ESRF	**Experiment title:** Site-selective XAS/XES on Fe complexes of H_2 production in hydrogenases and models	**Experiment number**: SC2859
Beamline: ID26	**Date of experiment**: from: 12.02.2010 to: 19.02.2010	**Date of report**: 04.01.2011
Shifts: 18	**Local contact(s)**: Dr. Tsu-Chien Weng	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*Dr. Michael Haumann, Freie Universität Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany,

*Dr. Ivelina Zaharieva, Freie Universität Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany,

*Dr. Kajsa Havelius, Freie Universität Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany,

*Nils Leidel, Freie Universität Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany,

*Jonathan Heidkamp, Freie Universität Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany.

Report: Experiment SC2859 is a continuation of our project on applying high-resolution XAS/XES to iron sites in proteins and models to study their molecular and electronic structure (see also report SC2733). In particular we explored the capabilities of site-selective XAS/XES approaches to study individual metal sites in binuclear centers and measured extended sets of K-beta satellite line spectra (valence-to-core transitions). In this run, we completed a series of iron reference compounds featuring Fe ions in various oxidation states, measured XAS/XES spectra of an FeFe hydrogenase enzyme (HydA1) and its maturation protein (HydF) from the alga *Chlamydomonas reinhardtii* [1-3], and performed an extended radiation damage study and aquired iron XAS/XES spectra on several ribonucleotide reductase (RNR) proteins containing high-valent FeFe or MnFe active sites for oxygen activation [4,5]. The results show that high-resolution XAS/XES is feasible also on high-valent metal sites in proteins. However, time-resolved approaches are required to avoid X-ray induced reduction of the metal centers. A first example of a time resolved measurement of the K-beta satellite line spectrum of RNR is presented. Implementation of a rapid-scan and/or continuous-scan mode of the XES spectrometer would be a major improvement of the facilities at ID26, which is intended in the near future. The group of M. Haumann will apply for a long-term proposal to participate in these developments.

Experimental: XAS/XES spectra were recorded in fluorescence mode using the Rowland-type XES spectrometer at ID26 equipped with 5 analyser crystals (R = 1 m, Fe K-alpha Ge440, Fe K-beta Ge620) and an avalanche photodiode (APD) or a silicon-drift detector for fluorescence detection. Si311 crystals of the monochromator were used. Samples were held in a liquid-helium cryostat of ID26 at 20 K. The emission detection resolution was ~1 eV (see also report SC2733). X-ray photoreduction of high-valent metal sites was investigated using a time-scan approach (excitation at a fixed energy in the Fe K-edge and following of the change in X-ray fluorescence level due to K-edge shifts over time) [6].

Results:

(1) Completion of a series of Fe reference spectra: Fe K-beta emission spectra (main lines and satellite lines) were measured on various iron oxides, sulfides, and cyanides containing Fe(II), Fe(III), or Fe(IV) and on model compounds for FeFe hydrogenase with their two Fe ions in different coordination environments (see report SC2733). Data evaluation was done by simulations of K-beta spectra using a density-functional theory (DFT) approach (Fig. 1). We are currently exploring the wealth of information on molecular (metal ligands) and electronic configuration of the sites emerging from the simulations. Our results show that even subtle features of FeFe sites in hydrogenases, such as the presence of metal-bridging carbon monoxide (CO) ligands and hydride binding, can be distinguished by evaluation of the valence-to-core transitions. A respective publication is in preparation [7].

(2) K-beta spectra of FeFe hydrogenase: For the first time K-beta emission spectra of FeFe hydrogenase HydA1 and of its crucial maturation protein HydF were obtained (Fig. 1). The results revealed differences in the spectral shape. However, their interpretation is hampered by the limited signal-to-noise ratio of spectra and by possible radiation damage effects. Radiation damage could be overcome by the implementation of a continuous scan mode of the XES spectrometer at ID26 and by using time-resolved approaches for XES (see below). We plan to continue these experiments in the future by measurements of changes in K-beta emission spectra due to oxidative modification of the FeFe active site. We expect that crucial information on the mechanism of oxygen inactivation of FeFe hydrogenase can be obtained.

(3) Site selective XAS/XES: XANES and EXAFS spectra of two model compounds for FeFe hydrogenase were measured using narrow-band K-beta emission detection at selected energies in the region of the K-beta main line. Data evaluation is still ongoing, but preliminary results show that relatively high discrimination with respect to bond lengths and coordination numbers at individual Fe ions is obtained (see also SC2733).

(4) XAS/XES on ribonucleotide reductase (RNR): An extended X-ray photoreduction study at the Fe K-edge was performed on RNR proteins from the bacterium *Chlamydia trachomatis* and from mouse (Fig. 2). It was found that X-ray induced reduction of Fe(III) and Fe(IV) sites is very rapid (i.e. completed in less than 10 s) under XES conditions even with samples held at liquid helium temperatures (20 K). Fe K-beta emission spectra were measured for 6 RNR samples containing Fe(III)Mn(IV), $Fe(III)_2$, and further oxidation states (Fig. 3). Photoreduction was avoided by beam attenuation. The data revealed spectral differences due to varying Fe coordination environments and respective results are included in a recent publication [4]. In an attempt to measure a K-beta satellite line spectrum of RNR with a $Fe(III)_2$ site, a time-resolved approach was tested (time-scans of X-ray fluorescence measured in a point-by-point approach in the K-beta line spectral region and reconstruction of spectra at certain points in time from the timescan data). A spectrum could be measured in ~5 s, proving the feasibility of measuring K-beta spectra for high-valent metal sites (Fig. 4).

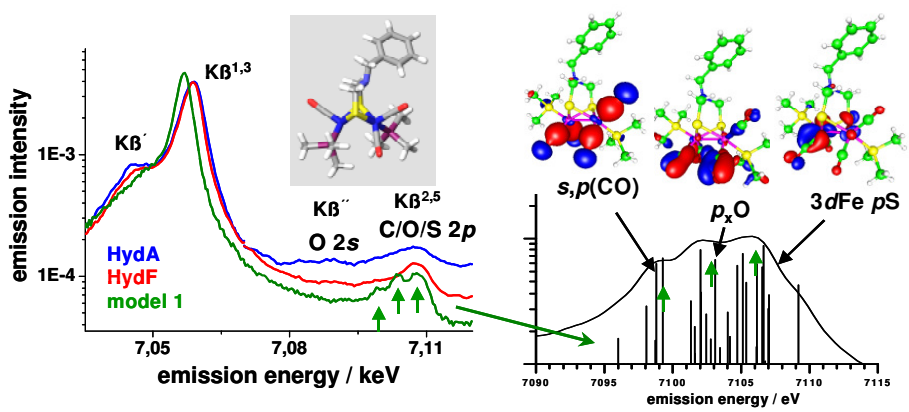


Figure 1: Left: K-beta spectra of FeFe hydrogenase HydA1 and maturation protein HydF and of a model (inset). Right: DFT structures, molecular orbitals and calculated valence-to-core spectrum of 1.

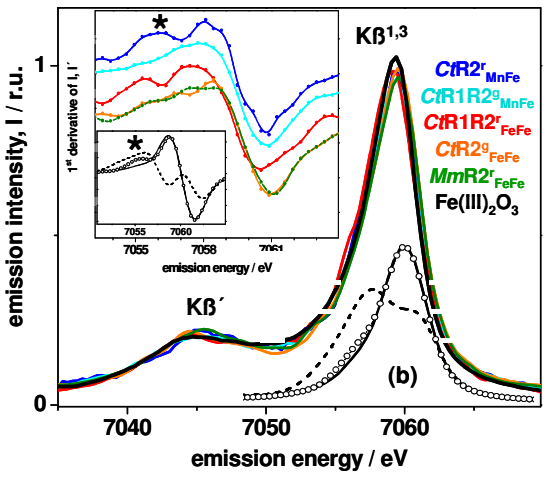


Figure 3: Fe K-beta emission spectra of RNR proteins containing high-valent FeFe or MnFe sites. Inset: 1st derivatives. Black lines, simulations by a multiplet approach.

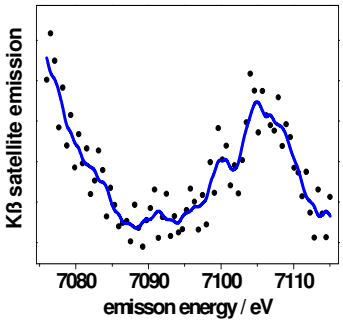
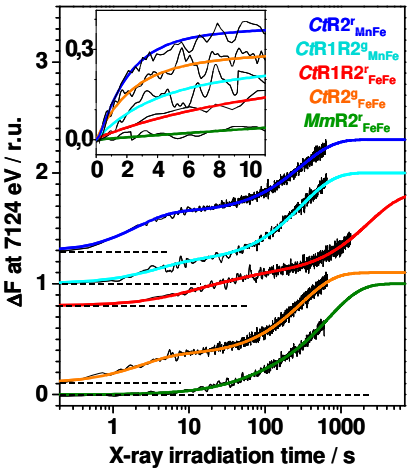


Figure 4: Fe valence-to-core transitions in Mn(IV)Fe(III) RNR measured within 5 s per data point in a time-resolved approach.



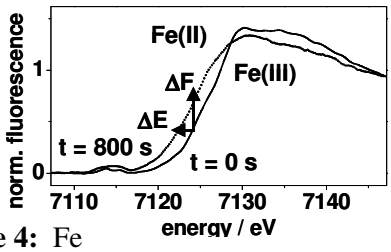


Figure 2: Rapid X-ray photoreduction under XES conditions of Fe sites in RNR (*C. trachomatis* and mouse) followed by a timescan approach.

Conclusions: We consider the run as highly successful. A series of emission spectra of Fe compounds was completed, first XES data were obtained for FeFe hydrogenase, further site-selective XAS data were obtained for FeFe hydrogenase model, and an extended XAS/XES study on metal sites in ribonucleotide reductase was performed, showing the feasibility of XES on high-valent metal sites in a time-resolved approach. Data evaluation is still ongoing and several results are included in currently prepared manuscripts [4,7]. We will apply for a long-term project to continue the XES work on high-valent metal sites in proteins

References:

[1] S. Stripp, O. Sanganas, T. Happe, M. Haumann, Biochemistry 48, 5042-5049 (2009)

[2] S. Stripp, G. Goldet, C. Brandmayr, O. Sanganas, K. Vincent, M. Haumann, F. Armstrong, T. Happe, Proc. Natl. Acad. Sci. USA 106, 17331-17336 (2009)

[3] I. Czech, S. Stripp, O. Sanganas, N. Leidel, T. Happe, M. Haumann, FEBS lett., DOI: 10.1016/j.febslet.2010.11. 052 (2010)

[4] N. Leidel, A. Popovic-Bijelic, K. Havelius, P. Chernev, N. Voevodskaya, A. Gräslund, M. Haumann, Redox intermediates of Mn-Fe and Fe-Fe sites in *Chlamydia trachomatis* ribonucleotide reductase, to be submitted (2011)

[5] N. Voevodskaya, F. Lendzian, O. Sanganas, A. Grundmeier, A. Gräslund, M. Haumann, J. Biol. Chem. 284, 4555-4566 (2009)

[6] P. Chernev, I. Zaharieva, H. Dau, M. Haumann, J. Biol. Chem., in press, DOI: 10.1074/jbc.M110.202879 (2010)

[7] N. Leidel, K. Havelius, P. Chernev, M. Haumann, Valence-to-core transitions in models for FeFe hydrogenase active site, in prep.