# Supporting Information

# Nanopore single-molecule investigation of aflatoxin B1-aptamer interactions for evolving the aptamer

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## EXPERIMENTAL SECTION

**Reagents.** Lipid 1, 2-diphytanoyl-snglycero-3-phosphocholine (DPhPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, U.S.A.). Wild-type $\alpha$-HL, KCl, $MgCl_2 \cdot 5H_2O$, $CaCl_2$, $BaCl_2$ and Trizma @hydrochloric acid were obtained from Sigma-Aldrich and used without further purification. Aflatoxin B1, ochratoxin A, and fumonisin B1 were purchased from Aladdin (Shanghai, China). A Teflon chamber with a 50 μm orifice was purchased from Warner Instruments (Hamden, CT). All solutions for analytical studies were prepared using ultrapure water (18.2 MΩ).

The DNA sequences were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences of the oligonucleotide probes used in this work are listed below:

Native aptamer: 5'-CACGTGTTGTCTCTCTGTGTCTCGTGT$_{15}$-3'

A5-T22 aptamer: 5'-CACG**A**GTTGTCTCTCTGTGTCTCGTGT$_{15}$-3'

G2-C25 aptamer: 5'-C**G**CGTGTTGTCTCTCTGTGTCTCG**C**GT$_{15}$-3'

The test buffer contains 1M KCl, 50 mM $MgCl_2$, 10 mM Tris-HCl and 1mM EDTA (pH=7.5). The final concentration of DNA was 200 nM. Before the combination

experiment, AFB1 and DNA were incubated in the experimental buffer at 25℃ for 30 minutes in advance, and then added to the cis chamber. The beer and grape juice used in the real sample test were purchased at a local store.

**Single-channel recording**

30 mg/mL of 1, 2-diphytanoyl-snglycero-3-phosphocholine (DPhPC) are coated on both sides of the detection chamber to form a phospholipid bilayer. $\alpha$-HL solution (5 μg/mL) was added to promote the formation of transmembrane channels after 1 mL of electrolyte solution was added to the cis and trans chambers. After forming a single protein nanopore on the lipid bilayer, a potential of + 160 mV was applied to the lipid bilayer with Ag/AgCl electrodes. Currents were recorded with a patch clamp amplifier (Axopatch 200B, Axon Instruments, Foster city, CA, USA). The signal was low-pass filtered at 5 kHz and sampled at a frequency of 100 kHz with a Dig data 1550A A/D converter (Axon Instruments, Foster city, CA, USA). All the recordings were carried out at 25 ± 2 ℃.

**Data Analysis.**

Current traces were analyzed with NANOPORE ANALYSIS which is designed by MATLAB (R2011b, MathWorks) software and OriginLab 9.0 (OriginLab Corp., Northampton, MA, USA). The mean dwell time was calculated from the logarithmically binned dwell time histograms using fitted Gaussian distributions. The mean current blockade was derived from the $I/I_0$ histograms, which were fitted by a Gaussian function. All the data are presented as mean ± standard deviation of three independent experiments.

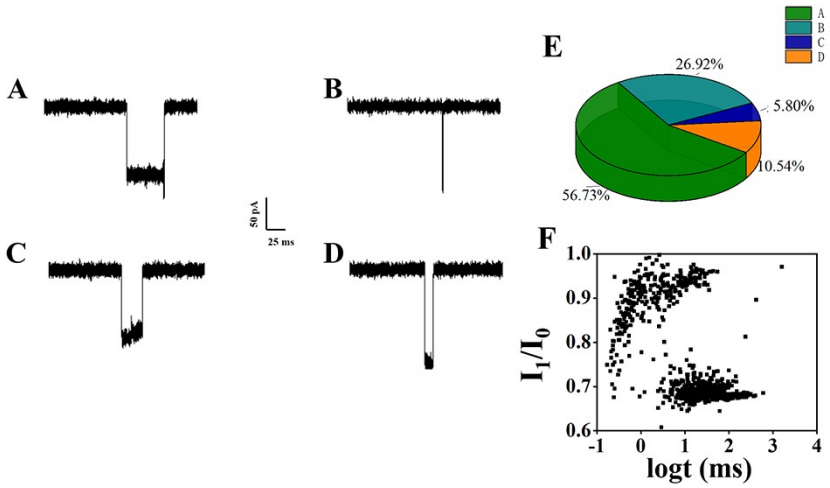


Fig. S1 (A)-(D) The four signal types generated by the aptamer. (E) Pie chart showing the proportions of the four signal types. (F) Scatter plot summarizing all observed signals.

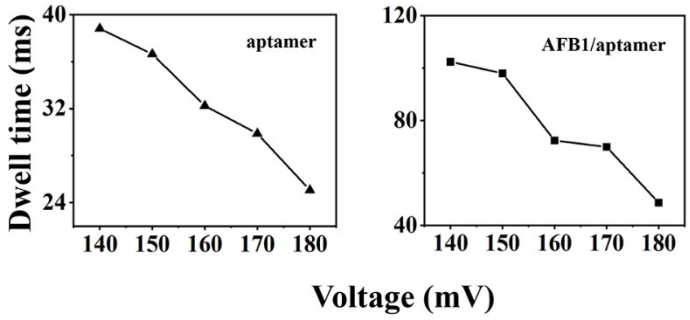


Fig. S2 Voltage dependence of the aptamer and the AFB1/aptamer complex as the voltage increases from +140 mV to +180 mV.

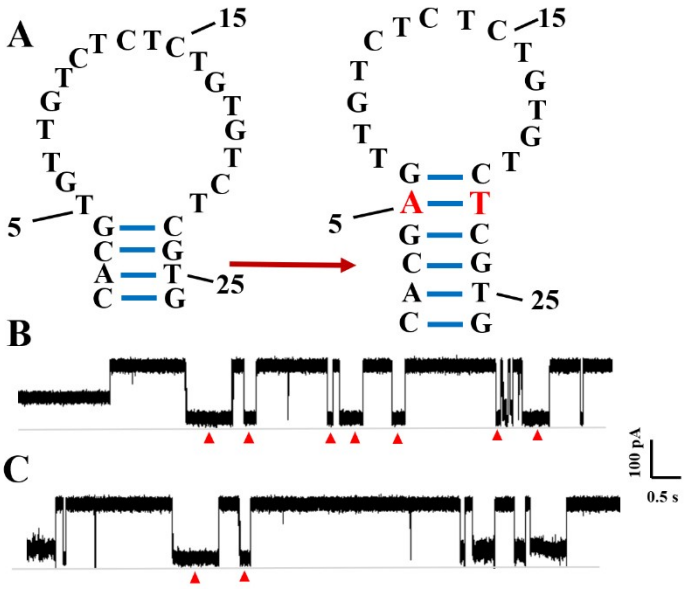


Fig. S3 (A) Secondary structure diagram of the aptamer after A5-T22 base mutation. (B) Current trace of the native aptamer interacting with AFB1. (C) Current trace of the A5-T22 mutant aptamer

interacting with AFB1. (The red triangle indicates the characteristic current signal of the AFB1/aptamer complex. The final concentrations of the aptamer and AFB1 in the experiment were 100 nM.)

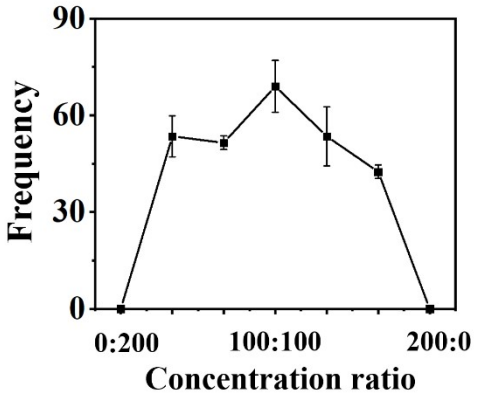


Fig. S4 The frequency of AFB1/aptamer complex formed under the different conditions (the number of signature events recorded in 5 minutes). The total concentration of AFB1 and the aptamer was maintained at 200 nM, while the concentration ratios were systematically varied as follows: 0:200, 50:150, 75:125, 100:100, 125:75, 150:50, and 200:0.

Table S1. $I/I_0$ and dwell time ($\tau_{off}$) of the aptamer and the complex under different pH conditions. The final concentration of both the aptamer and AFB1 was 200 nM. (n=3).

| | | **pH=6.0** | **pH=7.5** | **pH=9.0** |
|---|---|---|---|---|
| **Aptamer** | $I/I_0$ （%） | 68.07 ± 0.37 | 68.34 ± 0.16 | 68.68 ± 0.28 |
| | $\tau_{off}$（ms） | 34.86 ± 7.29 | 32.24 ± 7.41 | 30.48 ± 2.64 |
| **Complex** | $I/I_0$ （%） | 87.01 ± 0.06 | 87.39 ± 0.03 | 89.46 ± 0.28 |
| | $\tau_{off}$（ms） | 80.95 ± 13.60 | 72.35 ± 4.28 | 57.82 ± 11.29 |

Table S2. DNA sequences used in the experiment and their corresponding melting temperatures ($T_m$).

| **Name** | **Sequence** | **$T_m$ (°C)** |
|---|---|---|
| Native aptamer | 5’-CACGTGTTGTCTCTCTGTGTCTCGTGT$_{15}$-3’ | 50.6 |
| A5-T22 mutant | 5’-CACGAGTTGTCTCTCTGTGTCTCGTGT$_{15}$-3’ | 63.3 |
| G2-C25 mutant | 5’-CGCGTGTTGTCTCTCTGTGTCTCGCGT$_{15}$-3’ | 62.7 |

Table S3. $I/I_0$ and dwell time of the aptamer and AFB1/aptamer complex before and after the G2-C25 mutation (n=3).

| | **Native aptamer** | **G2-C25 aptamer** | **complex** | **G2-C25 complex** |
|---|---|---|---|---|
| $I/I_0$ （%） | 68.34 ± 0.16 | 69.27 ± 0.38 | 87.39 ± 0.03 | 88.65 ± 0.44 |
| $\tau_{off}$（ms） | 32.24 ± 7.41 | 131.03 ± 22.41 | 72.35 ± 4.28 | 766.56 ± 24.19 |

Table S4. Comparison of the linear range and detection limit of various methods for AFB1 detection.

| **Methods** | **Liner range** | **LOD** | **Reference** |
|---|---|---|---|
| Nanopore | 0.5 - 100 nM | 0.5 nM | This work |
| Fluorescence | 5 - 100 ng/mL | 1.6 ng/mL (5.1 nM) | 9 |
| Fluorescence | 125 pM - 31.2 nM | 125 pM | 10 |
| Electrochemical | 2 nM- 4 uM | 2 nM | 13 |
| Circular Dichroism | 0.6 nM - 2 uM | 0.6 nM | 14 |

Table S5. Detection results of AFB1 in real samples. (n=3).

| **Samples** | **Add (nM)** | **Found (nM)** | **Recovery (%)** | **RSD (%)** |
|---|---|---|---|---|
| Beer | 15.00 | 15.18 | 101.20 | 6.64 |
| | 35.00 | 34.08 | 97.37 | 4.35 |
| Grape juice | 5.00 | 4.27 | 85.40 | 2.34 |
| | 66.00 | 67.31 | 101.98 | 3.08 |