Nature Reviews Molecular Cell Biology | AOP, published online 28 October 2015; doi:10.1038/nrm4080

PERSPECTIVES

TECHNOLOGY AND TECHNIQUES — INNOVATION

The systematic analysis of protein–lipid interactions comes of age

Antoine-Emmanuel Saliba, Ivana Vonkova and Anne-Claude Gavin

Abstract | Lipids tailor membrane identities and function as molecular hubs in all cellular processes. However, the ways in which lipids modulate protein function and structure are poorly understood and still require systematic investigation. In this Innovation article, we summarize pioneering technologies, including lipid-overlay assays, lipid pull-down assays, affinity-purification lipidomics and the liposome microarray-based assay (LiMA), that will enable protein–lipid interactions to be deciphered on a systems level. We discuss how these technologies can be applied to the charting of system-wide networks and to the development of new pharmaceutical strategies.

Lipid localization is tightly regulated and shapes the unique function and properties of every cellular membrane[1,2]. The accumulation of discrete phosphoinositide, glycerophospholipid, sphingolipid or sterol species defines the identity of the organelle and locally regulates the organization of cellular membranes into microdomains. Over the past few years, much effort has been dedicated to cataloguing the 'lipidome', which is now estimated to contain more than 40,000 different lipid species (see the LIPID MAPS Structure Database (LMSD))[3]; however, the biological role of only a few of these lipids is known.

Lipids function predominantly through their interactions with proteins. Indeed, protein–lipid interactions are involved in all biological processes and are of paramount interest in pharmaceutical discovery, as 60% of drug targets are located at the cell surface or in other cellular membranes[4,5]. Integral and peripheral membrane proteins account for one-third of the full proteome[6]; however, research into the mechanisms that are used by lipids to regulate protein function and structure — and, ultimately, to shape cell physiology — has been restricted to a small number of proteins, with a strong focus on mechanistic insights. This gap in our knowledge is due to a lack of methods that are amenable to systematically charting protein–lipid interactions, and a technological effort similar to the one made for DNA–protein and RNA–protein interactions is now needed for lipids. We believe that a global atlas of protein–lipid interactions will benefit biology and medicine by shedding light on the role of lipids with 'orphan' bioactive activity; by deciphering novel modes of action of lipids through their interactions with proteins; and by understanding the misregulation of lipids in disease. In this Innovation article, we summarize the recent technological advances that will allow global protein–lipid interactions to be mapped on a systems level and open up new pharmaceutical strategies. We also discuss the future needs and challenges involved in the integration of diverse, orthogonal approaches for studying the regulation of proteins by lipids in biology and medicine.

The interaction landscape

Lipids are well known for their amphipathic nature and their capacity to form membrane bilayers. Their structures are diverse because of their distinct chemical backbones, which are composed of glycerol in the case of the glycerolipids and glycerophospholipids, sphingoid long-chain bases for the sphingolipids and isoprene for the sterols (BOX 1). Glycerophospholipids and sphingolipids also contain at least one fatty acid that can differ in length and/or in its level of unsaturation (which helps to determine membrane thickness and fluidity), as well as a hydrophilic head group that extends out of the hydrophobic bilayer into the aqueous phase and can function as a signalling hub. The combination of these various metabolic building blocks results in a diverse lipid landscape that is extensively exploited by proteins[7]. Proteins have adapted a variety of motifs and domains that can recognize and sense individual lipids and/or more general membrane properties such as curvature, thickness or specialized microdomains[7]. Furthermore, protein–lipid interactions can generally be divided into three categories, according to the mechanisms by which these molecules recognize each other and the environment in which they are found (FIG. 1).

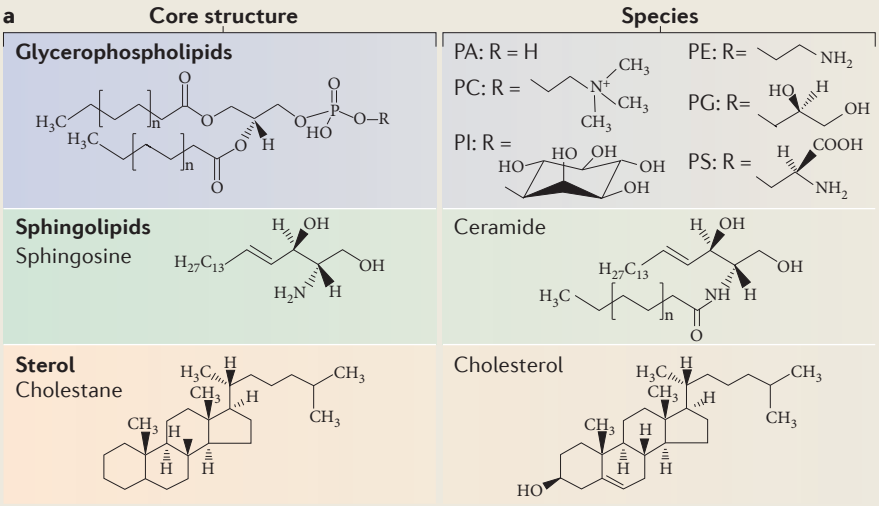
Integral membrane proteins. An essential type of protein–lipid interaction involves integral membrane proteins, which interact with specific lipids within the hydrophobic plane of the membrane. This type of interaction is involved in targeting proteins to specific organelles[8], and it can be used to modulate protein structure, activity and function[9,10]. For example, the protein p24, which is a component of the coat protein I (COPI) machinery, has a transmembrane segment that specifically and exclusively binds to one sphingomyelin species, SM18 (REF. 11). This interaction, which involves the head group and backbone of the sphingolipid and a signature sequence (VXXTLXXIY; where X indicates any amino acid) within the transmembrane domain of p24, induces p24 oligomerization and activation to regulate COPI-dependent vesicular transport. Integral membrane proteins, however, are challenging to study, and our current knowledge is limited to a few prototypic examples that have generally been investigated using advanced biochemical, biophysical or structural methods that cannot easily be scaled up. More generic approaches — that is, those that are applicable to all types of protein–lipid interactions — are needed (see below).

Box 1 | **Liposomes: surrogates of biological membranes**

Liposomes (also called phospholipid vesicles), which are closed vesicles comprising a bilayer of amphiphilic molecules, are *in vitro* mimics of natural membranes[82,83]. A large variety of lipids are available from multiple suppliers (see Supplementary information S1 (table)), notably glycerophospholipids, phosphoinositides, sphingolipids and sterols (the structures of the major lipid species are shown in the figure, part **a**), although the commercially available lipid repertoire is still far from covering the range of natural lipids. Each lipid category is hierarchically organized around a core structure that is defined in a species on the basis of a modification of the head group (for glycerophospholipids the modifications are labelled 'R' in the figure, part **a**) or a chemical modification in the core structure (for example, in the sphingolipids and sterols). Lipids dissolved in organic solvents can be stored in glass vials at -20 °C under argon or nitrogen, and the quality of the initial lipid composition and any further degradation over time can be monitored, conveniently, by matrix-assisted laser desorption/ionization–time of flight (MALDI–TOF) mass spectrometry. A thin-layer chromatography spotter can be used for the high-throughput and automatic handling of lipids, because they are resistant to organic solvents[16,39], but these robots are limited by poor spatial resolution.

Liposomes are normally classified by their size and the number of bilayers that they contain (unilamellar liposomes have a single bilayer; multilamellar liposomes have multiple bilayers)[82] and are usually designated as SUVs (small unilamellar vesicles, which have a diameter of <100 nm), LUVs (large unilamellar vesicles, which have a diameter of ≤1 μm) or GUVs (giant unilamellar vesicles, which have a diameter of >1 μm). The preparation of liposomes in general[82], and of GUVs specifically[83], has been reviewed elsewhere; several popular and successful methods are now used (see the figure, part **b**). These protocols are suitable for preparing a few liposome types, but they are difficult to scale up because of major technical limitations: the protocols are time-consuming and require large amounts of starting material; each lipid mixture needs to be optimized; and the liposomes cannot be stored long-term. The introduction of a method that allows GUVs to be produced upon the hydration of an agarose film[84] has opened up new possibilities, as this protocol can be integrated into a microarray format to form hundreds of liposome types in parallel[39] and can be further developed to create proteoliposome arrays[42] (that is, liposomes into which membrane proteins have been inserted). PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine.

a



b

Method to produce liposome	Size of liposome	Pros of method	Cons of method	Throughput (per day)	Refs
Extrusion	<500 nm	Curvature control	Short storage time (<1 day)	<10	82
Electroformation	>1 μm	Well-established	Formation in low salt	<10	83
Hydration on agarose-coated slide	>1 μm	Easy to set up, generic for any lipid mixture, physiological	Polydispersity	>1,000	39,84
Microfluidic jetting	>1 μm	Size control, encapsulation	Difficult to set up	<10	85

Peripheral membrane proteins. Another important type of interaction involves the recruitment of soluble proteins to the cell periphery of biological membranes. Several domains recognize specific lipid head groups[12] (for example, pleckstrin homology (PH) domains recognize phosphoinositide phosphates and the C2 domain of lactadherin recognizes phosphatidylserine) and/or specific membrane features (for example, epsin amino-terminal homology (ENTH) and BAR domains recognize membrane curvature)[12–14]. Thus, lipids and other membrane attributes function as second messengers to control the spatiotemporal recruitment and activation of specific protein effectors[14]. A key example is the Ser/Thr kinase AKT1, which is activated by its recruitment to membranes through its specialized PH domain that recognizes phosphatidylinositol-3,4,5-trisphosphate ($PtdIns(3,4,5)P_3$) or $PtdIns(3,4)P_2$; both lipids are produced locally by phosphoinositol 3-kinase (PI3K)[14]. Domains involved in the recruitment of proteins to the periphery of membranes were the focus of the first systematic protein–lipid interaction survey[15] (see below). However, despite these pioneering efforts, many signalling lipids still have an elusive mode of action, for example, only a handful of direct cellular effectors are known for sphingolipids and phosphatidylserine. In addition, the effects of mutations that have been observed in human biopsies, or of post-translational modifications (for example phosphorylation or Lys acetylation), on the lipid-binding affinity and specificity of proteins remain largely elusive. Another largely unexplored question is the role of 'coincidence sensing' (that is, the simultaneous detection of several membrane properties, for example, the simultaneous sensing of membrane curvature and the presence of phosphatidylserine), or 'cooperative mechanisms' (that is, when the binding affinity of a protein for a lipid X is changed upon the binding of a lipid Y), in the selective recruitment of peripheral proteins to specific membranes[16,17].

Soluble proteins. The third — and mostly overlooked — type of lipid–protein interaction involves soluble proteins that bind lipids outside cellular membranes. These proteins bind lipids in a hydrophobic pocket and, by carrying their hydrophobic cargo through the aqueous phase of the cell, act as transporters, lipid chaperones (for example, presenting lipids to metabolic enzymes) and/or signalling factors[18–20]. Two paramount examples are lipid-transfer proteins that transport various lipids between different organelles by a non-vesicular transfer mechanism[2],

and nuclear receptors[21]. This type of interaction has been the focus of surveys that were limited to two protein families: the kinases[22] and the lipid-transfer proteins[23] of *Saccharomyces cerevisiae*.

These different types of protein–lipid interaction are not mutually exclusive. Often, the same protein can simultaneously sense and bind to different membranes or lipids — through distinct mechanisms — and this creates molecular complexity and diversity. For example, some lipid-transfer proteins also contain a PH domain (these include oxysterol-binding protein (OSBP), OSBP-related protein 1 (ORP1L), ORP3, ORP4L, ORP5, ORP6, ORP7, ORP8, ORP9L, ORP10 and ORP11) or a transmembrane domain (these include Niemann-Pick C1 protein (NPC1), ORP3, ORP5 and ORP8). Evidently, then, proteins and lipids engage in pleiotropic and multifaceted relationships that are based on various biophysical principles and are traditionally studied by distinct scientific communities. The diversity of protein–lipid interactions implies a need for appropriate, specialized assays that are optimized to capture specific types of interactions (FIG. 1).

Classic methods

Several biochemical, biophysical and structural methods are traditionally used to study biomolecular interactions[24], and most of these have been adapted to the study of protein–lipid interactions, for example, X-ray crystallography, nuclear magnetic resonance, isothermal titration calorimetry, surface plasmon resonance (SPR) and microscale thermophoresis (MST) (BOX 2). These assays are generally low-throughput, but they have contributed important atomic or mechanistic models for the different types of protein–lipid interactions, and they remain the methods of choice for studying integral membrane proteins (reviewed in REF. 25). It is beyond the scope of this article to discuss all of the classic assays in depth, as they have been reviewed elsewhere[26,27]; rather, we will focus on the most prominent and widely used method, SPR.

SPR allows the direct and rapid measurement of lipid–protein association and dissociation rates without the need for protein or lipid labelling[28]. Interactions are studied on the surface of 'sensor chips', which are glass slides that are coated with a very thin layer of gold, to which an artificial membrane or liposome (BOX 1) is attached. The recruitment of an interacting protein to the artificial membrane or liposome changes the refractive index at the surface of the chip; this information is recorded. Many protocols have been designed to prepare mimetic membranes on sensor chips[28]. SPR studies can provide both quantitative and qualitative data on molecular interactions — for example, information on lipid specificity or on the effects of mutations on binding affinity — and this approach has been extensively used to study, for example, lipid-binding domains or proteins involved in signalling[15], pore-forming peptides, coagulation factors and enzymes[28].

An interesting and impressive newcomer to the lipid field is native protein mass spectrometry. This method was first used to study the dynamics and composition of soluble protein complexes[29] but has been adapted for the study of integral membrane proteins that are in complex with their lipid ligands[25,30]. The challenge in adapting this method was to preserve the native protein–membrane complexes as they entered the gas phase. For this purpose, innovative protocols — based on the use of detergent micelles[31], bicelles or nanodiscs[32] — were developed. This has enabled the identification of lipids that specifically bind to large complexes of integral membrane proteins, including ATP-binding cassette (ABC) transporters[33], mechanosensitive ion channels[31] and two rotary ATPases/synthases[34]. These studies have revealed complex stoichiometry, the identity of the bound lipid and the role of lipids in modulating protein folding and stability[31].

Novel *in vitro* methods

Biochemical and biophysical assays based on the use of artificial, surrogate membranes are popular (see above), as they allow lipids to be studied *in vitro* in controlled and defined environments (that is, independently of the other attributes or constituents of cellular membranes). Some of these assays have been adapted and applied to systematic analyses.

Lipid-overlay and lipid pull-down assays. Lipid-overlay and lipid pull-down assays (FIG. 1) are based on the same principle and involve the immobilization of individual lipids (or lipid head groups) on a solid support (for example, nitrocellulose membranes for lipid-overlay and magnetic beads for lipid pull-down). The proteins that bind to the immobilized lipids can be characterized using either immunodetection (for example, with fluorescent or chemiluminescent antibodies) or mass spectrometry[35,36]. The lipid-overlay assay is available commercially and is widely used to measure protein–lipid interactions *in vitro* (see Supplementary information S1 (table)). Both methods have been readily adapted to large surveys in model organisms such as *S. cerevisiae*[16] or *Dictyostelium discoideum*[35] (FIG. 2). However, a major drawback to these approaches is that the lipids are tested under highly artificial conditions, for example, outside a membrane bilayer and at high, non-physiological concentrations. Thus, they are prone to artefacts, and interactions need to be verified using orthogonal assays[26].

Protein-array assays. In a protein-array assay (FIG. 1), proteins are immobilized on a solid support, and the binding of fluorescent liposomes that comprise a carrier lipid (usually phosphatidylcholine) and a signalling lipid is measured. The advantage of this approach is that liposomes form a membrane bilayer and therefore closely mimic the *in vivo* situation. So far, this assay has been limited to two screens in *S. cerevisiae* (FIG. 2), an organism for which a protein microarray containing the majority of its proteome (80%; 5,800 proteins) is available[37,38]. The use of this approach is also limited by the fact that the protocols designed to produce, handle and store liposomes are labour-intensive and difficult to scale up (BOX 1), thus precluding the systematic analyses of different types of membrane.

Liposome microarray-based assay (LiMA). LiMA (FIG. 1) addresses many of the drawbacks mentioned above[39]. This assay measures the recruitment of proteins to membranes in a quantitative, multiplexed and high-throughput manner. It integrates biochemical principles — that is, the formation of liposomes with diameters of >1 μm (BOX 1) on a thin agarose layer — with quantitative fluorescence microscopy-based imaging and microfluidics. The procedure used to produce and array the liposomes is generic, and hundreds of liposomes of varying lipid composition (including the main lipid classes) can be simultaneously produced on a small (~1 cm^2) microfluidic chip. The recruitment of fluorescently labelled proteins is measured by high-content, quantitative fluorescence microscopy[40]. Importantly, the assay is quantitative and can measure discrete changes in binding affinities that are produced by protein mutations found in human biopsies or by the presence of additional lipids in the membrane (which trigger coincidence-sensing mechanisms)[39,41]. LiMA is scalable to the proteome or lipidome levels and takes advantage of the available genome-wide collections of cell lines expressing GFP fusions. Recently, LiMA was applied to ~10,000 experiments designed to quantify the role of phosphoinositides in the

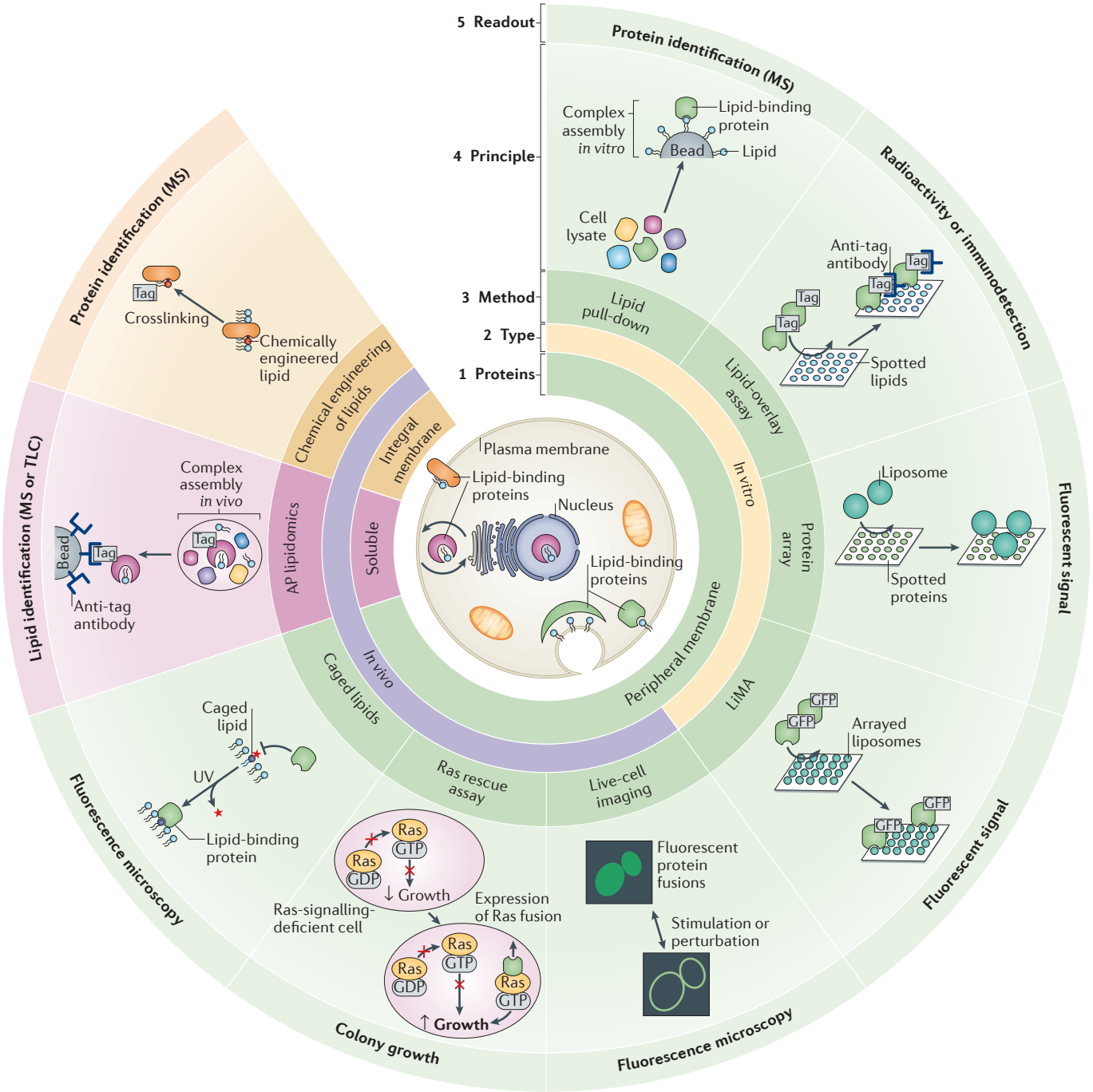
recruitment of PH domains to membranes; cooperativity between lipids was found to be a key mechanism for such recruitment[41]. The assay is also adaptable to other readouts, such as those from mass spectrometry or advanced optical methods, including total internal reflection fluorescence microscopy or two-photon excitation microscopy; the latter of these optical techniques enables equilibrium dissociation constants to be determined. Currently, LiMA is best suited to studying the recruitment of soluble proteins to the periphery of membranes. Going forward, protocols for the production of proteoliposomes and for the study of integral membrane proteins in the context of different lipids will need to be integrated[42].

Importantly, these *in vitro* assays produce invaluable data sets on the ability of proteins and/or different lipids or membrane environments to interact. The physiological relevance and functions of these interactions will need to be investigated through orthogonal and more physiological assays.

Novel *in vivo* methods

Recent technical advances have enabled the study of protein–lipid interactions *in vivo*; the key functional assays are discussed below.

In vivo *functional assays and live-cell imaging.* Many *in vivo* functional assays have been developed to characterize the roles of intracellular membranes and individual lipids in tuning the behaviour and function of effector proteins. These cellular assays are based on similar general principles, and they



involve the systematic, targeted perturbation of a membrane component (for example, the inhibition or mutation of enzymes in lipid metabolic pathways) followed by the measurement of the effects of these perturbations on specific phenotypic readouts (FIG. 1). Among these functional assays, genetic interaction screening is probably the oldest and most widely used technique for identifying functional relationships between cellular pathways that cannot be linked *a priori*[43]. The assay measures how two genes that are deleted simultaneously perturb a phenotype, for example, cell growth or, spurred on by the recent advances in high-throughput live-cell imaging, cell morphology[44]. Interaction screens were used in *S. cerevisiae* to build genetic interaction maps that are focused on lipid biology (termed lipid E-MAPs) and that revealed new components of the eisosome complex (a protein complex that marks the site of endocytosis at the plasma membrane in some eukaryotes), as well as novel molecular mechanisms linking protein degradation and fatty acid desaturation[45,46].

The Ras rescue assay (FIG. 1) is another *in vivo* functional assay that was developed in yeast. It is based on rescuing the growth of a thermosensitive mutant of the yeast Cdc25 (which is a membrane-bound guanine nucleotide exchange factor for Ras). Membrane-binding domains or proteins fused to a constitutively active Ha-Ras mutant can target active Ras to the membrane and thus rescue growth[47,48]. The role of specific lipids in recruiting PH domains to the membrane can be assessed by measuring the effect of targeted perturbations of lipid metabolism (for example, PH domains that bind PtdIns(4,5)P_2 will not be recruited to yeast membranes if the enzyme that produces PtdIns(4,5)P_2, the PtdIns(4)P 5-kinase, is mutated). Similarly, live-cell imaging (FIG. 1) has been used to observe the translocation of proteins or protein domains fused to a fluorescent tracer (usually GFP) to cellular membranes following cell stimulation or metabolic perturbations[15,16,49]. Generally, these cell-based assays are generic and compatible with large-scale and systematic surveys. They are very powerful tools for inferring functional relationships between proteins or measuring membrane binding directly inside cells. Their main drawback is that they do not necessarily measure direct physical associations, because they also capture functional links and indirect interactions. In addition, the effects of metabolic perturbations on the lipidome of specific subcellular membranes remain difficult to predict because of, among other factors, the so-called 'ripple effect' (REF. 50) (in which perturbing the level of one lipid induces indirect changes in the levels of many other lipids). In short, the approaches discussed in this subsection are not usually designed to capture the mechanisms of biomolecular recognition; this requires the integration of orthogonal methods, for example, biophysical, structural or large-scale biochemistry (see above).

Affinity-purification lipidomics. A series of new physiological assays have recently been developed that alleviate some of the drawbacks of the *in vivo* functional assays discussed above. An example is affinity-purification lipidomics, which relies on the homologous expression of affinity-tagged protein fusions to systematically purify and characterize protein–lipid complexes that have been assembled *in vivo*[22,23] (FIG. 1). The main advantage of this method is that endogenously expressed and native proteins are retrieved from cells or tissues, closely mirroring physiological conditions. The protein–lipid complexes are kept in their native states throughout the purification process, remain functional and are amenable to *in vitro* activity-based assays or structural analyses[23]. The co-eluting proteins and lipids are analysed by mass spectrometry and lipidomics methods. This protocol is not limited to one cell type and can, in principle, be used to quantify changes in complex composition between cell lines or following cell stimulations; it also benefits from being able to use the many available proteome-wide collections of cell lines that express a tagged fusion protein[51,52]. The assay is also readily

Box 2 | Classical methods for studying protein–lipid interactions

- Flotation assay: a method in which liposomes and proteins are mixed at the bottom of a sucrose gradient and ultracentrifuged. If proteins and lipids interact, the complex floats in the upper fractions of the centrifugation tube[27].
- ITC (isothermal titration calorimetry): a biophysical method to obtain thermodynamic parameters of the interaction between proteins and liposomes, which allows their molecular affinities to be calculated[86].
- SPR (surface plasmon resonance): in this approach, a lipid membrane is formed or liposomes are bound on top of a plasmon resonance sensor chip, and SPR is used to detect the binding and dissociation of proteins[26,28].
- MST (microscale thermophoresis): a method using a temperature field to perform biomolecular interaction studies, which can be applied to protein–lipid interaction studies[87].
- Native protein mass spectrometry: the analysis of intact protein complexes by mass spectrometry.

◀ Figure 1 | **System-wide methods for capturing protein–lipid interactions.** Protein–lipid interactions can be divided into three categories according to the type of protein involved in the interaction: peripheral membrane proteins, soluble proteins and integral membrane proteins (curve 1). Each protein–lipid interaction can be captured by specialized *in vitro* assays (using artificial membranes) or *in vivo* assays (using biological membranes) (curve 2). Each method (curve 3) and its associated principle (curve 4) is schematically shown. Finally, each method is associated with specific readout techniques (curve 5). To capture direct protein–lipid interactions of peripheral membrane proteins, four *in vitro*-based methods are available. These techniques are based on the immobilization of either the lipid or the liposome (lipid pull-down, lipid-overlay assay, liposome microarray-based assay (LiMA)) or of the protein (protein array) on a solid surface, followed by probing with the other ligand (protein or lipid or liposome) in solution. Direct protein–lipid interactions of peripheral membrane proteins can also be captured using *in vivo* methods. Live-cell imaging involves the observation of the recruitment of GFP-fused proteins to the cellular plasma membrane. Another *in vivo* method, caged lipids, which is based on a chemical biology approach, relies on the enrichment of a particular lipid in cellular membranes by 'uncaging' the chemically modified lipid using ultraviolet (UV) light; the recruitment of the protein to the membrane is then followed with fluorescence microscopy. Finally, among *in vivo* methods devoted to peripheral membrane proteins, the Ras rescue assay is based on a mutant yeast strain that is deficient in the Ras signalling pathway and has a growth deficiency as a result. Growth is rescued only when a membrane-targeted protein fused to constitutively active Ras protein is expressed. The second interaction mode between lipids and proteins involves soluble proteins, that is, those present in the cytoplasm that can tightly bind lipids. Such interactions are analysed by protein–lipid co-purification; this requires the purification of *in vivo* assembled protein–lipid complexes from a whole-cell lysate and analysis of the associated lipids with mass spectrometry (MS) or thin-layer chromatography (TLC). Finally, to capture direct interactions between lipids and an integral membrane protein, a crosslinking strategy is used that creates a covalent link between the protein and the lipids, forming complexes that can be purified and analysed by mass spectrometry. AP, affinity purification.

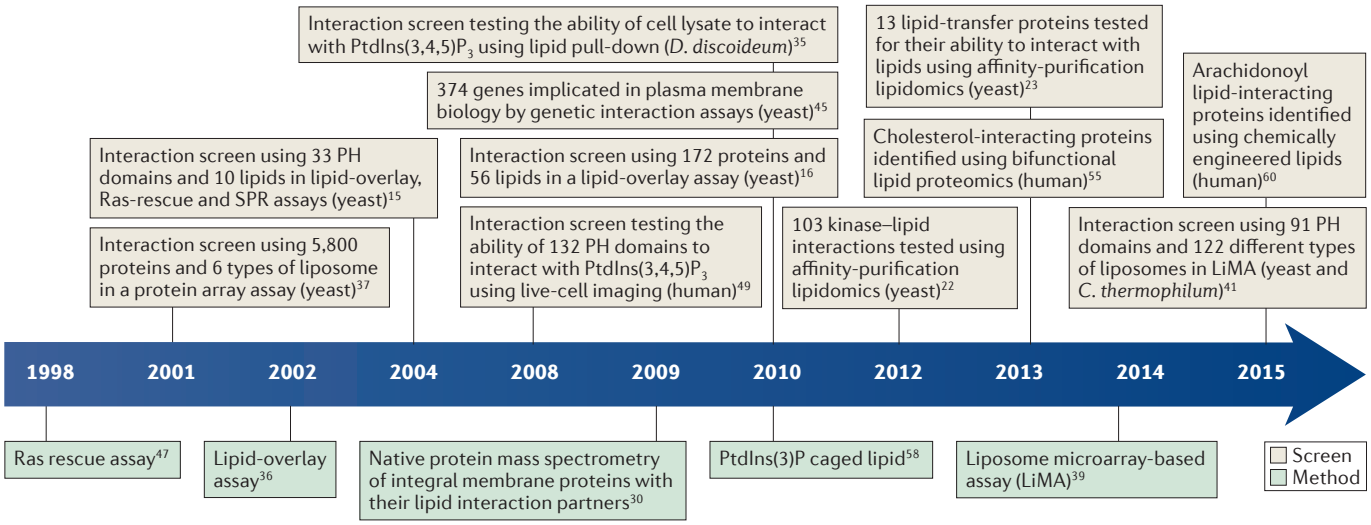


Figure 2 | **Timeline of key events in protein–lipid interaction research.** The development of key methods and their application to systematic protein–lipid interaction screens are listed here in chronological order. The systematic screens that have been conducted so far are shown above the timeline. The development of key techniques that are suitable for the large-scale detection of protein–lipid interactions are indicated below the timeline. The species in which the screen was carried out is shown in parentheses. *C. thermophilum*, *Chaetomium thermophilum*; *D. discoideum*, *Dictyostelium discoideum*; LiMA, liposome microarray-based assay; PH, pleckstrin homology; PtdIns(3)P, phosphatidylinositol-3-phosphate; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-trisphosphate; SPR, surface plasmon resonance.

scalable and has been applied to systematic surveys of yeast kinases[22] and lipid-transfer proteins, which revealed new mechanisms for the non-vesicular transport of phosphatidylserine between organelles[23] (FIG. 2). Another important outcome of these pilot studies is that they demonstrate the feasibility of affinity-purification lipidomics and the need for broader, proteome-wide efforts for studying protein–lipid interactions in higher eukaryotes. However, this approach remains limited to the characterization of relatively stable and soluble protein–lipid complexes.

Chemical engineering of lipids. The chemical engineering of lipids (FIG. 1) contributes invaluable tools for measuring, visualizing and studying lipids inside living cells, and these are broadly applicable to all types of protein–lipid interactions. For example, photoactivatable lipids have been developed that contain a chemical group (such as a diazirine ring) that enables them to form crosslinks (FIG. 1) with proteins that they interact with in response to ultraviolet light[11,53] (reviewed in REF. 54). These approaches, however, are limited in their scope, as they do not allow for affinity enrichment of the crosslinked protein–lipid complexes. This issue was recently addressed with the development of bifunctional lipids that, in addition to the photoreactive moiety, contain an alkyne group. This alkyne group enables the lipid to be coupled through click chemistry (that is, bio-orthogonal reactions that allow the selective coupling of two functional groups in biological samples[54]) to a reporter molecule, such as a fluorescent dye for visualization or biotin for affinity purification[54–57]. In combination with high-resolution mass spectrometry, the use of bifunctional lipids can provide global maps of protein–lipid interactions directly in living cells or organisms. The list of available bifunctional lipids is continuously increasing and spans all of the main lipid classes, and the approach is becoming generic[54]. The main drawback of this method is that the modified lipids are generally metabolized in living cells, which generates various bifunctional products that can, in principle, also react with proteins. As for the genetic methods described above, the characterization of the precise lipid or lipids involved requires the integration of orthogonal methods such as biophysical, structural or large-scale biochemistry approaches.

Other interesting advances include the recent development of caged lipids (FIG. 1), which bear a photoactivatable protective group that is intended to keep the lipid (whether it is a signalling or structural lipid) inactive until a light pulse removes the 'cage' (REFS 58–60). Also, alkyne-tagged lipids combined with Raman-scattering imaging (which is a method used to measure the specific vibrational signatures of chemical bonds) offer a promising approach for directly imaging the metabolic route of lipids in live cells[61,62]. These *in vivo* techniques allow lipids and the composition of cellular membranes to be precisely manipulated in both time and space and — coupled with some of the *in vivo* assays described above, such as live-cell imaging — hold great promise.

Towards multi-omics integration

Pioneering protein–lipid interaction screens were based on early technologies and focused on the study of soluble proteins or of specific lipid-binding domains[15,16]. Since then, more generic and physiological methods have been developed. These hold great potential for the next generation of screens, which will embrace entire proteomes and lipidomes in a range of cell types and in various physiological or pathological states (FIG. 3). We expect that novel screens will be used to discover novel modes of protein–lipid interaction that involve multiple lipids, lipids and proteins and, potentially, lipids and glycolipids (FIG. 3a). Although a few examples of lipid-binding domains that can recognize several lipids simultaneously have been described[12,16,17], a full repertoire of such interactions is not known. Also, other aspects of membrane biophysics, such as membrane electrostatics and bending, and how they are influenced by protein–lipid interactions, must be integrated into the design of future screens[63] (FIG. 3b).

As our understanding of the molecular and functional circuitry between proteins

and lipids unfolds, we must extend and complete these networks through the integration of orthogonal 'omics' data sets and develop comprehensive, multilevel models that depict the roles of lipids in cell biology and physiology[50]. There is, for example, a pressing need for a more systematic functional interpretation of the multitude of protein–lipid interactions that have been detected, which can be addressed through the *in silico* integration of complementary data sets derived from both *in vivo* and *in vitro* biochemical assays.

Another important advance is the ability to experimentally measure the function of lipid–protein interactions through the integration of different assays, so that binding events can be directly linked to, for example, their subcellular location, the enzymatic activity[64,65] or oligomerization state of the protein, or whether the local membrane is organized into nanodomains or microdomains[66,67] (FIG. 3b). Recently, it was demonstrated, using membrane-mimics (BOX 1), that cholesterol-dependent raft-like lipid domains have a pivotal role in the fusion of the human immunodeficiency virus with membranes; this illustrates the power of *in vitro* approaches in reconstituting complex protein–lipid interactions[68]. In this regard, LiMA holds great promise, and we envision that in the future this microfluidic platform will support new assays, including enzymatic assays (for example, for the detection of kinase, phosphatase and phospholipase activity), as well as assays for the activity of transport proteins, and fluorescence resonance energy transfer (FRET), atomic force, super-resolution and electron microscopy (FIG. 3c). Finally, the development of new chemical tools[54] and probes to measure protein and lipid binding and activity, or specific membrane attributes such as thickness, inside living cells will be instrumental in the move towards multi-omics integration.

Translational medicine challenges

Understanding the molecular and functional circuitry linking proteins and lipids in human cells and how they are affected in disease states will be an invaluable tool for medicine[69]. Mutations in lipid-binding domains that cause discrete changes in their affinity and specificity for lipids frequently affect cell fate and the physiology of whole organisms. Prominent examples include point mutations in the PtdIns(3,4,5)P_3- and PtdIns(3,4)P_2-specific PH domain of AKT1, which result in diseases such as colorectal cancers[70], Proteus syndrome (which is characterized by overgrowth of skin and skeleton)[71] or metabolic disorders (such as hypoglycaemia)[72]; mutations in the histone fold of Son of sevenless homologue 1 (SOS1; a guanine nucleotide exchange factor for RAS), which cause Noonan syndrome[73]; and mutations in the PX domain of p40phox (also known as NCF4; a subunit of NADPH oxidase), which underlie chronic granulomatous disease[74].

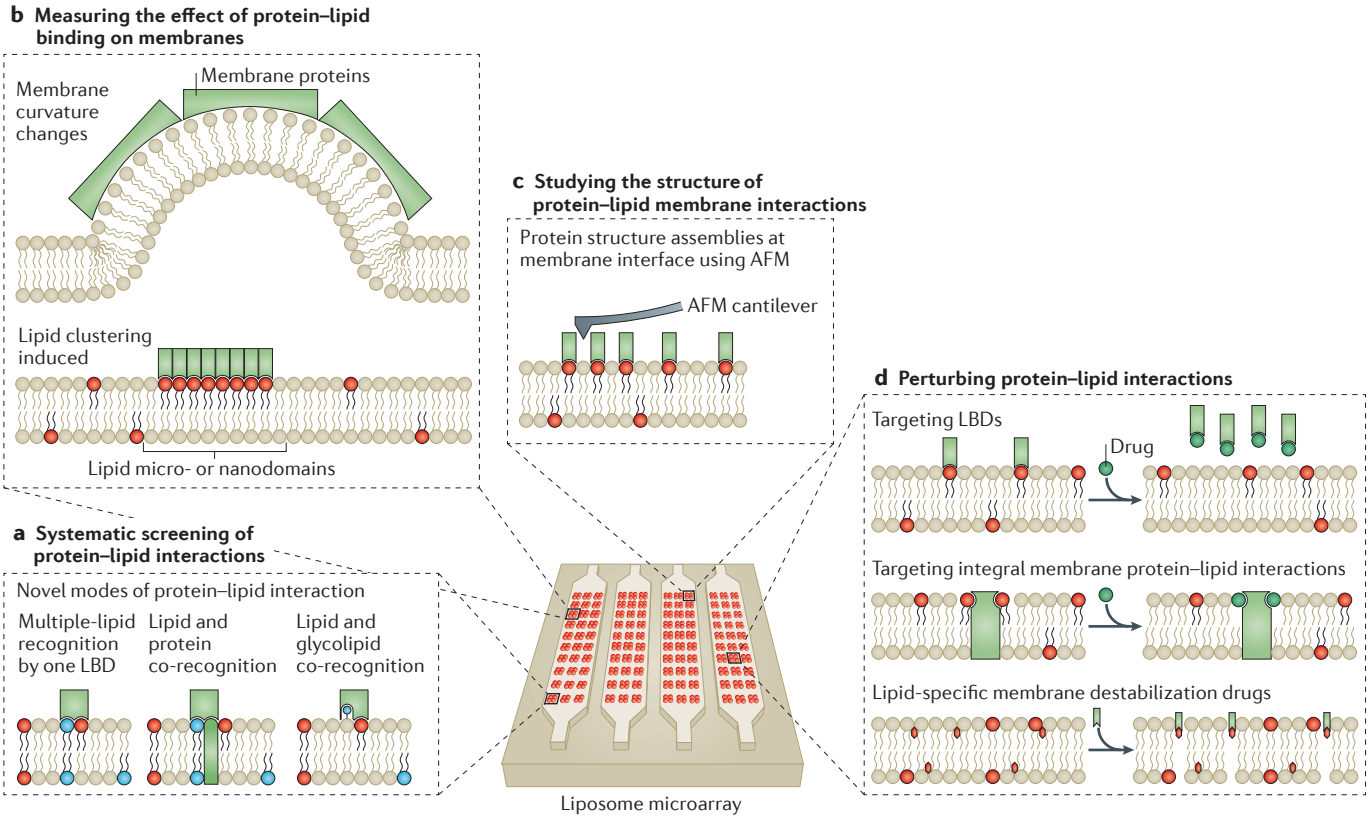


Figure 3 | **Future scientific and technological challenges for liposome microarray-based assays (LiMA). a** | In the future, LiMA will be used to investigate novel modes of protein–membrane interactions that imply multiple partners, for example, the coincident sensing of multiple lipids (left) and the co-recognition of lipid and proteins (middle) or lipids and glycolipids (right). **b** | Another field of investigation is the measurement of the effect of protein–lipid interactions on the biophysical properties of membranes, notably the ability of proteins to bend membranes (top) or to generate microdomains or nanodomains of lipids (bottom). **c** | Whereas this technology has been used so far in combination with fluorescence microscopy[39], further development will allow protein structure assemblies to be investigated at the membrane interface using techniques such as atomic force microscopy (AFM). **d** | LiMA is also an attractive method for developing novel pharmaceutical compounds that perturb protein–lipid interactions. Such compounds might target the lipid binding domains (LBDs) of peripheral membrane proteins (top), integral membrane proteins (middle) or compounds that kill pathogens by destabilizing membranes (bottom).

Globally, however, little is known about the functional consequences of single-nucleotide polymorphisms on lipid–protein interaction networks, and the recently developed high-throughput *in vitro* binding assays are likely to contribute to filling this gap.

Lipids are also taking centre stage in the field of infectious diseases, and pharmaceutical strategies targeting fungal or bacterial lipid metabolism or membranes are being proposed to counteract antibiotic resistance[75,76]. In addition, intracellular pathogens such as bacteria[77], viruses[78] and parasites[79] can hijack the host cell machineries that are responsible for lipid homeostasis, and targeting the mechanisms involved could lead to new therapeutic strategies.

Lipid metabolism and lipid-binding proteins have thus emerged as promising new targets for pharmacological intervention in various human diseases[80,81]. In addition, many of the new assays described here should enable the disruption of protein–lipid interactions by small molecules to be studied using, for example, LiMA (FIG. 3d).

Conclusion

In this Innovation article, we have summarized and illustrated the new approaches for investigating protein–lipid interactions on a large scale. The vast majority of lipids have not been assigned a functional biological role, thus these methods might allow novel biological insights into lipid biology. *In vitro* approaches are constantly progressing towards allowing the reconstitution of cellular membrane complexity, and they will allow the exact mechanisms by which proteins target membranes to be deciphered. In addition, the development of affinity- and chemical biology-based methods will shed light on the *in vivo* roles of lipids. More generally, these methods illustrate the value of multidisciplinary approaches that integrate biology, chemistry, engineering and the physical sciences.

Antoine-Emmanuel Saliba is at the Institute for Molecular Infection Biology and Core Unit Systems Medicine, University of Würzburg, Josef-Schneider-Strasse 2, D-97080 Würzburg, Germany.

Ivana Vonkova is at the European Molecular Biology Laboratory (EMBL), Structural and Computational Biology Unit, Meyerhofstrasse 1, D-69117 Heidelberg, Germany.

Anne-Claude Gavin is at the European Molecular Biology Laboratory (EMBL), Structural and Computational Biology Unit and Molecular Medicine Partnership Unit, Meyerhofstrasse 1, D-69117 Heidelberg, Germany.

Correspondence to A-C.G.
e-mail: gavin@embl.de

doi:10.1038/nrm4080
Published online 28 October 2015

1. van Meer, G., Voelker, D. R. & Feigenson, G. W. Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell. Biol.* **9**, 112–124 (2008).
2. Holthuis, J. C. & Menon, A. K. Lipid landscapes and pipelines in membrane homeostasis. *Nature* **510**, 48–57 (2014).
3. Fahy, E. *et al.* Update of the LIPID MAPS comprehensive classification system for lipids. *J. Lipid Res.* **50**, S9–S14 (2009).
4. Overington, J. P., Al-Lazikani, B. & Hopkins, A. L. How many drug targets are there? *Nat. Rev. Drug Discov.* **5**, 993–996 (2006).
5. Wymann, M. P. & Schneiter, R. Lipid signalling in disease. *Nat. Rev. Mol. Cell. Biol.* **9**, 162–176 (2008).
6. Babu, M. *et al.* Interaction landscape of membrane-protein complexes in *Saccharomyces cerevisiae*. *Nature* **489**, 585–589 (2012).
7. Capelluto, D. G. *Lipid-mediated Protein Signaling* (Springer, 2013).
8. Sharpe, H. J., Stevens, T. J. & Munro, S. A comprehensive comparison of transmembrane domains reveals organelle-specific properties. *Cell* **142**, 158–169 (2010).
9. Nury, H. *et al.* Structural basis for lipid-mediated interactions between mitochondrial ADP/ATP carrier monomers. *FEBS Lett.* **579**, 6031–6036 (2005).
10. Whorton, M. R. & MacKinnon, R. Crystal structure of the mammalian GIRK2 K^+ channel and gating regulation by G proteins, PIP2, and sodium. *Cell* **147**, 199–208 (2011).
11. Contreras, F. X. *et al.* Molecular recognition of a single sphingolipid species by a protein's transmembrane domain. *Nature* **481**, 525–529 (2012).
12. Lemmon, M. A. Membrane recognition by phospholipid-binding domains. *Nat. Rev. Mol. Cell. Biol.* **9**, 99–111 (2008).
13. Antonny, B. Mechanisms of membrane curvature sensing. *Annu. Rev. Biochem.* **80**, 101–123 (2011).
14. Scott, J. D. & Pawson, T. Cell signaling in space and time: where proteins come together and when they're apart. *Science* **326**, 1220–1224 (2009).
15. Yu, J. W. *et al.* Genome-wide analysis of membrane targeting by *S. cerevisiae* pleckstrin homology domains. *Mol. Cell* **13**, 677–688 (2004).
16. Gallego, O. *et al.* A systematic screen for protein–lipid interactions in *Saccharomyces cerevisiae*. *Mol. Syst. Biol.* **6**, 430 (2010).
17. Moravcevic, K., Oxley, C. L. & Lemmon, M. A. Conditional peripheral membrane proteins: facing up to limited specificity. *Structure* **20**, 15–27 (2012).
18. Lev, S. Non-vesicular lipid transport by lipid-transfer proteins and beyond. *Nat. Rev. Mol. Cell. Biol.* **11**, 739–750 (2010).
19. Forman, B. M., Chen, J. & Evans, R. M. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc. Natl Acad. Sci. USA* **94**, 4312–4317 (1997).
20. Grabon, A., Khan, D. & Bankaitis, V. A. Phosphatidylinositol transfer proteins and instructive regulation of lipid kinase biology. *Biochim. Biophys. Acta* **1851**, 724–735 (2015).
21. Musille, P. M., Kohn, J. A. & Ortlund, E. A. Phospholipid-driven gene regulation. *FEBS Lett.* **587**, 1238–1246 (2013).
22. Li, X., Gianoulis, T. A., Yip, K. Y., Gerstein, M. & Snyder, M. Extensive *in vivo* metabolite-protein interactions revealed by large-scale systematic analyses. *Cell* **143**, 639–650 (2010).
23. Maeda, K. *et al.* Interactome map uncovers phosphatidylserine transport by oxysterol-binding proteins. *Nature* **501**, 257–261 (2013).
24. Charbonnier, S., Gallego, O. & Gavin, A. C. The social network of a cell: recent advances in interactome mapping. *Biotechnol. Annu. Rev.* **14**, 1–28 (2008).
25. Barrera, N. P., Zhou, M. & Robinson, C. V. The role of lipids in defining membrane protein interactions: insights from mass spectrometry. *Trends Cell Biol.* **23**, 1–8 (2013).
26. Narayan, K. & Lemmon, M. A. Determining selectivity of phosphoinositide-binding domains. *Methods* **39**, 122–133 (2006).
27. Zhao, H. & Lappalainen, P. A simple guide to biochemical approaches for analyzing protein-lipid interactions. *Mol. Biol. Cell* **23**, 2823–2830 (2012).
28. Besenicar, M., Macek, P., Lakey, J. H. & Anderluh, G. Surface plasmon resonance in protein-membrane interactions. *Chem. Phys. Lipids* **141**, 169–178 (2006).
29. Zhang, Z., Wu, S., Stenoien, D. L. & Pasa-Tolic, L. High-throughput proteomics. *Annu. Rev. Anal. Chem.* **7**, 427–454 (2014).
30. Barrera, N. P. *et al.* Mass spectrometry of membrane transporters reveals subunit stoichiometry and interactions. *Nat. Methods* **6**, 585–587 (2009).
31. Laganowsky, A. *et al.* Membrane proteins bind lipids selectively to modulate their structure and function. *Nature* **510**, 172–175 (2014).
32. Hopper, J. T. *et al.* Detergent-free mass spectrometry of membrane protein complexes. *Nat. Methods* **10**, 1206–1208 (2013).
33. Bechara, C. *et al.* A subset of annular lipids is linked to the flippase activity of an ABC transporter. *Nat. Chem.* **7**, 255–262 (2015).
34. Zhou, M. *et al.* Mass spectrometry of intact V-type ATPases reveals bound lipids and the effects of nucleotide binding. *Science* **334**, 380–385 (2011).
35. Zhang, P., Wang, Y., Sesaki, H. & Iijima, M. Proteomic identification of phosphatidylinositol (3,4,5) triphosphate-binding proteins in *Dictyostelium discoideum*. *Proc. Natl Acad. Sci. USA* **107**, 11829–11834 (2010).
36. Dowler, S., Kular, G. & Alessi, D. R. Protein lipid overlay assay. *Sci. STKE* **2002**, pl6 (2002).
37. Zhu, H. *et al.* Global analysis of protein activities using proteome chips. *Science* **293**, 2101–2105 (2001).
38. Lu, K. Y. *et al.* Profiling lipid–protein interactions using nonquenched fluorescent liposomal nanovesicles and proteome microarrays. *Mol. Cell. Proteomics* **11**, 1177–1190 (2012).
39. Saliba, A. E. *et al.* A quantitative liposome microarray to systematically characterize protein-lipid interactions. *Nat. Methods* **11**, 47–50 (2014).
40. Pepperkok, R. & Ellenberg, J. High-throughput fluorescence microscopy for systems biology. *Nat. Rev. Mol. Cell. Biol.* **7**, 690–696 (2006).
41. Vonkova, I. *et al.* Lipid cooperativity as a general membrane-recruitment principle for PH domains. *Cell Rep.* **12**, 1519–1530 (2015).
42. Hansen, J. S., Thompson, J. R., Helix-Nielsen, C. & Malmstadt, N. Lipid directed intrinsic membrane protein segregation. *J. Am. Chem. Soc.* **135**, 17294–17297 (2013).
43. Costanzo, M. *et al.* The genetic landscape of a cell. *Science* **327**, 425–431 (2010).
44. Laufer, C., Fischer, B., Huber, W. & Boutros, M. Measuring genetic interactions in human cells by RNAi and imaging. *Nat. Protoc.* **9**, 2341–2353 (2014).
45. Aguilar, P. S. *et al.* A plasma-membrane E-MAP reveals links of the eisosome with sphingolipid metabolism and endosomal trafficking. *Nat. Struct. Mol. Biol.* **17**, 901–908 (2010).
46. Surma, M. A. *et al.* A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress. *Mol. Cell* **51**, 519–530 (2013).
47. Isakoff, S. J. *et al.* Identification and analysis of PH domain-containing targets of phosphatidylinositol 3-kinase using a novel *in vivo* assay in yeast. *EMBO J.* **17**, 5374–5387 (1998).
48. Moravcevic, K. *et al.* Kinase associated-1 domains drive MARK/PAR1 kinases to membrane targets by binding acidic phospholipids. *Cell* **143**, 966–977 (2010).
49. Park, W. S. *et al.* Comprehensive identification of PIP3-regulated PH domains from *C. elegans* to *H. sapiens* by model prediction and live imaging. *Mol. Cell* **30**, 381–392 (2008).
50. Cowart, L. A. *et al.* Revealing a signaling role of phytosphingosine-1-phosphate in yeast. *Mol. Syst. Biol.* **6**, 349 (2010).
51. Gavin, A. C. *et al.* Proteome survey reveals modularity of the yeast cell machinery. *Nature* **440**, 631–636 (2006).
52. Huh, W. K. *et al.* Global analysis of protein localization in budding yeast. *Nature* **425**, 686–691 (2003).
53. Thiele, C., Hannah, M. J., Fahrenholz, F. & Huttner, W. B. Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. *Nat. Cell Biol.* **2**, 42–49 (2000).
54. Haberkant, P. & Holthuis, J. C. Fat and fabulous: bifunctional lipids in the spotlight. *Biochim. Biophys. Acta* **1841**, 1022–1030 (2014).
55. Hulce, J. J., Cognetta, A. B., Niphakis, M. J., Tully, S. E. & Cravatt, B. F. Proteome-wide mapping of cholesterol-interacting proteins in mammalian cells. *Nat. Methods* **10**, 259–264 (2013).
56. Gubbens, J. *et al.* Photocrosslinking and click chemistry enable the specific detection of proteins interacting with phospholipids at the membrane interface. *Chem. Biol.* **16**, 3–14 (2009).

57. Peng, T. & Hang, H. C. Bifunctional fatty acid chemical reporter for analyzing *S*-palmitoylated membrane protein–protein interactions in mammalian cells. *J. Am. Chem. Soc.* **137**, 556–559 (2015).
58. Subramanian, D. *et al.* Activation of membrane-permeant caged PtdIns(3)P induces endosomal fusion in cells. *Nat. Chem. Biol.* **6**, 324–326 (2010).
59. Hoglinger, D., Nadler, A. & Schultz, C. Caged lipids as tools for investigating cellular signaling. *Biochim. Biophys. Acta* **1841**, 1085–1096 (2014).
60. Niphakis, M. J. *et al.* A global map of lipid-binding proteins and their ligandability in cells. *Cell* **161**, 1668–1680 (2015).
61. Wei, L. *et al.* Live-cell imaging of alkyne-tagged small biomolecules by stimulated Raman scattering. *Nat. Methods* **11**, 410–412 (2014).
62. Folick, A., Min, W. & Wang, M. C. Label-free imaging of lipid dynamics using coherent anti-stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) microscopy. *Curr. Opin. Genet. Dev.* **21**, 585–590 (2011).
63. McLaughlin, S. & Murray, D. Plasma membrane phosphoinositide organization by protein electrostatics. *Nature* **438**, 605–611 (2005).
64. Mouchlis, V. D., Bucher, D., McCammon, J. A. & Dennis, E. A. Membranes serve as allosteric activators of phospholipase A2, enabling it to extract, bind, and hydrolyze phospholipid substrates. *Proc. Natl Acad. Sci. USA* **112**, E516–E525 (2015).
65. Leonard, T. A., Rozycki, B., Saidi, L. F., Hummer, G. & Hurley, J. H. Crystal structure and allosteric activation of protein kinase C βII. *Cell* **144**, 55–66 (2011).
66. Groves, J. T. & Kuriyan, J. Molecular mechanisms in signal transduction at the membrane. *Nat. Struct. Mol. Biol.* **17**, 659–665 (2010).
67. van den Bogaart, G. *et al.* Membrane protein sequestering by ionic protein-lipid interactions. *Nature* **479**, 552–555 (2011).
68. Yang, S. T., Kiessling, V., Simmons, J. A., White, J. M. & Tamm, L. K. HIV gp41-mediated membrane fusion occurs at edges of cholesterol-rich lipid domains. *Nat. Chem. Biol.* **11**, 424–431 (2015).
69. Koberlin, M. S. *et al.* A conserved circular network of coregulated lipids modulates innate immune responses. *Cell* **162**, 170–183 (2015).
70. Carpten, J. D. *et al.* A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature* **448**, 439–444 (2007).
71. Lindhurst, M. J. *et al.* A mosaic activating mutation in *AKT1* associated with the Proteus syndrome. *N. Engl. J. Med.* **365**, 611–619 (2011).
72. Arya, V. B. *et al.* Activating AKT2 mutation: hypoinsulinemic hypoketotic hypoglycemia. *J. Clin. Endocrinol. Metab.* **99**, 391–394 (2014).
73. Yadav, K. K. & Bar-Sagi, D. Allosteric gating of Son of sevenless activity by the histone domain. *Proc. Natl Acad. Sci. USA* **107**, 3436–3440 (2010).
74. Matute, J. D. *et al.* A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40 phox and selective defects in neutrophil NADPH oxidase activity. *Blood* **114**, 3309–3315 (2009).
75. Ling, L. L. *et al.* A new antibiotic kills pathogens without detectable resistance. *Nature* **517**, 455–459 (2015).
76. Anderson, T. M. *et al.* Amphotericin forms an extramembranous and fungicidal sterol sponge. *Nat. Chem. Biol.* **10**, 400–406 (2014).
77. Salomon, D. *et al.* Effectors of animal and plant pathogens use a common domain to bind host phosphoinositides. *Nat. Commun.* **4**, 2973 (2013).
78. Hsu, N. Y. *et al.* Viral reorganization of the secretory pathway generates distinct organelles for RNA replication. *Cell* **141**, 799–811 (2010).
79. Bhattacharjee, S., Stahelin, R. V. & Haldar, K. Host targeting of virulence determinants and phosphoinositides in blood stage malaria parasites. *Trends Parasitol.* **28**, 555–562 (2012).
80. Miao, B. *et al.* Small molecule inhibition of phosphatidylinositol-3,4,5-triphosphate (PIP3) binding to pleckstrin homology domains. *Proc. Natl Acad. Sci. USA* **107**, 20126–20131 (2010).
81. Jo, H. *et al.* Deactivation of Akt by a small molecule inhibitor targeting pleckstrin homology domain and facilitating Akt ubiquitination. *Proc. Natl Acad. Sci. USA* **108**, 6486–6491 (2011).
82. Jesorka, A. & Orwar, O. Liposomes: technologies and analytical applications. *Annu. Rev. Anal. Chem.* **1**, 801–832 (2008).
83. Walde, P., Cosentino, K., Engel, H. & Stano, P. Giant vesicles: preparations and applications. *ChemBioChem* **11**, 848–865 (2010).
84. Horger, K. S., Estes, D. J., Capone, R. & Mayer, M. Films of agarose enable rapid formation of giant liposomes in solutions of physiologic ionic strength. *J. Am. Chem. Soc.* **131**, 1810–1819 (2009).
85. Stachowiak, J. C. *et al.* Unilamellar vesicle formation and encapsulation by microfluidic jetting. *Proc. Natl Acad. Sci. USA* **105**, 4697–4702 (2008).
86. Lemmon, M. A., Ferguson, K. M., O'Brien, R., Sigler, P. B. & Schlessinger, J. Specific and high-affinity binding of inositol phosphates to an isolated pleckstrin homology domain. *Proc. Natl Acad. Sci. USA* **92**, 10472–10476 (1995).
87. Jerabek-Willemsen, M., Wienken, C. J., Braun, D., Baaske, P. & Duhr, S. Molecular interaction studies using microscale thermophoresis. *Assay Drug Dev. Technol.* **9**, 342–353 (2011).

Acknowledgements

The authors thank all members of A.-C.G.'s groups for continuous discussions and support. This work was partially funded by the German Federal Ministry of Education and Research (BMBF; 01GS0865) in the framework of the IG-Cellular System Genomics project and the CellNetworks (Excellence Initiative of the University of Heidelberg) community to A.-C.G. A.-E.S. was supported by the European Molecular Biology Laboratory and the EU Marie Curie Actions Interdisciplinary Postdoctoral cofund Programme.

Competing interests statement

The authors declare competing interests: see Web version for details.

FURTHER INFORMATION

LIPID MAPS Structure Database: http://www.lipidmaps.org

SUPPLEMENTARY INFORMATION

See online article: S1 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF