SUPPLEMENTARY MATERIAL TO:

Metal-Induced DNA Translocation Leads to DNA Polymerase Conformational Activation

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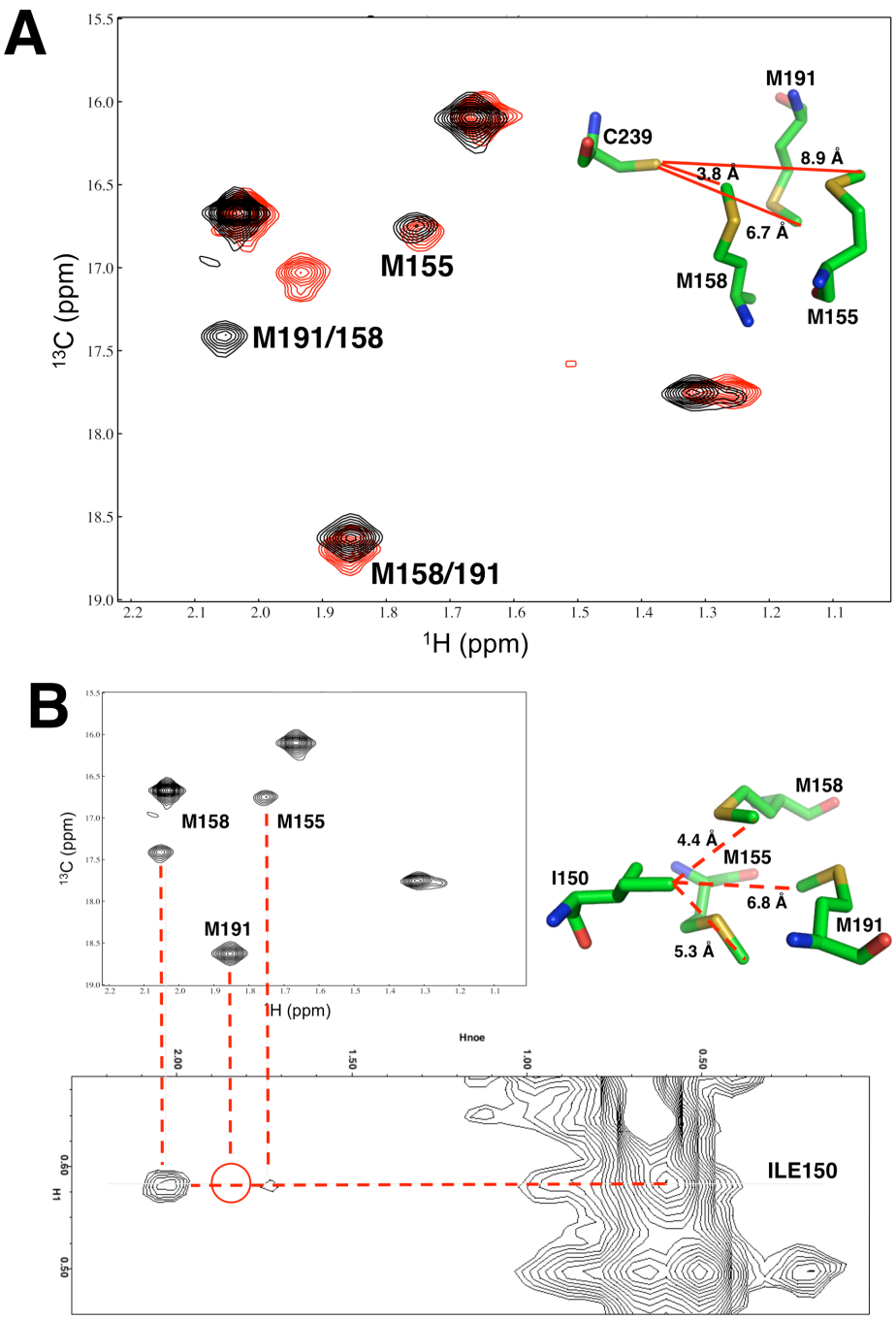
Reassignment of Met158Cε and Met191Cε. The assignments of methionine Cε-methyl resonances of residues Met158 and Met191 have been interchanged based on the studies outlined below. As discussed by Bose-Basu et al. (15), mutation of any of the three closely positioned Met155, Met158, and Met191 residues significantly alters the chemical shift of the two remaining methionine resonances. To circumvent this problem, we used several nudge mutations, i.e., mutations of nearby residues positioned so that they are expected to selectively perturb only a single methionine resonance. The most unequivocal nudge mutant identified was C239A, which, based on crystal structure data, e.g. PDB ID 3ISB, is expected to selectively perturb the Met158 resonance (Supplementary Figure S1*A*, inset). The resulting spectrum revealed a very specific perturbation of the resonance at $\delta(^{1}H,^{13}C)$ = (2.05,17.4) previously assigned to Met191, but now reassigned to Met158 (Fig. S1*A*). Additional assignment information was derived from a sample of pol β that was labeled with [*methyl*-^{13}C]methionine, U-^{13}C [3,3-$^{2}H_2$] α-ketobutyrate (to label the isoleucine Cδ), and ^{15}N. Based on the previously published ^{15}N amide assignments (41) the Ile Cδ could be unambiguously assigned. Supplementary Figure S1*B* shows the NOE strip of the ^{13}C separated NOESY corresponding to the Ile150Cδ. The strip shows NOE crosspeaks to two proton resonances at 2.05 ppm and 1.75 ppm. Based on examination of multiple crystal structures, the 2.05 ppm resonance should correspond to the closer, Met158 methyl, and the weaker cross peak at 1.75 should correspond to the somewhat more distant Met155 methyl. No cross peak was observed for the 1.85 ppm resonance, consistent with its assignment to Met191. Thus, both the C239A nudge mutation and the NOESY data support a reversal of the previous 158 and 191 assignments. The reassignment of these residues does not impact our interpretation of previous results (15,16).

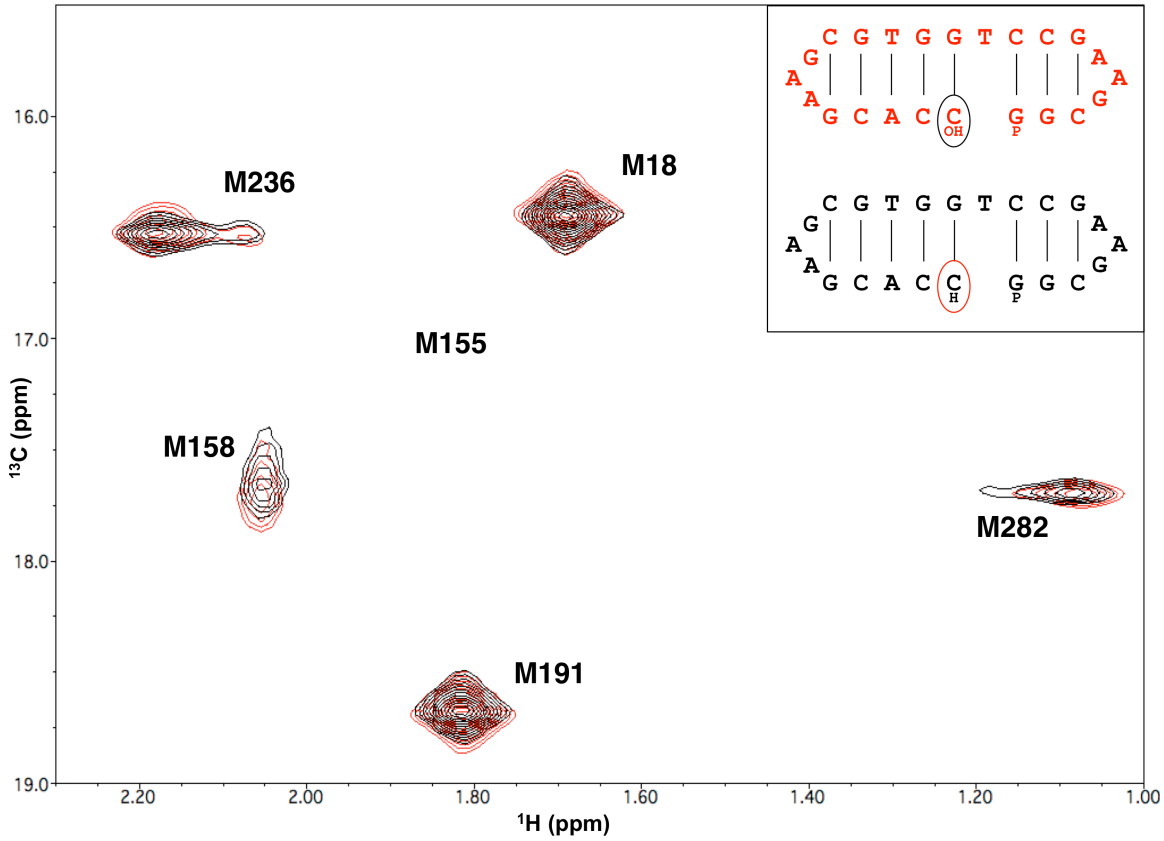


Supplementary Figure S1. Assignments of resonances for Met191 and Met158. (*A*)^{1}H-^{13}C HSQC spectra of [methyl-^{13}C]methionine pol β (black) and C239A (red). The inset shows the relative positions of the labeled amino acids in a crystal structure of a binary DNA complex of pol β (PDB ID 3ISB). (*B*) The NOE strip of the ^{13}C separated NOESY

corresponding to the Ile150Cδ (lower); ^{1}H-^{13}C HSQC spectra of [methyl-^{13}C]methionine pol β (upper left); the relative positions of the labeled amino acids is also shown.

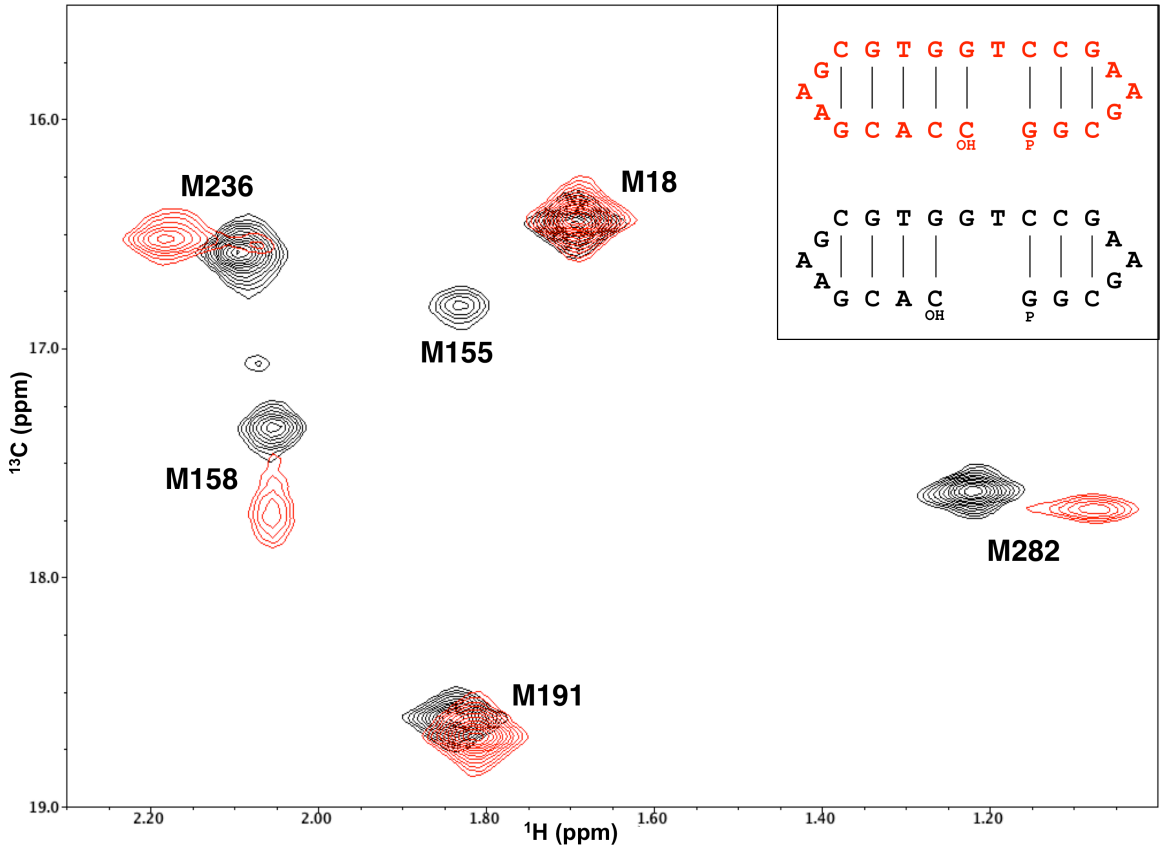
Dependence of Zn^{2+} Activation on the Presence of a Primer 3′-Hydroxyl Group and the Size of the Nucleotide Gap.

Supplementary Figure S2 shows the ^{1}H-^{13}C HSQC spectrum of [methyl-^{13}C]methionine pol β in complex with a double hairpin DNA substrate designed with a one-nucleotide gap and either a deoxycystosine (red) or a 2′,3′-dideoxycytosine (black) at the primer terminus. An essentially identical pattern of activation in the presence of 2.5 mM $ZnCl_2$ obtains with either substrate, indicating that the presence of a 3′-hydroxyl group is not required. Other conditions were 100 mM KP_i, pH 6.7.



Supplementary Figure S2. Dependence of Zn^{2+} activation on the presence of a primer 3′-hydroxyl. ^{1}H-^{13}C HSQC spectrum of [methyl-^{13}C]methionine pol β in complex with a double hairpin DNA substrate with a one-nucleotide gap with either deoxycystosine (red) or 2′,3′-dideoxycytosine (black) at the primer terminus. Other conditions were 100 mM KP_i, pH 6.7, 2.5 mM $ZnCl_2$.

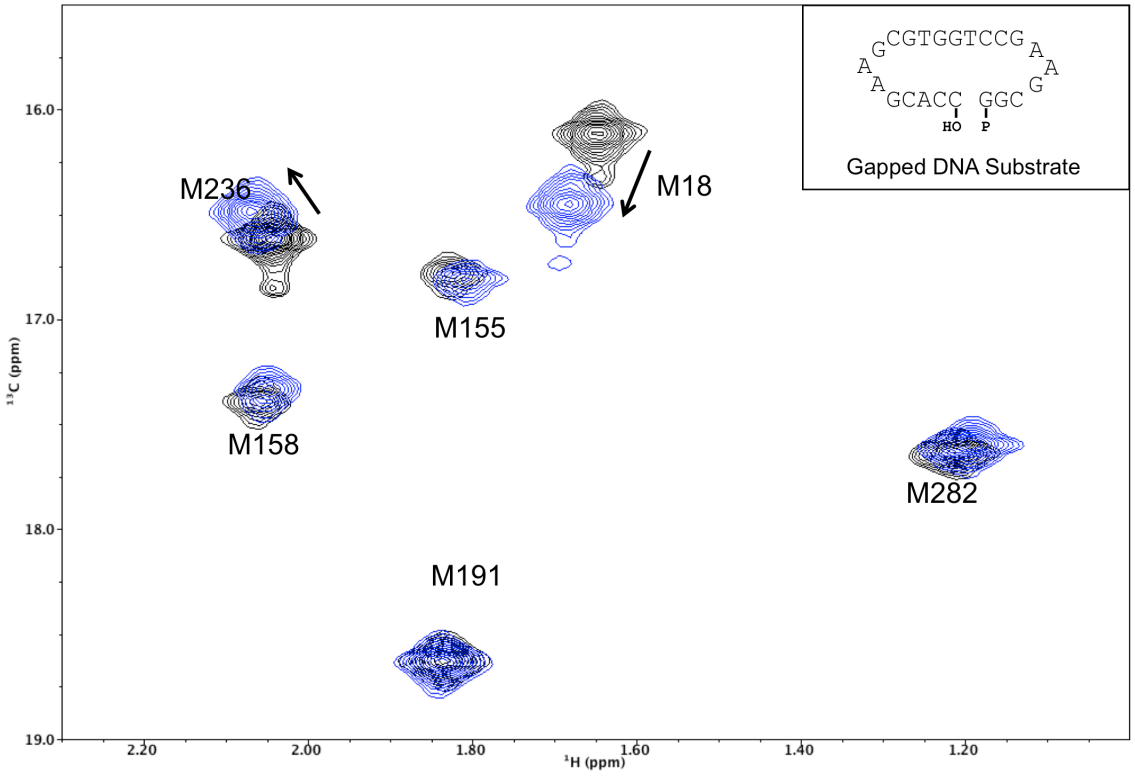
Supplementary Figure S3 shows the ^{1}H-^{13}C HSQC spectrum of [methyl-^{13}C]methionine pol β in complex with a double hairpin DNA substrate designed with a one-nucleotide gap (red) or a two-nucleotide gap (black). Other conditions were 100 mM KP_i, pH 6.7, 2.5 mM $ZnCl_2$. As is apparent from the figure, Zn^{2+} does not produce the conformational activation response in the presence of a two-nucleotide gapped substrate.



Supplementary Figure S3. Dependence of Zn^{2+} activation on the size of the nucleotide gap. ^{1}H-^{13}C HSQC spectrum of [methyl-^{13}C]methionine pol β in complex with a double hairpin DNA substrate with a one-nucleotide gap (red) or a two-nucleotide gap (black). Other conditions were 100 mM KP_i, pH 6.7, 2.5 mM $ZnCl_2$.

Lack of conformational activation of pol β E295K mutant.

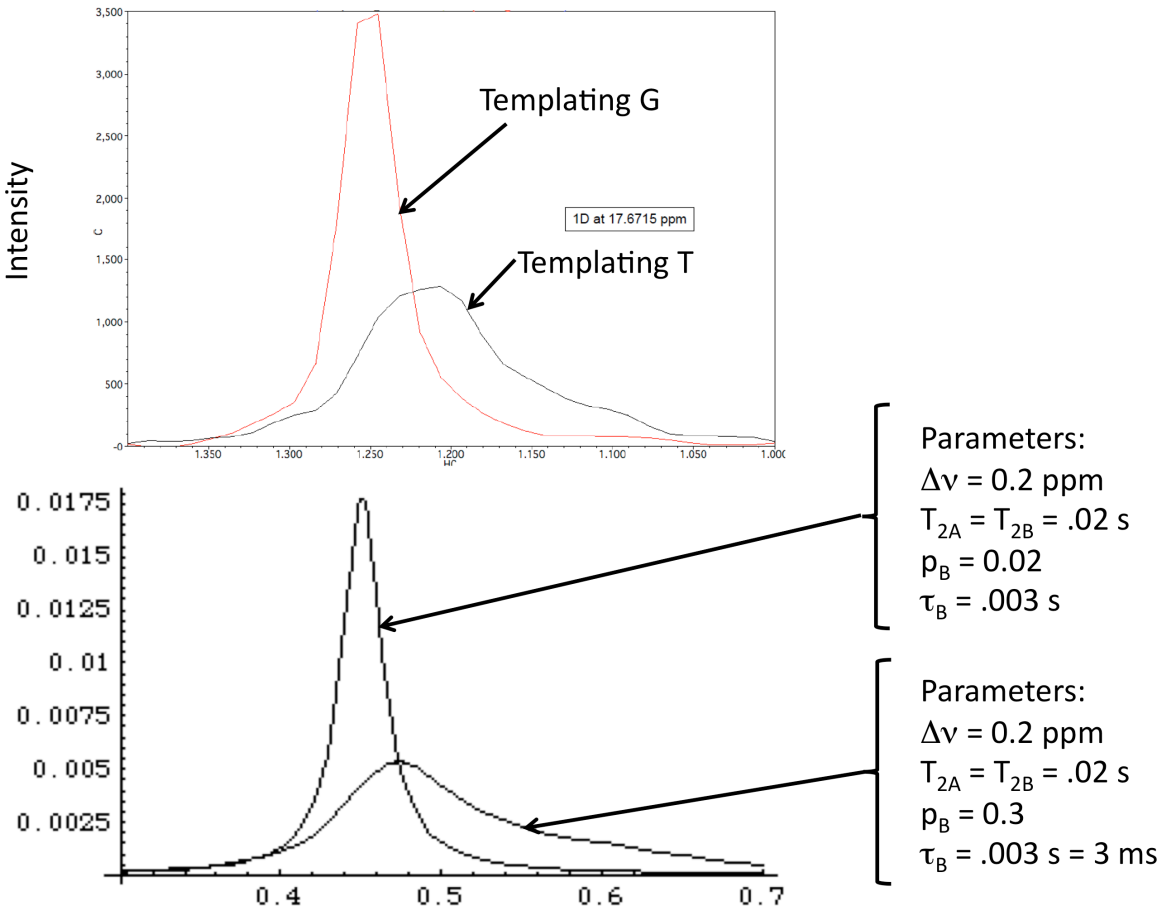
Supplementary Figure S4 shows the overlaid ^{1}H-^{13}C HSQC spectra of [methyl-^{13}C]methionine pol β E295K mutant protein in black, and the same protein in complex with a double hairpin DNA substrate designed with a one-nucleotide gap (templating thymidine) after the addition of the non-hydrolyzable deoxynucleoside triphosphate analog dAPCPP and 5 mM $MgCl_2$ (blue). The spectrum of the uncomplexed protein is essentially identical to that of wild-type pol β, indicating that the single amino acid change does not affect the protein's structure. For the complex, the indicated shifts of the M18 and M236 resonances are characteristic for binding of gapped DNA, but none of the characteristic shifts of an activated complex obtain (15, 16).



Supplementary Figure S4. Lack of conformational activation of pol β E295K mutant. ^{1}H-^{13}C HSQC spectra of labeled E295K pol β (black) and the ternary complex in the presence of $MgCl_2$ (blue). Other conditions were 100 mM potassium phosphate, pH 6.7. The DNA sequence of the double hairpin substrate is indicated in the inset.

Simulation of the ^{1}H Lineshape for Met282.

Supplementary Figure S5 shows a ^{1}H spectrum of the Met282 methyl resonance corresponding to $\delta^{13}C$ = 17.67 ppm from a sample of methionine-labeled pol β C267A bound to a one-nucleotide gapped double hairpin substrate with either a T (black) or a G (red) templating base. The spectra in the lower trace were simulated with a two state chemical exchange model using the relations of Rogers and Woodbrey (42) and a program written with Mathematica. The simulation parameters were: $\Delta\nu$ = 0.2 ppm; $1/T_{2A} = 1/T_{2B}$ = 0.02 s (corresponding to a linewidth of 16 Hz); τ_B = 3 ms. For the narrower line, the fraction in the "B" state, p_B, was taken as 0.02 (2 %), while the broader line corresponds to p_B = 0.3 (30 %). These parameter values are estimates derived from the spectra of activated and non-activated complexes.



Supplementary Figure S5. Simulation of the ^{1}H NMR lineshape for pol β Met282.

Supplementary Table S1

Divalent Metal	k_{cat}	$K_{m,dCTP}$	$k_{cat}/K_{m,dCTP}$	Relative Eff.
	s^{-1}	μM	$(s–\mu M)^{-1}$	
Mg^{2+}	0.10 (0.01)	1.0 (0.2)	0.10 (0.02)	1.0 (0.3)
Zn^{2+}	0.019 (0.001)	0.11 (0.01)	0.17 (0.02)	0.6 (0.1)

Effect of divalent metal on rat pol β-catalyzed dCTP insertion opposite guanine with one-nucleotide gapped DNA. Relative efficiency is $(k_{cat}/K_m)_{Mg(II)}/(k_{cat}/K_m)_{Zn(II)}$. The results represent the mean (SEM) of at least two independent determinations.

Supplementary Table S2

Enzyme	k_{cat}	$K_{m,dCTP}$	$k_{cat}/K_{m,dCTP}$	Relative Eff.
	s^{-1}	μM	$(s–\mu M)^{-1}$	
Wild type	1. 3 (0.3)	0.46 (0.06)	2.8 (0.7)	1.0 (0.4)
C267A	0.8 (0.12)	0.27 (0.05)	3.0 (0.7)	0.9 (0.3)

Steady-state kinetic summary for dCTP insertion opposite guanine with one-nucleotide gapped DNA. Relative efficiency is $(k_{cat}/K_m)_{Wild\ type}/(k_{cat}/K_m)_{Mutant}$. The results represent the mean (S.E.) of at least two independent determinations.

Supplementary References

15. Bose-Basu, B., DeRose, E.F., Kirby, T.W., Mueller, G.A., Beard, W.A., Wilson, S.H. and London, R.E. (2004) Dynamic characterization of a DNA repair enzyme: NMR studies of [*methyl*-^{13}C]methionine-labeled DNA polymerase β. *Biochemistry*, **43**, 8911-8922.
16. Kirby, T.W., DeRose, E.F., Beard, W.A., Wilson, S.H. and London, R.E. (2005) A thymine isostere in the templating position disrupts assembly of the closed DNA polymerase β ternary complex. *Biochemistry*, **44**, 15230-15237.
41. Mueller, G.A., DeRose, E.F., Kirby, T.W. and London, R.E. (2007) NMR assignment of polymerase beta labeled with H-2, C-13, and N-15 in complex with substrate DNA. *Biomol. NMR Assignments*, **1**, 33-35.
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