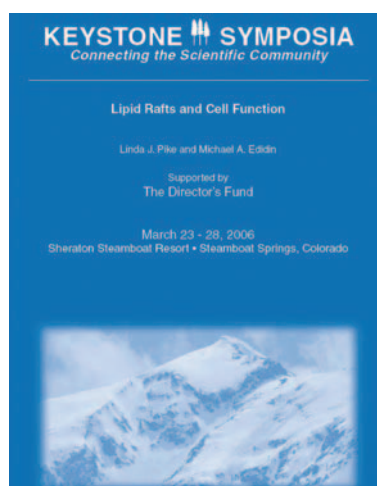


# Flying kites on slippery slopes at Keystone

## Symposium on Lipid Rafts and Cell Function

Satvajit Mayor<sup>1</sup>, Antonella Viola<sup>2</sup>, Radu V. Stan<sup>3</sup> & Miguel A. del Pozo<sup>4\*</sup>

<sup>1</sup>National Centre for Biological Science (NCBS), Bangalore, India, <sup>2</sup>Venetian Institute of Molecular Medicine and Istituto Clinico Humanitas, Rozzano, Italy, <sup>3</sup>Dartmouth Medical School, Lebanon, New Hampshire, USA, and <sup>4</sup>Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain



This Keystone Symposium on Lipid Rafts and Cell Function took place between 23 and 28 March 2006, in Steamboat Springs, Colorado, USA, and was organized by L.J. Pike and M.E. Edidin.

Keywords: membrane microdomains; lipid order; signalling; trafficking; cytoskeleton

EMBO reports (2006) 7, 1089–1093. doi:10.1038/sj.embor.7400836

### Introduction

The fluid-mosaic model of the plasma membrane, proposed by Singer and Nicolson in the early 1970s (Singer & Nicolson, 1972), has provided a powerful driving force for understanding the structure of the cell membrane. Studies of the temporal and spatial architecture of the plasma membrane have indicated that it consists of compartments, which are probably maintained through their interaction with the underlying cortical cytoskeleton meshwork (Edidin, 2003; Kusumi

*et al.*, 2005). The membrane raft hypothesis was originally proposed by Simons and van Meer to explain the segregation of lipids and membrane proteins during their delivery and distribution in polarized epithelium (Simons & van Meer, 1988). This hypothesis—according to which specific lipids dynamically associate with each other to form platforms that are important for membrane-protein sorting and the formation of signalling complexes—was then integrated with the observed compartments (Simons & Toomre, 2000). Subsequently, this type of compartmentalization was related to studies of artificial membranes showing phase segregation behaviour, in which lipids in a fluid bilayer separate into domains containing molecules that display more or less ordered organization in terms of correlation with the orientation of neighbouring molecules (Simons & Vaz, 2004).

This conference focused on lipid rafts and caveolar domains that occur in biomembranes. There are many models for lipid rafts, ranging from lipid shells and nanoscopic domains to the predominantly ordered plasma membrane (Anderson & Jacobson, 2002; Edidin, 2003; Hancock, 2006). However, many questions about lipid rafts remain, including the structure of these domains, their size and distribution in the membrane, their mechanism of formation and their functional significance, if any. In this report, we discuss new developments in the understanding of the lateral segregation of lipids that have been obtained from studies using artificial membranes and the parallel efforts to visualize lipid-dependent protein assemblies in living-cell membranes. We also report on new information regarding the various roles of lipid rafts in many cellular processes, such as membrane trafficking and signal transduction, the anchorage of rafts to the actin cytoskeleton, and the involvement of caveolae and caveolin-1 in cell function.

### New perspectives in biophysics of lipid domains

The physics of phase segregation is a relevant subject for discussion considering that the plasma membrane is a lipid bilayer. Several groups have focused on exploring the phase diagram of multi-component membrane systems in terms of the co-existence of liquid-ordered ( $L_o$ ) and -disordered ( $L_d$ ) domains (Edidin, 2003; Simons & Vaz, 2004), and its relationship to what occurs at the cell membrane (Silvius, 2006).

<sup>1</sup>National Centre for Biological Science (NCBS), Bellary Road, Bangalore 560 065, India

<sup>2</sup>Venetian Institute of Molecular Medicine and Istituto Clinico Humanitas, via Manzoni 56, 20098 Rozzano (MI), Italy

<sup>3</sup>Dartmouth Medical School, One Medical Center Drive, Lebanon, New Hampshire 03756, USA

<sup>4</sup>Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernández Almagro 3, 28029 Madrid, Spain

\*Corresponding author. Tel: +34 91 453 1212; Fax: +34 91 453 1245;

E-mail: madelpozo@cnic.es

Submitted 14 July 2006; accepted 15 September 2006;

published online 20 October 2006

The phase behaviour of cholesterol-containing membranes was described by S. Veatch (Vancouver, BC, Canada)—formerly of S. Keller's group (Veatch & Keller, 2005)—and J. Silvius (Montréal, QC, Canada). They have used the lateral distribution of fluorescent probes (Silvius and Veatch) and line broadening of proton nuclear magnetic resonance (Veatch) to study the co-existence of  $L_0$  and  $L_d$  phases in ternary mixtures of cholesterol-containing model membranes in giant unilamellar vesicles. The data suggest that there is a critical point in the phase diagram near equi-molar concentrations of cholesterol, sphingomyelin and phosphatidylcholine at standard temperature (30–33 °C). At or near the critical point, there could be wide fluctuations in phase behaviour in membranes, which could result in nanoscopic domains or domains with diverse shapes (Veatch). The implication for cell plasma membrane structure was explored with reference to the idea of nanoscale domains (Sharma *et al*, 2004) and that large-scale domains might be induced from nanoscopic domains if the membrane is held at or near the critical point. In this context, it is worth noting that nanoscopic domains also form near phase boundaries (Tokumasu *et al*, 2003), suggesting that the plasma membrane might be held at or near the critical point of an equilibrium phase diagram.

Silvius described a new method by which proteins that are linked to artificial lipid anchors of varying structure can be incorporated into the plasma membrane of living mammalian cells. Silvius illustrated the potential use of these probes to study the functional significance of raft formation by comparing the abilities of protein–lipid conjugates with  $L_0$ - and  $L_d$ -domain-preferring lipid anchors to trigger intracellular signalling in Jurkat T cells. Antibody-mediated crosslinking of such lipid–protein conjugates triggers signalling responses in Jurkat cells that closely resemble those induced by crosslinking of endogenous glycosylphosphatidylinositol (GPI)-anchored proteins or ganglioside GM1. Surprisingly, however, the signalling abilities of the exogenous lipid–protein conjugates were entirely independent of the degree of saturation of their acyl chains, and hence of their relative affinities for  $L_0$  and  $L_d$  domains. These results suggest that rafts might not mediate the signalling responses induced by crosslinking of glycosphingolipids or GPI-anchored proteins in Jurkat cells (Silvius, 2006), but Silvius cautioned that these results should not be generalized to all examples of raft-based signalling systems.

S. McLaughlin (Stony Brook, NY, USA) reported that lipid domains could form on the inner leaflet of the plasma membrane, owing to electrostatic interactions between the positively charged myristoylated alanine-rich C-kinase substrate (MARCKS) proteins and the surface charges of phosphatidylserine and phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>). Theoretical modelling of molecular interaction potentials and the inclusion of the effect of the hydrophobic patch on MARCKS—and other proteins—indicates that these regions could dramatically influence the local equilibrium structure and composition of domains on the inner leaflet (McLaughlin & Murray, 2005). McLaughlin also discussed the possibility that inclusion of PIP<sub>2</sub> at physiological levels could result in slower protein diffusion, possibly owing to the inclusion of cholesterol in these regions.

Many of the presentations on the functional organization of membrane rafts in living cells were devoted to the structure of domains formed by inner and outer leaflet lipid-anchored proteins and glycans. S. Mayor (Bangalore, India) presented data on the spatial distribution of nanoclusters of GPI-anchored proteins as visualized by fluorescence resonance energy transfer (FRET)-based imaging of fluorescently tagged GPI-anchored proteins in living cells (Sharma *et al*,

2004). The data suggest that the spatial distribution of nanoscale clusters is actively maintained by the actin cytoskeleton. Remarkably, these results were mirrored by the distribution of nanoclusters of green fluorescent protein (GFP), which were anchored to the inner leaflet by the H-Ras–lipid signal. J. Hancock (Brisbane, QLD, Australia) and colleagues have visualized the same length-scale and concentration-independence characteristics for inner leaflet lipid-tethered proteins using completely different techniques. They used a statistical analysis of gold-particle-identified protein species—visualized in rip-offs made from intact living cells—to identify nanoclusters of H-Ras and K-Ras proteins with differential sensitivity to actin. Mathematical modelling suggests that the scale of clustering is important for regulating the optimal functions of these domains (Nicolau *et al*, 2006).

T. Fujimoto (Nagoya, Japan) examined the distribution of GM1 and GM3 in cell membranes by immuno-electron microscopy using quick-frozen and freeze-fractured specimens. This method physically immobilizes molecules *in situ* and minimizes the possibility of artefactual perturbation. Through a point pattern analysis of immunogold labelling, GM1 formed clusters of less than 100 nm in diameter in normal mouse fibroblasts (considering the spacer length, they could be as small as about 64 nm). Cholesterol depletion or lowering the temperature reduced clustering of both endogenously and exogenously loaded GM1, but the distribution showed regional heterogeneity in the cells. GM3 showed similar cholesterol-dependent clustering, and these aggregates were not coincident with the GM1 clusters in most cases, suggesting the presence of heterogeneous microdomains. The results further support the use of such electron-microscopy-based high-resolution methodology to study the domain structure of cell membranes.

Transmembrane proteins also seem to be assembled in reversible cholesterol-sensitive clusters of a few proteins (<4). Using fluorescence intensity correlation analysis (FICA), L. Pike (St Louis, MO, USA) and colleagues showed that the epidermal growth factor receptor is present in a pre-clustered distribution that is sensitive to cholesterol and is altered on ligand activation. These results suggest that functional domains in cell membranes are assembled from pre-existing nanoscale lipid-sensitive complexes, as proposed recently (Mayor & Rao, 2004), much like small individual kites that come together when several kite-holders congregate (Fig 1).

### Linkers between the actin cytoskeleton and lipid domains

Several reports have identified molecules that participate in tethering lipid rafts to the actin cytoskeleton, including actin-binding proteins such as the ezrin-radixin-moesin (ERM) proteins talin and vinculin, which have been found associated with detergent-resistant membranes and PIP<sub>2</sub>. PIP<sub>2</sub> accumulates in membrane rafts, where it promotes the recruitment and activation of various signalling components. In addition, PIP<sub>2</sub> is one of the main regulators of actin polymerization by modulating the activity of Rac and Cdc42. Thus, rafts contain part of the elements involved in the regulation of F-actin rearrangements; conversely, the actin cytoskeleton can participate in inducing and sustaining raft polarization.

Several talks reported progress in identifying new linkers between rafts and the cytoskeleton. K. Jacobson (Chapel Hill, NC, USA) showed that by deliberately crosslinking several GPI-anchored proteins with antibody-conjugated 40-nm gold particles, transient anchorage of the gold-labelled clusters occurred for periods ranging from 300 ms to 10 s in a manner completely dependent on cholesterol and Src family kinases (SFKs). He called these transient anchorage



**Fig 1** | Kites as a model for rafts. Individual brown kites (lipid-based nanoclusters) come together by as yet ill-defined mechanisms. These might constitute a functional 'raft', in which the characteristics of the large yellow kites (proteins) that associate with these domains can change.

zones (TAZs), which are distinct from the transient confinement zones (TCZs) that his group previously observed for GPI-anchored proteins that have been deliberately crosslinked to different degrees (Dietrich *et al*, 2002). The TAZs are less mobile, longer-lived segments in the trajectory of the crosslinked GPI-anchored protein. Jacobson's working model suggests that crosslinking GPI-anchored proteins lead to transient anchorage in an induced, caveolin-1- and cholesterol-dependent nanodomain. This promotes an SFK-regulatable linkage between the transmembrane protein Csk-binding protein (Cbp), which senses the clusters and the cytoskeleton.

A role for actin in controlling raft dynamics in lymphocytes was suggested by N. Gupta (San Francisco, CA, USA) and A. Viola (Padova, Italy). In B cells, ligation of the B-cell antigen receptor (BCR) induces raft coalescence (Pierce, 2002). Gupta showed that BCR engagement induced the dissociation of ezrin from rafts, presumably through its dissociation from the raft-resident transmembrane protein Cbp, threonine dephosphorylation of ezrin and its concomitant detachment from actin. Expression of constitutively active ezrin chimaeras inhibited the BCR-induced coalescence of rafts (Gupta *et al*, 2006). Gupta thus proposed that ezrin acts as a linker between the actin cytoskeleton and Cbp, and that ezrin regulates raft dynamics in B cells.

Similar to B cells, T lymphocytes must undergo functional polarization to accomplish immune function. At the plasma membrane, T-cell polarization is accompanied by assemblies of domains with the characteristics of lipid rafts. One hypothesis is that small rafts in the plasma membrane of unstimulated cells migrate through the bilayer in an F-actin-dependent manner, to form large raft clusters in discrete regions on the cell surface. In resting T cells, CD28 co-stimulation induces selective recruitment of raft markers to the T-cell-receptor triggering site (Viola *et al*, 1999). Similarly, CD28 stimulation mediates major actin rearrangements at the T-cell immunological synapse. Viola presented data indicating a role for the actin-binding protein filamin A in concentrating and trapping rafts at the T-cell immunological synapse. Filamins are large cytoplasmic proteins that crosslink cortical

actin into a dynamic three-dimensional structure and, by interacting with functionally diverse proteins, might represent versatile signalling scaffolds. After physiological stimulation, CD28 recruited filamin A into the immunological synapse, and filamin A knockdown inhibited raft accumulation at the immunological synapse. Notably, a lack of raft accumulation at the immunological synapse selectively affected CD28 signalling and co-stimulation, leaving T-cell-receptor-induced T-cell activation unperturbed (Tavano *et al*, 2006).

### Lipid domains in endocytosis and trafficking

The participation of rafts in processes such as endocytosis and vesicular trafficking has been recognized for some time. Several endocytosis pathways through which lipid rafts are internalized have been documented in different cell types. In his keynote address, A. Helenius (Zurich, Switzerland) presented an up-to-date summary of the various endocytic pathways used by viruses to enter cells (Marsh & Helenius, 2006). Viruses seem to use eight putative endocytosis pathways in animal cells. He cautioned that several of these pathways might represent variations of the same pathway and that the differences could stem from using different cell types, endocytosis markers and inhibitors, or be induced by the virus. A need for a standardized set of markers and inhibitors is therefore clearly apparent. Helenius went into more detail about the modes of entry of SV40 and polyoma viruses, the latter of which seem to use a novel pathway through their receptor, the ganglioside GD1. This pathway is independent of clathrin, caveolin, dynamin, kinase and Arf6, but is dependent on actin.

D. Brown (Stony Brook, NY, USA) reported preliminary findings on ErbB2 internalization. ErbB2 is unique among epidermal growth factor receptor family members in that constitutive binding to the chaperone Hsp90 is required for its stability. The antibiotic geldanamycin causes dissociation of heat-shock protein 90 (Hsp90) from ErbB2, leading to internalization and degradation of the protein. On geldanamycin treatment, ErbB2 is transported to early endosomes where it accumulates in the internal vesicles of multivesicular

bodies. Brown reported that ErbB2 is internalized largely by a non-clathrin-mediated pathway that requires cholesterol but not dynamin or tyrosine kinase activity in geldanamycin-treated SKBr3 cells. After internalization, ErbB2 is delivered rapidly to early and then late endosomes, and finally to lysosomes for degradation.

Membrane cholesterol has an important role in human immunodeficiency virus-1 (HIV-1) particle production and infectivity, suggesting that rafts are involved in HIV infectivity (Ono & Freed, 2005). E. Freed (Frederick, MD, USA) showed that a cholesterol-binding polyene fungal antibiotic, amphotericin B methyl ester (AME), potently inhibits the replication of a highly divergent panel of HIV-1 isolates. AME causes profoundly impaired viral infectivity, as well as a defect in virus particle production. Freed selected for and characterized AME-resistant HIV-1 variants. Mutations responsible for AME resistance map to a highly conserved and functionally important endocytosis motif in the cytoplasmic tail of the transmembrane glycoprotein gp41. Truncation of the gp41 cytoplasmic tail in the context of either HIV-1 or a simian immunodeficiency virus also confers resistance to AME. These results support the concept that cholesterol-binding compounds should be pursued as anti-retrovirals to disrupt HIV-1 replication.

W. Lencer (Boston, MA, USA) gave a progress report on his group's work on the role of rafts in the internalization and trafficking of cholera toxin, a prototype for the toxins that have the AB5 domain structure (Lencer, 2004). Cholera toxin binds through its B subunit to GM1, which transports the toxin from the plasma membrane to the trans Golgi and the endoplasmic reticulum, a phenomenon known as the retrograde pathway. In most cells, only the ceramide-based glycolipids that have a strong affinity for partitioning into rafts internalize the AB5 toxins through this pathway. The wild-type toxin can simultaneously bind up to five gangliosides through its five B-chains, possibly providing a scaffold to stabilize or cluster raft microdomains. Lencer therefore introduced mutations into the B-chain to remove three or four of the five binding sites. These mutant toxins bound stably to GM1 on the cell surface but exhibited a decrease in endocytosis and toxicity. Lencer also showed that conversion of apical plasma membrane sphingomyelin to ceramide by sphingomyelinase attenuated cholera toxin toxicity. This effect is reversible, is specific to toxin entry through the apical surface of epithelial cells, and is recapitulated by cell loading with long-chain ceramides. The attenuation of toxicity is associated with a dramatic change in the apical cortical F-actin cytoskeleton, lipid domain structure and apical endocytosis.

### Caveolin-1 and lipid domains in signal transduction

The ability of lipid domains to compartmentalize cellular processes suggested that lipid rafts (Simons & Toomre, 2000) and, in particular, caveolae (Shaul & Anderson, 1998) can act as signalling platforms for many pathways, and much evidence has been provided to support this hypothesis. Several presentations highlighted an important role for tyrosine-14-phosphorylated caveolin-1 (pYcav-1) in signal transduction. P. Insel (La Jolla, CA, USA) provided evidence that, in cardiac fibroblasts, adenylyl cyclase localizes at focal adhesions together with pYcav-1 (Swaney *et al*, 2006). Activation of adenylyl cyclase stimulates protein tyrosine phosphatase 1B, which, in turn, triggers both increased caveolin-1 phosphorylation and decreased focal adhesion kinase (FAK) phosphorylation. Both pathways then contribute to disrupting actin polymerization, which is crucial for the transformation of cardiac fibroblasts into myofibroblasts. These results provide an example of caveolin-1 acting as a scaffolding protein outside caveolae, namely at the sites of focal adhesions.

C. Mastick (Reno, NV, USA) also noted the localization of pYcav-1 at focal adhesions where it acts as a scaffold to bind, recruit and activate carboxy-terminal Src kinase (Csk), a negative regulator of SFK (Cao *et al*, 2002). Mastick pointed out the importance of the upstream kinase regulating tyrosine 14 phosphorylation of caveolin-1. Phosphorylation by SFK results in a Csk-dependent attenuation of the kinase, which could be required for actin remodelling. However, phosphorylation by Abl, which is insensitive to Csk, results in stable caveolin-1 phosphorylation and permanent inhibition of SFK through Csk, which could negatively regulate actin turnover. Mastick also described six species of phosphocaveolin-1, depending on the number of serine residues that are phosphorylated. Together, these results highlight the importance of the stimuli inducing caveolin-1 phosphorylation, with specific consequences for different downstream signalling outcomes. M.A. del Pozo (Madrid, Spain) also noted a crucial role for pYcav-1 in inducing the endocytosis of lipid domains, which is important for integrin signalling and anchorage-dependent cell growth (del Pozo *et al*, 2005). After loss of anchorage to the extracellular matrix, the subsequent uncoupling of integrins from intracellular mediators releases caveolar endocytosis of lipid domains, shutting down the Rac-Pak, Ras-Erk and phosphatidylinositol-3-kinase-Akt growth regulatory pathways. Lipid domain internalization requires pYcav-1; del Pozo also described the implications of this mechanism in regulating actin cytoskeleton architecture and cell migration.

Caveolar endocytosis was also discussed by A. Choudhury from R. Pagano's group (Rochester, MN, USA). He showed that inhibition of syntaxin 6, a t-SNARE (target membrane soluble N-ethylmaleimide attachment protein receptor), specifically blocked caveolar endocytosis, but not other endocytic pathways (Choudhury *et al*, 2006). Under these conditions, the presence of caveolin-1, caveolae, GM1 and GPI-GFP at the plasma membrane was reduced, as were the total levels of pYcav-1. Intriguingly, the addition of GM1 restored the normal levels and distribution of these components, as well as caveolae uptake, suggesting that syntaxin 6 regulates caveolar endocytosis by controlling the delivery of essential membrane microdomain constituents to the cell surface. Elucidating the molecular mechanism of this function will be an interesting topic for investigation.

R. Anderson (Dallas, TX, USA) synthesized the work of several laboratories showing that caveolins are found in lipid droplets (Beckman, 2006) with his own proteomics analysis of these structures (Liu *et al*, 2004) to propose the name 'adiposome' for these organelles, highlighting their metabolically active status. Anderson showed that there is GTP- and Rab-dependent trafficking from early endosomes to adiposomes, although the specific Rab involved was not identified. He concluded that these organelles are much more complex than previously thought and that further research is needed to identify the molecules regulating membrane traffic to and from the adiposome.

Evidence of a role for rafts in antigen receptor signalling in lymphocytes has largely come from studies using detergent solubility to identify rafts. Using live-cell FRET imaging, S. Pierce (Rockville, MD, USA) presented evidence that within seconds of antigen-induced clustering, the BCR transiently and selectively associates with raft lipids. This association is prolonged by the engagement of activating co-receptors and is blocked by inhibitory co-receptors (Sohn *et al*, 2006). Pierce proposed that BCR-lipid raft interactions are important in the initiation of BCR signalling because they facilitate BCR interactions with signalling components, but that protein-protein interactions quickly dominate the signalling process.

### Concluding remarks

On the last day of this conference there was an *ad hoc* session that attempted to reach a consensus definition of 'membrane rafts' (see Pike, 2006). This attempt at definition highlights the idea—repeated in many of the talks—that cell membranes are heterogeneous in their lipid and protein composition, and that local control of composition, size, dynamics and lateral segregation of membrane microdomains remain open questions (Hancock, 2006). Emerging from this meeting was the suggestion that the actin cytoskeleton might have a more prominent role in the construction of rafts. It is hoped that new probes to examine local lipid order, such as laurdan or di-4-ANEPPDHQ (Gaus *et al*, 2005; Jin *et al*, 2006) that were presented by T. Magee (London, UK), new techniques, such as fluorescence correlation spectroscopy (Elson, 2004; Haustein & Schwille, 2003) introduced at the meeting by Pike, B. Baird (Ithaca, NY, USA) and E. Gratton (Irvine, CA, USA), and nanoscale patterning of cell substrates to examine the construction of local signalling processes (Senaratne *et al*, 2006) will help to answer these unsolved questions.

### ACKNOWLEDGEMENTS

We thank our colleagues for sharing information and allowing their work to be described, and apologize to those whose work could not be cited owing to space limitations. We are grateful to L. Pike and M. Edidin for organizing this stimulating meeting. S.M. is supported by intramural funds from the Tata Institute of Fundamental Research, and grants from the Department of Biotechnology (India) and from the Human Frontier Science Program (RGP0050/2005-C). M.A.dP. is supported by the European Molecular Biology Organization Young Investigator Programme, a European Young Investigator Award, a European Young Investigator (EURYI) Award, the European Union (grant MIRG-CT-2005-016427) and the Spanish Ministry of Science and Education (grants SAF2005-00493 and GEN2003-20239-C06-04).

### REFERENCES

- Anderson RG, Jacobson K (2002) A role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains. *Science* **296**: 1821–1825
- Beckman M (2006) Cell biology. Great balls of fat. *Science* **311**: 1232–1234
- Cao H, Courchesne WE, Mastick CC (2002) A phosphotyrosine-dependent protein interaction screen reveals a role for phosphorylation of caveolin-1 on tyrosine 14: recruitment of C-terminal Src kinase. *J Biol Chem* **277**: 8771–8774
- Choudhury A, Marks DL, Proctor KM, Gould GW, Pagano RE (2006) Regulation of caveolar endocytosis by syntaxin 6-dependent delivery of membrane components to the cell surface. *Nat Cell Biol* **8**: 317–328
- del Pozo MA, Balasubramanian N, Alderson NB, Kiosses WB, Grande-Garcia A, Anderson RG, Schwartz MA (2005) Phospho-caveolin-1 mediates integrin-regulated membrane domain internalization. *Nat Cell Biol* **7**: 901–908
- Dietrich C, Yang B, Fujiwara T, Kusumi A, Jacobson K (2002) Relationship of lipid rafts to transient confinement zones detected by single particle tracking. *Biophys J* **82**: 274–284
- Edidin M (2003) The state of lipid rafts: from model membranes to cells. *Annu Rev Biophys Biomol Struct* **32**: 257–283
- Elson EL (2004) Quick tour of fluorescence correlation spectroscopy from its inception. *J Biomed Opt* **9**: 857–864
- Gaus K, Chklovskaya E, Fazekas de St Groth B, Jessup W, Harder T (2005) Condensation of the plasma membrane at the site of T lymphocyte activation. *J Cell Biol* **171**: 121–131
- Gupta N, Wollscheid B, Watts JD, Scheer B, Aebersold R, DeFranco AL (2006) Quantitative proteomic analysis of B cell lipid rafts reveals that ezrin regulates antigen receptor-mediated lipid raft dynamics. *Nat Immunol* **7**: 625–633
- Hancock JF (2006) Lipid rafts: contentious only from simplistic standpoints. *Nat Rev Mol Cell Biol* **7**: 456–462
- Haustein E, Schwille P (2003) Ultrasensitive investigations of biological systems by fluorescence correlation spectroscopy. *Methods* **29**: 153–166
- Jin L, Millard AC, Wuskell JP, Dong X, Wu D, Clark HA, Loew LM (2006) Characterization and application of a new optical probe for membrane lipid domains. *Biophys J* **90**: 2563–2575
- Kusumi A, Nakada C, Ritchie K, Murase K, Suzuki K, Murakoshi H, Kasai RS, Kondo J, Fujiwara T (2005) Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned

- fluid: high-speed single-molecule tracking of membrane molecules. *Annu Rev Biophys Biomol Struct* **34**: 351–378
- Lencer WI (2004) Retrograde transport of cholera toxin into the ER of host cells. *Int J Med Microbiol* **293**: 491–494
- Liu P, Ying Y, Zhao Y, Mundy DI, Zhu M, Anderson RGW (2004) Chinese hamster ovary K2 cell lipid droplets appear to be metabolic organelles involved in membrane traffic. *J Biol Chem* **279**: 3787–3792
- Marsh M, Helenius A (2006) Virus entry: open sesame. *Cell* **124**: 729–740
- Mayor S, Rao M (2004) Rafts: scale-dependent, active lipid organization at the cell surface. *Traffic* **5**: 231–240
- McLaughlin S, Murray D (2005) Plasma membrane phosphoinositide organization by protein electrostatics. *Nature* **438**: 605–611
- Nicolau DV Jr, Burrage K, Parton RG, Hancock JF (2006) Identifying optimal lipid raft characteristics required to promote nanoscale protein–protein interactions on the plasma membrane. *Mol Cell Biol* **26**: 313–323
- Ono A, Freed EO (2005) Role of lipid rafts in virus replication. *Adv Virus Res* **64**: 311–358
- Pierce SK (2002) Lipid rafts and B-cell activation. *Nat Rev Immunol* **2**: 96–105
- Pike LJ (2006) Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. *J Lipid Res* **47**: 1597–1598
- Senaratne W, Sengupta P, Jakubek V, Holowka D, Ober CK, Baird B (2006) Functionalized surface arrays for spatial targeting of immune cell signaling. *J Am Chem Soc* **128**: 5594–5595
- Sharma P, Varma R, Sarasij RC, Ira, Gousset K, Krishnamoorthy G, Rao M, Mayor S (2004) Nanoscale organization of multiple GPI-anchored proteins in living cell membranes. *Cell* **116**: 577–589
- Shaul PW, Anderson RG (1998) Role of plasmalemmal caveolae in signal transduction. *Am J Physiol* **275**: L843–L851
- Silvius J (2006) Lipid microdomains in model and biological membranes: how strong are the connections? *Q Rev Biophys* **38**: 1–11
- Simons K, Toomre D (2000) Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* **1**: 31–39
- Simons K, van Meer G (1988) Lipid sorting in epithelial cells. *Biochemistry* **27**: 6197–6202
- Simons K, Vaz WL (2004) Model systems, lipid rafts, and cell membranes. *Annu Rev Biophys Biomol Struct* **33**: 269–295
- Singer SJ, Nicolson GL (1972) The fluid mosaic model of the structure of cell membranes. *Science* **175**: 720–731
- Sohn HW, Tolar P, Jin T, Pierce SK (2006) Fluorescence resonance energy transfer in living cells reveals dynamic membrane changes in the initiation of B cell signaling. *Proc Natl Acad Sci USA* **103**: 8143–8148
- Swaney JS, Patel HH, Yokoyama U, Head BP, Roth DM, Insel PA (2006) Focal adhesions in (myo)fibroblasts scaffold adenyl cyclase with phosphorylated caveolin. *J Biol Chem* **281**: 17173–17179
- Tavano R, Contento RL, Baranda SJ, Soligo M, Tuosto L, Manes S, Viola A (2006) CD28 interaction with filamin-A controls lipid raft accumulation at the T cell immunological synapse. *Nat Cell Biol*, in press
- Tokumasu F, Jin AJ, Feigenson GW, Dvorak JA (2003) Nanoscopic lipid domain dynamics revealed by atomic force microscopy. *Biophys J* **84**: 2609–2618
- Veatch SL, Keller SL (2005) Seeing spots: complex phase behavior in simple membranes. *Biochim Biophys Acta* **1746**: 172–185
- Viola A, Schroeder S, Sakakibara Y, Lanzavecchia A (1999) T lymphocyte costimulation mediated by reorganization of membrane microdomains. *Science* **283**: 680–682



**Miguel A. del Pozo, Radu V. Stan, Satvajit Mayor & Antonella Viola**