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Supplemental information

Force-dependent stimulation of RNA unwinding by SARS-CoV-2 nsp13 helicase

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Supplementary Materials and Methods

Protein expression and purification

Nsp13 was expressed and purified as reported previously with minimal changes (5). Briefly, the plasmid expressing the SARS-CoV-2 nsp13 helicase (SARS-CoV-2-nsp13 Pet28a or SARS-CoV-2-nsp13 Pet28a-PreScission) was transformed into BL21 Rosetta cells and a starter culture grown overnight at 37 ^{o}C was used to inoculate 4-6 L of LB by adding 10 mL of SARS-CoV-2-nsp13 Pet28a-PreScission starter culture per L. These cultures were grown at 37 ^{o}C until they reached an OD_{600} of 0.6. The temperature was then lowered to 18 ^{o}C and induced with 1 mL of 0.2M IPTG per liter of growth. The cultures were left overnight (~16 hrs) at 18 ^{o}C and harvested the next day by spinning down at ~5000 g for 10 minutes. Pellets were resuspended in affinity buffer (50 mM Hepes pH 7.0, 500 mM NaCl, 4 mM $MgCl_2$, 5% Glycerol, 1 mM PMSF, 20 mM Imidazole, 1 mM ATP, 1 mM BME) supplemented with 9 µL of benzonase and subsequently lysed using an Avestin Emulsiflex C5 with pressure exceeding 10,000 psi. The lysate was then centrifuged using a Beckman Coulter ultracentrifuge and a Type 70 Ti rotor at 15,000 rpm for 45 minutes at 4 ^{o}C. The supernatant was then added to 2 mL of Ni-NTA resin equilibrated with affinity buffer and incubated at 4 ^{o}C with rocking for 1 hr. The supernatant was then washed off and the resin was subsequently washed with ~900 mL of affinity buffer. Once complete, the protein was eluted with 15-20 mL of affinity buffer + 250 mM imidazole and dialyzed overnight with 100 uL of preScission protease (4 mg/mL) in dialysis buffer (50 mM Hepes pH 7.0, 100 mM NaCl, 4 mM MgCl2, 1 mM TCEP). The elution was diluted 2Xfold with milliQ-H_2O the next morning to give ion exchange A buffer (25 mM Hepes pH 7.0, 50 mM NaCl,2 mM $MgCl_2$, 0.5 mM TCEP) and loaded onto a CaptoS HP column (Cytiva) at a flow rate of 1 mL/min and then eluted with a gradient from 0% ion exchange B buffer (25 mM Hepes pH 7.0, 1 M NaCl, 2 mM $MgCl_2$, 0.5 mM TCEP to 50% ion exchange B buffer over 30 minutes. The protein eluted at around ~5-10% ion exchange B buffer. The protein was then concentrated to 500 µL using a 50 kDa cutoff Amicon Ultra-4 Centrifugal filter (Millipore UFC805008) and injected onto a Superdex 200 increase gel filtration column in sizing buffer (25 mM HEPES pH 7.0, 250 mM KCl, 1 mM $MgCl_2$, 5% glycerol, 1 mM TCEP). The protein was flash frozen in liquid N_2 and stored at -80 ^{o}C.

Bulk Helicase Activity Assays

Variable enzyme concentration assays were performed by combining a 4x substrate/capture oligo (6 µL) stock solution in helicase buffer with 2-fold serial dilution of 2x nsp13 (12 µL) in helicase buffer in reaction wells. Reactions were initiated by the addition of a 4x ATP solution in helicase buffer (6 µL). Final concentrations were 0-2 nM nsp13, 0.5 µM DNA/RNA substrate, 2.5 µM capture oligo and 2 mM ATP. Control reactions containing no ATP were included in the assay.

Variable KCl concentration assays were performed by combining a 4x nsp13/substrate/capture oligo (6 µL) stock solution in helicase buffer (-KCl) with 2-fold serial dilutions of 2x KCl solutions (12 µL) in helicase buffer in reaction wells. Reactions were initiated by the addition of a 4x ATP solution in helicase buffer (6 µL). Final concentrations were 10 nM nsp13, 0.5 µM DNA/RNA substrate, 2.5 µM capture oligo, 2 mM ATP and 0-250 mM KCl. Control reactions containing no ATP were included in the assay.

Gel-based Helicase Activity Assays

Time-course experiments were performed by mixing recombinant nsp13 (20 nM) with partial duplex DNA or RNA substrates (100 nM) in the presence of ATP (2 mM) and capture oligo (1000 nM) in 80 µL reaction volumes. Reactions were performed at 30^{o}C in a temperature controlled aluminum block. Nsp13/substrate/capture oligos were prepared as a combined 4x stock solution in helicase buffer (20 mM HEPES, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$, 1 mM TCEP, 0.005% triton X100 and 0.1 mg/mL BSA), and 20 µL was added to 40 µL helicase buffer in the reaction tube. Reactions were initiated by the addition of 20 µL of a 4x ATP stock solution in helicase buffer. 10 µL reaction time-points were taken at 1, 2.5, 5, 10, 20, 30 and 60 min, and quenched by the addition of 10 µL of a 100 mM EDTA, 20% glycerol solution. Time-point samples (5 µL) were resolved by 10% native-PAGE at room temperature, 200 V for 25 min. The gels were scanned using a Typhoon Trio Variable Mode Imager (GE Healthcare) in TAMRA dye mode to visualize fluorescence.

Variable KCl concentration assays were performed by mixing recombinant nsp13 (200 nM) with partial duplex DNA or RNA substrates (20 nM) in the presence of ATP (2 mM) and capture oligo (2000 nM) in 10 µL reaction volumes. Nsp13/substrate/capture oligo was prepared as a 4x stock solution in helicase buffer and 2.5 µL was added to 5 µL of 2x stocks of KCl in helicase buffer (2-fold serial dilutions). Reactions were initiated by the addition of 2.5 µL of a 4x ATP stock solution in helicase buffer and incubated at 37 ^{o}C for 30 min. Reactions were quenched and gel assays were performed as described above.

Supplementary Figures

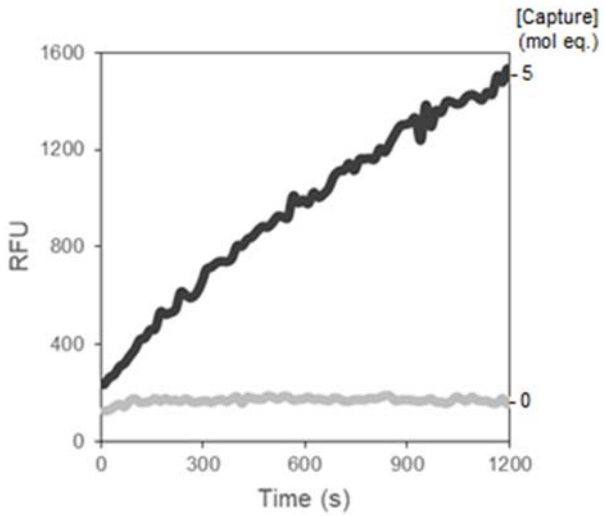

Figure S1. Effect of capture oligo in bulk nsp13 helicase assay
Bulk helicase assays in the absence (gray) or presecence (black) of capture DNA oligo (5 molar equivalents relative to substrate). Conditions: 0.25 nM nsp13, 150 μM ATP, 1 μM DNA substrate. Buffer: 20 mM HEPES, pH 7.5, 40 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM TCEP, 0.01% triton X100 and 0.1 mg/mL BSA.

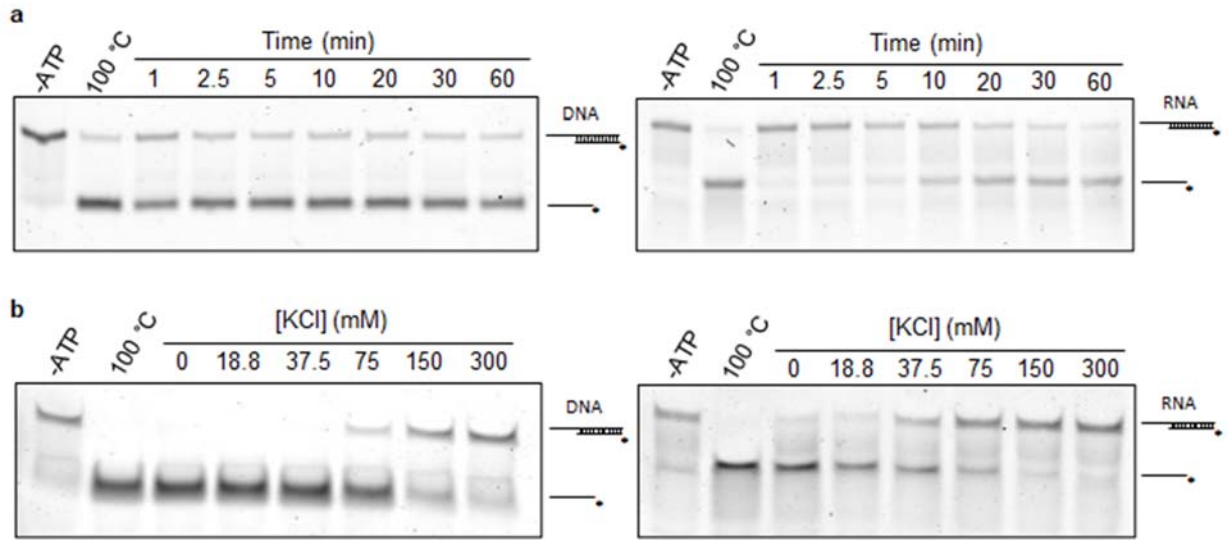

Figure S2. Gel-based nsp13 helicase activity assays.

a. Helicase assay time-courses show that SARS-CoV-2 nsp13 can unwind partial duplex DNA (*left*) or RNA (*right*) with a 5' overhang. TAMRA label is marked with an asterisk. Heat denatured and no ATP controls are indicated. Fluorescence was visualized using a Typhoon Trio Variable Mode Imager in TAMRA dye mode.

b. Effect of salt concentration on SARS-CoV-2 nsp13 DNA (*left*) or RNA (*right*) helicase activity.

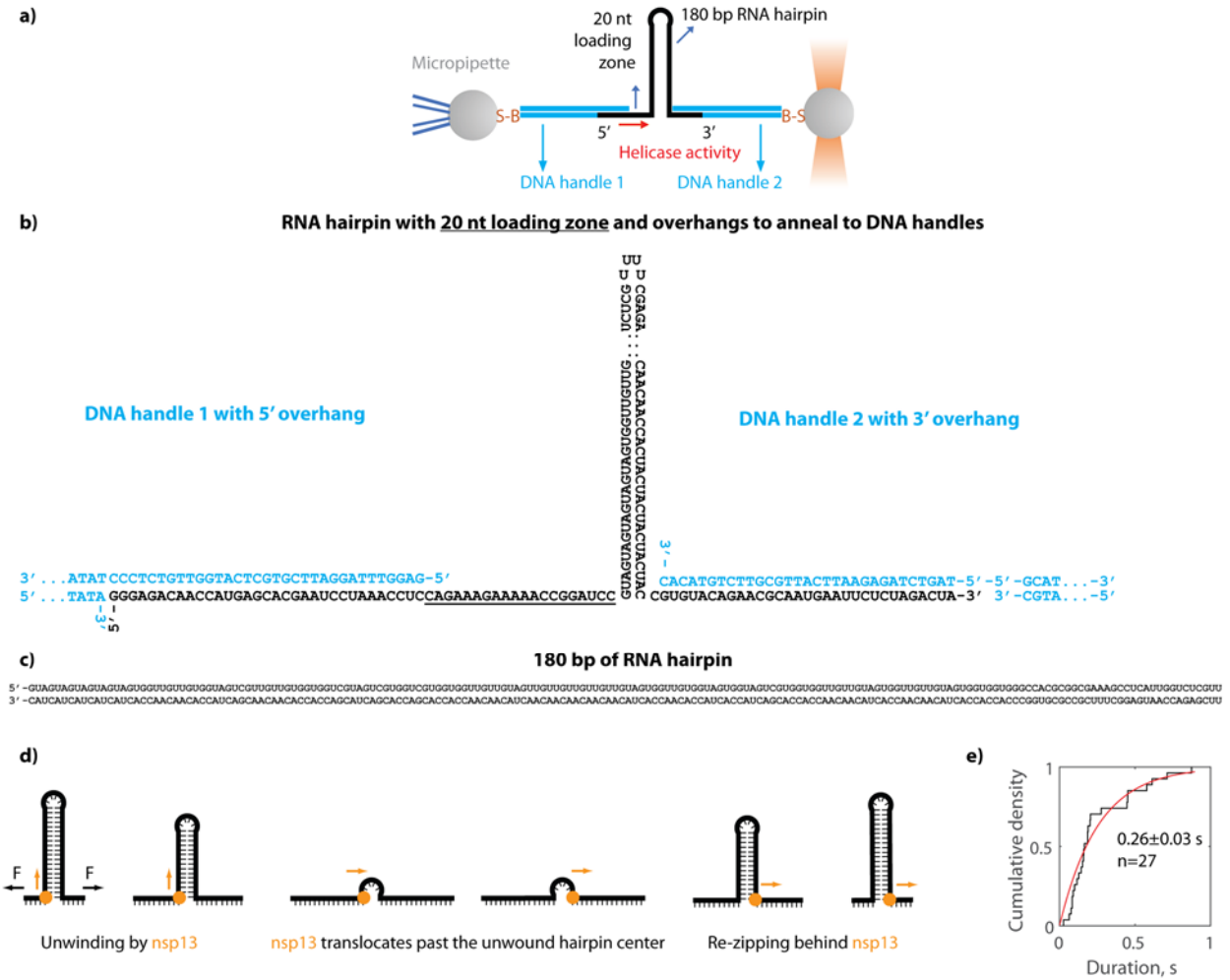

Figure S3. Design of RNA hairpin and nsp13 interactions at the end of the hairpin.

a. Diagram showing the single-molecule optical tweezers assay geometry. Color code matches panel b.

b. Detailed diagram of the DNA/RNA tether. DNA handle 1 has an ~30 nt 5' overhang, which hybridizes with the 5' region of the RNA. A 20 nt loading region (underlined) is the only single-stranded region where nsp13 can bind. DNA handle 2 has an ~30 nt 3' overhang generated using inverted base pairs, which hybridizes with the 3' region of the RNA. Due to the orientation and use of inverted base pairs, the DNA handles cannot be unwound by nsp13.

c. Complete sequence of the 180 bp duplex region of the RNA hairpin.

d. Diagram showing how the RNA hairpin could rezip behind translocating nsp13. Data in Fig. 3 shows that nsp13 likely pauses when the hairpin is fully unwound.

e. Distribution of pause durations at the 180 bp position preceding nsp13 detachment (18 pN, 1 mM ATP). Data (n=27) is fitted to the exponential distribution $F(x) = 1 - exp(-x/\tau)$, with characteristic duration τ (fit ± 95% confidence intervals) shown inset.

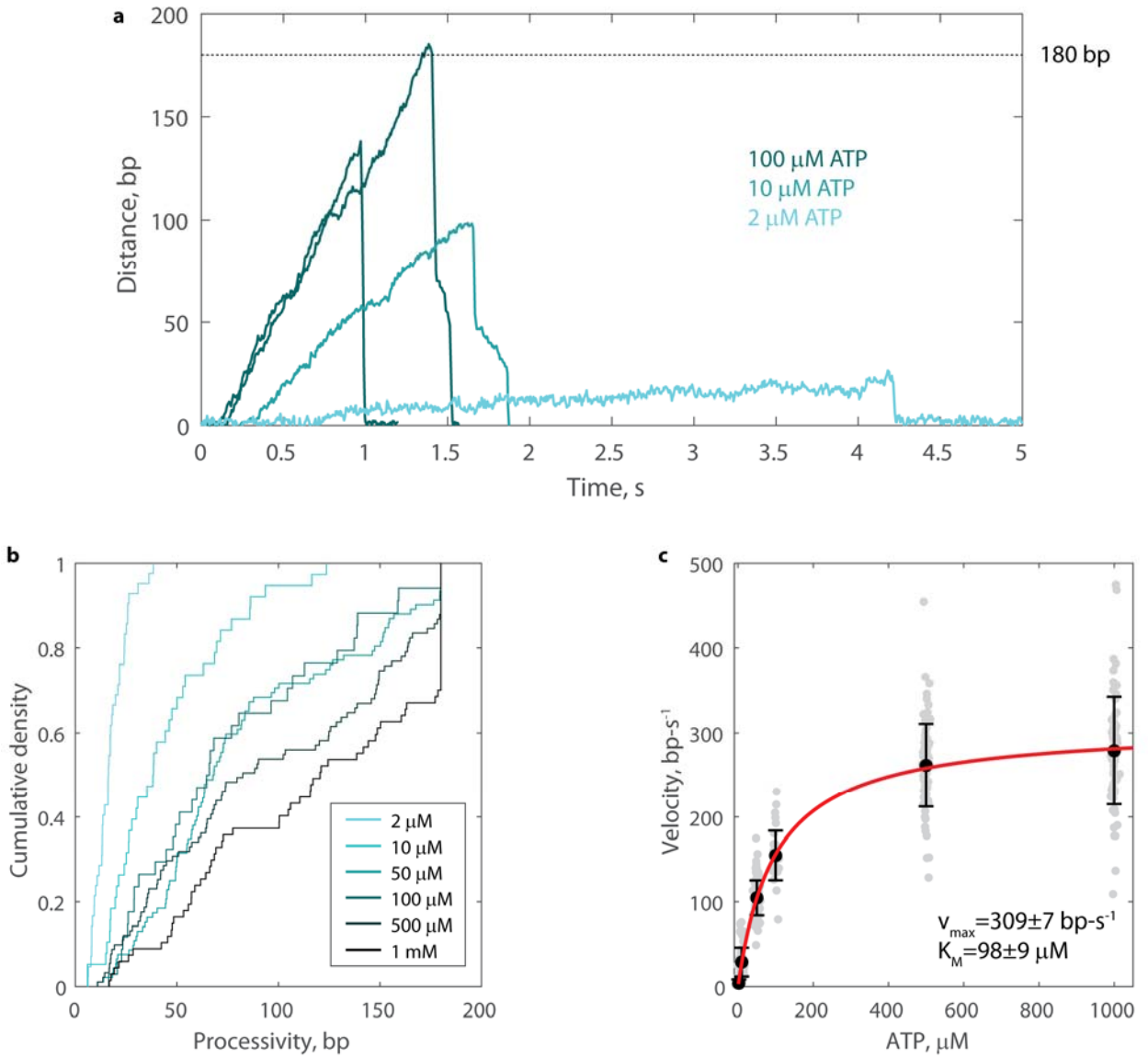

Figure S4. Single-molecule measurements of nsp13 at 18 pN and various ATP concentrations

a. Example traces of nsp13 unwinding activity on the same RNA hairpin as Fig. 3, but with different amounts of ATP.

b. The processivity distributions for nsp13 at 2 μM ATP (n=42), 10 μM ATP (n=38), 50 μM ATP (n=92), 100 μM ATP (n=34), 500 μM ATP (n=91), and 1000 μM ATP (n=67).

c. The velocities of nsp13 at 2 μM ATP (n=42), 10 μM ATP (n=44), 50 μM ATP (n=92), 100 μM ATP (n=34), 500 μM ATP (n=91), and 1000 μM ATP (n=67). Gray dots show individual measurements, black dots show mean ± standard deviation. Red line shows fit to the Michaelis-Menten equation $v(x) = \frac{v_{max}x}{K_M+x}$, with fitted parameters (fit ± 95% confidence intervals) shown inset.

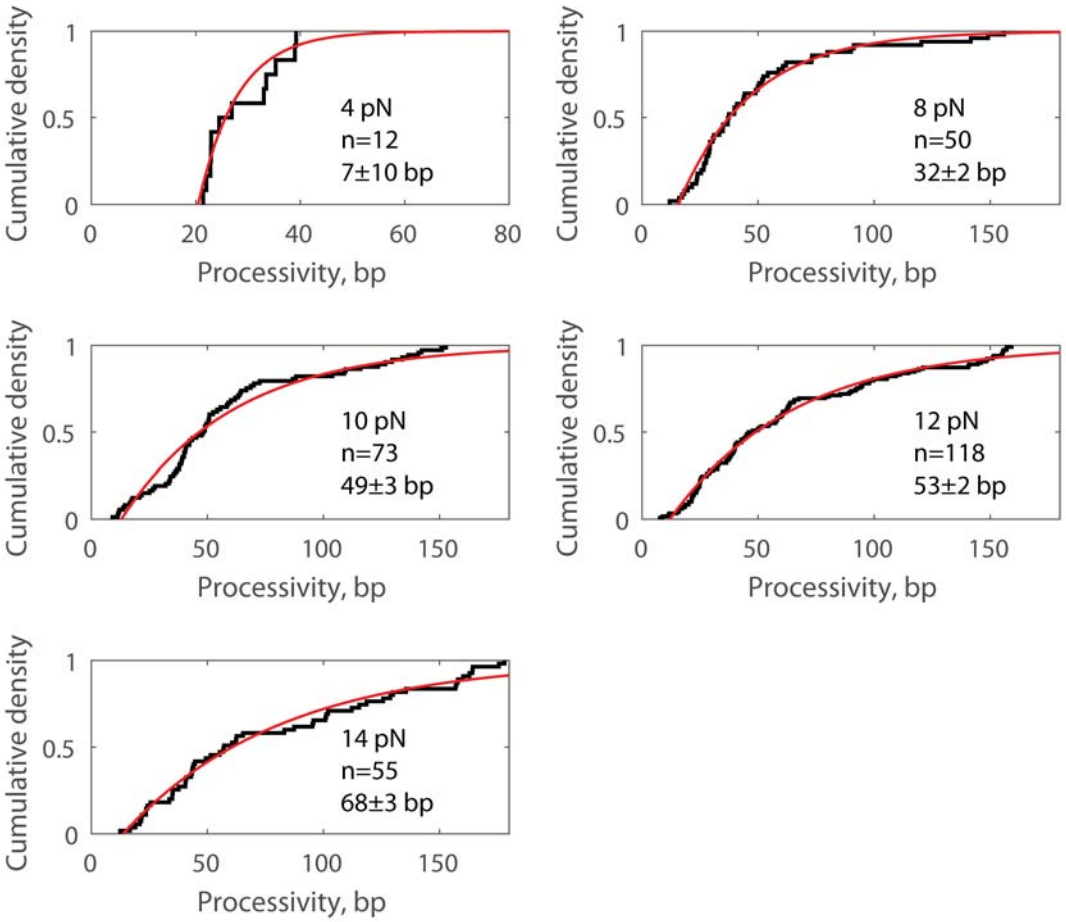

Figure S5. Single-molecule processivity data. Each panel shows the processivity distribution of nsp13 at 1 mM ATP and the indicated force, replotted from Fig. 4c. Red lines are fits to an offset exponential $F(x) = 1 - exp(\frac{-(x-x_{min})}{p})$ where x_{min} denotes the smallest possible processivity measurement and p denotes the average processivity. Fitted values for p are shown inset as fit ± 95% confidence intervals.