MX-141. Early intermediates of the PYP photocycle studied by ~100 ps time-resolved X-ray Laue diffraction

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Picosecond time-resolved Laue diffraction experiments were conducted on single crystals of the bacterial blue light photoreceptor, photoactive yellow protein (PYP), at the ID09B beamline during 4-bunch mode in December 2003. A total of 12 shifts were allocated to this project, which, for the first time, produced a ~100-ps time-resolved difference signal from photoactivated PYP.

In all previous picosecond time-resolved diffraction studies of PYP, photoactivation was triggered with a ~100 femtosecond optical pulse, but, no difference signal was observed. Time-resolved optical studies of PYP in protein crystals suggested that a stretched optical pulse would enhance the yield of the photogenerated PYP intermediates. The key experimental advance in this run was the implementation of a pulse stretcher to stretch femtosecond optical pulses to the picosecond regime. The pulses were first stretched by passing the femtosecond optical pulse through a pair of Brewster-cut 15-cm fused silica rods, with additional stretching achieved by passing through 3 m of 200 um core multimode optical fiber. In the first excitation scheme, 485 nm pulses were passed through the fused silica rods to broaden the femtosecond pulses to about 2 ps. Due to the high peak power achieved in the optical fiber, stimulated Raman scattering from 485 nm was severe, and the wavelength emerging from the fiber was significantly red-shifted, broadened, and unusable for exciting PYP. Consequently, when the laser was tuned to 485 nm, pulse stretching was accomplished solely by the fused silica rods. When the femtosecond laser was tuned to 400 nm, the stimulated Raman effect in the fiber was less severe, and a 418 nm pulse emerged from the fiber; the pulse duration was estimated to be of the order of 100 ps. Data sets at four time delays (-10 μs, 100 ps, 1 ns, 10 μs) with the two different excitation schemes were obtained and processed separately with the program suite *LaueView*.

Data collected using the first excitation scheme (485 nm, ~ 2 ps), showed no difference signal. However, data collected using the second excitation scheme (418 nm, ~ 100 ps) produced difference signals. Whereas data acquired at 100 ps time delay shows no difference signal, the 1ns and 10us time delays show noticeable signal. It is conceivable that time-zero shifted from where it was set prior to the data acquisition, so the 100-ps time delay might actually correspond to a negative time point. Observing a significant signal at 1 ns and 10 μs with ~ 100 ps, 418-nm excitation is important for the

following reasons. A) This is the first experimental result using sub-nanosecond excitation that showed a substantial difference signal. B) This is the first time that a difference signal has been observed when the PYP crystal was excited on the blue side of its absorption peak. C) Our success in launching a photocycle with light on the blue side of the PYP absorbance is contrary to predictions made by other spectroscopists (those predictions were based on spectroscopic measurements on the gas-phase chromophore). D) The laser-driven streakiness of the diffraction spots was minimal (with ns excitation, significant difference signals were always accompanied by spot streakiness).

In summary, when PYP was photoactivated with pulses stretched to ca. 100 ps significant difference signals were observed. This success paves the way for future studies where we will acquire sub-ns time-resolved diffraction data from PYP over a broader range of time delays, with an aim to characterize in detail the structure and dynamics of the primary intermediate in the PYP photocycle.