Supplementary Information

UV-Driven Self-Repair in Chimeric d(GAUU) Outcompetes Damage Formation

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Materials and Methods

Samples

To accumulate cis-syn cyclobutane pyrimidine dimer (CPD) lesions[1], d(GAU=U) (where "=" represents the CPD throughout the text), we first synthesized the undamaged d(GAUU) and then used acetone photosensitization.

The undamaged sequence, d(GAUU), was prepared using phosphoramidites and reagents from ChemGenes (Wilmington, MA) and Glen Research (Sterling, MA) on either the Expedite 8909 or the K&A H-6 DNA/RNA/LNA Synthesizer. Following synthesis, the oligonucleotides were then deprotected and purified by reverse phase flash chromatography using a 50 g C18Aq column with gradient elution over 10 minutes of 0-10% acetonitrile in 25 mM (pH 7.5) triethylamine bicarbonate (TEAB) buffer.

To generate CPDs, purified d(GAUU) was dissolved at 1 mM in LC-MS grade water (Supelco, OmniSolv) containing 25% v/v acetone (Sigma-Aldrich, 270725-2L, ≥ 99.9%). The solution was then photosensitized at 310 nm (LED, Thorlabs, M310L1) for 60 min in a fused silica cuvette (Starna Cells, 21-Q-10) at an average power of 13 mW (measured at the cuvette position) following the methods described in Crucilla et al. (2023)[2]. The sample was mixed continuously with a magnetic stir bar. The resulting d(GAU=U) was purified by high-performance liquid chromatography (HPLC) on a C18 reverse-phase column (ZORBAX Eclipse plus C18, 4.6 x 250mm, 5µm, 400 bar) with a 12-minute gradient from 5% to 20% acetonitrile (Sigma-Aldrich, 494445-2L) in 100 mM triethylammonium acetate (prepared from triethylamine [Sigma-Aldrich, 81101-2ML] and acetic acid [Sigma-Aldrich, 45754-100ML]). Purified samples were lyophilized and stored at -20 ℃ until use.

For the UV / Vis repair experiments, lyophilized d(GAU=U) was dissolved in 1.6 mL of 50 mM phosphate buffer (Sigma Aldrich: KH2PO4, P5655-100G, ≥ 99.0% and Na_2HPO_4, S7907-100G, ≥ 99.0%) at pH 6.9 (Hach, sensION+ PH3). To match prior published experiments[2], concentration was kept between 0.03 mM and 0.05 mM of d(GAU=U). For experimentation, the sample was separated into two 800 µL aliquots placed inside 1 mL cuvettes (Starna Cells 9B-Q-10-GL14-C). One unirradiated aliquot served as the reference. The other aliquot was irradiated at increasing dosages. Throughout the experiment, both cuvettes were measured simultaneously in the UV / Vis spectrophotometer (Shimadzu, UV-1900). All spectrophotometric measurements were performed in triplicate at room temperature (24 °C) under ambient oxygen conditions.

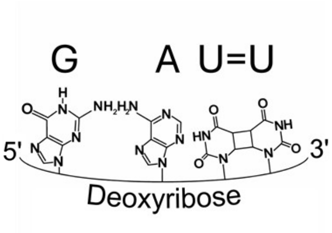

Figure S1. Chemical structure of d(GAUU) with a CPD lesion (represented by "="). dG and dA nucleotides are attached to the dUU dipyrimidine, two uridines that are connected to each other by a cyclobutane bond.

Photometric Setup and Irradiation

The schematic of the photometric irradiation setup used to irradiate and repair the damaged d(GAU=U) is described in detail in Crucilla et al. (2023)[2]. Sample cuvettes were irradiated using a 285 nm LED (Thorlabs, M285L5, 10 mm path length) with a bandwidth of ~15 nm (Figure S2, gray). This wavelength selectively excites the d(GA) of d(GAU=U) (Figure S1, purple), allowing for intervalence charge transfer to repair the damaged sample to d(GAUU) (Figure S1, red). During irradiation, the sample was mixed continuously with a magnetic stir bar. The LED power was continuously detected by a silicon photodetector (Newport, 918D-UV-OD3R) and a power meter (Newport, 1919-R).

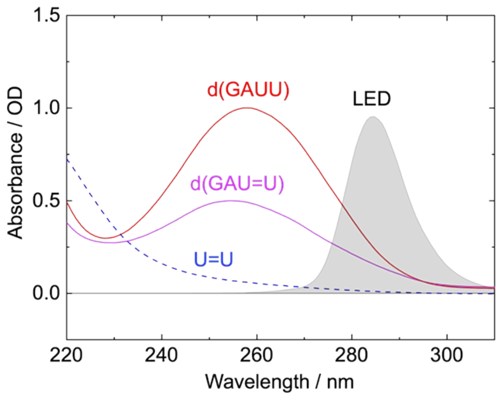

Figure S2. UV/Vis absorbance spectra of U=U (blue), d(GAUU) (red), CPD-damaged d(GAU=U) (purple), and an LED centered at 285 nm. The LED is able to selectively excite d(GA), as U=U is not spectroscopically active at 285 nm. This process allows for the charge transfer to occur, repairing d(GAU=U) to d(GAUU).

Quantum Yield Determination

The quantum yield, ϕ, is defined as the ratio of photoproducts formed to photons absorbed[3]. Here, we quantify ϕ as the number of d(GAUU) molecules repaired over the number of photons absorbed as measured by our silicon photodetector. To determine quantum yield, we use the derived equation (1) from Crucilla et al., which is based on [2–4] . We plug in our sample volume of 800 µL, cuvette depth of 10 mm and molar decadic absorption coefficients of $\varepsilon_{UU}|_{263\text{ nm}} = 22125\ M^{-1}\ cm^{-1}$ (determined from scaling to [5]) and $\varepsilon_{U=U|263\text{ nm}} = 495\ M^{-1}\ cm^{-1}$.

$$\varphi \approx 1.55 \cdot 10^{-2} \frac{J}{OD} \cdot \frac{\Delta A|_{263\ nm}[OD]}{E_{abs}|_{285\ nm}[J]} \qquad (1)$$

Data Evaluation

For direct comparison to previously published work, we performed identical data evaluation to Crucilla et al. (2023)[2] using d(GAUU) as our target sequence.

At each irradiation dose, UV/Vis spectra of the irradiated sample and an unirradiated reference were recorded in parallel. Difference spectra were obtained by subtracting the reference spectrum from the sample spectrum. Baseline offsets were corrected by subtracting the absorbance at 360 nm from each difference spectrum. The absorbed irradiation energy was calculated as the difference between the incident and transmitted power, multiplied by the irradiation time (s). Reflection losses were accounted for by applying a 0.9 scaling factor to the incident power. The change in absorbance at 263 nm was then plotted as a function of absorbed energy, and the slope from a linear fit was used to determine the quantum yield (1).

The statistical uncertainty in the d(GAUU) quantum yield was estimated as 7% from the standard deviation across triplicate experiments. Systematic uncertainty was estimated as ~32%, assuming contributions of 15% from spectrophotometric measurements, 25% from the LED spectral bandwidth (~15 nm; measured by oscilloscope), 5% from sample impurities (estimated from Fig. S4 and related HPLC traces), 8% from pipetting variability, 5% from sample positioning during irradiation, 3% from Fresnel reflection losses, 2% from timing precision, 1% from beam focusing, and 1% from power detection.

Table S1: Slopes and intercepts of difference spectra for d(GAT=T), d(T=TAG), GAU=U, and d(GAU=U) repair and dUU and dGAUU damage. For our measurements, the error bars on the repair slopes across triplicates are approximately ±0.05 mOD/mJ for d(GAU=U), ±0.003 mOD/mJ for d(GAUU), and ±0.05 mOD/mJ for dUU. Repair is indicated by a positive slope while damage is indicated by a negative slope.

Molecule	**Slope (mOD/mJ)**
d(GAT=T)	0.282 ± 0.006
d(T=TAG)	0.336 ± 0.004
GAU=U	0.122 ± 0.002

d(GAU=U)	0.687 ± 0.01
dUU	-0.507± 0.007
d(GAUU)	-0.105 ± 0.004

NMR Measurements

To identify damaged vs undamaged d(GAUU) and determine purity, NMR was performed. ^{1}H spectra (Figure S3) were acquired on a Bruker AVANCE NEO 400B (400 MHz for ^{1}H) at 25 °C. Chemical shifts are reported in parts per million (ppm) values using the δ scale. ^{1}H NMR was collected in D_2O for d(GAU=U) and in 1:10 D_2O to H_2O for d(GAUU). The unirradiated sample was run for 1 hour under water suppression mode. Because the d(GAU=U) sample was at a low concentration, it was dissolved in D_2O and lyophilized for three cycles prior to a 1-hour measurement in water suppression mode to achieve better resolution.

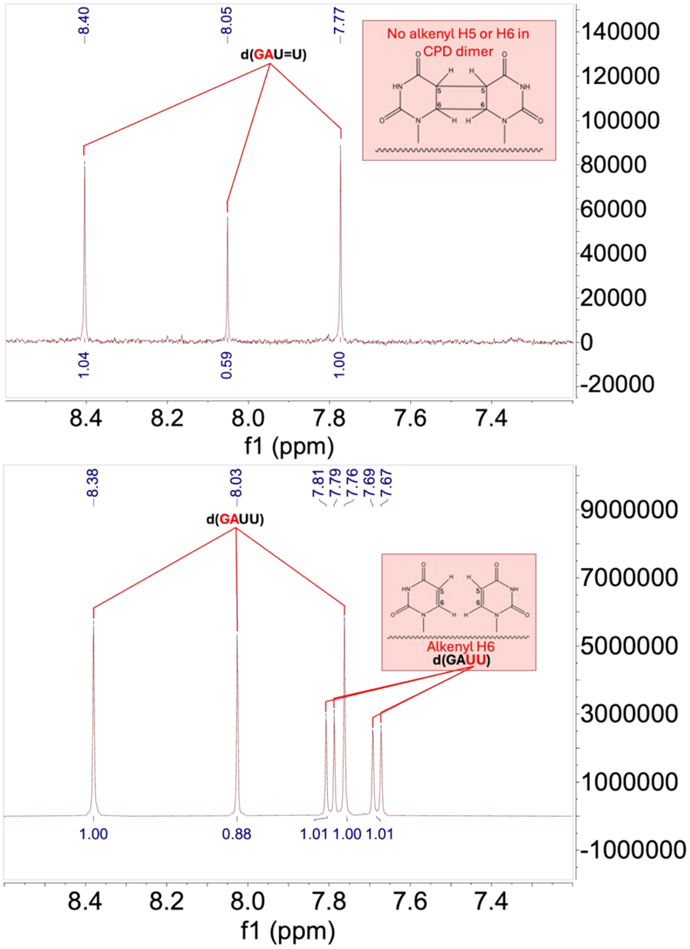

Figure S3. NMR of d(GAU=U) (top) and d(GAUU) (bottom) in D_2O. The uridine H6 aromatic proton peaks of d(GAUU) at 7.81 ppm (d, J = 8.1 Hz, 1H) and 7.69 ppm (d, J = 8.1 Hz, 1H) disappear in the d(GAU=U) spectrum, indicating the dimer formation. The chemical shifts and integrations of the proton peaks on adenine and guanine (7.67–8.38 ppm) are also indicated in both spectra.

HPLC Analysis

5 µL aliquots were taken from the irradiated sample after 0, 10, 20, and 30 min for HPLC analysis. The measurements were performed using a reverse phase column on an analytical HPLC (Agilent 1100, ZORBAX Eclipse plus C18, 4.6 x 250 mm, 5 µm, 400 bar). Samples were measured using a 12-minute gradient from 5% to 20% acetonitrile (Sigma-Aldrich, 494445-2L) in 100

mM triethylammonium acetate (prepared from triethylamine [Sigma-Aldrich, 81101-2ML] and acetic acid [Sigma-Aldrich, 45754-100ML]) at a column temperature of 25°C. The spectra are shown in Figure S4.

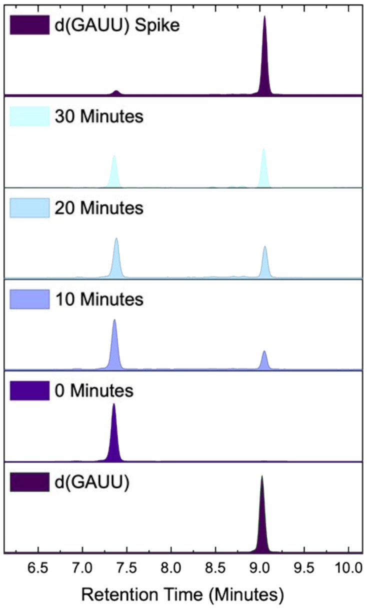

Figure S4. HPLC spectra of d(GAU=U) repair over time. At 0 minutes, the sample was pure d(GAU=U) (peak at ~7.4 minutes, pink). Over time, the repair peak returns at ~9.0 minutes. The repair peak matches the spectral shape and position of d(GAUU) (black, bottom). At 30 minutes, we spiked the sample with unirradiated d(GAUU) to ensure the repair peak was in the correct location (black, top).

Photostationary Equilibrium

Upon extended irradiation over several hours, the d(GAU=U) reaches a photostationary state in which the rates of self-repair and photodamage (including CPD formation and other photodamage) balance. This equilibrium is reflected as the plateau observed in Figure S5. As noted in this figure, d(GAU=U) (red) reaches 60% more repair compared to GAU=U (black). Details of the experimental approach have been previously described in Crucilla et al. (2023).

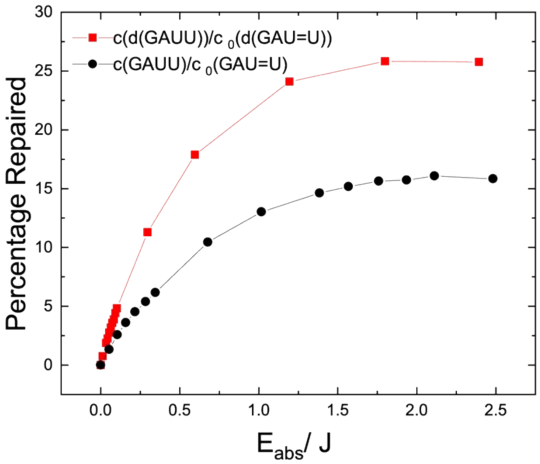

Figure S5. Long-term irradiations of the samples d(GAU=U) (red, this work) and GAU=U (black, Crucilla et al. (2023)[2]). The photostationary equilibrium of d(GAU=U) to d(GAUU) is reached after ~26% conversion and equilibrium of GAU=U to GAUU after ~16% conversion[2]. The experimental starting concentrations were c_0(d(GAU=U)) = 0.82 OD and c_0(GAU=U) = 0.054 OD[2] and the irradiation doses P_0 = 0.4 mW for d(GAU=U) and P_0 = 0.6 mW for GAU=U[2]. The absorbed dose of irradiation, E_{abs}, (x-axis), was calculated as explained in the "Data Evaluation" section of this SI. When comparing these long-term irradiations, there is an 11% difference in percentage repair, representing a 66% proportional increase overall in conversion of d(GAU=U) to d(GAUU) compared to GAU=U to GAUU.

Damage Yields

To determine the rate at which d(UU) and d(GAUU) damage, we ran triplicate experiments at 285 nm. Our results are summarized in Figure S6, where we show the removal of the absorbance at 265 nm over time, consistent with the formation of CPDs and other photodamage. dUU undergoes damage at a much greater yield compared to dGAUU, demonstrating dGAUU's photoprotective properties.

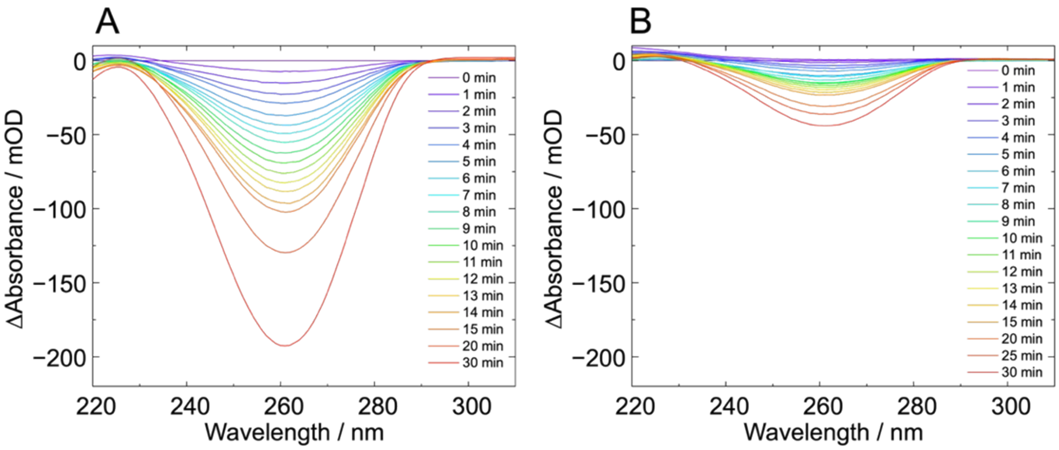

Figure S6. (A) The damage spectra of dUU over time at 285 nm. (B) The damage spectra of dGAUU over time at 285 nm. dUU undergoes damage at a much greater rate than dGAUU, likely due to the ability of the "GA" motif to repair the sample.

References

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3. *The IUPAC Compendium of Chemical Terminology: The Gold Book*. (International Union of Pure and Applied Chemistry (IUPAC), Research Triangle Park, NC, 2019). doi:10.1351/goldbook.
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