| | **Experiment title: Ability of new N-based thioether and thiol(ate) ligands (L[MSN], n = 2 or 3) to remove Cu(I) from the amyloid-beta peptide involved in Alzheimer's disease in presence of Zn (II)** | **Experiment number**: A30-2-1161 |
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| **Beamline**: BM30 | **Date of experiment**: from: February, 1st          to: February, 7th | **Date of report**: 23/02/2023 |
| **Shifts:** 18 | **Local contact(s)**: Denis Teste male | *Received at ESRF:* |

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**Report:**

One therapeutic approach explored in Alzheimer's disease consists to remove the redox active copper ions bound into the amyloidogenic peptide involved in the disease (amyloid-β peptide, Aβ). Despite several years of development, this strategy did not yield expected results in terms of therapeutic effects. While most of the ligands studied were thought to target the Cu(II), Cu(I) ligands have been overlooked and might be the missing piece of the puzzle for the strategy to be effective. In this context we have developed N-based thioether and thiolate ligands dedicated to the removal of Cu(I) from Aβ to fill this gap. The project aim to gain insights into the binding site of Cu(I) (and Zn(II)) with the designed ligands and to monitor the metal removal from the Cu(Aβ) species in presence and in absence of Zn(II). Zn(II) is also present in high amount in the synaptic cleft; its impacts on Cu detoxification by the designed ligands must thus be evaluated.
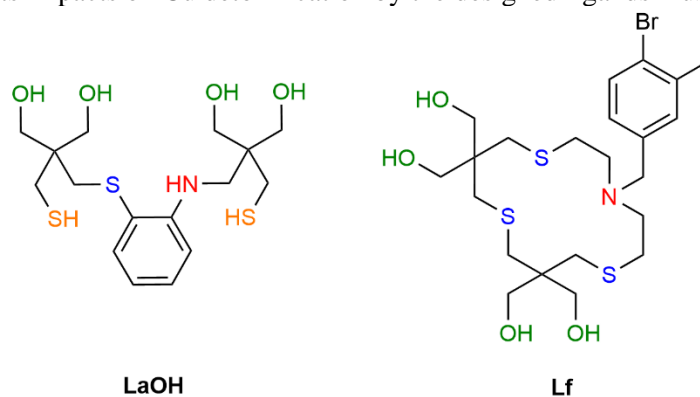


*Figure 1: Ligand studied during the beam-line session*

**Main results:**

The ability of Lf (Figure 1) to form complexes with Cu(I), Cu(II) and Zn(II) ions has been studied. It appears clearly that Lf is not able to chelate Zn (II) ions, indeed the Zn K-edges XANES spectra of a mixture of Lf and Zn(II) in stoichiometric proportion is the same than the free Zn(II) in the buffer (Figure 2). As we want a ligand with a good selectivity between Cu(I) and Zn(II), this is a very important result.
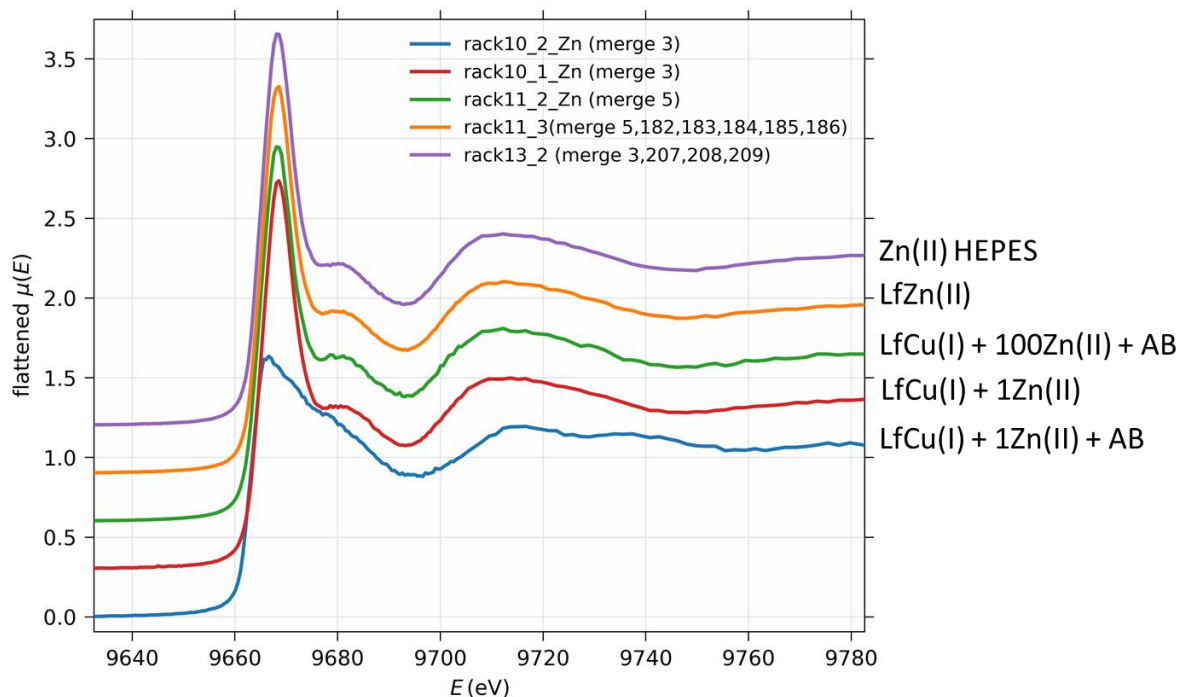
*Figure 2: Zn K-edges spectra of (violet) Zn, (orange) Zn(Lf), (green) 100 eq.Zn + (Lf) + Cu(I) + Aβ (red) 1 eq.Zn + (Lf) + Cu(I) and (blue) 1 eq.Zn + (Lf) + Cu(I) + Aβ . HEPES buffer 50 mM pH 7.4, 10 % DMSO, [Cu] = [Aβ16] = [Lf] = [Zn] = 1 mM, except in presence of 100 eq of Zn, [Zn] = 100 mM. 10 % glycerol are used as a cryoprotectant. T = 20 K.*

We have measured the XANES spectra of the LfCu(I) complex, and get a proof of its formation as the spectra is different from the one of Cu(I) without ligand (Figure 4). This information is in line with the preliminary ^{1}H NMR spectra of Cu(I)Lf recorded. Then, we have clearly highlighted the spontaneous reduction of the Cu(II)Lf complex to the Cu(I)Lf complex under ambient conditions ($O_2$). The pre-edge peak at 8985 eV characteristic of the presence of Cu(I) is growing over the time (Figure 3). The conversion is completed after 8h, and the Cu(I)Lf remains stable in solution for at least 24 h. To obtain more details about the coordination of the Cu(I) inside Lf, EXAFS spectra have been recorded and need to be fitted. From the XANES region it is not that easy to determine if the complex is coordinated by 3 or 4 donor atoms s.
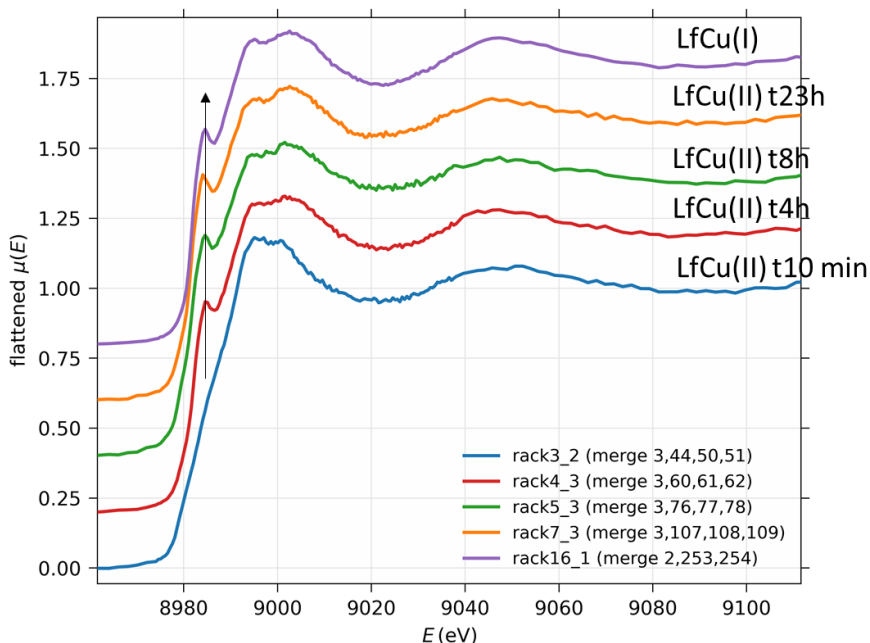


*Figure 3: Cu K-edges spectra of (blue) Cu(II)Lf, t = 10 min, (red) Cu(II)Lf, t = 4h, (green) Cu(II)Lf, t = 8h (orange) Cu(II)Lf, t = 23h and (violet) Cu(I)Lf. HEPES buffer 50 mM pH 7.4, 10 % DMSO, [Cu] = [Lf] = 0.5 mM. 10 % glycerol are used as a cryoprotectant. T = 20 K.*

We have then monitored the swap of metallic ions between Cu(Aβ) and (Lf) (Figures 2 and 4)

$$Cu(A\beta) + Zn(II) + (L) \longrightarrow Cu(L) + Zn(A\beta)$$

The spectra indicate that Cu(I) removal by Lf from Aβ is total even in presence of Zn(II) in a large excess (100 eq). For this ligand the Cu over Zn selectivity is not known and those experiments give us access to the relative affinities.

For Cu(I) : Lf > Aβ

For Zn(II) : Aβ > Lf

Those results are in line with the ^{1}H NMR results we obtained recently and with the ability of this ligand to inhibit the Cu(Aβ)-induced ROS production even in presence of Zn(II).
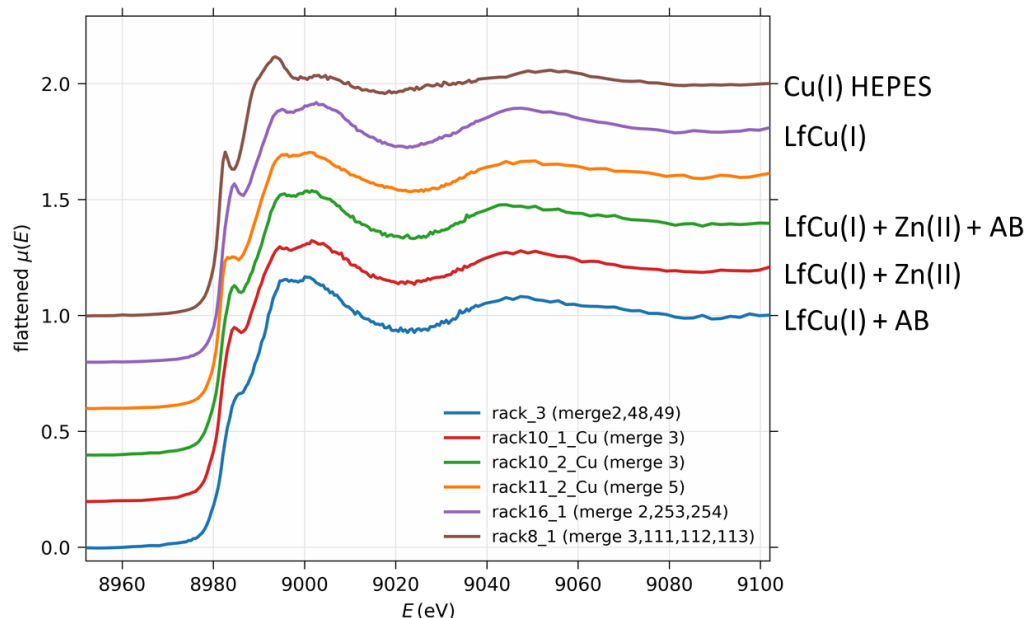


Figure 4: Cu K-edges spectra of (violet) Zn, (orange) Zn(Lf), (green) 100 eq.Zn + (Lf) + Cu(I) + Aβ (red) 1 eq.Zn + (Lf) + Cu(I) and (blue) 1 eq.Zn + (Lf) + Cu(I) + Aβ . HEPES buffer 50 mM pH 7.4, 10 % DMSO, [Cu] = [Aβ16] = [Lf] = [Zn] = 1 mM, except in presence of 100 eq of Zn, [Zn] = 100 mM. 10 % glycerol are used as a cryoprotectant. T = 20 K.

We also measured the Zn and Cu K-edges EXAFS spectra of an azathiamacrocycle (L1-5) series of ligand in order to get indication on the Cu(I) coordination inside the macrocycle.

**Conclusion:** During this beam-time we have studied the ability of Lf and LAOH to chelate the Cu(I) and Zn(II) metal ions in aqueous buffer and in DMSO respectively. The experiments in DMSO where difficult to set up du to problem to obtain regular frozen drops. The ability of Lf to remove Cu(I) from Aβ has been studied. We show that Lf is efficient in the removal of Cu(I) from Aβ, even in the presence of Zn(II). This is the first example of a chelator which is selective of Cu(I), inhibiting ROS production and stable under its reduced form even in presence of $O_2$. This makes this ligand a very interesting and significant candidate in the context of AD. Alongside, XANES is the method of choice to study such metal swap with Cu(I) and Zn(II) two $d^{10}$ metal ions which are silent with classical spectroscopies.

**Experimental details:** Zn and Cu K-edges XANES and EXAFS spectra were recorded on the FAME beamline (BM30) during a 18-shifts session in February 2023. The measurements were performed on ~mM solution 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 Cu foil spectra in transmission. For each sample, at least 3 XANES and 5 EXAFS spectra were recorded and averaged. We experiment several beam lost (6 h) and we lost 24 h of measurement du to spectra which appeared suddenly very glitched and unusable.

**Publications:** We expect to publish one paper on the Cu(I) removal from Aβ with the Lf.