ESRF	**Experiment title:** **Towards sub-picosecond temporal resolution in X-ray diffraction studies.**	**Experiment number:** **LS-922**
Beamline: ID14, EH3	**Date of Experiment:** from: 17 June 98 **to:** 23 June 98	**Date of Report:** 27 August 98
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Report:

This experiment was motivated by an experimental methodology, crossed beam topography (CBT) [l], which may eventually yield sub-ps temporal resolution in X-ray diffraction experiments at synchrotron sources. CBT will utilize fs laser photo-excitation in combination with X-ray topography to resolve extremely short lived photo-intermediates in crystals. As a first step, using a monochromatic X-ray beam and flash freezing methods for cryo-trapping protein reaction intermediates, this experiment explored the use of topography for recovering structural information from two distinct protein conformations *within the* same *crystal,* thus mimicking potential CBT experiments.

Building on an earlier experiment (LS506, 25/10/96) which established the viability of X-ray topography at ID09 (the dedicated time-resolved beamline) the aims of this experiment were to:

- establish that a currently available, large area X-ray detector has sufficient spatial resolution for X-ray topography applications;
- develop novel methods of reaction initiation within crystals for trapping, in an *inhomogeneous* way, both a reaction intermediate and the initial conformation of a protein; and
- obtain two structures (native and intermediate) of the same protein from a single crystal.

Using a wide (600 μm horizon x 150 μm vertical) and monochromatic X-ray beam, which entirely exposed an unfrozen elongated (needle like) crystal of elastase, high quality X-ray topogrammes were recorded on X-ray film, the Fuji FDL 5000 X-ray detector, and the Mar CCD. Fig. 1 gives a typical topography image from the Mar CCD, establishing that this detector has sufficient spatial resolution for future CBT applications, thereby achieving the first aim of this experiment.

Crystals of elastase with bound β-casomorphin-7 were triggered inhomogeneously by establishing a pH gradient across the crystal, and then flash frozen. The original buffer was at pH 5, and the pH jump buffer at pH 10. A pH gradient was monitored using micro-spectroscopy, where a pH sensitive dye (cresol red) was soaked into the crystal providing a pH sensitive optical signal. Fig. 2 give spectra recorded from the opposite end of the same crystal, indicating the existence of a pH gradient, which could also be seen under a microscope with one end of the crystal appearing red and the other yellow.

As the elastase:β-casomorphin-7 complex [2] undergoes ester hydrolysis when the pH is raised, it was hoped that the pH gradient would allow the realisation of the second aim of this experiment: to inhomogeneously trigger a catalytic reaction within elastase crystals.

X-ray topogrammes recorded from frozen crystals (either with or without inhomogeneous triggering) again showed 'projection like' images of the crystal, yet these were not always parallel to each other, nor parallel to the exposed crystal. This 'twisting' of the crystal X-ray projections was interpreted as a physical twisting of the crystal itself due to freezing, with different regions of the crystal satisfying the Bragg condition at slightly different values of θ. To test this, still using a wide X-ray beam, we collected two data sets from frozen crystals (one with and one without a pH gradient) using a small rotation angle ($\Delta\theta$ = 0.2 °). With the Mar CCD one could see (in real time) from one image to the next, a diffraction spot appear (as the Bragg condition was satisfied), move across the detector (as different regions of the same crystal came into the Bragg condition), and then disappear. To date there has not been sufficient time (as we will do) to develop software predicting how the diffraction condition changes with θ when a slightly twisted crystal is rotated within a wide X-ray beam.

We also used a narrow X-ray beam (50 μm horizontal x 150 μm vertical) and recorded diffraction data sets from opposite ends and the center of frozen crystals. Data sets were recorded both from crystals with and without inhomogeneous pH jumps. These crystals diffracted to $\approx$ 1.6 Å resolution and typically R_{merge} was $\approx$ 3.5 % for any one data set. Correlations between different data sets, however, showed very promising results: intensity correlations between two ends of **the** same **crystal without** a pH gradient give R = 4.5 % (ie. no systematic intensity differences); intensity correlations between two ends of the same **crystal with** a pH gradient give R = 10.8 % (ie. significant systematic intensity differences); and intensity correlations between difrerent crystals (one with and one without pH jumps present) gave R $\geq$ 20 %. To date (more work is required) these observations have not produced interpretable electron density differences. These results, however, indicate that significant improvements in intensity correlations for time-resolved diffraction experiments may arise when both the reference and the trapped intermediate state are obtained from the same crystal.

We anticipate that, with further analysis, our results from LS-922 will be publishable. Steps towards $\sim$ ps resolution in time-resolved experiments at the ESRF have also been taken (experiment CH-522, scheduled for November 1998) which is based upon an experimental and analysis protocol [3] related to (yet less technically challenging than) our earlier suggestion [l] which motivated this experiment.

References:

[l] *Femtosecond time resolution in X-ray diffraction* experiments, Neutze, R. and Hajdu, J., Proceedings of the National Academy of Science U.S.A. 94, 5651-5655 (1997).

[2] Structure of a specific acyl-enzyme complex formed *between β-casomorphin-7 and porcine pancreatic elastase*, Wilmouth, R. C., Clifton, I. J., Robinson, C. V., Roach, P. L., Aplin, R. T., Westwood, N. J., Hajdu, J., Schofield, C. J., Nature Structural Biology, 4, 456-462 (1997).

[3] Deconvoluting ultrafast structural dynamics: temporal *resolution* beyond *the pulse-length of synchrotron radiation,* Neutze, R. and Wouts, R., submitted (1998).

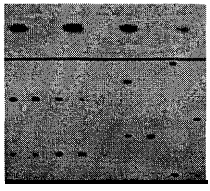

Fig. 1.

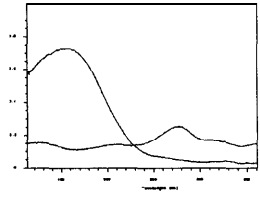

Fig. 2.