ESRF	**Experiment title:** **"Bromide-binding to the active-site of vanadate-dependent peroxidases"**	**Experiment number**: LS1986
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Names and affiliations of applicants (* indicates experimentalists):

Prof. Holger Dau, Freie Universität Berlin, Fachbereich Physik, Arnimallee 14, D-14195 Berlin, Germany
Prof. Dieter Rehder, Inst. f. Anorganische u. Angewandte Chemie, Universität Hamburg, D-20146 Hamburg, Germany
Dr. Michael Haumann*, FU Berlin, FB Physik
Dr. Carola Schulzke*, Christian-Albrechts-Universität Kiel, Institut für Anorganische Chemie, Olshausenstr. 40, D-24098 Kiel, Germany
Markus Grabolle*, FU Berlin, FB Physik
Peter Liebisch*, FU Berlin, FB Physik
Claudia Müller*, FU Berlin, FB Physik
§block allocation for LS1986 and SC924, separate report for SC924

Report. Haloperoxidases catalyse the formation of hypohaleous acids. They play a role in anti-bacterial medication [1,2]. To gain deeper insight into the catalytic mechanism, the binding of bromide in a vanadate-dependent peroxidase (bromoperoxidase, BP) was investigated using XAS at the vanadium K-edge.

Synthetic models for the mono-vanadium metal site in bromoperoxidases were investigated. XANES and EXAFS spectra (scan range 5300-6500 eV) of ten different vanadium model compounds (powder samples) containing either V(IV) or V(IV) oxidation states and bound bromide at distances ranging between 3 Å and > 6 Å from vanadium were measured at room temperature. XAS spectra were collected in absorption and fluorescence modes (using a photodiode as detector in the latter case).

On bromoperoxidase samples from *Ascophyllum nodosum* XAS measurements were performed on frozen solutions (~ 1 mM vanadium) [1,2]. Spectra were collected on samples containing vanadium-to-bromide (Br) concentration ratios of 1:0, 1:0.5, 1:1, and 1:3 and on a sample containing iodate (I) instead of Br (V:I = 1:3). Spectra were measured at 20 K in fluorescence mode using an energy-resolving multi-element detector. Due to the presence of trace amounts of cerium in the Kapton tape used for the windows of the sample holders (giving rise to Cer L-edge signals), the analysis of EXAFS spectra of enzyme samples at the V K-edge was limited to an upper energy of 5720 eV. For energy calibration the simultaneously recorded absorption spectrum of a vanadium metal foil was used.

The following results have been obtained:

(1) Good quality EXAFS spectra of ten vanadium model compounds have been measured. Pronounced differences in the shapes and positions of the vanadium K-edge are observed, depending on the coordination

and oxidation state of the V-atom (Fig. 1A). The bound Br-atom can be detected in the EXAFS spectra (Fig. 1B) up to a distance of ~5.5 Å from vanadium.

(2) XANES spectra of the bromoperoxidase differ from the ones of all studied model compounds, pointing to a different coordination of vanadium in the enzyme. The XANES spectra reveal only minor changes upon the addition of Br or I to the samples (not shown). In the EXAFS spectra minor changes were detected at reduced distances close to 3.2 Å (not shown). Comparison of the Fourier-transforms of EXAFS spectra calculated over the same limited energy range (20-5720 eV above E_0) for vanadium model compounds and enzyme samples (not documented) and preliminary simulations revealed that, if Br (or I) is bound to the enzyme, it is bound at a distances of about 3.7 Å to the vanadium atom, in line with previous results obtained by XAS at the Br K-edge [1,2].

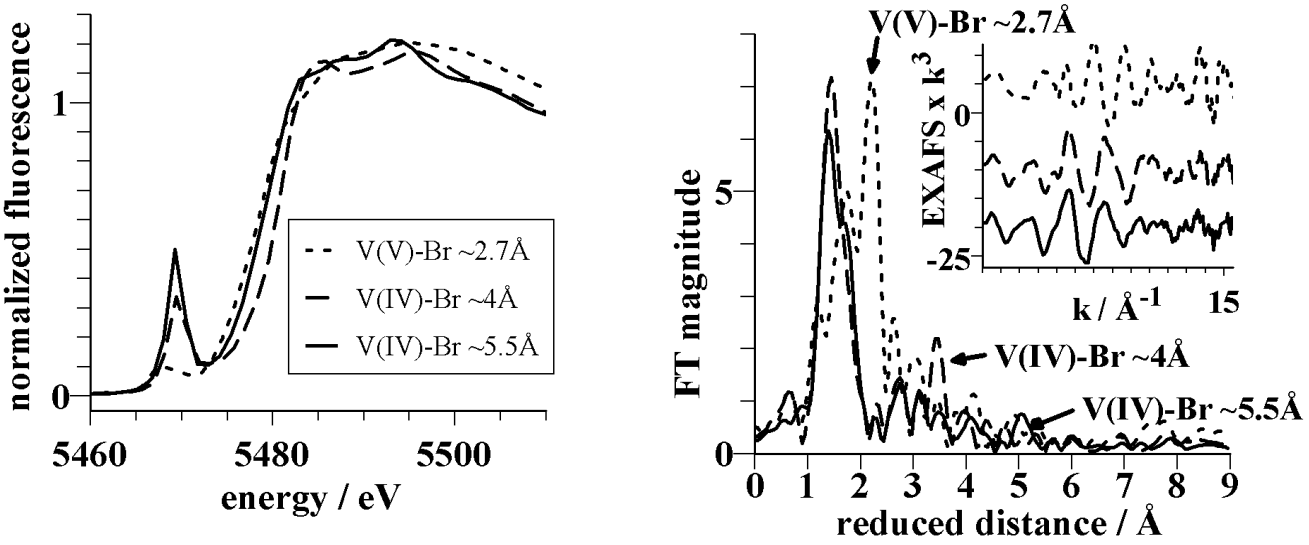

Fig. 1: (A) XANES spectra at the V K-edge for three selected synthetic vanadium model compounds with different V-Br distances. (B) EXAFS spectra of the models in (A). Fts calculated for energies ranging from 20 eV to 950 eV above E_0.

Summary and Conclusions.

(1) High-quality XANES and EXAFS spectra have been collected for ten synthetic compounds which model the potential ligand environment of vanadium in the enzyme. The thus obtained "library" of spectra is needed for the comparative analysis of XANES spectra of the enzyme as well as for a realistic assessment of the information content of the EXAFS spectra of the bromoperoxidase.

(2) For the enzyme system, we find that the substrate (Br or I) is not directly coordinated to the active-site vanadium. In conjunction with XAS data previously collected at the bromine K-edge, the EXAFS results points towards Br-binding at an amino acid residue resulting in a V-Br distance of about 3.7 Å. However, due to unforeseen experimental problems (cerium contaminations), evaluation of EXAFS was only possible in a limited *k*-range. Therefore, the obtained results do not represent unambiguous proof for Br-binding at a distance of 3.7 Å to the vanadium of the active site.

A publication on the obtained results is in preparation.

1. Dau, H., Dittmer, J., Epple, M., Hanss, J., Kiss, E., Rehder, D., Schulzke, C. and Vilter, H. (1999) FEBS Lett. 457, 237-240. Bromine K-edge EXAFS studies of bromide binding to bromoperoxidase from Ascophyllum nodosum.

2. Rehder, D., Schulzke, C., Dau, H., Meinke, C., Hanss, J. and Epple, M. (2000) J. Inorganic Biochem. 80, 115-121. Water and bromide in the active center of vanadate-dependent haloperoxidases.