Supporting information

The hepatitis C virus RNA-dependent RNA polymerase directs incoming nucleotides to its active site through magnesium-dependent dynamics of its motif F

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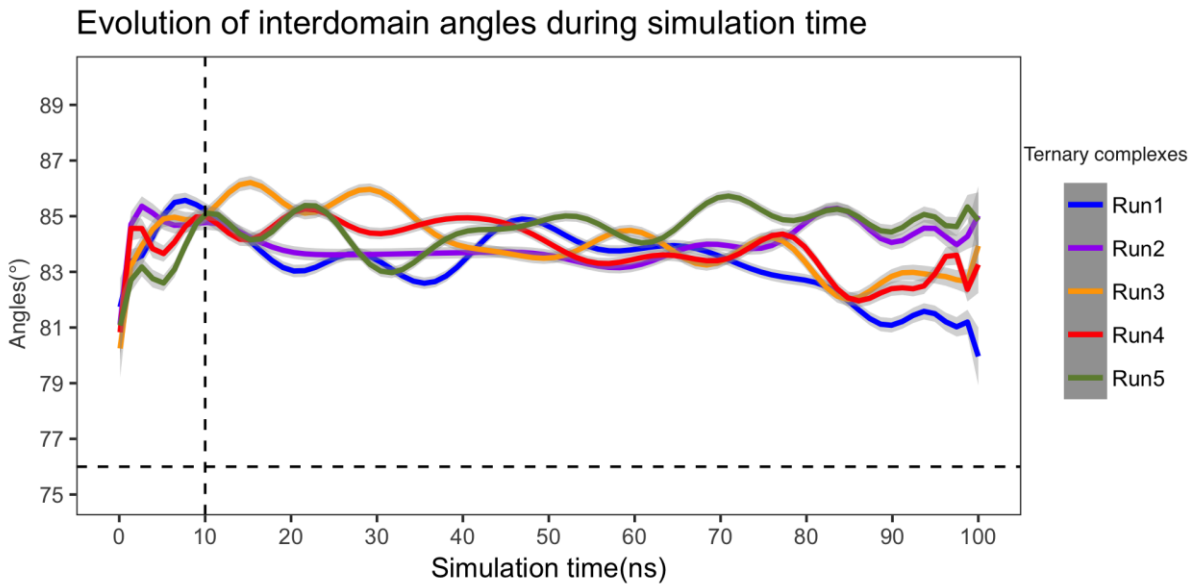
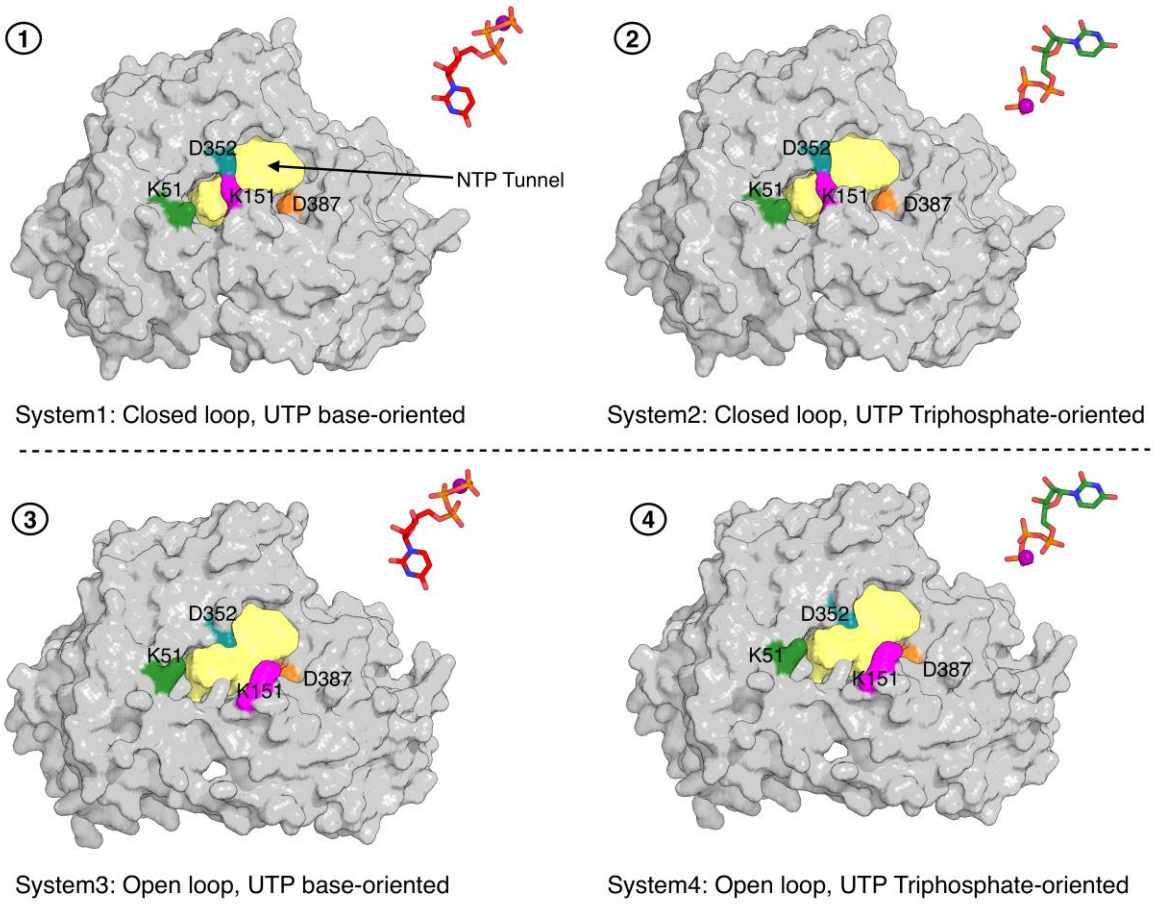
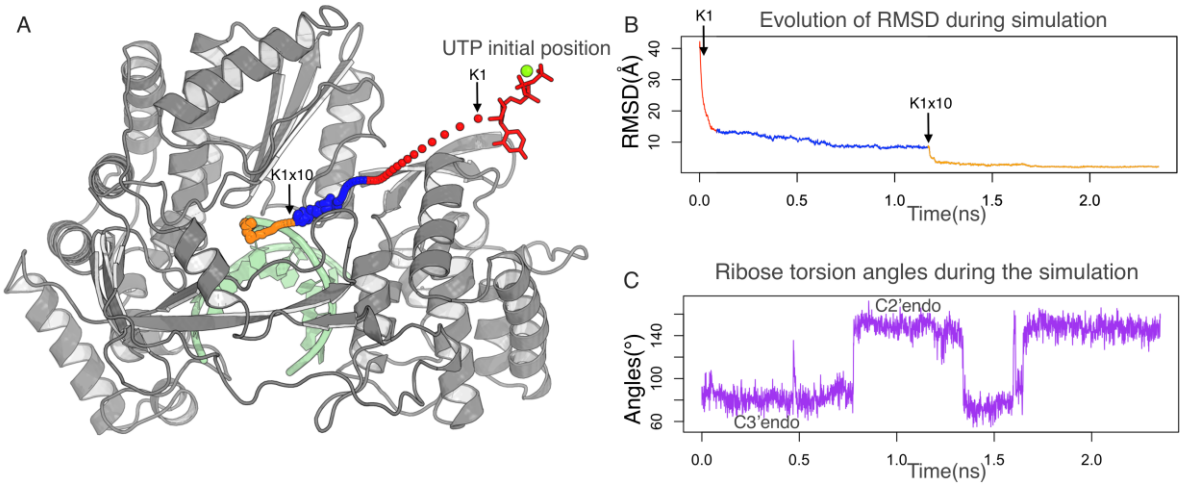
Supplementary figures



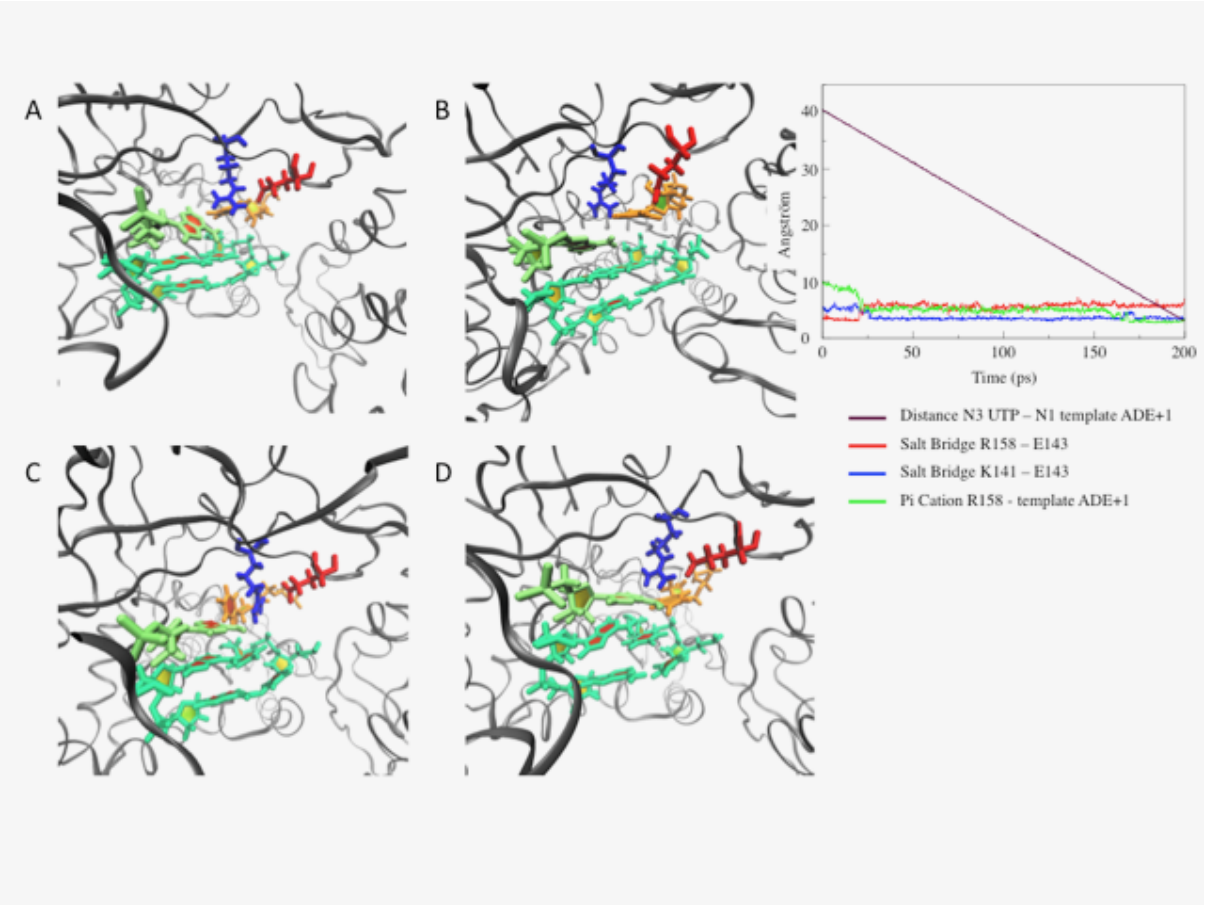
Supplementary Figure S1. NS5B interdomain angle as a function of time for 5 replicas of the ternary complex simulation derived from PDB 4WTA. The angle is computed as the angle between the two vectors connecting the centers of masses of palm and fingers on the one hand, palm and thumb on the other. Fingers: residues 1-188+227-287; Palm: residues 189-226+288-370; Thumb: residues 371-529. The horizontal dashed line indicates the 76° value for 4WTA. The vertical dashed line indicates the limit between pre-production and production in our analyses.



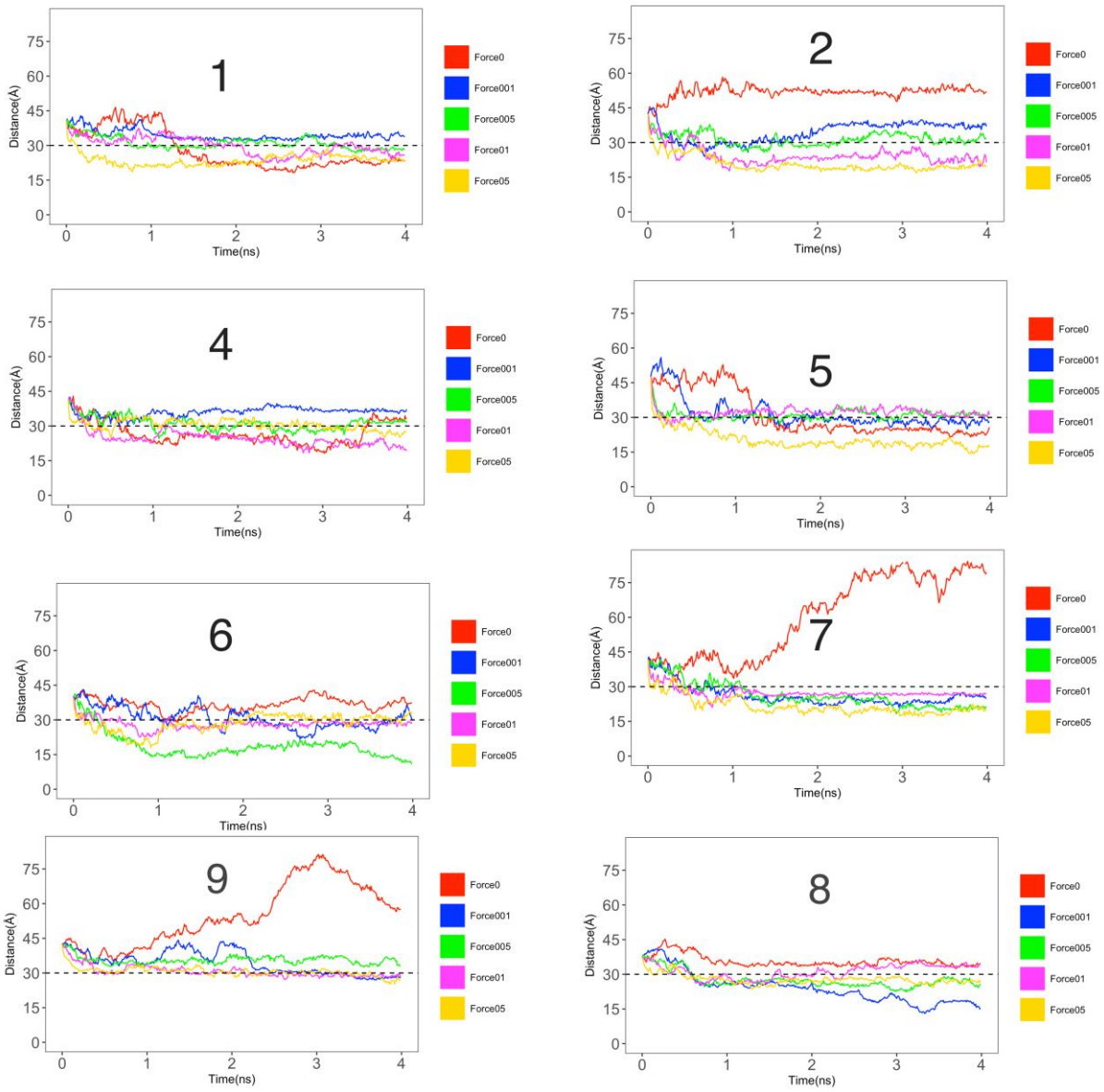
Supplementary Figure S2. The four combinations of entry loop conformations and UTP orientations used in entry TMD. NS5B is displayed in surface representation with the limits of the NTP tunnel as a yellow surface. K151 on the entry loop and D352 on the other side of the NTP tunnel are shown, as well as K51 and D387 on either side of the entry loop.



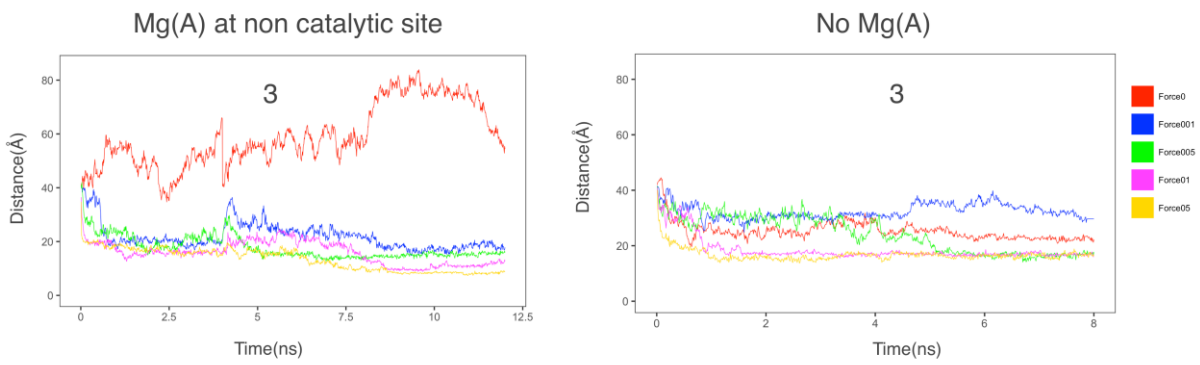
Supplementary Figure S3. Targeted molecular dynamics simulations of passage of UTP with Mg(B) along the NTP tunnel of the NS5B binary complex. In this figure results for the (closed entry loop, UTP base-oriented) system are shown (Supplementary Figure S2, panel 1). TMD requires calibration of the energy constant K for the particular system and RMSD subsystem (defining the energy penalty) considered. We tried a range of constants and monitored the system's response. An initial value of K_1=0.01 Kcal/mol/Å^2 was retained as one that allows the nucleotide to penetrate the entry tunnel with no apparent artefact (pushing aside of protein parts). (**A**) Trajectory of the center of mass of UTP against a representation of the binary complex. The initial position of UTP is shown (red sticks) together with Mg(B) (green sphere). Two successive energy constants K_1 (red and blue parts of curves) and 10 x K_1 (orange part) were applied. (**B**) RMSD between UTP during the simulations and UTP in the ternary complex of Figure 1. (**C**) Ribose torsion angle during the simulations. The characteristic values for the major conformations C3'-endo and C2'-endo are indicated. Note that the final small movement of the UTP center of mass under K_1 (blue part of the curve) is not due to a further advance but rather to a crunching up of UTP thanks to a switch in ribose conformation from C3'-endo (the preferred ribose conformation, and the one it assumes at the NS5B active site) to C2'-endo.



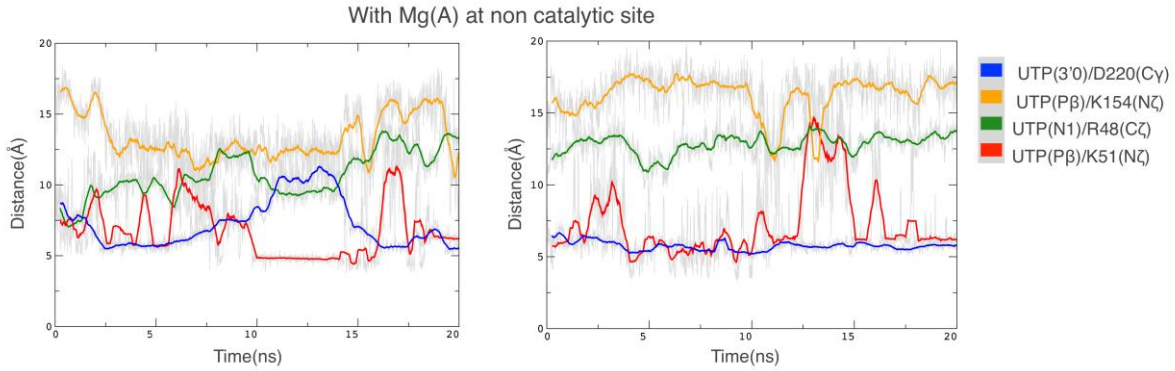
Supplementary Figure S4. Four typical scenarios obtained in entry SMD. The endpoints of four simulations with similar parameters are shown. The template/primer RNA is shown in bright green with the +1 base in lighter green. UTP is in orange, R158 in blue and E143 in red. (**A**) The progress of the nucleotide is stopped at the R158-E143 salt bridge. (**B**) The salt bridge R158-E143 breaks off before the nucleotide arrives, clearing the passage to the template +1 adenosine. The right panel shows that this occurs well in advance of UTP approach. (**C**) The nucleotide passes next to the salt bridge but misses the +1 base. (**D**) The nucleotide passes under the salt bridge and reaches the +1 base.



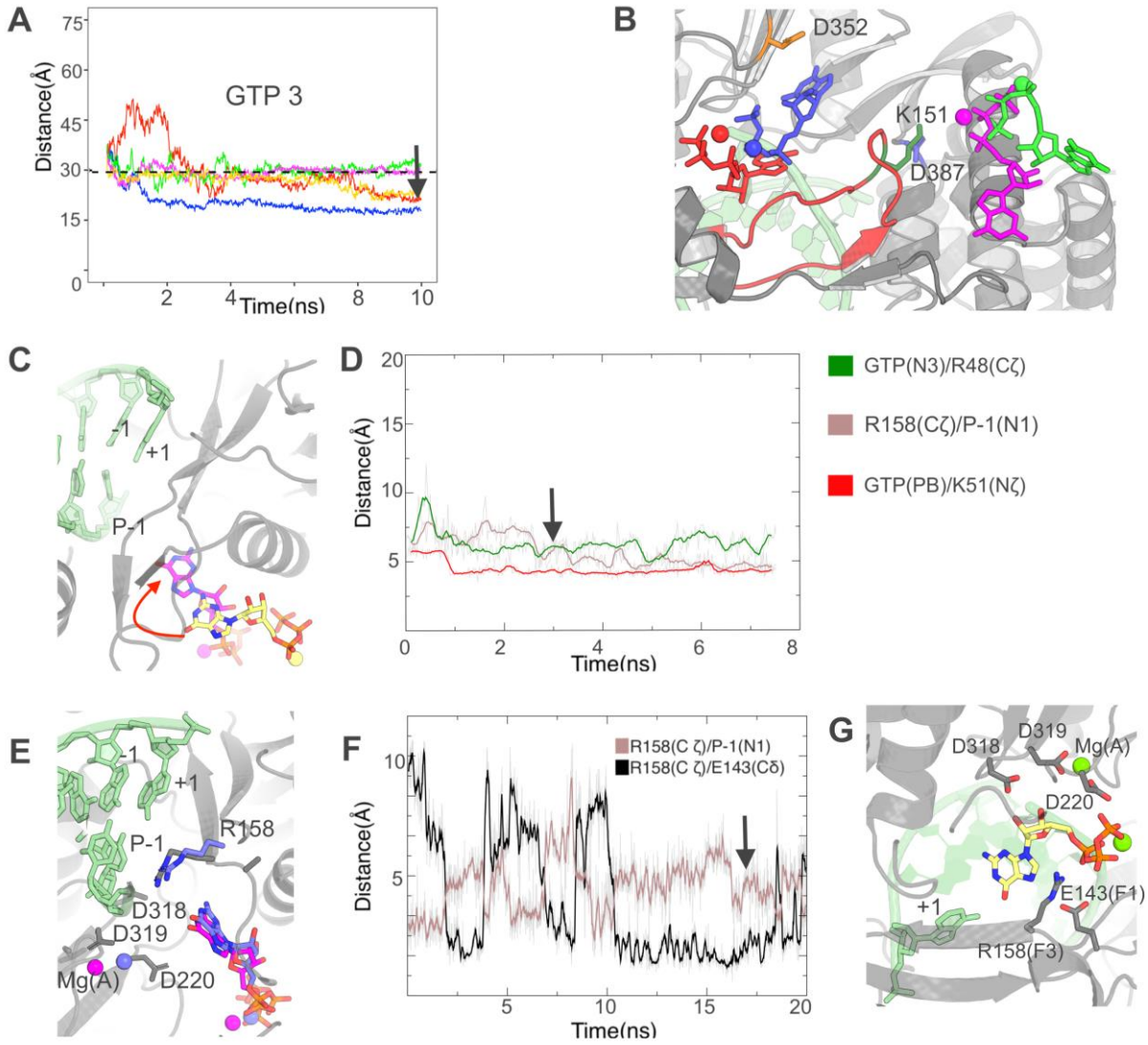
Supplementary Figure S5. Simulations with distance restraints applied. The relevant distance is between the UTP center of mass and Mg(A) at the active site, and harmonic restraints are applied if this distance is beyond 30 Å (indicated by the horizontal dashed line). Numbers 1-2 and 4-9 denote the respective starting positions shown on Figure 3A. The curves are colour coded by the energy constant applied.



Supplementary Figure S6. Simulations with distance restraints applied from position 3 of Figure 3A for systems with Mg(A) at the non catalytic site (left) or with no Mg(A) (right). Same colour code as in Supplementary Figure S4.



Supplementary Figure S7. Key distances between NS5B and UTP during aMD simulations starting from the two positions depicted in Figure 4C, middle, for systems with Mg(A) at the non catalytic site.



Supplementary Figure S8. GTP entry with an adenine as +1 base. (**A**), (**B**) Molecular dynamics simulations with distance restraints between the centers of masses of D319 and GTP starting from position 3. (**A**) Evolution of GTP distances from D319 during five 10-ns simulations with five energy constants 0 (no bias, red curve), 0.02 (blue), 0.05 (green), 0.1 (magenta) and 0.5 (yellow) Kcal/mol/Å^2. The distance of 30 Å below which no extra energy term is added is indicated. The black arrow denotes the snapshot that was selected for further simulations. (**B**) Four positions closest to D319 reached by GTP from initial position 3, coloured according to the energy constant applied beyond 30 Å. (**C**), (**D**) Accelerated molecular dynamics simulations show spontaneous GTP orientation at the NTP tunnel entry. (**C**) Positions of GTP at the start of simulations (yellow carbons) and at a stable position (magenta carbons). (**D**) Evolution of relevant distances between GTP and residues lining the NTP tunnel in one such simulation. The black arrow denotes the snapshot that was selected for further simulations. (**E**), (**F**) Simulation after addition of Mg(A) at the non catalytic site. (**E**) Initial GTP position (magenta carbons for GTP and R158) superimposed on the snapshot (purple carbons) defined by the arrow in (**F**). (**F**) Evolution of the same R158 distances as in Figure 6B

highlight the same dynamics of R158 as in the system with UTP and an adenine as +1 base. The black arrow denotes the snapshot that was selected for further simulations. (**G**) After targeted molecular dynamics simulations, preinsertion cannot be established.