Mitigation of Radiation Damage in Biological SAXS on High Brilliance Synchrotrons

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Small angle X-ray scattering (SAXS) is widely used to probe the structure of biological macromolecules in near-native solutions [1]. Radiation damage induced by the intense X-ray beams at modern synchrotrons is one of the major complications for biological SAXS studies. In macromolecular solutions, radiation damage occurs mostly indirectly through water radicals and solvated electrons formed by the X-ray photons, which can reduce the macromolecules and induce aggregation. Already a small amount of aggregates can render the SAXS curves uninterpretable. As the observable effect depends not only on the radiation dose deposited but also on the tendency of the solute to aggregate, it is not possible to reliably predict the magnitude of the expected effect prior to the measurements. Complicating matters, the purified biological samples are usually only available in limited quantities.

The high brilliance P12 bioSAXS beamline [2], dedicated and optimized for solution scattering, delivers 1 x 10^{13} photons/s in a 120 x 200 μm^2 beam at the sample position. With this flux, aggregates may be rapidly formed. Different approaches have been explored to mitigate radiation damage including beam attenuation, continuous sample flow, addition of 'scavenger' molecules, or cryo-cooling [3]. All this methods may help to reduce the damage but have own limitations.

Continuous flow measurements, where samples are measured under flow through a cell (typically, a capillary) constantly exposing fresh solutions to the beam, are employed at many beamlines. Here we show that in bioSAXS, capillaries with smaller diameters than those calculated from plain scattering/absorption criteria allow for a more effective use of the available sample amounts. This is demonstrated by comparing two capillaries (0.9 and 1.7 mm inner diameter) to study different protein solutions at various flow rates, where the smaller capillary yields higher quality SAXS data [4].

While criteria for accessing the presence of radiation damage for such batch measurements are successfully in use [5], this approach is limited for time-resolved measurements, in which the change of the SAXS curves are the actual aim of the experiment. We will discuss approaches used for stopped-flow measurements at P12 to deal with this problem.

The exploration of radiation damage is particularly important in the context of high flux operations at the P12 beamline using the recently commissioned double multilayer monochromator. With the flux of 5 x 10^{14} photons/s in 85 x 285 μm^2 beam, macroscopic effects such as bubble formation and very large aggregates are observed in protein solutions within a few millliseconds exposure time. Proper characterization and mitigation of radiation damage are required for the optimal use of such a powerful beam.

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[1] M.A. Schroer, D.I. Svergun, Emerg. Top. Life Sci. 2, 69 (2018).

[2] C.E. Blanchet et al, J. Appl. Cryst. 48, 431 (2015).

[3] C.M. Jeffries et al, J. Syn. Rad. 22, 273 (2015).

[4] M.A. Schroer et al, J. Syn. Rad. 25, 1113 (2018).

[5] D. Franke, C.M. Jeffries, D.I. Svergun, Nat. Methods 12, 419 (2015).