ESRF	**Experiment title:** **A study on how Zn(II) ions perturb Cu(II) coordination to Aβ peptides and modulate the peptide aggregation thus possibly affecting the Alzheimer disease progression**	**Experiment number**: LS 2332
Beamline: BM30B	**Date of experiment**: from: 13/11/2014 to: 18/11/2014	**Date of report**: 26/02/2015
Shifts: 15	**Local contact(s)**: Olivier Proux	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Emiliano De Santis * Physics Dept. and INFN - University of Roma 'Tor Vergata' - Italy

Velia Minicozzi Physics Dept. and INFN - University of Roma 'Tor Vergata' - Italy

Silvia Morante * Physics Dept. and INFN - University of Roma 'Tor Vergata' - Italy

Olivier Proux * OSUG, Observatoire des Sciences de l'Univers de Grenoble - France

Giancarlo Rossi Physics Dept. and INFN - University of Roma 'Tor Vergata' - Italy

Sunil S. Saxena Chemistry Dept. - University of Pittsburgh - U.S.A.

Ishara K. Silva Chemistry Dept. - University of Pittsburgh - U.S.A.

Francesco Stellato * Physics Dept. and INFN - University of Roma 'Tor Vergata' - Italy

Report:

In this experiment we have analyzed at a structural level the mechanism by which different metal ions compete in the binding to the beta-amyloid (Aβ) peptides involved in the Alzheimer's disease (AD). The hypothesis that metal ion competition in biological systems is critical for homeostasis is becoming more and more well established. Metal homeostasis is of particular relevance in the central nervous system, where ion imbalance has bee related to several, severe neurological diseases. In the context of AD [1,2], Cu and Zn have been the most studied metal ions [3-5]. Recent EPR data [6] and XAS [7] measurements, carried out in the related case of the prion protein (PrP), confirmed that there is a competition for PrP binding between the two ions, thus suggesting the existence of a general mechanism of fine regulation of metal binding possibly to prevent cell damage from accumulated free ions. In this general framework it appears to be of the utmost importance to understand and clarify whether and how Cu(II) and Zn(II) cross-interact with amyloidogenic peptides in general, and Aβ peptides in particular.

As a natural extension of the recent EPR experimental results [8] on Aβ-[Cu/Zn] complexes and those [7,9] obtained using XAS on the similar PrP-[Cu/Zn] complexes, we performed a XAS study of the Aβ-[Cu/Zn] complex with the aim of elucidating at the atomic level the cross-interaction dynamics when both ions are simultaneously present. We collected XAS spectra on the same set of samples studied in [8], containing Aβ with Cu and Zn at different concentration ratios. Furthermore to investigate the competition between Cu and Zn ions for peptide binding, we collected spectra from complexes prepared by dissolving in the peptide solution first one of the two ions, either Cu(II) or Zn(II), and then, after incubation, adding the other ion only a few minutes before measurement. Experiments have been carried out at the BM30b beamline. We were granted 15 shifts out of the 18 we had asked in the proposal.

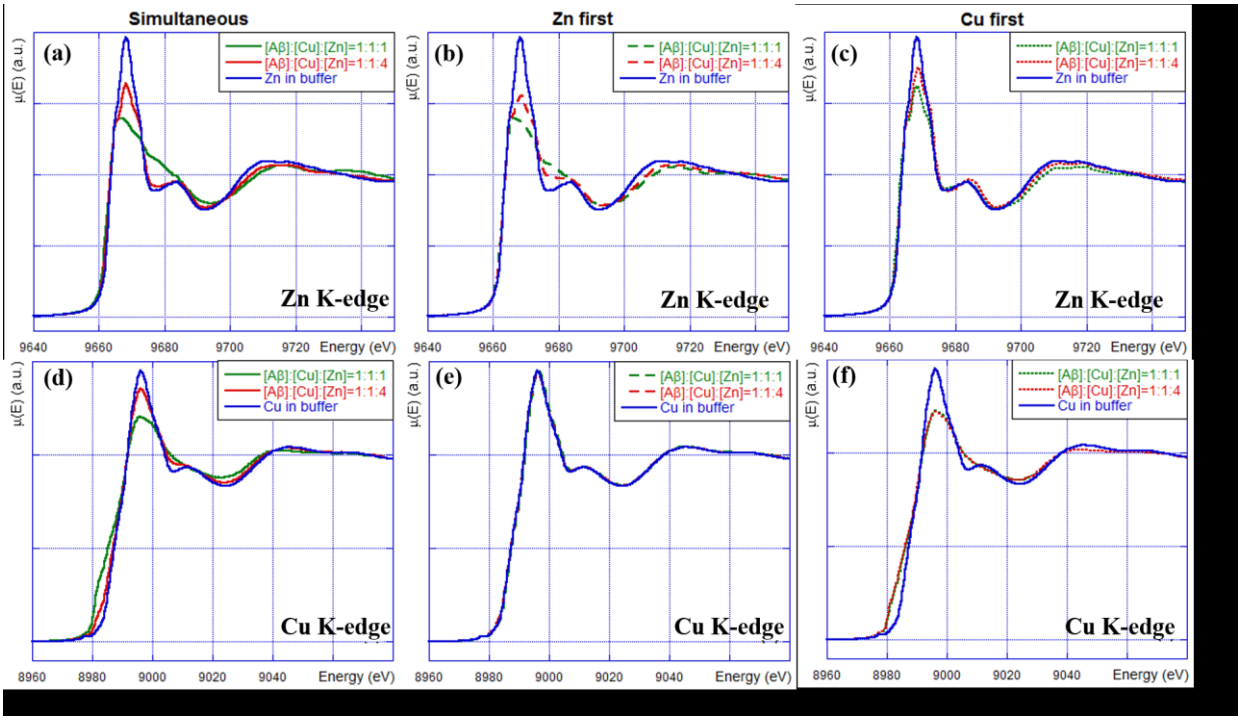

Fig. 1 – Comparison among spectra at the Zn (panels a-c) and Cu (panels d-f) K-edge. In each panel we group samples obtained from the same preparation procedure. Panels (a,d): simultaneously added metals; panels (b,e): Zn added first; panel (c,f): Cu added first. Concentration ratios are indicated in the legend.

The comparison among the acquired spectra (see **Fig.1**) suggests that the order in which metals are added to the peptide solution matters, but, the strongest effect is when Zn is added first, because apparently in this situation Cu binding practically does not occur. On the contrary, when Cu is added first, it does not (completely) prevent Zn binding. The effect of the relative metal concentration is more difficult to assess. First of all because it depends on the ordering by which the metals are added to the solution and secondly because, when the Zn concentration is increased, the spectrum at the Zn K-edge is most probably a combination of the spectrum of the Zn bound to the Aβ-peptide and that of Zn free in solution.

The quantitative analysis of EXAFS spectra is ongoing. Anyway we have preliminary results showing that Cu and Zn ions are coordinated to different numbers of histdine residues according to the relative [ions]:[peptide] concentration ratio and the order in which metal ions are added to the solution.

In the conclusion, our study exploited the unique chance offered by XAS to investigate the way the two metals interact with the peptide and clarify their cross-interaction when they are simultaneously present. Elucidating this issue will help shedding light on the role of metal dis-homeostasis in devastating neurodegenerative diseases, like AD.

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