

Investigating Nonadiabatic Photoisomerization Dynamics of Phytochrome Cph1 Using 2D ES

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We use high-sensitivity two-dimensional electronic spectroscopy (2D ES) to directly probe the conformational heterogeneity of the ground state populations in phytochromes. The sub-100 fs population dynamics of the P_{fr} to P_r reaction indicate photoisomerization proceeds through a conical intersection.

Phytochromes are chromophore-protein complexes found in plants and photosynthetic microorganisms. They serve as the “eyes” of the organism by monitoring the ratios and intensities of red and far-red light. Upon irradiation, phytochromes reversibly photoisomerize between an inert P_r state and a biologically active P_{fr} state via a ring-flip mechanism. Recently, there has been much interest in phytochromes within the biomedical engineering community due to their potential use as medical imaging probes and optogenetic actuators. Although the picosecond dynamics of phytochromes have been extensively studied, fundamental questions remain about sub-picosecond dynamics.

Of particular interest are the sub-100 fs dynamics of P_{fr} to P_r , as some have suggested that this photoreaction proceeds through a conical intersection or closely avoided crossing [1]. Additionally, the degree of homogeneity of the phytochrome ground states is still widely debated. Some studies have found each ground state to be homogeneous [2] while others have indicated heterogeneous conformations [3]. The inhomogeneous broadening limits the utility of transient absorption spectroscopy measurements.

Here we use high-sensitivity 2D ES and sub-7 fs laser pulses [4] to probe the initial dynamics of the P_{fr} to P_r photoisomerization reaction and directly measure the sub-100 fs population dynamics of phytochromes in the presence of conformational heterogeneity.

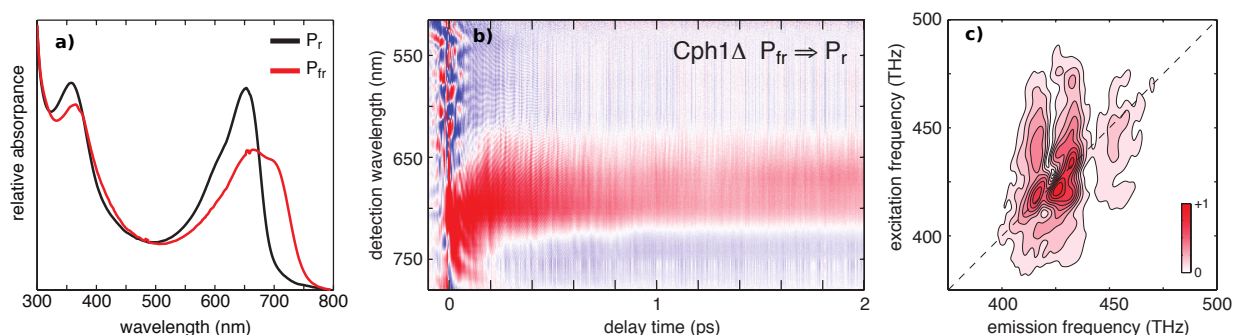


Fig. 1 a) The linear absorption spectra of P_{fr} (red) after five minute irradiation with 625 nm light, and P_r (black) after five minute irradiation with 735 nm light show reversible photoconversion of Cph1Δ. b) Transient absorption spectrum of Cph1Δ shows unusual and rapid blueshift for P_{fr} to P_r photoconversion. c) 2D ES of Cph1Δ at $\tau_2 = 50$ fs. Diagonal peaks show clear signatures of inhomogeneous broadening.

[1] Kim *et al.* *J. Phys. Chem. Lett.*, **4**, 2605 (2013)

[2] Dasgupta, J. *et al.* *Proc. Natl. Acad. Sci. USA*, **106**, 1784 (2009)

[3] Kim *et al.*, *Biochem.*, **53**, 2018 (2014)

[4] Bizimana *et al.*, *J. Chem. Phys.*, **143**, 164203 (2015)