



	Experiment title: Vibrational properties of iron sulfur proteins	Experiment number: SC-1217
Beamline: ID18	Date of experiment: from: 26-07-03 to: 01-08-03	Date of report: 25-02-04
Shifts: 12	Local contact(s): Dr. Aleksandr CHUMAKOV	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): V. Schünemann*, P. Wegner*, H. Winkler*, A.X. Trautwein, Institut für Physik, Universität Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany C. Schmidt Institut für Biochemie, Universität Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany		

Report:

Iron-sulfur proteins function as electron-transfer proteins in all living cells. They are involved in photosynthesis, cell respiration as well as in nitrogen fixation. Most iron-sulfur proteins have either single-, two-, three- or four-iron centers. Mössbauer spectroscopy has contributed a lot to the understanding of the electronic states of these centers. Their vibrational properties, however, are much less studied. This is partly due to the fact that the vibrational frequencies of those centers are masked by the vibrational frequencies of the protein backbone and, thus, conventional spectroscopy like Resonance Raman or infrared spectroscopy do not provide a clear picture of the vibrational properties of the iron centers. Nuclear Inelastic Scattering of synchrotron radiation (NIS) measurements allow to identify individual iron-modes and therefore are sensitive to iron-ligand bonds which are in turn related to the formal oxidation state of the iron. Vibrational properties of both $\text{Fe}^{\text{III}}\text{-S}_4$ and $\text{Fe}^{\text{II}}\text{-S}_4$ contribute to the reorganisation of these centers upon electron uptake or release and therefore modulate the electron transfer.

During SC1217 we have investigated at the beamline ID 18 of ESRF in Grenoble/France a rubredoxin-type iron-sulfur protein from *Pyrococcus abyssi* which has a single Fe-S center [1]. Protein samples have been prepared with ^{57}Fe concentrations of up to 10 mM. NIS measurements on the oxidized $\text{Fe}^{\text{III}}\text{-S}_4$ protein reveal a broad band around 15-25 meV and a broad band around 42-48 meV (fig. 1). The latter result confirms Resonance Raman studies on oxidized rubredoxin which indicate that there are three resonances (43.15, 45.01 and 46.62 meV) in the

region where asymmetric $\text{Fe}^{\text{III}}\text{-S}$ stretch modes are expected [1,2]. Upon reduction the asymmetric stretch modes observed by NIS shift to lower energies (36 – 42 meV). This can be rationalized by the fact that upon reduction the $\text{Fe}^{\text{II}}\text{-S}$ bond lengths increase, which in turn is accompanied by a decrease of $\text{Fe}^{\text{II}}\text{-S}$ binding energy and therefore by a decrease of the force constant of the $\text{Fe}^{\text{II}}\text{-S}$ bond. This correlation between iron-oxidation state and Fe-S stretch-mode position is in line with DFT calculations which have been performed on these systems (fig.1).

Resonance Raman data of the reduced rubredoxin with its iron in the $\text{Fe}^{\text{II}}\text{-S}_4$ ($S=2$) state are inaccessible, because the protein is colorless in this state [2]. Thus this study has provided first information about the vibrational properties of the reduced iron-sulfur center in rubredoxin.

In addition we have also obtained NIS data of $[\text{4Fe-4S}]^{2+}$ -centers from the Benzoyl-CoA reductase from *T. aromatica*. DFT calculations also on this system are in progress in order to appropriately describe its vibrational properties.

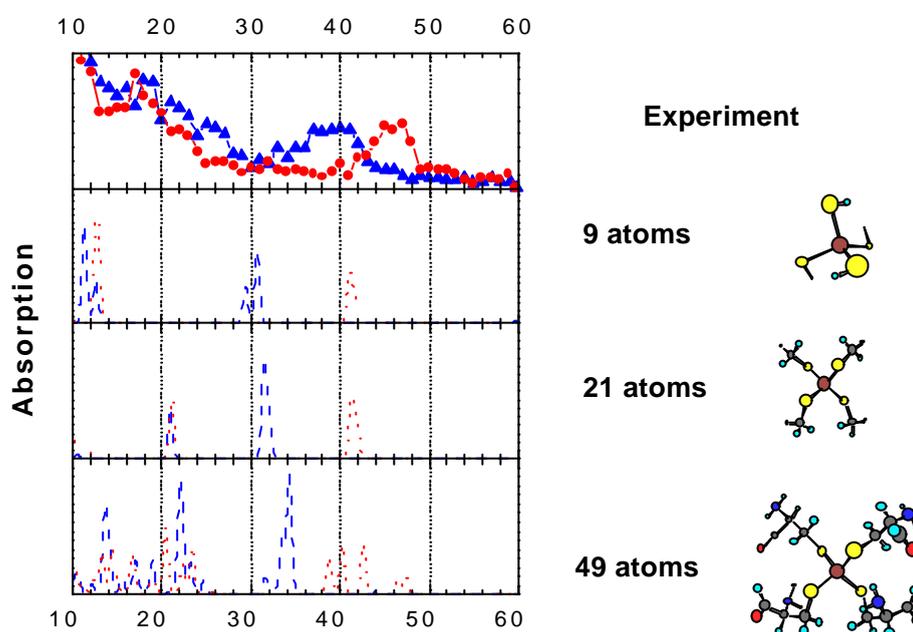


Figure 1: NIS-spectra of oxidized () and reduced rubredoxin () from *Pyrococcus abyssi* obtained at 25 K. Theoretically calculated NIS spectra based on DFT-calculations of 9, 21 and 49 atoms are shown below. The blue dashed lines represent calculated NIS spectra for the oxidized $\text{Fe}^{\text{III}}\text{-S}_4$ center and the red dotted line for the reduced $\text{Fe}^{\text{II}}\text{-S}_4$ center.

References

[1] Czernuszewicz R.S., LeGall J., Moura I. and Spiro T.G. (1986) *Inorg. Chem.* **25**: 696-700.

[2] Spiro T.G., Czernuszewicz R.S. and Han S. (1988) in Spiro T.G. (ed.) *Biological Applications of Raman Spectroscopy*, John Wiley & Sons, Inc, New York, pp. 523-554.