Display items

Boxes

Box 1 | **Model membranes to study formation and organization of lateral domains**

Combining a relatively saturated lipid, an unsaturated lipid and cholesterol in a model membrane, often results in liquid–liquid phase separation and the establishment of two distinct phases (still liquid in nature)[11]. One of these phases (liquid-ordered (Lo)) is more viscous that the other (liquid disordered (Ld)) owing to the tighter packing and higher molecular order of its constituent lipids[91]. This Lo phase is believed to be analogous to lipid rafts in cellular membranes.

Supported lipid bilayers (SLBs, see Figure panel **a**) are planar bilayers formed on glass or mica surfaces[29]. As these membranes are planar, they are highly amenable to microscopic imaging, either by light or Atomic Force Microscopy, allowing observations of the topology of nanodomains that are not resolvable with diffraction-limited optical microscopy (Supplementary Box S2). The artefacts caused by the solid support in SLBs are avoided by the use of free-standing membranes like giant unilamellar vesicles (GUVs) (see Figure panel **b**), which have been frequently used to investigate domain dynamics and morphologies[187]. The limitation of synthetic model systems is their simple composition, which does not fully recapitulate that of the cell membrane.

Giant plasma membrane vesicles (GPMVs) are obtained from cell membranes[37]. Like GUVs, these form micron-scale lateral liquid domains (confirming the capacity for liquid–liquid phase separation in biological membranes), but do so while maintaining the broad compositional features of the native plasma membrane. The most notable differences between GUVs and GPMVs are lipid complexity and the presence of abundant transmembrane proteins[37,38], incorporation of which into SLBs and GUVs remains technically challenging. The biophysical properties of GPMVs are somewhat distinct compared to artificial membranes[37,92,188]. For example, the difference in packing between Lo and Ld domains in GPMVs is much smaller than in GUVs (Figure Box panel **c;** generalized polarization is a relative index of lipid packing, with +1 representing maximally ordered and -1 representing maximally disordered membranes), which may explain why transmembrane proteins can associate with Lo phase in GPMVs[43] but not in GUVs[189]. Despite these differences, most of the core features of coexisting liquid-ordered and -disordered domains in these model systems are fundamentally similar[24].

SUPPLEMENTARY BOX 1

Supplementary Box 1 | **Diffraction limit and super resolution microscopy**

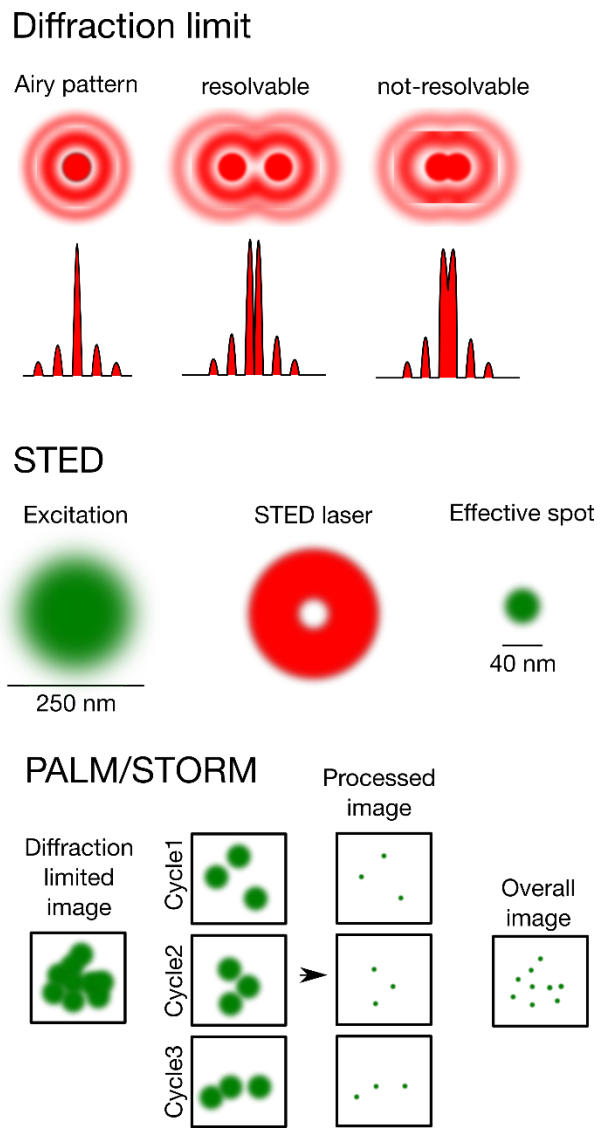
Membrane domains in cell membranes are assumed to measure below 20 nm in diameter[1], and consequently are not resolvable on a conventional optical microscope. The spatial resolution of any lens-based microscope is limited to about 200-250 nm for visible light. Since such a lens-based microscope employs focussed light, the light interacts with fringes of intricate objects, and it broadens to a diffraction pattern as it propagates. This diffraction pattern contains a central disk containing most of the information on the object (Airy disk), but becomes larger than the object masking the subtle details of it. This diffraction limit, as formulized by Ernst Abbe in 1873: $d = \lambda/2NA$ (where d is the diameter of the focal spot (or Airy disk at full-width-half-maximum of the focused light intensity), λ is the wavelength of the light and NA is the numerical aperture of the objective) prevents the separation of two close-by objects with the standard illumination (**see Figure below**). A remedy to this physical limit is the reversible inhibition of fluorescence, ensuring that the measured signal stems from a region of the sample that is much smaller than 200-250 nm, as realized in super-resolution optical microscopy or nanoscopy (for a review see ref[2]). Stimulated emission depletion nanoscopy (STED) nanoscopy uses a depletion laser whose focal spot exhibits at least one intensity zero such as a donut-shaped intensity distribution to inhibit spontaneous fluorescence emission at the periphery of the airy pattern, leaving the central fluorescence signal untouched (**see Figure below**). The resulting effective fluorescence spot is thus

reduced to sub-diffraction scales, and, consequently, the STED nanoscope produces images with <<200 nm spatial resolution. On the other hand, photoactivatable localization microscopy (PALM) or stochastic optical reconstruction microscopy (STORM) switches the employed fluorescence labels between dark and bright states in such a way that in a single camera image only a few molecules are bright and detected, allowing a precise determination (or localization) of the spatial positions of molecules (**see Figure below**). Switching on and off all fluorescence labels in subsequently recorded image frames allows establishing a final image out of all molecular positions. Since the localization precision is <<200 nm, the final PALM or STORM images feature sub-diffraction spatial resolution. Both methods have their own advantages and disadvantages. While STORM/PALM may deliver higher spatial resolution in day-to-day use, dynamic live-cell imaging is less straightforward and requires extensive post processing. In contrast, STED nanoscopy delivers direct images, and its spatial resolution can be continuously tuned by increasing the STED laser intensity, which offers unique possibilities such as combination with fluorescence correlation spectroscopy (STED-FCS). However, high laser power of the depletion laser is the main disadvantage of the STED technique. Further techniques such as structured-illumination microscopy achieve an up to 2-fold improvement in spatial resolution (in contrast to the in principle unlimited increase in STED and PALM/STORM microscopy) by employing spatial patterns in the illuminating laser light only and no reversible photoswitching of fluorescence emission.

References

1 Pathak, P. & London, E. The Effect of Membrane Lipid Composition on the Formation of Lipid Ultrananodomains. *Biophys J* **109**, 1630-1638, doi:10.1016/j.bpj.2015.08.029 (2015).

2 Eggeling, C., Willig, K. I., Sahl, S. J. & Hell, S. W. Lens-based fluorescence nanoscopy. *Quarterly reviews of biophysics* **48**, 178-243, doi:10.1017/s0033583514000146 (2015).



Box S2 | **Rafts in the tree of life**

Although the general fluid bilayer organization of biological membranes is nearly completely conserved throughout the kingdoms of life, the specific lipid and protein compositions of cellular membranes vary dramatically between various life forms. Recent findings suggest that membrane domains are prevalent throughout the evolutionary tree, suggesting that lateral heterogeneity is a broad organizing principle for biological membranes.

Rafts in prokaryotes – Most prokaryotes do not produce cholesterol or sphingomyelin, instead relying on sterol analogs[1] or Lipid A[2] that may function as physical surrogates of key raft constituents. Hopanoids, for instance, fluidize the bacterial membrane and drive formation of ordered membrane domains[2]. Borrelia, a genus of bacteria obtaining cholesterol from the host, shows distinct domains of high lipid order[3] that are required for bactericidal activity by host factors[4]. Comparing the DRM proteome of Borrelia[5] with that of mammalian cells has provided insight in how membrane domains composition has evolved from prokaryotes to eukaryotes. Membrane heterogeneity is specifically maintained in distinct parts of the bacterial cells. For instance, bacterial flagella are rich in saturated and depleted with unsaturated lipids, which supports the formation of ordered membrane environments in the flagella and this environment is vital for its function[6].

Rafts in fungi and plants – Fungal membranes possess broadly similar lipid classes to those found in metazoans. For instance, the dominant sterol is ergosterol[7], which like cholesterol has the ability to interact with sphingolipids and form ordered membrane domains[8]. Such domains have been implicated in several processes, including growth and death of the yeast cell[9]. In plants, on the other hand, a complex mixture of sterols (usually called phytosterols) is present and interactions of these sterols with lipids (mostly ceramides) and proteins can lead to membrane structuring[10].

Rafts in animals – As highlighted before, plasma membranes of mammalian cells are marvellously capable of undergoing microscopic phase separation in the absence of cortical actin cytoskeleton[11]. Recent labelling and observation technologies have also enabled us to probe lateral heterogeneity in the plasma membrane of live mammalian cells[12-14]. However, it is not the only structure accommodating domains. Very surprisingly, most direct evidence of liquid-liquid phase separation in live cells occurs in vacuoles[15]. Mitochondria or mitochondria-associated membranes[16] and exosomes[17] have recently been shown to be enriched in DRMs, as well.

1 Saenz, J. P. *et al.* Hopanoids as functional analogues of cholesterol in bacterial membranes. *Proc Natl Acad Sci U S A* **112**, 11971-11976 (2015).

2 Saenz, J. P., Sezgin, E., Schwille, P. & Simons, K. Functional convergence of hopanoids and sterols in membrane ordering. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 14236-14240 (2012).

3 LaRocca, T. J. *et al.* Proving Lipid Rafts Exist: Membrane Domains in the Prokaryote Borrelia burgdorferi Have the Same Properties as Eukaryotic Lipid Rafts. *Plos Pathogens* **9** (2013).

4 LaRocca, T. J. *et al.* Cholesterol lipids of Borrelia burgdorferi form lipid rafts and are required for the bactericidal activity of a complement-independent antibody. *Cell host & microbe* **8**, 331-342 (2010).

5 Toledo, A., Perez, A., Coleman, J. L. & Benach, J. L. The lipid raft proteome of Borrelia burgdorferi. *Proteomics* **15**, 3662-3675 (2015).

6 Serricchio, M. *et al.* Flagellar membranes are rich in raft-forming phospholipids. *Biology open* **4**, 1143-1153 (2015).

7 Ejsing, C. S. *et al.* Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. *Proc Natl Acad Sci U S A* **106**, 2136-2141 (2009).

8 Klose, C. *et al.* Yeast lipids can phase-separate into micrometer-scale membrane domains. *J Biol Chem* **285**, 30224-30232 (2010).

9 Mollinedo, F. Lipid raft involvement in yeast cell growth and death. *Frontiers in oncology* **2**, 140 (2012).

10 Zauber, H., Burgos, A., Garapati, P. & Schulze, W. X. Plasma membrane lipid-protein interactions affect signaling processes in sterol-biosynthesis mutants in Arabidopsis thaliana. *Frontiers in plant science* **5**, 78 (2014).

11 Baumgart, T. *et al.* Large-scale fluid/fluid phase separation of proteins and lipids in giant plasma membrane vesicles. *Proc Natl Acad Sci U S A* **104**, 3165-3170 (2007).

12 Eggeling, C. *et al.* Direct observation of the nanoscale dynamics of membrane lipids in a living cell. *Nature* **457**, 1159-1162 (2009).

13 Owen, D. M., Williamson, D. J., Magenau, A. & Gaus, K. Sub-resolution lipid domains exist in the plasma membrane and regulate protein diffusion and distribution. *Nature Communications* **3** (2012).
14 Sanchez, S. A., Tricerri, M. A. & Gratton, E. Laurdan generalized polarization fluctuations measures membrane packing micro-heterogeneity in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 7314-7319 (2012).
15 Toulmay, A. & Prinz, W. A. Direct imaging reveals stable, micrometer-scale lipid domains that segregate proteins in live cells. *Journal of Cell Biology* **202**, 35-44 (2013).
16 Poston, C. N., Duong, E., Cao, Y. & Bazemore-Walker, C. R. Proteomic analysis of lipid raft-enriched membranes isolated from internal organelles. *Biochemical and biophysical research communications* **415**, 355-360 (2011).
17 Dubois, L., Ronquist, K. K., Ek, B., Ronquist, G. & Larsson, A. Proteomic Profiling of Detergent Resistant Membranes (Lipid Rafts) of Prostasomes. *Molecular & cellular proteomics : MCP* **14**, 3015-3022 (2015).