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UV-Induced Charge Transfer States in DNA Promote Sequence Selective Self-Repair

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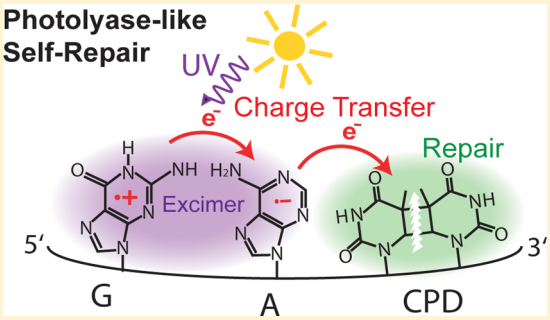
Supporting Information

ABSTRACT: Absorption of UV-radiation in nucleotides initiates a number of photophysical and photochemical processes, which may finally cause DNA damage. One major decay channel of photoexcited DNA leads to reactive charge transfer states. This study shows that these states trigger self-repair of DNA photolesions. The experiments were performed by UV spectroscopy and HPLC on different single and double stranded oligonucleotides containing a cyclobutane pyrimidine dimer (CPD) lesion. In a first experiment we show that photoexcitation of adenine adjacent to a CPD has no influence on this lesion. However, excitation of a guanine (G) adenine (A) sequence leads to reformation of the intact thymine (T) bases. The involvement of two bases for the repair points to a long-living charge transfer state between G and A to be responsible for the repair. The negatively charged A radical anion donates an electron to the CPD, inducing ring splitting and repair. In contrast, a TA sequence, having an inverted charge distribution (T radical anion, A radical cation), is not able to repair the CPD lesion. The investigations show that the presence of an adjacent radical ion is not sufficient for repair. More likely it is the driving power represented by the oxidation potential of the radical ion, which controls the repair. Thus, repair capacities are strongly sequence-dependent, creating DNA regions with different tendencies of self-repair. This self-healing activity represents the simplest sequence-dependent DNA repair system.



INTRODUCTION

Extended irradiation by UV light damages the genetic information stored in DNA. UV photons populate energy-rich excited states in the nucleobases promoting photochemical reactions. The high photostability of DNA has often been ascribed to the ultrashort excited state lifetime of the nucleotides in the subpicosecond range,[1] which leads to ultrafast deactivation of the reactive excited states. Indeed, this deactivation mechanism is realized for single nucleotides and may have played an important role during early stages of evolution when specific nucleotides were selected under strong ultraviolet irradiation as building blocks of the genetic code. However, in the real information carriers, the single or double stranded DNA, additional long-living excited states prevail with lifetimes in the 100 ps range.[2] Recently these states were identified as excimer/charge transfer states formed along DNA strands.[3] The direction of this charge separation between nucleobases is governed by the redox potential of the involved DNA bases.[3a] Additional experiments have shown that charge transfer states also exist between a DNA lesion and neighboring nucleobases, influencing photochemical reactivity.[4] However, the biological impact of these transient reactive radical states is unknown. On one hand, charged radicals induce a number of reactions in DNA and cause damage leading to mutations and cell death.[5] On the other hand, charged radicals are also able to reverse pre-existing photolesions. This DNA repair by charge transfer has been extensively investigated using charge injection from artificial donors.[6] In these experiments, chromophores are incorporated in DNA strands which are used to photoinject charges into DNA where they are able to split CPDs.[7] An important use of charged species for the repair of photolesions is realized in photolyases. In many organisms these proteins catalyze the reversion of DNA lesions using a photoinduced electron transfer mechanism.[8] The reaction cycle of photolyases (see Figure 1, left part) involves the absorption of visible light around 400 nm by a flavin, an electron transfer from the excited flavin cofactor to the cyclobutane pyrimidine dimer (CPD) photolesion, and the opening of the cyclobutane ring. After repair the electron is transferred back to the photolyase, and the original DNA is restored,[9] which closes the catalytic cycle.

There is an ongoing discussion on a related repair mechanism in purely nucleotide-based systems. Chinnapen and Sen have observed for the first time photoinduced self-

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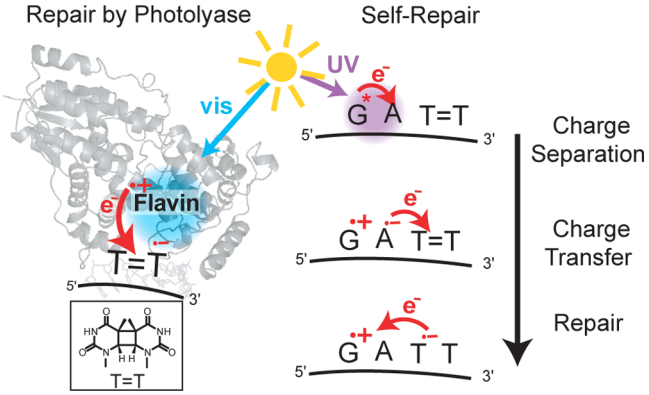

Figure 1. Repair schemes of a CPD lesion (T = T) in DNA via a photoinduced charge transfer. Left: Conventional enzymatic repair by a photolyase where the electron transfer occurs from the flavine excited by visible light to the T = T. Right: Self-repair by UV-induced charge transfer along the DNA strand. (Crystal structure prepared from Protein Data Bank entry 1TEZ.).[14]

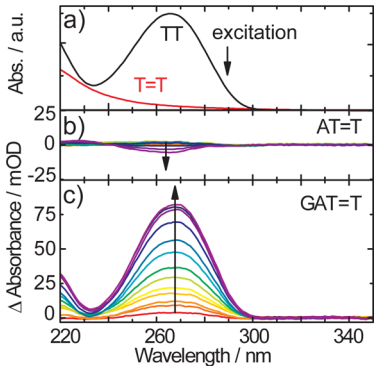

Figure 2. UV absorption of nucleotides, CPD lesion, and irradiation-induced difference spectra. Absorbance spectra of TT and T = T (a). Absorbance difference spectra of sequences AT = T (b) and GAT = T (c) upon stepwise increase of illumination dose. The same illumination conditions were used for both samples. The increase of absorption at 266 nm indicates repair of the GAT = T sequence with reformation of the T nucleotides. In contrast the AT = T sequence possesses no repair activity, and slight damage is seen with increasing illumination dose.

repair of TT-dimers in a deoxyribozyme system. As a possible mechanism they proposed that the excitation of a G-quadruplex donates an electron to reactivate the thymine dimer.[10] For small oligonucleotide model systems, it has been shown that photoexcited oxidatively damaged guanine (8-oxoG) transfers charges to an adjacent CPD lesion, leading to repair.[11] These observations were explained by a direct electron transfer from a photoexcited nucleobase to CPD. The same mechanism was proposed for self-repair of thymine dimer in duplex DNA.[12] In contrast, it has been shown recently that the excitation of a single nucleobase does not promote repair of a neighboring TT-dimer.[13]

In this study we show that self-repair in DNA indeed exists, and we propose a new mechanism based on excimer (charge transfer) states in DNA. We treat DNA strands where the TT-dimer is flanked by specific single bases and dinucleotides. The sequences have been selected according special charge distributions and lifetimes of excimer states previously characterized by femtosecond infrared spectroscopy.[3a,c,d] With these selected sequences we demonstrate that repair occurs when the intrastrand charge transfer leads to a suitably charged nucleotide adjacent to the lesion. The reaction follows the scheme given in Figure 1, right. The UV light induces an electron migration process from a guanine (G) to a nearby adenine (A) which gives rise to the formation of the zwitterion $G^{\bullet+}A^{\bullet-}$.[3a] Within the lifetime of this charge-separated state (300 ps) the negative charge located on the A base may migrate to the adjacent CPD lesion. Here an electron induces the opening of the cyclobutane ring, which leads to a repair of the CPD lesion in a photolyase-like manner.

RESULTS AND DISCUSSION

In Figure 2 the absorbance spectrum of a thymine (T) dinucleotide and the corresponding CPD lesion (T = T) is plotted. Nucleobases have strong absorbance bands around 266 nm, whereas the CPD lesion exhibits only a weak absorbance. It is known that absorption of UV radiation at short wavelengths by the lesion causes a direct photoreversal process with high efficiency.[15] However, this process plays only a minor role in nature, since UVC irradiance is negligible on the surface of the earth.[16] We performed our experiments with excitation in the UVB range at 290 nm. At this wavelength the extinction coefficient of the CPD lesion is very small, and direct photoreversion should be of minor importance. The DNA nucleotides, however, have much larger absorption than the CPD at this wavelength. CPD repair or lesion formation of DNA induced by light at 290 nm is tracked by the change of the thymine absorbance band around 266 nm (see Figure 2). First, we investigated the absorbance change of this band for the AT = T oligonucleotide upon excitation at 290 nm (illumination conditions see Materials and Methods in the SI). We observe a weak decrease in absorbance at 266 nm during illumination indicating damaging processes, which lead to bleaching of the nucleotide absorption. This result shows that a direct photoinduced repair, e.g., via charge transfer from photoexcited A, is negligible. We obtained similar results for the GT = TG sequence (SI). This is in line with other studies showing that neither A nor G in the vicinity of the CPD is able to photorepair the lesion.[17]

In a second experiment, the sequence GAT = T was illuminated under identical conditions. Now the absorption at 266 nm increases, directly indicating thymine recovery and base repair. The repair reaction was studied next by analytical HPLC experiments (Figure 3a). The peak at small retention times (left) shows that the concentration of the original oligomer GAT = T decreases while the repaired oligomer (right peak) increases with illumination time. Apparently the GA dinucleotide causes CPD repair via the charge-separation state $G^{\bullet+}A^{\bullet-}$ and charge transfer to the T = T lesion.[3a]

In order to quantify the contributions from a potential direct photoreversal of the CPD, we performed identical illumination experiments with the T = T containing nucleotides (Figure 4). For the T = T dinucleotide, we detected a very weak positive absorbance change around 266 nm representing CPD repair. However, the repair rate and the saturation value for long illumination times are a factor of 5 lower than those for the GAT = T oligonucleotide, emphasizing that self-repair at 290 nm is not due to direct absorption by the T = T lesion.

At low doses the absorbance increase is a linear function of illumination. Its slope gives a measure for the quantum yield of repair. The initial quantum yield of repair for the GAT = T sequence was determined to be ~0.25% which is in a similar

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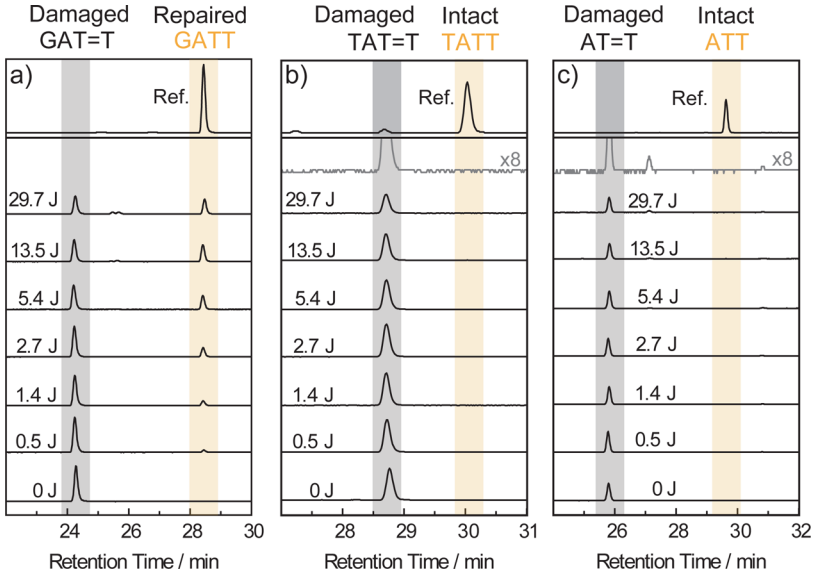

Figure 3. Analytical HPLC analysis of single DNA strands. HPLC analysis of illuminated GAT = T (a), TAT = T (b), and AT = T oligonucleotides (c) at different doses and the corresponding intact sequences (top traces). The repaired GAT = T sequence is clearly observed in part a. In cases b and c (see expanded traces in gray) no indication of the repaired product was obtained upon illumination. Additional peaks in the reference strand of part c are due to impurities of the commercial DNA sample.

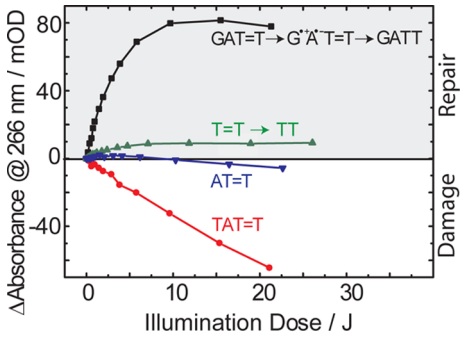

Figure 4. Absorbance changes recorded at 266 nm as a function of illumination dose for different CPD-containing sequences. Positive absorbance changes observed for GAT = T and T = T indicate repair, whereas bleach indicates damage formation for the AT = T and TAT = T sequences. The charge-transfer-induced repair in GAT = T is a factor of 7 more efficient than the direct photoreversal of T = T. The quantum yield is determined from the initial slope.

range compared to the UV-induced lesion formation (~1%).[2a] At strong illumination doses the absorption increase levels off due to accumulated damage formation (CPD from the repaired TT step and the AT photodimer).[18] Approximately 25% of the CPDs are repaired before reaching the photostationary equilibrium between repair and lesion formation.

It is known that CPD lesions can also be repaired by the transfer of a positive charge from an external chromophore.[7a,19] To address potential repair by positive charges we investigated the repair activity of the TAT = T oligonucleotide. UV-excitation of the TA sequence leads to a $T^{\bullet-}A^{\bullet+}$ charge transfer state,[3c,d] where the charge distribution is inverse to that of the GA sequence and where the positive charge is placed directly adjacent to the T = T lesion. For TAT = T a strong bleach of the 266 nm band is observed (dose dependence see Figure 4), indicating that damaging processes dominate, e.g., via formation of a TA photodimer.[18] The reduced lifetime of the charge transfer state $T^{\bullet-}A^{\bullet+}$ (50 ps)[3d] in comparison to that of $G^{\bullet+}A^{\bullet-}$ (300 ps)[3a] could also explain a reduction of the repair efficiency. However, in additional HPLC analyses (see Figure 3b), no indication for repaired T = T was found. This observation shows that the $T^{\bullet-}A^{\bullet+}$ charge transfer state does not repair the CPD lesion. This indicates that the driving force of electron transfer, i.e., the redox potential of the adjacent charged bases, is a crucial parameter for the repair process. The pure presence of a charged base plays a minor role. In the GA sequence, an electron transport from the $A^{\bullet-}$ to the T = T is indeed likely. Here the electron transfer is possible since the reduction potential of the $A^{\bullet-}$ is lower than that of T = T (−2.45 V vs −2.20 V).[20] In contrast, the oxidation potential of $A^{\bullet+}$ is similar to T = T (~2 V for both);[21] no distinct driving force is present, and repair should be weaker than in the GA sequence. In agreement, the experiments show that self-healing occurs in GA but not in TA sequences. Apparently, repair is driven by redox potential, which explains the sequence-dependent photoreactivity of DNA.[12,13,22]

To generalize this repair mechanism for the DNA double helix we addressed self-repair in a double strand consisting of GAT = TAG and the complementary CTAATC sequence. In such a system, damage formation and repair processes interfere. To allow comparison between oligomers with different absorbance characteristics in Figure 5, we plotted the absorbance changes versus absorbed dose instead of illumination dose as used in Figure 4. In a first experiment, the corresponding single strands were investigated separately. A reference strand AGT = TGA, where a cation G^{+} is found in the neighborhood of the TT-dimer, exhibits very weak repair (see SI, Table S1). As expected, the GAT = TAG oligonucleotide shows pronounced repair activity (Figure 5a, black). In contrast, one finds an absorbance decrease due to massive lesion formation in the complementary strand CTAATC (green). Apparently different photodimers can occur, such as CT− and TC−CPD and TA and AT photodimers. The sum of the spectroscopic traces of both single strands (black + green = blue) leads to a negative total absorbance change. The blue curve was calculated by summing up each absorbed dose and each absorbance change of the single strand curves point by

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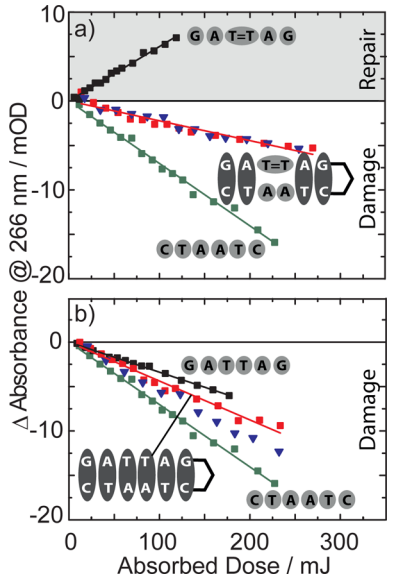

Figure 5. Self-repair in DNA single and double strands. (a) Illumination-induced absorbance changes at 266 nm of the single strands GAT = TAG (black) and CTAATC (green) and the calculated sum (blue). The absorption changes for the corresponding double strand (red) are close to the sum indicating a concerted change of damage formation and self-repair upon double strand formation. (b) Illumination experiment on originally intact DNA samples. Illumination (290 nm)-induced absorbance change at 266 nm of the single strands GATTAG (black) and CTAATC (green) and the calculated sum (blue) together with the absorbance change found for the double strand with the hexaethylenglycol linker (red). The illumination doses are kept at low level with linear response.

point. The sum curve gives a measure for the double strand absorbance without interstrand interaction. Now these results are compared to the double strand structure. For this purpose we linked GAT = TAG and CTAATC via a hexaethylenglycol linker leading to a stable double strand at room temperature (SI Appendix, Figure S1). In this double stranded sample we find the slope of the absorbance change at 266 nm (Figure 5a, red) similar to the slope of the sum of the single strands. For the investigated sequence the net nucleotide photoreaction, i.e., the sum of lesion formation and repair, is similar for single and double stranded DNA (Figure 5a).

The constant net photoreactivity alone does not prove that repair is unchanged when going from the single to the double strand. This observation could also be explained by a strongly reduced damage formation upon double strand formation. To exclude this possibility we performed illumination experiments on damage formation on initially intact single and double strands with the sequences used above (Figure 5b). In all three cases we find the bleach of the 260 nm band due to damage formation. The damage formation of the double strand (red) is very similar (within 20%) to that of the sum of the corresponding single strands (blue). Thus, the efficiency of damage formation is only weakly reduced in the double strand. This weak reduction shows that the self-repair activity in double strands is only somewhat lower than in the single stranded DNA. The reduction can be explained by the fact that charge transfer states are partly quenched in DNA double strands.[23]

CONCLUSIONS

In summary, we have shown that specific DNA sequences, particularly GA dinucleotides embedded in UV vulnerable T-tracts, possess intrinsic photolyase-like self-repair properties. We propose a mechanism based on charge transfer/excimer states within the DNA strand initiating CPD repair via electron transfer to a neighboring CPD lesion. Repair activity strongly depends on the redox potential of the involved charged nucleotides. The repair mechanism discovered here is an intrinsic photochemical property of natural DNA and exhibits pronounced sequence selectivity. The self-healing activity fueled by light is purely based on the DNA structure itself. It consequently leads to the simplest existing selective DNA repair system and may represent an early ancestor of today's complex photolyase enzymes. For the present life on earth where the ozone layer shields hard ultraviolet radiation below 300 nm the proposed repair mechanism has a smaller influence than the well-known highly optimized enzymatic repair mechanisms. However, one may assume that in the prebiotic era with strong UV-C irradiation such a compact and purely nucleotide-based intrinsic repair system has strongly influenced the selection of base sequences with implications on today's life.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b09753.

Detailed materials and methods, melting curve of linked GAT = TAG double strand, and repair activity in additional nucleotides (GT = TG, AGT = TGA compared to GAT = TAG) (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Crespo-Hernandez, C. E.; Cohen, B.; Hare, P. M.; Kohler, B. *Chem. Rev.* **2004**, *104* (4), 1977−2020.

(2) (a) Middleton, C. T.; de La Harpe, K.; Su, C.; Law, Y. K.; Crespo-Hernandez, C. E.; Kohler, B. *Annu. Rev. Phys. Chem.* **2009**, *60* (1), 217−239. (b) Schreier, W. J.; Gilch, P.; Zinth, W. *Annu. Rev. Phys. Chem.* **2015**, *66*, 497−519.

(3) (a) Bucher, D. B.; Pilles, B. M.; Carell, T.; Zinth, W. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (12), 4369−4374. (b) Zhang, Y.; Dood, J.; Beckstead, A. A.; Li, X.-B.; Nguyen, K. V.; Burrows, C. J.; Improta, R.; Kohler, B. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (32), 11612−11617.

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J. Am. Chem. Soc. 2016, 138, 186−190

(c) Doorley, G. W.; Wojdyla, M.; Watson, G. W.; Towrie, M.; Parker, A. W.; Kelly, J. M.; Quinn, S. J. *J. Phys. Chem. Lett.* **2013**, *4* (16), 2739−2744. (d) Pilles, B. M.; Bucher, D. B.; Liu, L.; Gilch, P.; Zinth, W.; Schreier, W. J. *Chem. Commun.* **2014**, *50* (98), 15623−15626.
(4) Bucher, D. B.; Pilles, B. M.; Carell, T.; Zinth, W. *J. Phys. Chem. B* **2015**, *119* (28), 8685−8692.
(5) Kanvah, S.; Joseph, J.; Schuster, G. B.; Barnett, R. N.; Cleveland, C. L.; Landman, U. *Acc. Chem. Res.* **2010**, *43* (2), 280−287.
(6) Genereux, J. C.; Barton, J. K. *Chem. Rev.* **2010**, *110* (3), 1642−1662.
(7) (a) Dandliker, P. J.; Holmlin, R. E.; Barton, J. K. *Science* **1997**, *275* (5305), 1465−1468. (b) Giese, B.; Carl, B.; Carl, T.; Carell, T.; Behrens, C.; Hennecke, U.; Schiemann, O.; Feresin, E. *Angew. Chem., Int. Ed.* **2004**, *43* (14), 1848−1851. (c) Schwoegler, A.; Burgdorf, L. T.; Carell, T. *Angew. Chem., Int. Ed.* **2000**, *39* (21), 3918−3920.
(8) (a) Kneuttinger, A. C.; Kashiwazaki, G.; Prill, S.; Heil, K.; Muller, M.; Carell, T. *Photochem. Photobiol.* **2014**, *90* (1), 1−14. (b) Sancar, A. *Chem. Rev.* **2003**, *103* (6), 2203−2238.
(9) (a) Liu, Z.; Tan, C.; Guo, X.; Kao, Y.-T.; Li, J.; Wang, L.; Sancar, A.; Zhong, D. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (36), 14831−14836. (b) Kao, Y.-T.; Saxena, C.; Wang, L.; Sancar, A.; Zhong, D. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (45), 16128−16132. (c) Thiagarajan, V.; Byrdin, M.; Eker, A. P. M.; Muller, P.; Brettel, K. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (23), 9402−9407.
(10) Chinnapen, D. J.-F.; Sen, D. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101* (1), 65−69.
(11) (a) Nguyen, K. V.; Burrows, C. J. *J. Am. Chem. Soc.* **2011**, *133* (37), 14586−14589. (b) Anusiewicz, I.; Swierszcz, I.; Skurski, P.; Simons, J. *J. Phys. Chem. A* **2013**, *117* (6), 1240−1253.
(12) Holman, M. R.; Ito, T.; Rokita, S. E. *J. Am. Chem. Soc.* **2007**, *129* (1), 6−7.
(13) (a) Law, Y. K.; Forties, R. A.; Liu, X.; Poirier, M. G.; Kohler, B. *Photochem. Photobiol. Sci.* **2013**, *12* (8), 1431−1439. (b) Pan, Z.; Hariharan, M.; Arkin, J. D.; Jalilov, A. S.; McCullagh, M.; Schatz, G. C.; Lewis, F. D. *J. Am. Chem. Soc.* **2011**, *133* (51), 20793−20798.
(14) Mees, A.; Klar, T.; Gnau, P.; Hennecke, U.; Eker, A. P. M.; Carell, T.; Essen, L.-O. *Science* **2004**, *306* (5702), 1789−1793.
(15) (a) Johns, H. E.; Rapaport, S. A.; Delbrueck, M. *J. Mol. Biol.* **1962**, *4* (2), 104−114. (b) Garcés, F.; Dávila, C. A. *Photochem. Photobiol.* **1982**, *35* (1), 9−16.
(16) Mayer, B.; Kylling, A. *Atmos. Chem. Phys.* **2005**, *5*, 1855−1877.
(17) Pan, Z.; Chen, J.; Schreier, W. J.; Kohler, B.; Lewis, F. D. *J. Phys. Chem. B* **2012**, *116* (1), 698−704.
(18) Zhao, X.; Nadji, S.; Kao, J. L.; Taylor, J. S. *Nucleic Acids Res.* **1996**, *24* (8), 1554−1560.
(19) Young, T.; Nieman, R.; Rose, S. D. *Photochem. Photobiol.* **1990**, *52* (4), 661−668.
(20) (a) Behrens, C.; Cichon, M. K.; Grolle, F.; Hennecke, U.; Carell, T. In *Long-Range Charge Transfer in DNA I*; Schuster, G. B.; Springer: Berlin, 2004. (b) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541−5553.
(21) (a) Boussicault, F.; Krueger, O.; Robert, M.; Wille, U. *Org. Biomol. Chem.* **2004**, *2* (19), 2742−2750. (b) Paukku, Y.; Hill, G. *J. Phys. Chem. A* **2011**, *115* (18), 4804−4810. (c) Steenken, S.; Telo, J. P.; Novais, H. M.; Candeias, L. P. *J. Am. Chem. Soc.* **1992**, *114* (12), 4701−4709.
(22) Hariharan, M.; Lewis, F. D. *J. Am. Chem. Soc.* **2008**, *130* (36), 11870−11871.
(23) (a) Bucher, D. B.; Schlueter, A.; Carell, T.; Zinth, W. *Angew. Chem., Int. Ed.* **2014**, *53* (42), 11366−11369. (b) Zhang, Y.; de La Harpe, K.; Beckstead, A. A.; Improta, R.; Kohler, B. *J. Am. Chem. Soc.* **2015**, *137* (22), 7059−7062.

DOI: 10.1021/jacs.5b09753
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