# Nuclear inelastic scattering investigations on high-valent iron-oxo intermediates in Myoglobin single crystals

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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 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 (Fig.1) [1]. Sample cooling is performed with a cryogenic gas stream which allows NRS measurements in the temperature range from 80 up to 400 K. We have investigated the nuclear inelastic scattering (NIS) pattern of a protein single crystal. As a test case we have chosen a hydrogen peroxide treated ferric $^{57}$Fe-Myoglobin (Mb) single crystal and we could indeed obtain orientation dependent NIS data sets. High-valent ferryl iron-oxo species can be formed in reactions of the oxygen storage protein myoglobin with hydrogen peroxide. Hydrogen peroxide is toxic to cells, but it is always formed in cells of oxygen dependent organisms. The ferryl state of Mb (Cpd II) is important because it is believed to be of physiological relevance through its involvement in oxidative stress reactions [2]. Diffraction patterns could be obtained for three orientations of the protein crystal using a 2-dimensional X-ray detector. These are currently used to determine the orientation of the protein crystal with respect to the synchrotron beam during the NIS experiment.

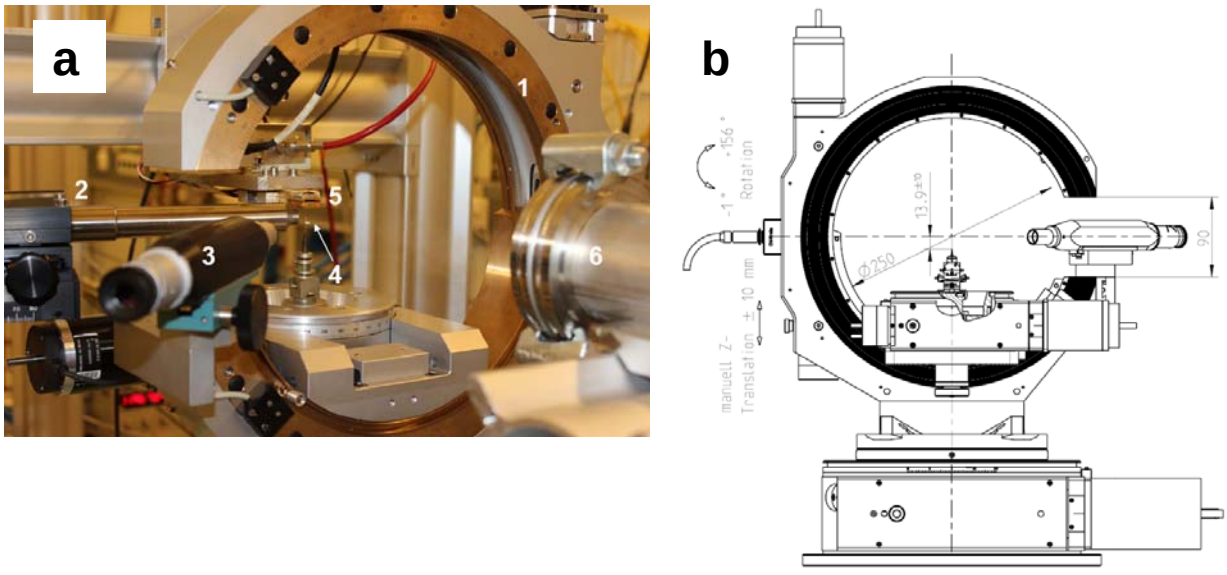


Figure 1: (a) Goniometer stage (1) and cryostream (2) now installed at P01, PETRA III. The microsample is mounted on a goniometer head (4) and alligned via a telescope (3). Also shown is the APD for NIS detection (5). (b) Technical drawing of the goniometer stage (1) with measures given in mm. [1]

Fig. 2a 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*350*300 µm. The NIS data presented in Fig. 2a clearly show one band at ~ 425 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° than for phi=0° 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 [3]. 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$^{-1}$ [4] cannot be identified unambiguously from the data presented in Fig. 2. 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. 2a 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 (see Fig. 2b). The evaluation of these data is currently undertaken in our laboratories.

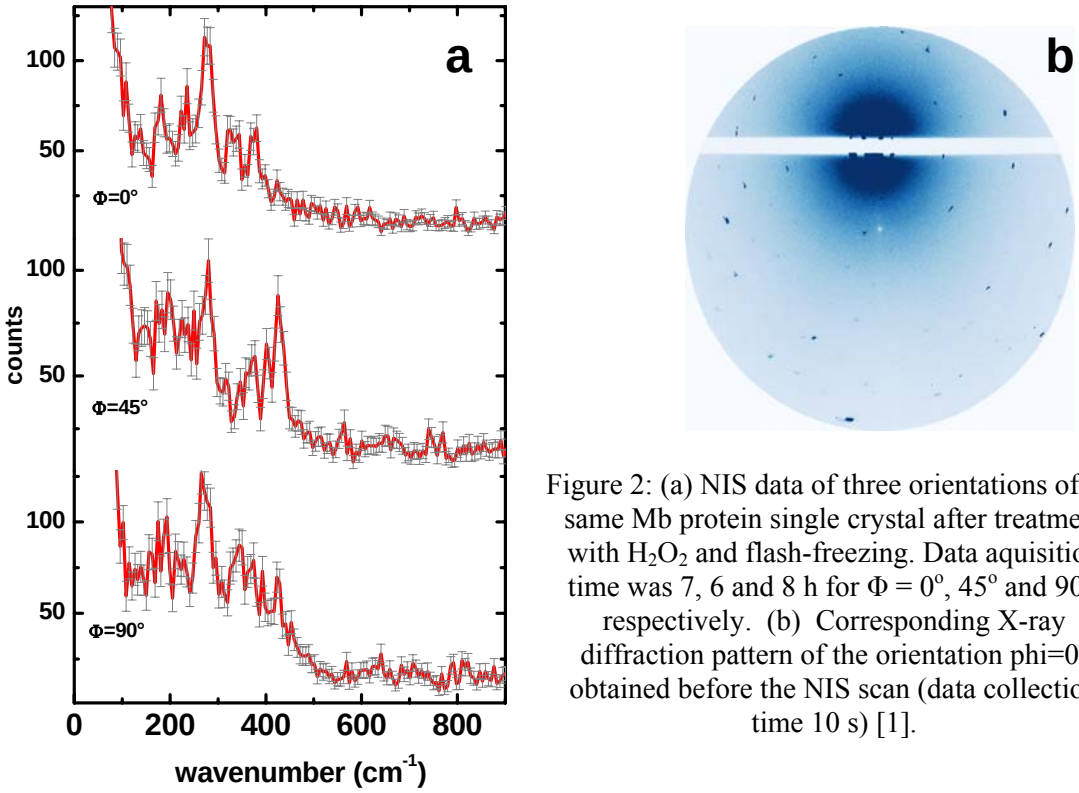


Figure 2: (a) NIS data of three orientations of the same Mb protein single crystal after treatment with $H_2O_2$ and flash-freezing. Data aquisition time was 7, 6 and 8 h for Φ = 0°, 45° and 90°, respectively. (b) Corresponding X-ray diffraction pattern of the orientation phi=0° obtained before the NIS scan (data collection time 10 s) [1].

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**References**

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