ESRF	**Experiment title:** X-ray Diffraction Study of a 12-heme cytochrome c from *D.desulfiricans* ATCC 27774 using MAD measurements near the Fe-edge at low T	**Experiment number:** LS-41
Beamline: D2AM	**Date of experiment:** from: 14 June 1995 07:00 to: 16 June 1995 07:00	**Date of report:** 19 July 1995
Shifts: 4	**Local contact(s):** Dr. Michel Roth	*Received at ESRF:* 2 2 AOUT 1997

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Report:

1. EXPERIMENTAL and DATA PROCESSING

400 difraction images were measured from a frozen crystal of 12-heme cytochrome cc3 from *D.desulfuricans* ATCC 27774, using a wavelength of 1.7367 Å (near the Fe absorption edge, chosen from an EXAFS scan of the crystal), a crystal to detector distance of 246 mm, a detector tilt of 14 degrees, an image angular width of 0.5 degrees and an exposure time of 20 seconds per image.

These data were processed to obtain 24762 measurements of 15513 unique reflections in resolution range 39.8 to 2.88 A. This represents about 70% of the number of theoretically possible reflections in this resolution range, but only 7318 unique reflections had anomalous measurements. The processing statistics were very good, Rsym(I) = 3.8 %, however Ranom(I) = 3.9 %, indicating only a weak Fe anomalous signal to be present.

A Patterson map calculated using the anomalous differences as coefficients has been calculated and work towards its interpretation is currently under way in order to locate as many Fe sites as possible.

2. EXPERIMENT ASSESSMENT

This experiment was **not** what had been intended originally. Rather, a **4-wavelength MAD experiment** had been intended. This became **impossible** for several reasons:

- The cryosystem used at D2AM developed a fault which forced its shutdown on the morning of June 15. This problem could not be fixed by the technical staff and another cryosystem had to be borrowed from another beam line. The latter only became available in the afternoon of June 15, **thus the available time for this experiment was only 15 hours instead of 48, clearly insufficient for a full MAD experiment.**
- The fact that the closest crystal to film distance usable was about 240 mm coupled with the wavelength range near 1.74 Å meant that the highest resolution at the edge of the untilted detector was about 5 A, clearly unsatisfactory, forced us to tilt the detector by 14 degrees, gaining resolution **at the expense of data completeness and redundancy.**
- Finally, in order to obtain the most accurate measurements of anomalous differences, the crystal has to be set very accurately. In our case, since the crystals are monoclinic, this implied aligning the crystal with its b-axis along the spindle of the goniometer. Although the goniometer system used is rather ingenious, it did not allow the proper alignment of the frozen crystals of cc3 since the arc rotation needed went **beyond the allowed range of values** and thus the data were measured from an unset crystal. In addition, there is at present no simple general means of determining the setting angles for a crystal.

3. ADDITIONAL REMARKS

We encountered problems in the data processing which were caused by an incorrect calibration file. This was only discovered after the beam time period was over.

The data collection geometry used also conflicted with some parameter settings of the software, implying additional loss of time while these problems were solved.

Although the CCD readout time is very fast compared to other area detector systems, we discovered it took almost as long to write the image to disk. Since the data collection system only starts the next exposure once the previous has been written to disk, it took about a minute to expose, read out and write to disk one image. According to Dr. Michel Roth, this abnormal situation was caused by some network malfunction during the period of our experiment.