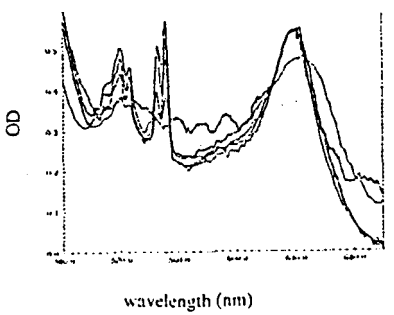
ESRF	Expcriment title: Dynamics of Nitrite Reductase along the the reaction pathway.	Experiment number: LS 898
Beamline: ID-14	Date of experiment: from: 30 Ap 98 to: 02 May 98	Date of report: 28 Sept 1998
Shifts: 6	Local contact(s): Wilhelm BURMEISTER	*Received at ESRF:* 1 SEP. 1998

Names and affiliations of applicants (* indicates experimentnlists):
Didier NURIZZO*
Mariella TEGONI*
Christian CAMBILLAU

Report:

Reaction intermediates of Nitrite Reductase from *Pseudomonas aeruginosa* (NiR) were obtained by soaking the oxidized crystals in the presence of ascorbate as reducing agent and cryoquenching the crystals at definite reaction times. The redox state of the enzyme was characterized using the microspectrophotometer installed on ID09.

Data were collected from crystals at 15%, 50% and 90% of c heme reduction (NiR-15, NiR-50 and NiR-90, respectively), previously cryoquenched and characterized by microspectrophotometry.



The crystals belonged to the space group $P2_12_12$, with unit cell (163.1x90.1x111.9) $Å^3$. A summary of the data collection statistics is shown in table 1. Datasets were indexed and integrated with DENZO (Otwinovski, Z. (1993) Yale University) and PrOW (Bourgeois et al. (1998)*J. Appl. Cryst. 31*, 22-35). They were merged with the CCP4 suite (Collaborative Computing Project 4, Daresbury Laboratory, UK, 1979) at a maximum resolution of 2.70 Å.

Table 1	NiR-15	NiR-50	NiR-90
resolution (in Å)	40.0-2.70	40.0-2.70	40.0-2.70
overall I/sI	5.6	8.9	3.7
overall R_{sym}	5.2	6.7	6.6
completeness (in %)	99.7	99.0	98.0

The oxidized model of NiR at 2.15 Å resolution without any ions or water molecules, was used as the starting model. In order to compare the NiR-15, NiR-50 and NiR-90 models with the fully reduced model diffracting between 12.0 to 2.90 Å, identical refinement procedures were used with the same resolution range between 12.0 and 2.90 Å and the same reflections sets for the R- free calculation. After several cycles of X-PLOR3.8 (Brunger et al.(1987)*Science 235*, 458-460; Brunger A. T. (1996), X-PLOR Manual. Yale University, New Haven, USA) rigid-body refinement. (2mFo-DFc and mFo-DFc) maps were calculated (Read, R. J. (1986) *Acta Cryst A42*, 140-149). The refinement and stereochemical data are given in Table 2.

Table 2	NiR-15	NiR-50	NiR-90
Rfree / R factor (in %)	24.7 / 20.3	24.6 / 20.8	24.1 / 20.4
rmsd on bonds (in Å)	0.009	0.011	0.009
on angle (in °)	1.660	1.750	1.673
on dihedral (in °)	25.294	25.186	25.218
on dihedral (in °)	1.431	1.517	1.358

The analysis of these results is in progress, in particular for the role of the redox state of the c heme on the structural re-arragements observed previously in the fully reduced NiR.