PHILOSOPHICAL TRANSACTIONS B

rstb.royalsocietypublishing.org

Review

Cite this article: Sych T, Mély Y, Römer W. 2018 Lipid self-assembly and lectin-induced reorganization of the plasma membrane. *Phil. Trans. R. Soc. B* **373**: 20170117.
http://dx.doi.org/10.1098/rstb.2017.0117

Accepted: 21 January 2018

One contribution of 15 to a theme issue 'Self-organization in cell biology'.

Subject Areas:
cellular biology, synthetic biology, biophysics

Keywords:
lectin, plasma membrane, clustering, lipid rafts, glycosphingolipid, lipid bilayer

Author for correspondence:
Winfried Römer
e-mail: winfried.roemer@bioss.uni-freiburg.de

Lipid self-assembly and lectin-induced reorganization of the plasma membrane

Taras Sych[1,2,3,4], Yves Mély[4] and Winfried Römer[1,2,3]

[1]Faculty of Biology, Albert-Ludwigs-University Freiburg, Schänzlestraße 1, 79104 Freiburg, Germany
[2]Centre for Biological Signalling Studies (BIOSS), Albert-Ludwigs-University Freiburg, Schänzlestraße 18, 79104 Freiburg, Germany
[3]Freiburg Center for Interactive Materials and Bioinspired Technology (FIT), Albert-Ludwigs-University Freiburg, Georges-Köhler-Allee 105, 79110 Freiburg, Germany
[4]Laboratoire de Bioimagerie et Pathologies, UMR 7021 CNRS, Faculté de Pharmacie, Université de Strasbourg, 67401 Illkirch Cedex, France

WR, 0000-0002-2847-246X

The plasma membrane represents an outstanding example of self-organization in biology. It plays a vital role in protecting the integrity of the cell interior and regulates meticulously the import and export of diverse substances. Its major building blocks are proteins and lipids, which self-assemble to a fluid lipid bilayer driven mainly by hydrophobic forces. Even if the plasma membrane appears—globally speaking—homogeneous at physiological temperatures, the existence of specialized nano- to micrometre-sized domains of raft-type character within cellular and synthetic membrane systems has been reported. It is hypothesized that these domains are the origin of a plethora of cellular processes, such as signalling or vesicular trafficking. This review intends to highlight the driving forces of lipid self-assembly into a bilayer membrane and the formation of small, transient domains within the plasma membrane. The mechanisms of self-assembly depend on several factors, such as the lipid composition of the membrane and the geometry of lipids. Moreover, the dynamics and organization of glycosphingolipids into nanometre-sized clusters will be discussed, also in the context of multivalent lectins, which cluster several glycosphingolipid receptor molecules and thus create an asymmetric stress between the two membrane leaflets, leading to tubular plasma membrane invaginations.

This article is part of the theme issue 'Self-organization in cell biology'.

1. The plasma membrane

The plasma membrane physically separates the cytoplasm of living cells from the extracellular environment and hence maintains the physical integrity of the cell. It acts as a barrier that is selectively permeable to ions and organic molecules, and regulates transport processes into and out of the cell [1]. The plasma membrane is involved in a multitude of cellular processes, such as signalling and adhesion, among others [2,3]. It also helps to hold the cytoskeleton in place to preserve the cell shape [4]. With respect to all these diverse functions, it sounds amazing that the plasma membrane represents a lipid matrix with a thickness of only 4–6 nm, with embedded integral and peripheral proteins [5,6]. The principles of how membrane lipids self-assemble into cell membranes and how lipid–lipid interactions lead to the formation of small, transient domains within the membrane will be outlined in the following.

2. Self-organization of lipids in aqueous solutions

The plasma membrane consists of a complex mixture of components, among which lipids are the major building blocks. Plasma membranes contain a large number of different types of lipids that form a lipid bilayer with an inner (cytosolic) and an outer (extracellular) membrane leaflet [7]. Lipids exhibit an impressive

THE ROYAL SOCIETY PUBLISHING

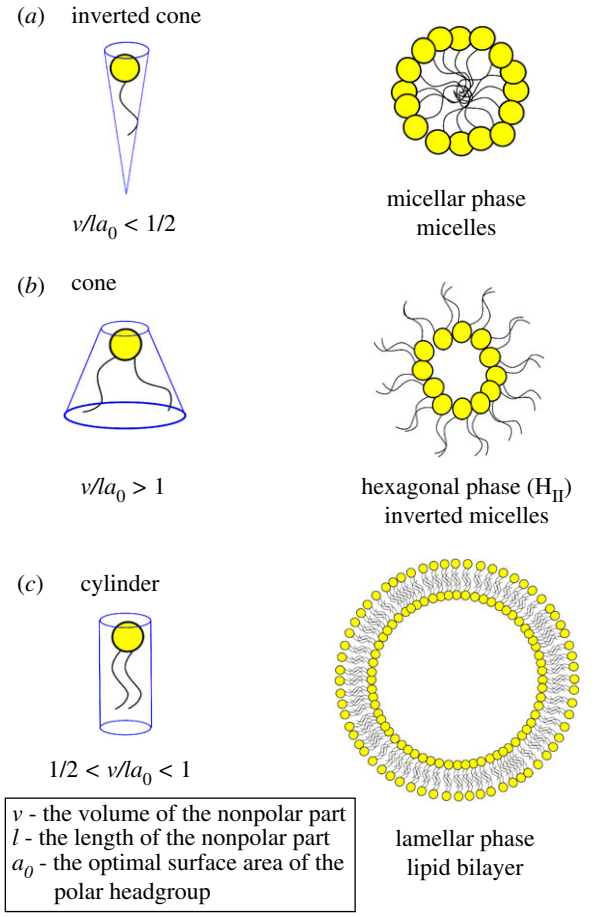

Figure 1. Self-assembly of lipids in contact with water. Lipids are characterized by their *critical packing parameter* v/la_0. (*a*) Inverted cone-shaped lipids form micelles (micellar phase) of positive curvature. (*b*) Cone-shaped lipids assemble into different structures of hexagonal (H_{II}) phase. (*c*) Cylinder-shaped lipids form a lamellar phase.

diversity of properties and can be classified by their geometry [8], as well as by the type of assembly they form in contact with water.

Since lipids contain a hydrophilic (polar head group) and a hydrophobic part (fatty acyl chain) they are considered as amphiphilic molecules. Exposed to water, lipids compact their hydrophobic parts in the energetically most favourable way, forming a hydrophobic core [9]. This simple principle underlies the self-organization of lipids in an aqueous environment, e.g. in living cells as well as in artificial membrane systems.

As indicated by Israelachvili *et al.* [10,11], the lipid behaviour in aqueous solution can be easily predicted by taking into consideration the geometrical parameters of its hydrophobic and hydrophilic parts. These are v, the volume of the nonpolar part, l, the length of the nonpolar part and a_0, the optimal surface area of the polar head group. The parameters v and l can be simply determined by the lipid size and shape [11,12]. The determination of the parameter a_0 is less straightforward. The possible repulsive interactions between head groups and parameters of the aqueous environment (ionic strength, pH) have to be taken into account. This factor is particularly critical for charged lipids, e.g. phosphatidylserine (PS) or phosphatidylglycerol (PG), where the head groups have to be far enough from each other that Coulomb's repulsive forces are equilibrated by lipid–lipid interactions.

One of the potential assembly modes of lipid molecules in water is the formation of spherical micelles (figure 1) [12]. If a spherical micelle is built up of N lipid molecules, the total micelle surface area a_M and the volume of the micelle V_M can be determined as

$$a_M = Na_0 = 4\pi R_m^2$$

and

$$V_M = Nv = \frac{4}{3\pi R_m^3}.$$

Hence, the radius of the micelle R_m can be expressed as

$$R = \frac{3v}{a_0}.$$

As $R_m \leq l$ for spherical micelles, we obtain the following equation:

$$\frac{v}{la_0} \leq \frac{1}{3}.$$

The v/la_0 expression is called the *critical packing parameter*.

The interconnection between these three parameters determines the geometry of the lipid molecule and its behaviour in water. Using the same logic, the value of the *critical packing parameter* for a lipid bilayer can be obtained.

Lipids can be classified by their shapes into three major groups (figure 1)

(a) Inverted cone-shaped lipids ($v/la_0 < 1/2$), which form micellar phases with positive curvature in water. This group comprises mainly lipids with a single fatty acyl chain, such as lysophospholipids. To assemble as spherical micelles, the *critical packing parameter* of lipids has to be below one-third, whereas lipids with $1/3 < v/la_0 < 1/2$ assemble as cylindrical micelles. The relevance of this type of lipids in native cell membranes is most obvious for pore formation [13] or vesicle budding [14].

(b) Cone-shaped lipids ($v/la_0 > 1$), which self-organize in so-called hexagonal micellar phases of negative curvature (in acidic environment). This group of lipids includes mainly unsaturated lipids, which participate in uptake processes where the cell membrane is bended inwards [15].

(c) Cylinder-shaped lipids ($1/2 < v/la_0 \leq 1$), which assemble into a lipid bilayer that forms a lamellar phase when assembled in stacks. These lipids that are characterized by two fatty acyl chains of different length and saturation degree represent probably the most important group of membrane lipids and form the fluid lipid matrix of the cell membrane. They can assemble into flexible lipid bilayers of liposomes ($1/2 < v/la_0 < 1$) as well as planar lipid bilayers ($v/la_0 \approx 1$).

In addition, factors such as the ion composition of aqueous solutions, pH or temperature can alter the lipid geometric parameters, and as a result, the assembly of lipids [11].

A lipid bilayer can undergo solid–liquid phase transition, also referred to as main phase transition or chain-melting transition [16,17]. Depending on the lipid, the main phase transition temperature varies from −17°C for unsaturated lipids, like dioleoylphosphatidylcholine (DOPC), up to 37°C for sphingomyelin (SM) with long saturated acyl chains [18]. Glycolipids with sugar moieties have even higher solid–liquid phase transition temperatures [19]. Lipid phase transitions have already been intensively studied for more than 50 years [20–22] using a large variety of techniques,

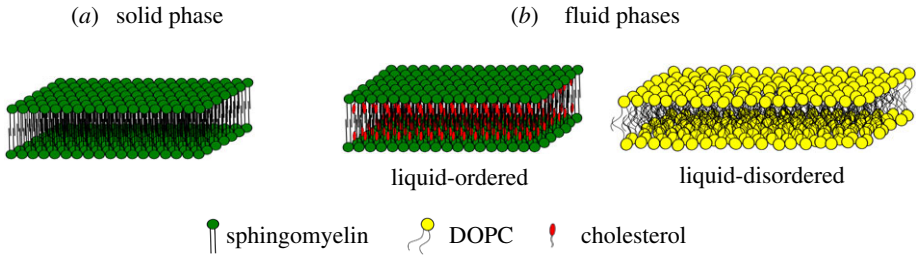

Figure 2. Lipid bilayer phases. Depending on the lipid composition, temperature and order parameters, the lamellar phase can adopt solid or fluid phases. (*a*) Lipid bilayer of solid phase composed of SM. (*b*) Lipid bilayer of fluid phases can exist as liquid-ordered (Lo) (composed of SM and cholesterol) or Ld (composed of DOPC).

such as X-ray diffraction [23], calorimetry [22], atomic force microscopy (AFM) [24] or fluorescence techniques on different membrane model systems [25,26]. The length and saturation degree of the fatty acyl chain determines not only the temperature of the solid–liquid phase transition but also the degree of packing in the liquid phase. Quantitatively, this degree of packing can be described using the so-called acyl chain order parameter. Lipids with a low value of order parameter form a bilayer phase of low order and high mobility, known as liquid-disordered (Ld) phase [7]. Lipids that form Ld phases are mainly unsaturated phospholipids (figure 2). By contrast, bilayers formed of long-chain saturated lipids, like SM, adopt a solid-like phase of high order and low mobility. Addition of sterols, such as cholesterol, renders the solid-like membrane more mobile, turning it into a liquid phase. This phase is called the liquid-ordered (Lo) phase as the order still remains high. Interestingly, the addition of cholesterol enhances acyl chain order in Ld membranes [7,27,28].

A lipid bilayer can contain spatially separated domains of different lipid phases. In binary mixtures composed of lipid species of low-melting temperature (e.g. DOPC) and of high-melting temperature (e.g. SM), the bilayer exhibits spatial phase separation into solid and Ld phase domains. The temperature is the crucial factor for this process; phase separation can occur only at temperatures in between the main phase transition temperatures of the two lipids. Moreover, the coexistence of all three—Ld, Lo and solid phase—domains is possible by the addition of cholesterol [29]. The preference of cholesterol to associate with sphingolipids and partition in SM-containing Lo phases is remarkable [30–32]. Furthermore, phase separation can be reversed by increasing the lipid entropy (e.g. by increasing the temperature).

3. Higher-order organization of lipids within the plasma membrane

To describe the structure of biological membranes, Singer & Nicolson introduced the fluid mosaic model in 1972 ([33]; see also [34]). According to this model, the cell membrane is considered as a two-dimensional liquid arranged as a lipid bilayer with homogeneously distributed proteins. Lipids in a lipid bilayer are in continuous motion. In addition to translational diffusion (microseconds) and rotational movement (picoseconds–nanoseconds), they can flip from one leaflet to the other (milliseconds–seconds) or exhibit undulatory movements (milliseconds–seconds), which are induced by transmembrane proteins or cytoskeleton [35–37]. Further studies of biological membranes based on the use of detergents revealed the separation of the so-called detergent-resistant membrane (DRM) fractions, enriched in particular with SM and cholesterol, from detergent-soluble membrane fractions enriched with rather unsaturated membrane phospholipids [38]. Based on these and other important findings, the 'lipid raft' theory was introduced by Simons & Ikonen in 1997 [39]. According to this theory, the membrane is no longer a homogeneous fluid where all components are randomly distributed, but contains highly ordered lipid (micro-) domains stabilized by SM and cholesterol (figure 3*a*). Lipid rafts are also enriched with (transmembrane) proteins and glycolipids, and are thought to play a key role in cell signalling, uptake and release [40–42]. Further studies of membrane asymmetry revealed even more complicated structures of raft and non-raft membranes [43]. SM-enriched rafts form mainly in the extracellular membrane leaflet while the cytosolic leaflet contains in total less than 10% of all membrane SM molecules [44–46]. Consequently, the cytosolic leaflet is less viscous and does not sustain the formation of lipid rafts [47]. In general, the cytosolic leaflet is enriched with lipids, such as phosphatidylinositol (PI), phosphatidylethanolamine (PE) and PS. Remarkably, lipid raft-embedded glycosylphosphatidylinositol (GPI)-anchored proteins in the extracellular leaflet interdigitate with PS clusters in the cytosolic membrane leaflet [48]. Saturated phosphatidylcholine (PC) lipids are found in the extracellular membrane leaflet.

The lipid raft theory is in line with data obtained from viral membranes. It was found that the HIV envelope is enriched in host cell sphingolipids and cholesterol, the typical lipid raft components [49]. This suggested that the assembly of HIV Gag proteins at the cytosolic leaflet of the cell membrane induces the accumulation of lipid rafts on the extracellular leaflet, so that the HIV envelope consists mainly of raft-like membrane after virus release [50]. This strengthens the significance of lipid rafts as important signalling platforms for diverse cellular processes [6,51].

The size and lifetime of lipid rafts are far below the spatial and temporal resolution of most conventional microscopy techniques, which makes a direct visualization of these domains in cell membranes nearly impossible [52]. Though impressive progress in fluorescence microscopy has been made with the development of advanced techniques that offer high temporal and spatial resolution [52–56], membrane nano-domains can hardly be visualized.

Plasma membrane organization can be also explored in less dynamic systems with compositions close to native ones. These types of membrane systems, e.g. giant plasma membrane vesicles [57] or plasma membrane sheets [58], can be extracted from living cells by cell membrane disruption.

For elucidating the role of distinct membrane components, synthetic membrane systems (figure 3*b*), such as unilamellar vesicles [59,60], supported [61] or pore-suspending [62] lipid bilayers are employed. The main advantage of

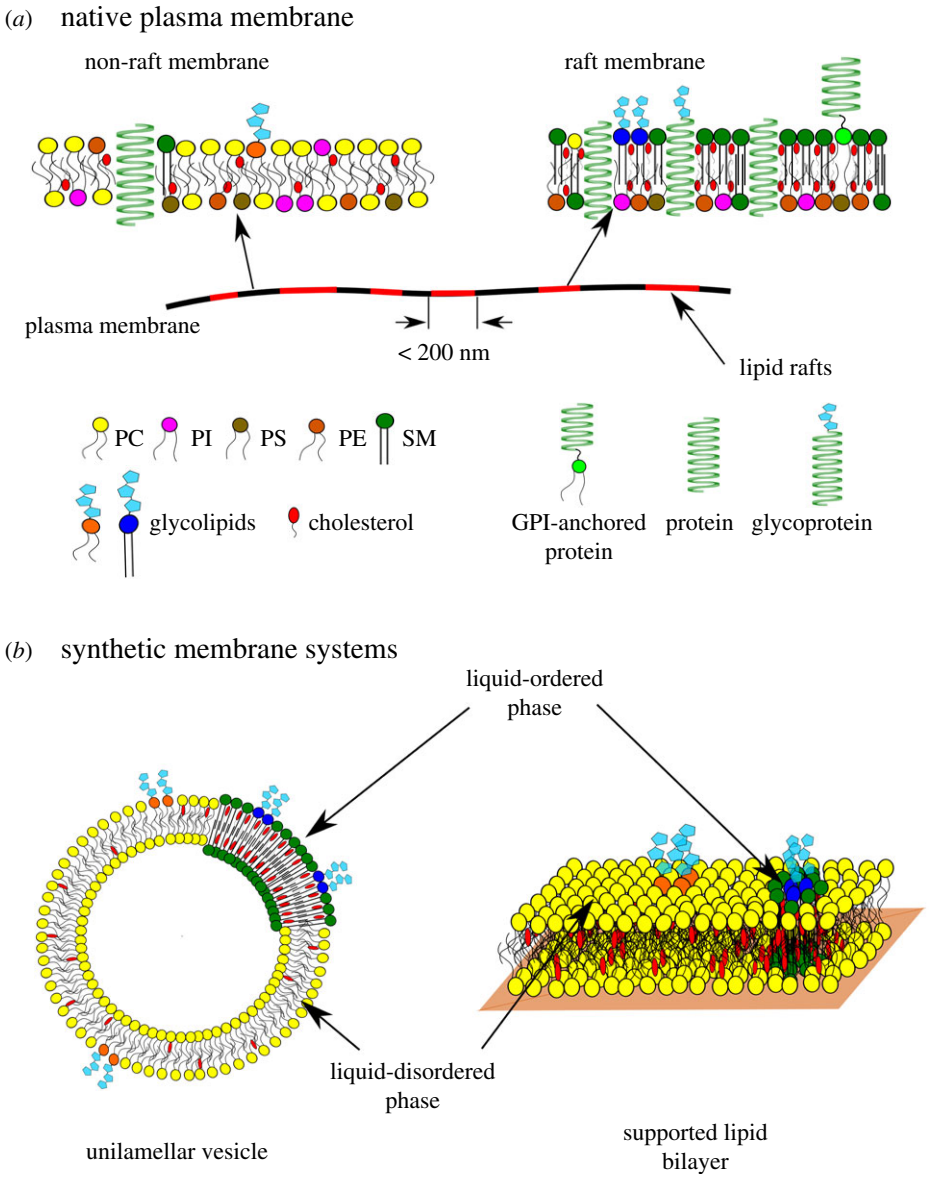

Figure 3. From lipid rafts in native plasma membranes to Lo phases in synthetic membrane systems. (*a*) Composition of raft and non-raft types of native plasma membranes. The membrane is asymmetrical—the extracellular membrane leaflet is enriched with SM and saturated phosphatidylcholine (PC) lipids, while phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidyl ethanolamine (PE) lipids associate mainly to the cytosolic leaflet. (*b*) Synthetic membrane systems composed of liquid membrane domains to mimic native membrane phases. The Lo domain mimics a lipid raft membrane and the Ld domain mimics a non-raft membrane. In synthetic membrane systems, lipids self-assemble into a lipid bilayer mainly in a symmetric manner. GPI, glycosylphosphatidylinositol; SM, sphingomyelin.

synthetic membrane systems is the possibility to adjust the lipid and/or protein content in order to prepare membranes of different properties. These systems are widely used to study the organization of lipid bilayer phases (e.g. Lo and Ld) [63], membrane pore formation [64] and uptake processes [65]. Reconstitution of Lo domains using lipids found in DRMs (e.g. SM and cholesterol, in distinct ratios) enables mimicking some properties of lipid rafts at the microscopic scale. Various cellular processes (e.g. transport across the plasma membrane or induction of signalling events) require local reorganization of the plasma membrane. For instance, extracellular (e.g. pore-forming proteins [66–68] and cell-penetrating peptides [69,70]) and cytosolic (e.g. actin [71,72], clathrin [73], BAR domains [74,75]) components can alter the lipid distribution within the lipid bilayer.

In this review, we focus on the membrane reorganization driven by specific interactions of carbohydrate-binding proteins (so-called lectins) with glycosphingolipids, their host cell receptors.

4. Glycosphingolipids self-organize in small clusters

Glycosphingolipids (GSLs) play crucial roles in intercellular communication and uptake processes, among others [76]. The GSL content in cell membranes varies from 5% to more than 20% [77]. GSLs are mainly found in the extracellular membrane leaflet—the hydrophobic ceramide backbone being embedded in the lipophilic part of the membrane and the carbohydrate moieties being exposed to the extracellular environment [78]. The majority of GSLs are reported to be enriched in lipid rafts [5,39,79–81], where they associate with SM and cholesterol. In addition, AFM studies as well as molecular dynamics simulations have revealed the capability of GSLs to form separate clusters in lipid raft-like membranes [82–85]. Variations in the length and saturation degree of the fatty acyl chain of the ceramide backbone affect the orientation and partitioning of GSLs in the lipid monolayer [86,87], and hence the exposure of the carbohydrate group rendering it

partially inaccessible for ligands [88,89]. GSL assemblies can locally modify membrane order and curvature, which is of high significance for various uptake processes. The long ceramide backbones of GSLs are also important elements for transbilayer coupling and communication since they interdigitate the two membrane leaflets [90].

5. Lectin-induced glycosphingolipid clustering reorganizes the plasma membrane

Besides their natural functions, GSLs are hijacked by various pathogens (e.g. bacteria and viruses) [91–93] and pathogenic products (e.g. toxins, lectins) [94,95]. Lectins [96] can either be soluble, prearranged as a viral capsid or attached to the outer bacterial membrane. They bind to the carbohydrate moieties of GSLs [97], either by targeting preformed GSL domains or by inducing domains by local recruitment of receptor molecules underneath their oligomeric structures [94]. Thereby, asymmetric stress between the extracellular and cytosolic membrane leaflets can lead to inward bending of the cell membrane and growth of tubular plasma membrane invaginations [94].

(a) Toxins/lectins act as clustering devices

Based on cellular as well as synthetic membrane systems, it has been demonstrated that oligomeric toxins/lectins bind to and cluster host cell GSLs, which leads to the formation of tubular plasma membrane invaginations as endocytic transport intermediates. For instance, the homopentameric B-subunit of Shiga toxin (StxB) from *Shigella dysenteriae* recognizes specifically the carbohydrate moieties of the GSL globotriaosylceramide (Gb3) on the extracellular membrane leaflet. Up to 15 Gb3 receptor molecules can interact with one toxin pentamer [98]. This multivalent binding leads to Gb3 clustering and membrane reorganization [99]. Thus, the entropy of the system decreases together with lipid mobility [100], while the membrane order increases locally [85,101]. StxB-induced Gb3 clustering modifies the area per lipid in the outer membrane leaflet. This creates asymmetric stress in the bilayer, and consequently a membrane tubule nucleates [102]. Such a mechanism is probably common for the cellular uptake of many oligomeric lectins [94,103,104]. Nevertheless, the mechanism of lectin clustering on the extracellular membrane surface remains debated. Different reports claim that this process is either driven by capillary effect [105], by entropy modification of the lipid bilayer hydrophobic bulk [102,106] or by local lectin-induced curvature [107]. However, Pezeshkian *et al*. [108] suggested that neither of these factors is critical and that multi-molecular lectin clustering is more likely related to membrane fluctuation-induced forces. Multiple carbohydrate-binding sites of lectins ensure their efficient binding to GSLs and clustering on the membrane surface to induce cellular signalling and uptake processes. In order to explore the impact of lectin valency and binding site geometry on the induction of cellular processes, distinct carbohydrate-binding sites of lectins have been modified by site-directed mutagenesis [94,99,103,109], and tailor-made neolectins have been designed [110]. In comparison to the natural *Ralstonia solanacearum* lectin (RSL), which is trimeric and hexavalent, a divalent, monomeric neolectin, which is highly similar in structure, was sufficient to induce tubular membrane invaginations on giant unilamellar vesicles by binding to a fucosylated glycolipid receptor. Amazingly, only the divalent neolectin with two carbohydrate-binding sites nearby was able to form membrane invaginations, whereas divalent neolectins with more distant binding sites did not, even though their binding constants were quite similar [110].

(b) Toxins/lectins generate membrane curvature

Membrane curvature changes are the response to external or internal perturbations of the membrane, such as osmotic effects [111], membrane fusion [112], vesicle budding [113] or endocytosis [113–115]. For instance, lectin–GSL interactions induce spontaneous curvature leading to tubular plasma membrane invaginations, which are energetically favourable. The efficiency of curvature induction and tubule formation depends on membrane rigidity and composition. In the case of membranes with low rigidity, small protein clusters can initiate tubule nucleation, while in the case of membranes with higher rigidity larger protein clusters are required to bend the membrane [116,117]. Once initiated, the endocytic membrane tubule grows and sequesters lectin molecules on its negatively bended surface. With increasing lectin concentrations inside the tubule, a highly curved bud forms [114] that finally undergoes scission. In living cells, bud formation and scission can be significantly facilitated by the assembly of clathrin, actin [115] or BAR proteins at the cytosolic leaflet of the membrane [75,118].

6. Lectin- and curvature-driven lipid sorting as a membrane reorganization process

Lipid sorting was reported for multicomponent lipid mixtures [119], where an interplay between membrane entropic forces and lipid–lipid interactions takes place. In such systems, like ternary mixtures close to phase separation [119,120], lipid sorting can be triggered by a change in temperature or lipid composition. When an oligomeric lectin binds to GSLs, it acts like a clustering device (figure 4*a*,*b*) that can activate lipid sorting and induce phase separation in otherwise homogeneously mixed membranes [101,120–123]. Local asymmetric stress imposed by lectin–GSL interactions and subsequent membrane deformation can change locally the lipid organization of the bilayer. Pinot *et al*. [124] highlighted the importance of polyunsaturated, cone-shaped phospholipids in those uptake processes. Such lipids can adapt their conformation to the membrane curvature and may support endocytosis [65,124]. Besides, curvature-driven lipid sorting may trigger the redistribution of membrane lipids based on their molecular geometry. However, numerous studies [119,125–129] demonstrated that such curvature-driven lipid sorting is effective only in the presence of membrane-bound or transmembrane proteins, otherwise entropy homogenizes the lipid distribution even in highly curved membranes. The lipid composition of plasma membrane tubules is still elusive. Pulling a membrane tether from giant unilamellar vesicles using a micropipette [119,120,130] showed that tethers are enriched with unsaturated lipids, strongly arguing for their implication in tubule formation [130]. However, as GSL clusters are associated with SM, lectin-induced tubules may also contain saturated sphingolipids [101,130]. Hence, successful tubule formation should involve an interplay between GSL clustering and attraction of saturated lipids (figure 4*c*).

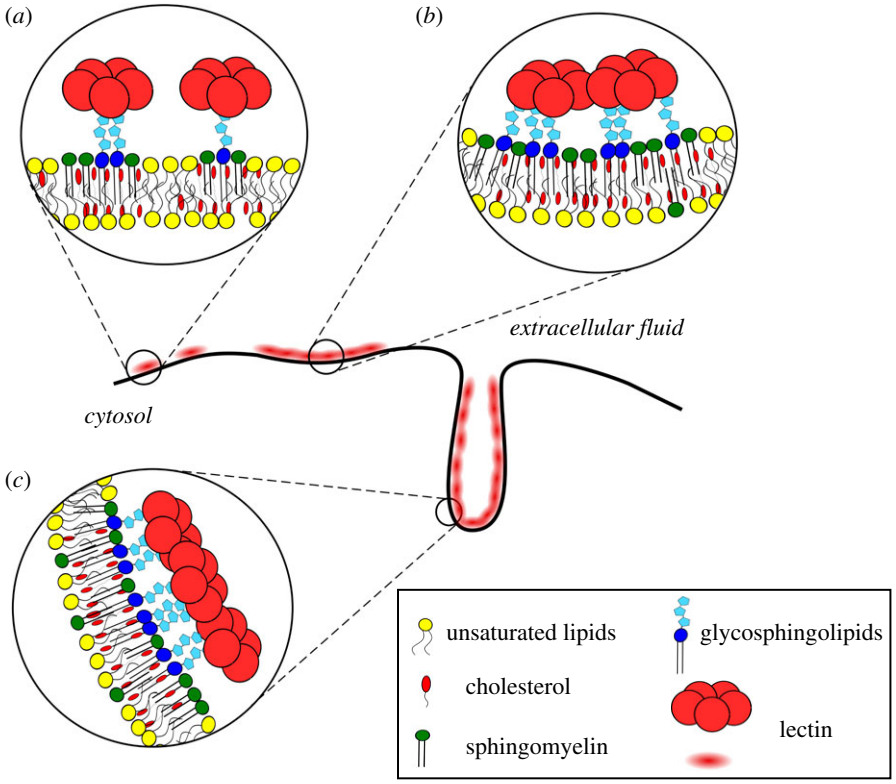

Figure 4. Lectin- and curvature-driven lipid sorting. (*a*) Lectins recognize GSL receptors and bind to the membrane surface. (*b*) Lectins cross-link the GSL-enriched clusters. These clusters induce an asymmetric stress in the membrane, which leads to tubule nucleation and plasma membrane invagination. (*c*) Lectin-induced plasma membrane tubules are enriched with sphingolipids due to their association with GSL clusters.

7. Conclusion

The plasma membrane is an exciting example of self-organization in nature. Although many discoveries have been made concerning lipid self-assembly into a bilayer and the organization of distinct lipids into functional units within the plasma membrane, the exact membrane organization and its dynamics are still ill-defined. Synthetic membrane models are helpful tools in mimicking, and hence understanding plasma membrane organization and dynamics, but one should not forget that the plasma membrane is a highly complex, asymmetric bilayer system consisting of a huge variety of lipid species that fulfil specific functions. Further improvements in microscopy techniques, in particular in super resolution live cell microscopy, as well as in the development of novel lipid labelling and detection methods are required to better decipher the principles of dynamic membrane organization.

Data accessibility. This article has no additional data.

Competing interests. We declare we have no competing interests.

Funding. The position of T.S. was funded by the German Research Foundation grant [IRTG 1642 - SoftMatter Science: Concepts for the design of functional materials].

Acknowledgements. This work was supported by the Excellence Initiative of the German Research Foundation [EXC 294]. T.S. acknowledges the support of the Franco-German University (PhD-track program 'Polymer Sciences' and the program 'Cotutelle de thèse') and the Collège doctoral européen (Programme doctoral international). We are grateful to Toshihide Kobayashi for proof-reading of the manuscript.

References

1. Conner SD, Schmid SL. 2003 Regulated portals of entry into the cell. *Nature* **422**, 37–44. (doi:10.1038/nature01451)
2. Hynes RO. 1992 Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11–25. (doi:10.1016/0092-8674(92)90115-S)
3. Schlessinger J. 2000 Cell signaling by receptor tyrosine kinases. *Cell* **103**, 211–225. (doi:10.1016/S0092-8674(00)00114-8)
4. Pollard TD, Cooper JA. 2009 Actin, a central player in cell shape and movement. *Science* **326**, 1208–1212. (doi:10.1126/science.1175862)
5. Simons K, Toomre D. 2001 Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **2**, 1038. (doi:10.1038/35103091)
6. Sezgin E, Levental I, Mayor S, Eggeling C. 2017 The mystery of membrane organization: composition, regulation and roles of lipid rafts. *Nat. Rev. Mol. Cell Biol.* **18**, 361–374. (doi:10.1038/nrm.2017.16)
7. Meer GV, Voelker DR, Feigenson GW. 2008 Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* **9**, 112–124. (doi:10.1038/nrm2330)
8. Cooke IR, Deserno M. 2006 Coupling between lipid shape and membrane curvature. *Biophys. J.* **91**, 487–495. (doi:10.1529/biophysj.105.078683)
9. Tanford C. 1974 Theory of micelle formation in aqueous solutions. *J. Phys. Chem.* **78**, 2469–2479. (doi:10.1021/j100617a012)
10. Israelachvili JN, Mitchell DJ, Ninham BW. 1977 Theory of self-assembly of lipid bilayers and vesicles. *Biochim. Biophys. Acta* **470**, 185–201. (doi:10.1016/0005-2736(77)90099-2)
11. Israelachvili JN. 2011 *Intermolecular and surface forces*. New York, NY: Academic Press.

12. Gennis RB. 2013 *Biomembranes: molecular structure and function*. Berlin, Germany: Springer Science & Business Media.
13. Reusch R. 2014 Biogenesis of ion channels. *J. Biochem. Biophys* **1**, 1–24.
14. Sprong H, van der Sluijs P, van Meer G. 2001 How proteins move lipids and lipids move proteins. *Nat. Rev. Mol. Cell Biol.* **2**, 504–513. (doi:10.1038/35080071)
15. Jouhet J. 2013 Importance of the hexagonal lipid phase in biological membrane organization. *Front. Plant Sci.* **4**, 494. (doi:10.3389/fpls.2013.00494)
16. Reinert JC, Steim JM. 1970 Calorimetric detection of a membrane-lipid phase transition in living cells. *Science* **168**, 1580–1582. (doi:10.1126/science.168.3939.1580)
17. Simons K, Vaz WL. 2004 Model systems, lipid rafts, and cell membranes. *Annu. Rev. Biophys. Biomol. Struct.* **33**, 269–295. (doi:10.1146/annurev.biophys.32.110601.141803)
18. Caffrey M, Hogan J. 1992 LIPIDAT: A database of lipid phase transition temperatures and enthalpy changes. *DMPC data subset analysis*. *Chem. Phys. Lipids* **61**, 1–109. (doi:10.1016/0009-3084(92)90002-7)
19. Kociurzynski R, Pannuzzo M, Böckmann RA. 2015 Phase transition of glycolipid membranes studied by coarse-grained simulations. *Langmuir* **31**, 9379–9387. (doi:10.1021/acs.langmuir.5b01617)
20. Lee AG. 1977 Lipid phase transitions and phase diagrams I. Lipid phase transitions. *Biochim. Biophys. Acta* **472**, 237–281. (doi:10.1016/0304-4157(77)90018-1)
21. Lee AG. 1977 Lipid phase transitions and phase diagrams II. Mixtures involving lipids. *Biochim. Biophys. Acta* **472**, 285–344. (doi:10.1016/0304-4157(77)90001-6)
22. Mabrey S, Sturtevant JM. 1976 Investigation of phase transitions of lipids and lipid mixtures by sensitivity differential scanning calorimetry. *Proc. Natl Acad. Sci. USA* **73**, 3862–3866. (doi:10.1073/pnas.73.11.3862)
23. Kjaer K, Als-Nielsen J, Helm CA, Laxhuber LA, Möhwald H. 1987 Ordering in lipid monolayers studied by synchrotron x-ray diffraction and fluorescence microscopy. *Phys. Rev. Lett.* **58**, 2224. (doi:10.1103/PhysRevLett.58.2224)
24. Leonenko ZV, Finot E, Ma H, Dahms TE, Cramb DT. 2004 Investigation of temperature-induced phase transitions in DOPC and DPPC phospholipid bilayers using temperature-controlled scanning force microscopy. *Biophys. J.* **86**, 3783–3793. (doi:10.1529/biophysj.103.036681)
25. Carravilla P, Nieva JL, Goñi FM, Requejo-Isidro J, Huarte N. 2015 Two-photon Laurdan studies of the ternary lipid mixture DOPC: SM: cholesterol reveal a single liquid phase at sphingomyelin: cholesterol ratios lower than 1. *Langmuir* **31**, 2808–2817. (doi:10.1021/la504251u)
26. De Almeida RF, Fedorov A, Prieto M. 2003 Sphingomyelin/phosphatidylcholine/cholesterol phase diagram: boundaries and composition of lipid rafts. *Biophys. J.* **85**, 2406–2416. (doi:10.1016/S0006-3495(03)74664-5)
27. Veatch SL, Keller SL. 2005 Miscibility phase diagrams of giant vesicles containing sphingomyelin. *Phys. Rev. Lett.* **94**, 148101. (doi:10.1103/PhysRevLett.94.148101)
28. Maxfield FR, Tabas I. 2005 Role of cholesterol and lipid organization in disease. *Nature* **438**, 612–621. (doi:10.1038/nature04399)
29. Aufderhorst-Roberts A, Chandra U, Connell SD. 2017 Three-phase coexistence in lipid membranes. *Biophys. J.* **112**, 313–324. (doi:10.1016/j.bpj.2016.12.025)
30. Busto JV, García-Arribas AB, Sot J, Torrecillas A, Gómez-Fernández JC, Goni FM, Alonso A. 2014 Lamellar gel (L_{β}) phases of ternary lipid composition containing ceramide and cholesterol. *Biophys. J.* **106**, 621–630. (doi:10.1016/j.bpj.2013.12.021)
31. Sodt AJ, Sandar ML, Gawrisch K, Pastor RW, Lyman E. 2014 The molecular structure of the liquid-ordered phase of lipid bilayers. *J. Am. Chem. Soc.* **136**, 725–732. (doi:10.1021/ja4105667)
32. Brown DA, London E. 1998 Structure and origin of ordered lipid domains in biological membranes. *J. Membr. Biol.* **164**, 103–114. (doi:10.1007/s002329900397)
33. Singer SJ, Nicolson GL. 1972 The fluid mosaic model of the structure of cell membranes. *Science* **175**, 720–731. (doi:10.1126/science.175.4023.720)
34. Nicolson GL. 2014 The fluid–mosaic model of membrane structure: still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years. *Biochim. Biophys. Acta* **1838**, 1451–1466. (doi:10.1016/j.bbamem.2013.10.019)
35. Vermeer LS, De Groot BL, Réat V, Milon A, Czaplicki J. 2007 Acyl chain order parameter profiles in phospholipid bilayers: computation from molecular dynamics simulations and comparison with 2H NMR experiments. *Eur. Biophys. J.* **36**, 919–931. (doi:10.1007/s00249-007-0192-9)
36. Gov N. 2004 Membrane undulations driven by force fluctuations of active proteins. *Phys. Rev. Lett.* **93**, 268104. (doi:10.1103/PhysRevLett.93.268104)
37. Sackmann E. 1996 Supported membranes: scientific and practical applications. *Science* **271**, 43–48. (doi:10.1126/science.271.5245.43)
38. Seddon AM, Curnow P, Booth PJ. 2004 Membrane proteins, lipids and detergents: not just a soap opera. *Biochim. Biophys. Acta* **1666**, 105–117. (doi:10.1016/j.bbamem.2004.04.011)
39. Simons K, Ikonen E. 1997 Functional rafts in cell membranes. *Nature* **387**, 569–572. (doi:10.1038/42408)
40. Simons K, Gerl MJ. 2010 Revitalizing membrane rafts: new tools and insights. *Nat. Rev. Mol. Cell Biol.* **11**, 688–699. (doi:10.1038/nrm2977)
41. Levental I, Grzybek M, Simons K. 2011 Raft domains of variable properties and compositions in plasma membrane vesicles. *Proc. Natl Acad. Sci. USA* **108**, 11 411–11 416. (doi:10.1073/pnas.1105996108)
42. Lingwood D, Simons K. 2010 Lipid rafts as a membrane-organizing principle. *Science* **327**, 46–50. (doi:10.1126/science.1174621)
43. van Meer G. 2002 The different hues of lipid rafts. *Science* **296**, 855–857. (doi:10.1126/science.1071491)
44. Rothman JE, Lenard J. 1977 Membrane asymmetry. *Science* **195**, 743–753. (doi:10.1126/science.402030)
45. Devaux PF. 1991 Static and dynamic lipid asymmetry in cell membranes. *Biochemistry* **30**, 1163–1173. (doi:10.1021/bi00219a001)
46. Ingólfsson HI *et al.* 2014 Lipid organization of the plasma membrane. *J. Am. Chem. Soc.* **136**, 14 554–14 559. (doi:10.1021/ja507832e)
47. Devaux PF, Morris R. 2004 Transmembrane asymmetry and lateral domains in biological membranes. *Traffic* **5**, 241–246. (doi:10.1111/j.1600-0854.2004.0170.x)
48. Raghupathy R *et al.* 2015 Transbilayer lipid interactions mediate nanoclustering of lipid-anchored proteins. *Cell* **161**, 581–594. (doi:10.1016/j.cell.2015.03.048)
49. Ono A, Freed EO. 2001 Plasma membrane rafts play a critical role in HIV-1 assembly and release. *Proc. Natl Acad. Sci. USA* **98**, 13 925–13 930. (doi:10.1073/pnas.241320298)
50. Vlach J, Saad JS. 2015 Structural and molecular determinants of HIV-1 Gag binding to the plasma membrane. *Front. Microbiol.* **6**, 232. (doi:10.3389/fmicb.2015.00232)
51. Rajendran L, Simons K. 2005 Lipid rafts and membrane dynamics. *J. Cell Sci.* **118**, 1099–1102. (doi:10.1242/jcs.01681)
52. Sezgin E. 2017 Super-resolution optical microscopy for studying membrane structure and dynamics. *J. Phys.: Condens. Matter* **29**, 273001. (doi:10.1088/1361-648X/aa7185)
53. Owen DM, Neil MA, French PM, Magee AI. 2007 Optical techniques for imaging membrane lipid microdomains in living cells. *Semin. Cell Dev. Biol* **18**, 591–598. (doi:10.1016/j.semcdb.2007.07.011)
54. Kwiatek JM, Hinde E, Gaus K. 2014 Microscopy approaches to investigate protein dynamics and lipid organization. *Mol. Membr. Biol.* **31**, 141–151. (doi:10.3109/09687688.2014.937469)
55. Klymchenko AS, Kreder R. 2014 Fluorescent probes for lipid rafts: from model membranes to living cells. *Chem. Biol.* **21**, 97–113. (doi:10.1016/j.chembiol.2013.11.009)
56. Eggeling C *et al.* 2009 Direct observation of the nanoscale dynamics of membrane lipids in a living cell. *Nature* **457**, 1159–1162. (doi:10.1038/nature07596)
57. Sezgin E, Kaiser HJ, Baumgart T, Schwille P, Simons K, Levental I. 2012 Elucidating membrane structure and protein behavior using giant plasma membrane vesicles. *Nat. Protoc.* **7**, 1042–1051. (doi:10.1038/nprot.2012.059)
58. Saka SK, Honigmann A, Eggeling C, Hell SW, Lang T, Rizzoli SO. 2014 Multi-protein assemblies underlie the mesoscale organization of the plasma membrane. *Nat. Commun.* **5**, 4509. (doi:10.1038/ncomms5509)
59. Hope MJ, Bally MB, Webb G, Cullis PR. 1985 Production of large unilamellar vesicles by a rapid

extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. *Biochim. Biophys. Acta* **812**, 55–65. (doi:10.1016/0005-2736(85)90521-8)

60. Madl J, Villringer S, Römer W. 2016 Delving into lipid-driven endocytic mechanisms using biomimetic membranes. In *Chemical and synthetic approaches in membrane biology* (ed. Arun K. Shukla), pp. 17–36. New York, NY: Humana Press.
61. Mingeot-Leclercq MP, Deleu M, Brasseur R, Dufrêne YF. 2008 Atomic force microscopy of supported lipid bilayers. *Nat. Protoc.* **3**, 1654–1659. (doi:10.1038/nprot.2008.149)
62. Römer W, Steinem C. 2004 Impedance analysis and single-channel recordings on nano-black lipid membranes based on porous alumina. *Biophys. J.* **86**, 955–965. (doi:10.1016/S0006-3495(04)74171-5)
63. Kaiser HJ, Lingwood D, Levental I, Sampaio JL, Kalvodova L, Rajendran L, Simons K. 2009 Order of lipid phases in model and plasma membranes. *Proc. Natl Acad. Sci. USA* **106**, 16 645–16 650. (doi:10.1073/pnas.0908987106)
64. Lee MT, Hung WC, Chen FY, Huang HW. 2008 Mechanism and kinetics of pore formation in membranes by water-soluble amphipathic peptides. *Proc. Natl Acad. Sci. USA* **105**, 5087–5092. (doi:10.1073/pnas.0710625105)
65. Schubert T, Römer W. 2015 How synthetic membrane systems contribute to the understanding of lipid-driven endocytosis. *Biochim. Biophys. Acta* **1853**, 2992–3005. (doi:10.1016/j.bbamcr.2015.07.014)
66. Phillips R, Ursell T, Wiggins P, Sens P. 2009 Emerging roles for lipids in shaping membrane-protein function. *Nature* **459**, 379–385. (doi:10.1038/nature08147)
67. Yilmaz N, Kobayashi T. 2015 Visualization of lipid membrane reorganization induced by a pore-forming toxin using high-speed atomic force microscopy. *ACS Nano* **9**, 7960–7967. (doi:10.1021/acsnano.5b01041)
68. Gilbert RJ, Dalla Serra M, Froelich CJ, Wallace MI, Anderluh G. 2014 Membrane pore formation at protein–lipid interfaces. *Trends Biochem. Sci.* **39**, 510–516. (doi:10.1016/j.tibs.2014.09.002)
69. Sodt AJ, Pastor RW. 2014 Molecular modeling of lipid membrane curvature induction by a peptide: more than simply shape. *Biophys. J.* **106**, 1958–1969. (doi:10.1016/j.bpj.2014.02.037)
70. Maniti O, Piao HR, Ayala-Sanmartin J. 2014 Basic cell penetrating peptides induce plasma membrane positive curvature, lipid domain separation and protein redistribution. *Int. J. Biochem. Cell Biol.* **50**, 73–81. (doi:10.1016/j.biocel.2014.02.017)
71. Römer W *et al.* 2010 Actin dynamics drive membrane reorganization and scission in clathrin-independent endocytosis. *Cell* **140**, 540–553. (doi:10.1016/j.cell.2010.01.010)
72. Head BP, Patel HH, Insel PA. 2014 Interaction of membrane/lipid rafts with the cytoskeleton: impact on signaling and function: membrane/lipid rafts, mediators of cytoskeletal arrangement and cell signaling. *Biochim. Biophys. Acta* **1838**, 532–545. (doi:10.1016/j.bbamem.2013.07.018)
73. Saleem M, Morlot S, Hohendahl A, Manzi J, Lenz M, Roux A. 2015 A balance between membrane elasticity and polymerization energy sets the shape of spherical clathrin coats. *Nat. Commun.* **6**, 6249. (doi:10.1038/ncomms7249)
74. Itoh T, Takenawa T. 2009 Mechanisms of membrane deformation by lipid-binding domains. *Prog. Lipid Res.* **48**, 298–305. (doi:10.1016/j.plipres.2009.05.002)
75. Johannes L, Parton RG, Bassereau P, Mayor S. 2015 Building endocytic pits without clathrin. *Nat. Rev. Mol. Cell Biol.* **16**, 311–321. (doi:10.1038/nrm3968)
76. Johannes L, Wunder C, Shafaq-Zadah M. 2016 Glycolipids and lectins in endocytic uptake processes. *J. Mol. Biol.* **428**, 4792–4818. (doi:10.1016/j.jmb.2016.10.027)
77. Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME. 2009 *Essentials of glycobiology*, 2nd edn. New York, NY: Cold Spring Harbour.
78. Manna M, Róg T, Vattulainen I. 2014 The challenges of understanding glycolipid functions: an open outlook based on molecular simulations. *Biochim. Biophys. Acta* **1841**, 1130–1145. (doi:10.1016/j.bbalip.2013.12.016)
79. López CA, Sovova Z, van Eerden FJ, de Vries AH, Marrink SJ. 2013 Martini force field parameters for glycolipids. *J. Chem. Theory Comput.* **9**, 1694–1708. (doi:10.1021/ct3009655)
80. Fra AM, Williamson E, Simons K, Parton RG. 1994 Detergent-insoluble glycolipid microdomains in lymphocytes in the absence of caveolae. *J. Biol. Chem.* **269**, 30 745–30 748.
81. Hooper NM. 1999 Detergent-insoluble glycosphingolipid/cholesterol-rich membrane domains, lipid rafts and caveolae. *Mol. Membr. Biol.* **16**, 145–156. (doi:10.1080/096876899294607)
82. Shi J, Yang T, Kataoka S, Zhang Y, Diaz AJ, Cremer PS. 2007 GM1 clustering inhibits cholera toxin binding in supported phospholipid membranes. *J. Am. Chem. Soc.* **129**, 5954–5961. (doi:10.1021/ja069375w)
83. Mondal S, Mukhopadhyay C. 2008 Molecular level investigation of organization in ternary lipid bilayer: a computational approach. *Langmuir* **24**, 10 298–10 305. (doi:10.1021/la8015589)
84. Koldsø H, Shorthouse D, Hélie J, Sansom MS. 2014 Lipid clustering correlates with membrane curvature as revealed by molecular simulations of complex lipid bilayers. *PLoS Comput. Biol.* **10**, e1003911. (doi:10.1371/journal.pcbi.1003911)
85. Solovyeva V, Johannes L, Simonsen AC. 2015 Shiga toxin induces membrane reorganization and formation of long range lipid order. *Soft Matter* **11**, 186–192. (doi:10.1039/C4SM01673D)
86. Chinnapen DJ *et al.* 2012 Lipid sorting by ceramide structure from plasma membrane to ER for the cholera toxin receptor ganglioside GM1. *Dev. Cell* **23**, 573–586. (doi:10.1016/j.devcel.2012.08.002)
87. Schütte OM, Ries A, Orth A, Patalag LJ, Römer W, Steinem C, Werz DB. 2014 Influence of Gb 3 glycosphingolipids differing in their fatty acid chain on the phase behaviour of solid supported membranes: chemical syntheses and impact of Shiga toxin binding. *Chem. Sci.* **5**, 3104–3114. (doi:10.1039/c4sc01290a)
88. Mahfoud R, Manis A, Lingwood CA. 2009 Fatty acid-dependent globotriaosyl ceramide receptor function in detergent resistant model membranes. *J. Lipid Res.* **50**, 1744–1755. (doi:10.1194/jlr.M800385-JLR200)
89. Schütte OM, Patalag LJ, Weber LM, Ries A, Römer W, Werz DB, Steinem C. 2015 2-Hydroxy fatty acid enantiomers of Gb_3 impact Shiga toxin binding and membrane organization. *Biophys. J.* **108**, 2775–2778. (doi:10.1016/j.bpj.2015.05.009)
90. Manna M, Javanainen M, Monne HM, Gabius HJ, Rog T, Vattulainen I. 2017 Long-chain GM1 gangliosides alter transmembrane domain registration through interdigitation. *Biochim. Biophys. Acta* **1859**, 870–878. (doi:10.1016/j.bbamem.2017.01.033)
91. Rydell GE, Svensson L, Larson G, Johannes L, Römer W. 2013 Human GII.4 norovirus VLP induces membrane invaginations on giant unilamellar vesicles containing secretor gene dependent α1, 2-fucosylated glycosphingolipids. *Biochim. Biophys. Acta* **1828**, 1840–1845. (doi:10.1016/j.bbamem.2013.03.016)
92. Ewers H *et al.* 2010 GM1 structure determines SV40-induced membrane invagination and infection. *Nat. Cell Biol.* **12**, 11–18. (doi:10.1038/ncb1999)
93. Eierhoff T *et al.* 2014 A lipid zipper triggers bacterial invasion. *Proc. Natl Acad. Sci. USA* **111**, 12 895–12 900. (doi:10.1073/pnas.1402637111)
94. Römer W *et al.* 2007 Shiga toxin induces tubular membrane invaginations for its uptake into cells. *Nature* **450**, 670–675. (doi:10.1038/nature05996)
95. Zheng S, Eierhoff T, Aigal S, Brandel A, Thuenauer R, de Bentzmann S, Imberty A, Römer W. 2017 The *Pseudomonas aeruginosa* lectin LecA triggers host cell signalling by glycosphingolipid-dependent phosphorylation of the adaptor protein CrkII. *Biochim. Biophys. Acta* **1864**, 1236–1245. (doi:10.1016/j.bbamcr.2017.04.005)
96. Sharon N. 2007 Lectins: carbohydrate-specific reagents and biological recognition molecules. *J. Biol. Chem.* **282**, 2753–2764. (doi:10.1074/JBC.X600004200)
97. Aigal S, Claudinon J, Römer W. 2015 Plasma membrane reorganization: a glycolipid gateway for microbes. *Biochim. Biophys. Acta* **1853**, 858–871. (doi:10.1016/j.bbamcr.2014.11.014)
98. Johannes L, Römer W. 2010 Shiga toxins—from cell biology to biomedical applications. *Nat. Rev. Microbiol.* **8**, 105–116. (doi:10.1038/nrmicro2279)
99. Windschiegl B, Orth A, Römer W, Berland L, Stechmann B, Bassereau P, Johannes L, Steinem C. 2009 Lipid reorganization induced by Shiga toxin clustering on planar membranes. *PLoS ONE* **4**, e6238. (doi:10.1371/journal.pone.0006238)
100. Johannes L, Mayor S. 2010 Induced domain formation in endocytic invagination, lipid sorting,

and scission. *Cell* **142**, 507–510. (doi:10.1016/j.cell.2010.08.007)

101. Safouane M, Berland L, Callan-Jones A, Sorre B, Römer W, Johannes L, Toombes GE, Bassereau P. 2010 Lipid cosorting mediated by shiga toxin induced tubulation. *Traffic* **11**, 1519–1529. (doi:10.1111/j.1600-0854.2010.01116.x)
102. Pezeshkian W, Hansen AG, Johannes L, Khandelia H, Shillcock JC, Kumar PS, Ipsen JH. 2016 Membrane invagination induced by Shiga toxin B-subunit: from molecular structure to tube formation. *Soft Matter* **12**, 5164–5171. (doi:10.1039/C6SM00464D)
103. Arnaud J *et al.* 2013 Reduction of lectin valency drastically changes glycolipid dynamics in membranes but not surface avidity. *ACS Chem. Biol.* **8**, 1918–1924. (doi:10.1021/cb400254b)
104. Lakshminarayan R *et al.* 2014 Galectin-3 drives glycosphingolipid-dependent biogenesis of clathrin-independent carriers. *Nat. Cell Biol.* **16**, 592–603. (doi:10.1038/ncb2970)
105. Gil T, Ipsen JH. 1997 Capillary condensation between disks in two dimensions. *Phys. Rev. E* **55**, 1713. (doi:10.1103/PhysRevE.55.1713)
106. Pezeshkian W, Chaban VV, Johannes L, Shillcock J, Ipsen JH, Khandelia H. 2015 The effects of globotriaosylceramide tail saturation level on bilayer phases. *Soft Matter* **11**, 1352–1361. (doi:10.1039/C4SM02456G)
107. Reynwar BJ, Illya G, Harmandaris VA, Müller MM, Kremer K, Deserno M. 2007 Aggregation and vesiculation of membrane proteins by curvature-mediated interactions. *Nature* **447**, 461–464. (doi:10.1038/nature05840)
108. Pezeshkian W, Gao H, Arumugam S, Becken U, Bassereau P, Florent JC, Ipsen JH, Johannes L, Shillcock JC. 2016 Mechanism of shiga toxin clustering on membranes. *ACS Nano* **11**, 314–324. (doi:10.1021/acsnano.6b05706)
109. Bast DJ, Banerjee L, Clark C, Read RJ, Brunton JL. 1999 The identification of three biologically relevant globotriaosyl ceramide receptor binding sites on the Verotoxin 1 B subunit. *Mol. Microbiol.* **32**, 953–960. (doi:10.1046/j.1365-2958.1999.01405.x)
110. Arnaud J, Tröndle K, Claudinon J, Audfray A, Varrot A, Römer W, Imberty A. 2014 Membrane deformation by neolectins with engineered glycolipid binding sites. *Angew. Chem. Int. Ed.* **53**, 9267–9270. (doi:10.1002/anie.201404568)
111. Shibly SU, Ghatak C, Karal MA, Moniruzzaman M, Yamazaki M. 2016 Experimental estimation of membrane tension induced by osmotic pressure. *Biophys. J.* **111**, 2190–2201. (doi:10.1016/j.bpj.2016.09.043)
112. Kozlov MM, Chernomordik LV. 2015 Membrane tension and membrane fusion. *Curr. Opin Struct. Biol.* **33**, 61–67. (doi:10.1016/j.sbi.2015.07.010)
113. Sens P, Turner MS. 2006 Budded membrane microdomains as tension regulators. *Phys. Rev. E* **73**, 031918. (doi:10.1103/PhysRevE.73.031918)
114. Liu J, Kaksonen M, Drubin DG, Oster G. 2006 Endocytic vesicle scission by lipid phase boundary forces. *Proc. Natl Acad. Sci. USA* **103**, 10 277–10 282. (doi:10.1073/pnas.0601045103)
115. Hassinger JE, Oster G, Drubin DG, Rangamani P. 2017 Design principles for robust vesiculation in clathrin-mediated endocytosis. *Proc. Natl Acad. Sci. USA* **114**, E1118–E1127. (doi:10.1073/pnas.1617705114)
116. Johannes L, Wunder C, Bassereau P. 2014 Bending 'on the rocks'—a cocktail of biophysical modules to build endocytic pathways. *Cold Spring Harb. Perspect. Biol.* **6**, a016741. (doi:10.1101/cshperspect.a016741)
117. Sens P, Johannes L, Bassereau P. 2008 Biophysical approaches to protein-induced membrane deformations in trafficking. *Curr. Opin Cell Biol.* **20**, 476–482. (doi:10.1016/j.ceb.2008.04.004)
118. Simunovic M *et al.* 2017 Friction mediates scission of tubular membranes scaffolded by BAR proteins. *Cell* **170**, 172–184. (doi:10.1016/j.cell.2017.05.047)
119. Roux A, Cuvelier D, Nassoy P, Prost J, Bassereau P, Goud B. 2005 Role of curvature and phase transition in lipid sorting and fission of membrane tubules. *EMBO J.* **24**, 1537–1545. (doi:10.1038/sj.emboj.7600631)
120. Sorre B, Callan-Jones A, Manneville JB, Nassoy P, Joanny JF, Prost J, Goud B, Bassereau P. 2009 Curvature-driven lipid sorting needs proximity to a demixing point and is aided by proteins. *Proc. Natl Acad. Sci. USA* **106**, 5622–5626. (doi:10.1073/pnas.0811243106)
121. Hammond AT, Heberle FA, Baumgart T, Holowka D, Baird B, Feigenson GW. 2005 Crosslinking a lipid raft component triggers liquid ordered–liquid disordered phase separation in model plasma membranes. *Proc. Natl Acad. Sci. USA* **102**, 6320–6325. (doi:10.1073/pnas.0405654102)
122. Lingwood D, Ries J, Schwille P, Simons K. 2008 Plasma membranes are poised for activation of raft phase coalescence at physiological temperature. *Proc. Natl Acad. Sci. USA* **105**, 10 005–10 010. (doi:10.1073/pnas.0804374105)
123. Bacia K, Schwille P, Kurzchalia T. 2005 Sterol structure determines the separation of phases and the curvature of the liquid-ordered phase in model membranes. *Proc. Natl Acad. Sci. USA* **102**, 3272–3277. (doi:10.1073/pnas.0408215102)
124. Pinot M *et al.* 2014 Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins. *Science* **345**, 693–697. (doi:10.1126/science.1255288)
125. Kamal MM, Mills D, Grzybek M, Howard J. 2009 Measurement of the membrane curvature preference of phospholipids reveals only weak coupling between lipid shape and leaflet curvature. *Proc. Natl Acad. Sci. USA* **106**, 22 245–22 250. (doi:10.1073/pnas.0907354106)
126. Tian A, Baumgart T. 2009 Sorting of lipids and proteins in membrane curvature gradients. *Biophys. J.* **96**, 2676–2688. (doi:10.1016/j.bpj.2008.11.067)
127. Auth T, Gompper G. 2009 Budding and vesiculation induced by conical membrane inclusions. *Phys. Rev. E* **80**, 031901. (doi:10.1103/PhysRevE.80.031901)
128. Tsafrir I, Sagi D, Arzi T, Guedeau-Boudeville MA, Frette V, Kandel D, Stavans J. 2001 Pearling instabilities of membrane tubes with anchored polymers. *Phys. Rev. Lett.* **86**, 1138. (doi:10.1103/PhysRevLett.86.1138)
129. Campelo F, McMahon HT, Kozlov MM. 2008 The hydrophobic insertion mechanism of membrane curvature generation by proteins. *Biophys. J.* **95**, 2325–2339. (doi:10.1529/biophysj.108.133173)
130. Callan-Jones A, Sorre B, Bassereau P. 2011 Curvature-driven lipid sorting in biomembranes. *Cold Spring Harb. Perspect. Biol.* **3**, a004648. (doi:10.1101/cshperspect.a004648)