BL38B1
RIKEN Structural Biology I

1. Introduction

BL38B1, whose light source is a bending magnet, is a small-angle X-ray scattering (SAXS) beamline dedicated to the structural analysis of biological macromolecules, such as proteins, nucleic acids, and their complexes in solution. In FY2020, a SAXS measurement system was installed, making BL38B1 available to RIKEN researchers and Basis for Supporting Innovative Drug Discovery and Life Science Research by AMED (BINDS) project users. Since 2021B, BL38B1 has been open to public use, focusing on biological SAXS, particularly size-exclusion chromatography-coupled SAXS (SEC-SAXS).

2. Recent activities

During the summer shutdown of FY2023, the focusing mirror in the optical hutch was upgraded to enhance SAXS/WAXS measurements at BL38B1. This mirror was designed to optimize the beamline capability by aligning the focal point with the detector downstream of the experimental hutch. The dimensions and specifications of the new mirror are detailed in Table 1. This upgrade has improved beam collimation and transport efficiency for SAXS measurements, with an increase in flux density achieved by enhancing the flux and reducing the beam size. Specifically, in the SEC-SAXS setup, the flux at the sample position increased to 1.1×10^{11} photons/s, which is 1.5 times higher than before, while the beam size at the focus position (full width at half-maximum: FWHM) was reduced to 0.12 mm (hor.) × 0.7 mm (ver.), that is, 30 % smaller in both dimensions (Fig. 1).

Table 1. Specifications of the mirror.

Manufacturer	Thales SESO SAS
Length along beam (Useful area)	> 980 mm
Width (Useful area)	> 20 mm
Glancing angle	3.7 mrad
Distance from the source	35.5 m
Distance to the focus	16.5 m
Longitudinal radius	Flat (> 20 km)
Sagittal radius	83.36 mm (+/- 1%)
Substrate material	Si
Coating material	Rh (Binding Cr)

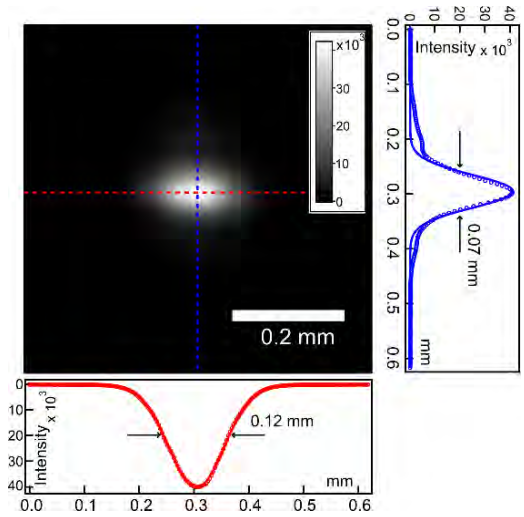

Fig. 1. Beam profiles at the focal point.

Previously, SEC-SAXS experiments using a 24 ml SEC column, such as the Superdex 200 Increase 10/300 GL (Cytiva), required a 3 s exposure per image owing to signal-to-noise (S/N) ratio considerations. This necessitated a pump flow rate of 0.3 ml/min, resulting in an 80 min

measurement time per sample [1]. Following the mirror upgrade, the increased flux density allowed the exposure time to be reduced to 2 s. Consequently, the pump flow rate could be increased to 0.45 ml/min, shortening the time to 53 min per measurement [2]. Figure 2 shows the zero-concentration extrapolation profiles for standard proteins (ALD: aldolase; BSA: bovine serum albumin; OVA: ovalbumin) measured using SEC-SAXS post-upgrade. The data retain the same level of analytical accuracy as before despite the shorter exposure time.

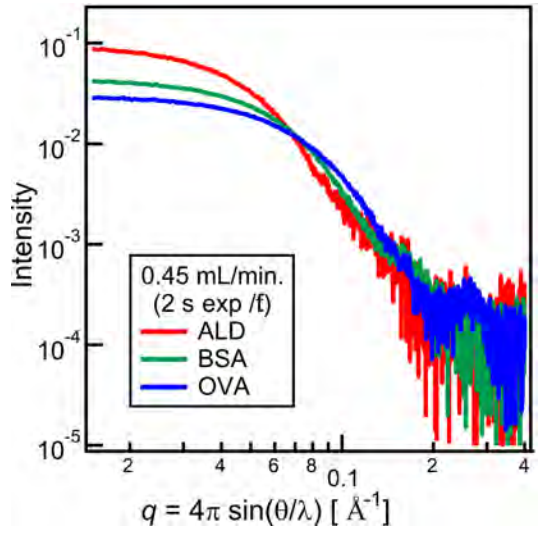

Fig. 2. SAXS profiles obtained by SEC-SAXS.

The current SEC-SAXS system employs a flat stainless-steel flow cell with a 0.02-mm-thick quartz glass window in the X-ray irradiation section. While this quartz glass effectively reduces background noise, it presents several challenges, including a sensitivity to pressure fluctuations within the flow path. Additionally, when quartz glass becomes fouled owing to radiation damage to the sample, it is difficult to clean it chemically because it is bonded to the stainless steel with epoxy resin. The issue of glass fouling has become particularly pronounced with the increased flux density resulting from the mirror upgrade. To address these challenges, we developed a new type of cell featuring a quartz capillary for the X-ray irradiation section and a specially designed cell holder (Fig. 3). The capillary is more resistant to pressure fluctuations, and the bonding method has been modified to allow for easier chemical cleaning. Additionally, the internal pathway of the cell has been refined and improved, reducing the solution volume within the cell to one-sixth of the previous amount. For UV-visible spectroscopy, which is conducted simultaneously with SAXS, the optical path has been reoriented vertically, perpendicular to the X-ray beam, thanks to the capillary in the measurement section, thereby enhancing measurement efficiency.

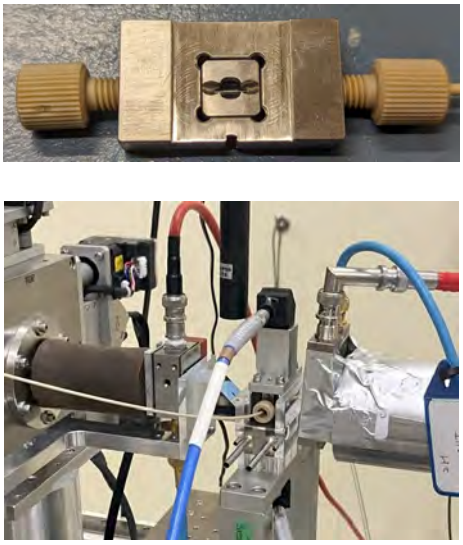

Fig. 3. New cell and cell holder.

Acknowledgment

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References:

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