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Coupling dTTP Hydrolysis with DNA Unwinding by the DNA Helicase of Bacteriophage T7

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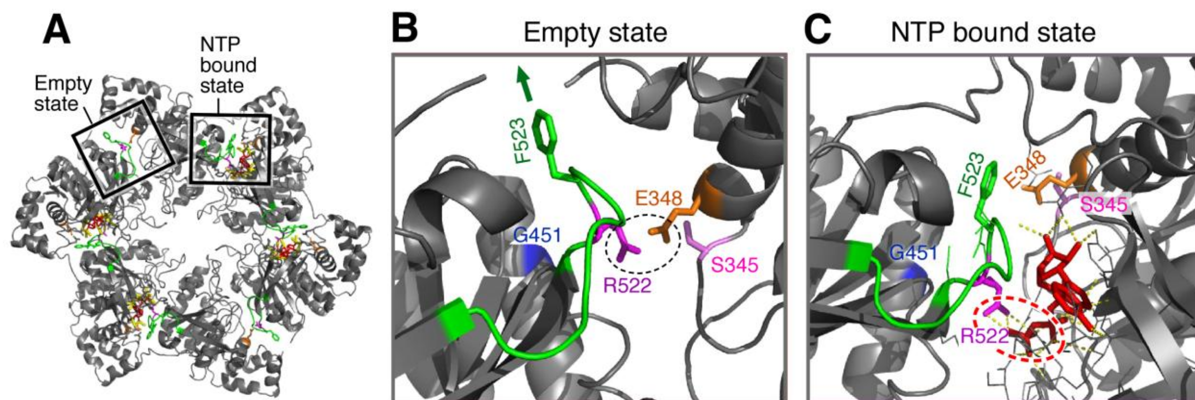
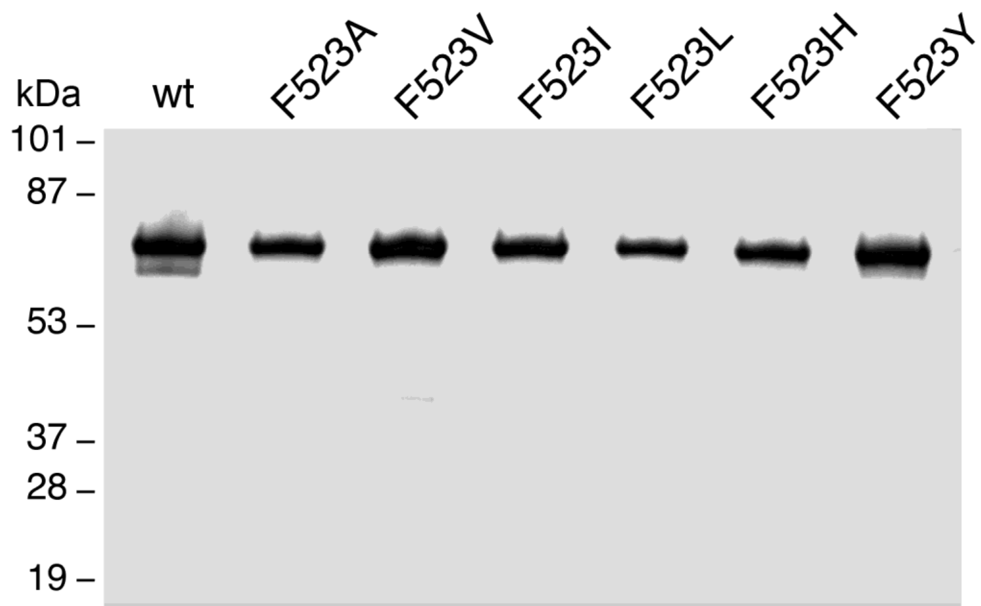
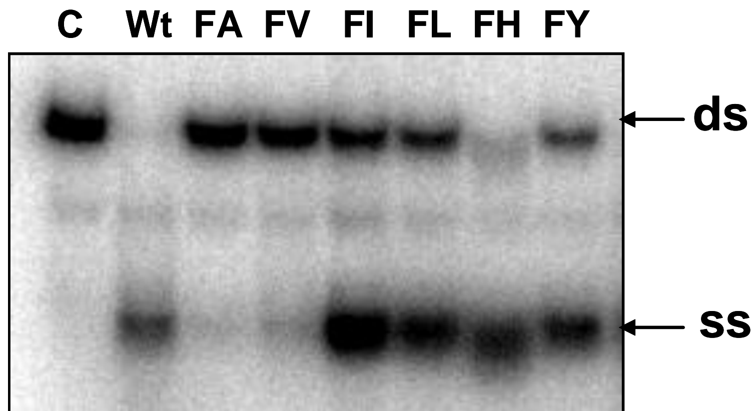


Supplementary Figure S1. **Location of Ser345 and Glu451 in the crystal structure of helicase domain of T7 gp4**. *A*, The crystal structure of T7 gp4 (PDB: 1E0J) drawn in Pymol. *B* & *C*, The magnified view of the empty state and NTP bound state of the nucleotide binding site. The location of Ser345, Gly451 along with Phe523, Arg522 and Glu348 are shown in sticks. Note that substitution of these residues independently, uncouples the dTTP hydrolysis activity of the protein from DNA unwinding.

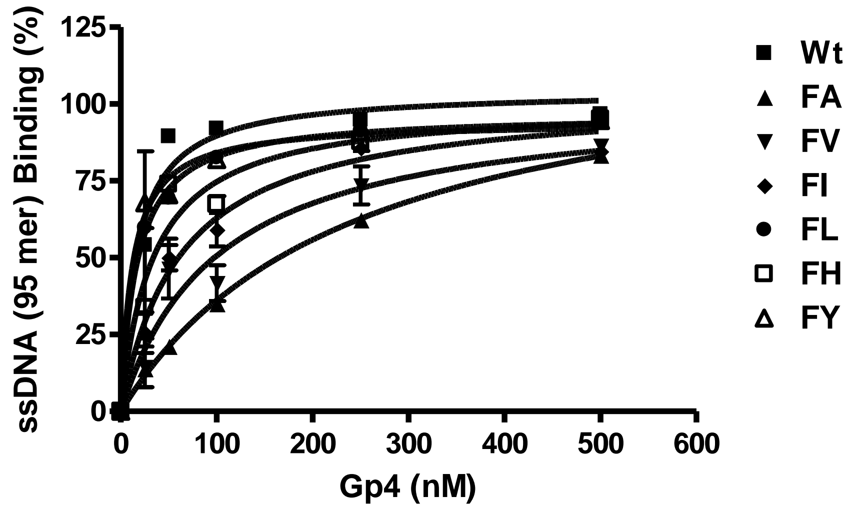


Supplementary Figure S2**. SDS-PAGE analysis of purified wild type and altered gp4**. 30 pico mole of wild-type or altered gp4 were loaded onto a 10% SDS-PAGE. The gel was stained with Coomassie blue.

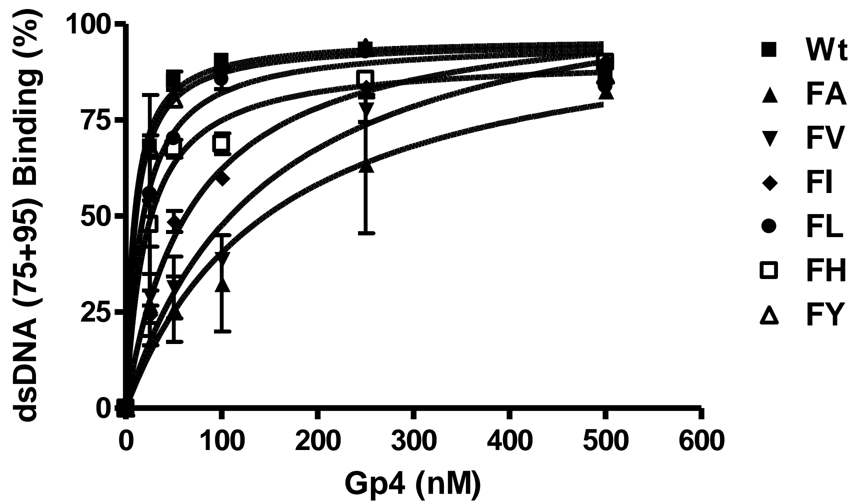


Supplementary Figure S3**. DNA unwinding activity by wild type and F523 altered gene 4 helicases**. The reactions were carried out as described in Experimental Procedures. The reactions contained 2uM gp4, 100nM DNA and 1mM dTTP and incubated at 37ºC for 10 minutes. The unwound ssDNA was separated from the dsDNA substrate in a 10% non-denaturing polyacrylamide gel. The lane labeled as "C" contained only the substrate with no proteins added. The identities of each of the gp4 variant are indicated. Wt, FA, FV, FI, FL, FY and FH in the picture are abbreviations for wild type gp4, gp4-F523A, gp4-F523V, gp4-F523I, gp4-F523L, gp4-F523Y and gp4-F523H respectively.

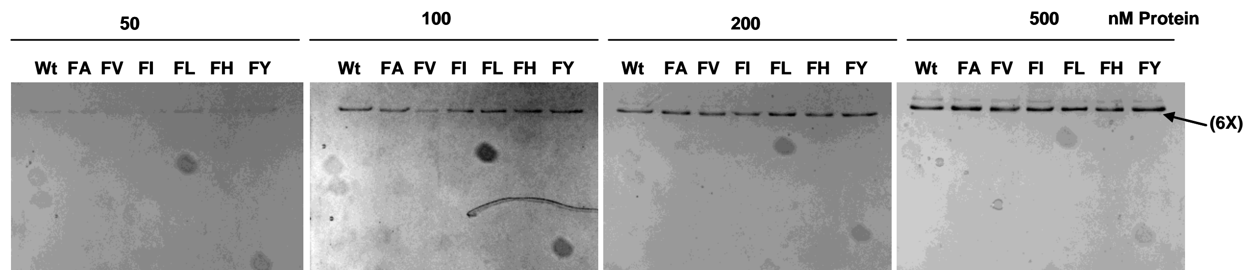
A



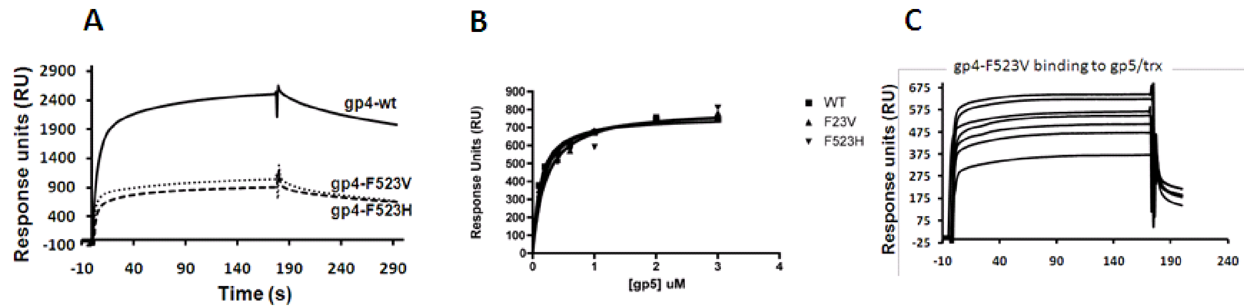
B



Supplementary Figure S4. **Binding of gp4 helicases to ssDNA and forked-end duplex DNA**. The DNA binding activity for the wild type or altered gp4s were determined in a nitrocellulose DNA-binding assay. The reactions were carried out in 20 μl volume containing indicated concentrations of gp4, 1nM 5' -^{32}P labeled 95-mer ssDNA or 1nM forked –end duplex DNA (5'-^{32}P labeled 75-mer annealed with a cold 95-mer), 1 mM β,γ methylene dTTP and incubated for 30 minutes at 37ºC as described in the Experimental procedures. The quantity of protein bound single-stranded DNA and free single stranded DNA was measured as described in the experimental procedures. The relative binding by the gp4 variants to both the substrates were compared with the wild type activity. The error bars represent the standard deviation obtained from three independent experiments.



Supplementary Figure S5**. Oligomerization of wild-type and altered gp4**. Oligomerization assays were carried out with different concentrations of proteins (50, 100, 200 and 500nM) in a 40μl reaction containing 1mM β, γ-methylene dTTP and 1μM 50 mer oligonucleotides. The reactions were carried out as described in the experimental procedures. The gel picture shows the mobility of hexamers of the indicated proteins. Wt, FA, FV, FI, FL, FY and FH in the picture are abbreviations for wild type gp4, gp4-F523A, gp4-F523V, gp4-F523I, gp4-F523L, gp4-F523Y and gp4-F523H respectively.



Supplementary Figure S6**. Interaction of Gp4 with T7 gp5/trx.** A. Interaction of wild type or altered gp4 with gp5/trx in the presence of primer/template. Binding studies were carried out as described in the Materials & Methods. One hundred response units of the biotinylated primer-template were coupled to the surface. Gp5/trx was injected at a concentration of 0.2 μM in a flow buffer containing 1 mM dGTP and 10 μM ddATP: a saturating 1:1 binding condition between gp5/trx and primer-template. The 100 response units resulting from the coupling of the primer-template was subtracted from the baseline. Gp4 was injected at a concentration of 0.7 μM (monomer) in flow buffer containing 0.1 mM ATP and 2 mM dGTP. B, Interaction of wild type or altered gp4 with gp5/trx in the absence of primer/template. Binding studies were carried out as described in the Material & Methods. Three thousand Response Units (RU) of gp4 are coupled to the chip, and the concentration of the gp5/trx in the flow buffer was 0.1 to 3 μM. A control flow cell lacking gp4 is used to subtract the RU resulting from nonspecific interaction. A representative figure is presented in figure C for the interaction of gp4-F523V with different concentrations of gp5/trx in the absence of primer/template.