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Current Opinion in Structural Biology

Deciphering the language of mingling lipids and proteins

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Each cell possesses a genetic and a proteolipid code that together convey molecular information in a perpetual cycle. One element of this cycle is the recognition of lipids that work together to specify subcellular locations for biochemical activity. These "lipidons" are now being resolved in protein structures from eukaryotic plasma membranes, endosomes, mitochondria, prokaryotes, and viruses with technologies like *in situ* cryo-electron imaging and membrane-active polymers. This adds to an expanding catalogue of codified protein-lipid interactions that are recontextualizing cell biology and drug discovery.

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Introduction

The ability to detect native protein-lipid interactions is revealing a previously hidden aspect of the biological program. Unlike nucleotide codons and their relationship to amino acids, the "lipid codons" (lipidons) that specify locational assembly of proteins into active complexes are only now being revealed [1] (Figure 1). Lipidons are sets of lipids that are locally enriched in specific zones throughout the cell membrane system and serve as primary triggers, secondary ligands, or tertiary supports for protein function. They can be detected by native mass spectrometry [2], visualized in cryo-electron microscopy (cryo-EM) maps, and communicated in machine-readable languages [3].

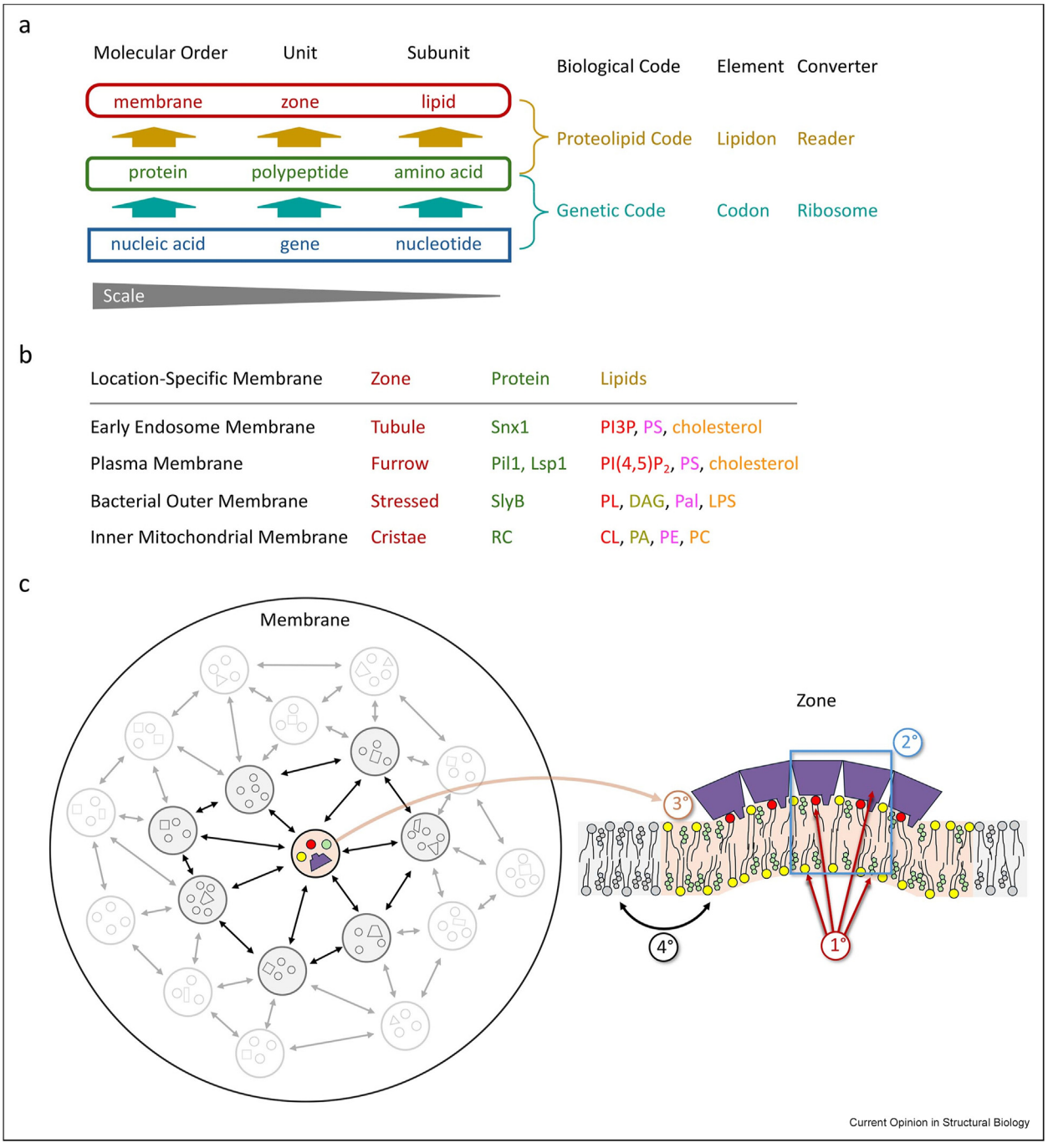
Previously, we proposed a solution to the enigma of membrane organization that explains how lipidons arrive at designated zones [4**]. Zones include proteins together with lipid fingerprints [5], protein clusters and their collective fingerprints, which we refer to as protein islands, and regions devoid of protein, which are termed voids [6]. Similar to proteins, zones are characterized by a hierarchy of structures. Primary structure is the set of lipids, proteins, and other components that make up the zone. Secondary structures are microstate features, such as contacts between molecules and their distributions, while tertiary features are macrostates such as curvature and electrostatic potential. Quaternary structure describes interactions between zones, allowing membranes to be formalized as graphs with many nodes (Figure 1c). Although our solution broadly describes the contributions of protein−protein, protein-lipid, and lipid−lipid interactions to membrane compartmentalization, these detailed structures must be experimentally or empirically resolved for specific cases.

Here we illustrate experimental progress on resolving lipidons within zones and explore their roles in membrane fission and fusion. The interactions are conveyed in COMPOSEL language, which concisely states the residues that bind ligands in membranes [3]. Technologies, including amphiphilic copolymers that extract proteins together with natively bound lipids are anticipated to enable future discoveries of zone states. Together with advances in *ex vivo* and *in situ* structural biology, this paves the way for deciphering the rules governing the organization of the proteome and lipidome.

Plasma membrane lipidons

Many eukaryotic proteins recognize the inner leaflet of the plasma membrane through lipidons containing phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$], phosphatidylserine (PS), and cholesterol. This is exemplified by the eisosome complex [8**] that compartmentalizes the yeast plasma membrane into furrows through two banana-shaped proteins called Pil1 and Lsp1, which contain Bin/Amphiphysin/Rvs (BAR)

Figure 1



Logic of the biological code. **(a)** The genetic and proteolipid codes convert levels of molecular order. Membrane readers include hundreds of phosphoinositide code detectors such as sorting nexin 1 (Snx1) [1], as well as extramembrane domains that specifically engage lipid headgroups [4] and other gene products such as RNA molecules that can also recognize lipids [7]. **(b)** Four examples of how lipidons within zones encode subcellular location are listed. **(c)** Depiction of the proteolipid code as a network of interdependent zones, each of which comprises primary, secondary, tertiary, and quaternary structures, with examples labeled 1°, 2°, 3°, and 4°, respectively.

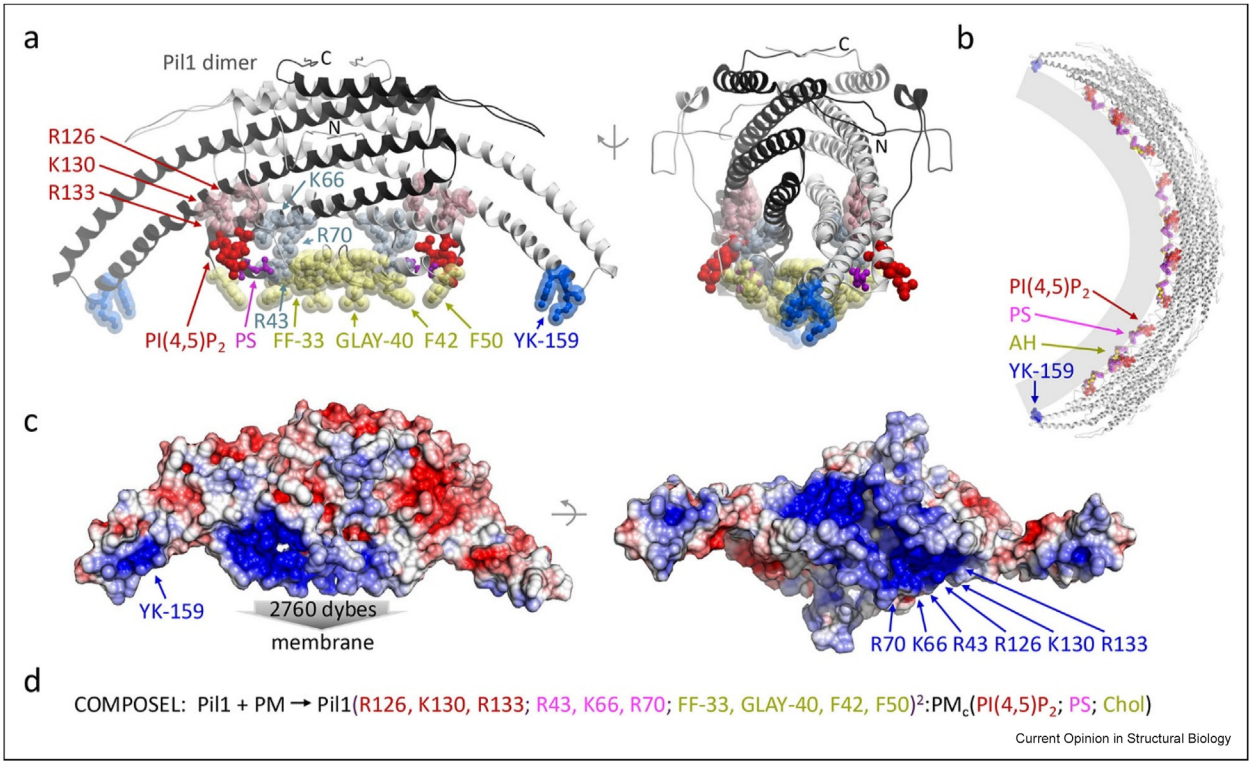
domains and sense and respond to mechanical stress through conformational rearrangements induced on membranes. Cryo-EM structures reveal how the lipidon is dynamically bound to the inner surface of a protein lattice formed by curved BAR domain dimers (Figure 2). The assembly buries an aligned amphipathic helix pair inside the inner leaflet. The lipid-protein complex is resolvable by reconstitution into various liposome compositions, including with brominated sterols. This shows how $PI(4,5)P_2$, PS, and cholesterol are bound by neighboring clusters of basic and hydrophobic residues on an amphipathic helix, with the two phospholipids being integrated within the protein lattice. Together these form a membrane-specific surface that is propagated by oligomeric arrays and allows spring-like stretching and compression to yield dynamic furrow zones.

Regulatory post-translational modifications are hallmarks of lipidon recognition [1]. The Pil1 lipidon binding area is populated by phosphoryated residues including S41, S44, and S131 [9]. Our prediction of lipid binding sites indicates that the BAR tip residues Y158 and K159 also insert and contribute to Pil1's membrane interacting lattice (Supplementary Figure 1). The proximal Y158 and S163 residues are phosphorylated during late mitosis [9] and stress response [10], inferring that multiple Pil1 phospholipid engagements are controlled by a network of phosphoswitches. Consequently, we propose that construction and deconstruction of plasma membrane zones is directed by specific protein kinases as well as phosphatases [11] that act on lipidon-binding surfaces.

Early endosome lipidons

The early endosome is marked by a lipidon formed by phosphatidylinositol 3-phosphate (PI3P), PS, and cholesterol [15*]. These lipids in the cytosolic leaflet are detected together by phox homology (PX) domains and curved BAR domains of membrane readers such as Snx1. Once this sorting nexin recognizes its lipidon, the protein forms rings that curve the membrane into tubules [16] and attracts retromers that sort cargo

Figure 2



Eisosome complex with a plasma membrane lipidon. **(a)** The complexed Pil1 dimer is seen in two views (PDB 8QBF) [8]. Residues R126, K130, and R133 bind $PI(4,5)P_2$; R43, K66, and R70 bind PS including its acyl tails; F32, F33, G37, L38, A39, Y40, F42, R43, and F50 accommodate cholesterol. The N- and C-termini are labeled "N" and "C". **(b)** The dimers assemble into a curved lattice with bound lipids on the concave side. Interacting ligands and motifs are shown as surfaces and are labeled (PDB entry: 8QBD). (**c**) Electrostatic surface potentials calculated using eF-surf [12] suggest that the YK-159 extremities also insert. Calculation of the dipole moment [13] of 2760 debyes shown by an arrow under the dimer indicates a strong attraction to the acidic phospholipid bilayer surface. Structures were visualized using PyMOL (The PyMOL Molecular Graphics System, Version 3.0 Schrodinger, LLC) and ICM software [14]. (**d**) The formation of the lipidon-bound Pil1 dimer is described in COMPOSEL [3].

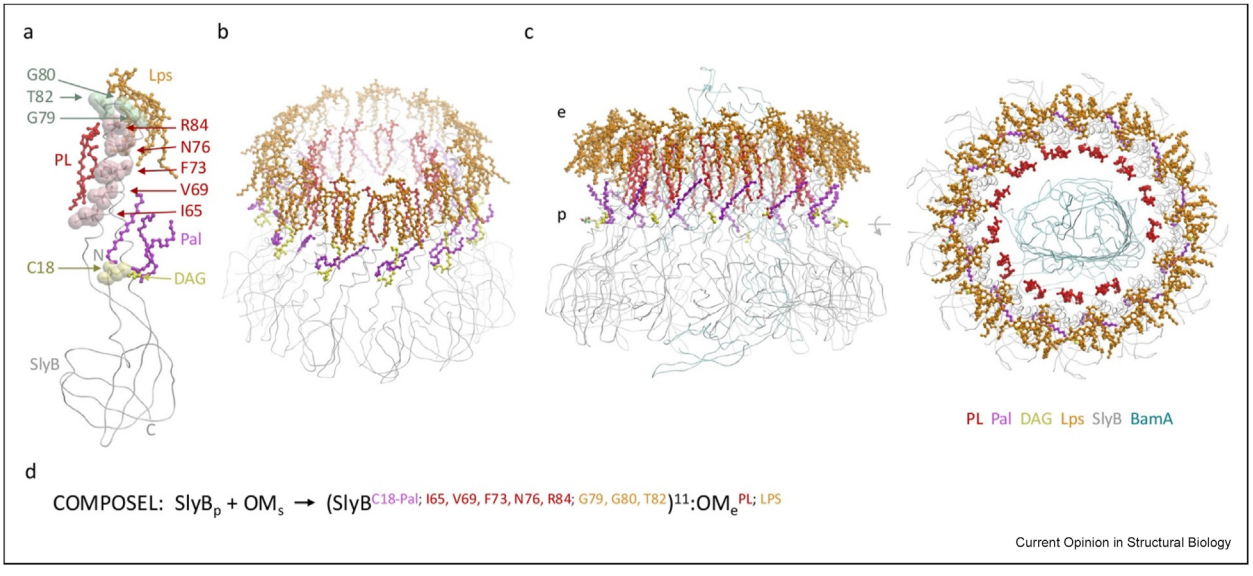
receptors [17,18]. The domains are attracted to negatively charged membranes by their respective dipole moments and then dock to each lipid ligand *via* proximal binding sites, thus stimulating conformational changes. Phosphorylation of recognition motifs as well as lysine acetylation and arginine methylation are predicted to regulate this process [15,19,20]. Elucidation of the conformational states, ligands, and modifications involved illustrates the journeys these proteins undergo to gather at endomembrane destinations and form trafficking tubules.

Lipidons across leaflets

Zonal lipid concentration is regulated by numerous mechanisms, including inter- and intra-leaflet lipid transport. Flippases transport lipids from the exoplasmic leaflet to the cytosolic one fueled by ATP [21]. They create and maintain lipid asymmetry in membranes of the late secretory pathway in eukaryotic cells, which is tightly linked to the membrane resistance to stresses, membrane trafficking, and the regulation of membrane protein activities. Most members belong to the P4-ATPase family that accommodates large structural rearrangements throughout their ATP-driven transport cycle, as highlighted by recent structures [22,23]. Their transport function has been shown to be tightly regulated by several mechanisms. For example, the yeast flippase Drs2 and the disease-related ATP8B1 and ATP8A2 have been shown to be regulated by their N- and C-terminal extensions, for which it has been hypothesized that activation involves specific interactions with binding partners and phosphorylation [24]. The precise localization and relative abundance of these phosphoinositides within the cell are strictly regulated, which in turn determines the types of membranes in which flippases should be active. This underscores the crucial role of lipids in controlling the composition of the membranes where these flippases are embedded and can themselves modify the membrane leaflet composition.

Extracellular lipid signals are sensed across membrane leaflets, emphasizing the range of proteolipid code detectors. This is demonstrated by the *E. coli* protein SlyB, which detects bilayer stress signals in the bacterial outer membrane to protect cell integrity [25**]. This zone is normally densely packed with β-barrel proteins and lipopolysaccharide, but rearranges to form a stress lipidon that contains exposed phospholipid. The periplasmic SlyB protein responds by employing glycine zipper domains, which oligomerize into ring-shaped transmembrane assemblies that encapsulate β-barrel proteins [25**] (Figure 3). The exposed lipids are bound by sets of SlyB residues (Supplementary Figure 2), thus triggering assembly of stable oligomeric rings that encircle selected β-barrel proteins to prevent shedding while protecting the outer membrane from further damage.

Figure 3

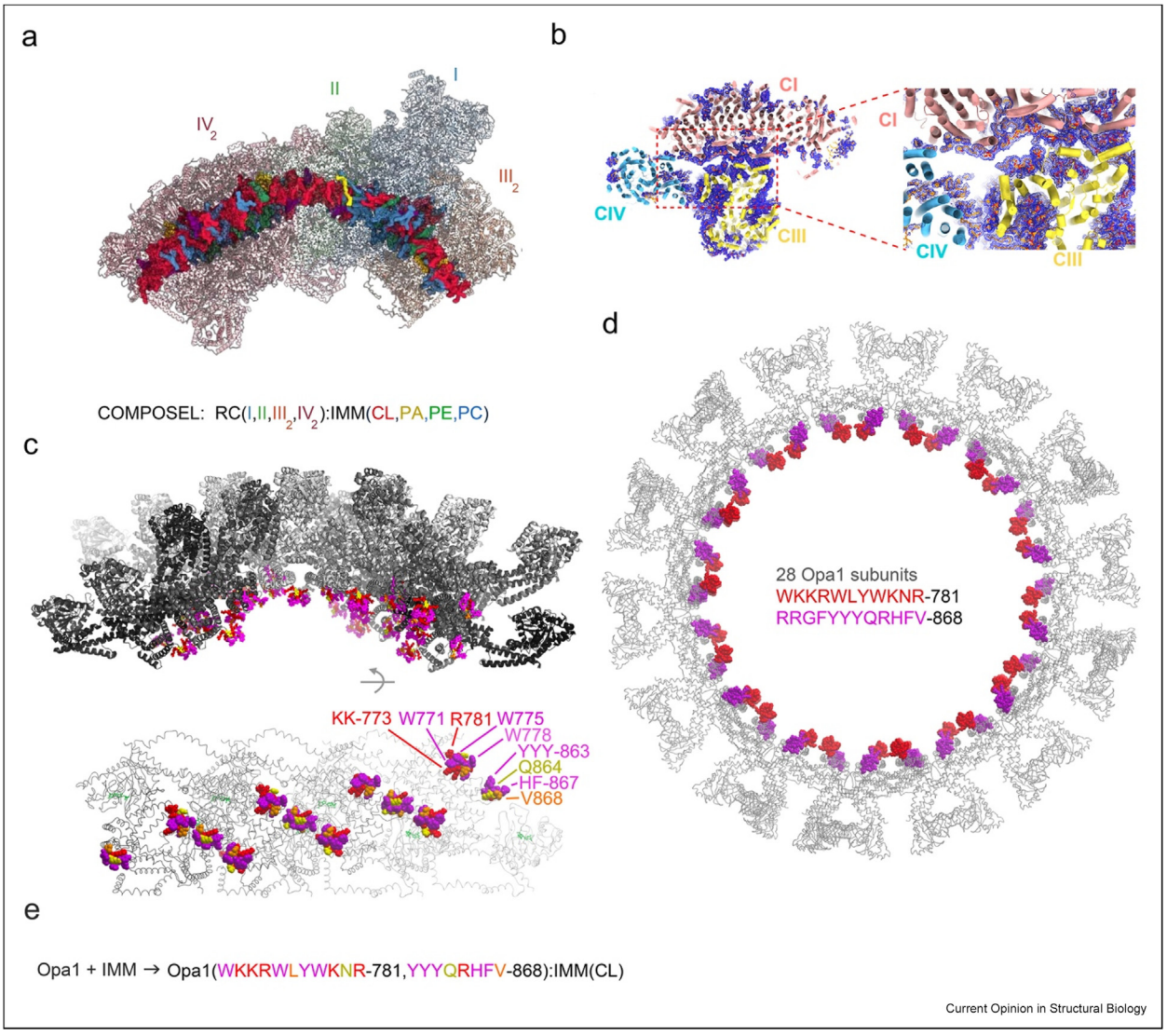


Structures of SlyB complexed with outer membrane lipids. **(a)** Protomer derived from SlyB oligomer (gray) with bound phospholipid (PL), lipopolysaccharide (LPS), diacylglycerol (DAG), and N-terminal palmitoyl (Pal) groups, which are shown as stick representations and colored red, orange, yellow, and magenta, respectively. Residues I65, V69, F73, N76, and R84 bind leaked outer leaflet PLs, G79, G80, and T82 bind Lps, and C18 is attached to a Pal group. The terminal residues are labeled "N" and "C." Rings formed by the SlyB 11-mer **(b)** and 13-mer enclosing the BamA β barrel (aqua) **(c)** are shown with color coded lipid ligands from various perspectives (PDB: 7OJG and 7OJF) [25]. **(d)** The COMPOSEL script summarizes the interaction of SlyB in the periplasm (subscript "p") with stressed outer membrane ("s") to form 11-mer transmembrane rings that recognize phospholipid (PL) pools in the extracellular leaflet ("e") [3]. The images were generated using ICM software [14].

Mitochondrial membrane lipidons

Lipidons are critical to energy generation and organization of membrane proteins in mitochondria. For example, analysis of cytochrome *c* oxidoreductase with attached fingerprint lipids, natively bound quinone, and a labile helical subunit required styrene maleic acid lipid particle (SMALP) encapsulation [26]. This reveals how oxidoreductase stability and activity are retained due to numerous lipids that occupy clefts in the transmembrane domain. Assembly of respiratory complexes is guided by protein phosphorylation and depends on membrane potential [27]. Resolution of a respiratory supercomplex structure [28**] reveals how 300 transmembrane helices orient in curved mitochondrial cristae, as represented by 311 bound lipids (Figure 4a). These include cardiolipin (CL), phosphatidic acid (PA), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) molecules, which line the 150-subunit complex, including subunit

Figure 4



Structures of membrane-bound mitochondrial assemblies. **(a)** The *Tetrahymena thermophila* respiratory complex with bound CL (red), PA (yellow) and PC (blue), PE (blue), lipid molecules, and ubiquinone-8 (purple) within the four color-coded components I, II, III_2, and IV_2 (PDB: 8BQS) (modified from Ref. [28]). **(b)** *In situ* cryo-EM images of mitochondrial respiratory complex showing lipids within the purple density mediating interactions between labeled subunits (modified from Ref. [30]). **(c)** Fusion of the inner mitochondrial membrane is mediated by Opa1 multimers that recognize curved CL-containing bilayers *via* basic, aromatic, and aliphatic residues within a pair of helices. These are expanded below for half of the structure (PDB: 8EFR), which is rotated to show color-coded binding residues [33]. **(d)** The 28 Opa1 protamers assemble into 49 nm diameter rings for which lipid binding motifs are color-coded (PDB: 8CT1) [34]. Images **(c)** and **(d)** were made using ICM [14]. (**e**) The Opa1 interactions with CL are described as a COMPOSEL script [3] with residues that interact color-coded. CL, cardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; cyro-EM, cyro electron microscopy.

interfaces and an internal pocket. Comparative analysis of mutant and wild-type respiratory complex structures from inner mitochondrial membranes shows that CL and phosphatidylglycerol (PG) can bind interchangeably and pack flexibly to allow for energy generation [29]. This suggests that lipidon recognition is not necessarily absolute, reminiscent of the wobbles found in codon recognition.

Visualization of respiratory supercomplexes within purified mitochondria reveals preservation of electrochemical proton gradients [30**]. The structure of the entire membrane-protein assembly shows 197 associated lipids stabilizing multiple states and mediating intersubunit interactions *in lieu* of protein−protein interactions (Figure 4b). The direct contribution of two CLs and one PE to the proton transfer reaction demonstrates that zone lipids, not just proteins, directly mediate chemical reactions in membranes. Mitochondrial cristae also employ adenosine triphosphate (ATP) synthase that forms helical stacks of lipid-bound dimers that rotate in the membrane as protons translocate [31*]. Assemblies of ATP synthases include pentameric scaffolds of hexamers that fold mitochondrial cristae in concert with curvature-inclined CL molecules [32]. Such studies demonstrate how zones operate cooperatively as extensive functional networks in asymmetric, curved membranes subject to proton gradients.

Fission and fusion lipidons

Fusion and fission are essential for introducing and removing membrane components. In mitochondria, this involves recognition of CL-containing lipidons by proteins such as the dynamin-related protein's variable domain, which inserts into the outer membrane [35]. Its GTPase activity then triggers assembly of oligomers with transmembrane partners to form constricted 16 nm rings inside the hemi-fission intermediate that splits the mitochondrion [36], with contributions from PI4P molecules imported *via* vesicles from the trans-Golgi network [37]. The fusion of inner mitochondrial membranes is carried out by the Opa1 protein, which inserts two helices into the bilayer and oligomerizes to present an array of membrane binding motifs (Figure 4c and d, Supplementary Figure 3). This leads to the formation of 49 nm rings [38] and the use of GTP *via* a distal pocket to fuse membranes [33,34], thus revealing how energy is generated and used to remodel zones.

Fusion to host cell membranes by viruses such as SARS-CoV-2 involves recognition of a PS lipidon that, once appearing on the extracellular leaflet, is bound by spike multimers [39] to trigger viral entry [40*]. Furthermore, the assembly and budding of enveloped viruses relies on zones containing PI, $PI(4,5)P_2$, and cholesterol [41*]. Mirroring infection, gamete fusion relies on recognition of exposed PS molecules on sperm membranes by oocyte receptor complexes [42]. While the critical concentrations and structural coordination of the various components that initiate and execute cell-specific fusion remain unclear, the identities of the key lipid and protein ligands in the multivalent complexes are becoming evident.

Cell death lipidons

Pathogen-induced host cell death, known as pyroptosis, is mediated by the formation of large pores in cellular membranes by gasdermin proteins. The preferential localization of gasdermin A to mitochondrial membranes is consistent with basic binding sites that appear to be occupied by CL in cryo-EM structures [38]. The related gasdermin D protein is recruited to the inner leaflet of plasma membranes by recognition of $PI(4,5)P_2$, and PS through three interconnected basic pockets that trigger multimerization [43]. Detergents induce the formation of stable non-native "slinky" conformations derived from the disruption of closed-ring pores consisting of 52 subunits [44*]. The formation of intact β sheet rings in these pores involves a palmitoyl group that is covalently attached to a cysteine and exits a hydrophobic sheath to insert into the membrane, showing how lipidation contributes to lipidon function. These processes show how specific lipid-protein interactions that disrupt vital organelles mediate organized cell death, complementing the formation, fusion, and fission processes used to construct new zones.

Lipidon-dependent channels

Transmembrane channels are sensitive to lipidons and colocated ions, which are recognized by their peripheral domains. This is evident in the Slo1 channel's proximal $PI(4,5)P_2$, and metal binding sites [4], and also drives gating of mechanosensitive channels. Visualization of biological lipids bound within MscS's heptameric structure is enabled by amphipathic polymers that preserve its functional state [45*]. The cycling of resting, open, and desensitized states of ligand-gated ion channels depends on neurotransmitter binding. The states of the glycine receptor channel in the presence of glycine agonist and partial agonists taurine and γ-amino butyric acid can be seen by cryo-EM using styrene maleic acid (SMA) polymer to form nanodiscs [46]. Not only are the brain lipids retained, but small neurotransmitter molecules are resolvable in their respective binding pockets, where they stabilize distinct conformations. In contrast, detergents induce unnaturally open states that do not reflect the channel's functional cycle. This emphasizes the fact that specific lipids determine channel function and preserve physiologically relevant states.

Polymer-based nanodiscs

Extraction of functional membrane proteins from cells for shotgun proteomics increasingly relies on nanodisc-forming polymers, which offer advantages over classical

detergents. Synthetic polymers, while rivaling detergents in solubilization efficiency, also preserve native lipids, which are critical for stability and activity [47]. Nonetheless, a polymer's chemical structure can bias the efficiency for solubilizing protein complexes and organellar membranes into native nanodiscs. This prompted development of fluorescence-based screens for extraction of thousands of unique mammalian membrane proteins and complexes [48], allowing more comprehensive functional screening of proteomes. Immobilization of fluorescent protein-tagged native nanodiscs on surfaces allows the oligomeric distributions of membrane-protein assemblies (memtein) to be detected [49]. Attachment of fluorescent dye molecules to specific positions can be accomplished by insertion of a noncanonical amino acid that is compatible with single-molecule studies, as shown for proteins involved in N-linked glycosylation [50].

Membranes can be spontaneously converted into nanodiscs containing functionally intact proteins and bound lipids by adding SMA-type polymers, although charge interactions can occur [51]. The solubilizing efficiency can be further increased by adding halogen substituents to styrene rings, with hydrophobic balance, polymer length and backbone rigidity also being key influences [52]. Addition of alkyl groups to the maleic acid subunits increases the sizes of native nanodiscs [53], which can deliver active prions for *in vivo* use [54]. Polymer conformational freedom can be optimized by using methyl-substituted stilbene as the hydrophobic subunit to preserve protein multimers in homogeneous nanodiscs [55], prompting development of improved synthesis routes [56]. Amphipols with cycloalkane rather than linear aliphatic chains can be used to extract membrane proteins more efficiently for resolving structures [57]. To reduce interaction bias, the net charge and excess hydrophobicity can be removed from polymers, thus improving coverage of membrane proteome analysis. A set of polymer derivatives that include sulfobetaine groups provides aqueous solubility through zwitterionic sidechains [58]. These are useful for microfluidic diffusional sizing assays of binding affinities and proteome preparations for native mass spectrometry. Zwitterionic amine oxide sidechains grafted onto alternating SMA minimize nonspecific binding to protein or ions; these fluorescent polymers form ~20 nm discs and efficiently solubilize β-barrel and G-protein coupled receptor (GPCR) proteins [59].

Drug discovery

The integrity of membrane-associated receptors and enzymes requires bound native lipids, retention of which is crucial for targeting physiological states [60,61]. In particular, GPCRs [62] are most stable in SMALPs that retain cholesterol, $PI(4,5)P_2$, and PS, and facilitate conformational changes for signaling and engaging β arrestin and G proteins [63–65*]. We propose that these three lipids synergize to ensure physiological activity of such receptors in plasma membranes, providing new opportunities for drug discovery through lipidon recognition and novel target sites.

Native membranes are crucial for studying interactions of drug molecules, which are routinely displaced from transmembrane proteins by detergents, as evidenced by purification of the transient receptor potential ion channel TRPM4 [66*]. Comparison of structures solved by cryo-EM reveals that selective and potent small molecule inhibitors are displaced by detergents such as cholesteryl hemisuccinate, which also disrupt lipid bilayer packing, while SMA-based purification retains the bound inhibitor, calcium, and endogenous lipid. Similarly, membrane solubilizing media also influence antibody interactions, as shown with the trimeric envelope glycoprotein of immunodeficiency virus in SMALPs [67*], emphasizing that membrane mimics should minimally perturb targets for drug screening [68].

The diverse enzymes that regulate recognition of the proteolipid codes represent major drug targets. Kinases form phosphatidylinositol phosphates (PIPs) and phosphorylate lipidon-binding surfaces to regulate signaling and assembly on membranes [69,70]. Similarly, methyl and acetyl transferases regulate lipidon function by modifying basic residues responsible for recognizing phospholipid headgroups [19]. The enzymes that carry out lipidation are of growing interest for therapeutic intervention [71], and membrane-bound states of conventional target classes are increasingly attractive [72]. Computer models that simulate how such targets operate in membranes [73] along with artificial intelligence tools could provide new avenues for deducing such mechanisms.

Author contributions

Conceptualization, analysis, visualization (MO, TAK) and writing (all coauthors).

Declaration of competing interest

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through the University of Birmingham. Michael Overduin has patent Functional derivatives of maleimide copolymers for nanodisc production pending to University of Alberta. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Glossary

Lipidon: set of proximal lipids encoding a location in a cell

Memtein: membrane-derived assembly containing a functional protein and bound lipids

Native nanodisc: nanometer scale disc of biologically intact membrane

Protein island: A zone including multiple proteins and associated lipid fingerprints

Proteolipid code: instructions within membrane components (e.g. proteins and lipids) that form zones

Void: zone devoid of protein

Zone: structurally and functionally distinct membrane region

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbi.2025.103061.

Data availability

See supplementary data

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