Annual BAG Progress Report - mx1932
Fundamental & Applied Aspects of Radiation Damage

1) Fundamental Mechanisms of Radiation Damage

Over the last year we have begun two studies addressing fundamental mechanisms of radiation damage.

a) What is the role of the aqueous solvent within a crystal in radiation damage?

Here we have used light cross linking (with glutaraldehyde) to stabilise crystals of lysozyme and thaumatin in a variety of organic solvents (incuding methanol, acetone and hexane). Dose series experiments were then carried out on both control crystals (aqueous buffer) and the crystals in organic solvents at 100 K and the data analysed for both global and specific damage using the range of density loss and damage metric tools that have been partly developed by members of the Raddam BAG. Data analysis is still on going, and we require some additional data (particularly at very low doses) but the initial analysis suggests that the organic solvents provide some measure of protection versus specific damage.

b) Investigating the role of the local environment on specific damage

An open question in the radiation damage field is how the local environment modulates the sensitivity of individual residues to radiation damage. Investigation of this question has been hampered by the lack of systematic studies of a wide range of local environments against the same background. To address this we decided to use a synthetic four helical bundle (Schafmeister *et al.*, 1997) that is comprised of only 7 different amino acids, with no aromatic or sulphur containing residues and very few charged residues (only glutamate). We expected this to be a relatively chemically (and radiation damage) inert scaffold into which we could insert combinations of specific mutations. To our very great surprise, initial X-ray diffraction series collected at ID23-1 on 4HB1 at 100 K showed the exact opposite of what we expected. We had infant created a highly radiation sensitive protein which showed considerable global damage within just 2.6 MGy of dose (Fig. 1).

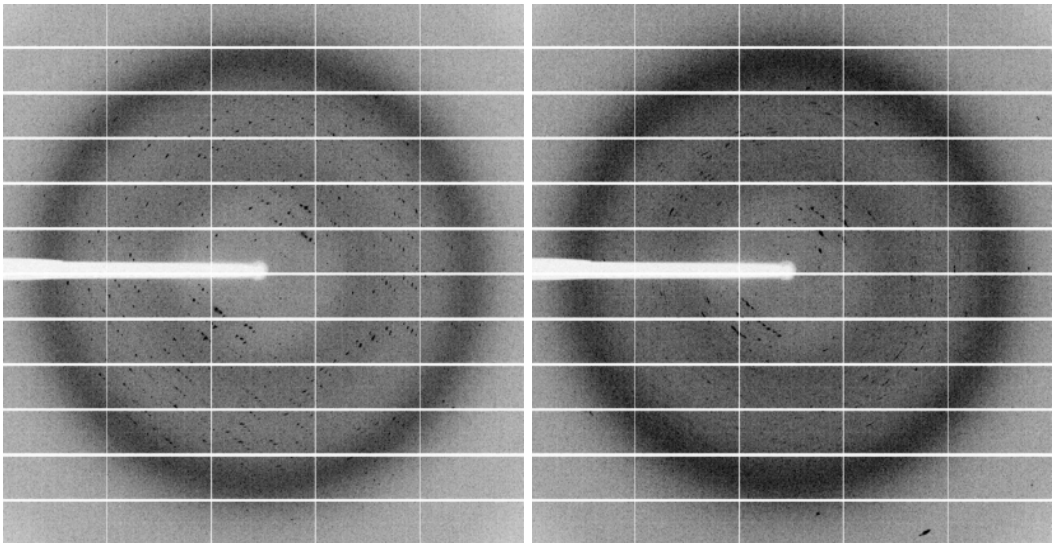

Fig. 1: Diffraction data from 4HB1 crystals from image one of dataset one (left) and image one of dataset two (right) collected at ID23-1 at 100 K. The total dose delivered during dataset 1 was 2.6 MGy.

Following this observation we wondered whether any of the amino acids we had purposely excluded could have a radiation damage protective effect. Therefore on MASSIF3 we investigated the effects of the presence of different amino acids on crystal lifetime by soaking the 4-helical bundle crystals with aspartate, histidine, serine or tryptophan, as well as other known electron scavengers like nitrite (NO_2). Initial results soaking 4HB1 crystals with 100 mM tryptophan showed a consistent and significant improvement in crystal lifetime. For example, it was possible to collect a dose series at 100 K where the crystal initially diffracted to 2.10 Å and still diffracted to 2.20 Å following around 50 MGy of dose. Analysis of this dose series using the Radiation-Induced Density Loss (RIDL) program (Bury *et al.,* 2016) shows specific damage only manifested after 10 datasets (22.40 MGy). These results suggest residues sensitive to specific damage may in fact be acting as

“Sacrificial Residues”, taking damage to prevent more significant damage spreading to the main chain of the protein, and raises the possibility these amino acids may be useful protective agents against radiation damage. Further experiments on BM30A in combination with single crystal spectroscopy on lysozyme crystals soaked with tryptophan indicate that formation of the characteristic disulphide radical anion absorption band at 400 nm was significantly reduced in the presence of tryptophan. We are currently completing the analysis of these data ready for publication.

2) Development of protocols to optimise data collection for radiation sensitive states - focus on photocages and photoactive groups.

A major effort during the last year has been to obtain good quality “dark” structures of intact photocages as a starting point for time-resolved structural studies. However, the photocages are extremely radiation labile, with even multi-wedge datasets at 35 kGy showing signs of cleavage. We have therefore been exploring the use of serial data collection methods to reduce the absorbed dose even further. As part of this effort we installed the translation stage and silicon nitride chip system that has been developed in collaboration with the Miller group at the Max Planck Institute for Structural Dynamics and Diamond Light Source (Oghaeby et al., 2016) on MASSIF-3 (Figure 2). Unfortunately, for this experiment the space restrictions around the sample position meant that we could not use the normal capillary and apertures and this resulted in images with a considerable amount of background artefacts that could not be successfully dealt with during processing, even with masking. Very recently (as part of the SSX BAG, mx1991) we have carried out a similar experiment (but with a different chip) with a shorter capillary and aperture that fit the space available and these data look much more promising.

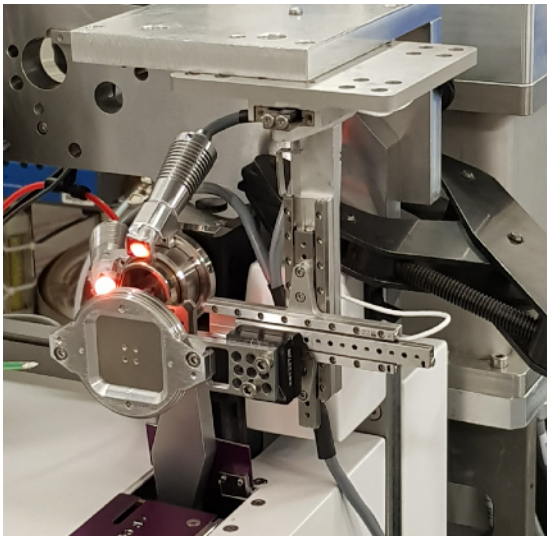

Figure 2: The translation stage and chip mounted on MASSIF3

In order to gain further insight into the stability of our different photocage scaffolds in the X-ray beam we have carried out combined X-ray diffraction/optical spectroscopy experiments on BM30A. Spectra were recorded at 100 K from both thin films of each photocage and from aspartate decarboxylase (ADC) crystals soaked with photocages (Figure 3). Spectra were recorded continuously during X-ray illumination and the changes observed. The data clearly indicate that the photocages are breaking down within only a few kGy and that a serial approach to data collection will be required to collect non-radiolysed “dark” structures.

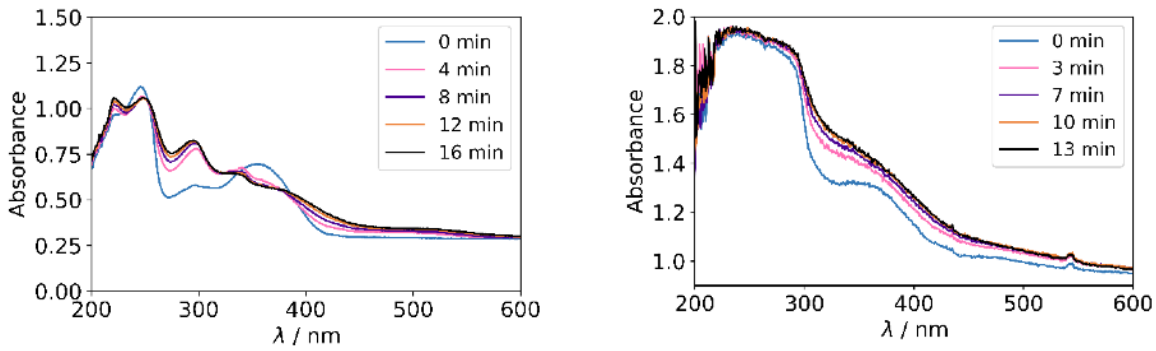

Figure 3: Left: Spectra from a thin film of CNA-Asp during X-ray exposure. Right: Spectra from an ADC crystal soaked with CNP-Asp during X-ray exposure. Estimated dose rate 2 kGy/min.

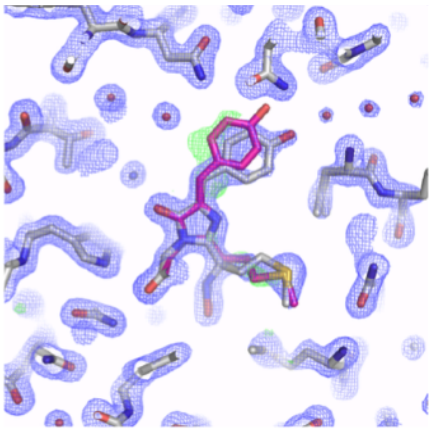

Figure 4: Alternative conformation of the chromophore (magenta) fitted on the positive electron density observed on the calculated F_o-F_c map upon refinement. $2F_o$-F_c (blue mesh) and F_o-F_c (green mesh) maps contoured at 1.2 and 3.0 σ.

In another set of experiments on MASSIF-3 we have determined the crystal structures of the fluorescent protein mGarnet2 (Matela *et al.*, 2017) at 1.60 Å and from a double mutant (unpublished data) at a resolution of 2.40 Å. The high-resolution structure has allowed us to determine the conformation of the *cis*-chromophore in the dark-state and to observe an unexpected alternative conformation (Figure 4) that explains its unique spectroscopic properties. As part of its characterisation we collected radiation damage series on MASSIF-3 to analyse the effect of radiation-induced damage and to extrapolate it to its photo-conversion mechanism and this data is under analysis.

Both structures will be deposited in the PDB and published in a paper focused on explaining the molecular basis of the spectroscopic properties of both proteins. Additionally, these results will serve to design future time-resolved experiments at both XFELs and Synchrotron sources as well to improve mGarnet2-derived markers for their implementation in super-resolution microscopy.

3) Use of radiation damage to probe biological mechanism

The remaining experiments this year have focussed on a number of biological systems where we are interested in either using X-ray derived aqueous electrons to drive a reductive chemical reaction, or to determine conditions where oxidised or partially oxidised states can be stabilised for structural analysis. These systems include integral membrane proteins as well as soluble enzymes. For several of these systems we are still identifying optimal crystallisation conditions for the subsequent mechanistic studies, for others we have had more success and initial datasets are collected. However, this year we have not carried out any detailed mechanistic experiments. These screening experiments included our first testing of MASSIF1 to automatically screen crystals (April 2017).

We also carried out a combined spectroscopic (cryobench) and X-ray RIP experiment on ID29 using a brominated GDP derivative to solve the structure of an AAA+ family member. This gave data that are currently under analysis. However, the C-Br stretch we were hoping to follow in the Raman spectra sits almost on top of the C-Se stretch, and unfortunately the crystals from our collaborators turned out to also be Se-Met derivatised. This meant we could not use the Raman spectra to identify the optimal X-ray dose to cleave the bromine as had been originally planned.

References

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Bury et al., Acta Cryst. (2016). D72 648-657
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