

BioSAXS-2000

Small Angle X-ray Scattering (SAXS)

Rigaku's BioSAXS-2000 SAXS camera is designed specifically to meet the needs of the structural biologist. In standard experiment, the intensity of scattered X-rays is recorded from biological sample in solution. SAXS is used for determination of the integral structural parameters, shape reconstruction, determination of the oligomeric state in solution, unraveling the quaternary structure of protein complexes, modeling of molecular flexibility and more. The BioSAXS AUTO configuration incorporates an Automatic Sample Changer for unattended overnight operation.

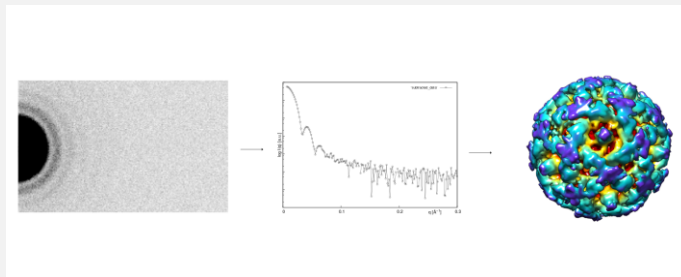
● Biological SAXS can be used for:

- particle size determination 1-60 nm range (R_g , D_{max} , V_{porod} , MW , etc.)
- sample characterization: stability, folding state, aggregation, oligomeric state, etc.
- *Ab initio* shape determination
- atomic models evaluation
- rigid body and hybrid modeling of macromolecular complexes
- modeling of flexible systems



● Technical specifications

Instrument: BioSAXS-2000 (Rigaku)



Features:

- Rigaku MicroMax-007HF - rotating anode generator of Cu K α X-ray ($\lambda=1.54 \text{ \AA}$)
- Confocal Max-Flux SAXS optic, specialized X-ray optic for use in SAXS applications
- 2-D Kratky collimation
- Rigaku HyPix-3000, compact photon counting X-ray detector
- ASC, Automatic Sample Changer with support for up to 96 samples
- Quartz glass capillary 1.0 mm outside diameter, wall thickness 0.01 mm
- Typical acquisition time 60 min per sample
- Sample stage temperature range: $-35 \text{ }^\circ\text{C}$ to $+60 \text{ }^\circ\text{C}$ (during measurement)
- Sample tray storage temperature range: $4 \text{ }^\circ\text{C}$ to $+60 \text{ }^\circ\text{C}$

Operational mode:

SAXS data acquisition is performed by core facility staff only

● Provided Services and Established Methodologies

● Provided services:

SAXS data measurement – user obtains radially averaged, buffer subtracted scattering curves. (2D images or unsubtracted data on request).

SAXS data analysis - user defines the biological problem/question and obtains report protocol with answer, comments, SAXS Table 5., fit plots, *ab initio*/rigid body models, presentation figures or further solution proposals.

● Established Methodologies:

SAXS data measurement - standard transmission mode X-ray scattering from biological samples in solution in concentration series, with radiation damage check and radial averaging of 2D scattering images. Radial averaging and subtraction is performed using SAXLab (Rigaku).

SAXS data analysis – analysis of biological SAXS data using ATSAS software package (EMBL Hamburg) depending on user specifications. Analysis covers integral structural parameters determination, *ab initio* shape reconstruction, rigid body/hybrid modeling, oligomeric state determination, analysis of flexible systems, etc.

● Sample requirements

- **Sample quality and purity is crucial for successful SAXS measurement** and data analysis. Macromolecular sample should be as pure as possible (close to 100%) - as any contaminant contributes to X-ray scattering.
- For evaluation of sample purity use the **SDS-PAGE** and/or **native-PAGE** gel. Especially, avoid the contaminants with MW higher than your molecule of interest.
- Use **DLS (Dynamic Light Scattering)** to evaluate of monodispersity, where sample should have monomodal distribution and MW as expected.
- Sample is measured at **concentration series**, usually 1.0-10 mg/ml (e.g. 1.0; 2.0 and 4.0 mg/ml).
- Sample volume: **60 µl per exposure**.
- The amount of the buffer: 120 µl per each sample (for concentration series of 3 samples bring 360 µl buffer).
- **The buffer** should be **identical** as in the sample, use dialysis or buffer fractions from gel filtration.
- Most of biological buffer compositions are suitable for SAXS. The use of higher concentration of salts (>0.5M), glycerol (>5%), sucrose (>10%) or organic solvents discuss with CF staff.

● **Contacts**

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