Improvements to Time-Resolved Structural Study using Mix- and-Quench Crystallography

John A Indergaard[1], Dr. Matthew McLeod[1], Ash Mahmood[1], Dr. Robert Thorne[1]
[1]*Cornell University*
jai55@cornell.edu

Time-resolved crystallography (TRX) is an emerging technique that allows the observation of proteins in action. During sample preparation for TRX a reaction is initiated *in crystallo* and, after some well-defined time delay, diffraction data is collected to observe the protein structure at that given time point with the hopes of seeing chemical intermediate states in the reaction pathway. Current time-resolved methods tend to require either complex setups at the synchrotron or XFEL beamline, or to yield inadequate time resolution. We recently developed a new and simplified TRX sample preparation method – millisecond mix-and-quench crystallography (MMQX)[1]. In this approach, the reaction is initiated by plunging the sample through a substrate-containing film and into liquid nitrogen. The travel time between film contact and entry into the liquid nitrogen determines the reaction time point and optimized cooling apparatus allows quenching in <2 ms and capture of intermediate states. X-ray data can then be collected remotely on any standard cryocrystallography beamline and far more data can be collected per crystal. Here we describe significant improvements to this method that improve application of substrate solution, allow use of samples on standard ALS or SPINE-compatible goniometer bases, and that should yield time resolutions of ~10 ms. This very simple approach with its very parsimonious use of crystals should allow time-resolved crystallography to become a mainstream technique applicable to probing structure-function relationships in a broad range of biological targets.

References

Clinger, J. A., Moreau, D. W., McLeod, M. J., Holyoak, T. & Thorne, R. E. (2021). IUCrJ, 8, 784–792