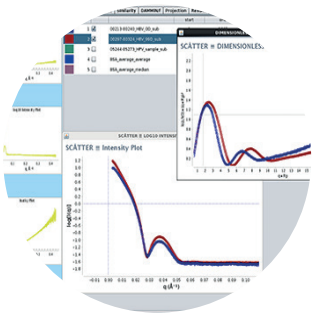
B21

Size Exclusion Chromatography (SEC) - SAXS

B21 is a dedicated solution state Small Angle X-ray Scattering (SAXS) beamline that can accommodate a wide variety of aqueous-based and some limited organic solvent-based samples.

In order to achieve the highest quality data, B21 offers online Size-Exclusion Chromatography (SEC) coupled SAXS. A range of Shodex and Superdex columns are available for use, controlled by an Agilent HPLC system. The eluate flows through a temperature controlled capillary for SAXS data collection.

The beamline users benefit from a fast streamlined data processing and data analysis pipeline so data can be analysed during the experiment. Multi-angle light scattering is also available for off-line sample analysis.



*Some solvents may be incompatible with robot/HPLC internal tubing. Please contact us for advice.

SEC-SAXS

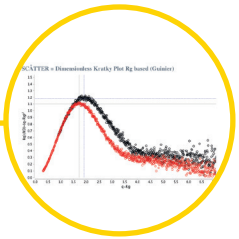
Sample volume	50 µl
Sample concentration	For 21 kDa proteins: up to 10 mg/ml For 200 kDa proteins: up to 3-4 mg/ml
Buffer	~250ml volume recommended. Less than 300 mM salt pH < 8 Less than 10% glycerol No detergent (unless own column supplied) No organic solvents*
Maximum protein molecular weight	Up to MDa if globular
Data collection time	~30 min per sample

Sample delivery method

Column	Vol. (ml)	Mass range (kDa)	Max pressure (bar)	Recommended flow rate (ml/min)
Superdex 200 (GE Healthcare)	2.4	10 to 600	35	0.075
Superose 6 (GE Healthcare)	2.4	5 to 5000	35	0.075
KW-402.5 (Shodex)	4.6	5 to 100	85	0.160
KW-403 (Shodex)	4.6	10 to 700	85	0.160
KW-404 (Shodex)	4.6	30 to 4000	85	0.160

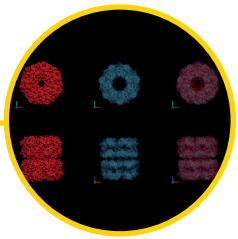
B21 APPLICATIONS

Flexible Proteins



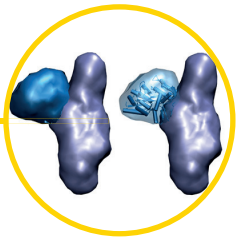
- Investigate proteins that are hard or impossible to crystallise;
- Screen buffer conditions to monitor folding;
- Domain structure analysis to determine suitability for crystallography.

Macromolecular Complexes



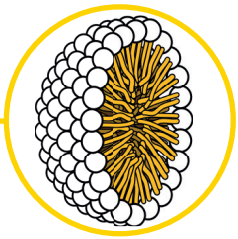
- Determine molecular weight of a protein or protein complex to determine oligomerisation state;
- Characterise multi-domain proteins using data from subcomponents of a modular protein or complex.

Ligand Binding



- Investigate conformational changes associated with ligand binding;
- Validate structures by comparing SAXS to crystallographic data.

Advanced modelling



- Generate highest quality data for advanced modelling of conformational changes or multi-subunit complexes.

For further information

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