

BEAMLINES

X17B1, X6A

PUBLICATION

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Anomalous Diffraction at Ultra High Energy for Macromolecular Crystallography

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Ultra high-energy x-rays were employed for the first time in a macromolecular crystallography experiment. High-resolution diffraction data recorded on frozen samples allowed for the determination of the crystal structure of a model protein. Phases were determined at high resolution using various methods such as Multiple-wavelength Anomalous Diffraction (MAD), Single-wavelength Anomalous Diffraction (SAD) and Single Isomorphous Replacement with Anomalous Scattering (SIRAS). 1.25 Å diffraction resolution was achieved for the best sample. No radiation damage was observed from the experimental data an indication that at ultra high-energy x-ray radiation damage is minimized as the mass-energy absorption coefficient is much smaller therefore resulting in a lower energy dose absorbed by the sample.

Radiation damage to biological crystals is a major challenge in the determination of the crystal structure of macromolecules at conventional x-ray energies. At 12 keV, the dominance of the photoelectric effect leads to energy deposition that causes premature radiation-induced death of the crystals. Typical sample lifetimes can be extended somewhat with the use of cryogenic temperatures, but it has been shown that it will be insufficient for third-generation, synchrotron-based beamlines. The use of higher energy x-rays, advocated by Helliwell and Fourme (Helliwell and Fourme, 1983), lowers the risk of radiation damage due to the lower photoelectric effect cross section while higher scattering cross section can be preserved by the use of heavy elements.

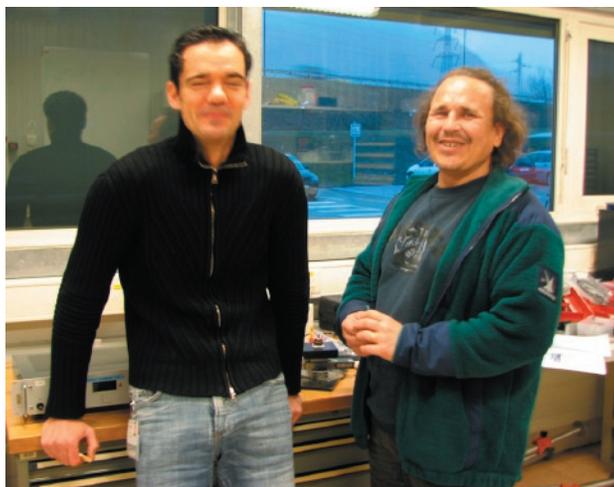
The purpose of the experiments pursued on X17B at the NSLS and ID15B at the ESRF, two beamlines dedicated to material sci-

ences, was to exploit the use of ultra high-energy x-rays, in the 55 keV range, for the crystal structure determination of macromolecules. Only a couple of experiments so far have been performed in the 35 keV range, mainly because this is the limit in the achievable energy for macromolecular crystallography beamlines at synchrotron facilities.

The data collected on a chicken lysozyme sample co-crystallized

with holmium allowed us to explore a large variety of phasing methods (**Figure 1**). A Bijvoet-difference Fourier map calculated with the high-energy remote data (SAD) approximately 1 keV below the holmium K edge (at 56.5 keV) is shown in **Figure 2**. The map, contoured at 11 σ level, clearly indicates three occupied sites. The Bijvoet ratio, calculated with the refined occupancies is 2.5 %, indicating the potential use for holmium as one of the standard heavy atoms for ultra high-energy measurements. The quality of the phases after density improvement (**Figure 1**) allowed us to automatically build the totality of the molecule for all the phasing methods tested.

Additional data collected at ID15B at the high-energy remote, 56.5 keV, corresponding to a total cumulated exposure of 93 min and an estimated cumulated dose of 1.7 x 10⁶ J.kg⁻¹, was compared to data collected on NSLS



Marco Di Michiel (left) and Veijo Honkimaki

beamline X6A at 12keV (total cumulated exposure 93 min and estimated dose $7 \times 10^6 \text{ J.kg}^{-1}$). Crystals grown under the same conditions, with similar sizes presenting the same diffracting resolution, 1.3

Å, exposed to 12keV, showed the onset of radiation damage after a cumulated exposure of 18 min, while no radiation damage was observed in the data collected at ultra high-energy x-ray for the to-

tal cumulated exposure. In conclusion, high- and ultra high-energy can be efficiently used to determine a crystal structure while minimizing radiation damage effects.

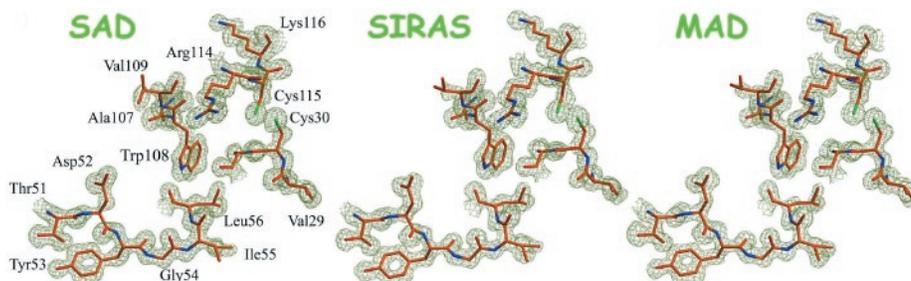


Figure 1. Experimental electron density maps after density improvement (before model building and refinement). All 3 maps are contoured at 1σ level and calculated around the same region, the residues indicated in the left panel.

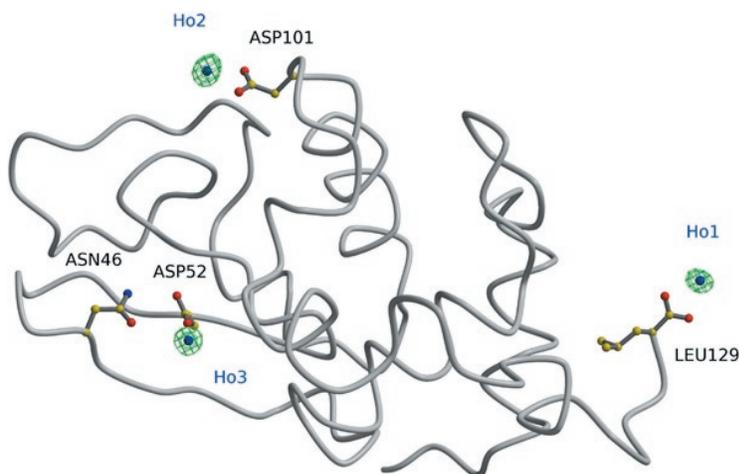


Figure 2. Bijvoet-difference Fourier map. The map is contoured at the 11σ level and calculated with Bijvoet pairs from the high-energy remote (56.5 keV) data collected on X17B1.