Macromolecular SAXS

Size • Shape • Flexibility • Assemblies • Solution State

Solution scattering from biological molecules

Rigaku

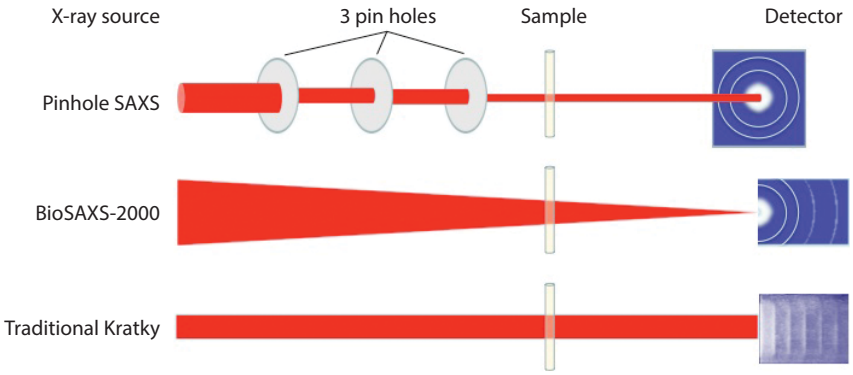
Better measurements. Better confidence. Better world.

Rigaku's BioSAXS-2000 System for Biological Solutio

SAXS cameras

There are two basic designs for SAXS cameras. The most common is referred to as a pinhole camera, which uses a series of apertures to collimate and reduce the parasitic scatter of the X-ray beam. These cameras tend to be 3 meters or more in length in order to measure the low resolution data that most experiments require, which can be restrictive for many labs. The design of a pinhole camera easily allows measurement of 360° of scattering data, which is useful for materials that scatter anisotropically, such as oriented fibers.

The second design for a SAXS camera is called a Kratky camera and is named after one of the pioneers of SAXS research, Dr. Otto Kratky. In this design, a special aperture device, or Kratky block, is used to collimate the X-ray beam, and allows for a variable beam size in one direction. Coupled with a beamstop that can be translated in the direction of the beam movement, a Kratky camera allows you to adjust the beam size and to use more flux for samples that do not require extremely low angle data. The traditional Kratky camera uses a line focus X-ray source, which allows higher flux on the samples with the downside that experimental data are smeared due to significant anisotropy of the line-shaped X-ray beam. As a result, these data often require special corrections.



Schematic comparison of pinhole and Kratky camera geometry

Rigaku's modern Kratky camera

Rigaku's modern Kratky camera, the BioSAXS-2000[1], eliminates the need for desmearing of SAXS data and has the advantage that it can be mounted on a variety of X-ray sources, including the open port of a rotating anode. The Rigaku BioSAXS system is a two dimensional Kratky (2D Kratky) camera. The main difference between it and the traditional Kratky camera lies with the optics. A doubly focusing multilayer optic provides a focused X-ray beam at the detector, with the result that data smearing is negligible compared to the traditional Kratky camera. The 2D Kratky design also has the advantage that higher flux is delivered to the sample in a much shorter camera length compared to a pinhole camera.

Comparison of SAXS camera geometries

	Pinhole camera	1D Kratky camera	2D Kratky camera
Size restrictions	≥3 meter length	Compact size	Compact size
X-ray sources	Microfocus sealed tube and rotating anode sources	Restricted to standard line focus sealed tube	Microfocus sealed tube and rotating anode sources
Alignment	Manual	Manual	Motorized with automatic tools
Desmearing of data	Not required	Required	Not required
Sample type	Randomly oriented and oriented samples	Randomly oriented samples only	Randomly oriented samples only
q_{min}	Fixed	Adjustable, manual	Adjustable, computer controlled

[1] Licai Jiang, Rigaku Innovative Technology US patent: 8,094,780., Japan patent: 5,214,442. Europe: patent pending

n Scattering

Designed for performance and ease of use

Rigaku's precision-milled and polished Kratky block is motor controlled for ease of alignment and adjustment of q_{min}. This flexibility means that you can either run in a standard configuration or in one that provides higher flux. Easily switch with one click in the control software and you are ready to collect.

The BioSAXS-2000 includes a sample holder that allows three capillaries to be mounted in the sample chamber along with one Ag-behenate standard. A provision for an optional flow cell and automatic sample changer is also provided. The beamstop is equipped with a PIN diode to assist in alignment, as well as in calculation of transmission factors. The detector is a DECTRIS® PILATUS 100K, a hybrid pixel array detector and photon counting device that is excellent at measuring the weak scattering data from macromolecules.

Outstanding flux performance

The BioSAXS-2000 includes the OptiSAXS optic, a double-bounce confocal optic that captures a larger angle of the X-ray source and thus provides significantly greater flux at the sample position. Moreover, the focal point of the OptiSAXS optic remains at the detector position so that data do not require desmearing, as is the case with SAXS cameras using line focus sources. These features result in faster data collection with higher signal to noise for your biological SAXS samples.

The OptiXAS optic with higher X-ray capture angle delivers outstanding flux performance and faster SAXS experiments

Specifications

BioSAXS-2000 system specifications		
X-ray generator	**MicroMax™-007 HF**	**FR-X**
Camera length	~500 mm	
Sample volume	20 – 30 µL	
Beam size	Variable width x 1500 µm (at sample), 530 µm (avg. at detector)	
Cu *K*α flux at sample	3.4 x 10^9 ph/s	8.4 x 10^9 ph/s
Incident beam optics	OptiSAXS	
Collimation	2D Kratky (X stage and tilt, motorized)	
Sample stage	3 capillaries (X,Y stage, motorized)	
Beam stop	PIN diode detector (motorized)	
q range	0.006 – 0.65 $Å^{-1}$	
Size	238 mm (width), 1392 mm (depth), 546 mm (height), 150 kg	
Detector DECTRIS PILATUS 100K	Sensor: Reversed biased silicon diode array Active area: 83.8 x 33.5 mm Pixel size: 172 µm x 172 µm Dynamic range: 10^6:1 max. count rate 2 x 10^6/sec Quantum efficiency: 99% at Cu *K*α Cooling: Air cooled	

Rigaku Structural Biology Workflow Center

The X-ray diffraction community now recognizes that SAXS can play an import of an iterative workflow in macromolecular crystallography.

SAXS data collected prior to setting up crystallization experiments can tell you something about the probability of success. For example, a non-linear Guinier plot will provide information as to whether the sample exhibits aggregation or interparticle interference, both of which suggest reduced propensity for crystal-lization. Also, a Kratky plot will provide information about whether your protein is unfolded or exhibits high structural flexibility. These analytical tools guide you towards better sample production rather than wasting your precious time trying to crystallize non-ideal samples.

SAXS is a useful tool for understanding macromolecules and assemblies in solution. For example, SAXS data are used to determine a number of structural parameters and to examine molecular interactions and biological assemblies. For monodispersed systems, SAXS data can provide real space information about macromolecules, as well as low resolution *ab initio* models. Moreover, SAXS can be used to monitor conformational changes in macromolecules and to investigate disorder or flexibility.

tant role in structural biology and, in particular, be part

SAXS experiments following structure determination by X-ray or NMR methods can be useful for many situations. For example, SAXS data can be used to model macromolecular complexes and assemblies. SAXS experiments also provide information about disordered regions within protein structures. In such cases, these disordered regions are absent from crystal structures.

Rigaku's MicroMax-007 HF rotating anode generator featuring a left port BioSAXS-2000 system and a right port AFC-11 4-circle goniometer and PILATUS 200K detector.

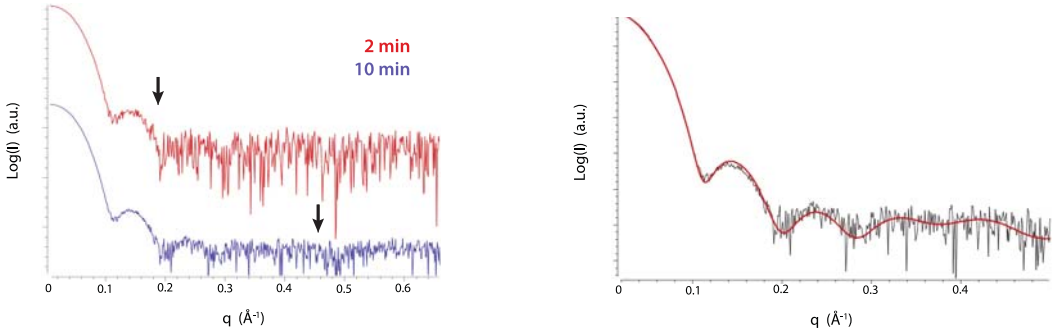
Rigaku offers all the components for an integrated system to explore protein structure and improve the throughput in a structural biology lab.

Experimental Example

The BioSAXS-2000 provides better than 2-fold higher flux at the sample position compared to its predecessor, which translates to shorter exposure times and faster SAXS experiments. To evaluate expected experimental times, data were collected for standard proteins using the BioSAXS-2000. These experimental examples demonstrate that data collections as fast as 1-2 minutes are often sufficient for determination of structural parameters.

Glucose isomerase

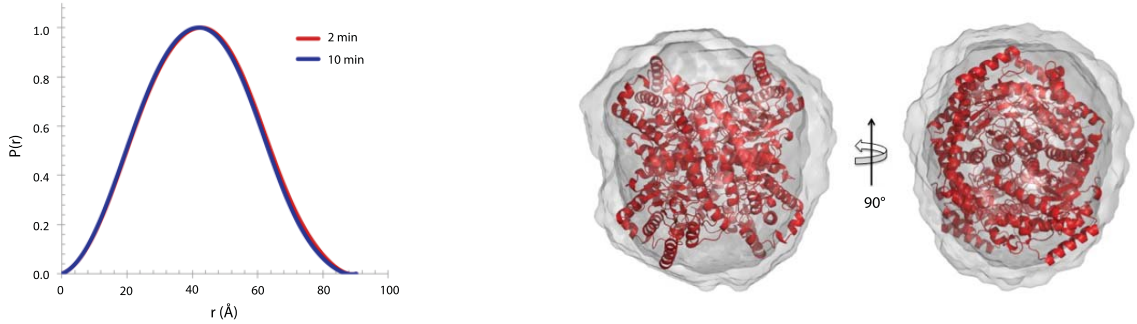
SAXS profiles for a 4 mg/mL solution of glucose isomerase (173 kDa) are presented below for exposures of 2 and 10 minutes. The arrows indicate the effective q_{max} value where the calculated I/σ(I) is determined to be 2.0. Keeping in mind that most software analysis algorithms use SAXS data less than 0.25 $Å^{-1}$, the plots below illustrate that data collected for 2 minutes is sufficient for most analyses. The plot on the right shows a fit of the experimental data to SAXS data calculated from the crystal structure of glucose isomerase.



Structural parameters calculated for three concentrations of glucose isomerase SAXS data are reported below. These results, which are consistent with published values, indicate that short exposures times are sufficient to determine basic structural parameters.

Conc.	Exposure time	Guinier R_g (Å) (error) AutoRg[1]	Guinier *I*(0)/c (a.u) AutoRg[1]	Real Space R_g (Å) (error) AutoGNOM[1]	Real Space D_{max} (Å) AutoGNOM[1]
1.0 mg/ml	2 min 10 min	31.7 (1.7) 32.8 (0.7)	3.02 3.13	32.8 (0.2) 32.2 (0.1)	101 100
2.0 mg/ml	2 min 10 min	32.0 (0.8) 32.4 (0.4)	3.10 3.16	32.5 (0.1) 32.5 (0.1)	95 99
4.0 mg/ml	2 min 10 min	30.3 (0.8) 30.6 (0.5)	3.13 3.18	32.1 (0.1) 32.1 (0.003)	90 91

The plot on the bottom left shows the pair distance distribution plot, or P(r) plot, calculated using 2 and 10 minute data for the 4.0 mg/mL sample. These plots are essentially superimposable and exhibit D_{max} of ~90Å. These same data can also yield accurate shape reconstructions. For example, an *ab initio* model using 10 minute SAXS data to 0.25 Å is shown on the bottom right.



[1] Petoukhov, M.V. et al., (2012). *J. Appl. Cryst.* **45**, 342-350.

BioSAXS AUTO – Automation

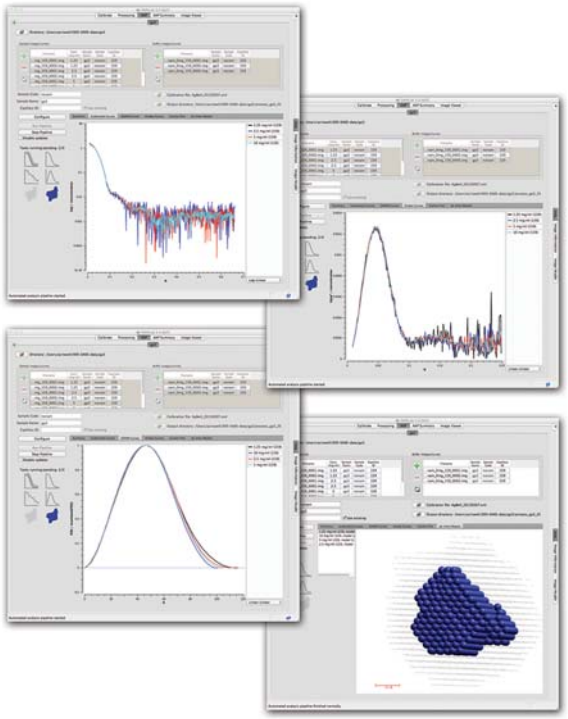
Automated Sample Changer

For those labs that desire unattended sample mounting and data collection, the BioSAXS-2000 includes an optional flow cell and Automatic Sample Changer (ASC). Together, these components add automated sample loading, washing and data collection capabilities to the BioSAXS-2000. The ASC supports samples supplied in 96-well plates and 0.2 mL PCR tube arrays. The ASC seamlessly integrates with the BioSAXS-2000 system and SAXSLab software to allow easy setup of unattended or overnight experiments so that you can focus more time where it counts, on SAXS data analysis and publication of results. Features of the BioSAXS-2000 ASC include:

- **Sample loading and flow control stations**
- **Flow cell for unattended sample loading from samples in microfuge tubes**
- **Support for foil-sealed samples to ensure that your samples won't evaporate prior to data collection**
- **Support for multiple cleaning solutions**
- **Temperature controlled sample storage, including option for separate storage versus data collection temperatures**

Automated data collection and analysis

The BioSAXS-2000 system is controlled by the Rigaku SAXSLab software, a single package that integrates data collection, data processing and data analysis. Rigaku offers an optional Automatic Analysis Pipeline (AAP) that utilizes the industry standard ATSAS package to provide the following automatic analyses for each sample:



- **Automatic sample evaluation and aggregation identification**
- **Automatic profile averaging and buffer subtraction**
- **Automatic Guinier plot generation and calculation of R_g and I(0)**
- **Automatic Kratky plot generation**
- **Automatic Porod volume and molecular weight calculation**
- **Automatic P(r) calculation**
- **Automatic envelope calculation, averaging and analysis**
- **PDF report generator**
- **Easy results review for previous AAP runs or data collections**

The AAP displays results for all concentrations of a sample with a comprehensive table of calculated structural parameters along with warnings to the user in cases of poor sample quality, aggregation or other problems. With the AAP, users get a gauge of sample quality as soon as data collection is complete.

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Photo left:
Dr. Y. Shimura (founder of Rigaku) and Professor A. Guinier. This photo was taken in 1959 at the front gate of Rigaku Science & Research Laboratory in Tokyo. Shimura and Guinier became friends when Rigaku undertook the project to publish the Japanese version of Guinier's famous X-ray book, "The Theory and Technique of X-ray Crystallography."

Front cover:
SAXS determined overlay of glucose isomerase and an ATSAS filtered bead model (damfilt.pdb), determined from an average of 15 superimposed bead models from DAMMIF.

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Our Passion

The determination of the first protein structure (myoglobin) in 1957 served as a watershed event for structural biology. Crystallography and NMR continue to provide accurate, high resolution structural information that can be used in understanding the function of biological molecules, but an additional technique (SAXS) is now becoming widely used as a complementary tool for the structural biologist. As larger and more complex molecular systems are being studied, SAXS provides the ability to determine the size and shape of macromolecules in solution. Moreover, SAXS can be useful for providing information about the molecular arrangements of assemblies, when combined with complementary biophysical techniques. The Life Sciences group of Rigaku is proud to play an integral part in developing and providing the tools that help advance the science of structural biology.

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