Supporting Information

Sampling a Biomarker of the Human Immunodeficiency Virus (HIV) across a Synthetic Nanopore

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1. Control experiments with NCp7 and SL3 RNA aptamers using small nanopores

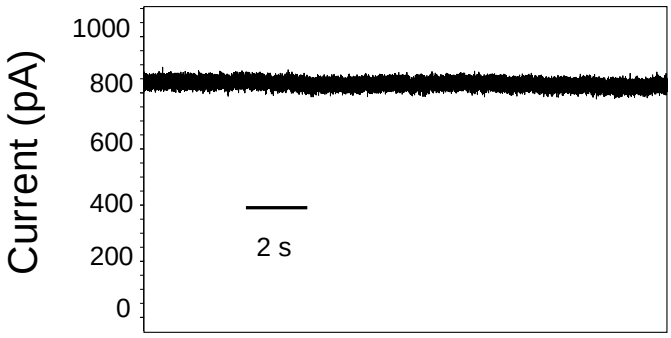


Figure S1: **The NCp7 protein did not produce any alteration in the single-channel electrical signature of a small nanopore.** Single-channel electrical trace demonstrates lack of current blockades at a positive bias of +100 mV for a nanopore after 1000 nM was added to the *cis* side. Solution in the chamber was 0.2 M NaCl, 5 mM NaH_2PO_4 on the *cis* side, and 1 M NaCl, 5 mM NaH_2PO_4 on the *trans* side. The diameter of the nanopore was 3.8 ± 0.3 nm.

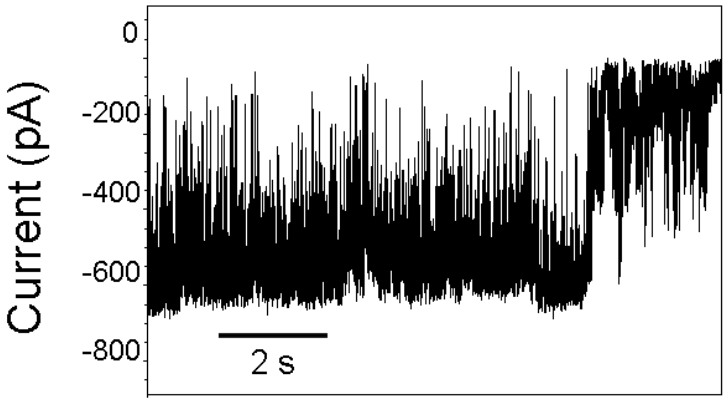


Figure S2: **NCp7 fouling of a nanopore**. The single-channel electrical trace demonstrates NCp7 events at a negative bias of -100 mV for a nanopore after 1 μM NCp7 was added to the *cis* side. Solution in the camber was 0.2 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *cis* side, and 1 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *trans* side. The diameter of the nanopore diameter was 3.8 ± 0.3 nm. Nanopores eventually clogged under these conditions.

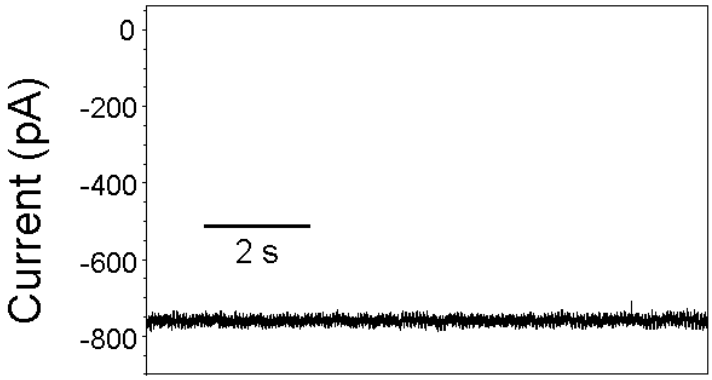


Figure S3: **The SL3 (GAG) aptamer does not produce current blockades at a negative voltage using a small nanopore**. Single-channel electrical trace demonstrates lack of events at a negative bias of -100 mV for a nanopore after 500 nM RNA SL3 (GAG) was added to the *cis* side. Solution in the chamber was 0.2 M NaCl, 5 mM NaH_2PO_4 on the *cis* side, and 1 M NaCl, 5 mM NaH_2PO_4 on the *trans* side. The diameter of this nanopore was 3.7 ± 0.3 nm.

2. Comparison of the values of the dwell time and amplitude of the current blockades recorded with a small-diameter nanopore before and after addition of NCp7 to solution

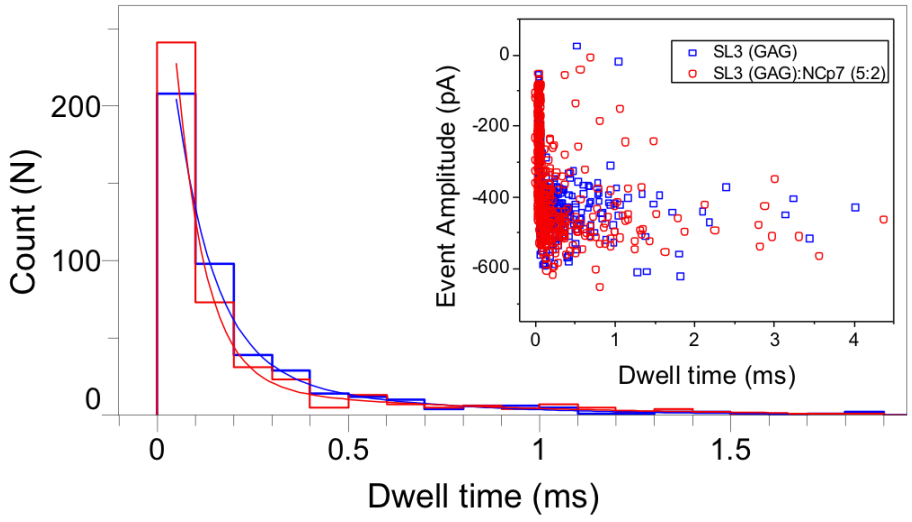


Figure S4: Comparison of the values of the dwell time and amplitude of the current blockades recorded with a small-diameter nanopore. Dwell time histograms for events in solution with 1000 nM SL3(GAG) RNA (blue) and after addition of 400 nM NCp7 (red). Fitting to a single term exponential gives dwell times of 210 ± 3 µs and 220 ± 3 µs, respectively. Event amplitude *versus* dwell time scatter plot is given in the inset. Solution was 0.2 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *cis* side, and 1 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *trans* side. 500 nM SL3 (GAG) aptamer was added to the *cis* side. The diameter of the nanopore was 4 nm.

3. Voltage dependence of the dwell time of the current blockades produced by SL3 RNA aptamers

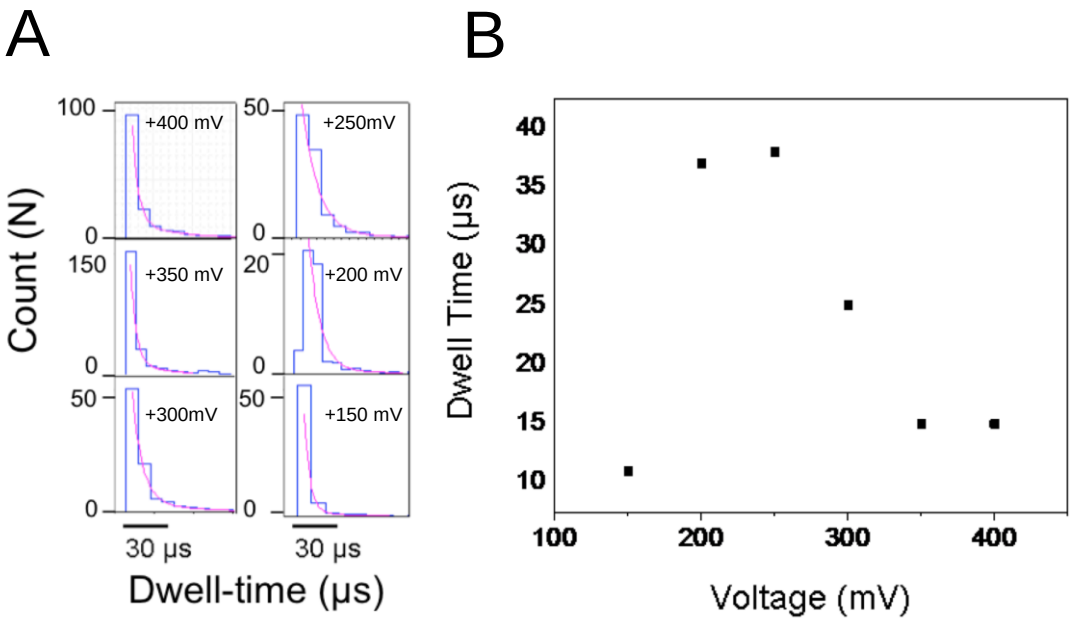


Figure S5: **Voltage dependence of the dwell time of the SL3 RNA aptamer-produced current blockades.** (**A**) Dwell-time histograms are shown; (**B**) The voltage dependence plots presenting that the dwell time displays a maximum value at applied voltages between 200 and 250 mV. Solution was 0.2 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *cis* side, and 1 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *trans* side.

500 nM SL3 (GAG) aptamer was added to the *cis* side. The diameter of the nanopore was 4.5 nm. Note that single-channel recordings with different nanopores displayed different dwell time durations.

4. Large event ratio with smaller events for all three SL3 RNA aptamers

Assuming that the capture of NCp7 and its complex in large nanopores is representative of the concentration of the complex and free SL3 RNA aptamer in solution and also assuming 1:1 complexes, we could use equation 2 (the main text) to find K_d, making the substitution of $f_{small\ events} + f_{large\ events}$ for f_0. **Fig. S6** shows a plot of the titration curves following this method. Using this approach, there is a substantial difference between the binding affinities of the high-affinity SL3 RNA aptamer to NCp7, as calculated with large nanopores and fluorescence (**Table S1**).

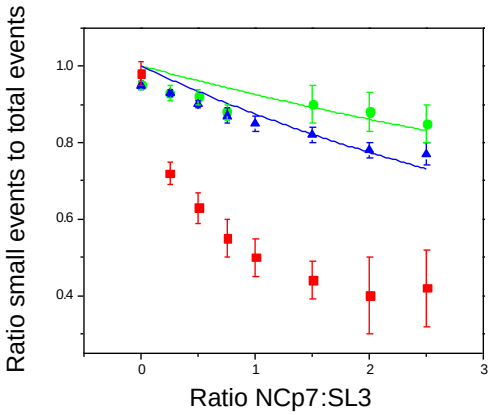
Table S1. The K_d values calculated with large nanopores and using the event ratios.[a]

The SL3 RNA aptamer	Large nanopore	Fluorescence[b]
GAG	1357±337 nM	28 ± 2 nM
CUG	607±67 nM	850 ± 250 nM
AUA	11400±2450 nM	20000 nM

[a]Note that it is the NCp7 protein - SL3 (GAG) aptamer complex that gives the great distinction values from fluorescence data. The SL3 RNA (CUG) and (AUA) fittings give fairly reasonable values, despite their slopes being shallower than expected.

[b]Fluorescence-based values are from the previously reported data.[2]

The most probable culprit is the assumption that the nanopore samples the ratio of events correctly. There are several reasons for thinking this might not be the case. Most theories of analytes entering a nanopore break the process into 3 regimes: the diffusion regime, the attraction regime and the entering regime. All three will affect the capture rate of the analyte and for all three we may expect that the complex will behave differently than RNA alone. In the first regime the bulkier complex will have a slower diffusion rate. In the second it will feel less force because of its lesser charge density. In the third, while larger pores appear to admit the complex, there still may be a substantial energetic penalty for the complex to enter the pore when compared with the RNA alone.



<u>Figure S6</u>: Titration curves for all three SL3 RNA aptamer variants using ratio of the number of small events to number of large events acquired with large nanopores. Squares represent the high-affinity SL3 (GAG) aptamer, triangles indicate the low-affinity SL3 (CUG) aptamer, and circles display the no-affinity SL3 (AUA) aptamer. The applied voltage was +200 mV. The solution in the chamber contained 200 mM NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *cis* side, and 1 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *trans* side. Curves are fits, as described in the main text.

5. Frequency of low-amplitude, SL3 RNA aptamer-induced current blockades observed with large nanopores

The success in determining K_d values by monitoring the reduction in event frequency with small nanopores suggests that a similar mode of analysis may be fruitful with large nanopores. However, monitoring the reduction in small-current amplitude events in larger nanopores does not lead to reasonable K_d values for NCp7-SL3 RNA (GAG) interaction. Analysis was performed in which the original frequency of small amplitude current events was measured and titrations with NCp7 were performed. The frequency of small amplitude events was measured at each NCp7 concentration and a ratio was made to the original SL3 RNA frequency. The main difficulty with this approach is the reproducibility of event reduction when NCp7 concentration exceeds that of SL3 RNA (**Fig. S7**). It may be the case that NCp7-SL3 complexes are causing short-lived bumping events that increase the apparent frequency of SL3 RNA (GAG) events.

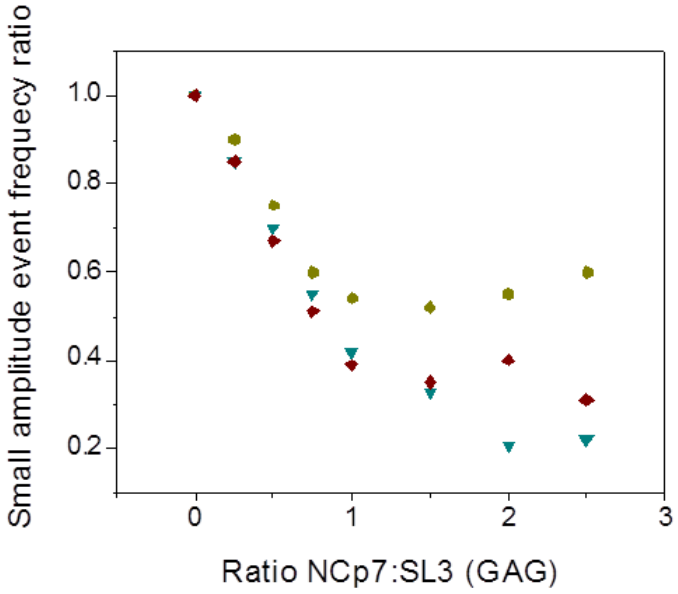


Figure S7: **Frequency reduction of short amplitude events in large nanopores**. The three scatter plots represent different measurements of ratio of measured small amplitude events ascribed to SL3 RNA (GAG) in a single nanopore 10 nm diameter nanopore. The reduction in short event frequency varied significantly even on the same pore. Solution was 0.2 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *cis* side, and 1 M NaCl, 5 mM NaH_2PO_4, pH 7.0 on the *trans* side. The original SL3 RNA (GAG) concentration was 1000 nM. Note that none of these curves show a reduction in frequency as great as that observed with small nanopores.

Reference List

1. Paoletti, A. C.; Shubsda, M. F.; Hudson, B. S.; Borer, P. N. Affinities of the Nucleocapsid Protein for Variants of SL3 RNA in HIV-1. *Biochemistry* **2002,** *41* (51), 15423-15428.