Development of pump-probe capabilities at B23: Investigating structural rearrangement kinetics of photoreceptor proteins.

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Photoreceptor proteins are a group of proteins sensitive to certain wavelengths of light, inducing conformational change resulting in signal transduction. They are a key part in the emerging field of optogenetics wherein selective gene expression is initiated through light exposure and also in understanding how photoreceptors work for increasing crop yields.

Here we look to investigate the bacterial phytochrome in order to deduce the kinetics of the alpha helix formation; key to the activation of the protein. Seen in **Figure 1** is the crystal structure of the chromophore binding and phytochrome domains in their light and dark states. It is observed that the 'tongue' region refolds from beta to helix in order to transduce the signal to the histidine kinase domain present on the full length protein.

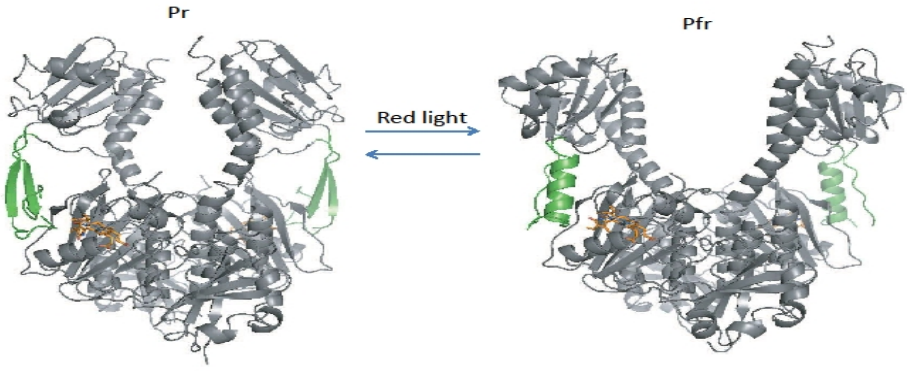

Figure 1: Crystal structure of CBD:PHY in the dark (Pr 780 nm illuminated) and light (Pfr, 660 nm illuminated) state. Highlighted in green is the 'tongue' which structurally rearranges from beta to helix upon exposure to 660 nm light. Image taken from doi:10.1038/nature13310

In order to investigate this, synchrotron radiation CD was required due to the flexibility of the sample compartment and high flux of the beam in order to gain the biggest signal to noise ratio possible – especially important with our low difference signal originating from a ~3% beta to helix conversion observed. We demonstrate pump-probe capabilities at B23 for the first time and also implemented a novel sample delivery system utilising a pump/capillary system to avoid excessive UV damage during measurements. Presented will be the process of setting up such an experiment, current limitations and data handling techniques.