



Small-angle X-ray scattering of biological samples (BioSAXS)

The technology at a glance

Small-angle X-ray scattering (SAXS) is a highly effective tool for determining low-resolution molecular envelopes of macromolecules (from a few kDa to 1MDa) in solutions. This powerful technique allows the study of macromolecules (proteins, nucleic acids, carbohydrates) and their complexes in solution whilst not requiring crystallisation. In addition, medium to large protein conformational changes can be monitored over a wide range of conditions.

What information can I obtain?

From SAXS data alone

- Monodispersity and behaviour of a macromolecule in solution (useful during optimisation of crystallisation conditions or protein stabilisation studies)
- Dimension of the particles, and hence oligomeric state
- Shape of a non-crystallisable protein (low-resolution phases obtained)
- Shape of a macromolecular assembly

From SAXS data coupled with crystallographic data

- Confirmation of the quaternary structure of a protein or a multiprotein complex
- Elucidation of the shape of a domain that is not present - or visible - in the crystal structure
- Monitoring of structural changes and domain movements upon ligand binding or complex formation.

The added value of the ESRF BioSAXS beamline

Easy to use, automated and requiring small amounts of sample

The dedicated ESRF BioSAXS beamline is simple and efficient to use. Its automatic liquid handler (sample changer) automates the entire cycle of sample loading, unloading and sample cell cleaning. Very small amounts of sample (typically 50 μ l at 10 mg/ml) are sufficient to perform a complete experiment. The simple point-and-click data collection interface is linked to an automated data analysis pipeline which handles all preliminary data treatment. Our dedicated scientists offer support and help for experiments and data analysis.

Practical Details

What do I need?

- Sample in solution which is mono-dispersive (no aggregation)
- 3 different dilutions of the sample with known concentrations (from 1 to 10 mg/ml)
- Buffer solution that matches exactly that of the samples

How long will it take?

Studying a single sample takes about 1 hour of beam time including preliminary data analysis* (initial beamline calibration by our scientists is not included).

*This includes data collection from 3 different concentrations of sample, verification of monodispersity, determination of radius of gyration and molecular weight of the particle.



Close-up view of ID14-3 sample changer with the sample storage compartment open

How do I access the technique?

Two modes of access are currently available: standard beam time access, with and without scientific assistance, and an express mail-in data collection service.

Beamline technical specifications

Q-range	0.05 - 5 nm ⁻¹
Sample cell	Quartz capillary
Volume of exposed sample	Typical 10-30 µl
Exposure time	Typically 10 seconds
Sample changer	PCR, eppendorf tubes and 96-well plates
Sample temperature range	2-60 °C

CASE STUDY

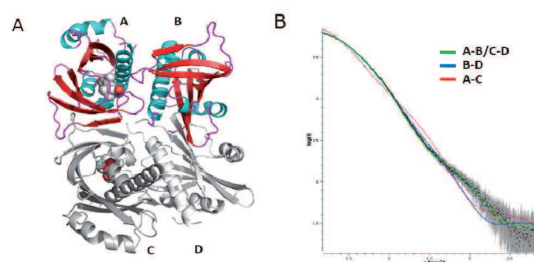
The intracellular receptor PYR1 from *A. Thaliana*.

The challenge: To identify the biologically active dimer of PYR1 present in solution and its relation to the crystal structure which had shown a tetrameric arrangement.

Background: The plant hormone abscisic acid (ABA) has a central role in the adaptive response to desiccation stress. In this study, the authors [1] determined the crystal structure of the *A. thaliana* PYR1 protein in complex with ABA. The refined crystallographic model of PYR1 contains four monomers in the asymmetric unit as shown in panel A. However, size-exclusion chromatography combined with multi-angle laser light scattering (MALLS) demonstrates that PYR1 is a dimer in solution.

Results: Small angle X-ray scattering (SAXS) data were collected at the ID14-3 BioSAXS beamline on PYR1 samples. The three putative dimers A-C, B-D and A-B were fitted into the SAXS data (panel B) showing a good fit only for the A-B dimer, confirming the latter as the biologically relevant dimer of the PYR1 protein.

How did the synchrotron help? Small angle X-ray scattering (SAXS) data collected at the ID14-3 BioSAXS beamline were essential to elucidate which is the biologically relevant dimer in solution.



[1] Santiago J, Dupeux F, Round A, Antoni R, Park SY, Jamin M, Cutler SR, Rodriguez PL, Márquez JA. "The abscisic acid receptor PYR1 in complex with abscisic acid", Nature 462, 665-668.