Proton-coupled electron transfer constitutes the photoactivation mechanism of the plant photoreceptor UVR8

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Supporting information

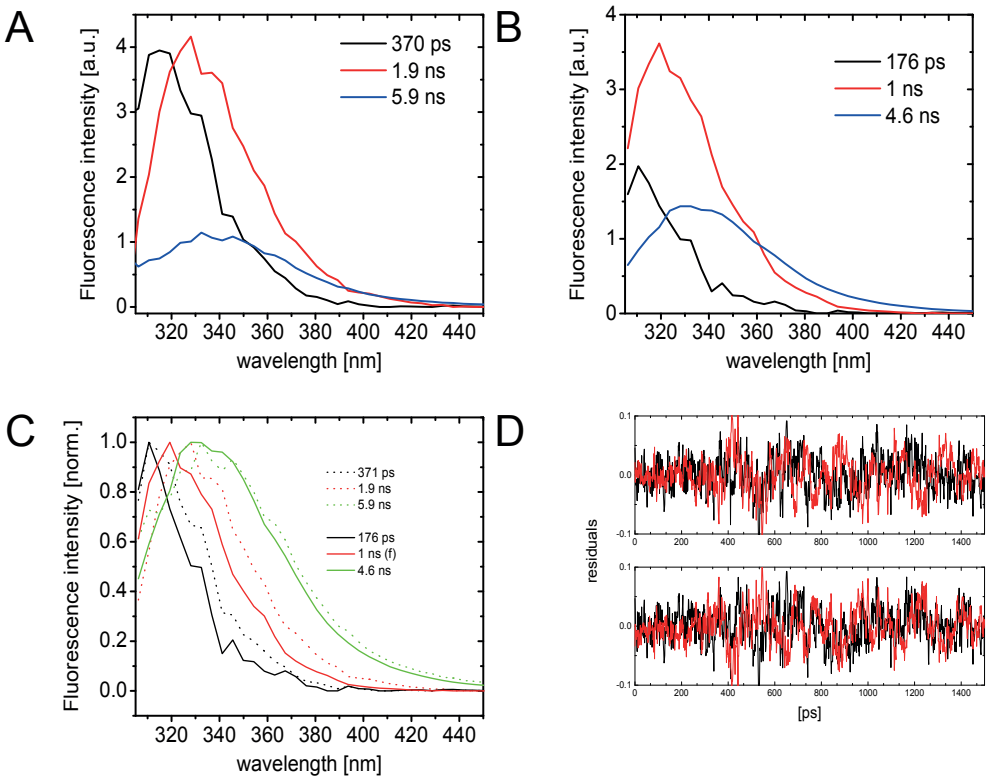

Fig. S1: Alternative fits for time resolved fluorescence data of UVR8 wild-type dimer (A, B). If the middle lifetime (red DAS) is fixed to 1 ns (B) the remaining lifetimes decrease to 176 ps (black DAS) and 4.6 ns (blue DAS) along with redistribution of their relative amplitudes. The DAS of the free model (dashed lines) differ only slightly from the alternative model (solid lines) (C). The first two left-singular vectors of the residual matrices (D) illustrate the identical quality of the free (right upper panel) fit and the constrained fit (right lower panel).

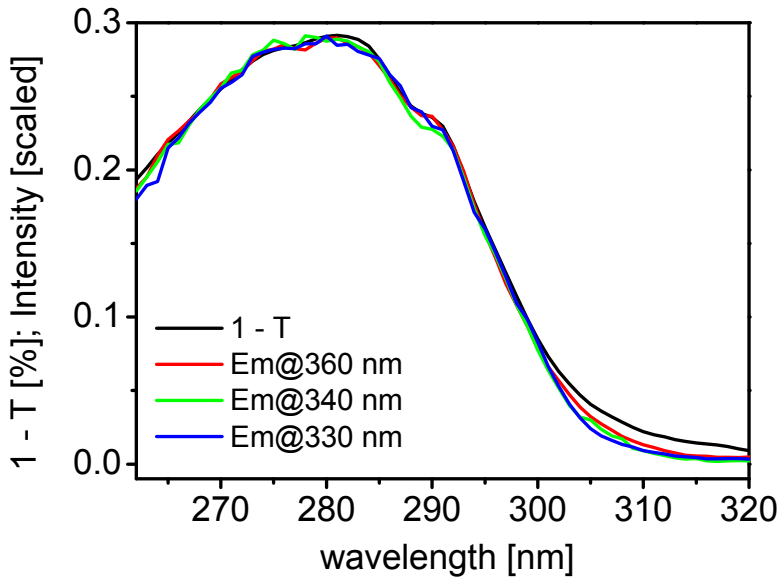

Fig. S2: Steady-state absorption and fluorescence excitation spectra of UVR8 dimer. The black curve corresponds to the (1-Transmission) spectrum and the colored traces to the fluorescence excitation spectra at the indicated detection wavelength. No significant differences between excitation and 1 – T spectra are observed, besides a small difference in the red tail above 300 nm which is considered too minor for interpretation.

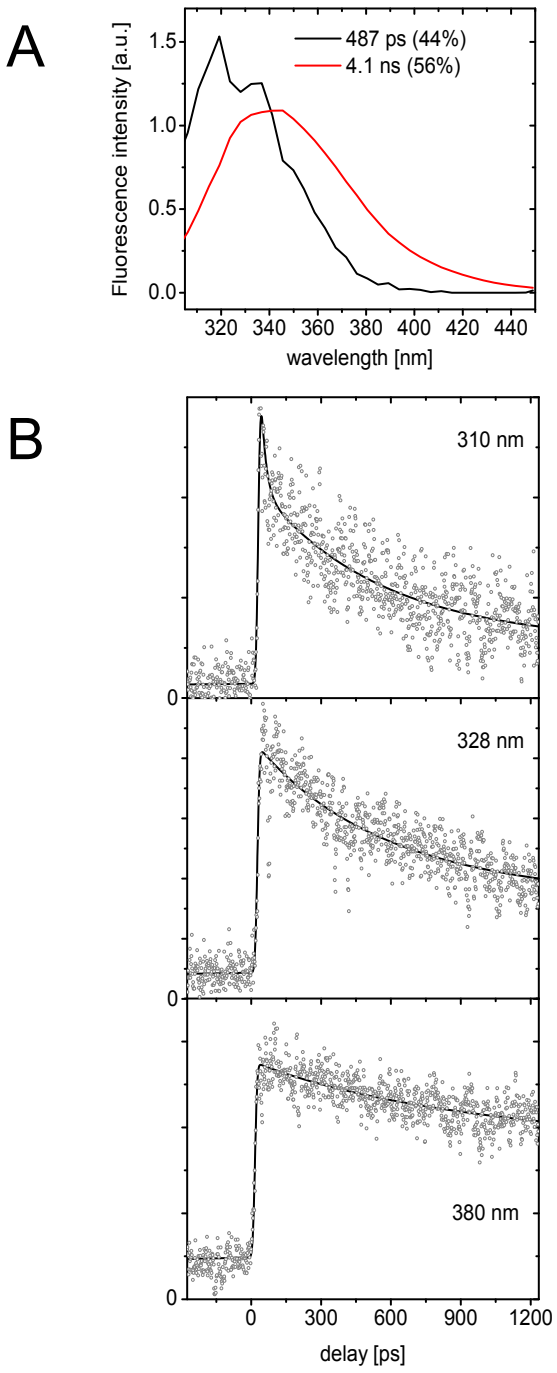

Fig. S3: Time resolved fluorescence of the constitutive monomeric UVR8 mutant R286A. (A) DAS and their corresponding lifetimes and fractions. (B) Fluorescence transients at selected wavelengths illustrating the decay of the corresponding DAS.

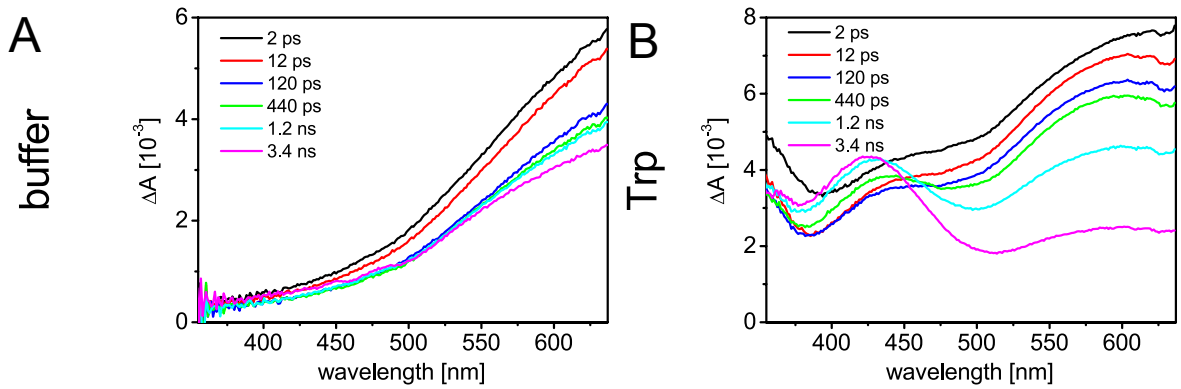

Fig. S4: Ultrafast photodynamics of buffer (A) and tryptophan in solution (B) after excitation at 266 nm illustrated by difference spectra at the indicated delays.

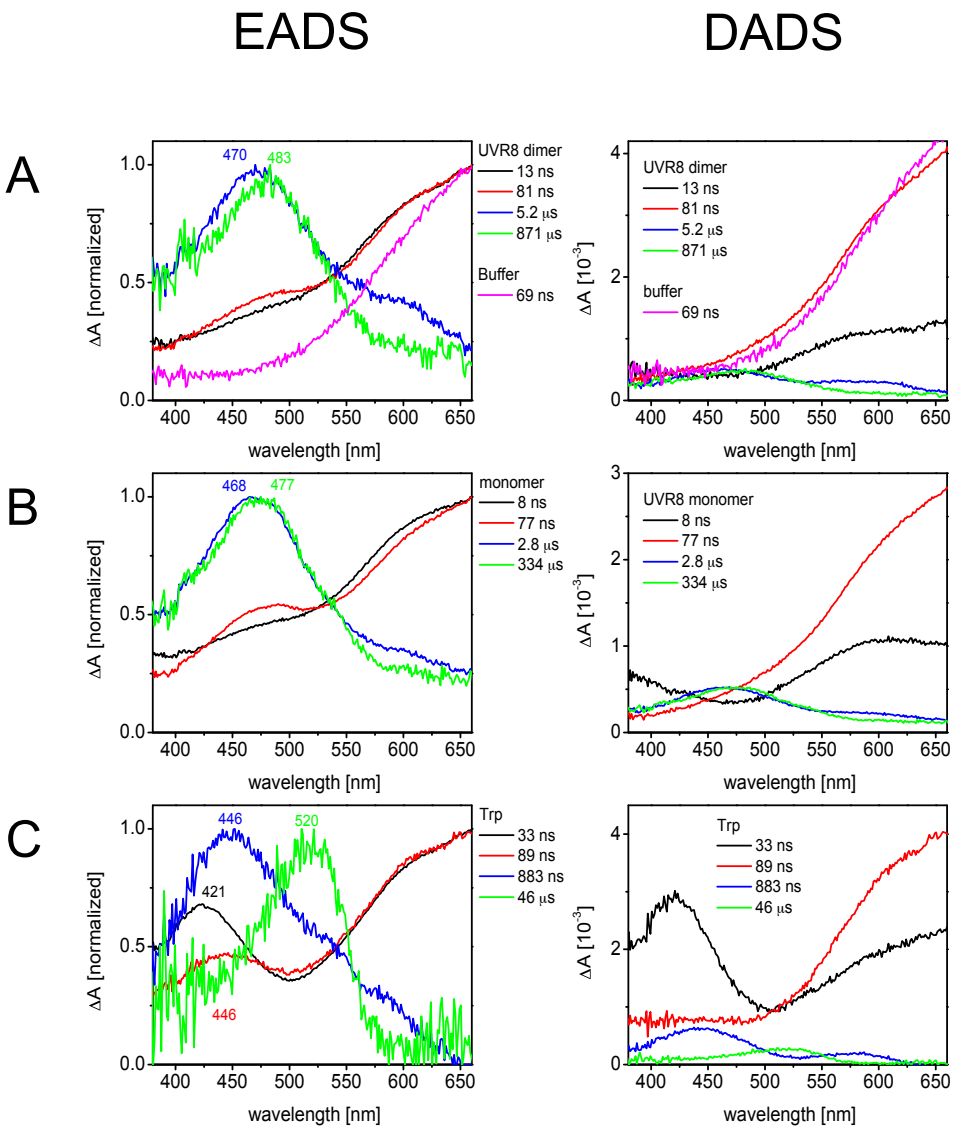

Fig. S5: Spectral evolution of UVR8 dimer (A), UVR8 monomer (B) and tryptophan in solution (C) in the ns to μs time domain illustrated by the normalized EADS (left column) and the DADS (right column).

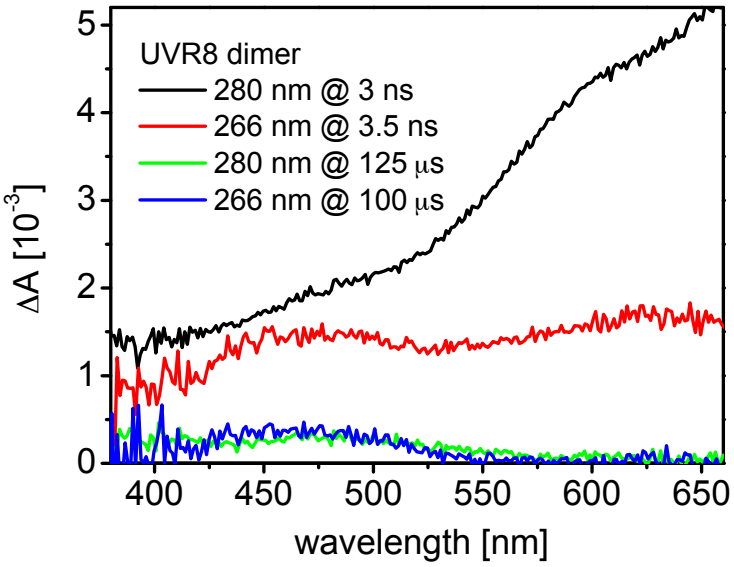

Fig. S6: Difference spectra of UVR8 WT dimer at the indicated time delay and excitation wavelength. The relative magnitude of the absorption at ~650 nm at ~3 ns compared to the photoproduct absorption at ~480 nm is significantly lower for excitation at 266 nm.

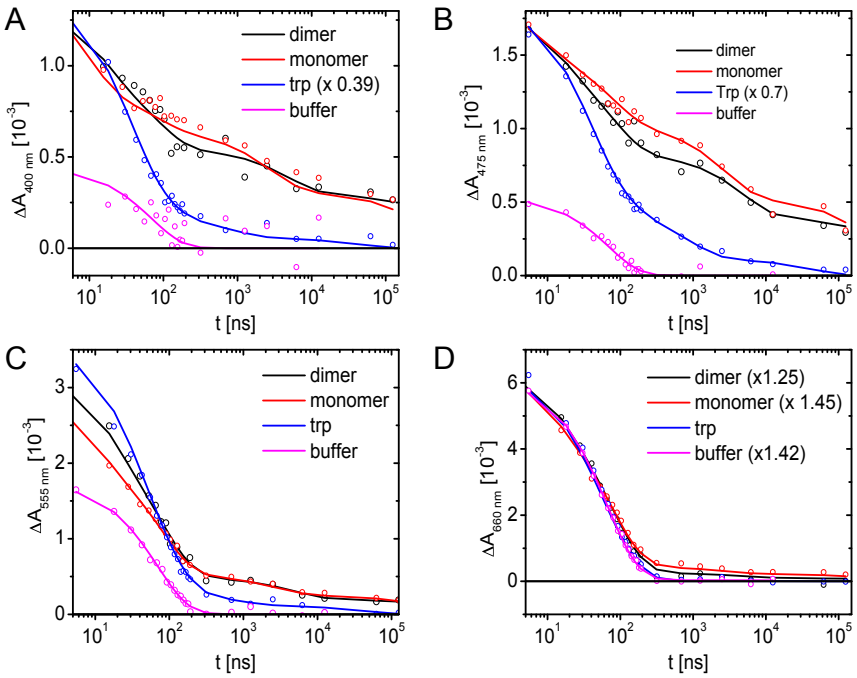

Fig. S7: Photodynamics of UVR8 dimer (black), UVR8 monomer (red), buffer (magenta) and tryptophan (blue) in solution in the ns to μs time domain after excitation at 280 nm. Transient absorption at 400 nm (A), 475 nm (B), 555 nm (C) and 660 nm (D). At 555 nm, the decay until 300 ns is biased by the solvated electron artifact (magenta). UVR8 dimer and monomer both show absorption in the tens of μs time domain that represents the formation and decay of the U600 absorption in this region. In tryptophan such a transient absorption belongs to the formation of the neutral radical, but is of relatively low amplitude (C). The absorption change at 475 nm represents the decay of U483 (B). Here, the solvated electron artifact is less strong. Compared to tryptophan in solution, both UVR8 dimer and monomer still contain significant absorption at the longest delay (125 μs), which corresponds to the long lifetime of the final species in these proteins. At 660 nm (D) all samples show almost identical absorption changes that are predominantly assigned to the solvated electron artifact.

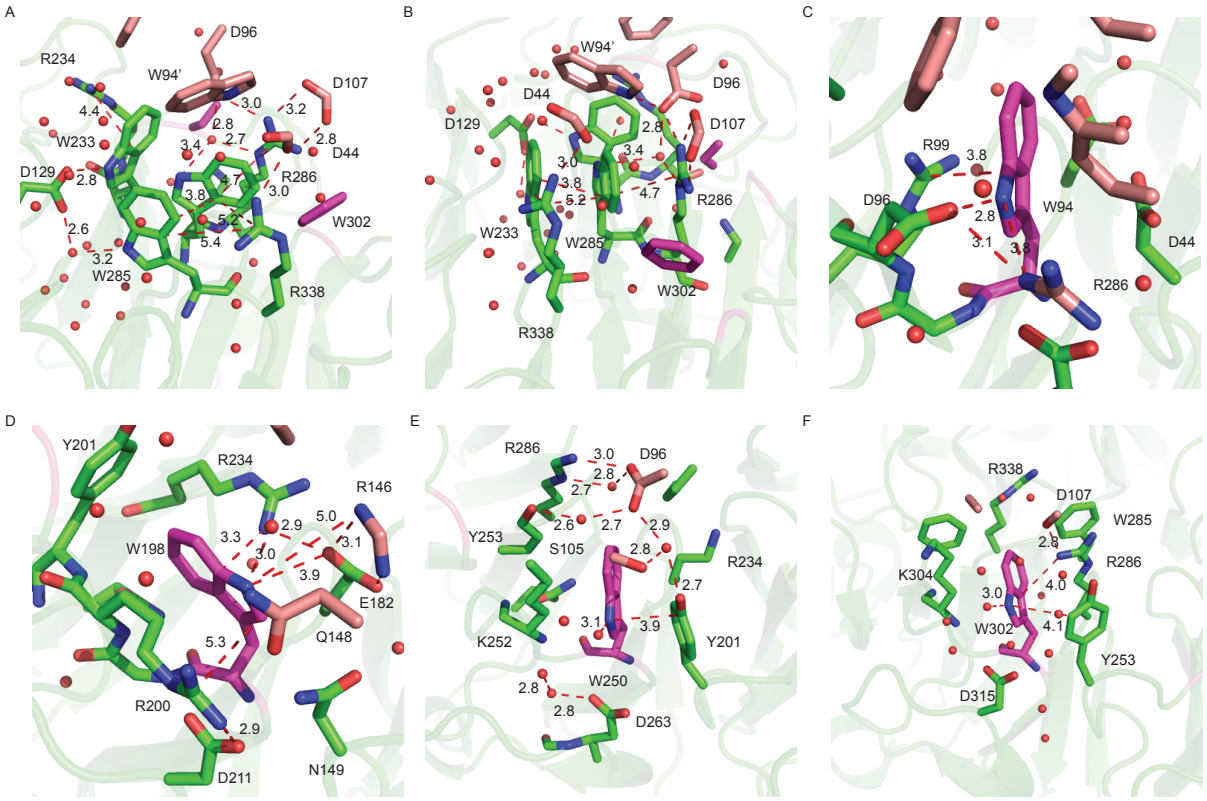

Fig. S8: Molecular environment of the interfacial tryptophan residues. Atoms of the protein and water (red dots) within a 6Å shell around the corresponding tryptophans are displayed. Residues belonging to the same monomer are colored either green/magenta or pink.

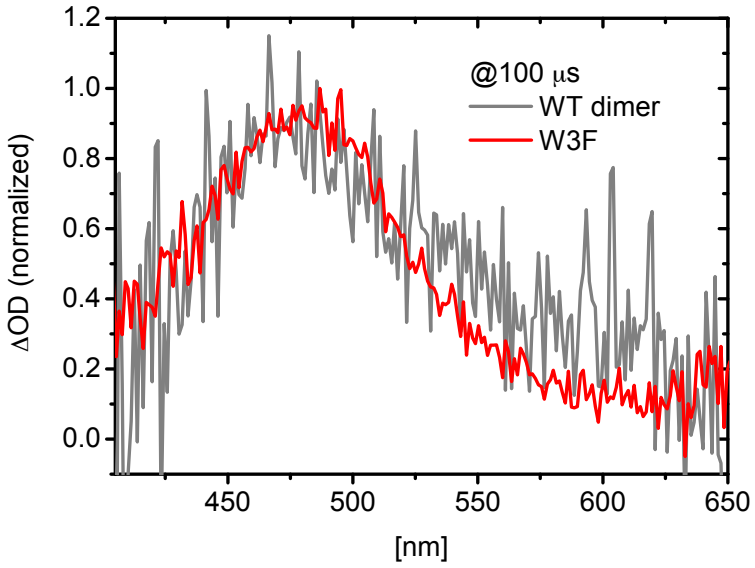

Fig. S9: Normalized difference spectrum 100 μs after excitation at 266 nm of WT (dimer) and a mutant (red) where W233, W285 and W337 have been replaced by phenylalanine.