SUPPLEMENTARY INFORMATION

Flexible Receptor Docking of Tgt to RNAP – The 4D-grid docking (1) and flexible receptor with refinement modules (2,3) of the ICM-Dock program (Molsoft, LLC; La Jolla, CA) were used to perform the computational molecular docking experiments. Tgt was docked to the *T. thermophilus* RNAP EC crystal structures [PDB IDs 2O5I and 2O5J; (4,5)] with and without the substrate analog and the Mg2 ion present. Energetically favorable conformations for Tgt in complex with the EC could not be obtained when docking was performed without the NTP, with Mg2, or with the TL in its crystallographic conformation. However, a promising structure was obtained when Tgt was docked to the EC using the following steps:

(**1**) The crystal structure of the *T. thermophilus* RNAP EC (PDB ID 2O5J, 3.00 Å) was imported into the software and converted into an ICM object. During this process, water molecules were deleted, coordinates were assigned to all hydrogens, heavy atoms missing from the PDB entry were added, the most isomeric form was assigned to histidine side chains, and the orientation and positions of glutamine and asparagine side chains, as well as polar hydrogens, were optimized and assigned the lowest energy conformation.

(**2**) The second Mg^{2+} ion (Mg2) was removed from the structure, as suggested by our earlier modeling efforts (data not shown). The ω and α chains were also removed from the structure prior to docking, as these are not thought to play a role in Tgt binding.

(**3**) An ensemble of receptor structures was generated by Monte Carlo sampling of possible conformations of the β' TL (residues T1234-L1256), using the software's default loop sampling options.

(**4**) The top ten conformations in the resultant conformational stack were used to pre-calculate a soft interaction energy function for the receptor on a "4D" grid. For this procedure, the binding site for Tgt was represented as a box surrounding the NTP substrate, the catalytic Mg^{2+} ion, and the active site.

(**5**) An initial three-dimensional structure for Tgt was built from its isomeric SMILES string, and docked to the ensemble 4D grid, using the software's default small-molecule docking options.

(**6**) The top ten docked conformations were then further refined by Biased Probability Monte Carlo sampling and minimization of the ligand and ligand-interacting receptor side chains as previously described (2,3). The top scoring conformation after refinement was evaluated for whether it was located at a biologically reasonable site on the EC in order to accept or reject the null hypothesis that a structurally and biologically reasonable site existed in the existing low-resolution views of the EC in the vicinity of the TL.

SUPPLEMENTAL FIGURES

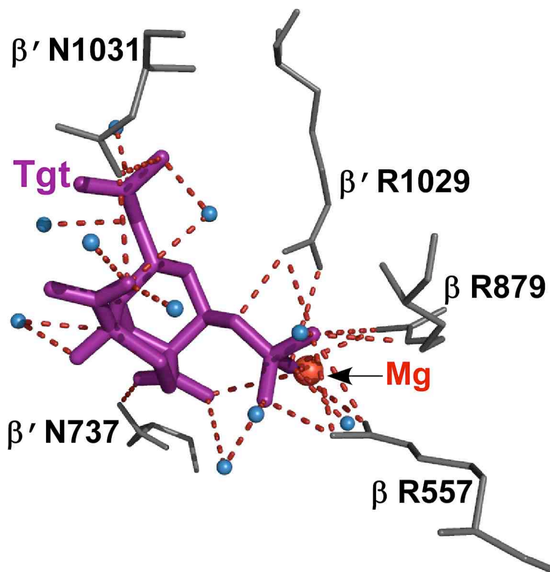
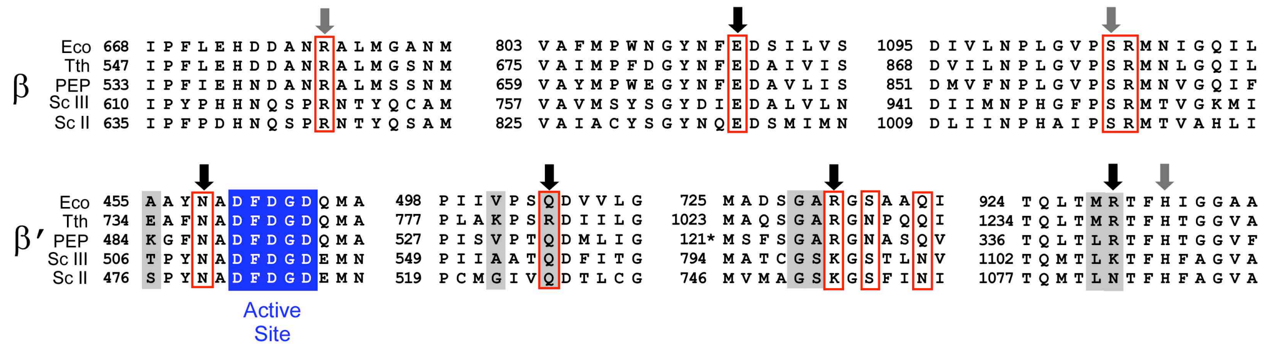


FIGURE S1**. Tgt contacts in the Tth holoenzyme co-crystal** (PDB ID: 2BE5). Tgt is shown as purple sticks, RNAP amino acids - as grey sticks, labeled, solvent – as blue spheres. Polar contacts are indicated by red dotted lines.

A



B

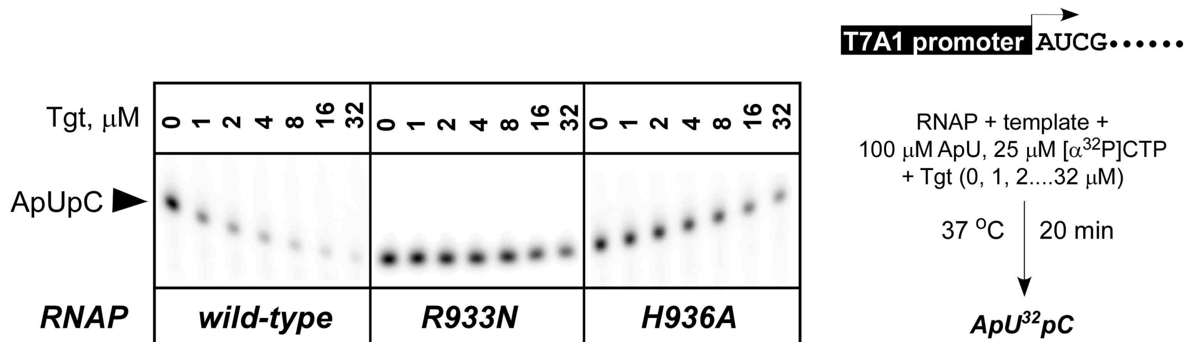


FIGURE S2. **Tgt interactions with RNAP.** (A) Sequence alignment of the β and β′ subunits from bacterial (*E. coli,* Eco*; T. thermophilus,* Tth), chloroplast (*Arabidopsis thaliana,* PEP), and yeast *Saccharomyces cerevisiae* (pol II, Sc II; and pol III, Sc III) enzymes in regions flanking the Tgt contact sites using DNAStar MegAlign. Active site is shown in blue. The Tgt-binding determinants in the holoenzyme complex (6) and in the EC model are shown by red boxes and gray shading, respectively. Residues substituted for Ala in the previous work (6) and in this study are indicated by arrows; black arrows indicate a high level of Tgt resistance *in vitro*, gray - intermediate resistance, white - low resistance. (B) An assay schematic and analysis of the TL mutants β'R933N and β'H936A resistance to Tgt.

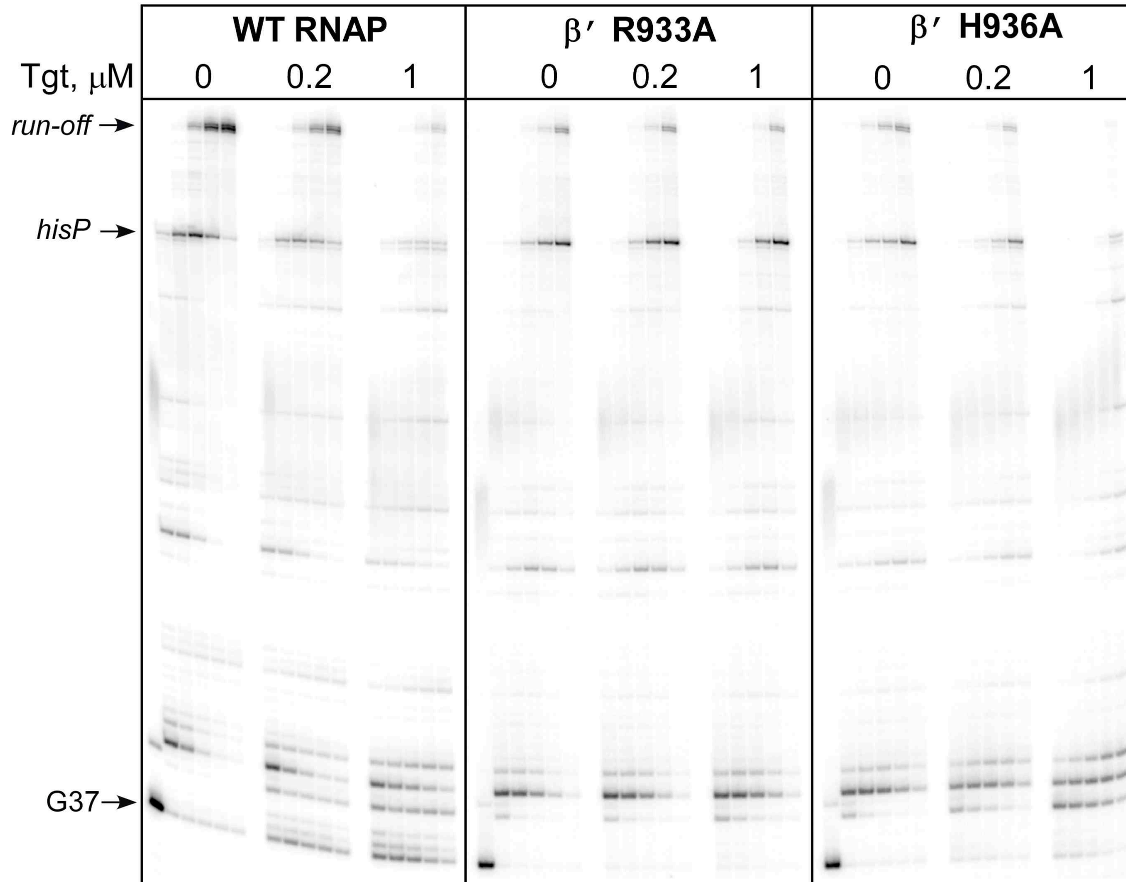


FIGURE S3. **Effect of Tgt on transcription elongation by β'R933A and β'H936A RNAPs**. Halted G37 complexes were formed on a linear pIA349 template (30 nM, shown on top) with holo RNAP (40 nM, WT of mutant), ApU (100 μM) and starting NTPs (10 μM CTP, ATP and GTP, 10 μCi [α^{32}P]-CTP, 3000 Ci/mmol) in TGA2 buffer (20 mM Tris-acetate, 20 mM Na acetate , 2 mM Mg acetate, 14 mM 2-mercaptoethanol, 0.1 mM EDTA, 5% glycerol, pH 7.9) for 15 minutes. Tgt (or an equal volume of water) was added at indicated concentrations, followed by a 2 min incubation at 37°C. Transcription was restarted by addition of NTPs (200 μM ATP, CTP and UTP, 10 μM GTP) and 10 μg/ml heparin. Reactions were quenched at 15, 30, 60, 120 and 240 sec by addition of an equal volume of STOP buffer (10 M urea, 50 mM EDTA, 45 mM Tris-borate; pH 8.3, 0.1% bromophenol blue, 0.1% xylene cyanol) and analyzed on a 10% denaturing urea-acrylamide gel.

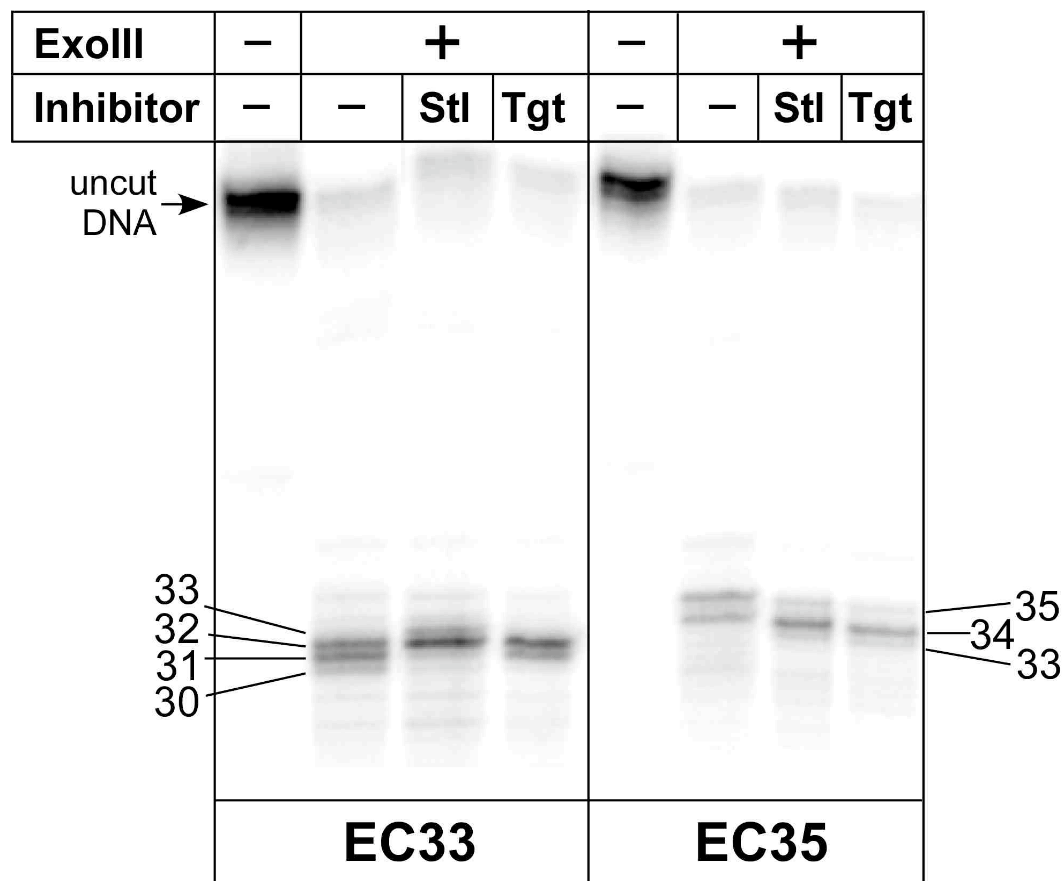


FIGURE S4. **Comparison of Tgt and Stl effects on RNAP translocation**. Halted U33 or G35 complexes were formed at A1 32 DNA (100nM) PCR fragment with holo RNAP (40 nM) as described before (7). The DNA fragment was labeled at the non-template strand. Briefly, halted ECs were incubated with 1.5 mg/ml heparin for 5 min, washed trice with TB1000 (40 mM Tris-HCl, pH 8.0,10 mM $MgCl_2$, 1M NaCl) and trice with TB100 (same as TB1000 but with 100 mM NaCl). The volume was adjusted to 10 µl and 30 units of ExoIII (NEB) were added, followed by a 5-minute incubation at room temperature. Where indicated, AMP (1 mM), Tgt (2U/µl), or Stl (50 ng/µl) were added to the EC 5 minutes prior to ExoIII. Reactions were stopped by addition of 8M Urea, 20 mM EDTA, 40 mM Tris-HCl, pH 8.0. Positions of the active center corresponding to the footprinting bands are indicated.

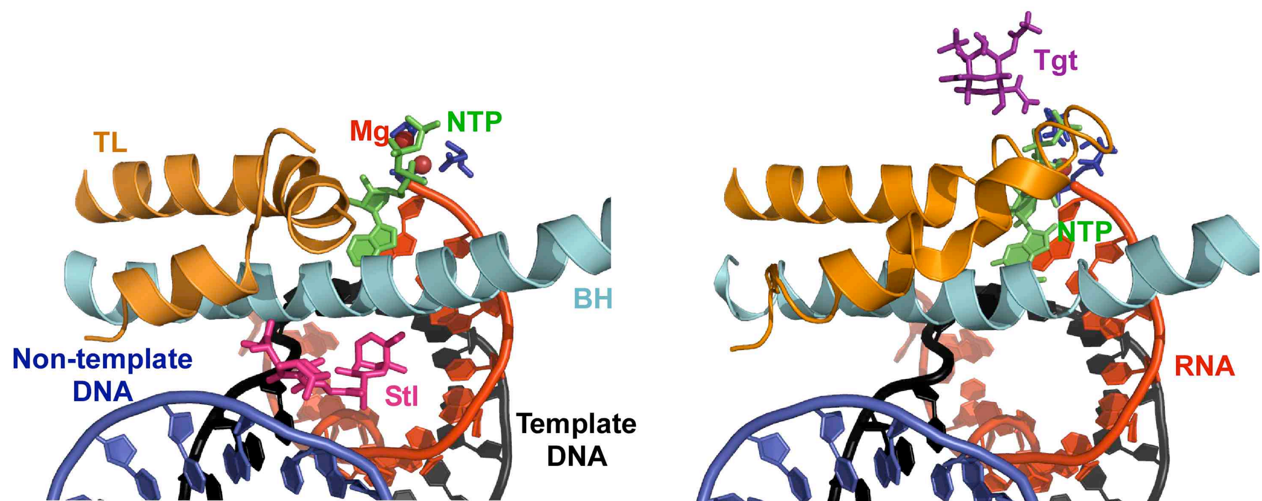


FIGURE S5. **Stl and Tgt trap different inactive states of the TL.** Comparison of Stl (PDB ID: 2PPB, left) and Tgt (model, right) binding poses relative to the RNAP elongation complex. Stl (pink sticks), Tgt (purple sticks), the β' bridge helix (BH, teal cartoon), the β' trigger loop (TL, orange cartoon), Mg^{2+} (red sphere), the catalytic Asp triad (blue sticks), the RNA transcript (red cartoon), the non-template DNA strand (blue cartoon), the template DNA strand (black cartoon), and the substrate NTP (green sticks) are shown.

SUPPLEMENTAL REFERENCES

1. Bottegoni, G., Kufareva, I., Totrov, M. & Abagyan, R. (2009) *J Med Chem* **52**, 397-406.
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