	Experiment title: Structure of enzymatic antioxidants and accelerated evolution of enzymes	**Experiment number**: 30-01-737
Beamline: BM30A	**Date of experiment**: from: 18-6-05 8h00 to: 20-6-05 8h00	**Date of report**: 26-9-05
Shifts: 6	**Local contact(s)**: Lilian Jacquamet	*Received at ESRF:*

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Report:

Development of new biological catalysts for oxidoreduction reactions.

H-chain ferritins (H-Ft) are members of the super-family of di-iron-oxo proteins, the di-iron centre is situated in the middle of the ferritin subunit and known as the “ferroxidase centre” because of its ability to oxidise iron. An unusually short Fe-Fe distance of 2.53 angstroms was found by EXAFS spectroscopy when compared to the other di-iron proteins (3.1 to 4.0 angstroms). In order to mimic the metal-binding site of the other di-iron proteins (like methane monooxygenase), and to increase the Fe-Fe distance, we have mutated independently and together the residues Glu107 and Glu27 which complex iron in the ferroxidase centre by two Asp residues.
We have used recombinant human H-chain ferritin in which Lys86 has been replaced by Gln (HuHF K86Q) in order to render the protein crystallisable. Since the iron centres in ferritins are relatively labile and, upon oxidation, the di-iron tends to move into the iron storage cavity, in order to study the metal binding site, we used Zn^{++} as a redox-stable alternative to Fe^{++}. Apoprotein is also analysed to determine if a conformational change occurs with metal incorporation.
Data were collected for four mutants: E107D, E27-107D and the same crystals soaked in a $ZnCl_2$ solution. Similar data for the E27D mutant and of the wild type protein (with and without soaking in $ZnCl_2$) had been previously obtained. The results show that the E27D mutant strongly binds two Zn^{2+} ions in the ferroxidase centre, with an interdistance of 3.4 Å, while no ions are observed in the E27-107D mutant. The distance

between the carboxylate oxygen atoms (O_{27} ... O_{107}) is 6.35 Å , comparable to 6.44 Å in the wild type, while the same distance becomes 8.79 Å in the E27-107D mutant.