Watching proteins function in real time via picosecond X-ray diffraction

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To understand how a protein functions, it is crucial to know the time-ordered sequence of structural changes associated with its function. To that end, we have developed numerous experimental techniques for characterizing structural changes in proteins over time scales ranging from femtoseconds to seconds. This talk will focus primarily on time-resolved X-ray studies performed on the BioCARS beamline at the Advanced Photon Source, which allowed us to characterize structural changes in proteins with 150-ps time resolution. We have used this capability to track the reversible photocycle of photoactive yellow protein following trans-to-cis photoisomerization of its p-coumaric acid (pCA) chromophore. Briefly, a picosecond laser pulse photoexcites pCA and triggers a structural change in the protein, which is probed with a suitably delayed picosecond X-ray pulse. When the protein is studied in a crystalline state, this “pump-probe” approach recovers time-resolved diffraction "snapshots" whose corresponding electron density maps can be stitched together into a real-time movie of the structural changes that ensue [1]. However, the actual signaling state is not accessible in the crystalline state due to crystal packing constraints. This state is accessible in time-resolved small- and wide-angle X-ray scattering studies, which probe changes in the size, shape, and structure of the protein [2,3]. These studies help provide a framework for understanding protein function, and for assessing and validating theoretical/computational approaches in protein biophysics [4]. This research was supported in part by the Intramural Research Program of the NIH, NIDDK.

References:


