Structure-Dependent DNA Damage and Repair in a DNA Trinucleotide Repeat Sequence

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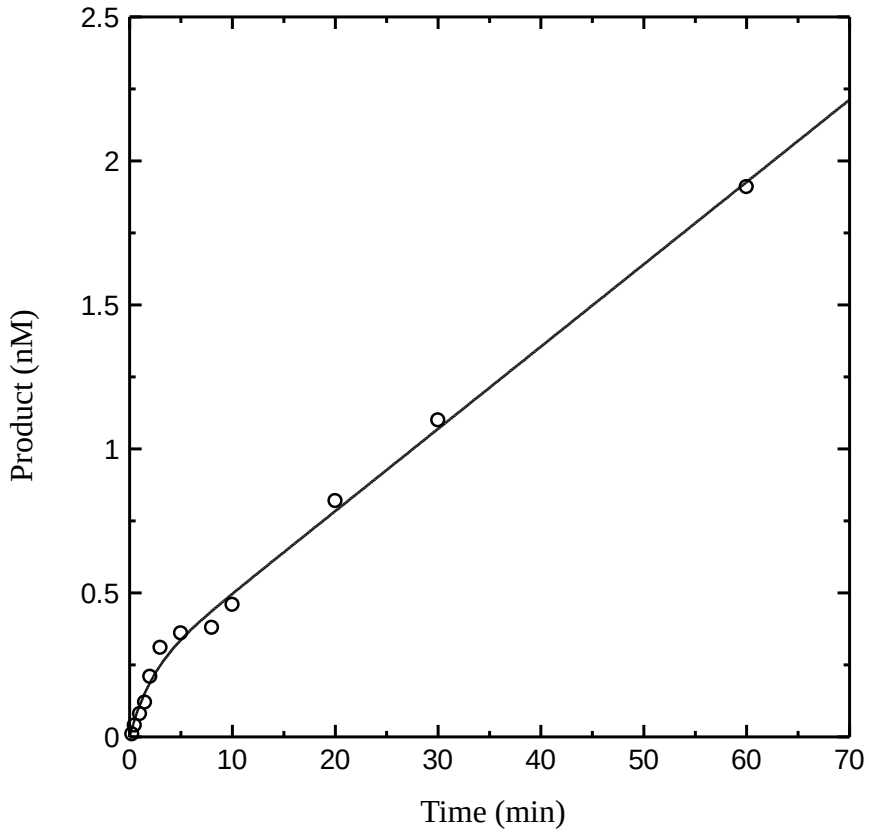
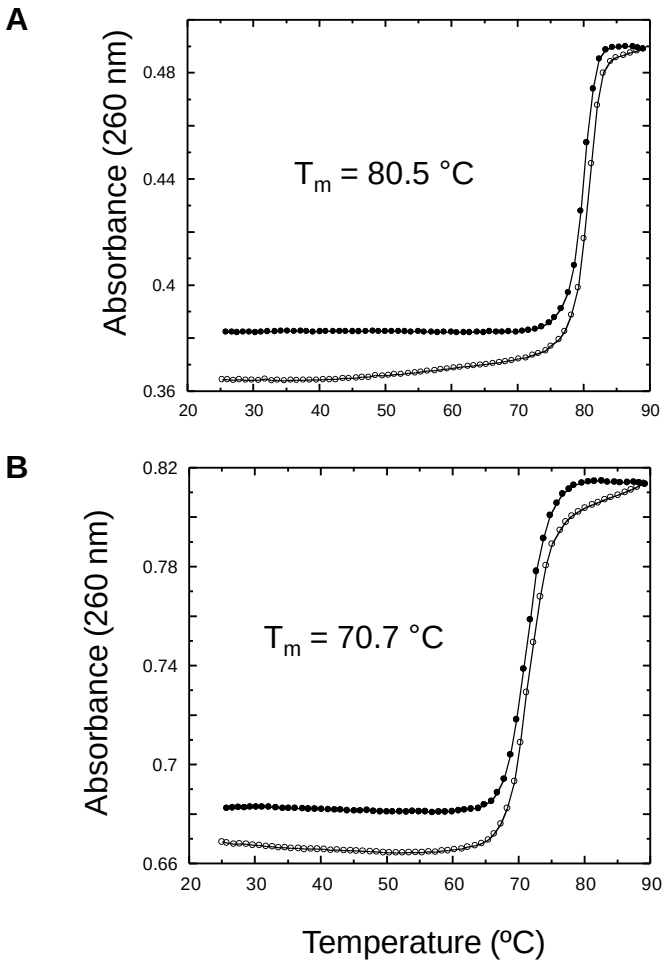
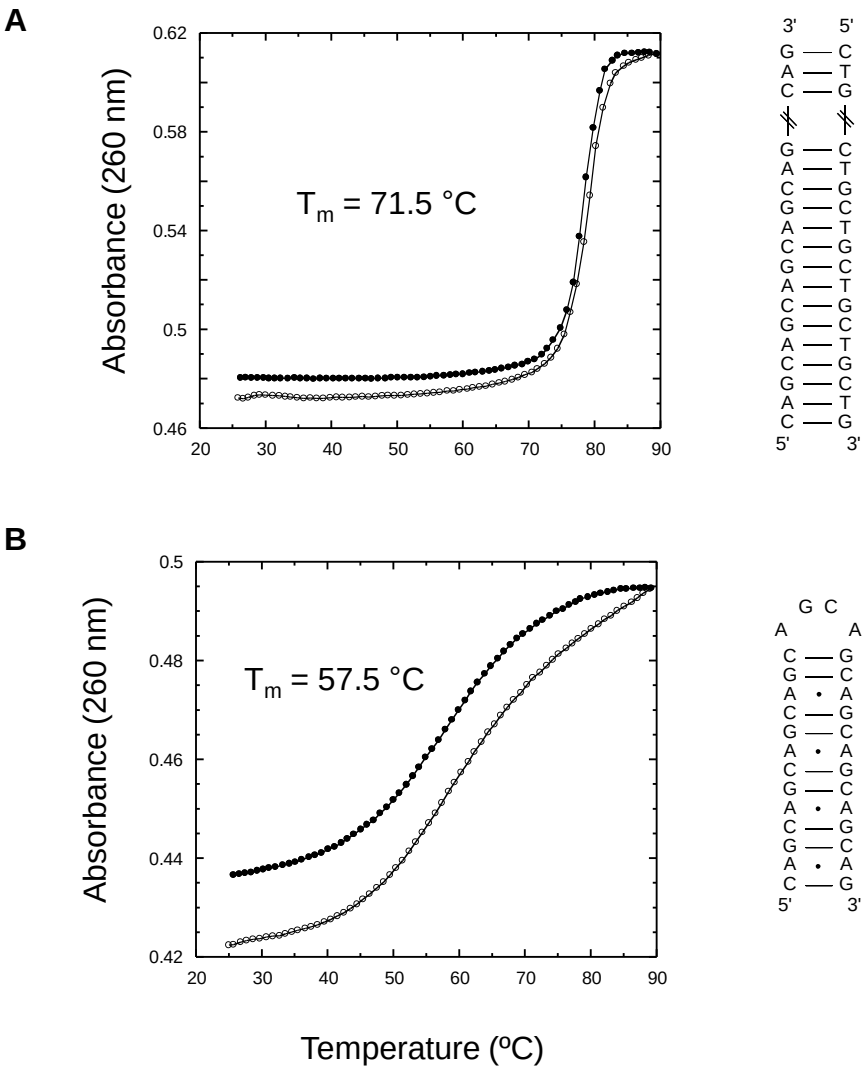
Contents



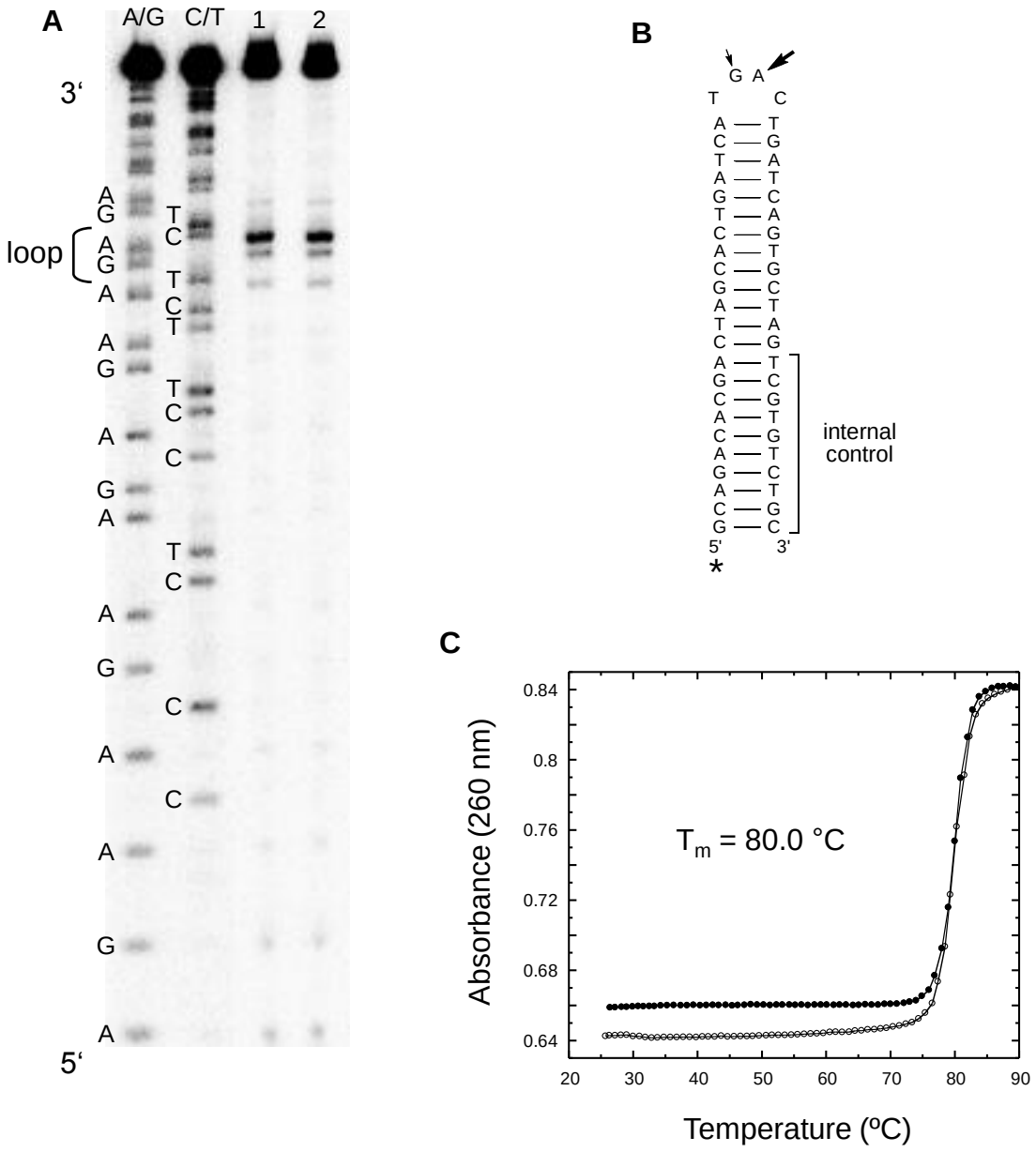
Supporting Information Figure 1. Graphical representation of active site titration using mixed-sequence duplex (20 nM) and hOGG1 (0.5 nM total enzyme). The linear portion of the data is extrapolated to the y-axis to determine the amplitude of the burst phase which relates directly to the active enzyme concentration (see Experimental Procedures).



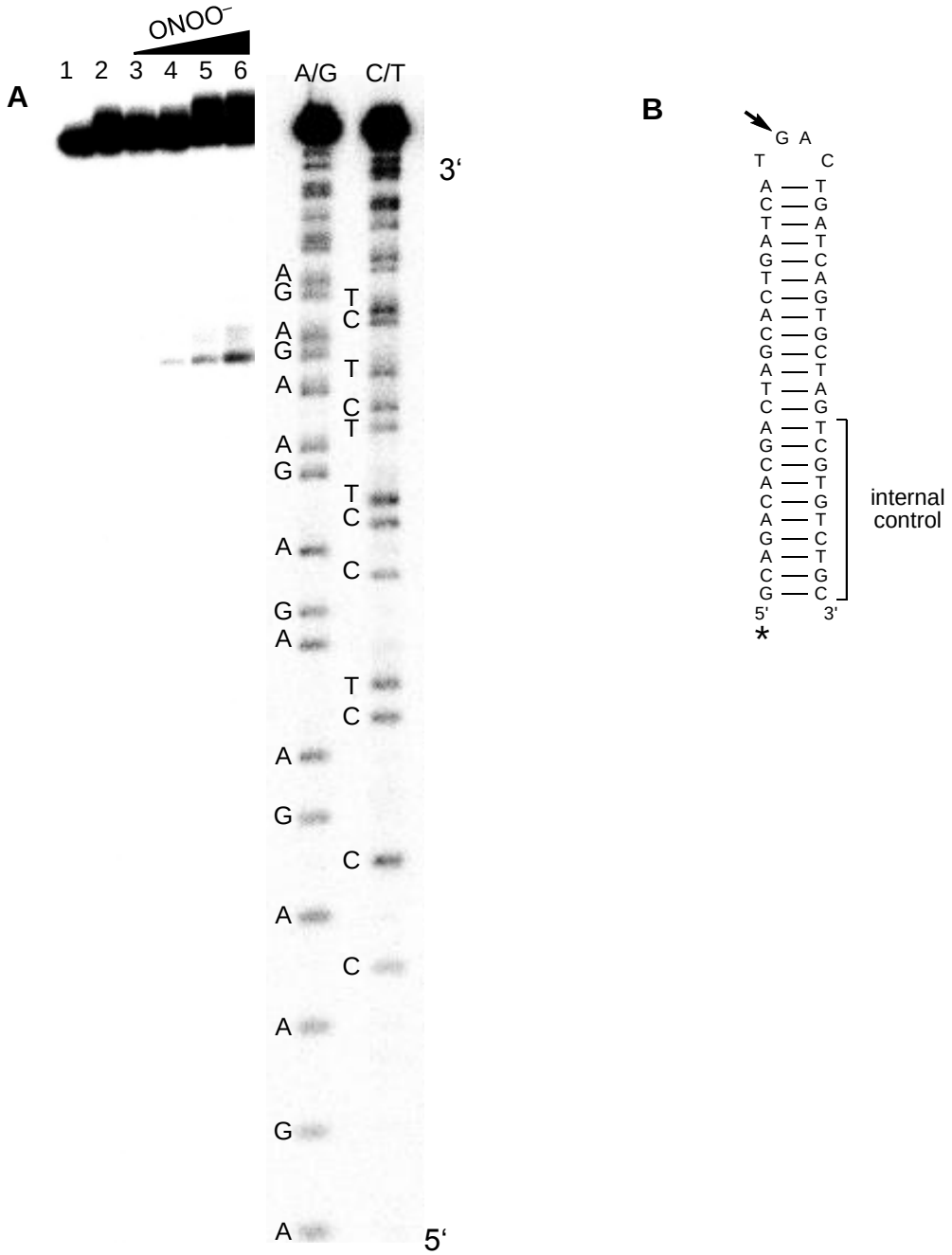
Supporting Information Figure 2. Optical melting analysis of $(CAG)_{10}/(CTG)_{10}$ duplex (A) and $(CAG)_{10}$ hairpin (B) assemblies. Conditions for (A) and (B) are 0.5 and 1.1 μM DNA assembly, respectively, in 50 mM sodium phosphate, 25 mM $NaHCO_3$, 10 mM NaCl, pH 7.5. Open circles represent data obtained while heating samples from 25 to 90 °C; closed circles represent data obtained while cooling samples from 90 to 25 °C.



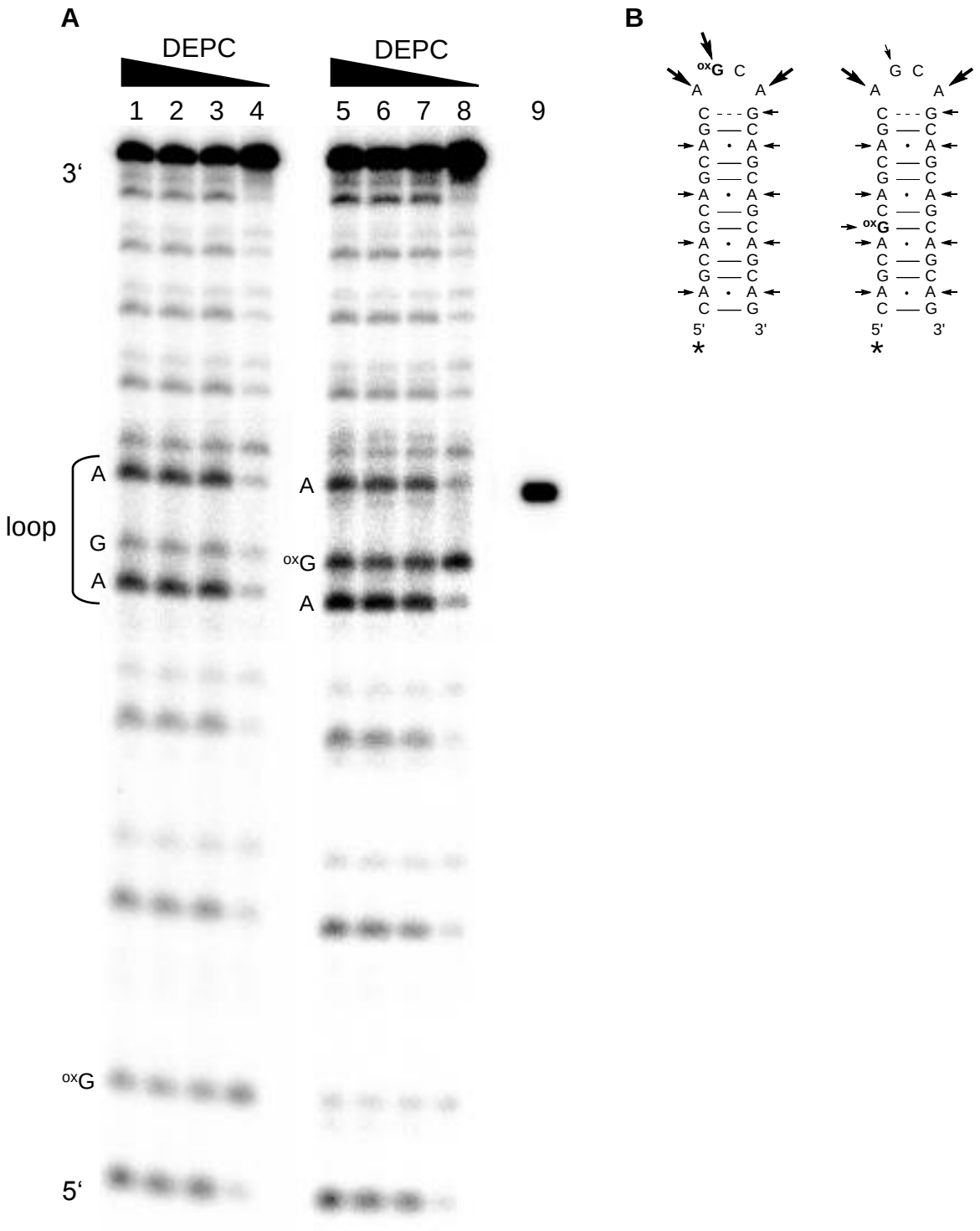
Supporting Information Figure 3. Optical melting analysis of $(CAG)_{10}/(CTG)_{10}$ duplex (A) and $(CAG)_{10}$ hairpin (B) assemblies lacking the internal control region. Conditions for (A) and (B) are 1.0 and 1.2 μM DNA assembly, respectively, in 50 mM sodium phosphate, 25 mM $NaHCO_3$, 10 mM NaCl, pH 7.5. Open circles represent data obtained while heating samples from 25 to 90 °C; closed circles represent data obtained while cooling samples from 90 to 25 °C.



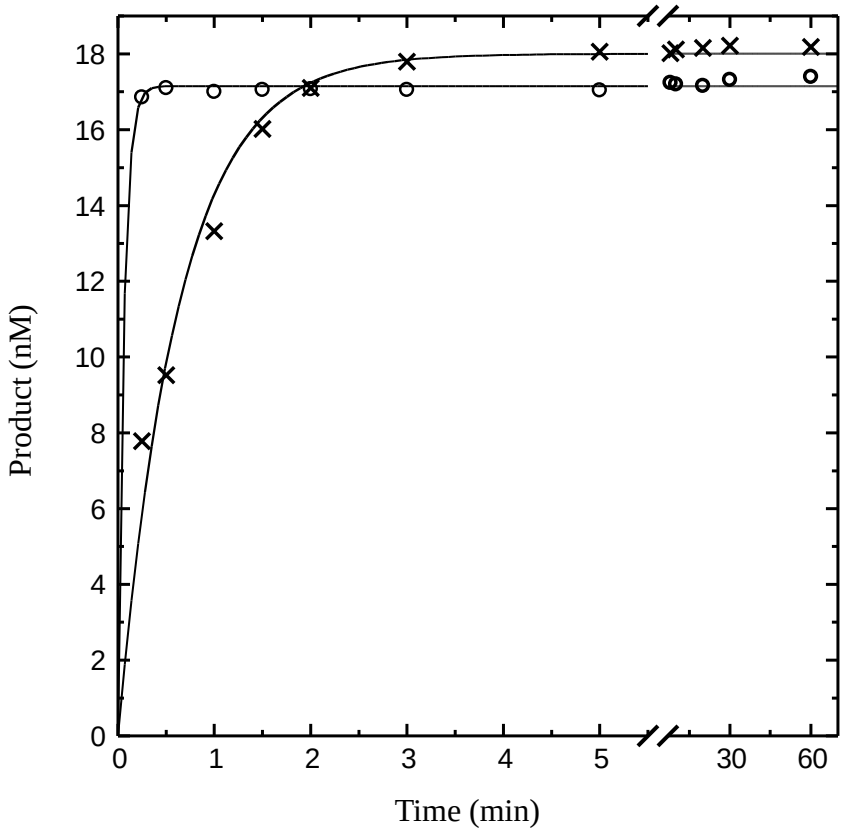
Supporting Information Figure 4. (A) Autoradiogram revealing damage of mixed-sequence hairpin assembly (5.0 μM) in 50 mM sodium phosphate, 25 mM $NaHCO_3$, 10 mM NaCl, pH 7.5 induced by DEPC (lanes 1 and 2 with 69 and 138 mM DEPC, respectively) following incubation for 30 min at 37 ºC. (B**)** Schematic representation of damage induced by DEPC. The arrows reflect the relative amount of strand cleavage at a given site. (C**)** Optical melting analysis of the mixed-sequence hairpin assembly (1.1 μM) in 50 mM sodium phosphate, 25 mM $NaHCO_3$, 10 mM NaCl, pH 7.5.



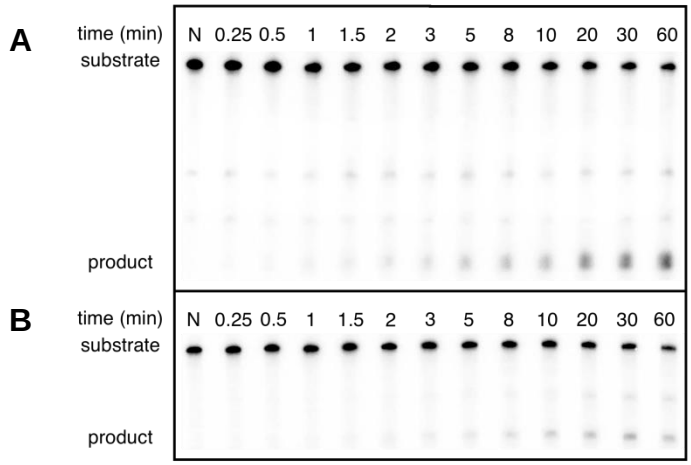
Supporting Information Figure 5. (A) Autoradiogram revealing peroxynitrite-induced damage of the mixed-sequence hairpin. Conditions are 5 μM DNA in 50 mM sodium phosphate, 25 mM $NaHCO_3$, 10 mM NaCl, pH 7.5. Lanes 1, 2 contain DNA alone and piperidine-treated DNA, respectively. Lanes 3-6 contain DNA incubated for 30 min at 37 °C in the presence of an increasing concentration of peroxynitrite (0, 100, 1000, 2500 μM, respectively) followed by piperidine treatment. A/G and C/T, Maxam-Gilbert sequencing reactions. (B) Schematic representation of DNA strand cleavage induced by peroxynitrite. The arrow reflects the relative amount of strand cleavage at a given site.



Supporting Information Figure 6. (A) Autoradiogram revealing DEPC-induced damage of loop-HP and stem-HP in 20 mM Tris-HCl, 1 mM Na_2EDTA, pH 8.0. Conditions are 20 nM DNA and DEPC (1-646 mM) with incubation for 30 min at 37 °C followed by piperidine treatment. Lane 9 is a $(CAG)_5$ size marker. (B) Schematic representation of damage induced by DEPC. The size of the arrow reflects the relative amount of strand cleavage at a given site.



Supporting Information Figure 7. Graphical representation of time course data for removal of 8-oxoG by hOGG1 from mixed-DUP with (open circles) and without (X) NaOH/heat treatment. Reaction conditions are 20 nM DNA and 100 nM hOGG1 in 20 mM Tris-HCl, 10 mM Na_2EDTA, 80 nM NaCl, 100 μg/ml BSA, pH 7.6. Samples were incubated for 0.25 to 60 min at 37 ˚C. The data are fitted to a single-exponential to determine the rate constant k_2.



Supporting Information Figure 8. Removal of 8-oxoG from hairpin substrates by hOGG1 in the absence of NaOH/heat treatment. Autoradiogram revealing strand cleavage upon treatment of (A) stem-HP and (B) loop-HP with hOGG1. Reaction conditions are 20 nM DNA and 100 nM hOGG1 in 20 mM Tris-HCl, 10 mM Na_2EDTA, 80 nM NaCl, 100 μg/ml BSA, pH 7.6. Samples were incubated for 0.25 to 60 min at 37 °C. N represents DNA alone with no hOGG1.