Keywords: neutron macromolecule crystallography, specific labeling, proton

MS.57.3

Acta Cryst. (2008). A**64**, C101

Neutron macromolecular crystallography using the Laue diffractometer LADI-III

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At the Institut Laue-Langevin a new neutron Laue diffractometer (LADI-III) has been fully operational since March 2007. LADI-III is dedicated to neutron macromolecular crystallography at high-resolution (1.5 Å - 2.5 Å) and is used to study key hydrogen atoms and water structure in macromolecular structures. An improved detector design and readout system has been incorporated so that a miniaturized reading head located inside the drum scans the image-plate. From comparisons of neutron detection efficiency (DQE) with the original LADI-I instrument, the transferal of the image-plates and readout system internally provides a 2- to 3-fold gain in neutron detection, allowing data collection to higher resolution (~1.5 Å), using shorter exposure times and smaller crystal volumes. The improved neutron detector efficiency of LADI-III combined with the use of perdeuterated biological samples has enabled neutron macromolecular crystallography to become more accessible to the structural biology community, extending the size and complexity of systems that can be studied (~150 Å on cell edge) while lowering the sample volumes required (~0.1mm^3). Current projects aim to address questions concerning enzymatic mechanism, ligand-binding interactions, solvent effects, structure dynamics and their implications. Examples of recent project highlights from LADI-III will be presented.

Keywords: neutron macromolecular crystallography, perdeuteration, Laue

MS.57.4

Acta Cryst. (2008). A**64**, C101

Seeing hydrogens: X-ray limitations and possibilities at 0.9 Å and synergy with neutron diffraction

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Protons and proton transfer pathways play a critical role in many enzyme mechanisms. Direct information on proton (hydrogen) positions may be obtained from neutron crystallographic data. Despite advances in neutron sources and detectors, the application of neutron diffraction has remained limited to large crystals with small unit cell dimensions. This has resulted in general in a paucity of information about the proton delivery mechanisms that are central to our understanding of many enzyme mechanisms. In our attempt to understand the underlying mechanism of proton transfer, we have adopted the approach of obtaining X-ray data to subatomic (<1 Å) resolution. Electron density maps from structures determined to such resolution can reveal the positions of crucial hydrogens within the active sites and on proton pathways. Details of hydrogen location in green nitrite reductase from *Achromobacter cycloclastes* (0.90 Å) [1], human superoxide dismutase (0.88 Å) [2] and cytochrome c' (CYTcp) from *Alcaligenes xylosoxidans* will be presented. The last of these, CYTcp is able to discriminate between CO and NO by binding them on opposite faces of the heme. Recently, we have determined the X-ray crystal structure of oxidised and reduced CYTcp to 0.9 Å resolution and a detailed analysis of the structures is in progress. Crystals of CYTcp have been grown to a size of 3000x500x500 microns and neutron diffraction studies are in progress. These data will allow us to explore the synergy between the two approaches for this important system.

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Keywords: neutron crystallography, high-resolution X-ray crystallography, metalloproteins

MS.57.5

Acta Cryst. (2008). A**64**, C101

Locating hydrogen atoms in enzymes: A neutron structure of D-xylose isomerase with bound D-xylulose

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The transfer of hydrogen is important in many enzyme reaction mechanisms, and yet hydrogen is difficult to visualize in proteins using X-ray crystallography because of its low X-ray scattering power. We have exploited the recently developed time-of-flight neutron Laue technique to determine the location of hydrogen atoms during the reaction of an enzyme of economic importance, D-xylose isomerase (XI). Neutron diffraction studies of this deuterated enzyme with bound perdeuterated substrate show, unexpectedly, that the terminal O5 hydroxyl group of D-xylulose, the product, is not protonated but is H-bonded to doubly protonated His54 in a sugar hydroxyl-histidine-aspartate triad such as is found in trypsin. In addition, the metal ion-bound water molecule (which may be important in the enzyme mechanism) that was found in neutron diffraction studies of the native enzyme, is deprotonated in this XI-xylulose structure and exists as a hydroxyl group. Furthermore, lysine 289, which has only two protons on its terminal nitrogen atom (NZ) in the native enzyme neutron structure, has three in the XI-xylulose complex. This indicates that there is a positive charge on this amino group. These findings lead to further insight into the mode of action of this enzyme. Support from the National Institutes of Health (GM071939, CA06927, CA10925), the Office of Biological and Environmental Research of the Department of Energy, and Los Alamos National Laboratory (20070131ER and 20080001DR) is gratefully acknowledged.

Keywords: neutron structure analysis, enzyme structure function, isomerases