Profiling the Chemistry and Confinement Controlled Sensing Capability of an Octameric Aerolysin-Like Protein

Xue-Yuan Wu†, Jie Jiang†, Jun-Ge Li, Meng-Yin Li * and Yi-Tao Long

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, 210023, Nanjing, China
† These authors contributed equally: Xue-Yuan Wu, Jie Jiang.
**Correspondence to: mengyinli@nju.edu.cn*

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Methods

Reagents and chemicals

Decane (anhydrous, ≥99%), trypsin-ethylenediaminetetraacetic, KCl (≥99%) Ethylenediaminetetraacetic acid (EDTA, ≥99%), Tris(hydroxymethyl)aminomethane (Tris, ≥99%) were all purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). 1, 2-diphytanoyl-sn-glycero-3-phosphocholine (chloroform, ≥99%) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). All the chemicals used are analytical grade. All solutions in our experiments were prepared using ultrapure water (18.2 MΩ cm at 25 °C) from a Milli-Q system (Billerica, MA, USA).

Nanopore experiments and data analysis.

10 μL Aep1 (0.4 mg/mL) was mixed with 10 μL mannan (1.5 mg/mL) at pH 5.5, and incubated at room temperature for 1 h. A lipid solution of 30 mg/mL was prepared by using 1, 2-diphytanoyl-sn-glycero-3-phosphocholine in decane. Nanopore experiments were performed in a Delrin bilayer cup (Warner Instruents), which was separated into two compartments (*cis* and *trans*) by a septum with a 50 μm aperture. 1 mL buffer (1.0 M KCl, 10 mM Tris and 1.0 mM EDTA) was added into both two compartments. The pH of buffer was set from symmetric 8.0/8.0 (*cis*/*trans*) and 5.0/5.0 (*cis*/*trans*), to asymmetric 8.0/5.0 (*cis*/*trans*). A pair of Ag/AgCl electrodes were immersed into the buffer in two compartments for applying voltage. Lipid solution was painted over the aperture for lipid bilayer formation. The incubated Aep1 was diluted 20 times and added to the *cis* chamber. Aep1 protein was self-assembled and inserted into the membrane nanopore to form a single nanopore. Target molecules were added to the *cis* side of the pore, and the ionic current trace in nanopore was amplified and recorded by a patch clamp amplifier (Axopatch 200B, Molecular Devices) and an analog-digital converter (DigiData 1440A, Molecular Devices) at a sampling frequency of 100 kHz and filter of 5 kHz. The final concentration of analytes was set at 200 μM. The data were analyzed by software Mosaic software[1,2] and OriginLab 8.0. The data were recorded at 24 ± 2 °C. Capture frequency was calculated by $f = 1/\tau_{on}$, where τ_{on} represents the inter-event intervals.

The possible sequence that forms the entire channel of octameric Aep1

EEIKSVSFENKTSVSVKQEQKVETSKKVIKTSSWSMTKSFSSTFSVEVSAGIPEIAEVSTGFSISFGVESTHSLEQTDEKNETLTTTVEV
PPKKK

Supplementary Notes, Tables and Figures

Table S1 The sequences of analytes used in this work.

DNAs/Peptides	Sequences
$(dA)_{10}$	5′-AAAAAAAAAA-3′
$(dA)_{45}$	5′-A_{45}-3′
dsDNA	5′-A_{20}TTAAAGCTCGCCATCAAATAGCTTTCCA$_{20}$-3′
His_{12}	HHHHHHHHHHHH

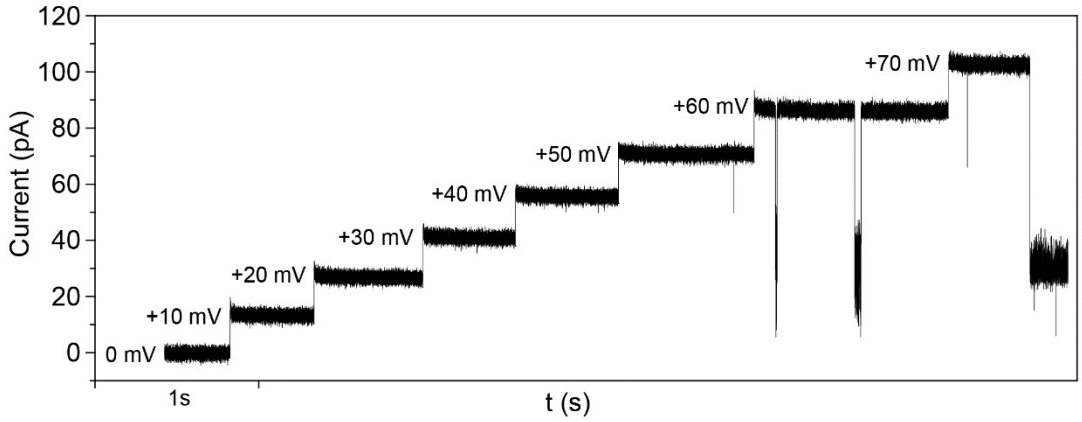

Fig. S1 The voltage-dependent gating events in Aep1 nanopore at pH 8.0/8.0 (*cis*/*trans*).

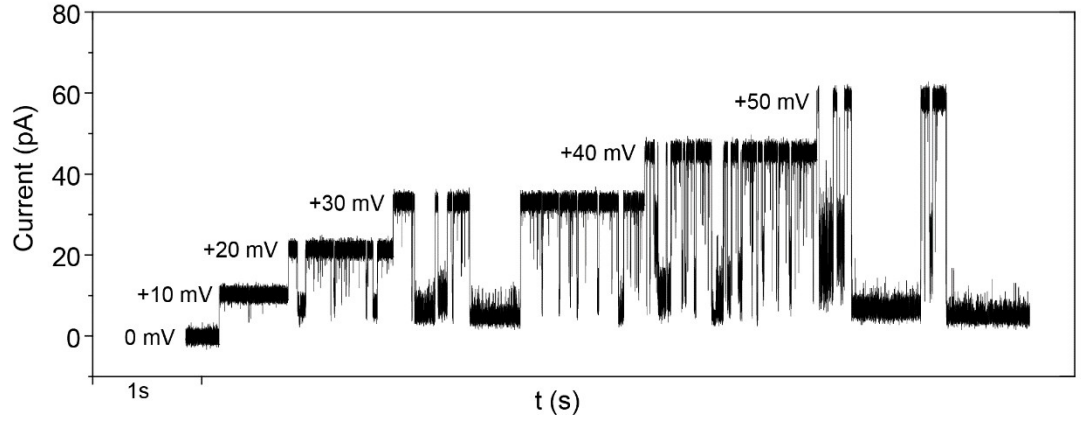

Fig. S2 The voltage-dependent gating events in Aep1 nanopore at pH 5.0/5.0 (*cis*/*trans*).

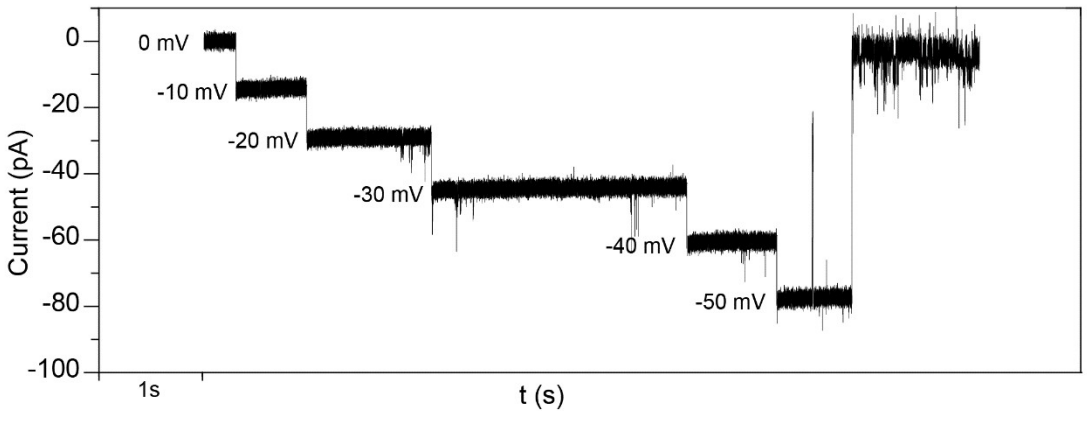

Fig. S3 The voltage-dependent gating events in Aep1 nanopore at pH 8.0/5.0 (*cis*/*trans*).

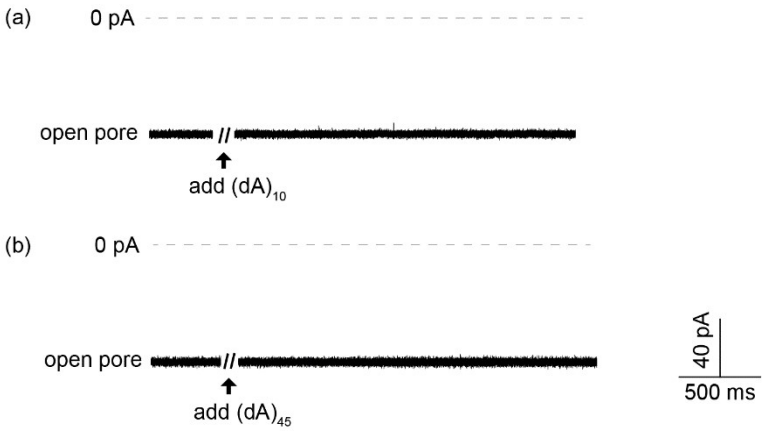

Fig. S4 The ionic current traces in Aep1 nanopore after the addition of $(dA)_{10}$ (a) or $(dA)_{45}$ (b) both into *cis* and *trans* side at - 80 mV.

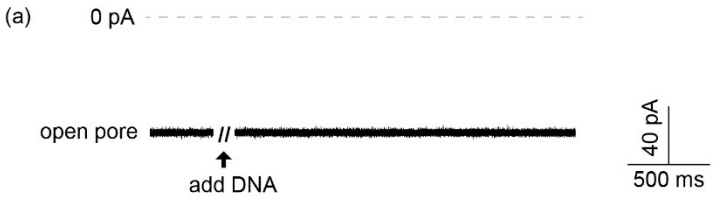

Fig. S5 The ionic current trace in Aep1 nanopore after the addition of DNA with a hairpin structure both into *cis* and *trans* side at - 80 mV.

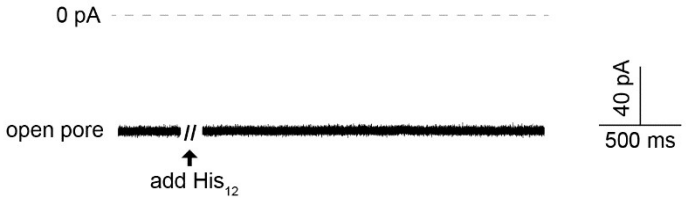

Fig. S6 The ionic current trace in Aep1 nanopore after the addition of His_{12} both into *cis* and *trans* side at - 80 mV.

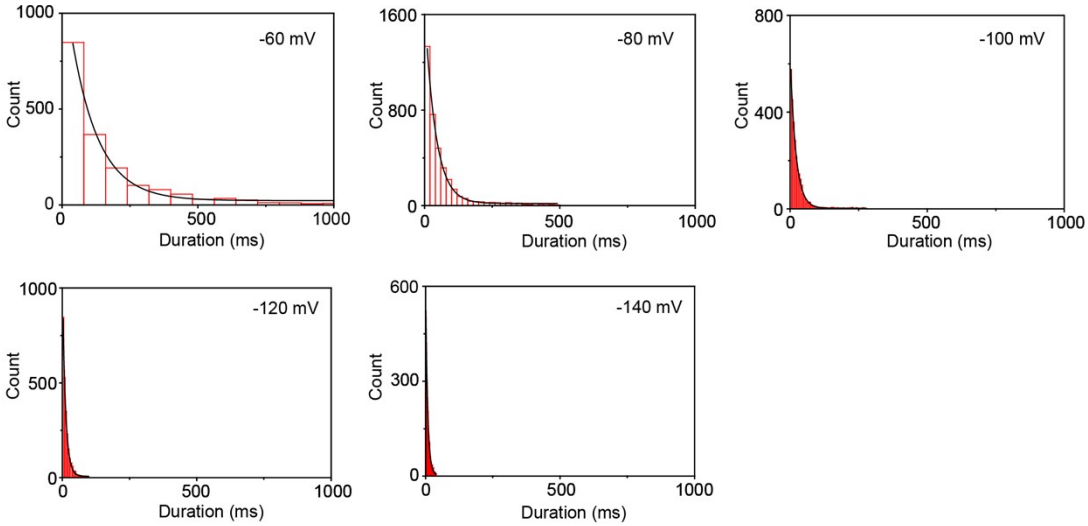

Fig. S7 The histograms of inter-event intervals for β-CD in Aep1 nanopore at different voltages.

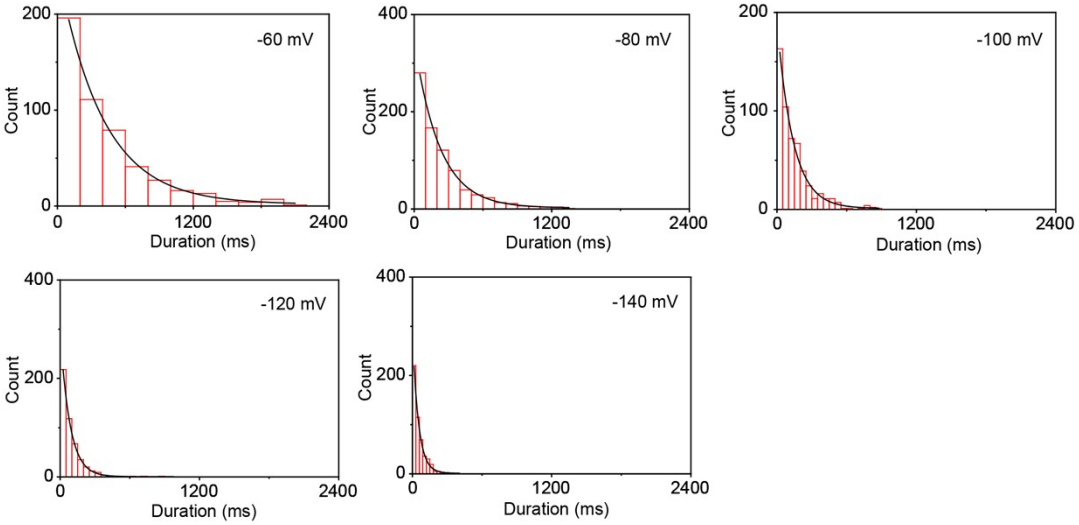

Fig. S8 The histograms of inter-event intervals for γ-CD in Aep1 nanopore at different voltages.

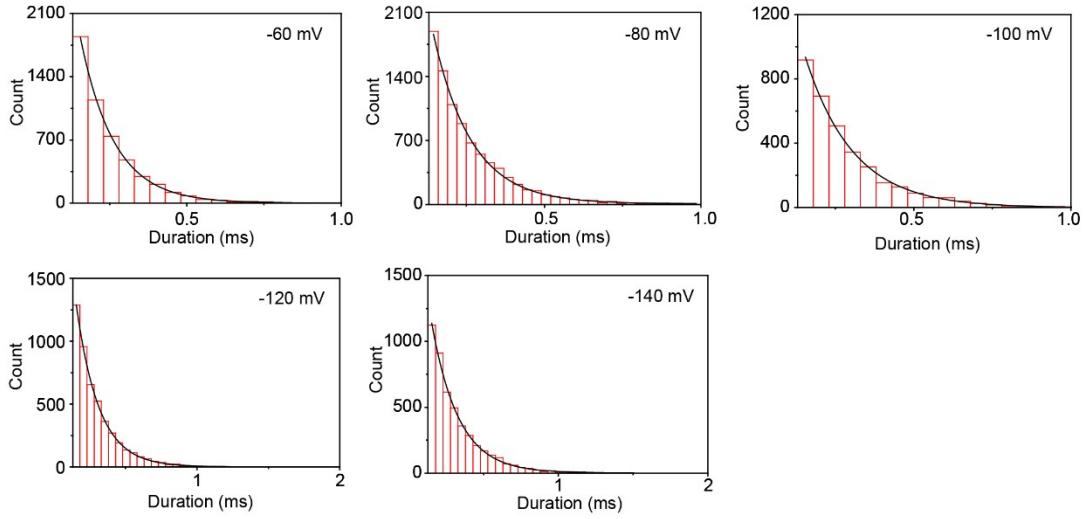

Fig. S9 The duration histograms of β-CD in Aep1 nanopore at different voltages.

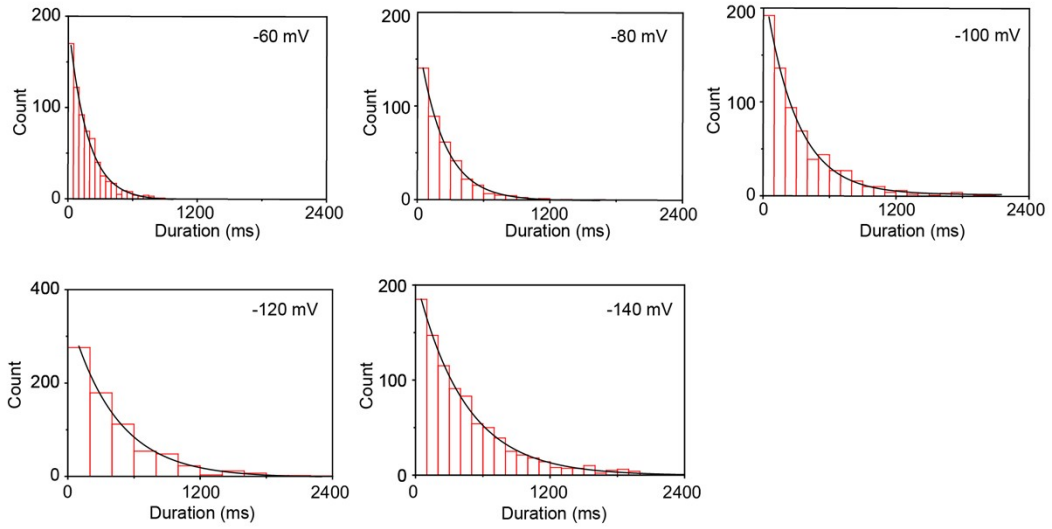

Fig. S10 The duration histograms of γ-CD in Aep1 nanopore at different voltages.

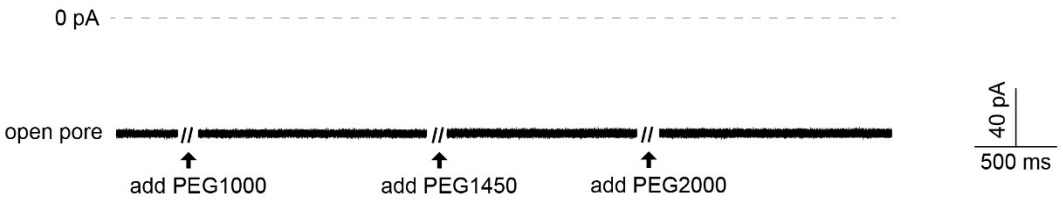

Fig. S11 The ionic current trace in Aep1 nanopore after the successive addition of PEG1000, PEG1450 and PEG2000 both into *cis* and *trans* side at - 80 mV.

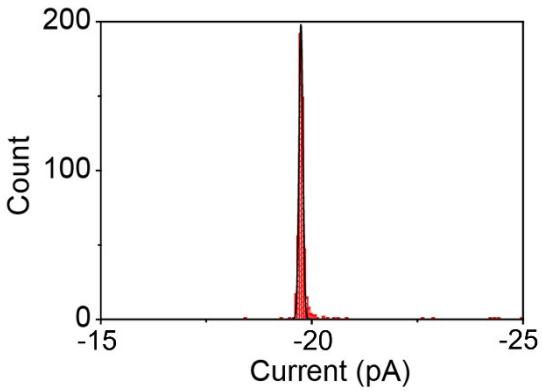

Fig. S12 The histogram of blockage current of γ-CD in Aep1 nanopore at -140 mV.

References

1. J. H. Forstater, K. Briggs, J. W. F. Robertson, J. Ettedgui, O. Marie-Rose, C. Vaz, J. J. Kasianowicz, V. Tabard-Cossa and A. Balijepalli, *Anal. Chem.*, 2016, **88**, 11900-11907.

2. A. Balijepalli, J. Ettedgui, A. T. Cornio, J. W. F. Robertson, K. P. Cheung, J. J. Kasianowicz and C. Vaz, *ACS Nano*, 2015, **9**, 12583-12583.