Structural Characterization of BSA Using SAXS at the PLS-II 4C Beamline

Dahee Jang, ID: 20252087, Div. of Advanced Nuclear Engineering, POSTECH, Pohang, Republic of Korea

Abstract

This report presents the results of a Small-Angle X-ray Scattering (SAXS) experiment conducted at the 4C beamline of PLS-II, focusing on bovine serum albumin (BSA) in aqueous solution. Measurements were carried out in both static and flow modes to assess differences in radiation sensitivity and data reliability. Background subtraction, theoretical modeling, and qualitative comparison of scattering profiles were used to evaluate sample integrity under different experimental conditions. The results demonstrate the practical importance of flow-mode SAXS in studies involving radiation-sensitive biomolecules.

1 Introduction

Small-Angle X-ray Scattering (SAXS) is a powerful non-destructive technique for characterizing nanometer-scale structures in soft matter and biological samples. It provides low-resolution structural information about size, shape, and aggregation state of macromolecules in solution without the need for crystallization. This study explores the effect of different measurement modes—static and flow—on the quality and reliability of SAXS data collected from BSA samples.

2 Principles of SAXS and SEC-SAXS

SAXS involves measuring the scattering intensity $I(q)$ as a function of the momentum transfer vector q, defined by the equation:

$$q = \frac{4\pi}{\lambda} \sin(\theta) \tag{1}$$

where λ is the X-ray wavelength and 2θ is the scattering angle.

The scattering intensity can be expressed as:

$$I(q) \propto Mc(\rho_1 - \rho_2)^2 |F(q)|^2 S(q) \tag{2}$$

where M is molecular weight, c is concentration, ρ_1 and ρ_2 are the scattering length densities of the solute and solvent, $F(q)$ is the form factor, and $S(q)$ is the structure factor.

SAXS data are typically interpreted in the low-q region to extract structural parameters such as the radius of gyration R_g and forward scattering intensity $I(0)$ using the Guinier approximation:

$$\ln I(q) = \ln I(0) - \frac{R_g^2 q^2}{3} \tag{3}$$

For spherical particles, the form factor can be analytically described, while for complex shapes, ab initio modeling or fitting to known structures is used. The pair-distance distribution function $P(r)$, obtained via indirect Fourier transformation, provides real-space insight into the particle shape and maximum dimension (D_{max}).

Size-Exclusion Chromatography coupled SAXS (SEC-SAXS) is a technique that combines chromatographic separation with SAXS to minimize radiation damage and improve sample purity.

3 Experimental Methodology

3.1 SAXS at PLS-II 4C Beamline

Experiments were conducted at the 4C SAXS beamline of the Pohang Light Source-II (PLS-II). The setup includes a double-crystal

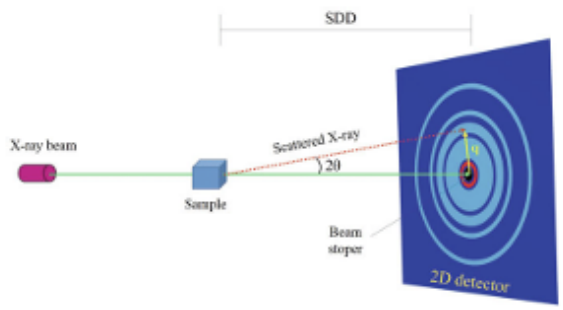

Figure 1: Schematic of the SAXS experimental setup showing the X-ray beam, sample stage, and 2D detector.

monochromator for energy selection and a focusing mirror to optimize the beam at the sample location. The sample-to-detector distance is adjustable to access a broad q-range.

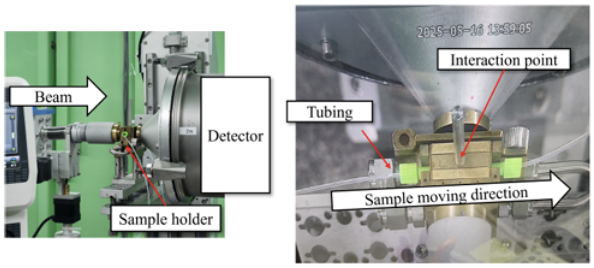

Figure 2: (Left) Schematic diagram of the SAXS beamline, illustrating the X-ray source, optical components, sample stage, and 2D detector. (Right) Detailed view of the SEC-SAXS setup, featuring the flow-through capillary cell, syringe pump, and sample delivery tubing used to continuously circulate the BSA solution during data acquisition.

3.2 Static vs. Flow Mode

Measurements were carried out in both static and flow configurations using BSA dissolved in deionized water. Static mode involved keeping the sample stationary, while flow mode used a syringe pump and tubing to circulate the sample through a capillary.

Both methods involved acquiring 10 frames at 8 seconds per exposure. Solvent-only scattering was measured and subtracted from sample data to obtain clean protein scattering curves.

4 Results and Discussion

The static mode yielded slightly noisier scattering profiles compared to flow mode, likely due to accumulated radiation damage in the exposed volume. Flow mode provided consistent and cleaner data by refreshing the sample volume continuously.

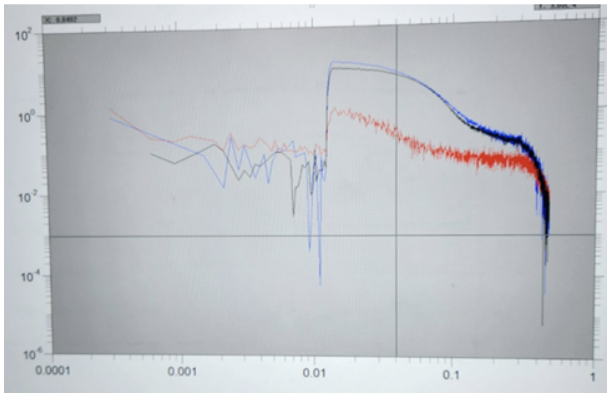

Figure 3: Scattering profile of BSA measured in static mode. Blue: BSA in water, Black: solvent, Red: subtracted profile.

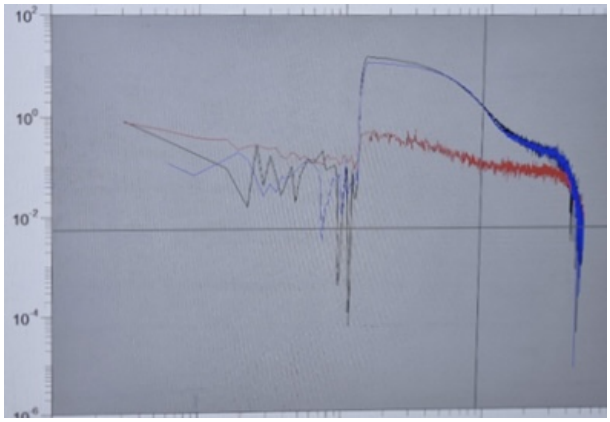

Figure 4: Scattering profile of BSA measured in flow mode. Red: subtracted result, Blue: background, Black: raw intensity.

The consistency of peak positions and general profile shape across both modes suggests minimal structural change. However, higher signal-to-noise ratio in flow mode confirms its suitability for sensitive samples like proteins.

5 Conclusion

This study validates the effectiveness of flow-mode SAXS data acquisition for protein samples. By preventing localized radiation dam-

age and reducing noise, flow mode enhances data quality without requiring significant instrumentation changes. The successful subtraction of buffer scattering and agreement of experimental profiles with theoretical expectations confirm sample monodispersity and structural integrity.

References

[1] K. Jin, "Small angle X-ray scattering overview and application", Lecture Notes, NUCE719P-01, Pohang, Republic of Korea, May, 2025.