Supplementary Information for "A critical residue selectively recruits nucleotides for T7 RNA polymerase transcription fidelity control"

Interaction (kcal/mol)	ATP			dATP		
	ele	vdw	non -bond	ele	vdw	non-bond
Tyr639-dNT	1.4 ± 0.9	-3.2 ± 0.8	-1.7 ± 0.9	-0.2 ± 0.8	-8.4 ± 0.9	-8.5 ± 1.2
Tyr639-rNT	1.0 ± 0.9	-1.9 ± 0.9	-0.9 ± 0.7	-0.4 ± 0.2	-0.3 ± 0.2	-0.7 ± 0.3
Phe639-dNT	1.2 ± 0.3	-0.7 ± 0.4	0.5 ± 0.4	1.3 ± 0.6	-2.4 ± 1.4	-1.1 ± 1.5
Phe639-rNT	-0.2± 0.1	-0.2 ± 0.1	-0.4 ± 0.2	-0.1 ± 0.2	-0.3 ± 0.1	-0.4 ± 0.2

Interaction (kcal/mol)	GTP			UTP			CTP		
	ele	vdw	non -bond	ele	vdw	non-bond	ele	vdw	non-bond
Tyr639-dNT	2.3 ± 1.1	-5.3 ± 1.1	-3.0 ± 1.5	1.1 ± 0.4	-2.2± 0.5	-1.1± 0.6	1.5± 0.7	-4.3± 2.4	-2.8± 2.2
Tyr639-rNT	-1.0± 2.6	-2.1 ± 1.2	-3.1 ± 2.8	-0.2± 0.3	-0.4±0.1	-0.6± 0.3	0.2± 0.6	-1.0 ± 0.5	-0.8± 0.6

Table S1 Interactions between Tyr639 and the end bp (the DNA nucleotide, dNT and the RNA nucleotide, rNT) in the wild type, in the case of the ATP, dATP, GTP, UTP, CTP pre-insertion, or interactions between Phe639 and the end bp (dNT and rNT) in the mutant Y639F, in the case of ATP and dATP pre-insertion.

Interaction (kcal/mol)	ATP			dATP		
	ele	vdw	non -bond	ele	vdw	non-bond
Tyr639	-1.6± 3.1	-1.3± 0.8	-2.9 ± 2.7	-3.5 ± 3.3	-0.3± 0.9	-3.9 ± 3.0
Phe639	-1.1± 0.3	-0.9± 0.4	-2.1±0.7	-2.7± 1.0	-1.9± 0.6	-4.6± 1.2

Interaction (kcal/mol)	GTP			UTP			CTP		
	ele	vdw	non -bond	ele	vdw	non-bond	ele	vdw	non-bond
Tyr639-dNT	-5.3± 3.6	-2.4± 1.3	-7.7 ± 4.2	-8.1± 1.6	-0.2± 1.3	-8.3± 1.1	-8.8± 5.3	-1.0± 1.2	-9.8± 5.1

Table S2 Interactions between Tyr639 and the pre-inserted ATP, dATP, GTP, UTP, CTP in the wild type, or interactions between Phe639 and the pre-inserted ATP or dATP in the mutant Y639F.

Interaction (kcal/mol)	ATP			dATP		
	ele	vdw	non -bond	ele	vdw	non-bond
wild-type dT	-1.4± 3.5	-1.0± 0.9	-2.4 ± 3.8	1.0± 0.4	-.02± .02	0.9 ± 0.4
Mutant dT	0.5± 2.0	-0.8± 0.9	-0.3± 1.7	-4.3± 4.6	-1.1±1.1	-5.4± 4.5

Interaction (kcal/mol)	GTP			UTP			CTP		
	ele	vdw	non -bond	ele	vdw	non-bond	ele	vdw	non-bond
Wild-type dT	-0.4± 2.2	-0.3± 0.4	-0.7 ± 2.3	-1.2± 2.1	-1.2± 1.1	-2.4± 1.7	1.5± 1.7	-0.5± 0.4	1.1± 1.8

Table S3 Interactions between the template dT (the one to pair with the right NTP) and the pre-inserted ATP, dATP, GTP, UTP, CTP in the wild type, or interactions between the template dT and the pre-inserted ATP or dATP in the mutant Y639F.

Non-bond Interaction (kcal/mol)	rATP-			dATP		
	2'-OH	rest part	sum	2'-H	rest part	sum
Y639-OH	0.7± 0.7	1.1 ± 2.5	1.8 ± 2.6	-0.1± 0.03	-0.05± 1.9	-0.2 ± 1.9
F639-H	-0.9± 0.4	-2.2± 0.6.	-3.1 ± 0.7	0.1± 0.05	-3.2± 1.0	-3.1 ± 1.0

Table S4 Interactions between the hydroxyl group in Tyr639 (Y639-OH) and rATP/dATP in the wild type, and interactions between the corresponding group (F639-H) and rATP/dATP in the mutant Y639F.

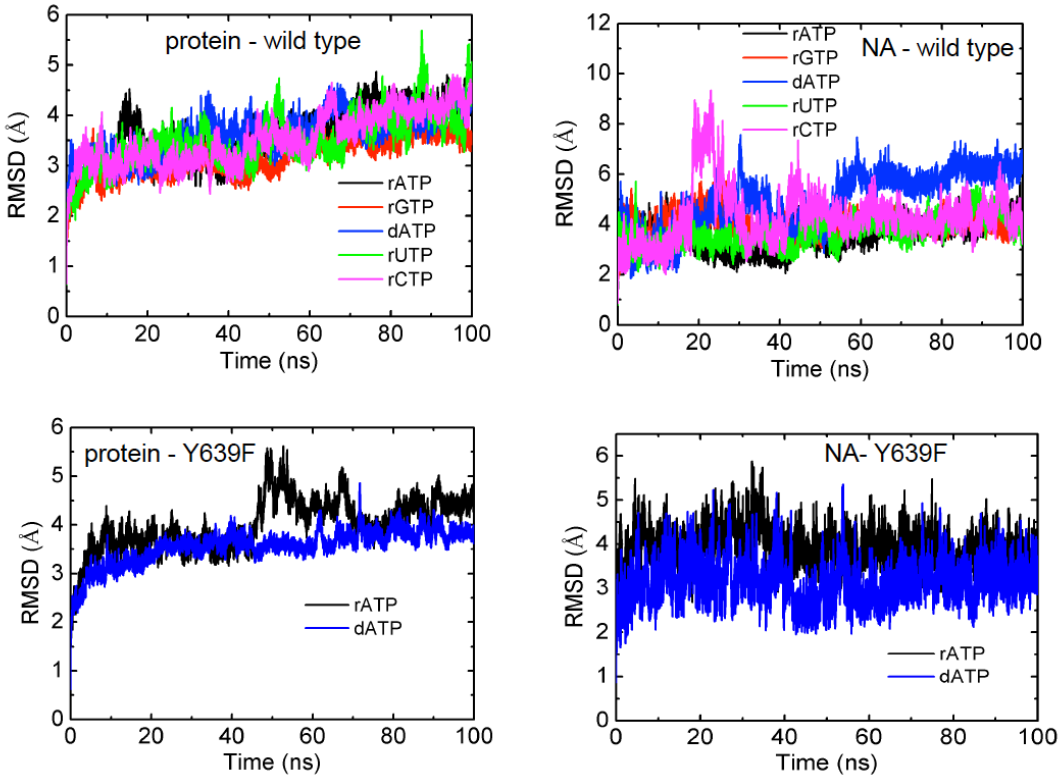


Fig S1 RMSDs for the pre-insertion structures. Upper row is for the wild type T7 RNAP: for protein (left; CA atoms) and NA (right; P atoms), and for pre-insertion of rATP, rGTP, rUTP, rCTP and dATP. Bottom row is for the mutant Y639F, pre-insertion of rATP and dATP, respectively.

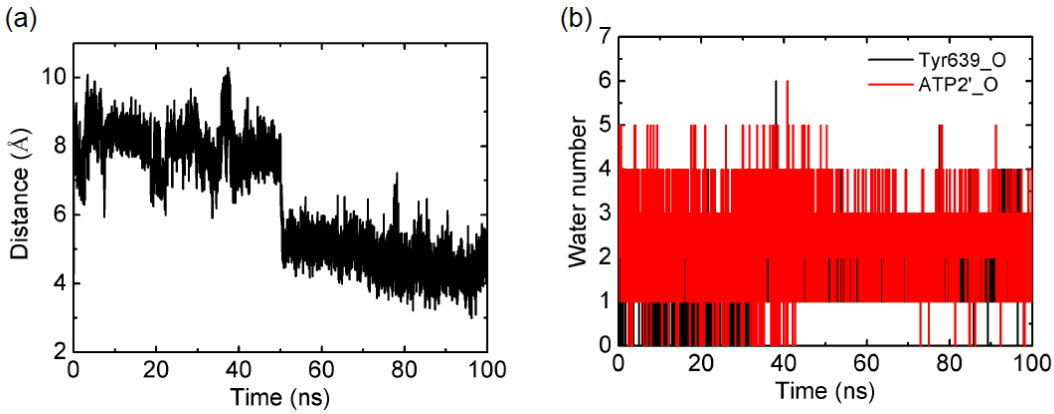


Fig S2 Distance measure between Tyr639-OH and 2'-OH of rATP and water counts around the two groups in the rATP pre-insertion simulation. (a) The distance measure vs. time, as the distance is defined between two oxygen atoms in the respective groups. (b) The number of water molecules were counted within ~3.5 Å of respective oxygen atoms.

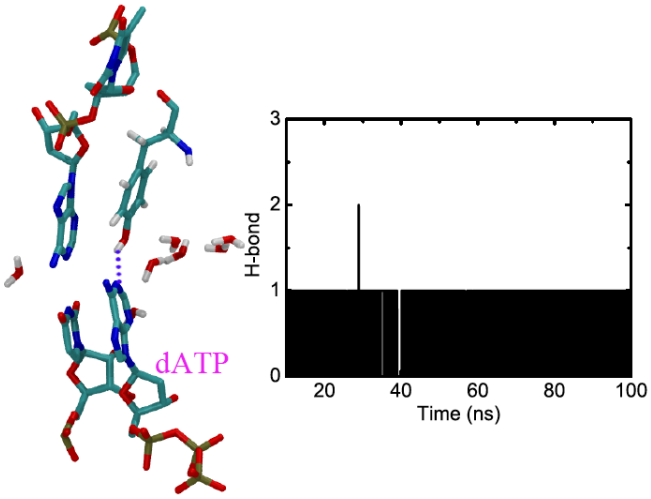


Fig S3 HB interaction between Tyr639-OH and 1-N of dATP base in the dATP pre-insertion simulation.

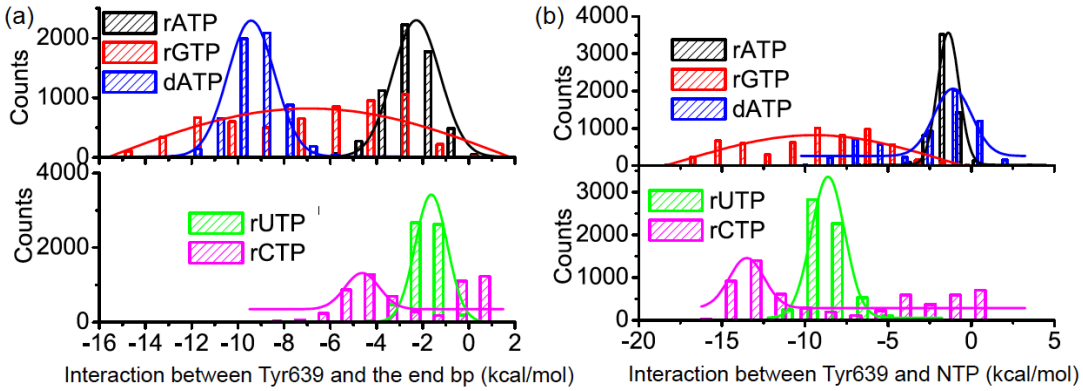


Fig S4 Interactions that stabilize Tyr639 in the active site of the pre-insertion complex with different nucleotides bound. The histograms are sampled from 40 ns – 100 ns in each individual simulation (comparing to **Fig 4 i**n main). (a) The interactions between Tyr639 and the end bp of the DNA-RNA hybrid, in case of different nucleotide at pre-insertion. (b) The interactions between Tyr639 the pre-inserted nucleotide (rATP, rGTP, dATP, rUTP and rCTP).

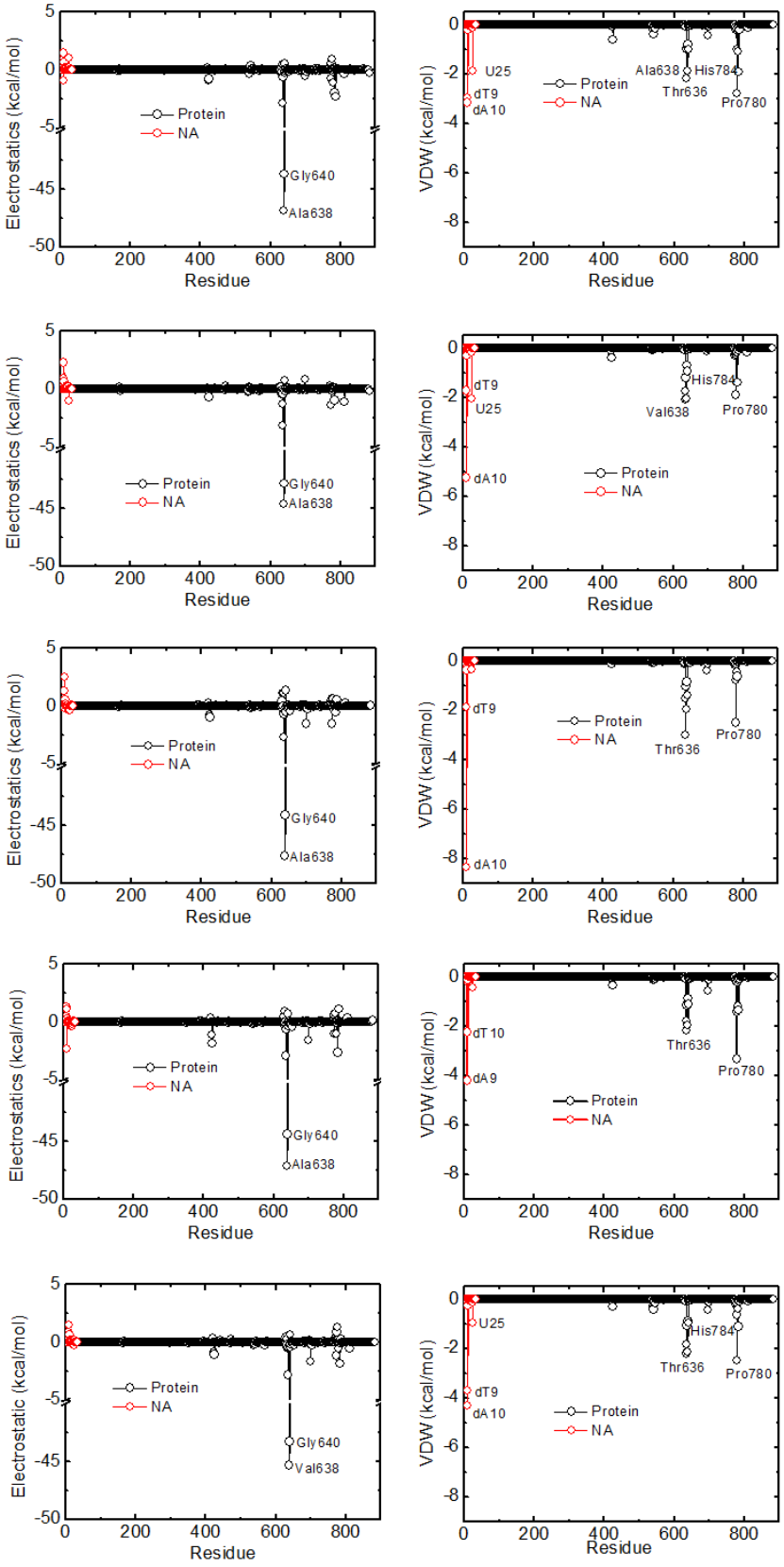


Fig S5 Average interactions between Tyr639 and any amino acid or nucleotide in the pre-insertion complex, in the case of ATP, GTP, dATP, UTP and CTP pre-insertion, from up to down (note dA10-U25 is the end bp, while dT9 is the template nt to pair with).

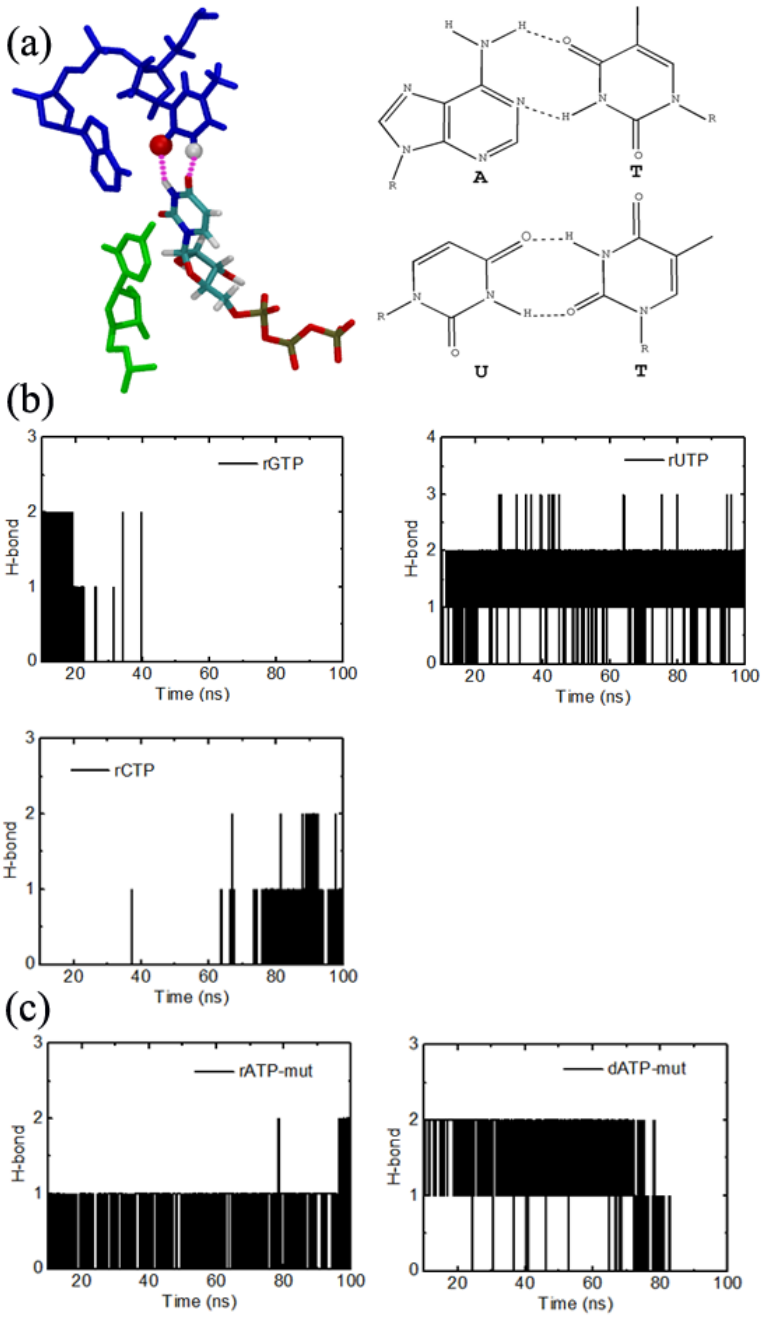
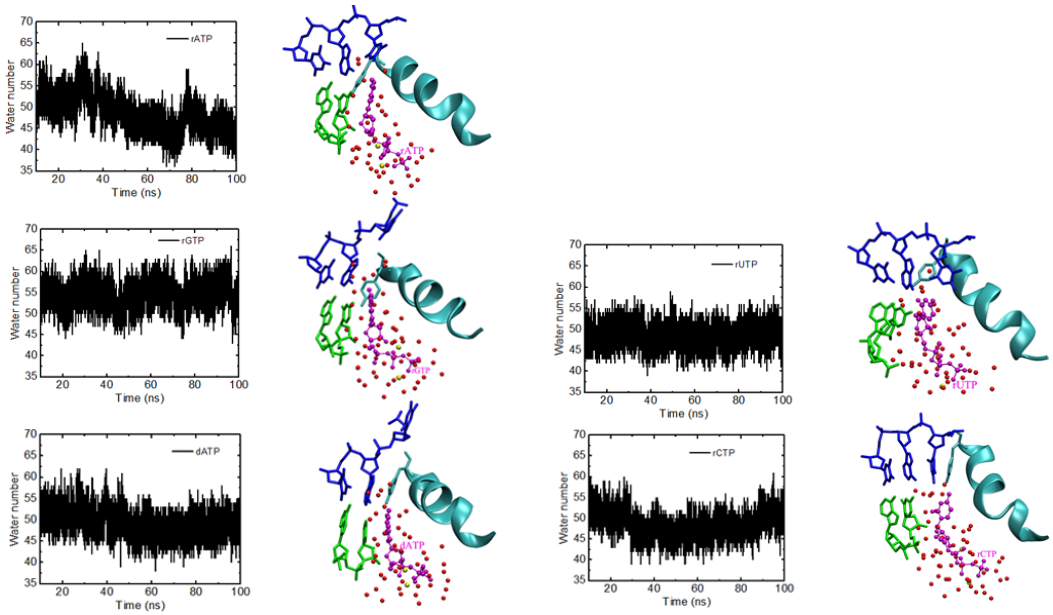


Fig S6 Hydrogen bonding interactions in the non-standard Watson-Crick base pairing or in the mismatched cases. (a) The structure view of the non-standard hydrogen bonding interactions between the template dT and the pre-inserted rU (left); the illustrations of the standard base pairing rA-dT and the non-standard rU-dT are shown for comparison (right). (b) The number of the hydrogen bonds formed between the template dT and the pre-inserted rG, rU, and rC in the wild type (note there is No hydrogen bonds formed at all in dA pre-insertion). (c) The number of the hydrogen bonds formed between the template dT and the pre-inserted rA and dA in the mutant polymerase Y639F.



(b)

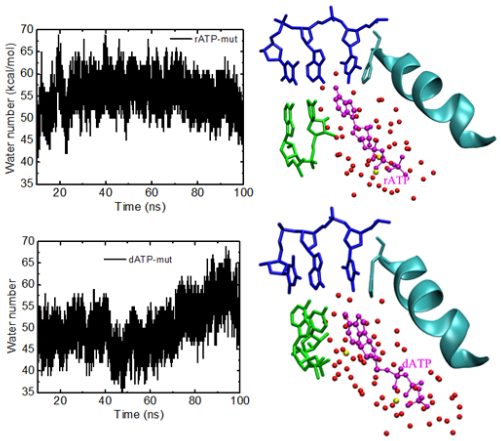


Fig S7 Hydration patterns in the active site of the pre-insertion complex, for the pre-insertion of rA, rG, dA, rU, and rC in the wild type (a), and for the pre-insertion of rA and dA in the mutant polymerase Y639F (b). The number of water molecules within 5 angstrom distance of the active site is calculated along the 100-ns trajectories (left column), while the water distribution (orange spheres) in the end of the simulation is shown in the active site (right column). Note that Tyr/Phe639 is shown in cyan, while the NTP molecule is shown in magenta.

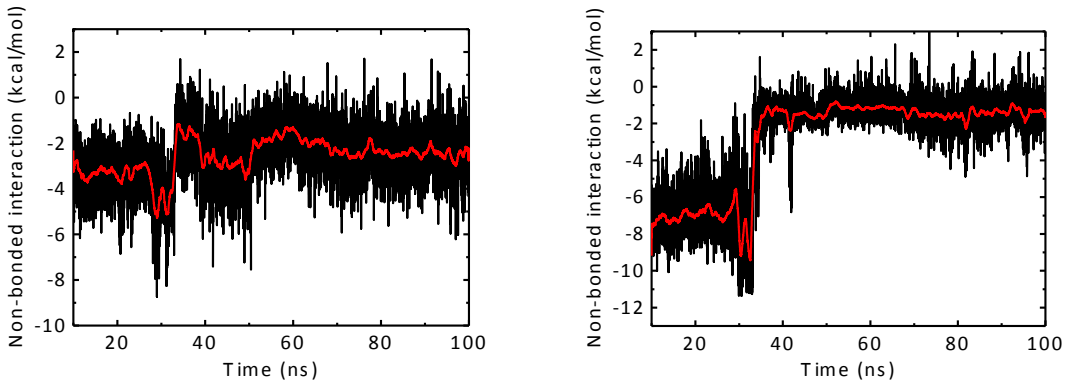
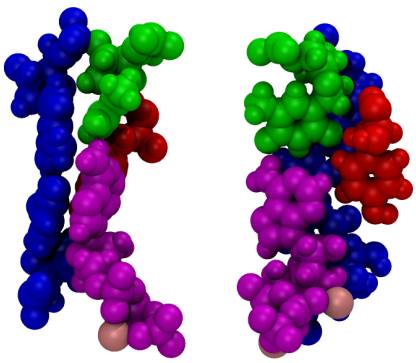
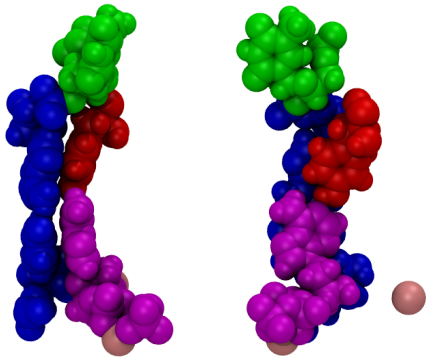


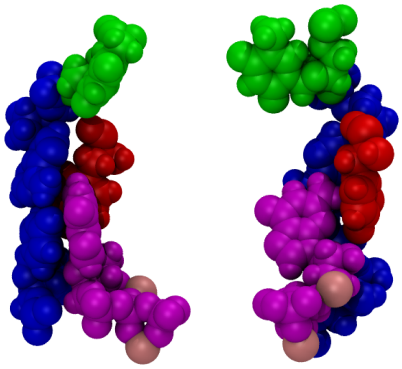
Fig S8 The local non-bond interactions between Tyr639 and the end bp of the DNA-RNA hybrid (left) and that between Tyr639 and rA in the 100 ns simulation of the pre-insertion complex. Prior to the Watson-Crick base pairing (after ~ 50 ns), both interactions appeared fairly strong that stabilize Tyr639 in the active site. The formation of base pairing 'frees' Tyr639 from the local stabilization.



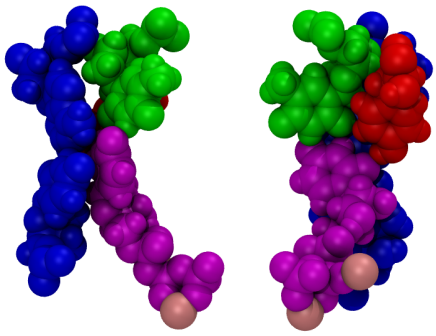
(a) rATP



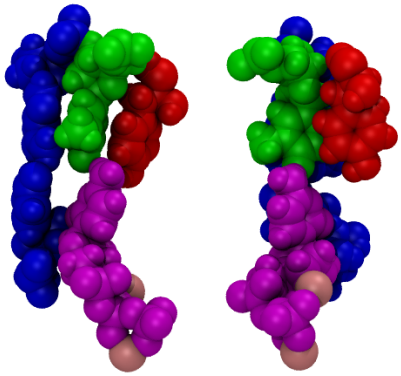
(b) dATP



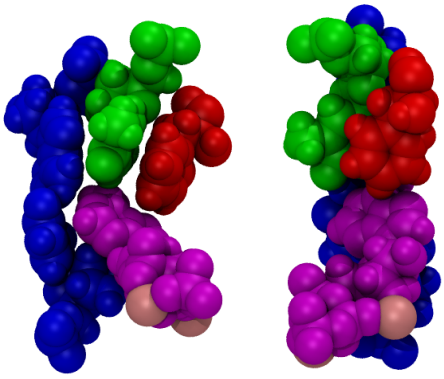
(c) rGTP



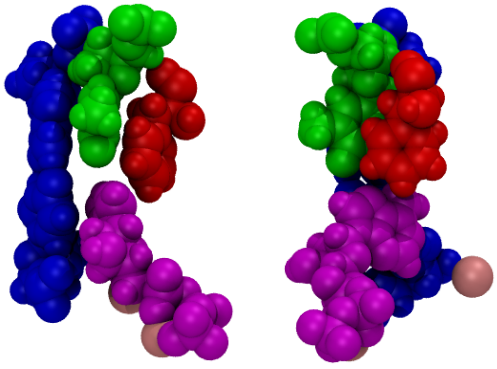
(d) rUTP



(e) rCTP



(f) rATP – Y639F



(g) dATP – Y639F

Fig S9 The front and side atomistic views of the active site, captured in each pre-insertion complex at the end of 100 ns simulation. Color scheme the same as Fig 6 in main (Y639/F639, red; template dT, green; the end bp, blue; the pre-inserted NTP, magenta; Mg^{2+}, pink).

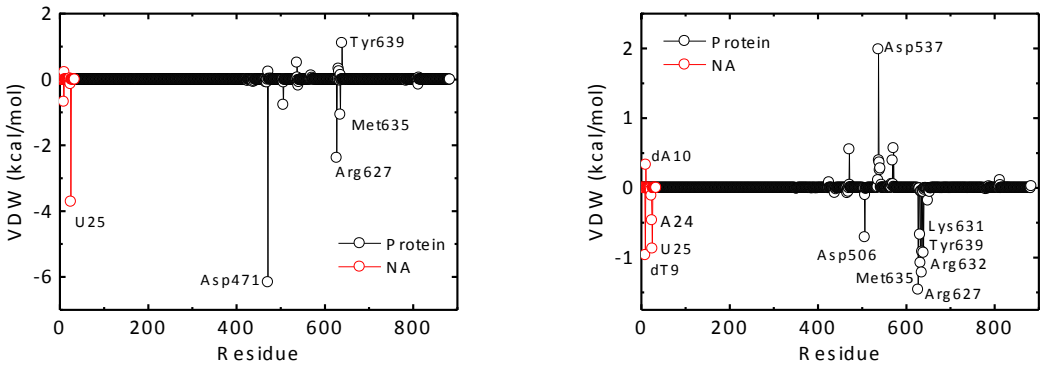
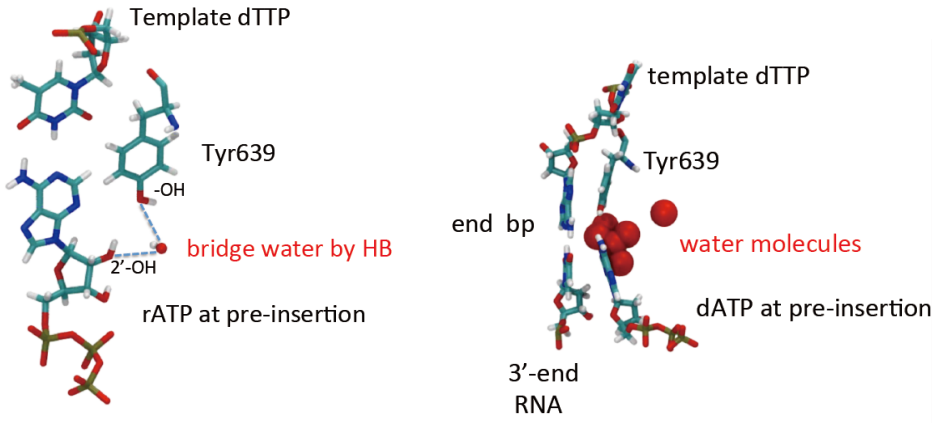


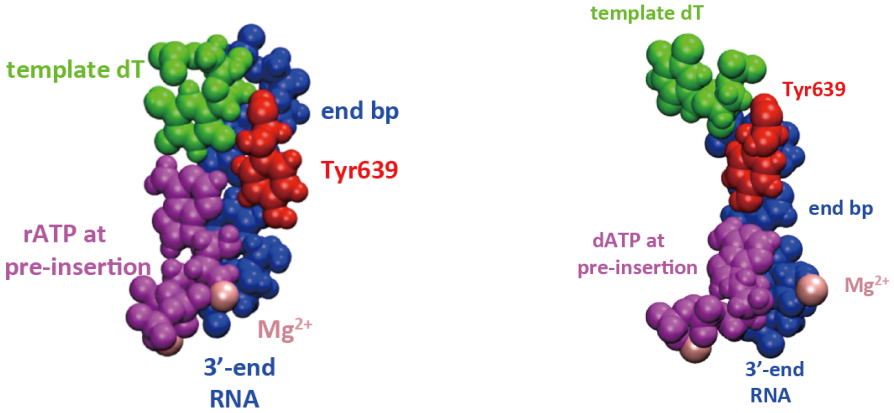
Fig S10 The vdW interaction contributions to relative stabilization of NTP at pre-insertion, coming from individual amino acids and nucleotides. Negative values of the energetic contribution indicate those that stabilize the right rA relative to rG (left) or dA (right). One can see that Tyr639 destabilizes rA more than rG for ~ 1 kcal/mol (left), and stabilizes rA more than dA for ~ 1 kcal/mol (right). Note that the electrostatic contribution from Tyr639 on the NTP stabilization is negligible.

Movie Illustrations (Movie S1 and S2)



Movie S1 (left): Transient water molecule bridges in between Y639-OH and 2'-OH of rATP in the rATP pre-insertion (made of 459 frames out of the 9000 frames of the 10 -100 ns simulation); **Movie S2** (right): Stabilized water molecules stay in between Tyr639 and dATP base in the dATP pre-insertion (made from the 10 – 100 ns MD simulation).

Movie Illustrations (Movie S3 and S4)



Movie S3 (left): Pre-insertion of rATP with the formation of the WC base pairing rA-dT (see water bridge in **Movie S1** as well); **Movie S4** (right): Pre-insertion of dATP with a pair association between Tyr639 and dATP (see immobilized water molecules in **Movie S2** as well). The color code and representation are the same as **Fig 6** in main and **Fig S9**. All movies were obtained from the 10 – 100 ns MD simulations, and were made using the graphic viewer Visual Molecular Dynamics or VMD (http://www.ks.uiuc.edu/Research/vmd/).