Capturing Enzymatic Intermediates via Mix-and-Inject Serial Crystallography

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The visualization of protein motions is one of the many exciting, cutting-edge uses of an X-ray free electron laser. While much work has been done towards understanding protein motions of photoactive proteins using an X-ray free electron laser, the ability to probe enzyme catalysis in non-photoactive proteins remains a challenge. Here, preliminary results are presented towards the visualization of enzymatic intermediates of beta-lactamase, an enzyme that confers antibiotic resistance to the organism *Mycobacterium tuberculosis*. An antibiotic substrate is mixed in high concentrations with micro-crystals in a microfluidic device, causing reaction initiation to occur as the substrate diffuses across the crystal. Data is then collected downstream from the mixing device at the X-ray interaction zone. The ultimate goal of this work is to generate multiple structures of the reaction from start to finish, in order to make a molecular movie of the enzyme in action. Understanding the motions an enzyme undergoes in catalysis and the time scales on which these occur has large implications in many fields, including protein engineering and drug discovery. As a proof-of-principle experiment, successfully using a mix-and-inject approach would open the doors to a broad spectrum of enzymes that could be investigated provided that the crystal sample is amenable to enzyme activation through substrate diffusion.

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