| | **Experiment title:** Early intermediates of the PYP photocycle studied by picosecond time-resolved X-ray Laue diffraction | **Experiment number**: MX-284 |
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| **Beamline**: | **Date of experiment**: from: 04/10/2004 to: 13/10/2004 | **Date of report**: 01/03/2005 |
| **Shifts:** | **Local contact(s)**: Michael Wulff | *Received at ESRF:* |


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## Report:

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 October 2004. A total of 8 shifts were allocated to this project, which, for the first time, produced time-resolved difference signal at 100 ps time delay from photoactivated PYP.

In typical, 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 excitation scheme, 400 nm pulses were passed through the fused silica rods to broaden the femtosecond pulses to the picosecond regime. Then the resulting pre-stretched pulse was further stretched through the optical fiber. The stimulated Raman effect in the fiber was not so 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 22 time delays from -20 ns to 1 s, evenly spaced in the logarithmic time scale, were obtained and processed with the program suite *LaueView*.

The difference electron density maps at several time delays (randomly chosen) are shown in the figures. Data acquired at 100 ps time delay shows clear difference signal, and data at all other time points

also show clear difference signals. In all our previous attempts, we failed to see any signal at 100 ps, and this is the first time that we see a clear signal at 100 ps.  We attributed our previous failures to the shifted time-zero and finally succeeded in capturing the signal at 100 ps. However, our results are not without any problems. Since we collected all the time delays from one single crystal, we expected to have a uniform photoexcitation throughout all the time delays. Contrary to our expectation, it appears that the degrees of photoactivation fluctuate with time. In addition, the data at 10 μs is worse than those obtained in previous experiments at APS.

In summary, when PYP was photoactivated with pulses stretched to ca. 100 ps significant difference signals were observed even at 100 ps time delay. We consider this as a triumph since this is the first time we see any noticeable signal at sub-ns time dealys. However, it seems that the experimental results are not optimal in that the photoactivation was not uniform throughout the whole data acquisition and the signal to noise ratio was a bit worse than our best ones. Once we acquire more data with better photoactivation and signal to noise ratio, it should allow us to characterize in detail the structure and dynamics of the primary intermediate in the PYP photocycle with the sub-ns time resolution.

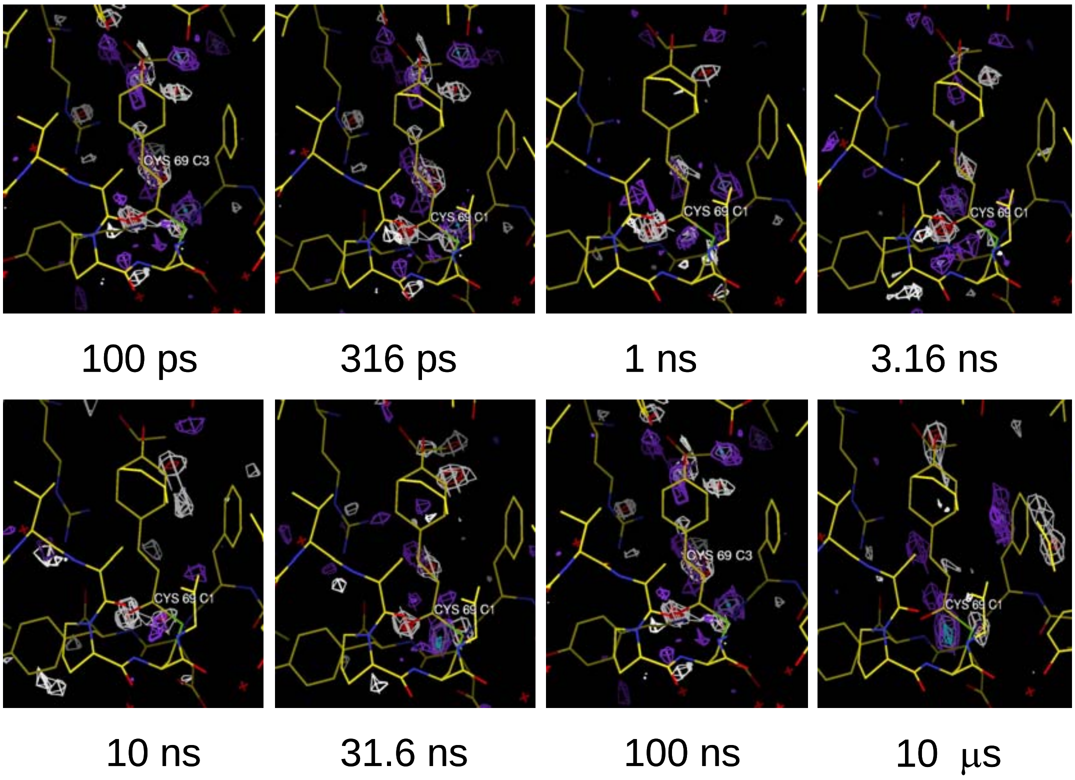


Figure 1. Difference electron density maps at several time delays from a single crystal of PYP excited with ~100 ps laser pulse at 418 nm, contour levels: white/red: -2.5σ/-3.5σ, purple/blue: 2.5σ/3.5σ.