**Oral presentation**

# Fixed-target time-resolved serial crystallography at a synchrotron and XFEL using photocages

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Time-resolved X-ray crystallography using the serial approach has received increased attention over the past years at synchrotron and XFEL sources worldwide. Developments in instrumentation, data collection strategies and data processing have lowered the entry barrier to a wider user community seeking insight to a wide range of biological targets. Existing methods have effectively been used to initiate reactions in naturally occurring light sensitive proteins or mixing methods have been applied to micro-crystalline slurries for observing the evolution of ligand binding in active sites. Despite these significant advances, there are remaining open questions: how can we initiate time-dependent processes where neither of the above approaches can be applied? How can we handle targets sensitive to environmental conditions, such as the presence/absence of oxygen?

A widely applicable approach to effectively initiate time-dependent reactions is the use of photocaged substrates which can be pre-mixed and soaked into the micro-crystals. Subsequently, a laser pulse can be used for releasing the caged compound providing near-uniform reaction initiation. Here, we will present the use of photocages with myoglobin, a challenging exemplar gas-binding enzyme. Serial diffraction data at ambient temperature were collected using silicon chips on a fixed-target setup both at beamline I24 [1] (Diamond Light Source) and at SACLA XFEL [2] (Japan). We will present recent developments on the optimisation of fixed-targets for anaerobic data collection, showcasing myoglobin as an oxygen sensitive enzyme. From sample preparation under anaerobic conditions to data collection, we have optimised the steps required to obtain oxygen-free static structures and to observe snapshots of photocaged gas release.

[1] Owen, R. L., Axford, D., Sherrell, D. A., Kuo, A., Ernst, O. P., Schulz, E. C., Miller, R. J. D. & Mueller-Werkmeister, H. M. (2017). *Acta Cryst. D,* **73**, 373-378.

[2] Sherrell, D. A., Foster, A. J., Hudson, L., Nutter, B., O'Hea, J., Nelson, S., Paré-Labrosse, O., Oghbaey, S., Miller, R. J. D. & Owen, R. L. (2015). *J. Synchrotron.Radiat.*, **22**, 1372-1378.