein kinase C is an enzyme active at membranes that requires charged lipids, like phosphatidylserine, to bind to the membrane and small amounts of diacylglycerol to become activated. It has been argued (Dibble et al., 1996; Hinderliter et al., 1997) that a reason why small amounts of diacylglycerol may be sufficient is that, due to the small-scale structure of the lipid bilayer substrate, the diacylglycerol is not randomly distributed, but rather accumulates into domains and that the protein kinase C activity is controlled by the interface between regions enriched in and poor in diacylglycerol.

Several other membrane-bound proteins and receptors have been investigated in order to unravel the relationship between function and the physical properties of the lipid membrane, in particular the possible small-scale structure. Examples, where recent experimental data have been obtained and taken to support this relationship, include the activity of the nicotinic acetylcholine receptor channel (Zanello et al., 1996) and the activation by phosphatidylglycerol of diglucosyldiacylglycerol synthase from *Acholeplasma laidlawii* membranes (Karlsson et al., 1996), controlled by accumulation of the activator into domains that are formed in response to a hydrophobic mismatch between the acyl chains of the activator and the host bilayer.

Small-Scale Lipid Domains — are They Really There?

Using glancing incidence neutron scattering, Gliss et al. (1997) have recently found evidence for lipid domain formation on the scale of about 50Å in supported lipid bilayer mixtures of $DC_{14}PC$ and $DC_{18}PC$ in their gel-fluid coexistence region. Scattering studies provide information in reciprocal space. A direct visualization of nano-meter scale domains would have to use ultra-sensitive surface probe techniques, like atomic force microscopy. Preliminary studies applying such techniques to lipid monolayers, prepared in thermodynamic state equivalent to that of a bilayer and transferred to a solid support, have lead to real-space pictures like the one in Fig. 2 (L. Kildemark, T. Bjørnholm, and O. G. Mouritsen, unpublished). This picture shows the presence of a small-scale domain structure characterized by a length scale of about 100-200Å. — Nano-scale lipid domains undoubtedly exist in lipid bilayer membranes. The existence of this type of nano-scale heterogeneity may be a way of compartmentalizing the membrane thereby beating the randomness of the fluid lipid bilayer.

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