ESRF	**Experiment title:** Crystallographic Studies of the H327A Mutant of Nitrite Reductase from *Pseudomonas aeruginosa*	**Experiment number:** LS1657
Beamline: ID14-2	**Date of experiment:** from: 10-4-00 to: 11-4-00	**Date of report:** Aug00
Shifts: 3	**Local contact(s):** Mark van RÅAIJ	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

A dataset to 2.7Å was collected on EH2 (Table 1) from crystals grown in space group $P4_322$ with cell dimensions a=b=70.523, c=281.23Å. The H327A mutant structure was solved by molecular replacement using AmoRe taking the c-heme domain and d1-heme domain of the wild-type reduced structure as two distinct starting models (Nurizzo *et al*, 1998). After iterative rounds of model building and both minimisation and B-factor refinement by CNS using bulk solvent correction, the c-heme domain Cα backbone could be traced from Val23 to Tyr543.

The refined crystal structure shows that the c-heme domain, whilst preserving its classical c-type cytochrome fold, has undergone a 60° rigid-body rotation about an axis parallel to the pseudo 8-fold axis of the β-propellor, and passing through residue Gln 115 (Figure 1). Even though the distance between the Fe ions of the c and d1-heme remains 21Å, the edge-to-edge distance between the two heme groups has increased by 5Å.

The occupancy of the d1-heme is only about 30%, a result which is confirmed in the uv/visible absorption spectrum from the crystals (measured using the in-house micro-spectrophotometer) which indicated that the d1-heme prosthetic group was almost absent (Figure 2).

Figure 1. Top (A) and side (B) view of the c-heme domain of the mutant structure H327A (yellow), compared with the wild-type (red). The d1-heme domain is coloured in grey

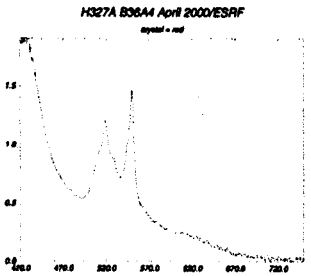

Figure 2. Visible absorption spectrum of the H327A mutant enzyme, showing the c-heme in the reduced state (peaks at 520 and 540nm) , and the small d1-heme peak (630nm).

Table 1 Structural Statistics

Data Collection		**Refinement Statistics**	
Beamline	ID14-EH2	Resolution (Å)	20-2.7
Space group	$P4_322$	Total reflections	19404
λ (Å)	0.9326	Total atoms/AU (protein/water)	4081/125
Resolution (Å)	2.7	R_{work}/R_{free} (%)	22.3/30.1
R_{sym} (%)	4.6 (32.1)	Mean B fact. (Å²) : main/side/solvent	57.9/61.1/54.1
I/σ	6.6 (2.3)	Rmsd B fact. : main/side	2.3/3.37
Completeness	95.8 (97.2)	Rmsd bonds (Å)	0.012
Redundancy	5.1	Rmsd angles,dihedrals,impropers (°)	1.7/25.2/1.5

References

Nurizzo D, Cutruzzolla F., Arese M., Bourgeois D., Brunori M., Cambillau C., Tegoni M. (1998) Biochemistry, 37, 13987

Publications arising from this work

Brown K, Cutruzzolla F., Wilson E.K., Bellelli A., Arese M., Brunori M., Tegoni M., Cambillau C (in preparation)

Cutruzzolla F., Brown K., Wilson E.K., Bellelli A., Arese M., Tegoni M., Cambillau C., Brunori M. Nitrite Reductase from Pseudomonas aeruginosa : Essential Role of Two Histidine Residues in Catalysis, PNAS (submitted)