Insights and advantages offered by coflow to high flux solution SAXS measurements.

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Small angle X-ray scattering (SAXS) is becoming an increasingly commonplace technique for structural biology. With ongoing advances in software and measurement techniques it is increasingly in demand for challenging samples, because of structural disorder, instability, rapid dynamics or low purification yields. One limitation that has long been detrimental to the measurement is radiation damage, particularly on high flux undulator based synchrotron beamlines. Many methods have been used to combat this problem, including the use of additives, X-ray attenuation and flowing the sample to distribute dose over a wider volume. Even with these approaches the amount of flux that can be used has been a major limiting factor in measurement quality and sample consumption. The presence of laminar flow in sample cells such as capillaries is widely known, and the slow flow near the walls which exacerbates radiation damage of biological samples has until recently remained unsolved.

We developed a sheath flow method well suited to routine, high throughput solution SAXS which allows greatly increased flux (at least 10-fold) to be used for solution analysis of biological and other samples [1]. This “coflow” approach gives many advantages over the traditional flowing solution SAXS measurement. Apart from the increased flux, and hence increased improved data quality, the technique allows for reduced sample volume in static measurements and abrogates capillary fouling by proteins being measured.

Results during the development of coflow have shown that aggregation due to radiation damage is typically not observed using the coflow technique. This suggests that the dwell time of the sample in the beam is sufficiently low and narrowly distributed to prevent slower radiation damage effects from contributing to scattering patterns. Coflow, in combination with the use of variable flow rates, dwell time, and high flux is thus a practical tool for investigating the kinetics of radiation damage on proteins, which we are exploiting to further advance our understanding of this complex process.

The high flux we can now apply, and the abrogation of protein fouling, are well suited to rapid SEC analysis using fast-flowing columns that have become available in recent years. We have done a major optimisation of our in-line SEC-SAXS measurement using the coflow approach, which has become the routine measurement approach on the Australian SAXS/WAXS beamline. It includes quantitative, full spectrum UV measurements within microliters of the x-ray beam, allowing accurate measurement (with knowledge of the extinction coefficient of the sample) of I0/concentration and, hence, molecular weight.

The development of coflow offers a perhaps unprecedented ability to quantify and understand radiation damage on synchrotron sources, while improving the utility, quality and accessibility of SAXS measurements on routine and challenging samples alike.

References

[1] N. Kirby, N. Cowieson, A.M. Hawley, S.T. Mudie, D.J. McGillivray, M. Kusel, V.Samardzic-Bobana and T.M. Ryan, “Improved radiation dose efficiency in solution SAXS using a sheath flow sample environment”, *Acta Cryst.* (2016) D72, 1254-1277. doi: 10.1107/S2059798316017174