Supplementary Material

Chemically Functionalized Conical PET Nanopore for Protein Detection at the Single-molecule Level

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Table S1. Properties of Proteins

Proteins	Size or Diameter (nm)	Molecular Mass (kDa)	Isoelectric Point (pI)	Net charge at pH 7.5
HIV-1 PR	4.5×2.3×2.5 [s1]	10.8	9.1 [s2]	+1.60
Trypsin	3.8×3.8×3.8 [s3]	24	10.1-10.5 [s4]	+3.78
BSA	4×4×14 [s5]	66.4 [s6]	5.4 [s6]	-22.06
HSA	3×8×8 [s7]	66.4 [s8]	4.7 [s8]	-16.48

*The net charges of the proteins were estimated using an online tool (https://www.protpi.ch/Calculator/ProteinTool).

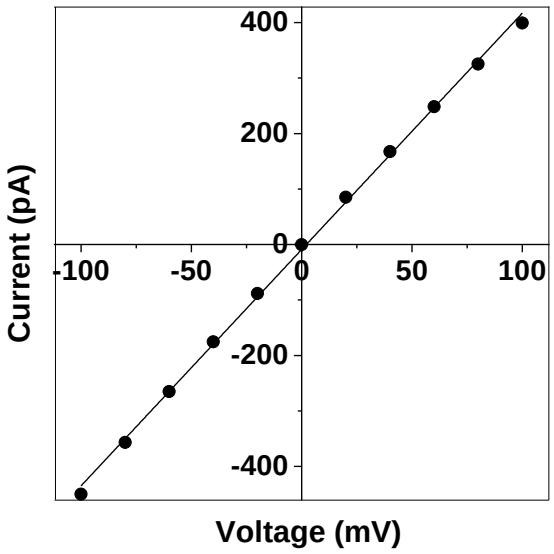

Figure S1. The current-voltage (I-V) curve of a single PET conical nanopore at an applied voltage bias ranging from -100 mV to +100 mV. The experiment was performed in an electrolyte solution containing 1 M KCl and 1 mM EDTA (pH 7.5). The diameters of the *tip* and *base* of the PET nanopore are ~ 5.6 and ~1000 nm, respectively. The linear regression equation for the current-voltage relationship is $y = 4.261x - 8.969$, $r^2 = 0.9988$.

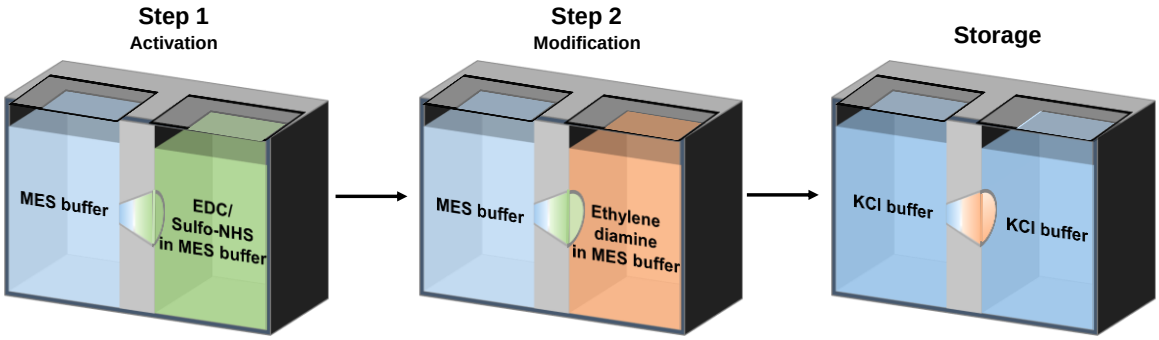

Figure S2. Schematic illustration of the 2-step coupling method for asymmetric modification of the single conical PET nanopore.

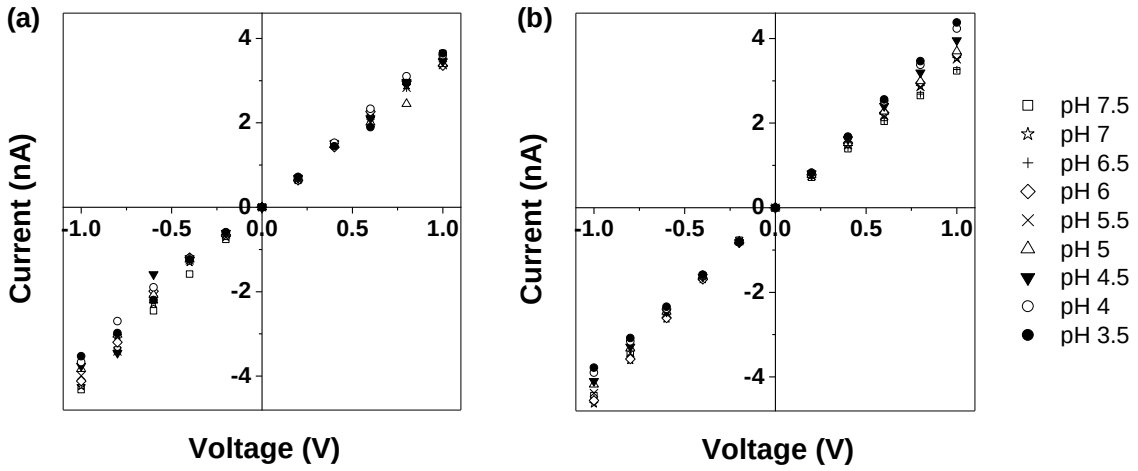

Figure S3. Current-voltage (I-V) curves of a single PET conical nanopore at different solution pHs: (a) before and (b) after chemical modification. Experiments were performed in electrolyte solutions containing 1 M KCl and 1 mM EDTA with different pH values ranging from 3.5 to 7.5.

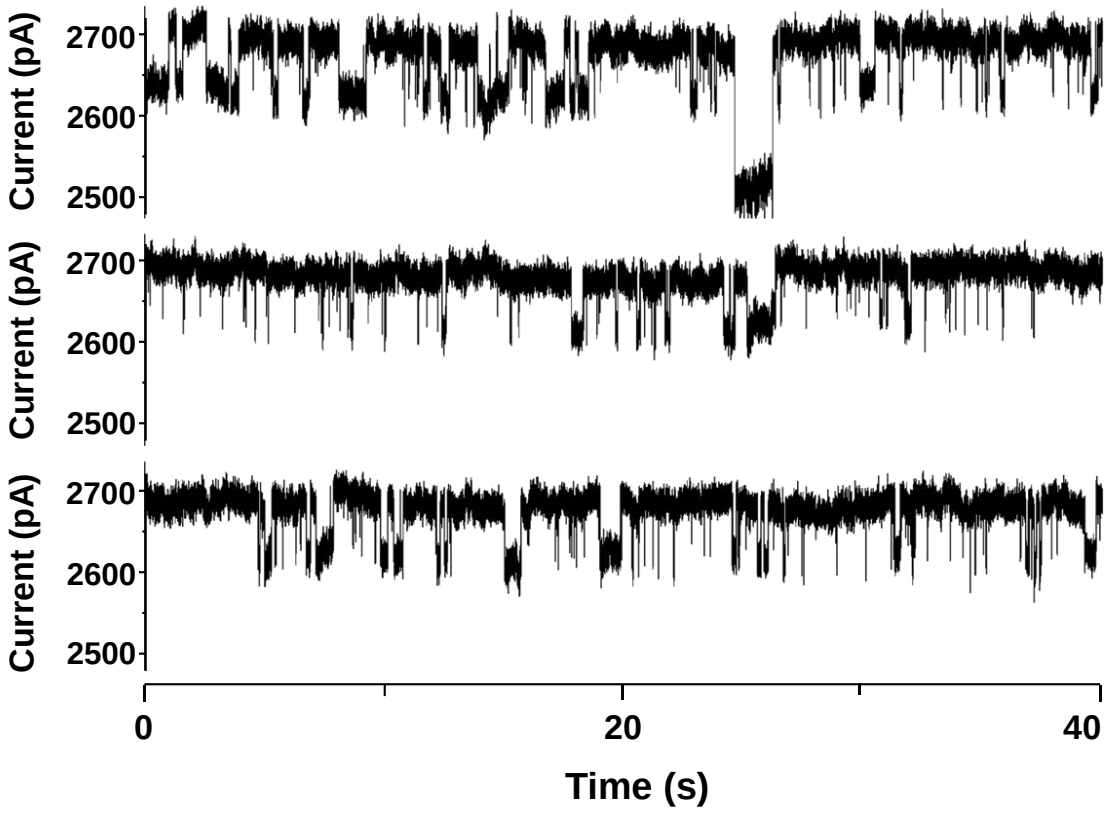

Figure S4. An uninterrupted 2-min single-channel recording trace segment of HIV-1 PR in the amine-modified PET nanopore. The experiment was performed at +800 mV in a solution comprising 1 M KCl and 10 mM Tris (pH 7.5). The concentration of HIV-1 PR used was 100 ng/mL.

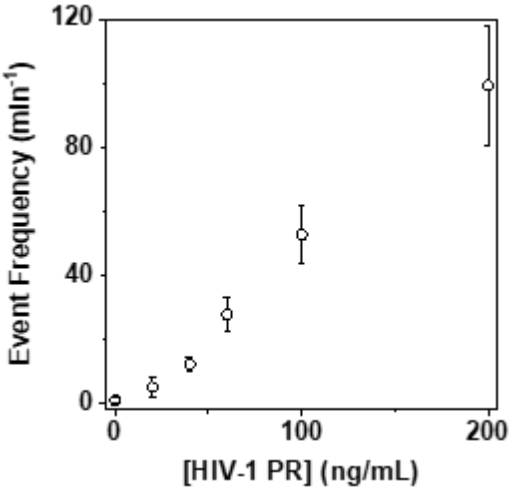

Figure S5. Plot of event frequency versus HIV-1 protease concentration. The experiments were performed at +800 mV using the amine modified PET nanopore in a solution comprising 1 M KCl and 10 mM Tris (pH 7.5).

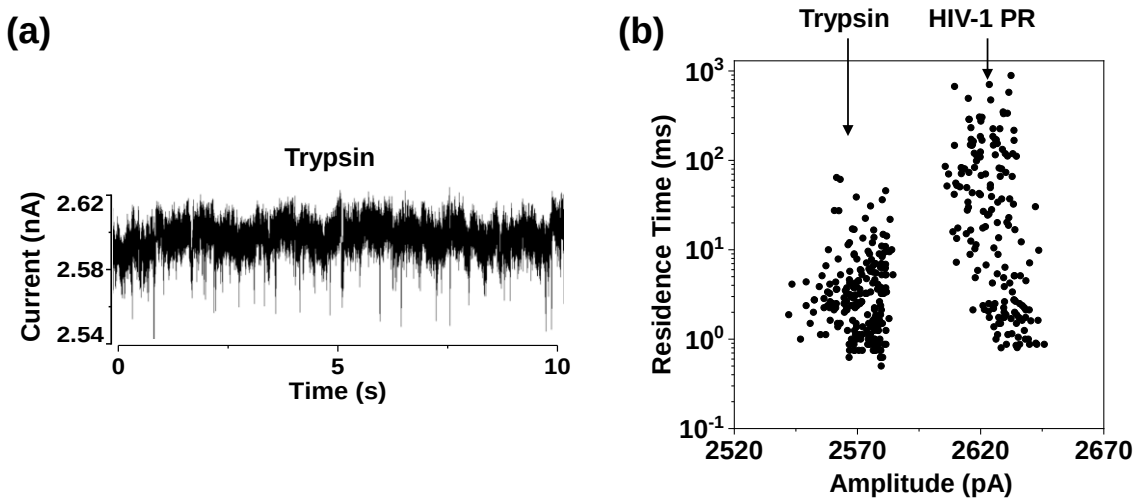

Figure S6. Selectivity study of the PET nanopore. (a) Typical single-channel recording trace segment of trypsin in the PET nanopore, and (b) the scatter plot of residence time vs. residual current of the trypsin and HIV-1 PR events, showing that these two protein species could be well differentiated. Both the experiments were performed at +800 mV in a solution comprising 1 M KCl and 10 mM Tris (pH 7.5). The concentrations of trypsin and HIV-1 PR were 100 ng/mL each.

References

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