Experimental report

25/04/2024

Proposal: 9-13-1060 **Council:** 10/2022

Title: SASMod: a new methodological approach for ShuA-DDM complex structure modeling

Research area: Biology

This proposal is a continuation of 9-13-984

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Samples: ShuA

Instrument	Requested days	Allocated days	From	To
D22	2	1	05/06/2023	06/06/2023

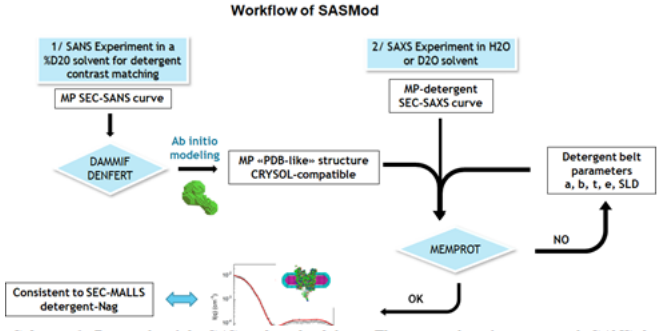
Abstract:

Structural studies of membrane proteins (MPs) are a challenge in biology because they highly depend on the amphiphilic environment (e.g. detergents) hat allow MP stabilization and proper folding. The description of the detergent behavior around a MP (corona steric hindrance, specific interactions with MP) could be useful to select the suitable detergents for MP biochemistry and structural biology. Up to now, detergent modeling was made possible from SAXS data only using the MP atomic 3D-structure. The aim of our project is to propose a methodology when no MP 3D structure is available.
We propose to make a proof of principle with ShuA-DDM complex. Based on SEC-SANS measurements, by contrast matching DDM, we will extract an ab initio low-resolution envelop for ShuA alone. With additional SEC-SAXS data of the whole complex, we will adapt MEMPROT or use molecular dynamics simulations to model the detergent corona around ShuA ansd will compare with results from Abel et al BBA 2021.

Proposal 9-13-1060: Experimental Report
SASMod: a new methodological approach for ShuA-DDM complex structure modeling
D22 - Anne Martel (LC)
05-06/06/2023

Structural studies of membrane proteins (MPs) are a challenge in structural biology because of both the necessity of using amphiphilic molecules to maintain them stable for experiments by NMR, crystallography (XRD), or cryo-EM and the absence of rules to choose suitable amphiphilic molecules. Molecular modeling has been developed (MemProt [1] or molecular dynamics [2]) to describe the detergent corona around a membrane protein, but their use needs the knowledge of the atomic structure of the MP [3, 4].

Our aim was to develop a new methodology to describe the amphiphilic corona even when the 3D-structure of the MP is unknown, by using the complementary of SAXS and SANS methods in combination with molecular modeling. The idea was to use the low-resolution MP envelope obtained from SANS by contrast matching the detergent and adapting the MemProt program to use this MP low-resolution model with SAXS or SANS curves of the whole MP-detergent complex (as described in schema 1).



Schema 1. *Principle of the SASmod methodology. The principle is first to couple SANS data and ab initio modeling (e.g. DAMMIF) to obtain a low resolution structure of the PM only (step 1). This envelope is then modified to be compatible with softwares such as CRYSOL ("PDB-like") to be used with SEC-SAXS data in MEMPROT-type software (hybrid ab initio/all-atom simulations), to obtain structural information of both the MP and its detergent belt (step 2).*

In our previous proposal 9-13-984, we studied ShuA, a stable beta-barrel model protein of 70 kDa, whose 3D-structure (3FHH.pdb) was obtained by XRD from crystals of the protein in octyl glucoside (OG) [5, 6]. We performed SEC-SANS experiments on the diffractometer D22, using 2 configurations (6 Å -1.4 m & 6 Å-5.6 m) simultaneously.

Shua was expressed in *E coli* and then solubilized and purified in octyl polyoxyethylene glycol (OPOE). The OPOE was exchanged with hOG, d_{24}-OG, or d_{inv}-OG (the "invisible" OG synthesized in ANSTO D-lab facility, Sydney) in H_2O buffer. The exchanges with appropriate D_2O:H_2O ratios were performed directly on a small size-exclusion (SEC) column (S200 Increase 5/150, 3.2mL).

3 samples were injected at about 10-15 mg/mL and eluted with 50 mM Tris, pH (ou pD) 8 with OG at 25 mM:

- ShuA + hOG 1.4% in 100% D_2O buffer to measure the whole ShuA-OG complex
- ShuA + d_{inv}-OG 1.4% in 100% D_2O buffer to measure only ShuA was expressed in E coli, solu
- ShuA + d_{24}-OG 1.4% in 45% D_2O buffer to measure specifically the OG corona around ShuA

Unfortunately we did not obtain the expected results. Detergent exchanges between hydrogenated (hOG) and deuterated detergents (d_{24}OG and d_{inv}OG) and solvent exchange from H_2O to D_2O were not optimized to obtain SANS curves of ShuA compatible with its crystallographic structure (3FHH.pdb).

A new experiment with ShuA-d_{inv}DDM (detergent from ANSTO) was performed (proposal 9-13-1060) to compare with results from Abel *et al*. (2021) [3].

Results

A new protocol of preparation was used: ShuA was expressed in *E coli*, solubilized and purified directly with hDDM. Exchange of hDDM to invDDM in100% D_2O buffer was done on a large SEC column and fractions at the maximum OD@280nm were collected. Reciprocal space (I0, R_G_Guinier) and real space (D_{max}, R_G_Gnom) analysis were done using Primus (from ATSAS) or BioXtas Raw. R_G and D_{max} (65 Å and 198 Å) of ShuA-invDDM suggest a large object compared to ShuA monomer from pdb file (R_G 24 Å).

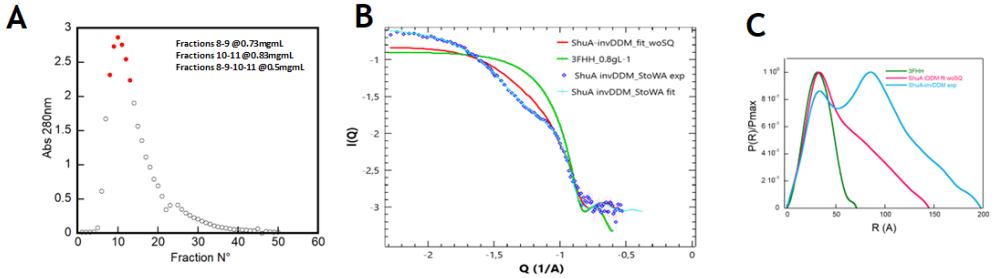
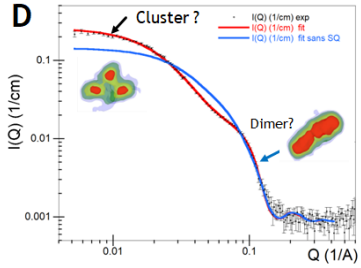


Figure A: *Chromatogram of ShuA in invDDM on S200;* ***Figure B****: SANS curve for the highest fraction (blue dot) compared with atomic structure 3FHH (green line).* ***Figure C:*** *Pair distance distribution function of ShuA-invDDM (blue) compared to 3FHH 'green)*

From these experimental results, it appears that ShuA in invDDM does not correspond to ShuA monomer from the atomic structure. Both deuterated detergent and D2O have an effect on the association state of the protein. A fitting

model from SasView was used to account for SANS data, *i.e.* a cylinder form factor and a sticky hard sphere structure factor. From the model form factor without structure factor (blue line in Fig D), we could calculate the 3D electron density and visualize a dimer of ShuA-invDDM.



	Guinier analysis		Sasview modeling
At 0.8 mg/mL	R_G, D_{max} (Å)	I0 (cm^{-1})	Cylinder (R, H) (Å)
ShuA 3FHH	24.9 ; 72 V_{por} 114930 $Å^3$	0.042	25 ; 59
ShuA-invDDM	64,9 ; 198 V_{por} 348104 $Å^3$	0.235	23 ; 150 V_{por} 180385 $Å^3$

Complementary experiments using SEC-SAXS and SEC-MALLS in hDDM and invDDM in H2O and D2O are still in progress to thoroughly describe the behavior of ShuA in DDM.

References

[1] Perez and Koutsioubas (2015), Acta Crystallographica D, https://doi.org/10.1107/s1399004714016678
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