Electronic Supplementary Information (ESI)

Synchronous Screening Multiplexed Biomarkers of Alzheimer's Disease by Length-encoded Aerolysin Nanopore-Integrated Triple-helix Molecular Switch

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EXPERIMENTAL SECTION

Chemicals. All deoxyribonucleic acids (DNAs) were synthesized by SangonBiotech Company, Ltd., (Shanghai, China) and their detailed sequence information is shown in Table S1. The oligonucleotides were purified by high-performance liquid chromatography (HPLC) and dissolved in ultrapure water as stock solutions. Trypsin-EDTA, trypsin-agarose, decane (anhydrous, ≥99%), and protein biomarkers of AD including alpha-1 antitrypsin (AAT), Tau protein (the Tau 381 isoform) and the purified human BACE1 extracellular domain was purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO). Immunoglobulin G (IgG), Human serum albumin (HSA), Glucose Oxidase (GOx), lysozyme, thrombin, and insulin were commercially obtained from Dingguo Biotechnology CO., Ltd (Beijing, China). Proaerolysin was kindly provided by Prof. Yi-Tao Long from East China University of Science and Technology.[1] 1,2-Diphytanoyl-sn-glycero-3-phosphocholine (powder,≥99%) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Other reagents and solvents were provided by Sinopharm Chemical Reagent Co., Ltd. (China), and used without further treatment. Ultrapure water (18.2 MΩ cm) from a Milli-Q system with a Pyrogard filter (Millipore, MA, USA) was used for preparation of solution.

Construction of aerolysin nanopore. The aerolysin nanopore was conducted according to previous reports.[2-4] Typically, monomeric aerolysin was obtained by trypsin-EDTA digestion of proaerolysin for 11 h at room temperature. The lipid bilayer membrane was formed spanning a 50 μm orifice in a Delrin bilayer cup

(Warner Instruments, USA). The aerolysin was injected adjacent to the aperture in the *cis* chamber, and the pore number was determined by a well-defined jump in the current value. The compartments on each side of the bilayer contained 1.0 ml of a buffer solution (1.0 M KCl, 10 mM Tris and 1.0 mM EDTA). The potential is applied using Ag/AgCl electrodes, and the cis compartment is defined as a virtual ground.

Nanopore Experiments and Data Analysis. All of the nanopore measurements were carried out at room temperature. The current traces were measured and amplified by an amplifier (DFCA-001) developed by Prof. Yi-Tao Long's group equipped with a Digidata 1440A A/D converter (Molecular Devices, Forest City, CA). The signals were low-pass filtered at 5 kHz and acquired at the sampling rate of 100 kHz by running Clampex 10.4 software (Molecular Devices, Forest City, CA). The data analysis was performed using the software from Yi-Tao Long's group (http://ytlong.ecust.edu.cn/9148/list.htm) based on MATLAB (R2011b, MathWorks[5, 6]). The statistical figures were constructed via and OriginLab 9.0 (Origin-Lab Corporation, Northampton, MA).

Table S1. Oligonucleotides used in this study.

Type	from 5' to 3'
Tau apt1	TCTTTTGCGGAGCGTGGCAGGTTTTCT
Tau apt2	TTCTTTTGCGGAGCGTGGCAGGTTTTCTT
Tau apt3	CTTCTTTTGCGGAGCGTGGCAGGTTTTCTTC
AAT apt1	TCTTTTGGGGCACGTACGGGCATCATAACAACAGGCGTGCCCCT TTTCT
AAT apt2	TTCTTTTGGGGCACGTACGGGCATCATAACAACAGGCGTGCCCC TTTTCTT
AAT apt3	CTTCTTTTGGGGCACGTACGGGCATCATAACAACAGGCGTGCCC CTTTTCTTC
BACE1 apt1	TCTTTTGCAATGGTACGGTACTTCCTGTGTTATTGTTATGTTTTTT CAGTGTAGTCAAAAGTGCACGCTACTTTGCTAATCTTTT
BACE1 apt2	TTCTTTTGCAATGGTACGGTACTTCCTGTGTTATTGTTATGTTTTT TCAGTGTAGTCAAAAGTGCACGCTACTTTGCTAATCTTTTT
BACE1 apt3	CTTCTTTTGCAATGGTACGGTACTTCCTGTGTTATTGTTATGTTTT TTCAGTGTAGTCAAAAGTGCACGCTACTTTGCTAATCTTTTTC
Stem 1	AAAAGA
Stem 2 (D7)	AAAAGAA
Stem 3	AAAAGAAG
Stem 4 (D10)	AAAAAAAGAA
Stem 5 (D13)	AAAAAAAAAAGAA
Stem 6	AAAAGAAAAAAAA

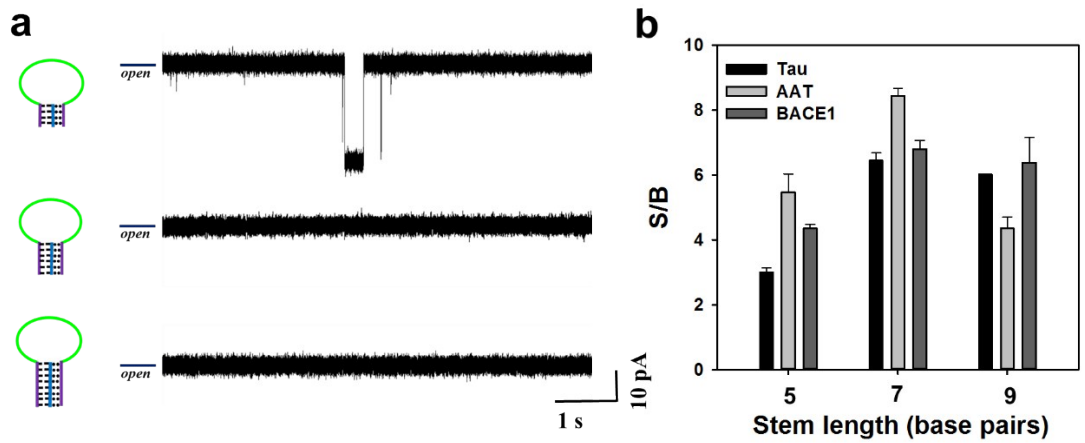

Figure S1. Determination of the stem length of TMS. The concentrations of three biomarkers are 10 nM. The channel recording time is 100 min/run. Each experiment was repeated three times.

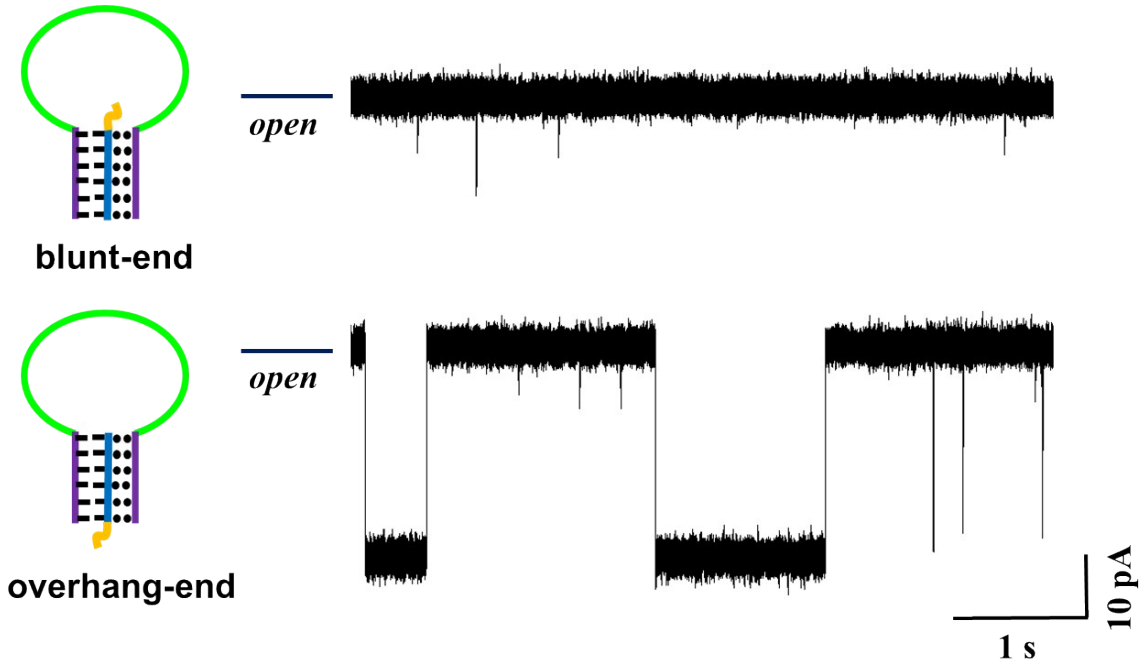

Figure S2. Comparison of the stability of overhang and a blunt-ended TMS. From the signature current event, it was obvious that the blunt-ended TMS is much more stable.

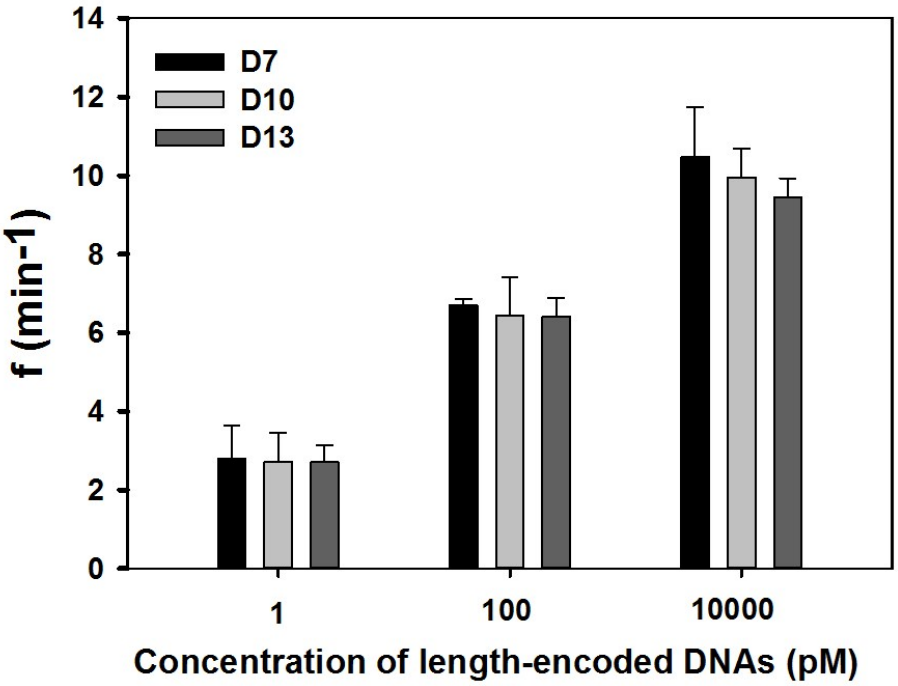

Figure S3. Signature frequencies at various concentrations of length-encoded DNAs.

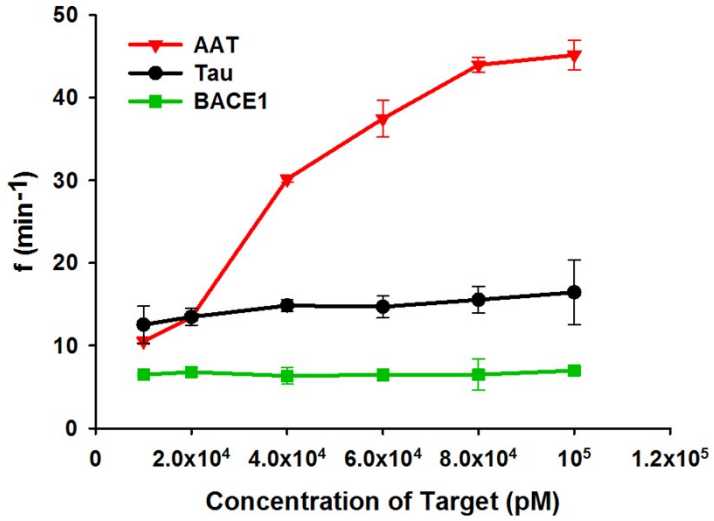

Figure S4. Signature frequencies at various AAT concentrations from 10 nM to 100 nM while concentrations of Tau and BACE1 proteins are fixed to 10 nM.

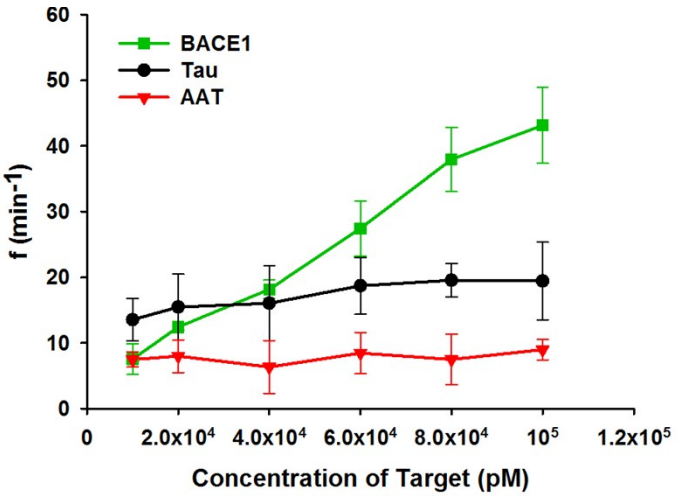

Figure S5. Simultaneous detection of multiple biomarkers. (a) current traces in presence of 10 nM Tau, AAT, and BACE. (b) Signature frequencies at various AAT concentrations from 10 nM to 100 nM while concentrations of Tau and BACE1 proteins are fixed to 10 nM.

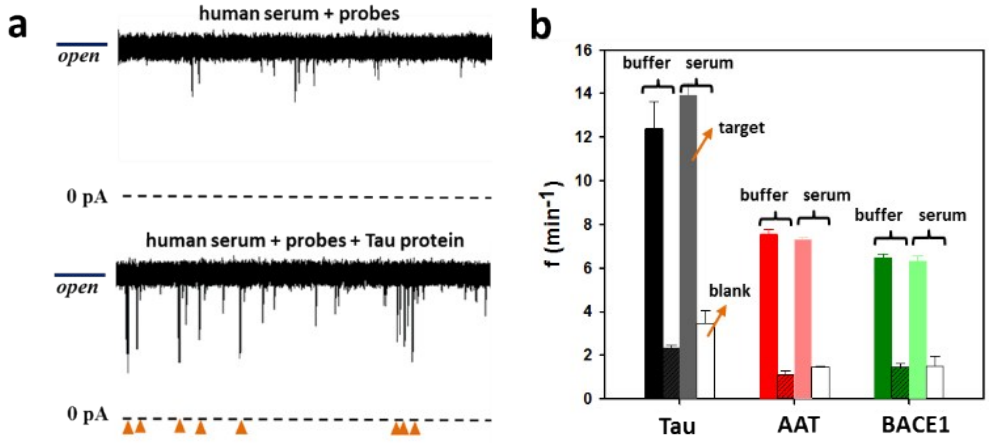

Figure S6. (a) Representative current traces for human serum sample in the absence and in the presence of Tau protein. (b) Comparison of multiple biomarker detection capability with and without human serum. The concentrations of the three biomarkers are 10 nM. The channel recording time is 100 min per run. Each experiment was repeated three times.

REFERENCES

[1] Y. Q. Wang, M. Y. Li, H. Qiu, C. Cao, M. B. Wang, X. Y. Wu, J. Huang, Y. L. Ying, Y. T. Long, *Anal. Chem*., 2018, **90**, 7790.

[2] C. Cao, Y. L. Ying, Z. L. Hu, D. F. Liao, H. Tian, Y. T. Long, *Nat. Nanotech*., 2016, **11**, 713.

[3] C. Cao, M. Y. Li, N. Cirauqui, Y. Q. Wang, M. D. Peraro, H. Tian, Y. T. Long, *Nat. Commun*., 2018, **9**, 2823.

[4] C. Cao, D. F. Liao, J. Yu, H. Tian, Y. T. Long, *Nat. Protoc*., 2017, **12**, 1901.

[5] Z. Gu, Y. L. Ying, C. Cao, P. He, Y. T. Long, *Anal. Chem*., 2015, **87**, 907.

[6] Z. L. Hu, Z. Y. Li, Y. L. Ying, J. Zhang, C. Cao, Y. T. Long, H. Tian, *Anal. Chem*., 2018, **90**, 4268.