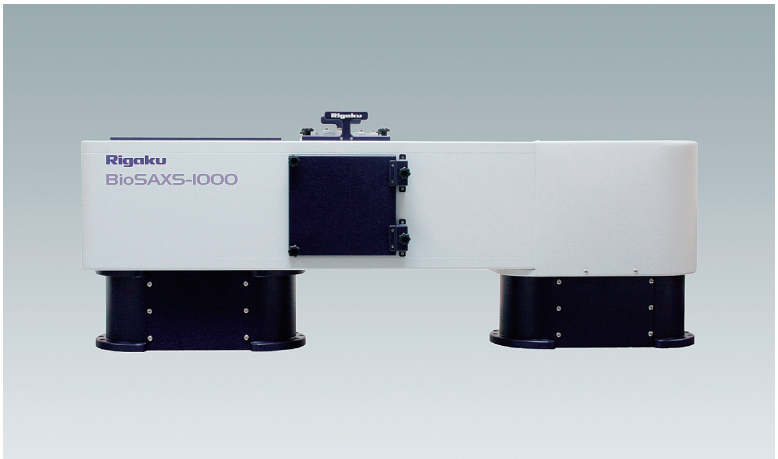
Small angle X-ray scattering Kratky camera system

BioSAXS-1000



1. Introduction

Small-angle X-ray scattering (SAXS) is an important technique for generating low resolution structures of proteins in native solution. Moreover, SAXS provides information on aggregation, folding, unfolding, assembly and conformational changes of proteins in solution despite of the low resolution. BioSAXS-1000 is a two dimensional compact Kratky camera for SAXS measurements of biological macromolecules. BioSAXS-1000 can be installed on a new Rigaku microfocus X-ray generator, such as the MicroMax-007 HF, FR-E+SuperBright or MicroMax-003, or on an existing Rigaku microfocus X-ray generator with an open port.

For the purpose of protein structure determination, one often uses X-ray crystallography, electron crystallography and NMR spectroscopy. X-ray and electron crystallography are methods for determining three dimensional structures of high molecular weight protein complexes at atomic resolution. However, X-ray and electron crystallography require single crystals or two-dimensional crystals, respectively. Furthermore, the crystal structures from X-ray and electron crystallography are static, restrained by the packing energy of the crystal. NMR spectroscopy requires stable isotope labeling and multi-dimensional experiments. Moreover, NMR spectroscopy has molecular weight limitations.

Proteins are highly dynamic, and conformational and ordered-disordered structural changes play a crucial role in their functions. Static structural information is not sufficient for investigating protein function. SAXS determines the dynamic three-dimensional solution structures of proteins in various solution conditions. The acquired diverse information is useful both pre- and post-structure determination by X-ray crystallography. SAXS provides complementary structure information for structural biology.

BioSAXS-1000 is a powerful tool to investigate dynamic structural biology.

2. Features

2.1. Two dimensional Kratky camera

A precision-milled and polished Kratky block is used to collimate the X-ray beam, and allows for a variable beam size in one direction. A double focused multi-layer optic provides a focused X-ray beam at the detector. This optic configuration means that desmearing of the data is not required and higher X-ray flux is delivered to the sample in a much shorter camera length. The Kratky block is motor-controlled for easy alignment and adjustment of q_{min}. One of the big advantages of the Kratky block collimation system is that you can adjust the size of the beam in one direction and reposition the beamstop to measure lower q value data. The beamstop is equipped with a PIN-diode to assist in alignment as well as in the calculation of transmission factors. The detector is a PILATUS 100K/R, a hybrid pixel array detector that is excellent at measuring weak data since it is a photon counter.

2.2. Software: SAXSLab

The BioSAXS-1000 system is controlled using the Rigaku SAXSLab software, a package that allows you to collect fast and accurate in-house SAXS data and to prepare it for input into ATSAS or other SAXS

processing packages. SAXSLab controls all aspects of the BioSAXS-1000 system, including the motorized beamstop, Kratky block, 4-jaw slit and a motorized sample holder. SAXSLab incorporates data collection and monitoring tools for viewing experimental scattering data. Moreover, SAXSLab includes calibration and data processing tools for 1D averaging SAXS profiles, for averaging multiple SAXS images or profiles and for buffer subtraction.

3. Applications

An assembly of atoms is described by the associated electron density distribution. In macromolecule solutions, the contrast in electron density between the macromolecule and the solvent is relevant for scattering. SAXS measurements of macromolecule solutions involve separate measurements of scattering from the macromolecular solution and the solvent. To acquire the macromolecular scattering, the solvent scattering must be subtracted.

$$I_{\text{Macromolecule solution}} - I_{\text{Solvent}} = I_{\text{Macromolecule}}$$

The solution structures of macromolecules from X-ray scattering were constructed by the *ab initio* method[1] using the ATSAS program package[2] (EMBL). Some applications are described below: analyses at short measurement times, a small molecular weight peptide, a very large macromolecular complex and a low concentration protein solution.

3.1. SAXS analysis at short measurement times

A scattering curve and the solution structure are shown in Fig. 1. The 14.8 mg/ml lysozyme and the equivalent buffer were exposed for 15 minutes at room temperature. Dummy residues modeling was performed on the experimental scattering using the program GASBOR (ATSAS), which can be used with high q scattering curves. Superposition of the solution structure on the three-dimensional crystal structure of 6LYZ was carried out using the program SUPCOMB13[3] (ATSAS). The solution structure fits well with the crystal structure. High quality scattering data is obtained by the BioSAXS-1000 even with measurement times as short as 15 minutes.

3.2. SAXS analysis of a low molecular weight peptide and a very large macromolecular complex

Details of the scattering measurement conditions are summarized in Table 1. Scattering curves and the corresponding solution structures are shown in Fig. 2. Dummy residues modeling was performed from the experimental scattering using the program GASBOR. The superpositions of each solution structure on the three-dimensional crystal structure revealed that a wide range of molecular weights, from small peptides to very large macromolecular complexes, are candidates for SAXS analysis.

3.3. SAXS analysis of a low concentration macromolecular solution

A scattering curve and the solution structure are shown in Fig. 3. The 0.5 mg/ml glucose isomerase and the equivalent buffer were exposed for 60 minutes at room temperature. Dummy residues modeling was performed on the experimental scattering using the program GASBOR. The superposition of the solution structure on the three-dimensional crystal structure of 1A0C was carried out using the program SUPCOMB13. The solution structure fits the crystal structure well. High quality scattering data is obtained using the BioSAXS-1000 even with low macromolecule concentration, such as 0.5 mg/ml.

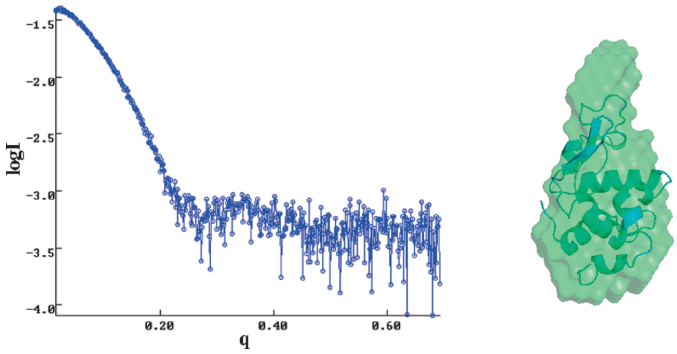


Fig. 1. Scattering curve of 14.8 mg/ml lysozyme at 15 minutes exposure (left), and an overlay of the lysozyme crystal structure (PDB ID 6LYZ) with the averaged SAXS determined solution structure (right).

Table 1. Experimental conditions.

Sample name	Molecular Weight	Protein concentration	Sample volume	Exposure time
Insulin	5.8 kDa	14.0 mg/ml	30 µl	60 minutes
20S Proteasome	750 kDa	2.5 mg/ml	15 µl	180 minutes

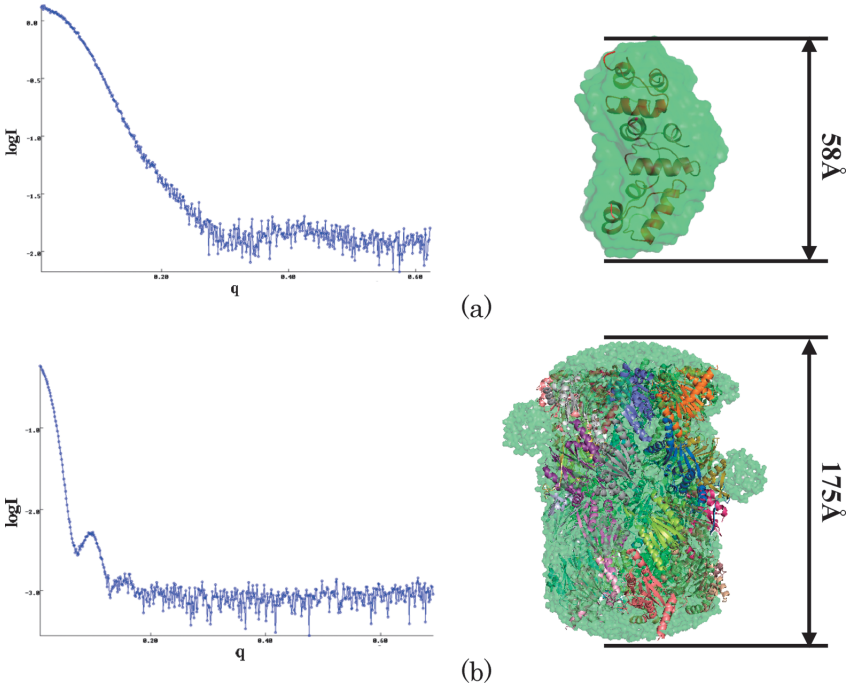


Fig. 2. Scattering curves (left) and overlay of crystal structures with SAXS determined solution structures (right) (a) Insulin (PDB ID: 9INS), (b) 20S Proteasome (PDB ID: 3NZJ).

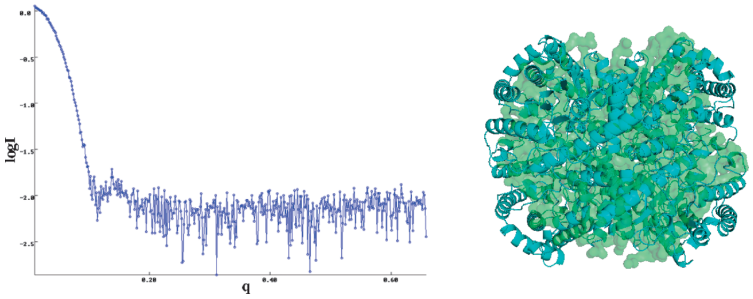


Fig. 3. Scattering curve of 0.5 mg/ml glucose isomerase at 60 minutes exposure (left), and an overlay of the glucose isomerase crystal structure (PDB ID 1A0C) with the averaged SAXS determined solution structure (right).

References

(1) D. I. Svergun, M. V. Petoukhov and M. H. J. Koch: *Biophys. J.*, **80** (2001), 2946−2953.
(2) M. V. Petoukhov, P. V. Konarev, A. G. Kikhney and D. I. Svergun: *J. Appl. Cryst.*, **40** (2007), s223−s228.
(3) M. B. Kozin, and D. I. Svergun: *J. Appl. Cryst.*, **34** (2000), 33−41.