Supporting Information

Label-free nanopore single-molecule measurement of trypsin activity

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Calculation of the Substrate Digestion Percentage. It should be noted that, in a proteolytic reaction, the concentration of the substrate breakdown product (e.g., LVFF in this work) produced in a given period of time t should be equal to that of the substrate consumed. Since the number of the peptide blockage events is proportional to its concentration in a nanopore, the digestion percentage of the substrate at a specific time t could be calculated by using the equation: Cleavage (%) = N_t / N_0 × 100%, where N_t represents the number of LVFF events produced after the tryptic digestion occurred for a period of time t, while N_0 is the number of LVFF events after all of the substrate peptide has been digested by trypsin in the protein pore. Note that our experimental results (Fig. S-4) showed that all of the substrate peptide A-β (10-20) was cleaved by trypsin in ~150 min. Therefore, the concentration of the product LVFF after 150 min enzymatic reaction would be equal to the initial concentration of the substrate.

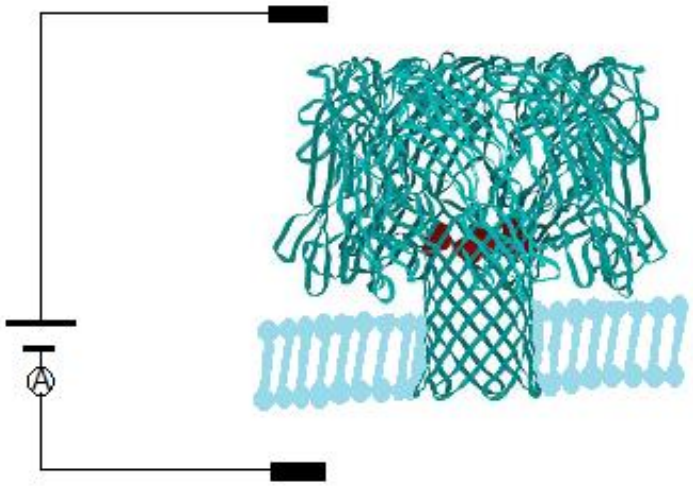

Figure S-1. Schematic illustration of the trypsin nanopore sensor.

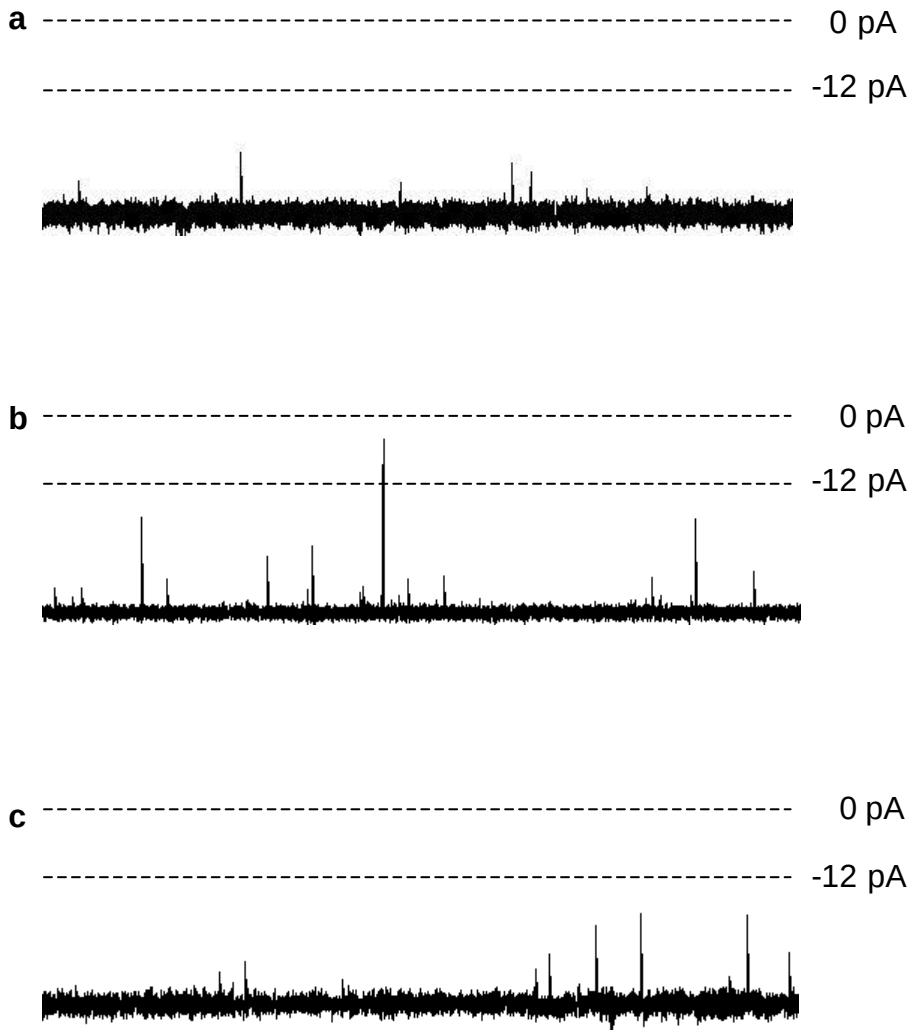

Figure S-2. Typical 10-second single-channel recording trace segments for (a) trypsin, (b) TIBP, and (c) bovine serum, suggesting that trypsin, TIBP, and serum would not interfere with the identification of the events attributed to the peptide substrate and its two cleavage products. Note that these three peptide events had residual currents at -2.25 pA, -5 pA, and -12 pA, respectively. The experiments were performed at -40 mV with the $(M113F)_7$ α-hemolysin protein pore in a buffer solution comprising 1.0 M NaCl and 10 mM Tris (pH 7.5). The concentrations of trypsin and TIBP were 100 ng/mL, and 0.125 nM, respectively. The amount of serum used was 5 μL.

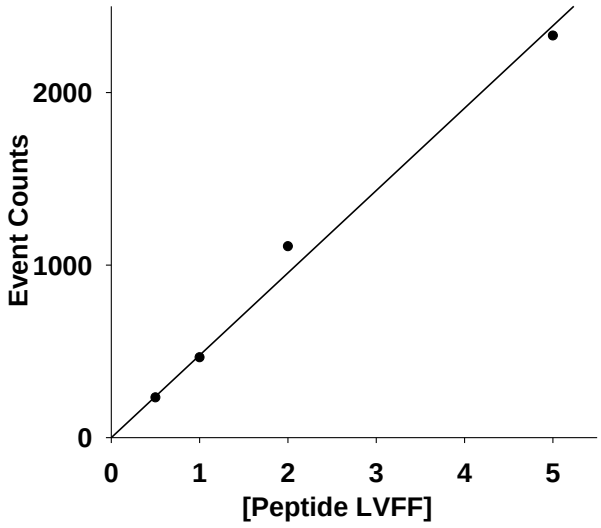

Figure S-3. Dose response curve for peptide LVFF. The experiments were performed at an applied potential bias of -40 mV using the $(M113F)_7$ α-hemolysin protein pore in a buffer solution comprising 1.0 M NaCl and 10 mM Tris (pH 7.5).

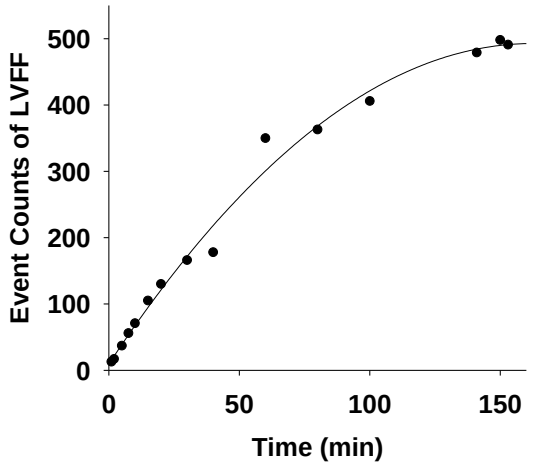

Figure S-4. Effect of digestion time on the number of events of the cleavage product LVFF. The experiment was performed at -40 mV with the $(M113F)_7$ α-hemolysin protein pore in a buffer solution comprising 1.0 M NaCl and 10 mM Tris (pH 7.5). The concentration of the substrate β-amyloid (10-20) peptide and trypsin were 5 μM and 100 ng/mL, respectively. The number of LVFF events was collected in a period of 1 min around a specific digestion time t. Figure S-4 showed that all of the substrate was cleaved in ~150 min.

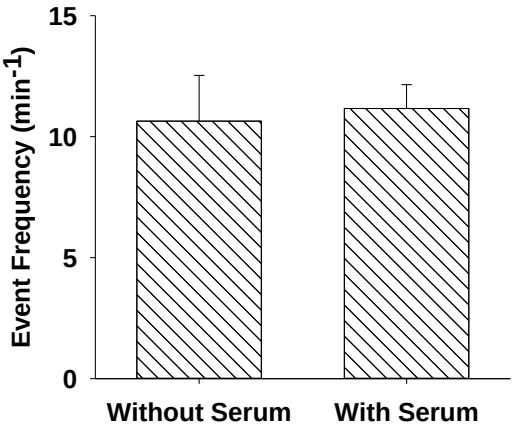

Figure S-5. Effect of bovine serum on trypsin detection. The experiments were performed using the $(M113F)_7$ α-hemolysin pore in a buffer solution comprising 1.0 M NaCl and 10 mM Tris (pH 7.5) at -40 mV. The substrate peptide and trypsin were incubated for 30 minutes at 22 °C in the absence and presence of bovine serum before the mixture solution was added to the nanopore sensing chamber compartment for single channel recording. The final concentrations of the substrate and trypsin were 5 μM and 50 ng/mL, respectively.