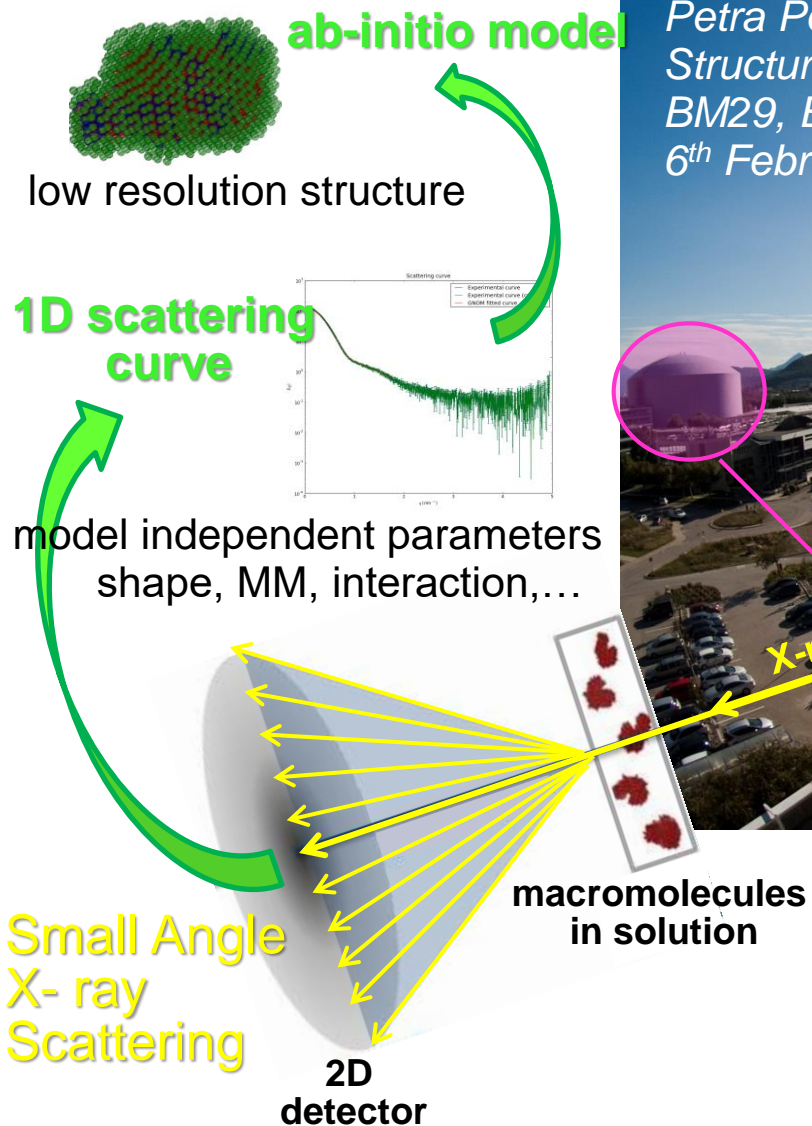
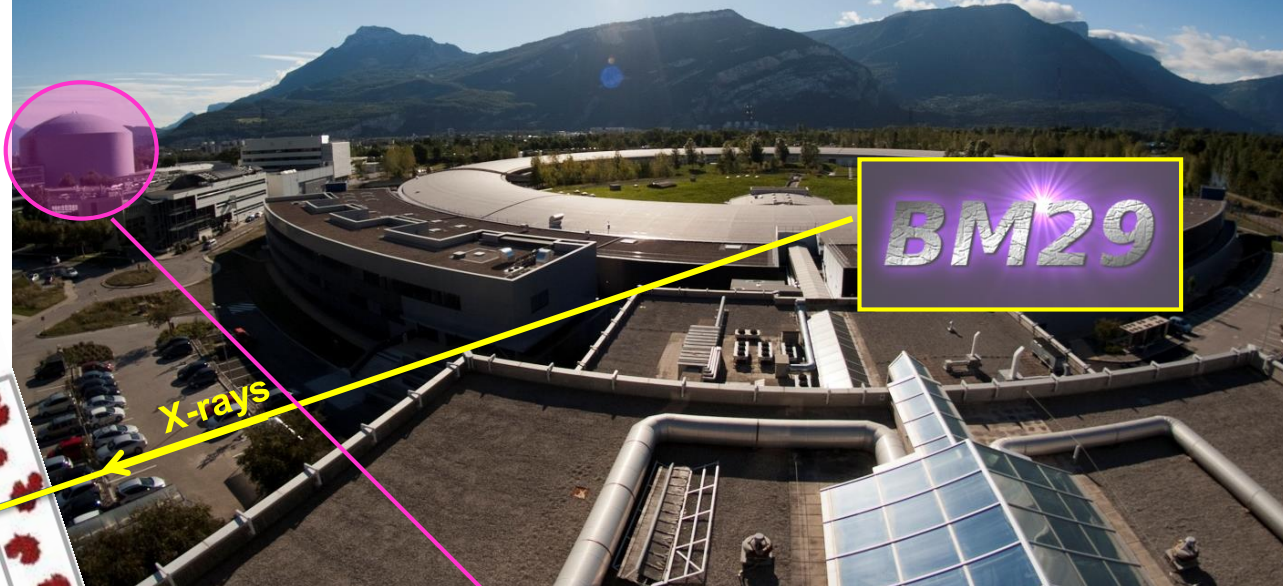




| The European Synchrotron



Petra Pernot  
Structural Biology Group  
BM29, ESRF  
6<sup>th</sup> February 2017



## OUTLINE

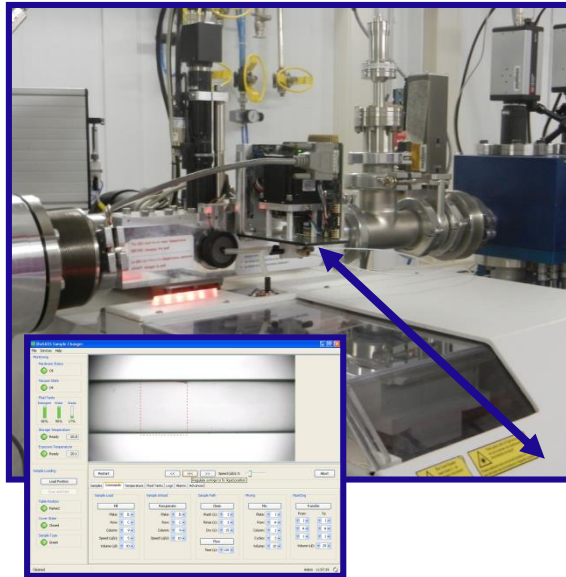
- ESRF BioSAXS beamline(s) key features, automated data collection and analysis;
- ion exchange chromatography;
- microfluidics: crystallisation in situ;
- complementarity with **SANS**: joint access
- new sample prep lab, EXI

# KEY FEATURES

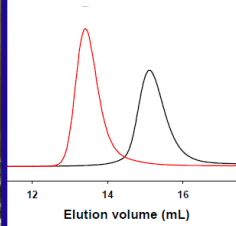
- beamline is dedicated exclusively to small-angle scattering experiments of biological macromolecules in solution;
- minimal sample requirements: 10  $\mu\text{L}$  with  $c \ll 1 \text{ mg/mL}$ ;
- high throughput facility “easy” to use in  
*typical data collection times*
  - a) **sample changer** mode 10 s (cycle loading-exposure-cleaning  $\sim 1.5 \text{ min}$ )
  - b) **HPLC on-line** mode 30 min (column elution time);
- providing automated data analysis and data tracking;
- access through Rolling procedure or BAG system: proposals any time and beam time in a few weeks (if successful and time available);
- SANSAXS BAG: one trip to Grenoble for ILL and ESRF experiments on same samples: SAS with neutrons and X-rays.

# BM29 OUTLOOK – AUTOMATISATION

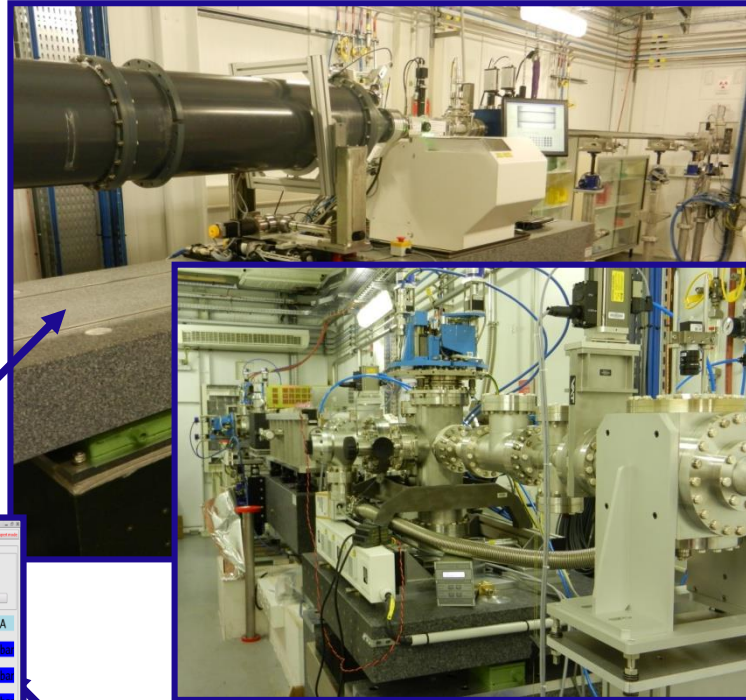
## ROBOT/HPLC



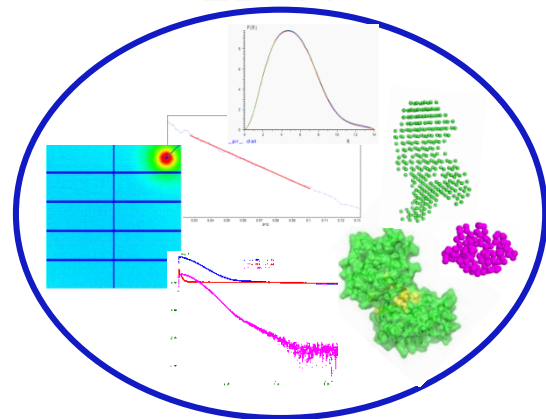
## EXPERIMENTAL parameters



BsxCuBE



## EDNA



ISPyBB  
sample tracking  
and data flow

Macromolecule	Conc. of Scattering	Wavelength	Frame (Overexposed/Total)	Exp. Det.	Frame	Quality (%)	Apprec. Guinier	Exp. Det.	Total	Conc. (%)	
ISPyBB	5.70	10.00	100/100	3.00	95-98/100	75.75	43.34 +1.00	No	3.00	0.54	10.20
ISPyBB	3.97	10.00	100/100	3.11	94-98/100	75.17	43.56 +1.00	No	3.11	0.56	10.41
ISPyBB	1.47	10.00	100/100	3.19	98-99/100	76.89	45.47 +1.00	No	3.20	0.60	11.18
ISPyBB	0.70	10.00	100/100	3.19	43-92/100	75.94	41.77 +1.00	No	3.18	0.60	10.84
ISPyBB	0.38	10.00	100/100	3.19	32-99/100	76.30	43.00 +1.00	No	3.20	0.60	10.67
ISPyBB	0.20	10.00	100/100	3.19	32-92/100	81.83	39.06 +1.00	No	3.17	0.64	10.86

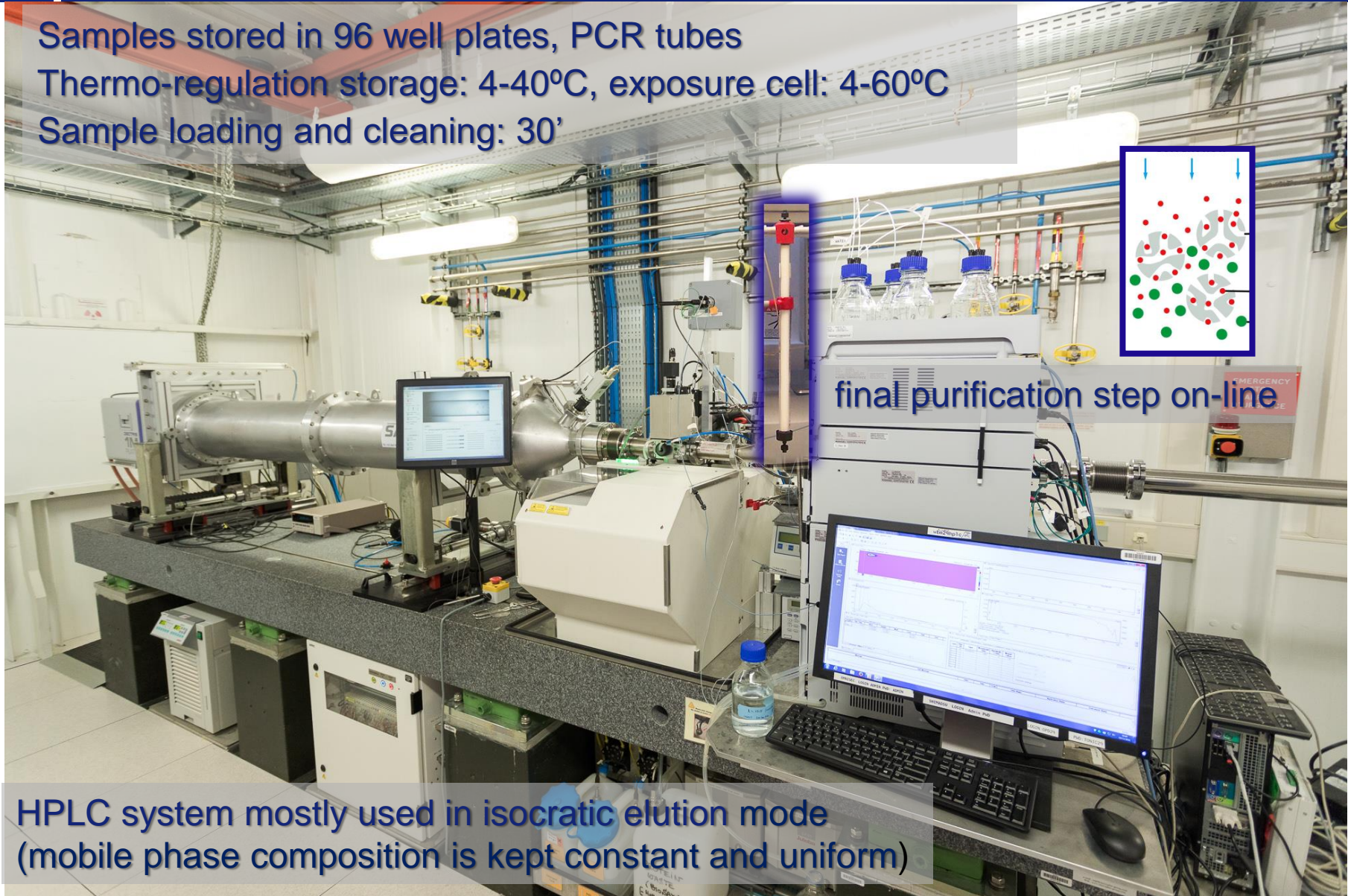
DATABASE

# BM29 OUTLOOK = SAMPLE CHANGER + HPLC SYSTEM

Samples stored in 96 well plates, PCR tubes

Thermo-regulation storage: 4-40°C, exposure cell: 4-60°C

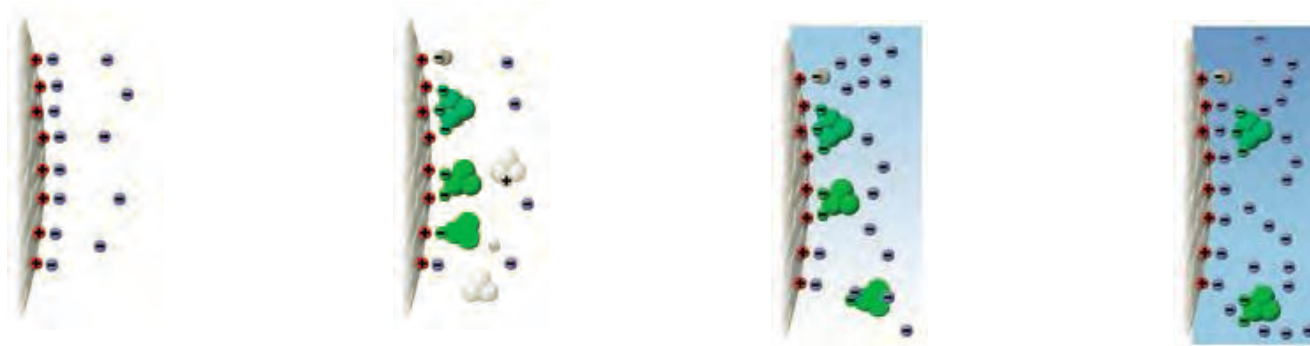
Sample loading and cleaning: 30'



HPLC system mostly used in isocratic elution mode  
(mobile phase composition is kept constant and uniform)

# ION EXCHANGE CROMATOGRAPHY

➔ Separation based on surface charge



Salt concentration (linear gradient)

## Advantage:

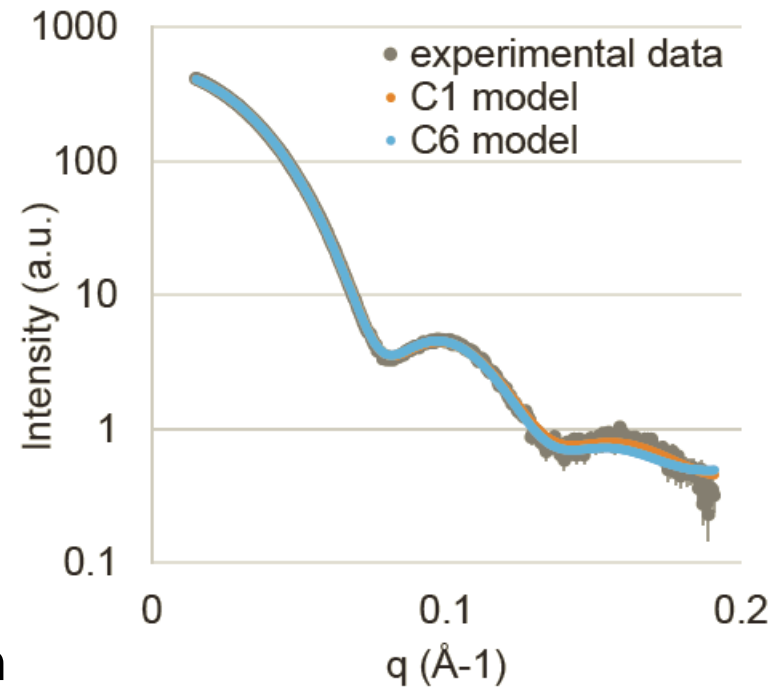
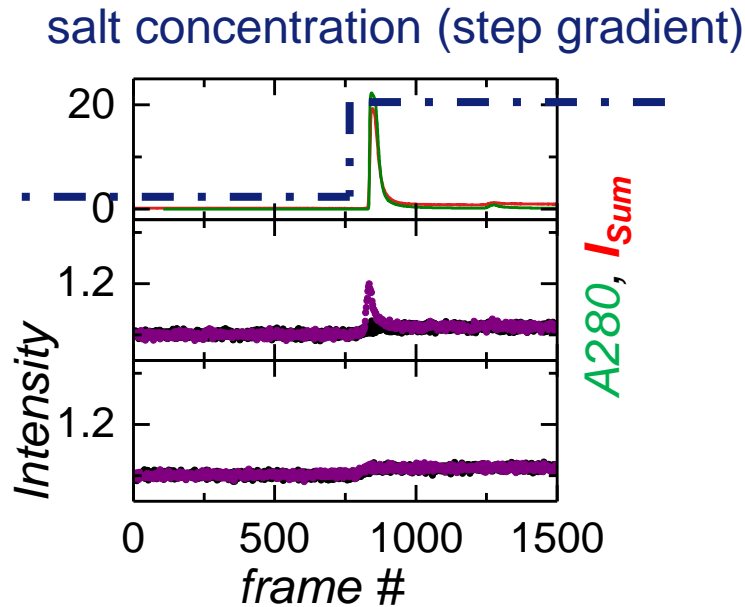
- sample can be loaded at low concentrations (avoids sample loss due to aggregation)
- relatively high flow rate (mL/min) - reduces the risk of radiation damage

## Disadvantage:

- buffer composition intrinsically unknown

*Collaboration:*  
*Martha Breninich (EMBL, Grenoble)*  
*Stephanie Hutin (IBS, Grenoble)*

Figures:  
GE Healthcare

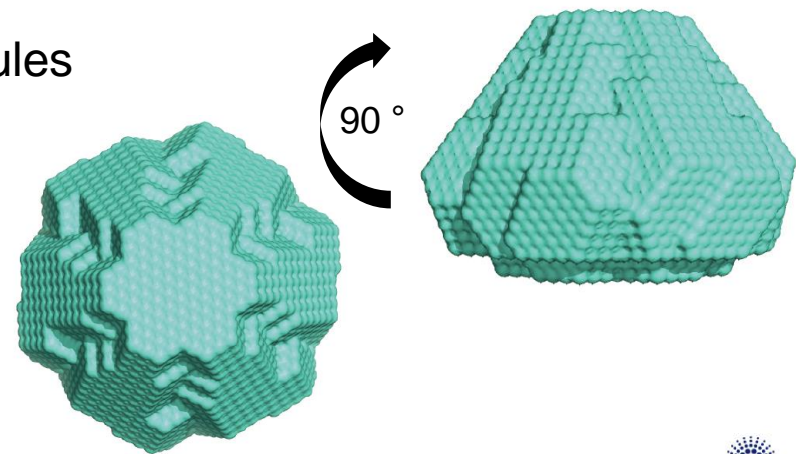


➔  $D5_{323-785}$  is hexameric in solution

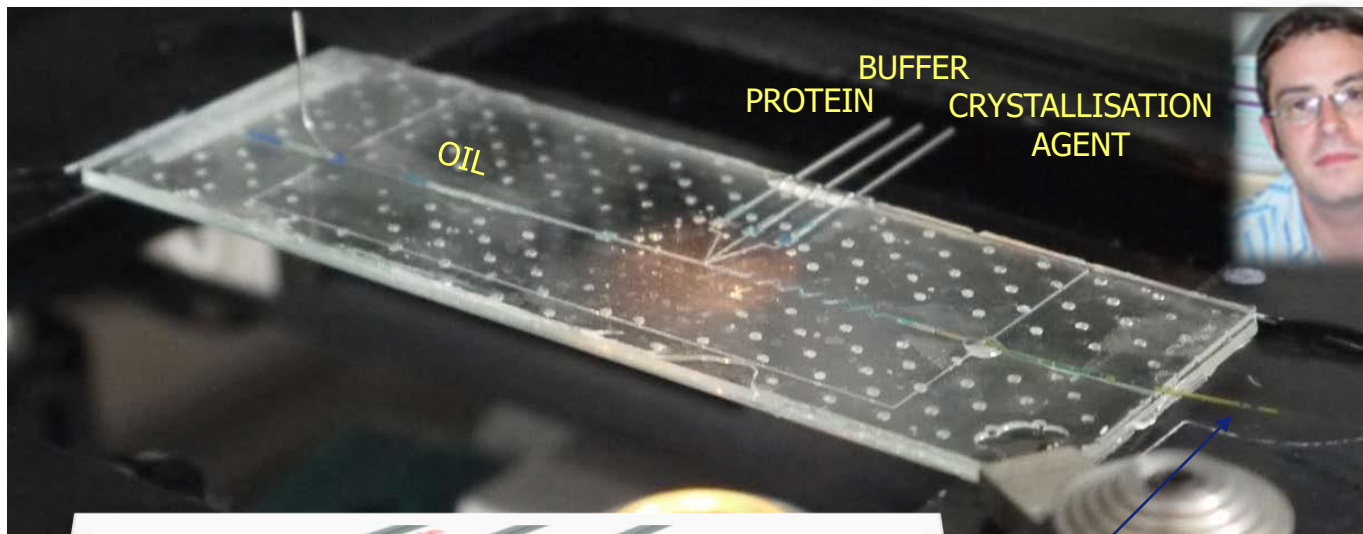
- IEC can separate similarly sized macromolecules
- using careful background correction, high quality SAXS data can be obtained

“Online ion-exchange chromatography for small-angle X-ray scattering”

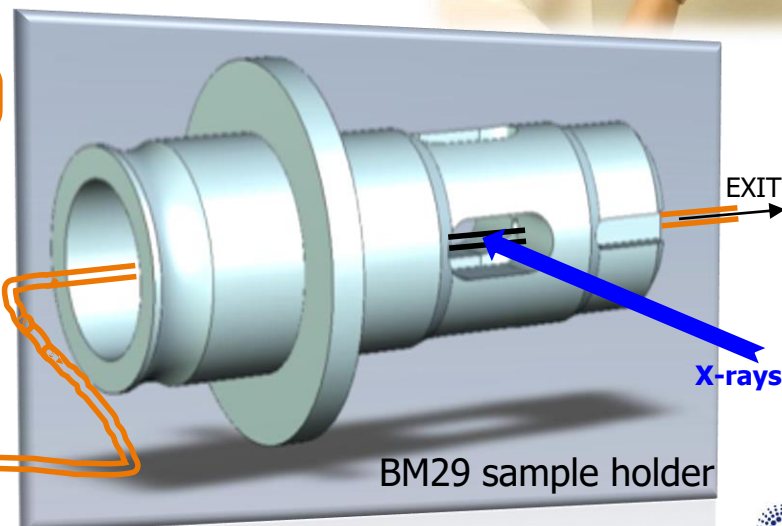
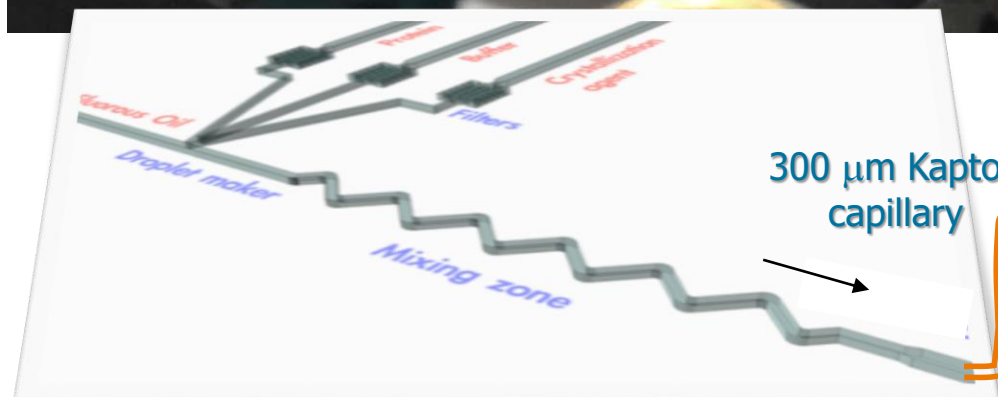
S. Hutin, M. Brennich, B. Maillot and A. Round  
*Acta Cryst.* (2016) D72, 1090–1099.



# MICROFLUIDICS INTEGRATION AT BM29



Collaboration within LTP  
Sébastien Teychené  
Laboratoire de Génie  
Chimique, Toulouse  
and  
Françoise Bonneté  
Institut des Biomolécules  
Max-Mousseron, Avignon



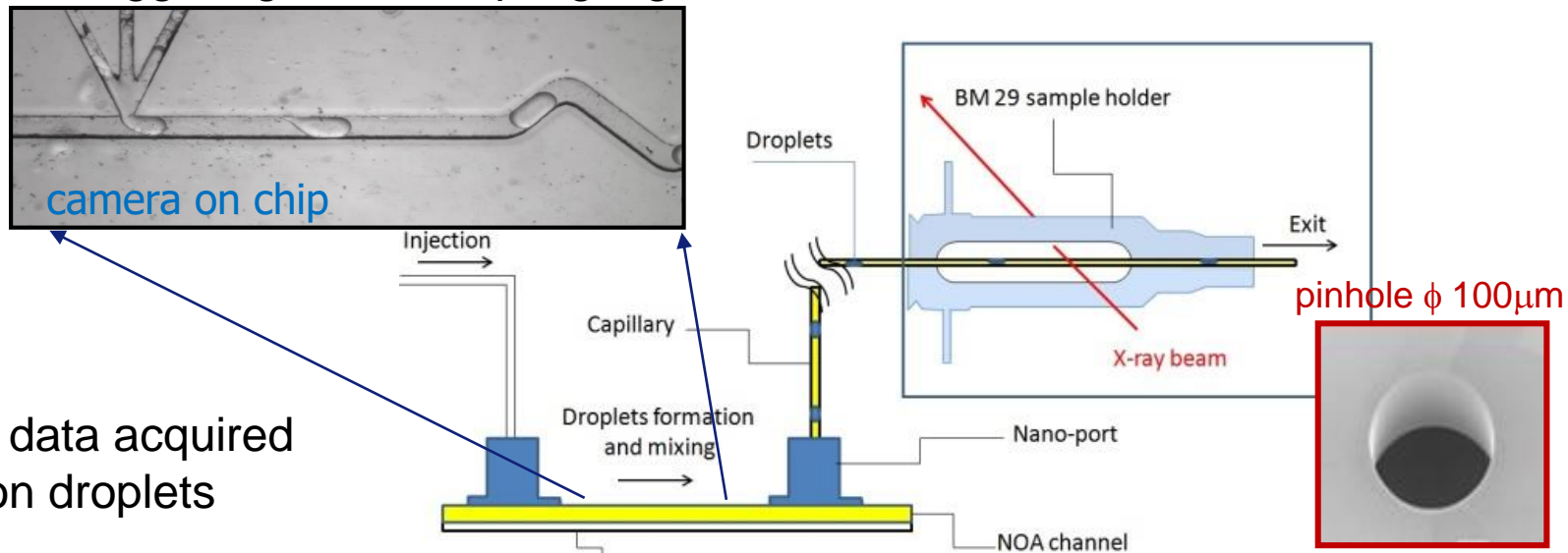
“Coupling digital microfluidics and Small-Angle X-ray scattering to study the whole crystallization process of proteins in solution”

N.V. Pham, D. Radajewski, P. Guillet, A. Round, M. Brennich, P. Pernot, B. Biscans, F. Bonneté and S. Teychené, *Analytical Chemistry* (2017) in press

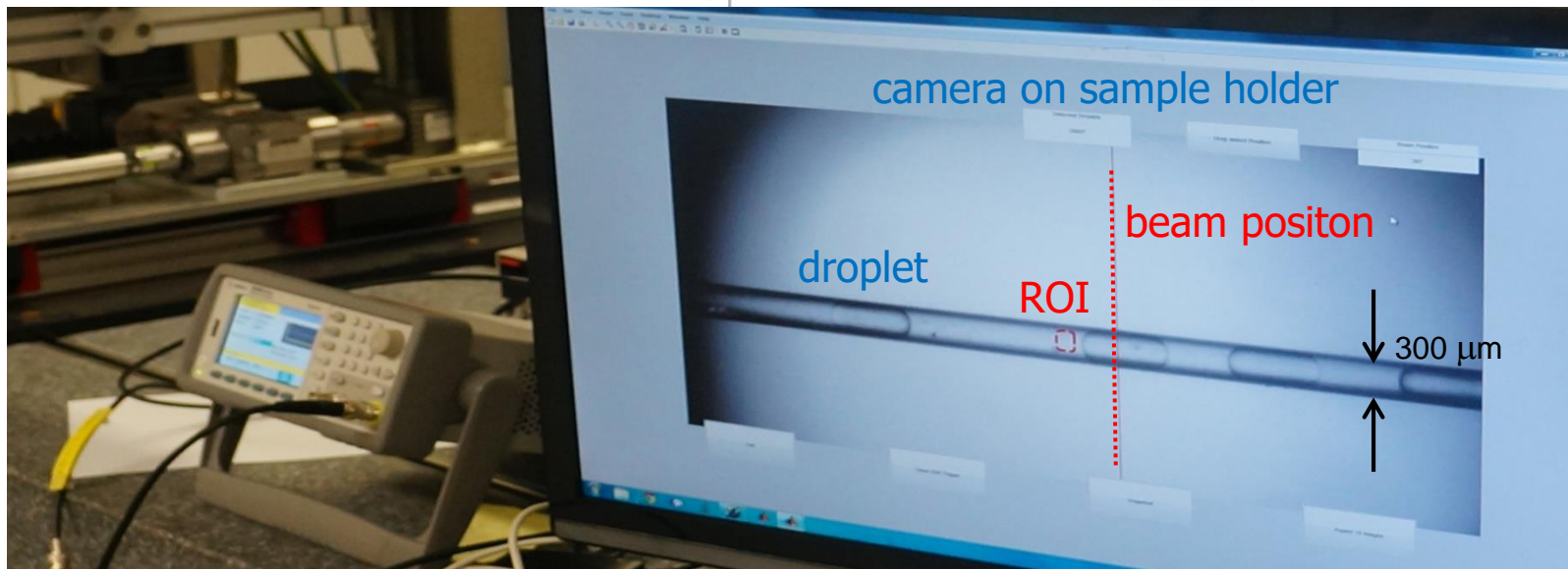


# COUPLING DIGITAL MICROFLUIDICS AND SAXS AT BM29

External triggering when coupling digital microfluidics and SAXS:



X-ray data acquired only on droplets

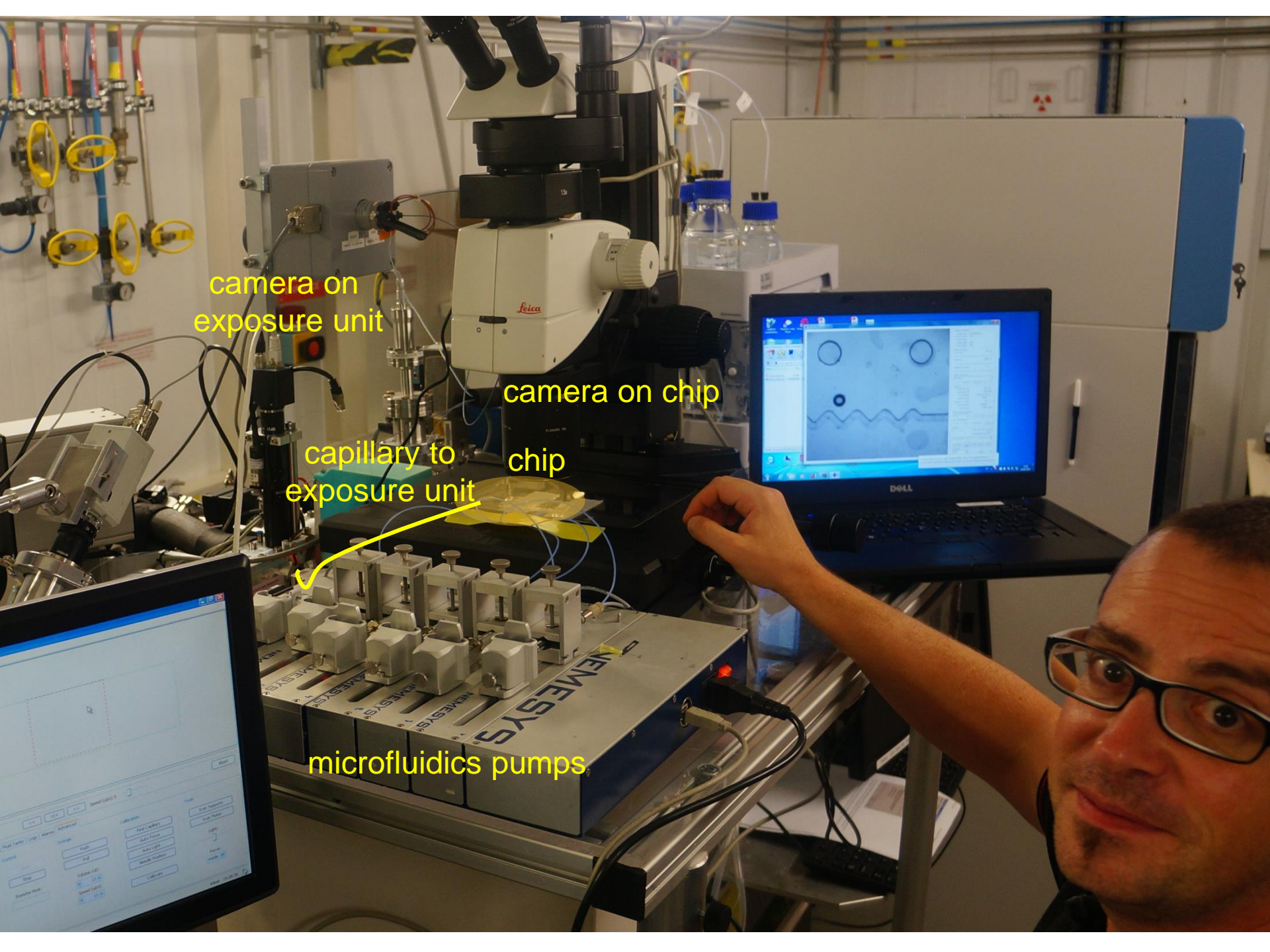


camera on exposure unit

camera on chip

capillary to exposure unit  
chip

microfluidics pumps



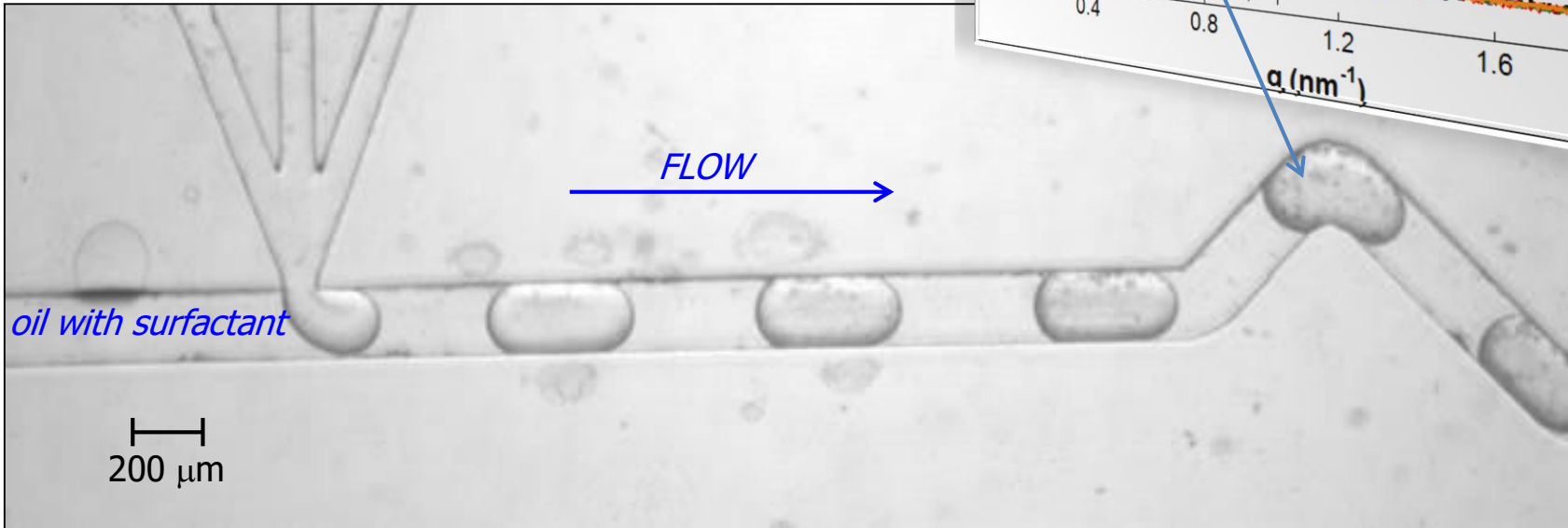
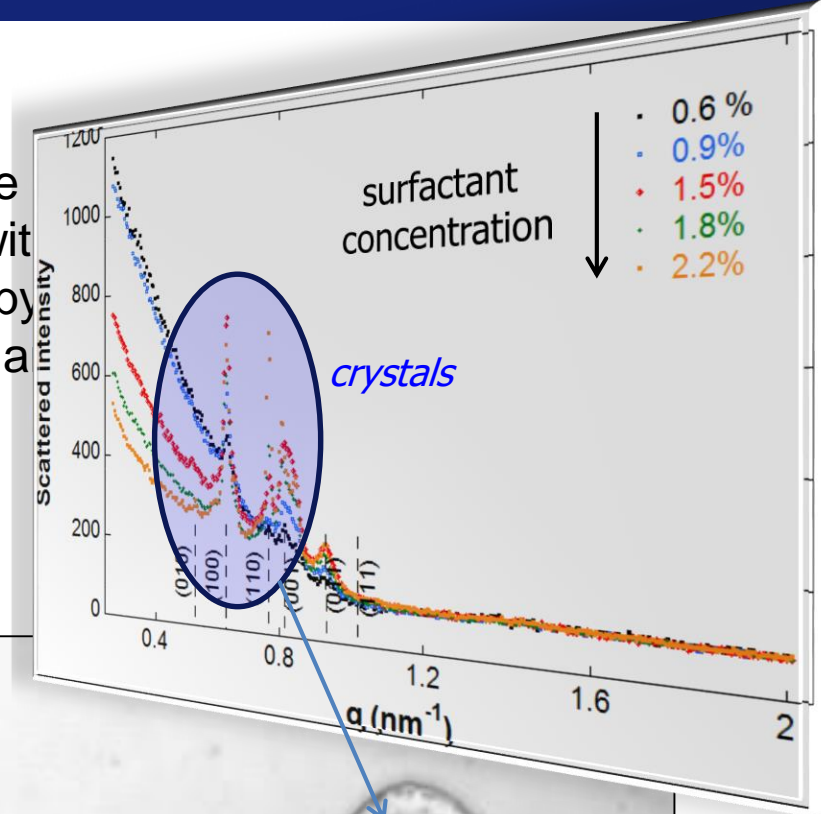
# CRYSTALLISATION IN SITU

Each droplet acts as a microreactor in which the pH, ligands, and additives) can be fine-tuned, with As the conditions can be adjusted dynamically by range of conditions can be investigated quickly a

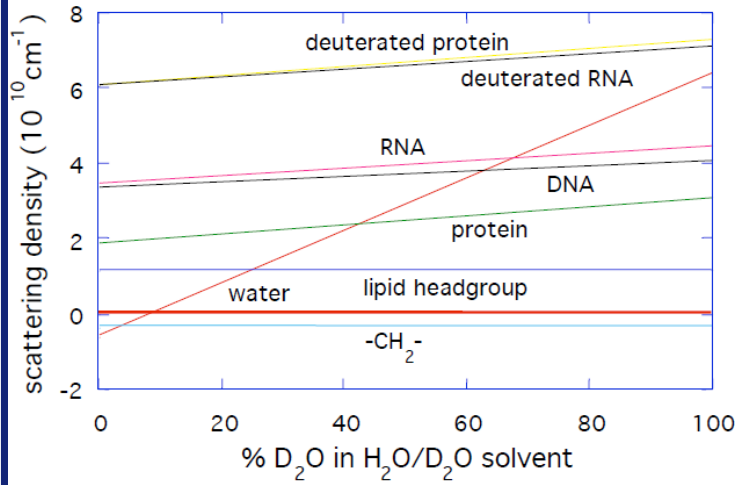
## Glucose isomerase + surfactant

*crystallisation*

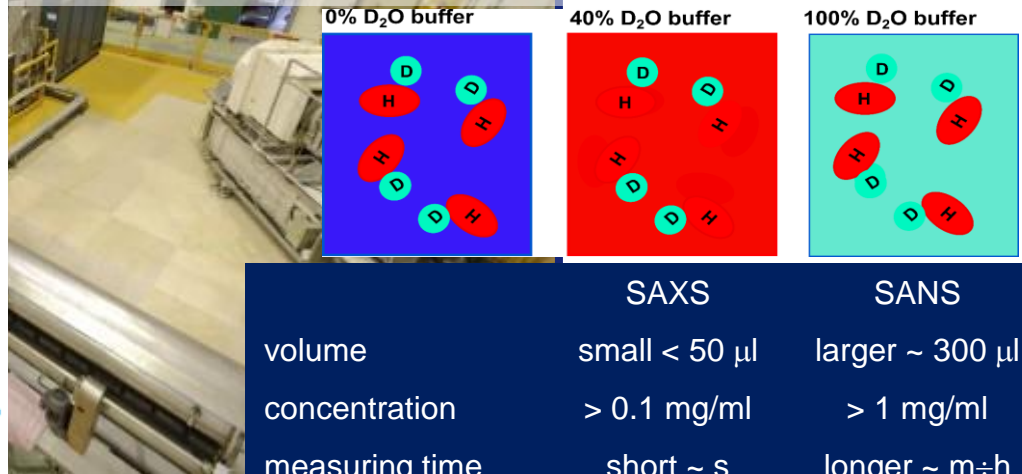
*agent buffer protein*



# JOINT SANSAXS EXPERIMENT



**SANS contrast variation** experiments obtained by exchanging the solvent for deuterated or partially deuterated solvent enhances the signal from one component of a complex.



**D22 at ILL**



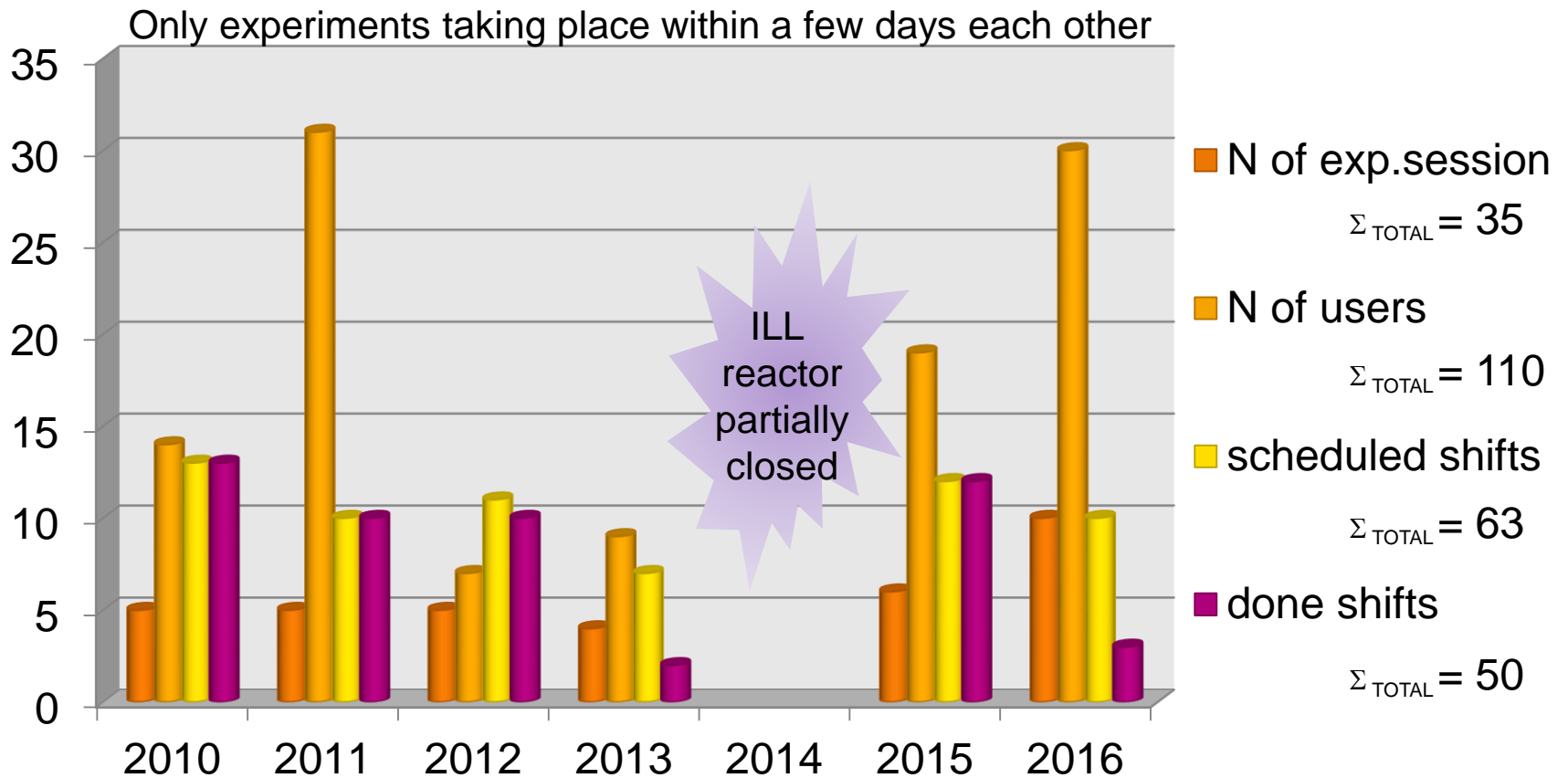
*sample changer*

	SAXS	SANS
volume	small < 50 $\mu$ l	larger ~ 300 $\mu$ l
concentration	> 0.1 mg/ml	> 1 mg/ml
measuring time	short ~ s	longer ~ m÷h
radiation damage	yes	no
contrast variation	no	yes
sensitive to salts, denaturants	yes	no

**Joint access** – ESRF and ILL SAS experiments during one trip to Grenoble.

**SANSAXS BAG** from June 2016: when SAN/XS proposal accepted (and scheduled) by ILL → beamtime (typically 1 shift) on BM29 within a few days of ILL experiment.

# JOINT SANSAXS STATS



## Sample environment for this experiment session

in A-form D<sub>2</sub>O

— Samples will contain —

Deuterium If checked, specify the volume per ml

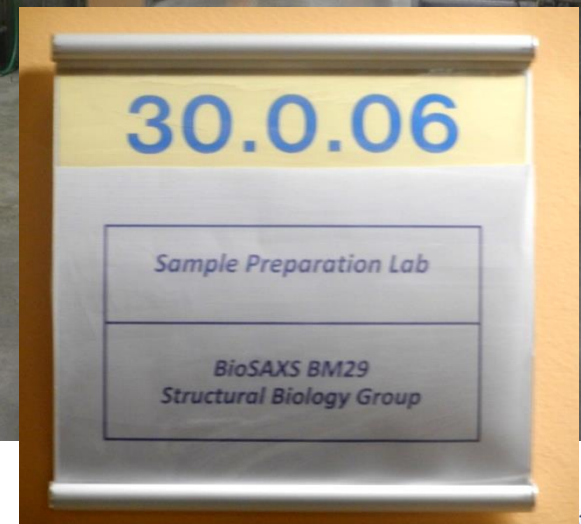
2-3

# BM29 NEWS: NEW SAMPLE PREPARATION LAB

new lab in front of ID30 MASSIF



old lab shared  
in and with ID29



# BM29 NEWS: NEW SAMPLE PREPARATION LAB



September 2016

# BM29 NEWS: NEW SAMPLE PREPARATION LAB







<https://exi.esrf.fr/saxs>

<http://ispyb.esrf.fr>

- new GUI, which separates database and user interface;
- will replace ISPyB, new features added only to EXI;
- in development, but in use already, accessible from outside;
- much quicker access, personalized accounts, searching by key word, LC, date

The screenshot shows the ExiSAXS web interface. The top navigation bar includes 'Home', 'Shipment', 'Prepare Experiment', 'Data Explorer', and 'Help'. A search bar for 'macromolecule' is present. The main content area displays 'Sessions for proposal opd29' with a table of data collections. The left sidebar shows experiment details for various sample changers and HPLC runs.

Start	Beamline	Proposal	Shifts	Local Contact	Pi	Data Collections	Comments
November 9th 2016	BM29	OPD29	3		operator on BM29, null	1 Calibration	session created by ISPyBB
October 31st 2016	BM29	OPD29	3		operator on BM29, null	1 Calibration	session created by ISPyBB
October 24th 2016	BM29	OPD29	3		operator on BM29, null	16 Calibration	session created by ISPyBB
October 20th 2016	BM29	OPD29	3		operator on BM29, null	4 Calibration 1 HPLC	session created by ISPyBB
October 18th 2016	BM29	OPD29	3		operator on BM29, null	7 Calibration	session created by ISPyBB
October 3rd 2016	BM29	OPD29	3		operator on BM29, null	2 Calibration	session created by ISPyBB
September 30th 2016	BM29	OPD29	3		operator on BM29, null	5 Calibration	session created by ISPyBB
September 22nd 2016	BM29	OPD29	3		operator on BM29, null	1 Calibration 3 Sample Changer	session created by ISPyBB
September 21st 2016	BM29	OPD29	3		operator on BM29, null	2 Calibration	session created by ISPyBB
September 19th 2016	BM29	OPD29	3		operator on BM29, null	13 Sample Changer 2 HPLC	session created by ISPyBB
September 18th 2016	BM29	OPD29	3		operator on BM29, null	9 Calibration 2 Sample Changer 11 HPLC	session created by ISPyBB
September 16th 2016	BM29	OPD29	3		operator on BM29, null	1 Calibration 13 HPLC	session created by ISPyBB
September 14th 2016	BM29	OPD29	3		operator on BM29, null	34 HPLC	session created by ISPyBB
September 13th 2016	BM29	OPD29	3		operator on BM29, null	2 Calibration 6 HPLC	session created by ISPyBB

Aknowledgment: Alejandro de Maria Antolinos

## Experiment

### SAMPLE CHANGER

Sep 19, 2016 8:46:32 PM

ND\_sucrose.xml

Samples	30 of 30
Averages	30 of 30
Subtractions	10 of 10

### HPLC

Sep 19, 2016 8:04:53 PM

name

Subtractions	0 of 0
--------------	--------

### HPLC

Sep 19, 2016 6:40:55 PM

name

Subtractions	0 of 0
--------------	--------

### SAMPLE CHANGER

Sep 19, 2016 4:55:00 PM

ND\_APOF\_LAMBDA\_2Ang.xml

Samples	12 of 12
Averages	12 of 12
Subtractions	4 of 4

### CALIBRATION

Sep 19, 2016 3:42:34 PM

Water.xml

Samples	3 of 3
Averages	3 of 3
Subtractions	1 of 1

### CALIBRATION

Sep 19, 2016 3:12:22 PM



Choose a Date ▾

enter search term (proposal or title)



## Sessions for proposal opd29



**BM29**

Start	Beamline
November 9th 2016	BM29
October 31st 2016	BM29
October 24th 2016	BM29
October 20th 2016	BM29
October 18th 2016	BM29
October 3rd 2016	BM29
September 30th 2016	BM29
September 22nd 2016	BM29
September 21st 2016	BM29
September 19th 2016	BM29
September 18th 2016	BM29

# EXTENDED ISPYB = EXI

Experiment  
 Nov 24, 2016 6:51:15 AM  
 Contrast\_var\_comp\_and\_bR\_try2.xml  
 Samples 57 of 57  
 Averages 57 of 57  
 Subtractions 19 of 19

**SAMPLE CHANGER**  
 Nov 24, 2016 6:40:33 AM  
 Contrast\_var\_comp\_and\_bR\_try2.xml  
 Samples 0 of 57  
 Averages 0 of 57  
 Subtractions 0 of 19

**SAMPLE CHANGER**  
 Nov 24, 2016 6:01:17 AM  
 Contrast\_var\_comp\_and\_bR.xml  
 Samples 16 of 72  
 Averages 17 of 72  
 Subtractions 5 of 24

**SAMPLE CHANGER**  
 Nov 24, 2016 4:38:24 AM  
 Raul\_samples.xml

Run	Sample	Frames (Average/Total)	Guinier			Gnom			Porod		Scattering	Kratky	Density	Guinier	Advanced
			Rg	Points	I0	Rg	Total	D <sub>max</sub>	Volume	MM Vol. est.					
#293	buf_9	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input type="checkbox"/> Abinitio <input type="checkbox"/> Fit <input type="checkbox"/> Superposition <input type="checkbox"/> Rigid Body	
#290	bigal9	1.000 mg/ml 19.97 C	2.363 nm	78 - 108 (30)	0.3 ± 1e-3	2.378 nm	0.543	8.272 nm	NA	NA					<input checked="" type="checkbox"/> Data Reduction <input type="checkbox"/> Abinitio <input type="checkbox"/> Fit <input type="checkbox"/> Superposition <input type="checkbox"/> Rigid Body
#289	buf_9	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input type="checkbox"/> Abinitio <input type="checkbox"/> Fit <input type="checkbox"/> Superposition <input type="checkbox"/> Rigid Body	
#288	big9	1.000 mg/ml 19.97 C	2.248 nm	36 - 115 (79)	0.3 ± 0e+0	2.264 nm	0.580	7.870 nm	NA	NA					<input checked="" type="checkbox"/> Data Reduction <input type="checkbox"/> Abinitio <input type="checkbox"/> Fit <input type="checkbox"/> Superposition <input type="checkbox"/> Rigid Body
#287	buf_9	10 / 10												<input checked="" type="checkbox"/> Data Reduction <input type="checkbox"/> Abinitio <input type="checkbox"/> Fit <input type="checkbox"/> Superposition <input type="checkbox"/> Rigid Body	

## Data Collection

Run	Sample	Frames (Average/Total)	Guinier			Gnom			Porod		Scattering	Kratky	Density	Guinier	Advanced
			Rg	Points	I0	Rg	Total	D <sub>max</sub>	Volume	MM Vol. est.					
#	HPLC_B	1497 / 1364												<input checked="" type="checkbox"/> Data Reduction <input type="checkbox"/> Abinitio <input type="checkbox"/> Fit <input type="checkbox"/> Superposition <input type="checkbox"/> Rigid Body	
#	HPLC_M 0.000 mg/ml C	1497 / 1364	3.530 nm	37 - 63 (26)	0.0 ± 0e+0	3.594 nm	0.718	12.356 nm	NA	NA					<input checked="" type="checkbox"/> Data Reduction <input type="checkbox"/> Abinitio <input type="checkbox"/> Fit <input type="checkbox"/> Superposition <input type="checkbox"/> Rigid Body
#	HPLC_B	1497 / 1364												<input checked="" type="checkbox"/> Data Reduction <input type="checkbox"/> Abinitio <input type="checkbox"/> Fit <input type="checkbox"/> Superposition <input type="checkbox"/> Rigid Body	

## Size-exclusion chromatography

