Supplementary Information

CRISPR/Cas12a-based competitive aptasensor for ochratoxin A detection

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Detection of AFB1 spiked in the complex samples

We detected AFB1 spiked in 50-fold diluted liqueur samples and 20-fold diluted milk samples. First, the liqueur and milk were treated separately with a 0.22 μm filter. Then, we obtained the OTA-spiked samples by adding various concentrations of OTA to the binding buffer containing 2% liqueur or 5% milk. Finally, the prepared samples were analyzed according to the procedure described in the section "OTA detection".

Gel eletrophoresis

The tested samples were prepared from the assay buffer. The concentrations of acDNA, biotinylated acDNA, and SA were 10 nM, and the Cas12a reporting system consisted of 12 nM Cas12a, 6 nM crRNA, and 1 μM FAM-ssDNA reporters. We obtained the SA-acDNA complex by assembling biotinylated acDNA and SA at room temperature for 20 min. The trans-cleavage reaction of Cas12a was performed at room temperature for 120 min. Then, the products were transferred to the wells of a 15% polyacrylamide gel, which was run in 0.5× TBE buffer at 100 V for 60 min. Finally, the gel was visualized with a gel imaging system (Bio-Rad, USA)

Table S1 Sequences of oligonucleotides used in this work.

Oligo name	**Sequence (5'-3')**
CRISPR RNA (crRNA)	UAA UUU CUA CUA AGU GUA GAU AAG GUU UGU GUG UUU ACC UG
F-Q reporter	[FAM]-TTT TTT TTT T-[BHQ1]
3'Bio-aptamer (biotinylated aptamer)	GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-[Biotin]
5'Bio-T15-acDNA (biotinylated acDNA)	[Biotin]-TTT TTT TTT TTT TTT **CCCAGGTAAACACACAAACCTT**
C7-O36-3'BioTEG (C7)	CCC GAT C-[Biotin]
C9-O36-3'BioTEG (C9)	CA CCC GAT C-[Biotin]
C11-O36-3'BioTEG (C11)	C ACA CCC GAT C-[Biotin]
C13-O36-3'BioTEG (C13)	CCC ACA CCC GAT C-[Biotin]
C15-O36-3'BioTEG (C15)	CA CCC ACA CCC GAT C-[Biotin]
C17-O36-3'BioTEG (C17)	G CCA CCC ACA CCC GAT C-[Biotin]
FAM-ssDNA reporter	[FAM]-CCC ATC TTT TTT TTT TTT TTT GAT GGG

The acDNA sequence is shown in bold format.

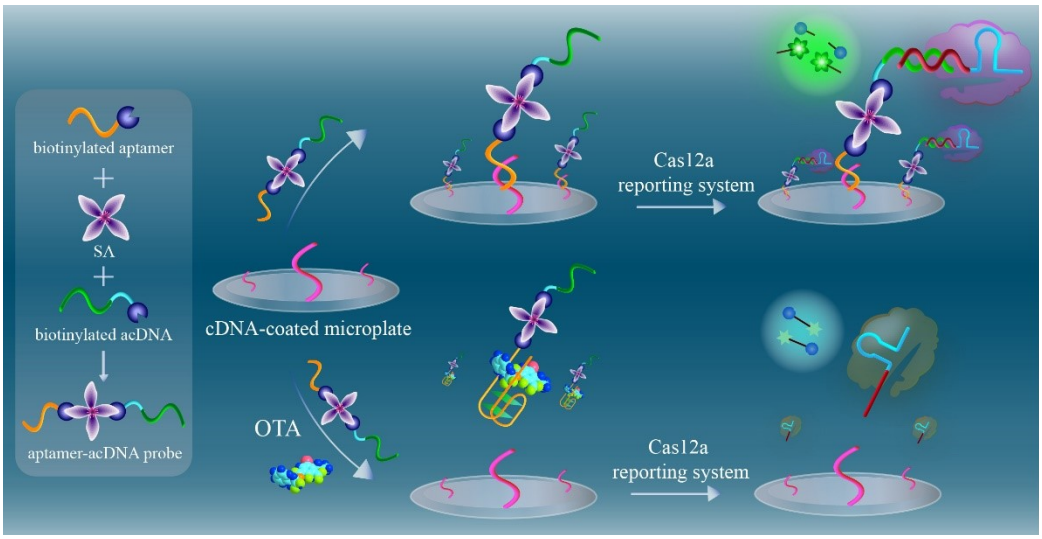

Fig. S1 Working principle of the CRISPR/Cas12a-based competitive aptasensor for OTA detection (one-step signal conversion mode).

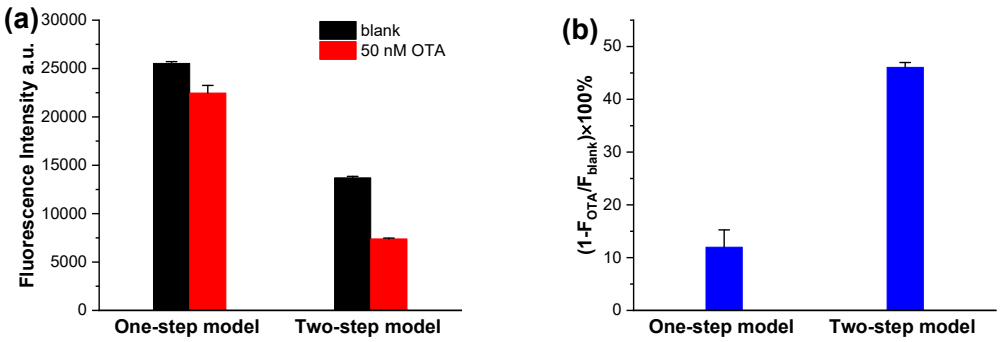

Fig. S2 Comparison of the developed methods for OTA detection using one-step signal conversion mode and two-step signal conversion mode. The fluorescence signal of samples was obtained from the 60 min reaction. The binding buffer consists of 10 mM Tris-HCl (pH 7.5), 20 mM $CaCl_2$, 120 mM NaCl, and 0.1% Tween 20.

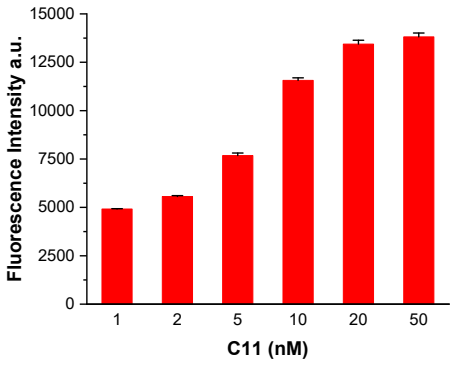

Fig. S3 Optimization of C11 concentrations for microplate coating. The fluorescence signal of samples was obtained from the 60 min reaction. The binding buffer consists of 10 mM Tris-HCl (pH 7.5), 20 mM $CaCl_2$, 120 mM NaCl, and 0.1% Tween 20.

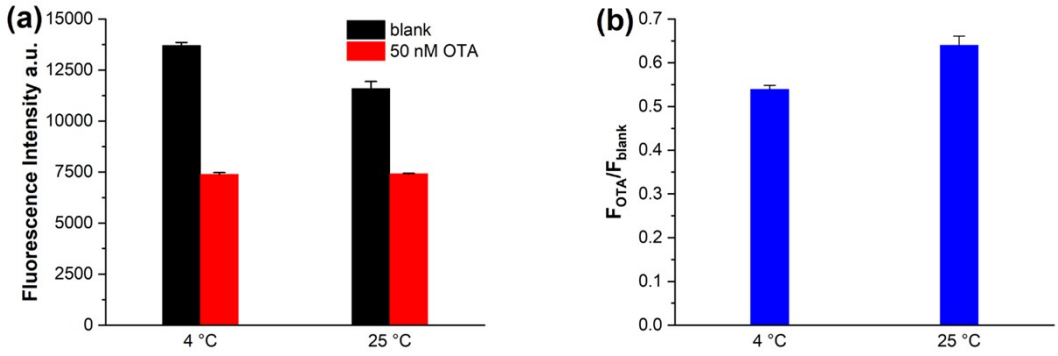

Fig. S4 Optimization of competitive reaction temperature. The fluorescence signal of samples was obtained from the 60 min reaction. The binding buffer consists of 10 mM Tris-HCl (pH 7.5), 20 mM $CaCl_2$, 120 mM NaCl, and 0.1% Tween 20.

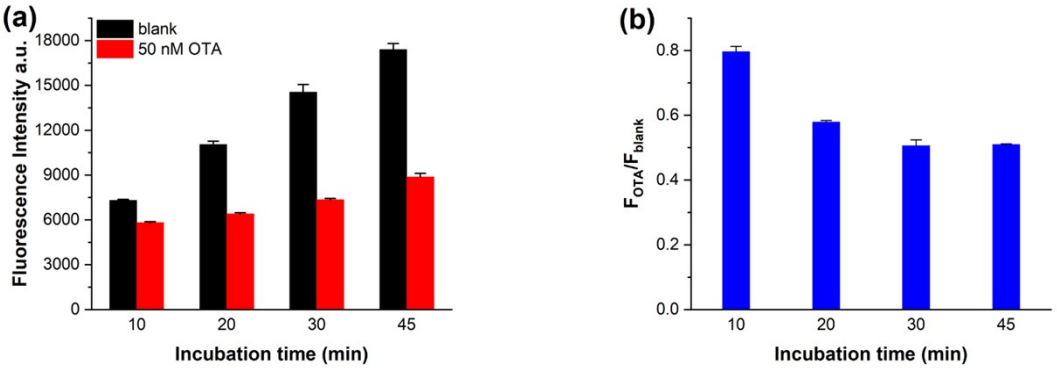

Fig. S5 Optimization of incubation time for competitive reactions. The fluorescence signal of samples was obtained from the 60 min reaction. The binding buffer consists of 10 mM Tris-HCl (pH 7.5), 20 mM $CaCl_2$, 120 mM NaCl, and 0.1% Tween 20.

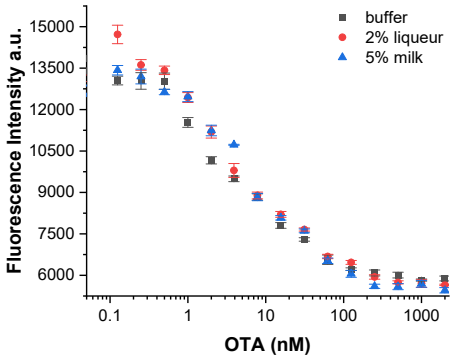

Fig. S6 Detection of OTA spiked in the real sample matrix containing 2% liqueur or 5% milk in comparison with the result of OTA detection in the binding buffer. The fluorescence signal of samples was obtained from the 60 min reaction. The binding buffer consists of 10 mM Tris-HCl (pH 7.5), 20 mM $CaCl_2$, 120 mM NaCl, and 0.1% Tween 20.

Table S2 Comparison of some recently reported aptasensors for OTA.

Method	Assay time	Linear range	LOD	Ref.
A fluorescent aptasensor based on multisite strand displacement amplification	195 min	1.2 nM-1.2 uM	0.44 nM	1
A label-free electrochemical aptasensor	140 min	0.12 nM-24.7 nM	0.12 nM	2
Colorimetric aptasensor based on enzyme-induced gold nanoparticle aggregation	160 min	6.25 nM-750 nM	5 nM	3
A highly sensitive and widely adaptable plasmonic aptasensor using berberine	60 min	1 nM-1 μM	0.56 pM	4
Cascade strand displacement reaction-assisted aptamer-based assay	230 min	2.4 nM-2.4 uM	1.5 nM	5
A label-free aptasensor based on SYBR gold and single walled carbon nanohorns	95 min	12.3 nM-1.2 uM	5.7 nM	6
Multicolor colorimetric aptasensor with enzyme-induced metallization of gold nanorods	70 min	12.5 nM-20 μM	9 nM	7
Upconversion-mediated CRISPR-Cas12a biosensor	55 min	12.4 nM-248 nM	2.1 nM	8
A electrochemical aptasensor based on peroxidase-like graphitic carbon nitride nanosheet	75 min	0.2 nM-500 nM	73 pM	9
CRISPR/Cas12a-based competitive aptasensor	130 min	0.5 nM-62.5 nM	0.5 nM	This work

1 D. Zhang, X. Cai, Q. Zhang and C. Zhang, *Chem. Commun.*, 2023, **59**, 13155-13158.

2 Y. Hou, N. Long, B. Jia, X. Liao, M. Yang, L. Fu, L. Zhou, P. Sheng and W. Kong, *Food Control*, 2022, **135**, 108833.

3 Y. He, F. Tian, J. Zhou, Q. Zhao, R. Fu and B. Jiao, *J. Hazard. Mater.*, 2020, **388**, 121758.

4 J.-H. Park, J.-Y. Byun, H. Jang, D. Hong and M.-G. Kim, *Biosens. Bioelectron.*, 2017, **97**, 292-298.

5 B. Han, C. Fang, L. Sha, M. Jalalah, M. S. Al-Assiri, F. A. Harraz and Y. Cao, *Food Chem.*, 2021, **338**, 127827.

6 Z. Guo, J. Tian, C. Cui, Y. Wang, H. Yang, M. Yuan and H. Yu, *Food Control*, 2021, **123**, 107741.

7 F. Tian, J. Zhou, R. Fu, Y. Cui, Q. Zhao, B. Jiao and Y. He, *Food Chem.*, 2020, **320**, 126607.

8 Z. Mao, X. Wang, R. Chen, Z. Zhou, S. Ren, J. Liang and Z. Gao, *Talanta*, 2022, **242**, 123232.

9 X. Zhu, F. Kou, H. Xu, Y. Han, G. Yang, X. Huang, W. Chen, Y. Chi and Z. Lin, *Sensor. Actuat. B-Chem.*, 2018, **270**, 263-269.