Published in final edited form as:
Integr Biol (Camb). 2012 March ; 4(3): 247–258. doi:10.1039/c2ib00143h.

Emerging methodologies to investigate lipid–protein interactions

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Abstract

Cellular membranes are composed of hundreds of different lipids, ion channels, receptors and scaffolding complexes that act as signalling and trafficking platforms for processes fundamental to life. Cellular signalling and membrane trafficking are often regulated by peripheral proteins, which reversibly interact with lipid molecules in highly regulated spatial and temporal fashions. In most cases, one or more modular lipid-binding domain(s) mediate recruitment of peripheral proteins to specific cellular membranes. These domains, of which more than 10 have been identified since 1989, harbour structurally selective lipid-binding sites. Traditional *in vitro* and *in vivo* studies have elucidated how these domains coordinate their cognate lipids and thus how the parent proteins associate with membranes. Cellular activities of peripheral proteins and subsequent physiological processes depend upon lipid binding affinities and selectivity. Thus, the development of novel sensitive and quantitative tools is essential in furthering our understanding of the function and regulation of these proteins. As this field expands into new areas such as computational biology, cellular lipid mapping, single molecule imaging, and lipidomics, there is an urgent need to integrate technologies to detail the molecular architecture and mechanisms of lipid signalling. This review surveys emerging cellular and *in vitro* approaches for studying protein–lipid interactions and provides perspective on how integration of methodologies directs the future development of the field.

Introduction

Cellular membranes provide dynamic barriers that anchor receptors, ion channels, carriers, and reversibly bound peripheral proteins.[1] Binding of numerous peripheral proteins to membranes is essential for cell growth, metabolism, response to exogenous signals, and general homeostasis.[2] Dysregulation of lipid binding is associated with various diseases including cancer[3] and metabolic syndromes[4] and thus there is a pressing need to better understand membrane-mediated processes. A detailed understanding of these processes should help identify new targets for therapeutic intervention.

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Biological membranes contain lipids of various size and charge that are organized into a bilayer comprising a hydrophobic core and highly polarized interfacial regions.[5] Membranes of cellular organelles are composed of phospholipids, glycerolipids, sphingolipids, sterols, and other lipid species in varying concentrations. This allows for control of signalling events on spatial and temporal levels through specific protein–lipid interactions. In eukaryotes these interactions are mediated by several conserved lipid-binding domains often found in signal transduction and membrane-trafficking proteins.[6]

Many of these domains have been well characterized for their lipid-binding properties, such as the C1 and C2 domains of Protein Kinase C (PKC), which were discovered in the late 1970's.[7] Despite rigorous biochemical and biophysical analysis of several lipid-binding domains and their host proteins there remains a paucity of predictive lipid-binding data. This necessitates ongoing *in vitro* and *in vivo* screening of specific protein–lipid interactions to discover new lipid-binding proteins. Meanwhile, the mechanisms by which these interactions regulate host-protein activity in disease pathologies are being unravelled. Thus, it is essential to advance our understanding of lipid targeting mechanisms through the use of novel technologies.

The aim of this review is to introduce general principles governing the lipid binding and cellular localization of the most well characterized lipid-binding domains and to discuss how emerging methodologies will shape the coming decade of research in this area.

Lipid binding

Since the discovery of the C1[8] and C2[9] domains, a number of conserved lipid-binding modules have been identified. The modules have been categorized into families and characterized for their lipid binding specificities. The majority of these domains have been found in trafficking proteins, metabolic enzymes, and proteins involved in growth and regulatory pathways. In general, these domains employ specific lipid head-group recognition complimented by additional hydrogen bonding[10] and non-specific electrostatic interactions to reversibly associate with the membrane bilayer. Some lipid-binding domains are involved in additional hydrophobic interactions and partially insert into the membrane interior.[11] At least 12 conserved lipid-binding domains have been identified to date, including C1,[12] C2,[13] PH,[14] FYVE,[14b,15] PX,[14b,15a] ENTH,[16] ANTH,[17] BAR,[18] FERM,[19] PDZ,[20] and TUBBY,[21] (See MeTaDoR: http://proteomics.bioengr.uic.edu/metador/MeTaDoR.html)[22] or The Pawson Lab: http://pawsonlab.mshri.on.ca/index.php?option=com_content&task=view&id=30&Itemid=63 for more information) domains.[23] Recently yeast and mammalian kinase-associated 1 (KA1) have been added to the list of proteins capable of binding to anionic phospholipids *in vitro* and *in vivo*.[24] This discovery brings to light the exciting possibility that there remain yet-to-be-identified lipid-binding modules.[25] Additionally, identification of some lipid-binding domain family members is a challenging task due in part to low sequence homology, particularly the PH[26] and C2[27] domains. To overcome these challenges proteomic methods are being developed to characterize new lipid-binders using mass spectrometry.[28] The integration of proteomics with lipid binding assays will be essential to discovering new lipid-binding modules or identifying subfamilies of previously characterized modules.

Biophysical analysis of lipid-binding

A number of well established biochemical and biophysical experiments have been employed to determine the lipid specificity of proteins. Centrifugal liposome assays[29] and antibody-based "lipid-blots"[30] have been used to rapidly assess selectivity. On the quantitative side, surface plasmon resonance (SPR)[31] remains the gold standard for measuring protein affinity for lipids and has been used successfully in determining the K_d values for interactions of

C2,[32] ENTH,[33] FYVE,[34] PH,[35] and PX[36] domains with lipid vesicles. Additionally, this technique has provided a quantitative assessment of lipid-binding proteins such as Protein Kinases C[37] and cPLA$_2\alpha$.[37b,38] However, it can be difficult to obtain reliable and reproducible binding data using SPR due to nonspecific binding to the sensor chip, mass transport effects, and protein stability.[39] Thus, it is essential to carefully optimize conditions to produce reliable SPR measurements.[40] Isothermal titration calorimetry using soluble lipid headgroup species has also been utilized, albeit less extensively.[41] To determine the ability of peripheral membrane proteins to insert into the hydrophobic core of the membrane the monolayer penetration technique[42] has been employed.[34b] Additionally, the depth of penetration and protein orientation at the membrane interface have been determined by electron paramagnetic resonance spectroscopy.[43] Taken together, biophysical assays allow for the thorough characterization of lipid binding specificities and mechanisms of membrane association of peripheral proteins. However, the inability to investigate lipid binding in a high-throughput fashion limits progress toward rapid identification of new lipid-binding proteins. A significant step in this direction was the synthesis of chemically modifiable phosphoinositide (PI) probes that can be used in high-throughput screening.[44] Chemically modifiable lipids hold much promise for proteomic studies in which they can be cross-linked to high affinity targets from cell lysates for protein identification.[45]

Cellular localization

Regulation of the spatial and temporal cellular distribution of lipid-binding domains has become an area of intense study in the past decade. The discovery that the lipid composition of cellular membranes is highly dynamic has aided in understanding the cellular localization and function of peripheral proteins. In addition, temporal modulation of lipids such as PIs and diacylglycerol (DAG) in response to an assortment of signals can dramatically regulate protein–lipid binding.[14b,23a] Targeting of proteins to a particular organelle can also be regulated by requiring a specific degree of membrane curvature for proper interaction. This property of the BAR domain-containing proteins[18c,46] was shown to be essential for endocytosis and recycling of plasma membrane proteins. Membrane targeting can be further mediated by secondary factors such as cytoplasmic calcium, which activates cPLA$_2\alpha$ in inflammatory processes[47] or by pH. For example, the PI(3)P-binding pocket of the FYVE domain contains conserved histidine residues, protonation of which at a lower pH is necessary for association with early endosomes.[48] Furthermore, the pH dependence is not limited to amino acids as protonation of the headgroup of the phosphomonoester phosphatidic acid (PA) is also pH dependent and can regulate effector proteins such as Opi1 in yeast.[49]

Coincidence detection

Finally, the requirement of two distinct lipid-binding sites within the C2, PH, and PX domains has been demonstrated for robust membrane localization of several proteins. This mode of dual lipid recognition termed “coincidence detection” is seen in the C2 domain of PKCα that associates with PS[32,50] and PIs,[35d] Akt1 and PDK-1 PH domains that bind to PS[51] and PI(3,4,5)P$_3$,[52] and the p47^{phox} PX domain that recognises PI(3,4)P$_2$ and PA[36a] (Fig. 1). Several PH domains exhibit coincidence detection through a protein–protein interaction site in addition to a protein–lipid interaction site. Four-phosphate adaptor protein 1 (FAPP1)[53] and OSBP[54] bind PI(4)P and small GTPases in the Golgi membrane (Fig. 1). As we discuss below, it is possible that some protein–lipid interactions require additional conditions that should be taken into consideration when investigating these specific interactions. For instance, sphingolipids such as ceramide,[55] ceramide-1-phosphate (C1P),[56] and sphingosine-1-phosphate(S1P)[57] have recently been identified to target a number of effector proteins. Because these lipids are often overlooked during screening of lipid-binding proteins there may be a number of unidentified receptors for these sphingolipids. In

general, lipid binding screens in search of cognate ligand usually employ anionic lipids such as PIPs, PS and PA, which may limit discovery of new lipid binding properties of peripheral proteins. A prime example of such is C1P,[58] which has been shown to associate with a cationic patch in the β-groove[13a] of the cPLA$_2$α C2 domain.[38] This is an example of coincidence detection where the C2 domain detects a glycerophospholipid (PC) through the hydrophobic loop regions[11,59] and a sphingolipid (C1P) through the adjacent cationic patch.[38] It is important to note the C1P binding properties of the C2 domain were discovered several years after the association with PC had been accepted as the main mode of lipid-binding. This underscores the importance of considering sphingolipid binding to well-known lipid-binding modules.

Lipid-dependent drug targets

Association with specific membranes is required for the proper biological function of various enzymes and non-catalytic components of cellular signalling. Dysregulation of these signalling pathways can lead to adverse consequences manifested in metabolic disorders and cancers.[60] For instance, an oncogenic E17K mutation in the PH domain of Akt1 has been found in human cancer tissue.[3a] This mutation appears to abolish specificity for PI(3,4,5)P_3 and promotes constitutive association of Akt1 with the PI(4,5)P_2-enriched plasma membrane.[61] Additionally, mutations in the PH domain of Dynamin that compromise GTPase activity result in Centronuclear Myopathy.[62] Clearly, peripheral proteins are potent drug targets for which therapeutic intervention at the level of protein–lipid binding can be invaluable.[63] Recently, seminal studies have demonstrated the feasibility of such an approach in which small molecules that inhibit lipid binding of C2[64] and PH domains[65] were generated and tested. Progress has been made in understanding the role of protein–lipid interactions in cellular processes such as *endo-* and exocytosis, cellular lipid trafficking and disease pathologies. However, there remains a great need for more detailed structural, biophysical and system-wide lipidomic analyses. Computational biology, theoretical and "omic" studies have already begun to play a leading role in the field and should be bolstered by a wealth of information gained in the next decade. In the following sections we evaluate recent technological advances in probing protein–lipid interactions.

Structural biology

The three dimensional structures of lipid-bound proteins provide invaluable information regarding the molecular mechanisms underlying the biological activities of these proteins. In the last three decades the atomic-resolution crystal and solution structures of various water-soluble domains have been determined; however, structural details of membrane-associated modules are challenging to obtain.[66] A number of relatively soluble PI-recognizing domains have been studied using conventional X-ray crystallographic and NMR spectroscopic approaches.[14b,23a] The mechanistic basis of the association with membranes has been elucidated from the structures of these domains in complex with lipid head groups or intact lipids containing short acyl chains. However, biochemical and structural characterization of less soluble peripheral membrane proteins requires additional manipulation and often necessitates the use of detergents during purification. In addition, the lipid system in which the protein can be reconstituted in a form that retains its biological function must also be identified and optimised.

Cryomicroscopy (CryoEM) techniques, i.e. electron crystallography of two-dimensional crystals and single particle analysis of detergent-solubilized protein complexes, provide an alternative method of structural characterization.[67] As with many other structural tools, both crystallographic approaches have limitations. The major concern is the crowding of protein molecules, particularly in two dimensional crystals, and a limited amount of lipids present and/or bound to the protein. Therefore, additional analysis is always necessary to confirm

the lipid binding sites and the relative orientation of the protein molecules. Nevertheless, despite the common view of X-ray crystallography as a technique that produces a static scaffold, it provides the most desirable insight into protein–ligand interactions and allows for determining the structures of large proteins and complexes. Recent notable advances in obtaining well-diffracting crystals of membrane proteins for X-ray crystallographic analysis and the electron crystallography structure of aquaporin determined at 1.9 Å resolution[68] reveal the great potential of these approaches in studying lipid-bound proteins.

NMR spectroscopy has developed as a powerful tool to determine the three-dimensional structures of membrane-associated proteins and to investigate protein dynamics and lipid binding properties.[66a,69] One of the major requirements, however, is that an NMR sample must contain large amounts of soluble protein and interacting lipid. This can be achieved by using water-soluble short chain lipids in the case where the protein is soluble, or alternatively a membrane mimetic system that solubilizes the hydrophobic protein and the lipid in an aqueous environment. A number of membrane mimetics have been developed for this purpose, including detergent-based and lipid-based spherical micelles, disk-like bicelles, amphipols and organic solvents.[70] The lipid-binding site can be identified from intermolecular nuclear Overhauser enhancements (NOEs) observed between the protein and lipid protons or through resonance perturbations occurred in the protein upon binding of the lipid. The ^{1}H, ^{15}N heteronuclear single quantum coherence (HSQC) technique is particularly useful in monitoring chemical shift changes in the ^{15}N-labeled protein as an unlabeled lipid is titrated in.[35b,c,71] These experiments not only allow for identification of residues that are most likely involved in the interaction and/or accompanying conformational changes but also provide an effective method for examining the stability and folding of the protein and assessing the strength of the interaction. In addition, the internal motions can be investigated through measuring relaxation times T_1 and T_2 and heteronuclear NOEs.[72] The depth of penetration and topology of the peripheral proteins can be measured by adding lipophilic or soluble paramagnetic probes and spin-labelled lipids.[73]

The size of a protein or a protein–lipid complex remains a major limitation of solution NMR, and it becomes increasingly difficult to obtain the atomic-resolution structure of a macromolecule of over 30–40 kDa. Another concern is that micelles and bicelles represent modest mimetics of lipid bilayers because of pronounced curvature and different surface tension. Therefore, data produced in these systems need to be thoroughly validated by lipid binding assays such as SPR or vesicle sedimentation assays. Furthermore, formation of a tight complex between the protein and membrane-mimetic particle significantly increases the effective size, which can easily reach the limit for NMR structure determination (for example, the size of commonly used dodecylphosphocholine (DPC) micelles is ~20 kDa). This results in slower tumbling and broadening of NMR resonances beyond detection. Nevertheless, remarkable breakthroughs recently seen in the application of NMR spectroscopy to structural analysis of biological macromolecules offer new tools and strategies that could be applicable in probing membrane proteins. These include measurements of residual dipolar couplings (RDCs) in bicelles, polyacrylamide gels and other aligned media, aiding in a more accurate structure refinement and assessment of relative orientation of multiple linked domains.[74] Paramagnetic relaxation enhancement (PRE) of NMR relaxation rates have been used as another long-range restraint.[74b,75] In these experiments, a paramagnetic spin-label is attached to the thiol group of a cysteine residue leading to distant-dependent line broadening of NMR resonances of the protein. Additionally, development of transverse relaxation optimized spectroscopy (TROSY)-based pulse sequences, deuteration of proteins, application of high dimensionality experiments and advanced labelling strategies allow for determination of structures of macromolecules as large as 100 kDa.[75b,76] The use of cryoprobes and ultra-high field solution NMR spectrometers as well as recent innovations of solid state NMR should significantly increase

sensitivity and resolution and further broaden the application of NMR to the structural characterization of lipid-binding proteins.

Computational biology

While significant progress has been made in characterizing protein–lipid interactions experimentally a number of limiting factors remain. Generally membrane proteins are difficult to express in bacterial systems or to obtain in a soluble form in amounts sufficient for biochemical experiments. On the other hand, design and expression of fluorescently labelled constructs *in vivo* is laborious and concerns remain regarding the physiological relevance of the data obtained with over-expressed probes. Some of these obstacles can be overcome using *in silico* approaches. Computational biology was first used as a tool to predict structures of membrane proteins based on previously determined structures[77] or to assess lipid binding properties as was shown for the PH and FYVE domains.[78] Additionally, electrostatic potential calculations complemented by an array of biophysical lipid-binding assays helped to elucidate the PI-induced docking mechanisms of the ENTH, FYVE, and PX domains.[33a,34b,36b]

Molecular dynamics

Molecular dynamics (MD) simulations have shed light on the molecular mechanisms of membrane anchoring. *In silico* studies of the FYVE, PH and PX domains have demonstrated how non-specific interactions of the protein residues with the hydrophobic core of the bilayers enhance binding to PI-containing membranes.[79] MD simulations of the C2 domain of PKCα combined with experimental lipid-binding studies, cell translocation assays, and mutagenesis suggested that the Ca^{2+}-induced binding to PS is augmented by $PI(4,5)P_2$ due to the increased membrane residence time of the domain.[35d] Subsequent biochemical studies coupled with MD simulations revealed the $PI(4,5)P_2$ stoichiometry.[80]

MD simulations have also contributed to a better understanding of the mechanisms underlying the protein-induced vesicle tubulation that has been observed experimentally with some lipid-binding domains. Studies have shown how amphyphysin N-BAR domains can induce local curvature upon association with a membrane, and do so with greater affinity when negatively-charged $PI(4,5)P_2$ is added to the membrane.[81] It has also been shown that when amphipathic α-helices adjacent to the N-BAR domain are removed this strong membrane association is lost. Interestingly, high concentrations of the amphipathic α-helices alone can drive membrane curvature independent of the BAR domain at higher concentrations.[82] MD studies have uncovered how the complementarity of N-BAR concentration, oligomerization, and membrane composition affect the level and type of curvature, tubulation, or vesiculation that can occur in different lipid models.[81b]

Bioinformatics

In addition to *in silico* modelling techniques, recently developed databases, algorithms, and statistical methods have broadened the toolkit for analysis of protein–lipid interactions. Computational approaches including sequence alignments and charge mapping also serve as an important platform to design cellular and biochemical assays. For instance, a recursive-learning algorithm was used to identify $PI(3,4,5)P_3$-specific PH domains, and these findings were confirmed experimentally through observation of the translocation of fluorescently labelled proteins to the plasma membrane upon $PI(3,4,5)P_3$ stimulation.[83] Recently, a new combinatorial computational approach has been applied in a genome-wide scale identification of ANTH domains.[84] This study not only defined the ANTH domain family but also annotated its members based upon structural features and biophysical properties

including membrane deforming activity. Additionally, a machine-learning algorithm has been used to predict lipid-binding abilities of a subset of C2 domains.[85]

Integration of computational work with genomic, proteomic, and lipidomic studies will be essential to rapidly identify novel lipid-binding proteins and may reveal new mechanisms of lipid recognition. Furthermore, linking identification of new lipid binding proteins[86] with lipid metabolic pathways[87] could uncover additional roles of lipid-binding proteins in health and disease. However, computational biology still has limitations as demonstrated by association of peripheral proteins with PA. Despite the experimental characterization of more than 22 PA binding proteins[88] bioinformatic approaches have not been able to identify a common motif or protein module that is able to specifically recognize PA.

Cellular imaging

Imaging of fluorescently labelled proteins including the C2, FYVE, PH, and PX domains have been used to estimate the subcellular distribution of PS, PI(3)P, PI(4)P, PI(4,5)P_2 and PI(3,4,5)P_3. (See Fig. 2).[89] However, there are concerns regarding the ability of these lipid-binding probes to accurately reach all organelles. These concerns were recently highlighted in a study where cellular PS distribution was assessed using confocal and electron microscopy. Electron microscopy detected PS which was not visible with a fluorescent C2 domain probe in subcellular compartments such as caveolae and the inner mitochondrial membranes.[90] Similar rigorous analysis will be essential for mapping the compartmentalization and distribution of other signalling lipids. In addition to conventional lipid-binding probes, unique sensors have been developed to estimate lipid concentrations. A C1 domain DAG sensor has been constructed, which undergoes a change in FRET upon binding DAG while an ENTH domain with an environmentally sensitive fluorophore that detects PI(4,5)P_2 was engineered.[91] The new strategy applied to the ENTH domain allows for quantification of PI(4,5)P_2 in different cellular compartments under physiological conditions. This sensor methodology should also be applicable to other specific lipid-binding domains to quantify the native state of lipids such as PI(3)P, PI(4)P and PI(3,4,5)P_3 in cells. Although monitoring dynamics of some lipids with fluorescently tagged lipid-binding domains has become more or less routine it is important to note that a significant gap remains for sensing glycerophospholipids and sphingolipids due to a lack of corresponding lipid-binding probes.

Tools to manipulate cellular lipid compositions such as reagents to deplete plasma membrane PI(4,5)P_2[92] allow for analysis of lipid-mediated signalling pathways. These types of assays are agonist-inducible where binding of a drug triggers association of cytosolic bait (the enzyme) with a protein receptor in a target membrane. Recently, this strategy has been applied to monitor the modulation of PI(4)P levels in the Golgi.[93] Such an approach holds promise for engineering targeted enzymes essential in sphingolipid metabolism, which is an underexplored area of lipid biology. Despite the progress in monitoring lipid dynamics, there remains a concern that the lipid probes act as dominant negative effectors when over-expressed. This overexpression may block downstream signalling events due to sequestration of target lipids. Small molecule sensors or chemically modified lipid-binding reporters that can sense lipids with subnanomolar affinities,[94] would alleviate these issues. This is admittedly a difficult problem but a step in the right direction has been the development of small fluorescent molecules that can recognize PS exposed on apoptotic cells[95] as well as small molecules that act as PI-binding mimetics of PH domains.[96] Lipid biosensors that could easily enter cells and readily map available pools of different lipids would be a great boon to the lipid community.

Single molecule imaging

Single molecule imaging enables highly sensitive detection of transient interactions between labelled proteins and lipid vesicles or cellular membranes.[97] One such example is total internal reflection fluorescence (TIRF) imaging, which has become a useful tool for measuring protein–lipid interactions with giant unilamellar vesicles or at the plasma membrane of live cells.[97,98] TIRF generates an evanescent wave focused approximately in the 100 nm between the cover slip and the sample; exciting fluorophores near the plasma membrane or vesicle-media interface. This technique significantly eliminates background noise from other fluorophores to allow accurate interpretation of single molecule binding events.[99] TIRF has been used to characterize the membrane binding affinity of peptides[97a] as well as the kinetics of PH domain association with $PI(3,4,5)P_3$-containing membranes.[99] Because TIRF can be combined with single molecule detection and analysis methods it is possible to monitor absolute protein concentrations with high sensitivity per unit area of membrane. TIRF is also robust when integrated with software that measures the number and brightness of the fluorescent molecules. This allows properties such as protein oligomerization to be observed in real time. However, the bleaching of fluorophores can complicate interpretation of TIRF results, as labelled proteins may be bound and photobleached simultaneously. To minimize this scenario, fluorophores with high quantum yield are often used and the laser power is carefully controlled.[97a] This technique has proved useful in monitoring the kinetics of assembly and egress of fluorescently labelled HIV-1,[100] which binds $PI(4,5)P_2$ and PS in the cytoplasmic leaflet of the plasma membrane.[101]

Lipidomics

Lipidomics has emerged as a platform to integrate system-wide cellular information. Enhanced extraction and separation protocols, improved analysis software, and the availability of integrated databases have led to a wealth of new information on cellular lipid compositions.[102] It is our hope that technological advances will help to elucidate the composition of cellular organelles and enable comparision of lipids between healthy and diseased state models. One can envision an integrated approach to build a cellular map of lipid distribution using lipidomics combined with lipid-binding probe studies that can resolve lipid compartmentalization.[90] The availability of lipidomic data will enhance our understanding of lipid–protein interactions and the biophysical characteristics of cellular membranes required to recruit effector proteins. While the basics of formation of lipid domains are understood to some extent, recent data demonstrate that lipid domains are heterogeneous in nature and can differentially attract specific lipid-binding proteins.[103] Robust analysis of lipid domain formation and composition of cell membranes will be indispensable for studying signalling platforms and trafficking pathways.

Lipidomics and disease

Several diseases are known to be linked to lipid imbalances including Alzheimer's,[104] diabetes,[105] atherosclerosis,[106] and lipid storage disorders.[107] Lipid metabolic changes can rapidly alter peripheral protein localization and activity, thus information regarding the temporal and spatial composition of organelle lipids in healthy and pathological states is invaluable. Lipidomic analysis can detect small but physiologically significant changes of signalling lipid concentrations and lead to a targeted search for enzymes responsible for these fluctuations. In addition, lipidomic studies of bacterial and viral pathogens can provide metabolic profiles of infection and replication of these organisms in human hosts.[108] In the last year lipidomic analysis of diseased tissue has emerged with the analysis of mitochondrial cardiolipins linked to diet and disease,[109] oxidative lipidomic analysis in traumatic brain injury,[110] analysis of human and mouse brain with Alzheimer's disease,[111] and eicosanoid release in inflamed synovial joints.[112] Taken together, these seminal studies

provide a template to build targeted approaches for specific lipid analysis of pathological conditions.

Lipidomics and systems biology

With the 'omics' revolution have come efforts to identify lipid-binding domains in a system-wide fashion. Using PI(3,4,5)P_3-coated beads and mass spectrometry, two new PI(3,4,5)P_3-binding PH domains were recently discovered.[28] Additionally, novel lipid-binding patterns were identified in yeast by a custom-made lipid array containing 56 lipid metabolites. Using this approach, 530 lipid–protein associations were detected, most of which were novel.[87] These examples illustrate significant progress in a field filled with challenges such as organelle separations, resolution, bilayer asymmetry, and lipid metabolism during purification. Additionally, three main databases have been assembled to collect lipidomic information including the Lipid Metabolite and Pathways Strategy (LIPID MAPS, http://www.lipidmaps.org/), METLIN (Metabolite and Tandem MS Database, http://metlin.scripps.edu/) and the Human Metabolome Database (http://www.hmdb.ca/). Integrating information through databases and cross-disciplinary research to rapidly discover druggable targets will be essential to moving the field forward. This is well exemplified in a study that mined the ovarian cancer literature integrating various lipid studies to identify potential drug targets.[113] Once again, this emphasizes the importance of cross-disciplinary research to expedite drug discovery.

Conclusion and perspectives

The lipid-binding field has undergone a technological revolution in the last decade and is well primed with available and evolving methodologies to fill in the aforementioned gaps. The implication of lipid–protein interactions in diverse disorders will continue to prompt intense investigation of specific protein targets. Lipidomics and bioinformatics should enable researchers to evaluate protein–lipid interactions in relation to cellular lipid profiles and irregularities in disease states. System-wide studies will unveil new lipid-binding proteins and uncover unknown intricacies of those already under study. Researchers are encouraged to partake in cross-disciplinary studies that will surely underlie future breakthroughs in the field.

Acknowledgments

Research in the Stahelin lab is supported by grants from the NIH (AI081077) and the NSF (1122068) and research in the Kutateladze lab is supported by the NIH (GM096863 and CA113472) and the AHA (10GRNT2600175) grants. J.L.S. is supported by a CBBI NIH training fellowship (T32GM075762), E.A.G. is supported by a Notre Dame Eck Institute for Global Health graduate training fellowship and C.A.M. is an NIH NRSA postdoctoral fellow (F32HL096399).

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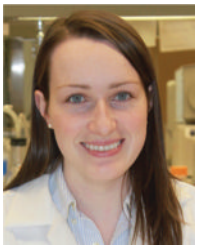

Jordan L. Scott received her BS in Biology from Calvin College where she studied the activation of the glucose transporter GLUT1 with Professor Larry Louters. She is currently a third year PhD student in the Stahelin lab in the Department of Chemistry and Biochemistry at the University of Notre Dame. At Notre Dame, she has focused her molecular mechanistic studies on the PI4P-binding protein FAPP1 and other phosphoinositide effectors.

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Insight, innovation, integration

In the last two decades significant progress has been made towards understanding the molecular mechanisms by which lipid-binding proteins are recruited to membranes. While investigating the most common biochemical properties of individual lipid-binding domains has become more or less routine, studies of intact proteins within membranes can be limited by the available technology. Lipid-binding proteins have been implicated in a number of diseases so as this field expands there is an urgent need for integration of data from newly emerging technologies into quantitative databases that will direct translational studies. This article presents emerging technologies in the field and provides perspectives on how these methodologies will address critical questions regarding cellular lipid-binding events.

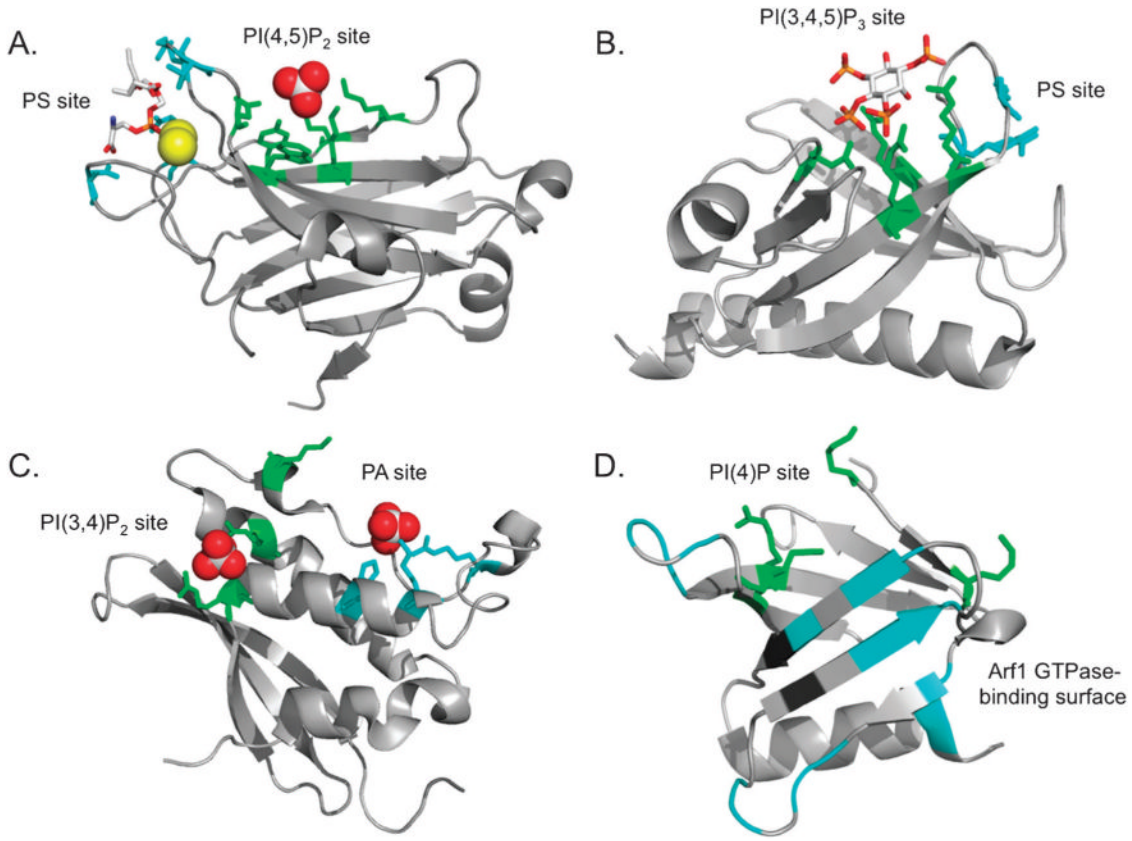

Fig. 1.

Lipid-binding domains termed “coincidence detectors” harbour two distinct binding sites. Residues involved in lipid-head group coordination or protein binding are highlighted. (A) Protein Kinase Cα (PKCα) C2 domain bound to phosphatidylserine (PS) (red and gray sticks) and two calcium ions (yellow) with PS-coordinating residues shown in cyan (Asn-189, Arg-216, Arg-249, and Thr-251). The PtdIns(4,5)P_2 headgroup is coordinated by Tyr-195, Lys-197, Lys-209, Lys-211, Trp-245 and Asn-253 shown in green. The sulfate group (red and gray) was bound in the inositol-binding site (Protein Data Bank (PDB) Code 1DSP). (B) The structure of the Akt1 PH domain (PDB 1UNQ) was determined in complex with the PI(3,4,5)P_3 headgroup, Ins(1,3,4,5)P_4 (red and gray). PI(3,4,5)P_3 is bound by Arg-23, Arg-25, Asn-52, and Arg-86 shown in green. Akt1-PH additionally recognizes PS through Arg-15 and Lys-20 (cyan) adjacent to the PI(3,4,5)P_3 pocket. (C) The p47^{phox} PX domain structure was obtained with two sulfate molecules bound in each lipid-binding site (PDB 1O7K). This domain coordinates PA by His-51, Lys-55, and Arg-70 (cyan), and PI(3,4)P_2 by Arg-43, Lys-79, and Arg-90 (green). (D) FAPP1-PH coordinates the PI(4)P headgroup at the bottom of the β-barrel with Lys-7, Arg-18, Lys-41 and Lys-45 (green) and co-localizes with Arf1 GTPase at the Golgi membrane (PDB 3RCP). The Arf1 binding surface, as determined by NMR resonance perturbation analysis, includes the β1–β2 and β5–β6 loops as well as the β5,6 and 7 strands (cyan).

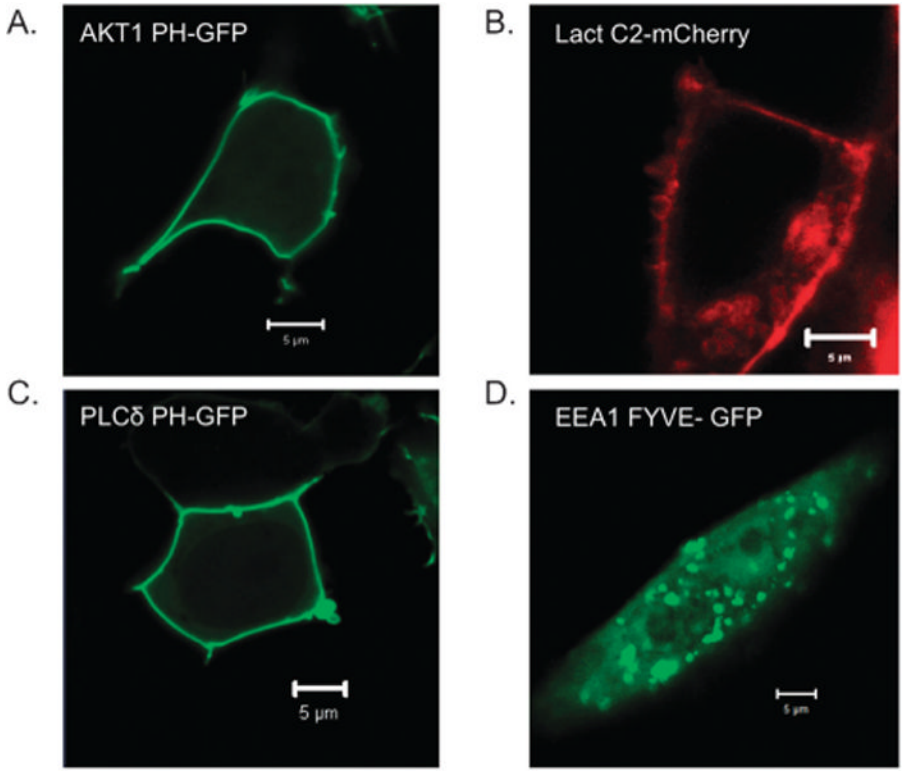

Fig. 2.

GFP- and mCherry-labelled lipid-binding domains serve as markers of cellular lipids. (A) Akt1 PH-GFP localizes to the plasma membrane in response to $PI(3,4)P_2$ or $PI(3,4,5)P_3$, (B) Lactadherin C2-mCherry marks PS-enriched membranes including the cytoplasmic leaflet of the plasma membrane, (C) PLCδ PH-GFP binds $PI(4,5)P_2$ most prominently in the plasma membrane and (D) EEA1 FYVE-GFP localizes to PI(3)P-containing early endosomes.