ESRF	**Experiment title:** Unveiling the kinetics of light-induced structural changes in phytochromes and cyanobacteriochromes by time resolved WAXS	**Experiment number**: LS2411
Beamline: ID09	**Date of experiment**: from: 03/02/2016 to: 08/02/2016	**Date of report**: 05/03/2018
Shifts: 15	**Local contact(s)**: Martin Pedersen	*Received at ESRF:*

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Report:

Photosensory proteins are the molecular machines which enable biological systems to detect light from the environment and to convert it into a biochemical output necessary for metabolism and environmental adaptation. Phytochromes and cyanobacteriochromes (CBCR) are an important class of photosensory proteins and work by interchanging between different states upon light absorption in a reaction that is coupled to large-scale conformational changes.

During the present experiment, we used time-resolved small and wide-angle X-ray scattering (TR-S/WAXS) to characterise these structural changes in two phytochromes (Cph1 – photosensory region and full-length protein - and DTen). The Cph1 and DTen solutions needed for this study were prepared in Derren Heyes' lab. We used a 5 ns laser pulse to photoexcite proteins inside a quartz capillary connected, via a pumping peristaltic system, to a reservoir under continuous illumination at 720 nm to let the sample recover the equilibrium "dark" form, and to acquire structural snapshots of the solution as a function of the time delay after laser pulse. The protein concentration was about 0.1 mM. We investigated time scales from us to s, with an energy of about 0.5 mJ focused to 0.4x1.2 mm^2. An orthogonal laser pump / x-ray probe geometry was used to match the two different penetration depths of laser and x-ray.

We collected a full dataset on both photosensory region and full-length form of Cph1 (Figure 1) and on DTen (Figure 2).

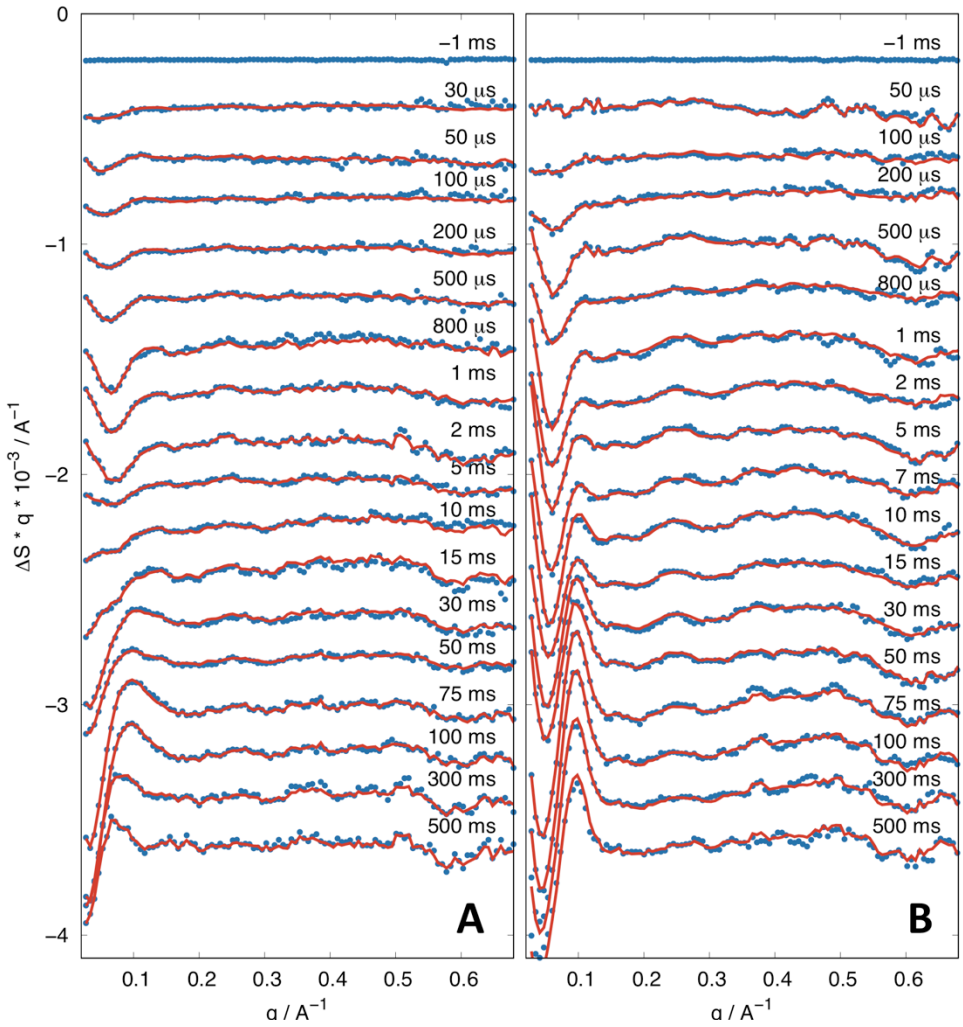

Figure 1. Light-induced time-resolved X-ray scattering difference patterns for the photosensory region of Cph1 (**A**) and the full-length Cph1 protein (**B**) in solution.

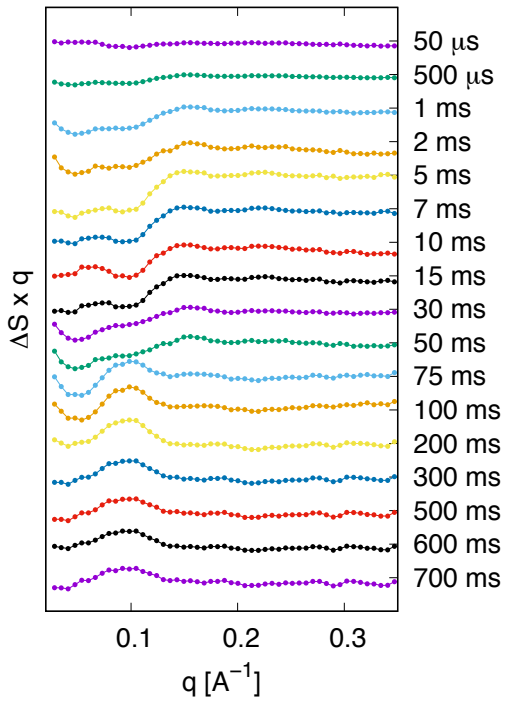

Figure 2. Laser-induced time-resolved X-ray scattering difference patterns for DTen in solution.