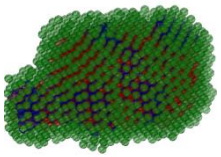
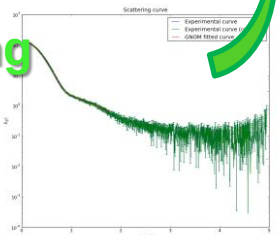

ESRF
The European Synchrotron

BM29 NEWS



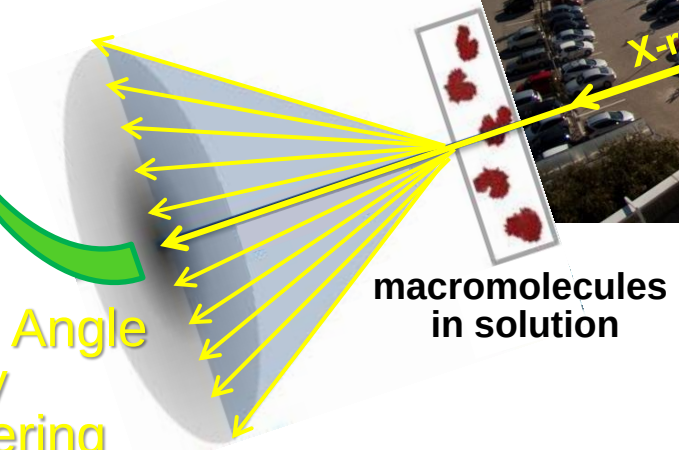
ab-initio model

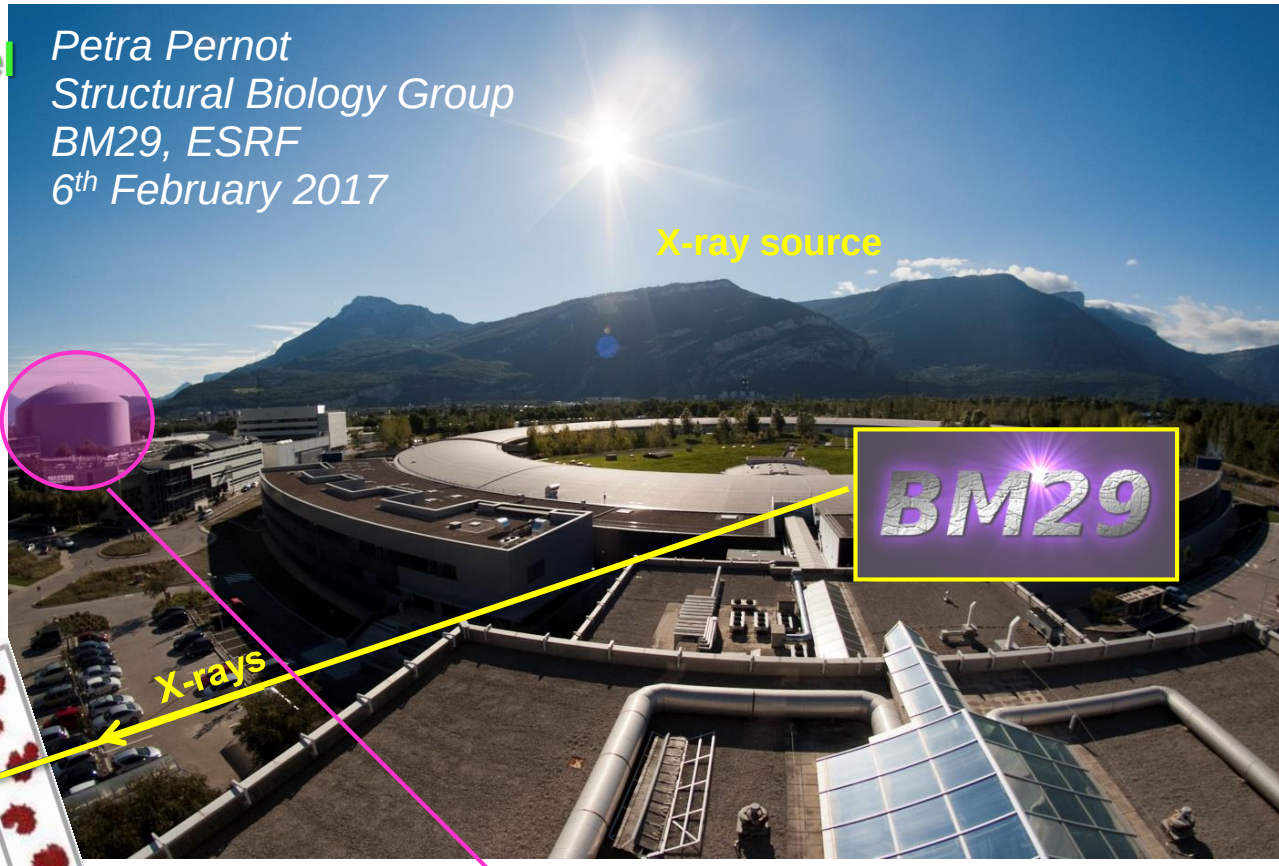
low resolution structure



1D scattering curve

model independent parameters
shape, MM, interaction,…





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 μL with c << 1 mg/mL;

• high throughput facility "easy" to use in

typical data collection times

a) **sample changer** mode 10 s (cycle loading-exposure-cleaning ~ 1.5 min)
b) **HPLC on-line** mode 30 min (column elution time);

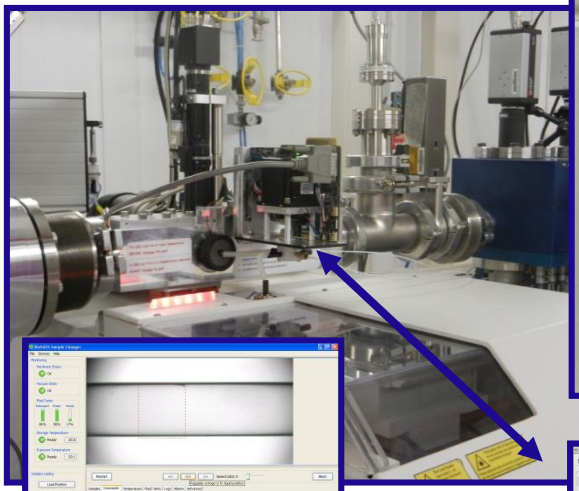
• providing automated data analysis and data tracking;

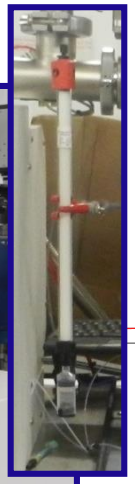
• access through Rolling procedure or BAG system: proposals any time and beam time in a few weeks (if successful and time available);

• SANSAXS BAG: one trip to Grenoble for ILL and ESRF experiments on same samples: SAS with neutrons and X-rays.

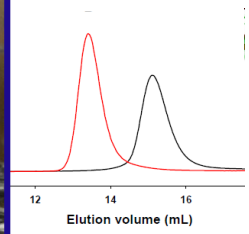
BM29 OUTLOOK – AUTOMATISATION

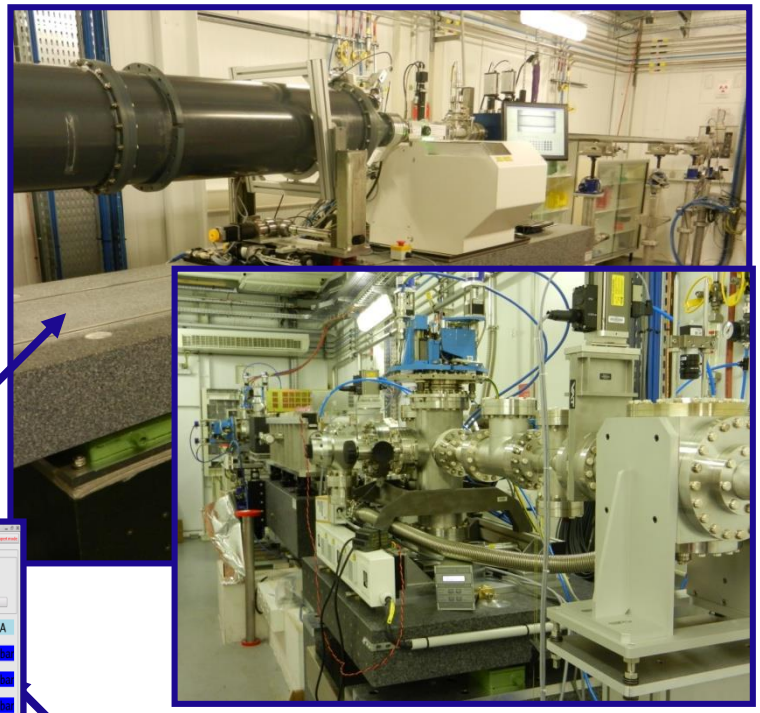
ROBOT/HPLC



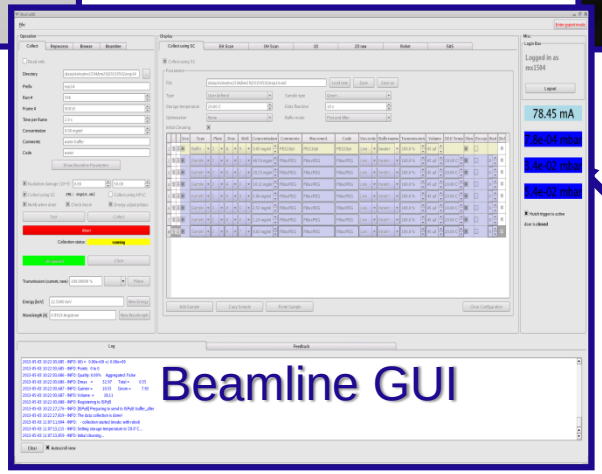


EXPERIMENTAL parameters



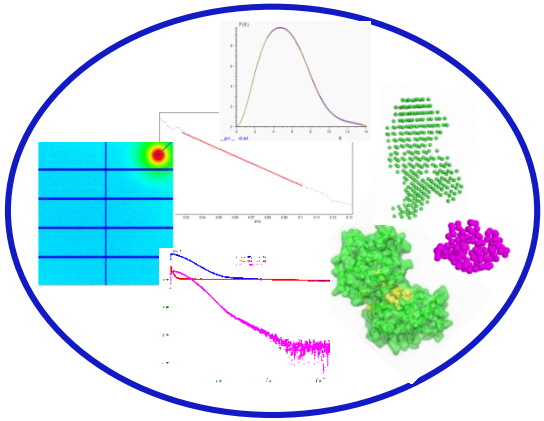


BsxCuBE

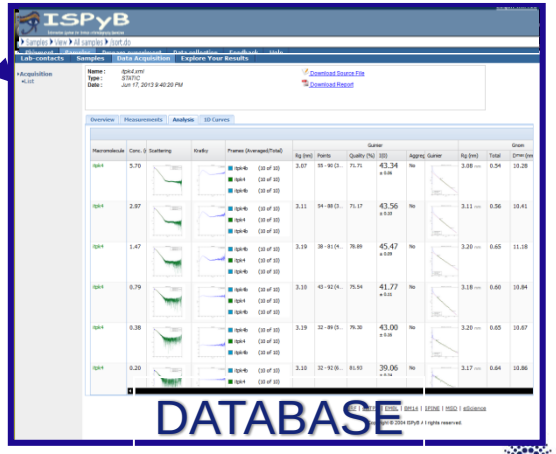


Beamline GUI

EDNA



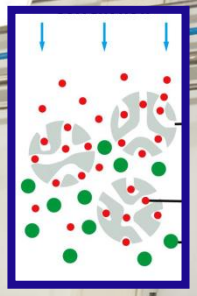
ISPyBB
sample tracking
and data flow



DATABASE

BM29 OUTLOOK = SAMPLE CHANGER + HPLC SYSTEM

Samples stored in 96 well plates, PCR tubes
Thermo-regulation storage: 4-40ºC, exposure cell: 4-60ºC
Sample loading and cleaning: 30’

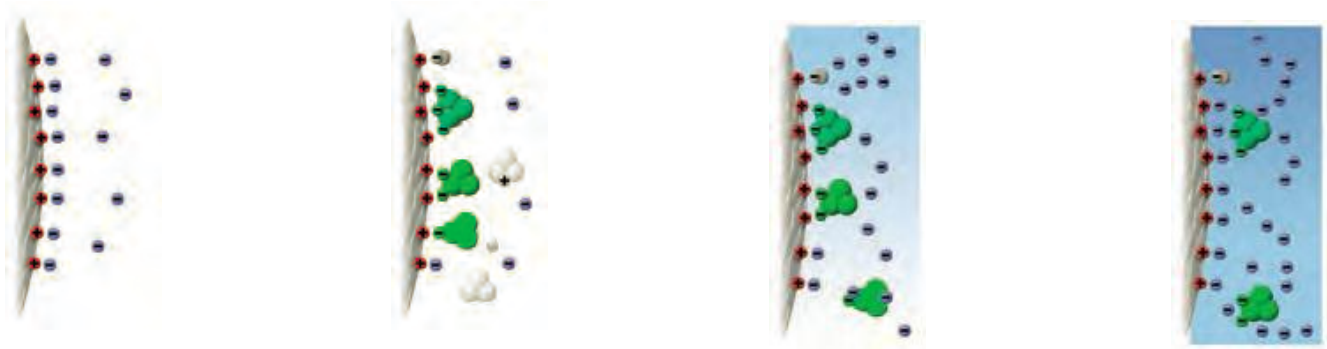


final purification step on-line

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:

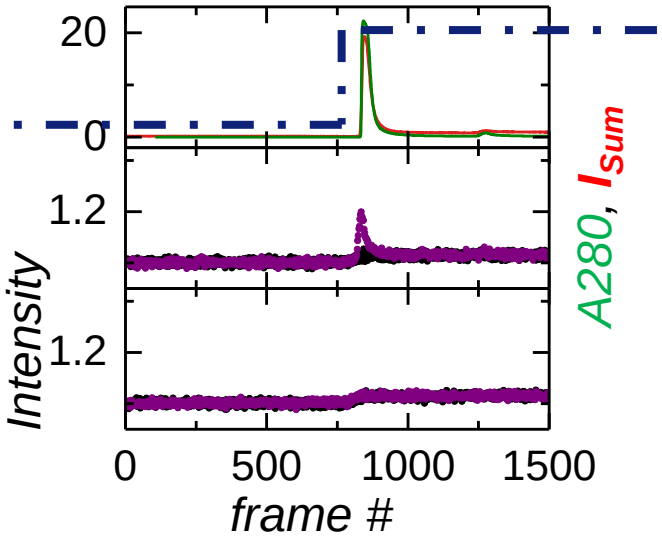
- buffer composition intrinsically unknown

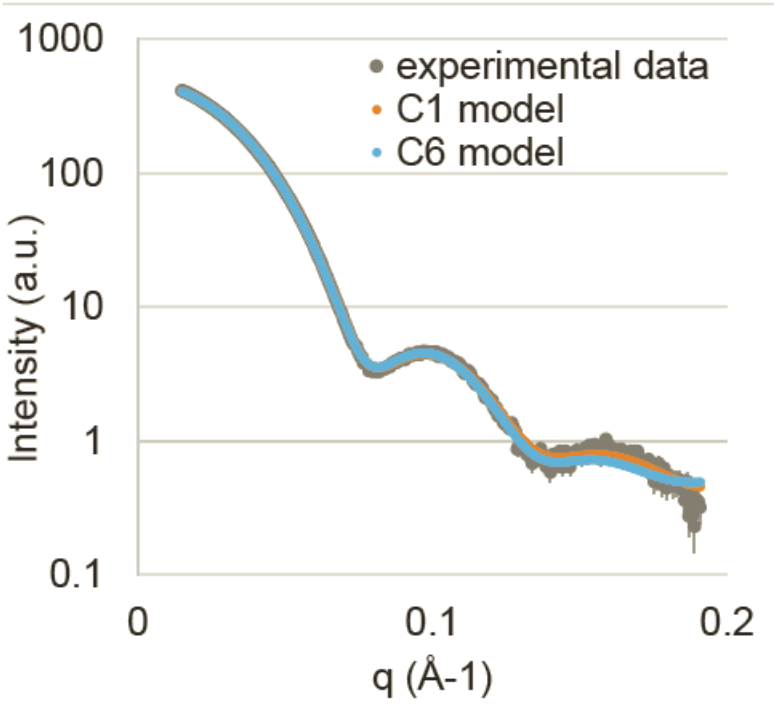
Collaboration:
Martha Breninich (EMBL, Grenoble)
Stephanie Hutin (IBS, Grenoble)

Figures:
GE Healthcare

IEC-SAXS online: Small-POX HELICASE-PRIMASE d5

salt concentration (step gradient)

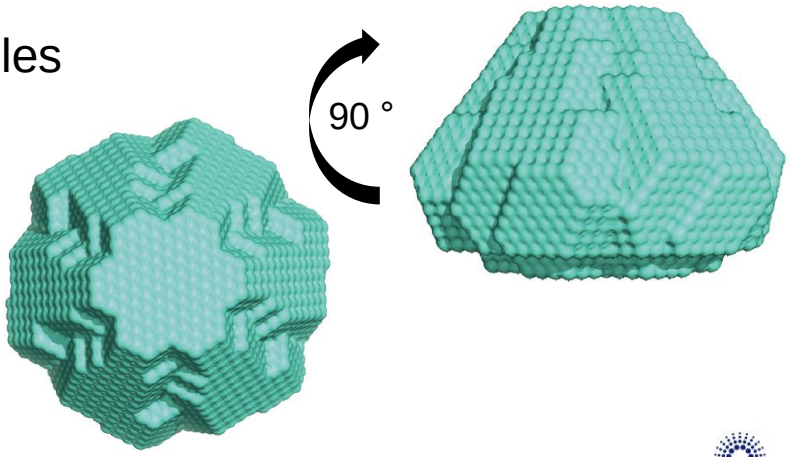




➡ $D5_{323-785}$ is hexameric in solution

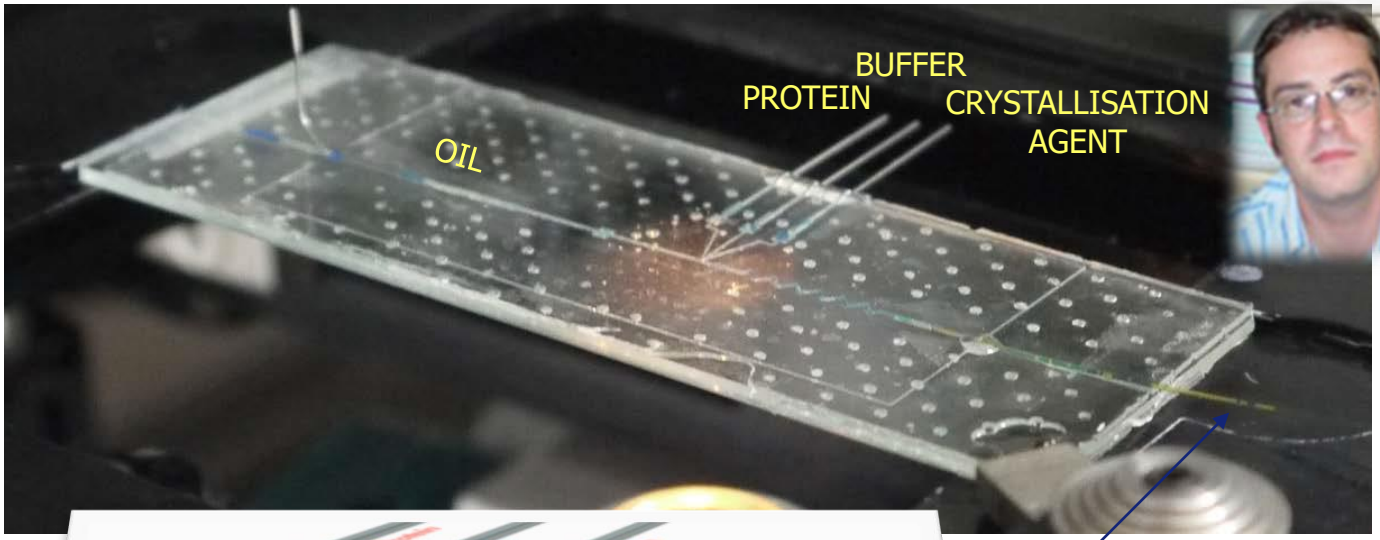
- IEC can separate similarly sized macromolecules
- using careful background correction,

high quality SAXS data can be obtained

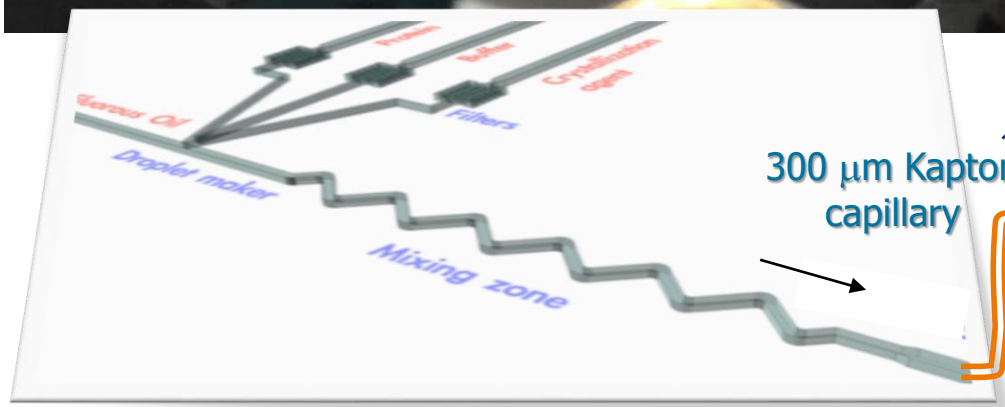


"Online ion-exchange chromatography for small-angle X-ray scattering"
S. Hutin, M. Brennich, B. Maillot and A. Round
Acta Cryst. (2016) D72, 1090–1099.

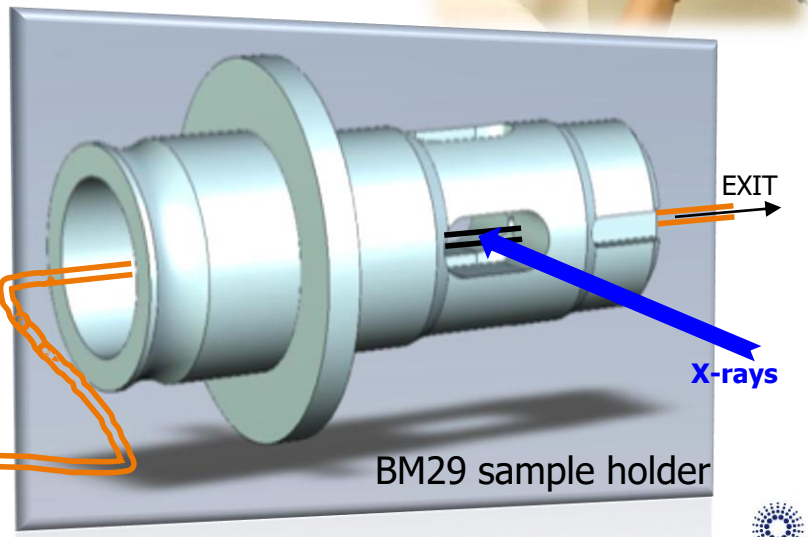
MICROFLUIDICS INTEGRATION AT BM29



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



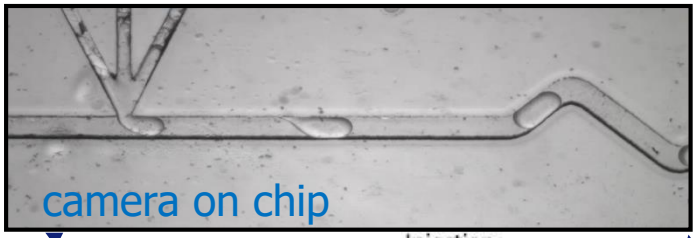
300 μm Kapton capillary

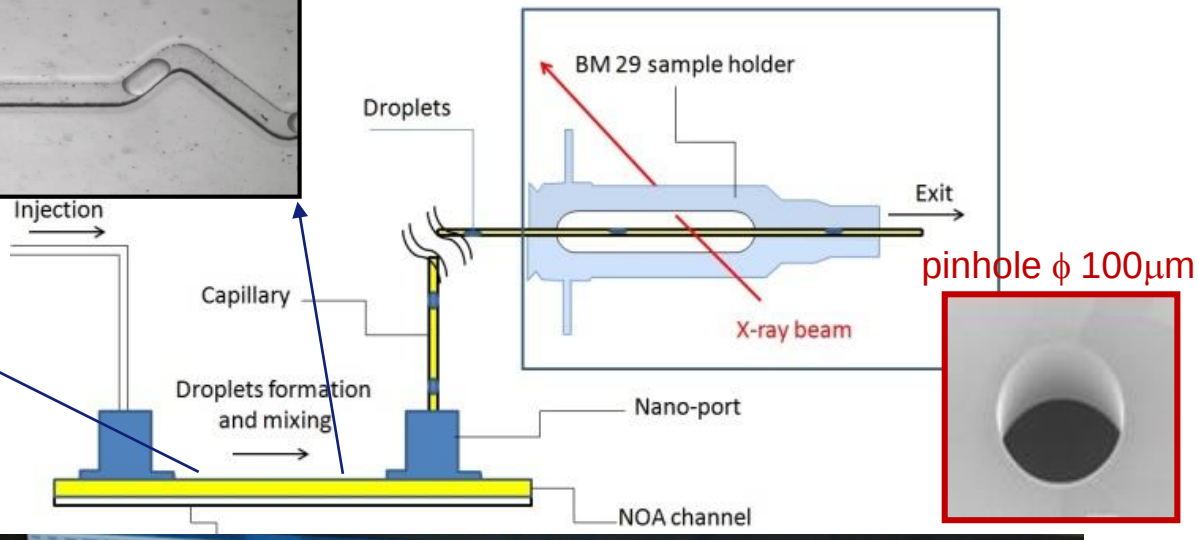


"Coupling digital microfluidics and Small-Angle X-ray scattering to study the whole crystallization process of proteins in solution"
N.V. Pham, D. Radajewski, P. Guillet, A. Round, M. Brennich, P. Pernot, B. Biscans, F. Bonneté and S. Teychené, *Analytical Chemistry* (2017) in press

COUPLING DIGITAL MICROFLUIDICS AND SAXS AT BM29

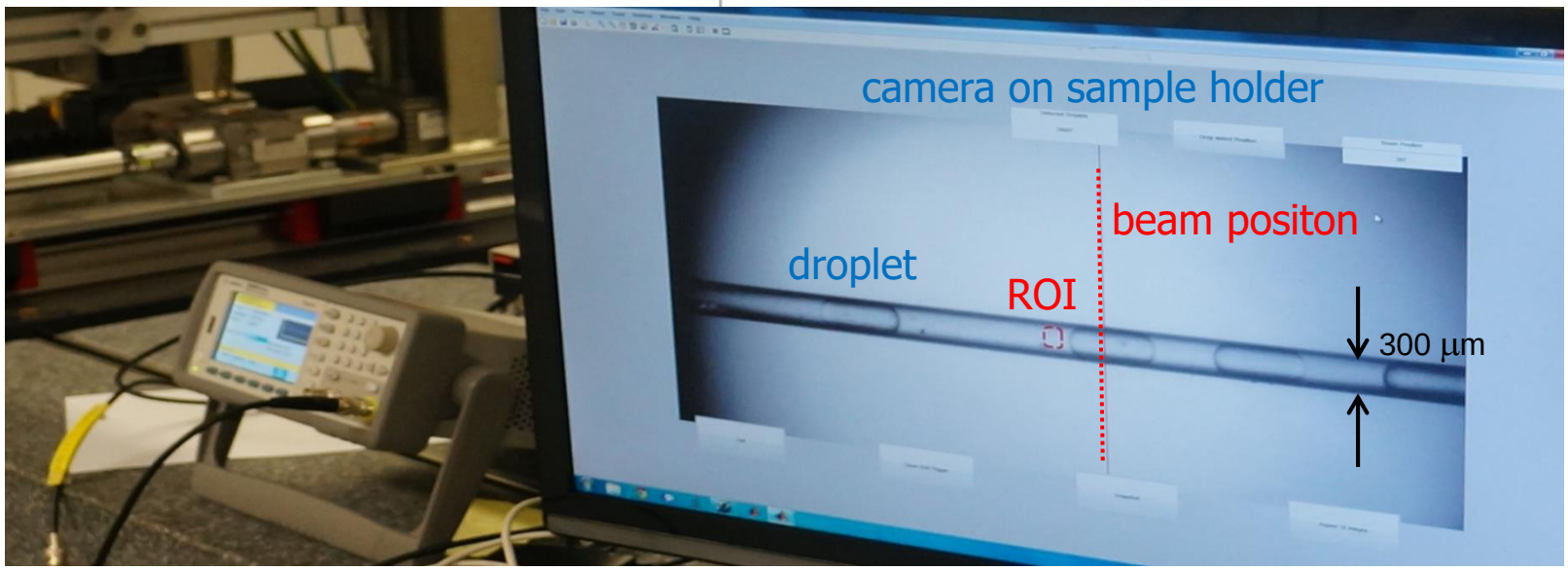
External triggering when coupling digital microfluidics and SAXS:

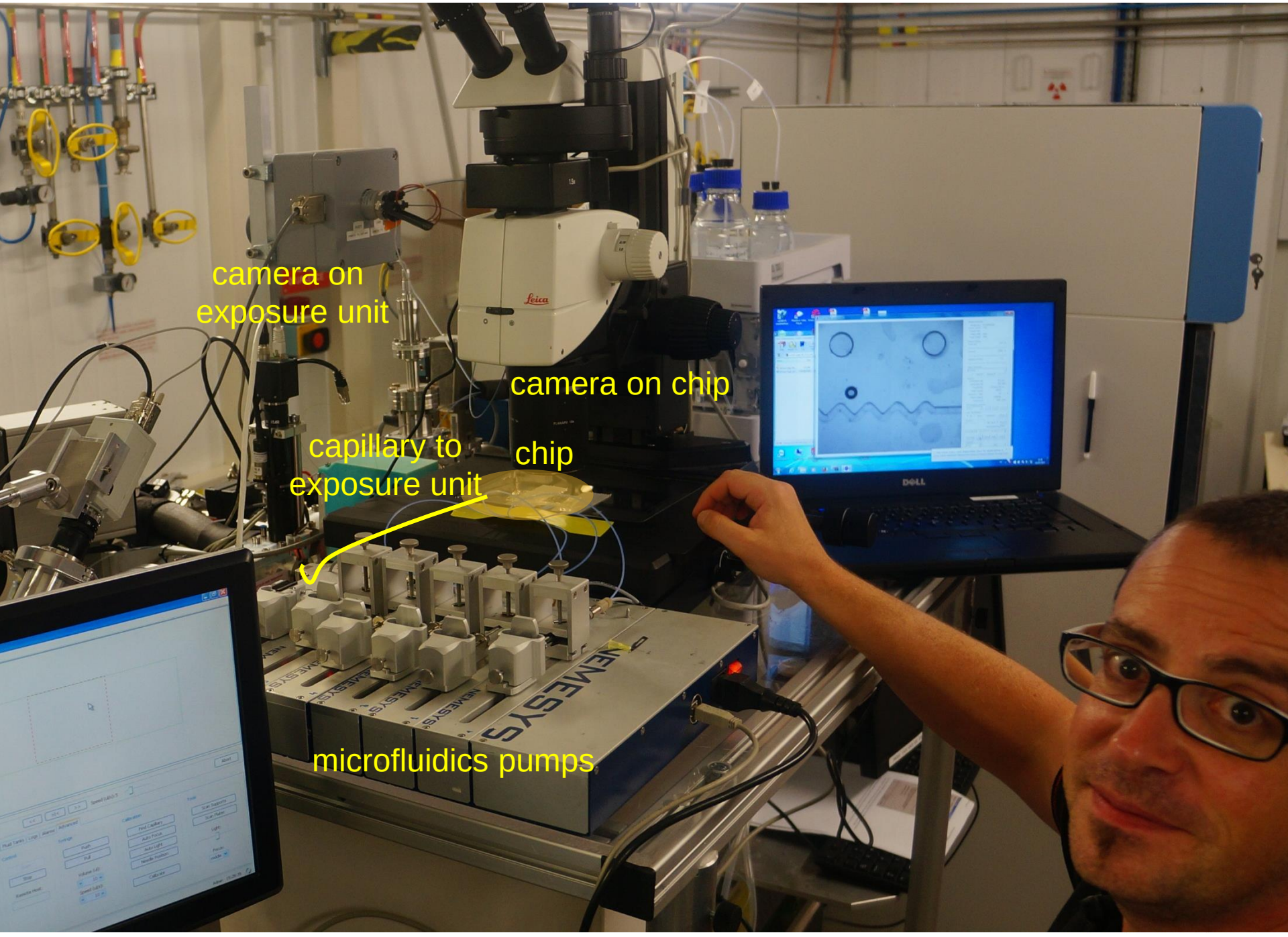




pinhole ϕ 100μm

X-ray data acquired only on droplets

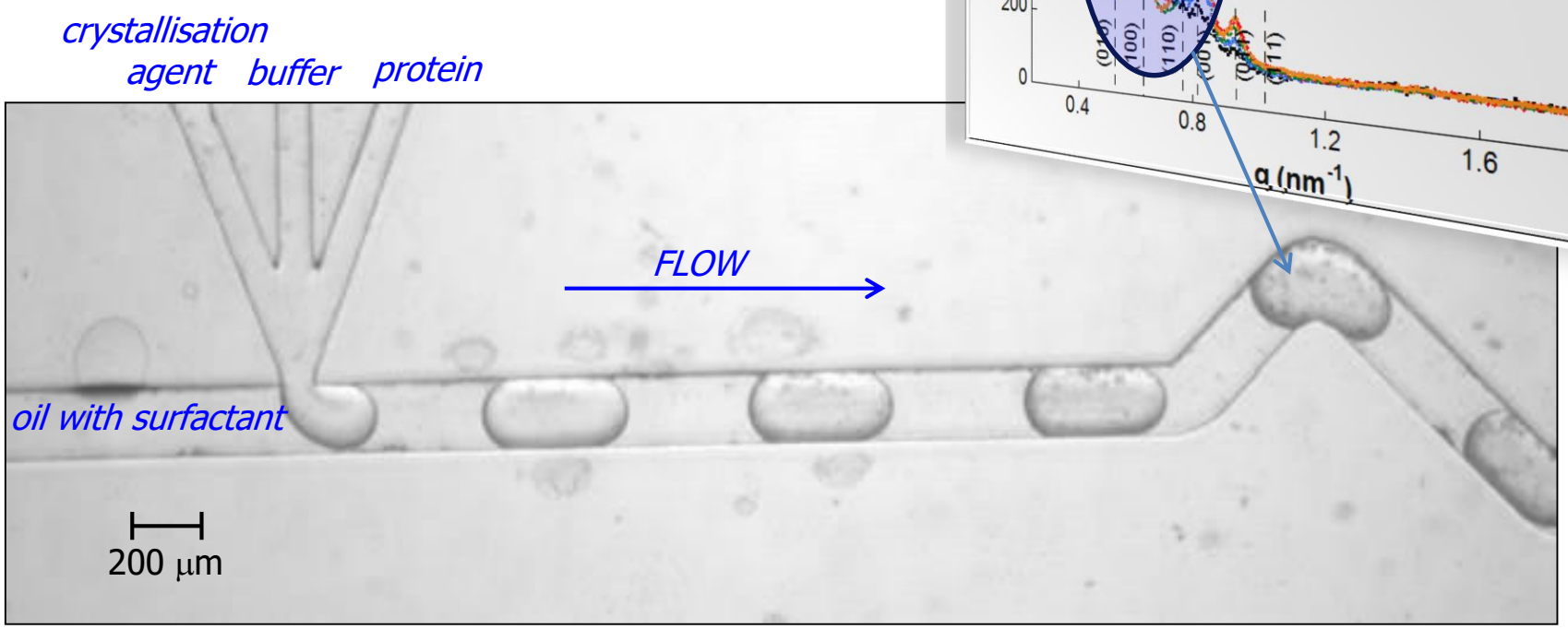



camera on
exposure unit
camera on chip
capillary to
exposure unit
chip
microfluidics pumps

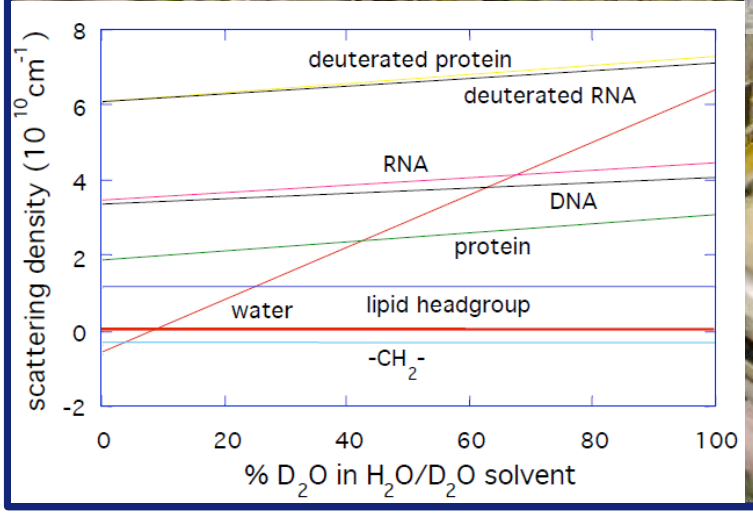
CRYSTALLISATION IN SITU

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

Glucose isomerase + surfactant

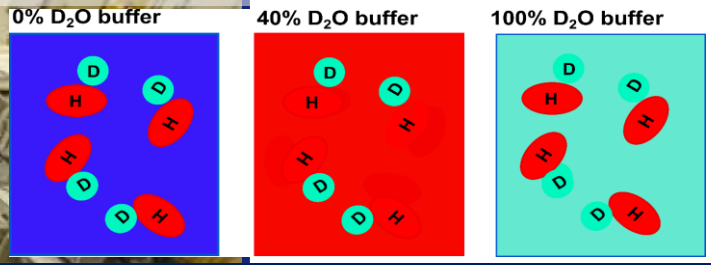


JOINT SANSAXS EXPERIMENT

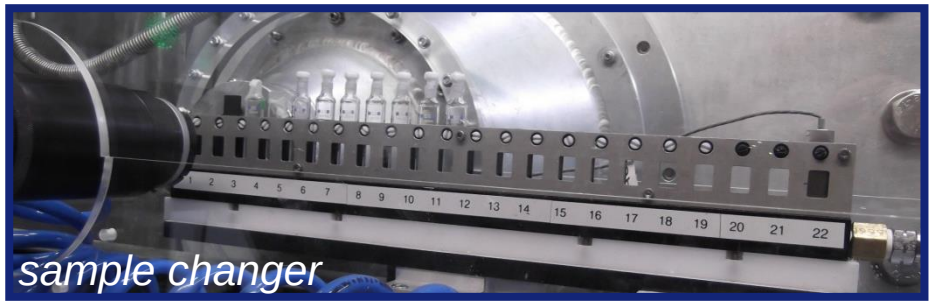


D22 at ILL

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



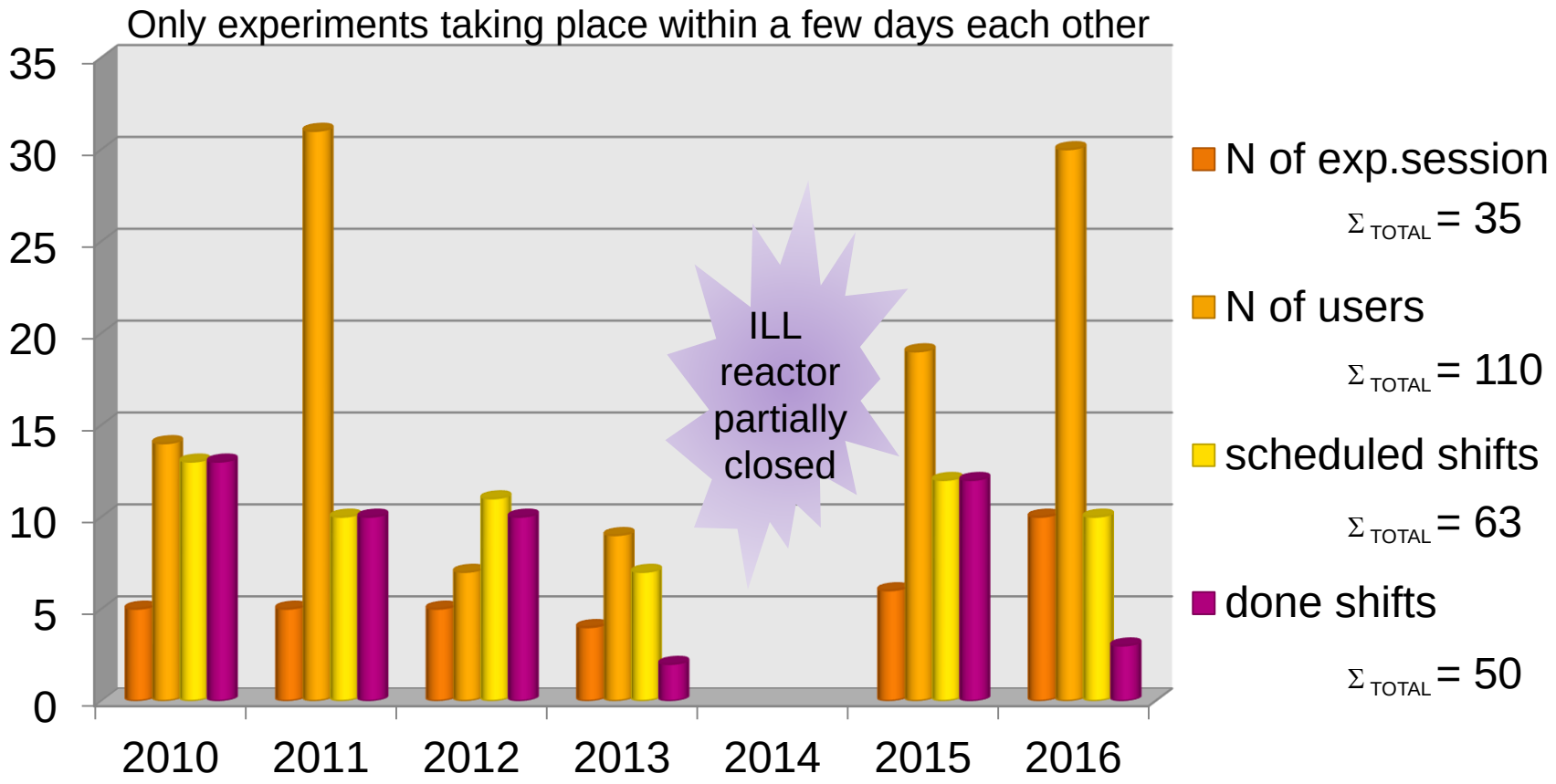
	SAXS	SANS
volume	small < 50 μl	larger ~ 300 μl
concentration	> 0.1 mg/ml	> 1 mg/ml
measuring time	short ~ s	longer ~ m÷h
radiation damage	yes	no
contrast variation	no	yes
sensitive to salts, denaturants	yes	no



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



in A-form D_2O

BM29 NEWS: NEW SAMPLE PREPARATION LAB

new lab in front of ID30 MASSIF

BM29 NEWS: NEW SAMPLE PREPARATION LAB

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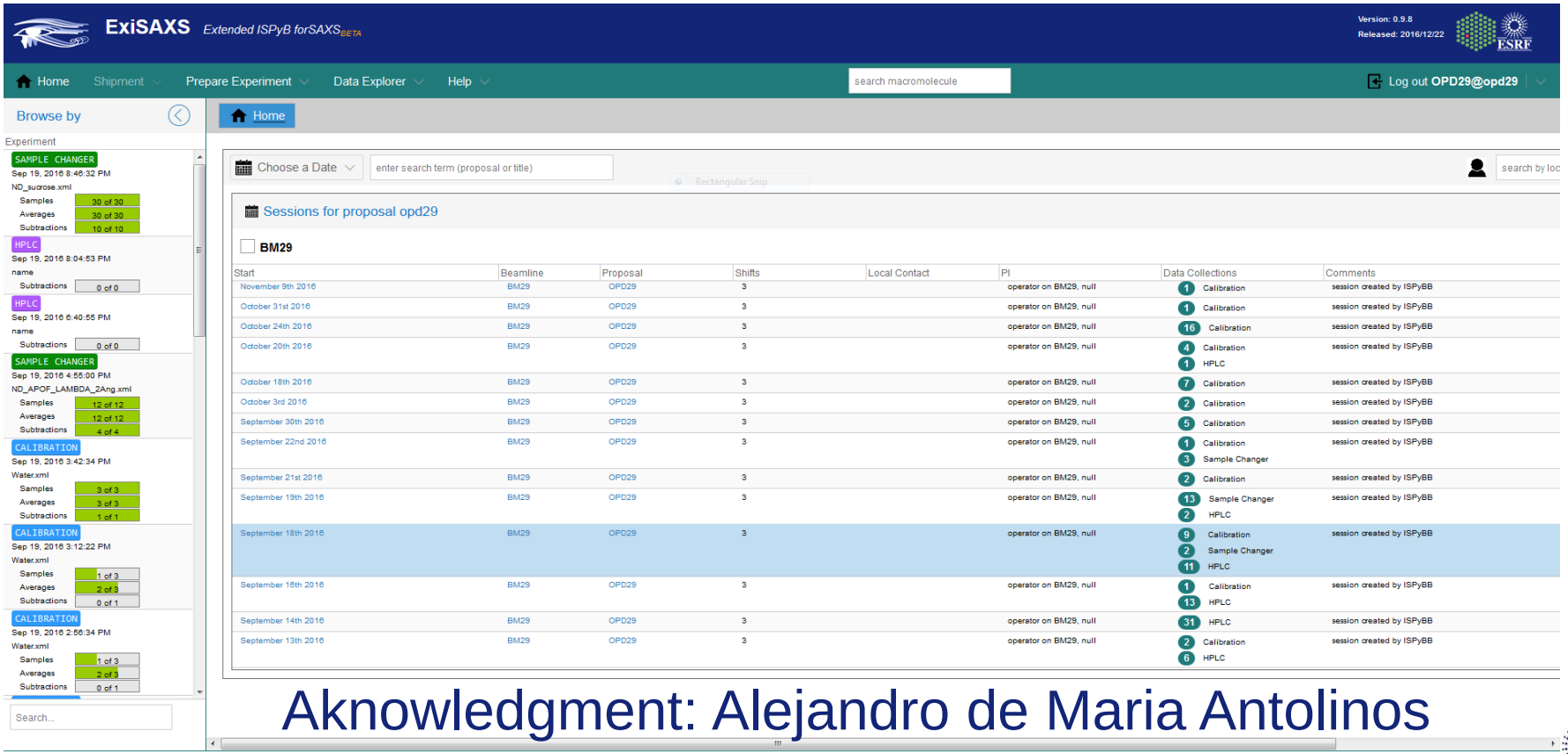
ExiSAXS *Extended ISPyB forSAXS*$_{BETA}$

ISPyB
Information System for Protein crystallographY Beamlines

https://exi.esrf.fr/saxs

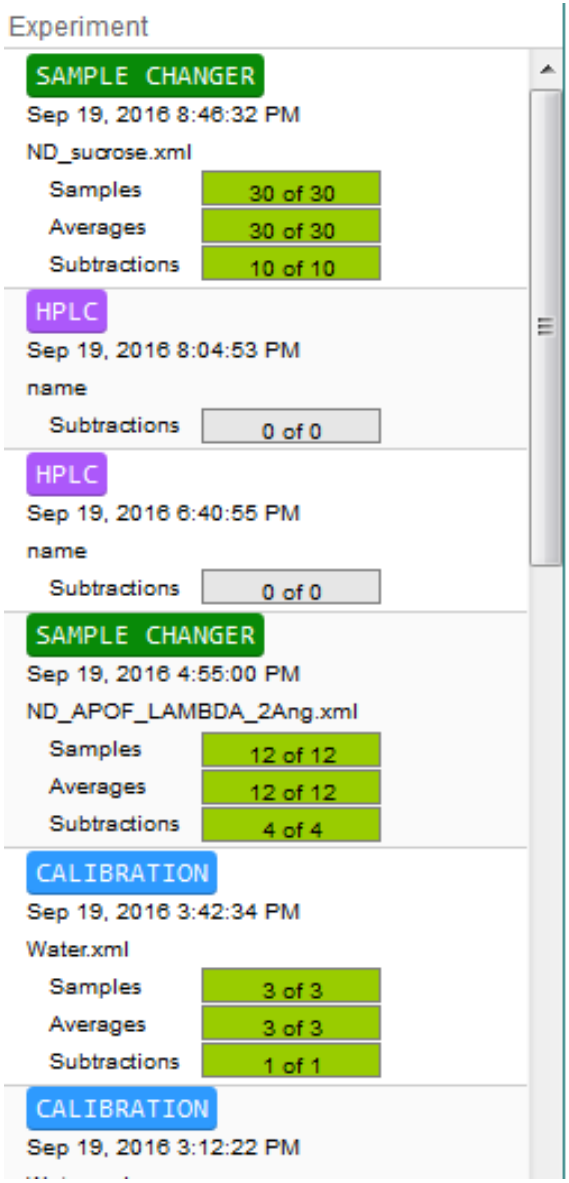
http://ispyb.esrf.fr

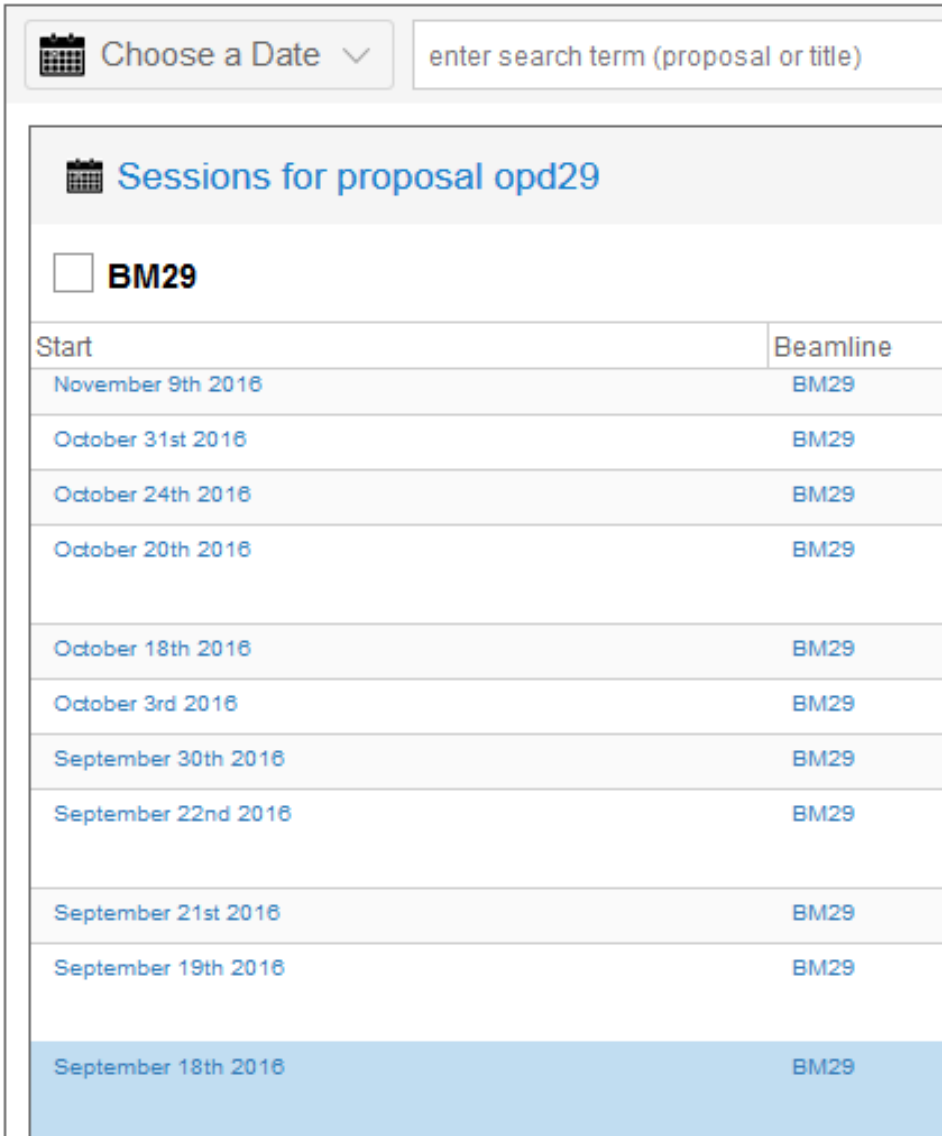
- new GUI, which separates database and user interface;
- will replace ISPyB, new features added only to EXI;
- in development, but in use already, accessible from outside;
- much quicker access, personalized accounts, searching by key word, LC, date



Aknowledgment: Alejandro de Maria Antolinos

EXTENDED ISPYB = EXI





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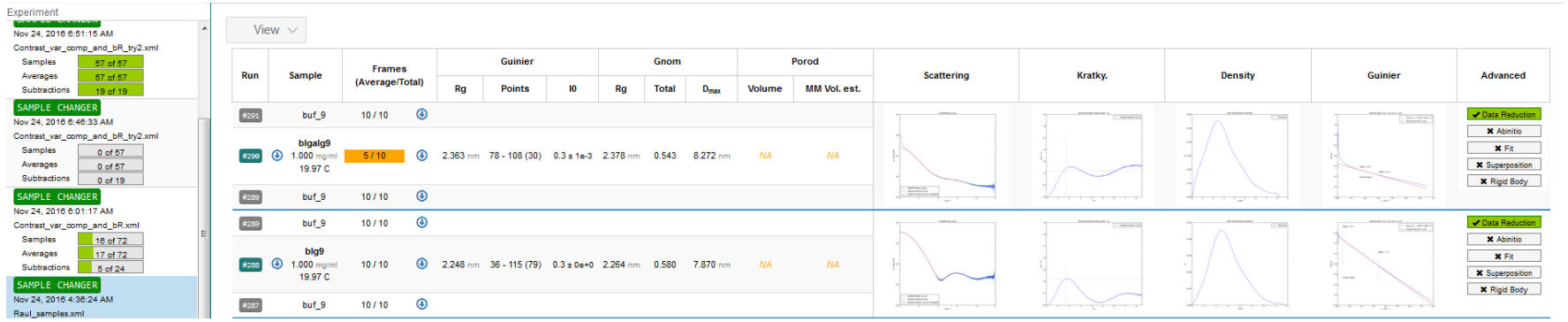

Data Collection

Run	Sample	Frames (Average/Total)	Guinier Rg	Guinier Points	Guinier I0	Gnom Rg	Gnom Total	Gnom D_{max}	Porod Volume	Porod MM Vol. est.	Scattering	Kratky.	Density	Guinier	Advanced
#	HPLC_B	1497 / 1364													Data Reduction, Abinitio, Fit, Superposition, 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					
#	HPLC_B	1497 / 1364													

Size-exclusion chromatography