Chemistry and Physics of Lipids 192 (2015) 87–99

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Lateral organization, bilayer asymmetry, and inter-leaflet coupling of biological membranes

Jonathan D. Nickels[a,b], Jeremy C. Smith[c,d], Xiaolin Cheng[c,d,*]

[a] Joint Institute for Neutron Sciences, Oak Ridge National Laboratory, Oak Ridge, TN, USA
[b] Department of Physics and Astronomy, University of Tennessee, Knoxville, TN, USA
[c] Center for Molecular Biophysics, Oak Ridge National Laboratory, Oak Ridge, TN, USA
[d] Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville TN, USA

ARTICLE INFO

Article history:
Received 27 May 2015
Received in revised form 20 July 2015
Accepted 25 July 2015
Available online 29 July 2015

Keywords:
Lateral organization
Bilayer asymmetry
Inter-leaflet coupling
Neutron scattering
Molecular dynamics

ABSTRACT

Understanding of cell membrane organization has evolved significantly from the classic fluid mosaic model. It is now recognized that biological membranes are highly organized structures, with differences in lipid compositions between inner and outer leaflets and in lateral structures within the bilayer plane, known as lipid rafts. These organizing principles are important for protein localization and function as well as cellular signaling. However, the mechanisms and biophysical basis of lipid raft formation, structure, dynamics and function are not clearly understood. One key question, which we focus on in this review, is how lateral organization and leaflet compositional asymmetry are coupled. Detailed information elucidating this question has been sparse because of the small size and transient nature of rafts and the experimental challenges in constructing asymmetric bilayers. Resolving this mystery will require advances in both experimentation and modeling. We discuss here the preparation of model systems along with experimental and computational approaches that have been applied in efforts to address this key question in membrane biology. We seek to place recent and future advances in experimental and computational techniques in context, providing insight into in-plane and transverse organization of biological membranes.

1. Introduction

The lipid raft hypothesis has fundamentally changed the way we look at the cell membrane and how it organizes proteins and lipids to accomplish its varied and vital functions (Simons and Ikonen, 1997). Through the selective partitioning of membrane proteins between rafts and their surroundings, rafts control protein–protein interactions, enhancing certain associations while suppressing others. In this manner, rafts are thought to mediate a range of cellular processes, including signal transduction (Simons and Toomre, 2000), apoptosis (Gajate and Mollinedo, 2001), cell adhesion and migration (del Pozo et al., 2004), cell recognition (Pierce, 2002), synaptic transmission (Hering et al., 2003), cytoskeletal organization (Villalba et al., 2001) and protein sorting (Lingwood and Simons, 2010; Jacobson et al., 2007). The rafts themselves are thought to be small (10–100 nm) (Pralle et al., 2000), heterogeneous (Pike, 2004) and highly dynamic (Samsonov et al., 2001) structures composed primarily of lipids but also rich in sterols, carbohydrates, and proteins (Brown and London, 2000). Rafts have been extensively studied in animal cells (Verkleij et al., 1973; Allen et al., 2007), and recent research has shown that they are also central to the organization and function of membranes in plants (Mongrand et al., 2004) and microbes (López and Kolter, 2010). They also play a role in bacterial and viral pathogenesis (van der Goot and Harder, 2001; Dick et al., 2012). The size, lifetime, and connectivity of rafts are crucial parameters for understanding their function (Lingwood and Simons, 2010). How these parameters are controlled by the cell is, at best, poorly understood and is the focus of intense current scientific interest.

Another key feature of biological membranes is that they are compositionally asymmetric (Van Meer et al., 2008). In fact, bilayer compositional asymmetry was well known even within five years (Verkleij et al., 1973; Bretscher, 1972, 1973; Steck and Dawson, 1974; Rothman and Lenard, 1977; Zwaal et al., 1977; Op den Kamp, 1979) of the publication of the fluid mosaic model (Singer and Nicolson, 1972). The majority of aminophospholipids reside on the membrane inner leaflet, with sphingomyelin and choline phospholipids primarily in the outer leaflet (Fig. 1) (Verkleij et al., 1973; Singer and Nicolson, 1972). Cholesterol is found in both bilayer

* Correspondence author at: Oak Ridge National Laboratory, P.O. Box 2008, MS-6309 Oak Ridge, TN 37831, USA.
E-mail address: chengx@ornl.gov (X. Cheng).

http://dx.doi.org/10.1016/j.chemphyslip.2015.07.012
0009-3084/

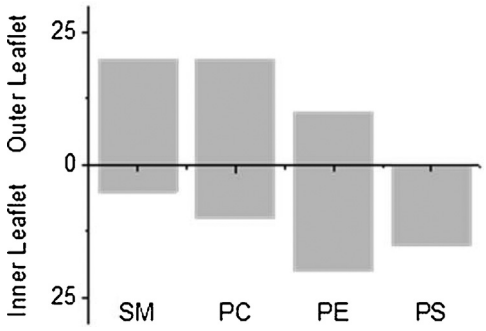

Fig. 1. The cell membrane of human red blood cells possess an asymmetric distribution of lipids (SM = sphingomyelin, PC = phosphatidylcholine, PE = phosphatidylethanolamine and PS = phosphatidylserine) between the inner leaflet (cytoplasmic) and outer leaflet (extracellular) (Verkleij et al., 1973).

leaflets, but distributed asymmetrically, with a greater concentration suggested for the inner leaflet (Gibson Wood et al., 2011). This asymmetry impacts a range of bilayer properties (Cheng et al., 2009; Chiantia and London, 2012; Devaux, 1991) and is tied to numerous biological functions, including signaling of apoptosis (Fadok et al., 1992; Martin et al., 1995; Bennett et al., 1995; Casciola-Rosen et al., 1996), thrombosis (Zwaal et al., 1977; Bevers et al., 1982), phagocytosis (Fadok et al., 1992; Tanaka and Schroit, 1983; Schroit et al., 1985; Fadok et al., 1993), and as an indicator of tumorigenic cells (Connor et al., 1989; Utsugi et al., 1991). *In vivo*, bilayer asymmetry is maintained by adenosine triphosphate (ATP)-dependent enzymes that transport lipids between the two leaflets (Seigneuret and Devaux, 1984; Bevers et al., 1999). However, generation of asymmetry in experimentally accessible model systems has, until very recently, proven elusive (Kiessling et al., 2009a; Devaux and Morris, 2004; May, 2009; Marquardt et al., 2015)—meaning that most work on membrane biophysics has made use of symmetric self-assembled bilayers.

In the context of membrane domains, the absence of asymmetry has been recognized as a significant deficiency (Devaux and Morris, 2004; May, 2009; Kiessling et al., 2009b) and leaves a number of important questions unanswered. Key among these is the role of inter-leaflet coupling (Fig. 2) in formation of lipid rafts. Current consensus is that domain formation in the membrane outer leaflet is coupled to the inner leaflet, and this interdependence is important for both the structure of the membrane and the communication of information across it (Devaux and Morris, 2004; May, 2009; Harder et al., 1998). It is known that symmetric model membranes made from lipid mixtures emulating the outer leaflet of mammalian cells exhibit nanoscopic phase behavior characteristic of lipid rafts. However, lipid mixtures emulating the inner leaflet fail to do so, remaining uniformly mixed (Wang and Silvius, 2003; Kiessling et al., 2006). This is intriguing because rafts are expected to exist in both leaflets of the bilayer (Harder et al., 1998; Zacharias et al., 2002; Nickels et al., 2015), implying a critical role of inter-leaflet coupling *in vivo*. Moreover, the mechanisms by which the outer leaflet of the bilayer could interact with the inner leaflet remain unknown.

Perhaps the most intuitive explanation for interleaflet coupling in cell membranes is the presence of membrane-spanning proteins that physically bridge the two bilayer leaflets. However, observations in protein-free model systems suggest that the coupling may be an intrinsic property of the lipids themselves (Chiantia and London, 2012). Experimental work has shown direct evidence of interleaflet coupling in the form of domain alignment between the bilayer leaflets in symmetric giant unilamellar vesicles (GUVs) and supported bilayers (Kiessling et al., 2006; Dietrich et al., 2001; Garg et al., 2007; Collins and Keller, 2008; Korlach et al., 1999). An alternative coupling mechanism has also been reported, manifested as anti-correlation in small (nm) or solid domains (Zhang et al., 2004; Almeida et al., 1992a; Khanna et al., 2006; Stevens, 2005; Perlmutter and Sachs, 2011). Owing to recent advance in generating asymmetric vesicle models, calorimetric measurements of melting transitions in these asymmetric models also supported the notion of interleaflet coupling (Cheng and London, 2011). A number of excellent reviews on inter-leaflet domain coupling have been written (Kiessling et al., 2009a; Devaux and Morris, 2004; May, 2009). The present review will focus on bringing this topic together with recent developments in three areas: generating asymmetric model bilayers, neutron scattering methods, and computer simulation techniques. Neutron scattering and computational methods are naturally compatible techniques, able to detect and analyze nanoscopic domains and lipid asymmetry. Together with recent developments in generating model asymmetric bilayers there is a tremendous opportunity to offer new insights into the mechanisms of raft formation and interleaflet coupling.

2. Experimental studies of cross-layer coupling

2.1. Preparation of asymmetric bilayers

Replicating the asymmetry of biological membranes is a critical starting point for studies of bilayer coupling. One might consider harvesting naturally occurring lipid bilayers in order to take advantage of the asymmetry native to biological membranes. In fact, Weik et al., (1998) have used neutron scattering on crystalline membrane structures known as purple membranes to demonstrate the asymmetric bilayer orientation of glycolipids with respect to membrane proteins. Unfortunately, most natural systems are non-crystalline, and moreover natural constructs typically lose asymmetry over time. It is advantageous then to produce model systems which replicate the asymmetry of biological membranes. Methods for generating asymmetric model bilayer systems have progressed in recent years, using passive mechanisms (cyclodextrin exchange, Langmuir–Blodgett), emulsification strategies (microfluidics), or active mechanisms (lipid flippase/floppase enzymes).

2.1.1. Supported lipid bilayers

Supported lipid bilayers are classic models for biological membranes. They allow membrane constituents to be studied in a controlled environment, and are compatible with many experimental probes, such as fluorescence microscopy, atomic

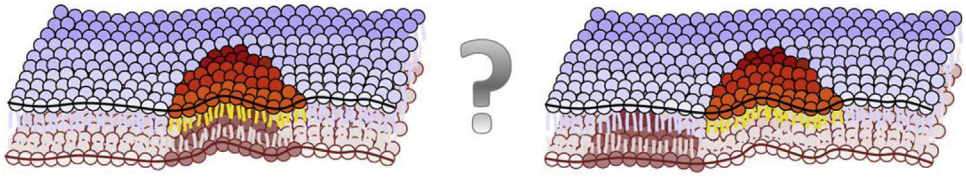

Fig. 2. Key outstanding questions in membrane biophysics center on the interplay of leaflet compositional asymmetry and lateral organization. Are lipid rafts 'in-registry'? Can leaflet coupling induce raft formation in the mammalian inner leaflet?