residues among homologous proteins, providing a working hypothesis for functional as well as structural studies. In contrast, the type of metal ion potentially bound to conserved residues is only rarely determined for individual proteins or even individual protein batches, although this information is essential for the understanding of element specificity of metal binding sites and has strong implications on potential functions of protein. Such data are easily obtainable by techniques such as PIXE [1] or TXRF [2] these days. They provide a solid basis for spectroscopic methods such as XAS [3] and structural studies by NMR or macromolecular crystallography.

Studying a metalloprotein by crystallography seems to be sufficient in many cases, but for example:

- metal specificity or stoichiometry of homologous enzymes vary,
- non-regular metal cores might be formed,
- crystallization conditions might influence the element types bound to proteins, and
- new metal binding motifs might require independent data.

Prominent examples of proteins with varying metal specificity are the metallo-beta lactamase superfamily [4] and metal regulators (e.g. of the FUR-type) [5], [6]. Here, metal-stoichiometry in protein crystals can reflect the crystallization conditions and thus independent evidence is essential to understand element specificity as well as the role of individual binding sites.

In other cases, such as Ferritins or Dps-like peroxide resistance proteins (Dpr) a metal containing core is formed that is frequently not well suited for crystallographic studies [7]. In other cases, such as CO dehydrogenase I and the mononuclear [Fe]- hydrogenase [8] independent evidence for a structural model is essential because of the large impact of the new metal binding motif on potential catalytic mechanisms and future research in bioinorganic chemistry.

Therefore, it is essential to incorporate available synergistic methods. XAS is perfectly suited to provide such input and fits very well into the above described pipeline for the analysis of metalloproteins. In this presentation some of the above mentioned examples will be discussed in more detail and synergism between crystallography and XAS is discussed.

[1] E. Garman, *Struct. Fold. Des.* **1999**, *7*, 291–299. [2] G. Wellenreuther, U.E.A. Fittschen, M.E.S. Achard, A. Faust, X. Kreplin, W. Meyer-Klaucke, *Spectrochimica Acta Part B* **2008**, 1461-1468. [3] I. Ascone, W. Meyer-Klaucke, L. Murphy, *J. Synchr. Rad.* **2003**, *10*, 16-22. [4] O. Schilling, N. Wenzel, M. Naylor, A. Vogel, M. Crowder, C. Makaroff, W. Meyer-Klaucke, *Biochem.* **2003**, *42*, 11777-11786. [5] E. Pohl, J.C. Haller, A.E. Mijovilovich, W. Meyer-Klaucke, E. Garman, M.L. Vasil, *Mol. Microbiol.* **2003**, *47*, 903-915. [6] D. Lucarelli, S. Russo, E. Garman, A. Milano, W. Meyer-Klaucke, E. Pohl, *J. of Biol. Chem.* **2007**, *282*, 9914-9922. [7] A. Kauko, A.T. Pulliainen, S. Haataja, W. Meyer-Klaucke, J. Finne, S.C. Papageorgiou, *J Mol Biol*, **2006**, *364*, 97-109. [8] M. Salomone-Stagni, S. Vogt, S. Shima, W. Meyer-Klaucke, *J. Phys.: Conf. Ser.* **2009**, *190*(012197)

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Single Crystal XAS Studies on Transient Metalloprotein Intermediates

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Synchrotron based metal K-edge XAS and EXAFS spectroscopy is a powerful tool for electronic and geometric structure determination of metalloprotein active sites and is dominantly applied to isotropic samples. The combined single crystal XAS and diffraction technique developed at SSRL on beam line 9-3 can be applied to anisotropic protein crystals to obtain direction specific metrical and electronic information about the active site. In addition, important electronic structure information on unstable, trapped intermediate and transient species can be obtained, which can guide the structure determination process or help develop a strategy for diffraction data collection.

Two recent studies on metalloprotein active sites will be presented. In the first study, single crystal XAS studies on the Ni containing active site of Methyl Coenzyme M Reductase was combined with solution XAS and EXAFS data. The data were used to determine the redox state of a putative Ni(III)-Me intermediate and coupled to structure determination. In the second study, the electronic structure of oxyhemoglobin was explored using both solution and single crystal Fe K-pre-edge and near-edge XAS studies to differentiate between two putative electronic structure descriptions. Other potential applications will be discussed.

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Structural studies of Amyloid-β oligomerization and metal binding in Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of dementia and is characterized by the presence of fibrillar protein depositions or plaques in the brain. The major constituent of AD plaques is the amyloid-β peptide (Aβ) which is cleaved from the membrane-bound amyloid precursor protein (APP). However, current evidence suggests that soluble non-fibrillar Aβ oligomers are the major drivers of Aβ-mediated neuronal dysfunction and a significant source of the neurotoxicity is mediated by the interaction of Aβ with transition metals (Cu, Fe and Zn) which leads to altered neuronal metal homeostasis, oxidative injury and accumulation of toxic Aβ oligomers. Determining the structure of Aβ oligomers and the details of the Aβ metal binding site are vital steps towards understanding why neurotoxic aggregates and plaques occur – knowledge that is important in the development of new treatments.

Interfering with metal binding to Aβ is an emerging target for the development of AD therapeutics [1]. We have analyzed *in vitro* the structure of Aβ(1-16) (metal-binding region) complexed with transition metals, Pt-based and other inhibitors [1] by combined X-ray absorption spectroscopy (EXAFS, XANES) and *ab initio* density functional calculations (DFT) [2,3]. XANES has been used to probe the substrate mediated reduction of Cu(II) to Cu(I) in Aβ-Cu complexes by indigenous reducing agents [4].

Here, we also describe the first atomic resolution x-ray crystallographic structure of an oligomeric Aβ(17-42) (p3) fragment [5] constrained within the CDR3 loop region of a shark IgNAR single variable domain antibody [6]. This discovery finally shows that the structure of oligomers is not like a piece of a fibril. The predominant oligomeric species is a tightly-associated Aβ dimer, with paired dimers forming a tetramer in the crystalline form. The general features of this oligomer match some recent predictions, thus potentially providing a