During the beam-time allocated at the BM30B beam line (Proposal # SC 1373) we collected a series of spectra at both the Cu and Zn edges. Carefully examining the collected spectra we reached the conclusion that the experiment is feasible at the concentration we are able to obtain with these samples. We also realized that, due to the necessary (for low concentrations) high intensity, the spectra must be collected at low temperature, in order to avoid spectra modification under the beam.
Here below are few comments about the spectra collected.

We measured four different types of peptides, namely:
1-40: [C1;C13] a peptide made of 40 amino-acids
1-28: [C3; C4; C5; C6;C9] a peptide made of 28 amino-acids
1-16: [C7;C8] a peptide made of 16 amino-acids
17-40: [C14] a peptide made of 24 amino-acids
the buffer: [C2;C11;C15]
buffer +TFA: [C12]

Cu samples.
The geometry around the absorber is expected to be the same for all the different peptides, furthermore, C3, C4, C5, C6 and C9 are, in principle, exactly the same sample. But the corresponding spectra look very different (with the exception of C3 and C9 that show identical spectra). We observed that the spectra were changing during the measurements.
In particular, C3 and C9 showed a spectrum equal to that of metallic Cu. When we looked at the sample C3, once taken away from the beam, a dark line in the middle of the sample holder with exactly the dimension and shape of the beam was clearly visible. Furthermore the intensity of the recorded spectra was growing with the exposition time. This phenomenon may possibly be explained by a sort of "burning" process over the sample due to ionization by radiation that promote Cu release from the peptide followed by the formation of a kind of metallic crystal. The increase of intensity with time can be explained by the fact that the total amount of Cu "encountered" by the beam (the "burned" one, concentrated in the middle of the sample holder) increases with exposition time.
The phenomenon of intensity increasing is generally observable (it is present, more or less, in all cases, also in the buffer – C2, C11 and C12 samples). A possible explanation is that the "burning" of samples, possibly due to a strong ionization, is always, more or less, in act and we always observe a mixed signal coming partially from the Cu bound to the peptide plus a variable fraction coming from the metallic Cu.

We can summarize our observation as follows: the consequences of the exposition to a high intensity beam are of two different kinds:

1. some samples "burn" very rapidly (C2 and C3): the spectra are very similar to the metallic Cu already at the very beginning of beam exposition.
2. other samples "burn" slowly (C1, C5 and C7): the intensity increases while the sample is under the beam, but the shape remains definitely different from that of metallic Cu.

Here we show an example of a spectrum collected at the Cu edge. It corresponds to the C1 sample (Fig.1).

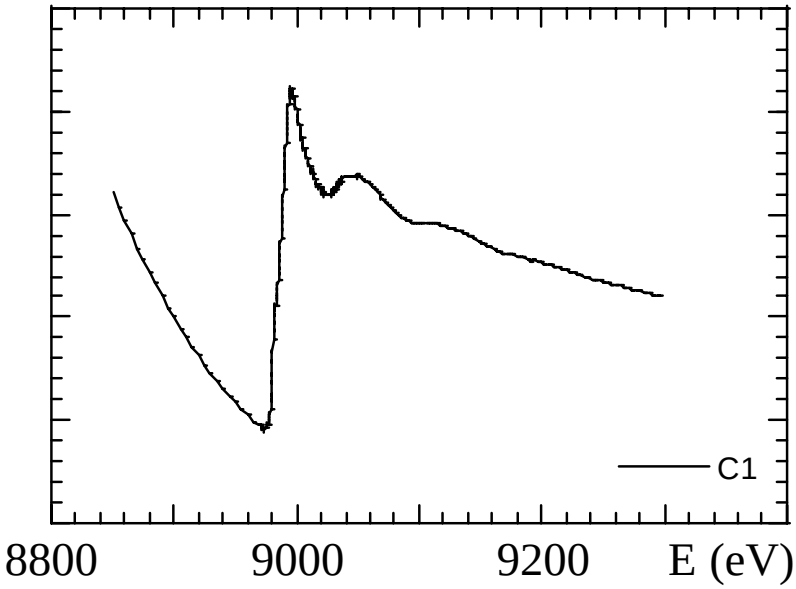

Fig.1: spectrum of C1 sample, collected at the Cu edge.

Zn samples.

The situation of Zn samples is much easier to describe, but in a sense much more puzzling. In fact, ALL the spectra of Zn samples, buffer and with any kind of different peptides, are identical and they didn't change with time. A possible explanation in this case is that the effect of beam intensity (if any) is very rapid and we are always in presence of "burned" samples. In Fig.2 an example of a spectrum collected at Zn edge is given.

We conclude that to perform measurements at temperatures around the freezing point or above is not enough to avoid the sample changing during measurements. Possibly very low temperature (e.g. liquid nitrogen) should be used.

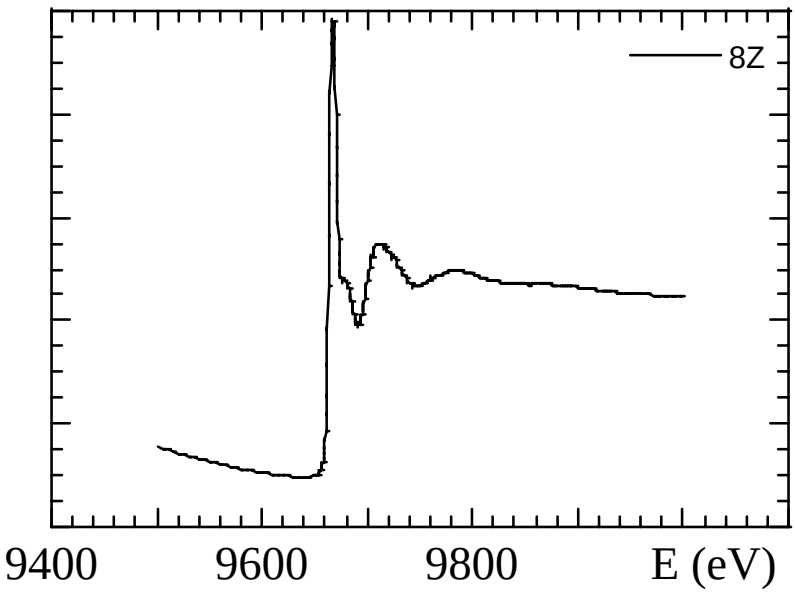

Fig.2: an example of a spectrum collected at the Zn edge.