Contribution ID : **28** Type : **not specified**

Current and future capabilities for serial and time resolved crystallography at Diamond microfocus beamline VMXi

Monday, 22 September 2025 17:00 (15)

There is increasing interest in obtaining room temperature, time resolved crystal structures of proteins carrying out their biological functions. The transition between conventional cryogenic macromolecular crystallography and serial crystallography involving microcrystals remains challenging for many projects. We have recently demonstrated the capability to measure good quality, low dose serial crystallography data from microcrystals within crystallisation plates [1]. This capability is available in the standard operation mode of the beamline and does not require any specific serial crystallography apparatus. In this approach, microcrystals are transferred into a crystallisation plate (typically 100 nL per drop) and each droplet is subjected to raster scanning with a still diffraction image measured every 10 μm. The resulting images are processed using standard serial data processing software such as xia2.multiplex. This approach enables straightforward structure determination and analysis of crystal quality and unit cell parameters from non-optimised crystallisation conditions, guiding users in their optimisation efforts. Very small quantities of protein are required, and the determination of a human peroxidase structure to 1.88 Angstrom resolution using only 1.2 μL microcrystal suspension.

Several approaches to sample delivery for time resolved crystallography have been developed including droplet-on-demand tape drive-based systems developed for XFEL experiments that have been combined with X-ray emission spectroscopy (XES) to monitor the redox and spin state of metal -containing cofactors within the proteins [2]. However, currently available systems require a large quantity of microcrystal sample as well as requiring multiple skilled staff to operate. A new system for serial crystallography at VMXi is currently under development. This incorporates a picolitre droplet-on-demand tape drive system capable of anaerobic operation together with an XES von Hamos spectrometer to enable spectroscopic validation in time resolved experiments of metalloproteins. A compact design was required due to the tight spatial constraints of the VMXi end station that was built for highly automated data collection from crystallisation plates, and the design incorporates automation to reduce the number of personnel required to more closely approach a typical synchrotron experiment.

Proof of concept data obtained during the development process of the tape drive and XES spectrometer will be presented, including a high-resolution protein structure determined using the tape drive system and XES data obtained from microcrystals of the copper enzyme nitrite reductase.

[1] A.J. Thompson, J. Sanchez-Weatherby, L.J. Williams, H. Mikolajek, J. Sandy, J.A.R. Worrall and M.A. Hough (2024) Efficient in situ screening of and data collection from microcrystals in crystallization plates Acta Cryst.D80, 279-288

[2] Butyrin, A. et al (2021) An on-demand, drop-on-drop method for studying enzyme catalysis by serial crystallography. Nature Methods 12, 4461.

Authors:

Juan Sanchez Weatherby, Pierre Aller, Amy Thompson, Abby Telfer, John Sutter, James Sandy, Halina Mikolajek, Matthew Rodrigues, Mike Hough, Allen Orville

Presenter(s) : SANCHEZ WEATHERBY, Juan (Diamond Light Source)

Session Classification : Monday