Recent developments in coflow and high flux solution SAXS measurements

Nigel Kirby*[1], Timothy Ryan[1], Stephen Mudie[1], Adrian Hawley[1], Haydyn Mertens[2], Andrey Gruzinov[2], Clement Blanchet[2], Stefan Fiedler[2], and Thomas Gehrmann[2]

[1]*Australian Synchrotron, Australia*

[2]*European Molecular Biology Laboratory (EMBL), Hamburg, Germany*

**nigel.kirby@synchrotron.org.au*

Small angle X-ray scattering (SAXS) analysis of purified biomacromolecular solutions is commonplace at most synchrotrons. 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 X-ray attenuation, the use of additives, and flowing the sample to distribute dose over a larger 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. We developed a sheath flow method which minimises radiation damage, by keeping the radiation sensitive protein sample away from the region of slow flow velocities near sample cell walls inherent to laminar flow conditions. Coflow is well suited to routine, high throughput solution SAXS and gives many advantages over the traditional flowing solution SAXS measurement for solution analysis of biological and other samples [1]. Efficient X-ray dosing maximises data quality per unit volume of sample and allows one to two orders of magnitude increase in X-ray flux at practical flow rates. Direct injection sample loading supports effective analysis of sample volumes of a few microliters. Capillary fouling by unstable or radiation damaged samples is prevented by their separation sample from the capillary wall by sheath flow of solvent.

Coflow is ideally suited to continuous flow methods, such as size exclusion chromatography. 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, particularly with fast running SEC columns. 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 [2].

Coflow allows the full flux of the Australian Synchrotron SAXS/WAXS beamline to be routinely applied to protein solution scattering experiments. The limiting factor most commonly observed is the radiation resistance of buffers, which can be managed by buffer selection, radical scavenging additives such as glycerol and/or sodium azide, care with buffer composition (with respect to salts, reducing agents, de-gassing) and beam focal size. In order to explore the full X-ray flux potential of coflow, we have conducted an initial experiment on the Petra III P12 beamline using a double multilayer monochromator. Results show that coflow allowed SEC measurements on model proteins up to 3.5×10^{14} ph/s at 10 keV at practical sample flow rates. The results support the design of a new high flux solution scattering beamline at the Australian Synchrotron.

References

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