

## Review Article

# Biological insights from SMA-extracted proteins

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In the twelve years since styrene maleic acid (SMA) was first used to extract and purify a membrane protein within a native lipid bilayer, this technological breakthrough has provided insight into the structural and functional details of protein–lipid interactions. Most recently, advances in cryo-EM have demonstrated that SMA-extracted membrane proteins are a rich-source of structural data. For example, it has been possible to resolve the details of annular lipids and protein–protein interactions within complexes, the nature of lipids within central cavities and binding pockets, regions involved in stabilising multimers, details of terminal residues that would otherwise remain unresolved and the identification of physiologically relevant states. Functionally, SMA extraction has allowed the analysis of membrane proteins that are unstable in detergents, the characterization of an ultrafast component in the kinetics of electron transfer that was not possible in detergent-solubilised samples and quantitative, real-time measurement of binding assays with low concentrations of purified protein. While the use of SMA comes with limitations such as its sensitivity to low pH and divalent cations, its major advantage is maintenance of a protein's lipid bilayer. This has enabled researchers to view and assay proteins in an environment close to their native ones, leading to new structural and mechanistic insights.

## Introduction

Membrane proteins carry out a wide range of critical functions and are key targets for drug discovery [1]. However, their location embedded within a lipid bilayer has made them more difficult to study than soluble proteins. To investigate structure or function in detail the protein typically needs to be removed from the complex environment of a biological membrane. Traditionally this has been carried out using detergents, which disrupt the lipids and form micelles around the hydrophobic regions of a membrane protein to maintain them in solution [2]. Dodecylmaltoside (DDM) is one of the most commonly used detergents for membrane proteins and has yielded much success [3]. However, detergents do pose certain challenges. There is a fine balance between effective solubilisation and denaturation/destabilisation of the protein, and finding the optimal detergent can be a lengthy process [4]. The detergent micelle does not fully mimic the environment of a lipid bilayer, meaning lateral pressure and/or key interactions between the protein and specific lipids are lost [5–7]. Therefore, much effort has been made to improve the detergents used to increase protein stability [4]. Alternative systems, such as amphipols or nanodiscs, have been developed which better mimic the natural environment of a protein, but these still require initial detergent solubilisation [8,9]. In 2009 the use of styrene maleic acid co-polymers (SMA) for the effective extraction/solubilisation of membrane proteins was first reported [10]. SMA is an amphipathic polymer comprising hydrophobic styrene moieties and hydrophilic maleic acid groups, typically in a ratio of 2 : 1 or 3 : 1 styrene: maleic acid. SMA inserts into membranes and forms small discs of membrane surrounded by the polymer, termed SMA lipid particles (SMALPs) [11]. They have also been referred to as lipodisks [12], native nanodiscs [13] and PoLiPa (polymer lipid particles) [14]. Membrane proteins extracted within SMALPs retain their natural lipid bilayer environment, yet are small and soluble and amenable to many different downstream techniques [15]. Encapsulated proteins with an affinity tag can be easily purified using affinity chromatography [16,17]. Once formed, the SMALPs are stable and do not require buffers to be supplemented with free SMA, unlike detergents where the concentration of detergent must be kept above the critical micelle concentration (CMC) for all downstream processes [15].

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SMA has been shown to be effective for membrane protein solubilisation from a wide range of different expression systems, including bacteria, insect cells, yeast, mammalian cells and plant cells (Table 1). Like any new technique, there are some limitations to the use of SMA. One of these is a sensitivity to pH below 7 or to divalent cations, which can be problematic for proteins which require more acidic conditions or divalent cation binding for function, such as ATP hydrolysis [18,19]. Another issue is encountered with free excess SMA which can interfere with effective binding to affinity resins or antibodies and other downstream procedures [15,20–22]. As a result of these limitations, numerous polymer modifications are being investigated [23,24], including DIBMA (diisobutylene maleic acid), which has an aliphatic group in place of styrene, and has been shown to generate slightly larger discs, which are more tolerant to divalent cations [25,26]. Despite the limitations, application of SMA polymer to the extraction and purification of membrane proteins has grown substantially over the last 10 years. From initial proof of concept studies, to the application to a wide range of different protein families and cell systems and numerous downstream analysis techniques. In this review, we will examine the biological insights that have been made using SMA (and related polymer)-extracted proteins. This will be divided into three main themes: structural insights, functional insights and protein–lipid interactions.

## Structural insights

Approaches for the determination of high-resolution protein structure include X-ray crystallography, NMR and cryo-electron microscopy (cryo-EM). Broecker et al. reported the first X-ray structure of an SMA-extracted membrane protein in 2017 using a lipidic cubic phase crystallisation of a bacterial rhodopsin [31] (Figure 1A). Near-atomic resolution (2.0 Å) was observed which was on a comparable level with the detergent-purified receptor in DDM micelles. The concept of being able to go from cell membrane to purified protein and then crystal formation, whilst always having a lipid environment, seemed highly promising, yet to date no further X-ray crystallography structures of SMALP purified proteins have been determined.

NMR based structural studies of membrane proteins can often be challenging simply due to their size, however solid-state NMR of proteins within SMALPs does show some promise for the future. It has been demonstrated that proteins encapsulated in SMALPs magnetically align well and can be used to determine structure [56]. However, it should be noted that this was for relatively small membrane proteins where the structure had previously been determined by NMR, and did not use SMA extraction direct from the expression system. Solid state NMR data has also been obtained for the zinc diffusion facilitator CzcD, but the structure could not be resolved due to challenges in assigning peaks [20].

The most successful approach for determining the structure of membrane proteins within SMALPs has undoubtedly been cryo-EM. The applicability of cryo-EM to SMALP purified proteins was first demonstrated with a low resolution (35 Å) structure of P-glycoprotein/ABCB1 [47] and this was followed by a negative stain EM 23 Å structure of AcrB [30], but these were both ‘proof of concept’ studies of proteins for which the structure had already been determined. The major breakthrough came with the structure of Alternative Complex III (ACIII) from *Flavobacterium johnsoniae* [38] (Figure 1B). This was the first time the structure of this protein, which is a key component of the respiratory and/or photosynthetic electron transport chains of many bacteria [57,58], had been determined. The 3.4 Å resolution cryo-EM map enabled the creation of an atomic model for its six subunits for more than 90% of the sequence. The structure showed, for the first time, the presence of N-terminal triacylated cysteine lipid anchors, as well as the presence of some ordered annular lipids. Both the single enzyme and the functional supercomplex with an *aa*<sub>3</sub>-type cytochrome *c* oxidase was determined, providing details of the protein–protein interactions in this supercomplex. With a size of 464 kDa, the supercomplex is to date the biggest protein extracted in SMALPs.

A 3.2 Å resolution structure of SMALP-encapsulated AcrB was also determined in 2018 by cryo-EM [21] (Figure 1C). Although the structure itself was already known, what this study showed for the first time was the presence of 24 lipids in a central cavity at the trimeric threefold axis, corresponding to a small triangular patch of lipid bilayer. Thus, the SMA approach was able to retain structural elements of the lipid bilayer that are lost when using detergents. This central cavity containing lipids has also been observed subsequently for AcrB reconstituted within a Saposin disc system [59].

A structure of the KUP (K<sup>+</sup> uptake) family transporter KimA at 3.7 Å resolution was determined within SMALPs [33] (Figure 1D). It was shown to form a homodimer, as expected, but highlighted the regions involved in stabilising this dimeric interaction as well as the likely locations of the potassium binding sites. This contributed to a proposed model for the mechanism of proton-coupled potassium transport by KimA. However, it should be noted that the structure displayed a tilting of the two transmembrane domains towards

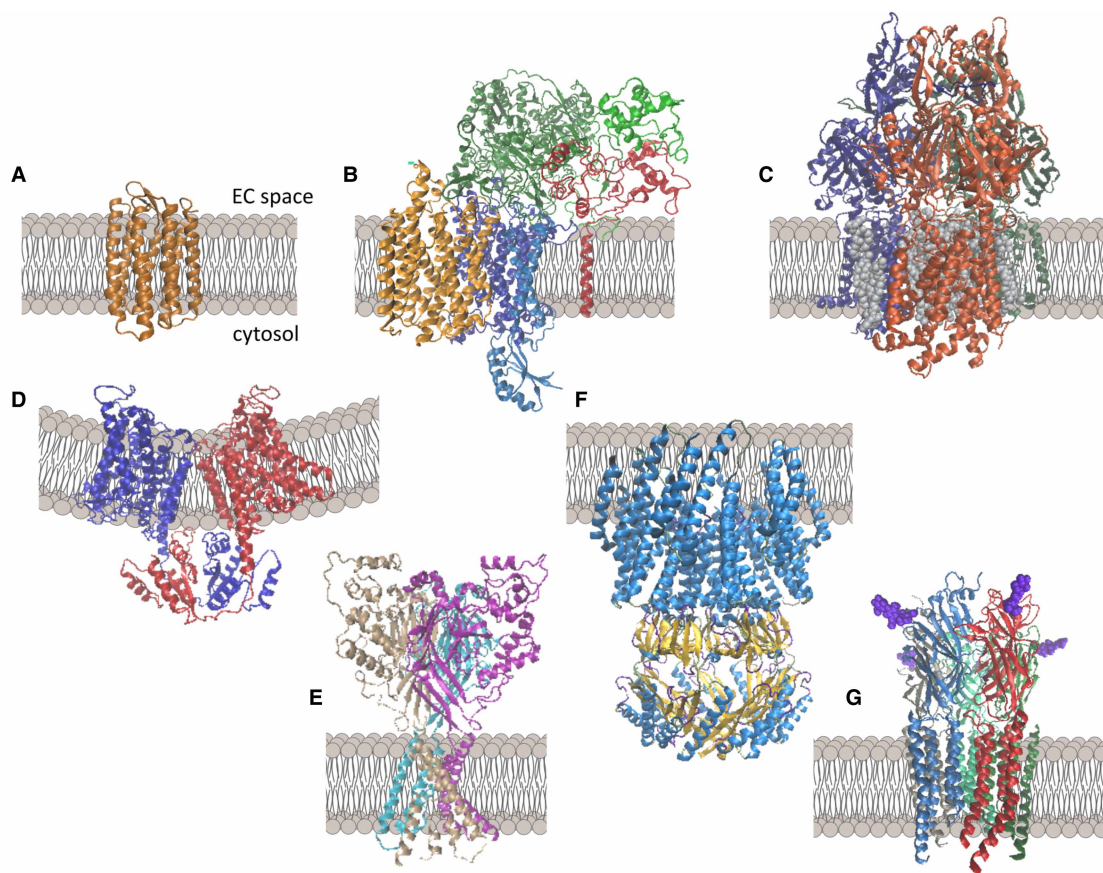
**Table 1** Examples of different proteins and expression systems successfully solubilised using polymers

Expression organism	Membrane type	Target protein	Polymer	Reference
<i>E. coli</i>	Total membrane	BmrA	SMA2000, SMA3000, SZ25010, SZ30010, DIBMA	[18,19,26]
		ZipA	SMA2000, SMA3000, SZ25010, SZ30010, DIBMA	[16,18,19,26,28]
		KcsA	SMA2000	[13]
		AcrB	SMA2000	[21,29,30]
		bacteriorhodopsin	SZ25010	[31]
		SecYEG	SMA3000	[32]
		KimA	SZ30010	[33]
		Ynal	DIBMA	[34]
		GlpG	DIBMA, SZ25010, SZ30010, SMA2000	[35,36]
<i>Rhodobacter sphaeroides</i>	Total membrane	Cytochrome bc1	SZ30010	[37]
<i>Flavobacterium johnsoniae</i>	Total membrane	Alternative complex III	SMA3000	[38]
<i>Thermosynechococcus elongatus</i>	Thylakoid	Photosystem I	SMA1440	[39]
<i>Saccharomyces cerevisiae</i>	Mitochondrial	Cytochrome oxidase	SMA EF30	[40]
	Total membrane	Wsc1	SMA3000	[41]
<i>Pichia pastoris</i>	Total membrane	Adenosine 2A receptor	SMA2000	[42]
		Melatonin receptor	SMA2000	[43]
		CD81	SMA2000	[22]
Sf9 insect cells	Total membrane	hENT1/SLC29A1	SZ30010	[44]
		MRP4/ABCC4	SMA2000	[45]
		$\alpha$ 1 glycine receptor	SZ30010	[46]
High five insect cells	Total membrane	P-glycoprotein/ABCB1	SMA2000	[47]
HEK293T cells	Total membrane	Dopamine receptor	SMA3000	[48]
		ABCG2	SMA2000	[49]
		Acid-sensing ion channel isoform 1	SZ30010	[50]
Hela cells	Whole cells	Total membrane solubilisation	SZ30010	[51]
Red blood cells	Red blood cell ghosts	Rh complexes	SMA3000	[52]
Jurkat cells	Total membrane	Numerous cell surface proteins	SMA3000	[53]
Hamster brain	Tissue homogenate	Prion protein	SZ25010, SZ30010	[54]
<i>Spinacia oleracea</i>	Chloroplast thylakoid	Total protein	SMA1440, SZ25010, SZ30010, SMA2000	[27]
<i>Sorghum bicolor</i>	Total membrane	Dhurrin catalysing metabolon	SMA2000	[55]

SMA2000, SMA3000, SMA EF30 and SMA1440 polymers are from Cray Valley. SZ30010 and SZ25010 are from Polyscope. DIBMA is from BASF. SMA3000, SMA EF30 and SZ25010 have a styrene:maelic acid ratio of ~3 : 1 whilst SMA2000 and SZ30010 have a ratio of 2 : 1 and 2.3 : 1, respectively. SMA1440 is a partially esterified variant of SMA [27]. DIBMA is a 1 : 1 alternating polymer of diisobutylene and maleic acid.

each other, which caused curvature of the SMALP/surrounding membrane (Figure 1D). Molecular dynamics simulations suggest the protein moves between this tilted state and a more upright state, but the upright state was not observed in the cryo-EM images. The authors postulate this is due to loss of lipid during purification, but it is unclear how or why lipid was lost from the SMALP, or if this is a problem observed elsewhere.

Cryo-EM structures of chicken acid-sensing ion channel (ASIC1) at both pH7 (low pH desensitised) and pH8 (high pH resting state) were determined, at resolutions of 2.8 Å and 3.7 Å respectively [50] (Figure 1E). Although the main structural architecture resembled that of previously determined detergent solubilised



**Figure 1. Example structures obtained using polymer extracted proteins.**

(A) *Haloquadratum Walsbyi* bacteriorhodopsin (PDB ID 5ITC) [31]. (B) Alternative complex III (PDB ID 6BTM) [38]. (C) AcrB (PDB ID 6BAJ) [21]. (D) KimA (PDB ID 6S3K) [33]. (E) ASIC1 in the low pH desensitized state (PDB ID 6VTK) [50]. (F) YnaI in the closed state (PDB ID 6ZYD) [34]. (G) Glycine receptor (GlyR) with bound glycine (purple) in the super-open state (PDB ID 6PM4) [46].

structures, the SMA-encapsulated proteins showed strong protein density corresponding to the amino terminal residues for the first time. The pre-transmembrane helix 1 region formed a reentrant loop which may be important for ion permeation. It seems that the retained lipid environment when using SMA was important for preserving the complete protein structure.

The mechanosensitive channel YnaI was isolated from *E. coli* using DIBMA rather than SMA, and its structure determined by cryo-EM at a resolution of 3.0 Å [34] (Figure 1F). The overall structure was conserved with respect to the related MscS protein, but it was shown to have two additional transmembrane helices per subunit that extend the sensor paddle compared with MscS, although it should be noted that the resolution of these helices was only sufficient for the backbone to be modelled. The structure of YnaI following opening in the presence of LPC was also investigated, and produced three different conformations, although these were all at lower resolution than the closed state structure. Additionally the protein was not obtained directly from *E. coli* but from purified, reconstituted proteoliposomes, which the authors acknowledge contained non-native lipids. The overall shape of the ‘open’ structure is quite different in appearance to the closed state and suggests that the gating mechanism and pore opening of YnaI occur in a completely different way from that seen for MscS. This is possibly related to the presence of lipids in a pocket, for which densities were detected.

Recently the mechanosensitive cell wall sensor Wsc1 was successfully extracted with SMA copolymer from *S. cerevisiae* [41]. The negative stain EM structures obtained were of low resolution but depicted two key states of the protein (extended or folded) and since the cell wall represents the main target in antifungal therapy this study provides the starting point for more extensive studies and functional assessments which will be crucial for future drug discoveries.

Finally, multiple structures of the glycine receptor, a ligand-gated ion channel, have been determined by cryo-EM following SMA extraction [46] (Figure 1G). Interestingly the SMALP encapsulated protein showed three different conformations in the presence of the agonist glycine, which matches with electrophysiology data, but contrasts with the protein in nanodiscs where just a single structure was observed. They were also able to obtain structures in the presence of partial agonists and observed a new, partial agonist-bound closed conformation of the receptor, which is proposed to match the long-lived shut states that are seen in single-channel recordings with partial agonists. These physiologically relevant different states of the protein provide insights to the mechanism of the receptor function.

These studies have shown that the combination of cryo-EM and polymer-extracted proteins enables visualisation of protein–protein complexes and protein–lipid interactions, in a range of different protein conformations, providing previously unknown details.

## Functional insights

Many studies of SMALP-encapsulated proteins have carried out established functional assays to show that the proteins within the SMALPs were indeed functioning, and that this was comparable to either detergent solubilised protein or within native membranes. Techniques included radioligand binding assays [42–44,47,60], spectroscopic binding assays [19,30,32,43,47], characteristic spectra [27,40,61], NMR based assays [10,62], and measurement of channel activity [13,63]. One limitation of SMALP structure, with both sides of the membrane freely accessible is the inability to measure vectorial transport. However, several studies have now demonstrated effective reconstitution of proteins from SMALPs or DIBMALPs into proteoliposomes for measurement of transport [60,64,65].

Purification of the rhomboid protease GlpG using DIBMA demonstrated activity more similar to that observed in membranes than could be measured in detergents [35]. Furthermore, the rhomboid protease from *Vibrio cholerae* VcROM, which is proteolytically unstable in detergents, remained intact in DIBMA. Similarly, the transport activity of reconstituted human serotonin transporter, hSERT, showed a 5-fold higher level of activity when purified within DIBMALPs than with detergent [60].

An example of novel functional activity, that has been demonstrated through the use of SMALPs, is the binding of the neurotransmitter neurotensin to the dopamine receptor, as measured by microscale thermophoresis [48]. Although prior *in vivo* studies had suggested this interaction may occur, this is the first time a specific interaction could be measured *in vitro*. Secondly, the isolation of cyanobacterial Photosystem I within SMALPs enabled the detection of a new ultrafast component in the kinetics of electron transfer, that has not previously been seen in detergent solubilised samples [39].

Polymer extraction of membrane proteins has also enabled the development of new tools to investigate biological processes and functions. Pellowe et al. [66] have utilised DIBMA extraction of *E. coli* membranes combined with SecM-facilitated nascent chain stalling, to develop a way to isolate ribosome-bound nascent chain complexes for membrane proteins within their native lipid environment, for the first time. This has the potential for future structural and biophysical analysis to gain further insight to the mechanism of co-translational membrane protein folding and membrane insertion. Recently, native-mass spectrometry of SMALP-encapsulated proteins has also been demonstrated, enabling investigation of post-translational modifications, association with specific lipids and protein maturation [67]. Fluorescence correlation spectroscopy of SMALP purified proteins has been utilised to develop a new method for measuring ligand binding to membrane proteins, including both an ABC transporter [49], and a GPCR [68]. This approach enables a quantitative, real-time measurement of binding assays with relatively low concentrations of purified protein. The use of SMA-solubilised bacteria as a tool to isolate and study phage-receptor interactions has been investigated [69]. It was found that specific interactions could be detected between different phage and their target bacteria, which led to DNA ejection by the phage. This has great potential for future work to identify phage receptors.

Finally, the ability to obtain structures of multiple states of some proteins via cryo-EM in polymer lipid particles, as described above, has led to a new understanding of the mechanism of proteins such as YnaI and the glycine receptor [34,46].

## Protein–lipid interactions

One of the advantages of the SMALP approach is the co-extraction of membrane proteins with their surrounding lipid bilayer. It is well established that the function of many membrane proteins is dependent on their lipid

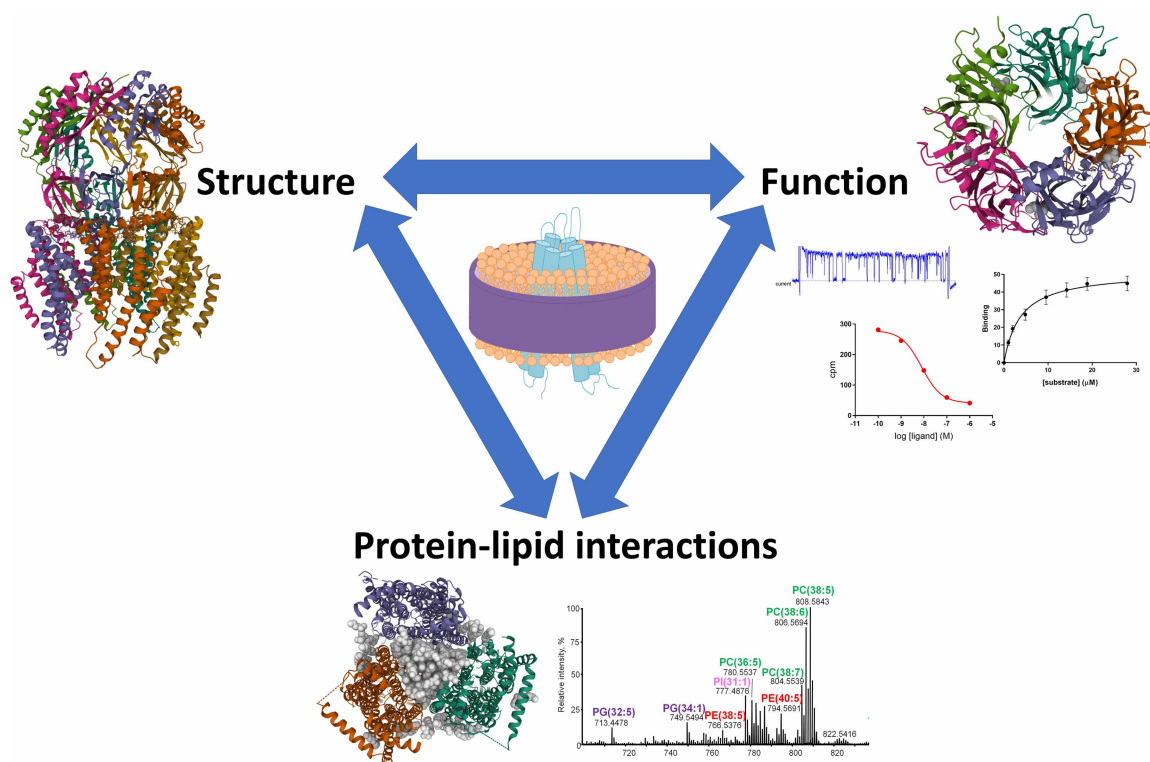
environment [5,6,70], and analysis of the lipids co-purified with a protein may be able to yield insight into the native local lipid environment of a protein. Although there are examples of lipids retained within detergent-solubilised structures, it is generally considered that most lipids get displaced by detergents. For example, native mass spectrometry of two different bacteriorhodopsins extracted using SMA showed the presence of native ether-linked lipids. For one of the proteins this was also observed when the detergent octyl glucoside was used, but for the other this lipid adduct was lost with detergent [67]. Thus, it seems more likely that lipid-interactions will be maintained when using SMA than conventional detergents. Several studies have reported analysis of the lipid content of purified protein SMALPs using techniques such as TLC [13,32,37,66,71], HPLC [54] and/or mass spectrometry [22,71,72]. Lipids co-purified from *E.coli* with SMA-extracted KcsA were found to be enriched in both cardiolipin (CL), and phosphatidylglycerol (PG) [13], both of which are negatively charged at physiological pH. Similarly, lipids co-purified with the *E. coli* translocon subunit SecYEG were enriched in CL and PG, yet lower in the zwitterionic phosphatidylethanolamine (PE) [32]. The *Rba. sphaeroides* cytochrome *bc*<sub>1</sub> (*cytbc*<sub>1</sub>) complex was purified using SMA, and the resulting SMALPs were enriched in CL, and low in sulfoquinovosyl diacylglycerol [37]. In contrast analysis of lipids co-purified from *E. coli* with ZipA suggest it preferentially localised to domains that are relatively enriched in PE and low in CL [72]. Similarly SMALPs of purified aquaporin Z from *E.coli* were low in CL [71]. Lipids co-purified with human CD81 expressed in *Pichia pastoris* were low in PC (phosphatidylcholine) and PE species compared with total and SMA-solubilised membranes, but there were no real differences in CL species. The major lipid that co-purified with prion proteins from infected mice and hamsters was PE, which might relate to a role in prion propagation [54]. PC and cholesterol were also high, but PS (phosphatidyl serine) and PI (phosphatidylinositol) were also detected, which can stimulate prion aggregation.

There have been questions about the reliability of identifying co-purified lipids due to the ability of lipids to diffuse between individual SMALPs [73,74]. However, it should be noted that these studies have utilised lipid-only SMALPs, and it is not yet known how the presence of a protein might affect this lipid exchange. Secondly, it would argue that if specific lipids are found with a given protein, that are distinct from the total solubilised membrane, these must represent only the higher-affinity protein–lipid interactions with slow off-rates. It is not yet clear whether SMA (or other polymers) has bias towards solubilising specific lipids. One study reported no differences in lipid content between SMALPs and the originating *E. coli* membrane [72], whereas subtle differences were observed between total membrane and SMA solubilised samples for *Pichia pastoris* expressing CD81 [22]. Lipidomic analysis of nanodiscs generated using a variety of polymers, including SMA and DIBMA, suggested that glycerolipids are strongly enriched and phospholipids de-enriched in polymer-solubilised samples compared with the originating *E. coli* and Jurkat membranes [75]. In model heterogenous phase-separated bilayers, SMA preferentially solubilised lipids in the fluid phase [76], suggesting that biological membrane microdomains with differing fluidity may be differentially soluble in SMA. It was noted when studying solubilisation of whole HeLa cells by SMA that organelle membranes were solubilised more quickly than plasma membranes, which might be explained by plasma membrane domains with different properties [51]. Solubilisation of Jurkat cells with SMA gave both large and small SMALPs, with the lipid composition of the large SMALPs suggested to be more representative of lipid rafts [53]. However, even if the polymers do preferentially solubilise certain lipids, this does not explain differences in lipid content between total SMA solubilised membranes and protein-specific SMALPs purified from this sample.

To date only a few studies have utilised SMALPs to investigate the effects of specific lipids on the function or dynamics of the protein. For SecYEG, which was identified in purified SMALPs to be enriched in CL and PG, reconstitution into proteoliposomes with varying lipid contents demonstrated that the presence of at least one of these anionic lipids was crucial for full protein translocation activity [32]. Reading et al. modulated the cellular lipid environment of GlpG by using slightly different *E.coli* strains and/or changing the temperature. This enabled SMA extraction and purification of GlpG with different co-purifying lipids and they then used hydrogen-deuterium exchange mass spectrometry to monitor difference in protein dynamics [36]. They found that differences in PE and PG content had no major impact on GlpG dynamics, however differences in chain length and unsaturation leading to more fluid bilayers did impact GlpG dynamics. SMALPs formed of defined lipid compositions were used to investigate the interactions between lipids and  $\alpha$ -synuclein, the main protein involved in Parkinson's disease [77]. The presence of cholesterol was found to both inhibit interaction of  $\alpha$ -synuclein with lipids and promote  $\alpha$ -synuclein aggregation. Using NMR they were able to start to pinpoint the specific effects of different lipids on specific regions of the protein, to better understand this complex

interaction, and found that the parts of the protein affected by cholesterol depended on which other lipids were also present. Finally, the EphA2 receptor tyrosine kinase can dimerise in two different binding modes with different transmembrane helix crossing angles, with distinct impacts on signalling. EphA2 transmembrane peptide was reconstituted into proteoliposomes with different bilayer thickness, to bias the dimerisation towards one of the two binding modes, and these proteoliposomes were solubilised with SMA for single molecule fluorescence studies. Addition of PIP<sub>2</sub> was found to increase the dimer:monomer ratio only in the thicker bilayer, suggesting that PIP signalling may modulate the pro-oncogenic ligand-independent signalling of EphA2 [78].

As described already, several of the cryo-EM structures of SMALP-encapsulated proteins contained densities corresponding to lipids. For the *E. coli* mechanosensitive channel YnaI solubilised with DIBMA, one lipid molecule was well-resolved, with the phosphate headgroup forming a salt bridge with a lysine residue (K108). Mutation of this lysine (K108L) significantly reduced the pressure required to open YnaI, suggested that lipid coordination at this site is functionally important for pressure sensing [34]. Also, in the cryo-EM structure of the trimeric *E. coli* multi-drug efflux transporter AcrB, the patch of lipids in the central cavity has been suggested to play a role in harmonising the conformational changes of the protein [21]. Previous structural studies of detergent-solubilised AcrB mutants suggested that large inward movements of transmembrane domains into the central cavity were associated with disruption of function. However, in the cryo-EM structure of one of these same mutants solubilised with SMA, the presence of the central bilayer patch was still present and restricted this inward motion. This suggests that these motions were exaggerated by the removal of the cavity lipids in structures of detergent-solubilised AcrB, and that the structural difference between wild-type and mutants of AcrB may be much more subtle [21].



**Figure 2. Polymer extraction of membrane proteins retains interactions between proteins and lipids which is important for both protein structure and function.**

Side view structure of YnaI (PDB ID 6ZYD) [34]. Top view structure of GlyR showing bound partial agonist taurine (space filling grey) (PDB ID 6PM0) [46], alongside representative images of various types of functional assays. Bottom view of AcrB trimer showing central lipid filled cavity (space filling grey) (PDB ID 6BAJ) [21], alongside a representative mass spectrum for lipids co-purified with a protein from yeast. Structural images made using Mol\* [79].

## Conclusions

The SMA approach is not without limitations, the sensitivity to low pH or divalent cations, the problems of free SMA interfering with binding or inhibiting activity, and it remains unclear whether the SMALP structure allows full dynamic function of all proteins or if it might be restrictive in some cases. Therefore, significant effort is being made to improve upon this, with many different polymers being made and tested. However, the maintenance of a lipid environment that SMA extraction of membrane proteins facilitates is arguably the biggest benefit of this approach, and this impacts on both structure and function of proteins (Figure 2). It has enabled researchers to view and assay proteins in a much closer to native format, which has led to new mechanistic insights. The investigation of the specific interactions between lipids and proteins is likely to be an area of interest for some time to come.

## Perspectives

- Extraction/solubilisation and purification of membrane proteins using SMA (and related polymers) overcomes several of the limitations associated with detergents, allows the lipid bilayer environment of the protein to be retained, and is amenable to many downstream techniques.
- High resolution structural determination by cryo-EM of polymer encapsulated proteins has been successful, enabling new knowledge on protein complex formation and the association with lipids.
- SMALPs allow study of the important interactions between membrane proteins and their lipid environment and this will likely be exploited further in the future.

## Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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## Author Contributions

L.U., A.R.C., P.K., R.M.B., and A.J.R. wrote the manuscript.

## Abbreviations

ABC, ATP Binding Cassette; CL, cardiolipin; CMC, critical micelle concentration; DDM, dodecylmaltoside; DIBMA, diisobutylene maleic acid; DIBMALP, DIBMA lipid particle; EM, electron microscopy; GPCR, G protein-coupled receptor; KUP, K<sup>+</sup> uptake transporters; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PoLiPa, polymer lipid particles; PS, phosphatidylserine; SMA, styrene maleic acid; SMALP, SMA lipid particle.

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