Supporting Information For

Microsecond Light-induced Proton Transfer to Flavin in the Blue Light Sensor Plant Cryptochrome

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1. Absorption spectrum of CPH1-PHR

Absorption spectra of the light-sensitive domain of the plant cryptochrome CPH1-PHR were recorded on a Specord S-100B diode array spectrophotometer (Analytic Jena) before the streak camera experiments (Figure S1). They show the typical fine-structured absorption of protein-bound flavin with maxima at around 370 and 450 nm.

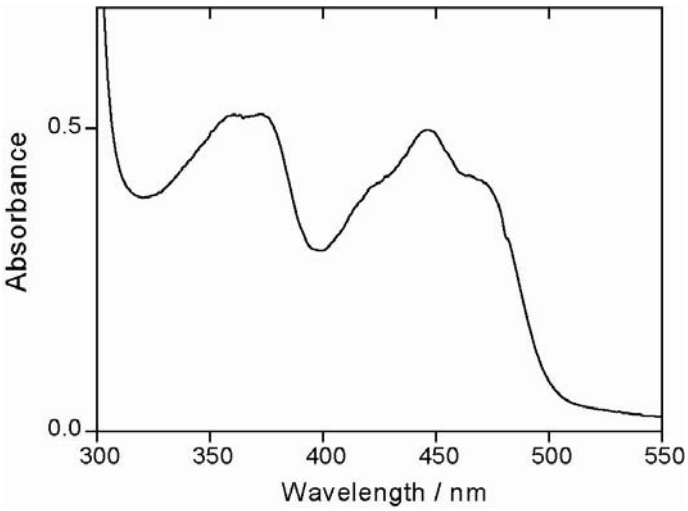

Figure S1. Representative absorption spectrum of CPH1-PHR before the streak camera experiments.

2. Streak camera image

Detection with a streak camera allowed for simultaneous recording of temporal and spectral information. The whole visible spectral range of 375-750 nm was covered. Analysis was limited to the spectral range >375 nm due to a cut off filter. The screenshot of a time window of 20 µs (Figure S2) shows the typical, temporal evolution of difference absorption after excitation of the plant cryptochrome CPH1-PHR. Negative and positive differences are both represented by the same color code. The negative difference absorption at 450 nm remains constant. Difference absorption at 400 nm and above 610 nm decays to a constant level within few microseconds, while simultaneously absorption at 500-600 nm increases.

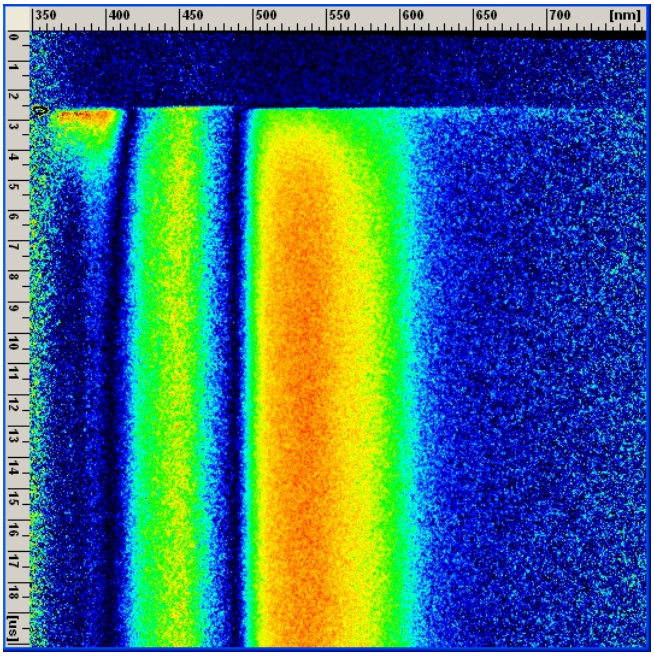

Figure S2. Screenshot from the streak camera showing a 20 µs time window.

3. Reference spectra of flavoprotein anion radicals and of a tryptophan radical

Absorption difference spectra of flavoprotein anion radicals minus oxidized state vary among different proteins (Figure S3). Difference spectra of *Drosophila* cryptochrome (dCRY) and of glucose oxidase (GOx) from *Aspergillus niger* were recorded after illumination with blue light (Berndt et al., 2007). Both anion radicals are characterized by a prominent, positive band at 400 nm and a broad absorption >550 nm. The spectra significantly differ in contributions to <410 nm and 500-550 nm. Tyrosine radicals show an absorption maximum at a similar position as flavin anion radicals, albeit rather at 405-410 nm than at 400 nm (Figure S3) (Bent and Hayon, 1975).

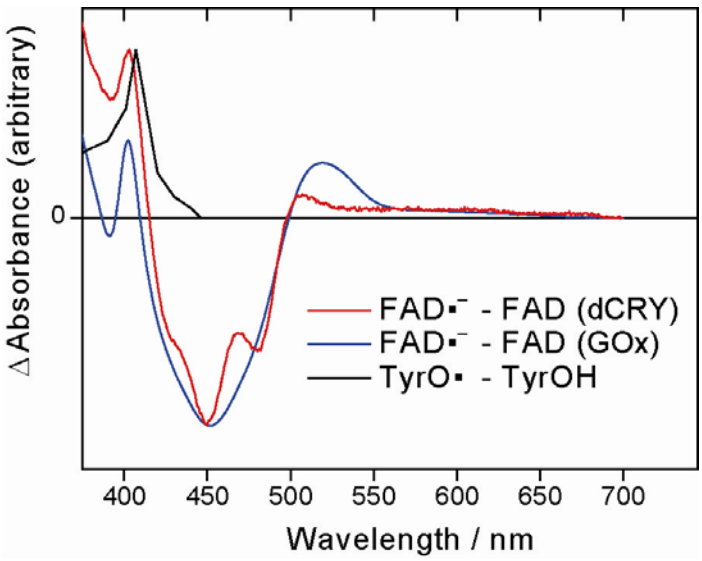

Figure S3. Difference spectra of the photoreduction of glucose oxidase (GOx) and *Drosophila* cryptochrome (dCRY) showing the reaction of oxidized flavin to the anion radical. Graphs were adapted from Berndt et al. (2007) and scaled at 450 nm. For comparison, the difference spectrum of a tyrosine radical minus tyrosine in water is presented (taken from Bent and Hayon, 1975).

References:

Bent, D. V.; Hayon, E. *J. Am. Chem. Soc.* **1975,** *97*, 2599-2606.

Berndt, A.; Kottke, T.; Breitkreuz, H.; Dvorsky, R.; Hennig, S.; Alexander, M.; Wolf, E. *J. Biol. Chem.* **2007**, *282*, 13011-13021.

4. Processes in the 200 μs time window

Difference absorption spectra of CPH1-PHR were recorded in the time window of 200 μs (Figure S4). Global analysis reveals a decaying component with a time constant >200 μs besides the contribution by the 1.7 μs decay. It is assigned to a decay of tryptophan neutral radical with a small contribution from the decay of FADH•.

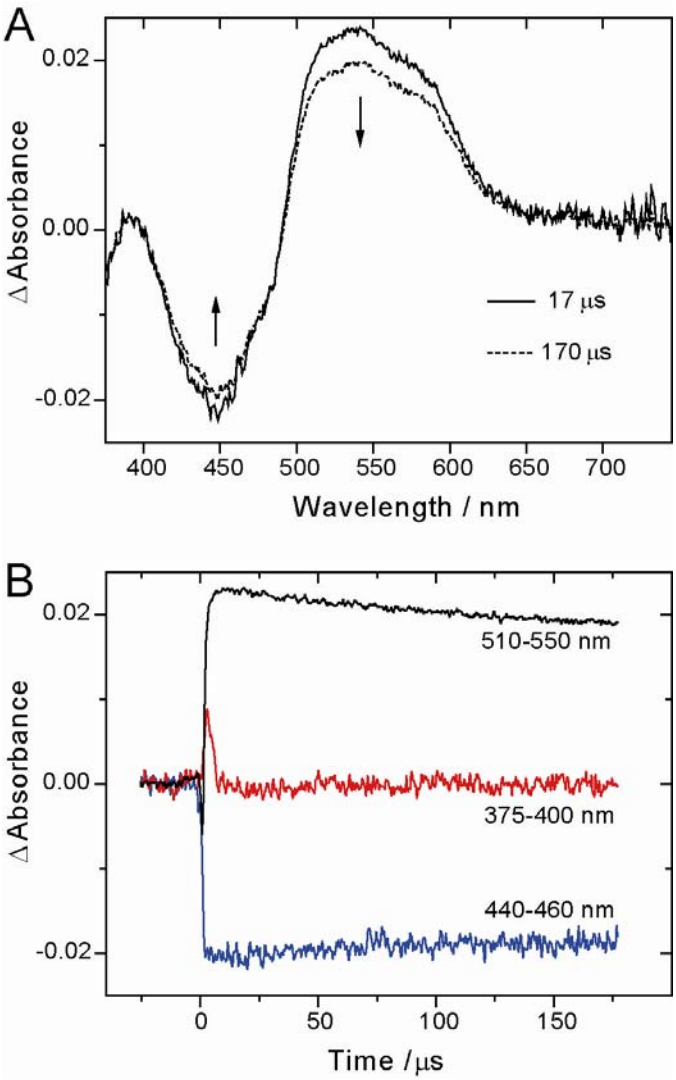

Figure S4. *(A)* Difference absorption spectra recorded at 17 and 170 μs after excitation of CPH1-PHR. Difference absorption decays at 500-610 nm and to a minor extend at about 450 nm. The decay process in the 200 μs time window is dominated by the tryptophan radical. *(B)* Kinetic traces extracted from indicated spectral ranges show a decay process with a time constant >200 μs.

5. Spectral fitting of contributions to the initial spectrum

A contribution of FADH• was included in the spectral fitting procedure to test if the initial spectrum can be reproduced without $TrpH^{\bullet+}$. As further species, a presence of $FAD^{\bullet-}$ and Trp• was assumed. When this fitting procedure was run without any constraints, the amount of FADH• was reduced almost to cero (data not shown). In a next step, the contribution of FADH• was fixed to account for the absorption in the spectral range of 550-600 nm. As a result, the fitted sum differed strongly from the initial spectrum at <415 nm, at around 450 nm, and at 520-560 nm (Figure S5). The absorption at 450 nm is mainly due to the sum of bleached FAD(ox). When the sum of difference absorption at 450 nm was fixed to the value of the initial spectrum, the other deviations increased even further due to the reduction in amount of $FAD^{\bullet-}$. It is concluded that FADH• does not significantly contribute to the initial spectrum.

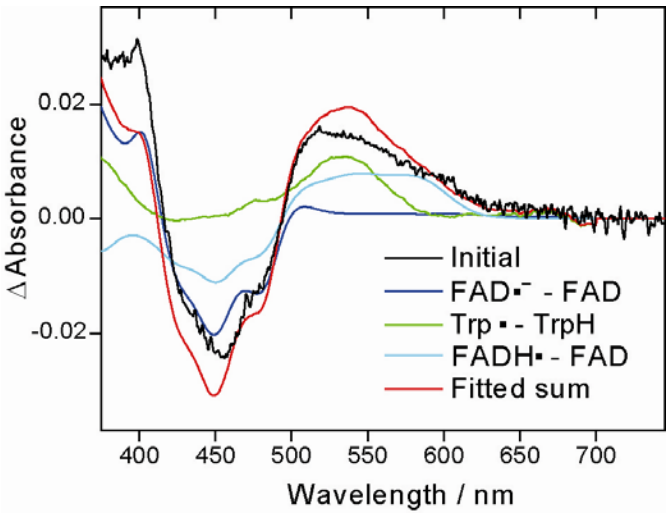

Figure S5. The initial spectrum was reproduced in a fitting procedure by including FAD neutral and anion radicals and the tryptophan neutral radical. Strong deviations of the fitted sum from the measured spectrum illustrate that the flavin neutral radical cannot account for the absorption at 550-600 nm.