

Report Beamtime use Gilda, Oct. 2013 (CH 3933 round 04/2013)

In the October 2013 shifts, assigned to us on the ESRF beamline Gilda (CH 3933 round 04/2013), we collected a total of 7 spectra on the following human α - and β -synuclein (AS and BS respectively) samples:

- a. whole construct for AS + Copper(I)
- b. whole construct for AS + Copper(II)
- c. AS N-terminal fragment 1-15 (AS1-15)+ Copper(I)
- d. BS N-terminal fragment 1-15 (BS1-15)+ Copper (I)
- e. BS N-terminal fragment 1-15 (BS1-15)+ Copper(II)
- f. Aggregated AS + Copper (II)
- g. Aggregated AS prepared by Copper(II) incubation

All XAS data were collected at the Cu K-edge in presence of Cu(I) or Cu(II), with a final concentration of about 1.0 mM and Cu:protein(peptide) stoichiometric ratio $\sim 0.9:1$.

Contrary to the experiment SC3507 (round 4/2012) the sample acquisition at the Cu K edge results in a noisy but good quality spectra, with the absence of residual glitches in the spectra. This was obtained through a particular sample preparation and through an accurate set up of the instrument which required some shifts for a good refinement. In particular, samples a and c were repeated with the addition of 20% ethylene glycol which largely improved the spectra.

A preliminary analysis of the data allow us to observe the following:

- Samples a and b are characterized by different EXFAS and FT spectra (Figure 1) strongly indicating different Cu(I) and Cu(II) coordination spheres. In particular the data of sample a suggest the presence of N/O donor atoms set, while those sample b suggest Cu(I) binding to S and O/N atoms.

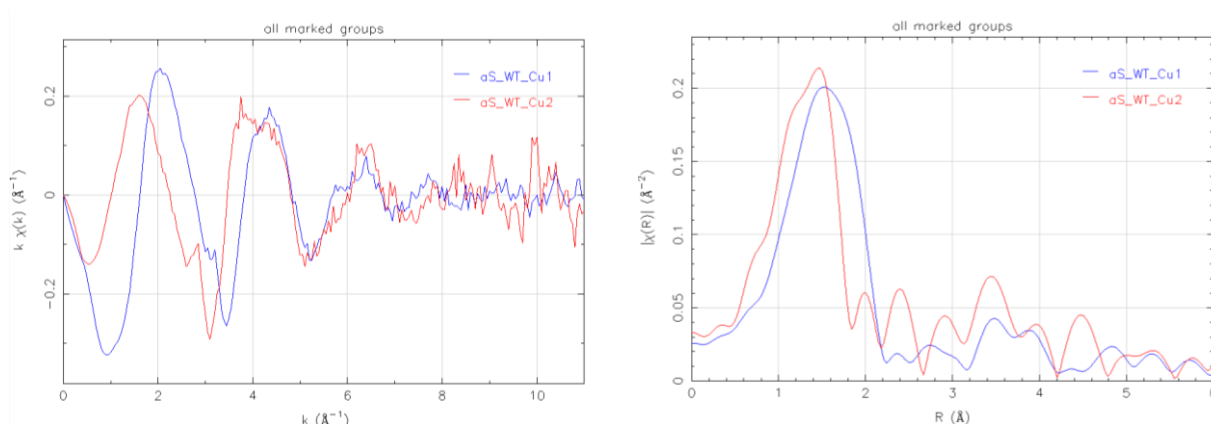


Figure 1 Superimposition of the EXAFS data (left) and of their FT (right) of samples a (blue) and b (red).

- As shown in Figure 2, the spectra obtained for AS1-15 (sample c) are comparable to those obtained for the full length AS protein (sample b), strongly suggesting a similar Cu(I) coordination environment for both systems.

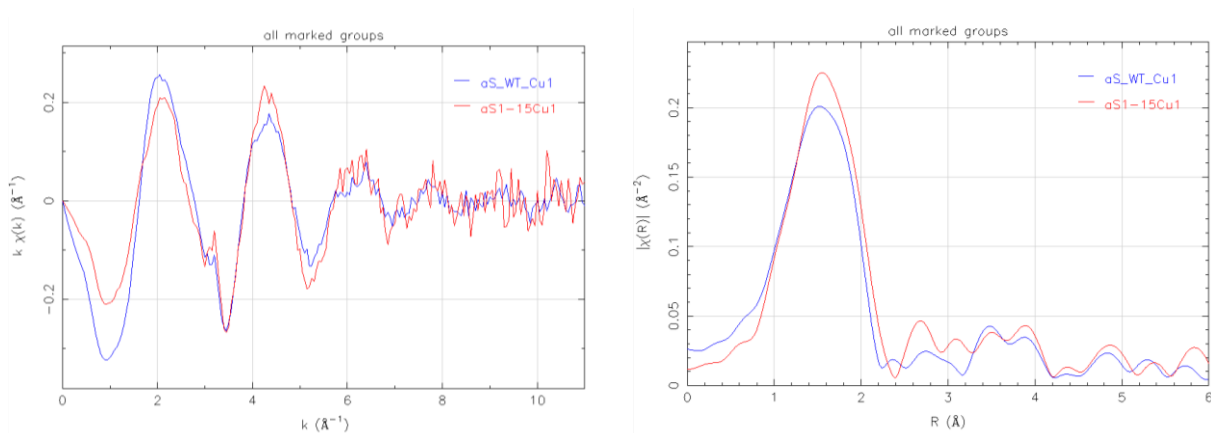


Figure 2 Superimposition of the EXAFS data (left) and of their FT (right) of samples a (blue) and c (red).

- Similarly to what obtained for AS, samples d and e exhibit completely different EXAFS and FT data, indicating the presence of diverse Cu(II) and Cu(I) binding modes. Moreover, the comparison between samples d and e, revealed that AS1-15 and BS1-15 constructs, differing in one amino acid (K10M), have different Cu(I) coordination sphere.

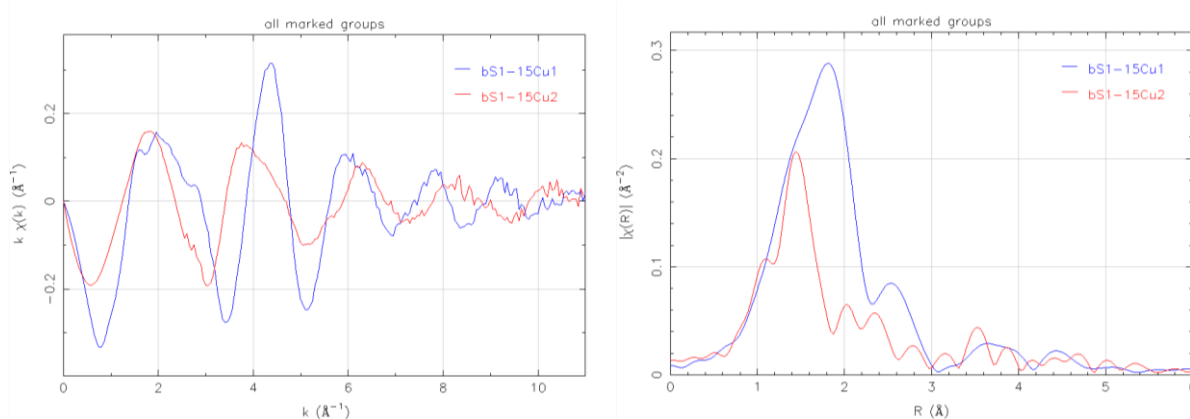


Figure 3 Superimposition of the EXAFS data (left) and of their FT (right) of samples D (blue) and E (red).

- Finally we examined the Cu(II) binding to aggregated AS forms. The two samples showed very similar spectra strongly suggesting that Cu(II) binding is not affected by sample preparation.

The study of all these data, by using Excurve and/or Artemis programs, is still in progress. From this analysis, we are confident to obtain more detailed information on the Cu(I) and Cu(II) coordination spheres of all the investigated systems. In addition the comparison between these

data and the ones taken at the Ag K-edge (experiment SC3507) will allow to definitely identify any difference in Ag(I) and Cu(I) binding to AS.