ESRF	**Experiment title:** MAD on *Paramecium caudatum* hemoglobin	**Experiment number:** LS1372
Beamline: BM14	**Date of experiment:** from: 18-02-99 to: 20-02-99 14-05-99 16-05-99	**Date of report:** 03-08-99
Shifts: 18 to BAG	**Local contact(s):** Vivian Stojanof, Ioan Petrescu	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(*) Alessandra Pesce, Martino Bolognesi
Advanced Biotechnology Center
INFM-University of Genova
Italy

Report:

Small hemoproteins, or truncated hemoglobins (Hbs) have been discovered rather recently in protozoa, unicellular algae and in bacteria. Truncated Hbs display no more than 116 to 130 amino acids per protein chain (thus being 20-35 residues shorter than most vertebrate Hbs) and their sequence relationship to vertebrate and non-vertebrate Hbs is weak. The function(s) of truncated Hbs is completely unknown.

In this context, we studied the three-dimensional structure of the truncated Hb from *Paramecium caudatum* (PMHb). Crystals of PMHb have been grown using 35% ammonium sulfate as precipitant, at pH 5.5. Molecular replacement techniques didn't allow to solve the structure, due to low sequence identity with other Hbs of known 3-D structures.

A three wavelength MAD experiment, run at BM14, ESRF (Grenoble), on the heme Fe-atom X-ray absorption edge, was successful in solving the PMHb structure.

Diffraction data were collected to 2.7 Å resolution at the wavelength corresponding to the absorption peak of the spectrum (λ = 1.739 Å) and to the inflection point (λ = 1.740 Å) and to 1.54 Å resolution at the remote wavelength (λ = 0.979 Å). The crystals belong to space group $P4_3$, with unit cell $a=b=61.2$ Å, $c=35.8$ Å, one molecule per asymmetric unit. The structure was solved with SOLVE (initial phases at 2.7 Å resolution); subsequently the wARP package was used to improve MAD phases and for autotracing (the whole protein main chain and side chains). The structure was refined with CNS, at 1.54 Å resolution (R-factor 0.13, R-free 0.18) and the final model contains 116 amino acids and 207 water molecules per asymmetric unit.

A preliminary analysis of the structure shows an overall globin fold, which is however affected by large structural perturbations, due to the lack of the A helix, to the lack of the CD loop and of the D helix, to a striking rearrangment of EF corner and to the presence of an extended polypeptide chain in the region of the expected F helix. Manuscript in preparation.