

The Dynamic Cell

Joint Biochemical Society and British Society for Cell Biology Focused Meeting held at Appleton Tower, University of Edinburgh, U.K., 1–4 April 2009. Organized and Edited by Ian Dransfield (Edinburgh, U.K.), Margarete Heck (Edinburgh, U.K.), Kairbaan Hodivala-Dilke (Cancer Research UK, London, U.K.), Robert Insall (Beatson Institute for Cancer Research, Glasgow, U.K.), Andrew McAinsh (Marie Curie Research Institute, Oxted, U.K.) and Barbara Reaves (Bath, U.K.).

Lipid rafts as functional heterogeneity in cell membranes

Daniel Lingwood, Hermann-Josef Kaiser, Ilya Levental and Kai Simons¹

Max Planck Institute for Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany

Abstract

Biological membranes are not structurally passive solvents of amphipathic proteins and lipids. Rather, it appears their constituents have evolved intrinsic characteristics that make homogeneous distribution of components unlikely. As a case in point, the concept of lipid rafts has received considerable attention from biologists and biophysicists since the formalization of the hypothesis more than 10 years ago. Today, it is clear that sphingolipid and cholesterol can self-associate into micron-scaled phases in model membranes and that these lipids are involved in the formation of highly dynamic nanoscale heterogeneity in the plasma membrane of living cells. However, it remains unclear whether these entities are manifestations of the same principle. A powerful means by which the molecular organization of rafts can be assessed is through analysis of their functionalized condition. Raft heterogeneity can be activated to coalesce and laterally reorganize/stabilize bioactivity in cell membranes. Evaluation of this property suggests that functional raft heterogeneity arises through principles of lipid-driven phase segregation coupled to additional chemical specificities, probably involving proteins.

Definitions

Lipid rafts are currently defined as dynamic sterol-sphingolipid-enriched nanoscale assemblies that associate and dissociate on a sub-second timescale [1,2]. Protein localization to these membrane environments is primarily mediated by a GPI (glycosylphosphatidylinositol) anchor, acylation or certain transmembrane domains [3]. Although still structurally elusive, biologically, it appears that rafts provide a means for cell membranes to form dynamic platforms within the bilayer, functioning in membrane trafficking, signal transduction and cell polarization [4,5]. In the present paper, we review the concept as it relates to our present understanding of functional heterogeneity in cell membranes.

Origins

The raft hypothesis was originally developed as an explanation for the enrichment of GSL (glycosphingolipid) in the apical membrane of polarized epithelial cells [6]. Unlike the specific signals used by proteins, there is no known soluble factor that can organize the lateral distribution of membrane lipids [7]. However, asymmetric enrichment of GSL implies that, during their journey to the largely immiscible apical and basolateral surfaces [8], lipids are somehow divided in the plane of the membrane. Indeed, lipodomies of post-Golgi carriers from yeast now reveals that glycerophospholipids are segregated away from GSL and sterol during trafficking to the plasma membrane [9]. Simons and van Meer [6] hypothesized that the self-associative properties of GSL *in vitro*, i.e. their capacity for hydrogen bonding [10–12], could lead to the formation of membrane domains as a basis for lipid sorting. The discovery of cholesterol-dependent L_o (liquid-ordered)–L_d (liquid-disordered) phase separation in wholly liquid model membranes [13–15] would later change the focus away from lateral heterogeneity by hydrogen-bonding to domain formation by differences in lipid order parameters [16,17]. In this case, L_o phase formation occurs

Key words: chemical interaction, cholesterol, lateral self-organization, membrane order, sphingolipid.

Abbreviations used: CTB, cholera toxin b-subunit; DRM, detergent-resistant membrane; GPI, glycosylphosphatidylinositol; GPMV, giant plasma membrane vesicle; GSL, glycosphingolipid; GUV, giant unilamellar vesicle; L_d, liquid-disordered; L_o, liquid-ordered; PMS, plasma membrane sphere; STED, stimulated emission depletion; TCR, T-cell receptor; TM, transmembrane.

¹To whom correspondence should be addressed (email simons@mpi-cbg.de).

via preferential association between cholesterol and longer saturated lipid hydrocarbon chains [18]. Although both forms of lipid assemblage potential now appear to be important (see below), it is worth mentioning an often forgotten fact: unlike previous descriptions of membrane domain formation [19,20], the raft hypothesis was uniquely geared to explain a functional biological phenomenon, i.e. lipids are sorted during membrane trafficking. Indeed, later discoveries of raft-mediated changes in lateral dimensionality and its consequent modulation of membrane bioactivity (e.g. signalling foci [21]) stress the need to relate principles of heterogeneous self-organization to the most likely evolutionarily selected need for functional association within cell membranes.

Detergent-resistance

The first working definition of lipid rafts was suggested by Brown and Rose [22] who reported that sphingolipids and GPI-anchored proteins from cell membranes were insoluble in Triton X-100 at 4°C and floated to a characteristic density following equilibrium density gradient centrifugation. Coupled with the observation that this detergent-insolubility was cholesterol-dependent and enriched for L_o phase constituents [23], these preparations (broadly defined as detergent-resistant membranes or DRMs) quickly became the ruling method for assigning lipid and protein raft affinity. However, using the standard Triton X-100 preparation, DRMs from animal cells are obtained at 4°C, but not 37°C, indicating that these Triton-insoluble complexes are not isolations of pre-existing membrane structures [24]. DRMs involve the artificial coalescence of raft constituents into an insoluble residue by process in which the physicochemical parameters are not fully understood [25]. Nevertheless, it is possible to work within the confines of this artefactual system via an analysis of 'paired' differences. Detergent-solubility/insolubility are not strict criteria in themselves; however, many of the major breakthroughs in the raft field originate from observations of changes in DRM association upon induction of physiologically relevant stimuli [26,27]. Such analyses of DRM partitioning changes have served as major workhorses for the evaluation of many raft-related phenomena, including assessing roles in disease pathogenesis [28], membrane trafficking [22] and identifying 'raftophilic' peptide moieties [24].

Membrane patching and the uncovering of raft activation and functionality

A major advance in the field came from the discovery that following antibody (homo-) cross-linking at the cell surface, the GPI-anchored placental alkaline phosphatase, the TM (transmembrane) raft protein haemagglutinin and the raft ganglioside G_{M1} (monosialotetrahexosylganglioside) exhibited a micron-scale co-patching distribution that excluded the transferrin receptor, a classical non-raft protein [29]. Similar selectivity in coalescence behaviour has now been demonstrated for a number of other laterally associating

raft proteins and lipids [30–32] and can be transmitted through to the cytosolic membrane leaflet [33–35]. Selective coalescence is taken as strong evidence that clustered membrane constituents occupied a similar lipid environment before multimerization. More specifically, it suggests a means through which raft lateral organizing potential is functionalized. In the context of membrane trafficking, multimerization is an important lateral factor that promotes recruitment of GPI-anchored proteins into sphingolipid- and cholesterol-enriched carriers, most notably in clathrin-independent endocytosis [36]. Selective raft-based domain clustering can also occur naturally, as in the case of T-cell activation where lymphocytes restructure their cell surface to form membrane domains at TCR (T-cell receptor) signalling foci and immunological synapses [37]. Lipidomics of immunisolated TCR activation domains now shows that, in comparison with membranes clustered by anti-transferrin antibodies, the activation site accumulates more cholesterol, sphingomyelin and saturated phosphocholine [38], all consistent with a raft-selectivity for lipid sorting in the membrane plane. Rafts in this 'activated' or coalesced condition are envisaged as being a more ordered membrane environment wherein lipid acyl chains are longer and more saturated. In combination with tighter cholesterol interdigitation, the result is a more condensed assemblage, a membrane environment that is separate from the surrounding more disordered unsaturated glycerophospholipid milieu [18].

The resting state

Rafts appear functionalized as lateral sorting platforms when activated to coalesce; however, of continual contention is the size and distribution of the so-called 'resting state' of lipid rafts. With the exception of caveolae, unperturbed lipid microdomains are too small and transient to be observed directly in unperturbed living cells [39]. As such, this debate has largely become the realm of researchers who employ high-resolution non-invasive microscopic techniques including single-particle tracking and microscopy [40–43], nanometric aperture-based FCS (fluorescence correlation spectroscopy) [44–46], high spatial and temporal resolution FRET (Förster resonance energy transfer) microscopy [47–49], and the recently applied STED (stimulated emission depletion) far-field fluorescence nanoscopy [50]. A synthesis of this literature paints a picture of small non-random GPI-anchored protein assemblies, residing in ~10–20 nm areas of the plasma membrane. These nanoscale assemblies are highly dynamic, fluctuating on a sub-second timescale. Assembly formation is cholesterol-dependent; however, in some cases, an obligate role of the underlying actin cytoskeleton has been assigned [41,51]. Other techniques indicate that this nanoheterogeneity is actin-independent [46]. Moreover, the situation for TM raft proteins is unknown, so a coherent picture of ultrafine membrane organization is still lacking. In the traditional view of lipid rafts, the selective associative properties of cholesterol and sphingolipid would play a major role in domain formation. Indeed, STED microscopy now

shows that that, unlike glycerophospholipid, sphingolipid exhibits cholesterol-dependent confined diffusive behaviour, transiently dwelling in <20 nm diameter areas of the plasma membrane [50]. Although the effect of lipid hydrocarbon chain length was not tested directly, the glycerophospholipid and sphingolipid probes of this study had similar (and sometimes identical) acyl chain length and saturation indices. The differences in diffusivity were therefore suggested as being due to the differential hydrogen-bonding capacities of glycerol- compared with sphingosine-based lipids.

Model membranes and the relevance of L_o phases

The re-emergence of hydrogen-bonding as a principle of raft organization raises an issue as to the degree to which lipid ordering, as it is understood in model membranes, accounts for raft-based membrane heterogeneity in the biological condition. In model membranes, it is clear that L_o phase separation depends on the interaction of cholesterol with longer saturated lipid [52–58]. This phase separation is often visualized by the macroscopic coexistence of two immiscible liquid phases in ternary lipid mixtures [59,60]. However, it is becoming apparent that nanoscale assemblies of L_o phase conformation exist well below the ~300 nm spatial resolution limit set by the diffraction of light [61,62]. An interesting example is the emerging concept of critical behaviour in biological membranes [63,64]. Before stable microscopic L_o – L_d phase separation, membranes can exist in a ‘supercritical state’, wherein compositional fluctuations of L_o phase within an optically uniform membrane can be observed by fluorescence microscopy. At higher temperatures above the critical point, sub-micron fluctuations are expected. Whereas the form of optically unresolvable L_o behaviour remains unclear, micron-scale phase separation is thought to result from coalesce of nanoscale L_o domains once a certain line tension (greater than thermal energy) has been reached [62]. In this situation, L_o – L_d phase miscibility decreases until it is no longer possible for L_o phase membrane to persist below optical resolution and microscopic phase separation results. Line-tension-driven L_o phase formation has been suggested as an explanation underlying coalescence of raft domains in cell membranes [65,66]. Indeed, GPMV (giant plasma membrane vesicle) isolated by a formaldehyde blebbing procedure can be induced to phase separate into L_o - and L_d -like domains by cooling [67]. Moreover, this system displays similar critical point behaviour to that of model membranes [68]. Now the question arises: does L_o formation behaviour account for raft assembly and functional clustering in cell membranes?

A contention of the lipid raft hypothesis is that the metastable raft resting state can be stimulated to coalesce into larger more stable raft domains by specific lipid–lipid, protein–lipid and protein–protein interactions [69]. When clustered, bilayer components are thought to be laterally stabilized according to their underlying affinity for pre-existing raft domains, i.e. clustering enhances the inclusion of proteins associating both strongly and weakly to rafts and

excludes further those that segregate away [18,69,70]. Indeed, a lipid basis for clustering has been identified in model membranes: uniform GUVs (giant unilamellar vesicles) containing sphingomyelin and cholesterol can be induced to macroscopically phase separate into G_{M1} -enriched L_o phases via cross-linking by quinquivalent CTB (cholera toxin b-subunit) [71]. However, in this situation (and for all cases of phase separation in model membranes) raft TM proteins are excluded from the L_o phase [72–74]. This phenomenon is also seen for the cold-induced L_o -like phase of the formaldehyde-isolated GMPVs [75]. It appears that TM proteins are excluded physically by the energetic constraints of packing membrane-spanning α -helices into rigid membrane phases. This property does not appear to correlate with the co-patching behaviour seen for clustered TM raft proteins and raft lipids at the cell surface. The discrepancy between TM protein inclusion in rafts and exclusion from ordered phases in model systems has been unclear for some time.

A possible answer to this question has come from recent work suggesting that L_o phases are not equivalent to stabilized raft domains at physiological temperature [76]. We have developed a cell-swelling procedure to separate PMSs (plasma membrane spheres) from the cytoskeletal/endocytic/exocytic influence in A431 cells, an epidermoid carcinoma cell line expressing the raft ganglioside G_{M1} . At 37°C, clustering of G_{M1} by quinquivalent CTB, induces the cholesterol-dependent micron-scale coalescence of G_{M1} domains on the surface of previously uniform PMS. The G_{M1} phase exhibits slower translational diffusion, enriches for cholesterol and recruits both TM and exoplasmic/cytoplasmic lipid-anchored raft proteins, but not the transferrin receptor. These data indicate that, at physiological temperature, biological membranes possess the compositional capacity to access underlying raft-based connectivity and amplify it to the level of a distinct membrane phase. Moreover, the selective inclusion of TM proteins suggests that this phase possesses a quality in addition to the lipid basis for L_o – L_d phase separation seen in model membranes. Indeed, the membrane-order-sensing dye Laurdan has now indicated that although this G_{M1} phase (TM protein selective membrane) is more ordered than the surrounding PMS membrane, the level of condensation is far below that which is seen in the L_o phase (TM protein excluding membrane) regions of phase-separated GUVs [77]. Therefore phase separation, as it is understood in model membranes, does not completely account for raft coalescence behaviour at 37°C, at least in activated PMSs.

Perspectives: rafts as assemblies functionalized by both physical and chemical specificities

The context for heterogeneity by rafts must be understood within the complex chemical background of biological membranes. In model systems, phase separation arises from interactions between different lipids that leads to a liquid–liquid

immiscibility in the membrane plane, a concept valid for a number of lipid species and simple lipid/protein preparations [18]. However, cell membranes encompass a multitude of specific biochemical structure–function relationships that generate a lateral interaction diversity not described by these systems. Occupying over 20% of cell membrane area [78], proteinaceous sources of membrane heterogeneity are also potentiated: oligomeric protein assemblies dominate over monomers [79]; variations in hydrophobic domain dimensionality create membrane regions of varying thickness and composition [80,81]; and large ectodomains cover lipid and produce steric restrictions [79,82]. A particularly interesting area of this complexity is the specific binding of lipid to protein. Here, the discovery of the peptide boundary layer unveiled the capacity for direct lipid sorting by protein [83]. Moreover, X-ray crystallography now shows that specific lipids are selected as integral components of the quaternary structure of many membrane protein complexes [84–89]. These data underscore the capacity for a coherent membrane structure formed through both chemical and physical parameters. Lipid–protein interactions alone cannot account for lipid rafts as has been suggested [90], because this excludes the possibility for selective lipid–lipid association. Both lipids and proteins have the potential to specifically organize features of the membrane plane. In this respect, coalesced rafts are likely to be the products of lipid phase separation principles coupled to specific lateral associations governed by standard biochemical ligand interactions, i.e. hydrogen bonds, van der Waals attractions, hydrophobic/hydrophilic interactions and electrostatic forces. Under this scheme, functional raft-based membrane heterogeneity depends on both lipid physical parameters and specific interactions that may include or even require TM proteins. The importance of raft functionality was highlighted in the opening of the present review: heterogeneity in cell membranes did not evolve to laterally sort membrane constituents through a purely physical lipid phase separation that is devoid of protein, but through raft phase separation that is selectively and functionally inclusive. Here the principles of lipid phase separation are united or ‘wetted’ to specific lipid–protein–protein interactions, not just as cross-linkers of lipid domains, but also as structural features of the domain itself. Consequently, the entity produced upon raft clustering at 37°C is not exclusively a domain in which proteins and lipids partition according to order preferences, but is a chemical complex scaffolded by specific chemistries of association. In this view, protein-based heterogeneity joins with cholesterol–sphingolipid assemblage-potential to functionalize rafts in living organisms. Rafts, as we define the term, refers to the operational assembly present in living cells, assemblages wherein bioactivity is dependent on lipid and protein.

Acknowledgements

We thank the Simons laboratory for helpful discussion.

References

- Pike, L.J. (2006) Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. *J. Lipid Res.* **46**, 1597–1598
- Hancock, J.F. (2006) Lipid rafts: contentious only from simplistic standpoints. *Nat. Rev. Mol. Cell Biol.* **7**, 456–462
- Lucero, H.A. and Robbins, P.W. (2004) Lipid rafts–protein association and the regulation of protein activity. *Arch. Biochem. Biophys.* **426**, 208–224
- Simons, K. and Ikonen, E. (1997) Functional rafts in cell membranes. *Nature* **387**, 569–572
- Rajendran, L. and Simons, K. (2005) Lipid rafts and membrane dynamics. *J. Cell Sci.* **116**, 1099–1102
- Simons, K. and van Meer, G. (1988) Lipid sorting in epithelial cells. *Biochemistry* **27**, 6197–6202
- van Meer, G. and Sprong, H. (2004) Membrane lipids and vesicular traffic. *Curr. Opin. Cell Biol.* **16**, 373–378
- van Meer, G., Gumbiner, B. and Simons, K. (1986) The tight junction does not allow lipid molecules to diffuse from one epithelial cell to the next. *Nature* **322**, 639–641
- Klemm, R.W., Ejsing, C.S., Surma, M.A., Kaiser, H.J., Gerl, M.J., Sampaio, J.L., de Bollivar, Q., Ferguson, C., Proszynski, T.J., Shevchenko, A. and Simons, K. (2009) Segregation of sphingolipids and sterols during formation of secretory vesicles at the *trans*-Golgi network. *J. Cell Biol.* **185**, 601–612
- Karlsson, K.-A. (1977) Aspects on structure and function of sphingolipids in cell surface membranes. In *Structure of Biological Membranes* (Abrahamsson, S. and Pascher, I., eds), pp. 245–274, Plenum Press, New York
- Abrahamsson, S., Dahlén, B., Löfgren, H., Pascher, I. and Sundell, S. (1977) Molecular arrangement and conformation of lipids of relevance to membrane structure. In *Structure of biological membranes* (Abrahamsson, S. and Pascher, I., eds), pp. 1–21, Plenum Press, New York
- Thompson, T.E. and Tillack, T.W. (1985) Organization of glycosphingolipids in bilayers and plasma membrane of mammalian cells. *Annu. Rev. Biophys. Chem.* **14**, 361–386
- Ipsen, J.H., Karlstrom, G., Mouritsen, O.G., Wennerstrom, H. and Zuckermann, M.J. (1987) Phase equilibria in the phosphatidylcholine–cholesterol system. *Biochim. Biophys. Acta* **905**, 162–172
- Ipsen, J.H., Mouritsen, O.G. and Zuckermann, M.J. (1989) Theory of thermal anomalies in the specific heat of lipid bilayers containing cholesterol. *Biophys. J.* **56**, 661–667
- Vist, M.R. and Davis, J.H. (1990) Phase equilibria of cholesterol/dipalmitoylphosphatidylcholine mixtures: ²H nuclear magnetic resonance and differential scanning calorimetry. *Biochemistry* **29**, 451–464
- Brown, D.A. and London, E. (1998) Functions of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.* **14**, 111–136
- Brown, D.A. and London, E. (1998) Structure and origin of ordered lipid domains in biological membranes. *J. Membr. Biol.* **15**, 103–114
- Simons, K. and Vaz, W.L. (2004) Model systems, lipid rafts, and cell membranes. *Annu. Rev. Biophys. Biomol. Struct.* **33**, 269–295
- Karnovsky, M.J., Kleinfeld, A.M., Hoover, R.L. and Klausner, R.D. (1982) The concept of lipid domains in membranes. *J. Cell Biol.* **94**, 1–6
- Thompson, T.E., Barenholz, Y., Brown, R.E., Correa-Freire, M., Young, W.W. and Tillack, T.W. (1986) Molecular organization of glycosphingolipids in phosphatidylcholine bilayers and biological membranes. In *Enzymes of Lipid Metabolism II* (Freysz, L., Dreyfus, H., Massarelli, H. and Gatt, S., eds), pp. 387–396, Plenum Press, New York
- Simons, K. and Toomre, D. (2000) Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1**, 31–39
- Brown, D.A. and Rose, K.J. (1992) Sorting of GPI-anchored proteins to glycolipid enriched membrane subdomains during transport to the apical cell surface. *Cell* **68**, 533–544
- Schroeder, R., London, E. and Brown, D. (1994) Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12130–12134
- Brown, D.A. (2006) Lipid rafts, detergent-resistant membranes, and raft targeting signals. *Physiology* **21**, 430–439
- Lingwood, D. and Simons, K. (2007) Detergent resistance as a tool in membrane research. *Nat. Protoc.* **2**, 2159–2165
- Field, K.A., Holowka, D. and Baird, B. (1995) FcεRI-mediated recruitment of p53/56^{lyn} to detergent-resistant membrane domains accompanies cellular signaling. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9201–9205

- 27 Montixi, C., Langlet, C., Bernard, A.M., Thimonier, J., Dubois, C., Wurbel, M.A., Chauvin, J.P., Pierres, M. and He, H.T. (1998) Engagement of T-cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. *EMBO J.* **17**, 5334–5348
- 28 Simons, K. and Ehelhalt, R. (2002) Cholesterol, lipid rafts and disease. *J. Clin. Invest.* **110**, 597–603
- 29 Harder, T., Scheiffele, P., Verkade, P. and Simons, K. (1998) Lipid domain structure of the plasma membrane revealed by patching of membrane components. *J. Cell Biol.* **141**, 929–942
- 30 Janes, P.W., Ley, S.C. and Magee, A.I. (1999) Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. *J. Cell Biol.* **147**, 447–461
- 31 Harder, T. and Simons, K. (1999) Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: accumulation of actin regulated by local tyrosine phosphorylation. *Eur. J. Immunol.* **29**, 556–562
- 32 Rajendran, L., Masilamani, M., Solomon, S., Tikkanen, R., Stuermer, C.A., Plattner, H. and Illges, H. (2003) Asymmetric localization of flotillins/reggies in preassembled platforms confers inherent polarity to hematopoietic cells. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 8241–8246
- 33 Prior, I.A., Muncke, C., Parton, R.G. and Hancock, J.F. (2003) Direct visualization of Ras proteins in spatially distinct cell surface microdomains. *J. Cell Biol.* **160**, 165–170
- 34 Gri, G., Molon, B., Manes, S., Pozzan, T. and Viola, A. (2004) The inner side of T cell lipid rafts. *Immunol. Lett.* **94**, 247–252
- 35 Eisenberg, S., Shvartsman, D.E., Ehrlich, M. and Henis, Y.I. (2006) Clustering of raft-associated proteins in the external membrane leaflet modulates internal leaflet H-Ras diffusion and signaling. *Mol. Cell. Biol.* **26**, 7190–7200
- 36 Mayor, S. and Pagano, R.E. (2007) Pathways of clathrin-independent endocytosis. *Nat. Rev. Mol. Cell Biol.* **8**, 603–612
- 37 Harder, T. and Engelhardt, K.R. (2004) Membrane domains in lymphocytes: from lipid rafts to protein scaffolds. *Traffic* **5**, 265–275
- 38 Zech, T., Ejsing, C.S., Gaus, K., de Wet, B., Shevchenko, A., Simons, K. and Harder, T. (2009) Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signaling. *EMBO J.* **28**, 466–476
- 39 Lai, E. (2003) Lipid rafts make for slippery platforms. *J. Cell Biol.* **162**, 365–370
- 40 Kusumi, A. and Suzuki, K. (2005) Toward understanding the dynamics of membrane-raft-based molecular interactions. *Biochim. Biophys. Acta* **1746**, 234–251
- 41 Suzuki, K.G., Fujiwara, T.K., Sanematsu, F., Iino, R., Edidin, M. and Kusumi, A. (2007) GPI-anchored receptor clusters transiently recruit Lyn and G_q for temporary cluster immobilization and Lyn activation: single-molecule tracking study 1. *J. Cell Biol.* **177**, 717–730
- 42 Suzuki, K.G., Fujiwara, T.K., Edidin, M. and Kusumi, A. (2007) Dynamic recruitment of phospholipase C_γ at transiently immobilized GPI-anchored receptor clusters induces IP₃-Ca²⁺ signaling: single molecule tracking study 2. *J. Cell Biol.* **177**, 731–742
- 43 Drbal, K., Moertelmaier, M., Holzhauser, C., Muhammad, A., Fuertbauer, E., Howorka, S., Hinterberger, M., Stockinger, H. and Schütz, G.J. (2007) Single-molecule microscopy reveals heterogeneous dynamics of lipid raft components upon TCR engagement. *Int. Immunol.* **19**, 675–684
- 44 Lenne, P.F., Wawrezinieck, L., Conchonaud, F., Wurtz, O., Boned, A., Guo, X.J., Rigneault, H., He, H.T. and Marguet, D. (2006) Dynamic molecular confinement in the plasma membrane by microdomains and the cytoskeleton meshwork. *EMBO J.* **25**, 3245–3256
- 45 Wenger, J., Conchonaud, F., Dintinger, J., Wawrezinieck, L., Ebbesen, T.W., Rigneault, H., Marguet, D. and Lenne, P.F. (2007) Diffusion analysis within single nanometric apertures reveals the ultrafine cell membrane organization. *Biophys. J.* **92**, 913–919
- 46 Lasserre, R., Guo, X.J., Conchonaud, F., Hamon, Y., Hawchar, O., Bernard, A.M., Soudja, S.M., Lenne, P.F., Rigneault, H., Olive, D. et al. (2008) Raft nanodomains contribute to Akt/PKB plasma membrane recruitment and activation. *Nat. Chem. Biol.* **4**, 538–547
- 47 Varma, R. and Mayor, S. (2004) GPI-anchored proteins are organized in submicron domains at the cell surface. *Nature* **394**, 798–801
- 48 Sharma, P., Varma, R., Sarasij, R.C., Ira, Gousset, K., Krishnamoorthy, G., Rao, M. and Mayor, S. (2004) Nanoscale organization of multiple GPI-anchored proteins in living cell membranes. *Cell* **116**, 577–589
- 49 Rao, M. and Mayor, S. (2005) Use of Förster's resonance energy transfer microscopy to study lipid rafts. *Biochim. Biophys. Acta* **1746**, 221–233
- 50 Eggeling, C., Ringemann, C., Medda, R., Schwarzmann, G., Sandhoff, K., Polyakova, S., Belov, V.N., Hein, B., von Middendorff, C., Schönle, A. and Hell, S.W. (2009) Direct observation of the nanoscale dynamics of membrane lipids in a living cell. *Nature* **457**, 1159–1162
- 51 Goswami, D., Gowrishankar, K., Bilgrami, S., Ghosh, S., Raghupathy, R., Chadda, R., Vishwakarma, R., Rao, M. and Mayor, S. (2008) Nanoclusters of GPI-anchored proteins are formed by cortical actin-driven activity. *Cell* **135**, 1085–1097
- 52 Ahmed, S.N., Brown, D.A. and London, E. (1997) On the origin of sphingolipid/cholesterol-rich detergent-insoluble cell membranes: physiological concentrations of cholesterol and sphingolipid induce formation of a detergent-insoluble, liquid-ordered lipid phase in model membranes. *Biochemistry* **39**, 10944–10953
- 53 Brown, R.E. (1998) Sphingolipid organization in biomembranes: what physical studies of model membranes reveal. *J. Cell Sci.* **111**, 1–9
- 54 Patra, S.K., Alonso, A., Arrondo, J. and Goñi, F. (1999) Liposomes containing sphingomyelin and cholesterol: detergent solubilization and infrared spectroscopic studies. *J. Liposome Res.* **9**, 247–260
- 55 London, E. and Brown, D.A. (2000) Insolubility of lipids in Triton X-100: physical origin and relationship to sphingolipid/cholesterol membrane domains (rafts). *Biochim. Biophys. Acta* **1508**, 182–195
- 56 Veiga, M.P., Arrondo, J.L., Goni, F.M., Alonso, A. and Marsh, D. (2001) Interaction of cholesterol with sphingomyelin in mixed membranes containing phosphatidylcholine, studied by spin-label ESR and IR spectroscopies: a possible stabilization of gel-phase sphingolipid domains by cholesterol. *Biochemistry* **29**, 451–464
- 57 Xu, X., Bittman, R., Duportail, G., Heissler, D., Vilcheze, C. and London, E. (2001) Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts): comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and ceramide. *J. Biol. Chem.* **276**, 33540–33546
- 58 Edidin, M. (2003) The state of lipid rafts: from model membranes to cells. *Annu. Rev. Biophys. Biomol. Struct.* **32**, 257–283
- 59 Baumgart, T., Hess, S.T. and Webb, W.W. (2003) Imaging coexisting fluid domains in biomembrane models coupling curvature and line tension. *Nature* **425**, 821–824
- 60 Veatch, S.L. and Keller, S.L. (2003) Separation of liquid phases in giant vesicles of ternary mixtures of phospholipids and cholesterol. *Biophys. J.* **85**, 3074–3083
- 61 Niemelä, P.S., Hyvönen, M.T. and Vattulainen, I. (2009) Atom-scale molecular interactions in lipid raft mixtures. *Biochim. Biophys. Acta* **1788**, 122–135
- 62 Feigenson, G.W. (2009) Phase diagrams and lipid domains in multicomponent lipid bilayer mixtures. *Biochim. Biophys. Acta* **1788**, 47–52
- 63 Veatch, S.L., Soubias, O., Keller, S.L. and Gawrisch, K. (2007) Critical fluctuations in domain-forming lipid mixtures. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 17650–17655
- 64 Honerkamp-Smith, A.R., Veatch, S.L. and Keller, S.L. (2009) An introduction to critical points for biophysicists; observations of compositional heterogeneity in lipid membranes. *Biochim. Biophys. Acta* **1788**, 53–63
- 65 Almeida, P.F., Pokorny, A. and Hinderliter, A. (2005) Thermodynamics of membrane domains. *Biochim. Biophys. Acta* **1720**, 1–13
- 66 Risselada, H.J. and Marrink, S.J. (2008) The molecular face of lipid rafts in model membranes. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 17367–17372
- 67 Baumgart, T., Hammond, A.T., Sengupta, P., Hess, S.T., Holowka, D.A., Baird, B.A. and Webb, W.W. (2007) Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 3165–3170
- 68 Veatch, S.L., Cicuta, P., Sengupta, P., Honerkamp-Smith, A., Holowka, D. and Baird, B. (2008) Critical fluctuations in plasma membrane vesicles. *ACS Chem. Biol.* **3**, 287–293
- 69 Meder, D. and Simons, K. (2006) Lipid rafts, caveolae and membrane traffic. In *Lipid Rafts and Caveolae: from Membrane Biophysics to Cell Biology* (Fielding, C.J., ed.), pp. 1–23. Wiley-Vch, Weinheim
- 70 Schuck, S. and Simons, K. (2004) Polarized sorting in epithelial cells: raft clustering and the biogenesis of the apical membrane. *J. Cell Sci.* **117**, 5955–5964
- 71 Hammond, A.T., Heberle, F.A., Baumgart, T., Holowka, D., Baird, B. and Feigenson, G.W. (2005) Crosslinking a lipid raft component triggers liquid ordered-liquid disordered phase separation in model plasma membranes. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6320–6325
- 72 de Planque, M.R. and Killian, J.A. (2003) Protein-lipid interactions studied with designed transmembrane peptides: role of hydrophobic matching and interfacial anchoring. *Mol. Membr. Biol.* **20**, 271–284

- 73 Shogomori, H., Hammond, A.T., Ostermeyer-Fay, A.G., Barr, D.J., Feigenson, G.W., London, E. and Brown, D.A. (2005) Palmitoylation and intracellular domain interactions both contribute to raft targeting of linker for activation of T cells. *J. Biol. Chem.* **280**, 18931–18942
- 74 Kalvodova, L., Kahya, N., Schwille, P., Ehehalt, R., Verkade, P., Drechsel, D. and Simons, K. (2005) Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids and anionic phospholipids *in vitro*. *J. Biol. Chem.* **280**, 36815–36823
- 75 Sengupta, P., Hammond, A.T., Holowka, D. and Baird, B. (2008) Structural determinants for partitioning of lipids and proteins between coexisting fluid phases in giant plasma membrane vesicles. *Biochim. Biophys. Acta* **1778**, 20–32
- 76 Lingwood, D., Ries, J., Schwille, P. and Simons, K. (2008) Plasma membranes are poised for activation of raft phase coalescence at physiological temperature. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 10005–10010
- 77 Kaiser, H.-J., Lingwood, D., Levental, I., Sampaio, J., Kalvodova, L., Rajendran, L. and Simons, K. (2009) Order of lipid phases in model and plasma membranes. *Proc. Natl. Acad. Sci. U.S.A.*, in the press
- 78 Dupuy, A.D. and Engelman, D.M. (2008) Protein area occupancy at the center of the red blood cell membrane. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2848–2852
- 79 Engelman, D.M. (2005) Membranes are more mosaic than fluid. *Nature* **438**, 578–580
- 80 Mouritsen, O.G. and Bloom, M. (1993) Models of lipid-protein interactions in membranes. *Annu. Rev. Biophys. Biomol. Struct.* **22**, 145–171
- 81 Mitra, K., Ubarretxena-Belandia, I., Taguchi, T., Warren, G. and Engelman, D.M. (2004) Modulation of the bilayer thickness of exocytic pathway membranes by membrane proteins rather than cholesterol. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 4083–4088
- 82 Ryan, T.A., Meyers, J., Holowka, D., Baird, B. and Webb, W.W. (1988) Molecular crowding on the cell surface. *Science* **239**, 61–64
- 83 Marsh, D. and Horváth, L.I. (1998) Structure, dynamics and composition of the lipid-protein interface: perspectives from spin-labelling. *Biochim. Biophys. Acta* **1376**, 267–296
- 84 Belrhali, H., Nollert, P., Royant, A., Menzel, C., Rosenbusch, J.P., Landau, E.M. and Pebay-Peyroula, E. (1999) Protein, lipid and water organization in bacteriorhodopsin crystals: a molecular view of the purple membrane at 1.9 Å resolution. *Structure* **7**, 909–917
- 85 Lee, A.G. (2003) Lipid-protein interactions in biological membranes: a structural perspective. *Biochim. Biophys. Acta* **1612**, 1–40
- 86 Shinzawa-Itoh, K., Aoyama, H., Muramoto, K., Terada, H., Kurauchi, T., Tadehara, Y., Yamasaki, A., Sugimura, T., Kurono, S., Tsujimoto, K. et al. (2007) Structures and physiological roles of 13 integral lipids of bovine heart cytochrome *c* oxidase. *EMBO J.* **26**, 1713–1725
- 87 Cherezov, V., Rosenbaum, D.M., Hanson, M.A., Rasmussen, S.G., Thian, F.S., Kobilka, T.S., Choi, H.J., Kuhn, P., Weis, W.I., Kobilka, B.K. and Stevens, R.C. (2007) High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science* **318**, 1258–1265
- 88 Hanson, M.A., Cherezov, V., Griffith, M.T., Roth, C.B., Jaakola, V.P., Chien, E.Y., Velasquez, J., Kuhn, P. and Stevens, R.C. (2008) A specific cholesterol binding site is established by the 2.8 Å structure of the human β_2 -adrenergic receptor. *Structure* **16**, 897–905
- 89 Wenz, T., Hielscher, R., Hellwig, P., Schägger, H., Richers, S. and Hunte, C. (2009) Role of phospholipids in respiratory cytochrome *bc₁* complex catalysis and supercomplex formation. *Biochim. Biophys. Acta* **1787**, 609–616
- 90 Anderson, R.G. and Jacobson, K. (2002) A role for lipid shells in targeting proteins to caveolae, rafts and other lipid domains. *Science* **296**, 1821–1825

Received 27 April 2009
doi:10.1042/BST0370955