MS07-03 | Watching an Enzyme at Work: Breaking the Strongest Single Bond in Organic Chemistry

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The functional characterization of biomolecular catalysis requires a correlation of the three-dimensional structural ensemble with time-dependent changes. The advent of serial femtosecond time-resolved crystallography (TR-SFX) has provided insight into ultrafast time-scales of biomolecular function. This renaissance triggered the emergence of time-resolved serial synchrotron crystallography (TR-SSX), which simplifies accessing prevalent biological time-scales (> ns). To this end we have applied the recently developed the *hit-and return* (HARE)[1] approach which allows to collect several time points along the reaction coordinate of an enzyme during a single synchrotron beamtime. This contrasts conventional methods, which require several hours up to several beamtimes per time-point. We used the HARE approach to capture 18 time points from 30 milliseconds to 30 seconds during the non-reversible turnover cycle of fluoroacetate dehalogenase (FAcD). These time points include all key states involved in enzymatic C-F bond cleavage: substrate binding and reorientation, covalent-intermediate formation, location of the water nucleophile and product release. In total four substrate turnovers can be observed between the two subunits, which are highly coupled but display different conformations. Reactivity is coupled to molecular breathing, expressed by dynamic changes in lateral FAcD dimensions and modulation in water content pointing at an allosteric communication pathway between the two subunits. These results demonstrate the excellent suitability of TR-SSX to unravel biomolecular catalysis and provide key insights into protein dynamics.

[1]Schulz, E. C., Mehrabi P., Müller-Werkmeister H. *et al.* The hit-and-return system enables efficient time-resolved serial synchrotron crystallography. *Nature Method*s, 2018, doi:10.1038/s41592-018-0180-2