ESRF	**Experiment title:** **Comparative study of the binding sites of Zn: a proton transfer inhibitor in respiratory enzymes.**	**Experiment number**: SC-1853
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Report:

The results of the experiment have been published in three papers of which we report in the following the reference details and abstracts.

L. Giachini, F. Francia, G. Veronesi, D.-W. Lee, F. Daldal, L.-S. Huang, E. A. Berry, T. Cocco, S. Papa, F. Boscherini, G. Venturoli
X-ray absorption studies of Zn^{2+} binding sites in bacterial, avian and bovine cytochrome bc_1 complexes.
***Biophysical Journal* 93 (2007) 2934-2951.**

Abstract
Binding of Zn^{2+} has been shown previously to inhibit the ubiquinol cytochrome *c* oxidoreductase (cyt bc_1 complex). X-ray diffraction data in Zn-treated crystals of the avian cyt bc_1 complex identified two binding sites, located close to the catalytic Q_o site of the enzyme. One of them (Zn01) might interfere with the egress of protons from the Q_o site to the aqueous phase. Using Zn K-edge X-ray absorption fine structure spectroscopy (XAFS) we report here on the local structure of Zn^{2+} bound stoichiometrically to non crystallized cyt bc_1 complexes. We performed a comparative XAFS study by examining the avian, the bovine and the bacterial enzymes. A large number of putative clusters, built by combining information from first-shell analysis and metalloprotein databases, were fitted to the experimental spectra by using *ab initio* simulations. This procedure led us to identify the binding clusters with high levels of confidence. In both the avian and bovine enzyme a tetrahedral ligand cluster formed by two His, one Lys and one carboxylic residue was found, and this ligand attribution fit the crystallographic Zn01 location of the avian enzyme. In the chicken enzyme the ligands were the His121, His268, Lys270 and Asp253 residues, and in the homologous

bovine enzyme they were the His 121, His267, Lys269 and Asp254 residues. Zn^{2+} bound to the bacterial cyt bc_1 complex exhibited quite different spectral features, consistent with a coordination number of six. The best fitting octahedral cluster was formed by one His, two carboxylic acids, one Gln or Asn residue and two water molecules. Interestingly, by aligning the crystallographic structures of the bacterial and avian enzyme, this group of residues was found located in the region homologous to that of the Zn01 site. This cluster included the His276, Asp278, Glu295 and Asn279 residues of the cyt *b* subunit. The conserved location of the Zn^{2+} binding sites at the entrance of the putative proton release pathways, and the presence of His residues point out to a common mechanism of inhibition. As previously shown for the photosynthetic bacterial reaction center, zinc would compete with protons for binding to the His residues, thus impairing their function as proton donor/acceptors.

F. Francia, L. Giachini, F. Boscherini, G. venturoli, G. Capitanio, P.L. Martino, S. Papa
The inhibitory binding site(s) of Zn^{2+} in cytochrome c oxidase.
***FEBS Letters* 581 (2007) 611-6161.**

Abstract
EXAFS analysis of Zn binding site(s) in bovine-heart cytochrome c oxidase and characterization of the inhibitory effect of internal zinc on respiratory activity and proton pumping of the liposome reconstituted oxidase are presented.
EXAFS indentifies tetrahedral coordination site(s) for Zn^{2+} with two N-histidine imidazoles, one N-histidine imidazol or N-lysine and one O-COOH (glutamate or aspartate), possibly located at the entry site of the proton conducting D pathway in the oxidase and involved in inhibition of the oxygen reduction catalysis and proton pumping by internally trapped zinc.

L. Giachini, F. Francia, F. Boscherini, C. Pacelli, T. Cocco, S. Papa, G. Venturoli
EXAFS reveals a structural zinc binding site in the bovine NADH-Q oxidoreductase.
***FEBS Letters* 581 (2007) 5645-5684.**

Abstract
The metal content of bovine NADH-Q oxidoreductase determined by inductively-coupled plasma atomic-emission spectroscopy reveals the presence of about one atom of Zinc per molecule of FMN. We applied Zn K-edge extended X-ray absorption fine structure spectroscopy (EXAFS) to investigate the local structure of the bound zinc ion and to identify the nature of the coordinating residues. The EXAFS spectrum is consistent with a structured zinc binding site. By combining information from first-shell analysis and from metalloprotein data bases putative binding clusters have been built and fitted to the experimental spectrum using *ab initio* simulations. The best fitting binding cluster is formed by 2 histidine and 2 cysteine residues arranged in a tetrahedral geometry.