ESRF	**Experiment title:** Crystallographic analysis of the CuA center of a soluble domain from *Thermus thermophilus* cytochrome ba_3	**Experiment number:** LS-982
Beamline: BM14	**Date of experiment:** from: 08-May- 1998 to: lo-May-1998	**Date of report:** 1st Sept 1998
Shifts: 6	**Local contact(s):** Andy Thompson	*Received at ESRF:* 02 SEP. 1998

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Report:

At the time of data collection, we had already collected MAD data on the CuA center of cytochrome ba_3 and had made some progress towards determining the structure of the protein. We therefore turned our attention to another project ongoing in our laboratory, the determination of the structure of progesterone alpha-hydroxylase. This is an integral-membrane protein that has been engineered to enhance solubility to facilitate the structure determination. Over a number of years various forms of the protein had been used in crystallisation trials, but recent trials had produced crystals which diffracted X-rays weakly using a rotating anode source. We therefore decided to collect MAD data on derivatised crystals of alpha-hydroxylase and to investigate the possibility of utilising the signal from the single iron present in the protein as a source of phasing information.

An EXAFS scan of a large native crystal indicated the presence of a weak iron anomalous signal, and thus four datasets were collected so as to maximise this signal, one at the peak, one at the point of inflection and two at remote wavelengths. The data statistics are given in table 1. Patterson maps calculated at the beamline confirmed the presence of a single

iron, and preliminary electron density maps revealed the presence of many alpha helices, as predicted by secondary structure predictions and homology studies.

While the analysis of the iron-MAD experiment was being performed, a number of derivatives were screened. The Patterson map of a dataset collected from a gold derivative revealed some signal but a large amount of non-specific binding, and thus a shorter soak with a back soak was set up. A MAD dataset was collected from this gold crystal, again after an EXAFS scan had been performed to determine the wavelengths at which to collect data. Patterson maps indicated a single gold atom bound. In addition a number of other crystals were screened, but none of them proved to be derivatives.

Wavelength	PI: 1.740Å	PK: 1.739Å	RE: 1.6314Å	RE2: 0.9536Å
No of obs.	48684	48619	49067	49599
No of obs I/σ > 1	48241	48193	48718	49326
Mean I/σ	21.7	21.6	22.0	22.4
Completeness 50-3.3Å	98.7%	98.5%	98.7%	98.0%
Completeness 3.4-3.3Å	96.3%	93.6%	99.0%	99.7%
Rmerge 50-3.3Å	6.6%	6.5%	6.4%	5.9%

Table 1 ESRF Fe-MAD data collection statistics, May 1998

The six shifts of time which we were allocated proved sufficient to collect two complete MAD experiments. The electron density maps resulting from the data collected has enabled the tracing of the majority of the backbone of the protein. We have encounted some difficulties in obtaining more isomorphous derivatives due to large cell changes observed when compounds are introduced into the crystals. We therefore plan to collect additional MAD datasets on multi-site derivatives - it is possible to screen for these derivatives by calculating cross Fouriers between derivative data and the initial phasing information we obtained from the iron and gold MAD experiments.