**From Polymer Chemistry to Structural Biology: The Development of SMA and Related Amphipathic Polymers for Membrane Protein Extraction and Solubilisation**

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**Abbreviations**

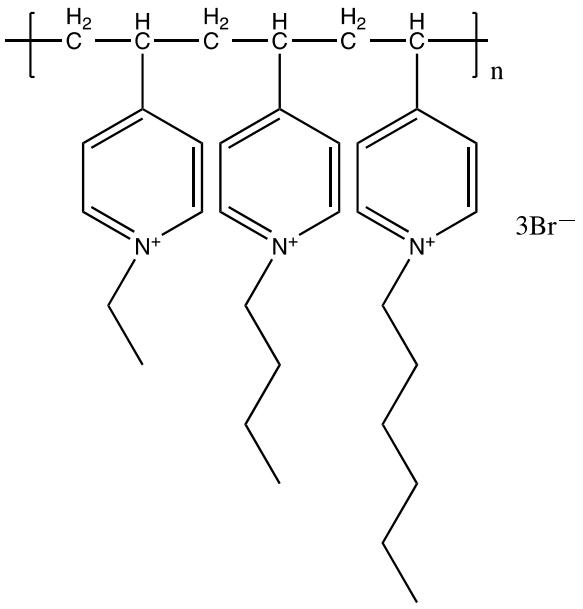
ABC: ATP Binding Cassette
alt-SMA: Alternating hydrolysed copolymer of styrene and maleic anhydride
CTC: Charge-transfer complex
DIBMA: Hydrolysed copolymer of diisobutylene and maleic anhydride
DMPC: 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine
DPPC: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine
EM: Electron microscopy
HDL: High-density lipoprotein
LCP: Lipidic cubic phase
MA: Maleic acid
MSP: Membrane scaffold protein
PEAA: Poly(2-ethacrylic acid) polymer
PMA: poly(methacrylate)
SMA: Hydrolysed copolymer of styrene and maleic anhydride
SMAnh: Non-hydrolysed copolymer of styrene and maleic anhydride
SMA-SH: Thiol derivative of SMA

**Abstract**

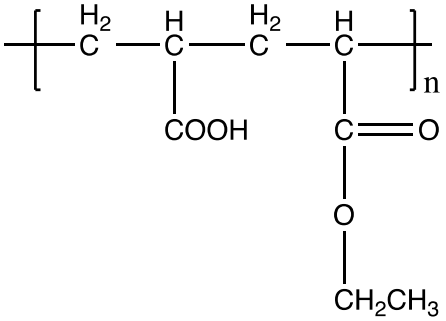
Nanoparticles assembled with poly(styrene-maleic acid) copolymers, identified in the literature as Lipodisq, SMALPs or Native Nanodisc, are routinely used as membrane mimetics to stabilise protein structures in their native conformation. To date, transmembrane proteins of varying complexity (up to 8 beta strands or 48 alpha helices) and of a range of molecular weights (from 27 kDa up to 500 kDa) have been incorporated into this particle system for structural and functional studies. SMA and related amphipathic polymers have become versatile components of the biochemist's tool kit for the stabilisation, extraction and structural characterization of membrane proteins by techniques including cryo-EM and X-ray crystallography. Lipodisq formation does not require the use of conventional detergents and thus avoids their associated detrimental consequences. Here the development of this technology, from its fundamental concept and design to the diverse range of experimental methodologies to which it can now be applied, will be reviewed.

**Interactions between lipid membranes and amphipathic polymers**

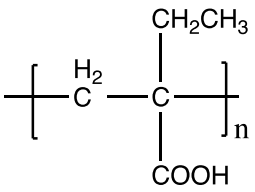
The amphipathic homopolymer poly(2-ethacrylic acid) (PEAA) (Figure 1) was shown by Tirrell and colleagues in the late 1980s to disrupt liposomes of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and to release their contents in a pH-dependent manner[1]. Using electron microscopy (EM), the authors were able to observe the formation of disc-like nanoparticles, of similar appearance to the lipid-containing structures formed by lipoproteins, specifically HDL[1]. The potential that these discoidal macromolecular assemblies could have as drug-delivery vehicles, was proposed by Tonge & Tighe[2]. Initial investigations focused on producing an alternative lipid platform to liposomes both for drug delivery and also for use as a synthetic lung surfactant, (Surfactant Protein B, an amphipathic helical apoprotein that enables alveoli to inflate, had been proposed to form phospholipid bilayer discs in the mammalian lung[3]).



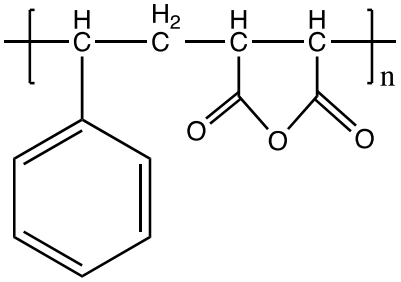

Quaternized poly(4-vinylpyridine)




Copolymer of acrylic acid and ethyl acrylate




Poly(2-ethacrylic acid)



Poly(styrene maleic anhydride)

*Figure 1: Structural formulae of anionic and cationic polymers. Note that the product produced by free-radical polymerisation is heterogeneous (in both length and in the case of copolymers sequence) and contains molecules with a wide range of molecular weights (Table 1).*

Table 1: Typical degree of polymerization number (n) and molecular weight (Mw) of the different polymers presented in Figure 1.

| Polymers | Degree of polymerisation (n) | Mw (g/mol) |
|---|---|---|
| Quaternized poly(4-vinylpyridine) | 6000 | $9.7x10^5$ |
| Poly(2-ethacrylic acid) | 310 | $3x10^4$ |
| Poly(styrene maleic anhydride) | 12-18 | $5\text{-}11x10^3$ |
| Copolymer of acrylic acid and ethyl acrylate | 500 | $1.1x10^5$ |

In the 1950-60s, studies of cationic polysoaps, especially halide salts of alkyl-quaternized pyridine such as poly(4-vinylpyridine) (Figure 1), showed that synthetic polymers with a carbon-carbon backbone and hydrophilic and hydrophobic pendant groups, could fold into compact, surface-active structures with polar and non-polar domains[4]. Anionic polysoaps, such as alternating copolymers of maleic acid (MA, formed by the hydrolysis of maleic anhydride) and *n*-alkyl vinyl ethers, were found to exhibit similar behaviour in aqueous solution and to transition from an expanded polyelectrolyte to a compact polysoap in response to changes in pH and charge density[5]. Hydrolysed copolymers of styrene and maleic anhydride (SMA) (Figure 1) were also able to form compact hydrophobic domains, a behaviour that Dannhauser termed 'hypercoiling'[6]. Solutions of these hypercoiled polymers exhibited a greatly reduced intrinsic viscosity, approximately four times less than that predicted by the Flory equation[6].

As a result of the so-called hydrophobic effect described by Tanford, hypercoiling polymers in aqueous solution adopt a collapsed state, in which hydrophobic parts are crowded together to form nanodomains[7]. The hydrophilic groups interact with the aqueous phase, creating an amphipathic structure with surface activity that is dependent on the protonation states of the pendant carboxylic acid groups. Potentiometric titration of SMA copolymers indicates that when around 20%-50% of the primary ($\alpha_1$) carboxylic acid groups of MA are ionised, an apparent increase in $pK_a$ is observed as the polyanion starts to behave as a weaker acid, consistent with a conformational change in the polymer. The transition to a compact polymer structure occurs over the so-called *collapse pH* range, when 100%-20% of the $\alpha_1$ groups are negatively charged and the secondary ($\alpha_2$) groups of MA are fully uncharged[8–10].

As a result of the observations of Tirrell, and widespread literature covering hypercoiling polymers, the Biomaterials Group at Aston University, led by Tighe, started a test programme in the early/mid 1990s to produce discoidal polymer-phospholipid nanostructures or macromolecular assemblies, based on PEAA and similar polymers. The programme also sought, more broadly, to investigate whether synthetic vinyl polymers could be made to function in a manner analogous to HDL apoproteins. A range of polymers (including copolymers of acrylic or methacrylic acids with short-chain acrylates (Figure 1), or copolymers of styrene or alkyl vinyl ethers with maleic anhydride and their corresponding hydrolysed maleic acid salts) that readily associate with hydrophobic species such as lipids were synthesised. As part of this work, the 1:1 alt-SMA copolymer was found to interact with

DPPC phospholipids to form discoidal nanoparticles in aqueous solution at pH ~4 or below, corresponding to the lower end of the *collapse pH* range for this polymer[2].

Amphipathic helices (in which hydrophobic and hydrophilic groups are presented on opposite faces[11]) are common in native apoproteins, as exemplified by apolipophorin III[12]. Initially it was proposed that it would only be possible to form analogous structures with SMA copolymers, if the polar and non-polar groups alternated along the carbon-carbon backbone, as in the SMA 1:1 alt-copolymer (alt-SMA).

SMA is somewhat unusual for a free-radical initiated polymer, in that it tends to perfect alternation between the styrene and maleic acid (MA) moieties. It has been proposed that this results from the formation of a preformed charge-transfer complex (CTC) by electron transfer from a donor radical to an acceptor monomer and vice versa, in which MA forms one of the monomers; alternatively, a CTC of this type could also be formed between the propagating radical and the monomer; another view considers alternation to be the result of repeat combinations between monomers of differing polarity, which produces a regular sequence distribution of monomer units[13].

Despite the 1:1 ratio of styrene to MA, alt-SMA lacks the torsion angle constraints, imposed by the amide backbone, that are inherent in polypeptides and therefore exhibits only a limited degree of structural regularity. Furthermore, the chirality of the polymer cannot be controlled during synthesis. Other techniques such as RAFT polymerisation have also been employed in an attempt to control polymer architecture, but their precision is not comparable with the high-fidelity template synthesis approach used by ribosomes to build proteins.

**The development of SMA polymers for protein extraction from biological membranes**

Experiments on detergent-solubilized integral membrane proteins have played a fundamental role in helping to understand structures and functional mechanisms. Nevertheless, micelles are poor membrane mimetics as their lateral pressure profile differs considerably from that of a bilayer environment[14,15]. Moreover, detergents may remove or perturb annular lipids, that have a direct influence on protein function[16]. The identification of detergents that maintain protein activity and structure is a time-consuming and expensive task, as no single detergent is appropriate for all membrane proteins[17–19]. High concentrations of detergent may result in the occlusion of binding sites or denaturation of hydrophobic binding domains. Other ligands may bind non-specifically to micelles, reducing their effective concentration in solution[20–22].

In order to mitigate the limitations and complications inherent in the biophysical characterisation of detergent-solubilised samples, extracted membrane proteins may be inserted into reconstituted model membranes (typically containing only a few lipid types). Discoidal lipid structures, including bicelles, have been extensively used, however their size is somewhat variable (and is dependent on dilution) and may be unstable over longer durations[23–27]. It was proposed that SMA-phospholipid nanostructures might offer a solution, by providing a stabilising polymer annulus around the phospholipid bilayer disc; the compact polymer structures formed over a narrow pH range, exhibit a subtle yet significantly increased surface activity that enables them to solubilise DPPC membranes, but not so high as to denature membrane-associated proteins. Early work was performed on lipid

nanoparticles formed with a 1:1 alt-SMA , which were termed “Astosomes” (1997; US 6436905[28]). Unfortunately, the 1:1 alt-SMA copolymer has an apparent $pK_a$ around 4, constraining the pH at which the polymer collapses, and hence at which Astosomes form, to an acidic range far below that generally encountered in biological systems. It was therefore clear that a technology that was only operable below pH 4, would have very limited biochemical and biomedical applications.

Malvern Cosmeceutics Ltd, founded in 2005, investigated SMA block copolymers with a high styrene content. These second-generation SMA polymers require a greater degree of ionization of the carboxylate groups to remain in solution and they therefore collapse into an amphipathic structure in pH ranges closer to physiological conditions. By conducting titrations of various SMA copolymers with styrene:MA ratios of 2:1, 3:1 and 4:1, the apparent $pK_a$ values of the different polymers were determined as pH 5.5-8, pH 6-9 and pH 8.5-10.5 respectively; the 2:1 and 3:1 copolymers collapse in the physiological pH range to form nanostructures in the presence of phospholipids. These apparent $pK_a$ values have subsequently been confirmed by other groups, although it should be noted that the precise value measured is dependent on several factors including the ionic strength[29], the styrene block size, the method of manufacture and the use of end-capping agents.

It is important to note that the apparent $pK_a$ values reported typically refer to the net contribution of all ionisable groups within the polymer, rather than for individual carboxylic acid groups. Broadly speaking, the maximum surface activity of 1:1 SMA polymers occurs when 20—50% of the $\alpha_1$ carboxylic acid groups are ionised[30], which corresponds to the lower end of the *collapse pH* range (potentiometric)[8].

A patent covering non-alt-SMA copolymers “as a means of solubilising membrane peptides or proteins for the investigation of their structure” (US 8623414[31]) was submitted in 2005 by the Malvern group, and commercial products, containing 3:1 and 2:1 SMA in hydrolysed and non-hydrolysed form, were launched in 2007 under the tradename Lipodisq®.

3:1 SMA, when combined with phospholipids, forms discoidal nanostructures with a typical size of around 11 nm in diameter (Figure 2a). This property has been exploited for lipidomics studies[32–34], in particular in eukaryotic systems in which lipid extraction does not result in a loss of organism viability (see, Bada Juarez *et al.*, this volume[35]). Aqueous suspensions of these Lipodisq nanoparticles (also known as SMALPs) are monodisperse[36,37], however at high concentrations they may form stacks (rouleaux), especially when deposited onto surfaces (Figure 2b).

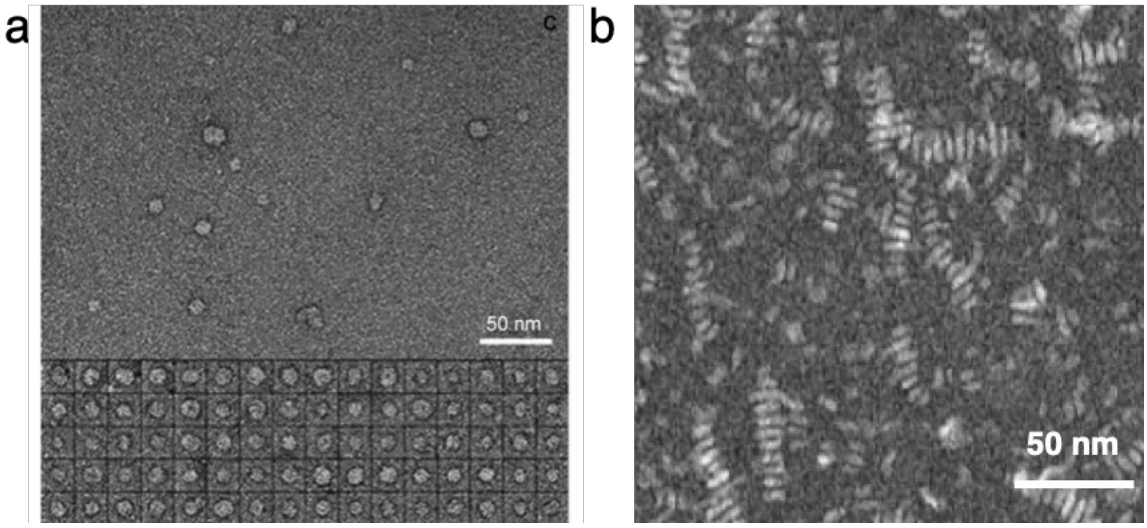



*Figure 2a (left): TEM images of Lipodisq nanoparticles (containing SMA polymer and 1,2-dimyristoyl-sn-glycero-3-phosphocholine, DMPC) reveal that the diameter of the nanoparticles varies between 5–15 nm (adapted from reference [36]).*

*Figure 2b (right): Negative-stain TEM showing the formation of stacks or rouleaux (worm-like structures) of Lipodisq nanoparticles containing bacteriorhodopsin protein from Halobacterium salinarum (unpublished data) revealed using phosphotungstate. Scale bar is 50 nm. (For a full description of the methodology, please see SI.)*

Extraction of membrane proteins by SMA polymers has a number of distinct advantages over more conventional methods of protein solubilisation. Firstly, unlike the processes of bicelle[38], Saposin-nanodisc[39,40] or MSP-nanodisc[41,42] formation, detergent solubilisation is not required as an initial step and so disruption of lipid-protein interactions is minimised[34,43–45]. Like other discoidal lipid nanoparticles, both faces of an embedded membrane protein are accessible to ligands and substrates, and the bilayer structure of the core of the disc reproduces the lateral pressure profile of the native membrane more faithfully than detergent micelles[36,46–48].

**Applications of SMA-lipid nanoparticles for membrane protein characterisation**

In the autumn of 2005, the Malvern group began a collaborative project with Overduin's group at the University of Birmingham (CTB-TTF project[49]) to investigate the ability of SMA copolymers to solubilise membrane proteins (in particular PagP, an eight-stranded beta-barrel outer membrane enzyme) and to characterise biophysical and biochemical properties of the resultant structures using high-field NMR spectroscopy. This work depended upon identification of a low-temperature technique to prepare the nanoparticles without denaturing the protein and was successfully achieved as demonstrated by Knowles and colleagues[37]. As a result, the group at Birmingham observed that 2:1 SMA is able to interact directly with cellular membranes to form protein-containing nanostructures that retain the folding and the oligomeric state present in the native membrane environment. This work subsequently led to a patent (US 8754168[50]) issued to the Birmingham group.

A great strength of membrane protein extraction by SMA is its applicability to proteins from a wide variety of membranes from a diversity of different organisms. To date, proteins from bacteria[51], archaea[52], yeast[53], insect[54] and human cell[54] membranes have been purified for biophysical studies.

Membranes with a high protein content (such as the purple membrane from *Halobacterium salinarum*, which contains bacteriorhodopsin; Figure 2b and 3) may require the addition of exogenous lipid in order to permit the formation of SMA nanoparticles[47].

Protein-containing Lipodisq nanoparticles may be purified using many common chromatographic methods (including size exclusion and $Ni^{2+}$-affinity) with only minor modifications to standard detergent-based purification protocols[55,56]. A human nucleoside transporter and a bacterial zinc-diffusion facilitator protein solubilised in SMA-lipid nanoparticles have also been purified for structural and functional characterisation, using FLAG-tag[57] and Strep-tag[58] affinity chromatography respectively.

Furthermore, proteins embedded in Lipodisq nanoparticles are suitable for a wide range of biophysical experiments (Figure 3). Circular dichroism spectroscopy has been used to study protein folding and thermo-stability[43,56,59]. (Pulsed-)EPR and NMR studies of ion channels and transporters have been performed and can show an enhancement in data output when compared to other lipid platforms such as liposomes[36,45,47,60–62]. Ligand-binding experiments using both radioactive and fluorescent probes have been used successfully for a number of ABC transporters[54] and receptors including GPCRs[54,63].

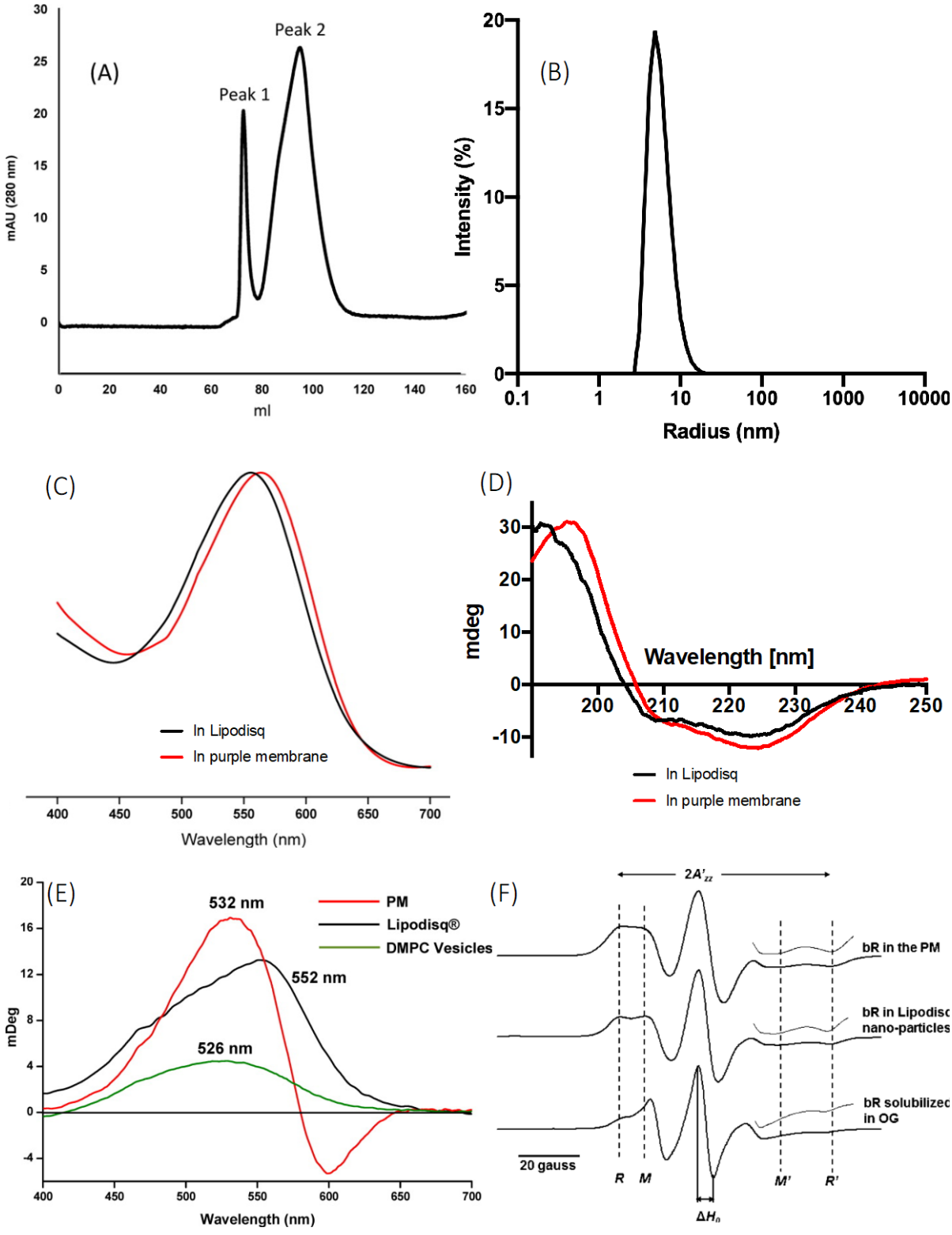



***Figure 3:*** *Bacteriorhodopsin (a trimer in its native purple membrane) was solubilized using SMA with the addition of DMPC (ratio 1:172 protein-to-lipid ratio (mol/mol)) to form Lipodisq nanoparticles containing monomeric protein for subsequent biophysical characterisation*[47]*. Size exclusion chromatography (A) was used for the purification of bR-Lipodisq nanoparticles, with Peak 1 being the aggregation peak (bR in purple membrane proteoliposomes) and Peak 2 being the bR DMPC Lipodisq. (B) DLS data for Peak 2 (in A) with a measured diameter of 12 ± 2 nm. For a full description of the method, please see SI. (C) Absorption spectra for Peak 1 (in A), with* $A_{max}$ *at 565 nm (red) and for Peak 2 with* $A_{max}$ *at 555 nm (black), showing the hypsochromic shift characteristic of the change in oligomeric state from purple membrane (trimer) to Lipodisq (monomer)*[47]*.*

*(D) UV CD spectra showing that the protein is folded both in the purple membrane and in Lipodisq nanoparticles (J. F. Bada Juarez, unpublished data). For a full description of the method and analysis of the secondary structure composition, please see SI. (E) Visible CD spectra collected for bR in the PM (red line), in Lipodisq nanoparticles (black line), and reconstituted into DMPC vesicles (green line), with absorbance maxima indicated in each spectrum, demonstrating a change of oligomeric state from the purple membrane (trimer) to Lipodisq (monomer)[47]. (F) cw EPR spectra of bR double mutant (D38R1/F156R1) in the purple membrane (top), in Lipodisq nanoparticles (middle), and solubilized into detergent micelles (OG, bottom). The motionally informative spectral components (RR') and rigid (MM') are expanded in the high field region. The solid lines mark the line width of the central peak, $\Delta H_0$.*

Structural studies of membrane proteins purified using SMA polymers, have also been performed using X-ray crystallography and cryo-EM. Bacteriorhodopsin has been crystallised using lipidic cubic phase (LCP) from Lipodisq nanoparticles in a detergent-free environment and its structure has been solved at 2.2 Å resolution[64]. The structures of two other proteins, AcrB[65,66] and Complex III from bacteria[67] have been solved by cryo-EM to 8 Å, 3.2 Å and 3.2 Å resolution respectively. In the Complex III structure, several lipids and post-translational modifications have been resolved and the authors note that SMA nanoparticles are ideally suited for future cryo-EM studies of protein-protein and protein-lipid interactions[67].

**Mechanism of lipid membrane solubilisation by SMA polymers**

Existing models for the process of formation of SMA nanoparticles envisage the polymer in the form of an extended chain conformation which interacts with a portion of lipid bilayer in a similar manner to that of a "cookie-cutter" in which rings of elongated polymer strands encircle 100-200 lipids, allowing the hydrophobic groups to cut out a discoidal section[68].

A recent computational study has sought to simulate the initial interaction between polymer and model membrane, using coarse-grained molecular dynamics, in which polymers of approximate molecular weight of 7.4 kDa with a 2:1 ratio of styrene to maleic acid are shown to bind first to the lipids via the styrene groups and to be fully adsorbed onto the membrane surface within 400 ns[69]. The simulations were allowed to continue, and the authors observed membrane poration by the polymers. Disk formation was also observed, and elongated polymer molecules were seen to be bound to the rim of the nanoparticle[69].

We would note that, in these simulations[69], the polymer annulus observed (composed of elongated chains one molecule thick) would appear to be somewhat thinner than has previously been measured for SMA-lipid nanoparticles by SANS experiments, in which a radial shell thickness of 9±1 Å (corresponding to the thickness of the polymer layer) is modelled, compared to a core radius of 38±2 Å (corresponding to the radius of the encircled lipid)[45].

We would also note that the SMA model used in these simulations[69] is fully deprotonated (two negatively charged carboxylate groups on each maleic acid moiety) and assumes an ideal 2:1 styrene-styrene-maleic acid alternation. Considering the stochastic nature of free-radical polymerizations, which tend to produce blocky atactic copolymers consisting of -SS- blocks followed by blocks of alternating SMA with varying -SS- block size and a range of Mws (depending on the polydispersity of the polymer used), this regular alternation seems somewhat unrealistic. SMA polymer cannot be

considered as a pure material with a fixed Mw and sequence distribution[29], as predicted by the Alfrey-Price Q-e method[70] or the penultimate unit model[71].

The physical characteristics of SMA in the *collapse pH* range would also suggest that some modification of this model is required to account for both (i) bilayer disruption and (ii) particle formation, and this may more closely resemble interaction with conventional polymeric surfactants.

At the lower end of the *collapse pH* range, when the surface activity is at its maximum, the polymer is not in the form of an extended chain or worm-like random coil of the type envisaged in the "cookie-cutter" model. Measurements of mean-square molecular radius of gyration of 1:1 alt-SMA copolymer indicate that the collapsed polymer dimensions at low pH are approximately half of those reported for the extended chain, and that the extent of this collapse is consistent across a range of molecular weights[8]. In the same pH range (when 50—20% of the $\alpha_1$ carboxylic acid groups of MA are ionised), surface tension is markedly reduced from 65 mN/m to 54 mN/m[30] over a range of molecular weights. It also appears that this polymer exhibits the same concentration-dependent behaviour as detergents, with a critical micelle concentration that indicates the formation of a surface-active conformation in which hydrophobic and hydrophilic groups are spatially separated within the collapsed coil[30]. This behaviour is consistent with the formation of a (dynamic) amphipathic, globular structure in aqueous solution.

A second recent computational study, by Orekhov and colleagues[72], using partially charged SMA models (i.e. the maleic acid groups were not fully ionised), suggests that the polymers have a tendency to aggregate in solution to form clusters and that it is these clusters that interact with membranes. The authors were able to observe the extraction of lipid patches by 3:1 SMA, whereas 2:1 SMA was observed to induce pore formation. The authors also note that the behaviour is dependent on the sequence of the polymer and that nanoparticle formation appears to require the presence of three or four styrene moieties in sequence, to drive the interaction with the hydrophobic regions of lipid bilayers[72].

We therefore propose that, in the pH range required for membrane lipid and protein extraction, SMA polymers form dynamic collapsed structures (as simulated by Orekov and colleagues[72]), which then interact individually with the bilayer. We suggest that, as multiple polymer molecules bind to the membrane surface, nanoparticle formation proceeds in a manner analogous to pore formation by the alpha-hemolysin toxin secreted by *Staphylococcus* species (in which soluble monomers bind to the surface of the erythrocyte membrane, forming a heptameric ring, before a hydrophobic beta-barrel is lowered cooperatively into the core of the membrane, each monomer providing two beta strands to form a 14-stranded barrel[73]). The interaction of several SMA molecules with the membrane then leads to blebbing, distortion of the bilayer and bud formation[1]. Partial uncoiling of the polymer may also occur as the styrene groups are inserted into the acyl chain region of the bilayer, thus eliminating the hydrophobic forces acting to collapse the SMA and favouring electrostatic expansion of the polymer backbone (driven in part by interactions between the maleic acid moieties and the phospholipid headgroups).

Turbidity measurements by Scheidelaar and co-workers suggest that membrane solubilisation by SMA begins by deposition of the negatively charged polymer on the surface of the bilayer, a process that may be hindered by the lipid packing, the bilayer thickness, the incorporation of anionic lipids (such as phosphatidyl glycerol) or salt concentration[46]. The insertion of the polymer into the hydrophobic

core of the lipid bilayer depends strongly on the lipid packing. Indeed, membrane fluidity, lateral pressure and bilayer thickness have an impact on the insertion of the polymer as tight packing or thick membranes have detrimental effects on SMA insertion[55,74]. Scheidelaar and colleagues have further shown that lipids in the fluid phase are solubilized more rapidly than those in the gel phase[46], however from a thermodynamic standpoint, gel-phase lipids are solubilised more efficiently[75].

**Salt- and pH-tolerant polymers for membrane protein extraction**

A limitation of SMA is its incompatibility with high concentrations of divalent cations including $Ca^{2+}$ and $Mg^{2+}$, which may be chelated by the carboxylate groups thereby causing the polymer to precipitate[46,55,56]. Similarly, a decrease in pH below the apparent $pK_a$ values of the carboxylic acid groups may also result in nanoparticle aggregation and polymer precipitation in the case of 2:1 and 3:1 copolymers of SMA, a limitation that can be largely overcome by using the 1:1 copolymer to extend the working pH range to lower values. Although the threshold concentration of ions is dependent on polymer length and charge density, precipitation is generally observed at $M^{2+}$ ion concentrations above 1 mM[76]. The use of SMA is therefore restricted for solubilisation of membrane proteins requiring high concentrations of divalent ions such as proteins that bind and/or hydrolyse ATP (ABC transporters are typically purified in buffers containing a minimum concentration of 50 mM $Mg^{2+}$) or some ion channels, for which divalent cations can promote oligomerization and function[77]. Scheidelaar and colleagues have also noted that, even in the absence of these divalent cations, the solubility of SMA is dependent on the buffer ionic strength and the amount of SMA added to the membrane[46,55].

A new generation of more salt-resistant polymers has recently been developed. SMA-EA (Figure 4) is an SMA derivative in which the carboxylic acid groups have been covalently conjugated to ethanolamine groups via amide bonds[78]. Lipid nanoparticles formed by SMA-EA are typically larger than those formed under equivalent conditions with SMA 2:1 or 3:1 and have a diameter of 10 nm to 50 nm. Recently SMA-EA nanoparticles have been characterised by both NMR and EPR and, after the addition of paramagnetic ions such as $Yb^{3+}$, they may be aligned in magnetic fields to facilitate the study of helix tilt angles[76].

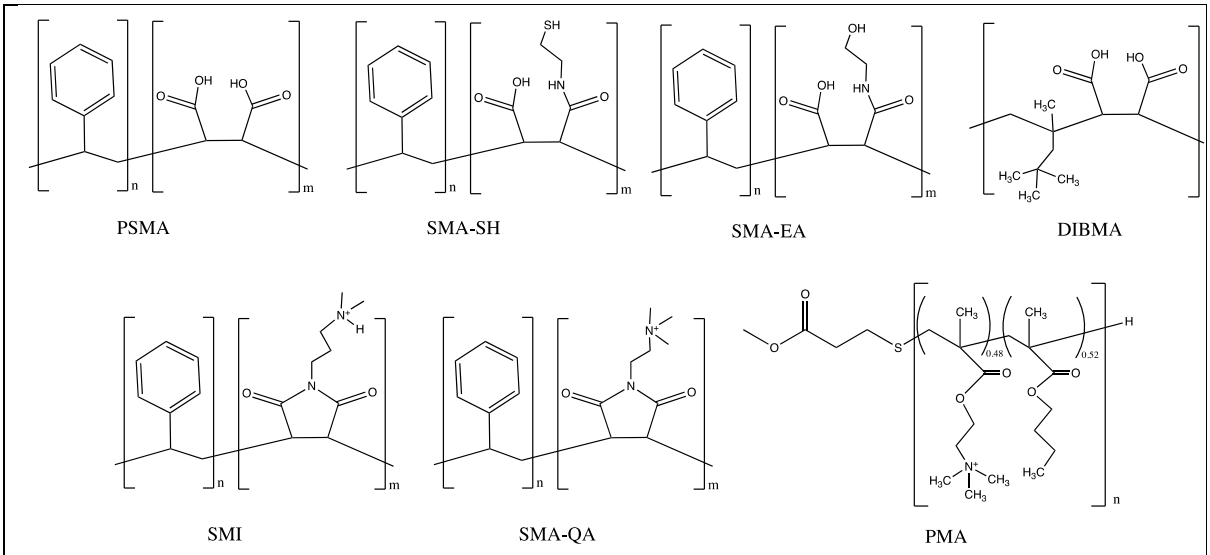



*Figure 4: Chemical structures of the different types of polymer found in the literature which form discoidal nanoparticles with lipid of different sizes (10-50 nm). With the exception of SMA, all polymers are compatible with high concentrations of divalent cation. Extraction of membrane proteins is possible with SMA, DIBMA and SMI, the other polymers have not yet been tested with biological membranes. DIBMA and PMA do not possess styrene, which strongly absorbs in the UV region, and are therefore more suitable for some types of spectroscopy. Note that the products produced by free-radical polymerisation are heterogeneous (in both length and sequence) and contain molecules with a wide range of molecular weights.*

SMA-QA-stabilized nanodiscs[79] have been shown to remain soluble over a broad pH range (pH 2.5 to pH 10), offering a larger stability range compared to SMA Lipodisq (pH 4 to pH 10 depending on grade used) but with larger disc size (approximately 30 nm) dependant on the amount of polymer added. As in the case of SMA-EA, SMA-QA nanoparticles are tolerant to high concentrations of divalent cation and can be used for NMR experiments as shown by Ravula and colleagues[79]. Unfortunately, no experimental data are available concerning its ability to solubilize membrane proteins directly from native membranes.

Polymers made of poly(methacrylate), PMA (Figure 4), have been designed to mimic the membrane scaffold protein (MSP), responsible for Nanodisc formation[80]. PMA nanoparticles are larger than SMA nanoparticles (approximately 15 nm diameter) and have been used to stabilize intermediates of amyloid proteins. Currently, PMA efficiency to solubilize biological membranes has not been demonstrated.

SMI, a polymer of styrene and dimethylaminopropylamine maleimide in a 2:1 ratio, available commercially, is able to form nanoparticles slightly smaller than Lipodisq[81]. SMILPs (SMI nanoparticles) have the advantage of working in an acidic pH range and at a high concentration of divalent cations, where 3:1 and 2:1 SMA nanoparticles usually aggregate. SMI, like SMA, is able to extract membrane proteins from biological membranes as demonstrated by Hall and colleagues[81].

A hydrolysed copolymer of diisobutylene and maleic anhydride (DIBMA, Figure 4) has also been shown to form nanodiscs (12 nm to 30 nm diameter) in the presence of lipids. Similar to other SMA-derivatives (like SMA-EA), DIBMA shows improved salt tolerance to high concentrations of divalent cations, but it lacks the styrene groups which give rise to the absorbance of SMA in the UV spectrum. DIBMA may be used more easily for circular dichroism and other forms of absorption spectroscopy in the UV range[82–84].

**Lipodisq nanoparticles for drug delivery**

Lipodisq nanoparticles have potential for drug delivery purposes, as a consequence of their stability upon dilution (unlike conventional micelles or bicelles) and their nano-molecular dimensions, make them ideal candidates for interaction with sub-cellular organelles. Recently, Tanaka and colleagues have performed a biodistribution study of SMA nanoparticles using radiolabelled probes showing that lipid-only Lipodisq nanoparticles behave similarly to apolipoprotein-based lipid nanodiscs[85], suggesting that Lipodisq could be used as an effective nanocarrier for hydrophobic drugs. Additionally, SMA is the only polymer of those so far investigated for producing polymer-phospholipid nanodiscs that has been used as an injectable in clinical studies. A butyl half ester known as SMANCS[86], which degrades *in vivo* into SMA, has been marketed as a commercial pharmaceutical product.

**Labelling of a thiol derivative of SMA (SMA-SH)**

Biodistribution studies of nanoparticles often require the addition of dye markers and the modular structure of a Lipodisq nanoparticle enables fluorophores and other labels to be attached by covalent conjugation (either to lipids or to the polymer itself) without altering their size. For polymer labelling, SMA-SH (an SMA analogue carrying thiol groups) allows conjugation via maleimide groups for fluorescence microscopy and biophysical experiments such as FRET[87]. Briefly, synthesis of SMA-SH begins with the conjugation of cysteamine to the non-hydrolysed copolymer of styrene and maleic anhydride (SMAnh). The resulting thiol derivative is then hydrolysed at pH 8.5 (Figure 5), as for non-derivatised SMA. The fluorophore is typically conjugated to SMA-SH once Lipodisq particles have been formed and the excess dye is removed by size-exclusion chromatography.

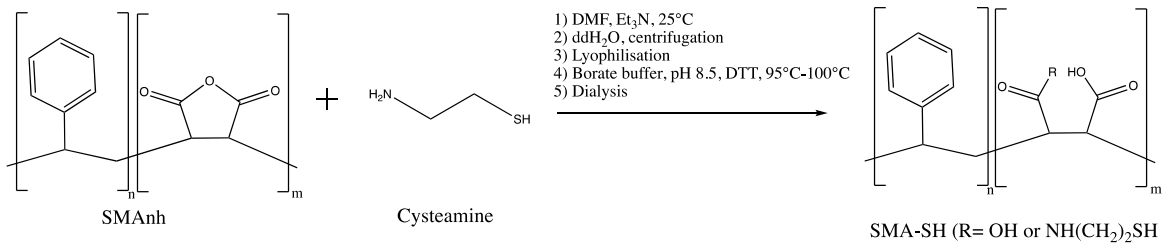



***Figure 5:*** *Chemical reaction scheme from SMAnh to SMA-SH. The cysteamine is conjugated to SMAnh in organic solvents in the presence of triethylamine (step 1). Removal of organic solvents and excess of cysteamine are performed by several centrifugation steps (step 2) and the polymer is lyophilized (step 3) prior to its hydrolysis (step 4). Excess of DTT and buffer exchange is performed by dialysis (step 5). Adapted from*[87].

**Conclusion**

SMA-based Lipodisq nanoparticles (also known as SMALPs) are highly versatile self-assembling membrane mimetics, suitable for a wide range of structural and functional biomembrane studies. The lipid environment may be changed and designed as required, and further development will undoubtedly result in improved formulations based on the initial concepts described here. The monodisperse nature of Lipodisq nanoparticles allows them to be used in spectroscopy (NMR, ESR, UV-Vis, CD), diffraction and scattering (x-rays and neutrons), chemical identification and applications in which sample homogeneity is of great importance.

The ability of SMA (and related polymers) to extract and encapsulate a small number (or only one) membrane protein within a lipid environment, without the use of detergent is an important step forward (if not a landmark) in membrane structural biology, not least because protein functionality can be retained and examined. Added dimensions for this platform are for topical and intravenous drug delivery. One formulation will not be the universal panacea, but through thoughtful chemical design, suitable polymer characteristics can be produced. Much is still to be resolved with respect to the types of lipids, and types of extramembranous chemical interactions permissible, (including the accessibility of the pH range), but this new technology marks a major advance.

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