# Towards Structure Based Enzymology at an X-ray Free Electron Laser

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Time-resolved protein crystallography relies on the uniform initiation of reaction across the protein crystal. There are various examples of optical triggering as a means of reaction initiation in crystals; however, many proteins, more specifically, enzymes, are not intrinsically susceptible to reaction initiation by this method. As a proof-of-concept, this work aims to expand the types of proteins that can be investigated in a time-resolved manner by diffusion and rapid mixing. Here, initial results towards serial time-resolved crystallography experiments of β-lactamase at an X-ray Free Electron Laser (XFEL) in a mix-and-inject approach are presented. The enzymatic target being investigated, β-lactamase, is an enzyme from *Mycobacterium tuberculosis* that confers antibiotic resistance for a broad spectrum of antibiotics to the bacterium. It achieves this through hydrolysis of the lactam ring. By using relatively small crystals and rapidly mixing ceftriaxone antibiotic substrate, we aim to probe the crystals with an XFEL to collect structural reaction intermediates and obtain information about the dynamics and kinetics of the molecule undergoing catalysis. In a widely collaborative effort involving the BioXFEL science and technology center, these results show promise towards structure-based enzymology using an XFEL. We hope that our work will establish the use of diffusion and a mix-and-inject approach for optimized time-resolved studies of a broad variety of interesting enzymes.