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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Keywords: alzheimer's, amyloid-β oligomer, structure

MS.42.5

Acta Cryst. (2011) A**67,** C103

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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Keywords: XAS, copper-proteins, stability

MS.43.1

Acta Cryst. (2011) A**67,** C103

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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Keywords: validation, checkCIF, PLATON