Supporting Information For

Photoreaction of Plant and DASH Cryptochromes Probed by Infrared Spectroscopy: The Neutral Radical State of Flavoproteins

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Representative Infrared Absorption Spectra

The infrared absorption spectrum of LOV1-C57S shows the typical features of a protein spectrum with amide I and amide II bands of the backbone at 1651 and 1549 cm\(^{-1}\), respectively (Figure S1). Water absorbs in the region of 2900-3700 cm\(^{-1}\) and at 1650 cm\(^{-1}\), overlapping with the absorption of the amide I vibration. Accordingly, the ratio of absorbance \(A(1650) / A(1550)\) serves as an indicator for the hydration of the sample. For LOV1-C57S, this ratio is 1.8, indicating a low hydration. At lower water content, the protein did not produce a light-induced difference spectrum anymore.

![Infrared absorption spectrum of LOV1-C57S](image)

Figure S1: Infrared absorption spectrum of LOV1-C57S
In contrast, the preparation of CPH1-PHR resulted in a ratio of 2.4, evidencing a very well hydrated sample (Figure S2). The difference between the preparations becomes even more pronounced, if the molecular weight is taken into account with CPH1-PHR being about four times the size of LOV1-C57S.

The exchange of water to D$_2$O by lyophilization of CPH1-PHR resulted in a loss of the broad band at 2900-3700 cm$^{-1}$ (Figure S2). Hidden contributions by the N-H-stretching (amide A) vibrations of the protein backbone become visible at around 3400 cm$^{-1}$. Bands originating from D$_2$O are found at 2100-2800 cm$^{-1}$ and 1210 cm$^{-1}$. The amide I band was downshifted by ~5 cm$^{-1}$ to 1647 cm$^{-1}$, whereas the amide II band exhibited a stronger shift of more than 90 cm$^{-1}$ to 1456 cm$^{-1}$, where it might overlap with contributions from HDO. Taken together, these bands evidence a replacement of exchangeable hydrogen by deuterium in the sample.

![Figure S2: Infrared absorption spectra of CPH1-PHR in H$_2$O (solid line) and after exchange to D$_2$O (dashed line)](image-url)
The absorption spectrum of *AtCry3* shows a ratio of absorbance $R = 2.4$ at about the same size of the protein as CPH1-PHR, which demonstrates a well hydration. An additional absorption band at 1047 cm$^{-1}$ originates from glycerol in the buffer.

Figure S3: Infrared absorption spectrum of *AtCry3*