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Measuring a vibrational spectrum for the Single-Molecule Interactions with A Confined Nanopore

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Experiential section

1.1 Reagents and chemicals

Trypsin-EDTA, trypsin-agarose, decane (anhydrous, ≥99%), NaCl, Na_3PO_4, HCl and imidazole were 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). The poly(dA)$_4$ was synthesized and HPLC-purified by Sangon Biotech Co., Ltd. (Shanghai, China). Yeast extract and peptone were purchased from OXOID Co., Ltd. (Basingstoke, UK). Glycerinum was purchased from Amresco, Inc. (Solon, OH, USA). Isopropyl-β-D-1-thiogalactopyranoside (IPTG) were purchased from Inalco SpA, Inc. (Milano, Italy). BL21 [DE3] pLysS E. coli was purchased from TIANGEN Co., Ltd. (Beijing, China). The pET22b-proaerolysin plasmid were synthesized and HPLC-purified by Genewiz, Inc. (Suzhou, China). All reagents and materials are of analytical grade. All solutions were prepared using ultrapure water (18.2 MΩ cm at 25 °C) from a Milli-Q system (Billerica, MA, USA).

1.2 Proaerolysin production

The gene of proaerolysin (WT or K238E) was retrieved from PDB (3G40) and synthesized by Genewiz, Inc. (Suzhou, China). Target gene was introduced to pET22b vector. BL21 [DE3] pLysS E. coli was transformed with the pET22b-proaerolysin plasmid, which ensures periplasmic expression of the protein with a His6 tag on the C-terminus. BL21 [DE3] pLysS E. coli harbouring the WT aerolysin expression plasmid was grown at 37 ºC in 1 L cultures to an OD600 of 0.6. The IPTG (0.25 mM) was added and the temperature was shifted to 16 ºC for proaerolysin production. Cells were harvested after about 2 h, reaching an OD600 = 1.2. The E. coil cells were collected from the LB medium by centrifugation (3000 rpm for 30 min at 4 ºC). 50 mL buffer contained 0.5 M NaCl, 20 mM Na_3PO_4, pH 7.4 was added into the sediment and made it resuspended. After sonication on ice for 10 min, samples were then centrifuged for 30 min at 4 °C and 10000 rpm. After that, the supernatant was load onto a Ni-NTA sorbent by a gravity flow column. In order to remove the non-specifically bound proteins, the column was washed with buffer (20 mM Na_3PO_4, 0.5 M NaCl, pH 7.4) with a linear gradient of imidazole of 0 - 0.5 M. The protein was finally purified by the size exclusion chromatography through a Superdex 200 16/600 PG column (GE Healthcare USA) equilibrated in 20 mM Tris-HCl, pH 7.4, and 500 mM NaCl. The purity of preaerolysin was confirmed by SDS-PAGE (Fig. S1). Equivoluminal 30% (v/v) glycerinum was added into the protein. The proaerlysin was stored at -80 ºC.

1.3 Formation of aerolysin nanopores

Monomeric aerolysin were acquired from proaerolysin by digesting it with trypsin-EDTA for 1 h at room temperature. Lipid bilayers were formed by 1, 2-diphytanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Alabaster, AL, USA) and spanning in a 50 μm orifice in a Delrin bilayer cup (Warner Instruments, Hamden, CT, USA). Both compartments are assigned as *cis* and *trans* chambers containing 1.0 mL of 1 M KCl, 10 mM Tris, pH 8.0, with 1 mM EDTA. The bias potential is applied through Ag/AgCl electrodes. ~ 1.0 μl monomeric aerolysin (~1.5 μg/ml) was added to the *cis* chamber to form the aerolysin nanopore.[1] The poly(dA)$_4$ was added to the *cis* chamber at a final concentration of 2.0 μM. All of the nanopore experiments were conducted at 24 ± 2 °C with an applied bias potential of +100 mV. The electric response induced by the poly(dA)$_4$ traversing through the WT aerolysin and mutant K238E aerolysin nanopore are acquired in the same conditions.

1.4 Data acquisition and analysis

As described in our previous studies,[2] the current recordings were performed with a current amplifier (Axon 200B equipped with a Digital1440A A/D converter, Molecular Devices, USA) with the *cis* compartment connected to ground. The amplified signal was low-pass filtered at 5 kHz and sampled at 100 kHz. The data was recorded by running the Clampex 10.4 software (Molecular Devices, Foster City, CA, USA).

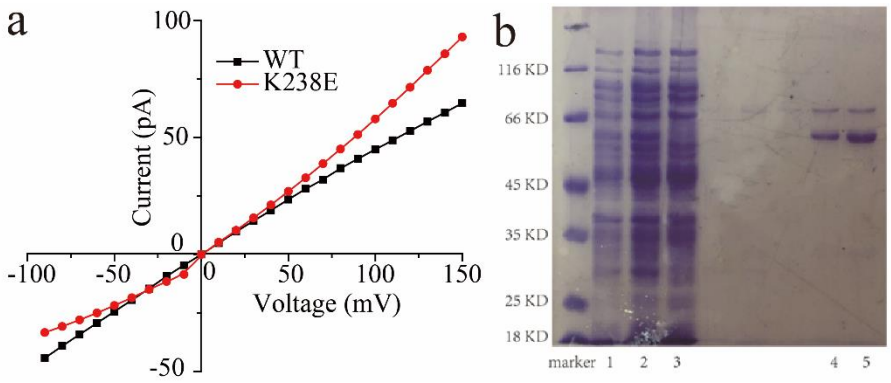

Fig S1. Characterizations of K238E aerolysin nanopore. **(a).** The I-V curve of wild type aerolysin and mutant K238E aerolysin. **(b)**. SDS-PAGE analysis of K238E proaerolysin. (1) Cell extracts, (2) supernatant, (3) supernatant through the column, (4) (5) K238E proaerolysin

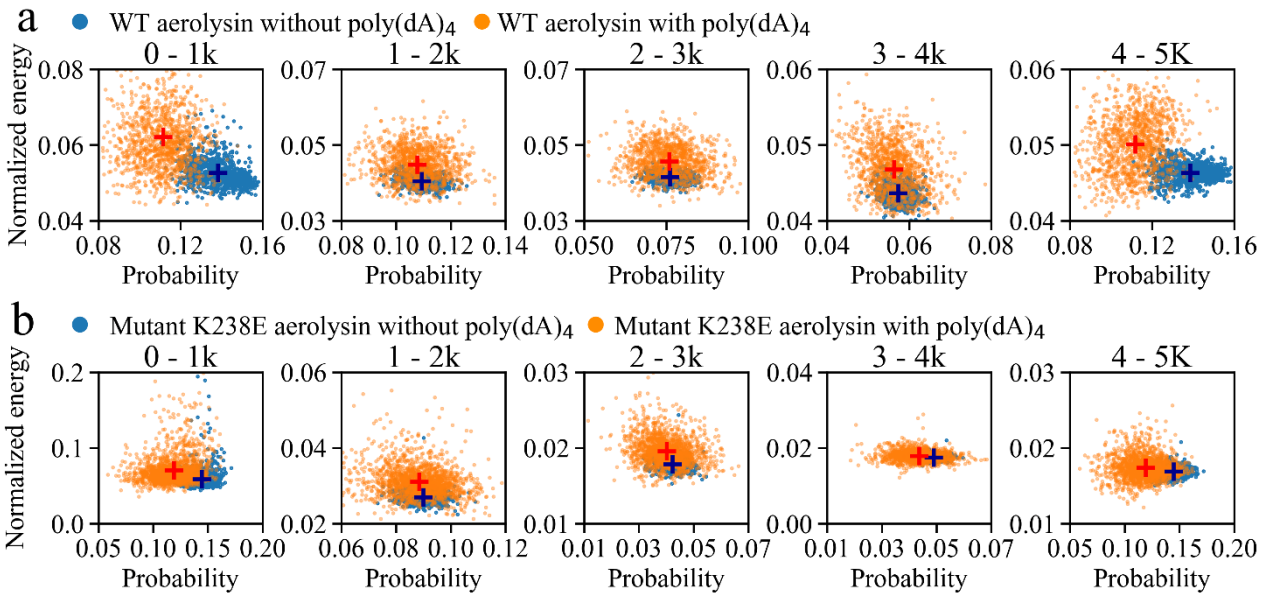

Fig S2. Normalized energy-probability distribution by statistical analysis of the probability of the frequency with amplitude/energy greater than zero in a specific frequency range from 0 – 5 kHz at the total duration time. **(a)** The WT aerolysin without (orange) and with the presence (blue) of poly(dA)$_4$. **(b)** The Mutant K238E aerolysion without (orange) and with the presence (blue) of poly(dA)$_4$.

Table S1. The cluster centre and standard deviation (SD) of the normalized energy-probability distribution as shown in Fig. 4.*

Frequency range	0 - 10k		10 - 20k		20 - 30k		30 - 40k		40 - 50K	
	center	SD	center	SD	center	SD	center	SD	center	SD
WT baseline	[0.278, 0.068]	0.0020	[0.093, 0.044]	0.0017	[0.048, 0.040]	0.0016	[0.048, 0.040]	0.0039	[0.279, 0.038]	0.0032
WT+DNA	[0.270, 0.069]	0.0052	[0.093, 0.044]	0.0025	[0.043, 0.045]	0.0029	[0.050, 0.041]	0.0061	[0.271, 0.039]	0.0055
K238E baseline	[0.263, 0.051]	0.0039	[0.074, 0.027]	0.0012	[0.043, 0.022]	0.0020	[0.047, 0.026]	0.0065	[0.264, 0.029]	0.0031
K238E+DNA	[0.253, 0.054]	0.0086	[0.074, 0.027]	0.0025	[0.043 0.021]	0.0028	[0.047, 0.026]	0.0060	[0.254, 0.029]	0.0137
Frequency range	0 - 1k		1 - 2k		2 - 3k		3 - 4k		4 - 5K	
	center	SD	center	SD	center	SD	center	SD	center	SD
WT baseline	[0.138, 0.053]	0.0053	[0.109, 0.04]	0.0026	[0.076, 0.042]	0.0019	[0.057, 0.044]	0.0014	[0.139, 0.046]	0.0054
WT+DNA	[0.111, 0.062]	0.0100	[0.108, 0.045]	0.0051	[0.076, 0.046]	0.0041	[0.056, 0.047]	0.0032	[0.112, 0.05]	0.0066
K238E baseline	[0.144, 0.059]	0.0102	[0.09, 0.027]	0.0027	[0.042, 0.018]	0.0020	[0.049, 0.017]	0.0016	[0.145, 0.017]	0.0053
K238E+DNA	[0.119, 0.071]	0.0176	[0.088, 0.031]	0.0063	[0.04, 0.02]	0.0044	[0.044, 0.018]	0.0042	[0.119, 0.017]	0.0099

* The cluster centre is acquired by kmeans algorithm. The SD is represented by the distance between the point and the cluster center. The SD value represent the dispersion degree of the signal in this frequency range.

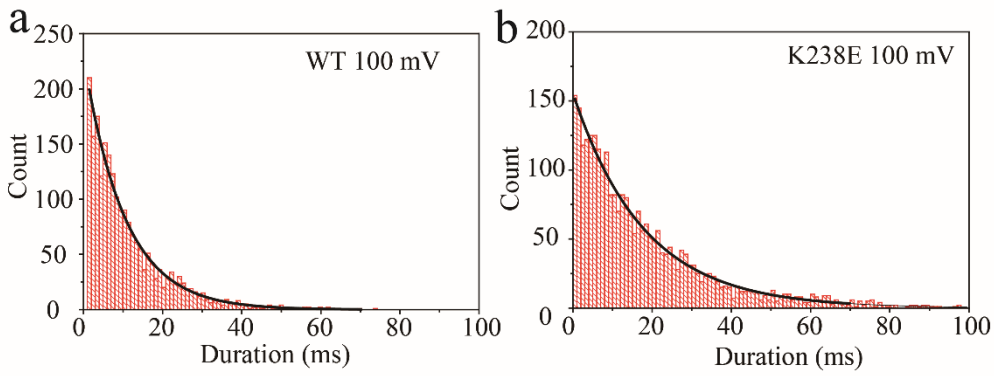

Fig S3. Duration time histograms of Poly(dA)$_4$ by (a) WT aerolysin and (b) K238E aerolysin at potential of 100 mv. The data were acquired in 1.0 M KCl, 10 mM Tris, 1.0 mM EDTA, pH 8.0 and in the presence of 2.0 µM Poly(dA)$_4$.

References

1. C. Cao, D.-F. Liao, J. Yu, H. Tian and Y.-T. Long, *Nat. Protoc.*, 2017, **12**, 1901–1911.
2. C. Cao, Y.-L. Ying, Z.-L. Hu, D.-F. Liao, H. Tian and Y.-T. Long, *Nat. Nanotechnol.*, 2016, **11**, 713–718.