Supplementary Information

Electrochemical Kinetic Fingerprinting of Single-Molecule Cooridations in the Confined Nanopores

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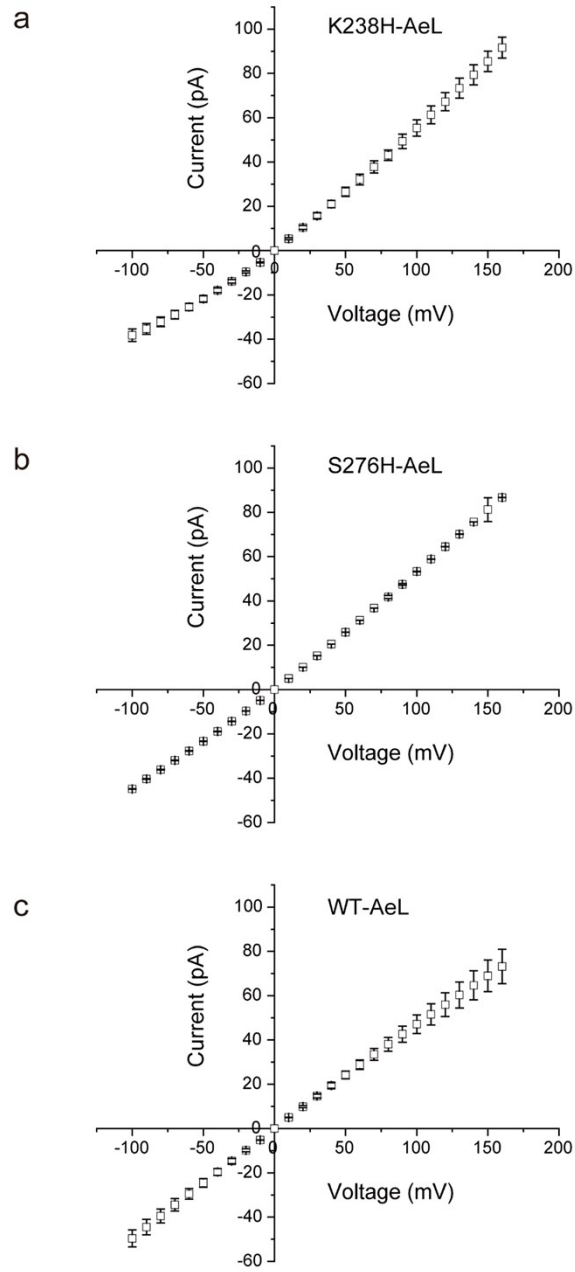
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Fig. S1. Current-voltage (*I-V*) curves of (a) K238H-AeL, (b) S276H-AeL and (c) WT-AeL nanopore. The data were acquired in the condition of 1.0 M KCl, 10 mM Tris, and 1.0 mM EDTA at pH 8.0. The error bars are the standard error of the mean (s.e.m) for 3 independent experiments.
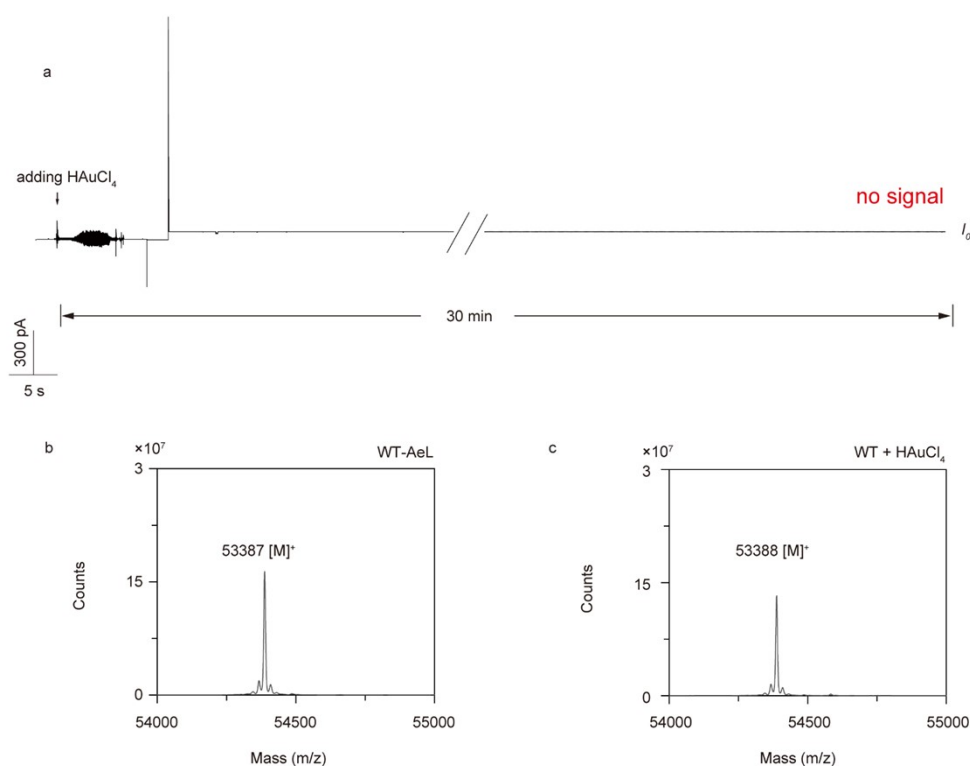
Fig. S2. Control experiment of WT-AeL nanopore. (a) The current trace of WT-AeL nanopore treated with the 0.5 mM HAuCl$_4$, the current trace was acquired in the condition of 1.0 M KCl, 10 mM Tris, and 1.0 mM EDTA at pH 8.0. The applied voltage is +100 mV. No reaction event occurs between WT-AeL nanopore and the HAuCl$_4$ after recording within 30 min; (b) The mass spectrometry of WT Pro-aerolysin; (c) The mass spectrometry of WT Pro-aerolysin incubated with Au(III). The [M]$^+$ peaks at m/z = 53387 ± 1 and m/z = 53388 ± 1 are both assigned to unreacted WT Pro-aerolysin. The concentration of Au(III) is 0.5 mM, and the experiments were performed in the buffer of 10 mM Tris, at 22 ± 2 °C.
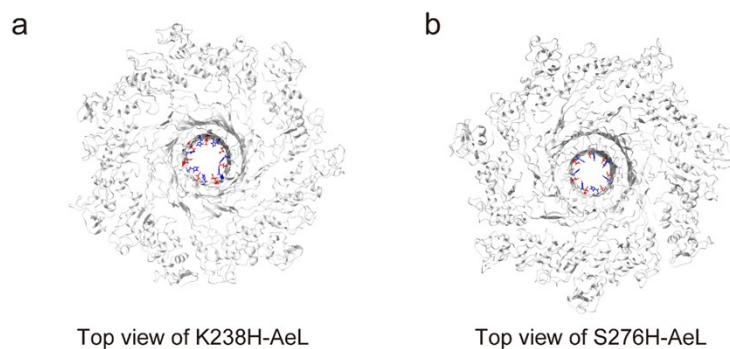
Top view of K238H-AeL   Top view of S276H-AeL

Fig. S3. MD simulations of K238H-AeL nanopore and S276H-AeL nanopore. (a) The top view of K238H-AeL nanopore. K238H is shown in blue, E258 site is shown in red. (b) The top view of S276H-AeL nanopore. K238H is shown in blue, D222 site is shown in red. The equilibrated time is 5 ns.

Table. S1. Distance of N atom to O atom in K238H-AeL or S276H-AeL

Distance of the N atom to O atom	d(K238H-AeL) / Å [*]	d(S276H-AeL) / Å [&]
d_1	4.4	6.1
d_2	4.3	4.9
d_3	4.4	6.6
d_4	5.3	4.8
d_5	4.9	5.0
d_6	4.6	7.3
d_7	4.4	7.1
Average d[$]	4.6±0.3	6.0±1.0

[*] The distance of N atom of the imidazole ring in K238H site to O atom of carboxyl in E258 site under K238H-AeL nanopore.

[&] The distance of N atom of the imidazole ring in S276H site to O atom of carboxyl in D222 site under S276H-AeL nanopore.

[$] The average $d = (d_1 + d_2 + d_3 + d_4 + d_5 + d_6 + d_7)/7$

Author Contributions

Y.-L. Ying and Y.-T. Long conceived idea. C.-N. Yang and conducted the experiment. C.-N. Yang analyzed the data. C.-N. Yang, W. Liu, H.-T. Liu and J.-C. Zhang prepared the manuscript. Y.-L. Ying and Y.-T. Long wrote the manuscript.