ESRF	**Experiment title: Structural studies of catalytic intermediates in copper-containing amine oxidase.**	**Experiment number: LS956**
Beamline: BM14	**Date of experiment:** from: 6/5/'98, 7.00 to: 8/6/'98, 7.00	**Date of report:** August '98
Shifts: 6	**Local contact(s):** Vivian Stojanoff	*Received at ESRF:*

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Report:

Four complete data sets were collected during this beam time allocation from crystals of amine oxidase intermediates trapped at various stages of the catalytic cycle by freezing in liquid nitrogen. The data statistics are given in Table 1. All the data sets have been processed and maps calculated. The structures of the enzyme active sites are currently being analysed.

In the first crystal, the enzyme was trapped by flash freezing, under aerobic conditions in a reduced intermediate state as judged by spectroscopy. The active site shows more than one conformation suggesting that the timing of the trapping needs further optimisation.

Table 1

Crystal	Resolution(Å)	Completeness(%)	R_{merge}(%)	Redundancy
Intermediate	2.3	88.0	6.5	3.4
cyanide	2.25	97.8	7.6	3.4
azide	1.95	94.1	5.7	2.4
nitric oxide	2.4	92.5	7.4	2.9

In the three other crystals, anerobically substrate reduced crystals had been exposeded to cyanide, azide and nitric oxide respectively, each of which competitively inhibits the role of molecular oxygen in the reaction pathway. In other metal containing redox enzymes, these molecules ligand to the metal at the oxygen binding site. Each of the inhibitors unexpectedly affects the active site in a different way. In the cyanide treated crystals, the ion is not liganded to the copper, however, the modified active site tyrosine binds to the copper in a similar manner to a previously observed "inactive" enzyme form. Density for both the azide and nitric oxide is visible and unexpectedly they occupy different copper ligand positions. A preliminary map of the active site of the substrate reduced/azide crystal form is illustrated in figure 1.

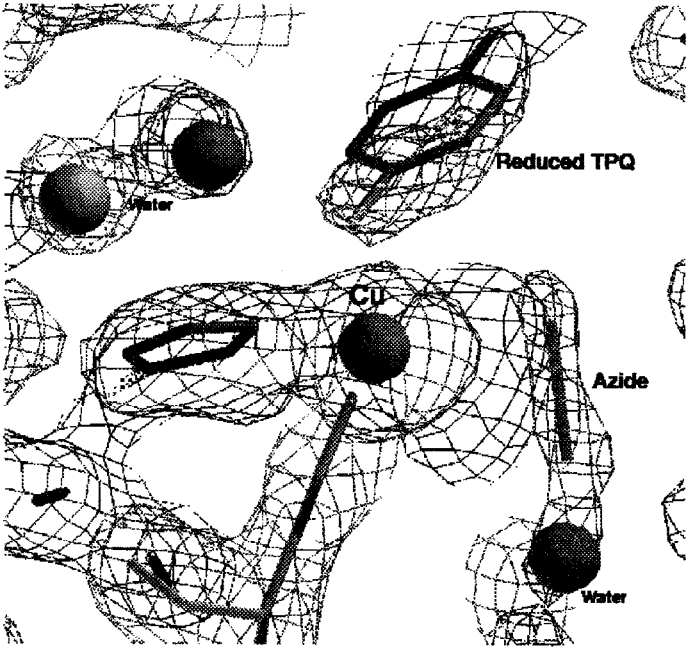

Figure 1

A MAD data set at three wavelengths was also collected from crystals of RepDC a replication initiator protein. These data have been processed (3.8 Å and R_{merge} of 14% for native, 20% λ_{remote} and 17% λ_{edge}) and structure solution attempts are ongoing.