	Experiment title: Nitric oxide heme interactions in soluble and membrane bound nitrophorin 7	**Experiment number**: LS-2214
Beamline: ID-18	**Date of experiment**: from: 27/02/2013 to: 02/03/2013	**Date of report**: 02/04/2013
Shifts: 9	**Local contact(s)**: Aleksandr Chumakov	*Received at ESRF:*

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Report:

The nitrophorins (NPs) comprise an unusual family of proteins that use ferric (Fe(III)) heme to transport highly reactive nitric oxide (NO) from the salivary gland of a blood sucking bug to the victim, resulting in vasodilation and reduced blood coagulation. The NPs are found in the salivary glands of the Amazon river-based kissing bug *Rhodnius prolixus* in seven isoforms called NP1 to 7, and represent the first examples of proteins with stable Fe(III)-NO complexes, where the NO can be stored for a long period of time. *Rhodnius prolixus* also spreads the parasite *Trypanosoma cruzi* which in turn causes Chagas' disease, an incurable heart disease afflicting 16-18 million Latin Americans **[1].** Therefore it is highly desirable to have an effective pesticide against *Rhodnius prolixus*. Chemical substances which block the ability of the NPs to bind NO might be regarded as good candidates. In addition the study of the interaction of NO with the NPs can also lead to a better understanding of physiological NO-heme interactions in humans which is essential for the regulation of our bloodstream. All these facts explain the considerable interest and the efforts to obtain a detailed understanding of the NO-binding mechanism in NPs.

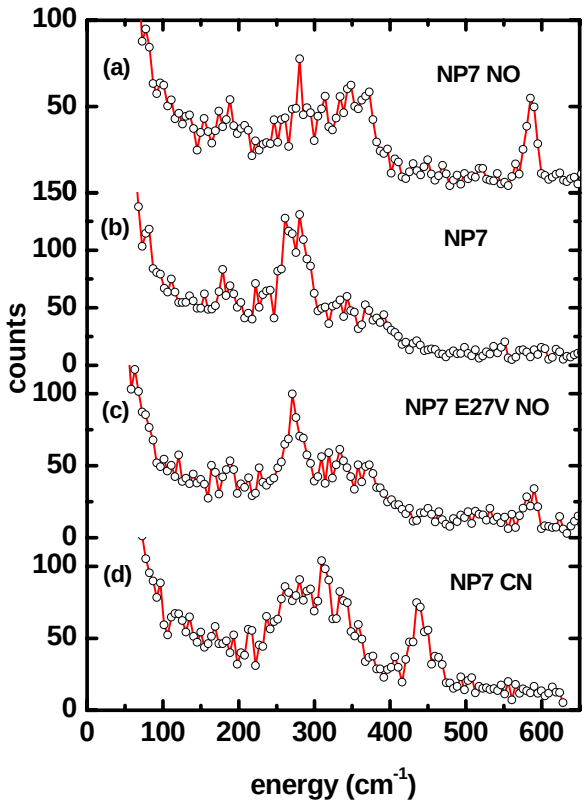
NP7, in contrast to the other nitrophorins cannot sequester histamine in vivo due to the low binding constant of histamine to NP7 **[2]**. However NP7 is able to bind to L-phosphatidyl-L-serine (PS) containing phospholipid membranes which other isoforms like NP1-4 do not. Thus, NP7 recognizes PS-bearing membrane surfaces as an indicator of activation and uses this as a means of targeting the surfaces of activated platelets and degranulating mast cells. Once bound on an activated platelet, NP7 can release NO to inhibit platelet aggregation and act as an anticoagulant by blocking coagulation factor binding sites **[2]**.

Along the line of our investigations on NO-heme interactions in proteins, we have used nuclear inelastic scattering (NIS) in order to detect directly iron-ligand vibrations in NP7. We wanted to use NIS, because only this method is able to detect functionally-relevant Fe(III)-NO, as well as low energy protein modes **[3]**.

Within experiment LS-2214 we have obtained NIS data sets for the following Nitrophorin samples: (i) NP7 with no additional ligands, (ii) NP7 complexed with NO, (iii) a mutant NP7(E27V) complexed with NO, (iv) NP7 complexed with CN, (v) a mutant NP2(L132V) with no additional ligands and (vi) NP2(L132V)

complexed with NO. As an example the obtained data sets for the NP7 samples are shown in Figure 1. For the NP7 complexed with NO a strong band at 587 cm^{-1} is observed which is due to a modes with significant Fe-NO stretching contribution also observed in NP2 [3]. What is striking is the significant peak at ~ 270 -280 cm^{-1} in the region where the heme modes occure. Surprisingly this mode seems to be quenched after adition of CN. Also the NP2 mutant NP2L132V which has a much less ruffled heme than the native NP2 does not have a significant single band feature around 270-280 cm^{-1}. In order to explain this effect NIS data are being presently calculated by means of combined quantum chemical and molecular mechanics (QM/MM) calculations based on the crystal structures of these complexes.

Figure 1: NIS data of (a) the NO-ligated form of NP7; (b) the substrate free NP7; (c) the NO-ligated form of the NP7E27V mutant and (d) the CN^--ligated form of NP7. The data have been measured at ID-18 of ESRF during LS-2214 with an energy resolution of 1 meV (~ 8 cm^{-1}) during hybrid mode and a temperature setting of 20 K.



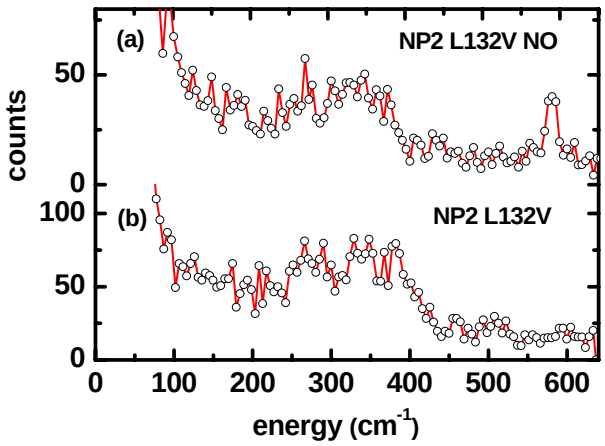


Figure 2: NIS data of (a) the NO-ligated form of the NP2 mutant NP2L132V and (b) its the substrate free form without NO addition. The data have been measured at ID-18 of ESRF during LS-2214 with an energy resolution of 1 meV (~ 8 cm^{-1}) during hybrid mode and a temperature setting of 20 K.

1. L.V. Kirchhoff, *N. Engl. J. Med.* 329 (1993) 639.
2. M. Knipp, F. Yang, R.E. Berry, H. Zhang, M.N. Shokhirev, F.A. Walker, *Biochemistry* 46 (2007) 13254.
3. B. Moeser, A. Janoschka, J.A. Wolny, H. Paulsen, I. Fillipov, R.E. Berry, H. Zhang , A.I. Chumakov, F.A. Walker, V. Schünemann, *J. Am. Chem. Soc.* 134(9) (2012) 4216.