Supplementary Information (SI) for Analytical Methods.
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Supplementary Information

A label-free and universal CRISPR/Cas12a platform for the detection of hazardous substances in food

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Experimental section

Materials and reagents

The DNA and RNA oligonucleotides utilized in this investigation were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China), with their sequences detailed in Table S1. Mycotoxins, including aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), ochratoxin A (OTA), ochratoxin B (OTB), and zearalenone (ZEN), were obtained from Qingdao Prebon BioEngineering Co., Ltd. (Qingdao, China). These samples are all quality control samples and comply with the ISO/IEC 17025, ISO 17034, and ISO 9001 quality management system certifications. The pesticides acetamiprid (ACE), malathion (MAL), decamethrin (DEC), fenpropathrin (FEN), and imidacloprid (IMI) were supplied by Beijing Putian Tongchuang Biotechnology Co., Ltd. (Beijing, China). EnGen LbaCas12a (1.0 μM) and bovine serum albumin (BSA, 10 mg/mL) were procured from New England Biolabs Beijing Ltd. (Beijing, China). N-methyl mesoporphyrin IX (NMM) was purchased from J&K Scientific Ltd. (Beijing, China). Additionally, Acry/Bis 40% solution (19:1), tris(hydroxymethyl)aminomethane (Tris, ≥99.0%), and DEPC-treated water were sourced from Sangon Biotech Co., Ltd. (Shanghai, China). Streptavidin magnetic beads (MNB, 50 mg/mL, 0.5 μM), ammonium persulfate (APS, ≥98%), and N,N,N',N'-tetramethylethylenediamine (TEMED, ≥99.0%) were acquired from BBI Co., Ltd. (Shanghai, China). The 20 bp DNA ladder and 6× loading buffer were provided by TaKaRa Biotech Co., Ltd. (Beijing, China). SYBR™ Gold nucleic acid gel stain (10,000× concentration in DMSO) was obtained from Thermo Fisher Scientific Co., Ltd. (Shanghai, China). The magnetic frame (BeyoMag™) was purchased from Beyotime Biotechnology Ltd. (Shanghai, China). Laboratory-grade Triton™ X-100, ethylenediaminetetraacetic acid disodium dihydrate ($Na_2EDTA \cdot H_2O$, ≥99.0%), boric acid (H_3BO_3, ≥99.5%), sodium chloride (NaCl, ≥99.0%), potassium chloride (KCl, ≥99.0%), and hydrochloric acid (HCl, 1.2 g/mL at 25 °C) were supplied by Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). All buffer solutions employed throughout the experiments were prepared using DEPC-treated water.

Table S1 Oligonucleotide sequences used in this study

	Name	Sequence (5' → 3')
AFB1	Aptamer sequence	ACAC CCGG ATCG CTTC CCGT GCTC TGTG TCTC TCTG TTGT GCAC GGGT TG
	Trigger sequence	AAGC GATC CGGG TGTC ACAC TC
	H1	GAGC GTGA CACC CGGA TCGC TT TTTA GATC GTTA CGCT AACT ATGA AAGC GATC CGGG TGTC
	H2	ATCG CTTT CATA G TTAG CGTA ACGA TCTA AA AAGC GATC CGGG TGTC AC A GATC GTTA CGCT AACT ATGA
ACE	Aptamer sequence	TGTA ATTT GTCT GCAG CGGT TCTT GATC GCTG ACAC CATA TTAT GAAG A
	Trigger sequence	GTGT CAGC GATC AAGA ACCG CTGC
	H1	GCAG CGGT TCTT GATC GCTG ACTC TTTA GATC GTTA CGCT AACT ATGA GAGT CAGC GATC AAGA AC
	H2	GCTG ACTC TCAT AGTT AGCG TAAC GATC TAAA GAGT CAGC GATC AAGA ACCG G ATCG TTAC GCTA ACTA TGAG
CRISPR/ Cas12a	CrRNA	UAAUU UCUAC UAAGU GUAGA UGAUC GUUAC GCUAA CUAUG A
	G4 reporter	GGGT AGGG CGGG TTGG GTAA AAAA ACCC AACC C

Gel electrophoresis

Gel electrophoresis was performed utilizing a 15% nondenaturing polyacrylamide gel, which was prepared by combining 9.4 mL of 40% Acry/Bis solution (19:1 ratio), 5 mL of 5× TBE buffer (445 mM Tris, 10 mM EDTA, and 445 mM boric acid, pH 8.3), 10.6 mL of DEPC-treated water, 180 μL of 0.01% (w/v) APS solution, and 18 μL of N,N,N',N'-TEMED. This mixture was subsequently cast into an electrophoresis chamber (JY-CZ-BL, Beijing, China) and allowed to polymerize at ambient temperature for approximately four hours. Following polymerization, samples were combined with loading buffer at a volumetric ratio of 5:1 and applied to the gel. Electrophoresis was conducted for approximately 100 min at 15 °C under a constant current of 45 mA. Finally, the gel was stained with a 1× SYBR Gold solution for 40 mi and visualized using an ultraviolet (UV) imaging system (JY04S-3E, Junyi, Beijing, China).

Results and discussion

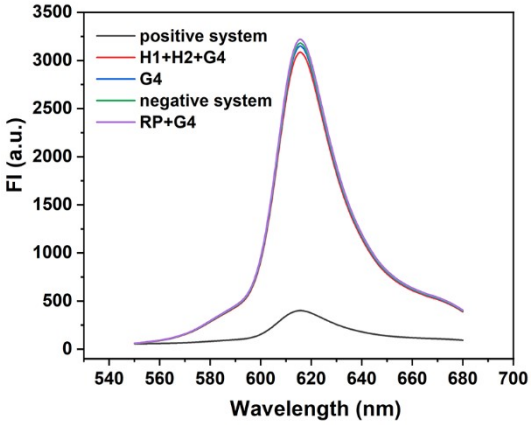

Fig. S1 Fluorescence spectra of the proposed method for AFB1 detection under different conditions.

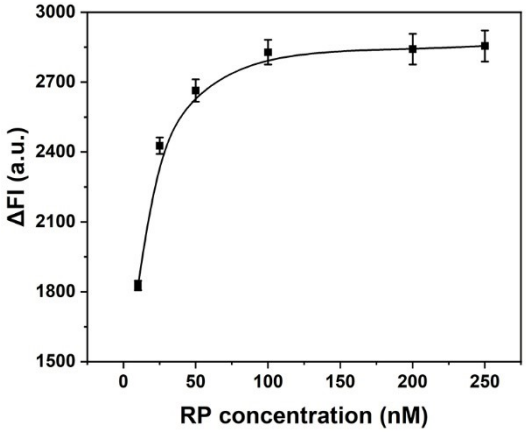

Fig. S2 Fluorescence responses concerning various concentrations of the RP.

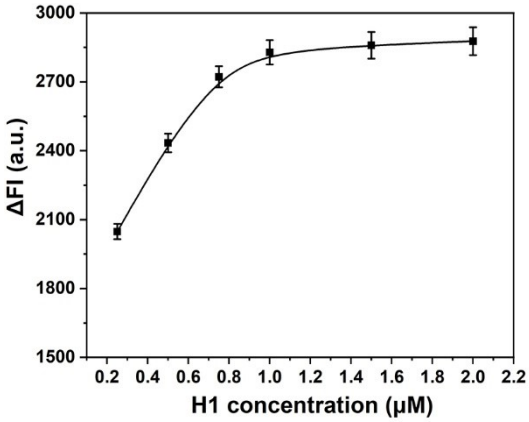

Fig. S3 Fluorescence responses concerning various concentrations of the H1.

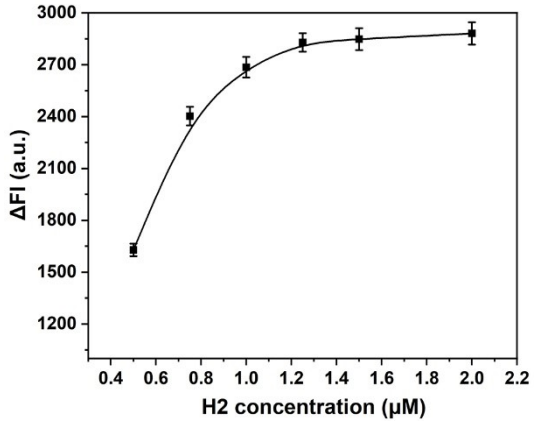

Fig. S4 Fluorescence responses concerning various concentrations of the H2.

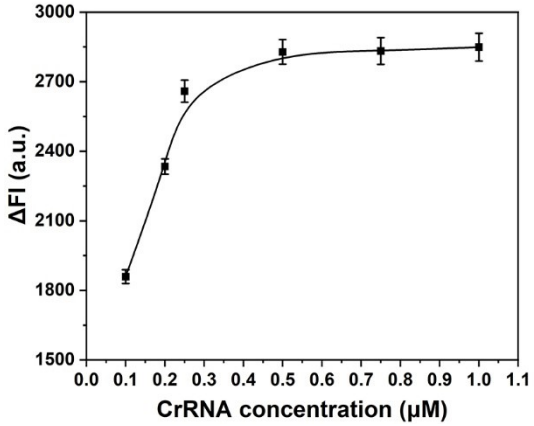

Fig. S5 Fluorescence responses concerning various concentrations of the crRNA.

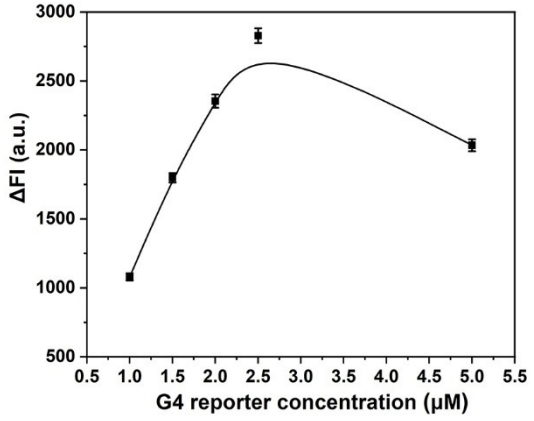

Fig. S6 Fluorescence responses concerning various concentrations of the G4 reporter.

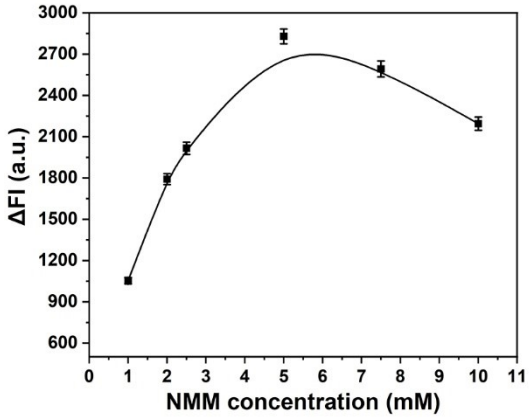

Fig. S7 Fluorescence responses concerning various concentrations of the NMM.

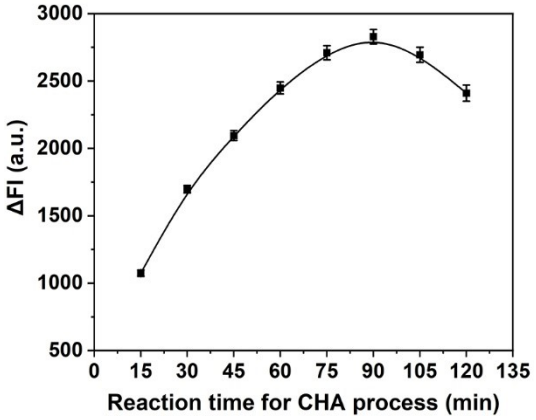

Fig. S8 Fluorescence responses concerning various reaction times for the CHA process.

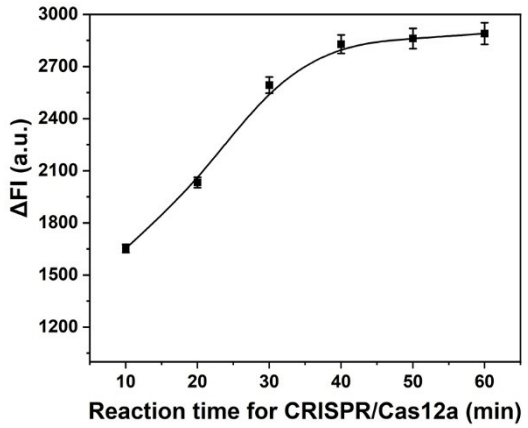

Fig. S9 Fluorescence responses concerning various reaction times for the CRISPR/Cas12a system.

Table S2 Comparison of the analytical performance for AFB1 detection

Mechanism	Linear range	LOD
Nb_2C MXene based aptasensor [1]	–	0.095 ng/mL
DNA tetrahedral scaffold-corbelled 3D DNAzyme walker [2]	1.0 fg/mL – 10 ng/mL	0.58 fg/mL
DNAzyme-driven tripedal DNA Walker [3]	0.1 pg/mL – 10 ng/mL	61 fg/mL
Aptamer probe-assisted SDA-CRISPR/Cas12a biosensor [4]	0.01 – 100 ng/mL	3.6 pg/mL
High-affinity and selectivity AFB1 circular aptamer-based biosensor [5]	–	265 pM
The proposed method	10 fg/mL – 5.0 ng/mL	3.0 fg/mL

Table S3 Results of the repeatability experiment for AFB1 detection

Concentration	ΔFI				RSD%
	Sample 1	Sample 2	Sample 3	Average	
1.0 ng/mL	2828	2764	2885	2825.7	2.14
10 pg/mL	1861	1845	1903	1869.7	1.60
0.1 pg/mL	1004	1039	1011	1018	1.82

Table S4 Results of the reproducibility experiment for AFB1 detection

Concentration	ΔFI				RSD%
	Sample 1	Sample 2	Sample 3	Average	
1.0 ng/mL	2828	2851	2732	2803.7	2.25
10 pg/mL	1861	1941	1834	1878.7	2.96
0.1 pg/mL	1004	1057	995	1018.7	3.29

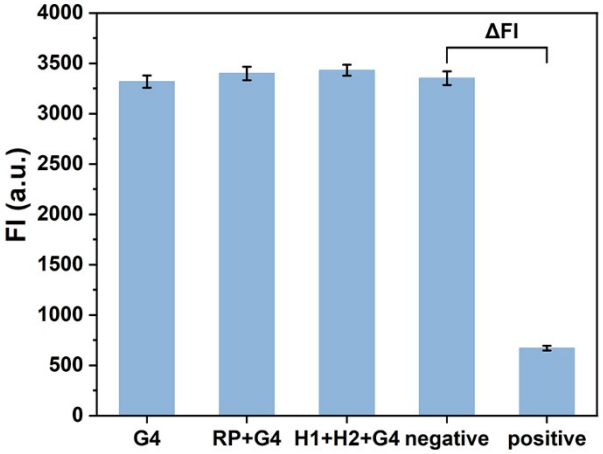

Fig. S10 Fluorescence responses for the detection of ACE under various conditions.

Table S5 Results of the repeatability experiment for ACE detection

Concentration	ΔFI				RSD%
	Sample 1	Sample 2	Sample 3	Average	
1.0 ng/mL	2673	2741	2648	2687.3	1.79
10 pg/mL	1447	1478	1521	1482	2.51
0.1 pg/mL	551.9	526.1	511.9	530.0	3.83

Table S6 Results of the reproducibility experiment for ACE detection

Concentration	ΔFI				RSD%
	Sample 1	Sample 2	Sample 3	Average	
1.0 ng/mL	2673	2655	2762	2696.7	2.12
10 pg/mL	1447	1463	1551	1487	3.77
0.1 pg/mL	551.9	523.1	504.6	526.5	4.53

The limits of detection (LODs) in the proposed method were determined based on the triple standard deviation (3σ) criterion. Taking AFB1 as an example, the relationship $\Delta FI = 3\sigma$ was employed. Using the regression equation $\Delta FI = 1378.7 + 458.2 \lg C$ and the standard deviation value $\sigma = 75.8546$, the concentration (C) corresponding to the LOD was calculated to be 3.0 pg/mL for AFB1. Similarly, for ACE, the equation $\Delta FI = 1042.1 + 518.6 \log C = 3\sigma$ was used, with $\sigma = 68.6216$, allowing for the calculation of the LOD for ACE.

Table S7 Detected ACE in the spiked agricultural products using the proposed method and HPLC

Sample	Spiked	This method		HPLC	
		Detected	Recovery (%)	Detected	Recovery (%)
Milk	0	– [a]	–	–	–
	0.1 pg/mL	0.0982 pg/mL	98.3	–	–
	10 pg/mL	9.91 pg/mL	99.1	–	–
	1.0 ng/mL	1.002 ng/mL	100.2	0.969 ng/mL	96.9
Wheat flour	0	–	–	–	–
	0.1 pg/mL	0.1011 pg/mL	101.1	–	–
	10 pg/mL	9.84 pg/mL	98.4	–	–
	1.0 ng/mL	1.012 ng/mL	101.2	0.981 ng/mL	98.1
Corn flour	0	–	–	–	–
	0.1 pg/mL	0.1035 pg/mL	103.5	–	–
	10 pg/mL	9.49 pg/mL	94.9	–	–
	1.0 ng/mL	0.996 ng/mL	99.6	0.944 ng/mL	94.4

[a] "–" means not detected.

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