Metal binding sites in the mature and C-terminal regions of wild-type and mutant human frataxin.

Frataxin (FXN) is a highly conserved protein found in prokaryotes and eukaryotes. Its deficiency or mutations cause the neurodegenerative disease known as Friedreich's ataxia and are also associated to increased tumorigenesis. FXN functions are still under debate, however FXN is considered to be involved as a component of the Fe–S cluster assembly and in iron binding. Three iron binding sites have been identified in human FXN, with the highest-affinity binding site located in the disordered N-terminal region and the two lower-affinity ones located on the protein surface in the C-terminal region of the protein. It has also been observed that the presence of iron promotes the formation of FXN oligomers.
The X-ray Absorption Spectroscopy (XAS) measurements described here were aimed at characterizing the high-affinity iron binding site in the wild-type (wt) human FXN (in the mature form of the protein, 81-210) and the effect of point mutations, found in tumour cells, on the two lower affinity binding sites (limiting the study to the C-terminal form of the protein, 90-210). XAS experiments at the Co K-edge on samples kept at 10 K were performed at the BM30 of ESRF.
Co(II) was used as a model of Fe(II) and the measurements have been performed at different [FXN]:[Co] concentration ratios in order to obtain atomic resolution information on the Co binding sites in different binding sites occupancy conditions. In all cases, the concentration of Co was 0.8 times the concentration of the protein, in order to minimize the amount of Co free in solution.

The first important finding of our experiments is that, by comparing the XANES data for the wt81-210 sample (wt_81_0.8) with those of the wt90-210 (wt_90_0.8) sample and the buffer (see Fig. 1), one sees that the spectrum of the longer fragment, wt_81_0.8 sample, looks much more similar to the bugger than the spectrum of the wt_90_0.8 sample. This feature suggests that in the case of wt_81_0.8 a non-negligible amount of Co(II) remains free in solution.

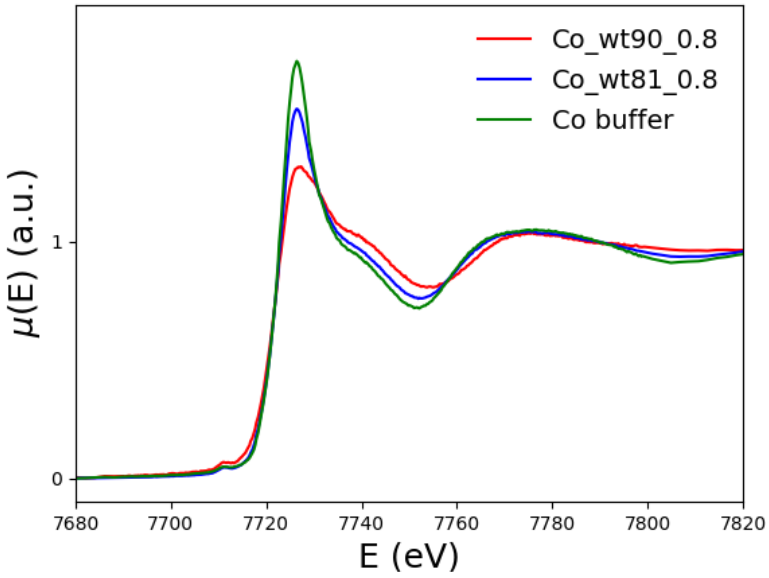

Figure 1: XANES spectra of the FXN samples including (wt_81_0.8 - blue curve) and not-including (wt_90_0.8 - red curve) the N-terminal 81-90 disordered region, compared with the buffer spectrum (green curve).

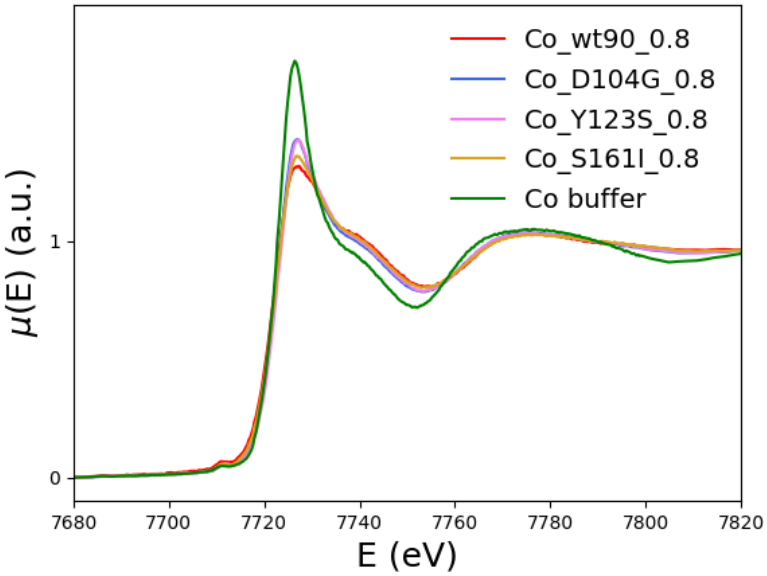

Figure 2: XANES spectra of the three selected mutants, D104G (blue), Y123S (violet) and S161I (gold), are plotted together with the wt-FXN (red) and the buffer (green) spectra.

Then, looking at the mutants (see Fig. 2), we observe that the buffer spectrum appears to be significantly different from the spectra of all the mutants thus allowing to conclude that all the mutants are able to bind Co(II). Qualitatively we see that the most significant difference with the wt-FXN spectrum occurs in the case of mutants in which the destabilizing effect due to metal binding (measured, in complementary experiments, by the difference of the melting temperature with and without metal) is larger. While destabilization upon metal binding is not surprising in the case of the D104G mutation, as the latter just occurs well inside the alleged metal binding acidic ridge, the other two mutations, Y123S and S161I, are located far away from it and the reasons at the basis of the metal-induced destabilization are more difficult to identify.