Supporting Information

For

Conformational Control of Thymine Photodimerization in Single Strand and Duplex DNA Containing Locked Nucleic Acid TT Steps

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Experimental Section

T-LNA phosphoramidite, C12 linker, and other reagents required for oligonucleotide synthesis were purchased from Glen Research, Sterling, VA. Oligonucleotide conjugates containing $T_L T_L$ steps (Chart 1) were prepared using standard phosphoramidite chemistry starting from 5'-phosphate CPG as solid support using a Millipore Expedite DNA synthesizer and following the procedure of Letsinger and Wu.¹ Following synthesis, the conjugates were isolated as trityl-on derivatives by reverse phase (RP) HPLC, detritylated in 80 % acetic acid for 30 min, and repurified by RP-HPLC as needed. RP-HPLC analysis was carried out on a Dionex chromatograph with a Hewlett-Packard Hypersil ODS-5 column (4.6 x 250 mm) and a 1% gradient of acetonitrile in 0.03 M triethylammonium acetate buffer (pH 7.0) with a flow rate of 1.0 mL/min. Molecular weights were determined following desalting by means of MALDI-TOF mass spectroscopy (Tables S1, Supporting Information).

Solutions containing ca. 1-1.2 μ M hairpin in 10 mM phosphate buffer (pH 7.2) with 1.0 M NaCl were irradiated at 280 nm using a Xenon arc lamp and monochromator (ca. 2 mW) at 10 °C in 1 cm path-length quartz cuvettes. Aliquots irradiated for different time intervals were analyzed by high performance liquid chromatography (HPLC; Waters 600) on a C18 reversed phase column (MICROSORB-MV 100-5 C18, 250 $\times$ 4.6 MM VALCO) equipped with a diode array detector (Waters PDA 996) using a column temperature of 60 °C with a UV detection wavelength of 260 nm, corresponding to the absorption maxima of the base pairs. A linear gradient of 20 mM ammonium acetate containing 0-30% CH₃CN with a flow rate of 1 mL/min for 40 min was used. Under these conditions, starting material and the product eluted with different characteristic retention time. The assignments of product peaks to cyclobutane adduct has been previously described.² Product yields were determined from the initial slopes of plots of peak area vs irradiation time. Light intensities were determined using ferrioxalate actinometry.³

Computational Section

Simulations of all species were carried out using the NAMD program.⁴ All systems were neutralized with sodium ions and solvated with an 8 Å shell of TIP3P water. Constant pressure and temperature were employed using a Langevin thermostat and barostat respectively. An initial equilibration and production run was done for each species at 350 K. 10 geometries were randomly chosen from the 350 K production run as starting geometries for runs at 300 K. These systems were briefly minimized and then equilibrated for 2 ns followed by a 6ns production run. Snapshots were saved every 2 ps yielding 30000 snapshots per system.

The resulting trajectories were all analyzed by monitoring the distance, d , between the C5-C6 double bonds and the C5-C6-C6-C6 dihedral angle, η , of the TT step. The dimerization cutoff used was $d < 3.52$ Å.

All possible pairs of thymines were considered in determining the final quantum yields. Non-neighboring TT dimers were only found in structures **2** and **2L** and were minor in comparison to the neighboring TT dimers. The trajectories were also analyzed using 3DNA to determine their helical parameters.⁵ For the inter basepair parameters (rise, shift, slide, roll, twist and tilt), only the strand containing the TT steps was analyzed as if it were single stranded DNA. This was done to remain consistent between single and double stranded systems.

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Table S1. m/z values for single strand sequences **1L** and **2L** and hairpin sequences **3L-7L** determined by MALDI-TOF mass spectrometry.

Sequence	m/z Calculated	m/z Found
1L	602.4	601.1
2L	1836.8	1835.7
3L	4568.2	4568.8
4L	4264.6	4263.7
5L	3958.6	3958.9
6L	3958.6	3958.8
7L	3958.6	3958.1

Table S2. DNA melting temperature (T_m) of the hairpin sequences **3L-7L** and **3-7**.

Hairpin Sequence	$T_L T_L$ Melting point $T_m, ^\circ\text{C}$	$T T$ Melting point $T_m, ^\circ\text{C}$
3L	53.4	51.0
4L	61.5	55.1
5L	57.9	48.5
6L	71.5	50.5
7L	60.6	49.5

Table S3. 6-4 quantum yields from MD simulation calculated with the single cutoff of $g < 2.87 \text{ \AA}$ for sequences **1L**, **2L** and **5L-7L** in comparison with **1**, **2** and **5-7**.

	1	2	5	6	7
$10^{-3} \Phi_{TT}$	0	0	0.3	0.3	1.4
$10^{-3} \Phi_{T_L T_L}$	0	0.5	0.3	0.3	0.2
$\Phi_{T_L T_L} / \Phi_{TT}$	-	-	1	1	0.1

Figure S1. (A) UV/Visible absorption and (B) circular dichroism spectrum of hairpin sequences **7** (black) and **7L** (red) in 10 mM phosphate buffer (pH 7.2) containing 100 mM phosphate buffer.

Figure S2. a) HPLC trace for irradiation of hairpin **7L** at 280 nm with increasing irradiation time a) 0; b) 10; c) 20; d) 30 and e) 40 minutes. Solutions were analyzed by high temperature reverse phase HPLC on a Microsorb MV C₁₈ reverse phase column maintained at 60 °C using a gradient of 20 mM ammonium acetate containing 7% to 12% acetonitrile.

Figure S3. Relative percentage dimer formation of sequences **1L-7L** and **1-7** as determined using HPLC with increasing time of irradiation at 280 nm as monitored at 260 nm.

Figure S4. Probability densities as a function of A) rise distance; B) tilt angle; C) slide distance; D) shift distance; E) twist angle and F) roll angle in **1L** (red line) versus **1** (blue dotted line).

Figure S5. Probability densities of thymine dimerisation for the A) rise distance; B) tilt angle; C) slide distance; D) shift distance; E) twist angle and F) roll angle in single strand sequences **2L** (blue dotted line) vs. **2** (red line) and double strand sequences **6L** (cyan dotted line) vs. **6** (black dotted line).

Figure S6. Probability densities for A) buckle, B) propeller, and C) opening angles at the 5'- and 3'- T of the TT step in **6** (red line, black dotted line) and at the 5'- and 3'- T_L of the T_LT_L step in **6L** (blue dotted line, cyan dotted line).