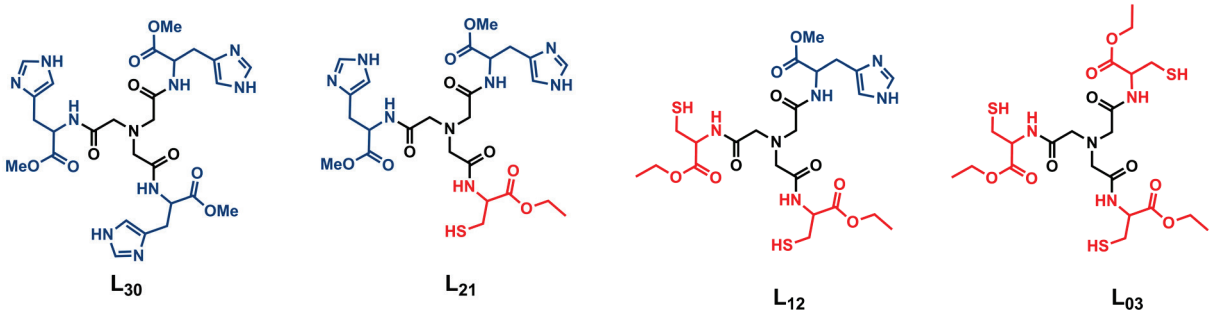
EUROPEAN SYNCHROTRON RADIATION FACILITY
ESRF User Office
Experimental Report for 20140210

Copper(I) removal from the amyloid-β peptide involved in Alzheimer's disease by [N,S⁻] ligands.

Scope of the project:

(A) The initial aim of the project was to make use of XAS (XANES and EXAFS) to (i) characterize the coordination of Cu(I) of [N,S⁻] ligands and (ii) to test their ability to remove Cu(I) from the Aβ peptide, where the Cu-Aβ interaction is considered deleterious in the Alzheimer's disease (AD) context, (B) In addition to the former planned study, we have been able to perform another study on the impact of oxidative modifications of the Aβ peptide on its ability to bind Cu(I), Cu(II) and Zn(II) ions. This is a key issue since Reactive Oxygen Species (ROS) produced by Cu(Aβ) itself do oxidize the Aβ peptide, thus changing its metal ions binding ability, leading to a runaway of the ROS production and impacts on Aβ aggregation, the two deleterious events in the aetiology of AD.



Results:

(A.i) The first EXAFS simulation results obtained on Cu(I) coordination to the [N,S⁻] ligands (L_{03}, L_{21} and L_{12}, see scheme above) are shown in Figure 1 and summarized in Table 1 (For L_{03}, Inorg. Chem., 2013, 52, p9954).

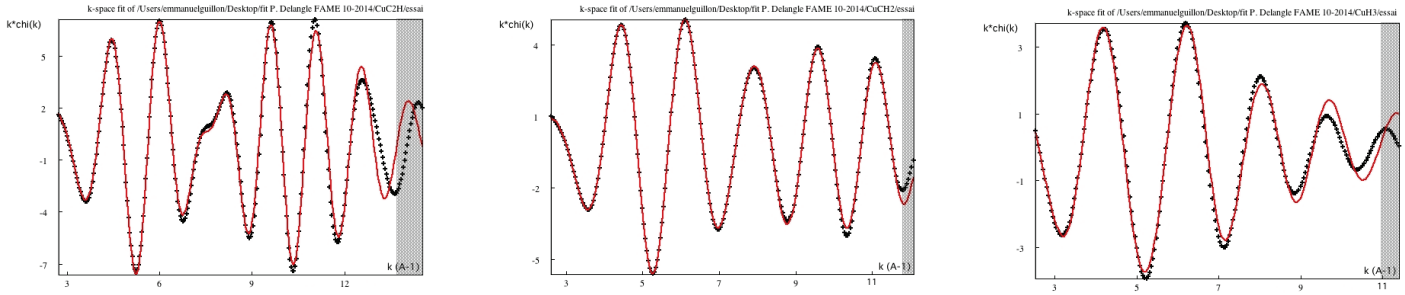


Figure 1. k-space fit of [Cu(L_{ns})] complexes, left: L_{ns}=L_{12}, middle: L_{ns}=L_{21}, right: L_{ns}=L_{30}. Recording conditions: phosphate buffer 50 mM pH 7.4, [Cu] = 1 mM, [L_{ns}] = 1.1 mM. T = 20 K.

Ligand	Cu-N (Å)	N	σ^2 (Å^2)	Cu-S (Å)	N	σ^2 (Å^2)	Cu-Cu (Å)	N	σ^2 (Å^2)	ref.
L_{03} (mononuclear)				2.23	3	0.005				Inorg. Chem., 2013, 52, p9954
L_{03} (cluster)				2.26	3	0.005	2.73	3	0.015	
L_{12} (cluster) fit 1	2.39	4	0.0099	2.27	3	0.0038	2.66	4	0.01	This work
L_{12} (cluster) fit 2	2.44	3	0.0099	2.27	3	0.0033	2.67	4	0.01	
L_{21} (cluster)	2.31	3	0.01	2.27	2	0.0042	2.63	1	0.01	
				Cu-N/O (Å)						
L_{30} (mononuclear)	1.96	3	0.00496	2.08	1	0.00264				

Table 1. EXAFS fitting results.

These results are in line with other characterizations (NMR, CD...) obtained in the group of P. Delangle. In particular, they confirm the high nuclearity (N=5) of the $[Cu(I)L_{ns}]_N$ species when $L_{ns} = L_{12}$ as determined by DOSY experiments. The characteristic beat due to Cu-Cu interactions is clearly observed at 8 $Å^{-1}$ (Figure 1, left).

(A.ii) Then, we have probed the Cu(I) removal from the Aβ peptide by ligands L_{30}, L_{21}, and L_{12}, as pictured in Figure 2. In agreement, with the known affinities of the L_{ns} ligands and Aβ peptide for Cu(I), we have observed a total removal when $L_{ns} = L_{12}, L_{21}$ and a partial removal when $L_{ns} = L_{30}$.

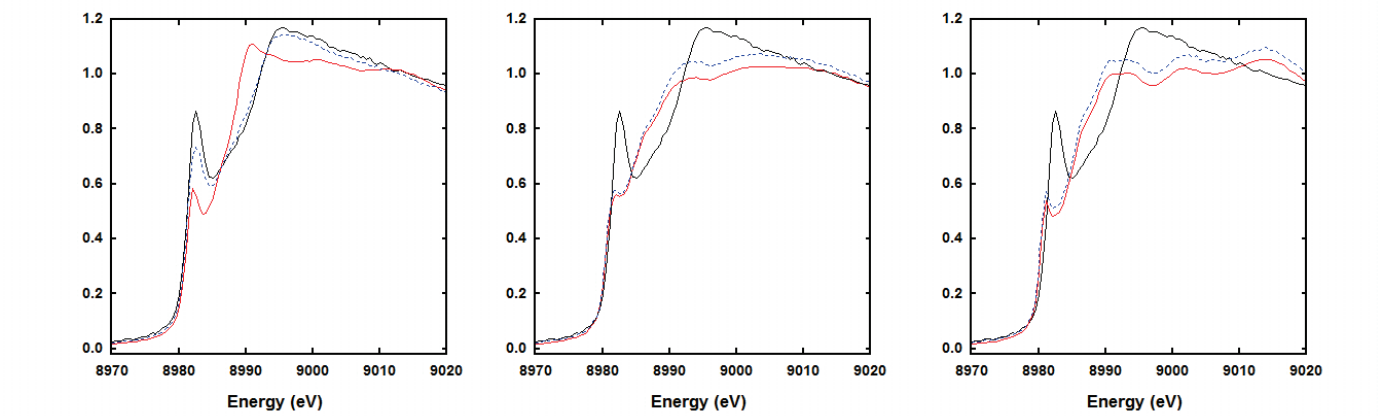


Figure 2. Normalized XANES spectra of a mixture of Cu(I)(Aβ) and of ligands $L_{ns} = L_{30}$ (left), L_{21} (middle) and L_{21} (right). Recording conditions: in phosphate buffer 50 mM pH 7.4, [Cu(Aβ)] = 1 mM, $[L_{ns}] = 1$ mM. $T = 20$ K.

(B) In this second kind of the experiments, we want to determine the impact of Aβ peptide oxidations (for the protocol of Aβ oxidation leading to $Aβ^{ox}$, see Angew. Chem., 2013, 52, p11110) on Cu and Zn coordination. As can be seen in the first series of experiments pictured in Figure 3, the impact of Aβ oxydation depends on the metal ion. Regarding Cu(I) and Zn(II), oxidation leads to a partial decoordination of the metal ion indicating that when the peptide is oxidized it is no more able to bind neither the Cu(I) nor the Zn(II) ion, but that oxidation is only partial impacting only a portion of the available peptide. Regarding Cu(II), the situation is more complex and Cu(II) is not released in the buffer (Fig. 3, panel B). This indicates that oxidation of the peptide leads to a reorganization of the Cu(II) coordination sphere. For comparison, note that a similar trend is also observed when the terminal amine, the most prominent binding group of the Cu(II) in the native peptide, has been acetylated and thus, its Cu(II) binding prevented (Fig. 3, panel C).

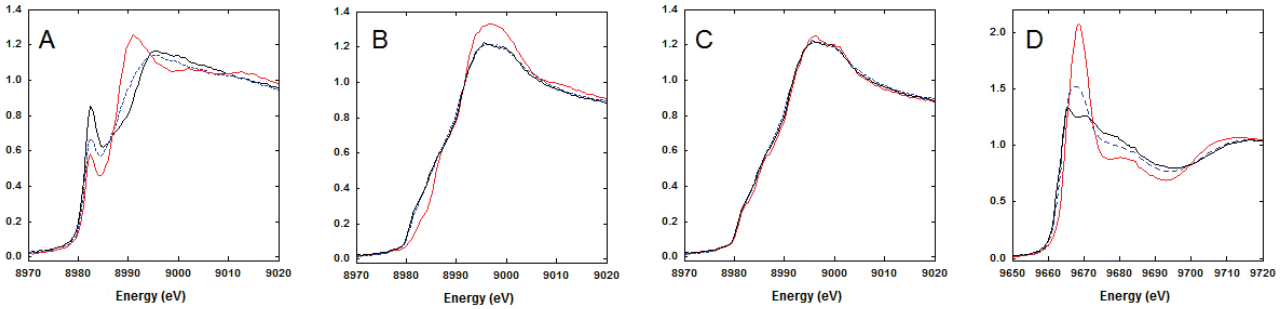


Figure 3. Normalized XANES spectra of Cu(I) (PanelA, in buffer: red line, bound to Aβ: black line and bound to $Aβ^{ox}$: dashed blue line), of Cu(II) (Panels B and C, in buffer (panel B) or bound to Ac-Aβ (panel C): red line, bound to Aβ: black line and bound to $Aβ^{ox}$: dashed blue line) and of Zn(II) (Panel D, in buffer: red line, bound to Aβ: black line and bound to $Aβ^{ox}$: dashed blue line). Phosphate buffer 50 mM pH 7.4, [M] = 1 mM, [peptide] = 1.1 mM. $T = 20$ K.

Experimental details: Zn and Cu K-edges XANES spectra were recorded on the FAME beamline during a 18-shifts session in October 2014. 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 Zn and Cu foil spectra in transmission. For each sample, about 4 to 6 XANES or EXAFS spectra were recorded and averaged.

Publications: We expect to publish two papers on the Cu(I) removal from Aβ study, a first one with the L_{30} ligand, which has the same range of affinity for Cu(I) than Aβ, thus being a quite "weak" ligand and a second one with the higher Cu(I) affinity ligands (L_{12} and L_{21}). A third publication on the Aβ oxidation story could be foreseen although completion with several kinds of other oxidized peptides could be helpful.