

Supplementary Information

Study of light-induced formation of photodimers in the *i*-motif nucleic acid structure by rapid-scan FTIR difference spectroscopy and hybrid hard- and soft-modelling

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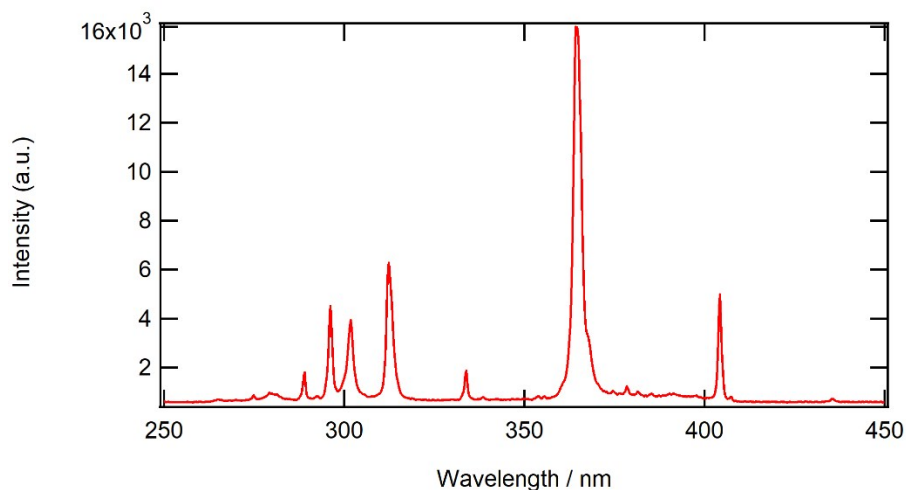
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S1. Additional information on procedures

The extinction coefficients were calculated from <https://eu.idtdna.com/calc/analyzer>. The values were 192,300, 204,100, 279,400, and 274,200 L·mole⁻¹·cm⁻¹ for TT, AA, nmyc and nmycM sequences, respectively.

The spectrum of the lamp used in the irradiation experiments is the following:



CD and absorption spectra were recorded on a Jasco J-810 spectropolarimeter equipped with a Julabo F-25/HD temperature control unit. Quartz cells (1 or 10 mm path length, 300, 1400 and 3000 µl volume) were used (Hellma, Germany).

Electrospray ionization mass spectrometry (ESI-MS) spectra were recorded with an Agilent 2006 LC/MSD TOF Mass Spectrometer (Agilent Technologies, Santa Clara, CA, USA). The capillary voltage was set to 3.5 kV, the voltage range to 200 or 300 V, the gas temperature to 325 °C, the pressure of nebulizer N₂ gas to 15 psi, and the N₂ flow rate for drying was set to 7.0 L·min⁻¹. The sample (10 µL volume, 150 mM NH₄AcO, 1% NH₃) was introduced into the source by means of an Agilent 1100 HPLC pump. The flow rate was set to 200 µL·min⁻¹ (H₂O:CH₃CN, 1:1).

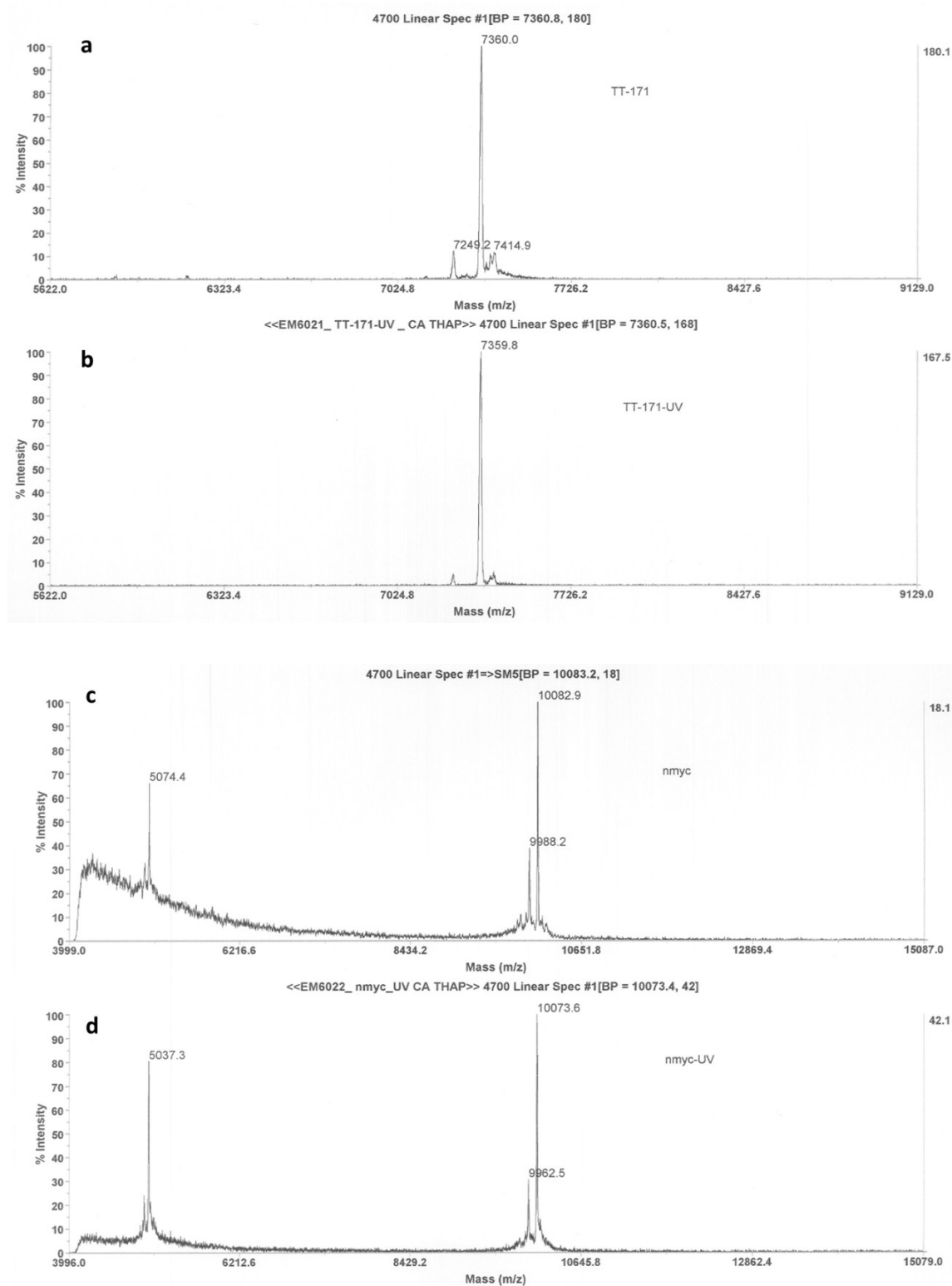
Matrix-Assisted Laser Desorption Ionization (MALDI-TOF) spectra recorded with 4800 *Plus* MALDI TOF/TOF (ABSciex – 2010). Solid state laser was used (Nd: YAG) (355nm, 200Hz, pulses of 3-7ns). The matrix was AC (ammonium citrate) (50mg/mL H₂O) i THPA (Trihydroxyacetophenone) (50mg/ml H₂O:CH₃CN 1:1). 1µL of the sample is mixed with 1µL AC. 1µL of this mixture is mixed with 1µL of THAP and 1 µL of this final mixture is deposited on the plate and allowed to dry before its analysis.

Enzymatic analysis of oligonucleotides. Oligonucleotides TT and nmyc were subjected to enzymatic digestion followed by HPLC analysis. Briefly, 0.5-1 OD₂₆₀ units of the oligonucleotide were dissolved in 92 µl of water, 5 µl of 1 M aqueous Tris-HCl solution (pH 8.0) and 1 µL of 1 M MgCl₂ solution. Then 1 µl of phosphodiesterase I from *rotalus adamanteus* venom (USB) and 1 µl of phosphatase alkaline (Roche) were added. The digestion was incubated overnight at 37 °C.

HPLC analysis of the digestion mixture was done using a 20 min linear gradient from 0 to 25 % B in a Nucleosil C18, 10 μ m, 250x4mm column (Buffer A: 5% ACN 0.1 M TEAAc and Buffer B: 70% ACN 0.1 M TEAAc). The retention times of the unmodified nucleosides were: dC, 3.2 min; T: 4.1 min; dG, 4.6 min and dA, 6.1 min. In the case of the compounds obtained after UV-photolysis we observed additional peaks corresponding to nucleoside photoadducts in the range of 9-13 min.. Oligonucleotides were also analyzed before and after UV irradiation using the same HPLC column and following a 0 to 50 % B gradient.

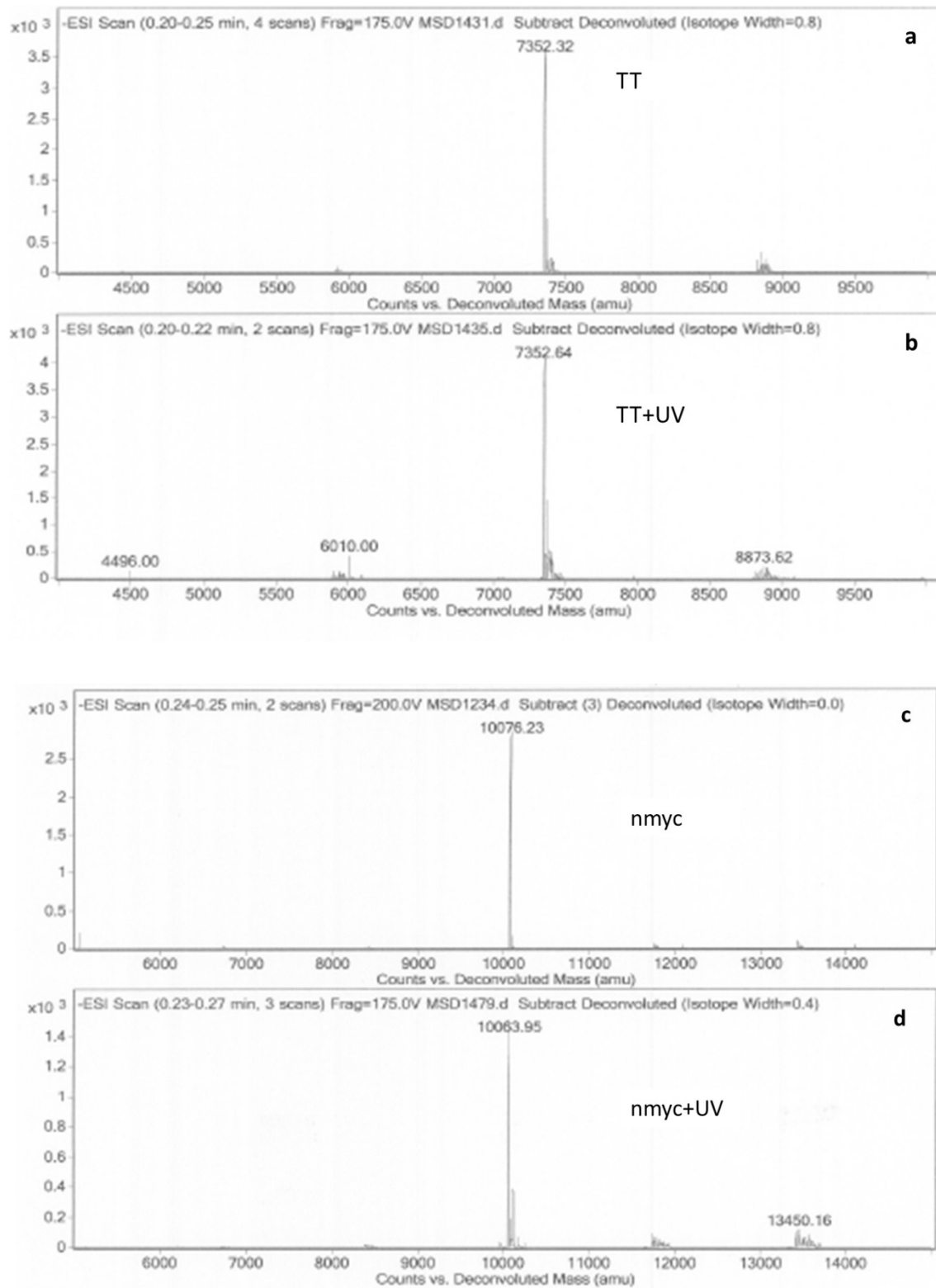
S2. MALDI-TOF spectra

MALDI-TOF spectra before and after 66 seconds of UV irradiation. a) TT before UV irradiation, b) TT after UV irradiation, c) nmyc before UV irradiation and d) nmyc after UV irradiation. DNA concentration: 5 μ M in ammonium acetate.



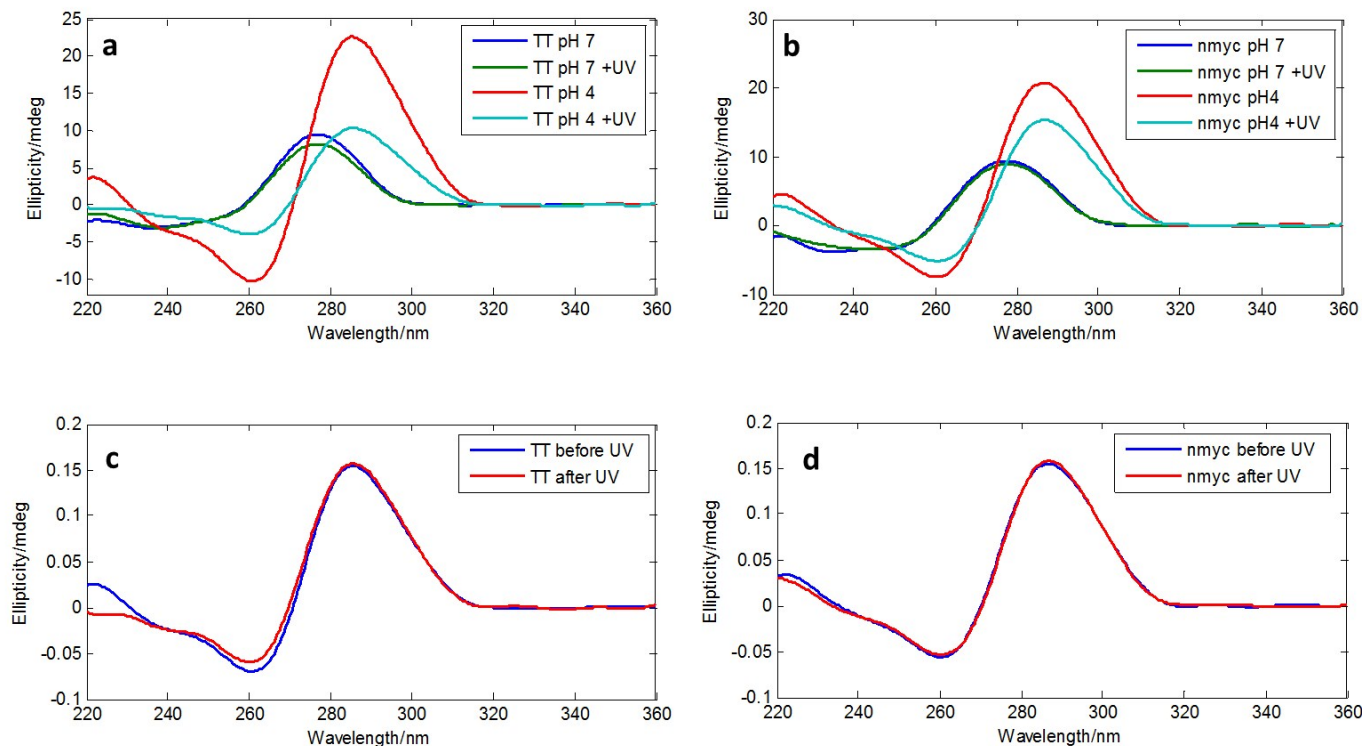
S3. ESI spectra

ESI spectra before and after 66 seconds of UV irradiation. a) TT before UV irradiation, b) TT after UV irradiation, c) nmyc before UV irradiation and d) nmyc after UV irradiation. DNA concentration: 5 μ M in ammonium acetate.



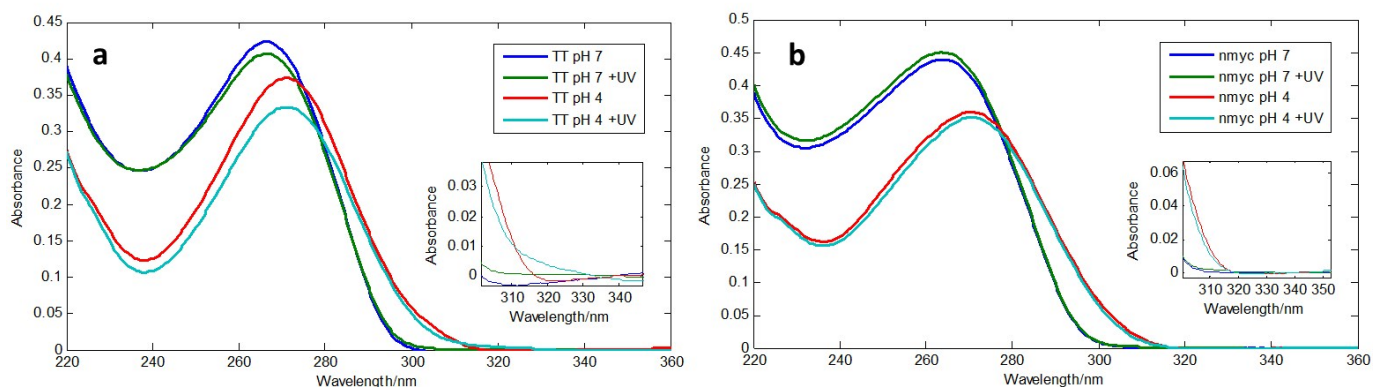
S4. CD spectra

CD spectra at pH 4 and 7 before and after 66 seconds of UV irradiation. a) TT, b) nmyc, c) normalized spectra of TT at pH 4 and d) normalized spectra of nmyc at pH 4. DNA concentration: 2 μ M. Spectra were acquired at pH 4.0 (50 mM acetate buffer, 100 mM KCl) and pH 7.0 (50 mM phosphate buffer, 100 mM KCl).



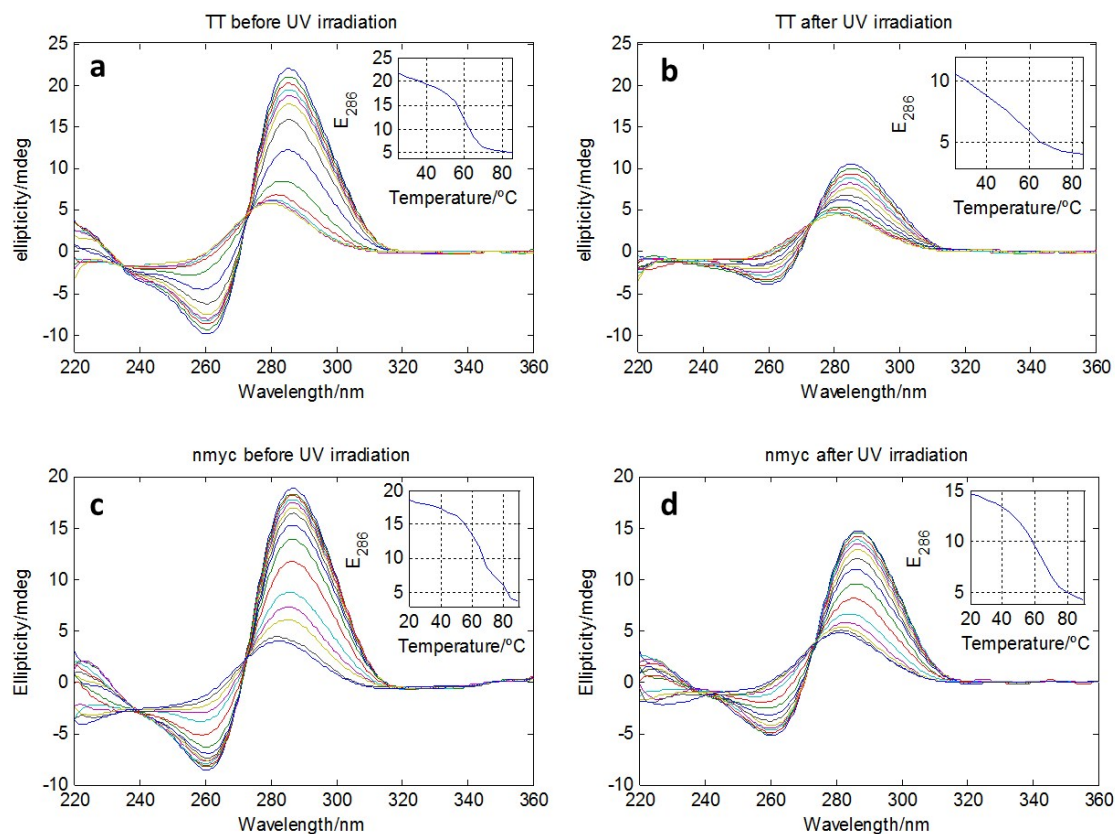
S5. Molecular absorption spectra

Absorbance spectra at pH 4 and 7 before and after 66 seconds of UV irradiation. a) TT and b) nmyc. DNA concentration: 2 μ M. Spectra were acquired at pH 4.0 (50 mM acetate buffer, 100 mM KCl) and pH 7.0 (50 mM phosphate buffer, 100 mM KCl).

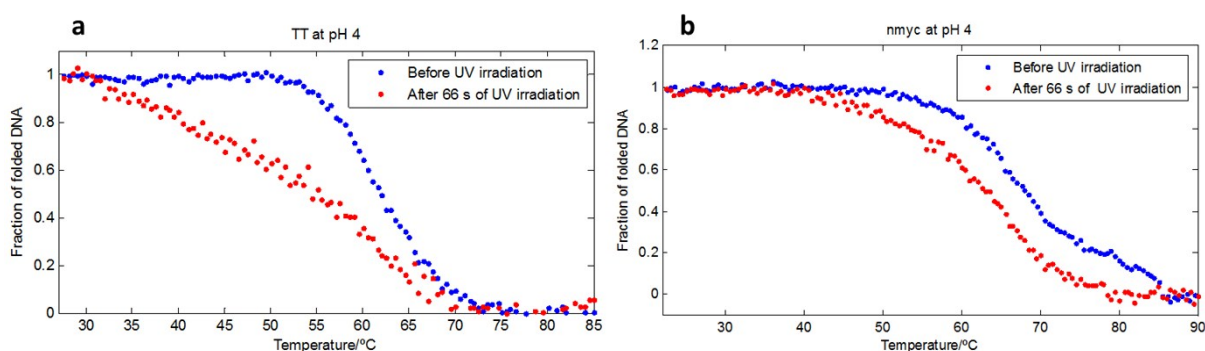


S6. CD-monitored melting experiments

S6.1. Melting experiment recorded by CD before and after 66 seconds of UV irradiation at pH 4. Inset: ellipticity trace at 286 nm. a) TT before UV irradiation, b) TT after UV irradiation, c) nmyc before UV irradiation and nmyc after UV irradiation. DNA concentration: 2 μ M. Spectra were acquired at pH 4.0 (50 mM acetate buffer, 100 mM KCl).



S6.2. Normalized ellipticity trace at 286 nm before and after 66 seconds of UV irradiation at pH 4. a) TT and b) nmyc. DNA concentration: 2 μ M. Spectra were acquired at pH 4.0 (50 mM acetate buffer, 100 mM KCl).



S7. HPLC analysis of the enzymatic digestions of oligonucleotides

HPLC chromatograms of the enzymatic digestion of the samples before and after 66 seconds of UV irradiation. a) TTat pH 7 i, b) nmyc at pH 7, c) TT at pH 7 after UV irradiation, d) nmyc at pH 7 after UV irradiation, e) TT at pH 4 after UV irradiation and f) nmyc at pH 4 after UV irradiation. DNA concentration: 5 μ M in acetate or phosphate buffer (50 mM buffer, 100 mM KCl).

