


Experiment title:

Combining Nuclear Resonance Scattering and Raman Microscopy in order to study spin-state switching and iron dynamics in monocrystals and microstructures.

Experiment number:
CH-2839

Beamline: ID18	Date of experiment: from: 06-05-09 to: 13-05-09 (set-up time: 18-04-09 to 21-04-09)	Date of report: 01-09-09
Shifts: 15	Local contact(s): Dr. Rudolf RÜFFER	<i>Received at ESRF:</i>

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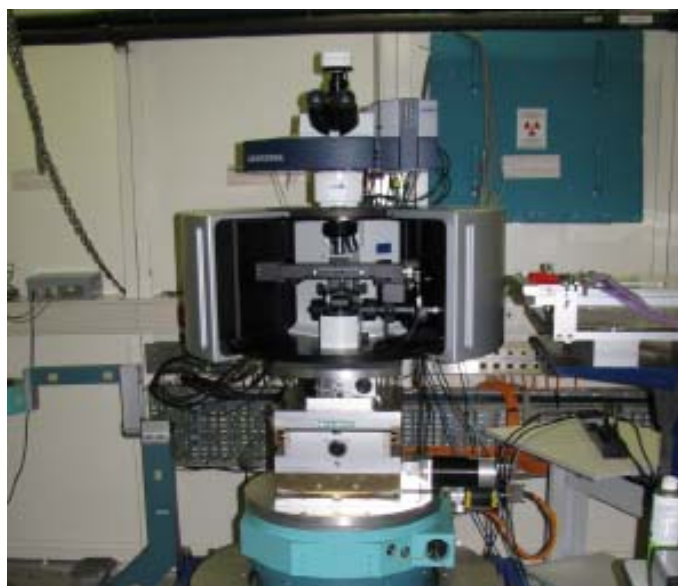
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Report:

There is an increasing demand for the investigation of micro-structured iron-containing samples not only from biogenous material but also from synthetic inorganic and organic compounds by application of synchrotron radiation simultaneously with vibrational spectroscopic methods such Raman spectroscopy. During exp. CH-2839 the set-up of the first combined Raman-microscope and NFS/NIS measuring station at a synchrotron radiation facility was installed (Fig.1). This experiment enables the simultaneous measurement of vibrational nuclear scattering techniques while the sample is additionally probed by optical methods with an applied microscope.

We have characterized high valent iron(IV) myoglobin crystals by the combination of NIS and Raman spectroscopy. Due to the high reactivity of the high valent iron(IV) myoglobin crystals the NIS measurements have been performed at 110 K.

Figure 1. Set-up of the Raman microscope (Senterra, Bruker Optik GmbH, Germany) inside experimental hutch of beamline ID 18 during experiment CH 2839; the microscope is mounted on a goniometer with an adequate adapter plate. The figure shows the microscope with opened safety doors.



For that purpose a cryostream (Oxford Ltd.) (Fig. 2) was used. Fig. 3 shows the NIS spectra obtained at 110 K of a ^{57}Fe -Myoglobin single crystal ($700 \times 150 \times 150 \mu\text{m}^3$) after addition of $\text{H}_2^{18}\text{O}_2$ and subsequent freezing. It should be noted that these experiments are the first NIS experiments on protein single crystals which are small enough also to be investigated by X-ray crystallography.

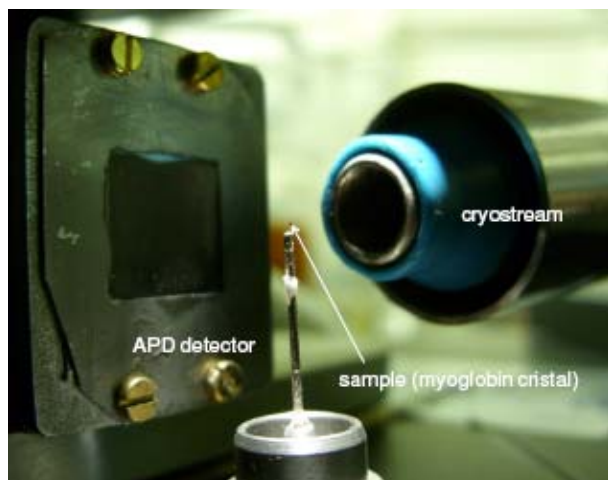


Figure 2. Experimental set-up of the cryostream and APD detector for probing the myoglobin crystal. The pin of the crystal mount has a diameter of 0.65 mm.

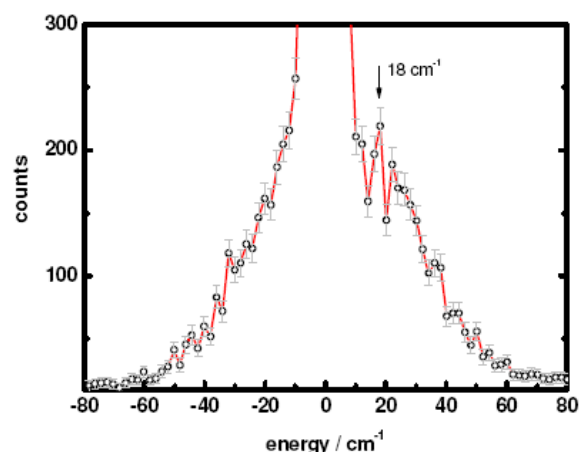


Figure 3. NIS spectrum up to 80 cm^{-1} obtained at 110 K of a ^{57}Fe -Myoglobin single crystal (crystal size: $700 \times 150 \times 150 \mu\text{m}^3$) after addition of $\text{H}_2^{18}\text{O}_2$ and subsequent freezing.

The single crystal data identify a localized iron mode of 18 cm^{-1} . Modes in this region have also been observed near 25 cm^{-1} in MbCO and deoxyMb and have been assigned to translational modes in response to torsional oscillations of the polypeptide backbone and side chains [1]. This assignment was confirmed by a NIS study of millimeter-sized metmyoglobin single crystals which reports haem sliding motions in the region of $32 - 40 \text{ cm}^{-1}$ [2]. The reason why in the case of $\text{H}_2^{18}\text{O}_2$ treated myoglobin a localized mode is seen at lower energy is unclear and subject to further investigations by combined quantum mechanical and molecular mechanics calculations. Unfortunately the obtained Raman spectra do only show a significant band at 975 cm^{-1} using an excitation wavelength of 785 nm which corresponds to the ammonium sulfate (usually found at 983 cm^{-1}) within the crystal. Clearly more measurements also at 488 and 532 nm excitation wavelength as well as directly in the Soret band e.g. at 407 or 413 nm have to be performed to increase the up to now non-significant scattering signals which are related to the protein vibrations. However, the present NIS-data might be correlated with recently published synchrotron-derived results concerning investigations on different protonation states of oxidized myoglobin [3].

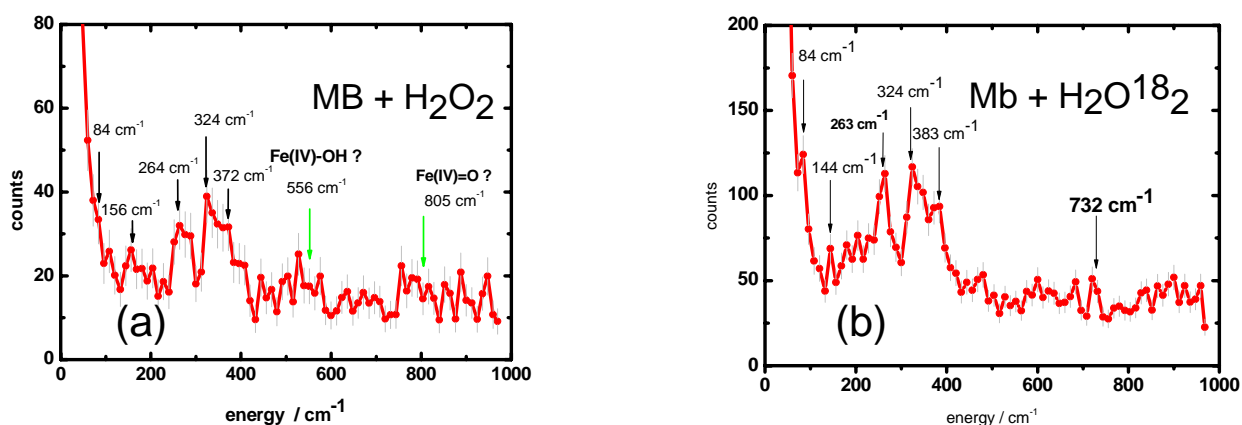


Figure 4. NIS spectra up to 965 cm^{-1} obtained at 110 K of a ^{57}Fe -Myoglobin single crystal after addition of unlabeled H_2O_2 (a) and $\text{H}_2^{18}\text{O}_2$ (b) and subsequent freezing.

The evaluation of the NIS spectra obtained up to 965 cm^{-1} (Fig. 4) is in progress, but due to the low count rate indications for the presence of Fe(IV)-OH (556 cm^{-1}) and Fe(IV)=O (805 cm^{-1}) stretching modes [3] have to be taken with care.

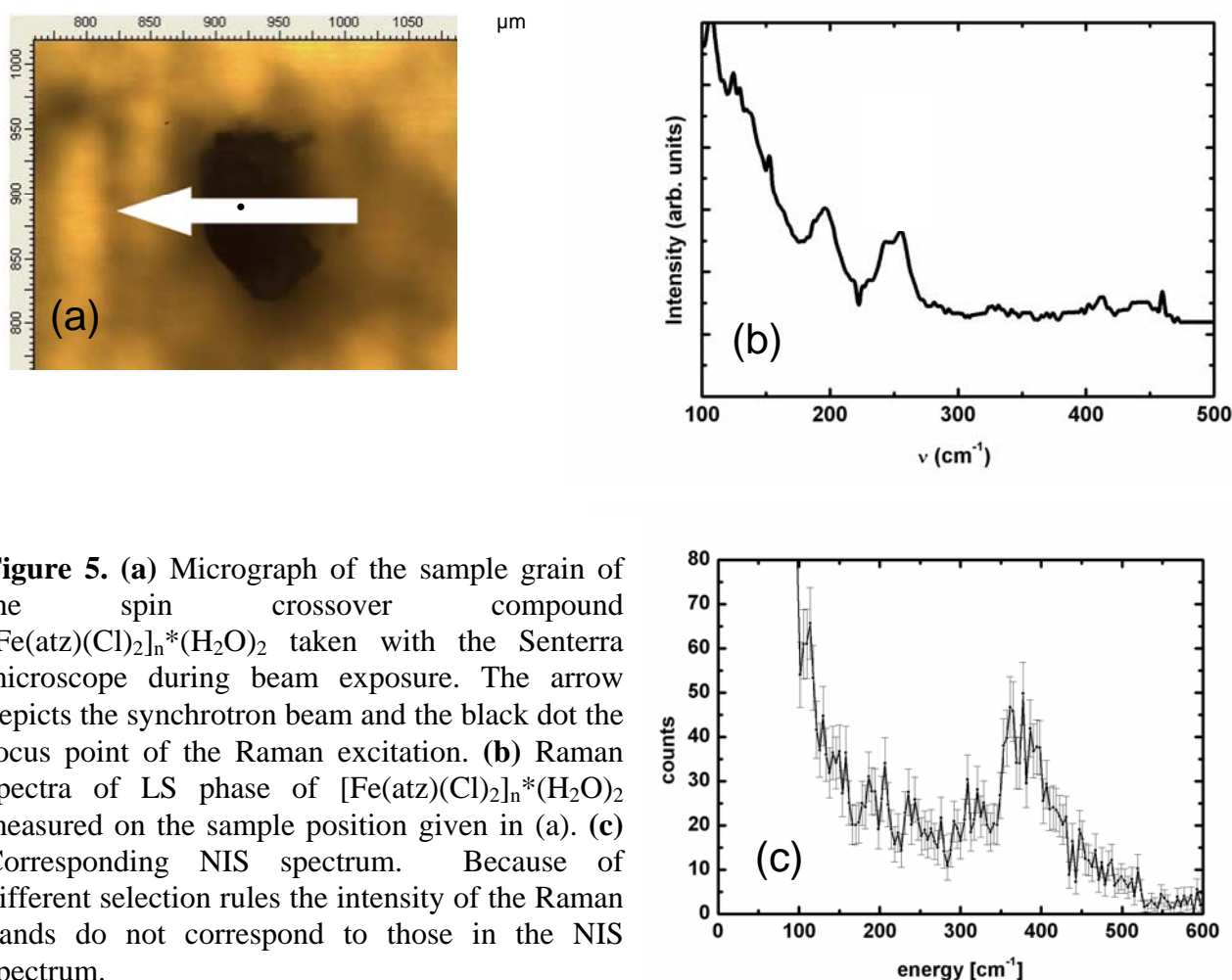


Figure 5. (a) Micrograph of the sample grain of the spin crossover compound $[\text{Fe}(\text{atz})(\text{Cl})_2]_n \cdot (\text{H}_2\text{O})_2$ taken with the Senterra microscope during beam exposure. The arrow depicts the synchrotron beam and the black dot the focus point of the Raman excitation. (b) Raman spectra of LS phase of $[\text{Fe}(\text{atz})(\text{Cl})_2]_n \cdot (\text{H}_2\text{O})_2$ measured on the sample position given in (a). (c) Corresponding NIS spectrum. Because of different selection rules the intensity of the Raman bands do not correspond to those in the NIS spectrum.

In addition we have performed simultaneous NIS and Raman microspectrometry on a sample grain of the spin crossover compound $[\text{Fe}(\text{atz})(\text{Cl})_2]_n \cdot (\text{H}_2\text{O})_2$ which had a diameter in the order of $100\text{ }\mu\text{m}$. With the focus of the Senterra Raman microscope (less than $1\text{ }\mu\text{m}$ diameter spot size) in the middle of the sample we oriented the whole microscope with respect to the focused synchrotron beam (area $15 \times 8\text{ }\mu\text{m}$) in such a way that the beam was passing the short axis of the sample grain (Fig. 5a). The corresponding Raman spectrum (Fig. 5b) and the corresponding NIS spectrum (Fig. 5c) reveals a signature typical for the low-spin ($S=0$) form. Quantum chemical density functional calculations for mode assignments [4] are in progress in our laboratory in Kaiserslautern. These preliminary experiments show that future NIS measurement with a beam size of less than $1\text{ }\mu\text{m}$ are very promising in order to answer the question whether spin domains are really present in SCO systems during spin crossover.

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3. Zeng W, Barabanschikov A, Zhang Y, Zhao J, Sturhahn W, Alp E E, Sage J T 2008 *J. Am. Chem. Soc.* **130** (6) 1816
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