# Supplementary Information

**Title:** Real-time label-free measurement of HIV-1 protease activity by nanopore analysis

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**Serum sample analysis.** 60 ng HIV-1 PR was spiked into 5 µL human serum (Sigma-Aldrich, St. Louis, MO). 10 µL of reaction buffer, which contained 1 M NaCl, 1 mM EDTA, and 1 mM $NaH_2PO_4$ (pH 4.7), was added to the sample, followed by addition of 10 nmoles of the substrate peptide. The total volume of the solution mixture was 20 µL. After a 20 min protease-substrate reaction, the mixture was added to the nanopore sensor for analysis. The single channel recording experiment was performed with the $(M113F)_7$ α-hemolysin pore at -40 mV in a 2 mL of 1 M NaCl solution buffered with 1 mM EDTA and 1 mM $NaH_2PO_4$ (pH 4.7).

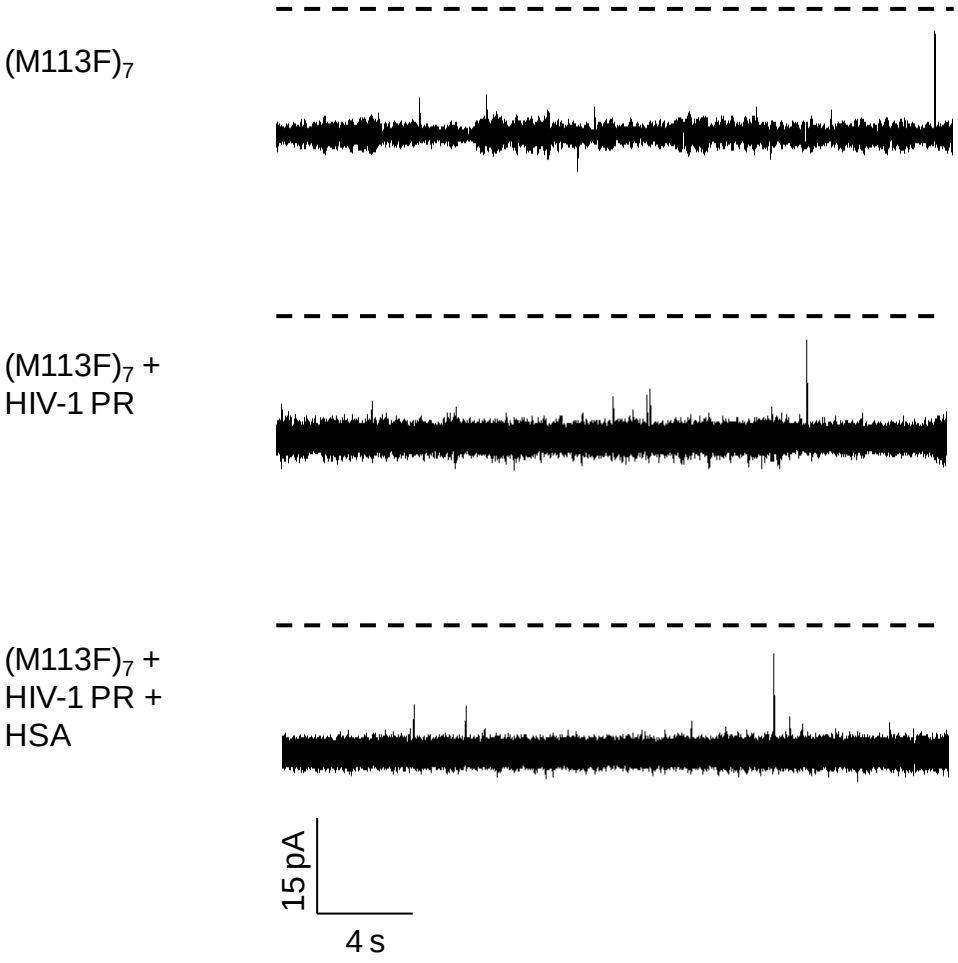


**Figure S1.** Typical single-channel recording trace segments of the mutant αHL protein $(M113F)_7$ pore with the HIV-1 protease and HSA protein. The experiments were performed at an applied voltage bias of -40 mV in a 1 M NaCl solution buffered with 1 mM EDTA and 1 mM $NaH_2PO_4$ (pH 4.7). The concentrations of the HIV-1 protease and HSA were 300 ng/mL, and 20 μM, respectively.

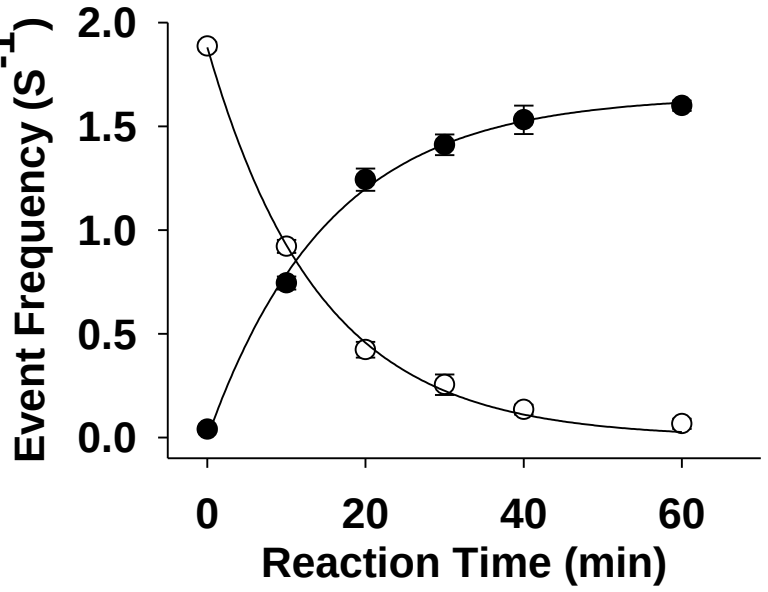


**Figure S2.** The effect of reaction time on the event frequency of the peptide substrate (○) and its degradation product (●). The experiment was performed by real-time monitoring of the substrate-protease interaction continuously for 1 h at an applied voltage bias of -40 mV in a 1 M NaCl solution buffered with 1 mM EDTA and 1 mM $NaH_2PO_4$ (pH 4.7). The concentrations of the peptide substrate and HIV-1 protease were 5 μM, and 300 ng/mL, respectively. The event frequency at a particular time point t (min) was obtained based on the statistical analysis of all the events collected during the period from time point t-10 to time point t.

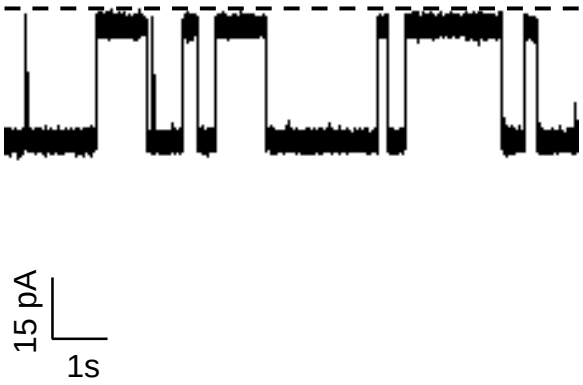


**Figure S3.** Typical single-channel recording trace segment, showing the interaction between human serum and the nanopore. The experiment was performed at -40 mV with the mutant αHL protein $(M113F)_7$ pore in a 2-mL 1 M NaCl solution buffered with 1 mM EDTA and 1 mM $NaH_2PO_4$ (pH 4.7) in the presence of 5μL human serum. The dashed line represents the level of zero current.

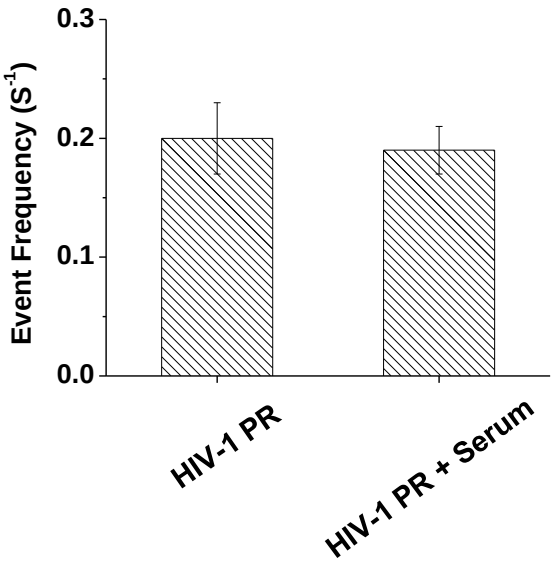


**Figure S4.** The effect of human serum on HIV-1 PR detection. The substrate FFSQNYPIVQ and HIV-1 PR were incubated in the absence and presence of human serum for 20 minutes before addition to the nanopore sensor for single channel recording (refer to the "serum sample analysis" section for the detailed sample preparation procedure and nanopore sensing experiment conditions). Although human serum also caused current modulations in the nanopore (Fig. S3), these events showed significantly longer residence times and larger blockage amplitudes than those of the substrate degradation products, allowing them to be readily differentiated. The values of event frequency (*f*) shown in Fig. S4 were obtained after blank (water and serum, respectively) subtraction and correction for the channel gating caused by serum. Specifically, t was calculated by using the equation $f = N / t$, where N is the total number of collected peptide events having a mean residual current of -9.5 pA, while t is the effective recoding time (t = single-channel trace length – the sum of the residence times of other types of events).