

# Instrumentation and data collection procedures for BioSAXS

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# SAXS INSTRUMENTS



 Offline or home source

 Synchrotron beamline



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#### All past, present and future synchrotrons with a SAXS beamline

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



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

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#### Solution SAXS experiment – simple view





#### OPTICS



Page 5 I SAXS instrumentation and data collection I 20th June 2022 I Mark Tully

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#### **EXPERIMENTAL HUTCH**





- 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<sub>0</sub>, Y<sub>0</sub>]: direct beam spot recorded (strongly attenuated, beamstop out)
- sample-to detector distance powder diffraction of Silver Behenate AgC<sub>22</sub>H<sub>43</sub>O<sub>2</sub>







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





Snapshots close to beamstop



**pinhole** for cleaning "small beams" = 100  $\mu$ m diameter hole





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#### **INTENSTY CALIBRATION (PARTICLE MASS)**

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







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

SAS invariant (protein-only integral converges for folded complex,...) and unfolded particles

Page 10 I SAXS instrumentation and data collection I 20th June 2022 I Mark Tully

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- 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 Volume

- minimum 10  $\mu$ L per exposure
- 50  $\mu L$  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

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#### SAMPLE ENVIRONMENTS – SEC-SAXS

- Measurement of instable samples such as complexes, membrane proteins,...
- Speed limited by chromatography column
- Robot measurement possible when column is ٠ equilibrating: easy and quick switch



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# 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 – 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** 

X-ray windows materials

100 μm thick COC film
1 μm thick Silicon Nitride



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

Page 21 | SAXS instrumentation and data collection | 20th June 2022 | Mark Tully

#### 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.



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





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# 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.

ISPyB/EXI

The European Synchrotron

• When studying a complex do you really have a complex or is it a mixture

# **BsxCUBE**



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
  - $\checkmark\,$  data processing, analysis and feedback
  - $\checkmark$  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)

Thorough data analysis

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



(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

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

You should know Your proteins!

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 <a href="https://www.esrf.fr/UsersAndScience/UserGuide/Applying/ProposalGuidelines/MXRollingCrystalloAndBioSAXS">https://www.esrf.fr/UsersAndScience/UserGuide/Applying/ProposalGuidelines/MXRollingCrystalloAndBioSAXS</a>

3<sup>rd</sup> 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.

4<sup>th</sup> 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**





# THANKS TO WHOM BIOSAXS BEAMLINE EXISTS IN ITS CURRENT SHAPE



*Technicians*: Mario Lentini, John Surr, Franck Felisaz, Julien Huet, Hugo Caserotto, Fabien Dobias, Jonathan Gigmes.

Software developers: Staffan Ohlsson, Jerome Kiefer, Alejandro De Maria Antolinos, Alexandre Gobbo, Matias Guijarro, Antonia Beteva, Vicente Rey-Bakaikoa, Olof Svensson, Marcus Oskarsson, Jean-Baptiste Florial.

Instrument support division: Dean Gibson, Philippe Retout.

Engineers/scientists: Petra Pernot, Pascal Theveneau, Werner Schmid, Ray Barrett, Muriel, Mattenet, Christian Morawe, François Torrecillas, Adam Round, Florent Cipriani, Louiza Zerrad, Martha Brennich, Mark Tully.





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