BioSAXS @ ESRF

Life after ID14eh3 Current status and future possibilities for BM29

Adam Round

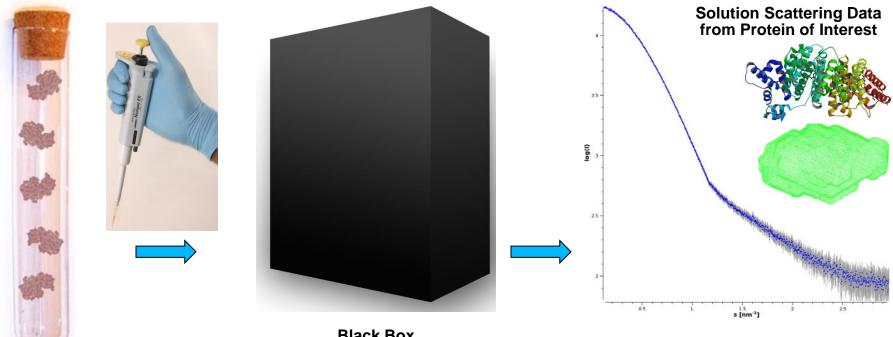


Contents

- 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



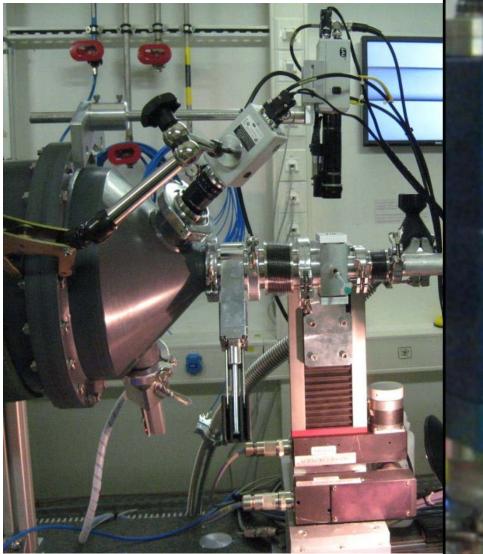
Idealised Solution SAS Experiment



Black Box



Experimental Procedure





<u>Clean</u> Water Detergent Water Dry

Load New Sample/Buffer

Interlock Measure



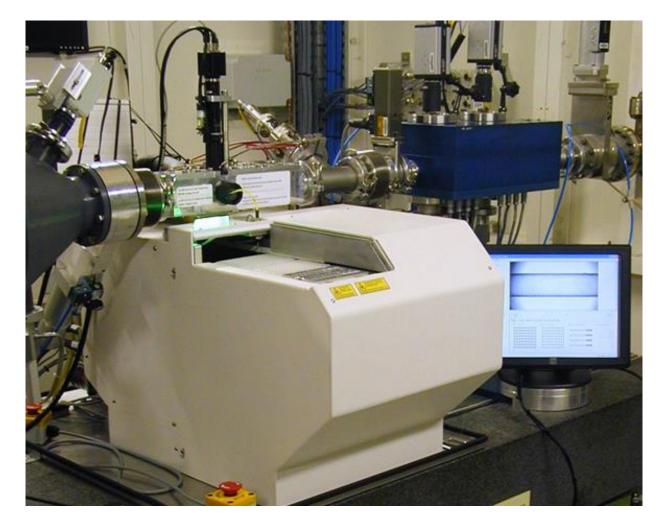
2nd generation SC (evaluation setup)







2nd generation SC @ ID14eh3



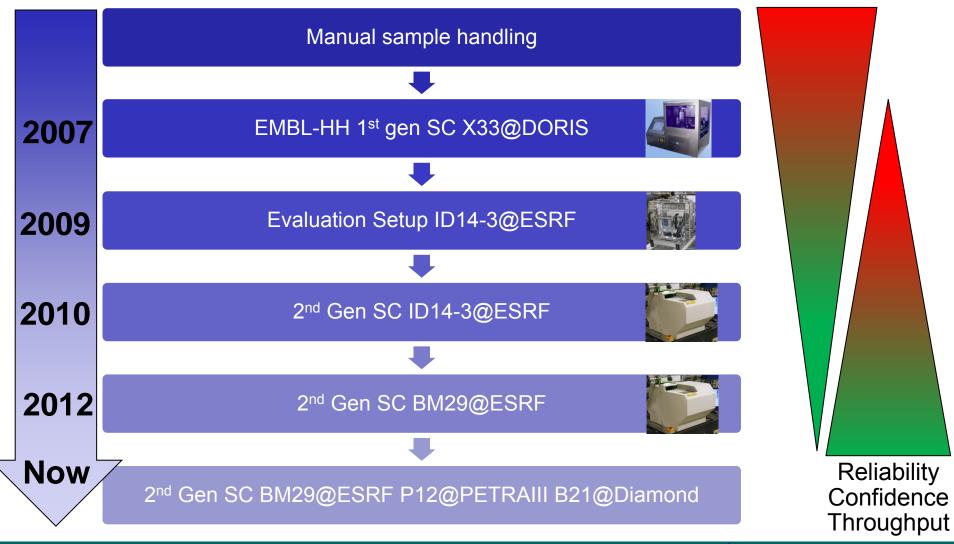
Developed by EMBL-GR, HH and ESRF

- 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

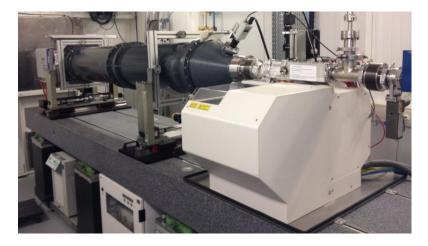
Sample Volume Cleaning time Total cycle time





Reliability

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)



Automated data collection

Par	ame	ers		_																					
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3	4	×	Sample	-	2	-	A	-	2	-	2.50 mg/ml	*	Construct A	A	Low	-	Buf1	-	100.0 %	30 u/l 💂	5.00 C	×	×		•
4	1	×	Sample	-	2	-	A	-	3	-	5.00 mg/ml	+	Construct A	A	Low	-	Buf1	-	100.0 %	30 u/l 🌲	5.00 C	×	×	0 sec	-
5		×	Sample	-	2	-	A	-	4	-	1.50 mg/ml	4	Construct B	в	Low	-	Buf1	-	100.0 %	30 u/l 🗘	5.00 C	×	×	0 sec	•
6	Ŷ.	×	Sample	-	2	-	A	-	5	-	3.00 mg/ml	•	Construct B	в	Low	-	Buf1	-	100.0 %	30 u/l 🛔	5.00 C	×	×	0 sec	•
7	4	×	Sample	-	2	-	A	-	6	-	6.00 mg/ml	*	Construct B	в	Low	-	Buf1	-	100.0 %	30 u/l 🗘	5.00 C	×	×	0 sec	•
8	4	×	Sample	-	2	-	в		1	-	1.00 mg/ml	•	Construct AB	AB	Low	-	Buf1	-	100.0 %	30 u/l 🗘	5.00 C	×	×	0 sec	•
9	分.	×	Sample	-	2	-	в	-	1	-	2.00 mg/ml	4	Construct AB	AB	Low	-	Buf1	-	100.0 %	30 u/l 🛔	5.00 C	×	×	0 sec	•
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11	1	×	Sample	-	2	-	в	+	1	-	8.00 mg/ml	+	Construct AB	АВ	Low	-	Buf1	-	100.0 %	30 u/l 🌲	5.00 C	×	×	0 sec	•
12	1	×	Buffer	-	2	-	с	Ŧ	9	-	0.00 mg/ml	4	pes ph7 ATP	BUFF2	Low	-	Buf2	-	100.0 %	30 u/l 🛔	-	×			1
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14	<u>.</u>	×	Sample	-	2	-	с	-	2	-	2.00 mg/ml		AB + ATP	ABATP	Low	-	Buf2	-	100.0 %	30 u/l	5.00 C	×	×	0.000	•
15	4	×	Sample	-	2	-	с	-	3	-	4.00 mg/ml		AB + ATP	ABATP	Low	-	Buf2	-	100.0 %	30 u/l	5.00 C	×	×		•
16	<u></u>	×	Sample	-	2	-	с	-	4	-	8.00 mg/ml		AB + ATP	ABATP	Low	-	Buf2	-	100.0 %	30 u/l	5.00 C	×	×		÷
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Beamline data acquisition interface initially used to define samples and experiments

Effective but:

Time consuming

Error Prone

No logging



SAXS Experiment	t Designer									
Define Measurer Define only th buffers' mea	ne macromolecul	e's measurem d for substrac	ent you want to ma tion automatically	ke. This w	izard will a	dd				
Single Measure	ment Concent	ration Series								
Macromolecules	: PGK	•	Buffer:	AMP	*]				
How many unkn	ow concentratio	ns do you have	?:	3	~]				
Exposure. Temp	.: 4	* *	Vol. To Load (µ	l): 50	~]	Tra (%	ansmissio):	on 100	* *
Wait Time:	0	* *	Viscosity:	low	*		Flo	ow:	\checkmark	
				Add						
Measurements										
	Specimen				Paramete	rs			C	
Macromo.	Conc. (mg/ml)	Buffer	Exp. Temp.	Vol. Load	Trans.	Wait T.	Flow	Viscosity	Comments	
PGK	1.000	AMP	4.00 c	50.00 μΙ	100 %		yes	low		REMOVE
PGK	2.000	AMP	4.00 c	50.00 μΙ	100 %		yes	low		REMOVE

50.00 µl 100 %

yes

low

4.00 c

3.000

AMP

PGK



REMOVE

IIOSAXS 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 Measurem	ent Concentration Series				
Macromolecules:	PGK 👻	Buffer:	ATP.		
How many unknow	w concentrations do you have?	:	3		
Exposure. Temp.:	4	Vol. To Load (µl):	50	Transmission (%):	100
Wait Time:	0	Viscosity:	low 👻	Flow:	
			Add		

	Specimen					Paramete	ers			Commente	
Macromo.	Conc. (mg/ml)		Buffer	Exp. Temp.	Vol. Load	Trans.	Wait T.	Flow	Viscosity		
PGK	1.000		AMP	4.00 c	50.00 μΙ	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	$\textbf{50.00} \; \mu \textbf{I}$	100 %		yes	low		REMOVE
	PGK PGK PGK PGK	Macromo. Conc. (mg/ml) PGK 1.000 PGK 2.000 PGK 3.000 PGK 1.000 PGK 2.000	Macromo. Conc. (mg/ml) PGK 1.000 PGK 2.000 PGK 3.000 PGK 1.000 PGK 2.000	Macromo. Conc. (mg/m) I Buffer PGK 1.000 I AMP PGK 2.000 I AMP PGK 3.000 I AMP PGK 1.000 I AMP PGK 2.000 I AMP PGK 1.000 I ATP	Macromo. Conc. (mg/ml) Image: Buffer Exp. Temp. PGK 1.000 Image: AMP 4.00 c PGK 2.000 Image: AMP 4.00 c PGK 3.000 Image: AMP 4.00 c PGK 1.000 Image: AMP 4.00 c PGK 2.000 Image: AMP 4.00 c PGK 2.000 Image: ATP 4.00 c	Macromo. Conc. (mg/ml) Image: Macromolic mark Exp. Temp. Vol. Load PGK 1.000 Image: Mark AMP 4.00 c 50.00 μl PGK 2.000 Image: Mark AMP 4.00 c 50.00 μl PGK 3.000 Image: Mark AMP 4.00 c 50.00 μl PGK 1.000 Image: Mark AMP 4.00 c 50.00 μl PGK 2.000 Image: Mark ATP 4.00 c 50.00 μl	Macromo. Conc. (mg/ml) Buffer Exp. Temp. Vol. Load Trans. PGK 1.000 AMP 4.00 c 50.00 µl 100 % PGK 2.000 AMP 4.00 c 50.00 µl 100 % PGK 3.000 AMP 4.00 c 50.00 µl 100 % PGK 1.000 AMP 4.00 c 50.00 µl 100 % PGK 1.000 ATP 4.00 c 50.00 µl 100 % PGK 2.000 ATP 4.00 c 50.00 µl 100 %	Macromo. Conc. (mg/m) I Buffer Exp. Temp. Vol. Load Trans. Wait T. PGK 1.000 I AMP 4.00 c 50.00 μl 100 % Image: Simple state sta	Macromo. Conc. (mg/m) I Buffer Exp. Temp. Vol. Load Trans. Wait T. Flow PGK 1.000 I AMP 4.00 c 50.00 μl 100 % yes PGK 2.000 I AMP 4.00 c 50.00 μl 100 % yes PGK 3.000 I AMP 4.00 c 50.00 μl 100 % yes PGK 1.0000 I AMP 4.00 c 50.00 μl 100 % yes PGK 3.000 I AMP 4.00 c 50.00 μl 100 % yes PGK 2.000 I ATP 4.00 c 50.00 μl 100 % yes	Macromo. Conc. (mg/ml) Buffer Exp. Temp. Vol. Load Trans. Wait T. Flow Viscosity PGK 1.000 Image: AMP 4.00 c 50.00 μl 100 % yes low PGK 2.000 Image: AMP 4.00 c 50.00 μl 100 % yes low PGK 3.000 Image: AMP 4.00 c 50.00 μl 100 % yes low PGK 3.000 Image: AMP 4.00 c 50.00 μl 100 % yes low PGK 3.000 Image: AMP 4.00 c 50.00 μl 100 % yes low PGK 1.0000 Image: ATP 4.00 c 50.00 μl 100 % yes low PGK 2.000 Image: ATP 4.00 c 50.00 μl 100 % yes low	Macromo. Conc. (mg/ml) Buffer Exp. Temp. Vol. Load Trans. Wait T. Flow Viscosity Comments PGK 1.000 AMP 4.00 c 50.00 μl 100 % yes low



Estima	ation of required Volume	
🗙 Go	to Shipment	
	Specimen 🔺	Estimated Volume
	ATP	300.00 µl
	PGK + ATP	150.00 µI
	PGK + common buffer	150.00 µl
•	PGK + p38buffer	150.00 µl
-	common buffer	300.00 µl
	p38buffer	300.00 µl



🖃 PGK						
E PGK	ATP	1.000 mg/ml	50.00 μΙ	2	Α	1
PGK	common buffer	1.000 mg/ml	50.00 μΙ	2	В	1
PGK	p38buffer	1.000 mg/ml	50.00 μΙ	2	с	1
PGK	ATP	2.000 mg/ml	50.00 μΙ	2	А	2
PGK	common buffer	2.000 mg/ml	50.00 μΙ	2	В	2
PGK	p38buffer	2.000 mg/ml	50.00 μΙ	2	с	2
PGK	ATP	3.000 mg/ml	50.00 μΙ	2	А	3
PGK	common buffer	3.000 mg/ml	50.00 μΙ	2	В	3
PGK	p38buffer	3.000 mg/ml	50.00 μΙ	2	с	3

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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

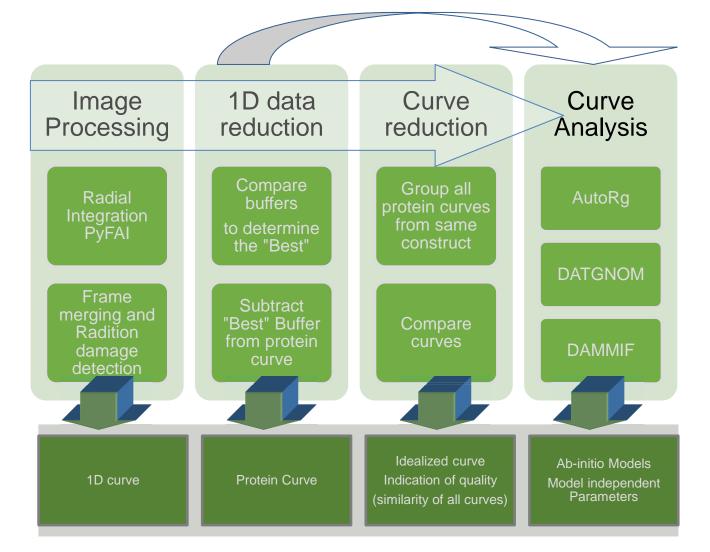
Without automation significant experimental time can be lost to data reduction

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



Data Processing: ATSAS tools in EDNA







	Concentration	Contractor			Gu	inier			Gnom			Porod
Macromolecule	Concentration	Scattering	Frames (Averaged/Tota	Rg (nm)	Points	Quality (%)	l(0)	Rg (nm)	Total	Dmax (nm)	Volume (nm ³)	MM (kD) Vol. es
3												
taHEFD33	14.00 mg/ml		D33 (10 of taHEFD33 (10 of D33 (1 of 1	10)	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 taHEFD33 (5 of D33 (5 of	.0)	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 1 taHEFD33 (10 of D33 (10 of	10)	50 - 77 (27)	72.77	59.53 ±6.55654	3.44 nm	0.53	11.81	95.25	47.6 - 63.5
ta <mark>HEF</mark> D33	1.25 mg/ml		D33 (10 of taHEFD33 (10 of D33 (10 of	10)	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 taHEFD33 (10 of D33 (10 of	10)	27 - 78 (51)	86.16	78.86 ±9.98563	3.20 nm	0.75	11.06	84.35	42.2 - 56.2



1D Scattering Curves Visualizer × Criteria ~ List Ξ 7 -File 🔺 6 taHEFD33_044_sub.dat taHEFD33_044_sub.out 5 taHEFD33_045_00001.dat taHEFD33_045_00002.dat taHEFD33_045_00003.dat 4 taHEFD33_045_00004.dat taHEFD33_045_00005.dat 3 taHEFD33_045_00006.dat 2 taHEFD33 045 00007.dat taHEFD33_045_00008.dat taHEFD33_045_00009.dat 1 taHEFD33_045_00010.dat March taHEFD33_045_ave.dat 0 taHEFD33_045_ave.dat taHEFD33_046_00001.dat -1 taHEFD33_046_00002.dat taHEFD33_046_00003.dat -2 taHEFD33_046_00004.dat taHEFD33_046_00005.dat -3 taHEFD33_046_00006.dat ⊡ -4 Macromolecules + Tree Ð -5 Save Cancel



USR

	Concentration	Scattering	Frames (Averaged/Total)		Gu	nier			Gnore			Porod
lacromolecule	Concernment	scaterry	(names (Averages (coa)	Pg (nm)	Points	Quality (%)	1(0)	Pg (nm)	Total	Dmax (nm)	Volume (nmg)	MM (kD) Vol. es
HEFD33	1.25 report	~	D33 (10 of 10) twikEFD33 (10 of 10) D33 (10 of 10) D33 (10 of 10)	3.23 nm	40 - 82 (42)	78.58	78.16 ±8.02344+-2	3.26 m	0.59	10.81	90.31	452-602
	rfit Denmin Me	erged denmin pob + Ra	da: 3 🛟									
							()					
						- A.S.	223.37	1. 1/3	and the second			
							**	¥9	1	10 B		
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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

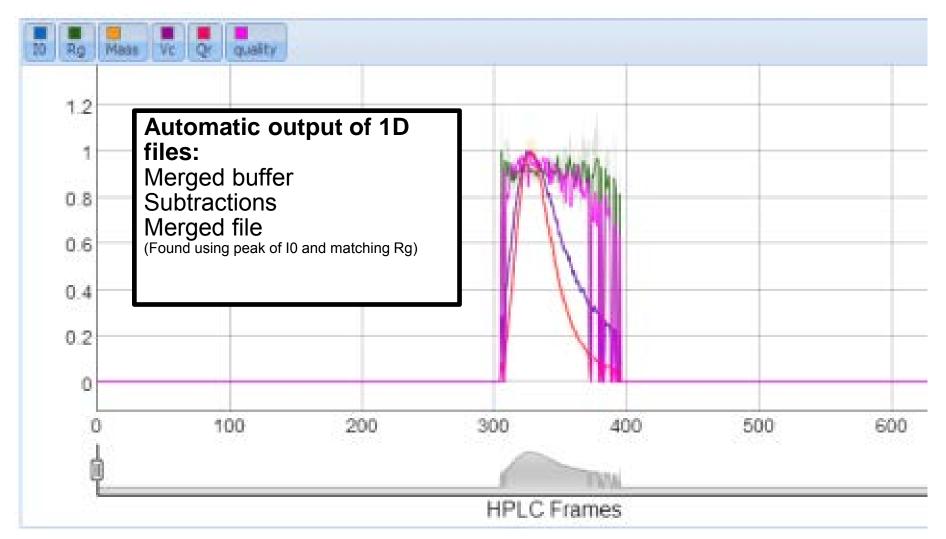
Summary

 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

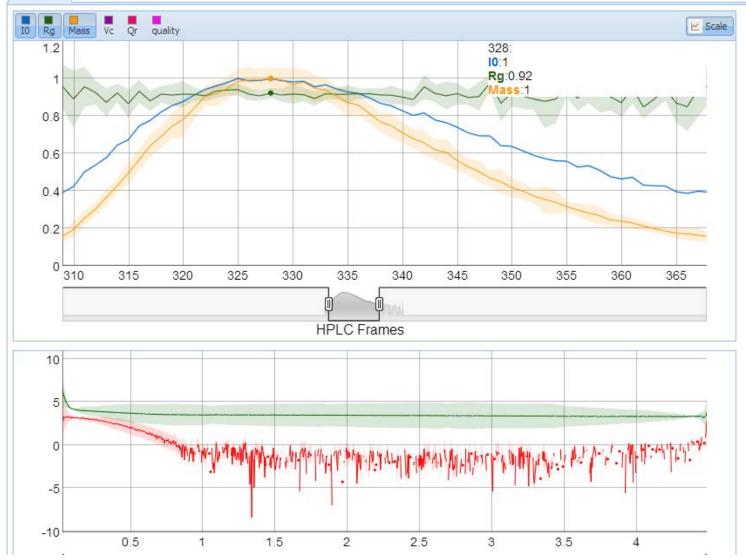


Online SEC in ISPyB



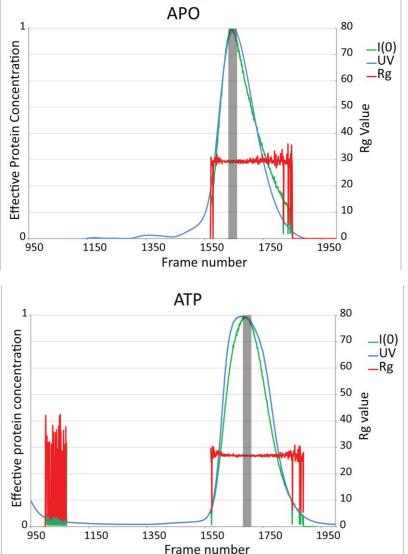


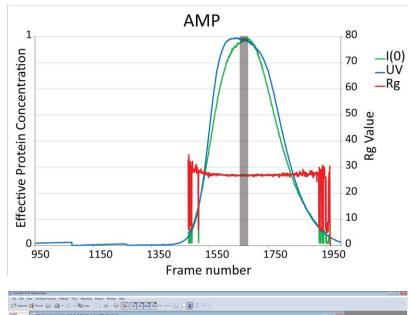
Online SEC in ISPyB

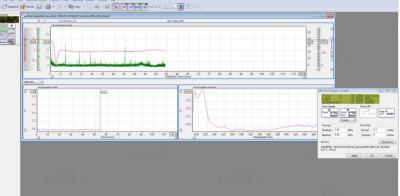




Online SEC







Biophysical characterization



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

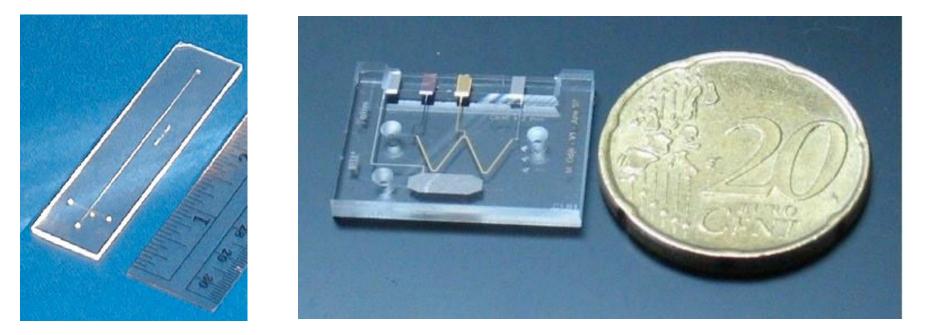
ISPyB Ongoing addition of logic for crosschecking Intuitive feedback on data quality within GUI Automated optimisation of data acquisition Link to HTX CRIMS database Automated sample preparation

Smaller beam (scatterless pinhole) Optimised for smaller capillaries Minimise sample volume requirements Enable microfluidics

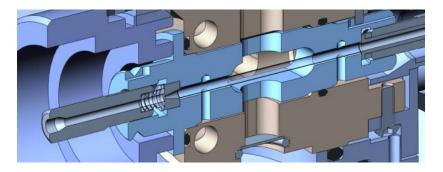
Redesign SEU



SC compatible Microfluidics

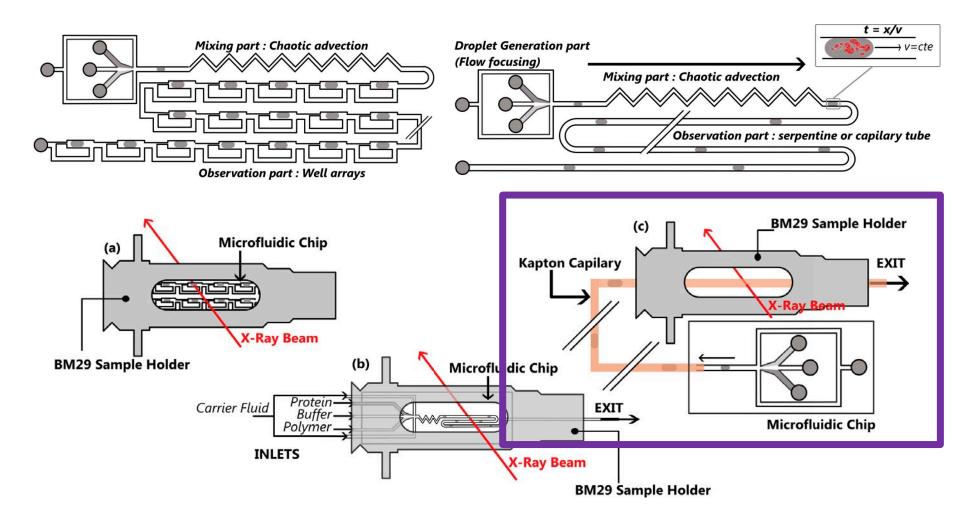








SC compatible Microfluidics





SC compatible Microfluidics

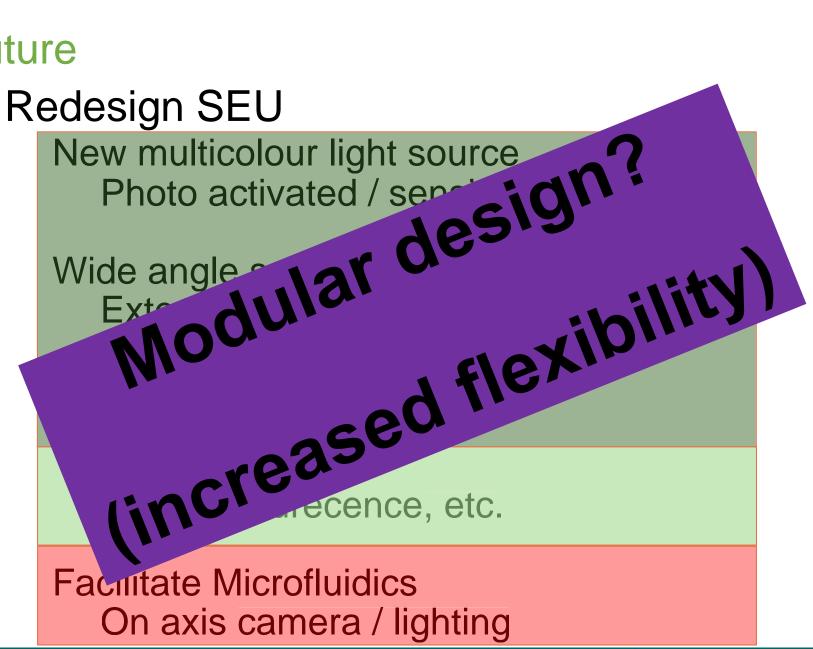
Potential use in:

Mixing studies

Time resolved measurements (seconds) Dynamic screening of buffer conditions









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





Acknowledgments











