# Lipid Sorting by Membrane Proteins and Membrane Organization

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*Abstract*

In this contribution it is shown that a mismatch between the hydrophobic lengths of transmembrane proteins and the hydrophobic thickness of the surrounding lipid bilayer may generate physical forces which can promote molecular mechanisms of lipid sorting by the proteins and then contribute to the supramolecular organization of biological membranes.

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Biol. Skr. Dan. Vid. Selsk. 1998, **49**:127–131

Biological membranes are complex assemblies composed of a wide variety of lipid and protein molecular species. In current models of membranes, the lipid bilayer is considered as a two-dimensional fluid in which lipids and intrinsic proteins are free to diffuse. As a direct consequence, one would expect both types of molecules to be randomly distributed within the membrane. In fact, membrane organization is certainly more complex and evidence is accumulating to indicate the occurrence of both a transverse (Devaux, 1993) and lateral (Tocanne, 1992; Glaser, 1993) compartmentation of membranes which can be described in terms of lipid and protein macro- and microdomains. Macrodomains, a few micrometers in size, have been recognized in the plasma membrane of specialized cells such as spermatozoa, eggs and epithelia (Tocanne et al., 1994a). The two leaflets of the lipid bilayer can also be considered as two large and nearly independent membrane domains but with the possibility of transbilayer lipid redistribution mediated by a specific and energy-dependent aminophospholipid transporter (Devaux, 1993). Microdomains extending from the submicron to the molecular scale have been recognized in the plasma membrane of many cell types and by means of a quite wide variety of approaches (Tocanne, 1992) among which Fluorescence Recovery After Photobleaching (FRAP) (Tocanne et al., 1994) and Single Particle Track-

ing (SPT) (Saxton and Jacobson, 1997) techniques have proved very useful.

At this stage, one can have the feeling that membranes are structurally well known and that the concept of membrane domain is firmly established. In fact, and as emphasized by other contributors, one has to realize that the word 'domain' is generic and applies to a wide variety of structures and that the view one can have of these domains still strongly depends on the technique and the lipid or the protein probes used for their detection. There is certainly not a unique definition of membrane domains and as a matter of fact, the size of these domains, their composition, the time- and space-scale over which they exist may vary from one cellular system to another. The nature of the interactions and forces responsible for their formation have also to be clearly identified and remains, in addition to the previous points, one of the many challenging problems in membranology.

With respect to proteins, one current opinion is to consider their confinement in domains is due principally to interactions of their cytosolic part with membrane skeleton and/or cytoskeleton elements (Sheets et al., 1995; Kusumi and Yako, 1996). In this model, the barriers to protein diffusion are located outside the membrane, not within the membrane.

Like proteins, lipids exhibit a wide diversity in structure, at the level of both their polar headgroups and acyl chains. The causal structure-function relationship which may easily be put forward for membrane proteins is not so obvious in the case of lipids. From a physical point of view, the structural diversity they display is not required to maintain bilayer assembly and fluidity. Gel-fluid phase lateral separations have been suggested as being responsible for lateral heterogeneities in membranes (Vaz, 1992). Such a possibility, which can occur in some specific cases, like in ram sperm plasma membrane (Wolf et al., 1990), does not seem to be of general relevance to natural membranes. In many cases, the lateral heterogeneities in lipid distribution detected in natural membranes are no longer observed in the corresponding protein-free lipid bilayers (Tocanne et al., 1994b) and there is increasing evidence that proteins are responsible for membrane organization (Edidin, 1997). From another point of view, we recently arrived at the conclusion that FRAP experiments conducted at variable beam radius provide a way to detect the presence of domains in membranes and to evaluate their size. When applied to published data obtained for lipids and proteins on various cellular membranes, this approach indicates the presence of closed protein and lipid domains in these membranes, with nearly the same size (Salomé et al., 1997). This implies that in addition to interactions of membrane proteins with the membrane skeleton, protein-protein interactions occur within the membrane. They lead to the formation of intramembrane barriers which prevent lipids and proteins from long-range lateral diffusion. However, if protein-protein interactions seem to play an important role, we still have no direct information on the molecular organization of lipids in contact with or far from the proteins, nor on the potential specificity of lipid distribution around membrane proteins, nor on the contribution of protein-lipid interactions to protein agregation.

The possibility of specific interactions between lipids and transmembrane proteins is not to be discarded (Tocanne et al., 1994b; see also D. Marsh's contribution in the present issue). However, this kind of interaction remains limited to specific cases and is not expected to contribute in any great extent to membrane organization. In contrast, and because of the long-range dispersion of elastic forces in membranes (principle of cooperativity), the consequences of non specific protein-lipid interactions on lipid dynamics may extend over a few lipid layers around each protein and therefore may contribute on a large scale to membrane organization. One particularly attractive case is the existence of a mismatch between the hydrophobic thicknesses of a transmembrane protein and the supporting lipid bilayer. As is now well evidenced by theoretical and experimental data, a hydrophobic mismatch between lipids and proteins can generate physical forces capable of promoting an aggregation of proteins within the membrane, changes in the organization and dynamics of those lipid molecules which are in contact with proteins,

and a sorting of lipids by the proteins.

First developed from a theoretical point of view (Mouritsen and Bloom, 1984), the concept of hydrophobic mismatch has been checked experimentally with the reaction center and the light-harvesting antenna from *Rhodobacter sphaeroides* (Riegler and Möhwald, 1986; Peschke et al., 1987) and bacteriorhodopsin from *Halobacterium halobium* (Piknova et al., 1993) reconstituted in phosphatidylcholines with different acyl chain lengths. Depending on the relative hydrophobic thickness of the proteins and the supporting lipid bilayers, upward or downward shifts in the phase transition temperature of the lipids were detected with the three proteins which could be accounted for in the light of the current theories, thus providing strong experimental support to the concept that hydrophobic mismatch can be at the origin of great changes in the physical state of lipids. Coherence lengths of 12 - 15 Åwere determined, which means that at least three to four lipid layers around the proteins are significantly affected by the hydrophobic mismatch.

The Ca-ATPase from muscle sarcoplasmic reticulum reconstituted in phosphatidylcholines substituted by various monounsaturated fatty acids has long been shown to exhibit a bell-shape activity versus acyl chain length curve, with maximum activity for oleic acid (Starling et al., 1993). As recently shown, these changes in activity are closely related to the aggregation state of the protein, the lower the activity of the protein in the short or long chain PC species, the higher its agregation state in the proteoliposomes (Cornea and Thomas, 1994).

In this contribution, it is pointed out that a mismatch between the hydrophobic lengths of transmembrane proteins and the hydrophobic thickness of the surrounding lipid bilayer may generate physical forces which can promote molecular mechanisms of lipid sorting by the proteins and then contribute to the supramolecular organization of biological membranes.

In mixtures of lipids with different acyl chain lengths, a transmembrane protein is expected to be solvated by the lipid species capable of best matching its hydrophobic surface (Sperotto and Mouritsen, 1993). This theoretical prediction is now supported experimentally by data obtained with the pulmonary surfactant protein SP-C and bacteriorhodopsin (BR), through fluorescence energy transfer experiments. SP-C, reconstituted in surfactant lipids, is excluded from gel phase palmitoyl lipids and prefers shorter chain and unsaturated lipids below the bulk lipid phase transition (Horowitz, 1995). BR was reconstituted in DLPC/DSPC mixtures and its behaviour was analyzed quantitatively by comparing simulated and experimental data. In a quite consistent way, the theoretical and experimental approaches show that at low temperature, when all the lipids are in the gel state, BR is embedded in the nearly pure DLPC phase. At high temperature, when all the lipids are in the fluid state, the protein is preferentially surrounded by DSPC at the expense of DLPC. Quite interestingly, at moderate temperature, when DLPC is in the fluid phase while DSPC is still in the gel phase, BR is found to be located at the gel-fluid phase boundary (Dumas et al., 1997). This last conclusion is also supported by indirect arguments developed by Schram and Thompson who studied the phase behaviour and dynamics of BR/DMPC/DSPC mixtures (Schram and Thompson, 1997).

Even in the absence of hydrophobic mismatch, the dynamics of lipids may also be affected by interactions with membrane proteins. Thus, in the course of our studies on the influence of proteins on the lateral diffusion of lipids, comparison of simulated and experimental FRAP data led us to conclude that in egg yolk phosphatidylcholine, bacteriorhodopsin is not to be viewed as naked but rather as surrounded by about two layers of lipids with restricted lateral mobility (Schram et al., 1994).

From a biophysical point of view, the hydrophobic matching condition can be considered as a very useful and operational concept. From a biological point of view, one can argue that this concept has nothing to do with membrane organization because in a given membrane, proteins and lipids show, on the average, similar hydrophobic thicknesses. The influence of cholesterol, which is known to have a buffering effect on the dynam-

ics of the lipid acyl chains, should also be considered. However, this concept has been recently introduced in cellular biology to explain some aspects of the sorting of membrane proteins from the reticulum to the plasmalemma, through the Golgi apparatus (Bretscher and Munro, 1993). Moreover, careful inspection of protein sequences and structures show that the various transmembrane segments of membrane proteins may differ in hydrophobic length (by at least a few angstrom) and orientation with respect to the bilayer normal. In other words, the concept of hydrophobic mismatch and lipid sorting by proteins can be extended to a submolecular level. A membrane protein may be seen now surrounded by various lipid species (including cholesterol), each being selected to match best its various hydrophobic segments (Dumas et al., 1997).

Protein-lipid interactions are believed to contribute important molecular forces which, on account of the relatively high protein surface density in membranes, can propagate over long distances. In the presence of too strong protein-lipid hydrophobic mismatch, protein aggregation provides a way for the system to decrease the importance of protein-lipid interactions and therefore to relieve an excess of energy. Another and not contradictory possibility is to generalize the concept of lipid sorting by proteins to the whole system and to consider now the membrane as a supramolecular assembly in which microdomains are the consequence of a good structural matching of the various protein and lipid components. However, the final organization of the membrane and in particular the structure of the protein fences are also expected to depend critically on protein-protein interactions. These various interaction parameters, their relative extent and interplay need to be firmly established before any clear picture of membrane organization can be drawn. Models of membranes should not be thought of as being static and in equilibrium. They should include the dynamics of the protein and lipid components and of the membrane itself.

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