SUPPLEMENTARY TABLE

Table S1. Crystallographic Statistics for Data Collection and Structure Refinement of dQTP/dT-Containing Ternary Complexes of RB69pol triple mutant

RB69pol Ternary Complex	tm/dQTP/dT
Space group	$P2_12_12_1$
Unit cell dimensions [a, b, c (Å)]	78.16, 118.51, 130.67
Resolution range (Å)	50.0-2.04 (2.08-2.04)
Number of reflections	
Unique	78,654
Redundancy	5.9 (5.1)
Completeness (%)	99.9 (98.3)
R_{merge} (%)	9.8 (85.7)
I/σ	17.7 (1.3)
Final model	
Amino acid residues	903
Water molecules	504
Metal ions	5
Template nucleotides	18
Primer nucleotides	13
dNTP molecules	1
Refinement Statistics	
Reflections	74,440
R_{work} (%)	17.9 (26.4)
R_{free} (%)	21.8 (29.3)
r.m.s.d.	
Bond length (Å)	0.007
Bond angle (°)	1.188
PDB access code	4E3S

Footnotes:

a, Statistics for the highest resolution shell are in parenthesis.

b, $R_{merge} = \Sigma_{hkl}\Sigma_j |I_j(hkl)-\langle I_j(hkl)\rangle|/\Sigma_{hkl} \Sigma_j \langle I(hkl)\rangle$, statistics for merging all observations for given

reflections.

c, $R = \Sigma_{hkl} |F_{obs}(hkl)-F_{calc}(hkl)|/\Sigma_{hkl} F_{obs}(hkl)$, statistics for crystallographic agreement between the measured and model-calculated amplitudes. Rfree is the agreement for cross-validation data set.

e, Root mean squares deviations (rmsd) to ideal values.

SUPPLEMENTARY FIGURES

Figure S1.

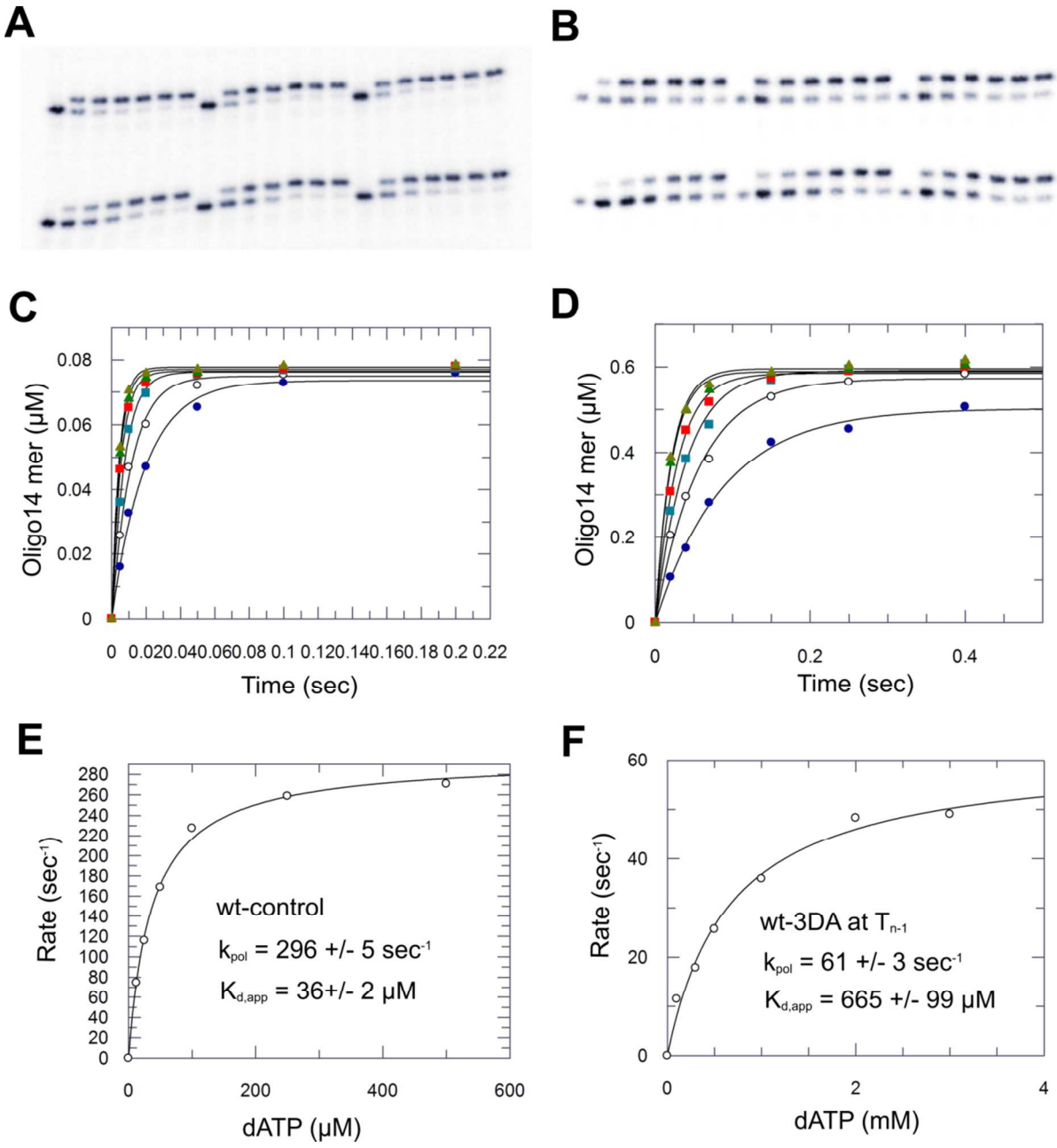

Figure S2.

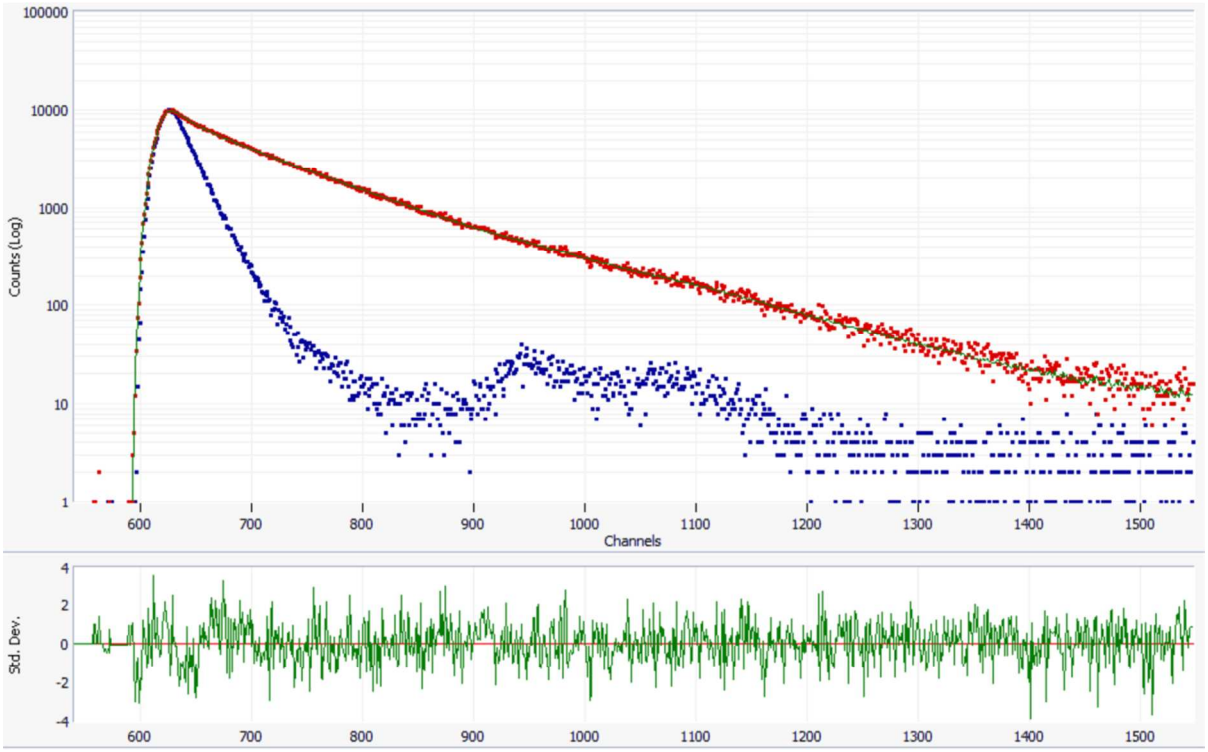

Figure S3.

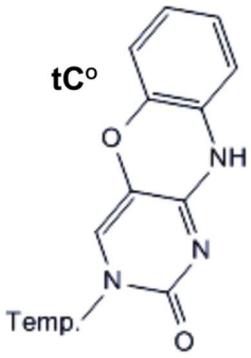

Figure legends:

Figure S1. Raw dat and plots for the insertion of dAMP opposite dT by wt RB69 pol with SeqA1 and SeqA2. (A) A denaturing PAGE gel showing the time course of dAMP incorporation with different [dATP]: top lane (100μM, 250μM, 500μM from left to right) and bottom lane (12.5μM , 25μM , 50μM from left to right) by wt RB69pol with SeqA1. (B) A denaturing PAGE gel showing the time course of dAMP incorporation with different [dATP]: top lane (1mM, 2mM, 3mM from left to right) and bottom lane (0.1mM , 0.3mM , 0.5mM from left to right) by wt RB69pol with SeqA2. (C) Progress curves at various dATP concentrations, 12.5, 25, 50, 100, 250 and 500 μM (from bottom to top), fit to single-exponential equations (SeqA1). (D) Progress curves at various dATP concentrations, 0.1, 0.3, 0.5, 1, 2, and 3mM (from bottom to top), fit to single-exponential equations (SeqA2). (E) Plot of k_{obs} vs [dATP] fit to a hyperbola equation to yield k_{pol} and $K_{d,app}$ (SeqA1). (F) Plot of k_{obs} vs [dATP] fit to a hyperbola equation to yield k_{pol} and $K_{d,app}$ (SeqA2).

Figure S2. Screen-shot of fluorescence decay of wt RB69pol with SeqB2 fitting with 3 exponentials using DAS6.0 (HORIBA Scientific). In the upper panel, the red dots are the fluorescence decay, the blue dots are the prompt, and the green line buried in the red dots is the fitted curve. The residuals from the fitting are shown in the lower panel.

Figure S3. Chemical structure of tC^o.