BioSAS in Grenoble

What can be done and how to do it

Adam Round

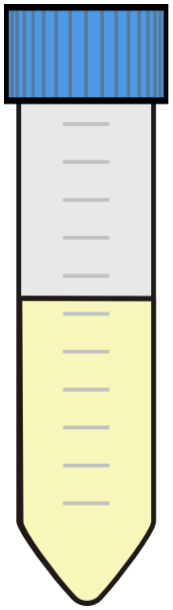
EMBL
40 YEARS | 1974–2014

Contents

- What can you learn from solution BioSAS
  - Basics of what can be obtained
  - Extending interpretation using complementary information
  - Examples

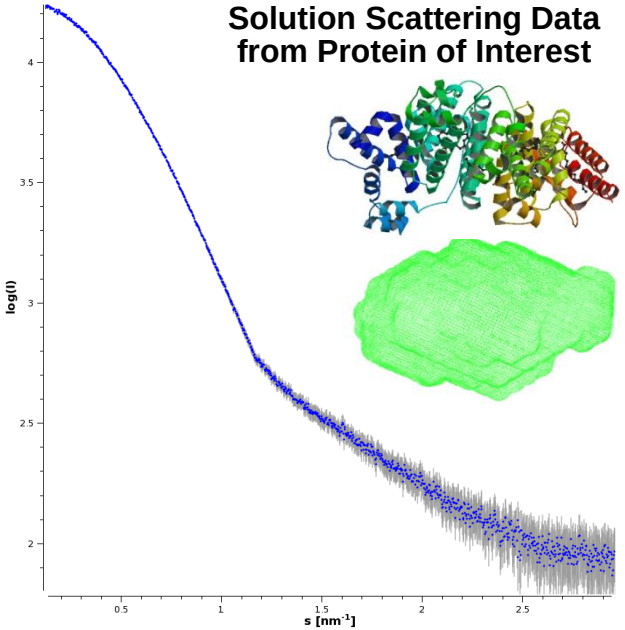
- How to collect data
  - Automation
    - Data collection and analysis
  - Feedback

Idealised Solution SAS Experiment



Black Box

Neutron or X-ray source



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 effects or flexibility could be preventing crystallisation (find optimum conditions)

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

What can be done if you know more!

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)

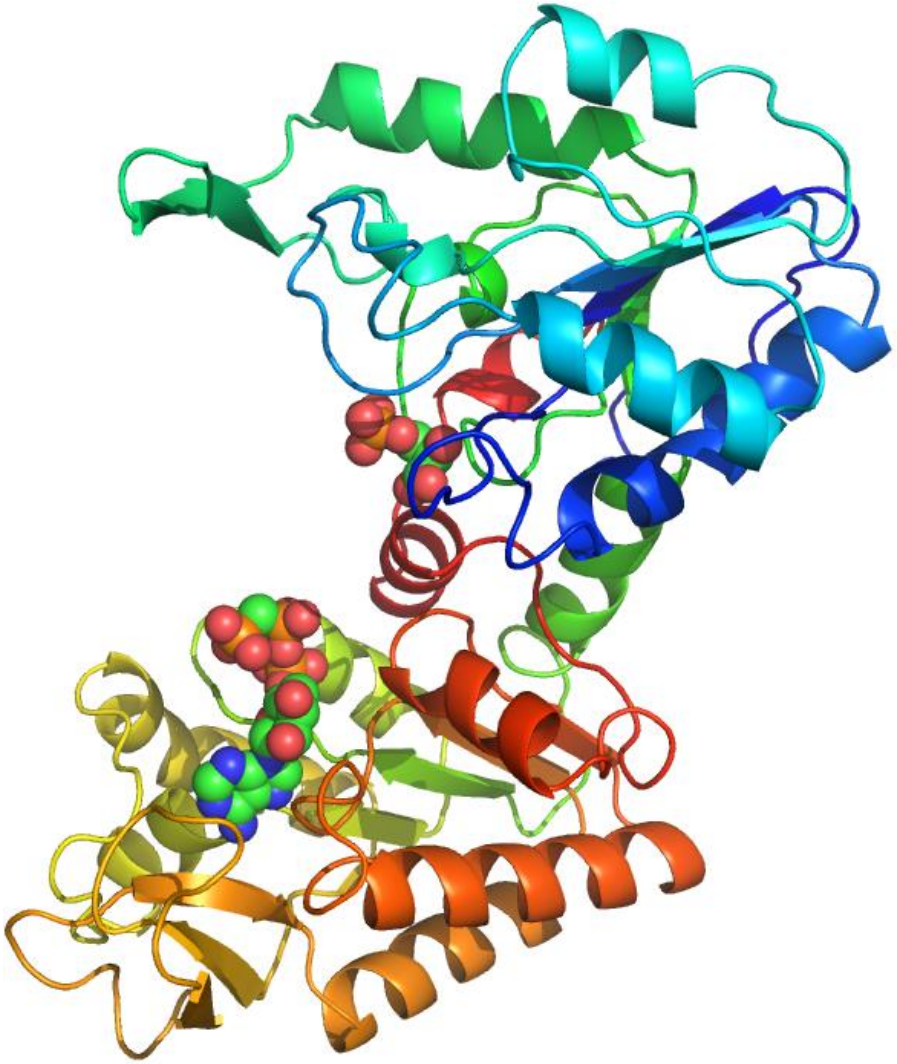
SAXS demonstrated that the AB ensemble corresponds to the biologically relevant form found under physiological conditions.

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

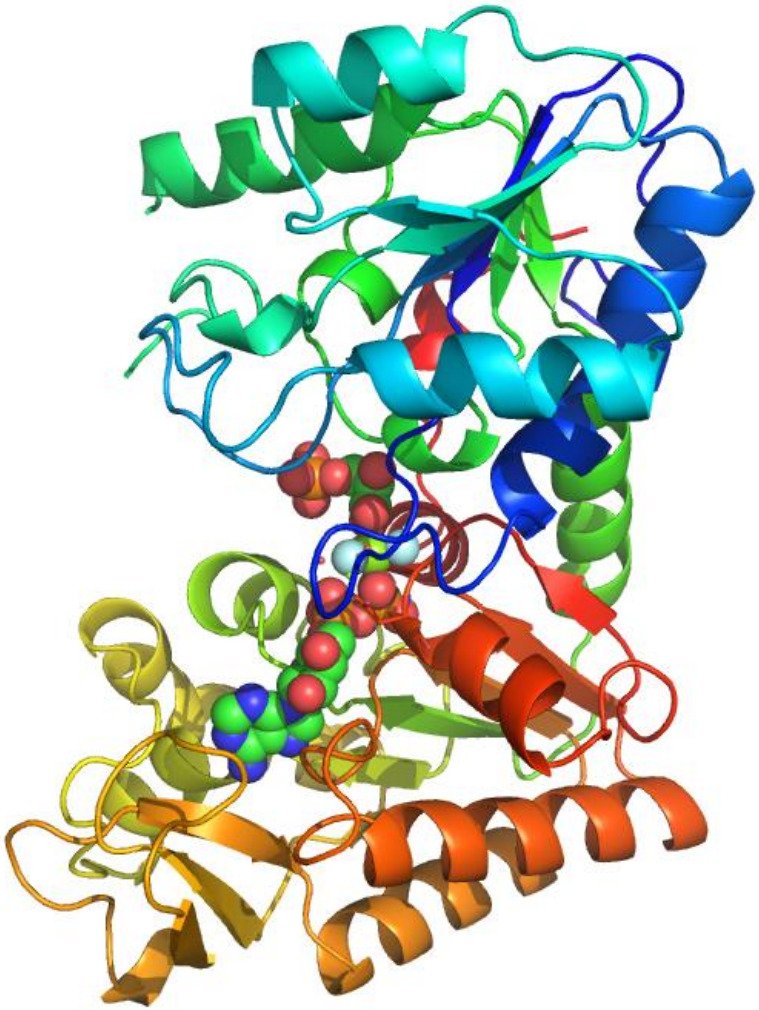
Functional study of Phosphoglycerate Kinase (PGK)

- Catalyses 7th step in Glycolosis
- It activates L-nucleoside analogue drugs in the treatment of AIDS and Hepititis
- Implicated in oncogenesis
- Drug target for obligate anaerobic pathogens

Phosphoglycerate Kinase (PGK)



Rg Open 2.33 nm

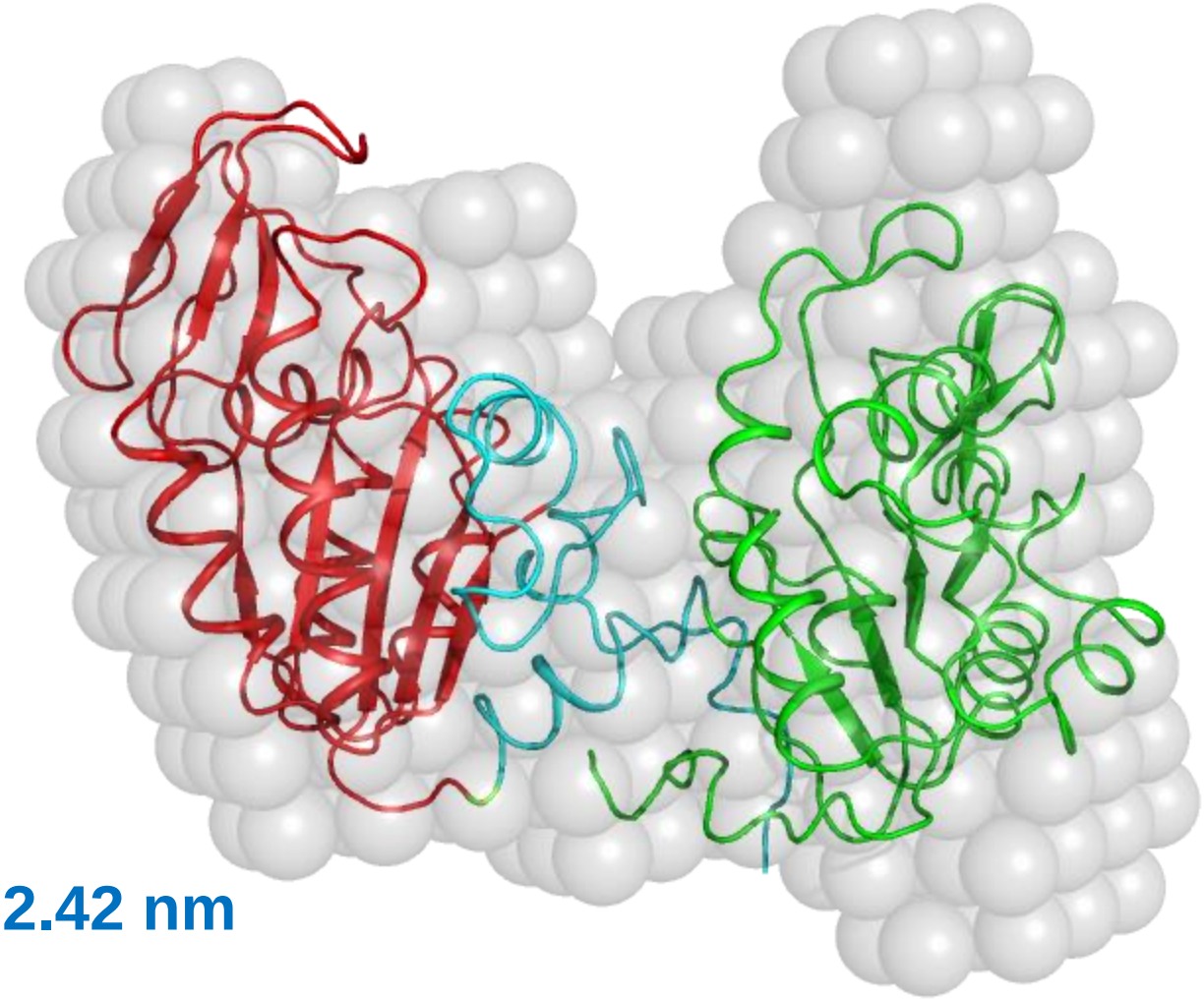


Rg Closed 2.17 nm

Banks RD, Blake CC, Evans PR, Haser R, Rice DW, Hardy GW, Merrett M, & Phillips AW (1979) *Nature* **279,** 773-777.

Cliff, *et al.* (2010) *J. Am. Chem. Soc.*, **132**, 6507-6516

SAXS analysis of PGK: **APO (open) from significantly more open**



Rg SAXS 2.42 nm

DEN refinement used to allow flexibility in the linkers to overcome clashes and provide an atomic model

Zerrad, *et al.* (2011) *J. Biol. Chem.*, **286,** 14040-14048

ESRF

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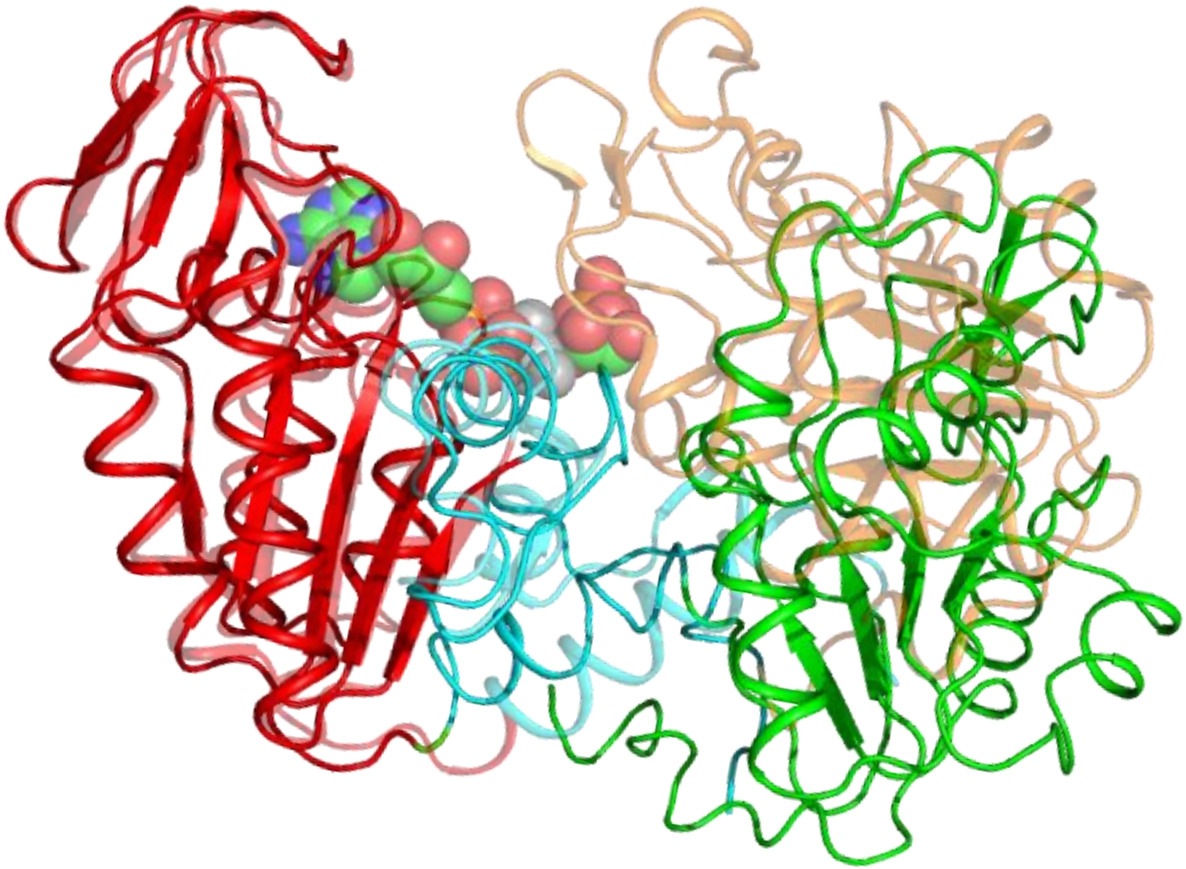
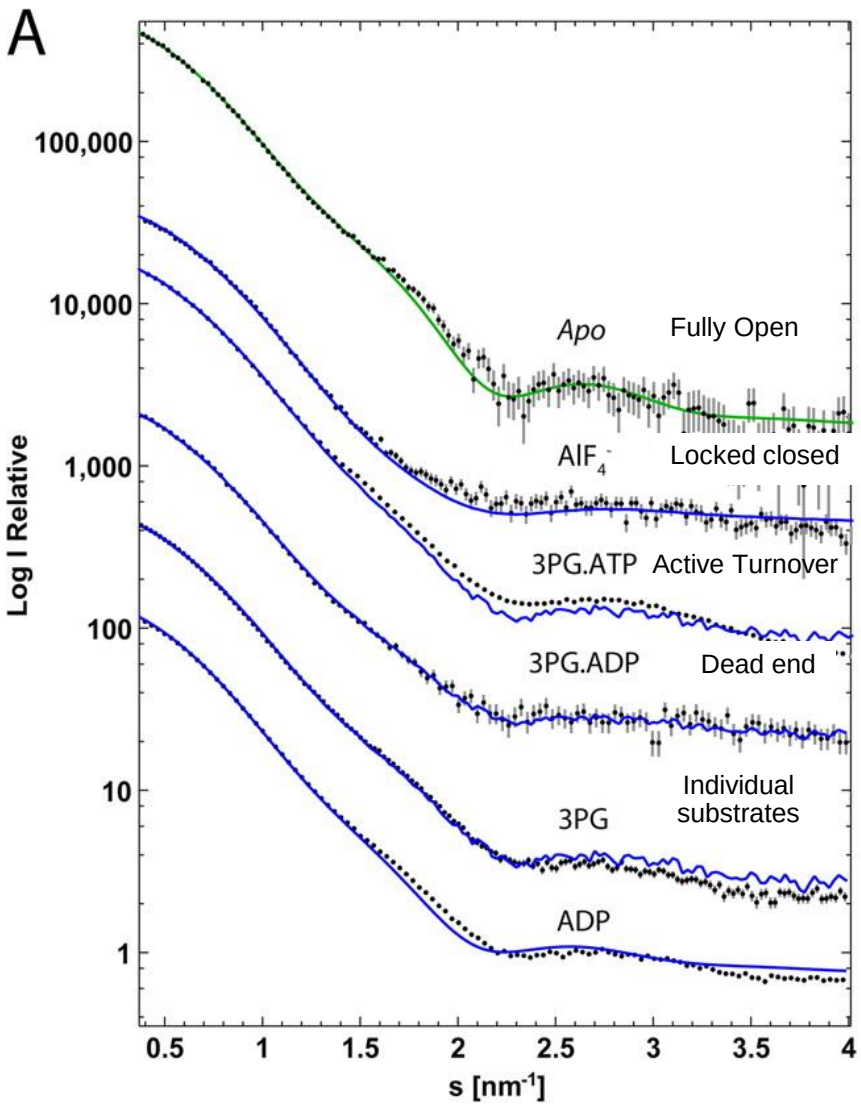
SAXS analysis of PGK: **Shows larger domain movement than expected**



50° rotation from open to closed

Zerrad, *et al.* (2011) *J. Biol. Chem.*, **286,** 14040-14048

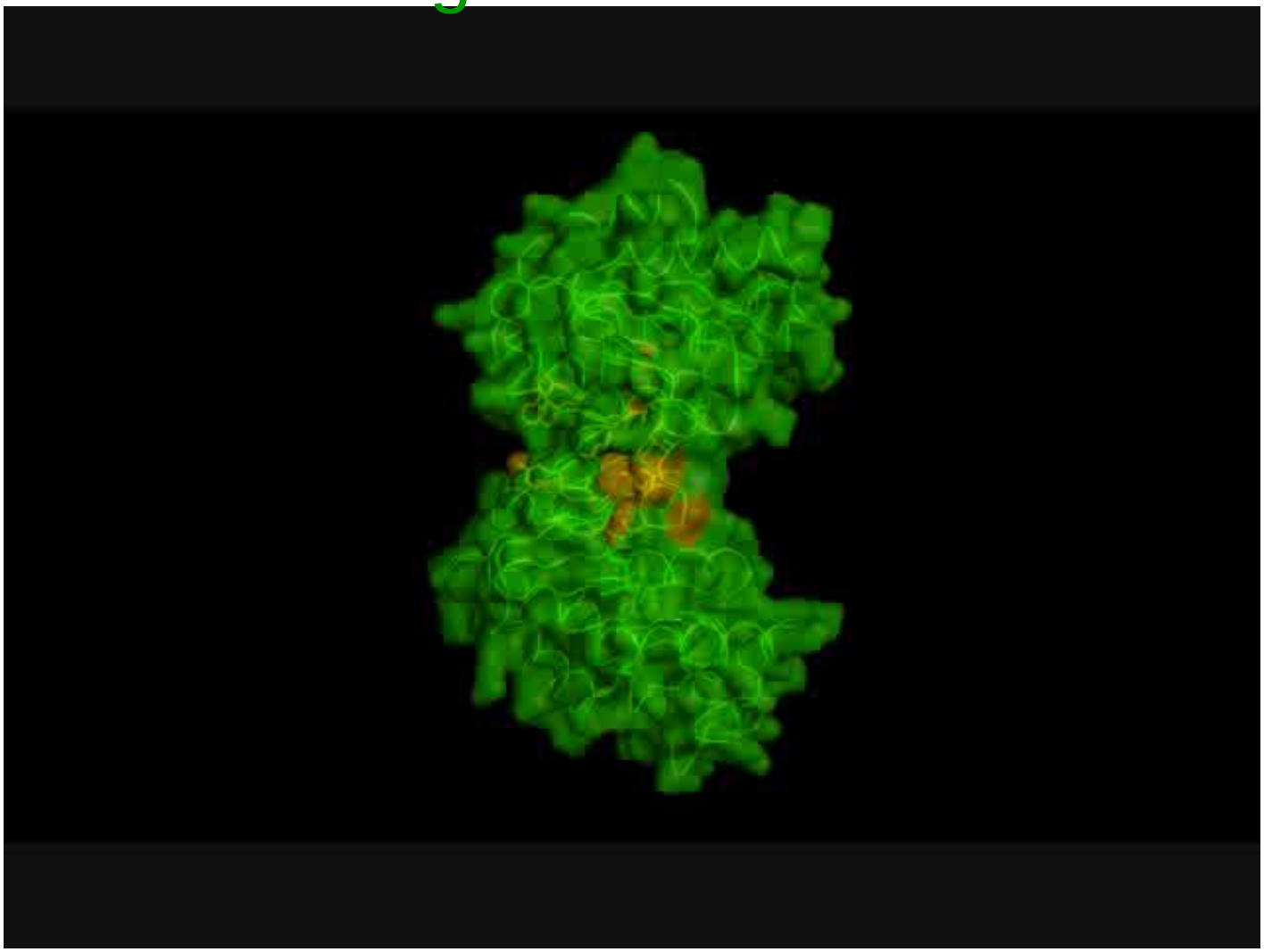
SAXS analysis of PGK: **Shows the domain movement during catalysis**



Protein complex	R_g (nm)	% Open	% Closed	Chi-square of fit
Apo	2.42	N.D	N.D	1.08 (DEN model)
3PG-AlF_4^--ADP	2.24	39.3	60.6	1.36
3PG-ATP	2.34	92.5	7.5	7.55
3PG-ADP	2.37	96.5	3.5	0.9
3PG	2.40	96.6	3.4	2.75
ADP	2.42	96.7	3.3	5.15
crystal structures				
Open	**2.33**			
Closed	**2.17**			

Zerrad, *et al.* (2011) *J. Biol. Chem.*, **286,** 14040-14048

Refinement of Rigid Domains

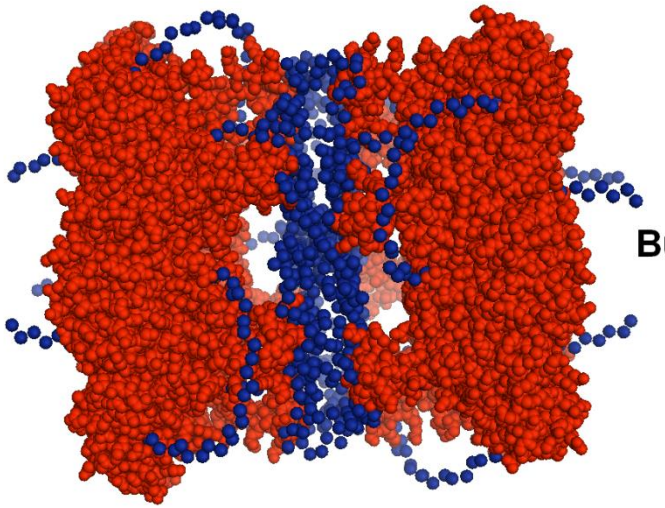


DEN refinement using SAXS and MX data allows visualization of domain movement

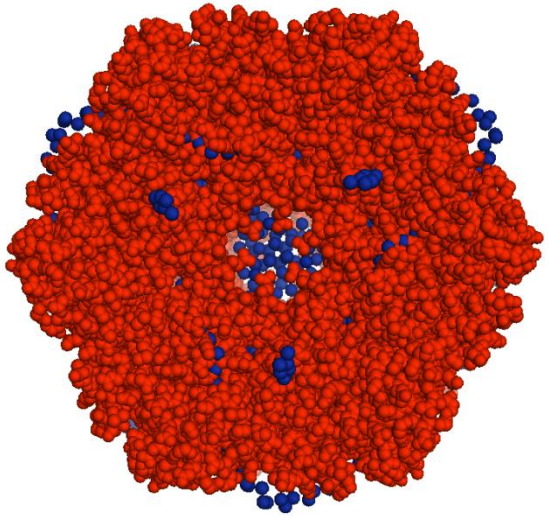
Zerrad, *et al.* (2011) *J. Biol. Chem.,* **286,** 14040-14048

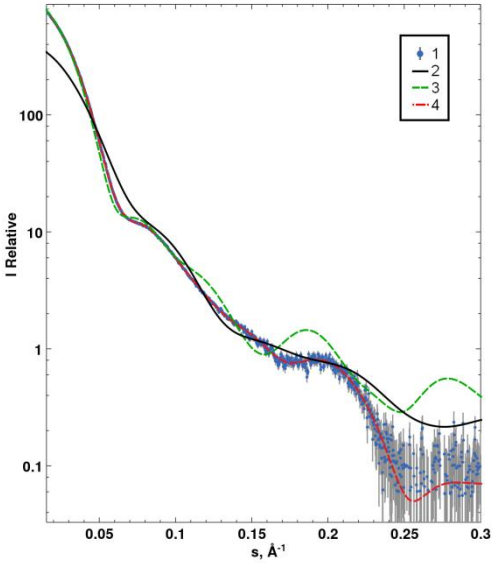
Adding Missing Linkers

Modelling of proteins from high resolution fragments/constructs
replacing missing portions BUNCH or CORAL



Bunch P32





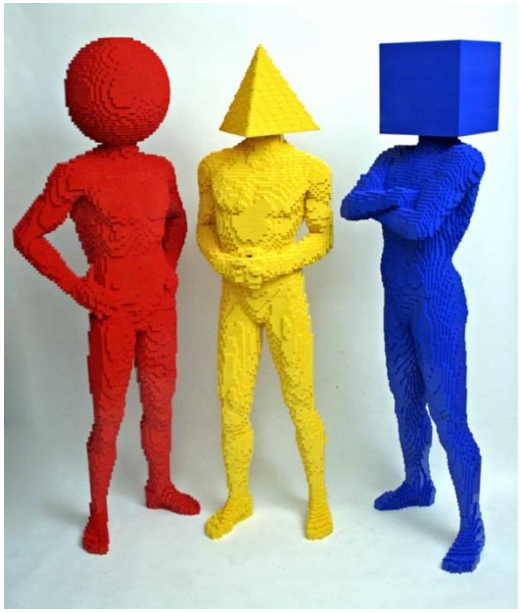
High resolution structure from X-ray crystallography showed missing portions due to disorder

Questions:
is it hexamer or dodecamer in solution?
Where are the missing residues

Ab-initio Modelling

Modelling of proteins 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

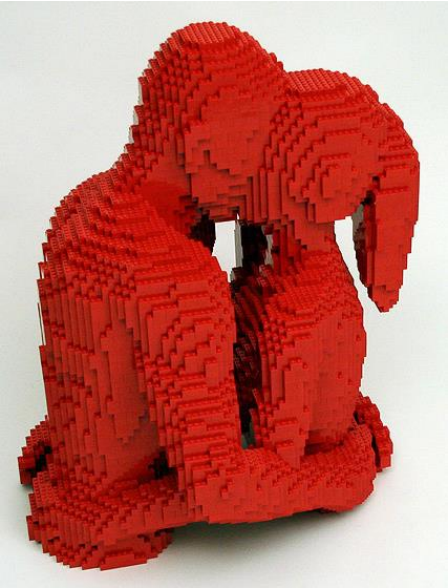
Occupies a known position in space

Has a known scattering pattern (Solvent or Particle)

Ab-initio Modelling

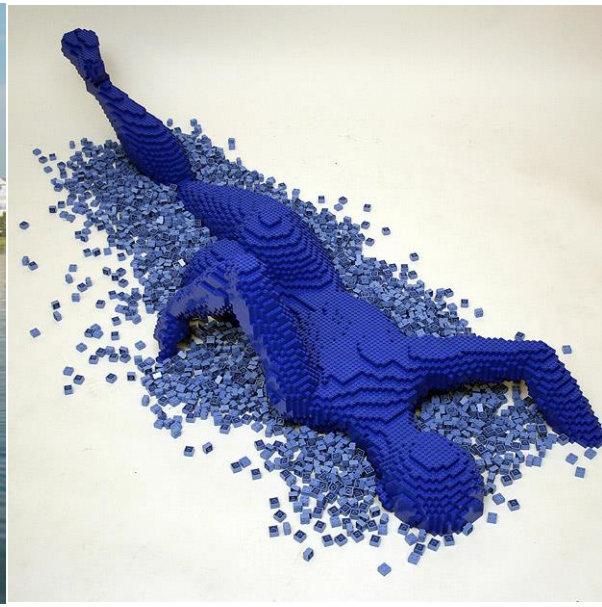
Modelling of proteins with no prior information

Build complex models from uniform blocks



Must be
Compact

Must NOT be
disconnected



Must NOT be
Loose

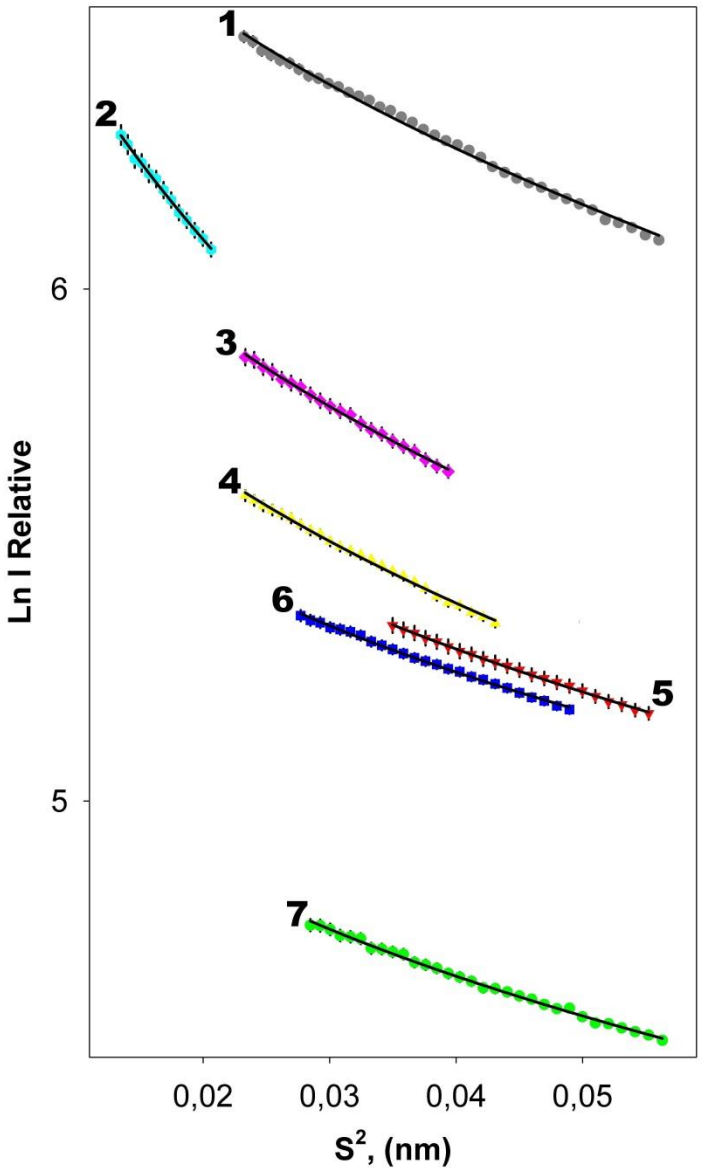
Ab-initio Modelling

With multiple data sets multi phase models can be produced

Points in the search grid can be assigned to Protein 1 Protein 2 or solvent

MONSA

SAXS analysis of yeast Arc1p-complex



P(r) Plot

1) MetRS:Arc1p:GluRS + tRNA

2) MetRS:Arc1p:GluRS

3) GluRS:Arc1p

4) MetRS:Arc1p

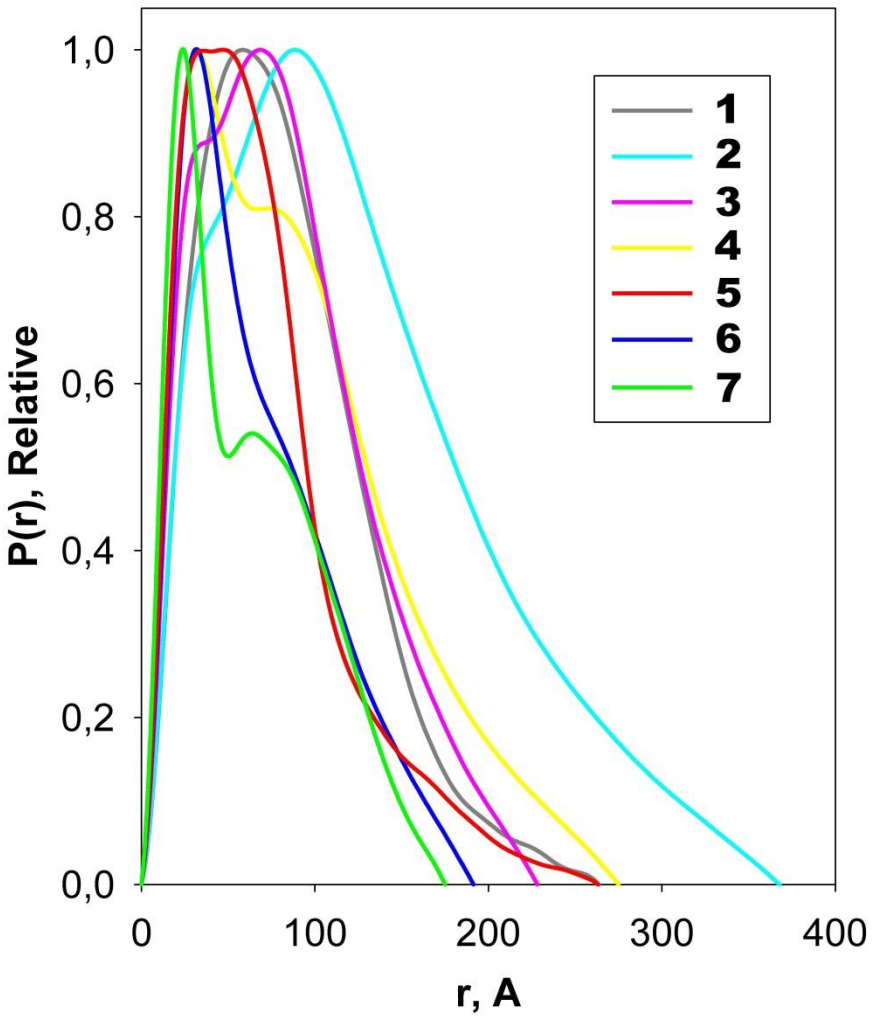
5) GluRS

6) MetRS

7) Arc1p

Nucleic Acids Res. 2013 Jan 7;41(1):667-76

SAXS analysis of yeast Arc1p-complex



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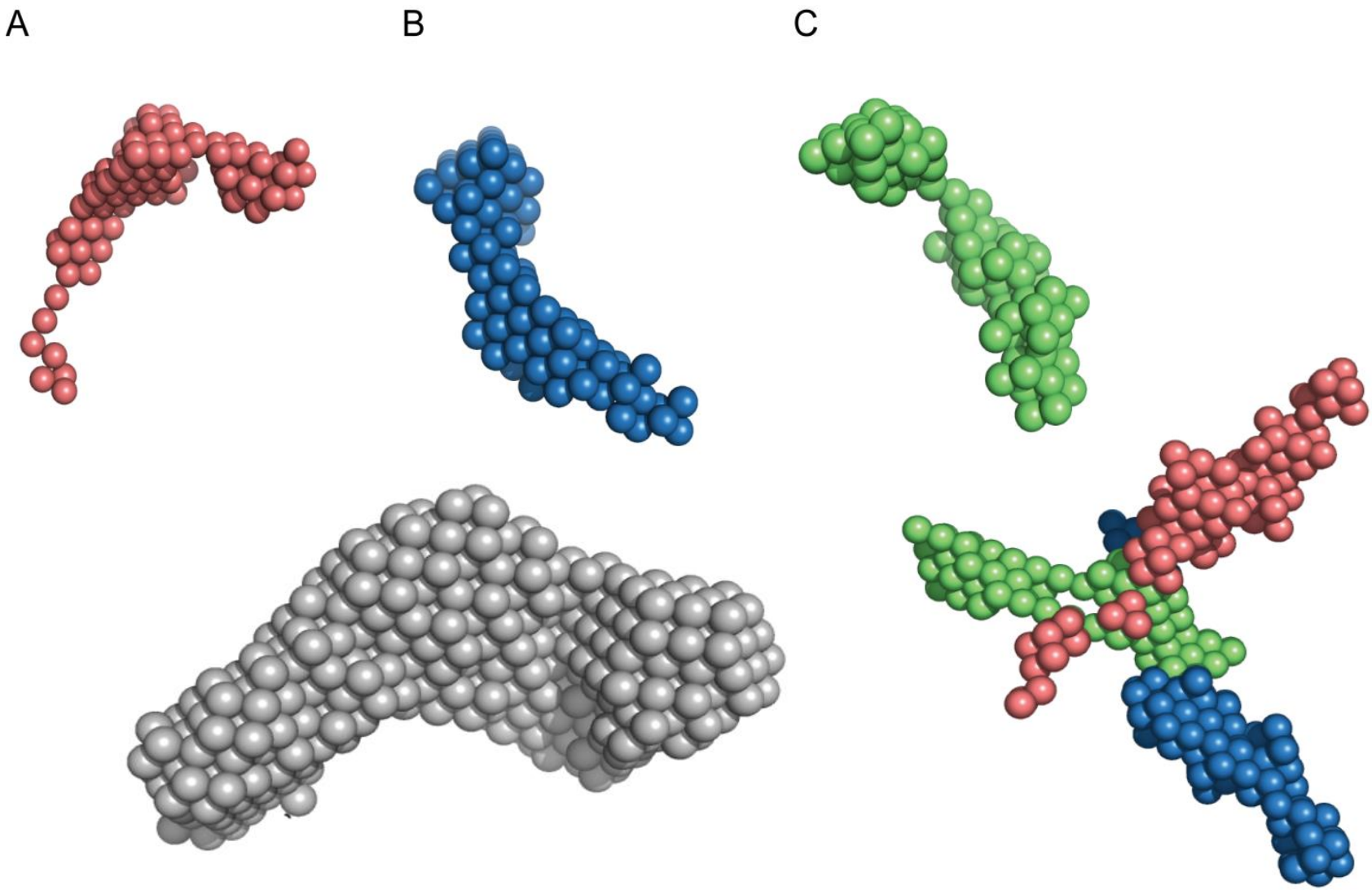

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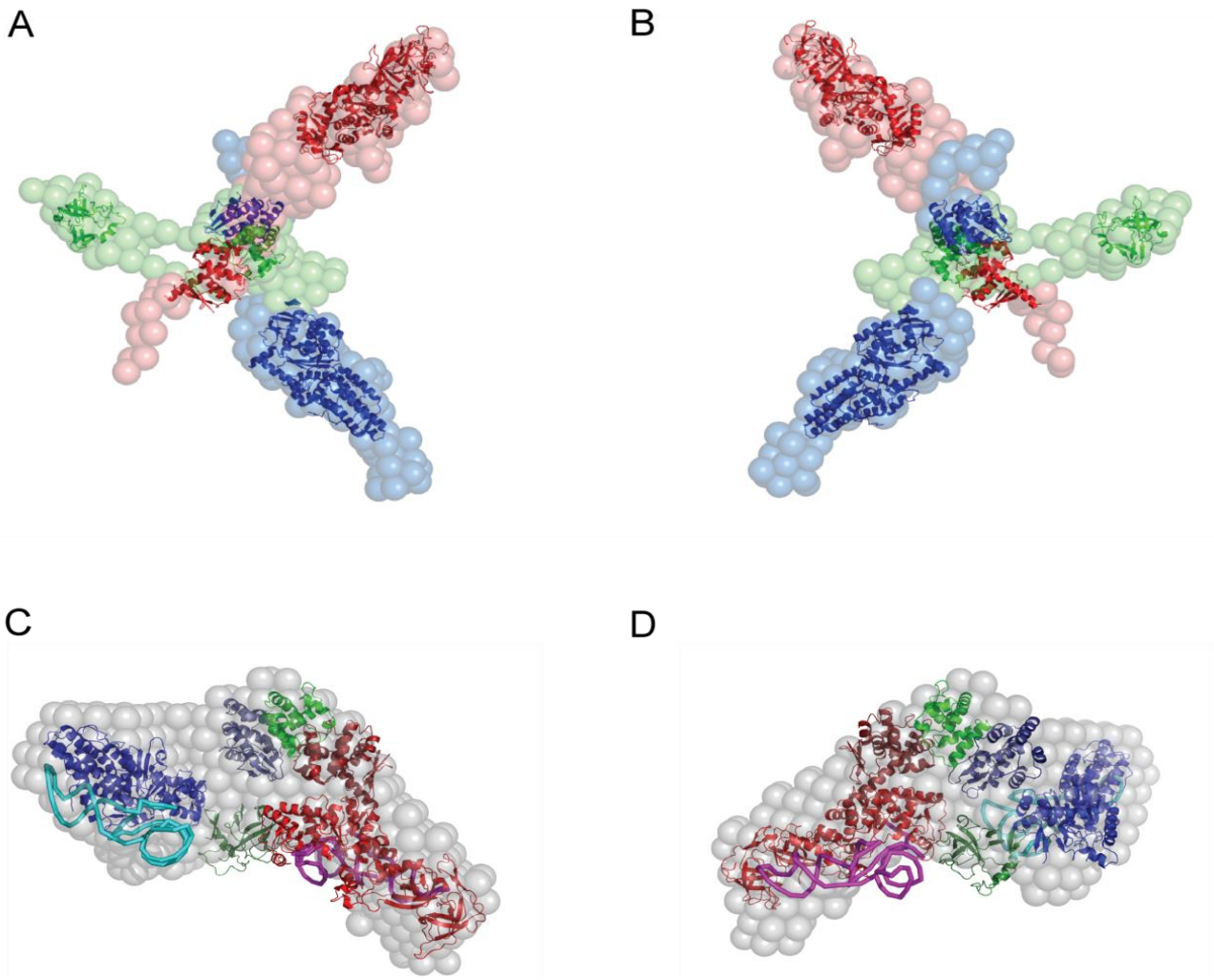

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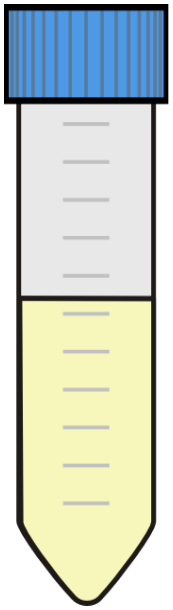
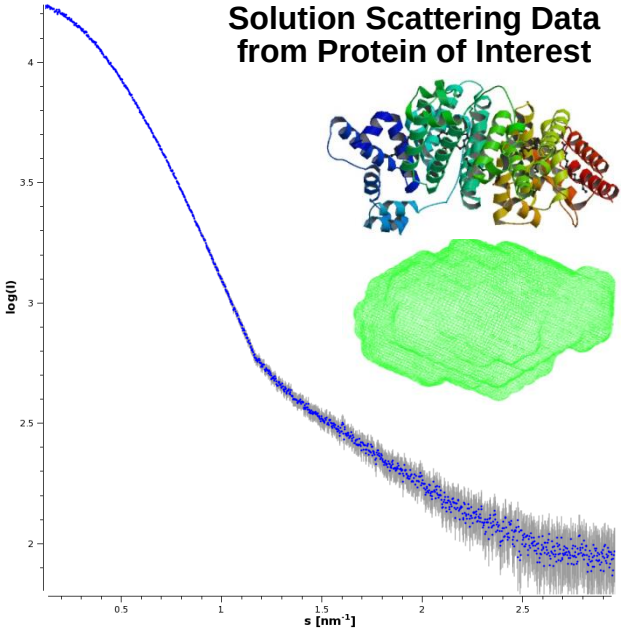
Idealised Solution SAS Experiment



Black Box

Neutron or X-ray source



What exactly is the Black box?

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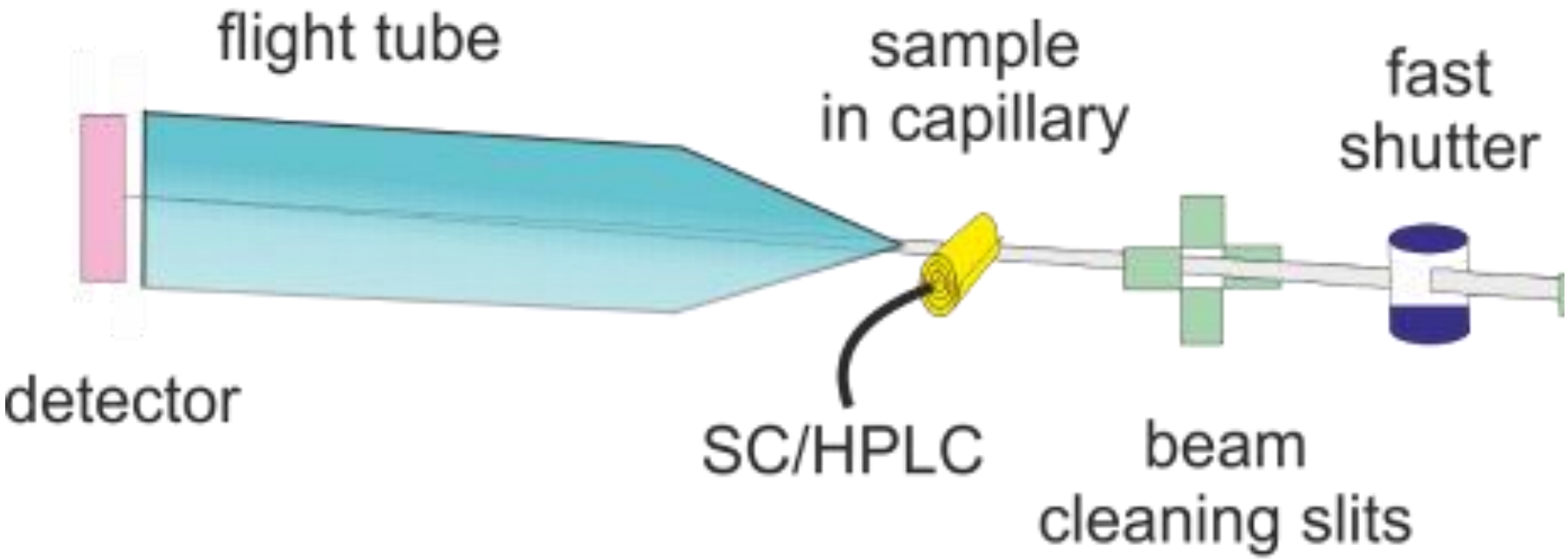

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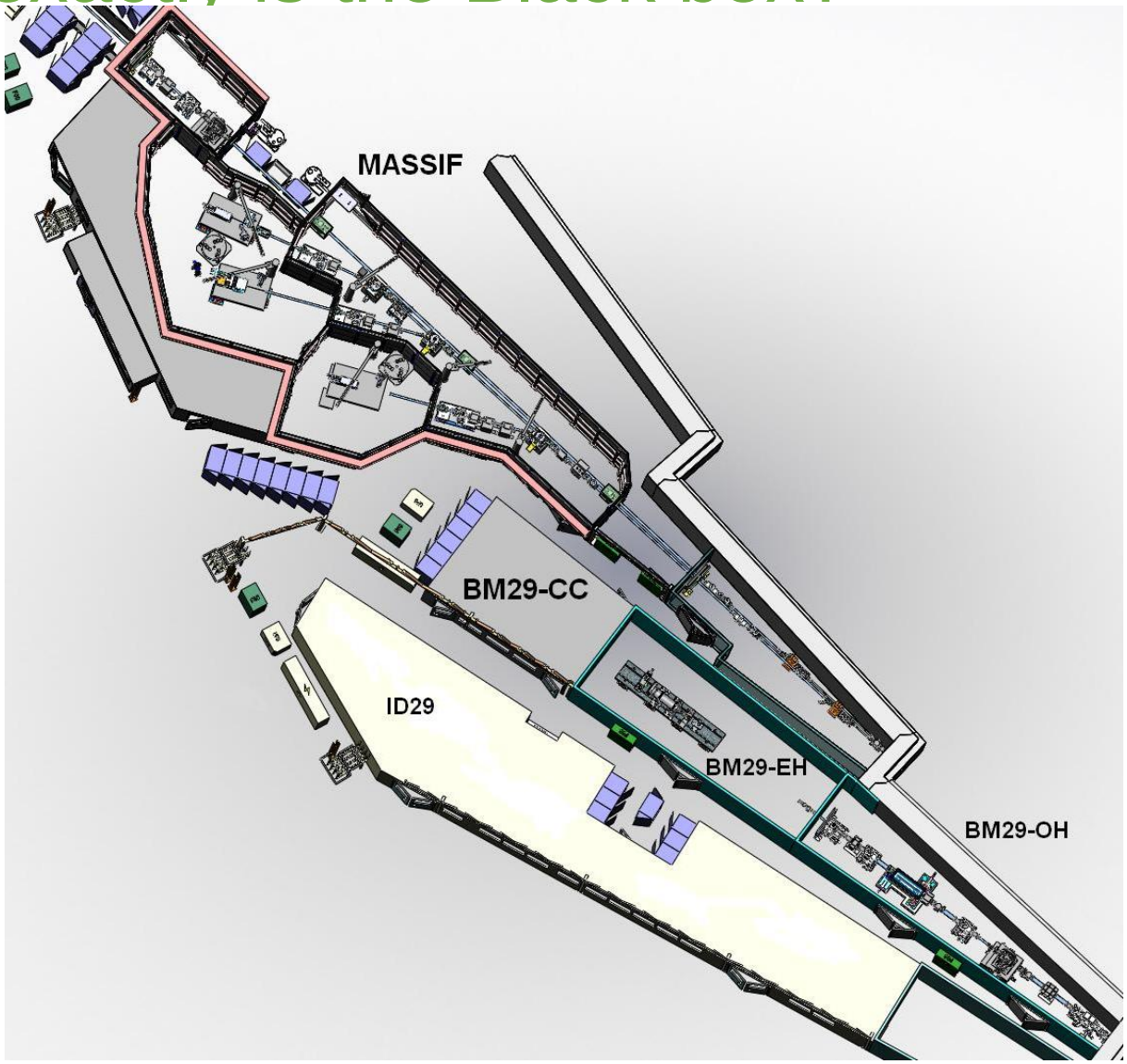

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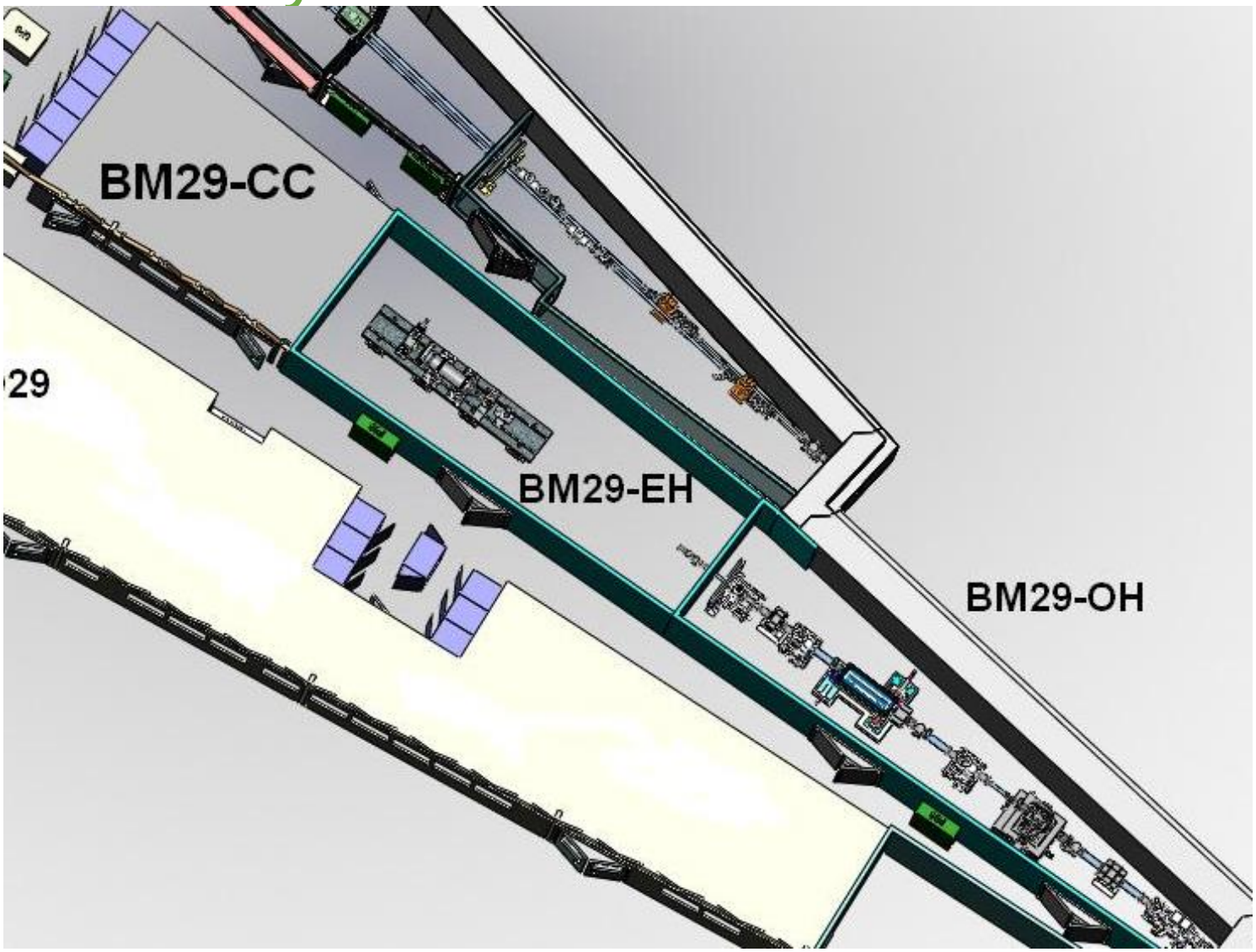

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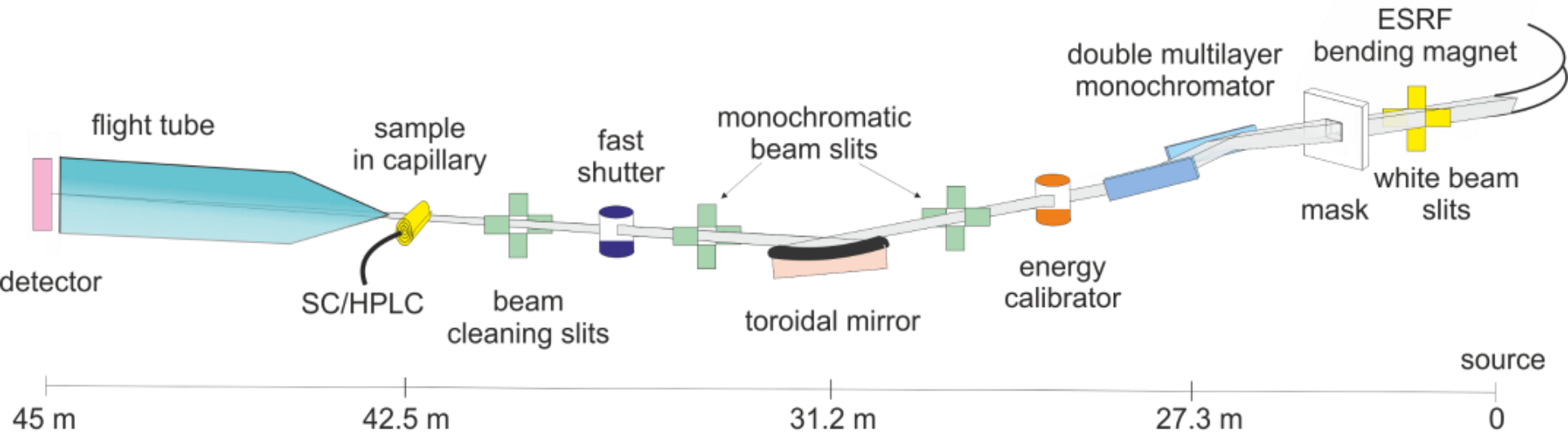

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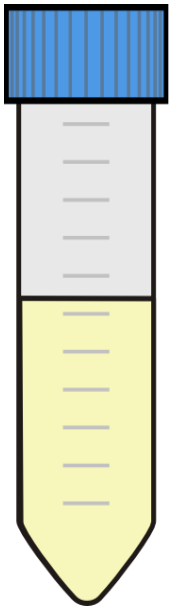

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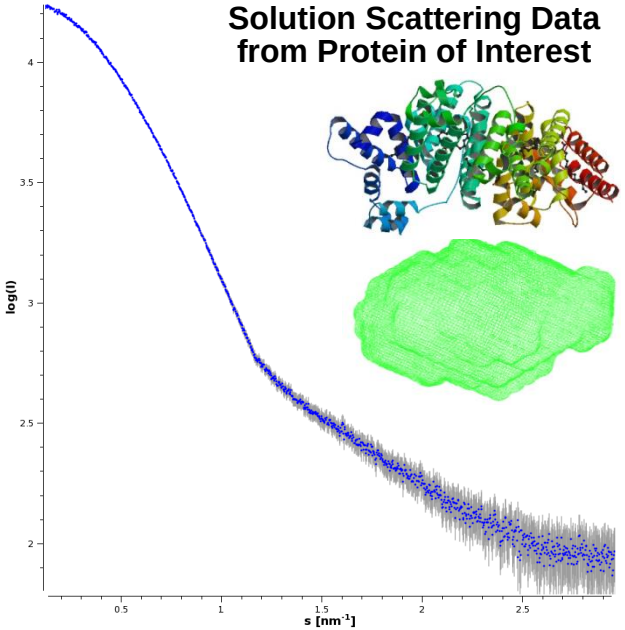
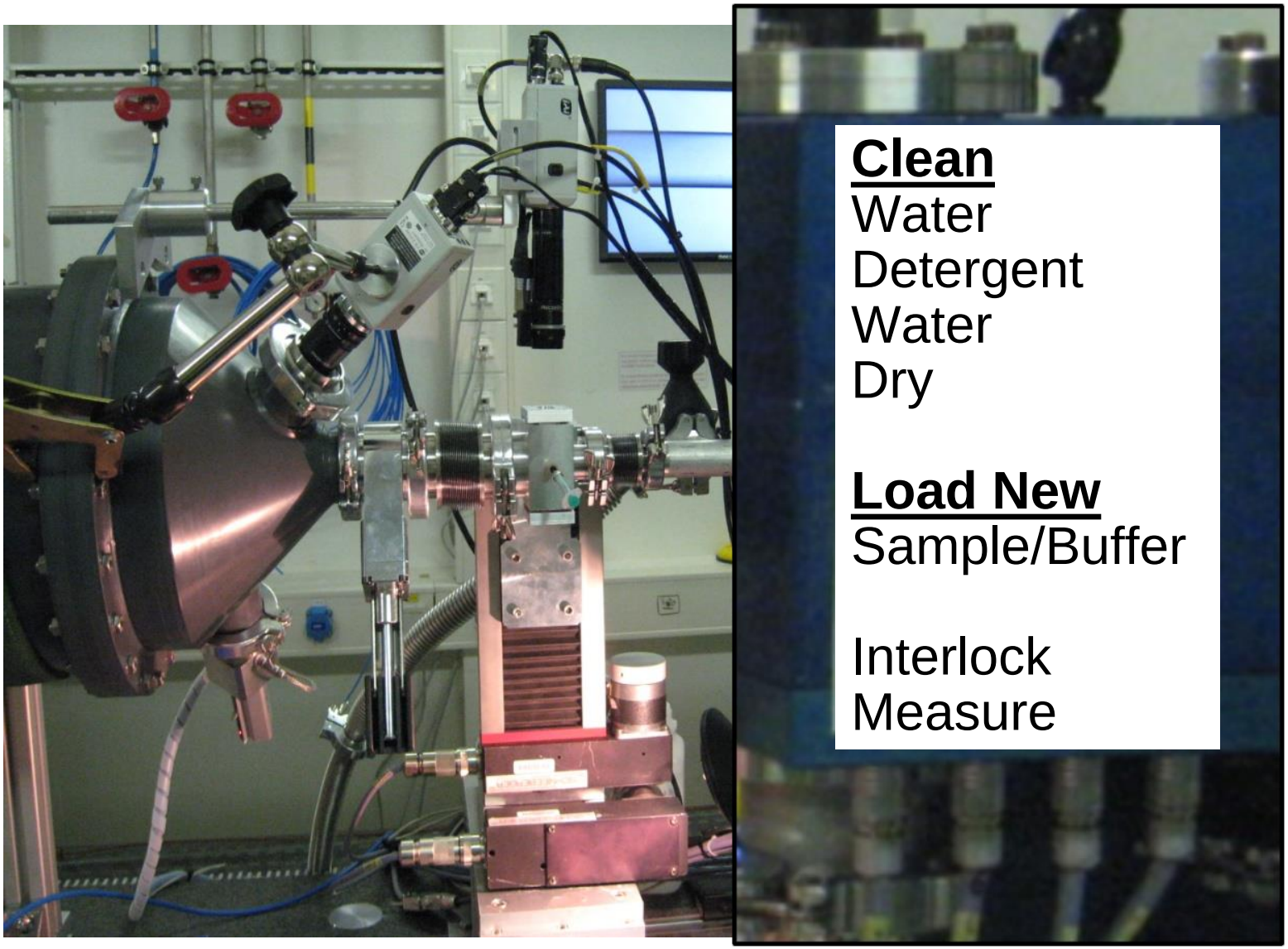


Experimental Procedure



SC development

Sample Volume
Cleaning time
Total cycle time

2007 — Manual sample handling

2007 — 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

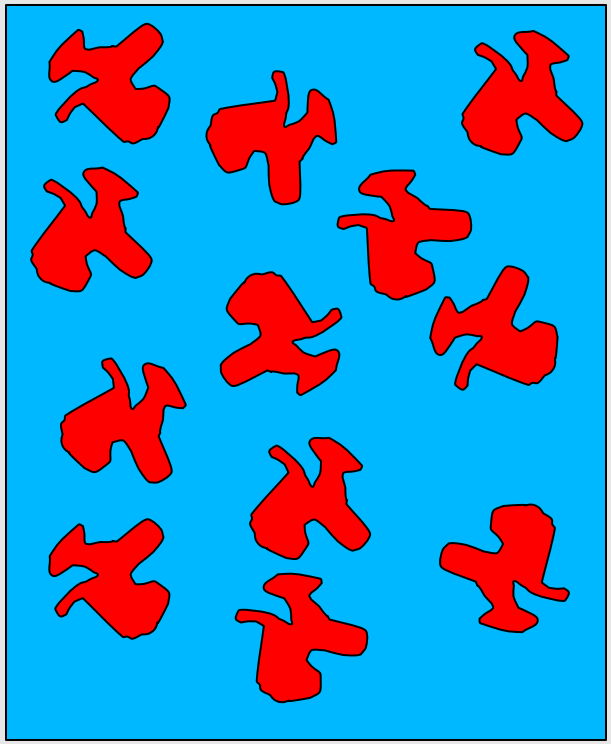
Now — 2nd Gen SC BM29@ESRF P12@PETRAIII B21@Diamond

Reliability
Confidence
Throughput

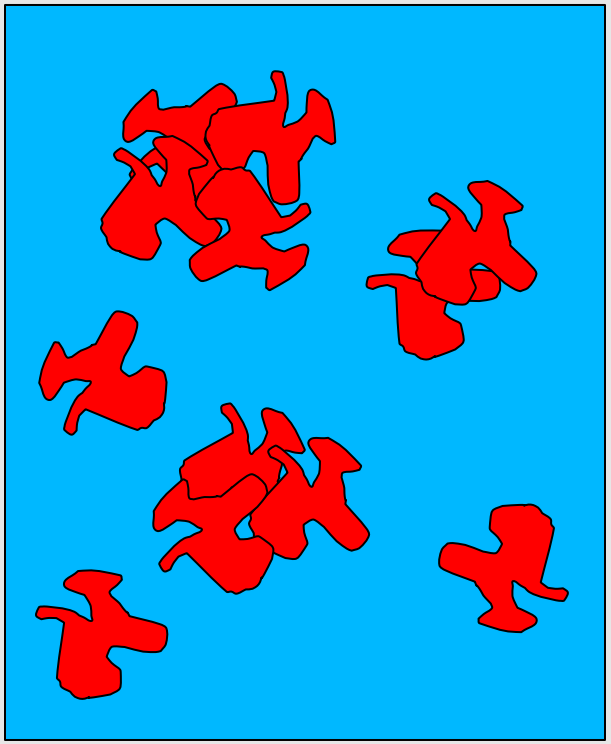
Sample Preparation

In solution SAXS we observe the **Average**

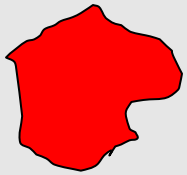
Monodisperse



Mixture



Average



Sample Preparation

Shape reconstruction requires:

MONODISPERSE!

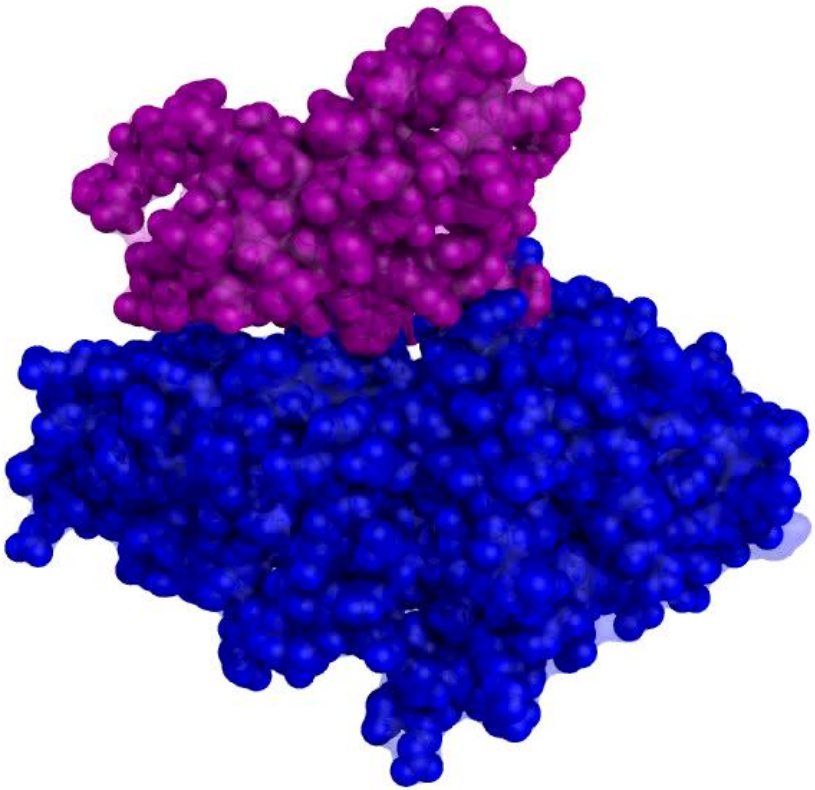
samples in solution

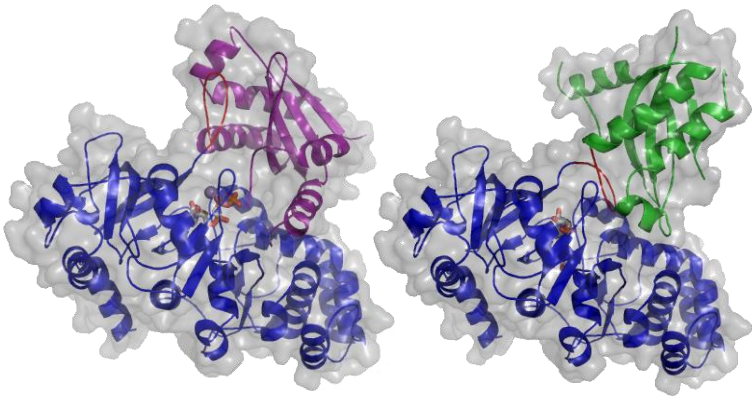
Pure protein (>90%)!
In only 1 oligomeric state!
NO aggregation!
Free from interparticle effects!

Before going to the beamline users are encouraged to use:

HPLC/FPLC purification
MALS/DLS
Analytical ultra centrifugation

Online SEC GH3-12



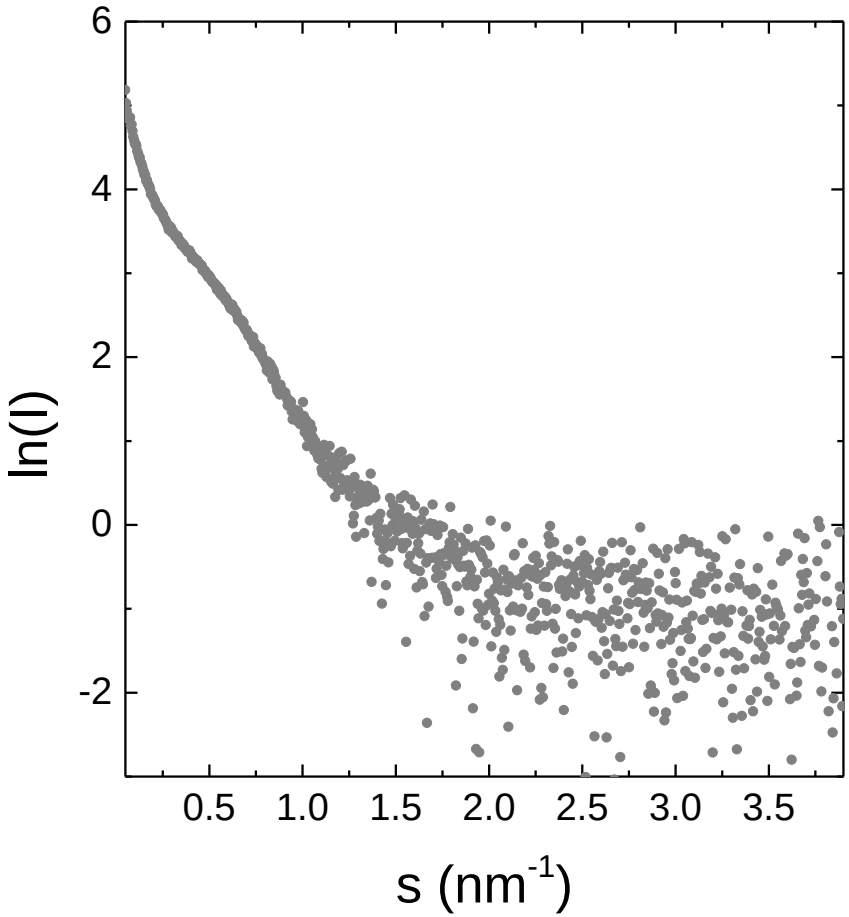


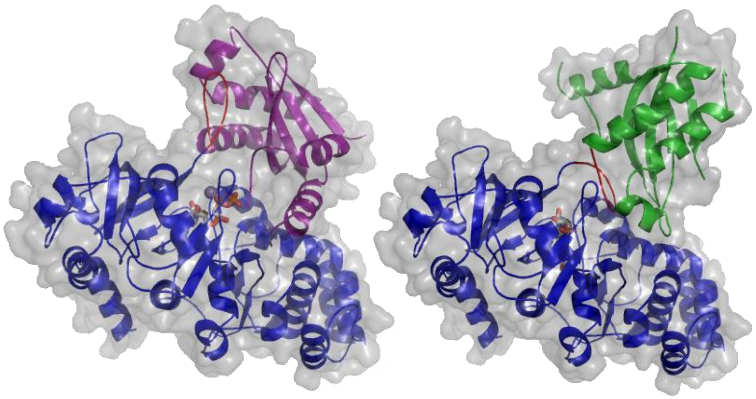
open closed

$\Delta R_G = 0.35$ nm

Round A, Brown E, Kapp U, Westfall CS, Jez JM, Zubieta C. (2013) Acta D

Online SEC GH3-12





open closed

$\Delta R_G = 0.35$ nm

Aggregation!
Standard experiment
gives no answer

Round A, Brown E, Kapp U, Westfall CS, Jez JM, Zubieta C. (2013) Acta D

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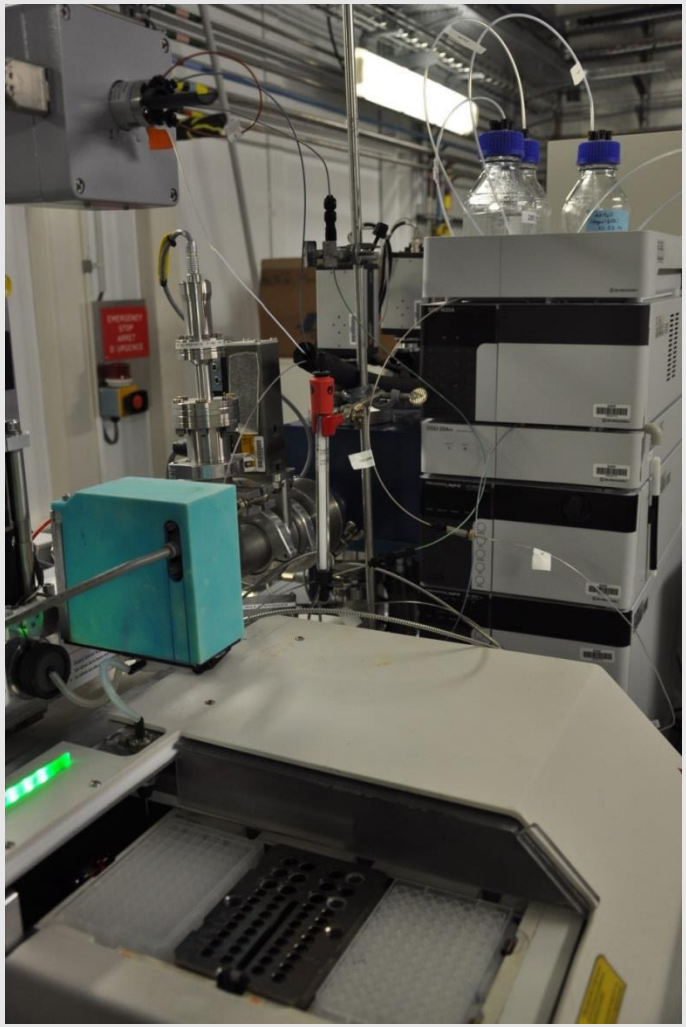
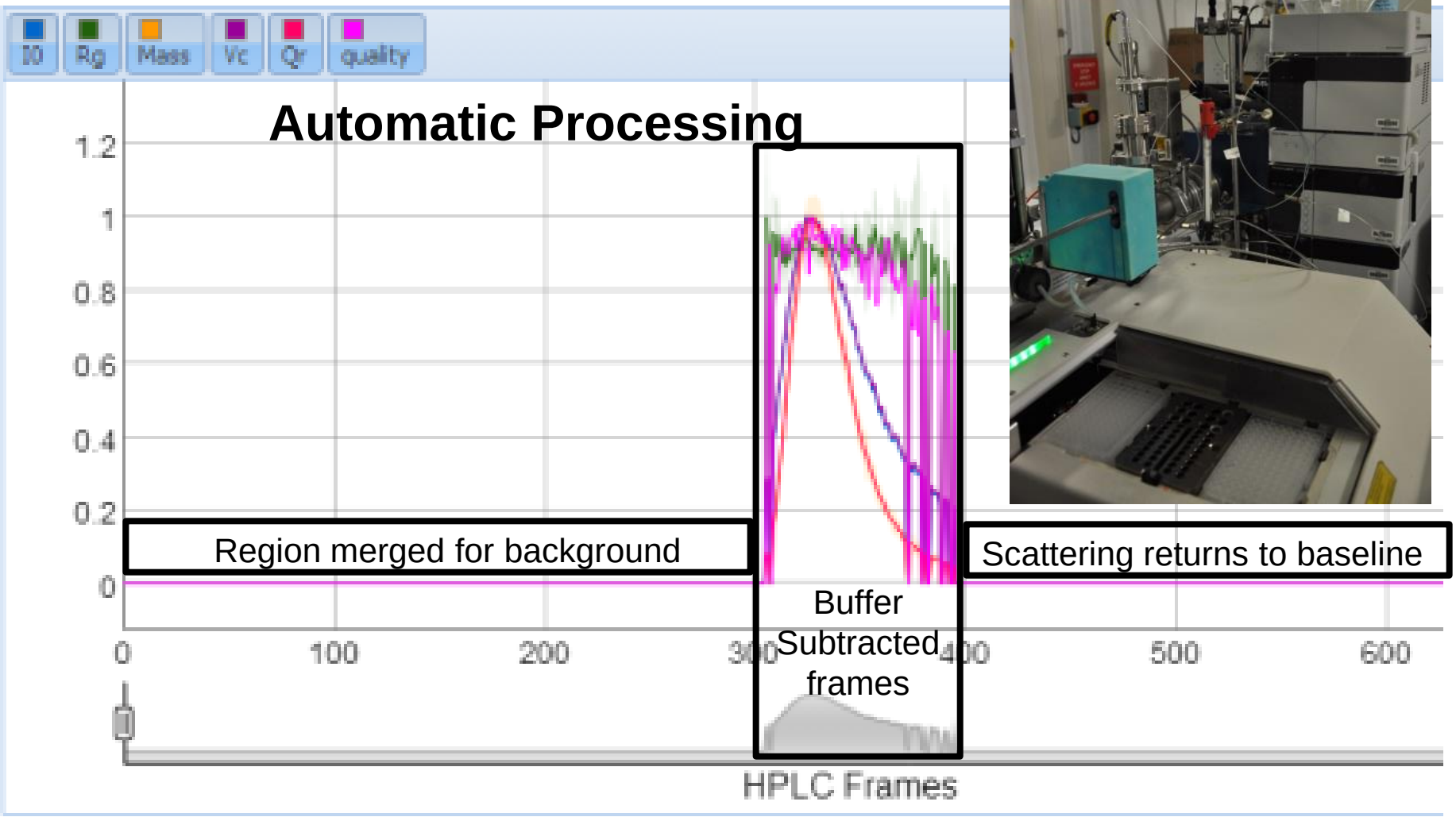
Current status of BM29: New online SEC

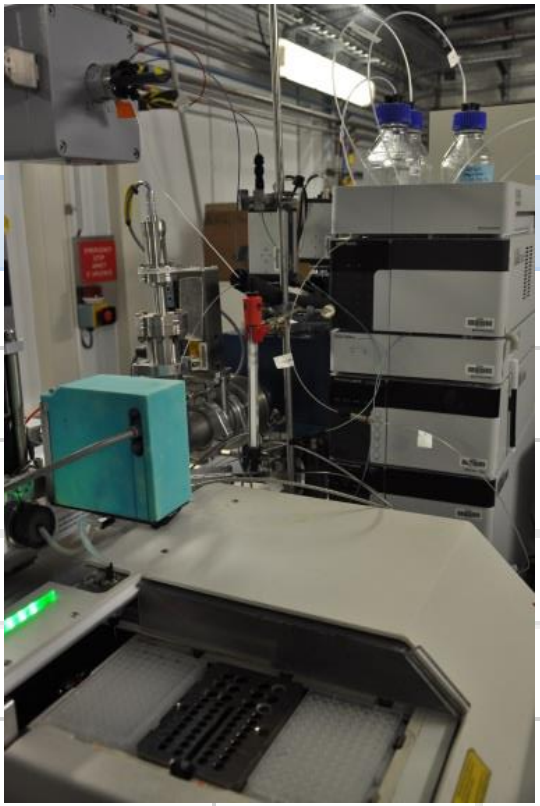
Malvern currently in place
but will be moved to prep lab



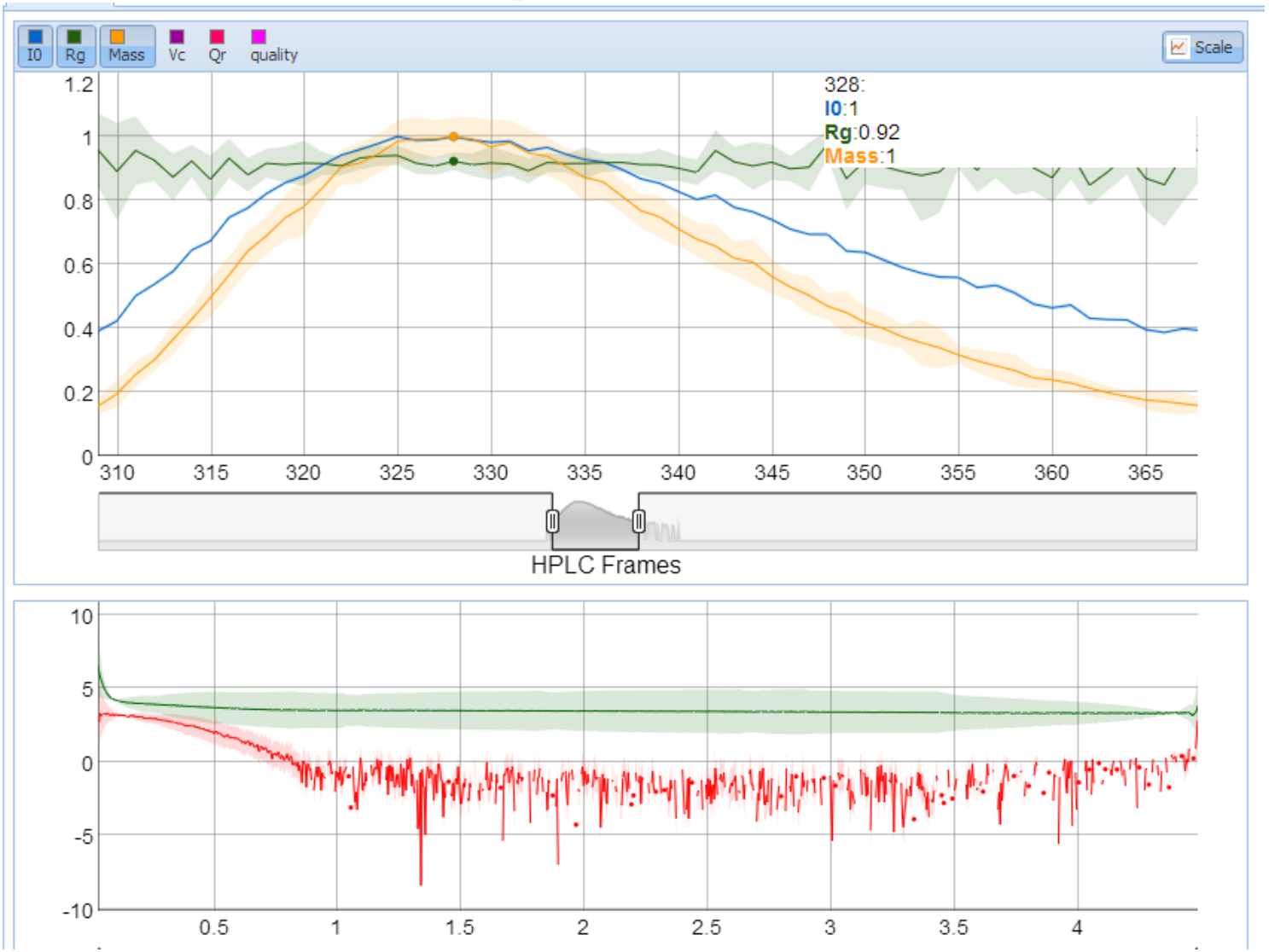
Shimadzu installed over Easter
and is already available to users

Online SEC in ISPyB





Online SEC in ISPyB



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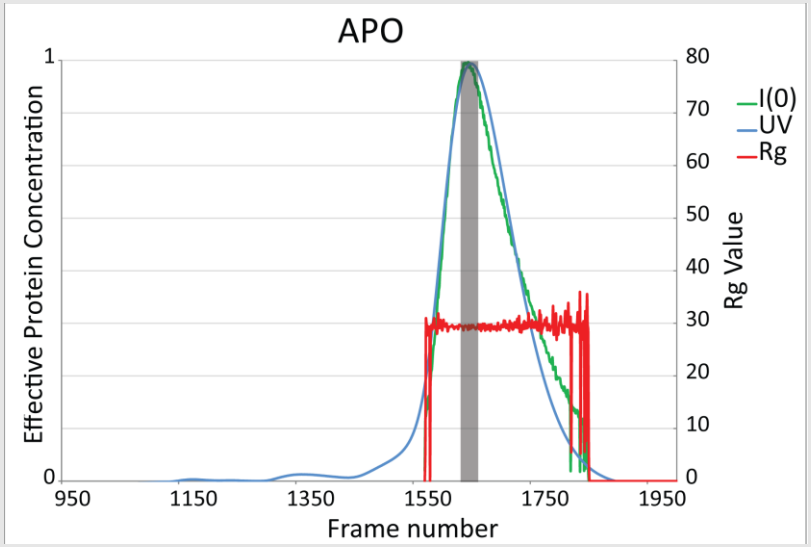
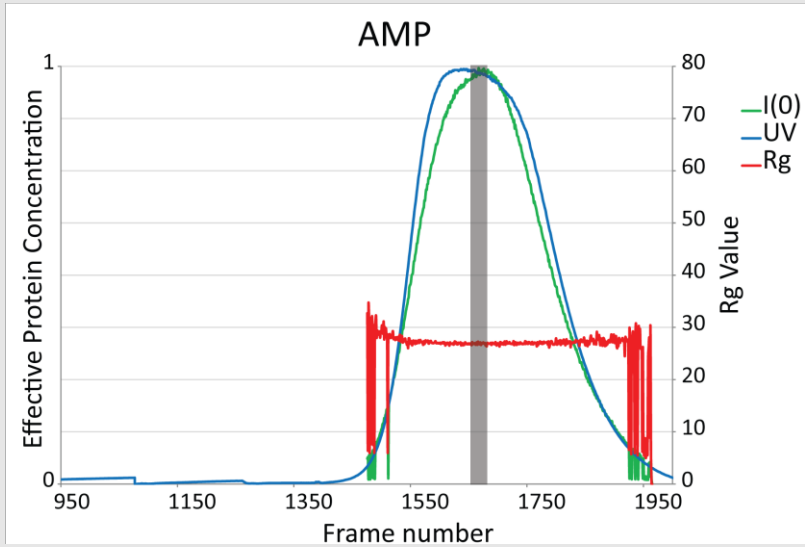
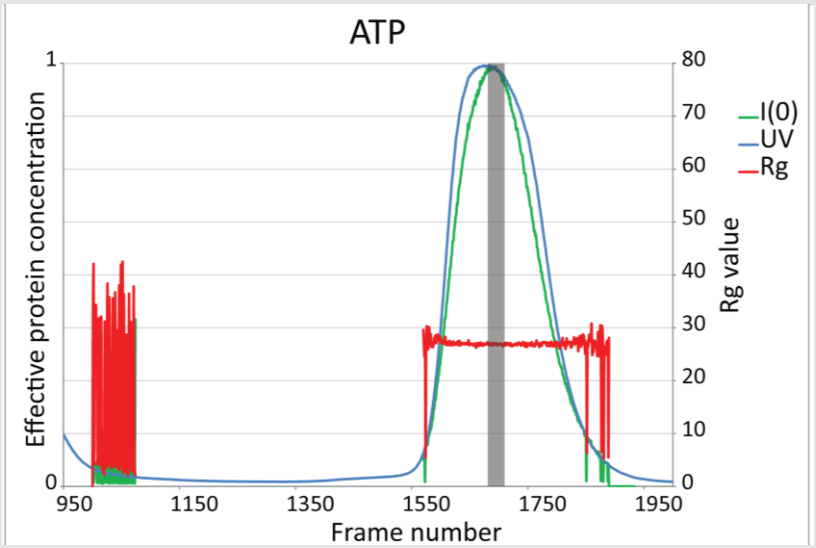
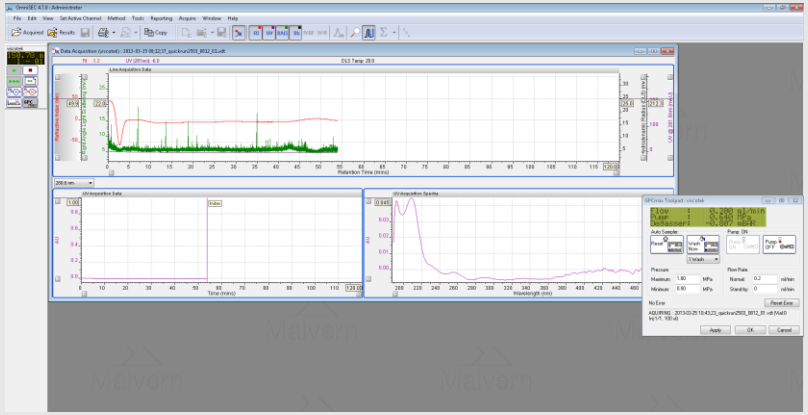








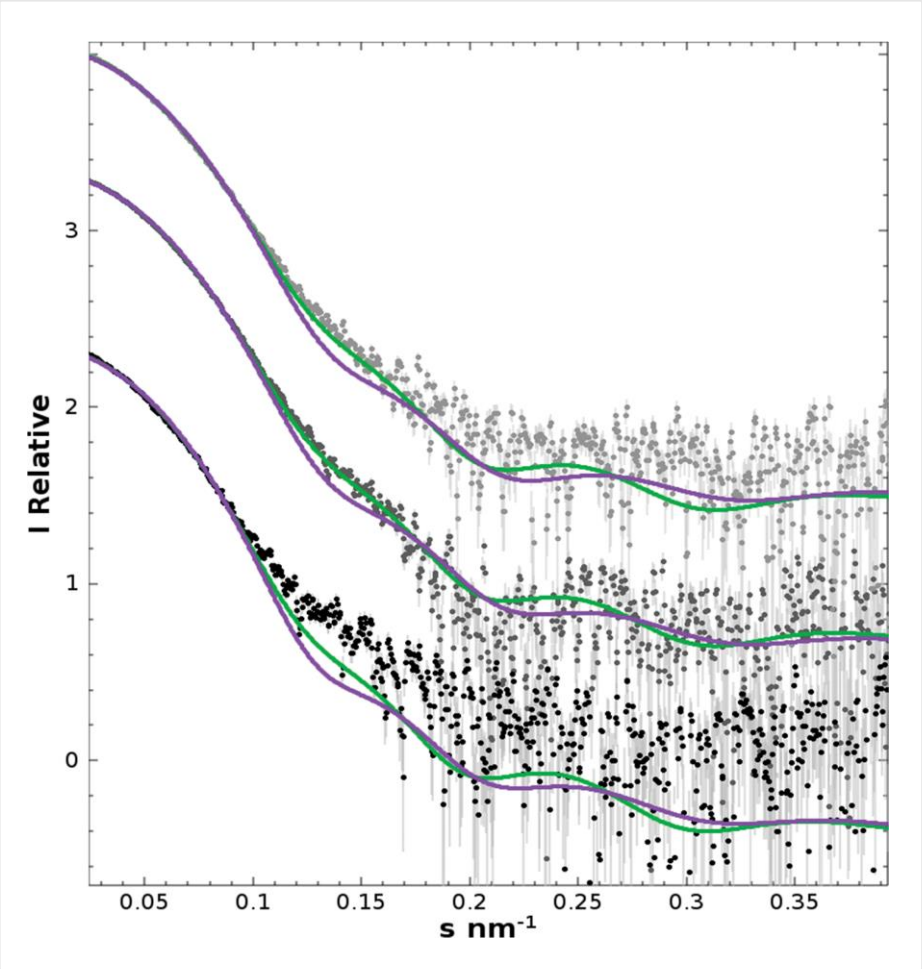
Biophysical characterization

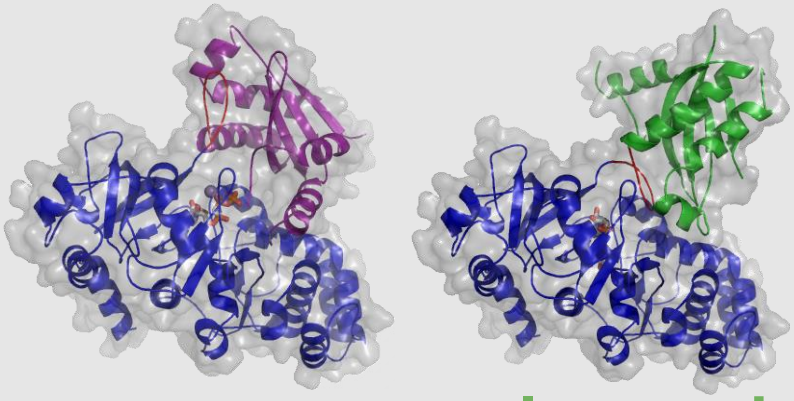
Round A, Brown E, Kapp U, Westfall CS, Jez JM, Zubieta C. (2013) Acta D

ESRF

EMBL

Online SEC





open closed

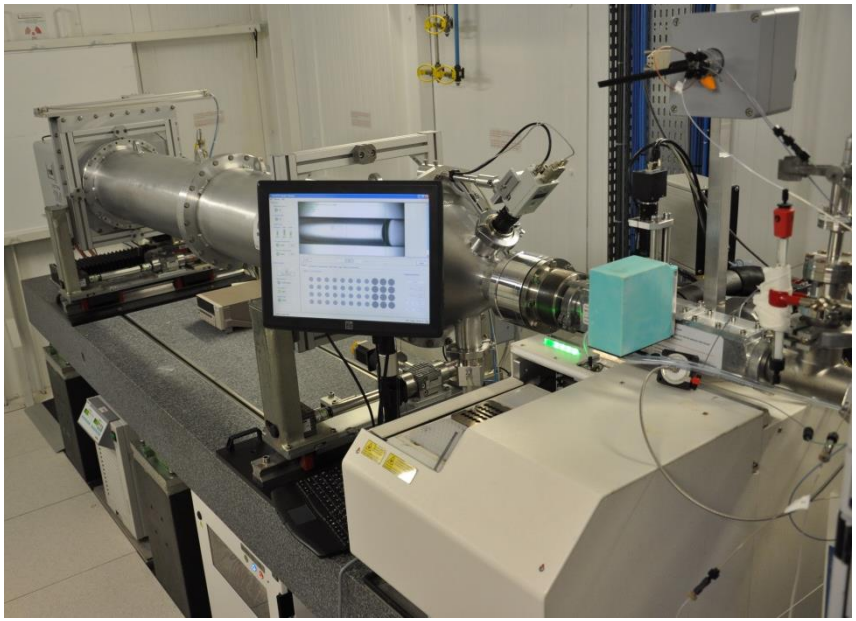
	R_G (nm)	D_{max} (nm)
ATP	2.75 ± 0.05	9.0 ± 0.3
AMP	2.75 ± 0.05	9.0 ± 0.3
APO	3.04 ± 0.04	11.0 ± 0.5

ATP/AMP: closed state *APO: flexibility*

Round A, Brown E, Kapp U, Westfall CS, Jez JM, Zubieta C. (2013) Acta D

ESRF EMBL

Current status of data collection:



Temperature

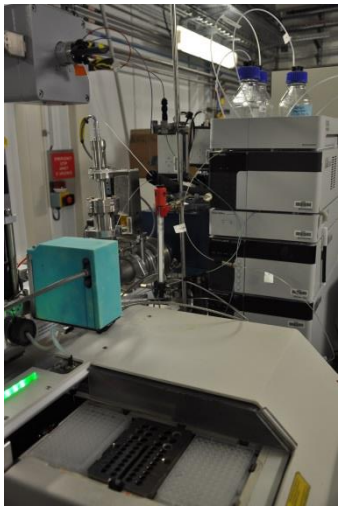
- Data acquisition between 4 and 60 C
- SEC operation at 4 or 20 degrees C

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



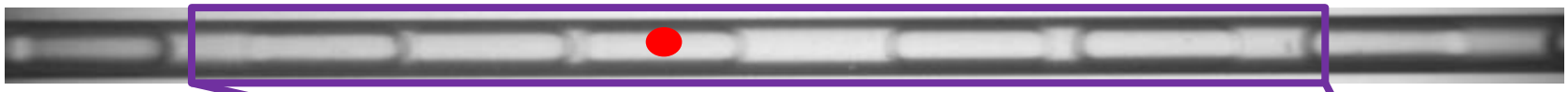
Automated valve

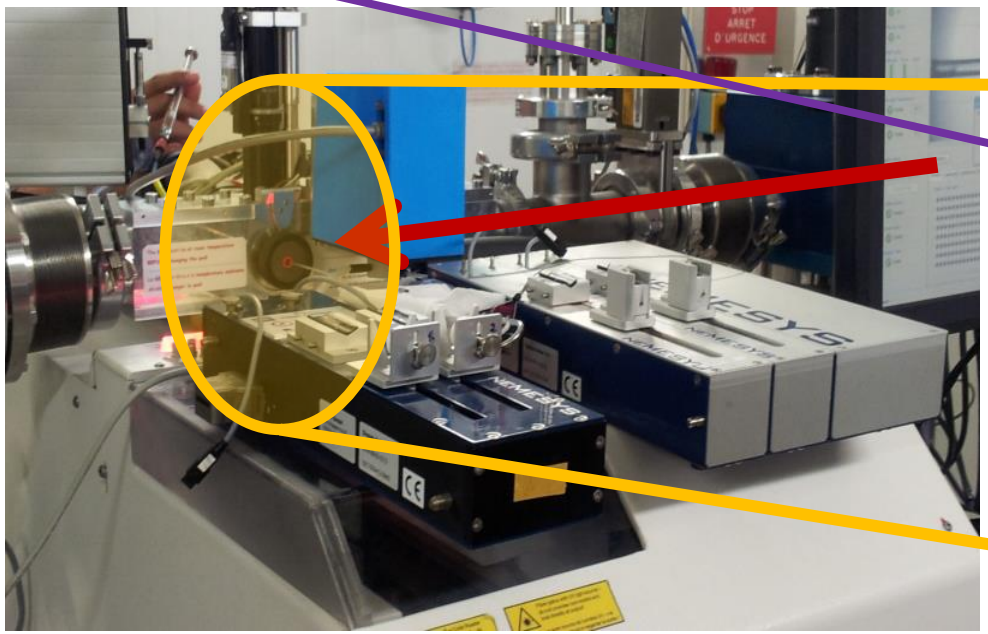
To switch between
SEC and Static modes

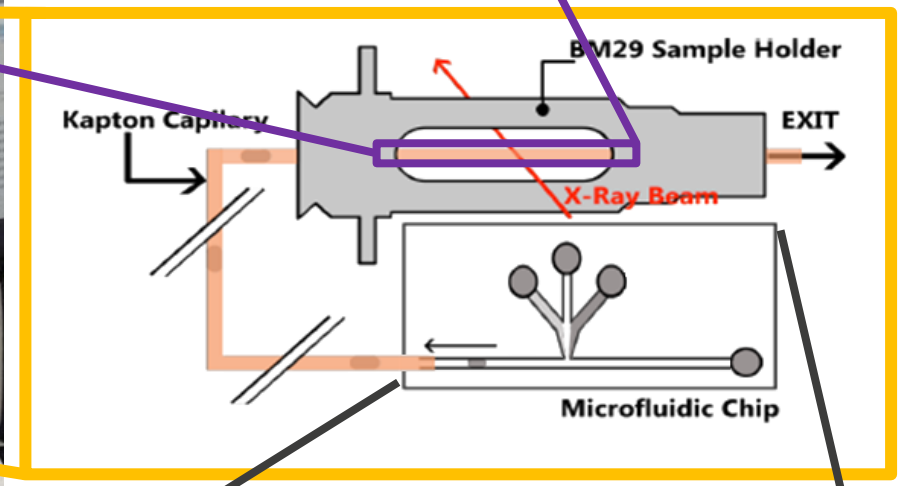
Gives users control
Safe and reliable switching
Maximises efficiency
cleaning between SEC runs

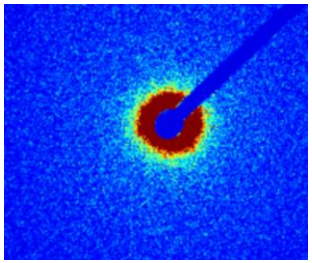
SC compatible Microfluidics: First results

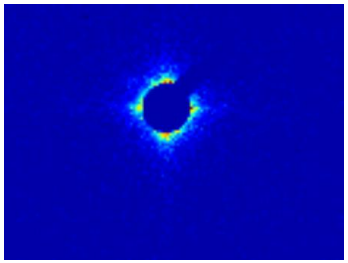
Proof of Principle achieved



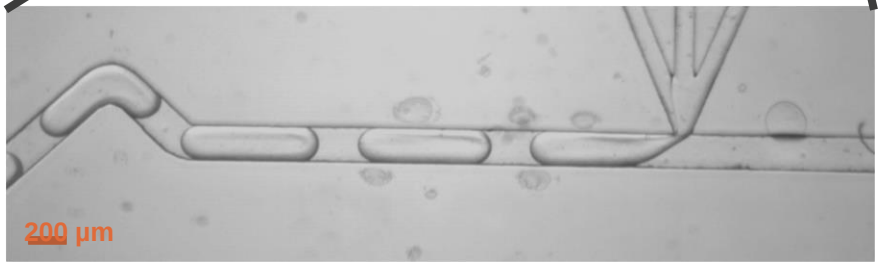




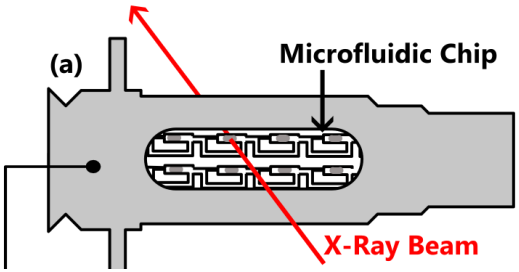


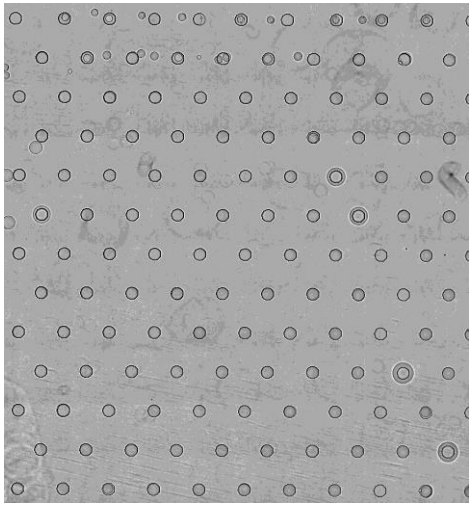


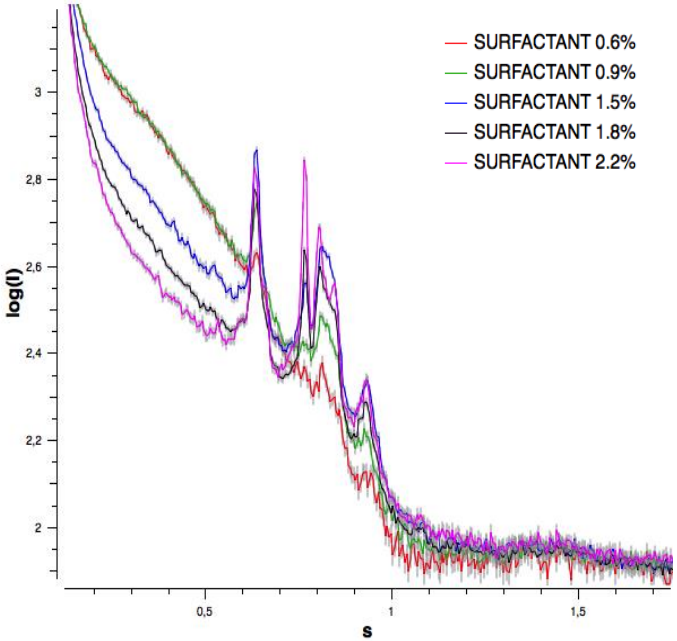
Background improved with scatterless pinhole

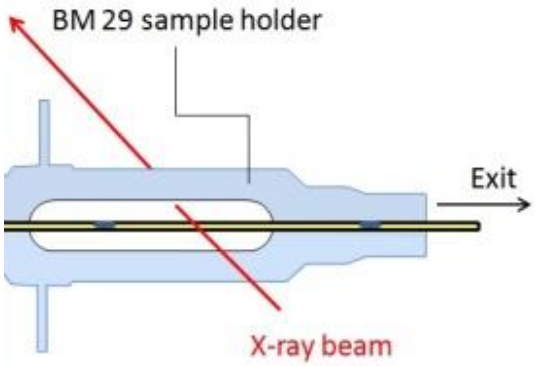


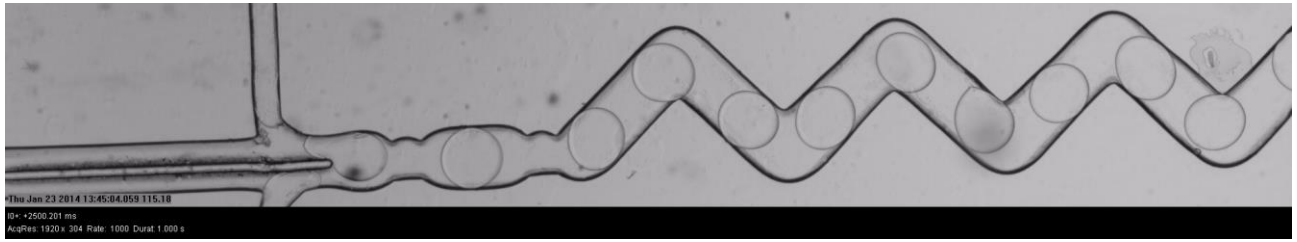
SC compatible Microfluidics











Experimental preparation and efficient use of beamtime facilitated through ISPyB

Improved feedback for experimental preparation

BIOSAXS Experiment Designer

Define Measurements

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

Single Measurement | Concentration Series

Macromolecules: PGK

Buffer: AMP

How many unknow 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	Viscosity		
	PGK	1.000		AMP	4.00 c	50.00 µl	100 %		yes	low		REMOVE
	PGK	2.000		AMP	4.00 c	50.00 µl	100 %		yes	low		REMOVE
	PGK	3.000		AMP	4.00 c	50.00 µl	100 %		yes	low		REMOVE

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 substraction automatically.**

Single Measurement | Concentration Series

Macromolecules: PGK

Buffer: ATP

How many unknow 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	Viscosity		
	PGK	1.000		AMP	4.00 c	50.00 µl	100 %		yes	low		REMOVE
	PGK	2.000		AMP	4.00 c	50.00 µl	100 %		yes	low		REMOVE
	PGK	3.000		AMP	4.00 c	50.00 µl	100 %		yes	low		REMOVE
	PGK	1.000		ATP	4.00 c	50.00 µl	100 %		yes	low		REMOVE
	PGK	2.000		ATP	4.00 c	50.00 µl	100 %		yes	low		REMOVE
	PGK	3.000		ATP	4.00 c	50.00 µl	100 %		yes	low		REMOVE

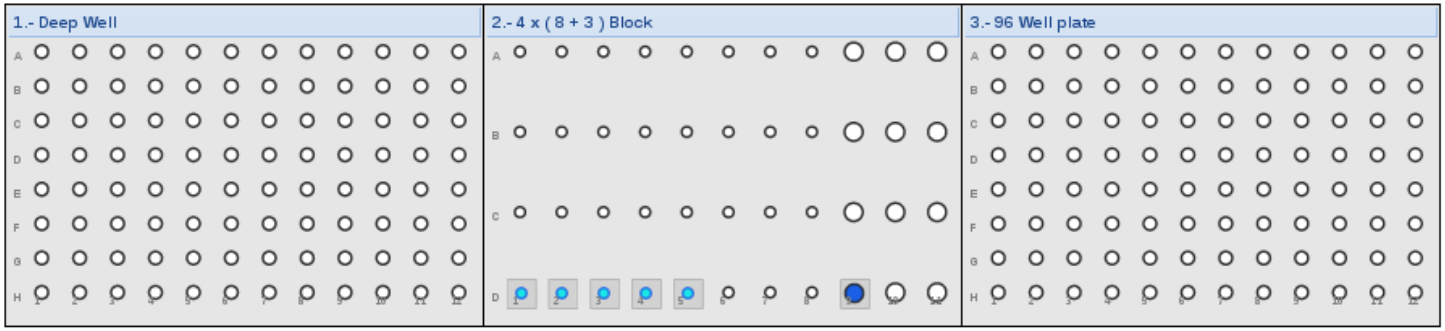
Improved feedback with ISPyB

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

Collapse buffers

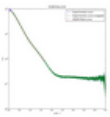
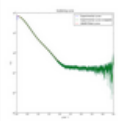
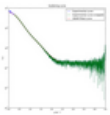
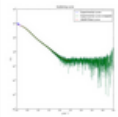
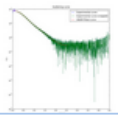
Specimen					Parameters						Status	Time	Comments
	Macromo.	Conc. (mg/ml)		Buffer	Exp. Temp.	Vol. Load	Trans.	Wait T.	Flow	Viscosity			
☐			■	D33	20.00 c	100.0...	100 %		yes	Low	DONE	06:47:07 pm	buffer
■	taHEFD33	14.000	■	D33	20.00 c	150.0...	100 %		yes	Low	DONE	06:48:23 pm	[1] tahef d33 truncation
☐			■	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

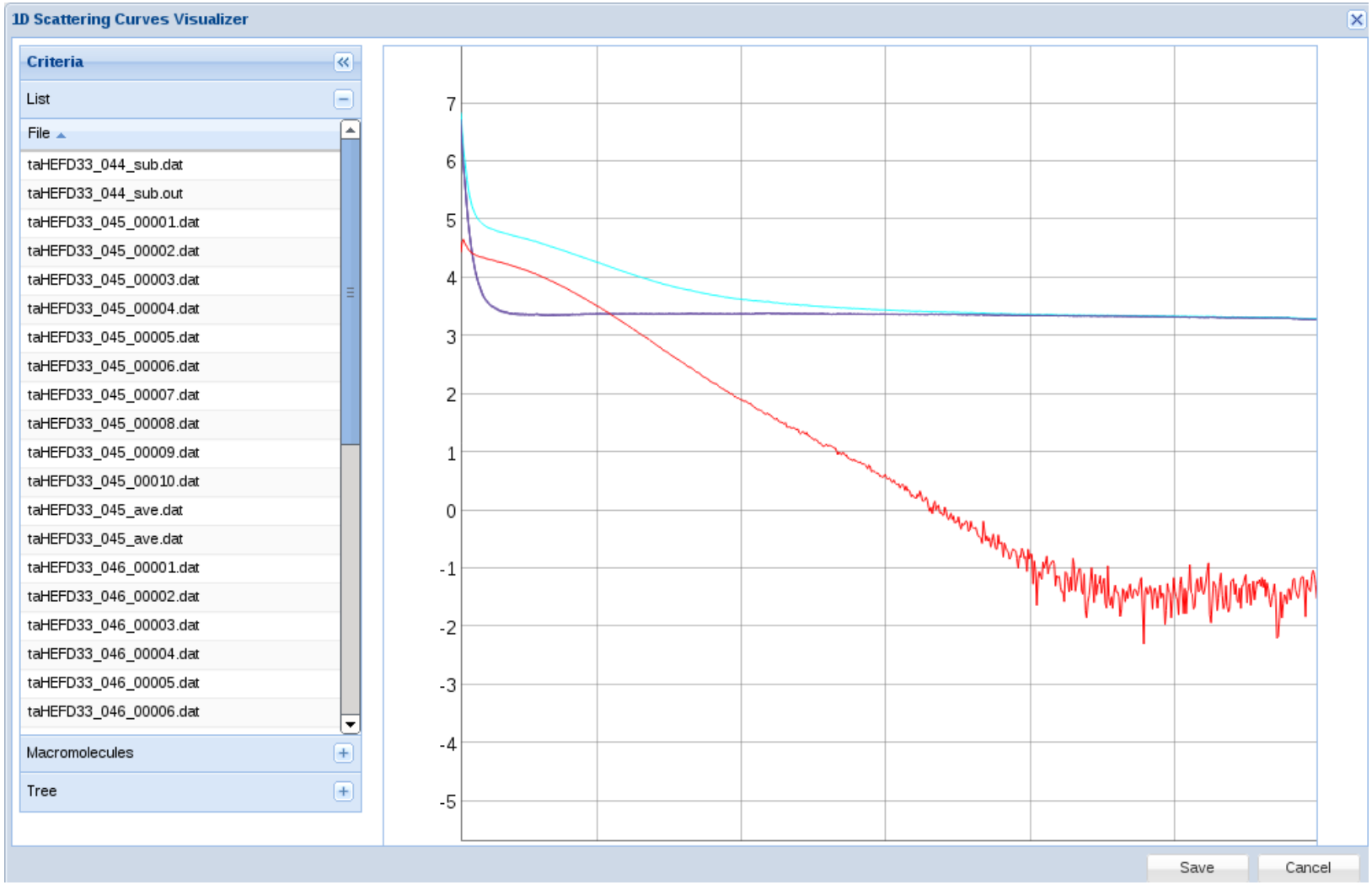


Improved feedback with ISPyB

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

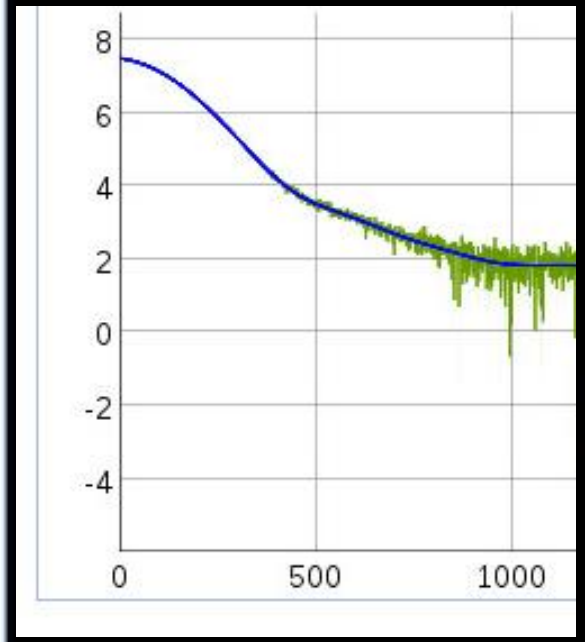


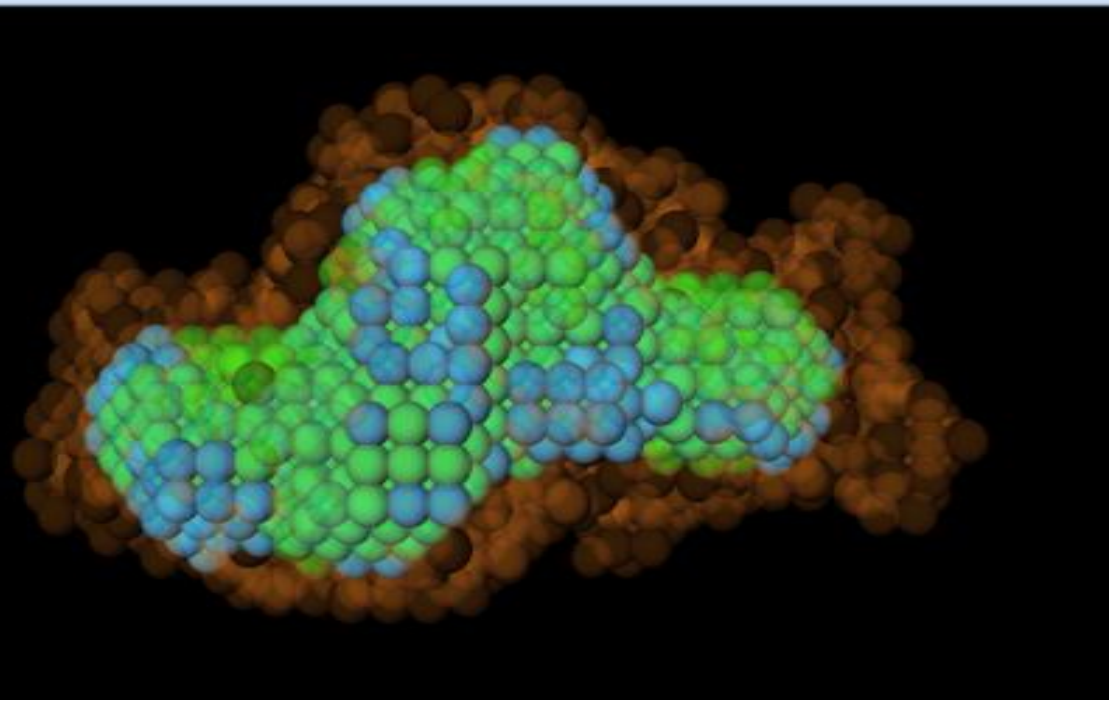
Improved feedback with ISPyB

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

Damaver Damfilt Dammin Merged

damaver_fss.pdb damfilt.pdb dammin.pdb Radius: 3

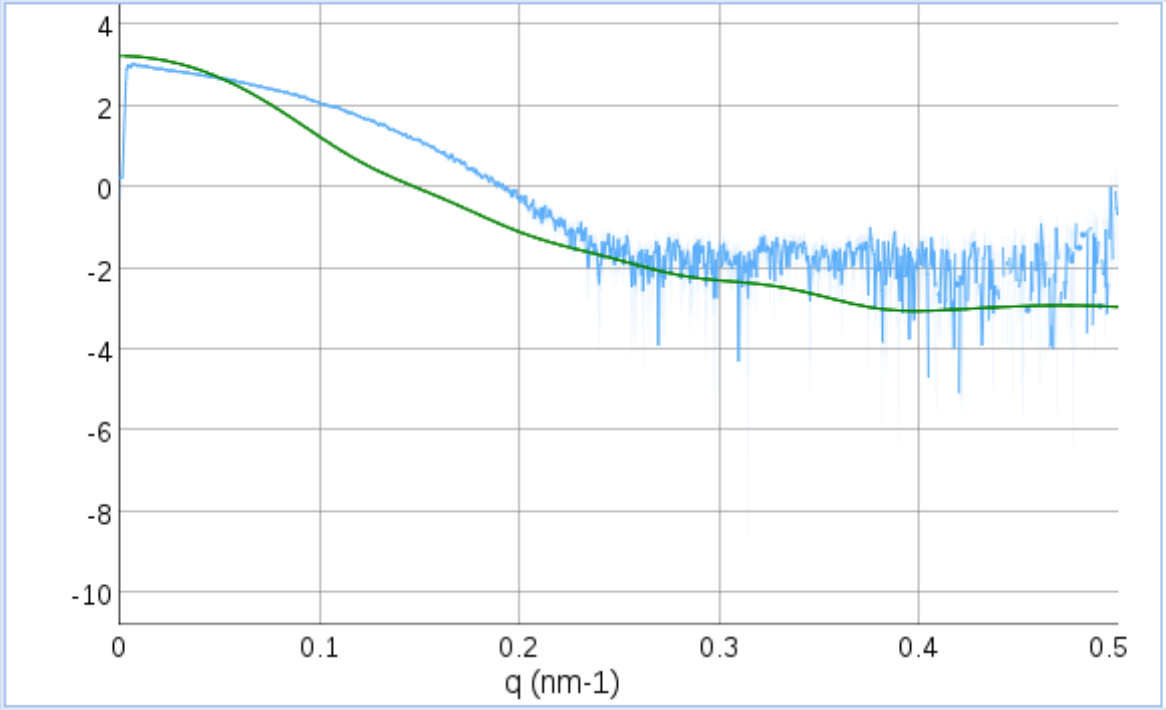




Automated processing is being extended:

Additional information such as PDB's of possible conformations

Fit Structure to Data



Created by CRYSOL v. 28 on 23-Dec-2014 11:06:02

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Data file name ..... : /data/pyarch/bm29/opd29/2604/1d/Data_050_sub.dat
Model: /tmp/biosaxsworkflow Rg: 24.90 Run: 00 Chi: 14.01

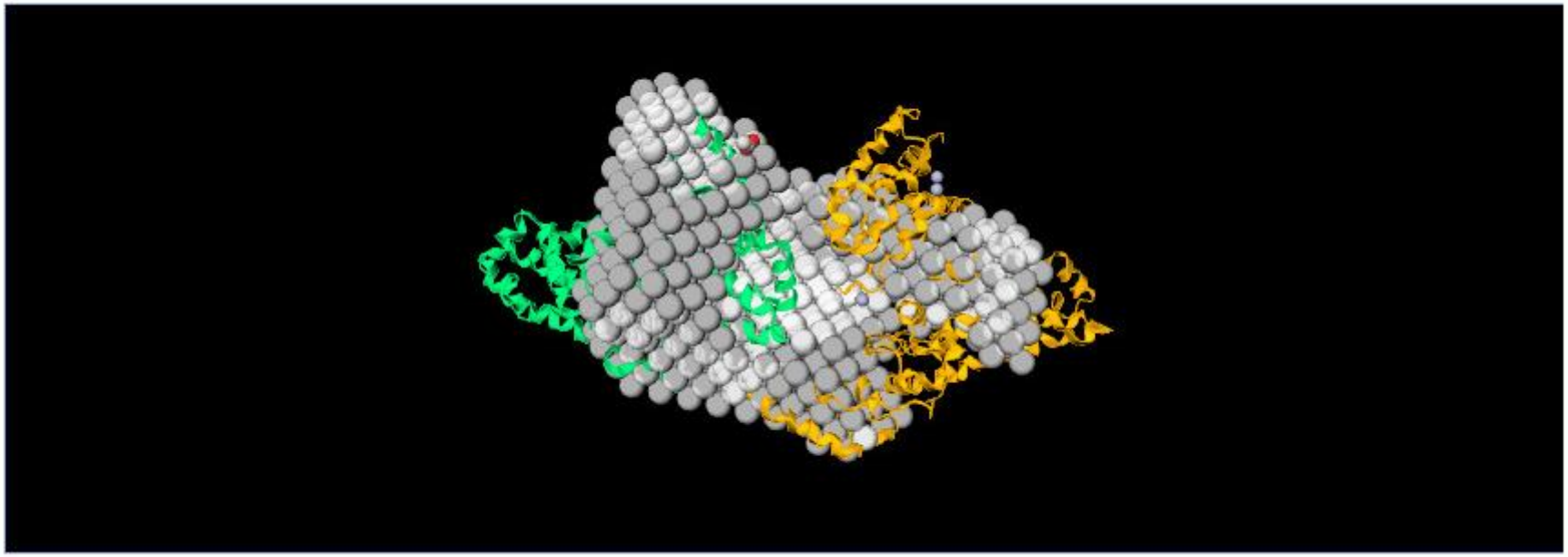
Automated processing is being extended:

Additional information such as PDB's of possible conformations

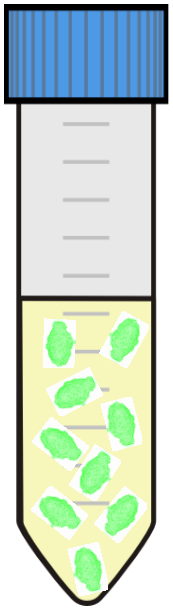
Superposition

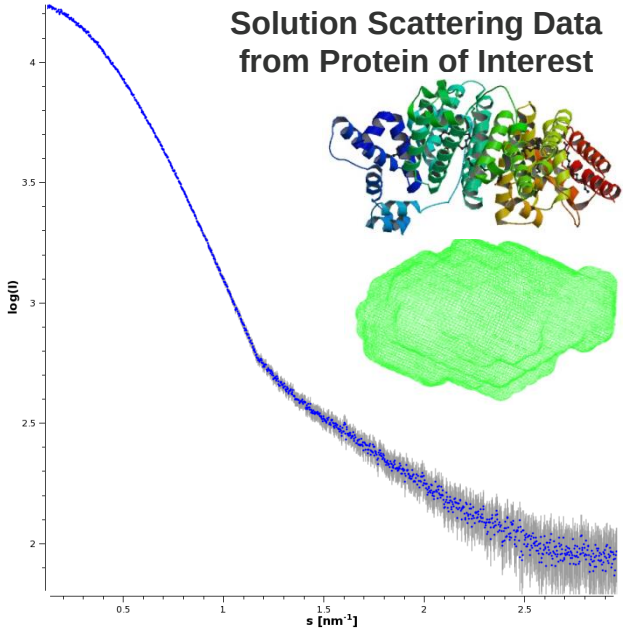
INLINE HELP: To be updated

Abinitio	Apriori	Aligned
BSA_0008-1-1.pdb	dimer.pdb	BSA_0008-1-1_dimer.pdb
BSA_0008-1-1.pdb	monomer.pdb	BSA_0008-1-1_monomer.pdb



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

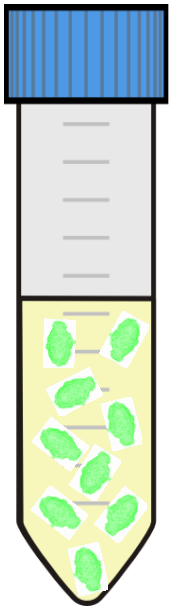


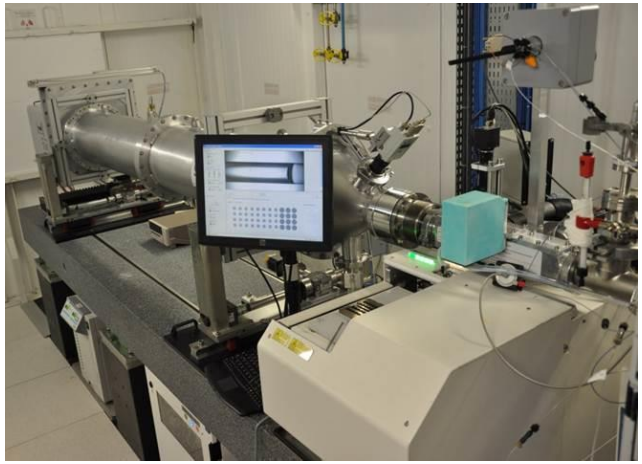


Black Box

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

EMBL GRENOBLE

EMBL HAMBURG

ESRF

BioStructX

PSB

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Institut Laue-Langevin