



	Experiment title: Structure determination of <i>M.tuberculosis</i> Hb	Experiment number: LS-1664
Beamline: ID14-3	Date of experiment: from: 26-04-2000 to: 28-04-2000	Date of report: 01-08-00
Shifts to BAG: 6	Local contact(s): Stephanie Monaco	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): (*) Alessandra Pesce, Mario Milani and Martino Bolognesi Advanced Biotechnology Center INFM-University of Genova Italy		

Report:

Two genes, glbN and glbO, encoding hemoglobin-like proteins (HbN and HbO, respectively) were recently discovered in the complete genome sequence of the virulent *M.tuberculosis* strain. In particular, HbN was suggested to be involved in the protection of the bacilli against reactive nitrogen species produced by the host macrophage during the initial infection or during latency. HbN and HbO belong to a distinct hemoglobin family, called truncated hemoglobins, displaying unique structural rearrangements of the classical globin fold.

We obtained very thin crystals of HbN (complexed with O₂) by vapour diffusion method, using phosphate as precipitant.

During these shifts, we collected a native data set at 1.9 Å (R_{symm} 5.3%, completeness 95%) and four possible heavy atom derivatives (platinum, gold and lutetium, respectively). A mercury derivative did not show diffraction. The crystals belong to the orthorhombic space group P2₁2₁2₁ (unit cell $a=45$ Å, $b=62$ Å, $c=91$ Å, with 2 molecules of about 16kDa each, per a.u.).

After an extensive check of Patterson maps, we couldn't find good peaks, indicating that these heavy atom derivatives are not bound to the protein.

We are waiting for beamtime for a MAD experiment on heme Fe atom absorption edge.

N.B.about 6 hours were lost because of a shutter problem.

- Pesce, A., Couture, M., Dewilde, S., Guertin, M., Yamauchi, K., Ascenzi, P., Moens, L., Bolognesi, M.
A NOVEL TWO-OVER-TWO α -HELICAL SANDWICH FOLD IS CHARACTERISTIC OF THE TRUNCATED HEMOGLOBIN FAMILY. *EMBO J.* (2000), **19**, 1-11.

Summary of HbNO₂ data collections

	Resolution (Å)	Completeness (%)	R _{symm} (%)	Comment
Native	40-1.9	95.1	5.3	-
Pt	40-2.4	84.6	10.8	not a derivative
Au	50-2.75	98.9	9.3	" "
Lu	50-2.6	99.3	4.7	" "