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Intramolecular Signal Progression of the Blue Light Receptor Plant Cryptochrome

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Plant flowering is a light-dependent process requiring activation of the photoreceptor plant cryptochrome (CRY) by excitation of the chromophore flavin adenine dinucleotide (FAD). Further, CRY is applied as optogenetic tool for light-switchable protein clustering in vivo. However, biophysical signaling in CRY from the photoreaction of FAD to the structural protein response is unclear. We identified several point mutants with dark constitutive activity including D393S. This aspartic acid protonates the FAD anion radical to the neutral radical during the FAD photoreaction, as a key step of CRY activation. Biophysical characterization of D393S by time-resolved spectroscopy showed formation of a FAD anion radical only stabilized for hundreds of microseconds after the laser pulse. Constitutive activity of D393S is driven structurally as the FAD redox potential is seemingly not affected by the mutation. In contrast to monomeric wild-type, D393S is already homomerized in the dark, suggesting a decoupling of the protein structure from the FAD. For wild-type CRY activation we propose a charge-driven disruption of the hydrogen bonding network, emulated by D393S. Structurally analyzing all constitutively active mutants allowed us to identify an activation via two distinct pathways. Our work provides new insight into the mechanism of CRY activation relevant for physiology and optogenetics.