SAXS and SANS facilities and experimental practice

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Small Angle Scattering experiment

The beam hits the sample, X-rays/neutrons interact with the sample and are scattered, providing structural information on the sample. Same formalism but different scattered particles $\rightarrow$ Different instrument.
Outline

• X-rays / neutrons

• SAS instruments

• Sample environment

• Sample requirements and collection strategy
X-rays and neutrons
X-rays

Roentgen, 1895
Electromagnetic wave
How are X-ray produced?

- Brehmstrahlung
  - When a charge is accelerated charge, electromagnetic radiation is produced (from Maxwell equation)
X-ray sources - Synchrotron

- Synchrotrons
X-ray sources - synchrotron

• Synchrotron radiation – Insertion devices
Insertion devices

Dipole bending magnet (APS)

Undulator (PetraIII)
Synchrotrons around the world
X-ray sources - FEL

• Free electron laser
  – Electrons are accelerated and send to a long undulator (several 100s meters)
  – Self amplified spontaneous emission: electrons group themselves into small bunches.
  – Production of very short and intense X-ray pulses
X-ray sources - FEL

• Free electron laser
Lab sources
Lab sources

- Principle: electrons, produced by heating a cathode are accelerated in an electric field and projected on a metallic anode.
  - Brehmstrahlung
  - Fluorescence
X-ray sources

- Lab source (rotating anode, liquid jet)
Neutron

\[ \lambda = \frac{h}{mv} \]

James Chadwick
Neutron production

• Nuclear reaction
Neutron production

• Spallation source
  – Accelerated protons hit a heavy metal target.
Neutrons Facilities
SAXS and SANS Instruments
Optics

Polychromatic divergent beam from the source

Monochromatic focused (parallel) beam for SAS
Monochromatic X-ray

- Bragg diffraction on a crystal
Monochromator

- Before
  - Polychromatic
- After
  - One wavelength + harmonics
Focusing/low divergence

- Small beam at the detector position
- Small beam at the sample position
Focusing X-ray

- Compound refractive lenses
  - Series of concave lenses
  - Cylindrical holes

- X-ray mirrors
Focusing X-ray

- Focussing mirror

- Reflectivity
Focussing mirror – harmonics filter

Monochromatic, focused x-ray beam
Monochromatic neutrons

- De Broglie equation: $\lambda = \frac{h}{mv}$
  The wavelength of a neutron is related to its velocity.
- Velocity selector
  $\frac{\Delta \lambda}{\lambda} = 5\text{--}10\%$
- For pulsed source, TOF
Collimation neutrons

• The collimator is used to obtain a parallel beam
Get rid of parasitic scattering:
slits

Beam defining slits
Guard or anti-scatter slits
Hybrid slits

- Idea: use a crystal for the tip of the blade:
  → no scattering but diffraction
Hybrid slits

- On the P12 beamline
Sample environment
Flight tube
Beamstop

• Prevent the direct beam from hitting the detector
  – Big enough to stop the direct beam
  – Small enough to collect the small angle

• Measure transmitted beam
Active Beamstop

• SAXS images needs to be accurately scaled to allow for proper buffer subtraction and extraction of the solute SAXS pattern
Detectors
CCD detector
Single photon counting detector principle
Single photon counting detector
Pilatus

- High dynamic range
- No background noise
- (relatively) Fast framing

→ Ideal for SAXS
Neutron detection

- He3 detector:

\[ n + ^3\text{He} \rightarrow ^3\text{H} + ^1\text{H} + 0.764 \text{ MeV} \]
Sample environment
Samples

SAS applicable to many type of samples.

Metal alloys  Sufactants  Tissues  Polymers

Nanomagnetic materials  Bio-macromolecules in solution
Sample environment

Magnetic field system

Heating stages

Sample changers

Rapid mixing device

Example ID02 (ESRF) multipurpose beamline
Sample environment

• Bio-macromolecules in solution are weakly scattering sample.

• For biological macromolecules in solution:
  – fragile
  – Preferably in vacuum
  – Thermostated
Sample cell

- Cell material: low absorption and scattering
  - Mica, quartz, polycarbonate
- Sample thickness (t): compromise between scattering and absorption
  - Scattering $\alpha t$
  - absorption $\alpha \exp(-ut)$

- For neutron, cell are rather thin (<1mm to avoid multiple scattering)
Solution SAXS

10 years ago:
Manual sample loading

- Buffer and sample should be measured in the same cell
- Difficult to implement in vacuum
- 10-15 minutes per measurement
- High sample consumption
- Non-optimized cleaning procedure
- Tedious, energy and attention consuming
SAXS sample changer @EMBL Hamburg

Fraunhofer

EMBL

ESRF
SAXS sample changer
Sample changer performances

- Large storage capacity
- Full cycle time (loading, exposure, flushing, cleaning, drying) ≈ 1 min
- Volume 5-20 microliter
- Very efficient cleaning
- Flow measurement
Online size exclusion column

UV \sim c \varepsilon \\
LS \sim c (dn/dc)^2 MW \\
R_i \sim c dn/dc

6/20/2016
SEC + SAXS
Experimental practice
Buffer subtraction
Buffer subtraction

• Biological sample scatters very weakly, SAXS curves collected on the buffer should be carefully subtracted
  – Exactly matching buffer (dialysis, elution buffer)
  – Sample and buffer measured in the same cell
Monodispersity

- SAS is very sensible to aggregation, the sample should be monodisperse
Monodispersity

- Check the monodispersity of your sample before coming to the beamline.
  (native gel, dynamic light scattering, ultracentrifugation,...)

- Use online chromatography
Inter-particle interactions

- Ideal solution of particles
- Repulsive particle interactions
- Attractive particle interactions
Inter-particle interactions

• Change solution (pH, salt concentration) to limit interactions

• Measure different concentrations and extrapolate
Measure also water and/or standard protein...

• ... to estimate the molecular mass of your sample using the forward scattering

  – For data on an absolute scale (water measurement)
    • \( M = I(0) \cdot N_A / (C \cdot \nu \cdot \Delta \rho) \)

  – Using a protein standard
    • \( M = M_{\text{BSA}} \cdot I(0) / I_{\text{BSA}}(0) \)
X-rays - Radiation damage!!!

• With intense synchrotron beam: radiation damage:

\[ \text{H}_2\text{O} \rightarrow \text{H} \cdot + \text{OH} \cdot \]

Free radicals: oxidize proteins which leads to their aggregation

• Monitor radiation damage: collect several frames and compare them.

• Limit the radiation damage
X-rays - Radiation damage!!!

Rnase – Static measurement

Flow measurement

Beam attenuation

Use of additives

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Contrast in neutron

- Neutrons interact with the nucleus of atoms
- Each atom has its own scattering length:

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<th>C</th>
<th>N</th>
<th>O</th>
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Labeling

- Protein and nucleic acids have different scattering length densities and the different components can readily be studied by SANS
- To study protein-protein complex, one of the components needs to be deuterated (hydrogen exchanged with deuterium) to changed its scattering length density
- A deuterated protein is obtained by producing the protein in a deuterated medium.
Neutrong collection strategy

• Solvent matching
  – Find the solvent composition that match the contrast of the component you want to hide
  – Measure the sample in this solvent

• Contrast variation
  – Measure the sample in solvent with different D/H ratio, different scattering length.
  – Using Stuhrmann analysis you can access the curves of the different components.
Summary: SAS sample

• Protein concentration: 0.1-10 mg/ml

• Volume: 5-50 microliter (SAXS), 200-300 microliter (SANS)

• Time:
  • lab source: 5-60 min
  • Synchrotron: seconds
  • Neutrons: 30 minutes - hours
Summary: SAS sample

- Pure and monodisperse sample
- Exactly matching buffer
- Measure concentration series
- For SAXS:
  - Be aware of radiation damage
- For SANS:
  - Carefully design your experiment, think of your collection strategy.
Conclusion

• To optimally use your beamtime: carefully plan your experiment, prepare your sample and characterize them before coming to the beamline

• SAXS is now widely used. Dedicated instruments with low background and high level of automation, high quality lab instruments.

• SANS more difficult: consume more time and sample, requires good planning of your experiments and collection strategy, but can provides unique information.