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Method development for protein crystallography

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This year our research has concentrated on two areas: the structure solution of the ²F1 ³F1 module pair from human fibronectin (in collaboration with Jen Potts and Uli Schwarz-Linek) and further techniques development for macromolecular crystallography.

Fibronectin crystals of high diffraction quality (1.7 Å) in P4 or P422 were obtained but exhibited symptoms of possible twinning (1). We attempted to solve the structure by molecular replacement using different ensembles of the NMR models, but after many different strategies failed, we moved on to MIR methods. Data from crystals of platinum, gold and xenon derivatives were collected, and all showed partially occupied sites, but there was not enough phase information to give interpretable maps. In-house data was collected for the sulphur SAD method, but this also failed. RIP (radiation damage induced phasing) was tried next, and although a large RIP signal from the breakage of the 4 disulphide bonds in the structure was obtained from data collected at ID14-4 at the ESRF, the maps were uninterpretable. Highly redundant SAD data (1500 images) were then collected at 1.7Å incident X-ray energy on BM14 (ESRF) and a sulphur signal which should have been large enough to solve the structure was measured, but again the maps were uninterpretable. The structure was eventually solved when the phase information from the S-SAD and RIP data were combined by George Sheldrick and Raimond Ravelli. The structure of the modules is as expected from the NMR models, although the relative orientations of the modules differ. We are now working to obtain crystals with a bacterial (*Staphylococcus aureus*) peptide bound to ²F1 ³F1.

The structure of influenza A neuraminidase subtype N6 (English duck), solved by us some years ago, has been further refined to 1.85Å and deposited in the PDB. Unlike N2 and N9, it has no crystallographic four-fold axis down the centre of the functional tetramer, so differences between the monomers have been observed. A series of experiments to find the second sialic acid binding site, postulated to be present on bird flu influenza neuraminidases, has shown that indeed there is such a site in N6, but that unlike that reported in N9 following a 4°C sialic acid soak, it can be clearly seen after an 18°C soak.

Our techniques development work this year has involved further investigation of the use of the Metripol birefringence microscope (Oxford Cryosystems) to possibly pre-determine crystal diffraction quality prior to X-ray irradiation. The Metripol was used to measure variations in the Slow Optical Axis Position (SOAP) in Hen Egg White Lysozyme (HEWL), glucose isomerase and fibronectin crystals. The magnitude of variations in SOAP were then compared with the diffractive properties of each crystal both at room temperature and 100K. The results so far 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 (2). Work is ongoing to both identify crystalline material and assess the quality of crystals in crystallization drops. A study of the effect of crystal annealing on the SOAP was inconclusive (3).

We have continued to work with various collaborators on the analysis of proteins by microPIXE, using the proton microbeam at the National Ion Beam Centre at the University of Surrey in Guildford (4, 5). We have just completed a major account of the method which includes a summary of all the analyses in the literature so far (6).

In the study of X-ray radiation damage to cryocooled crystals, we have published a description of our work on RADDOSE (7) which is a computer programme (available from the authors) to calculate the absorbed dose in a crystal of specified composition and in defined beam conditions (flux density, energy, size and profile). The time to reach the so called Henderson limit of 2×10^7 Gy (J/kg) is output, as well as other times and parameters of interest to the experimenter for optimising the diffraction intensity per absorbed

dose (the 'diffraction dose-efficiency'). We have made a number of assumptions in the calculations, and we are currently working on improving the programme, which will ultimately be incorporated into the automated pipeline software 'DNA'. We have now undertaken a series of experiments at the ESRF designed to enable us to validate the RADDOSE calculations and also to test whether the theoretical dose Henderson limit of 2×10^7 Gy for the diffraction intensity to be halved is a reasonable estimate.

References

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