Electronic Supplementary Information

Rapid and label-free detection of aflatoxin B1 by using a rationally truncated aptamer and via circular dichroism measurement

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Experiment

1. Materials and methods

Assay buffer containing 10 mM Tris-HCl (pH 7.4), 20 mM $MgCl_2$, 150 mM NaCl and 0.01% (v/v) tween20 was used. All DNA strands were synthesized by Sangon Bioteh (Shanghai, China, http://www.sangon.com), and purified through HPLC. Their sequences were listed in **Table S1**. Mycotoxins including aflatoxin B1 (AFB1), fumonisins B1 (FB1), ochratoxin A (OTA), zearalenone (ZAE) and deoxynivalenol (DON) were purchased from Sangon Bioteh (Shanghai, China). Beer was bought from a local supermarket. All aqueous solutions were prepared with ultrapure water with an electric resistivity of 18.2 MΩ·cm.

The CD measurements were performed on a circular dichroism spectropolarimeter (JASCO J-1500, Japan) with a thermostatic cell holder and nitrogen-purging facility. Following parameters were set, data acquisition interval 0.5 nm, response time 1 s, bandwidth 5 nm, scan speed 200 nm/min, accumulations times 3. One quartz cuvette with 1 cm path length was used for holding sample solution. Spectra were baseline subtracted with the CD signals from the buffer only. The data were collected from the triplicate scans, and all experiments were performed in duplicate.

2. Detection of AFB1

We mixed aptamer with AFB1 together in the assay buffer. The final aptamer concentration was 4 μM. After an incubation for 1 min, 400 μL of the mixture solution was transferred into a microsample quartz cuvette, and circular dichroism (CD) measurements were performed immediately. The CD signal value at 280 nm was recorded and used for AFB1 analysis.

3. Specificity test

To assess specificity of the short aptamer towards AFB1, some no-target mycotoxins (OTA, FB1, ZAE and DON), which probably co-existed with AFB1, were detected by using the short aptamer. Concentrations of the no-target mycotoxins were 1 μM. Other assay conditions were same with that for AFB1 detection.

4. Real samples analysis

We used 26nt to detected AFB1 spiked in methanol and beer samples, respectively. The beer was ultrasonicated to degas, and then filtered through a syringe filter (0.22 μm) before dilution with the assay buffer. Finally, different concentrations of AFB1 spiked in 20-fold diluted methanol and 50-fold diluted beer were detected, respectively, following above AFB1 detection procedures.

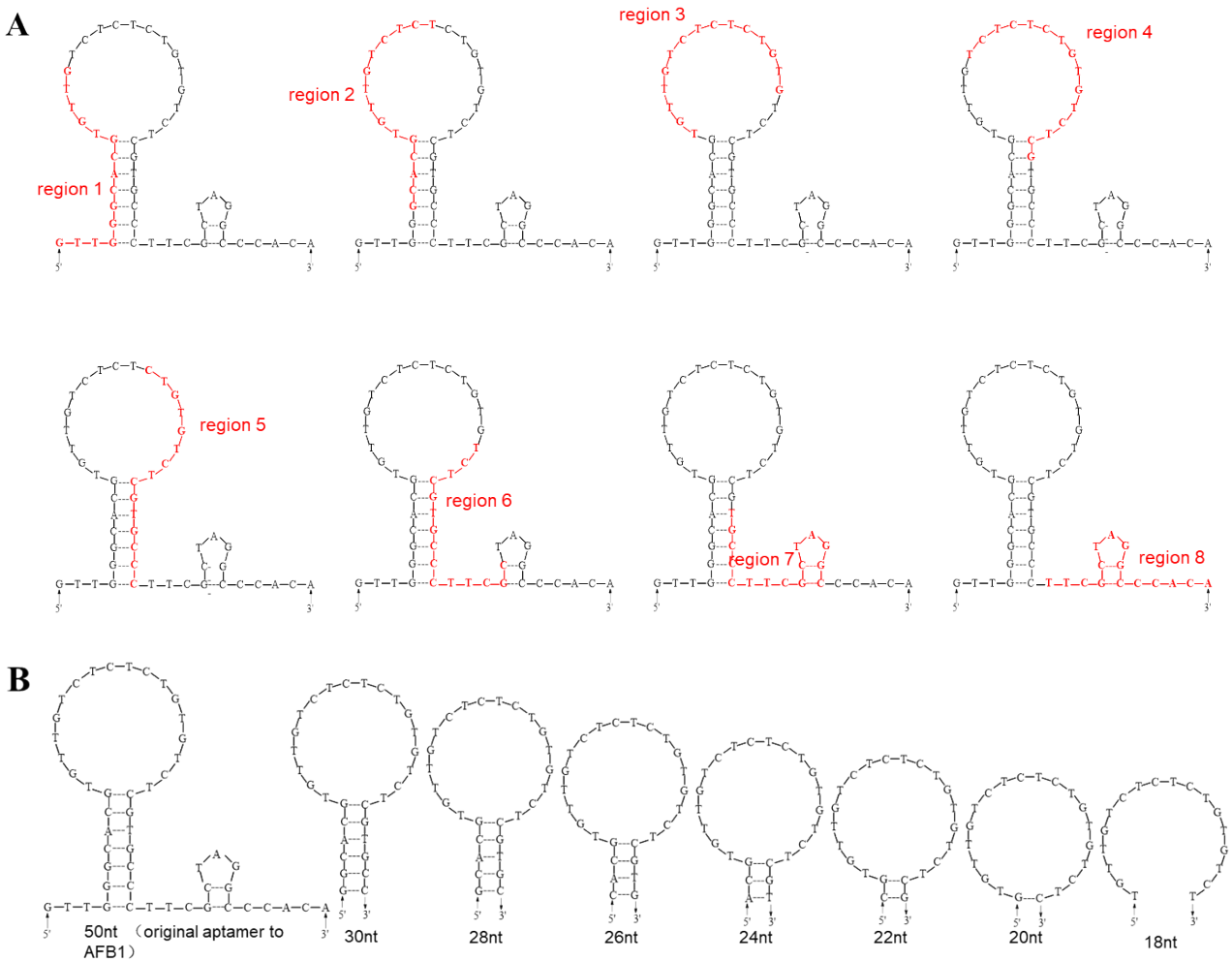

Fig. S1. (A) Different regions (region 1 to region 8) of the original aptamer 50nt, corresponding to hybridization regions with different cDNAs (C1 to C8). (B) Secondary structures corresponding to the original anti-AFB1 aptamer (50nt) and short aptamers (30nt, 28nt, 26nt, 24nt, 22nt, 20nt and 18nt), predicted by the UNAFold web server (http://www.unafold.org/).

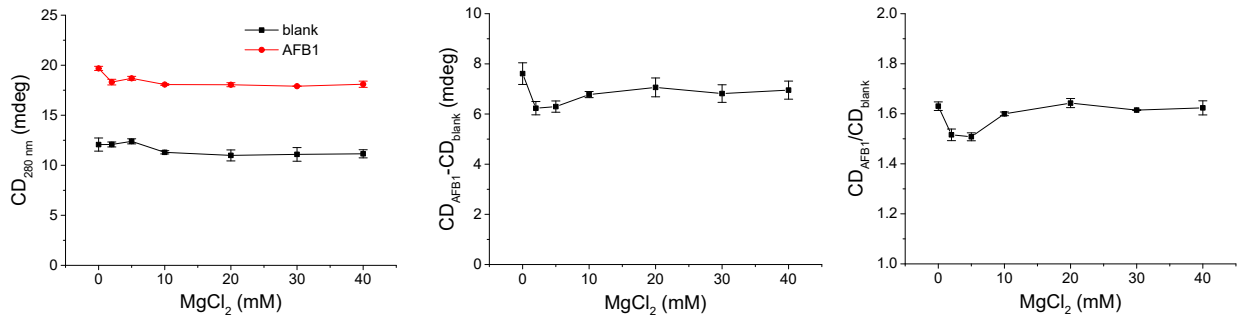

Fig. S2. Effects of different $MgCl_2$ concentrations in assay buffer on detection performance. Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 150 mM NaCl, 0.01 % tween20 and different concentrations of $MgCl_2$. AFB1 concentration was 500 nM, concentration of 26nt was 4 μM.

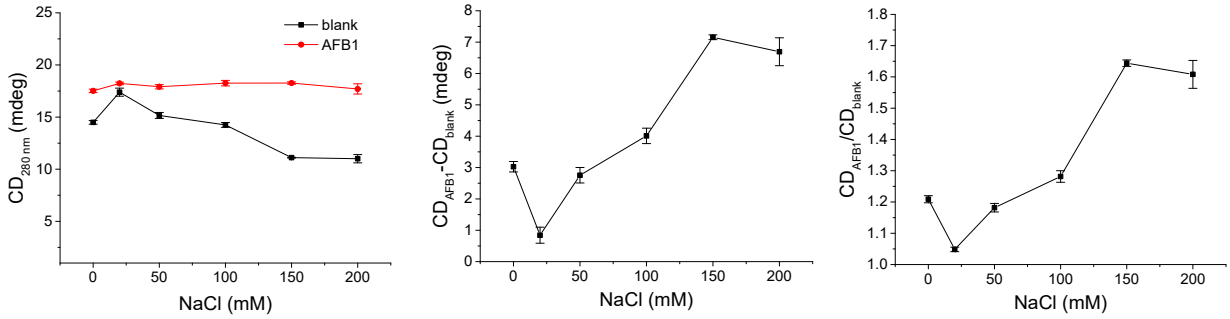

Fig. S3. Effects of NaCl concentrations in assay buffer on detection performance. Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 20 mM $MgCl_2$, 0.01 % tween20 and different concentrations of NaCl. AFB1 concentration was 500 nM, concentration of 26nt was 4 μM.

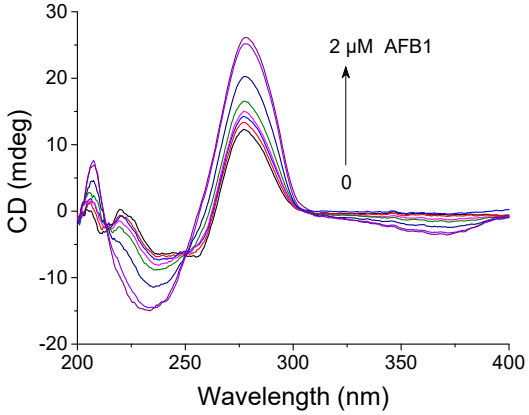

Fig. S4. CD spectra of this 26nt aptamer responses to different AFB1 concentrations. Assay buffer was 10 mM Tris-HCl (pH 7.5) containing 20 mM $MgCl_2$, 150 mM NaCl and 0.01 % tween20 at 25 ^{o}C. Concentration of 26nt was 4 μM.

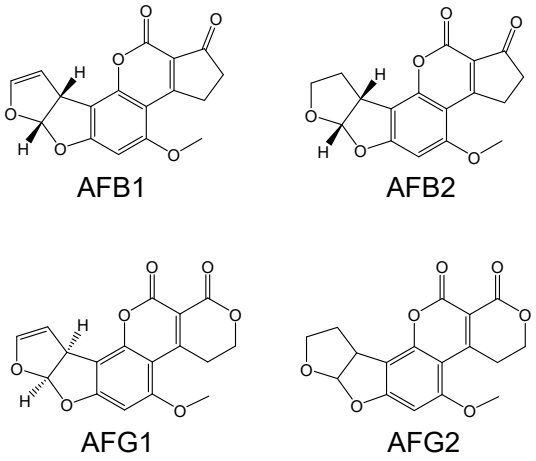

Fig.S5. Molecule structures corresponding to AFB1, AFB2, AFG1 and AFG2.

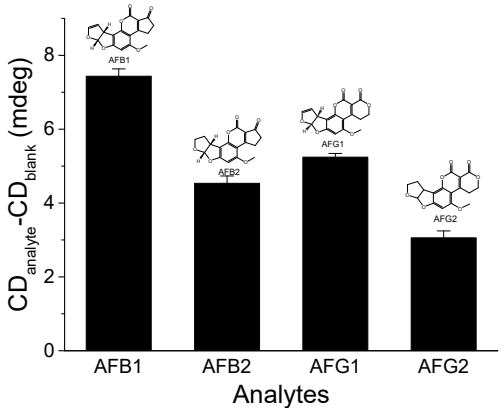

Fig.S6. Detections of AFB1 and its analogues AFB2, AFG1 and AFG2, by using this method. Concentrations of AFB1, AFB2, AFG1 and AFG2 were 500 nM.

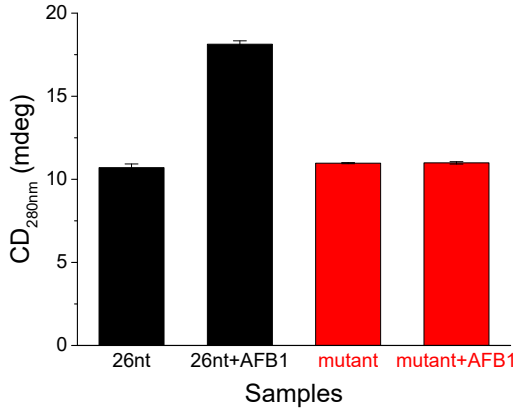

Fig.S7. Testing sequences specificity of this truncated aptamer (26nt) for AFB1 detection, using a mutational aptamer sequence (The ninth G was mutated to T in the sequence).

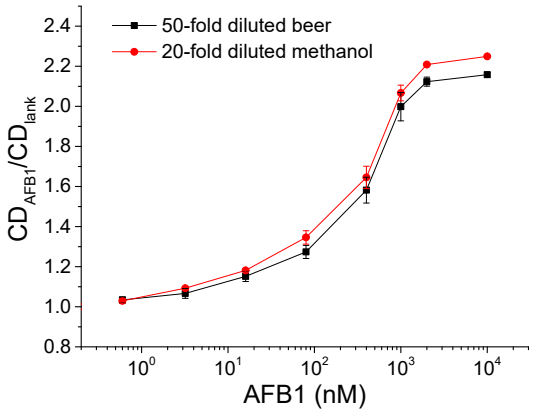

Fig.S8. Detection of different concentrations of AFB1 spiked in 20-fold diluted methanol and 50-fold diluted beer, respectively.

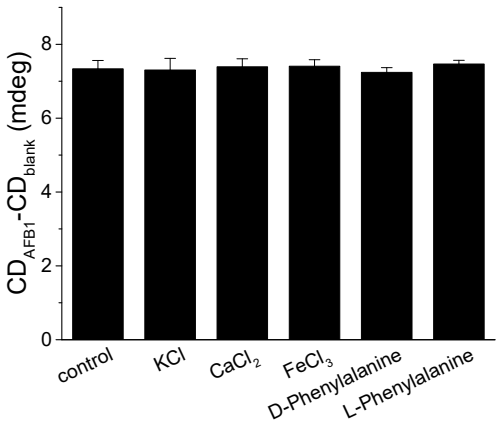

Fig.S9. Assessment of interferences from KCl (100 mM), $CaCl_2$ (20 mM) and $FeCl_3$ (5 mM), D-phenylalanine (10 μg/mL) and L-phenylalanine (10 μg/mL) on detection of AFB1 in 20-fold diluted methanol.

Table S1. Sequences of different DNA strands used in this work

Category	Name	Sequence (5’ to 3’)
Original aptamer	50nt	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCC CTTCGCTAGGCCCACA
Short aptamer	30nt	GGCACGTGTTGTCTCTCTGTGTCTCGTGCC
	28nt	GCACGTGTTGTCTCTCTGTGTCTCGTGC
	26nt	CACGTGTTGTCTCTCTGTGTCTCGTG
	24nt	ACGTGTTGTCTCTCTGTGTCTCGT
	22nt	CGTGTTGTCTCTCTGTGTCTCG
	20nt	GTGTTGTCTCTCTGTGTCTC
	18nt	TGTTGTCTCTCTGTGTCT
Mutational DNA	mutant	CACGTGTT***T***TCTCTCTGTGTCTCGTG
Complementary DNA (cDNA)	C1	CAACACGTGCCCAAC
	C2	AGAGACAACACGTGC
	C3	CACAGAGAGACAACA
	C4	CGAGACACAGAGAGA
	C5	GGGCACGAGACACAG
	C6	GCGAAGGGCACGAGA
	C7	GCCTAGCGAAGGGCA
	C8	TGTGGGCCTAGCGAA

Table S2. Comparison of a few aptamer based assays for AFB1 with respect to dynamic range, analysis time, limit of detection (LOD) and label-free.

Detection method	Dynamic range	LOD	Analysis time	Label-free	References
Fluorescence	1.6 nM-160 μM	1.4 nM	>30 min	No	[1]
Fluorescence	10-400 nM	3.4 nM	45 min	No	[2]
Fluorescence	3.1-976 nM	1.1 nM	>25 min	No	[3]
Fluorescence	15.6-312 nM	5 nM	>35 min	No	[4]
Colorimetric	80-270 nM	7 nM	10 min	Yes	[5]
Colorimetric	0.3 nM-32 μM	0.32 nM	70 min	Yes	[6]
Electrochemical	5.0 pM-10 nM	0.4 pM	180 min	No	[7]
Electrochemical	20 pM-1.6 nM	6.4 pM	135 min	No	[8]
SERS sensor	3.2 fM-3.2 nM	1.3 fM	120 min	No	[9]
RT-PCR	0.15 pM-15 nM	0.08 pM	>87 min	Yes	[10]
Circular dichroism	0.6 nM-2 μM	0.6 nM	3 min	Yes	This work

References cited in Table S2**:**

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