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EXPLORING PROTEIN STRUCTURAL DYNAMICS WITH TIME-RESOLVED X-RAY DIFFRACTION IN BOTH CRYSTALLINE AND SOLUTION PHASE

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Determining 3-dimensional intermediate structures during the biological action of proteins in real time under ambient conditions is essential for understanding how proteins function. Here we show two examples where this goal has been achieved. In the first example, we use time-resolved Laue crystallography to extract short-lived intermediate structures and thereby unveil signal transduction in the blue light photoreceptor photoactive yellow protein (PYP) from *Halorhodospira halophila*. By analyzing a comprehensive set of Laue data during the PYP photocycle (forty-seven time points from one nanosecond to one second), we track all atoms in PYP during its photocycle and directly observe how absorption of a blue light photon by its p-coumaric acid chromophore triggers a reversible photocycle. We identify a complex chemical mechanism characterized by five distinct structural intermediates. Structural changes at the chromophore in the early, red-shifted intermediates are transduced to the exterior of the proteinin the late, blue-shifted intermediates through an initial "volume-conserving" isomerization of the chromophore and the progressive disruption of hydrogen bonds between the chromophore and its surrounding binding pocket. In the second example, quaternary structural changes in hemolgobin was captured in solution phase by time-resolved x-ray diffraction. Contrary to previous spectroscopic measurements, the quaternary structural change occurs as early as 316 ns accoring to our preliminary results.

(This research is supported by grants from KRF and KOSEF)