Experiment title: Picosecond structures of the early PYP photocycle		Experiment number: MX-382
Beamline:	**Date of experiment**: from: 22/05/2005 to: 25/05/2005	**Date of report**: 15/09/2005
Shifts:	**Local contact(s)**: Michael Wulff	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Hyotcherl Ihee (*), Department of Chemistry and School of Molecular Science, KAIST, Deajeon, Republic of Korea

Philip Anfinrud (*), NIH, USA

Michael Wulff (*), ESRF, Grenoble, France

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 May 2005. A total of 12 shifts were allocated to this project, which produced time-resolved difference signal at 100 ps time delay from photoactivated PYP.

Previous spectroscopic and diffractional studies of PYP suggested that the first intermidiate I_{cp} forms in 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 palce in sub-nanosecond time scale while the width of exicitation laser pulse was several nanoseconds. To remedy this problem, in this run, 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 400 μm 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 6 time delays (-20 ns, 100 ps, 316 ps, 1 ns, 1 μs, 1 ms) were obtained and 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 signal quality and signal to noise ratio have improved compared with the previous data. Combined with data aquired in October 2004, we can obtain the insight into early events in the photo-cycle of PYP. Due to this success of observering the difference electron density signal at picosecond, we can investigate this early events or intermediates of PYP photocycle at sub-nanoseconds level. In addition, new intermediates between pG and I_{cp} may be discovered for the first time.

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.

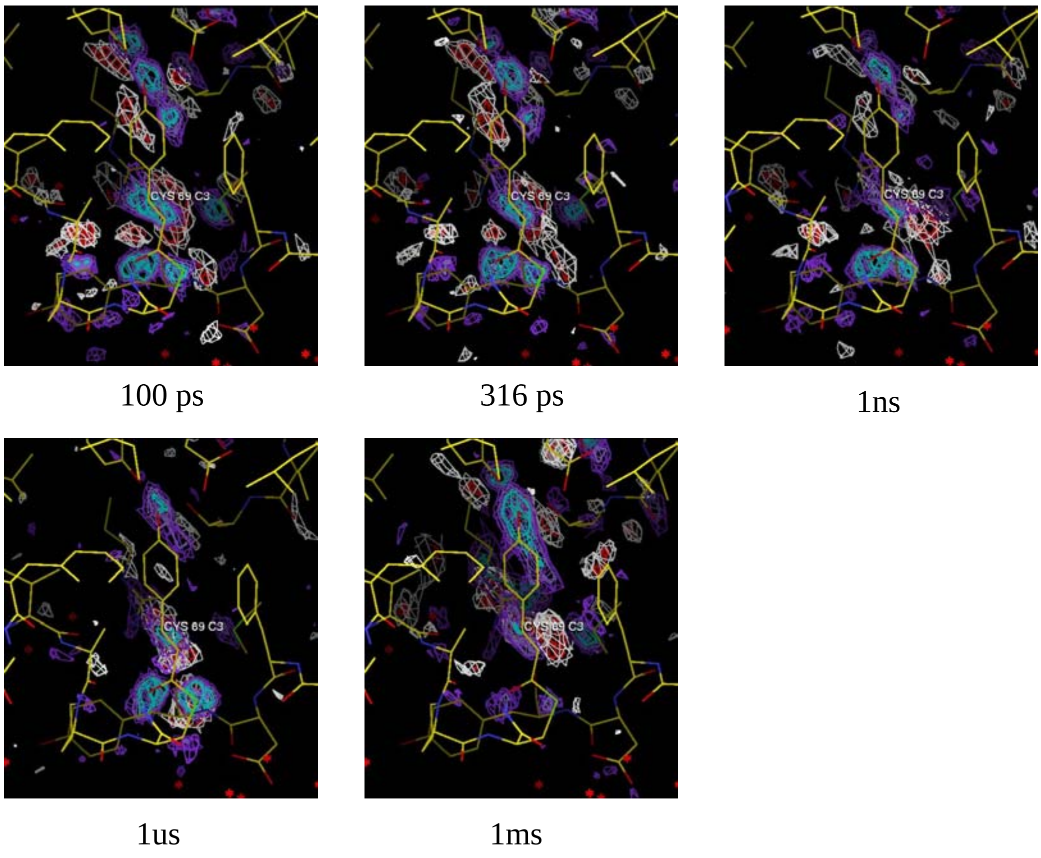

Figure 1. Difference electron density maps of PYP at several time point in each experiment excited with ~100ps laser pulse at 418nm, contour levels: white/red: -2.5σ /-3.5 σ , purple/blue: 2.5σ /3.5 σ .