Experiment LS-2323
Unveiling the kinetics of light-induced structural changes in phytochromes and cyanobacteriochromes by time resolved WAXS

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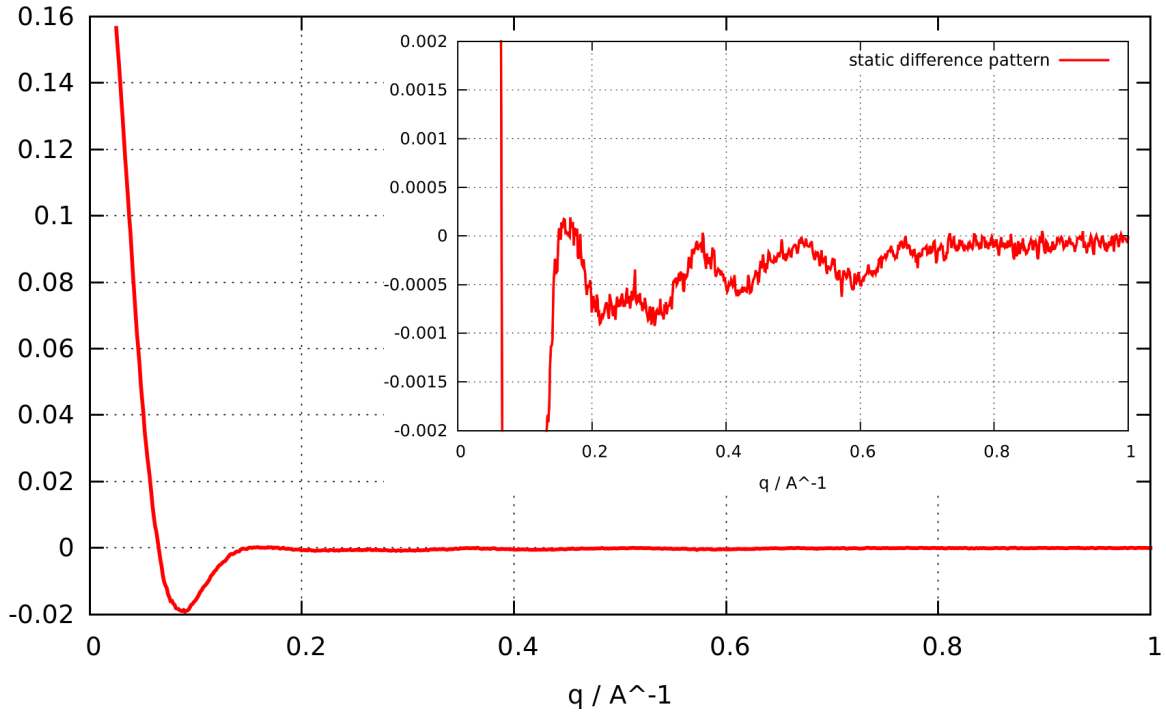
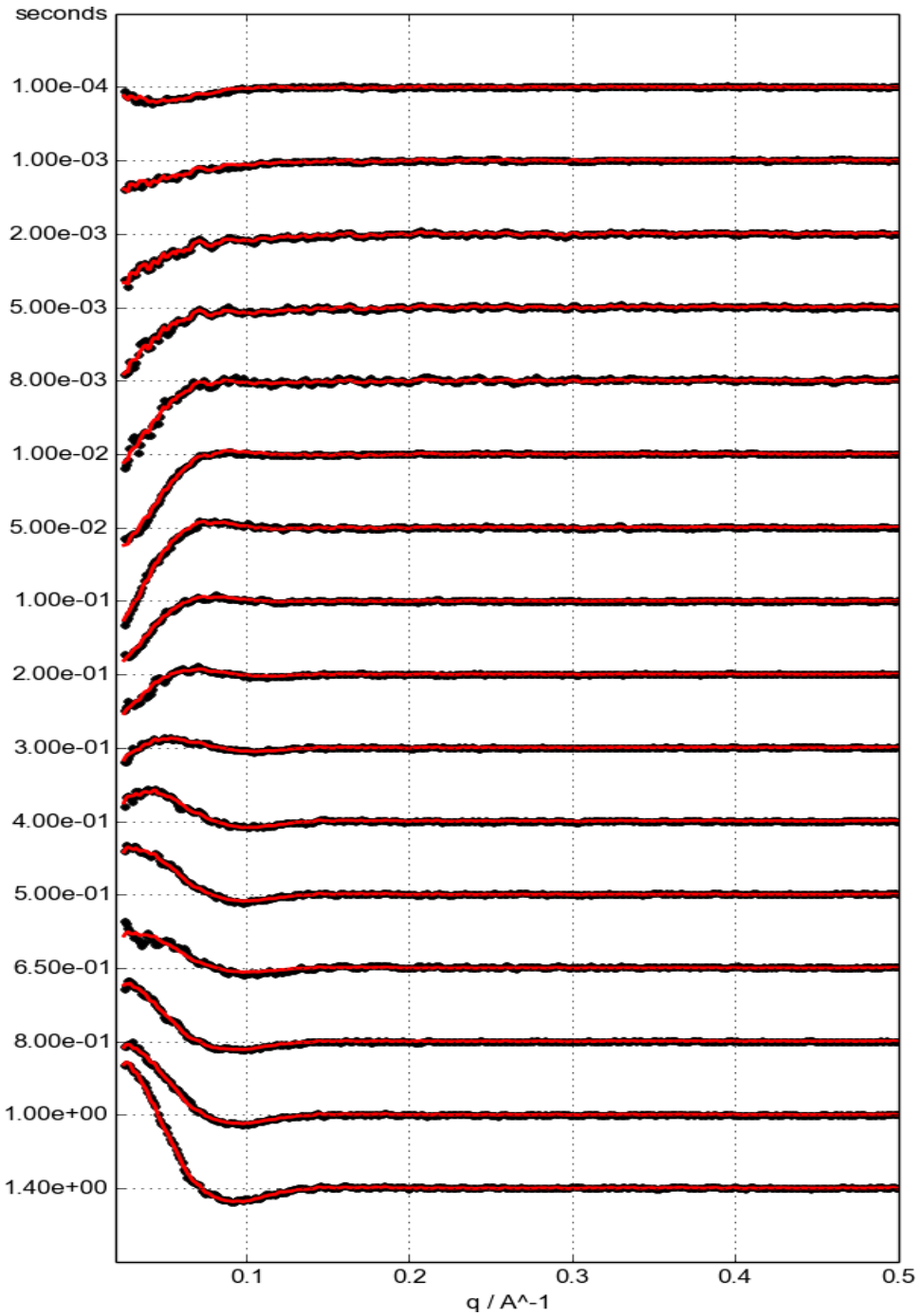
Photosensory proteins are the molecular machines which enable biological systems to detect light from the environment and to convert it into a biochemical output necessary for metabolism and environmental adaptation. Phytochromes and cyanobacteriochromes (CBCR) are an important class of photosensory 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 wide-angle X-ray scattering (TR-WAXS) to characterise these structural changes in a phytochrome (Cph1) and a CBCR (Tlr0924) protein. The Cph1 and Tlr0924 solutions needed for this study were prepared in Derren Heyes' lab. We used a 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 0.2x1 mm2. An orthogonal laser pump / x-ray probe geometry was used to match the two different penetration depths of laser and x-ray.

Cph1: static difference pattern (below) and time resolved data (next page) after excitation at 660 nm (5 ns pulse).




seconds
1.00e-04
1.00e-03
2.00e-03
5.00e-03
8.00e-03
1.00e-02
5.00e-02
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2.00e-01
3.00e-01
4.00e-01
5.00e-01
6.50e-01
8.00e-01
1.00e+00
1.40e+00
0.1
0.2
0.3
0.4
0.5
q / A^-1

TIr0924: time resolved data at 430 and 530 nm excitation (5 ns pulses).