Experimental report

19/08/2019

Proposal: 8-03-969 **Council:** 10/2018

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

Research area: Biology

This proposal is a continuation of 8-03-894

Main proposer: Henning TIDOW

Experimental team: Dominique Maurice KEHLENBECK
Trevor FORSYTH

Local contacts: Trevor FORSYTH

Samples: MsbA
PMCA
saposin A

Instrument	Requested days	Allocated days	From	To
D11	2	0		
D22	2	1	20/07/2019	21/07/2019

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). 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.

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Structural studies of integral membrane proteins (IMPs) are challenging, as most of them are inactive or insoluble in the absence of a lipid environment. Reconstitution of IMPs in nanodiscs or Salipro (saposin-lipid-protein) complexes can overcome these obstacles and allow low-resolution structural investigation in membrane proteins in a native-like solution environment. Nanodiscs are lipid domains encased within an apolipoprotein A1-derivative scaffold protein. Salipros are saposin-lipoprotein particles that can also be exploited as scaffold protein to reconstitute integral membrane proteins in a lipid environment.

Within this proposal, we generated fractionally deuterium labelled 'stealth Salipro' particles consisting of deuterated phopatidylcholine and deuterated saposin A that are effectively invisible to low-resolution neutron diffraction. We incorporated the ABC transporter MsbA as model protein into these carrier systems and acquired SANS data on 'empty' stealth Salipro as well as on the complex with incorporated IMP.

The SANS data allow for a direct observation of the signal from the solubilized protein without contribution from the surrounding Salipro particle (Fig. 1). In the future, we plan to extend these proof-of-priciple studies to invstigate the structure of MsbA in different conformational states (trapped by nucleotide/anologs) and combine these SANS data with cryo-EM data acquired on the same samples.

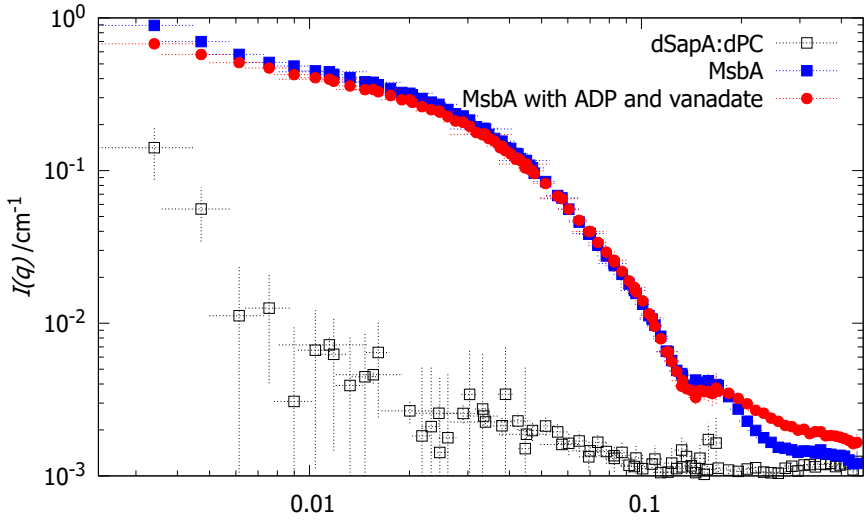

Fig. 1: SANS scattering data of an ABC transporter (MsbA) incorporated in stealth Salipro. The IMP contribution can be clearly distinguished from the negligible scattering of the empty stealth Salipro particles (SapA:dPC). SANS data were measured at D22 (ILL).