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Study and mitigation of radiation damage on the P12BioSAXS beamline

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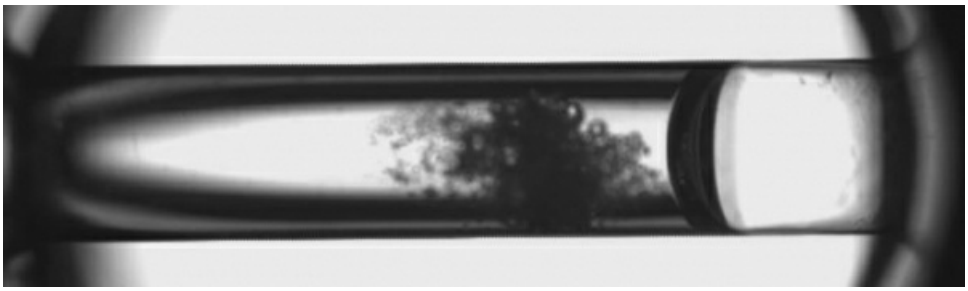
With the progress in X-ray sources and increasing X-ray beam flux, radiation damage is a major challenge for biological SAXS measurements on modern synchrotron beamlines. In macromolecular solutions, radiation damage occurs mostly indirectly. Water molecules are dissociated into hydrogen- and hydroxyl-radicals by the X-ray beam. In turn, these free radicals oxidise the macromolecules, which eventually start to aggregate. Since the SAXS intensity scales with the square of the particle size, a few large aggregates may quickly spoil the scattering pattern of the intact molecules.
The high brilliance P12 BioSAXS beamline at Petra-3 storage ring (DESY, Hamburg) [1], dedicated and optimised for solution scattering, delivers 5*10^12 photons/s in a 120*200 µm^2 beam (full width half maximum) at the sample position. With this flux, aggregates may be rapidly formed and the data become unusable after exposure of a few tens of ms. Different approaches have been explored to reduce radiation damage [2]: attenuation of the beam, "in-flow" sample measurement, addition of free radicals scavengers or additives that stabilizes proteins and prevent their aggregation. All these methods help to reduce the damage, but all have their limits, and their action can vary depending on the nature of the sample.
The "in-flow" measurement, where fresh sample is continuously flowed in the beam path during the exposure, is routinely used at many beamlines and may be the most versatile approach using no additives. On the downside, more sample is required compared to the measurement on a fixed sample. To improve the efficiency of in-flow measurements, radiation sensitive samples were measured in capillaries of different diameter (Schreoer et al., 2017, in preparation). SAXS cells are typically dimensioned such that the signal of aqueous sample is maximised, while the limited lifetime of the sample in the beam and the sample consumption are sometimes ignored. Using smaller capillaries (e.g. 1 mm instead of 2 mm diameter), the scattering signal is still decent and can be detected on low background beamline, but the sample volume required drops significantly (with the square of the diameter). In practice, for a given sample volume, in-flow measurement in small capillaries leads to less noisy data compared to the standard capillaries.
This exploration of radiation damage is particularly important in the context of high flux operations on the P12 beamline (Blanchet et al., 2017, in preparation) using the recently commissioned double multilayer monochromator. With the intense flux of 4*10^14 photons/s in 85 x 285 µm^2 at the sample position delivered in this setup, proteins aggregate in a couple of ms and macroscopic effects such as bubble formation and very large aggregates are present in less than a second of exposure. Proper characterization and mitigation of radiation damage are required to make optimal use of this powerful beam.
[1] Blanchet, C.E., et al., Versatile sample environments and automation for biological solution X-ray scattering experiments at the P12 beamline (PETRA III, DESY). Journal of Applied Crystallography, 2015. 48(2): p. 431-443.
[2] Jeffries, C.M., et al., Limiting radiation damage for high-brilliance biological solution scattering: practical experience at the EMBL P12 beamline PETRAIII. Journal of synchrotron radiation, 2015. 22(2): p. 273-279.



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