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Supplemental Information

T7 RNA Polymerase Discriminates Correct and Incorrect Nucleoside Triphosphates by Free Energy

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Supporting information

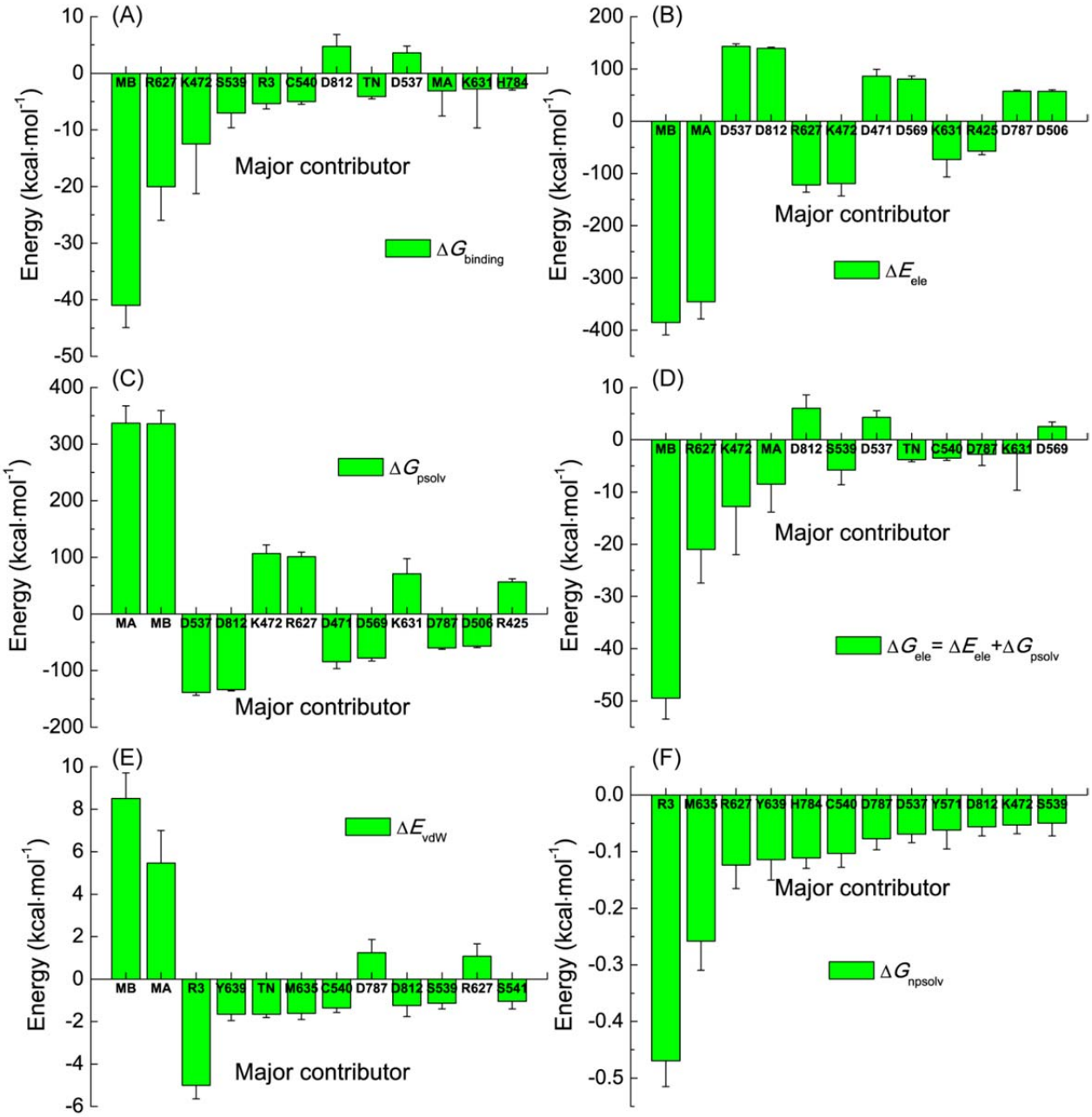

Fig.S1. Details of binding free energy for ATP-T7 RNAP complex in the Insertion state. (A) Per-residue binding free energy ($\Delta G_{binding}$); (B) electrostatic energy (ΔE_{ele}); (C) polar solvation free energy calculated by GB (ΔG_{psolv}); (D) total electrostatic energy contribution ($\Delta G_{ele} = \Delta E_{ele} + \Delta G_{psolv}$); (E) van der Waals contribution (ΔE_{vdw}); (F) nonpolar solvation free energy (ΔG_{npsolv}). Only the top 12 major contributors were present in each individual energy term.

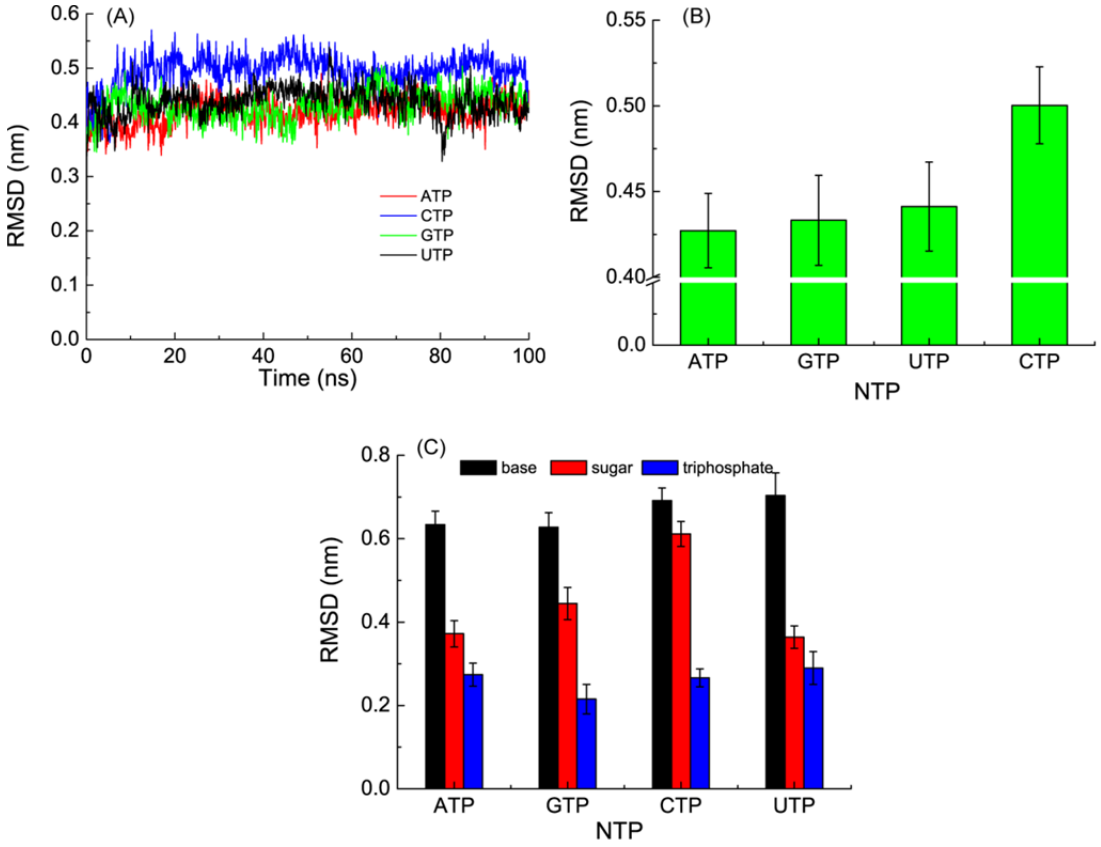

Fig.S2. Comparison of RMSD for the four NTPs in 100 ns unbiased MD simulations. (A) Time evolution of RMSD for the four NTPs with CA atoms of the T7 RNAP as reference; (B) Average RMSD values; (C) RMSD contributions from base, sugar and triphosphate.

Fig.S2A displays the time dependence of root-mean-square-deviation (RMSD) values of the four NTPs incorporated in the insertion site, which were determined using CA atoms of the T7 RNAP to align simulation snapshots. It can be seen that all the simulations almost reach equilibriums after 20 ns. We performed statistics on the RMSD data of the later 80 ns, as shown in Fig.S2B. The average RMSD values for the four NTPs are in ascending order: ATP < GTP < UTP < CTP. It is clear that ATP has the smallest average RMSD value, indicating the correct NTP is most stable in the active site. We further decomposed the RMSDs into contributions from triphosphate, sugar and base, as

shown in Fig.S2C. The result shows that the three components with contributions from low to high are: triphosphate < sugar < base. As mentioned above, most of the charges of NTP molecule are distributed on the triphosphate. Therefore, the movement of triphosphate is constraint by electrostatic interaction network, leading to the smallest RMSD as compared with that of base and sugar. Sugar part, connecting triphosphate and base of NTP, has the middle RMSD values. Base part has the largest RMSD values. Careful examination suggests ATP and GTP bases have relatively smaller RMSD values than UTP and CTP bases, indicating the correct substrate binds to the active site with favorable contacts; GTP base, structurally similar to ATP base, has the close RMSD value. From the RMSD results, we found that the inserted correct substrate is well stabilized in the active site due to favorable contacts.