

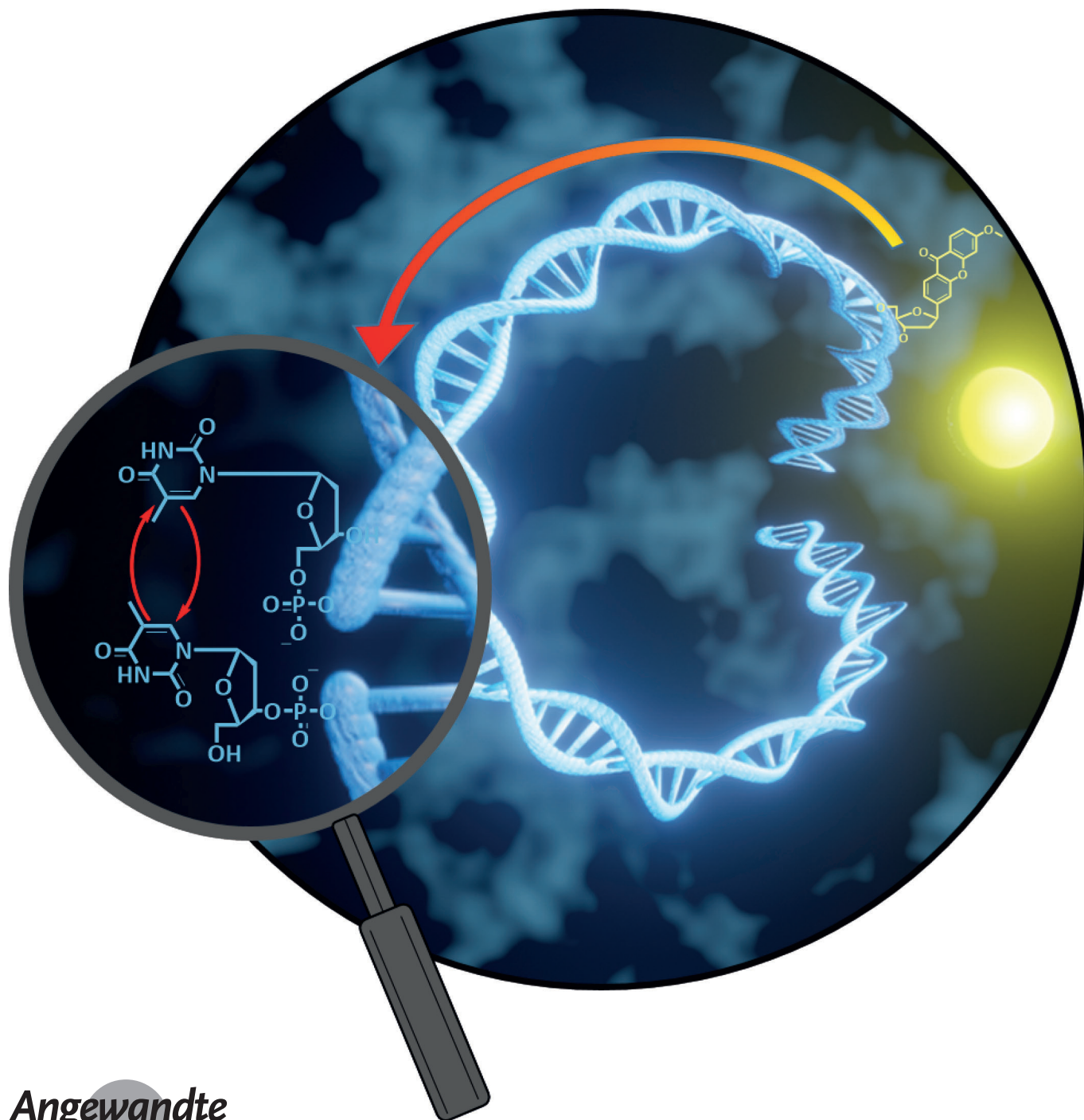
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How Far Does Energy Migrate in DNA and Cause Damage? Evidence for Long-Range Photodamage to DNA

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Abstract: A new DNA architecture addresses the question, how far energy migrates in DNA and forms cyclobutane pyrimidine dimers (CPDs) as photodamages causing skin cancer. The 3-methoxyxanthone nucleoside allows site-selective photoenergy injection into DNA. The designated CPD site lacks the phosphodiester bond and can be placed in defined distances. The CPD formation links two oligonucleotides together and allows probing by gel electrophoresis. We obtained a sigmoidal distance dependence with R_0 of 25 ± 3 Å. Below R_0 , short-range energy migration occurs with high CPD yields and shallow distance dependence, characteristic for a coherent process. 5-methyl-C as epigenetic modification on the 3'-side facilitates CPD formation. Above R_0 , long-range incoherent energy migration occurs over 30 A-T pairs (105.4 Å). The evidence of long-range CPD formation is fundamental for our understanding of DNA photodamaging. Open access funding enabled and organized by Projekt DEAL.

Exposure of DNA to solar UV light is dangerous for the integrity of genetic information by the formation of DNA photodamages. Hence, the understanding of excited state dynamics in DNA is fundamentally important. UV-B excitation of DNA leads to charge separated states with charges delocalized over several base pairs and finally to charge recombination in a few hundred ps.^[1] These extremely fast photophysical processes protect DNA from UV damages. Cyclobutane pyrimidine dimers (CPDs) are the main DNA lesion caused by UV radiation, which are a molecular origin of skin cancer.^[2] The CPD formation occurs within 1 ps in a nearly barrierless reaction.^[3] The “collective excitation” of several stacked base pairs by UV-A radiation causes CPDs, too.^[4] Triplet sensitization by for example, ketoprofen,^[5] other phenones,^[6] 6,4-DNA photoproduct as “Trojan Horse”,^[7] and formyl-dU as epigenetic marker^[8] also yields CPDs. In contrast to these established short-range pathways of DNA photochemistry, the CPD damaging by energy migration over long range in DNA has not yet been studied.^[9] Time-resolved spectroscopy revealed that energy transfer between nucleobases of natural DNA is mediated by dark states, but the migration distances were not determined.^[10] The key question is how far excited state energy migrates in DNA. We recently demonstrated that triplet energy migration occurs with an exponential distance dependence and has a limit of 10 A-T base pairs (37.4 Å).^[11] Triplet energy hopping with small

exciton delocalization may explain this.^[12] Herein, we present a new DNA architecture that tackles the question of long-range CPD formation (Figure 1). It consists of the 3-methoxyxanthone C-nucleoside **X** for site-selective excitation and energy injection, and of two adjacent pyrimidines as designated site for CPD formation. In comparison to xanthone (triplet energy $E_{00} = 311 \text{ kJ mol}^{-1}$)^[13] 3-methoxyxanthone has a singlet energy of $E_{00} = 332 \text{ kJ mol}^{-1}$, shows fluorescence and only little triplet photochemistry, if at all.^[14] Due to the additional endocyclic oxygen, xanthenes are less twisted than benzophenones^[11] and allow better stacking inside DNA. The designated site for CPD formation consists of two pyrimidines (C or T) that are placed next to each other but lack the phosphodiester bond. It probes energy migration directly by the formed damage, because the cyclobutane links the two oligonucleotides together and thereby changes their electrophoretic mobility. This allows PAGE analysis of the chemical CPD yields.^[11] With this DNA architecture, the distance dependence can be studied because both the site of photoenergy injection **X** and the damage site are well defined and can be placed in distinct distances. We synthesized the hybrids **DNA n -TT** that differ by the number n of pairs of alternating A-T pairs, ranging from $n = 0$ (direct neighborhood) in **DNA0-TT** to $n = 15$ (30 intervening A-T pairs) in **DNA15-TT**.

The methoxyxanthone C-nucleoside **X** was incorporated into DNA single strands by automated solid-phase synthesis (Scheme S1, Tables S1, Figures. S2–S14). The counterstrands to these **X**-modified oligonucleotides consist of two pieces. The 5'-terminus of the oligonucleotide piece on the 5'-side of the reaction site was marked by the photostable atto550 dye (**At**), the other piece on the 3'-side was not marked. The CPD formation links the two oligonucleotide pieces together and was quantified by PAGE analysis of aliquots taken during the irradiations (see gel image in Figure 1). All irradiations were performed (i) with a 369 nm LED, (ii) at 10 °C to ensure complete annealing of the three oligonucleotides in each hybrid **DNA n -TT** (e.g. the hybrid **DNA15-TT** shows two melting temperatures at $T_m = 14$ °C and $T_m = 60$ °C due to its ternary composition, Figure S1, for the other melting temperatures see Table S2), (iii) under strict exclusion of oxygen to prevent other damages, and (iv) at least three times to elucidate standard deviations of 2–4 % (Table S4). We ruled out potential background reactions^[4,15] by negative control experiments with a DNA hybrid equivalent to **DNA0-TT** but with a T instead of **X**. After 96 h irradiation, the gels did not show any CPD formation (Figures S63–S64). Expectedly, the extinction of unmodified DNA at 369 nm is too small. The **At** dye showed 35 ± 5 % bleaching (Figure S65), which is a sufficient photostability. We assume from our PAGE analysis that photobleaching of the **At** dye occurs independently from the DNA hybrid and equally in the starting materials and products. This allows using **At** as internal standard for quantitative PAGE analyses. **DNA0-TT** bears **X** directly adjacent to the reaction site and serves as positive control. After 96 h irradiation, the CPD-linked product was obtained in 80 % yield, which was evidenced additionally by ESI mass spectrometry (Table S3, Figures. S15–S62). These results clearly show that only **X** as energy injector induces CPD

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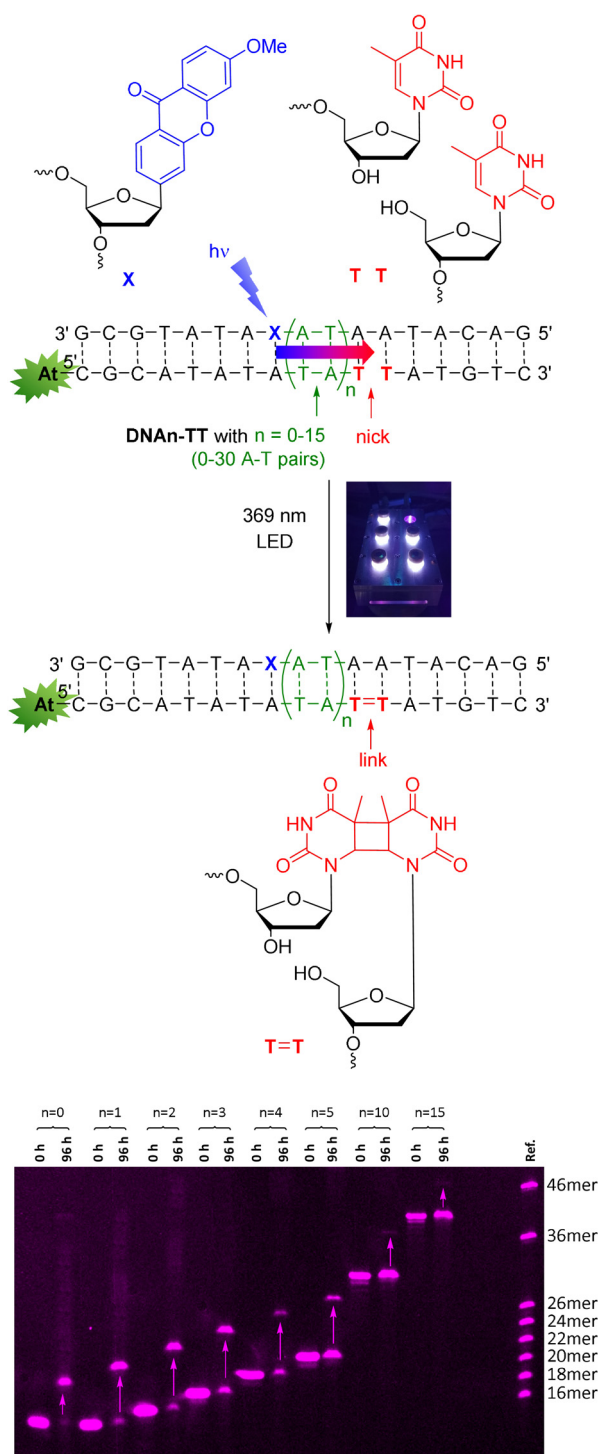


Figure 1. DNA hybrids **DNAn-TT** with $n = 0-15$ for the distance-dependent investigation of CPD formation by energy migration through DNA. The 3-methoxyxanthone C-nucleoside serves as energy injector **X** upon excitation by the 369 nm LED (see lower image). The fluorescently marked counterstrand (**At** = Atto550 dye) is elongated by the CPD formation between two adjacent thymidines lacking the phosphodiester bond. The CPD (representatively T=T) yield can be analyzed by PAGE (see lower image).

formation. We tried to measure the reaction quantum yields by chemical actinometry.^[16] But the values are too small ($< 0.1\%$) to be accurately determined by this method. After

60 h irradiation of the hybrids **DNAn-TT**, the CPD yields seem to plateau at different values depending on distance to **X**. There is, however, still a small increase of CPD yields observable. Nevertheless, the time-dependent CPD yields indicate a contribution from reversibility; in principal, reopening of CPDs may occur by energy or charge transfer. The yields in the other DNA hybrids range from 75% for **DNA1-TT** (short distance) to 9% for **DNA15-TT** (long distance) (Figures S66–S74). It is clearly not the exponential distance dependence that we previously observed for triplet energy migration,^[10] which excludes also an electron transfer mechanism. Instead, the distance dependence of the CPD yields shows a sigmoidal behavior, similarly at every snapshot between 6 h and 96 h irradiation (Figure 2). The data was fitted according to the Boltzmann sigmoid function depending on the distance R , $y = A_2 + (A_2 - A_1) / (1 + \exp((R - R_0) / dR))$, with A_1 = plateau before the sigmoidal transition, A_2 = plateau after the sigmoidal transition and $R_0 = 50\%$ threshold. The most important parameter describing the energy migration is $R_0 = 25 \pm 3 \text{ \AA}$. It is the turning point between short-range and long-range damaging. Interestingly, our R_0 agrees well with the Förster radius of 27 \AA described by Wilhelmsson et al. for energy transfer between two fluorescent DNA base analogs.^[17] This further supports that the CPD formation is the result of energy migration from **X** over the DNA base stack. Based on the ultrafast T-T dimerization rate of $\approx 1 \text{ ps}$,^[3] we assume that the preceding energy migration is the rate-limiting step. Over distances below the critical R_0 CPDs are formed in high yields, thus the energy migration occurs extremely fast, therefore coherently, and shows a shallow distance dependence. However, we cannot disentangle a Förster-like energy transfer from a mechanism that involves the intervening DNA bases. At longer distances than R_0 , the yields of CPD formation significantly drop, but not to zero. The second most important parameter is A_2 ranging from 0.9% after 6 h to 9.4% after 96 h irradiation. It describes the CPD yields over long ranges ($> 56 \text{ \AA}$, $> 2R_0$). In particular, CPD formation in **DNA10-TT** and **DNA15-TT** was observed in remarkable 10% and 9% yield over distances of 71.4 \AA and 105.4 \AA , respectively. It was not expected that the energy may migrate over such long distances to form sufficient CPD damages to allow their detection above the experimental error. This result evidences a second mechanism beyond the R_0 limit and its distance dependence seems to be extremely shallow. In contrast to the energy migration over distances below R_0 , we assign this additional long-range pathway to an incoherent energy hopping process as it has been previously proposed for triplet energy hopping^[12] or experimentally observed for long-range charge hopping through DNA.

CPDs are preferably formed between Ts; UV-B irradiation of human skin yields CPDs in the order $\text{TT} > \text{TC} > \text{CT} \gg \text{CC}$.^[18] 5-Methyl-C (mC) is an important epigenetic marker^[19] and, in contrast to C, a preferred target for CPD formation.^[20] Our DNA architecture allows to directly compare the influence of $\text{Y} = \text{T}$, C and mC in the hybrids **DNA1-YZ** ($\text{Z} = \text{T}$, C mC) with two intervening A-T pairs between **X** and the site of CPD formation (Figures 3, S75–S84). The mixed CPD yields are generally higher if T is on the

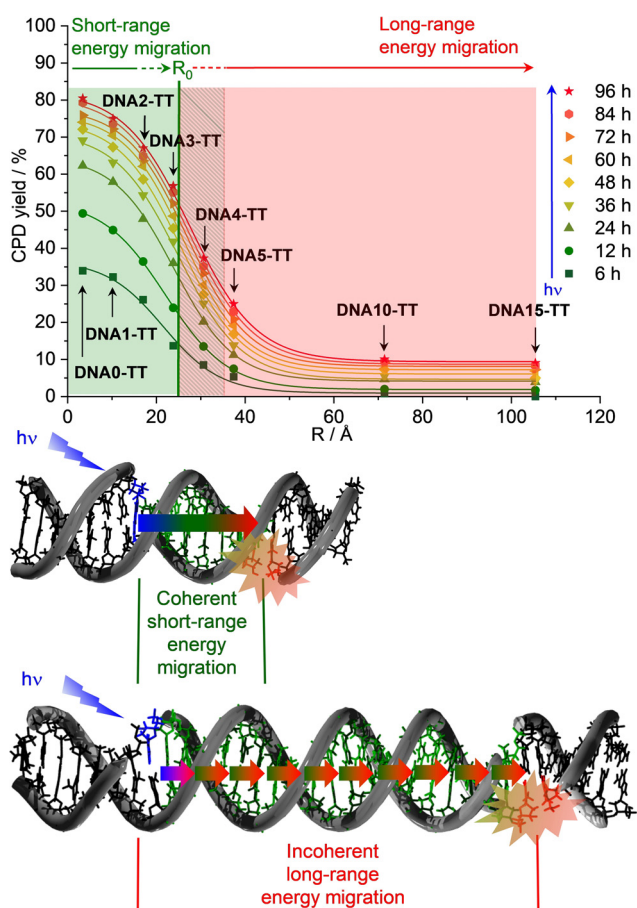


Figure 2. Short- and long-range CPD damages to DNA by energy migration: Top: Distance dependence of the CPD yields in the hybrids **DNA_n-TT** after 6 h–96 h irradiation by the 369 nm LED. Bottom: Model of hybrids **DNA3-TT** and **DNA10-TT** with X as internal energy donor (blue), 6 or 20 intervening A-T pairs (green), and the CPD formation site (red).

5'-side and lower if T is on the 3'-side. **DNA1-TT** serves as reference and shows $75 \pm 5\%$ CPD yield after 96 h irradiation. C on the 3'-side (**DNA1-TC**) decreases the yield to $71 \pm 5\%$, while mC (**DNA1-TmC**) increases the yield to $82 \pm 5\%$. mC and C on the 5'-side drop the yields to $59 \pm 4\%$ (**DNA1-mCT**) and $18 \pm 4\%$ (**DNA1-CT**). In contrast, no CPD is formed at all between two Cs in **DNA1-CC** as expected. The methyl groups increase the yields slightly to $5 \pm 3\%$ and $4 \pm 3\%$ in **DNA1-mCC** and **DNA1-CmC**, and more significantly to $29 \pm 4\%$ in **DNA1-mCmC**. It has been proposed that the methyl group of mC enhances the stacking interactions by altering the sugar pucker which properly orientates the two pyrimidines.^[21] Our results give experimental support for this proposal: Since there is no phosphodiester bond between the pyrimidines, the CPD formation in our DNA architectures exclusively relies on stacking and relative orientation. mC on the 3'-side of T facilitates CPD formation, whereas mC on the 5'-side lowers the CPD yields. These directional differences were not yet experimentally evidenced. If a G is placed on the 3'-side of T-mC or T-C, the CPD yields are lower due to a known photoinduced repair mechanism by charge transfer.^[22] Taken together these are important results because

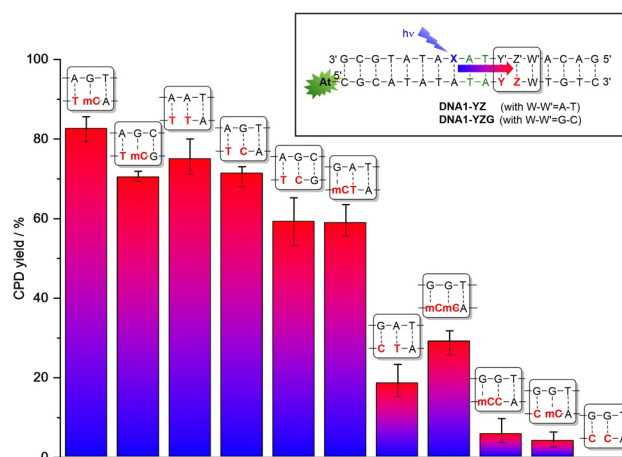


Figure 3. DNA architectures to probe the influence of 5-methyl C (mC) as epigenetic marker for CPD damaging: CPD yields after 96 h irradiation of hybrids **DNA1-YZ** (with Y-Y'/Z-Z'=T-A, C-G, mC-G and W-W'=A-T) and **DNA1-YZG** (with W-W'=G-C).

TCG sites are subject to CPD formation and subsequent mutational events to cancer.

Our DNA architecture evidences short-range and long-range energy migration directly by the CPD formation as characteristic photodamage. The preceding energy migration is the rate-limiting step since the T-T dimerization rate occurs on ultrafast timescale in ≈ 1 ps.^[3] Our results contradict triplet energy and electron transfer by the absence of exponential distance dependencies of the CPD yields. We obtained a sigmoidal distance dependence with a critical R_0 of 25 ± 3 Å. Below R_0 , short-range energy migration occurs with high yields and shallow distance dependence which is characteristic for a coherent and fast process. Energy migration between natural DNA bases occurs on the ultrafast timescale (fs).^[23] This agrees well with our experiments and is likely the reason why we do not see any significant distance dependence for CPD formation. The formation of mixed CPDs (T, C and mC) revealed sequential differences that were not evidenced previously in such completeness but have been proposed to be important for mutational events to cancer. Above R_0 , long-range energy migration is observed up to 30 A-T pairs (105.4 Å). The distance dependence is very shallow. Taken together these are typical characteristics of an incoherent hopping process with the intervening A-T pairs as intermediate energy carriers (“steppingstones”). For charge transfer, the mechanistic change from coherent superexchange to incoherent hopping occurs at distances of 4–5 base pairs (17–20 Å).^[24] However, CPD formation, including those with mC, takes place via exciton states delocalized over the two reacting pyrimidines, and exciton states migrate differently in DNA than charges.^[20d,25] For such energy migration, our experiments reveal a the mechanistic change between short and long range at distances of 6–8 base pairs (24–31 Å). The distance limit of the long-range energy migration may be beyond 105 Å. However, even more extended DNA architectures would require longer irradiation times than 96 h; the applied **At** marker is considered to be one of the most photostable dyes, but it is not stable enough for such

experiments. In general, the evidence of long-range CPD formation is important for our understanding of DNA photodamaging. Future research will focus on the detailed mechanisms.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: cycloaddition · epigenetics · oligonucleotide · photochemistry · xanthone

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