Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

The Structure of an Archaeal B-Family DNA Polymerase in Complex with a Chemically Modified Nucleotide

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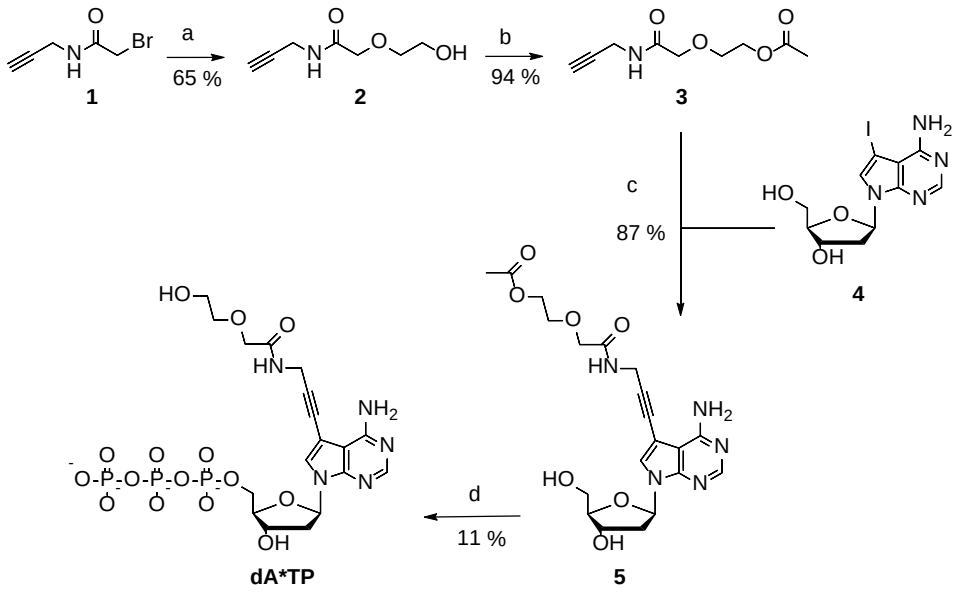
General procedures

Thin-layer chromatography (TLC) was performed on TLC aluminium sheets covered with silica gel 60 F254 (0.2 mm, Merck, Germany). Column flash chromatography (FC) was carried out on silica gel 60 (Merck, Germany) at 0.3 bar. High-resolution ESI-TOF mass spectrometric analysis was performed on a micrOTOF II (Bruker Daltonics, Germany). NMR spectra were recorded at 298 K using Avance 400 (1H =400 MHz, Bruker, Germany). Reverse phase MPLC was performed using a PrepChrom C-700 (Büchi, Germany) instrument equipped with a SVP D40-RP18 25-40 µm 90 g (Götec Labortechnik GmbH, Germany) column with a flow rate of 20 mL/min. Reverse phase HPLC was performed using a SIL-10 AP system (Shimadzu, Japan) instrumented with a VP 250/21 Nucleodur C18 HTec, 5 µm (Macharey-Nagel, Germany) column. Ion exchange chromatography was performed using a PrepChrom C-700 (Büchi, Germany) instrument equipped with a 250 /22 DNAPac® PA-100 (Dionex, USA).

Chemical synthesis



Scheme 1.: Synthesis of dA*TP. **a**: ethylene glycol, Na; **b**: Ac_2O, pyridine; **c**: CuI, Et_3N, $Pd(PPh_3)_4$, DMF; **d**: 1. $POCl_3$, TMP; 2. pyrophosphate, tributylamine; 3. 30 % NH_3 aq. solution.

2-(2-hydroxyethoxy)-N-(prop-2-yn-1-yl)acetamide (2)

Sodium (453 mg, 19.7 mmol) was dissolved under nitrogen atmosphere in 15 mL ethyleneglycol. 2-bromo-N-(2-propyn-1-yl)acetamide (3.3 g, 18.7 mmol) was added and the reaction mixture was stirred at RT for 6 h. Excess of ethylene glycol was distilled of at high vacuum (0.02 mbar, 35-40 °C). The residue was purified by column chromatography (4 % MeOH/DCM, R_f = 0.2) to obtain the desired product as a yellow oil (1.92 g, 65 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.09 (s, 1H, N**H**), 4.10 (dd, 2H, J = 2.5 Hz, 5.5 Hz, HC≡C-C**H$_2$**), 4.04 (s, 2H, O=C**H$_2$**-O), 3.81 (m, 2H, O-C**H$_2$**-CH$_2$-O-C), 3.67 (m, 2H, O-CH$_2$-C**H$_2$**-O-C), 2.23 (t, 1H, J = 2.5 Hz, **H**C≡C-CH$_2$).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 169.66 (NH-**C**=O), 79.43 (HC≡**C**-CH$_2$), 73.17 (O-**C**H$_2$-CH$_2$-O-C), 71.71 (H**C**≡C-CH$_2$), 70.52 (O=**C**H$_2$-O), 61.75 (O-CH$_2$-**C**H$_2$-O-C), 28.69 (HC≡C-**C**H$_2$).

HRMS: m/z calculated for [$C_7H_{12}NO_3^+$]: 158.0812; found: 158.0817

2-(2-oxo-2-(prop-2-yn-1-ylamino)ethoxy)ethyl acetate (3)

2-(2-hydroxyethoxy)-N-(prop-2-yn-1-yl)acetamide (7.54 g, 48 mmol) was dissolved in 75 mL pyridine. Acetic anhydride (13.6 mL, 144 mmol) was added dropwise and the reaction was stirred for 1.5 h at RT. The solvent was evaporated and the residue was dissolved in DCM. The organic layer was washed with 1 M HCl (5x 50 mL) and the aqueous layer was back extracted with DCM. The combined organic layers were once washed with sat. $NaHCO_3$ and water, dried over $MgSO_4$ and the solvent was evaporated to obtain 2-(2-oxo-2-(prop-2-yn-1-ylamino)ethoxy)ethyl acetate as an orange oil (9.01 g, 45.2 mmol, 94 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.81 (bs, 1H, NH), 4.29 – 4.16 (m, 2H, O-CH_2-C**H_2**-O-C), 4.08 (dd, 2H, J = 5.5, 2.5 Hz, HC≡C-C**H_2**), 4.00 (s, 2H, O=C**H_2**-O), 3.80 – 3.63 (m, 2H, O-C**H_2**-CH_2-O-C), 2.23 (t, 1H, J = 2.6 Hz, **H**C≡C-CH_2), 2.09 (s, 3H, C**H_3**).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.89 (O**=C**-CH_3), 169.16 (NH-**C**=O), 79.31 (HC≡**C**-CH_2), 71.77 (H**C**≡C-CH_2), 70.47 (O=**C**H_2-O), 69.79 (O-**C**H_2-CH_2-O-C), 63.30 (O-CH_2-**C**H_2-O-C), 28.62 (HC≡C-**C**H_2), 20.99 (**C**H_3).

HRMS: m/z calculated for [$C_9H_{14}NO_4^+$]: 200.0917; found: 200.0928

7-deaza-7-(2-(2-oxo-2-(prop-2-yn-1-ylamino)ethoxy)ethyl acetate)-2'-deoxy-adeonsine (5)

Compound 5 (100 mg, 0.27 mmol), CuI (10 mg, 53 µmol), $Pd(PPh_3)_4$ (31.2 mg, 27 µmol) were dried at high vacuum for 30 min. 2-(2-oxo-2-(prop-2-yn-1-ylamino)ethoxy)ethyl acetate (106 mg, 0.53 mmol) was dissolved in dry, degassed DMF (4 mL) and was added to the solid components. Et_3N (112 µL, 0.81 mmol) was added and the reaction mixture was stirred under nitrogen at RT overnight. The solvent was evaporated and the residue was applied to column chromatography (0 – 10 % MeOH in DCM, R_f (10%) = 0.3). The solvent was again evaporated and the residue was dissolved in 10 mL 5 % MecN in H_2O and applied to reverse phase column chromatography (solvent A = H_2O, solvent B = MeCN, gradient: 0-5 min 0 % B, 5-10 min 5 % B, 10-40 min, 5-40 % B, 40-45 min 40-100 % B, 45-50 min 100% B, 50-55 min 100-5 % B, R_t = 32 min (31 % B)). After evaporation of the solvent, the product was obtained as a colorless solid (104 mg, 87 %).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.08 (s, 1H, H-2), 7.56 (s, 1H, H-8), 6.46 (dd, *J* = 7.9, 6.1 Hz, 1H, H1′), 4.51 (dt, *J* = 5.7, 2.7 Hz, 1H, H3′), 4.29 – 4.21 (m, 4H, O-CH_2-C**H_2**-O-C, HC≡C-C**H_2**), 4.06 (s, 2H, O=C**H_2**-O) 4.00 (q, *J* = 3.3 Hz, 1H, H4′), 3.83 – 3.74 (m, 3H, H5′, O-C**H_2**-CH_2-O-C), 3.73 (dd, *J* = 3.7 Hz, 1H, H5′), 2.61 (ddd, *J* = 13.8, 8.1, 5.9 Hz, 1H, H2′), 2.32 (ddd, *J* = 13.4, 6.0, 2.8 Hz, 1H, H2′), 2.05 (s, 3H, CH_3).

^{13}C NMR (101 MHz, Methanol-d_4) δ 172.75 (O=**C**-CH_3), 172.67 (NH-**C**=O), 159.14 (C6), 153.25 (C2), 149.95 (C4), 127.99 (C8), 104.74 (C5), 96.84 (C7), 89.20 (**C**≡C-CH_2), 89.15 (C4′), 86.63 (C1′), 76.14 (C≡**C**-CH_2), 72.96 (C3′), 71.28(O=**C**H_2-O), 70.73 (O-**C**H_2-CH_2-O-C), 64.56 (O-CH_2-**C**H_2-O-C), 63.61 (C5′), 41.56 (C2′) , 30.24 (C≡C-C**H_2**), 20.78 (CH_3).

HRMS: m/z calculated for [$C_{20}H_{24}N_5O_7^-$]: 446.1681; found: 446.1689

Extinction coefficients were determined by absorbance using the Lambert-Beer-Law and a solution of known concentration: ε_{260nm} = 7200 M^{-1} cm^{-1}, ε_{280nm} = 11500 M^{-1} cm^{-1}

7-deaza-7-(2-(2-hydroxyethoxy)-N-(prop-2-yn-1-yl)acetamide)-2'-deoxy-adeonsine triphosphate (dA*TP)

Compound 6 (50 mg, 0.112 mmol) was dried at high vaccum for 1 h and dissolved in trimethylphosphate (1mL). Phosphoryl chloride (12.2 µL, 0.134 mmol) was added under ice bath cooling and the reaction mixture was stirred at 0 °C for 1.5 h. $(Bu_3N)_2H_2P_2O_7$ (307 mg, 0.56 mmol) and freshly distilled tributyl amine (111 µL, 0.47 mmol) were dissolved in 1 mL dry DMF and added to the reaction mixture at 0°C. The reaction mixture was allowed to warm to RT and was stirred for 30 min, followed by the addition of 2 mL 0.1 M TEAB and again stirring at RT for 30 min. The reaction mixture was diluted with 10 mL H_2O and was extracted three times with 10 mL ethyl acetate. The aqueous layer was concentrated and purified by HPLC (solvent A = 50 mM TEAB buffer, solvent B = MeCN, Rt = 24 min, 21 % B, gradient: 0-10 min 5 % B, 10-40 min 5-40 % B, 40-45 min 40-100 % B, 45-50 min 100 % B, 50-55 min 100-5 % B). The fractions containing product were pooled and the solvent was evaporated. The residue was dissolved in 3 mL 30 % NH_3/H_20 and stirred at RT for 2 h. The solvent was evaporated, the residue was dissolved in H_2O and purified by HPLC (5.2 min, 5 % B), ion exchange MPLC (IEX) (solvent A = 25 mM Tris pH 8.0, 5 % MeCN, solvent B = 25 mM Tris pH 8.0, 5 % MeCN, 0.5 M $NaClO_4$, Rt = 19.2 min, 17 % B, gradient: 0-5 min 0 % B, 5-35 min 0-40 % B, 35-40 min 40-100 % B, 40-45 min 100 % B, 45-

50 min 100-0% B) and again HPLC (Rt = 21.3 min, 18 % B). The product was obtained in 11 % yield (12 µmol).

^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.21 (s, 1H, H2), 7.73 (s, 1H, H8), 6.58 (t, *J* = 6.7 Hz, 1H, H1′), 4.75 (s, 1H , H3′), 4.35 – 4.16 (m, 7H, H4′, H5′,C≡C-C**H$_2$**, O=C**H$_2$**-O), 3.81 (m, 2H, O-CH$_2$-**C**H$_2$-O-C), 3.74 (m, 2H, O-**C**H$_2$-CH$_2$-O-C), 2.71 – 2.47 (m, 2H, H2').

^{31}P NMR (162 MHz, Deuterium Oxide) δ -10.54 (Pγ) , -11.20 (Pβ) , -22.98 (Pα).

HRMS: m/z calculated for [$C_{18}H_{25}N_5O_{15}P_3^-$]: 644.0565; found: 644.0588

Crystallization

2′,3′-dideoxy-cytidine 5′-triphosphate (ddCTP) and 2′-deoxy-adenosine-5′-triphosphate (dATP) were purchased from Jena Bioscience.
Unmodified primers were purchased HPLC purified from Biomers.

Protein Expression

KlenTaq DNA pol was expressed and purified as described by Betz et. al. [1].

KOD DNA pol was expressed and purified as described by Kropp et. al. [2].

KOD and KlenTaq DNA pol were crystallized using sitting drop vapor diffusion as described by Kropp et. al. [1-2]. In brief for KOD DNA pol:

2.18 µL primer (5′-d(GAC CAC GGC CAC)-3′, 6mM) and 2.18 µL template (5′-d(AAC T GTG GCC GTG GTC)-3′, 6mM) were annealed at 95 °C and stepwise cooled to 4 °C, followed by the addition of 3.27 µL ddCTP (10 mM), 1.06 µL $MgCl_2$ (1 M), 1.06 µL $MnCl_2$ (1 M) and 85 µL 7.8 mg/mL KOD DNA pol. The sample was incubated at 55 °C for 45 min, followed by the addition of 10.89 µL dA*TP (10 mM), resulting in a final protein concentration of 6.3 mg/mL. Again incubation at 30 °C for 45 min and cooling to 16 °C. The protein was mixed with the reservoir solution in ratios 1:2, 1:1 and 2:1 giving a final drop size of 0.6 µL.

Crystallization trials

Diffracting crystals of KOD-dA*TP grew in the C12 condition of the Morpheus MD1-46 screen (Molecular Dimensions) (12.5 % PEG 1000, 12.5 % PEG 3350, 12.5 % MPD, 0.1 M Tris, 0.1 M

BICINE pH 8.5, 0.09 M sodium nitrate, 0.09 M sodium phosphate dibasic, 0.09 M ammonium sulfate)

Diffracting crystals of KlenTaq-dA*TP were found in a condition containing 14 % PEG 8000, 0.1 M Tris pH 8.0, 10 % glycerol, 0.2 M magnesium formate.

Diffracting crystals of KlenTaq-dATP were found in a condition containing 27 % PEG 4000, 0.2 M ammonium acetate, 0.1 M magnesium acetate, 0.1 M MES pH 6.5.

Prior to freezing all crystals were cryoprotected using 20 % ethylene glycol in the reservoir solution.

Structure Determination

For data collection crystals were kept at 100 K. Data were collected at the Swiss Light Source (SLS) of the Paul Scherrer Institute (PSI) in Villigen, Switzerland, at the beamline PXI (X06SA). Data reduction was performed with the XDS package [1, 3]. The structures were solved using difference Fourier techniques using the KOD (PDB: 5OMF) and KlenTaq DNA pol (PDB: 3RTV) wildtype structures. Refinement was performed with PHENIX [4], model building was performed in COOT [5] and figures were generated using PyMOL [6].

The structure are uploaded to the PDB data base.

Primer extension experiments

The primers used for the single nucleotide incorporation assay, were radioactively labeled as described before [7]. Primer extensions were carried out on a PCR-Thermocycler system (BIOMETRA, Germany). PAGE gels were visualized by phosphorimaging using the Molecular Imager FX (Biorad, UK). Quantification of the bands was done using the Image Lab software (Biorad, UK).

DNA sequences

3′-C-primer: 5′-d(GAC CAC GGC CAC)-3′
template: 5′-d(AAC T GTG GCC GTG GTC)-3′

3′-T-primer: 5′-d(GAC CAC GGC CAT)-3′

template: 5′-d(AAC T ATG GCC GTG GTC)-3′

Competitive single nucleotide incorporation assay

The reactions were performed in an analogue way as published before [7-8].

A typical reaction (6µL) contained 1 x reaction buffer (50 mM Tris-HCl pH 8.0, 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1 % (v/v) Tween20), 100 nM primer, 150 nM template, 0.1 nM KlenTaq or KOD DNA pol and 10 µM dA*TP or dATP. The primer and template annealed in 1x reaction buffer, followed by the addition of DNA pol. The reaction mixture was incubated at 55 °C and the reaction was started by the addition of 3 µL dNTP. The reaction was quenched after 5 min for the 3′-C-primer and 1 min for the 3′-T-primer by the addition of 12 µL PAGE gel loading buffer (80 % (v/v) formamide, 20 mM EDTA, 0.25 % (w/v)). The reaction mixture was analyzed using a 16 % denaturing polyacrylamide gel and subjected to autoradiography. The incorporation in % of dA*TP and dATP was plotted against the employed concentrations using Origin 2015. All reactions were done in triplicates.

dA*TP/ dATP: 1/0, 10/1, 6/1, 4/1, 2/1, 1/1, 1/2, 1/4, 1/6, 1/10, 0/1.

SI Tables

Table SI1.: Data processing and refinement statistics. * Numbers in brackets refer to highest resolution shell. p: primer, t: template, dNTP: dA*TP or dATP

	KOD-dA*TP	**KlenTaq-dA*TP**	**KlenTaq-dATP**
PDB ID	**6Q4T**	**6Q4U**	**6Q4V**
Wavelength (Å)	1.0	1.0	1.0
Space group	$P2_12_12$	$P3_121$	$P3_121$
Cell dimensions			
a, b, c (Å)	107.94, 146.42, 71.48	109.36, 109.36, 90.89	109.48, 109.48, 91.25
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 120.00	90.00, 90.00, 120.00
Resolution (Å)*	48.808 – 1.997 (2.007 – 1.997)	47.354 – 2.005 (2.016 – 2.005)	47.407 – 2.006 (2.016 – 2.006)
Total no. of reflections	468430 (40014)	428101 (70423)	424793 (66787)
No. of unique reflections	138630 (16466)	81414 (13148)	81683 (12970)
R_{meas} (%)	18.9 (254.2)	9.0 (84.9)	7.6 (81.6)
I / σ	5.60 (0.35)	11.21 (1.66)	11.59 (1.53)
Completeness (%)	93.3 (68.5)	99.9 (99.8)	99.6 (97.8)
Redundancy	3.4 (2.4)	5.2 (5.4)	5.2 (5.1)
$CC_{1/2}$ (%)	99.4 (19.5)	99.8 (77.4)	99.9 (80.7)
ISa [9]	24.20	20.93	18.03
Refinement			
Resolution (Å)	46.219 – 1.997	47.354 – 2.005	47.407 – 2.006
No. of reflections	138318	81381	81638
R_{work}/ R_{free}	20.23 / 23.11	18.49 / 21.97	18.57 / 21.86
Coordinate error	0.37	0.28	0.27
No. of atoms			
Protein	12555	8747	8608
DNA (p/t/dNTP)	373 / 511 / 67	373 / 508 / 67	373 / 509 / 42
Water	277	266	191
Average B-factors (Å)			
Protein	52.52	59.43	66.15
DNA (p/t/dNTP)	53.94 / 57.26 / 41.64	47.55 / 51.74 / 48.48	52.74 / 56.92 / 46.95
Water	46.94	46.07	46.81
R.m.s. deviations			
Bond lengths (Å)	0.004	0.003	0.003
Bond angles (°)	0.701	0.615	0.623
Ramachandran (%)			
Favored/ Allowed/ Outlier	97.48 / 2.52 / 0.00	96.65 / 3.17 / 0.19	96.83 / 2.99 / 0.19

Table S2.: P-values of sugar moieties in primer of KOD-dA*TP and KlenTaq-dA*TP, determined using the 3DNA server [10]. The P-value was not restraint during refinement in phenix.

primer strand of KOD-dA*TP

base	v0	v1	v2	v3	v4	tm	P	Puckering	DNA form
C	-3.5	-13.0	23.1	-25.8	19.0	25.8	26.5	C3′-endo	A
A	-13.7	25.6	-27.2	20.0	-4.2	27.7	169.8	C2′-endo	B
C	-40.2	28.7	-7.5	-15.2	34.9	39.4	101.0	O4′-endo	B
C	-38.0	38.6	-24.8	3.7	21.4	39.4	129.0	C1′-exo	B
G	-32.1	25.0	-9.4	-8.7	25.7	31.2	107.6	O4′-endo	B
G	-32.5	37.8	-28.8	10.8	13.5	37.3	140.5	C1′-exo	B
C	-36.1	35.3	-22.0	1.9	21.4	36.9	126.7	C1′-exo	B
A	-15.1	33.5	-38.4	30.5	-10.0	38.5	176.1	C2′-endo	B
C	-26.7	29.6	-21.5	6.6	12.5	29.5	136.7	C1′-exo	B
C	-38.8	25.9	-4.8	-17.2	35.3	38.4	97.2	O4′-endo	B
A	-16.7	29.4	-30.3	21.3	-3.1	31.1	166.9	C2′-endo	B
G	3.0	-13.0	17.5	-16.2	8.4	17.7	9.0	C3′-endo	A

primer strand of KlenTaq-dA*TP

base	v0	v1	v2	v3	v4	tm	P	Puckering	DNA form
C	-5.8	-9.9	20.2	-24.4	19.5	24.0	32.6	C3′-endo	A
A	-6.5	17.4	33.1	-37.6	28.0	37.6	28.2	C3′-endo	A
C	0.7	-24.6	37.7	-38.1	23.6	39.5	17.4	C3′-endo	A
C	-32.6	44.1	-37.8	20.0	7.7	43.2	151.0	C2′-endo	B
G	-43.3	35.2	-14.6	-9.7	33.1	42.0	110.3	C1′-exo	B
G	-31.7	36.2	-27.0	9.5	13.7	35.7	139.1	C1′-exo	B
C	-38.1	26.4	-6.2	-15.4	33.9	37.5	99.5	O4′-endo	B
A	-14.4	28.4	-31.1	23.4	-5.9	31.4	172.0	C2′-endo	B
C	-28.2	11.5	8.1	-24.5	33.4	32.7	75.6	O4′-endo	A
C	-10.3	-0.8	10.7	-16.9	17.3	17.8	53.0	C4′-exo	A
A	-24.8	18.5	-6.0	-8.0	20.6	24.1	104.4	O4′-endo	B
G	-15.2	-3.7	19.8	-29.0	28.1	29.8	48.5	C4′-exo	A

v0: C4′-O4′-C1′-C2′, v1: O4′-C1′-C2′-C3′, v2: C1′-C2′-C3′-C4′, v3: C2′-C3′-C4′-O4′, v4: C3′-C4′-O4′-C1′, tm: the amplitude of pucker, P: the phase angle of pseudorotation.

SI Figures

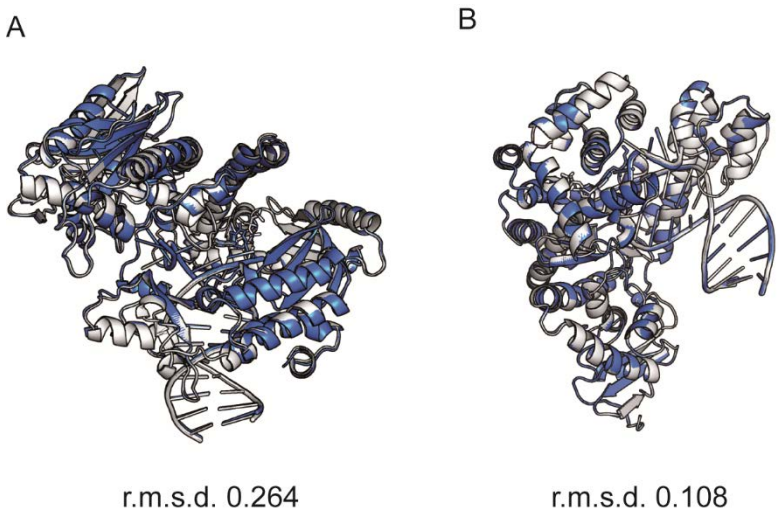


Fig. S1: Superimpositions of KOD-dA*TP (blue) and KOD-dATP (white) (A) as well as KlenTaq-dA*TP (blue) and KlenTaq-dATP (white) (B).

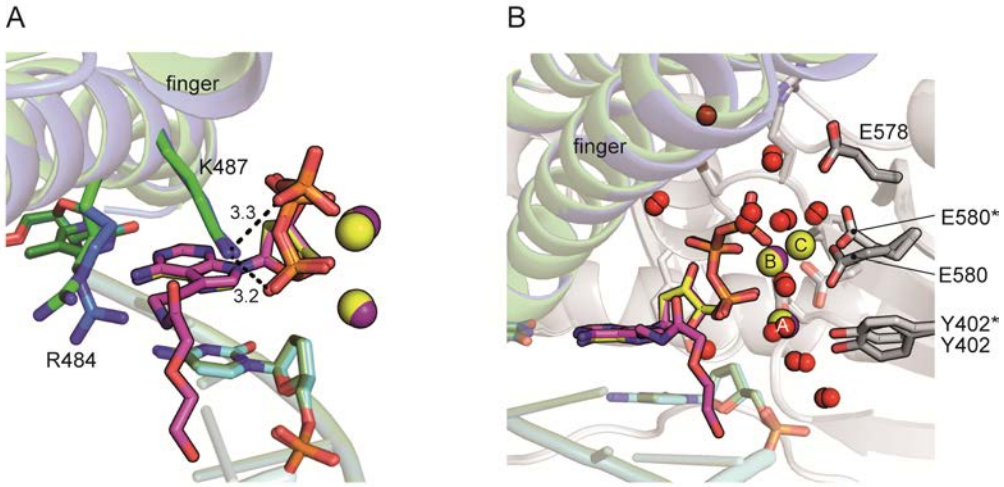


Fig. S2: Superimposition of the active site of KOD-dA*TP and KOD-dATP. For KOD-dA*TP the finger domain is shown in blue, dA*TP in purple, metal ions in purple. For KOD-dATP the finger domain is shown in green, dATP in yellow, metal ions in yellow. (A) K487 in KOD-dATP form hydrogen bonds to the α- (3.2 Å) and β- (3.3 Å) phosphate. (B) The conformations of E580 and Y402 in KOD.dA*TP are indicated by an asterisk. E580 adopts an “open” conformation in KOD-dA*TP opening space for Y402 to move in the direction of E580.

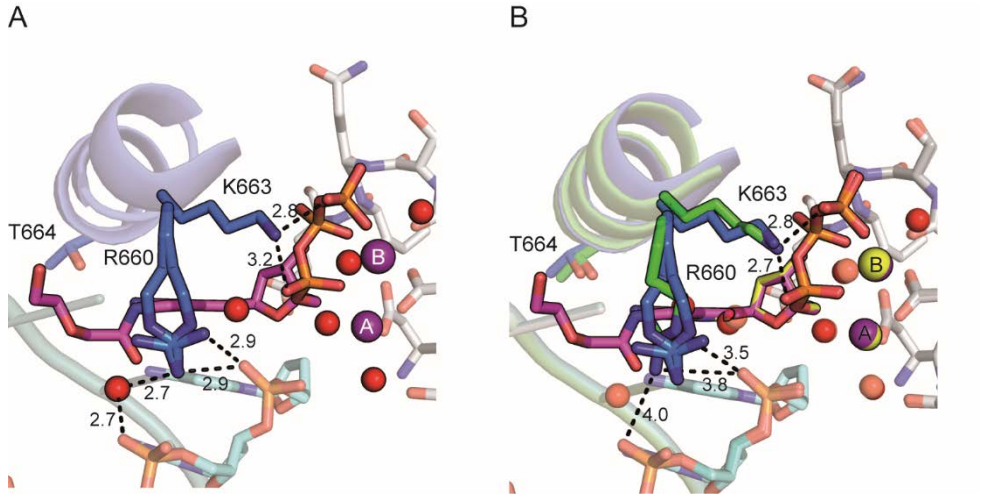


Fig. S3: Zoom into the active site of KlenTaq-dA*TP. (A) K663 forms hydrogen bonds to the α- (2.8 Å) and β-phosphate (2.7 Å) of dA*TP. (B) superimposition of KlenTaq-dA*TP and KlenTaq-dATP. dATP and the corresponding metal ions are shown in yellow, the O-helix of KlenTaq-dATP and its residues are shown in green.

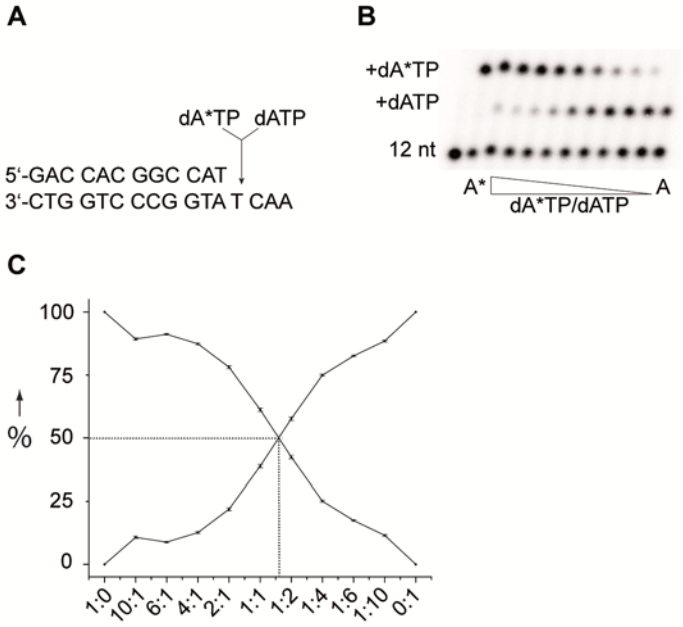


Fig. S4: Competitive primer extension experiments employing the 3′-dTMP primer. (A) Sequence of the primer/template complex. (B) PAGE analysis of the competition experiment using KlenTaq DNA pol. (C) Graphical readout of the PAGE analysis. The point of 50 % incorporation is indicated with a dashed line.

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