MS1-O4 High throughput and time resolved BioSAXS at the P12 beamline of EMBL Hamburg

Clement E. Blanchet[1], Alessandro Spilotros[1], Florian D.C. Wieland[1], Martin A. Schroer[1], Cy M. Jeffries[1], Melissa A. Graewert[1], Daniel Franke[1], Nelly Hajizadeh[1], Stefan Fiedler[1], Dmitri I. Svergun[1]

1. EMBL Hamburg

email: clement.blanchet@embl-hamburg.de

Last decades saw a growing interest for SAXS from the structural biology community, underlining the need for dedicated instruments able to rapidly collect accurate SAXS data on weakly scattering, sensitive, and scarce samples. The EMBL BioSAXS beamline P12 (PETRA-III ring, Hamburg) is tailored for biological solution SAXS and offers services to about 100 user groups from the entire world every year.

The undulator and double crystal monochromator deliver a beam of energy tunable between 4 and 20keV with up two 10^{13} photons per seconds focused by bimorph mirrors down to the size of 200x100 µm^2.

High throughput solution SAXS measurements are performed in an in vacuum flow through capillary. The samples are automatically loaded by a robotic sample changer, which also cleans and dries the capillary between measurements. The typical exposure time is one second and the full loading/cleaning cycle finished within 1 minute. Alternatively, an on-line size exclusion chromatography mode is available with additional spectrometers (UV/Vis, refractive index and RALS) attached for online purification and characterization.

For these experiments, particular care was taken to automate the measurements such that they can be performed with a minimal input from the user. Fully automated data collection by the sample changer robot is followed by the computation of the overall parameters of the solute (Rg, p(r), MW and 3D low resolution shape) by the data analysis pipeline SASFLOW within minutes after data collection. This high level of automation allows one to conduct and analyze over 1000 measurements per day and also allows for permit remote and mail-in operation.

The sample environment can be rapidly exchanged to conduct "non-standard" SAXS experiments such as scanning SAXS, microfluidic chips, etc. The beamline is further being developed to allow for fast time resolved measurements. A multilayer monochromator, presently in commissioning, delivers the flux 5x10^{14} photons per seconds allowing for data collection on biological samples within a few ms, and using the newly installed EIGER 4M detector, data can be collected at 750 Hz frame rate. A stopped flow device, already available at the beamline, allows time resolved data collection with a dead time of a few ms. Continuous flow chip and laser triggering devices are developed to further reduce the dead time and allow sub-ms time resolved SAXS experiments. Pilot time-resolved experiments conducted at P12 will be presented.

Keywords: SAXS, Biological macromolecules, time resolved experiments, automation, high brilliance synchrotron beamline

MS1-O5 Structural insight into host cell surface retention of a 1.5-MDa bacterial ice-binding adhesin

Shuaiqi (Phil) Guo[1], Luuk Olijve[2], David Langelaan[1], Sean Phippen[1], Robert Campbell[1], Steven Smith[1], Ilja Voets[2], Peter Davies[1]

1. Queen's University, Kingston, Canada
2. Eindhoven University of Technology, The Netherlands

email: 9sg52@queensu.ca

Repeats-In-Toxin (RTX) adhesins are a member of the biofilm-associated protein (Bap) family used by many Gram-negative bacteria to form multicellular communities. The largest RTX adhesin known to date is the 1.5-MDa *Mp*AFP produced by the Antarctic bacterium *Marinomonas primoryensis*. The giant adhesin includes ice- and sugar-binding domains close to its C terminus, and ~120 extender domains that project the ligand-binding region ~ 0.6 µm away from the host. The role of *Mp*AFP is to help its strictly aerobic host remain in the upper reaches of ice-covered seas and lakes, perhaps to gain better access to oxygen and other nutrients produced by photosynthetic microorganisms. Here we have focused on *Mp*AFP's ~ 50-kDa N-terminal region 1 (RI) that likely serves as the cell surface anchoring point for the adhesin. Bioinformatic analyses indicated that RI crosses the bacterium's outer membrane (OM), with its N terminus (RIN) localized in the periplasmic space, its C terminus (RIC) in the extracellular environment, while the intervening domain (RIM) spans the OM. RTX adhesins are exported to the cell surface via the Type I Secretion Pathway (T1SS), however, the mechanism of surface retention is unclear due to a lack of detailed structural information. Here we solved the 30-kDa crystal structure of RIC to 1.9 Å. It has an extended shape with three tandemly linked Ca^{2+}-dependent Immunoglobulin-like domains. We also determined the NMR structure of the 8-kDa periplasmic RIN, which has a novel β-sandwich fold with a triangular cross section. The SAXS envelope of the whole RI is an elongated, kinked rod, whose two ends are in good agreement with the RIN and RIC structures. By subtraction, the structure of the ~ 12-kDa intervening RIM is that of a thin cylinder with a diameter of ~18 Å and a height of ~ 40 Å. This shape is similar to the internal dimension of the T1SS pore (TolC) embedded in the OM. As TolC restricts passage of folded proteins, all T1SS substrates must stay unstructured until they enter the Ca^{2+}-rich extracellular environment. Indeed, all *Mp*AFP domains but RIN and RIM require millimolar Ca^{2+} levels to fold. While RIM might interact with the interior of TolC, RIN cannot pass TolC due to steric hindrance, which prevents the total release of *Mp*AFP from the cell surface. Since the RIN fold is conserved in many T1SS Baps, this could be a general mechanism for these adhesins to stay attached to their hosts.

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