Nanopore based detection of *Bacillus thuringiensis* HD-73 spores using aptamers and versatile DNA hairpins

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Fig. S1. (a) Example of current trace and (b) blocking event rate of unbound complementary DNA hairpin after the reaction 6.67nM complementary hairpin/aptamer and BT spore in symmetric buffer condition.



Fig. S2. Blocking event rates in symmetric and asymmetric buffer. (a) Current trace and (b) blocking rate of complementary DNA hairpin (6.67 nM) to pass through alpha-hemolysin when using 1M KCl symmetric buffer and asymmetric concentration of KCl buffer (*cis* / *trans* : 0.25M/3M).



Fig. S3. Microscopic image and fluorescent intensity of HEX conjugated with original and modified aptamer binding to BT spores. (a) The predicted DNA structure of single strands DNA attached *Bacillus thuringiensis* HD-73 spore binding aptamer at the 3' ends. (b) Fluorescence microscope images of modified aptamer followed by washing with PBS buffer for three times. Scale bars are 5 μ m. (c) Fluorescent intensity of HEX conjugated with original (red) and modified (blue) aptamer binding to BT spores after washing by PBS buffer for three times, and without aptamer (black) in response to various concentration of BT spores. Fluorescent intensity was obtained using fluorescence plate reader at an excitation wavelength of 535 nm and emission wavelength of 556 nm.



Fig. S4. Measurements of blockade currents of the aptamer and the modified aptamer. Current traces show blockades by (a) the aptamer and (b) the modified aptamer. Deep block means that is translocation of aptamer and modified aptamer through alpha-hemolysin. Scattering plot of (c) the translocation event of aptamer and (d) modified aptamer shows passing characteristics through alpha-hemolysin. Inset plot of c shows that is expanding in the red circle. Red dotted lines of c and d mean dwell time of 500 ms. I/I_0 means that it normalized ratio of current blockade than full open current of alpha-hemolysin.



Fig. S5. Comparison of aptamer blocking signals with/without spores. Schematic image and blocking signal of the DNA aptamer (a) without (b) with spore. (c) histogram of blocking signal of DNA aptamer without (Blue), with spore (Red) for 50 sec. The concentration of spore was 1.2×10^8 CFU / mL.



Fig. S6. Electrical measurement of spore-unbound aptamers. (a) Concept of measurement of aptamers not bound to spores. (b) Blocking rate of aptamers not bound to spores.



Fig. S7. Detection of spores in asymmetric 0.25 M/3 M (cis/trans) KCl buffer ($3 \le n \le 9$)

Table S1. The sequences of DNAs used in this research. The DNA sequence of Original,modified aptamer and complementary DNA hairpin.

| Name | Sequence |
|------------------------------|---|
| Original aptamer | 5'- |
| | CATCCGTCACACCTGCTCTGGCCACTAACATGGGGACC |
| | AGGTGGTGTTGGCTCCCGTATC-3' |
| Modified aptamer | 5'- |
| | CATCCGTCACACCTGCTCTGGCCACTAACATGGGGACC |
| | AGGTGGTGTTGGCTCCCGTATCAAAAAAAAAAAAAAAAA |
| | AAAAAAAAAAAAAAAAAA |
| Complementary DNA hairpin | 5'-CTGAGCTTTTTTTTGCTCAGTTTTGATACGGG-3' |