BIODIFF: First installations and detector concepts

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In collaboration between the Forschungszentrum Jülich (FZJ) and the Forschungsneutronenquelle Heinz Maier-Leibnitz (FRM II) a single crystal diffractometer optimised for crystals with large unit cells is being built. Its scientific focus will be the determination of the exact location and bonding properties of hydrogen atoms in protein or DNA crystals. With this information the hydration pattern of proteins can be studied, the protonation state of critical amino acid side chains can be determined and even dynamical information can be obtained by determining the hydrogen-deuterium exchange of the protein backbone. In order to achieve these results two detector concepts are foreseen: a) an image plate detector covering a large solid angle and b) a neutron scintillator imaged onto a CCD-camera providing a fast read out for a timely alignment of the crystals and additional detection abilities.

Crystallography is a powerful tool to determine the structure of biopolymers like DNA or proteins. Knowing the structure of proteins for example is a prerequisite for understanding their function. Often hydrogen atoms play a crucial role in the functioning of proteins, be it as liable protons in acidic side chains near the active core or as part of a water molecule involved in the catalytic reaction of the protein. Unfortunately, x-ray crystallography can only resolve the exact position of hydrogen atoms at very high resolutions which are not readily reached with protein crystals. Here, neutrons as probes for crystallography offer the advantage to make the hydrogen positions in a protein crystal accessible even at moderate resolutions. In particular neutrons allow distinguishing between hydrogen and deuterium. Thereby acidity and exposure of hydrogens in a protein may be classified. Figure 1 shows an example where neutron scattering gave important insights into the H/D exchange pattern in the protein myoglobin. The corresponding data set was recorded at the monochromatic BIX-3 beam line at the JRR-3M reactor at the JAEA - a beam line similar in design with the BioDiff instrument. The degree of hydrogen exchange of the protein backbone was determined for the 8 α-helices of myoglobin using a special refinement method. Obviously, the hydrogen bond between amino acid 67 and 71 is more susceptible to hydrogen/deuterium exchange than the H-bond between amino acid 72 and 76. This means that the packing of the protein is tighter or less flexible at the latter position.

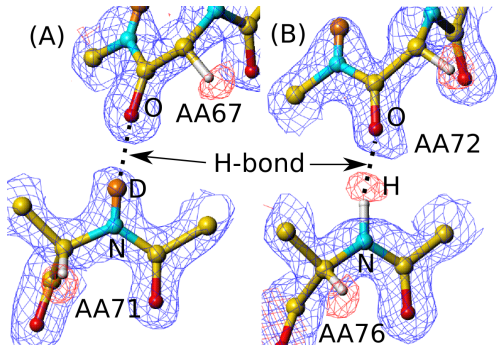

FIG. 1: Monitoring hydrogen/deuterium in the E-Helix of myoglobin. (A) Here, complete exchange of hydrogen atoms with deuterium is observed. This indicates a dynamical opening and reformation of the H-bond between amino acid 67 and amino acid 71.(B) Only very little exchange is seen in the H-bond between amino acid number 72 and 76 showing that this H-bond is not so much accessible to solvent molecules. From Ostermann, A. and Parak, F. G. in "Hydration- and Hydration-Sensitive Structural Biology", Nimura, N. et al. (Eds.) Tokyo, Japan (2005) pp. 111-122.

The BioDiff instrument (see Fig. 2) being built at the neutron guide NL1 in the neutron guide hall west of the FRM II is designed to perform neutron crystallographic experiments on protein crystals with large unit cells. The wavelengths of the monochromatic neutron beam can be adjusted to the size of the unit cell between 2.4 Å and 5.6 Å. A pyrolytic graphite crystal will be used to deflect the neutrons out of the neutron guide NL1. Higher order contaminations will be removed by a velocity selector.

Whereas the monochromator shielding has been already installed in the neutron guide hall (see Fig. 3 for a recent picture), the detector housing is still in its design phase. The requirements on this housing are fourfold:

- Proper shielding should be provided to the γ-radiation background from outside.
- One should be able to position both detectors accurately and reproducibly relative to the sample position.

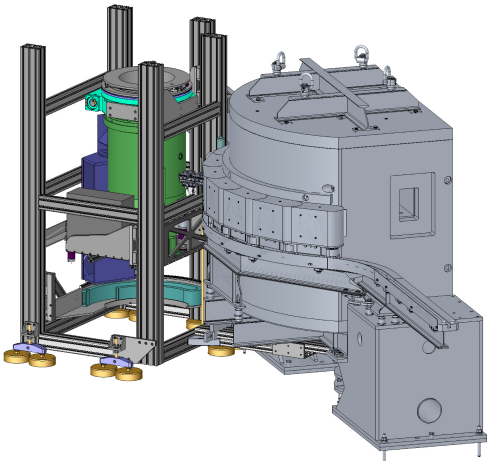

FIG. 2: Overview over the complete BioDiff instrument: monochromator shielding on the neutron guide NL1 at the FRM II (grey), image plate detector (green) and CCD-detector assembly (blue).

- The whole detector housing has to move when another neutron wavelength is desired.
- Enough space should be provided to accommodate the sample environment for the crystals under investigation.

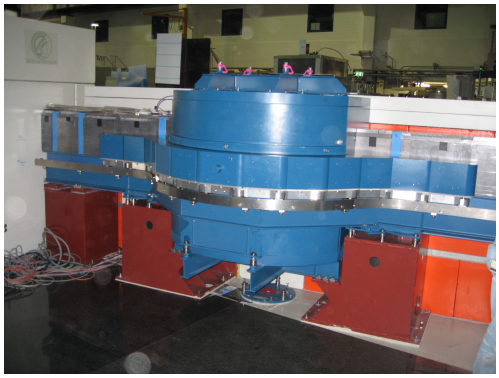

FIG. 3: The monochromator shielding as it was installed on the neutron guide NL1 in the neutron guide hall west of the FRM II in 2009. As the desired wavelength changes the monochromator crystal will be turned and with it the neutron beam and the whole detector unit moves. This requires the lead "curtain" γ-ray shielding to move with it.

The γ-radiation shielding is provided by 5 cm thick lead walls surrounding the detector housing (not shown in Fig. 2). The sample environment will enter the detector housing from the top, here only the goniometer for the sample environment is indicated in Fig. 2 (light blue). When changing to another neutron wavelength the whole detector housing will be lifted by pressurizing the air cushions. A friction wheel will drive it to the new position where the air pressure is then released again.

A sophisticated mechanical design has been developed to be able to switch between both detectors (see Fig. 4). The neutron image plate detector (depicted in green in Fig. 4) can be moved downwards to give way for the neutron scintillator. The converted light of the scintillator is bent by an aluminium mirror and imaged with a commercial objective onto the CCD-chip (blue assembly in Fig. 4). Special attention is paid to avoid any possibility for stray light to enter the CCD assembly.

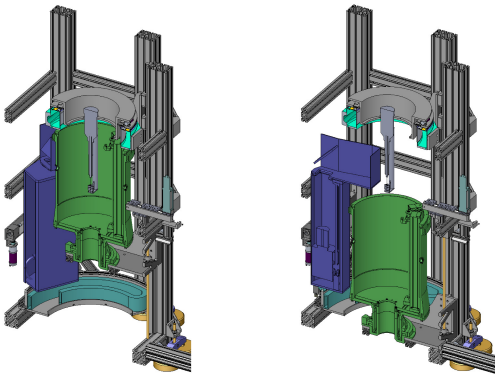

FIG. 4: Cut through the middle plane of the detector housing of the BioDiff instrument: (left) The image plate detector is positioned for recording data. (right) When the image plate detector is moved to the bottom, the CCD-detector assembly can be used to take data.

The scintillator of the CCD-assembly (dimensions: 20 cm by 20 cm) is closer to the sample location (10 cm distance) as the neutron image plate (20 cm radius). Additionally it can be dislocated in height by 10 cm upwards and downwards relative to the neutron beam. Furthermore, it can be swung around up to an angle of 113° with respect to the neutron beam.

The neutron image plate detector provided by Maatel (Voreppe, France) will have a software selectable spatial resolution between 125 μm and 500 μm. It covers a solid angle of approximately 2π. The spatial resolution of the CCD-assembly is limited by the thickness of the scintillator which smears out the recorded Bragg reflexes to about 300 μm. A solid angle of ca. 0.7π can be covered at once by the CCD-assembly and 2π is accessible in total.