Sample Preparation and Pratical Consideration

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BioSAXS2019, Dec 2, 2019

Basic prerequisites for SAXS analysis

- **Sample is Monodisperse**, i.e. identical particles

$$i_j(q) = i_1(q) \qquad \forall j$$

Check using SEC, DLS, Electrophoresis

- **The Particles/Molecules are Uncorrelated**, i.e. no inter-molecular interactions present

$$I(q) = \sum_j n_j i_j(q)$$

Dilute or change buffer composition if necessary

$$I(q) = N i_1(q)$$

A good SAXS experiment starts in your home lab

- arguably sample preparation is the most important part in your SAXS experiment
- use other biochemical / biophysical methods to characterize your sample and optimize sample purification and handling protocols
- think about special constructs (truncations, cross-linking) to optimize sample stability
- Avoid detergents, if absolutely needed use well below CMC
- simulate transport to beam line to check if sample is getting there uncompromised (e.g. run through a freeze-thaw cycle and check for sample quality after)
- Know your numbers: MW, concentration of stock solution …
- talk to the beam line scientist for advise

A good SAXS experiment starts in your home lab

- **every protein has its own "personality"**

  the more you know about your protein the better you can select the data acquisition parameters (buffer composition, pH, additives ….) or make informed decisions at the beamline on possible ways to make your protein cooperate to get the best possible SAXS data

Sample requirements for solution scattering

- size: >5kD
- purity: **highly monodisperse !**
- concentration range: 0.25 – 10mg/ml

  Rule of thumb for max. concentration:

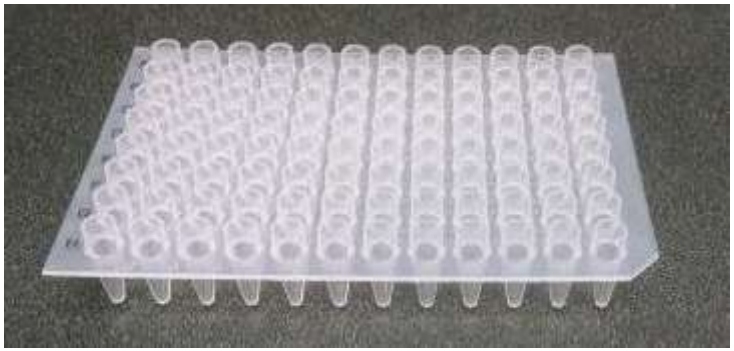
  *Molecular weight [kD] * concentration [mg/ml] ≈ 100*

- enough material for at least 3 concentrations
- Best one high concentration and several lower (e.g. 10 – 2 – 1 – 0.5 mg/ml)
- sample volume 15-30 µl per concentration (for Autosampler data collection)
- exact **matching buffer** solution is very important (lower salt preferable)
- most buffer components tolerated (e.g. glycerol (<15%) and salt (<0.5M))
- radical scavengers (DTT, TCEP …) freshly added (2-5mM) can protect the protein form rad. Damage
- also glycerol (~5%) might be useful to keep your protein from aggregating

Sample are pipetted out into ….

- PCR strip tubes (one for each concentration series)

- or 96 well microplates



Guidelines for good Buffer conditions

- use the buffer your protein is most “happy” in
- if you have a choice: use a lower salt concentration
- for high salt (>500mM) conditions you need higher protein conc.
- variety of buffer possible: Tris, HEPES, PBS …
- consider additives to prevent radiation damage: such as DTT, TCEP or BME (2-5mM, added freshly)
- glycerol (~5%) will help prevent rad. damage but makes buffer matching difficult; also decreases contrast
- if detergents are absolutely needed use well below CMC (and be aware that it be somewhere on your sample)
- bring plenty of matched buffer

The "matched" buffer

- correct buffer subtraction is crucial for good SAXS data
- **recall:** at intermediate to high q the protein signal is way below the buffer background so even small differences in the subtracted buffer with respect to the buffer the protein is sitting in will distort the protein signal

- How to match your buffer
  - Buffer from the last size exclusion filtration
  - flow-through from concentrator
  - Dialysis (allow 20h+)

Monodispersity

- **carefully check your samples:**
  - Good solubility (clear solution), no obvious precipitates
  - Single species on native gels
  - SDS-PAGE should show no contamination
  - Single symmetric peak on SEC column

- **use other complementary analytical techniques:**
  - static and dynamic light scattering (MALLS, DLS)
  - Analytical ultracentrifugation
  - Mass spectrometry

Typical Sample Quality issues

- **Aggregation of sample**
  even a few percent of aggregates in your sample will render your low angle data unusable

- **Sample not monodisperse**
  e.g. dimerization (or multimerization) of the protein, partial dissociation of complex, other contaminations

- **Buffer mismatch**
  buffer blank not exactly same as the buffer of the protein solution; blank buffer should be dialized or the run-off from the final SEC step

- **Strong particle interaction**
  particles have strong interaction even at low concentration due to unscreened charge or hydrophobic interactions

more sample issues (mostly at the beamline)

- **radiation damage**
  the X-ray radiation will cause some damage that most often leads to sample aggregation

- **Aggregation, dissociation or multimerization due to shipment or freezing-thawing**
  the freeze-thaw cycle necessary for shipping the samples to the synchrotron is often problematic; shipment at 4C can be a possible solution but is not without problems either

- **data unexpectedly noisy**
  sample concentration too low (because protein lost in filter or centrifuged out), salt concentration too high

So … what do I do if my sample is bad?

- centrifuge your sample and pipette from the top
- dilute and centrifuge
- filter (syringe or spin filter)
- add more/fresh DTT or TCEP if radiation damage is the problem
- run sample through SEC column if time and material permits
- change buffer condition (if you have enough material)
- change data collection protocol: shorter exposure, larger sample volume and oscillation, unidirectional flow, lower temperature
- consider SEC-SAXS data collection

Before coming to SSRL

- provide accurate information in the beam time request form ask beam line staff if you are unsure or have questions
- contact your beam line staff anyways before experiment just in case something changed

At the beamline

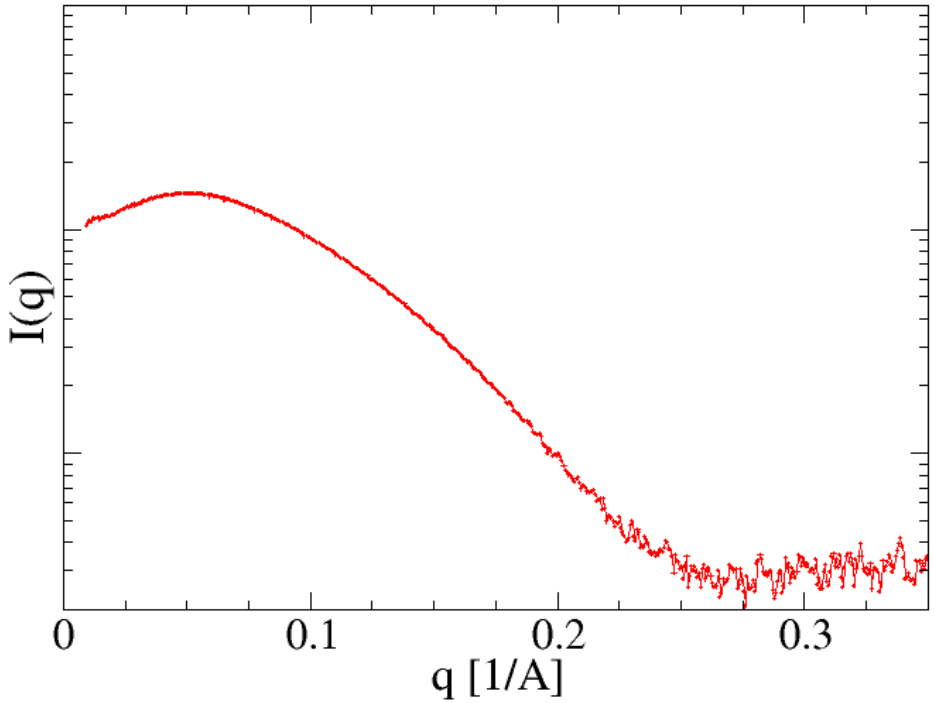
- understand how the data collection works and how to load your samples
- take plenty of buffer data and check for consistency
- take advantage of the online data reduction: pipeline

  monitor what's happening!
- consider sample recovery for post exposure analysis
- bring additional radical scavengers in case of unexpected rad. damage

Additional requirements for TR-SAXS

- lots of sample (at least 10mg, better more); the faster the reaction the more sample you need;
- concentration should be as high as possible, but without noticable interparticle interactions
- plenty of matched buffer (200 – 500 ml)
- sample well pre-characterized by static SAXS with sufficiently large change (e.g. several Angstrom in Rg) between initial and final state
- if possible pre-characterization of kinetics by other techniques to have a general idea of interesting time scale
- glycerol can help, but will likely slow down kinetics

What does "good" SAXS data look like?



- dipping down at low q
- repulsive interaction (S(q) < 1 at low q)
- concentration too high or salt concentration too low

Not good!

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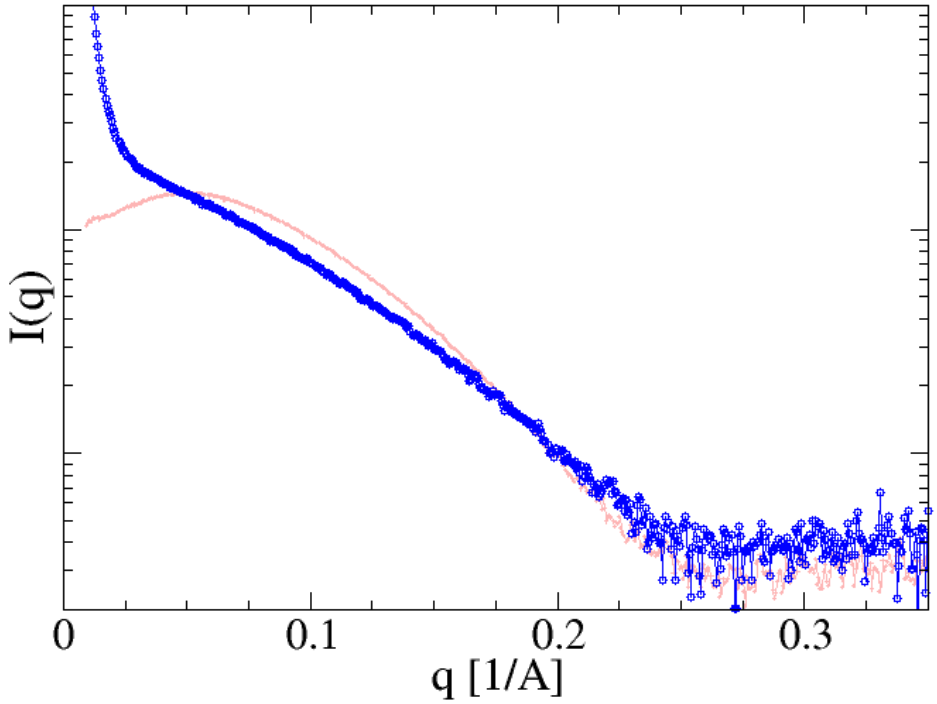
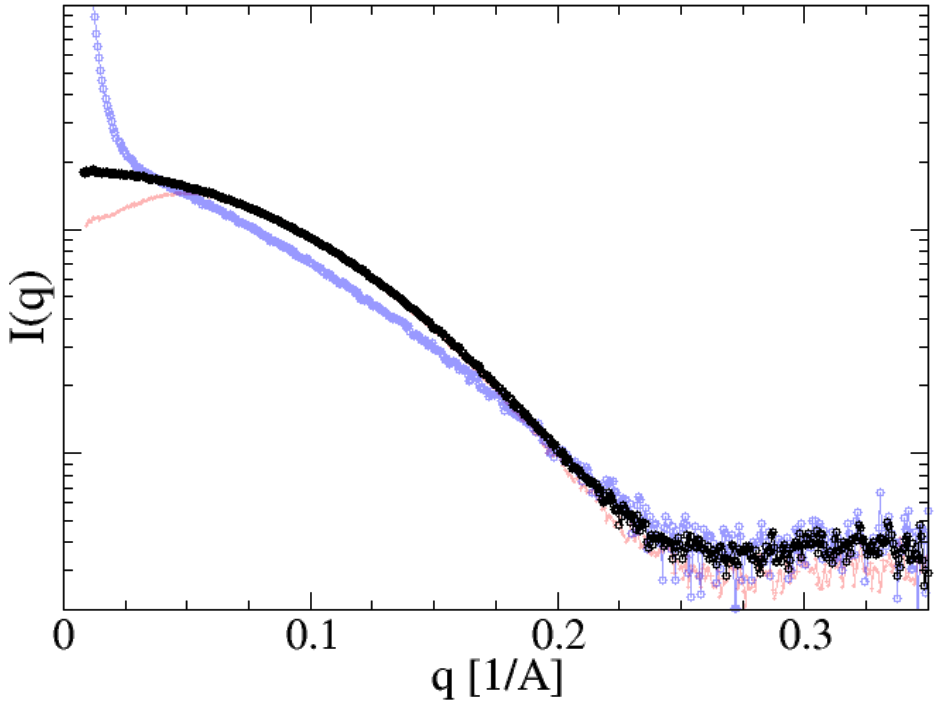


- steep increase at low q
- aggregation
  - Radiation damage
    - add glycerol, TCEP, DTT
  - sample not clean
    - Filter, centrifuge

Not good!

What does "good" SAXS data look like?



- looks good, but will need to check carefully if Guinier is straight, P(r) makes sense, …

Thank you