BioSAXS @ ESRF

Life after ID14eh3

Current status and future possibilities for BM29

Adam Round

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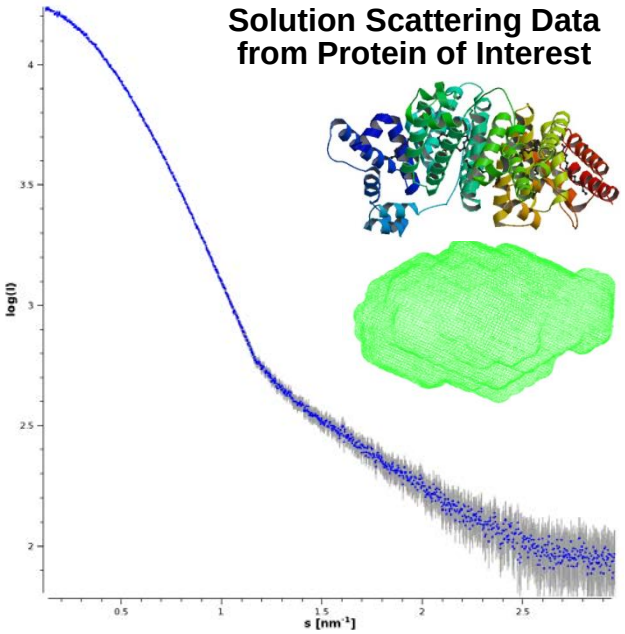
Contents

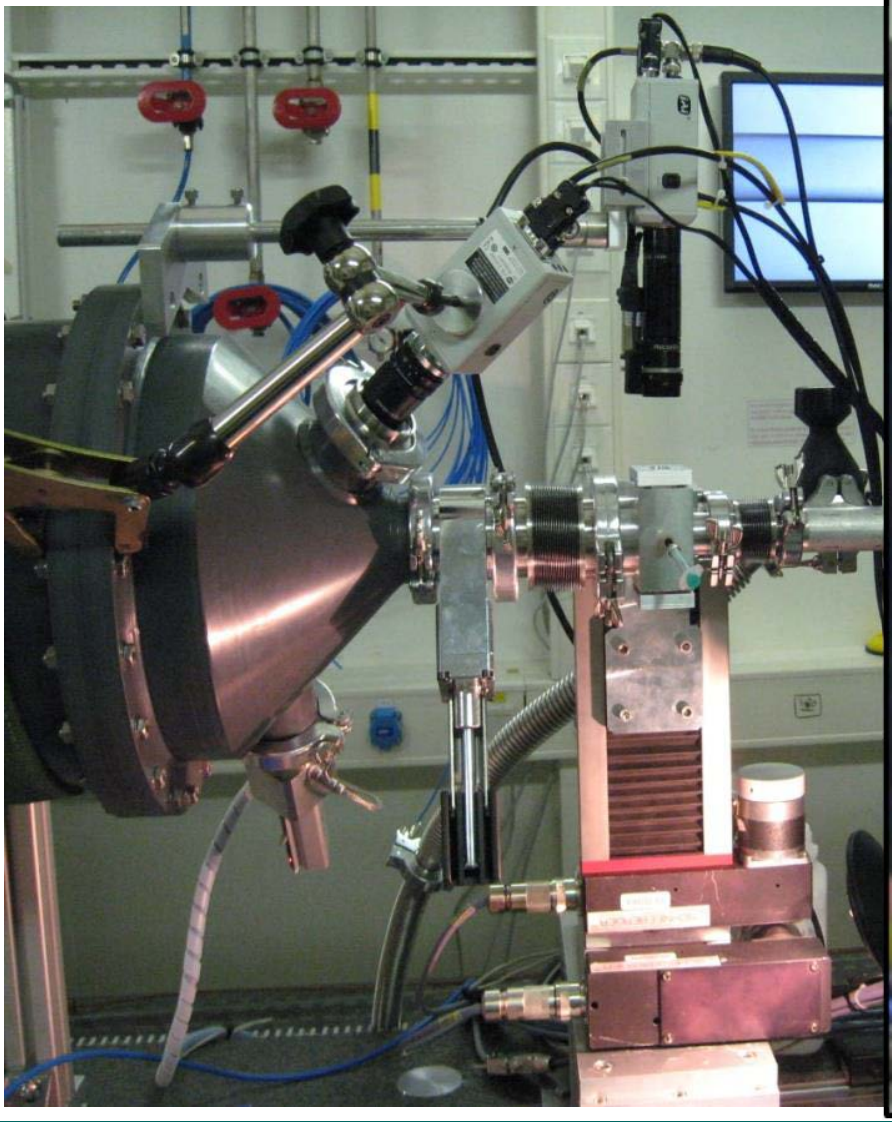
- BioSAXS on ID14eh3
- Current Status of BM29
  - Data collection
    - BioSAXS sample changer
    - Online SEC
  - ISPyB
    - Sample preparation
    - Experimental logging
    - Data reduction and processing
    - Analysis and Interpretation
- Future possibilities for BM29

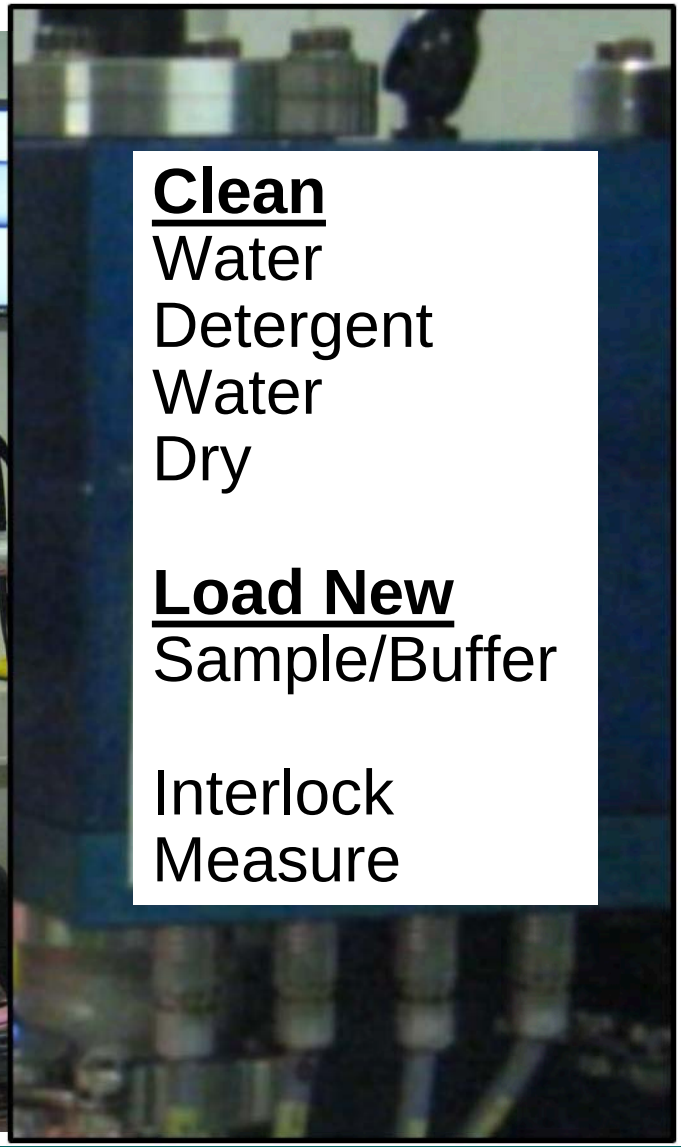
Idealised Solution SAS Experiment

Black Box



Experimental Procedure





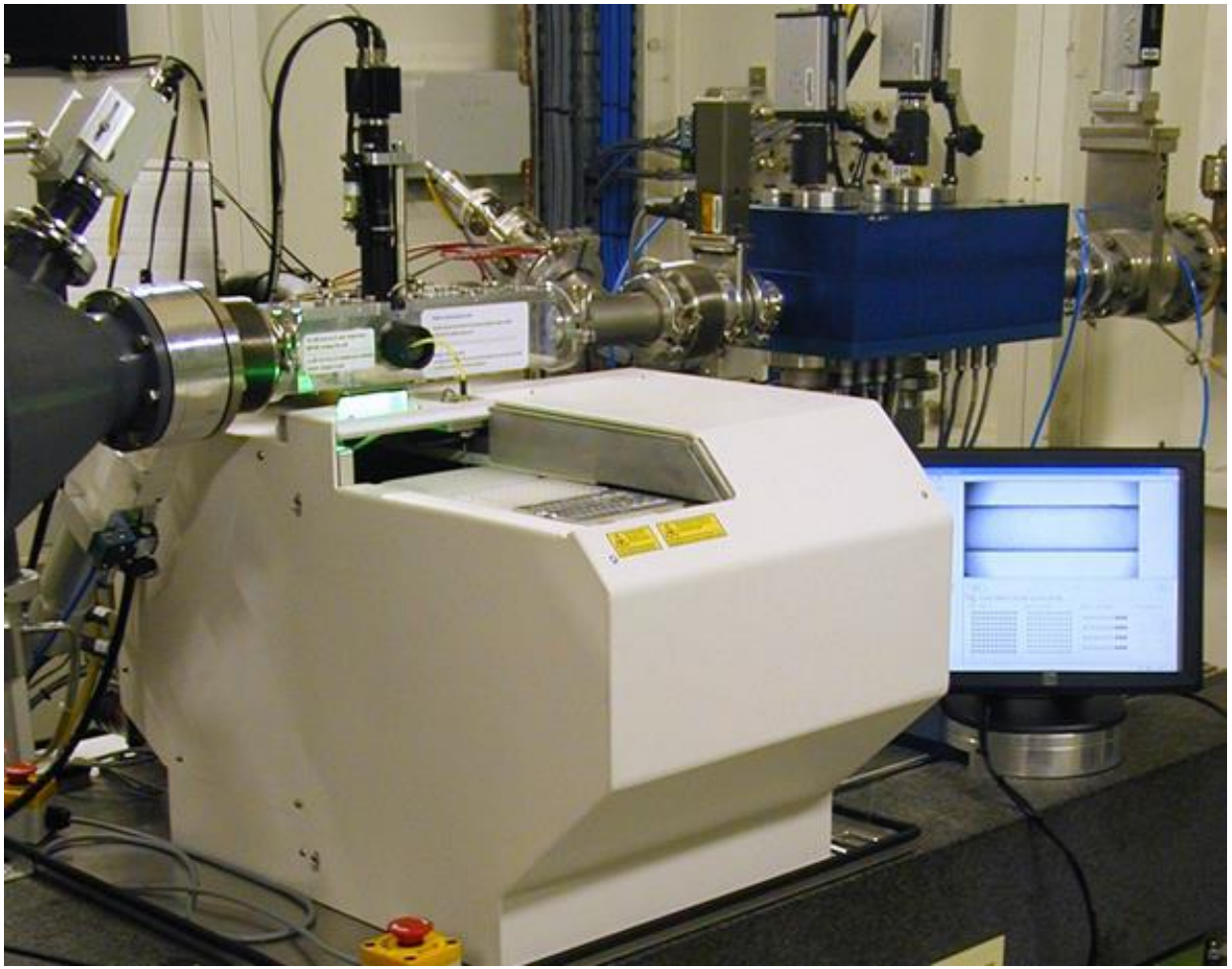
Clean
Water
Detergent
Water
Dry

Load New
Sample/Buffer

Interlock
Measure

2nd generation SC (evaluation setup)

2nd generation SC @ ID14eh3



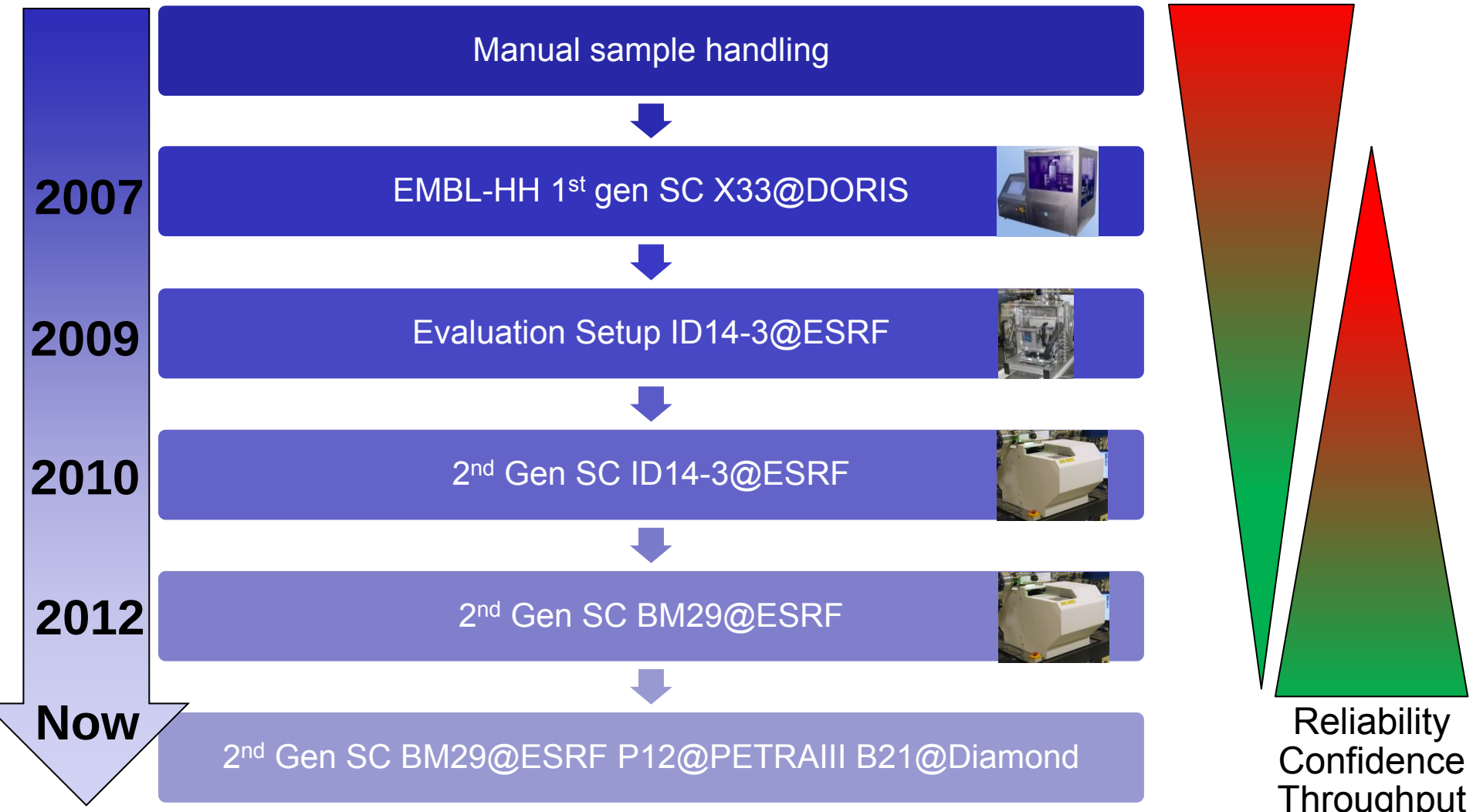
Developed by

EMBL-GR, HH and ESRF

- In use since September 2010 at ESRF
- Sister units at:
  P12@PETRAIII
  B21@Diamond
- Sample capacity
  up to 3x96 well plates
  from 0.2 to 2 mL
- Pipetting and mixing enables remote data collection

SC development

Sample Volume
Cleaning time
Total cycle time



Reliability
Confidence
Throughput

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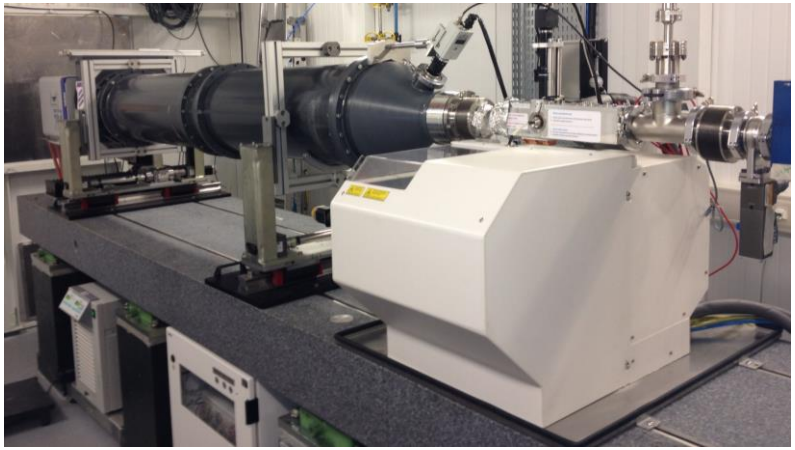
Data collection protocols on BM29:



Temperature

- Independent temperature regulation for
- Storage 4-40 degrees C
- Measurement 4-60 degrees C

Exposure Time

- Standard starting time (10 s)
  - Easily modifiable in case of SNR or Radiation issues

Additives

- No strict limitations but best to minimise where possible to avoid complications
- Recommended
  - < 0.5 M salt
  - < 5% glycerol

Sample Volume

- Minimum 10 µL per exposure
  - 30 µL recommended
- Minimum 3 concentrations required per construct
  - Approx. 1-20 mg/mL
- Plus buffer measurement for background subtractions

Summary

- **Users recommended to bring total volume of 100 µL of stock (Ideally > 10 mg/mL) solution per construct**
  (plus approx. 1 ml buffer for dilutions/background measurements)

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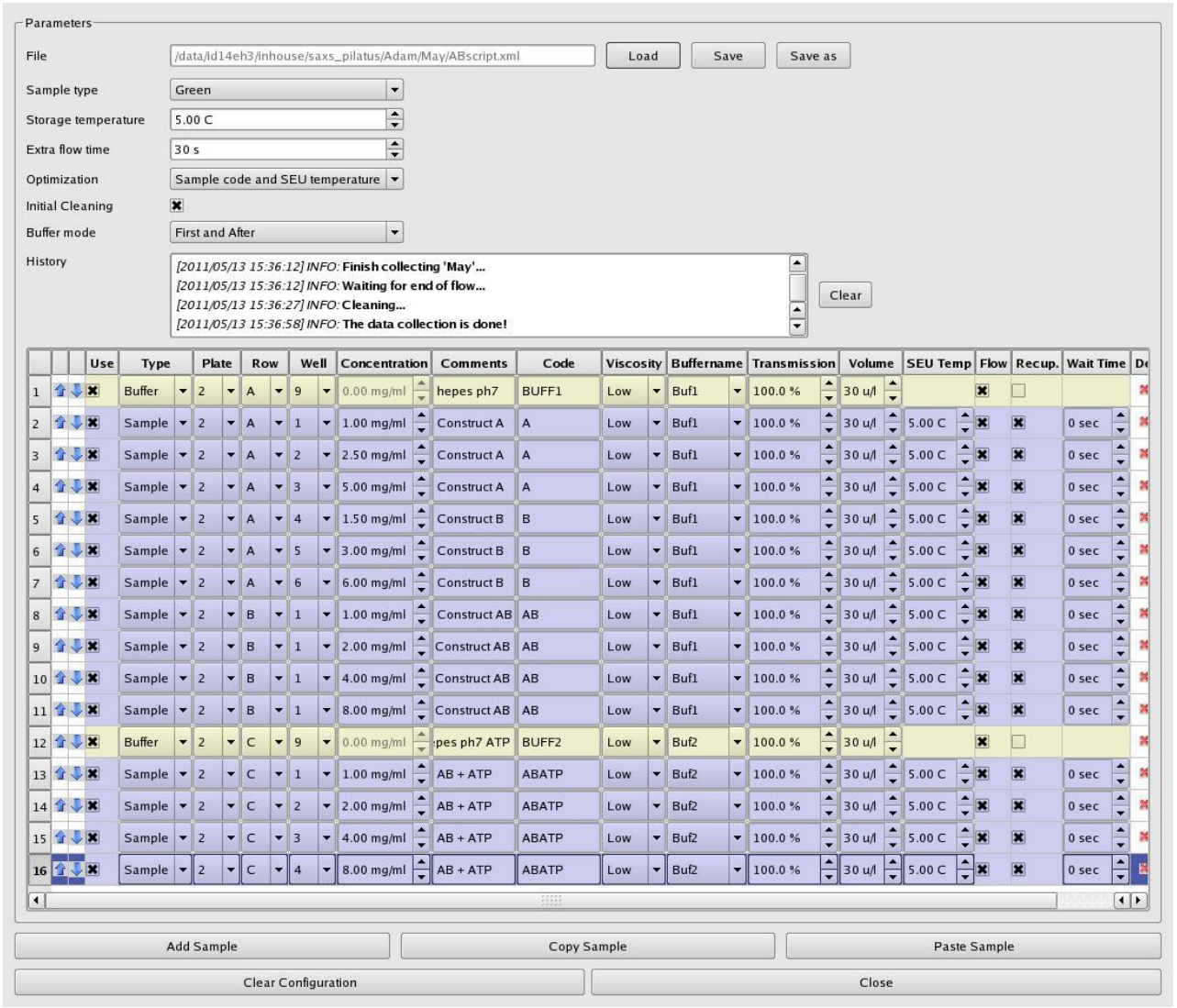
Automated data collection



Beamline data acquisition interface initially used to define samples and experiments

Effective but:

Time consuming

Error Prone

No logging

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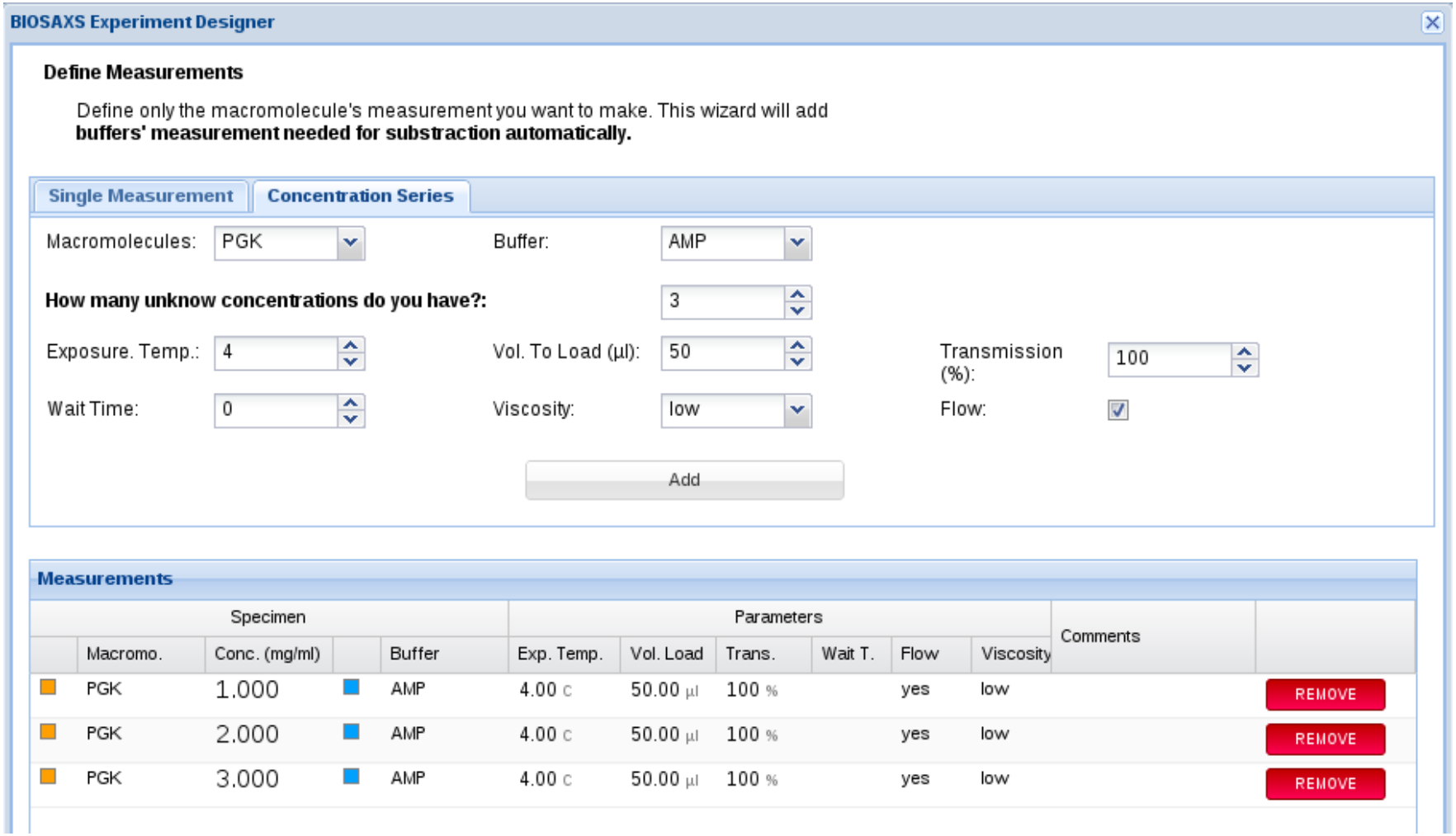
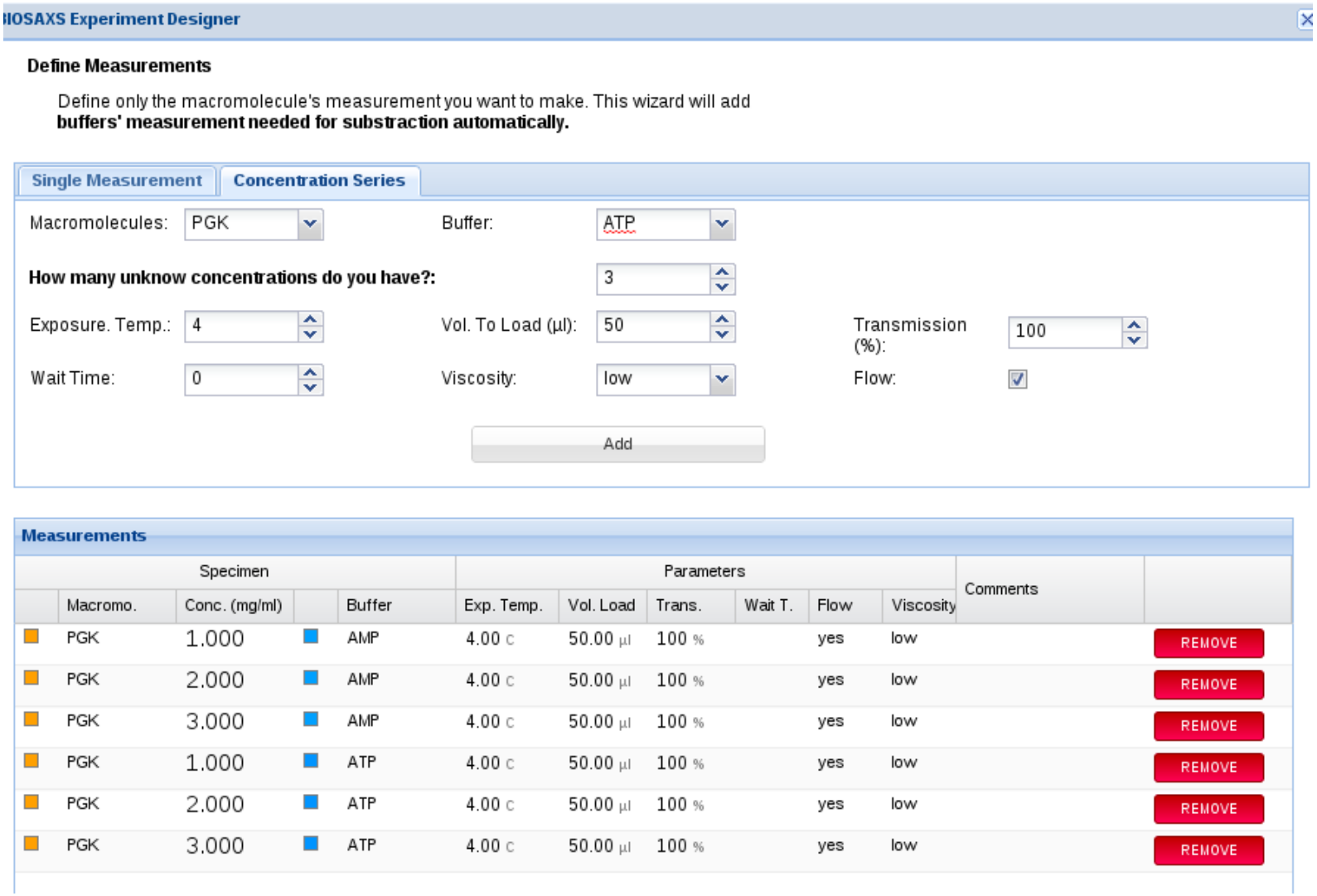
Improved feedback for experimental preparation



Improved feedback for experimental preparation



Improved feedback for experimental preparation

Estimation of required Volume

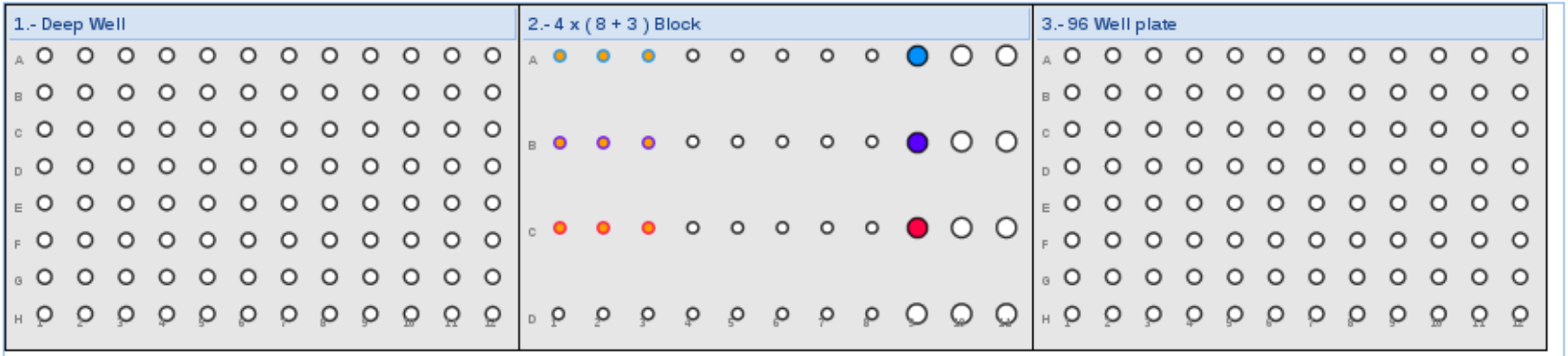
Go to Shipment

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 for experimental preparation

PGK

PGK	ATP	1.000 mg/ml	50.00 µl	2	A	1
PGK	common buffer	1.000 mg/ml	50.00 µl	2	B	1
PGK	p38buffer	1.000 mg/ml	50.00 µl	2	C	1
PGK	ATP	2.000 mg/ml	50.00 µl	2	A	2
PGK	common buffer	2.000 mg/ml	50.00 µl	2	B	2
PGK	p38buffer	2.000 mg/ml	50.00 µl	2	C	2
PGK	ATP	3.000 mg/ml	50.00 µl	2	A	3
PGK	common buffer	3.000 mg/ml	50.00 µl	2	B	3
PGK	p38buffer	3.000 mg/ml	50.00 µl	2	C	3



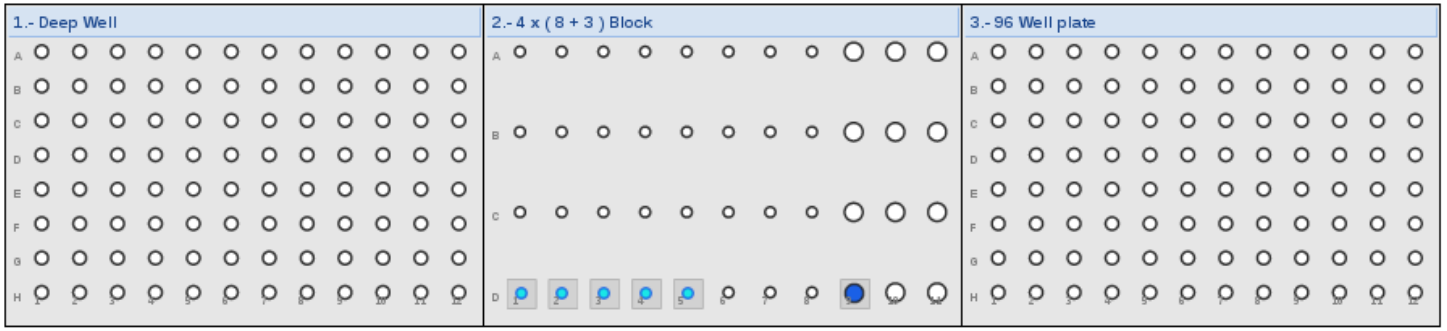
Improved feedback with ISPyB

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



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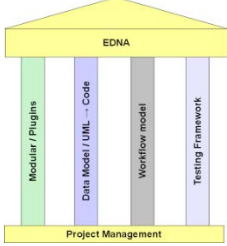
Automatic aquisition = big data

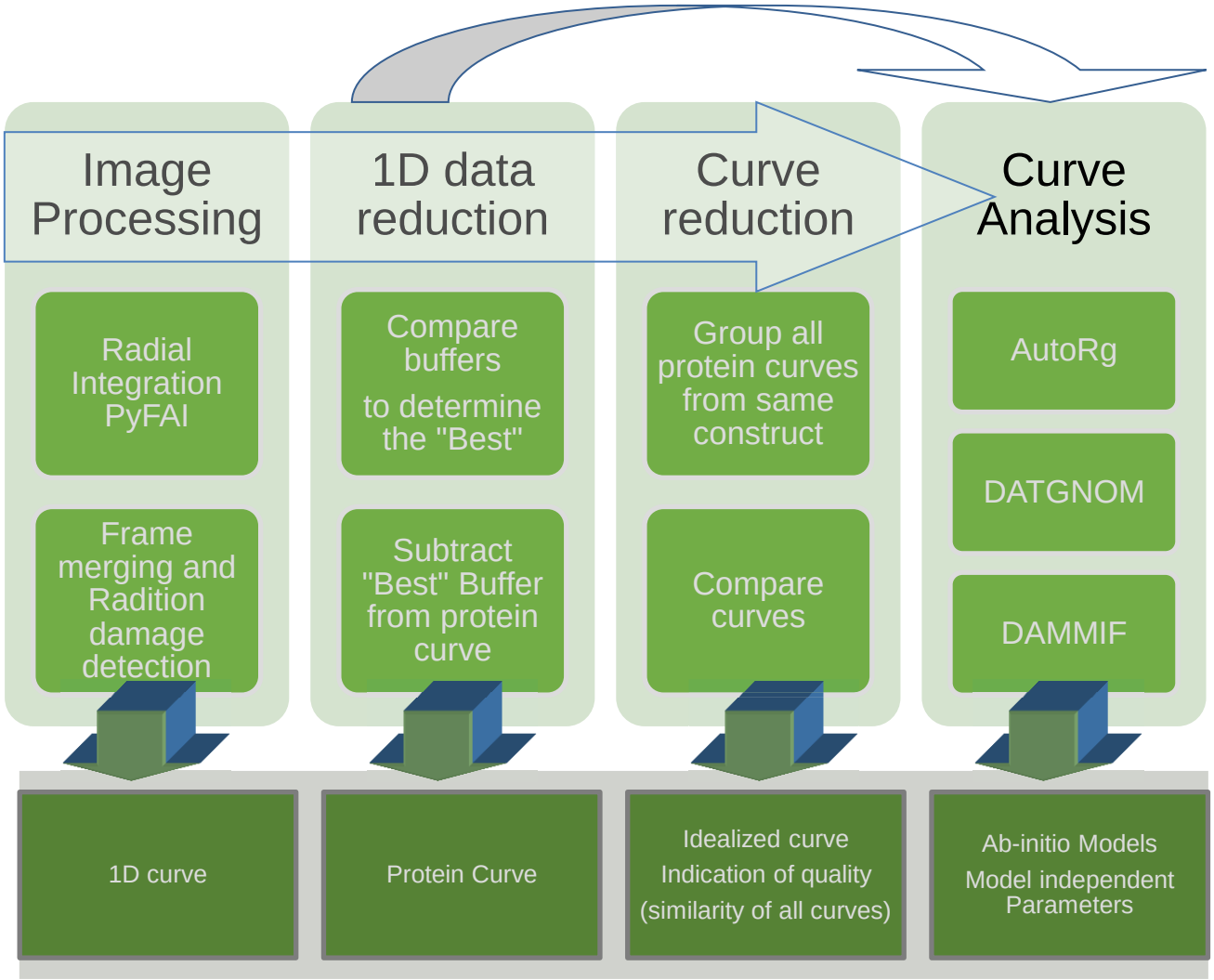
Preliminary analysis is required to obtain feedback on sample behaviour and data quality to ensure experimental aims are met

Without automation significant experimental time can be lost to data reduction

Online analysis is essential to help users work better at the beamline

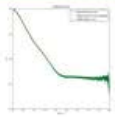
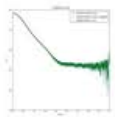
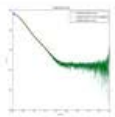
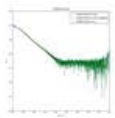
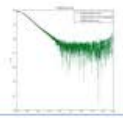
Data Processing: ATSAS tools in EDNA



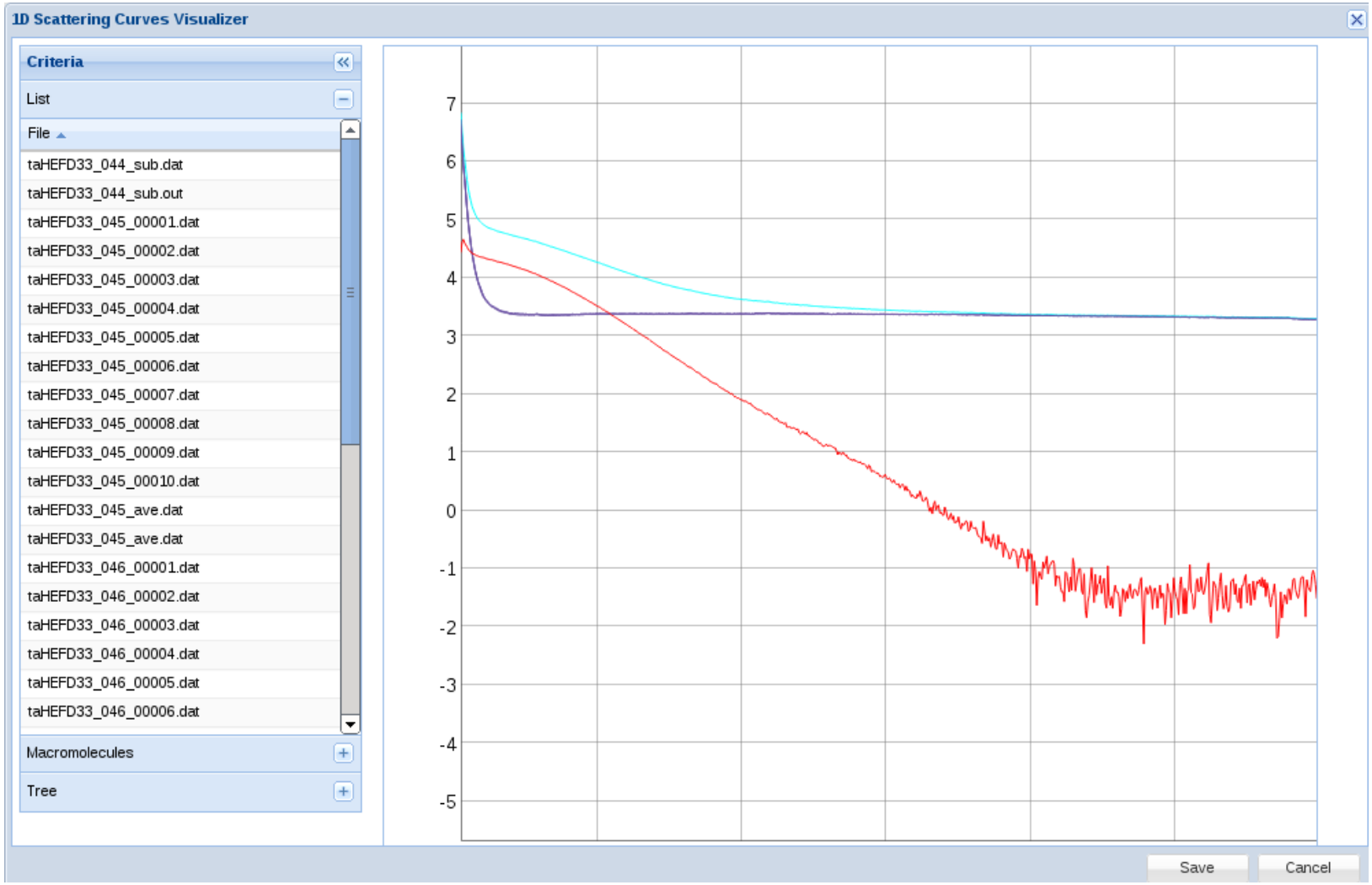


Improved feedback with ISPyB

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

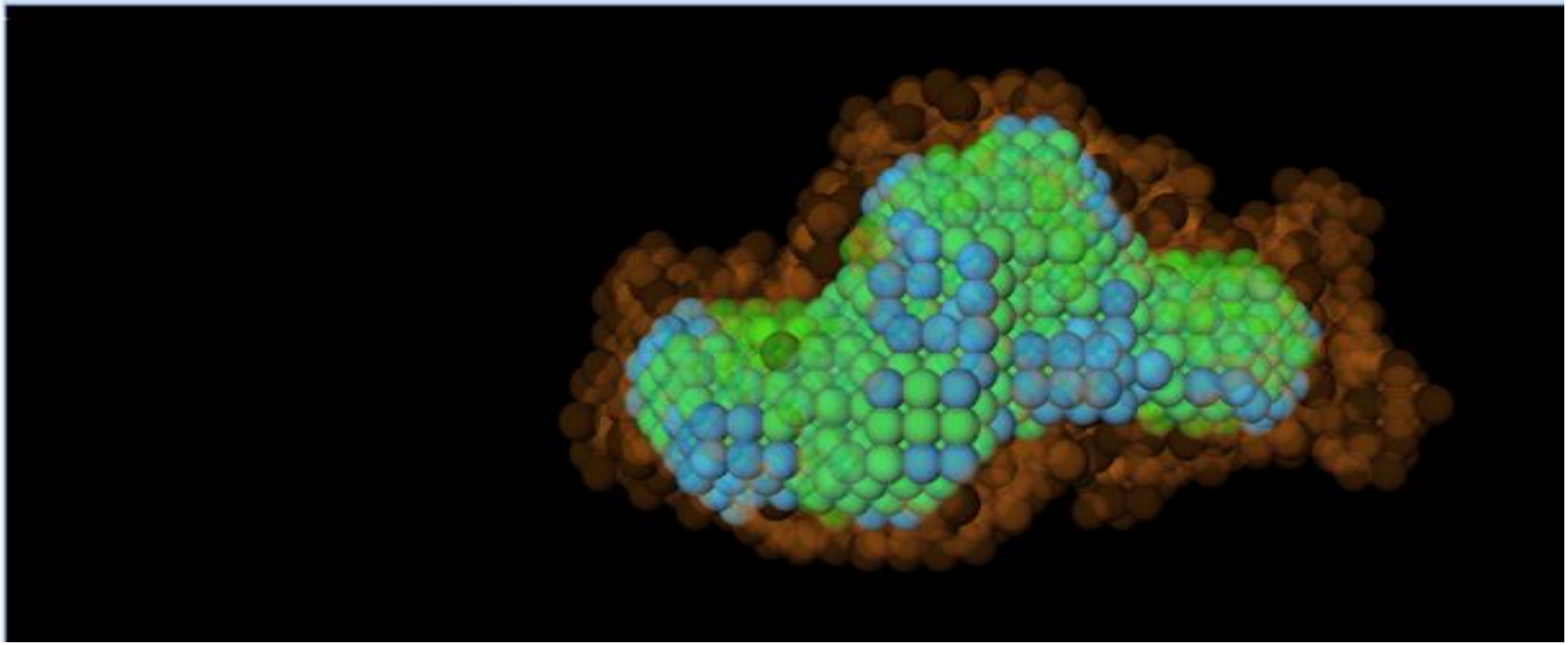


Improved feedback with ISPyB

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

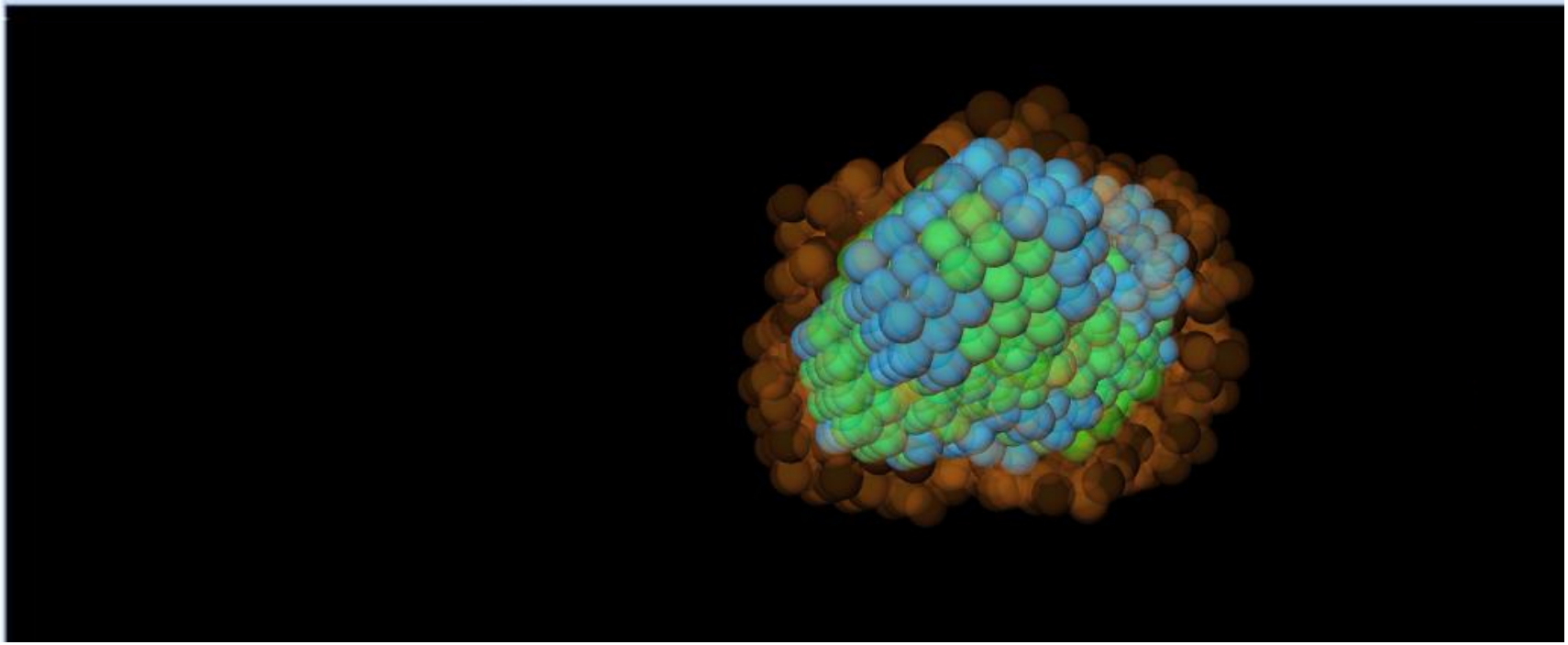


Improved feedback with ISPyB

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

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



Summary of SC experiments

Easy, fast, reliable, fully automated

Online results and immediate feedback for better experiments

Pipetting and mixing enable remote experiments

Sample characterisation and preparation are key

Mixtures, buffer mismatches, aggregation will destroy any chance of finding an answer

Online purification and biophysical characterisation have been implemented to help overcome these issues

Addition of Online SEC

Addition of Online SEC

4-way valve

Fast automatic switching between sample changer and HPLC modes

Mounted in Fridge

Operation at 4 ° C or 20 ° C

SEC data collection protocols:

Temperature

- SEC operation at 4 or 20 degrees C

Exposure Time

- Standard 1 FPS (5 FPS max)
  - S200 column ~1 hour
  - Increase column ~10 mins

Additives

- No strict limitations but best to minimise where possible to avoid complications
- Recommended
  - < 0.5 M salt
  - < 5% glycerol

Sample Volume

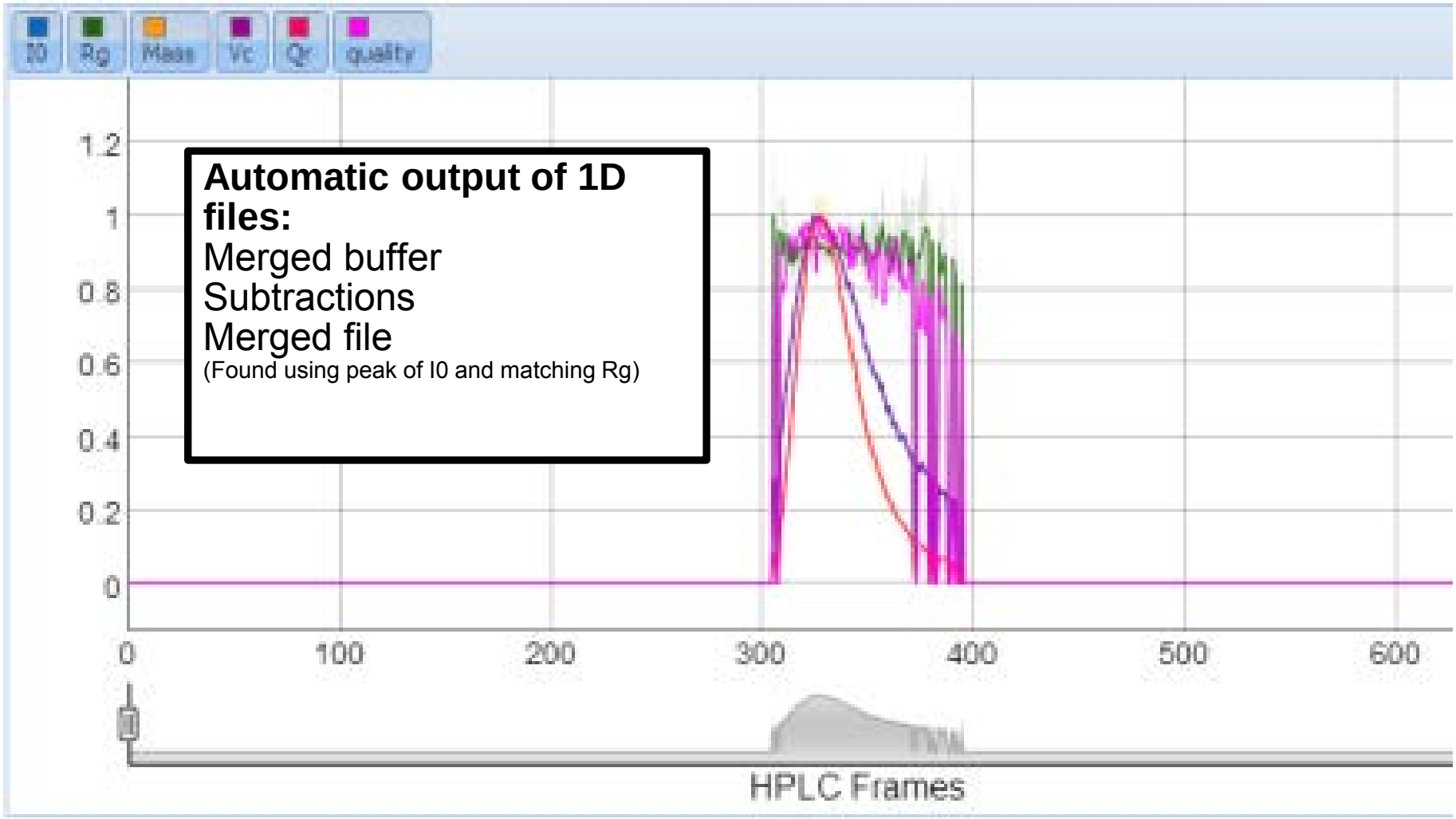
- Minimum 50 µL per injection
  - 100 µL recommended
  - Approx. 5 mg/mL
- Plus 0.5 L buffer per injection and equilibration

Summary

- **Users recommended to bring own column(s)!**
  100 µL of stock protein per run
  minimum 1 L buffer

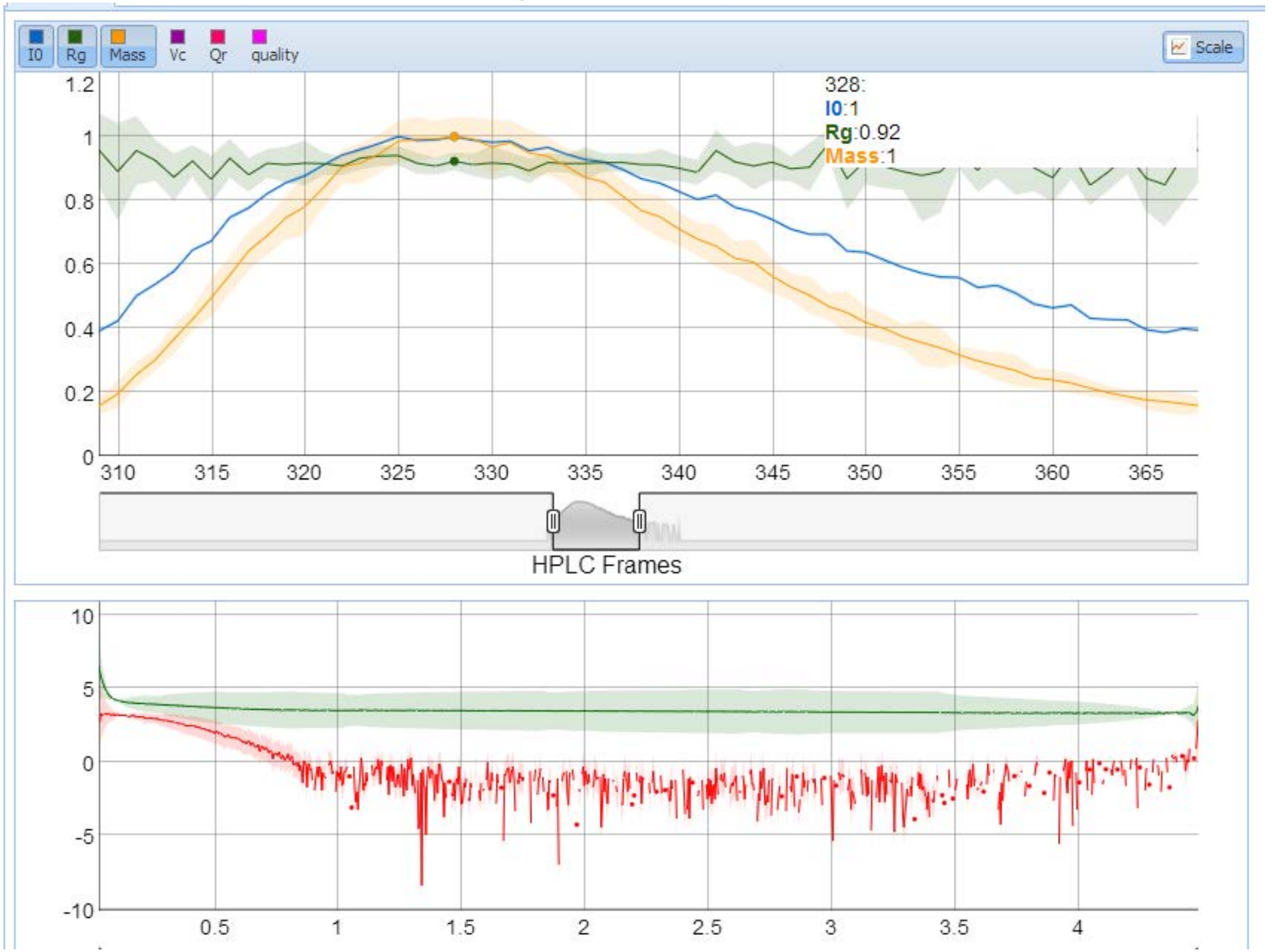
Buffers can be prepares onsite in EMBL user support lab upon request

Online SEC in ISPyB

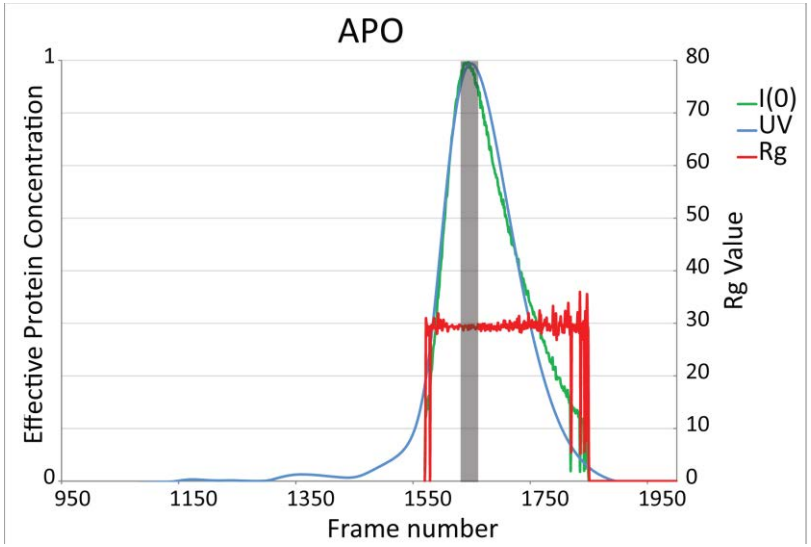


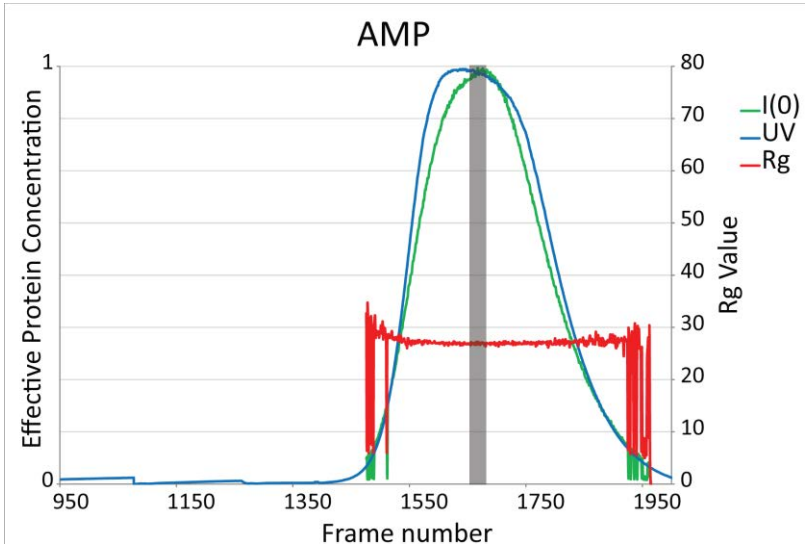
Automatic output of 1D files:
Merged buffer
Subtractions
Merged file
(Found using peak of I0 and matching Rg)

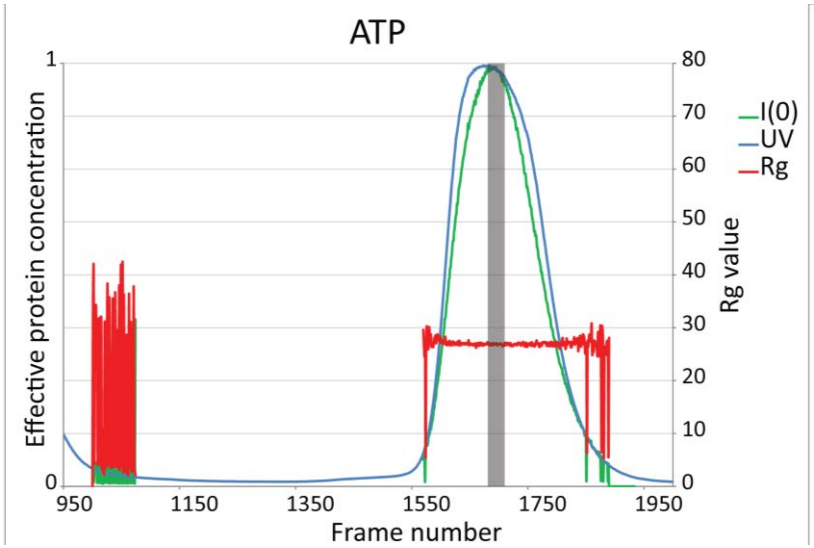
Online SEC in ISPyB

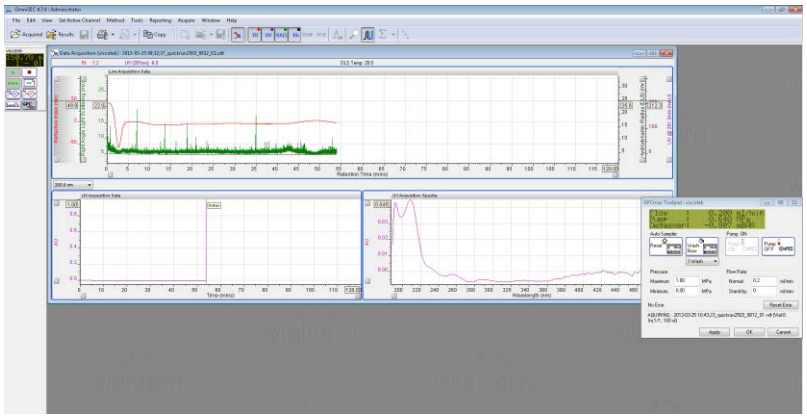


Online SEC









Biophysical characterization

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

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Summary of online SEC experiments

Easy, automated switching to maximise efficiency

Online results and immediate feedback for better experiments

Online sample characterisation
- DLS
- RALS
- UV
- RI

Mixtures can be separated

Buffer mismatches can be overcome

Aggregation can be removed

Future of online SEC experiments

Online SEC is increasing in popularity and requires improved efficiency

New columns!
- Superdex 200 increase columns
- enable injections every 10 minutes

Offline system for equilibrating columns in parallel

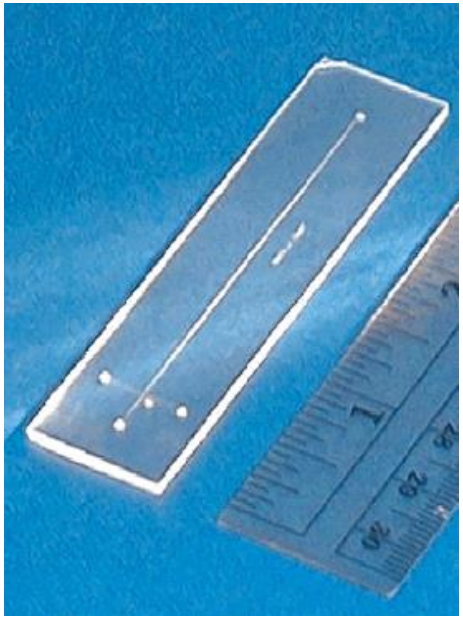
Upgraded online system with multiple columns
- Multiplexing

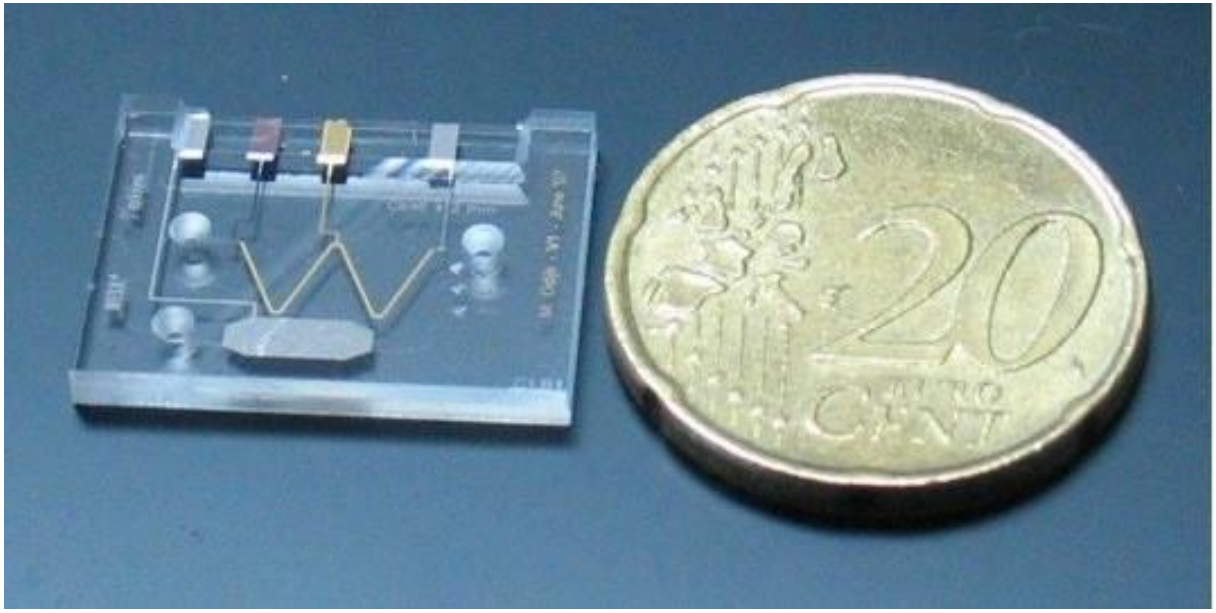
Automation of:
- Injection sequences
- Buffer switching
- Data acquisition based on peak sensing?

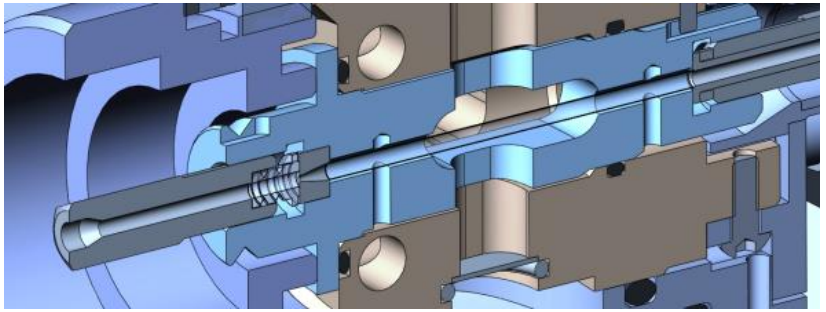
Current developments

- ISPyB
  - Ongoing addition of logic for crosschecking
  - Intuitive feedback on data quality within GUI
  - Automated optimisation of data acquisition
  - Link to HTX CRIMS database
    - Automated sample preparation
- Smaller beam (scatterless pinhole)
  - Optimised for smaller capillaries
    - Minimise sample volume requirements
  - Enable microfluidics
- Redesign SEU

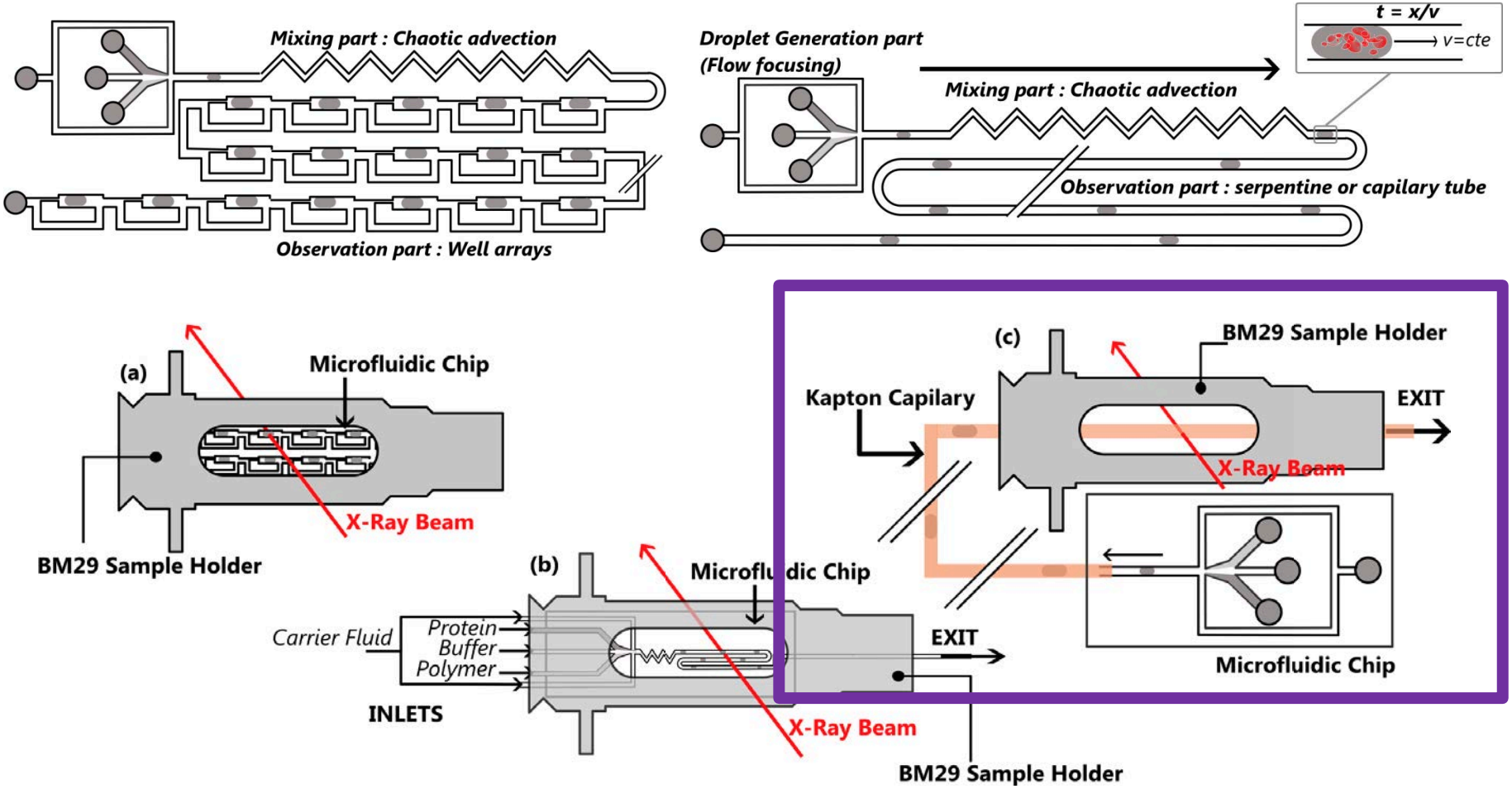
SC compatible Microfluidics







SC compatible Microfluidics



SC compatible Microfluidics

Potential use in:

- Mixing studies
- Time resolved measurements (seconds)
- Dynamic screening of buffer conditions

Future

Redesign SEU

New multicolour light source

Photo activated / sensi

Wide angle s

Exte

ecence, etc.

Facilitate Microfluidics

On axis camera / lighting

Modular design?

(increased flexibility)

Summary of current operation

Automation of data acquisition and analysis for both Static (SC) and online SEC (HPLC) gives:

Reliability, Confidence, Independence, High Throughput and Efficiency

Easy switching between experiments is required to maintain efficiency and reliability

Frequent training courses in data acquisition and analysis (EMBO, Hercules, BAG training)

SAS validation Workshop

Commitment to Data Quality and integrity

28 Experts in Neutron and X-ray scattering

From 15 institutes in 7 different countries

EMBL GRENOBLE

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NEUTRONS FOR SCIENCE® Institut Laue-Langevin

ibs Institut de Biologie Structurale

Acknowledgments