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Biochimica et Biophysica Acta 1746 (2005) 193 – 202

BIOCHIMICA ET BIOPHYSICA ACTA

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Review

Partitioning of membrane molecules between raft and non-raft domains: Insights from model-membrane studies

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Received 2 June 2005; received in revised form 2 September 2005; accepted 7 September 2005
Available online 23 September 2005

Abstract

The special physical and functional properties ascribed to lipid rafts in biological membranes reflect their distinctive organization and composition, properties that are hypothesized to rest in part on the differential partitioning of various membrane components between liquid-ordered and liquid-disordered lipid environments. This review describes the principles and findings of recently developed methods to monitor the partitioning of membrane proteins and lipids between liquid-ordered and liquid-disordered domains in model membranes, and how these approaches can aid in elucidating the properties of rafts in biological membranes.

Keywords: Membrane domain; Cholesterol; Cellular membrane; Lipid raft; Fluorescence spectroscopy; Fluorescence microscopy; Electron spin resonance; Sphingolipid

1. Introduction

A central element in our current picture of 'lipid rafts' and related membrane domains is the concept that their lipid components exist in a liquid-ordered (l_o) state distinct from that found in the coexisting liquid-disordered (l_d) regions of the bilayer [1–7]. While this concept may be only approximately correct (it remains to be established, for example, precisely what factors could induce the lipids in the inner leaflet of a raft to exist in a liquid-ordered state), it has proven useful to rationalize a variety of experimental observations concerning the properties of rafts, including evidence that they exhibit protein and lipid compositions quite different from those of the membrane as a whole.

When assessing the possibility that a given membrane component is associated with or excluded from raft domains, two questions immediately arise (beyond that of the functional significance of such localization): First, how, and how clearly, can the raft association or exclusion of the species of interest be established experimentally? Second, does the enrichment or depletion of a particular species in rafts rest on its intrinsic affinity (or lack of affinity) for a liquid-ordered lipid environment or on other factors, such as specific interactions with membrane proteins?

Studies using lipid and lipid–protein model membranes that exhibit segregation of l_o and l_d domains can usefully contribute toward answering both of the questions just noted. In regard to the first, model-system studies can aid in developing novel approaches to assess the association of particular membrane components with rafts in biological membranes. In regard to the second, studies using model membrane systems can allow the investigator to determine directly, in a well-controlled experimental context, the intrinsic affinity (or lack of it) that a given membrane component exhibits for liquid-ordered lipid domains. Achieving these objectives of course requires methods suitable to monitor reliably and quantitatively the partitioning of membrane-associated molecules between liquid-ordered and liquid-disordered lipid environments. This review will describe recent progress in the development and exploitation of such methods in model membranes, some of the insights that these methods have yielded, and current prospects to extend them to cellular membranes.

Abbreviations: AFM, atomic force microscopy; CTB, cholera toxin B-subunit; DM-, dimyristoyl; DO-, dioleoyl; GPI-, glycosylphosphatidylinositol-; l_d, liquid-disordered; l_o, liquid-ordered; NBD-, 7-nitrobenz-2-oxa-1,3-diazol-4-yl-; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PLAP, placental alkaline phosphatase; POPC, 1-palmitoyl-2-oleoyl phosphatidylcholine

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doi:10.1016/j.bbamcr.2005.09.003

2. Basic thermodynamic principles

The molar partition coefficient $K_p(i)$ describing the distribution of a membrane-associated component i between two coexisting phases α and β at equilibrium is defined as

$$K_p(i) = x_i(\alpha)/x_i(\beta) \quad (1)$$

where $x_i(\alpha)$ and $x_i(\beta)$ are the mol fractions of species i in the two phases. It is often convenient (though not always feasible) to determine the value of $K_p(i)$ under conditions where $x_i(\alpha)$ and $x_i(\beta)$ are very small, so that the composition and properties of each phase are not significantly dependent on the distribution of i.

Many experimental methods, including most forms of spectroscopy, do not measure directly the mol fractions of component i in the two coexisting phases but rather report the relative fractions of the total pool of i that are present in each phase, $f_i(\alpha)$ and $f_i(\beta)$, where $(f_i(\alpha)+f_i(\beta)=1)$. In order to determine the molar partition coefficient from such data, it is necessary also to know the relative proportions of the two phases, i.e., the relative molar proportions of the total pool of bilayer constituents (other than water) that exist in the two phases, $f(\alpha)$ and $f(\beta)$:

$$K_p(i) = \frac{(f_i(\alpha)/f(\alpha))}{(f_i(\beta)/f(\beta))} \quad (2)$$

In such cases, quantitative assessment of the partitioning of component i between coexisting bilayer domains requires separate determination of both the relative amounts of i that are present in the two phases and the relative proportions of the two phases themselves.

In binary mixtures of lipid components A and B (in the presence of excess water), the proportions of two coexisting phases at a given temperature can be determined directly from the relevant phase boundaries in the temperature–composition phase diagram, as illustrated in Fig. 1A. For such pseudobinary systems, at a given temperature, the tie line in a region of two-phase coexistence runs horizontal to the composition axis, and the relative proportions of the two phases for any lipid composition that falls with this region are given by simple application of the lever rule:

$$\frac{f(\alpha)}{f(\beta)} = \frac{(x_B(\beta) - x_B)}{(x_B - x_B(\alpha))} \quad (3)$$

where x_i is the mol fraction of component B in the total lipid fraction and the compositions $x_B(\alpha)$ and $x_B(\beta)$ represent the compositions at the ends of the tie line (which define the compositions of phases α and β, respectively, for any composition within the two-phase region at the temperature of interest). These relationships are shown schematically in Fig. 1A.

While coexistence of liquid-ordered and liquid-disordered domains can be demonstrated in binary mixtures of cholesterol and phospho- or sphingolipids [8–14], model systems for l_o/l_d domain segregation frequently combine cholesterol with two other lipid species, one a long-chain saturated phospho- or sphingolipid and the second an unsaturated phospholipid. The determination and interpretation of phase diagrams for such ternary lipid systems are discussed in the articles by Feigenson [15] and Keller [16] in this issue. Most importantly for present purposes, in the phase diagrams for ternary lipid mixtures, the compositions and proportions of two coexisting phases cannot be determined simply from the boundaries of the two-phase region but also require separate experimental determination of the orientations of the tie lines within this region (Fig. 1B). Only very recently have efforts been reported to determine information of this latter type for any lipid/lipid/sterol ternary system [17–21]. Once tie lines have been accurately deter-

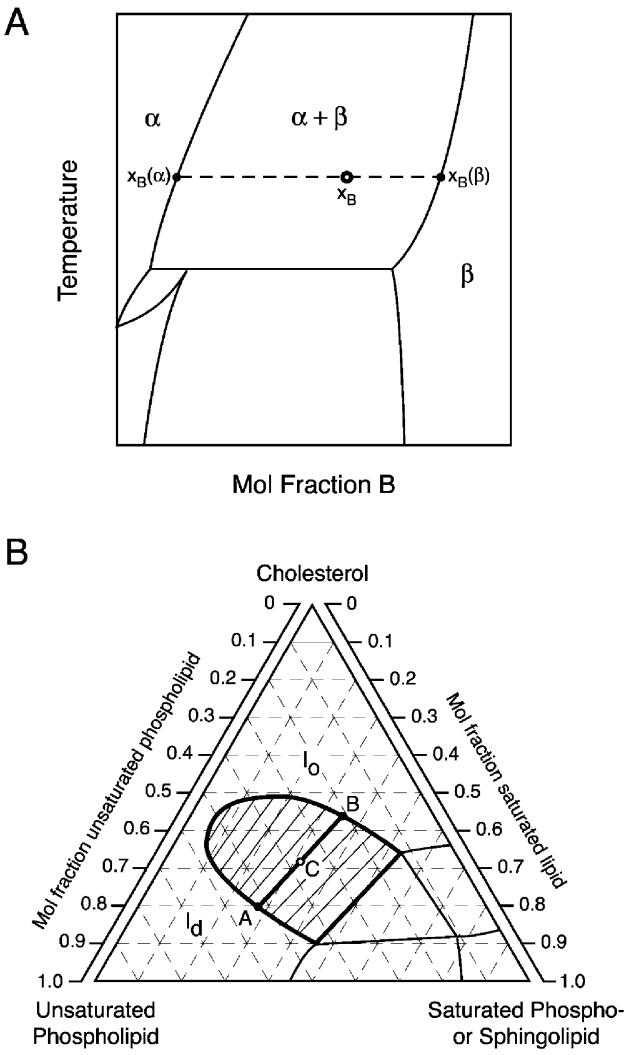

Fig. 1. Schematic illustrations of tie lines in two-phase regions in (A) binary and (B) ternary (saturated lipid/unsaturated lipid/cholesterol) lipid mixtures in excess water. For the lipid composition x_B shown in the binary (temperature/composition) phase diagram in panel A, the relative amounts of phases a and b are given by the expressions $f(\alpha)=(x_B(\beta)-x_B)/(x_B(\beta)-x_B(\alpha))$ and $f(\beta)=(x_B-x_B(\alpha))/(x_B(\beta)-x_B(\alpha))$. For the ternary phase diagram shown in panel B, the relative amounts of the l_o and l_d phases for a lipid mixture with composition C are given by the expressions $f(l_o)$=AC/AB and $f(l_d)$=BC/AB, where AB, AC and BC are the lengths of the corresponding line segments shown in the phase diagram, and the compositions of the coexisting l_d and l_o phases are those corresponding to points A and B, the ends of the relevant tie line.

mined for a ternary system in a region of two-phase coexistence, one can determine the compositions and the relative proportions of the coexisting phases for any composition that lies along a given tie line, using the lever rule in a manner very similar to that described above for binary systems (see Fig. 1B).

As discussed later, liquid-ordered domains in model systems may in some cases be of nanoscopic dimensions, as appears to be the case for rafts in biological membranes. For a given system of this type, it may or may not be strictly justified to describe the ensemble of coexisting domains in terms of a classical phase separation (see [15,16] for further discussion). We will nonetheless use the term 'partitioning' to describe the distribution of a given membrane component between such coexisting domains, even though in such cases the assumptions of the thermodynamic analysis described above may not always be rigorously applicable.

3. Direct measurements of amphiphile partitioning between liquid-ordered and liquid-disordered lipid vesicles

An appealingly straightforward approach to determine the relative affinities of a membrane-binding molecule for a liquid-ordered vs. a liquid-disordered bilayer environment is to measure its distribution between two populations of lipid vesicles which exist entirely in the l_o and the l_d phases, respectively. This method is applicable only to amphiphiles that redistribute between different vesicles much more rapidly than do any of the other vesicle components, so that the compositions (and physical states) of the vesicles remain distinct and well defined throughout the experiment. We have used this approach [22] to demonstrate that fluorescent phospho- and sphingolipid probes bearing short NBD- or Bodipy-labeled acyl chains partition poorly into l_o-phase compared to l_d-phase environments. The utility of this method can be extended to membrane components that (like most constituents of biological membranes) normally transfer very slowly between bilayers if a catalyst is available that selectively enhances the rate of interbilayer diffusion of the component of interest. Cyclodextrins have been used for this purpose to investigate the equilibrium partitioning of cholesterol between different lipid environments [23], allowing direct confirmation of previous conclusions that the sterol associates preferentially with sphingolipids and saturated phospholipids, and very poorly with highly unsaturated phospholipids, in l_o- or l_d-phase bilayer environments [24–28]. Transfer proteins have been identified that selectively enhance the interbilayer transfer of ceramides [29,30] or glycolipids [31] and could be used in a similar manner to determine the equilibrium partitioning of such components between lipid vesicles that exist in the l_o and l_d phases, respectively.

A related approach to compare the relative affinities of a given amphiphile for a liquid-ordered vs. a liquid-disordered lipid environment is to compare the affinities of partitioning of the species of interest between the aqueous phase, or a water-soluble carrier, and l_o- vs. l_d-phase lipid vesicles. Niu and Litman [32] used cyclodextrin in this way to measure the relative affinities of cholesterol for lipid vesicles of different compositions, supporting and extending the conclusions noted above based on direct measurements of cyclodextrin-accelerated equilibration of cholesterol between different vesicle populations. Abreu et al. [33], using serum albumin as a soluble reservoir for NBD-labeled dimyristoyl phosphatidylethanolamine, found that the fluorescent lipid partitioned with only 4- to 6-fold lower affinity into liquid-ordered sphingomyelin/cholesterol vesicles than into liquid-disordered POPC vesicles. This result agrees with the finding discussed later that saturated diacyl phospholipids with shorter hydrocarbon chains (e.g., myristoyl) partition to a detectable, albeit modest, extent into liquid-ordered domains.

Pokorny et al. [34] reported that a cationic coumarinyl-labeled probe with a C_{14}-saturated alkyl chain partitioned into l_o-phase vesicles (DMPC/cholesterol, 65:35 mol/mol, 30 °C) with an affinity only about 7-fold lower affinity than it partitioned into l_d-phase vesicles (DMPC) at the same temperature. By contrast, Abreu et al. [35] found that a Rhodamine Green-labeled amphiphile with a C_{14}-saturated chain showed a 2,000- to 10,000-fold lower affinity for liquid-ordered DMPC/cholesterol or sphingomyelin/cholesterol vesicles than for liquid-disordered DMPC or POPC vesicles under the same conditions. These results indicate that the partitioning of an amphiphilic molecule between l_o and l_d phases can be strongly affected by the structure of the polar, as well as the apolar, portions of the molecule. The authors of the above studies suggested that differential interactions between the dipoles of the amphiphilic species and of the host lipid matrix may strongly influence the partitioning of molecules between l_o and l_d domains, a possibility that merits systematic investigation.

To date, studies like those described above have measured the equilibrium distributions of amphiphiles between populations of vesicles whose compositions do not closely replicate those of l_d and l_o domains that could coexist within a single bilayer at equilibrium. As more detailed information becomes available to define the complete phase diagrams for different cholesterol-containing ternary model systems, it will become possible to apply the above approach to monitor the distribution of exchangeable amphiphiles between vesicles whose compositions accurately match those of coexisting l_d and l_o domains in a given ternary lipid mixture, and hence to estimate with greater precision how such amphiphiles will distribute between such domains within the plane of an individual phase-separated bilayer.

4. Partitioning of membrane components between laterally segregated l_o and l_d domains in lipid bilayers: microscopic measurements

Most membrane lipids and integral membrane proteins transfer between different bilayers, or between bilayers and the aqueous phase, if at all far too slowly to compare their affinities for different bilayer environments using the methods discussed in the previous section. Fortunately, an increasing number of methods is becoming available to monitor the partitioning of

membrane molecules between coexisting lipid phases within the bilayer plane. Direct microscopic imaging of the distribution of a bilayer-incorporated species between coexisting lipid domains is feasible when the domains are readily resolved and the concentration of the species of interest within each type of domain can be determined without perturbing its lateral distribution. For reasons discussed below, bilayer-incorporated proteins are particularly amenable to this approach.

As discussed elsewhere in this issue [15,16], various lipid/lipid/cholesterol ternary mixtures form coexisting l_o and l_d bilayer domains that are large enough to be imaged by fluorescence microscopy, at temperatures and cholesterol contents that approach the physiological range [36–39]. When low concentrations of fluorescent-labeled lipids or proteins are incorporated into such systems, fluorescence intensity measurements can be used to examine the distribution of the fluorescent species between the coexisting l_o and l_d domains. In principle, such measurements can provide quantitative estimates of the partition coefficient $K_p(l_o/l_d)$ for the labeled species. However, in order to be quantitative such determinations require accurate correction of the raw intensity data for a number of factors. Some of the requisite corrections (e.g., for background fluorescence and photobleaching) are obvious, though not necessarily trivial in practice. As well, however, accurate quantitation of the relative concentrations of a given fluorescent species in coexisting lipid domains requires either that the normalized fluorescence yield per molecule, and the average area per 'host' lipid molecule, are identical in both coexisting phases or that any differences in these parameters between the two phases can be accurately determined and corrected for. Additional complications arise (e.g., from possible orientation-dependent photoselection effects) in applying this approach to curved structures such as freestanding (giant) lipid vesicles. For these reasons, at present fluorescence microscopy provides a semiquantitative tool to determine the distributions of membrane components between l_d and l_o domains. However, with further refinements, this approach should be able to provide a truly quantitative picture of the partitioning of fluorescent molecules between coexisting lipid domains.

Using fluorescence microscopy Dietrich et al. [36,40] showed that ganglioside GM1, when complexed to pentavalent (fluorescent-labeled) cholera toxin B-subunit (CTB), partitions with a strong preference into liquid-ordered domains that coexist with more disordered fluid domains in supported DOPC/sphingomyelin/cholesterol mono- and bilayers. In the same system, a fluorescent-labeled form of the glycosylphosphatidylinositol (GPI)-anchored-protein Thy-1 also associated significantly with the more ordered phase, albeit with a partition coefficient ($K_p(l_o/l_d)$) estimated as ca. 0.7, suggesting a slight net preference for liquid-disordered domains. Kahya et al. [41] have recently reported similar findings for the GPI-anchored placental alkaline phosphatase (PLAP), which distributes between l_o and l_d phases in DOPC/sphingomyelin/cholesterol giant unilamellar vesicles with an estimated partition coefficient $K_p(l_o/l_d)$ of roughly 0.25. In the same experimental system the transmembrane SNARE proteins syntaxin 1 and synaptobrevin 2 and the polytopic membrane protein bacteriorhodopsin showed negligible partitioning into liquid-ordered domains [41,42]. A peptide corresponding to the transmembrane domain of the T-lymphocyte adaptor protein LAT likewise was found to partition strongly in favor of liquid-disordered domains in DOPC/sphingomyelin/cholesterol giant unilamellar vesicles [43]. By contrast, NAP-22, a myristoylated neuronal protein that binds selectively to cholesterol- or phosphatidylethanolamine-containing bilayers, exhibited a substantial preference for l_o-like over l_d-like domains in supported DOPC/sphingomyelin/cholesterol monolayers [44].[1]

Fluorescence-microscopic studies of the distributions of lipids and lipid-anchored proteins between l_o and l_d domains in lipid model membranes have provided two further findings of possible relevance to the observed behavior of GPI-anchored proteins and other raft components in cellular membranes. First, Kahya et al. [41] found that the affinity of partitioning of PLAP into l_o-domains in DOPC/sphingomyelin/cholesterol vesicles increased by roughly 2-fold upon antibody-mediated crosslinking, echoing an earlier report by Dietrich et al. [40] that antibody-mediated crosslinking of a saturated fluoresceinyl-phosphatidylethanolamine enhanced by roughly 4-fold its partitioning into l_o domains in supported monolayers of the same composition. These findings are consistent with predictions that oligomerization of proteins that as monomers exhibit modest affinities for rafts can significantly enhance their raft partitioning [7][2]. CTB-mediated oligomerization of ganglioside GM1 has likewise been reported to induce its partitioning into liquid-ordered domains in giant unilamellar DOPC/sphingomyelin/cholesterol vesicles [45]. However, a headgroup-labeled GM1 was found to partition preferentially into l_o-domains in the absence of CTB in bilayer lipid membranes of the same composition spread from squalene [37]. It is possible that this apparent discrepancy arises from an effect of headgroup labeling on GM1 behavior or the reported ability of CTB to alter the phase behavior of cholesterol-containing ternary lipid mixtures containing even low mol fractions of GM1 [43].

[1] As indicated, the discussion in this section encompasses results obtained using supported monolayers, supported bilayers and freestanding bilayers (lipid vesicles). It is quite possible that the interdomain partitioning of a given protein could vary at least quantitatively, if not qualitatively, among these different types of model systems.

[2] In the simplest analysis, crosslinking-induced multimerization of a membrane-bound protein or lipid that as a monomer exhibits a $K_p(l_o/l_d)$ value of α would shift the effective value of $K_p(l_o/l_d)$ to α^N, where N is the number of monomers of the membrane-anchored species in the crosslinked aggregate. Molecules that as monomers show a net preference for l_o-domains ($\alpha > 1$) are thus predicted to exhibit a still greater preference for these domains when crosslinked, while molecules that as monomers show a net preference for l_d-domains ($\alpha < 1$) should show still lower abilities to partition into l_o-domains upon crosslinking. These simple predictions run contrary to the reports cited in the text that saturated fluorescent lipids and PLAP give estimated $K_p(l_o/l_d)$ values less than unity which nonetheless *increase* upon antibody-mediated crosslinking. It is not clear whether this apparent conundrum is a real one or whether it simply reflects the likelihood that current fluorescence-microscopic analyses provide only semiquantitative estimates of the absolute value of the partition coefficient.

However, if (as is likely) monomeric GM1 molecules distribute between l_d and l_o domains in such mixtures, it is also conceivable that cholera toxin may bind with markedly faster kinetics to GM1 molecules present in l_d vs. l_o domains. In this case, cholera toxin could be initially recruited to l_d domains, consistent with the findings reported in [45], even if a majority of monomeric GM1 molecules is actually present in l_o domains prior to, as well as after, toxin addition.

A second intriguing behavior of GPI-proteins in model membranes was reported by Dietrich et al. [40], who found that the presence of 1 mol% ganglioside GM1 significantly reduced the partitioning of Thy-1 into l_o-like domains in supported DOPC/sphingoymyelin/cholesterol monolayers (decreasing the estimated value of $K_p(l_o/l_d)$ from ca. 0.7 to 0.4). This result agrees with the finding that addition of exogenous gangliosides to mammalian cells reduced the association of GPI-proteins with lipid rafts [46]. The physical basis for this effect has not been determined, but the availability of a relatively simple model system in which the effect can be replicated should facilitate its elucidation.

The findings of Dietrich et al. [40] and of Kahya et al. [41] that GPI-anchored Thy-1 and PLAP are partially but not predominantly localized to l_o domains in l_o/l_d mixed-phase bilayers contrast with earlier findings that GPI-anchored proteins are largely associated with the detergent-insoluble 'raft' fraction obtained when mammalian cells or model membranes exhibiting l_o/l_d phase coexistence are fractionated using cold Triton X-100, a classical biochemical approach to isolate raft-associated membrane components [47,48]. This discrepancy may arise in part from current limitations in determining quantitatively the distributions of fluorescent molecules between l_o and l_d domains using fluorescence microscopy, as noted above. It may also however illustrate the limitations of detergent fractionation-based assays at least for quantitative determination of the affinities of different membrane components for liquid-ordered domains (for further discussion, see [49–51]).

Atomic force microscopy (AFM) offers a potentially attractive alternative to fluorescence microscopy to examine the distribution of specific molecular species between coexisting phases in supported mono- or bilayers, as it can image domains with dimensions below the optical resolution limit so long as these domains exhibit adequate contrast in height [52]. Application of AFM to monitor molecular distributions in bilayers requires additionally that the species to be imaged exhibits detectable height contrast with the surrounding lipid matrix. This requirement can be met both by bilayer-associated proteins and by complex glycolipids with sufficiently large, protruding headgroups. Near-field scanning optical microscopy (NSOM) has also been proposed as a potential complementary tool to explore the distributions of fluorescent-labeled bilayer components between coexisting domains that are too small to be distinguished by conventional fluorescence microscopy. This technique may be particularly powerful when combined with AFM measurements [53].

Yuan et al. [54] used AFM to image the distributions of ganglioside GM1 between l_o and l_d domains in supported DOPC/sphingomyelin/cholesterol bilayers, in the absence or presence of CTB. GM1 was found to partition strongly in favor of liquid-ordered domains in this system, both in the free state and when bound to CTB. Interestingly, under either condition, the ganglioside was not homogeneously distributed but rather formed clusters within the liquid-ordered domains. AFM has also been used to show that the VacA toxin of *H. pylori* is enriched in l_o domains in DOPC/sphingomyelin/cholesterol supported bilayers [55]. Interestingly, inclusion in the bilayers of unsaturated phosphatidylserine, to which the VacA toxin binds but which should partition poorly into l_o-domains, shifted the localization of the bilayer-associated toxin from l_o to l_d domains. The SNARE protein syntaxin 1a was found by AFM to be entirely excluded from l_o-domains in supported DOPC/sphingomyelin/cholesterol bilayers [56], consistent with results obtained for this protein using giant unilamellar vesicles of the same composition [42].

Two AFM studies [57,58] found that placental alkaline phosphatase (PLAP) partitioned strongly in favor of liquid-ordered domains in supported DOPC/sphingomyelin/cholesterol bilayers. This result contrasts with the finding of Khaya et al. [41] that PLAP partitioned with only modest affinity into l_o domains in freestanding giant unilamellar vesicles with the same lipid composition. As noted by the authors of the latter study, this discrepancy suggests that the presence of the solid substrate used to prepare samples for AFM may significantly alter the physical properties and phase equilibria of coexisting l_o and l_d domains in lipid bilayers, as Tokumasu et al. [59] have demonstrated directly for the dilauroyl PC/dipalmitoyl PC/cholesterol ternary lipid system. The use of alternative supports in AFM studies (e.g., a second bilayer interposed between the substrate and the bilayer under examination [60]) may allow such effects to be minimized.

A potential technical concern in studies of the partitioning of transmembrane proteins between l_o and l_d domains in lipid model membranes is that such membranes typically have identical lipid compositions in both bilayer leaflets, while plasma and at least some other cellular membranes exhibit markedly different lipid compositions in their cytoplasmic and extracytoplasmic leaflets. It is possible that some transmembrane proteins might adopt quite different distributions between coexisting domains in asymmetric bilayers, with compositions resembling those of the leaflets of the proteins' native membrane, than they adopt in symmetric model membranes whose composition models that of the outer leaflet of the plasma membrane. Supported bilayers (spread either on a surface or across an aperture as 'black' lipid membranes) can be more readily prepared with asymmetric lipid compositions than can freestanding lipid vesicles and may offer a means to address this important issue.

5. Spectroscopic measurements of molecular partitioning between liquid-ordered and liquid-disordered domains

Microscopic methods like those discussed above cannot always be used to monitor the distributions of membrane components between liquid-ordered and liquid-disordered

bilayer domains. Many membrane lipids, for example, have headgroups too small to resolve from the 'host' bilayer matrix by atomic force microscopy and cannot be labeled with fluorescent groups suitable for microscopy without substantially altering their physical properties. As well, recent NMR and fluorescence studies have shown that cholesterol-containing bilayers can exhibit 'nanoscopic' domain segregation, with coexisting domains too small to resolve by fluorescence microscopy (and with thicknesses too similar to be well resolved by AFM), over a wide range of compositions and temperatures [19,61–65]. Membrane components and systems such as these can often be better characterized by spectroscopic than by microscopic methods, particularly as more complete information becomes available to describe the phase behavior of pertinent model systems.

Feigenson and his colleagues [66–69] first described over 20 years ago a fluorescence-based method to determine the partition coefficient of a fluorescent species between coexisting lipid phases in binary and, with appropriate modifications, more complex lipid mixtures. The principle of this approach is illustrated in Fig. 2A. A series of lipid samples is prepared in which the relative proportions of the two major lipid components are varied along a tie line spanning a region of two-phase coexistence in the system's phase diagram. Each sample contains a trace amount of a fluorescent-labeled molecule whose normalized (per-molecule) fluorescence intensity F_N is measured and plotted as a function of sample composition as shown in Fig. 2B. The resulting 'quenching curve' can be fit to a relatively simple theoretical equation to yield the partition coefficient $K_p(\alpha/\beta)$ describing the relative

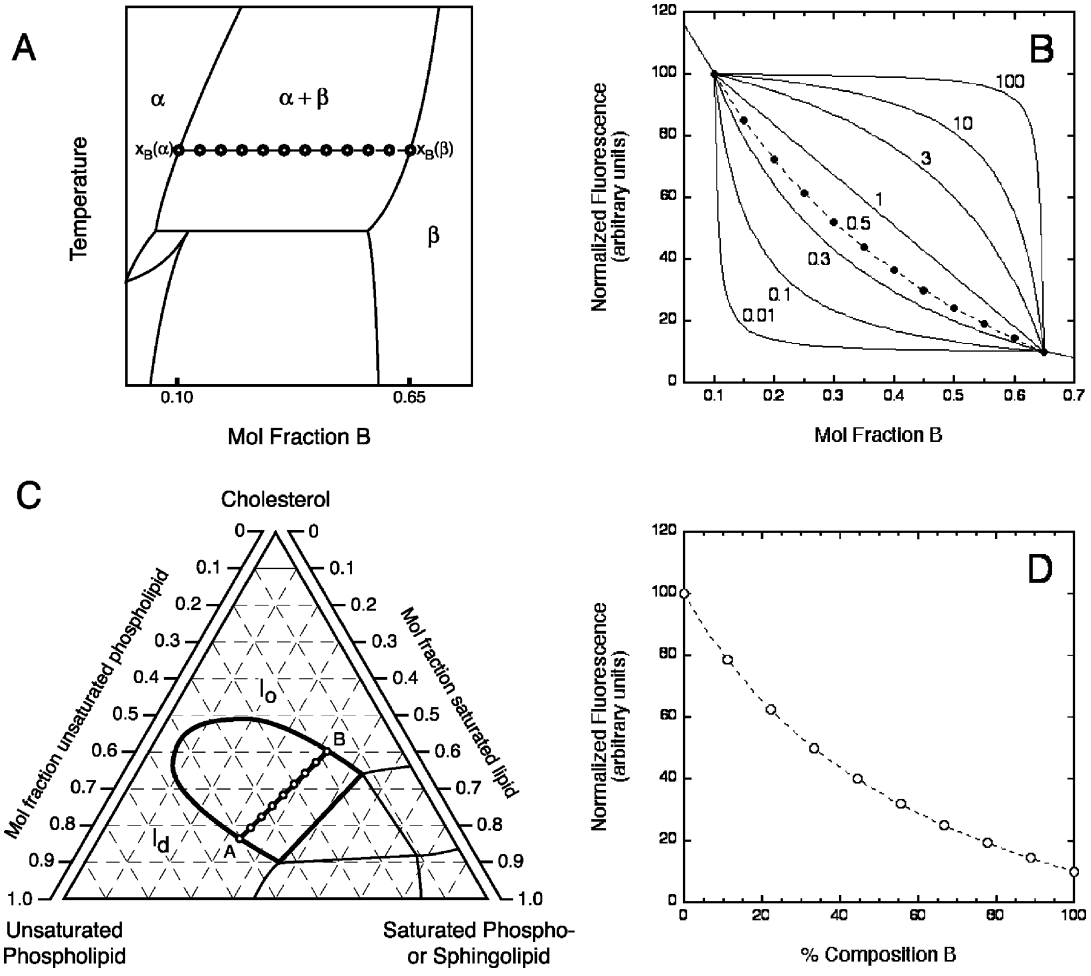

Fig. 2. Illustration of the determination of the partition coefficient of a fluorescent species between two coexisting lipid phases in (A, B) a binary and (C, D) a ternary (lipid/lipid/sterol) lipid mixture. (A) A series of lipid mixtures is prepared with compositions (open circles) spanning the full length of the tie line for the region of two-phase coexistence at the temperature of interest. (B) The normalized fluorescence F_N for each sample (solid data points) is plotted as a function of composition and fitted to an equation of the form $F_N=(K_p(\alpha/\beta)F_N(\alpha)(x_B(\beta)-x_B)+F_N(\beta)(x_B-x_B(\alpha))/(K_p(\alpha/\beta)(x_B(\beta)-x_B)+(x_B-x_B(\alpha))$, where $F_N(\alpha)$ and $F_N(\beta)$ represent the normalized fluorescence values for probe molecules present in the phases α and β, respectively, the compositions $x_B(\alpha)$ and $x_B(\beta)$ define the ends of the tie line (and hence the limits of the region of phase separation at the indicated temperature) and $K_p(\alpha/\beta)$ is the partition coefficient for the fluorescent species ($K_p(\alpha/\beta)>1$ indicates a net preference for phase α). Other terms are as described in the text. Dashed curve — fitted curve for the indicated data ($K_p(\alpha/\beta)=0.5$); solid curves, predicted quenching curves for fixed values of $F_N(\alpha)$ and $F_N(\beta)$ and the indicated values of $K_p(\alpha/\beta)$. (C) For a ternary system, a series of lipid mixtures is prepared with compositions (open circles) spanning the full length of the tie line AB in the region of (l_o/l_d) two-phase coexistence. (D) The normalized fluorescence is plotted as a function of composition (left-hand extreme=composition A, right-hand extreme=composition B) and analyzed to determine the partition coefficient as described above for panel (B). As noted in the text, this basic approach can be applied to data obtained from a variety of techniques that yield distinct (resolvable or averaged) signals from probe molecules present in the two coexisting phases.

affinity of the labeled molecule for phase α over phase β. This basic approach can readily be adapted to the analysis of other spectroscopic data, such as fluorescence lifetime or anisotropy measurements or ESR or NMR spectra of suitably labeled molecules, so long as the method used provides either resolvable and proportionate signals from the populations of probe molecules present in the two phases or a composite signal in which the contributions from probe molecules present in each phase are properly number-averaged. Loura et al. [70] used this approach to estimate that NBD-labeled cholesterol distributes in favor of l_d domains ($K_p(l_o/l_d)=0.35-0.39$) in DMPC/cholesterol bilayers at 30–40 °C. Mesquita et al. [71] used a similar approach to determine qualitatively that NBD-labeled distearoylphosphatidylethanolamine partitions into l_o-domains with greater affinity than does the analogous 1-palmitoyl-2-oleoyl species in DMPC/cholesterol bilayers at 30 °C. When applying the above approach to measurements of fluorescence properties such as intensity or lifetime, a fluorescence-quenching spin-labeled or brominated lipid is frequently used as one of the two major lipid components in order to enhance the 'contrast' between the signals measured from labeled molecules in the two different lipid phases. Since the quencher species will be present at different mol fractions in the two coexisting phases, the extent of fluorescence quenching, and hence the normalized fluorescence F_N and the fluorescence lifetime for the labeled molecule, will differ markedly for probe molecules present in the two phases.

The rigorous approach discussed above for (pseudo-) binary lipid mixtures can in theory be extended to ternary mixtures within a region of two-phase separation, so long as all compositions examined fall on a single tie line, as illustrated in Fig. 2C and D. This approach has not yet been applied to ternary lipid systems, since it requires accurate knowledge not only of the phase boundaries but also of at least one tie line within the region of l_d/l_o phase coexistence for the ternary system under study, information that is only now becoming available. In the interim, modifications of the above approach have been used to obtain information about the affinities of fluorescent-labeled molecules for l_o vs. l_d domains in cholesterol-containing ternary systems. Wang et al. [22] described a method that allows quantitative comparison of the l_o/l_d phase-partition coefficient coefficients for related fluorescent-labeled molecules in cholesterol-containing ternary lipid mixtures, and that in favorable cases can provide qualitative information concerning the absolute magnitudes of these partition coefficients. Using this method, it was confirmed directly that in bilayers combining cholesterol, a spin-labeled PC and either dipalmitoyl phosphatidylcholine or bovine brain sphingomyelin, phospholipid derivatives with long saturated acyl chains partition with substantial affinities into liquid-ordered domains, while polyunsaturated species are effectively excluded. Interestingly, however, at temperatures near physiological, phospholipid derivatives with shorter (14-carbon) saturated chains or containing a single unsaturated bond were also found to partition detectably into l_o-domains, albeit with lower affinities than were measured for analogous longer-chain saturated species. Markedly different results were obtained from parallel analyses of the same lipid mixtures using the cold Triton-fractionation assay, which reported essentially no partitioning of shorter-chain or monounsaturated phospholipids into l_o-domains. This disparity appears to result at least in part from a tendency of shorter-chain saturated and monounsaturated lipids to show progressively lower affinities for l_o domains, relative to their longer-chain saturated counterparts, as the temperature decreases [22].

Using the above fluorescence-quenching approach, different sphingolipids have been shown to partition into l_o domains in sphingolipid/unsaturated phospholipid/cholesterol bilayers with affinities that for most species vary only modestly (over a range of roughly 3-fold) with changes in the size and structure of the polar headgroup [72,73]. However, ceramide was found to partition into l_o domains with a much higher partition coefficient, achieving concentrations within l_o domains that far exceeded those found in coexisting l_d domains. In agreement with these conclusions, London and colleagues [74,75] have shown that ceramide not only concentrates in ordered lipid domains but can actually displace cholesterol from them. Ceramide generated metabolically from sphingomyelin during the course of cellular signaling at the plasma membrane is thus expected to concentrate strongly in l_o domains, a property that may explain reports that ceramide-mediated signaling at the plasma membrane is localized to such domains [76,77]. Interestingly, the uniquely high affinity of ceramide (compared to other sphingolipids) for l_o domains was observed for ceramides bearing long saturated N-acyl chains, or long saturated chains labeled at their methyl termini with an N-indolyl residue, but not for ceramides labeled with diphenylhexatrienylpropanoyl-(DPH-3:0-) chains, even though DPH-3:0-labeled sphingolipids also partition with significant affinity into l_o domains [72,78]. This result underscores the fact that fluorescent lipid analogues must be chosen with great care if they are intended to reflect accurately the tendencies of the corresponding natural membrane lipids to partition into liquid-ordered domains.

The experimental approach just described has also been applied to compare the partitioning of different lipidated and bilayer-spanning peptides between l_o and l_d domains in ternary lipid mixtures containing cholesterol. Short peptide sequences bearing two saturated acyl groups (N-myristoyl/S-palmitoyl or di-S-palmitoyl) attached to adjacent amino acids were shown to exhibit substantial affinities for l_o-domains [79], confirming previous suggestions that such motifs can confer l_o-domain association on proteins of the $G\alpha_{i/o}$ and src families, among others, through favorable interactions between l_o-domain lipids and the peptide-coupled acyl chains. Interestingly, however, the l_o-domain affinity of peptides modified with two saturated acyl chains was strongly affected by the positions of attachment of the two acyl groups. This finding may be correlated with reports that for example the $G\alpha_{q13}$ protein, which is doubly palmitoylated on nonadjacent cysteine residues, shows a comparatively weak association with 'raft' domains in mammalian cell membranes [80], and that the cysteine string protein is modified on a number of cysteine residues yet is excluded from rafts in adipocytes [81]. Peptides combining an S-

palmitoyl and an S-farnesyl or -geranylgeranyl modification, as found at the carboxyl-terminus of various proteins of the ras superfamily, were found to show negligible affinity for l_o domains [22,79]. These findings agree qualitatively with those reported by Moffett et al. [82], who showed using the cold Triton-fractionation method that binding of geranylgeranylated G$\beta\gamma$ to palmitoylated Gα_s reduced the affinity of the latter for l_o domains in reconstituted lipid–protein bilayers.

London and colleagues [83,84] have used the fluorescence-quenching approach described above to examine the abilities of two types of bilayer-spanning peptides to partition into liquid-ordered domains in cholesterol-containing ternary lipid mixtures. Bilayer-spanning peptides with a polyleucine hydrophobic core sequence were shown to be effectively excluded from l_o domains, a finding that may be correlated with inferences from other studies that the majority of transmembrane proteins are underrepresented in or absent from lipid rafts in biological membranes [83]. A transbilayer peptide derived from the raft-associating lymphocyte protein LAT was also largely excluded from liquid-ordered domains [84], even when a doubly palmitoylated flanking sequence was included that is required for association of LAT with raft domains in the lymphocyte cell membrane [85–87]. A full-length but palmitoylation-defective version of the LAT protein also partitioned poorly into the detergent-resistant membrane fraction isolated after reconstitution of the protein into mixed-phase l_o/l_d lipid vesicles [84], consistent with the conclusion from the above fluorescence-quenching experiments that the transmembrane domain of LAT exhibits an intrinsically low affinity for l_o domains.

Electron spin resonance can provide a useful alternative to fluorescence measurements to monitor the distributions of appropriately (spin-) labeled molecules between liquid-ordered and liquid-disordered domains in model membranes. Chiang et al. [20,21] showed that 1-palmitoyl-2-(16-doxylstearoyl) phosphatidylcholine partitions with roughly equal affinity between ordered and disordered domains in dilauroyl PC/dipalmitoyl PC/cholesterol bilayers and can be used to good effect to map phase boundaries and tie lines in this system. ^{2}H-NMR of a deuterated species in bilayers whose other components are protonated also allows resolution and separate quantitation of the spectral components arising from deuterated molecules in l_d and l_o domains when the domains are large enough to avoid spectral averaging by lateral diffusion on the ^{2}H-NMR time scale (ca. >160 nm [19]). Combining such data with independent information concerning the relative proportions of l_o and l_d domains that are present in the lipid mixture(s) under study, the mol fraction of the labeled species in each type of domain, and hence the value of $K_p(l_o/l_d)$, can be determined. When domain sizes are smaller (ca. <80 nm at physiological temperatures [19]), the ^{2}H-NMR spectral contributions from labeled molecules in l_o and l_d domains are averaged, and the above approach cannot be employed [19,63,88]. Nonetheless, under such conditions, qualitative inferences can still be drawn concerning the distributions of different bilayer components between coexisting domains [63].

A new potential approach to compare the distributions of molecules between coexisting l_o and l_d domains is plasmon-waveguide resonance spectroscopy. This optical technique, which does not require labeling of the species of interest, has been used to demonstrate preferential insertion of the GPI-protein PLAP into sphingomyelin-enriched gel-state domains in sphingomyelin/DOPC supported bilayers [89]. This approach has not yet been extended to examine cholesterol-containing ternary lipid mixtures.

6. Extension to biological membranes

Knowledge gained from model-system studies like those described above may be transferable in at least three ways to understand better the organizing principles of raft domains in cellular membranes. First, as already noted, by measuring the intrinsic affinity of a raft-associated membrane protein or lipid for liquid-ordered domains in model membranes, we can determine whether this affinity alone could account for the raft association of the species of interest in biological membranes or whether other, more specific interactions (e.g., protein–protein) must be invoked. An interesting case in point is that of the small G-protein H-ras. The carboxy-terminal membrane-targeting domain of H-ras incorporates a terminal farnesyl group that is strongly excluded from liquid-ordered domains in model membranes [22,29,90], yet in the plasma membrane H-ras appears to be raft-associated in its GDP-bound, though not its GTP-bound form [91,92]. These observations together suggest the existence of a membrane-associated protein that either sequesters the farnesyl group of H-ras(GDP) or binds another region of H-ras(GDP) within rafts with a very high affinity, in either case overcoming the intrinsic raft-avoiding tendency of the farnesyl group and permitting selective association of the GDP-bound form of H-ras with raft domains. Observations that transmembrane domains from several membrane proteins, including the raft-localizing lymphocyte protein LAT, exhibit low intrinsic affinities for l_o-domains in model systems likewise suggest that other factors (e.g., strong interactions with raft-resident or cytoskeletal proteins) must be sought to explain how some bilayer-spanning proteins associate with rafts in biological membranes. More generally, it is interesting to note that few of the membrane proteins studied to date in model systems, including GPI-anchored proteins, show a marked net preference to partition into l_o-domains. Such findings may be correlated with results suggesting that while raft domains in membranes are of nanoscopic dimensions [93–95], only a minority fraction of GPI-proteins show spectroscopic evidence for clustering within such small domains (reviewed in [96]).

A second useful contribution of model-system studies to investigate rafts in biological systems is the potential to develop and to test probes that exhibit substantial affinities for liquid-ordered domains and that may be useful to detect and to characterize raft domains in cellular membranes. Such species can provide a useful complement to endogenous raft markers, whose behavior may in principle be affected not only by their raft association per se but also by biospecific interactions with other membrane components. Laurdan, an amphiphilic fluorescent probe shown in model systems to

partition into both l_o and l_d domains and to emit distinguishable fluorescence signals from the two types of domains [36], has recently been used in efforts to map the overall surface distribution of rafts (which as already noted are too small to be resolved as separate entities) in intact mammalian cells [97,98]. Other fluorescent molecules, including chrysene [43] and fluorescent saturated tetraacyl lipid analogues [62] have also been identified that in model systems show substantial affinities for liquid-ordered lipid domains and that if suitably modified may be adaptable for use to probe raft domains in biological membranes. By contrast, model-system studies have shown that some exogenous probes that a priori might seem attractive candidates to label membrane rafts, such as acyl-chain-labeled fluorescent gangliosides or long-chain saturated indocarbocyanine derivatives, in fact associate poorly with liquid-ordered domains in sphingolipid-containing model membranes [62,78,99].

Finally and more broadly, the fact that model systems can exhibit some of the key physical behaviors ascribed to rafts (e.g., fluid–fluid domain segregation, formation of nanoscale domains under physiological conditions [19,61,62] and a strong sensitivity of l_o-domain organization to modest perturbations of the bilayer under certain conditions [40,43]) allows them to be used to refine new technologies, as well as new probes, that may be useful to investigate rafts in biological membranes. It can certainly be argued that this potential is overdue to be realized. However, most of the methods discussed in this review to monitor molecular distributions between membrane domains (and other emerging approaches, such as conventional or scanning fluorescence correlation spectroscopy [99–102]) have been developed or adapted only in the last few years for use to study small-scale liquid-ordered domains in model membranes, and some are now beginning to see application to biological systems [100,103]. The technical and other limitations of lipid and lipid/protein membranes as models for biological membrane rafts are clear. Nonetheless, as the examples discussed above illustrate, a healthy dialogue between model and biological systems can be both informative and provocative in advancing our understanding of the properties, organization and functional significance of rafts in biological membranes.

Acknowledgments

Research in the author's laboratory was supported by an Operating Grant from the Canadian Institutes of Health Research. I thank Dr. Ken Jacobson (University of North Carolina) for helpful discussions during the preparation of the review.

References

[1] K. Simons, E. Ikonen, Nature 387 (1997) 569–572.
[2] R.E. Brown, J. Cell Sci. 111 (1998) 1–11.
[3] D.A. Brown, E. London, Annu. Rev. Cell Dev. Biol. 14 (1998) 111–136.
[4] A. Rietveld, K. Simons, Biochim. Biophys. Acta 1376 (1998) 467–479.
[5] E. London, Curr. Opin. Struct. Biol 12 (2002) 480–486.
[6] M. Edidin, Annu. Rev. Biophys. Biomol. Struct. 32 (2003) 257–283.
[7] K. Simons, W.L. Vaz, Annu. Rev. Biophys. Biomol. Struct. 33 (2004) 269–295.
[8] M.R. Vist, J.H. Davis, Biochemistry 29 (1990) 451–464.
[9] M.B. Sankaram, T.E. Thompson, Biochemistry 29 (1990) 10676–10684.
[10] M.B. Sankaram, T.E. Thompson, Proc. Nat. Acad. Sci. U. S. A. 88 (1991) 8686–8690.
[11] J.L. Thewalt, M. Bloom, Biophys. J. 63 (1992) 1176–1181.
[12] T.-H. Huang, C.W.B. Lee, K. Das Gupta, A. Blume, R.G. Griffin, Biochemistry 32 (1993) 13277–13287.
[13] L.M. Loura, A. Fedorov, M. Prieto, Biochim. Biophys. Acta 1511 (2001) 236–243.
[14] M.I. Collado, F.M. Goñi, A. Alonso, D. Marsh, Biochemistry 44 (2005) 4911–4918.
[15] G.W. Feigenson, Biochim. Biophys. Acta 1746 (2005) 186–192 (accompanying chapter in this issue).
[16] S.L. Keller, Biochim. Biophys. Acta 1746 (2005) 172–185 (accompanying chapter in this issue).
[17] R.F. de Almeida, A. Fedorov, M. Prieto, Biophys. J. 85 (2003) 2406–2416.
[18] S.L. Veatch, S.L. Keller, Biophys. J. 85 (2003) 3074–3083.
[19] S.L. Veatch, I.V. Polozov, K. Gawrisch, S.L. Keller, Biophys. J. 86 (2004) 2910–2922.
[20] Y.W. Chiang, Y. Shimoyama, G.W. Feigenson, J.H. Freed, Biophys. J. 87 (2004) 2483–2496.
[21] Y.W. Chiang, J. Zhao, J. Wu, Y. Shimoyama, J.H. Freed, G.W. Feigenson, Biochim. Biophy. Acta 1668 (2005) 99–105.
[22] T.-Y. Wang, R. Leventis, J.R. Silvius, Biophys. J. 79 (2000) 919–933.
[23] R. Leventis, J.R. Silvius, Biophys. J. 81 (2001) 2257–2267.
[24] Y. Lange, J.S. D'Alessandro, D.M. Small, Biochim. Biophys. Acta 556 (1979) 388–398.
[25] Y. Nakagawa, K. Inoue, S. Nojima, Biochim. Biophys. Acta 553 (1979) 307–319.
[26] B.W. Wattenberg, D.F. Silbert, J. Biol. Chem. 258 (1983) 2284–2289.
[27] C. Rujanavech, D.F. Silbert, J. Biol. Chem. 261 (1986) 7215–7219.
[28] P.L. Yeagle, J.E. Young, J. Biol. Chem. 261 (1986) 8175–8181.
[29] K. Hanada, K. Kumagai, S. Yasuda, Y. Miura, M. Kawano, M. Fukasawa, M. Nishijima, Nature 426 (2003) 803–809.
[30] K. Kumagai, S. Yasuda, K. Okemoto, M. Nishijima, S. Kobayashi, K. Hanada, J. Biol. Chem. 280 (2005) 6488–6495.
[31] L. Malinina, M.L. Malakhova, A. Teplov, R.E. Brown, D. Patel, J. Nat. 430 (2004) 1048–1053.
[32] S.L. Niu, B.J. Litman, Biophys. J. 83 (2002) 3408–3415.
[33] M.S. Abreu, M. Moreno, W.L. Vaz, Biophys. J. 87 (2004) 353–365.
[34] A. Pokorny, P.F. Almeida, E.C. Melo, W.L. Vaz, Biophys. J. 78 (2000) 267–280.
[35] M.S. Abreu, L.M. Estronca, M. Moreno, W.L. Vaz, Biophys. J. 84 (2003) 386–399.
[36] C. Dietrich, L.A. Bagatolli, Z.N. Volovyk, N.L. Thompson, M. Levi, K. Jacobson, E. Gratton, Biophys. J. 80 (2001) 1417–1428.
[37] A.V. Samsonov, I. Mihalyov, F.S. Cohen, Biophys. J. 81 (2001) 1486–1500.
[38] S.L. Veatch, S.L. Keller, Phys. Rev. Lett. 89 (2002) 268101.
[39] S.L. Veatch, S.L. Keller, Biophys. J. 84 (2003) 725–726.
[40] C. Dietrich, Z.N. Volovyk, M. Levi, N.L. Thompson, K. Jacobson, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 10642–10647.
[41] N. Kahya, D.A. Brown, P. Schwille, Biochemistry 44 (2005) 7479–7489.
[42] K. Bacia, C.G. Schuette, N. Kahya, R. Jahn, P. Schwille, J. Biol. Chem. 279 (2004) 37951–37955.
[43] A.T. Hammond, F.A. Heberle, T. Baumgart, D. Holowka, B. Baird, G.W. Feigenson, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 6320–6325.
[44] T.K. Khan, B. Yang, N.L. Thompson, S. Maekawa, R.M. Epand, K. Jacobson, Biochemistry 42 (2003) 4780–4786.
[45] K. Bacia, P. Schwille, T. Kurzchalia, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 3272–3277.
[46] M. Simons, T. Friedrichson, J.B. Schulz, M. Pitto, M. Masserini, T.V. Kurzchalia, Mol. Biol. Cell 10 (1999) 3187–3196.

[47] R. Schroeder, E. London, D. Brown, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 12130–12134.
[48] R.J. Schroeder, S.N. Ahmed, Y. Zhu, E. London, D.A. Brown, J. Biol. Chem. 273 (1998) 1150–1157.
[49] E. London, D.A. Brown, Biochim. Biophys. Acta 1508 (2000) 182–195.
[50] H. Heerklotz, H. Szadkowska, T. Anderson, J. Seelig, J. Mol. Biol. 329 (2003) 793–799.
[51] E. London, Biochim. Biophys. Acta 1746 (2005) 203–220 (accompanying article in this issue).
[52] H.A. Rinia, B. de Kruijff, FEBS Lett. 504 (2001) 194–199.
[53] C. Yuan, L.J. Johnston, J. Microsc. 205 (2002) 136–146.
[54] C. Yuan, J. Furlong, P. Burgos, L.J. Johnston, Biophys. J. 82 (2002) 2526–2535.
[55] N.A. Geisse, T.L. Cover, R.M. Henderson, J.M. Edwardson, Biochem. J. 381 (2004) 911–917.
[56] D.E. Saslowsky, J. Lawrence, R.M. Henderson, J.M. Edwardson, J. Membr. Biol. 194 (2003) 153–164.
[57] D.E. Saslowsky, J. Lawrence, X. Ren, D.A. Brown, R.M. Henderson, J.M. Edwardson, J. Biol. Chem. 277 (2002) 26966–26970.
[58] P.E. Milhiet, M.C. Giocondi, O. Baghdadi, F. Ronzon, B. Roux, C. Le Grimellec, EMBO Rep. 3 (2002) 485–490.
[59] F. Tokumasu, A.J. Jin, G.W. Feigenson, J.A. Dvorak, Biophys. J. 84 (2003) 2609–2618.
[60] C. Leidy, T. Kaasgaard, J.H. Crowe, O.G. Mouritsen, K. Jorgensen, Biophys. J. 83 (2002) 2625–2633.
[61] G.W. Feigenson, J.T. Buboltz, Biophys. J. 80 (2001) 2775–2788.
[62] J.R. Silvius, Biophys. J. 85 (2003) 1034–1045.
[63] B.Y. van Duyl, D. Ganchev, V. Chupin, B. de Kruijff, J.A. Killian, FEBS Lett. 547 (2003) 101–106.
[64] R.F. de Almeida, L.M. Loura, A. Fedorov, M. Prieto, J. Mol. Biol. 346 (2005) 1109–1120.
[65] J. Korlach, T. Baumgart, W.W. Webb, G.W. Feigenson, Biochim. Biophys. Acta 1668 (2005) 158–163.
[66] E. London, G.W. Feigenson, Biochemistry 20 (1981) 1932–1938.
[67] K. Florine-Casteel, G.W. Feigenson, Biochim. Biophys. Acta 941 (1988) 102–106.
[68] N.N. Huang, K. Florine-Casteel, G.W. Feigenson, C. Spink, Biochim. Biophys. Acta 939 (1988) 124–130.
[69] C.H. Spink, M.D. Yeager, G.W. Feigenson, Biochim. Biophys. Acta 1023 (1990) 25–33.
[70] L.M. Loura, A. Fedorov, M. Prieto, Biochim. Biophys. Acta 1511 (2001) 236–243.
[71] R.M. Mesquita, E. Melo, T.E. Thompson, W.L. Vaz, Biophys. J. 78 (2000) 3019–3025.
[72] T.-Y. Wang, J.R. Silvius, Biophys. J. 84 (2003) 367–378.
[73] M. Koivusalo, J. Alvesalo, J.A. Virtanen, P. Somerharju, Biophys. J. 86 (2004) 923–935.
[74] X. Xu, R. Bittman, G. Duportail, D. Heissler, C. Vilcheze, E. London, J. Biol. Chem. 276 (2001) 33540–33546.
[75] M. London, E. London, J. Biol. Chem. 279 (2004) 9997–10004.
[76] P. Liu, R.G. Anderson, J. Biol. Chem. 270 (1995) 27179–27185.
[77] E. Gulbens, Biochim. Biophys. Acta 1746 (2005) 284–294 (accompanying article in this issue).
[78] T.-Y. Wang, J.R. Silvius, Biophys. J. 79 (2000) 1478–1489.
[79] T.-Y. Wang, R. Leventis, J.R. Silvius, Biochemistry 40 (2001) 13031–13040.
[80] A.A. Waheed, T.L. Jones, J. Biol. Chem. 277 (2002) 32409–32412.
[81] L.H. Chamberlain, G.W. Gould, J. Biol. Chem. 277 (2002) 49750–49754.
[82] S. Moffett, D.A. Brown, M.E. Linder, J. Biol. Chem. 275 (2000) 2191–2198.
[83] M.E. Fastenberg, H. Shogomori, X. Xu, D.A. Brown, E. London, Biochemistry 42 (2003) 12376–12390.
[84] H. Shogomori, A.T. Hammond, A.G. Ostermeyer-Fay, D.J. Barr, G.W. Feigenson, E. London, D.A. Brown, J. Biol. Chem. 280 (2005) 18931–18942.
[85] W. Zhang, R.P. Trible, L.E. Samelson, Immunity 9 (1998) 239–246.
[86] T. Harder, M. Kuhn, J. Cell Biol. 151 (2000) 199–208.
[87] Y. Webb, L. Hermida-Matsumoto, M.D. Resh, J. Biol. Chem. 275 (2000) 261–270.
[88] F. Aussenac, M. Tovares, E. Dufourc, J. Biochem. 42 (2003) 1383–1390.
[89] Z. Salamon, S. Devanathan, I.D. Alves, G. Tollin, J. Biol. Chem. 280 (2005) 11175–11184.
[90] S. Janosch, C. Nicolini, B. Ludolph, C. Peters, M. Volkert, T.L. Hazlet, E. Gratton, H. Waldmann, R. Winter, J. Am. Chem. Soc. 126 (2004) 7496–7503.
[91] I. Prior, I.A. Prior, A. Harding, J. Yan, J. Sluimer, R.G. Parton, J.F. Hancock, Nat. Cell Biol. 3 (2001) 368–375.
[92] J.F. Hancock, Biochim. Biophys. Acta 1746 (2005) 274–283 (accompanying paper in this issue).
[93] R. Varma, S. Mayor, Nature 394 (1998) 798–801.
[94] A. Pralle, P. Keller, E.L. Florin, K. Simons, J.K. Horber, J. Cell Biol. 148 (2000) 997–1008.
[95] P. Sharma, R. Varma, R.C. Sarasij, K. Gousset, G. Krishnamoorthy, M. Rao, S. Mayor, Cell 116 (2004) 577–589.
[96] S. Mayor, Biochim. Biophys. Acta 1746 (2005) 221–233 (accompanying paper in this issue).
[97] K. Gaus, E. Gratton, E.P. Kable, A.S. Jones, I. Gelissen, L. Kritharides, W. Jessup, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15554–15559.
[98] A.L. Kindzelskii, R.G. Sitrin, H.R. Petty, J. Immunol. 172 (2004) 4681–4685.
[99] N. Kahya, D. Scherfeld, K. Bacia, B. Poolman, P. Schwille, J. Biol. Chem. 278 (2003) 28109–28115.
[100] K. Bacia, D. Scherfeld, N. Kahya, P. Schwille, Biophys. J. 87 (2004) 1034–1043.
[101] Q. Ruan, M.A. Cheng, M. Levi, E. Gratton, W.W. Mantulin, Biophys. J. 87 (2004) 1260–1267.
[102] J. Korlach, T. Baumgart, W.W. Webb, G.W. Feigenson, Biochim. Biophys. Acta 1668 (2005) 158–163.
[103] M. Ge, A. Gidwani, H.A. Brown, D. Holowka, B. Baird, J.H. Freed, Biophys. J. 85 (2003) 1278–1288.