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Supporting Information

Learning from Directed Evolution: *Thermus aquaticus* DNA Polymerase Mutants with Translesion Synthesis Activity

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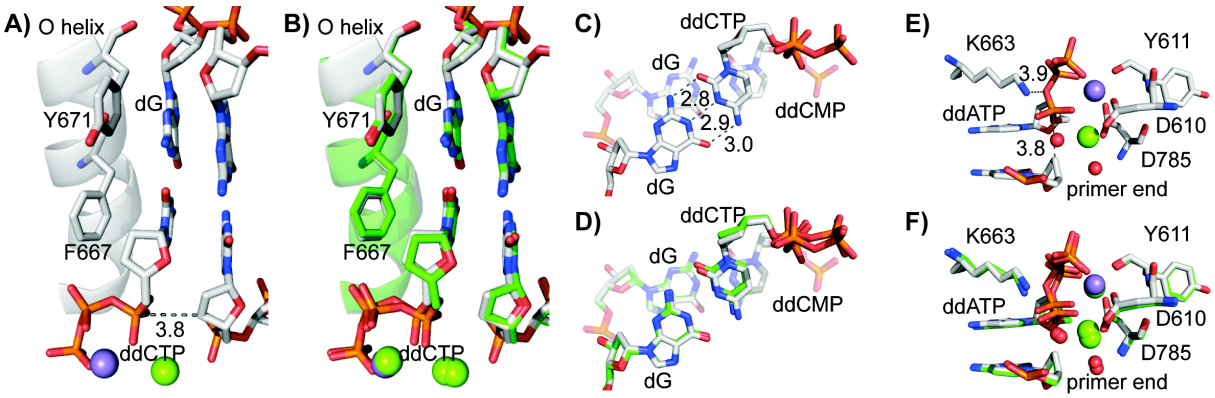
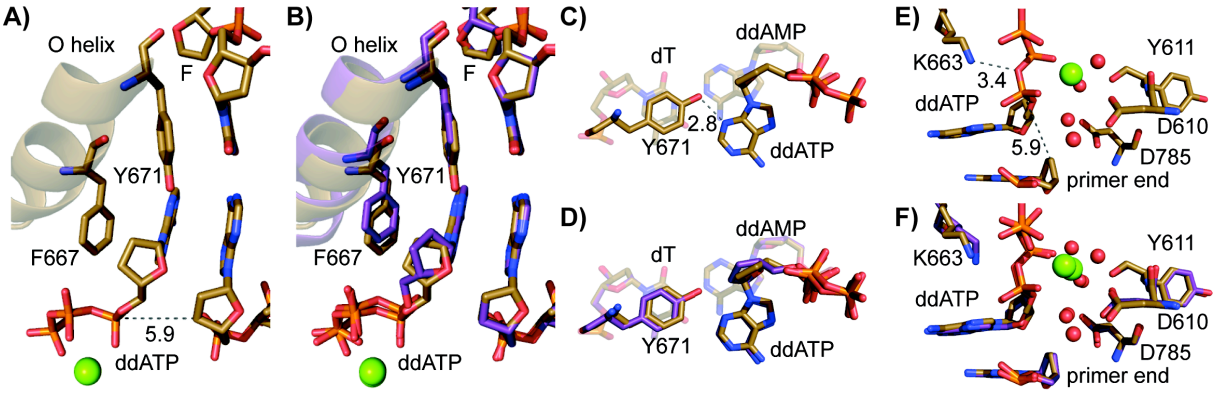
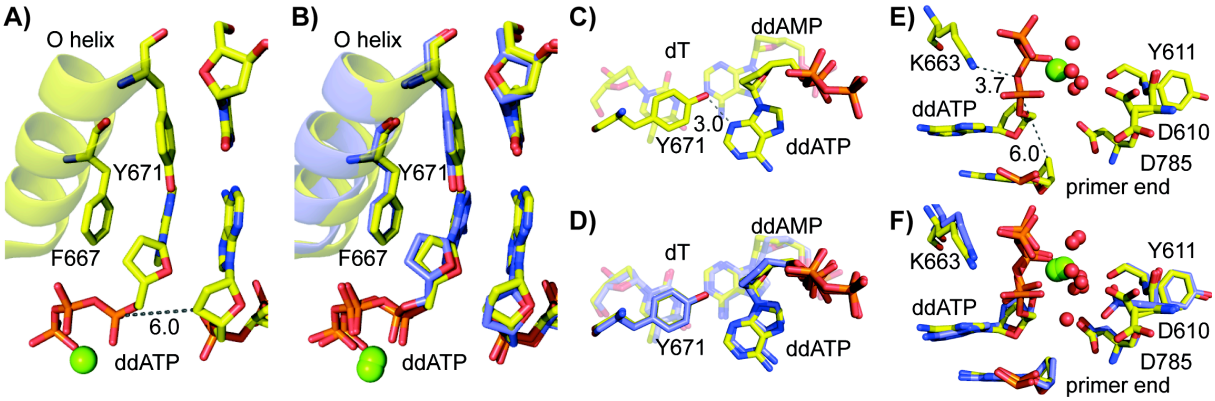


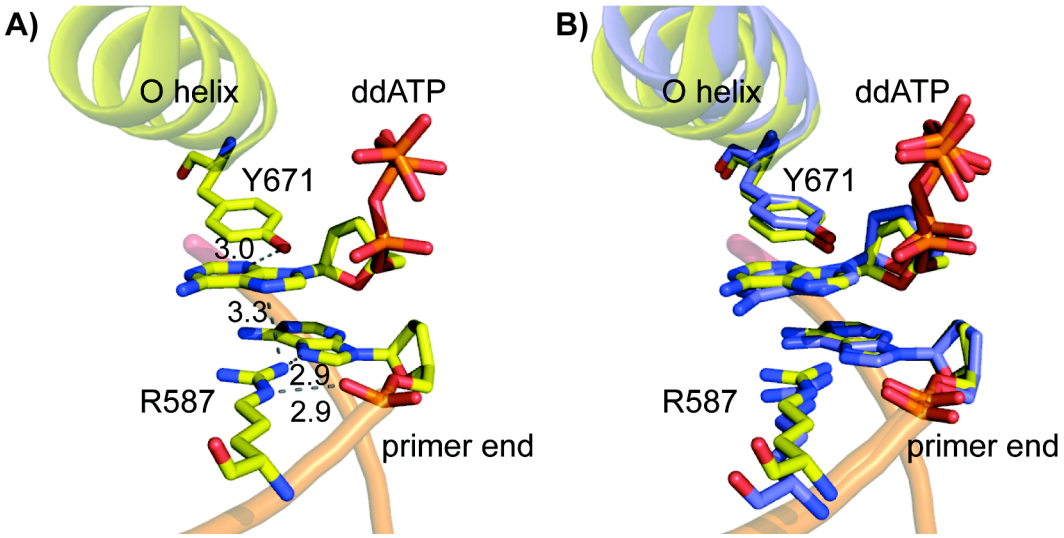
S1. Active site and nascent base pair assemblies in a canonical case. A) Close-up view of *KlenTaq* DM_{ddCTP} (grey) active site processing ddCTP opposite a template dG. Shown are O helix, residues Y671, and F667, the respective template residues and incoming ddCTP. B) Superimposed structures of *KlenTaq* DM_{ddCTP} (grey) and *KlenTaq* wild-type (green, PDB 3KTQ) showing the same residues as in A). C) Top view to the nascent base pair of *KlenTaq* DM_{ddCTP}. In front the incoming ddCTP opposite a template dG. The hydrogen bonding between the incoming ddCTP and the templating dG is shown in dashed lines. In transparent the first nucleobase pair of the primer template terminus is shown. D) Overlay of top view of the nascent base pair from *KlenTaq* DM_{ddCTP} and *KlenTaq* wild-type (PDB 3KTQ). The primer template terminus is illustrated in transparent. E) Inner coordination spheres showing metal ion in *KlenTaq* DM_{ddCTP}. A Mn^{2+} ion (purple sphere) and Mg^{2+} ion (green sphere) are coordinated by the triphosphate moiety of ddCTP and catalytically essential carboxylates. Residue K663, recently, discussed to act as general acid in catalysis, is shown. The distance of the α-phosphate to the primer 3'-terminus constitutes 3.8 Å. F) Superimposition of *KlenTaq* DM_{ddCTP} (grey) and *KlenTaq* wild-type (green, PDB 3KTQ) showing the same residues as in E). All distances are in Å.



S2. Active site and nascent base pair assemblies in an abasic site (F) context. A) Close-up view of *KlenTaq* DM_{AP} (sand) active site processing ddATP opposite an abasic site F. Shown are O helix, residues Y671, and F667, the respective template residues and incoming ddATP. B) Superimposed structures of *KlenTaq* DM_{AP} (sand) and *KlenTaq* wild-type in complex with a F containing template (purple, PDB 3LWL) showing the same residues as in A). C) Top view to the nascent base pair of *KlenTaq* DM_{AP} (sand). In front the incoming ddATP opposite an abasic site F. The hydrogen bonding between the incoming ddATP and the amino acid side chain Y671 is shown in dashed lines. In transparent the first nucleobase pair of the primer template terminus is shown. D) Overlay of top view of the nascent base pair from *KlenTaq* DM_{AP} (sand) and *KlenTaq* wild-type (purple, PDB 3LWL). The primer template terminus is illustrated in transparent. E) Inner coordination spheres showing metal ion in *KlenTaq* DM_{AP}. A Mg^{2+} ion (green sphere) is coordinated by the triphosphate moiety of ddATP and two water molecules (red spheres). Two additional water molecules form hydrogen bonds to residues D610, Y611, and D785. Residue K663, recently, discussed to act as general acid in catalysis, is shown. The distance of the α-phosphate to the primer 3'-terminus constitutes 5.9 Å. F) Superimposition of *KlenTaq* DM_{AP} (sand) and *KlenTaq* wild-type (purple, PDB 3LWL) showing the same residues as in E). All distances are in Å.



S3. Active site and nascent base pair assemblies in a blunt-end context. A) Close-up view of *KlenTaq* DM_{BE} (yellow) active site processing ddATP at a blunt-end primer template duplex. Shown are O helix, residues Y671, and F667, the respective template residues and incoming ddATP. B) Superimposed structures of *KlenTaq* DM_{BE} (yellow) and *KlenTaq* wild-type in complex with a blunt-end primer template duplex (blue, PDB 3LWM). C) Top view to the nascent base pair of *KlenTaq* DM_{BE}. In front the incoming ddATP opposite the amino acid side chain Y671. The hydrogen bonding between the incoming ddATP and Y671 is shown in dashed lines. In transparent the first nucleobase pair of the primer template terminus is shown. D) Overlay of top view of the nascent base pair from *KlenTaq* DM_{BE} (yellow) and *KlenTaq* wild-type (blue, PDB 3LWM). The primer template terminus is illustrated in transparent. E) Inner coordination spheres showing metal ion in *KlenTaq* DM_{BE}. A Mg^{2+} ion (green sphere) is coordinated by the triphosphate moiety of ddATP and two water molecules (red spheres). Residue K663, recently, discussed to act as general acid in catalysis, is shown. The distance of the α-phosphate to the primer 3'-terminus constitutes 6.0 Å. F) Superimposition of *KlenTaq* DM_{BE} (yellow) and *KlenTaq* wild-type (blue, PDB 3LWM) showing the same residues as in E). All distances are in Å.



S 4. Interaction network of incoming ddATP at a blunt-end primer template duplex. A) Hydrogen bond network stabilizing ddATP at a blunt-end primer template duplex in *KlenTaq* DM_{BE} (yellow). Labelled are the amino acid side chains R587 and Y671. B) Superimposed structures of *KlenTaq* DM_{BE} (yellow) and *KlenTaq* wild-type in complex with a blunt-end DNA substrate (blue, PDB 3LWM).

Table S1 Data collection and refinement statistics (molecular replacement)

	KlenTaq DM_{ddCTP} PDB ID: 3PY8	*KlenTaq* DM_{AP} PDB ID: 3PO5	*KlenTaq* DM_{BE} PDB ID: 3PO4
Data collection			
Space group	P 3_1 2 1	P 3_1 2 1	P 3_1 2 1
Cell dimensions			
a, *b*, *c* (Å)	108.6, 108.6, 90.6	109.4, 109.4, 90.4	113.5, 113.5, 91.3
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Resolution (Å)	47.00-1.74 (1.79-1.74)*	47.38-2.38 (2.38-2.53)*	49.16-1.80 (1.91-1.80)*
R_{meas}	12.0 (166.4)*	11.0 (89.2)*	7.2 (76.7)*
I / σ	18.7 (1.5)*	14.1 (1.9)*	16.9 (2.6)*
Completeness (%)	98.9 (86.0)*	99.6 (97.7)*	99.7 (98.5)*
Redundancy	24.6 (9.9)*#	9.5 (7.5)*	10.0 (10.1)*
Refinement			
Resolution (Å)	41.74-1.74 (1.77-1.74)*	47.33-2.39 (2.49-2.39)*	48.20-1.80 (1.83-1.80)*
No. reflections	63416 (2572)*	24927 (2569)*	63101 (2672)*
R_{work} / R_{free}	16.8/19.8 (27.5/30.1)*	21.0/26.6 (28.5/32.8)*	16.4/19.4 (23.4/25.0)*
No. Atoms/ average *B*-factor (Å^2)			
Protein	4287 / 31.7	4197 / 49.5	4313 / 35.1
DNA	528 / 25.4	499 / 34.2	485 / 28.7
Triphosphate	27 / 27.9	29 / 76.8	29 / 46.6
Ion	5 Mn^{2+} / 39.0 Mg^{2+} / 29.3	Mg^{2+} / 64.3	3 Mg^{2+} / 36.3 Na^{+} / 50.7
Water	525 / 43.0	191 / 36.5	548 / 43.8
Ramachandran statistics (%)§			
Most favored	94.2	88.7	92.9
Additional allowed	5.5	10.9	6.6
Generously allowed	0.0	0.2	0.2
disallowed	0.2	0.2	0.2
R.m.s. deviations			
Bond lengths (Å)	0.006	0.006	0.007
Bond angles (°)	1.064	0.921	1.088

* Values in parentheses are for highest-resolution shell.
# 3 datasets were merged with XSCALE
§ Ramachandran statistics as defined by PROCHECK