Developing Temperature-Jump X-ray Crystallography to Study Dynamic Biosynthetic Enzymes at Synchrotrons and XFELs

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Molecular motions underlie many important biochemical functions, such as enzyme catalysis, but these motions are challenging to study because they involve distances of less than a nanometer, and timescales ranging from picoseconds to milliseconds. Modern X-ray light sources, including synchrotrons and X-ray free electron lasers (XFELs) built and maintained by the DOE, offer new opportunities to study molecular motion with atomic resolution imaging. These instruments produce ultrafast, high-brilliance X-ray pulses that act as a "molecular high-speed camera," enabling the observation of molecular motions in real time using a technique known as time-resolved crystallography. A major caveat to these experiments is that the measured samples contain billions of individual molecules, and to study the motions of interest, the molecules must be synchronized, which is not a trivial task. A variety of rapid perturbations have been explored for this purpose. To make time-resolved crystallography a general tool that can be applied to study the dynamics of any protein of interest, researchers are exploiting the inextricable link between temperature and molecular motion. Specifically, the team is developing the use of infrared laser-induced temperature-jump (T-jump), wherein the molecules of interest are rapidly heated with a nanosecond pulsed infrared laser to stimulate and synchronize their motions for time-resolved imaging. In this proposal, the team describes a collaborative effort to pilot the use of new hardware and software for imaging protein motions across DOE structural biology facilities at SLAC National Laboratory. Because temperature is a universal means by which to perturb protein motions, researchers expect the methods developed will be broadly applicable to the study of many different biochemical systems, and the tools developed will be made available to the large user base of these facilities. To demonstrate the power of these new time-resolved molecular imaging methods that utilize T-jump, the team will study lipoxygenase enzymes that are found in plants and animals, which catalyze important biosynthetic reactions that also have broad industrial applications. The catalytic function of lipoxygenase enzymes is hypothesized to be linked to a specific set of molecular motions within the enzyme. Researchers will test this hypothesis by using T-jump to map molecular motions in several lipoxygenase variants with different catalytic properties. This comparative analysis will lend insight into which of the observed motions are functional in catalysis. Experiments performed at both synchrotron (SSRL) and XFEL (LCLS) facilities will allow us to observe molecular motions across broad timescales ranging from nanoseconds to milliseconds. This work will help shed light on the fundamental relationship between molecular motions and catalytic function in enzymes. This knowledge will inform the future engineering of artificial enzymes with designer catalytic properties that can act as environmentally friendly chemical reagents for industry.