

Supplementary Information for

Selective and Sensitive Detection of Methylcytosine by Aerolysin

Nanopore under Serum Condition

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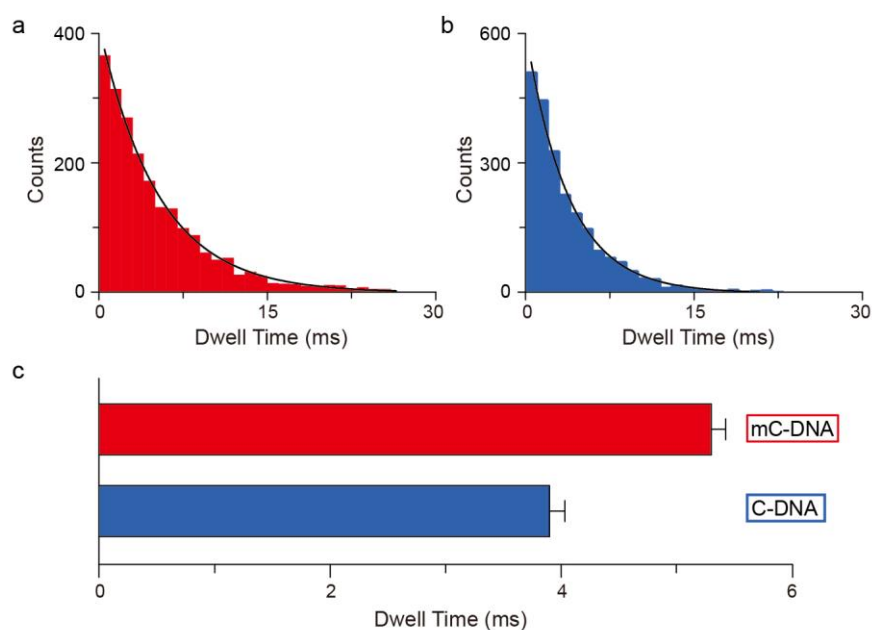


Figure S1. Dwell time of DNA oligomers traversing aerolysin nanopore. The dwell time histograms of (a) mC-DNA and (b) C-DNA were all fitted to single exponential function. (c) Mean values of dwell time of mC-DNA and C-DNA. The values were 5.3 ± 0.1 and 3.9 ± 0.1 ms, respectively. Data were based on at least three separate experiments and were recorded in 1 M KCl, 10 mM Tris, 1 mM EDTA, pH 8.0 at +80 mV.

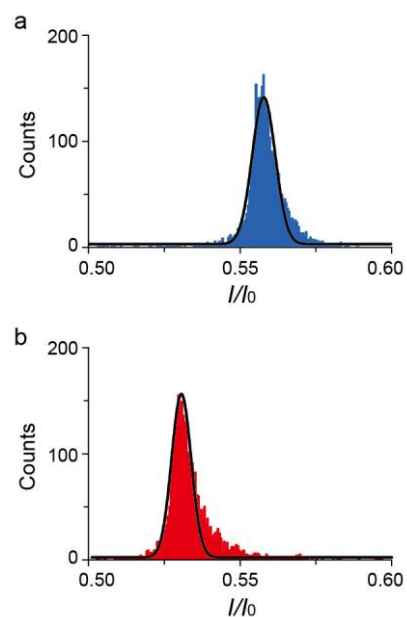


Figure S2. Histograms of the residual current blockade for (a) C-DNA and (b) mC-DNA. Values were obtained by fitting the current distribution with the Gaussian function. C-DNA and mC-DNA generated a current blockade of $I/I_0 = 0.56$ and $I/I_0 = 0.53$, respectively. Data were recorded in 1 M KCl, 10 mM Tris, 1 mM EDTA, pH 8.0 at +80 mV.

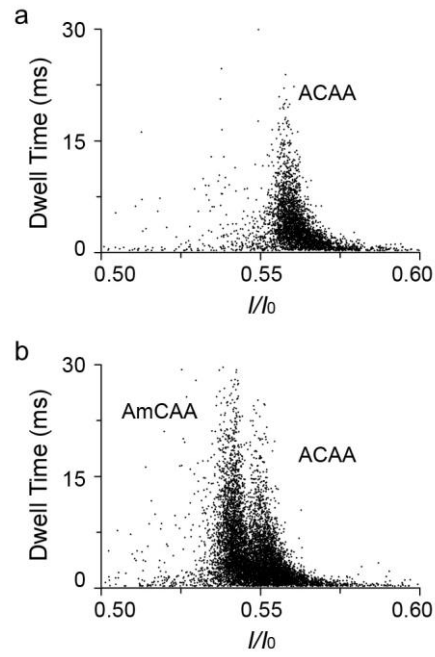


Figure S3. Scatter plots induced by the addition of DNA oligomers. ACAA and AmCAA were added into *cis* chamber in succession. Distributions for the addition of (a) ACAA, (b) ACAA and AmCAA were shown. Statistical results shown that ACAA could be distinguished from AmCAA. Data were recorded in 1 M KCl, 10 mM Tris, 1 mM EDTA, pH 8.0 at +80 mV.

Table S1. Mean values of event frequency for DNA oligomers at different applied potentials. Values were obtained from the slopes that linear fitted to number-versus-time curves. Data were recorded for 5 min using a single aerolysin nanopore. Experiments were performed in triplicate and the standard deviation were shown.

Potential (mV)	Event Frequency (s⁻¹)	
	mC-DNA	C-DNA
60	2.39 ± 0.04	1.74 ± 0.06
80	7.10 ± 0.02	5.93 ± 0.05
100	17.72 ± 0.20	14.76 ± 0.06
120	30.17 ± 0.06	28.24 ± 0.07
140	42.11 ± 0.21	40.77 ± 0.12

Table S2. Comparison of mean event frequency of DNA oligomers with different concentration ratios. Values were obtained from the slopes that linear fitted to number-versus-time curves. Data were recorded for 5 min using a single aerolysin nanopore. Experiments were performed in triplicate and the standard deviation were shown.

Ratio (mC-DNA : C-DNA)	Event Frequency (s ⁻¹)	
	mC-DNA	C-DNA
0 : 1	0	5.93 ± 0.05
1 : 0	7.10 ± 0.02	0
1 : 1	6.63 ± 0.05	4.98 ± 0.08
2 : 1	12.23 ± 0.13	4.98 ± 0.03
3 : 1	16.99 ± 0.07	6.46 ± 0.08
3 : 2	20.00 ± 0.07	10.70 ± 0.05