Better Data with SEC-SAXS

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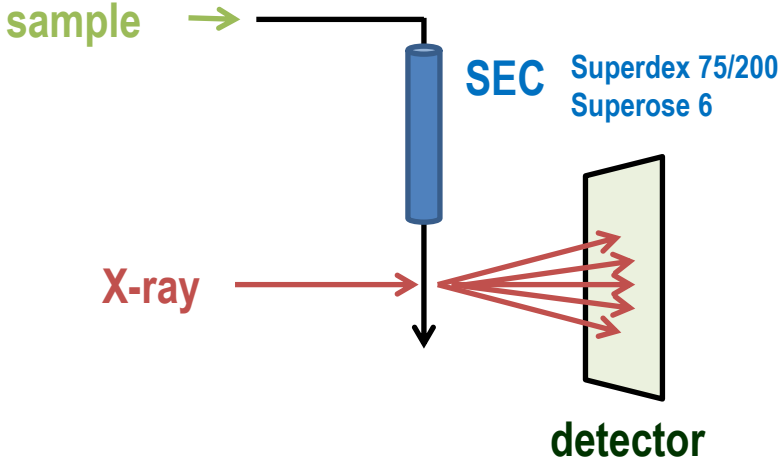
SSRL

Structural Molecular Biology

Online Size Exclusion Chromatography SAXS (SEC-SAXS, FPLC-SAXS)

- Very powerful tool for problematic sample
- Column volume: 2.4ml
- Required Sample volume: 25-100µl
- Sample concentration: 5-20mg/ml
- Flow rate: 50µl/min
- 1 sec exposure per image (5sec repetitions)



Standard setup at BL4-2

- 11keV, 500mA
- 0.3x0.3mm beam
- 1.5 mm cap
- 1.7m distance
  (q_{max}=0.4-0.55)
  (R_g <~75Å)
- Room temperature

Blu-Ice SEC-SAXS tab

Why SEC-SAXS (FPLC-SAXS)?

- Very powerful tool for problematic sample having:
  - Aggregates which are frequently generated at re-concentration of sample
  - Mixture or multimer
  - Complex with weak kd
- Less inter-molecular (inter-particle) interactions at the same concentration. (Less concentration-dependence)
- Accurate background subtraction.

Difficulty

- Accumulation of radiation-damaged sample on capillary (sample cell).
- Large sample consumption.
- Concentration issue.
- Needs exhaustive cleaning of the capillary after data collection.

SEC-SAXS setup at BL4-2

SSRL BL4-2: Bio-SAXS/D Station

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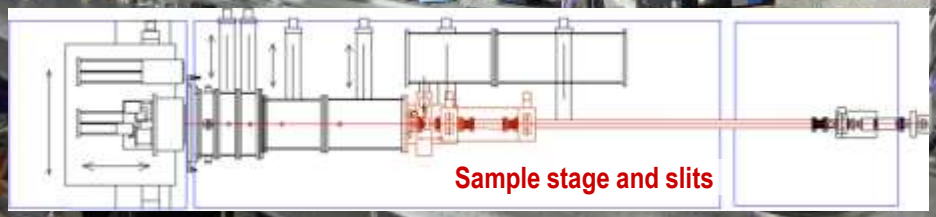


Pilatus 300K
(200Hz PAD)

Rayonix MX225-HE
(wide area CCD)

- Interchangeable 7 sample-detector distances: 0.25m to 3.5m (Semi-automatic)
- Rapidly interchangeable monochromators: Si(111) and Mo/B_4C multilayers
- Extended energy range: 5-17keV (Si(111) + multilayers)
- Beamstop with integrated photodiode.
- Pinhole camera
- Two interchangeable detectors: Rayonix MX 225-HE (for static) and Pilatus 300K (for TR)
- Customized Blu-ICE control system for the whole instrumental hardware
  (including optics, detectors and sample environments)

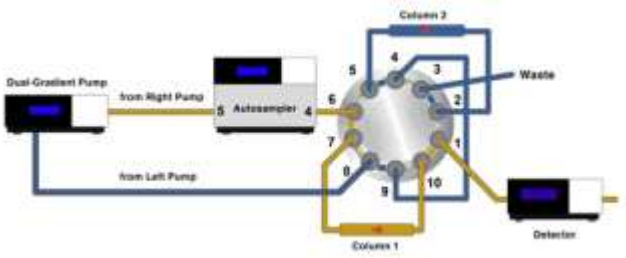
Chromatography at BL4-2

Akta Ettan FPLC (GE Healthcare Life Science)

- 2 pumps (2 solutions)
- 3 UV/Vis detection
- 50-100ul sample loop
- Software: Unicorn

UltiMate 3000 UHPLC (Thermo Scientific)

Coming soon!



- 2 pumps (6 solutions) w/ degasser
- 4 UV-vis channels (up to 200 Hz)
- Auto-sample changer
- Tandem analyses for HPLC high throughput
- Refractive index detector
- Software: Chromeleon

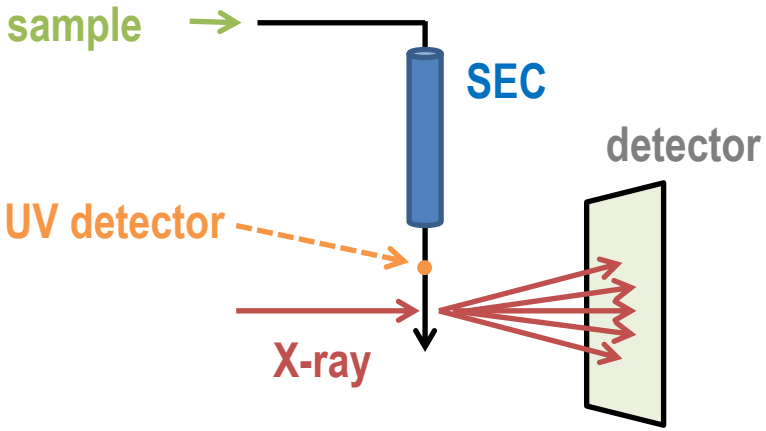
Available SEC column at BL4-2

	Mw range (kDa)	Sample volume (ul)	Bed Volume (ml)	Standard flow rate at BL4-2 (ml/min)
Superdex 200 PC 3.2/30 (old)	10 – 600	2-25	2.4	0.05
Superdex 200 PC 3.2/30 (new)	10 – 600	4-50	2.4	0.05
Superdex 200 Increase 3.2/300	10 – 600	4-50	2.4	0.05
Superdex 75 PC 3.2/300	3 - 70	4-50	2.4	0.05
Superose 6 PC 3.2/30	5-5000	2-25	2.4	0.05

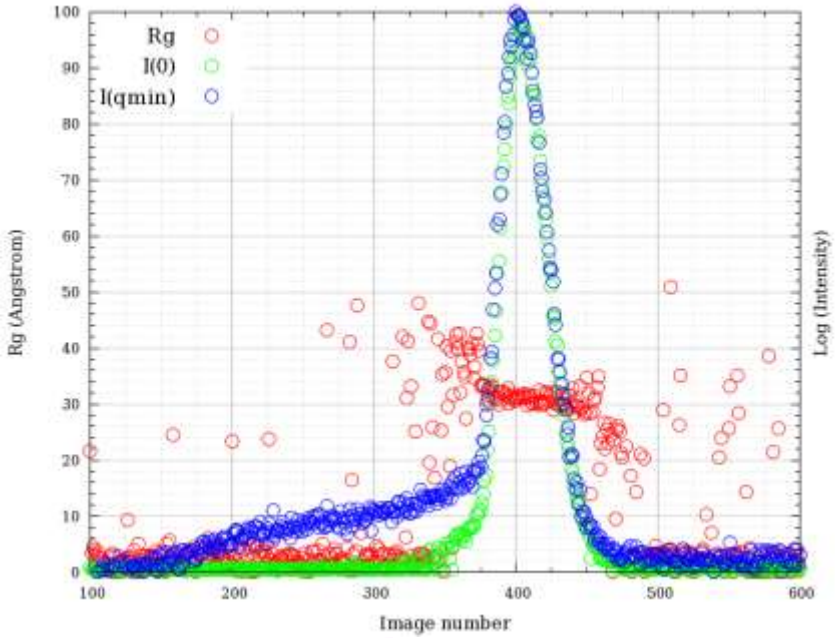
- Small column (2.4ml) is used to minimize dilution.
- Elution profile would be different from that with large column.
- Just to remove aggregates from monodisperse & monomodal sample (single peak), 100ul injection generally works great.
  - Dilution factor at X-ray position: ~60-70%
  - Good for low concentration sample.

Data Collection and analysis at BL4-2

SEC-SAXS: UV and X-ray profiles

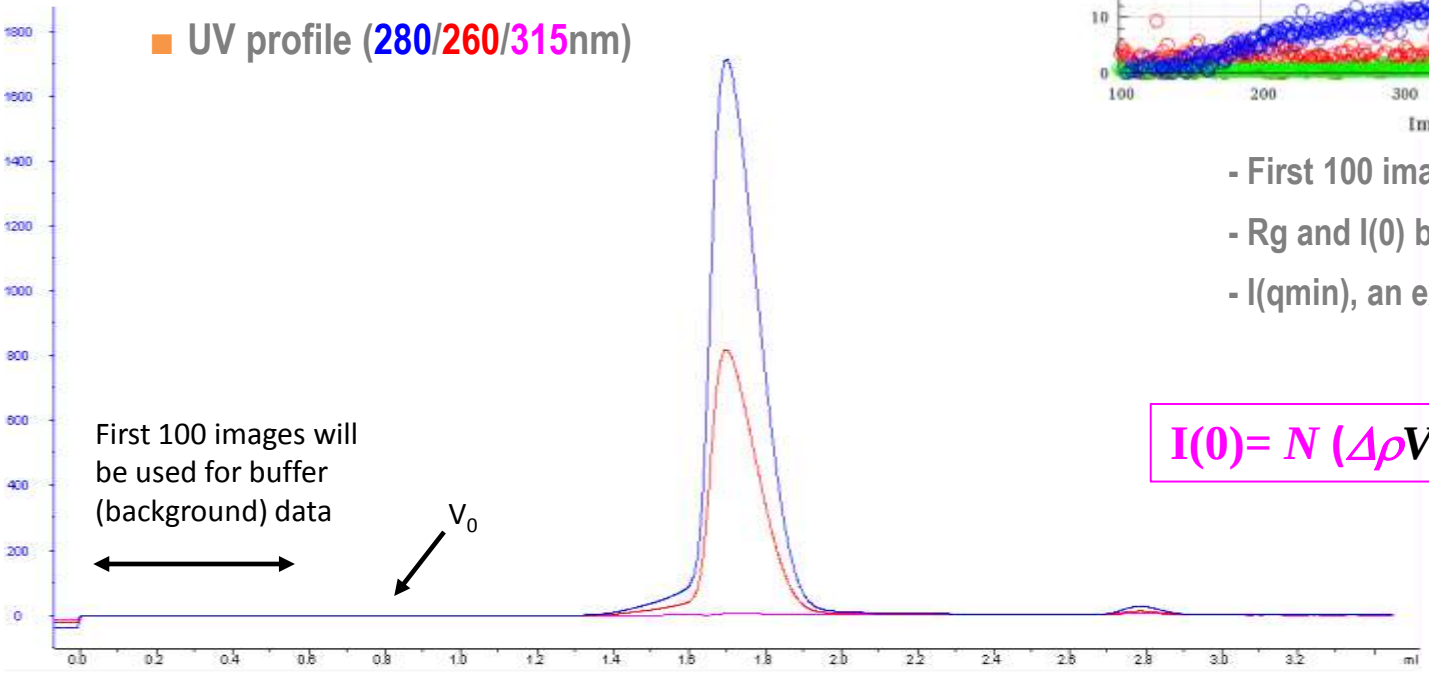


■ X-ray profile



- First 100 images are used for blank.
- Rg and I(0) by Guinier analysis.
- I(qmin), an experimental intensity at low q.

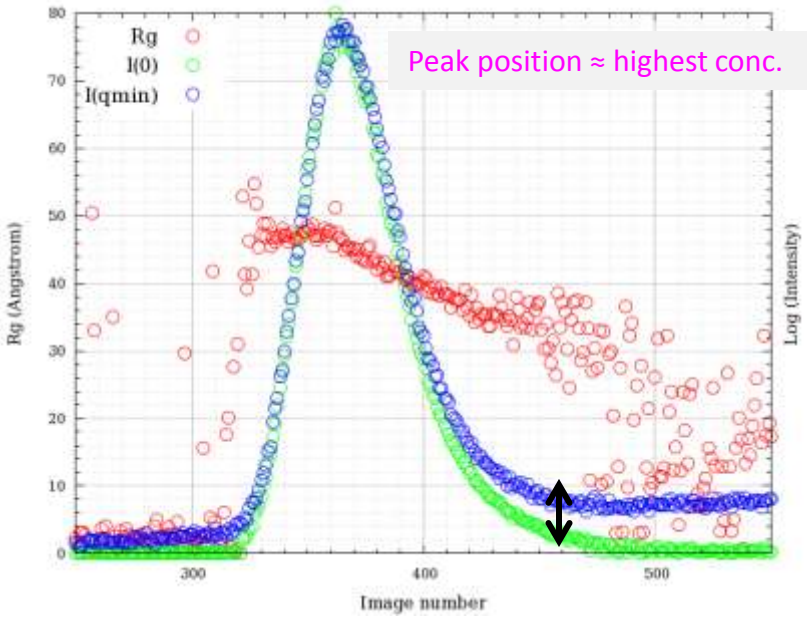
■ UV profile (280/260/315nm)

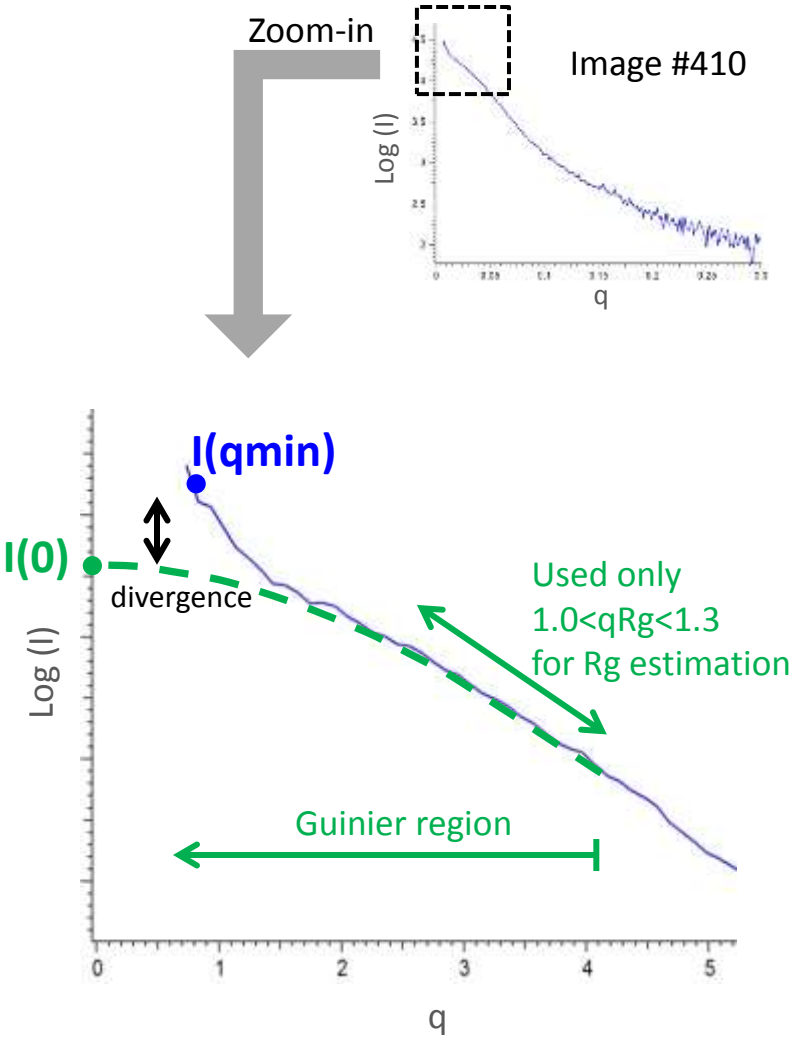


$$I(0) = N(\Delta\rho V)^2 = C\Delta\rho^2 v^2 Mw/N_A$$

SEC-SAXS: Detection of Radiation Damages

- SEC-SAXS X-ray profile

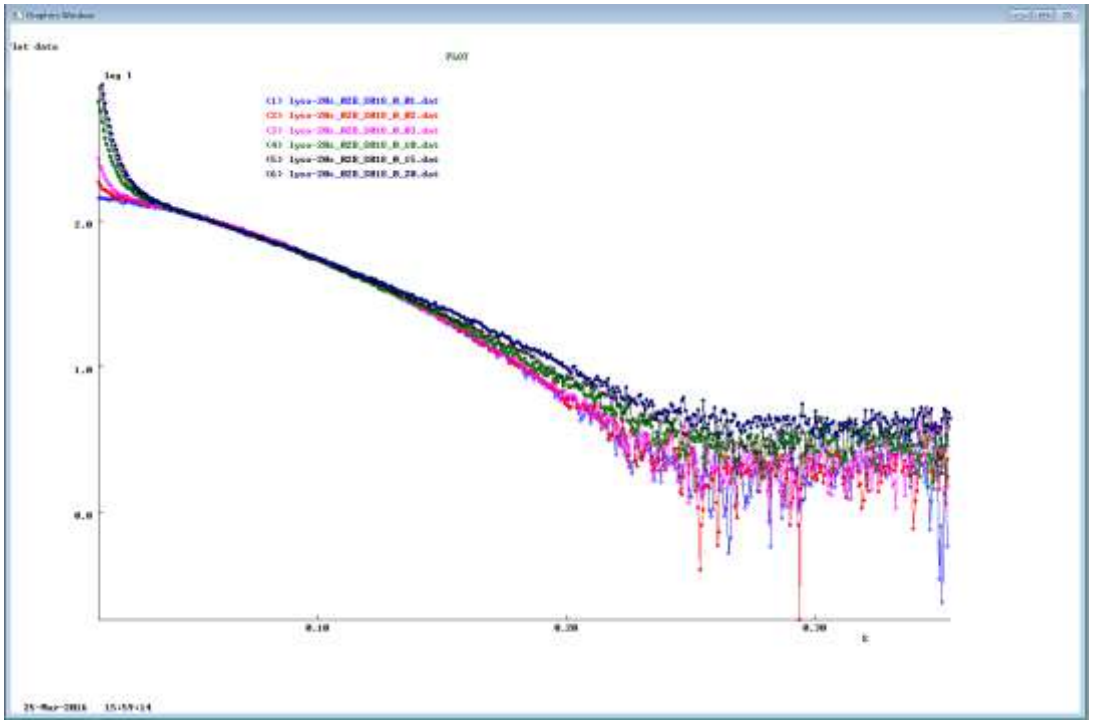




- Radiation-damaged sample makes capillary dirty.
- Accumulations of such a dirt mess all data.
- Needs powerful cleaning after data collection.
- 1.0<qRg<1.3 is used for Guinier analysis.
- Script is available at BL4-2
- This is only used for a quick data check at beamline. Needs careful inspection when you analyze data.

Radiation damage

- Lysozyme: 1sec x 20 images. No DTT in buffer.



- Lysozyme with multilayer beam (flux: approx. x30 higher)

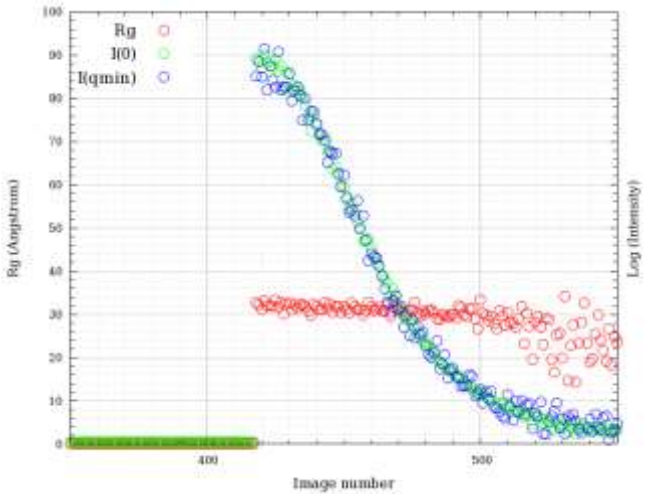
- Radiation damaged sample frequently adheres to capillary cell.
- Add radical scavenger (e.g. 5mM DTT) in sample and buffer.

SEC-SAXS: Minimize Radiation Damages during Data Collection

Capillary dirt by radiation-damaged sample is the biggest problem for SEC-SAXS.

Very effective:

- Radical scavenger. (e.g. 5mM DTT)
- Higher energy beam .
- Shutter control.
- No re-concentration after purification.
  (aggregates easily make capillary dirty)



Worth a try:

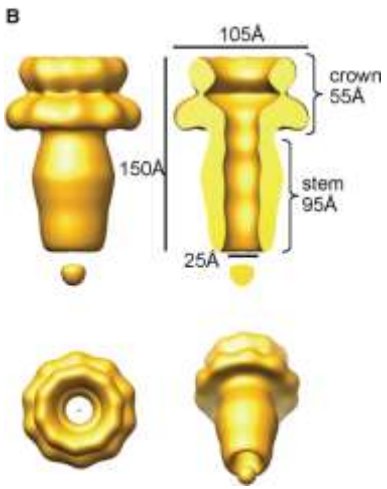
- Faster flow rate.*
- Longer interval between shots.*
- Lower sample concentration.

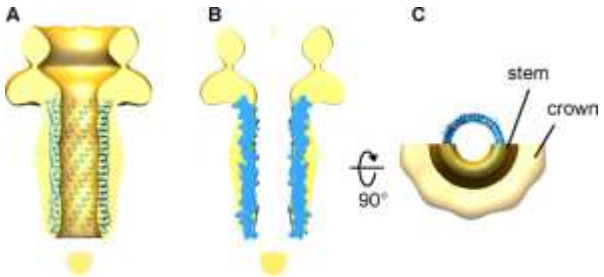
* Would be limited by detector mode

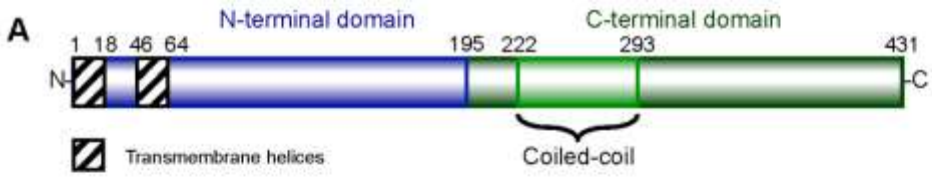
Example: Data Interpretation

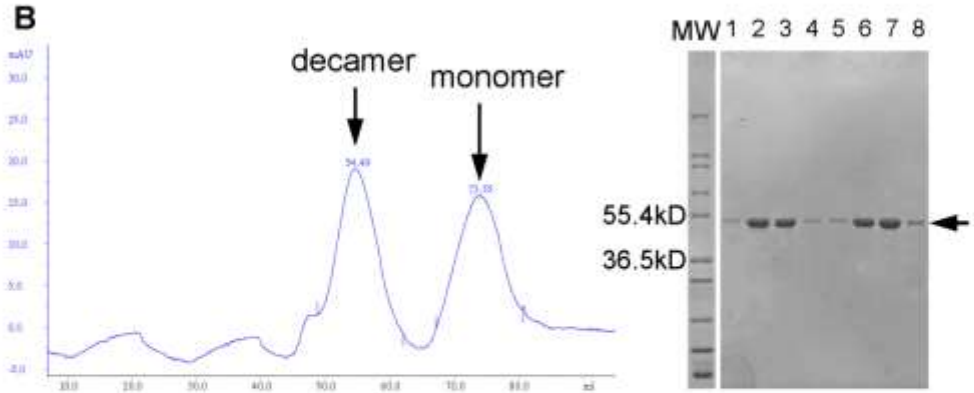
Almost all SEC-SAXS data are confidential and unpublished.

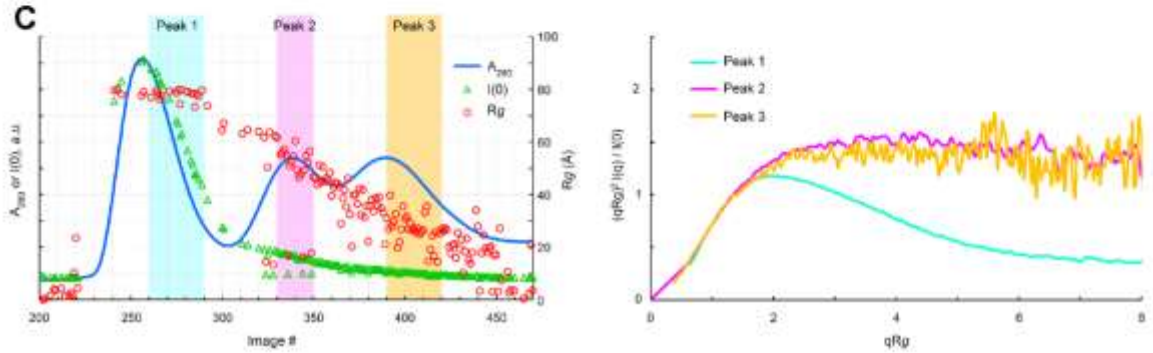
SEC-SAXS: DNA injection protein complex of bacteriophage







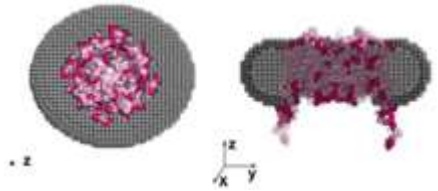


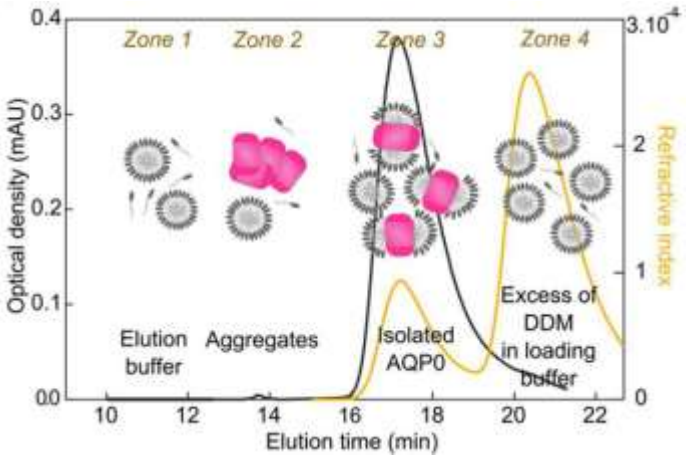


Zhao, et al., 2016, PLOS One,
DOI: 10.1371/journal.pone.0149337

SEC-SAXS: Complemental techniques with SEC-SAXS

- RI detector (Refractive Index)
- MALS detector (Multi Angle Light Scattering)
- CD (Circular dichroism)
- Viscometer
- Fluorescence Detector





Berthaud, et al., (2012) J. Am. Chem. Soc

Acknowledgements

SSRL BL4-2

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