DEVELOPMENT AND EVALUATION OF A NEW SAXS SYSTEM FOR BIOLOGICAL SAMPLES

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ABSTRACT

SAXS (Small Angle X-ray Scattering) is a well-known technique for studying the size and shape of particles in solution. More recently, SAXS has been recognized as a complementary tool for extracting structural information from biological systems where X-ray crystallographic methods have failed. SAXS is also considered a useful tool to study behavior of a macromolecule under varied chemical conditions.

However, to get reliable data from SAXS, the sample should be monodisperse in the solution at low concentration. Conventional home laboratory systems require two or more hours per exposure due to the weak signal from the sample. Access to synchrotron facilities is also very limited, thus there is a demand for a fast system for the home laboratory.

Here we introduce the first commercial system specifically designed for high-throughput solution scattering experiments with macromolecules, the BioSAXS-1000. Comprised of specially designed focusing optics with Kratky collimation, the BioSAXS-1000 system eliminates smearing issues common to traditional Kratky cameras and provides sufficient flux for measuring scattering data for low concentration biological samples. This paper will summarize data collection and analyses for data collected on the BioSAXS-1000 for several protein samples, including ab initio protein envelopes.

INTRODUCTION

SAXS (Small Angle X-ray Scattering) is a well-known technique for studying the size and the shape of particles in solution. More recently, SAXS has undergone a resurgence as a useful tool to study biological samples.

X-ray crystallography is a fundamental technique for obtaining atomic resolution structural information for macromolecules. However, the requirement for diffraction quality crystals often limits its efficacy for studying protein complexes or systems with inherent structural disorder. In cases where crystallographic methods have failed, SAXS provides a complementary tool for extracting structural information from biological systems (Hura et al. 2009). In particular, SAXS proves ideal for studying partially disordered macromolecules, for monitoring structural changes in response to environmental perturbations, and for monitoring conformational changes due to ligand binding. It is also a useful tool for probing the crystallization slot.

Here we introduce the system specifically designed for high-throughput solution scattering experiments for macromolecules in solution, the BioSAXS-1000. Comprised of specially designed focusing optics and a Kratky block, the BioSAXS-1000 system eliminates smearing issues common to traditional Kratky cameras (Jiang, 2006). The system features a
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high sensitivity pixel array detector and intuitive data collection software that includes full
automation of the Kratky alignment hardware. Together, these features of the BioSAXS-1000
system allow for synchrotron-quality SAXS data from a home laboratory source.

![Fig.1 The BioSAXS-1000 System](image)

DEVELOPMENT OF THE SYSTEM

(a) Optics

Conventional SAXS systems use the three pinhole design, employing a
monochromator or Confocal Multi Layer optic (CMF) as a beam conditioner. Advances in
X-ray optics over the last two decades have offered great improvement in the quality of X-ray
beams from home labs in terms of flux and spectral purity. The advent of CMF optics during
the 1990s provided significant increases in X-ray flux for home laboratory systems (Verman
et al., 1998) and . These advances have greatly enabled the expansion of both X-ray
crystallographic and SAXS experiments in home laboratories.

A three pinhole system is ideal for measuring anisotropic samples but the requisite
parallel beam has relatively low flux, making it difficult to measure weakly scattering
samples, especially at low concentration. Another collimation system uses a Kratky block
with a line-shaped beam. This design produces much higher flux but suffers from a distortion
to the image data known as smearing.

Here we introduce a new system that consists of a specially designed focusing CMF,
with the focus is on the detector surface and a Kratky block. The Kratky block is designed
such that the bottom surface of one block is parallel to the top surface of the other, and the
blocks are rotatable about the beam around a pivot. The advantage of this design is that the
beam intensity distribution at the detector position is independent of the collimation produced
by the block.

Furthermore, Kratky cameras significantly outperform pinhole cameras for
experiments on large particles, as in the case of macromolecules. Figure 2 shows a schematic
of the the flux reduction in the tightly collimated pinhole system, limiting the beam “seen” by
the solution sample. This decrease in intensity directly translates to a reduction in scattering
signal at the detector.

A unique feature of the Kratky camera is the ability to change the q value in cases
where low q measurements are required. Specifically, a Kratky block limits the converging
beam by virtue of rotation and translations. As illustrated Figure 3, in the “high qmin” mode,
the Kratky block does not occlude the beam. At the sample position, the beam size is more
quadrate. When the Kratky block is rotated to occlude the X-ray beam, the beam shape
becomes more rectangular. These changes to the overall shape of the beam at the sample have
a direct impact on the accessible qmin for data collection. Thus, the Kratky camera is ideal
for measuring large particles, as in the case of macromolecules and macromolecular
complexes. Though changes to the Kratky angle reduce the flux at the sample position,
Kratky cameras produce greater flux in “low q” mode than pinhole cameras when installed on an identical source.

(b) Sample Holder

The sample holder has 3 positions for sample capillary cells and one for a standard sample for calibration of q and the beam center. Liquid samples can be injected using Pipetman® or a syringe; a typical amount is 30 μl.

The three samples are automatically measured using motorized X Y movements. This sample holder also has temperature control, with a range of 4° C to 90° C when water is used as the coolant.

During the exchange of a sample, the entire sample holder assembly can be pulled to the outside of the instrument as show in Figure 4 and an operator can easily load a freshly charged capillary cell, then push it back to the inside.

Figure 2: Schematic comparison of pinhole and Kratky camera geometries

Figure 3: Schematic of beam shape changes with increasing Kratky block angle

Figure 4: Photograph of the capillary cell and sample stage with temperature control
(c) Beam Stop and Detector

The beam stop has a built in pin-diode and the continuously measured intensity is automatically saved to the log file. Although the laboratory source does not show intensity fluctuations, the transmission factor can be calculated and used for the scaling of sample data and buffer subtraction.

For the detector, the most advanced pixel array detector is used with a dynamic range of more than $10^6$ photon counting capability. Both lower q, high intensity data and high q, low intensity can be measured concurrently. The maximum q value is better than 0.7 Å$^{-1}$.

RESULTS

Scattering data were collected for glucose isomerase (GI) on the BioSAXS-1000 and averaged one dimensionally using SAXSGUI then processed using the ATSAS2.1 software package[4]. Followed by buffer subtraction with primus[4]. Output from primus was used to create PDDFs(Particle Distance Distribution Function), with gnom[4], to a qmax of $\sim$0.3 Å$^{-1}$ ("medium q" data) and 0.64 Å$^{-1}$ ("high q" data), respectively. Fifteen $ab\ initio$ envelopes were generated with dammif[4] and gasbor[4] for the $\sim$0.3 Å$^{-1}$ and 0.64 Å$^{-1}$ data, respectively. The fifteen envelopes were superimposed and averaged for each data set with damaver[4].

As shown Figure 5 the envelopes determined with high q data are more comparable to the size of the protein molecule and exhibit greater faithfulness to the surface of the protein compared to the envelope determined using "medium q" data.

![Medium q](image1)

![High q](image2)

Fig.5 Average envelopes 5mg/ml Glucose Isomerase determined from ‘medium q’ and ‘high q’ Rigaku FR-E+ X-ray generator 45KV, 55mA, Exposure 30min

The Figure 6 shows several protein envelopes obtained using the BioSAXS-1000. Data were processed using the same method as described above.
CONCLUSIONS

In this paper we present a new instrument, the BioSAXS-1000, which provides complementary structural information to X-ray crystallography and NMR. The system utilizes a motorized Kratky block for adjustments to qmin for measurement of large particles, such as macromolecular complexes and viral particles. The BioSAXS-1000 is the highest brilliance SAXS instrument available on a home source and provides a powerful platform for maximizing the experimental workflow for structural biology laboratories.

REFERENCES


Figure 6: SAXS analysis for several proteins: lysozyme, glucose isomerase (GI), thaumatin, human serum albumin (HSA), insulin