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talking point

Have we become overly reliant on lipid rafts?

Talking Point on the involvement of lipid rafts in T-cell activation

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During the past decade, the lipid-raft hypothesis has focused attention on the role of membrane domains in controlling cellular functions. Among the best-studied roles of lipid rafts is the regulation of T-cell signalling. In particular, a model has emerged in which lipid rafts regulate protein–protein interactions during signalling in a cholesterol-dependent manner. Does this model provide the best description of what is happening in living cell membranes? Alternatively, has our ability to evaluate this question critically become compromised by the influential nature of the lipid-raft model itself? Here, this issue is explored in the context of two of the major tenets of the lipid-raft model.

Keywords: cholesterol; lipid rafts; membrane microdomains; palmitoylation; signalling

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Introduction

Since it was first proposed in 1997, the lipid-raft hypothesis has focused attention on the role of membrane domains in controlling cellular functions (Simons & Ikonen, 1997). Building on biochemical evidence for the interactions of glycosylphosphatidylinositol (GPI)-anchored proteins and protein tyrosine kinases with glycosphingolipids and cholesterol (Stefanova *et al*, 1991; Brown & Rose, 1992), the term lipid rafts was developed to convey the idea that these molecules form small platforms within the plane of the membrane, in which they function as transport and signalling organizers (Simons & Ikonen, 1997). The earlier discovery that purified lipids can be used to generate detergent-resistant membranes (DRMs) that equate with lipid rafts (Schroeder *et al,* 1994), and the subsequent proposal that the lipids that comprise the rafts are in a liquid-ordered (L_o) state (Brown & London, 1998), pointed to a crucial role for cholesterol in raft formation.

Among the best-studied functions of lipid rafts is their role in the regulation of signalling in immune cells, especially through the T-cell receptor (Harder & Engelhardt, 2004; He *et al,* 2005; Horejsi, 2005; Kabouridis, 2006; Zeyda & Stulnig, 2006; Jury *et al,* 2007). In these and other signalling pathways (Pike, 2003), a general model has emerged in which lipid rafts regulate protein–protein interactions in a cholesterol-dependent manner (Sidebar A). Yet, despite the now-pervasive nature of the lipid-raft hypothesis, its validity has been questioned (Munro, 2003; Shaw, 2006). Criticism stems largely from concerns about the detergent-based assays that are widely used to define raft-associated proteins and events (Lichtenberg *et al,* 2005; Munro, 2003; Shaw, 2006). In addition, biophysical studies indicate that raft domains are typically small and short-lived under steady-state conditions (He *et al,* 2005; Hancock, 2006; Marguet *et al,* 2006; Jacobson *et al,* 2007). Therefore, current models evoke a requirement for crosslinking or stabilization of small rafts to facilitate their function (Kusumi *et al,* 2004; Mayor & Rao, 2004; Hancock, 2006).

The companion to this Talking Point article discusses evidence in support of a role for lipid rafts in T-cell receptor signalling (He & Marguet, 2008). Here, I discuss evidence that indicates that we should not be so quick to ascribe this and other cellular functions to lipid rafts. I frame this discussion in the context of two of the major tenets of the lipid-raft model.

Tenet 1: lipid rafts require cholesterol

Lipid–lipid interactions are thought to provide the underlying basis for lipid-raft formation, with cholesterol having a crucial role in this process (Brown & London, 1998). In particular, lipid-raft domains are thought to be composed of lipids that exist in a cholesterol-enriched L_o state and that coexist with cholesterol-poor liquid-disordered (L_d) domains within the plane of the membrane. Therefore, in cells, cholesterol manipulations have become a standard tool for studying the structure and function of lipid rafts (Simons & Toomre, 2000; Zidovetzki & Levitan, 2007). Specific treatments include acute or chronic cholesterol depletion (for example, through the use of methyl-β-cyclodextrin; Xavier *et al,* 1998; Ike *et al,* 2003; Monjas *et al,* 2004; Zidovetzki & Levitan, 2007), cholesterol-sequestration agents such as saponin and filipin (Xavier *et al,* 1998; Sharma *et al,* 2004; Chichili & Rodgers, 2007), cholesterol oxidase (Drevot *et al,* 2002; Lenne *et al,* 2006) and depletion of serum lipoproteins in conjunction with inhibition of cholesterol biosynthesis (Shvartsman *et al,* 2006).

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Sidebar A | Examples of proposed mechanisms by which lipid rafts regulate T-cell signalling

Regulated assembly of incomplete signalling pathways
On ligation, the T-cell receptor is translocated to lipid rafts, where the linker for activation of T cells (LAT) and LCK are located (Montixi *et al*, 1998; Xavier *et al*, 1998; Pizzo *et al*, 2004).

Requirement for raft association for protein function
The association of LCK with lipid rafts is required for its function (Kabouridis *et al*, 1997; Stulnig *et al*, 1998; Hawash *et al*, 2002).
The association of LAT with lipid rafts is required for its function (Zhang *et al*, 1998; Lin *et al*, 1999; Zeyda *et al*, 2002; but also see Zhu *et al*, 2005; Tanimura *et al*, 2006).

Regulation of protein activity within a raft
LCK activity is downregulated in detergent-resistant membranes compared with non-raft fractions (Rodgers & Rose, 1996; Kabouridis *et al*, 2000).

Segregation/sequestration of signalling pathway components
CD45, which is a transmembrane tyrosine phosphatase, is sequestered in non-raft regions of the membrane, leading to hyperphosphorylation and, hence, inactivation of LCK in raft fractions (Rodgers & Rose, 1996); this sequestration occurs in a cholesterol-dependent and actin-dependent manner (Chichili & Rodgers, 2007).

Using these manipulations, numerous studies have described the functional consequences of cholesterol depletion in a range of signalling pathways. In many cases, the effects of cholesterol depletion are consistent with a model in which depletion causes loss of raft domains and, subsequently, misregulated signalling (Kabouridis *et al*, 2000).

Perhaps even more interesting, however, are those studies in which unexpected cellular responses to cholesterol depletion have been observed. For example, cholesterol depletion causes plasma-membrane depolarization and non-specific depletion of intracellular Ca^{2+} stores in T cells (Pizzo *et al*, 2004), and the induction of autophagy (Cheng *et al*, 2006). Intriguingly, cholesterol depletion can also slow the diffusion of both raft and non-raft proteins at the cell surface, and/or induce protein immobilization, as well as increase membrane stiffness (Kwik *et al*, 2003; Kenworthy *et al*, 2004; Vrljic *et al*, 2005; Shvartsman *et al*, 2006; Sun *et al*, 2007).

Given these findings, many recent reviews have rightly pointed out the need to interpret the results of cholesterol-depletion experiments with caution. However, these data also beg the question of whether we can learn something new about the way in which cholesterol influences membrane structure and signalling transduction from these phenotypes. Although the mechanisms underlying these changes are not yet fully understood, at least some of these effects could occur as a result of changes in actin organization, some from the mobilization of phosphatidylinositol-4,5-bisphosphate and others from perturbed cell-division cycle 42 recruitment to the plasma membrane (Ike *et al*, 2003; Kwik *et al*, 2003; Chadda *et al*, 2007). Intriguingly, there are indications that some of these effects might not be owing to cholesterol removal (Shvartsman *et al*, 2006), raising further questions about the way in which cells sense and respond to these treatments. Therefore, efforts to define raft structure and function by changing cholesterol might have inadvertently given us clues about other ways that cells regulate signalling, if we are willing to explore these alternative mechanisms further.

Sidebar B | Palmitolyation of LAT and T-cell signalling: evidence for rafts?

One of the main mechanisms by which proteins are thought to be targeted to lipid rafts is through palmitoylation, which is the covalent attachment of the 16-carbon saturated fatty acid palmitate to a cysteine by a thioester linkage (Brown, 2006). Palmitoylation is thought to help increase the affinity of transmembrane proteins for lipid rafts, as transmembrane domains are predicted to pack poorly within the ordered environment of a lipid raft (Brown, 2006). The functional consequences of palmitoylation have been well studied for linker for activation of T cells (LAT), which is palmitoylated on two cysteines close to the cytoplasmic face of the membrane. Several early studies documented a role for palmitoylation in targeting the protein to DRMs that were isolated from cell extracts, and a requirement for raft association for LAT function (Zhang *et al*, 1998; Lin *et al*, 1999). However, palmitoylation is not sufficient to enable targeting of the LAT transmembrane domain to either liquid-ordered domains or detergent-resistant membranes *in vitro* (Shogomori *et al*, 2005). In addition, more recent studies indicate that palmitoylation of LAT is required for the protein to be transported to the plasma membrane and that, in the absence of palmitoylation, LAT is susceptible to degradation (Tanimura *et al*, 2006). The low abundance of a LAT palmitoylation mutant on the cell surface was also noted in another study (Douglass & Vale, 2005). Furthermore, when a LAT fusion protein that is targeted to non-raft domains was used to replace LAT in LAT-deficient Jurkat cells or $LAT^{-/-}$ mice, many functions of the native protein were restored (Zhu *et al*, 2005). These data raise the possibility that at least some of the signalling defects observed for a LAT palmitoylation mutant might result from defects in its delivery to the plasma membrane, rather than its mislocalization from plasma membrane lipid rafts.

Tenet 2: rafts regulate protein interactions

One of the main attractions of the lipid-raft model is that it provides a mechanism for regulating protein–protein interactions (Sidebar A). Early studies in T-cell signalling postulated that lipid rafts function to concentrate certain proteins within rafts, as well as to segregate raft and non-raft proteins, based on the association of some proteins with DRMs (Montixi *et al*, 1998; Xavier *et al*, 1998; Zhang *et al*, 1998; Lin *et al*, 1999). Moreover, many of the proteins involved in T-cell signalling are palmitoylated, which has led to the intense study of the role of palmitoylation in targeting them to DRMs and their ability to signal correctly (Sidebar B). However, as the limitations of biochemical assays for rafts have been increasingly recognized (Lichtenberg *et al*, 2005), new strategies to examine raft structure and dynamics, and in turn to infer their function, have evolved.

One such approach has been to explore the process of L_o/L_d domain formation in artificial membranes (Veatch & Keller, 2005). As lipid domains are often micrometre-sized and can be easily visualized by doping them with fluorescent lipid probes, the preference of purified peptides or membrane proteins for an L_o or an L_d environment can be investigated with these *in vitro* systems (Shogomori *et al*, 2005). In support of this model, the presence of coexisting L_o and L_d domains in polarized epithelial cells was inferred from recent fluorescence-recovery after photobleaching (FRAP) measurements (Meder *et al*, 2006). Furthermore, visible fluid–fluid phase coexistence was recently shown to occur in plasma-membrane blebs, and these lipid domains have the ability to sort proteins (Baumgart *et al*, 2007). In intact cells, however, lipids rarely exhibit this large-scale lipid-domain separation. It has therefore been proposed that in cells, L_o domains are normally small and transient, but can be stabilized when captured by proteins (Hancock, 2006). The formation of large

Table 1 | Comparison of specific predictions of three major models of lipid rafts

	L_o/L_d phase coexistence model	Lipid-shell model	Actively maintained domains model
Basis of domain formation	Lipid–lipid interactions sufficient	Protein–lipid interactions	Might involve lipid–lipid interactions
	Proteins distribute by partitioning	Raft proteins act as nucleation sites	Might involve protein–lipid interactions
	–	Can be targeted to other domains	Additional cellular machinery required
Number of proteins per raft	Depends on protein concentration	Single protein/shell	Depends on number of available sites
	Depends on partition coefficient	Can interact in regulated manner	Might be saturable
Protein interactions in raft	Non-competitive	Non-competitive	Possibly competitive

Predictions are extrapolated from published descriptions of the liquid-ordered (L_o)/liquid-disordered (L_d) phase-coexistence model (Meder *et al*, 2006), the lipid-shell model (Anderson & Jacobson, 2002; Jacobson *et al*, 2007) and the actively maintained domains model (Mayor & Rao, 2004).

L_o domains might be prevented by an active, energy-dependent process (Hancock, 2006). However, others have questioned whether L_o/L_d phase separation is a good model for the basis of domain formation in cell membranes (Mayor & Rao, 2004; Jacobson *et al,* 2007).

In fact, biophysical studies in cells indicate that the distribution of putative raft proteins in cells is more consistent with a random distribution or a model of active organization than with passive partitioning into L_o domains (Glebov & Nichols, 2004; Sharma *et al,* 2004; Hess *et al,* 2005; Plowman *et al,* 2005). In one such study, fluorescence-resonance energy transfer (FRET) was used to test the hypothesis that raft-associated proteins are concentrated within lipid rafts in T cells, and showed that they are instead randomly distributed (Glebov & Nichols, 2004). However, another FRET study in fibroblasts provided strong evidence of the presence of a small but significant fraction of GPI-anchored proteins in small (~4–5 nm) cholesterol-sensitive clusters (Sharma *et al,* 2004). Remarkably, although the clusters coexist with a large monomer fraction, the two populations are not in equilibrium with each other, indicating that the fraction of clustered proteins is actively regulated by the cell (Table 1). This provides a view of rafts as pre-existing and actively maintained structures that can be organized into larger and more stable structures, for example by crosslinking (Mayor & Rao, 2004; Sharma *et al,* 2004). Interestingly, measurements of protein diffusion by fluorescence-correlation spectroscopy (FCS) have also provided evidence for the dynamic lateral confinement of GPI-anchored proteins within cholesterol-dependent domains (Lenne *et al,* 2006).

Given such findings, several recent reviews indicate that lipid rafts are normally small and dynamic structures, and evoke mechanisms by which cells organize and stabilize these small domains on an 'as-needed' basis (Harder & Engelhardt, 2004; Kusumi *et al,* 2004; Mayor & Rao, 2004; Hancock, 2006; Viola & Gupta, 2007). By doing so, they address a crucial assumption of the model: the idea that lipid rafts need to have a finite size and lifetime to bring proteins together or to keep them apart in a functionally meaningful way.

How do lipid rafts do this? Given differing opinions about the definition of a lipid raft, this is not a trivial question. The lipid-shell model, the L_o/L_d phase coexistence model and the actively maintained domains model make substantially different predictions about what drives domain formation, how the number of proteins that associate with a given raft is regulated and how proteins interact with one another within a raft (Table 1). Recent studies also vary in their depiction of how proteins diffuse within and between raft and non-raft domains (Kenworthy *et al,* 2004; Douglass & Vale, 2005; Lenne *et al,* 2006; Meder *et al,* 2006). Even the area fraction of rafts within the plasma membrane is debated (Shaw, 2006). It is also clear that rafts cannot be doing all the work themselves, especially in immune-cell signalling during which protein networks and the actin-binding proteins also have an important role (Harder, 2004; Douglass & Vale, 2005; Viola & Gupta, 2007). Therefore, although models of raft function abound, the details of how these events are accomplished remain far from certain.

Looking beyond rafts

The lipid-raft field has reached a crucial juncture. There is a plethora of biochemical and functional data in support of the lipid-raft model. Yet, much of this evidence comes from assays, such as DRM-isolation and cholesterol-depletion assays, the validity of which is increasingly being questioned. At the same time, models of how rafts function are growing progressively more complex, reflecting fundamental uncertainties about the nature of rafts. This has placed the field in a tenuous position in which the relevance of the model has been strongly criticized (Munro, 2003; Shaw, 2006). Much emphasis has been placed on the importance of developing new tools with which to study rafts, in order to address some of these concerns. Indeed, it was recently proposed that the lipid-raft field is at a technical impasse (Jacobson *et al,* 2007).

I propose that the field has also reached a conceptual impasse by becoming overly reliant on the lipid-raft model itself. Most recent studies have focused on determining whether lipid rafts are involved in a particular process. However, much less effort has been devoted to considering alternative models. Indeed, the absence of a strong competing model might be the main reason why we are so reluctant to reject the lipid-raft model altogether, despite its clear limitations. Further investigation of the physiological origin of the effects of cholesterol depletion would be an excellent starting point for identifying alternative mechanisms. Another approach that shows great promise is the direct visualization of signalling in action (Bunnell *et al,* 2002; Ike *et al,* 2003; Douglass & Vale, 2005; Larson *et al,* 2005; Chen *et al,* 2006; Sohn *et al,* 2006; Suzuki *et al,* 2007). By doing so, one can take an unbiased view of the movements and interactions of various proteins in a pathway. Such experiments have already begun to reveal viable alternatives to the lipid-raft model, such as the formation of microdomains by protein–protein networks through a diffusional trapping and exclusion mechanism (Douglass & Vale, 2005).

The fluid nature of the lipid-raft model has also made it difficult to evaluate critically, as the definition of a lipid raft is still in

flux (Table 1). Of the current raft models in the literature, the L_o/L_d model is the most clearly defined, owing to the ease of generating and characterizing the properties of these domains in artificial and well-controlled systems. However, this does not necessarily mean that this model provides the best description of what occurs in cell membranes. More work is needed to determine the robustness of the various models of lipid rafts reported in the literature. The properties of 'non-raft' domains also deserve much greater attention than they currently receive (Shaikh & Edidin, 2006).

Finally, to validate or dispute the lipid-raft model, more efforts are needed to develop mechanistic models linking raft structure and function. A good place to begin would be to evaluate systematically how a particular set of structural and dynamic features of lipid rafts theoretically influence protein diffusion or reaction kinetics. Some efforts in this direction are under way (Nicolau *et al,* 2006). Ultimately, one would like to be able to put current understanding of the relationship between raft structure and function directly to the test by building quantitative models and validating their predictions experimentally (Tian *et al,* 2007). More quantitative efforts of this type are desperately needed to move the field beyond simple descriptions of the actions of lipid rafts (that is, "lipid rafts are involved in…") towards a mechanistic understanding of how they act.

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REFERENCES

Anderson RGW, Jacobson K (2002) A role for lipid shells in targeting proteins to caveolae, rafts and other lipid domains. *Science* **296:** 1821–1825

Baumgart T, Hammond AT, Sengupta P, Hess ST, Holowka DA, Baird BA, Webb WW (2007) Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles. *Proc Natl Acad Sci USA* **104:** 3165–3170

Brown DA (2006) Lipid rafts, detergent-resistant membranes, and raft targeting signals. *Physiology (Bethesda)* **21:** 430–439

Brown DA, London E (1998) Structure and origin of ordered lipid domains in biological membranes. *J Membr Biol* **164:** 103–114

Brown DA, Rose JK (1992) Sorting of GPI-anchored proteins to glycolipid-enriched membrane subdomains during transport to the apical cell surface. *Cell* **68:** 533–544

Bunnell SC, Hong DI, Kardon JR, Yamazaki T, McGlade CJ, Barr VA, Samelson LE (2002) T cell receptor ligation induces the formation of dynamically regulated signalling assemblies. *J Cell Biol* **158:** 1263–1275

Chadda R, Howes MT, Plowman SJ, Hancock JF, Parton RG, Mayor S (2007) Cholesterol-sensitive Cdc42 activation regulates actin polymerization for endocytosis via the GEEC pathway. *Traffic* **8:** 702–717

Chen Y, Thelin WR, Yang B, Milgram SL, Jacobson K (2006) Transient anchorage of cross-linked glycosyl-phosphatidylinositol-anchored proteins depends on cholesterol, Src family kinases, caveolin, and phosphoinositides. *J Cell Biol* **175:** 169–178

Cheng J, Ohsaki Y, Tauchi-Sato K, Fujita A, Fujimoto T (2006) Cholesterol depletion induces autophagy. *Biochem Biophys Res Commun* **351:** 246–252

Chichili GR, Rodgers W (2007) Clustering of membrane raft proteins by the actin cytoskeleton. *J Biol Chem* **282:** 36682–36691

Douglass AD, Vale RD (2005) Single-molecule microscopy reveals plasma membrane microdomains created by protein–protein networks that exclude or trap signalling molecules in T cells. *Cell* **121:** 937–950

Drevot P, Langlet C, Guo XJ, Bernard AM, Colard O, Chauvin JP, Lasserre R, He HT (2002) TCR signal initiation machinery is pre-assembled and activated in a subset of membrane rafts. *EMBO J* **21:** 1899–1908

Glebov OO, Nichols BJ (2004) Lipid raft proteins have a random distribution during localized activation of the T-cell receptor. *Nat Cell Biol* **6:** 238–243

Hancock JF (2006) Lipid rafts: contentious only from simplistic standpoints. *Nat Rev Mol Cell Biol* **7:** 456–462

Harder T (2004) Lipid raft domains and protein networks in T-cell receptor signal transduction. *Curr Opin Immunol* **16:** 353–359

Harder T, Engelhardt KR (2004) Membrane domains in lymphocytes: from lipid rafts to protein scaffolds. *Traffic* **5:** 265–275

Hawash IY, Hu XE, Adal A, Cassady JM, Geahlen RL, Harrison ML (2002) The oxygen-substituted palmitic acid analogue, 13-oxypalmitic acid, inhibits Lck localization to lipid rafts and T cell signalling. *Biochim Biophys Acta* **1589:** 140–150

He H-T, Marguet D (2008) T-cell antigen receptor triggering and lipid rafts: a matter of space and time scales. *EMBO Rep* **9:** 525–530

He H-T, Lellouch A, Marguet D (2005) Lipid rafts and the initiation of T cell receptor signalling. *Semin Immunol* **17:** 23–33

Hess ST, Kumar M, Verma A, Farrington J, Kenworthy A, Zimmerberg J (2005) Quantitative electron microscopy and fluorescence spectroscopy of the membrane distribution of influenza hemagglutinin. *J Cell Biol* **169:** 965–976

Horejsi V (2005) Lipid rafts and their roles in T-cell activation. *Microbes Infect* **7:** 310–316

Ike H, Kosugi A, Kato A, Iino R, Hirano H, Fujiwara T, Ritchie K, Kusumi A (2003) Mechanism of Lck recruitment to the T-cell receptor cluster as studied by single-molecule-fluorescence video imaging. *Chemphyschem* **4:** 620–626

Jacobson K, Mouritsen OG, Anderson RG (2007) Lipid rafts: at a crossroad between cell biology and physics. *Nat Cell Biol* **9:** 7–14

Jury EC, Flores-Borja F, Kabouridis PS (2007) Lipid rafts in T cell signalling and disease. *Semin Cell Dev Biol* **18:** 608–615

Kabouridis PS (2006) Lipid rafts in T cell receptor signalling. *Mol Membr Biol* **23:** 49–57

Kabouridis PS, Magee AI, Ley SC (1997) S-acylation of LCK protein tyrosine kinase is essential for its signalling function in T lymphocytes. *EMBO J* **16:** 4983–4998

Kabouridis PS, Janzen J, Magee AL, Ley SC (2000) Cholesterol depletion disrupts lipid rafts and modulates the activity of multiple signalling pathways in T lymphocytes. *Eur J Immunol* **30:** 954–963

Kenworthy AK, Nichols BJ, Remmert CL, Hendrix GM, Kumar M, Zimmerberg J, Lippincott-Schwartz J (2004) Dynamics of putative raft-associated proteins at the cell surface. *J Cell Biol* **165:** 735–746

Kusumi A, Koyama-Honda I, Suzuki K (2004) Molecular dynamics and interactions for creation of stimulation-induced stabilized rafts from small unstable steady-state rafts. *Traffic* **5:** 213–230

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

Larson DR, Gosse JA, Holowka DA, Baird BA, Webb WW (2005) Temporally resolved interactions between antigen-stimulated IgE receptors and Lyn kinase on living cells. *J Cell Biol* **171:** 527–536

Lenne PF, Wawrezinieck L, Conchonaud F, Wurtz O, Boned A, Guo XJ, Rigneault H, He HT, Marguet D (2006) Dynamic molecular confinement in the plasma membrane by microdomains and the cytoskeleton meshwork. *EMBO J* **25:** 3245–3256

Lichtenberg D, Goni FM, Heerklotz H (2005) Detergent-resistant membranes should not be identified with membrane rafts. *Trends Biochem Sci* **30:** 430–436

Lin J, Weiss A, Finco TS (1999) Localization of LAT in glycolipid-enriched microdomains is required for T cell activation. *J Biol Chem* **274:** 28861–28864

Marguet D, Lenne PF, Rigneault H, He HT (2006) Dynamics in the plasma membrane: how to combine fluidity and order. *EMBO J* **25:** 3446–3457

Mayor S, Rao M (2004) Rafts: scale-dependent, active lipid organization at the cell surface. *Traffic* **5:** 231–240

Meder D, Moreno MJ, Verkade P, Vaz WL, Simons K (2006) Phase coexistence and connectivity in the apical membrane of polarized epithelial cells. *Proc Natl Acad Sci USA* **103:** 329–334

Monjas A, Alcover A, Alarcon B (2004) Engaged and bystander T cell receptors are down-modulated by different endocytotic pathways. *J Biol Chem* **279:** 55376–55384

Montixi C, Langlet C, Bernard AM, Thimonier J, Dubois C, Wurbel MA, Chauvin JP, Pierres M, He HT (1998) Engagement of T cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. *EMBO J* **17:** 5334–5348

Munro S (2003) Lipid rafts: elusive or illusive? *Cell* **115:** 377–388

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

Pike LJ (2003) Lipid rafts: bringing order to chaos. *J Lipid Res* **44:** 655–667

Pizzo P, Giurisato E, Bigsten A, Tassi M, Tavano R, Shaw A, Viola A (2004) Physiological T cell activation starts and propagates in lipid rafts. *Immunol Lett* **91:** 3–9

Plowman SJ, Muncke C, Parton RG, Hancock JF (2005) H-ras, K-ras, and inner plasma membrane raft proteins operate in nanoclusters with differential dependence on the actin cytoskeleton. *Proc Natl Acad Sci USA* **102:** 15500–15505

Rodgers W, Rose JK (1996) Exclusion of CD45 inhibits activity of p56lck associated with glycolipid-enriched membrane domains. *J Cell Biol* **135:** 1515–1523

Schroeder R, London E, 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 USA* **91:** 12130–12134

Shaikh SR, Edidin MA (2006) Membranes are not just rafts. *Chem Phys Lipids* **144:** 1–3

Sharma P, Varma R, Sarasij RC, Gousset IK, Krishnamoorthy G, Rao M, Mayor S (2004) Nanoscale organization of multiple GPI-anchored proteins in living cell membranes. *Cell* **116:** 577–589

Shaw AS (2006) Lipid rafts: now you see them, now you don't. *Nat Immunol* **7:** 1139–1142

Shogomori H, Hammond AT, Ostermeyer-Fay AG, Barr DJ, Feigenson GW, London E, Brown DA (2005) Palmitoylation and intracellular domain interactions both contribute to raft targeting of linker for activation of T cells. *J Biol Chem* **280:** 18931–18942

Shvartsman DE, Gutman O, Tietz A, Henis YI (2006) Cyclodextrins but not compactin inhibit the lateral diffusion of membrane proteins independent of cholesterol. *Traffic* **7:** 917–926

Simons K, Ikonen E (1997) Functional rafts in cell membranes. *Nature* **387:** 569–572

Simons K, Toomre D (2000) Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* **1:** 31–41

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 signalling. *Proc Natl Acad Sci USA* **103:** 8143–8148

Stefanova I, Horejsi V, Ansotegui IJ, Knapp W, Stockinger H (1991) GPI-anchored cell-surface molecules complexed to protein tyrosine kinases. *Science* **254:** 1016–1019

Stulnig TM, Berger M, Sigmund T, Raederstorff D, Stockinger H, Waldhausl W (1998) Polyunsaturated fatty acids inhibit T cell signal transduction by modification of detergent-insoluble membrane domains. *J Cell Biol* **143:** 637–644

Sun M, Northup N, Marga F, Huber T, Byfield FJ, Levitan I, Forgacs G (2007) The effect of cellular cholesterol on membrane–cytoskeleton adhesion. *J Cell Sci* **120:** 2223–2231

Suzuki KG, Fujiwara TK, Sanematsu F, Iino R, Edidin M, Kusumi A (2007) GPI-anchored receptor clusters transiently recruit Lyn and Gα for temporary cluster immobilization and Lyn activation: single-molecule tracking study 1. *J Cell Biol* **177:** 717–730

Tanimura N, Saitoh S, Kawano S, Kosugi A, Miyake K (2006) Palmitoylation of LAT contributes to its subcellular localization and stability. *Biochem Biophys Res Commun* **341:** 1177–1183

Tian T, Harding A, Inder K, Plowman S, Parton RG, Hancock JF (2007) Plasma membrane nanoswitches generate high-fidelity Ras signal transduction. *Nat Cell Biol* **9:** 905–914

Veatch SL, Keller SL (2005) Seeing spots: complex phase behavior in simple membranes. *Biochim Biophys Acta* **1746:** 172–185

Viola A, Gupta N (2007) Tether and trap: regulation of membrane-raft dynamics by actin-binding proteins. *Nat Rev Immunol* **7:** 889–896

Vrljic M, Nishimura SY, Moerner WE, McConnell HM (2005) Cholesterol depletion suppresses the translational diffusion of class II major histocompatibility complex proteins in the plasma membrane. *Biophys J* **88:** 334–347

Xavier R, Brennan T, Li QQ, McCormack C, Seed B (1998) Membrane compartmentation Is required for efficient T cell activation. *Immunity* **8:** 723–732

Zeyda M, Stulnig TM (2006) Lipid Rafts & Co.: an integrated model of membrane organization in T cell activation. *Prog Lipid Res* **45:** 187–202

Zeyda M, Staffler G, Horejsi V, Waldhausl W, Stulnig TM (2002) LAT displacement from lipid rafts as a molecular mechanism for the inhibition of T cell signalling by polyunsaturated fatty acids. *J Biol Chem* **277:** 28418–28423

Zhang WG, Trible RP, Samelson LE (1998) LAT palmitoylation—its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. *Immunity* **9:** 239–246

Zhu M, Shen S, Liu Y, Granillo O, Zhang W (2005) Cutting edge: localization of linker for activation of T cells to lipid rafts is not essential in T cell activation and development. *J Immunol* **174:** 31–35

Zidovetzki R, Levitan I (2007) Use of cyclodextrins to manipulate plasma membrane cholesterol content: evidence, misconceptions and control strategies. *Biochim Biophys Acta* **1768:** 1311–1324

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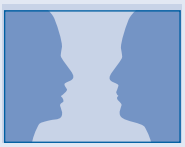

For more discussion on this topic, see also
De Wet B, Harder T (2008) Are rafts involved in T-cell receptor signalling? This issue p523. doi:10.1038/embor.2008.91
He H-T, Marguet D (2008) T-cell antigen receptor triggering and lipid rafts: a matter of space and time scales. This issue p525. doi:10.1038/embor.2008.78