MS03-O4

Solving the phase problem with long wavelength X-ray diffraction

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Harnessing the anomalous signal natively present in proteins, to solve their crystal structures, requires an experimental setup optimised for the tender X-ray regime. Macromolecular crystallography experiments on the long-wavelength beamline I23 at Diamond Light Source, UK, target the absorption edges of anomalously scattering atoms in proteins by collecting diffraction data in a high vacuum environment. This unique experimental setup enables a multitude of phasing opportunities, including Sulphur/Phosphorus single wavelength anomalous dispersion (S/P-SAD), and also by accessing the absorption edges of metal ions commonly bound by proteins. Examples will be shown of structures solved on the beamline based on the anomalous signal from Sulphur, Iodine, Cadmium, Potassium, Calcium, Gold, Vanadium, among others. It will also be shown how the combination of a vacuum environment and a large curved Pilatus 12M detector yields superior signal-to-noise diffraction data, leading to sub-atomic resolution characterisation of certain structural targets.

References:

A. Wagner, R. Duman, K. Henderson, V. Mykhaylyk In-vacuum long-wavelength macromolecular crystallography Acta Cryst. D72 (2016) 430-439.

Aurelius, O., et al. (2017). "Long-wavelength macromolecular crystallography – First successful native SAD experiment close to the sulfur edge." Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 411: 12-16.

Austin, H. P., et al. (2018). "Characterization and engineering of a plastic-degrading aromatic polyesterase." Proceedings of the National Academy of Sciences.

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MS03-O5

Sample triggering and delivery for time-resolved studies of proteins

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Dynamically resolved, or better known time-resolved, structural studies of proteins rely on specific sample environments at both synchrotrons and XFELs due to two main requirements: the continuous delivery of fresh sample to the X-ray beam and the need to trigger the protein activity. The two most widely used methods for protein triggering are the diffusion of actuators by rapid mixing and light activation using laser pulses. Within collaborative efforts between the Trebbin group and other groups at the University of Hamburg, DESY and ESRF, we have developed sample environments and methods for these complex studies. Specifically, we have recently performed a light-triggered solution scattering experiment employing photocaging to follow a protein dimerisation event at the white-beam station ID09 (ESRF). We have also developed different X-ray compatible devices for serial synchrotron crystallography (SSX) data collection in flow with mixing for which I will present an example using 3D-printed microfluidic devices at beam line ID303-A (ESRF). These versatile methods can be tailored to the requirements of the protein target characteristics such as time scales of reaction and sample form (solution or microcrystalline slurry). Therefore, the developing general and user-friendly sample environments is an important step in making these experiments more widely available.

Keywords: time-resolved, microfluidics, photocaging