

The XIPHOS system in Durham is now operational, allowing crystal structures to be determined routinely at temperatures down to 2K. [1] In order to reach these temperatures, the sample is isolated from the environment using vacuum shrouds made from beryllium. These shrouds contain unevenly distributed crystalline parts, resulting in powder rings of non-uniform intensity. When collecting data with a weakly diffracting crystal where the reflections from the beryllium are strong in comparison to those reflections from the crystal, the uneven intensities from the rings can distort the intensities calculated by integration programs.

This can be avoided, and improved data quality obtained by masking out the regions of the diffraction image which are affected by beryllium scattering. Any reflections which overlap with the masked region are discarded by the integration program. As the scattering from the beryllium shrouds and the crystal originates from different positions, overlapping reflections can be separated by changing the detector distance. By using masks and recovering the lost data with these different detector distances, data quality can be improved compared with data obtained at one distance and without masking.

Masquerade has been written to generate the mask files required for this technique quickly and accurately. The positions of the beryllium rings are calculated using an *ab-initio* model of the position of the shrouds in 3D space, and include goniometer rotations as well as centring offsets for the shrouds themselves. The program is written in C++ and uses multithreading to make full use of modern multi-core processors. It runs on a variety of platforms and can generate thousands of mask images for a full data collection in minutes.

The use of this program along with the data collection at multiple detector distances has been found to improve the data quality obtained from weakly diffracting crystals, showing clear improvements in R_1 , wR_2 and R_{sigma} values over data integrated without the masks.

[1] M.R. Probert, C.M. Robertson, J.A. Coome, J.A.K. Howard, B.C. Michell, A. E. Goeta, *J. Appl. Cryst.* **2010**, *43*, 1415-1418.

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X-ray-induced cooperative atomic movements in protein crystals
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A specially designed diffraction experiment with controlled radiation damage to proteins makes it possible to investigate X-ray-induced changes in protein molecules and their crystals at the atomic level. We present the results of several experiments in which multiple data sets corresponding to increasing absorbed doses were collected from the same crystals at atomic resolution ([1], [2]). A comparison of the refined models of a protein molecule that corresponded to different levels of damage allowed us to study progressively increasing local and overall radiation damage effects. It was shown that irradiation causes displacements of big parts of the protein molecule. These movements have a cooperative character: big parts of protein molecules are displaced approximately as rigid bodies. The collective movement of the atoms of the protein leads to the expansion of the protein globule,

which occurs synchronously with, and in the same direction as, the expansion of the unit cell.

It was also shown that water molecules in the vicinity of protein surface move in the same direction and in concert with the nearest protein atoms. It appears as if the atoms of the expanding protein molecule pull the hydrogen-bonded network.

Displacements of protein domains occur simultaneously with the X-ray-induced damage to protein residues that participate in the contacts between different domains (decarboxylation of Asp and Glu residues, disruption of S-S bridges). An analysis of atomic ADPs revealed that, for the atoms with initially isotropic ADPs, an increase in anisotropy of ADPs occurs in the same directions as atomic displacements. This indicates that radiation-induced atomic displacements occur with different speed in different cells of the crystal and lead to an additional increase in crystal static disorder.

We studied the radiation-induced local and global damage not only at 100K, which is a usual temperature of data collection at modern synchrotrons, but also at as low as 15K. In the last case, crystals were cooled by a cold helium stream. Both kinds of radiation damage, local and overall, evolve on approximately the same time scale and similarly diminish as temperature decreases from 100K to 15K. Lowering the temperature from 100 to 15K decreases the disulfide-bond deterioration and atomic displacements, the decrease being somewhat greater than twofold.

[1]. T. Petrova, V.Y. Lunin, S. Ginell, I. Hazemann, K. Lazarski, A. Mitschler, A. Podjarny, A. Joachimiak, *J. Mol. Biol.* **2009**, *387(5)*, 1092-1105. [2]. T. Petrova, S. Ginell, A. Mitschler, Y. Kim, V.Y. Lunin, G. Joachimiak, A. Cousido-Siah, I. Hazemann, A. Podjarny, K. Lazarski, A. Joachimiak. *Acta Cryst.* **2010**, *D66*, 1075-1091.

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Characterization of radiation damage in macromolecular crystals at cryo and room temperature

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Radiation damage caused by ionising radiation is a constant source of concern when collecting X-ray data from biological samples. Its effects are sample dependent and are proportional to the absorbed dose. Accurate dose estimation is essential to determining the crystal dose tolerance and data collection strategy. A reliable and reproducible method to automatically characterise the radiation sensitivity of macromolecular crystals based on a preliminary experiment sacrificing a whole or part of a sample has been recently developed [1]. The method was implemented at the ESRF beamlines through an automated procedure using the EDNA online data analysis framework [2] and the MxCuBE data collection control interface [3]. The information extracted from this procedure is directly used for optimal planning of data collection by strategy software program BEST [4]. Using test crystals with well known radiation sensitivity, the procedure can also be used at the beamlines to verify and calibrate X-ray flux and beam size.

The method has been recently applied to a systematic study of radiation damage at room temperature. Reproducibility of the measurements, dose rate effects, radiation damage correlation with crystal properties and the diffraction intensity degradation model have been investigated, analysed and compared with the results obtained at cryo-temperature.