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Computational Approaches to Investigate and Design Lipid-binding Domains for Membrane Biosensing

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Abstract: Association of proteins with cellular membranes is critical for signaling and membrane trafficking processes. Many peripheral lipid-binding domains have been identified in the last few decades and have been investigated for their specific lipid-sensing properties using traditional *in vivo* and *in vitro* studies. However, several knowledge gaps remain owing to intrinsic limitations of these methodologies. Thus, novel approaches are necessary to further our understanding in lipid–protein biology. This review briefly discusses lipid-binding domains that act as specific lipid biosensors and provides a broad perspective on the computational approaches such as molecular dynamics (MD) simulations and machine learning (ML)-based techniques that can be used to study protein–membrane interactions. We also highlight the need for *de novo* design of proteins that elicit specific lipid-binding properties.

Keywords: *de novo* Design · Lipid-binding domains · Lipid-sensing · MD simulations

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Introduction

Lipid bilayers are functional barriers that compartmentalize cell organelles and define boundaries between the internal constituents of a cell and its external environment. In addition to serving as structural components of membranes, lipids also act as reservoirs for energy storage[1] and as messengers in signal transduction and molecular recognition processes.[2] Membranes of cellular organelles are typically composed of fatty acids, glycerolipids, phospholipids, sphingolipids, sterols, and other lipid species in different concentrations. The chemical architecture of most lipids is characterized by a distinct polar headgroup (*e.g.* phosphocholine, phosphoethanolamine, phosphoinositide, phosphoserine), one or more acyl chains of different lengths, that could be saturated, mono-, or poly-unsaturated, and a backbone (such as: glycerol for glycerolipids and glycerophospholipids, sphingosine for sphingolipids).[3] Ongoing lipidome-cataloguing efforts indicate that, as a consequence of the combinatorial possibilities of different head groups, backbones, acyl chain lengths, and chain saturations, there are more than 46000 different lipid types in total.[4] The lipid composition varies not only between different organelles of the cell, but also between leaflets of the same bilayer as well as within the same leaflet.[5]

Several intriguing questions have entranced lipid biology researchers over the last few decades – Why does the cell invest a substantial amount of resources in producing and maintaining this large repertoire of lipid species? What is the evolutionary significance of this heterogeneity? Where are each of these lipids produced? By what enzymatic reactions? Where are they redistributed to? And by what mechanism?

Hence, it is no surprise that several experimental techniques have also been developed[6] in the last few decades in the attempt to solve these long-standing challenges. Fluorescent lipid-tagging is one such widely used method to study the localization and movement of lipids within the cell. In this approach, a fluorophore is conjugated to either the lipid head group (example - Rhodamine-labelled PE[7]) or to the hydrophobic tail (as in the case of BODIPY-cholesterol[8]). However, some drawbacks of this method are that the labelled lipid could have different physicochemical properties, thus resulting in different membrane characteristics. For example, they could follow different metabolic routes compared to that of their endogenous counterparts, and they could potentially metabolize into species that cannot be distinguished *via* fluorescence or that cannot be transported by the same non-vesicular pathways. Another approach to probe lipids is the use of antibodies,[9,10] such as anti-LysoBisPhosphatidic Acid

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(LBPA).[11] A key disadvantage of this method is that their visualization requires cells to be fixed. However, lipids do not fix very well and remain mobile, which could result in false deductions. A third strategy to determine cellular distribution of lipids capitalizes on proteins that possess specific lipid-binding domains, such as the pleckstrin homology (PH) or C2 domains. Two main advantages of this method are that it allows visualization of lipids in live cells and that it has minimal effects on altering lipid distribution and metabolism.

In this review, we focus on the use of peripheral membrane proteins that contain lipid-binding domains as lipid sensors. We summarize the different lipid-binding domains in the cell that are used to probe cellular localization and function of lipids, the experimental approaches in place, their caveats, and the *in silico* techniques such as molecular dynamics (MD) simulations, machine learning (ML), and *de novo* protein design, that can help fill the gaps.

Lipid-binding Domains as Biosensors

Lipid-binding domains are extensively used as genetically encoded lipid biosensors for visualizing lipids in cells.[12,13] They are typically expressed as fusions with fluorescent proteins such as GFP or mCherry and can be visualized *via* fluorescence microscopy. While some domains are highly stereospecific and have distinct lipid-binding partners, as shown in Table 1, some others are non-specific and sense general physical properties of the membrane such as charge,[14] curvature[15] or lipid-packing defects.[16] Fig. 1 illustrates the diversity of lipid-binding folds for some representative lipid-binding proteins.

A closer look at Table 1 reveals that while acidic phospholipids, such as PA and many PIPs, have several corresponding lipid-binding domains, many lipids (such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), or ceramides) have few or no corresponding proteins that can bind with suitable affinity. While assays such as the LiMA[49]

Table 1. A non-exhaustive list of lipid-binding domains commonly used to visualize lipids and their localization.

Lipid	Protein-Domain/ Motif	Cellular Localization	Reference
Cholesterol	Perfringolysin-O D4+ its mutants	PM	[17]
	WCR-eOsh4	Inner PM	[18]
DAG	PKCe-C1	ER, NE	[19]
PA	Opi1p-PABD	ER, PM	[20]
	Sos1-PH	PM	[21]
	Spo20p-PABD+PASS[a]	ER, PM	[22]
$PI(3,4,5)P_3$	Akt-PH[a]	PM	[23]
	ARNO-PH[a]	PM	[24]
	Btk-PH[a]	PM	[25]
	Cytohesin1-PH	PM	[24]
	GRP1-PH[a]	PM	[23,24,26]
$PI(3,4)P_2$	TAPP1-PH	PM	[27]
$PI(3,5)P_2$	Cti6	Yeast nucleus	[28]
	Ent3p-ENTH[a]	Yeast endosomes	[29]
$PI(4,5)P_2$	AP180-ENTH[a]	PM	[30]
	PLCd1-PH[a]	PM	[31]
	PLCd4-PH[a]	PM	[32]
	Tubby domain[a]	PM	[33]
PI3P	EEA1-FYVE	EE	[34]
	Hrs-FYVE	EE	[34]
	p40phox-PX	EE	[35]
PI4P	FAPP1-PH[a]	Golgi	[36,37]
	OSBP1-PH	Golgi	[38]
	Osh2-PH[a]	PM	[39]
	SidM-P4M	Golgi, PM, endosomes	[40]
	SidC-P4C	Golgi, PM, endosomes	[41,42]
PI5P	ING2-PHD	PM, nucleus	[43]
PS	Lactadherin-C2[a]	PM, endosomes	[44]
SM	Lysenin	PM	[45]
	Equinatoxin II-SM	Golgi, PM	[46]
	OlyAb	PM	[47]

[a]Also binds selectively to other lipids; [b]Detects sphingomyelin complexed with cholesterol; Abbreviations used: DAG, diacylglycerol; PA, phosphatidic acid; $PI(3,4,5)P_3$, phosphatidylinositol 3,4,5- trisphosphate; $PI(3,4)P_2$, phosphatidylinositol 3,4-bisphosphate; $PI(3,5)P_2$, phosphatidylinositol 3,5-bisphosphate; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; PI3P, phosphatidylinositol 3-phosphate; PI4P, phosphatidylinositol 4-phosphate; PI5P, phosphatidylinositol 5-phosphate; PS, phosphatidylserine; SM, sphingomyelin; EE, early endosome; ER, endoplasmic reticulum; NE, nuclear envelope; PM, plasma membrane.

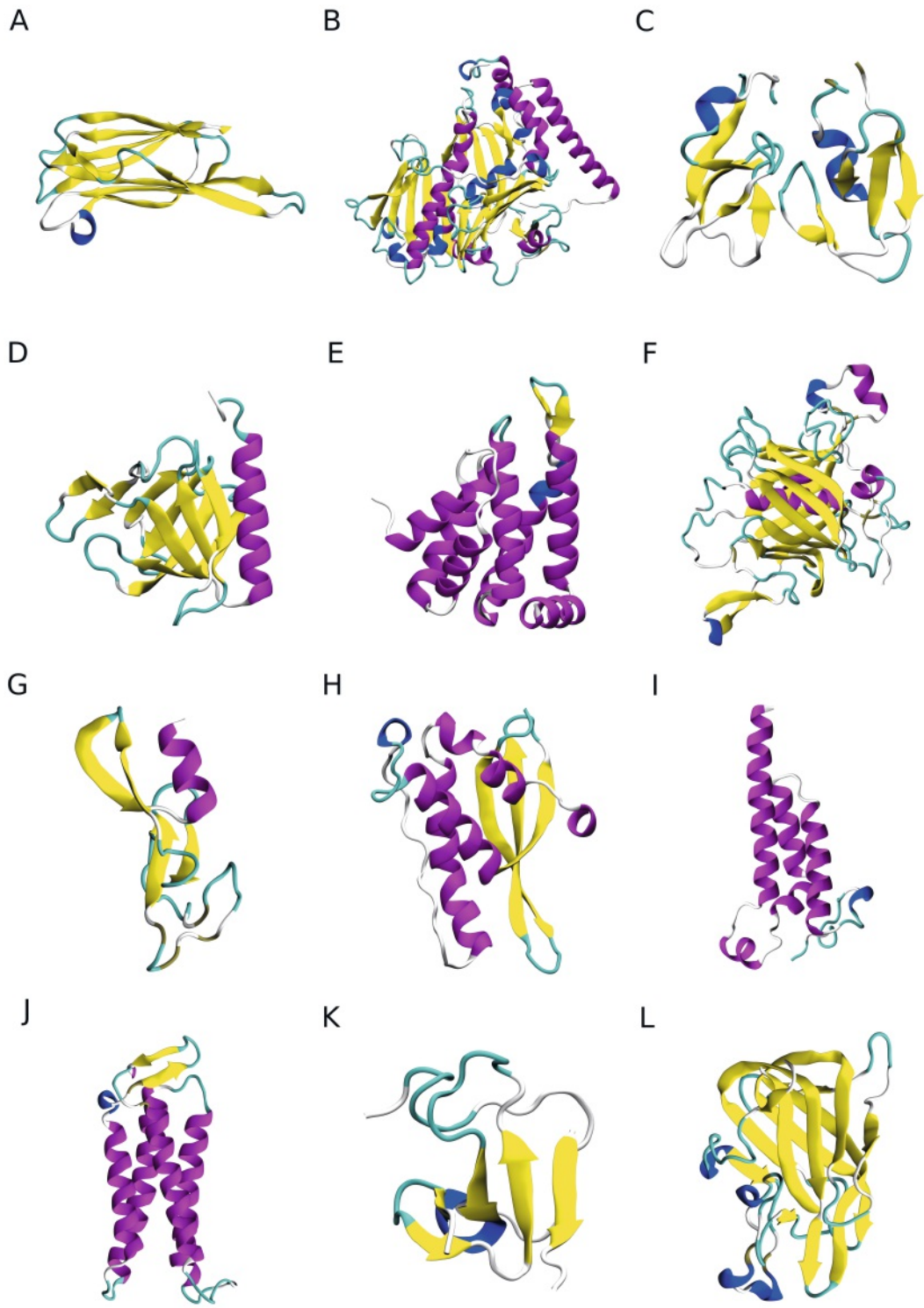

Fig. 1. Secondary structures of some lipid-binding domains, indicating the diversity of their folds. (A) Domain-4 of Perfringolysin-O (PDB ID: 1M31) (B) OSBP domain of yeast Osh4 (PDB ID: 1ZHX) (C) C1A and C1B domains of PKCe (structure from AlphaFold) (D) PH domain of GRP1 (PDB ID: 1FGZ) (E) ENTH domain of Ent3p (PDB ID: 3ONK) (F) Tubby domain (structure from AlphaFold) (G) FYVE domain of EEA1 (PDB ID: 1HYI) (H) PX domain of p40phox (PDB ID: 1H6H) (I) P4M domain of SidM (PDB ID: 4MXP) (J) P4C domain of SidC (PDB ID: 4ZUZ) (K) PHD domain of ING2 (PDB ID: 2G6Q) (L) C2 domain of Lactadherin (PDB ID: 3BN6). Alpha helices and beta-strands are colored violet and yellow respectively. Protein images were rendered using VMD.[48]

(Liposome-Microarray-based Array) that screen and quantify protein–membrane binding *in vitro*, can potentially also be used to identify novel specific probes for lipids, progress remains to be made on this front.

A second drawback is that many of these proteins could act as coincidence detectors, *i.e.* they could bind to additional lipids or other factors at a second binding site, thus biasing their localization. For example, the PH domains of OSBP, FAPP1, and yeast Osh2, all have a lipid-binding site and can serve as a PI4P probe.[50] Yet, the PH domains of OSBP and FAPP1 localize to the Golgi apparatus since they also bind to Arf1 GTPase,[37] while that of the Osh2 exhibits strong plasma membrane localization due to its interaction with $PI(4,5)P_2$.[39] Several proteins also exhibit dual lipid recognition as in the case of Akt1- and PDK1-PH domains that bind to PS[51] as well as $PI(3,4,5)P_3$.[52] Hence care must be taken when using lipid-binding domains as probes for lipids. These caveats highlight the pressing need for tools and methodologies that can (1) determine all lipid-binding sites and interfaces of proteins, as well as their lipid specificity, and (2) design artificial membrane-binding proteins that exhibit specific lipid-interacting properties. In this context, several computational approaches have emerged as promising strategies and have been extensively used in the last two decades, some of which we discuss here.

Computational Approaches to Study Protein–Lipid Interactions

Peripheral membrane proteins often interact transiently and reversibly with membranes.[53] Hence, the 3D structures of most peripheral membrane proteins are generally determined in their soluble, membrane-less conformation. This makes it impossible to determine, based solely on the solved 3D structure, their membrane-binding interface. In addition, protein dynamical properties such as membrane binding-driven conformational changes or energetic and kinetic details of the binding process, are missing.

In this context, MD simulations have emerged as a promising approach to study protein–membrane interactions and have fueled our understanding of how peripheral membrane proteins bind to bilayers and which specific lipids or membrane properties they sense.[16,54–59] To this extent, in the last few decades, all atom (AA) MD simulations have been useful in determining the interactions between proteins and bound lipids as well as characterizing protein–membrane interactions.[57,59–63] However, in many instances, membrane binding takes place on timescales that are longer than those accessible to unbiased AA-MD simulations.[64]

To address this limitation, techniques to enhance the sampling of this process have been put forward. Most notably, two techniques that have been used extensively for this purpose are (i) the highly mobile membrane-mimetic model (HMMM)[65] and (ii) coarse grain (CG) simulations. In HMMM, the hydrophobic core of the bilayer is replaced by an organic solvent layer, while the polar heads are represented by short-tail phospholipids using an AA force-field (CHARMM36). This workaround strongly accelerates the lateral diffusion of lipids, in turn promoting membrane binding by peripheral proteins.[66] However, due to the overstabilization of aromatic, polar, and charged side chains in the membrane core,[67] as well as to a general over-binding of proteins to the bilayer,[65] this methodology is not well-suited to study the specificity of proteins to bilayers of different lipid compositions.

CG force-fields, on the other hand, allow the study of long timescale and large sizescale processes by treating three or four heavy atoms as a single interaction site, thus drastically reducing the computational cost. While CG approaches possess intrinsic limitations arising from a decrease in the resolution of the model, such as the absence of H-bonds and inaccurate entropy-dependent properties,[68] force-fields such as the MARTINI[69,70] have been shown to hold enormous potential towards identifying membrane binding regions of proteins as well as characterizing their sensitivity for membrane properties such as electrostatics or lipid packing defects.[71] Some noteworthy examples include the use of MARTINI to determine the interaction modes of several PIP-binding PH domains,[54,55,58,72] PTEN domains,[73] proteins that bind *via* their aromatic residues,[74] and lipid-transfer proteins such as cholesteryl ester transfer protein (CETP)[75] and α-tocopherol transfer protein (α-TTP),[76] to name a few.

In silico Design of Lipid-binding Proteins

Until recently, a major limitation of using MD simulations to investigate membrane–protein binding was the unavailability of 3D structures for many proteins. However, with the advent of AlphaFold2 which predicts protein structures from their amino acid sequence with very high accuracy,[77] this problem has now been alleviated. This opens the possibility to potentially screen multiple protein structures to predict their lipid-binding specificity and membrane-binding interface. This approach could help attenuate a major limitation of *in vitro* liposome-binding experiments, where genome-wide identification of peripheral membrane proteins *via* experimental methods remains a laborious and expensive endeavor. In fact, although sequence similarities with other peripheral membrane proteins can be predicted, the results are not always reliable as in the case of several PH domains that exhibit stark differences in membrane binding.[78] This MD-based screening approach could also be partnered by ML-based techniques. Recently, in fact, ML studies have shown to be particularly useful in identifying peripheral membrane-binding proteins with an accuracy greater than 90%[79] as well as in predicting their membrane-binding interfaces.[80] Another recent *in silico* approach to identify peripheral membrane proteins is based on the discovery that hydrophobic residues are more frequently found in protruding sites on peripheral membrane proteins than in other proteins, and that these residues cluster at sites that constitute the membrane-binding interface.[81]

A serious hiccup in using proteins as lipid biosensors, as discussed earlier, is that there exist only a few proteins that have been experimentally characterized to bind lipids such as PC, PE, and ceramides. Thus, a potentially interesting approach would be the computational design of proteins and peptide sequences that fold

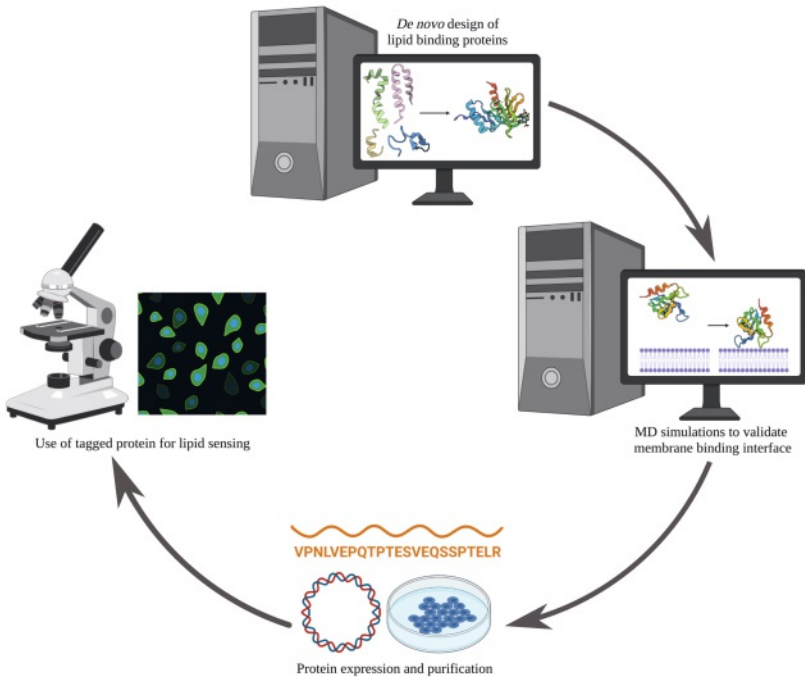

Fig. 2. Potential pipeline for the *de novo* design of lipid biosensors. Image created with BioRender.com

into desired 3D structures and elicit specific lipid-binding properties. Over the last two decades, several proteins have been either successfully redesigned from existing protein structures,[82,83] or *de* novo designed[84,85] to perform specific functions. Protein redesign approaches are limited to sampling the conformational space around an existing structure, thus enabling one to rigorously sample backbone and side chain conformations at the functional site. On the other hand, *de novo* design approaches lack an experimental structure to start with, thus making it possible to incorporate structural features not found in naturally occurring proteins. A typical *de novo* protein design pipeline involves:

1. Sampling the backbone structure space to identify backbones compatible with the desired function. This can be achieved by using fragment libraries derived from existing protein data bank (PDB)[86] structures (as in the case of methods such as SEWING[87] (structure extension with native-substructure graphs) or AbDesign[88]) or by training machine learning models based on existing protein structures, including the most recent method of neural network (NN) hallucination which uses the NN from transform-restrained (TR)-Rosetta.[89]
2. Optimizing the sequence composition and side chain rotamers to stabilize the backbone structure. Successful strategies include (a) layer design, which restricts amino acid possibilities at each residue position based on their solvent accessibility,[90] or (b) the HBNet[91] method, that involves identifying residues and side chain rotamers that support hydrogen bonded networks.

Designed proteins with tailor-made properties and functions have begun to make their mark in several areas; some noteworthy examples include the design of immunogens,[92] targeted therapeutics,[93] and biosensors.[94] However, most of these protein design studies focus on water-soluble proteins, and no peripheral membrane proteins with specific lipid-binding properties have been designed so far to the best of our knowledge. We foresee that the design of proteins that specifically bind lipids such as PC, PE, or ceramides, for example, could help better understand the cellular localization and distribution pathways of these lipids.

Conclusions and Outlook

In this short review, we focused on lipid-binding proteins that can assist in visualizing lipid pools in cells, and some caveats of current methodologies such as the lack of probes for some common lipids. We propose that MD simulations and *in silico* design of lipid-binding proteins could help identify and validate membrane binding modes and lipid specificities of natural and artificially designed proteins. This will help elucidate lipid distribution and trafficking in cells when expressed *in vitro* or *in vivo*. We expect these methodologies to be paramount in filling crucial knowledge gaps in lipid biology.

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