Membrane Proteins from Amino Acids to Zinc

Membrane proteins: is the future disc shaped?

Sarah C. Lee*[1, 2] and Naomi L. Pollock*[1]
*School of Biosciences, University of Birmingham, Birmingham, Edgbaston B15 2TT, U.K.

Abstract
The use of styrene maleic acid lipid particles (SMALPs) for the purification of membrane proteins (MPs) is a rapidly developing technology. The amphiphilic copolymer of styrene and maleic acid (SMA) disrupts biological membranes and can extract membrane proteins in nanodiscs of approximately 10 nm diameter. These discs contain SMA, protein and membrane lipids. There is evidence that MPs in SMALPs retain their native structures and functions, in some cases with enhanced thermal stability. In addition, the method is compatible with biological buffers and a wide variety of biophysical and structural analysis techniques. The use of SMALPs to solubilize and stabilize MPs offers a new approach in our attempts to understand, and influence, the structure and function of MPs and biological membranes. In this review, we critically assess progress with this method, address some of the associated technical challenges, and discuss opportunities for exploiting SMA and SMALPs to expand our understanding of MP biology.

Introduction
Membrane proteins (MPs) are not only numerous, contributing 20–30 % of the proteome in all genomes [1], but they also undertake complex processes mediating between cells and their environment. As gate-keepers of the cell, they act as channels, receive and transmit signals across membranes, and import or export substrates including metals, sugars and lipids. Many MPs are also enzymes (e.g. cytochrome P450s, oxidases and reductases) [2]; some have even specialized to combine the roles of transporter, ATPase and enzyme in one polypeptide chain [3]. As well as these individual functions, many MPs are also constituents of large dynamic assemblies, choreographing cellular processes including endocytosis [4], cell division [5,6] and migration.

Despite their abundance and importance, relatively few integral MPs have been purified and characterized functionally and structurally. Just 2.3 % of all depositions in the protein data bank (PDB) are of MPs, which rightly reflects the challenges in purifying MPs in sufficient quantity and quality for detailed study. Indeed, MPs are often represented as difficult to express and purify, and unstable once purified. Large protein assemblies are especially difficult to isolate, particularly those that contain both membrane-bound and soluble components.

Using detergents is currently the first choice of strategy for MP purification [7,8]. Although head-and-tail detergents (such as sodium dodecyl sulfate, octyl glucoside and others) have enabled vital and pioneering discoveries to be made throughout biochemistry, it is well-known that detergents are not an ideal solvent for MPs [9–11]. Now, a new reagent promises to provide benefits beyond those currently in use: the amphipathic polymer styrene maleic acid (SMA). This polymer can solubilize biological membranes, forming styrene maleic acid lipid particles (SMALPs) containing MPs. Bacterial, plant, fungal and mammalian proteins have been successfully purified and studied using this polymer, without the use of traditional detergents [12–18]. The success of this method is shown by an increasing number of publications describing a wide variety of architectures and functions (Figure 1). These include membrane-anchored and membrane-associated proteins, and membrane-spanning proteins with transmembranes domains containing both alpha helices and beta barrels.

In this review, we describe the properties of SMALPs, and their successful use to-date in MP purification. We argue that exposure to high concentrations of detergent, rather than their intrinsic properties, are to blame for the challenging behaviours of MPs. Furthermore, we propose that nanodisc assemblies, with particular reference to SMALPs, provide a

Key words: biophysical studies, detergent-free, membrane proteins, protein purification, SMA, SMALPs, styrene maleic acid.

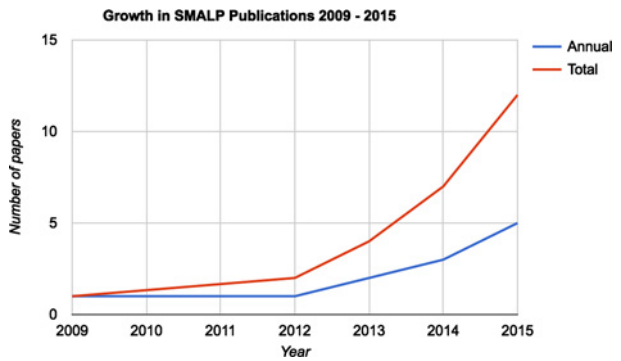
Abbreviations: AP, amphipol; MP, membrane protein; MSP, membrane scaffold protein; PDB, protein data bank; SMA, styrene maleic acid copolymer; SMALP, styrene maleic acid copolymer lipid particle.

[1] **These authors contributed equally.**

[2] **To whom correspondence should be addressed (email S.Lee.5@bham.ac.uk).**

Figure 1 | Growth of publications describing MPs purified using styrene:maleic acid co-polymer

Using ratio of 2:1 or 3:1 SMA [12,13,15–24].



solution to many of these challenges. As a new technology, the properties and applications of SMALPs are yet to be fully characterized but even at this early stage, the opportunities offered by SMALPs for MP research are exciting and likely to be far-reaching.

Understanding SMALPs as a tool for membrane research

What are SMALPs and how are they used?

The styrene maleic acid (SMA) copolymer is a well-characterized industrial copolymer of styrene and maleic anhydride, produced by free radical polymerization followed by acid hydrolysis to the styrene maleic acid form (Figure 2a). Different ratios of styrene and maleic anhydride produce different polymers. In their simplest form, SMALPs are stable, self-assembling, water-soluble particles consisting of SMA and approximately 140 lipids (lipid-only SMALPs) [25,26]. Adding SMA to a suspension of lipids at pH 6.5 or above leads to spontaneous and immediate clearing of the suspension due to the formation of SMA/lipid particles of ∼10 nm diameter. These particles can be detected using light scattering techniques. Therefore, SMALP discs are not dependent on proteins for their formation, but can also form protein-free assemblies [25,26]. Hereafter, we principally discuss the purification of MPs using the 2:1 co-polymer of styrene and maleic acid. Other SMA co-polymers (e.g. 3:1 SMA, sometimes called lipodisq) exist and have been successfully used for MP purification [17,20,23].

The key to this method is the ability of SMA to partition into lipid bilayers. The SMA surrounds patches of lipid that may or may not contain MPs, and removes them into aqueous solution (Figure 2b). SMA is highly efficient at solubilizing biological membranes of many kinds [27], and stabilizing MPs in aqueous solutions [28,16]. Unlike most detergents, SMA removes proteins from the membrane along with a significant quantity of native lipids, arguably with no direct interaction between the SMA and the MP [14,25]. This promotes the retention of correct protein folding and activity therefore making samples amenable to functional and structural characterization [14,25]. MPs overexpressed with affinity tags in heterologous systems can be chromatographically purified as SMALPs and used in standard biophysical and biochemical analyses [12].

Do we need SMALPs?

Several approaches have already contributed to improving MP purification and stability. For example, amphipathic polymers (amphipols/APs) first reported by Popot and co-workers, entered the scientific literature in the mid-1990s [31]. In addition the work of Sligar and co-workers describes MPs in self assembling lipid-containing nanodiscs using membrane scaffold proteins (MSPs) to encircle the lipid discs [32]. These tools have been developed as a response not only to frustrations encountered with purification and analysis of MPs with detergents, but also out of a growing understanding of the complexity of the lipid bilayer and its importance for MP function. The existence and success of these methods prompts a question: what additional benefits do SMALPs offer?

The most mentioned, and perhaps the most important, advantage of using SMALPs over other methods is that it circumvents the use of traditional detergents which disturb lipid–protein interactions, and instead allows MPs to retain their native lipids. Both APs and MSPs are generally added after a standard detergent-based purification. By contrast, SMA solubilizes membranes by spontaneously assembling SMALPs with minimal perturbation of the proteins. This increases the chance of extracting proteins in their entirety, including complexes that may be disrupted by detergents.

In contrast with MSPs, SMALPs do not require any additional protein components in the nanodisc. This property can be important for downstream applications (e.g. CD and SANS), as scattering or UV absorption by SMA in the SMALP is either minimal, or distinct from that of the MP, allowing the signals to be easily deconvoluted or phased out [28]. Unlike detergents, SMA does not form micelles, and after purification is only present in solution as part of the SMALPs. This further simplifies data collection and processing.

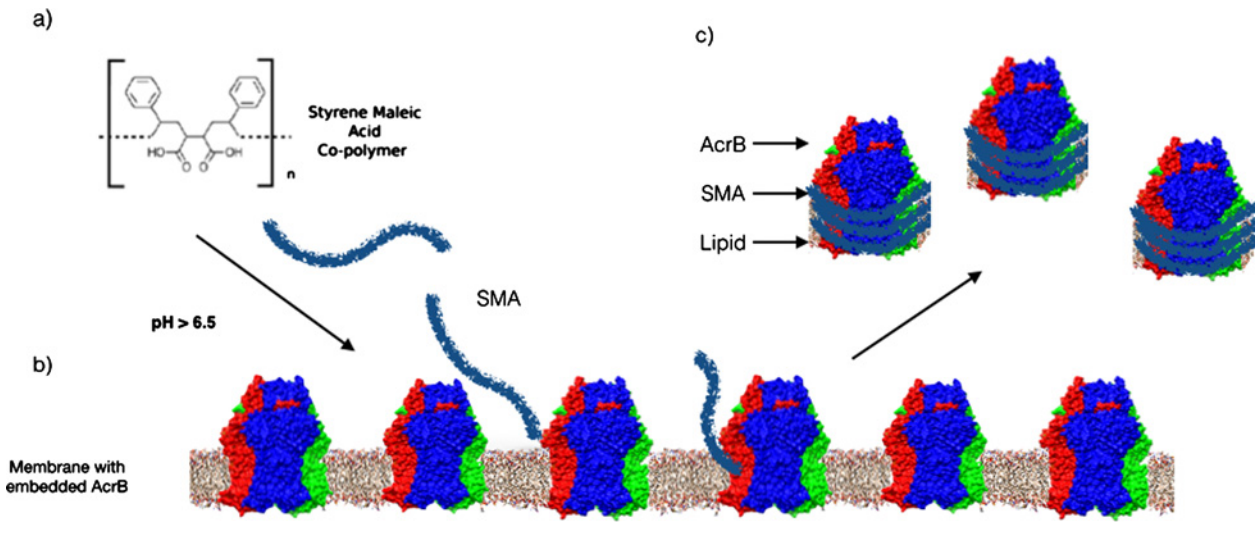
A prosaic advantage of SMALPs over other systems is simply the low cost of the styrene maleic anhydride copolymer and the ease with which it can be converted to styrene maleic acid. Only a small quantity is required to solubilize and subsequently stabilize proteins. SMALPs are stable, self-assembling particles, so buffers do not need to be supplemented with excess SMA to maintain their stability.

Do SMALPs resemble true bilayers?

There are several compelling reasons for studying MPs within a lipid environment. Above all, evolution has tuned these proteins to be stable and functional in their native membrane. Of course the limitation to studying their function and structure in membranes is interference from numerous other proteins, hence the need to purify proteins from the lipid bilayer. Prior to functional analysis, purified MPs are often

Figure 2 | **Schematic of SMALP formation using AcrB as an example**

(**a**) The chemical structure of styrene maleic acid copolymer with a 2:1 ratio. SMA has a molecular weight of ∼7.5 kDa SMALPs. At neutral/basic pH the SMA inserts into lipid bilayers (adapted from [40]: Lee, S.C., Knowles, T.J., Postis, V.L., Jamshad, M., Parslow, R.A., Lin, Y.P., Goldman, A., Sridhar, P., Overduin, M., Muench, S.P. and Dafforn, T.R. (2016) A method for detergent-free isolation of membrane proteins in their local lipid environment. Nat. Protoc. **11**, 1149–1162). (**b**) AcrB (PDB 2gif [30]) is represented overexpressed embedded in membrane, when SMA is added to membrane it begins to encapsulate the MP. (**c**) AcrB is excised from the membrane in the form of soluble particles containing SMA, lipid from the bilayer and AcrB.



reconstituted into artificial bilayers, such as liposomes. As a direct method for purifying MPs in bilayers, the use of SMA bypasses this step in the protocol. However, as the SMA purification method develops, it is important to establish the extent to which the MPs within SMALPs experience a native environment.

Most importantly, several studies have established that MPs in SMALPs do retain lipids [16,28,33]. Using thin-layer chromatography and phosphate assays, the number and composition of these lipids has been established, and appears to be protein-dependent. More studies will be needed to confirm the dependence of the lipid content on the protein and the membrane of origin.

A recent publication has addressed the thermodynamics of SMALP formation, albeit on a model system using suspensions of 3:1 styrene:maleic acid and POPC, a bilayer-forming lipid [26]. This study found that SMA had a preference for residing in SMALPs rather than free in solution, or within the bilayer and there was a low free-energy cost for the transfer of lipids from bilayer to SMALP. This provided reassuring evidence the SMALPs not only act as strong solubilizing agents but also, from a thermodynamic perspective, represent a native-like environment for lipids. Likewise, the phase transition temperature for lipid in SMALPs is close to that observed for lipids in bilayers [25].

Two other important features of membranes are their curvature and lateral pressure. Whether the bilayer segment within SMALPs is sufficiently large for membrane curvature to be apparent or important is not known. It may be relevant only for proteins that induce extreme curvature of membranes, such as the mitochondrial ATPase [34]. In this case extreme curvature of the mitochondrial inner membrane is seen over distances of 17 nm [35], which exceeds the size of SMALPs formed from 2:1 or 3:1 SMA. Similarly, the lateral pressure of the lipid bilayer is often cited as an important aspect of MP structure and stability [36–38]. A truly successful membrane mimetic would ideally be able to recapitulate these features; it remains to be seen whether SMALPs will offer these properties.

Current challenges of using SMALPs

The SMA purification method is sufficiently new that applications, protocols and techniques are appearing all the time. Nonetheless, there is much more to learn and some limitations to the use of SMALPs are currently apparent:

Size limitations

Analysis of nanodiscs containing 2:1 SMA and lipids seem to agree that the discs have a fixed diameter of ∼10 nm [25]. It has been calculated that this surface area could contain at most 40 transmembrane helices (TMHs) [25]. Indeed this seems to be the case, with a low-resolution structure of AcrB recently having been published [15]. This showed that the 36 TMHs of AcrB could be accommodated, but with relatively little surrounding lipid [33] (Figure 3), suggesting that the system may be better suited to smaller proteins such as GPCRs (7 TMHs) [18], ABC transporters [13] (12 TMHs) and potassium channels [39] (8 TMHs). Given the

defined size of the discs, there is justifiable concern that they may restrict the conformational changes that some transport proteins undergo during their transport cycle, rendering them non-functional. Perhaps the inactivation would be reversible upon the reinsertion of the protein to a more extensive bilayer, though no such protocol has yet been developed. Conversely, locking proteins in a fixed conformation can be a desirable step in some analyses, or one that may improve the resolution in structural techniques which rely on averaging the signal over a large number of molecules (e.g. SAXS, SANS, EM). It is likely that these considerations will apply on a case-by-case basis for different proteins.

Buffer compatibility

The 2:1 SMA copolymer is aliphatic with both hydrophilic and hydrophobic groups, and a pK_a of 6.5. Purification and experimental procedures are normally carried out above pH 7.5 to ensure the solubility of the SMA [40]. Below pH 6.5 the 2:1 SMA precipitates, leading to disassembly of the SMALP and precipitation of the protein. This may present a problem if downstream applications include assays that require the MP to be at different pH.

Divalent cations, especially magnesium ions (Mg^{2+}), affect the solubility of the SMA copolymer, causing it to dissociate from the lipid and destabilizing the particle. MPs that bind nucleotides, especially ATPases, require Mg^{2+} as a co-factor, limiting the use of SMALPs in some functional assays. Fortunately, SMA can withstand moderate levels of Mg^{2+}: SMALPs remain intact at concentrations below 5 mM magnesium [40].

Lack of sidedness and need for reconstitution

Characterizing the function of membrane receptors like GPCRs can be achieved with binding assays, which are possible in SMALPs [18,41]. For channels and transporters it relies on measuring changes in the concentration of the substrate on either side of the protein, which generally requires reconstitution of the protein into proteoliposomes or another bilayer system. SMALPs are very stable particles, and although it is well-known that the SMA can be precipitated out of solution using divalent cations and pH changes, a method for the controlled release of proteins from SMALPs is yet to be developed. For full functional characterization of some proteins it will be critical to establish methods for reconstitution of MPs into bilayers from SMALPs.

Future potential for the use of SMALPs

Since the first MPs were purified in SMALPs [42,28], the field has grown quickly (Figure 1) but it is a technique still in its very early days. Although the concept of using amphipathic polymers for protein solubilization is not new [31], use of SMA co-polymers is a novel and relatively unexplored approach. This means that there are many unanswered questions about the nature and uses of SMA and SMALPs for the solubilization and characterization of membrane and membrane-associated proteins.

Exploring and improving styrene copolymers

One obvious approach to expanding the applications of SMA would be to produce and assess a family of styrene co-polymers with varying properties. Current publications describe just two variants of SMA for protein purification, those with 3:1 and 2:1 ratios of styrene:maleic acid [28,42]. Given that it is a copolymer there are many possible variations in the ratio of S:MA in the polymerization mixture. Different ratios of the constituents, or altering the different constituent chemicals, could create polymers that allow the formation of discs with different properties (i.e. solubility, size, charge). Furthermore, functionalization of SMA polymers, perhaps with purification tags or fluorescent moieties, could open up new applications and approaches to purification.

It is also noteworthy that the polymerization of SMA is random, giving rise to a heterogeneous mixture of polymers of different lengths and compositions [43]. This seems shocking to those accustomed to using detergents purified to >99%, with maltoside detergents even purified to differentiate between their α- and β-isomers [44]. However, the synthesis of detergents has developed very much over the last decades, and it is reasonable to expect that this will also be possible for amphipathic polymers. One place to start will be improving the chemistry of the polymerization reaction to standardize the composition of the polymers, for instance to ensure controlled alternation between the styrene and maleic acid components. Improved quality control is also a priority. Since SMA copolymers have broad distributions of molecular weights, it could also be instructive to discover whether all lengths of the polymer insert into the lipid bilayer and extract protein, or whether this property is restricted to a subset of molecules within the mixture.

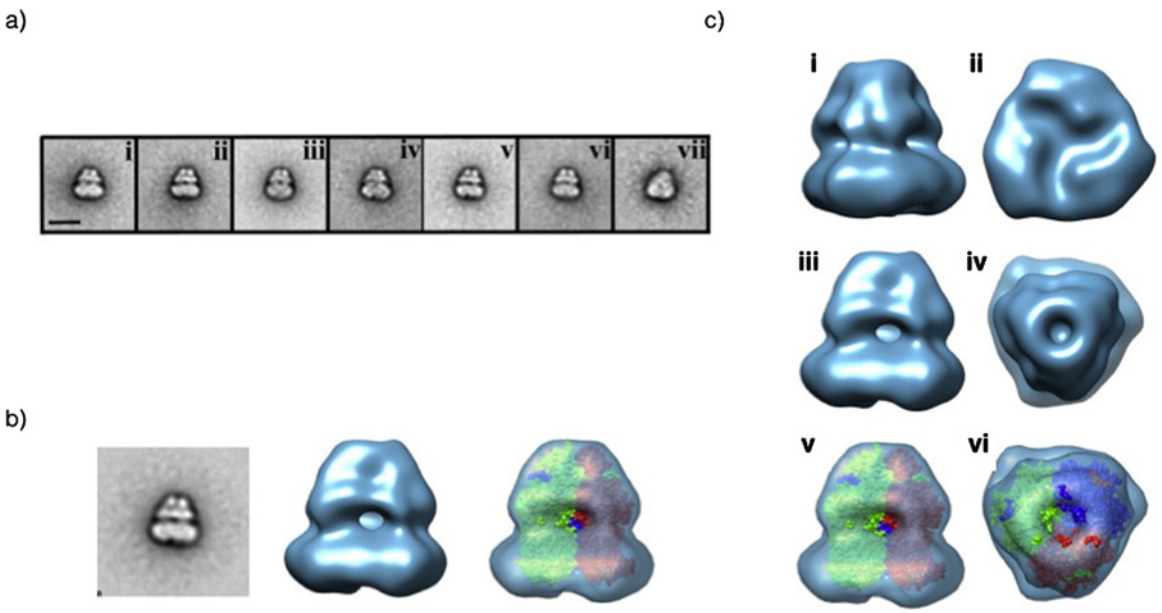
High-resolution structures of proteins within SMALPs

The emergence of nanodisc-based methods for protein purification has coincided fortuitously with technological advances in EM [45]. Enhanced data recording has increased the resolution that can be achieved by cryo-EM to rival that of crystallographic methods [46,47]. With MP crystallization remaining an unpredictable process [48,49], by-passing it altogether while still producing highly detailed structures, would be a huge step forward. The presence of lipid and SMA may bring smaller proteins into a useful size-range for EM (currently the lower size limit of proteins for accurate structure determination is approximately 100 kDa). EM has long been a useful tool for considering the structures of large assemblies [50], so the combination of SMALPs and high-resolution EM could also prove fruitful for examining the structures of complexes consisting of both soluble and membrane-bound proteins. Indeed, the structures of several proteins in SMALPs have already been solved at moderate resolution using EM [16,33].

A major unanswered question is whether it will be possible to use X-ray crystallography to determine very high-resolution structures of MP-SMALPs. It seems unlikely that

Figure 3 | Demonstrates the use of negative stain electron microscopy (EM) reconstruction of SMALP AcrB (adapted from Postis et al. [15])

(**a**) Single particle reconstruction shows side views of classes equivalent to viewing AcrB from the plan of the bilayer. (i–vi) and base view vii. (**b**) Side view of AcrB from (**a**) i next to SMALP AcrB reconstruction showing crystal structure with excellent fit. (**c**) Six different views of the SMALP AcrB from the reconstruction demonstrate three-fold symmetry. (i) and (iii) side views (ii) cytoplasmic face (iv) periplasmic face, identifying three-fold symmetry. (v) Side and (vi) top view showing the crystal structure fitted into the reconstruction. SMA/phospholipid envelope can be seen as extra density surrounding the base of the structure.



proteins within SMALPs could form ordered 3D arrays due to the lack of exposed protein to provide crystal contacts. Whether it is possible to remove SMA in a controlled manner to allow nucleation of crystal growth, without destabilizing the protein is unknown, and requires investigation.

Lipidomics of MPs

Understanding the lipidomics of MP could transform our understanding of membrane dynamics. It is increasingly apparent that MPs form specific interactions with lipids, and lipids may mediate MP interactions. Analysis of MPs reconstituted into different lipid mixtures has shown the general influence of lipid composition on their function [51–54] and a few high-resolution structures exist showing lipids in specific locations in the MPs [55]. Mass spectrometry is also emerging as a robust method to analyse the lipids bound to purified MPs [56–58].

There is much still to be learned about the details of these protein:lipid interactions. For example, the interactions are likely to be dynamic, and may vary through the lifetime of the cell, or under different environmental conditions. SMA could provide a method to extract MPs from cells membrane together with their local lipids after varying growth conditions, and identify differences in the lipid composition. Moreover, questions about the existence of lipid rafts continue to divide membrane biochemists, with contradictory findings arguing for both their existence [59–61] and non-existence [62]. By extracting small segments of membranes, SMA may prove to be another method to test these ideas.

One obstacle to understanding the details and implications of membrane–protein interactions with their native lipids is the prevalence of heterologous expression and over-expression. The molecular biology revolution of the 1970s opened up huge opportunities for protein biochemistry, allowing overexpression and subsequent purification of many proteins. However, heterologous overexpression has the drawback of thrusting MPs into non-native environments even before extraction from the membrane. Prokarya and eukarya differ radically in their cellular substructures, protein trafficking signals [63] and the lipid and sterol compositions of their membranes [64]. Common expression systems such a *Escherichia coli* lack any membrane sterols, whereas yeasts produce ergosterol rather than the cholesterol found in mammalian cells. Phospholipid types and distribution also vary between organisms, cell types, specific membranes and even membrane leaflets [65].

Biotechnology can attempt to address these deficiencies by engineering laboratory strains of microorganisms able to synthesize and traffic membrane constituents which they naturally lack, for example yeast that produce cholesterol-like sterols [66]. If we aspire to accurately identify the

annular lipids surrounding MPs, and perhaps also their wider lipid context, SMALPs may be a useful tool. For instance, antibody-based purification is a powerful way to isolate specific proteins from cells and tissues [67] and co-immunoprecipitation allows interacting proteins to be identified without the need for large-scale purification. Combined with SMA solubilization this offers exciting opportunities to examine proteins purified from their native membrane with minimal perturbation to their immediate environment.

At present the possibility that lipids can exchange into and out of SMALPs cannot be excluded, and indeed seems likely from an energetic perspective [68]. Until we understand it better, this possibility undermines the argument that SMALPs can be used to examine the native lipid environment of proteins. On the other hand, if this does prove to be the case, it could be also an opportunity to determine whether specific lipids exchange into discs containing certain proteins. This is an indirect method of assessing the preferred lipid environment of the protein, but it could nonetheless provide useful information. By similar logic, lipids labelled by fluorescence or radioactivity could be added in a controlled way into SMALPs for particular studies.

Conclusions

Although our fundamental knowledge of the architecture and activity of MP has increased in recent years, our understanding of how the functions of individual MPs are integrated into the wider context of the cell remains limited. We believe that SMAs and SMALPs will play a vital role in this expansion of knowledge by enabling us to stabilize difficult target proteins, extract and characterize protein assemblies and better understand the relationship between MPs and membrane lipids. Bypassing detergents may speed up this process, producing more reliable structural and functional descriptions of MPs.

In describing the uses of SMA, the aim is not to promote it as a generic fail-safe method for all circumstances and all experiments, but to highlight its potential to expand our methodological toolbox. The literature describing proteins purified in SMALPs remains in its infancy, as does our understanding of the mechanism of SMALP formation. The possibilities for formulating and addressing important questions seem almost endless, as alluded to in the previous section. Therefore the future of MP purification, if not exclusively disc-shaped seems certain to benefit from nanodiscs, hopefully of many types, sizes and properties. Perhaps more importantly, it seems that the complex interplay between proteins and their associated lipids will dominate membrane research in years to come and SMALPs give us a window into that world.

Acknowledgements

The authors thank the contribution to this work by the late Professor Stephen Baldwin in whose honour the Biochemical Society meeting MPs from A to Z was held. We also acknowledge discussion with colleagues and collaborators working with SMALPs, especially those who have attend our meetings at the University of Birmingham.

Funding

This work was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/J017310/1].

References

1 Krogh, A., Larsson, B., von Heijne, G. and Sonnhammer, E.L. (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. **305**, 567–580 CrossRef PubMed
2 Werck-Reichhart, D. and Feyereisen, R. (2000) Cytochromes P450: a success story. Genome Biol **1**,, REVIEWS3003 CrossRef
3 De Marcos Lousa, C., van Roermund, C.W., Postis, V.L., Dietrich, D., Kerr, I.D., Wanders, R.J., Baldwin, S.A., Baker, A. and Theodoulou, F.L. (2013) Intrinsic acyl-CoA thioesterase activity of a peroxisomal ATP binding cassette transporter is required for transport and metabolism of fatty acids. Proc. Natl. Acad. Sci. U.S.A. **110**, 1279–1284 CrossRef PubMed
4 Hicke, L. and Dunn, R. (2003) Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. Annu. Rev. Cell Dev. Biol. **19**, 141–172 CrossRef PubMed
5 Broughton, C.E., Roper, D.I., Van Den Berg, H.A. and Rodger, A. (2015) Bacterial cell division: experimental and theoretical approaches to the divisome. Sci. Prog. **98**, 313–345 CrossRef PubMed
6 Rowlett, V.W. and Margolin, W. (2015) The bacterial divisome: ready for its close-up. Philos. Trans. R. Soc. Lond. B Biol. Sci. **370**, 1679 CrossRef
7 le Maire, M., Philippe, C. and Møller, J.V. (2000) Interaction of membrane proteins and lipids with solubilizing detergents. Biochim. Biophys. Acta **1508**, 86–111 CrossRef PubMed
8 Seddon, A.M., Paul, C. and Booth, P.J. (2004) Membrane proteins, lipids and detergents: not just a soap opera. Biochim. Biophys. Acta **1666**, 105–117 CrossRef PubMed
9 Yang, Z., Wang, C., Zhou, Q., An, J., Hildebrandt, E., Aleksandrov, L.A., Kappes, J.C., DeLucas, L.J., Riordan, J.R., Urbatsch, I.L. et al. (2014) Membrane protein stability can be compromised by detergent interactions with the extramembranous soluble domains. Protein Sci. **23**, 769–789 CrossRef PubMed
10 Errey, J.C., Doré, A.S., Zhukov, A., Marshall, F.H. and Cooke, R.M. (2015) Purification of stabilized GPCRs for structural and biophysical analyses. Methods Mol. Biol. **1335**, 1–15 CrossRef PubMed
11 Crichton, P.G., Lee, Y., Ruprecht, J.J., Cerson, E., Thangaratnarajah, C., King, M.S. and Kunji, E.R. (2015) Trends in thermostability provide information on the nature of substrate, inhibitor, and lipid interactions with mitochondrial carriers. J. Biol. Chem. **290**, 8206–8217 CrossRef PubMed
12 Knowles, T.J., Finka, R., Smith, C., Lin, Y.P., Dafforn, T. and Overduin, M. (2009) Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer. J. Am. Chem. Soc. **131**, 7484–7485 CrossRef PubMed
13 Gulati, S., Jamshad, M., Knowles, T.J., Morrison, K.A., Downing, R., Cant, N., Collins, R., Koenderink, J.B., Ford, R.C., Overduin, M. et al. (2014) Detergent-free purification of ABC (ATP-binding-cassette) transporters. Biochem. J. **461**, 269–278 CrossRef PubMed
14 Jamshad, M., Lin, Y.P., Knowles, T.J., Parslow, R.A., Harris, C., Wheatley, M., Poyner, D.R., Bill, R.M., Thomas, O.R., Overduin, M. and Dafforn, T.R. (2011) Surfactant-free purification of membrane proteins with intact native membrane environment. Biochem. Soc. Trans. **39**, 813–818 CrossRef PubMed
15 Postis, V., Rawson, S., Mitchell, J.K., Lee, S.C., Parslow, R.A., Dafforn, T.R., Baldwin, S.A. and 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 **1848**, 496–501 CrossRef PubMed

16 Dörr, J.M., Koorengevel, M.C., Schäfer, M., Prokofyev, A.V., Scheidelaar, S., van der Cruijsen, E.A., Dafforn, T.R., Baldus, M. and Killian, J.A. (2014) Detergent-free isolation, characterization, and functional reconstitution of a tetrameric K+ channel: the power of native nanodiscs. Proc. Natl. Acad. Sci. U.S.A. **111**, 18607–18612 CrossRef PubMed
17 Orwick-Rydmark, M., Lovett, J.E., Graziadei, A., Lindholm, L., Hicks, M.R. and Watts, A. (2012) Detergent-free incorporation of a seven-transmembrane receptor protein into nanosized bilayer Lipodisq particles for functional and biophysical studies. Nano Lett. **12**, 4687–4692 CrossRef PubMed
18 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. et al. (2015) G-protein coupled receptor solubilization and purification for biophysical analysis and functional studies, in the total absence of detergent. Biosci. Rep. **35**, CrossRef
19 Long, A.R., O'Brien, C.C., Malhotra, K., Schwall, C.T., Albert, A.D., Watts, A. and 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 CrossRef PubMed
20 Sahu, I.D., McCarrick, R.M., Troxel, K.R., Zhang, R., Smith, H.J., Dunagan, M.M., Swartz, M.S., Rajan, P.V., Kroncke, B.M., Sanders, C.R. and Lorigan, G.A. (2013) DEER EPR measurements for membrane protein structures via bifunctional spin labels and lipodisq nanoparticles. Biochemistry **52**, 6627–6632 CrossRef PubMed
21 Paulin, S., Jamshad, M., Dafforn, T.R., Garcia-Lara, J., Foster, S.J., Galley, N.F., Roper, D.I., Rosado, H. and 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 CrossRef PubMed
22 Swainsbury, D.J.K., Scheidelaar, S., van Grondelle, R., Killian, J.A. and Jones, M.R. (2014) Bacterial reaction centers purified with styrene maleic acid copolymer retain native membrane functional properties and display enhanced stability. Angew. Chem. Int. Ed. **53**, 11803 CrossRef
23 Prabudiansyah, I., Kusters, I., Caforio, A. and Driessen, A.J.M. (2015) Characterization of the annular lipid shell of the Sec translocon. Biochim. Biophys. Acta **1848**, 2050–2056 CrossRef PubMed
24 Li, D., Li, J., Zhuang, Y., Zhang, L., Xiong, Y., Shi, P. and Tian, C. (2015) Nano-size uni-lamellar lipodisq improved *in situ* auto-phosphorylation analysis of E. coli tyrosine kinase using (19)F nuclear magnetic resonance. Protein Cell **6**, 229–233 CrossRef PubMed
25 Jamshad, M., Grimard, V., Idini, I., Knowles, T.J., Dowle, M.R., Schofield, N., Sridhar, P., Lin, Y., Finka, R., Wheatley, M. et al. (2014) Structural analysis of a nanoparticle containing a lipid bilayer used for detergent-free extraction of membrane proteins. Nano Res. **8**, 774–789 CrossRef
26 Vargas, C., Carolyn, V., Arenas, R.C., Erik, F. and Sandro, K. (2015) Nanoparticle self-assembly in mixtures of phospholipids with styrene/maleic acid copolymers or fluorinated surfactants. Nanoscale **7**, 20685–20696 CrossRef PubMed
27 Gulati, S., Jamshad, M., Knowles, T.J., Morrison, K.A., Downing, R., Cant, N., Collins, R., Koenderink, J.B., Ford, R.C., Overduin, M. et al. (2014) Detergent-free purification of ABC (ATP-binding-cassette) transporters. Biochem. J **461**, 269–278 CrossRef PubMed
28 Knowles, T.J., Finka, R., Smith, C., Lin, Y.P., Dafforn, T. and Overduin, M. (2009) Membrane proteins solubilized intact in lipid containing nanoparticles bounded by styrene maleic acid copolymer. J. Am. Chem. Soc. **131**, 7484–7485 CrossRef PubMed
29 Reference deleted PubMed
30 Pos, K.M. (2009) Drug transport mechanism of the AcrB efflux pump. Biochim. Biophys. Acta **1794**, 782–793 CrossRef PubMed
31 Tribet, C., Audebert, R. and Popot, J.L. (1996) Amphipols: polymers that keep membrane proteins soluble in aqueous solutions. Proc. Natl. Acad. Sci. U.S.A. **93**, 15047–15050 CrossRef PubMed
32 Bayburt, T.H., Grinkova, Y.V. and Sligar, S.G. (2002) Self-assembly of discoidal phospholipid bilayer nanoparticles with membrane scaffold proteins. Nano Lett **2**, 853–856 CrossRef
33 Postis, V., Rawson, S., Mitchell, J.K., Lee, S.C., Parslow, R.A., Dafforn, T.R., Baldwin, S.A. and 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 **1848**, 496–501 CrossRef PubMed
34 Kühlbrandt, W. (2015) Structure and function of mitochondrial membrane protein complexes. BMC Biol **13**, 89 CrossRef PubMed
35 Strauss, M., Hofhaus, G., Schröder, R.R. and Kühlbrandt, W. (2008) Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. EMBO J. **27**, 1154–1160 CrossRef PubMed
36 van den Brink-van der Laan, E., Chupin, V., Killian, J.A. and de Kruijff, B. (2004) Stability of KcsA tetramer depends on membrane lateral pressure. Biochemistry **43**, 4240–4250 CrossRef PubMed
37 Drozdova, A.A. and Mukhin, S.I. (2012) Lateral pressure profile in a lipid membrane with curvature: analytical expression. Biophys. J. **102**, 503a CrossRef
38 Zhan, H., Huan, Z. and Themis, L. (2013) Inclusion of lateral pressure/curvature stress effects in implicit membrane models. Biophys. J. **104**, 643–654 CrossRef PubMed
39 Doerr, J.M., Koorengevel, M.C., Schäfer, M., Prokofyev, A.V., Scheidelaar, S., van der Cruijsen, E.A., Dafforn, T.R., Baldus, M. and Killian, J.A. (2014) Solubilization, purification and characterization of the potassium channel Kcsa in its native lipid environment: the power of native nanodiscs. Biophys. J. **106**, 298a CrossRef
40 Lee, S.C., Knowles, T.J., Postis, V.L., Jamshad, M., Parslow, R.A., Lin, Y.P., Goldman, A., Sridhar, P., Overduin, M., Muench, S.P. and Dafforn, T.R. (2016) A method for detergent-free isolation of membrane proteins in their local lipid environment. Nat. Protoc. **11**, 1149–1162 CrossRef PubMed
41 Wheatley, M., Charlton, J., Jamshad, M., Routledge, S.J., Bailey, S., La-Borde, P.J., Azam, M.T., Logan, R.T., Bill, R.M., Dafforn, T.R. and Poyner, D.R. (2016) GPCR-styrene maleic acid lipid particles (GPCR-SMALPs): their nature and potential. Biochem. Soc. Trans. **44**, 619–623 CrossRef PubMed
42 Orwick-Rydmark, M., Lovett, J.E., Graziadei, A., Lindholm, L., Hicks, M.R. and Watts, A. (2012) Detergent-free incorporation of a seven-transmembrane receptor protein into nanosized bilayer Lipodisq particles for functional and biophysical studies. Nano Lett. **12**, 4687–4692 CrossRef PubMed
43 Klumperman, B. and Bert, K. (2010) Mechanistic considerations on styrene–maleic anhydride copolymerization reactions. Polym. Chem. **1**, 558 CrossRef
44 Pagliano, C., Barera, S., Chimirri, F., Saracco, G. and Barber, J. (2012) Comparison of the α and β isomeric forms of the detergent n-dodecyl-D-maltoside for solubilizing photosynthetic complexes from pea thylakoid membranes. Biochim. Biophys. Acta **1817**, 1506–1515 CrossRef PubMed
45 Ruskin, R.S., Yu, Z. and Grigorieff, N. (2013) Quantitative characterization of electron detectors for transmission electron microscopy. J. Struct. Biol. **184**, 385–393 CrossRef PubMed
46 Cong, Y., Yao, C. and Ludtke, S.J. (2010), Methods in Enzymology, pp. 211–235
47 Doerr, A. and Allison, D. (2015) Structural biology: Cryo-EM goes high-resolution. Nat. Methods **12**, 598–599 CrossRef PubMed
48 Carpenter, E.P., Beis, K., Cameron, A.D. and Iwata, S. (2008) Overcoming the challenges of membrane protein crystallography. Curr. Opin. Struct. Biol. **18**, 581–586 CrossRef PubMed
49 Tate, C.G. (2009), in Methods in Molecular Biology 187–203
50 Booth, D.S., Avila-Sakar, A. and Cheng, Y. (2011) Visualizing proteins and macromolecular complexes by negative stain EM: from grid preparation to image acquisition. J. Vis. Exp. **58**, e3227 CrossRef
51 Xie, X.S., Tsai, S.J. and Stone, D.K. (1986) Lipid requirements for reconstitution of the proton-translocating complex of clathrin-coated vesicles. Proc. Natl. Acad. Sci. U.S.A. **83**, 8913–8917 CrossRef PubMed
52 Kirilovsky, J., Jorge, K., Sonia, S.-M., Zvi, S. and Michael, S. (1985) Lipid requirements for reconstitution of the delipidated β-adrenergic receptor and the regulatory protein. FEBS Lett. **183**, 75–80 CrossRef PubMed
53 Doolittle, G.M., Reconstitution and partial purification of acyl-coenzyme A:cholesterol acyltransferase and investigations of the enzyme's activity in cultured cells after reconstitution in lipid vesicles, doi:10.1349/ddlp.1576 PubMed
54 Klingenberg, M. (2009) Cardiolipin and mitochondrial carriers. Biochim. Biophys. Acta **1788**, 2048–2058 CrossRef PubMed
55 Long, S.B., Xiao, T., Campbell, E.B. and Roderick, M. (2007) Atomic structure of a voltage-dependent K channel in a lipid membrane-like environment. Nature **450**, 376–382 CrossRef PubMed

56 Laganowsky, A., Reading, E., Allison, T.M., Ulmschneider, M.B., Degiacomi, M.T., Baldwin, A.J. and Robinson, C.V. (2014) Membrane proteins bind lipids selectively to modulate their structure and function. Nature **510**, 172–175 CrossRef PubMed

57 Reading, E., Walton, T.A., Liko, I., Marty, M.T., Laganowsky, A., Rees, D.C. and Robinson, C.V. (2015) The effect of detergent, temperature, and lipid on the oligomeric state of MscL constructs: insights from mass spectrometry. Chem. Biol. **22**, 593–603 CrossRef PubMed

58 Bechara, C., Bolbach, G., Bazzaco, P., Sharma, K.S., Durand, G., Popot, J.L., Zito, F. and Sagan, S. (2012) MALDI-TOF mass spectrometry analysis of amphipol-trapped membrane proteins. Anal. Chem. **84**, 6128–6135 CrossRef PubMed

59 Leslie, M. (2013) A lifeline for lipid rafts? J. Cell Biol. **202**, 3–3 CrossRef

60 LaRocca, T.J., Pathak, P., Chiantia, S., Toledo, A., Silvius, J.R., Benach, J.L. and London, E. (2013) Proving lipid rafts exist: membrane domains in the prokaryote Borrelia burgdorferi have the same properties as eukaryotic lipid rafts. PLoS Pathog **9**, e1003353 CrossRef PubMed

61 Bramkamp, M. and Lopez, D. (2015) Exploring the existence of lipid rafts in bacteria. Microbiol. Mol. Biol. Rev. **79**, 81–100 CrossRef PubMed

62 Sevcsik, E., Brameshuber, M., Fölser, M., Weghuber, J., Honigmann, A. and Schütz, G.J. (2015) GPI-anchored proteins do not reside in ordered domains in the live cell plasma membrane. Nat. Commun. **6**, 6969 CrossRef PubMed

63 Dacks, J.B. and Field, M.C. (2007) Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode. J. Cell Sci. **120**, 2977–2985 CrossRef PubMed

64 van Meer, G., Voelker, D.R. and Feigenson, G.W. (2008) Membrane lipids: where they are and how they behave. Nat. Rev. Mol. Cell Biol. **9**, 112–124 CrossRef PubMed

65 van Meer, G. and de Kroon, A.I.P.M. (2010) Lipid map of the mammalian cell. J. Cell Sci. **124**, 5–8 CrossRef

66 Kitson, S.M., Mullen, W., Cogdell, R.J., Bill, R.M. and Fraser, N.J. (2011) GPCR production in a novel yeast strain that makes cholesterol-like sterols. Methods **55**, 287–292 CrossRef PubMed

67 Schneider, C., Newman, R.A., Sutherland, D.R., Asser, U. and Greaves, M.F. (1982) A one-step purification of membrane proteins using a high efficiency immunomatrix. J. Biol. Chem. **257**, 10766–10769 PubMed

68 Vargas, C., Arenas, R.C., Frotscher, E. and Keller, S. (2015) Nanoparticle self-assembly in mixtures of phospholipids with styrene/maleic acid copolymers or fluorinated surfactants. Nanoscale **7**, 20685–20696 CrossRef PubMed

Received 15 January 2016
doi:10.1042/BST20160015