EUROPEAN SYNCHROTRON RADIATION FACILITY
ESRF User Office
BP 220, F-38043 GRENOBLE CEDEX, France
Delivery address: 6 rue Jules Horowitz, 38043 GRENOBLE, France
Tel: +33 (0)4 7688 2552; fax: +33 (0)4 7688 2020; email: useroff@esrf.fr; web: http://www.esrf.fr

Application for beam time at ESRF – Experimental Report for CH-3245

X-ray Absorption Spectroscopy studies of the simultaneous interaction of copper and zinc ions with peptides relevant for Alzheimer's disease.

Scope of the project: The initial aim of the project was to make use of XAS (XANES and EXAFS) to gain insights into the coordination of Zn^{2+} and to both Cu^{+} and Zn^{2+} to the amyloid-β (Aβ) peptide involved in Alzheimer disease (AD). Due to partial precipitation of our samples that preclude spectra recording in high metal ion concentration (not above 1 mM) and to the deficiency of two among the nine detectors (including the central one), we were not able to record the number of samples we have initially planned. Indeed, during the 18-shifts session, we recorded about ten workable XAS spectra. As a consequence, we decided to favour the study of Zn^{2+} binding to Aβ. Indeed, only few studies are reported dealing with Zn^{2+} binding to Aβ and XAS are the method of choice for such studies. To determine the amino-acids involved in the Zn^{2+} coordination (and not only the first coordination sphere of the metal centre), we study 8 pertinent mutants (thus also completing our previous study on FAME, experiment CH 3015). First analysis of XANES and EXAFS data has shown interesting results and will allow the **proposition of a structural Zn^{2+} binding model to Aβ.** These results will thus help better describing the metal centre environment when bound to the Aβ peptide, which is still an open debate and which is an important issue regarding the importance of metal ions in the development of AD. Moreover, we obtained **very promising first results** (XANES only) **regarding the Zn^{2+} pH dependent binding to Aβ.**

Results: The results obtained on Zn^{2+} coordination to Aβ and to a wide series of relevant mutants are shown in Figure 1 (left panel) where the XANES spectra of Zn^{2+} bound to the human Aβ and to 8 Aβ mutants are depicted. In Figure 1 (right panel), the XANES spectra of Zn^{2+} bound to the human Aβ at 3 pH values are also compared.

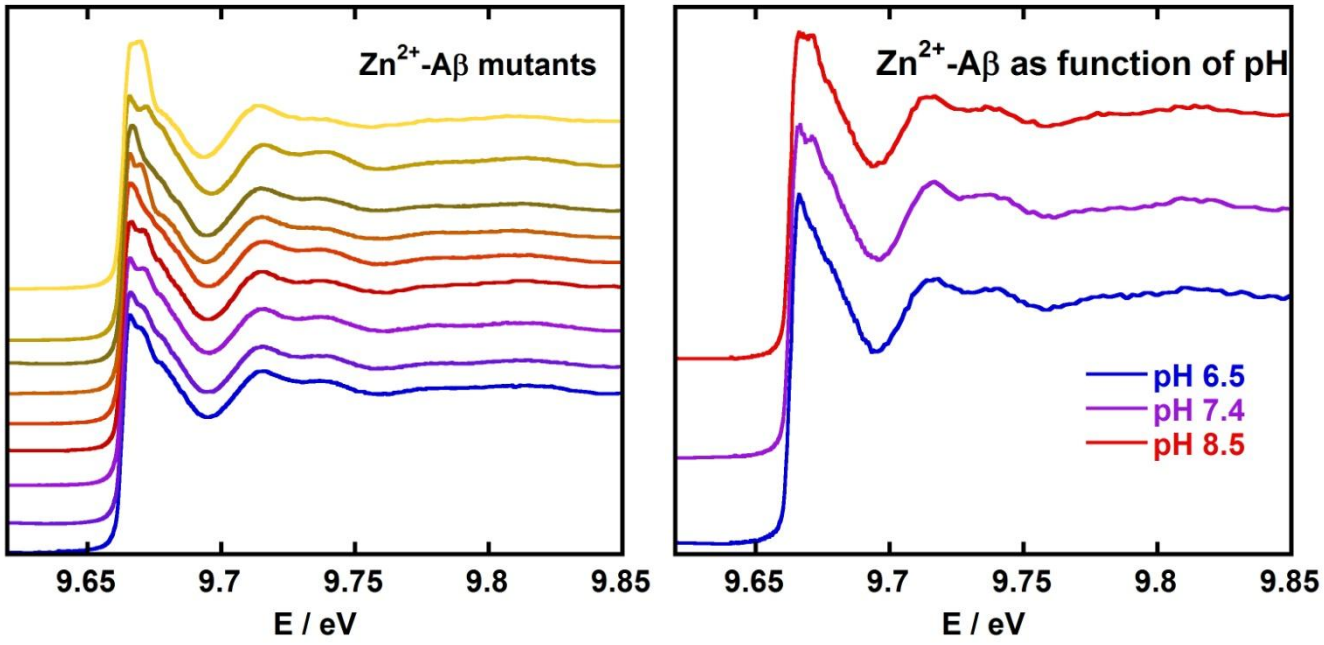

Figure 1. XANES spectra of Zn^{2+} bound to the human Aβ (blue line) and to mutants and XANES spectra of Zn^{2+} bound to Aβ as a function of pH (right panel).

In the examples chosen in Figure 1, there are significant differences between the spectroscopic signatures of Zn^{2+} bound to human Aβ and mutants. By studying a series of mutants, it is then possible to discriminate which are the amino-acids residues involved in the metal center coordination and we have thus identified several key residues for the coordination of Zn^{2+} to human Aβ and exclude other residues previously proposed in the literature, e.g. the Tyr10. Full analysis of the EXAFS data will bring some crucial insights into the structural modification encountered in the mutants compared to the human Aβ. Moreover, it has been shown that the XANES signature of Zn^{2+} bond to human Aβ is pH dependent thus indicating that around physiological pH at least two Zn-Aβ complexes are present (as was previously demonstrated for the Cu^{2+} centre). Effect of pH on the Zn^{2+} coordination to Aβ is very intersting and we hope that we will have the opportunity to study it in a forthcoming session.

Experimental details: Zn K-edge XAS spectra were recorded on the BM26A beamline during a 18-shifts session in December 2010. The measurements were performed on ~mM solution or aggregated samples at low temperature (He-cryostat) in the fluorescence mode using a 9-element high-purity Ge detector. The energy was calibrated by the simultaneous measurement of a Zn foil spectrum in transmission. For each samples, about ten EXAFS spectra were recorded and averaged.

Publication: On the basis of the data we obtained during this session, we expect to publish one paper where the XAS results (XANES and EXAFS on Zn^{2+} coordination to human Aβ and mutants) will be the principal topic of the paper.