8. Jasper van Thor

Light-induced processes in photosensitive proteins

Wellcome Trust 1st year student: Stephen Moran
Research assistant: Jenny Gibson

We work with light-sensitive proteins and are interested both in the light-induced structural response on the ultrafast timescale as well as slower and (meta-) stable transitions. These processes are investigated in various light-sensitive proteins and photoreceptors, such as Green Fluorescent Protein, Phytochrome, Photoactive Yellow Protein and Cryptochrome, using structural and spectroscopic techniques.

Phytochrome Light Receptors

^{13}C and ^{15}N isotope labelling of the phycocyanobilin chromophore of the Cph1 bacteriophytochrome from *Synechocystis* 6803 has enabled specific assignments of the light-induced FTIR difference spectra showing vibrational modes of the 'Pr' and 'Pfr' states. This assignment work has been carried out in order to evaluate the spectroscopy data in light of the Z-E photoisomerisation model for the Pr-Pfr phototransformation reaction of this light receptor. The isotopically substituted materials are also used for NMR investigations of the chromophore structure and the light-induced structural changes. NMR spectroscopy studies of uniformly labelled photoactive fragments of Cph1 and other phytochrome light receptors are done to probe the light-induced structural changes.

We pursue crystallisation of photoactive fragments of Cph1 having developed a method to produce photoactive, holo-protein core-fragments that are better folded than the full length protein.

Photoactive yellow protein

The 14 kDa photoreceptor protein Photoactive Yellow Protein (PYP) from the bacterium *E. halophila*.shows light-dependent partial unfolding, which is transient and fully reversible. A meta-stable state, called 'pB' is formed with light-excitation that exists for ms-s before the protein subsequently refolds to re-form the ground state, called 'pG'. The rate and free energy of the refolding reaction can be manipulated by specific mutations and also by changing the pH. From NMR studies there is some indication of which parts of the protein become unfolded in the 'pB' state. The NMR data indicate that in the meta-stable pB state significant parts of the polypeptide undergo conformational exchange on the s-ms timescale, broadening the ^{1}H-^{15}N crosspeaks in HSQC spectra. Many optical and vibrational spectroscopy studies support the unfolding model which occurs in solution (but not in crystals) but no detailed experimental evidence is available with regard to the unfolded structures in the pB state. We are mapping the light-induced conformational changes by attaching probes for optical and EPR studies to determine couplings and distances as well as local dynamics. Site directed mutagenesis and chemical modification influences the light-induced structural changes which are evident from NMR spectroscopy measurements.

Green Fluorescent Protein

A light-induced electron transfer reaction in the wild type Green Fluorescent Protein causes the green ground state to be irreversibly converted into a highly fluorescent yellow product (van Thor et al., 2002). An electron transfer reaction occurs with low quantum efficiency between the buried ionised glutamate 222 carboxylate and the electron deficient singlet excited state of the chromophore, causing the sidechain to decarboxylate. The edge-to-edge distance between donor and acceptor atoms is 3.5 Å, close to van der Waals contact. However, the forward rate for the electron transfer is estimated to be approximately $2.4.10^{8}$ s^{-1}, suggesting a low or endoergonic free energy difference between reactant and product states and probably a large solvent reorganisation energy. We are engineering wild type GFP through site directed

mutagenesis to manipulate the free energy and reorganisation energy of the electron transfer reaction. We have created mutants belonging to two different classes where the free energy has been changed resulting in a modified photochemical quantum yield for phototransformation. Such data is used for Marcus analysis to obtain the thermodynamic parameters.

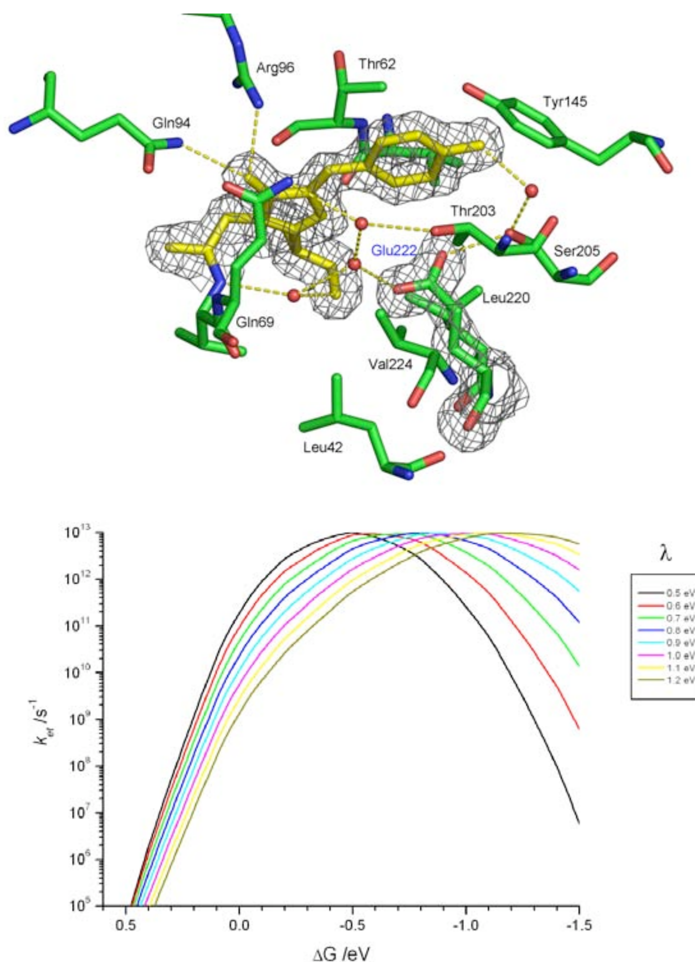

Figure 1. 2Fo-Fc electron density maps of wild type GFP, with contouring of the electron donor (Glu222) and the acceptor (chromophore). The right panel shows theoretical calculations of the electron transfer rate k_{et} in GFP depending on the free energy G assuming a van der Waals distance between acceptor and donor groups for various reorganisation energy values λ ranging from 0.5 eV to 1.2 eV, according to Marcus theory.

Time-resolved spectroscopic and structural studies make use of the ps-transient absorption facilities at the Central Laser Facilities at Rutherford Appleton Laboratories, Chilton. These studies probe the ultrafast excited state proton transfer events as well as chromophore and protein structural perturbations.

Time-resolved structural studies on GFP have been initiated by pump-probe Laue X-ray diffraction at ID09B, ESRF, in collaboration with Philip Anfinrud (NIH/NIDDK, Bethesda).