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.

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MS.42.3

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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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MS.42.4

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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

model system for non-fibrillar oligomer formation in AD.

Studies [7] indicated that yeast may be a tractable model system for screening metals re-distribution and toxicity caused by A β *in vivo*. The intracellular A β was produced through fusion with a green fluorescent protein (GFP) in yeast [7]. Here, we present X-ray florescence microscopy (XFM) imaging of these yeast cells which suggests that toxic A β species promote stress and enhance copper uptake. This correlates with overexpression of APP and increased copper content in human cells [8].

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Metal-protein interplay in protein function and stability

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Metalloproteins account for one third of the proteins encoded in cell genomes. The metal atoms within these proteins can play either structural or functional roles. The functionality of any metal site is very sensitive to tiny changes in its geometry. Here, we illustrate how small changes in the metal site can affect protein function and stability.

Plastocyanin (Pc) is an essential electron carrier in oxygenic photosynthesis. It is a well-known representative of the blue copper protein family, which has a high technological interest. EXAFS shows how the geometry of the copper site changes during the transient binding between Pc and its physiological partners, thereby modulating the electronic coupling between the donor and its acceptor [1].

Further, we faced up the relations between the stability of Pc and its oxidation state. In most Pcs, the reduced state shows a higher melting point than the oxidised. However, the Pc from *Phormidium* (a thermophilic cyanobacteria) is more stable in its this state, despite the high sequence identity between the different proteins. In fact, the entire metal site environment is identical in the compared Pcs. Hence, we designed mutations of a thermo-resistant protein that affected this behavior. Notably, a remote aminoacid substitution in a loop around 20 Å from the copper centre reversed the above relation. Withal, the secondary structure and the macroscopic redox potential of the protein remained unaltered. Moreover, the mutation barely affected the geometry of the copper site: changes in metal-ligand distances were within EXAFS error [2].

Nevertheless, the edge of the XAS spectra of the mutant was shifted with respect to that of the WT, thermo-resistant protein, thereby matching that of a non resistant protein used as control. Analysis of the XANES region, using the CONTINUUM approach, allowed us to determine that a single bond – that between copper and the gamma sulphur of a cysteine – correlated with the different thermal resistances of the proteins, including their reduced states [3].

We are currently analysing XRD data from two distinct Pc mutants. While XANES gives a very detailed picture of the electronic structure within the copper environment, XRD probes the overall structure necessary to understand how the introduced mutations can affect the long range interactions within Pcs and to explain their different thermal stabilities.

In summary, combining XRD with spectroscopic approaches like XAS is indispensable to tackle subtle changes in metal sites, which are critical to modulate the functionality and stability of metalloproteins.

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Structure Validation Challenges in Chemical Crystallography

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Structure validation was pioneered by the IUCr journals [1], [2]. Today validation reports are required by all major journals when a crystal structure is included in a paper. Such a report itemizes as ALERTS the issues that need special attention of the author and referees. Currently, more than 400 ALERTS have been implemented. New ALERTS are regularly added to address additional problems that came to our attention. ALERTS come in various forms and are not necessarily synonymous with ERRORS. They may indicate missing data, inconsistencies, poor or good quality data and results, errors and potentially interesting issues to look at in some detail. Most ALERTS are reported as a short one line message. More details about an ALERT and possible solutions can be found in reference [3]. Structure validation is possible either through the IUCr CheckCIF Web-based service or with an in-house implementation of the program PLATON. Recently FCF-validation has been introduced [4] for a more detailed analysis with the supporting reflection data. Unfortunately this is only possible with the deposited data for Acta Cryst. journals. Contrary to standard practices in the bio-crystallography world, this is not standard practice yet in chemical crystallography. One of the problems with the current validation practice is that most validation criteria are set to the standards required for publications in the Acta Cryst. journals with its expressed intension to publish the best attainable crystallographic results. Meeting some of those criteria in the case of a structure determination that is presented in a chemical journal in support of the reported chemistry might seem less important. The obvious challenge is currently the conflict between 'the-best-attainable' against 'sufficient-for-the-purpose'. In the end all results go largely unqualified in data bases as supposedly solid information.

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