New and Notable

Stepping between Membrane Microdomains

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Lateral membrane microdomains are postulated to be involved in a number of important functions in mammalian cells, such as membrane trafficking, exocytosis, endocytosis, signal transduction, and protein activity (1,2). These putative cell microdomains have been the subject of extensive research using a variety of biophysical and biochemical techniques, including detergent extraction (2), fluorescence microscopy (3), electron microscopy (4), fluorescence resonance energy transfer microscopy (5), fluorescence correlation spectroscopy (6), fluorescence recovery after photobleaching (7), and single-molecule tracking (8). Roles for lipid-lipid, lipid-protein, and protein-protein interactions have been invoked as critical factors in the formation and maintenance of membrane microdomains (1,2,8). There is now strong experimental evidence for nanoscale heterogeneity in cell plasma membranes, with microdomains (often called rafts) enriched in cholesterol and phospholipids with saturated hydrocarbon chains such as sphingomyelin (SM), together with surrounding (nonraft) microdomains enriched in phospholipids containing more unsaturated hydrocarbon chains.

At least in part due to their transient nature (3,5), as well as their small size in cell membranes (4,5), there are still many fundamental unanswered questions concerning microdomain formation and organization. One of the most important open questions concerns the thickness and elasticity of raft and nonraft structures. Both theory (9) and experiments (10) indicate that size and morphology of lipid microdomains depends on the line tension between these domains, which is a function of the difference in bilayer thickness, as well as the elastic properties of the domains. In addition, bilayer thickness is thought to be a critical factor in determining protein-lipid interactions in general, and specifically in protein trafficking through the Golgi apparatus (1). Moreover, bilayer thickness and elastic properties are key to the sorting of proteins between microdomains (11).

A tremendous boon to understanding microdomain formation, structure, and elastic properties has been the use of lipid bilayer systems, particularly bilayers containing mixtures of the lipids characteristic of raft and nonraft microdomains. In such bilayers the initial lipid compositions can be precisely measured, the organization of the microdomains can be analyzed, and the roles of lipid-lipid interactions can be addressed. With bilayers systems, critical observations (12) are that microdomains can be observed in mixtures of 1) Cholesterol; 2) PC (phosphatidylcholine) with two monounsaturated hydrocarbon chains, such as (C18:1)(C18:1)PC; and 3) PCs with two saturated hydrocarbon chains such as (C16:0)(C16:0)PC, or SM, which also contains saturated hydrocarbon chains.

These studies show that the presence of each of these three lipid classes is critical to microdomain formation; that resulting microdomains can be up to micrometers in size (much larger than raft microdomains observed in a biological membrane); and that both microdomains are liquid. Specifically, in these lipid systems one microdomain is a liquid-disordered (L_d) phase, characteristic of fully hydrated unsaturated phospholipid bilayers, whereas the other microdomain is a liquid-ordered (L_o) phase, characteristic of bilayers containing saturated phospholipids with relatively large concentrations of cholesterol. Although both the L_d and L_o phases are liquid, the L_o phase has more conformationally ordered hydrocarbon chains due to short-range phospholipid-cholesterol interactions. Fluorescence spectroscopy and microscopy have been used to assess lipid-packing order, demonstrating the importance of L_d and L_o phase separation in both bilayer and plasma membrane systems (13).

In this issue of the *Biophysical Journal*, Heftberger et al. (14) present small-angle x-ray scattering (SAXS) data from multilamellar lipid vesicles containing both liquid-ordered and liquid-disordered microdomains to obtain data on microdomain thickness and elastic properties. These vesicles are free of detergents and fluorescent labels that could potentially modify bilayer thickness or elasticity. Their global SAXS analysis uses both discrete Bragg reflections and diffuse scattering to obtain high-resolution electron density profiles of the coexisting L_o and L_d phases. This elegant analysis provides the bilayer thicknesses, areas per lipid molecule, and bending fluctuations of each phase for lipid systems containing cholesterol, a fully saturated PC (either (C16:0)(C16:0)PC or (C18:0)(C18:0)PC), and (C18:1)(C18:1)PC. They find that the bilayer thickness for the L_o microdomain is ~10 Å greater than that of the L_d microdomain, the area per lipid molecule is ~20 Å^2 greater for L_o microdomain, and the Caillé parameters associated with bending fluctuations are ~3 times bigger for the L_d microdomain. Thus these studies precisely quantify the effects of cholesterol and hydrocarbon chain unsaturation on bilayer structure and elasticity. This outstanding study provides fundamental and detailed information that should be extremely helpful in understanding the roles of

Submitted December 2, 2014, and accepted for publication December 23, 2014.

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Editor: Anne Kenworthy.

0006-3495/15/02/0783/2 $2.00

http://dx.doi.org/10.1016/j.bpj.2014.12.039

lipid-lipid interactions in microdomain formation.

The authors (14) explicitly describe several issues that could be addressed in the future by their sophisticated SAXS analysis, including the critical behavior of microdomain mixtures across the transition into a homogeneous phase and predictions of protein activity and partitioning in microdomains. Hopefully in the future this analysis could be also extended to lipid mixtures more similar to those found in cell plasma membranes, for instance those containing cholesterol, SM, and a phospholipid with one saturated and one unsaturated hydrocarbon chain, such as (C18:0)(C18:1)PC.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grant No. GM27278.

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