ESRF	**Experiment title:** Time-resolved WAXS of bacterial phytochromes	**Experiment number**: CH-4006
Beamline: ID09B	**Date of experiment**: from: 28 Nov 2013 to: 04 Dec 2013	**Date of report**: 28 Feb 2014
Shifts: 17	**Local contact(s)**: Dmitry Khakhulin	*Received at ESRF:*

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Report:

Phytochromes are photoswitchable signaling proteins in plants, cyanobacteria and fungi. They regulate for example flowering, the circadian rhythm and the germination of seeds. The resting state structures of some bacterial phytochrome fragments are reported (Yang 2009, Essen 2008, Wagner 2007) and the quaternary arrangement of the wild-type protein has been modeled based on cryo-electron micrographs (Li 2010). However, structural information about the signaling state and the sequence of changes that lead to the active state is still sparse.

Prior to this experiment, we had collected excellent difference WAXS data of the bacterial phytochrome from *D. radiodurans* with millisecond time-resolution at the beamline cSAXS at the Swiss Light Source. We had also collected time-resolved data of the same samples at BioCARS at the Advanced Photon Source. The samples proved stable against radiation damage and significant difference WAXS signals appeared on a millisecond time scale. Furthermore, the data indicated that conformational changes in the smallest protein fragment amplified when downstream domains were added, revealing the role of each domain in signal amplification. These results are submitted to a high-impact journal and currently under review (Takala, Björling 2014).

This experiment aimed **(i)** to record solution scattering fingerprints from structural intermediates of the photoconversion process and **(ii)** to verify the previously observed findings and to record data of higher quality and under improved conditions.

Figure 1 shows example data collected. The figure illustrates how the difference X-ray scattering relative to the resting state evolves over time as a result of laser excitation at $t = 0$. Similar data were acquired both for the forward (resting-to-active) and backward (active-to-resting) transitions, with three different protein constructs.

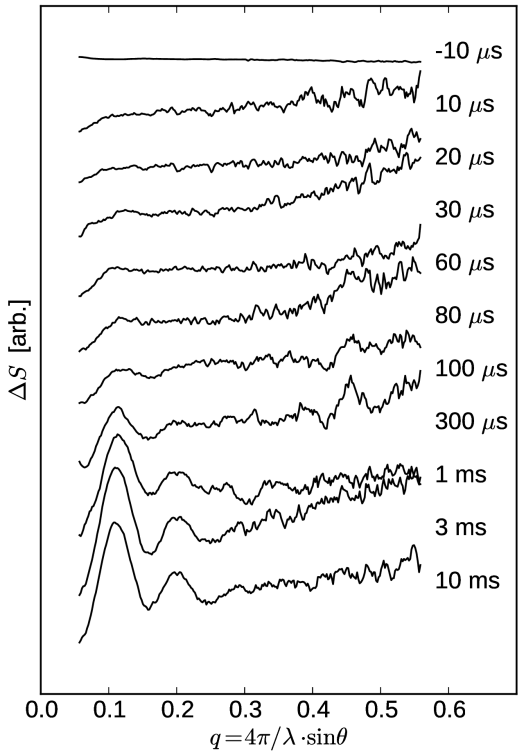

Figure 1: Time-resolved X-ray solution scattering from the photosensory core of D. radiodurans after excitation at t=0 with a short laser pulse.

The data has yet to be definitively analyzed, but it is clear that the signal-to-noise ratio is significantly improved compared to previous experiments. This is crucial with respect to the first aim of identifying intermediate structures, as any transient species are likely to be visible at intermediate or high q. The data in Figure 1 contain coherent oscillations at $0.4/\text{Å} < q < 0.5/\text{Å}$ which, with careful analysis and at this improved signal-to-noise level, may carry valuable information on the structural mechanism of photoconversion.

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