Time-resolved Serial Synchrotron Crystallography: an efficient interlacing system enables milliseconds to seconds time delays

Pedram Mehrabi,[1] Eike C. Schulz, Henrike M. Müller-Werkmeister, Friedjof Tellkamp, William Stuart, Elke Persch, Raoul De Gasparo, Francois Diederich, Emil F. Pai & R.J. Dwayne Miller

[1] Max Planck Institute for the Structure and Dynamics of Matter
pedram.mehrabi@mpsd.mpg.de

Time-resolved crystallography has recently seen a resurgence with the advent of very bright X-ray sources. However, canonical methods for time-resolved studies of enzymatic reactions are often impaired by difficulties of acquiring enough time points to span the entire reaction coordinate pathway. To examine chemically triggered reactions, we used fluoroacetate dehalogenase as a model system. In a proof-of-principle approach we combined an established fixed target mount (chip), with a novel "*interlacing"* approach for (?) data acquisition. The interlacing approach allows for the acquisition of both a dark and light image for every crystal at a wide range of time points that go into the minutes range to enable capturing of the entire reaction pathway of even the slowest systems.

By these means one can efficiently collect almost 100k images per hour and hence to acquire several timepoints ranging from ms to many seconds within a single beamtime. Near homogenous reaction initiation was achieved by photolytic cleavage via a femtosecond laser pulse of caged fluoroacetate (2(4-hydroxyphenyl)-2-oxoethyl fluoroacetate). Data collection was performed at both the P11 and P14 beamlines at the PETRA III synchrotron on sub-25-micron crystals. Multiple time points were collected spanning a range from milliseconds to several seconds producing well-resolved electron density maps ranging from 1.7-2 Å in resolution. Preliminary results showed significant structural changes in both dynamic fluctuations of the cap domain as well as in the active site, supporting previous results obtained by a mutant trapping approach. This newly developed method is highly flexible and can be used on numerous different enzymatic systems and can be adapted for use at XFELs as well as synchrotrons with bright microfocus beamlines.