ESRF	**Experiment title:** NRS on FeFe and MnFe cofactors in a ligand-binding oxidase protein (*Gk*lox)	**Experiment number**: SC3923
Beamline: ID18	**Date of experiment**: from: 26.11.2014 to: 29.11.2014	**Date of report**: 26.02.2015
Shifts: 8	**Local contact**: Dr. Alexander Chumakov	*Received at ESRF:*

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Report: Prototypic dimetal-carboxylate cofactors (DMC) are typical for ferritin-like enzymes. They catalyze chemically most challenging reactions [1,2]. We have recently been granted a new research project in the Röntgen-Angström-Cluster ("*Dimetal-carboxylate complexes in enzymes and biomimetic materials studied by novel X-ray crystallography and spectroscopy techniques*") funded by the German BMBF and the Swedish VR, which is dedicated to the study of DMC-enzymes by advanced X-ray spectroscopy techniques. First NRS experiments on a new DMC-enzyme (*Gk*lox) [3] have been carried out by us in 2013 at ID18. However, they were performed using 7/8+1 filling mode, allowing only to use 2 bunches for NRS. Now we have carried out improved measurements using the hybrid (24x8+1) ring mode. This has lead to significantly improved data quality for the protein samples. We found that hybrid mode is as well suited as 16 bunch mode for NRS on diluted but fully 57Fe labelled protein samples, extending the useful beamtime periods at ESRF for this type of demanding experiments. The significantly improved NIS data are presented in the following.

The new *Geobacillus kaustophilus* ligand-binding oxidase (*Gk*Lox) binds a DMC cofactor (Fig. 1), which after metal reconstitution can be of the FeFe or MnFe type. High-valent metal species are formed after reaction of initial M(II) ions with O_2 [3]. Crystal structures have revealed oxygen species bound to the metals, the chemical nature of which is unclear. The goal of this project is to obtain information from NRS on the origin (from O_2 or water) and the protonation state (OH_x) of metal-bound oxygen species in the dimetal cofactor using ^{57}Fe labelled *Gk*Lox and isotopic substitution (H/D, $^{16/18}O_2$. $H_2{}^{16/18}O$). The vibrational dynamics were probed using NIS [4] at ID18. *The results prove that relatively small isotopic shifts of vibrational bands can be detected in the relatively dilute (3-6 mM 57Fe) protein samples.*

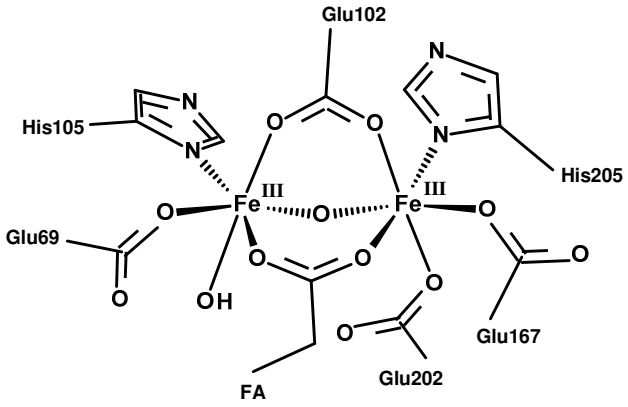

Figure 1: FeFe cofactor in the crystal structure of *Gk*Lox protein [3]. FA denotes a fatty acid ligand. Here the cofactor was labelled to ~100 % with ^{57}Fe for NIS and NFS.

Experimental: $^{57}FeCl_2$ was synthesized from metallic ^{57}Fe powder. Aqueous solutions of $^{57}FeCl_2$ (50 mM) using H_2O, D_2O, and $H_2{}^{18}O$ were prepared, which contained hexaquo-Fe(II) ions. The metal-free apo-*Gk*Lox protein was reconstituted with ^{57}Fe in H_2O or D_2O and using $^{16}O_2$ as the oxidant to form the Fe(III)Fe(III) cofactor in the laboratory of M. Högbom (U-Stockholm). NIS and NFS measurements were carried out using the set-up at ID18 (~0.5 meV high-resolution monochromator, avalanche photodiode detectors) and a liquid-helium cryostat. Up to 20 NIS scans (a ~30 min) were accumulated on each sample. Partial-vibrational-density-of-state (PVDOS) spectra were generated from raw emission scan data using the software packages available at the beamline.

Results:

(A) NIS and NFS on isotope substituted hexaquo-^{57}Fe. High quality NIS (and NFS) spectra were obtained for ^{57}Fe(II)(H/D$_2$$^{16/18}$O)$_6$ containing samples (Fig. 2, left). The NFS spectra revealed the exclusive presence of Fe(II). The NIS spectra showed clear vibrational bands and significant band shifts due to the isotopic substitutions. As expected the band shifts are similar for H/D or 16O/18/O substitution.

(B) NIS on GkLox protein. The Fe(III)Fe(III) and Mn(III)Fe(III) cofactors were studied by NIS (and NFS) in ~100 % ^{57}Fe labeled *Gk*Lox samples (Fig. 2, right). Spectra were measured for samples prepared with H$_2$^{16}O or D$_2$^{16}O. Spectra with satisfying signal-to-noise ratio were obtained after ~10-20 scans (5-10 h). Clear band shifts are observed for isotopically substituted samples. DFT calculations on the PVDOS spectra of *Gk*Lox protein for band assignment are underway in our laboratories and a manuscript is in preparation [6].

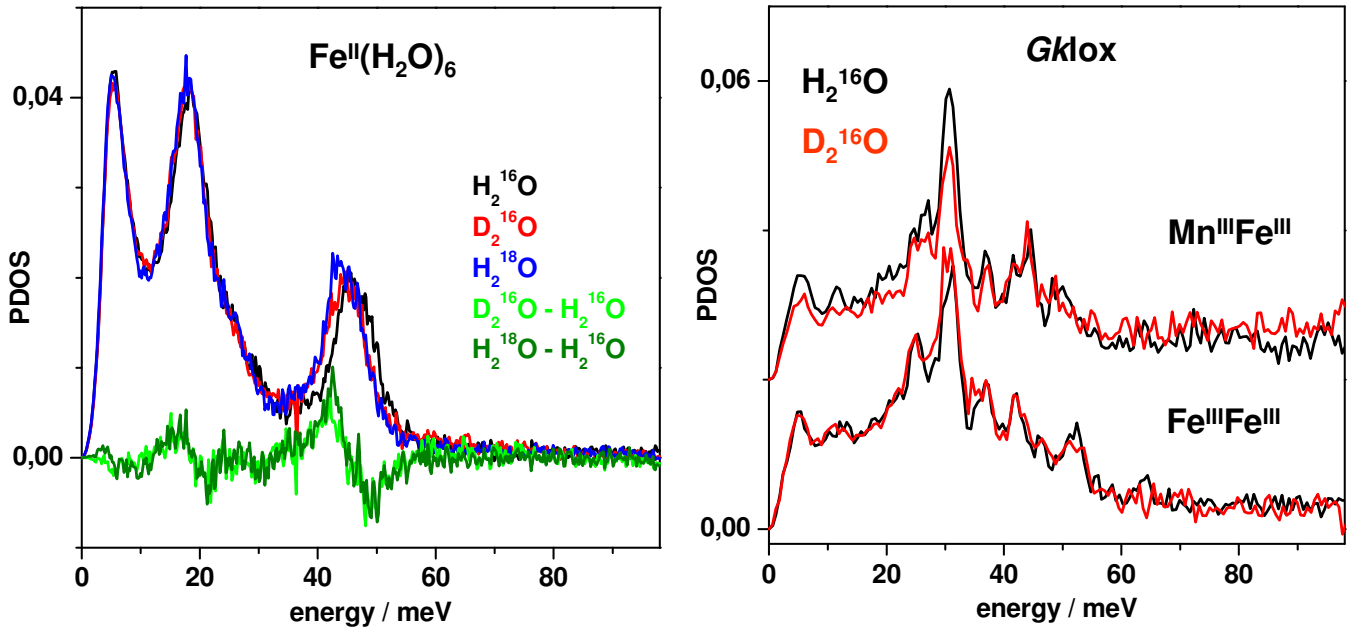

Figure 2: Raw PVDOS spectra from NIS data of hexaquo-57Fe(II) (left) and *Gk*lox ligand-binding-oxidase protein (right) containing 57FeMn or 57Fe57Fe cofactors in the III,III oxidation state in different solvents with isotopic substitutions. Clear band shifts due to isotopic substitutions are observed, which are analyzed using DFT calculations on model structures based on crystal data.

Conclusions: Improved NIS (and NFS) spectra were obtained for hexaquo-^{57}Fe(II) and ^{57}Fe-labelled *Gk*Lox protein with FeFe or MnFe cofactors in samples with isotopic substitutions. We show that H/D and $^{16/18}$O isotopic shifts were clearly resolved by NIS, for the first time in the *Gk*lox enzyme. Significantly improved data quality at much shorter measuring times was obtained for the protein samples using the hybrid ring mode. Preliminary data analysis and DFT calculations of NIS spectra suggest a specific protonation state (OH) of one of the bridging oxides in both FeFe and MnFe cofactors in *Gk*lox. We aim at continuation of this promising project on *Gk*Lox protein and other DMC-enzymes in a forthcoming measuring period at ID18. A publication including the present NIS data is in preparation [6].

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References:

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