Supporting Information

The peroxidation-derived DNA adduct, 6-oxo-M_1dG, is a strong block to replication by human DNA polymerase η: structural and functional evaluation

Robyn Richie-Jannetta[1], Pradeep Pallan[2], Philip J. Kingsley[1], Nikhil Kamdar[1], Martin Egli[2], and Lawrence J. Marnett[1,*]
[1] A. B. Hancock, Jr., Memorial Laboratory for Cancer Research, Departments of Biochemistry, Chemistry and Pharmacology, Vanderbilt Institute of Chemical Biology, Center for Structural Biology and Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA
[2] Department of Biochemistry, Vanderbilt Institute of Chemical Biology, Center for Structural Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA

*Corresponding author:
Lawrence J. Marnett
Vanderbilt University
Tel.: 615-322-0907
Email: larry.marnett@vanderbilt.edu

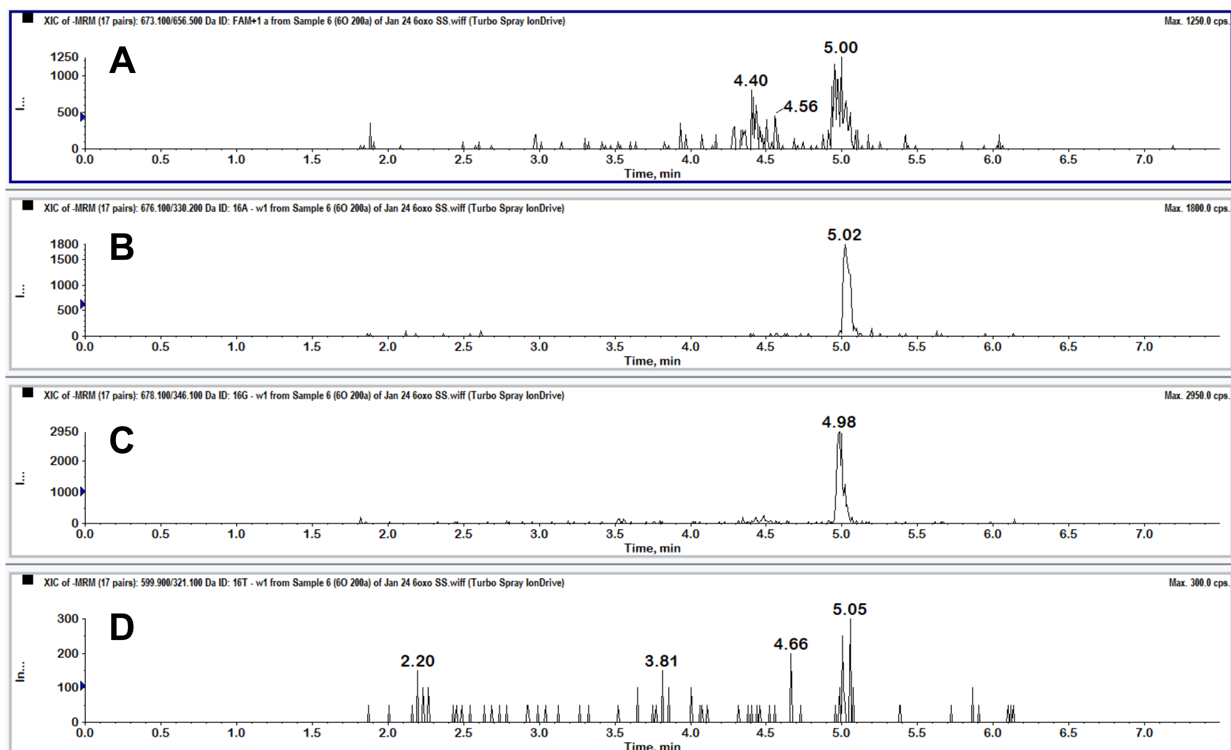


Figure S1. LC-MS chromatogram of 16 nucleotide products from extension of 6-oxo-M$_1$dG template-primer duplex by hPol η. HPol η (200 nM) was incubated for 40 min. with 5 µM DNA duplex and a 500 µM mix of all four dNTPs in a 10 µl reaction volume. The FAM-labeled complementary primer (5'-FAM-CGC TCG TAA GGA TTC-3') extended by a dCMP on the 3' end was detected by SRM in positive ion mode with the following transition: 673.1$\rightarrow$656.5 (A). Extension by a dAMP (transition 676.1$\rightarrow$330.2), dGMP (transition 678.1$\rightarrow$346.1), or dTMP (transition 599.9$\rightarrow$321.1) on the 3' end is shown in panels B-D, respectively.

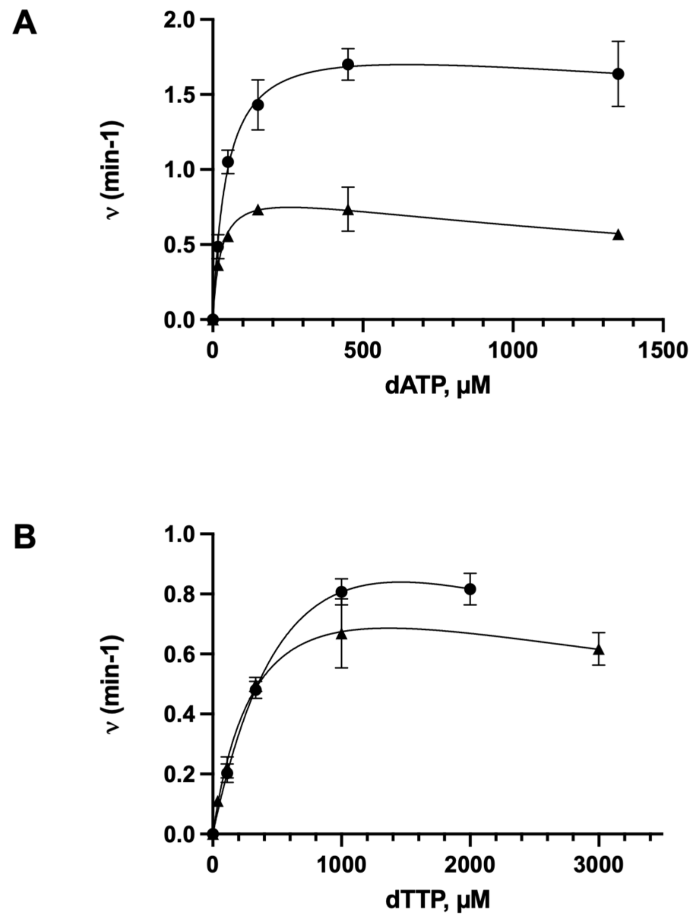


Figure S2. Steady state kinetics data for dATP (A) and dTTP (B) incorporation opposite dG or 6-oxo-M_1dG by hPol η. Control (●) and 6-oxo-M_1dG (▲) oligonucleotide duplexes (5 µM) were incubated with 25 nM or 100 nM hPol η, respectively, and increasing concentrations of dATP for 5 min. (A). Control (●) and 6-oxo-M_1dG (▲) oligonucleotide duplexes (5 µM) were incubated with 40 nM hPol η and increasing concentrations of dTTP for 5 min. Each point represents the mean and standard deviation of triplicate determinations. Data were analyzed using the Substrate Inhibition model in GraphPad Prism.

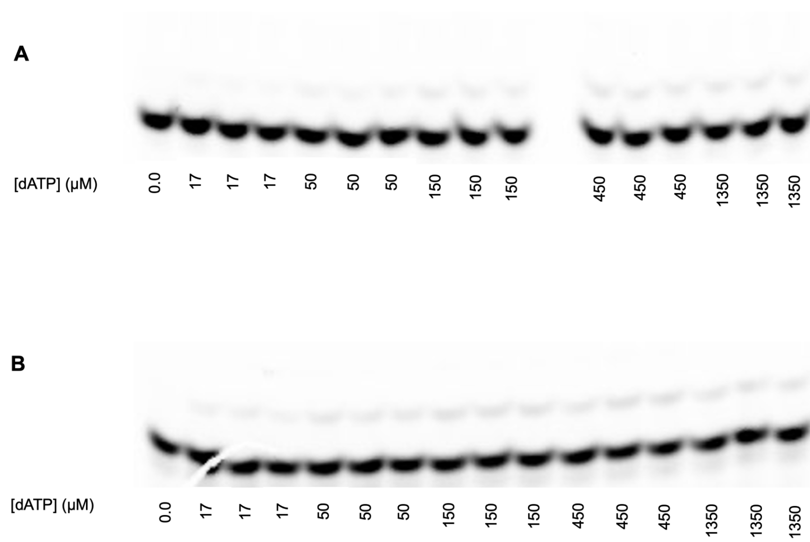


Figure S3. Incorporation of dATP opposite dG (control template, A) and 6-oxo-M_1dG (B). hPol η (25 nM for control duplex or 100 nM for 6-oxo-M_1dG duplex) was incubated with 5 μM DNA duplex and increasing concentrations of nucleotides. The reaction was stopped at 5 min.

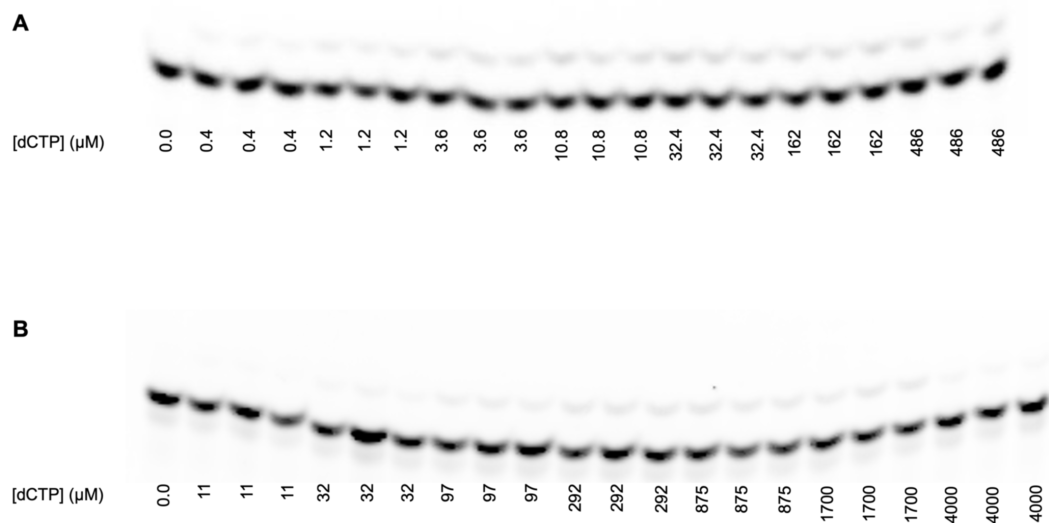
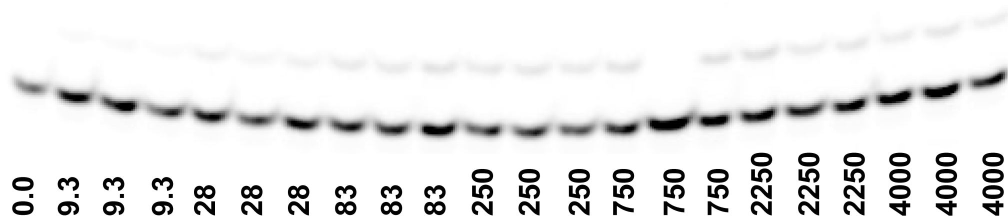


Figure S4. Incorporation of dCTP opposite dG (control template, A) and 6-oxo-M_1dG (B). hPol η (10 nM for control duplex or 200 nM for 6-oxo-M_1dG duplex) was incubated with 5 μM DNA duplex and increasing concentrations of nucleotides. The reaction was stopped at 6 min for control duplex or 10 min for 6-oxo-M_1dG duplex.

A



B

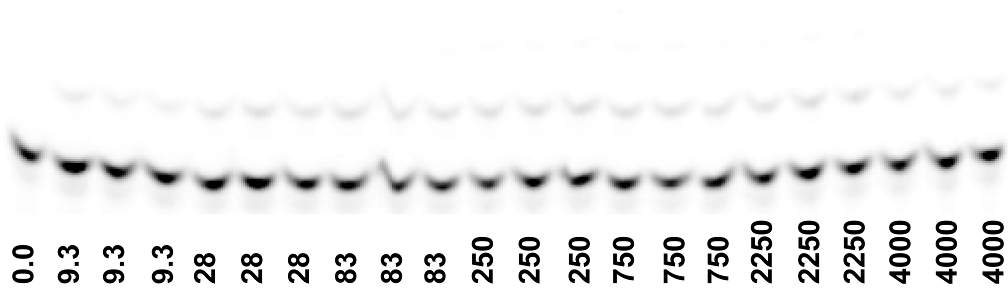


Figure S5. Incorporation of dGTP opposite dG (control template, A) and 6-oxo-M_1dG (B). hPol η (50 nM for control duplex or 200 nM for 6-oxo-M_1dG duplex) was incubated with 5 μM DNA duplex and increasing concentrations of nucleotides. The reaction was stopped at 10 min.

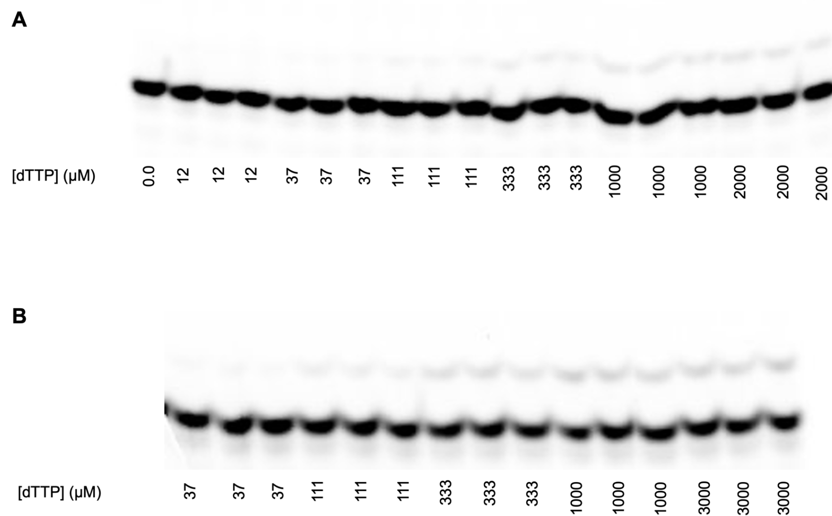


Figure S6 Incorporation of dTTP opposite dG (control template, A) and 6-oxo-M_1dG (B). hPol η (40 nM) was incubated with 5 μM DNA duplex and increasing concentrations of nucleotides. The reaction was stopped at 5 min.

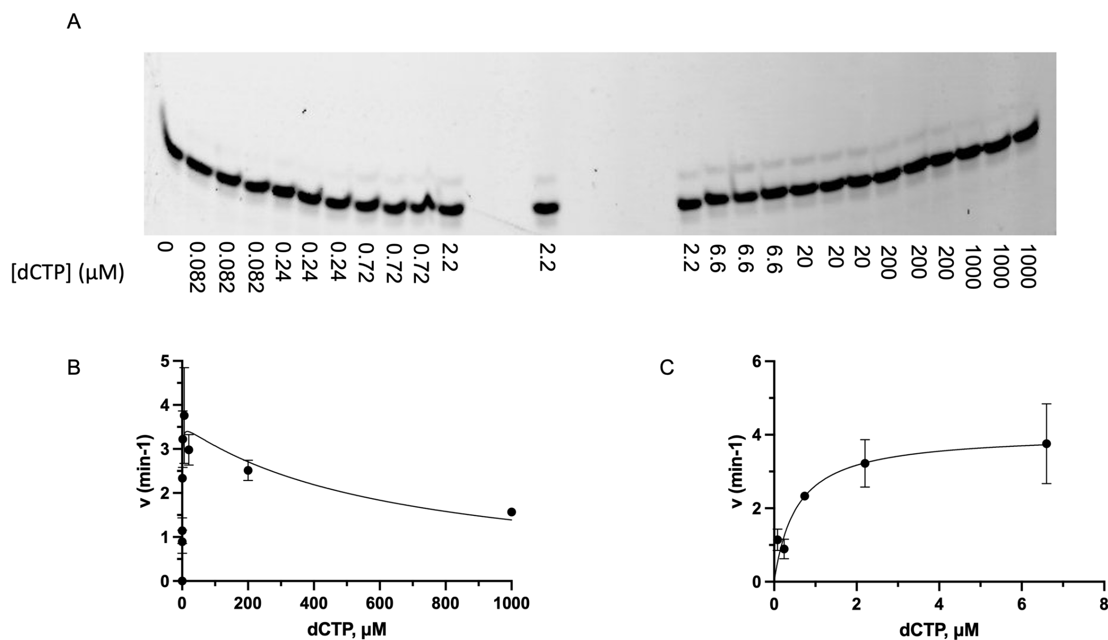


Figure S7. Steady state kinetics for dCTP, analyzed using gel electrophoresis. A, incorporation of dCTP opposite dG (control template). HPol η (1.6 nM) was incubated with 80 nM DNA duplex and increasing concentrations of nucleotides. The reaction was stopped at 1 min. B, each point represents the mean and standard deviation of triplicate determinations. Data was analyzed using the Substrate Inhibition model in GraphPad Prism. C, dCTP concentrations from 0-6.6 μM from panel B are shown. Data was analyzed using the Michaelis-Menten model in GraphPad Prism.

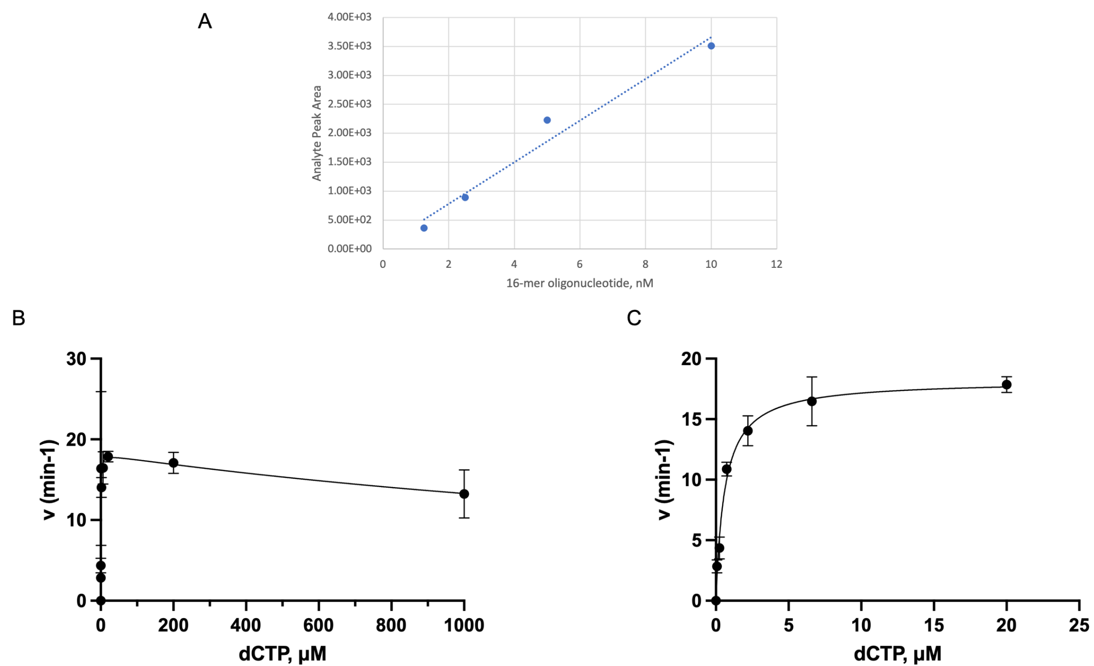


Figure S8. Steady state kinetics for dCTP, analyzed using LC-MS/MS analysis. A, standard curve of 16-mer oligonucleotide product using concentrations of 1.25 nM, 2.5 nM, 5 nM, and 10 nM. HPol η (1.6 nM) was incubated with 80 nM DNA duplex and increasing concentrations of nucleotides. The reaction was stopped at 1 min. B, each point represents the mean and standard deviation of triplicate determinations. Data was analyzed using the Substrate Inhibition model in GraphPad Prism. C, dCTP concentrations from 0-20 µM from panel B are shown. Data was analyzed using the Michaelis-Menten model in GraphPad Prism.