ESRF	**Experiment title:** EXAFS analysis for studying metal ion cross linkinking in polypeptide model systems, with relevance to biomaterials	**Experiment number**: CS 3819
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Report:

We investigated the local structure around selected metal ions at different coordination states in order to draw conclusions regarding the metal ion cross-linking of protein matrices in biomaterials. In addition to synthetic polymers and minerals we studied two biological structures from the spider *Cupiennius salei* – the fangs and the metatarsal claws. The fangs are used by the spider as injection needle to inject venom into its prey, while the claws are used to attach to rough surfaces. The Fang tips are reinforced with Zn metal ions which are cross-linking His residues in the protein matrix of the cuticle, while the claws are reinforced with Mn. The oxidation state of Mn in the claw was previously not known.

The experiments were carried out at the beamline BM08 (GILDA) during July 2014.The first days were dedicated to optimzing sample preparation as well as measuement conditions. We first studied Mn enviroment, followed by Zn and lastly we obtained XAS data on Cu and Ni cross-linked peptides and minerals standards.

The collected absorption data allowes us to obtain detailed structural information on our different materials. This are now being correlated to the mechanical properties of the materials as well as other spectropsscopic data (from EELS and Raman spectroscopies). First important information presented in figure 1 is the

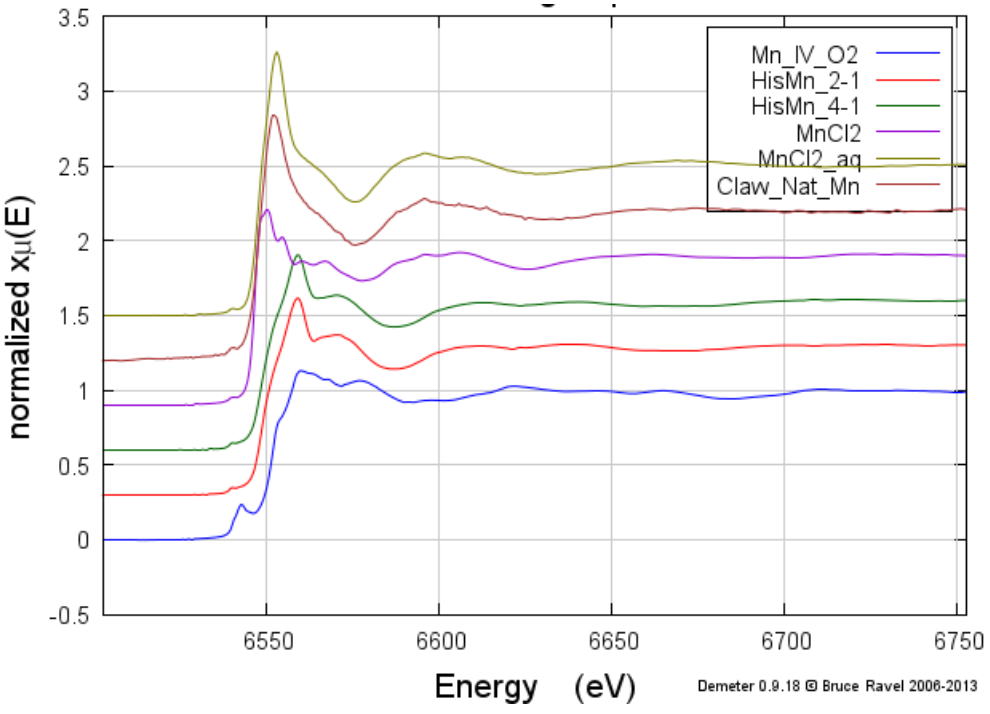
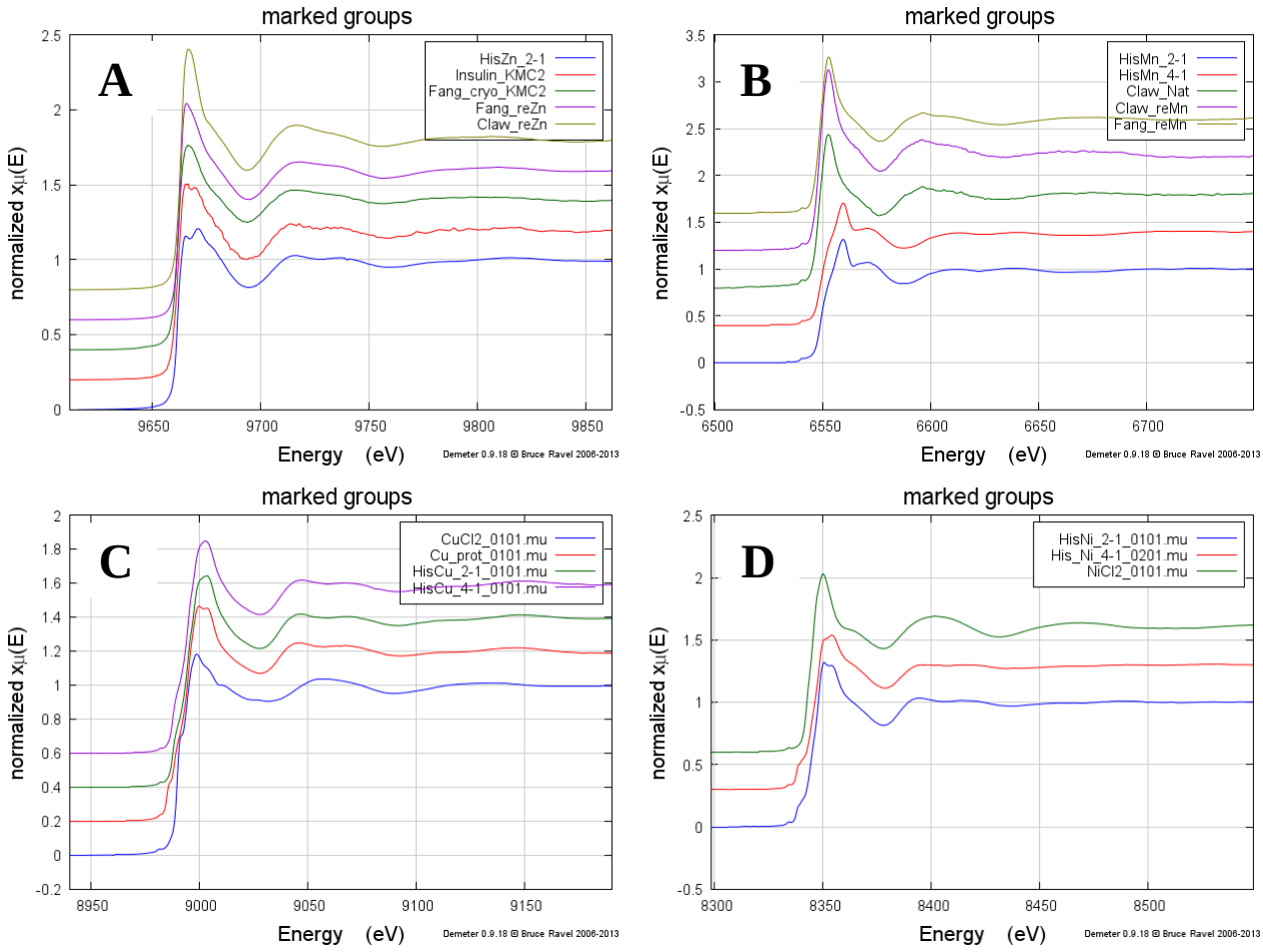


Figure 1. Mn K-edge XANES data on Mn salt (Mn VI oxide) (blue curve), polyhistidine corss-linked with Mn at raion of 2:1 (red) and 4:1 (green). MnCl2 salt (Purple), MnCl2 aquaous (brown) solution and the native Mn-rich spider claw (olive-green).

determination of the oxydation state in the spider claws. As can be seen the claw edge energy is similar to that of MnCl2 suggesting +2 oxidation state. Polyhistidine peptides coordinated by Mn show similar coordination enviorment regardless of the Mn:His ratio, with oxidation state most likely +4. Similar behaviour is seen when Exchanging the metal ion cross-link in polyhis to Cu. As can be seen in figure 2C. When Ni is used as the coordinating ion, we observe a different behavour -
the local structure dependns strongly on the ratio between Ni and the His residues (Fig. 2D).
We also compared the metal binding of the claw and the fang to both Zn and Mn by removing the original matal ion using EDTA, reintroducing it (to verify that the binding is maintained) or relacing it by the reciprocal metal ions (i.e. Zn into the claws and Mn into the fang). The results show that the metals are being re-incorporated and with minor changes in the coordination geometry of Zn and Mn in the claw and the fang. These results are also compared elsewhere to mechanical data of the modified skeletal elements.



XAS data of different meral ion corss-linked biomaterials. (A) Zn K-edge ZANES of polyhistidine-Zn (blue), Insulin* – Zn cross-linked protein hexamer (red), spider fang* (green), and spidr fang (purple) and spider claws (olive) reintroduced with Zn. *these data is measured at beamline KMC2 at Bessy II, berlin. Presented for comparison). (B) Mn K-edge XANES of polyhistidine cross linked with Mn and the claw and fang replaced with Mn. (C) Cu X-edge ZANES of polyhistidine cross linked with Cu at two concentrations conpared to CuCl2 mineral and to the protein tyrosiase which contains at Cu center. (D) Ni K-edge XAS of polyhistidine cross linked with Ni at two concentrations and aquaous solution of Ni Cl2.