Supplementary Materials

Supplementary figure and movie legends

Supplementary Figure 1. Difference distance matrix plots comparing the open (1L3U) and ajar conformations (A), and ajar and closed (2HVI) conformations (B) of Bacillus fragment. Red color indicates a relative decrease in Cα interatomic distances in the second structure versus the first; blue color indicates a relative increase. Key structural motifs are bracketed. Distance decreases between open and ajar conformations indicate movement of N and O helices of the fingers subdomain (residues 672-710) towards the thumb subdomain (residues 508-580) as well as minor movement in the thumb. Distance decreases between ajar and closed conformations indicate a large movement of N and O helices towards the thumb, palm, and exonuclease domains. Plots were generated using DDMP from the Center for Structural Biology at Yale University, New Haven, CT. Insets show aligned structures 1L3U (gray), ajar (BF(F710Y)•DNA(dG)•ddTTP-Mg^{2+}; cyan) mismatch, and 2HVI (magenta), noting the locations and residue numbers of thumb and fingers subdomains.

Supplementary Figure 2. The crystal structure of the BF(Y714S)•DNA(dG)•dTTP-Mg^{2+} mispair complex. (A) The nucleotide binding site of Bacillus fragment from the BF(Y714S)•DNA(dG)•dTTP-Mg^{2+} crystal structure is shown from the side in blue. The DNA, dTTP, and side chains along fingers subdomain surface that contact the dTTP are represented in ball and stick. The $2F_o$-F_c electron density map at 1.0 σ is shown as a mesh around the template dG and dTTP. Hydrogen bonds are shown as dashed lines. (B) Structural superimposition of the BF(Y714S)•DNA(dG)•dTTP-Mg^{2+} (blue) and the BF•DNA(dG)•ddTTP-Mg^{2+} (cyan) crystal structures. Both structures are shown in ribbons. The nascent base pair and residues 710 and 714 are shown in sticks.

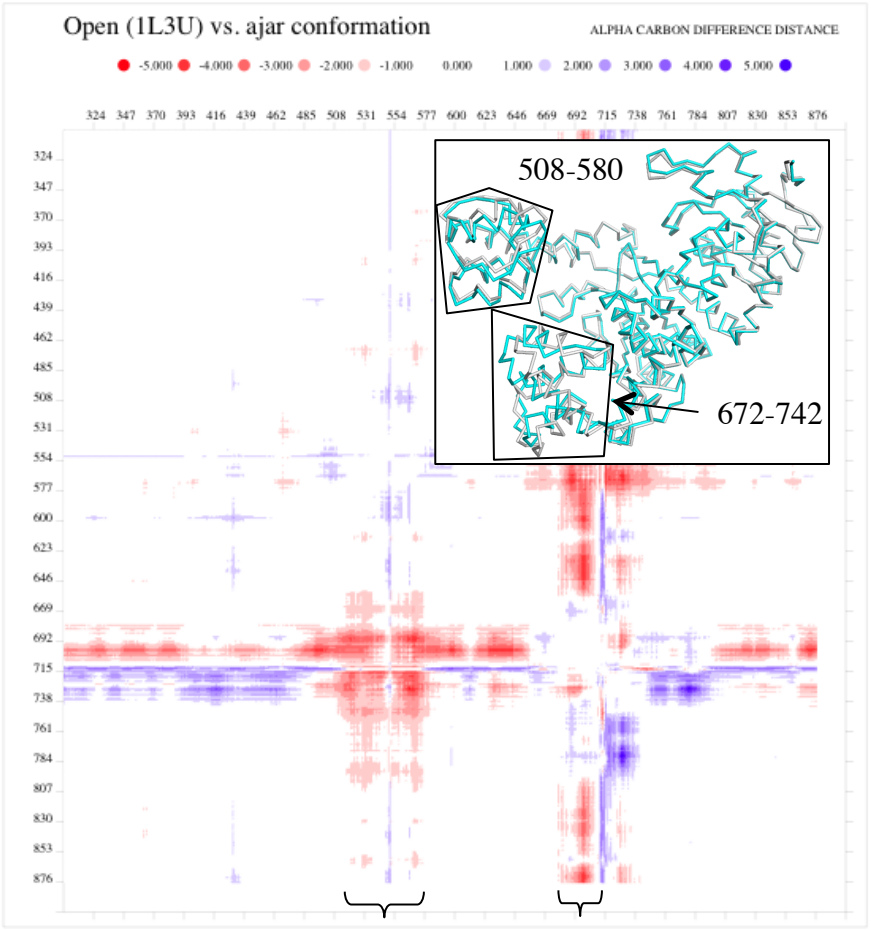
Supplementary Figure 3. Fluorescein-Cy3 FRET in BF. Molecular model for fluorophore locations in Bacillus fragment (A). The primer strand was labeled at the n-6 position (green sphere) with fluorescein and BF C388S/C845S/Q691 mutant. The structures of the O helices in the open (gray), ajar (cyan), and closed (tan) conformations are shown with a sphere of the corresponding color to mark the location of residue 691. The fluorescence spectra of fluorescein-labeled, dideoxy-terminated DNA (FlDNA^{dd}G; blue line), Cy3-labeled BF (Cy3BF; cyan line), and the two mixed together at equimolar amounts (orange line) are shown (B). When the FlDNA^{dd}G spectrum is normalized to the same level as the FlDNA^{dd}G:Cy3BF spectrum (magenta line) and subtracted from the same spectrum to remove the strong fluorescein contribution (dark purple line), the resultant Cy3 spectrum shows an increased fluorescence relative to Cy3BF alone, indicating energy transfer from fluorescein to Cy3 in the complex. The FlDNAddG:Cy3BF (at 1:5 ratio) fluorescence spectra in the presence of various concentrations of the correct (dCTP; C) or the incorrect (dTTP; D) nucleotide are shown. The total Cy3 fluorescence in Fig. 3C is the sum of all measurements between 511 and 530 nm (shown in black lines). Arrows indicate the direction of change in fluorescence as [dNTP] increases.

Supplementary Figure 4. Pre-steady state kinetics of nucleotide incorporation in BF (A and B) and V713P mutant (C and D). 0.1 μM T75:P67 duplex DNA (template base dG) complexed with 0.5 μM BF (final concentrations) were mixed at room temperature with dCTP and dTTP for various times prior to quenching by excess formamide. The fractions of extended primer strand, as determined by peak areas of capillary electropherograms, are plotted against time for the incorporation of the complementary dCTP (A and C) or the incorrect dTTP (B and D). Fits to the data are shown in colored lines. Rates derived from these curve fits are plotted against dNTP

concentrations and fit to the Michaelis-Menton equation to determine pre-steady state kinetic parameters (Inset).

Movie S1. The transition from the open to the closed conformation in DNA polymerase I as it encounters the correct nucleotide. The coordinates of the Bacillus fragment in open conformation, ajar conformation, and closed conformation were interpolated using PyMol (Delano Scientific LLC) and are shown successively. For a description of the proposed mechanism, see the text. The nascent base pair between the template base and incoming nucleotide is shown in cyan. The magenta sphere represents a magnesium ion. The polymerase and inactive exonuclease domains are shown in yellow and blue, respectively. The O and O1 helices that comprise the pre-insertion site are colored in copper. The template and primer oligonucleotides are colored in red and green, respectively. The side chains of key residues (I716 in the pre-insertion site, Y714 in the insertions site, and active site residues D653 and D830) are shown in stick representation and in gray with red oxygen atoms.

A


Open (1L3U) vs. ajar conformation
ALPHA CARBON DIFFERENCE DISTANCE
-5.000 -4.000 -3.000 -2.000 -1.000 0.000 1.000 2.000 3.000 4.000 5.000
324 347 370 393 416 439 462 485 508 531 554 577 600 623 646 669 692 715 738 761 784 807 830 853 876
508-580
672-742
Thumb subdomain
N & O helices

B

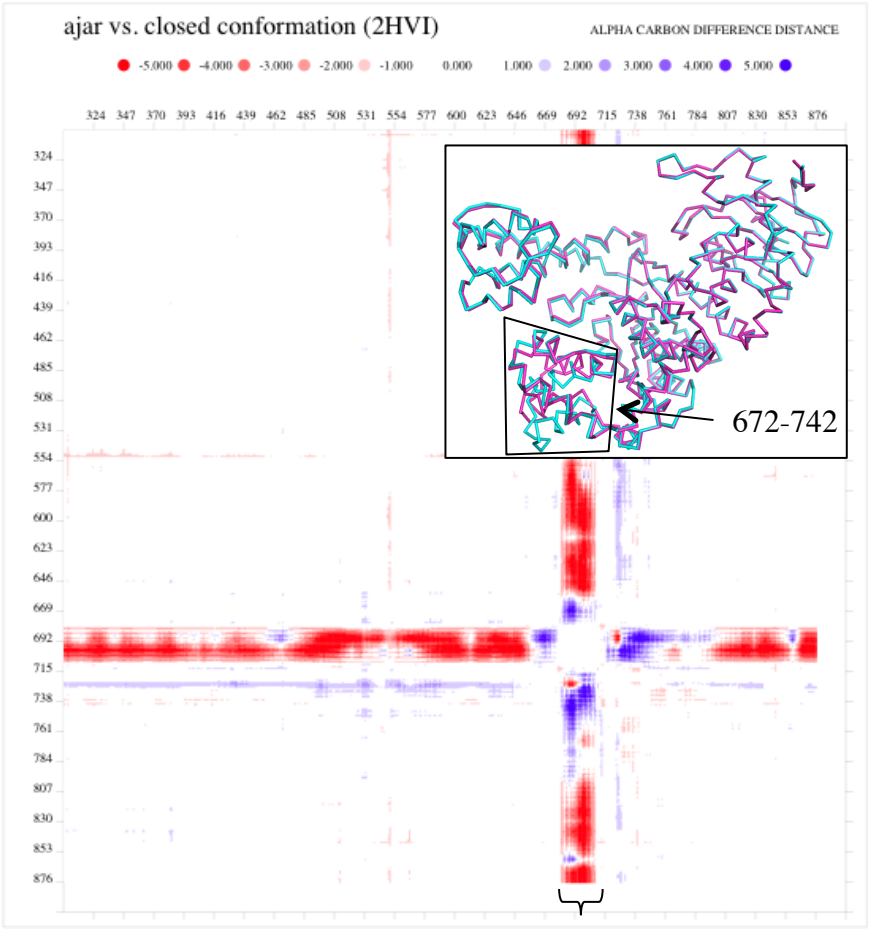

ajar vs. closed conformation (2HVI)
ALPHA CARBON DIFFERENCE DISTANCE
-5.000 -4.000 -3.000 -2.000 -1.000 0.000 1.000 2.000 3.000 4.000 5.000
324 347 370 393 416 439 462 485 508 531 554 577 600 623 646 669 692 715 738 761 784 807 830 853 876
672-742
N & O helices

Figure S1

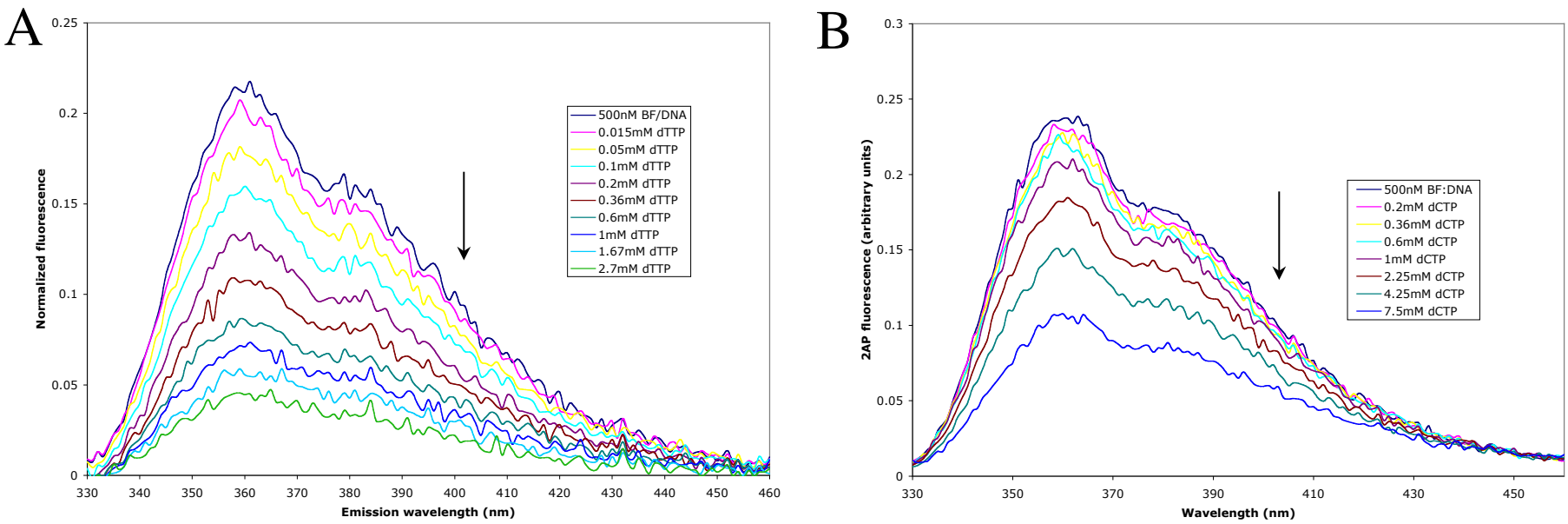

A
Normalized fluorescence
Emission wavelength (nm)
500nM BF/DNA
0.015mM dTTP
0.05mM dTTP
0.1mM dTTP
0.2mM dTTP
0.36mM dTTP
0.6mM dTTP
1mM dTTP
1.67mM dTTP
2.7mM dTTP
B
2AP fluorescence (arbitrary units)
Wavelength (nm)
500nM BF:DNA
0.2mM dCTP
0.36mM dCTP
0.6mM dCTP
1mM dCTP
2.25mM dCTP
4.25mM dCTP
7.5mM dCTP

Fig. S2

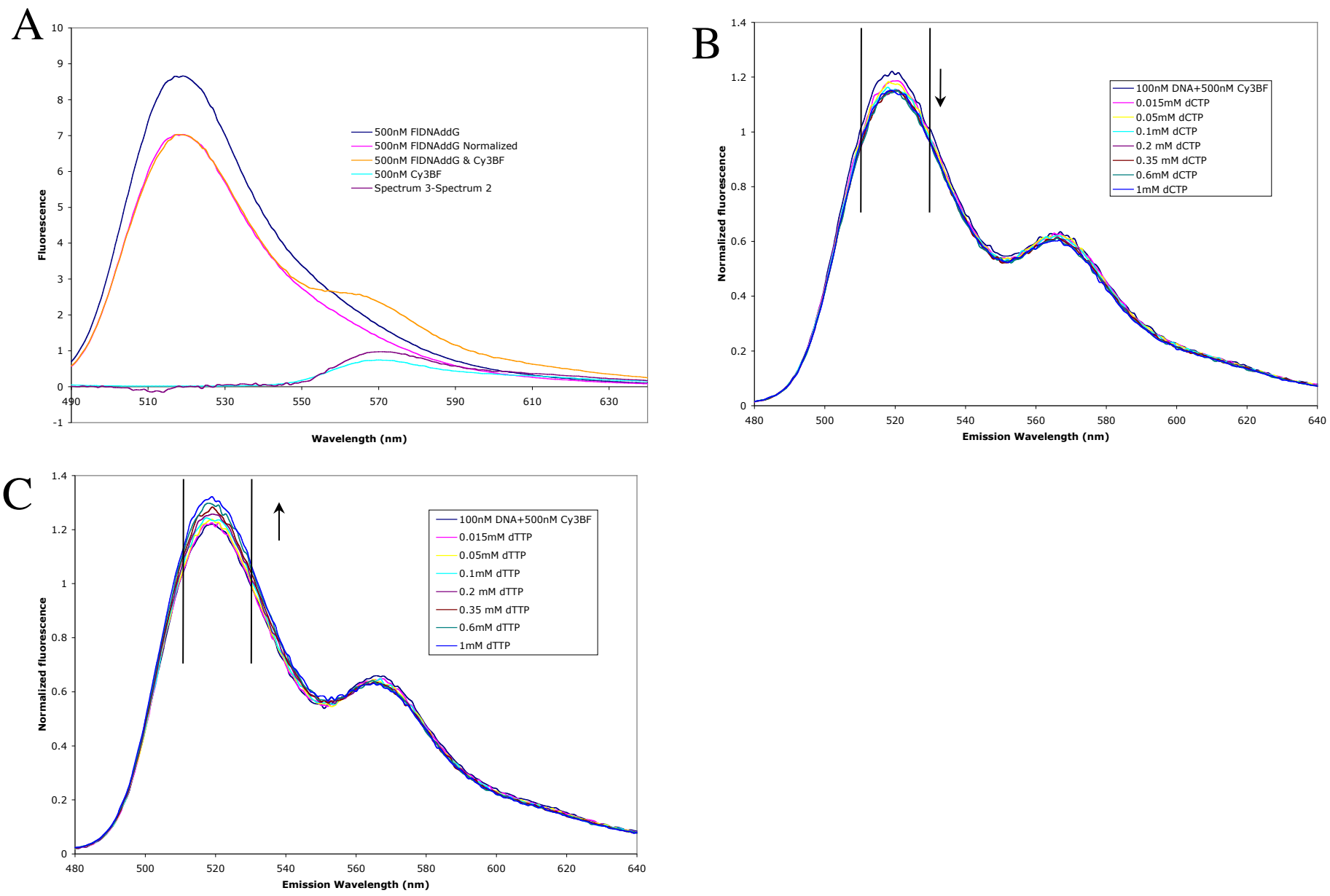

A
Fluorescence
Wavelength (nm)
500nM FlDNAddG
500nM FlDNAddG Normalized
500nM FlDNAddG & Cy3BF
500nM Cy3BF
Spectrum 3-Spectrum 2
B
Normalized fluorescence
Emission Wavelength (nm)
100nM DNA+500nM Cy3BF
0.015mM dCTP
0.05mM dCTP
0.1mM dCTP
0.2 mM dCTP
0.35 mM dCTP
0.6mM dCTP
1mM dCTP
C
Normalized fluorescence
Emission Wavelength (nm)
100nM DNA+500nM Cy3BF
0.015mM dTTP
0.05mM dTTP
0.1mM dTTP
0.2 mM dTTP
0.35 mM dTTP
0.6mM dTTP
1mM dTTP

Fig. S3

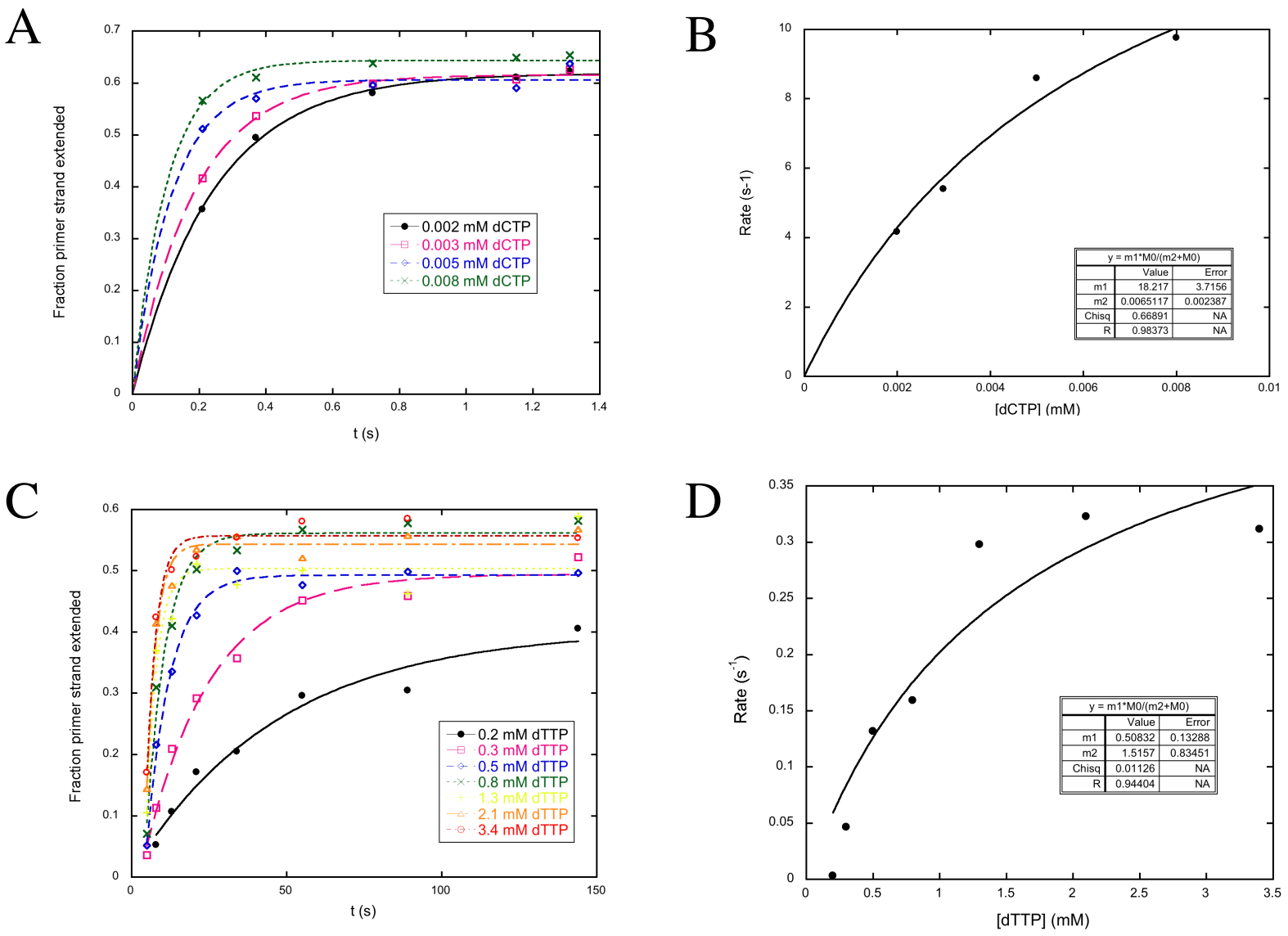

A
Fraction primer strand extended
t (s)
0.002 mM dCTP
0.003 mM dCTP
0.005 mM dCTP
0.008 mM dCTP
B
Rate (s-1)
[dCTP] (mM)
y = m1*M0/(m2+M0)
Value Error
m1 18.217 3.7156
m2 0.0065117 0.002387
Chisq 0.66891 NA
R 0.98373 NA
C
Fraction primer strand extended
t (s)
0.2 mM dTTP
0.3 mM dTTP
0.5 mM dTTP
0.8 mM dTTP
1.3 mM dTTP
2.1 mM dTTP
3.4 mM dTTP
D
Rate (s-1)
[dTTP] (mM)
y = m1*M0/(m2+M0)
Value Error
m1 0.50832 0.13288
m2 1.5157 0.83451
Chisq 0.01126 NA
R 0.94404 NA

Fig. S3