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Coflow and Fast-SEC Improvements on the SAXS/WAXS Beamline

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Small angle X-ray scattering (SAXS) is an extremely useful tool for analysing protein structures that is becoming increasingly popular. SAXS displays a number of advantages over other techniques, but radiation damage and sample consumption limit the utility of the technique to the wider protein community. Of the various avenues being pursued to circumvent radiation damage, we have focused on the dynamics of laminar flow inside capillaries because of the well-known zero flow velocity at the edge of the capillary that causes elevated radiation damage. We have modelled the dose-distribution under laminar flow in the x-ray beam profile, showing the edges receive severe over-dosing if the sample fills the capillary, which requires overall under-dosing of the sample to manage radiation damage.

We have developed a new method for SAXS which avoids this problem by limiting the sample flow to the centre of a sheath fluid which avoids the boundary condition for the radiation sensitive protein component, and acts as a barrier between the sample and the capillary wall. In practice, this approach is very effective in reducing radiation damage, allowing sensitive protein solutions to be exposed to at least 10 fold greater flux despite much lower sample flow rates. Up until this development, protein experiments had to be run at a low flux, but now the full flux of the beamline is a key limitation. There are a number of other advantages, including that biomolecule samples do not come into contact with the capillary at all, and hence do not stick, and less samples is needed for the same degree of sensitivity, improving the efficiency of measurements. At the same time, significant improvements have been made to the size elution chromatography (SEC) setup that push the capability essentially to the limitation of SEC columns themselves. For routine samples, this can halve the measurement time, reduces the dilution in the system to that of the column itself, removes peak broadening of the SAXS measurement, allows quantitative UV measurement for normalising SEC traces by concentration during SAXS, and reduces the delay between elution off the column to SAXS to only a few seconds reducing the potential for post-column recombination to a minimum. These developments represent a major advance for the current and future solution scattering beamlines that unlocks the full capability of current beamline technology for challenging and high throughput applications for solution samples.

Keywords or phrases (comma separated)

Solution SAXS, radiation damage

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No

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No

What is your gender?

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