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Review

Capturing the nanoscale complexity of cellular membranes in supported lipid bilayers

Lance C. Kam

Department of Biomedical Engineering, Columbia University, 351 Engineering Terrace, 500 W 120th St., New York, NY 10027, USA

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ABSTRACT

The lateral mobility of cell membranes plays an important role in cell signaling, governing the rate at which embedded proteins can interact with other biomolecules. The past two decades have seen a dramatic transformation in understanding of this environment, as the mechanisms and potential implications of nanoscale structure of these systems has become accessible to theoretical and experimental investigation. In particular, emerging micro- and nano-scale fabrication techniques have made possible the direct manipulation of model membranes at the scales relevant to these biological processes. This review focuses on recent advances in nanopatterning of supported lipid bilayers, capturing the impact of membrane nanostructure on molecular diffusion and providing a powerful platform for further investigation of the role of this spatial complexity on cell signaling.

1. Introduction

Lipid membranes are powerful organizational components of living cells, delineating and separating the various components of these systems. Somewhat paradoxically, lipid membranes are largely fluid, allowing motion of membrane biomolecules along the structure. This fluidity arises from the concept that membranes are held together by hydrophobic/hydrophilic interactions between lipid molecules, an idea that was the foundation for the Fluid Mosaic model formalized by Singer and Nicolson (1972). Since that time, new experimental tools and conceptual frameworks have allowed exploration of the more subtle regions of this model, revealing short-range organization within membranes of living cells. What has emerged is a complex and not completely understood mix of interactions that give rise to a membrane nanostructure that modulates diffusion and mobility of membrane biomolecules. These effects are often invoked as modulators of cell signaling over a range of spatial scales. Development of sufficiently quantitative models of these effects would be greatly accelerated by the ability to capture the nanoscale complexity of the proposed membrane structure in controllable, reductionist systems. In this direction, substrate-supported lipid bilayers have provided elegant insights into the role of membranes as matrices for cell signaling by capturing this system in format that is compatible with contemporary, high-resolution microscopy (Chan et al., 1991; Dean et al., 2003; Grakoui et al., 1999; Groves and Dustin, 2003). Techniques for micropatterning these lipid systems hold much promise for capturing nanoscale membrane organization (Cremer et al., 1999; Groves and Boxer, 2002; Groves et al., 1997; Kam and Boxer, 2000; Kung et al., 2000a,b; Ulman et al., 1997). However, the underlying mechanisms that give rise to membrane structure in cells are dynamic, fugitive, and very different than the manipulations that are commonly done to pattern supported lipid bilayers. We review here recent progress in the design of supported lipid bilayers systems that seek to capture this nanoscale complexity.

2. Nanoscale membrane structure

The recognition that cell membranes exhibit nanoscale structure has had a profound impact on the study of cell signaling and membrane physiology. Current research focuses on the formulation of general, guiding principles that govern membrane structure and identification of how these rules act in specific signaling proteins and pathways. Within this wide field, we focus here on capturing two general types of nanoscale organization in substrate-supported lipid bilayers.

The first is the concept of microdomains, localized aggregates of membrane biomolecules (suggested by the red islands in Fig. 1A) that dynamically assemble, reorganize, and dissolve against a background of otherwise fluid-phase membrane. The archetypical model of this concept, the lipid raft, was proposed as a mechanism for trafficking of membrane proteins from synthesis to delivery on the cell surface, mediated by local, liquid-ordered regions rich in sphingolipids and cholesterol (Simons and Ikonen, 1997), interspersed throughout a liquid-disordered matrix. The concept of lipid rafts and microdomains in general as spatial modulators of

E-mail address: lk2141@columbia.edu

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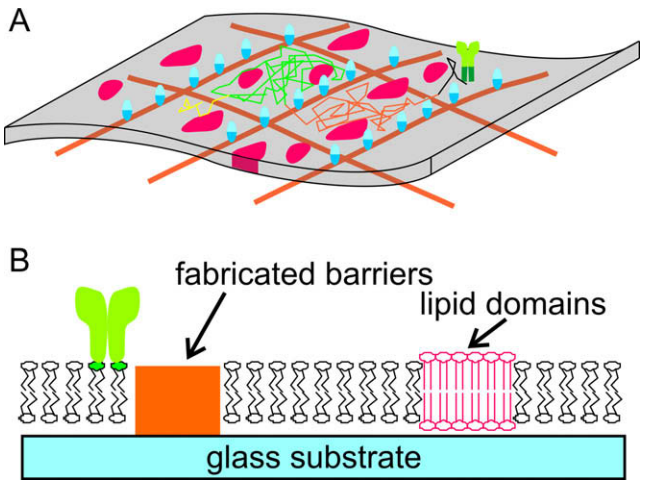

Fig. 1. Nanoscale organization of cell membranes. (A) Two mechanisms for this organization in living cells are the presence of microdomains (red forms) and cytoskeletal structures (orange grid and blue transmembrane proteins) both of which limit long-range diffusion. The random-walk trajectory of a cell signaling molecule (green) that is excluded from these domains and restricted by the cytoskeletal elements is illustrated in this panel. (B) Strategies for capturing nanoscale membrane complexity in supported lipid bilayers include the patterning of non- compatible materials (orange) onto the substrate, or the use of lipid mixtures that demix to form stable analogs of the native microdomains.

cell signaling has become a major organizational principle (Dustin, 2002; Edidin, 2003; Gomez-Mouton et al., 2004; Jury et al., 2007; Shaw, 2006; Simons and Toomre, 2000) that is applied across multiple cell types and a wide range of signaling protein pathways. In each situation, important questions include the size and duration of domain formation, domain composition, and inclusion/exclusion of the molecule of interest as well as associated signaling partners into the microdomain.

The second source of nanoscale order we will focus on is the cell cytoskeleton that underlies the cell membrane, which modulates molecular diffusion of membrane components (Fujiwara et al., 2002; Iino et al., 2001; Kusumi et al., 1993, 1999; Ritchie and Kusumi, 2004; Ritchie et al., 2003). The original observation driving this concept is that membrane proteins exhibit diffusion coefficients that are often much smaller than that predicted from the aggregate properties of the membranes. High-resolution microscopy revealed that this slower diffusion is consistent with a model in which diffusing membrane molecules interact either directly with the cytoskeleton or through intermediate proteins (Fig. 1A) tethered to the cytoskeleton; these two complementary models are termed the "skeleton fence" and "picket fence" models, respectively. In both cases, the cytoskeleton forms semi-permeable barriers that delineate corrals of the membrane, leading to a process of diffusion along the surface characterized by a relatively slow "hop diffusion" across multiple corrals (suggested by the multicolor trajectory shown in Fig. 1A).

These two complementary sources of membrane organization share several properties. First, both act to reduce diffusion along the cell membrane. Second, both involve structures that are generally believed to be nanoscale. Microdomains are composed of clusters as small as a countable number of lipids or up to several hundred nanometers in extent; the specific size and dynamics vary tremendously as a function of signaling mechanism, physical conditions, and experimental analysis method. The size of corrals estimated to be present in a variety of cells ranges over a similar range, tens to hundreds of nanometers (Kusumi et al., 2005a,b). Third, both sources of nanoscale organization are based on phenomena that are difficult to fully verify and demonstrate; the formation and stability of microdomains, as well as the nature of interactions between the cytoskeleton and membrane proteins, are transient and sensitive to the experimental techniques. In particular, the presence of semi-permeable barriers has not been observed concurrently with molecular diffusion. As such, the term "hop diffusion" will be applied to diffusion in the presence of explicit corrals; more generally, the term "hindered diffusion" will be used to expresses the concept of non-Brownian subdiffusion.

Capturing these sources of membrane nanostructure in substrate-supported lipid bilayers would provide a powerful tool for investigating the impact of these mechanisms on cell signaling. The basic structure of the supported membrane system is a single bilayer of lipids closely apposed with but not attached to an underlying substrate. This separation between the membrane and support imparts lateral fluidity to the membrane. Proteins tethered to the membrane are also laterally mobile, forming a system for studying the role of protein mobility on cell signaling. This approach has been highly successful in the context of immune cell function, and is finding application in an increasing range of cellular systems (Baksh et al., 2005; Dori et al., 2000; Groves et al., 2001; Kam and Boxer, 2001; Lenz et al., 2004; Orth et al., 2003; Pautot et al., 2005; Perez et al., 2005; Ratto and Longo, 2003; Sackmann, 1996; Torres et al., 2008). Moreover, the development of approaches to micropatterning these bilayers has set much promise for imposing nanoscale order on these systems. A widely-used method of forming lipid bilayers is through fusion of lipid vesicles (essentially lipid bilayers patches rolled on themselves) onto a support. This fusion process occurs on only a few, select materials, including silicon oxide surfaces such as glass and quartz but also mica and, perhaps surprisingly, oxidized polydimethyl siloxane (Brian and McConnell, 1984; Hafeman et al., 1981; Hovis and Boxer, 2001). Micropatterning of metals, plastics, or even proteins onto these oxide surfaces prior to vesicle exposure serves to restrict, limit, and pattern lipid bilayers formation (Fig. 1B) (Boxer, 2000; Groves and Boxer, 2002; Groves et al., 1997; Kam and Boxer, 2000; Kung et al., 2000b; van Oudenaarden and Boxer, 1999), and extensions of these methods into the nanoscale has allowed capture of both microdomains and cytoskeletal barriers (Furukawa et al., 2007; Lenhert et al., 2007; Nabika et al., 2005, 2009; Shi et al., 2008; Tsai et al., 2008; Werner et al., 2009). While these methods offer a high degree of control over the barrier and substrate layout, they are expensive to use over large areas. An alternative approach, inspired by the microdomain concept, is to form bilayers from binary and ternary mixture of lipids, each with physical/chemical properties that lead to demixing or phase separation in the final structure (Johnston, 2007; Ratto and Longo, 2002,2003). The resulting structures tend to be less well-defined than fabricated systems, but are highly effective in capturing the concept of microdomains on supported bilayers. In particular, these surfaces have had recent success as a platform for understanding the targeting and segregation of specific classes of biomolecules into the gel phase domains, which are relatively immobile (Alves et al., 2005; Devanathan et al., 2006; Giocondi et al., 2007; Ira and Johnston, 2008; Saslowsky et al., 2002).

Notably, this review will focus on diffusion of molecules that do not preferentially associate with raft or microdomain lipids. The motion of raft-associated protein is expected to be tied strongly to those lipid structures, a very rich topic that is beyond the scope of this review.

3. Observation and quantification of diffusion in complex environments

To understand the impact of these local membrane and cytoskeleton structures on molecular diffusion, it is useful to review