Experimental report

13/04/2022

Proposal: 8-03-1018 **Council:** 4/2020

Title: Structural studies of integral membrane proteins in Salipro stealth carriers

Research area: Biology

This proposal is a continuation of 8-03-969

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Samples: MsbA
saposin A
YddA

Instrument	Requested days	Allocated days	From	To
D11	2	2	18/05/2021	20/05/2021

Abstract:

Reconstitution of integral membrane proteins (IMPs) in nanodiscs or Salipro (saposin-lipid-protein) complexes allows low-resolution structural investigation of membrane proteins in a native-like environment. In order to fully contrast-match-out the nanodisc carriers, we have recently incorporated our IMP targets in stealth carriers consisting of deuterated MSP1 belt protein and deuterated lipids. This allowed the structural characterization of our IMPs of interest in a lipid environment without the often-dominating scattering contribution of the nanodisc (Josts et al, 2018, Structure)(Nitsche et al, 2018, Communications Biology). We now further plan to adapt the stealth carrier system to the Salipro system by selectively deuterating saposin A and thus being able to contrast match out the scaffold proteins/lipids also in the Salipro system. These SANS data shall then be combined with cryo-EM data on the identical samples.

Experimental report for proposal 8-03-1018

Most integral membrane proteins (IMPs) lose their natural folding and activity in the absence of a lipid environment. Reconstitution of IMPs in Salipro nanoparticles (saposin-lipid-protein) complexes can overcome these obstacles and allow low-resolution structural investigation of membrane proteins in a native-like environment. The Salipro system uses the lipid-binding protein Saposin A (SapA) to form a discoidal disc to provide the IMP incorporated in a lipidic environment.

We incorporated the ABC transporter MsbA into fractional deuterium labelled 'stealth Salipro' particles which are effectively invisible to low-resolution neutron diffraction. We acquired SANS data of MsbA in the stealth Salipro system trapped in distinct conformational states (apo vs ADP+vanadate). Orthovanadate is used to mimic the transition state after ATP hydrolysis by trapping the Mg^{2+}-ADP-Vi complex in the catalytic site. Additionally, we performed a solvent contrast-variation experiment by measuring MsbA in fractionally deuterated and hydrogenated Salipro nanoparticles (combination of labelled and unlabelled SapA and lipids) in buffers with different D_2O levels.

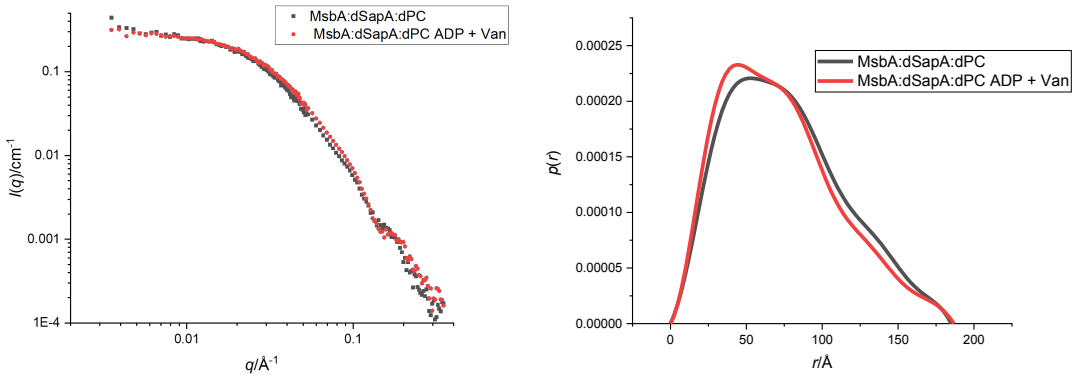

Fig. 1: SANS scattering data of MsbA incorporated in stealth Salipro nanoparticles comparing the apo (black) and ADP-Vi trapped state (red). Left: Scattering shows minor differences in the mid-q range. Right: Distance distribution plots indicating structural differences between the two states of MsbA. SANS data were measured at D11 (ILL).