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Review

Using small-angle neutron scattering to detect nanoscopic lipid domains

Jianjun Pan[a,*], Frederick A. Heberle[a], Robin S. Petruzielo[b], John Katsaras[a,c,d,**]

[a] Biology and Soft Matter Division, Neutron Sciences Directorate, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States
[b] Department of Physics, Cornell University, Ithaca, NY 14853, United States
[c] Canadian Neutron Beam Centre, National Research Council, Chalk River, Ontario K0J 1J0, Canada
[d] Joint Institute for Neutron Sciences, Oak Ridge National Laboratory, Oak Ridge, TN 37831, United States

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ABSTRACT

The cell plasma membrane is a complex system, which is thought to be capable of exhibiting non-random lateral organization. Studies of live cells and model membranes have yielded mechanisms responsible for the formation, growth, and maintenance of nanoscopic heterogeneities, although the existence and mechanisms that give rise to these heterogeneities remain controversial. Small-angle neutron scattering (SANS) is a tool ideally suited to interrogate lateral heterogeneity in model membranes, primarily due to its unique spatial resolution (*i.e.*, ~5–100 nm) and its ability to resolve structure with minimal perturbation to the membrane. In this review we examine several methods used to analyze the SANS signal arising from freely suspended unilamellar vesicles containing lateral heterogeneity. Specifically, we discuss an analytical model for a single, round domain on a spherical vesicle. We then discuss a numerical method that uses Monte Carlo simulation to describe systems with multiple domains and/or more complicated morphologies. Also discussed are several model-independent approaches that are sensitive to membrane heterogeneity. The review concludes with several recent applications of SANS to the study of membrane raft mixtures.

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* Corresponding author at: Oak Ridge National Laboratory, P.O. Box 2008 MS-6453, Oak Ridge, TN 37831, United States. Tel.: +1 865 576 5841.
** Corresponding author. Tel.: +1 865 274 8824.
E-mail addresses: jianjunp@gmail.com, panj@ornl.gov (J. Pan), katsarasj@ornl.gov (J. Katsaras).

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1. Introduction

The cell plasma membrane (PM) is composed of proteins, sterols, and amphiphilic lipids. It is therefore not surprising that such a complex system is capable of exhibiting non-random lateral organization. Indeed, heterogeneities in multiple-component lipid mixtures have been known since the early 1970s. However, in the intervening years a rather surprising hypothesis has taken shape around those observations, namely that the non-random organization of lipids and proteins has explicit functionality in biological membranes. These functional lipid/protein microdomains, or membrane rafts, are enriched in sphingomyelin (SM) and cholesterol (Chol). In the current view, rafts effectively compartmentalize the cell PM into ordered and disordered regions, separating proteins based on their preferential interaction with certain types of lipids. Nevertheless, despite nearly two decades of intense research, the existence of rafts remains controversial, primarily due to their small size and possible transient nature.

1.1. The roadmap for rafts

In 1972, Singer and Nicolson proposed the fluid mosaic model, which postulated that the PM is a homogeneous, relatively inert, two-dimensional fluid matrix that provides a medium for protein diffusion, and a substrate for protein interactions and protein-directed processes (Singer and Nicolson, 1972). In short, lipids were treated as passive structural components, and proteins were thought to control membrane architecture and organization, possibly through interactions with the cytoskeleton. Soon after, studies of various lipid-only systems proposed the possible existence of temperature-dependent lipid clustering in the membrane (Lee et al., 1974). The lipid clusters were thought to exist in an ordered fluid state (Wunderlich et al., 1978) relative to their surrounding disordered liquid crystalline lipids. In 1982, Karnovsky and coworkers advanced the concept of membrane domains (Karnovsky et al., 1982) after observing heterogeneous fluorescence lifetime decay in both model lipid bilayer mixtures and isolated cell membranes. They proposed several lines of inquiry, some of which are still being investigated today, including the forces behind the formation, maintenance, and fluctuation of lipid domains.

Interest in the roles of lipids and sterols in the lateral organization and chemical functions of the PM followed these observations. For example, sphingolipids and cholesterol were found to be enriched in the apical membranes of epithelial cells (Simons and Vanmeer, 1988). It was also observed that protein partitioning and function were affected by lipid composition in model systems (Dibble et al., 1993; Florine and Feigenson, 1987). Brown and Rose found that certain proteins localized with sphingolipids and cholesterol in detergent-resistant membrane fractions, which were presumed to exist in the membrane as ordered domains prior to detergent solubilization (Brown and Rose, 1992). In model membranes made up of three lipid components, liquid–liquid immiscibility was first reported in 1996 (Silvius et al., 1996).

The findings from live cells and model membranes led to the membrane raft description of the PM (Ahmed et al., 1997; Simons and Ikonen, 1997). The raft hypothesis postulated that the PM is organized into functional domains with different average compositions, compared to the bulk lipids (presumably due to the preferential association of SM with cholesterol). These domains organize proteins and control their function by influencing protein diffusion and local concentration. Analysis of lipids by mass spectrometry has elucidated the lipid composition of rafts. For example, compared to the bulk PM, SM levels in rafts are elevated by about 50% (Fridriksson et al., 1999), cholesterol levels are double (Pike et al., 2002), while phosphatidylcholine (PC) levels are similar. Since the raft concept was formalized, membrane domains have been implicated in many cellular functions, including cell signaling pathways (Foster et al., 2003; Simons and Toomre, 2000), protein sorting (Anderson and Jacobson, 2002), protein activity modulation (Jensen and Mouritsen, 2004), and cytoskeletal connections (Su et al., 2012).

A continuing source of controversy is the lack of visual (*e.g.*, fluorescence micrograph) evidence for rafts in resting (unstimulated) cells. Rafts are currently thought to be ordered domains of nanoscopic dimensions that can coalesce to form larger, stable platforms upon cell stimulation (*e.g.*, external crosslinking of a membrane component), and exclude certain proteins (Lingwood and Simons, 2010). Evidence for such nanoscale heterogeneities in the resting PM is accumulating. For example, Sharma and coworkers used homo and hetero-FRET (Förster resonance energy transfer) to detect small clusters (<5 nm) of certain proteins in the PM (Sharma et al., 2004). FRET between fluorescent lipid analogs in the PM's outer leaflet revealed nanoscale heterogeneity in cells (Sengupta et al., 2007). Electron spin resonance (ESR) studies in live cells found order and rotational diffusion parameters consistent with distinct liquid-ordered (Lo) and liquid-disordered (Ld) phases (Swamy et al., 2006). New tools with enhanced spatial resolution are also proving helpful (Simons and Gerl, 2010). Super resolution microscopy methods, including fluorescence photoactivation localization microscopy (FPALM), are capable of detecting nanoscale heterogeneities in cells (Hess et al., 2006). Moreover, stimulated emission depletion (STED) far-field nanoscopy inferred the presence of nanoscopic domains in live cells through the detection of sphingolipids with hindered diffusion (Eggeling et al., 2009).

1.2. Phase separation in model membranes

Complementing the research with live cells, chemically simplified model systems with well-defined lipid compositions have proven to be powerful tools for elucidating the thermodynamics governing the lateral heterogeneity of membrane lipids. Lipids in the outer leaflet of the mammalian PM can be broadly grouped based on fluidity: high-melting (high-T_M) lipids, including SM, undergo a transition from the gel to the fluid phase near or above physiological temperatures, while low-T_M lipids in the outer leaflet are fluid at ambient temperature. A three-component model for the cell membrane outer leaflet can be constructed using one component from each of these categories, together with cholesterol, the most abundant lipid in the PM by mole fraction. These mixtures often exhibit lateral segregation of the Ld and Lo phases over a broad range of composition and temperature. Importantly, the phase diagrams of these model systems provide critical information for understanding lipid–lipid interactions governing raft formation.