Supplementary Material

Analysis of Single Cysteine Molecule with an Aerolysin Nanopore

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Table of Contents

Materials and Methods

1.1   Chemicals and Reagents

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP, ≥ 98%), L-Asparagine (≥ 98%) and L-Glutamine (≥ 99%), Trypsin-EDTA, Trypsin-agarose, decane (anhydrous, ≥ 99%) and potassium chloride (KCl, anhydrous, ≥ 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris (hydroxymethyl) aminomethane (Tris, > 99.9%), L-Cysteine (99%) and ethylenediaminetetraacetic acid (EDTA, > 99.9%) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). 1,2-Diphytanoyl-sn-glycero-3-phosphocholine (DPhPC in chloroform, ≥ 99%) was purchased from Avanti Polar Lipids, Inc. (Alabaster, Al, USA). Proaerolysin was synthesized and HPLC-purified according to our previous researches[1,2]. All the reagents and chemicals are of analytical grade and were used without further purification. All solutions were prepared with ultrapure water (18.2 MΩ·cm at 25 °C) using a Milli-Q System (EMD Millipore, Billerica, MA, USA).

1.2   Single molecule measurement

The nanopore experiments were performed according to our previous studies[3]. Proaerolysin was activated by digestion with trypsin-agarose for 6 h at room temperature to get the monomeric aerolysin. The cysteine was dissolved in the electrolyte solution with 1.0 M KCl, 10.0 mM TCEP, 10 mM Tris, pH 8.0 to form the 50 mM cysteine solution. A planar lipid bilayer was formed by DPhPC solution on an orifice with 50 μm diameter of the delrin cup (Warner Instruments, Hamden, CT, USA). The cup was separated into two chambers (*cis* and *trans*). 1 mL electrolyte solution (1.0 M KCl, 1.0 mM EDTA, 10 mM Tris, pH 8.0) was added into each chamber. The WT AeL was added to the *cis* chamber. Once a stable single nanopore was formed, the cysteine solution was added to the *cis* chamber at a final concentration of 4 mM. The analyte was driven to enter into the nanopore through a pair of Ag/AgCl electrodes for conducting the applied positive voltage. The *cis* chamber was grounded. All the nanopore experiments were carried out at 22.5 ± 1.0 °C.

Due to the high concentration of Cys (4 mM and 6 mM) used in our experiments, the concentration of reduce agent should reach to millimolar level to ensure an effective thiol reduction. Our control experiments show that the DTT with the concentration as low as 10 nM would generate the blockages by using WT AeL (Figure S3), much less its high concentration at millimolar level. The high concentration of DTT clearly shows the blockages with the I/I_0 ranging from 0.0 to 0.8, which is seriously overlapped with characteristic distribution of Cys (I/I_0 = 0.64 ± 0.02). Moreover, the events from 4 mM Cys with the addition of 0.8 mM DTT are widely distributed in the scatter plots with I/I_0 ranging from 0.0 to 0.8 on account of the DTT events influencing the distribution of Cys. Therefore, TCEP is a preferred reduce reagent in our measurements.

Data acquisition and analysis

The Current traces were measured by a patch-clamp amplifier (Axopatch 200B, Axon Instruments, Forest City, CA, USA), coupled with a Digidata 1440A A/D converter (Molecular Devices, USA). The current events were low-pass filtered at 5 kHz and sampled at 100 kHz by running the Clampex 10.4 software (Molecular Devices, Forest City, CA, USA). The data analysis was performed by Mosaic software[4,5], and then processed by OriginLab 2018 (OriginLab Corporation, Northampton, MA, USA). MOSAIC recovers the attenuated blockades to their steady-state convergence value, and to minimize the fitting errors of MOSAIC, the short blockades that last < 64 μs at bandwidth of 5 kHz was excluded.
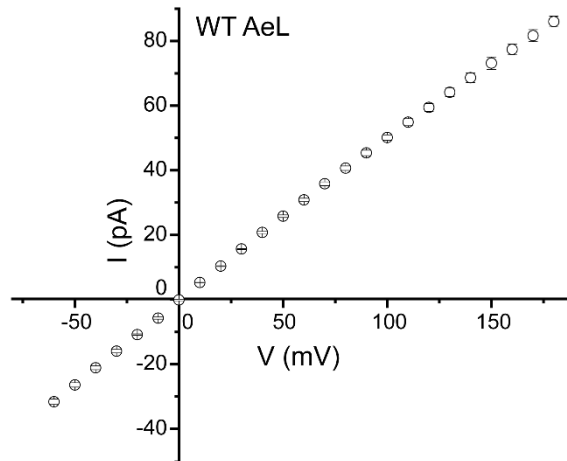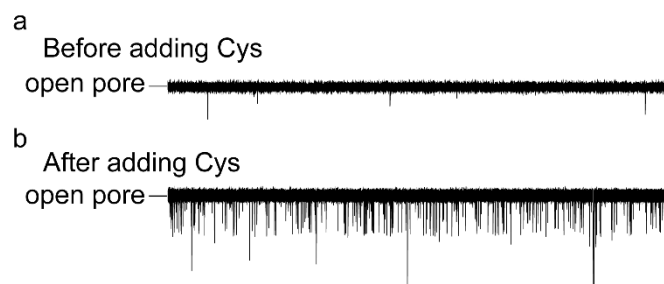
Supplementary Figure 1. The current-voltage curve of WT AeL nanopore. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA, pH 8.0 and at 22.5 ± 1.0 ˚C.
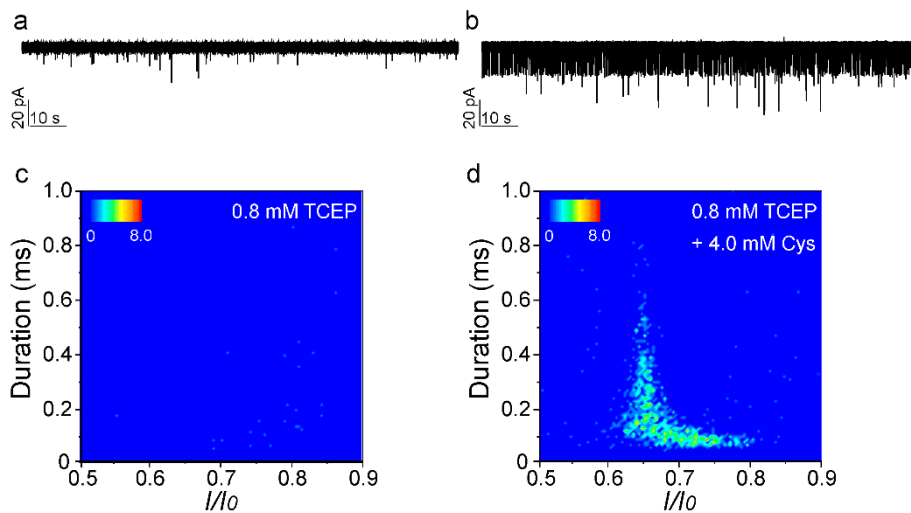

Supplementary Figure 2. The current traces of 0.8 mM TCEP (a) and 4.0 mM Cys with 0.8 mM TCEP (b) analyzed by the AeL nanopore. Two-dimensional (2D) contour plots of the 0.8 mM TCEP (c) and 4.0 mM Cys with 0.8 mM TCEP (d) analyzed by the AeL nanopore. The 2D contour plots present the current and display the events density distribution. The Cys was dissolved in 1.0 M KCl, 10.0 mM Tris, 10.0 mM TCEP and pH 8.0. The TCEP was dissolved in 1.0 M KCl, 10.0 mM Tris and pH 8.0. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA and pH 8.0 at the potential of + 120 mV.
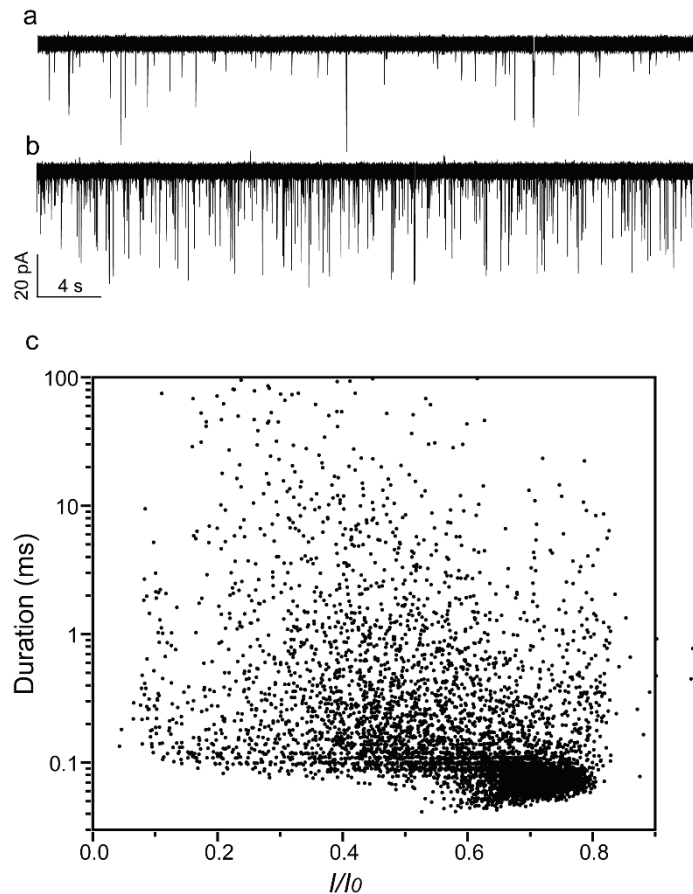
Supplementary Figure 3. Effects of the DTT solution on the sensing of a WT AeL nanopore. The concentration of DTT in the *cis* chamber is 10 nM (a) and 0.8 mM (b). (c) The scatter plots of DTT with 0.8 mM. The DTT was dissolved in 1.0 M KCl, 10.0 mM Tris, 1.0 mM EDTA and pH 8.0. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA and pH 8.0 at the potential of + 120 mV.


Supplementary Figure 4. The current signals of the open pore before adding Cys solution (a) and after adding Cys solution (b) analyzed by the AeL nanopore. The open pore current is 57.50 pA and 55.70 pA before and after adding Cys solution respectively. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA and pH 8.0 at the potential of + 120 mV.
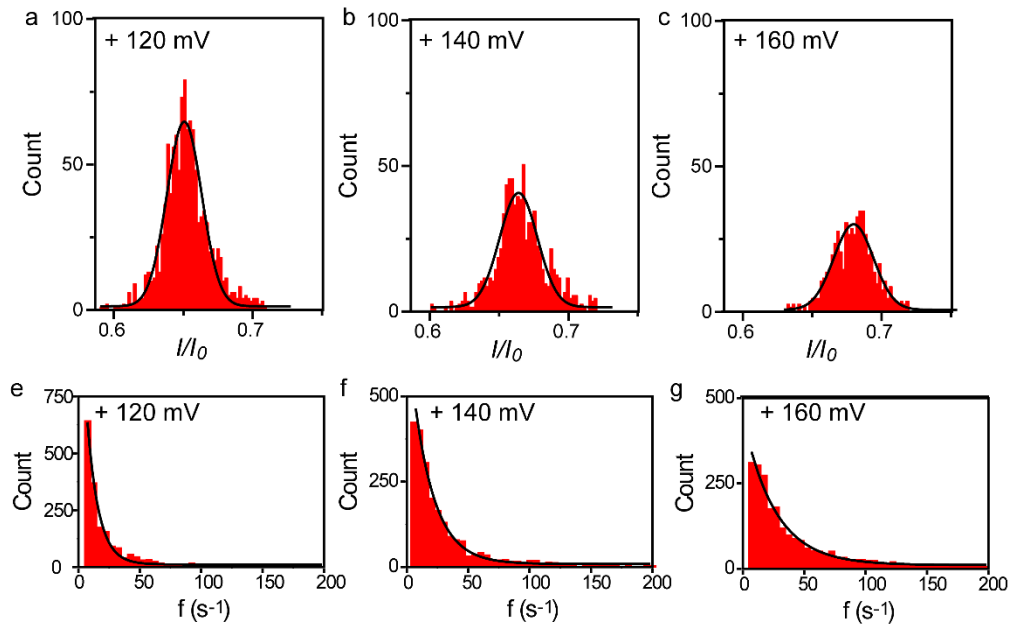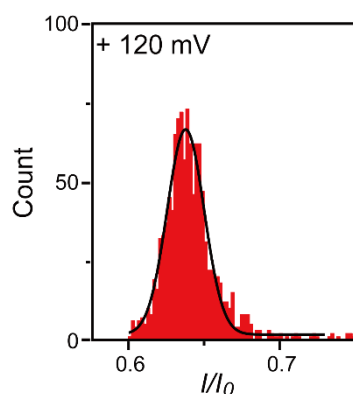
Supplementary Figure 5. The I/I_0 histograms of PI population for Cys analyzed by the AeL nanopore at potential of (a) + 120 mV, (b) + 140 mV, (c) + 160 mV. The frequency histograms of Cys analyzed by the AeL nanopore at potential of (a) + 120 mV, (b) + 140 mV, (c) + 160 mV. All of the I/I_0 histograms were fitted to single Gauss function and all of the frequency histograms were fitted to exponential function. The Cys was dissolved in 1.0 M KCl, 10.0 mM Tris, 10.0 mM TCEP and pH 8.0. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA, pH 8.0 and in the presence of 4.0 mM Cys.
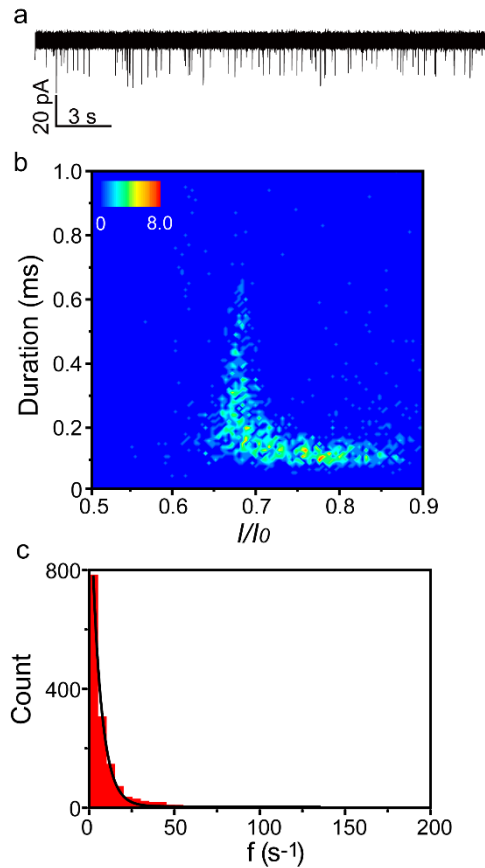
Supplementary Figure 6. (a) The current traces of 4.0 mM Cys in absence of TCEP. (b) Two-dimensional (2D) contour plots of 4.0 mM Cys in absence of TCEP analyzed by the AeL nanopore. The 2D contour plots present the current and display the events density distribution. (c) The frequency histograms of Cys analyzed by the AeL nanopore. The Cys was dissolved in 1.0 M KCl, 10.0 mM Tris, 1.0 mM EDTA and pH 8.0. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA, pH 8.0 at the potential of + 140 mV and in the presence of 4.0 mM Cys.
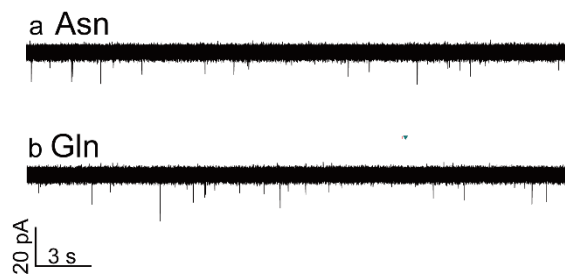
Supplementary Figure 7. The recorded raw current traces in the presence of Asn (a) and Gln (b) analyzed by the AeL nanopore. The concentration of the analyte in *cis* chamber is 4 mM for Asn and Gln, respectively. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA and pH 8.0 at the potential of + 120 mV.

Supplementary Figure 8. The I/I_0 histograms of PI population for Cys analysed by the AeL nanopore at the potential of + 120 mV. The histogram was fitted to the single Gauss function. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA, pH 8.0 and in the presence of 6.0 mM Cys.

References

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