Chemistry and Physics of Lipids 218 (2019) 73–84

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Review

Memtein: The fundamental unit of membrane-protein structure and function

Michael Overduin*, Mansoore Esmaili

Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7, Canada

ARTICLE INFO

Keywords:
Membrane structure
Memtein
Native nanodisc
Styrene maleic acid
SMALP
Transmembrane protein

ABSTRACT

The concept of a memtein as the minimal unit of membrane function is proposed here, and refers to the complex of a membrane protein together with a continuous layer of biological lipid molecules. The elucidation of the atomic resolution structures and specific interactions within memteins remains technically challenging. Nonetheless, we argue that these entities are critical endpoints for the postgenomic era, being essential units of cellular function that mediate signal transduction and trafficking. Their biological mechanisms and molecular compositions can be resolved using native nanodiscs formed by poly(styrene-co-maleic acid) (SMA) copolymers. These amphipathic polymers rapidly and spontaneously fragment membranes into water-soluble discs holding a section of bilayer. This allows structures of complexes found *in vivo* to be prepared without resorting to synthetic detergents or artificial lipids. The *ex situ* structures of memteins can be resolved by methods including cryo-electron microscopy (cEM), X-ray crystallography (XRC), NMR spectroscopy and mass spectrometry (MS). Progress in the field demonstrates that memteins are better representations of how biology actually works in membranes than naked proteins devoid of lipid, spurring on further advances in polymer chemistry to resolve their details.

1. Introduction

Membrane protein structural and functional integrity depends on a layer of lipids that surround much of their surface. However, available membrane protein structures typically lack this biological lipid layer, despite its requirement for their correct operation. In fact, there is no word for the biologically intact unit formed by complexes of the lipid-coated proteins in a membrane. This gap persists despite the growing evidence that direct interactions with structured lipids are widespread and integral to membrane protein folding, structure, stability, ligand binding, activation, signal transduction and trafficking (Overduin et al., 2001; Phillips et al., 2009). Our language has not caught up with the tremendous science being done in the field of membrane structural biology. The lack of an ability to name the concept not only creates unnecessary ambiguity and confusion, but also limits our ability to focus on the important challenges and tasks ahead. Recombinant technology cannot necessarily solve these problems, as memteins cannot necessarily be formed by recombining the isolated parts. Instead memteins are assembled through the stepwise trafficking and processing of lipids and proteins in a series of subcellular compartments that are difficult to recapitulate. Here we propose the word memtein to describe the functionally intact unit of a membrane protein bound to a continuous layer of biologically relevant, structured lipids. As such, memteins represent minimal and stable operational states of membrane proteins packed against a perimeter of endogenous lipids that engage as they would *in vivo*. We argue that the stereospecifically packed and dynamically restricted lipid headgroups and tails are as integral to these entities as a protein's residues, being key determinants of physiologically relevant folding, stability, specificity, dynamics, structure and function as well as being required for moving towards rational drug design.

The development of styrene maleic acid (SMA) polymers for making

Abbreviations: ATP, adenosine triphosphate; Cem, cryo-electrom microscopy; CL, cardiolipin; DIBMA, poly(diisobutylene-*alt*-maleic acid); DDM, n-dodecyl-β-D-maltopyranoside; DLS, dynamic light scattering; DMPC, dimyristoyl phosphatidylcholine; DMPG, dimyristoyl phosphatidylglycerol; DPC, dodecylphosphocholine; EM, electron microscopy; FRET, Förster Resonance Energy Transfer; GPCR, G protein-coupled receptor; HEK, human embryonic kidney; LCP, lipidic cubic phase; LDAO, lauryldimethylamine N-oxide; LPS, lipopolysaccharide; LHC, light harvesting chlorophyll; MA, maleic acid; MI, maleimide; MSP, membrane scaffold protein NMR: nuclear magnetic resonance spectroscopy; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIP, phosphatidylinositol phosphate; PG, phosphatidyglycerol; S, styrene; SDS, sodium dodecyl sulfate; SMA, poly(styrene-co-maleic acid); SMAd-A, dehydrated styrene maleic acid ethylenediamine; SMA-EA, styrene maleic acid ethanolamine; SMA-ED, styrene maleic acid ethylenediamine; SMA-QA, styrene maleimide quaternary ammonium; SMALP, styrene maleic acid lipid particle; SMA-SH, SMA with sulfhydrils; SMI, poly(styrene-co-maleimide); TSPAN, tetraspanin; zSMA, zwitterionic SMA

* Corresponding author.
E-mail address: overduin@ualberta.ca (M. Overduin).

https://doi.org/10.1016/j.chemphyslip.2018.11.008
Received 6 October 2018; Received in revised form 24 November 2018; Accepted 28 November 2018
Available online 30 November 2018

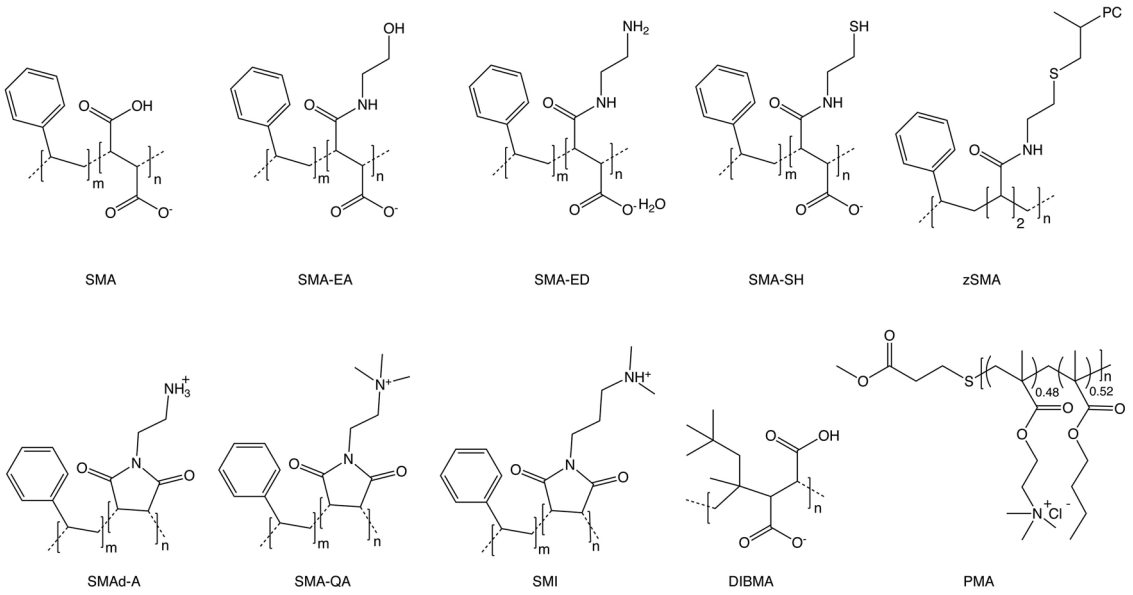

Fig. 1. Chemical structures of polymer-based solubilizing systems. Different synthetic polymers that have been used for the preparation of membrane protein nanodiscs are depicted. SMA-related copolymers which form native nanodiscs include SMA, SMA-EA, SMA-ED, SMAd-A, SMA-QA, SMI, SMA-SH, zSMA, DIBMA and PMA.

native nanodiscs (Fig. 1) has allowed memteins to be isolated directly from membranes, cells and tissues without conventional detergents. Such detergents strip away endogenous lipids and destabilize membrane proteins, thus leading to a variety of documented artifacts in membrane protein structures (Zhou and Cross, 2013). SMA copolymers are designed to bypass these problems and provide a simpler and less expensive approach to memtein solubilization, and have led to an expanding set of membrane types being incorporated into nanodiscs. Generally applicable methods are being developed by an open innovation community known as the SMALP Network, and show compatibility with most biophysical tools and biochemical methods, although limitations with pH ranges, polyvalent cations and absorbance have been identified and are being overcome with new formulations. These developments are revealing new membrane complexed structures including large ligand-bound machines and post-translational modifications that could not be resolved with any other technology, suggesting an avalanche of potential impacts and applications. However, this field is still young. Further acceleration of the field rests on the design of new polymer chemistries that improve performance and progress our understanding of how native nanodiscs work. The drive to further refine the materials and tools (needed to prepare and study memteins) results from several observations and known limitations. These are described below, and are considered in light of the properties of the SMALP system and allied technologies.

2. Memtein properties

Membranes are clearly integral to the development and behavior of cells, and may be reduced to fundamental functional units known as memteins which accurately mimic their properties for detailed analysis. Bilayer thickness varies to complement protein interfaces, and lipids segregate to proteins to facilitate hydrophobic matching of their long tails and polar headgroups with transmembrane regions in a manner that can dramatically influence activity (Dumas et al., 1997). Retaining such a pool of closely packed lipids within an adaptable nanoparticle of variable dimensions would be desirable, given the diversity of protein and lipid sizes and shapes. The lateral pressure profile across a bilayer depends on the mix of contained molecules, which can be included when SMA polymers wrap around a section of membrane. This pressure differential exerts significant forces across the membrane, influencing the stability and conformational equilibria of contained proteins and lipids (Cantor, 2002; Marsh, 1996). Membrane proteins with between 1 and 48 transmembrane helices have now been isolated *via* SMALPs from many eukaryotic and prokaryotic cell types (Lee et al., 2016; Sun et al., 2018). Such memteins maintain their native-like stability and ligand binding activity as well as a surrounding layer of lipids, while those in detergent micelles are typically destabilized to a significant degree and have lost their endogenous lipid content (Jamshad et al., 2015a). Hence, SMALPs appear to be advantageous for the isolation of intact memteins, especially those that are unstable or depend on having a lipid bilayer that assumes the correct thickness and pressure profile.

The orientation of protein elements at the membrane-water interface is inherently dependent on the local distribution of the surrounding lipids. Belts of Tyr and Trp residues offer aromatic groups that engage lipid carbonyls at membrane-water interfaces (Killian and Von Heijne, 2000). Positively charged sidechains of Arg and Lys residues snorkle out of the hydrophobic layer to contact negatively charged phosphate groups. These interactions continue around the perimeter on each side of transmembrane proteins, providing balanced anchoring within the bilayer. The presence of this continuous shell of lipid in nanodiscs allows such interactions to be preserved as the system moves and works. This can be seen by the packing of the aromatic residues of the ActE subunit of the respiratory Alternative Complex III (ACIII) into the lipid bilayer of a SMALP (Fig. 2), as resolved by cEM (EMD-7286, EMD-7448) (Sun et al., 2018). This *ex situ* structure shows how a native-state complex composed of six subunits, bound cofactors and attached lipids mediates electron transport. Its purification and structure determination had previously proven elusive but could be accomplished with either of two different SMA types that yielded more stable and active forms than could be prepared with detergents such as dodecylmaltoside (DDM). Structural interactions of a triacylated cysteine residue are evident, showing how post-translational modifications can be resolved in native nanodiscs. This would be very difficult, if not impossible, to re-assemble from isolated component parts. Hence it is best to directly isolate memteins from biological sources, which can be readily and cost-effectively accomplished in mass quantities using SMA (Lee et al., 2016). Preparing large amounts of many diverse memtein types with their amino acid residues engaging a network of bound biological lipids, co-factors and post-translationally modified subunits may currently only be possible with SMALP methods. It is hoped that this

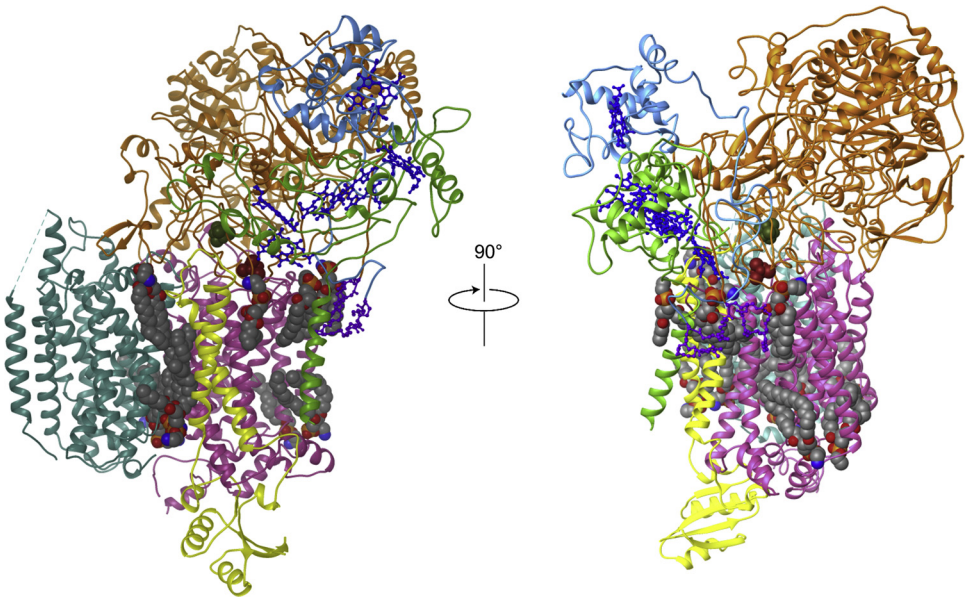

Fig. 2. Photosystem complex structure elucidated in a SMALP. Side views of the *ex situ* structure of respiratory Alternative Complex III (ACIII) into the lipid bilayer of a SMALP, as resolved by cryo-EM (EMD-7286, EMD-7448) (Sun et al., 2018). The subunits are color coded as ActA (green), ActB (orange), ActC (red), ActD (yellow), ActE (light blue) and ActF (cyan) subunits, haem groups (dark blue), lipid anchors (purple), and bound phospholipids (space filling with grey acyl tails). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

nanodisc technology will lead to a much wider universe of complex membrane machineries becoming experimentally accessible.

The folding of outer membrane proteins depends on local disordering and thinning of the lipid bilayer due to hydrophobic mismatch with the β-barrel machine (BAM) structure (Noinaj et al., 2013). When the BamA subunit is present in membrane mimetics such as micelles, bicelles or nanodiscs bounded by membrane-scaffolding protein (MSP) it is unable to correctly fold its juxtamembrane domains (Morgado et al., 2015), suggesting a need for further accessory factors. Indeed, BAM-mediated assembly requires phosphatidylethanolamine (PE) to correctly assemble outer membrane proteins into their folded structures, which are otherwise kinetically trapped (Gessmann et al., 2014). Hence biologically relevant lipids such as PE are required to guide membrane proteins through their folding pathways into native states, and are integral to BAM memteins.

Lipids have chiral carbon centers, and are made as various enantiomeric and diastereomeric forms with important biological significance that would ideally be resolvable. The phospholipids from biological sources are typically a single stereoisomer with different acyl chains and unsaturated groups, and differ from racemic mixtures or synthetic versions with identical chains. This results in established differences in membrane ordering and lipid bilayer structure (Böhm et al., 1993) as well as a variety of effects on membrane protein activity (Epand et al., 2005). Membrane-associated enzyme function depends on stereospecific lipid interactions, as is evident in the cases of phosphatidylinositol (PI)-specific phospholipase C (Bruzik et al., 1994) and the phosphatases PP1 and PP2A (Chalfant et al., 1999). There are also clear effects of different lipid bilayer systems on the influenza A M2 protein (Saotome et al., 2015) and phospholipase A2 (Høyrup et al., 2002). Thus lipids are not innocent bystanders in the membrane. Rather their stereospecific interactions make vital contributions to signaling and metabolic processes. Membrane protein structures are approaching the atomic resolution needed to show how stereospecific lipid recognition occurs. Further optimization of chemical properties and dispersity of SMA polymers, as well as covalent circularization of membrane scaffolds would enhance the structural resolution of memtine nanodiscs (Nasr and Wagner, 2018).

Once removed, sets of natural lipid ligands cannot typically be replaced into their *in situ* positions in memteins. Multiple lipid molecules dynamically associate with inducible pockets and dynamic surfaces that are found within unique protein conformers and multimer interfaces, and rely on the membrane's lateral pressure profile. Lipids behave as cooperative groups that both exhibit repulsive and attractive forces between headgroups and acyl chains. They can be stereospecifically layered onto proteins in non-random and non-uniform ways (Bagatolli et al., 2010). The only way to see such cooperative lipid-protein assemblies at high resolution may well be to isolate the entire memtein directly without removing the lipids *via* detergent extraction or any other chemical or physical means.

The identities of lipids bound to proteins can be discerned by ion mobility MS in the gas phase (Laganowsky et al., 2014). For example, aquaporin Z is stabilized by lipids including cardiolipin (CL), which modulates its ability to transport water molecules. Crystals of aquaporin AQPO show nine nonspecifically attached lipids lying in grooves on each monomer's hydrophobic surface (Gonen et al., 2005), while simulations indicate a single dynamic layer of ~70 lipid molecules nonspecifically associated with these tetrameric channels (Stansfeld et al., 2013). The ammonia channel AmtB from *E. coli* binds phosphatidylglycerol (PG) molecules, 8 of which are seen to decorate the periplasmic leaflet of the 2.3 Å resolution trimeric structure. The lipids induce conformational changes by structuring the binding loops that would normally engage the local bilayer. The mechanosensitive channel of large conductance (MscL) from *Mycobacterium tuberculosis* interacts non-selectively with lipids such as PI's, which stabilize it and may play functional roles (Laganowsky et al., 2014). The *E. coli* MscS channel associates most tightly with PE lipids, and its 3.0 Å resolution structure reveals interhelical packing of bound lipid-like acyl chains which decrease in number upon channel opening and can be exchange with the bilayer (Pliotas et al., 2015). The structure of TRAAK potassium channel suggests that the binding of lipid-like acyl chains induce structural changes which regulate the channel (Brohawn et al., 2014). Current structural tools have not yet revealed the stereospecific contacts of individual lipids with proteins let alone complete bilayer shells, and usually cannot even determine whether a bound molecule is a detergent or particular lipid species. Hence retention of a layer of lipids and concomitant avoidance of harsh detergents are needed in order to see and understand memteins.

The studies described below consistently demonstrate that SMA polymers efficiently convert diverse types of membranes into nanodiscs without requiring any conventional detergent at any step. This differs fundamentally from other methods such as those based on amphipols or membrane scaffold proteins, in which lipid must be re-introduced and thus cannot prepare memteins *ex situ*. Decades of study of SMA polymers attached to anticancer agents have demonstrated their biocompatibility (Maeda et al., 1985). These SMA conjugates can penetrate

into tumours to deliver their payload (Li et al., 2018). They are inexpensive to produce at scale, thus allowing potentially large amounts of plant, yeast, mammalian or bacterial biomass to be turned into nanoparticles for industrial applications.

3. Original SMA polymers used for memtein production

The discovery that SMA polymers could spontaneously liberate functionally intact membrane proteins (including helical bacteriorhodopsin and the PagP β-barrel protein) into nanodiscs was reported in 2009 (Knowles et al., 2009). Since then hundreds of groups have used the SMALP system and over 115 publications have described how SMA can be used to prepare nanodiscs of proteins, liposomes and membranes. The interested scientific community coalesced into the SMALP network in order to share tools and resources, and are pushing through remaining technical limitations. This open innovation approach effort has learned from the foundation laid by decades of progress on MSP-based nanodiscs (Bayburt and Sligar, 2010), bicelles (Dorr et al., 2013) and amphipols (Tribet et al., 1996). This builds on a century of experience with conventional detergents, with SMA offering a fundamental advantage by incurring a much lower free-energy penalty for membrane dissolution (Cuevas Arenas et al., 2016). Although each system offers unique strengths and weaknesses, all can be used to generate high resolution structures of stably overexpressed membrane proteins. However, only the SMA approach offers a detergent-free and scalable way to isolate and resolve memteins from any cell, tissue or organism.

Different types of SMA can be used to isolate memteins from various environments by inserting into and removing only the unstructured lipids. These copolymers all containnon-alternating sequences of styrene (S) and maleic acid (MA) residues which appear in statistically defined patterns within their linear chains. The most widely used ratios of S to MA groups are between 2:1 and 3:1. This pattern offers enough hydrophobicity to allow rapid insertion into lipid bilayers as well as sufficient polarity and dynamics to fragment membranes at critical polymer concentrations of around 1% (w/v). The MA residues ensure pH-dependent solubility due to their single negative charge at neutral pH values. The SMA polymers are synthesized commercially by conventional radical copolymerization, which provides a narrow chemical composition distribution as well as a chain length dispersity of about 2. The SMA polymerization reaction can also be carried out by reversible addition fragmentation chain transfer (RAFT) methods, which reduce chain length dispersity but result in co-monomer gradients along the chain. Acid hydrolysis is used to form the charged maleic acid copolymer which is water soluble and able to insert into membranes. They spontaneously form nanodiscs when a copolymer solution and membrane suspension are mixed to yield a clear emulsion. The resulting SMALPs are very stable in aqueous solutions, and can be freeze-dried and resuspended.

The first wave of papers in the SMALP field utilized hydrolyzed versions of SMA2000 and SMA3000 (Total Cray Valley). These have 2:1 and 3:1 styrene to maleic acid ratios, respectively, as do related Lipodisq™ reagents. Polyscope offers XIRAN 30010 and 25010 reagents to the research community, and these have similar activities and S:MA ratios of 2.3:1 and 3:1, respectively, while lacking cumene endgroups and offering a wider distribution of distinct molecular sizes. Comparison of many studies indicates that the commercially available XIRAN reagents and SMA2000 versions are the most useful for solubilizing diverse membrane proteins, including those that contain single transmembrane helices (Paulin et al., 2014) to multimers with large bundles of 36 or 48 helices, to which the polymers can adapt to form larger disc-like particles (Lee et al., 2016; Sun et al., 2018).

Comparative studies have optimized solubilization of different membrane proteins after overexpression in *E. coli*, Sf9 insect cells and H69AR cancer cells (Morrison et al., 2016). The proteins include ZipA, which spans the membrane once, the homodimeric BmrA multidrug efflux pump with six transmembrane helices, and the LeuT symporter which contains 12 transmembrane helices. Approximately ~55% of total protein is recovered into nanodiscs, which is comparable to DDM and better than octyl glucoside detergents, with SMA(2:1) giving the highest yields, purities, and activities. Smaller discs with 5 nm diameters were found using SMA(3:1) polymers. Divalent cations are better tolerated by the SMA(2:1)-based nanodiscs, with precipitation observed at calcium concentrations over 4 mM. Such studies conclude that the SMA(2:1) copolymer having an average molecular mass of 7.5–10 kDa generally offers the best performance.

The photoreaction center can be isolated from of the *Rhodobacter sphaeroides* membranes along with ~150 CL, PC, PE, PG, and sulphoquinovosyl diacylglycerol lipids using SMA2000. This contrasts with DDM and lauryldimethylamine N-oxide, which have destabilizing effects and strip away the lipids (Swainsbury et al., 2014). The SMA treatment results in elliptical particles with 12–15 nm diameters containing stably folded memteins which can be recognized by gold nanoparticles and seen by negative stain transmission EM. A survey of eight SMA polymers shows that 10 kDa range polymers with S:MA ratios of 2:1 and 3:1 perform best, while larger oligomers or tighter packing of proteins reduces solubilization efficiency. Yields can be increased by fusing cellular membranes with synthetic or biological source lipid, and lower concentrations of SMA can increase solubilization of large oligomers into discs having diameters of 50–100 nm.

A limitation of the original SMA series is their restricted pH range. The optimal pH varies for each SMA type, but is generally between 7 to 9. Lower pH levels lead to polymer aggregation, although keeping salt conditions low helps maintain SMA solubility (Scheidelaar et al., 2016). SMA polymers with lower relative styrene content are less perturbing of membranes upon insertion (Jamshad et al., 2015b). Lipids can exchange through diffusion of monomeric lipids between nanodiscs, particularly if their acyl chains are short. The molecular exchange also occurs *via* disc collisions, especially at high disc concentrations (Cuevas Arenas et al., 2017). Due to their inherent dynamics, lipids can be introduced or removed from a SMALP, and proteins can also be moved back into a liposome or membrane (Broecker et al., 2017).

The lipid interactions of various SMAs are promiscuous, showing comparable solubilization activities with different bilayer compositions and various biological source material. This is a critical advantage for unbiased preparation of memteins from the diversity membranes that proteins traffic through in cells. There is, however, a clear preference for fluid phases, which are more loosely packed and allow easier insertion of polymer groups (Zhao et al., 2016). The rates of SMA-mediated solubilization differ for lipid bilayers exhibiting short, unsaturated or cylindrical acyl chains, which facilitate insertion (Scheidelaar et al., 2015). The fragmentation of plasma and intracellular membranes occurs at discrete rates due to different levels of order (Dörr et al., 2017). The plasma membrane perforates first when XIRAN 30010 is added to HeLa cell cultures, followed by intracellular membranes, which then release contained fluorescent test proteins. All organelles in the cell are equally vulnerable, although more fluid membranes are dispersed sooner. This indicates that SMA first binds and then penetrates into fluid sections of the outer bilayer, allowing leakage of cytosolic contents, and affording entry to intracellular membranes. The rate of memtein release then presumably depends on whether the surrounding lipid is disordered or ordered. Solubilization of highly ordered memteins can require fluidizing lipids such as dimyristoyl phosphatidylcholine (DMPC) or elevated temperatures, thus providing methods for selective protein release from various compartments. Further handles are provided by derivatized SMA. A thiol group can be added to make SMA-SH (Fig. 1) to which fluorescent dyes and molecular tags can be attached (Lindhoud et al., 2016). Such Alexa Fluor 488 groups on the polymer allow measurement of distances to lipids labelled with an acceptor based on the transfer of excitation energy from a donor fluorophore to an acceptor chromophore by Förster Resonance Energy Transfer (FRET) methods. The biotin tags allow SMALPs to be affinity-

purified, as well as enabling measurement of intermolecular distances.

Memteins in rafts can be purified from cells using SMA, obviating the concerns with detergent-based extraction. Fragments of T cell membranes formed by adding 1% (w/v) concentrations of SMA(3:1) can be immunoprecipitated to collect those with glycosylphosphatidylinositol-anchored proteins and Src family kinases (Angelisová et al., 2018). Being over 250 nm in diameter, they are the largest SMALPs seen to date. Their lipids are more ordered and enriched in cholesterol, PS, sphingomyelins, ceramides and monohexosylceramides than smaller particles of under 20 nm, which have more PI, PG, PC, PE, and their ether-linked lipids. Thus SMA appears to provide a detergent-free approach to solubilize even large native rafts and structured nanodomains of lipids and receptors.

More homogeneous disc populations with diameters of approximately 28 and 10 nm can be made using reduced polydispersity SMA versions having 2:1 and 3:1 subunit ratios (Craig et al., 2016). Increasing the relative amount of styrene compromises aqueous solubility, while decreasing styrene content compromises solubilization activity. In this case a 3:1 subunit ratio of S:MA appears preferable for the formation of discs from liposomes, while 2:1 ratio more effectively solubilizes larger protein complexes. In this study, the length of the polymer does not affect disc size significantly. Steep gradients of styrene and maleic acid monomers in a short (3 kDa) SMA polymers increase solubilization efficiency (Smith et al., 2017).

4. SMA variants designed for solubilizing under diverse conditions

Zwitterionic SMA (zSMA) polymers offer utility under a broader range of buffer, pH and polycation ranges, and have solubilization activities comparable to regular SMAs (Fiori et al., 2017). These PC lipid-headgroup containing polymers are designed for analysis of proteins which require calcium or magnesium or extreme pH values. The sizes of the resulting nanoparticles are relatively uniform with diameters that range from 10 to 30 nm depending on the length of the polymer used, thus allowing small or large proteins to be contained.

Nanodiscs with diameters between 10 and 50 nm can be generated using a short SMA(1.3:1) polymer derivative bearing ethanolamines on their polar sidechains (Ravula et al., 2017a). The resulting SMA-EA nanodiscs are stable under a wide range of pH values, temperatures, and divalent cation and salt concentrations, yielding resolvable solution NMR signals of ^{15}N-labelled, folded cytochrome *b5* protein. Larger SMA-EA nanodiscs can be aligned in magnetic fields through addition of lanthanide ions such as Yb^{3+}, allowing transmembrane helix tilt angles to be measured by solid state NMR method. In particular, characteristics like pattern of resonances are observed in the 2D PISEMA (Polarization Inversion and Spin Exchange at Magic Angle) spectra of ^{15}N-labeled cytochrome b5 that predict similar angles of helices oriented in bicelles or SMALPs (Dürr et al., 2007; Ravula et al., 2017b). Adding ethylenediamine (ED) groups to this SMA yields a zwitterionic copolymer, SMA-ED, which solubilizes multilamellar vesicles outside the pH range of 5–7 (Ravula et al., 2017a). Upon dehydration, this polymer, which is termed SMAd-A, solubilizes DMPC vesicles at pH values under 6. Both of these latter derivatives tolerate high salt and divalent cation levels.

A positively charged derivative with a quaternary ammonium group termed SMA-QA can also form nanodiscs. This copolymer turns vesicles into nanodiscs with 10 to 30 nm diameters at pH values from 2 to 10 with metal ions present at up to 200 mM (Ravula et al., 2018). Another positively charged dimethylaminopropyl sidechain has been incorporated within the styrene maleimide to form “SMI” coploymers at pH values under 7.8 (Hall et al., 2018). The resulting nanodiscs have diameters of 6 nm, are stable up to 80 °C, and do not bind divalent cations. The *E. coli* cell division protein ZipA can be isolated with SMI albeit at lower yields than SMA, while the human adenosine A_{2A} and V1a vasopressin receptors purified from human embryonic kidney (HEK) 293 cells with SMI remain able to specifically bind their ligands. Thus, the charge state of SMA-related copolymers can be varied, thus allowing a broader range of polar groups, solution conditions and disc sizes to be explored, while avoiding any nonspecific electrostatic interactions.

The aromatic groups of SMA can be replaced by aliphatic chains while maintaining solubilization capability, thus further increasing the chemical universe available for nanodisc production. Using alternating diisobutylene and maleic acid sidechains result in a “DIBMA” polymer (Fig. 1) that is milder and offers advantages for isolating large labile protein complexes with native nanodiscs having diameters of 12–29 nm (Oluwole et al., 2017). Advantages of DIBMA include its transparency in ultraviolet and circular dichroism spectra (used to measure protein concentrations and secondary structures), and its compatibility with higher levels of divalent cations.

A polymethacrylate (PMA) random copolymer series has been developed as an alternative to SMA and converts DMPC liposomes into ~17 nm discs (Yasuhara et al., 2017). This polymer lacks a light absorbing aromatic group and hence works well in circular dichroism, UV/vis, and fluorescence spectroscopy experiments. Nanodiscs made of PMA as well as DMPC and DMPG in a ratio of 9:1 stabilize a helical structural intermediate state of the islet amyloid polypeptide (IAPP), rather than allowing the formation of beta amyloid fibrils. Together this constitutes a growing family of amphipathic polymers for solubilizing virtually any memtein under physiological conditions into nanodiscs of various sizes.

5. Lipid composition of memteins

SMALPs are designed to co-purify any memtein-associated molecules, with the short styrene sidechains intended to be nonspecific solubilizers of bound hydrophobic material. The retained lipid species can be identified by MS. For example, SMALPs containing the equilibrative nucleoside transporter-1 (hENT1) overexpressed in insect cells show that it holds 16 PC and 2 PE molecules but does not bind polyunsaturated lipids based on electrospray ionization MS analysis (Rehan et al., 2017). Approximately 50 PG, PE, and CL molecules of different degrees of saturation and chain length associate with each monomer of the GlpG rhomboid protease, but vary by cell type and temperature, as detected by electrospray ionization and collision-induced dissociation MS (Reading et al., 2017). The ^{13}C signals of lipid substrates and products of PagP could be resolved by solution state although the ^{1}H NMR signals of SMA polymer and contained proteins are broadened due to polydispersity and complex sizes (Knowles et al., 2009). The conversion of liposomes into lipid nanodiscs during polymer titrations can be readily tracked by NMR, as conversion into rapidly tumbling nanodiscs sharpens lipid ^{31}P resonances to reveal the critical polymer concentrations (Oluwole et al., 2017).

6. Structures of memteins in SMALPs

Recent developments in cEM technology have led to an avalanche of high resolution structures, including of memteins in SMALPs. The structure of the 464 kDa ACIII supercomplex (Sun et al., 2018), discussed in Section 2 and Fig. 2, represents a landmark in the field. In particular this study demonstrates how SMA can be used to prepare active complexes of native state transmembrane protein assemblies for determination of atomic resolution structures of post-translationally modified subunits bound to multiple lipids and cofactors. This was preceded by the 8.8 Å cEM structure of AcrB in SMA(2:1) nanodisc, with its soluble domain showing higher resolution than its transmembrane portion (Parmar et al., 2017). Eukaryotic ATP-binding-cassette transporters in SMALP also exhibit improved activity, purity and stability than those in traditional detergents, and reveal the molecular envelope of the dimeric P-glycoprotein (Gulati et al., 2014). The trimeric AcrB multidrug transporter can be purified in SMALPS *via* a His_8 tag and low salt to prevent undesirable particle association (Postis et al.,

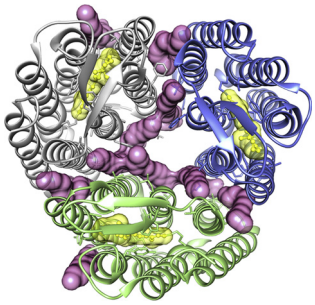

Fig. 3. Structure of a rhodopsin trimer bound to monoolein determined using SMA. a) A top view of the first crystal structure of a membrane protein, that of the seven-transmembrane microbial rhodopsin (PDB ID: 5ITC) obtained *via* SMA-LCP-mediated approach, shows three proteins of the trimer (white, blue and green) with nine monoolein lipids (in pink) occupying the inter-monomer spaces and three retinal molecules in yellow (Broecker et al., 2017). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2015). Dimers and trimers are apparent by sedimentation velocity, and negative stain EM and 3D reconstructions show an annulus of > 40 lipid molecules and polymer encircling the inner vestibule of the protein (Fig. 4).

The first 3D structure of a protein isolated using SMA was resolved by XRC with a resolution of 2.0 Å, which is superior to that of comparable crystals prepared using detergents (Broecker et al., 2017). The process involved using of DMPC to liberate the protein from tightly packed membrane into XIRAN 25010-based SMALPs, and inclusion of a pair of histidine tags for improved purification. The seven transmembrane helices of the microbial rhodopsin bind *trans*-retinal and form a trimer (Fig. 3). Their hydrophobic surfaces are shown to bind to monoolein molecules, which were used to form lipidic cubic phases for *in meso* crystallization. Bound bacterial lipids could not be resolved, presumably due to their displacement by the excess monoolein molecules.

Nanodiscs constructed with SMA typically have diameters of 10 nm but can range from 6 to 30 nm, depending on the polymer and method. Use of XIRAN 25010 polymer and synthetic lipids can yield smaller (6–10 nm) nanodiscs (Dominguez Pardo et al., 2017). An inner section of the bilayer is surrounded by an annulus of polymer, which can be contrasted by using hydrogenated and deuterated lipids in small angle neutron scattering (SANS) experiments (Jamshad et al., 2015b). The styrene groups pack against lipid acyl chains based on distances detected by NMR (Jamshad et al., 2015b; Orwick et al., 2012). The integral membrane protein KCNE1 solubilized in SMA(3:1) nanodiscs has been studied by electron paramagnetic resonance (EPR) experiments (Sahu et al., 2017). These EPR studies show that sidechains of the spin-labeled residues which located in the aqueous phase are more mobile than those which are located within the bilayer. Such studies indicate that SMA boosts the relative signal to noise and phase memory time in double electron–electron resonance (DEER) spectra, providing more precise measurements of distances.

NMR can be used to characterize structures, dynamics and interactions of transmembrane proteins in SMALPs. A stable helical structure is formed by the Pf1 bacteriophage coat protein in magnetically-oriented -30 nm discs composed of XIRAN 25010, DMPC and DMPG (Radoicic et al., 2018). The ^{15}N-resolved solid state NMR signals are sharper than that can be obtained in bicelles or peptide-based discs, and indicate larger order parameters. The solid state NMR spectra of the magnetically aligned ^{15}N-labeled cytochrome b_5 protein in ~50 nm discs formed by SMA-EA and DMPC indicate a stable transmembrane helix while the signals of the soluble domain are motionally averaged out (Ravula et al., 2017b). The bacterial zinc diffusion facilitator CzcD isa 34 kDa protein solubilized with XIRAN 25010 or XIRAN 30010 retains a layer of bound lipid (Bersch et al., 2017). The resulting 10–15 nm nanodiscs exhibit resolvable solid-state NMR signals for amide or methyl groups that are deuterated and selectively re-protonated. Resonance assignments can be transferred by comparison with the NMR spectra of the solution state of the isolated cytoplasmic domain. The elucidation of novel 3D structures of proteins in SMALPs by solution NMR methods remains a challenge due to polymer polydispersity and broad linewidths. New formulations are being actively developed to reduce heterogeneity and aid in the assignment and structural characterization of transmembrane and attached soluble domains.

7. Functions of memteins in SMALPs

Enzymes which work together are often organized as co-localized units in membranes. Isolating them intact has been technically difficult in the past. The plant metabolon that produces the glucoside dhurrin is a case in point. It comprises three membrane proteins along with a soluble glycotransferase protein. The entire complex can be isolated from microsome membranes by treating with SMA2000 to form nanodiscs of diameter of 10–25 nm and yields of 80%, while the complex is broken down by traditional detergents such as cholate (Laursen et al., 2016). The soluble subunit binds to and modulates the nanodisc-based

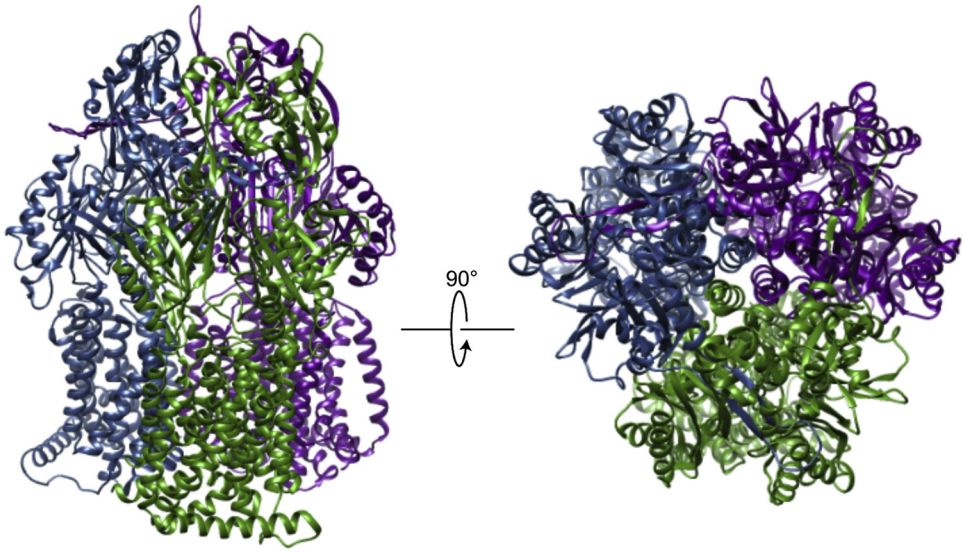

Fig. 4. Trimeric transporter structure elucidated using SMA. Top and side views of the secondary transporter AcrB trimer in SMALPs resolved by EM (EMD: 3887) (Postis et al., 2015) using the crystal structure obtained with DDM (PDB ID:4ZLJ), with the three subunits colored green, blue and purple. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

memtein, the activity of which depends on bound negatively charged phospholipids.

As memteins containing G protein-coupled receptors (GPCRs) remain the highest value superfamily of therapeutic targets, their solubilization in SMALPs has been a major goal. The adenosine receptor is solubilized by SMA(2:1) treatment of expression hosts including *Pichia pastoris* and HEK 293 T cells, and is stable and binds ligand normally. This GPCR can be stored and freeze-thawed in SMALPs, and is stable for seven times the length of time as detergent preparations (Jamshad et al., 2015a). The melatonin and ghrelin receptors can be placed into 13 nm nanodiscs by applying either SMA(2:1) or SMA(3:1) to liposomes or *Pichia pastoris* membranes, and still bind their ligands and transduce signals as expected (Logez et al., 2016). The nucleoside transporter hENT1, which also transport chemotherapeutic agents, can be isolated from insect cells using XIRAN 30010 (Rehan et al., 2017). The polymer was added at low levels (0.25% w/v) with cholesteryl hemisuccinate at low temperature in order to avoid protein degradation and inactivation. The SMALP'd protein displays the expected level of inhibitor binding, while it is destabilized by conventional detergents such as decyl maltoside. Together this indicates that SMALPs are a viable way to present drug targets, including those that may be too unstable, scarce or dependent on lipids for detergent based extraction.

Tetraspanins are challenging targets due to their small sizes, oligomeric states, disulfide bonds, glycosylation and palmitoylation sites and the paucity of biochemical activity assays. Five human members of this family were expressed in *S. cerevisiae* and solubilized by three different SMA copolymers. The yield of TSPAN7 was comparable to conventional detergents, while CO-029, TSPAN12 and TSPAN18 yields were higher in detergents, and CD63 was resistant to any approach (Skaar et al., 2015). The organized superstructure of such memteins emphasize the need for further development of tools for efficient dispersal of very large, ordered networks.

The mitochondrial cytochrome *c* oxidase from yeast contains 19 transmembrane helices. This multicomponent complex can be isolated from *Saccharomyces cerevisiae* using SMA(3:1). The resulting complexes are active in the ~12 nm discs, which contain PC, PE and CL, and display the expected ligand binding and reaction kinetics (Long et al., 2013). However, free polymer acts as an inhibitor, and thus ways to remove it after disc formation would be desirable, leading to plans to develop affinity tags. The entire biological assembly contains two more weakly bound respiratory supercomplex factors proteins that dissociate upon exposure to detergent but are retained in SMALPs (Smirnova et al., 2016). The solubilized particles exhibit dimensions of 11 nm and 14 nm, which can fit in the entire supercomplex including 11 protein subunits and assorted mitochondrial lipids in order to pump protons across the membrane.

Proteins are secreted through bacterial inner membranes by the holo-translocon assembly, which comprises SecYEG, SecDF, YajC and YidC subunits. All these components in addition to associated bacterial lipids are solubilized together from the *E. coli* membrane by SMA(2:1) copolymer and can be detected with available antibodies (Komar et al., 2016). The biologically relevant complex of the SecYEG translocon copurifies with the motor protein SecA and essential CL, PE and PG lipids when treated with SMA(3:1) polymer but not with conventional detergents (Prabudiansyah et al., 2015). When this memtein is transferred into proteoliposomes using Bio-Beads the complex is able to translocate transmembrane proteins and interact with the ribosome.

The physiological structures and functions of α-synuclein remain mysteries, yet are important for tackling Parkinson's disease. The protein assumes unstructured or helical conformations as monomers and tetramers and is present on membranes and in cytosol. Its biological states can be delineated by incubating with PC-containing SMALPs. In so doing the protein disaggregates and maintains a helical conformation, but loses the ferrireductase activity associated with its form in biological membranes (McDowall et al., 2017). This infers that copolymers with reduced protein affinity would be beneficial, and highlights the need to compare SMALPs with other systems used to study memteins.

8. Memteins in detergent

Membrane protein structures in detergent-lipid mixtures continue to provide useful insights into the structures and interactions of memteins, as exemplified by the series of structures of complexes that follows. Detergents are particularly useful for structural analysis of stable and abundant recombinant membrane proteins that have assayable biochemical activities that can be monitored. Careful optimization of the LDAO and dodecylphosphocholine (DPC) detergent-based purification of the photosynthetic LHC- reaction centre supercomplex from a thermophilic purple sulfur bacterium yielded its calcium-stabilized crystal structure (Yu et al., 2018). As seen at a resolution of 1.9 Å, 16 heterodimers surround the reaction center, which is comprised of 4 subunits, as well as six quinone cofactors in the midst of what appear to be ten PG, nine CL, and two PE molecules. The lipids are asymmetrically distributed, with CL molecules lining the cytoplasmic side of the memtein and head groups positioned towards the membrane surface. The other two lipid types are present on leaflet surfaces and engage with Arg and Lys residues on the cytoplasmic interface. SMALPs have also been used to characterize the photosystem I (PSI) complex that is found in spinach thylakoids, which exhibits dimensions of 140 × 180 Å, *i.e.* bigger than the typical 10 nm nanodiscs. Nonetheless this complex solubilizes with SMA3000 with its 17 protein subunits interfacing with LHC protein trimers that deliver excitation energy (Bell et al., 2015), demonstrating the adaptability of the polymer encasement.

The mammalian two-pore channel TPC1 protein is modulated by its endolysosomal ligand PI(3,5)P_2. This phosphoinositide can be added back after extraction with DDM and cholesteryl hemisuccinate from overexpressing HEK 293 T cells. The resulting 3.2 Å cEM structure shows a pair of 6 transmembrane bundles along with the positions of acetylglucosamine groups attached to a pair of asparagines (She et al., 2018). A set of Lys and Arg residues mediate binding of a single PtdIns (3,5)P_2 molecule which induces channel opening. The *Gloeobacter violaceus* ligand-gated ion channel GLIC4 can be overexpressed in *E. coli* and extracted with DDM. There are 6 DDM and 15 partial PC lipid molecules arranged in a bilayer type fashion inside the channel and perimeter of the apparently open pentameric channel, as seen in its 2.9 Å resolution crystal structure (Bocquet et al., 2009). Hence the use of detergents can be inferred to block channel function, with some exposed lipid headgroups being disordered and difficult to resolve. The detailed conformations of such memteins including their channels remain of great interest.

The *Saccharomyces cerevisiae* oligosaccharyltransferase (OST) resides in the endoplasmic reticulum membrane. This complex of eight proteins has been resolved by cEM following solubilization with digitonin (Bai et al., 2018). This 3.5 Å resolution structure shows how one phospholipid sits at the substrate-binding surface while seven others mediate inter-subunit interactions *via* lipid headgroup interactions with aromatic and polar amino acid residues while their acyl chains are nestled among hydrophobic sidechains. An N-glycan also "glues" subunits together, indicating a structural role for lipids in this complex that mediates co-translational protein N-glycosylation.

Structures of the PagP phospholipase are available in three different detergent systems, providing insights from many angles. The 1.4 Å XRC structure of PagP crystallizes directly from a cosolvent system composed of 2-methyl-2,4-pentanediol (MPD) and sodium dodecyl sulfate (SDS) (Cuesta-Seijo et al., 2010). Its eight-stranded barrel structure is similar to that solved in LDAO (Ahn et al., 2004) or DPC and octyl glucoside (OG) (Hwang et al., 2002). However, the conformations of the four extracellular loops differ, reflecting their flexibility and involvement in substrate binding and catalysis as well as their crystal lattice contacts. The interior pocket that provides access to palmitate

chains in the sn-1 position of substrate access is occluded by detergent molecules, which hence are inhibitory. Five SDS molecules are arrayed across the hydrophobic surface of the PagP protein, with aliphatic chain being more ordered than headgroups and packed into similar crevices occupied by LDAO. When solubilized into 11 nm SMALPs, PagP co-purifies with 11 bound DMPC molecules and is more stably folded than in micelles, and is active in phospholipase assays, consistent with the absence of inhibitory detergents that could occlude the active site (Knowles et al., 2009). Thus the stability of this cooperatively folded barrel generally withstands the destabilizing forces of detergents, while its flexible loops and active site are vulnerable. A similar barrel, OmpF, forms very stable trimers that pack tightly in the outer membrane of bacteria *in vivo*. However, unlike in detergent, this protein is entirely wrapped in lipid molecules in two different *in meso* crystal forms which were determined to resolutions of 1.9 and 2.0 Å (Efremov and Sazanov, 2012). While monoolein molecules form extensive van der Waals contacts and engage with exposed Trp and Tyr residues, there are no direct inter-protein contacts, and physiological lipids had been eliminated. In a further development, a detergent-solubilized OmpF relative forms trimers that bind four lipopolysaccharide (LPS) molecules, as seen in a XRC structure of OmpE36 solved to 1.45-Å resolution (Arunmanee et al., 2016). Although not required for folding, the LPS molecules form extensive van der Waals and polar interactions with intramembrane and interfacial residues, respectively. These lipid-protein interactions recur across the densely packed outer membrane, allowing these memteins to form close-knit networks on gram-negative bacterial surfaces to ensure an impenetrable barrier.

Mixed micelles can be used as realistic mimics of endogenous membrane surfaces to deduce stereospecific organelle recognition mechanisms by stable peripheral membrane domains. This approach has been demonstrated with (Fab1, YOTB, Vac1 and EEA1) FYVE, phox homology (PX) and pleckstrin homology (PH) domains. The FYVE domains are the most structurally efficient phosphatidylinositol phosphate (PIP) binding domains, consisting of only 65 residues in a fold stabilized by two zinc binding clusters that almost exclusively recognizes phosphatidylinositol 3-phosphate (PI3P) molecules on endosomes. The recognition of early endocytic membranes by the FYVE domain of the EEA1 protein depends on stereospecific binding of the PI3P headgroup and simultaneous insertion of proximal hydrophobic residues into the bilayer (Dumas et al., 2001; Kutateladze and Overduin, 2001). These interactions are supported by binding of accessory phosphatidylserine (PS) and PC headgroups, and are strengthened by the low pH that is encountered around these compartments (Dancea et al., 2008; Kutateladze et al., 2004; Lee et al., 2005). The insertion of the FYVE domain into PI3P-containing nanodiscs involves a slightly more extensive surface than seen in micelles, potentially reflecting attraction to a flat rather than convex bilayer (Yokogawa et al., 2012). The dimers of FYVE domain-containing proteins employ a similar orientation for membrane docking, and this then provides avidity and opportunities for downstream membrane fusion events which are regulated by partner proteins (Dumas et al., 2001; Hayakawa et al., 2004).

PX domains generally recognize PI3P in endocytic membranes but utilize a fold that differs from FYVE domains. Their ligand binding mode is apparent from the structure of the $p40^{phox}$ PX domain bound to a short chain PI3P molecule (Bravo et al., 2001), as well as by hydrophobic insertion of a neighbouring loop into mixed micelles, as shown with the Vam7p PX domain (Cheever et al., 2001). Phosphoinositide binding occurs within a pocket containing conserved Arg, Lys and Tyr residues. The Grd19p PX structure contains a bound PI3P molecule in the same pocket (Zhou et al., 2003). A similar binding mode is found in the Snx9 PX domain, which can accommodate either PI3P or $PI(4,5)P_2$ through a larger pocket, with the attached BAR domain offering a broad concave basic surface that recognizes curved bilayers (Pylypenko et al., 2007). A secondary site is separated from the first pocket that binds PIPs by the membrane insertion loop, and can simultaneously accommodate acidic phospholipids, as demonstrated for the $p47^{phox}$ PX domain. This multivalent interaction with proximal lipids boosts membrane avidity (Kanai et al., 2001; Karathanassis et al., 2002). The mixed micelle-inserted Vam7p PX domain displays extensive interactions with headgroups and tails of several PI3P and phosphocholine molecules, as resolved by NMR (Dancea et al., 2008). The Snx3 protein utilizes a similar mechanism. It dips a membrane insertion loop non-specifically into the bilayer, being guided by its electrostatic polarity that exposes its retromer-binding termini. It can then diffuse laterally in the membrane until a PI3P ligand is encountered and stereospecifically recognized, leading to deeper insertion as well as synergistic contacts with neighbouring lipid molecules. Its PX domain, as well as those of many other sorting nexins, also exhibit a so-called PIP stop. This regulatory feature consists of a conserved Ser or Thr residue at the rim of the PI3P binding site that becomes phosphorylated in order to release the protein from the membrane and into the cytosol. Solution NMR structures of lipid and mixed micelle-complexed states of Snx3 reveal how this switch operates to determine localization of the associated retromer complex, thus controlling receptor traffic in cells (Lenoir et al., 2018).

The PH superfamily comprises 285 human proteins that contain 334 PH domains, about 61% of which associate with membranes based on Membrane Optimal Docking Area (MODA) analysis (Lenoir et al., 2015). These interactions involve insertion by the first β hairpin loop and lipid docking to either one or both sides of this protruding loop *via* basic and hydrophobic residues. Lipid, micelle and bicelle binding by the PH domain of FAPP1 has been studied by biophysical, NMR and computational methods (Lenoir et al., 2014, 2010; Overduin et al., 2015). This pinpoints the conserved aromatic and aliphatic sidechains that insert into the membrane and provide PIP specificity. A two pronged network of electrostatic interactions and hydrogen bonds bind a patch of lipid, as is reminiscent of FYVE and PX domains, which also are attracted to negatively charged lipid bilayers such as those that include PS. The FAPP1 PH domain and related FAPP2 protein both insert selectively into disordered PI4P-containing membranes, suggesting that the bilayer must be deformable to allow ready access to the PI4P headgroup. The dynamin protein binds to lipid bilayers *via* its PH domain and self-assembles through its activated guanine nucleotide-binding (GTPase) domain to form a membrane fission machine. The 3.75 Å resolution cEM structure of the protein assembly formed on PS-containing liposomes displays different interdomain orientations than the lipid-free state, with the PH and helical stalk domains positioned within membranes in order to mediate tubulation reactions (Kong et al., 2018), highlighting the complex interplay between lipid-protein and protein-protein interactions in memtein assembly and activity.

A different mode of membrane binding *via* a pair of surfaces is exhibited by extracellular metalloproteases. These enzymes flip like pancakes on the surface of a cell using two separate membrane binding surfaces, thus localizing themselves by their sites of action. The MMP-12 catalytic domain prefers membranes with negatively charged lipids or unsaturated lipids like palmitoyloleoyl (POPC), forming an extensive array of hydrogen bonding, salt bridges and hydrophobic contacts with the lipid head groups (Koppisetti et al., 2014). This ambidextrous binding process does not occlude the active site, but rather excludes binding of a protein inhibitor. The MMP-7 zymogen binds zwitterionic bicelles superficially *via* loops around the edges of its β sheet, leading to allosteric modulation of the active site (Prior et al., 2015). The presence of anionic lipids such as cholesterol 3-sulfate draws the protein deeper into the bilayer, altering its insertion angle and broadening its interface. By doing so the rocking of protein in the membrane becomes more restricted, while affording access to pericellular protein substrates. These studies show parallels between the membrane binding mechanisms of cytosolic and extracellular proteins, and suggest that common principles will emerge that could also provide insight into how transmembrane proteins behave.

9. Lipidic cubic phase-derived structures

Over 525 *in meso* structures of proteins have been solved using LCP methods to date. These show how memteins could be modelled using crystal structures although physiological lipid ligands are typically displaced by, for example, added monoacylglycerol molecules. The 1.95 Å structure of the cobalamin transporter, BtuB, as solved by LCP methods, contains 11 monoolein molecules in a bilayer arrangement (Cherezov et al., 2006). No native *E. coli* lipids are evident due to extraction in OG detergent and the use of LDAO detergent during purification. One set of five lipids is arrayed where the outer leaflet would lie against the barrel, while another five are arrayed where the inner leaflet would lay. The headgroups are oriented toward the expected membrane-water interfaces of the protein, while the acyl chains interdigitate there termini and align across the hydrophobic interface of the barrel perpendicular to the β-strands. Another lipid molecule lies near the mid-plane of the bilayer, suggesting an unnatural pose, illustrating an inherent risk of artifacts resulting from membrane protein crystallization.

The crystal structure of bacteriorhodopsin reveals that native purple membrane lipids occupy the central region within its trimeric complex. Lipids are arranged within crevices in the hydrophobic surface around the trimer, indicative of their positions within a bilayer (Luecke et al., 1999). The 1.8 Å resolution structure of the light-driven chloride pump halorhodopsin also shows bound monoolein molecules (Kolbe et al., 2000). Ten lipids collect on the periplasmic half of this helical protein in the trimer interface with spacings consistent with their packing density, which differs from that of the branched, phytanol-containing lipids of haloarchaea. Three palmitates are deep inside the cytoplasmic side of the trimer's transport site, with their C_{10}-C_{16} acyl tails lying within a channel lined by Ala, Ile, Pro, and Thr residues. Hence this demonstrates how endogenous lipids can be displaced and how active sites can become occupied by monoolein, thus compromising function.

Undecaprenyl pyrophosphate phosphatase (UppP) recycles the lipid carrier that is used to build the bacterial cell wall. Its crystal structure determined at a resolution of 2.0 Å shows all ten transmembrane α-helices that form an active site deep in the membrane (Workman et al., 2018). A pair of monoolein molecules are oriented at the cytoplasmic side of the dimer interface, potentially stabilizing it, while another monoolein sits in the substrate binding cleft and two more decorate the portal to the active site. These examples demonstrate how physiological lipids can be displaced by monoolein. This limits the insights that can be gathered about the mechanism of stereospecific ligand recognition.

10. Amphipol-solubilized membrane proteins

Amphipols are a set of amphipathic polymers that are based on a hydrophilic backbone with hydrophobic sidechains that are intended to associate with hydrophobic surfaces of membrane proteins, stabilizing them for solubilization in aqueous environments (Popot et al., 2011). However, they require that proteins go through a detergent phase, and issues regarding polymer aggregation and scaffold-function interference have been raised (Picard et al., 2006; Zhang et al., 2017). Nonetheless, several structures have been resolved by cEM using amphipols and reveal positions of bound lipids. The human γ-secretase complex resolved to 3.4 Å in amphipols reveals the positions of several N-linked glycans and lipid molecules. One lipid is found at the interface between catalytic subunit presenilin 1 (PS1) and scaffolding protein Aph-1 subunits, and another bridges the transmembrane elements of the nicastrin and Aph-1 subunits *via* acyl chain contacts with several hydrophobic residues and hydrogen bonding of its phosphate group *via* Arg and Gln residues (Bai et al., 2015). The Polycystic Kidney Disease 2 (Pkd2) protein overexpressed in HEK 293 cells, extracted with DDM and stabilized by either amphipol A8-35 or MSP nanodiscs yields similar cEM structures of the homotetrameric ion channel. An annulus of lipid bilayer is evident around the protein, and the positions of several ordered lipids can be seen between the crevices at subunit interfaces, as well as structured N-acetylglucosamine groups attached to three asparagines (Shen et al., 2016). The amphipol-stabilized trimer retains 12 tightly bound lipids, while its structure in MSP nanodiscs could be resolved to 3 Å resolution, indicating the complementarity of the two solubilization methods.

11. Nanodiscs formed by membrane-scaffolding proteins

The first nanodisc system developed for biochemical applications employs membrane scaffold protein (MSP) constructs of different sequences and lengths (Bayburt et al. 2002) that are originally derived from apolipoprotein A-1 structures (Brouillette et al. 1984). A pair of largely helical MSPs encircle the lipid bilayer and are zippered together by a series of salt bridges and cation-π interactions (Bibow et al., 2017). The resulting discs are stable particles in aqueous solution and possess diameters ranging from 6 to 17 nm (Grinkova et al., 2010; Hagn et al., 2013; Wang et al., 2015). They can hold in the range of 65 POPC or 90 DPPC molecules in the case of 9.6 nm nanodiscs made by MSP1D1, or 85 POPCs or 115 DPPCs in the case of 10.5 nm diameter nanodiscs made by MSP1E1D1 polypeptides (Ritchie et al. 2009). The stability of MSP-based nanodiscs and the ability to attach affinity tags have led to their use for functional and structural analysis of many membrane proteins (Denisov and Sligar, 2017) including oligomers or complexes between membrane proteins (Boldog et al. 2006; Shih et al. 2005). The optimization of MSP systems for both solution NMR (Hagn and Wagner, 2015; Zhang et al., 2016) and solid state NMR applications (Li et al., 2006; Opella and Marassi, 2017) is also benefiting other applications. Cryo-EM is increasingly used to structurally characterize membrane proteins in nanodiscs. Several cEM structures solved using nanodiscs reveal positions of individual lipids. For example, the *E. coli* SecYEG complex reconstituted into nanodiscs reveals phospholipid headgroups that appear to directly contact ribosomal protein L24 and rRNA helix H59, suggesting an integral role of lipids in ribosome function (Frauenfeld et al., 2011).

High resolution insights into memtein function are given by the TRPV1 ion channel (Gao et al., 2016). The recombinant protein overexpressed in mammalian cells could be solubilized with DDM, which needed to be replaced with soybean polar lipids while being incorporated in nanodiscs. Both annular and regulatory lipids form ordered contacts with the TRPV1 as resolved in the ~150 Å nanodiscs formed by MSP2N. The 2.9 Å resolution of TRPV1 tetramer reveals how Arg, Phe, Tyr and Trp sidechains from the channel and toxin molecules bind to PC lipid headgroups and acyl tails, which must reorient from bilayer poses to become engaged. Importantly, bridging contacts between PC molecules and the channel contact the toxin, which inserts almost halfway through the position of the outer leaflet of the bilayer. This shows that antagonists are directly recognized by both lipid and protein moieties in memteins, and underscores the integral role of lipids in protein function. A PIP headgroup engages a charged pocket inside the channel which is lined by Arg, Lys and Glu residues. This supports a key role of this signaling lipid as a competitive antagonist, negative allosteric modulator and positively acting co-factor that primes the channel for activation. Hence protein and lipid function are interdependent based on an increasing number of structures, and point to the crucial role that memteins play in biology.

12. Summary and prospects

In the past decade, the field of membrane structural biology has grown tremendously through the emergence of SMA-related polymers and native nanodiscs to isolate sections of membrane, and the concurrent development of cEM, NMR, XRC and MS technologies to resolve their structures. Their confluence has led to this proposal that much of biology is fundamentally driven by the specific activities of proteins in contact with a layer of biological lipid molecules, *i.e.* memteins. A

wealth of accumulated data from many research groups and methods has been considered here, and is distilled into a single new word. Without such a word it is difficult to discuss, focus on and refine this concept. It is hoped that giving complexes of a continuous layer of biological lipids with proteins an intuitive label could spark further interest and studies that allow the research community to more fully explore how many more memteins work at an atomic resolution under a wide spectrum of different timescales and cellular contexts. New approaches for exploring the lipidomics and protemics of membrane compartments could expose the intricacies of biological machines such as those responsible for fibril and cytoskeletal assembly, cell adhesion and organelle biogenesis. As the tools become adapted for high throughput screening of native nanodiscs, novel ligands including lead molecules and lipid modulators may emerge. As much of drug discovery and pharmacology depend on an accurate understanding of proteins situated inside membranes, there is the possibility of entirely new classes of therapeutic agents arising from the discovery of memtein targets. There is much room for further improvement, given that the physical mechanisms of how the polymers bind, concentrate and disperse membranes into consistently sized discs with a layer of lipid surrounding a protein remain largely unknown. The chemical optimization of SMA is increasingly guided by the knowledge gained from synthesis and testing of an ever growing family of polymers. This is starting to reveal what appear to be optimal properties such as polymer sizes, residue charge and hydrophobicity, steric restraints, blockiness and polydispersity. However, the universe of synthetic polymers is larger than that of proteins, and hence we have probably not yet converged on the best system, nor is there likely going to be a single best solution for the array of possible biological systems, target types and assays. Already the dimensions of reported SMALPs span 5–100 nm with shapes varying from circular to ellipsoid to irregular, depending on the polymer used, conditions and cargo. How these nanodiscs could be deployed as protein- and lipid-containing sensors, transducers and delivery vehicles to harness various biological machines remains largely unexplored. Designing and making new polymers and tags is technically demanding, and scaling production of standardized polymers for reproducible results requires having industry on board. Hence an open innovation approach that involves academic researchers, pharmaceutical companies, and manufacturers of novel chemical products, analytical equipment and assay kits is needed. The SMALP Network is a grass roots community that has attempted to bring these parties together and has fertilized the field with new ideas, collaborations and polymers, and is intended to continue foster support of the field as it enters a new decade and becomes increasingly mainstream.

Author contributions

M.O. and M.E. wrote the manuscript and prepared the figures.

Acknowledgments

This project has been supported by the Campus Alberta Innovation Program (RCP-12-002C), NSERC Discovery (RGPIN-2018-04994) and Alberta Prion Research Institute / Alberta Innovates Bio Solutions (201600018) grants awarded to M.O. and TMIC which is supported by the Canada Foundation for Innovation and Genome Canada. We thank the members of the SMALP network including Stefan Scheidelaar for discussions and for sharing their polymers and methods. We apologize to anyone whose results could not be cited due to space limitations.

References

Ahn, V.E., Lo, E.I., Engel, C.K., Chen, L., Hwang, P.M., Kay, L.E., Bishop, R.E., Privé, G.G., 2004. A hydrocarbon ruler measures palmitate in the enzymatic acylation of endotoxin. EMBO J. 23, 2931–2941.

Angelisová, P., Ballek, O., Sýkora, J., Benada, O., Čajka, T., Pokorná, J., Pinkas, D., Hořejší, V., 2018. The use of styrene-maleic acid copolymer (SMA) for studies on T cell membrane rafts. Biochim. Biophys. Acta Biomembr. 1861 (1), 130–141.

Arunmanee, W., Pathania, M., Solovyova, A.S., Le Brun, A.P., Ridley, H., Baslé, A., van den Berg, B., Lakey, J.H., 2016. Gram-negative trimeric porins have specific LPS binding sites that are essential for porin biogenesis. Proc. Natl. Acad. Sci. 113 (34), E5034–43.

Bagatolli, L.A., Ipsen, J.H., Simonsen, A.C., Mouritsen, O.G., 2010. An outlook on organization of lipids in membranes: searching for a realistic connection with the organization of biological membranes. Prog. Lipid Res. 49 (4), 378–389.

Bai, L., Wang, T., Zhao, G., Kovach, A., Li, H., 2018. The atomic structure of a eukaryotic oligosaccharyltransferase complex. Nature 555, 328–333.

Bai, X., Yan, C., Yang, G., Lu, P., Ma, D., Sun, L., Zhou, R., Scheres, S.H.W., Shi, Y., 2015. An atomic structure of human γ-secretase. Nature 525, 212–217.

Bayburt, T.H., Sligar, S.G., 2010. Membrane protein assembly into Nanodiscs. FEBS Lett. 584 (9), 1721–1727.

Bell, A.J., Frankel, L.K., Bricker, T.M., 2015. High yield non-detergent isolation of photosystem I-light-harvesting chlorophyll II membranes from spinach thylakoids: implications for the organization of the PS I antennae in higher plants. J. Biol. Chem. 290, 18429–18437.

Bersch, B., Dörr, J.M., Hessel, A., Killian, J.A., Schanda, P., 2017. Proton-detected solid-state NMR spectroscopy of a zinc diffusion facilitator protein in native nanodiscs. Angew. Chemie - Int. Ed. 56, 2508–2512.

Bibow, S., Polyhach, Y., Eichmann, C., Chi, C.N., Kowal, J., Albiez, S., McLeod, R.A., Stahlberg, H., Jeschke, G., Güntert, P., Riek, R., 2017. Solution structure of discoidal high-density lipoprotein particles with a shortened apolipoprotein A-I. Nat. Struct. Mol. Biol. 24, 187–193.

Bocquet, N., Nury, H., Baaden, M., Le Poupon, C., Changeux, J.P., Delarue, M., Corringer, P.J., 2009. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. Nature 457, 111–114.

Böhm, C., Möhwald, H., Leiserowitz, L., Als-Nielsen, J., Kjaer, K., 1993. Influence of chirality on the structure of phospholipid monolayers. Biophys. J. 64, 553–559.

Bravo, J., Karathanassis, D., Pacold, C.M., Pacold, M.E., Ellson, C.D., Anderson, K.E., Butler, P.J.G., Lavenir, I., Perisic, O., Hawkins, P.T., Stephens, L., Williams, R.L., 2001. The crystal structure of the PX domain from p40phox bound to phosphatidylinositol 3-phosphate. Mol. Cell 8, 829–839.

Broecker, J., Eger, B.T., Ernst, O.P., 2017. Crystallogenesis of membrane proteins mediated by polymer-bounded lipid nanodiscs. Structure 25, 384–392.

Brohawn, S.G., Campbell, E.B., MacKinnon, R., 2014. Physical mechanism for gating and mechanosensitivity of the human TRAAK K+ channel. Nature 516, 126–130.

Bruzik, K.S., Hakeem, A.A., Tsai, M.D., 1994. Are D- and L-chiro-Phosphoinositides Substrates of Phosphatidylinositol-Specific Phospholipase C? Biochemistry 33, 8367–8374.

Cantor, R.S., 2002. Size distribution of Barrel-Stave aggregates of membrane peptides: influence of the bilayer lateral pressure profile. Biophys. J. 82, 2520–2525.

Chalfant, C.E., Kishikawal, K., Mumby, M.C., Kamibayashi, C., Bielawska, A., Hannun, Y.A., 1999. Long chain ceramides activate protein phosphatase-1 and protein phosphatase-2A. Activation is stereospecific and regulated by phosphatidic acid. J. Biol. Chem. 274, 20313–20317.

Cheever, M.L., Sato, T.K., De Beer, T., Kutateladze, T.G., Emr, S.D., Overduin, M., 2001. Phox domain interaction with Ptdlns(3)P targets the Vam7 t-SNARE to vacuole membranes. Nat. Cell Biol. 3 (7), 613–618.

Cherezov, V., Yamashita, E., Liu, W., Zhalnina, M., Cramer, W.A., Caffrey, M., 2006. In meso structure of the cobalamin transporter, BtuB, at 1.95 Å resolution. J. Mol. Biol. 364, 716–734.

Craig, A.F., Clark, E.E., Sahu, I.D., Zhang, R., Frantz, N.D., Al-Abdul-Wahid, M.S., Dabney-Smith, C., Konkolewicz, D., Lorigan, G.A., 2016. Tuning the size of styrene-maleic acid copolymer-lipid nanoparticles (SMALPs) using RAFT polymerization for biophysical studies. Biochim. Biophys. Acta Biomembr. 1858, 2931–2939.

Cuesta-Seijo, J.A., Neale, C., Khan, M.A., Moktar, J., Tran, C.D., Bishop, R.E., Pomès, R., Privé, G.G., 2010. PagP crystallized from SDS/Cosolvent reveals the route for phospholipid access to the hydrocarbon ruler. Structure 18, 1210–1219.

Cuevas Arenas, R., Danielczak, B., Martel, A., Porcar, L., Breyton, C., Ebel, C., Keller, S., 2017. Fast Collisional Lipid Transfer Among Polymer-Bounded Nanodiscs. Sci. Rep. 7, 45875.

Cuevas Arenas, R., Klingler, J., Vargas, C., Keller, S., 2016. Influence of lipid bilayer properties on nanodisc formation mediated by styrene/maleic acid copolymers. Nanoscale 8, 15016–15026.

Dancea, F., Kami, K., Overduin, M., 2008. Lipid interaction networks of peripheral membrane proteins revealed by data-driven micelle docking. Biophys. J. 94, 515–524.

Dominguez Pardo, J.J., Dorr, J.M., Iyer, A., Cox, R.C., Scheidelaar, S., Koorengevel, M.C., Subramaniam, V., Killian, J.A., 2017. Solubilization of lipids and lipid phases by the styrene maleic acid copolymer. Eur. Biophys. J. 46, 91–101.

Dörr, J.M., van Coevorden-Hameete, M.H., Hoogenraad, C.C., Killian, J.A., 2017. Solubilization of human cells by the styrene-maleic acid copolymer: insights from fluorescence microscopy. Biochim. Biophys. Acta 2736, 30260–30262.

Dorr, U.H.N., Soong, R., Ramamoorthy, A., 2013. When detergent meets bilayer: birth and coming of age of lipid bicelles. Prog. Nucl. Magn. Reson. Spectrosc. 69, 1–22.

Dumas, F., Sperotto, M.M., Lebrun, M.C., Tocanne, J.F., Mouritsen, O.G., 1997. Molecular sorting of lipids by bacteriorhodopsin in dilauroylphosphatidylcholine/ distearoylphosphatidylcholine lipid bilayers. Biophys. J. 73, 1940–1953.

Dumas, J.J., Merithew, E., Sudharshan, E., Rajamani, D., Hayes, S., Lawe, D., Corvera, S., Lambright, D.G., 2001. Multivalent endosome targeting by homodimeric EEA1. Mol. Cell 8, 947–958.

Dürr, U.H.N., Yamamoto, K., Im, S.C., Waskell, L., Ramamoorthy, A., 2007. Solid-state NMR reveals structural and dynamical properties of a membrane-anchored electron-

carrier protein, cytochrome b5. J. Am. Chem. Soc. 129 (21), 6670–6671.
Efremov, R.G., Sazanov, L.A., 2012. Structure of Escherichia coli OmpF porin from lipidic mesophase. J. Struct. Biol. 178 (3), 311–318.
Epand, R.M., Rychnovsky, S.D., Belani, J.D., Epand, R.F., 2005. Role of chirality in peptide-induced formation of cholesterol-rich domains. Biochem. J. 390, 541–548.
Fiori, M.C., Jiang, Y., Altenberg, G.A., Liang, H., 2017. Polymer-encased nanodiscs with improved buffer compatibility. Sci. Rep. 7, 7432.
Frauenfeld, J., Gumbart, J., Sluis, E.O.V.Der, Funes, S., Gartmann, M., Beatrix, B., Mielke, T., Berninghausen, O., Becker, T., Schulten, K., Beckmann, R., 2011. Cryo-EM structure of the ribosome-SecYE complex in the membrane environment. Nat. Struct. Mol. Biol. 18, 614–621.
Gao, Y., Cao, E., Julius, D., Cheng, Y., 2016. TRPV1 structures in nanodiscs reveal mechanisms of ligand and lipid action. Nature 534, 347–351.
Gessmann, D., Chung, Y.H., Danoff, E.J., Plummer, A.M., Sandlin, C.W., Zaccai, N.R., Fleming, K.G., 2014. Outer membrane β-barrel protein folding is physically controlled by periplasmic lipid head groups and BamA. Proc. Natl. Acad. Sci. 111, 5878–5883.
Gonen, T., Cheng, Y., Sliz, P., Hiroaki, Y., Fujiyoshi, Y., Harrison, S.C., Walz, T., 2005. Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. Nature 438, 633–638.
Grinkova, Y.V., Denisov, I.G., Sligar, S.G., 2010. Functional reconstitution of monomeric CYP3A4 with multiple cytochrome P450 reductase molecules in nanodiscs. Biochem. Biophys. Res. Commun. 398 (2), 194–198.
Gulati, S., Jamshad, M., Knowles, T.J., Morrison, K.A., Downing, R., Cant, N., Collins, R., Koenderink, J.B., Ford, R.C., Overduin, M., Kerr, I.D., Dafforn, T.R., Rothnie, A.J., 2014. Detergent-free purification of ABC (ATP-binding-cassette) transporters. Biochem. J. 461.
Hagn, F., Etzkorn, M., Raschle, T., Wagner, G., 2013. Optimized phospholipid bilayer nanodiscs facilitate high-resolution structure determination of membrane proteins. J. Am. Chem. Soc. 135 (5), 1919–1925.
Hagn, F., Wagner, G., 2015. Structure refinement and membrane positioning of selectively labeled OmpX in phospholipid nanodiscs. J. Biomol. NMR 61 (3-4), 249–260.
Hall, S., Tognoloni, C., Charlton, J., Bragginton, É., Rothnie, A., Sridhar, P., Wheatley, M., Knowles, T., Arnold, T., Edler, K., Dafforn, T.R., 2018. An acid-compatible co-polymer for the solubilization of membranes and proteins into lipid bilayer-containing nanoparticles. Nanoscale 10 (22), 10609–10619.
Hayakawa, A., Hayes, S.J., Lawe, D.C., Sudharshan, E., Tuft, R., Fogarty, K., Lambright, D., Corvera, S., 2004. Structural basis for endosomal targeting by FYVE domains. J. Biol. Chem. 279, 5958–5966.
Høyrup, P., Jørgensen, K., Mouritsen, O.G., 2002. Phospholipase A2- an enzyme that is sensitive to the physics of its substrate. Europhys. Lett. 57, 464–470.
Hwang, P.M., Choy, W.-Y., Lo, E.I., Chen, L., Forman-Kay, J.D., Raetz, C.R.H., Prive, G.G., Bishop, R.E., Kay, L.E., 2002. Solution structure and dynamics of the outer membrane enzyme PagP by NMR. Proc. Natl. Acad. Sci. 99, 13560–13565.
Jamshad, M., Charlton, J., Lin, Y.-P., Routledge, S.J., Bawa, Z., Knowles, T.J., Overduin, M., Dekker, N., Dafforn, T.R., Bill, R.M., Poyner, D.R., Wheatley, M., 2015a. G-protein coupled receptor solubilization and purification for biophysical analysis and functional studies, in the total absence of detergent. Biosci. Rep. 35 (2) pii: e00188.
Jamshad, M., Grimard, V., Idini, I., Knowles, T.J., Dowle, M.R., Schofield, N., Sridhar, P., Lin, Y., Finka, R., Wheatley, M., Thomas, O.R.T., Palmer, R.E., Overduin, M., Govaerts, C., Ruysschaert, J.-M., Edler, K.J., Dafforn, T.R., 2015b. Structural analysis of a nanoparticle containing a lipid bilayer used for detergent-free extraction of membrane proteins. Nano Res. 8 (3), 774–789.
Kanai, F., Liu, H., Field, S.J., Akbary, H., Matsuo, T., Brown, G.E., Cantley, L.C., Yaffe, M.B., 2001. The PX domains of p47phox and p40phox bind to lipid products of PI(3) K. Nat. Cell Biol. 3, 675–678.
Karathanassis, D., Stahelin, R.V., Bravo, J., Perisic, O., Pacold, C.M., Cho, W., Williams, R.L., 2002. Binding of the PX domain of p47phoxto phosphatidylinositol 3, 4-bisphosphate and phosphatidic acid is masked by an intramolecular interaction. EMBO J. 21, 5057–5068.
Killian, J.A., Von Heijne, G., 2000. How proteins adapt to a membrane-water interface. Trends Biochem. Sci. 25 (9), 429–434.
Knowles, T.J., Finka, R., Smith, C., Lin, Y.-P., Dafforn, T., Overduin, M., 2009. Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer. J. Am. Chem. Soc. 131 (22), 7484–7485.
Kolbe, M., Besir, H., Essen, L.O., Oesterhelt, D., 2000. Structure of the light-driven chloride pump halorhodopsin at 1.8 Å Resolution. Science 288 (5470), 1390–1396.
Komar, J., Alvira, S., Schulze, R.J., Martin, R., Lycklama a Nijeholt, J.A., Lee, S.C., Dafforn, T.R., Deckers-Hebestreit, G., Berger, I., Schaffitzel, C., Collinson, I., 2016. Membrane protein insertion and assembly by the bacterial holo-translocon SecYEG-SecDF-YajC-YidC. Biochem. J. 473, 3341–3354.
Kong, L., Sochacki, K.A., Wang, H., Fang, S., Canagarajah, B., Kehr, A.D., Rice, W.J., Strub, M.P., Taraska, J.W., Hinshaw, J.E., 2018. Cryo-EM of the dynamin polymer assembled on lipid membrane. Nature 560 (7717), 258–262.
Koppisetti, R.K., Fulcher, Y.G., Jurkevich, A., Prior, S.H., Xu, J., Lenoir, M., Overduin, M., Van Doren, S.R., 2014. Ambidextrous binding of cell and membrane bilayers by soluble matrix metalloproteinase-12. Nat. Commun. 5, 5552.
Kutateladze, T., Overduin, M., 2001. Structural mechanism of endosome docking by the FYVE domain. Science 291 (5509), 1793–1796.
Kutateladze, T.G., Capelluto, D.G.S., Ferguson, C.G., Cheever, M.L., Kutateladze, A.G., Prestwich, G.D., Overduin, M., 2004. Multivalent mechanism of membrane insertion by the FYVE domain. J. Biol. Chem. 279, 3050–3057.
Laganowsky, A., Reading, E., Allison, T.M., Ulmschneider, M.B., Degiacomi, M.T., Baldwin, A.J., Robinson, C.V., 2014. Membrane proteins bind lipids selectively to modulate their structure and function. Nature 510, 172–175.
Laursen, T., Borch, J., Knudsen, C., Bavishi, K., Torta, F., Martens, H.J., Silvestro, D., Hatzakis, N.S., Wenk, M.R., Dafforn, T.R., Olsen, C.E., Motawia, M.S., Hamberger, B., Møller, B.L., Bassard, J.-E., 2016. Characterization of a dynamic metabolon producing the defense compound dhurrin in sorghum. Science 354, 890–893.
Lee, S.A., Eyeson, R., Cheever, M.L., Geng, J., Verkhusha, V.V., Burd, C., Overduin, M., Kutateladze, T.G., 2005. Targeting of the FYVE domain to endosomal membranes is regulated by a histidine switch. Proc. Natl. Acad. Sci. U. S. A. 102 (37), 13052–13057.
Lee, S.C., Knowles, T.J., Postis, V.L.G., Jamshad, M., Parslow, R.A., Lin, Y.-P., Goldman, A., Sridhar, P., Overduin, M., Muench, S.P., Dafforn, T.R., 2016. A method for detergent-free isolation of membrane proteins in their local lipid environment. Nat. Protoc. 11 (7), 1149–1162.
Lenoir, M., Coskun, U., Grzybek, M., Cao, X., Buschhorn, S.B., James, J., Simons, K., Overduin, M., 2010. Structural basis of wedging the Golgi membrane by FAPP pleckstrin homology domains. EMBO Rep. 11 (4), 279–284.
Lenoir, M., Grzybek, M., Majkowski, M., Rajesh, S., Kaur, J., Whittaker, S.B.-M., Coskun, U., Overduin, M., 2014. Structural basis of dynamic membrane recognition by trans-golgi network specific FAPP proteins. J. Mol. Biol. 427 (4), 966–981.
Lenoir, M., Kufareva, I., Abagyan, R., Overduin, M., 2015. Membrane and protein interactions of the pleckstrin homology domain superfamily. Membranes (Basel). 5 (4), 646–663.
Lenoir, M., Ustunel, C., Rajesh, S., Kaur, J., Moreau, D., Gruenberg, J., Overduin, M., 2018. Phosphorylation of conserved phosphoinositide binding pocket regulates sorting nexin membrane targeting. Nat. Commun. 9 (1), 993.
Li, Y., Kijac, A.Z., Sligar, S.G., Rienstra, C.M., 2006. Structural analysis of nanoscale self-assembled discoidal lipid bilayers by solid-state NMR spectroscopy. Biophys J. 91 (10), 3819–3828.
Li, X., Sambi, M., DeCarlo, A., Burov, S.V., Akasov, R., Markvicheva, E., Malardier-Jugroot, C., Szewczuk, M.R., 2018. Functionalized folic acid-conjugated amphiphilic alternating copolymer actively targets 3D multicellular tumour spheroids and delivers the hydrophobic drug to the inner core. Nanomaterials 8, 588.
Lindhoud, S., Carvalho, V., Pronk, J.W., Aubin-Tam, M.E., 2016. SMA-SH: modified styrene-maleic acid copolymer for functionalization of lipid nanodiscs. Biomacromolecules 17, 1516–1522.
Logez, C., Damian, M., Legros, C., Dupré, C., Guéry, M., Mary, S., Wagner, R., M'Kadmi, C., Nosjean, O., Fould, B., Marie, J., Fehrentz, J.A., Martinez, J., Ferry, G., Boutin, J.A., Banères, J.L., 2016. Detergent-free isolation of functional g protein-coupled receptors into nanometric lipid particles. Biochemistry 55, 38–48.
Long, A.R., O'Brien, C.C., Malhotra, K., Schwall, C.T., Albert, A.D., Watts, A., Alder, N.N., 2013. A detergent-free strategy for the reconstitution of active enzyme complexes from native biological membranes into nanoscale discs. BMC Biotechnol. 13, 41.
Luecke, H., Schobert, B., Richter, H.T., Cartailler, J.P., Lanyi, J.K., 1999. Structure of bacteriorhodopsin at 1.55 Å resolution. J. Mol. Biol. 291, 899–911.
Maeda, H., Ueda, M., Morinaga, T., Matsumoto, T., 1985. Conjugation of poly(styrene-co-maleic acid) derivatives to the antitumor protein neocarzinostatin: pronounced improvements in pharmacological properties. J. Med. Chem. 28 (4), 455–461.
Marsh, D., 1996. Lateral pressure in membranes. Biochim. Biophys. Acta - Rev. Biomembr. 93 (11), 3884–3899.
McDowall, J.S., Ntai, I., Hake, J., Whitley, P.R., Mason, J.M., Pudney, C.R., Brown, D.R., 2017. Steady-state kinetics of α-synuclein ferrireductase activity identifies the catalytically competent species. Biochemistry 56, 2497–2505.
Morgado, L., Zeth, K., Burmann, B.M., Maier, T., Hiller, S., 2015. Characterization of the insertase BamA in three different membrane mimetics by solution NMR spectroscopy. J. Biomol. NMR 61, 333–345.
Morrison, K.A., Akram, A., Mathews, A., Khan, Z.A., Patel, J.H., Zhou, C., Hardy, D.J., Moore-Kelly, C., Patel, R., Odiba, V., Knowles, T.J., Javed, M.-u.-H., Chmel, N.P., Dafforn, T.R., Rothnie, A.J., 2016. Membrane protein extraction and purification using styrene-maleic acid (SMA) copolymer: effect of variations in polymer structure. Biochem. J. 473, 4349–4360.
Nasr, M.L., Wagner, G., 2018. Covalently circularized nanodiscs; challenges and applications. Curr. Opin. Struct. Biol. 51, 129–134.
Noinaj, N., Kuszak, A.J., Gumbart, J.C., Lukacik, P., Chang, H., Easley, N.C., Lithgow, T., Buchanan, S.K., 2013. Structural insight into the biogenesis of β-barrel membrane proteins. Nature 501, 385–390.
Oluwole, A.O., Danielczak, B., Meister, A., Babalola, J.O., Vargas, C., Keller, S., 2017. Solubilization of membrane proteins into functional lipid-bilayer nanodiscs using a Diisobutylene/Maleic acid copolymer. Angew. Chemie - Int. Ed. 56, 1919–1924.
Opella, S.J., Marassi, F.M., 2017. Applications of NMR to membrane proteins. Arch. Biochem. Biophys. 628, 92–101.
Orwick, M.C., Judge, P.J., Procek, J., Lindholm, L., Graziadei, A., Engel, A., Gröbner, G., Watts, A., 2012. Detergent-free formation and physicochemical characterization of nanosized lipid-polymer complexes: lipodisq. Angew. Chemie - Int. Ed. 51, 4653–4657.
Overduin, M., Cheever, M.L., Kutateladze, T.G., 2001. Signaling with phosphoinositides: better than binary. Mol. Interv. 1 (3), 150–159.
Overduin, M., Rajesh, S., Gruenberg, J., Lenoir, M., 2015. Secondary structure and 1H, 13C, 15N resonance assignments of the endosomal sorting protein sorting nexin 3. Biomol. NMR Assign. 9, 355–358.
Parmar, M., Rawson, S., Scarff, C.A., Goldman, A., Dafforn, T.R., Muench, S.P., Postis, V.L., 2017. Using a SMALP platform to determine a sub-nm single particle cryo-EM membrane protein structure. BBA Biomembr. 1860 (2), 378–383.
Paulin, S., Jamshad, M., Dafforn, T.R., Garcia-Lara, J., Foster, S.J., Galley, N.F., Roper, D.I., Rosado, H., Taylor, P.W., 2014. Surfactant-free purification of membrane protein complexes from bacteria: application to the staphylococcal penicillin-binding protein complex PBP2/PBP2a. Nanotechnology 25, 285101.
Phillips, R., Ursell, T., Wiggins, P., Sens, P., 2009. Emerging roles for lipids in shaping membrane-protein function. Nature 459 (7245), 379–385.
Picard, M., Dahmane, T., Garrigos, M., Gauron, C., Giusti, F., Le Maire, M., Popot, J.L.,

Champeil, P., 2006. Protective and inhibitory effects of various types of amphipols on the Ca2 + -ATPase from sarcoplasmic reticulum: a comparative study. Biochemistry 45, 1861–1869.
Pliotas, C., Dahl, A.C.E., Rasmussen, T., Mahendran, K.R., Smith, T.K., Marius, P., Gault, J., Banda, T., Rasmussen, A., Miller, S., Robinson, C.V., Bayley, H., Sansom, M.S.P., Booth, I.R., Naismith, J.H., 2015. The role of lipids in mechanosensation. Nat. Struct. Mol. Biol. 22, 991–998.
Popot, J.-L., Althoff, T., Bagnard, D., Banères, J.-L., Bazzacco, P., Billon-Denis, E., Catoire, L.J., Champeil, P., Charvolin, D., Cocco, M.J., Crémel, G., Dahmane, T., de la Maza, L.M., Ebel, C., Gabel, F., Giusti, F., Gohon, Y., Goormaghtigh, E., Guittet, E., Kleinschmidt, J.H., Kühlbrandt, W., Le Bon, C., Martinez, K.L., Picard, M., Pucci, B., Sachs, J.N., Tribet, C., van Heijenoort, C., Wien, F., Zito, F., Zoonens, M., 2011. Amphipols From A to Z*. Annu. Rev. Biophys. 40, 379–408.
Postis, V., Rawson, S., Mitchell, J.K., Lee, S.C., Parslow, R.A., Dafforn, T.R., Baldwin, S.A., Muench, S.P., 2015. The use of SMALPs as a novel membrane protein scaffold for structure study by negative stain electron microscopy. Biochim. Biophys. Acta Biomembr. 1848, 496–501.
Prabudiansyah, I., Kusters, I., Caforio, A., Driessen, A.J.M., 2015. Characterization of the annular lipid shell of the Sec translocon. Biochim. Biophys. Acta Biomembr. 1848, 2050–2056.
Prior, S.H., Fulcher, Y.G., Koppisetti, R.K., Jurkevich, A., Van Doren, S.R., 2015. Charge-triggered membrane insertion of matrix Metalloproteinase-7, supporter of innate immunity and tumors. Structure 23 (11), 2099–2110.
Pylypenko, O., Lundmark, R., Rasmuson, E., Carlsson, S.R., Rak, A., 2007. The PX-BAR membrane-remodeling unit of sorting nexin 9. EMBO J. 26, 4788–4800.
Radoicic, J., Park, S.H., Opella, S.J., 2018. Macrodiscs comprising SMALPs for oriented sample solid-state NMR spectroscopy of membrane proteins. Biophys. J. 115, 22–25.
Ravula, T., Hardin, N.Z., Ramadugu, S.K., Cox, S.J., Ramamoorthy, A., 2018. Formation of pH-resistant monodispersed polymer–Lipid nanodiscs. Angew. Chemie - Int. Ed. 57, 1342–1345.
Ravula, T., Hardin, N.Z., Ramadugu, S.K., Ramamoorthy, A., 2017a. PH tunable and divalent metal ion tolerant polymer lipid nanodiscs. Langmuir 33, 10655–10662.
Ravula, T., Ramadugu, S.K., Di Mauro, G., Ramamoorthy, A., 2017b. Bioinspired, size-tunable self-assembly of polymer–lipid bilayer nanodiscs. Angew. Chem. Int. Ed. Engl. 56, 11466–11470.
Reading, E., Hall, Z., Martens, C., Haghighi, T., Findlay, H., Ahdash, Z., Politis, A., Booth, P.J., 2017. Interrogating membrane protein conformational dynamics within native lipid compositions. Angew. Chemie - Int. Ed. 56, 15654–15657.
Rehan, S., Paavilainen, V.O., Jaakola, V.P., 2017. Functional reconstitution of human equilibrative nucleoside transporter-1 into styrene maleic acid co-polymer lipid particles. Biochim. Biophys. Acta Biomembr. 1859, 1059–1065.
Sahu, I.D., Zhang, R., Dunagan, M.M., Craig, A.F., Lorigan, G.A., 2017. Characterization of KCNE1 inside lipodisq nanoparticles for EPR spectroscopic studies of membrane proteins. J. Phys. Chem. B 121, 5312–5321.
Saotome, K., Duong-Ly, K.C., Howard, K.P., 2015. Influenza A M2 protein conformation depends on choice of model membrane. Biopolymers 104, 405–411.
Scheidelaar, S., Koorengevel, M.C., Pardo, J.D., Meeldijk, J.D., Breukink, E., Killian, J.A., 2015. Molecular Model for the solubilization of membranes into nanodisks by styrene maleic acid copolymers. Biophys. J. 108, 279–290.
Scheidelaar, S., Koorengevel, M.C., van Walree, C.A., Dominguez, J.J., Dörr, J.M., Killian, J.A., 2016. Effect of polymer composition and pH on membrane solubilization by styrene-maleic acid copolymers. Biophys. J. 111, 1974–1986.
She, J., Guo, J., Chen, Q., Zeng, W., Jiang, Y., Bai, X.C., 2018. Structural insights into the voltage and phospholipid activation of the mammalian TPC1 channel. Nature 556, 130–134.
Shen, P.S., Yang, X., DeCaen, P.G., Liu, X., Bulkley, D., Clapham, D.E., Cao, E., 2016. The structure of the polycystic kidney disease channel PKD2 in lipid nanodiscs. Cell 167, 763–773.
Skaar, K., Korza, H.J., Tarry, M., Sekyrova, P., Högbom, M., 2015. Expression and subcellular distribution of GFP-Tagged human tetraspanin proteins in saccharomyces cerevisiae. PLoS One 10 (7), e0134041.
Smirnova, I.A., Sjostrand, D., Li, F., Bjorck, M., Schafer, J., Ostbye, H., Hogbom, M., von Ballmoos, C., Lander, G.C., Adelroth, P., Brzezinski, P., 2016. Isolation of yeast complex IV in native lipid nanodiscs. Biochim. Biophys. Acta Biomembr. 1858, 2984–2992.
Smith, A.A.A., Autzen, H.E., Laursen, T., Wu, V., Yen, M., Hall, A., Hansen, S.D., Cheng, Y., Xu, T., 2017. Controlling styrene maleic acid lipid particles through RAFT. Biomacromolecules 18, 3706–3713.
Stansfeld, P.J., Jefferys, E.E., Sansom, M.S.P., 2013. Multiscale simulations reveal conserved patterns of lipid interactions with aquaporins. Structure 21, 810–819.
Sun, C., Benlekbir, S., Venkatakrishnan, P., Wang, Y., Hong, S., Hosler, J., Tajkhorshid, E., Rubinstein, J., Gennis, R., 2018. Structure of the alternative complex III in a supercomplex with cytochrome oxidase. Nature 557, 123–126.
Swainsbury, D.J.K., Scheidelaar, S., Van Grondelle, R., Killian, J.A., Jones, M.R., 2014. Bacterial reaction centers purified with styrene maleic acid copolymer retain native membrane functional properties and display enhanced stability. Angew. Chemie - Int. Ed. 53, 11803–11807.
Tribet, C., Audebert, R., Popot, J.-L., 1996. Amphipols: polymers that keep membrane proteins soluble in aqueous solutions. Proc. Natl. Acad. Sci. 93, 15047–15050.
Wang, X., Mu, Z., Li, Y., Bi, Y., Wang, Y., 2015. Smaller nanodiscs are suitable for studying protein lipid interactions by solution NMR. Protein J. 34 (3), 205–211.
Workman, S.D., Worrall, L.J., Strynadka, N.C.J., 2018. Crystal structure of an intramembranal phosphatase central to bacterial cell-wall peptidoglycan biosynthesis and lipid recycling. Nat. Commun. 9 (1), 1159.
Yasuhara, K., Arakida, J., Ravula, T., Ramadugu, S.K., Sahoo, B., Kikuchi, J.I., Ramamoorthy, A., 2017. Spontaneous lipid nanodisc fomation by amphiphilic polymethacrylate copolymers. J. Am. Chem. Soc. 18657–18663.
Yokogawa, M., Kobashigawa, Y., Yoshida, N., Ogura, K., Harada, K., Inagaki, F., 2012. NMR analyses of the interaction between the FYVE domain of Early Endosome Antigen 1 (EEA1) and phosphoinositide embedded in a lipid bilayer. J. Biol. Chem. 287, 34936–34945.
Yu, L.J., Suga, M., Wang-Otomo, Z.Y., Shen, J.R., 2018. Structure of photosynthetic LH1-RC supercomplex at 1.9 Å resolution. Nature 556 (7700), 209–213.
Zhang, M., Huang, R., Ackermann, R., Im, S.C., Waskell, L., Schwendeman, A., Ramamoorthy, A., 2016. Reconstitution of the Cytb5-CytP450 complex in nanodiscs for structural studies using NMR spectroscopy. Angew Chem. Int. Ed. Engl. 55 (14), 4497–4499.
Zhang, S., Li, N., Zeng, W., Gao, N., Yang, M., 2017. Cryo-EM structures of the mammalian endo-lysosomal TRPML1 channel elucidate the combined regulation mechanism. Protein Cell 8, 834–847.
Zhao, Y., et al., 2016. Molecular Model for the solubilization of membranes into nanodisks by styrene maleic acid copolymers. Colloids Surf. B Biointerfaces 7, 3779–3788. https://doi.org/10.1039/C6RA12971D.
Zhou, C.Z., De La Sierra-Gallay, I.L., Quevillon-Cheruel, S., Collinet, B., Minard, P., Blondeau, K., Henckes, G., Aufrère, R., Leulliot, N., Graille, M., Sorel, I., Savarin, P., De La Torre, F., Poupon, A., Janin, J., Van Tilbeurgh, H., 2003. Crystal structure of the yeast phox homology (PX) domain protein Grd19p complexed to Phosphatidylinositol-3-phosphate. J. Biol. Chem. 278, 50371–50376.
Zhou, H.-X., Cross, T.A., 2013. Influences of membrane mimetic environments on membrane protein structures. Annu. Rev. Biophys. 42, 361–392.