Solution SAXS in Grenoble

What can be done and how to do it

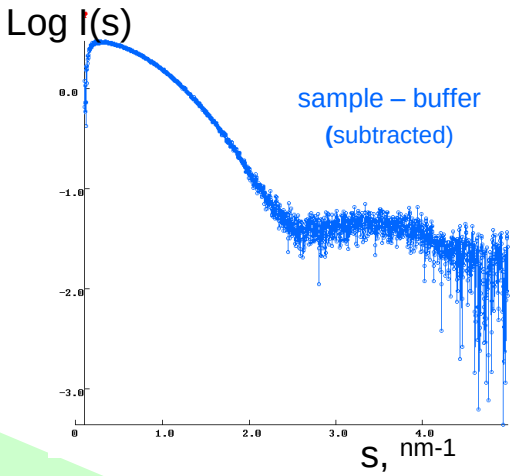
Adam Round

EMBL
40 YEARS | 1974–2014

Contents

- What is Solution SAS
- What can you learn from solution SAS
  - Basics of what can be obtained
  - Analytical analysis
  - Modelling
  - Examples
- How to collect data
  - Automation
    - Data collection and analysis

Solution scattering data collection



X-ray Beam

X-ray Scattering

Sample

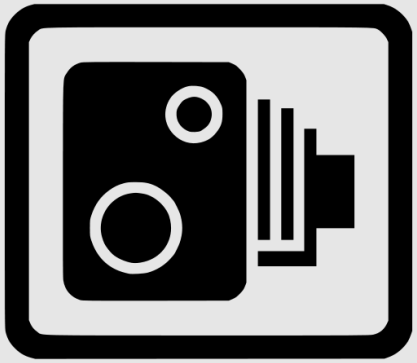
X-ray detector

Thanks to A. Kikhney for this slide

Experimental Equipment: The Basics

Source of Radiation
Neutrons / X-rays

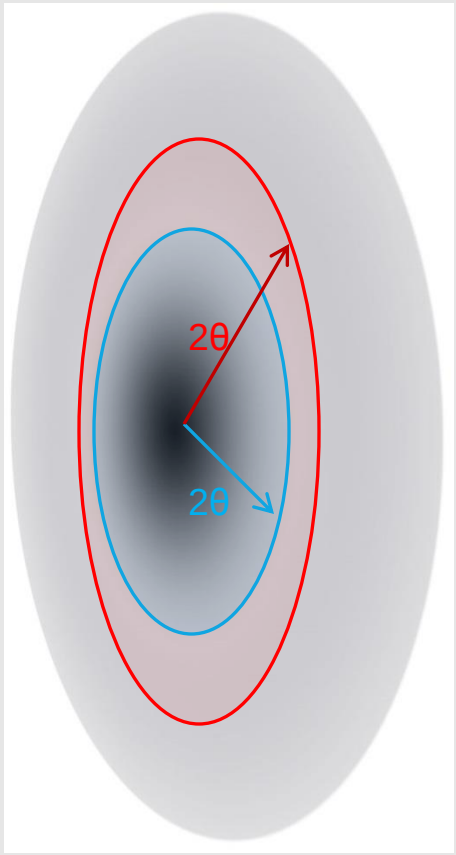
Sample Holder



Detector

Calibration

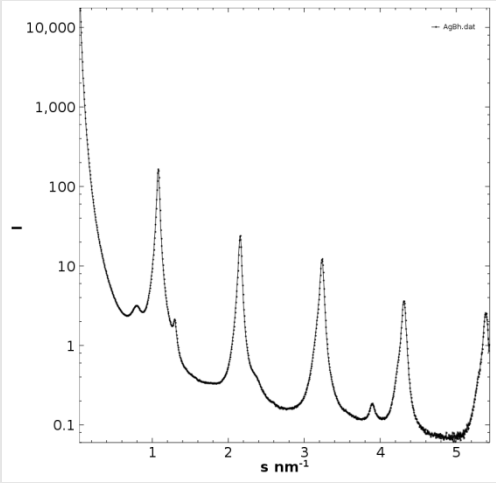
Conversion 2D to 1D

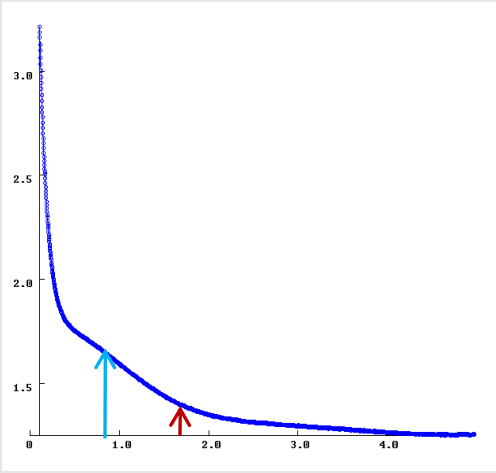


All pixel positions with equal 2θ are averaged

θ converted to s based on known beamline parameters for geometry and λ

Data are scaled for intensity Based on measurement of intensity of direct beam





Performed automatically by Beamline software based on calibration by LC's

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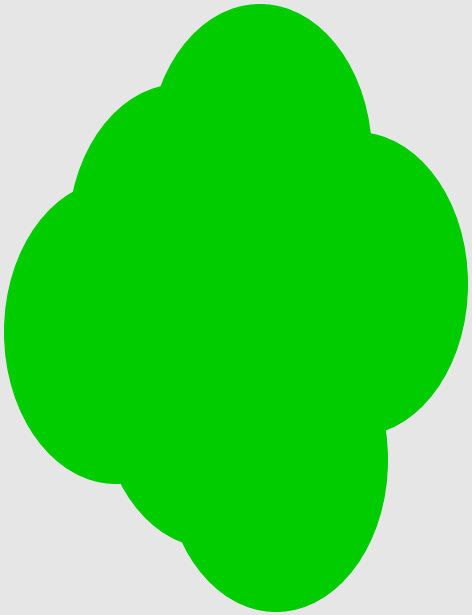
Calibration: Y

Intensity is proportional to: The number of interactions between

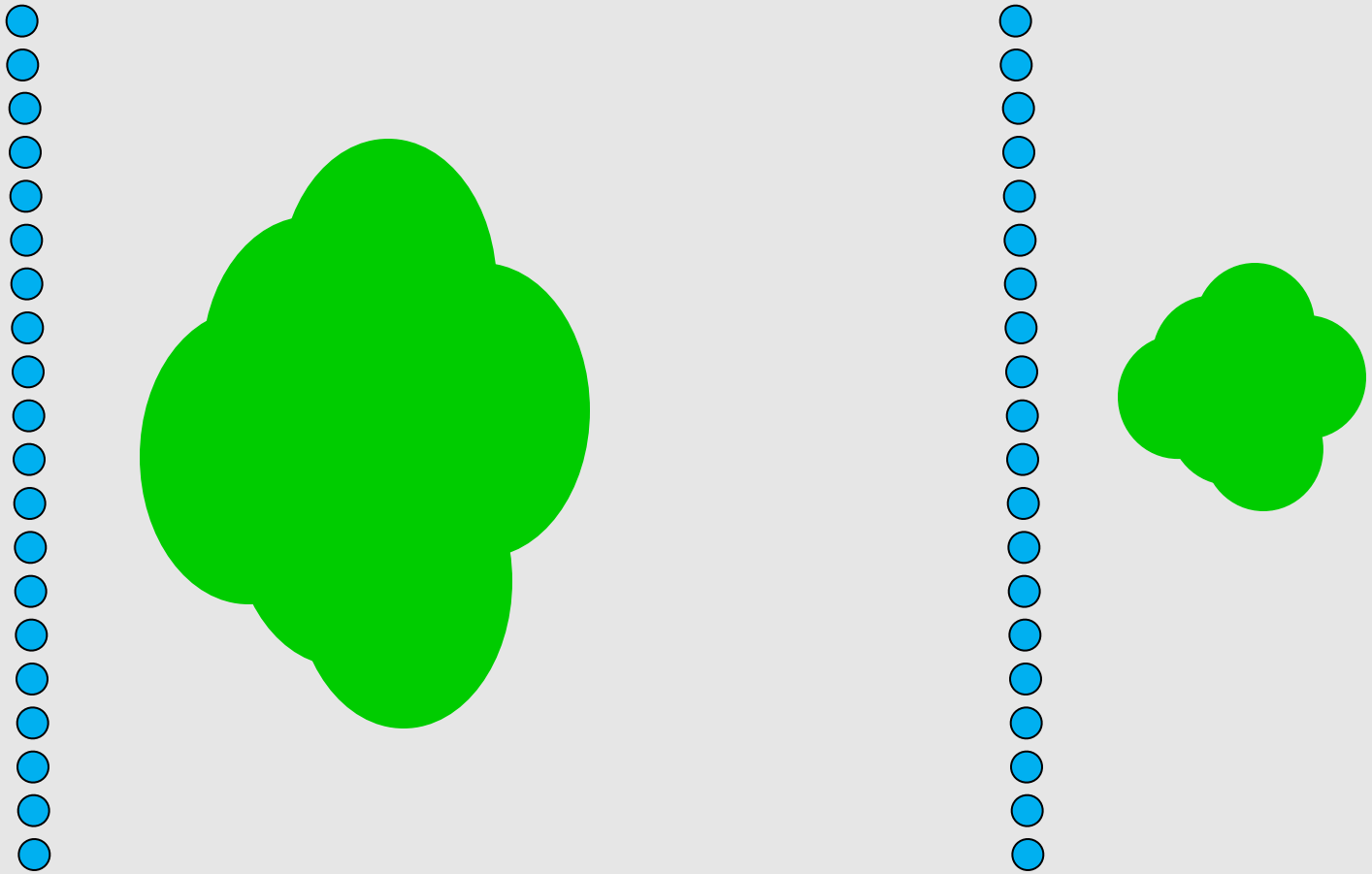
Scattered particles
(Neutrons/X-rays)

And

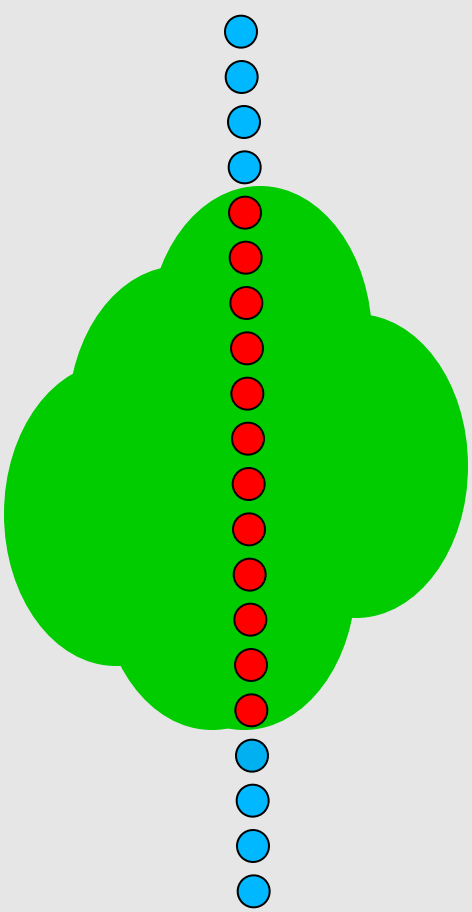


Scattering object
(Biological Macromolecule)

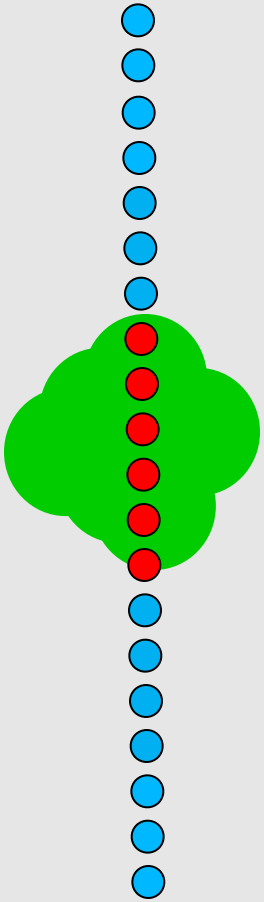
Calibration: Y



Calibration: Y

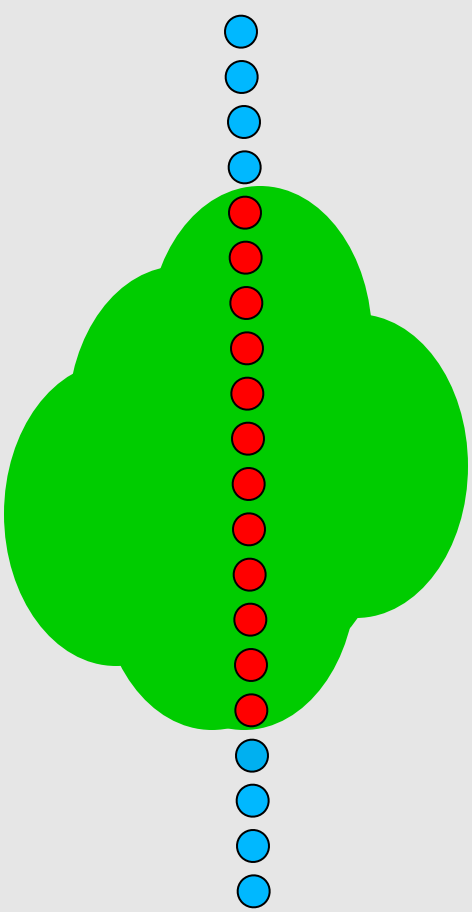


20 12



20 6

Calibration: Y



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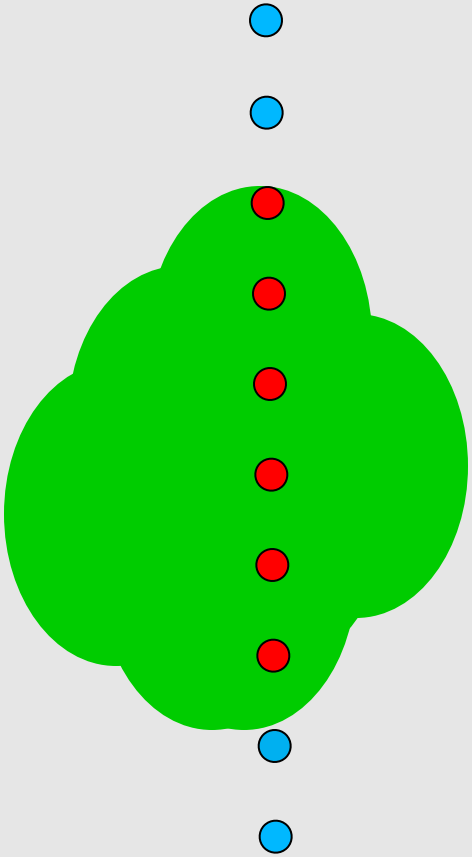
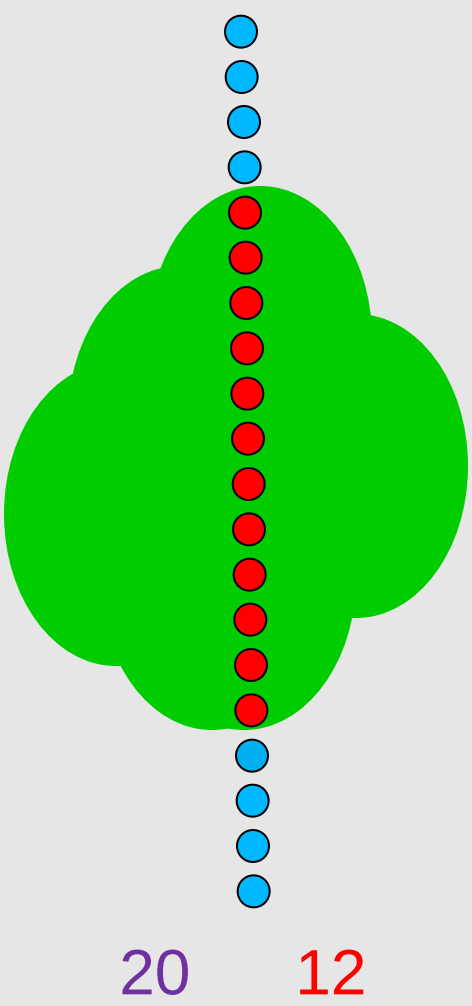
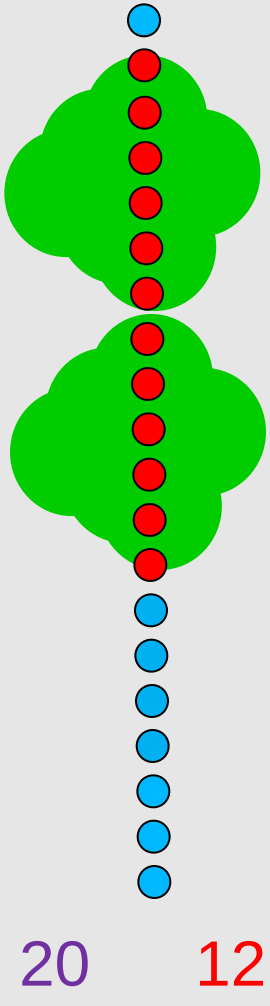


10 6

Calibration: Y





Scattering intensity

Is proportional to: The number of interactions between
Scattered particles (Neutrons/X-rays)
and
Scattering object (Biological Macromolecule)

Therefore if:
Neutron/X-ray flux is normalised

And

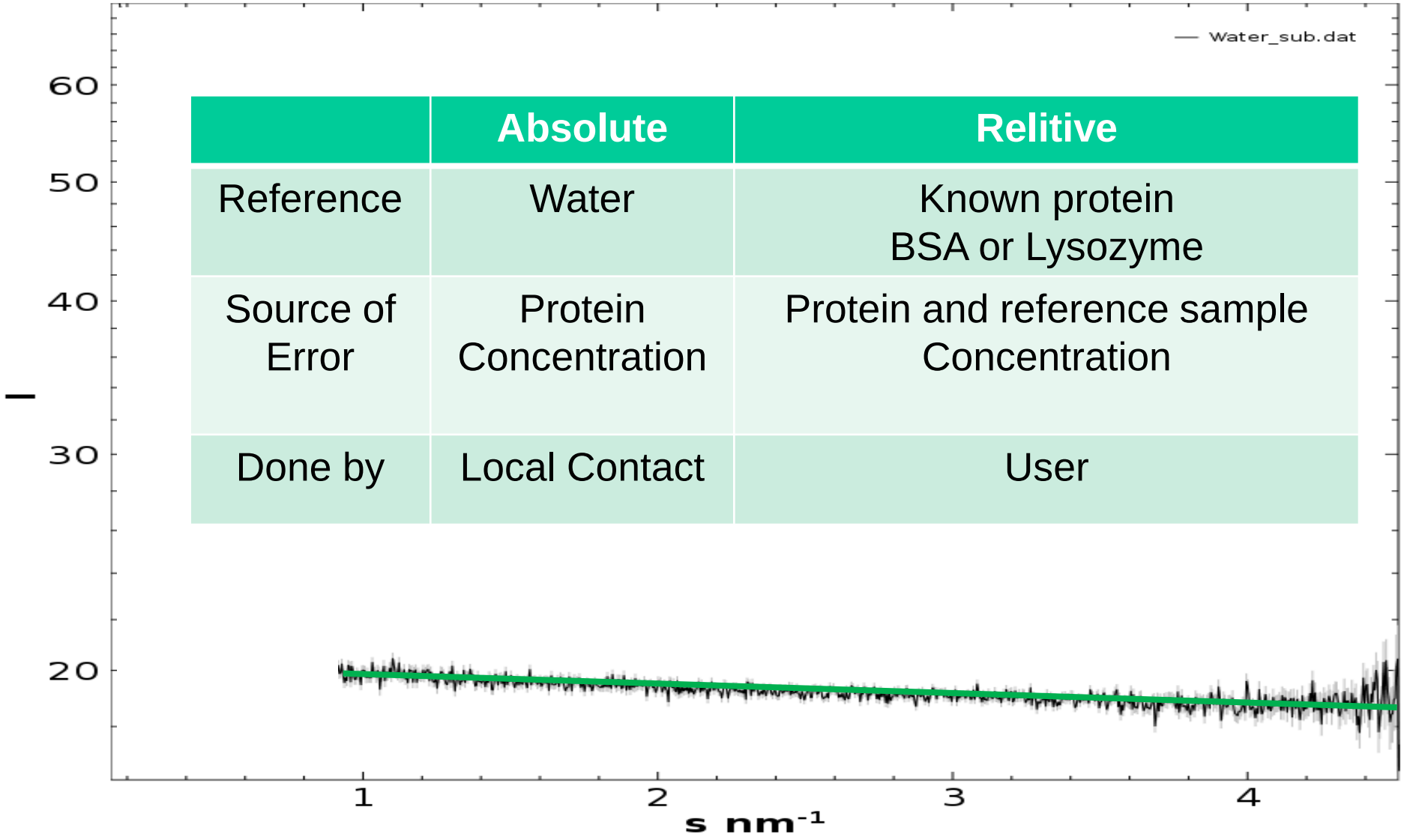
The observed scattering intensity is corrected for the number of scatterers (proportional to concentration)

The resulting measured intensity is proportional to the **size** and contrast of the scattering particles under investigation

Warning! Concentration measurements might not be accurate

Intensity Calibration

	Absolute	Relitive
Reference	Water	Known protein BSA or Lysozyme
Source of Error	Protein Concentration	Protein and reference sample Concentration
Done by	Local Contact	User



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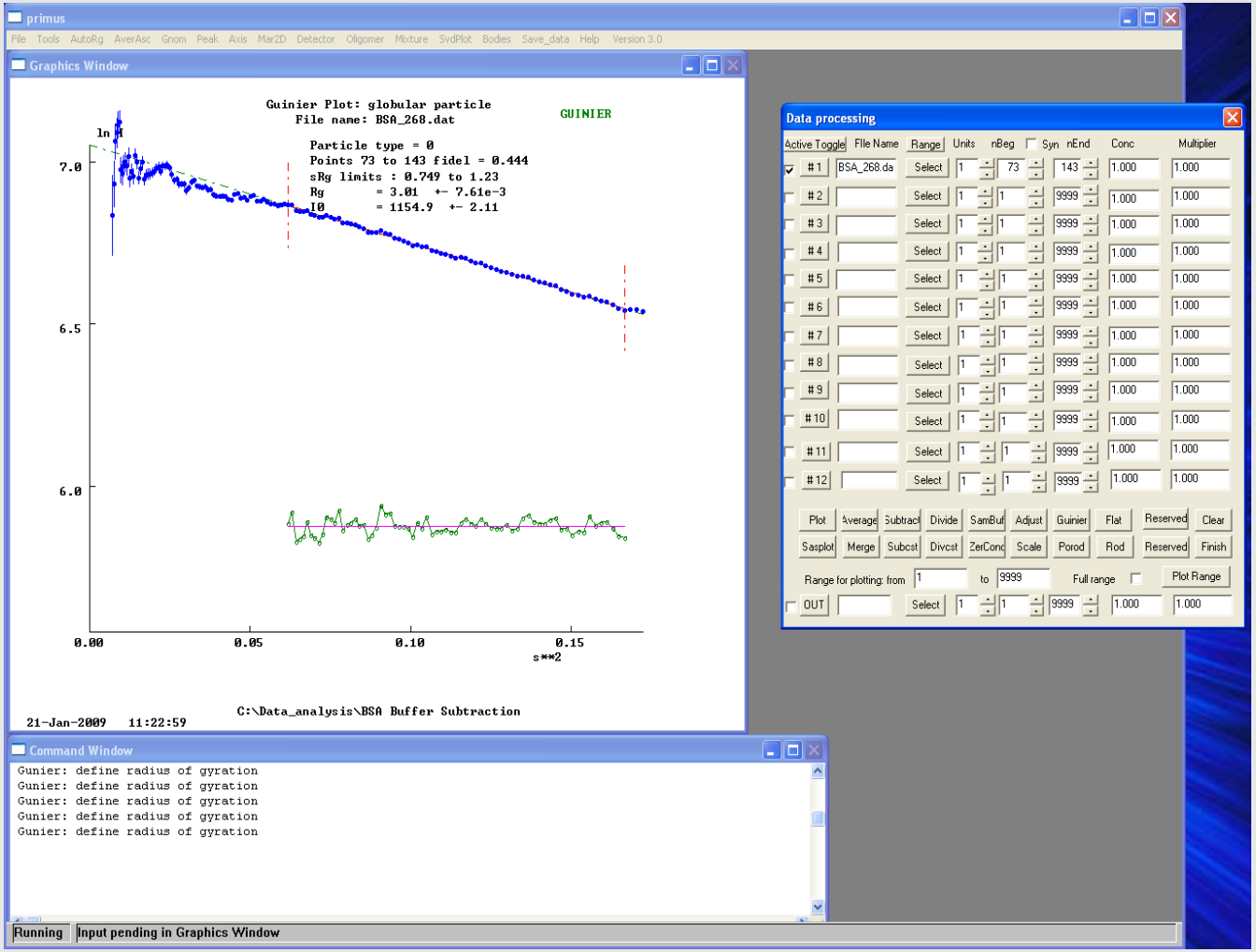
Radius of Gyration and Zero Angle Intensity



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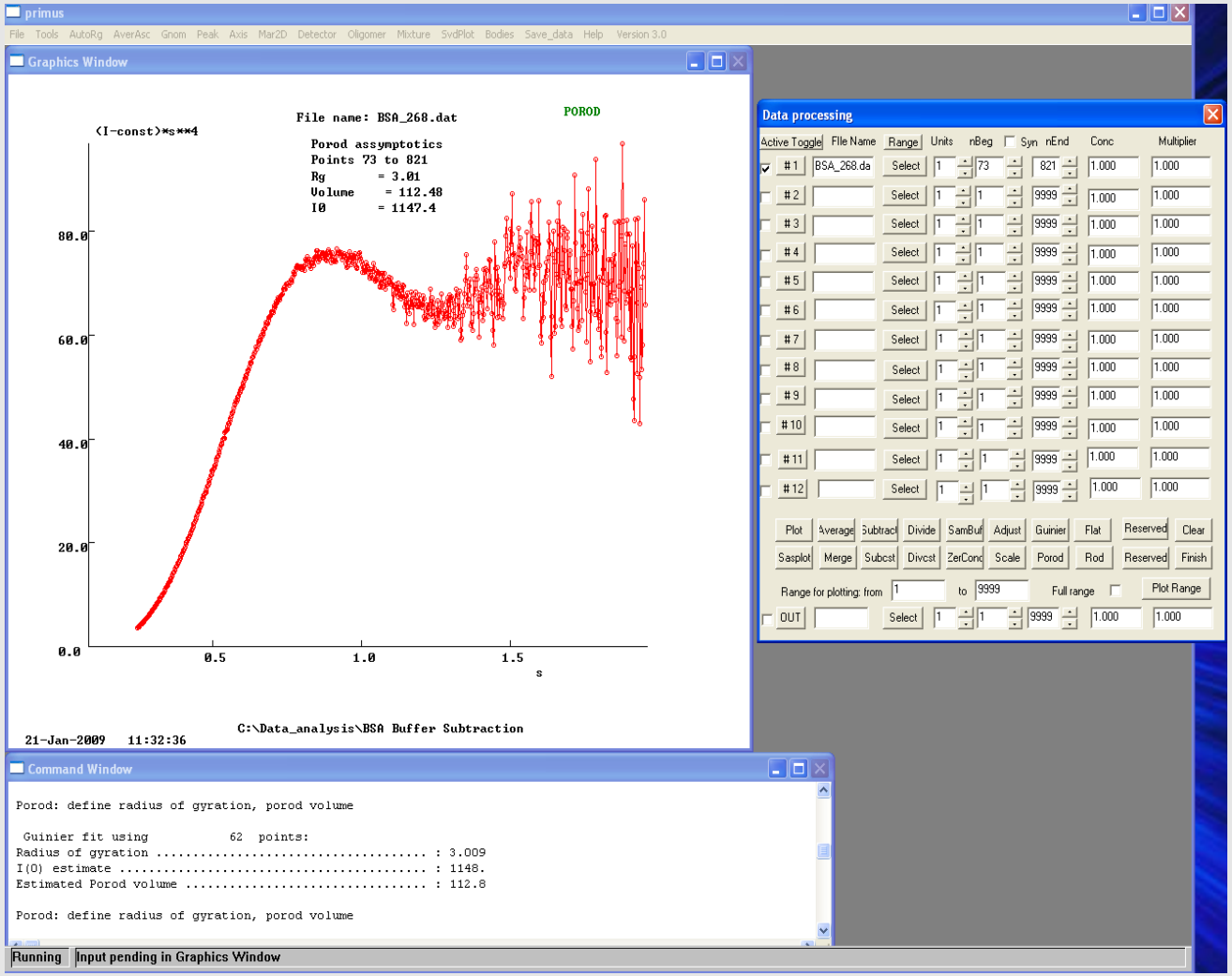
Porod Analysis in Primus gives excluded volume



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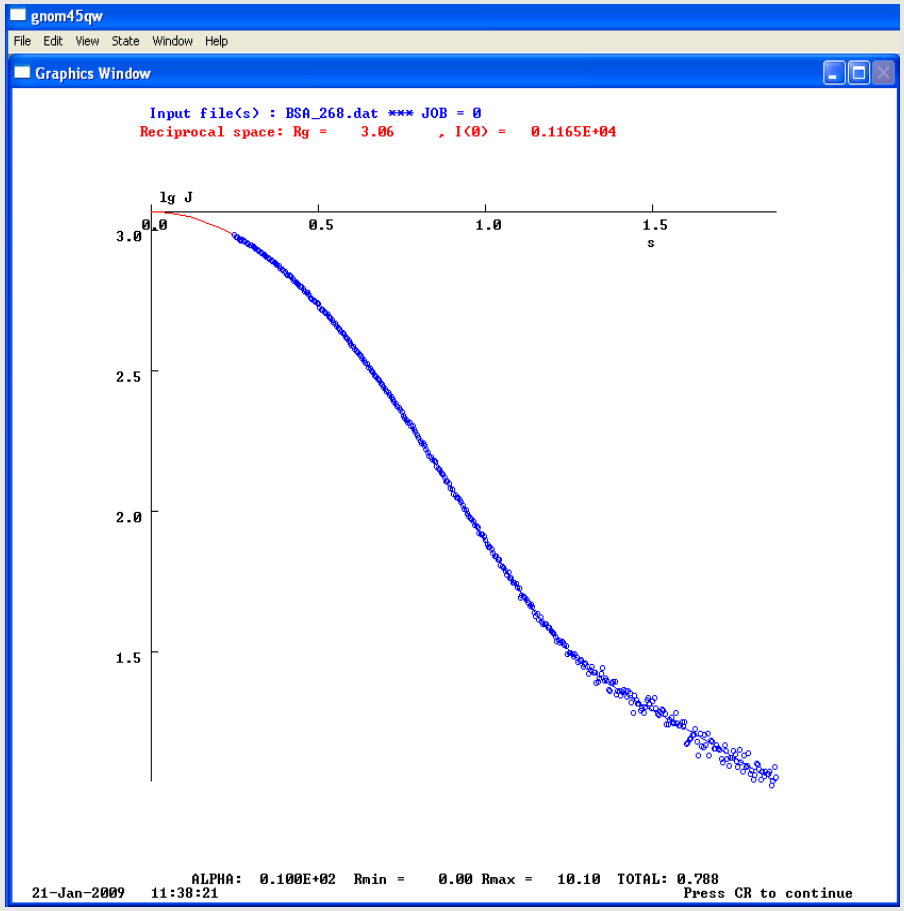
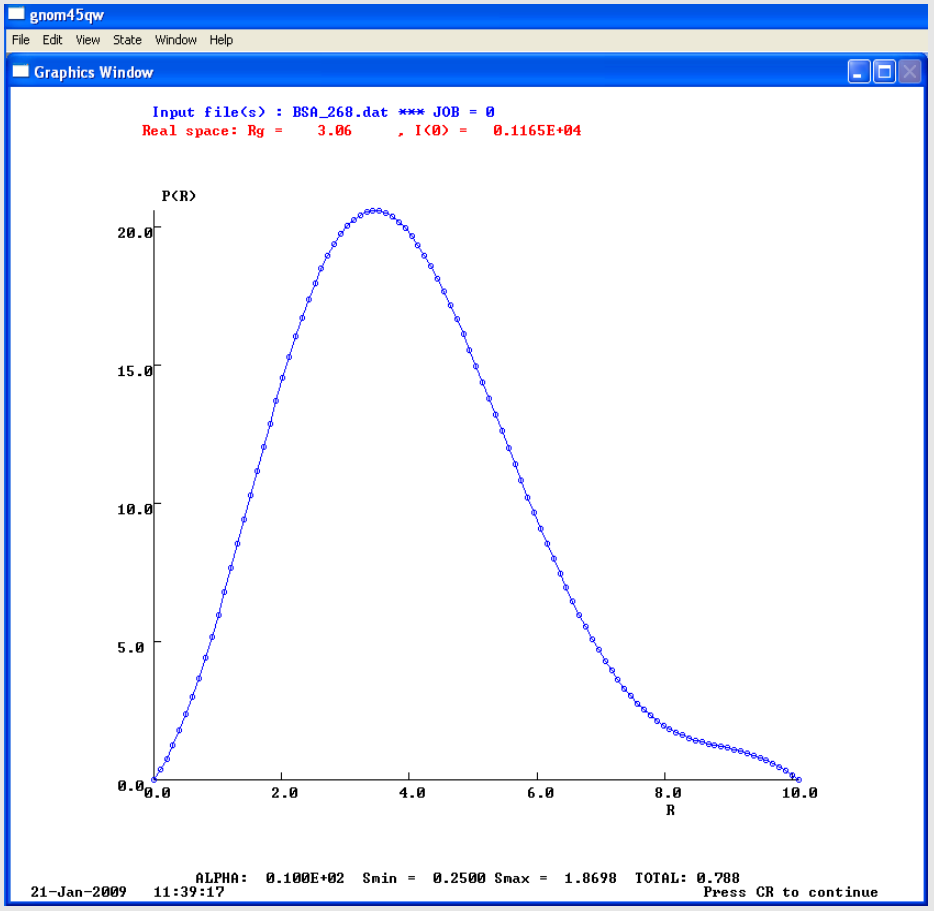
Distance Distribution P(r) Function

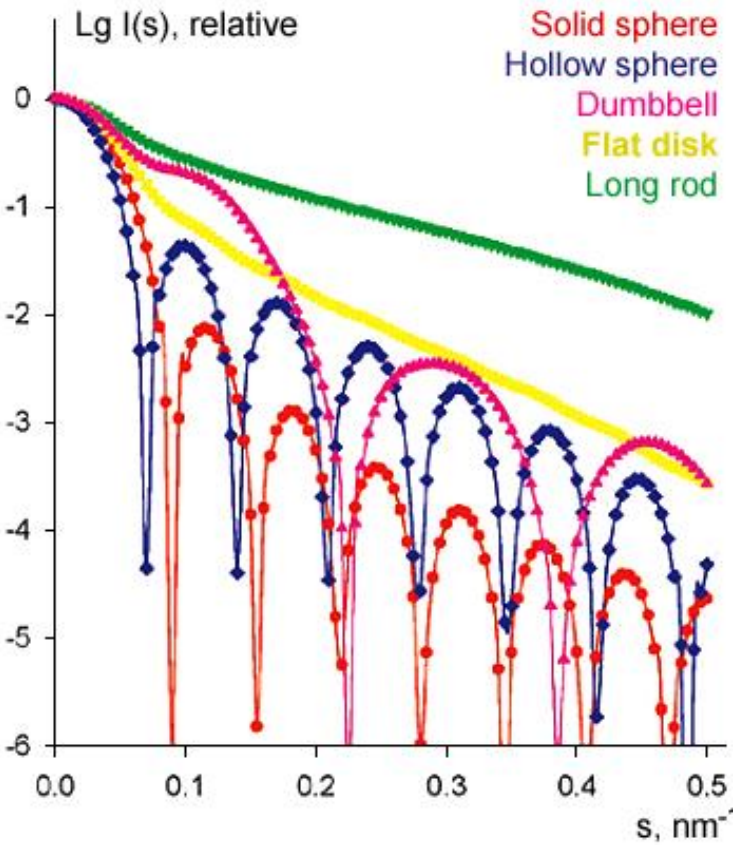
Calculated by GNOM give the Dmax and the input file required for Ab-initio modeling

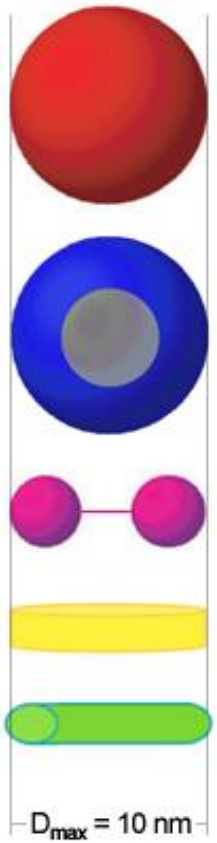


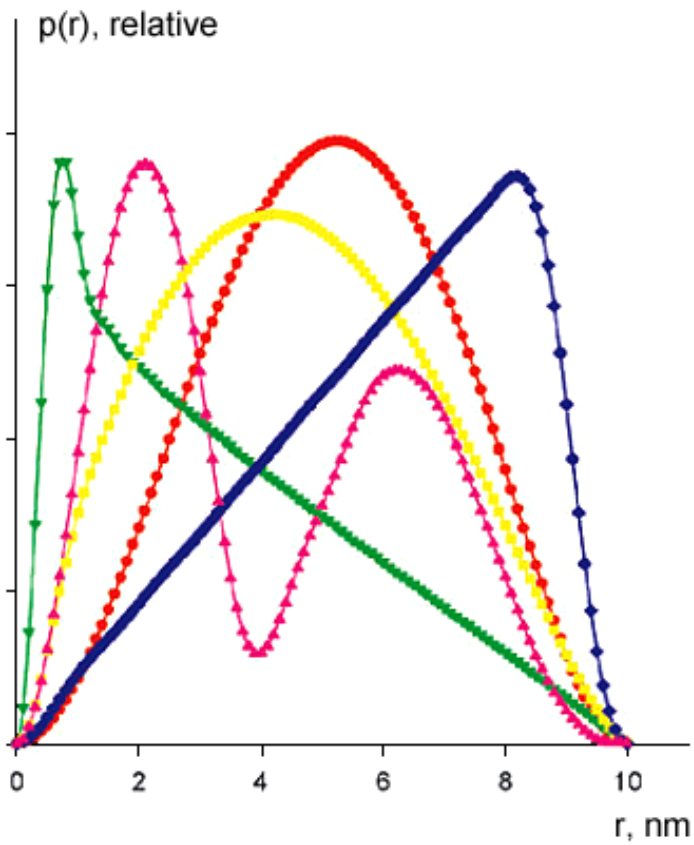


Indirect Fourier Transform!

1D curve and P(r) Function are effected by shape







Indirect Fourier Transform!

What can we learn from solution SAXS

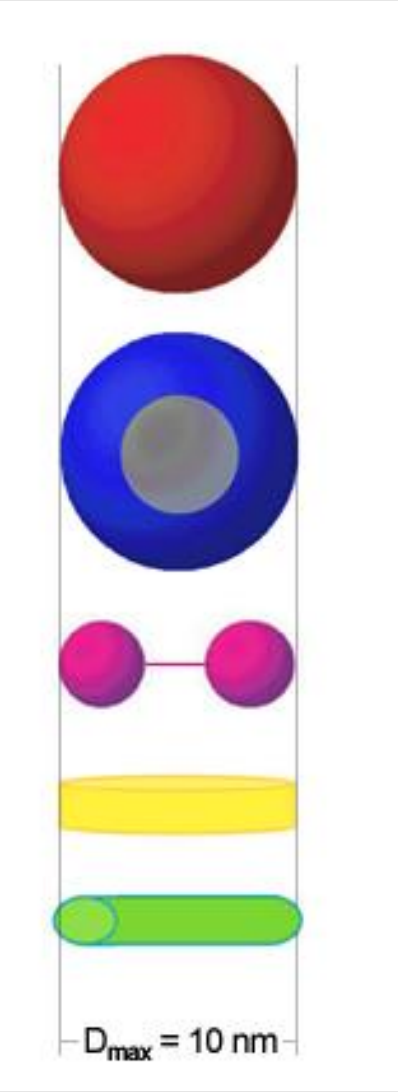
- Model independent parameters:
- Size! Rg, Dmax, Volume and MM estimates.
  Basic shape (Extended or Globular)

- Behaviour in different buffer conditions:
- To assess
  - effects of different buffer formulations
  - if interparticle scattering which shows structural effects (find optimum conditions – monitor behaviour)

- Dynamic investigations under physiological conditions:
  Conformational changes with
  temperature, pH, binding etc.

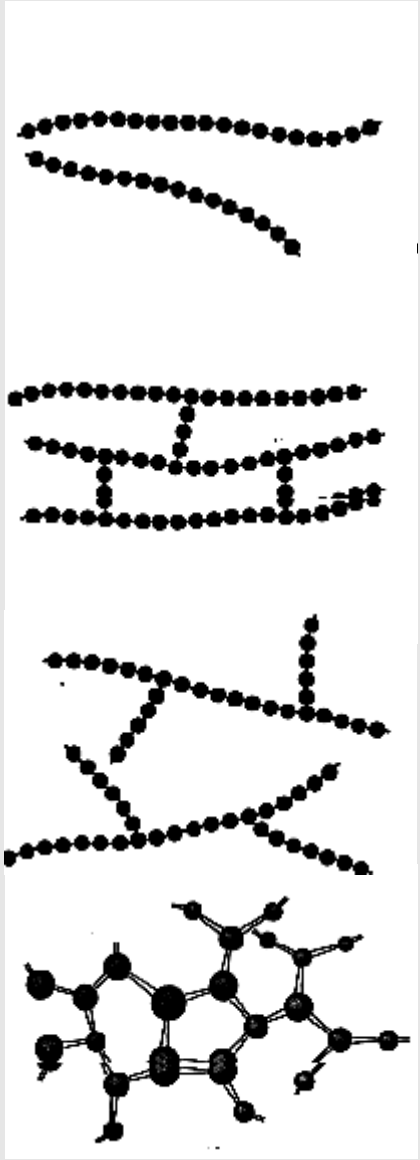
Form Factor fitting of basic shapes

1. Homogeneous sphere
2. Spherical shell
3. Spherical concentric shells
4. Particles consisting of spherical subunits
5. Ellipsoid of revolution
6. Tri-axial ellipsoid
7. Cube and rectangular parallelepipedons
8. Truncated octahedra
9. Faceted Sphere
10. Cube with terraces
11. Cylinder
12. Cylinder with elliptical cross section
13. Toroid
14. Infinitely thin rod
15. Infinitely thin circular disk

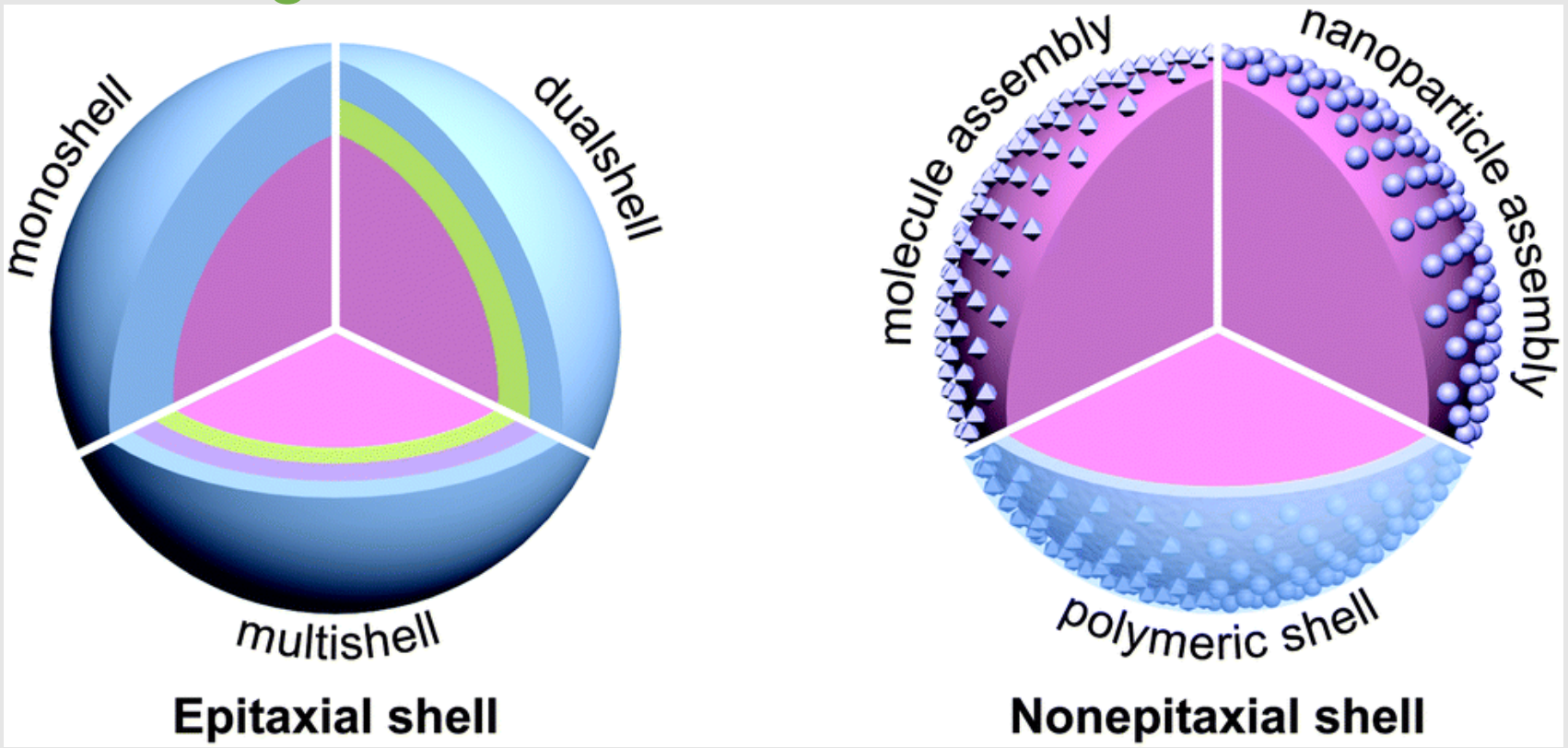


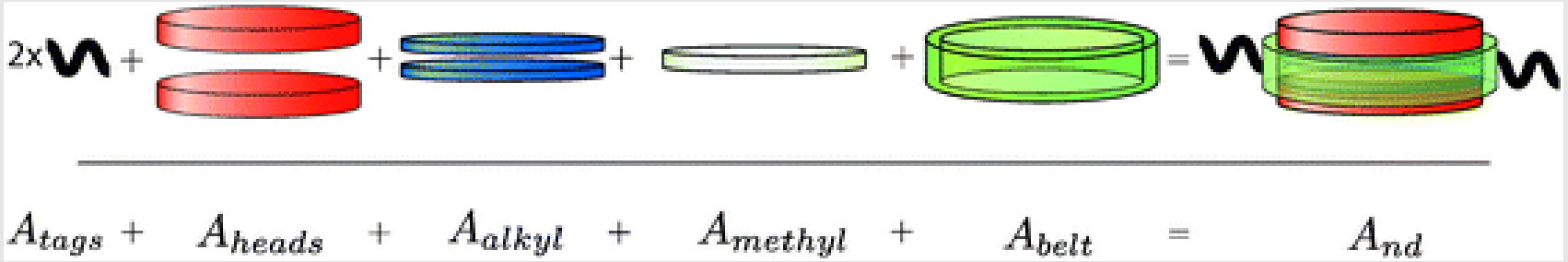
Form Factor fitting of polymers

1. Flexible polymers with Gaussian statistics
2. Polydisperse flexible polymers with Gaussian statistics
3. Flexible ring polymers with Gaussian statistics
4. Flexible self-avoiding polymers
5. Polydisperse flexible self-avoiding polymers
6. Semi-flexible polymers without self-avoidance
7. Semi-flexible polymers with self-avoidance:
8. Polyelectrolyte Semi-flexible polymers with self-avoidance:
9. Star polymer with Gaussian statistics
10. Polydisperse star polymer with Gaussian statistics
11. Regular star-burst polymer (dendrimer) with Gaussian statistics
12. Regular comb polymer with Gaussian statistics
13. Arbitrarily branched polymers with Gaussian statistics
14. Arbitrarily branched semi-flexible polymers
15. Arbitrarily branched self-avoiding polymers



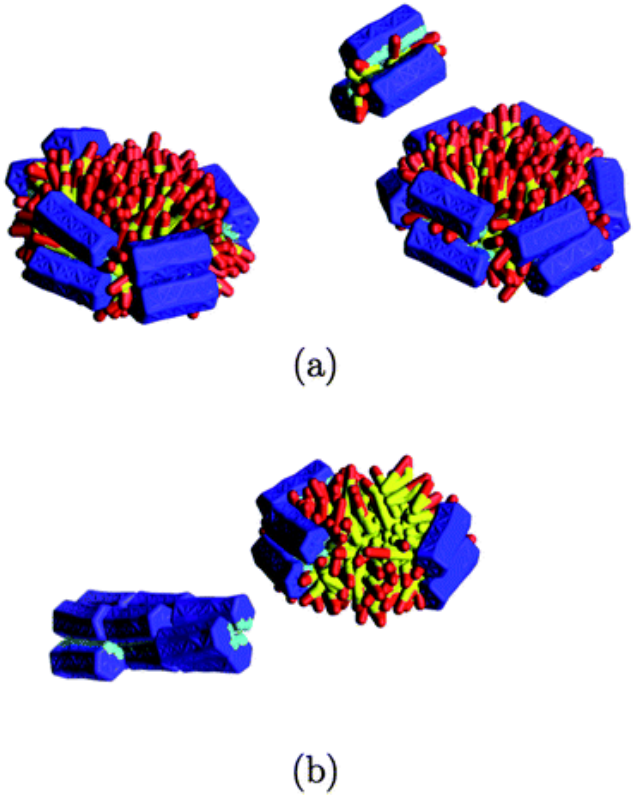
Combining Form Factors

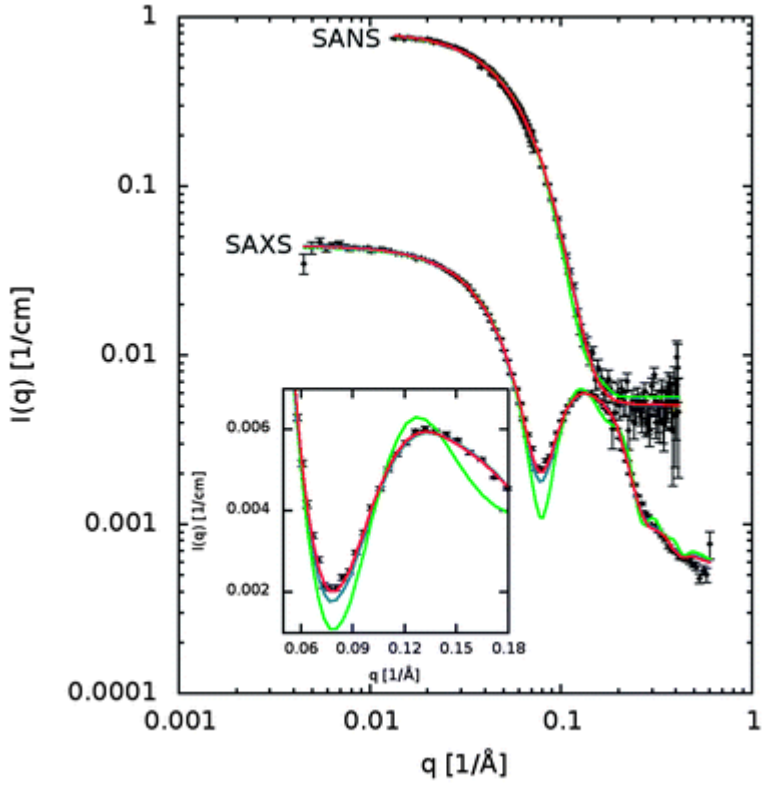




Self-assembling peptides forming nanodiscs

$$A_{tags} + A_{heads} + A_{alkyl} + A_{methyl} + A_{belt} = A_{nd}$$





S. R. Midtgaard, M. C. Pedersen et al. Soft Matter, 2014, 10, 738-752

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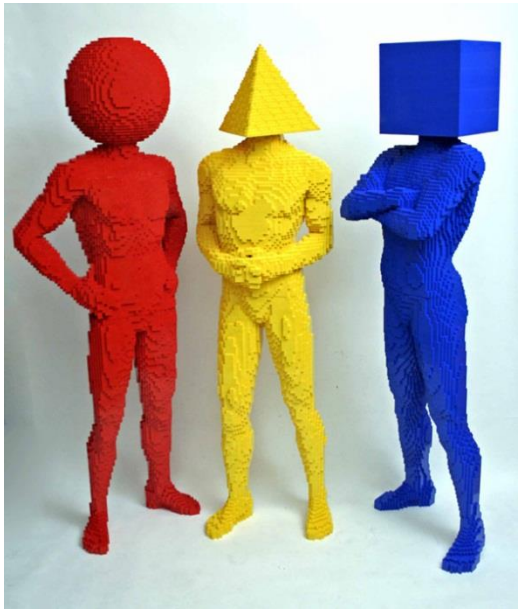
Ab-initio Modelling

Modelling of particles with no prior information

Build complex models from uniform blocks



like LEGO but we use Dummy Atoms

Acts as a placeholder for, but does not resemble, a real atom

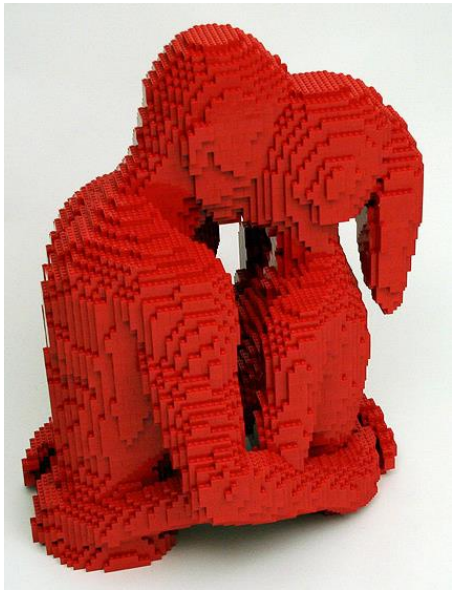
Occupies a known position in space

Has a known scattering pattern (Solvent or Particle)

Ab-initio Modelling

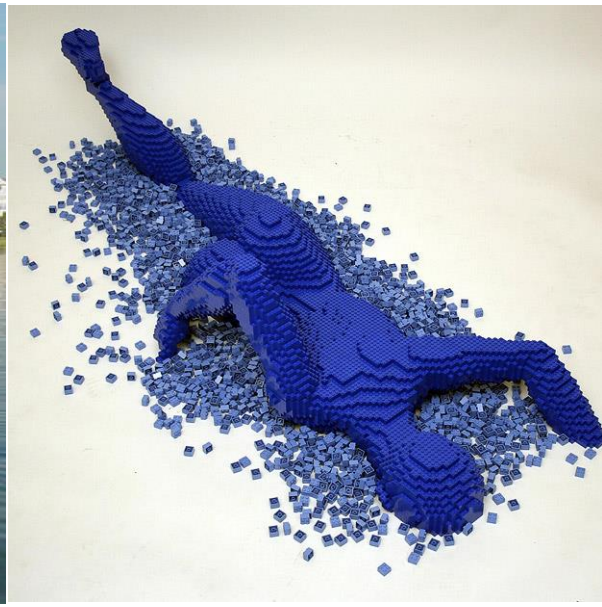
Modelling of particles with no prior information

Build complex models from uniform blocks



Must be
Compact

Must NOT be
disconnected



Must NOT be
Loose

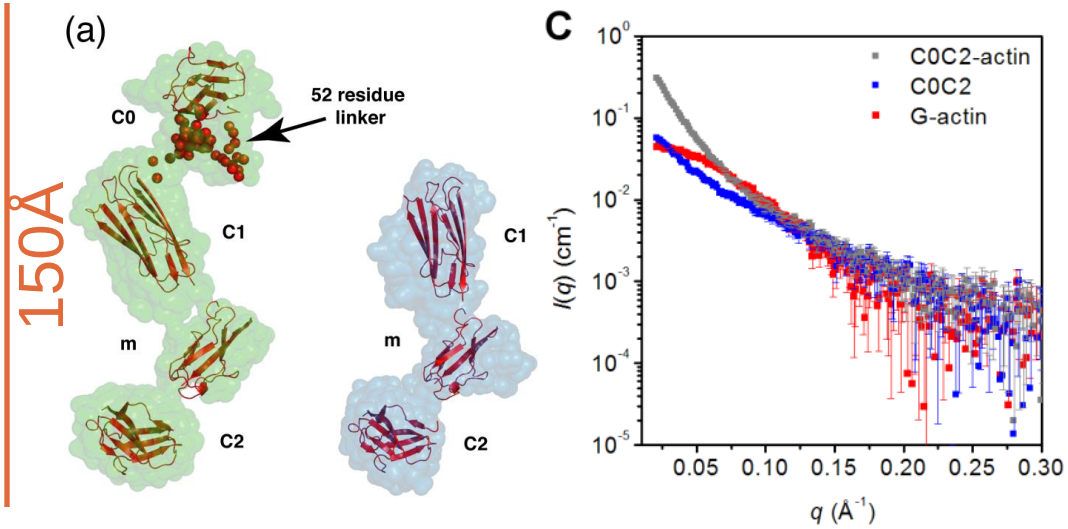
Ab-initio Modelling

With multiple data sets multi phase models can be produced

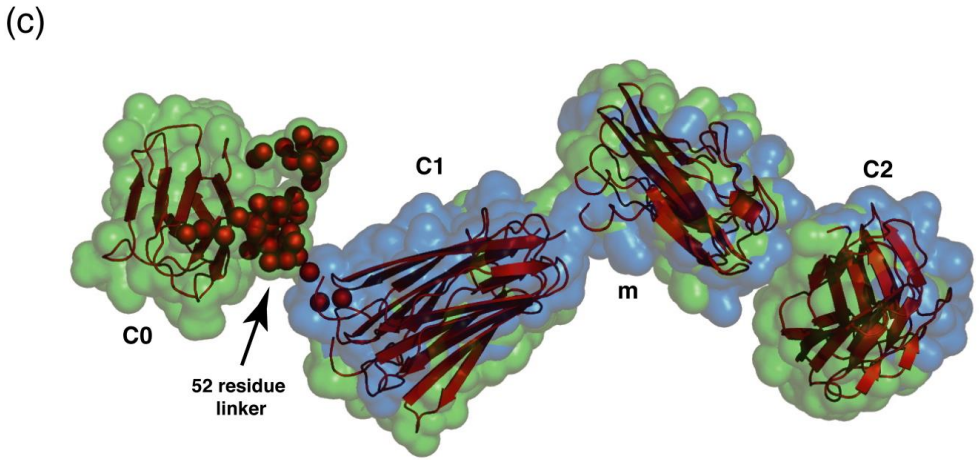
Points in the search grid can be assigned to Different phases

MONSA

Ab-initio Example



SAXS data + crystal and NMR structures show the N-terminal domains of mouse cMyBP-C form an extended structure with a defined disposition of the modules

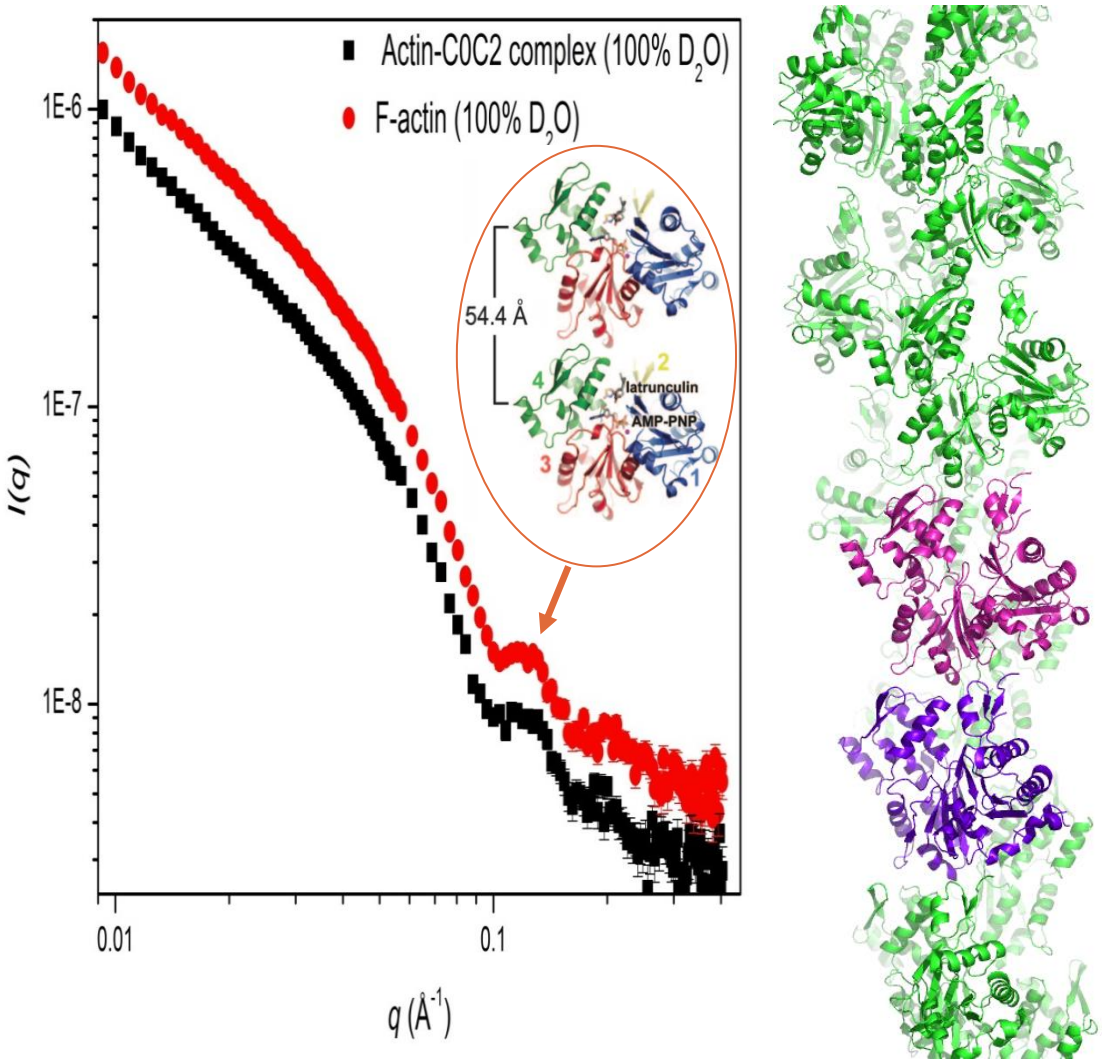


However mixing with actin results in a dramatic increase in scattering signal due to the formation of a large, rod-shaped assembly

Thanks to J Trewhella for these slides

Whitten, Jeffries et al. (2008) *PNAS 105*, 18360

Ab-initio Example



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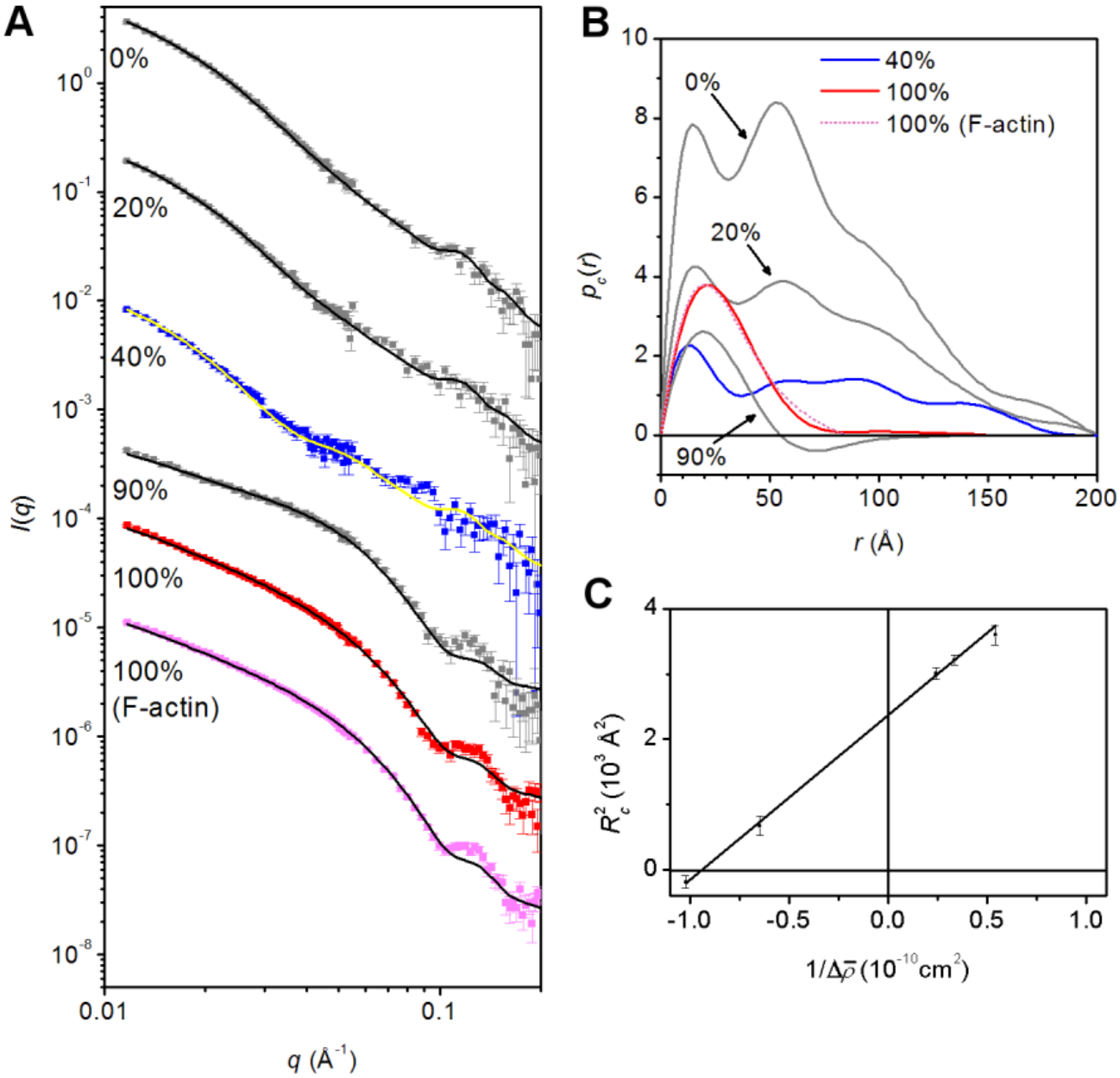

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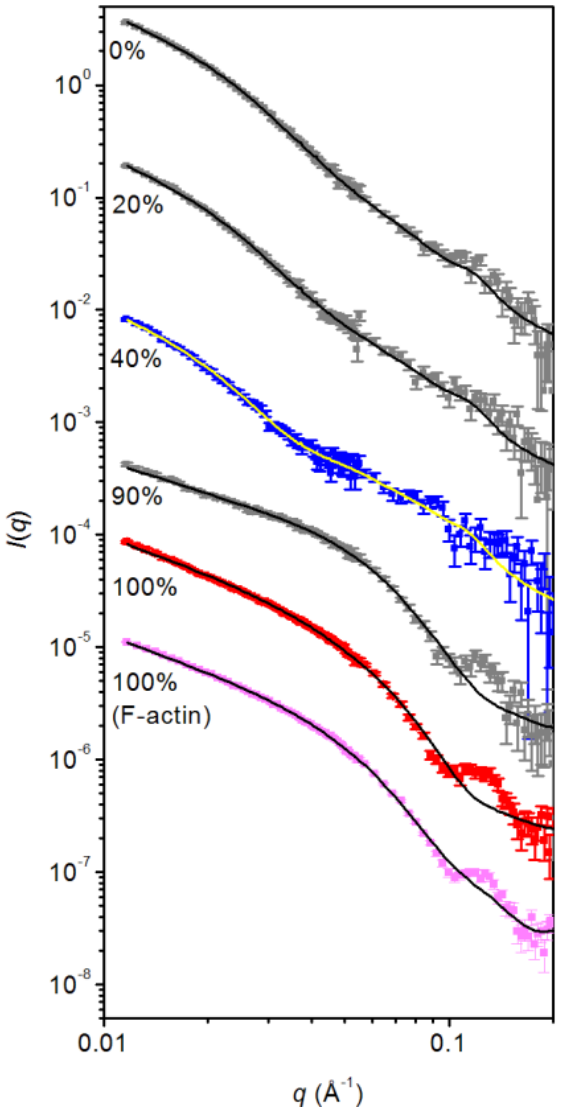

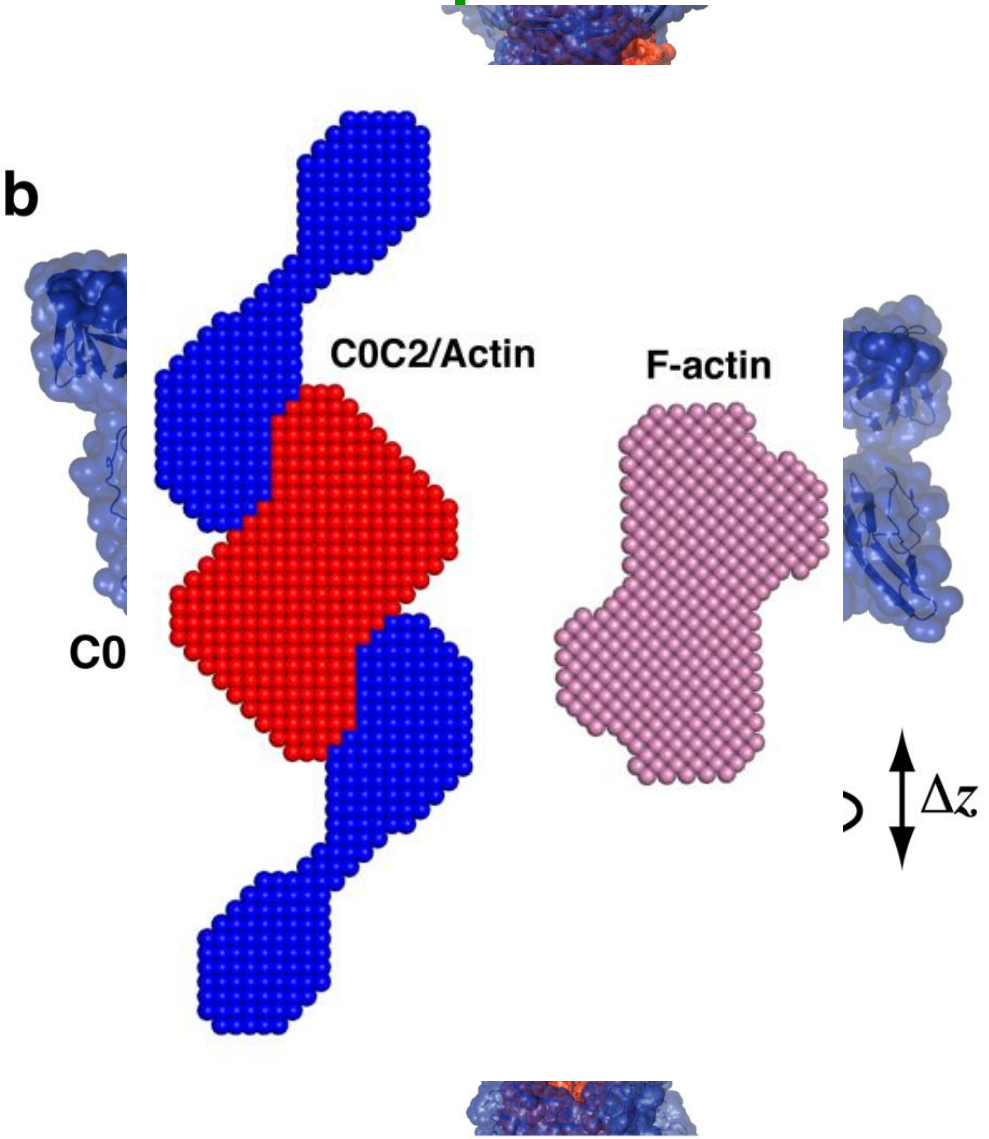

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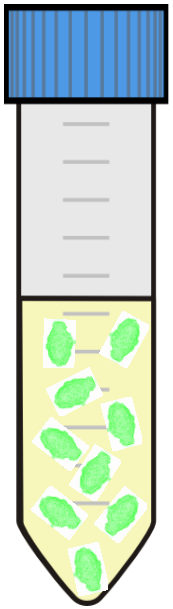
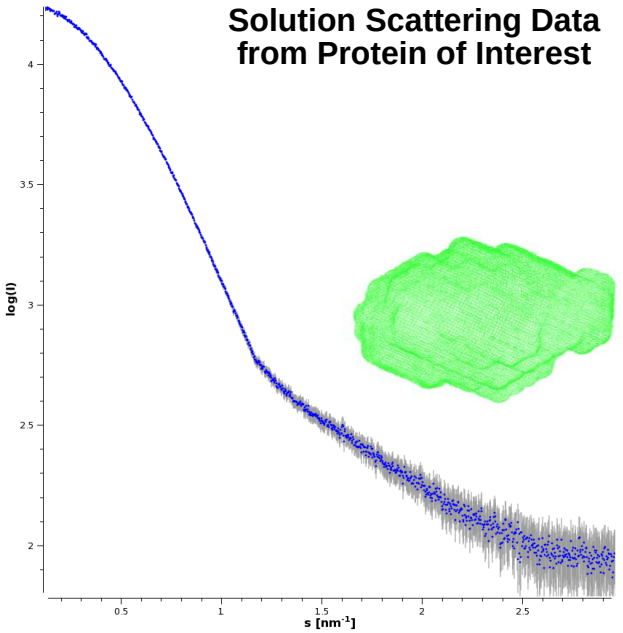
Idealised Solution SAS Experiment





Black Box

Neutron or X-ray source

What exactly is the Black box?

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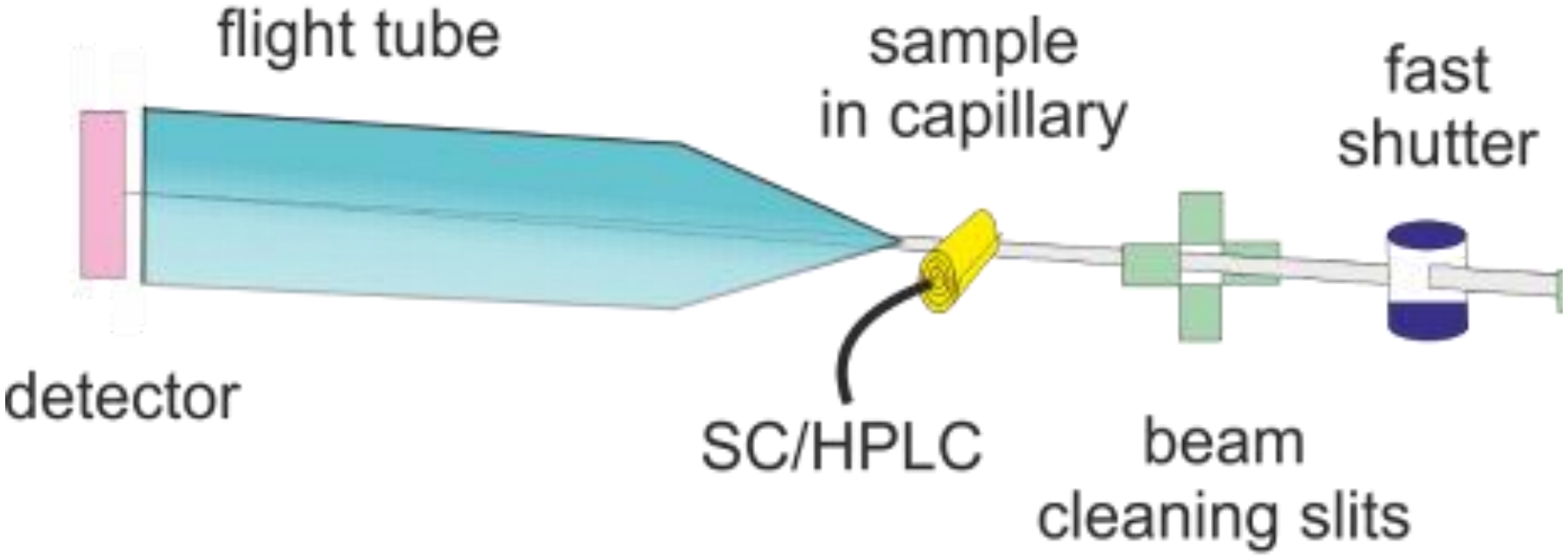
What exactly is the Black box?



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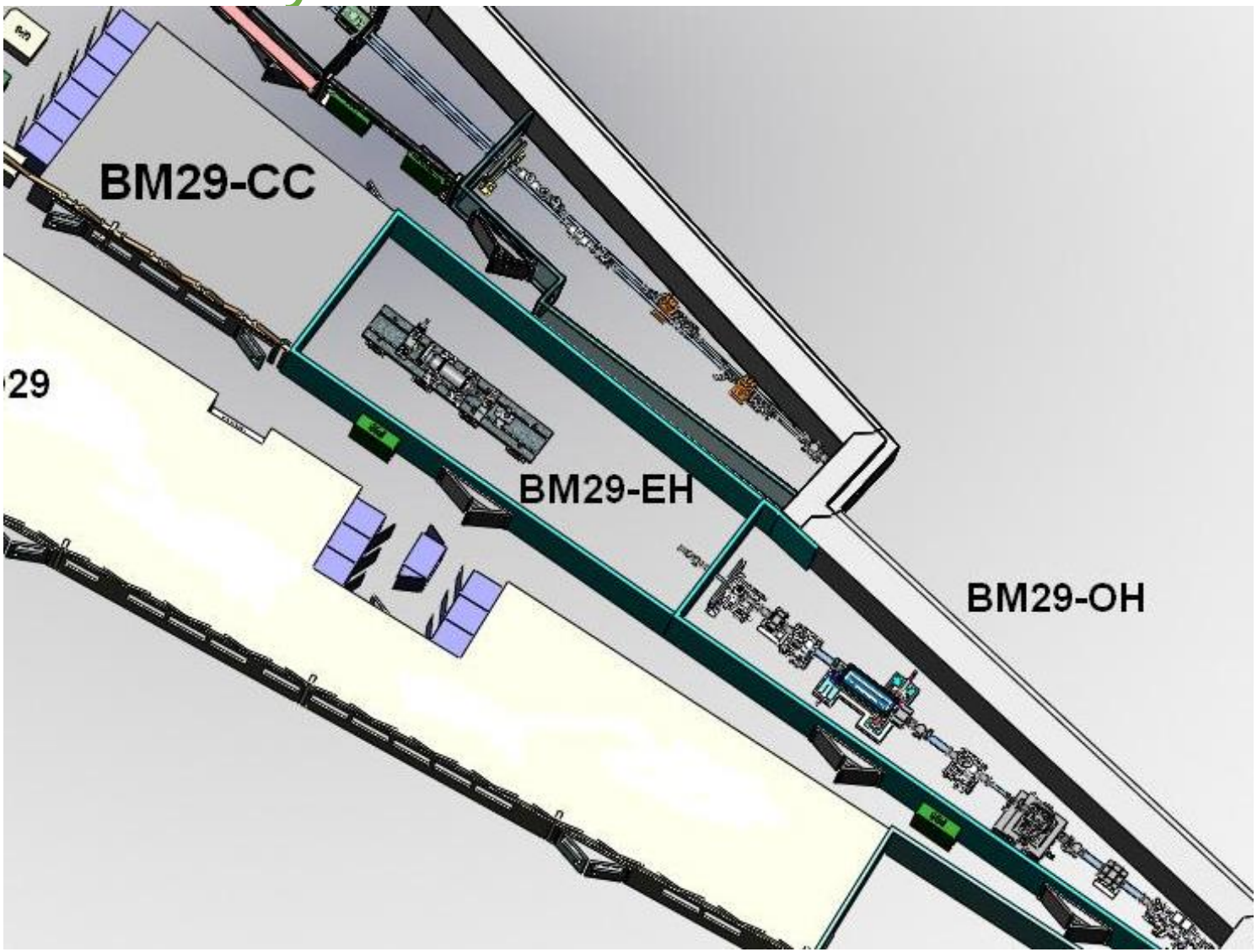

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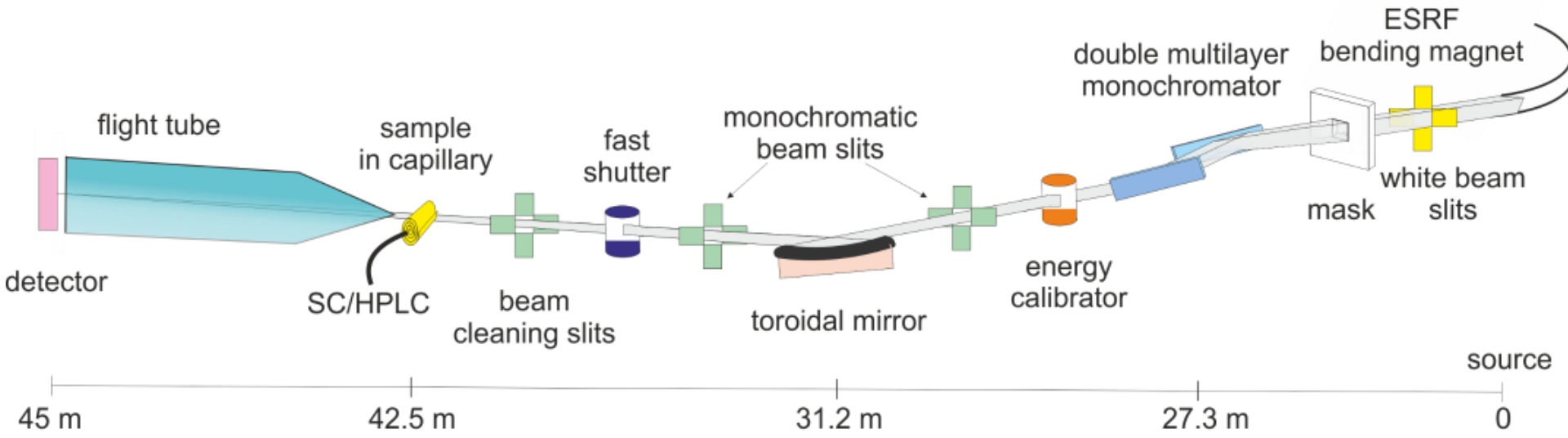
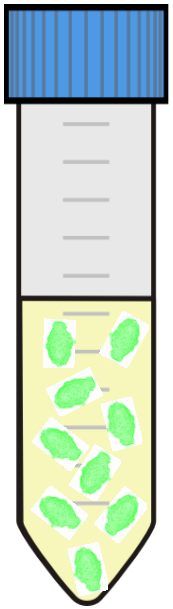
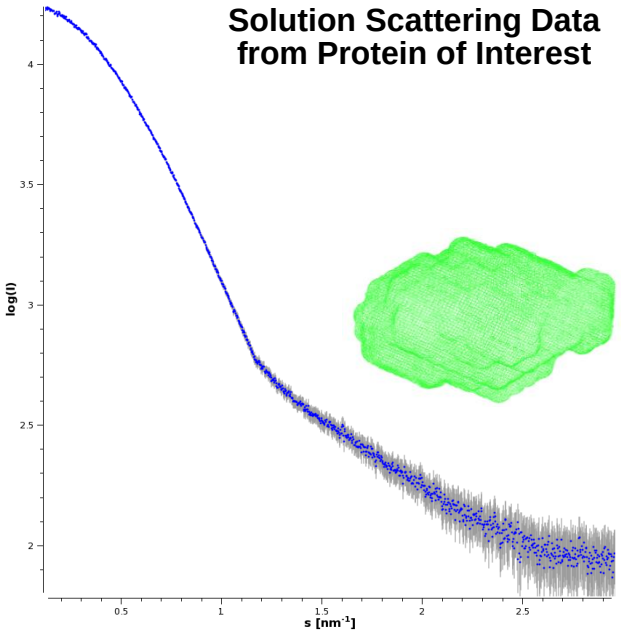


Energy	7-15 keV, standard: 12.5 keV
q-range	0.020 - 5 nm^{-1}
beam size at sample	about 700 µm x 700 µm
flux at sample	3 x 10^{12} photons/s
detector distance	2.867 m

Idealised Solution SAS Experiment

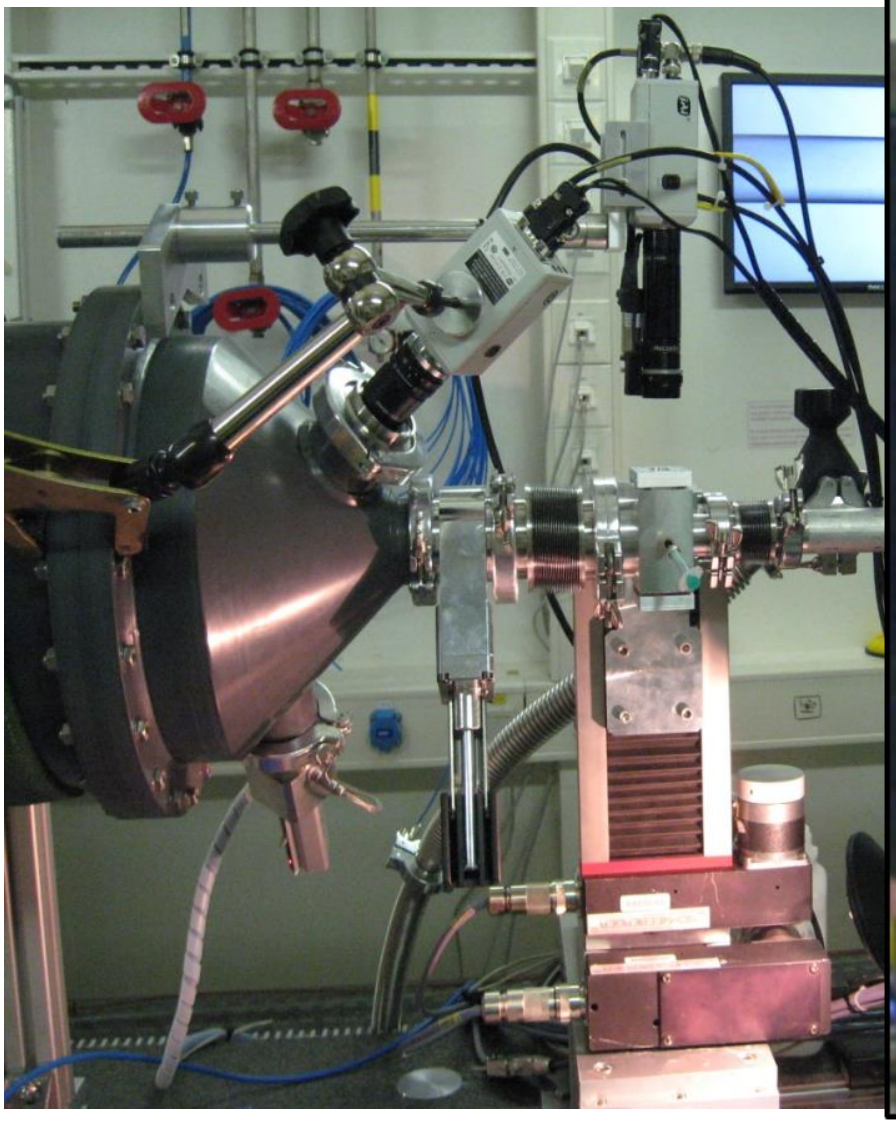


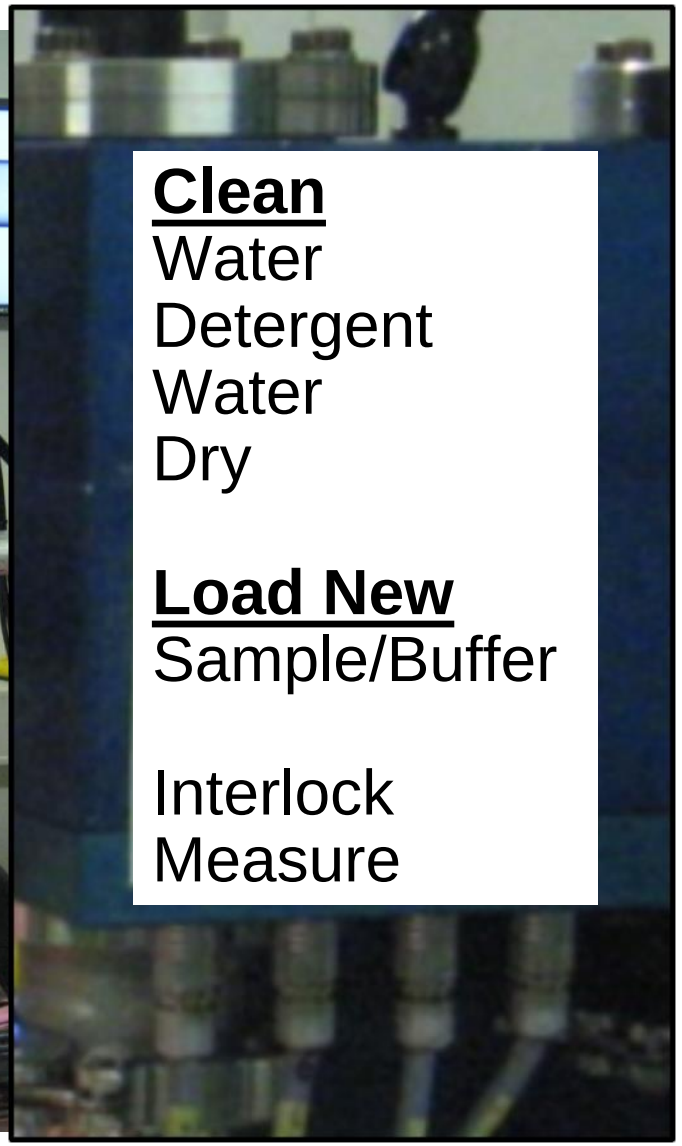


Black Box

Neutron or X-ray source

Experimental Procedure





Clean
Water
Detergent
Water
Dry

Load New
Sample/Buffer

Interlock
Measure

SC development

Sample Volume
Cleaning time
Total cycle time

2007 Manual sample handling


EMBL-HH 1st gen SC X33@DORIS

2009


Evaluation Setup ID14-3@ESRF

2010


2nd Gen SC ID14-3@ESRF

2012


2nd Gen SC BM29@ESRF

Now

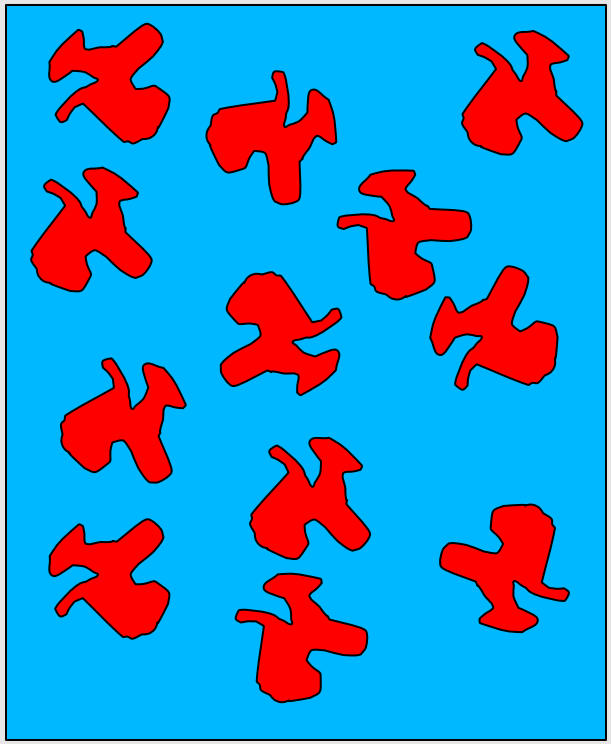
2nd Gen SC BM29@ESRF P12@PETRAIII B21@Diamond

Reliability
Confidence
Throughput

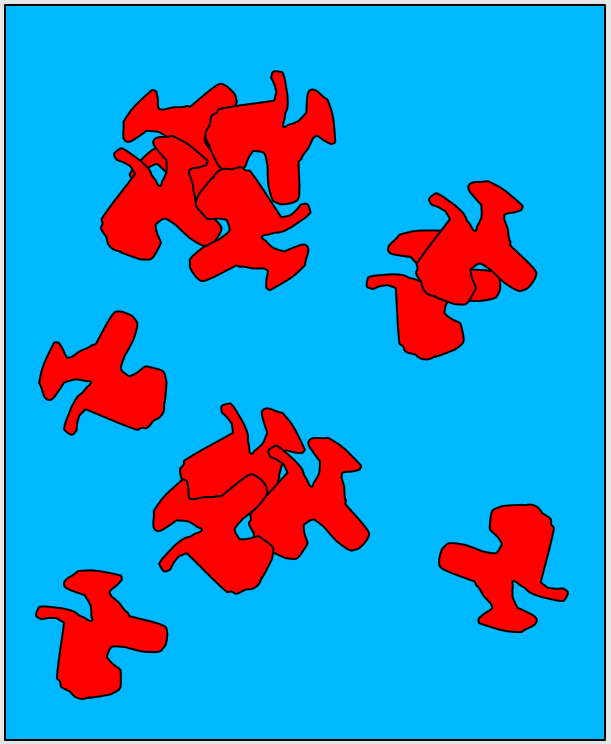
Sample Preparation

In solution SAXS we observe the **Average**

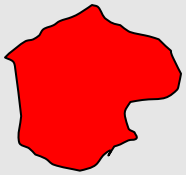
Monodisperse



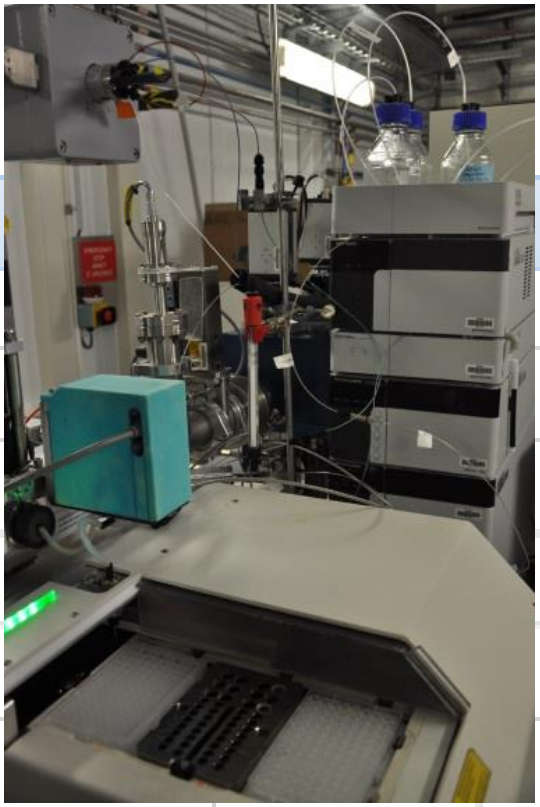
Mixture



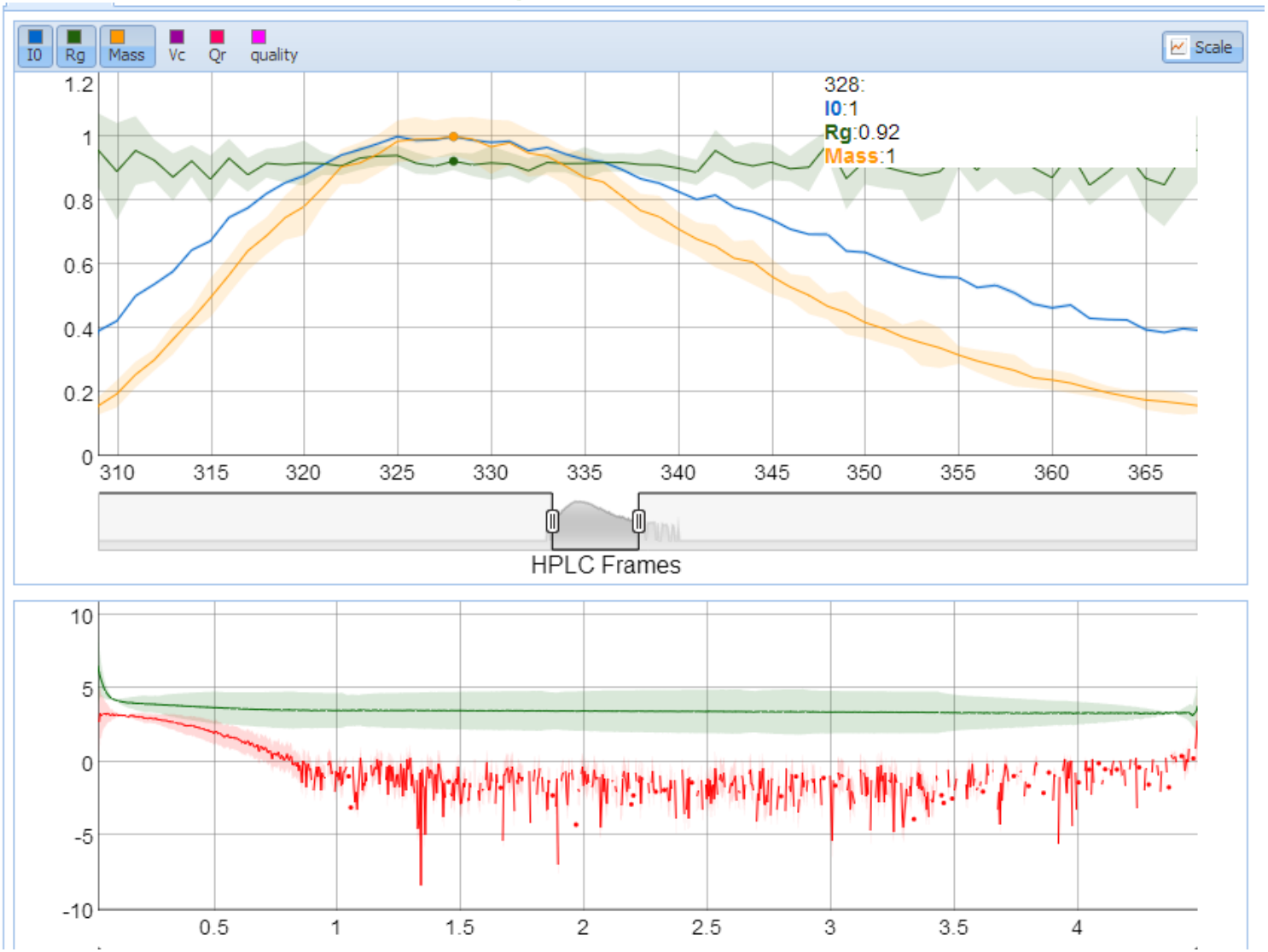
Average



Online SEC in ISPyB



Online SEC in ISPyB



Current status of data collection:

Temperature

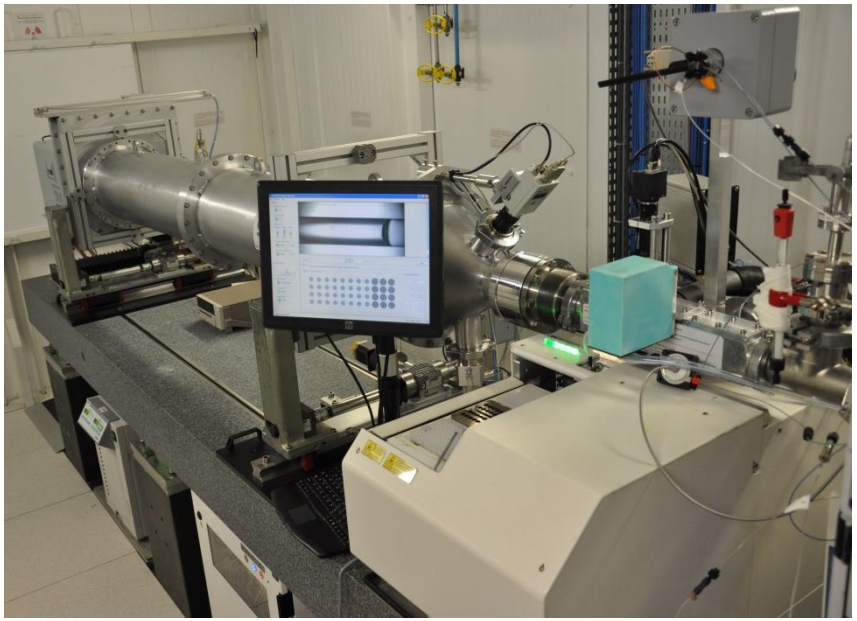
- Data acquisition between 4 and 60 C
- SEC operation at 4 or 20 degrees C

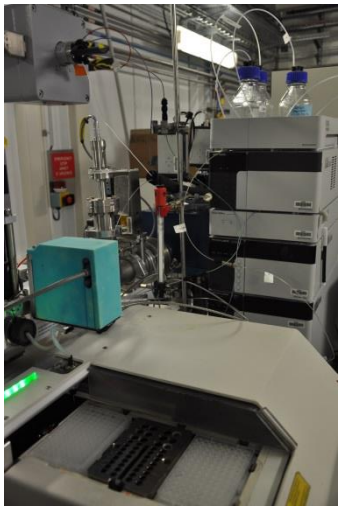
Exposure Time

- 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





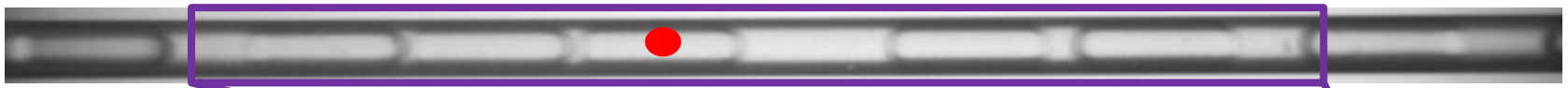
Automated valve

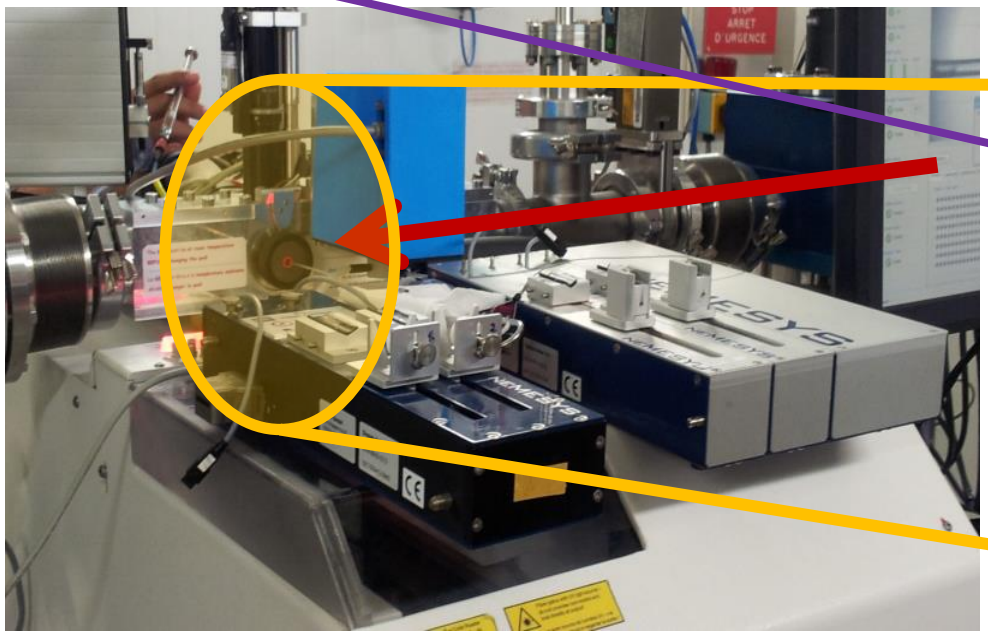
To switch between
SEC and Static modes

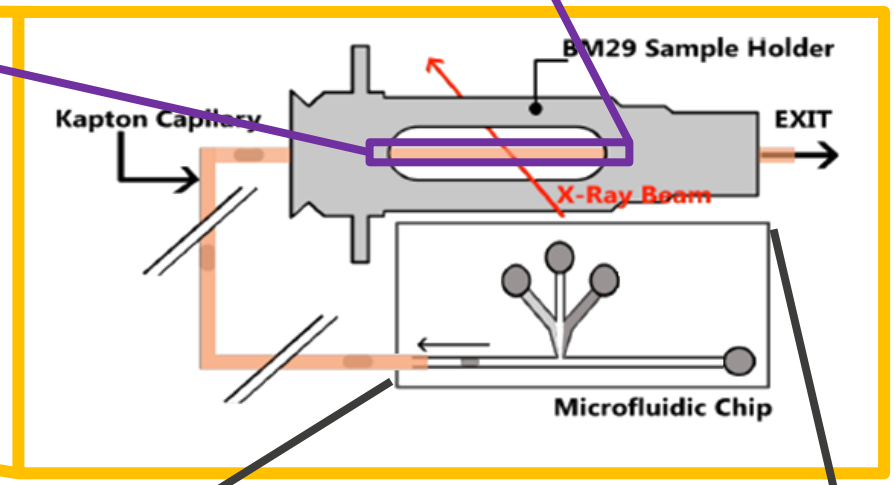
Gives users control
Safe and reliable switching
Maximises efficiency
cleaning between SEC runs

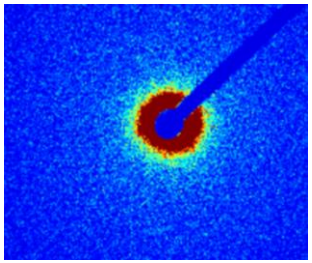
SC compatible Microfluidics: First results

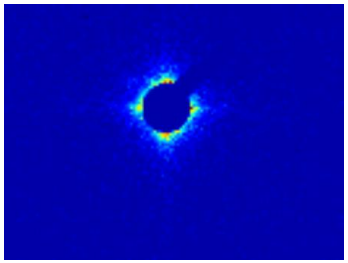
Proof of Principle achieved



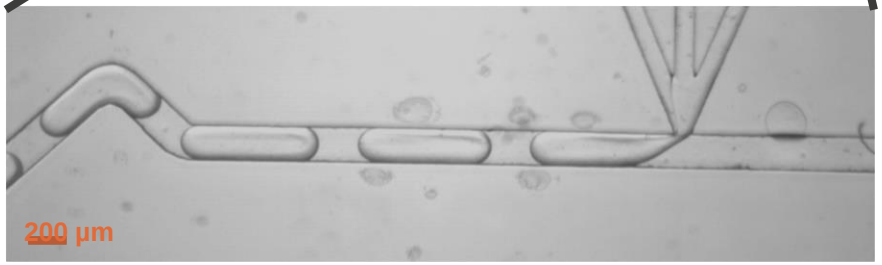




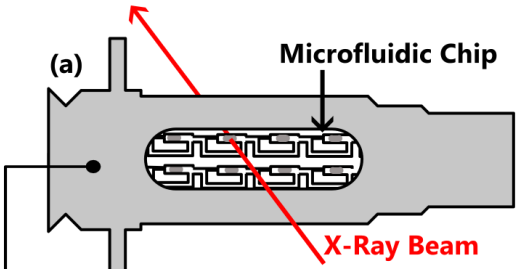


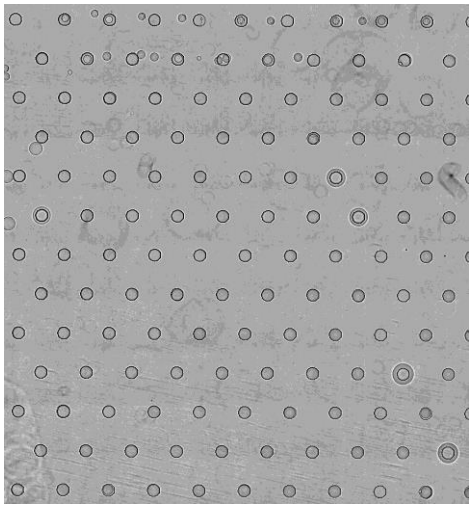


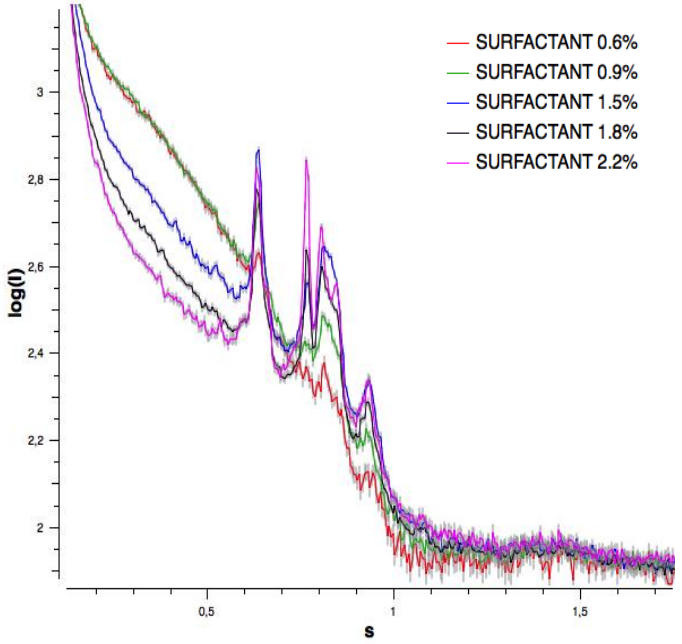
Background improved with scatterless pinhole

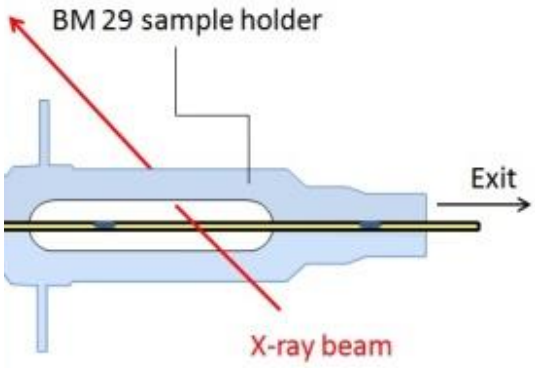


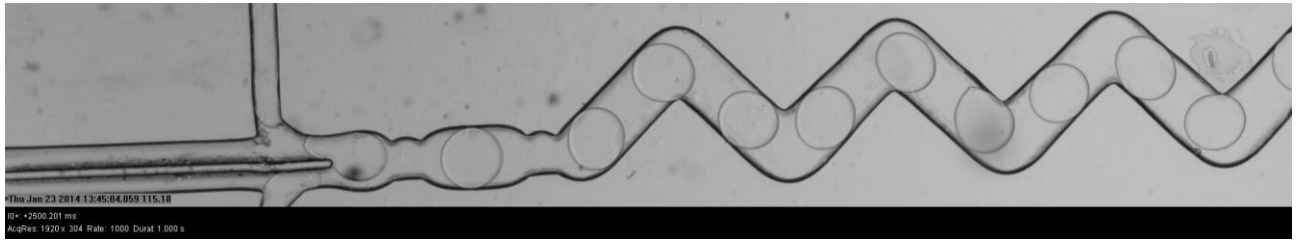
SC compatible Microfluidics





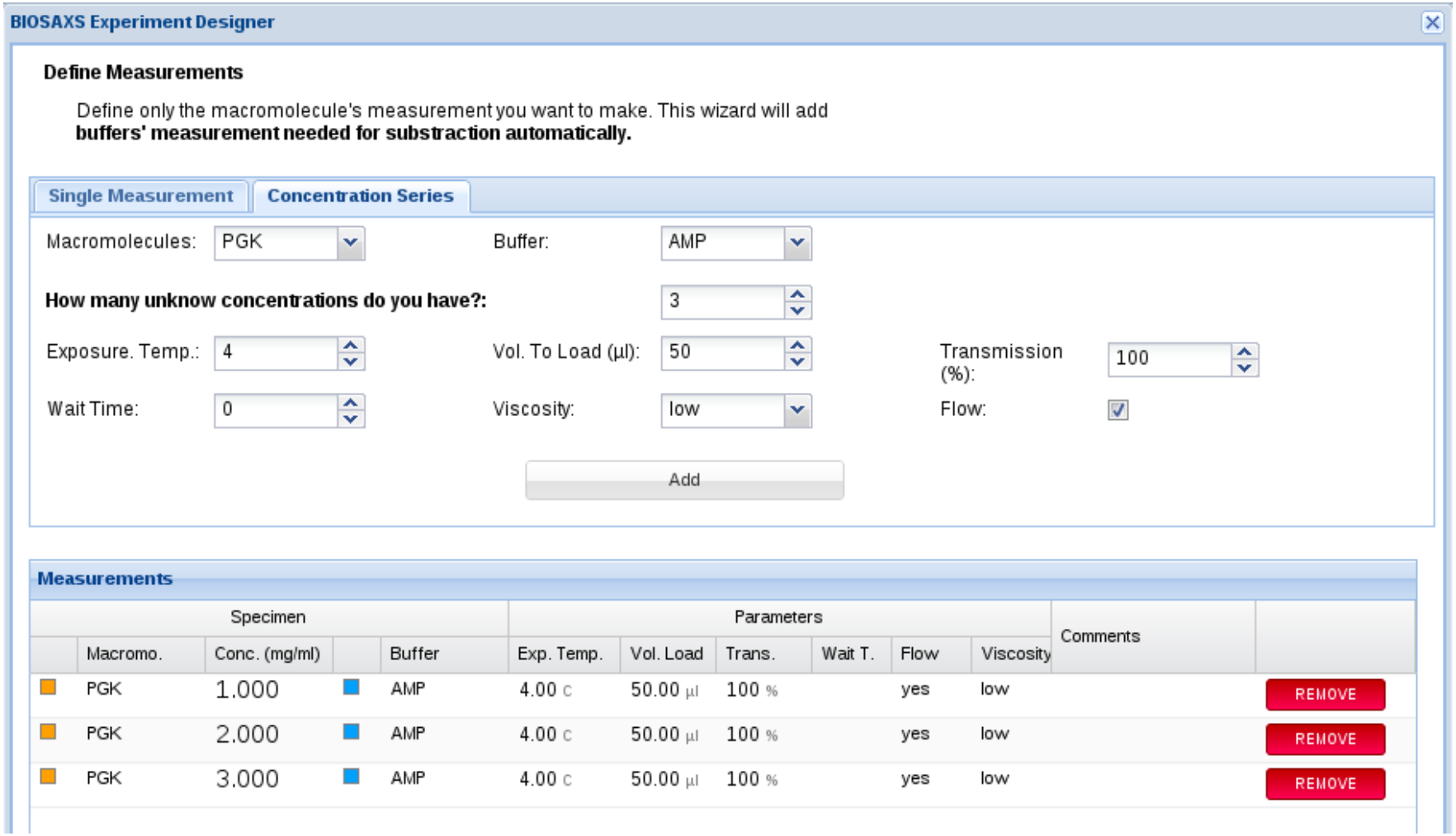




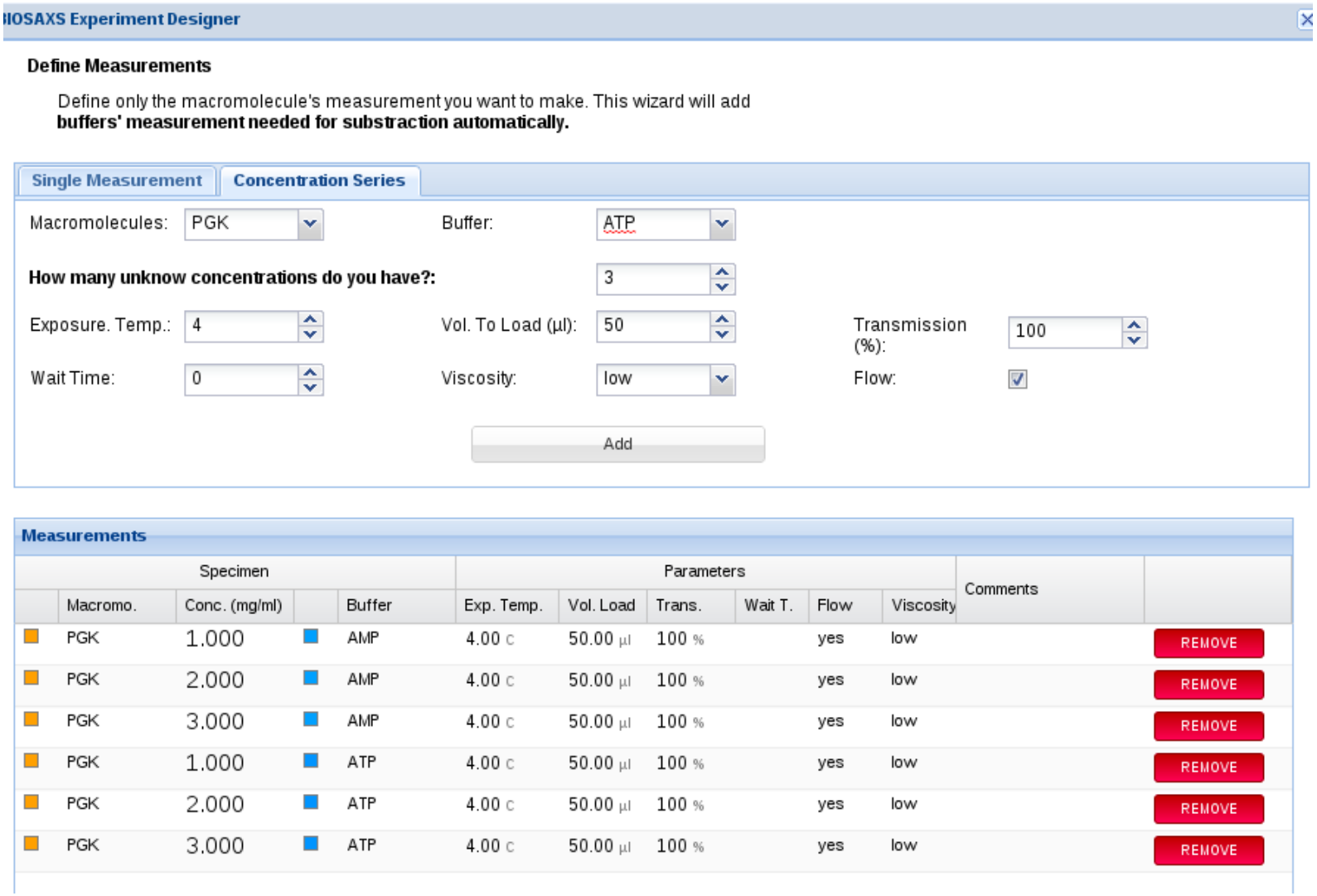


Experimental preparation and efficient use of beamtime facilitated through ISPyB

Improved feedback for experimental preparation



Improved feedback for experimental preparation



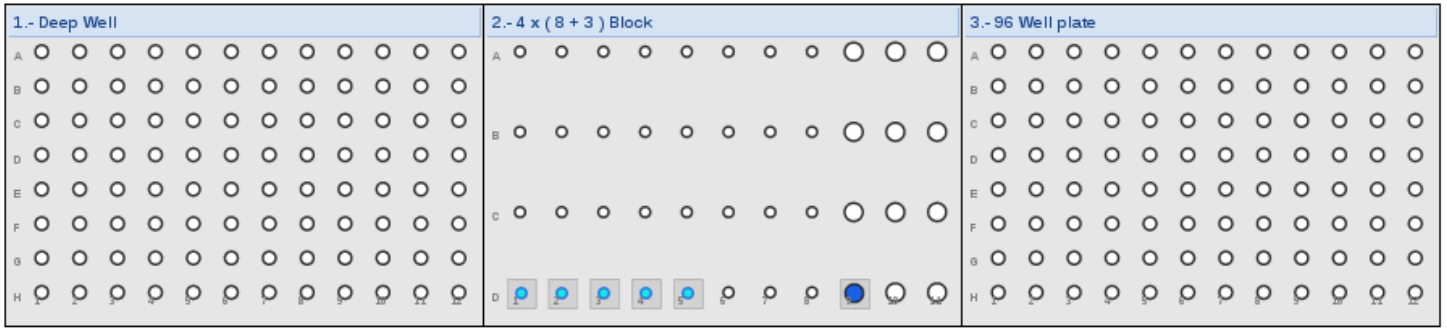
Improved feedback with ISPyB

Overview | **Measurements** | Analysis | 1D Viewer

Collapse buffers

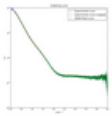
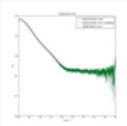
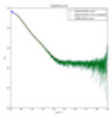
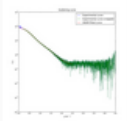
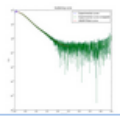
Specimen					Parameters						Status	Time	Comments
	Macromo.	Conc. (mg/ml)		Buffer	Exp. Temp.	Vol. Load	Trans.	Wait T.	Flow	Viscosity			
☐			■	D33	20.00 c	100.0…	100 %		yes	Low	DONE	06:47:07 pm	buffer
☐	taHEFD33	14.000	■	D33	20.00 c	150.0…	100 %		yes	Low	DONE	06:48:23 pm	[1] tahef d33 truncation
☐			■	D33	20.00 c	100.0…	100 %		yes	Low	DONE	06:49:41 pm	buffer
☐	taHEFD33	7.000	■	D33	20.00 c	90.00 µl	100 %		yes	Low	DONE	06:50:54 pm	[2] tahef d33 truncation
☐			■	D33	20.00 c	100.0…	100 %		yes	Low	DONE	06:52:09 pm	buffer
☐	taHEFD33	3.500	■	D33	20.00 c	90.00 µl	100 %		yes	Low	DONE	06:53:25 pm	[3] tahef d33 truncation
☐			■	D33	20.00 c	100.0…	100 %		yes	Low	DONE	06:54:40 pm	buffer
☐	taHEFD33	1.250	■	D33	20.00 c	90.00 µl	100 %		yes	Low	DONE	06:55:56 pm	[4] tahef d33 truncation
☐			■	D33	20.00 c	100.0…	100 %		yes	Low	DONE	06:57:11 pm	buffer
☐	taHEFD33	0.610	■	D33	20.00 c	90.00 µl	100 %		yes	Low	DONE	06:58:28 pm	[5] tahef d33 truncation
☐			■	D33	20.00 c	100.0…	100 %		yes	Low	DONE	06:59:43 pm	buffer

Ready

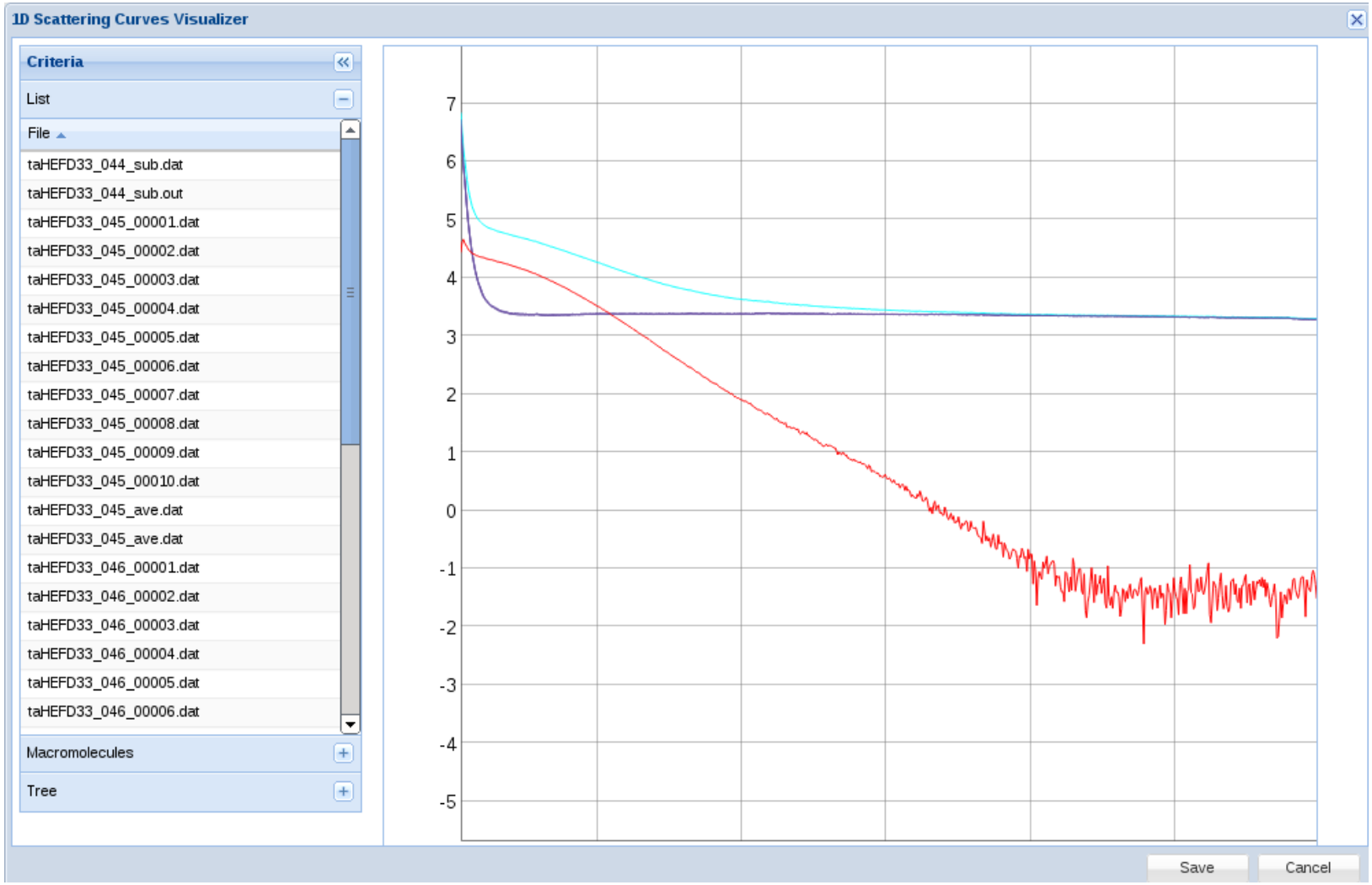


Improved feedback with ISPyB

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

Improved feedback with ISPyB

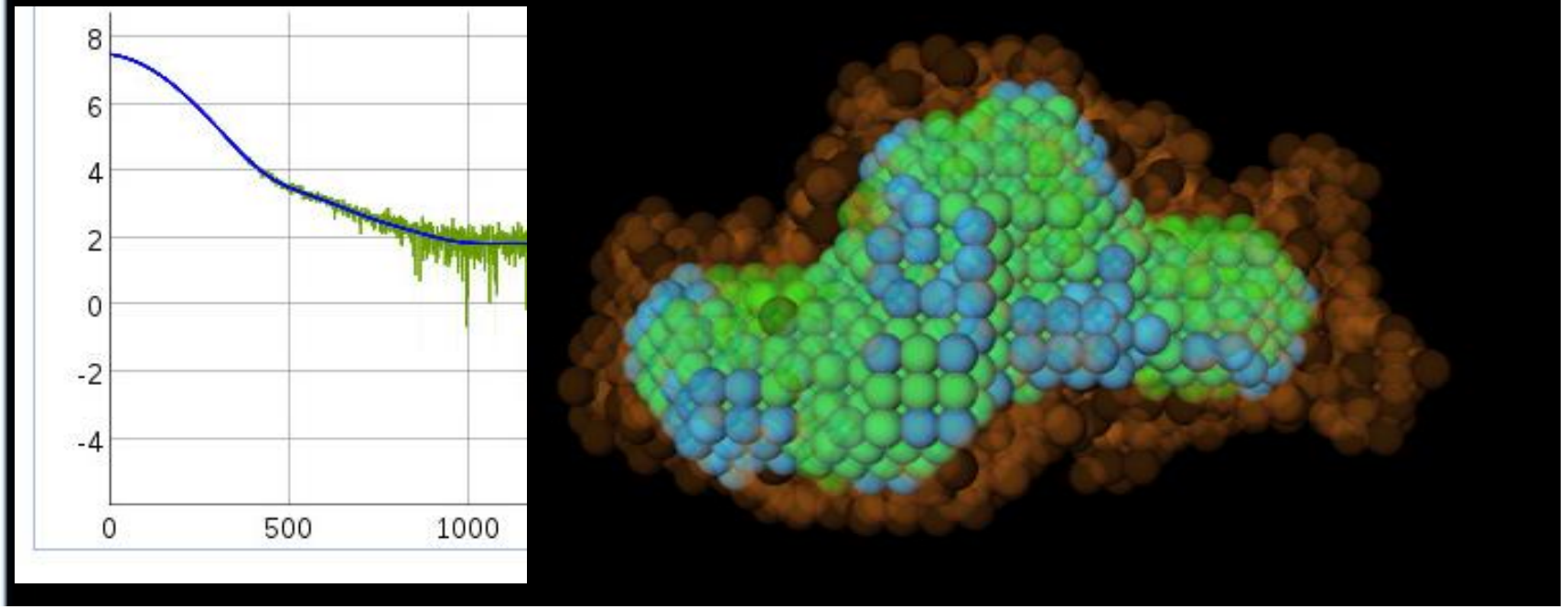


Improved feedback with ISPyB

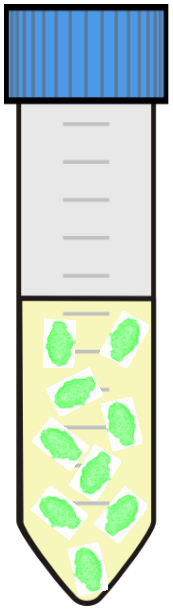
Macromolecule	Concentration	Scattering	Frames (Averaged/Total)	Guinier				Gnom			Porod	
				Rg (nm)	Points	Quality (%)	I(0)	Rg (nm)	Total	Dmax (nm)	Volume (nm3)	MM (kD) Vol. est.
taHEFD33	1.25 mg/ml		☐ D33 (10 of 10) ☐ taHEFD33 (10 of 10) ☐ D33 (10 of 10)	3.23 nm	40 - 82 (42)	78.58	78.16 ±8.02344e-2	3.26 nm	0.59	10.81	90.31	45.2 - 60.2

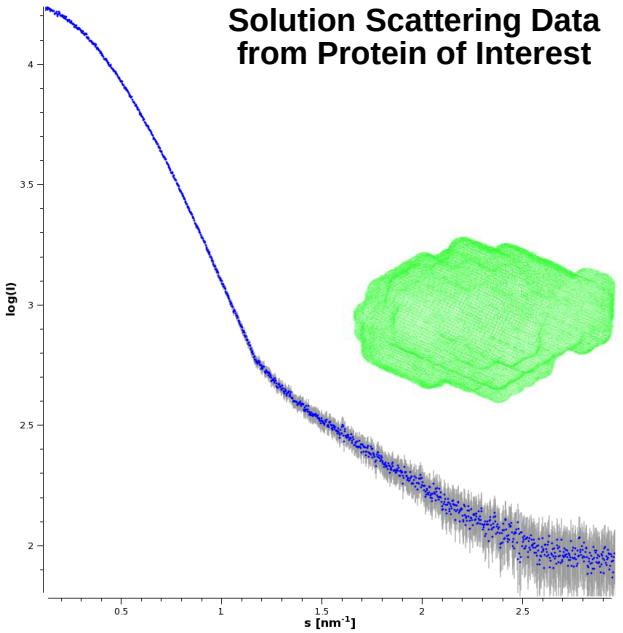
Dammaver | Damfilt | Dammin | Merged

damaver_fhs.pdb | damfilt.pdb | dammin.pdb | Radius: 3



Idealised Solution SAS Experiment

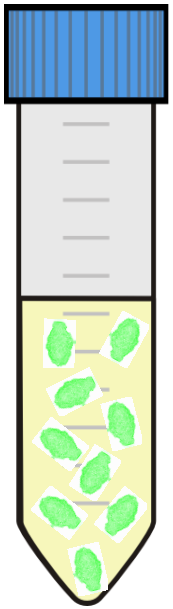


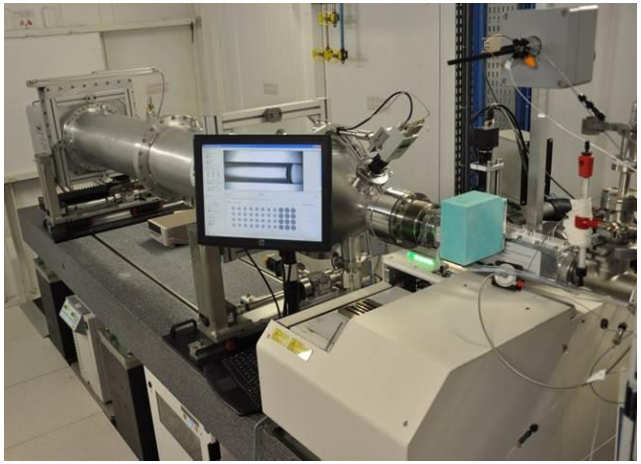


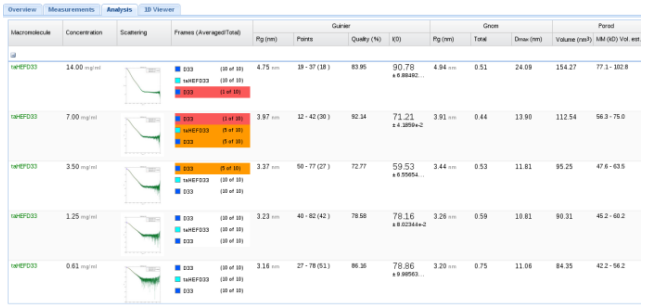
Black Box

Neutron or X-ray source

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







Automated data acquisition

With feedback

Acknowledgments

EMBL GRENOBLE

EMBL HAMBURG

ESRF

PSB

BioStruct-X

NEUTRONS FOR SCIENCE
Institut Laue-Langevin