ESRF	**Experiment title: "**Understanding radiation damage on proteins in solution scattering: efficacy of scavengers and functional implications of SAXS irradiation on enzyme function".	**Experiment number**: MX-1621
Beamline: BM-29	**Date of experiment**: from: 07/07/2014 to: 08/07/2014	**Date of report**: 21/03/2017
Shifts: 3 shifts (24h)	**Local contact(s)**: Adam Round	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

[1]Eva Crosas* (main proposer)

[1]Marc Malfois* (co-proposer)

[1]Jordi Juanhuix (co-proposer)

[1]Albert Castellví*

[1]Christina Kamma-Lorger*

[1]*ALBA Synchrotron*

Carrer de la Llum, 2-26, 08290 Cerdanyola del Vallès, Barcelona (Spain)

Report:

Scientific background: Radiation damage has become a serious problem in third-generation synchrotron light sources, especially for biological SAXS (BioSAXS), where macromolecules are analysed in solution and usually at room temperature. In these conditions, hydroxyl and hydroperoxyl radicals, and superoxide anions, are produced due to the radiolysis of water present in the buffer solution. These radicals lead to the modification of amino acid side chains, which could result in protein aggregation, unfolding, and/or fragmentation of the polypeptide chain. Typical additives used in SAXS against radiation damage are reducing agents like dithiothreitol (DTT), which are not advisable in proteins with disulphide bridges, or cryoprotectants like glycerol, at the cost of worsening the signal-to-noise ratio. Interestingly, synergistic action of hydroxyl radical scavenging compounds like uridine, and superoxide anion scavenging compounds, like manganese and orthophosphate, have been described to show radioprotective properties on enzyme function up to 17.5 kGy exposure [1]. These compounds are found in high concentrations in *Deinococcus radiodurans*, an extremely radioresistant bacteria that can withstand up to 12,000 Gy [1]. Nevertheless, these mixtures have not been investigated as potential scavengers for SAXS.

Proposal Summary: The aim of this proposal was to characterize the radiation damage on proteins in solution using Small-Angle X-ray Scattering (SAXS) experiments. The efficacy of common additives used to limit radiation damage in SAXS, such as dithiothreitol (DTT) and glycerol was compared to uridine, which has shown radioprotective properties [1], but has not been tested as a potential additive to decrease radiation damage on proteins in SAXS. Radiation damage is an issue not completely solved at third-generation synchrotrons, and it is a limiting factor specially for proteins in solution. For this purpose, a well-characterized model protein, lysozyme, and human aldose reductase (ALDR1), were analysed in presence and absence of different additives used to decrease radiation damage.

Experimental methods: The efficacy of uridine as a scavenger for SAXS experiments was compared to DTT. SAXS data were collected at BM29 Beamline using a DECTRIS PILATUS 1M photon counting detector. The SAXS intensity data [I(q) versus q, where $q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle] were acquired at 12.5 keV. Measurements were carried out at 25ºC by using the sample changer robot in the static mode, in order to speed-up radiation damage. SAXS data were collected for lysozyme at 1 and 2.5 mg/ml, in the presence of increasing concentrations of DTT and uridine (1, 5 and 10 mM), at pH 5 (50 mM di-ammonium citrate, pH 5.0) and pH 7.5 (50 mM HEPES, pH 7.5). Unfortunately, aldose reductase (ALDR1) precipitated after thawing, and it was impossible to collect data from this sample as the protein was aggregated. In addition, samples of lysozyme containing glycerol and control samples without additive were loaded by the robot but not exposed to the beam, due to an under-estimation of the time needed to collect all the images. This underestimation led to a mistake in the programming of the automated collection mode.

Results: The efficacy of uridine as a scavenger for SAXS experiments was compared to DTT, a common additive used to limit radiation damage. As radius of gyration (R_g) increases proportionally to radiation-induced protein aggregation [2], the dependence of R_g on the accumulated dose was determined for lysozyme in the presence of the different additives. Figure 1 shows a representative result of the experiment, corresponding to the increase of the R_g of lysozyme at 1 mg/ml (Figure 1A, top) and 2.5 mg/ml (Figure 1B, bottom) at different absorbed doses, in presence of increasing concentrations of DTT (left panels) and uridine (right panels).

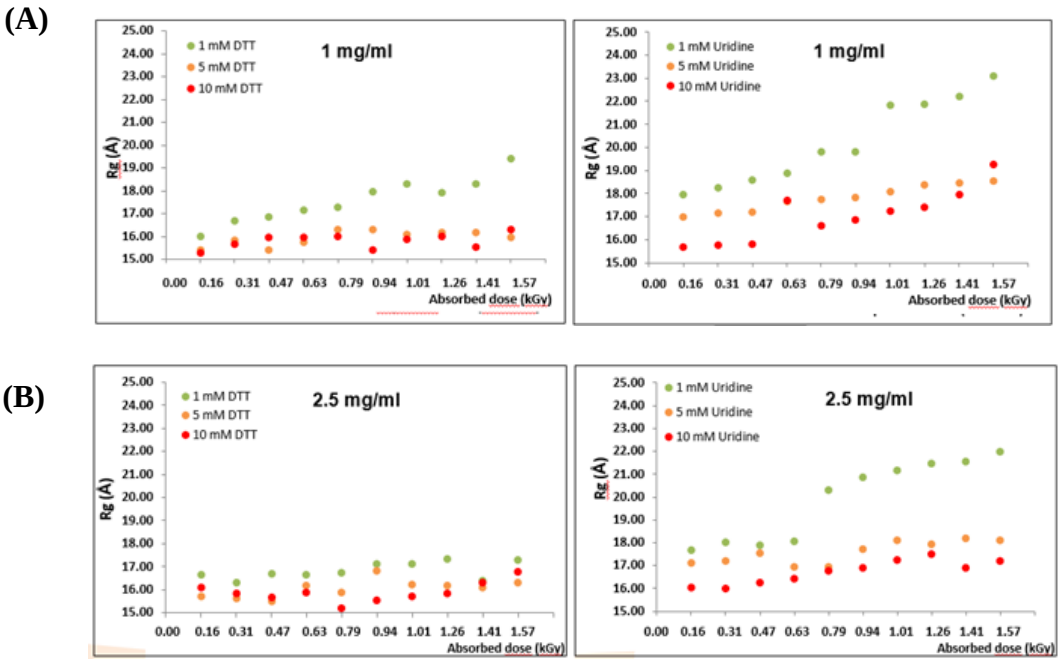

Figure 1. Dependence of the R_g of lysozyme on the accumulated dose, in presence of increasing concentrations of uridine and DTT (1, 5 and 10 mM). Lysozyme was measured in 50 mM di-ammonium citrate, pH 5.0. The nominal radius of gyration of lysozyme is 14.8 ± 0.5 Å.

Conclusions and future work: Although a slight initial aggregation (prior to irradiation) is present in all the samples, the results show that uridine between 5 and 10 mM decreases radiation damage with a similar efficiency as DTT, which could maintain the R_g of lysozyme 2.5 mg/ml until 1.57 kGy even at 1 mM concentration. Samples containing 2.5 mg/ml lysozyme show in general less radiation-induced aggregation than 1 mg/ml samples, as it is known that protein concentration also influences radiation damage [2]. We consider that the results obtained suggest that uridine could be a possible scavenger for SAXS experiments, although it would be necessary to study the increase of R_g in presence of the different additives until higher absorbed doses, and most probably higher uridine concentrations should also be tested.

References:

[1] Daly MJ et al. 2010. PLoS One. 5(9):e12570.
[2] Kuwamoto S et al. 2004. J Synchrotron Radiat. 11(Pt 6):462-8.