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Chemical bonds in aluminium

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Recent advances in quantitative convergent beam electron diffraction (QCBED) [1], [2], [3] have led to the solution of an 82 year old problem – determination of the bonding electron density in aluminium. Until the present determination [4], [5], there was considerable disparity between a large number of experimental and theoretical determinations. These disagreements culminated in two recent theoretical studies where one asserted that bonding in aluminium is octahedrally coordinated [6] and the other that it is tetrahedrally coordinated [7].

In addition to the present QCBED determination, a density functional theory (DFT) first-principles calculation of the ground state electron distribution was performed. The QCBED and DFT determinations are in close agreement and show that the bonding electron density is concentrated exclusively in the tetrahedral interstices.

The detailed understanding of bonding in aluminium resulting from the present work has led to a new understanding of the influence of interatomic bonding on the mechanical anisotropy of aluminium. Correlations between bonding and the morphologies of precipitates that form in aluminium alloys have also been identified.

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Polarized XAS of photosystem II and relevant Mn model complexes

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The Mn₄Ca catalytic cluster of the oxygen-evolving complex (OEC) in Photosystem II (PS II) cycles through five oxidation states (S_i-states, i=0-4) coupling the one electron photochemistry of the reaction center with the 4 electron redox chemistry of water oxidation. The structure of the Mn₄Ca cluster has been extensively studied by X-ray [1] and other spectroscopic methods and by X-ray

crystallography between 3.8-2.9 Å resolution [2-5]. The most recent X-ray crystallography structure is at 1.9 Å resolution [6]. Radiation damage to the Mn₄Ca cluster, although much reduced in the most recent structure at the highest resolution, still remains a limiting factor [7].

We have previously reported detailed X-ray absorption spectroscopy studies from solution and single crystal XANES and EXAFS data from the native S₁ (dark) state and proposed structural models of the Mn₄Ca cluster of PS II within the context of the protein environment [1, 8]. This study used PS II dimer crystals obtained from *T. elongatus*, in which the crystal unit cell contains four PS II dimers. The structural parameters derived from X-ray spectroscopy in relation to the X-ray crystallography structure at 1.9 Å will be discussed. We have also collected polarized spectra of monomer PS II single crystals, which have been recently crystallized [9]. Monomer crystals have a favorable orientation for deriving a unique structure for the Mn₄Ca cluster.

We have extended the study to the higher S-states by illuminating the S₁ state solution and single crystals either by continuous illumination or by laser flashes to create intermediate S-states (S₂ and S₃). The solution state spectra show changes in the structure of the catalytic cluster as it advances through the intermediate states. Polarized XANES and EXAFS spectra from these crystals show unique orientation dependence and provide additional information about the structural changes taking place in these intermediate states. The relevance of the geometric and electronic structural changes derived from the XAS studies to the mechanism of water oxidation will be discussed.

The polarized XAS data from crystals of many multinuclear oxo-bridged Mn complexes have been collected. The polarized spectra have been used to understand the electronic structure of the Mn complexes, and these studies provide information that will be useful for understanding the structure and the mechanism of the Mn₄Ca cluster present in PS II.

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The synergism of XAS and crystallography

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In recent years, research on metalloproteins has benefited from synergism between bioinformatics, sample characterization, spectroscopy, and structural methods. The combination of these methods results in a pipeline that helps to establish an excellent basis for understanding necessities and variability of metal binding.

Bioinformatics is the established method to identify conserved

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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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 ($A\beta$) which is cleaved from the membrane-bound amyloid precursor protein (APP). However, current evidence suggests that soluble non-fibrillar $A\beta$ oligomers are the major drivers of $A\beta$ -mediated neuronal dysfunction and a significant source of the neurotoxicity is mediated by the interaction of $A\beta$ with transition metals (Cu, Fe and Zn) which leads to altered neuronal metal homeostasis, oxidative injury and accumulation of toxic $A\beta$ oligomers. Determining the structure of $A\beta$ oligomers and the details of the $A\beta$ 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 $A\beta$ is an emerging target for the development of AD therapeutics [1]. We have analyzed *in vitro* the structure of $A\beta$ (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 $A\beta$ -Cu complexes by indigenous reducing agents [4].

Here, we also describe the first atomic resolution x-ray crystallographic structure of an oligomeric $A\beta$ (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 $A\beta$ 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