

A new sample environment for cryogenic nuclear resonance scattering experiments on single crystals and microsamples at P01, PETRA III

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Abstract In order to carry out orientation dependent nuclear resonance scattering (NRS) experiments on small single crystals of e.g. iron proteins and/or chemical complexes but also on surfaces and other micrometer-sized samples a 2-circle goniometer including sample positioning optics has been installed at beamline P01, PETRA III, DESY, Hamburg. This sample environment is now available for all users of this beamline. Sample cooling is performed with a cryogenic gas stream which allows NRS measurements in the temperature range from 80 up to 400 K. In a first test this new sample environment has been used in order to investigate the orientation dependence of the nuclear inelastic scattering (NIS) signature of (i) a dinuclear iron(II) spin crossover (SCO) system and (ii) a hydrogen peroxide treated metmyoglobin single crystal.

Keywords Nuclear resonance scattering · Nuclear inelastic scattering · Single crystals · Spin crossover complexes · Iron proteins · Myoglobin

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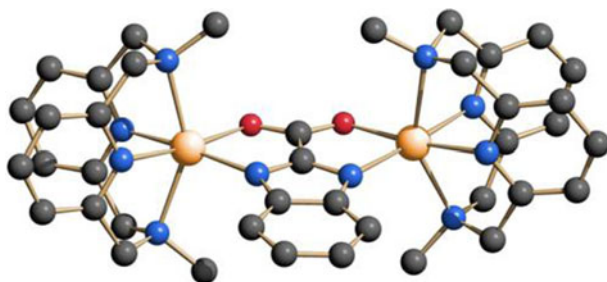


Fig. 2 Perspective view of $[\{\text{Fe}(\text{L-N}_4\text{Me}_2)\}_2(\text{BzImCOO})]^{2+}$ [3, 5]

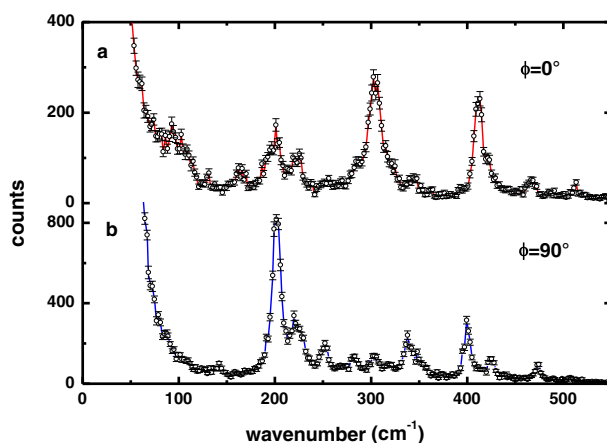


Fig. 3 NIS data of the HS-LS form obtained from two perpendicular orientations of a single crystal of $[\{\text{Fe}(\text{L-N}_4\text{Me}_2)\}_2(\text{BzImCOO})]^{2+}$ measured at 80 K. Data acquisition time was 4.6 h for $\Phi = 0^\circ$ and 2.7 h for $\Phi = 90^\circ$

2 Materials and methods

NIS experiments were performed at P01, Petra III, DESY in Hamburg, Germany under experiment I-20120310. The 6 GeV storage ring was operated in 60 bunch mode with a bunch separation of 123 ns. The beam was monochromatized to an energetic width of 0.4 eV by a pair of Si 311 crystals and further down to 1 meV by a high resolution monochromator using two pairs of asymmetrically cut silicon crystals. The used reflections are twice Si (4 0 0) and twice Si (10 6 4). The total asymmetry of the system is $b = 20$. The set-up displayed in Fig. 1 consists of a 2-circle goniometer including sample positioning optics (single-circle goniometer stage 420-X2W1 plus Euler cradle 511.5 and a z-table 5103.A20-90, all from Huber Diffraktionstechnik GmbH & Co. KG) and a Nitrogen Gas Cryostream Cooler which provides a N₂ gas stream, the temperature of which can be set from 80 up to 400 K (700 Series, Oxford Cryosystems Ltd.). The N₂ stream has a diameter of ~ 8 mm and is shielded by a shell of N₂ streaming at room temperature. This set-up allows mounting an avalanche photo diode (APD) detector for the detection of inelastically scattered radiation as close as ≤ 1 cm from the sample with the APD detector still being at room temperature.

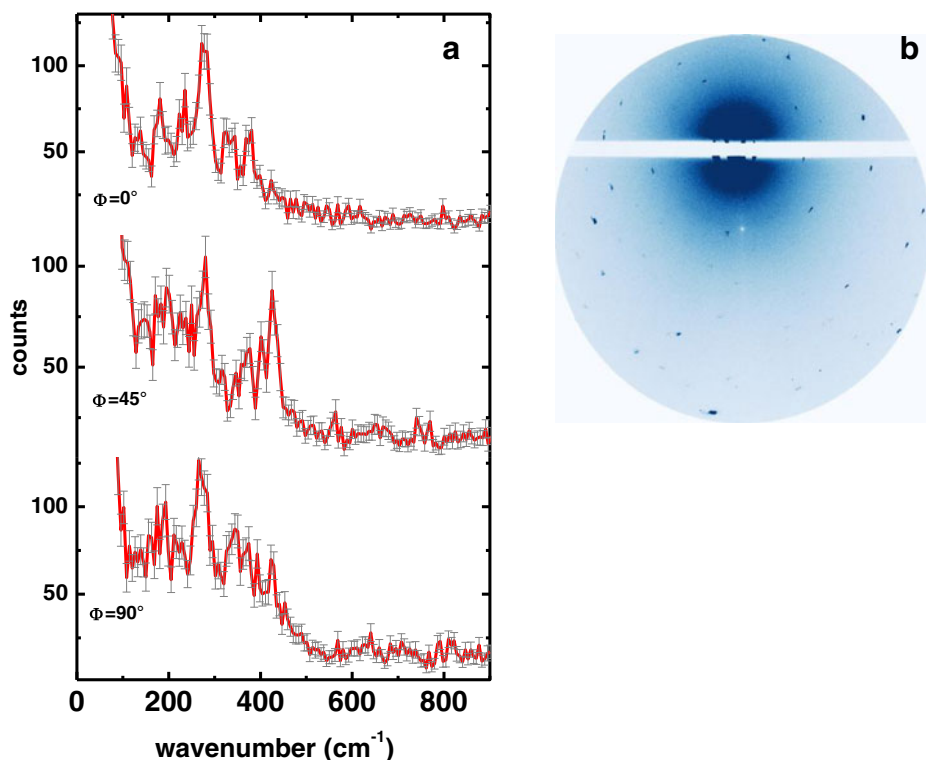


Fig. 4 **a** NIS data of three orientation of the same Mb protein single crystal after treatment with H_2O_2 and flash-freezing. Data acquisition time was 7, 6 and 8 h for $\Phi = 0^\circ$, 45° and 90° , respectively. **b** Corresponding X-ray diffraction pattern of the orientation $\phi = 0^\circ$ obtained before the NIS scan (data collection time 10 s)

In a first test experiment the vibrational pattern of a single crystal of the dinuclear SCO complex $[\{\text{Fe}(\text{L-N}_4\text{Me}_2)\}_2(\text{BzImCOO})](\text{ClO}_4)_2 \cdot 0.5(\text{CH}_3)_2\text{CO}$ [3, 4] further denoted as $[\{\text{Fe}(\text{L-N}_4\text{Me}_2)\}_2(\text{BzImCOO})]^{2+}$ was determined via NIS (Fig. 2). The single crystal (size $1500 \times 1000 \times 100 \mu\text{m}$) was fixed on a thin glass fiber with superglue and mounted on a goniometer head. For taking NIS data the scattered radiation was detected by an avalanche photodiode (APD) mounted 1 cm above the sample (see Fig. 1a).

NIS data were collected during several scans within the energy range -20 to 70 meV with a 0.25 meV step size and 3 s measuring time. The experimental energy resolution of the set-up was ~ 1 meV ($\sim 8.06 \text{ cm}^{-1}$). The experimentally determined partial density of vibrational states (pDOS) presented in this study was generated with a binning of 0.5 meV.

3 Results and discussion

Figure 3 shows NIS data of the dinuclear SCO complex $[\{\text{Fe}(\text{L-N}_4\text{Me}_2)\}_2(\text{BzImCOO})]^{2+}$ for two crystal orientations. The temperature controller was set to 80 K and the temperature determined from the detailed balance of the Stokes and anti-Stokes bands was

determined to 83 ± 12 K. At this temperature one of the two iron(II) centers of $[\{\text{Fe}(\text{L-N}_4\text{Me}_2)\}_2(\text{BzImCOO})\}^{2+}$ has a HS d-electron configuration and the other iron(II) center has a LS d-electron configuration [3].

The two bands at 300 and 410 cm^{-1} which dominate the NIS data shown in Fig. 3a disappear upon turning the crystal around an angle of 90° (see Fig. 3b). Preliminary DFT calculations indicate that the band at 300 cm^{-1} is caused by multiple modes where the HS iron moves along the iron-iron axis. Figure 3b shows a dominating band at $\sim 200\text{ cm}^{-1}$ which can be assigned to a mode where the HS iron moves perpendicular to the iron-iron axis of the complex. These preliminary assignments are consistent with the experimental observation. It should be noted that the data sets displayed in Fig. 3 have been obtained by the summation of 12 scans (Fig. 3a) respectively 7 scans (Fig. 3b) measured in 6 respectively 4 h.

Figure 4a shows the NIS data obtained at three different orientations of a ferric Myoglobin single crystal treated with hydrogen peroxide in order to generate a ferryl Fe(IV) = O unit also termed Cpd II. The size of the Mb crystal was $500 \times 350 \times 300\ \mu\text{m}$. The NIS data presented in Fig. 4a clearly show one band at $\sim 425\text{ cm}^{-1}$. Its intensity depends on the orientation of the crystal. The intensity of the second significant band around 275 cm^{-1} is less influenced by changing the orientation of the Mb single crystal, but it is much sharper at the orientation $\Phi = 45^\circ$ than for $\Phi = 0^\circ$ and 90° . It should be noted that the quality of the NIS data has been improved considerably compared to a previous NIS study of a ^{57}Fe -Myoglobin single crystal after addition of H_2O_2 and subsequent freezing performed at 110 K [6].

Unfortunately, vibrational modes involving Fe(III)-OH and Fe(IV) = O stretching modes which for Myoglobin in solution have been shown by NIS to occur for Fe(III)-OH at 556 cm^{-1} and for Fe(IV) = O (Cpd II) at 805 cm^{-1} [7] cannot be identified unambiguously from the data presented in Fig. 4. This may have two reasons: (i) MbCpd (II) has not been formed in large quantities inside the protein single crystal during the addition of hydrogen peroxide or (ii) since the Mb crystal could not be treated with hydrogen peroxide in a preoriented form it could well be that the data presented in Fig. 4a correspond to heme orientations with the Fe(IV) = O bond nearly perpendicular to the synchrotron beam. Therefore X-ray diffraction pattern have been taken from the protein crystals just before the NIS measurements in the same set-up using an image plate detector (mar345 from Marresearch GmbH; as an example see Fig. 4b). The evaluation of these data is currently undertaken in our laboratories.

In conclusion we have shown that NRS experiments with protein crystals are now conveniently possible with the new sample environment installed at beamline P01, PETRA III. But this sample environment can not only be used for single crystal experiments, it has also been used by us in order to perform NRS measurements on only small amounts of a powder sample mounted on the tip of a magnetic base plate, because sample change in the cryogenic gas stream is much faster than sample mounting in a conventional cryostat. The same holds for the sample alignment.

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