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Salipro technology in membrane protein research

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Reconstitution and direct extraction of membrane proteins using saposins is an emerging technique for solubilizing and stabilizing membrane proteins. The Salipro technology offers several advantages over traditional detergent solubilization, including a more native lipid environment, increased protein stability, and maintenance of functionality. This review covers recent studies that have used Salipros to characterize membrane proteins, as well as advances in direct extraction methods that have enabled the structural and functional characterization of a variety of targets.

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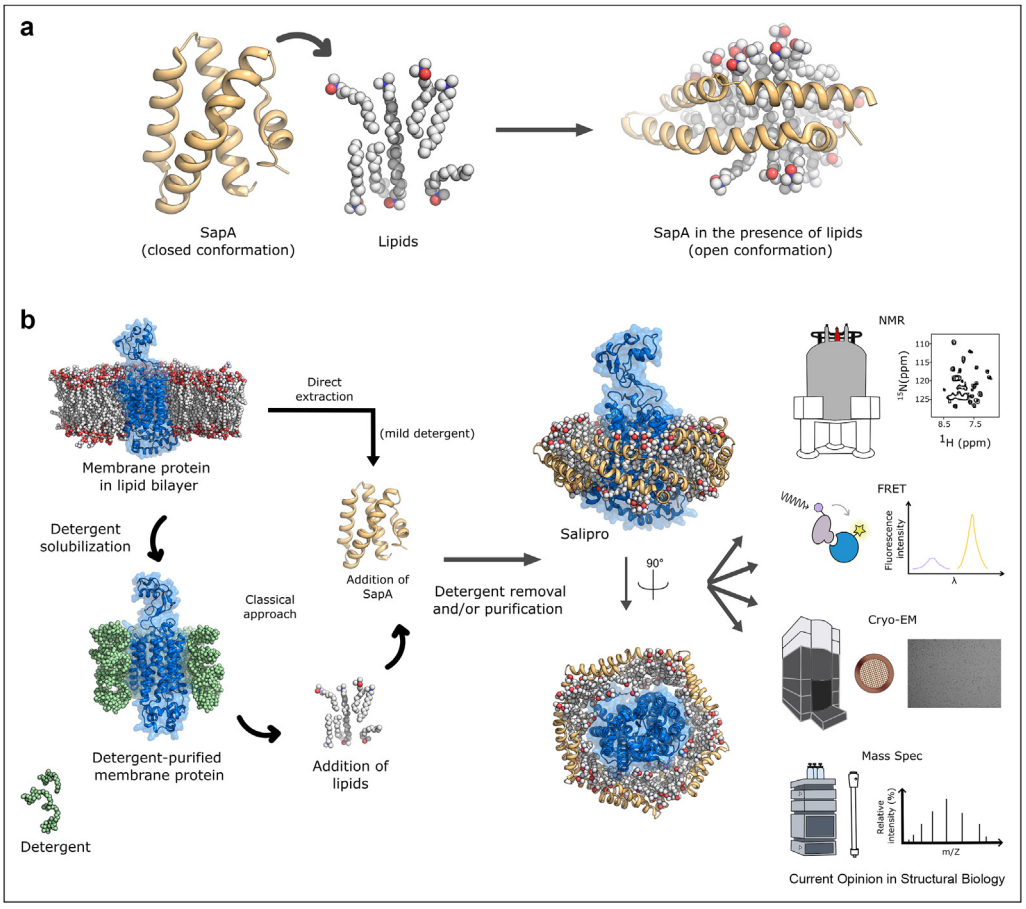
Introduction

Integral membrane proteins (IMPs) are essential to many biological processes but are notoriously difficult to study due to their hydrophobic nature and the challenges in isolating them in a functional, water-soluble state. To overcome these challenges, various membrane protein reconstitution systems have been developed [1], with the Salipro technology emerging as a highly promising and versatile approach. The Salipro membrane mimetic system (also referred to as Saposin nanoparticles and Saposin nanodiscs) has been established by Frauenfeld and colleagues [2] and uses the mammalian lipid-binding protein saposin A (SapA) to stabilize and reconstitute IMPs into a lipid bilayer-like environment.

Saposins belong to a family of small glycoproteins that play a critical role in lipid metabolism, particularly in the breakdown of sphingolipids, which are key components of cell membranes. They are synthesized as part of a larger precursor protein called prosaposin (Uniprot: P07602), that is later processed into the individual saposins (SapA, SapB, SapC, and SapD), each of which is associated with specific enzymes that facilitate the degradation of complex lipids in the lysosome. Earlier work has already demonstrated that saposins can exist in different conformations [3]. In the absence of lipids, SapA, a nine kilodalton fragment of prosaposin spanning residues 60-140, adopts a closed monomeric conformation, while the structure opens in the presence of detergents or lipids and exposes a hydrophobic surface (Figure 1a). The X-ray structure of such a complex revealed two chains of SapA encapsulating 40 bound detergent molecules organized in an ordered bilayer-like hydrophobic core [4]. This lipoprotein structure is mainly stabilized by lipid self-assembly. Later, such an assembly was further confirmed with branched detergents such as lauryl maltose neopentyl glycol (LMNG) [5].

The Salipro system is based on these binding properties of SapA. SapA serves as a flexible scaffold that surrounds an IMP embedded in a small lipid bilayer patch (Figure 1b). This flexibility eliminates the need to screen multiple scaffolds. The membrane mimetic structure allows IMPs to maintain their native conformation, a critical feature for structural biology, drug discovery, and functional assays. The classical workflow for reconstituting IMPs into Salipros is similar to that of other systems, such as nanodiscs, where a detergent-purified membrane protein is combined with SapA and phospholipids. After detergent removal, the resulting Salipro can be purified and analysed to confirm successful reconstitution (Figure 1). But recent advances in this technology now allow the reconstitution of IMPs into Salipros while keeping the endogenous lipids bound [6]. Here we review the latest developments of this technique, highlight applications and provide examples where this technology led to new insights into the structure and function of IMPs.

Figure 1



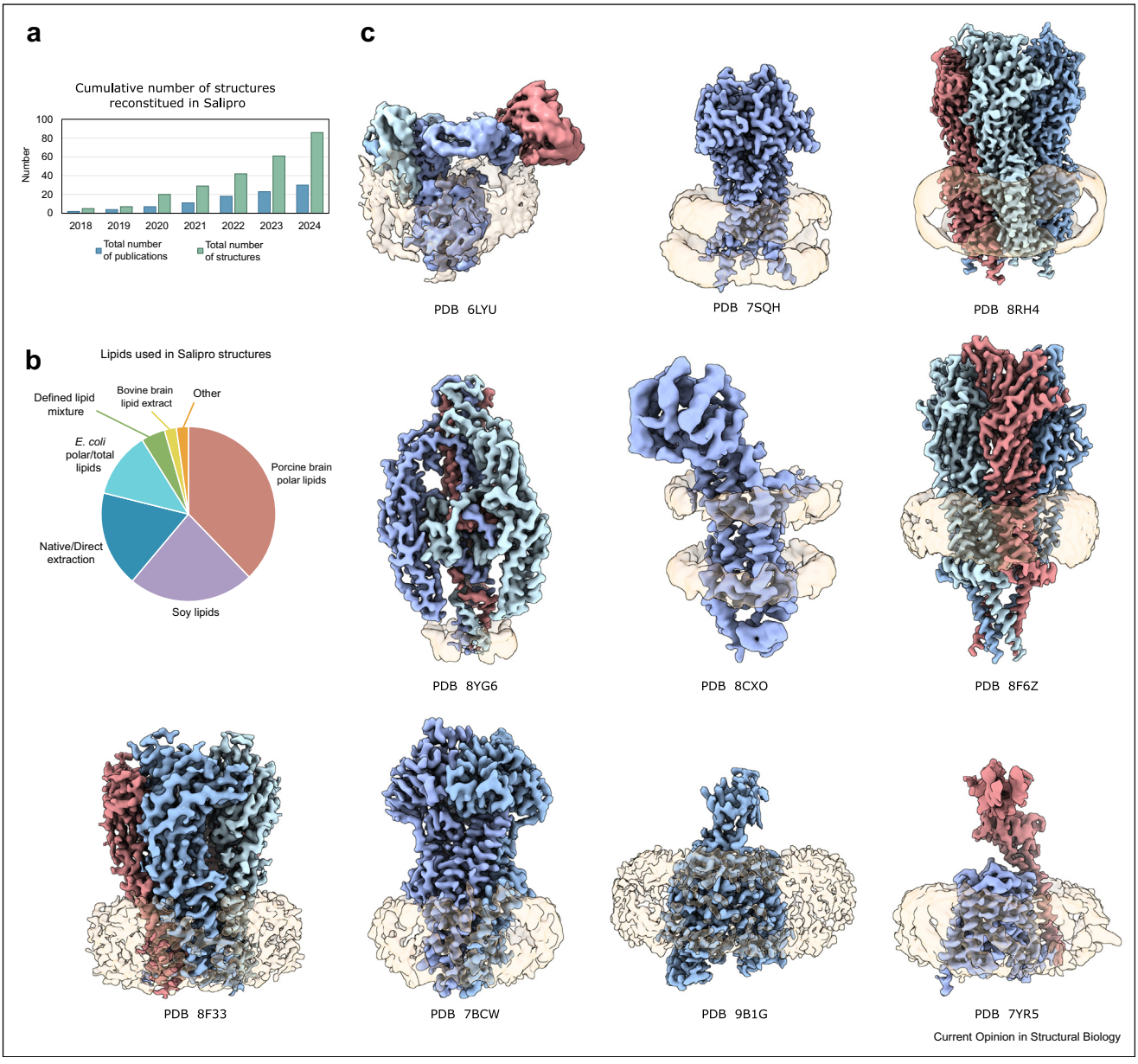
(a) Schematic diagram of SapA binding to lipids. Upon the addition of lipids, SapA undergoes a conformational change to reveal the hydrophobic core, which then binds to lipid molecules. **(b)** highlights the general process of Salipro reconstitution. IMPs are either first solubilized and purified in detergent, then mixed with SapA and lipids. After detergent removal, SapA and lipids combine to form a scaffold around the IMP. Alternatively, membranes are partially solubilized using Digitonin or mild detergents, followed by the addition of SapA. In this scenario SapA forms a small belt around the IMPs bound to its endogenous lipids.

Applications of Salipros for structure determination by cryo electron microscopy

Among the advantages offered by Salipros are preservation of bound lipids [7–11], improved protein stability [12,13], stabilization of oligomeric [14,15] or conformational states [10,16] and improved structure resolution by cryo electron microscopy (cryo-EM) [17]. All properties that make this reconstitution technology attractive for structure determination. Within the last few years, there have been almost a hundred Salipro-reconstituted structures deposited in the Protein Data Bank (Figure 2, Supplementary Table S1). Of these, about 25 % are unique structures, while the rest represent structures with different ligands or conformational states. A small gallery of selected structures is presented in Figure 2, and a subset where the SapA density is resolved are shown in Figure 3, highlighting that this technology can be applied to IMPs of various sizes, oligomeric states and origins. The saposin density in these structures shows that SapA molecules arrange in a tilted array around the IMP and lipid belt (Figure 3).

Several of these structures highlight well-resolved densities corresponding to lipid molecules tightly interacting with the IMP. These bound lipids can alter the activity or conformation of the protein, sometimes revealing previously unknown states or even mediating ligand binding. For example, the reconstitution of the ACA8 Calcium Pump from *Arabidopsis thaliana* enabled the structural characterization of lipid-mediated regulation [18]. A series of structures of the human ligand-gated ion channel $\alpha1\beta2\gamma2$ $GABA_A$ receptor reconstituted in Salipros and bound to various ligands show well-resolved lipid densities [7,8,19,20]. In some cases, the lipids appear to be involved in modulating neurosteroid binding [8], while in other cases, such as when the receptor is bound to methaqualone and its analog 2-

Figure 2

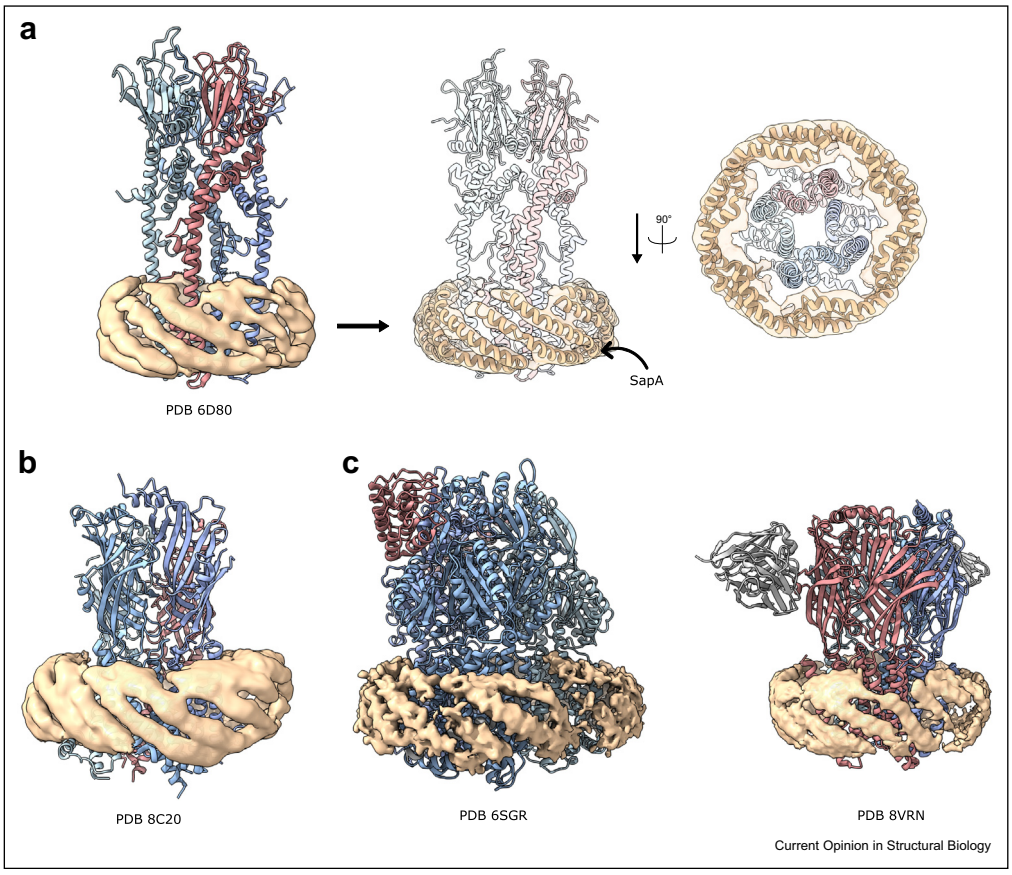


(a) The cumulative number of publications utilizing Salipros for structural studies, as well as the cumulative number of structures published every year which shows an accelerating growth in the use of saposin reconstitution. **(b)** The choice of lipids for SapA reconstituted structures within the last years. **(c)** A gallery of selected, recently published structures of IMPs in Salipros. The density corresponding to the scaffold protein SapA and lipids has been filtered to aid in visualization.

phenyl-3-(p-tolyl)quinazolin-4(3H)-one (PPTQ), the lipids stabilize the bound drug molecule [7].

In the case of the human ρ1 $GABA_A$ receptor, endogenous neurosteroids such as β-estradiol and pregnenolone sulfate can modulate receptor response to GABA binding [21]. A structure of this receptor reconstituted in Salipros and bound to β-estradiol revealed a previously unknown binding pocket for the neurosteroid at the interface between the extracellular domain (ECD) and the transmembrane domain (TMD) [21]. Interestingly, β-estradiol binding did not induce any significant conformational changes, however the presence of β-estradiol blocked allosteric conformational changes that link GABA binding at the ECD and pore opening within the TMD [21]. This inhibitory mechanism appears to be specific to ρ subtypes of the $GABA_A$ receptor. In contrast, the mechanism of pregnenolone sulfate-induced inhibition was shown to occur via binding within the pore and inhibition of ion permeation, which

Figure 3



(a, b) Examples of structures where the SapA scaffold protein is well-resolved, highlighting the alpha-helical organization of the scaffold protein, as well as demonstrating the low degree of flexibility within the scaffold. (c**)** By low-pass filtering the density corresponding to the disc, we were able to find additional examples of structures where the SapA scaffold is well-resolved.

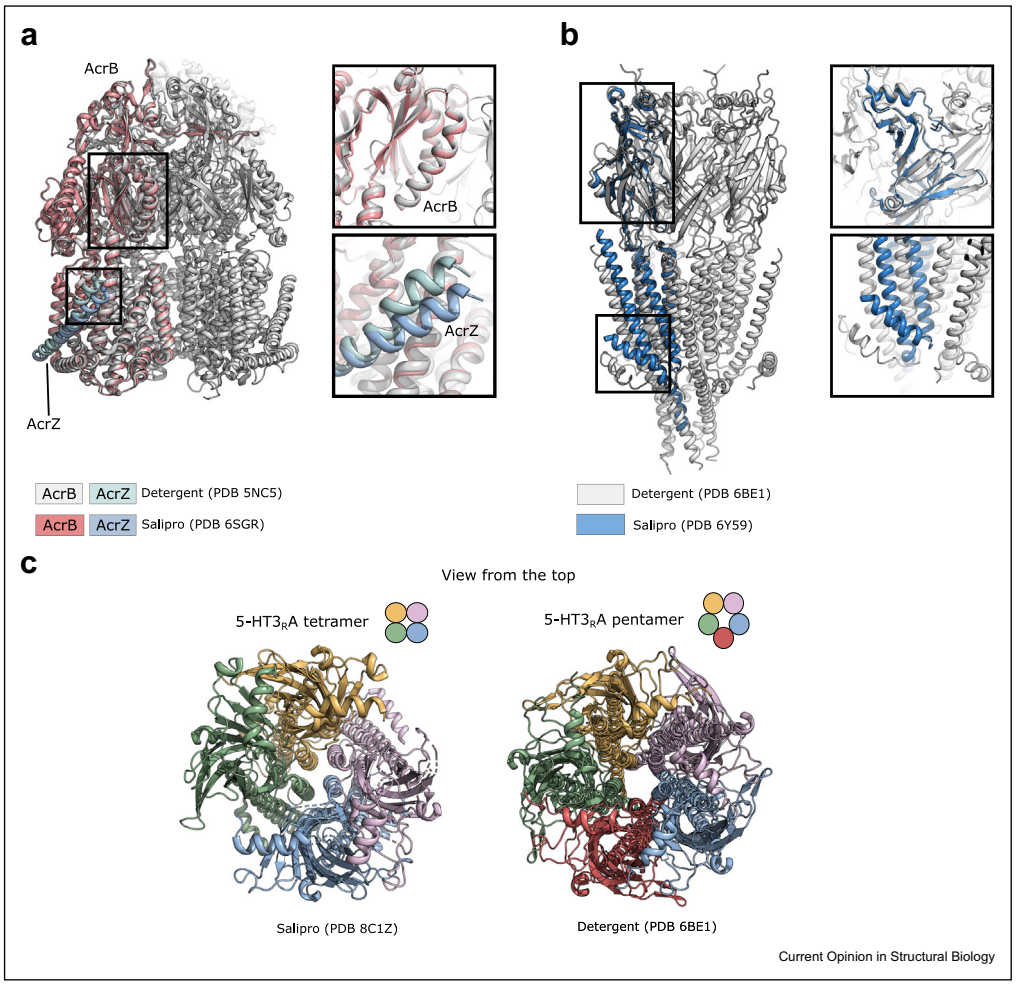
is similar to the inhibitory mechanism of other steroids in the $\alpha1\beta2\gamma2$ $GABA_A$ receptor [21].

Lipids are known to affect the equilibrium and transitions between the various conformational states of nicotinic acetylcholine receptors (nAChR) [22], and a structure of the nAChR from the electric ray *Torpedo torpedo* in Salipros revealed a new lipid binding site within a helical bundle previously hypothesized to be important for lipid sensing by the receptor [23]. Similarly, in a structure of the protein Smoothened, a component of the hedgehog signalling pathway, reconstituted in Salipros and representing an inactive state, a previously unknown sterol binding site could be identified, as well as accompanying conformational differences compared to previous structures that may have important consequences for the hedgehog signalling pathway [10].

In addition to the presence of specifically bound lipids, a lipidic environment can have additional effects on the overall conformation of a protein and may stabilize physiologically more relevant states compared to detergent. An example of this is the *Escherichia coli* multidrug efflux pump AcrB. Binding of the small protein AcrZ is known to alter the transporter's substrate selectivity [24]. A structure of the complex determined in Salipros revealed the structural mechanism of how cardiolipin and AcrZ cooperate to allosterically modulate the transporter's selectivity [24]. Notably, the structure differs from previously determined detergent solubilized crystal structures of the complex, and showed a strong bending of AcrZ towards the binding groove of AcrB (Figure 4a).

In the case of the murine 5-HT_{3A} serotonin receptor in Salipros, its apo form adopts a more tightly packed 'coupled' state that extends to the boundary between the extracellular and transmembrane domains, compared to the detergent-solubilized structure [25] (Figure 4b). This region contains highly conserved residues important for gating. In addition, due to the lipid bilayer-like environment, the receptor shows a widened

Figure 4



Comparisons of detergent and Salipro reconstituted structures. **(a)** In the AcrBZ complex, most of the AcrB subunit does not show large differences (upper panel), but the AcrZ subunit is more bent towards the membrane in the Salipro reconstituted structure. **(b)** There are major conformational differences within the transmembrane domains (lower panel), but not in the extracellular part of the 5-HT$_{3A}$ receptor (upper panel) when solubilized in detergent compared to the Salipro reconstituted receptor. **(c)** Reconstitution of the 5-HT$_{3A}$ receptor in Salipros also revealed an additional tetrameric, as opposed to pentameric, assembly state.

open pore upon serotonin binding compared to the detergent-solubilized structure [25]. The saposin-reconstituted receptor bound to serotonin is also present in an asymmetric form, speculated by the authors to be an intermediate state that represents the conformational changes due to sequential ligand binding [25]. Another study on the same receptor in Salipros showed the presence of a tetrameric assembly by cryo-EM and cryo-ET of secreted extracellular vesicles (Figure 4c) [15]. The authors suggest that this tetrameric state represents an assembly intermediate [15].

A series of structures of the human proton-activated chloride channel ASOR, determined at neutral or acidic pH in Salipros as well as the detergents glycodiosgenin (GDN) and Digitonin also highlight the effect of a lipid environment on protein conformation. The channel can exist in resting, activated, and desensitized states. While the authors were able to capture the resting and activated states in detergent by altering the pH, the saposin-reconstituted sample contained both states at acidic pH, along with a previously reported desensitized state [16]. Another example is the β-barrel assembly machine (BAM complex) from *Escherichia coli*, where reconstitution of the complex into SapA revealed that the BamA subunit's beta barrel domain adopts a laterally closed conformation, in contrast to the structure of the detergent solubilized complex [26].

While reconstitution of an IMP in other nanodisc systems may have similar effects on protein structure and

function as described above, Salipros have the additional advantage of auto-tuning the nanodisc size to accommodate the target IMP [2]. This property not only simplifies the reconstitution procedure; it may also result in substantially improved reconstruction resolution in cases where transmembrane domains show substantial flexibility. An example of this is the structure of the mitochondrial calcium uniporter, which was determined to 3.8 Å resolution after reconstitution in Salipros, but only to 15 Å resolution when reconstituted in other nanodisc systems [17].

Applications of Salipros beyond structural studies

Beyond their use in structure determination, Salipros have been proven to be valuable tools for studying the conformational changes and dynamics of membrane proteins using advanced techniques such as single-molecule Förster resonance energy transfer (smFRET) [27], nuclear magnetic resonance (NMR) [28], and mass spectrometry (MS) [29].

A key example is the dynamic study of the proton-coupled oligopeptide transporter, DtpA [27]. During a single transport cycle, this transporter transitions between at least three distinct conformational states. Using smFRET, researchers demonstrated that detergents tend to trap DtpA in a single conformational state, whereas in more native-like lipid environments, populations encompassing all functional states were observed. Furthermore, varying the lipid composition allowed for fine-tuning of the abundance of specific states [27].

In NMR studies, the stability and adaptability of Salipros have addressed long-standing challenges in membrane protein solubilization and spectral resolution. This size-tuneable nanoparticle maintains a high lipid content to mimic realistic membrane environment while enabling stable, long-term solution NMR studies. This has been demonstrated with proteins like OmpX, pSRII, and β1AR, the latter retaining functional interactions with binding partners [28]. The Salipro technology has also been used in mass spectrometry (MS) applications, in particular in the field of native MS. Zhou *et al.* demonstrated how Salipros maintain ferroportin's native structure and functionality during footprinting, achieving high sequence coverage [29].

Further developments: direct extraction method

Conventionally, reconstitution into Salipros is achieved by mixing a detergent-solubilized membrane protein with SapA and lipids of choice. However, this approach requires the target protein to be purified and stable for an extended period under detergent-solubilized conditions. Prior information or optimization is necessary to choose the ideal lipid composition to promote stabilization of an active membrane protein within the Salipro assembly. To overcome these obstacles, direct extraction of IMPs using Salipros, also known as DirectMX, was introduced to preserve native lipids bound to the IMP of choice [6]. However, the technique does not fully exclude the addition of detergents since a brief incubation step with digitonin or other mild detergents is required to disrupt the cellular membrane to allow for Salipro reconstitution (Figure 1b). However, after reconstitution, detergent is no longer necessary during subsequent steps. This reduces the time and efforts spent to identify a suitable detergent. DirectMX has been successfully applied in purifying the human solute carrier Excitatory Amino Acid Transporter 1 (EAAT1) [6] and in obtaining a high-resolution cryo-EM structure of the mouse ion channel Pannexin-1 (PANX1) [30]. For the latter, human and mouse PANX1 were directly isolated from Sf9 and HEK293 cells and purified to homogeneity. Moreover, the authors used His-tagged or biotinylated SapA to perform surface plasmon resonance measurements of ligand binding to reconstituted PANX1 [30]. This highlights the versatility of using Salipros beyond structure determination as a tool for biophysical analysis of IMPs where additional labeling could hamper activity measurements of the protein of interest.

Conclusions and perspective

In recent years, the Salipro method has emerged as an attractive alternative to detergents and other nanodisc systems. In comparison to detergents, Salipros enable the structural and functional characterization of IMPs in a more lipid-like environment and preserve bound lipids. In addition, the recombinant production of Saposin A is simple and cost effective, and expression constructs are available. Salipros have been a great boon to structure determination by cryo-EM, as evidenced by the fact that all recently released Salipro structures were determined by cryo-EM (Supplementary Table S1) as opposed to X-ray crystallography or NMR [31–42]. They share these advantages over detergents with other nanodisc systems such as the Membrane Scaffold Proteins (MSPs). However, Salipros self-tune their size depending on the target, which simplifies sample preparation but might be detrimental for the conformational flexibility of the IMP in case the interaction of the IMP with the scaffold protein is too tight. With the advent of the DirectMX technology, saposin reconstitution can also bypass the need for detergent solubilization prior to classical Salipro reconstitution. They share this property with synthetic nanodisc reagents such as styrene maleic anhydride lipid particles (SMALPs) [43]. However, SMALPs and related reagents may not be suitable for every IMP target and may require expensive and time-consuming screening and optimization. Taken together, Salipros combine many of the desirable

properties of other IMP reconstitution methods due to their biochemical properties, versatility, and simplicity.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbi.2025.103050.

Data availability

The PDB files used for this Mini-Review are stated. IMP structures reconstituted in Salipros with a PDB release date before December 2024 were considered.

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