Investigating Photoreception, and Bio-catalysis using Time-resolved Serial Femtosecond Crystallography

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Time-resolved serial femtosecond crystallography (TR-SFX) plays an important role in the understanding of structure-function relationships. With TR-SFX, protein structure and dynamics can be determined in real-time and at the atomic length scale. For a TR-SFX experiment, a reaction must be triggered inside protein crystals using various methods such as light activation, diffusion of a substrate, temperature jump, etc. Subsequent changes in the structure are probed by X-ray pulses in a time-resolved fashion. These time-resolved structural changes are used to gain insight into the mechanism of how proteins work.

X-ray free electron lasers (XFELs) are exceptionally well suited for these experiments. XFELs produce ultra-short, femtosecond X-ray pulses with more than 10^12 photons per pulse. Hence, they allow femtosecond time resolution. A single exposure by one of these pulses damages the crystals. However, since the X-ray pulses are extremely short, the diffraction patterns are recorded right before the crystals are damaged. This is called the "diffraction before destruction" principle. This allows the collection of high-quality/high-resolution X-ray data from µm and sub-µm sized crystals.

In this talk, I will present and discuss results from pioneering time-resolved studies on two classes of proteins: (i) an enzyme produced by M. tuberculosis called ß-lactamase (Kupitz et al. 2017, Olmos et al. 2018, and Pandey et al. 2021), and (ii) a light-sensing protein called photoactive yellow protein (Pandey et al, 2020).

• S. Pandey et al. "Observation of Substrate Diffusion and Ligand Binding in Enzyme Crystals using High Repetition Rate Mix-and-Inject Serial Crystallography", (Under Review, Nature Chemistry)

• S. Pandey et al. "Time Resolved Serial Femtosecond Crystallography at the EuXFEL", Nat Methods 17,73-78 (2020)

• J.L. Olmos, S. Pandey, J.M. Garcia et al. "Enzyme Intermediates Captured 'on-the-fly' by Mix-and-Inject Serial Crystallography", BMC Biology 16:59 (2018)

• C. Kupitz et al. "Structural Enzymology Using X-ray Free Electron Lasers", Structural Dynamics 4, 044003 (2017,