Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science

Supporting Information for Accumulation of Thymine Dimer in Defined Sequences of Free and Nucleosomal DNA

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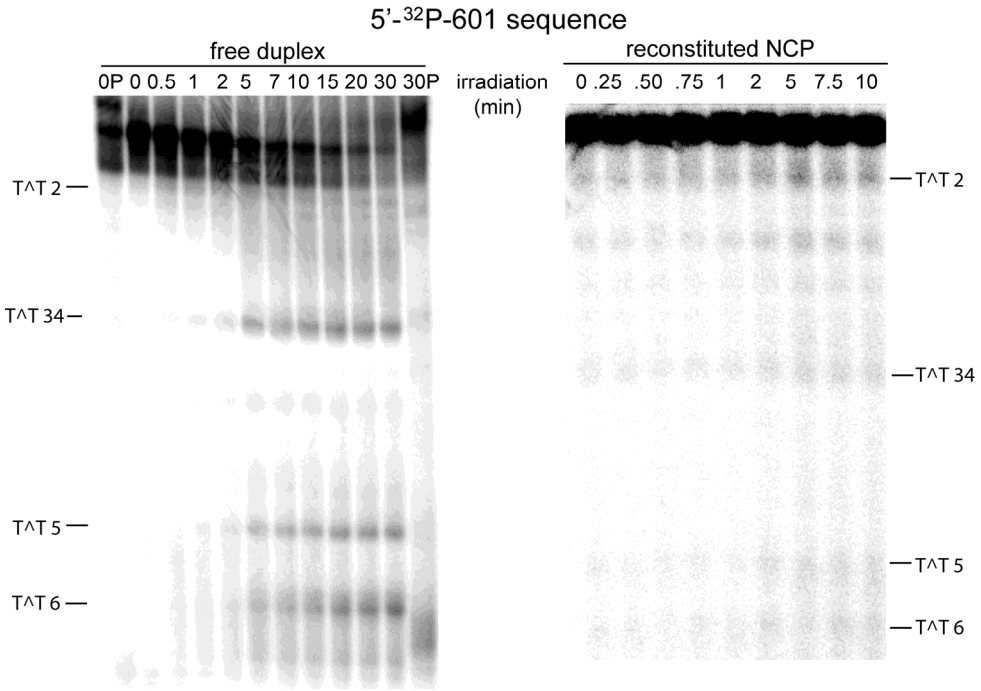

Figure S1. Detection of cyclobutane thymine dimer within the 601 sequence of duplex DNA. Dimer yields and distribution were determined after irradiation by strand fragmentation using T4 endonuclease V, polyacrylamide gel electrophoresis (8%) and phosphoimage analysis. The radiolabeled 601 sequence as the free duplex and reconstituted in a NCP as illustrated in Figure 5 were analyzed after the indicated time of irradiation (254 nm). Equivalent samples before (OP) and after irradiation (30 min) (30P) were heated with piperidine (1 M) for 30 min at 90 ^{o}C.

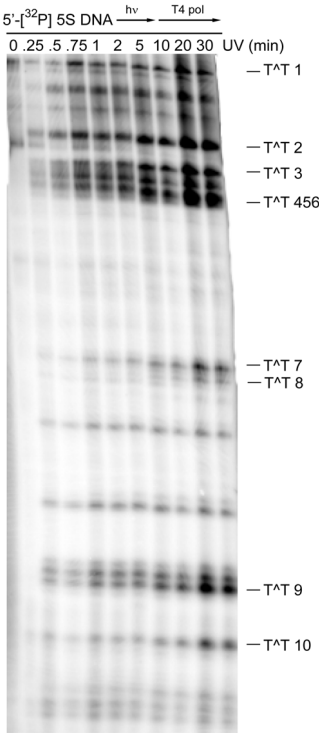

Figure S2. Photoproduct detection using the 3'-5' exonuclease activity of T4 polymerase. Radiolabeled 5S DNA in 100 mM NaCl and 10 mM potassium phosphate pH 7.0 was irradiated (254 nm) for the indicated time, digested with T4 polymerase as recommended by the manufacturer (New England Biolabs) for 2 hrs at 37 ºC. Samples were then extracted with chloroform-phenol, precipitated with ethanol, dried under reduced pressure, suspended with standard loading buffer, separated by denaturing polyacrylamide gel electrophoresis (8 %) and detected by phosphoimagery.

AGCTACCATGCCTGCACGAA**T^T**AGCCGATGCGACATGACTCCAGTGC "**T^T**"
TCGATCCTACGGACGTGCTTA ATCGGCTACGCTGTACTGAGGTCACG

(a) hν ⇌ (b) hν

AGCTACCATGCCTGCACGAA**TT**AGCCGATGCGACATGACTCCAGTGC "**TT**"
TCGATCCTACGGACGTGCTTAATCGGCTACGCTGTACTGAGGTCACG

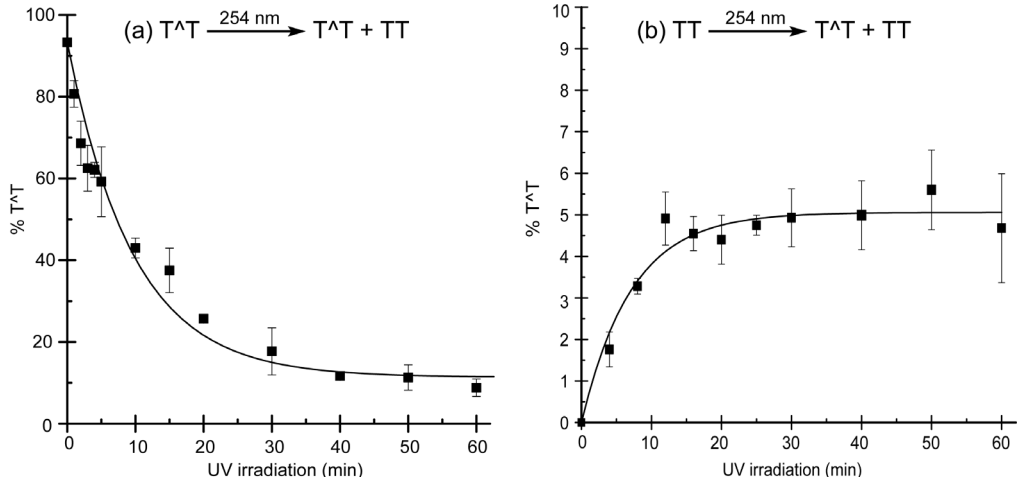

Figure S3. Approach to a photostationary level of the cyclobutane thymine dimer (T^T). (a) The T^T dimer-containing duplex (T^T) was prepared using a published method[1] and (b) the parent duplex lacking the dimer (TT) was purchased commercially. These were individually irradiated (254 nm) in the presence of 50 mM NaCl and 50 mM Tris pH 7.5 for the indicated time in siliconized centrifuge tubes (1.5 ml). The fractional population containing the dimer was measured by strand fragmentation using T4 endonuclease V, polyacrylamide gel electrophoresis (8%) and phosphoimage analysis.

1. P. Ordoukhanian and J.-S. Taylor, Solid phase-supported thymine dimers for the construction of dimer-containing DNA by combined chemical and enzymatic synthesis: a potentially general method for the efficient incorporation of modified nucleotides into DNA, *Nucleic Acids Res.*, 1997, **25**, 3783-3786.