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 dyes[1] 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 Sequence[3]

Cy5 strand

AAGC**TT**GCAT GCAGATCTAT GTCGGGCTCG TCC**TT**ATCGA GCTCGGTCCG ACAGGATGTA
TATATCTGAC ACGTGCCTGG AGACTAGGGA GTAATCCCC**T** **T**GGCG**TTT**AA AACGCGGGGG
ACAGCGCGTA CG**TT**CGATCA AGCGGATCCA GAGC**TT**GCTA CGACCAA**TT**G AGCGGCCCCG
GCACCAAGC**T** **T**CTGCAGTGA TCATCCCCGC ACCTCGTCAA CCGGAA**TT**CA CTGGCCGTCG
TTTTACA

Cy3 strand

TGTAAAACGA CGGCCAGTGA A**TT**CCGG**TT**G ACGAGGTGCG GGGATGATCA CTGCAGAAGC
TTGGTGCCGG GGCCGCTCAA **TT**GGTCGTAG CAAGCTCTGG ATCCGC**TT**GA TCGAACGTAC
GCGCTGTCCC CCGCG**TTTT**A AACGCCAAGG GGA**TT**ACTCC CTAGTCTCCA GGCACGTGTC
AGATATATAC ATCCTGTCGG ACCGAGCTCG ATAAGGACGA GCCCGACATA GATCTGCATG
CAAGC**TT**

b. 5S+50 Nucleosome Binding Sequence[4]

Cy5 strand

AAGC**TT**GCAT GCAGATCTAT GTCGGGCTCG TCC**TT**ATCGA GCTCGGTCCG AACCGAGCCC
TATGCTGC**TT** GAC**TT**CGGTG ATCGGACGAG AACCGGTATA **TT**CAGCATGG TATGGTCGTA
GGCTC**TT**GC**T** **T**GATGAAAG**T** **T**AAGCTA**TTT** AAAGGGTCAG GGATG**TT**ATG ACGTCATCGG
C**TT**ATAAATC CCTGGAATGA TCATCCCCGC ACCTCGTCAA CCGGAA**TT**CA CTGGCCGTCG
TTTTACA

Cy3 strand

TGTAAAACGA CGGCCAGTGA A**TT**CCGG**TT**G ACGAGGTGCG GGGATGATCA **TT**CCAGGGA**T**
TTATAAGCCG ATGACGTCAT AACATCCCTG ACCC**TTT**AAA TAGC**TT**AAC**T** **TT**CATCAAGC
AAGAGCCTAC GACCATACCA TGCTGAATAT ACCGG**TT**CTC GTCCGATCAC CGAAGTCAAG
CAGCATAGGG CTCGG**TT**CGG ACCGAGCTCG ATAAGGACGA GCCCGACATA GATCTGCATG
CAAGC**TT**

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.

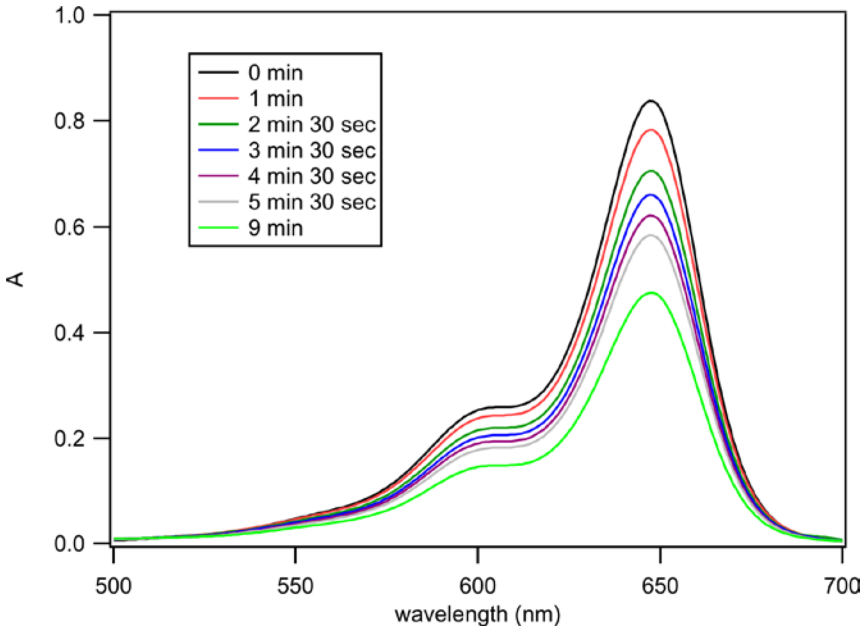

Fig. S2. Visible absorption spectra of Cy5 dye vs. irradiation time at 254 nm.

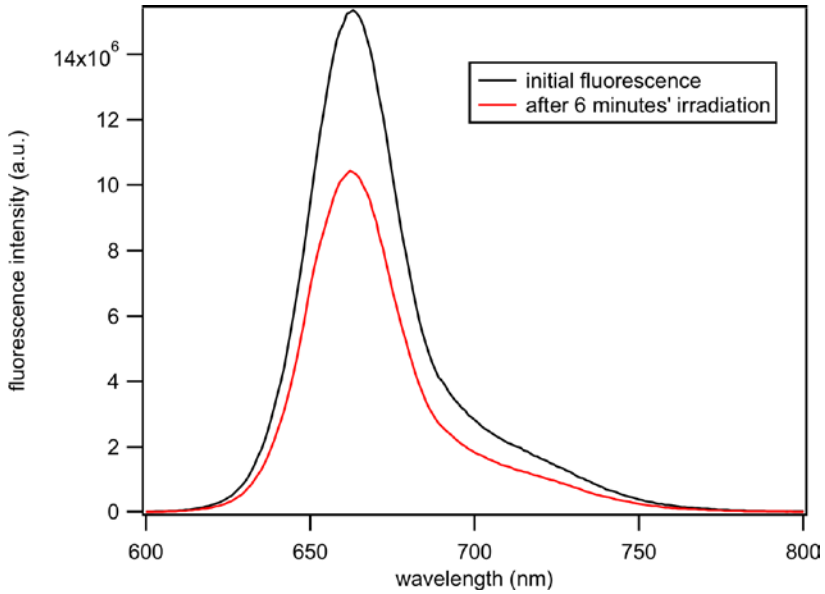

Fig. S3. Fluorescence spectra of Cy5 dye before and after irradiation at 254 nm.

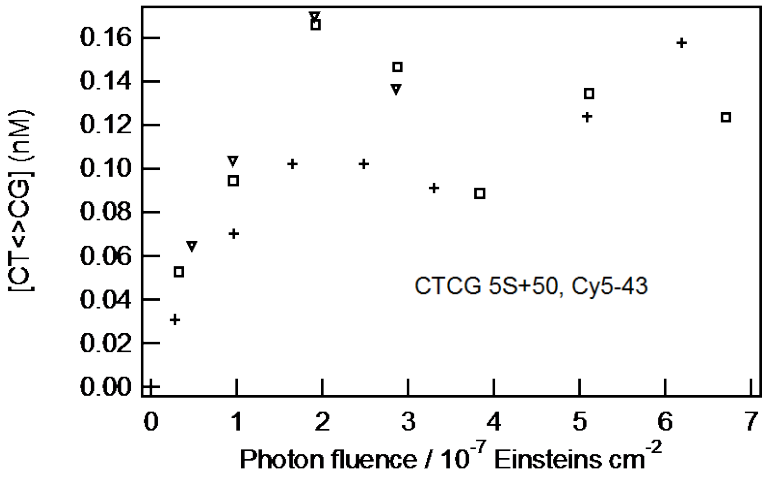

Fig. S4. Strand scission kinetics at the CTCG tetrad located at Cy5-43 on the 5S+50 sequence. Different symbols indicate separate trials.

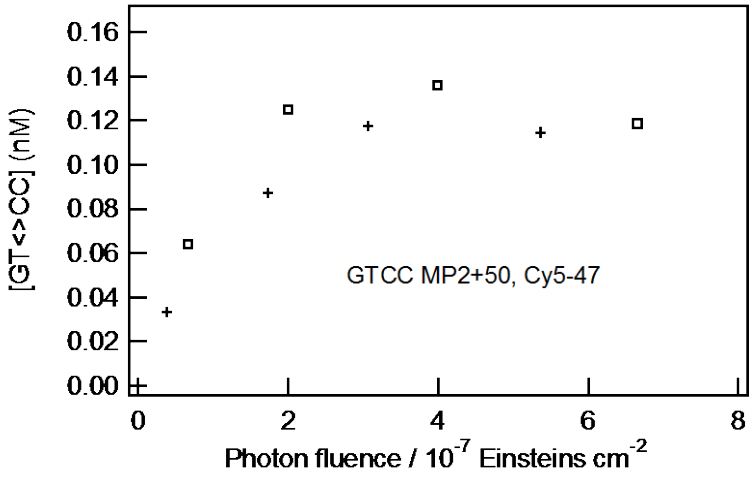

Fig. S4. Strand scission kinetics at the CTCG tetrad located at Cy5-43 on the 5S+50 sequence. Different symbols indicate separate trials.

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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