

## **XANES analysis of the [CuSMo] active site cluster in CO dehydrogenase – Extending EXAFS models to 3D**

*Manuel Gnida and Wolfram Meyer-Klaucke*

European Molecular Biology Laboratory, Outstation at DESY, D-22603 Hamburg, Germany

X-ray absorption spectroscopy (XAS) is well established as a tool for structure determination. Most of the structural information is derived from a quantitative analysis of the oscillatory part of the spectrum well above the absorption edge, the extended X-ray absorption fine structure (EXAFS). In general, three-dimensional (3D) information is not obtained, since the EXAFS regime of the XAS spectrum is dominated by single scattering processes of the photoelectron released after the X-ray absorption. In contrast, the spectral region of the absorption edge itself is dominated by multiple scattering events. The X-ray absorption near edge structure (XANES) therefore depends on the 3D arrangement of atoms around the absorbing atom. Consequently, 3D structural information can be deduced by simulating experimental XANES spectra.

Here, we present Mo-*K*-edge simulations for the enzyme CO dehydrogenase employing the program FEFF8 [Ankudinov *et al.*, Phys. Rev. B 58, 7565 (1998)]. In its active form CO dehydrogenase carries a novel [CuSMo] active site cluster. Using the EXAFS technique it has been shown previously that three, two, or a single oxo group Mo=O is coordinated to the Mo center in the various forms of the protein [Gnida *et al.*, Biochemistry 42, 222 (2003) and Gnida *et al.*, HASYLAB annual report 2, 435 (2002)].

Our XANES results show that experimental trends observed for the different protein forms are well reproduced in the edge simulations, even when using a minimal structural model. Furthermore, our data demonstrate that XANES is much more sensitive to weakly bound ligands (e.g. water) as compared to EXAFS. Thus, XANES can be used to verify the presence and nature of such ligands assumed in the EXAFS model. Finally and most importantly, modeling the absorption edges allows distinguishing between equatorial and axial positions of oxo groups at the Mo site, thereby contributing valuable 3D structural information that cannot be deduced from EXAFS.