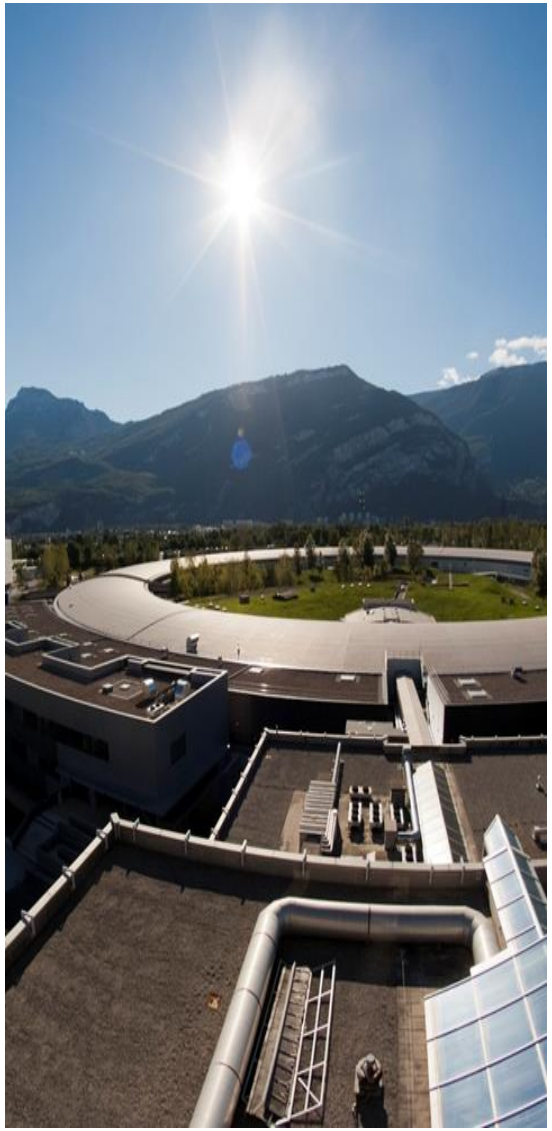


Instrumentation and data collection procedures for BioSAXS

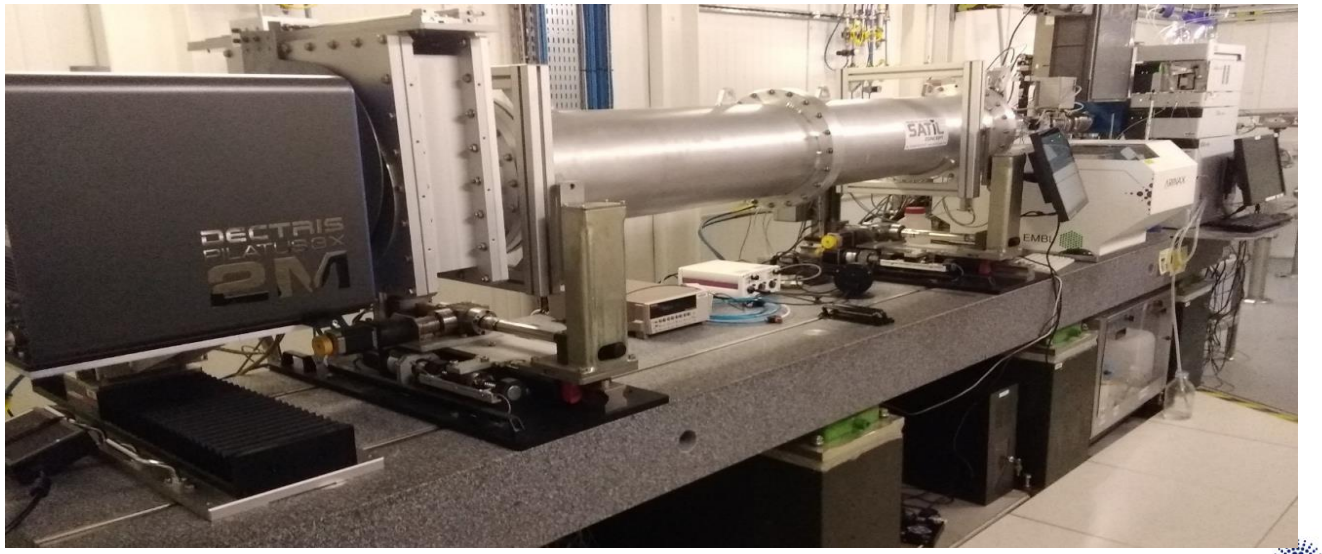
By Mark Tully

Structural Biology Group
BM29, ESRF

SAXS INSTRUMENTS

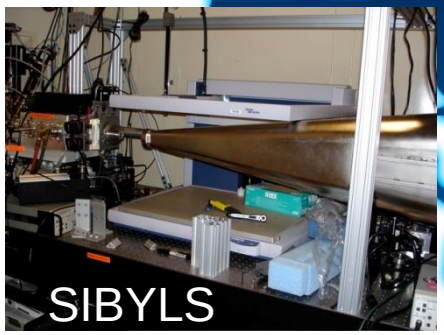
- Offline or home source

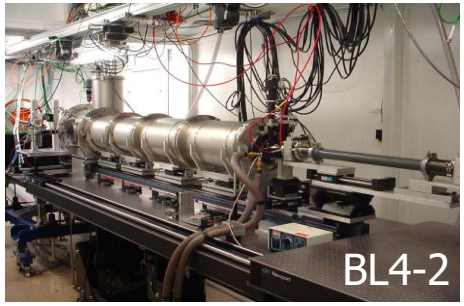
- Synchrotron beamline



All past, present and future synchrotrons with a SAXS beamline

- broad scientific applications soft matter, nano-materials, fibers,…





Campinas LNLS
SAXS-2

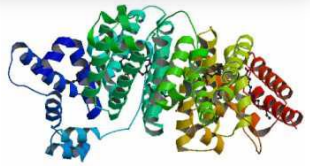
Australian synchrotron
SAXS/WAXS

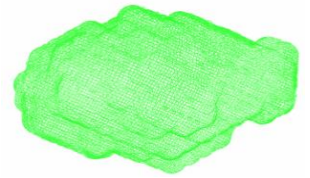
Only few dedicated (nearly) exclusively to macromolecules in solution = BioSAXS

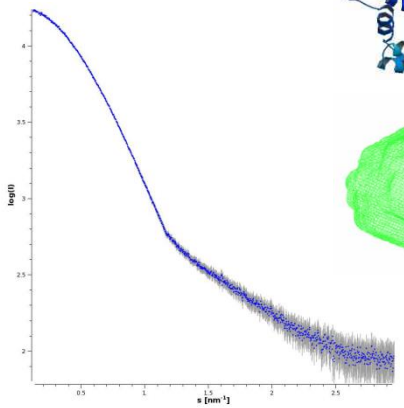
Solution SAXS experiment – simple view

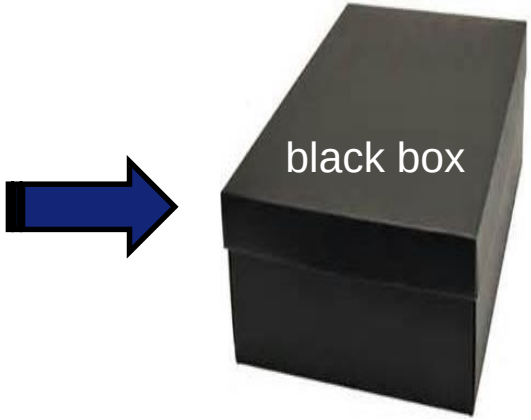
synchrotron beamline
or home source set-up

solution scattering data
from protein of interest

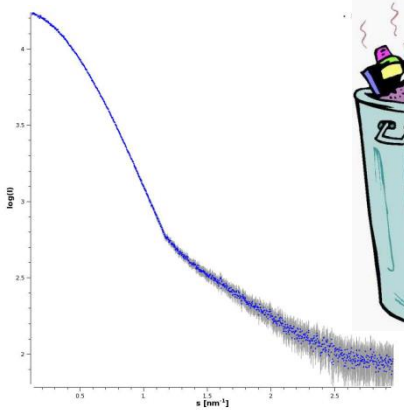




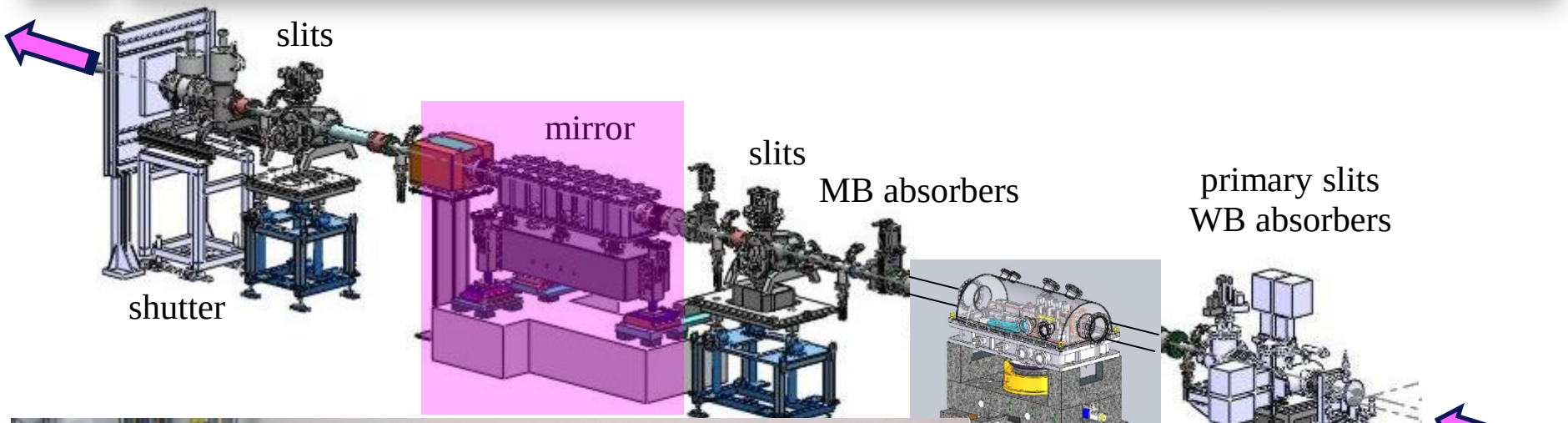


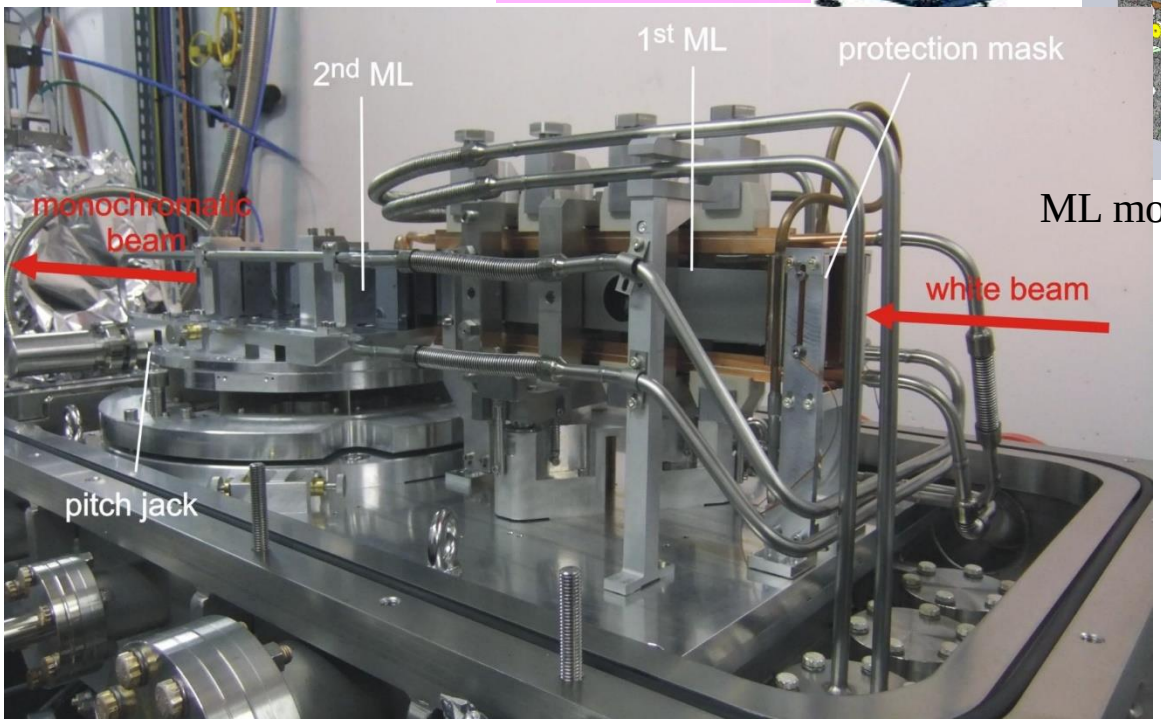


potentially interestingly looking
solution scattering data from garbage



OPTICS



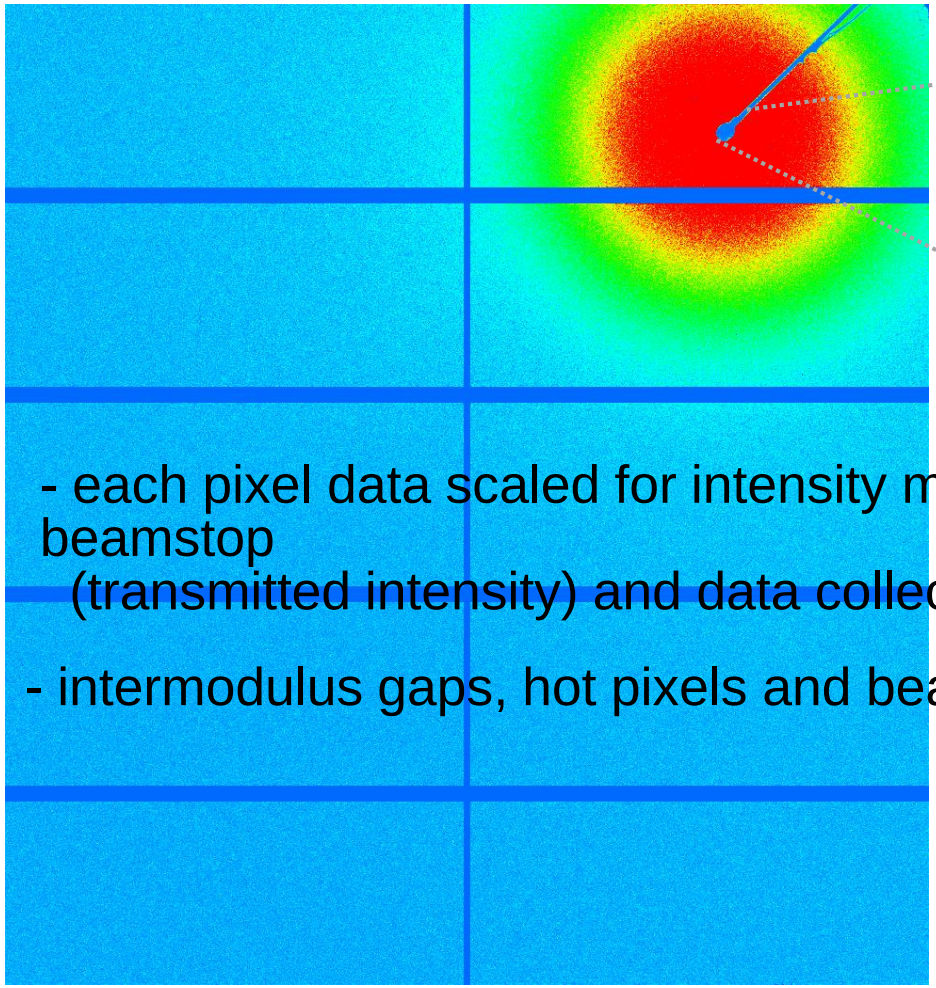


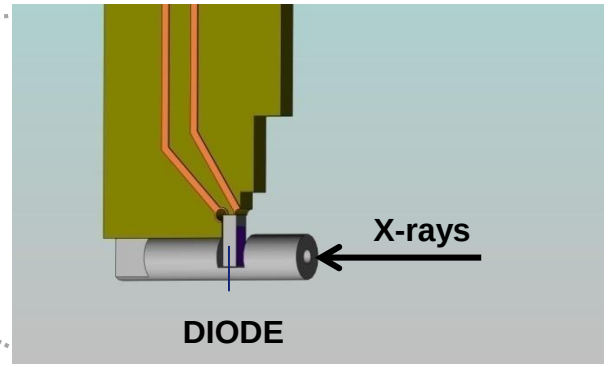
Focusing:
1.1m long Rh coated cylindrical-torioid mirror reflecting vertically upwards at a glancing angle of 4 mrad

EXPERIMENTAL HUTCH

BEAMSTOP COLLECTION OF 2D DATA

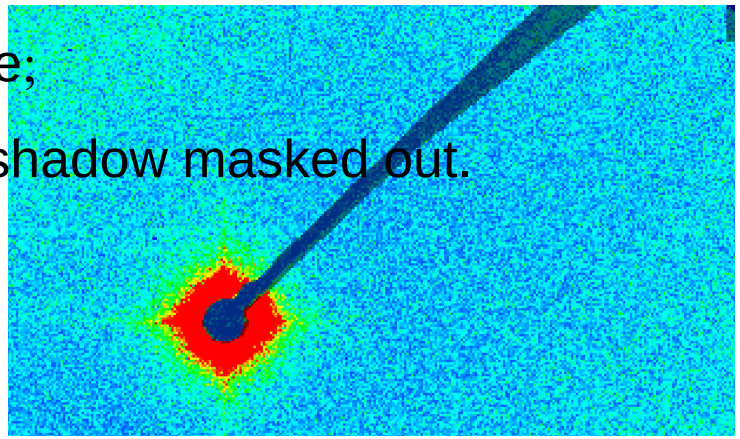
- detector set offset to increase the maximal angular (q)-range





- each pixel data scaled for intensity measured in diode incorporated in beamstop
  (transmitted intensity) and data collection time;

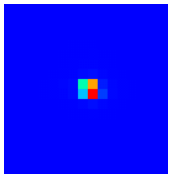
- intermodulus gaps, hot pixels and beamstop shadow masked out.



CALIBRATING BEAMLINE - CONVERSION 2D TO 1D

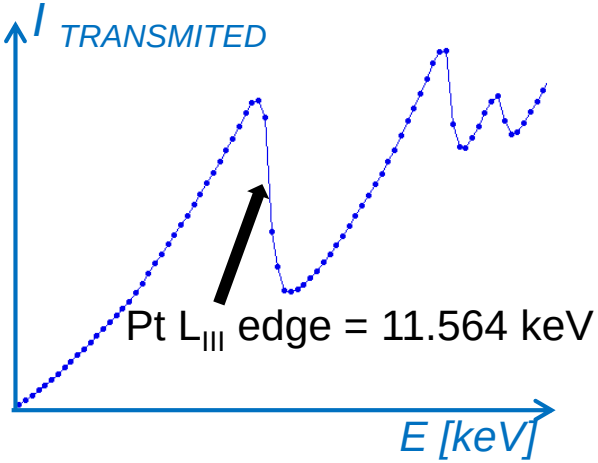
- Radial averaging = all pixels with equal angle are averaged
- Angle converted to $q = 4\pi\sin\Theta/\lambda$ ⟺ calibrated beamline parameters needed

- energy (energy scan with a metal foil – ex. Pt)
- beam position [X_0, Y_0]: direct beam spot recorded (strongly attenuated, beamstop out)

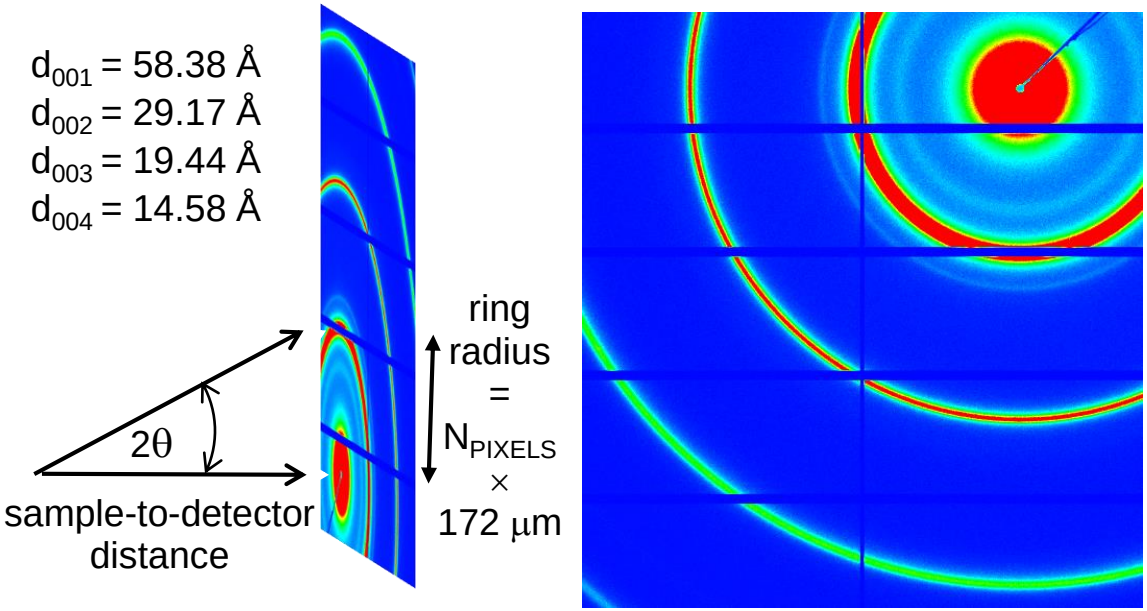


- sample-to detector distance

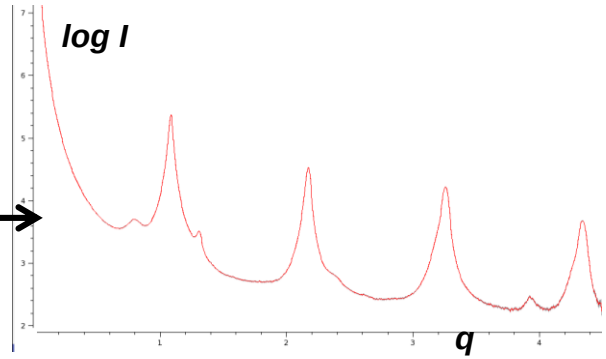
powder diffraction of Silver Behenate $AgC_{22}H_{43}O_2$



d_{001} = 58.38 Å
d_{002} = 29.17 Å
d_{003} = 19.44 Å
d_{004} = 14.58 Å



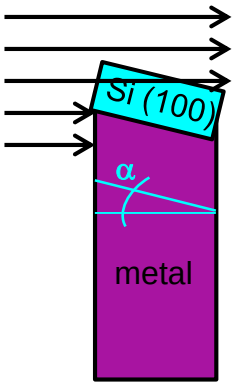
1D



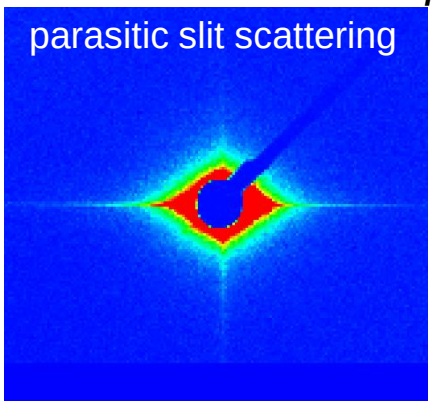
$q = 4\pi \sin\Theta/\lambda$

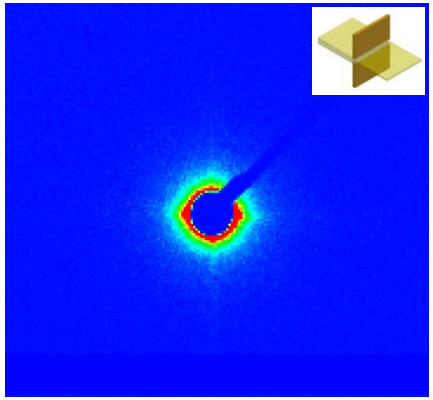
PARASITIC SCATTERING (produced by anything in Beam not Sample)

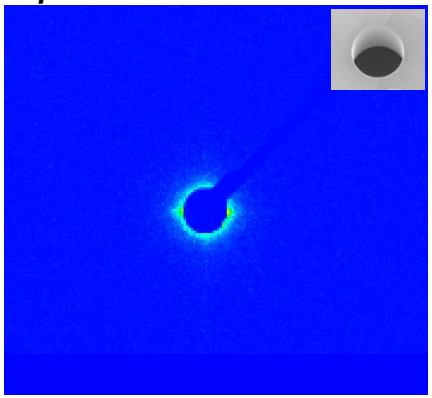
Goal = to minimize parasitic scattering: sandwich/hybrid metal - single crystal blades



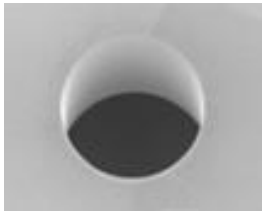
Snapshots close to beamstop

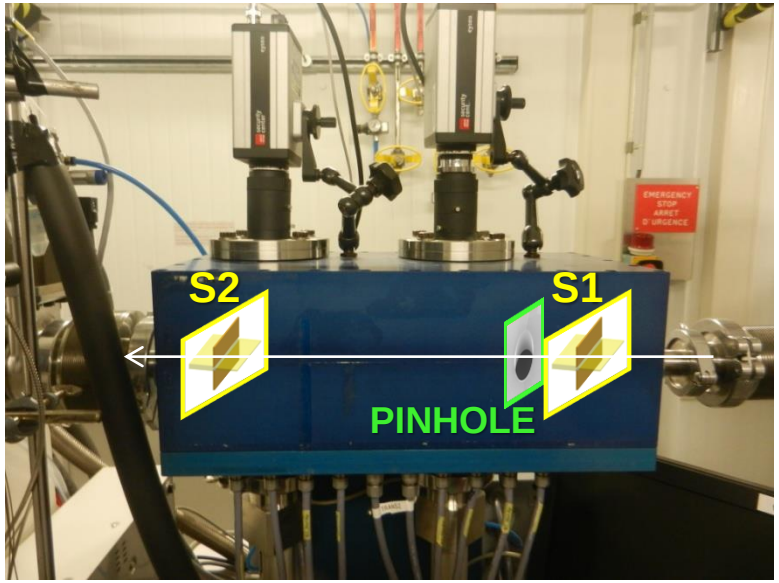






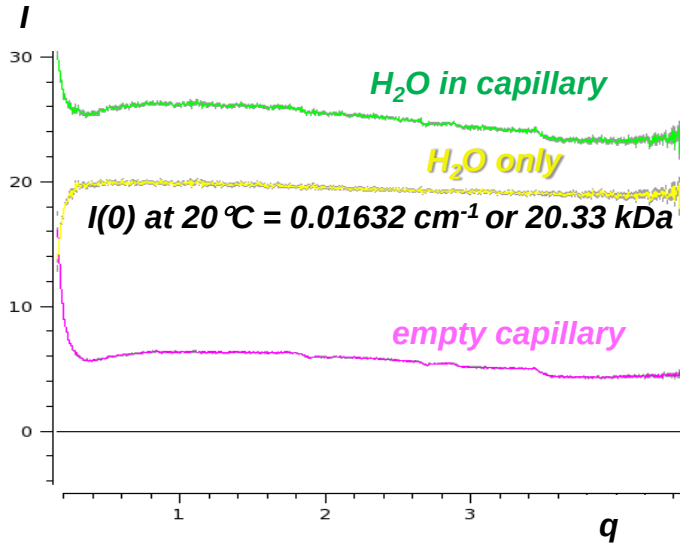
pinhole for cleaning “small beams”
= 100 μm diameter hole





INTENSTY CALIBRATION (PARTICLE MASS)

	absolute	relative	empirical
reference	water	known protein (BSA or lysozyme)	Ex. Rambo & Tainer (2013) Nature 496, 477-481. from Q_R ratio
source of error	protein concentration	protein and reference sample concentration	independent on particle concentration and folding
done by	local contact	user	software



$$Q_R = \frac{V_C^2}{R_g}$$

$$V_C = \frac{I_{PART}(0)}{\int qI(q)dq}$$

SAS invariant

integral converges for folded and unfolded particles

$$M_{PART} = \left(\frac{Q_R}{e^c}\right)^{\frac{1}{k}}$$

paramaters ***k*** and ***c*** empirically determined and specific to the class of macromolecular particle (protein-only, RNA, complex,…)

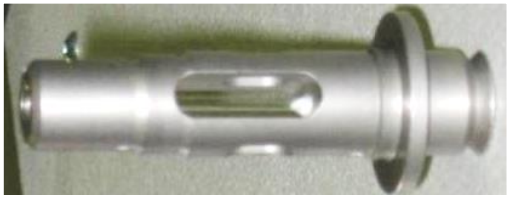
SAMPLE ENVIRONMENTS

- Automated Robot – Batch method
- In-line Size Exclusion Chromatography – SEC-SAXS
- Microfluidics platform – CHIP-SAXS

SAMPLE ENVIRONMENTS – BATCH

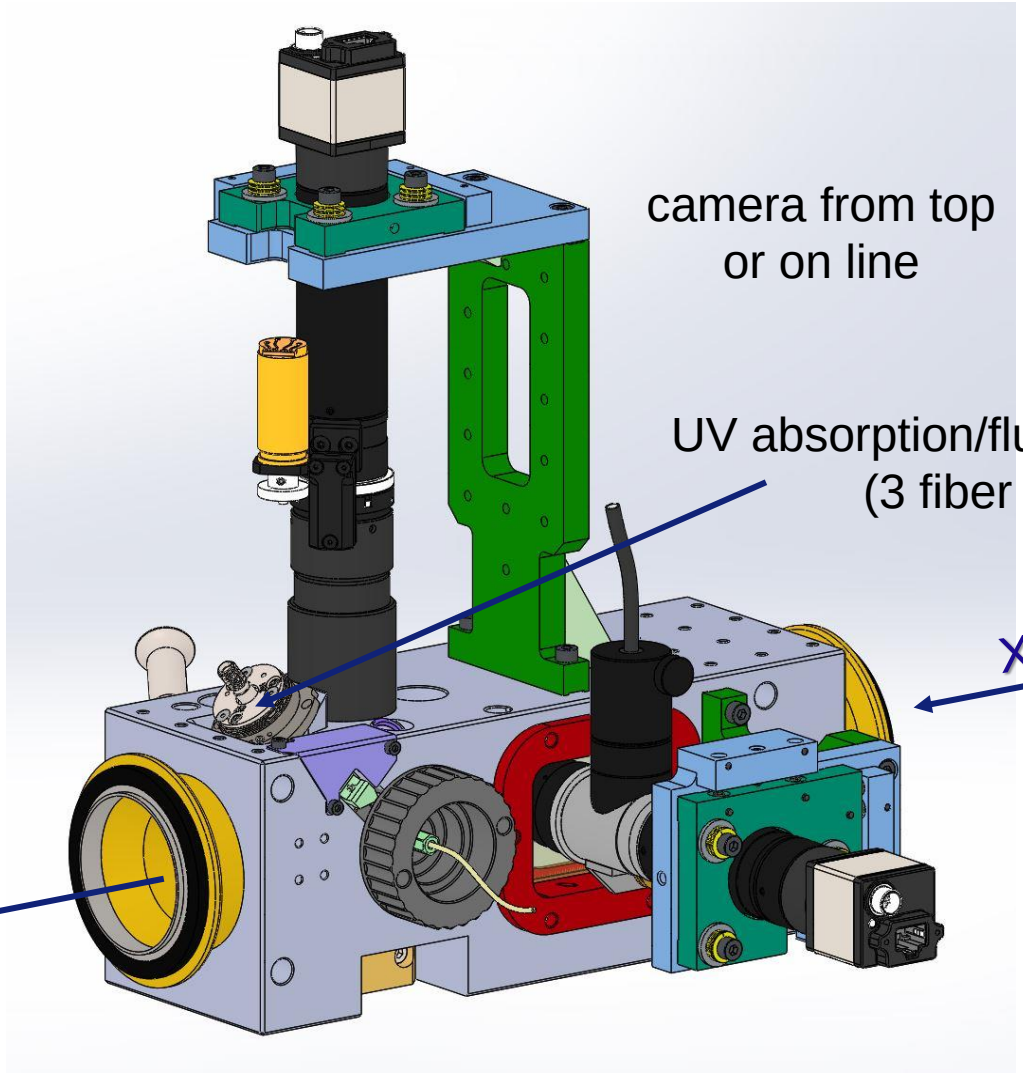
SAMPLE ENVIRONMENTS – BATCH SAMPLE CELL

- capillary pods



Robot and SEU

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



SAMPLE ENVIRONMENTS – BATCH DATA COLLECTION

Sample Volume

- minimum 10 µL per exposure
- 50 µL recommended (flow)
- minimum 3 concentrations required per construct
- approx. 0.5 – 20 mg/mL
- plus buffer measurement for background subtractions

Temperature

- Independent temperature regulation for - storage: 4-40°C
  - exposure: 4-60°C

Exposure Time

- Standard starting time (10 x 1s)
- Easily modifiable in case of SNR or Radiation damage issues

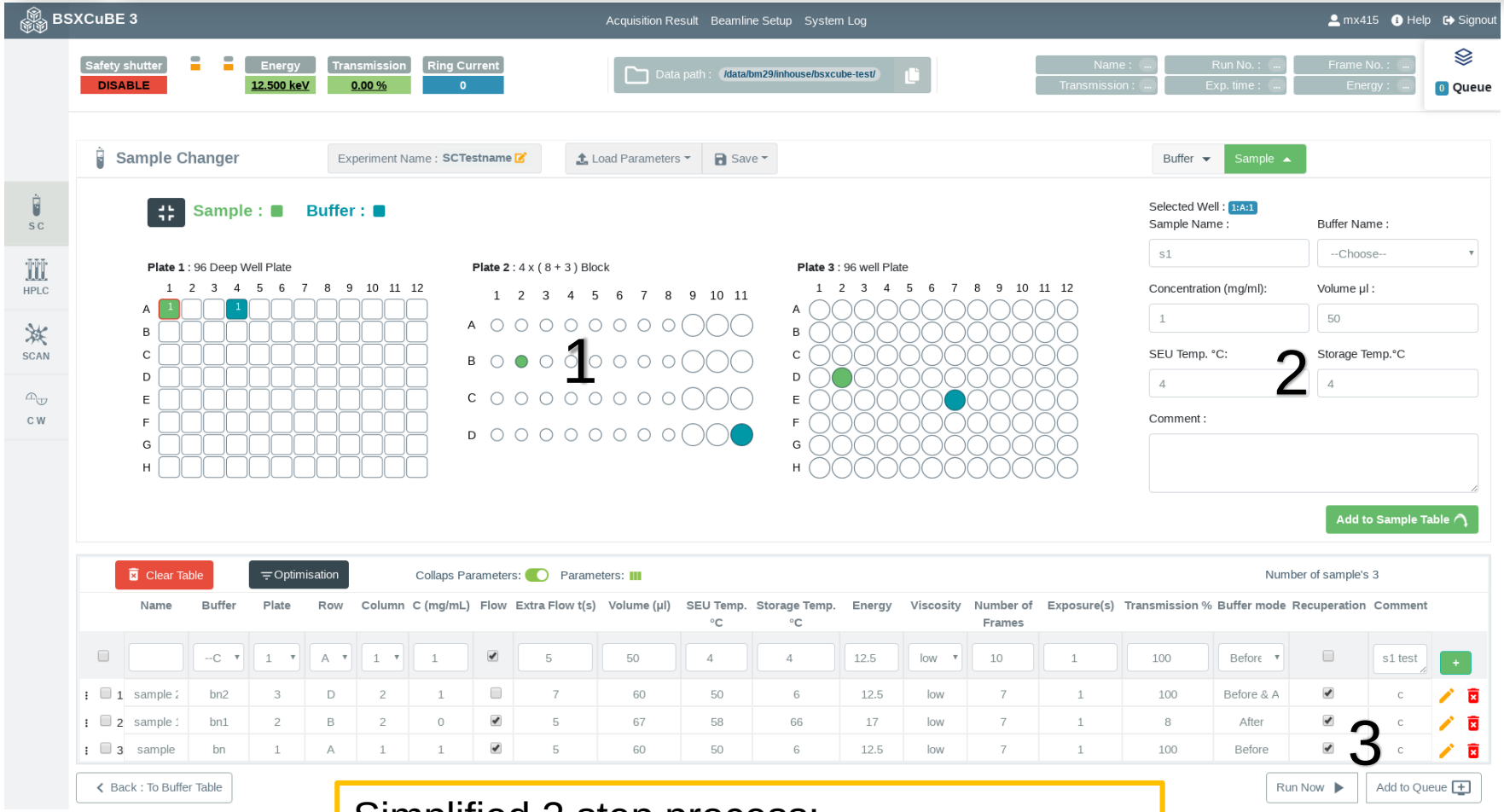
Summary

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

Additives

- no strict limitations but best to minimise where possible to avoid complications
- recommended < 0.5 M salt < 5% glycerol (use sucrose if you can)

SAMPLE ENVIRONMENTS – COLLECTION SOFTWARE – BSXCUBE3

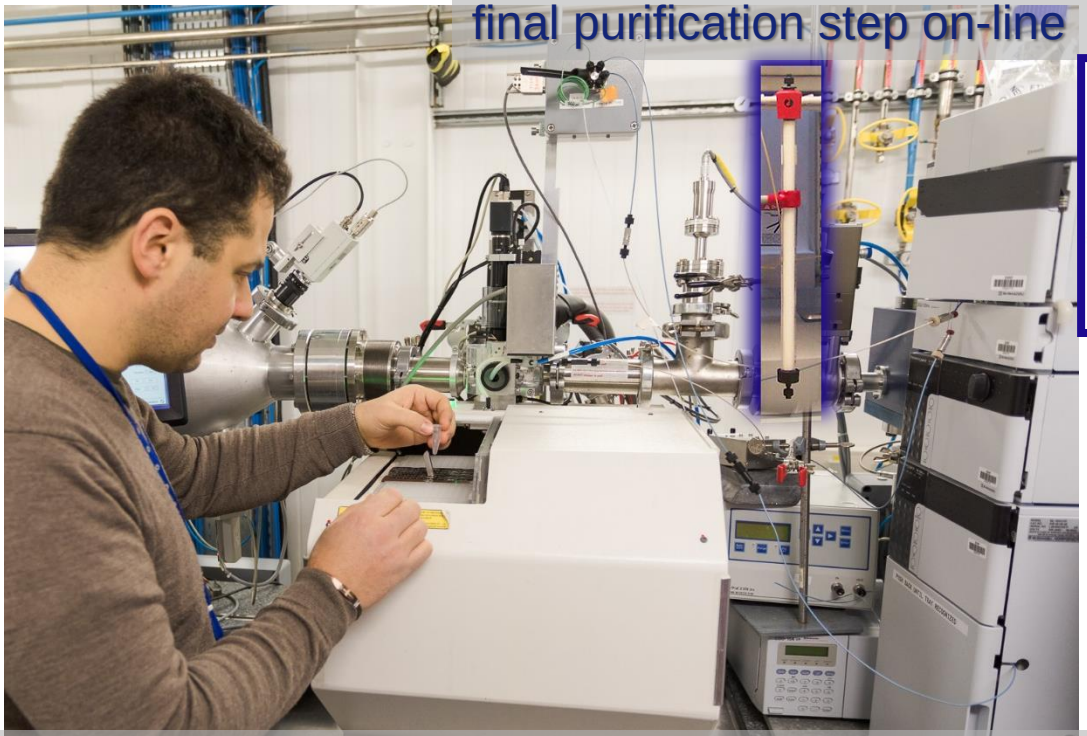


Simplified 3 step process;

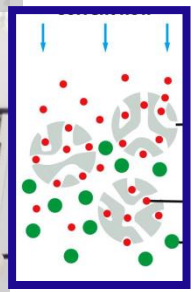
1. Select your sample position
2. Name your sample, concentration, etc.
3. Select “Run” or “Add to Queue”

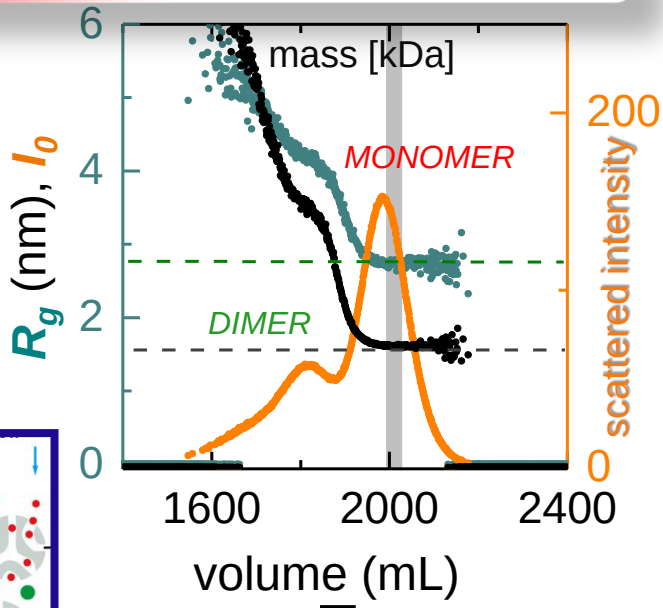
SAMPLE ENVIRONMENTS – SEC-SAXS

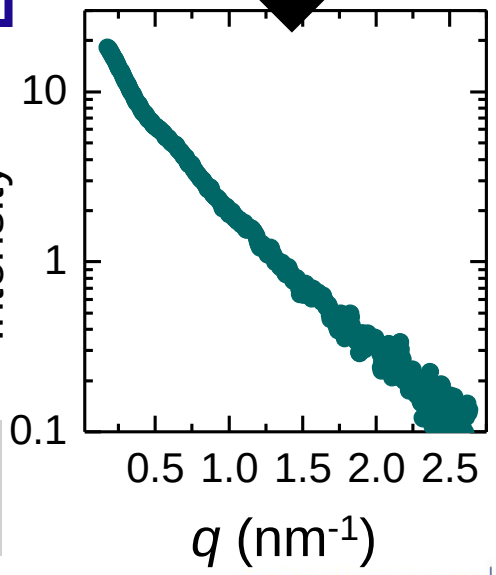
- Measurement of instable samples such as complexes, membrane proteins,…
- Speed limited by chromatography column
- Robot measurement possible when column is equilibrating: easy and quick switch



final purification step on-line







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

SAMPLE ENVIRONMENTS – SEC-SAXS DATA COLLECTION

Sample Volume

- 10 – 100 µL (can use greater volume with injection loop)
- approx. 5 – 10 mg/mL
- Buffer measurement included in column run

Temperature

- Independent temperature regulation for - storage: 4-40°C
  - column cooler 4-20°C

Exposure Time

- 2 second / frames for entire column run (between 15 – 60 minutes)

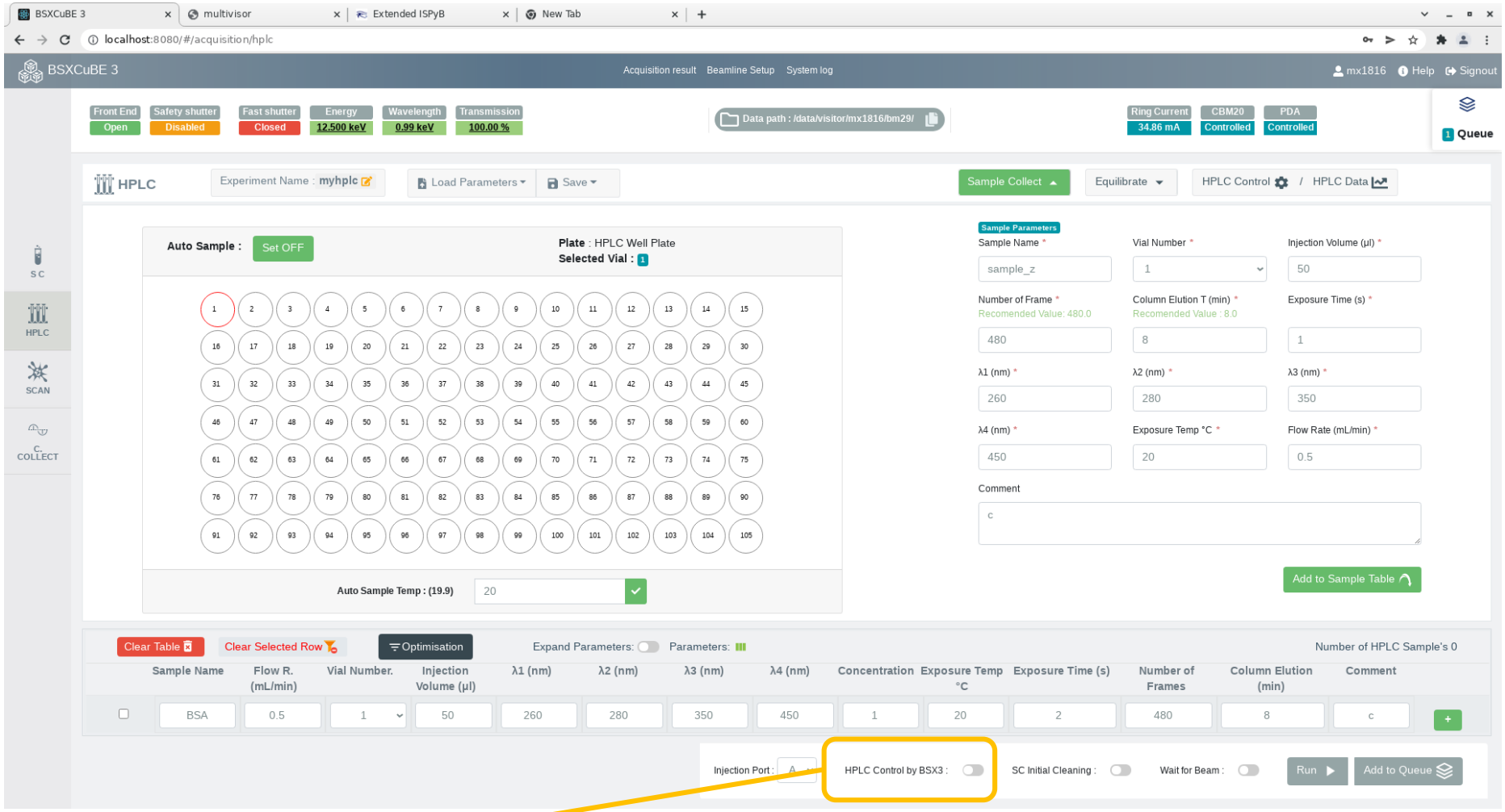
Columns

- Bring your own column
- Use beamline columns (Superdex and HPLC specific BioSEC columns)

Buffers

- no strict limitations but best to minimise where possible to avoid complications
- > 2 mM heavy metal ions.
- recommended < 0.5 M salt < 5% glycerol (use sucrose if you can)

SAMPLE ENVIRONMENTS – COLLECTION SOFTWARE – BSXCUBE3

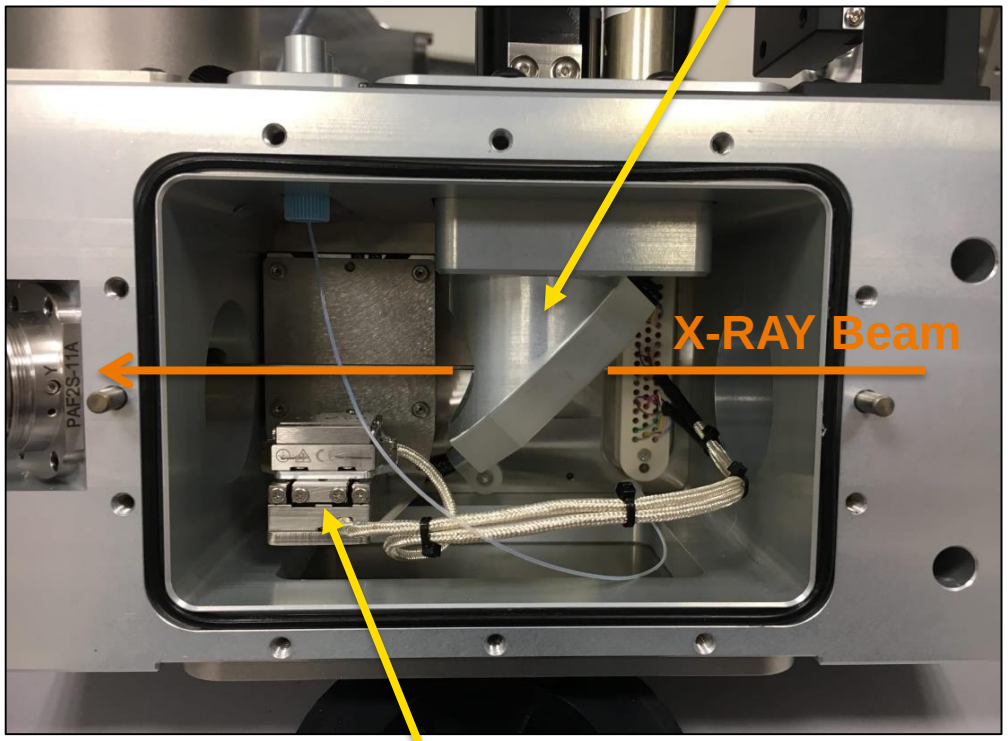


HPLC now integrated

SAMPLE ENVIRONMENTS – MICROFLUIDIC SAMPLE HOLDER

Vacuum chamber with piezo X, Y, Z stage

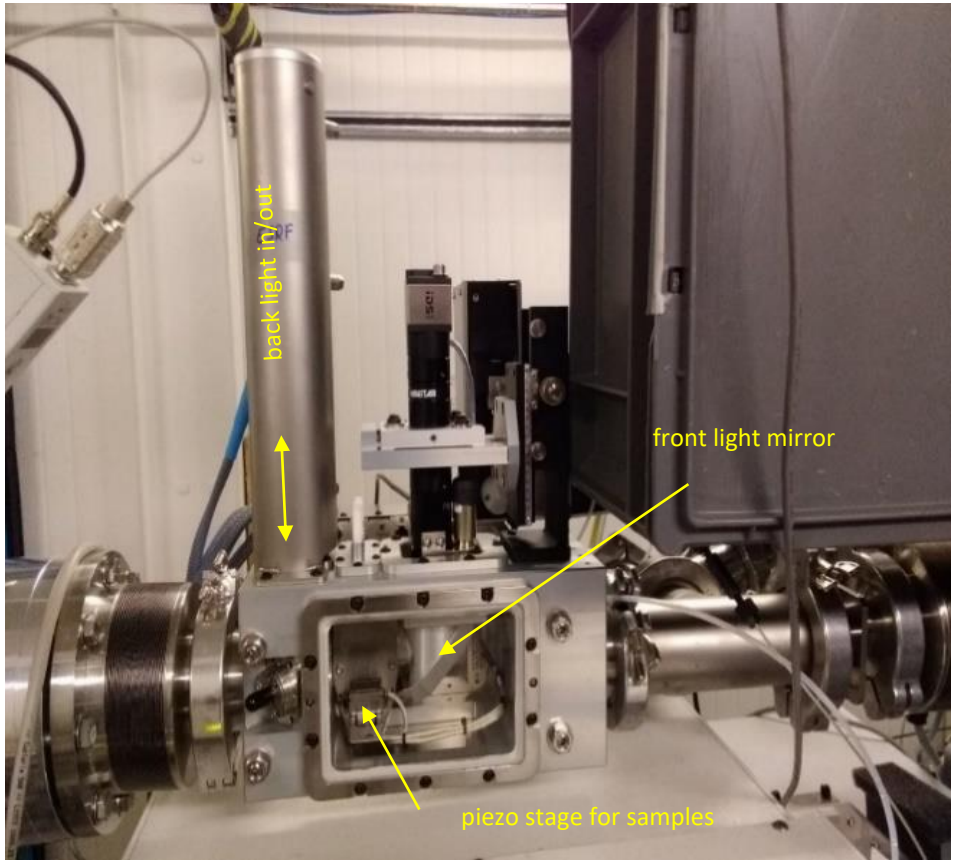
Front light mirror



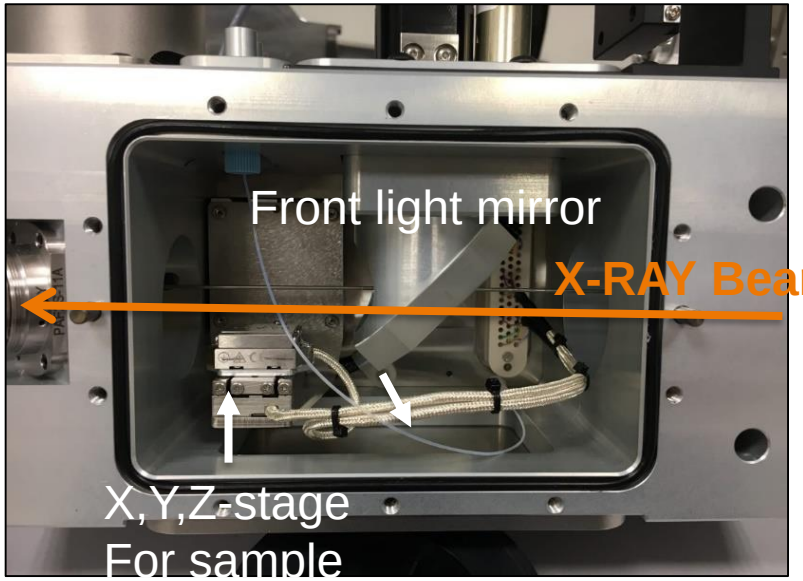
X,Y,Z-stage
For sample position

MICROFLUIDIC SAMPLE HOLDER

BM29 Sample Exposure Unit (SEU)

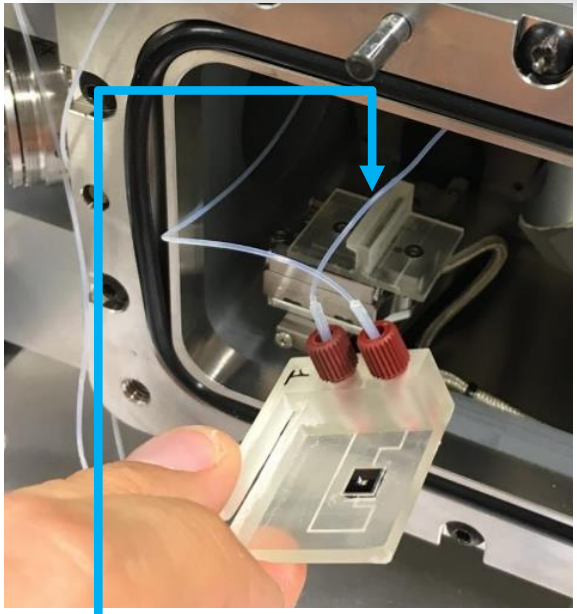


SEU Interior



- SEU developed at ESRF-BM29 in collaboration with EMBL Grenoble and EMBL Hamburg

SAMPLE ENVIRONMENTS – CHIP-SAXS



Fixed Chip Holder

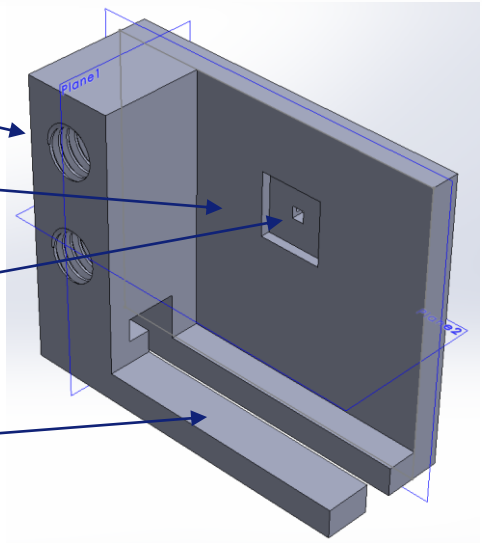
Standardized Chip Design

Standard fluid connectors

Microfluidic region

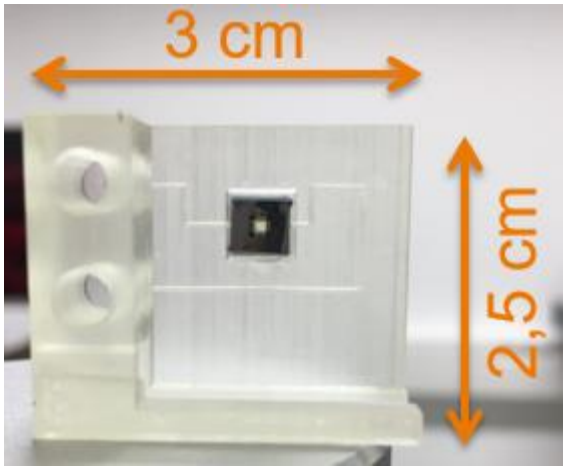
X-ray region

Sliding chip fixation



X-ray windows materials

- 100 μm thick COC film
- 1 μm thick Silicon Nitride



Developed by Anton Popov with help from PSCM and Peter Van Den Linden

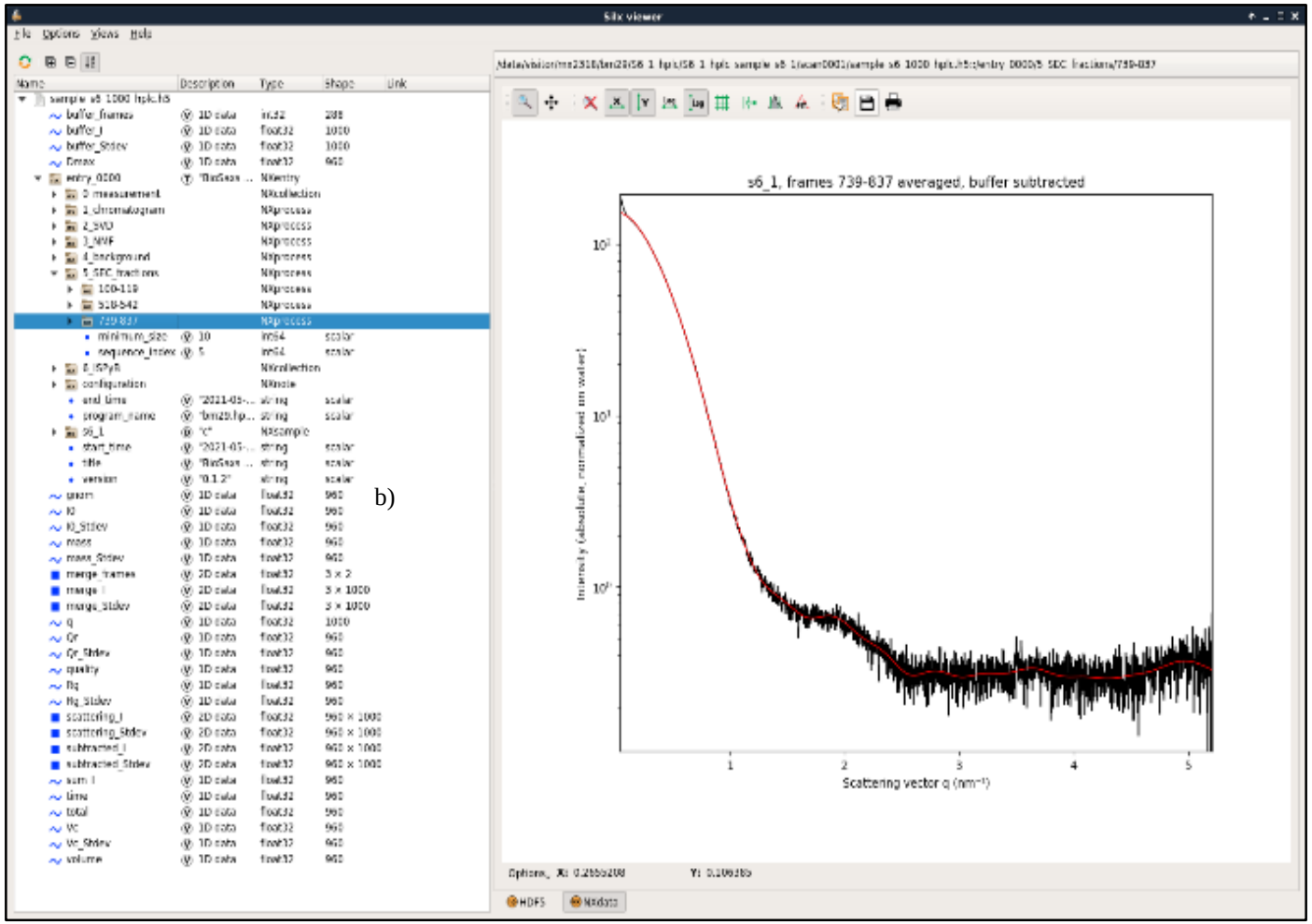
Microfluidic platform

Designing several different chips for different experiments;

- Mixing – ligand, metal ion, salt, pH,
- In situ Phase transition,
- Gels and other soft condensed matter too viscous for robot,
- High throughput ligand screening (use piezo stage to raster through samples),
- User specific applications.

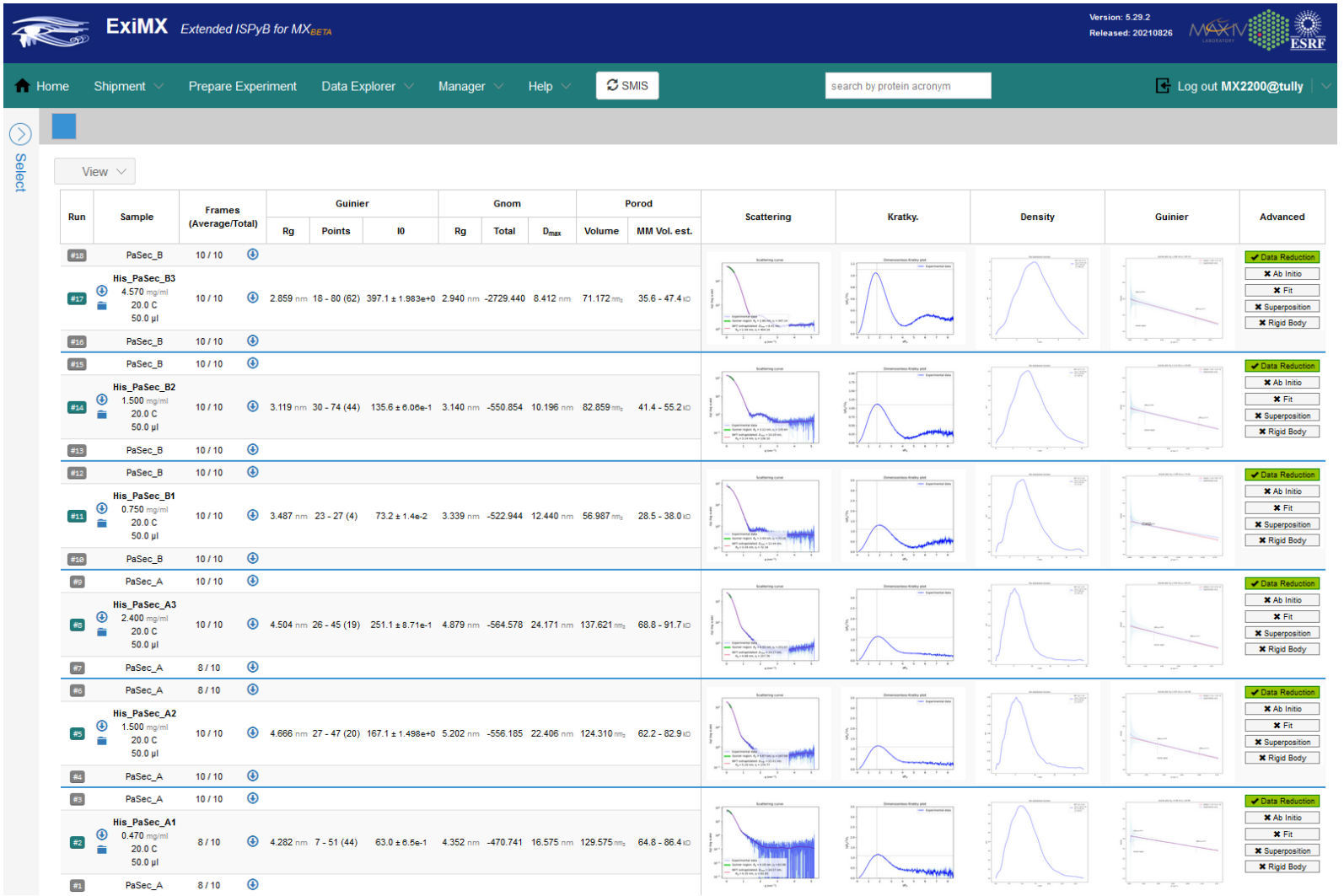
AUTOMATED DATA ANAYLSIS - FREESAS

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



b)

WEB BASED DATABASE – ISPYB EXI



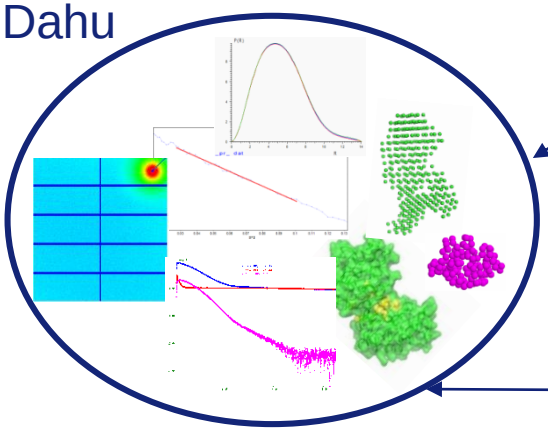
DATA ANAYLSIS - STRATADGY

Analyse data at the beamline!
Automation is very useful to help and save valuable time!

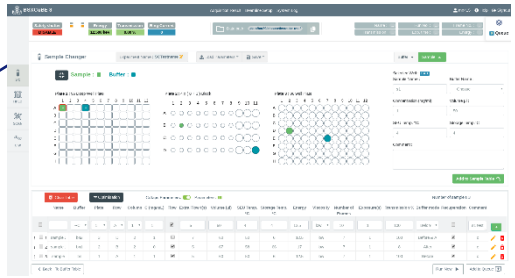
Preliminary data analysis does not have to be perfect (publication quality) but it should be used to tell you if your questions can be answered with the data you have or do you need to think.

- The standard three concentrations might not be enough!
- Samples could be in less than ideal condition after shipping.
- Radiation damage maybe present and the data collection parameters altered to account for this.
- When studying a complex do you **really** have a **complex** or is it a **mixture**

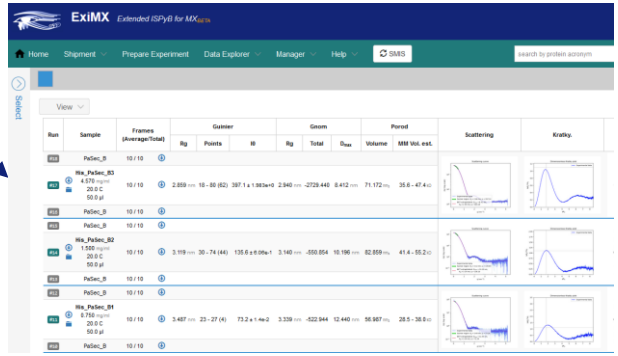
Dahu



BsxCUBE



ISPyB/EXI



beamline GUI

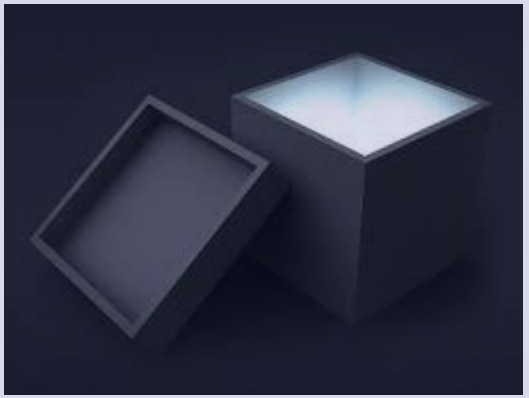
automated data analysis

data/sample tracking

OVERVIEW OF SAXS EXPERIMENT

Basic requirements (calibrated set-up)

- radiation source (monochromatic with appropriate collimation and focusing)
- detector (low noise, ideally photon counting)
- sample holder (exposure unit)
- routine experimental procedures ~ automation:
  - ✓ good background measurements for subtraction
  - ✓ repetition, no cross contamination (cleaning)
  - ✓ sample changer
  - ✓ data processing, analysis and feedback
  - ✓ sample and data tracking data base



Preparation to undertake a good experiment

- sample characterisation
- ensure you have well behaved samples (transport and timescale for experiment)

HOME

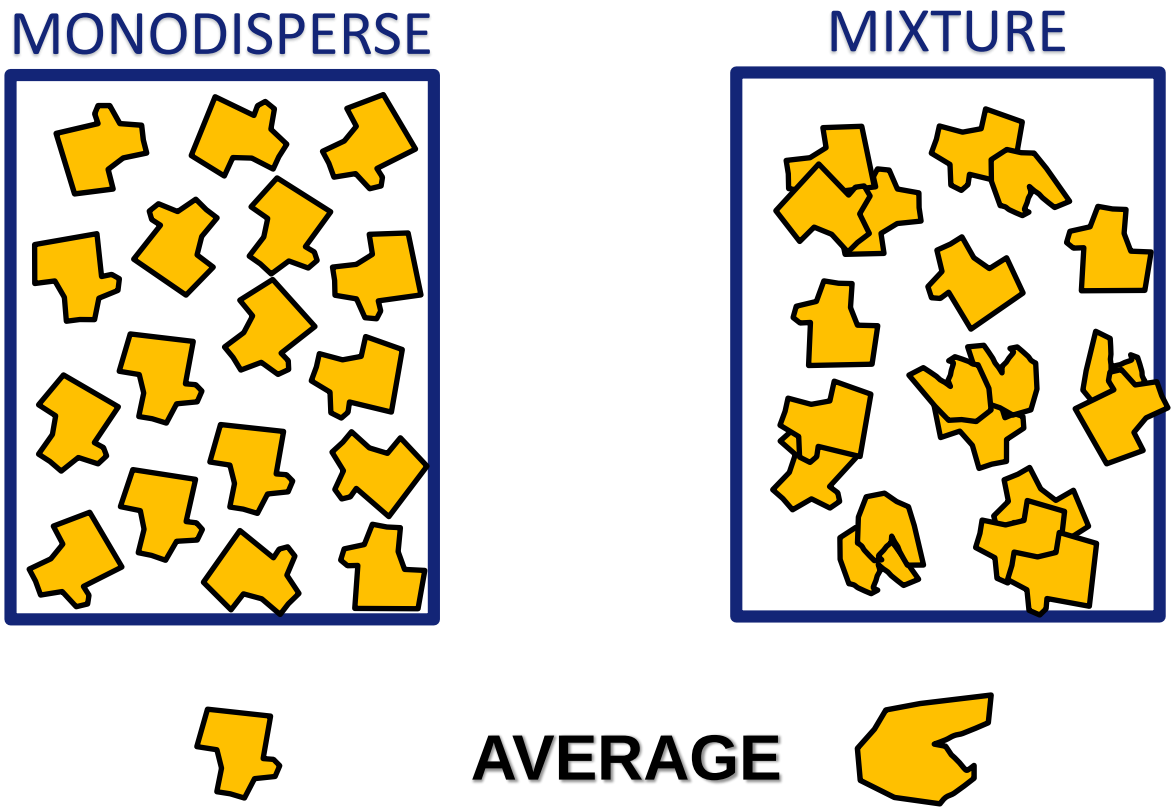
Thorough data analysis

- crosschecks and verification of consistency in all model independent parameters
- utilise information from complimentary techniques wherever possible to allow validation

SAMPLE PREPARATION

(garbage IN garbage OUT)

In solution SAXS we observe the **AVERAGE**



SAMPLE PREPARATION – MONODISPERSE AND IDEAL

Shape reconstruction requires:

MONODISPERSE!

samples in solution

Pure protein (>95%)!
In only 1 oligomeric state!
NO aggregation!
Free from interparticle effects (ideal solution)

Before going to the beamline users are encouraged to use:

- HPLC/FPLC purification
- MALS/DLS
- Mass photometry
- Analytical ultra centrifugation

You should know Your proteins!

Knowing as much as you can before visiting a synchrotron will give you the best chance to obtain good data

… and can save more time and effort than trying to recover information from non ideal samples

HOW DO YOU GET TIME – ACCESS?

2 main options for applying for time.

- Rapid Access Proposal – Can be submitted at any time during the year
- Rolling Access – Submitted for specific allocation periods (AP)

- These require a 2 page summary of your experiment, including why you want to use the beamline? What do you expect to achieve/ how will the results help you?
- Proposals peer reviewed by SAXS experts

At ESRF Rolling Proposals take ~ 1 month to review and will receive time in next period, ~ 3 months from submission.
See https://www.esrf.fr/UsersAndScience/UserGuide/Applying/ProposalGuidelines/MXRollingCrystalloAndBioSAXS

3rd option at ESRF many universities are associated with Block Allocation Group (BAG) Proposal. These are given a set amount of beam time/ year. Contact the main proposer who will organise your experiment.

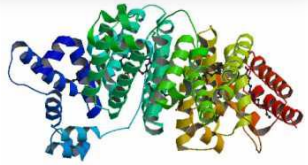
4th option – less used - form a collaboration with a beamline scientist/Post doc and they will "find time" far more quickly in return for being named on the paper.

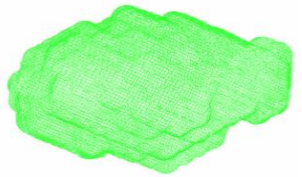
COMPLIMENTARY WITH SANS – SAXS/SANS BAG

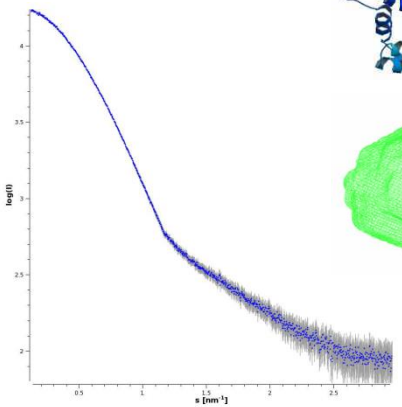
OVERVIEW OF SAXS EXPERIMENT – SIMPLE VIEW

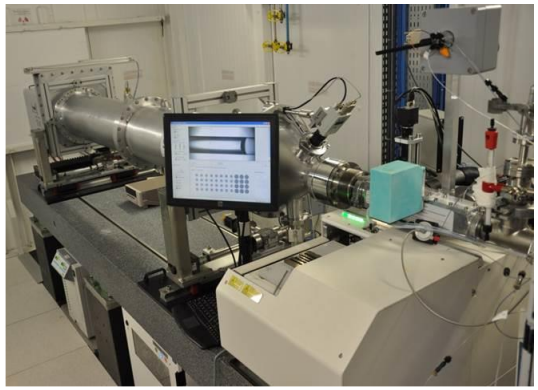
synchrotron beamline
or home source set-up

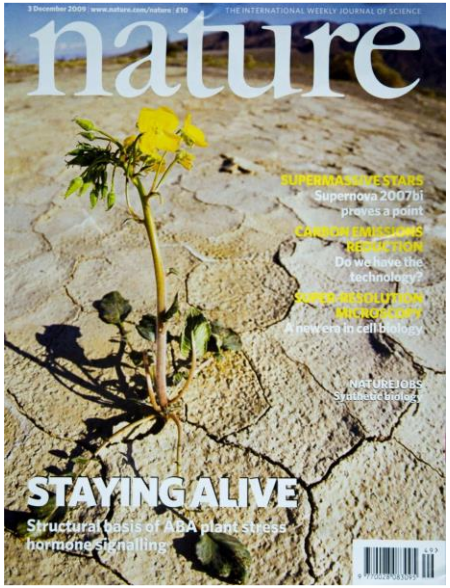
solution scattering data
from protein of interest











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BM29

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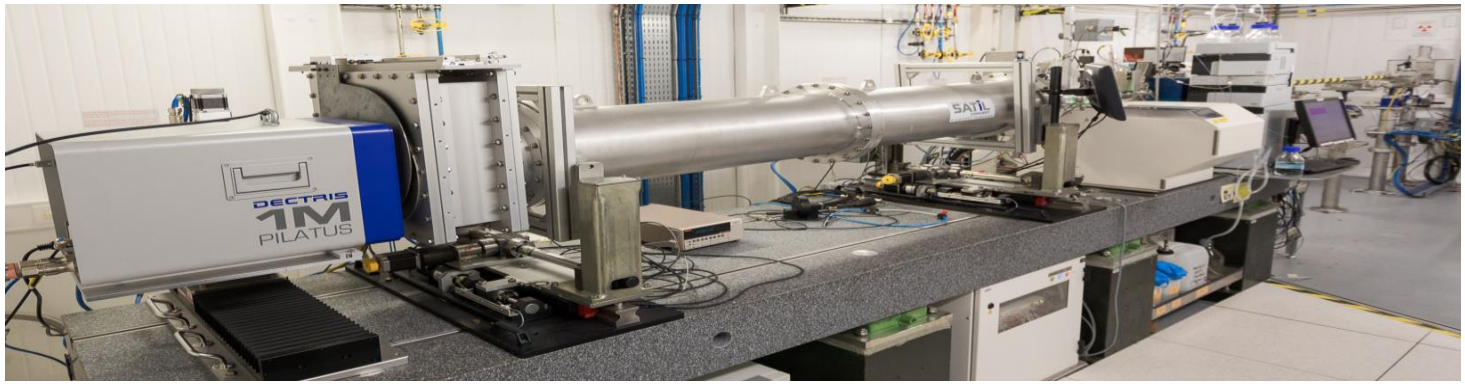
EMBL



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