In-lab SEC-SAXS for structural investigation of complex samples

SAXS data and 3D envelopes of small and large protein species are obtained through in-line gel filtration

Small-angle X-ray scattering (SAXS) is commonly used to investigate the solution structure of biological macromolecules. Highly accurate results can be obtained with monodisperse solutions devoid of aggregates and impurities.

Many biological processes, however, involve structurally heterogeneous multi-domain proteins[1] and heterogeneous protein complexes in dynamic equilibria[2-6]. While highly relevant, structural investigation of such samples is hampered by their transient or unstable nature. Size-Exclusion Chromatography (SEC) directly coupled with SAXS (SEC-SAXS) has emerged as a tool to mitigate these difficulties[7-9].

In the BioXolver setup, samples eluting from a SEC column flows directly through a SAXS exposure capillary, allowing continuous data collection immediately following separation of individual components. SEC-SAXS furthermore ensures clean samples and removes potential work steps of sample fractionation and subsequent loading on the SAXS instrument for very stable samples, thus reducing sample consumption and agitation while facilitating and expediting the work-flow.

Measurements & result

We show SEC-SAXS results obtained on a Xenocs BioXolver L for three proteins: bovine serum albumin (BSA, 66 kDa), ribonuclease A (14 kDa) and ferritin (476 kDa). Please refer to the publication from Bucciarelli *et al.*[10] for details of the study, as well as additional data sets.

Using two different stock concentrations of BSA (1 and 8 mg/ml) and loading volumes of 0.5 ml (Figure 1(a) and (b)), we demonstrate that data of good quality can be obtained using only a few milligrams (0.5 to 4 mg) of protein. It is even possible to obtain structural information from minor solution components, such as a small fraction of dimers (Figure 1(c)) in a mostly monomeric solution.

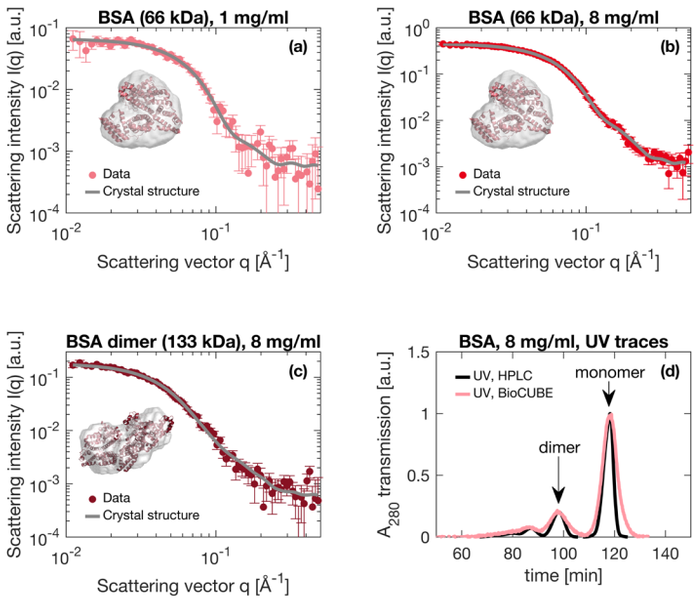

Figure 1. SEC-SAXS results of monomeric (a) and (b) and dimeric (c) BSA from stock concentrations of 8 mg/ml (b) and (c) and 1 mg/ml (a).

Panel (d) shows the UV traces of the HPLC unit and the BioCUBE over the SEC-SAXS run.

Utilizing the flexibility of the BioXolver L in terms of sample-detector distance (i.e. covered q-range), small (Figure 2(a)), as well as large (Figure 2(b)) macromolecules can be investigated. In all instances, the experimental results are in agreement with the known crystal structures of the proteins.

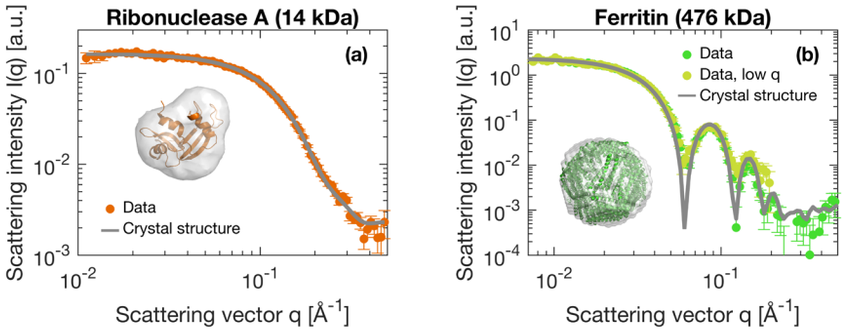

Figure 2. SEC-SAXS results of monomeric ribonuclease A ((a), stock concentration: 7.5 mg/ml) and ferritin ((b), stock concentration: 11.1 mg/ml).

The crystal structure fit and *ab initio* model in panel (b) correspond to merged data from two different sample-detector distances.

The BioCUBE, an integral part of the BioXolver, with its in-line UV/Vis exposing the exact same volume as the X-ray beam, is able to accurately monitor the evolution of the protein concentration during the elution (Figure 1(d)), enabling absolute scale SAXS intensity and correct molecular weight determination.

Conclusion

Using the BioXolver, it is possible to perform SEC-SAXS experiments directly in-house, allowing structural investigation of transient and unstable macromolecules in solution. Even in the case of more stable molecules, SEC-SAXS can be beneficial to the work-flow, as it removes additional steps associated with off-line chromatography filtration and purification prior to SAXS.

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