ESRF	**Experiment title:** Vibrational properties of the NO-carrier protein nitrophorin	**Experiment number**: SC-2122
Beamline: ID18	**Date of experiment**: from: 03-02-07 to: 06-02-07	**Date of report**: 28-02-07
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Report:

The protein nitrophorin (NP) is found in the salivary glands of the Amazon river-based kissing bug *Rhodnius prolixus*. For obtaining a sufficient blood-meal, these proteins are injected into the victim by the insect prior to feeding. When the pH value suddenly rises from 5 in the salivary glands of the kissing bug to pH 7.5 in the tissues of the victim, the binding affinity is changed and thus the signaling molecule nitric oxide (NO), which is bound to the iron in the active heme-center of the protein, is released. The NO can migrate through the victim's tissue to the capillaries to dilate them to allow more blood to flow to the site of the bite.

The *Rhodnius* NP's have a molecular weight of 20 kDa. Crystallographic data show that the tertiary structure of the NP's exhibit a β-barrel with a histidine residue (His-59) that serves as the proximal ligand to the heme [1]. The NP's represent the first examples of proteins with stable Fe(III)-NO complexes, where the NO can be stored for a long period of time.

We have performed nuclear inelastic scattering of synchroton radiation (NIS) to detect iron centered molecular vibrations of NO-ligated nitrophorins. Due to the high protein concentration of 10mM as well as the extraordinary beam stability at ID-18 during our experiment we have been able to measure the isoform nitrophorin 2 under four different conditions: (i) ligand-free (NP2-HS), (ii) NO-bound (NP2-NO), (iii) histamine bound (NP2-HIS), and (iv) CN- bound (NP2-CN) (see Figure 1). A striking feature of the vibrational spectrum obtained from NP2-NO is the well resolved and intense vibration at 73.5 meV (594 cm^{-1}

) with a shoulder at 72 meV (581 cm^{-1}). The former mode has been assigned to a NO-Fe stretching mode and the latter to a Fe-NO bending mode by Resonance Raman spectroscopy [2]. However these bands are only hardly visible in the Resonance Raman spectra and isotope labelling was used for the assignments. Currently theoretical QM/MM-calculations are undertaken to understand and assign all iron related stretching modes visible in Fig. 1. After these calculations are finished we foresee to publish this study as soon as possible. Already now it can be said that the Fe-axial ligand interaction is strongest for NO, decreases for CN- and decreases even more in the case of Fe-Histamine binding in nitrophorin. We consider this study as a textbook example of how NIS can be used to study the interaction of an active iron site in a protein with different ligands without the need of isotope labelling experiments. In fact these results may be very promissing for future studies on pharmacological relevant high-valent iron intermediates in enzymes.

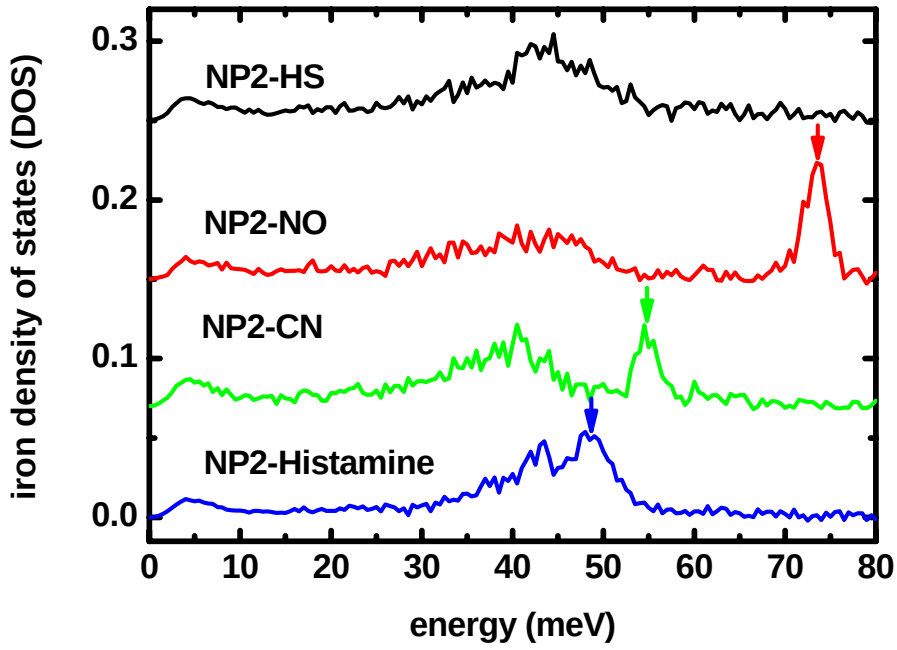

Figure 1: Density of states (DOS) obtained from NIS spectra obtained from NP-2 under four different conditions, from top to bottom: ligand-free (NP2-HS), NO-bound (NP2-NO), CN- bound (NP2-CN). Histamine bound (NP2-Histamine). The arrows denote the iron-ligand stretching modes.

References

[1] Andersen JF, Montfort, WR, (2000) J. Biol. Chem., **275**, 30496.

[2] Maes EM, Walker FA, Montfort WR, Czernuszewicz RS, (2001), J. Am. Chem. Soc.,**123**, 11664.