ESRF	**Engineered metallo-peptides as antioxidant enzymes mimics: XAFS investigation of the active site**	**Experiment number**: **CH-4354**
Beamline: BM23	**Date of experiment**: from: 09/07/2015 to: 14/07/2015	**Date of report**:
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Report:

Aim of the experiment

The design of proteins with novel chemical structures and biological functions is a powerful tool to study the principles and mechanism of protein folding and bioactivity, and to develop original catalysts and materials with unique chemical properties. In this context, the precise knowledge of the coordination chemistry of the metal site is one of the topmost issues in the study of such biomacromolecules, which would allow improving the design in terms of metal-binding affinity and optimized coordination geometry to carry out specific functions. A recent challenging example is represented by the use of biomimetic complexes involving Fe-redox metabolism[1] as growth inhibitor for bacteria infections (as example the Pseudomonas aeruginosa[2]). The main goal of our project was to exploit XAFS to carefully probe the electronic nature and coordination chemistry of newly synthesized cono-peptides coordinating Fe, Cu and or Mn [1] and biomimetic molecules: Pyochelin in which the metal-binding site, normally Fe, can be replaced by Ga [2]. Conopetides are a class of highly rigid disulphide constrained bioactive macromolecules, used as a scaffold to design metallo-peptides that are able to carry out redox reactions. Ga-Pyochelin is used as Iron uptake pathways inhibitor.

Experiment: *XAS measurements* were performed on Iron-conopeptides, Copper-conopetide, Pyochelin-Fe(III) and Pyochelin-Ga(III) samples at the BM23 XAFS beamline. Spectra were acquired in fluorescence geometry from liquid samples. The beam energy was calibrated and monitored during the measurements determining the absorption spectra of a Fe, Cu and Ga reference metal foil placed after the sample. To perform XAS measurements from liquid-phase samples, aqueous solutions of Fe-conopeptides(5.6 mM), Cu-conopetide(0.3 mM), Pyochelin-Fe(III) and Pyochelin-Ga(III) (2 mMol) were enclosed in a plexiglass cell with Kapton windows: 5 mm (vertical) 8 mm (horizontal). The solutions were cooled around 20 K to preserve the samples from Fe(III) photo-reduction, and to reduce the thermal contribution to the structural disorder. Water crystallize during cooling producing Bragg peaks on the spectra.

In order to reduce the effect of Bragg peaks we found better a slow cooling procedure within the XAS cryostat instead of fast cooling (that is wetting the sample in LN before closing into the XAS cryostat) because fast cooling produces sharper peaks (larger crystallites) which are simply individuated in the spectra, instead fast cooling produces more Bragg peaks and broader (many small crystallites) making difficult to treat the data. Many (5-10) scand were collected tilting the sample of ± 5 deg. To move the Bragg peaks along the spectra. This allows a reliable treatment of the data and final spectra suitable for accurate quantitative analysis.

Preliminary results:

Example of good quality Cu K-edge normalized XANES spectra on Cu-conopeptide are shown in figure. The analysis is in progress: the experimental XANES spectra have to be compared with model XANES calculated acordingly to calculated models for Cu local structure.

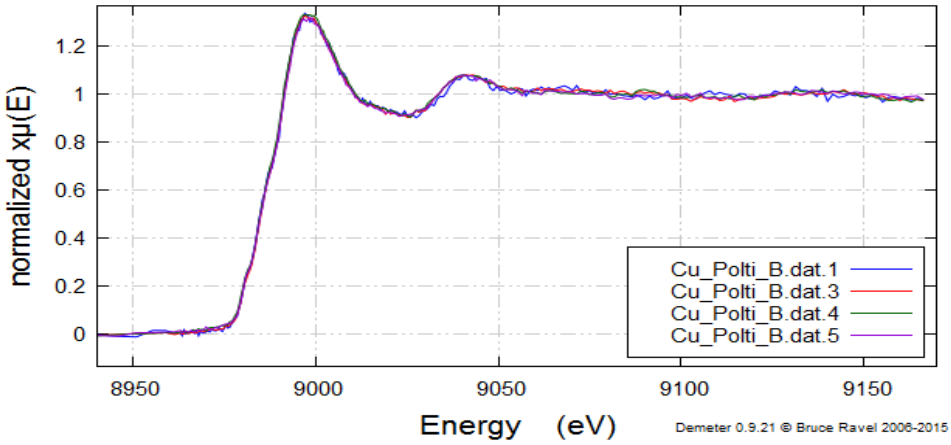

Figure of Cu-K edge xafs spectra on Cu-conopeptide, normalized and Bragg peak cleaned.

Regarding the Pyochelin, the good quality of the measurements allowed us to acquire the extended region of Absorption Spectrum. A preliminary analysis confirmed the active site structural stability after Ga substitution.

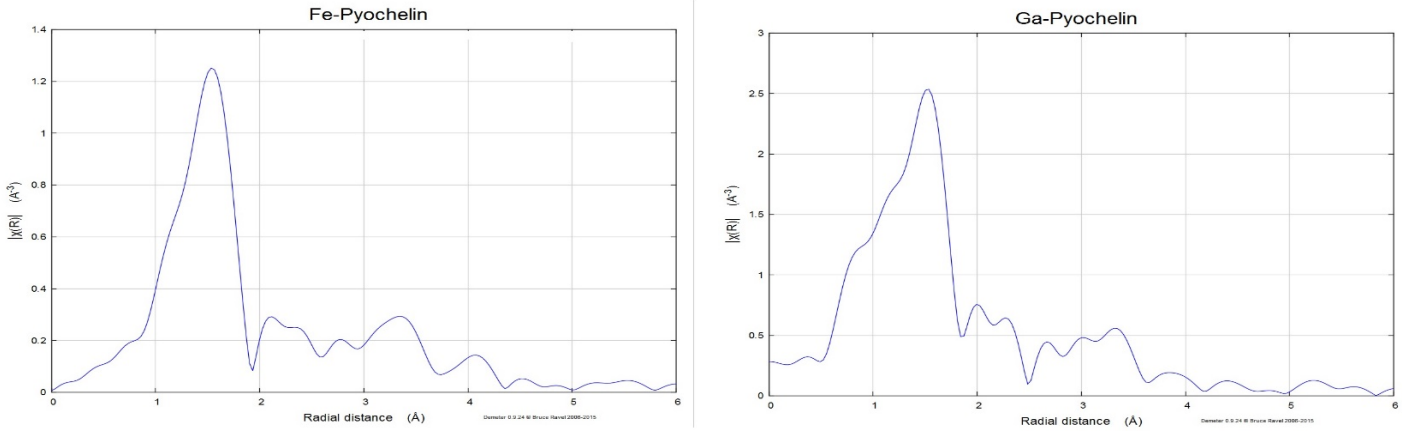

Figure of Fe-Pyochelin(left) and Ga-Pyochelin(left) Fourier Transformed absorption spectra.

References:

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[2] Frangipani, E; Bonchi, C; Minandri, F Imperi, F; Visca, P Antimicrob. Agents Chemother 58,9 5572-5575 (2014)