

Development of an Alternative Approach to Time-Resolved X-ray Crystallography

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Development of X-ray free electron laser sources (XFELs) and methods for serial crystallography have driven major advances in time-resolved (TR) protein crystallography. TR-crystallography is a wonderful tool for understanding protein dynamics and catalysis, allowing much greater understanding of structural motions and intermediate states in proteins. Early TR work was largely limited to proteins whose conformational changes could be triggered optically using an intrinsic chromophore, and whose motions were reversible in the crystal, so that a single crystal could be pumped and reset multiple times to generate the diffraction data. Serial crystallography using microcrystals has enabled non-reversible motions to be observed by utilizing large amounts of sample and getting one diffraction image per crystal. These experiments wouldn't have been possible without the extraordinary brightness of XFELs and the newest generation of synchrotron sources. More recently, serial crystallography has incorporated chemical triggering via diffusion on millisecond timescales, short enough to reveal biologically important intermediate states. However, the barrier to entry for current optically or chemically triggered TR crystallography techniques at XFELs and synchrotrons is high. Very large numbers of similar size and morphology crystals must be generated. Optical excitation and/or crystal mixing and delivery systems are complex and must be integrated into the beamline. Fine tuning for a given protein crystal system and efficient serial data collection using these methods requires multiple collaborators, knowledgeable beamline staff, and often large amounts of instrument time at the very few available beamlines suited to these experiments. This complexity puts TR crystallography beyond the reach of most investigators in the wider structural biology community. Alternative methods are needed to allow TR crystallography to be exploited fully by the field. Ideally, these methods should require far fewer crystals, allow data collection from standard MX beamlines without need for special sample delivery apparatus, and allow multiple routes to reaction initiation. We have been developing an alternative method to time-resolved serial crystallography that decouples the sample preparation and reaction evolution times from the data collection time, allows both optical and chemical triggering, and requires only remote data collection at standard synchrotron beamlines. Here we present preliminary data for our new TR-crystallography technique.