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What neutrons can do for you: The single crystal neutron diffractometer BIODIFF at the Heinz Maier-Leibnitz Zentrum (MLZ) and a short excursion to Small-angle neutron diffraction

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PURPOSE OF THE ABSTRACT

Neutrons are scattered from the nuclei and x-rays are scattered from the electrons of the atoms in a protein crystal. This renders these two scattering probes as being complementary to each other. The neutrons can see the hydrogen atom positions in a protein crystal. This allows to determine protonation states of crucial amino acid residues in the active centre of an enzyme or one can detect water clusters and proton paths to the active centre by locating water molecules and their exact orientation and hydrogen bonding.

Using the fact that hydrogen has a negative scattering length and deuterium has a positive one, proteins or DNA can be matched out at different solvent compositions of heavy and light water. This is heavily used in Small-angle neutron scattering where for example protein-DNA complexes can be disentangled in solution phase that way. In this contribution neutron protein crystallography is introduced using the example of alcohol dehydrogenase from the organism Lactobacillus brevis (LbADH), an enzyme which catalyzes the reduction of prochiral ketones to the corresponding secondary alcohols [1]. The data set for this project was taken with the instrument BIODIFF. The neutron single crystal diffractometer BIODIFF at the research reactor Heinz Maier-Leibnitz (FRM II) is especially designed to collect data from crystals with large unit cells. The main field of application is the structural analysis of proteins, especially the determination of hydrogen atom positions. BIODIFF is a joint project of the Jülich Centre for Neutron Science (JCNS) and the FRM II. BIODIFF is designed as a monochromatic instrument with a narrow wavelength spread of less than 3 %. To cover a large solid angle the main detector of BIODIFF consists of a neutron imaging plate in a cylindrical geometry with online read-out capability.

BIODFF is equipped with a standard Oxford Cryosystem “Cryostream 700+” which allows measurements at 100 K. But in case of the alcohol dehydrogenase project, room temperature data collection was chosen. Here, the crystal was kept in a glass capillary.

The resulting data led to a better understanding of the role of the Magensium ion in substrate binding and it showed a new hydrogen bonding network close to the active centre of the enzyme. It also showed nicely the complementary nature of x-ray and neutron protein crystallography. The metal ion in Figure 1 has not been detected by neutron scattering but it was easily seen by x-ray scattering. The reason for this lies in a cancellation effect between the negative scattering length of Manganese ions and the average positive scattering lengths of Magnesium ions which just cancel to zero in this position. The Magnesium ions were present in the crystallization condition, but the Manganese Ions must stem from the expression of the protein in the E.coli expression system.

Time permitting, I will show some results on protein crystal growth based on Small-angle neutron scattering data where radiation damage did not play a role [2]. So, the complete time and length scale from the monomeric protein molecules to the final protein crystals could be followed without disturbing the growth of the protein crystals with radiation damage which would not have been possible using SAXS measurements.

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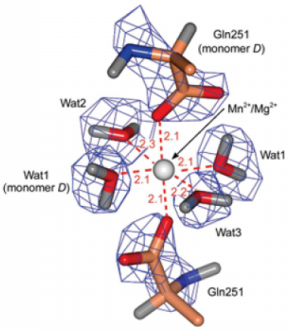
FIGURES



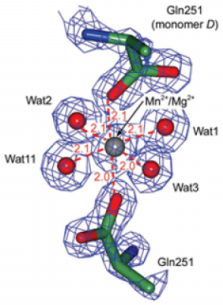


FIGURE 1

Figure 1

An neutron omit map of the involved amino acid residues from two copies of the protein Alcohol dehydrogenase and the water molecules. No density is observed for the central metal ion.

FIGURE 2

Figure 2

The same spot as in Figure 1 in the crystal as seen by x-rays: Here the metal atom is clearly observed. Figures taken from [1].

KEYWORDS

neutron | Small-angle | crystallography | scattering

BIBLIOGRAPHY

[1] Hermann et al., Acta Cryst. (2018). F74, 754

[2] Heigl et al., Cryst. Growth Des. 2018, 18, 1483