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Crystallographic and functional characterization of the effects of the radiation damage on proteins and prospection of new scavengers

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Biological material, such as proteins, is highly sensitive to radiation damage (RD) coming from the intense ionizing radiation, which causes severe structural and functional damage to organic matter either directly by the generated free electrons or indirectly by formation of reactive oxygen species (free radicals). Synchrotron radiation is a suitable tool to both generate the radiation and study its effects on biological material.

The first step to characterize the effects of the radiation on biological material is to establish the appropriate metrics of the damage. The functional effects are commonly measured by means of activity assays upon the absorbed dose. The structural effects can be measured in different ways, depending on the technique used. Structural changes studied by Small angle X-ray scattering (SAXS) are often measured by means of the radius of gyration Rg of the protein in solution. Much more controversy is generated on the correct metrics to measure the structural changes induced by radiation studied by macromolecular crystallography (MX). Suggested metrics are R-factors and I/Sigma, among others.

The correlation between the RD effects measured in functional assays and these measured in structural studies, both SAXS and MX, have not been explored extensively. Here we report an initial study aiming to correlate the metrics of these studies. The effects of RD have been explored in diverse soluble proteins, namely, Lysozyme (Lys), Aldose Reductase (AK1RB1) and Catalase (BLC).

Added small molecules to mitigate RD are extensively used in SAXS studies, like DTT or glycerol, which act primarily as free-radical scavengers of secondary radiation damage. The use of scavengers is also common in MX studies. Several putative scavengers have been tested in functional assays and in structural studies using Lys and AK1RB1 proteins. All experiments are performed at room temperature to highlight the effect of radiation damage. The different metrics to quantify the RD in the different studies are checked to correlate the results using scavengers.

This project is being carried out as collaboration between NCD-BL11 and XALOC-BL13, the beamlines at Alba dedicated to SAXS and MX, respectively.

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