ESRF	**Experiment title:** Early intermediates of the PYP photocycles studied by picosecond time-resolved X-ray Laue crystallography	**Experiment number**: MX-429
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Report:

We have recently conducted picosecond time-resolved Laue diffraction experiments on single crystals of a bacterial blue light photoreceptor, photoactive yellow protein (PYP), at the ID09B beamline during 4 bunch mode in October 2005. A total of 12 shifts were allocated to this project, which produced a time-resolved difference signal with a 100 ps time delay from photoactivated PYP.

Previous spectroscopic and diffractional studies of PYP suggested that the first intermediate I_{cp} forms in the nanosecond regime, and two co-existing pR-like intermediates (pR_{CW} and pR_{E46Q}) follow the first intermediate. However the exact mechanism of early conversion from pG state to I_{cp} state has been uncertain because this conversion took place in sub-nanosecond time scale while the width of exicitation laser pulse was several nanoseconds. To remedy this problem, in this trial, we used laser pulses with a pulse width of about 100 ps for the initiation of the PYP photocycle. Although femtosecond laser pulses were easily available, we stretched the femtosecond pulses to picosecond pulses to increase the degree of photoactivation.

The femtosecond laser 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 directed 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 the pulse emerged from the fiber with the center wavelength shifted to 418 nm; the pulse duration was estimated to be on the order of 100 ps. Data sets at 6 time delays (-20 ns, 100 ps, 316 ps, 1 ns,

1 μs, 1 ms) were obtained and the data at all time delays have been processed with the program suite *LaueView*.

The difference electron density maps at several time delays are shown in Figure 1. Data obtained at sub-nanoseconds shows clear difference signals, and the degree of photoactivation and signal-to-noise ratio have improved compared with the previous data. Combined with data aquired in October 2004, we can gain insight into early events in the photo-cycle of PYP at sub-nanosecond time resolution. In addition, the structure of a new intermediates between pG and I_{cp} has been observed for the first time.

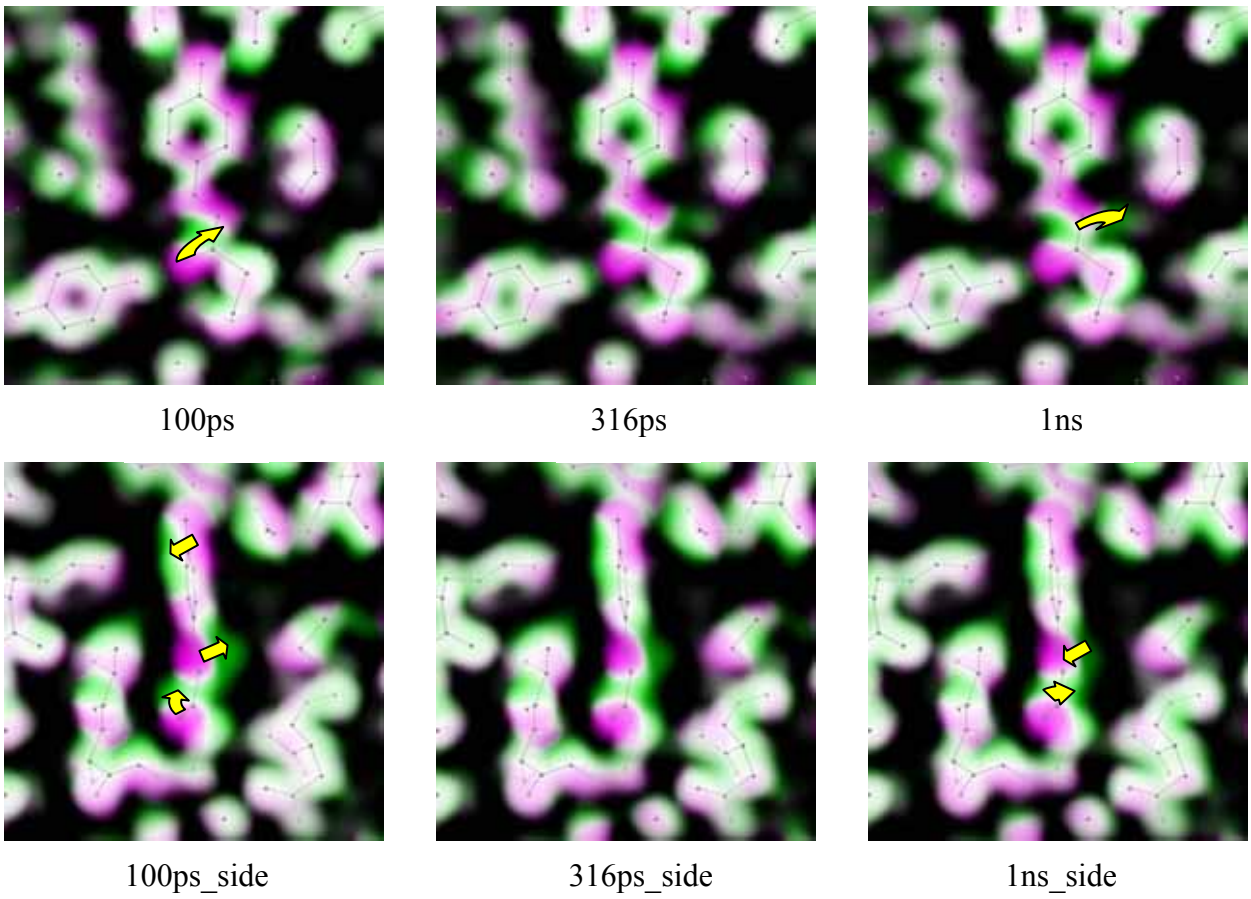

Figure 1. Color-coded electron density map recorded for the ground state (magenta) and photolyzed state(green) at several time points
magenta: $2mF_d$-DF_c, green: $2mF_d$ - DF_c + 0.1 w ΔF, size 16 x 16 A, depth 6.5 A

In Figure 1, significant differences between the ground state and photolyzed state can be observed. The major movement within sub-nanoseconds regime is the flip-flop movement of the carbonyl oxygen in the chromophore toward the opposite position from trans to cis. Considering that the previous experiments and theories proposed that the middle oxygen would move within the nanoseond regime, such movement of the middle oxygen in 100 ps and 316 ps would be appropriate. Another featured structural changes within the picsecond regime might be the backward movement of the double bond in the chromophore. After some picoseconds, the double bond came back to the original position within 1 nanosecond. This movement was discovered in this experiment for the first time, and would be the main driving force of the flip-flop movement of the middle oxygen of the chromophore. Also as the double bond moved backward, the phenyl ring of the chromophore partially moved forward. Because the chromophore connected to three surrounding

residues (E46, Y42, and C69) through the hydrogen bond, these surrounding residues also moved toward the chromophore to maintain the length of the hydrogen bond.

In summary, when PYP was photoactivated with pulses stretched to *ca.* 100 ps, significant difference signals were observed even at 100 ps time delay. This data with better photoactivation and signal-to-noise ratio can provide us to characterize in detail the structure and dynamics of the primary intermediate in the PYP photocycle with the sub-ns time resolution. In addition, E46Q PYP, which is a well-known mutant of PYP and has shorter lifetime of intermediates than wild-type PYP, or other mutant of PYP study would be an alternative choice to investigate the structual change of PYP photocycle in detail, because these mutants might affect the main initial movement of chromophore through the hydrogen bond linkage.