Supporting Information

Unzipping of A-Form DNA-RNA, A-Form DNA-PNA, and B-Form DNA-DNA in the α-Hemolysin Nanopore

Rukshan T. Perera,[1] Aaron M. Fleming,[1] Amberlyn M. Peterson,[1] Jennifer M. Heemstra,[1] Cynthia J. Burrows,[1,*] and Henry S. White[1,*]

[1]Department of Chemistry, University of Utah, Salt Lake City, Utah

*To whom correspondence should be addressed.

E-mail: burrows@chem.utah.edu or white@chem.utah.edu

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1. Sample *i-t* trace of a mixture containing B-form duplex.

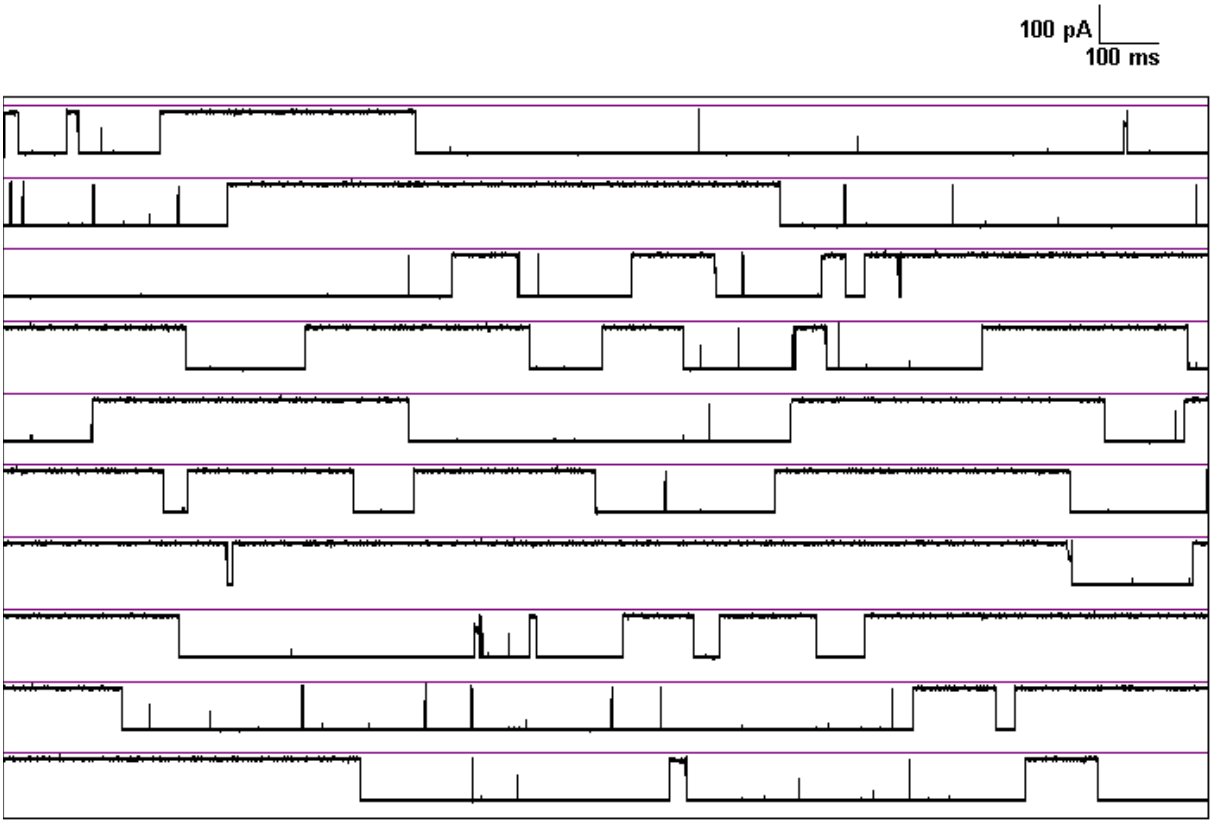


Figure SI1. A sample *i-t* trace showing uninterrupted data collected at 10 kHz for 20 s at 120 mV. The mixture contained 8 μM B-form duplex in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C.

2. Sample *i-t* trace of a mixture containing A-form duplex.

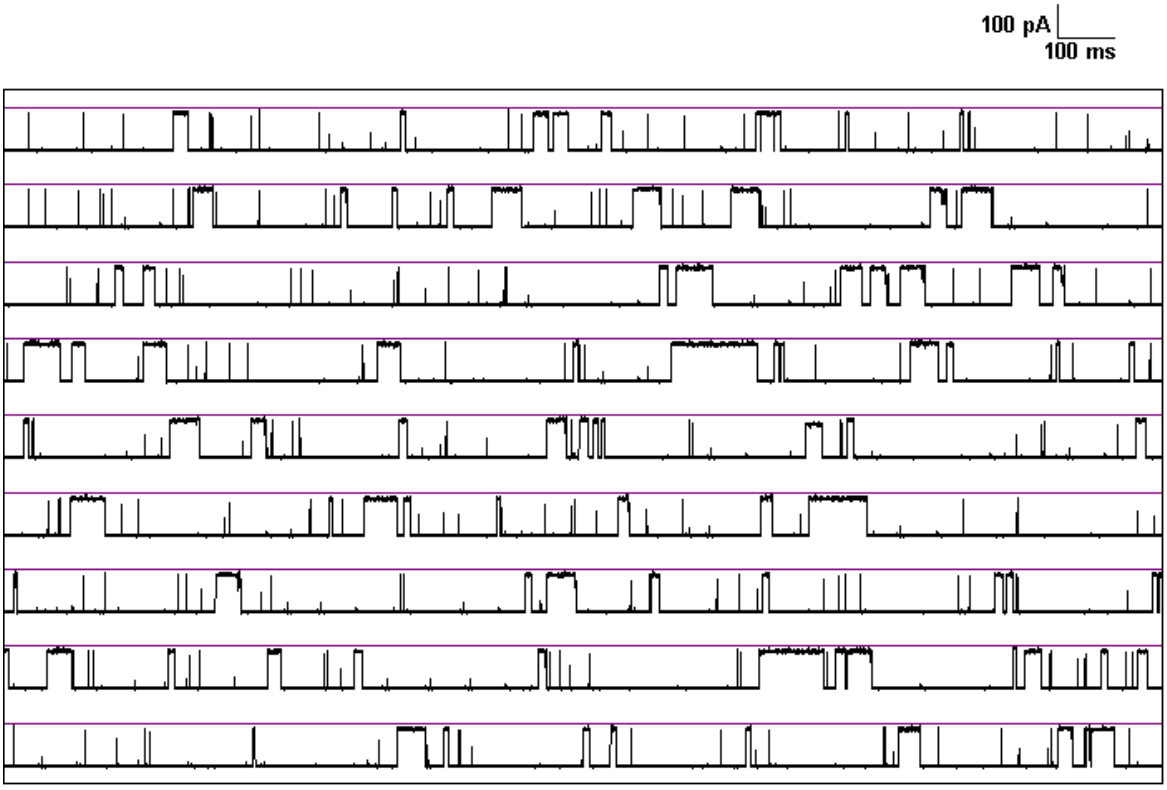


Figure SI2. A sample *i-t* trace showing uninterrupted data collected at 10 kHz for 20 s at 120 mV. The mixture contained 8 μM A-form duplex in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C.

3. Sample *i-t* trace of a mixture containing both A- and B-form.

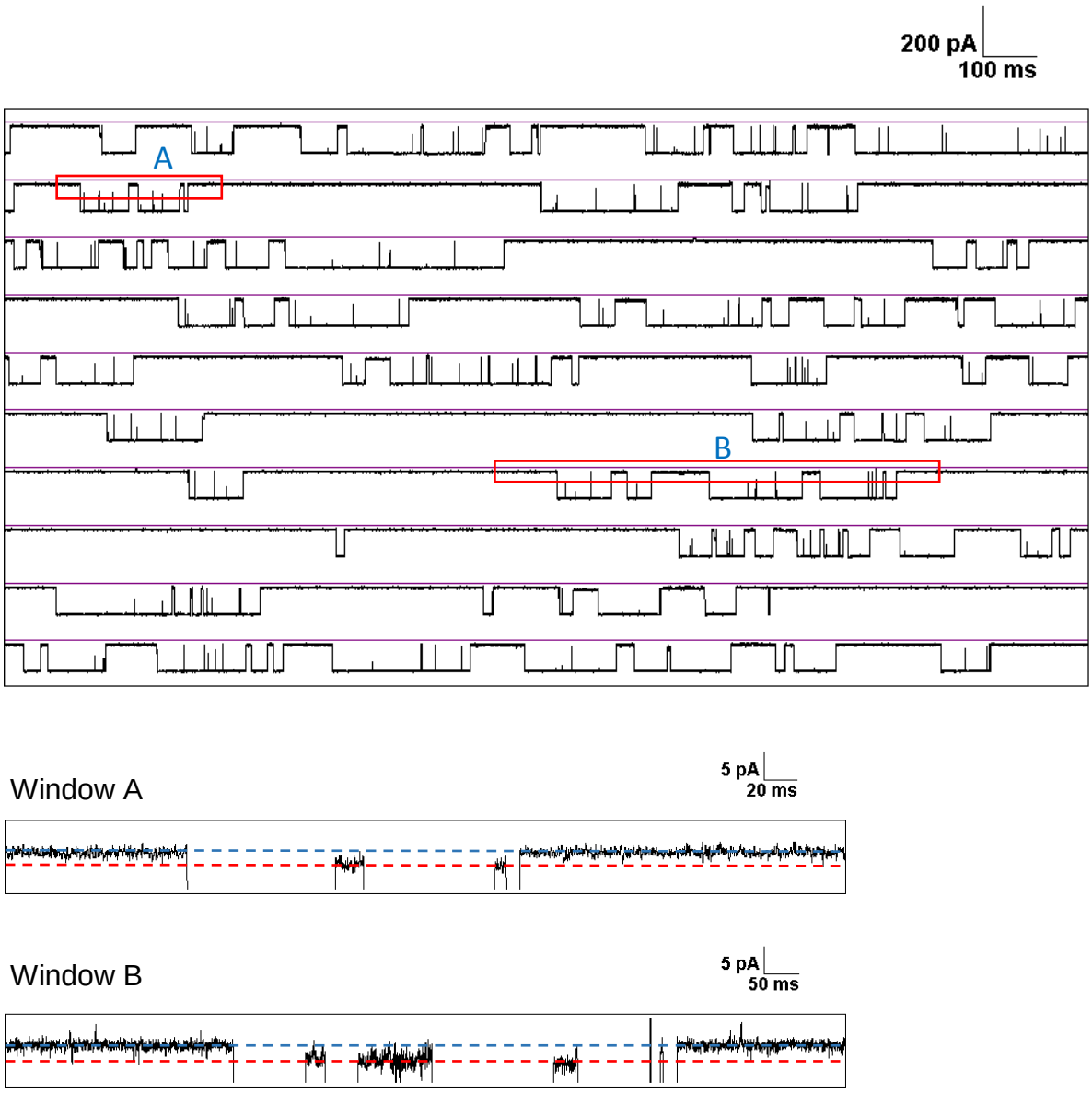


Figure SI3. A sample *i-t* trace showing uninterrupted data collected at 10 kHz for 20 s at 120 mV. The mixture contained 8 μM A- and B-form duplexes in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C. The two expanded windows, A and B, show the deep block current differences between A- and B-form duplexes. The expanded area is filtered to 1 kHz for presentation purpose.

4. Voltage dependence of unzipping times for DNA-RNA and DNA-DNA duplexes

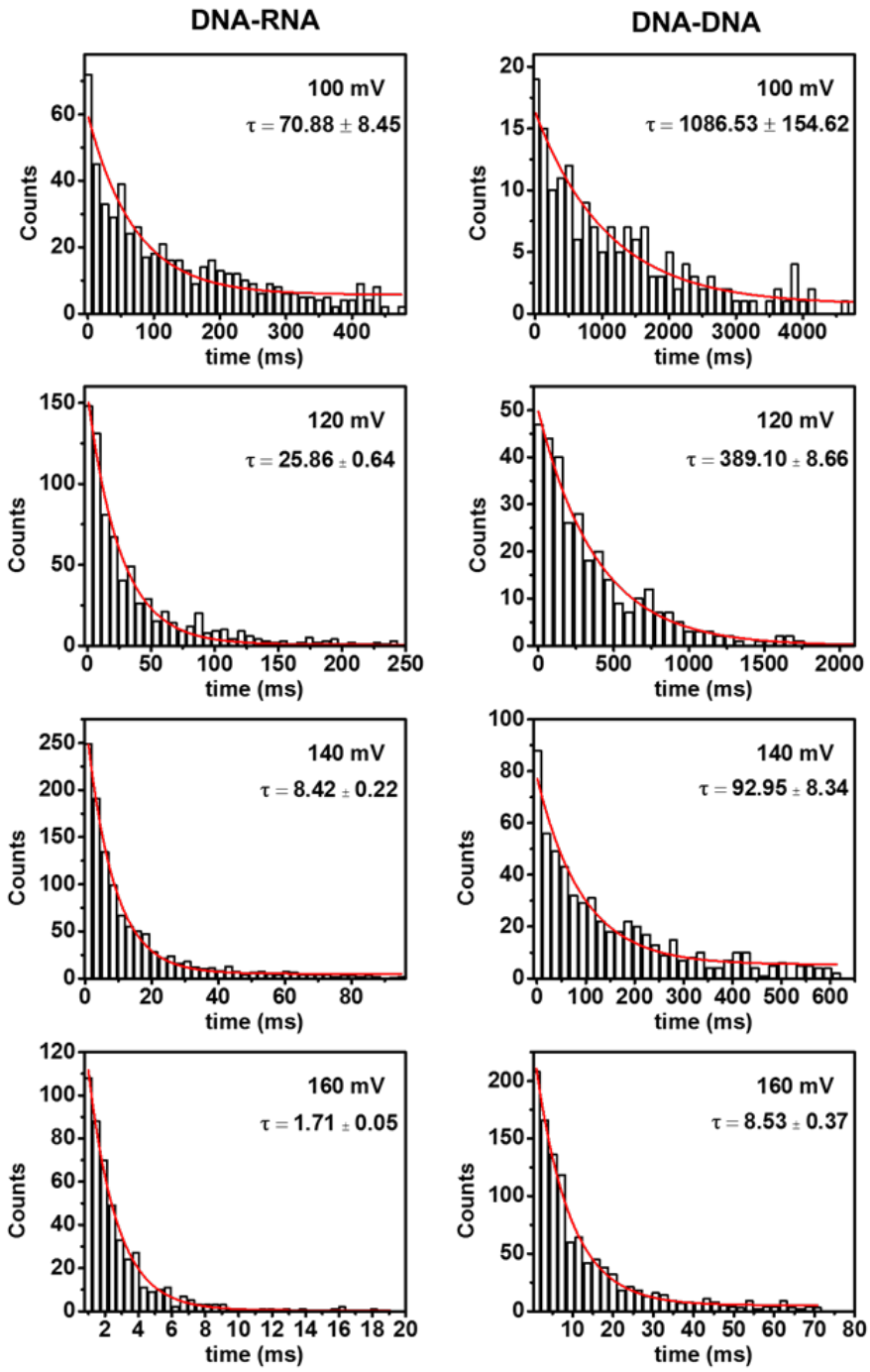


Figure SI4. Unzipping duration histograms as a function of voltage for DNA-RNA (left) and DNA-DNA (right) duplexes. The data were recorded at 20 °C in 1 M KCl, 10 mM PBS, pH 7.4. An exponential decay was fit to the data to obtain the unzipping time.

5. Unzipping of DNA-RNA duplex with 40-nt overhang.

Sequence of DNA-RNA duplex with 40–nt overhang.

DNA-RNA 5`-TCA TCA GTA GAA CTC AGA AAC $TCC_n$-3` n = 40

3`-AGU AGU CAU CUU GAG UCU UUG AG-5`

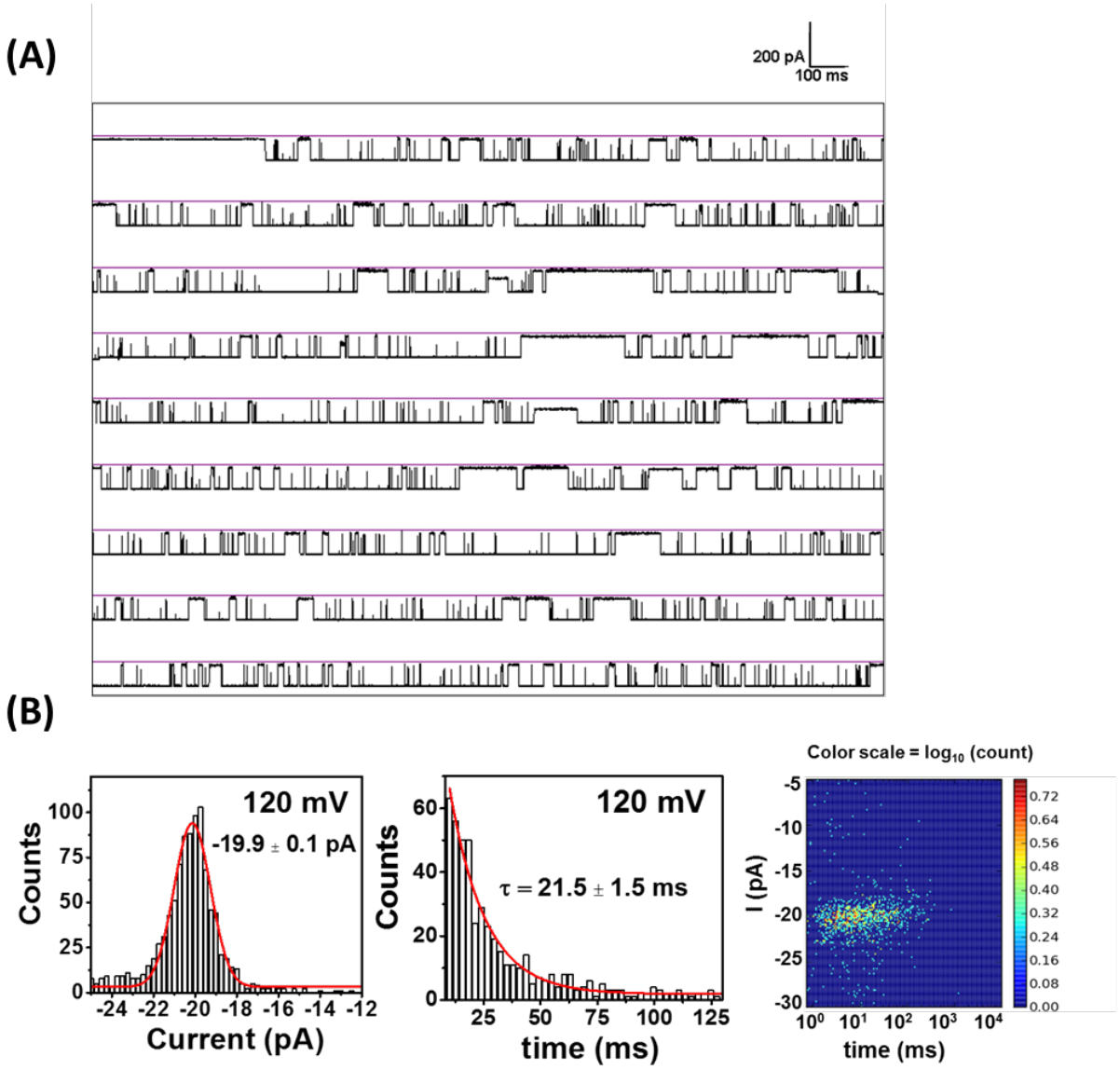
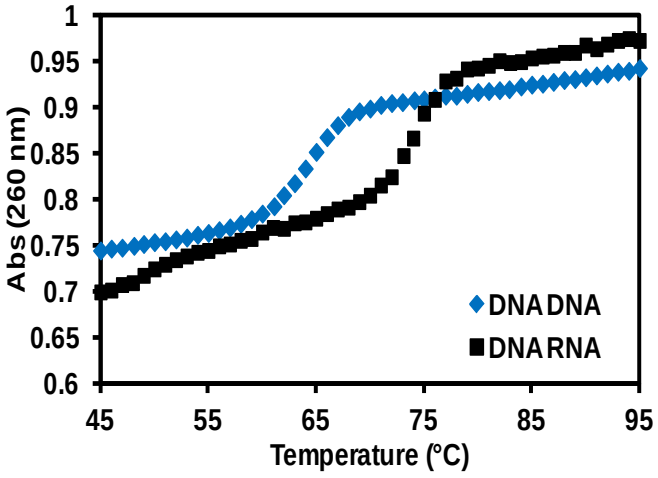


Figure SI5. (*A*) A sample *i-t* trace showing uninterrupted data collected at 10 kHz for 20 s at 120 mV. The mixture contained 10 μM DNA-RNA duplex with 40-nt overhang in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C. (*B*) Current blockage, unzipping time duration, and *i-t* density plots for DNA-RNA duplex with 40–nt overhang.

6. Thermal melting analysis of A-and B-form duplexes.



Sequences
DNA-DNA Duplex 5`-TCA TCA GTA GAA CTC AGA AAC TC-3` 3`-AGT AGT CAT CTT GAG TCT TTG AG-5`
DNA-RNA Duplex 5`-TCA TCA GTA GAA CTC AGA AAC TC-3` 3'-AGU AGU CAU CUU GAG UCU UUG AG-5`

Figure SI6. Thermal melting analysis of the DNA-DNA and DNA-RNA duplexes. All measurements were performed in 10 mM PBS, pH 7.4. The absorbance at 260 nm, $Abs_{260\ nm}$, was monitored as the temperature was increased from 20 °C to 100 °C at a ramp rate of 1 °C/min. At each time interval, the temperature was equilibrated for 30 s prior to making each absorbance measurement. Each experiment was conducted in triplicate.

7. Continuous *i-t* trace of DNA-RNA duplex with 10-nt overhang at 160 mV.

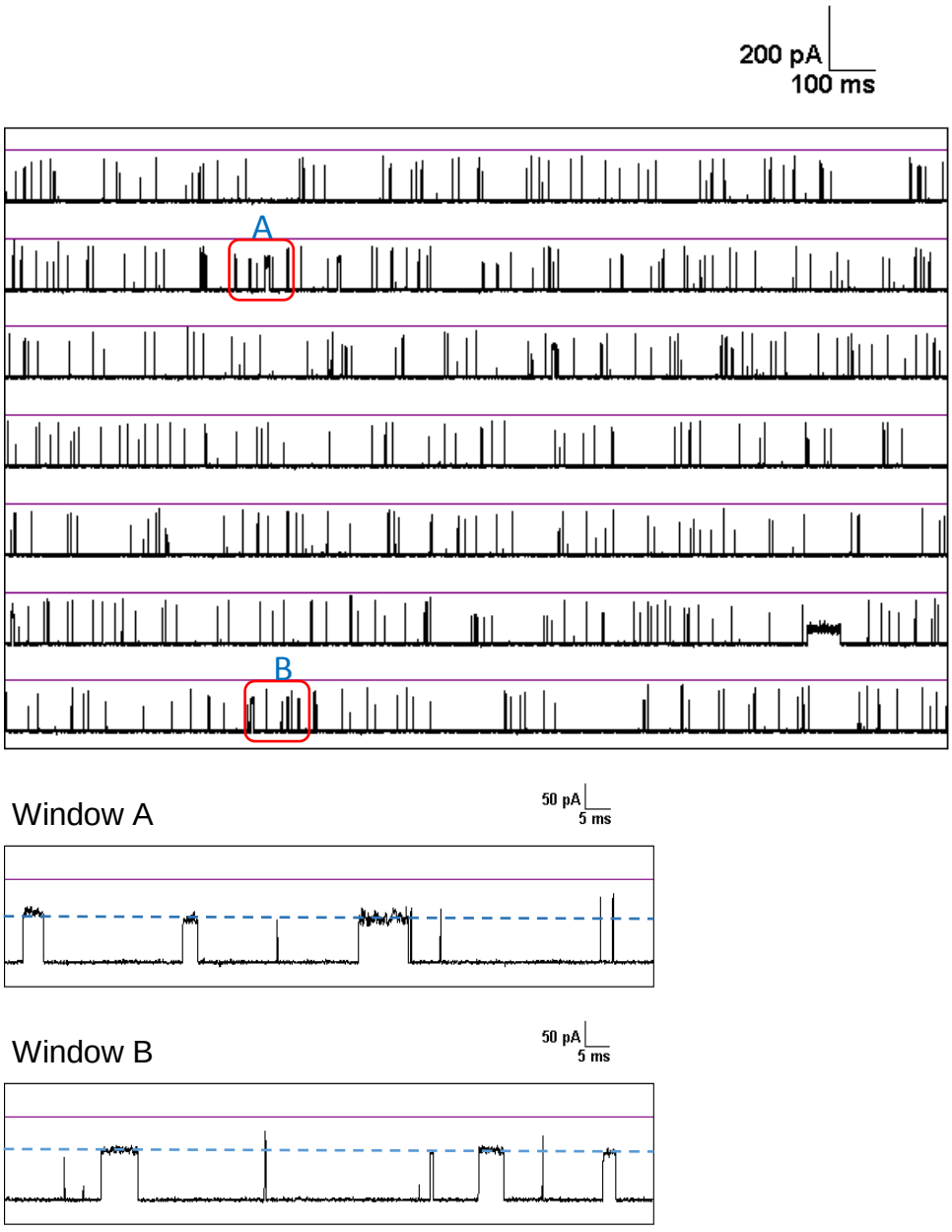


Figure SI7. A sample *i-t* trace showing uninterrupted data collection at 10 kHz for 20 s at 120 mV. The mixture contained 8 μM of DNA-RNA duplex with 10-nt overhang in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C. The two expanded windows (A and B) show the blockage due to occupation of the 10-nt overhang in the vestibule.

8. Voltage dependent trapping time of the DNA-RNA duplex with 10-nt overhang.

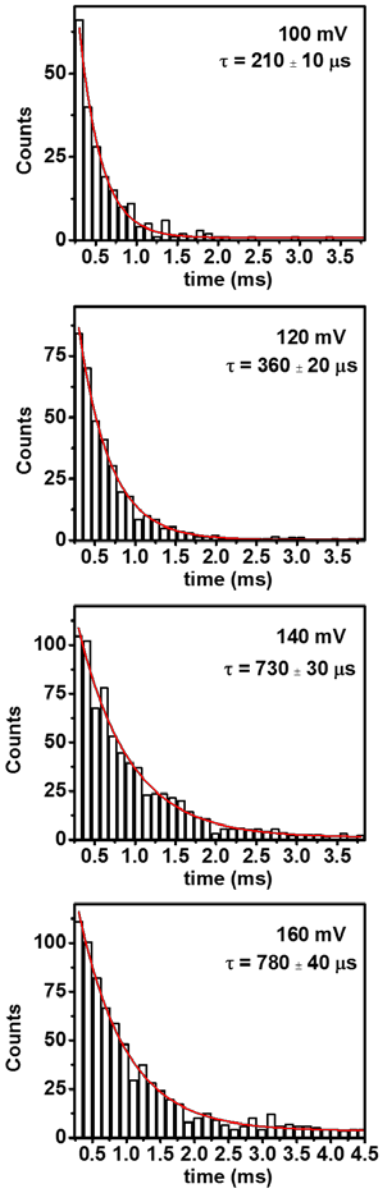


Figure SI8. Trapping time duration histograms as a function of voltage for DNA-RNA duplex with a 10-nt overhang. Only the events with $\%I/I_o$ between 20 and 80 and $\tau >$ 200 μs were analyzed as duplex unzipping events (single strand translocation is much faster). Data were recorded at 20 °C in 1 M KCl, 10 mM PBS, pH 7.4. An exponential decay was fit to the data to obtain the unzipping time.

9. Sample *i-t* trace of DNA-DNA duplex with 10-nt overhang at 160 mV.

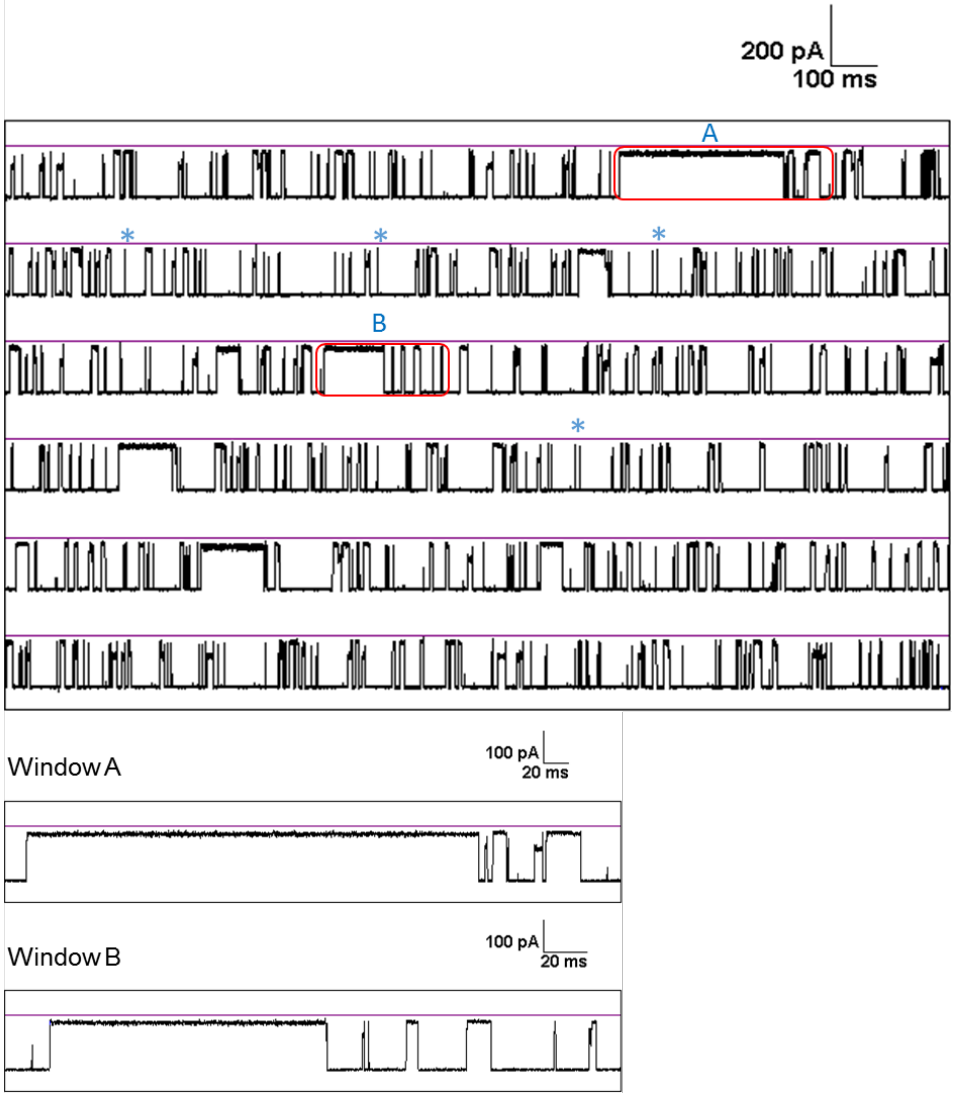


Figure SI9. A sample *i-t* trace showing uninterrupted data collection at 10 kHz for 20 s at 160 mV. The mixture contained 8 μM of the DNA-DNA duplex with 10-nt overhang in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C. The two expanded windows (A and B) show long-current blocks are due to unzipping of the duplex and the shorter blocks (less than 1 ms denoted by asterisks) are from translocation of the excess ssDNA.

10. Sample *i-t* trace of DNA-RNA duplex with no overhang.

Figure SI10. A continuous *i-t* trace showing uninterrupted data collection at 10 kHz for 20 s at 160 mV. The mixture contained 8 μM DNA-RNA blunt end duplex in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C. The expanded window A shows short translocation events (less than 500 μs) that are due to excess single strands present in the mixture.

11. Sample *i-t* trace of DNA-DNA duplex with no overhang.

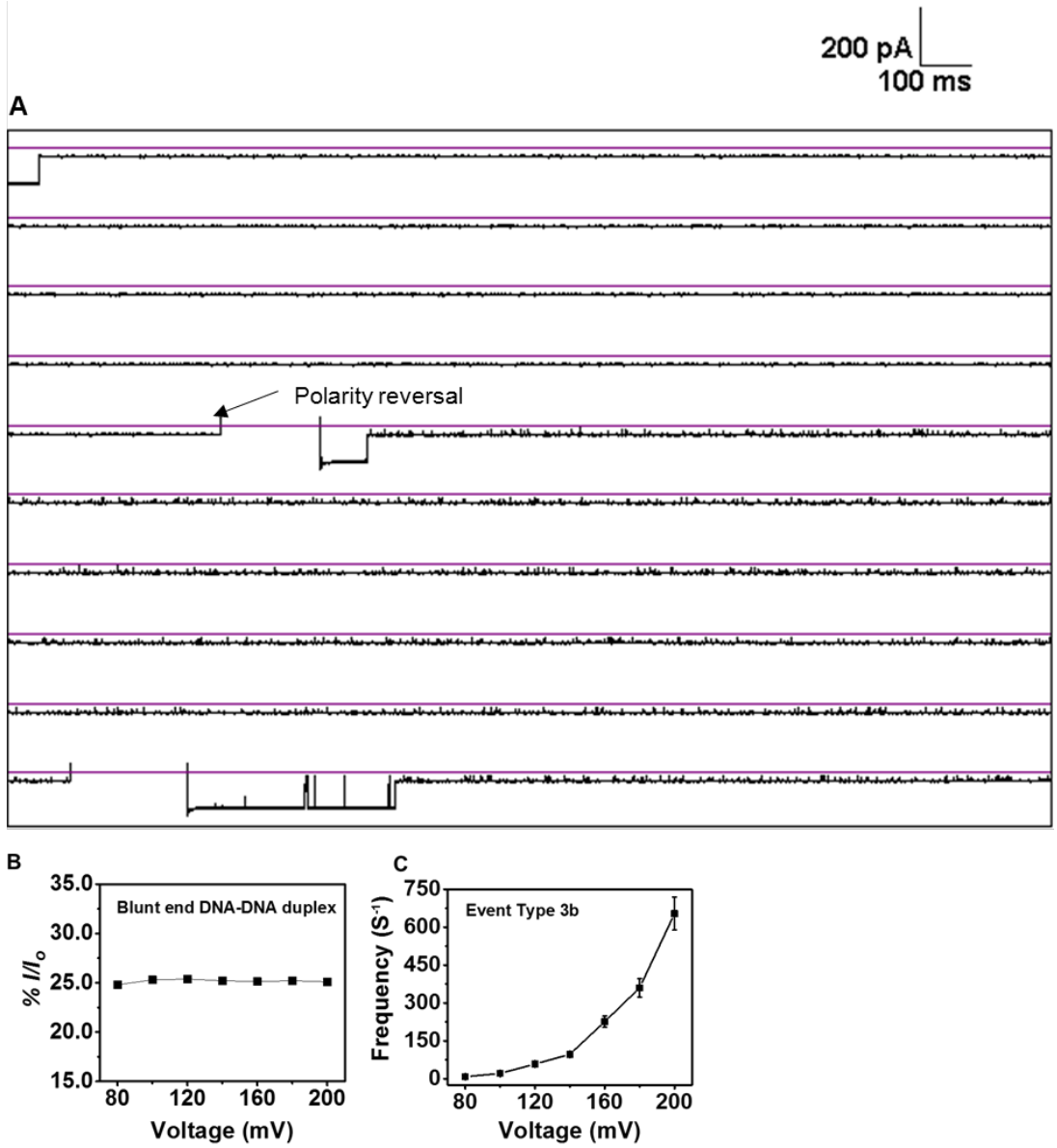


Figure SI11. (*A*) A continuous *i-t* trace showing uninterrupted data collection at 10 kHz for 10 s at 120 mV. The *cis* side contained 8 μM DNA-DNA blunt end duplex in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C. Long-current blockages show the duplex occupying the vestibule and the short events (less than 1 ms) are due to translocation of excess single strands. Interruption of the current blockage was due to the polarity reversal of the channel to remove the duplex in the nanopore. (*B*) Residual current when a blunt end duplex is inside the vestibule as a function of voltage. (*C*) Frequency of the events between two current levels shown in Event Type 3b in the main text Fig. 5B.

12. Sample *i-t* trace of DNA-DNA duplex with no overhang at 200 mV.

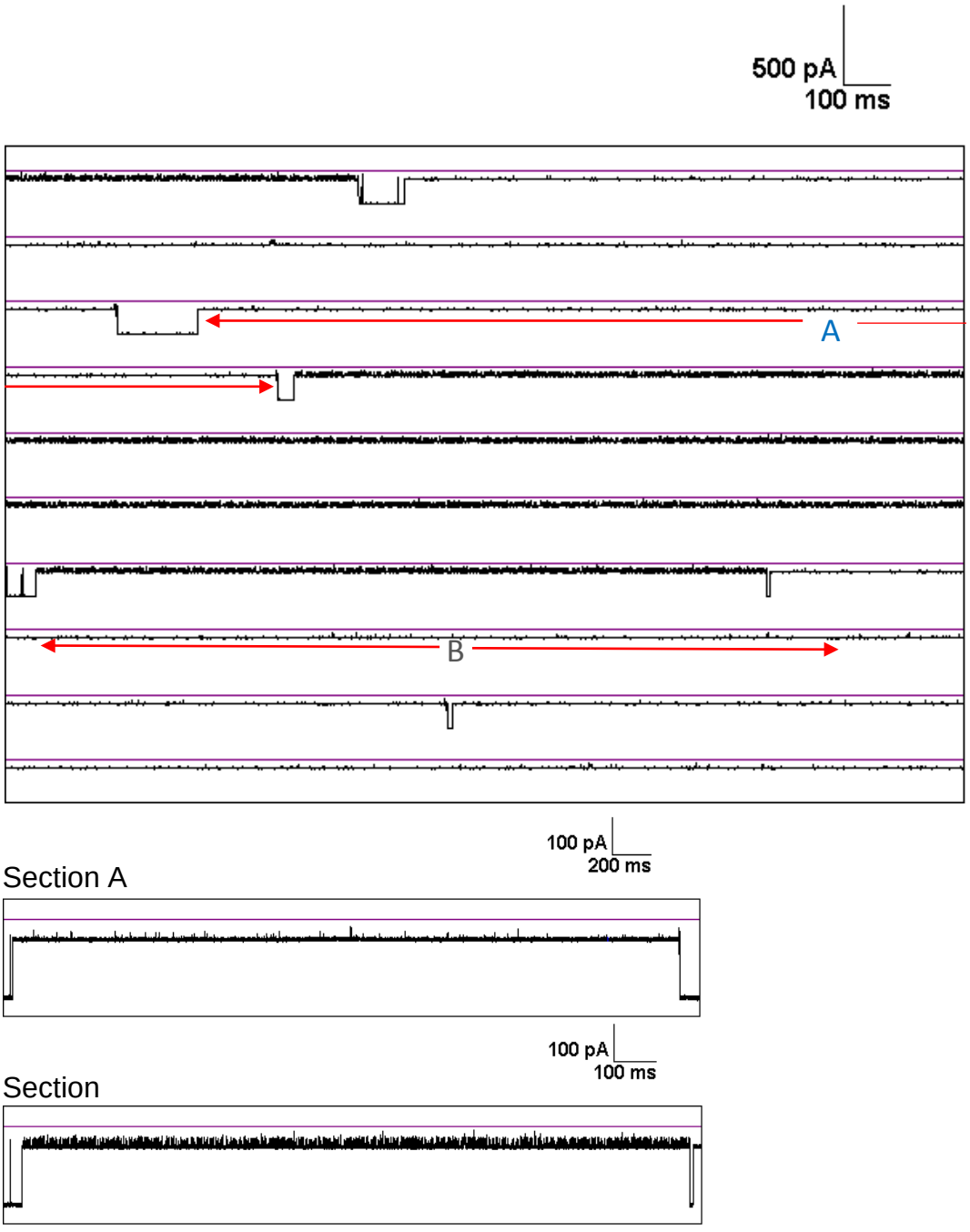


Figure SI12. A sample *i-t* trace showing uninterrupted data collection at 10 kHz for 20 s at 200 mV. The blunt end duplex unzips at 200 mV but not at 120 mV (see preceding section). The mixture contained 8 μM of the DNA-DNA duplex with 10-nt overhang in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C. The two expanded windows (A and B) shows long-current blocks are due to unzipping of the duplex.

13. Sample *i-t* trace of a mixture containing DNA-PNA duplex.

Sequence of DNA-PNA duplex

DNA-PNA 5`-N  GTA  GAT CAC T-Lys -3`

3`-CAT  CTA GTG $A_{24}$-5`

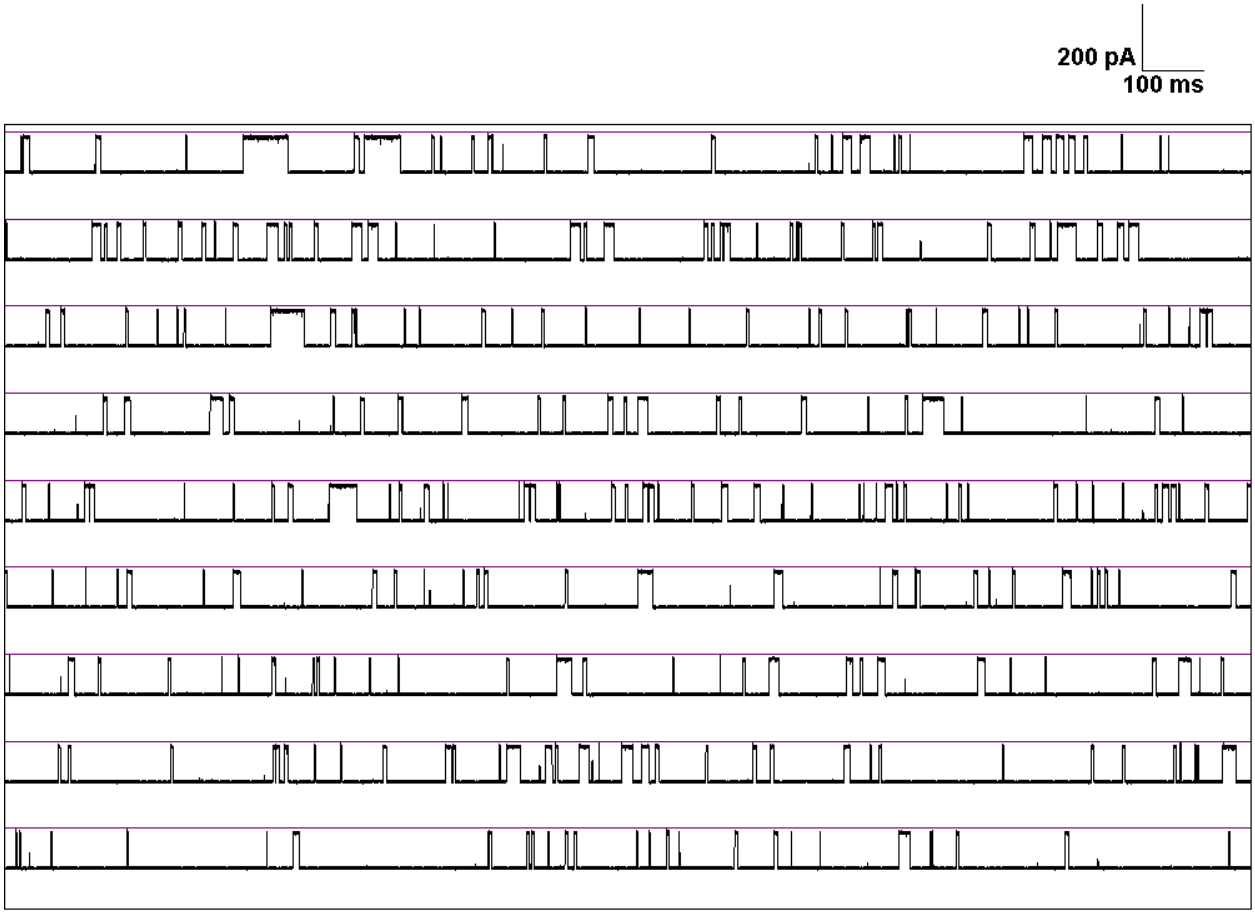


Figure SI13. A sample *i-t* trace showing uninterrupted data collected at 10 kHz for 20 s at 120 mV. The mixture contained 8 μM DNA-PNA duplex in 1 M KCl, 10 mM PBS, pH 7.4 at 20 °C.

14. Unzipping time and voltage dependent unzipping for DNA-PNA duplexes.

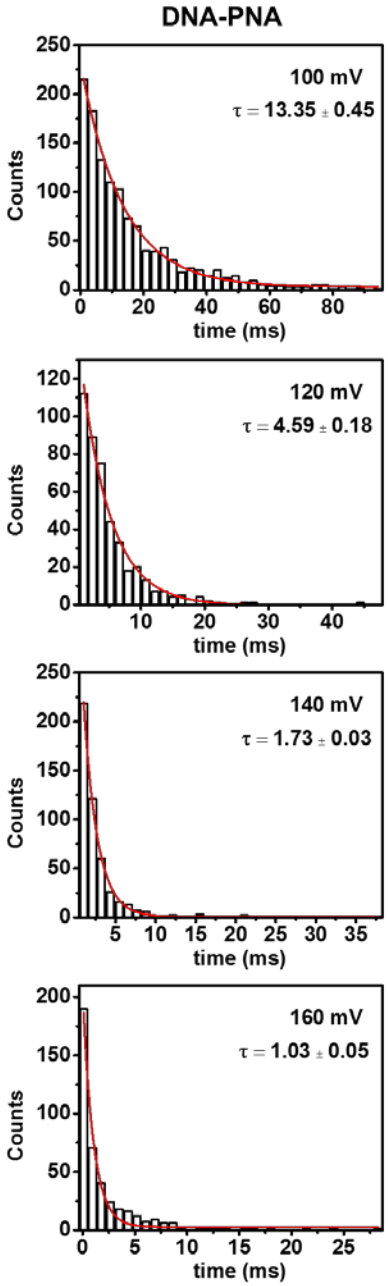


Figure SI14. Unzipping duration histograms as a function of voltage for the DNA-PNA duplexes. Data were recorded at 20 °C in 1 M KCl, 10 mM PBS, pH 7.4. An exponential decay was fit to the data to obtain the unzipping time. The *cis* side of the protein channel contained 8 μM of DNA-PNA sample.

15. Comparison of unzipping times of DNA-RNA duplexes with 3' and 5' overhangs.

In order to investigate if the orientation of the overhang influences the unzipping mechanism, we performed unzipping experiments using 5'-end poly C (24-nt) to compare with the 3'-end poly C (24-nt). The sequence used is given below.

DNA-RNA 5`-TCA TCA GTA GAA CTC AGA AAC $TCC_n$-3` n = 24

3`-AGU AGU CAU CUU GAG UCU UUG AG-5`

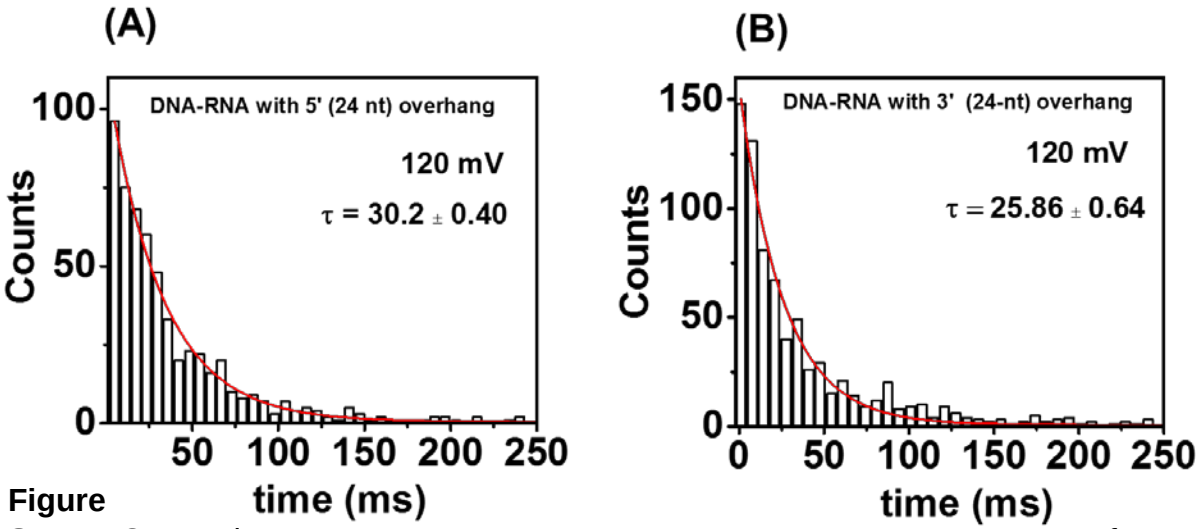


Figure SI15. Comparison of the unzipping duration histograms for the DNA-RNA duplexes. (*A*) 5' poly C overhang. (*B*) 3' poly C overhang. Data were recorded at 20 °C in 1 M KCl, 10 mM PBS, pH 7.4. An exponential decay was fit to the data to obtain the unzipping times. The *cis* side of the protein channel contained 8 μM of DNA-RNA in each case.