BioSAXS BM29

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ESRF

EMBL

Overview

- **Beamline optimised for**
  - Stability
  - Reliability
  - Usability
- **Ease of use through automation of**
  - Data collection
  - Data reduction
  - Data processing
  - Data analysis
- **Confidence in experiments through feedback**
  - User oriented focus to guide users
    - In experiment preparation
    - Data acquisition

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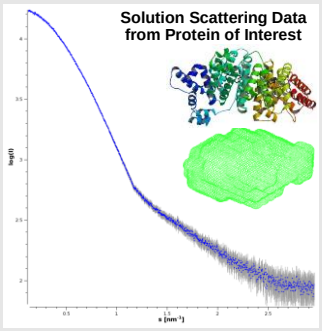
Inexperienced User‘s view of an experiment

Black Box

Neutron/X-ray beamline
Home source



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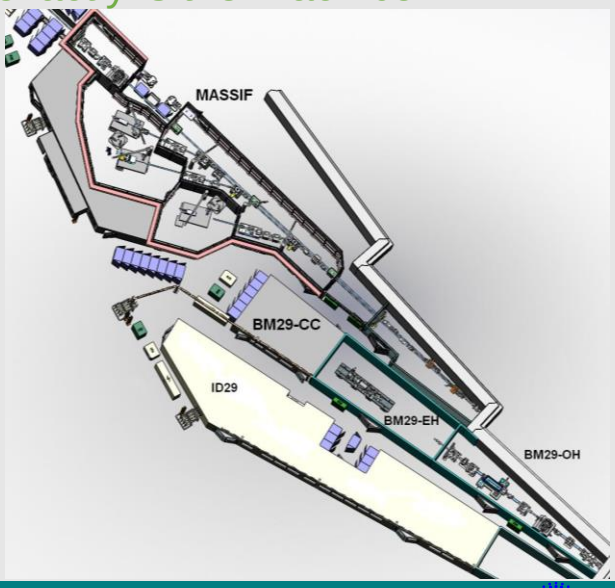
What exactly is the Black box?



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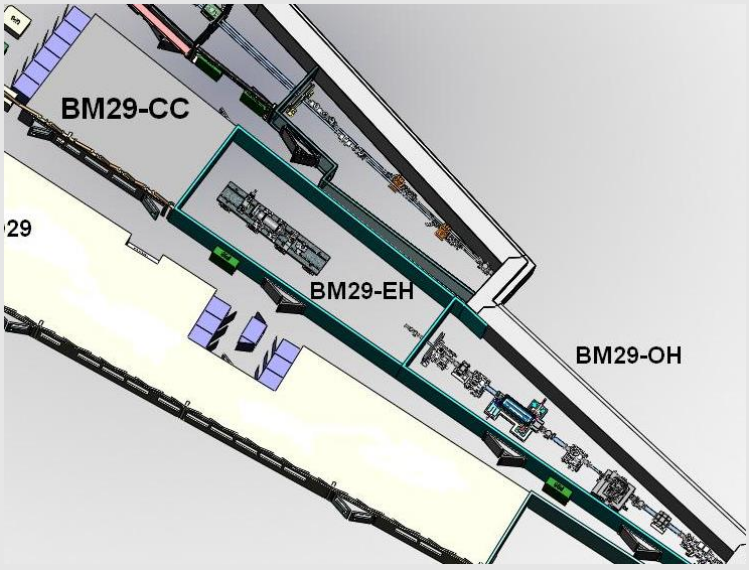


Current status of data collection:

Temperature

- Data acquisition between 4 and 60 C
- SEC operation at 4 or 20 degrees C

Exposure Time

- Standard 1 FPS (10 frames for Static)
  - S200 column ~1 hour (3600 frames)
  - Increase column ~10 mins (600 frames)

Sample Volume

- Minimum recommended 30 µL per measurement
  - Approx. 5 mg/mL
  - 100 µL stock recommended
    - for static and SEC

Automated valve
To switch between
SEC and Static modes

Gives users control
Safe and reliable switching
Maximises efficiency
cleaning between SEC runs

Fully Automated Data Collection

ISPyB

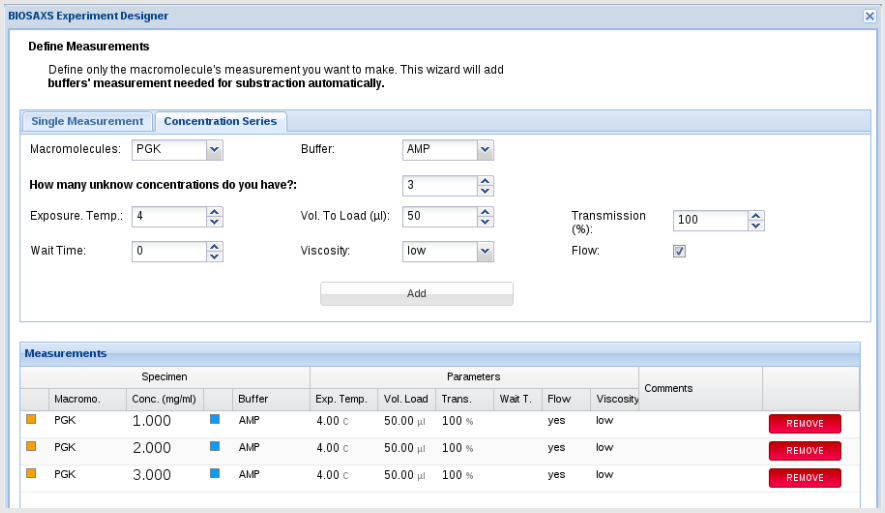
Information System for Protein crystallography Beamlines

LIMS database to store:

- Sample definitions
- Shipping details
- Experimental data
- Processing results

The ISPyB data base was designed to for MX and was adapted for bioSAXS

Improved feedback for experimental preparation



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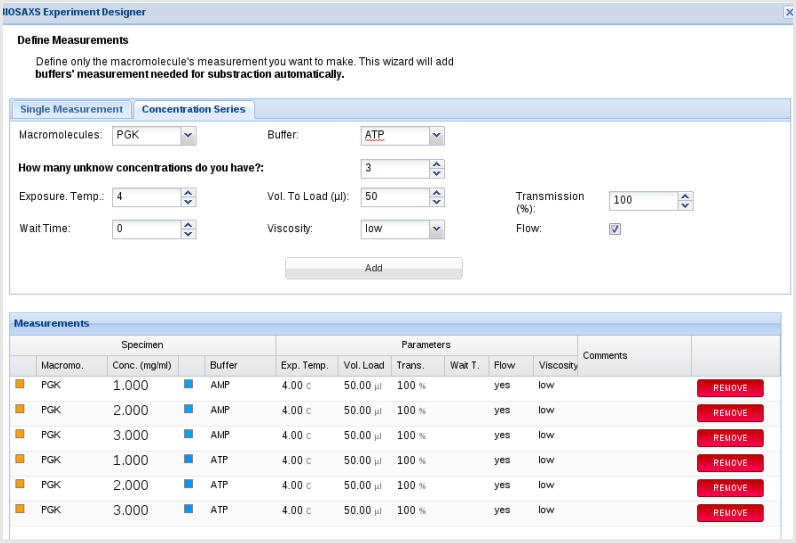

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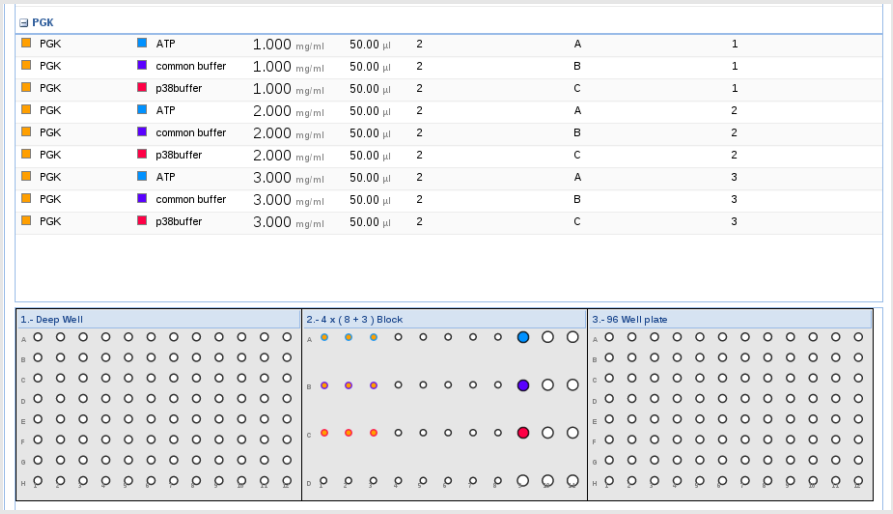


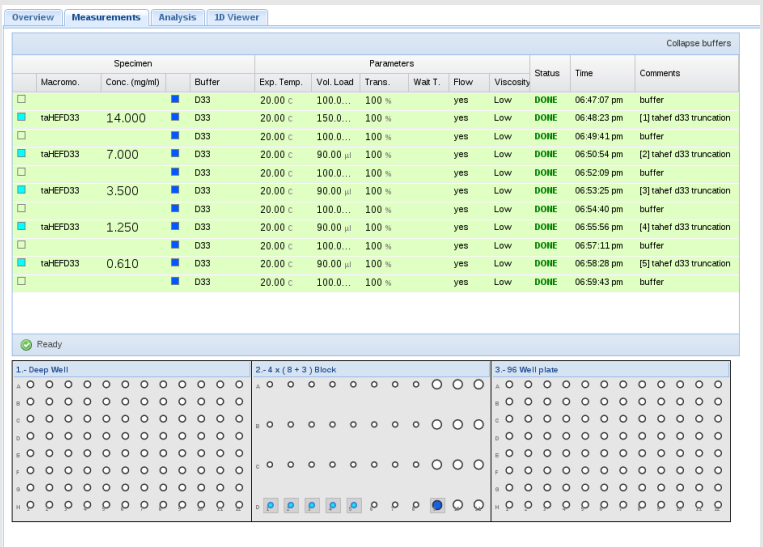
Improved feedback for experimental preparation

Estimation of required Volume

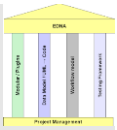
Go to Shipment

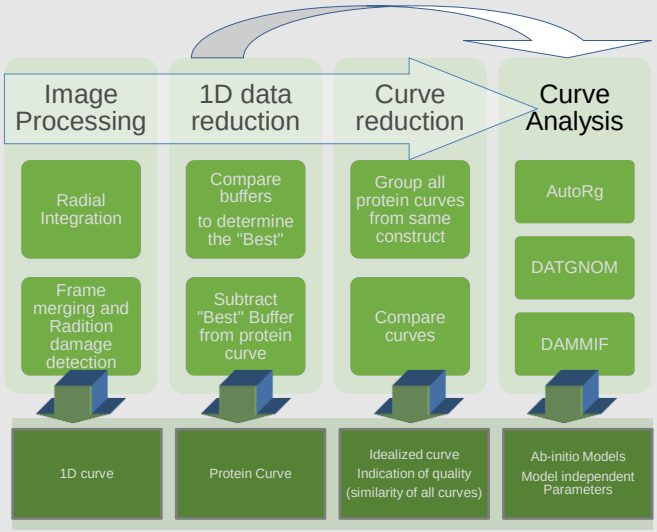
Specimen	Estimated Volume
ATP	300.00 µl
PGK + ATP	150.00 µl
PGK + common buffer	150.00 µl
PGK + p38buffer	150.00 µl
common buffer	300.00 µl
p38buffer	300.00 µl

Improved feedback with ISPyB



Data Processing: ATSAS tools in EDNA





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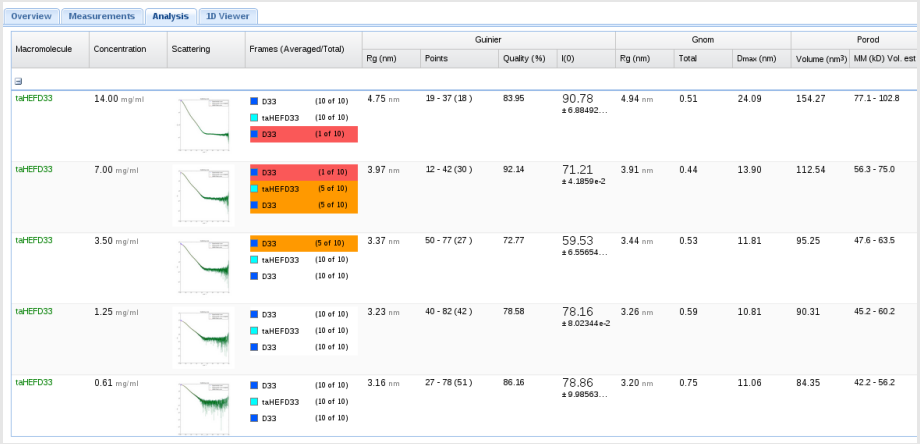
Improved feedback with ISPyB



Overview | Measurements | Analysis | 1D Viewer

Macromolecule	Concentration	Scattering	Frames (Averaged/Total)	Guinier				Gnom			Porod	
				Rg (nm)	Points	Quality (%)	I(0)	Rg (nm)	Total	Dmax (nm)	Volume (nm³)	MM (kD) Vol. est
taHEFD33	14.00 mg/ml		D33 (10 of 10) taHEFD33 (10 of 10) D33 (1 of 10)	4.75 nm	19 - 37 (18)	83.95	90.78 ± 6.88492…	4.94 nm	0.51	24.09	154.27	77.1 - 102.8
taHEFD33	7.00 mg/ml		D33 (1 of 10) taHEFD33 (5 of 10) D33 (5 of 10)	3.97 nm	12 - 42 (30)	92.14	71.21 ± 4.1859e-2	3.91 nm	0.44	13.90	112.54	56.3 - 75.0
taHEFD33	3.50 mg/ml		D33 (5 of 10) taHEFD33 (10 of 10) D33 (10 of 10)	3.37 nm	50 - 77 (27)	72.77	59.53 ± 6.55654…	3.44 nm	0.53	11.81	95.25	47.6 - 63.5
taHEFD33	1.25 mg/ml		D33 (10 of 10) taHEFD33 (10 of 10) D33 (10 of 10)	3.23 nm	40 - 82 (42)	78.58	78.16 ± 8.02344e-2	3.26 nm	0.59	10.81	90.31	45.2 - 60.2
taHEFD33	0.61 mg/ml		D33 (10 of 10) taHEFD33 (10 of 10) D33 (10 of 10)	3.16 nm	27 - 78 (51)	86.16	78.86 ± 9.98563…	3.20 nm	0.75	11.06	84.35	42.2 - 56.2

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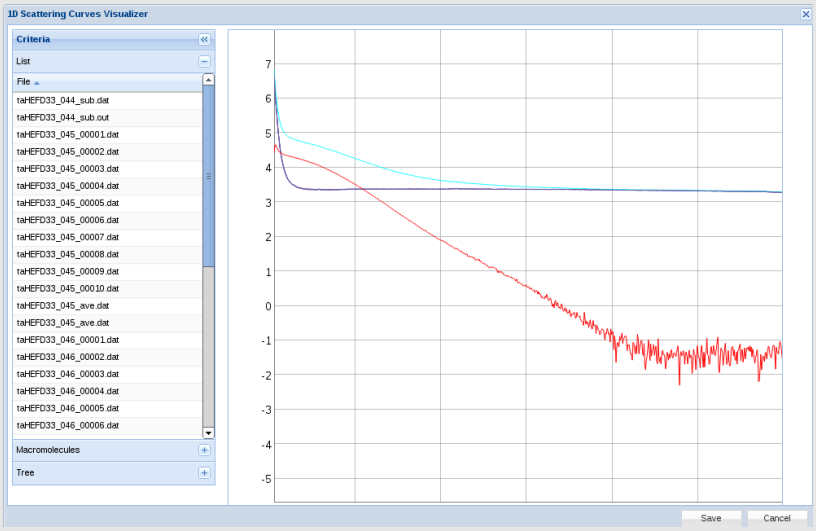
Improved feedback with ISPyB



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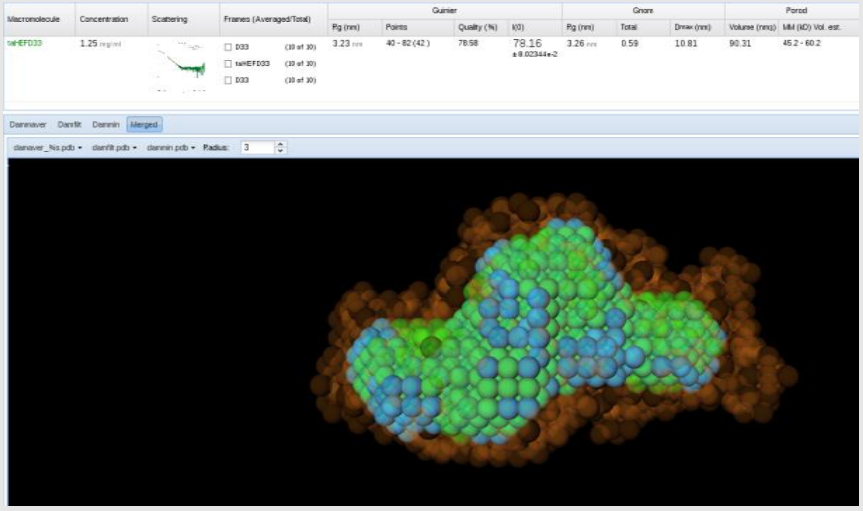

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Advanced analysis with aditional information

A simple example of what can be done with information from complimentary techniques!

Fitting to known structures!

Automated processing can be extended:

Additional information such as PDB's of possible conformations

Experimental X-ray scattering of the PYR1 protein in solution in the presence of 1mM (+) ABA.

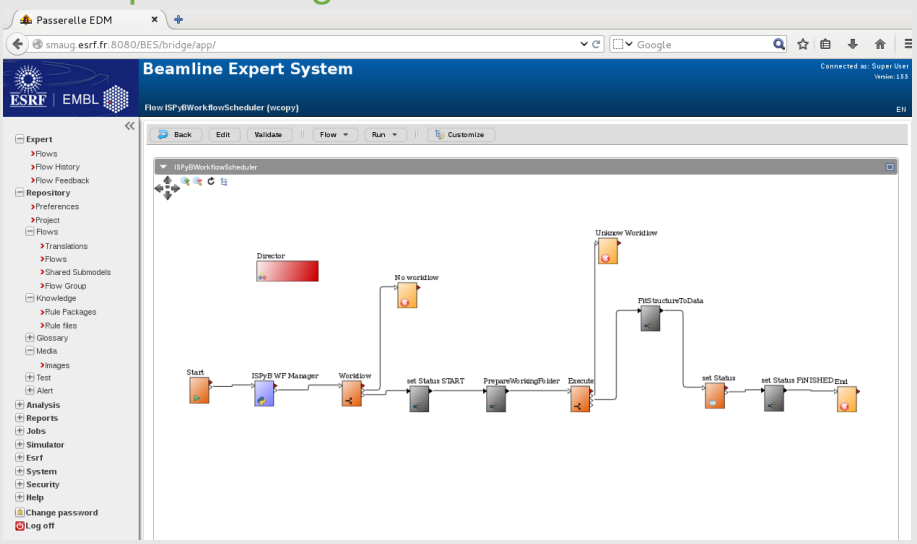
Scattering curves for possible ensembles were calculated.

Only the curve for ensembles AB/CD produced a good fit to the experimental data (χ=0.72)

SAXS demonstrated that the AB ensemble corresponds to the biologically relevant form found under physiological conditions.

J Santiago *et al. Nature* **462**, 665-668 (3 December 2009)

Data processing with *A Priori* information



Online / Offline Data processing

Online Processing essential for fast feedback
- check for data integrity
  - radiation damage
  - concentration effects
  - appropriate background measurements

Reprocessing datasets if needed

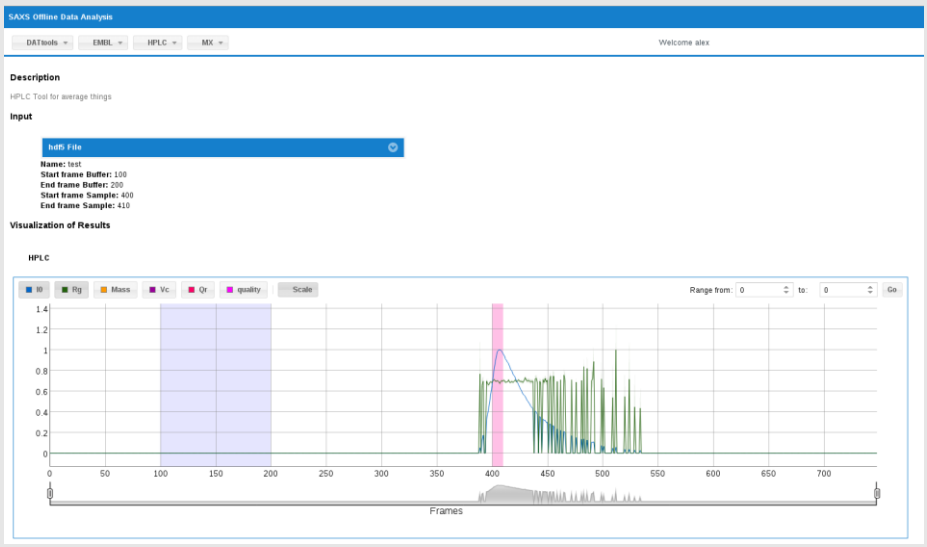
More in-depth analysis can be done but is more time consuming

More comprehensive feedback

Use of a priori information for advanced analysis

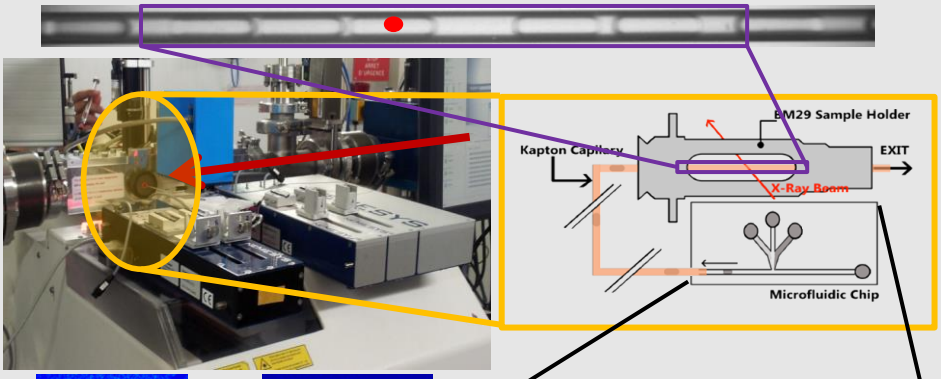
Online / Offline Data processing

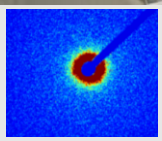
Online / Offline Data processing

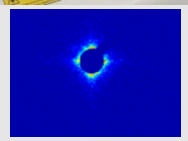


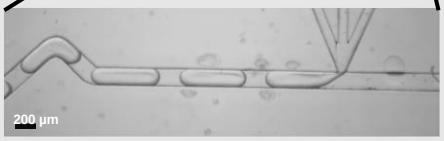
SC compatible Microfluidics: First results

Proof of Principle achieved



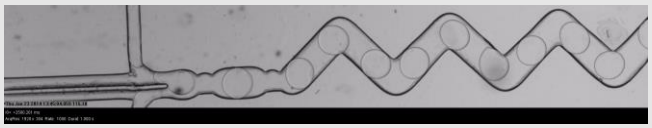


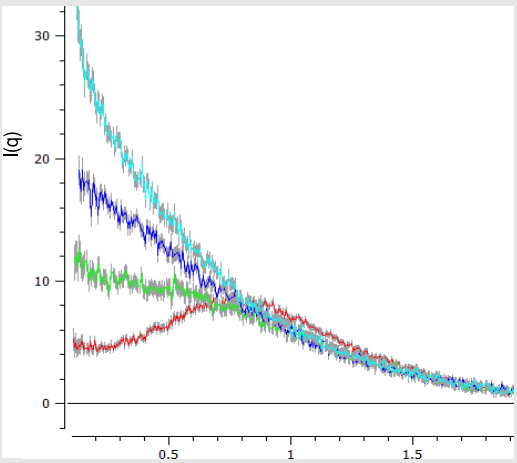


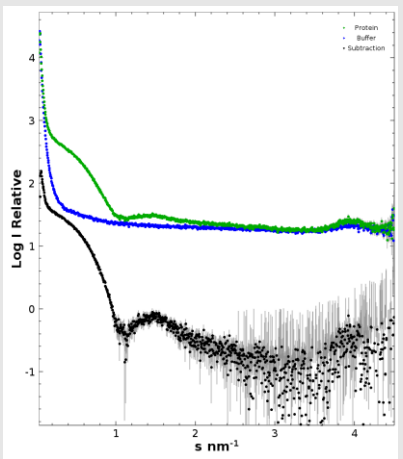


Background improved with scatterless pinhole

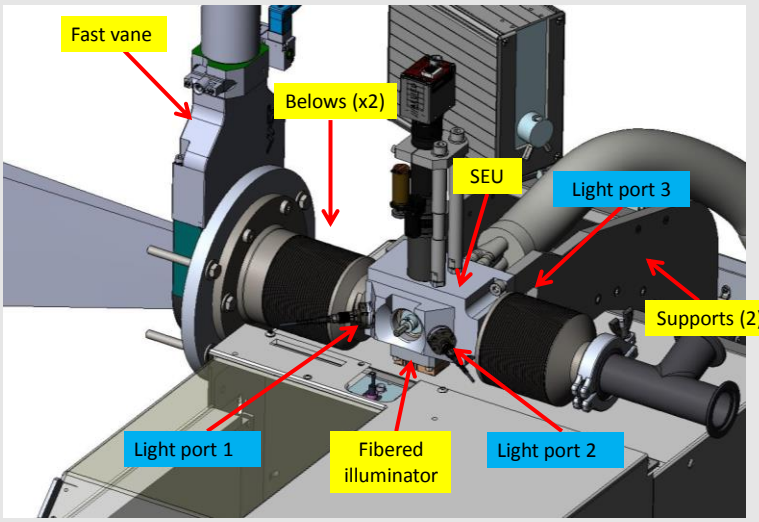
Microfluidics







SEU Upgrade



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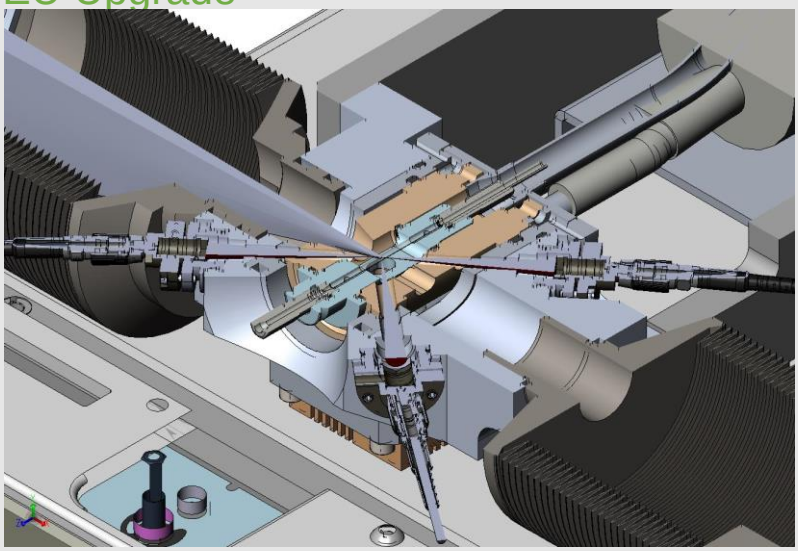

Light ports for DLS, Raman, absorption spectrometry, fluorescence, photo activation

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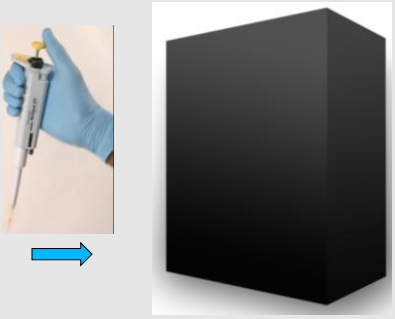
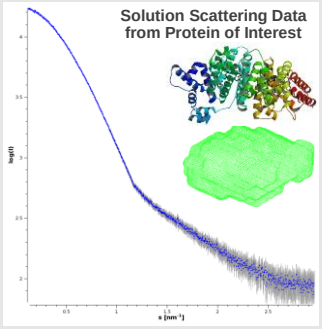
The black box should not be scary

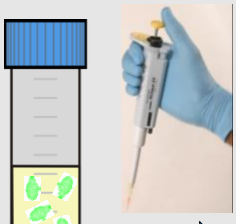




Black Box

Neutron/X-ray beamline
Home source

The black box should not be scary
It should be reasuring that it is accessible



Automated data acquisition

With feedback

Acknowledgments

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LABORATOIRE DE GÉNIE CHIMIQUE TOULOUSE • UMR 5503
Université de Toulouse
NEUTRONS FOR SCIENCE
Institut Laue-Langevin
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