Supplementary information

Amino acid templating mechanisms in selection of nucleotides opposite abasic sites by a family A DNA polymerase

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CONTENT

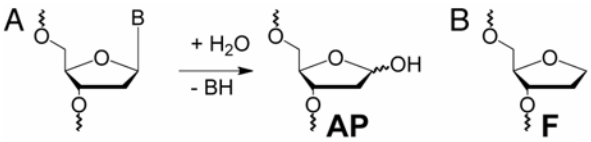

FIGURE S1. Formation of an abasic site. A. Hydrolysis of the glycosidic bond leading to nucleobase loss and formation of abasic site AP. B: nucleobase. B Structure of the abasic site analogue F.

TABLE S1. Data collection and refinement statistics

	KlenTaq$_{F\text{-}G\text{-}I}$	*KlenTaq*$_{F\text{-}G\text{-}II}$	*KlenTaq*$_{F\text{-}T}$	*KlenTaq*$_{F\text{-}binary\text{-}II}$	*KlenTaq*$_{F\text{-}binary}$	*KlenTaq*$_{F\text{-}NI}$
Data collection						
Space group	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$
Cell dim.						
a, *b*, *c* (Å)	109.0, 109.0, 90.4	110.3, 110.3, 91.0	108.3, 108.3, 90.2	108.3, 108.3, 89.7	109.5, 109.5, 90.4	109.8, 109.8, 91.2
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	47.2-2.38 (2.53-2.38)	47.8-2.30 (2.44-2.30)	46.9-1.79 (1.90-1.79)	46.9-1.79 (1.90-1.79)	47.4-1.94 (2.06-1.94)	47.5-1.90 (2.01-1.90)
R_{meas} (%)	14.4 (106.6)	14.6 (138.5)	9.9 (120.0)	13.8 (121.7)	10.9 (94.2)	9.7 (133.2)
I / σ_I	12.6 (1.61)	13.7 (1.52)	15.2 (2.12)	9.43 (1.35)	10.29 (1.27)	15.04 (1.58)
Completeness (%)	99.5 (97.2)	99.8 (98.6)	99.5 (96.9)	99.7 (98.1)	98.9 (93.6)	99.6 (98.3)
Redundancy	8.54	9.95	9.95	9.88	5.11	10.10
Refinement						
Resolution (Å)	47.2-2.40 (2.49-2.40)	47.8-2.30 (2.34-2.30)	46.9-1.80 (1.82-1.80)	46.4-1.80 (1.82-1.80)	47.4-1.95 (1.97-1.95)	42.2-1.90 (1.94-1.90)
No. reflections	24603	54951	109630	109029	88190	50201
R_{work} / R_{free}	20.07 (26.24) / 25.59 (32.79)	19.76 (31.32) / 24.98 (32.11)	16.64 (26.10) / 20.06 (29.27)	17.66 (30.25) / 21.16 (34.74)	19.32 (31.52) / 23.18 (34.83)	17.01 (27.58) / 20.65 (32.64)
No. residues / B-factors (Å^2)						
Protein	534 / 38.3	535 / 49.1	538 / 24.5	527 / 40.8	520 / 43.6	533 / 39.6
Primer,Template	12,14 / 25.6,35.7	12,13 / 35.3,33.9	12,14 / 21.9,24.6	12,15 /36.7,46.6	12,15 / 34.9,40.1	12,13 / 30.1,29.1
Triphosphate	ddGTP / 107.8	ddGTP / 108.3	ddTTP / 30.3	--	--	dNITP / 48.1
Ion (active site)	--	--	1 Mg^{2+} / 39.5	--	--	2 Mg^{2+} / 45.7
Water	101 / 37.2	140 / 37.6	362/ 31.3	428 / 41.0	350 / 39.9	309 / 42.3
R.m.s. deviations						
Bond lengths (Å)	0.002	0.003	0.006	0.005	0.002	0.014
Bond angles (°)	0.681	0.828	1.003	0.968	0.739	1.194
Ramachandran statistics*						
Most favored	89.4	91.2	92.8	90.9	91.6	91.9
Additionally allowed	9.7	8.2	6.6	8.5	8.0	7.3
Generously allowed	0.4	0.2	0.2	0.4	0.0	0.4
Disallowed	0.4	0.4	0.4	0.2	0.4	0.4
PDB ID	3RR8	3RRG	3RRH	--	3RR7	3T3F

Values in parentheses are for highest-resolution shell.

*PROCKECK V 3.4.4: Laskowski RA, MacArthur MW, Moss DS and Thornton JM (1993) PROCHECK: a program to check the stereochemical quality of protein structures. *J. Appl. Cryst*. 26:283-291.

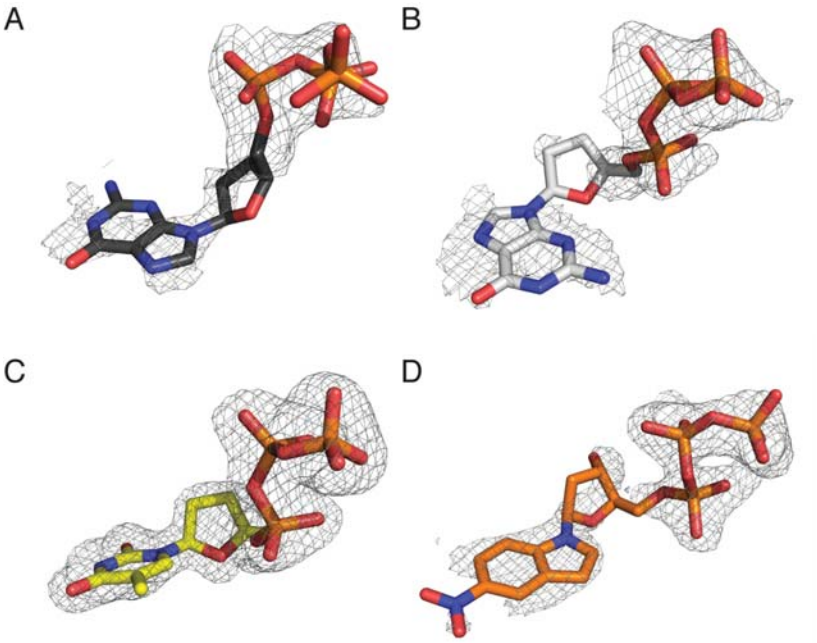

FIGURE S2. Incoming ddNTPs with the respective simulated annealing omit map mFo-DFc at 3σ. A. incoming ddGTP from *KlenTaq*$_{F\text{-}G\text{-}I}$. B. incoming ddGTP from *KlenTaq*$_{F\text{-}G\text{-}II}$. C. incoming ddTTP from *KlenTaq*$_{F\text{-}T}$. D. incoming dNITP from *KlenTaq*$_{F\text{-}NI}$.

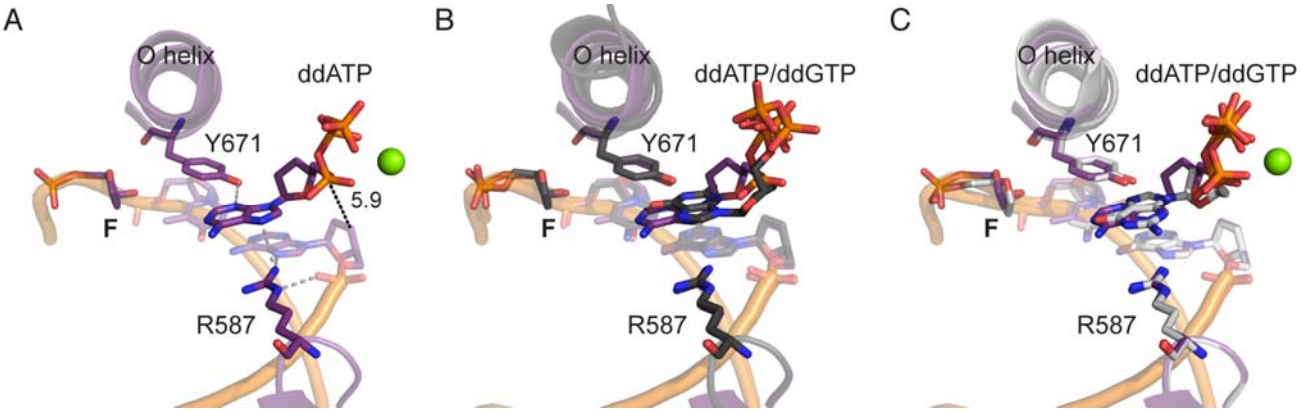

FIGURE S3. Structure of *KlenTaq*$_{F\text{-}A}$ in comparison with *KlenTaq*$_{F\text{-}G\text{-}I}$ and *KlenTaq*$_{F\text{-}G\text{-}II}$. A. Stabilization network of ddATP in *KlenTaq*$_{F\text{-}A}$ (purple). Labelled are the amino acid side chain R587 and Y671. Gray and black dashed lines indicate hydrogen-bonding interactions and distance (Å), respectively. B. The overlay of *KlenTaq*$_{F\text{-}A}$ (purple) and *KlenTaq*$_{F\text{-}G\text{-}I}$ (black). C. The overlay of *KlenTaq*$_{F\text{-}A}$ (purple) and *KlenTaq*$_{F\text{-}G\text{-}II}$ (gray).

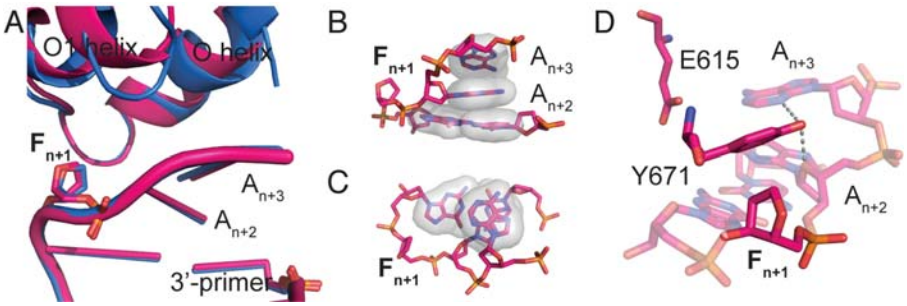

FIGURE S4. Structure of *KlenTaq*$_{\text{F-binary-II}}$. A. Template stacking assembly of *KlenTaq*$_{\text{F-binary-II}}$ (pink) superimposed with *KlenTaq*$_{\text{F-binary}}$ (blue). The abasic site analog F is located extrahelically. B. The stick and surface depiction highlights the template stacking arrangement. C. Top view of the primer template stacking arrangement. D. Hydrogen bonding network of the amino acid side chain Y671 with the template strand.

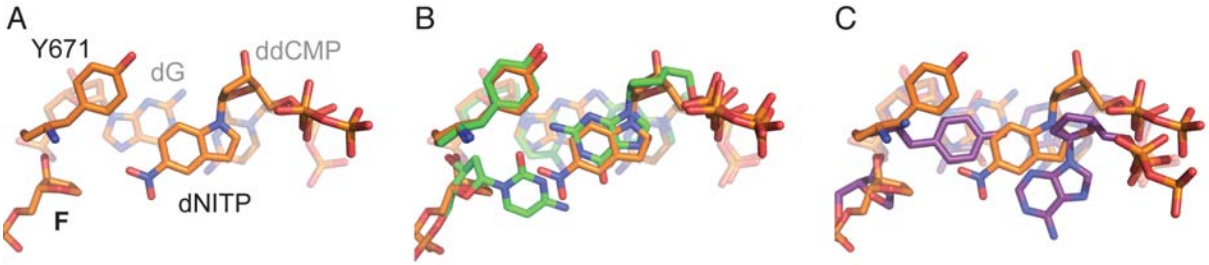

FIGURE S5. Stacking arrangement of the incoming dNTP in *KlenTaq*$_{F\text{-}NI}$. A. Top view to the incoming dNITPs of *KlenTaq*$_{F\text{-}NI}$ (orange). In the front the incoming dNITP opposite the abasic site analog F and the released Y671. B. Superimposition of the nascent base pair from *KlenTaq*$_{F\text{-}NI}$ (orange) and *KlenTaq*$_{C\text{-}G}$ (green). C. Same as B. for *KlenTaq*$_{F\text{-}NI}$ (orange) and *KlenTaq*$_{F\text{-}A}$ (purple),

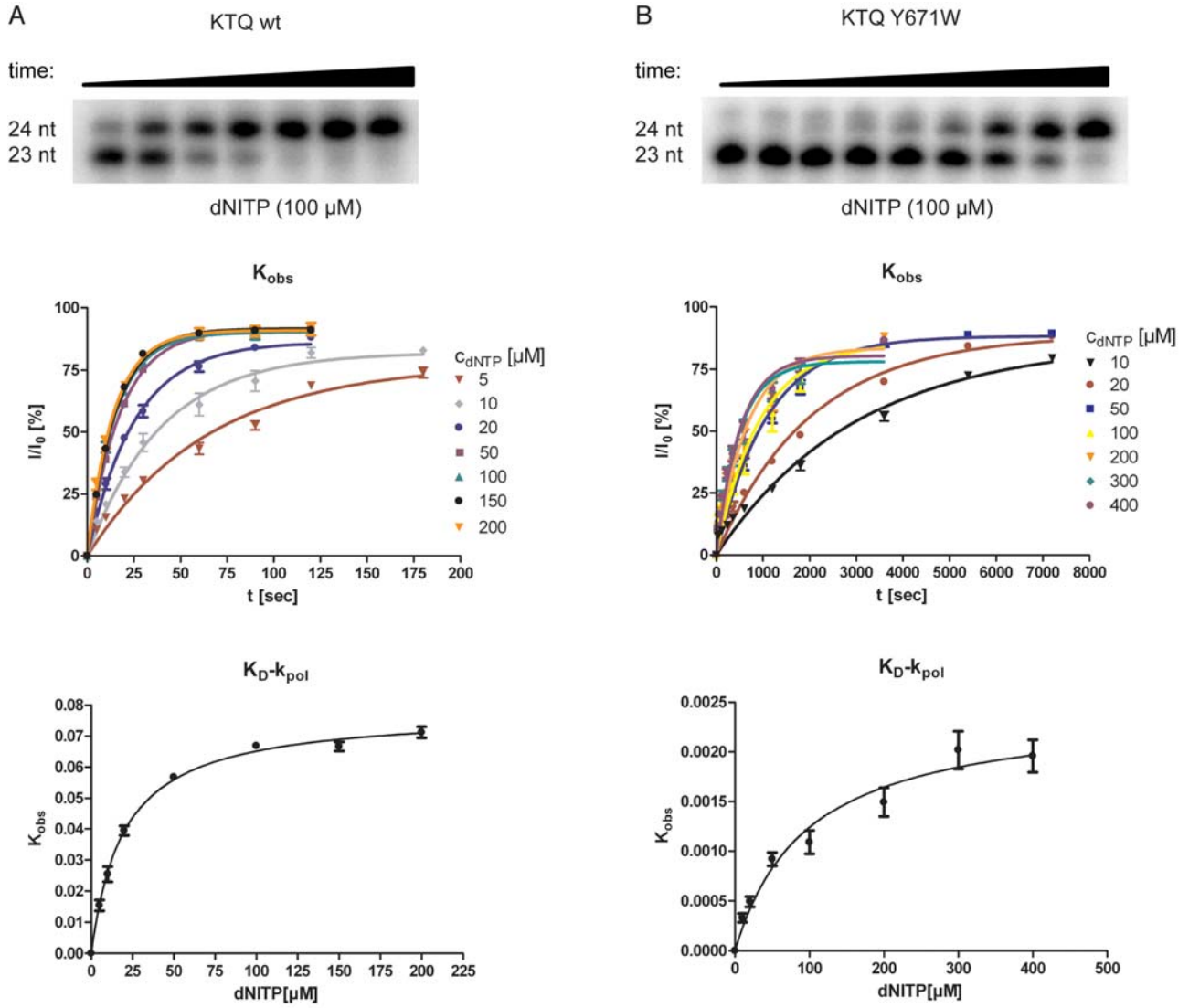

FIGURE S6. Pre-steady state kinetics of dNITP. A. Pre-steady-state kinetics of *KlenTaq*. A representative PAGE analysis of the single nucleotide incorporation of dNITP (100 µM) opposite abasic site analog F for 5, 10, 20, 30, 60, 90, or 120 sec is shown. Below the corresponding K_{obs} graph show the single nucleotide incorporation in dependence of time and dNITP concentration. The single turnover is plotted against the time for various dNITP concentrations in µM (color code on the right side). Beneath the K_D-k_{pol} curve show dependence of the observed pre-steady-state rates (k_{obs}) on dNITP concentration and fitted to a hyperbolic equation. B. The same as A. using *KlenTaq*Y671W mutant instead of *KlenTaq*. A representative PAGE analysis of the single nucleotide incorporation of dNITP (100 µM) opposite abasic site analog F for 0.5, 1, 2, 4, 6, 10, 20, 30 or 60 min is shown.

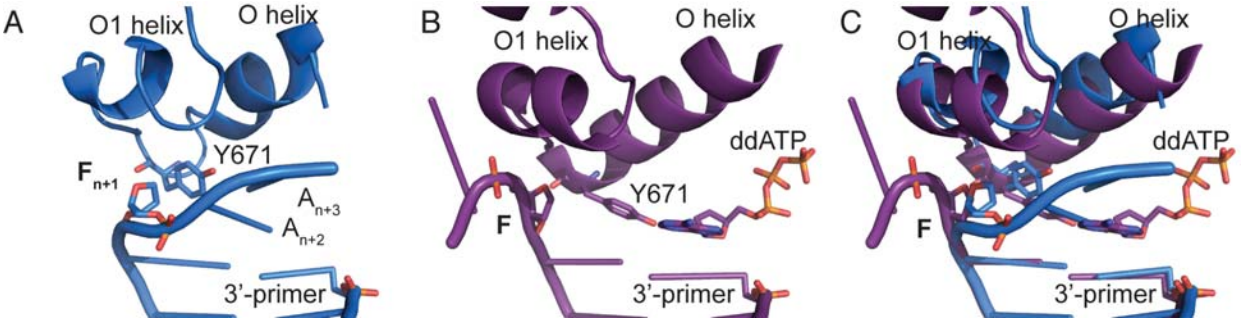

FIGURE S7. Transfer from the binary (*KlenTaq*$_{F\text{-}binary}$; blue) to the ternary (*KlenTaq*$_{F\text{-}A}$; purple) structure. A. Template stacking assembly of *KlenTaq*$_{F\text{-}binary}$. The abasic site analog F is located extrahelically. B. The active site arrangement of the ternary structure of *KlenTaq*$_{F\text{-}A}$. Highlighted are the incoming ddATP and the amino acid side chain Y671. The abasic site analog F is located intrahelically. C. Superimposition of the active site assembly of *KlenTaq*$_{F\text{-}binary}$ and *KlenTaq*$_{F\text{-}A}$.