Supplementary Information

Crystallographic Analysis of Engineered Polymerases Synthesizing Phosphonomethylthreosyl Nucleic Acid

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Supplementary Table S1. Oligonucleotide Sequences

Name	Oligonucleotide sequence (5' – 3')	Comments
P_f	ATCCATATGATCCTCGACACTGACTAC	Cloning
P_r	ACGCATGCGGCCGCTCAAGTTCCCTTCGGCTTCAGCCA	
P_1	/5IRD/ GGATACCACC	Primer extensions
T_1	TCTCTATAGTGAGTCGTATAGGTGGTATCC	
P_c	CGCGAACTGCG	Crystal complex
T_c	/Cy5/ AAATTCGCAGTTCGCG	

Supplementary Table S2. Buffer Compositions

Name	Buffer Composition	Comments
Kod low salt/lysis buffer	10 mM Tris.Cl pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol, 0.125 mg mL^{-1} egg hen lysozyme when lysing cells	Purification
Kod high salt buffer	10 mM Tris.Cl pH 7.5, 1 M NaCl, 0.1 mM EDTA, 1 mM DTT, 10% glycerol	
Kod buffer	50 mM Tris.Cl pH 8.5, 200 mM NaCl, 0.1 mM EDTA, 1 mM DTT	Protein storage; Crystallization
Thermopol® (NEB)	20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton® X-100 pH 8.8	Primer extensions
Stop Buffer	95% formamide, 25 mM EDTA, pH 8.0	

Supplementary Table S3. Data Collection and Refinement Statistics. The statistics for the highest-resolution shell are shown in parentheses.

	Kod-RI DNA	Kod-RI pTNA	Kod-RSGA pTNA
Data Collection			
Space group	*P 2 2$_1$ 2$_1$*	*P 2 2$_1$ 2$_1$*	*P 2 2$_1$ 2$_1$*
Cell Dimensions			
a, *b*, *c* (Å)	66.3, 112.0, 149.2	65.8, 111.6, 149.0	64.1, 111.5, 144.3
a, b, g (º)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	57.03-2.71 (2.81-2.71)	40.92-1.98 (2.05-1.98)	44.04-3.01 (3.12-3.01)
Total reflections	61392 (6062)	552026 (55933)	68272 (6983)
Unique reflections	30746 (3037)	77125 (7617)	19530 (1955)
Redundancy	2.0 (2.0)	7.2 (7.3)	3.5 (3.6)
Completeness (%)	99.36 (99.80)	99.90 (99.97)	91.96 (94.51)
I / σI	13.14 (4.74)	17.86 (2.46)	10.33 (2.44)
Wilson B-factor	47.10	36.59	54.99
R_{merge}	0.026 (0.137)	0.074 (1.204)	0.1458 (0.7314)
CC1/2	0.998 (0.949)	0.999 (0.741)	0.991 (0.810)
CC*	1 (0.987)	1 (0.923)	0.998 (0.946)
Refinement			
Resolution (Å)	2.71	1.98	3.01
No. of reflections	30731 (3037)	77092 (7615)	19492 (1944)
R_{work}/R_{free}	0.161/0.226 (0.188/0.254)	0.176/0.212 (0.249/0.243)	0.176/0.270 (0.258/0.365)
No. atoms	6883	7052	6777
Protein/DNA	6798	6767	6735
ptA 12 & 13	-	42	42
Water	85	243	-
B-factors	52.35	52.71	54.24
Protein/DNA	52.47	52.91	54.22
ptA 12 & 13	N/A	34.71	55.84
Water	42.42	50.37	-
Clashscores	5.38	4.48	12.09
RMS dev. (Bonds; Å)	0.008	0.008	0.010
RMS dev. (Angles; º)	1.22	1.16	1.32
PDB ID	7RSS	7RSR	7TQW

Supplementary Table S4. Local Base-Pair Parameters.

BASE PAIR	Shear (Å)	Stretch (Å)	Stagger (Å)	Buckle (°)	Propeller (°)	Opening (°)
RI/DNA						
dA13 - dT4	-0.21	-0.12	0.35	-24.29	-10.10	8.20
dA12 - dT5	0.20	-0.19	-0.20	-3.79	-2.24	0.36
dG11 - dC6	0.12	-0.21	0.16	-1.57	-13.73	4.38
RI/TNA						
tA13 - dT4	-0.25	-0.37	0.29	-16.69	-15.14	1.70
tA12 - dT5	-0.30	-0.20	-0.32	-4.28	-4.75	1.76
dG11 - dC6	0.37	-0.25	-0.22	-5.48	-4.14	1.47
RI/pTNA						
ptA13 - dT4	-0.15	-0.20	0.24	-11.36	-15.97	3.96
ptA12 - dT5	-0.04	-0.15	-0.38	2.77	-3.40	-1.06
dG11 - dC6	0.31	-0.21	0.34	-10.99	-13.44	3.51
RSGA/pTNA						
ptA13 - dT4	-0.19	-0.15	0.29	-16.7	-25.13	1.18
ptA12 - dT5	-0.44	-0.10	0.42	-8.42	-13.20	-6.01
dG11 - dC6	0.55	-0.15	-0.01	-9.00	-0.43	4.82

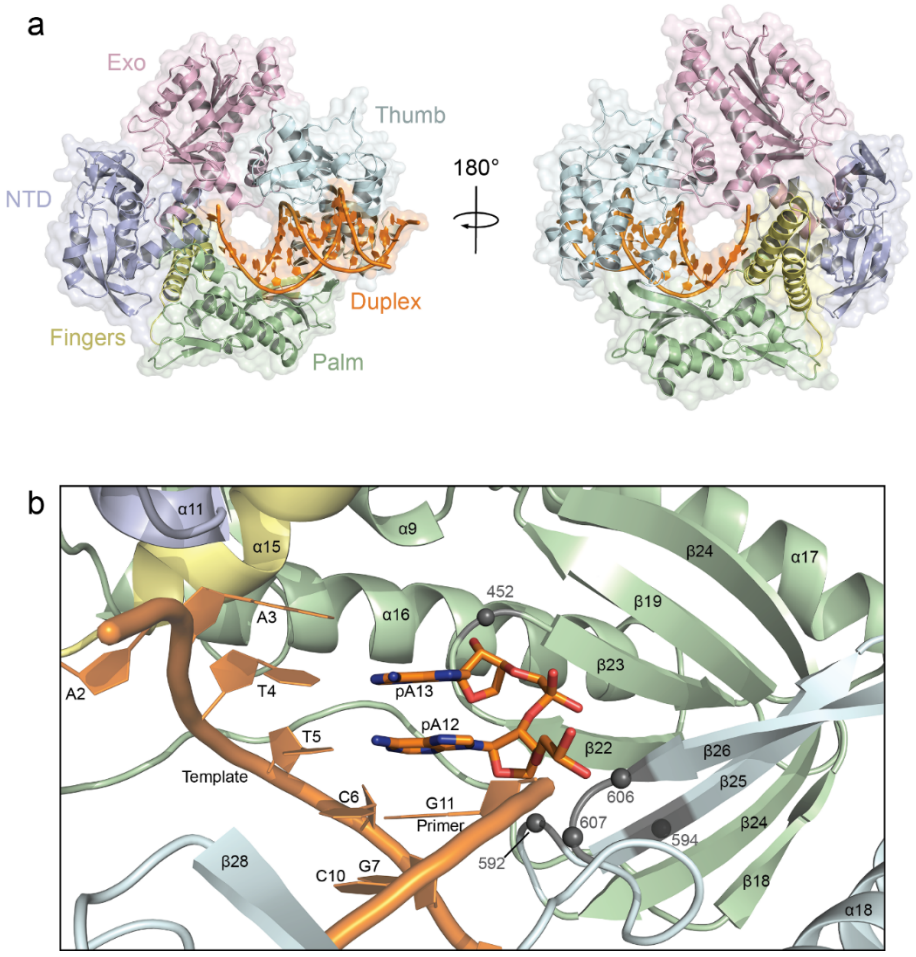
Supplementary Table S5. Local Base-Pair Step Parameters.

STEP	Shift (Å)	Slide (Å)	Rise (Å)	Tilt (°)	Roll (°)	Twist (°)
RI/DNA						
dT4 dT5 / dA12 dA13	-0.50	-0.08	2.83	5.82	8.19	29.75
dT5 dC6 / dG11 dA12	0.66	-0.55	3.31	-4.09	7.12	40.23
RI/TNA						
dT4 dT5 / tA12 tA13	-0.29	-0.41	3.02	6.36	5.35	28.08
dT5 dC6 / dG11 tA12	0.41	-0.29	3.39	0.33	0.77	43.30
RI/pTNA						
dT4 dT5 / ptA12 ptA13	-0.19	-0.30	2.97	6.93	6.15	31.35
dT5 dC6 / dG11 ptA12	0.82	-0.69	3.64	-4.63	0.07	42.36
RSGA/pTNA						
dT4 dT5 / ptA12 ptA13	-0.70	-0.48	3.16	-0.28	10.07	30.50
dT5 dC6 / dG11 ptA12	1.04	-0.57	3.47	-0.37	-9.14	44.77

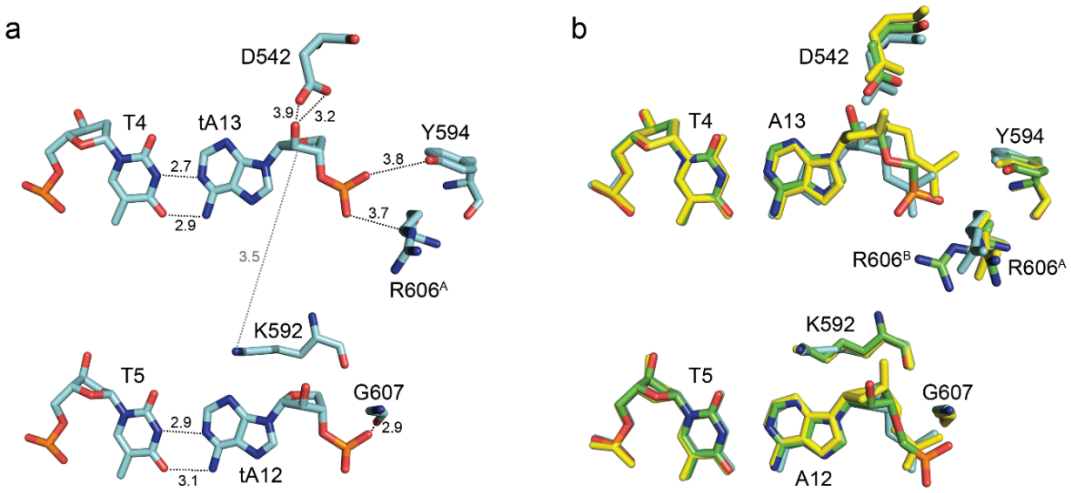
Supplementary Table S6. Torsion Angles and Pseudorotation Parameters.

	α	β	γ	δ	ε	ζ	χ	ν_0	ν_1	ν_2	ν_3	ν_4	ν_{max}	*P*
RI/DNA														
dA13	-77.7	167.4	51.5	91.2	N/A	N/A	-121.7	-24.9	2.9	18.4	-33.1	36.5	36.6	59.9
dA12	-71.7	-151.6	42.0	145.0	-167.5	-92.3	-103.5	-8.9	23.6	-28.8	24.5	-10.0	28.8	181.3
dG11	84.1	-118.6	-160.3	94.2	-150.5	-67.4	-172.3	-9.3	-7.7	20.7	-26.5	22.4	26.4	38.5
RI/TNA														
tA13	-88.9	N/A	168.3	159.0	N/A	N/A	-101.1	-5.4	-17.7	33.8	-36.6	24.7	37.3	25.3
tA12	-126.5	N/A	-135	161.4	-108.4	-83.2	-85.3	2.6	-21.3	31.88	-30.0	16.1	32.6	12.8
dG11	-56.6	161.9	49.0	130.1	177.1	-88.8	-126.3	-35.5	40.7	-30.3	10.9	15.1	40.0	139.3
RI/pTNA														
ptA13	66.0	-149.9	176.4	162.0	N/A	N/A	-119.1	-14.4	-12.7	32.3	-41.1	32.6	40.5	37.2
ptA12	-39.2	-147.4	-63.4	163.2	-84.6	-160.3	-92.7	-20.0	-4.9	26.5	-37.8	35.1	39.0	47.2
dG11	86.0	-124.8	-157.0	113.6	-134.6	-75.3	-166.7	-22.4	17.8	-7.0	-5.8	17.7	21.8	108.6
RSGA/pTNA														
ptA13	68.9	-160.1	-177.8	166.8	N/A	N/A	-107.2	-17.3	-11.7	34.0	-44.6	36.1	44.1	39.5
ptA12	-36.2	-148.4	-68.9	145.5	-93.3	-149.2	-78.2	-11.3	-6.4	20.3	-27.1	22.7	27.0	41.2
dG11	95.1	-125.6	-170.5	129.9	-135.5	-87.3	-154.9	-26.8	32.9	-26.3	11.7	9.4	32.2	144.7

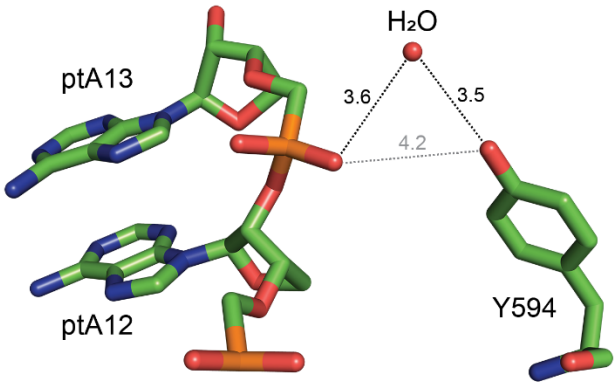
The torsion angles are reported in degrees for the two incorporated adenosine nucleotides, A12 and A13, and the preceding nucleotide of the primer, dG11. A pictorial definition of the torsion angles is provided in SI Fig 2. The angles α, β, γ, δ, ε, and ζ are assigned to the exocyclic backbone of the genetic polymers. The β angle is omitted for TNA. The ε and ζ angles non-computable for the terminal A13 nucleotides. The χ angle is torsion angle about the glycosidic bond. The angles ν 0 through 4 describe the sugar moiety's endocyclic torsion angles. The ν_{max} is the maximum torsion angle and is reflective of the degree of puckering; a ν_{max} of 0° reflects a planar furanose ring. The pseudorotation phase angle, *P*, ranges from 0° to 360° and $P = 0°$ is defined such that the torsion angle ν_2 is maximally positive.



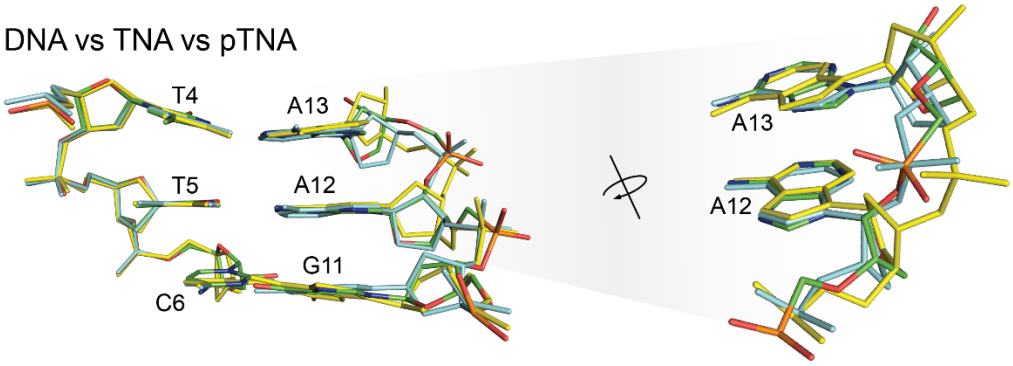
Supplementary Figure S1. Kod Structural Overview (a) The global architecture of Kod bound to a primer-template duplex. The front and back view showing a surface rendering overlayed on a cartoon representation is colored by protein subdomain. The primer-template duplex is shown in orange. (b) A view of the active site is shown with the exonuclease domain hidden to avoid view obstruction. Positions with residues that interact with the incorporated pTNA are shown as gray spheres.



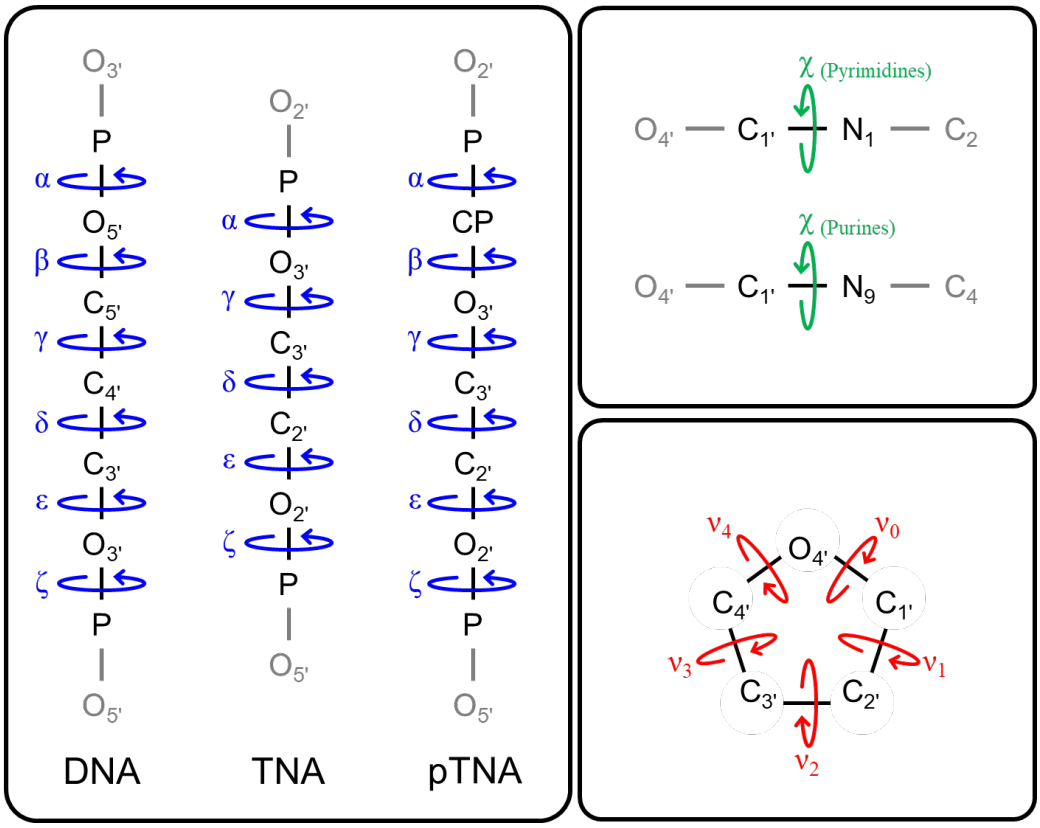
Supplementary Figure S2. Kod-RI Incorporating TNA and Active Site Overlays. (a) A panel equivalent to main text Figure 2b-d for TNA incorporations showing base pairing and protein interactions in angstroms. (b) An overlay of the incorporated DNA, TNA, pTNA is shown, highlighting the backbone variation in context of the active site.



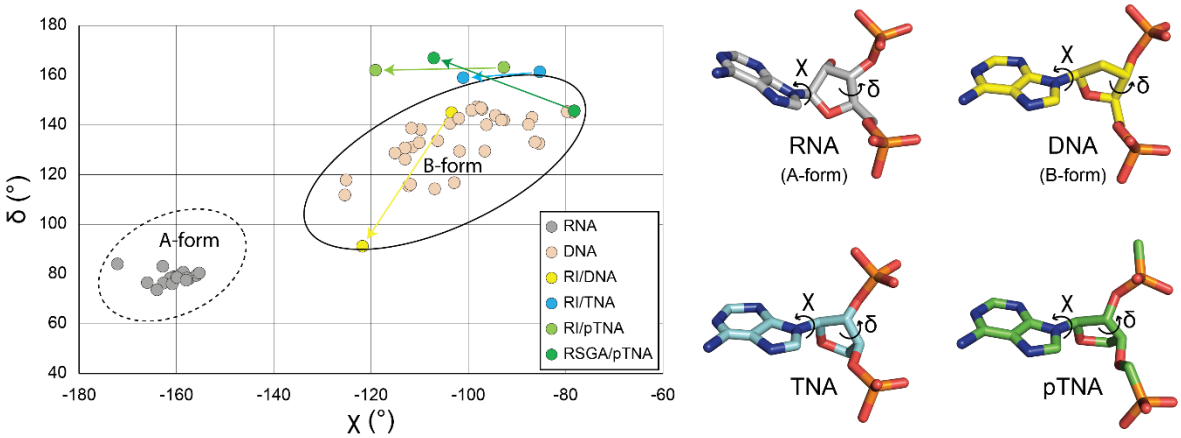
Supplementary Figure S3. Tyrosine 594 Forms a Bridging Water Interaction. In the structure of Kod-RI incorporating pTNA, tyrosine 594 is 4.2 Å away from the phosphonate backbone. Instead of hydrogen bonding, compensatory water coordination is observed.



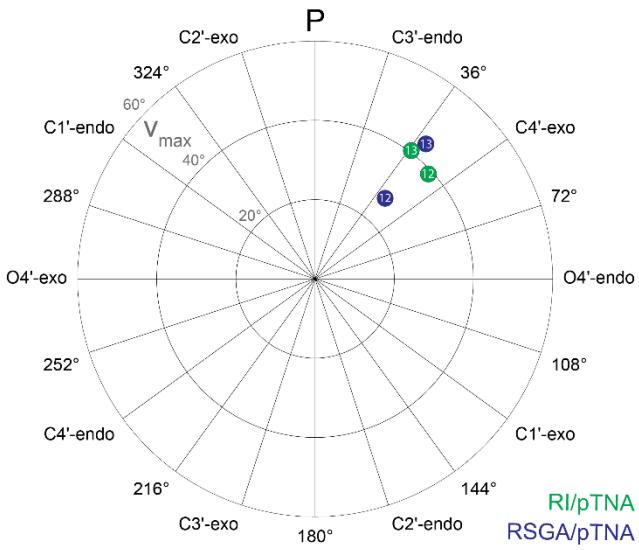
Supplementary Figure 4. A Backbone Comparison of Newly Added Nucleotides . A view identical to that of Figure 3, overlaying RI/pTNA (green, colored by element), RI/DNA (solid yellow), and RI/TNA (solid cyan) at once.



Supplementary Figure 5. Torsion Angles Definition. A torsion angle about a bond is defined by 4 atoms, the two bonding atoms, and an additional atom bonded to each. Pictorial definitions of the exocyclic backbone torsion angles in the backbones of DNA, TNA, and pTNA are shown in blue. TNA's shortened backbone lacks the β angle. For all genetic systems, the torsion angles about the glycosidic bond for pyrimidines and purines are defined in green, and the sugar endocyclic torsion angles are defined in red.



Supplementary Figure 6. Incorporated Nucleotides (χ,δ) Covariance. The (χ,δ) covariance is used to analyze the helical geometry of the TNA and pTNA nucleotides with canonical A-form RNA (PDB ID: 3ND4) and B-form DNA (PDB ID: 3BSE) nucleotides as a reference. Arrows drawn on the plot represent the shift in (χ,δ) covariance between the first (A12; n+1) and the second incorporations (A13; n+2). On the right, representative monomers, that is, A12 for DNA, TNA, and pTNA, are shown in comparison to a canonical A-form RNA monomer.



Supplementary Figure 7. pTNA's Sugar Puckering in Kod-RSGA. The pseudorotation parameters of pTNA in RI/pTNA (green) and RSGA/pTNA (blue) are plotted, depicting the slight increase in the extent of puckering (ν_{max}).