ESRF	**Experiment title:** **NRVS on oxygen binding at iron sites in synthetic and biological complexes**	**Experiment number**: LS-3049
Beamline: ID18	**Date of experiment**: from: 01.12.2021 to: 06.12.2021	**Date of report**: 02.02.2022
Shifts: 15	**Local contact(s)**: Sergey Yaroslavtsev	*Received at ESRF:*

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Report: Iron complexes play a central role in many catalytic reactions in chemistry related to sustainable energy supply generation. Important examples are hydrogen producing and oxygen activating cofactors in biological enzymes as well as respective synthetic counterparts in chemical catalysis. High-valent iron-oxygen sites, i.e., featuring a Fe(IV)=O bond, are suggested as reactive sites for, e.g., hydrogen abstraction in heme- and non-heme enzymes and can result from oxygen activation. An ongoing attempt of systematic characterization of Fe(IV)=O species is performed in the frame of the UniSysCat Cluster of Excellence Berlin. While crystal structures of Fe(IV)=O species are generally rare, information on compounds in solution so far has come mostly from functional studies as well as Raman, Mössbauer and other spectroscopic methods. Here, we use nuclear resonant scattering spectroscopy on ^{57}Fe labelled complexes, namely nuclear forward scattering (NFS) and nuclear resonance vibrational spectroscopy (NRVS) to study Fe(IV)=O species in solution samples.[1-3] Data were obtained for 9 synthetic complexes and for ^{16}O or ^{18}O conditions, enabling systematic access to vibrational frequency changes and Mössbauer parameters in response to ligand variation. In addition, data were obtained for samples of an oxidase enzyme containing a diiron cofactor, binding oxygen species from O_2 reduction, in D_2O or H_2O solutions.[4]

Experimental. Iron complexes were synthesized using ^{57}Fe precursors and oxidized samples in organic solvents (6-8 mM iron) were prepared in the laboratory of K. Ray (Humboldt Universität zu Berlin, Chemistry Department). Starting and oxidized complexes have been mostly characterized earlier by other methods (e.g., refs.**). Oxidase protein samples in aqueous solution were provided by the group of H. Dobbek (Humboldt Universität zu Berlin, Biology Department). NRS experiments were carried out at beamline ID18 of ESRF using our earlier established protocols[5-6] and samples held in a LHe cryostat at 20-30 K. NRVS and NFS data were collected using the high-resolution (~0.6 eV) monochromator and APD detectors at ID18. 12-20 NRVS scans of 20 min duration were averaged for signal-to-noise ratio improvement. NRS data were processed, e.g., with the software package available at the beamline.

Results.

(A) ***NRS data of synthetic iron complexes.*** NFS and NRVS data were obtained for 10 solution samples of synthetic complexes expected to contain Fe(IV)=O species (Fig. 1). The NFS time traces differ among complexes and their simulation provides the Mössbauer quadrupole splitting energy (ΔE_Q). The NRVS spectra show the Fe(IV)=O vibration in most cases clearly and suggest mixtures opf iron species. Qunatitative analysis of the data is underway, including quantum chemical calculations.

(B) ***NRS data of an oxidase enzyme.*** NRS data were obtained for an oxidase protein in H_2O or D_2O solution prior and after treatment with peroxide (H_2O_2), to adress the origin of potential oxygen ligands at the iron

centers (Fig. 2). The NFS data indicated near-quatitative H_2O_2 reaction at the cofactor. The NRVS data differ pronouncedly amoung cofactor species. Quantitative analysis of the spectral differences is underway.

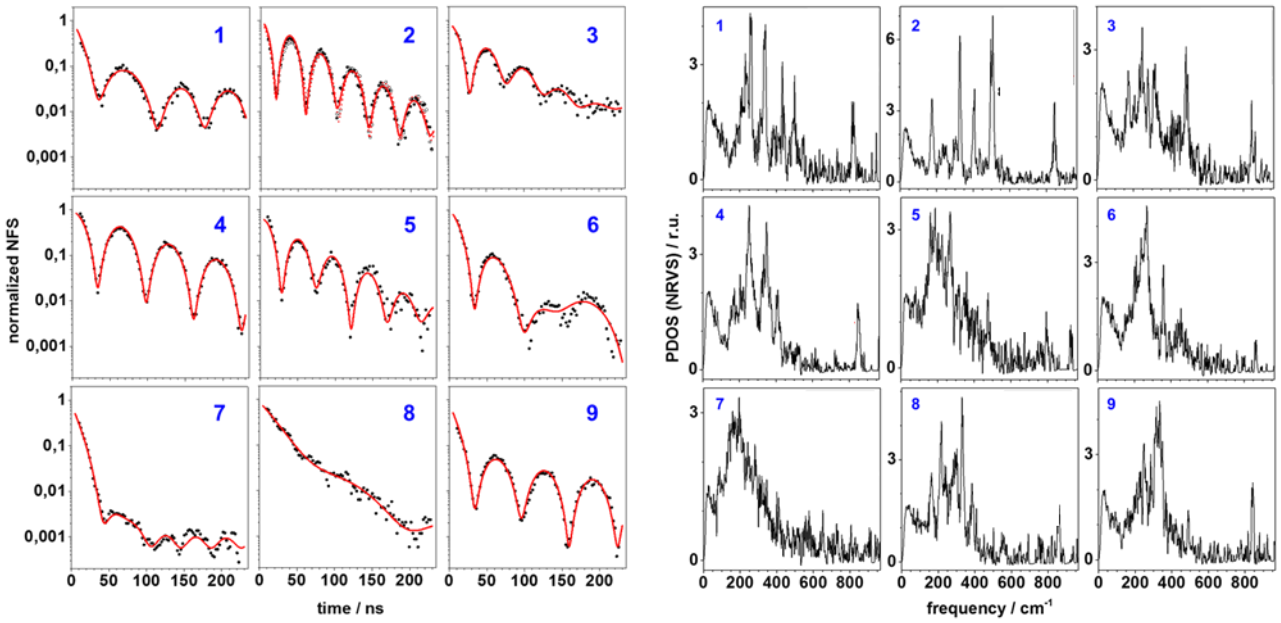

Figure 1: NRS data of synthetic complexes in solution containing Fe(IV)=O species. (Left) NFS time traces (black lines) and simulations (red lines). (Right) NRVS spectra (PDOS) and Fe(IV)=O vibrational bands in the insets.

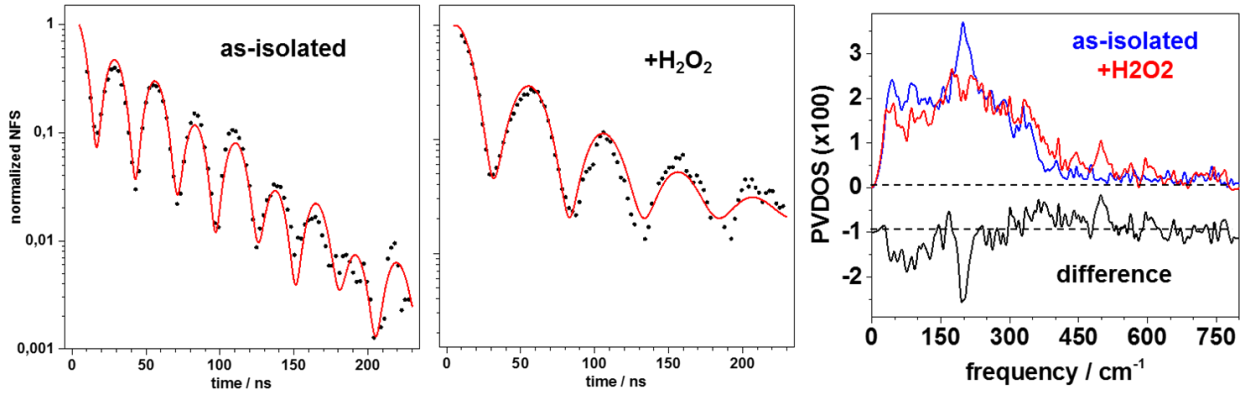

Figure 2: NRS data of an oxidase protein with a diiron cofactor prior to and after H_2O_2 exposure.

Conclusions.

We consider the December run at ID18 as very successful. An extended data set of NFS time traces and NRVS spectra was obtained for the first time for synthetic complexes containing ^{57}Fe(IV)=O species in organic solvents as well as for the non-heme cofactor in an oxidase enzyme. H/D and ^{16}O/^{18}O isotope effects were studied. (We note that the originally planned experiments, see original proposal title, on [FeFe]-hydrogenase could not be performed due to major changes in the collaborating groups.) The obtained data are of good quality and facilitate quantitative analysis by simulation and quantum chemical methods, which is underway in our laboratories. We expect that respective publications will emerge in the (near) future.

References.

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