

Shining Light on Phytochromes: Ultrafast Photosignalling Captured by Time-Resolved Serial Crystallography

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Phytochromes are photoreceptor proteins found in plants, fungi, and bacteria that sense light by switching between red- and far-red-absorbing states. Photoconversion begins with isomerization of the bilin chromophore, yet despite extensive study, the earliest structural events following photoexcitation and how they initiate proteinwide responses have remained incompletely understood.

Here, we investigate the photoactivation reactions across two phytochrome families using femtosecond to millisecond time-resolved serial crystallography, supported by complementary spectroscopy and molecular modeling.

Our first study captures a structural movie of a prototypical phytochrome from *Deinococcus Radiodurans* and reveals the earliest structural response to photoexcitation. Within three hundred femtoseconds, the biliverdin chromophore begins to isomerize through a space conserving rotation while a conserved hydrogen bond network surrounding the biliverdin chromophore reorganizes and induces a protonation shift on a conserved nearby histidine.¹

The second work, resolves the structural evolution from femtoseconds to milliseconds in a bathy phytochrome from *Pseudomonas Aeruginosa*, capturing the structural trajectory of chromophore photoisomerization. Our data reveal a space conserving rotation of the biliverdin chromophore and indicate branching within the excited state pathway, leading to distinct early intermediates during the first steps of photoconversion.²

Together, these studies reveal how phytochromes exquisitely sense light, coupling ultrafast chemical events to coordinated protein structural changes that initiate biological signalling.

1. Shankar M*, Grunewald L*, Wahlgren W* et al. Ultrafast, remote-controlled protonation reaction enables structural changes in a phytochrome. *Sci. Adv.* **11**, 2025.

2. Meszaros P*, Grunewald L* et al. Structural photoactivation of a bathy phytochrome resolved by time-resolved X-ray crystallography. *In review*.