ESRF	**Experiment title:** Time-resolved Laue diffraction experiments from the enzyme dienelactone hydrolase (DLH)	**Experiment number:** LS-269
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Report:

Dienelactone hydrolase (DLH) is an important enzyme involved in the biodegradation of aromatic compounds. The structure has been determined to 1.8Å resolution using conventional methods (Pathak & Ollis, 1990). Mechanistic models have been proposed based on the structure and inhibitor binding studies (Cheah *et al.,* 1993). Two alternative models have been proposed, they differ mainly in the means of deacylation of an enolate intermediate.

The long term aim of this project is to use synchrotrons Laue diffraction to observe the short-lived enolate intermediate, and in particular to locate the position of a hydrolytic water molecule, which differs significantly between the two models.

To enable us to demonstrate that small changes in electron density in the active site of DLH can be observed using Laue data, we collected data from crystals of both native and mutant forms of DLH in an unmodified state and after soaking in an inhibitor (dienelactam). Flow cell experiments were also performed to gather information on the

time course of substrate turnover in the solid state using a natural substrate (dienelactone) and a slower non-natural substrate (methyl dienelactone). Disordering/reordering phenomena were observed to occur more quickly in the natural substrate.

In conventional X-ray experiments on a rotating anode laboratory source, using wild type crystals, the cysteine residue Cys123 appears to become oxidised by the incident X-rays during the course of data collection *(ca* 24 hours) (Pathak & Ollis, 1990). As synchrotrons Laue diffraction is an exceedingly rapid data collection process, with exposure times for these experiments typically in the millisecond and sub-millisecond time regimes we proposed to study the effects of oxidation of cys123 as a function of X-ray dose from wild type crystals. Although this work is incomplete we have been able to observe electron density corresponding to increased oxidation of cys123 proving that the technique is sensitive enough to observe small changes in electron density within the , active site of this enzyme.

References:

Pathak, D & Ollis, D.L. (1990) *J. Mol. Biol., 214,497-525*

Cheah, E., Ashley, G. W., Gary, J. & Ollis, D.L. (1993) *Proteins: Structure, Function and Genetics, 16, 64-78*