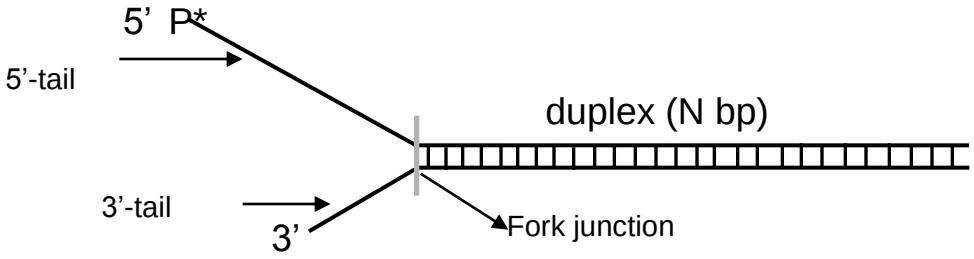
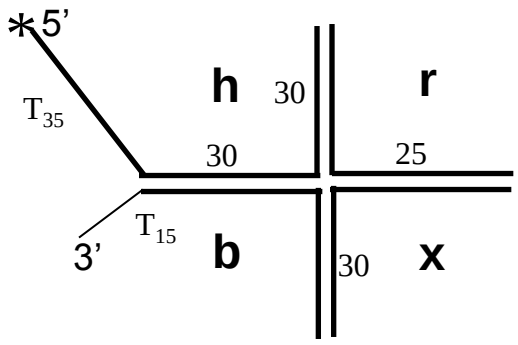
Supplementary Table 1. Forked DNA substrates



Substrate	Structure	5’-Strand Sequence
ds30	5’-(T_{35}) 30 3’-(T_{15})	$^{5'}T_{35}$GAGCGGATTACTATACTACATTAGAATTCA
ds90	5’-(T_{35}) 90 3’-(T_{15})	$^{5'}T_{35}$GAGCGGATTACTATACTACATTAGAATTCAGAGTGTAG AGATTCGGTAAGTAGGATCATGTAGACCAGAGATGTAGTA TGTAGCCGAAGA
ds30-5T	5’-(T_{35}) 30 3’-(T_5)	Same as ds30
ds30-10T	5’-(T_{35}) 30 3’-(T_{10})	Same as ds30
ds30-hp	5’-(T_{35}) 30	3’-strand sequence: $^{5'}$TGATTTAATGTAGTATAGTAATCCGCTCAGA TGGTTACTACGTAACCATCT
ds30-B·S	5’-(T_{35}) 30 3’-(T_0-^{a}B*^{b}S)	Same as ds30 ^{a}B = Biotin linked to 3'-end ^{b}S = Streptavidin
ts60	5’-(T_{35}) 60 30 29 3’-(T_{15})	$^{5'}T_{35}$GAGCGGATTACTATACTACATTAGAATTCAGAGTGTAG AGATTCGGTAAGTAGGATCATG
5’-35T30GC+30		5’– T_{35}GCGGGGCGGGCGGGGCGGCGGGCGCGGGCG TTTCGGTAAGTAGGATCATGTAGACCAGAG –3’
Morpholino-oligo		5’–CCTCTTACCTCAGTTACAATTTATA

Supplementary Table 2. Forked Holliday junctions



Substrate HJgcr	Sequence
h	5'-T_{35} GCGGGGCGGGCGGGGCGGCGGGCGCGGGCACGAGCGAAGGGCGAACGCTTATGAGCTCAT
r	5'-ATGAGCTCATAAGCGTTCGCCCTTCGCTCGCCTCAACTGGGACCGTTTCGTGACC
x	5'-GGTCACGAAACGGTCCCAGTTGAGGACTTGCAAGGGAAGCGGCGTCTGCTGTACC
b	5'-ATGAGCTCATAAGCGTTCGCCCTTCGCTCCTGCCCGCGCCCGCCGCCCCGCCCGCCCCGCT$_{15}$

Substrate HJ	Sequence
h	5'-T_{35} GAGCGGATTACTATACTACATTAGAATTCACGAGCGAAGGGCGAACGCTTATGAGCTCAT
r	5'-ATGAGCTCATAAGCGTTCGCCCTTCGCTCGCCTCAACTGGGACCGTTTCGTGACC
x	5'-GGTCACGAAACGGTCCCAGTTGAGGACTTGCAAGGGAAGCGGCGTCTGCTGTACC
b	5'-ATGAGCTCATAAGCGTTCGCCCTTCGCTCCTGAATTCTAATGTAGTATAGTAATCCGCTCT$_{15}$

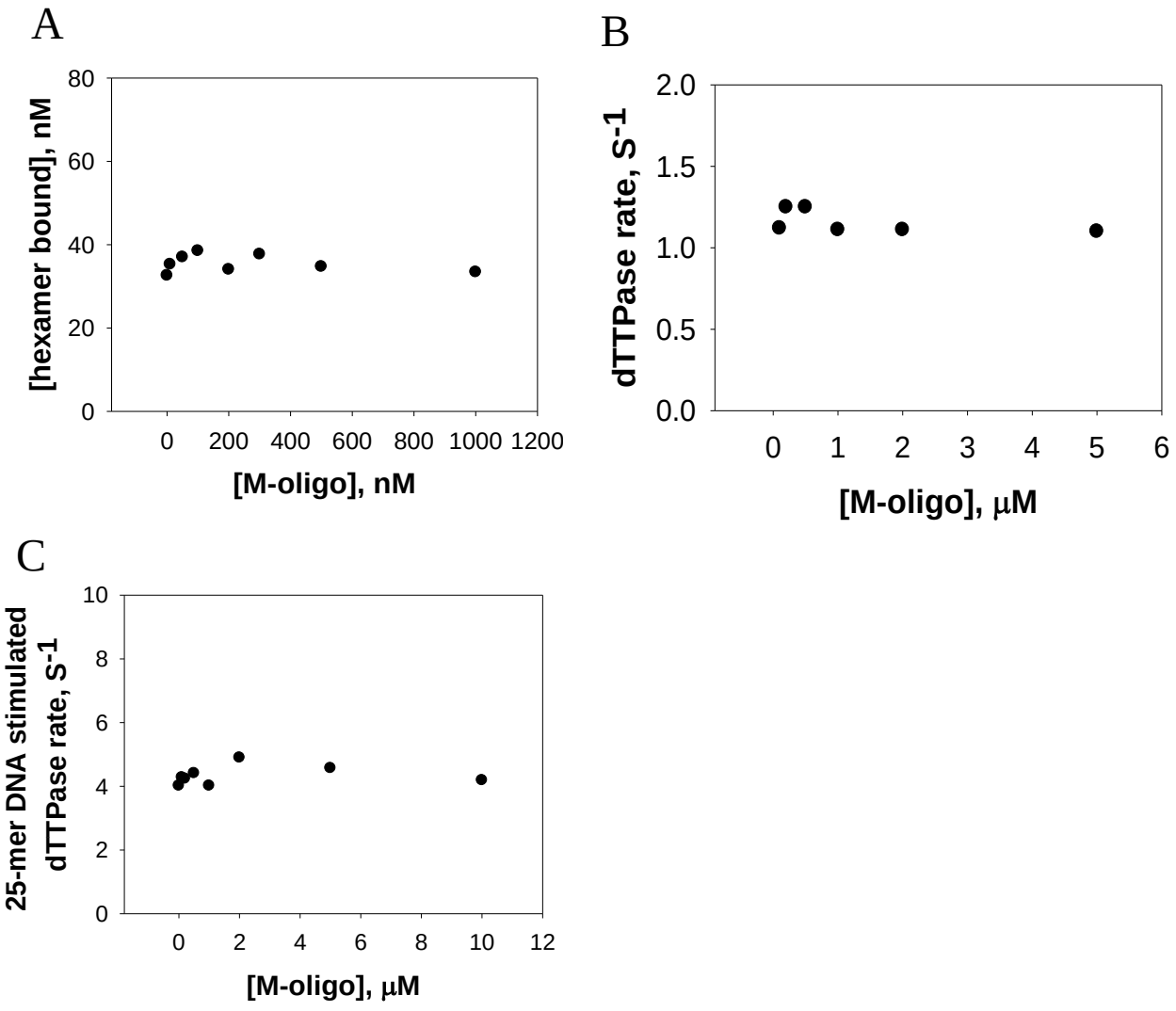
Supplementary Methods

DNA binding assay

The nitrocelluose-DEAE (NC-DEAE) filter binding assay was used to quantitate DNA binding to T7 helicase. The NC membrane circles (25 mm) were washed with 0.5 M NaOH for 3 min, rinsed with water, and equilibrated with membrane wash buffer (50 mM TrisCl (pH 7.5), 5 mM NaCl). T7 helicase (100 nM hexamer) was added to a mixture of radiolabeled 25-mer DNA oligo of the same sequence as M-oligo (100 nM) and M-oligo (0 – 1000 nM) in the presence of dTMPPCP (5 mM) and $MgCl_2$ (7 mM) in a total volume of 15 μl. The solution was incubated for 20 min at room temperature before filtering 10 μl aliquots through the NC membrane assembly. The membranes were washed before and after filtration with the membrane wash buffer. 1 μl aliquots were spotted on a separate NC membrane to measure the total radioactivity. The radioactivity on the membrane was quantitated using a PhosphoImager (Molecular Dynamics).

Steady state dTTP hydrolysis assay

T7 helicase (200 nM hexamer) was mixed with the 25-mer DNA oligo (0.5 μM) and M-oligo (0 – 10 μM) in the presence of [α-^{32}P]dTTP plus dTTP (2 mM) and $MgCl_2$ (4 mM) at room temperature. After various times, the reactions were quenched with 4 M formic acid. An aliquot of the quenched reactions was spotted on polyethyleneimine-cellulose thin layer chromatography and developed in 0.4 M potassium phosphate (pH 3.4) solution. Unreacted dTTP and product dTDP were quantitated using a PhosphorImager and ImageQuant (Molecular Dynamics). The molar concentration of dTDP was plotted versus the time of reaction, and the slope of the plot provided the steady state reaction rate. The experiment was repeated with various concentration of M-oligo and the steady state rate of each reaction was plotted versus M-oligo concentration.



Supplementary Figure 1. The morpholino oligo (M-oligo) does not interact with the T7 helicase. (A) The binding of a radiolabeled 25-mer DNA oligo to T7 helicase was measured by the nitrocellulose-DEAE filter binding assay and it is unaffected by the presence of increasing concentration of the M-oligo. (B) The dTTPase activity of T7 helicase under steady state conditions is not stimulated by the addition of increasing concentration of the M-oligo. (C) Increasing concentration of the M-oligo does not affect the DNA-stimulated (25-mer DNA oligo (0.5 μM)) dTTPase activity of T7 helicase.