

**JOURNAL:** Protein Science

**TITLE:** Unraveling ShuA detergent-induced colloidal behavior in solution: a comprehensive SEC-MALS, SAXS and SANS study.

**Running title:** ShuA Detergent-Induced Colloidal Behavior: A Biophysical Study

**AUTHORS:** Pozza A<sup>1</sup>, Martel A<sup>2</sup>, Moir M<sup>3,4</sup>, Darwish TA<sup>3</sup>, Wimalan K<sup>5</sup>, Koutsioubas A<sup>6</sup>, Combet S<sup>5</sup> and Bonneté F<sup>1\*</sup>

**Affiliations:**

<sup>1</sup> Université Paris Cité, CNRS, Laboratoire de Biochimie des Protéines Membranaires, Institut de Biologie Physico-Chimique, Paris, France.

<sup>2</sup> Large Scale Structures, ILL Neutrons for Society, Institute Laue-Langevin, Grenoble, France

<sup>3</sup> National Deuteration Facility (NDF), Australian Nuclear Science and Technology Organization (ANSTO), Lucas Heights, NSW 2234, Australia.

<sup>4</sup> School of Chemistry, The University of Sydney, Sydney, Australia

<sup>5</sup> Laboratoire Léon-Brillouin, UMR12 CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France

<sup>6</sup> Jülich Centre for Neutron Science at Heinz Maier-Leibnitz Zentrum (MLZ), Forschungszentrum Jülich, Garching, Germany

\*Corresponding authors: F Bonneté ([bonnete@ibpc.fr](mailto:bonnete@ibpc.fr)) and A Pozza ([pozza@ibpc.fr](mailto:pozza@ibpc.fr))

**ABSTRACT:**

In this study, we investigate the detergent-induced behavior of the integral membrane protein ShuA in solution, focusing on its interactions with octyl polyoxyethylene (OPOE) and *n*-dodecyl- $\beta$ -D-maltoside (DDM). Using a combination of size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS), and small-angle scattering techniques (SAXS and SANS), we provide a detailed characterization of protein-detergent complex (PDC) behavior under varying conditions. Our results reveal that ShuA remains monomeric in 1% OPOE, whereas in 0.5 mM DDM, it undergoes a reversible monomer/dimer equilibrium that shifts towards a monodisperse, monomeric state with increasing DDM concentration to 7.5 mM, highlighting the significant influence of detergent type and concentration on protein colloidal stability.

These findings have direct implications for membrane protein purification and structural studies, particularly in crystallization and cryo-EM sample preparation. The study emphasizes the necessity of optimizing detergent conditions to ensure monodispersity and structural integrity, preventing detergent-induced artifacts that could affect structural interpretations. Importantly, our results highlight the power of the SEC-MALS technique in determining oligomeric or association equilibrium states, detecting weak intermolecular interactions often overlooked in conventional SEC, and achieving this even in the particularly complex case of MPs.

By integrating advanced scattering techniques, this work contributes valuable insights into MP colloidal behavior, refining strategies for structural characterization and providing a framework for optimizing detergent conditions in biochemical and biophysical studies.

**Statement:** This study investigates the detergent-induced behavior of ShuA, revealing its monomeric state in OPOE and a reversible monomer/dimer equilibrium in DDM. Using SEC-MALS, SAXS, and SANS, we highlight the critical role of detergent selection in maintaining protein stability for membrane protein purification, structural studies, and crystallization.

**Keywords:** Membrane protein, detergent, small-angle X-ray and neutron scattering, light scattering, size-exclusion chromatography, oligomerization, colloidal interactions, crystallization.

## 1 | INTRODUCTION

Membrane proteins (MPs) play crucial roles in cells, acting as receptors for external signals, transporters of ions and molecules across the membrane, and performing other vital functions. Their involvement in various diseases makes them important drug targets. Consequently, an in-depth understanding of their structure-function relationship is essential for developing new therapeutic treatments. Over the last few decades, research on MPs has made significant progress, thanks to advances in technologies and methodologies, particularly in crystallography (XRD), cryo-electron microscopy (cryo-EM), computational methods and machine learning (Allen 2019; Healey *et al.* 2021; Sun *et al.* 2023; Thangaratnarajah *et al.* 2022). This progress is reflected in the explosion of unique structures in the “MPs of Known 3D Structure” database (<https://blanco.biomol.uci.edu/mpstruc/>), which grew more than tenfold, from 175 structures in 2007 to 1,803 in 2024 (Birch *et al.* 2020). Despite these successes, the study of MPs remains challenging, primarily due to difficulties in sample preparation, which range from the expression of the protein gene in homologous or heterologous systems to the solubilization of the host lipid membrane and the purification of the protein using appropriate amphiphilic molecules (usually detergents). Furthermore, maintaining the long-term stability of purified membrane protein-amphiphile complexes for biophysical and structural studies is a remaining issue.

A critical factor in sample preparation is the selection of detergent molecules used during the purification process. The nature of the detergent (non-ionic, ionic, zwitterionic) is crucial as it can influence the stability and proper folding of the MP. Despite some attempts at rationalizing detergent selection (Newstead *et al.* 2008; Urner *et al.* 2023; Yoon *et al.* 2024), there is no standardized approach, while the physico-chemical interactions between detergents and MPs may provide useful insights into sample preparation or potentially explain unexpected results. Among the standard detergents used, and despite the development of newer detergent and non-detergent molecules, *n*-dodecyl- $\beta$ -D-maltoside (DDM), *n*-decyl- $\beta$ -D-maltoside (DM), and *n*-octyl- $\beta$ -D-glucoside (OG) remain the most widely used for protein extraction, purification, and structural studies (Choy *et al.* 2021; Stetsenko and Guskov 2017). Due to its low critical micelle concentration (CMC < 0.01%) and its low cost, DDM is often considered the gold standard in biochemistry and structural biology of MPs. While it is extensively used for solubilization (accounting for over 80 % of total maltoside usage), its use in structural analysis depends on the method used to solve the protein structures. For example, when structures are determined using X-ray crystallography from *in-surf*o crystals (*i.e.* in surfactant micelles), it may be beneficial to exchange long-chain detergents for short-chain detergents to form smaller detergent micelles around the hydrophobic transmembrane domain, thus allowing crystal contacts between hydrophilic domains of MP, particularly in the case of integral membrane proteins (IMPs). However, this may be at the expense of protein stability. Indeed, short-chain detergents (C7–C10) have been shown to destabilize or inactivate MPs (Lee *et al.* 2016), whereas long-chain detergents (C12–C14) are better suited to accommodate the protein hydrophobic domain, thereby maintaining its structure and function (Tate 2010). Whether long- or short-

chain detergents, with sugar-based (mono or di-glycoside) or poly(ethylene glycol)-based head group, IMPs are not always stable and functional in detergents. Detergents can induce conformational changes, oligomerization or aggregation through specific interactions with flexible protein regions (Yang *et al.* 2014), or non-specific interactions between detergent micelles and protein-detergent complexes (PDCs). To address these issues, extensive efforts have been made over several decades to develop new stabilizing surfactants and membrane-mimetic systems (Woubshete *et al.* 2024; Yoon *et al.* 2024). However, less attention has been given to understand the effects of detergent micelles on the behavior of PDCs in solution, especially during sample preparation for biophysical and structural studies, such as crystallization screens or cryo-EM grid preparation. The oligomeric state of MPs, which is crucial for their stability and physiological function, can be altered during solubilization or purification, especially when detergents are used at high concentrations (Breyton *et al.* 1997) or when different detergents are applied (Fisher *et al.* 2003; Guo 2020). However, distinguishing between MP oligomers embedded in detergent micelles and oligomers of PDCs can be challenging (Heuberger *et al.* 2002). Controlling the structure of MPs in detergent is key for preparing suitable samples for structural studies. While the influence of detergents on the colloidal behavior of PDCs is often overlooked, these phenomena are well-studied in the context of nanoparticles for industrial applications such as drug delivery and cosmetics (Ray *et al.* 2018). Understanding these effects could be valuable for improving sample preparation for MP structural studies.

To describe the behavior of MPs in detergent solutions, we employed a combination of scattering techniques: multi-angle light scattering (MALS), small-angle X-ray (SAXS) and neutron (SANS) scattering. These methods, when coupled in-line with size-exclusion chromatography (SEC), offer valuable insights into the macromolecular structure in detergent solutions (Graewert *et al.* 2020). Specifically, SEC-MALS allows determination of macromolecule molar mass and oligomeric state, while SEC-SAXS and SEC-SANS provide information on the shape and size of the macromolecule. Additionally, these techniques help characterize weak interactions between macromolecules in solution via structure factor (*SF*) analysis (Spinozzi *et al.* 2002). Despite differences in sample preparation (*e.g.* concentration, sample volume, isotopic solvent), SAXS and SANS are highly complementary. For instance, both SAXS and SANS provide low-resolution structural information on the entire PDC, but SANS can exploit the contrast-variation technique to isolate protein information by matching out detergent contrast (Combet *et al.* 2023) using deuterium-labeled detergents (Midtgaard *et al.* 2018) and D<sub>2</sub>O solvents. Once the detergent is matched-out, *ab initio* shape reconstruction of the protein can be performed using programs such as DAMMIF/N (Svergun 1999) or DENFERT (Koutsoubas *et al.* 2016), or *ab initio* electron density map calculation using DENSS (Grant 2018). Analytical modeling using SasView (<http://www.sasview.org/>) can also be performed on 1D-scattering data to describe the geometry of the protein (Cleveland *et al.* 2018).

While SAXS and SANS provide similar information, each has its advantages and limitations. SANS, unlike SAXS, is not prone to radiation damage. However, the lower neutron

flux requires higher sample concentrations, larger volumes, and longer exposure times than synchrotron SAXS. Additionally, replacing H<sub>2</sub>O with D<sub>2</sub>O in sample buffers can improve the SANS signal-to-noise ratio, but this substitution may also promote protein aggregation, even as it enhances conformational stability (Giubertoni *et al.* 2023; Reslan and Kayser 2018). These effects are primarily due to the increased strength of hydrophobic interactions and the strengthening of intra- and intermolecular bonds, resulting from hydrogen–deuterium exchange (Haidar and Konermann 2023).

As a rule of thumb, structural studies using XRD or cryoEM require pure, monodisperse samples that remain structurally stable during crystallization or grid preparation, for successful results. The behavior of PDCs in terms of oligomeric state and protein-bound detergent amount depends on the protein concentration, but also on the type and concentration of detergent. In a previous study, we performed a molecular modeling of ShuA in DDM by combining SEC-SAXS, SEC-MALS, and molecular dynamics (MD) simulations (Abel *et al.* 2021). ShuA, a 70 kDa TonB-dependent receptor from the outer membrane of *Shigella dysenteriae*, was crystallized in OG (Brillet *et al.* 2009), a detergent commonly used for  $\beta$ -barrel MPs due to its ability to form small micelles, thus allowing crystal contacts and crystal growth. The high-resolution structure of ShuA was solved at 2.6 Å by X-ray diffraction (Cobessi *et al.* 2010), revealing a monomeric MP composed of 22  $\beta$ -strands. Although the SEC-MALS profile revealed a polydisperse population of ShuA in DDM (monomeric ShuA-DDM complex and higher molecular-weight complexes), we could model the DDM belt around the monomeric form of ShuA in diluted condition (Abel *et al.* 2021) using the atomic 3D-structure (3FHH.pdb), without, however, clarifying the origin of ShuA heterogeneity in DDM (oligomers of monomeric PDC, DDM-complexes of ShuA oligomers).

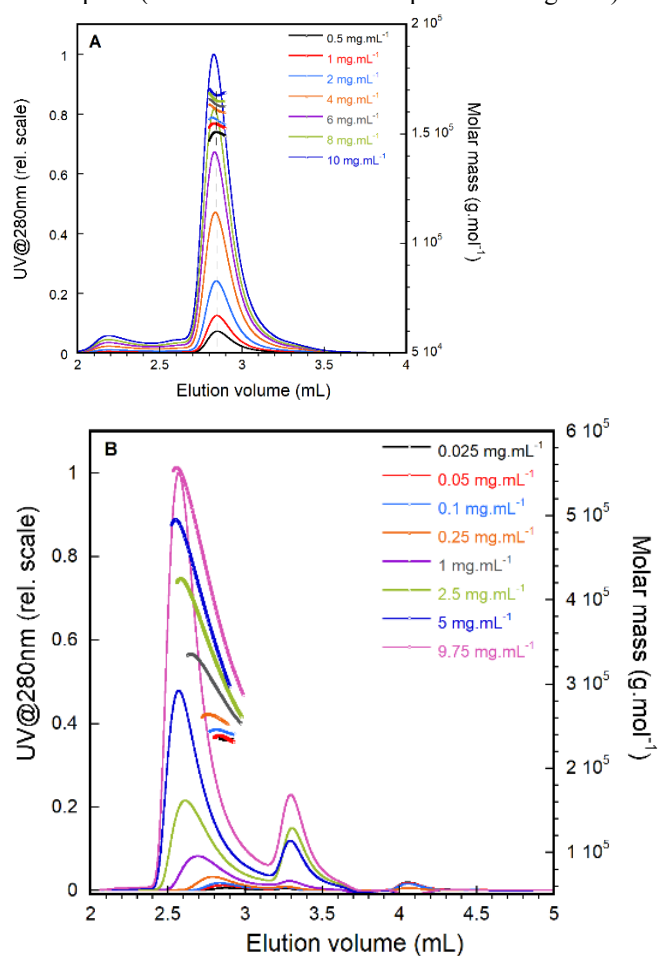
In the present study, we re-examine the behavior of ShuA in DDM and compare the results to those obtained using OPOE, the detergent originally used to solubilize and purify the protein from the outer membrane. ShuA was studied at concentrations ranging from 0.1 to 10 mg.mL<sup>-1</sup> using SEC-MALS and SEC-SAXS techniques. SAXS is particularly useful for investigating non-ideal solutions of semi-dilute or concentrated macromolecules (Zhang *et al.* 2007), while SEC-MALS, which usually needs lower sample concentration than SAXS, is considered as a “dilute-regime” technique, allowing weak interaction effects between particles to be overcome. Additionally, SANS experiments were conducted to further elucidate the specific behavior of ShuA-DDM in concentrated solutions of protein.

## 2 | RESULTS and DISCUSSION

### 2.1 | SEC-MALS analysis of ShuA-detergent complex in OPOE and DDM

Figure 1A shows the SEC profiles of ShuA in 1% OPOE at different protein concentrations ranging from 0.5 to 10 mg.mL<sup>-1</sup> injected onto a BioZen dSEC-2 column (5 mL) and monitored by UV absorbance at 280 nm. A single peak appears at  $V_e \sim 2.8$  mL whatever the amount of ShuA injected, with a very slight peak shift towards larger elution volumes as ShuA concentration increases. The extra protein-free micelles, which

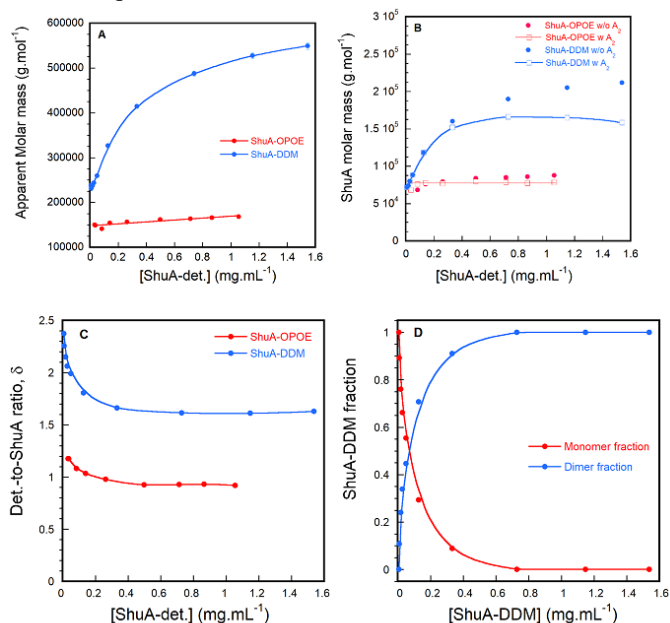
are not visible in UV profile, are well separated from the ShuA-OPOE peak (see the refractive index profile in Fig SI-1).



**Figure 1.** A) SEC-MALS elution profiles of ShuA-OPOE, 20  $\mu$ L injected between 0.5 and 10 mg.mL<sup>-1</sup> in 1% OPOE-containing buffer, eluted on a silica-gel column (BioZen, Phenomenex) at a flowrate of 300  $\mu$ L.min<sup>-1</sup>, with 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% OPOE: UV absorbance at 280 nm in solid line; symbols represent the calculated apparent molar masses for the protein-detergent complex at the different concentration injected. B) SEC-MALS elution profiles of ShuA-DDM, 20  $\mu$ L injected between 0.025 and 9.75 mg.mL<sup>-1</sup> in 0.5 mM DDM-containing buffer, eluted on a silica-gel column (BioZen) at a flowrate of 300 $\mu$ L.min<sup>-1</sup>, with 20 mM Tris-HCl pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.5 mM DDM: UV absorbance at 280 nm in solid line; symbols represent the calculated apparent molar masses for the protein-detergent complex at the different concentrations injected.

This good separation allows for the calculation of the PDC molar mass without contribution of protein-free micelle signals. The calculated molar mass of the ShuA-OPOE complex increases linearly as the injected concentration increases, from 150 kDa to about 170 kDa (Fig. 2A, Table SI-1). The small, regular and linear increase in the “apparent” molar mass of the complex suggests attractive interactions between ShuA-OPOE complexes rather than uncertainties from light scattering measurements (the LS baselines are stable and the signal fluctuations are low) or oligomerization of the complex (the calculated mass throughout the peak is constant). A negative second virial coefficient ( $A_2$ ), calculated from the Zimm equation (Eq.1) was found to about  $-3.3 \cdot 10^{-4}$  mol.mL.g<sup>-2</sup>, which is characteristic of attractive interactions between ShuA-OPOE complexes in 1% OPOE containing buffer. The molar mass of ShuA was calculated both with and without considering this second virial coefficient (Fig. 2B). With an expected molar mass of ShuA of 70,860 Da from its His<sub>6</sub>-

tagged sequence, ShuA can be described as a monomer in OPOE, binding approximately 210-230 OPOE molecules. This is in agreement with the value of ShuA molar mass determined in OPOE by SEC-MALS just after the affinity column, prior to the concentration step (Fig. SI-2A, Table SI-2). The detergent-to-protein mass ratio ( $\delta$ ) was calculated at all concentrations (Fig. 2C) and found  $\sim 0.9$  g/g for ShuA-OPOE for the highest concentrations, showing that the ShuA-OPOE complex forms a stable complex suitable for further structural studies.



**Figure 2.** A) Apparent molar masses of ShuA-detergent complex (○) (red for OPOE, blue for DDM) B) Calculated molar mass of ShuA taking (●) or not taking (○) into account the second virial coefficient (red for OPOE, blue for DDM); C) Bound detergent to ShuA ratio ( $\delta$ ) (red for OPOE, blue for DDM); D) ShuA-DDM fraction into monomer and dimer form. All data are plotted as a function of PDC concentration taken at the maximum of the elution peak.

Figure 1B shows a distinct behavior for ShuA solubilized and purified in DDM, and analyzed in 0.5 mM DDM. The UV elution profiles reveal a peak corresponding to ShuA-DDM complex, which shifts significantly from  $V_e \sim 2.9$  mL to  $\sim 2.5$  mL as the ShuA concentration increases, the peak at 3.3 mL corresponding to extra DDM micelles (also observed by refractometry, Fig. SI-3A). As a control at low concentration just after the affinity column and prior to concentration, the SEC-MALS analysis shows that ShuA in 0.5 mM DDM is monomeric, with approximately 300 molecules of DDM bound to the protein (Fig. SI-2B, Table SI-2). In contrast to ShuA-OPOE, the calculated apparent molar mass of ShuA-DDM complex increases asymptotically as ShuA concentration rises. Additionally, as the peak moves toward lower elution volumes (*i.e.* higher molar masses), the ShuA-DDM solution displays heterogeneous behavior throughout the peak (non-horizontal line) characteristic of variations in the apparent molar mass. Finally, for the highest concentrations injected, the SEC profiles overlap, while the complex apparent molar mass continues to increase with the ShuA concentration (Fig. SI-3B). As for OPOE, this suggests the presence of attractive interactions between oligomers of ShuA-DDM complexes. From the calculated mass fractions of ShuA and DDM (Table SI-3) and the mass ratio  $\delta$  (Fig. 2C), the complex at high concentrations appears structurally stable, with a constant

DDM-to-ShuA ratio of  $\sim 1.6$  g/g. A second virial coefficient was determined from the high concentration range of complex, *i.e.* where the complex appears stable, and was found to be approximately  $-1.97 \cdot 10^{-4}$  mol.mL.g<sup>-2</sup>. By accounting for these attractive interactions in the “*Protein Conjugate analysis*” procedure (Eq. 1), the molar mass of ShuA without interaction was calculated in 0.5 mM DDM (Fig. 2B). The values ranging from about 71 to 165 kDa suggest that ShuA transitions from a monomer at low concentrations to a dimer at high concentrations ( $> 5$  mg.mL<sup>-1</sup> injected). The monomer and dimer mass fractions were calculated (detailed equations in SI, Table SI-4) and plotted as a function of ShuA-DDM concentration taken at the maximum of the elution peak (Fig. 2D). The inhomogeneity observed across the elution peak can therefore be interpreted as a monomer/dimer equilibrium that occurs during the elution.

## 2.2 | SEC-SAXS analysis of ShuA-detergent complex in OPOE and DDM

In order to thoroughly describe the structure and the behavior of ShuA-detergent complex in solution as a function of ShuA concentration in 1% OPOE and 0.5 mM DDM, two concentration-series between 1 and 15 mg.mL<sup>-1</sup> (*i.e.* between 50 and 300  $\mu$ g injected on the BioZen column) have been measured by SEC-SAXS on the beamline SWING from synchrotron SOLEIL (St-Aubin, France). These concentrations correspond to the highest concentration range in the SEC-MALS experiments, where ShuA-detergent complex is stable.

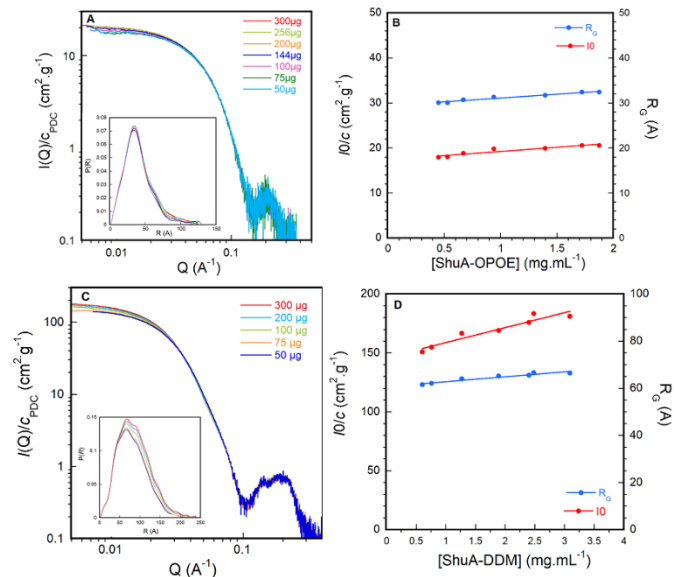
Figure 3A depicts the SAXS data analysis for ShuA in 1% OPOE. The absolute intensities have been normalized to ShuA-OPOE complex concentrations. All curves superimpose for  $Q > 0.05$  Å<sup>-1</sup>, whereas at low  $Q$ -values, the intensity increases as the concentration of complex increases. The Guinier approximation in the low  $Q$ -region allows to determine both the forward intensity,  $I(0)$ , and an “apparent” radius of gyration,  $R_G$ , for all concentrations injected on the SEC column. The increase in  $I(0)/c_{PDC}$  as a function of ShuA-OPOE concentration (Fig. 3B) confirms the presence of attractive interactions between monodisperse complexes as it was observed by SEC-MALS. This is also confirmed by the increase in the “apparent”  $R_G$  with concentration and the pair distance distribution function (PDDF) plot (Fig. 3A insert), whose bell shape is characteristic of a globular complex, and also by the Kratky representation (Fig. SI-4B), which shows a compact complex without shape evolution. The variation in  $P(R)$  for  $R \sim D_{max}$  is likely due to attractive interactions. A second virial coefficient was calculated using Eq.11 and found equal to  $\sim -3.5 \cdot 10^{-4}$  mol.mL.g<sup>-2</sup> in agreement with the value from SEC-MALS. From the SAXS intensities, an “ideal” form factor ( $FF$ ) was built by merging the low-concentration curve (50  $\mu$ g) at low  $Q$  to avoid weak interaction effects and the high-concentration curve (300  $\mu$ g) less noisy at high  $Q$ -values. Structural parameters ( $R_G$ ,  $D_{max}$ ) of ShuA-OPOE complex were determined (Table 1), which show a smaller complex in OPOE compared to the model previously described in DDM (Abel *et al.* 2021) and slightly larger than the atomic structure (3FHH.pdb). This is due to a low electron density contrast of polyoxyethylene head group with solvent as seen by SAXS, which reduces the X-ray scattering intensity of micelle at low  $Q$ -values (Fig. SI-5A).

**Table 1.** Structural parameters obtained from SEC-SAXS analysis: radius of gyration ( $R_G$ ), maximum dimension ( $D_{max}$ ) for ShuA-detergent complex in OPOE/H<sub>2</sub>O, DDM/H<sub>2</sub>O and moDDM/D<sub>2</sub>O ( $FF$  = form factor).

Sample	$R_G / D_{max}$ ( $\text{\AA} / \text{\AA}$ )
ShuA-1% OPOE ( $FF$ )	30.1 / ~95
ShuA-0.5mM DDM ( $FF$ )	61.8 / ~208
ShuA-0.5mM DDM(ABEL <i>ET AL.</i> 2021)( $FF$ )	42.2 / ~132
ShuA-0.5mM moDDM/D <sub>2</sub> O (exp. 10mg.mL <sup>-1</sup> )	68.9 / ~230
ShuA-0.5mM DDM/H <sub>2</sub> O (exp. 15mg.mL <sup>-1</sup> )	69.4 / ~235
ShuA-7.5mM DDM (exp. 1mg.mL <sup>-1</sup> )	48.7 / ~160

The main contribution of detergent signal in the ShuA-OPOE complex originates from the detergent tails, reducing the values of  $R_G$  and  $D_{max}$  of the whole complex.

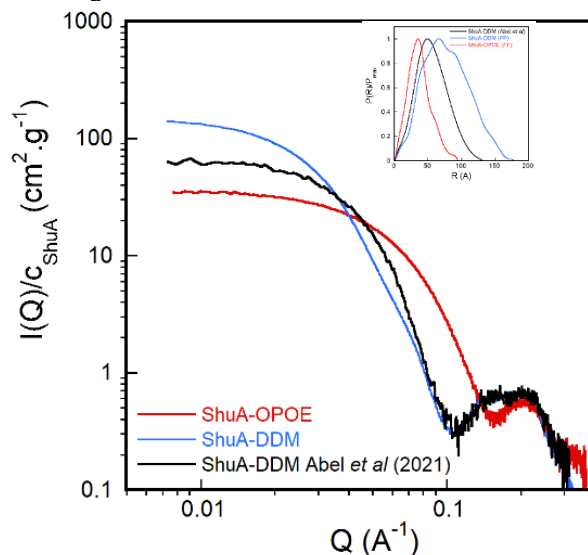
Figure 3C shows the SAXS analysis for ShuA-DDM complex. As for ShuA in OPOE, all SAXS curves superimpose at high  $Q$ -values between 50 and 300  $\mu\text{g}$  injected into the column in agreement with SEC-MALS analysis, and exhibit an increase in  $I(0)$  and  $R_G$  as the concentration of ShuA increases (Fig. 3D). However, the PDDF plot (Fig. 3C insert) shows evolution in shape as a function of concentration with the appearance of a shoulder at  $\sim 100 \text{\AA}$  from 100  $\mu\text{g}$  injected ShuA-DDM and that does not evolve as the concentration increases (Fig. SI-4C). This shoulder could be the signature of parallel dimer of ShuA complex (Glatter and Kratky 1982). This is consistent with the results from SEC-MALS that show an increase in molar mass up to 100  $\mu\text{g}$  and then a stabilization in the mass of the complex. As for SEC-MALS data analysis, a second virial coefficient was determined for concentrations between 50 and 300  $\mu\text{g}$  injected and found  $\sim -1.33 \cdot 10^{-4} \text{ mol.mL.g}^{-2}$ , slightly different from the one found with SEC-MALS ( $\sim -1.97 \cdot 10^{-4} \text{ mol.mL.g}^{-2}$ ) but still negative suggesting attractive interactions between ShuA-DDM complexes.



**Figure 3.** A) Concentration-normalized scattering intensities of ShuA in OPOE at different injected amounts as a function of  $Q$ ; PDDF plot in insert; B) Forward intensity  $I(0)/C_{PDC}$  and radius of gyration  $R_G$  as a function of ShuA-OPOE complex concentration; C) Concentration-normalized scattering intensities of ShuA in DDM at different concentrations as a function of  $Q$ ; PDDF plot in insert; D)  $I(0)/C_{PDC}$  and  $R_G$  as a function of ShuA-DDM complex concentration.

The dimensions of ShuA-DDM in this high range of concentration are larger ( $D_{max}$  between 210 – 240  $\text{\AA}$ ) than those found previously described in Abel *et al.* In this previous study,

the concentration of ShuA-DDM complex in the peak was between 0.03 and 0.06  $\text{mg.mL}^{-1}$  and the apparent molar mass of the complex between 220 and 235 kDa, which corresponds to the low concentration range in the present SEC-MALS study. Regardless of the detergent used, ShuA-detergent complex appears as a globular assembly as evidenced by the double logarithmic plot, where  $I(Q)$  is constant at small  $Q$ -values and decreases rapidly at intermediate  $Q$ -values, or the bell shape of both the PDDF and the Kratky plot. ShuA-OPOE forms a smaller complex than ShuA-DDM, as observed from the shift of the second maximum ( $\sim 0.2 \text{\AA}^{-1}$ ) towards larger  $Q$ -values (Fig. 4), and from the maximum dimension ( $D_{max} \sim 95 \text{\AA}$ ) in the PDDF plot (Fig. 4 insert). In DDM, ShuA exhibits distinct behavior as compared to the previous study. While both curves superimpose at large  $Q$ -values, suggesting a common feature, the forward intensity for ShuA-DDM at higher concentrations increases, as well as  $R_G$  and  $D_{max}$ . This could be consistent with the formation of a dimeric complex, as observed by SEC-MALS at high concentrations.



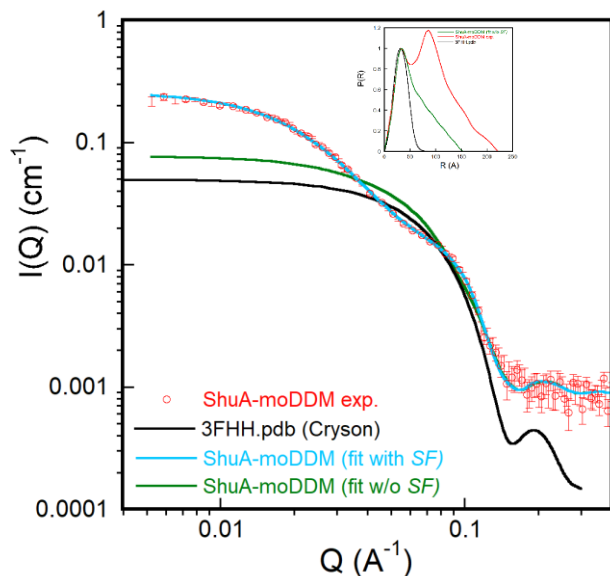
**Figure 4.** Experimental SAXS form factors of ShuA-OPOE (red line) and ShuA-DDM (blue line), obtained after merging low-ShuA concentration curve at small  $Q$ -values and high-ShuA concentration curve at large  $Q$ -values, compared to ShuA-DDM complex data from Abel *et al.* (Abel *et al.* 2021) (black line).

### 2.3 | SANS analysis of ShuA-moDDM complex

In order to decipher the oligomeric state of ShuA at high concentrations and its spatial organization in DDM, we performed SANS in a matched-out DDM (moDDM), *i.e.* a specifically deuterated DDM (in the head and tail regions) that is totally contrast matched in a 100% D<sub>2</sub>O buffer solution (Midtgaard *et al.* 2018). The SANS experiment was performed on the QUOKKA instrument<sup>a</sup> at ANSTO (Sydney, Australia) with ShuA initially prepared, *i.e.* solubilized from outer membrane and purified with 1% OPOE, then exchanged with 1% DDM-H<sub>2</sub>O on concentrator device and finally exchanged with 0.5 mM moDDM/D<sub>2</sub>O on a 24 mL-SEC column (S200 Increase 10/30). The fractions with maximum absorbance were collected (Fig. SI-7) and their SANS signal measured. The atomic structure of ShuA being available (3FHH.pdb), the theoretical scattering curve was calculated using CRYSON (Svergun *et al.* 1998) and compared to ShuA in moDDM/D<sub>2</sub>O

<sup>a</sup> During the long ILL shutdown, <sup>b</sup> after the ILL shutdown

buffer (Fig. 5). The monomeric structure of ShuA clearly does not coincide with the experimental SANS curve. To verify the results and the efficient exchange of detergent from OPOE to DDM and moDDM, the SANS experiment was reproduced on the D22 instrument<sup>b</sup> at ILL (Grenoble, France) with ShuA directly solubilized and purified using DDM and then exchanged with 0.5 mM moDDM in a 100 % D<sub>2</sub>O buffer on a 24 mL-S200 column as previously.



**Figure 5.** Experimental SANS curve of ShuA-moDDM (red dot) in 100% D<sub>2</sub>O buffer (50mM Tris, pH 7.5, 100mM NaCl, 0.5 mM moDDM/D<sub>2</sub>O). The curve is obtained by merging data from 3 sample-detector distances; SasView fitted curve of ShuA-moDDM (blue line) using a cylinder as form factor (*FF*) and a sticky hard-sphere structure factor (*SF*). The green curve represents the cylinder form factor alone. **Insert**) Pair-distance distribution functions, *P(R)*, of ShuA 3FHH structure (black line).

The SANS signal of ShuA-moDDM at the highest concentration is similar to result from QUOKKA (Fig SI-8A). The expected  $I(0)$  for a monomer of ShuA at 0.8 mg.mL<sup>-1</sup> is ~ 0.047 cm<sup>-1</sup>, whereas the experimental  $I(0)$  for ShuA-moDDM in 100% D<sub>2</sub>O buffer is found ~ 0.235 cm<sup>-1</sup>. The  $R_G$  of both curves, as well as the  $D_{max}$  from the PDDF (Insert Fig. 5, Table 2), suggest a large oligomer or a cluster of dimers (Valadez-Perez *et al.* 2021; Zhang *et al.* 2012) of ShuA in the matched-out DDM in D<sub>2</sub>O, which could corroborate the SEC-MALS results, where attractive interactions between dimers of ShuA-DDM complex were characterized. This cluster or large oligomer could be due to the effect of deuterated DDM and/or D<sub>2</sub>O that could induce stronger attraction between moDDM micelles (Fig. SI-5B) compared to DDM micelles and therefore could increase attractions between ShuA-moDDM complexes as suggested by Hitscherich and co-workers (Hitscherich *et al.*

2000). To decipher the behavior of ShuA in moDDM and take into account attractive interactions, the SANS curve was fitted, by using the program SasView (<http://www.sasview.org/>), as the product of a form factor  $P(Q)$  and a structure factor  $S(Q)$ , which enables to account for weak interactions. The theoretical SANS curve of ShuA 3D-structure was also fitted using SasView with either an ellipsoid or a cylinder model form factor. By using the scattering length density calculator from BioISIS (<http://pslde.isis.rl.ac.uk/Pslde/>) with the ShuA sequence in 100% D<sub>2</sub>O ( $\rho_{ShuA} = 3.273 \cdot 10^{-6} \text{ \AA}^{-2}$  and  $\rho_{buf} = 6.376 \cdot 10^{-6} \text{ \AA}^{-2}$ ), the best fit model for the 3FHH-structure was a prolate ellipsoid with an equatorial radius of ~27 Å and a polar radius of ~39 Å, which agrees well with the total dimensions of the  $\beta$ -barrel shape of ShuA (*i.e.* ~ 50 Å diameter and ~ 80 Å height) (Fig. SI-9). For ShuA-moDDM in D<sub>2</sub>O buffer, a cylinder model with a sticky hard-sphere (SHS) structure factor successfully accounts for the experimental SANS curve (Fig. 5). The dimensions of the cylinder were found equal to ~ 23.6 Å in radius and ~151 Å in height, suggesting the formation of a linear dimer with two ShuA molecules in close attraction. This is confirmed by the PDDF (Insert Fig. 5), which, in the small distance range, displays the characteristic feature of ShuA in its monomeric form. The PDDF of ShuA-moDDM, calculated without the contribution of  $S(Q)$ , displays the form factor of a ShuA dimer, with a maximum dimension and Porod volume approximately twice those of the ShuA monomer.

#### 2.4 | Macromolecular modeling of ShuA in detergent from SAXS and SANS data.

Small-angle scattering (SAS) is a powerful technique for probing the structure of biological macromolecules in solution. SAS is particularly useful for modeling dilute and non-interacting biomolecules in solution. It complements high-resolution methods like XRD, cryoEM and nuclear magnetic resonance (NMR), especially when suitable samples are challenging to obtain for these high-resolution techniques. Several software programs enable the *ab initio* reconstruction of biomolecules from 1D scattering data, including DAMMIF/N, MONSA, and DENFERT (Franke and Svergun 2009; Koutsioubas *et al.* 2016; Molodenskiy *et al.* 2020; Svergun 1999). These programs use collections of beads or dummy atoms, which are arranged in a shape that best fits the scattering profile of the biomolecule. A more recent approach (DENSS) directly calculates electron density maps from the SANS data (Grant 2018; Sumner and Qian 2022), without relying on predefined models or shapes, offering a more realistic 3D view of macromolecules, even complexes, in solution.

**Table 2.** Structural parameters obtained from SANS analysis († ellipsoid model; ‡ cylinder model; *SF* = structure factor)

Sample	$R_G$ (Å)   $D_{max}$ (Å)   $V_{Porod}$ (Å <sup>3</sup> )	$I(0)$ (cm <sup>-1</sup> )	SasView model dimensions (Å / Å)
3FHH 0.8 mg.mL <sup>-1</sup> (CRYSON)	24   ~76   ~94,600	0.047	$R_{eq.} 26 / R_{pol.} 39$ †
ShuA-moDDM (exp.)	66   ~220   ~350,000	0.235	
ShuA-moDDM (fit w/o <i>SF</i> )	46   ~155   ~176,000	0.142	$R 23.6 / H 151$ ‡

Other approaches enable modeling the detergent belt around a MP of known structure. For example, MemProt (Perez and Koutsioubas 2015) uses a collection of dummy beads to generate a coarse-grained model by fitting an experimental SAXS curve knowing the MP all-atom 3D-structure. In contrast, the Det.belt server (Zampieri *et al.* 2021) approximates the detergent belt as a hollow cylinder, based solely on the number of detergent molecules surrounding the MP. Although schematic, these representations of PDCs provide biochemists with valuable visual insights into their samples.

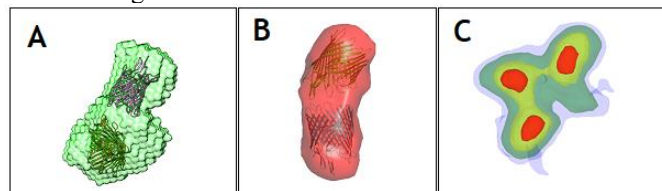
To expand MemProt's applicability to a wider range of detergents and belt geometries, Combet and collaborators are developing SASbelt, a new Python-based implementation (to be published), that adds a graphical interface, automated MP orientation, and a webserver dashboard (<https://llb.cnrs.fr/sasbelt/>).

In the present study, we combined two complementary strategies. First, we modeled SANS data using DAMMIF and DENSS to reconstruct the overall shape of ShuA in moDDM. Second, we applied SASbelt to SAXS data to visualize the detergent belt surrounding ShuA in OPOE and DDM. For the DAMMIF analysis, the overall shape of the ShuA-moDDM complex was reconstructed using the  $P(R)$  function derived from the SasView fitting SANS curve, without including the SHS structure factor. The reconstruction was carried out through the Web server interface (<https://www.embl-hamburg.de/biosaxs/atsas-online/>) with 20 iterations completed and the resulting envelopes aligned and averaged using DAMAVER (Volkov and Svergun 2003). For the DENSS analysis, 3D electron density maps were generated, either from the scattering intensity  $I(Q)$  or the pair-distance distribution function  $P(R)$  via the Web server (<https://denss.ccr.buffalo.edu/>). Both experimental SANS data and the SasView form factor were analyzed. A total of 20 electron density maps were computed, aligned, and averaged to produce the 3D reconstructions.

Figures 6A and 6B present the *ab initio* models obtained for the SasView  $FF$  with DAMMIF and DENSS, respectively. Each suggests that ShuA forms a rather linear dimeric structure with close interactions in 0.5 mM moDDM. Additionally, a DENSS reconstruction of the experimental SANS curve suggests that the solution at very low  $Q$ -values may consist of a cluster of three ShuA assemblies (Fig. 6C). This aligns with forward intensity values suggesting a hexamer or a cluster of three dimers (Zhang *et al.* 2012). This behavior, which was not observed in SAXS data with the protiated ShuA-DDM complex in H<sub>2</sub>O buffer, could originate from the presence of moDDM in D<sub>2</sub>O.

Figure 7 displays the ellipsoidal core-shell torus models generated by SASbelt, where blue and yellow beads represent the hydrophilic and hydrophobic regions of the detergent belt, respectively. As expected, the belt dimensions are smaller in OPOE than in DDM, consistent with a reduced PDC in the OPOE condition. The slightly greater ellipticity of the ShuA/OPOE model ( $e = 1.3$ ), as shown in Figs. 7 and SI-13A, may indicate increased shape variability or structural disorder in the OPOE belt compared to the more uniform DDM cases ( $e = 1.15$  and  $1.2$  for 0.5 and 7.5 mM DDM, respectively).

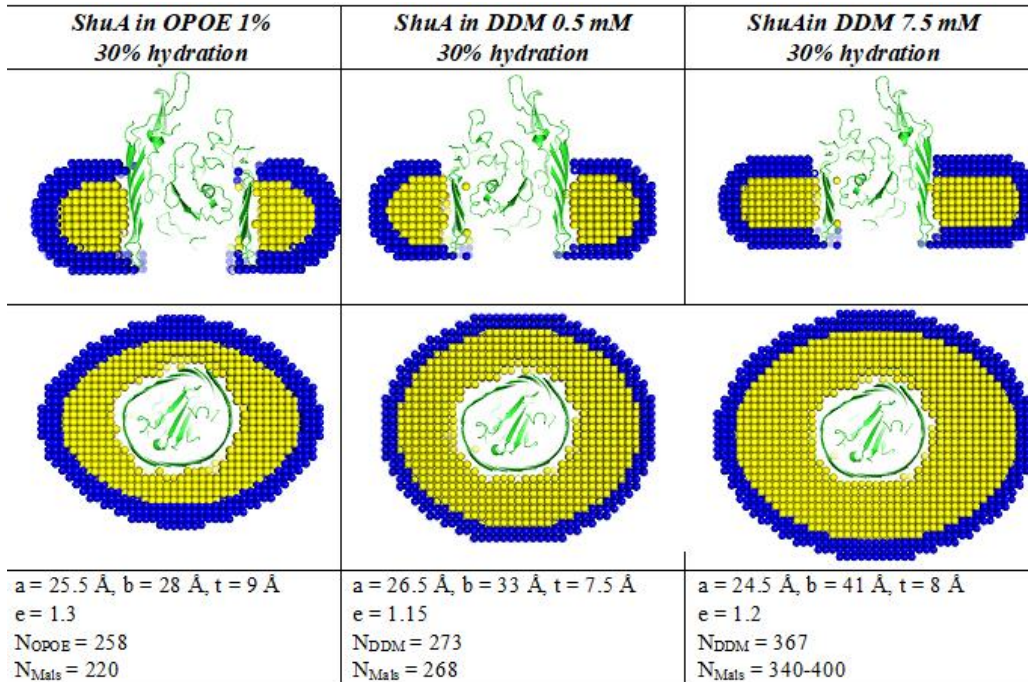
Using 30% hydration for the detergent heads yielded excellent fits (Fig. SI-13) with  $\chi^2 = 0.7$  for 0.5 mM DDM,  $\chi^2 = 1.8$  for 1% OPOE and a poorer but acceptable fit ( $\chi^2 = 10.7$ ) at 7.5 mM DDM. The head-to-tail ratios - ideally unity - were 1.14 (0.5 mM DDM), 1.01 (1% OPOE), and 0.90 (7.5 mM DDM). The aggregation numbers in OPOE and DDM from SASbelt agree well with those from SEC-MALS, confirming the reliability of the modeling.



**Figure 6.** **A)** *Ab initio* shape reconstruction from pair-distance distribution function of ShuA-moDDM using DAMMIF; **B)** Electron density reconstruction from fitted scattering data of ShuA-moDDM using DENSS. Electron densities are shown as volumes colored according to electron density. In both cases, models were performed using the fitted curve without  $SF$ . The two *ab initio* models can be overlaid with two molecules of ShuA; **C)** Electron density reconstruction from experimental SANS scattering data of ShuA-moDDM using DENSS

## 2.5 | SAXS comparison of ShuA-DDM/H<sub>2</sub>O and ShuA-moDDM/D<sub>2</sub>O complexes.

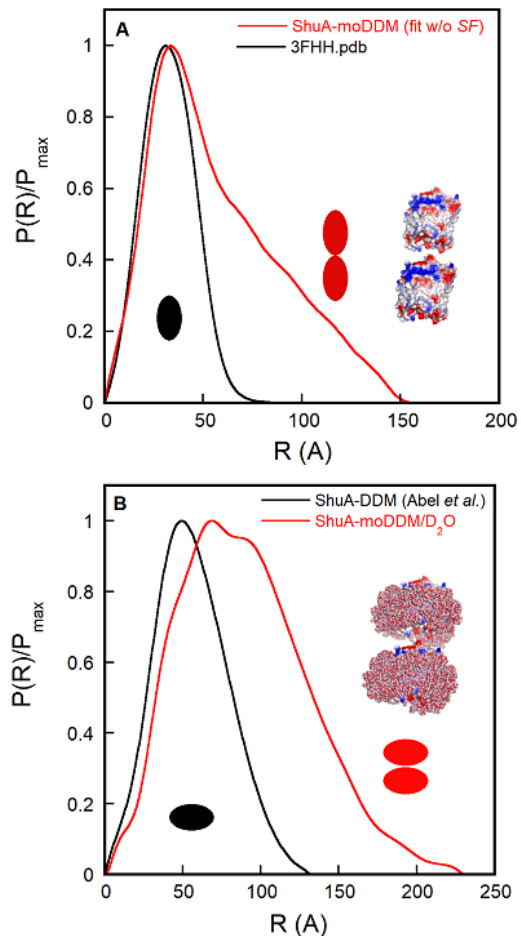
Samples prepared for SANS experiments typically require deuterated compounds such as (per)deuterated proteins or (per)deuterated detergents, in H<sub>2</sub>O/D<sub>2</sub>O solvent mixtures. In this study, ShuA was prepared using moDDM in 100% D<sub>2</sub>O buffer, allowing us to focus on characterizing the protein structure while matching out the contribution from the detergent. To evaluate the influence of moDDM and D<sub>2</sub>O on the structure of the ShuA-DDM complex in solution, we compared by SAXS, ShuA-moDDM in 100 % D<sub>2</sub>O buffer with ShuA-DDM in H<sub>2</sub>O buffer. The ShuA-moDDM/D<sub>2</sub>O sample preparation for SAXS was identical to that for the SANS experiment: ShuA-DDM was injected onto a 24 mL-S200 column pre-equilibrated with a deuterated moDDM-containing D<sub>2</sub>O buffer solution. The UV-elution profile of ShuA-moDDM/D<sub>2</sub>O was compared to the elution profiles on the same column of a concentrated ShuA-DDM/H<sub>2</sub>O sample (15 mg·mL<sup>-1</sup>) and a diluted ShuA-DDM sample after the affinity column (Fig. SI-10). Both concentrated ShuA-DDM (15 mg·mL<sup>-1</sup>) and ShuA-moDDM (10 mg·mL<sup>-1</sup>) eluted at  $V_e \sim 9$  mL whereas the monomeric ShuA eluted at  $V_e \sim 11$  mL confirming the dimerization of ShuA at high concentrations. The concentrated ShuA-DDM sample (15 mg·mL<sup>-1</sup>) was analyzed by SEC-SAXS on SWING at SOLEIL, whereas all fractions through the peak of ShuA-moDDM were collected and analyzed by SAXS in batch mode on the BM29 beamline at ESRF. For each fraction,  $I(0)$ ,  $R_G$  and  $D_{max}$  were determined (Table SI-6). Fractions 15 to 19, which exhibited similar shapes and values of  $R_G$ , were averaged for further analysis. The scattering curves  $I(Q)/c_{ShuA}$  and  $P(R)/P_{max}$  for both ShuA-DDM/H<sub>2</sub>O buffer and ShuA-moDDM/D<sub>2</sub>O buffer at the maximum absorbance show similar shapes and dimensions ( $R_G$ ,  $D_{max}$ ) (Fig. SI-8B, Table 1), indicating that the presence of the deuterated detergent and the use of D<sub>2</sub>O in the buffer neither affect the detergent belt surrounding ShuA nor the oligomeric state of the protein. However, the strength of the attractive interactions between dimers of ShuA-moDDM complexes could not be evaluated as this would have required injecting a series of more concentrated samples onto the column, which could not be achieved.



**Figure 7.** SasBelt modeling of ShuA in OPOE 1% and DDM 0.5 and 7.5 mM: Side view, top view, geometric parameters (Input parameters and output fits are in SI).

## 2.6 | SAXS/SANS comparison of ShuA-moDDM for dimer organization.

Analysis of SEC-MALS, SEC-SAXS and SANS experiments as a function of ShuA concentration suggests that the ShuA-DDM complex in a buffer containing 100 mM NaCl, 0.5 mM DDM at pH 7.5 forms a dimer at concentrations above  $5 \text{ mg}\cdot\text{mL}^{-1}$  (concentration measured prior to injection onto SEC column). However, the precise arrangement of this dimer - whether parallel, linear, or an intermediate form, and whether involving weak or strong interactions - remains unclear. By comparing the PDDFs of ShuA-moDDM from SAXS and SANS experiments, we propose a dimer structure for ShuA and its complex in DDM. Based on the values of maximum dimension and Porod volume (Tables 1 & 2) as well as the shape of the PDDFs (Fig. 8A) for the 3FHH-structure of ShuA and the fitted SANS curve of ShuA-moDDM without the SHS structure factor, we observe that both  $D_{\text{max}}$  and  $V_{\text{Porod}}$  double. Moreover, the  $P(R)$  of ShuA-moDDM dimer closely superimposes with that of the ShuA monomer for  $R < 50 \text{ \AA}$ , and then decreases linearly up to twice the  $D_{\text{max}}$  of ShuA monomer, suggesting a linear arrangement of ShuA dimers (Glatter and Kratky 1982). Comparing the PDDFs of ShuA-DDM complexes at low and high concentrations (Fig. 8B),  $D_{\text{max}}$  does not double for concentrated ShuA, and a shoulder appears that could be consistent with formation of parallel prolate dimers of ShuA-DDM complex (Glatter and Kratky 1982). Both results from SANS and SAXS are consistent with an aligned dimer of ShuA when regarding the complex with the matched-out belt and with a parallel dimer of ShuA complex when regarding the whole complex (schematic models in Fig.8). ShuA in 0.5 mM DDM therefore seems to form a colloidal oligomer rather than a dimer of ShuA embedded in a DDM belt (SASbelt model, data not shown). This is consistent with the fact that the majority of known 3D-structures of 22- stranded transmembrane  $\beta$ -barrels are monomeric (Tamm *et al.* 2004).



**Figure 8** A) Representative view of ShuA as linear dimer as suggested by the SANS PDDF; B) Representative view of ShuA-DDM complex as parallel dimer as suggested by the SAXS PDDF.

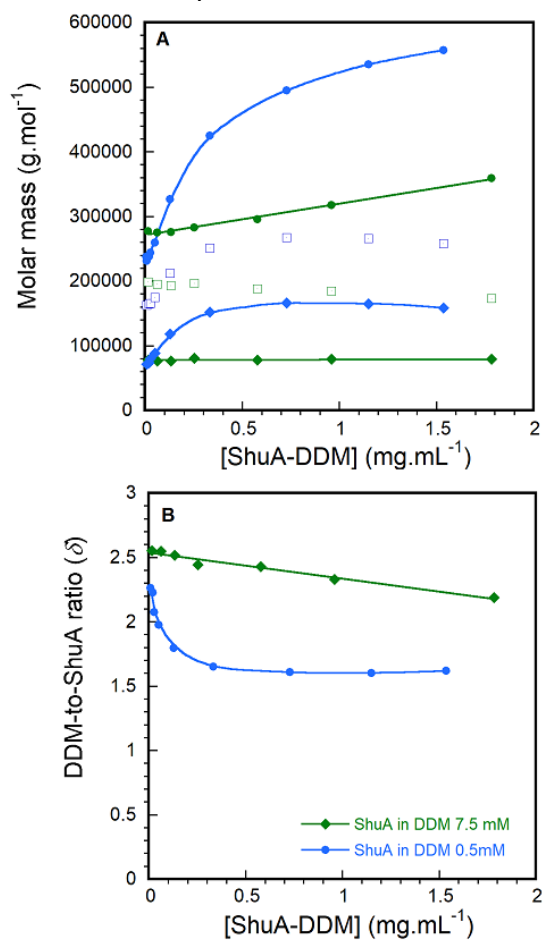
## 2.7 | Colloidal behavior of ShuA in detergent solution.

The present study of ShuA in OPOE and DDM using SEC-MALS and SAS techniques reveals two distinct behaviors regarding the oligomerization of the ShuA-detergent complex. In 1% OPOE ( $\sim 4 \times \text{CMC}$ ), the ShuA-detergent complex remains monomeric, exhibiting inter-complex attractive interactions that induce an increase in the apparent molar mass of the complex. However, this does not lead to the formation of PDC dimers. In contrast, in 0.5 mM DDM ( $\sim 3 \times \text{CMC}$ ), the ShuA-DDM complex in solution evolves into a reversible monomer/dimer equilibrium as a function of ShuA concentration, with ShuA-DDM dimers displaying attractive interactions at highest ShuA concentrations.

To assess whether the observed behaviors are influenced by the nature or by the concentration of detergent in the ShuA solution, SEC-MALS experiments were conducted by modifying the composition of the ShuA-DDM solution. Ionic strength, pH, and DDM concentration were tested individually. Changing the NaCl concentration from 100 mM to 300 mM, or lowering the pH from 7.5 to 5.0 (close to ShuA's pI) did not significantly affect the behavior of the ShuA-DDM complex with 0.5 mM DDM (*i.e.* complex dimer formation) (Fig. SI-11A). Modulating the ionic strength with NaCl or reducing the net charge of ShuA increased the apparent molar mass, likely due to stronger attractions between dimers. In contrast, increasing the DDM concentration in the mobile phase from 0.5 mM to 7.5 mM seemed to prevent dimer formation. A linear increase in apparent molar mass of ShuA-DDM complex as the injected concentration of sample increases, similar to that observed with 1% OPOE, suggested attractive interactions between ShuA-DDM monomeric complexes. A similar trend was observed for the DDM-to-ShuA ratio ( $\delta$ ), which was not significantly affected by changes in NaCl concentration or pH, but increased with higher DDM concentrations (Fig. SI-11B) and gradually decreased as the ShuA-DDM complex concentration increased. The increase in the DDM-to-ShuA ratio, indicating a larger detergent belt with increasing DDM concentration, as observed with the SASbelt modeling, has already been described in other studies (Kaufmann *et al.* 2006; Wolfe *et al.* 2019) and also modeled by MD simulations in our previous study (Abel *et al.* 2021). This can be explained by the tendency of DDM to energetically favor adsorption onto the protein surface rather than formation of micelles in the bulk (Polidori *et al.* 2016), whereas the leakage of the detergent from the belt to the bulk could result from the increase in attractive interactions between ShuA-DDM complexes (Gelbart *et al.* 1984).

To understand the mechanisms controlling ShuA-detergent behavior in the two detergents, we therefore analyze the composition of the ShuA solution in each detergent. It is well known that a solution of MPs in detergents is complex, consisting of free detergent molecules, detergent micelles, and MPs embedded in a detergent belt (PDCs). While the concentration of free detergent can be considered approximately equal to the critical micellar concentration (CMC), the concentrations of detergent micelles and PDCs depend on the specific experimental conditions used. For OPOE, the detergent concentration used in the mobile phase for purification, SEC-MALS and SAS experiments was 1% ( $\sim 28.6$  mM). With a CMC of 6.6 mM and an aggregation number of

approximately  $120 \pm 10\%$  molecules per micelle (from our SEC-MALS experiments), the molar concentration of OPOE micelles is  $\sim 170$   $\mu\text{M}$ . In our study, ShuA concentrations injected onto the SEC column ranged from 1 to 10  $\text{mg}\cdot\text{mL}^{-1}$ , resulting in ShuA-OPOE complex molar concentrations between 2 and 8  $\mu\text{M}$  at the maximum of the elution peak, which is much lower than the OPOE micelle molar concentration. For DDM, the concentration used in all SEC experiments was 0.5 mM, which corresponds (with  $N_{\text{ag}} \sim 135$  molecules per micelle and  $\text{CMC} \sim 0.17$  mM) to  $\sim 2.3$   $\mu\text{M}$  of DDM micelles, which is almost one hundred times less than OPOE. The molar concentrations of ShuA-DDM dimers ranged from 0.9 to 4.2  $\mu\text{M}$  in the elution peak, which is roughly the same order of magnitude as the DDM micelle concentration. In contrast, the molar concentrations of ShuA-DDM monomer complexes ranged from 0.02 to 0.08  $\mu\text{M}$ , which is much lower than the DDM micelle molarity. These results are in line with the works of Fleming *et al.* (Fleming 2002), which show that when the protein is diluted in a detergent micelle phase, it moves towards a dissociated state. Thus, in 1% OPOE, the detergent micelle concentration been greater than the PDC concentration, the ShuA-detergent complex remains in a monomeric form. This behavior is further confirmed by SEC-MALS and SEC-SAXS experiments conducted with a higher concentration of 7.5 mM DDM in the elution mobile phase.



**Figure 9.** A) Apparent molar masses of ShuA-detergent complex (●) calculated from SEC-MALS experiments with different detergent concentrations in the mobile phase (blue for 0.5 mM DDM, green for 7.5 mM DDM); calculated molar mass for ShuA (◆) and bound DDM (□) by taking  $A_2$  into account; B) Bound detergent to ShuA ratio ( $\delta$ ) (blue for 0.5 mM DDM, green for 7.5 mM DDM).

Figure 9 shows the calculated molar mass for the ShuA complex, the protein alone, and the protein-bound detergent at 0.5 mM and 7.5 mM DDM from SEC-MALS analysis. At 7.5 mM DDM, the apparent molar mass of the ShuA-DDM complex increases linearly with ShuA concentration, similar to observations made for ShuA in 1% OPOE, suggesting a comparable behavior. A second virial coefficient, calculated from MALS data ( $A_2 \sim -3.3 \times 10^{-4} \text{ mol}\cdot\text{mL}\cdot\text{g}^{-2}$ ), indicates attractive interactions between ShuA-DDM complexes. The corrected molar mass of the ShuA-DDM complex (Table SI-3B), derived from  $A_2$ , suggests a monomer binding approximately 340–400 DDM molecules at 7.5 mM DDM, with the number of bound DDM molecules decreasing as ShuA concentration increases. This behavior is confirmed by the SAXS experiments. A concentration-series between 1 and 6  $\text{mg}\cdot\text{mL}^{-1}$  (*i.e.* between 75 and 300  $\mu\text{g}$  injected on the BioZen column) was measured by SEC-SAXS on SWING (SOLEIL). The complex concentration-normalized intensities (Fig. SI-12) show an increase in  $I(0)/c$  and a decrease in the second maximum as ShuA concentration increases. The decrease in the second maximum ( $Q \sim 0.2 \text{ \AA}^{-1}$ ) correlates with a release of DDM molecules from the protein belt to the detergent bulk solution as observed in SEC-MALS experiment (Fig. SI-11B) and already reported in Abel *et al.* (2021), whereas the increase in  $I(0)/c$  can be attributed to the attractions between ShuA-DDM complexes. This attraction between complexes could originate from the presence of the large amount of extra detergent micelles in the bulk solution (Jia *et al.* 2005). Indeed, in the latter experiment with 7.5 mM DDM, the molar concentration of DDM micelles ( $\sim 54 \mu\text{M}$ ) is larger than the ShuA-DDM molarity, which ranged from 0.3 to 7.9  $\mu\text{M}$ . The observed outcomes can be understood by considering the complex interplay of inter-particle interactions within the solution. In PDC solutions, several types of interactions are at work: those between detergent micelles, between PDCs, and between protein-free micelles and PDCs. These include both attractive forces - such as van der Waals interactions, hydrophobic interactions, and depletion interactions - and repulsive forces, including excluded volume effects, electrostatic repulsion, and hydration forces. While proteins experience repulsion due to excluded volume, net charge, and hydration shells, the repulsive interactions between micelles can paradoxically lead to attractive forces between PDCs, like the polymer-induced depletion attractions seen in colloid or macromolecular systems (Asakura and Oosawa 1958; Marenduzzo *et al.* 2006).

If it is relatively straightforward to study and characterize weak interactions between soluble proteins in salts, PEGs or detergents (Bonneté *et al.* 1999; Jia *et al.* 2005; Kulkarni *et al.* 2000), characterizing the interactions between PDCs in solution is more challenging due to the presence of micelles (Hitscherich *et al.* 2000). This can be overcome by using SAS techniques coupled in-line with SEC, which permits the separation of protein-detergent complexes from extra detergent micelles and the subtraction of surrounding micelle signal. It is also possible to assess the nature of inter-complex interactions for integral MPs (IMPs) like ShuA by looking at the interactions between protein-free micelles. Indeed, in 2000's, Hitscherich and co-workers have shown that for IMPs embedded in a detergent belt, the interactive behavior of IMP-detergent complexes

follows that of protein-free detergent micelles, suggesting that the detergent contribution dominates in the behavior of the PDC (Hitscherich *et al.* 2000).

In our study, we characterized by SAXS the size, shape, and inter-micelle interactions of DDM, OPOE and moDDM in solution (Fig.SI-5). As previously described (Barret *et al.* 2013), the DDM micelle is stable in shape and presents an oblate core-shell ellipsoid structure, with  $R_G$  of  $\sim 32 \text{ \AA}$  and  $D_{\text{max}}$  of  $\sim 80 \text{ \AA}$ , exhibiting slight repulsive interactions as seen by the decrease in forward intensity as the detergent concentration increases. For OPOE, to our knowledge, there are few or no studies addressing the structural parameters of OPOE micelles. Our SAXS experiments do not allow for a clear characterization of OPOE micelle interactions at small  $Q$ -values, however we could model the OPOE micelle at 1% using SasView. A spherical core-shell model allows to fit the SAXS data (Fig. SI-6), yielding a hydrophobic radius of  $\sim 9.3 \text{ \AA}$ , which agrees rather well with the C8-alkyl chain length using the Tanford's formula (Tanford 1980), and a shell thickness of  $\sim 23.3 \text{ \AA}$ . The shape of the scattering curve  $I(Q)$  at low  $Q$ -values and the shell scattering length density from SasView fit ( $\text{Sld}_{\text{shell}} \approx \text{Sld}_{\text{water}}$ ) suggest that the OPOE micelles are strongly hydrated, which could induce repulsive inter-micellar interactions. For moDDM in  $\text{D}_2\text{O}$ , the micelles can be modeled by an oblate core-shell ellipsoid structure with a radius of gyration  $R_G \sim 36 \text{ \AA}$  and a maximum dimension  $D_{\text{max}} \sim 85 \text{ \AA}$ , exhibiting strong attractive interactions as seen by the increase in forward intensity as the detergent concentration increases (Fig. SI-5B) and the value of  $A_2 \sim -8.7 \cdot 10^{-4} \text{ mol}\cdot\text{mL}\cdot\text{g}^{-2}$  (Table SI-7), the form factor remaining constant for  $Q > 0.7 \text{ \AA}^{-1}$ . Although DDM and moDDM micelles have similar shapes and dimensions, the presence of the deuterated chain in moDDM enhances hydrophobic interactions between chains inside the micelles, thereby increasing overall inter-micelle interactions. Additionally,  $\text{D}_2\text{O}$  can influence these inter-micelle interactions by altering the hydration shell - owing to the stronger deuterium bonding - further affecting the interactions between ShuA-moDDM complexes in a  $\text{D}_2\text{O}$  buffer (Reslan and Kayser 2018). The presence of deuterated chains, if it neither alters the detergent belt around ShuA nor affects the dimer shape (Fig. SI-8) induces a strong attraction (SHS) between dimers as seen in the SANS experiment fitting model. These deuteration effects observed with a ShuA clustering at small  $Q$ -values could possibly explain the results on SERCA or LamB in the paper of Midtgaard *et al.* (Midtgaard *et al.* 2018), where combination of different oligomers and a minor fraction of aggregates were necessary to fit experimental data.

To explain the behavior of ShuA in detergent, one has to consider the protein embedded in its detergent belt and the effect of protein-free extra micelles. For low detergent micelle concentrations such as 0.5 mM DDM, the detergent adsorbs on the hydrophobic transmembrane domain of ShuA favoring repulsions between complexes, while keeping enough charged patches of the extra cellular surfaces accessible for van der Waals attractions. When ShuA concentration increases, attractions can occur between charged patches of ShuA leading to alignment of ShuA into dimers. When DDM in  $\text{H}_2\text{O}$  buffer is replaced with moDDM in  $\text{D}_2\text{O}$  buffer, the combination of the deuterated DDM belt and the presence of  $\text{D}_2\text{O}$  strengthens the attractive forces between dimers, leading to clustering of ShuA-

moDDM dimers (Fig. 6C). In contrast, for high concentration of detergents, *e.g.* 1 % OPOE or 7.5 mM DDM, ShuA is embedded in a strongly hydrated belt or a larger detergent belt, which prevents attraction between attractive patches of the protein and keeps the complex in a monomeric state, while the large amount of protein-free detergent micelles induces a depletion attraction between ShuA-detergent monomer complexes (Jia *et al.* 2005).

These results demonstrate the critical influence of detergent type and concentration on the colloidal stability and oligomeric state of MPs in solution. The contrasting behaviors of ShuA in OPOE and DDM highlight the importance of optimizing detergent conditions to maintain monodispersity (monomer or dimer) and structural integrity, particularly for structural studies such as X-ray crystallography and cryo-EM. A key finding is that the oligomeric state of ShuA is not solely dictated by detergent type but also by the protein-to-detergent ratio. While ShuA remains monomeric in OPOE (and in Octyl Glucoside (OG), data not shown), it exhibits a concentration-dependent monomer/dimer equilibrium in DDM. This suggests that free detergent micelle content can modulate protein-protein interactions in a manner similar to polymer in protein crystallization, a factor often overlooked in structural studies and which can influence crystallization mechanisms. Surprisingly, the formation of dimers did not prevent the crystallization of ShuA at 5 mg.mL<sup>-1</sup> in 0.5 mM DDM-containing buffer, induced by the depletion attraction of PEG present as crystallizing agent, as seen in Figure 10. Improvements in the crystallization of ShuA in DDM is still in progress in order to decrease the supersaturation and improve the crystal size and then determine if a dimer exists in the asymmetric unit or if it only acts as a precursor of crystallization.

Finally, by integrating SEC-MALS, SAXS, and SANS, our study provides a robust framework for characterizing protein-detergent interactions. These findings emphasize the need for a quantitative approach to detergent selection, ensuring that membrane protein samples are optimally prepared for structural and functional investigations.

### 3 | CONCLUSIONS

In this study, we explored the influence of detergents OPOE and DDM on the colloidal behavior of ShuA-detergent complex in solution. By combining SEC-MALS, SAXS, and SANS techniques, we revealed significant differences in the oligomeric state of the complex depending on the type and concentration of detergent. Our findings demonstrate that ShuA complex remains monomeric in the presence of OPOE, with weak attractive interactions between PDCs. In contrast, in DDM, ShuA exhibits a reversible monomer/dimer equilibrium, influenced by protein concentration and protein-free detergent micelle concentration in solution. Increasing the DDM concentration tends to stabilize the monomeric form, highlighting the critical role of the protein-to-detergent ratio in complex structuring.

These results have direct implications for membrane protein crystallization. Since successful crystallization requires monodisperse and stable protein samples, our study underscores the necessity of optimizing detergent selection and concentration to prevent unwanted oligomerization or

aggregation. Furthermore, the weak attractive interactions between PDCs, as observed in our experiments, suggest that detergent micelles can modulate protein-protein interactions, potentially influencing nucleation mechanism and crystal packing. Additionally, our findings highlight the importance of distinguishing true physiological oligomeric states from detergent-induced associations, as misinterpretations could lead to inaccurate structural models. Ultimately, by carefully controlling detergent conditions, future studies can enhance the success rate of membrane protein crystallization and improve the accuracy of structural interpretations.



**Figure 10.** Crystallization trial of ShuA at 5 mg.mL<sup>-1</sup> in 50 mM Tris pH 7.5, 150 mM NaCl, 0.5 mM DDM using the vapor-diffusion technique (reservoir solution: 35% PEG 300, 100 mM NaCl, 100 mM Na-Citrate pH 5 ; hanging droplet: 1μL ShuA-DDM)

## 4 | MATERIALS and METHODS

### 4.1 | Detergents and protein solutions.

All salt and buffer reagents were purchased from Sigma. Octyl PolyOxyEthylene (OPOE) was purchased from Bachem (Octyl-POE, formula CH<sub>3</sub>(CH<sub>2</sub>)<sub>6</sub>CH<sub>2</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>n</sub>OH, CAS number 27252-75-1; CMC (critical micelle concentration) ≈ 6.6 mM / 0.23 %; MW ≈ 350 g.mol<sup>-1</sup> (Rosenbusch *et al.* 2001)) and n-Dodecyl-β-D-Maltoside (DDM) from Anatrace (CAS number 69227-93-6; CMC ≈ 0.17 mM / 0.0087 %; MW ≈ 510.6 g.mol<sup>-1</sup>). The match-out DDM (moDDM, formula C<sub>24</sub>H<sub>15.8</sub>D<sub>30.2</sub>O<sub>11</sub>; MW ≈ 540.87 g.mol<sup>-1</sup>) used for SANS experiments on DDM-solubilized MPs (Midtgaard *et al.* 2018) was synthesized at the National Deuteration Facility (NDF-ANSTO, Sydney) as described in the supplementary information.

The recombinant ShuA was prepared either in OPOE or DDM-containing buffers, for SEC-MALS and SAS experiments, as described below.

### 4.2 | ShuA preparation.

The ShuA plasmid was a kind gift of Dr K Brillet (Strasbourg). Expression, solubilization and purification in OPOE were done on the basis of Brillet *et al.* (Brillet *et al.* 2009) with some improvements to reduce the number of steps. A new protocol was established with solubilization and purification in DDM.

*ShuA overexpression and crude bacterial membrane fraction preparation.* To increase the amount of ShuA targeted to the outer membrane, overexpression was performed using the strain *E. coli* BL21 (DE3) Gold ΔABCF, in which the genes encoding the most abundant porins (OmpA, OmpC, OmpF and LamB) have been deleted (Meuskens *et al.* 2017). Bacteria

transformed with the pET20b-ShuA plasmid were grown at 30°C in Difco LB Lennox broth supplemented with 0.1 mg.mL<sup>-1</sup> ampicillin. When the OD<sub>600</sub> reached about 0.5, induction was started with 1 mM isopropyl-d-1-thio-galactopyranoside (IPTG) for 3 h at 30°C. At the end of induction (OD<sub>600</sub> around 0.8), bacteria were harvested by centrifugation at 3 500 g and resuspended in 0.5 M sucrose, 50 mM Tris HCl pH 8 buffer at a ratio of 4.5 mL.L<sup>-1</sup> culture. After resuspension of the bacteria, 1 mg.mL<sup>-1</sup> lysozyme and 2.5 U.mL<sup>-1</sup> Benzonase were added for 20 min at 4°C under magnetic stirring. An equal volume of 50 mM Tris-HCl pH 8 and 1.5 mM EDTA was then added and kept under magnetic stirring for 20 min at 4°C. At the end of this step, the spheroplasts were harvested by centrifugation at 3 500 g for 45 min at 4°C. The spheroplasts were resuspended in 0.2 M sucrose, 50 mM Tris-HCl pH 8, 2.5 U.mL<sup>-1</sup> Benzonase, 1% (v/v) PMSF, 1% (v/v) Halt™ protease inhibitor cocktail. Lysis was performed by two passages through a French press (20 000 PSI) and the unbroken material was removed by centrifugation (45 min at 3 500 g, 4°C). The crude membranes were harvested by ultracentrifugation (100 000 g for 90 min at 4°C) and resuspended in 50 mM Tris-HCl pH 8, 1mM EDTA. Finally, a second ultracentrifugation was performed, the crude membrane fraction was resuspended at approximately 20 mg.mL<sup>-1</sup> and the material stored at -80°C after freezing in liquid nitrogen.

**Solubilization and Purification of ShuA in OPOE.** The crude bacterial membrane fraction was solubilized at 5 mg.mL<sup>-1</sup> in 50 mM Tris-HCl pH 8, 1 mM EDTA, 1% (w/v) N-lauroyl-sarcosine for 60 min at 25 °C. Then, an ultracentrifugation at 100 000 g for 60 min was performed and the sedimented fraction was solubilized for 60 min at 25 °C in 50 mM Tris-HCl pH 8, 150 mM NaCl, 20 mM imidazole, 0.1 mM EDTA, 1% (v/v) OPOE. After a last ultracentrifugation at 100 000 g for 60 min at 25°C, the supernatant fraction was used for a one-step purification on Ni-NTA column (from Cytiva). The eluate fraction was dialyzed against 50 mM Tris-HCl pH 8, 150 mM NaCl, 1% (v/v) OPOE to remove imidazole and concentrated to 10 mg.mL<sup>-1</sup> by ultrafiltration using 100 kDa MWCO Vivaspin 6 concentrators and frozen at -80°C for further experiments.

**Exchange of OPOE for DDM.** ShuA at 10 mg.mL<sup>-1</sup> in 1% OPOE was diluted at 0.2 mg.mL<sup>-1</sup> in 50 mM Tris HCl pH 7.5, 1% (w/v) DDM. The solution was then concentrated using 100 kDa MWCO Vivaspin 6 to a volume of 0.1 mL. This step was repeated three times. After exchange, the ShuA-DDM complex was frozen at -80°C at 10 mg.mL<sup>-1</sup>.

**Solubilization and purification of ShuA in DDM.** The same protocol as for OPOE was used with minor modifications. The solubilization with DDM was carried out in 50 mM Tris-HCl pH 8, 150 mM NaCl, 20 mM imidazole, 0.1 mM EDTA, 1.2 % (w/v) DDM for two hours at 25°C. The purification step on Ni-NTA was performed after a 12-fold dilution in 50 mM Tris-HCl pH 8, 150 mM NaCl, 20 mM imidazole to obtain a final concentration of 0.1 % DDM. This step was necessary for the binding of ShuA to the Ni-NTA resin. Finally, the eluted fractions were dialyzed against 20 mM Tris-HCl, pH 8, 100 mM NaCl, 1 mM EDTA, 0.5 mM DDM and concentrated at 10 mg.mL<sup>-1</sup> for further experiments.

### 4.3 | SEC-MALS experiments on ShuA-detergent complexes.

SEC-MALS (*i.e.* size exclusion chromatography coupled to multi-angle light scattering) experiments were carried out on a Shimadzu HPLC-UV system coupled to an Optilab™ T-rEX refractometer and a miniDawn™ TREOS MALS detector (Wyatt Technology). Two types of SEC column connected to a 20 μL-loop were used, *i.e.* either a 5 mL-silica column (BioZen dSEC-2 from Phenomenex) or a 24 mL-superdex column (S200 increase 10/300 from Cytiva). Weight-average molar mass (noted  $M$  or  $M_{app}$ ) of molecules passing through the SEC column are determined without column calibration by measuring at the maximum of the elution peak, their static light scattering at three angles (44, 90, and 136°), knowing their concentration  $c$  from UV and/or refractive index, by using the Zimm equation (Zimm 1948):

$$\frac{Kc}{\Delta R_{\theta}} = \frac{1}{M_{app}} = \frac{1}{M} + 2A_2c \quad (1)$$

with  $K = \frac{4\pi^2 n_0^2}{N_A \lambda^4} \left(\frac{\partial n}{\partial c}\right)^2$  and  $\Delta R_{\theta} = R_{\theta, sample} - R_{\theta, solvent}$

$K$  is an optical constant, which depends on the refractive index (RI) of the buffer ( $n_0$ ), the wavelength  $\lambda$  of the laser light (658 nm in the MiniDawn TREOS and Optilab T-rEX apparatus), the refractive index increment  $\partial n/\partial c$  of the scattering molecule and  $N_A$  the Avogadro's number.  $\theta$  is the angle between the incident and the scattered light.  $\Delta R_{\theta}$  is the excess Rayleigh ratio.  $A_2$  is the second virial coefficient, characterizing pairwise interaction forces between particles (*e.g.* PDC-PDC or detergent inter-micelle interactions), which can influence the value of the expected molar mass to a greater or lesser extent depending on the nature of the interactions. For attractive (*resp.* repulsive) interactions,  $A_2$  is negative (*resp.* positive) and the apparent molar mass ( $M_{app}$ ) will be higher (*resp.* lower) than expected.

For a PDC composed of a protein mass fraction ( $a$ ) and a protein-bound detergent mass fraction ( $b$ ), the concentration and molar mass of the complex are expressed, as a function of the protein concentration and molar mass, by:

$$c_{PDC} = (1 + \delta)c_{MP} \quad (2)$$

$$M_{PDC} = (1 + \delta)M_{MP} \quad (3)$$

with  $\delta = b/a$ , the bound detergent-to-protein mass ratio. Considering that the PDC concentration is similar whatever the detector used (UV or RI), that is to say,  $c_{PDC} = \frac{Abs_{280}}{\epsilon_{PDC}} =$

$\frac{dRI}{\partial n/\partial c_{PDC}}$ , we can determine the mass ratio  $\delta$  and the respective mass fractions  $a$  and  $b$  by the expressions:

$$\delta = \frac{dRI \epsilon_{MP} - Abs_{280} \partial n/\partial c_{MP}}{Abs_{280} \partial n/\partial c_{Det} - dRI \epsilon_{Det}} \quad (4)$$

$$a = \frac{1}{1 + \delta} \text{ and } b = \frac{\delta}{1 + \delta} \quad (5)$$

with  $Abs_{280}$  and  $dRI$  the measured absorbance at 280 nm and the differential refractive index of the complex, respectively, and  $\epsilon_{MP}$ ,  $\epsilon_{Det}$ ,  $(\partial n/\partial c)_{MP}$  and  $(\partial n/\partial c)_{Det}$ , the respective extinction coefficients and RI increments of the protein and the detergent. The value of  $\delta$  is then used to calculate  $\epsilon_{PDC}$  and  $\frac{\partial n}{\partial c_{PDC}}$  by applying the additivity rules for both absorbance and RI increment with:

$$\epsilon_{PDC} = a \epsilon_{MP} + b \epsilon_{Det} \quad (6)$$

and  $\frac{\partial n}{\partial c_{PDC}} = a \left(\frac{\partial n}{\partial c_{MP}}\right) + b \left(\frac{\partial n}{\partial c_{Det}}\right) \quad (7)$

Finally, the inverse of the weight-average PDC molar mass is obtained by extrapolating the Zimm equation at zero angle.

An automatic procedure “*Protein Conjugate analysis*” from Astra (Wyatt Technology) permits the direct calculation of complex, detergent and protein (apparent) molar masses, complex concentration, complex extinction coefficient ( $\epsilon_{\text{PDC}}$ ) and complex RI increment ( $(\partial n/\partial c)_{\text{PDC}}$ ), using for ShuA  $\epsilon^{0.1\%} = 1.664 \text{ L}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}$  at 280 nm and  $\partial n/\partial c = 0.185 \text{ mL}\cdot\text{g}^{-1}$  and for both detergents,  $\epsilon^{0.1\%} \approx 0$  at 280 nm and  $\partial n/\partial c = 0.141 \text{ mL}\cdot\text{g}^{-1}$  for DDM and  $\partial n/\partial c = 0.123 \text{ mL}\cdot\text{g}^{-1}$  for OPOE, measured in their respective mobile phase, using the Optilab T-rEX refractometer. The methodology is thoroughly described in recent articles (Abel *et al.* 2021; Mégret-Cavalier *et al.* 2024; Zuniga *et al.* 2024).

In the case of a monomer/dimer equilibrium (mix) of ShuA-DDM complex, the respective fraction of monomer of complex,  $\phi_{\text{PDCM}}$ , and dimer of complex,  $\phi_{\text{PDCD}}$ , can be calculated from the parameters  $\delta_{\text{PDCmix}}$ ,  $\frac{\partial n}{\partial c_{\text{PDCmix}}}$ ,  $\epsilon_{\text{PDCmix}}$  and  $c_{\text{PDCmix}}$  obtained from the “Protein conjugate” module, knowing the values of  $\epsilon_{\text{MP}}$ ,  $\epsilon_{\text{Det}}$ ,  $\frac{\partial n}{\partial c_{\text{MP}}}$ ,  $\frac{\partial n}{\partial c_{\text{Det}}}$  (See details in SI) with the following equations:

$$\phi_{\text{PDCM}} = \frac{\frac{\epsilon_{\text{PDCmix}} \delta_{\text{PDCmix}}}{\epsilon_{\text{MP}}} - \frac{\delta_{\text{PDCD}}}{1 + \delta_{\text{PDCD}}}}{\frac{\delta_{\text{PDCM}}}{1 + \delta_{\text{PDCM}}} - \frac{\delta_{\text{PDCD}}}{1 + \delta_{\text{PDCD}}}} \quad (8)$$

$$\text{and} \quad \phi_{\text{PDCM}} + \phi_{\text{PDCD}} = 1 \quad (9)$$

#### 4.4 | In-line SEC-SAXS experiments on ShuA-detergent complexes

SAXS experiments on ShuA-detergent complexes (OPOE and DDM) were performed on the SWING beamline at the French synchrotron facility (SOLEIL, St-Aubin, France). With a wavelength of 1.033 Å and a sample-to-detector distance of 2 m, the achievable  $Q$ -range was 0.0036 to 0.5538 Å<sup>-1</sup>, with  $Q=4\pi\sin\theta/\lambda$  and  $2\theta$  the scattering angle. ShuA sample in OPOE or DDM-containing buffer were injected through the same size exclusion columns as used for SEC-MALS on an Agilent HPLC system, which includes an automatic injector (5 – 100 µL), a UV-Vis cell to measure protein concentration at 280 nm and the SAXS flow cell at a flow rate of 300 µL·min<sup>-1</sup>. For data reduction, buffer acquisition was performed at the beginning of the elution profile on the 5 mL-SEC column for 120 sec with 180 frames and sample acquisition through peak elution for 380 sec with 620 frames. When using a 24-mL SEC column, buffer acquisition was for 300 sec with 300 frames and sample acquisition, for 1700 sec with 1033 frames. The 2D-SAXS patterns were normalized to the transmitted intensity and azimuthally averaged by using the Foxtrot program (Girardot *et al.* 2015). Identical frames corresponding to elution buffer signal were averaged to provide a buffer signal for subtraction of individual sample frames. All SAXS analysis were done using BioXTAS RAW, an open-source software (Hopkins 2024). Both forward intensity at  $Q = 0$  ( $I(0)$ ), and radius of gyration ( $R_G$ ) were determined from the Guinier approximation  $I(Q) = I(0)\exp(-Q^2 R_G^2/3)$  at very small angles, assuming that  $QR_G < 1.3$  and then were plotted as a function of sample frames in order to determine ranges of frames where the radius of gyration presents no significant variation. The forward intensity

in absolute units is related to the PDC molar mass by the expression:

$$I(0) = \frac{cM}{N_A} r_0^2 \left( \frac{n_{\text{SA}}}{M} - \rho_s v \right)^2 \quad (10)$$

with  $c$ ,  $M$ ,  $n$ ,  $v$ , respectively, the concentration, the molar mass, the number of electrons and the partial specific volume of the complex,  $N_A$  the Avogadro’s number,  $\rho_s$  the electron scattering length densitie of the solvent and  $r_0$  the classical electron radius equal to  $0.28179 \cdot 10^{-12} \text{ cm/e}$ .

For each injected sample, the averaged-subtracted intensity was normalized to the PDC concentration. A Guinier analysis allowed us to plot  $S(c,0)$ , the structure factor at  $Q = 0$  as a function of PDC concentration,  $c$ , following the expression:

$$S(c, 0) = \frac{I(c,0)}{I(0,0)} = 1 - 2MA_2c \quad (11)$$

$A_2$ , the second virial coefficient, is positive for repulsive interactions and negative for attractive ones and is expressed in mol·mL·g<sup>-2</sup>. Conditions where the second virial coefficient is negative have long been known to favor crystallization of soluble and MPs (Berger *et al.* 2005; George and Wilson 1994; Hitscherich *et al.* 2000).

#### 4.5 | SANS experiments on ShuA-moDDM complex.

The SANS measurements on ShuA in solution with moDDM were performed at the QUOKKA diffractometer (ANSTO, Sydney) and at D22 instrument (ILL, Grenoble, France). SANS measurements at QUOKKA (ANSTO, Sydney) were performed using neutrons with a wavelength of 6.0 Å with a wavelength spread of 10% (FWHM) at two sample-to-detector distances: 12 m and 1.33 m. These settings covered  $q$ -ranges of 0.005-0.0592 Å<sup>-1</sup> and 0.00251-0.4896 Å<sup>-1</sup>, respectively. Samples were placed in Hellma banjo suprasil quartz cuvettes of 1 mm thickness with a beam of 13 mm diameter.

SANS measurements at D22 (ILL, Grenoble) were performed using a wavelength of 6Å ± 10%, a collimation length and central detector distance of 11.2 m and second detector distance of 1.4 m. Samples were placed in Hellma rectangular cuvettes of 1 mm thickness, with a beam of 10 mm x 7 mm.

Data from both Quokka and D22 were reduced using Grasp software (Dewhurst 2023), including transmission and thickness normalization, empty cell and blocked beam subtraction as well as calibration to absolutely scale by normalization with the direct beam intensity.

In both cases, 240 µL of ShuA at *ca.* 10 mg·mL<sup>-1</sup> purified in Tris HCl 20 mM pH 8, NaCl 100 mM, EDTA 1 mM, DDM-H<sub>2</sub>O 0.5 mM was injected on a Superdex 200 increase 10/300 (24 mL column), pre-washed with pure D<sub>2</sub>O and pre-equilibrated with Tris 50 mM, pD 7.5, NaCl 100 mM, moDDM 0.5 mM in 100 % D<sub>2</sub>O and then eluted at 150 µL·min<sup>-1</sup> to ensure optimal exchange of deuterated detergent around ShuA. Eluted fractions of ShuA (300 µL per fraction at ANSTO and 120 µL at ILL) were collected at the maximum of absorbance (*ca.* 2 500 mAU) of the elution peak and the scattering signal measured in a 1 mm thickness quartz cell. For the subsequent data reduction, the buffer flow-through from the column was collected, measured and used for background subtraction.

#### 4.6 | SAXS experiments on ShuA-moDDM/D<sub>2</sub>O complex.

The ShuA-moDDM/D<sub>2</sub>O samples for SAXS experiments were prepared as for the SANS experiments: 200  $\mu\text{L}$  of ShuA at *ca.* 10  $\text{mg}\cdot\text{mL}^{-1}$  in 20 mM Tris HCl pH 8, 100 mM NaCl, 1 mM EDTA, 0.5 mM DDM/H<sub>2</sub>O was injected onto a Superdex 200 increase 10/300, pre-washed with pure D<sub>2</sub>O and pre-equilibrated with 50 mM Tris, pH 7.5, 100 mM NaCl, 0.5 mM moDDM in 100 % D<sub>2</sub>O and then eluted at 150  $\mu\text{L}\cdot\text{min}^{-1}$ . Fractions of 150  $\mu\text{L}$  were collected throughout the whole elution peak. The SAXS measurements on each fraction were performed in batch mode on the bioSAXS beamline BM29 at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). The sample-detector distance was 2.812 m and the X-ray wavelength  $\lambda = 0.992 \text{ \AA}$  leading to a  $Q$ -range of 0.00628 - 0.5209  $\text{\AA}^{-1}$ . The beamline was equipped with a 2D-detector (Pilatus3 2M) and an automated sample changer (Round *et al.* 2015). The sample storage and measurement temperatures were fixed at 20.0°C. To prevent radiation damage during the scattering experiments, data were collected in 10 successive 1-s frames, and the solution was moved in the capillary during exposure. All data manipulations were performed using a standard procedure via the ISPyB interface (Delagenière *et al.* 2011) and the program package PRIMUS (Konarev *et al.* 2003). All SAXS analysis were done using BioXTAS RAW. Forward intensity  $I(0)$  and radius of gyration ( $R_G$ ) of ShuA-moDDM/D<sub>2</sub>O fractions were determined from Guinier analysis. The pair-distance distribution functions (PDDF) and the maximum particle dimensions ( $D_{\text{max}}$ ) were determined by Inverse Fourier Transformation (IFT) of scattering intensity using the program GNOM (Svergun 1992) from the ATSAS package.

#### 4.7 | Crystallization experiment

Crystallization trials were tested with ShuA solubilized with DDM protocol and freshly purified in 50 mM Tris-HCl pH 7.5, 100mM NaCl, 1 mM EDTA, 0.5 mM DDM. The crystallization was performed using the hanging-drop vapor-diffusion method (Linbro plate) by mixing 1  $\mu\text{L}$  of ShuA-DDM at 5  $\text{mg}\cdot\text{mL}^{-1}$  with 1  $\mu\text{L}$  of crystallization solution (35 % PEG 300, 100 mM NaCl, 100 mM Na-citrate pH 5). The reservoir volume was 1 mL and the incubation temperature 20°C. Crystals were observed by optical microscopy (Carl Zeiss SteREO Discovery V12 microscope).

#### Supplementary Information

Two supplementary information files “Supplementary data.docx” and “DDM synthesis and characterization.docx” include additional data (SEC-MALS profiles, PDDF and Kratky plots, detergent SAXS curves, ShuA-moDDM SANS profiles, 3D-structure of ShuA, Tables on SEC-MALS and SAXS analysis) and synthesis of matched-out *n*-dodecyl- $\beta$ -D-maltoside. BM29 SAXS data are available at <https://doi.esrf.fr/10.1515/ESRF-ES-1118672762> and <https://doi.esrf.fr/10.1515/ESRF-ES-1830135339>. D22 SANS data are available here: <https://dx.doi.org/10.5291/ILL-DATA.9-13-1060>.

#### Acknowledgements.

This project received financial support from the CNRS through the MITI interdisciplinary program “Modélisation du

vivant” (2021-2022) and ANR through LABEX DYNAMO (ANR-11-LABX 0011). We acknowledge the framework CRISTECH-CNRS for financial support through an ITC (Initiative au Transfert Technologique) grant. We would like to warmly thank Dr Monika Budayova-Spano and Dr Samuel Hjorth-Jensen for their welcome at IBS (Grenoble, France) for this ITC and for sharing their know-how and expertise in crystallization techniques. We also thank Dr N. Dautin from LBPM at IBPC for the gift of the bacterial strain *E. coli* BL21 (DE3) Gold  $\Delta$ ABCF.

We gratefully acknowledge ESRF (Grenoble, France), SOLEIL (St Aubin, France), ILL (Grenoble, France) large-scale facilities for beamtime allocations on beamlines BM29 (BAG proposals MX-2456 and MX-2652)(Bonneté and Pozza 2027; Bonneté *et al.* 2026), SWING (BAG proposal 20231081), D22 (proposal 9-13-1060)(Bonneté *et al.* 2023), respectively. We acknowledge the support of the Australian Government in provision of access to ANSTO’s National Deuteration Facility which is partly funded through the National Collaborative Research Infrastructure Strategy (NCRIS) via NDF proposal DN13858. We thank local contacts, A Popov and A Calio (BM29), A Thureau (Swing), A Martel and L Porcar (D22) and A Whitten and K Wood (QUOKKA) for their assistance during experiments. We also thank Dr Martina Sandroni, responsible of ILL Chemistry Lab as well as Dr Deborah Wakeham and Dr Anthony Duff (NDF-ANSTO) for technical assistance.

This work benefited from the use of the SasView application, originally developed under NSF award DMR-0520547. SasView contains code developed with funding from the European Union’s Horizon 2020 research and innovation program under the SINE2020 project, grant agreement No 654000.

#### CRedit (Contribution Roles Taxonomy).

F Bonneté: Conceptualization, Project administration, Investigation, Formal analysis, Writing original draft, Review & editing

A Pozza : Investigation, Resources, Formal analysis, writing review

A Martel: Data acquisition and treatment, writing review

T. Darwish and M. Moir: sample preparation, writing review

S. Combet and K. Wimalan: SASbelt modeling, writing review.

A. Koutsioubas: SASbelt modeling.

#### REFERENCES

- Abel S, Marchi M *et al.* (2021) Structural insights into the membrane receptor ShuA in DDM micelles and in a model of gram-negative bacteria outer membrane as seen by SAXS and MD simulations. *BBA Biomembrane*. 1863(2): 183504. DOI: 10.1016/j.bbmem.2020.183504.
- Allen JP (2019) Recent innovations in membrane-protein structural biology. *F1000Research*. 8. DOI: 10.12688/f1000research.16234.1.
- Asakura S and Oosawa F (1958) Interaction between particles suspended in solutions of macromolecules. *J. Polymer Science*. 33(126): 183. DOI: 10.1002/pol.1958.1203312618.
- Barret L-A, Barrot-Ivolot C *et al.* (2013) Influence of Hydrophobic Micelle Structure on Crystallization of the Photosynthetic RC-LHI-PufX Complex from Rhodobacter

- blasticus. *J Phys Chem B*. 117(29): 8770. DOI: 10.1021/jp403483q.
- Berger BW, Gendron CM *et al.* (2005) The role of protein and surfactant interactions in membrane-protein crystallization. *Acta Cryst D*. 61: 724. DOI: 10.1107/S0907444904029063.
- Birch J, Cheruvara H *et al.* (2020) Changes in Membrane Protein Structural Biology. *Biology*. 9(11): 401. DOI: 10.3390/biology9110401.
- Bonneté F, Combet S *et al.* (2023) SASMod: a new methodological approach for ShuA-DDM complex structure modeling. ILL, Grenoble, France. DOI: 10.5291/ILL-DATA.9-13-1060.
- Bonneté F, Finet S *et al.* (1999) Second virial coefficient: variations with lysozyme crystallization conditions. *J. Crystal Growth*. 196: 403. DOI: 10.1016/S0022-0248(98)00826-4.
- Bonneté F and Pozza A (2027) From structure to drug design. ESRF, Grenoble, France. DOI: 10.15151/ESRF-ES-1830135339.
- Bonneté F, Pozza A *et al.* (2026) From structure to drug design. ESRF, Grenoble, France. DOI: 10.15151/ESRF-ES-1118672762.
- Breyton C, Tribet C *et al.* (1997) Dimer to monomer conversion of the cytochrome b6 f complex. Causes and consequences. *J Biol Chem*. 272(35): 21892. DOI: 10.1074/jbc.272.35.21892.
- Brillet K, Meksem A *et al.* (2009) Expression, purification, crystallization and preliminary X-ray diffraction analysis of the TonB-dependent haem outer membrane transporter ShuA from *Shigella dysenteriae*. *Acta Cryst F*. 65(Pt 4): 402. DOI: 10.1107/S1744309109008148.
- Choy BC, Cater RJ *et al.* (2021) A 10-year meta-analysis of membrane protein structural biology: Detergents, membrane mimetics, and structure determination techniques. *BBA Biomembrane*. 1863(3): 183533. DOI: 10.1016/j.bbamem.2020.183533.
- Cleveland TE, He W *et al.* (2018) Small-angle X-ray and neutron scattering demonstrates that cell-free expression produces properly formed disc-shaped nanolipoprotein particles. *Protein Sci*. 27(3): 780. DOI: 10.1002/pro.3365.
- Cobessi D, Meksem A *et al.* (2010) Structure of the heme/hemoglobin outer membrane receptor ShuA from *Shigella dysenteriae*: Heme binding by an induced fit mechanism. *Proteins-Structure Function and Bioinformatics*. 78(2): 286. DOI: 10.1002/prot.22539.
- Combet S, Bonneté F *et al.* (2023) Effect of amphiphilic environment on the solution structure of mouse TSPO translocator protein. *Biochimie*. 205: 61. DOI: 10.1016/j.biochi.2022.11.014.
- Delagenière S, Brenchereau P *et al.* (2011) ISPyB: an Information Management System for Synchrotron Macromolecular Crystallography. *Bioinformatics*. DOI: 10.1093/bioinformatics/btr535.
- Dewhurst CD (2023) Graphical reduction and analysis small-angle neutron scattering program: GRASP. *J Appl Cryst*. 56(5): 1595. DOI: 10.1107/s1600576723007379.
- Fisher LE, Engelman DM *et al.* (2003) Effect of Detergents on the Association of the Glycophorin A Transmembrane Helix. *Biophysical J*. 85(5): 3097. DOI: 10.1016/S0006-3495(03)74728-6.
- Fleming KG (2002) Standardizing the free energy change of transmembrane helix-helix interactions. *J Mol Biol*. 323(3): 563. DOI: 10.1016/s0022-2836(02)00920-8.
- Franke D and Svergun DI (2009) DAMMIF, a program for rapid ab-initio shape determination in small-angle scattering. *JApplied Cryst*. 42(2): 342. DOI: doi:10.1107/S0021889809000338.
- Gelbart WM, Ben-Shaul A *et al.* (1984) Micellar growth due to interaggregate interactions. *The Journal of Physical Chemistry*. 88(5): 861. DOI: 10.1021/j150649a008.
- George A and Wilson WW (1994) Predicting protein crystallization from a dilute solution property. *Acta Cryst*. D. 50: 361. DOI: 10.1107/S0907444994001216.
- Girardot R, Viguier G *et al.* (2015). FOXTROT: A JAVA-Based Application to Reduce and Analyse SAXS and WAXS Piles of 2D Data at Synchrotron Soleil. canSAS-VIII, Tokai, Japan.
- Giubertoni G, Bonn M *et al.* (2023) D<sub>2</sub>O as an Imperfect Replacement for H<sub>2</sub>O: Problem or Opportunity for Protein Research? *J Phys Chem B*. 127(38): 8086. DOI: 10.1021/acs.jpcc.3c04385.
- Glatter O and Kratky O (1982) *Small Angle X-ray Scattering*. London, New York, Academic Press. ISBN: 978-0-12-286280-9.
- Graewert MA, Da Vela S *et al.* (2020) Adding Size Exclusion Chromatography (SEC) and Light Scattering (LS) Devices to Obtain High-Quality Small Angle X-Ray Scattering (SAXS) Data. *Crystals*. 10(11): 975. DOI: 10.3390/cryst10110975.
- Grant TD (2018) Ab initio electron density determination directly from solution scattering data. *Nature Methods*. 15(3): 191. DOI: 10.1038/nmeth.4581.
- Guo Y (2020) Be Cautious with Crystal Structures of Membrane Proteins or Complexes Prepared in Detergents. *Crystals*. 10(2): 86. DOI: 10.3390/cryst10020086.
- Haidar Y and Konermann L (2023) Effects of Hydrogen/Deuterium Exchange on Protein Stability in Solution and in the Gas Phase. *J. Am. Soc. for Mass Spectrometry*. 34(7): 1447. DOI: 10.1021/jasms.3c00130.
- Healey RD, Basu S *et al.* (2021) An automated platform for structural analysis of membrane proteins through serial crystallography. *Cell Reports Methods*. 1(6): 100102. DOI: 10.1016/j.crmeth.2021.100102.
- Heuberger EH, Veenhoff LM *et al.* (2002) Oligomeric state of membrane transport proteins analyzed with blue native electrophoresis and analytical ultracentrifugation. *J Mol Biol*. 317(4): 591. DOI: 10.1006/jmbi.2002.5416.
- Hitscherich C, Kaplan J *et al.* (2000) Static light scattering studies of OmpF porin: Implications for integral membrane protein crystallization. *Protein Sci*. 9(8): 1559. DOI: 10.1110/ps.9.8.1559.
- Hopkins J (2024) BioXTAS RAW 2: new developments for a free open-source program for small-angle scattering data reduction and analysis. *J. Applied Cryst*. 57(1): 194. DOI: 10.1107/S1600576723011019.
- Jia Y, Narayanan J *et al.* (2005) Investigation on the mechanism of crystallization of soluble protein in the presence of nonionic surfactant. *Biophysical J*. 89(6): 4245. DOI: 10.1529/biophysj.105.066449.

- Kaufmann TC, Engel A *et al.* (2006) A novel method for detergent concentration determination. *Biophysical J.* 90(1): 310. DOI: 10.1529/biophysj.105.070193.
- Konarev PV, Volkov VV *et al.* (2003) PRIMUS: a Windows PC-based system for small-angle scattering data analysis. *J. Appl. Crystallogr.* 36: 1277.
- Koutsioubas A, Jaksch S *et al.* (2016) DENFERT version 2: extension of ab initio structural modelling of hydrated biomolecules to the case of small-angle neutron scattering data. *J. Applied Cryst.* 49(2): 690. DOI: 10.1107/S1600576716003393.
- Kulkarni AM, Chatterjee AP *et al.* (2000) Effects of polyethylene glycol on protein interactions. *J. Chem. Phys.* 113(21): 9863. DOI: 10.1063/1.1321042.
- Lee S, Mao A *et al.* (2016) How Do Short Chain Nonionic Detergents Destabilize G-Protein-Coupled Receptors? *J. Am. Chem. Society.* 138(47): 15425. DOI: 10.1021/jacs.6b08742.
- Marenduzzo D, Finan K *et al.* (2006) The depletion attraction: an underappreciated force driving cellular organization. *J Cell Biol.* 175(5): 681. DOI: 10.1083/jcb.200609066.
- Mégret-Cavalier M, Pozza A *et al.* (2024) Starting with an Integral Membrane Protein Project for Structural Biology: Production, Purification, Detergent Quantification, and Buffer Optimization-Case Study of the Exporter CntI from *Pseudomonas aeruginosa*. *Bacterial Secretion Systems (MIMB)*, Springer Natue. DOI: 10.1007/978-1-0716-3445-5\_26. ISBN: 1064-3745.
- Meuskens I, Michalik M *et al.* (2017) A New Strain Collection for Improved Expression of Outer Membrane Proteins. *Front Cell Infect Microbiol.* 7: 464. DOI: 10.3389/fcimb.2017.00464.
- Midtgaard SR, Darwish TA *et al.* (2018) Invisible detergents for structure determination of membrane proteins by small-angle neutron scattering. *FEBS.* 285(2): 357. DOI: 10.1111/febs.14345.
- Molodenskiy DS, Mertens HDT *et al.* (2020) An automated data processing and analysis pipeline for transmembrane proteins in detergent solutions. *Sci Rep.* 10(1): 8081. DOI: 10.1038/s41598-020-64933-1.
- Newstead S, Ferrandon S *et al.* (2008) Rationalizing  $\alpha$ -helical membrane protein crystallization. *Protein Sci.* 17(3): 466. DOI: 10.1110/ps.073263108.
- Perez J and Koutsioubas A (2015) Memprot: a program to model the detergent corona around a membrane protein based on SEC-SAXS data. *Acta Cryst D.* 71: 86. DOI: 10.1107/s1399004714016678.
- Polidori A, Raynal S *et al.* (2016) Sparingly fluorinated maltoside-based surfactants for membrane-protein stabilization. *New J Chem.* 40: 5364. DOI: 10.1039/C5NJ03502C.
- Ray D, Kumar S *et al.* (2018) Tuning Nanoparticle–Micelle Interactions and Resultant Phase Behavior. *Langmuir.* 34(1): 259. DOI: 10.1021/acs.langmuir.7b03429.
- Reslan M and Kayser V (2018) The effect of deuterium oxide on the conformational stability and aggregation of bovine serum albumin. *Pharm Dev Technol.* 23(10): 1030. DOI: 10.1080/10837450.2016.1268157.
- Rosenbusch JP, Lustig A *et al.* (2001) Approaches to determining membrane protein structures to high resolution: do selections of subpopulations occur? *Micron.* 32(1): 75. DOI: 10.1016/S0968-4328(00)00021-4.
- Round A, Felisaz F *et al.* (2015) BioSAXS Sample Changer: a robotic sample changer for rapid and reliable high-throughput X-ray solution scattering experiments. *Acta Cryst D.* 71(1): 67. DOI: 10.1107/s1399004714026959.
- Spinozzi F, Gazzillo D *et al.* (2002) Interaction of Proteins in Solution from Small-Angle Scattering: A Perturbative Approach. *Biophysical J.* 82(4): 2165. DOI: 10.1016/S0006-3495(02)75563-X.
- Stetsenko A and Guskov A (2017) An Overview of the Top Ten Detergents Used for Membrane Protein Crystallization. *Crystals.* 7(7). DOI: 10.3390/cryst7070197.
- Sumner J and Qian S (2022) DENSS-multiple: A structure reconstruction method using contrast variation of small-angle neutron scattering based on the DENSS algorithm. *BBA Advances.* 2: 100063. DOI: 10.1016/j.bbadv.2022.100063.
- Sun J, Kulandaisamy A *et al.* (2023) Machine learning in computational modelling of membrane protein sequences and structures: From methodologies to applications. *Comput Struct Biotechnol J.* 21: 1205. DOI: 10.1016/j.csbj.2023.01.036.
- Svergun DI (1992) Determination of the Regularization Parameter in Indirect-Transform Methods Using Perceptual Criteria. *J. Appl. Crystallogr.* 25: 495.
- Svergun DI (1999) Restoring Low Resolution Structure of Biological Macromolecules from Solution Scattering Using Simulated Annealing. *Biophysical J.* 76(6): 2879. DOI: 10.1016/S0006-3495(99)77443-6.
- Svergun DI, Richard S *et al.* (1998) Protein hydration in solution: experimental observation by x-ray and neutron scattering. *PNAS.* 95(5): 2267. DOI: 10.1073/pnas.95.5.2267.
- Tamm LK, Hong H *et al.* (2004) Folding and assembly of  $\beta$ -barrel membrane proteins. *BBA Biomembrane.* 1666(1): 250. DOI: 10.1016/j.bbamem.2004.06.011.
- Tanford C (1980) The hydrophobic effect: Formation of micelles and biological membranes. New York, John Wiley & Sons, Inc. ISBN: 1543-0472.
- Tate CG (2010) Practical considerations of membrane protein instability during purification and crystallisation. *Heterologous Expression of Membrane Proteins Springer Nature.* Chapter 12. DOI: 10.1007/978-1-60761-344-2\_12. ISBN: 978-1-4939-6132-0.
- Thangaratnarajah C, Rheinberger J *et al.* (2022) Cryo-EM studies of membrane proteins at 200 keV. *Curr Opin Struct Biol.* 76: 102440. DOI: 10.1016/j.sbi.2022.102440.
- Urner LH, Junge F *et al.* (2023) Rationalizing the Optimization of Detergents for Membrane Protein Purification. *Chemistry.* 29(30): e202300159. DOI: 10.1002/chem.202300159.
- Valadez-Perez N, Liu Y *et al.* (2021) Cluster Morphology of Colloidal Systems with Competing Interactions. *Frontiers of Physics.* 9. DOI: 10.3389/fphy.2021.637138.
- Volkov VV and Svergun DI (2003) Uniqueness of ab initio shape determination in small-angle scattering. *J. Applied Cryst.* 36(3 Part 1): 860. DOI: 10.1107/S0021889803000268.

- Wolfe AJ, Parella KJ *et al.* (2019) High-Throughput Screening of Protein-Detergent Complexes Using Fluorescence Polarization Spectroscopy. *Current Protocols in Protein Science*. 97(1): e96. DOI: 10.1002/cpps.96.
- Woubshete M, Cioccolo S *et al.* (2024) Advances in Membrane Mimetic Systems for Manipulation and Analysis of Membrane Proteins: Detergents, Polymers, Lipids and Scaffolds. *ChemPlusChem*. 89(6): e202300678. DOI: 10.1002/cplu.202300678.
- Yang Z, Wang C *et al.* (2014) Membrane protein stability can be compromised by detergent interactions with the extramembranous soluble domains. *Protein Sci*. 23(6): 769. DOI: 10.1002/pro.2460.
- Yoon S, Bae HE *et al.* (2024) Rational Approach to Improve Detergent Efficacy for Membrane Protein Stabilization. *Bioconjugate Chemistry*. 35(2): 223. DOI: 10.1021/acs.bioconjchem.3c00507.
- Zampieri V, Hilpert C *et al.* (2021) The Det.Belt Server: A Tool to Visualize and Estimate Amphipathic Solvent Belts around Membrane Proteins. *Membranes*. 11(7). DOI: 10.3390/membranes11070459.
- Zhang F, Roosen-Runge F *et al.* (2012) The role of cluster formation and metastable liquid—liquid phase separation in protein crystallization. *Faraday Discussions*. 159(0): 313. DOI: 10.1039/C2FD20021J.
- Zhang F, Skoda MWA *et al.* (2007) Protein Interactions Studied by SAXS: Effect of Ionic Strength and Protein Concentration for BSA in Aqueous Solutions. *The Journal of Physical Chemistry B*. 111(1): 251. DOI: 10.1021/jp0649955.
- Zimm BH (1948) The Scattering of Light and the Radial Distribution Function of High Polymer Solutions. *J. Chem. Physics*. 16(12): 1093. DOI: 10.1063/1.1746738.
- Zuniga D, Zoumpoulakis A *et al.* (2024) Biochemical, biophysical, and structural investigations of two mutants (C154Y and R312H) of the human Kir2.1 channel involved in the Andersen-Tawil syndrome. *FASEB Journal*. 38: e70146. DOI: 10.1096/fj.202401567R.