ESRF	**Experiment title:** Copper(I) removal from the amyloid-β peptide involved in Alzheimer's disease by [N,S$^-$] ligands in presence of Zn(II)	**Experiment number**: 30-02-1125
Beamline: BM30B	**Date of experiment**: from: 19th April 2017 to: 25th April 2017	**Date of report**: 20/12/2017
Shifts: 18	**Local contact(s)**: Denis Testemale (email: denis.testemale@neel.cnrs.fr)	*Received at ESRF:*

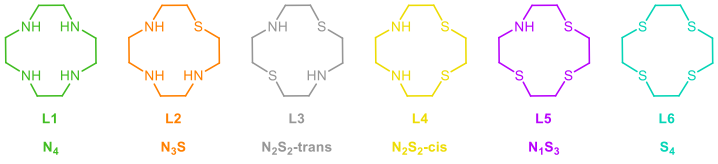
Names and affiliations of applicants (* indicates experimentalists):

* Dr Elena Atrián-Blasco (Laboratoire de Chimie de Coordination-LCC, UPR 8241, Toulouse, France)
* Dr. Clémence Cheignon (LCC)
Dr Fabrice Collin (LCC)
* Amandine Conte-Daban (LCC)
Pr. Emmanuel Guillon (Institut de Chimie Moléculaire de Reims-ICMR, CMR UMR CNRS 7312, Reims, France)
Dr. Christelle Hureau (LCC)
* Dr. Laurent Sabater (LCC)

Scope of the project and results:

The amyloid-β peptide, which can be found forming the senile plaques in Alzheimer's diseases brains, can coordinate both Cu and Zn metal ions. These are found naturally in the brain, but their dishomeostasis could be in part responsible of the development of this neurodegenerative disease. Particulary interesting is the case of Cu which could be implicated in the production of reactive oxygen species (ROS) and in the oxidative stress found in AD. One of the strategies for the treatment of the disease relies on the ability of different ligands to chelate Cu, forming stable complexes, thus, avoiding the production of ROS and their deleterious effects. However, Zn(II) is present in the sypnaptic cleft at high concentrations, and could affect the efficacy of the ligands to chelate Cu. Another key point is that the redox state of Cu ions in the brain remains unknown. Therefore, the effort should not only be focused on the development of Cu(II)-chelating ligands, but also Cu(I). As 1,3,5-triaza-7-phosphaadamantane (PTA) a phosphine capable of reducing Cu(II) to Cu(I) and forming and stable complex: $[Cu^{I}(PTA)_4]^+$. The ability of this ligand to remove Cu from Aβ peptides was already investigated by our team using the beamline BM30B at the ESRF during the experiment 20130207.[1] Indeed, XANES has been a powerful spectroscopy in our case, due to the intrinsically disordered nature of the Aβ peptide which impedes the caracterization of the metal-complexes of Aβ. Furthermore, XANES spectroscopy is the most appropriate method to study both Cu(I) and Zn(II) complexes, which have a d^{10} electron configuration.

During experiment 20150466 several Cu(II) chelators were studied, as well as their effect in presence of Zn(II).[2] For this experiment 30-02-1125, four different series of ligands were proposed. However, due to problems in the synthesis and purification of some of the products, two families of ligands were finally studied: (A) a series of cyclen and thiacyclen macrocycles (see below) and (B) the effectiveness of 1,3,5-triaza-7-phosphoadamante was reevaluated, this time in presence of Zn(II).



A) This series of cyclen and thiacyclen derivatives have been synthetized by Nora Kulak's group in Berlin. They can be of interest in this project due to their different affinities for Cu(II), Cu(I) and Zn(II). The

formation of Cu(II) complexes can be studied by EPR and UV-vis spectroscopies, but the use of XANES spectroscopy was required to obtain knowledge about their coordination to Cu(I) and Zn(II) and their abilities to remove these ions from Aβ peptide. The Zn K-edges data show that only L1 and L2 have a higher affinity for Zn(II) than Aβ, and only L1, L2, L4 and to some extent L3 can coordinate Zn(II). From the Cu K-edge data we can observe that the lack of sulfur atoms in the structure of the macrocycle precludes the coordination of Cu(I). Thus, L1 can not remove Cu(I) from Aβ, while the rest of the ligands are able to and form a Cu(I) complex. These results agree with the affinities reported for the ligands for both metal ions. In figure 1 the spectra acquired for L5 are shown as an example.

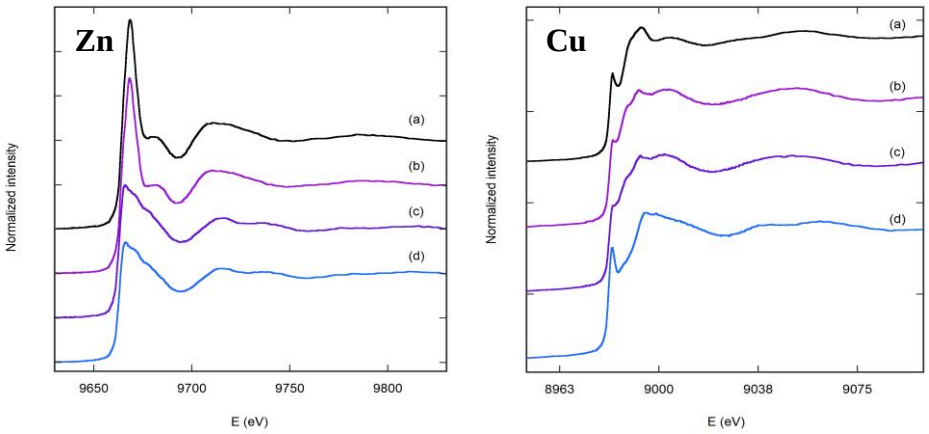


Figure 1. Zn and Cu K-edges spectra of (a) Zn^{II} or Cu^{I} in buffer, (b) Zn^{II} or Cu^{I} + L5, (c) Zn^{II}(Aβ) or Cu^{I}(Aβ) + L5, (d) Zn^{II}(Aβ) or Cu^{I}(Aβ). HEPES buffer 50 mM pH 7.4, [Cu] = [Zn] = 0.9 mM, [$Aβ_{16}$] = [L] = 1 mM. Copper was reduced with dithionite at 10 mM, and the solution was kept under an Ar atmosphere. Glycerol 10% v/v was used as a cryoprotectant. T = 20 K.

B) The experiments performed using PTA in this session have shown that the presence of Zn does not impede the removal of Cu from Aβ peptides and the formation of the complex $[Cu^{I}(PTA)_4]^{+}$. Furthermore, Zn remains coordinated to Aβ, after the removal of Cu by PTA. Figure 2 shows the spectra recorded at Zn K-edge.

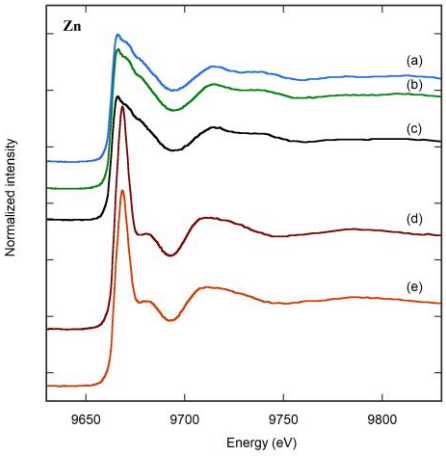


Figure 2. Zn K-edge spectra of (a) Zn(Aβ), (b) Zn(Aβ) + 5 equiv. PTA, (c) Cu,Zn(Aβ) + 5 equiv. PTA, (d) Zn + 5 equiv. PTA, (e) Zn in buffer. HEPES buffer 50 mM pH 7.4, [Cu] = [Zn] = 0.9 mM, [$Aβ_{16}$] = 1 mM, [PTA] = 5 mM. Glycerol 10% v/v was used as a cryoprotectant. T = 20 K.

Experimental details: Zn and Cu-K edges XANES spectra were recorded on the FAME beamline during a 18-shifts session in April 2017. The measurements were performed on mM solutions at low temperature (He-cryostat) in the fluorescence mode using a 30-element high-purity Ge detector. The energy was calibrated by the measurement of Zn and Cu foil spectra in transmission. For each sample, at least 3 XANES spectra were recorded and averaged.

Conclusions: In this experiment session we have tested different ligands: cyclen derivatives (L1-L6) and the phosphane PTA. For the first ligands, they show different abilities to remove Cu(I) or Zn(II) from the Abeta peptide, in accordance to thir affinities. For PTA, we could check that it is effective in copper removal even in presence of Zn(II). These results complete and confirm previous experiments on these ligands.

Publications: we expect to publish 2 papers on the removal of Cu(II/I) from Aβ in presence of Zn(II): one for the series of cyclen and thiacyclen ligands and another one for the phosphine PTA which would be a continuation of the communication published in 2015.[1]

References:

1 E. Atrián-Blasco, E. Cerrada, A. Conte-Daban, D. Testemale, P. Faller, M. Laguna and C. Hureau, *Metallomics*, 2015, 7, 1229–1232.

2 A. Conte-Daban, A. Day, P. Faller and C. Hureau, *Dalton Trans.*, 2016, **45**, 15671–15678.