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Supplemental Data

Structural Basis of Transcription: Role of the Trigger Loop in Substrate Specificity and Catalysis

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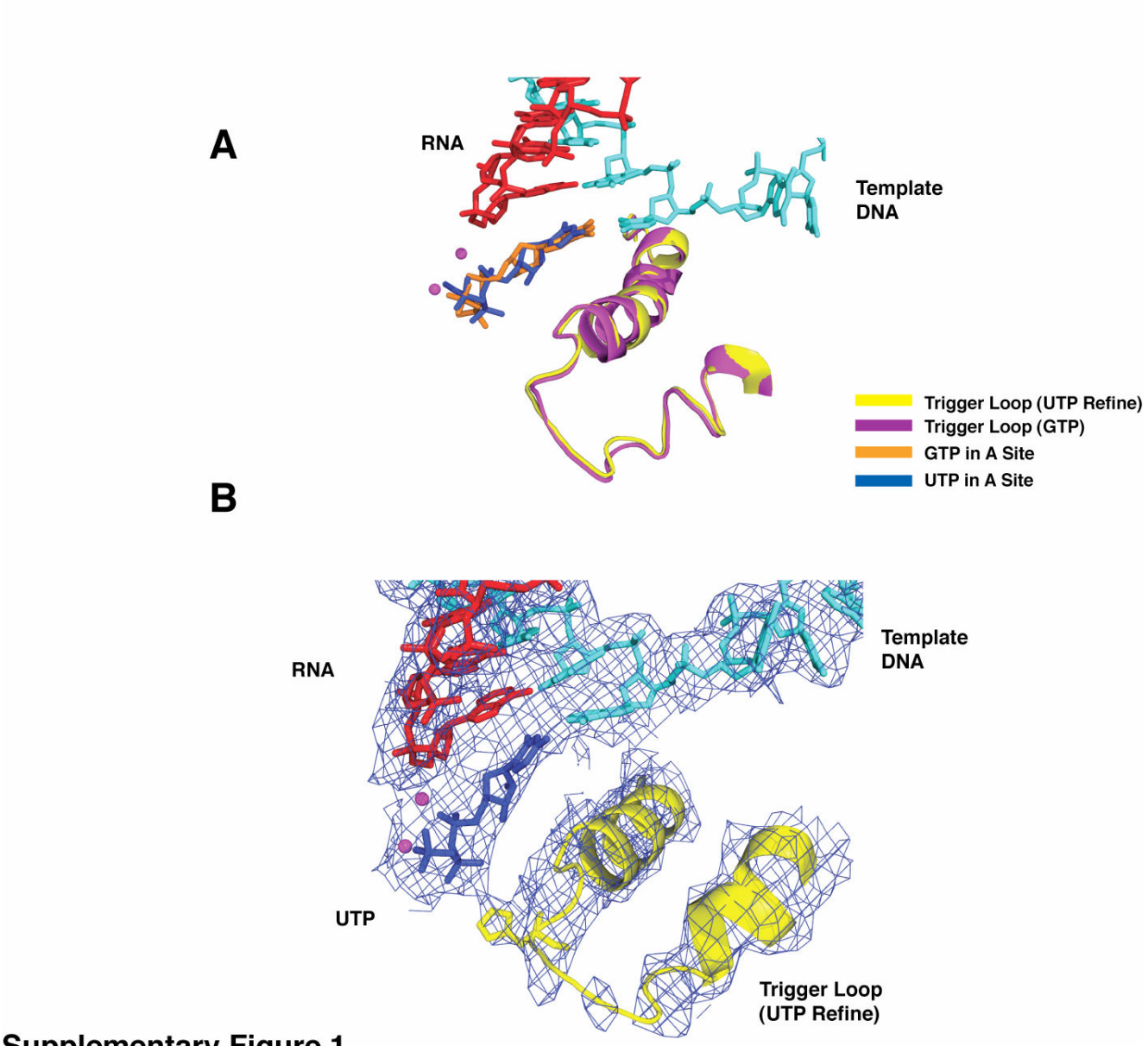
UTP Refinement

The previously published UTP matched nucleotide data (pdb code 1R9S) was reprocessed with TLS with 5 defined TLS domains using Refmac5. The resulting difference map (Fo-Fc) was examined and evidence was found for partial ordering of the trigger loop. Previously based on the refinement from CNS we have left the trigger loop as disordered as was observed in previous higher resolution structures. In the improved difference map there was positive difference density greater than 3 sigma for the trigger loop in an orientation similar to that observed in the matched GTP data. In addition in the difference map there was negative density around Rpb1 residues 1080-1081. The positive and negative densities observed in the improved Fo-Fc maps indicated the initial model may not be the best interpretation of the data. With the improved maps from Refmac with TLS the trigger loop was built into the matched UTP data and found to be in an orientation similar to the position observed in the GTP data. It is likely given the difficulty in seeing the trigger loop in the matched UTP data that the absolute occupancy of the trigger loop in the catalytic position is less than one. However, given the resolution constraints of the current data we are unable to determine the true occupancy of the trigger loop in the catalytic position.



Supplementary Figure 1

Figure S1. Refined UTP Data Reveals Similar Trigger Loop Location as GTP Data
(A) Similar conformations of the trigger loop in pol II transcribing complexes with matched GTP and UTP. Template DNA, RNA, UTP and GTP are shown in cyan, red, blue and orange, respectively. The trigger loops from GTP data and UTP data are shown in magenta and yellow respectively. Mg^{2+} ions are shown as magenta spheres.

(B) DNA, RNA, trigger loop, and UTP in the A site. The 2Fo-Fc map contoured at 1.0 sigma is shown in blue mesh. Template DNA, RNA and UTP are shown in cyan, red, and blue, respectively. The trigger loop is shown in yellow and Mg^{2+} ions are shown as magenta spheres.

Supplementary Figure 2

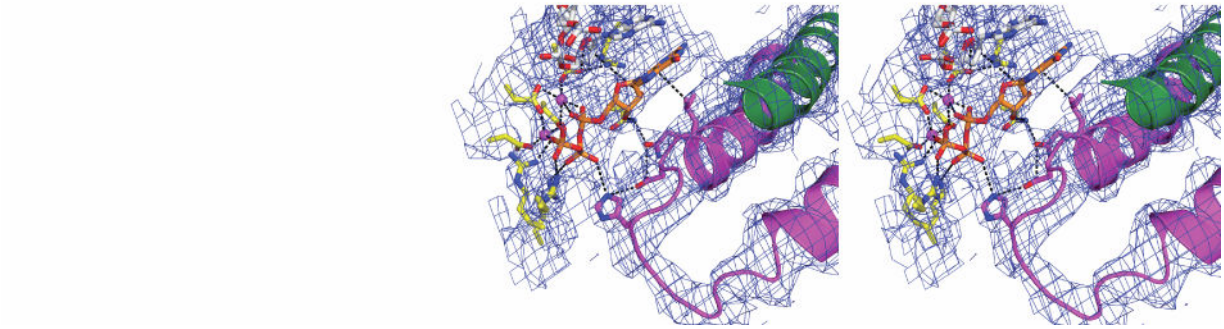
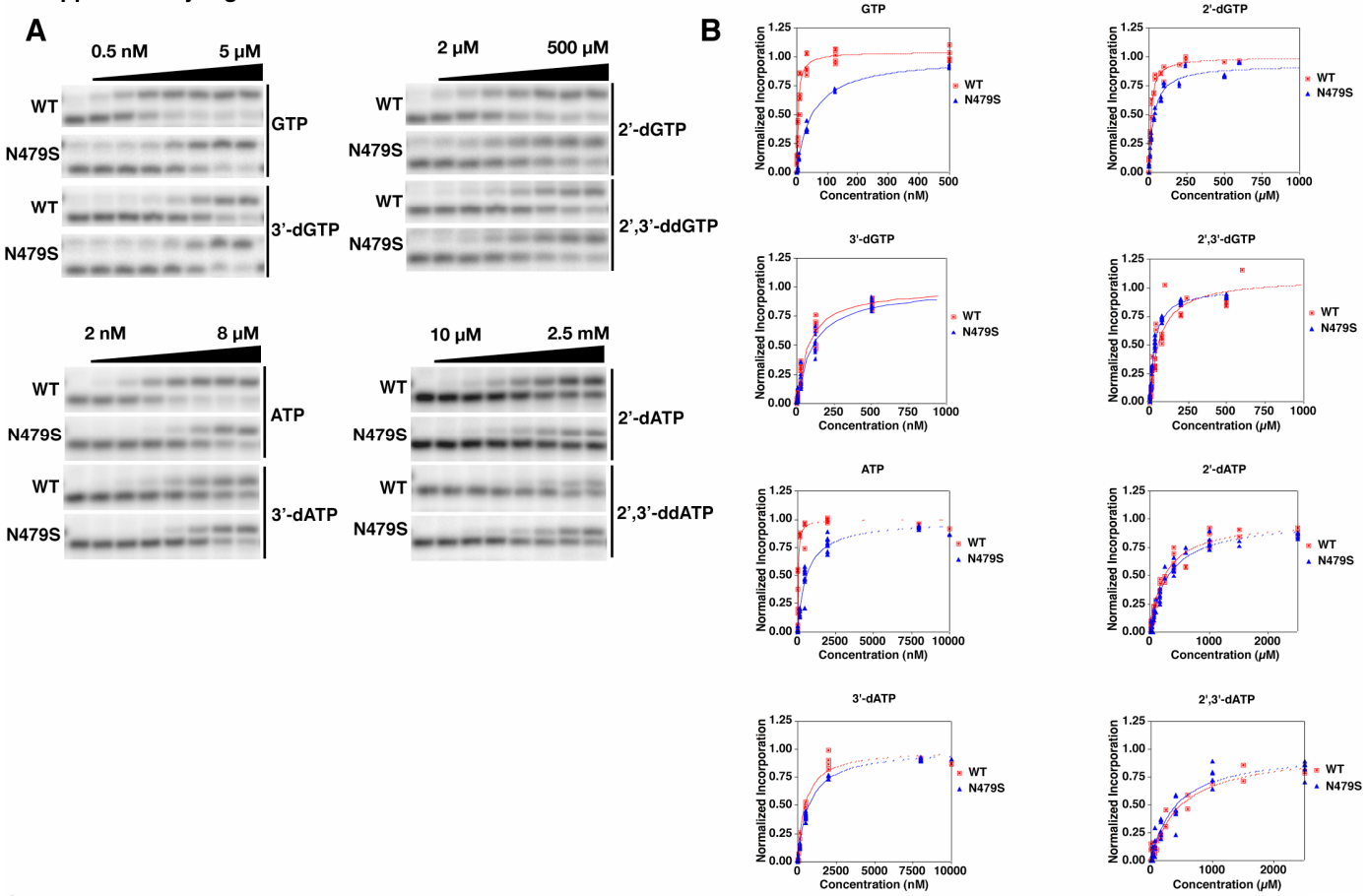


Figure S2. Stereo View of DNA, RNA, Trigger Loop, and GTP in the A Site
The 2Fo-Fc map contoured at 0.8 sigma is shown in blue mesh. Other colors as in Figure S3B.

Supplementary Figure 3



Supplementary Figure 3 (continued)

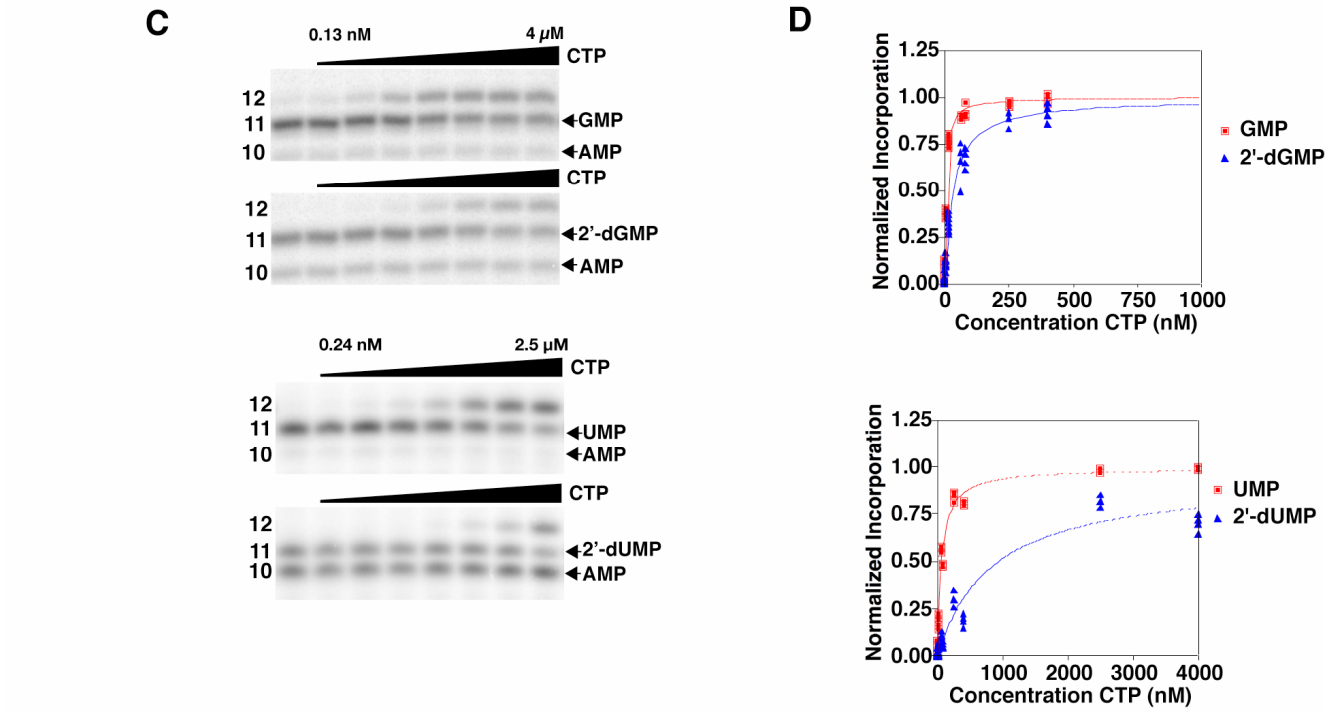


Figure S3. Determination of Apparent K_M Values for Wild-Type and N479S pol II
(A) Examples of the data used to determine apparent K_M values for wild type and N479S pol II, graphically represented in Figure 3A. Incorporation was determined by PhosphorImager analysis as the signal at position 11 divided by the signal at positions 10 and 11, following subtraction of background.
(B) Examples of determination of apparent K_M from plots of nucleotide incorporation vs. substrate concentration for wild type and N479S pol II for rNTP and dNTP substrates from experiments as in (A). The plotted curves were fit by non-linear regression of the data using GraphPad Prism. The maximal level of incorporation for each titration of substrate was taken as the value upon which the data converged by non-linear regression. GraphPad Prism then determined the concentration of substrate in each titration experiment that gave 50% incorporation of this maximal value. Maximal incorporation varied between experiments and was generally between 0.5 and 0.85. This variation between experiments, most likely due to loss of active elongation complexes during experimental manipulation and radio-nucleotide clean up, was greater than any differences in maximal incorporation between wild type and N479S enzymes (data not shown). Reactions for all substrates went to completion at higher concentrations of substrate within the 5 min reaction time except those for 2'-dATP and 2',3'-dATP, indicating the rates of incorporation of these substrates were near V_{max} and that substrate was not the limiting factor in incorporation. Upon longer incubation with these substrates, reactions could achieve similar maximal incorporation values as other substrates (data not shown). Each substrate titration was normalized to the experimentally determined maximal incorporation value for that experiment for presentation purposes. Normalization has no effect on apparent K_M determination. Apparent K_M values were determined from between 4 and 10 individually plotted substrate titrations done on at least two different days.
(C) Data used to determine apparent K_M for wild type pol II incorporation of CTP after incorporation of NMP or 2'-dNMP substrates in the previous position. Transcribing complexes were formed and extended to 10 residues as in Supplementary Figure 3A, and were subsequently extended to 11 residues with UTP, 2'-dUTP, GTP or 2'-dGTP. After removal of unincorporated substrates, complexes were challenged with increasing amounts of template-specified CTP and extension to 12 residues was measured after 5 min. Incorporation values were quantified by PhosphorImager analysis (Molecular Dynamics) as the signal present at position 12 divided by the signal present at positions 11 and 12 after subtraction of background and plotted to determine apparent K_M as above.
(D) Example of plotted data from (C) with non-linear regression curve-fitting (GraphPad Prism).

Supplementary Figure 4

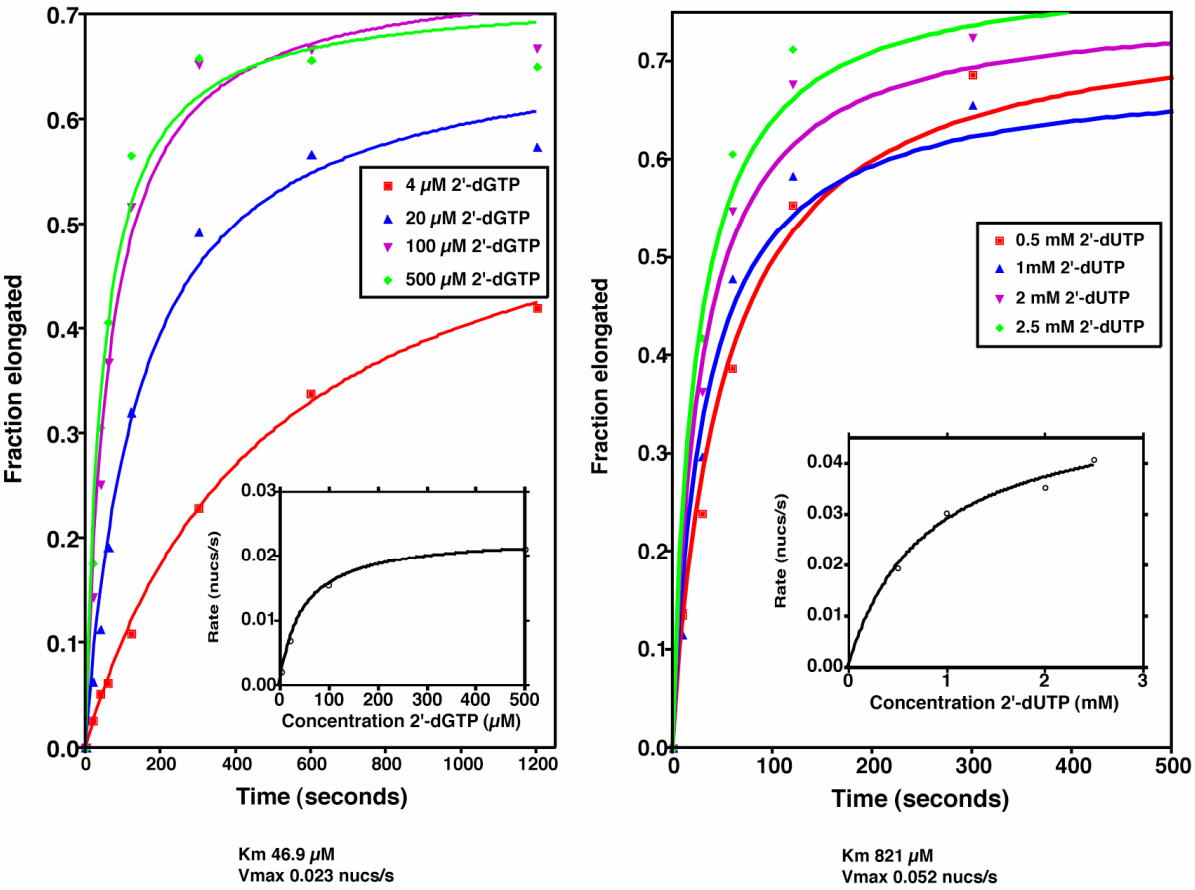


Figure S4. Kinetic Analysis of 2'-dGTP (left) and 2'-dUTP (Right) Incorporation by Wild-Ttype Pol II.

In vitro transcription with pre-formed elongation complexes was performed as in Supplementary Figure 3A. Rates of incorporation for four different concentrations of 2'-dGTP or 2'-dUTP by pol II were determined by plotting substrate incorporation against time. Substrate incorporation was determined by PhosphorImager analysis as the signal present at position 11 divided by the signal at positions 10 and 11 after background subtraction. Rate was determined as the reciprocal of the half-time for maximal incorporation as determined by curve fits of the data using non-linear regression (GraphPad Prism). Elongation rates were plotted versus substrate concentration (graph insets) and curve fitting with non-linear regression was used to determine maximum rate (V_{max}) and the Michaelis-Menten constant for the each substrate (K_M). Values for 2'-dGTP (left) and 2'-dUTP (right) are presented below the data plots for each substrate.