Conformational Homogeneity in Phytochrome Cph1 P_r, Wild Type and Single-Site Mutants

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Phytochromes, chromophore-protein complexes in plants and some photosynthetic microorganisms, serve as the organism's "eyes" by monitoring the ratio of red to far-red light. They transduce this optical information into a biological signal by reversibly photoisomerizing between a biologically-inactive P_r (red-absorbing) form and a biologically-active P_{fr} (far-red-absorbing) form. Phytochromes are of great interest to biomedical technologists because of their potential as optogenetic actuators and deep-tissue imaging probes.

Many studies of phytochrome's picosecond dynamics have been performed, and all measured multiphasic kinetics. However, these studies have led to conflicting conclusions about the

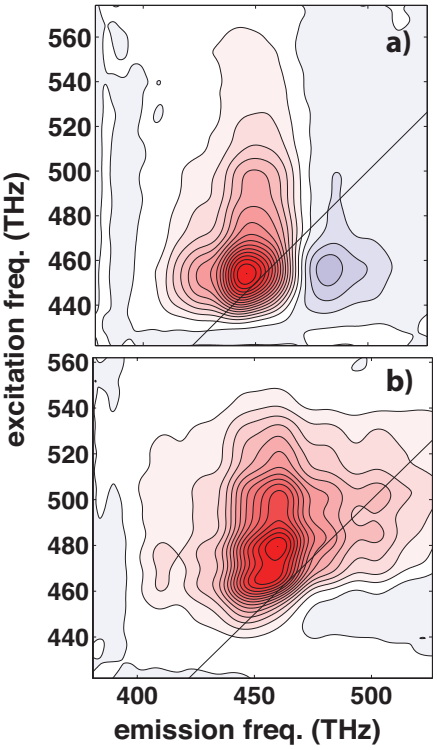

Figure 1. 2DES of a) wild type Cph1 P_r and b) Cph1 P_r Y176H at $\tau 2$ = 9 ps. The mutant shows significantly less excited-state absorption signal as well as increased spectroscopic heterogeneity, visible as peak elongation along the diagonal.

origins of such kinetics. Some studies have proposed a "heterogeneous model" in which the multiple lifetimes arise from distinct ground-state conformations with different photochemical transitions;[1,2,3,4] while some have proposed a "homogeneous model" — where multiphasic dynamics arise from a single ground-state population which bifurcates on the excited state.[5,6]

As a step toward resolving this controversy, we have directly probed the ground-state heterogeneity of Cph1 P_r with high-sensitivity two-dimensional electronic spectroscopy using visible broadband sub-6 fs pulses.[7,8] The 2D ES of Cph1 P_r WT, shown in Figure 1a, shows negligible inhomogeneous broadening, supporting the homogeneous model. However the 2D ES of Cph1 P_r Y176H shows at least two distinct subpopulations- visible as two peaks along the diagonal. We attribute this spectral heterogeneity to the ability of the histidine residue to stabilize several chromophore conformations through hydrogen-bonding. To complement our studies of spectral inhomogeneity, we measure the spectral dependence of the dynamics using 2D ES.

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