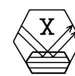

Friday, March 15, Session V

L17

BRINGING THE BEAMLINE HOME: INCREASING PRODUCTIVITY AND REDUCING COSTS WITH THE D8 VENTURE WITH METALJET X-RAY SOURCE

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The METALJET X-ray source delivers a beam of flux over three times greater than any home-source previously available. In the D8 VENTURE, the METALJET is coupled with the PHOTON 100 CMOS detector which provides greater sensitivity for collecting data from weakly diffracting crystals or those with very large unit cells. The PHOTON 100 also enables continuous data collection which improves data quality and leads to vast reductions in data collection times.

As expected, initial results from the system demonstrate that the performance of the new D8 VENTURE with METALJET will enable much of the work previously requiring synchrotron beam time to be done in the home-lab - screening of weakly diffracting crystals, high quality data collection and high-speed data collection with automated sample changing are now possible.

This enables much more work to be done in the home-lab, reducing the number of synchrotron trips and associated time and financial expenses. High-productivity results not only high sample throughput; but also considers the quality and the value of the results.

L18

METALS AND IONS IN PROTEIN STRUCTURES – FROM ESSENTIAL TO MARGINAL QUESTIONS IN IDENTIFICATION OF IONS IN ENZYMES AND RECEPTORS

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Biological macromolecules, such as proteins, nucleic acids, and their complexes, frequently require presence of metal or other ions to perform their native functions. In single crystal studies of biological macromolecules identification of ligands or generally solvent molecules very often represents an uneasy task. The studied molecules undergo a lengthy process of expression, modification (and secretion), purification and sometimes even special pre-crystallization treatment. Proteins and nucleic acids are commonly crystallized in solutions of salts and presence of unwanted metal ions in the used chemicals is not excluded. Even "trace" impurities in chemicals of known identity can represent a sufficient source of potential ligands of studied molecules. Therefore the resulting set of potential "binders" for a given protein or nucleic acid encompasses both the natural cofactors and the complete cohort of chemicals that were encountered on the long journey to the studied state.

If structure resolution allows, the details of an X-ray structure of an enzyme or receptor can tell us more than a pure coincidence of localization of an ion in a certain protein site. When carefully analyzed, such sites can bring better insight into the structure-function relationship and also a better understanding of the relevance of the analyzed state of a given protein. Metal binding to an enzyme can be on one hand a pure consequence of a particular crystallization condition and can be viewed as such. On the other hand it can indicate a real binding site where a metal ion is necessary for proper function or stability (extracellular hydrolases requiring sodium or calcium ions to maintain their stability, copper, zinc or other metal storage and transport proteins, *etc.*).

For many standard cases typical metal-ligand distances and standard coordination are observed. For example an observation of the typical octahedral coordination of Ca^{2+} ion mostly by oxygen atoms with a majority of the O-Ca^{2+} distances in the range of 2.3-2.4 Å (1500 observations in the PDB) does not leave much space for speculation and identity of such an ion for an extracellular enzyme with added calcium in the crystallization condition is far from doubtful. In spite of that even dependence of a given enzyme say on manganese does not necessarily imply that an ion localized in its X-ray structure is the same. Many protein metal binding sites are promiscuous and can bind for example a few different types of metals of the fourth period. For some of them activity towards the same substrate can be measured with different divalent metals occupying the relevant sites. There are serious implications following from this knowledge: 1) Not all structural data in the PDB (or indeed in your local set of solved structures) contain

correct information on metal or ion identity, 2) Special attention should be paid to ion identification in all structural studies of metal-binding or ion-binding, and especially metal-dependent proteins.

A majority of protein crystals do not provide diffraction data to atomic or subatomic diffraction limits (1.2 Å or better). To date some 76 thousand X-ray structures deposited in the Protein Data Bank have the high diffraction limit of data 1.2 Å or worse (98% of all X-ray structures) and about 1800 1.2 Å and better (2%). Therefore protein crystallographers must mostly rely on other indicators of the nature of an ion than just the height of an electron density maximum. Moreover, protein X-ray structures very often provide an accurate picture of a mixture of structural states and this can be true even for a metal or ion binding site. Therefore, essential features of an observation (relative heights of unbiased electron density maxima, presence of anomalous signal, the shortest interatomic distances, stability of atomic positions in refinement, nature of coordinating atoms/ions) must be distinguished from marginal signs which can be easily smeared by worse quality of the diffraction data, resolution limits, position in the protein chain (termini, surface) or local disorder (atomic displacement parameters, occurrence of some longer coordination distances, missing vertices of the first coordination sphere).

In our structural studies of enzymes (chitinases, nucleases, glycosyl hydrolases, anhydrolases, oxidases *etc.*) and natural killer cell receptors we regularly apply the described approach to help us identify a particular protein ligand. Examples include identification of metal ions such as Cu^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Ca^{2+}, Mg^{2+}, Na^{+} and other ionic ligands, for instance Cl^{-}, PO_4^{3-}, SO_4^{2-} [1-6].

The sum of the available tools for identification of metals/ions in protein structures includes anomalous scattering signal, typical coordination and bonding distances [7], statistical evaluation of typical cases, assessment of the local environment, experimental conditions such as pH, and other. Access to tunable X-ray sources with fluorescence detectors enables absorption edge checks and fluorescence analysis in some cases [4] and availability of a micrometer high energy proton beam allows element identification by microbeam Proton Induced X-ray Emission (microPIXE) [8].

Lighter ions, such as Na^{+}, Mg^{2+} and Cl^{-}, belong to a special category as their presence in protein structures either remains unnoticed or is misinterpreted. In such cases the correct assignment of an ion type and its distinction from a water molecule rely on sufficient evidence from all available information sources.

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L19

STRUCTURAL INSIGHT INTO THE CONFORMATION OF ONE OF THE MICROTUBULE BINDING MOTIFS ON THE ALZHEIMER'S DISEASE-ASSOCIATED PROTEIN TAU

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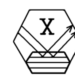
The Alzheimer's disease-associated protein tau is a typical representative of intrinsically disordered proteins (IDPs), existing as a conformational ensemble. Under physiological conditions, tau associates with microtubules and regulates their dynamics, whereas during the progression of neurodegeneration tau dissociates from microtubules, misfolds and creates highly insoluble deposits.

To obtain an insight into the atomic structure of tau, specific monoclonal antibodies can be used as surrogate tau protein binding partners to form complex crystals amenable to X-ray analysis [1]. Here we present the study with the monoclonal antibody Tau5, which has its epitope in the proline rich region of tau [2]. The Fab fragment of Tau5 has been crystallized alone and in complex with 30 amino acid long tau peptide Gly(201)-Arg(230) [3] and the structures were solved to the 1.69 Å resolution. 13 residues from the tau peptide can be modeled in the complex structure, but only some of them make contact with the antibody combining site. The structure of tau polypeptide reveals several important features for which only propensities were previously observed by NMR [4].



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Friday, March 15, Session VI

L20

INHIBITORS OF GLUTAMINYL CYCLASES AGAINST ALZHEIMER`S DISEASE

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N-terminal formation of pyroglutamate (pGlu) is catalyzed by glutaminyl cyclases (QCs) [1,2]. This form of post-translational modification is observed on numerous bioactive peptides. Formation of pGlu-amyloid has been also linked with Alzheimer`s disease [3-5]. Inhibitors of human QCs are currently the subject of intense development and testing [6,7].

We have currently solved crystal structures of two isoforms of QCs from *Drosophila melanogaster* [7]. Comparative study of structures of human, murine, and insect QCs from the PDB [8] allowed us reasonable evaluation of various binding modes of inhibitor PBD150. Structural observations are in concert with our inhibition studies.

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