

Routine room temperature protein structure determination in situ at Diamond beamline VMXi: current status and recent developments

The need for rapid turnaround times from the appearance of protein crystals to the determination of high quality crystal structures has driven many aspects of automation. Moreover, it is increasingly recognised that room temperature structures allow access to protein dynamics, may reveal ligand binding modes not apparent at cryogenic temperatures and allow for time resolved structures to be determined. We present the current pipeline of the Crystallisation Facility at Harwell and the Micro Focus, pink beam Diamond Beamline VMXi [1] and the capability of the pipeline to produce high quality structures at room temperature from small numbers of crystals in situ, for example within crystallisation plates. Protein samples in solution are provided by users, with automated crystallisation and data collection offering datasets and structure solution in many cases within days. In situ data collection allows for rapid feedback on crystallisation conditions for further optimisation and is particularly suitable for crystals challenging to cryo-protect or that are not robust to mechanical handling or other stresses. Operation is typically completely remote with data measured using a queueing system, providing convenience for the scientist. Recent developments in sample delivery, crystal identification and multi-crystal data analysis [2] will also be described. Finally, we will present recent developments in combining serial crystallography using a tape drive sample delivery method (with drop on drop mixing) and X-ray emission spectroscopy at VMXi.

[1] VMXi: a fully automated, fully remote, high-flux in situ macromolecular crystallography beamline (2019) Sanchez-Weatherby, J., Sandy, J., Mikolajek, H., Lobley, C., Mazzorana, M., Kelly, J., Preece, G., Littlewood, R. and Sørensen, T.L.M. *Journal of Synchrotron Radiation*. 26, 291- 301.

[2] xia2. multiplex: a multi-crystal data-analysis pipeline (2022) Gildea. R.J., et al. *Acta Cryst. D*78, 752-769.

Primary authors: HOUGH, Mike (Diamond Light Source); Dr THOMPSON, Amy (Diamond light source); Dr PFALZGRAF, Hans (Diamond light source); Dr GILDEA, Richard (Diamond light source); Dr MIKOLAJEK, Halina (Diamond light source); Dr SANDY, James (Diamond light source); Dr SANCHEZ-WEATHERBY, Juan (Diamond light source)

Presenter: HOUGH, Mike (Diamond Light Source)