

# 10. Jasper J. van Thor

## Signal transduction in photoreceptor proteins

Absorption of light by photoreceptor proteins leads to structural changes that are important for biological signal transduction. These structural events have their origin in photochemical events, which occur on a fast time-scale. The resulting protein conformational changes are usually much longer-lived and can be studied by various experimental techniques. Both functional spectroscopy and structural biology is used to study these 'activation processes'. A major goal is to understand these events in full molecular detail on fast as well as slow time-scales.

Photoreceptors of particular interest are Phytochromes, Cryptochromes and Photoactive Yellow Proteins (PYPs). These receptors are distinct with respect to their signaling mode and spectroscopic behavior. Whereas signaling in Phytochromes and PYPs is initiated by photoisomerisation, in Cryptochromes this is proposed to result from light-induced electron transport. Also photosensitive proteins, such as the Green Fluorescent Protein (GFP), can be used as generally more simple models to look at light-induced changes in proteins.

### 10.1 Phytochrome light receptors

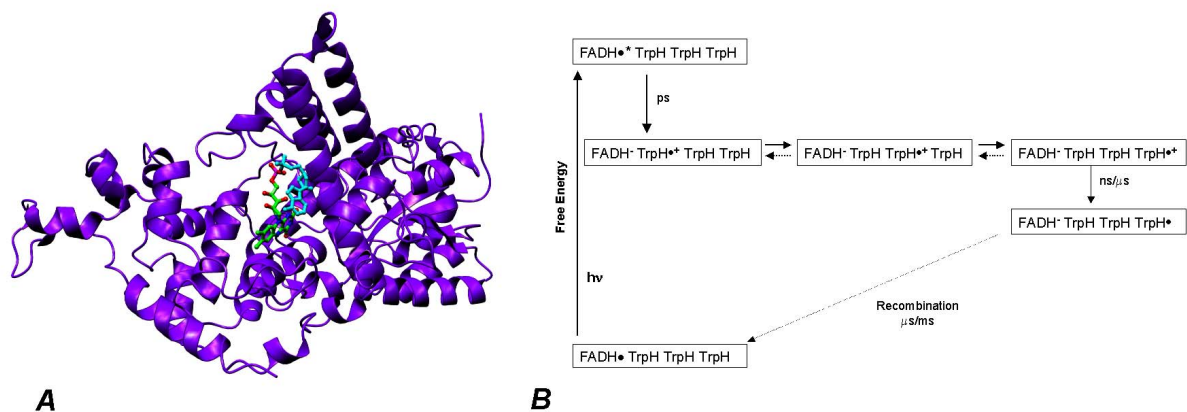
Phytochromes were initially discovered in plants, but have now also been found in bacteria. The bacterial proteins are light-regulated histidine-kinases of the two-component type, that show far-red and red light induced optical transitions between the 'Pfr' ('far-red' light absorbing) and 'Pr' ('red' light absorbing) states. Phototransformation from Pr to Pfr is known to involve a Z,E photoisomerisation around the 15, 16-double bond connecting the C- and D-rings of the linear tetrapyrrole chromophore. From optical and vibrational spectroscopy a relatively good understanding of the photochemistry of phytochromes has been developed, but structural data is still lacking.

We pursue both X-ray crystallography and NMR spectroscopy of bacterial phytochromes. These proteins are more simple when compared to the plant photoreceptors, and expression and reconstitution to produce workable amounts of apo-phytochromes has been achieved.

Functional spectroscopy studies have focused on the kinetics and complexity of the spectroscopic transitions, and proton transfer reactions have been time-resolved by flash photolysis experiments using exogenously added pH indicators. Presently, our efforts are directed towards time-resolving vibrational absorption difference features by FTIR techniques, together with Professor Peter Rich, of UCL, London. From steady state difference FTIR measurements in the amide region it is clear that very large conformational changes are associated with the transition between the Pr and Pfr states. Initial 'rapid-scan' ms-time-resolved FTIR measurements have already shown that for the Pr → Pfr transition, the difference FTIR spectrum 100 ms after flash excitation was already identical to the steady state difference spectrum. This is surprising in light of the slower transients observed in optical spectroscopy and from measurements of proton transfer reactions (van Thor et al., 2001). It is therefore important to pursue time-resolved FTIR measurements for comparison with laser flash photolysis measurements.

### 10.2 Cryptochromes

Circadian rhythms are oscillations in physiological and behavioural functions with daily periods that are generated by an internal time-keeping mechanism referred to as the Biological Clock. This clock is controlled by blue light in mammals, flies and plants. According to recently developed models, in mammals and flies the core oscillator driving this clock consists of an auto-regulatory transcription-translation based feedback loop involving a set of 'clock' genes (encoding Cryptochrome(s), PERIOD, TIMELESS, CLOCK, BMAL and other proteins). The Cryptochromes are blue light/UV-A absorbing proteins that have been experimentally shown to act as the photoreceptors for the circadian clock in flies (*Drosophila*) and plants (*Arabidopsis*). No structure is available for cryptochrome light receptors, and not much is known with respect to the basic signaling mechanism. In analogy to what is known about photolyase function, it is expected that light-induced electron transfer is important for signaling in these blue light receptors. We pursue structural and spectroscopic studies of *Drosophila* cryptochrome.



**Figure 1.** Model and possible photochemical mechanism for *Drosophila* Cryptochrome.

A. Model of DmCry, based on the photolyase structures 1DNP (*E. coli*) and 1QNF (*A. nidulans*). The cofactor FAD, which was specifically included in this model, is shown. B. Possible light-induced reactions in DmCry, involving radical transfer along W422, W397 and W342. This scheme is proposed in analogy to the photochemistry observed in *E. coli* photolyase, where light-activation leads to oxidation and deprotonation of a surface exposed Trp residue (Aubert et al., 2000).

### 10.3 The Photoactive Yellow Protein

The photoactive yellow protein (PYP) from the bacterium *Ectothiorhodospira halophila* is a 14 kDa cytoplasmic photoreceptor protein that shows large conformational changes associated with the formation of a long-lived photocycle intermediate state called  $I_2$ , which is considered to be the biologically active signaling state. The chromophore, responsible for light-activation of PYP, is *p*-coumaric acid linked to Cys69 via a thio-ester bond. Light activation of PYP leads to *trans/cis* isomerisation and protonation of this chromophore in the  $I_2$  state, which subsequently triggers these large conformational changes in the protein. The exact nature of these structural changes has been investigated by time-resolved X-ray crystallography (Genick et al., 1997) and by NMR spectroscopy (Rubinstenn et al., 1998; Craven et al., 2000). Interestingly, the results from X-ray crystallography and solution NMR studies were distinctly different. Whereas solution studies suggest a partial unfolding of the protein after light-activation, in the crystalline state less dramatic conformational changes were detected. Fourier Transform Infrared (FTIR) spectroscopy was later used to show that indeed, in the crystalline state smaller global conformational changes are associated with the  $I_2$  state (Xie et al., 2001). Also, binding of a hydrophobic fluorescent probe to the  $I_2$  state was used to detect the transient exposure of hydrophobic surface, which showed properties of the  $I_2$  state in solution to be different from the crystalline  $I_2$  state (Hendriks et al., 2002). New approaches using both X-ray crystallography and NMR spectroscopy can shed more light on the conformational changes that occur, depending on the specific environment the protein is in.

### 10.4 The Green Fluorescent Protein

The Green Fluorescent Protein is a light sensitive protein with some remarkable properties. The fluorescence mechanism as well as the photochromic behavior of the wild-type protein are of particular interest.

A light induced electron transfer mechanism was discovered in wild-type GFP that leads to decarboxylation of a buried Glutamate sidechain. This light-activated mechanism occurs with low probability and leads to a dramatic color change from green to yellow. This shows an example of light-induced structural changes in photosensitive proteins (van Thor et al., 2002).

The fluorescence mechanism of the wild-type GFP is intricately linked with proton-transfer events. Optical excitation of GFP leads to photocycling with a number of intermediate states. Recently, in collaboration with the Biophysics group at the Free University in Amsterdam, ps-time-resolved studies have resolved the properties of these spectral intermediates.

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