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Review

# Recent progress on lipid lateral heterogeneity in plasma membranes: From rafts to submicrometric domains

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ABSTRACT

The concept of transient nanometric domains known as lipid rafts has brought interest to reassess the validity of the Singer–Nicolson model of a fluid bilayer for cell membranes. However, this new view is still insufficient to explain the cellular control of surface lipid diversity or membrane deformability. During the past decades, the hypothesis that some lipids form large (submicrometric/mesoscale *vs* nanometric rafts) and stable (>min *vs* s) membrane domains has emerged, largely based on indirect methods. Morphological evidence for stable submicrometric lipid domains, well-accepted for artificial and highly specialized biological membranes, was further reported for a variety of living cells from prokaryot es to yeast and mammalian cells. However, results remained questioned based on limitations of available fluorescent tools, use of poor lipid fixatives, and imaging artifacts due to non-resolved membrane projections. In this review, we will discuss recent evidence generated using powerful and innovative approaches such as lipid-specific toxin fragments that support the existence of submicrometric domains. We will integrate documented mechanisms involved in the formation and maintenance of these domains, and provide a perspective on their relevance on membrane deformability and regulation of membrane protein distribution.



**Contents**



*Abbreviations:* AFM, atomic force microscopy; BODIPY, 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene; BSA, bovine serum albumin; Cer, ceramide; CTxB, cholera toxin B subunit; DHE, dehydroergosterol; DiI, dialkylindocarbocyanine; DPH, diphenylhexatriene; FRAP, fluorescence recovery after photobleaching; GPMV, giant plasma membrane vesicle; GPI, glycosylphosphatidylinositol; GSL, glycosphingolipid; GUV, giant unilamellar vesicle; LacCer, lactosylceramide; Ld, liquid-disordered; Lo, liquid-ordered; mAb, monoclonal antibody; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PH, pleckstrin homology; PI, phosphatidylinositol; $PIP_2$, phosphatidylinositol-4,5-bisphosphate; PM, plasma membrane; PS, phosphatidylserine; RBC, red blood cell; SIMS, secondary ion mass spectrometry; SL, sphingolipid; SM, sphingomyelin; SMase, sphingomyelinase; So, solid-ordered; TIRF, total internal reflection fluorescence; *Tm*, melting temperature.

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## 1. Introduction: key concepts and significance of lipid lateral heterogeneity

Even though the protein/lipid ratio of purified stripped membranes is close to the unity on a mass basis, their large difference in molecular weight makes ~50 lipid molecules per membrane protein a reasonable general estimate, underlining that membrane lipids actually cover most of the plasma membrane (PM) [1,2]. In addition, combinatorial variations in head-groups and aliphatic tails allow eukaryotic cells to synthesize thousands of different membrane lipids [3] by using ~5% of their genes (for a review, see [4]). It seems reasonable that due to the intrinsic complexity of their lipids, cell membranes are arranged in far more intricate structures than simple homogenous fluid bilayers. Membrane heterogeneity is illustrated by unequal lipid distribution among (i) different PMs, (ii) distinct intracellular compartments, (iii) inner *vs* outer membrane leaflets, and (iv) the same leaflet. Whereas the three first levels of membrane heterogeneity are well accepted by the scientific community, the fourth level is still disputed. Limited availability of fluorescent tools, use of poor lipid fixatives, imaging of artifacts due to non-resolved membrane projections, and description of unclassified membrane domains have intensified the debate in this rapidly growing area of research.

In this Introduction section, we will provide a historical review of the different types of domains evidenced at the PM of eukaryotes. Current views on structural and dynamical aspects of biological membranes have been strongly influenced by the homogenous fluid mosaic model proposed by Singer and Nicolson in 1972 [5]. In this model, proteins are dispersed and individually embedded in a more or less randomly organized fluid lipid bilayer. In 1987, Simons and Van Meer discovered that glycosphingolipids (GSLs) cluster in the Golgi apparatus before being sorted to the apical surface of polarized epithelial cells [6]. In 1997, Simons and coll. proposed the lipid raft theory [7], where GSLs form detergent-resistant membranes (DRMs) enriched in cholesterol and glycosylphosphatidylinositol (GPI)-anchored proteins in cold non-ionic detergents such as Triton. Such theory was however questioned for several reasons. Among others, it has been shown that Triton can promote domain formation and may even create domains in a homogenous fluid lipid mixture, arguing against an identification of DRMs with functional rafts [8]. In 2006, lipid rafts were redefined as: "small (20–100 nm), heterogeneous, highly dynamic, sterol- and sphingolipid (SL)-enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions" [9]. In addition to rafts, other nanoscale domains, *i.e.* <100 nm in diameter (also mis-called microdomains), have been described at the PM of eukaryotes: caveolae [10] and tetraspanin-rich domains [11]. Caveolae are defined as 60–80 nm invaginations of the PM and are especially abundant in endothelial cells and adipocytes [12]. Tetraspanins are structural proteins bearing four transmembrane domains, which control the formation of membrane tubules. They can oligomerize and recruit various proteins to establish functional domains [13]. There are several reasons to consider lipid rafts, caveolae and tetraspanin-enriched domains as distinct types of domains (reviewed in [11,14]). However, they share similarities such as small size, instability and governance by the liquid-ordered (Lo)/liquid-disordered (Ld) phase partitioning described in purified lipid systems (Section 2.1).

Besides nanometric lipid domains, morphological evidence for stable (min *vs* s) submicrometric (*i.e.* >200 nm in diameter *vs* 20–100 nm) lipid domains was first reported in artificial [15–17] and highly specialized biological membranes, such as lung surfactant and skin stratum corneum [16,18]. Such submicrometric domains, which are sometimes referred to as platforms, were first inferred in cells by dynamic studies [19–21]. However, morphological evidence was only occasionally reported and most of the time upon fixation [22–25]. In the past decade, owed to the development of new probes and new imaging methods, several groups have presented evidence for submicrometric domains in a variety of living cells from prokaryotes to yeast and mammalian cells [26–32]. Other examples include the large ceramide-containing domains, formed upon degradation of sphingomyelin (SM) by sphingomyelinase (SMase) into ceramide (Cer) in response to stress [33–35].

However, despite the above morphological arguments for lipid rafts and submicrometric domains at PMs, their real existence is still debated. This can be explained by several reasons. First, lipid domains have often been reported under non-physiological conditions. For example, they have been inferred on unfixed ghosts by high-resolution atomic force microscopy (AFM) upon cholesterol extraction by methyl-β-cyclodextrin [36]. Second, lipid or protein clustering into domains can be controlled by other mechanisms than cohesive interaction with Lo domains, thus not in line with the lipid phase behavior/raft hypothesis (see also Section 5). As an example, Kraft and coll. have recently found at the PM of fibroblasts submicrometric hemagglutinin clusters that are not