SMALL-ANGLE X-RAY SCATTERING EXPERIMENT AT THE 4C BEAMLINE OF PLS-II*

Y. Park†, POSTECH, Pohang, Korea

Abstract

Small-angle X-ray scattering (SAXS) experiments were conducted at the 4C beamline of PLS-II to investigate the structural characteristics of bovine serum albumin (BSA) in solution. Both theoretical modeling and experimental analysis were performed to interpret the scattering behavior of BSA. Measurements were carried out in static and flow modes to examine the influence of sample delivery conditions and radiation exposure. The comparison highlights differences in scattering profiles and data stability, emphasizing the importance of experimental mode selection in SAXS studies involving radiation-sensitive biomolecules.

INTRODUCTION

SAXS is a powerful technique for investigating the overall structure, size, and shape of macromolecules in solution. By analyzing the intensity of X-rays scattered at small angles, SAXS provides low-resolution structural information without requiring crystallization or labeling, making it particularly useful for studying biological samples under near-native conditions.

In this study, SAXS was used to examine the structural properties of BSA, a standard reference protein commonly used in solution-based scattering experiments. Measurements were conducted under both static and flow modes to evaluate how sample delivery conditions influence data quality and structural interpretation. The results were analyzed through both theoretical modeling and experimental data fitting to assess the effects of each measurement mode.

The following section provides an overview of the theoretical principles underlying SAXS and outlines the key equations used in the analysis of scattering data.

BASIC THEORY

When X-rays interact with matter, they are scattered by the electrons of atoms within the sample. The resulting scattering pattern encodes information about the spatial distribution of electron density, making X-ray scattering a powerful tool for structural analysis over a wide range of length scales.

In small-angle scattering, the key quantity is the scattering intensity $I(q)$, measured as a function of the momentum transfer vector q, which is defined by the scattering angle θ and the X-ray wavelength λ as

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

The scattering intensity depends on both the intrinsic structure of the particle and its interaction with neighboring particles. It can be expressed as:

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

where M is the molecular weight, c is the concentration, ρ_1 and ρ_2 are the scattering length densities of the particle and the solvent, respectively, $F(q)$ is the form factor describing the molecular shape, and $S(q)$ is the structure factor accounting for inter-molecular interactions.

X-ray scattering techniques are categorized by the scattering angle: wide-angle X-ray scattering (WAXS) probes short-range (sub-nanometer) order such as atomic lattices, while SAXS is sensitive to larger structures. A schematic of a typical SAXS experimental geometry is shown in Fig. 1, where the incident beam is scattered by the sample and captured on a 2D detector.

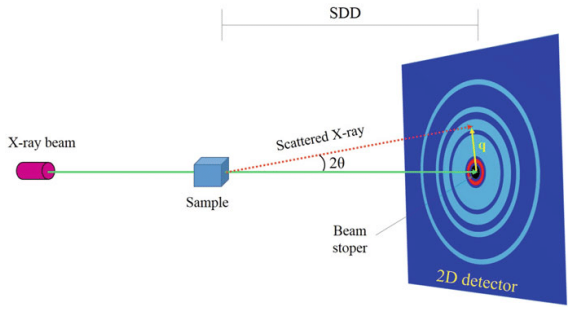

Figure 1: Schematic diagram of a SAXS setup [1]. The X-ray beam passes through the sample, and scattered X-rays are collected by a 2D detector. The scattering angle 2θ and momentum transfer q are defined by the geometry.

For isotropic samples such as proteins in solution, the 2D scattering image is radially symmetric. This allows for azimuthal averaging of the 2D pattern to produce a 1D scattering profile $I(q)$. The transformation from the 3D structure of the sample to a 2D detector image and finally to a 1D curve is illustrated in Fig. 2.

The real-space resolution scale d that can be probed in SAXS is approximately related to the minimum measurable q value:

$$d \sim \frac{2\pi}{q} \tag{3}$$

With a typical SAXS q-range of 0.01–1 nm^{-1}, SAXS enables structural analysis in the 6–600 nm range. This makes

* WORK SUPPORTED BY POHANG ACCELERATOR LABORATORY(PAL).

† pym102048@postech.ac.kr

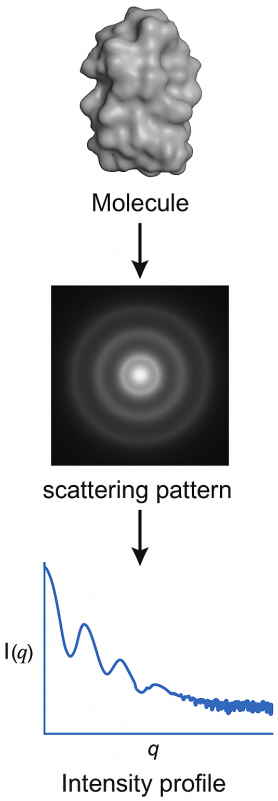

Figure 2: Data reduction in SAXS. The 3D structure of the sample produces a 2D scattering image on the detector, which can be azimuthally averaged to yield a 1D intensity profile $I(q)$.

it highly suitable for studying the size and shape of biomacromolecules, nanomaterials, and soft matter systems.

SAXS MEASUREMENT AT PLS-II 4C BEAMLINE

PLS-II 4C Beamline

The SAXS experiment was conducted at the 4C beamline of PLS-II [2]. In the front-end section, the X-ray beam is extracted and shaped using components such as a beam stopper and a movable mask to obtain the desired beam profile. In the optical hutch, key optical elements including a double-crystal monochromator (DCM) and a focusing mirror (FM) are installed. The DCM is used to select the desired photon energy with high resolution, while the FM focuses the X-ray beam onto the sample position. In the experimental hutch, the sample-to-detector distance can be adjusted to access a wide range of q, enabling flexible SAXS measurements. The overall beamline layout is illustrated in Fig. 3.

Traditional SAXS experiments are typically performed in static mode, where the sample remains stationary during X-ray exposure. However, this approach can be unsuitable for radiation-sensitive or aggregating samples, as prolonged exposure may lead to structural damage.

To address these limitations, size-exclusion chromatography coupled SAXS (SEC-SAXS) has been developed. In this method, the sample is first separated by molecular size using an inline SEC column and then continuously delivered to the SAXS flow cell. This flow-based approach ensures uniform sample delivery and significantly reduces radiation damage, making it particularly suitable for fragile or complex biomolecules [3].

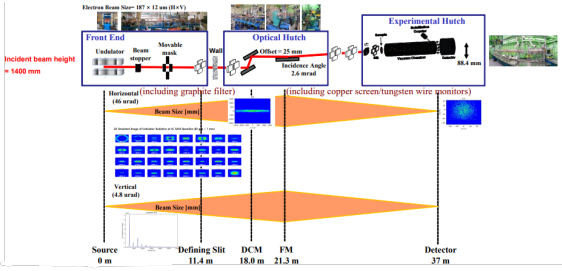

Figure 3: Schematic layout of the PLS-II 4C SAXS beamline, consisting of a front-end section (beam stopper and movable mask), an optical hutch (DCM and focusing mirror), and an experimental hutch where the sample-to-detector distance can be adjusted for SAXS measurements.

Experiments method

To investigate the structural properties of bovine serum albumin (BSA) in its solvated form, SAXS measurements were conducted at the PLS-II 4C beamline using both static and flow modes. The BSA sample was dissolved in deionized water to prepare an aqueous solution suitable for small-angle scattering. During the experiment, two main parameters were controlled: the exposure time per frame and the number of frames acquired. For both measurement modes, an exposure time of 8 seconds was used per frame, and a total of 10 frames were collected to improve statistical reliability through averaging.

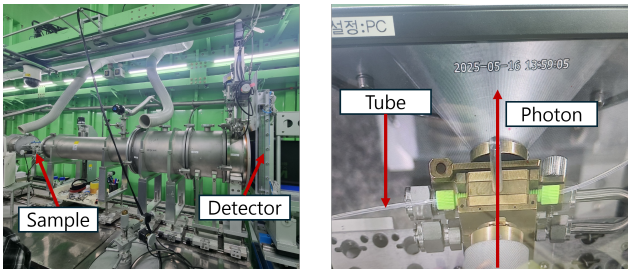

Figure 4: (Left) Overall schematic of the SAXS beamline, including the X-ray source, optical components, sample stage, and detector. (Right) Detailed configuration of the SEC-SAXS setup showing the flow-through capillary cell, syringe pump, and sample delivery tubing used to circulate solvated BSA during measurement.

The experimental setup for flow-based SAXS measurements is illustrated in Fig. 4. The sample solution was loaded into a syringe pump and delivered to the X-ray beam path through a flow-through capillary cell connected via tubing. The X-ray beam interacts with the flowing sample, and the

scattered X-rays are recorded by a two-dimensional detector located downstream. This setup mimics the SEC-SAXS geometry, although no chromatographic separation was applied in this experiment.

Because the sample is in a solvated state, proper background subtraction is essential. To isolate the scattering contribution from the BSA molecules, an initial measurement was performed using solvent only (pure water), under the same exposure conditions. The scattering intensity from the solvent was subtracted from the BSA measurements frame by frame, yielding the net scattering profile of the protein sample.

Result

The experiment was first performed in static mode, where the sample remained stationary in the capillary cell during exposure. This method is simple but can lead to radiation damage in sensitive samples due to prolonged beam exposure. The resulting scattering curve for the static measurement is shown in Fig. 5. Subsequently, the same sample was measured in flow mode. In this configuration, the sample was continuously circulated during exposure to reduce potential radiation damage and improve data reliability. The scattering curve obtained from the flow-mode measurement is presented in Fig. 6.

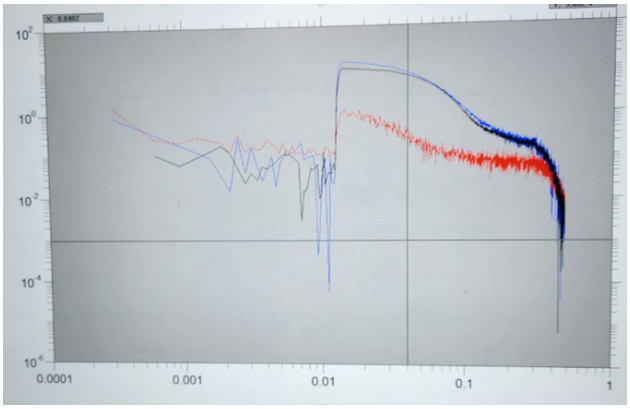

Figure 5: Scattering intensity profile of BSA measured in static mode. The blue curve represents the scattering from BSA in water, the black curve corresponds to the water background, and the red curve shows the subtracted result representing the scattering from BSA.

A comparison of the two results shows that the scattering intensities are generally consistent between the static and flow modes. However, the red curve in Fig. 5 (static mode) exhibits slightly higher noise levels compared to Fig. 6. This is likely due to radiation-induced structural perturbations, which are more pronounced in the absence of sample flow. While BSA is known to be relatively stable against radiation damage, minor structural changes may still occur during prolonged exposure in static conditions.

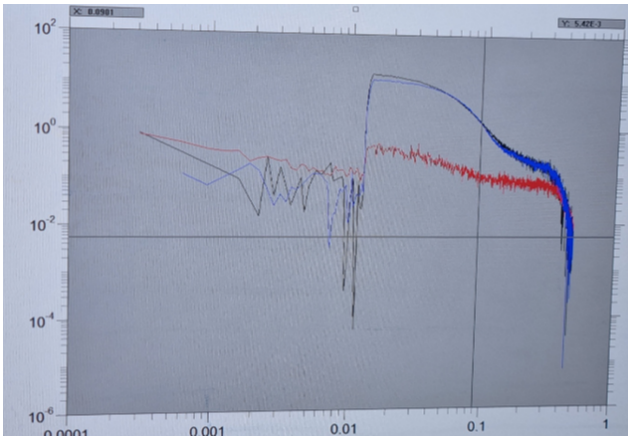

Figure 6: Scattering intensity profile of BSA measured in flow mode. The black curve represents the scattering from BSA in water, the blue curve corresponds to the water background, and the red curve shows the subtracted result representing the scattering from BSA.

CONCLUSION

In this study, SAXS measurements of BSA in aqueous solution were performed using both static and flow modes at the PLS-II 4C beamline. The overall scattering profiles obtained from both modes were consistent, indicating that the structural features of BSA were reliably captured under both conditions. However, the static mode exhibited slightly higher noise levels in the subtracted intensity curve, suggesting minor radiation-induced perturbations. This is attributed to the lack of sample flow, which allows prolonged exposure of the same volume to the X-ray beam. In contrast, the flow mode effectively minimized such effects by continuously refreshing the sample volume during exposure. Although BSA is known to be relatively resistant to radiation damage, this comparison highlights the advantage of flow-based measurements in improving data quality, especially for radiation-sensitive samples. These results confirm that flow mode SAXS, including SEC-SAXS configurations, provides a more robust platform for solution-state structural analysis of biomolecules.

REFERENCES

[1] O. Moscoso Londoño, P. Tancredi, P. Rivas Rojas, D. Muraca, L. Socolovsky, and M. Knobel, *Small-Angle X-Ray Scattering to Analyze the Morphological Properties of Nanoparticulated Systems*, 09 2018, pp. 37–75.

[2] K.-W. Kim, K. Jehan, Y. D. Yun, H. Ahn, B. Min, N. H. Kim, S. Rah, H.-Y. Kim, C.-S. Lee, I. D. Seo, W.-W. Lee, H. J. Choi, and K. S. Jin, "Small-angle x-ray scattering beamline bl4c saxs at pohang light source ii," *Biodesign*, vol. 5, no. 1, pp. 24–29, 2017.

[3] J. H. Kim, B. Min, Y. Yun, H. Choi, and K. Jin, "Size-exclusion chromatography coupled with small-angle x-ray scattering on the 4c small-angle x-ray scattering beamline at pohang light source ii," *Bulletin of the Korean Chemical Society*, vol. 41, 10 2020.