



BM29

Bio-Small Angle X-ray Scattering

By Mark Tully

BM29 is jointly operated by Petra Pernot and Mark Tully.

TALK STRUCTURE

- INTRODUCTION: WHY DO SAXS?

- BEAMLINER UPDATES

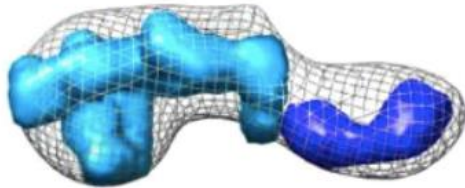
- SOFTWARE UPDATES

- USER INFORMATION

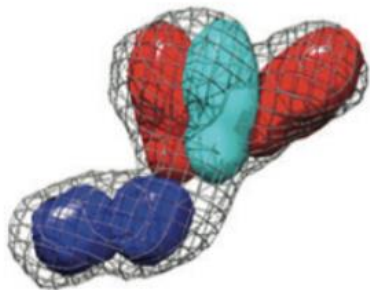
WHY DO SAXS?

- In structural biology, the aim is to visualize structures that unveil critical functional mechanisms.
- This is too often not realized due to missing or incomplete data of function conformations.
- SAXS is a high throughput technique that can directly measure; thermodynamic solution state, conformational states and assemblies albeit at lower resolution.
- SAXS is complementary to all other Structural biology techniques;
 - MX
 - Cryo-EM
 - Molecular modelling (Alpha fold)

1. subunit organization



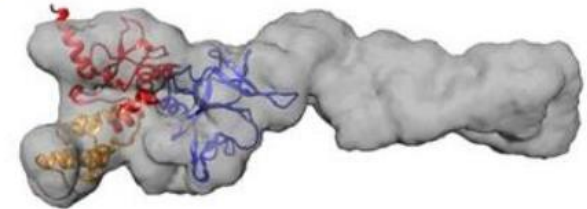
extracellular matrix proteins
(*Baldock Univ. Manchester*)



regulator bacterial
cell division
(*Lewis Univ. Newcastle*)

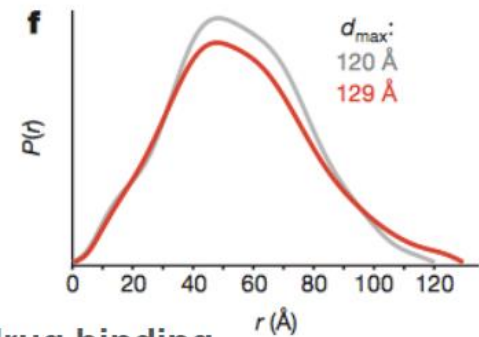
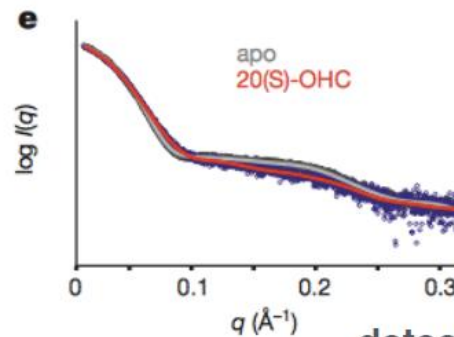
SAXS
experiments

2. low-resolution shapes



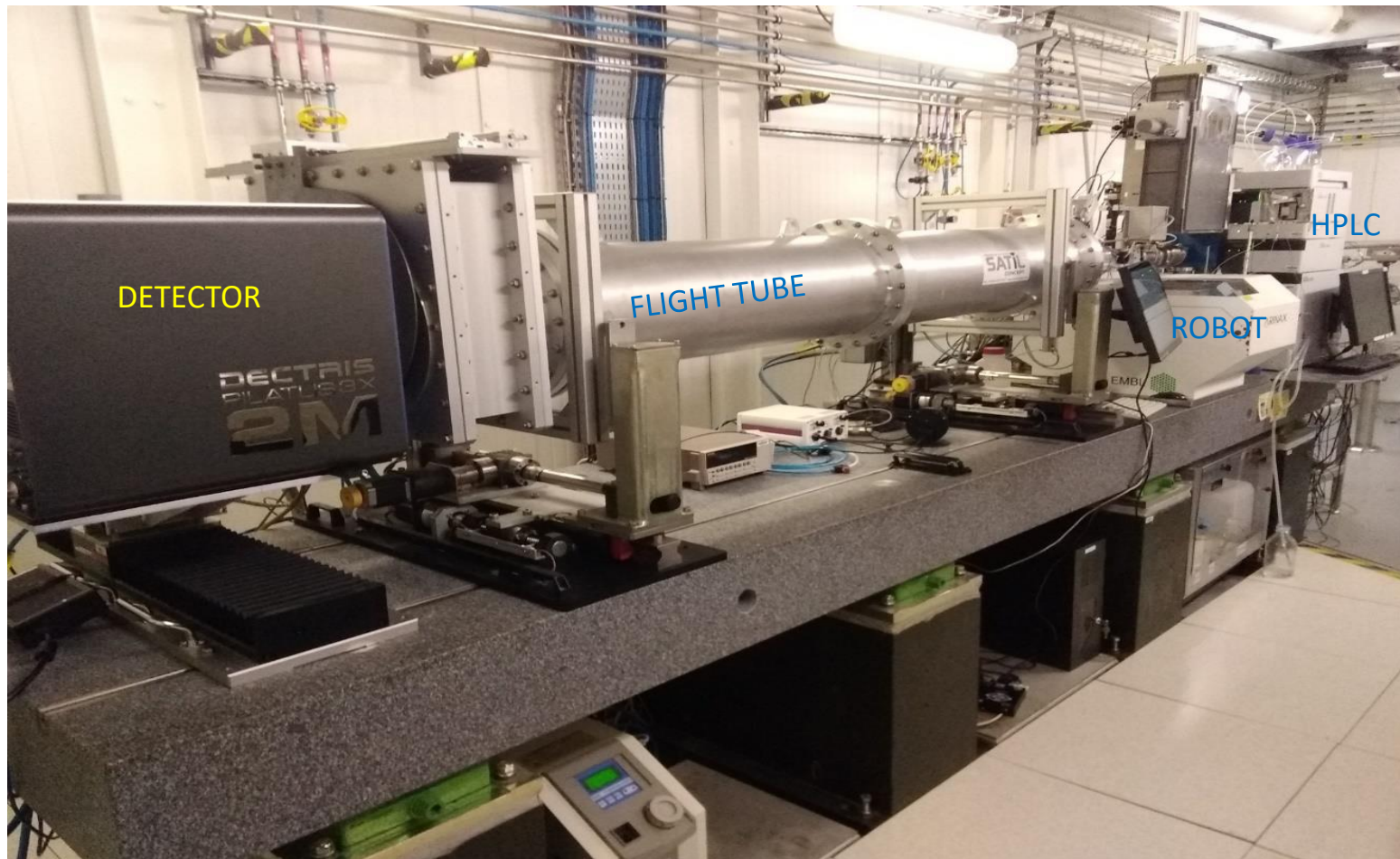
protein antibiotic
(*Kleanthous Univ. Oxford*)

3. Monitor changes in conformational state



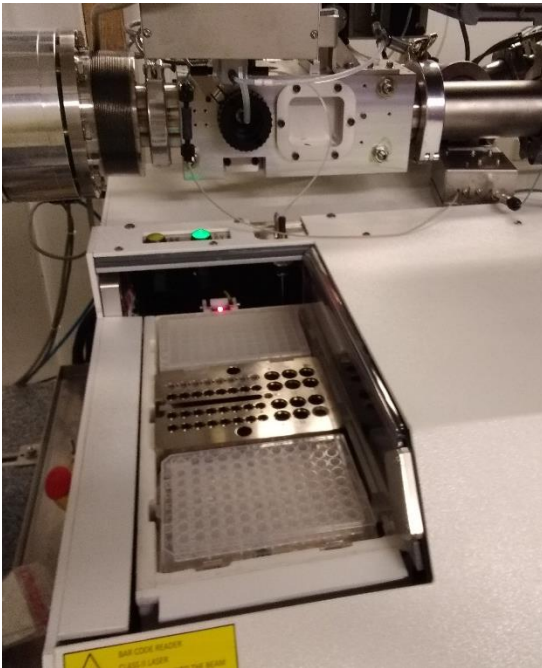
detect drug binding
(*Siebold Univ. Oxford*)

BEAMLINE – EXPERIMENTAL HUTCH



BEAMLINE – SAMPLE CHANGER ROBOT

- sample transfer: 5 to 200 μL ;
- cycle time (load-clean) $\sim 50\text{s}$
- less height: more place for sample environment



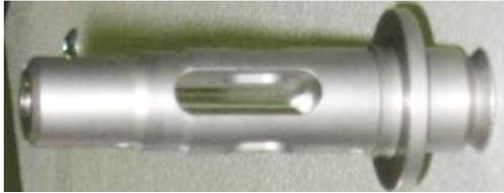
Samples stored in 96 well plates
or PCR tubes

Thermo-regulation

- storage: 4 – 40 $^{\circ}\text{C}$,
- exposure cell: 4 – 60 $^{\circ}\text{C}$

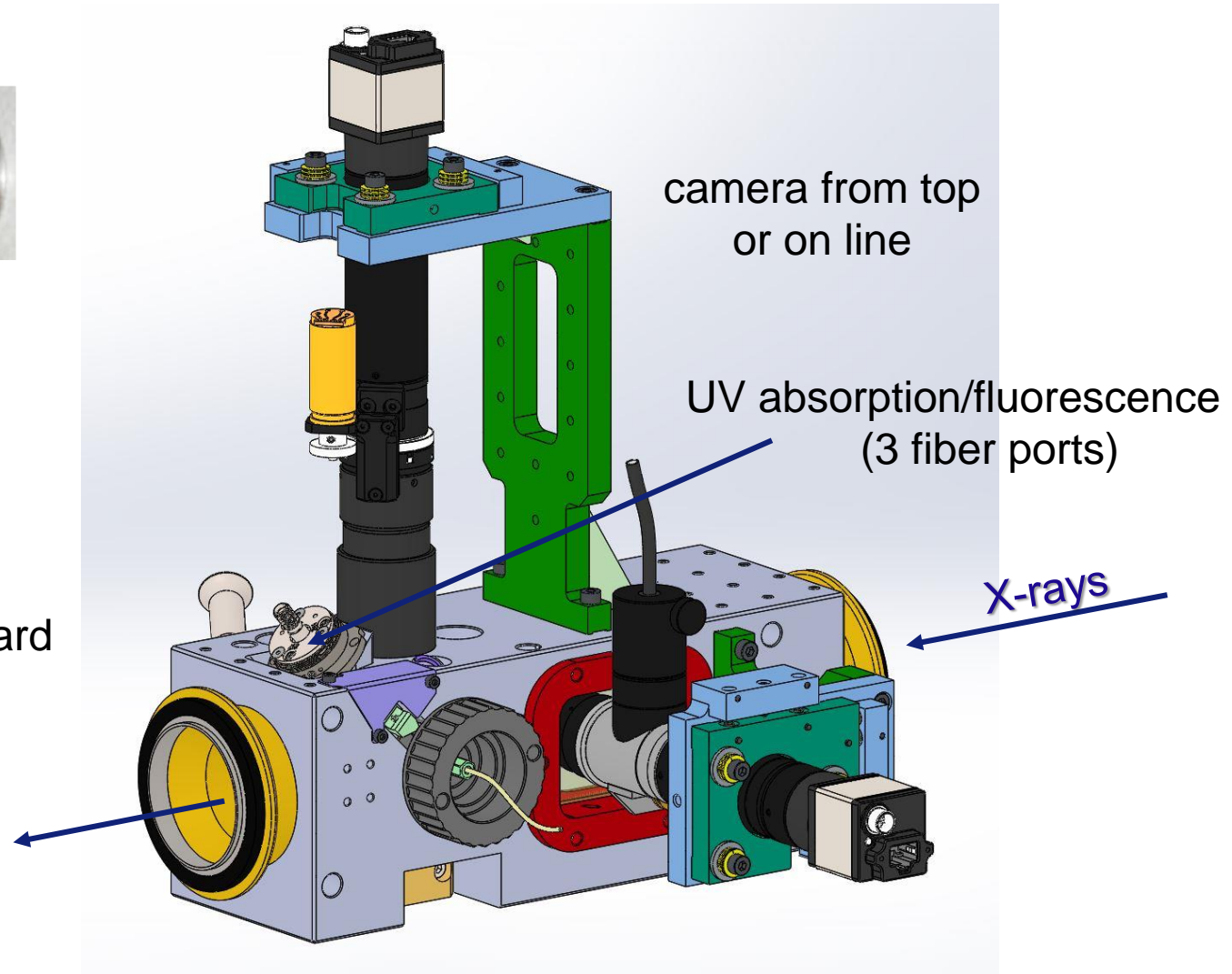
EBS UPGRADE – SAMPLE EXPOSURE UNIT

- capillary pods

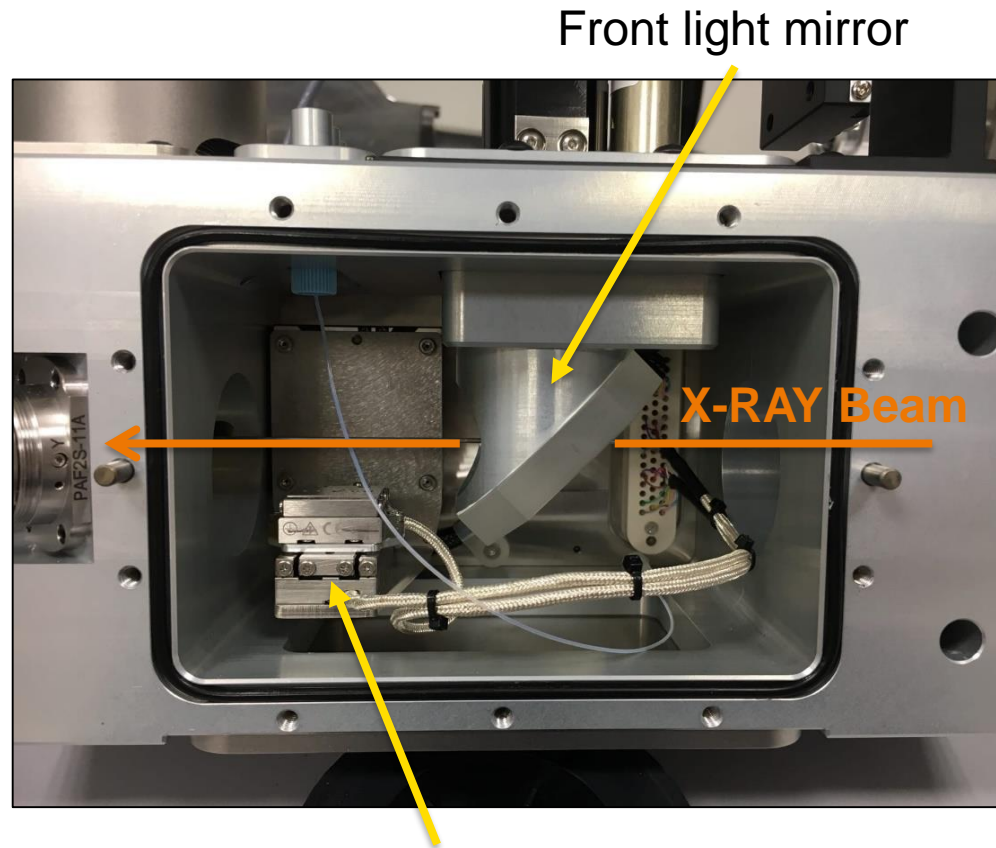


Robot and SEU

- optimized for smaller capillaries: 1mm standard (used to be 2mm)
- more precise syringe for loading used



Vacuum chamber with piezo X, Y, Z stage

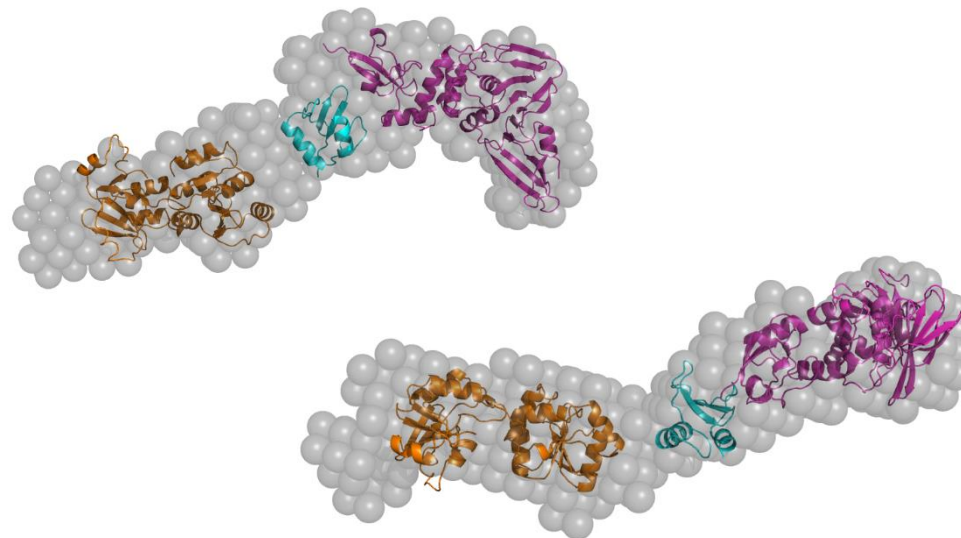
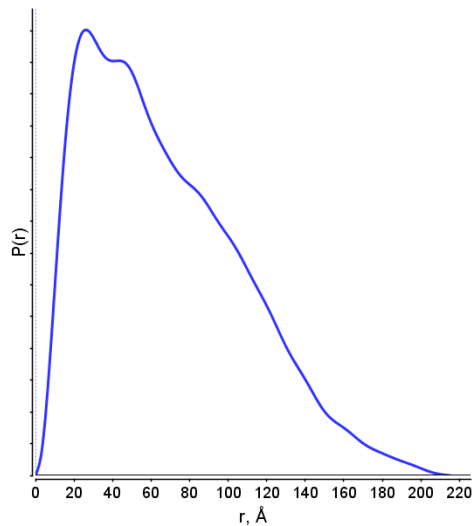
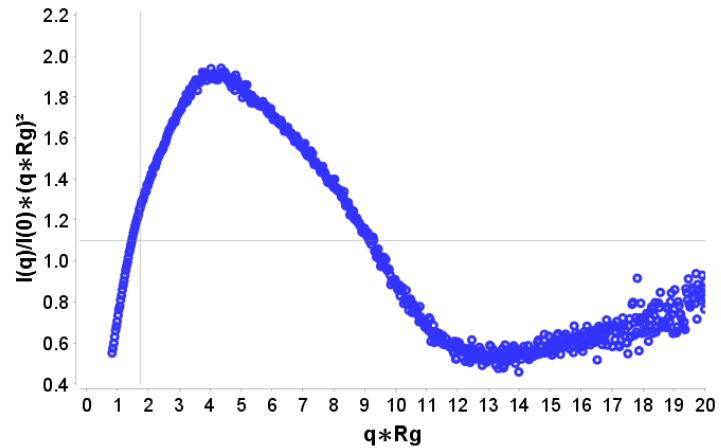
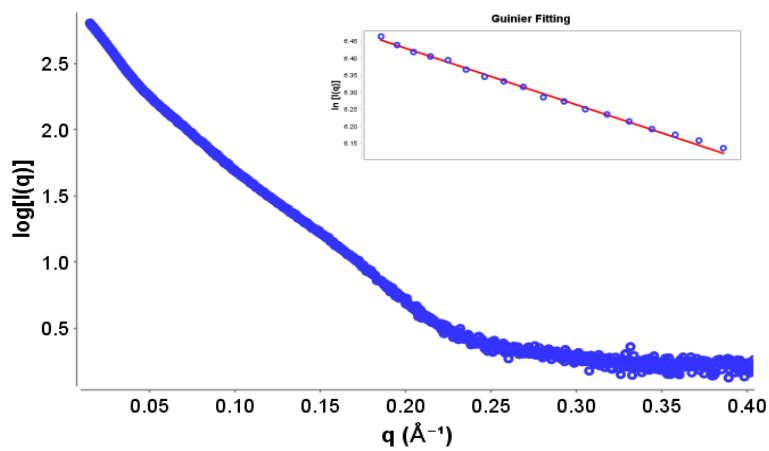


Front light mirror

X-RAY Beam

X,Y,Z-stage
For sample position

User Data – Post EBS SEC-SAXS



SEC-SAXS using Superdex200 10/300 GL 50 μ l 8.8mg/ml loaded.

SOFTWARE UPDATE – BSXCUBE3

BSXCuBE 3 Acquisition Result Beamline Setup System Log mx415 Help Signout

Safety shutter: **DISABLE** Energy: 12.500 keV Transmission: 0.00 % Ring Current: 0

Data path: /data/bm29/inhouse/bsxcube-test/ Name: Run No.: Frame No.: Transmission: Exp. time: Energy: Queue

Sample Changer Experiment Name: SCTestname Load Parameters Save

Buffer Sample

Sample: Buffer:

Plate 1: 96 Deep Well Plate Plate 2: 4 x (8 + 3) Block Plate 3: 96 well Plate

Selected Well: 1:A:1 Sample Name: s1 Buffer Name: --Choose-- Concentration (mg/ml): 1 Volume µl: 50 SEU Temp. °C: 4 Storage Temp. °C: 4 Comment:

Add to Sample Table

Clear Table Optimisation Collaps Parameters: Parameters: Number of sample's 3

Name	Buffer	Plate	Row	Column	C (mg/mL)	Flow	Extra Flow t(s)	Volume (µl)	SEU Temp. °C	Storage Temp. °C	Energy	Viscosity	Number of Frames	Exposure(s)	Transmission %	Buffer mode	Recuperation	Comment
1 sample	bn2	3	D	2	1		7	60	50	6	12.5	low	7	1	100	Before & A	<input checked="" type="checkbox"/>	c
2 sample	bn1	2	B	2	0		5	67	58	66	17	low	7	1	8	After	<input checked="" type="checkbox"/>	c
3 sample	bn	1	A	1	1		5	60	50	6	12.5	low	7	1	100	Before	<input checked="" type="checkbox"/>	c

Back: To Buffer table Run Now Add to Queue

Sample environment page selection

SOFTWARE UPDATE – BSXCUBE3

BSXCuBE 3 Acquisition Result Beamline Setup System Log mx415 Help Signed Out

Safety shutter: **DISABLE** Energy: **12.500 keV** Transmission: **0.00 %** Ring Current: **0**

Data path: /data/bm29/inhouse/bsxcube-test/

Name: Run No.: Frame No.: Transmission: Exp. time: Energy:

Queue

Sample Changer Experiment Name: SCTestname Load Parameters Save

Buffer Sample

Sample: Buffer:

Plate 1: 96 Deep Well Plate

	1	2	3	4	5	6	7	8	9	10	11	12	
A	1	1											
B													
C													
D													
E													
F													
G													
H													

Plate 2: 4 x (8 + 3) Block

	1	2	3	4	5	6	7	8	9	10	11	
A												
B												
C												
D												

Plate 3: 96 well Plate

	1	2	3	4	5	6	7	8	9	10	11	12	
A													
B													
C													
D													
E													
F													
G													
H													

Selected Well: 1:A:1

Sample Name: s1 Buffer Name: --Choose--

Concentration (mg/ml): 1 Volume µl: 50

SEU Temp. °C: 4 Storage Temp. °C: 4

Comment:

Add to Sample Table

Clear Table Optimisation Collaps Parameters: Parameters: Number of sample's 3

Name	Buffer	Plate	Row	Column	C (mg/mL)	Flow	Extra Flow t(s)	Volume (µl)	SEU Temp. °C	Storage Temp. °C	Energy	Viscosity	Number of Frames	Exposure(s)	Transmission %	Buffer mode	Recuperation	Comment	
1	sample :	bn2	3	D	2	1		7	60	50	6	12.5	low	7	1	100	Before		c
2	sample :	bn1	2	B	2	0		5	67	58	66	17	low	7	1	8	After		c
3	sample	bn	1	A	1	1		5	60	50	6	12.5	low	7	1	100	Before		c

Back : To Buffer Table Run Now Add to Queue

Addition of "Queue" System for integrated Sample changer and HPLC

SOFTWARE UPDATE – BSXCUBE3

BSXCuBE 3 Acquisition Result Beamline Setup System Log mx415 Help Signout

Safety shutter: **DISABLE** Energy: 12.500 keV Transmission: 0.00 % Ring Current: 0

Data path: /data/bm29/inhouse/bsxcube-test/ Name: Run No.: Frame No.: Transmission: Exp. time: Energy: Queue

Sample Changer Experiment Name: SCTestname Load Parameters Save

Sample: Buffer:

Plate 1: 96 Deep Well Plate Plate 2: 4 x (8 + 3) Block Plate 3: 96 well Plate

Selected Well: 1:A:1 Sample Name: s1 Buffer Name: --Choose-- Concentration (mg/ml): 1 Volume µl: 50 SEU Temp. °C: 4 Storage Temp. °C: 4 Comment:

Add to Sample Table

Clear Table Optimisation Collaps Parameters: Parameters: Number of sample's 3

Name	Buffer	Plate	Row	Column	C (mg/mL)	Flow	Extra Flow t(s)	Volume (µl)	SEU Temp. °C	Storage Temp. °C	Energy	Viscosity	Number of Frames	Exposure(s)	Transmission %	Buffer mode	Recuperation	Comment
1	sample :	bn2	3	D	2	1	7	60	50	6	12.5	low	7	1	100	Before & A	☑	c
2	sample :	bn1	2	B	2	0	5	67	58	66	17	low	7	1	8	After	☑	c
3	sample	bn	1	A	1	1	5	60	50	6	12.5	low	7	1	100	Before	☑	c

Run Now Add to Queue

Simplified 3 step process;

1. Select your sample position
2. Name your sample, concentration, etc.
3. Select "Run" or "Add to Queue"

SOFTWARE UPDATE – BSXCUBE3

The screenshot displays the BSXCUBE 3 software interface. At the top, there are system status indicators for Front End (Open), Safety shutter (Disabled), Fast shutter (Closed), Energy (12,500 keV), Wavelength (0.99 keV), and Transmission (100.00 %). The data path is shown as /data/visitor/mx1816/bm29/. System parameters include Ring Current (34.86 mA), CBM20 (Controlled), and PDA (Controlled). The HPLC section shows an experiment named 'myhplc' with a selected vial of 1. A 10x10 grid of vial positions is visible, with vial 1 highlighted. The 'Auto Sample' is set to OFF, and the 'Auto Sample Temp' is 20. The 'Sample Parameters' section includes fields for Sample Name (sample_z), Vial Number (1), Injection Volume (50), Number of Frame (480), Column Elution T (8), Exposure Time (1), λ1 (260), λ2 (280), λ3 (350), λ4 (450), Exposure Temp (20), and Flow Rate (0.5). A table at the bottom lists the sample parameters for the current run. The 'HPLC Control by BSX3' toggle is highlighted with a yellow box and a callout arrow pointing to a text box below.

Auto Sample : Set OFF

Plate : HPLC Well Plate
Selected Vial : 1

Auto Sample Temp : (19.9) 20

Sample Parameters

Sample Name * sample_z

Vial Number * 1

Injection Volume (μl) * 50

Number of Frame * 480
Recommended Value : 480.0

Column Elution T (min) * 8
Recommended Value : 8.0

Exposure Time (s) * 1

λ1 (nm) * 260

λ2 (nm) * 280

λ3 (nm) * 350

λ4 (nm) * 450

Exposure Temp °C * 20

Flow Rate (mL/min) * 0.5

Comment
c

Add to Sample Table

Sample Name	Flow R. (mL/min)	Vial Number	Injection Volume (μl)	λ1 (nm)	λ2 (nm)	λ3 (nm)	λ4 (nm)	Concentration	Exposure Temp °C	Exposure Time (s)	Number of Frames	Column Elution (min)	Comment
BSA	0.5	1	50	260	280	350	450	1	20	2	480	8	c

Injection Port : A

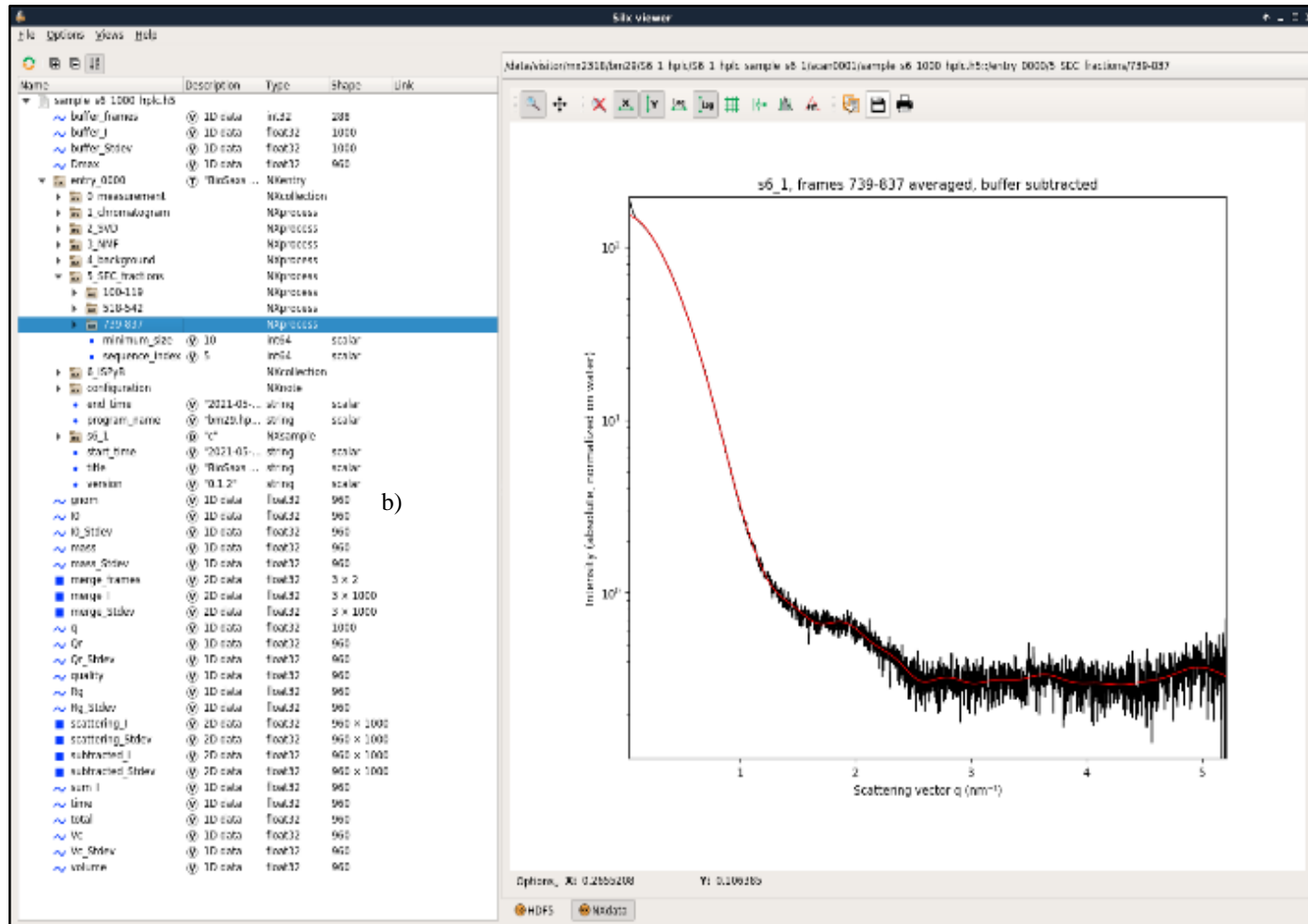
HPLC Control by BSX3 :

SC Initial Cleaning : Wait for Beam :

Run Add to Queue

HPLC now integrated

.H5 files encompass opensource data reduction pipeline, FreeSAS by Jerome Kieffer



b)

SOFTWARE UPDATE - ISPYB

Select

Run	Sample	Frames (Average/Total)	Guinier			Gnom			Porod		Scattering	Kratky	Density	Guinier	Advanced
			Rg	Points	I0	Rg	Total	D _{max}	Volume	MM Vol. est.					
#18	PaSec_B	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#17	His_PaSec_B3 4.570 mg/ml 20.0 C 50.0 µl	10 / 10	2.859 nm	18 - 80 (62)	397.1 ± 1.983e+0	2.940 nm	-2729.440	8.412 nm	71.172 nm ³	35.6 - 47.4 Å				<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#16	PaSec_B	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#15	PaSec_B	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#14	His_PaSec_B2 1.500 mg/ml 20.0 C 50.0 µl	10 / 10	3.119 nm	30 - 74 (44)	135.6 ± 6.06e-1	3.140 nm	-550.854	10.196 nm	82.859 nm ³	41.4 - 55.2 Å				<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#13	PaSec_B	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#12	PaSec_B	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#11	His_PaSec_B1 0.750 mg/ml 20.0 C 50.0 µl	10 / 10	3.487 nm	23 - 27 (4)	73.2 ± 1.4e-2	3.339 nm	-522.944	12.440 nm	56.987 nm ³	28.5 - 38.0 Å				<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#10	PaSec_B	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#9	PaSec_A	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#8	His_PaSec_A3 2.400 mg/ml 20.0 C 50.0 µl	10 / 10	4.504 nm	26 - 45 (19)	251.1 ± 8.71e-1	4.879 nm	-564.578	24.171 nm	137.621 nm ³	68.8 - 91.7 Å				<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#7	PaSec_A	8 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#6	PaSec_A	8 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#5	His_PaSec_A2 1.500 mg/ml 20.0 C 50.0 µl	10 / 10	4.666 nm	27 - 47 (20)	167.1 ± 1.498e+0	5.202 nm	-556.185	22.406 nm	124.310 nm ³	62.2 - 82.9 Å				<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#4	PaSec_A	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#3	PaSec_A	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#2	His_PaSec_A1 0.470 mg/ml 20.0 C 50.0 µl	8 / 10	4.282 nm	7 - 51 (44)	63.0 ± 6.6e-1	4.352 nm	-470.741	16.575 nm	129.575 nm ³	64.8 - 86.4 Å				<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	
#1	PaSec_A	8 / 10												<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigid Body	

We are open to in-person visits!

- If you request mail-in SAXS we can physically only run 8-10 hours samples.
- If several labs in a BAG send too many samples we will not be able to accommodate all of them.
- If you visit in person we can teach you how to use the beamline and also the processing software to enable you to analyse your data (ScÅtter, ATSAS).

How to Download Your Data

- *.dat files downloaded from <https://exi.esrf.fr>
- *.H5 files downloaded from <https://data.esrf.fr>
- Any other files (or large files > 2 GB) especially reintegrated files use <https://app.globus.org> (see link from <https://data.esrf.fr> to learn how to download and use)

How to Download Your Data

- *.dat files downloaded from <https://exi.esrf.fr>

The screenshot displays the EXI data analysis interface. On the left, there is a sidebar with 'Experiment' information and 'SAMPLE CHANGER' buttons. The main area shows a table of data for various samples. Below the table, there is a 'Data Collection' section and a 'Size-exclusion chromatography' plot.

Run	Sample	Frames (Average/Total)	Guinier			Gnom		Porod		MM	Scattering	Kratky	Density	Guinier
			Rg	Points	I0	Rg	Total	D _{max}	Volume					
#9	Buffer	10 / 10												
#8	Sample_three	7 / 10	3.883	35 - 50	108.9 ± 3.698	12.6								
#7	Buffer	7 / 10												
#6	Buffer	7 / 10												
#5	Sample_two	8 / 10	3.749	4 - 61	323.2 ± 3.970	16.3								
#4	Buffer	10 / 10												
#3	Buffer	10 / 10												
#2	sample_1	9 / 10	3.438	10 - 67	895.2 ± 3.562	14.5								
#1	Buffer	9 / 10												

The 'Data Collection' table shows a row for 'Sample' with values 'NA / NA' and a download icon.

The 'Size-exclusion chromatography' plot shows a chromatogram with a peak at approximately 200 frames. The x-axis is labeled 'Frames' and the y-axis is labeled 'sum_I'. A 'Download Range' button is visible at the bottom right of the plot area.

All data from all samples in the block.

Individual frames from individual sample.

Download button

How to Download Your Data

- *.H5 files downloaded from <https://data.esrf.fr>

🔍	📅 Date ↕	📄 Sample ↕	📁 Dataset ↕	📄 Definition	📄 Files	📄 Size	📄 Download
🔍	🕒 17:22 21 Nov 2021	📄 hplc_TNC_NP246_2_hplc	📁 sample_NP246_2		22	785.6 MB	📄 Download
🔍	🕒 18:10 21 Nov 2021	📄 hplc_TNC_NP246_2_hplc	📁 sample_DDTir [2021-11-21 18:10:27]		22	783.8 MB	📄 Download

Summary Instrument Files **22** Metadata List

Search

📄	👁️	📄 Preview	📄 Location	📄 Size
📄	👁️		/scan0001/sample_DDTir0005.h5	29.5 MB
📄	👁️		/scan0001/sample_DDTir000_hplch5	12.4 MB

20 Showing rows 21 to 22 of 22

< 1 2

/scan0001/sample_DDTir000_hplch5

sample_DDTir000_hplch5 Display Inspect Feedback

NX Spectrum X Linear Y Linear

520
x D0

Summed Intensity

frame index

Open viewer

📄 /data/visitor/mx2376/bm29/hplc_TNC_NP246_2_hplc/hplc_TNC_NP246_2_hplc_sample_DDTir 📄 Download

How to Download Your Data

- Any other files (or large files > 2 GB) especially reintegrated files use <https://app.globus.org> (see link from <https://data.esrf.fr> to learn how to download and use)

Data Portal My Data Open Data Closed Data Shipping ▾ Log out Mark TULLY

My Data

Dataset List 411 Logbook Shipping Proposal

For users that want to download large volume of experimental data (>2GB), ESRF users can access the Globus service, please read the documentation for proceeding: <https://confluence.esrf.fr/display/SCKB/Globus>

Search

	<input type="checkbox"/>	Date ▾▲	Sample ▾▲	Dataset ▾▲	Definition	Files	Size	Download
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How to Download Your Data

- Any other files (or large files > 2 GB) especially reintegrated files use <https://app.globus.org> (see link from <https://data.esrf.fr> to learn how to download and use)

Please view our updated [Privacy Policy](#) and [Terms of Service](#).

File Manager

Collection: ESRF Experimental Data

Path: /visitor/mx1816/bm29/bsa_19thJan_1_hplc/bsa_19thJan_1_hplc_sample_bsa_1/scan0001/

NAME	LAST MODIFIED	SIZE
<input checked="" type="checkbox"/> summary-hplc.h5	1/19/2022, 11:34 AM	7.45 MB
<input type="checkbox"/> hplc.json	1/19/2022, 11:34 AM	186 B
<input type="checkbox"/> hplc.json~	1/19/2022, 11:32 AM	429 B
<input type="checkbox"/> sample_bsa_10002-integrate.h5	1/19/2022, 11:18 AM	900.76 KB
<input type="checkbox"/> sample_bsa_10002-integrate.json	1/19/2022, 11:18 AM	9.23 KB
<input type="checkbox"/> sample_bsa_10002.h5	1/19/2022, 11:18 AM	138.31 MB
<input type="checkbox"/> sample_bsa_10001-integrate.h5	1/19/2022, 11:16 AM	900.80 KB
<input type="checkbox"/> sample_bsa_10001-integrate.json	1/19/2022, 11:16 AM	9.27 KB

Beamline Staff and Local Contacts

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Marcus Oskarsson

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BLISS:

Antonia Beteva

Marcus Oskarsson

Databases:

Alejandro De Maria Antolinos

Maxime Chaillet

