Supporting Online Information
Cryogenic and laser photoexcitation studies identify multiple roles for active site residues in the light-driven enzyme protochlorophyllide oxidoreductase

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Supplementary figures

Figure S1. 1D ^{1}H NMR spectra of wild-type (lower) and the Y193F variant (upper) of protochlorophyllide oxidoreductase (POR). Both samples comprise 0.15 mM protein in 100% D_2O containing 50 mM Tris and 1 mM DTT at pH 7.5. The spectra were recorded at 298 K on a Bruker DRX600 spectrometer equipped with a ^{1}H observe cryoprobe. Residual solvent resonance signals were suppressed using WATERGATE. 512 transients were recorded, each with an acquisition time of 2.6 s, and an exponential line-broadening of 2 Hz was applied prior to Fourier transformation. The spectra are virtually superimposable indicating that the mutation has no measureable effect on protein conformation. Inset is an expansion of the same spectra showing the region containing resonances from aromatic ring protons (6.0 - 8.0 ppm). Minor changes in resonances near to 6.6 and to 7.0 ppm are consistent with the mutation in a background of an otherwise conformationally unchanged protein.

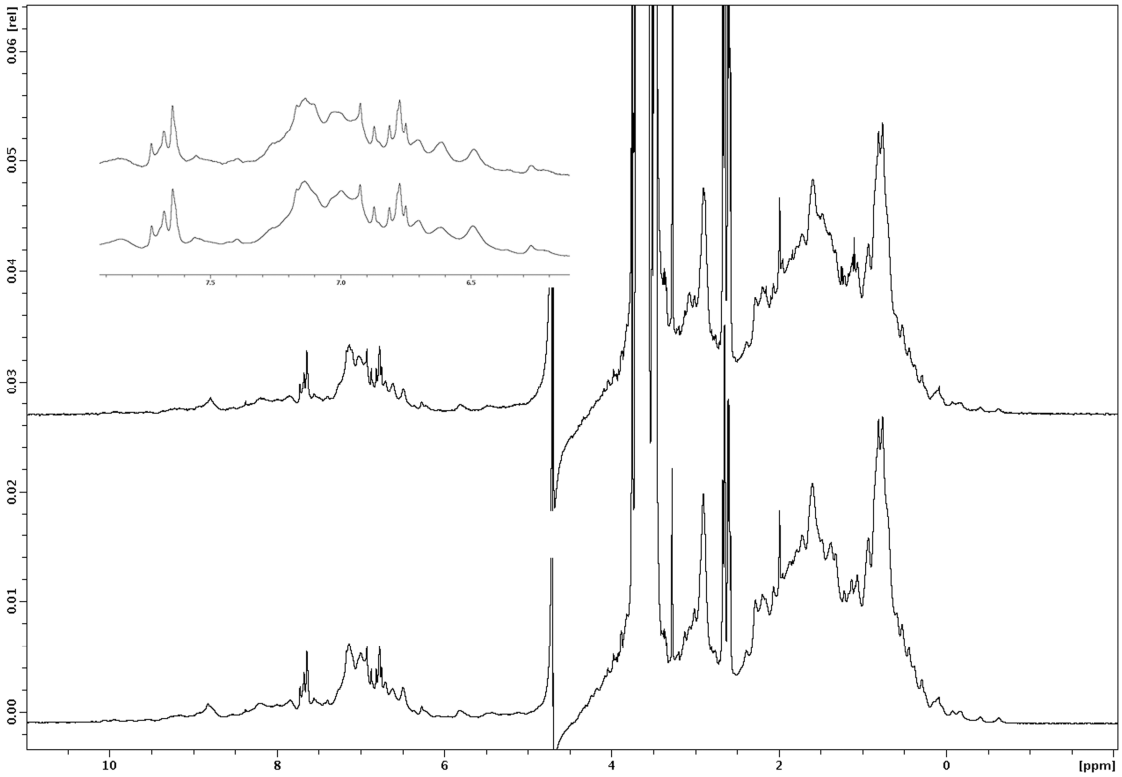

Figure S2. The binding of NADPH measured by fluorescence energy transfer for wild-type POR (A) and the Y193F mutant (B). The fluorescence emission of 0.25 μM enzyme was measured at 454 nm at increasing NADPH concentrations following excitation at 295 nm. The inset shows the typical fluorescence spectra that were measured for the experiments. The error bars were calculated from the average of at least 3 measurements.

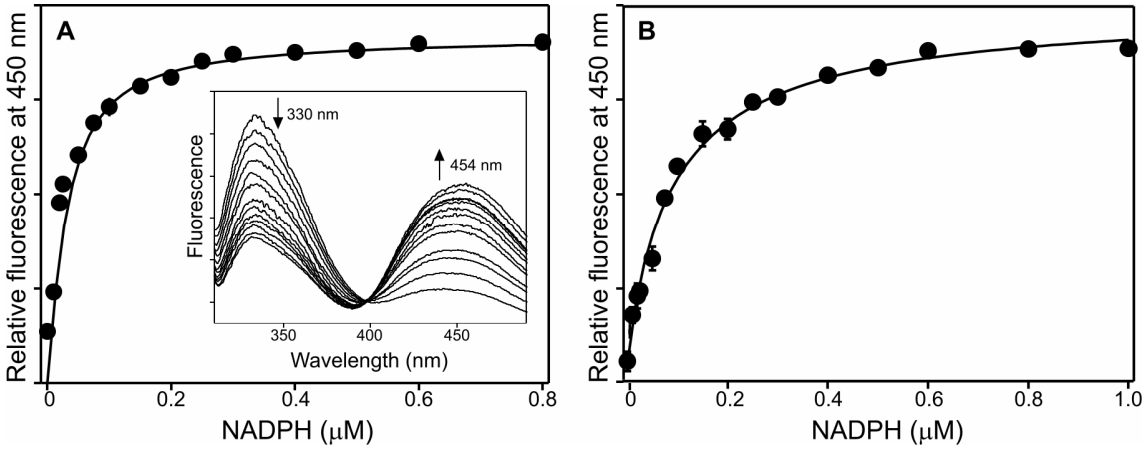

Figure S3. The binding of Pchlide measured by absorbance spectroscopy for wild-type POR (A) and the Y193F mutant (B). The ratio of absorbance at 642 nm to 630 nm for 0.25 μM Pchlide is measured at varyious POR concentrations in the presence of 250 μM NADPH. The insets show typical absorbance spectra of Pchlide and reveal the red-shift in the absorbance maximum upon binding to POR-NADPH. The error bars were calculated from the average of at least 3 measurements.