MX-11. Early structures of the PYP photocycle studied by time-resolved x-ray Laue diffraction in sub-ns time resolution

Hyotcherl Ihee, Philip Anfinrud, Michael Wulff, Keith Moffat

We proposed to conduct time-resolved Laue diffraction experiments on single crystals of the bacterial blue light photoreceptor, photoactive yellow protein (PYP), using femtosecond (fs) laser pulses at the ID09 beamline and 100 picosecond (ps) x-ray pulses in single-bunch mode. A total of 21 shifts were allocated to three groups for both nanosecond (ns) and fs experiments during October 6 to October 17, 2002, and we had 7 shifts for this project.

Our original plan was to employ fs laser pulses to activate the photocycle in PYP crystals, and obtain data sets at time delays between 0 ps to 10 ns. However, it soon became evident that the energy delivered to sample was too low at 485 nm, which is the desired excitation wavelength for PYP. The major reason was that the fs pulse stretcher was set up to optimize red light for a parallel experiment on myoglobin, and the right stretcher for our wavelength was not available (we are building a pulse stretcher for 485 nm for the next fs experiment). So we decided to use the beam time for an alternative experiment using ns laser pulses instead of fs laser pulses, and to obtain time delays between 1 ns and 10 μs.

In this experiment, we tried a few novel experimental schemes. First, we applied a new data collection scheme, where the time delay rather than the angular setting of the crystal is the fast variable. Our previous data collection scheme covered the whole angular range at a fixed time point and subsequently moved to another time point; angle was the fast variable. In that experimental scheme, laser intensity fluctuation and crystal-to-crystal variation between time points introduces a systematic error which ultimately has a detrimental effect on the accurate determination of the time constants. In the new scheme, a range of time delays were swept at a fixed angular setting, and subsequently moved to a new angular setting for another time sweep, and so on. To achieve a high coverage in reciprocal space, data from several crystals has to be merged. Second, in favorable cases, multiple data sets were obtained from one single crystal. This was done in an attempt to reduce the crystal-to-crystal variation. Third, a negative time point (-20 ns) as well as the usual dark data (the data without any laser illumination) was obtained with other positive time points evenly spaced in logarithmic scale (1 ns, 3 ns, 10 ns, 30 ns, 100 ns, 300 ns, 1 μs, 3 μs, 10 μs). A negative time point means that the probing x-ray pulse arrives earlier than the pumping laser pulse. Collecting a negative time point and

using it as a reference is a common practice in time-resolved spectroscopy, but had been rarely applied to time-resolved crystallography. This proved critical as explained later. Fourth, a rather high repetition rate of 1 Hz instead of the usual 0.2 Hz was used to reduce the total data acquisition time considerably. All previous results had indicated that the photocycle is essentially complete within 1 second. Even if some residual remains at 1 second, it should be recorded at the negative time point, and removed by subtracting the negative time point from other positive time points. This reasoning proved to be correct as explained later.

Eventually, we managed to obtain 10 Laue data sets. One data set in this case means 9 angular settings with 10 degree spacing and 11 time points (one negative, one dark, nine positive time delays), resulting in a total of 99 images. Each data set was processed separately with the program suite *LaueView* and the data at the same time delay were merged later to obtain a final good quality data set. Some of the data sets are still undergoing the processing procedure and the merging strategy has not yet been finalized. However, a preliminary analysis using the difference Fourier method shows two important results. First, the negative time point contains a considerable amount of residual signal. This means that some population lasting up to 1 second is apparently accumulated in our experimental conditions. This result was rather surprising to us because all previous results had pointed to a complete recovery of the photocycle within 1 second. We tentatively assigned the origin of this residual to a dehydration effect in the crystal caused by poor cooling conditions. Optimal cooling conditions can be achieved when the capillary holding the crystal is completely within the cooling stream. These ideal conditions were not met and the heat generated by a high repetition rate of 1 Hz could not be effectively dissipated, resulting in dehydration. Second, using the negative time point as a reference successfully removed the systematic residual from other positive time points as we correctly expected. For example, the difference maps at 1 μs, 3 μs, and 10 μs are consistent with our earlier map at 6 μs obtained at APS.

In summary, data show a rather large residual at the negative time point, probably because the cooling was not optimal, and the ESRF data and APS data show a consistent picture around the microsecond range if the negative time point is used as a reference for the ESRF data. The caveat is that the kinetics may not be the same in the partially dehydrated conditions. A concrete and robust conclusion will require further analysis.