

Parameters affecting crystal lifetime in MX and possible radiation damage mitigation strategies.

Elsbeth F. Garman and Karthik S. Paithankar

Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, U.K. [elsbeth.garman@bioch.ox.ac.uk]

Radiation damage continues to present a problem to crystallographers using cryo-cooled protein crystals at third generation synchrotrons [1]. Attempts to understand the physical and chemical processes affecting the damage rate, and thus to find strategies to reduce it, have become an active area of research in the last 10 years.

This talk will cover three aspects of our recent investigations with particular reference to their possible application to diffraction experiments on microcrystals. The first of these reports further developments of the program RADDPOSE [2,3], widely used to compute the dose absorbed by a macromolecule crystal and hence the irradiation time available to reach the experimental dose limit of 30 MGy [4] under specified experimental conditions. This program now takes into account of both the probability of fluorescent X-ray escape following excitation of an atom by a photoelectric absorption event, and also the energy deposition caused by incoherent (Compton) scattering. The former is significant for heavy atom containing protein crystals. The latter phenomenon becomes significant only above 20 keV incident X-ray energy. The predicted diffraction dose efficiency (scattered intensity/dose) is also now correctly output and can be a useful parameter to consider in the optimisation of the diffraction experiment.

Secondly, the possibility of reducing the rate of radiation damage at both room temperature (RT) and 100K by the addition of free radical scavengers and radioprotectants will be discussed [5,6,7]. In new work [8] to search for RT radiation damage mitigation strategies, three putative radioprotectants identified in reference [6] were tested. The results indicated that ascorbate offered a 2× enhancement of crystal lifetime, whereas 1,4-benzoquinone offered a >9× increase at the dose-rates used. Most interestingly, both scavengers modified the form of the RT dose dependent intensity decay from first order (exponential) to zeroth order (linear). This may give insight into the chemical processes which inflict damage at 100K, since at cryotemperatures the intensity decay is also predominantly linear.

Finally, the possibility of data collection by exposing multiple protein crystals simultaneously will be discussed. Using techniques adapted from material sciences [9], we have been able to index up to 7 lattices of HEWL on the same diffraction pattern and subsequently to integrate datasets of up to 4 of them. Merging of data from the first part of the decay curve of many crystals could be a useful strategy for some rapidly decaying microcrystals which are hard to physically mount one at a time.

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