Experimental report

28/01/2018

Proposal: 8-03-894 **Council:** 10/2016

Title: Structural studies of integral membrane proteins in stealth carrier nanodiscs

Research area: Biology

This proposal is a new proposal

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Samples: nanodiscs
AcrB
MsbA
PMCA

Instrument	Requested days	Allocated days	From	To
D11	3	2	06/03/2017	08/03/2017

Abstract:

Integral membrane proteins (IMPs) are usually extracted from their native lipid environment by detergent solubilisation in order to be used for structural studies. SAXS and SANS studies of membrane proteins in detergent micelles are problematic due to the dominating contribution of detergent and background scattering of empty micelles. Reconstitution of IMPs in nanodiscs can overcome these obstacles and allow low-resolution structural investigation in membrane proteins in a native-like solution environment. In order to fully contrast-match-out the nanodisc carriers, we plan to incorporate our IMP targets in stealth carriers consisting of deuterated MSP1 belt protein and deuterated lipids. This would allow the low-resolution structural characterization of our IMPs of interest in a native-like lipid environment without nanodisc contribution.

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Structural studies of IMPs are challenging, as most of them are inactive or insoluble in the absence of a lipid environment. Reconstitution of IMPs in discoidal nanoparticles (nanodiscs) enables structural studies in a lipidic native-like solution environment, however, complicated deconvolution of the contributions from protein and from the lipid scaffold is required. Within this proposal, we used fractionally deuterium labelled 'stealth carriers' that renders these nanodiscs effectively invisible to low-resolution neutron diffraction. We illustrate the potential of the method in a joint small-angle neutron scattering (SANS) and X-ray scattering (SAXS) study of the ATP-binding cassette (ABC) transporter protein MsbA solubilized in the stealth nanodiscs. The SANS data allow for a direct observation of the signal from the solubilized protein without contribution from the surrounding lipid nanodisc, while the SAXS data contain the contribution from the nanodiscs and provide valuable control information. Not only the overall shape but also differences between conformational states of MsbA can be reliably detected from the scattering data, demonstrating the sensitivity of the approach and its general applicability to structural studies of IMPs (Fig. 1).

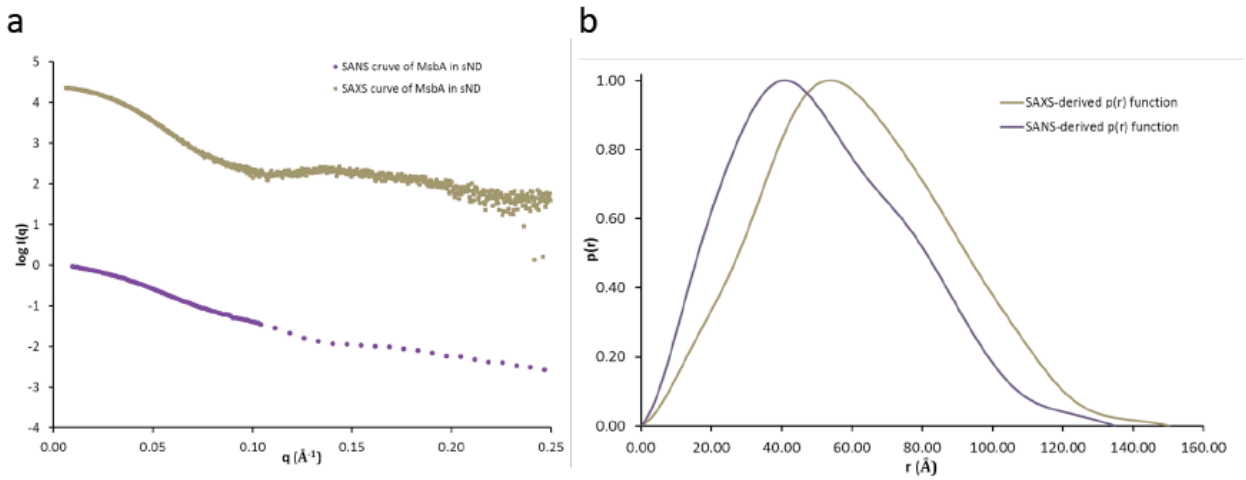

Fig. 1: **Comparison of neutron and x-ray scattering with integral membrane proteins incorporated in stealth carrier nanodiscs.** A) Comparison of SANS (violet) and SAXS (gold) scattering profiles of identical MsbA-sND samples. B) Comparison of SANS-derived (violet) and SAXS-derived (gold) distance distribution plots of identical MsbA-sND samples.

These results are currently in the publication process with ILL beamline scientists and D-lab members as co-authors.