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# Chapter 7
## General Discussion

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7.1 Structural changes during the PYP photocycle

In aqueous solution, in the later stages of its photocycle, PYP undergoes conformational changes that have characteristics of protein unfolding (Van Brederode et al., 1996; Rubinstenn et al., 1998; Hoff et al., 1999). In Chapter 2, it was shown that these large structural changes occur particularly in the N-terminus of the protein. This could be concluded from the “Arrhenius-behavior” in a PYP variant in which 25 N-terminal residues have genetically been truncated (Δ25PYP), whereas full-length PYP shows deviation from normal Arrhenius behavior (Van der Horst et al., 2001).

The photocycle characteristics of Δ25PYP, e.g. a slow groundstate recovery and less structural changes during its photocycle, allowed us to do structural studies on its pB intermediate. These, and other studies of Δ25PYP, are discussed in the next paragraphs.

7.1.1 Transient exposure of hydrophobic surface in the Photoactive Yellow Protein monitored with Nile Red.

As described above and in Chapter 2, we used Arrhenius plots to analyze changes in protein structure, that are accompanied by changes in the heat capacity (∆C_p). Another method to study protein unfolding is to use a probe of which a specific (spectroscopic) property changes with an altered molecular environment. We used a fluorescent hydrophobicity probe to study structural changes in both WT PYP and Δ25PYP (Hendriks et al., 2002). The fluorescence of the probe that was used, Nile Red, is sensitive to the local polarity. In a polar environment it has a low quantum yield, whereas a hydrophobic environment both increases the fluorescence quantum yield and shifts its emission maximum (Dutta et al., 1996; Hou et al., 2000). When Nile Red is added to a PYP solution in the dark, its fluorescence characteristics are the same as in an aqueous solution, indicating that the probe does not bind to the protein in its groundstate. However, after blue-light excitation, binding of Nile Red to PYP is shown by its fluorescence characteristics (see Figure 1). Surprisingly, Δ25PYP shows exactly the same fluorescence properties, i.e. no binding of Nile Red in the groundstate of the protein, and binding after blue-light excitation (Figure 1). There are two hydrophobic cores in PYP that can in principle act as binding sites for Nile Red; one contains the chromophore binding pocket, the other is formed by the two N-terminal α-helices and the central β-sheet. The fact that Δ25PYP behaves similarly to WT PYP with regards to Nile Red binding shows that (i) the Nile Red probe binds in the hydrophobic site that contains the chromophore binding pocket, and (ii) apart from the structural changes in the N-terminus during
the photocycle, other structural changes take place near the chromophore binding pocket.

The only currently known PAS domains that possess the N-terminal cap belong to the xanthopsin family. By removing the 25 amino-terminal residues of PYP, we constructed a minimal PAS domain. The structure of this minimal PAS domain was determined using X-ray crystallography (Vreede et al., 2003). The structure of Δ25PYP was solved by molecular replacement and refined to a 1.14-Å resolution (see Figure 2). In the crystals of Δ25 PYP, there are two protein molecules present in the asymmetric unit cell, with very similar conformations (a rmsd of 0.77 Å on the Cα atoms). Removal of the first 25 residues does not significantly affect the overall fold of the protein. Small differences are observed in the N-terminus and the loops consisting of residues 84-88, 98-101 and 111-117, presumably due to “crystal contacts”. Deletion of the N-terminus does result in solvent exposure of several hydrophobic residues (Phe28, Trp119 and Phe121).

The Δ25PYP structure was used in computer simulations, along with FixL, HERG and LOV2, other PAS domains of which the 3D structure has been solved. Essential dynamics analysis showed that certain segments of these PAS domains show movements in a concerted fashion, despite the absence of sequence conservation. As will be discussed below (Chapter 7.4 and Figure 10), the PAS domains may use a common mechanism for signaling.

**Figure 1: Nile Red emission and excitation spectra for WT PYP and Δ25 PYP.**
Fluorescence excitation spectra of NR, with detection at 600 nm (dashed line) and 659 nm (dotted line), selectively monitoring pB-associated NR and free NR, respectively. The emission spectrum (solid line) was recorded with excitation at 540 nm. A: steady-state mixture of pG and pB (0.7: 0.3) of WT PYP at pH 8.0. B: steady-state mixture of pG and pB (0.1: 0.9) of Δ25 PYP at pH 8.0.

7.1.2 PAS domains, common structure and common flexibility
7.1.3 NMR studies on the solution structure of the pB intermediate of PYP, using E46Q PYP and Δ25 PYP

Whereas the structure of the long-lived photocycle intermediate pB has been determined using X-ray crystallography, several spectroscopic studies have shown that the structural changes in solution are much larger than those seen in the pB structure in the crystalline state. Xie et al. have shown that the structural changes in solution are indeed larger than those in crystals, using one and the same technique, time-resolved FTIR, in two different environments (Xie et al., 2001). Because of this difference, it is of paramount importance to get detailed information on the structure of pB in solution. Whereas NMR would be the technique to achieve this, attempts to use this technique have failed until recently mostly due to two reasons: (i) the unstructured parts present in pB obscure – by broadening - also peaks that stem from structured parts, making assignments very difficult, and (ii) not enough pB state can be accumulated to obtain enough information on its structure. Using protein variants with properties that circumvent these problems makes it possible to obtain structural information on the pB intermediate of PYP-derivatives in aqueous solution. The E46Q variant of PYP shows less structural unfolding, but has the disadvantage that its recovery step is faster than in WT PYP, resulting in less accumulation of pB under continuous illumination. In Δ25PYP, the part of the protein that shows most unfolding has been removed, and its photocycle is slower than in WT PYP, making it an ideal system to

Figure 2: Structure and maps of Δ25 PYP.
Ribbons representation of the crystal structure of Δ25 PYP. The asymmetric unit cell contains two proteins. Secondary structure elements are marked on the structures. The \( F_o - F_c, \Delta \text{calc} \) map just before including the chromophore is shown, contoured at 2.5 \( \sigma \). Hydrophobic residues that have become solvent-exposed because of the deletion of residues 1-25 are shown as sticks. Picture taken from (Vreede et al., 2003).
study the pH structure in solution. Both variants have been used in NMR studies ((Derix et al., 2003), Bernard, C. et al., unpublished results). It was shown by multidimensional NMR experiments that the structural changes during the photocycle are less pronounced in E46Q PYP than in WT PYP (see Figure 3). A low-resolution structure of pH showed that in the groundstate, the structure of E46Q PYP closely resembles that of WT PYP. Structural changes after excitation were followed by comparing NOESY-spectra of pH and pB. Although there was less line-broadening upon illumination than in WT PYP, illustrating the smaller extent of unfolding, peak overlap still made it impossible to assign 60% of the protons in the pB state. The absence of E46 prevents the formation of a negative charge in the hydrophobic chromophore pocket, thereby making the "protein quake" as described by Xie et al. less disturbing (Xie et al., 2001). However, parts of the protein, i.e. the N-terminus and the chromophore binding region still cannot be assigned, illustrating that these parts show structural changes even in this mutant. The solution structure of the groundstate of Δ25PYP has been solved with a backbone root mean square deviation (rmsd) of 1.02 Å (Derix, 2004). It closely resembles the crystal structure, with the largest displacements in the regions comprising residues Ile49 to Arg52 and Met100 to Thr103. H/D exchange experiments showed

Figure 3: Chemical shift difference between pH and pB for WT and E46Q.
Chemical shift differences are superimposed on the structure of WT PYP for WT (a) and for E46Q (b). Peaks are colored lighter with increasing chemical shift difference. Peaks that were no longer visible in pB are also colored ligh-grey. Peaks that could not be detected due to ambiguity or spectral overlap are colored gray. An arrow indicates the mutation.
that the protection factors in Δ25PYP are very similar to WT PYP, except for the helix consisting of residues Asn43 to Thr50, which showed no protection in Δ25PYP. HSQC spectra of the illuminated sample show much better resolved cross-peaks than in WT, indicating the smaller degree of disorder that is left in Δ25PYP. This enables a detailed structural study of the pB state of this protein. So far, assignment is not completed, but a low resolution structure has been determined (see Figure 4). Preliminary results show that at least the β-sheet core of the protein remains intact in pB.

7.2 A combination of site-directed mutagenesis and chromophore analogs to study the PYP photocycle

In Chapters 4 and 5, site-directed mutagenesis and chromophore derivatives are shown to be important tools to understand the mechanism of the PYP photocycle in atomic detail. Some protein variants and chromophore derivatives that were prepared are not discussed in these two chapters; they will be discussed in the following paragraphs.

7.2.1 Locked Chromophores to study the isomerization process

A derivative of p-coumaric acid, 7-hydroxy-coumarin-3-carboxylic acid, has been used to study the effect of effectively locking the chromophore in its trans configuration (Cordfunke et al., 1998), Chapter 4). As can be expected, PYP reconstituted with this chromophore is strongly impaired in its ability to photocycle and shows a highly increased quantum yield of fluorescence. Two other chromophore derivatives have been synthesized that are locked in a specific configuration. The chemical structures of the used chromophores are shown in Figure 5. In rot-lock, the rotation of the phenol-ring along the long axis of the chromophore has been locked. The resulting protein shows an absorption spectrum similar to WT PYP, as can be seen in Figure 6.
Genera l discussio n

Figur ee 5: Overvie w  o f  chromophor e  derivative s  
use dd  i n  thi s  study .  
Chromophor e  ss  wer e  use d  t o  construc t  hybri d  PYP' s  
in  whic h  th e  chromophor e  i s  eithe r  locke d  i n  it s  is-  
configuratio nn  (b,  cis-lock),  o r  wh e r e  th e  phenoli c  
rin gg  o f  th e  chromophor e  ca n  no rtat e  wit h  respec t  
too  th e  coumaryl  tai l  (c,  rot-lock).  A  show s  th e  nativ e  
chromophor e  ,, pCA .  
Afte rr  photoexcitation , th e  protei n  enter s  a  
photocycl ee  an d  recove rs  it s  groundstat e  wit h  a  
rate  constan t  k  =  1.5  s~¹ , simila r  t o  th e  rate  
constan t  i n  wild-typ e  PYP  wit h  a  pCA  
chromophor e  .  Ultrafast  measurements  o n  th e  
free  chromophor e  show  th at  al so  th e  primar y  
photochemistry  in  thi s  system  is  hardly  
affected  b y  locki ng  th e  phenoli c  rin g  (Larsen  
et al.,  unpublished  results).  Th e  othe r  
chromophor e ,  cis-lock,  is  effectivel y  locke d  i n  
it s  cis  configuratio n  (see  Figure 5).  Th is  
modification  doe s  hav e  a  pronounce d  effec t  o n  
the  absorp tio n  spectrum ,  a s  ca n  b e  see n  i n  

Figure 6. Presumably, the chromophore is  
protonated, resulting in the large blue-shift in  
the spectrum. A pKₐ could not be determined  
yet; at pH 10, the spectrum looks the same as  
at pH 8, suggesting a very high pKₐ in this  
chromophore. This chromophore can be of use  
in both structural research of the pB  
intermediate (in solution), and in studying the  
signaling partner of PYP, since this  
chromophore presumably induces a pB-like  
conformation in the protein backbone.

Figure 6: Spectra of PYP hybrids reconstituted  
with chromophore derivatives.  
Spectra were taken in the dark in 10 mM Tris/HCl,  
PpH 8.0. Solid: pCA, dash: rot-lock, dotted: cis-lock

7.2.2 Site-directed mutagenesis

Specific roles of amino acid residues of PYP  
can be proposed on the basis of structural  
information obtained by X-ray  
crystallography. In these cases, site directed  
mutagenesis can test these hypotheses. Below,  
three of these PYP variants are discussed:  
R52A PYP, R124A PYP and F96L PYP.
Arg52 is believed to play an important role in PYP structure and functioning. The residue is said to "shield" the chromophore from the solvent, which becomes clear from the 3D crystal structure. After photoexcitation, the arginine residue swings out of its pocket, thereby exposing the chromophore to the solvent. Furthermore, the positively charged arginine may at some point interact through a salt-bridge with the negatively charged chromophore. Quantum mechanical calculations have indicated another role that the arginine residue may play: *i.e.* stabilizing the negative charge on the chromophore (upon excitation; Groenhof *et al.*, 2002). Genick *et al.* showed that the R52A variant of PYP behaves very similar to WT PYP (Genick *et al.*, 1997b). We constructed both R52A, R52G, and R124A variants. The latter was constructed because X-ray crystallography had shown that Arg124, along with Arg52, is among the residues that show the largest degree of movement after photoexcitation of the protein. Also a double mutation, *i.e.* R52A/R124A was made, because of the possibility that one of the arginine residues takes over the function of the other when that is replaced by a non-charged side-chain. Interestingly, both R52A and R52G PYP are yellow proteins at neutral pH, in other words water molecules can not penetrate into the active site to protonate the chromophore. The proteins have absorption maxima at 450 nm (R52A PYP) and 444 nm (R52G PYP). So truncation of the sidechain of residue 52 does not open the pocket to solvent, possibly because the protein folding around this residue has slightly changed. The pKₐ of protonation does increase upon changing the residue to an alanine or a glycine: in R52A, two pKₐ's were found: 3.7 and 4.4, and one pKₐ of 4.7 was found in R52G. When looking at the pH dependent absorption spectra in these mutants, it is clear that there are two pB-like species: one with an absorption maximum at 365 nm, existing between pH 3.5 and 4.5, and one with an absorption maximum at 355 nm, existing below pH 3.5 (see Figure 7). Since the maxima are so close together, it is not possible to determine pKₐ values for both transitions from these data. Presumably, the higher pKₐ value corresponds to protonation of the phenolic oxygen of the chromophore in the folded protein, whereas the lower is a direct result in low pH-induced denaturation of the protein.

Also the photocycle, as reflected by the rate constants of the specific reactions and quantum yields, has not drastically changed; the R52A variant shows a slight decrease in the rate of groundstate recovery, this effect is more pronounced in the R52G variant, which shows a rate of groundstate recovery of 0.02 s⁻¹, ~ 20-fold slower than WT PYP. Surprisingly, the R124A protein has the same spectroscopic characteristics as WT PYP, and the R52A/R124A double mutant has the same properties as the R52A single mutant. This
indicates that an Arg-residue at position 124 is functionally not very important for PYP. It remains to be determined whether the functionality of the Arg52 residue can be taken over by another nearby residue, when the former is replaced.

Figure 7: pH titration of R52A PYP
A: Dependence of the absorption spectra on pH. B: absorbance at the absorption maximum as a function of pH. The solid line was obtained by fitting the data to the Henderson-Hasselbalch equation.

Phenylalanine 96 is one of the hydrophobic amino acids that make up the chromophore-binding pocket. Furthermore, Phe96 has been postulated to play an important role during the early events in the PYP photocycle; it has been speculated to be part of a transient pericyclic reaction involving the chromophore and this residue (see Figure 8; Radding et al., 1999).

If this hypothesis is correct, changing the phenylalanine residue to a non-aromatic one would be expected to greatly effect primary events in the photocycle of PYP. To test this, we constructed the F96L variant of PYP. Surprisingly, this variant behaved very similar to wild-type PYP with respect to absorption maximum, photocycle kinetics, and quantum yield of fluorescence. Although it may be that differences in the primary photochemistry in this variant can be seen using ultrafast spectroscopy, the above mentioned similarities with wild-type PYP suggest that the residue does not play the role as postulated by Radding et al.

7.3 Primary events in the photocycle of PYP

The primary photochemistry in PYP is based on trans/cis isomerization of the vinyl double bond in the chromophore. However, the exact details about isomerization are not observed easily. Questions like: via which mechanism
does the isomerization proceed, and what is the exact role and time of the chromophore carbonyl flipping, i.e. breaking its hydrogen bond with the cysteine backbone amide, are difficult to answer. Several recent experiments have tried to shed light on these questions, as is described below.

By determination of the so-called Stark effect, it was concluded that a charge translocation over the chromophore takes place upon excitation (Premvardhan et al., 2003). Using Stark effect spectroscopy, the field-induced change in an absorption spectrum is measured. From this spectrum, the magnitude of the change in the permanent dipole moment $\Delta \mu$ and the (average) change in polarizability $\Delta \alpha$, can be deduced, which are a measure of the degree of charge motion upon photoexcitation.

It was found that in PYP, upon excitation, there are large changes both in the permanent dipole moment, ($|\Delta \mu| = 26$ Debye) and in the (average) change in polarizability ($\Delta \alpha = 1000\text{Å}^3$). From the value of the dipole moment change the extent of charge separation in the molecule can be estimated, where one unit of electronic charge separated by 1 Å corresponds to $\sim 4.8$ Debye in vacuo. This implies that upon excitation there is charge separation of one unit of electronic charge over a distance of $\sim 5$ to 6 Å in WT-PYP. This estimated charge-transfer distance implies that the negative charge presumably localized on the phenolate oxygen atom, O1, in the ground state, is displaced toward the thio-ester moiety (see Figure 9). This proposal is further substantiated by calculations on model pCA systems (Groenhof et al., 2002; Molina and Merchan, 2001; Sergi et al., 2001).

**Figure 9:** Model for charge redistribution on excitation

The possible route of charge translocation from the phenolic oxygen towards the carbonyl group is indicated
We hypothesize that this charge motion would consequently increase the flexibility of the thioester tail, by decreasing the activation barrier for the rotation of the C=\(C\) bond in this moiety in the excited state.

Another powerful technique that is used to study early events in the PYP photocycle is infrared spectroscopy, e.g. visible pump/mid-infrared spectroscopy as was used by Groot et al. (Groot et al., 2003). Using this technique, changes in the chromophore and nearby amino acids could be followed with \(~200\) fsec. time resolution. It has been shown that upon photoexcitation the trans bands are bleached, and shifts of the phenol ring bands are observed. The latter are explained as a charge translocation that enables the isomerization process, as is described above. The isomerization was shown to have occurred on a 2 ps time scale, and is accompanied by breaking of the hydrogen bond of the carbonyl oxygen to the cysteine backbone amide. Both techniques described above show that upon photoexcitation, charge translocation takes place, presumably from the phenolic oxygen towards the coumaryl tail of the chromophore. A recent paper, on the analysis of a high-resolution structure of PYP, suggests that already in the groundstate the chromophore adopts a hybrid electronic configuration, combining a phenolic configuration with a quinonic configuration (Getzoff et al., 2003), but this has not been confirmed by other techniques, or computational studies.

Ultrafast dynamics of the PYP chromophore (or, rather, model compounds such as TMpCA, described in Chapter 5 of this thesis) in solution have been determined using pump-probe experiments (Changenet-Barret et al., 2001; Larsen et al., 2003). Recently, these pump-probe experiments have been extended towards so called pump-dump-probe experiments. With this novel technique, multiple pathways dynamics could be separated, both in TMpCA and in PYP (Larsen et al., unpublished results). One of the most surprising findings was a resonantly enhanced ionization pathway after LASER excitation, that generated detached electrons and radicals. This ionization of the chromophore was found both in the protein-bound chromophore and in solution.

7.4 Concluding remarks

We are heading towards a situation where we can understand the process of signal perception and subsequent transduction in photoreceptor proteins on an atomic scale, with time resolution down to the femtosecond range. With the discovery of previously unknown photoreceptors, for example from genome sequencing projects, similarities between different types of photoreceptor proteins are found, but also new questions
arise. With the finding of the flavin-type photoreceptor proteins, it turned out that not in all cases the photochemistry can be based on \textit{trans/cis} isomerization. The types of chemistry that are responsible for formation of a signaling state in these proteins are just beginning to become clear: for example electron transfer in the cryptochromes, proton transfer, possibly accompanied by changes in chromophore stacking, in AppA, and transient cysteinyll adduct formation in phototropin LOV domains. In all cases, a configurational change in the chromophore results in a conformational change in the protein backbone. A striking feature of many photoreceptors is that subsequent to photo-excitation of the chromophore, an intra-molecular proton transfer takes place. This may be an important (electrostatic) feature required for the subsequent conformational change that drives a photoreceptor protein into its signaling state.

In PYP, the early photocycle events are well understood, with one of the most important newly found aspects the above described charge separation that precedes and presumably makes possible \textit{trans/cis} isomerization. However, there are still ambiguities, such as the precise sequence of events regarding isomerization and rotation of the chromophore carbonyl group, as described above. However, together with the rhodopsins, the xanthopsins have the best characterized primary photocycle events.

Another question that is surfacing in organisms that have multiple photoreceptor proteins, such as plants, concerns the intensity of their mutual interactions. Whereas the (two) phototropins function in more or less linear response pathways, the redundant cryptochromes and phytochromes jointly regulate (Lin, 2002a) - through a very complex network - a variety of responses, at levels varying from the transcriptional to the post-translational (Quail, 2002). Functional redundancy of the phytochromes and their interaction with several blue light photoreceptors enhance sensitivity to light signals, facilitating the accurate detection of, and response to, environmental fluctuations (see for a recent review about the interactions among the phytochromes and the integration of light signals with directional and temperature sensing mechanisms (Franklin and Whitelam, 2004)). It remains a challenge to rationalize the underlying mutual interactions from knowledge about the dynamical changes in the structure of the photoreceptor proteins that are initiated by light. In micro-organisms, where far less different types of photoreceptor proteins have been found in a single organism, this problem of complex networks has not (yet) been an issue.

Many photoreceptor proteins furthermore display light-induced branching reactions in their photocycle, particularly originating from long-lived blue-shifted
intermediates, bringing the protein back in its receptor state. This has been shown for many (archaeal) rhodopsins (e.g. (Balashov et al., 2000)), xanthopsins (e.g. (Miller et al., 1993)), and now also for a LOV domain (Kennis et al., Free University Amsterdam, unpublished observations). Particularly for photoreceptor families with extremely low recovery rates ($<10^{-3}$ s$^{-1}$, like some phototropins and BLUF proteins) this has important functional consequences: Under many conditions they may actually operate as 'two-photon switches', such that short-wavelength irradiation negates the effect of visible light.

Considering the events in the second half of the photocycle of PYP (the formation of $pB$ and the recovery to $pG$), a lot of information has already been obtained from X-ray crystallography studies, but we are now close to understand the events as they take place in solution, using multi-dimensional NMR (Chapter 7.1.3) and infrared techniques (Xie et al., 2001). Interesting will be to see how interaction with a signal transduction partner influences the structure and the kinetics of the $pB$ state. In general, knowledge on the downstream signal transduction cascade is of utmost importance to have an in vivo assay to test PYP function. Possibly, the outcome of several genome sequencing projects will help with the identification of this partner; because in bacteria, functionally related genes are often clustered together in the genome, downstream genes, such as those described in Chapter 6, may very well be involved in PYP signaling. An important, but still poorly understood aspect during the late events in the photocycle is how the protein backbone functions as a catalyst in the reisomerization of the chromophore. M100 is postulated to play an important role in this process; PYP variants in which M100 has been replaced by another amino acid shows extremely decelerated ground state recovery. Kumauchi and coworkers studied the pK$_a$ of the $pG$ recovery process and the activation enthalpy $\Delta H^+$ (calculated from the temperature dependence of the rate of $pG$ recovery). From the linear correlation between the two they conclude that M100 reduces the energy barrier of the $pB$ decay process (Kumauchi et al., 2002). In this model M100 would donate electron density towards the chromophore, thereby weakening the interaction between the chromophore and Arg52. Direct evidence for this model is not available at this moment; possibly this issue can be settled when the recovery of $pG$ can be followed in solution using NMR spectroscopy. Another important question that has been solved during the last few years, with PYP in a leading role, was how the mesoscopic context of a protein can influence its structure and dynamics. That it does, was elegantly shown in (Xie et al., 2001), but the exact mechanism remains unclear. Possibly the finding of the influence of hydration on the photocycle, as described in Chapter 3 of this thesis, will help us further in
understanding this influence of the mesoscopic context in more detail.

An obvious common feature among many photoreceptor proteins is the involvement of one or more PAS domains. Therefore, this class of molecules may also significantly further our basic understanding of this very important signal-transduction module. Through detailed analysis of the structural alterations induced by light-activation of LOV domains from phototropins, Moffat and colleagues (Crosson et al., 2003) recently discovered a structural element that is conserved in many PAS domains: A salt bridge linking the αB helix with the βC/βD loop. The hydrogen bond between Arg52 and the backbone carbonyl of Tyr-98 may be its xanthopsin equivalent (in E-PYP as well as in Prr-PYP; see also (Rajagopal and Moffat, 2003a). Signal-induced disruption of this bridge, and the resulting flexibility of the βC/βD (or: M100) loop, may constitute a universal signal transfer mechanism, to a downstream signal transduction partner, among PAS-domain containing proteins (see Figure 10). The flexible αC/βC loop of the recently characterized human PAS kinase (Amezcue et al., 2002) could then be a nucleotide recognition surface, required for signal input. In more recent work from the same group, light-induced structural changes in a C-terminal helix of the LOV2 domain of phototropin were found using NMR spectroscopy. After unfolding, one face of the central β-sheet becomes solvent-exposed. This parallel with PYP suggests that both photoreceptor proteins use comparable signal transduction mechanisms, despite of the large difference between the two regarding cofactor and photochemistry. Possibly, this can even be extended to other, non-photoreceptor PAS

Figure 10: Conserved pathway of structural connectivity in PAS domains. (A) Detail of the structural position of the salt bridge and flanking aromatic side chains in LOV2, FixL PAS, and HERG. Salt bridges are shown as dashed lines. (B) Residues that are part of the structurally interconnected pathway leading from the FMN cofactor to the conserved surface salt bridge. All residues that are shown are in van der Waals contact with adjacent residues (±0.2 Å). The large gray arrow shows the pathway of structural connectivity from the flavin cofactor to the salt bridge. Picture taken from (Crosson et al., 2003).
domains. Once this type of extrapolation can be made, the wealth of information obtained in studies of photoreceptor proteins can help in the understanding of the mechanism of signaling in other proteins.