Uncovering the polymerase-induced cytotoxicity of an oxidized nucleotide

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Oxidative stress promotes genomic instability and human diseases. A common oxidized nucleoside is 8-oxo-7,8-dihydro-2'-deoxyguanosine, which is found both in DNA (8-oxo-G) and as a free nucleotide (8-oxo-dGTP). Nucleotide pools are especially vulnerable to oxidative damage. Therefore cells encode an enzyme (MutT/MTH1) that removes free oxidized nucleotides. This cleansing function is required for cancer cell survival and to modulate Escherichia coli antibiotic sensitivity. Discrimination for the preferred nucleotide is indicated.

Figure 1 | 8-Oxo-dGTP specificity and insertion opposite adenine.

(a) 8-Oxo-dGTP base pairing with Cy or Ad. (b) Pathways associated with 8-oxo-G DNA repair for the A to C transversion. Dashed lines are pol β insertion events. (c) Pol β insertion efficiency of 8-oxo-dGTP and dGTP opposite either templating Cy or Ad. Discrimination for the preferred nucleotide is indicated. (d) 8-Oxo-dGTP(syn):Ad pre-catalytic complex. (e) Phosphodiester bond formation after a 20 s soak in MgCl₂, with the reactant (green) and product (yellow) states. (f) Closed 8-oxo-dGMP:Ad product complex after a 40 s soak in MgCl₂. (g) Open 8-oxo-dGMP:Ad product complex after a 90 s soak. Closed conformation is shown in green (Protein Data Bank accession number 2FMS). F₀ − F₁ maps (3σ) are in green. Ca²⁺, Mg²⁺, and Na⁺ are orange, red, and purple spheres respectively. The catalytic, nucleotide, and product metals are denoted with subscripts c, n, and p, respectively.

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(Mg\textsubscript{c}) metals\textsuperscript{16–18}. This complex is optimized for nucleotidyl transfer, forming pyrophosphate (PP\textsubscript{i}), and following catalysis, pol β reopens, releasing PP\textsubscript{i}.

Soaking open binary crystals of pol β bound to DNA containing a templating Ad in a cryosolution with 8-oxo-dGTP and CaCl\textsubscript{2} results in a closed pre-catalytic ground state ternary complex (Extended Data Table 1). The incoming 8-oxo-dGTP\textsubscript{(syn)} Hoogsteen base pairs with Ad (Fig. 1d). The active site is in a similar conformation as previously observed using a dideoxy-terminated primer (Extended Data Fig. 1a)\textsuperscript{19}, with a change in the primer terminus sugar pucker to C\textsuperscript{3'}-endo (Extended Data Table 2). Unique active site interactions include Asn 279 hydrogen bonding to O\textsubscript{8} of 8-oxo-dGTP\textsubscript{(syn)} and an intramolecular hydrogen bond between N2 and the pro-\textsubscript{S} oxygen on P\textsubscript{2} of 8-oxo-dGTP\textsubscript{(syn)} (Fig. 1d).

This is consistent with previous studies identifying a role of Asn 279 in stabilizing 8-oxo-dGTP\textsubscript{(syn)}\textsuperscript{20}.

To observe catalysis the ground state crystals were transferred to a solution containing MgCl\textsubscript{2} for 20 s (Extended Data Table 1). Density corresponding to both the reactant and product was observed (Fig. 1e and Extended Data Fig. 1b), and on the basis of occupancy refinement the reaction was 60% complete\textsuperscript{c}. Compared with the ground state there is only moderate movement in the active site, at P\textsubscript{2} and O\textsubscript{3}’ (Extended Data Fig. 1b, c). The Hoogsteen base pairing and hydrogen bonding interactions that stabilize the planar syn-conformation are maintained, and the enzyme remains in the closed conformation. These observations are consistent with 8-oxo-dGTP insertion opposite Ad exhibiting a high catalytic efficiency (Fig. 1c).

Extending the MgCl\textsubscript{2} soak time to 40 s resulted in complete turnover of the reactant 8-oxo-dGTP\textsubscript{(syn)} in the closed polymerase conformation (Extended Data Table 1 and Fig. 1f). This post-chemistry complex shows the Hoogsteen base pairing and the intramolecular hydrogen bond at N2 are maintained, and Asn 279 hydrogen bonds with O\textsubscript{8}. The catalytic Mg\textsuperscript{2+} has been replaced by Na\textsuperscript{+}, while the nucleotide Mg\textsuperscript{2+} remains in the active site coordinating PP\textsubscript{i} (Extended Data Fig. 1d, e).

The closed product complex contains a new Mg\textsuperscript{2+} product metal (M\textsubscript{gp}) that bridges the backbone phosphate of 8-oxo-dGMP\textsubscript{(syn)} and PP\textsubscript{i}.

Extending the MgCl\textsubscript{2} soak to 90 s results in a closed-to-open conformational change (Fig. 1g). The PP\textsubscript{i} and associated metals have dissociated, and the inserted 8-oxo-dGMP\textsubscript{(anti)} has lost the interaction between Asn 279 and O\textsubscript{8}, promoting destabilization of the Hoogsteen base pairing. The inserted 8-oxo-dGMP has a high B-factor (57 Å\textsuperscript{2}; Extended Data Table 1), with the most stable position displaced into the major groove and a weak hydrogen bond formed between N6 of Ad and N3 of 8-oxo-dGMP (Extended Data Fig. 1f).

The insertion efficiency of 8-oxo-dGTP opposite Cy is much less than opposite Ad (Fig. 1c); this may arise from a clash between O\textsubscript{8} and P\textsubscript{2} of 8-oxo-dGTP\textsubscript{(anti)} (Fig. 1a)\textsuperscript{19,21}. To probe how this clash is accommodated, we soaked open binary pol β complex crystals with a templating Cy in a cryosolution containing 8-oxo-dGTP/CaCl\textsubscript{2}. The resulting closed ternary ground state complex contains 8-oxo-dGTP\textsubscript{(anti)} Watson–Crick base pairing with the templating Cy (Extended Data Table 3 and Fig. 2a).

The clash at O\textsubscript{8} is partly eased by an altered sugar pucker, glycosidic angle, buckle, and shear compared with dGTP\textsubscript{(anti)} (Extended Data Table 2).

Figure 2 | 8-Oxo-dGTP insertion opposite cytosine. a, 8-Oxo-dGTP:Cy pre-catalytic active site. b, A 90° rotation relative to a. Coordinating waters (blue) with distances (in ångströms) are indicated. Clash at O\textsubscript{8} is shown with red dashes. The Cy backbone shift is indicated. c, Overlay of 8-oxo-dGTP\textsubscript{(anti)} and dGTP\textsubscript{(anti)} opposite Cy is shown in yellow or green, respectively. The shift from the addeduct O\textsubscript{8} is indicated. d, Catalytic efficiency of 8-oxo-dGTP insertion opposite Cy or Ad for pol β R283K and R283A.

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The incoming 8-oxo-dGTP (*anti*) N3 and N2 atoms are within hydrogen-bonding distance of Asn 279 and Arg 283, respectively (Fig. 2b). To determine whether these are unique contacts, we solved the structure of dGTP (*anti*) Watson–Crick base pairing with Cy in the presence of CaCl₂ (Extended Data Table 3). Comparing these two structures indicates the contact with Arg 283 may be unique to the incoming 8-oxo-dGTP (*anti*) because O8 causes the triphosphate and base to move 1.1 Å apart (Fig. 2c). This shift promotes the phosphate backbone of Cy to move 2.6 Å into the minor groove (Fig. 2b). Mutating Arg 283 to lysine or alanine reduced the 8-oxo-dGTP specificity from favouring 8-oxo-dGTP(*anti*) insertion opposite Ad by 40-fold for wild-type, to sixfold and twofold for the R283K and R283A mutants, respectively (Fig. 2d). This loss of discrimination against insertion of 8-oxo-dGTP (*anti*) opposite Cy indicates Arg 283 promotes the mutagenic insertion of 8-oxo-dGTP (*anti*) by acting as a steric gate to prevent insertion opposite Cy. Interestingly, during the bypass of 8-oxo-G in the templating position, Watson–Crick base pairing is lost, 8-oxo-dGMP stacks over the templating base with only the product-associated metal is present (Fig. 3c). Occupancy refinement indicates the reaction is 40% complete. The Watson–Crick base pairing interactions are maintained with only moderate movement at the reacting atoms (P2 and O3); Fig. 2e). The phosphate backbone of the templating Cy is observed in two conformations corresponding to reactant and product. Surprisingly, density for both ground state and product metals can be observed, indicating there are two distinct populations within the crystal (Extended Data Fig. 3).

Soaking pre-catalytic complex crystals in MgCl₂ for 60 s results in a closed product complex (Extended Data Table 3). 8-Oxo-dGTP (*anti*) has been completely inserted with the sugar pucker shifting from a C4'-exo (ground state) to C3'-endo (Extended Data Table 2 and Fig. 3a). Importantly, there is no density corresponding to the ground state metal and only the product-associated metal is present (Fig. 3a, b). The clash at O8 has not been fully alleviated in the product complex and is probably being mediated by the product Mg²⁺ (Fig. 3c). Overlaying the closed ground (0 s) and product states (60 s) indicates that ground state and product metal-binding sites are in distinct positions separated by 2.0 Å that are dependent on having either substrate or product present (Fig. 3d). Extending the soak time in MgCl₂ to 120 s resulted in pol β transitioning to an open conformation (Extended Data Table 3). Watson–Crick base pairing is lost, 8-oxo-dGMP stacks over the template base with 8-oxo-dGTP(*anti*) insertion we soaked a ground state crystal in MgCl₂ for 40 s (Extended Data Table 3). Pol β remained in the closed conformation with density corresponding to both product and reactant species (Fig. 2e). Occupancy refinement indicates the reaction is 40% complete. The Watson–Crick base pairing interactions are maintained with only moderate movement at the reacting atoms (P2 and O3); Fig. 2e). The phosphate backbone of the templating Cy is observed in two conformations corresponding to reactant and product. Surprisingly, density for both ground state and product metals can be observed, indicating there are two distinct populations within the crystal (Extended Data Fig. 3).

![Figure 3](image-url)

**Figure 3 | Product complex with 8-oxo-dGMP(*anti*)/cytosine.** a. Closed 8-oxo-dGMP-Cy product complex after a 60 s soak in MgCl₂. b. Active site of the closed 8-oxo-dGMP-Ad product complex with coordination distances (in ångströms) and waters (blue). c. A 90° rotation relative to a. The clash at O8 is shown with red dashed lines. d. Overlay of the ground (green) and product (yellow) 8-oxo-dGTP(*anti*)/Cy complexes. The distance (in ångströms) between the ground and product metal sites is shown with a dashed line. e. Open 8-oxo-dGMP-Cy product complex after a 120 s soak in MgCl₂. Closed conformation is shown in green (Protein Data Bank accession number 2FMS). ΔFo–Fc maps (3σ) are in green. Ca²⁺, Mg²⁺, and Na⁺ are orange, red, and purple spheres, respectively.
a high B-factor (65.4 Å²), and the remaining PPi and associated metals have dissociated (Fig. 3e).

Quantum mechanical analysis allows the point charge on each atom of dGTP(anti) and 8-oxo-dGTP(anti) to be calculated (Extended Data Table 4 and Extended Data Fig. 4). Figure 4a illustrates the charge difference between 8-oxo-dGTP and dGTP mapped onto each atom of dGTP. The added dGTP causes the sugar moiety and triphosphate (OS) to become more positive. Likewise, the pro-S oxygen of Pz becomes more negative and may facilitate recruitment of the ground state metal. We also calculated the charge on each atom of 8-oxo-dGTP(anti) with all three metals (Extended Data Table 4 and Extended Data Fig. 4). Figure 4b shows the difference in charge of 8-oxo-dGTP(anti) with three metals from that with two metals (Ca and Cb) mapped onto the structure of 8-oxo-dGTP(anti). The largest electronegative change is localized on the triphosphate at key catalytic atoms. This includes making Pz and Pβ more positive, while their bridging oxygen becomes more negative.

Molecular dynamics simulations using the pre-catalytic 8-oxo-dGTP (anti) or dGTP(anti) opposite Cy structures indicate a stable Mg coordination sphere (Extended Data Table 5). With 8-oxo-dGTP(anti), one of the Mg-coordinating water molecules forms a hydrogen bond with the O8 oxygen of Pz to become more positive. Likewise, the pro-S oxygen of Pz becomes more negative and may facilitate recruitment of the ground state metal. We also calculated the charge on each atom of 8-oxo-dGTP(anti) with all three metals (Extended Data Table 4 and Extended Data Fig. 4). Figure 4b shows the difference in charge of 8-oxo-dGTP(anti) with three metals from that with two metals (Ca and Cb) mapped onto the structure of 8-oxo-dGTP(anti). The largest electronegative change is localized on the triphosphate at key catalytic atoms. This includes making Pz and Pβ more positive, while their bridging oxygen becomes more negative.

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Figure 4 | Charge modulation of the polymerase active site. a. The charge difference for each atom of dGTP(anti) and 8-oxo-dGTP(anti) is plotted onto dGTP with a colour key shown. The only value that does not fall within the indicated range is C8 (0.4e⁻). b. The charge difference for each atom of 8-oxo-dGTP(anti) with three and two metals is mapped onto 8-oxo-dGTP with a colour key shown. c. Proposed model for the catalytic mechanism of nucleotide insertion. The primer terminus is shown in grey with O3' in red and the incoming nucleotide in yellow. The transition state includes the catalytic (Mgα), nucleotide (Mgβ), and transition (Mgγ) metals. The transition metal is in the same location as the previous ground-state metal-binding site. The localized relative charges are indicated.

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We can infer a mechanistic model for the role of these adjunct metal ions during catalysis (Fig. 4c). In the pre-catalytic ground state the primer terminus, Mgα, Mgβ, and incoming nucleotide are bound. De-protonation of O3' initiates nucleophilic attack at Pz and as O3' approaches Pz it sterically clashes with the non-bridging oxygens of Pz. This results in a transition state and localized charges on O3', Pz, and OPα that recruit a metal ion to polarize Pz, thus facilitating O3' attack by making Pz more positive and OPα,β more negative. Such a role has been postulated for a
basic side chain in A- and B-family DNA polymerases. The accumulating negative charge on O_{2\cdot}, also promotes protonation of PP, which has been proposed to be a key rate-limiting step. Following product formation this metal could transfer to the nearby product metal-binding site, while the catalytic metal rapidly dissociates owing to the loss of a coordinating ligand (O3·). Accordingly, the appearance/disappearance of cations around the active site represents an elegant ballet of electrons during DNA synthesis.

The structures captured here reveal how 8-oxo-dGTP escapes general polymerase discrimination checkpoints by modulating the highly charged DNA polymerase active site. Importantly, if 8-oxo-dGTP were to be inserted into a single-nucleotide gap, DNA ligase would be responsible for sealing the nick with a modified base pair. Abnormalities at the general polymerase discrimination checkpoints by modulating the highly charged DNA polymerase active site. Importantly, if 8-oxo-dGTP were to be inserted into a single-nucleotide gap, DNA ligase would be responsible for sealing the nick with a modified base pair. Abnormalities at the

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 7 July; accepted 23 September 2014.

Published online 17 November 2014.

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Acknowledgements We thank the Collaborative Crystallography group at the National Institute of Environmental Health Sciences for help with data collection and analysis. We thank L. Pedersen for discussions. Use of the advanced Photon Source was supported by the US Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract W-31-109-Eng-38. This research was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences (project numbers Z01-ES050161 (to S.W.), Z01-ES050158 (to S.W.), Z01-ES050161 (to S.W.), and ZIC-ES043010 (to L.P.)) and in association with National Institutes of Health grant 1U19CA105010. We are grateful for computational support for the molecular dynamics simulations from the HPC clusters at NYU as well as the Blue Gene at CCNI. Support from Philip Morris USA Inc. and Philip Morris International to T.S. is gratefully acknowledged.

Author Contributions B.F., W.B., and S.W. designed the project. B.F. performed crystallography. D.S. did the kinetic analyses. T.K. and T.S. did the molecular dynamics simulations. L.P. did the quantum mechanical analysis. B.F., W.B., and S.W. prepared the manuscript. All authors discussed the results and commented on the manuscript.

Author Information Atomic coordinates and structure factors for the reported crystal structures have been deposited in the Protein Data Bank under accession numbers 4UAW, 4UAY, 4U4Z, 4UB1, 4UB2, 4UB3, 4UB4, 4UB5, 4UBB, and 4UBC. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.W.H. (wilson5@niehs.nih.gov).
METHODS

DNA sequences. To generate the 16-base oligonucleotide the following DNA sequences were used for crystallization (coding nucleotide is underlined): template, 5'--CCG ACA/G GGG CAT GAG C-3'; primer, 5'--GCT GAT GGG C-3'; downstream, 5'--GTC GGG--3'. The downstream sequence was 5'-phosphorylated. The kinetic studies required extending the downstream and upstream sequences to employ a 34-base oligonucleotide DNA substrate. The sequence of the template strand was

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\text{kinetic studies required extending the downstream and upstream sequences to employ}
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and products were quantified using a GE Typhoon 8600 phosphorimager in fluorescence mode. Two reference systems were simulated by molecular dynamics calculations. The systems were then equilibrated for 500 ps at constant pressure and temperature. The temperature was maintained at 300 K using weakly coupled Langevin dynamics, while keeping all the heavy atoms of pol β and DNA fixed. This was followed by unconstrained minimization consisting of 20,000 steps. The systems were then equilibrated for 500 ps at constant pressure and temperature. Pressure was maintained at 1 atmosphere using the Langevin piston method. The minimization, equilibration, and production molecular dynamics simulations were performed by the NAMD simulation package with the CHARMM27 all-atom force field. The force field parameters for 8-oxo-G were adopted from earlier works16,18. The average distances and standard deviations were calculated using molecular dynamics trajectories in the 50–80 ns time range.

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Extended Data Figure 1 | Mutagenic 8-oxo-dGTP insertion opposite adenine. a, Overlay of the ternary complex for 8-oxo-dGTP(syn):Ad generated with Ca\(^{2+}\) or a dideoxy-terminated primer (Protein Data Bank accession number 3MBY) is shown in yellow and green, respectively (root mean squared deviation of 0.17 Å). b, The pol β active site is shown with a 2Fo – Fc map contoured at 1.5σ after a 20 s soak. Key active site residues are indicated and Mg\(^{2+}\) ions are shown as red spheres. The reactant 8-oxo-dGTP and product 8-oxo-dGMP are shown in green and yellow respectively. c, A focused view of b with the density removed. Coordinating waters (blue) and their distances (in ångströms) to active site metals are shown. d, The active site following a 40 s soak is shown with an omit map (3σ) for the Mg\(_n\) and coordinating waters. e, The coordination distances (in ångströms) for the Na\(_{c}\), Mg\(_{gp}\), and Mg\(_{n}\) metals are indicated for the closed product complex after a 40 s soak. f, The 8-oxo-dGMP(anti):Ad contact between N3 and N6 of 8-oxo-dGMP and Ad respectively is shown for the open product complex after a 90 s soak.
Extended Data Figure 2 | Pre-catalytic ground state with 8-oxo-dGTP and templating cytosine after a 5 s soak in MnCl₂. a. The pre-catalytic pol β active site is shown with an omit map (3σ). The ground state metal (Mn₉) has been removed for clarity. b. The view is a 90° rotation relative to a. An overlay of the 8-oxo-dGTP(anti) with Ca²⁺ and Mn²⁺ is shown in yellow and purple respectively. The anomalous density map contoured at 5σ for the Mn²⁺ ions is shown in purple. The Mn₉ coordinating water molecules are shown in blue and the distances (in ångströms) are indicated.
Extended Data Figure 3 | Reaction with 8-oxo-dGTP opposite templating cytosine. a, Focused view of the active site following a 40 s soak is shown with key residues indicated; density has been removed for clarity (see Fig. 2e for density). b, An omit map (3σ) for Ca is shown. Coordinating waters are shown in blue (distances in Ångströms). c, An omit map (3σ) for Mg is shown.
Extended Data Figure 4 | Quantum mechanical computational models for 8-oxo-dGTP(anti) and dGTP(anti). The models used for the quantum mechanical computational studies with the calcium ions, oxygen, phosphates, carbon, nitrogen, and protons shown in green, red, orange, grey, blue, and white, respectively. The key atoms and Asp 190, Asp 192, and Asp 256 mimics are indicated. a, The 8-oxo-dGTP(anti) with three calcium ions and eight water molecules. b, The 8-oxo-dGTP(anti) with two calcium ions and three water molecules. c, The dGTP(anti) with two calcium ions and three water molecules.
Extended Data Figure 5 | Molecular dynamics simulation analysis of 8-oxo-dGTP(anti) and dGTP(anti) opposite Cy. 

a, The 8-oxo-dGTP(anti) opposite Cy at 80 ns superimposed upon the initial structure. A multicolour code based on atom type is used for the final molecular dynamics structure, whereas the reference initial structure is shown in light grey. The catalytic (Mg_c), nucleotide (Mg_n), and ground (Mg_g) magnesium metal ions are shown in green, and average distances over the course of the simulation are indicated for Pα-O3’ and Mg_g-O3’. 

b, Distance distributions between hydrogen atoms in the water shell and O8 in the 8-oxo-dGTP(anti):Cy simulation. A snapshot of the 8-oxo-dGTP, Mg_g, and water shell (W(g1–g5)) is plotted at top. Black and red dotted lines indicate Mg_g coordination and a hydrogen-bonding interaction between a water molecule and O8, respectively. Four of the five water molecules in the water shell (W(g1–g4)) contribute to hydrogen-bonding interactions with O8. Blue and orange lines indicate distances between hydrogen atoms in each water molecule and O8. The red line in the bottom plot indicates the minimum distance between hydrogen atoms in the water shell and O8. 

c, The dGTP(anti) opposite Cy at 80 ns superimposed upon the initial structure (grey). Distances and ion labelling are as for a. 

d, Root mean squared deviation of the evolving molecular dynamics structure for the entire polymerase/DNA complex (top) and for the active site only (bottom), with respect to the crystal structure.
## Extended Data Table 1 | Data collection and refinement statistics of pol β 8-oxo-dGTP insertion opposite adenine

| Templating Base | 0 s Ground state | 20 s Reactant state | 40 s Product state | 90 s Product state |
|-----------------|------------------|---------------------|-------------------|-------------------|
| **Data Collection** |                  |                     |                   |                   |
| Wavelength      | 1.00             | 1.00                | 1.00              | 1.00              |
| Space group     | P2₁              | P2₁                 | P2₁               | P2₁               |
| Cell dimensions (Å)     | 50.9,79.9,55.5  | 50.8,79.9,55.4     | 50.9,80.4,55.4   | 55.1,80.8,55.5   |
| a, b, c (Å)     | 90.107.6,90      | 90.107.6,90        | 90.107.7,90      | 90.109.6,90      |
| Resolution (Å)  | 50.0-1.90        | 50.0-1.88           | 50.0-1.98        | 50.0-2.35        |
| R_{sym} or R_{merge} (%) | 6.8 (52.8) | 5.5 (34.2)      | 6.0 (28.4)       | 5.2 (33.4)       |
| I/sI            | 28.9 (4.4)       | 23.3 (2.3)          | 24.2 (2.8)       | 24.3 (2.3)       |
| Completeness (%)| 98.0 (97.7)      | 95.6 (68.9)        | 96.6 (72.1)      | 97.3 (77.0)      |
| Redundancy      | 5.7 (4.0)        | 3.3 (1.8)           | 3.5 (2.3)        | 3.5 (2.5)        |
| **Refinement**   |                  |                     |                   |                   |
| Resolution (Å)  | 1.90             | 1.88                | 1.98              | 2.35              |
| No. reflections | 57079            | 56411               | 51562             | 34952             |
| R_{work}, R_{free} | 17.5/23.2      | 17.4/21.4           | 17.7/23.9        | 20.8/26.4        |
| No. atoms       | Protein          | 2677                | 2673             | 2673              | 2593              |
|                 | DNA              | 659                 | 681              | 681               | 681               |
|                 | Water            | 341                 | 278              | 274               | 86                |
| B-factors (Å²)  | Protein          | 25.9                | 29.2             | 26.2              | 40.5              |
|                 | DNA/8oxo/PPᵢ     | 26.2/18.7/-        | 36.5/18.2/25.1   | 35.0/20.9/28.1   | 38.2/57/-        |
|                 | Water            | 27.8                | 33.1             | 31.57             | 30.4              |
| R.m.s deviations | Bond length (Å)  | 0.01                | 0.01             | 0.01              | 0.01              |
|                 | Bond angles (°)  | 1.20                | 1.00             | 1.09              | 1.16              |
| **Reaction Ratio** |                  |                     |                   |                   |
| Pol β conformation | closed            | closed             | closed           | open              |
| Ratio of RS/PSⁱ | 1.00             | 0.4/0.6             | 0/1.0            | 0/1.0             |
| Occupancy       | Metal C/N/G/Pⁱ   | 1.0/1.0/-          | 1.0/1.0/-/-      | -/1.0/-/-/-      | -                 |
|                 | PPᵢ             | 0.6                 | 1.0              | -                 |
| PDB ID          | 4VAW             | 4VAZ               | 4UAY             | 4UB1             |

*Highest resolution shell is shown in parentheses.

ⁱRS and PS represent the reactant state and product states, respectively.

ⁱⁱMetal C/N/G/P refers to the catalytic, nucleotide, ground, and product metal-binding sites, respectively.
## Extended Data Table 2 | Key DNA and structural parameters near the pol β active site

| Complex          | Sugar pucker | Buckle (NBP) \(^*\) | Shear (NBP) \(^*\) |
|------------------|--------------|----------------------|--------------------|
|                  | Primer \(^*\) | Incoming             |                    |
| dC–dGTP          | C3’-endo     | C3’-endo             | 8.5                | -0.17              |
| dC–8oxodGTP      | C3’-endo     | C4’-exo              | -13.1              | 0.43               |
| dA–8oxodGTP      | C3’-endo     | C3’-endo             | 0.7                | 0.03               |
| dG–dATP\(^\text{II}\) | C2’-endo     | C3’-endo             | 43.2               | 0.61               |
| dA–8oxodGTP\(^\text{II}\) | C2’-endo     | C4’-exo              | -13.9              | -0.06              |

\(^*\) Parameters extracted with 3DNA.

\(^\dagger\) Primer terminus.

\(^\ddagger\) Nascent base pair.

\(^\text{III}\) Protein Data Bank accession number 4LVS.

\(^{\text{II}}\) Protein Data Bank accession number 3MBY.
Extended Data Table 3 | Data collection and refinement statistics of pol β insertion opposite cytosine with 8-oxo-dGTP and dGTP

| Templating Base | 0 s | 40 s | 60 s | 120 s | 5 s (MnCl₂) | 0 s (dGTP) |
|-----------------|-----|-----|-----|-------|------------|-----------|
|                 | Ground state | Reactant state | Product state | Ground state | Ground state | Ground state |
| Data Collection | Cytosine | Cytosine | Cytosine | Cytosine | Cytosine | Cytosine |
| Wavelength      | 1.54 | 1.00 | 1.54 | 1.54 | 1.54 | 1.54 |
| Space group     | P2₁ | P2₁ | P2₁ | P2₁ | P2₁ | P2₁ |
| Cell dimensions | 50.7, 79.8, 55.5 | 50.8, 79.8, 55.6 | 50.7, 80.1, 55.4 | 55.1, 79.6, 55.7 | 55.1, 77.7, 55.1 | 50.6, 79.4, 55.5 |
| a, b, c (Å)     | 90,107.5, 90 | 90,107.6, 90 | 90,107.8, 90 | 90,109.7, 90 | 90,114.2, 90 | 90,107.5, 90 |
| Resolution (Å)  | 50.0-1.96 | 50.0-1.85 | 50.0-2.06 | 50.0-2.51 | 50.0-2.15 | 50.0-1.96 |
| Rsym or Rmerge (%) | 6.0 (42.1) | 5.4 (27.5) | 8.3 (51.1) | 7.6 (62.3) | 6.9 (51.6) | 6.8 (51.3) |
| l/σf            | 20.4 (2.1) | 20.9 (2.9) | 16.3 (2.1) | 17.6 (2.2) | 13.3 (2.3) | 19.6 (2.2) |
| Completeness (%) | 98.4 (87.7) | 98.1 (79.0) | 97.4 (91.2) | 99.5 (99.7) | 99.6 (99.5) | 99.5 (95.8) |
| Redundancy      | 3.0 (1.7) | 3.2 (1.9) | 4.7 (3.3) | 3.6 (3.5) | 3.2 (2.9) | 4.8 (2.3) |
| Refinement      |                |                |                |                |                |                |
| Resolution (Å)  | 2.0 | 1.90 | 2.06 | 2.51 | 2.15 | 1.96 |
| No. reflections | 51032 | 63744 | 21786 | 25634 | 39111 | 49693 |
| Rmerge/Rsym     | 18.2/25.4 | 17.6/22.3 | 19.1/25.2 | 20.5/27.5 | 21.2/27.4 | 17.9/22.8 |
| No. atoms       |                |                |                |                |                |                |
| Protein         | 2673 | 2673 | 2673 | 2593 | 2673 | 2674 |
| DNA             | 627 | 661 | 661 | 661 | 627 | 627 |
| Water           | 304 | 293 | 172 | 63 | 101 | 235 |
| B-factors (Å²)  |                |                |                |                |                |                |
| Protein         | 27.7 | 28.8 | 32.3 | 38.9 | 42.1 | 36.1 |
| DNA/8oxo/PP     | 36.6/18.8/− | 38.0/21.0/20.5 | 41.4/25.8/28.1 | 34.8/65.4/− | 44.1/38.3/− | 42.6/25.0/− |
| Water           | 29.4 | 39.5 | 30.0 | 20.0 | 31.4 | 32.8 |
| R.m.s deviations |                |                |                |                |                |                |
| Bond length (Å) | 0.01 | 0.01 | 0.006 | 0.01 | 0.007 | 0.009 |
| Bond angles (°) | 1.17 | 1.08 | 1.20 | 1.26 | 1.1 | 1.17 |
| Reaction Ratio  |                |                |                |                |                |                |
| Pol β conformation | closed | closed | closed | open | closed | closed |
| Ratio of RS/PS | 1.0/0 | 0.6/0.4 | 0/1.0 | 0/1.0 | 1/0.0 | 1.0/0 |
| Occupancy Metal C/N/G/P | 1.0/1.0/1.0/− | 1.0/1.0/0.6/0.4 | −/1.0/−/0.6 | − | 1.0/1.0/1.0/− | 1.0/1.0/− |
| PDB ID          | 4UBC | 4UBB | 4UB3 | 4UB2 | 4UB5 | 4UB4 |

*Highest resolution shell is shown in parentheses.
†RS and PS represent the reactant state and product states, respectively.
‡Metal C/N/G/P refers to the catalytic, nucleotide, ground, and product metal-binding sites, respectively.
Extended Data Table 4 | Quantum mechanical point charges for each atom of 8-oxo-dGTP(anti) and dGTP(anti) with either two or three calcium ions

| Number | Atom | 8-oxodGTP 3 metals | 8-oxodGTP 2 metals | dGTP 2 metals | 8-oxodGTP (MD) |
|--------|------|---------------------|---------------------|---------------|----------------|
| 1      | H    | 0.41                | 0.39                | 0.4           | N/A            |
| 2      | O    | -0.67               | -0.74               | -0.68         | -0.9           |
| 3      | P    | 1.49                | 1.4                 | 1.42          | 1.10           |
| 4      | O    | -0.9                | -0.82               | -0.87         | -0.9           |
| 5      | O    | -0.83               | -0.84               | -0.82         | -0.9           |
| 6      | O    | -0.62               | -0.56               | -0.58         | -0.86          |
| 7      | P    | 1.49                | 1.33                | 1.32          | 1.50           |
| 8      | O    | -0.81               | -0.83               | -0.79         | -0.82          |
| 9      | O    | -0.88               | -0.89               | -0.82         | -0.82          |
| 10     | O    | -0.75               | -0.41               | -0.20         | -0.74          |
| 11     | P    | 1.44                | 1.32                | 1.33          | 1.5            |
| 12     | O    | -0.91               | -0.86               | -0.91         | -0.82          |
| 13     | O    | -0.97               | -0.89               | -0.77         | -0.82          |
| 14     | O    | -0.22               | -0.17               | -0.33         | -0.62          |
| 15     | C    | 0.03                | 0.01                | 0             | -0.08          |
| 16     | H    | 0.11                | 0.06                | 0.14          | 0.09           |
| 17     | H    | 0.05                | 0.04                | 0.05          | 0.09           |
| 18     | C    | 0.14                | 0.25                | 0.13          | 0.16           |
| 19     | H    | 0.03                | -0.04               | 0.02          | 0.09           |
| 20     | C    | 0.63                | 0.68                | 0.72          | 0.14           |
| 21     | H    | -0.12               | -0.08               | -0.14         | 0.09           |
| 22     | O    | -0.82               | -0.88               | -0.85         | -0.66          |
| 23     | H    | 0.48                | 0.49                | 0.49          | 0.43           |
| 24     | C    | -0.16               | -0.16               | -0.20         | -0.18          |
| 25     | H    | 0.05                | 0.01                | 0.01          | 0.09           |
| 26     | H    | -0.01               | -0.02               | 0.04          | 0.09           |
| 27     | C    | 0.59                | 0.60                | 0.44          | 0.16           |
| 28     | H    | -0.05               | -0.08               | 0.02          | 0.09           |
| 29     | O    | -0.56               | -0.58               | -0.55         | -0.5           |
| 30     | N    | -0.24               | -0.26               | -0.14         | 0.16           |
| 31     | C    | 0.66                | 0.71                | 0.33          | 0.41           |
| 32     | O/H  | -0.63               | -0.61               | 0.08          | -0.64          |
| 33     | N    | -0.51               | -0.54               | -0.57         | -0.34          |
| 34     | H    | 0.37                | 0.38                | -             | 0.38           |
| 35     | C    | -0.19               | -0.18               | -0.02         | 0              |
| 36     | C    | 0.77                | 0.77                | 0.78          | 0.33           |
| 37     | O    | -0.62               | -0.66               | -0.63         | -0.58          |
| 38     | N    | -0.81               | -0.83               | -0.88         | -0.23          |
| 39     | H    | 0.42                | 0.41                | 0.42          | 0.32           |
| 40     | C    | 0.91                | 0.92                | 0.97          | 0.57           |
| 41     | N    | -0.86               | -0.89               | -0.88         | -0.87          |
| 42     | H    | 0.38                | 0.37                | 0.36          | 0.41           |
| 43     | H    | 0.36                | 0.35                | 0.33          | 0.42           |
| 44     | N    | -0.72               | -0.72               | -0.78         | -0.57          |
| 45     | C    | 0.44                | 0.43                | 0.39          | 0.23           |

The molecular dynamics point charges used with three magnesium ions are shown for reference in the last column. The units are in electron charge (e⁻) and the key is shown for reference.

* The position of each atom is shown in the chemical structure cartoon below the table.

† The oxygen or proton corresponds to 8-oxo-dGTP and dGTP respectively.

‡ Proton corresponds to 8-oxo-dGTP only.
## Extended Data Table 5 | Average distances in the active sites in 8-oxo-dGTP(anti) and dGTP(anti)

| Distance                  | 8-oxodGTP (Å) | dGTP (Å) |
|---------------------------|--------------|----------|
| Mg₂− OD2 (ASP190)         | 1.81 ± 0.04  | 1.81 ± 0.04 |
| Mg₂− OD1 (ASP192)         | 1.80 ± 0.04  | 1.81 ± 0.04 |
| Mg₂− OD2 (ASP256)         | 1.80 ± 0.04  | 1.81 ± 0.04 |
| Mg₂− O1A (Pa)             | 3.18 ± 0.13  | 3.34 ± 0.25 |
| Mg₂− O3′ (C10)            | 2.19 ± 0.13  | 4.78 ± 0.42 |
| Mg₂− W(c)                 | 1.95 ± 0.05  | 2.00 ± 0.07 |
| Mg₂− W(c)*                | N/A          | 1.98 ± 0.07 |
| Pa − O3′ (C10)            | 3.67 ± 0.13  | 5.25 ± 1.01 |

| Mg₂− OD1 (ASP190)         | 1.86 ± 0.05  | 1.86 ± 0.05 |
| Mg₂− OD2 (ASP192)         | 1.87 ± 0.05  | 1.88 ± 0.05 |
| Mg₂− O1A (Pa)             | 1.90 ± 0.06  | 1.92 ± 0.06 |
| Mg₂− O2B (Pβ)             | 1.90 ± 0.06  | 1.91 ± 0.06 |
| Mg₂− O3G (Py)             | 1.85 ± 0.05  | 1.84 ± 0.05 |
| Mg₂− W(n)                 | 2.00 ± 0.06  | 2.03 ± 0.07 |

| Mg₂− O2A (Pa)             | 1.82 ± 0.04  | 1.82 ± 0.04 |
| Mg₂− W(g1)                | 1.98 ± 0.06  | 1.99 ± 0.06 |
| Mg₂− W(g2)                | 1.98 ± 0.06  | 1.99 ± 0.06 |
| Mg₂− W(g3)                | 2.00 ± 0.07  | 1.99 ± 0.06 |
| Mg₂− W(g4)                | 2.00 ± 0.07  | 2.01 ± 0.07 |
| Mg₂− W(g5)                | 1.99 ± 0.07  | 1.98 ± 0.06 |

| W(g) − O8 (8-oxodGTP)     | 1.73 ± 0.11  | N/A      |

| W(g) − N7 (dGTP)          | N/A          | 3.9 ± 0.34 Å |

W(c), W(n), W(g1−g5) are water molecules bound to the Mg₆, Mg₅, and Mg₄, respectively. The Mg₂ in the dGTP(anti) system establishes a new coordination with a water molecular (W(c)*) after 40 ns. The average values are calculated using 50–80 ns range of molecular dynamics trajectories.