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Detergent-free extraction polymers in membrane protein structure determination

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Membrane proteins (MPs) underpin essential cellular processes, representing ~60% of drug targets, but their structural characterisation remains challenging with bottlenecks across production, solubilisation, stabilisation and biophysical analysis. Extraction with detergents can disrupt important protein-lipid interactions, impacting structural and mechanistic characterisation. Alternatively, amphiphilic polymers enable direct extraction of MPs with surrounding endogenous lipids forming polymer-lipid particles (PLPs), preserving aspects of the native membrane context compatible with downstream biophysical techniques. Single-particle cryo-electron microscopy (cryo-EM) has become a principal method for MP structure determination. Here, we review progress in the development and application of extraction polymers for MP structural biology, highlighting studies that demonstrate how polymer chemistry can influence protein behaviour, lipid retention and conformational sampling sometimes revealing biologically relevant states unseen when using detergents and/or systems such as nanodiscs. Finally, we discuss current limitations, outlining future directions towards rational polymer design to advance MP structural and mechanistic understanding while supporting improved drug discovery.

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Introduction

Membranes define cellular boundaries and comprise a dynamic mixture of lipids and membrane proteins (MPs). MPs mediate essential processes such as transport and signalling and account for over 60% of drug targets [1]. Beyond acting as a permeability barrier, membrane lipids actively influence MP structure, function, and regulation through specific lipid–protein interactions. To study MPs using biophysical techniques, they typically must be extracted from their native lipid environment and into aqueous solution [2]. This is primarily done using detergents, which are used to strip away the surrounding lipids. While convenient, detergents often poorly mimic lipid bilayers and can disrupt key protein-lipid interactions, sometimes raising concerns about structural relevance [3,4]. To address these limitations, lipid reconstitution approaches such as membrane scaffold protein (MSP) based nanodiscs were developed, enabling MPs to be studied in lipid bilayers [5]. However, these systems still involve initial extraction in detergent and simplified lipid compositions that fail to capture the native membrane complexity. More recently, amphiphilic polymers capable of directly extracting MPs with their surrounding native lipids have emerged, offering a more physiologically relevant alternative [6–14].

Structural biology seeks high-resolution protein structures to elucidate biological mechanisms. X-ray crystallography has historically dominated MP structure determination and remains important for ligand studies, but is challenged by low MP stability, protein yields, and issues in forming crystal contacts due to the size of detergent micelles or other solubilisation approaches [15,16]. Advances in single-particle cryo-electron microscopy (cryo-EM) have transformed MP structural biology for proteins typically >70 kDa, offering lower sample requirements and eliminating the need for crystallisation [17]. Cryo-EM is also compatible with native-like environments via polymer extraction, though such applications remain limited [18–22]. This combination of cryo-EM with native extraction of proteins in lipids is potentially powerful as ideally MPs should be characterised in a native-like lipid environment.

In this review, we will summarise recent advances in the application of extraction polymers in the structural studies of MPs. Due to the current dominance of cryo-EM in MP structural biology, and its applications in the study of the structure of proteins in polymer-lipid particles (PLPs), cryo-EM case studies are the primary focus. We also discuss some limitations of existing extraction polymers and highlight areas where further optimisation may enhance their utility for cryo-EM.

Detergents to polymers

Detergents have been the principal tools for MP extraction and underpin most MP structures determined to date [16]. They solubilise lipid bilayers, forming micelles around proteins and releasing them into solution [23]. While this approach has been vital, extensive delipidation often reduces protein stability, alters conformational equilibria, and disrupts important protein-lipid interactions, complicating structural and mechanistic understanding.

Lipid reconstitution strategies such as amphipols, bicelles and MSP-based nanodiscs partially address these issues by restoring proteins to bilayer environments, often improving structural tractability. In recent years, ~20% of cryo-EM structures were determined in nanodiscs (Figure 1) [2,24,25]. However, these approaches typically require prior detergent solubilisation and yield assemblies with simplified lipid compositions, so the native lipid context and critical interactions are only partially recovered. While more physiologically relevant than detergents, nanodiscs also fail to recapitulate the physical properties of native membranes, such as lateral pressure and lipid packing [25–29]. There have also been instances where direct interactions between the protein and the nanodisc scaffold have altered the structure of membrane proteins [31].

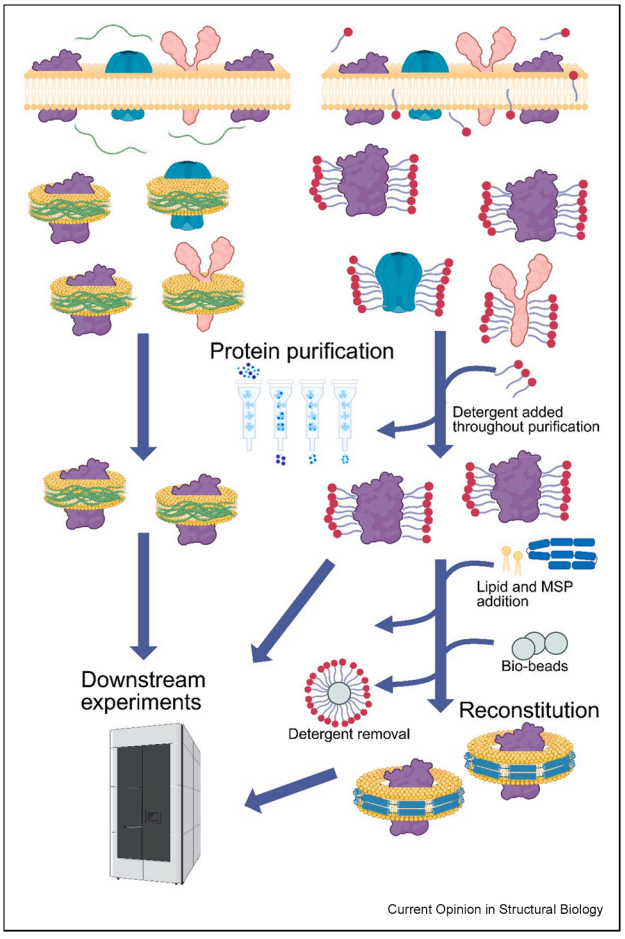
Amphiphilic polymers that directly extract MPs from biological membranes offer a powerful alternative [32,33,38–40]. Instead of replacing membranes with artificial lipids, these polymers excise nanoscale bilayer discs containing the target protein and associated native lipids (Figure 1). Styrene maleic-acid (SMA) copolymers and their derivatives have demonstrated that detergent-free extraction is both feasible and broadly applicable to a variety of protein classes. These systems have greater potential to preserve important protein-lipid interactions during solubilisation, enabling studies in a more native-like lipid environment [20,21,34–36].

Polymer Classes and Chemical Properties

Styrene maleic-acids and derivatives

The ability of amphiphilic polymers to directly extract MPs from biological membranes arises from a balance of hydrophobic and hydrophilic chemical features. The most widely used are SMA-based copolymers, in which hydrophobic styrene units intercalate into lipid bilayers while anionic maleic acid groups confer aqueous solubility [28–30]. SMA and its derivatives have limitations, which have motivated alternative designs, such as improved tolerance to divalent cation and pH sensitivity [7,10]. SMA is commonly modified via nucleophilic ring

Figure 1



Overview of different workflows for solubilising membrane proteins. Polymers and related amphipols can solubilise MPs directly from the membrane. Following purification of the polymer-extracted protein of interest, these native nanodiscs can be used in a variety of downstream processes, such as cryo-EM. MPs are traditionally extracted in detergents, and these solubilised proteins go through a similar protein purification workflow. At this point, the detergent-solubilised proteins can be used in downstream techniques or reconstituted in lipid-mimetic systems such as MSP nanodiscs and liposomes. During the reconstitution process, different lipid mixes can be introduced, though these are often simplified relative to the native membrane environment of MPs. The process for MP reconstitution into nanodiscs, amphipols, and bicelles can be found here [37]. Figure generated in BioRender with some images imported from BioIcons, which were provided by DBCLS or Sasha Sundstrom under a CC-BY 4.0 or a CC0 licence. MPs, membrane proteins; cryo-EM, cryo-electron microscopy.

opening of the maleic anhydride (SMAnh) repeat units, generating cationic, anionic, and zwitterionic polymers. Kuyler et al. have reviewed reported SMA derivatives and modification strategies [33]. Among all polymer classes, SMA2000 is the most widely used in cryo-EM and biochemical analysis, although this observation is based on a limited number of cryo-EM structures, and several MPs extracted in SMA have also been studied by solid-state nuclear magnetic resonance (NMR) [41,42]. SMA was also used to extract an MP into polymer nanodiscs before transferring the protein into the lipidic cubic phase for crystallisation [43].

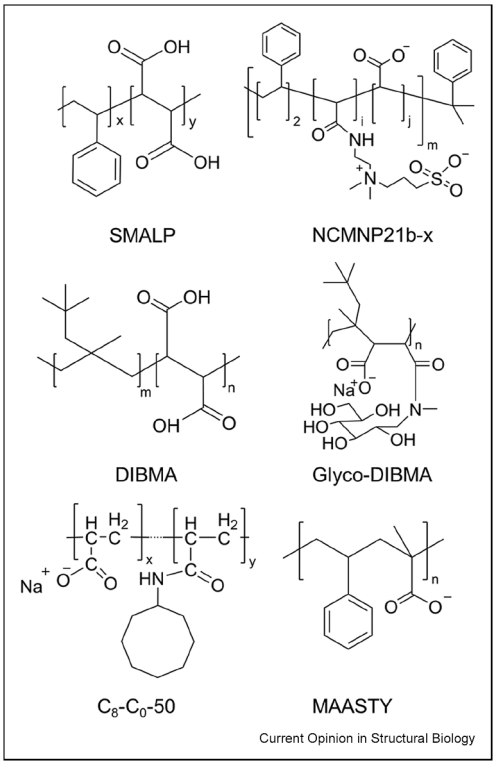
Extraction polymers have been produced by opening SMAnh rings with nucleophiles, followed by hydrolysis of the remaining maleic anhydrides to enhance functionality [33]. Substituting maleic acid carboxyl groups with hydrophilic moieties such as Tris (NCMNP2a-x series) improves pH stability (from >5 to 2-3) and divalent cation tolerance [21]. NCMNP21b-x polymers incorporate zwitterionic propane-1-sulfonate units and amine-modified side chains, reducing aggregation via electrostatic stabilisation (Figure 2) [20]. Both NCMNs mentioned here have been used in cryo-EM studies of AcrB, producing monodisperse nanoparticles that retain endogenous lipids and enable high-resolution structural characterisation [20,21].

DIBMAs

Poly(diisobutylene-alt-maleic acid) (DIBMA), after SMA, is the most utilised amphiphilic polymer for MP extraction and biophysical studies [44,45]. DIBMA has short-chain branched aliphatic diisobutylene units alternating with maleic acid, giving it properties distinct from SMA. DIBMALPs form larger discs than SMA and show greater stability in divalent cations, tolerating up to 20 mM Ca^{2+} or Mg^{2+} compared with 2 mM Ca^{2+} or 4 mM Mg^{2+} for SMA [45,46]. Although performance depends on protein class, DIBMA generally solubilises membranes less efficiently than SMA, but can be advantageous for certain biophysical methods, including reduced spectroscopic interference due to its aliphatic diisobutylene units [44,45,47]. DIBMA has been used in cryo-EM characterisation of the YnaI mechanosensitive ion channel [48].

Many derivatives have been generated from the DIBMA backbone. Glyco-DIBMA has partial amidation with the amino sugar N-methyl-D-glucamine, which increases hydrophobicity [49]. This derivative also forms smaller, more uniform nanodiscs. It enabled structural characterisation of the full MscS channel; the transmembrane domain could not be visualised using SMA2000 or a cycloalkane-modified amphipol [34]. Carboxy-DIBMA, another derivative, was used to show that the curvature inferred from the organisation of the FtsH-HflK/C complex in detergent is reproduced by the lipid bilayer, demonstrating that this curvature is a genuine property of the assembly and not a detergent-induced artefact [50].

Figure 2



A range of polymers that have been used in structural biology studies of membrane proteins. SMA, styrene-maleic acid; NCMNP21b-x, native cell membrane nanoparticle 21b-x; where x, 5, 30, or 30%; DIBMA, poly(diisobutylene-alt-maleic acid); Glyco-DIBMA, poly(diisobutylene-alt-maleic acid) where maleic acid modified with N-methyl-D-glucamine; C8-C0-50, cycloalkane-modified amphiphile polymer with cyclooctylamine; MAASTY, poly(methacrylic acid-co-styrene).

AASTYs

Poly(acrylic acid-co-styrene) (AASTY) has been shown to successfully solubilise MPs [51]. AASTY is a copolymer where the maleic groups in SMA are replaced by acrylic acid, giving reduced sensitivity to divalent cations [52]. AASTY showed potential in structural biology applications, with AASTY-solubilised human transient melastatin type 4 (hTRPM4) being analysed by cryo-EM and showing ideal particle distribution while yielding a low-resolution reconstruction [51]. Building on this, Pugh et al. generated MAASTY, an anionic methacrylic acid-styrene copolymer and showed that it effectively solubilises diverse lipid species and eukaryotic MPs from mammalian cells [9]. Structural analysis of MAASTY-solubilised hTRPM4 further demonstrated its suitability for high-resolution cryo-EM structure determination [9].

Amphipols

These polymers are typically based on a polyacrylate backbone with hydrophobic alkyl chains and hydrophilic, often anionic, groups [53]. The most widely used amphipol, A8-35, has been extensively applied in biophysical and structural studies, including cryo-EM [24]. Unlike SMA-based polymers, they exhibit high tolerance to divalent cations and minimal interference with spectroscopic techniques. Typically, amphipols do not directly extract MPs from membranes and are used to stabilise proteins after an initial detergent solubilisation step. Recently, amphipol derivatives, and to a lesser extent, A8-35, have been shown to directly extract proteins from the membrane, negating the need for detergent [6,18]. Marconnet et al. synthesised poly(-acrylic acid) (PAA) derivatives with cyclic side groups, known as cycloalkane-modified amphipols (CyclAPols), which can successfully solubilise MPs within a native-like lipid environment, and have been used successfully in cryo-EM [6,18].

Applications in structural biology and key case studies

While MP structures have been determined using polymers (Figure 3), polymer-based approaches remain strikingly underutilised. In 2021–2022, 98.4% of cryo-EM structures at <3 Å resolution were extracted with detergents [24]. During this period, only five MP structures were solved following native polymer extraction, all using SMA. A more recent survey of membrane protein cryo-EM structures resolved to better than 3 Å and published between January 2023 and June 2025 found that only a single structure used a SMA nanodisc system at the vitrification stage [25]. However, the authors note that many polymer-based structures have lower resolutions than 3 Å leading to potential underrepresentation [25]. Additionally, the growing chemical diversity of polymers, along with the different degrees to which researchers annotate their Protein Data Bank (PDB) depositions, means that some examples may be missed in large-scale surveys.

Although alternative copolymers such as DIBMA and CyclAPols have emerged, their use remains limited, and no polymer system has yet shown broad applicability across diverse MP classes [18,48]. Nevertheless, several structures solved in PLPs have yielded biological insights not observed with other solubilising agents. For example, multiple cryo-EM structures of the glycine receptor (GlyR), a ligand-gated ion channel, were determined after SMA extraction [54]. SMA-extracted GlyR particles revealed physiologically relevant open and desensitised conformations, together with a previously unseen agonist-bound closed state associated with partial agonism. These intermediate states were not observed in nanodisc preparations, and the structures provided direct structural evidence for pre-open conformations that had previously only been inferred from electrophysiological studies, demonstrating how polymer-based approaches can preserve functionally relevant conformational landscapes and enable the capture of biologically important states [54].

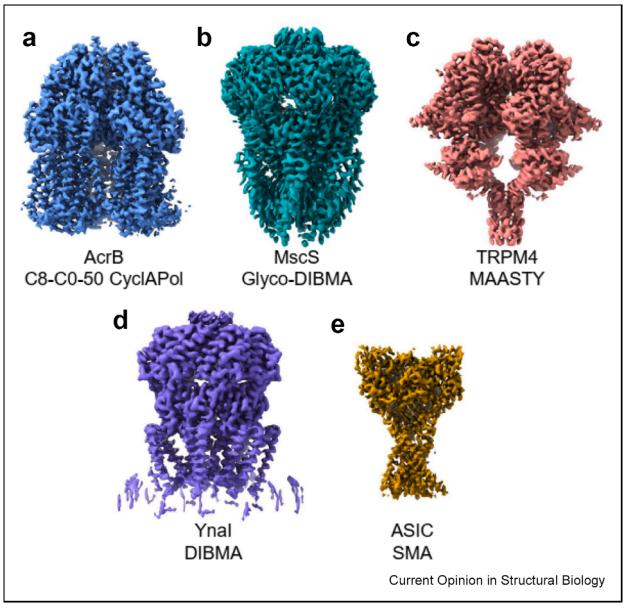
Another example is provided by the bacterial division motor TolQRA, where SMA solubilisation revealed multiple conformational states showing rotary motions in the proton motive force (PMF) driven motor complex [55]. Comparison of SMA-extracted and nanodisc structures enabled reconstruction of a proton-coupled rotational cycle, illustrating how direct extraction into polymer-lipid particles can preserve functionally relevant conformational heterogeneity and support mechanistic understanding [55].

To explore the application of polymers in understanding protein structure and interactions further, we focus on three systems: P2X4, AcrB and mechanosensitive channels.

P2X4

Polymer extraction enabled high-resolution cryo-EM analysis of the human P2X4 receptor, revealing how copolymer nanodiscs affect structural preservation and conformational sampling [56]. P2X4 particles extracted into different polymers retained their native trimeric architecture and triangular appearance, confirming

Figure 3



Examples of cryo-EM density maps of membrane proteins characterised in polymer-lipid-particles. (a) *E. coli* AcrB (EMDB: EMD-12043). **(b)** *E. coli* MscS (EMDB: EMD-71088). **(c)** Human TRPM4 (EMDB: EMD-52611). **(d)** *E. coli* YnaI (EMDB: EMD-11557). **(e)** Chicken ASIC1 (EMDB: EMD-21380). cryo-EM, cryo-electron microscopy.

preservation of receptor assembly. Systematic screening showed that particle orientation and resolution depended strongly on polymer identity [56]. While several copolymers produced high-quality 2D classes, Glyco-DIBMA-solubilised P2X4 exhibited near-complete preferential orientation. In contrast, Cubipol derivatives yielded more balanced angular distributions and reconstructions ranging from ~7 Å to 2.9 Å, highlighting substantial effects of polymer chemistry on structural outcomes [56].

A 3.6 Å P2X4 structure in Glycerol-Cubipol revealed clear ATP density at the orthosteric site, indicating a functional receptor [56]. Compared with previous structures, it displayed an open extracellular domain and a distinct transmembrane arrangement, differing from canonical closed or desensitised states and potentially representing an alternative conformation. Further optimisation with Sulfo-Cubipol produced near-atomic resolution structures of another P2X4 construct, with apo and ligand-bound reconstructions at ~3.5 Å and 2.9 Å. In both cases, transmembrane helices were well resolved and the pore was closed. MgATP density was observed in the agonist-bound structure, whereas the antagonist 5-BDBD could not be confidently assigned, likely due to partial occupancy or poorly resolved regions [56].

Overall, this work shows that polymer nanodiscs support cryo-EM studies of P2X4 while preserving ligand binding and native-like conformations. It also demonstrates that copolymer chemistry strongly influences particle orientation and structural outcomes, highlighting polymer choice as a critical factor in PLP-based MP studies.

Mechanosensitive ion channels

Mechanosensitive ion channels have an intimate relationship with the membrane environment through protein-lipid interactions, and several have been characterised in PLPs [34,48]. DIBMA was used to determine structures of the mechanosensitive channel YnaI in both closed and open states [48]. In the open form, the transmembrane (TM) domain showed poor density and could not be unambiguously assigned, and no lipid density was observed despite the presence of lipids. In the closed state, the full TM domain was visualised, but outer helices TM1 and TM2 were poorly resolved, again with no lipid density. By contrast, all TM helices were resolved in a cryo-EM structure of YnaI in LMNG detergent, with differences in TM1 and TM2 compared with DIBMA (PDB: 6URT). YnaI was also characterised in SMA2000, revealing only the two central TM helices, one of which differed from detergent structures [57]. These differences have been attributed to PLP-induced distortions; however, given that many bacterial and MscS-like channels exhibit multiple subconducting states, YnaI may instead adopt a distinct conformation rather than this necessarily reflecting PLP artefacts [5,58–61].

More recently, polymers were applied to the structural characterisation of MscS. Extraction from *E. coli* membranes using three copolymers produced mixed cryo-EM results [34]. In SMA2000 and C8–C0-50, only the cytoplasmic domain was fully visualised, whereas Glyco-DIBMA yielded the complete structure. The Glyco-DIBMA structure revealed bound endogenous phospholipids, including one at a previously unobserved site relative to detergent and nanodisc structures [34]. Lipidomic analysis of annular lipids further showed a 2–3-fold enrichment of specific 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylglycerol (POPG) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE) species [34].

AcrB

AcrB has been structurally characterised in multiple polymers, as well as extensively in nanodiscs and detergents [18,19,21,22,62–65]. Despite near-atomic resolution data collection, detergent-extracted samples showed no discernible lipid bilayer features [65]. The earliest single-particle cryo-EM analysis using SMA yielded an 8.8 Å reconstruction lacking lipid density [19]. Subsequently, SMA enabled a high-resolution cryo-EM structure capturing a native-like lipid bilayer around AcrB, with density corresponding to endogenous lipids [35]. More recently, CyclAPol (C8–C0-50) resolved AcrB at 3.2 Å, but again without lipid features [18]. A 4.6 Å cryo-EM structure of Salmonella AcrB using SMA also lacked lipid density [22]. In contrast, NCMN polymers NCMNP21b-20, NCMNP2a-50 and NCMNP2a-5 have all produced AcrB structures with visible endogenous lipids across diverse pH and ionic conditions [20,21]. A 2.7 Å cryo-EM structure of a homologous AcrB-family transporter obtained in nanodiscs reconstituted with total *E. coli* lipids revealed partial hexagonal packing of hydrocarbon chains in the inner leaflet, but incomplete density and a poorly defined outer leaflet [66]. However, these features are missing from higher-resolution nanodisc structures of *E. coli* AcrB [62], indicating that further optimisation is required and highlighting the need for a range of tools for structure determination.

A variety of other MP systems have been characterised in PLPs by cryo-EM (Figure 3), including the alternative complex III-cytochrome oxidase supercomplex and the chicken acid-sensing ion channel (ASIC1) [67,68], some of which are reviewed elsewhere [32].

Limitations and challenges

As discussed, PLPs have several limitations, including the fact that many are pH and divalent cation-sensitive, which restricts compatibility with some protein systems and assays. Although chemical modification has alleviated some issues, challenges remain [33].

Concerns also persist over how accurately PLPs reflect native lipid environments. Lipid exchange has been demonstrated in lipid-only styrene maleic acid lipid particles (SMALPs), though protein effects remain unclear [69,70]. Several studies report enrichment of specific lipid species in the presence of proteins, likely reflecting higher-affinity protein-lipid interactions [34,36,71]. A study of cyclodextrin-mediated lipid removal from MscS-containing nanodiscs showed that lipid removal occurred more slowly when MscS was present [59]. Polymer nanodiscs may also alter lipid packing, with increased headgroup packing relative to large unilamellar vesicles (LUVs) observed using fluorescent probes, although LUVs are themselves non-native [72]. Preferential solubilisation of certain lipids has been reported, with mixed results. While some lipidomic studies found no differences between SMALPs and native *E. coli* membranes [36], another study observed subtle glycerolipid enrichment and phospholipid depletion in polymer nanodiscs [73]. A study also suggests that SMA favours fluid-phase lipids in model membranes [74]. Nonetheless, protein-dependent lipid enrichment seen in several studies suggests that PLPs are potential tools for probing specific protein-lipid associations.

PLPs are generally underutilised in cryo-EM structure determination, with limited successful MP examples in comparison to detergents and systems such as protein-based nanodiscs [24]. Although alternative copolymers such as DIBMA and CyclAPols have emerged, their adoption remains limited, and no polymer has shown broad applicability across MP classes. While low extraction efficiency is often cited as a limitation, we have successfully applied multiple polymers to diverse MPs and have experienced high-quality SMA-extracted protein samples that have not translated to high-quality cryo-EM grids. There are several factors that may contribute to the limited uptake, including particle heterogeneity and flexibility, preferred orientation, and a propensity for air-water interface effects, all of which will hinder their use in structural studies. Finally, it has been suggested that polymers may occasionally interfere with TM domains, indicating that, like detergents and nanodiscs, they may not be universally benign [57].

Future outlook

The convergence of native polymer extraction with high-resolution cryo-EM offers a powerful opportunity to study MP structure in environments that more closely resemble their physiological context. Continued development of chemically diverse and tunable polymers will be essential to overcome potential limitations in capturing membrane properties, pH tolerance, cation sensitivity, particle heterogeneity, and preferred orientation, while expanding compatibility across protein classes and applications. From a structural perspective, polymers compatible with cryo-EM that preserve critical protein-lipid interactions and capture functionally relevant conformational states will be important and could reveal protein complexes lost during detergent extraction. Systematic structure-property studies of extraction polymers, integrated with single-particle cryo-EM analysis, will be central to building the experimental understanding needed for theory-driven and ultimately rational polymer design for structural biology. Additionally, other tools that can directly extract MPs, such as peptides, are emerging [75]. Benchmarking polymers and these other systems against detergents and reconstitution systems, combined with lipidomic and biophysical analysis, will clarify how faithfully they reflect native membranes. At present, no membrane-mimetic system is universally effective, requiring screening and optimisation to preserve native-like structure and function. As polymer-based approaches mature, they have the potential to move into mainstream structural workflows, deepening mechanistic understanding of MP function and disease, and ultimately enabling improved structure-based drug discovery.

These polymer and other mimetic systems will likely be complementary to other techniques that seek to preserve the native membrane context, such as cryo-electron tomography (cryoET). When combined with subtomogram averaging, cryoET allows the structural characterisation of MPs in their native membrane environment. Its increasing use provides an opportunity to compare existing structures determined in polymers and other membrane-mimetic systems with those in native membranes. While rapid developments in the field are increasing the achievable resolution of these *in situ* reconstructions, single particle cryo-EM will still be required to achieve the near-atomic models needed to resolve fine mechanistic details or study ligand binding. Ultimately, a range of complementary approaches will be required to understand the structure and mechanisms of MPs within their native membrane context.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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** of outstanding interest

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