Supporting Information for:

Electron Donor-Acceptor Interactions with Flanking Purines Influence the Efficiency of Thymine Photodimerization

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Experimental Methods

All single strands and hairpins were prepared using previously reported methods on an Expedite 8909 DNA synthesizer using standard phosphoramidite chemistry, followed by purification with reversed-phase HPLC, and characterization by MALDI-TOF mass spectrometry (Table S1).¹ UV-visible spectra were recorded on an Agilent 8453 spectrophotometer.

The standard buffer solution (pH 7.1) contains 10 mM phosphate buffer and 100 mM sodium chloride. Dilute solutions of single strands or hairpins (ca. 4 μ M) were irradiated at 280 nm in 1 cm path length quartz cuvettes using a 150 Watt Xenon arc lamp (PTI) and the irradiation wavelength was controlled by monochromator (PTI Model 101). The total energy incident on the sample solution was 2 mW as determined by potassium ferrioxalate actinometry.²

Aliquots were taken from the irradiated solution at different time intervals and analyzed by HPLC using a Microsorb-MV C₁₈ reversed-phase column (250 $\times$ 4.6 mm). For single strands, the optimal eluting condition was found to be a column temperature of 25°C and a 20-min gradient increase of acetonitrile from 5 to 15% in 20 mM NH₄OAc solution at a flow rate of 1 mL min⁻¹. However, a higher column temperature of 60°C and a slower increase of acetonitrile from 5 to 15% in 40 min were found necessary to separate hairpins from corresponding thymine dimers. The slopes of conversion% vs. irradiation time (min) were obtained by integrating HPLC peak areas of reactant and product at 260 nm. The quantum yields of photodimerization were calculated from the initial slopes using the methods described in the text and Table S3.

Oxidation potentials for the nucleosides (0.2 mM) were obtained for 10 ml phosphate buffer solutions (PBS buffer, pH 7.4) using a 3-electrode cell. A 1.5 mm glassy carbon electrode (GCE) served as working with platinum wire as a counter electrode, and Ag/AgCl as the

reference electrode. Squarewave voltammograms (SWV) were obtained with 50 mV pulse amplitude, 70 ms pulse width and 5 mV step potential. Similar results were obtained from differential pulse voltammograms (DPV) using a 50 mV pulse amplitude, 70 ms pulse width, and 5 mV step potential.

Investigations of ground state conformation employed molecular dynamics simulations done using the NAMD program³ with the CHARMM27 force field,⁴ as previously described.⁵

Table S1. m/z values determined by MALDI-TOF mass spectrometry for single strand (subscript S) and hairpin (subscript H) systems and T_m values for hairpins obtained from the first derivative of 260 nm UV thermal dissociation profiles. See Chart 1b for structures.

	m/z Calculated	m/z Found	T _m (°C)
II _S	1783.3	1780.8	
IA _S	1782.3	1780.4	
AI _S	1782.3	1780.4	
AA _S	1781.3	1778.9	
AG _S	1797.3	1793.7	
GA _S	1797.3	1793.5	
AZ _S	1796.3	1793.0	
ZA _S	1796.3	1793.4	
II _H	3876.8	3878.0	43.5
IA _H	3890.8	3892.2	49.3
AI _H	3890.8	3891.3	46.6
AA _H	3904.8	3902.9	50.5
AG _H	3905.8	3906.5	61.0
GA _H	3905.8	3906.6	61.5
GG _H	3906.8	3911.1	
AZ _H	3904.8	3899.2	60.5
ZA _H	3904.8	3899.2	60.5
ZZ _H	3905.8	3902.1	58.6

Table S2. Molar absorption coefficients (m²/mol) of single nucleic bases and for T<>T used in the quantum yield calculations.

Base	260 nm	280 nm
T ^a	856	624
A ^a	1506	207
G ^a	1218	813
I ^b	747	168
Z ^b	1232	816
C ^a	699	692
T<>T ^c	40	10

^a Data from ref. 1.⁶

^b Data from this work. See also ref. 2.⁷

^c Data from ref. 3.⁸

Table S3. Data and methods used for the calculation of quantum yields of TT photodimerization in Table 1.

	Slope 1 (Con%/min) ^a	Slope 2 (Con%/min) ^b	Slope 3 (Con%/min) ^c	TT% at 280 nm ^d
II _S	0.44 ± 0.02	0.64	1.44	44
IA _S	0.37 ± 0.02	0.52	1.19	44
AI _S	0.36 ± 0.02	0.49	1.14	44
AA _S	0.25 ± 0.02	0.34	0.78	43
AG _S	0.20 ± 0.02	0.27	0.75	36
GA _S	0.14 ± 0.01	0.19	0.54	36
AZ _S	0.077 ± 0.01	0.11	0.30	36
ZA _S	0.040 ± 0.005	0.056	0.16	36
II _H	0.86 ± 0.04	0.97	3.9	25
IA _H	0.63 ± 0.03	0.71	2.87	25
AI _H	0.53 ± 0.03	0.60	2.41	25
AA _H	0.52 ± 0.03	0.58	2.32	25
AG _H	0.25 ± 0.02	0.28	1.27	22
GA _H	0.096 ± 0.01	0.11	0.50	22
GG _H	n.d. ^e	n.d. ^e	n.d. ^e	20
AZ _H	0.048 ± 0.005	0.047	0.21	22
ZA _H	0.014 ± 0.005	0.016	0.073	22
ZZ _H	n.d. ^e	n.d. ^e	n.d. ^e	20

^a Plot slope of conversion% vs. irradiation time (min) by integrating HPLC peak areas of reactant and product with UV detection at 260 nm.

^b Modified Slope 1 with the consideration of different molar absorption coefficients of reactant and product at 260 nm (see Table S2 and text).

^c Corrected Slope 2 based on the competitive absorption of other nucleic bases at the irradiation wavelength of 280 nm: Slope 3 = Slope 2 / TT% at 280 nm.

^d Percentage of light absorbed by TT step at 280 nm (estimated error 5%).

^e No photodimerization product detected.

Figure S1. Probability densities for the distance between centers of T-T double bonds for hairpins AA_H, GA_H and AG_H.

Figure S2. Normalized UV spectrum of AZ_H (black) and ZA_H (red) in 10 mM phosphate buffer (pH 7.1) containing 100 mM sodium chloride.

Figure S3. Normalized UV spectrum of AA_S (black) and AA_H (red) in 10 mM phosphate buffer (pH 7.1) containing 100 mM sodium chloride.

Figure S4. HPLC trace for irradiation of II_H at 280 nm with increasing irradiation time (from top to bottom: 0, 15, 30, 45 and 60 minutes). Solutions were analyzed by high temperature reverse phase HPLC on a Microsorb MV C_{18} reverse phase column maintained at 60 °C using a gradient of 20 mM ammonium acetate containing 6% to 12% acetonitrile.

References for Supporting Information

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