Available online at www.sciencedirect.com

SCIENCE DIRECT®

Biochimica et Biophysica Acta 1666 (2004) 227–238

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

Review

Do proteins facilitate the formation of cholesterol-rich domains?

Richard M. Epand*

Department of Biochemistry, McMaster University Hamilton, ON L8N 3Z5, Canada

Received 16 April 2004; received in revised form 21 July 2004; accepted 23 July 2004
Available online 9 August 2004

Abstract

Both biological and model membranes can exhibit the formation of domains. A brief review of some of the diverse methodologies used to identify the presence of domains in membranes is given. Some of these domains are enriched in cholesterol. The segregation of lipids into cholesterol-rich domains can occur in both pure lipid systems as well as membranes containing peptides and proteins. Peptides and proteins can promote the formation of cholesterol-rich domains not only by preferentially interacting with cholesterol and being sequestered into these regions of the membrane, but also indirectly as a consequence of being excluded from cholesterol-rich domains. The redistribution of components is dictated by the thermodynamics of the system. The formation of domains in a biological membrane is a consequence of all of the intermolecular interactions including those among lipid molecules as well as between lipids and proteins.

Keywords: Cholesterol; Rafts; Membrane domain

Contents

Abbreviations: 4F, *N*-acetyl-DWFKAFYDKVAEKFKEAF-amide; DOPC, dioleoyl phosphatidylcholine; DSC, differential scanning calorimetry; GFP, green fluorescent protein; GPI, glycosylphosphatidylinositol; GUV, giant unilamellar vesicles; LUVs, large unilamellar vesicles; MAS, magic angle spinning; NSOM, near field scanning optical microscopy; PIP2, phosphatidylinositol (4,5) bisphosphate; RET, resonance energy transfer; SANS, small angle neutron scattering; SAXS, small angle X-ray scattering; SOPC, 1-stearoyl-2-oleoyl phosphatidylcholine; TIRF, total internal reflectance fluorescence

* Tel.: +1 905 525 9140x22073; fax: +1 905 521 1397.
E-mail address: epand@mcmaster.ca.

0005-2736/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2004.07.004

1. Introduction

There is considerable current interest and research activity focused on characterizing and understanding the role of cholesterol-rich domains in membranes. There have been many reviews describing the chemical composition and physical properties of cholesterol-rich "raft" domains [1–7]. One of the reasons for the particular interest in raft domains is their suggested roles in signal transduction pathways [8–10], possibly including modulation of the activity of ion channels [11]. Raft domains may also be important in certain disease processes [12]. There is evidence that raft domains have a particular lipid composition that is enriched in sphingomyelin as well as cholesterol, resulting in these domains having the physical properties of a liquid ordered state. Molecules in a liquid-ordered state are characterized by having liquid-like mobility in the plane of the membrane but a solid-like conformation of the phospholipid acyl chains.

The presence of domains in model membranes, including liquid-ordered domains, has been well established, but it is uncertain how this phenomenon relates to the possible existence of comparable domains in biological membranes [13–16]. One of the major difficulties is the size of domains in model membranes compared with biological membranes, in which domains are found to be much smaller. It has been suggested that lectins and glycoprotein-binding proteins may have an important role in promoting the clustering of small raft-like domains to allow the formation of larger domains in biological membranes under certain conditions [17]. The absence of protein in most model membrane studies, as well as their lack of transmembrane asymmetry, makes difficult the extrapolation from the model membrane system to biological membranes. This is particularly the case for the cytoplasmic leaflet of the cell surface membrane where many signal transduction events occur. Furthermore, there is evidence that biological raft domains are heterogeneous [18,19], meaning that no single model lipid system would be appropriate for all types of biological raft domains.

In addition to the role of lipids in the formation of domains, there is increasing evidence that proteins also facilitate their formation and stabilization. The role of protein–protein interactions and their interplay with lipid segregation has been discussed [20]. Small differences of just a few hundred calories per mole in the energy of interaction among components in a membrane can lead to dramatic changes in the lateral organization of a lipid bilayer [21,22]. Differences in the interaction of proteins or peptides with particular lipid components of the membrane can lead to domain formation [22,23]. Many proteins partition unequally between raft and non-raft domains. There is thus a thermodynamic requirement that proteins affect raft stability.

The field of "rafts" has been very active and there are many excellent reviews of the topic. The present review focuses on two aspects that have not received as much attention. Although there are several more comprehensive discussions of individual methods for studying domains in membranes, there are fewer reviews that survey a variety of methodologies that are available for these studies. In the present article, we provide an overview of several of the methods that have been developed to study membrane domains in both model systems and in biological membranes. In addition, we consider how the interaction of proteins or peptides with lipids can result in the sequestering of cholesterol. Most of the studies in this area focus on the lateral phase separation of lipids resulting in the sequestering of proteins into a lipid domain. In this review, we emphasize the inter-relationship between the effect of lipids on the distribution of proteins in a membrane and the converse, i.e., the effect of proteins on the distribution of lipids. This leads to the novel concept that cholesterol-rich membrane domains can be formed both as a result of proteins interacting with cholesterol-rich domains as well as by being excluded from such domains.

2. Methods

2.1. Fluorescence

Fluorescence methods are quite versatile in determining the distribution of molecules in a membrane. It is a method that can be applied to many different types of specimens including whole cells, liposomes, supported bilayers and monolayers. There are also many different kinds of fluorescence phenomena that can be used to study domain formation, including quenching, resonance energy transfer (RET), anisotropy and imaging. Fluorescence methods are sensitive, even to the extent of allowing single molecule detection. The phenomenon occurs on a nanosecond time scale enabling detection of individual species, not averaged by exchange. The method also allows for acquisition of time-resolved phenomenon, such as reorientation and diffusion.

2.1.1. Quenching

A method in which a nitroxide-labeled lipid is used to quench a fluorescently-labeled species has been developed [24]. The method is based on the finding that the quenching agent will preferentially partition into disordered domains

and hence more efficiently quench molecules that are in this domain. The method has been extended to assess the size of domains and the sequestering of molecules to domain interfaces [25]. The study showed that a model transmembrane helical peptide was largely excluded from ordered domains [25], similar to what had been previously found with the integral membrane protein, rhodopsin [26].

2.1.2. Resonance energy transfer

Transfer of energy from an excited state fluorophore to an acceptor can occur if there is spectral overlap between the fluorescence emission spectrum of the donor and the excitation spectrum of the acceptor. The efficiency of this transfer is strongly dependent on distance, falling off as the 6th power of the distance, and is therefore more efficient when donor and acceptor are sequestered in the same domain. This neglects the orientational factor that is not usually important for fluid liquid-crystalline systems. RET has been used to monitor nanometer scale domain formation in model lipid mixtures that would be too small to be seen by fluorescence microscopy [27,28]. Using GFP-labeled proteins, it was shown that acylated, but not prenylated proteins, were sequestered into raft domains in cell membranes [29]. A recent study using RET has shown that the majority of glycosylphosphatidylinositol (GPI)-linked proteins are present as monomers, but that 20–40% are in cholesterol-sensitive clusters smaller than 5 nm [30].

2.1.3. Anisotropy

Fluorescence anisotropy has been used to show the partitioning of certain proteins to the more rigid liquid ordered domain of rafts [31]. Using time-resolved measurements, the anisotropy could be analyzed in terms of the rotatory diffusion rate and limiting anisotropy [32]. This study demonstrated the important role of proteins in maintaining raft domains in biological membranes.

2.1.4. Fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy is based on the time-dependent fluctuation of concentration of a fluorophore in a small volume. Analysis of the results from this technique can yield a diffusion constant. This method has been used to determine rates of lipid lateral diffusion in giant unilamellar vesicles (GUVs) having a lipid composition chosen to form raft-like domains [33–36].

2.1.5. Imaging

All of the fluorescence methods discussed above can be used in conjunction with imaging. Microscopic imaging can be used to obtain spatial resolution of the distribution of fluorophores and their properties. Since the methods are based on light microscopy, resolution is limited to particles of large size. Three types of membrane arrangements have been used, monolayers, GUVs and planar bilayers deposited on a solid support, in addition to intact cells. Pure lipid systems have been studied to demonstrate the formation of domains in lipid mixtures similar to those expected to form rafts in biological membranes [37]. The temperature and lipid composition dependence of domain formation has been used to determine a phase diagram [38]. There have also been many applications to studies of fluorescently labeled proteins in both model and cell membranes. For example, the spatial location of the protein caveolin has been determined both by using GFP-caveolin [39] as well as with caveolin antibodies [40]. It is found that this protein partitions into invaginated plasma membrane domains termed caveolae.

Single particle tracking has been used to study the rate and uniformity of protein diffusion in membranes [41]. This method has also been applied to biological membranes. Although the resolution of light microscopy is low, detecting domains of the order of hundreds of nanometers or larger, single particle tracking can provide evidence for the existence of much smaller domains as a consequence of the presence of transient confinement zones. The results suggest that domains may be as small as five molecules [42]. One of the criteria often used to identify a domain as being enriched in cholesterol is to demonstrate that the domain disappears upon extraction of cholesterol from the membrane. However, other changes occur in the lateral mobility of proteins when cholesterol is extracted as a result of actin reorganization [43]. Thus, not all affects of cholesterol depletion are directly related to the loss of domains. In addition, not all cell membrane domains involve cholesterol. It has been shown using single particle tracking that h-Ras sequesters into a membrane domain that is not affected by cholesterol depletion [44].

A powerful approach for studying supported planar lipid bilayers, total internal reflectance fluorescence (TIRF) microscopy, relies on the propagation of an evanescent wave to excite fluorophores within ~100 nm from an interface [45,46]. Since fluorophores distributed in the bulk of the solution are not excited by the evanescent excitation wave, the resulting images are largely free of any background fluorescence. Recently TIRF microscopy has been combined with atomic force microscopy (AFM) to image domains in membranes [46].

2.2. AFM/NSOM

AFM itself is a high-resolution method of imaging membrane surface topology [47] that has been applied to detect the formation of raft domains [48]. AFM has shown the presence of small cholesterol-rich domains of dimensions 25 to 48 nm that have a height difference of 1.4 nm from the rest of the membrane [49]. Mixtures of sphingomyelin with cholesterol and dioleoyl phosphatidylcholine (DOPC) form larger domains when sphingomyelin is replaced by saturated phosphatidylcholine [50]. The higher domains have been shown to contain cholesterol on the basis of the localization of filipin [51]. It should be noted that the cholesterol-rich, high domains can be the “raft”

islands or they can be the sea in which the domains of the liquid disordered phase are floating. The difference depends on the lipid composition and can be described by percolation theory [52]. AFM has also been used to study raft domain formation in membranes composed of lipopolysaccharides and bacterial phospholipids [53]. The role of cholesterol in domain formation has been studied by AFM, by measuring the kinetics of morphological changes following cholesterol extraction with methyl-β-cyclodextrin [54]. However, another work indicates that the interaction of lipids with solid supports, such as mica, can affect phase behavior and domain size [55]. AFM has also been used to show that GPI-linked alkaline phosphatase localizes in the so-called raft domains of model membranes [56].

Another developing high-resolution microscopy method is near-field scanning optical microscopy (NSOM) [57]. Conventional far-field optical microscopy is diffraction-limited in resolution so that only domain structures in the hundreds of nanometers length scale can be resolved. NSOM has been applied to visualize submicron size domain structure in lung surfactant monolayers. Both AFM and NSOM have potentially high resolving power and will be especially useful for studying model membrane systems.

2.2.1. Fluorescent cholesterol probes

A limitation of fluorescence studies of cholesterol-rich domains is the difficulty of finding a cholesterol analog that is both fluorescent and has physical properties and miscibility with other lipids that are identical to that of cholesterol. This is a particular difficulty because the structure of cholesterol, with its fused ring system, makes it a rigid molecule. Nevertheless, several fluorescent derivatives of cholesterol have been useful in studying membrane domains. They include dehydroergosterol [58,59], NBD-cholesterol [60] and cholestatrienol [61–65]. Recently, the properties of several fluorescent analogs of cholesterol have been compared and it was concluded that cholestatrienol has properties most closely related to that of natural cholesterol [66].

2.3. Differential scanning calorimetry (DSC)

There have been many studies using DSC to establish phase diagrams for lipid mixtures including components of cholesterol-rich domains. Some of this work is summarized in a recent article that integrates observations from DSC studies with those from fluorescent probes [67]. This study outlines some of the complexities of establishing a phase diagram for a ternary lipid mixture.

With regard to cholesterol-rich domains induced by peptides or proteins, DSC can be used in a more direct and simpler fashion. The formation of crystals of cholesterol monohydrate in the presence of the raft protein, NAP-22 [68], or the formation of anhydrous cholesterol crystals in the presence of small peptides [69,70] can readily be detected by DSC. There are two criteria that can be used as an indication of phase separation of cholesterol-rich domains (Fig. 1). One is the appearance of cholesterol crystallites in lipid mixtures in which cholesterol is miscible in the absence of peptide. We have used this to study the effects of peptides on the phase behavior of mixtures of 1-stearoyl-2-oleoyl phosphatidylcholine (SOPC) and cholesterol. In cooling scans, at a scan rate of 2 K/min, the polymorphic transition of anhydrous cholesterol crystals appears at about 21 °C. It is not present in the absence of peptide (Fig. 1, curve 1), but appears in the presence of *N*-acetyl-LWYIK-amide (Fig. 1, curve 2) and even more prominently with the peptide 4F (Fig. 1, curve 3). The other criterion is the cooperativity and magnitude of the chain melting transition of the phospholipid. It is well known that cholesterol broadens and lowers the enthalpy of the gel to liquid crystalline phase transition of phospholipids. This is observed in cooling scans of SOPC/cholesterol

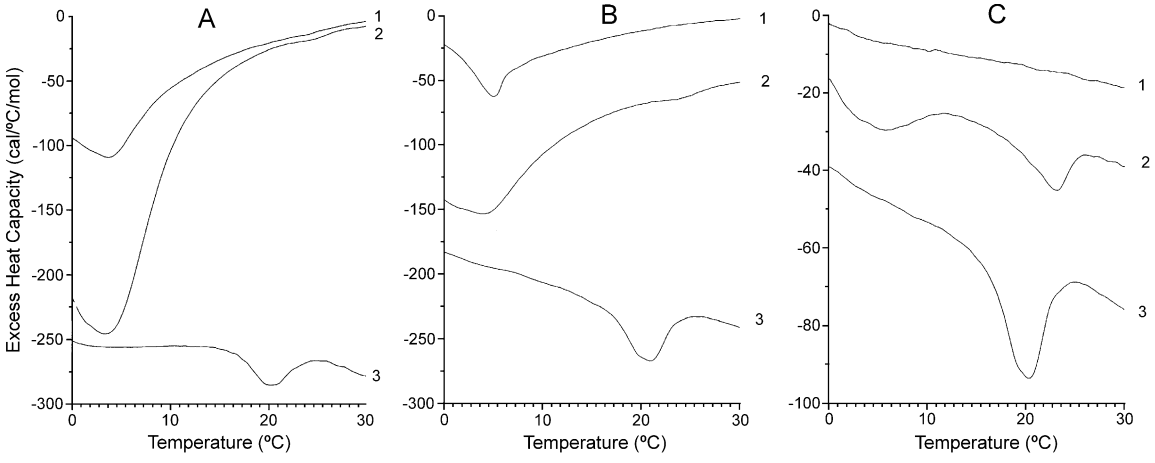

Fig. 1. DSC curves of SOPC/cholesterol mixtures with and without the addition of peptides. Panel A: 30% cholesterol, 70% SOPC; Panel B: 40% cholesterol, 60% SOPC; Panel C: 50% cholesterol, 50% SOPC (note that the a different scale is used for the *y*-axis in this panel). For all three panels, curve 1 corresponds to lipid alone; curve 2: lipid with the addition of 15 mol% *N*-acetyl-LWYIK-amide; curve 3: lipid with the addition of 15 mol% *N*-acetyl-DWFKAFYDKVAEKFKEAF-amide (referred to as peptide 4F). Cooling scans at 2 K/min.

mixtures at about 4 °C (Fig. 1, curve 1), eventually becoming essentially undetectable at 50% cholesterol (Fig. 1C, curve 1). Peptides that preferentially interact with cholesterol-rich domains will stabilize these domains resulting in other parts of the membrane being depleted of cholesterol. As a consequence, the cholesterol-poor regions will exhibit a more cooperative chain melting transition of higher enthalpy, compared with lipid alone, as occurs in the presence of *N*-acetyl-LWYIK-amide (Fig. 1, curve 2). Conversely, peptides that interact with the cholesterol-depleted regions will decrease the cooperativity of lipid phase transitions in these domains, resulting in broader transitions of lower enthalpy, as occurs in the presence of the 4F peptide (Fig. 1, curve 3).

2.4. NMR

NMR phenomenon occurs over a longer time scale, so that exchange between domains is generally more rapid than nuclear relaxation. Nevertheless, the NMR method is very versatile and can discriminate among atoms at different positions as well as being sensitive to the presence of domains with altered properties. ^{2}H NMR was used to establish the phase diagram of mixtures of dipalmitoyl phosphatidylcholine and cholesterol and to describe the particular physical properties of the liquid ordered phase [71]. This technique has also been extended to studies of the ternary lipid system of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC)/sphingomyelin/cholesterol using deuterated cholesterol, as well as ^{31}P NMR [72]. A particularly detailed NMR study of the ternary lipid system of DOPC/sphingomyelin/cholesterol has been able to establish the size of the domain, in addition to the phase diagram [73].

Magic angle spinning (MAS)/NMR has also been used to detect crystalline domains of cholesterol in membranes as an indication that cholesterol has passed its solubility limit [74,75]. In addition, a variety of NMR methods have been employed to study preferential interactions of peptides or proteins with specific lipid components of membranes. Many of these applications rely on measuring the strength of through-space dipolar interactions between an atom on the peptide and one on the lipid, such as the MAS/NMR method called REDOR [76,77]. Another MAS/NMR method is based on changes in the chemical shifts of lipid resonances caused by the insertion of aromatic groups into the membrane and the resultant ring current shifts in the lipid resonances [69,78]. In addition, one can acquire 2D-NOESY spectra using NMR. In particular, the proton resonances from the aromatic residues have a chemical shift that is removed from the bulk of the resonance lines. Cross peaks between aromatic protons and those from the lipid can be used to estimate the extent of insertion of a peptide into a membrane [79]. This method has been applied to compare the extent of peptide insertion into membranes with and without cholesterol [69,70].

2.5. Small angle X-ray scattering (SAXS) and small angle neutron scattering (SANS)

X-ray diffraction has been used to demonstrate that bilayers with the lipid composition of rafts are thicker than those in the liquid crystalline phase [80,81].

SANS has an important additional advantage for studying the distribution of molecular components in membranes. This results from the fact that protonated and deuterated components can be distinguished because of their difference in neutron scattering lengths. Hence, specific lipid components can be labeled with deuterium without substantially affecting the properties of the system. SANS is also sensitive to smaller length scales than light microscopy. SANS has been used to study calcium-induced domain formation in bilayers containing both anionic and zwitterionic lipids [82] and in membranes in a phase co-existence region with both gel and liquid crystalline phases [83]. There is currently work in progress (Pencer, Katsaras, Krueger and Epand) using SANS that provides evidence for submicron-sized domains in large unilamellar vesicles (LUVs) having the ternary lipid mixture of DOPC, DPPC and cholesterol.

3. Domains in membranes

3.1. Liposomes

There is good evidence, from a variety of techniques, that domains can exist in simple lipid mixtures. For example, fluorescence microscopy studies clearly show that large domains, several microns in diameter, are present in mixtures of lipids of the type found in the low density, detergent-insoluble fraction of biological membranes, i.e., sphingomyelin, cholesterol and phosphatidylcholine containing at least one unsaturated acyl chain [37]. The phase diagram of these lipid mixtures has been determined using fluorescence microscopy [84]. There have been several descriptions of the physical basis for this domain formation. It has been described as bulk phase separation caused by liquid–liquid immiscibility between a liquid disordered and a liquid ordered phase. In addition, however, there is evidence from monolayer studies that cholesterol forms discrete complexes with phospholipids [85,86]. It has been pointed out that some of the properties of lipid mixtures with such complexes resemble those of bulk lipid phase immiscibility [86]. In addition, certain properties of cholesterol–phospholipid mixtures exhibit abrupt changes at specific mole fractions of cholesterol. This has led to the formulation of a lattice model, suggesting the regular distribution of cholesterol in the membrane [87–92]. Although there are fundamental aspects of the molecular description of phase separation in mixtures of cholesterol and phospholipids that remain to be resolved, it is clear that cholesterol

does not mix uniformly with many phospholipid mixtures.

There are features of biological membranes that are not mimicked in most studies of model membranes. One of these is transbilayer asymmetry. In addition, domain formation is sensitive to the acyl chain composition of the phosphatidylcholine component [93]. DOPC is often used to promote the segregation of cholesterol-rich domains, but this lipid is not typical of the forms of phosphatidylcholine found in biological membranes. The other major lipid component of raft domains is cholesterol. Surprisingly, it is still not well established how cholesterol is distributed with regard to membrane sidedness. This problem impinges not only on the question of comparing model and biological membranes, but also on the biological function of "rafts" in cell membranes. One of the major biological functions suggested for rafts is in signal transduction. In most cases, this involves changes in the activity of proteins on the cytoplasmic surface of the membrane that has a low content of sphingolipids.

3.2. Liposomes with peptides and proteins

Proteins are a major component of biological membranes, corresponding to about half the weight of the plasma membrane. Proteins can preferentially associate with certain lipids and promote the formation of domains. It has been suggested that cholesterol-rich domains can form in membranes as a consequence of preferential sequestration of particular lipids as a shell surrounding proteins [2]. This shell of lipid is similar to boundary lipid that had been discussed in other contexts in earlier literature. The novel application of this concept to raft domains helps to explain why it has been difficult to image these domains in resting cells.

There is likely to be a range of affinities and specificity of the interaction of particular peptides and proteins with the lipid components of rafts. There are a few proteins that bind specifically to lipid components of rafts. Gangliosides are among the sphingolipids found in rafts. The B chain of cholera toxin has specific affinity for the ganglioside GM_1 [94] and has been used as a marker for raft domains [95]. Lysenin is an sphingomyelin-specific toxin [96] that would also be expected to locate in rafts [97]. In addition, there are cholesterol-binding peptides and proteins, including a toxic peptide perfringolysin O that binds to cholesterol-rich domains in membranes [98–100].

There also may exist less specific recognition of cholesterol by some proteins. The existence of a consensus sequence having the pattern -L/V-(X)(1–5)-Y-(X)(1–5)-R/K-, in which (X)(1–5) represents between one to five residues of any amino acid has been suggested to be a cholesterol recognition motif [101]. The HIV-1 fusion protein, gp41, has a segment corresponding to this consensus sequence that is adjacent to the transmembrane anchor. This segment contains the sequence LWYIK and has been shown to promote membrane fusion both by mutational studies of the intact viral protein [102] as well as with the use of a synthetic peptide [103]. However, it is unlikely that the very general motif proposed by Li et al. [104] is sufficient to predict whether or not a particular protein will sequester into rafts. The sequence is so general that there are proteins that contain the sequence that are not found in the detergent-insoluble fraction of membranes. In addition, although the LWYIK sequence is conserved in HIV-1; HIV-2 and SIV have very similar sequences that likely play the same role as LWYIK of HIV-1 [105], but generally do not have a Tyr (Y) residue, and thus they do not strictly conform to the proposed consensus sequence (Table 1).

We propose that most proteins and peptides will affect the distribution of cholesterol in membranes because, in general, there will be a difference in their affinity for bilayers devoid of cholesterol compared with bilayers that are enriched in cholesterol. We have demonstrated that the peptide *N*-acetyl-LWYIK-amide preferentially partitions into domains of the bilayer enriched in cholesterol [69]. This results in a cholesterol-depleted domain that exhibits a phospholipid chain-melting transition of higher enthalpy that is observed by DSC (Fig. 1). In addition, MAS/NMR NOESY studies indicate there is deeper insertion of the peptide into bilayers containing cholesterol than into pure phosphatidylcholine membranes [69]. The formation of these domains is peptide-driven, occurring in membranes devoid of high melting lipids that do not form domains in the absence of peptides.

Table 1
Comparison of the conserved tryptophan-rich motifs in the membrane-proximal regions from related retroviruses

HIV-1	
92/BR025-9	WQNLWTWFGITNWLWYIK
GB8/C4	ANLWNWFDITNWLWYIK
NL4-3	WASLWNWFNITNWLWYIK
MN	WASLWNWFDITNWLWYIK
HXB2	WASLWNWFNITNWLWYIK
92/UG024-2	WASLWNWFDITNWLWYIK
SIV	
SIVcpzant	WSSLWNWFDITQWLWYIK
SIVcpz(Q88004)	LNSWDVFGNWFDLASWIK
SIVcpz	LNSWDVFGNWFD***LASWIR***
SIVmac251	LNSWDVFGNWFDLASWIK
SIVagm	LNSWDVFGNWFDLASWIK
SIVmac	LNSWDVFGNWFDLTSWIK
SIVsm	LNSWDIFGNWFDLTSWIK
SIVsm84	LNSWDIFGNWFDLASWIR
HIV-2	
HIV2CBL24	LNSWDVFGNWFDLASWIK
HIV-2ST	LNSWDVFGNWFDLTSWIK
HIV2CBL21	LNSWDVFGNWFDLTSWIR

The putative cholesterol binding sites are underlined.
Taken from Vincent et al. [105].

The protein segments discussed above would be expected to be at or close to the membrane interface. In addition, it is known that integral membrane proteins tend to be segregated either into or excluded from raft domains. These integral membrane proteins generally have transmembrane helices that also affect the location of the protein in the membrane. It is possible that a smooth, uniform surface contour of a transmembrane helix would more readily mix with a rigid cholesterol-rich domain. However, many transmembrane helices are excluded from raft-like domains because of the increased area compressibility modulus in the cholesterol-rich domains, rather than the increase in length [80,81]. Nevertheless, the length of the hydrophobic segment of the bilayer has been suggested to be a factor in favoring interaction with transmembrane helices of specific lengths, both for certain model peptides [106] as well as with the M2 channel protein from influenza virus [107]. However, even with transmembrane proteins, the juxtamembrane region will also influence the lateral distribution of the protein in the membrane, as discussed above for the LWYIK segment from the transmembrane gp41 protein of HIV.

Lipidation is another feature that can result in the translocation of proteins to raft domains. Many proteins that are acylated with saturated fatty acids, particularly palmitic acid, are found in membrane rafts [3]. This is not the case with prenylated proteins that are commonly excluded from raft domains [108]. Another form of protein lipidation is by attachment to a GPI-anchor that results in the protein being found in the low density detergent-insoluble fraction [109,110], suggesting incorporation into rafts. Alkaline phosphatase is a relatively abundant GPI-anchored protein. AFM studies have demonstrated its sequestering into raft domains [111]. Quantitative affinity purification has been used to demonstrate that different GPI-anchored proteins are sequestered into different raft-like domains [112], indicating a greater specificity for domain formation in biological systems than simple recognition of a lipidic moiety.

An interesting example of a myristoylated protein that preferentially binds to cholesterol-rich domains is the neuronal protein, NAP-22. NAP-22 itself is a highly acidic protein that is water-soluble. It exhibits the property of binding to liposomes containing cholesterol but not to pure phospholipid liposomes [68,113]. Calorimetry results indicate that the protein induces the segregation of cholesterol into domains [68]. In membranes with pre-existing domains, it has been shown by fluorescence microscopy that NAP-22 partitions into raft-like domains [114].

In addition, there are peptides and proteins that are preferentially excluded from cholesterol-rich domains. It is anticipated that this would be a common situation for many peptides and proteins that do not penetrate deeply into the membrane, since cholesterol will condense the liquid crystalline phase, making the insertion of substances into such membranes more energetically costly. We have recently demonstrated that an apolipoprotein A-I mimetic peptide, 4F, will induce the formation of cholesterol-rich regions in membranes by preferentially sequestering into bilayers depleted of cholesterol [70]. This results in segregating cholesterol into crystalline domains (Fig. 1). Even though this peptide is largely excluded from cholesterol-containing membranes, it nevertheless promotes the formation of cholesterol-rich regions. Thus, rather than directly stabilizing the cholesterol-rich domain, this amphipathic helical peptide indirectly causes the clustering of cholesterol by facilitating the formation of a cholesterol-depleted domain.

Another lipid component of raft domains is phosphatidylinositol 4,5-bisphosphate (PIP2) [115–118]. Although PIP2 is a minor lipid component of these domains, it has an important functional role in signal transduction as a precursor phosphatidylinositol 3,4,5-triphosphate as well as being a precursor for inositol triphosphate and the important lipid secondary messenger, diacylglycerol. In addition, PIP2 plays an important role in the attachment between the membrane and the actin cytoskeleton and is involved in the rearrangement of the cytoskeleton [119,120]. The function of PIP2 is modulated by binding to peptides and proteins. This interaction is largely electrostatic in nature and results in the formation of domains [121–123]. Proteins such as GAP-43, MARCKS and CAP-23/NAP-22 have a cluster of cationic residues in their amino acid sequence. CAP-23, a protein with a high sequence homology to NAP-22 and likely with very similar properties, was first identified by Widmer and Caroni [124]. Along with GAP-43 and MARCKS, CAP-23 accumulates in rafts, where it co-localizes with PIP2 [125]. It has been suggested that proteins with clusters of cationic residues bind a significant fraction of the PIP2 in a cell, helping to sequester it in lateral membrane domains, then release this lipid in response to local signals such as an increased concentration of Ca^{2+}/calmodulin or activation of protein kinase C [123]. Assuming that equilibrium exists between anionic lipids contained within domains and those distributed randomly in the bilayer, a polycationic peptide will preferentially bind to the anionic lipids in domains, driving the equilibrium toward domain formation. Entropy of mixing will oppose this segregation. In addition, the counterions bound to the domains of charged lipids will result in an additional energy term that will be reduced by the binding of oppositely charged peptides [121]. In the case of the MARCKS peptide, it has been shown that in liposomes the sequestering of PIP2 is not cholesterol-dependent [122]. However, the sequestering of PIP2 by a NAP-22 peptide is cholesterol-dependent [126]. Compared with MARCKS, NAP-22 has fewer cationic residues in the cationic cluster, and thus may be less effective in promoting the segregation of PIP2. We suggest that the role of cholesterol, in the case of the NAP-22 peptide, is related to its effect of increasing line tension [127,128]. An increase in line tension will favor the growth of domains so as to minimize the interfacial boundary. This would also be a factor for the MARCKS peptide, but

apparently the electrostatic interactions in this case are sufficiently strong that this factor is not required for the sequestering of PIP2 by MARCKS. PIP2 stimulates actin polymerization by causing the dissociation of gelsolin–actin complexes [129], establishing a linkage between the plasma membrane and the cytoskeleton. The model helps to explain the physiological action of NAP-22, a protein found in rafts [130], in reorganizing the actin cytoskeleton [119].

4. Conclusions

There are many methods for determining the presence of domains in membranes. Many of these methods are based on the use of fluorescent probes because of their specificity and sensitivity of detection. Methods such as DSC and NMR are useful for model membrane studies, but for most questions biological membranes are too heterogeneous for these methods to be useful. However, with fluorescence methods there is a potential danger that the large fluorophore will alter the properties of the molecule of interest. This is particularly a problem with cholesterol that has a conformationally restricted fused ring structure. In addition, even in cases where the fluorescent probe itself does not incorporate into the membrane, it can alter the distribution of molecules in the membrane by specifically interacting with certain components. An example of this is the clustering of the ganglioside, GM_1, by a fluorescently labeled cholera toxin [131–133], which is often used as a marker for cholesterol-rich domains.

The distribution of cholesterol in membranes is an important aspect of domain formation. There have been several studies characterizing the formation of domains and their physical properties in lipid mixtures of sphingomyelin, cholesterol and DOPC. There are two important features of biological membranes that are not incorporated into this simple lipid mixture. One is the transbilayer asymmetry of lipids of a biological membrane. There is very little, if any, sphingomyelin on the cytoplasmic surface of the cell membrane. Sphingomyelin–cholesterol-rich domains are in the liquid ordered phase. There may be domains in the extracellular leaflet of the plasma membranes that are cholesterol- and sphingomyelin-rich and are likely in the liquid ordered phase, but there is little information about the chemical or physical properties of the cytoplasmic side of these domains.

Another important component of biological membranes are the proteins that comprise about half the weight of the plasma membrane. There is evidence that certain proteins are found in the raft fraction of membranes, while other proteins are excluded from these domains. A direct thermodynamic consequence of the unequal distribution of proteins between domains is that this will affect the distribution of lipids between the domains. We discuss above how certain peptides will sequester into cholesterol-rich domains and stabilize these domains. This alters the cholesterol distribution in the membrane, even for bilayers not containing high-melting lipids. Sequestering of proteins to these domains likely has biological importance such as allowing signal transduction to proceed more efficiently by concentrating the interacting proteins in a single domain as well as making the fusion and assembly of certain enveloped viruses more efficient by concentrating the viral proteins.

However, cholesterol-rich domains will also be formed as a consequence of the inability of peptides and proteins to sequester into bilayers containing a high mole fraction of cholesterol. These substances will stabilize cholesterol-depleted domains, forcing the cholesterol to be concentrated in other domains. Thus, one would anticipate that most proteins and peptide would promote the formation of cholesterol-rich domains because they do not partition equally between cholesterol-rich and cholesterol-poor domains. It would be the exception that a protein would not discriminate between these domains. Pure lipid mixtures can also segregate into domains. The formation of cholesterol-rich domains in protein-containing biological membranes will be a consequence of the redistribution of molecular components in a manner that lowers the energy of the system. Describing the formation of domains as proteins partitioning between raft and non-raft regions is incomplete, since this partitioning will also affect the distribution of lipids. Similarly, describing the formation of lipid domains as being a consequence of their affinity for peptides and proteins does not take into account the fact that the lipids themselves will have a tendency to segregate or that domains will also form from the lipids that do not interact with the peptides or proteins.

With regard to rafts of biological membranes, there are several alternative definitions used in the literature based on their chemical composition, their detergent insolubility or their physical properties. Whether they are called rafts or not, all cholesterol-rich domains probably do not have identical physical properties. Domains of sphingomyelin and cholesterol or of saturated phosphatidylcholine and cholesterol have been characterized in model membranes as being in the liquid ordered phase. However, cholesterol-rich domains are also formed from lipids that do not have a high melting temperature. The acyl chains of the phospholipids in these domains are probably not as extended as those having high melting lipids, although they can still have properties similar to the liquid ordered phase [134]. Thus, the distribution of cholesterol in membranes is a consequence of all of the intermolecular interactions among lipid molecules, as well as between lipids and proteins.

Acknowledgement

The author is grateful to Drs. Christopher M. Yip and Raquel F. Epand for their comments and helpful suggestions.

References

[1] M.A. Alonso, J. Millan, The role of lipid rafts in signalling and membrane trafficking in T lymphocytes, J. Cell. Sci. 114 (2001) 3957–3965.

[2] R.G. Anderson, K. Jacobson, A role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains, Science 296 (2002) 1821–1825.

[3] D.A. Brown, E. London, Structure and function of sphingolipid- and cholesterol-rich membrane rafts, J. Biol. Chem. 275 (2000) 17221–17224.

[4] M. Edidin, The state of lipid rafts: from model membranes to cells, Annu. Rev. Biophys. Biomol. Struct. 32 (2003) 257–283.

[5] F. Galbiati, B. Razani, M.P. Lisanti, Emerging themes in lipid rafts and caveolae, Cell 106 (2001) 403–411.

[6] B. Razani, S.E. Woodman, M.P. Lisanti, Caveolae: from cell biology to animal physiology, Pharmacol. Rev. 54 (2002) 431–467.

[7] M.J. Zuckermann, J.H. Ipsen, L. Miao, O.G. Mouritsen, M. Nielsen, J. Polson, J. Thewalt, I. Vattulainen, H. Zhu, Modeling lipid–sterol bilayers: applications to structural evolution, lateral diffusion, and rafts, Methods Enzymol. 383 (2004) 198–229.

[8] L.J. Pike, Lipid rafts: bringing order to chaos, J. Lipid Res. 44 (2003) 655–667.

[9] K. Simons, D. Toomre, Lipid rafts and signal transduction, Nat. Rev., Mol. Cell Biol. 1 (2000) 31–39.

[10] R.M. Young, D. Holowka, B. Baird, A lipid raft environment enhances Lyn kinase activity by protecting the active site tyrosine from dephosphorylation, J. Biol. Chem. 278 (2003) 20746–20752.

[11] J.R. Martens, K. O'Connell, M. Tamkun, Targeting of ion channels to membrane microdomains: localization of KV channels to lipid rafts, Trends Pharmacol. Sci. 25 (2004) 16–21.

[12] K. Simons, R. Ehehalt, Cholesterol, lipid rafts, and disease, J. Clin. Invest. 110 (2002) 597–603.

[13] T.P.W. McMullen, R.N.A.H. Lewis, R.N. McElhaney, Cholesterol–phospholipid interactions, the liquid-ordered phase and lipid rafts in model and biological membranes, Curr. Opin. Coll. Interface Sci. 8 (2004) 459–468.

[14] E.C. Lai, Lipid rafts make for slippery platforms, J. Cell Biol. 162 (2003) 365–370.

[15] S. Munro, Lipid rafts: elusive or illusive? Cell 115 (2003) 377–388.

[16] H. Heerklotz, Triton promotes domain formation in lipid raft mixtures, Biophys. J. 83 (2002) 2693–2701.

[17] J. Fullekrug, K. Simons, Lipid rafts and apical membrane traffic, Ann. N.Y. Acad. Sci. 1014 (2004) 164–169.

[18] L.H. Chamberlain, Detergents as tools for the purification and classification of lipid rafts, FEBS Lett. 559 (2004) 1–5.

[19] L.J. Pike, Lipid rafts: heterogeneity on the high seas, Biochem. J. 378 (2004) 281–292.

[20] T. Harder, Formation of functional cell membrane domains: the interplay of lipid- and protein-mediated interactions, Philos. Trans. R. Soc. Lond. B Biol. Sci. 358 (2003) 863–868.

[21] J. Risbo, M.M. Sperotto, O.G. Mouritsen, Theory of phase-equilibria and critical mixing points in binary lipid bilayers, J. Chem. Phys. 103 (1995) 3643–3656.

[22] A. Hinderliter, P.F. Almeida, C.E. Creutz, R.L. Biltonen, Domain formation in a fluid mixed lipid bilayer modulated through binding of the C2 protein motif, Biochemistry 40 (2001) 4181–4191.

[23] A. Hinderliter, R.L. Biltonen, P.F. Almeida, Lipid modulation of protein-induced membrane domains as a mechanism for controlling signal transduction, Biochemistry 43 (2004) 7102–7110.

[24] E. London, D.A. Brown, X.L. Xu, Fluorescence quenching assay of sphingolipid/phospholipid phase separation in model membranes, Methods Enzymol. 312 (2000) 272–290.

[25] M.E. Fastenberg, H. Shogomori, X. Xu, D.A. Brown, E. London, Exclusion of a transmembrane-type peptide from ordered-lipid domains (rafts) detected by fluorescence quenching: extension of quenching analysis to account for the effects of domain size and domain boundaries, Biochemistry 42 (2003) 12376–12390.

[26] A. Polozova, B.J. Litman, Cholesterol dependent recruitment of di22:6-PC by a G protein-coupled receptor into lateral domains, Biophys. J. 79 (2000) 2632–2643.

[27] G.W. Feigenson, J.T. Buboltz, Ternary phase diagram of dipalmitoyl-PC/dilauroyl-PC/cholesterol: nanoscopic domain formation driven by cholesterol, Biophys. J. 80 (2001) 2775–2788.

[28] J.R. Silvius, Fluorescence energy transfer reveals microdomain formation at physiological temperatures in lipid mixtures modeling the outer leaflet of the plasma membrane, Biophys. J. 85 (2003) 1034–1045.

[29] D.A. Zacharias, J.D. Violin, A.C. Newton, R.Y. Tsien, Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells, Science 296 (2002) 913–916.

[30] P. Sharma, R. Varma, R.C. Sarasij, X.X. Ira, K. Gousset, G. Krishnamoorthy, M. Rao, S. Mayor, Nanoscale organization of multiple GPI-anchored proteins in living cell membranes, Cell 116 (2004) 577–589.

[31] A. Gidwani, D. Holowka, B. Baird, Fluorescence anisotropy measurements of lipid order in plasma membranes and lipid rafts from RBL-2H3 mast cells, Biochemistry 40 (2001) 12422–12429.

[32] M. Sinha, S. Mishra, P.G. Joshi, Liquid-ordered microdomains in lipid rafts and plasma membrane of U-87 MG cells: a time-resolved fluorescence study, Eur. Biophys. J. 32 (2003) 381–391.

[33] N. Kahya, D. Scherfeld, K. Bacia, B. Poolman, P. Schwille, Probing lipid mobility of raft-exhibiting model membranes by fluorescence correlation spectroscopy, J. Biol. Chem. 278 (2003) 28109–28115.

[34] J. Korlach, P. Schwille, W.W. Webb, G.W. Feigenson, Characterization of lipid bilayer phases by confocal microscopy and fluorescence correlation spectroscopy, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 8461–8466.

[35] D. Scherfeld, N. Kahya, P. Schwille, Lipid dynamics and domain formation in model membranes composed of ternary mixtures of unsaturated and saturated phosphatidylcholines and cholesterol, Biophys. J. 85 (2003) 3758–3768.

[36] N. Kahya, D. Scherfeld, K. Bacia, P. Schwille, Lipid domain formation and dynamics in giant unilamellar vesicles explored by fluorescence correlation spectroscopy, J. Struct. Biol. 147 (2004) 77–89.

[37] C. Dietrich, L.A. Bagatolli, Z.N. Volovyk, N.L. Thompson, M. Levi, K. Jacobson, E. Gratton, Lipid rafts reconstituted in model membranes, Biophys. J. 80 (2001) 1417–1428.

[38] S.L. Veatch, S.L. Keller, Separation of liquid phases in giant vesicles of ternary mixtures of phospholipids and cholesterol, Biophys. J. 85 (2003) 3074–3083.

[39] D.I. Mundy, T. Machleidt, Y.S. Ying, R.G. Anderson, G.S. Bloom, Dual control of caveolar membrane traffic by microtubules and the actin cytoskeleton, J. Cell. Sci. 115 (2002) 4327–4339.

[40] U. Lahtinen, M. Honsho, R.G. Parton, K. Simons, P. Verkade, Involvement of caveolin-2 in caveolar biogenesis in MDCK cells, FEBS Lett. 538 (2003) 85–88.

[41] C. Dietrich, B. Yang, T. Fujiwara, A. Kusumi, K. Jacobson, Relationship of lipid rafts to transient confinement zones detected by single particle tracking, Biophys. J. 82 (2002) 274–284.

[42] W.K. Subczynski, A. Kusumi, Dynamics of raft molecules in the cell and artificial membranes: approaches by pulse EPR spin labeling and single molecule optical microscopy, Biochim. Biophys. Acta 1610 (2003) 231–243.

[43] J. Kwik, S. Boyle, D. Fooksman, L. Margolis, M.P. Sheetz, M. Edidin, Membrane cholesterol, lateral mobility, and the phosphatidylinositol 4,5-bisphosphate-dependent organization of cell actin, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13964–13969.

[44] P.H. Lommerse, G.A. Blab, L. Cognet, G.S. Harms, B.E. Snaar-Jagalska, H.P. Spaink, T. Schmidt, Single-molecule imaging of the h-

ras membrane-anchor reveals domains in the cytoplasmic leaflet of the cell membrane, Biophys. J. 86 (2004) 609–616.
[45] D. Axelrod, Total internal reflection fluorescence microscopy in cell biology, Traffic 2 (2001) 764–774.
[46] J.E. Shaw, A. Slade, C.M. Yip, Simultaneous in situ total internal reflectance fluorescence/atomic force microscopy studies of DPPC/dPOPC microdomains in supported planar lipid bilayers, J. Am. Chem. Soc. 125 (2003) 11838–11839.
[47] C.M. Yip, Atomic force microscopy of macromolecular interactions, Curr. Opin. Struct. Biol. 11 (2001) 567–572.
[49] F. Tokumasu, A.J. Jin, G.W. Feigenson, J.A. Dvorak, Atomic force microscopy of nanometric liposome adsorption and nanoscopic membrane domain formation, Ultramicroscopy 97 (2003) 217–227.
[50] B.Y. van Duyl, D. Ganchev, V. Chupin, B. de Kruijff, J.A. Killian, Sphingomyelin is much more effective than saturated phosphatidylcholine in excluding unsaturated phosphatidylcholine from domains formed with cholesterol, FEBS Lett. 547 (2003) 101–106.
[51] J.C. Lawrence, D.E. Saslowsky, J.M. Edwardson, R.M. Henderson, Real-time analysis of the effects of cholesterol on lipid raft behavior using atomic force microscopy, Biophys. J. 84 (2003) 1827–1832.
[52] J.M. Crane, L.K. Tamm, Role of cholesterol in the formation and nature of lipid rafts in planar and spherical model membranes, Biophys. J. 86 (2004) 2965–2979.
[53] J. Tong, T.J. McIntosh, Structure of supported bilayers composed of lipopolysaccharides and bacterial phospholipids: raft formation and implications for bacterial resistance, Biophys. J. 86 (2004) 3759–3771.
[54] M.C. Giocondi, P.E. Milhiet, P. Dosset, C. Le Grimellec, Use of cyclodextrin for AFM monitoring of model raft formation, Biophys. J. 86 (2004) 861–869.
[55] B.L. Stottrup, S.L. Veatch, S.L. Keller, Nonequilibrium behavior in supported lipid membranes containing cholesterol, Biophys. J. 86 (2004) 2942–2950.
[56] D.E. Saslowsky, J. Lawrence, X. Ren, D.A. Brown, R.M. Henderson, J.M. Edwardson, Placental alkaline phosphatase is efficiently targeted to rafts in supported lipid bilayers, J. Biol. Chem. 277 (2002) 26966–26970.
[57] P. Burgos, Z. Lu, A. Ianoul, C. Hnatovsky, M.L. Viriot, L.J. Johnston, R.S. Taylor, Near-field scanning optical microscopy probes: a comparison of pulled and double-etched bent NSOM probes for fluorescence imaging of biological samples, J. Microsc. 211 (2003) 37–47.
[58] J. Kavecansky, C.H. Joiner, F. Schroeder, Erythrocyte membrane lateral sterol domains: a dehydroergosterol fluorescence polarization study, Biochemistry 33 (1994) 2880–2890.
[59] A.L. McIntosh, A.M. Gallegos, B.P. Atshaves, S.M. Storey, D. Kannoju, F. Schroeder, Fluorescence and multiphoton imaging resolve unique structural forms of sterol in membranes of living cells, J. Biol. Chem. 278 (2003) 6384–6403.
[60] R. Rukmini, S.S. Rawat, S.C. Biswas, A. Chattopadhyay, Cholesterol organization in membranes at low concentrations: effects of curvature stress and membrane thickness, Biophys. J. 81 (2001) 2122–2134.
[61] F. Schroeder, G. Nemecz, E. Gratton, Y. Barenholz, T.E. Thompson, Fluorescence properties of cholestatrienol in phosphatidylcholine bilayer vesicles, Biophys. Chemist. 32 (1988) 57–72.
[62] A.D. Albert, J.E. Young, P.L. Yeagle, Rhodopsin-cholesterol interactions in bovine rod outer segment disk membranes, Biochim. Biophys. Acta 1285 (1996) 47–55.
[63] G. Smutzer, B.F. Crawford, P.L. Yeagle, Physical properties of the fluorescent sterol probe dehydroergosterol, Biochim. Biophys. Acta 862 (1986) 361–371.
[64] P.L. Yeagle, A.D. Albert, K. Boesze-Battaglia, J. Young, J. Frye, Cholesterol dynamics in membranes, Biophys. J. 57 (1990) 413–424.
[65] K. Boesze-Battaglia, S.T. Clayton, R.J. Schimmel, Cholesterol redistribution within human platelet plasma membrane: evidence for a stimulus-dependent event, Biochemistry 35 (1996) 6664–6673.
[66] H.A. Scheidt, P. Muller, A. Herrmann, D. Huster, The potential of fluorescent and spin-labeled steroid analogs to mimic natural cholesterol, J. Biol. Chem. 278 (2003) 45563–45569.
[67] R.F. de Almeida, A. Fedorov, M. Prieto, Sphingomyelin/phosphatidylcholine/cholesterol phase diagram: boundaries and composition of lipid rafts, Biophys. J. 85 (2003) 2406–2416.
[68] R.M. Epand, S. Maekawa, C.M. Yip, R.F. Epand, Protein-induced formation of cholesterol-rich domains, Biochemistry 40 (2001) 10514–10521.
[69] R.M. Epand, B.G. Sayer, R.F. Epand, Peptide-induced formation of cholesterol-rich domains, Biochemistry 42 (2003) 14677–14689.
[70] R.M. Epand, R.F. Epand, B.G. Sayer, G. Melacini, M.N. Palgulachari, J.P. Segrest, G.M. Anantharamaiah, An apolipoprotein AI mimetic peptide: membrane interactions and the role of cholesterol, Biochemistry 43 (2004) 5073–5083.
[71] M.R. Vist, J.H. Davis, Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: ^{2}H nuclear magnetic resonance and differential scanning calorimetry, Biochemistry 29 (1990) 451–464.
[72] F. Aussenac, M. Tavares, E.J. Dufourc, Cholesterol dynamics in membranes of raft composition: a molecular point of view from ^{2}H and ^{31}P solid-state NMR, Biochemistry 42 (2003) 1383–1390.
[73] S.L. Veatch, I.V. Polozov, K. Gawrisch, S.L. Keller, Liquid domains in vesicles investigated by NMR and fluorescence microscopy, Biophys. J. 86 (2004) 2910–2922.
[74] W. Guo, J.A. Hamilton, ^{13}C MAS NMR studies of crystalline cholesterol and lipid mixtures modeling atherosclerotic plaques, Biophys. J. 71 (1996) 2857–2868.
[75] R.M. Epand, A.D. Bain, B.G. Sayer, D. Bach, E. Wachtel, Properties of mixtures of cholesterol with phosphatidylcholine or with phosphatidylserine studied by ^{13}C magic angle spinning nuclear magnetic resonance, Biophys. J. 83 (2002) 2053–2063.
[76] S.L. Grage, J.A. Watts, A. Watts, $^{2}H[^{19}F]$ REDOR for distance measurements in biological solids using a double resonance spectrometer, J. Magn. Reson. 166 (2004) 1–10.
[77] A.K. Mehta, L. Cegelski, R.D. O'Connor, J. Schaefer, REDOR with a relative full-echo reference, J. Magn. Reson. 163 (2003) 182–187.
[78] W.M. Yau, W.C. Wimley, K. Gawrisch, S.H. White, The preference of tryptophan for membrane interfaces, Biochemistry 37 (1998) 14713–14718.
[79] W. Zhang, E. Crocker, S. McLaughlin, S.O. Smith, Binding of peptides with basic and aromatic residues to bilayer membranes: phenylalanine in the myristoylated Alanine-rich C kinase substrate effector domain penetrates into the hydrophobic core of the bilayer, J. Biol. Chem. 278 (2003) 21459–21466.
[80] T.J. McIntosh, The 2004 Biophysical Society-Avanti Award in Lipids address: roles of bilayer structure and elastic properties in peptide localization in membranes, Chem. Phys. Lipids 130 (2004) 83–98.
[81] T.J. McIntosh, A. Vidal, S.A. Simon, Sorting of lipids and transmembrane peptides between detergent-soluble bilayers and detergent-resistant rafts, Biophys. J. 85 (2003) 1656–1666.
[82] W. Knoll, G. Schmidt, H. Rotzer, T. Henkel, W. Pfeiffer, E. Sackmann, S. Mittler-Neher, J. Spinke, Lateral order in binary lipid alloys and its coupling to membrane functions, Chem. Phys. Lipids 57 (1991) 363–374.
[83] S. Fahsel, E.M. Pospiech, M. Zein, T.L. Hazlet, E. Gratton, R. Winter, Modulation of concentration fluctuations in phase-separated lipid membranes by polypeptide insertion, Biophys. J. 83 (2002) 334–344.
[84] S.L. Veatch, S.L. Keller, A closer look at the canonical 'Raft Mixture' in model membrane studies, Biophys. J. 84 (2003) 725–726.

[85] H.M. McConnell, A. Radhakrishnan, Condensed complexes of cholesterol and phospholipids, Biochim. Biophys. Acta 1610 (2003) 159–173.
[86] H.M. McConnell, M. Vrljic, Liquid–liquid immiscibility in membranes, Annu. Rev. Biophys. Biomol. Struct. 32 (2003) 469–492.
[87] M.M. Wang, M. Olsher, I.P. Sugar, P.L. Chong, Cholesterol superlattice modulates the activity of cholesterol oxidase in lipid membranes, Biochemistry 43 (2004) 2159–2166.
[88] B. Cannon, G. Heath, J. Huang, P. Somerharju, J.A. Virtanen, K.H. Cheng, Time-resolved fluorescence and Fourier transform infrared spectroscopic investigations of lateral packing defects and superlattice domains in compositionally uniform cholesterol/phosphatidylcholine bilayers, Biophys. J. 84 (2003) 3777–3791.
[89] J. Huang, Exploration of molecular interactions in cholesterol superlattices: effect of multibody interactions, Biophys. J. 83 (2002) 1014–1025.
[90] P.L. Chong, I.P. Sugar, Fluorescence studies of lipid regular distribution in membranes, Chem. Phys. Lipids 116 (2002) 153–175.
[91] F. Liu, P.L. Chong, Evidence for a regulatory role of cholesterol superlattices in the hydrolytic activity of secretory phospholipase A2 in lipid membranes, Biochemistry 38 (1999) 3867–3873.
[92] F.T. Presti, R.J. Pace, S.I. Chan, Cholesterol–phospholipid interaction in membranes: 2. Stoichiometry and molecular packing of cholesterol-rich domains, Biochemistry 21 (1982) 3831–3835.
[93] S.L. Veatch, S.L. Keller, Separation of liquid phases in giant vesicles of ternary mixtures of phospholipids and cholesterol, Biophys. J. 85 (2003) 3074–3083.
[94] B. Lanne, B. Schierbeck, J. Angstrom, Binding of cholera toxin B-subunits to derivatives of the natural ganglioside receptor, GM1, J. Biochem. (Tokyo) 126 (1999) 226–234.
[95] A.K. Kenworthy, N. Petranova, M. Edidin, High-resolution FRET microscopy of cholera toxin B-subunit and GPI-anchored proteins in cell plasma membranes, Mol. Biol. Cell 11 (2000) 1645–1655.
[96] A. Yamaji-Hasegawa, A. Makino, T. Baba, Y. Senoh, H. Kimura-Suda, S.B. Sato, N. Terada, S. Ohno, E. Kiyokawa, M. Umeda, T. Kobayashi, Oligomerization and pore formation of a sphingomyelin-specific toxin, lysenin, J. Biol. Chem. 278 (2003) 22762–22770.
[97] A.B.A. Shakor, E.A. Czurylo, A. Sobota, Lysenin, a unique sphingomyelin-binding protein, FEBS Lett. 542 (2003) 1–6.
[98] R. Ramachandran, A.P. Heuck, R.K. Tweten, A.E. Johnson, Structural insights into the membrane-anchoring mechanism of a cholesterol-dependent cytolysin, Nat. Struct. Biol. 9 (2002) 823–827.
[99] Y. Shimada, M. Maruya, S. Iwashita, Y. Ohno-Iwashita, The C-terminal domain of perfringolysin O is an essential cholesterol-binding unit targeting to cholesterol-rich microdomains, Eur. J. Biochem. 269 (2002) 6195–6203.
[100] A.A. Waheed, Y. Shimada, H.F. Heijnen, M. Nakamura, M. Inomata, M. Hayashi, S. Iwashita, J.W. Slot, Y. Ohno-Iwashita, Selective binding of perfringolysin O derivative to cholesterol-rich membrane microdomains (rafts), Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4926–4931.
[101] H. Li, V. Papadopoulos, Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern, Endocrinology 139 (1998) 4991–4997.
[102] K. Salzwedel, J.T. West, E. Hunter, A conserved tryptophan-rich motif in the membrane-proximal region of the human immunodeficiency virus type 1 gp41 ectodomain is important for Env-mediated fusion and virus infectivity, J. Virol. 73 (1999) 2469–2480.
[103] A. Saez-Cirion, S. Nir, M. Lorizate, A. Agirre, A. Cruz, J. Perez-Gil, J.L. Nieva, Sphingomyelin and cholesterol promote HIV-1 gp41 pretransmembrane sequence surface aggregation and membrane restructuring, J. Biol. Chem. 277 (2002) 21776–21785.
[104] H. Li, Z. Yao, B. Degenhardt, G. Teper, V. Papadopoulos, Cholesterol binding at the cholesterol recognition/interaction amino acid consensus (CRAC) of the peripheral-type benzodiazepine receptor and inhibition of steroidogenesis by an HIV TAT-CRAC peptide, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 1267–1272.
[105] N. Vincent, C. Genin, E. Malvoisin, Identification of a conserved domain of the HIV-1 transmembrane protein gp41 which interacts with cholesteryl groups, Biochim. Biophys. Acta 1567 (2002) 157–164.
[106] J. Ren, S. Lew, Z. Wang, E. London, Transmembrane orientation of hydrophobic alpha-helices is regulated both by the relationship of helix length to bilayer thickness and by the cholesterol concentration, Biochemistry 36 (1997) 10213–10220.
[107] L. Cristian, J.D. Lear, W.F. DeGrado, Use of thiol-disulfide equilibria to measure the energetics of assembly of transmembrane helices in phospholipid bilayers, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 14772–14777.
[108] K.A. Melkonian, A.G. Ostermeyer, J.Z. Chen, M.G. Roth, D.A. Brown, Role of lipid modifications in targeting proteins to detergent-resistant membrane rafts. Many raft proteins are acylated, while few are prenylated, J. Biol. Chem. 274 (1999) 3910–3917.
[109] S. Morandat, M. Bortolato, B. Roux, Cholesterol-dependent insertion of glycosylphosphatidylinositol-anchored enzyme, Biochim. Biophys. Acta 1564 (2002) 473–478.
[110] F.J. Sharom, M.T. Lehto, Glycosylphosphatidylinositol-anchored proteins: structure, function, and cleavage by phosphatidylinositol-specific phospholipase C, Biochem. Cell. Biol. 80 (2002) 535–549.
[111] P.E. Milhiet, M.C. Giocondi, C. Le Grimellec, Cholesterol is not crucial for the existence of microdomains in kidney brush-border membrane models, J. Biol. Chem. 277 (2002) 875–878.
[112] J. Wang, W. Gunning, K.M. Kelley, M. Ratnam, Evidence for segregation of heterologous GPI-anchored proteins into separate lipid rafts within the plasma membrane, J. Membr. Biol. 189 (2002) 35–43.
[113] S. Maekawa, C. Sato, K. Kitajima, N. Funatsu, H. Kumanogoh, Y. Sokawa, Cholesterol-dependent localization of NAP-22 on a neuronal membrane microdomain (raft), J. Biol. Chem. 274 (1999) 21369–21374.
[114] T.K. Khan, B. Yang, N.L. Thompson, S. Maekawa, R.M. Epand, K. Jacobson, Binding of NAP-22, a calmodulin-binding neuronal protein, to raft-like domains in model membranes, Biochemistry 42 (2003) 4780–4786.
[115] I. Parmryd, J. Adler, R. Patel, A.I. Magee, Imaging metabolism of phosphatidylinositol 4,5-bisphosphate in T-cell GM1-enriched domains containing Ras proteins, Exp. Cell Res. 285 (2003) 27–38.
[116] D.R. Klopfenstein, M. Tomishige, N. Stuurman, R.D. Vale, Role of phosphatidylinositol(4,5)bisphosphate organization in membrane transport by the Unc104 kinesin motor, Cell 109 (2002) 347–358.
[117] L.J. Pike, L. Casey, Localization and turnover of phosphatidylinositol 4,5-bisphosphate in caveolin-enriched membrane domains, J. Biol. Chem. 271 (1996) 26453–26456.
[118] Y. Liu, L. Casey, L.J. Pike, Compartmentalization of phosphatidylinositol 4,5-bisphosphate in low-density membrane domains in the absence of caveolin, Biochem. Biophys. Res. Commun. 245 (1998) 684–690.
[119] P. Caroni, New EMBO members' review: actin cytoskeleton regulation through modulation of PI(4,5)P(2) rafts, EMBO J. 20 (2001) 4332–4336.
[120] A.L. Rozelle, L.M. Machesky, M. Yamamoto, M.H. Driessens, R.H. Insall, M.G. Roth, K. Luby-Phelps, G. Marriott, A. Hall, H.L. Yin, Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP-Arp2/3, Curr. Biol. 10 (2000) 311–320.
[121] G. Denisov, S. Wanaski, P. Luan, M. Glaser, S. McLaughlin, Binding of basic peptides to membranes produces lateral domains enriched in the acidic lipids phosphatidylserine and phosphatidylinositol 4,5-bisphosphate: an electrostatic model and experimental results, Biophys. J. 74 (1998) 731–744.

[122] A. Gambhir, G. Hangyas-Mihalyne, I. Zaitseva, D.S. Cafiso, J. Wang, D. Murray, S.N. Pentyala, S.O. Smith, S. McLaughlin, Electrostatic sequestration of PIP2 on phospholipid membranes by basic/aromatic regions of proteins, Biophys. J. 86 (2004) 2188–2207.
[123] S. McLaughlin, J. Wang, A. Gambhir, D. Murray, PIP(2) and proteins: interactions, organization, and information flow, Annu. Rev. Biophys. Biomol. Struct. 31 (2002) 151–175.
[124] F. Widmer, P. Caroni, Identification, localization, and primary structure of CAP-23, a particle-bound cytosolic protein of early development, J. Cell Biol. 111 (1990) 3035–3047.
[125] T. Laux, K. Fukami, M. Thelen, T. Golub, D. Frey, P. Caroni, GAP43, MARCKS, and CAP23 modulate PI(4,5)P(2) at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism, J. Cell Biol. 149 (2000) 1455–1472.
[126] R.M. Epand, P. Vuong, C.M. Yip, S. Maekawa, R.F. Epand, Cholesterol-dependent partitioning of PtdIns(4,5)P2 into membrane domains by the N-terminal fragment of NAP-22 (neuronal axonal myristoylated membrane protein of 22 kDa), Biochem. J. 379 (2004) 527–532.
[127] E. Karatekin, O. Sandre, H. Guitouni, N. Borghi, P.H. Puech, F. Brochard-Wyart, Cascades of transient pores in giant vesicles: line tension and transport, Biophys. J. 84 (2003) 1734–1749.
[128] D.V. Zhelev, D. Needham, Tension-stabilized pores in giant vesicles: determination of pore size and pore line tension, Biochim. Biophys. Acta 1147 (1993) 89–104.
[129] P.A. Janmey, T.P. Stossel, Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate, Nature 325 (1987) 362–364.
[130] A. Terashita, N. Funatsu, M. Umeda, Y. Shimada, Y. Ohno-Iwashita, R.M. Epand, S. Maekawa, Lipid binding activity of a neuron-specific protein NAP-22 studied in vivo and in vitro, J. Neurosci. Res. 70 (2002) 172–179.
[131] P. Antes, G. Schwarzmann, K. Sandhoff, Detection of protein mediated glycosphingolipid clustering by the use of resonance energy transfer between fluorescent labelled lipids. A method established by applying the system ganglioside GM1 and cholera toxin B subunit, Chem. Phys. Lipids 62 (1992) 269–280.
[132] J.S. Mitchell, O. Kanca, B.W. McIntyre, Lipid microdomain clustering induces a redistribution of antigen recognition and adhesion molecules on human T lymphocytes, J. Immunol. 168 (2002) 2737–2744.
[133] P. Nagy, G. Vereb, Z. Sebestyen, G. Horvath, S.J. Lockett, S. Damjanovich, J.W. Park, T.M. Jovin, J. Szollosi, Lipid rafts and the local density of ErbB proteins influence the biological role of homo- and heteroassociations of ErbB2, J. Cell. Sci. 115 (2002) 4251–4262.
[134] C.R. Mateo, A.A. Ulises, J.C. Brochon, Liquid–crystalline phases of cholesterol/lipid bilayers as revealed by the fluorescence of *trans*-parinaric acid, Biophys. J. 68 (1995) 978–987.