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Revealing an Internal Stabilization Deficiency in the DNA Polymerase β K289M Cancer Variant through the Combined Use of Chemical Biology and X-ray Crystallography

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Abstract

The human DNA polymerase (pol) β cancer variant K289M has altered polymerase activity *in vitro*, and the structure of wild-type pol β reveals that the K289 side chain contributes to a network of stabilizing interactions in a C-terminal region of the enzyme distal to the active site. Here, we probed the capacity of the K289M variant to tolerate strain introduced within the C-terminal

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Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.biochem.9b01072>.

Crystallographic statistics (Tables S1–S3) and “hotspot” sequences (Figures S1–S6) (PDF)

Accession Codes

The atomic coordinates and structure factors have been deposited in the PDB as entries 6NKR, 6NKS, 6NKT, 6NKU, 6NKV, 6NKW, 6NKX, 6NKY, 6NKZ, and 6NLO.

region and active site. Strain was imposed by making use of a dGTP analogue containing a CF₂ group substitution for the β - γ bridging oxygen atom. The ternary complex structure of the K289M variant displays an alteration in the C-terminal region, whereas the structure of wild-type pol β is not altered in the presence of the dGTP CF₂ analogue. The alteration in the K289M variant impacts the active site, because the enzyme in the ternary complex fails to adopt the normal open to closed conformational change and assembly of the catalytically competent active site. These results reveal the importance of the K289-mediated stabilizing network in the C-terminal region of pol β and suggest an explanation for why the K289M cancer variant is deficient in polymerase activity even though the position 289 side chain is distal to the active site.

GRAPHICAL ABSTRACT

DNA polymerase (pol) β is an X-family polymerase that is important in the maintenance of genomic integrity through its roles in the base excision repair (BER) pathway. Pol β and the BER pathway have recently been reviewed.^{1–3} Briefly, pol β is a bifunctional enzyme consisting of two globular domains that contribute lyase and polymerase activities, respectively, to the BER pathway. The preferred DNA substrate for pol β is a repair intermediate in double-stranded DNA with a single-nucleotide gap containing 3'-OH and 5'-phosphate or 5'-deoxyribose phosphate (dRP) groups at the gap margins. Pol β catalyzes lyase removal of the 5'-dRP group generated by AP endonuclease 1 incision of the AP site during BER, and pol β fills the single-nucleotide gap through its polymerase function.^{4–8} After DNA substrate binding, pol β -catalyzed gap filling begins with bending and sculpting of the DNA during the formation of a binary complex, dNTP and divalent metal ion binding to the binary complex. This enables chemical synthesis: deprotonation of the 3'-OH group with nucleophilic attack by the 3' oxygen on the incoming dNTP's P α and concomitant cleavage of the P α -OP β bond with departure of the pyrophosphate leaving group (PP_i), followed by release of PP_i and the product DNA.^{9–11}

Multiple factors and individual steps involved in the BER pathway have been revealed.^{7,12–14} In general, the BER pathway cleanses genomic DNA by removing oxidatively induced lesions¹⁵ and other nonbulky lesions caused by endogenous compounds and environmental exposure.¹⁶ The BER pathway involves a series of sequential steps from damaged base recognition by a glycosylase, removal of the damaged base, strand incision

and gap tailoring, template-directed replacement of the base, and finally ligation to restore the integrity of the double-stranded DNA. Throughout this repair process, DNA intermediates are generated that are potentially cytotoxic.¹⁷ It is, therefore, imperative that the repair machinery in this multistage repair pathway function at an optimal level; a defect(s) in any of the enzymes and/or cofactor(s) involved in the repair pathway could stall the process, leading to accumulation of a toxic repair intermediate and eventually to genomic instability and cell death.

Multiple variants in the POLB gene have been identified in some human cancers.^{18–20} These include colon, gastric, and bladder cancers. Several variants (e.g., L22P, R137G, D160N, K167I, G231D, I260Q, I260M, S229L, Y265C, E288K, K289M, and E295K) have been expressed and purified as recombinant proteins, and some have been reported to exhibit deficient *in vitro* polymerase activity. The K289M variant is a relatively well-studied variant of pol β .^{21,22} Crystallography of the wild-type enzyme has revealed that the position 289 side chain is located in the C-terminal region of the enzyme at the end of α -helix N. This α helix is an important active site component of the enzyme, involved in recognizing the correct incoming dNTP during DNA synthesis. The K289M variant was found to be associated with increased spontaneous mutation frequency *in vivo* and reduced fidelity *in vitro* by virtue of misincorporating dCTP opposite template C in a sequence context-dependent manner.¹⁹ However, the mechanism by which DNA synthesis abnormalities are generated by K289M structural features has not yet been elucidated.

We previously reported a series of structures of wild-type pol β in a ternary complex with incoming dNTP β , γ -bisphosphonate analogues in which the oxygen atom bridging the β - and γ -phosphate groups has been replaced by a methylene group (CXY)^{23–25} These analogues mimic dNTP as pol β substrates, but the leaving group is the corresponding bisphosphonate pCXYp instead of PP_i. By appropriately choosing electron-donating (e.g., X, Y = CH₃) or electron-withdrawing (e.g., X, Y = F) substituents on the methylene carbon atom, one can significantly modulate its electro-negativity. This makes it possible to investigate systematically the effect of changing the acidity and/or basicity of the leaving group on the kinetic properties of the nucleotide incorporation reaction. In general, the ternary complex crystal structures of pol β with the various dNTP analogues were found to be similar to the reference structure with a normal incoming dNTP. This observation facilitated interpretations regarding the acidity modification effects on the nucleotidyl transferase reaction versus other influences such as conformational effects.²³ Interestingly, an exception to the structural conservation was found with one type of incoming dNTP analogue, the dATP analogue with the CF₂ group substitution. In this case, the CF₂ group appeared to introduce strain into the structure preventing the open to closed conformational change and distorting the position of α -helix N in the active site.²³

In the work presented here, we evaluated the possibility that the change at residue 289 from lysine to methionine, removing a stabilizing contact in a C-terminal region network of the enzyme, might destabilize the structure of this region and in turn alter the active site, as well. Because K289 is located in a surface region of the enzyme distal to the active site, one might expect the modification at this residue to have little impact on functions many angstroms away in the active site. However, as noted above, the active site remote position K289 side

chain has a connection to the active site through interactions in a C-terminal network. In this way, it is evident that K289 could have a stabilizing effect within the active site. Thus, we were curious to know whether the loss of K289-mediated stabilization might result in an alteration of the ternary complex structure of the K289M variant.

We initially conducted experiments with the K289M enzyme using our traditionally employed DNA and a normal base-pair complementary incoming dNTP, dGTP. Surprisingly, the structure of the K289M enzyme was found to be similar to that of the wild-type enzyme. Therefore, we decided to test whether the K289M enzyme can tolerate an internal strain introduced by making use of a CF₂-containing analogue of an incoming dNTP, dGTP CF₂. The results reveal an internal stabilization deficiency in the K289M variant enzyme. New structures of wild-type pol β and the K289M variant are described.

MATERIALS AND METHODS

Protein Expression and Purification.

The K289M variant of pol β was obtained with a site-directed mutagenesis kit using the pGEx4T3 plasmid containing GST-TEV-pol β , and the recombinant enzyme was purified as previously described for wild-type pol β .²⁶ Briefly, the K289M variant was overexpressed at 18 °C in Rosetta2 (DE3) cells. After sonication and centrifugation, the lysate was incubated with glutathione 4B resin, washed, and then treated with TEV protease overnight at 4 °C. Eluted K289M protein was further purified by ion exchange chromatography (resource S) followed by size-exclusion (S-200) chromatography. The purified protein was buffer-exchanged and concentrated in 20 mM Bis-tris buffer (pH 7.0). The protein concentration was determined by the absorbance at 280 nm using an extinction coefficient of 23380 M⁻¹ cm⁻¹.

Synthesis and Characterization of dGTP Analogues.

The detailed synthesis procedures and biochemical and structural characterization of the dGTP analogues have been reported previously.^{24,25,27,28}

Crystallization of Pol β Substrate Complexes.

Binary complex crystals of the K289M variant with one-nucleotide gapped DNA were grown as described previously.¹⁰ The template strand (16-mer), primer strand (9-mer), and phosphorylated downstream primer strand (5-mer) were 5'-CCGACCGCGCATCAGC-3', 5'-GCTGATGCG-3', and 5'-pGTCCG-3', respectively. The oligonucleotides were annealed in a 1:1:1 ratio by being heated at 90 °C for 10 min and cooled to 4 °C (1 °C/min) using a polymerase chain reaction thermocycler, resulting in a 1 mM mixture of two-nucleotide gapped duplex DNA. This annealed mixture was further incubated with an equal volume of DNA polymerase β (14 mg/mL) or K289M variant. These mixtures were further incubated with ddCTP, resulting in one-nucleotide gapped oligonucleotides with dC as the templating base. Pol β variant-DNA complexes were crystallized by sitting-drop vapor diffusion at 18 °C by mixing 2 μ L of the complex with 2 μ L of the crystallization buffer. The crystallization buffer consisted of 14–16% PEG2000 monomethyl ether, 350 mM sodium acetate, and 50 mM imidazole (pH 7.5). Crystals grew in approximately 2–4 days after

seeding. The ternary complexes were obtained by soaking crystals of the binary one-nucleotide gapped DNA complexes in artificial mother liquor with 50 mM MgCl₂, 20% PEG2000 monomethyl ether, 15% ethylene glycol, and 2–4 mM dGTP, or an analogue, for 1–2 h. The crystals were then flash-frozen at 100 K in a nitrogen stream. Diffraction quality data were collected for the ternary complex crystals as described below.

Data Collection and Structure Determinations.

Data were collected at 100 K on a CCD detector system mounted on a MiraMax-007HF (Rigaku Corp.) rotating anode generator. Data were integrated and reduced with HKL2000 software.²⁹ All crystals belong to space group *P*2₁. The ternary complex structure was determined by molecular replacement using 2FMS as a reference model. The structures were refined using PHENIX³⁰ and manual model building using Coot. The crystallographic statistics are listed in Tables S1–S3.

RESULTS

Orientation and Surprises from the K289M Structures.

Crystal structures of ternary complexes of wild-type pol β and the K289M variant were obtained. Each structure of the K289M variant is compared with a known ternary complex reference structure of the wild-type enzyme [Protein Data Bank (PDB) entry 2FMS]. As will be summarized in detail below, an overlay of the reference structure of the wild-type enzyme and two structures of the K289M variant with incoming dGTP and the CHF analogue, respectively, were virtually identical to each other (Figure 1). In these three structures, the positions of the position 289 side chain are illustrated at the C-terminal end of α -helix N (helix N).

In contrast, as one can see in Figure 2, the structure of the K289M variant in combination with a different incoming dNTP analogue, dGTP CF₂, revealed major differences compared to either the reference structure (PDB entry 2FMS) or the structure of the wild-type enzyme with incoming dGTP CF₂ (PDB entry 2ISO).

The structure of the K289M variant with the incoming dGTP CF₂ does not overlay well with the wild-type enzyme structures (Figure 2A), and inspection of the structure of the K289M variant reveals that the variant's C-terminal region (residues 276–335) is different (Figure 2B). As described below, the K289 residue makes an interaction in a network in the C-terminal region of the enzyme, and this is disrupted in the K289M variant. These structural differences compared with the wild-type enzyme indicate that the K289M variant enzyme is deficient in handling the dGTP CF₂ analogue, whereas the wild-type enzyme accommodates the dGTP CF₂-imposed strain and is not distorted. An explanation for this difference in the K289M enzyme will be discussed in detail below.

Detailed Features of the New Structures.

A DNA duplex with a single-nucleotide gap was used for all of the structures discussed, and the template base in the gap was dC. The template-primer terminus was the G:C base pair, and the dCMP primer terminus sugar residue was dideoxyribose, used to block the insertion

reaction during crystal soaking with dGTP or a hydrolyzable analogue. As expected with a dideoxy primer, the metals in the nucleotide and catalytic metal binding sites are Mg^{2+} and Na^{1+} , respectively.¹⁰ Statistics for the new crystal structures of the K289M variant and wild-type enzyme are shown in Tables S1 and S2, respectively. Finally, in these ternary complex structures, the enzymes are in the closed conformation, with the exception of the difference noted for the K289M variant enzyme with the incoming dGTP CF_2 (Figure 2). Thus, the structures of the ternary complexes with different incoming bases are similar to each other and the reference structure with the incoming dGTP. In each case, the incoming base is Watson–Crick base paired with the template base, and the primer sugar and P_α groups are in positions similar to that seen in the reference structure.

Ternary Complex Structures of the K289M Variant Enzyme dGTP and the dGTP CHF Analogue.

Detailed assessments of contacts in the active sites of the K289M variant with incoming dGTP and the dGTP CHF analogue are shown in the top and bottom panels of Figure 3, respectively.

These structural features of the prechemistry active sites are similar to those found earlier for the wild-type enzyme. The CHF analogue structure was determined here as a reference toward verifying the special effect of the CF_2 analogue, to be described below. The orientation shown in Figure 3A illustrates coordination of magnesium in the nucleotide metal site, coordination of sodium in the catalytic metal site, the contact of the R183 guanidinium group N1 with a P_β oxygen, the R283 and Y271 contacts in the minor groove, and the network of interactions around the P_γ oxygens. A water molecule bridging the guanidinium group N atoms of R149 to oxygens of P_γ is present in the structure with dGTP, but not in the structure with the CHF analogue. This water molecule has a relatively low *B*-factor and contributes to stabilizing P_γ ; a water molecule in this position is also seen in the wild-type enzyme (not shown). Other P_γ oxygen contacts illustrated in Figure 3A are with the main chain at G189 and the side-chain oxygen of S180, both stabilizing the P_γ oxygen, O3G. In the vicinity of the primer terminus, R254 has close contacts with D256 and an oxygen in the primer terminus DNA backbone.

For the diastereomeric mixture structure of the incoming dGTP CHF analogue (shown in Figure 3B), stereoselective binding of the *R* isomer is observed with 100% occupancy. The fluoride atom makes a contact with the guanidinium N of R183, as shown, which is not observed when oxygen occupies the CHF position. These K289M ternary complex structures with the incoming dGTP and dGTP CHF are similar, except in the vicinity of the P_γ oxygens. In addition, an important stabilizing contact distal to the incoming dNTP binding pocket is noted in panels A and B of Figure 3, i.e., between R182 near the binding pocket and E316 in the C-terminal region. As illustrated, this interaction is observed in the presence of either dGTP or the CHF analogue.

Structure of the K289M Variant with the Incoming dGTP CF₂.

The structure of the active site, including helix N, in the K289M variant with the incoming dGTP CF₂ is illustrated in Figure 4 and compared with the structure of the reference wild-type enzyme.

The orientation shown illustrates that the K289M variant fails to form the closed ternary complex in the presence of the incoming dNTP. The position of helix N in the wild-type enzyme closed ternary complex is colored blue, and distortions in the active site contacts for the K289M variant are noted for R283 and Y271, along with the obvious difference in closure of helix N, as compared with the wild-type enzyme. Thus, the structure of the K289M variant (gold) reveals major distortions compared with the wild-type enzyme (blue), such that (1) the stabilizing interaction between O4' of the sugar of the penultimate templating nucleotide and the R283 guanidinium group N1 is lost (distance of 8.8 Å), (2) the stabilizing interaction between the O2 atom of the primer terminus base and the OH of the Y271 side chain is lost (distance of 7.6 Å), and (3) several other interatomic distances within the active site are longer than in the wild-type enzyme as summarized in Figure 5.

As noted previously, when the nucleotide binds to the binary complex, the wild-type enzyme undergoes a transition from the “open” conformation to the “closed” conformation. During this transition, several contacts that stabilize the ternary complex structures are formed. Summaries of key contact distances are provided in Figure 5. In the panels, the individual contact is specified at the top of each panel and the various incoming dNTPs are listed at the bottom, in the order of reference structure(s) on the left to the halogen analogues on the right. The distances that are >0.2 Å (1/10th of the average resolution) longer or shorter than those in the reference structure are considered as significantly different compared with the reference structure and are colored red or green, respectively. For example, in the K289M structure (Figure 5), some interactions have shorter distances (colored green) than in the reference structure and other interactions have longer distances (colored red); interactions that are similar to those of the reference structure are colored black. In discussing the structures, we have given special consideration to the interactions summarized in Figure 6, including those of four active site arginine side chains: R254, R283, R149, and R183.

Alterations Distal to the Active Site.

The K289M structure, although similar to the wild-type enzyme spanning from the N-terminal region to residue D276, is different from that of the wild-type enzyme from residue 277 extending on to the C-terminal end of the molecule, i.e., residue 335 (Figures 2B and 6). The explanation for this difference appears to be as follows. As illustrated in Figures 6 and 7A, in the wild-type enzyme, K289 is in a C-terminal region network of stabilizing interactions and has a close interaction (2.84 Å) with the side-chain oxygen of Q324 (Figure 7A).

An effect of this contact is stabilization of the structure locally, and this network stabilizing effect extends to E316, then to R182, and finally to R183 that has a contact with an oxygen of the dNTP P_β group of the incoming nucleotide. In addition to the Q324 contact in the wild-type enzyme, there is a close crystal lattice contact (2.75 Å) between K289 and E249 of

the adjacent enzyme molecule in the crystal lattice (see Figure 7). As illustrated in panels B and C of Figure 7, these contacts are not present in the K289M variant. In summary, K289 makes interactions in a network that appears to stabilize the structure in the C-terminal region of the wild-type enzyme; this facilitates the critical open to closed transition, and K289 also contributes to the crystallization properties.

Structures of Ternary Complexes with the Hot-Spot Sequence.

We compared structures mimicking the hot-spot sequence with wild-type pol β and with the K289M variant enzyme; the incoming nucleotide was either a dGTP analogue or dGTP. As shown in Figure S1, the overall structures are similar to the reference structure (2FMS) with root-mean-square deviation values of 0.201–0.314 Å (Figure S1A,B).

Wild-Type Enzyme.

With the hot-spot sequence, the active site of wild-type pol β with incoming dGTP and three of the dGTP analogues is shown in Figure S2.

The water molecule bridging the guanidinium group N atoms of R149 to oxygens of P_{γ} is present in the structure with dGTP and the CHF analogue, but not in the structures with the CH_2 or $CHCl$ analogues. As discussed above, this water molecule helps in stabilizing P_{γ} in the respective structures. For the structure of the diastereomeric mixture of the CHF analogue, stereoselective binding of the *R* isomer is observed with 100% occupancy. The fluoride atom interacts with the guanidinium N1 atom of residue R183, as shown in Figure S2.

Another critical residue in the active site, R283, stabilizes the templating base in the minor groove by interacting with $O4'$ of the sugar of the penultimate nucleotide in the template strand. The distances between the N atom of R283 and the $O4'$ atom of the penultimate templating bases are comparatively shorter in all of these wild-type enzyme structures with the hot-spot sequence (Figures S1–S3). These shorter interactions could influence the catalytic cycle and, therefore, could be linked to altered enzymatic kinetic properties.

K289M Variant Enzyme.

The active sites of K289M structures with different dGTP analogues are illustrated in Figure S4.

The water molecule bridging the guanidinium group N atoms of R149 and oxygens of P_{γ} is present in the structures with dGTP and the CHF and CH_2 analogues (Figure S4), but not in the structure with the CF_2 analogue (Figure S5). The incoming dNTP is stabilized in the active site by interaction of the nonbridging oxygen ($O2B$) atom of P_{β} with the N1 atom of R183. However, the distances between $O2B$ and N1 are shorter (Figure S6) than in the reference structure (2FMS) and in the structures determined here with the wild-type enzyme. Overall, the structure with the hot-spot sequence and dGTP CF_2 analogue is similar to the other structures and, interestingly, lacks the distortion in the C-terminal region, in striking contrast to the structure observed with regular sequence (see the overlay in Figure S5).

DISCUSSION

The loss of network interactions (Figure 6) in the K289M variant enzyme coupled with a difluoro destabilizing effect on R183 is responsible for the distortion observed in the C-terminal region of the K289M enzyme. The basis for the dGTP CF₂ analogue effect involves a displacement in the position of the R183 side chain (Figure 2). This creates strain in the C-terminal region mediated through R183 and contacts between R182 and E316. The resulting distortion interferes with closure of α -helix N, displacing key side chains from the active site, e.g., R283 and Y271; in the wild-type enzyme ternary complex, these residues are important in contributing minor groove-stabilizing interactions with the penultimate template strand nucleotide and the primer terminus base, respectively (Figure 6).

Correlations between Structures and the Activity of the K289M Cancer Variant.

Previous studies of pol β revealed that the pK_a of the pyrophosphate leaving group of various dNTP analogues is correlated with the observed gap filling enzymatic rate constant k_{pol} . In addition, these studies with dNTP analogues revealed that differences in the “local environment” in the prechemistry active site of the wild-type enzyme are associated with differences in the k_{pol} values. In the work presented here, we found that the ternary complex crystal structure of the pol β K289M variant is altered with the incoming dGTP CF₂ analogue. The alterations include many of the key side-chain contacts in the enzyme’s active site along with a major conformational deficiency in the C-terminal region of the enzyme. Thus, this conformational deficiency involves a destabilizing effect in the K289M enzyme preventing the “closing” transition required for positioning of O3’ and the proper assembly of the active site in mediating the nucleotidyl transfer reaction.¹⁰ Even though the distortion in the C-terminal region structure was not observed with the incoming dGTP, it is likely that a distortion is present at some level in solution because the insertion properties of the wild-type and K289M enzymes are different.

Structures with “Hot-Spot” Sequence DNA.

Finally, in addition to the structures described with the DNA routinely used in our studies, we obtained structures of the wild type (Figures S1A, S2, and S3) and K289M variant in ternary complex with DNA containing the “AT-rich” region of the “hot-spot sequence” (Figures S1B, S4, and S5). We found that these K289M structures are similar to those of the wild-type enzyme. Moreover, introduction of strain by the use of the dGTP CF₂ analogue as the incoming nucleotide failed to produce an alteration in the structure (Figures S1–S5). Thus, with DNA containing the “AT-rich” region of the hot-spot sequence, only subtle alterations are noted in the structure of the K289M active site (Figures S5 and S6). These alterations involve subtle, tighter interactions between the template strand and the guanidinium N1 atom of R283 (Figure S5). These differences in substrate interactions could potentially impede steps in the catalytic cycle, resulting in changes in the observed enzymatic properties.

CONCLUSIONS

We demonstrated a functional deficiency in the DNA polymerase β K289M cancer variant using X-ray crystallography. Prechemistry ternary complexes of the wild-type and K289M enzymes were crystallized, and the structures compared. The K289M substitution results in loss of a stabilizing network of interactions in the C-terminal region of the enzyme. Nevertheless, the K289M enzyme can assemble a normal prechemistry closed complex in the presence of the normal incoming nucleotide dGTP. In contrast, when an analogue of dGTP that introduces strain into the active site is used as the incoming nucleotide, the K289M enzyme is unable to overcome the strain and assemble the catalytically relevant active site, whereas the wild-type enzyme handles the dGTP analogue and assembles the optimal prechemistry active site. Further studies are required to reveal factors involved in the dGTP analogue-induced strain, but we suggest it is likely that substrate combinations encountered by the K289M variant *in vivo* could impose strain and compromise the variant's ability to assemble the optimal active site.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Overlay of ribbon diagrams of structures of wild-type human pol β (gold) and the K289M variant both with incoming dGTP (cyan) and a CHF analogue (pink).

Figure 2.

(A) Overlay of ribbon diagrams of structures of wild-type pol β (2FMS, gold), wild-type pol β with the incoming dGTP CF₂ analogue (pink), and the K289M variant (green) with the incoming dGTP CF₂ analogue. The overlays were made with residues 10–335 of the enzyme and with the wild-type enzyme in complex with the regular sequence. The boxed area shows the distorted region of the K289M mutant structure. (B) Magnified view of the distorted region (residues 274–325) as shown in panel A.

Figure 3.

Active site of the K289M variant of pol β with the regular sequence and with the incoming dGTP, termed O (top) and the CHF (bottom) analogue of dGTP. The K289M enzymes in the closed conformation are very similar to the reference structure (2FMS) with root-mean-square deviation values of 0.256 and 0.294 for O and the CHF analogue, respectively. Magnesium is illustrated in the nucleotide metal site (green sphere), and sodium in the catalytic metal site. Watson-Crick base pairs are shown for the template-primer base pair and also for the template base in the gap (C) and the incoming dGTP and dGTP CHF (i.e., the *R* isomer of the monofluorinated analogue was selectively bound in the active site). Amino acid side chains are labeled. The Met 289 side chain is not in position to interact with E316.

There is an obvious void in these K289M variant structures in the position occupied by K289 in the wild-type enzyme.

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Figure 4.
 Overlay illustrating the active site distortion in the K289M variant of pol β with the incoming dGTP CF₂ analogue.

Figure 5.

Bar diagram of the main interatomic distances for dGTP and the dGTP analogues. The distances that are similar to that of the reference structure (2FMS) are colored black; distances that are at least 0.2 Å longer or shorter than that of reference structure are colored red and green, respectively. The red horizontal line shows the distance in angstroms for the reference structure.

Figure 6. Illustration of the distortion of the distal site of the K289M variant of pol β in ternary complex with the CF₂ analogue of dGTP.

Figure 7.

(A) Crystal symmetry contact of K289 with E249 and the salt bridge with Q324 from 2FMS. An $F_o - F_c$ map contoured at 2σ is also shown. (B) Loss of the crystal symmetry contact in the structure with the CF₂ analogue with the K289M variant. (C) Overlay of panels A and B.