Supplementary Materials

A novel strategy for analyzing aptamer dominated sites and detecting AFB1 based on CRISPR-Cas12a

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1. Supplementary figures

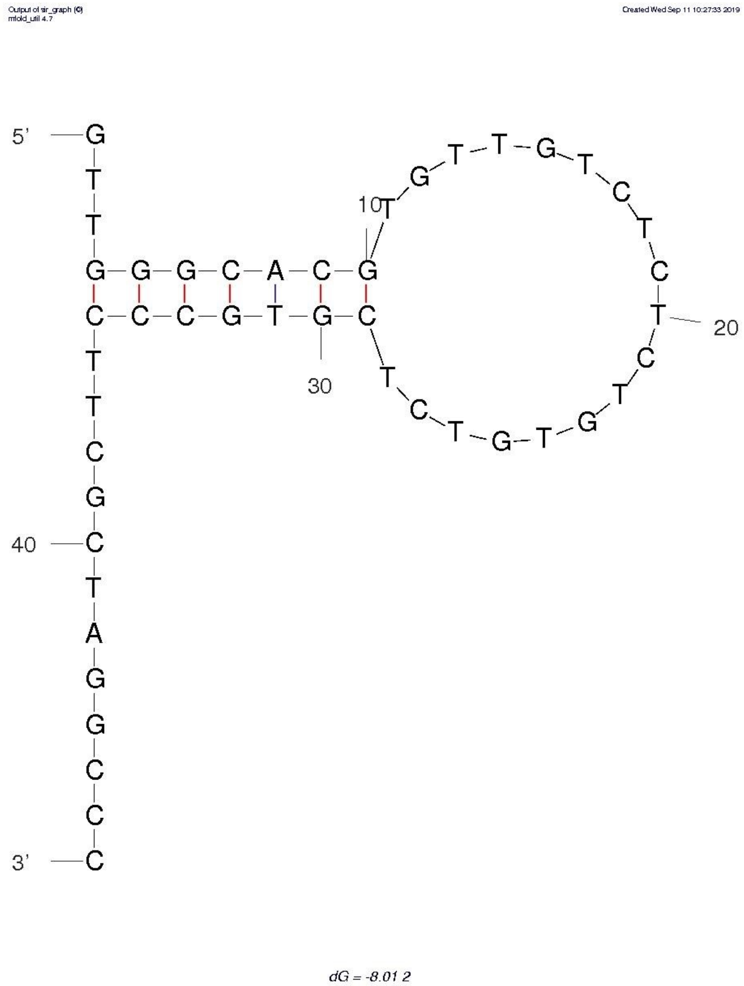

Figure S1. The secondary structure (a simple stem-loop structure) of 47-nt AFB1 aptamer from oligo analysis website Mfold (http://unafold.rna.albany.edu/)

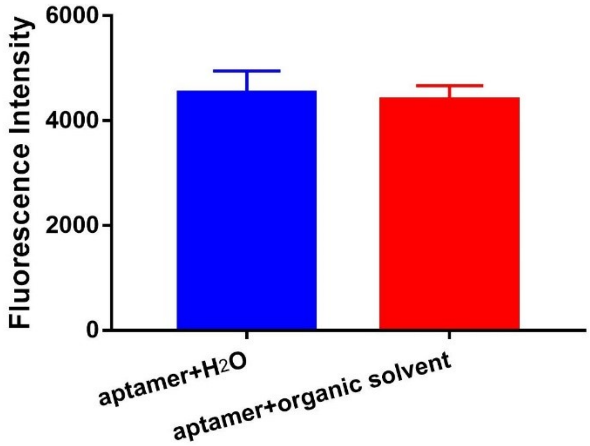

Figure S2. Effect of organic solvent (acetonitrile) on the trans-cleavage activity of Cas12a.

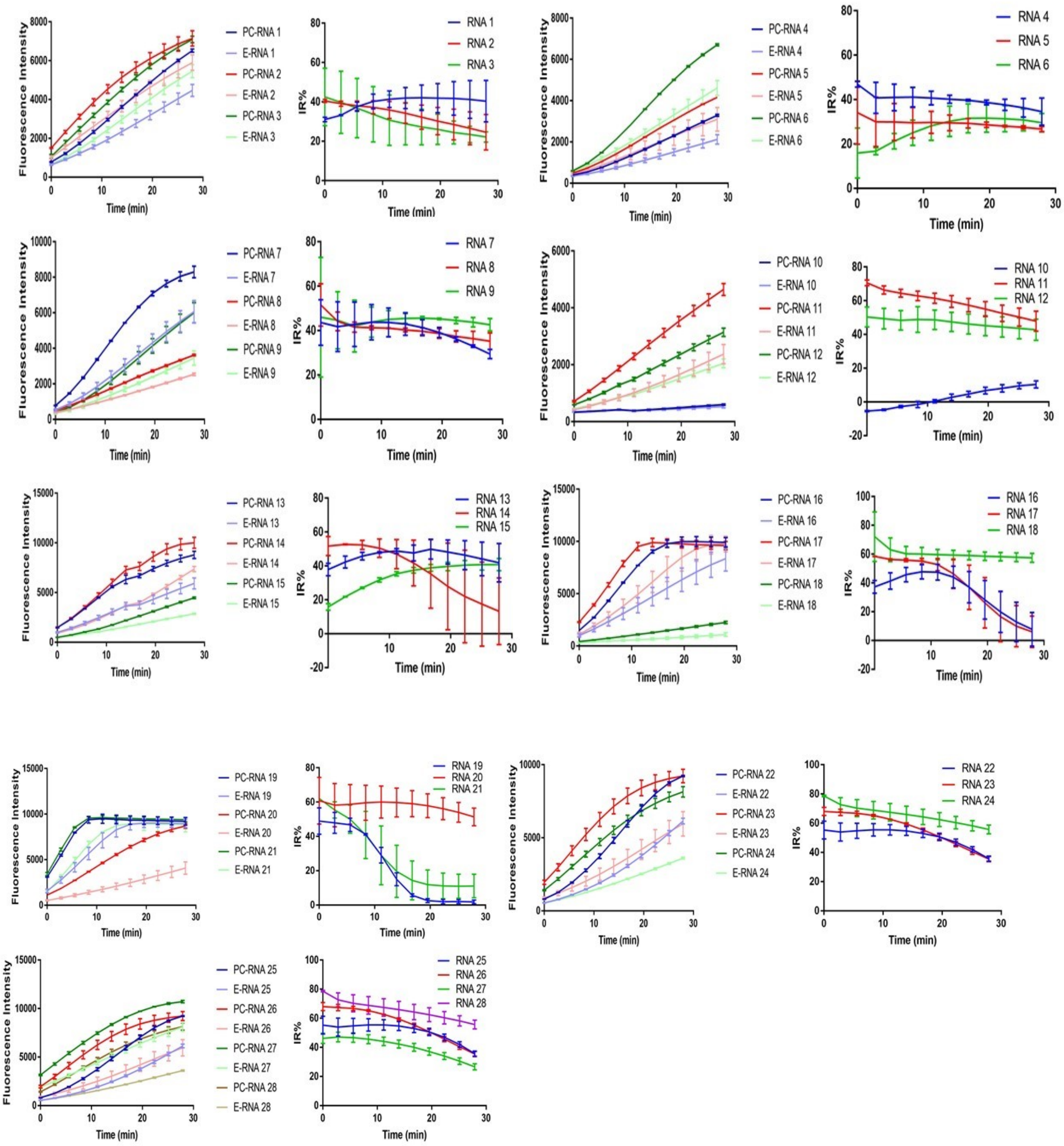

Figure S3. Kinetic fluorescence results of 28 IR% of 28 RNAs

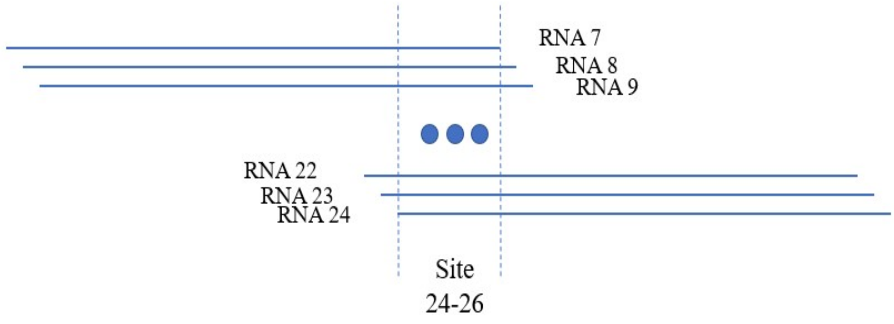

Figure S4. Repeat site in crRNA 7-24 (site 24-26 from 5’ end to 3’ end in AFB1 ssDNA aptamer) was speculated to be the dominated site.

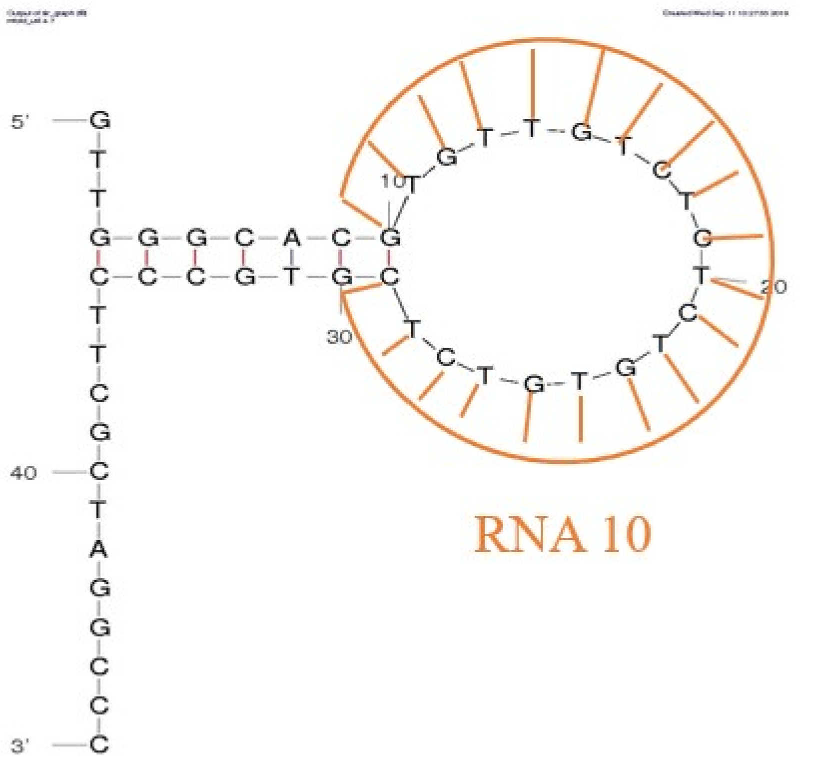

Figure S5. RNA 10 hybridization with the whole loop sequence from site 10-29 of aptamer.

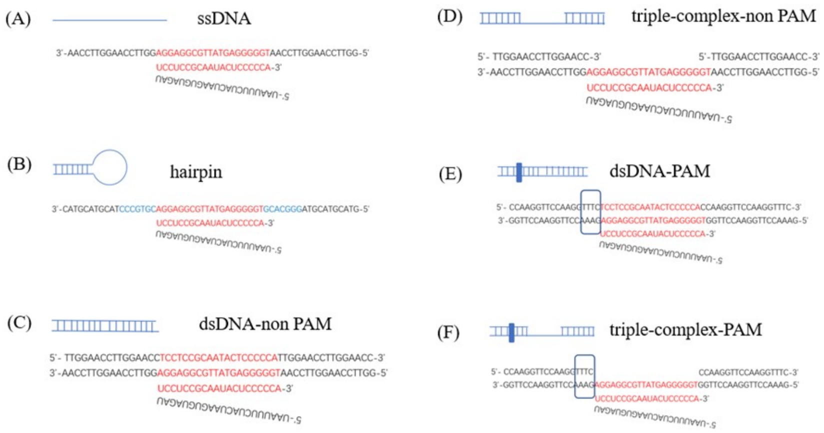

Figure S6. Sequences and symbols of Six structures. The below sequence with the twist is crRNA. Region in red is the hybridization region. Region in blue is the same sequence as the stem sequence in 47-nt AFB1 aptamer. Region in a black box is the PAM sequence (TTTN), which is required in the dsDNA hybridization for Cas12a.

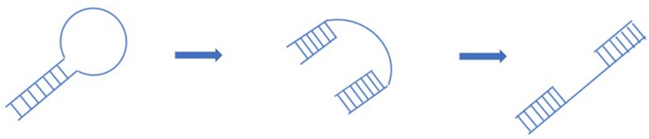

Figure S7. The predicted process of recognition of hairpin structure.

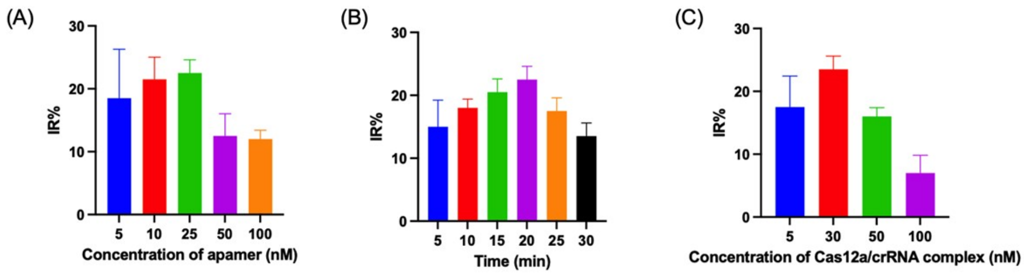

Figure S8. Optimization of the detection of AFB1. (A) The concentration of AFB1 aptamer. (B) The cutting time of Cas12a. (C) The concentration of Cas12a/crRNA complex. The error bars indicate the standard deviation of three repeats.

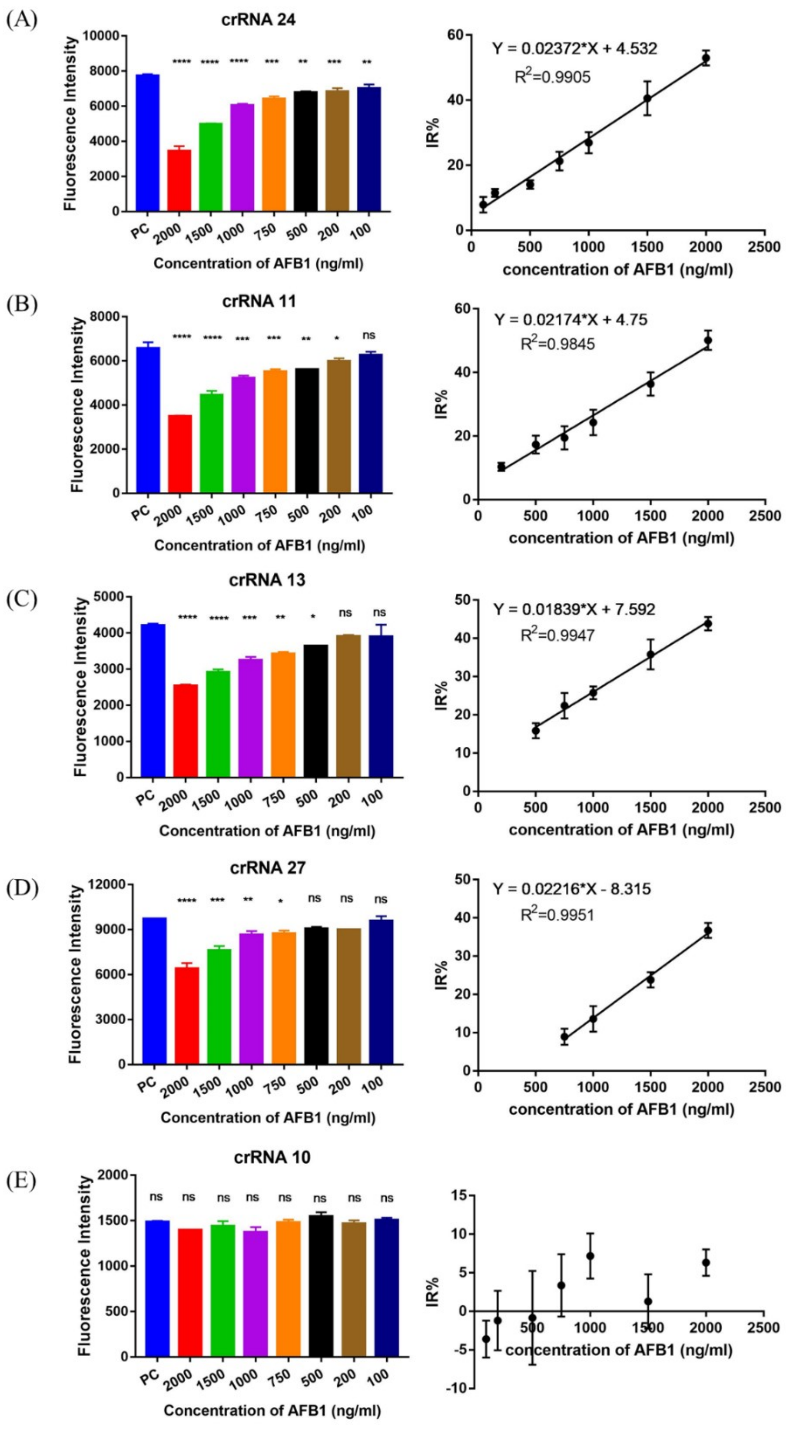

Figure S9. Sensitivity test for 5 different crRNAs. A) Kinetics of the fluorescence change at various concentrations of AFB1 for crRNA 24 and the linear calibration curve. B) Kinetics of the fluorescence change at various concentrations of AFB1 for crRNA 11 and the linear calibration curve. C) Kinetics of the fluorescence change at various concentrations of AFB1 for crRNA 13 and the linear calibration curve. D) Kinetics of the fluorescence change at various concentrations of AFB1 for crRNA 27 and the linear calibration curve. E) Kinetics of the fluorescence change at various concentrations of AFB1 for crRNA 10 and the linear calibration curve. The data were obtained from three independent measurements. The error bars indicate the standard deviation. ****: P < 0.0001. ***: P < 0.001. **: P < 0.01. *: P < 0.05. ns: no significant difference. PC: control group.

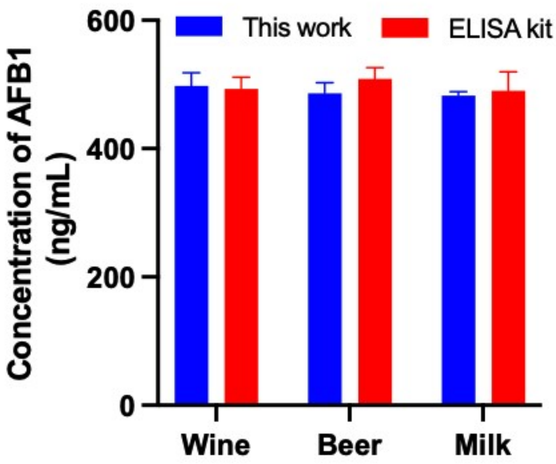

Figure S10. The detection results of AFB1 in the complex matrix sample test using the ELISA kit and the proposed method in this work.

2. Supplementary tables

Table S1. Oligonucleotides sequence

Name	Sequence (5’-3’)
AFB1-crRNA-1	UAAUUUCUACUAAGUGUAGAU AGAGACAACACGUGCCCAAC
AFB1-crRNA-2	UAAUUUCUACUAAGUGUAGAU GAGAGACAACACGUGCCCAA
AFB1-crRNA-3	UAAUUUCUACUAAGUGUAGAU AGAGAGACAACACGUGCCCA

AFB1-crRNA-4	UAAUUUCUACUAAGUGUAGAU CAGAGAGACAACACGUGCCC
AFB1-crRNA-5	UAAUUUCUACUAAGUGUAGAU ACAGAGAGACAACACGUGCC
AFB1-crRNA-6	UAAUUUCUACUAAGUGUAGAU CACAGAGAGACAACACGUGC
AFB1-crRNA-7	UAAUUUCUACUAAGUGUAGAU ACACAGAGAGACAACACGUG
AFB1-crRNA-8	UAAUUUCUACUAAGUGUAGAU GACACAGAGAGACAACACGU
AFB1-crRNA-9	UAAUUUCUACUAAGUGUAGAU AGACACAGAGAGACAACACG
AFB1-crRNA-10	UAAUUUCUACUAAGUGUAGAU GAGACACAGAGAGACAACAC
AFB1-crRNA-11	UAAUUUCUACUAAGUGUAGAU CGAGACACAGAGAGACAACA
AFB1-crRNA-12	UAAUUUCUACUAAGUGUAGAU ACGAGACACAGAGAGACAAC
AFB1-crRNA-13	UAAUUUCUACUAAGUGUAGAU CACGAGACACAGAGAGACAA
AFB1-crRNA-14	UAAUUUCUACUAAGUGUAGAU GCACGAGACACAGAGAGACA
AFB1-crRNA-15	UAAUUUCUACUAAGUGUAGAU GGCACGAGACACAGAGAGAC
AFB1-crRNA-16	UAAUUUCUACUAAGUGUAGAU GGGCACGAGACACAGAGAGA
AFB1-crRNA-17	UAAUUUCUACUAAGUGUAGAU AGGGCACGAGACACAGAGAG
AFB1-crRNA-18	UAAUUUCUACUAAGUGUAGAU AAGGGCACGAGACACAGAGA
AFB1-crRNA-19	UAAUUUCUACUAAGUGUAGAU GAAGGGCACGAGACACAGAG
AFB1-crRNA-20	UAAUUUCUACUAAGUGUAGAU CGAAGGGCACGAGACACAGA
AFB1-crRNA-21	UAAUUUCUACUAAGUGUAGAU GCGAAGGGCACGAGACACAG
AFB1-crRNA-22	UAAUUUCUACUAAGUGUAGAU AGCGAAGGGCACGAGACACA
AFB1-crRNA-23	UAAUUUCUACUAAGUGUAGAU UAGCGAAGGGCACGAGACAC
AFB1-crRNA-24	UAAUUUCUACUAAGUGUAGAU CUAGCGAAGGGCACGAGACA
AFB1-crRNA-25	UAAUUUCUACUAAGUGUAGAU CCUAGCGAAGGGCACGAGAC

AFB1-crRNA-26	UAAUUUCUACUAAGUGUAGAU GCCUAGCGAAGGGCACGAGA
AFB1-crRNA-27	UAAUUUCUACUAAGUGUAGAU GGCCUAGCGAAGGGCACGAG
AFB1-crRNA-28	UAAUUUCUACUAAGUGUAGAU GGGCCUAGCGAAGGGCACGA
AFB1-47 nt-aptamer	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCGCTAGGCCC
ssDNA	GGTTCCAAGGTTCCAATGGGGGAGTATTGCGGAGGAGGTTCCAAGGTTCCAA
dsDNA-nonPAM	TTGGAACCTTGGAACCTCCTCCGCAATACTCCCCCATTGGAACCTTGGAACC
Tri-complex-nonPAM	TTGGAACCTTGGAACC
PAM-down	GAAACCTTGGAACCTTGGTGGGGGAGTATTGCGGAGGAGAAACCTTGGAACCTTGG
dsDNA-PAM-UP	CCAAGGTTCCAAGGTTTCTCCTCCGCAATACTCCCCCACCAAGGTTCCAAGGTTTC
Tri-complex-PAM	CCAAGGTTCCAAGGTTTC
hairpin	GTACGTACGTAGGGCACGTGGGGGAGTATTGCGGAGGACGTGCCCTACGTACGTAC
crRNA-special structure DNA	UAAUUUCUACUAAGUGUAGAU UCCUCCGCAAUACUCCCCCA
F-Q probe	FAM-TTTTTT-BHQ1

Table S2. Comparison of sensitivities of different biosensor for AFB1.

No.	Signal output	Characteristic of the method	LOD	Working range	Detection time	Reference
	Visual	Lateral Flow Immunoassay based on multicolour gold nanoparticles	1 ng/mL	Not mentioned	Not mentioned	(Di Nardo et al. 2019)
2	Fluorescence	Aptamer-conjugated Quantum dots (QDs) based FRET	3.4 nM	10 to 400 nM	45 min	(Sabet et al. 2017)
3	Fluorescence	Structure-switching aptamer for enzyme-free amplification.	0.91 ng/mL	1 to 200 ng/mL	40 min	(Xia et al. 2019)
4	Raman signals	Surface enhanced Raman scattering (SERS) with self-assembly of gold nanobipyramids	0.5 ng/mL	1.5 ng/mL to 1.5 µg/mL	Several min	(Lin et al. 2020)
5	Fluorescence	Structure-switching aptamer for competition assay	1.6 ng/mL	5 to100 ng/mL	40 min	(Chen et al. 2017)
6	Temperature	Photothermal immunoassay (PTIA) based on the PTEs of plasmonic Cu2–xSe nanocrystals (NCs)	1 ng/mL	1 to 30 ng/mL	1-2 h	(Li et al. 2019)
7	Fluorescence	Dual-terminal stemmed aptamer beacon and aggregation-induced emission (AIE) effects	27.3 ng/mL	40–300 ng/mL	160 min	(Xia et al. 2018)
8	pH	Combination of AFB1-responsive aptamer-cross-linked hydrogel.	0.1 µM	0.2–20 µM	80 min	(Zhao et al. 2018)

9	Fluorescence	FRET, nanomaterial, Cas12a	0.2 nM	500 fM to 100 nM	1.5 h	(Cheng et al. 2021)
10	Fluorescence	SERS, nanomaterial, Cas12a	0.14	0.2 to 20,000 ng/mL	50 min	(He et al. 2023)
9	Fluorescence	Aptamer inhibition based on Cas12a	0.8 ng/mL (2.6 nM)	100 ng/ml to 2μg/ml	20 min	This work

Table S3. Recovery experiments of AFB1 spiked in 5% red wine, beer and milk.

Matrix	Added (ng/mL)	Found ((ng/mL)	Recovery (%)
5% red wine	500	472.33±35.41	94.47±7.08
5% red wine	250	229.00±12.36	91.6±4.94
5% beer	500	524.00±28.89	104.80±5.78
5% beer	250	275.00±12.83	110.00±5.13
5% milk	500	451.00±52.56	90.20±10.51
5% milk	250	219.67±6.80	87.87±2.72

Reference:

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