Experimental report

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Proposal: 9-13-984 **Council:** 10/2020

Title: New methodological approach for MP/detergent structure modeling

Research area: Biology

This proposal is a new proposal

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Samples: ShuA
OG
dOG

Instrument	Requested days	Allocated days	From	To
D22	2	2	15/06/2021	17/06/2021

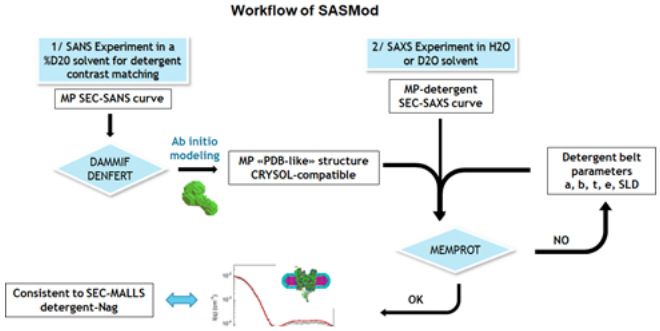
Abstract:

The structural study of membrane proteins (MPs) is a challenge in biology because it is highly dependent on the amphiphilic environment (e.g. detergents) allowing MP stabilization and crystallization, especially in crystallography. The description of the detergent behavior around a MP (corona steric hindrance, specific interactions with MPs) could be useful to select the suitable detergents for MP biochemistry and structural biology.
Up to now, detergent modeling is possible from SAXS data only using the MP atomic 3D-structure. The aim of our project is to propose a new methodological approach whe the MP atomic structure is unknown, which is generally the case. We propose to make a proof of principle on the ShuA/OG complex. Based on SEC-SANS measurements at multiple contrasts of the complex, we will be able, (i) to run DANVILLE and MONSA in order to get a low-resolution model of the whole complex; (ii) by contrast matching OG, to extract an ab initio low-resolution envelop for ShuA alone. These two strategies should allow us, with additional SEC-SAXS data of the whole complex, to use MEMPROT and/or molecular dynamics simulations to model the detergent corona around any MP structure

Proposal 9-13-984: Experimental Report
New methodological approach for MP/detergent structure modeling
D22 - Anne Martel (LC)
15-17/06/2021

Structural studies of membrane proteins (MPs) are still 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 is 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 is 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 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 purified in octyl polytoxyethylene glycol (OPOE) and exchanged with hOG, d_{24}-OG, or d_{inv}-OG (the "invisible" OG synthesized in ANSTO D-lab facility) in H_2O buffer. The exchanges with appropriate D_2O:H_2O ratios were performed directly on the size-exclusion (SEC) column (S200 Increase 5/150).

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
- ShuA + d_{24}-OG 1.4% in 45% D_2O buffer to measure specifically the OG corona around ShuA

Because of the high CMC (critical micelle concentration) of OG and the high costs of d_{24}-OG and d_{inv}-OG, we used a small SEC column of 3.2mL. The SEC environment on D22 is supposed to separate the protein-detergent complex from both aggregates and protein-free detergent micelles to enable the perfect subtraction of the solvent.

- ***1st injection of ShuA-hOG in 100% D_2O***

30 µL of ShuA-hOG-H_2O at 16 mg/mL was injected on the column and eluted with the solvent containing h-OG in 100% D_2O. The exchange H_2O to D_2O was achieved during the elution. We observed a single pic corresponding to ShuA-hOG (Figure 1, left). The SANS curve obtained after solvent subtraction and averaging of the frames (30 sec *per* frame) where ShuA-R_G is stable is plotted on Figure 1 (right).

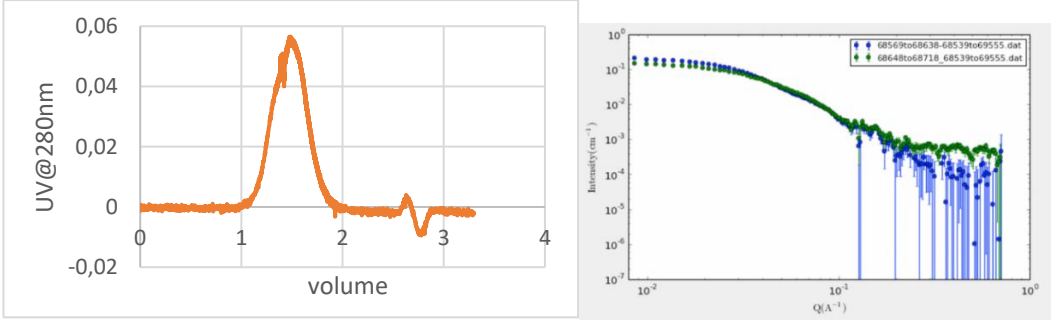


Figure 1*: (Left) Chromatogram of ShuA-hOG eluted on Superdex 200i 5/150 (3.2 mL) column (Tris buffer, 25mM hOG-100%D2O, pD 8); (right) SANS profil of ShuA-hOG in 100% D_2O buffer.*

Two zones in the scattergram have been studied: files from #68569 to 68638 and #68548 to 68718, which give for ShuA-hOG complex, respectively, R_G = 54 ± 2 Å; D_{max} = 182 Å and R_G = 48 ± 1 Å; D_{max} = 162 Å. These values are larger than the ones obtained from SEC-SAXS (R_G = 34 Å; D_{max} = 125 Å). This discrepancy suggests an aggregation of ShuA-hOG during buffer exchange.

- ***2nd injection of ShuA-d24OG in 45% D2O***

Two samples were injected on the column after equilibration with d_{24}OG-45% D_2O buffer (Figure 2). The first injection failed and the second one did not give any scattering signal: both signal of ShuA and detergent corona seem to be matched at 45% D_2O, suggesting a problem with the exchange from OPOE to d_{24}OG detergents that could produce a mixed OPOE/ d_{24}OG detergent corona.

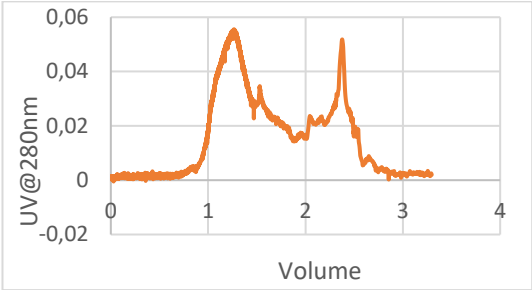


Figure 2: *Chromatogram of ShuA-d_{24}OG eluted on S200i 5/150 (3.2 mL) column (Tris buffer, 25 mM d_{24}OG -45% D_2O, pD 8).*

- ***3rd injection of ShuA-dinvOG in 100% D2O***

Three samples were injected on the column after equilibration with d_{inv}OG-100% D_2O buffer,. As for d_{24}OG, the first injection failed probably due to a too small equilibration volume (1.5 volume column, CV) before sample injection. After a new equilibration with 15 CV, an elution profile was obtained. The ShuA-d_{inv}OG complex in 100% D_2O eluted later than ShuA-hOG complex (Figure 3, left). The chromatogram was analyzed in two zones (files #68871-6894 and #69126-691716). Figure 3 (right) shows a profile more characteristic of an elongated complex/aggregate as compared to the SANS curve obtained with Cryson software (ATSAS) using the pdb file of ShuA (3FHH). The 3rd injection also failed.

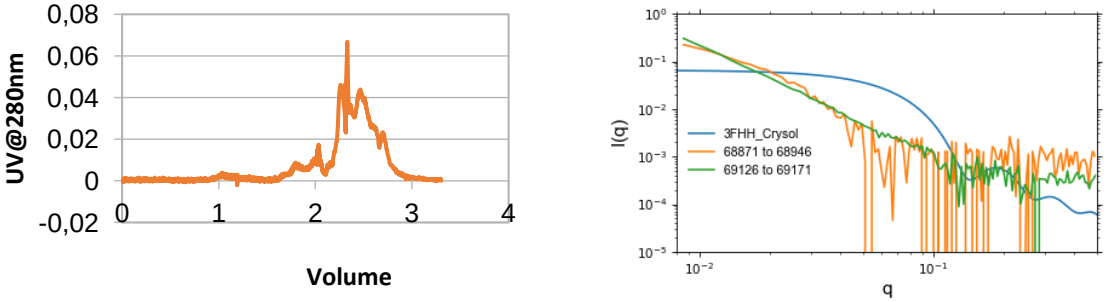


Figure 3*: (Left) Chromatogram of ShuA-d_{inv}OG eluted on Superdex 200i 5/150 (3.2 mL) column (Tris buffer, 25 mM d_{inv}OG-100% D_2O, pD 8); (right) SANS profil of ShuA-d_{inv}OG in 100% D_2O buffer compared with Cryson curve from 3D structure of ShuA (3FHH.pdb).*

Conclusions & Perspectives

In order to save the expensive detergent d_{inv}OG), we used a SEC column of low volume (3.2 mL, 5/150 mm), which did not allow us to obtain the expected results. Detergent exchanges between hydrogenated (hOG) and deuterated detergents (d_{24}OG and d_{inv}OG) and solvent exchange (CV) from H_2O to D_2O were not optimized to obtain SANS curves of ShuA compatible with its crystallographic structure (3FHH.pdb).

This experience has taught us that the exchange of detergents and solvents are dynamic and kinetic mechanisms and that they cannot be done on too small volumes. Also, a knowledge of the CMCs as a function of temperature is necessary to optimize the preparation (in cold room) of the samples to be injected on column. SEC-SANS experiments have to be adapted as a function of the MP studied and the detergent used.

A next experiment with ShuA-d_{inv}DDM (cheaper detergent from ANSTO) is envisaged to compare with results from Abel *et al*. (2021) [3].

References

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