Supplementary Information

ROTATIONAL POSITION OF A 5-METHYLCYTOSINE-CONTAINING CYCLOBUTANE PYRIMIDINE DIMER IN A NUCLEOSOME GREATLY AFFECTS ITS DEAMINATION RATE

Qian Song, Vincent J. Cannistraro, John-Stephen Taylor*
Department of Chemistry, Washington University, St. Louis, MO 63130

Address correspondence to: John-Stephen Taylor, PhD, Department of Chemistry, Campus Box 1134, Washington University, St. Louis, MO 63141. Fax: 314-935-4481; E-mail: taylor@wustl.edu.

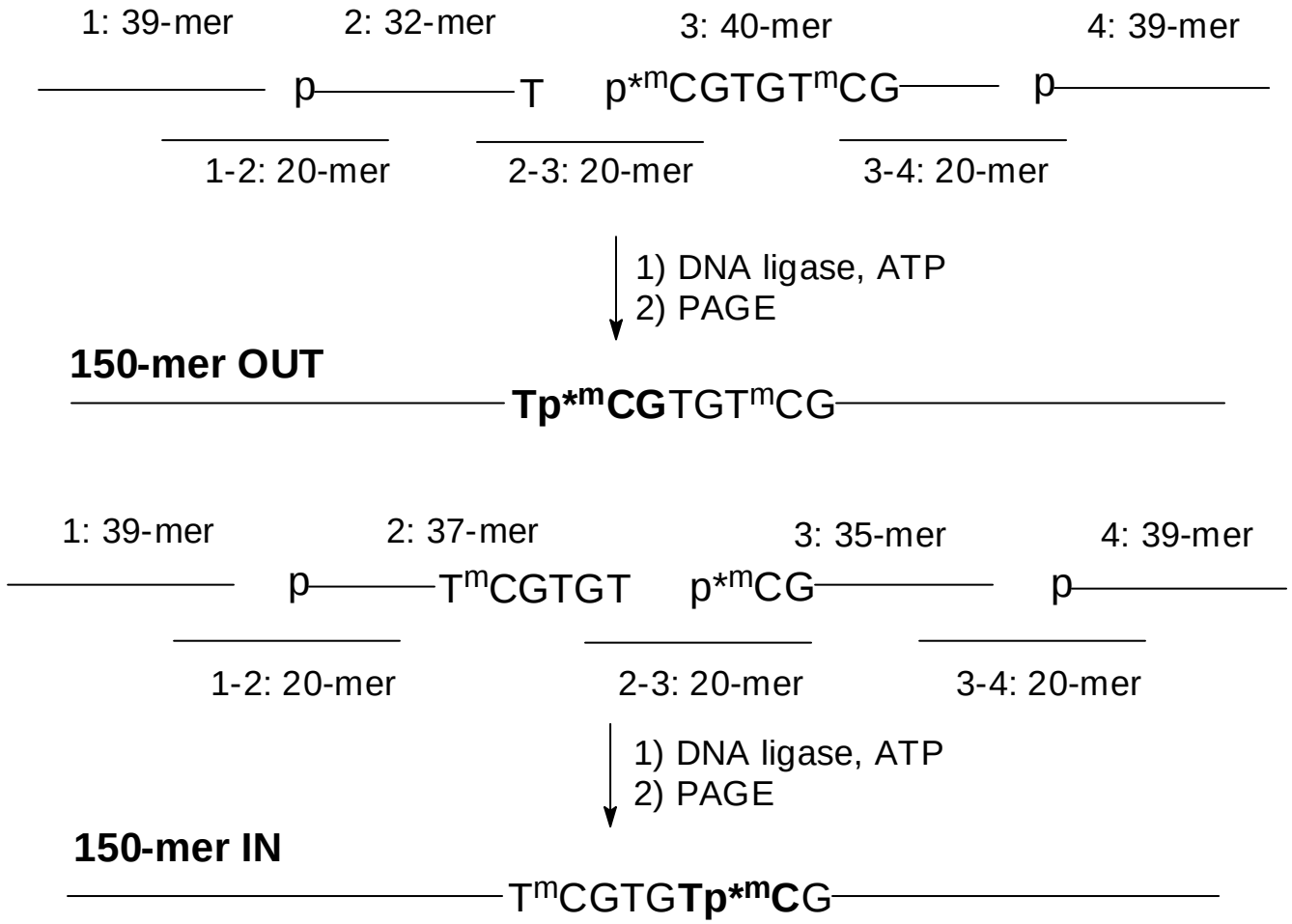

Fig. S1. Ligation strategy for assembly of the 150-mer top strand substrates. The control top strand was prepared in the same way as ds-IN. Specific sequences are shown in Fig. S2, along with those for the bottom strand.

Top Strand Sequences

ds-OUT

1: 5'-TGT TAG AGC CTG TAA CTC GGT GTT AGA GCC TGT AAC TCG

2-OUT: GTG TTA GAG CCT GTA ACT CGG TGA TTG TAC A

3-OUT : T^mC GTG T^mCG TAG CCT GTA ACA GCC TGT TAG AGC CTG TAA CTC

4: GGT GTT AGA GCC TGT AAC TCG GTG TTA GAG CCT GTA ACT-3'

ds-IN and ds-control

5'-TGT TAG AGC CTG TAA CTC GGT GTT AGA GCC TGT AAC TCG

2-IN: GTG TTA GAG CCT GTA ACT CGG TGA TTG TAC A T^mC GTG

3-IN: T^mCG TAG CCT GTA ACA GCC TGT TAG AGC CTG TAA CTC

GGT GTT AGA GCC TGT AAC TCG GTG TTA GAG CCT GTA ACT-3'

Ligation Scaffolds

***1-2* GCT CTA ACA CCG AGT TAC AG**

***2-3-OUT* CTA CGA CAC GAT GTA CAA TC**

***2-3-IN* ACA GGC TAC GAC ACG ATG TA**

***3-4* CTC TAA CAC CGA GTT ACA GG**

Bottom Strand Sequence

1-AGT TAC AGG CTC TAA CAC CGA GTT ACA GGC TCT AA

2-CAC CGA GTT ACA GGC TCT AAC AGG CTG TTA CAG GCT ACG

3-ACA CGA TGT ACA ATC ACC GAG TTA CAG GCT CTA ACA C

4-CGA GTT ACA GGC TCT AAC ACC GAG TTA CAG GCT CTA ACA

Ligation Scaffolds

1-2: TAA CTC GGT GTT AGA GCC TG

2-3: TAC ATC GTG TCG TAG CCT GT

3-4: CTG TAA CTC GGT GTT AGA GC

Fig. S2. Sequences used to assemble the 150-mer top and bottom strands as shown in Fig. S1.

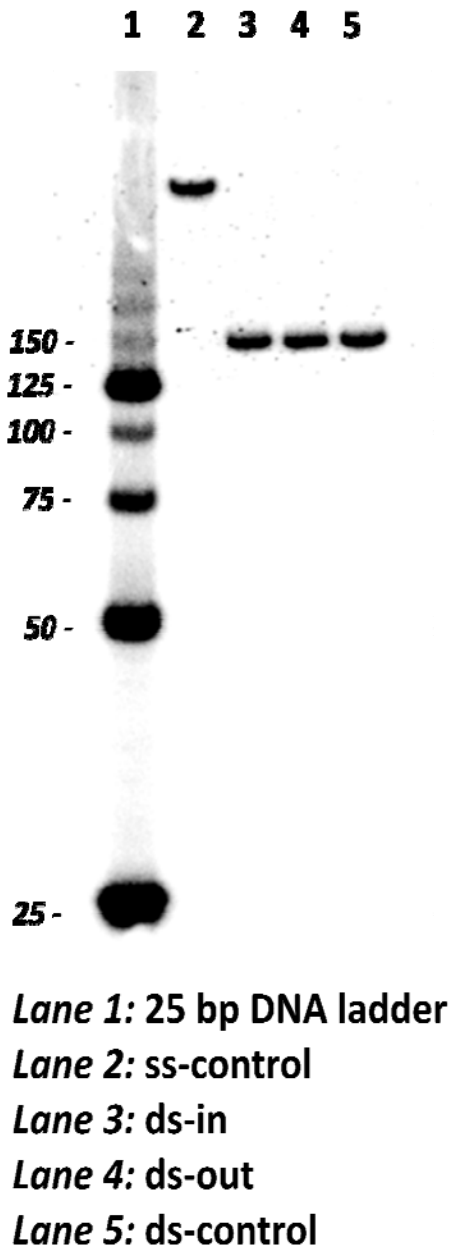

Fig. S3. Characterization and purification of the 150-mer DNA duplexes by PAGE. The PAGE-purified single strand 150-mer substrates were annealed together to form duplexes and characterized by native gel electrophoresis on a 10% acrylamide, 0.3% bisacrylamide polyacrylamide gel in TBE. Lane 1: 25 bp DNA ladder, lane 2: 5'-endlabeled single strand 150-mer, lane 3: 150-mer duplex with internally ^{32}P-labeled facing out ^{m}C, lane 4: 150-mer duplex with internally ^{32}P-labeled facing in ^{m}C, lane 5: 5'-end labeled 150-mer. Each duplex substrate was isolated from the gel for further studies.

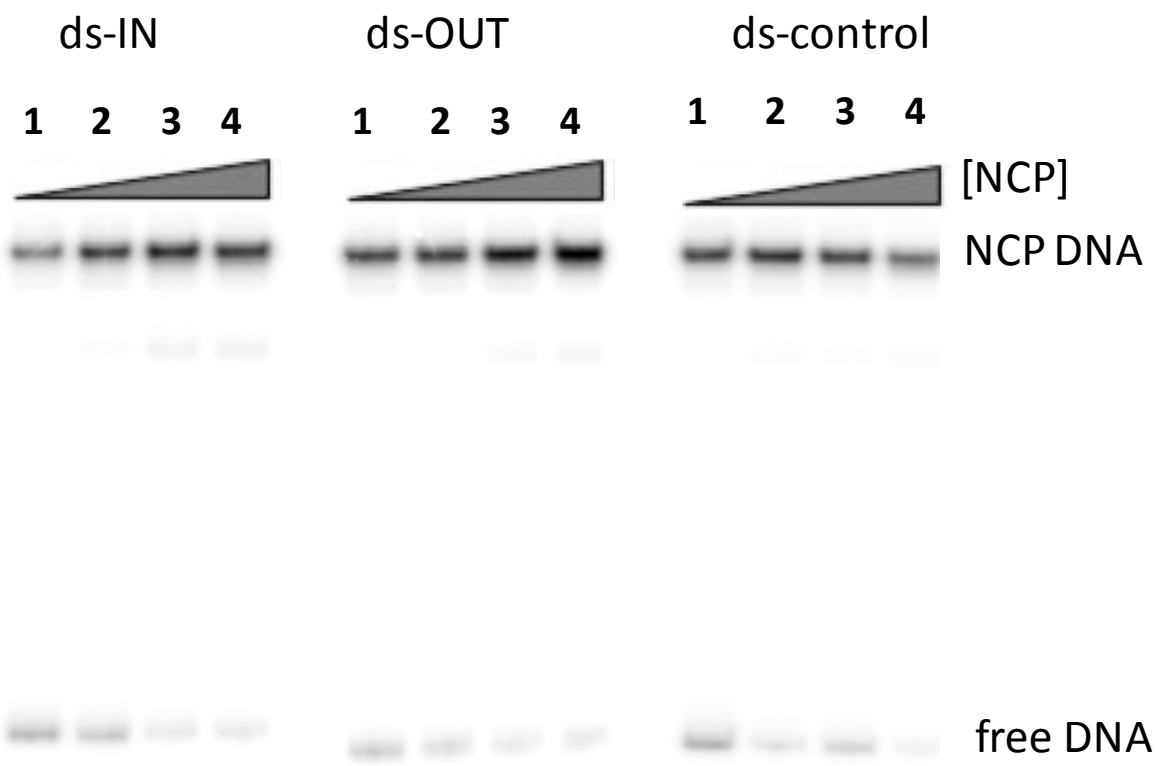

Fig. S4. Reconstitution of the nucleosome core particles with the 150-mer duplexes. The 150-mer DNA duplexes (10 nM) were incubated with increasing amounts of chicken erythrocyte nucleosome core particles (NCP) (lanes 1-4: 100, 200, 300, 500 nM) at room temperature in 2 M NaCl at pH 7.5 for 2 h followed by dialysis overnight at 4 °C in 50 mM NaCl, with final equilibration at 55°C for 2 h. The reconstituted NCP were then electrophoresed on a native polyacrylamide gel (6% acrylamide, 0.2% bisacrylamide in TBE).

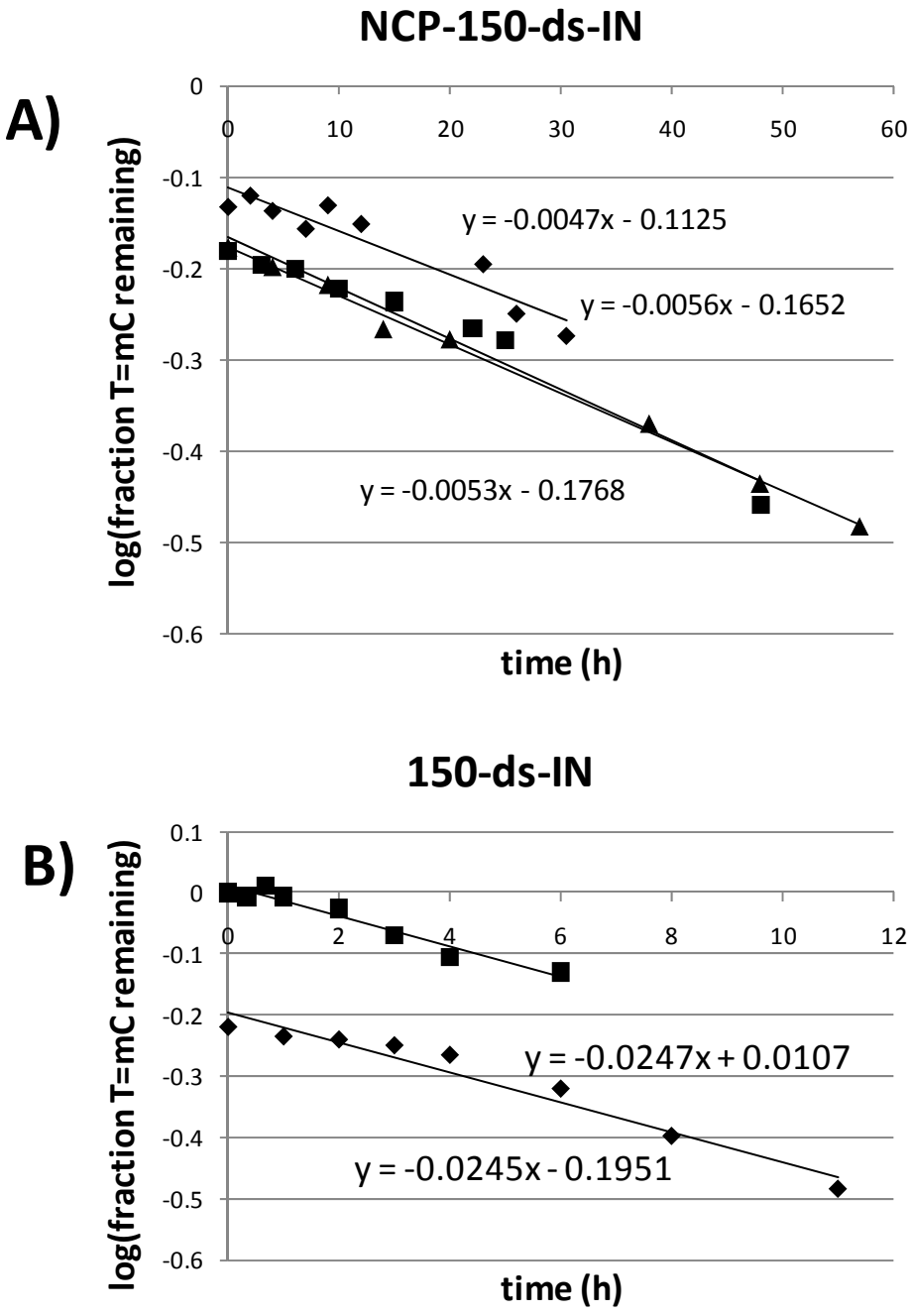

Fig. S5. Linear regression analysis of the deamination rate data for CPD-IN. Plots of the individual deamination rate data as log(fraction T=^{m}C remaining) vs time for A) the NCP-ds-IN, and B) free ds-IN. The non-zero intercept is due to contamination from ^{32}pdT resulting from labeling the 5'-end of the DNA 150-mer as a result of incomplete heat inactivation of the kinase prior to ligation.

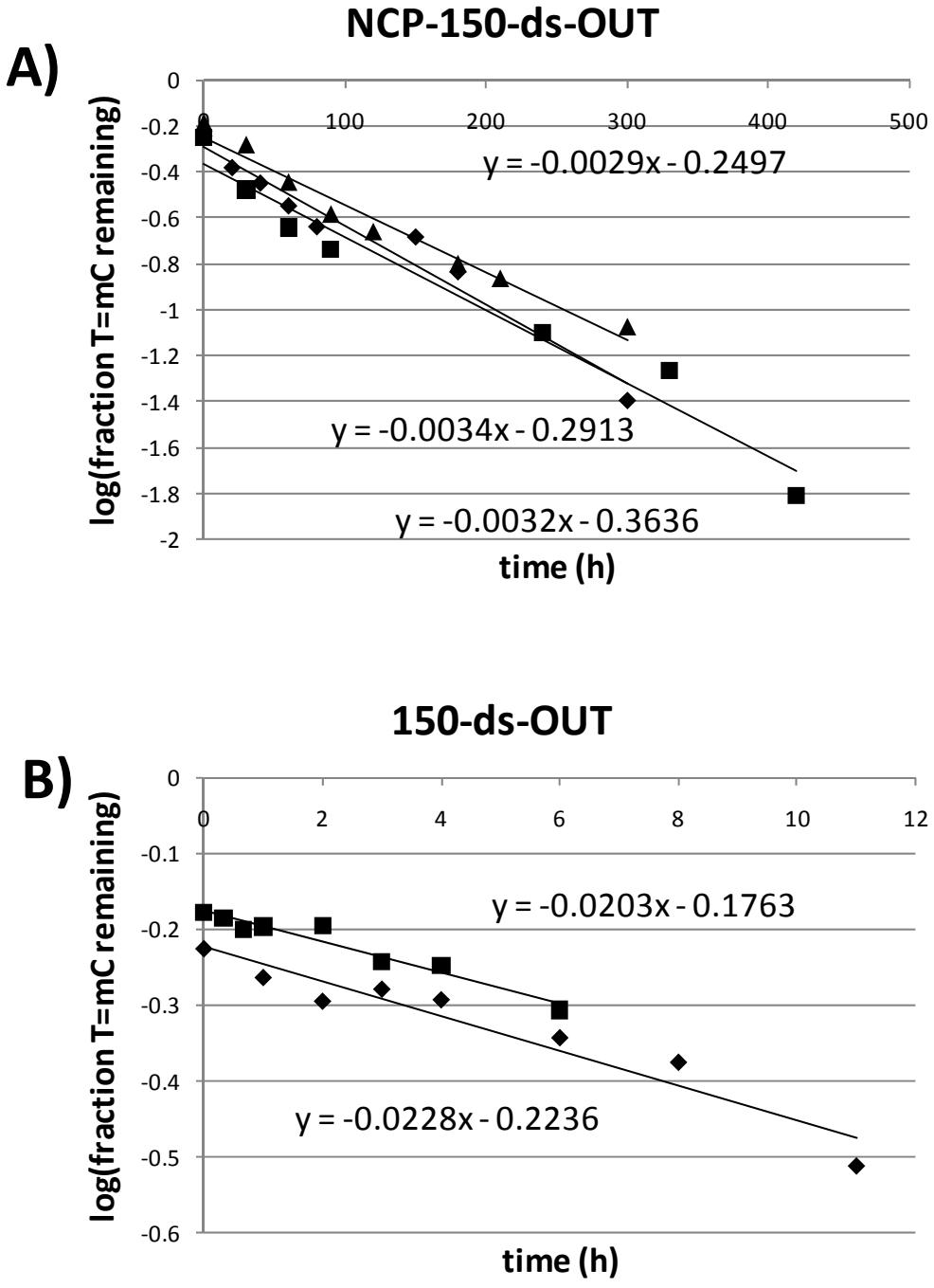

Fig. S6. Linear regression analysis of the deamination rate data for CPD-OUT. Plots of the individual deamination rate data as log(fraction T=^{m}C remaining) vs time or A) NCP-ds-OUT, and B) free ds-OUT. The non-zero intercept is due to contamination from ^{32}pdT resulting from labeling the 5'-end of the DNA 150-mer as a result of incomplete heat inactivation of the kinase prior to ligation.