ESRF	**Experiment title:Time-resolved Laue** Crystallography Structure determination of light cycle intermediates of wildtype and mutant forms of PYP	**Experiment number:** LS-633
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Report: During the last experiments we faced a number of technical and organizational difficulties that were beyond our control and the control of the beamline personnel.

1) The fast "Juelich''-shutter was not available due to technical problems

2)Permission to use the "Chicago''-shutter which is permanently installed on ID09 was withheld at very short notice by the BIOCARS consortium. Reconfiguration of the beamline to accommodate the fastest available alternative shutter limited the time resolution to approximately 150 microsecond and required time-consuming equipment rearrangement and reprogramming.

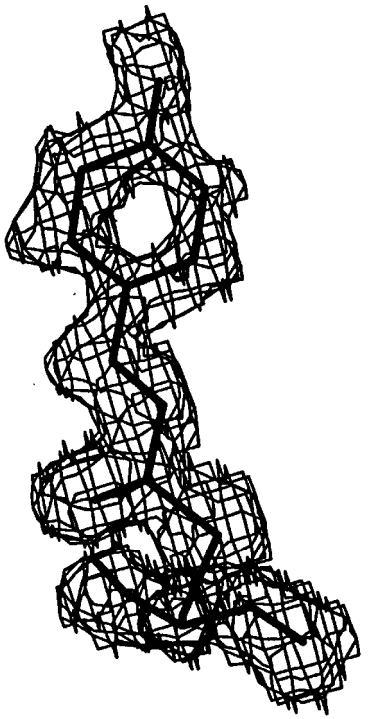
3) The microspectrophotometer's spot size was too large for our experiments. This and the failure of a detector chip made the optical monitoring of reaction initiation impossible.

Only the skill and hard work of Michael Wulff and Friederich Schotte made data collection possible at all.

Data collection: We collected data sets for both the ground and light-activated states of PYP crystals. For light cycle initiation we used 7.5 nanosecond laser pulses of the third harmonic output of a Nd:YAG laser. The laser delivered 4-5 mJ into a focal spot of lmm^2. For X-ray exposures we used the maximum time resolution available (150 micro seconds), To compensate for the reduced time resolution, we attempted to decrease the speed of the light cycle by cooling and the addition of glycerol to the crystal storage solution.

However the available Oxford cryosystem was unsuitable for operation near room temperature and the addition of glycerol reduced crystal lifetime.

Data processing: The three best data sets have been processed with the Daresbury software package. The best data set had a maximum resolution of 1.75 Angstrom and a completeness of 76% (51% in the 1.83- 1.88 A shell). Integration and merging of this data set resulted in R_{merge} of 10.7 % of the ground state and13.5% for the light activated state. We inspected both difference electron density maps and omit maps of the chromophore region for all three processed data sets, but no electron density changes stood out above the noise level.



1.75 A sa_omit map of chromophore region in ground state

Problems and solutions: We propose the following improvements:

1) Shorten the X-ray pulse below 10 microseconds to ensure that the vat majority (>90%) of light activated molecules still occupy the early intermediate state Il. This will be easily accomplished with the "Juelich''-shutter.

2) Reduce the focal spot of the micro-spectrophotometer to allow optical measurements on our crystals. We have constructed our own microspectrophotometer to give a spot size well below the required diameter and acquired a suitable laser source to perform laser initiation in our home laboratory.

3) Make the laser beam approximately co-linear, rather than perpendicular, to the X-ray beam to ensure that the laser and X-ray beam travel though the same volume of the crystal.

Conclusion: The reported results demonstrate that Laue diffraction data collection of PYP crystals with microsecond time resolution yield data sets that result in electron density maps of excellent overall quality. We were also able to identify experimental problems that will either be remedied by the upgrades of the shutter system at beamline ID09 or can be avoided by adjustments of the experiments setup during our next experiments