Structural Insights into Photoactivation of Plant Cryptochrome-2

Malathy Palayam†, Jagadeesan Ganapathy†, Angelica M. Guercio†, Lior Tal†, Samuel L. Deck†, and Nitzan Shabek†

Supplementary Information

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Supplementary Figure 1. Multiple sequence alignment and conservation analysis of selected cryptochromes and photolyase ortholog. Amino acid alignment of 12 plant cryptochrome proteins with photolyase used as outgroup for comparison. Intensity of red behind residues shows degree of divergence. Numbers on residues refer to position in *At*CRY2-PHR sequence. Key *At*CRY2-PHR subdomains N-terminal α/β domain (dark green), C-terminal α- domain (bright green), and Linker Region (red line) are indicated above alignment. Secondary structure of *At*CRY2-PHR sequence is shown below sequence in blue as alpha helices (α1- α20), beta sheets (β1- β6), 3_10 helices (3_10) and non-secondary structure-containing loops (L1-L26). Black arrow indicates residues involved in Trp triad. Magenta ovals indicate residues involved in formation of H-T interface and Cyan ovals indicate residues involved in formation of H-H interface. In naming scheme *At* represents *Arabidopsis thaliana*, Tomato *Solanum lycopersicum*, Soy *Glycine max*, Rice *Oryza sativa*, Fern *Asplenium yunnanense*, Zmays *Zea mays*, and Agro *Agrobacterium tumafaciens*. 
Supplementary Figure 2. AtCRY2-PHR protein characterization. (a) Size exclusion analysis of purified AtCRY2 PHR domain. MW were estimated by biomolecule standard markers. Elution fractions were resolved by SDS-PAGE and Coomassie staining (i) and UV absorption spectra (ii). (b) SEC-MALS analysis of AtCRY2-PHR domain. (c) Absorption spectra of AtCRY2-PHR proteins in solution and in crystals was measured at the range of 300 nm to 700 nm wavelength. Sample of the purified AtCRY2-PHR used for crystallization trials was resolved by SDS-PAGE and visualized via Coomassie staining.
**Supplementary Figure 3. Structural analysis of CRYs.** (a) Superposition of monomers A (cyan), B (pink), C (purple), D (green) of *AtCRY2-PHR* tetramer determined in this study. The r.m.s deviation is calculated for the superposition between monomers A and D (0.59 Å), monomer B and C (0.59 Å) and monomer B and D (0.62 Å) chains respectively. (b-c) Superposition of *AtCRY2-PHR* tetramer (cyan) with mutant *ZmCRY1C* mutant (blue, PDB: 6LZ3) resulting in a r.m.s deviation of 1.0 Å. Top view of tetramers superposition with close up view on oligomeric interface (b) and side view of monomers (c). (d) Dimers superposition of *AtCRY2-PHR* tetramer (cyan, monomer A and monomer D) with the two copies of *AtCRY2-PHR* monomer, (copy A’ and copy B’ in the Asymmetrical Unit, ASU, shown in yellow and grey respectively, PDB: 6K8I). The two distinct rearrangements resulting in r.m.s.d of 0.88 Å for all the Cα atoms for the alignment in (i), and r.m.s.d of 0.86 Å for the alignment in (ii). (iii) Superposition of *AtCRY2* tetramer (cyan) with the *AtCRY2* monomer (yellow, PDB: 6K8I) and its symmetrical mate generated from the crystal structure (crystallographic two-fold symmetry in C222₁ space group, *AtCRY2* monomer grey). (e) Superposition of *AtCRY2-PHR* tetramer (monomer A, cyan) and CRY2-PHR monomer (copy A’, yellow, PDB: 6K8I). Side chains with distinct structural deviation are labeled and shown as sticks, close ups of key residues are shown with black arrows (i). (ii) Electron density maps of represented rotamers are shown as 2Fo-Fc mesh (σ = 1.2).
Supplementary Figure 4. Characterization of CRYs tryptophan triad and FAD pocket. (a) 2Fo-Fc electron density map (1σ cut-off) (mesh light blue) of FAD (yellow) binding pocket within \( AtCRY2\)-PHR\_tetramer (cyan) with the direct interacting amino acids residues shown as sticks. (b) 2Fo-Fc electron density map (1σ cut-off) (mesh light blue) of FAD (yellow). (c) Close up view on the superposition of \( AtCRY2\)-PHR\_tetramer with \( AtCRY2\)-PHR\_monomer (PDB: 6K8I) within the FAD binding pocket. r.m.s.d of 0.4 Å. (d) Sequence alignment and conservation of cryptochrome FAD binding pocket across plant species. Colors indicate amino acid polarity. (e) Structural analysis of \( AtCRY2\)-PHR\_tetramer (light blue) tryptophan triad superposition with \( AtCRY1\) (green, PDB: 1U3C), \( AtCRY2\)-PHR\_monomer (yellow, PDB: 6K8I), \( AtCRY2\)-PHR\_inactive (Magenta, PDB:6K8K), \( ZmCRY1\)C mutant (Blue, PDB:6LZ3).
Supplementary Table 1. Detailed summary of PHR photo-induced conformational changes

| Major structural changes          | Residue positions       | Proposed Function                  | References |
|----------------------------------|-------------------------|-----------------------------------|------------|
| Presence of a short helix         | 189-192 (AtCRY2-PHR\textsubscript{inactive})*  
181-184 (AtCRY2-PHR\textsubscript{inactive})**  
189-192 (AtCRY2-PHR\textsubscript{monomer})**  
187-190 (AtCRY1-PHR)  
144-146 (AtCRY1-PHR) | Photo-inactivation       | This study and 1-3 |
| Absence of a short helix          | 189-192 (AtCRY2-PHR\textsubscript{tetramer})  
181-184 (AtCRY2-PHR\textsubscript{tetramer}) | Photo-activation and oligomerization | This study and 1-3 |
| Presence of 3\textsubscript{10} helix | 448-450 (AtCRY2-PHR\textsubscript{tetramer}) | Photo-activation and oligomerization | This study |
| \(\alpha\)9 helix                | Change of 0.32Å deviation 453-459 (AtCRY2-PHR\textsubscript{inactive})  
Change of 0.41Å deviation 453-459 (AtCRY2-PHR\textsubscript{monomer})  
Change of 0.39 deviation 455-463 (AtCRY1-PHR) | Participate in oligomeric interface | This study and 1-3 |
| \(\alpha\)8 helix                | Change of 0.32Å deviation 427-433 (AtCRY2-PHR\textsubscript{inactive})  
Change of 0.32Å deviation 427-433 (AtCRY2-PHR\textsubscript{monomer})  
Change of 0.32Å deviation 430-437 (AtCRY1-PHR) | Participate in oligomeric interface | This study and 1-3 |
| \(\alpha\)3 helix                | Change of 0.20Å deviation 340-350 (AtCRY2-PHR\textsubscript{inactive})  
Change of 0.19Å deviation 340-350 (AtCRY2-PHR\textsubscript{monomer})  
Change of 0.21Å deviation 342-354 (AtCRY1-PHR) | Participate in oligomeric interface | This study and 1-3 |
| \(\alpha\)2 helix                | Change of 0.24Å deviation 326-333 (AtCRY2-PHR\textsubscript{inactive})  
Change of 0.20Å deviation 326-333 (AtCRY2-PHR\textsubscript{monomer})  
Change of 0.15Å deviation 328-337 (AtCRY1-PHR) | Participate in oligomeric interface | This study and 1-3 |
| \(\alpha\)6 helix                | Change of 0.4Å deviation 201-209 (AtCRY2-PHR\textsubscript{inactive})  
Change of 1.3Å deviation 201-299 (AtCRY2-PHR\textsubscript{monomer})  
Change of 1.0Å deviation 199-213 (AtCRY1-PHR) | Participate in oligomeric interface | This study and 1-4 |

* AtCRY2-PHR\textsubscript{inactive} represents the molecular structure of CRY2-PHR in complex with BIC2  
** AtCRY2-PHR\textsubscript{monomer} represents the molecular structure of CRY2-PHR in monomeric form 

Supplementary References

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