SUPPORTING INFORMATION FOR PUBLICATION

Melting of duplex DNA in the absence of ATP by NS3 helicase domain through specific interaction with a single-strand/double-strand junction

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SUPPORTING EXPERIMENTAL PROCEDURES

$KMnO_4$ Footprinting of DNA. All concentrations indicated are after mixing. DNA footprinting was used to probe NS3h binding to $T_{15(bio\cdot dT\text{-}12)}$-22 bp, which features a biotin-thymidine analogue in the 3'-ssDNA tail at position 12 containing a total of 15 thymidine residues (see Table 1 for DNA sequences), in the presence and absence of streptavidin. The streptavidin bound DNA complex was formed by nutation of 10 nM $T_{15(bio\cdot dT\text{-}12)}$-22 bp with 0.1 mg/mL Dynabeads® M-280 Streptavidin (Invitrogen, Oslo, Norway) for 15 min. The DNA was then incubated with 1 μM NS3h for 3 min at 37 °C in the presence of 25 mM MOPS (pH 7.0), 10 mM $MgCl_2$, 20 mM NaCl, 0.1 mM EDTA, and 0.1 mg/mL BSA. The footprinting reaction was initiated by the addition of 8 mM $KMnO_4$ and subsequently quenched after 5 sec by the addition of 1.5 M βME and 200 mM EDTA. Cleavage products of the samples were then captured on Dynabeads (only added to the samples not already containing them) then magnetically isolated from the rest of the mixture. The DNA products attached to the Dynabeads were then incubated at 90 °C for 30 min in a solution containing 1 M piperidine and 1 mM biotin to separate the cleavage products from the Dynabeads. The resulting DNA fragments were isolated and purified via lyophilization followed by two wash cycles in water and lyophilization. The cleavage products were then re-suspended in denaturing loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol, and 6% glycerol in 95% formamide), heated at 95 °C for 10 min, and loaded onto a denaturing 20% acrylamide/ 7 M urea gel. The gel was run at 22 mA for 2.5 hr. Detection of the cleaved DNA fragments utilized a PhosphorImager (GE Healthcare, Piscataway, NJ), and quantitation was performed using ImageQuant software (GE Healthcare, Piscataway, NJ). The

reactivity of each thymidine in three identical samples containing protein was normalized to the average reactivity of the thymidine in the absence of protein with corrections for background subtraction and potential lane loading differences. The average of these three experiments was calculated and plotted as a function of thymidine position using Kaleidagraph (Synergy Software, Reading, PA). Error bars represent the standard deviation.

Figure S1. NS3h dissociation from increasing lengths of DNA to examine diffusion on ssDNA. Dissociation of NS3h from oligonucleotides of increasing lengths (T_8, T_{10}, T_{13}, T_{15}, T_{18}, T_{20}, T_{30}, T_{40}, or T_{55}) was measured by using stopped-flow fluorescence spectroscopy. All concentrations reported are final after mixing. Data were collected upon rapid mixing of 200 nM NS3h bound to 1.2 μM strands ssDNA with a solution containing 10 mM $MgCl_2$ and 4 mg/mL heparin, which prevented re-association of NS3h to the ssDNA. Dissociation of NS3h from ssDNA results in an increase in fluorescence. Data were fit to a single exponential to obtain observed dissociation rates ($k_{d,obs}$, s^{-1}).

Figure S2. Streptavidin does not influence unwinding by NS3h in the absence of a substrate with a biotin-modified nucleotide. To ensure that the presence of excess streptavidin in the unwinding reaction does not interfere with the ability of NS3h to interact with and unwind DNA, 2 nM T_{15}-22 bp (no biotin-dT analogue) was pre-incubated with 120 nM streptavidin prior to initiation of ATP-independent unwinding reaction by the addition of 500 nM NS3h in the presence (⊞) or absence of streptavidin (♦).

Figure S3. NS3h binds the biotinylated DNA substrate in the presence of the streptavidin protein block. $KMnO_4$ footprinting was performed on $T_{15(bio\cdot dT\text{-}12)}$-22 bp in the presence and absence of streptavidin by binding 10 nM $T_{15(bio\cdot dT\text{-}12)}$-22 bp with 0.1 mg/mL Dynabeads M-280 Streptavidin

for 15 min prior to the incubation with 1 μM NS3h at 37 °C. After 3 min, samples were treated with 8 mM $KMnO_4$, and the resulting cleavage products were treated, isolated, and quantified as described in the Supplemental Experimental Procedures. The average reactivity of each residue in the presence of NS3h relative to in the absence of the protein is shown for each thymidine in the 3'-ssDNA overhang, which is made up of 15 thymidine residues with a biotin-thymidine analogue at position 12. In the absence of streptavidin (●), two NS3h molecules bind resulting in protection from cleavage with an area sensitive the $KMnO_4$-mediated cleavage found between the two bound proteins peaking at thymidine position 8. In the presence of streptavidin (■), there is an initial protected region attributed to NS3h binding the first 6-7 thymidine residues, followed by a peak that is shifted to the thymidine at position 7, followed by a slightly broader region of protection likely due to the bound streptavidin.

Figure S4. The directional polarity in unwinding by NS3 is restored by the presence of ATP and Mg^{2+}. ATP-dependent unwinding of the DNA substrate was initiated by mixing the pre-bound NS3-DNA complex with a solution containing 5 mM ATP at 37 °C following a 5 min pre-incubation also at 37 °C. Unwinding of 2 nM 22 bp-T_{15} which contains a 5'-ssDNA tail by 500 nM NS3 revealed that the presence of ATP and Mg^{2+} (●) results in no observed strand separating activity.

Figure S5. Streptavidin does not influence unwinding by full-length NS3 in the absence of a substrate with a biotin-modified nucleotide. No major decrease in product formation was detected due solely to the presence of excess streptavidin. Unwinding of 2 nM T_{15}-22 bp (no biotin-dT analogue) pre-incubated with 120 nM streptavidin prior to initiation of ATP-independent unwinding reaction by the addition of 500 nM NS3 in the presence (⊞) or absence of streptavidin (●).

Figure S6. Full-length NS3 binds to the biotinylated DNA substrate in the presence of the streptavidin protein block. DNA footprinting with $KMnO_4$ was performed as described in Supplemental Figure 3 using NS3 in place of NS3h. Binding was examined in the presence (■) or absence (●) of streptavidin. The most reactive thymidine was located at position 7 with flanking regions of protection due to binding of NS3.

Figure S1

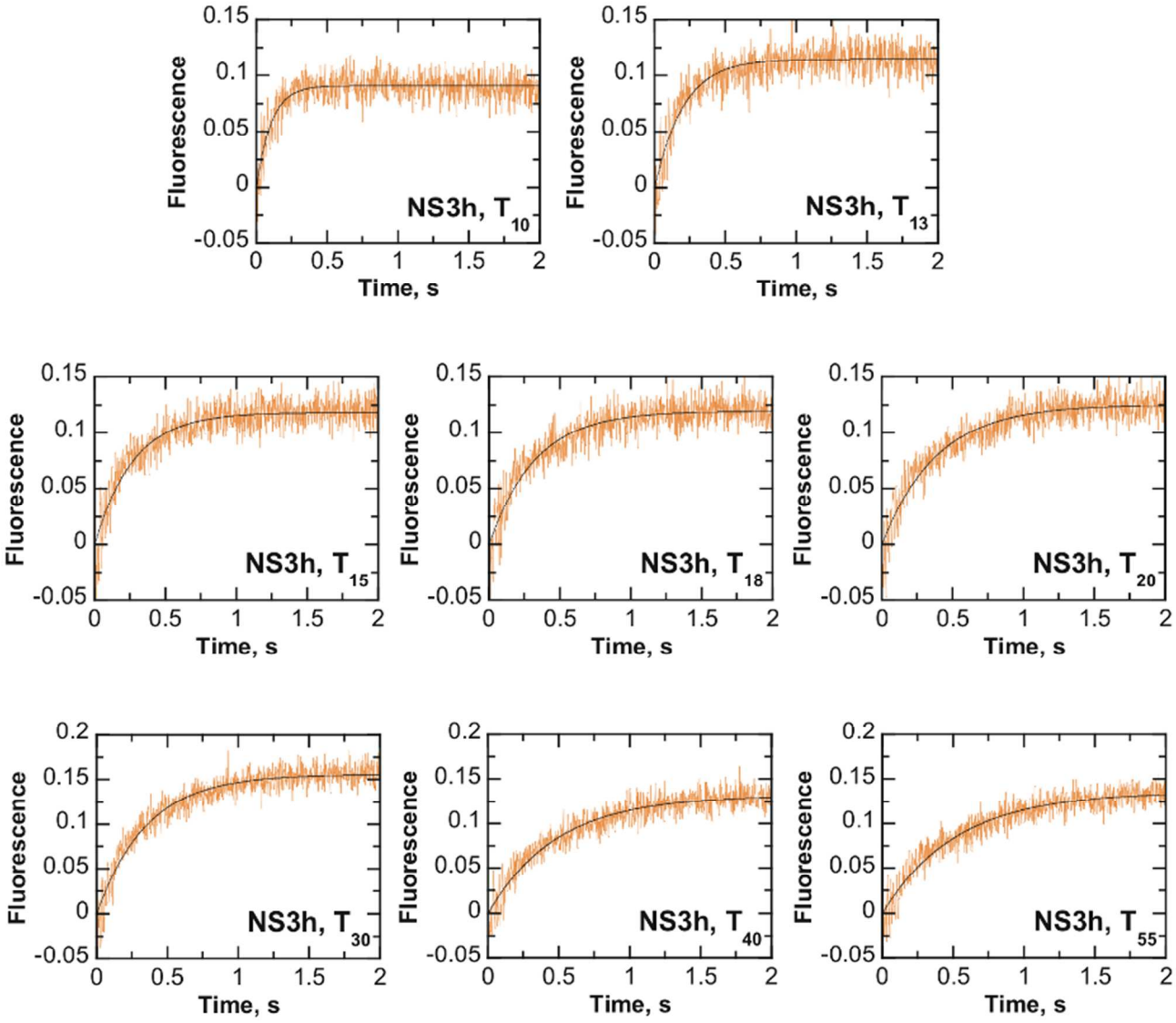

Fluorescence
Time, s
NS3h, T10
NS3h, T13
NS3h, T15
NS3h, T18
NS3h, T20
NS3h, T30
NS3h, T40
NS3h, T55

Figure S2

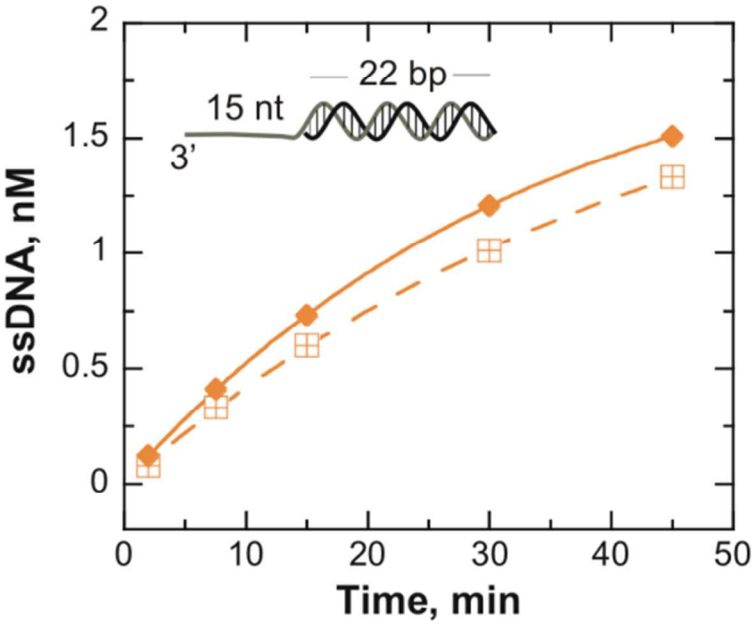


Figure S3

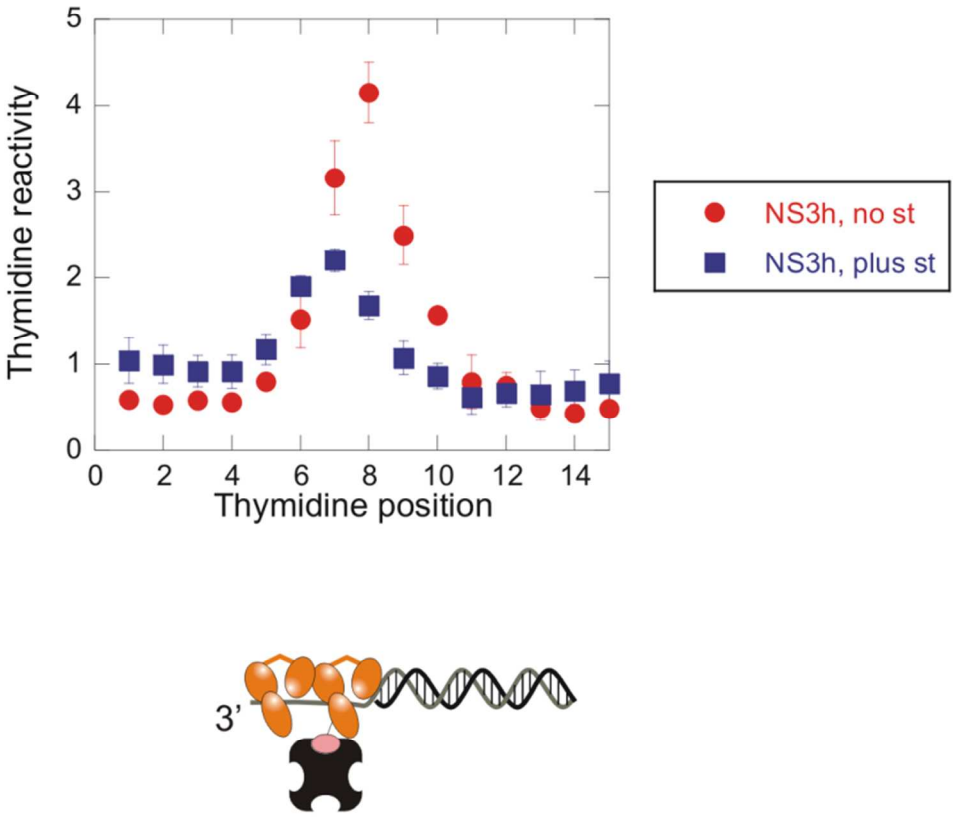

Thymidine reactivity
Thymidine position
0
1
2
3
4
5
0
2
4
6
8
10
12
14
NS3h, no st
NS3h, plus st
3'

Figure S4

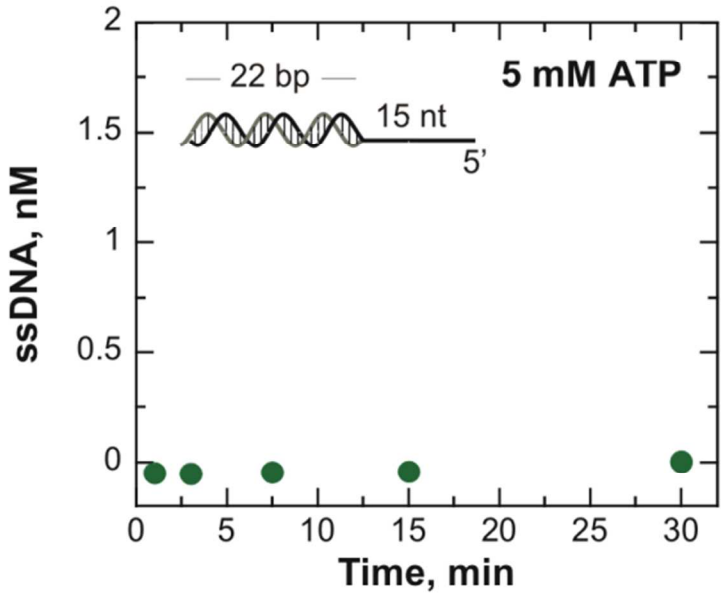


Figure S5

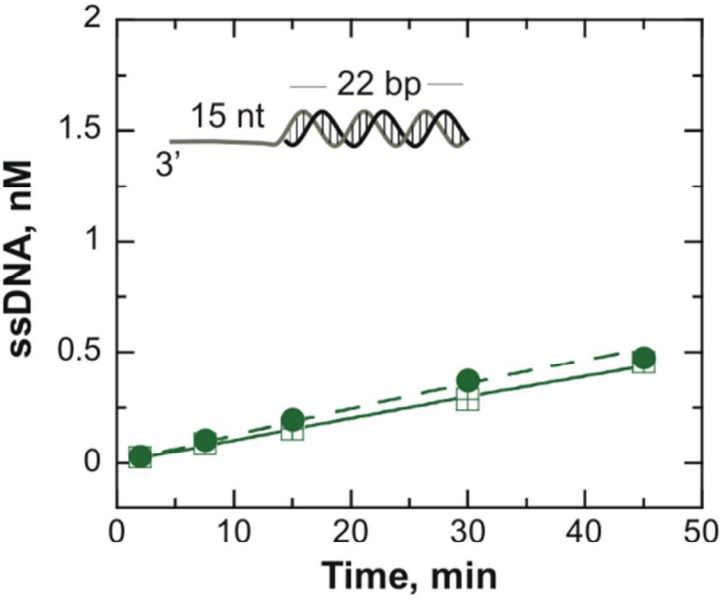
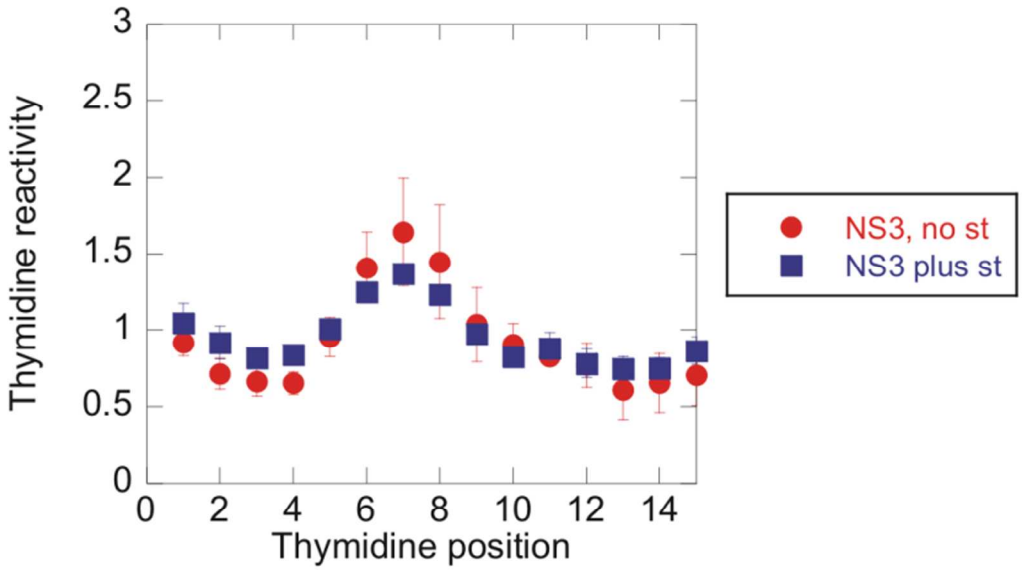


Figure S6


Thymidine reactivity
3
2.5
2
1.5
1
0.5
0
0
2
4
6
8
10
12
14
Thymidine position
NS3, no st
NS3 plus st