SWING

Insight into membrane profeins' structure

A hybrid modeling based on SAXS data

Membrane proteins play crucial roles in transport and signaling, and are often key targets for efficient drugs. Their specific localization and their amphiphilic properties are an obstacle to structural studies. On SWING beamline, a strategy has been developed to tackle with those refractory proteins.

Membrane proteins are proteins which are functional within the lipidic membranes surrounding biological cells or cellular compartments. Being simultaneously in contact with both the interior and the exterior of the cell compartment, they play crucial roles in transport and signaling, and are often key targets for efficient drugs. All membrane proteins specifically share a structural intermediate domain whose surface is hydrophobic, allowing their insertion into the membrane interior, itself constituted by hydrophobic lipidic tails. For this reason mainly, they are notoriously difficult to tackle. Despite a continuous increase, the number of known structures of membrane proteins (monitored at http://blanco.biomol.uci.edu/Membrane_Proteins_xtal.html) remains less than 2% of the total number of known protein structures, while their occurrence in the genome is about 30%.

Small Angle X-Ray Scattering (SAXS) is a mature and powerful technique able to precisely distinguish different protein structural models in solution, and therefore is a strong complementary support to all structural studies, in particular those from protein crystallography. However, studying membrane proteins with SAXS is not either a simple task, as for other techniques. Not only are these proteins difficult to overexpress, extract and purify, but because they must be maintained in an amphiphilic environment, they can never be studied in isolation. To be maintained folded in solution, membrane proteins have to be associated with amphiphilic molecules (most often detergents, but more recently lipidic surroundings are increasingly used), whose hydrophobic tails cover the hydrophobic transmembrane surface of the protein, thus hiding it from the (aqueous) solvent. However, when their concentration is raised to values needed to dissolve membrane proteins (i.e. higher than their critical micellar concentration, cmc), detergent molecules themselves spontaneously associate into micelles, with X-ray scattering power comparable to those of the proteins. The resulting coexistence of different particles in the sample then requires a specific strategy to analyze the protein structure, as the one designed on the SWING beamline [1].

Online Size exclusion chromatography

Size exclusion chromatography (SEC)-SAXS must be used as the basis of this strategy. The membrane protein solution (concentrated using a protocol that most of the time also concentrates the detergent micelles), is eluted through a column pre-equilibrated with a buffer containing the detergent. The SEC process then ensures both that the level of free micelles around the protein is the same as in the buffer, and that the surplus of free micelles elutes with a different retention volume, usually higher, than the protein-detergent complex. The SAXS data collected under the protein peak can then be reduced afterwards in the same way as for a soluble protein, i.e. by experimental subtraction of the detergent buffer data collected before the column void volume.

Producing a detergent/ protein hybrid model with Memprot

The first step in the strategy requires modeling the detergent moiety around a known construct of the protein, e.g. using the program Memprot developed on SWING that models a detergent corona shaped as an elliptical torus using a coarsed grain approach [Fig.1 up]. The program was built on experimental data collected on the SWING beamline from Aquaporin-0 solubilized in DoDecylMaltoside (DDM), a transmembrane protein of known crystal-

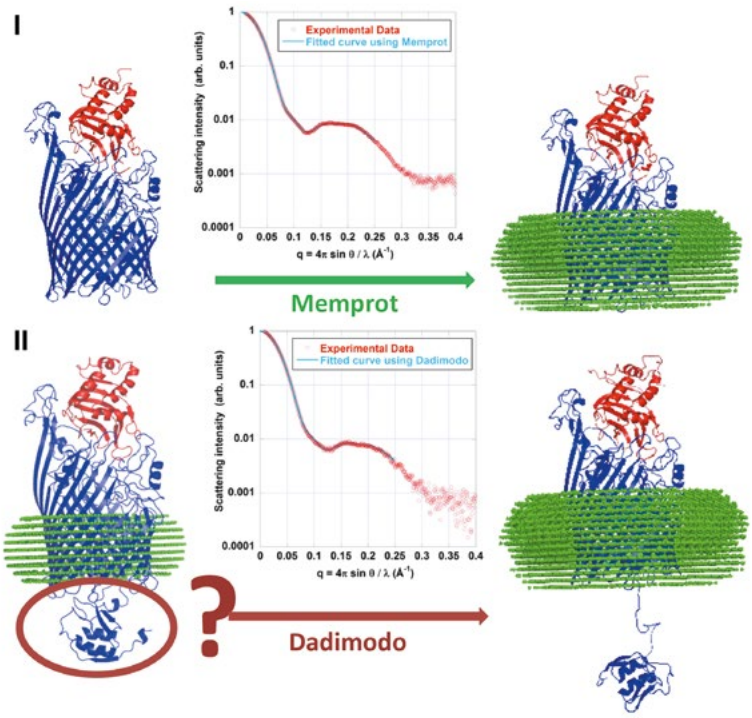

Figure 1. Two-steps strategy to model the conformation of a transmembrane protein based on partial high-resolution structural information and SEC-SAXS data.

lographic structure. It was shown that there is an optimal set of the parameters for which the experimental curve was perfectly fitted, suggesting that the hybrid structure (coarse grain / atomic structure) can be considered as a reliable modeling basis [2].

Interactions study, with Dadimodo

The second step consists in putting to good use the known hybrid structure to analyze its (unknown) interactions with soluble protein partners, whether these are additional domains within the same chain than the known construct or separate chains that make a complex with the membrane protein (Fig. 1 down). An example of such a study was recently published by Wojtowicz *et al* (3). In this study, the interactions between the periplasmic signaling domain of the transmembrane heme transporter HasR and its transmembrane domain could be directly investigated in the presence or not of the outer hemophore HasA. Given that HasR has no symmetry, the design of the detergent corona was improved to mimic the shape of the protein in the transmembrane plane, without increasing the number of parameters. The optimization of the signaling domain position was performed using a specifically modified version of the docking program Dadimodo, also developed at SOLEIL, which should be shortly open to users via a web access. The output of the study points to a position of the signaling domain closer to the main body when the latter is associated to HasA.

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References:
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