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Review

Membrane biology visualized in nanometer-sized discs formed by styrene maleic acid polymers

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ABSTRACT

Discovering how membrane proteins recognize signals and passage molecules remains challenging. Life depends on compartmentalizing these processes into dynamic lipid bilayers that are technically difficult to work with. Several polymers have proven adept at separating the responsible machines intact for detailed analysis of their structures and interactions. Styrene maleic acid (SMA) co-polymers efficiently solubilize membranes into native nanodiscs and, unlike amphipols and membrane scaffold proteins, require no potentially destabilizing detergents. Here we review progress with the SMA lipid particle (SMALP) system and its impacts including three dimensional structures and biochemical functions of peripheral and transmembrane proteins. Polymers systems are emerging to tackle the remaining challenges for wider use and future applications including in membrane proteomics, structural biology of transient or unstable states, and discovery of ligand and drug-like molecules specific for native lipid-bound states.

1. Introduction to polymer-based membrane solubilization

1.1. Polymers used for direct extraction of membrane proteins

Detailed analysis of molecular assemblies in their membrane environment requires isolation of the desired states. While detergents have proven adept at removing lipids, they can compromise protein stability and are inadequate at mimicking the true chemical and physical properties of biological bilayers. Just as soluble proteins are necessarily surrounded by water to retain stability and functionality, membrane proteins are coated by lipids that help preserve their biological activity. Hence there has been a search for molecular “scissors” that can excise a working section of membrane while leaving protein:lipid complexes intact. Since their discovery as efficient extractors of functionally intact proteins including the PagP palmitoyl transferase and bacteriorhodopsin [1] from membranes, SMA has appeared in over 80 publications (Fig. 1), and a grassroots SMALP network has formed to push the potential of this new field of native nanodisc technology. This community effort is harnessing the unique abilities of SMA polymers and overcoming technical barriers, and builds on decades of work on amphipols [2], bicelles [3] and membrane scaffold protein (MSP)-encircled nanodiscs [4] (Fig. 2). These systems each offer a growing array of new possibilities for membrane protein preparation and analysis, thus driving their rise in popularity and the emergence of practical solutions.

Several formulations of SMA have been shown to be effective at liberating active membrane protein assemblies. All contain styrene and maleic acid subunits arranged in a semi-randomly alternating pattern in a linear chain (Fig. 2). They offer differing ratios of hydrophobic groups, which insert into lipid bilayers. The polar moieties maintain pH-dependent solubility of the polymer. At a critical concentration, these polymers fragment membranes, yielding nanometer-sized discs.

Various free radical syntheses are used to generate these polymers, resulting in a variable number of styrene groups separated by single maleic anhydride groups. Acid hydrolysis is used to form maleic acid from the maleic anhydride group, yielding an active amphipathic polymer that is highly soluble in aqueous solution. They spontaneously form styrene maleic acid lipid particles (SMALPs) when the polymer solution and a membrane fraction or whole cells are mixed, yielding a clear emulsion (Fig. 3). These particles are stable in aqueous solutions, and can be freeze-dried after incorporation and then readily reconstituted. Initial studies reported the use of hydrolyzed versions of SMA2000 and SMA3000 from Total Cray Valley, which contain styrene to maleic acid ratios of 2:1 and 3:1, respectively, as do the related Lipodisq™ products. Polyscope offers XIRAN 30010 and 25,010 reagents, which have comparable activities and styrene to maleic acid subunit ratios of 2.3:1 and 3:1, respectively, as well as lacking cumene end-groups and offering distinct molecular size distributions. Based on comparative data from many studies, the commercially available XIRAN reagents and the various SMA2000 versions are the most

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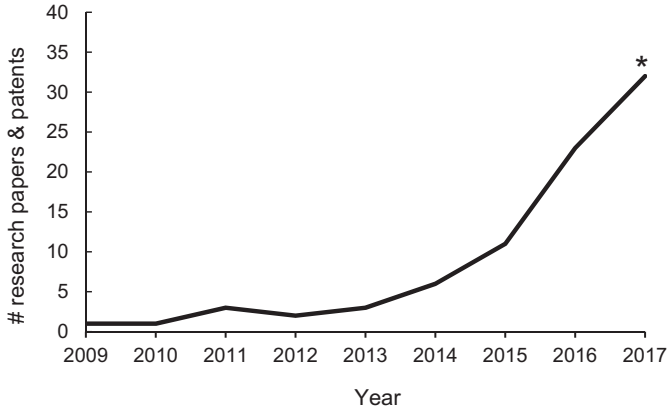

Fig. 1. Growth in the SMALP literature. The number of publications and patents describing SMA since the first paper describing their discovery appeared in 2009. The asterisk for 2017 indicates a partial year count.

effective and widely used SMA polymers for solubilizing a range of membrane proteins from single-transmembrane α-helices [5] to oligomeric complexes containing 36 transmembrane helices [6] and even larger assemblies.

For comparison, a range of SMA types have been tested for solubilization of three different membrane proteins expressed in *Escherichia* (*E.*) *coli* [7]. These included the homodimeric BmrA multidrug efflux pump which contains six transmembrane helices and a nucleotide binding domain, the LeuT symporter with 12 transmembrane helices, and ZipA, which spans the membrane once and has a large extramembrane domain. Yields of ~55% from total protein were purified, with SMA(2:1) producing 8–9 nm diameter nanodiscs with the highest protein yields, purities, and ligand binding activities. In contrast, smaller nanodiscs with diameters of about 5 nm could be produced by various SMA(3:1) polymers. The SMA(2:1) nanodiscs tolerated up to 4 mM magnesium chloride, while the varieties with 3:1 ratios of styrene to maleic acid show precipitation at cation concentrations over 1 mM due to cation chelation by the maleic acid groups. Altogether, the SMA(2:1) polymers with an average molecular mass of 7.5–10 kDa appear optimal for isolating these *E. coli*-expressed assemblies.

1.2. Replacement of detergents with SMA to solubilize and purify membrane assemblies

Conventional detergents work by sequestering lipid molecules within micelles. They dramatically disrupt bilayers, thus perturbing the stability and functionality of proteins. Hence only stable systems and those with reliable assays have been tractable. In contrast, SMA is much less disruptive and acts as a mild solubilizing agent by inserting into bilayers and, at a critical polymer concentration, spontaneously forming nanodiscs [8,9]. The optimal pH depends on the SMA polymer, but is in the range of 7.0 to 9.0, with lower pH values causing polymer aggregation, while low salt conditions generally help keep the SMA soluble [10]. The lipid bilayer structure appears to be less affected by polymers containing a lower amount of styrene to maleic acid, with a 2:1 ratio being less disruptive than a 3:1 ratio [11]. The exchange of lipids between particles is relatively rapid compared to other nanodisc systems, and is mediated by diffusion of monomeric lipid molecules out of discs and, particularly at high concentrations, by collisions between discs [12]. The dynamic nature of the SMA presumably allows lipids to be introduced or removed from a SMALP relatively easily, and also allows proteins to be transferred back into a liposome or other bilayer system [13].

The specificity of SMA for lipids has been investigated by multiple studies. They all show promiscuous interactions with various phospholipid bilayers and native biological membranes. Nonetheless, there is strong selectivity for fluid phase bilayers, while densely packed or ordered bilayers resist insertion of SMA [14]. There are differences in the rates of SMA-mediated solubilization, with easier insertion and disruption of bilayers formed of lipids having acyl chains that are shorter or unsaturated or having cylindrical shapes [15].

Eight SMA types have been surveyed for their abilities to solubilize monomeric, dimeric, trimeric and tetrameric forms of the *Rhodobacter sphaeroides* reaction center [16]. The most effective are those with lower molecular weights in the 10 kDa range and having 2:1 and 3:1 ratios of styrene to maleic acid. Increasing oligomer size or tight protein packing results in lower solubilization efficiencies. Fusing cellular membranes with synthetic or biological source lipid can boost protein yields, and large oligomers can be better solubilized in longer chain SMA forms in the 80–120 kDa range. In addition to small nanodiscs, larger membrane fragments with diameters of 50–100 nm can also be discerned by transmission electron microscopy (EM).

SMA perforates and solubilizes plasma and intracellular membranes at different rates, as seen by live cell fluorescence microscopy [17]. Addition of XIRAN 30010 to HeLa cell cultures causes the plasma membrane to perforate first. The polymer can then perforate intracellular membranes next, releasing fluorescent test proteins contained in their organelles shortly after those in the cytosol. There is no apparent preference of SMA for particular organelles in the cell,

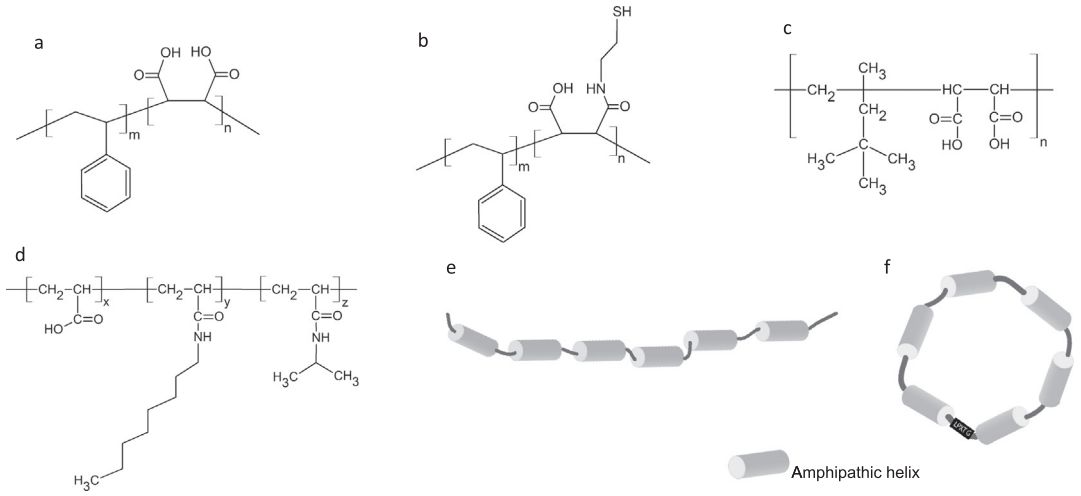

Fig. 2. Chemical structures of polymer-based solubilizing systems. Different synthetic polymers and schematic diagram of protein-based polymers that have been used for the preparation of membrane protein nanodiscs. a) SMA, b) SMA-SH, c) DIBMA, d) amphipols (A8-35), e) linear MSP and f) circularized MSP.

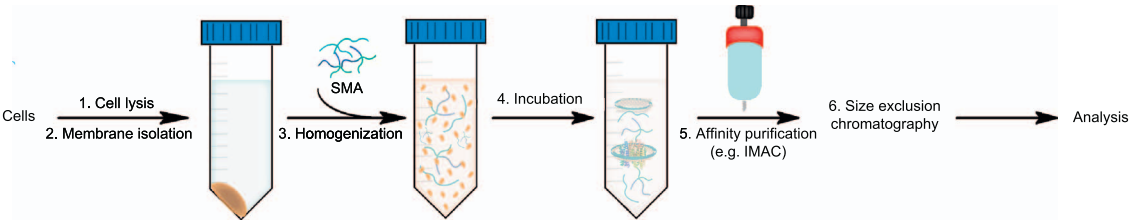

Fig. 3. Schematic diagram of the SMALP protocol. Left: cells are broken open and membrane fractions are suspended in buffer before the addition of SMA. After incubation, the membrane solution with SMA at first appears cloudy and then clarifies. The protein-containing SMALPs can be purified by immobilized metal affinity and size exclusion chromatography for analysis by a variety of biochemical and structural assays.

although proteins in more fluid membranes are liberated faster than those in ordered domains. These results suggest that SMA initially binds to and then penetrates through fluid sections of the plasma membrane, allowing cytosolic molecules to leak out. The polymers thus gain entry to the cell, and after a delay, bind to and perforate the available intracellular membranes. Different protein complexes are solubilized within nanodiscs at various rates that presumably depend on whether they are surrounded by disordered lipid or tightly packed together or engaged in cytoskeletal structures. Liberating the more inaccessible proteins may require addition of lipids such as dimyristoyl phosphatidylcholine (DMPC) or raising the temperature. This, along with polymer concentrations, provides potential ways to selectively access proteins within outer and inner membranes or from ordered or disordered bilayers, as well as de-lipidating or solubilizing compartmentalized transmembrane machines.

1.3. Development of alternative polymers for membrane analysis

The growth of new applications for SMA polymers has generated a flurry of derivative chemistries and comparative studies. For example, a thiol version, SMA-SH (Fig. 2), can be further functionalized by fluorescent dyes and molecular tags [18]. The attachment of Alexa Fluor 488 and biotin tags to the polar subunits enhances the capacity of SMALPs for affinity purification and detection. The Alexa group can be used as donor fluorophore for Förster Resonance Energy Transfer (FRET) experiments that provide distances to an acceptor, such as can be included in a lipid molecule. The attachment of biotin to the maleimide group allows purification of the particles using avidin, and provides an avenue for purifying untagged and endogenous membrane proteins in native nanodiscs.

The controlled synthesis of SMA polymers with low dispersity allows more homogeneous discs of specific sizes to be generated. Reversible addition-fragmentation chain transfer (RAFT) methods to produce 2:1 and 3:1 subunit ratio SMA polymers, which convert liposomes into nanodiscs with diameters of approximately 28 and 10 nm, respectively, as seen by dynamic light scattering (DLS) and EM methods [19]. The polymer length, on the other hand, has no apparent effect on particle size. Higher ratios of styrene to maleic acid render the polymer insufficiently soluble in aqueous solution, while lower ratios are too hydrophilic to solubilize membranes. This analysis indicates that RAFT-SMA with alternating 3:1 subunit ratios are optimal for rapid and complete dissolution of synthetic liposomes into small discs, while larger protein complexes may be better solubilized by RAFT-SMA(2:1) polymers. A recent study of RAFT polymerization shows that greatest solubilization is achieved using a steep gradient of styrene and maleic acid monomers in a small (3 kDa) polymer, while inclusion of a long homoblock of styrene is detrimental [20].

A new family of zwitterionic SMA (zSMA) RAFT polymers have been designed that offer compatibility with a broad range of buffers [21]. They show solubilization efficiencies that are similar to SMA polymers but contain zwitterionic PC rather than maleic acid groups. In contrast to conventional SMA polymers, zSMAs demonstrate compatibility with low pH buffers and polycationic solutions, and hence are uniquely suited to studying proteins that depend on cations such as Mg^{2+} or acidic conditions. Moreover, the zSMA lipid particles appear to be more uniform in size, and their diameters of 10–30 nm correlate with RAFT polymer length, thus providing a way to accommodate cargo of diverse sizes.

Ethanolamine modification of the carboxylic groups of a short SMA(1.3:1) polymer yields a product, SMA-EA, that can spontaneously solubilize multilamellar vesicles into discs of diameters between 10 and 50 nm [22]. Static light scattering data indicate high stability of the resulting nanodiscs under a range of different pH values, temperatures, and divalent cation and salt concentrations. Incorporating nitrogen-15 labelled 16 kDa cytochrome *b5* protein into small SMA-EA nanodiscs allows their backbone signals to be resolved by solution state nuclear magnetic resonance spectroscopy (NMR) spectroscopy, revealing the expected folded state. Moreover, the alignment of large SMA-EA disks in magnetic fields can be controlled by addition of lanthanide ions such as Yb^{3+}. This allows tilt angles of transmembrane helices to be determined by solid state NMR experiments, providing structural insights into protein orientations.

An alternative to SMA polymers with aliphatic rather than aromatic moieties was recently found by Sandro Keller and colleagues [23] to more gently extract membrane proteins. It offers alternating diisobutylene and maleic acid sidechains that are less perturbing to native nanodiscs and hence may be advantageous for solubilizing particularly labile protein complexes. Depending on the polymer concentration, treatment with DIBMA polymer (Fig. 2), which is a BASF product known as Sokalan CP9, yields native nanodiscs with diameters of 12 to 29 nm. They can hold a wide range of proteins, with a bias towards those that are generally larger than those solubilized by SMA treatment. Advantages of DIBMA over SMA polymers include their transparency in the UV and circular dichroism spectra used to quantify protein concentrations and secondary structures, respectively. While DIBMA is a less efficient solubilizer than SMA, it is compatible with higher concentrations of divalent cations such as calcium and magnesium, which are important for activities of some proteins, such as kinases and nucleotide transporters.

2. Composition of polymer-lipid particles

Native nanodiscs can contain virtually any molecules found within the original membrane environment. Lipid phosphate levels can be measured [1], and specific lipid types can be identified by thin layer chromatography or by mass spectrometry, confirming that lipid molecules in the immediate vicinity of the solubilized protein are retained. The conversion of liposomes into lipid nanodiscs during polymer titrations can be readily monitored by NMR spectroscopy. Due to their large sizes, liposomes exhibit completely broadened phosphorus-31 NMR signals, while rapidly tumbling nanodiscs yield sharp signals for contained phospholipids. The transition is sharp, moving rapidly from onset to completion of membrane solubilization with only a neglible concentration of free polymer DIBMA [23]. The proton NMR spectra of the polymer and contained proteins are broadened due to the polydispersity of the polymers and heterogeneity of the assemblies [1].

Nonetheless, the carbon-13 NMR signals of lipid substrates and products of transmembrane enzymes can be resolved [1], suggesting that interacting ligand signals may also be discernible. Despite this progress, mapping the ligand binding and lipid interaction sites within solubilized proteins remains technically difficult and may require more homogeneous nanodiscs.

3. Structures of membrane assemblies

The bilayer discs formed using SMA polymers range from 6 to 30 nm in diameter, as seen by EM. Those formed from synthetic lipids may be smaller, with sizes of 6–10 nm reported using XIRAN 25010 polymer [24]. The discs comprise an inner patch of bilayer surrounded by a belt of polymer, as visualized by small angle neutron scattering using hydrogenated and deuterated lipids for contrast [11]. The packing of the styrene groups against lipid acyl chains is evidenced by close intermolecular distances observed by NMR [11,25]. The elucidation of novel structures of proteins in SMALPs by NMR spectroscopy has not yet proven to be feasible. This limitation is presumably due to heterogeneity within nanodisc samples, as is evident by EM even after removal of the excessive polymer by size exclusion chromatography. More homogeneous polymer formulations or transfer of isotope labelled proteins into other membrane mimicking systems may be required to achieve the necessary spectral quality.

The structure of the membrane-spanning KCNE1 protein, which modulates a voltage-gated potassium ion channel, has been characterized in SMALPs. The diameters of the particles containing monomeric KCNE1 depend on the SMA:lipid ratio, and range from 14 to 30 nm when slightly more polymer is present than lipid by weight, as observed by dynamic light scattering and transmission EM, while using insufficient SMA yields larger aggregates [26]. Site-directed mutagenesis was used to introduce spin labels, allowing identification of residues located inside and outside of the membrane environment by electron paramagnetic resonance [27].

A functional nucleoside transporter has been prepared from insect cells using XIRAN 30010 for solubilization [28]. A lower polymer concentration of only 0.25% (rather than the typical 1–3%) was added at low temperature to avoid protein degradation and inactivation, and cholesteryl hemisuccinate was added as a stabilizing agent. The resulting protein complex in SMALPs possesses native inhibitor binding properties, while conventional detergents such as decyl maltoside destabilize the protein. Protein-free SMALPs display negligible inhibitor binding, which bodes well for ligand binding and screening experiments.

A major breakthrough occurred with the report of the first high resolution structure of a SMA-solubilized, lipid-embedded protein [13]. The seven membrane-spanning α-helices of a microbial rhodopsin could be resolved with an increased resolution of 2.0 Å (Fig. 4a). This involved use of DMPC to loosen the tightly packed membrane before XIRAN 25010-based liberation. Addition of a second poly-histidine tag was needed to achieve the requisite purity, and monoolein was used form lipidic cubic phases for protein crystallization. The structure of the trimeric protein includes a bound *trans*-retinal molecule, although native lipids lacked sufficient avidity to be detected.

The growing popularity of EM for structural determination of membrane proteins has prompted analysis of native-state proteins in SMALPs. Preparation of eukaryotic ATP-binding-cassette transporters from a variety of expression systems using SMA reveals better activity, purity and stability than conventional detergents, and yields low resolution envelopes of the *P*-glycoprotein dimer [29]. The AcrB multidrug transporter forms a trimer in the *E. coli* inner membrane that can be purified intact *via* a His_8 tag using SMA, with low salt preventing spurious particle association [30]. Sedimentation velocity experiment reveals the presence of dimeric and trimeric forms, while negative stain EM and 3D reconstruction reveals an inner vestibule surrounded by a belt of at least 40 lipid molecules and polymer around the trimeric protein (Fig. 4b). The global structure of AcrB extracted, purified and visualized in SMA(2:1) is resolvable by cryo-EM at 8.8 Å, with higher resolution for its soluble domain while two exposed helices appear dynamic [31]. Together with recent progress on visualizing NMR signals of proteins in SMA-derived nanodiscs [22] and polymer developments, this bodes well for further structural studies of native membrane states.

The potential of NMR spectroscopy to resolve integral membrane proteins in SMALPs is demonstrated by a recent study of the bacterial zinc diffusion facilitator CzcD [32]. This 34 kDa protein could be similarly solubilized with either XIRAN 25010 or 30010, retaining a single layer of bound lipid, and purifies well with Strep tag. The helical dimers within the 10–15 nm nanodiscs display resolvable signals by solid-state NMR methods when deuterated and selectively re-protonated at amide or methyl sites. Comparison with the spectra of the solution state of isolated cytoplasmic domain allows assignments to be transferred. Thus, while the polydispersity and size of these SMALPs prevents detection of the transmembrane helices, signals and structural and dynamic data can be obtained from the attached soluble domain.

4. Functions of native membrane assemblies

The concerted action of multiple enzymes and cofactors in diverse biological processes including metabolic pathways requires precise molecular organization within membranes. Many of these assemblies have been difficult to solubilize and purify intact. The metabolic pathway that catalyzes the production of a glucoside known as dhurrin is mediated by the metabolon. This complex is formed by a soluble glycotransferase and three membrane-anchored proteins along with associated lipids. Treatment of microsome membranes with SMA2000 transfers the entire endogenous complex into SMALPs with 10–25 nm diameters and yields of 80%, while conventional detergents such as cholate dissociate the complex [33]. The soluble subunit can be added to the transmembrane assembly in the SMALP to modulate the activity of the transmembrane components. The metabolon function also depends on the presence of negatively charged phospholipids which co-associate with isolated proteins, as detected by gas chromatography mass spectrometry. Together this demonstrates that large endogenous complexes of transmembrane and peripherally associated proteins can be solubilized intact using SMA, suggesting that the dynamic organization of other macromolecular machines will increasingly become accessible.

The most valuable class of drug targets is the G protein-coupled receptors (GPCRs), and hence they have been a subject of intense study. The adenosine receptor expressed in *Pichia pastoris* and mammalian HEK cells and solubilized with SMA(2:1) in native nanodiscs exhibits the expected ligand binding activity and thermostability. It can be repeatedly stored and freeze-thawed in SMALPs, and exhibits a half-life seven times longer than when isolated with conventional detergents [34]. Extraction of the melatonin and ghrelin receptors from liposomes or *Pichia pastoris* membranes using SMA with either a 2:1 or 3:1 ratio of styrene to maleic acid yields 13 nm nanodiscs. These solubilized proteins recognize their respective ligands and transmit signals while exhibiting the expected conformational changes [35]. Moreover, upon agonist stimulation, these GPCRs activate G proteins and recruit arrestins. Thus, SMALPs appear to be an attractive vehicle for preparing native states of therapeutic targets, although the yields of proteins in various hosts vary and can require optimization.

A comparative study of 15 human tetraspanins expressed in *S. cerevisiae* shows that these proteins, which form oligomeric networks, can be purified in SMALPs [36]. In particular, the CO-029, TSPAN7, TSPAN12 and TSPAN18 proteins could be extracted from isolated membranes using either conventional detergents or three SMA types. Despite this success, glycosylation and palmitoylation patterns of tetraspanins vary and their yields in intracellular granules and membranes differ. These complexities, along with the lack of ready biochemical

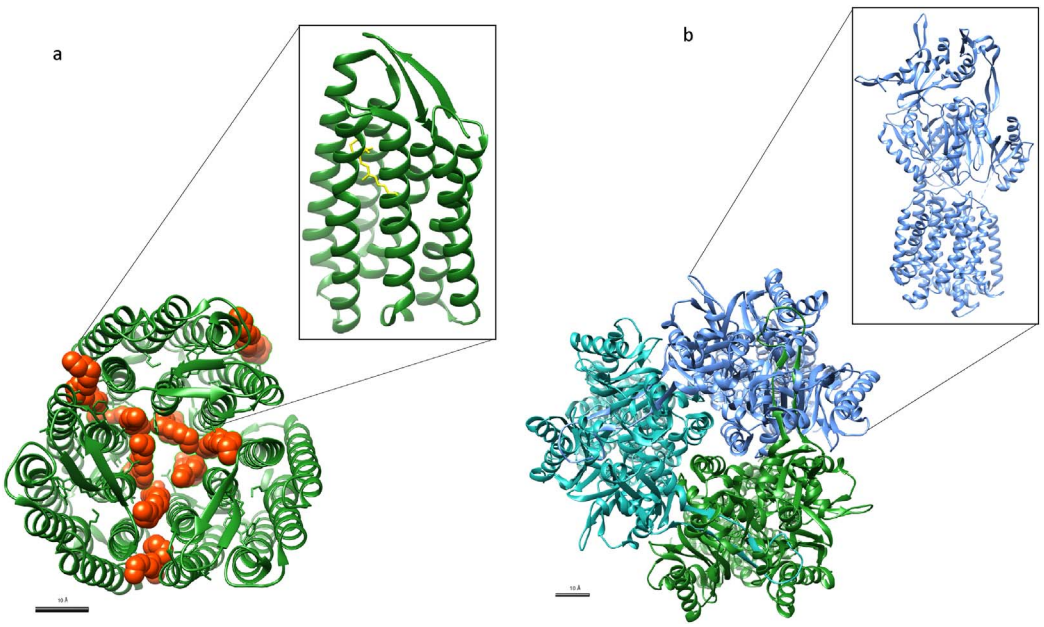

Fig. 4. Macromolecular structures elucidated using SMA. a) The first crystal structure of a membrane protein, that of the seven-transmembrane microbial rhodopsin (PDB ID: 5ITC) obtained *via* SMA-LCP-mediated approach, shows a trimer with nine monoolein lipids (in red) occupying the inter-monomer spaces [13]. b) Top and side views of the secondary transporter AcrB trimer in SMALPs resolved by EM using the crystal structure obtained with DDM detergent (PDB ID: 1IWG).

assays, multimeric networks and small, disulfide rich extramembrane domains, render these targets particularly challenging to characterize by any method. Nonetheless the ability to solubilize the intact tetraspanin web may be key to deciphering their detailed mechanisms.

Extraction of cytochrome *c* oxidase protein from *Saccharomyces cerevisiae* mitochondrial membrane using SMA(3:1) yields functional complexes with bound phospholipids in native nanodiscs [37]. The recombinant constituent proteins can also be purified using C-terminal His_7 tags [38]. The latter particles do not resemble round discs, instead displaying oblong dimensions of 11 nm and 14 nm by negative stain EM. This shape is presumably needed to accommodate the complex, which is composed of 11 subunits which have asymmetric charge and act as a transmembrane proton pump. The particles contain the same major phospholipids found in the mitochondrial inner membrane, associate with respiratory supercomplex factors, and exhibit the expected O_2-reduction activity. Excess SMA appears to reversibly inhibit the catalytic activity, possibly due to spurious protein binding, and hence should be removed after membrane protein extraction.

Spinach thylakoids contain a photosystem I complex of 17 protein subunits that receive excitation energy from light harvesting chlorophyll (LHC) proteins [39]. These monomeric complexes can be solubilized intact with SMA3000 and are purified using sucrose density gradients and gel filtration [39]. The high yield is remarkable given that the PS I complex has dimensions that exceed the size of the bilayer in most SMALPs, suggesting that SMA can adapt to solubilize larger assemblies. Moreover, SMA treatment yields complexes of photosystem I with typically one, three or five LHC II trimers. These proteins are functionally coupled, delivering excitation energy to the photosystem within the nanoparticles. The photoreaction center of the purple bacterium *Rhodobacter sphaeroides* has also been purified with a stable, intact immediate lipid environment using SMA polymer [40]. This yielded elliptical structures with 12–15 nm diameters, to which gold nanoparticles recognizing the His_{10} tags can be seen positioned away from the center of each nanodisc.

The holo-translocon secretes proteins through bacterial inner membranes. It consists of SecYEG–SecDF–YajC–YidC components that can be solubilized together from the *E. coli* membrane using SMA(2:1) polymer [41]. The functional complex of SecYEG and SecA along with essential negatively charged lipids can also be isolated using SMA(3:1) polymer but not conventional detergents [42]. The component subunits of the holo-translocon are identifiable in the SMALPs using specific antibodies in immunoprecipitation experiments. Transferring the complex into proteoliposomes using Bio-Beads allows the accurate translocation of a variety of transmembrane proteins. Moreover, the intact translocon also interacts with the ribosome, indicating that the entire complex is needed to form a functional interface.

The physiological structure and function of α-synuclein represent long-standing challenges, yet are central to understanding its contributions to Parkinson's disease. It is found in membrane-associated and cytosolic locations that are inhabited by either monomeric or tetrameric states, which are either unstructured or helical. To address the quandaries about its biological states the purified recombinant protein was incubated with PC-containing SMALPs. This detangles large aggregates, and eliminates the ferrireductase activity associated with α synuclein [43]. With these and other membrane assemblies pushing the limits of SMA and nanodisc technology, new biochemical tools and biological insights will continue to be generated to resolve dynamic states of membrane complexes.

5. Conclusions and future prospects

The past decade has witnessed the emergence of a new field of study of native membrane assemblies using a remarkable family of polymers that spontaneously transform biological membranes into soluble nanoparticles. The dimensions of SMA-bounded nanoparticles span 5–100 nm with shapes varying from circular to ellipsoid to irregular fragments, depending on the polymer used and the complexes contained. The determinants of nanodisc formation by synthetic polymers are beginning to be understood, and can now be used to engineered specific properties and behaviors. Challenges including incorporation of even larger and more labile complexes while keeping the functional integrity and dynamic states intact are being addressed by new polymer formulations. A variety of sidechains and affinity and fluorescent tags can now be attached to SMA, expanding their utility as purification and imaging vehicles.

The generation of smaller, more homogeneous nanodiscs and the transfer of the resulting native protein:lipid complexes into liposomes and LCP systems are allowing high resolution structures of complexes and multimers to be determined. The working pH range of SMA allows convenient solubilization of most biological membrane systems, while new derivatives such as zSMA and SMA-EA operate in a broader range to allow analysis of acidophiles and lysosomal complexes. Use of

alternative polymers like DIBMA allows the study of membrane proteins that are particularly labile or depend on divalent cations for activity. The interaction biases of the different polymers are becoming better understood, with novel polymers emerging that could allow novel high resolution structures to be determined of heteromultimeric complexes. Modelling the molecular dynamics within nanodiscs and the design of directionally oriented states could yield further functional insights.

In the future, screening for novel ligands such as drug-like molecules or lipid modulators could deliver valuable lead molecules specific for previously inaccessible membrane-embedded states which are stabilized by SMALPs. Exploring and exploiting utility of native nanodiscs through lipidomic and proteomic analysis of solubilized organelles, cells and tissues is now more feasible given the cost-effectiveness of available SMA polymers. Together with the development of new experimental polymers and associated methods this provides a wealth of further opportunities for isolating and studying the wider diversity of native molecular complexes that mediate biological processes.

Transparency document

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Abbreviations

ATP	adenosine triphosphate
DIBMA	diisobutylene maleic acid
DLS	dynamic light scattering
DMPC	dimyristoyl phosphatidylcholine
EM	electron microscopy
FRET	Förster Resonance Energy Transfer
GPCR	G protein-coupled receptor
HEK	human embryonic kidney
LCP	lipidic cubic phase
LHC	light harvesting chlorophyll
MSP	membrane scaffold protein
NMR	nuclear magnetic resonance spectroscopy
RAFT	reversible addition fragmentation chain transfer
SMA	styrene maleic acid
SMA-EA	SMA-ethanolamine
SMALP	styrene maleic acid lipid particle
SMA-SH	SMA with sulfhydrils
TSPAN	tetraspanin
zSMA	zwitterionic SMA

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Author contributions

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

Conflicts of interest

The authors declare no conflict of interest.

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