Characterization of protein solutions using SAXS on a multipurpose laboratory X-ray diffractometer

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Summary

Small-angle X-ray scattering (SAXS) applied to dilute protein solutions has become an accepted and rapidly growing structural biology technique. It provides information e.g. about the overall protein size and shape, folding and unfolding, aggregation behavior, stability and molecular weight. Measurements can be done under native conditions, and under variation of e.g., protein concentration, pH, ionic strength or temperature. SAXS ultimately also allows for a low resolution molecular shape envelope reconstruction and delivers information that is complementary to that obtained from XRD or NMR.

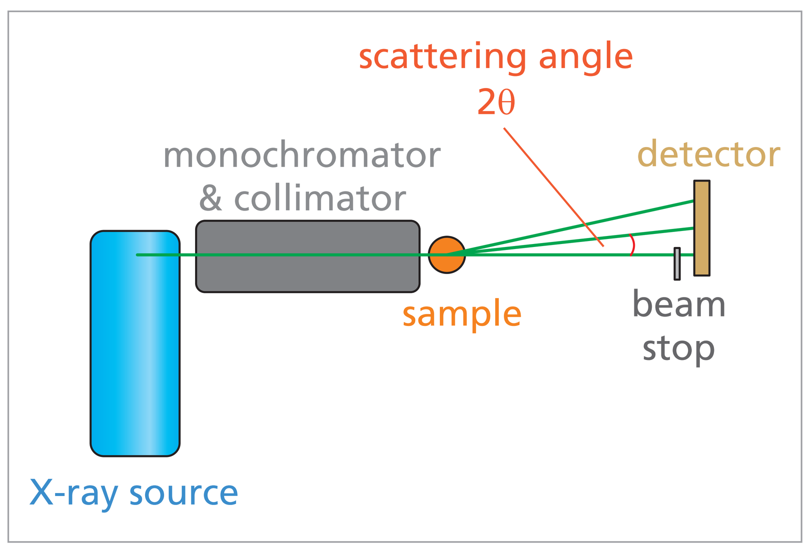
Although the SAXS technique is conceptually rather simple, it is demanding with respect to the instrumentation and thus required dedicated, costly lab instruments or the usage of synchrotron beamlines. The technique has thus not been readily accessible. We recently developed an economical solution that allows to easily configure a multipurpose XRD platform (Empyrean with ScatterX^{78}; PANalytical) for protein SAXS measurements. The Empyrean is widely used in labs for general material research and characterization.

Here we will give an introduction to SAXS, explain how a general XRD platform can be configured for SAXS experiments, and demonstrate its performance on glucose isomerase as a standard protein.

About small-angle X-ray scattering (SAXS)

SAXS is one of the most versatile tools to analyze nanoscale structures and dimensions on a variety of sample types (liquids, powders, solids, gels...) having an amorphous, crystalline or semi-crystalline structure. Typical samples that are investigated with SAXS include colloidal dispersions, surfactants, polymers, **biomacromolecules**, membranes, nanocomposites, nanopowders and porous materials.

An intense, monochromatic and highly collimated X-ray beam is directed on a capillary that contains the sample (e.g. a dilute protein solution). The intensity that is scattered by the sample as a function of the scattering angle, typically up to 5 deg, is recorded with an X-ray detector.



Traditionally SAXS experiments have been done on dedicated laboratory instruments or at synchrotron radiation facilities.

SAXS applied to biological macromolecules (Bio-SAXS)

SAXS yields complementary information to e.g. single crystal X-ray diffraction, NMR and cryo-EM:

- Overall size and shape of the protein molecule
- Folding and unfolding
- Protein-protein interaction
- Protein stability; detection of aggregates
- Molecular weight
- 3D envelope structure (low resolution) in solution

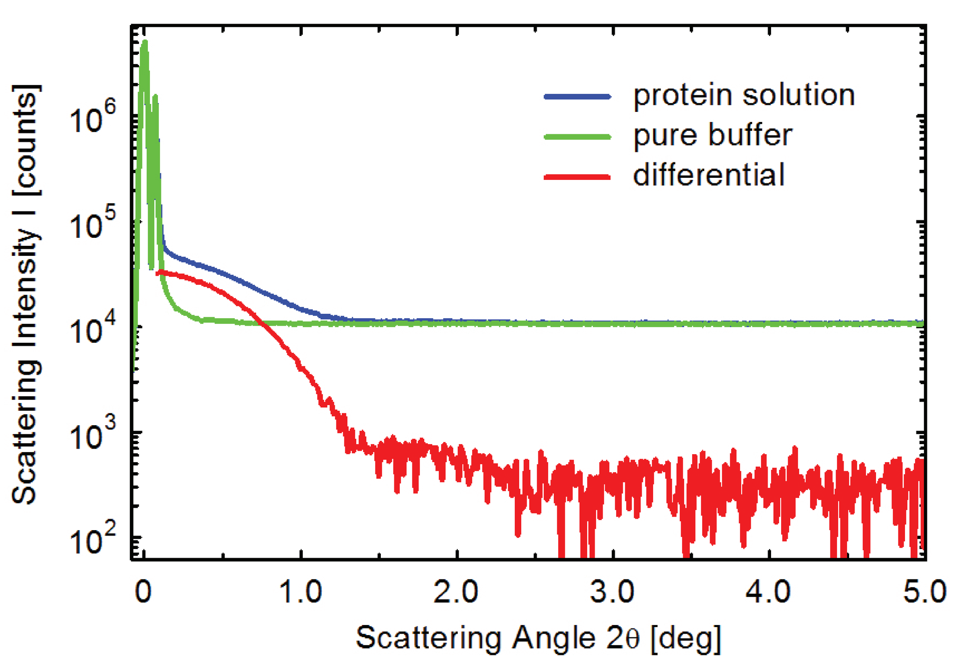
All properties are obtainable *in situ*, with the proteins under near-physiological conditions and as a function of e.g. pH, salt concentration, temperature, etc.

Protein sample and buffer requirements

- Pure and monodisperse (> 95%)
- Remove non-specific aggregates (filtration, ultracentrifugation); confirm by DLS
- Avoid oligomeric mixtures (separate by GPC)
- Protein concentration: 5-15 mg/ml; determine by spectrophotometer @ 280 nm or refractometer
- Sample amount: > 30 µl (can be recovered after the experiment)
- Buffer: 100-500 mM salt; pH buffer 10-100 mM; surfactants only if < 2 CMC; max. 5% glycerol
- Use last dialysis buffer for best match with sample
- Buffer amount: several ml

Beside the measurement of the protein solution it is also necessary to measure the pure buffer solution. The weak scattering signal due to the protein molecules is given by the differential. The latter is used for further data analysis to obtain information about the protein structure and dimensions.

Example: glucose isomerase
The figure on the right shows such measurements from a 11 mg/ml solution of glucose isomerase in a buffer made of 100 mM Tris and 1 mM $MgCl_2$ at pH 8.0.



Bio-SAXS experiments on a multipurpose XRD platform

Based on a commercial laboratory X-ray diffractometer platform (Empyrean, PANalytical), we developed an experimental configuration that allows to acquire high-quality SAXS data with a good small-angle resolution ($2\theta_{min}$ = 0.08 deg) , high intensity and a low background signal. It enables sensitive measurements even of weakly scattering samples, such as dilute protein solutions.

The configuration uses a Cu X-ray tube with a focusing X-ray mirror, an evacuated beam path module with beam conditioning elements (ScatterX^{78}) and a line detector that measures scattering intensities simultaneously at the different angles. The sample is confined in a 1 mm disposable quartz capillary and inserted in the center of the diffractometer. All modules that are used on this platform are pre-aligned and easy to use.

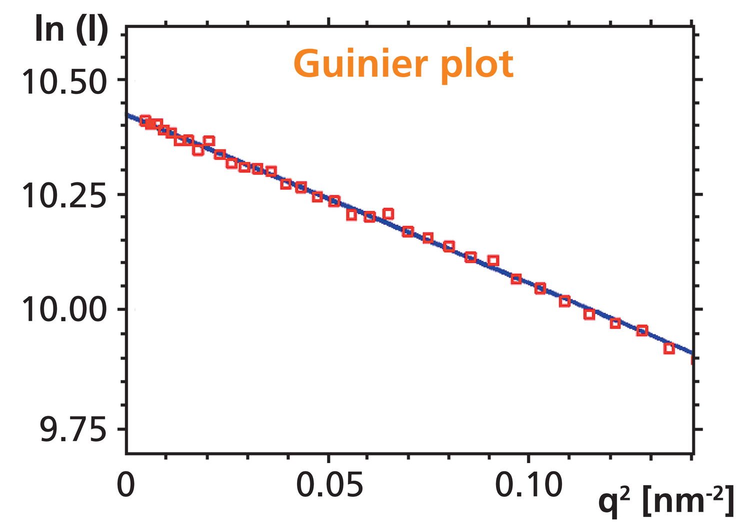
Data analysis was done with EasySAXS software (PANalytical) as well as with some software modules [1,3] developed by D. Svergun et al.

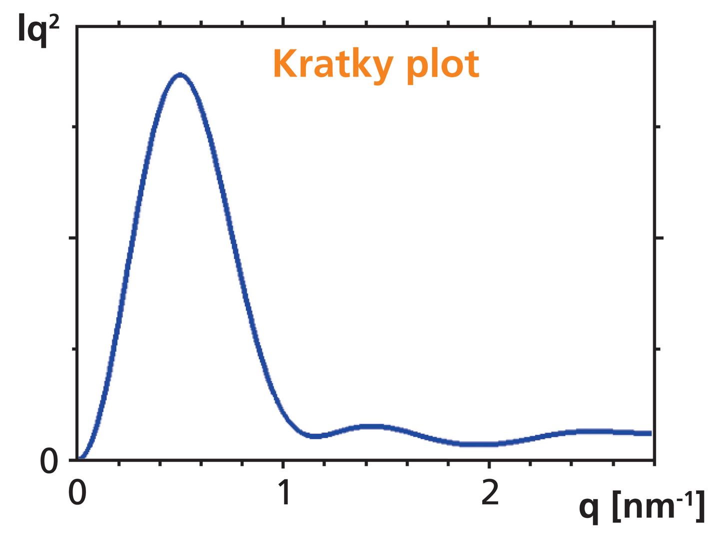
The same XRD platform can be quickly and easily reconfigured for other techniques, such as powder diffraction, X-ray reflectivity, stress and texture analyses, and even computed tomography.

SAXS results from glucose isomerase

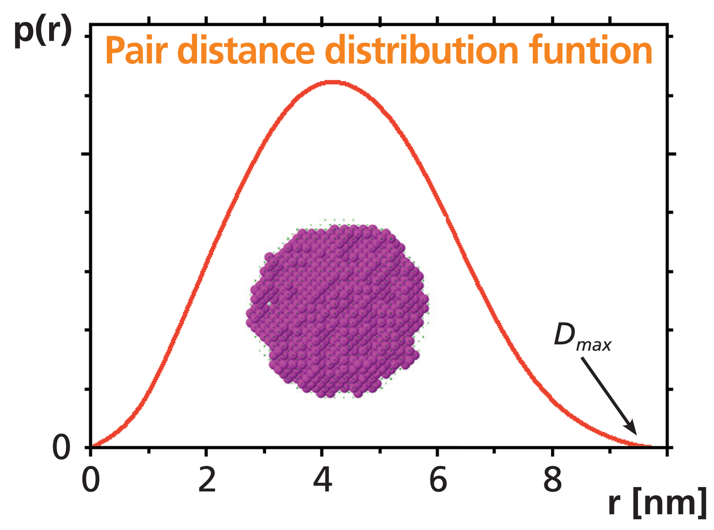
To validate the performance of our SAXS setup and data analysis we have used a solution of glucose isomerase as a model protein (measured data and sample details given at the bottom left of the poster).

From the straight line in the **Guinier plot** (ln*I* vs. q^2) of the data at the smallest angles one may determine the **radius of gyration R_g** of the protein molecules to 33 Å and conclude that no larger (non-specific) aggregates are present. The bell-shaped curve observed in the **Kratky plot** (Iq^2 vs. q) indicates that the protein has a **compact, folded structure**.



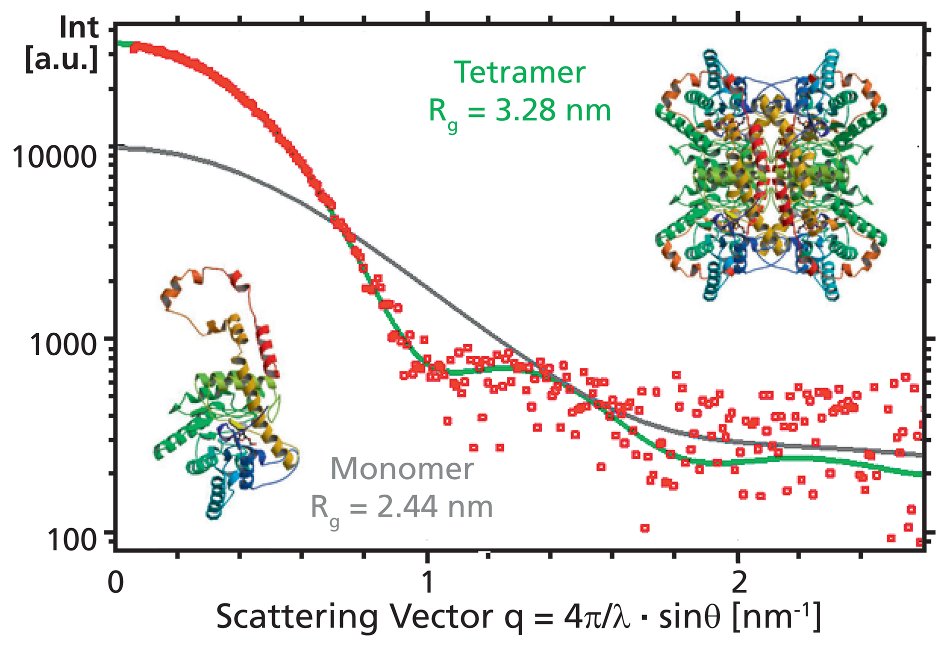


The **pair distance distribution function *p(r)***, calculated by a FT of the experimental data, is symmetric, which indicates that the protein has an overall **symmetric structure**. *P(r)* reaches zero at around 9.7 nm, which is the **maximum dimension D_{max}** of the protein molecule.



Ab initio **3D envelope shape reconstruction** by bead modeling using simulated annealing with DAMMIN software [1] confirms the overall symmetrical shape of this globular protein.

Based on the published protein structure determined by single crystal XRD [2] it is possible to simulate the corresponding solution scattering (SAXS) data. This was done both for the monomeric unit and for the tetramer by using CRYSOL [3]. Comparison with the experimental SAXS data shows that there is a good agreement with the simulation that was done for the tetramer. From this it can be confirmed that in solution the protein forms a **tetramer** rather than a monomer, and that **its structure in solution is very similar to its crystal structure.**



The obtained results are consistent and in good agreement with synchrotron data, even with a measurement time of only 10 min:

	R_g from Guinier plot	R_g from *p(r)*	D_{max} from *p(r)*
Lab instrument (60 min)	3.31 nm	3.32 nm	9.7 nm
Lab instrument (10 min)	3.33 nm	3.27 nm	9.5 nm
Synchrotron	3.25 nm [4]	n.a.	9.7 nm [5]

References

[1] D. I. Svergun, *Biophys J.* **76** (1999), 2879-2886.
[2] Images from the RCSB PDB of PDB ID 1XID (H. L. Carrell et al. (1994), *Acta Cryst.*, Sect. D 50:113-123).
[3] D.I. Svergun, C. Barberato, M.H.J. Koch, *J. Appl. Cryst.* **28** (1995),768-773.
[4] E. Mylonas and D. Svergun, *J. Appl. Cryst.* **40** (2007), 245-249.
[5] M. Kozak, *J. Appl. Cryst.* **38** (2005), 355-358.

Conclusion

The described XRD platform delivers high-quality SAXS data within short measurement times, even from dilute protein solutions. Its performance on glucose isomerase was successfully validated, yielding consistent results that agree with published data. Whereas BioSAXS data acquisition usually required the use of dedicated SAXS instrumentation, with ScatterX^{78} it is now for the first time possible to perform such measurements also on a multipurpose XRD platform.