Chapter 2

Introduction: Membrane Properties (Good) for Life

Marek Cebecauer

Abstract

Membranes protect cells from the surrounding environment but also provide a means for the optimization of processes such as metabolism, signalling, or mitogenesis. Membrane structure and function is determined by its molecular composition. How lipid species define membrane properties is discussed in this introductory chapter.

Key words Bilayer, Membrane functions, Membrane proteins, Biological roles

In living cells, lipids predominantly form lamellar structures; bilayers. The lipid bilayer represents a two-dimensional (2D) amphipathic structure with hydrophilic surfaces on both sides of a hydrophobic core. The core is largely impermeable for ions and other polar molecules, and to some extent for water molecules. These properties endow lipid bilayers with an ideal structure to function as cellular membranes which protect the cell from the surrounding environment and function in the organization of molecules. Membranes also form the surface of intracellular organelles, such as mitochondria or the nucleus, providing significant compartmentalization of eukaryotic cells. I will focus on membranes of higher eukaryotes such as humans and mice, but the herein described basic principles can be considered (to some level) also for membranes of prokaryotes, fungi, and plants [1–3]. Later chapters of this book describe useful techniques and tools which can be employed in membrane studies. In this introductory chapter I will try to outline a few aspects of membranes which are important for cellular function and are, therefore, in the center of membrane biology. In some cases, future directions of research are suggested.

Lipids define the basic lamellar structure of cellular membranes but proteins also significantly contribute to its form and function. Due to their hydrophilic surface, proteins normally do not integrate into the hydrophobic core of membranes but there are some exceptions. Proteins with hydrophobic helical segments (one or more), called transmembrane domains (TMDs), can integrate into

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the lipid bilayer. We call these integral membrane proteins. Other examples of membrane proteins are glycosphingolipid-anchored proteins (GPI-AP) of the outer plasma membrane leaflet and intracellular lipid-modified proteins which are anchored to the cytosolic leaflet of cellular membranes. All these proteins are directly influenced by the properties of membrane. Another group is peripheral membrane proteins possessing a specific domain (e.g., pleckstrin-homology (PH) domain) which can associate with the hydrophilic surface of the membrane based on their affinity for the lipid headgroups (e.g., negatively charged phosphatidylinositols). The function of such proteins is also regulated by membrane properties and organization. The list of membrane "associated" proteins can be further extended to include those which interact, directly or indirectly, with membrane components and, in some cases, can assemble into large supramolecular structures important for metabolism, signalling, cell motility, and other cellular processes. The formation and function of these complexes depends on membrane properties. It is therefore important to investigate the relations between individual membrane components and how these cooperate for the optimal local environment.

Only a few lipid species are required to form a lipid bilayer under physiological conditions [4]. Surprisingly, hundreds of different lipid species were found to form cellular membranes [5]. It is now well established that specific membrane lipid compositions can modulate its physical properties and function. In cells, a good example is mitochondria which are surrounded by two membranes, inner and outer. A unique lipid composition of mitochondrial inner membrane (increased presence of negatively charged lipids and large content of cardiolipin (1,3-bis(sn-3′-phosphatidyl)-sn-glycerol)) provides a specific environment for reactions exclusively localized in these metabolic factories of the cell. On the other hand, we do not have detailed information on lipid composition of cellular membranes and their subcompartments. The current development of techniques with dramatically improved sensitivity of such analysis (*see* Chapter 9) can help to better understand why cells use such a variety of lipid species for their function.

In parallel, it is important to investigate how lipid species modulate membrane properties and, as a consequence, how membrane properties regulate cellular processes. Phospholipids of cellular membranes are formed by a polar headgroup and attached acyl chains. Charge, size and the precise conformation of the headgroup as well as its local density can directly influence the association of peripheral molecules with membranes. A nice example of lipid headgroup-determined protein localization was shown for phosphatidylserine (PS) and the C2 domain of lactadherin [6]. PS is the abundant lipid of the cytosolic leaflet of plasma membrane but is present at lower level in other intracellular membranes, endosomes and lysosomes. In untreated cells, fluorescently labelled C2 domain

locates specifically to these membranes of murine macrophages. Such localization is exclusively due to its interaction with PS since no changes were observed after depletion of other negatively charged lipids (various phosphatidylinositols). On the other hand, interference with the presence of PS in membranes led to a rapid relocation of C2 domain of lactadherin to the cytosol [7]. Therefore, the PS headgroup with its charge, size and conformation determined the localization of C2 domain of lactadherin. An analogous effect was observed for other cellular proteins including signalling molecules c-Src, Rac1, and K-Ras [6]. In similar manner, the localization of phosphoinositide-binding signalling proteins interacting specifically with headgroups of phosphatidylinositol-4, 5-bisphosphate (phospholipase C-d1; [8]) or phosphatidylinositol-3,4,5-trisphosphate (Grp1; [9]) at membranes are regulated by lipases, kinases, and phosphatases providing mechanism for the control of their activity. An increasing number of available headgroup-specific biosensors [10, 11] and labelled lipids [12, 13] will be important for a better understanding of lipid changes in membranes of living cells [14].

Acyl chains of phospholipids define the rigidity of hydrophobic core of the membrane. Rigidity of membrane can also be described as a lipid packing density, meaning it determines how tightly lipid molecules (their acyl chains) are packed within a defined space of a membrane. Such a property has a dramatic effect on the freedom with which molecules (including proteins) can move and interact and was recognized as important for cells as early as the 1980s [15]. Membrane rigidity can also influence the orientation of membrane molecules, potentially causing change(s) in their conformation. Longer, saturated acyl chains of lipids support higher ordering, whereas increased presence of unsaturated bonds and acyl chain shortening decreases lipid ordering in membranes. This is well studied in model membranes with separated lipid phases. It is important to note here that the implementation of knowledge acquired from model systems to cellular membranes has to be performed with care (*see* Appendix).

In cells, an increased content of phospholipids with long and saturated acyl chains was detected in vesicles of trans-Golgi network (TGN), exo/endosomes, and plasma membrane [16, 17]. Increased rigidity of membranes towards the cell surface was expected (Fig. 1). Indeed, live cell imaging using environmentally sensitive membrane dyes (Laurdan or di-4-ANEPPDHQ; Chapter 10) supports this view with low lipid ordering observed for nuclear membrane and the endoplasmic reticulum (ER) and higher ordering in the plasma membrane and associated membrane structures [18, 19]. This may have consequences: (1) it can provide help for sorting of molecules (e.g., proteins) synthesized at the surface of intracellular membranes (ER) but targeted to the plasma membrane [20], and (2) increased membrane rigidity towards the cell surface can provide

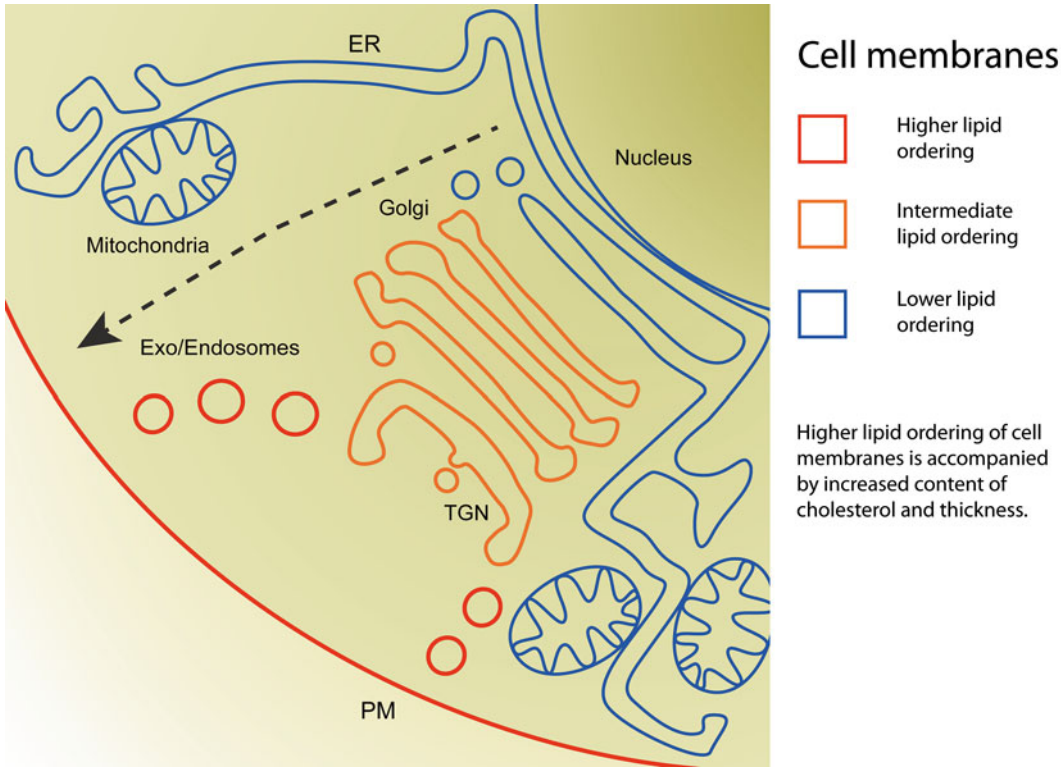

Fig. 1 Membrane order in living cells. Experiments using Laurdan and di-4-ANEPPDHQ fluorescent membrane probes indicate lower lipid ordering of intracellular membranes such as the endoplasmic reticulum (ER) and nucleus but higher ordering at the surface and in endosomal compartments. Somewhat intermediate state is believed for Golgi apparatus and trans-Golgi network (TGN). No clear prediction exists for mitochondria but no similar membrane structure with higher lipid ordering was observed in living cells. *Dashed arrow line* indicates anterograde traffic and sorting of membrane molecules

a specific environment for assembly of larger multimolecular entities [21, 22]. Such assemblies have recently been demonstrated at the surface of immune cells and neurons [23, 24] but also bacteria [25]. Techniques for characterization of multimolecular complexes and protein clusters in cellular membranes are undergoing a renaissance and a rapid development (Chapters 11 and 12). Such studies open new insights into membrane organization of cellular processes such as immune or neurological response, mitogenesis, and cell migration, to name just a few.

Cholesterol is essential component of cellular membranes. Its content is increased in the plasma membrane, endosomes, and membranes of TGN [16, 26]. One can speculate that cholesterol, in addition to its other functions, maintains fluidity of membranes with high content of gel-forming lipids such as phospholipids and sphingolipids with long saturated acyl chains (*see* Appendix). The fluid character of such ordered membranes is essential for the regulation of cellular processes by diffusion. The metabolism and important properties of cholesterol and other sterols in model and

cell membranes are described in detail in excellent recent reviews [27–29]. Sphingolipids can be glycosylated to generate glycosphingolipids (GSLs) of the outer plasma membrane leaflet. High density of hydroxyl groups in the glyco-structure of GSLs can lead to their aggregation and membrane reorganization (e.g., GM1 and GM3 clustering) adding to the heterogeneity of membranes. For example, GM3 clustering induces caveolin-1 redistribution and inhibition of EGFR activation [30]. Of note, GSLs have high affinity for cholesterol-rich membrane domains and the effect of their clustering can be indirect [31]. Both cholesterol and GSL levels can be modulated in cells by drugs or by the inhibition of metabolic pathways (Chapter 8 and [32]). Such experiments are very useful for our understanding of the importance of these lipids for cell function. But one has to be very careful when interpreting data from such experiments since these treatments are rather systemic [33, 34] and do not reduce cholesterol or GSL levels preferentially from cholesterol- and glycosphingolipid-rich domains, frequently called lipid rafts.

Another property, membrane thickness, also varies due to a lipid packing, acyl chain length, and the presence of cholesterol. This was suggested to have an impact on sorting of integral membrane proteins throughout cellular membranes due to a mismatch between the length of the TMD and membrane thickness. Different sorting of integral membrane proteins due to a mismatch has been observed in cells [35, 36]: proteins with short TMD preferentially reside in the ER having relatively thin membrane and those with longer TMD are sorted to Golgi apparatus and plasma membrane with thicker membrane and higher cholesterol content. On the other hand, membrane protein localization can be also influenced by its affinity for specific lipids [6, 37, 38] and there are proteins with long TMD which were shown to reside in the ER [39]. So it is probably a concert of various protein and lipid features which defines final destination of membrane molecules. It is also important to note here that our knowledge of mechanisms behind sorting and localization of membrane molecules is still very limited and requires further studies in silico, in vitro and in vivo.

Improved imaging techniques and analytical tools (Chapters 14, 15, 18 and 19) recently enabled us to investigate lateral membrane diffusion in living cells. Ensemble (FRAP, FCS, sptPALM) and single-molecule (SPT) techniques provide useful information on mobility of membrane molecules. Even though membrane dynamics studies are still in their early phase, it is already clear that membrane molecules in cells exhibit anomalous diffusion and are frequently constrained in mesoscale (20–200 nm) domains [40–42]. High molecular crowding in cellular membranes is certainly an important factor causing anomalous diffusion but membrane heterogeneities due to the interactions of lipids and proteins are hypothesized to add to this effect. The cytoskeleton further extends the complexity of cell membrane environment by providing

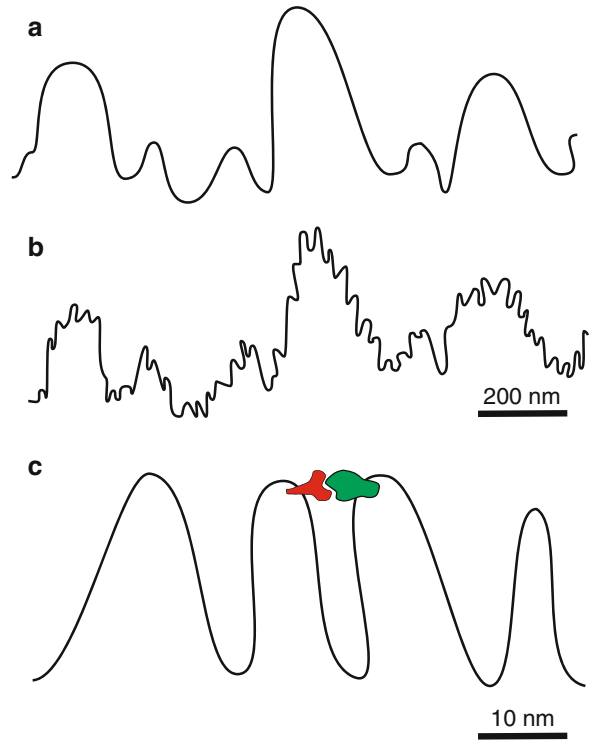

Fig. 2 Plasma membrane ruffling. Surface scanning techniques uncovered large ruffles at the surface of all studied cells. Scheme **A** indicates membrane curvature as observed by AFM or SICM. Fluorescence measured in membrane blebs suggested even more extensive folding of the plasma membrane. Scheme **B** illustrates membrane with low nanoscale ruffles which may support interaction of molecules in *cis* (Scheme **C**). Such intense membrane folding cannot be detected using current surface scanning techniques in living cells

"pickets-and-fences"(as suggested by Aki Kusumi's group; [43]) which were demonstrated to limit the movement of lipids and proteins in cellular membranes [44, 45]. Moreover, the cytoskeleton modulates mechanical forces affecting membranes which can have direct impact on cell function [46, 47]. Mechanical forces of membranes can be studied by atomic force microscopy (AFM; Chapter 13), a method which will certainly provide novel insights on cell membranes. AFM, but also scanning ion conductance microscopy (SICM; [48, 49]) and polarization microscopy [50, 51] can uncover the topology of cell surface which is far from being flat as observed by fluorescence imaging techniques [52]. Such observations have dramatic consequences for the interpretation of single particle tracking and membrane super-resolution image analysis [53]. In addition, if extensive folding and ruffling exists at mesoscale (<100 nm; Fig. 2), this would mean significant curvature of membranes [54], including the plasma membrane. Curvature was previously reported to cause lipid (and protein) sorting and has

impact on rigidity of membranes [55, 56], again adding to the heterogeneity of cell membranes. Further studies are required to describe the topology and curvature of plasma and intracellular membranes in higher detail for more general conclusions.

Finally, the exchange of lipid molecules between individual leaflets of a bilayer caused by transbilayer diffusion and transfer was observed in model and cell membranes. This is important due to the fact that a large part of lipid synthesis machinery localizes to the cytosolic leaflet of ER but various lipid species are found in both leaflets throughout the cell membrane compartments [57]. In addition, the distribution of lipids between membrane leaflets varies and in the membranes of the Golgi apparatus and plasma membrane we observe a lipid asymmetry with respect to transbilayer distribution [15]. It is, therefore, important to understand the mechanism(s) responsible for heterogeneous distribution of lipids in cell membranes. In model membranes, which are usually symmetric, transbilayer diffusion was found to be a very slow process in comparison to the pace demonstrated for similar lipids in cell membranes [57]. Asymmetric model membranes are currently available (*see* Chapter 7) but, to my knowledge, transbilayer mobility of lipids has not been investigated yet.

In summary, the organization and local properties of membranes dramatically affects cellular processes and function. The high speed of membrane-associated events frequently makes it difficult to perform all experiments in living cells at the physiological temperature. The current development of advanced imaging techniques and various tools for membrane studies, a good part of which is summarized in this book, will certainly accelerate membrane research. In a few years' time we may know, for example, what is behind the heterogeneities observed in the plasma membrane of all studied cells—is it an artifact, topology, or membrane domains?

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Appendix: Lipid Phases and Acyl Chain Ordering in Model Membranes

Physical properties of membranes are important factors for cell function but due to current technical limitations cannot be studied in living cells with high precision or, in some cases, not at all. Model membranes with well-defined composition (e.g., giant unilamellar vesicles; GUVs) provide an excellent subject for

biophysical studies and enable characterization of physical properties with good accuracy and reproducibility. In model membranes, lipids can form at least three phases which differ in freedom with which lipids can move around: the most rigid—solid (So), the most relaxed—liquid disordered (Ld), and somewhat intermediate—liquid ordered (Lo) phase. Phase behavior of lipids depends on the melting temperature (Tm) of lipids in a mixture and the presence of cholesterol. Temperature-gradient experiments with individual lipids and phase diagrams for bi- and ternary lipid mixtures uncovered some interesting complexities in lipid phase behavior and, especially, the importance of cholesterol (and other sterols) for the liquid character of cellular membranes [58, 59]. Cholesterol has rigidifying effect on lipids with low Tm, but it also prevents formation of solid phase by high Tm lipids. Currently, a phase diagram for 4-component lipid mixture was reported [60]. Data generated with even more complex lipid mixtures are difficult to interpret and we may wait another 5–10 years for phase diagram of 5-component mixture. In addition, no classical lipid phases can be expected in non-equilibrium system of living cells. Therefore, a more general property of lipid mixtures, lipid ordering or conformational order of acyl chains, was suggested for studies of complex lipid mixtures and cell membranes in order to better characterize the rigidity of a local membrane environment [61]. Reporters of lipid ordering have been established—fluorescent probes which can sense the presence or absence of water molecules in membranes, solvatochromic membrane dyes. Higher penetration of water molecules to the hydrophobic core indicates reduced rigidity of the membrane. To date, the best membrane order probe is probably Laurdan and its derivative, C-Laurdan (*see* Chapters 3 and 10; [62]). More ordered membranes can be easily distinguished from disordered parts in phase separated monolayers and GUVs [61, 63]. Since solid (or gel)-like phase is not expected to last for long in living cells, there is no problem that these probes cannot distinguish Lo from So phase. In cells, differences in lipid ordering are expected to be less pronounced due to a high complexity of lipid mixture and the presence of proteins. Indeed, data from cell-derived vesicles, GPMVs or cell-derived blebs (Chapter 6), show significant but small difference in values detected by C-Laurdan [64]. Even smaller differences are observed in living cells for intracellular membranes compared to the plasma membrane at physiological temperature [19, 65]. This indicates that organization of membranes in living cells depends more on subtle variation of local membrane composition and properties than on a large segregation of molecules due to their physical properties. It is, therefore, important to focus on a local membrane environments and changes therein. But model membranes represent an excellent tool for studies of molecules in a defined environment and adjusting our

methods for future cell experiments. Cell-derived vesicles then function as a bridge between these two worlds, artificial and natural. In addition, in silico simulations provide unprecedented tool to investigate local relations before doing costly experiments (Chapters 20 and 21).

References

1. Goldfine H (1984) Bacterial membranes and lipid packing theory. J Lipid Res 25:1501–1507
2. Cronan JE (2003) Bacterial membrane lipids: where do we stand? Annu Rev Microbiol 57: 203–224
3. Malinsky J, Opekarova M, Grossmann G, Tanner W (2013) Membrane microdomains, rafts, and detergent-resistant membranes in plants and fungi. Annu Rev Plant Biol 64: 501–529
4. Montes LR, Alonso A, Goni FM, Bagatolli LA (2007) Giant unilamellar vesicles electroformed from native membranes and organic lipid mixtures under physiological conditions. Biophys J 93:3548–3554
5. Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K, Shevchenko A (2009) Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci U S A 106: 2136–2141
6. Yeung T, Gilbert GE, Shi J, Silvius J, Kapus A, Grinstein S (2008) Membrane phosphatidylserine regulates surface charge and protein localization. Science 319:210–213
7. Yeung T, Terebiznik M, Yu L, Silvius J, Abidi WM, Philips M, Levine T, Kapus A, Grinstein S (2006) Receptor activation alters inner surface potential during phagocytosis. Science 313: 347–351
8. Lomasney JW, Cheng HF, Wang LP, Kuan Y, Liu S, Fesik SW, King K (1996) Phosphatidylinositol 4,5-bisphosphate binding to the pleckstrin homology domain of phospholipase C-delta1 enhances enzyme activity. J Biol Chem 271:25316–25326
9. Klarlund JK, Rameh LE, Cantley LC, Buxton JM, Holik JJ, Sakelis C, Patki V, Corvera S, Czech MP (1998) Regulation of GRP1-catalyzed ADP ribosylation factor guanine nucleotide exchange by phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem 273: 1859–1862
10. Kay JG, Grinstein S (2011) Sensing phosphatidylserine in cellular membranes. Sensors (Basel) 11:1744–1755
11. Nahorski SR, Young KW, John Challiss RA, Nash MS (2003) Visualizing phosphoinositide signalling in single neurons gets a green light. Trends Neurosci 26:444–452
12. Laketa V, Zarbakhsh S, Morbier E, Subramanian D, Dinkel C, Brumbaugh J, Zimmermann P, Pepperkok R, Schultz C (2009) Membrane-permeant phosphoinositide derivatives as modulators of growth factor signaling and neurite outgrowth. Chem Biol 16:1190–1196
13. Neef AB, Schultz C (2009) Selective fluorescence labeling of lipids in living cells. Angew Chem Int Ed Engl 48:1498–1500
14. Schultz C (2010) Challenges in studying phospholipid signaling. Nat Chem Biol 6:473–475
15. Spector AA, Yorek MA (1985) Membrane lipid composition and cellular function. J Lipid Res 26:1015–1035
16. van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9: 112–124
17. van Meer G, de Kroon AI (2011) Lipid map of the mammalian cell. J Cell Sci 124:5–8
18. Gaus K, Gratton E, Kable EP, Jones AS, Gelissen I, Kritharides L, Jessup W (2003) Visualizing lipid structure and raft domains in living cells with two-photon microscopy. Proc Natl Acad Sci U S A 100:15554–15559
19. Owen DM, Oddos S, Kumar S, Davis DM, Neil MA, French PM, Dustin ML, Magee AI, Cebecauer M (2010) High plasma membrane lipid order imaged at the immunological synapse periphery in live T cells. Mol Membr Biol 27(4–6):178–189
20. Surma MA, Klose C, Simons K (2012) Lipid-dependent protein sorting at the trans-Golgi network. Biochim Biophys Acta 1821:1059–1067
21. Gould GW, Lippincott-Schwartz J (2009) New roles for endosomes: from vesicular carriers to multi-purpose platforms. Nat Rev Mol Cell Biol 10:287–292
22. Cebecauer M, Spitaler M, Serge A, Magee AI (2010) Signalling complexes and clusters: functional advantages and methodological hurdles. J Cell Sci 123:309–320

23. Renner M, Specht CG, Triller A (2008) Molecular dynamics of postsynaptic receptors and scaffold proteins. Curr Opin Neurobiol 18:532–540
24. Varma R, Campi G, Yokosuka T, Saito T, Dustin ML (2006) T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. Immunity 25:117–127
25. Greenfield D, McEvoy AL, Shroff H, Crooks GE, Wingreen NS, Betzig E, Liphardt J (2009) Self-organization of the escherichia coli chemotaxis network imaged with super-resolution light microscopy. PLoS Biol 7:e1000137
26. Mukherjee S, Zha X, Tabas I, Maxfield FR (1998) Cholesterol distribution in living cells: fluorescence imaging using dehydroergosterol as a fluorescent cholesterol analog. Biophys J 75:1915–1925
27. Nes WD (2011) Biosynthesis of cholesterol and other sterols. Chem Rev 111:6423–6451
28. Olsen BN, Schlesinger PH, Ory DS, Baker NA (2012) Side-chain oxysterols: from cells to membranes to molecules. Biochim Biophys Acta 1818:330–336
29. Maxfield FR, van Meer G (2010) Cholesterol, the central lipid of mammalian cells. Curr Opin Cell Biol 22:422–429
30. Wang XQ, Sun P, Paller AS (2002) Ganglioside induces caveolin-1 redistribution and interaction with the epidermal growth factor receptor. J Biol Chem 277:47028–47034
31. Degroote S, Wolthoorn J, van Meer G (2004) The cell biology of glycosphingolipids. Semin Cell Dev Biol 15:375–387
32. Zha X, Pierini LM, Leopold PL, Skiba PJ, Tabas I, Maxfield FR (1998) Sphingomyelinase treatment induces ATP-independent endocytosis. J Cell Biol 140:39–47
33. Bijl N, van Roomen CP, Triantis V, Sokolovic M, Ottenhoff R, Scheij S, van Eijk M, Boot RG, Aerts JM, Groen AK (2009) Reduction of glycosphingolipid biosynthesis stimulates biliary lipid secretion in mice. Hepatology 49: 637–645
34. Mahammad S, Parmryd I (2008) Cholesterol homeostasis in T cells. Methyl-beta-cyclodextrin treatment results in equal loss of cholesterol from Triton X-100 soluble and insoluble fractions. Biochim Biophys Acta 1778:1251–1258
35. Sharpe HJ, Stevens TJ, Munro S (2010) A comprehensive comparison of transmembrane domains reveals organelle-specific properties. Cell 142:158–169
36. Bulbarelli A, Sprocati T, Barberi M, Pedrazzini E, Borgese N (2002) Trafficking of tail-anchored proteins: transport from the endoplasmic reticulum to the plasma membrane and sorting between surface domains in polarised epithelial cells. J Cell Sci 115:1689–1702
37. Haberkant P, Schmitt O, Contreras FX, Thiele C, Hanada K, Sprong H, Reinhard C, Wieland FT, Brugger B (2008) Protein-sphingolipid interactions within cellular membranes. J Lipid Res 49:251–262
38. Contreras FX, Ernst AM, Haberkant P, Bjorkholm P, Lindahl E, Gonen B, Tischer C, Elofsson A, von Heijne G, Thiele C et al (2012) Molecular recognition of a single sphingolipid species by a protein's transmembrane domain. Nature 481:525–529
39. Hennecke S, Cosson P (1993) Role of transmembrane domains in assembly and intracellular transport of the CD8 molecule. J Biol Chem 268:26607–26612
40. Eggeling C, Ringemann C, Medda R, Schwarzmann G, Sandhoff K, Polyakova S, Belov VN, Hein B, von Middendorff C, Schonle A et al (2009) Direct observation of the nanoscale dynamics of membrane lipids in a living cell. Nature 457:1159–1162
41. Ritchie K, Shan XY, Kondo J, Iwasawa K, Fujiwara T, Kusumi A (2005) Detection of non-Brownian diffusion in the cell membrane in single molecule tracking. Biophys J 88:2266–2277
42. Lasserre R, Guo XJ, Conchonaud F, Hamon Y, Hawchar O, Bernard AM, Soudja SM, Lenne PF, Rigneault H, Olive D et al (2008) Raft nanodomains contribute to Akt/PKB plasma membrane recruitment and activation. Nat Chem Biol 4:538–547
43. Ritchie K, Iino R, Fujiwara T, Murase K, Kusumi A (2003) The fence and picket structure of the plasma membrane of live cells as revealed by single molecule techniques. Mol Membr Biol 20:13–18
44. Fujiwara T, Ritchie K, Murakoshi H, Jacobson K, Kusumi A (2002) Phospholipids undergo hop diffusion in compartmentalized cell membrane. J Cell Biol 157:1071–1081
45. Murase K, Fujiwara T, Umemura Y, Suzuki K, Iino R, Yamashita H, Saito M, Murakoshi H, Ritchie K, Kusumi A (2004) Ultrafine membrane compartments for molecular diffusion as revealed by single molecule techniques. Biophys J 86:4075–4093
46. Alsteens D, Garcia MC, Lipke PN, Dufrene YF (2010) Force-induced formation and propagation of adhesion nanodomains in living fungal cells. Proc Natl Acad Sci U S A 107:20744–20749
47. Natkanski E, Lee WY, Mistry B, Casal A, Molloy JE, Tolar P (2013) B cells use

mechanical energy to discriminate antigen affinities. Science 340:1587–1590

48. Korchev YE, Bashford CL, Milovanovic M, Vodyanoy I, Lab MJ (1997) Scanning ion conductance microscopy of living cells. Biophys J 73:653–658
49. Rheinlaender J, Geisse NA, Proksch R, Schaffer TE (2011) Comparison of scanning ion conductance microscopy with atomic force microscopy for cell imaging. Langmuir 27:697–704
50. Benninger RK, Onfelt B, Neil MA, Davis DM, French PM (2005) Fluorescence imaging of two-photon linear dichroism: cholesterol depletion disrupts molecular orientation in cell membranes. Biophys J 88:609–622
51. Lazar J, Bondar A, Timr S, Firestein SJ (2011) Two-photon polarization microscopy reveals protein structure and function. Nat Methods 8:684–690
52. Parmryd I, Onfelt B (2013) Consequences of membrane topography. FEBS J 280:2775–2784
53. Adler J, Shevchuk AI, Novak P, Korchev YE, Parmryd I (2010) Plasma membrane topography and interpretation of single-particle tracks. Nat Methods 7:170–171
54. Drin G, Casella JF, Gautier R, Boehmer T, Schwartz TU, Antonny B (2007) A general amphipathic alpha-helical motif for sensing membrane curvature. Nat Struct Mol Biol 14:138–146
55. Tian A, Baumgart T (2009) Sorting of lipids and proteins in membrane curvature gradients. Biophys J 96:2676–2688
56. Ogunyankin MO, Huber DL, Sasaki DY, Longo ML (2013) Nanoscale patterning of membrane-bound proteins formed through curvature-induced partitioning of phase-specific receptor lipids. Langmuir 29: 6109–6115
57. Holthuis JC, Levine TP (2005) Lipid traffic: floppy drives and a superhighway. Nat Rev Mol Cell Biol 6:209–220
58. Veatch SL, Keller SL (2005) Miscibility phase diagrams of giant vesicles containing sphingomyelin. Phys Rev Lett 94:148101
59. Veatch SL, Keller SL (2005) Seeing spots: complex phase behavior in simple membranes. Biochim Biophys Acta 1746:172–185
60. Konyakhina TM, Wu J, Mastroianni JD, Heberle FA, Feigenson GW (2013) Phase diagram of a 4-component lipid mixture: DSPC/DOPC/POPC/chol. Biochim Biophys Acta 1828:2204–2214
61. Parasassi T, Gratton E, Yu WM, Wilson P, Levi M (1997) Two-photon fluorescence microscopy of laurdan generalized polarization domains in model and natural membranes. Biophys J 72:2413–2429
62. Kim HM, Choo HJ, Jung SY, Ko YG, Park WH, Jeon SJ, Kim CH, Joo TH, Cho BR (2007) A two-photon fluorescent probe for lipid raft imaging: C-laurdan. Chembiochem 8:553–559
63. Bagatolli LA, Gratton E (2000) Two photon fluorescence microscopy of coexisting lipid domains in giant unilamellar vesicles of binary phospholipid mixtures. Biophys J 78:290–305
64. Sezgin E, Levental I, Grzybek M, Schwarzmann G, Mueller V, Honigmann A, Belov VN, Eggeling C, Coskun U, Simons K et al (2012) Partitioning, diffusion, and ligand binding of raft lipid analogs in model and cellular plasma membranes. Biochim Biophys Acta 1818: 1777–1784
65. Dinic J, Ashrafzadeh P, Parmryd I (2013) Actin filaments attachment at the plasma membrane in live cells cause the formation of ordered lipid domains. Biochim Biophys Acta 1828:1102–1111

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