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Kinetics of Electron Returns in Successive Two-Photon DNA Repair by (6-4) Photolyase

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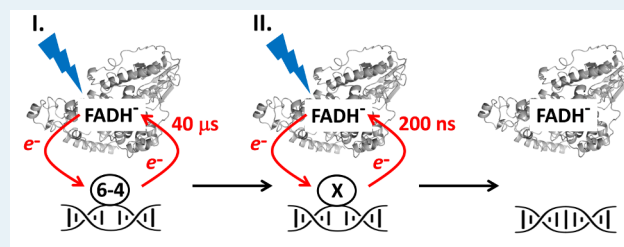
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Supporting Information

ABSTRACT: (6-4) photolyase is a flavoenzyme that catalyzes the chemically challenging repair of carcinogenic (6-4) photoproduct lesions in DNA using sunlight. Nature may have overcome the challenge by combining two distinct successive photoreactions, each starting with electron transfer from an excited flavin to the lesion. We herewith report a transient absorption study of electron returns to flavin following the chemical transformations of the lesion. We have successfully dissected the kinetics of the electron returns that finalize the first and second photoreactions ($\sim 40 \mu\text{s}$ and $\sim 200 \text{ ns}$, respectively) in the (6-4) photolyase from *Xenopus laevis*, corroborating and detailing the two-photon reaction model.

KEYWORDS: DNA repair, (6-4) photolyase, (6-4) photoproduct, electron transfer, transient absorption spectroscopy



Photolyases (PLs) are flavoproteins repairing carcinogenic UV-induced DNA damage in a light-dependent manner. PLs exist in all kingdoms of life except for placental mammals and specifically recognize and repair cyclobutane pyrimidine dimers (CPDs) or pyrimidine(6-4)pyrimidone photoproducts ((6-4)PPs); they are called CPD PLs and (6-4)PLs, respectively.¹ Although the chemical structures of CPDs and (6-4)PPs are remarkably different (Figure 1a), the photorepair of both DNA lesions by their respective photolyases is initiated by a light-driven electron transfer (ET) from the excited state of a fully reduced flavin adenine dinucleotide (FADH⁻) to the lesion (forward ET in $\sim 100 \text{ ps}$), leading to a rearrangement of the chemical structures of the lesions into an intact pair of nucleotides (reviewed in ref 2). In the CPD repair by PL, bond breakage of the cyclobutane ring takes place within 1 ns after the ET.^{3,4} Upon restoration of the intact nucleobases, the excess electron returns to the transiently formed FADH[•] within a few nanoseconds,^{3,4} regenerating FADH⁻, the catalytically active form of FAD (Figure 1a). While the mechanism of CPD repair is fully established, the reaction steps following the initial ET in (6-4)PP repair are still under debate.² In contrast to the simple cleavage of the covalent bonds in the CPD repair, a rearrangement of the $-\text{OH}$ (thymine) or $-\text{NH}_2$ (cytosine) functional group is required for the (6-4)PP repair. The reported repair quantum yields for (6-4)PPs (5–10%) are remarkably lower than for CPDs (50–100%).^{1,3–5}

While several reaction mechanisms of (6-4)PP photorepair have been proposed and computationally explored on the basis of a single photon reaction in analogy with the CPD repair,^{6–12} one computational study¹³ suggested a mechanism based on two consecutive photoreactions (Figure 1a). Intermediate species formed upon the first photoreaction were suggested to

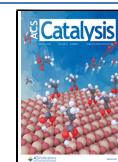
be oxetane- or azetidine-linked pyrimidine dimers, species known to be involved in the formation of (6-4)PPs.² A later study monitoring the repair yield upon a series of single turnover flashes provided experimental evidence for a two-photon mechanism (with estimated quantum yields of 5.4–11.8% and $>27\%$ for the first and the second photoreactions, respectively).¹⁴ Yet, this study did not monitor kinetics or the intermediates of the repair reactions. From an ultrafast spectroscopic analysis of the primary reactions of (6-4)PP repair under repetitive excitation,¹⁵ it was concluded that forward ET took place in 225 ps; most of the thus-formed FADH[•] decayed by futile back ET with an estimated intrinsic time constant of $\sim 50 \text{ ps}$ (in competition with productive bond rearrangement), and about 10% of FADH[•] survived until at least 3 ns (upper limit of the experimental time window), indicating that the photoreaction triggered by the excitation light would still be in progress beyond that time window. The authors anticipated that the repair was completed by electron return (ER) to FADH[•] in tens of nanoseconds,¹⁵ but no experimental proof has been provided so far.

Given that the DNA repair by (6-4)PL involves two distinct photoreactions, it is conceivable that the respective electron returns to FADH[•], ER1 and ER2, exhibit different kinetics. In this work, we have monitored ER on nano- and microsecond time scales and have indeed identified such different kinetics

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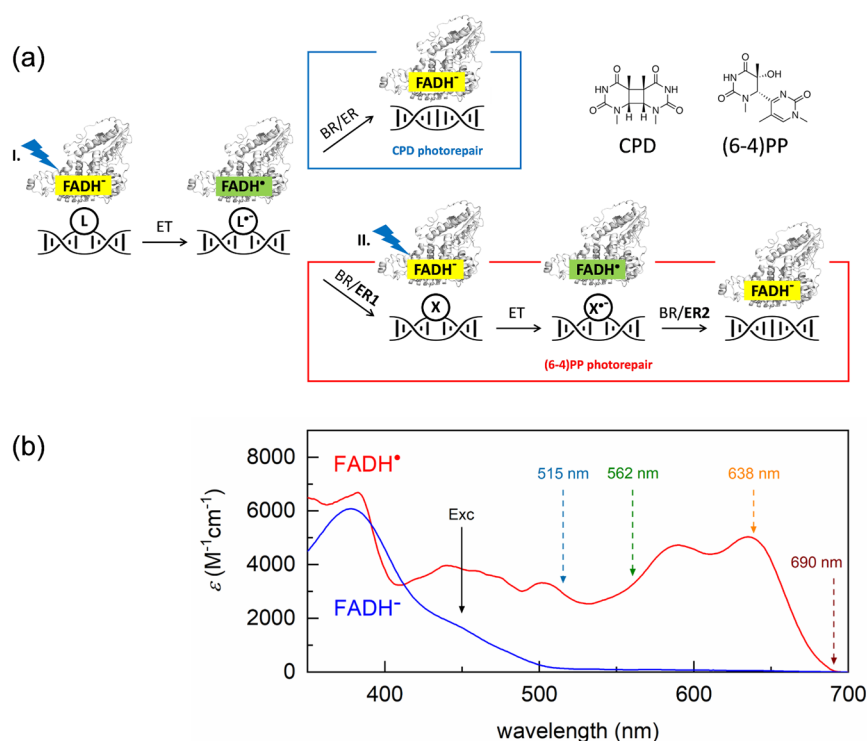


Figure 1. (a) Schematic illustration of photorepair of the most common UV-induced DNA lesions L (CPDs and (6-4)PPs) by photolyases. The excited FADH^- transfers an electron to the lesion, resulting in the transient formation of FADH^\bullet and an anion radical state of the lesion. Depending on the lesion type, the following bond rearrangement (BR) and electron return (ER) to the flavin result either in the repaired DNA (the case of CPD repair) or in the formation of an unknown intermediate X (the case of (6-4)PP repair and ER1). Complete DNA repair in the latter case requires another photoexcitation of FADH^- , leading to ET to the intermediate X, further bond rearrangement, and electron return ER2. (b) Absorption spectra of FADH^\bullet (red) and FADH^- (blue) in *Xl64*.¹⁷ The excitation and detection wavelengths used in the present study are indicated.

upon excitation with a single laser flash (ER1) or with repetitive flashes (ER1 and ER2).

Photoreduced *Xl64* in the presence of a substrate containing a T(6-4)T lesion was first excited repetitively (at 2 Hz) with 450 nm laser flashes, and the fate of FADH^\bullet formed by the forward ET to the substrate was monitored by transient absorption spectroscopy (see the Supporting Information for details and ref 16 for general guidelines) at four wavelengths between 500 and 700 nm, where the characteristic absorption band of FADH^\bullet is located (Figure 1b). The sample was pre-excited by 20 laser flashes in order to obtain a steady-state mixture of enzymes containing the (6-4)PP and the anticipated¹⁴ unknown intermediate X (formed by the first photoreaction in a low yield). Transient absorption signals from the following 32 flashes were averaged to improve the signal to noise ratio (Figure 2, top). At all studied wavelengths, the signals exhibit an instrument-limited (~ 5 ns) initial spike¹⁸ followed by a decay of nearly half of the amplitude within hundreds of nanoseconds. The remaining absorption changes decayed within tens of microseconds (Figure 2, top), indicating two distinct electron return processes occurring on two clearly distinct time scales, one of them orders of magnitude slower than that suggested previously.¹⁵ As a control, transient absorption signals were also recorded in the same manner for samples without substrate. These signals (Figure 2, bottom) exhibit pronounced initial spikes followed by a decay in ~ 1 μs and a small residual absorption change (both also visible in the signals recorded in the presence of the substrate). These features are hence not to be associated with

the repair process. After the initial spike, the signals from the repetitive experiment in the presence of the substrate could be globally fitted with three exponentials, yielding time constants of 178 ns, 1.74 μs , and 44.0 μs . A monoexponential global fit of the traces recorded in the absence of the substrate yielded a time constant of 1.32 μs .

In order to resolve the kinetics of electron return in the first photoreaction alone (ER1 in Figure 1a), transient absorption signals were recorded upon single-flash excitation of dark-adapted samples containing the enzyme with the substrate. Improvement in the signal to noise ratio was achieved by averaging eight signals, which were recorded with 15 min dark adaptation between flashes to ensure recovery of the initial state (the intermediate X formed by the first photoreaction reverts back to the initial (6-4)PP in 1.7 min;¹⁴ indeed, no significant repair was detected under these excitation conditions (see Figure S1), confirming that the first photoreaction (completed by ER1) alone does not lead to the restoration of intact pyrimidines). After the initial spike and the ~ 1 μs phase, the signals decayed in a few tens of microseconds (36.4 μs according to a global fit): i.e., with roughly the same kinetics as the slowest decay phase observed in the experiment with repetitive excitation flashes. The hundreds of nanoseconds kinetics observed under repetitive excitation was missing. This result suggests that the tens of microseconds phase reflects the electron return in the first photoreaction (ER1 in Figure 1a, forming the intermediate X) and the hundreds of nanoseconds phase reflects the electron

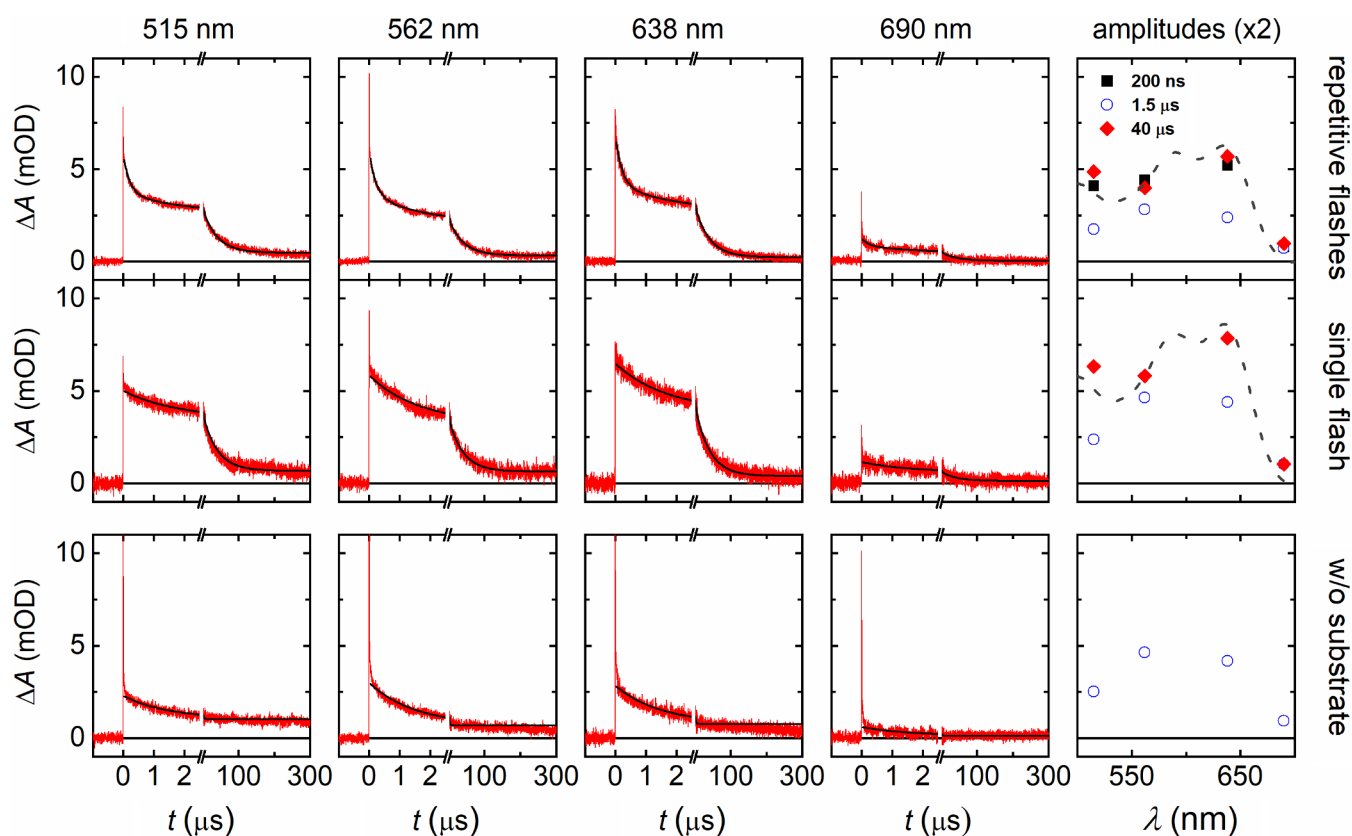


Figure 2. Transient absorption signals (red) recorded at selected wavelengths upon photoexcitation of XI64 containing fully reduced flavin (FADH^{-}) in the presence (top and center rows) and in the absence (bottom row) of the substrate. Black lines represent the global fit of all data with three exponential phases (plus an offset). Amplitudes of the phases allowed to be nonzero for the given data subset are shown in the rightmost column (multiplied by a factor of 2 for better visibility). Scaled $\text{FADH}^{\bullet} - \text{FADH}^{-}$ difference spectra (dashed dark gray lines) are shown to guide the eye.

return in the second photoreaction (ER2 in Figure 1a, restoring intact nucleobases).

Searching for the simplest coherent description of the data, we performed a global fit of all signals shown in Figure 2 (after the initial spike), allowing for three exponential decays (plus offset) and fixing the amplitudes of the fastest phase to zero in the single-flash signals and allowing only the $\sim 1 \mu\text{s}$ phase in the signals without the substrate. This approach yielded a reasonable fit (black solid lines in Figure 2) with time constants of $\sim 200 \text{ ns}$, $\sim 1.5 \mu\text{s}$, and $\sim 40 \mu\text{s}$ (precisely 206 ns, 1.49 μs , and 40.2 μs) and the amplitude spectra of the individual phases depicted in Figure 2 (right column). The amplitude spectra of the $\sim 200 \text{ ns}$ phase (Figure 2, top right) and the $\sim 40 \mu\text{s}$ phases in both experiments with the substrate (Figure 2, top right and middle right) are similar and consistent with the $\text{FADH}^{\bullet} - \text{FADH}^{-}$ difference spectrum (as expected for electron returns to FADH^{\bullet}). The absolute amplitudes of the $\sim 200 \text{ ns}$ phase and of the $\sim 40 \mu\text{s}$ phase under repetitive excitation are essentially equal but significantly ($\sim 30\%$) smaller than the amplitudes of the $\sim 40 \mu\text{s}$ phase in the single-flash experiments. These effects can be explained by the establishment of a quasi-steady state under repetitive excitation, in which a fraction ($\sim 30\%$) of the photolyases is bound to the intermediate X (PL-X) and the remainder is bound to the (6-4)PP (PL-(6-4)PP), so that only $\sim 70\%$ can undergo the first photoreaction (ending with ER1). In the quasi-steady state, the amount of X formed by the first photoreaction should equal the amount of its depletion by the

second photoreaction and hence ER1 and ER2 should indeed have equal amplitudes.

The $\sim 1.5 \mu\text{s}$ phase (present also in the absence of the substrate) differs spectrally from the other two phases and hence reflects a different process. The maximum at 562 nm and the time constant suggest that the underlying process could be a deprotonation of a tryptophan cation radical formed by photoactivation of a small fraction of proteins with oxidized or semireduced flavin cofactor. The resulting long-lived neutral tryptophan radical Trp^{\bullet} may account for the small residual absorption changes (offset in the fits) lasting for $>300 \mu\text{s}$ that were most pronounced at 515 nm, the absorption maximum of Trp^{\bullet} .¹⁹

Pure contributions of ER1 and ER2 (Figure 3) were isolated from the data as follows: for ER1, the signals obtained in the absence of the substrate were subtracted from the single flash experiment signals, and for ER2, the single-flash signals (rescaled by a factor of 0.7) were subtracted from the signals recorded under repetitive flashes. In both cases, the signals at the three most significant wavelengths (515, 562, and 638 nm) were averaged to improve the signal to noise ratio. The ER2 signal is also presented with rescaled amplitude to mimic the (hypothetical) situation of 100% PL-X complexes (gray signal in Figure 3). Monoexponential fits (solid black lines in Figure 3) of the averaged signals yielded time constants of 31 μs (ER1) and 192 ns (ER2).

For an estimation of the quantum yields (η_1 and η_2) of the (productive) electron returns (ER1 and ER2) from our data,

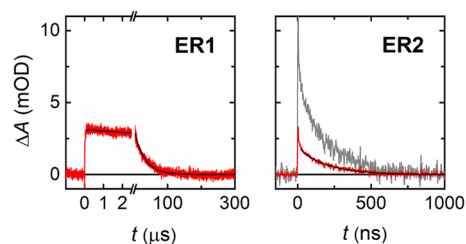


Figure 3. Isolated contributions of the $\sim 40 \mu\text{s}$ (ER1) and the $\sim 200 \text{ ns}$ (ER2) phases (red; constructed from averaged signals at 515, 562, and 638 nm as described in the text) and their fits (black). The red ER1 signal corresponds to the excitation of a sample containing 100% PL-(6-4)PP. The gray ER2 signal is scaled up to correspond to excitation of a hypothetical sample containing 100% PL-X.

we assumed that no further futile back ET occurs on the nanosecond to microsecond time scales. Using the incident energy of the excitation laser flashes at 450 nm of $18(\pm 3) \text{ mJ}$ per cm^2 (determined using a $[\text{Ru}(\text{bpy})_3]^{2+}$ actinometer;^{20,21} see the Supporting Information and Figure S2) and the absorbance of the sample at 450 nm over the 2 mm path, $\sim 25\%$ of all FADH^- ($\sim 8.8 \mu\text{M}$ in the $\sim 35 \mu\text{M}$ enzyme) was excited by each flash. Flash-induced absorption changes reflecting ER1 and ER2 were converted to concentrations of FADH^\bullet using its molar absorption coefficient at 638 nm of $5000 \text{ M}^{-1} \text{ cm}^{-1}$ (Figure 1b).

For ER1, the amplitude of the $\sim 40 \mu\text{s}$ phase (3.92 mOD at 638 nm) for the single-flash experiment corresponds to $0.78 \mu\text{M}$ FADH^\bullet and hence a quantum yield η_1 of $\sim 9\%$. Admitting possible errors of $\pm 10\%$ for the absorption coefficients and $\pm 15\%$ for the excitation energy, we obtain $7\% < \eta_1 < 12\%$.¹⁴ A similar approach exploiting the bleaching of the 325 nm band of T(6-4)T in single-flash experiments yielded $5.4\% < \eta_1 < 11.8\%$.

For ER2, the estimation of the quantum yield from the amplitudes of the $\sim 200 \text{ ns}$ phase under repetitive flashes (2.74 mOD at 638 nm, corresponding to $0.55 \mu\text{M}$ FADH^\bullet) has to take into account the quasi-steady state concentration of PL-X. With the assumption of an accumulation of $\sim 30\%$ PL-X (see above), $\sim 2.6 \mu\text{M}$ PL-X would have been excited and an η_2 value of $\sim 21\%$ ($=70/30 \eta_1$) would be obtained (accounting for the possible errors mentioned above gives $16\% < \eta_2 < 28\%$: i.e., less than the previously¹⁴ suggested 27–100%).

The present estimation of η_2 is based on the assumption that the observed 30% decrease in the amplitude of ER1 under repetitive flash excitation in comparison to single flashes was entirely due to the accumulation of PL-X. Part of the decrease may, however, be due to the accumulation of PLs not bound to DNA if rebinding of the substrate was (partially) limiting under our experimental conditions. In this case, less than 30% of PL-X would have accumulated and the η_2 value would be higher. In any case, the η_2 value is at least twice as high as the η_1 value, indicating that productive bond rearrangements in the second photoreaction compete more efficiently with futile back ET in comparison with the first photoreaction.

In summary, following up the work of Li et al.¹⁵ and looking beyond their experimental time window of $\sim 3 \text{ ns}$, we have resolved the kinetics of the electron returns (ER1 and ER2 in Figure 1a) in the two separate photoreactions leading to the photorepair of (6-4) photoproducts: ER1 leading to a formation of the intermediate X occurs in $\sim 40 \mu\text{s}$, and ER2 restoring the intact pair of nucleobases occurs in $\sim 200 \text{ ns}$.

Furthermore, our results confirm that the enzymatic photorepair of (6-4)PPs does indeed require absorption of not one but two photons. Finally, yet very importantly, even though the structure of the intermediate X remains elusive, we have determined the time scales on which it is formed and subsequently (upon a second photoexcitation of FADH^-) transformed into a pair of intact nucleobases. The kinetics and the quantum yields of the two photoreactions reported here provide essential input parameters for the computational modeling of possible reaction pathways, and above all, they are crucial for the right choice of the data collection time points and excitation conditions in time-resolved crystallographic and/or IR experiments, which should hopefully lead to the ultimate and unambiguous identification of the so-far enigmatic intermediate X.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acscatal.2c00492>.

Materials and methods, absorption spectra of the photoreduced *Xl64* in the presence of substrate before and after the transient absorption measurement, and an estimation of incident energy of the laser flashes using a $[\text{Ru}(\text{bpy})_3]^{2+}$ actinometer (PDF)

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Author Contributions

The manuscript was written through contributions of all authors and all authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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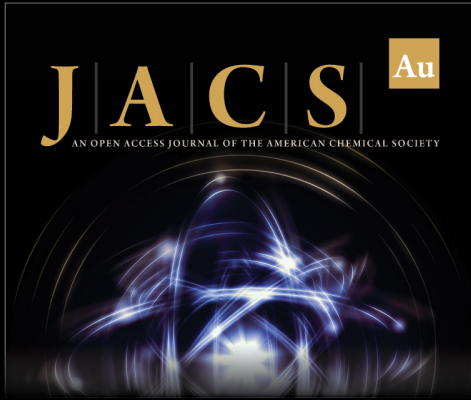
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ABBREVIATIONS

ET, electron transfer; ER, electron return; BR, bond rearrangement; FAD, flavin adenine dinucleotide; FADH[•]/FADH⁻, semireduced/fully reduced FAD; CPD, cyclobutane pyrimidine dimer; (6-4)PP, pyrimidine(6-4)pyrimidone photoproduct; T(6-4)T, (6-4)PP formed from two adjacent thymine nucleobases; PL, photolyase; *Xl64*, (6-4) photolyase from *Xenopus laevis*


REFERENCES


- (1) Sancar, A. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. *Chem. Rev.* **2003**, *103* (6), 2203–2237.
- (2) Yamamoto, J.; Plaza, P.; Brettel, K. Repair of (6-4) Lesions in DNA by (6-4) Photolyase: 20 Years of Quest for the Photoreaction Mechanism. *Photochem. Photobiol.* **2017**, *93* (1), 51–66.
- (3) Thiagarajan, V.; Byrdin, M.; Eker, A. P. M.; Müller, P.; Brettel, K. Kinetics of cyclobutane thymine dimer splitting by DNA photolyase directly monitored in the UV. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (23), 9402–9407.
- (4) Liu, Z.; Tan, C.; Guo, X.; Kao, Y.-T.; Li, J.; Wang, L.; Sancar, A.; Zhong, D. Dynamics and mechanism of cyclobutane pyrimidine dimer repair by DNA photolyase. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108* (36), 14831–14836.
- (5) Thiagarajan, V.; Villette, S.; Espagne, A.; Eker, A. P. M.; Brettel, K.; Byrdin, M. DNA Repair by Photolyase: A Novel Substrate with Low Background Absorption around 265 nm for Transient Absorption Studies in the UV. *Biochemistry* **2010**, *49* (2), 297–303.
- (6) Kim, S. T.; Malhotra, K.; Smith, C. A.; Taylor, J. S.; Sancar, A. Characterization of (6-4) photoproduct DNA photolyase. *J. Biol. Chem.* **1994**, *269* (11), 8535–8540.
- (7) Hitomi, K.; Nakamura, H.; Kim, S.-T.; Mizukoshi, T.; Ishikawa, T.; Iwai, S.; Todo, T. Role of Two Histidines in the (6-4) Photolyase Reaction. *J. Biol. Chem.* **2001**, *276* (13), 10103–10109.
- (8) Maul, M. J.; Barends, T. R. M.; Glas, A. F.; Cryle, M. J.; Domratcheva, T.; Schneider, S.; Schlichting, I.; Carell, T. Crystal structure and mechanism of a DNA (6-4) photolyase. *Angew. Chem., Int. Ed.* **2008**, *47* (52), 10076–10080.
- (9) Domratcheva, T.; Schlichting, I. Electronic structure of (6-4) DNA photoproduct repair involving a non-oxetane pathway. *J. Am. Chem. Soc.* **2009**, *131* (49), 17793–17799.
- (10) Domratcheva, T. Neutral Histidine and Photoinduced Electron Transfer in DNA Photolyases. *J. Am. Chem. Soc.* **2011**, *133* (45), 18172–18182.
- (11) Faraji, S.; Groenhof, G.; Dreuw, A. Combined QM/MM investigation on the light-driven electron-induced repair of the (6-4) thymine dimer catalyzed by DNA photolyase. *J. Phys. Chem. B* **2013**, *117* (35), 10071–10079.
- (12) Faraji, S.; Zhong, D.; Dreuw, A. Characterization of the Intermediate in and Identification of the Repair Mechanism of (6-4) Photolesions by Photolyases. *Angew. Chem., Int. Ed.* **2016**, *55* (17), 5175–5178.
- (13) Sadeghian, K.; Bocola, M.; Merz, T.; Schütz, M. Theoretical Study on the Repair Mechanism of the (6-4) Photolesion by the (6-4) Photolyase. *J. Am. Chem. Soc.* **2010**, *132* (45), 16285–16295.
- (14) Yamamoto, J.; Martin, R.; Iwai, S.; Plaza, P.; Brettel, K. Repair of the (6-4) photoproduct by DNA photolyase requires two photons. *Angew. Chem., Int. Ed.* **2013**, *52* (29), 7432–7436.
- (15) Li, J.; Liu, Z.; Tan, C.; Guo, X.; Wang, L.; Sancar, A.; Zhong, D. Dynamics and mechanism of repair of ultraviolet-induced (6-4) photoproduct by photolyase. *Nature* **2010**, *466* (7308), 887–890.
- (16) Brettel, K. Flash absorption spectroscopy. Principle and applications in plant photosynthesis research. *Bulletin de la Société Royale des Science de Liège* **1996**, *65* (4–5), 262–268.
- (17) Yamamoto, J.; Shimizu, K.; Kanda, T.; Hosokawa, Y.; Iwai, S.; Plaza, P.; Müller, P. Loss of Fourth Electron-Transferring Tryptophan in Animal (6-4) Photolyase Impairs DNA Repair Activity in Bacterial Cells. *Biochemistry* **2017**, *56* (40), 5356–5364.
- (18) The ~5 ns spike likely reflects the formation and decay of the excited state of FADH[•] and, in the presence of substrate, possibly also the formation of the FADH[•] L^{•-} pairs and the futile back ET.
- (19) Müller, P.; Yamamoto, J.; Martin, R.; Iwai, S.; Brettel, K. Discovery and functional analysis of a 4th electron-transferring tryptophan conserved exclusively in animal cryptochromes and (6-4) photolyases. *Chem. Commun.* **2015**, *51*, 15502–15505.
- (20) Byrdin, M.; Thiagarajan, V.; Villette, S.; Espagne, A.; Brettel, K. Use of ruthenium dyes for subnanosecond detector fidelity testing in real time transient absorption. *Rev. Sci. Instrum.* **2009**, *80* (4), 043102.
- (21) Müller, P.; Brettel, K. [Ru(bpy)₃]²⁺ as a reference in transient absorption spectroscopy: differential absorption coefficients for formation of the long-lived ³MLCT excited state. *Photochem. Photobiol. Sci.* **2012**, *11* (4), 632–636.



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