

2. Elspeth Garman

Method development for protein crystallography

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Our research this year has seen the culmination of two projects on techniques development which have been ongoing in the group for the last few years.

Dose Limit for Protein Crystals

In the first, we have completed work on ID14-4 at the ESRF designed to enable us to measure the experimental dose limit which reduces cryo-cooled crystals to half their initial diffraction intensities and compare it with the limit of 2×10^7 Gy calculated by Henderson from observations made in electron microscopy (1). The measurements have also allowed us to validate the dose calculations made by our computer programme, RADDPOSE, published last year. Up to 15 successive datasets were collected from four holo and three apoferritin crystals. The absorbed dose for each crystal was calculated using RADDPOSE after measurement of the incident photon flux (involving calibration of the beamline internal pin diode for each run with an external diode), and determination of the elemental crystal composition by microPIXE (see below). Degradation in diffraction quality and specific structural changes induced by synchrotron radiation could then be compared directly with absorbed dose for different dose/dose rate regimes. Remarkable agreement both between different crystals of the same type, and between apo and holoferritin (which has twice the absorption coefficient of apo) was observed for the dose required to reduce the diffracted intensity by half ($D_{1/2}$). From these measurements, a dose limit of $D_{1/2} = 4.3 (\pm 0.3) \times 10^7$ Gy has been obtained. However, by considering other data quality indicators, an intensity reduction to $I_{ln 2} = \ln 2 \times I_0$, corresponding to an absorbed dose of 3.0×10^7 Gy, is recommended as an appropriate dose limit for typical macromolecular crystallography experiments. A dose rate effect is observed. The results were presented at the IUCr Congress in Florence August as a poster (2), and they have been accepted for publication.

Birefringence as Predictor of Crystal Quality

In the second area of development, the results of our experimentation on the possibility of using the Metripol birefringence microscope (Oxford Cryosystems) to pre-determine crystal diffraction quality prior to X-ray irradiation, were published (3), with an image of the optical anisotropy, $|\sin \delta|$, of a cryocooled 2F1 3F1 human fibronectin crystal taken using the microscope on the front cover of the same Journal. For the three different proteins investigated (Hen Egg White Lysozyme, glucose isomerase and fibronectin), a correlation has been observed between the slow optical axis position (SOAP) and the diffractive quality of room temperature and cryocooled crystals. The results suggest that variations in SOAP increase as the crystal quality decreases, but that there is no such correlation between the mosaicity or crystal volume and SOAP. Currently we are working to identify non-birefringent crystallization trays, so that the quality of crystals in crystallization drops could be pre-assessed prior to selection for X-ray diffraction experiments.

Other topics of investigation being undertaken by members of the group have also developed further during the year.

Free Radical Scavengers

The investigation of possible radical scavengers for cryo-cooled protein crystals has constituted a large part of our ESRF synchrotron use in Radiation Damage BAG MX438 this year. We have used an on-line microspectrophotometer on ID14-4 at the ESRF, Grenoble, built in collaboration with Dr. Raimond Ravelli, EMBL, Grenoble and funded by a Royal Society Equipment Grant to EFG, with additional contributions and effort from the ESRF and EMBL, to monitor the production of a radical produced on disulphide bond breakage, which gives a peak at around 400nm, from solutions containing more than 20 different putative scavengers. We have written a Perl script which enables us to import the many spectra from the microspectrophotometer Ocean Optics software into MATLAB, and this gives us the potential to more easily extract lifetimes of transients (e.g. hydrated electrons) from the data. An example of a resulting plot is shown below for PEG400 + cystine solutions with and without ascorbate, which has previously found by us to be an effective suppressor of disulphide bond breakage (4). We are now drawing this work to some preliminary conclusions.

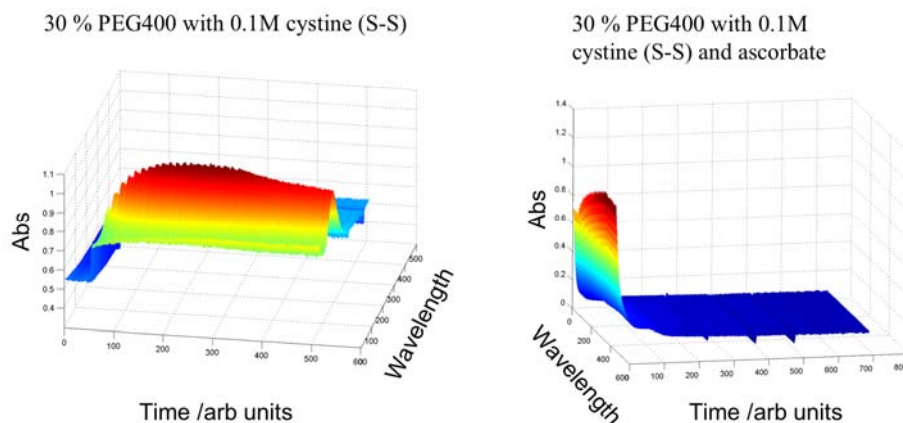


Figure 1: Series of microspectrophotometer spectra from solutions of PEG400 and cystine without (left) and with (right) ascorbate, showing suppression of 390nm disulphide radical peak by the scavenger. The wavelength range is 300 to 600nm and 20 x 1s beam exposures were used.

MicroPIXE for Elemental Analysis of Proteins

Our use of microPIXE to carry out elemental analysis of proteins, which allows us to obtain stoichiometric ratios of the number of atoms of heavier elements of interest per protein monomer to an accuracy of around $\pm 10\%$, has continued and we have a number of new collaborators with ongoing projects. We use the proton microbeam at the National Ion Beam Centre at the University of Surrey in Guildford. Experiments on proteins from the following groups were performed during the year of this report: Dr. Ben Luisi (Biochemistry Department, Cambridge University, U.K.) and previous investigations in collaboration with this group on zinc binding to endoribonuclease RNase E were published (5), Drs. Stephen Cusack (EMBL, Grenoble), Christophe Mueller (EMBL, Grenoble), Wolfram Meyer-Klaucke (EMBL, Grenoble), Susan Lea (LMB, Oxford) and Professor Chris Schofield (Chemistry Department, Oxford). We also used microPIXE to determine the elemental composition of the holo and apo-ferritin enzyme and crystals used in our determination of the experimental dose limit for cryo-cooled protein crystals (see above). Two different batches of horse spleen holo-ferritin gave an average of 1750 and 1766 iron atoms per 24-mer ferritin ball respectively; comparing very favourably with previous work which reported an average of 2000 iron atoms per 24mer for this type of ferritin.

In parallel, we investigated new cleaner microPIXE sample backings to replace the 2 μ m thick mylar, which was the optimum we had found until now. This film contains trace impurities of calcium and phosphorus from the manufacturing process, which we have previously characterised (6). We have identified a 4 μ m prolene film which when tested was completely clean (no elements other than carbon at greater concentration than the lower detectable limit). Using this new film, we are thus now able to obtain more accurate calcium and phosphorus concentration measurements.

Our major account of the microPIXE method applied to protein analysis, written in collaboration with Dr. Geoff Grime at the University of Surrey, includes a summary of all the measurements in the literature so far and was published this year (7).

Organic Superconductors and Radiation Damage

The investigations of the effects on X-ray irradiation damage on organic superconductors, lead by Dr. Arzhang Ardavan's correlated electron systems group from the Clarendon Laboratory in collaboration us, has reached fruition with some very interesting and novel observations on the possible mechanisms of superconductivity in these materials. The results have been prepared for publication and submitted to Physics Review Letters (8).

Human Fibronectin and other proteins

Work of the structure of the 2F1 3F1 module pair from human fibronectin (in collaboration with Dr. Jen Potts, University of York) in complex with a bacterial peptide will resume in January 2006, when Dr. Enrique Rudiño-Piñera from the National University of Mexico in Cuernavaca will visit the group again, this time for 3 months on a Royal Society Funded Grant for visiting scientists. Our four

structures of influenza A neuraminidase subtype N6 (English duck, native, sialic acid bound at different temperatures showing different occupancy of the second binding site), have now all been deposited in the PDB, and this work is being prepared for publication.

International Radiation Damage Workshop

Following the Third International Workshop on X-ray Damage to Biological Crystalline Samples held in Grenoble in November 2003, nine papers presented by participants at the Workshop on various aspects of radiation damage were published in the *Journal of Synchrotron Radiation* in 2005 (guest edited and introduced by Colin Nave and Elspeth Garman (9)), and a paper on the group's observations on the important physical and chemical factors affecting the rate of damage to cryo-cooled crystals was among them (10).

The Fourth International Workshop on X-ray Damage to Biological Crystalline Samples, organized in collaboration with Drs. Sean McSweeney (ESRF, Grenoble), Colin Nave (SRS, Daresbury), Raimond Ravelli (EMBL, Grenoble), Gerd Rosenbaum (University of Georgia, Athens, U.S.A.), Soichi Wakatsuki (Photon Factory, Japan) and Masaki Yamamoto (SPring8, Japan) will take place at SPring8 in Japan on the 7th and 8th March 2006, and we look forward to stimulating discussions of the problems still to be addressed in this area.

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