

Supplemental Data for electronic publication

Investigation of translocation, DNA unwinding, and protein displacement by NS3h, the helicase domain from the Hepatitis C Virus helicase

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Supplemental Figure 1. Size exclusion chromatography of NS3h in the presence of a 15mer oligonucleotide. NS3h (10 μ M) was incubated with dT15 oligonucleotide (20 μ M) prior to injecting 25 ml onto a Bio-Sil SEC 250-5 column (Bio-Rad). The mobile phase was 0.1M Potassium phosphate buffer (pH 7.0). For comparison, NS3h (10 μ M) or the dT15 oligonucleotide (20 μ M) were injected onto the column in separate experiments. NS3h alone eluted at 9.05 min which corresponds to the molecular weight of 52 kDa and DNA at 9.93 min (22 kDa). NS3h with DNA eluted at 8.78 min which corresponds to the molecular weight of NS3h with DNA (71kDa). NS3h does not dimerize in the presence of ssDNA.

Supplemental Figure 2. NS3 translocation kinetics monitored by stopped-flow experiments with fluorescein labeled ssDNA. NS3 (100 nM) was pre-incubated with 200 nM ssDNA labeled at the 5' end with fluorescein (5'-F-(dT)_L) at 37 °C in assay buffer and translocation was initiated by the addition of ATP:Mg²⁺ and heparin to a final concentration of 5 mM, 10 mM, and 4mg/ml, respectively. Fluorescence time courses were obtained with 5'-F-(dT)₄₀ (●), 5'-F-(dT)₇₂ (●), and 5'-F-(dT)₈₈ (●).

Supplemental Figure 3: NS3 translocation along poly(dT)/M13 monitored by stopped-flow fluorescence spectroscopy of the intrinsic tryptophan fluorescence of NS3 with ATP (A) and with out ATP (B). NS3 (50 nM) was pre-bound to poly (dT) or M13 (5 μ M nucleotide) in assay buffer, and translocation was initiated by mixing with buffer containing ATP (5 mM), Mg(OAc)₂ (10 mM), and Heparin (4mg/ml).

Supplemental Figure 4. NS3h translocation was monitored by using Cy3-labeled oligonucleotides and stopped-flow fluorescence spectroscopy. NS3h (50 nM) was pre-incubated with 200 nM ssDNA labeled at the 5' end with Cy3 (5'-Cy3-(dT)_L) at 37 °C in assay buffer and translocation was initiated by the addition of ATP:Mg²⁺ and heparin to a final concentration of 5 mM, 10 mM, and 4mg/ml, respectively. Fluorescence time courses were obtained with 5'-Cy3-(dT)₄₀ (●), 5'-Cy3-(dT)₆₀ (●), 5'-Cy3-(dT)₇₂ (●), and 5'-Cy3-(dT)₈₈ (●). Solid lines are the best fits from global NLLS analysis according to Scheme I. Kinetic constants resulting from the data analysis are shown in Supplemental Table 3.

Supplemental Figure 5. NS3h single-stranded DNA binding and translocation kinetics monitored by stopped-flow spectroscopy with fluorescein labeled ssDNA. NS3h (100nM) in assay buffer was rapidly mixed with 200 nM 5'-F-(dT)₆₀, 5 mM ATP, 10 mM Mg(OAc)₂ and various concentrations of heparin at 37 °C. Fluorescence time courses obtained with final heparin concentrations of 0 mg/ml (●), 2 mg/ml (●), 4 mg/ml (●), and 6 mg/ml (●). Fluorescence intensities were adjusted to zero intensity (or zero volts) at long times in order to simplify the data analysis. Thus, in these plots, an intensity of zero corresponds to the fluorescence of DNA in the absence of bound protein. To verify that heparin is able to effectively compete with single-stranded DNA for NS3h binding, an experiment was performed in which NS3h was rapidly mixed with a solution containing 5'-F-(dT)₆₀ and varying concentrations of heparin. As shown in Supplemental Figure 5, in the absence of heparin there is a large, rapid and persistent decrease in fluorescein fluorescence following mixing with NS3h. This fluorescence change is consistent with the binding of NS3h to the single-stranded DNA and the subsequent multiple rounds of single-stranded DNA translocation, dissociation and DNA rebinding by NS3h. In the presence of heparin, however, the magnitude of the fluorescence signal change following NS3h mixing is greatly diminished. Based upon our results, we conclude that heparin concentrations of 2 mg/ml, 4 mg/ml and 6 mg/ml are all able to effectively compete with 5'-F-(dT)₆₀ for NS3h binding under these conditions. All subsequent translocation experiments were therefore conducted with a heparin concentration of 4 mg/ml to ensure that trapping was complete for all DNA lengths considered.

Supplemental Figure 6. NS3h (100 nM) was pre-bound to 3'T58BiodTG5' substrate (200 nM) in assay buffer in the presence or absence of streptavidin (1μM) and translocation was initiated by mixing with buffer containing ATP (5 mM), Mg(OAc)₂ (10 mM), and heparin (4 mg/ml). Fluorescence data was fit to the sum of two exponentials resulting in a rate constant of $1.1 \pm 0.04 \text{ s}^{-1}$ for the first exponential phase and $0.001 \pm 2.4 \text{ s}^{-1}$ for the second exponential phase of the progress curve. Reaction carried out with streptavidin resulted in a rate constant of $1.4 \pm 0.01 \text{ s}^{-1}$ for the first exponential phase and $0.0002 \pm 0.9 \text{ s}^{-1}$ for the second exponential phase of the progress curve.

Supplemental Table 1.

k_t (steps/s)	2.7 (fixed)	27 (fixed)	270 (fixed)	48.45
k_d (s^{-1})	0.014	0.22	0.27	0.092
k_c (s^{-1})	2.77	1.80	1.81	1.87
k_{end} (s^{-1})	4.13	6.41	6.02	6.63
r	0.20	0.027	0.0029	0.016
rn	0.96	0.21	0.053	0.023
m (nt/step)	23.68	1.67	0.16	1.013
d (nt)	12.1	19.2	15.6	8 (fixed)
B	3.35	8.29	7.94	10.69
$m*k_t$ (nt/s)	64	46	43	49
$m/(1-P)$ (nt)	4500	230	160	530
Variance	5.97×10^{-10}	4.39×10^{-10}	4.43×10^{-10}	4.81×10^{-10}

Supplemental Table 1. Data from Figure 6 were analyzed according to equation 1 (derived from Scheme 1). The correlation between sets of parameters determined from the global nonlinear least squares analysis is illustrated by holding the parameter k_t constant and allowing other parameters to float. The macroscopic translocation rate remains similar over a range of fixed values for k_t .

Supplemental Table 2.

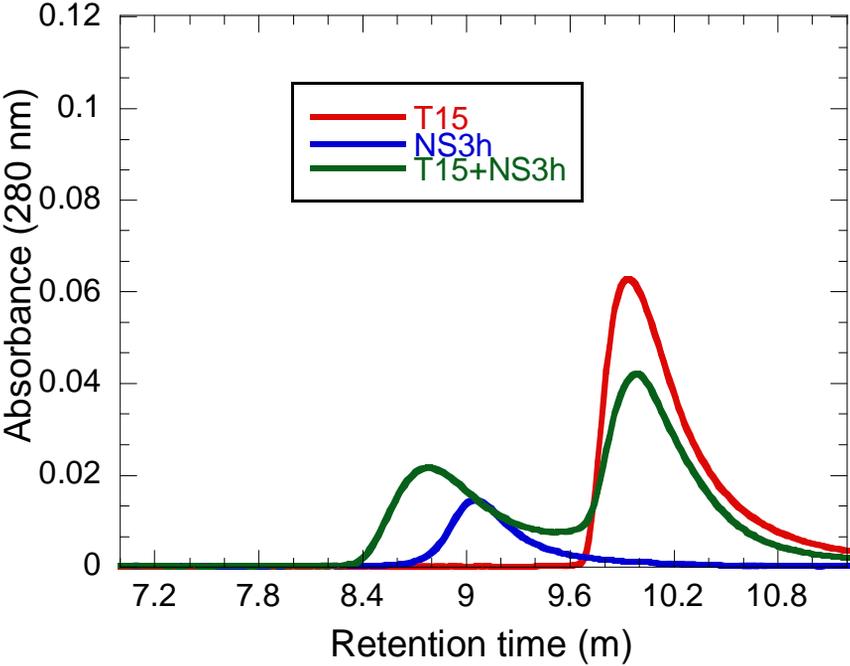
Scheme 1		Scheme 2	
k_t (steps/s)	31.7	k_t (steps/s)	32.2
k_d (s^{-1})	0.42 (fixed)	k_d (s^{-1})	0.42 (fixed)
k_c (s^{-1})	7.3	k_i (s^{-1})	7.3
k_{end} (s^{-1})	1.8	k_{end} (s^{-1})	1.8
m (nt/step)	1.4	m (nt/step)	1.3
d (nt)	19	d (nt)	21
r	0.03	r	0.03
rn	0.2	rn	0.2
B	3.1	C	0.3
$m*k_t$ (nt/s)	43.3	$m*k_t$ (nt/s)	43.3
$m/(1-P)$ (nt)	104	$m/(1-P)$ (nt)	104
Variance	4.45×10^{-10}	Variance	4.45×10^{-10}

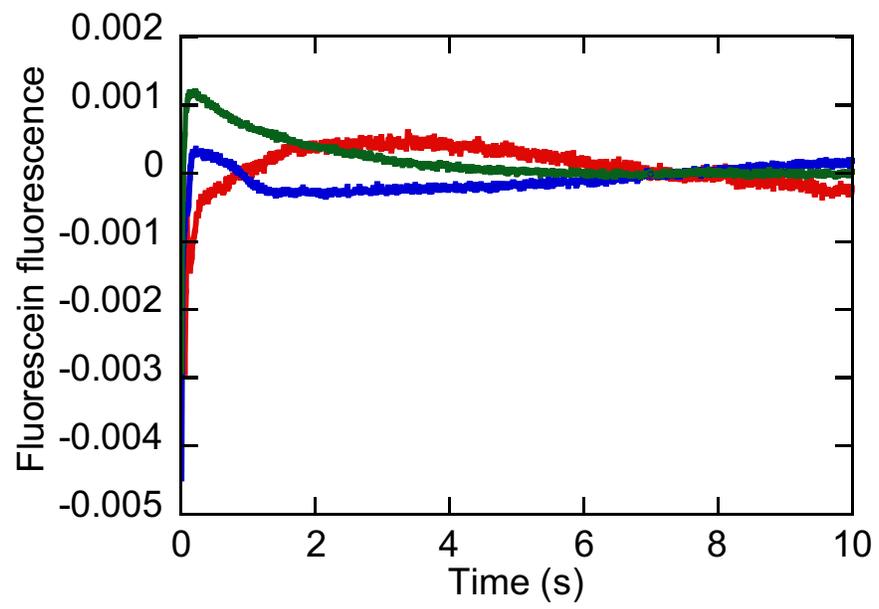
Supplemental Table 2. Data from Figure 6 were analyzed after fixing the value for the dissociation constant, k_d (Supplemental Table 2). The global nonlinear least squares analysis was performed using equation 1 (derived from Scheme 1) or equation 4 (derived from Scheme 2) while fixing the value for k_d at $0.42 s^{-1}$. This value for k_d was taken from the first exponential of the fit of the data in Figure 8, in which dissociation of NS3h from poly dT was measured by stopped-flow fluorescence. The macroscopic rate constant for translocation ($m*k_t$) remains similar to the value obtained when k_d was allowed to float (43 nt/s compared to 46 nt/s).

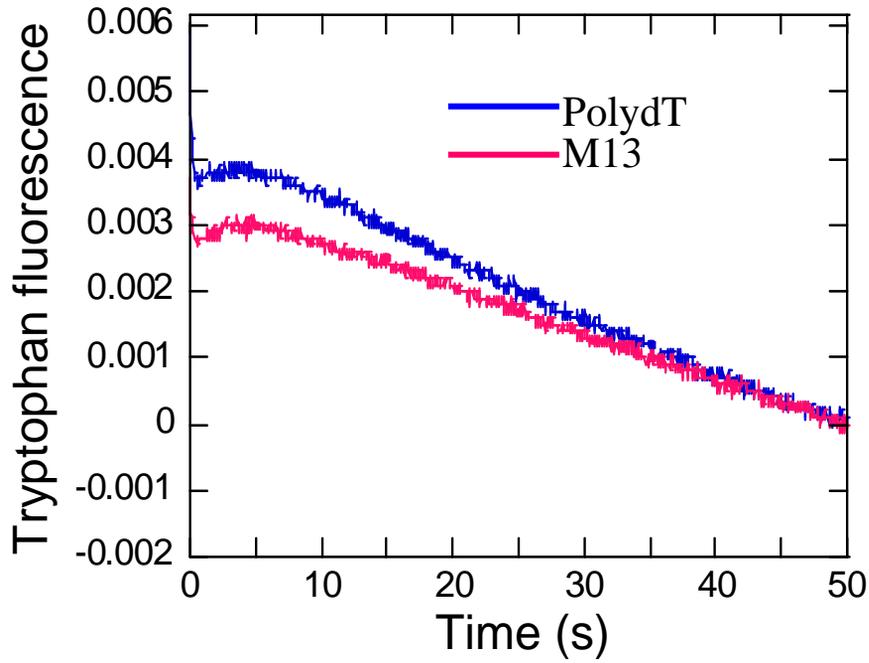
Supplemental Table 3.

Scheme 1		Scheme 2	
k_t (steps/s)	10.5 ± 0.9	k_t (steps/s)	13.6 ± 1.6
k_d (s^{-1})	0.32 ± 0.02	k_d (s^{-1})	0.33 ± 0.02
k_c (s^{-1})	26.0 ± 0.9	k_i (s^{-1})	25.5 ± 0.9
k_{end} (s^{-1})	3.15 ± 0.08	k_{end} (s^{-1})	2.90 ± 0.02
m (nt/step)	3.0 ± 0.3	m (nt/step)	2.3 ± 0.3
d (nt)	31.9 ± 0.4	d (nt)	36.1 ± 0.2
r	0.097 ± 0.009	r	0.08 ± 0.01
rn	0.55 ± 0.03	rn	0.60 ± 0.03
B	0.277 ± 0.007	C	3.24 ± 0.06
$m*k_t$ (nt/s)	31.8 ± 0.4	$m*k_t$ (nt/s)	31.6 ± 0.4
$mP/(1-P)$ (nt)	98 ± 7	$mP/(1-P)$ (nt)	95 ± 6
Variance	2.79×10^{-5}	Variance	2.79×10^{-5}

Supplemental Table 3. Kinetic constants derived by fitting data in supplemental figure 4 obtained from observed changes in Cy3 fluorescence as a function of oligonucleotide length. Kinetic constants are shown for fitting data to Scheme I and Scheme 2. The macroscopic rate for translocation ($m*k_t$) was 31.8 ± 0.4 nt/s and 31.6 ± 0.4 nt/s, for Scheme 1 and Scheme 2, respectively. These values for macroscopic translocation are similar to the value obtained from analysis of the fluorescein-labeled oligonucleotides, which was 46 nt/s.





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