ESRF	**Experiment title:** Copper binding effect on Zinc coordination mode of synthetic peptides mimicking Prion Protein sequence.	**Experiment number**: SC 3104
Beamline: BM30B	**Date of experiment**: from: 10/11/2010 to: 16/11/2010	**Date of report**: 15/8/2013
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Report:

In these experiments we used x-ray absorption spectroscopy (XAS) to study the binding of Zn(II) and Cu(II) ions to the tetra-octa-repeat portion of the prion.
The prion protein is a membrane-bound protein mainly expressed in the brain tissue of various organisms. It occurs in two alternative conformers: a cellular native harmless conformer, rich in a-helix, and a pathogenic conformer that has self-replicating properties and is characterized by larger b-sheet content. This latter form is involved in the pathogenensis of the so called transmissible spongiform encephalopathies. It has been shown that the presence of metal ions such as Cu(II) and Zn(II) can bind to the prion protein and that they have an influence on its folding (1).
In order to shed light on the effect of Zn(II) ions on the Cu(II) coordination modes we had already performed XAS measurements (2) that allowed us to demonstrate that Zn(II) acts by directly interacting with the peptide, in this way competing with Cu(II) for binding with histidine.
In these new measurements we studied the Zn(II) binding mode at different [Zn(II)]:[peptide] concentration ratios, both in the absence and in the presence of Cu(II) ions. We therefore prepared samples at different Cu(II) and Zn(II) concentration and acquired the XAS spectra at both Cu and Zn K-edge (see Table 1).

Sample	Zn(II) concentration (eq)	Cu(II) concentration (eq)
S_1	0.8	0
S_2	0.8	0.8
S_3	0.8	3.2
S_4	2	0
S_5	3	3
bCu	10	0
bZn	0	10

Table 1 – Samples overview. Concentrations are given in peptide equivalent (eq), so 1 eq corresponds to 0.2 mM.

XAS experiments were performed at the BM30B beamline (3) and the spectra were recorded in fluorescence mode using a 30-element solid-state Ge detector.

In Fig. 1 (left panel) a comparison of the several Zn K-edge XANES spectra we acquired is shown. It is clear that the buffer spectrum is different from all the other ones, thus indicating that in all the samples at least a fraction of the Zn(II) is bound to the peptide. Comparing the peptide containing samples, one can see that sample S_1, which contains the lowest concentration of Zn(II) and no Cu(II) at all, has the most different spectrum in comparison with that of bZn. Samples S_2 and S_3, in which Cu(II) has been added in different concentration, have very similar spectra thus suggesting that the effect of Cu(II) is already saturated at low Cu(II) concentration. Moreover, the spectra of samples S_5 and S_4, that are characterized by very different Cu(II) concentration, but have high Zn(II) concentration, are the most similar to that of bZn, thus suggesting that in these conditions there are Zn(II) ions free in solution.
An analogous comparison of the spectra acquired at the Cu K-edge is then made (Fig. 1, right panel). First of all, since the spectrum of bCu is different from all the others, it is clear that Cu(II) ions always bind to the peptide, even when Zn(II) is present. It is also evident that the Cu(II) coordination mode depends on its concentration.

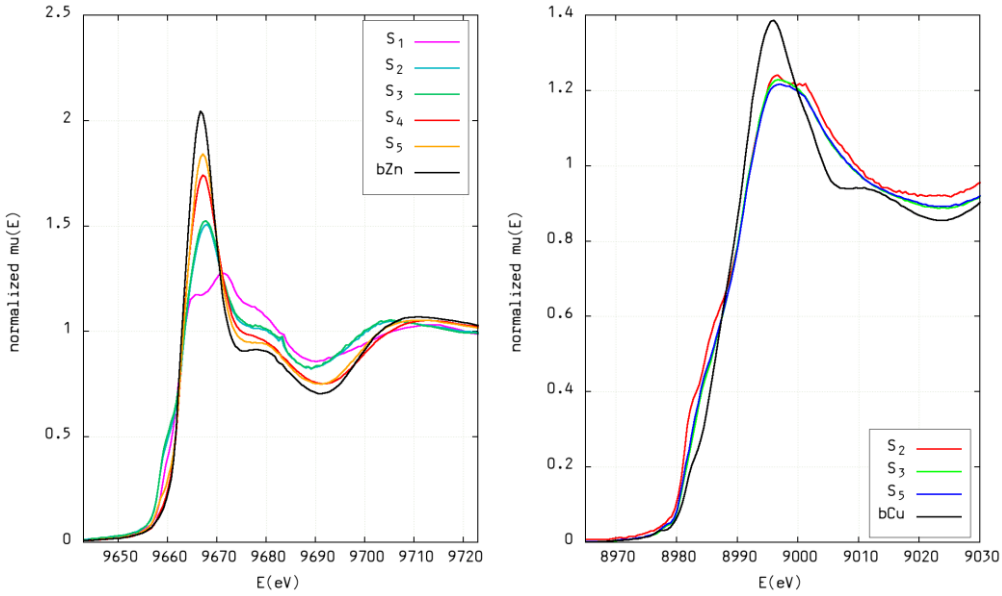

Figure 1 – Comparison among XANES spectra aquired at the Zn (left panel) and Cu (right panel) K-edge

Our measurements show (4) that the binding mode of Zn(II) in the absence of Cu(II) promotes the formation of small peptide clusters bridged by Zn ions that are disrupted by the presence of Cu(II), even if Cu(II) is not able to completely remove the Zn(II) from the peptide.
On the other side, we show that the Cu coordination mode is strongly dependent on the relative Cu(II)/peptide concentration. In agreement with previous EPR and XAS studies we observe that at increasing Cu(II) concentration the number of histidine residues bound to the Cu decreases. This behaviour is conserved also when Zn ions are present.
Our results confirm that metal binding competition can play an important role in the metal homeostasis mechanism.

References

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