

Sequence-dependent Thymine Dimer Formation and Photoreversal Rates in Double-stranded DNA

Supplementary Information

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Supplementary Methods and Figures

1. The Effect of Photobleaching of Fluorescent Dye Labels. Irradiation of Cy5 and Cy3 dyes present by themselves in aqueous solution in the photochemical reactor using 254 nm light resulted in photobleaching of the dyes, as evidenced by lowered absorbance at their respective absorption peaks (Figure S3) and an accompanying decrease in total fluorescence intensity (Figure S4). For labeled DNA, the extent of photobleaching is reduced due to the much greater absorbance at 254 nm by the nucleobases. Nonetheless, the total fluorescence intensity from all bands in an irradiated sample was observed to decrease slightly with increasing irradiation time. By normalizing the fluorescence of all bands in each lane separately, the observed kinetics are unaffected by the small amount of dose-dependent photobleaching.

Because of the low triplet quantum yield for the Cy3 and Cy5 dyes¹ and because CPD damage was followed at sites at least 30 bp away from the labeled end, no additional CPD formation is expected

due to triplet sensitization by a photoexcited dye molecule. In an earlier study, the use of a different fluorescent dye (6-carboxyfluorescein) as a labeling chromophore was shown to not affect the kinetics of dimer formation.²

a. MP2+50 Nucleosome Binding Sequence³

Cy5 strand

AAG CTT GCAT	GCAGATCTAT	GTCGGGCTCG	TC CTT ATCGA	GCTCGGTCCG	ACAGGATGTA
TATATCTGAC	ACGTGCCTGG	AGACTAGGGA	GTAATCCC CT	TGGCGTTTAA	AACGCGGGGG
ACAGCGCGTA	CGTT CGATCA	AGCGGATCCA	GAG CTT GCTA	CGACCA ATTG	AGCGGCCCCG
GCACCAAG CT	TCTG CAGTGA	TCATCCCCGC	ACCTCGTCAA	CCGGA ATTCA	CTGGCCGTCG
TTTTACA					

Cy3 strand

TGTAAACGA	CGGCCAGTGA	ATTCCGGTTG	ACGAGGTGCG	GGGATGATCA	CTGCAGAAGC
TTGGT GCCGG	GGCCGCTCAA	TTGGT CGTAG	CAAGCTCTGG	ATCCG CTTGA	TCGAACGTAC
GCGCTGTCCC	CCGCGTTTTA	AACGCCAAGG	GG ATT ACTCC	CTAGTCTCCA	GGCACGTGTC
AGATATATAC	ATCCTGTCTGG	ACCGAGCTCG	ATAAGGACGA	GCCCACATA	GATCTGCATG
CAAGCTT					

b. 5S+50 Nucleosome Binding Sequence⁴

Cy5 strand

AAG CTT GCAT	GCAGATCTAT	GTCGGGCTCG	TC CTT ATCGA	GCTCGGTCCG	AACCGAGCCC
TATGCTG CTT	GAC TT CGGTG	ATCGGACGAG	AACCGGTATA	TT CAGCATGG	TATGGTCGTA
GGCTC TTGCT	TGATGAAAG T	TAAGCTATTT	AAAGGGTCAG	GGATG TTATG	ACGTCATCGG
CTT ATAAATC	CCTGGAATGA	TCATCCCCGC	ACCTCGTCAA	CCGGA ATTCA	CTGGCCGTCG
TTTTACA					

Cy3 strand

TGTAAACGA	CGGCCAGTGA	ATTCCGGTTG	ACGAGGTGCG	GGGATGATCA	TTCCAGGGAT
TT ATAAGCCG	ATGACGTCAT	AACATCCCTG	ACC CTTT AAA	TAG CTT AACT	TT CATCAAGC
AAGAGCCTAC	GACCATACCA	TGCTGAATAT	ACCG GT TCTC	GTCCGATCAC	CGAAGTCAAG
CAGCATAGGG	CTCG GT TCTGG	ACCGAGCTCG	ATAAGGACGA	GCCCACATA	GATCTGCATG
CAAGCTT					

Fig. S1. 5S+50 and MP2+50 DNA sequences in 5' to 3' order. The strands are denoted by the dye (Cy5 or Cy3) attached to the 5'-end. The first fifty and final fifty base pairs are identical in both sequences. All thymine-thymine base steps are highlighted in bold.

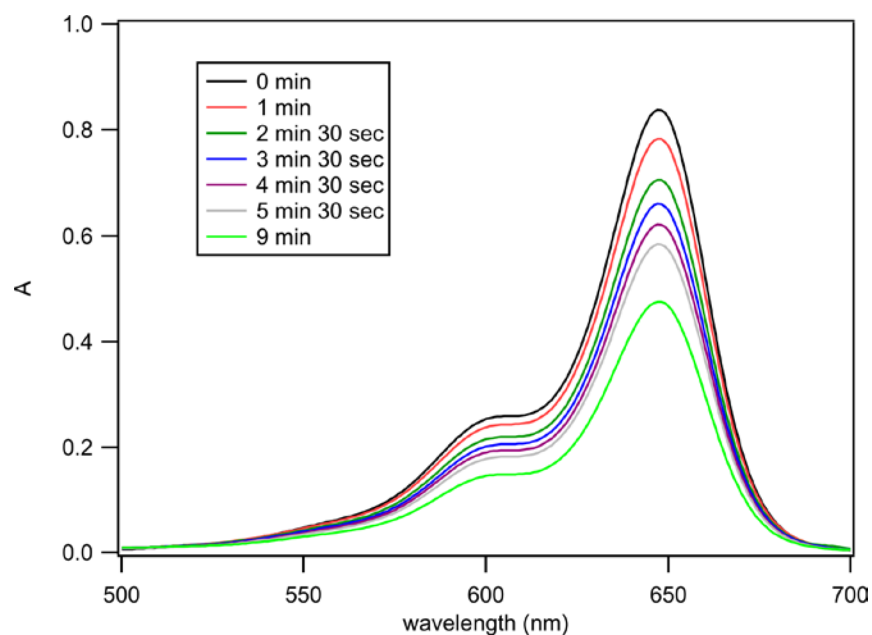


Fig. S2. Visible absorption spectra of Cy5 dye vs. irradiation time at 254 nm.

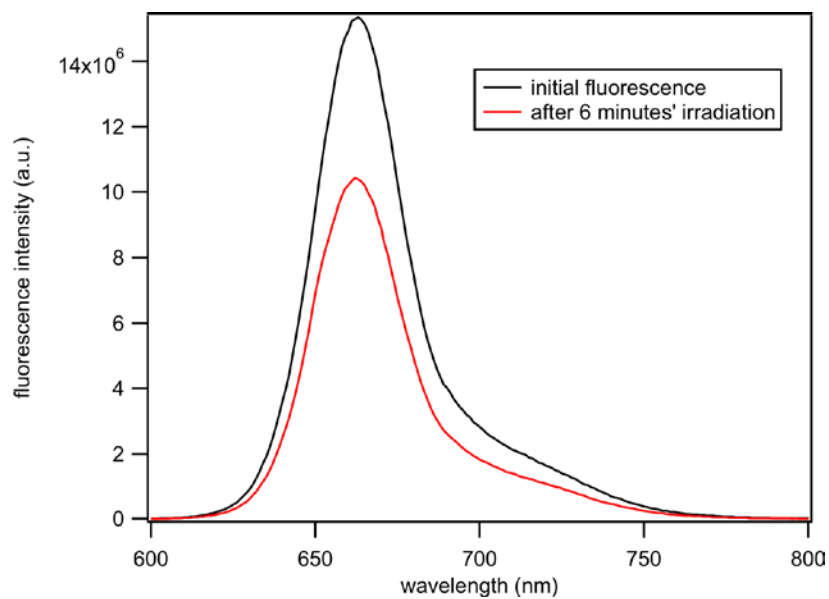


Fig. S3. Fluorescence spectra of Cy5 dye before and after irradiation at 254 nm.

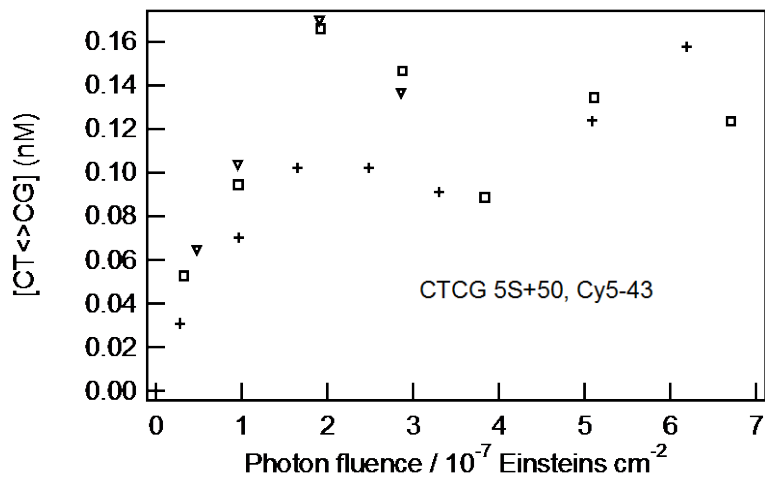


Fig. S4. Strand scission kinetics at the CTCG tetrad located at Cy5-43 on the 5S+50 sequence. Different symbols indicate separate trials.

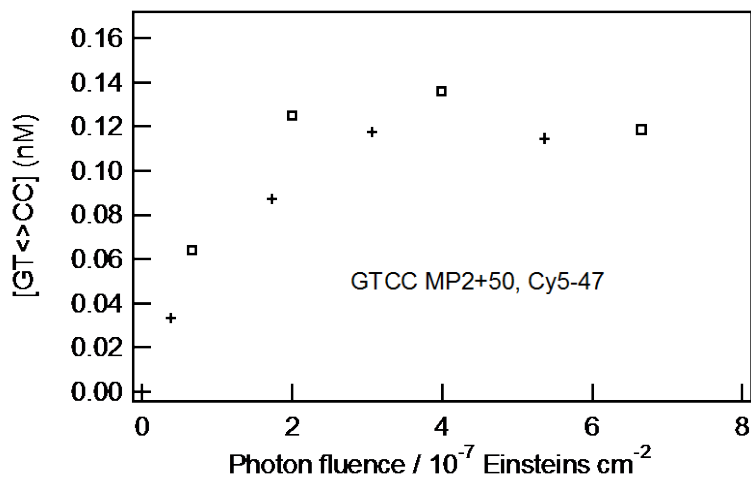


Fig. S4. Strand scission kinetics at the CTCG tetrad located at Cy5-43 on the 5S+50 sequence. Different symbols indicate separate trials.

Table S1. Relative Rates of TC Dimer Formation and Photoreversal relative to k_f for CTTA at Cy5-34.

Tetrad	Sequence	Position	k_f	k_r
ATCA	MP2+50	Cy3-48	0.09 ± 0.02	5 ± 2
	5S+50	Cy3-167	0.14 ± 0.08	10 ± 6
	Average		0.12 ± 0.04	7 ± 4
ATCC	MP2+50	Cy5-148	0.73 ± 0.22	24 ± 9
	MP2+50	Cy5-204	0.44 ± 0.19	9 ± 5
	MP2+50	Cy3-102	0.29 ± 0.11	8 ± 4
	5S+50	Cy5-189	0.26 ± 0.10	9 ± 4
	5S+50	Cy5-204	0.53 ± 0.32	18 ± 12
	5S+50	Cy3-85	0.59 ± 0.22	18 ± 7
	Average		0.47 ± 0.18	14 ± 7
ATCG	5S+50	Cy5-82	0.06 ± 0.02	7 ± 3
CTCC	MP2+50	Cy3-158	0.48 ± 0.17	8 ± 4
CTCG	MP2+50	Cy5-43	0.31 ± 0.09	11 ± 4
	MP2+50	Cy5-214	0.76 ± 0.42	20 ± 12
	5S+50	Cy5-43	0.20 ± 0.06	15 ± 5
	5S+50	Cy5-214	0.19 ± 0.09	10 ± 5
	5S+50	Cy3-191	0.22 ± 0.10	14 ± 7
	5S+50	Cy3-208	0.18 ± 0.08	10 ± 5
	Average		0.31 ± 0.22	13 ± 4
GTCC	MP2+50	Cy5-47	0.11 ± 0.03	5 ± 2
	MP2+50	Cy3-127	0.20 ± 0.04	4 ± 1
	5S+50	Cy5-47	0.07 ± 0.02	11 ± 4
	Average		0.13 ± 0.07	7 ± 4

References

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