SAXS MEASUREMENT IN STATIC AND FLOW MODES: A COMPARATIVE STUDY

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Abstract

This study compares Small-Angle X-ray Scattering (SAXS) measurements in static and flow modes at the PLS-II 4C SAXS II beamline using Bovine Serum Albumin (BSA) as a model protein. We focused on practical aspects of data collection with emphasis on background subtraction and radiation damage mitigation. While static mode offered simplicity, the Size-Exclusion Chromatography coupled SAXS (SEC-SAXS) flow mode showed better performance for radiation-sensitive samples through continuous sample renewal. This work shows how measurement strategy selection influences experimental outcomes, providing insights into the relationship between synchrotron beam properties and structural biology applications.

1. INTRODUCTION

Understanding beamline operations is essential for optimizing synchrotron facilities to meet diverse user requirements. Designing beamlines that deliver tailored X-ray beams requires knowledge of both accelerator physics and experimental techniques, particularly when considering beam energy, flux, divergence, and temporal resolution. To gain practical insights, we conducted studies on Small-Angle X-ray Scattering (SAXS), a widely utilized technique at synchrotron beamlines [2].

SAXS provides structural analysis of nanoscale structures (1 nm to 1 μm) in materials including polymers and biomacromolecules. Unlike crystallography methods, SAXS requires minimal sample preparation, enabling studies under various conditions [2]. Third-generation synchrotron sources have enhanced SAXS capabilities through high-flux, low-divergence beams. At PLS-II, the 4C SAXS II beamline offers Size-Exclusion Chromatography coupled with SAXS (SEC-SAXS), enabling real-time structural analysis while minimizing radiation damage through continuous sample flow.

In this study, we performed measurements using water and Bovine Serum Albumin (BSA) samples at PLS-II to investigate practical aspects of beamline operation (see Fig. 1). Experiments were conducted in both flow and static modes, allowing comparison of different data collection strategies regarding sample integrity, measurement time, and data quality.

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Figure 1: Group photograph of participants during the SAXS experimental.

2. PRINCIPLE OF SAXS AND SEC-SAXS

2-1. SAXS

Small-Angle X-ray Scattering (SAXS) is a good analytical technique that provides structural information about materials at the nm scale by measuring the intensity of X-rays scattered at very small angles (0.1° to 5°). The technique is particularly useful for studying systems with characteristic length scales from approximately 1 nm to 1 μm, including biological molecules, polymers, and colloidal systems. Unlike other structural methods, SAXS can analyze samples in their natural state without requiring crystallization or special preparation.

In SAXS measurements, the scattering intensity $I(q)$ is measured as a function of the scattering vector q, defined as:

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

where θ is half the scattering angle and λ is the X-ray wavelength detailed in Fig. 2. The scattering curve provides information about the size, shape, and internal structure of particles in solution.

Fig. 2 shows the basic experimental setup for SAXS measurements. The key components include a collimated X-ray beam, sample holder, and two-dimensional detector positioned at a specific sample-to-detector distance (SDD). The scattered X-rays form characteristic patterns on the detector, with intensity and position directly related to the sample's structural characteristics. A beam stopper blocks the direct beam to prevent detector saturation, and data is typically presented as intensity versus scattering vector q.

Category	SAXS	SEC-SAXS
Sample handling	Direct sample injection into cell	Sample flows through separation column first
Suitable samples	Pure samples with uniform size	Mixed samples with different-sized particles
Data mode	Single snapshot measurements	Continuous measurements during flow
Advantages	Fast measurements, simple setup	Better sample purity, less radiation damage
Disadvantages	Sample may clump or get damaged	Requires more time and complex equipment
Applications	Analysis of pure prepared samples	Analysis of mixtures and sensitive samples

Table 1: Comparison between SAXS and SEC-SAXS techniques

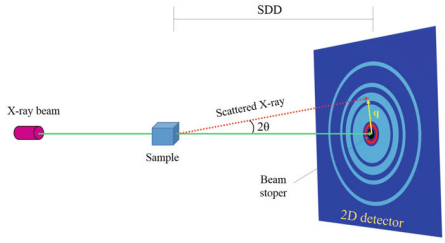

Figure 2: Basic schematic diagram of SAXS experimental setup showing the key components: X-ray beam, sample position, Sample-to-Detector Distance (SDD), and 2D detector with characteristic scattering pattern.

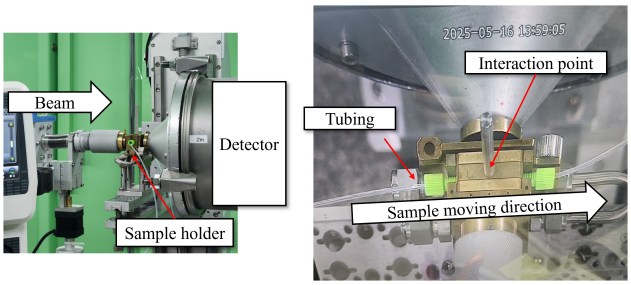

Figure 3: Experimental setup at the 4C SAXS II beamline. (Left) Side view of configuration showing the sample holder, beam path, and detector positioning for conventional SAXS measurements in static mode. (Right) Top view of experimental setup with chromatography column and flow cell for SEC-SAXS; sample moving direction is from left to right as indicated by the arrows.

2-2. SEC-SAXS

Size-Exclusion Chromatography coupled with SAXS (SEC-SAXS) is an advanced measurement technique that combines size-based separation with real-time structure analysis. In this method, samples are separated by molecular size as they flow through a separation column, and SAXS data is collected continuously as different components pass through the X-ray beam. This approach offers several advantages: it provides pure, uniform samples for analysis, prevents radiation damage through continuous sample movement.

In SEC-SAXS, the scattering data are collected as a function of both q and elution time, which is the time it takes for a sample to travel through a chromatographic column and be detected by the detector. This results in a series of scattering curves corresponding to different fractions of the sample as they elute from the column. By monitoring how the scattering intensity changes over time, researchers can identify different components in a mixture and analyze their structures separately.

3. EXPERIMENTAL METHODS

Our experimental procedures consisted of the following steps: background calibration, static mode measurement, and flow mode measurement. Fig. 3 illustrates the experimental setup for both measurement modes.

Our experiments at the PLS-II 4C SAXS II beamline employed two complementary measurement approaches: conventional SAXS in static mode and SEC-SAXS in flow mode. Fig. 3 shows the experimental setup for both configurations. Before sample measurements, initial calibration was performed using air as a reference to establish baseline transmission values and detector response calibration. For all measurements, the 360-degree azimuthal averaging method was utilized to convert two-dimensional detector images into one-dimensional scattering curves.

3-1. Static mode measurement

In the conventional SAXS approach, we conducted static mode measurements where samples remained in a fixed position throughout the data acquisition period. The sample-to-detector distance was optimized to achieve the q-range appropriate for our target size range of interest before experimental.

To assess potential radiation damage, each measurement consisted of 10 sequential exposure frames with individual exposure times of 8 seconds. This sequential acquisition allowed us to monitor sample integrity by comparing consecutive frames for any signs of radiation-induced structural changes.

3-2. Flow mode measurement

For radiation-sensitive samples and to achieve better sample homogeneity, we implemented SEC-SAXS measurements in flow mode. This advanced configuration integrated a liquid chromatography system with the SAXS beamline, including a liquid pump, sample injector, and a size-exclusion column connected to a specialized flow cell positioned in the X-ray beam path.

BSA samples were injected into the chromatography system and carried by solvent solution through the column, where they were separated by molecular size before reaching the measurement cell. The primary advantage of this

approach is that fresh sample continuously moves through the beam, preventing radiation damage by limiting exposure time for any specific portion of the sample. Additionally, the size-exclusion process separates different molecular species, allowing for the analysis of purified components within a heterogeneous sample.

4. RESULTS

Before analyzing the SAXS data, proper background subtraction was essential to identify the sample-specific scattering signal from experimental noise and solvent contributions. Some data has difference scattering pattern which may be caused by radiation effects. Thus, we exclude outlier data. Fig. 4 illustrates the data acquisition procedures both solvent and BSA we implemented for both static and flow mode measurements.

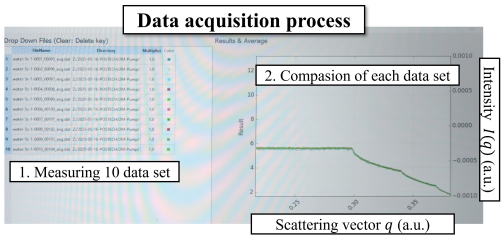

Figure 4: Scattering data taking process with respect to scattering vector q. 10 data set were measured to compared each data set during same exposure time.

The raw 2D scattering patterns were first azimuthally averaged to produce 1D intensity profiles. Then, the solvent solution scattering profile was subtracted from the sample scattering profile to eliminate contributions from the solvent, sample cell, and air path. This subtraction process significantly improved the signal-to-noise ratio and allowed for more accurate structural analysis of the BSA protein (see Fig. 5).

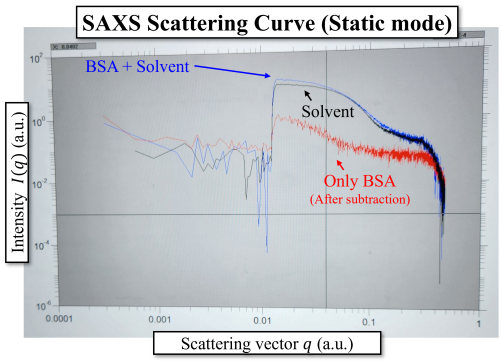

Figure 5: SAXS scattering profiles from static mode measurements of BSA samples. The main plot shows scattering intensity $I(q)$ versus scattering vector q for three different measurements: BSA sample (red), solvent solution (black), and BSA with solvent contribution (blue).

Fig. 5 presents three different scattering profiles obtained from static mode measurements of BSA samples. The distinct difference between the sample and solvent scattering curves is evident, with the BSA sample showing characteristic features.

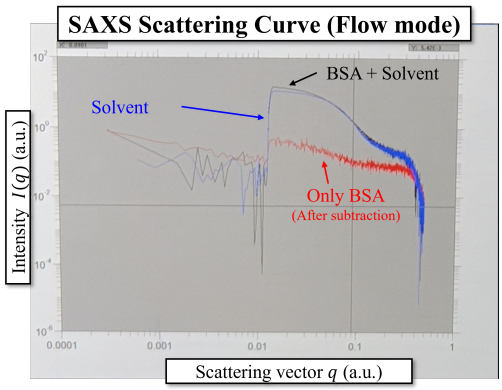

Figure 6: SEC-SAXS results from flow mode measurements of BSA samples.

Fig. 6 shows the basic configuration of SEC-SAXS flow mode measurements. The overall data plot profiles appear similar to static mode, but detailed values show differences. However, since quantitative analysis was not performed in this experiment, it is possible to analyze that radiation effects contributed to some extent to the measurement value differences between static and flow modes.

5. SUMMARY

Through this study, we gained a step-by-step understanding of the SAXS measurement process, comparing both static and flow mode approaches while learning the importance of background subtraction and radiation damage mitigation. This hands-on experience bridged the gap between accelerator physics concepts and experimental applications at beamlines, demonstrating how synchrotron radiation facilities enable sophisticated experimental techniques for structural biology research.

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