

# High-resolution Crystal Structures of Transient Intermediates in the Phytochrome Photocycle

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Phytochromes are red/far-red light photoreceptor enzymes in bacteria to plants, which elicit a variety of important physiological responses. They display a reversible photocycle between the resting (dark) Pr state and the light-activated Pfr state. The light signals are transduced as structural change through the entire protein to modulate the enzymatic activity. It is unknown how the Pr-to-Pfr interconversion occurs as the structure of intermediates remain elusive. Here, we present crystal structures of the bacteriophytochrome from myxobacterium *Stigmatella aurantiaca* captured by two different X-ray Free Electron Lasers, the Spring-8 Angstrom Compact free electron LAser (SACLA) and the Linac Coherent Light Source (LCLS). The structures were determined at early time points after light illumination of the Pr state. We observe large structural displacements of the covalently bound bilin chromophore, which trigger a bifurcated signaling pathway. The snapshots show with atomic precision how the signal progresses from the chromophore towards the output domains, explaining how plants, bacteria and fungi sense red light.

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