

Building and Optimizing a SAXS Instrument from the Basics—Lessons Learned



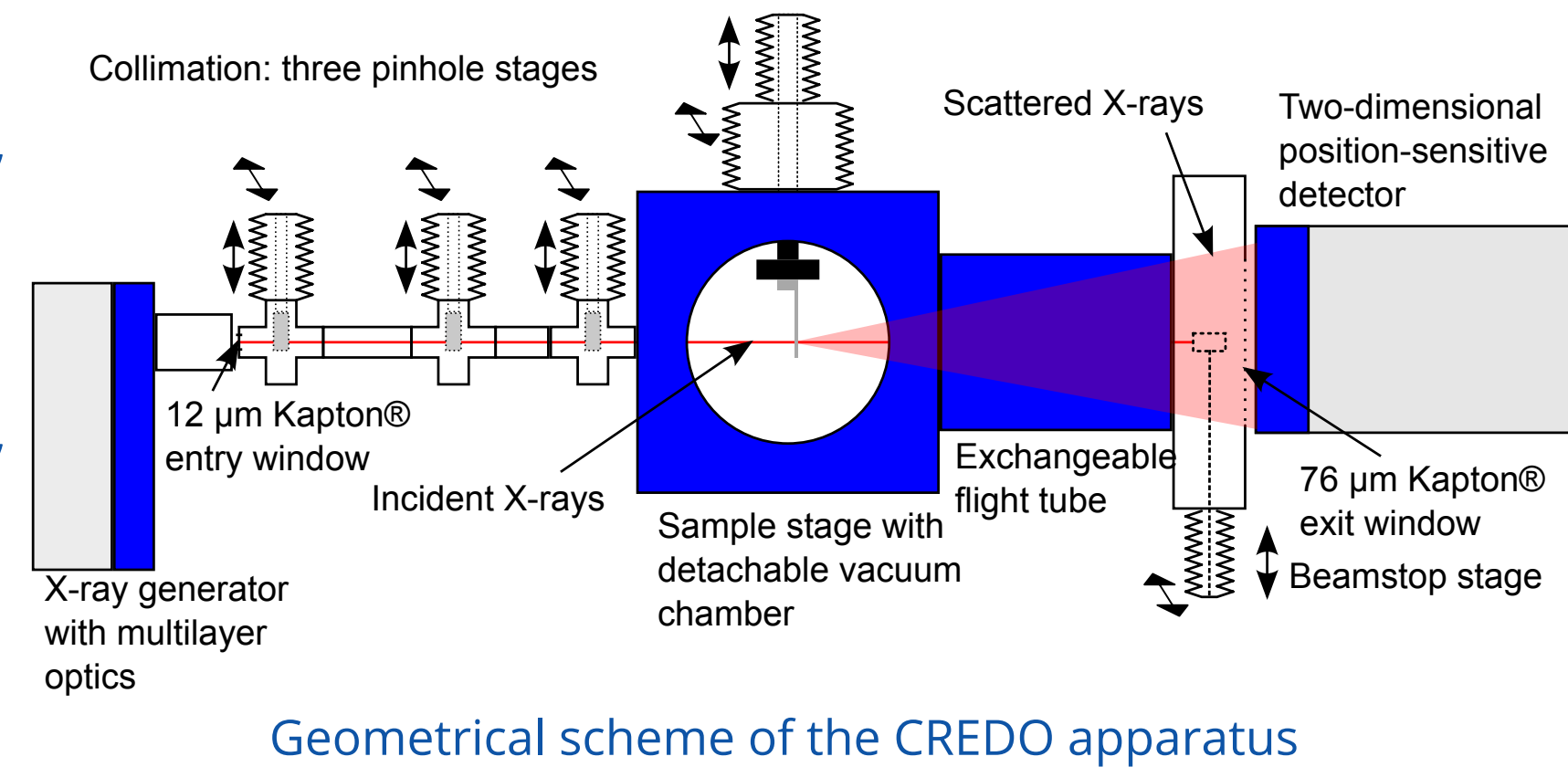
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Because the low X-ray flux in laboratory apparatuses, it is essential to do the experiments under optimal geometrical conditions, enabling measurement in a given range of the scattering variable (defined as $q = 4\pi \sin\theta / \lambda$) in the shortest time possible and with the least instrumental distortion. Furthermore, as most of the frequently studied systems (e.g. biological macromolecules or lipid systems) are weak scatterers, the signal-to-noise ratio has to be drastically improved by increasing the beam intensity and lowering the instrumental background.

Here we present CREDO (Creative Research Equipment for Diffraction), our recently constructed in-house SAXS facility, as well as some useful ideas to make the most from a laboratory small-angle scattering apparatus. More information about the facility is available at its homepage, <http://credo.ttk.mta.hu>.

Overview

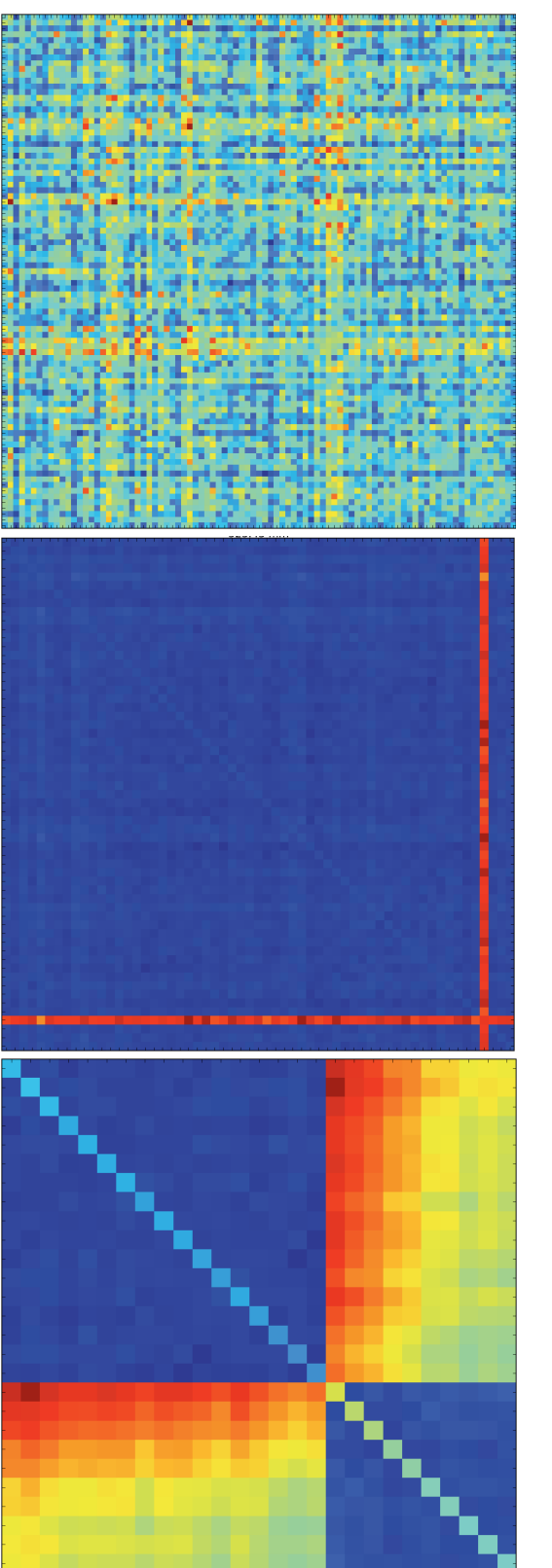
- X-ray source: GeniX^{3D} Cu ULD (Xenocs, France), 30W microfocus, Cu K α
- Detector: Pilatus-300k (Dectris, Switzerland), two-dimensional, CMOS hybrid pixel
- Attainable q-range: 0.019 nm⁻¹ to 30 nm⁻¹
- Sample-to-detector distance: 72 mm to 2.5 m in discrete steps
- Sample requirement <5 μ l (aqueous)
- Typical beam size at the sample: 0.8 mm (min. 0.2 mm)
- On-line data reduction with calibration into absolute intensity units against a pre-calibrated glassy carbon



Sample and instrumental stability

- Many short exposures with frequent sample change and frequent measurement of absolute intensity standard (glassy carbon)
 - following changes in flux: monitor counter can be spared
 - monitoring changes with **correlation matrices**
 - time-resolved experiments
- Processing of reduced data in a browser-based notebook interface (IPython, jupyter):
 - on-line: instant feedback during measurement
 - reusable code: plotting, fitting, data interpretation can be re-executed as more data are obtained
 - self-documenting workflow

$$\Delta_{ij} = \frac{\sum_k [I_i(q_k) - I_j(q_k)]^2}{\sum_k [\sigma_i(q_k)^2 + \sigma_j(q_k)^2]}$$

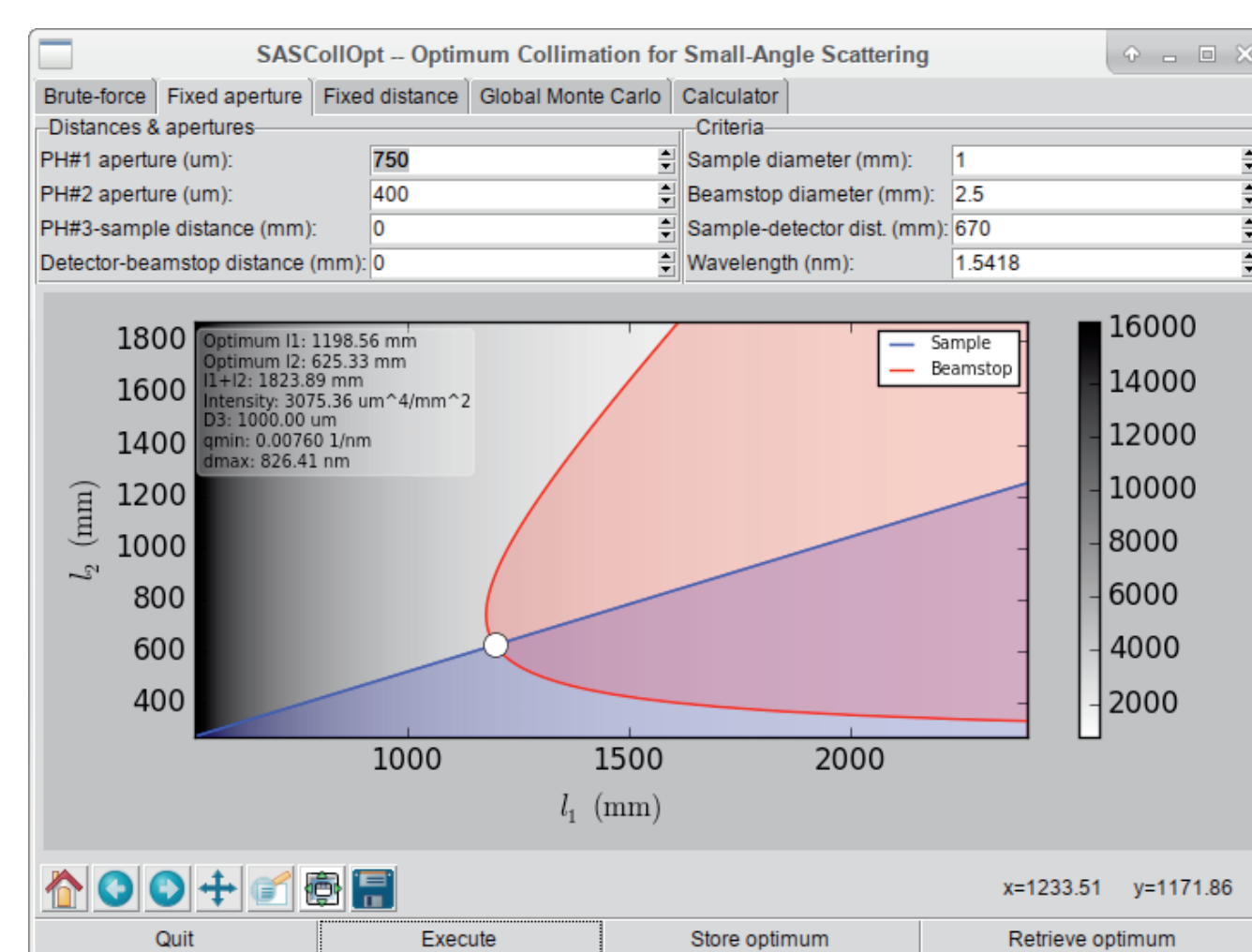


Correlation matrices of a sample stable throughout the measurement (top), a series of exposures, one of which is affected by cosmic radiation (center) and a sample undergoing a sudden change (bottom)

Optimal collimation

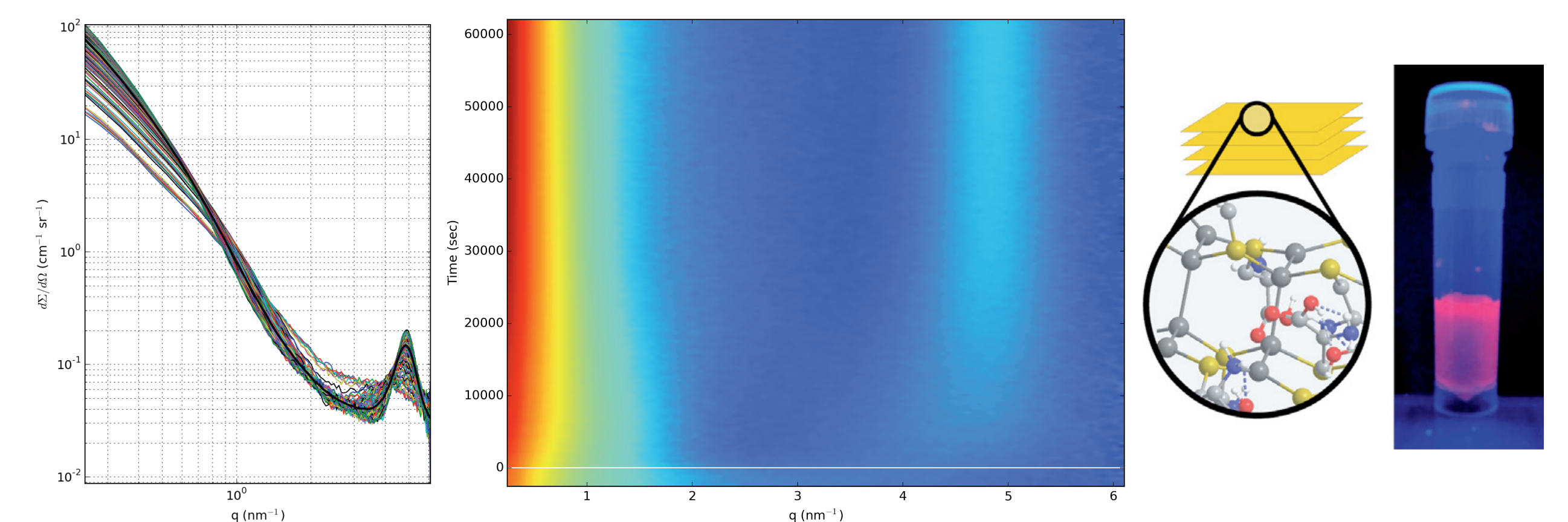
Analytical approach for determining the optimum collimation geometry of the three-pinhole scheme:

1. give constraints on **sample and beamstop diameter** (lowest q),
2. list setups (pinhole sizes and spacings) with **no parasitic scattering** outside the beamstop,
3. select the one with the **highest flux**.



Screenshot of the SASCollOpt.py program

The procedure has been implemented in a stand-alone program. Straightforward alignment: independent XY motorized positioning for each pinhole

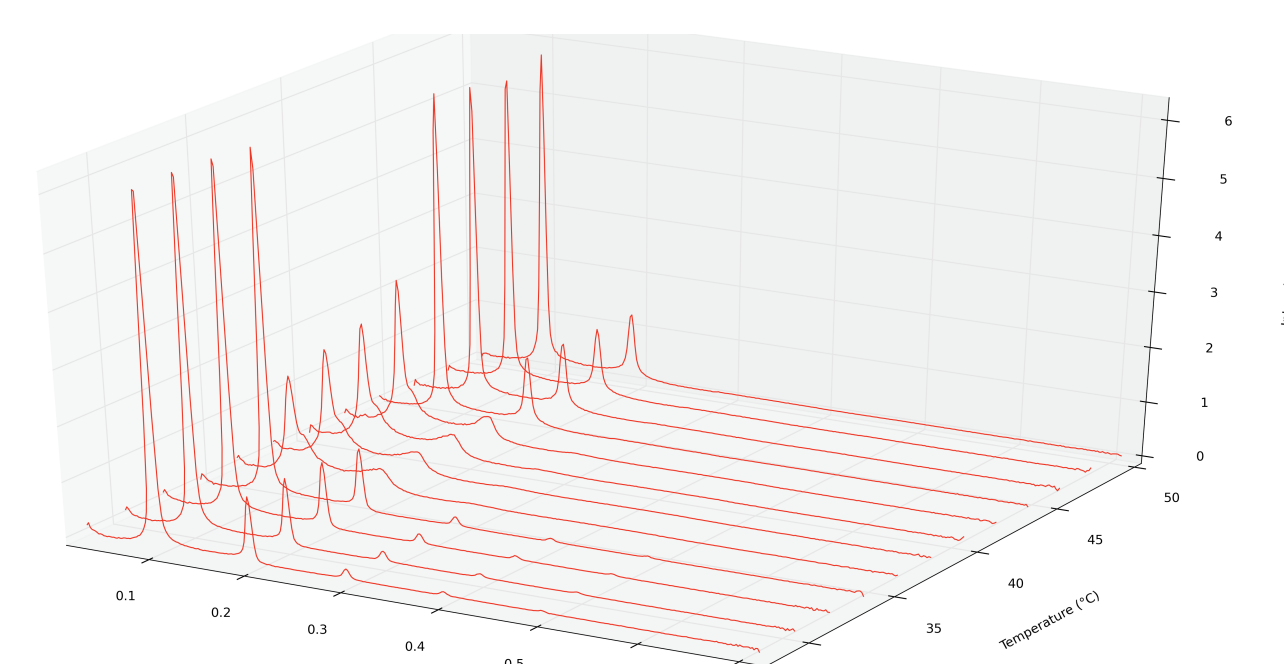


In situ time-resolved study of the self-assembly of a red-emitting gold-cysteine supramolecular complex

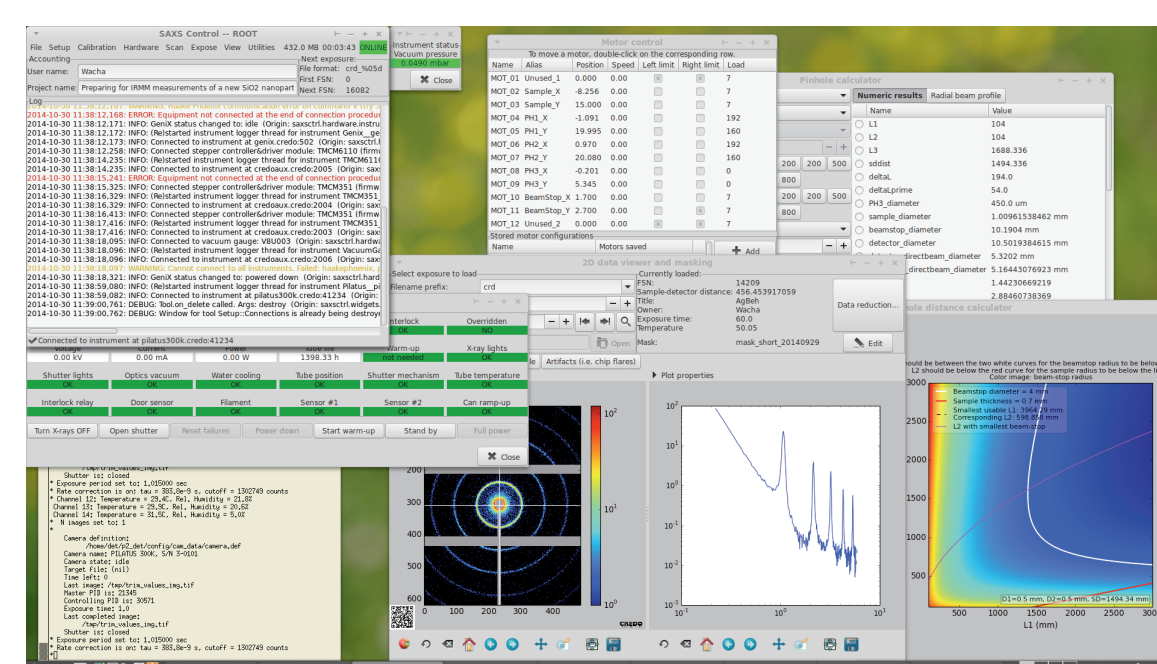
Extensive automation

Computer-controlled hardware (X-ray source, detector, motors, vacuum gauge, heater/cooler stage etc.)

- Motorized XY sample stage
 - sequential measurement of **several samples**: minimizing the need for user interaction
 - **position-resolved** experiments
 - frequent automatic recalibration
- Motorized collimation (pinholes, beamstop): **easy re-alignment**
- **In situ** measurements with tunable external parameters (temperature, shear strain etc.)
- **Logging** of all possible quantities: post-hoc diagnostics, reproducibility
- After loading the samples, the instrument **can be controlled remotely**, even over the internet
- In-house developed instrument control software (SAXSctrl)
 - intuitive **graphical user interface**
 - simple **command language**
 - interactive, command-line use
 - **scripts** for unattended operation



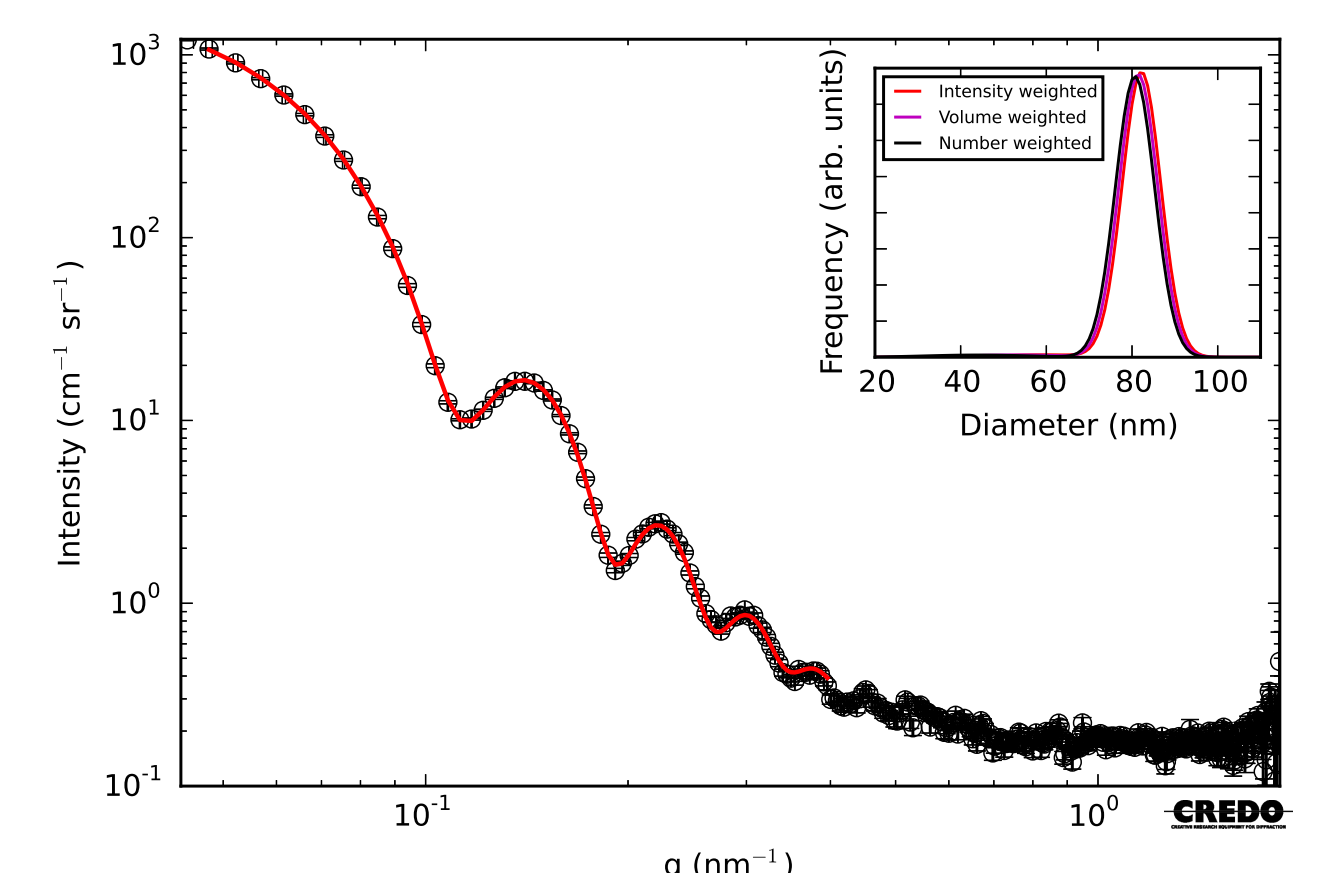
Effects of guest molecules and other external parameters on the thermotropic phase transitions in multilamellar phospholipid vesicle systems



Further examples

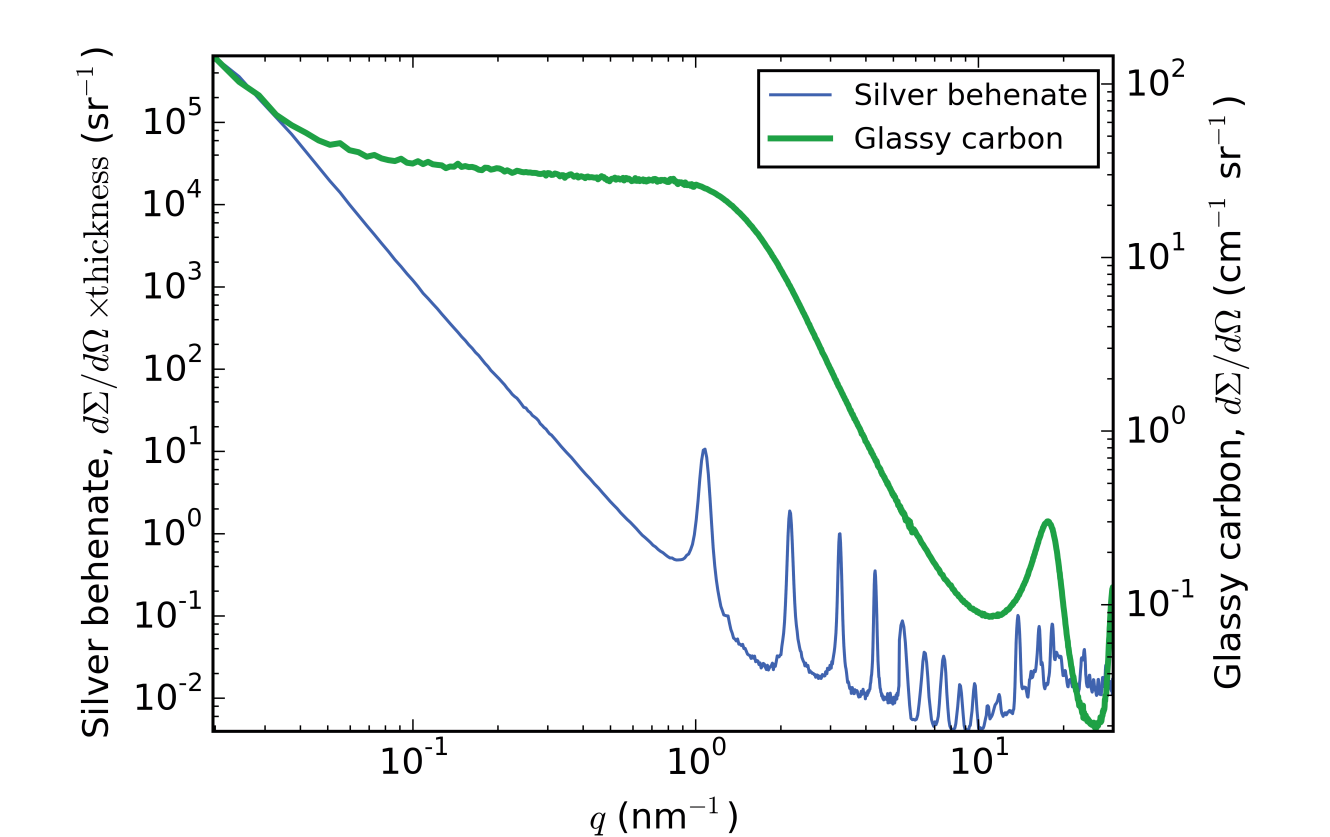
Traceable determination of the size-distribution of nanoparticles

- Interlaboratory comparison study of a new reference material
- Determination of size distribution: Guinier, monodisperse sphere, Gaussian ensemble of spheres, Monte Carlo
- CREDO has been certified by IRMM, Joint Research Centre, EC



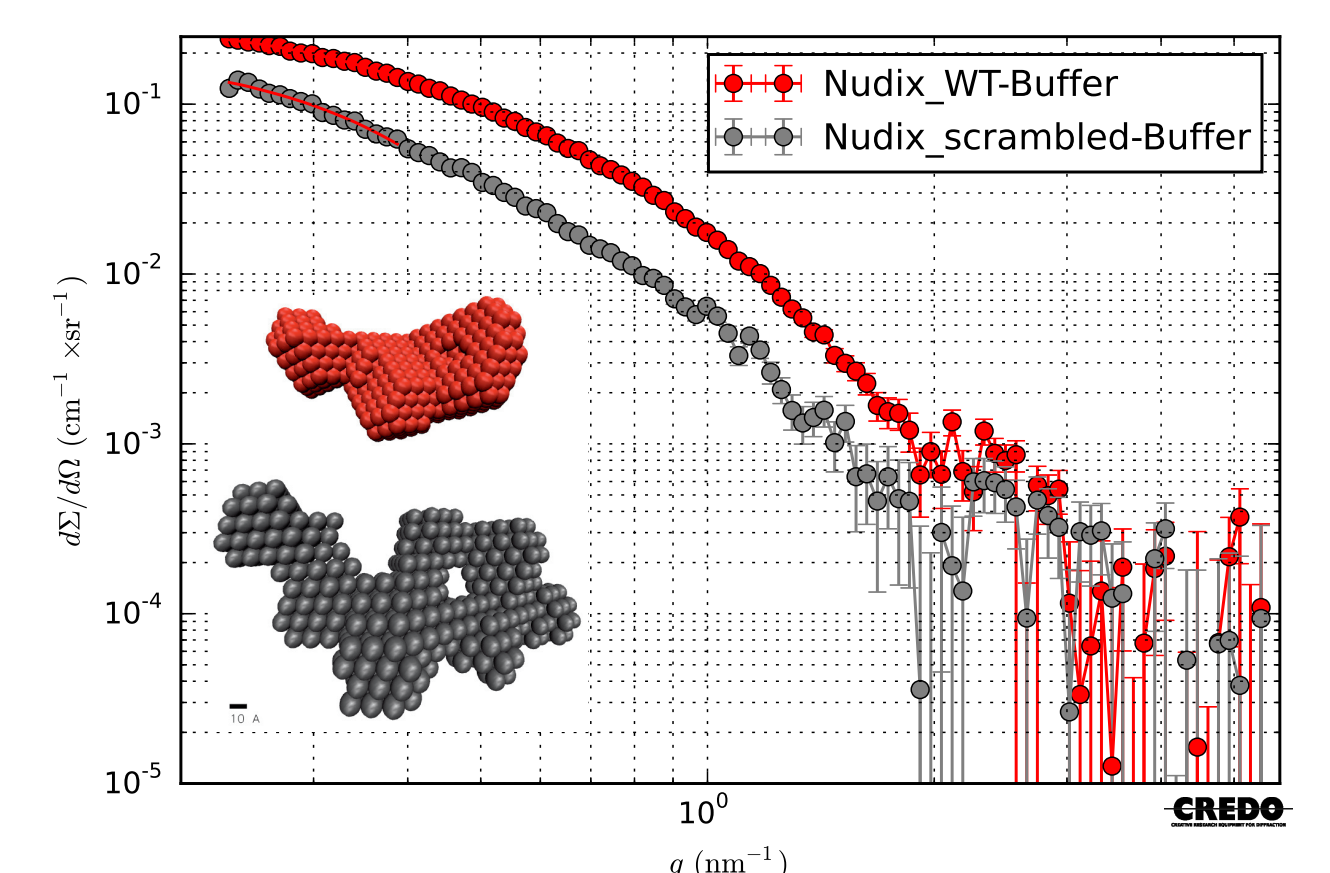
Full accessible angular range

- Continuous measurement in the Bragg-size-range of 0.2 nm to 340 nm (to be increased)
- The full range can be covered in three set-ups (incl. WAXS)
- Usually no scaling needed for merging curves



Weakly scattering samples

- First in-house BioSAXS: de-anchoring of the N-terminal tail of a Nudix hydrolase enzyme
- Shape reconstruction via dummy atom model (DAMMIN): still experimental
- Measurement time: 6-8 h + buffer



References

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Realization of the facility has been funded by Gedeon Richter Plc, by the Hungarian National Scientific Research Fund (grant nr. CNK 81052) and by the Central Hungarian Operative Program (KMOP-1.1.2-07/1-2008-0002). Studies on proteins were supported by the MEDINPROT grant (A. Bóta, 2014-2015). The help of Attila Mészáros, Ágnes Tantos, Balázs Söptei, as well as all our numerous former and present colleagues in Hungary and worldwide is gratefully acknowledged.

CREDO
CREATIVE RESEARCH EQUIPMENT FOR DIFFRACTION



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