

# BioSAXS BM29

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EMBL-Grenoble



## Overview

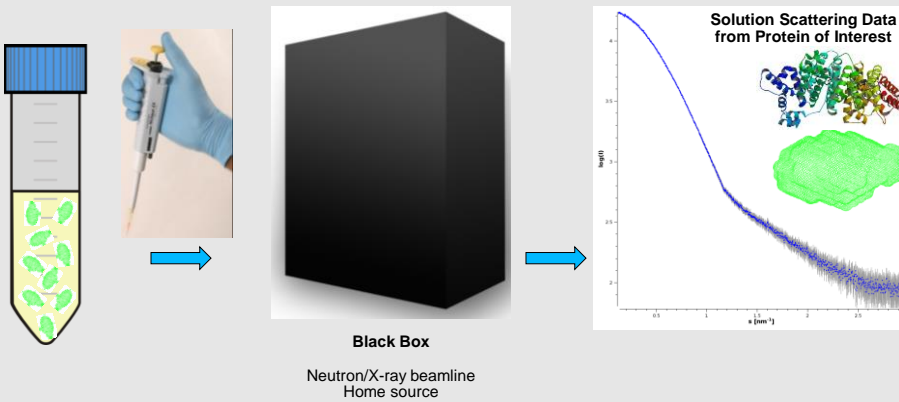
- **Beamline optimised for**
  - Stability
  - Reliability
  - Usability
- **Ease of use through automation of**
  - Data collection
  - Data reduction
  - Data processing
  - Data analysis
- **Confidence in experiments through feedback**
  - User oriented focus to guide users
    - In experiment preparation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



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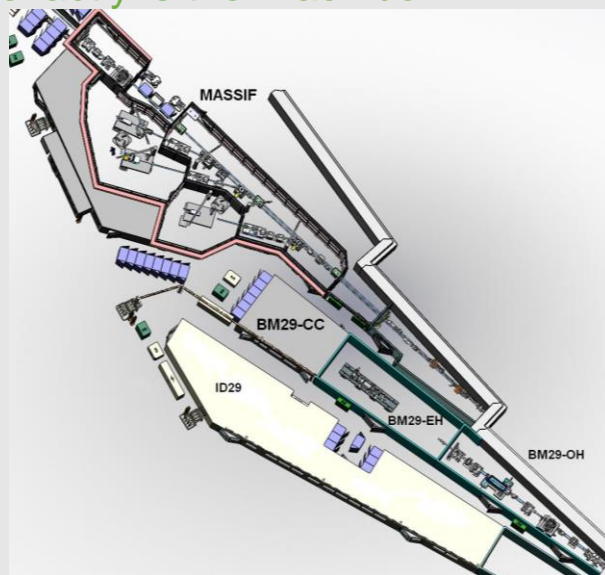
## Inexperienced User's view of an experiment



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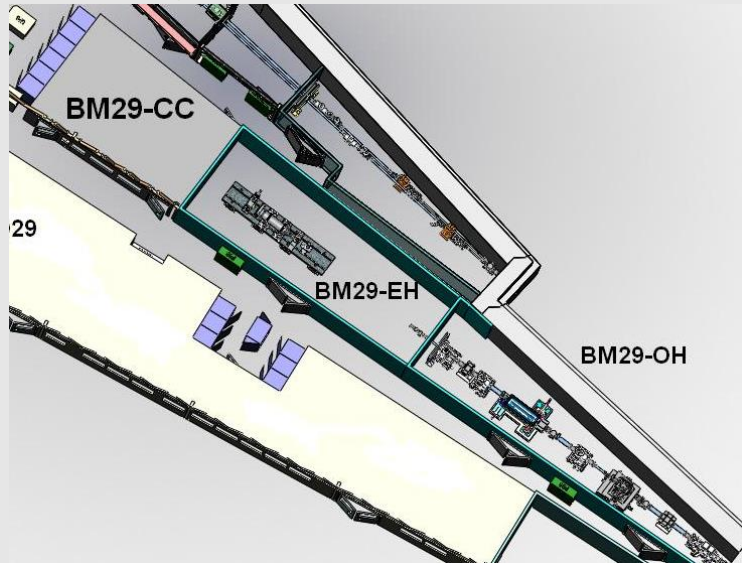
## What exactly is the Black box?



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## Current status of data collection:

### Temperature

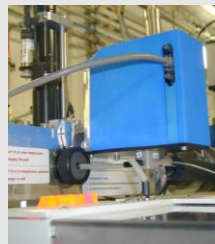
- Data acquisition between 4 and 60 C
- SEC operation at 4 or 20 degrees C

### 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  $\mu\text{L}$  per measurement
  - Approx. 5 mg/mL
  - 100  $\mu\text{L}$  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



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## Fully Automated Data Collection



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

Collaboration between EMBL (GR and HH) and Diamond funded by BioSTRUCT-X



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## Improved feedback for experimental preparation

BIOSAXS Experiment Designer

**Define Measurements**  
Define only the macromolecule's measurement you want to make. This wizard will add buffers' measurement needed for subtraction automatically.

Single Measurement | Concentration Series

Macromolecules: PGK Buffer: AMP

How many unknown concentrations do you have?: 3

Exposure. Temp.: 4 Vol. To Load (µl): 50 Transmission (%): 100

Wait Time: 0 Viscosity: low Flow:

Add

**Measurements**

	Specimen			Parameters					Comments	
	Macromo.	Conc. (mg/ml)	Buffer	Exp. Temp.	Vol. Load	Trans.	Wait T.	Flow		
<input checked="" type="checkbox"/>	PGK	1.000	<input checked="" type="checkbox"/> AMP	4.00 c	50.00 µl	100 %		yes	low	<input type="button" value="REMOVE"/>
<input checked="" type="checkbox"/>	PGK	2.000	<input checked="" type="checkbox"/> AMP	4.00 c	50.00 µl	100 %		yes	low	<input type="button" value="REMOVE"/>
<input checked="" type="checkbox"/>	PGK	3.000	<input checked="" type="checkbox"/> AMP	4.00 c	50.00 µl	100 %		yes	low	<input type="button" value="REMOVE"/>



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## Improved feedback for experimental preparation

Estimation of required Volume	
Go to Shipment	
Specimen	Estimated Volume
ATP	300.00 $\mu$ l
PGK + ATP	150.00 $\mu$ l
PGK + common buffer	150.00 $\mu$ l
PGK + p38buffer	150.00 $\mu$ l
common buffer	300.00 $\mu$ l
p38buffer	300.00 $\mu$ l



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## Improved feedback with ISPyB

Specimen		Parameters							Status	Time	Comments
Macro.	Conc. (ng/ml)	Buffer	Exp. Temp.	Vol. Load	Trans.	Wat. T.	Flow	Viscosity			
<input type="checkbox"/>		D33	20.00 c	100.0...	100 %		yes	Low	<b>DONE</b>	06:47:07 pm	buffer
<input checked="" type="checkbox"/>	14.000	D33	20.00 c	150.0...	100 %		yes	Low	<b>DONE</b>	06:48:23 pm	[1] tahef d33 truncation
<input type="checkbox"/>		D33	20.00 c	100.0...	100 %		yes	Low	<b>DONE</b>	06:49:41 pm	buffer
<input checked="" type="checkbox"/>	7.000	D33	20.00 c	90.00 $\mu$ l	100 %		yes	Low	<b>DONE</b>	06:50:54 pm	[2] tahef d33 truncation
<input type="checkbox"/>		D33	20.00 c	100.0...	100 %		yes	Low	<b>DONE</b>	06:52:09 pm	buffer
<input checked="" type="checkbox"/>	3.500	D33	20.00 c	90.00 $\mu$ l	100 %		yes	Low	<b>DONE</b>	06:53:25 pm	[3] tahef d33 truncation
<input type="checkbox"/>		D33	20.00 c	100.0...	100 %		yes	Low	<b>DONE</b>	06:54:40 pm	buffer
<input checked="" type="checkbox"/>	1.250	D33	20.00 c	90.00 $\mu$ l	100 %		yes	Low	<b>DONE</b>	06:55:56 pm	[4] tahef d33 truncation
<input type="checkbox"/>		D33	20.00 c	100.0...	100 %		yes	Low	<b>DONE</b>	06:57:11 pm	buffer
<input checked="" type="checkbox"/>	0.610	D33	20.00 c	90.00 $\mu$ l	100 %		yes	Low	<b>DONE</b>	06:58:28 pm	[5] tahef d33 truncation
<input type="checkbox"/>		D33	20.00 c	100.0...	100 %		yes	Low	<b>DONE</b>	06:59:43 pm	buffer

Ready

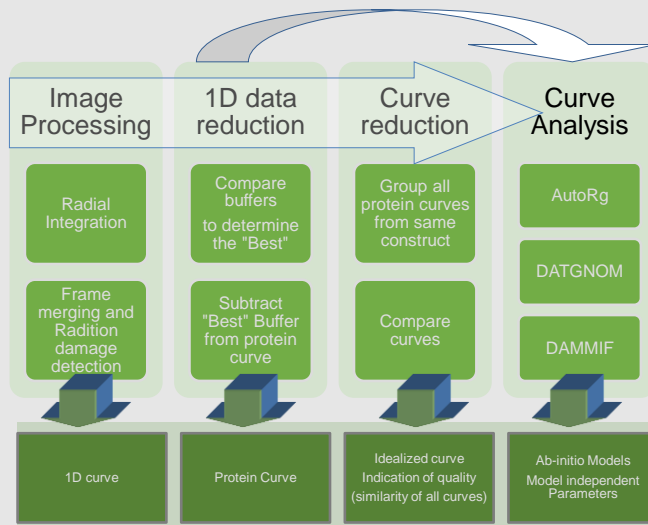
1- Deep Well	2- 4 x (8 + 3) Block	3- 96 Well plate



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# Data Processing: ATSAS tools in EDNA



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# Improved feedback with ISPyB

Macromolecule	Concentration	Scattering	Frames (Averaged/Total)	Rg (nm)	Points	Quality (%)	I(0)	Rg (nm)	Total	Dmax (nm)	Volume (nm <sup>3</sup> )	MM (kD)	Vol. est
taHEFD33	14.00 mg/ml		<ul style="list-style-type: none"> <li>033 (10 of 10)</li> <li>taHEFD33 (10 of 10)</li> <li>033 (1 of 10)</li> </ul>	4.75 nm	19 - 37 (18)	83.95	90.78 ± 6.88462...	4.94 nm	0.51	24.09	154.27	77.1 - 102.8	
taHEFD33	7.00 mg/ml		<ul style="list-style-type: none"> <li>033 (11 of 10)</li> <li>taHEFD33 (5 of 10)</li> <li>033 (5 of 10)</li> </ul>	3.97 nm	12 - 42 (30)	92.14	71.21 ± 4.3899e+2	3.91 nm	0.44	13.90	112.54	56.3 - 75.0	
taHEFD33	3.50 mg/ml		<ul style="list-style-type: none"> <li>033 (5 of 10)</li> <li>taHEFD33 (10 of 10)</li> <li>033 (10 of 10)</li> </ul>	3.37 nm	50 - 77 (27)	72.77	59.53 ± 6.59654...	3.44 nm	0.53	11.81	95.25	47.6 - 63.5	
taHEFD33	1.25 mg/ml		<ul style="list-style-type: none"> <li>033 (10 of 10)</li> <li>taHEFD33 (10 of 10)</li> <li>033 (10 of 10)</li> </ul>	3.23 nm	40 - 82 (42)	78.58	78.16 ± 9.02344e+2	3.26 nm	0.59	10.81	90.31	45.2 - 60.2	
taHEFD33	0.61 mg/ml		<ul style="list-style-type: none"> <li>033 (10 of 10)</li> <li>taHEFD33 (10 of 10)</li> <li>033 (10 of 10)</li> </ul>	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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## Advanced analysis with additional information

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

# Fitting to known structures!



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## 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 ( $\chi=0.72$ )

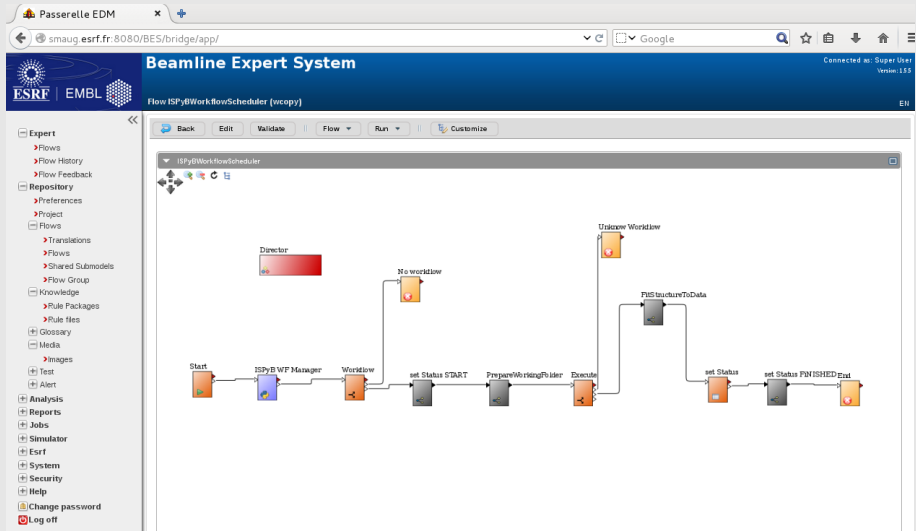
**SAXS demonstrated that the AB ensemble corresponds to the biologically relevant form found under physiological conditions.**



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## 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

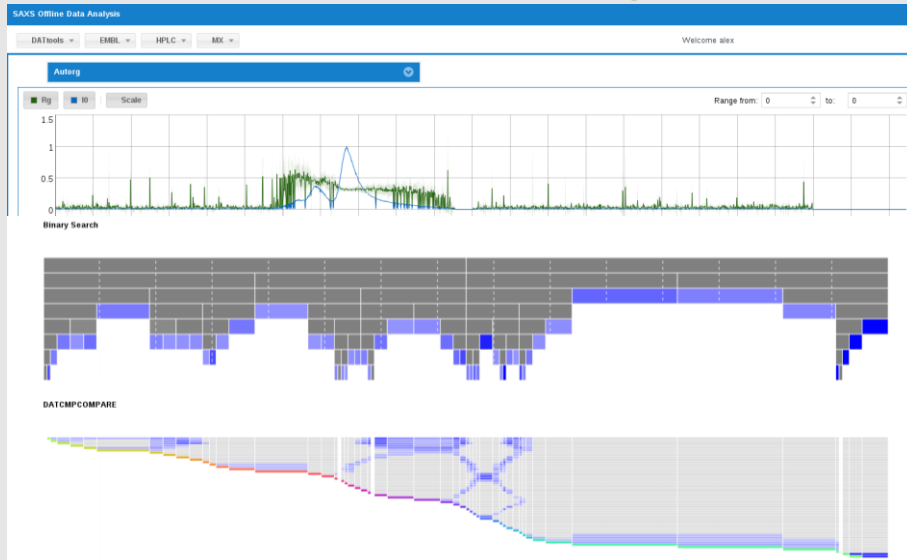
More in-depth analysis can be done but is more time consuming

More comprehensive feedback

Use of a priori information for advanced analysis



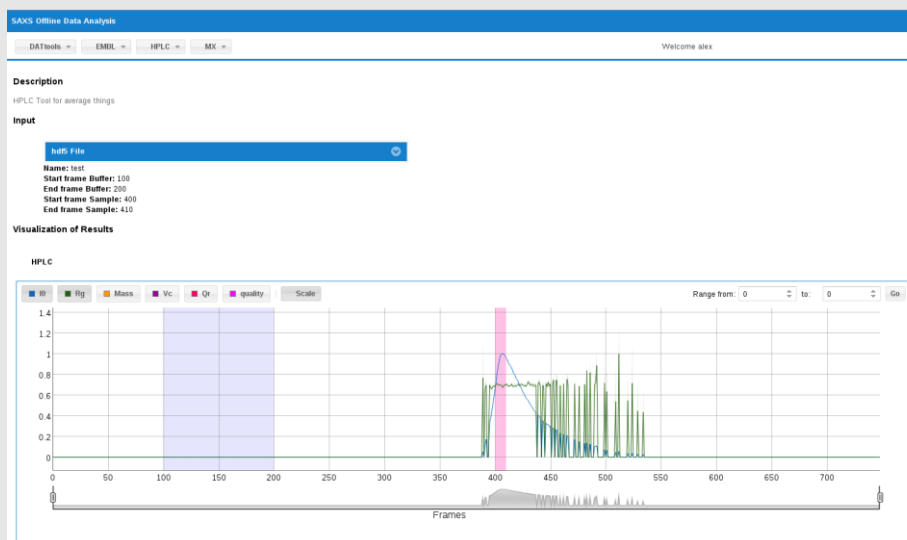
# Online / Offline Data processing



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# Online / Offline Data processing

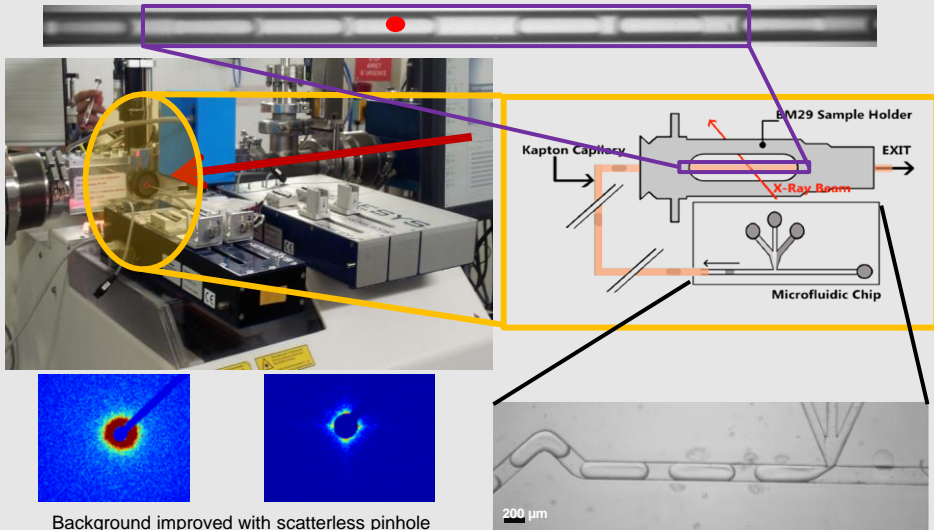


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# SC compatible Microfluidics: First results

Proof of Principle achieved



Background improved with scatterless pinhole

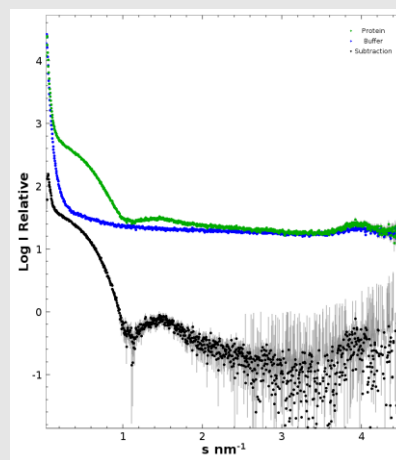
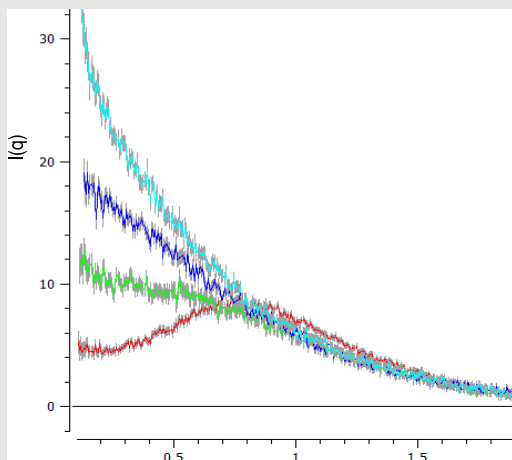
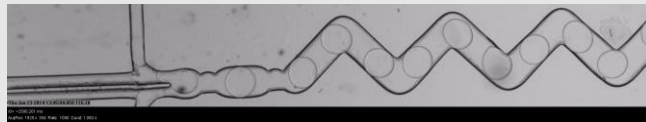
200 μm



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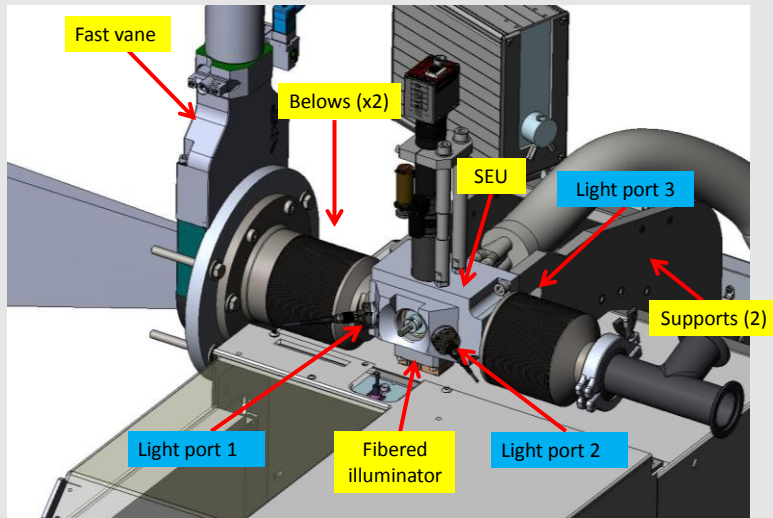
## Microfluidics



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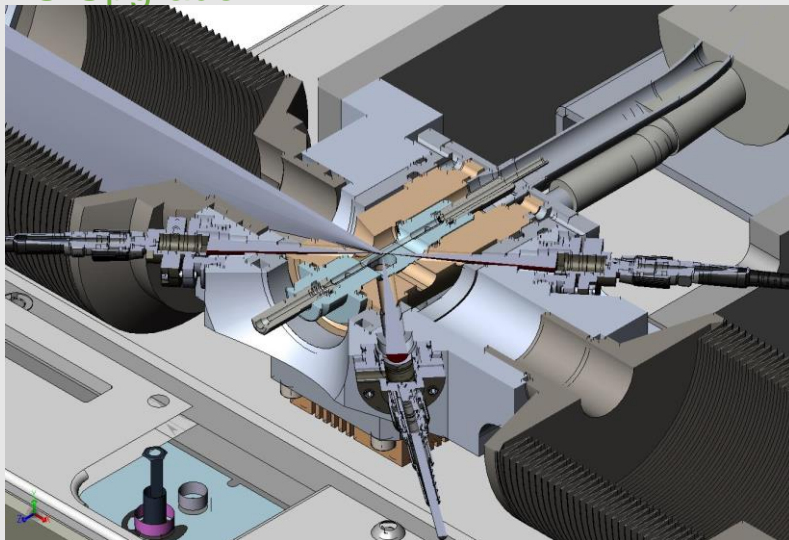
## SEU Upgrade



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## SEU Upgrade



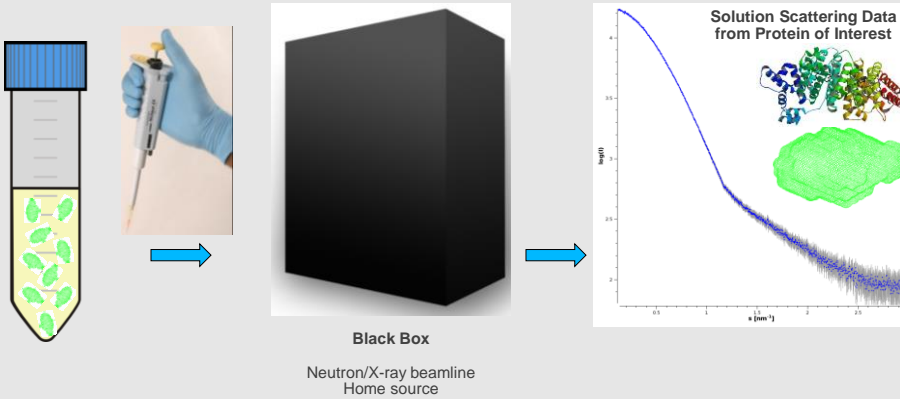
Light ports for DLS, Raman, absorption spectrometry, fluorescence, photo activation



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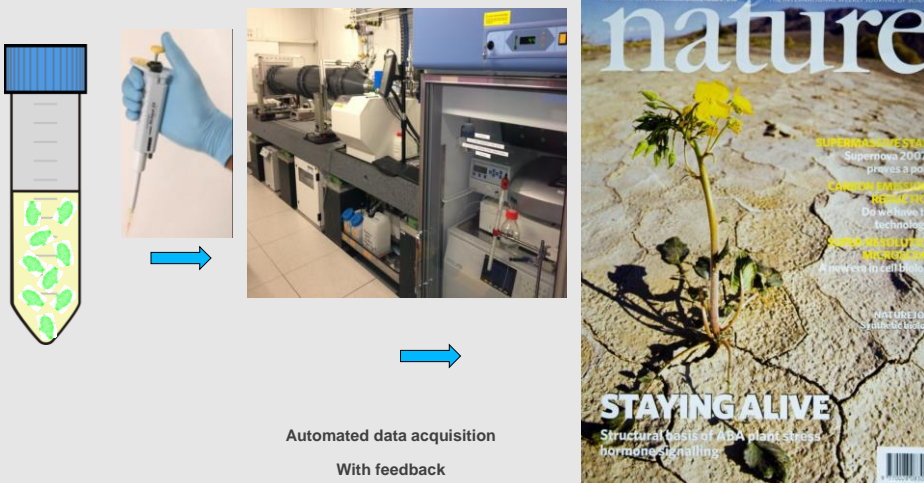
# The black box should not be scary



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



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## Acknowledgments



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An aerial photograph of the ESRF Grenoble facility at night, showing the large white dome and various buildings illuminated against a dark sky with mountains in the background.

**EMBO Practical Course**  
The programme for this event was reviewed and approved by the EMBO Course Committee.

**Small Angle Neutron and X-ray Scattering from proteins in solution**

18 – 22 May 2015 | Grenoble | France



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