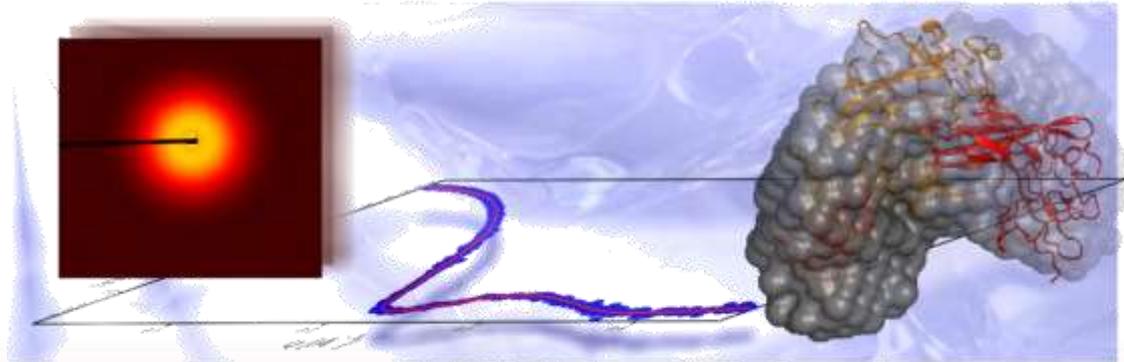


BioSAXS: Practical Considerations



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BioSAXS Workshop, March 28-30, 2016

Why SAXS?

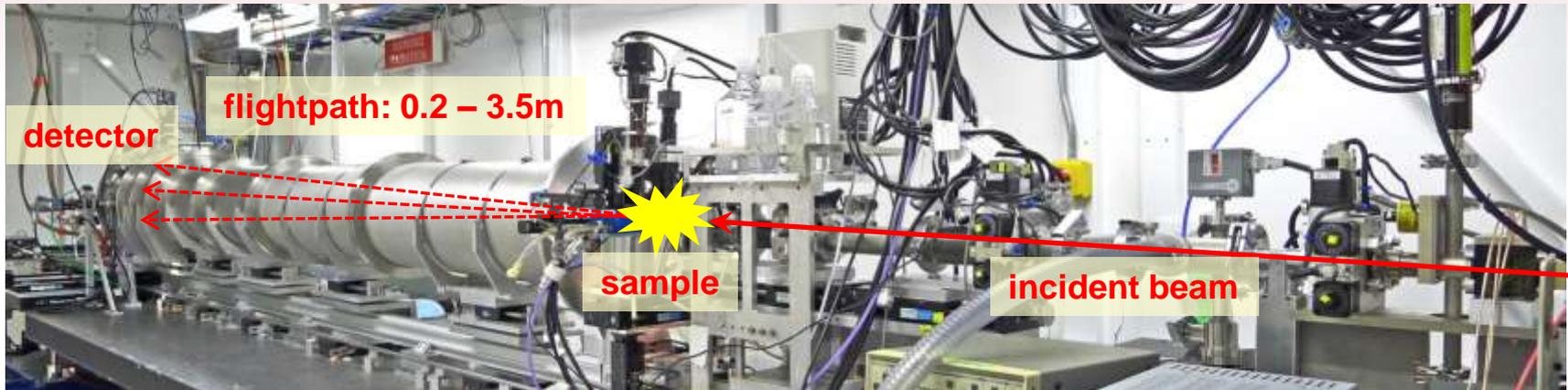
Structural information obtainable from SAXS

- Radius of gyration (globular, cross-sectional etc.) and Dmax
- molecular weight (monomer, dimer, multimer ..)
- pair-distance distribution function (real space representation)
- low-resolution envelope of molecule and ab-initio structures (about 1nm resolution)
- Rigid body (pseudo-atomic) models with high resolution components
- unfolded vs folded (Kratky plot)
- interaction potentials

Systems that can be studied by SAXS

- study protein at physiological conditions in solution (no crystals!)
- time-resolved studies possible (reaction kinetics)
- large protein complexes (no need for crystals)
- unfolded or partially folded proteins
- complex systems (protein-DNA, protein-lipid ...)

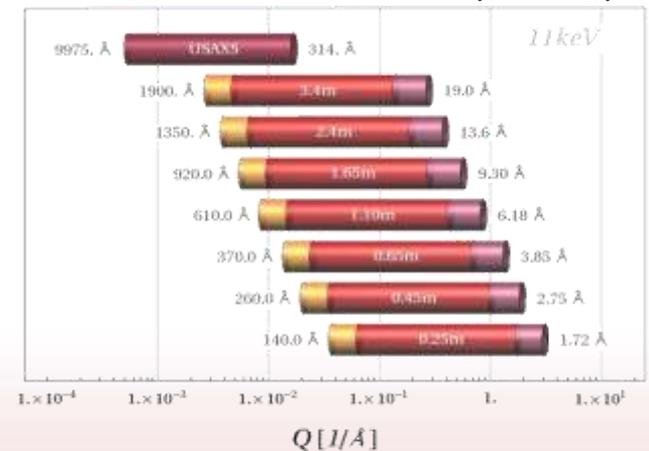
BioSAXS instrument at SSRL BL 4-2



- widely re-configurable instrument for
 - static and time-resolved solution scattering
 - lipid/fiber diffraction
 - grazing incidence scattering
 - anomalous scattering
- variety of advanced sample environments
 - solution scattering robot with attached analysis pipeline
 - in-line size-exclusion chromatography setup
 - stopped-flow mixer with low sample consumption
 - humidity chamber for lipid studies
 - high-throughput LCP screening setup

Q-range

Q = 0.003/Å ... 4.2/Å



- 7mm Beamsstop (Detector centered)
- 7mm Beamsstop (Detector off-center)
- 4mm Beamsstop

$$Q = \frac{4\pi \sin\theta}{\lambda}$$

$$D = \frac{2\lambda}{Q}$$

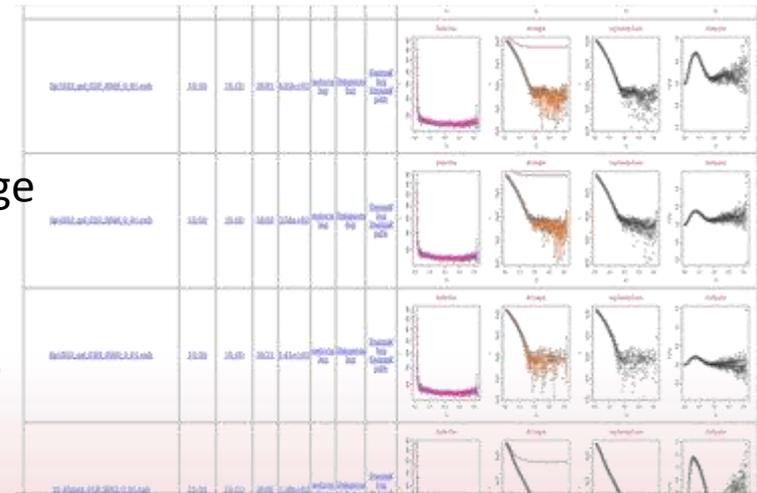
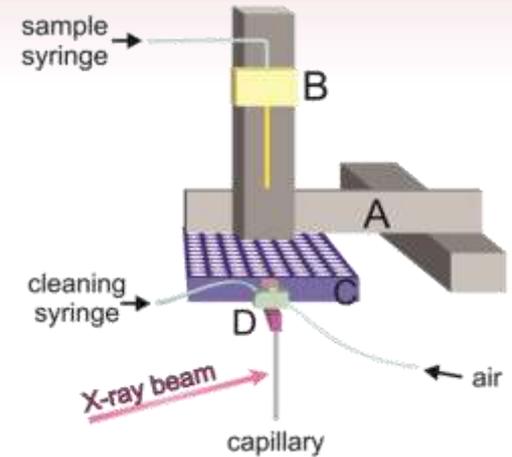
Automated Solution SAXS

Sample delivery robotics: autosampler

- 96-well microplate format (temperature controlled)
- compatibility to DLS plate reader
- *In-situ* fiber-optic UV/Vis spectrophotometer
- all hardware under Blu-Ice control (SolSAXS – tab)
- ~30 μ l sample aliquot, 3.5min per sample

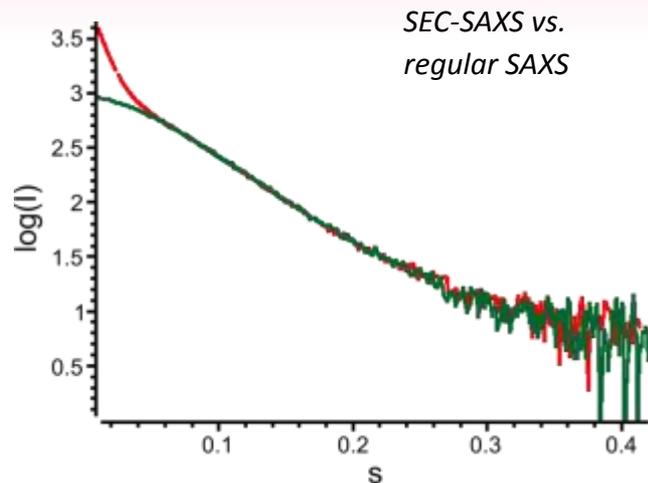
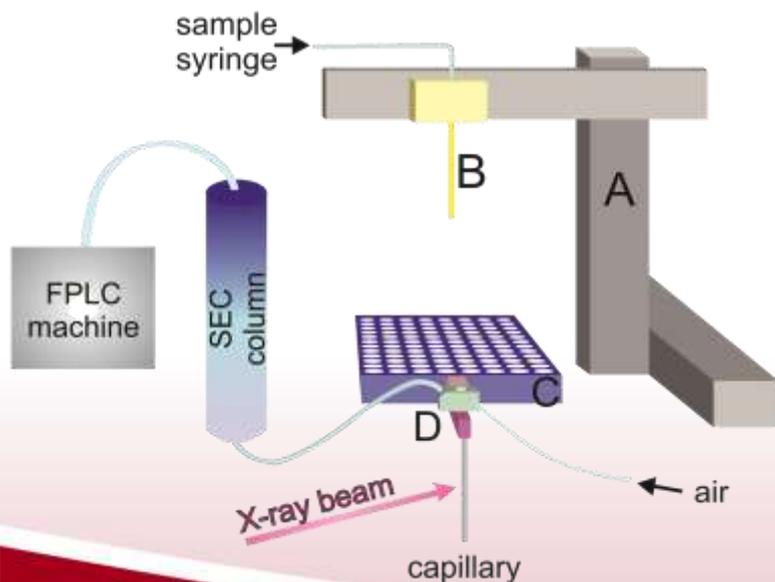
automated data analysis pipeline: SAXSPipe

- runs in the background without user input
- does buffer subtraction, checks for radiation damage
- basic analysis (R_g, I₀, Guinier and Kratky plot, P(r) calculation)
- output displayed in (clickable) html-table (viewable with any html-browser)

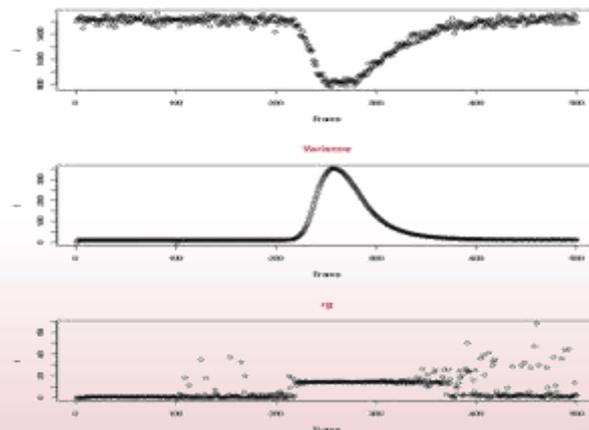


Chromatography coupled SAXS

- sample measured directly off the column
- typically 100 μ l sample at 4mg/ml
- about 40 min per sample
- 6-8 x-ray exposures (1s) in central peak
- UV/vis integration
- automatic R_g and $I(0)$ determination
- compatible with autosampler hardware

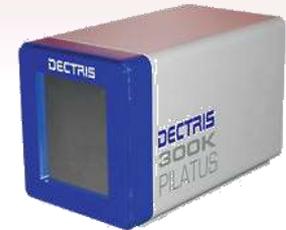
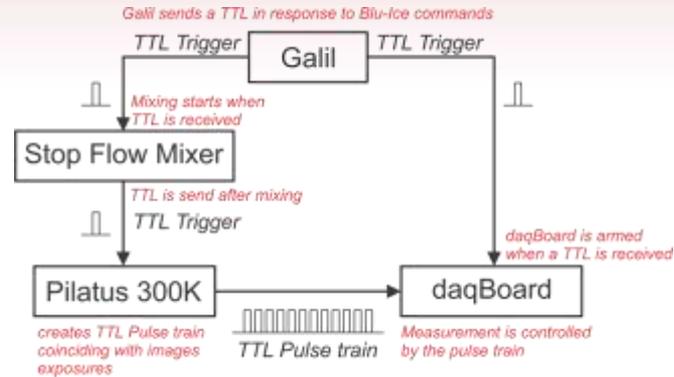


online monitoring: UV, $I(0)$, R_g



Time-resolved Solution SAXS

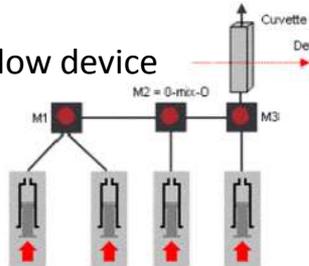
- high flux multilayer Monochromator
- using PCI data acquisition boards for detector trigger and intensity monitoring
- all hardware under Blu-ICE control
- dedicated Blu-ICE interface for TR- experiments
- fast PAD detector Pilatus 300k
- currently: time resolution 5ms



- 200Hz frame rate
- photon counting

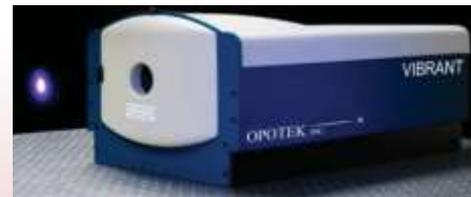
Stopped-flow Mixer

- Biologic SFM-400 stopped-flow device
- 30 μ l min injection
- Variable flowrate
- >0.25ms deadtime
- Variable mixing ratios



Photoreactions and T-jump (in preparation)

- Opotek Vibrant 355HE tunable laser
- wavelength range: 410 nm- 2400 nm
- peak energy 45mJ, 5ns pulse duration
- computerized control
- photoreactions and T-jump experiments



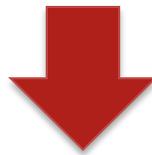
Basic prerequisites for SAXS analysis

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

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

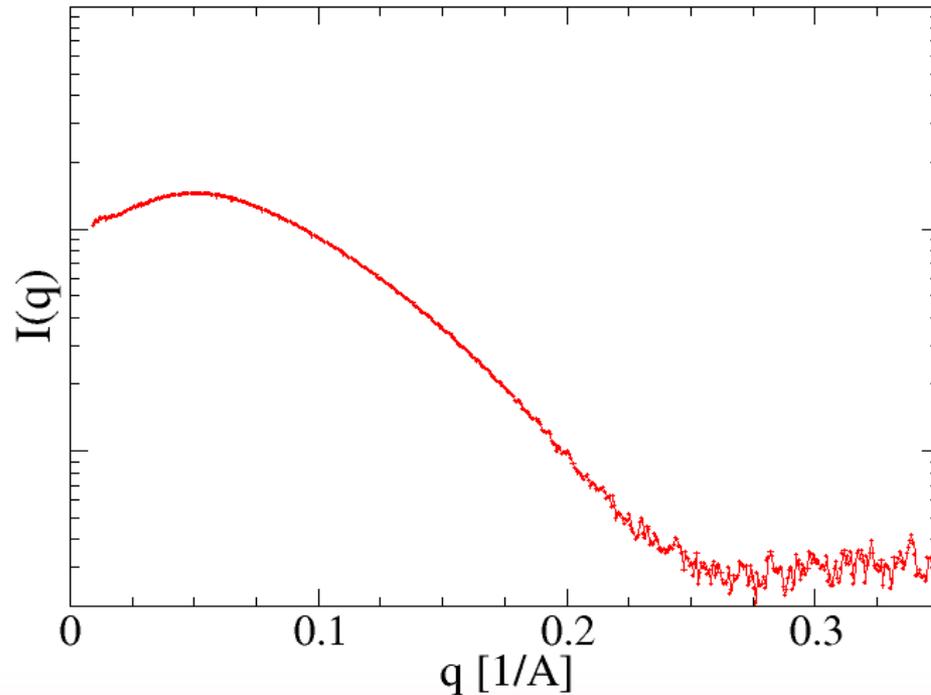
- **Particles Uncorrelated**, i.e. no inter-molecular interactions present

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



$$I(q) = Ni_1(q)$$

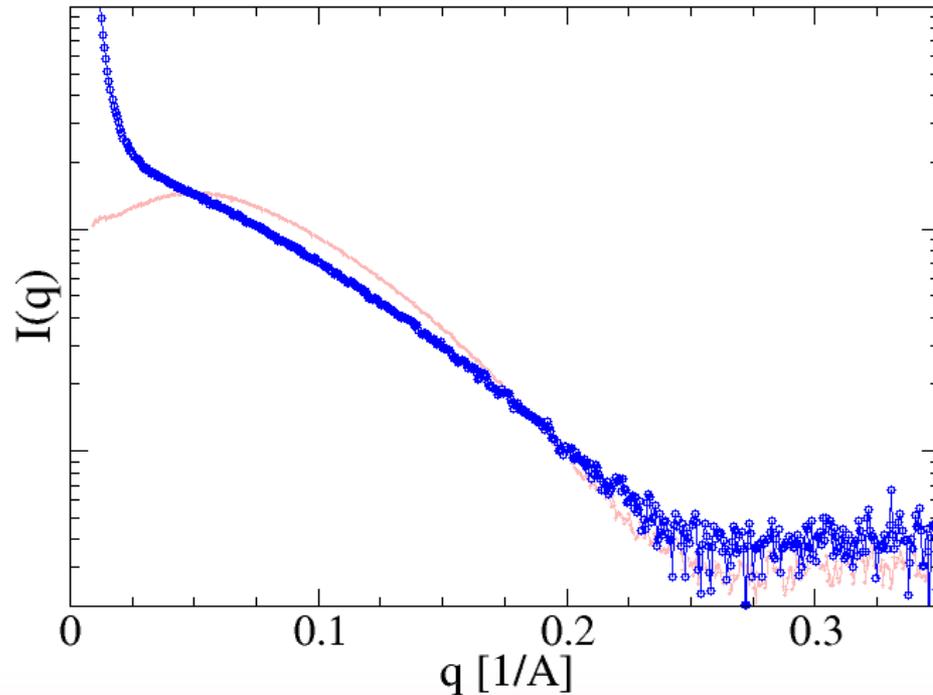
What is “good” SAXS data?



not this ...

- dipping down at low q
- repulsive interaction
- concentration too high or salt concentration too low

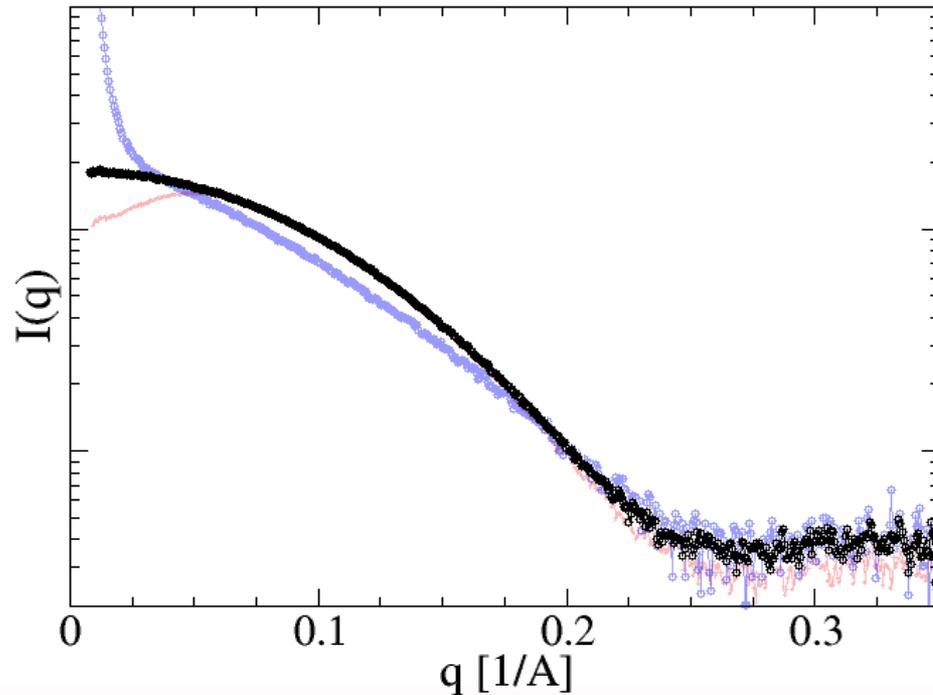
What is good SAXS data?



... neither that!

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

What is good SAXS data?



... this (maybe)

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

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 at all possible, if absolutely needed use well below CMC
- simulate transport to beamline to check what is the best way to ship
- Know your numbers: MW, concentration of stock solution ...
- talk to the beamline 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 take informed decisions at the beamline on possible ways to make your protein cooperate

Sample requirements for solution scattering

- size: >5kD
- purity: **highly monodisperse !**
- concentration range: 0.25 – 10mg/ml
 - **Rule of thumb:** Molecular weight [kD] * concentration [mg/ml] \approx 100
- sample volume 15-30 μ l per concentration (for Autosampler data collection)
- enough material for at least 3 concentrations
- 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 from rad. damage
- also glycerol (~5%) might be useful to keep your protein from aggregating

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
- Avoid detergents; if absolutely needed use well below CMC
- bring plenty of matched buffer

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 noticeable 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 R_g) 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

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 and 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 dialyzed 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 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

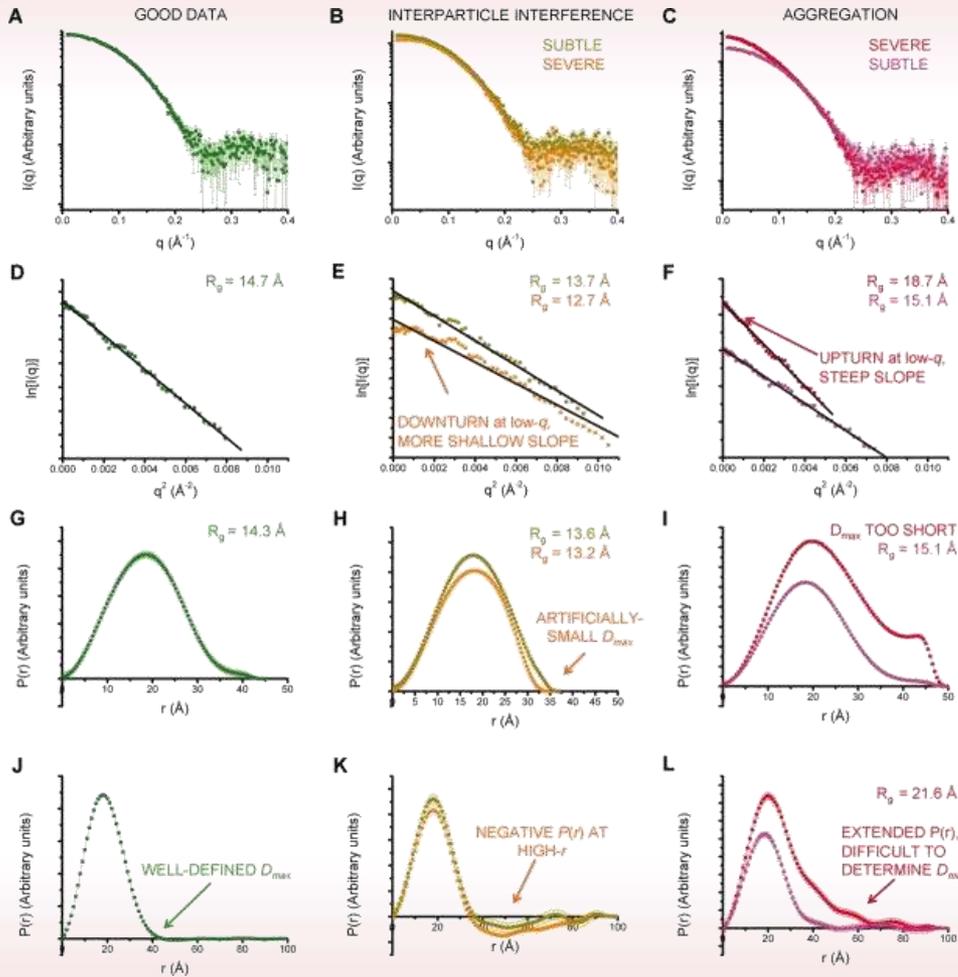
Before coming to SSRL

- provide accurate information in the beamtime request form ask beamline staff if you are unsure or have questions
- contact your beamline 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

Data quality at the beamline



David A Jacques and Jill Trehwella
 Protein Sci. 2010 April; 19(4): 642–657.

Immediate data quality checks:

- aggregation:
 - upturn at low q
 - residuals in guinier plot will show upward curvature
- interparticle repulsion:
 - downturn at low q
 - residuals in guinier plot will show downward curvature
 - will increase with concentration

Checks with the $P(r)$ function

- determine D_{max}
 - no “nose-diving” !
 - no excessive oscillation around 0
 - rule of thumb:
 - $D_{max} \approx 3 * R_g$
 - Switch off $P(d_{max})=0$ and use large D_{max} to estimate
- determine R_g
 - should compare well with R_g from Guinier

Thank you