Electronic Supplementary Information

Programmability of dual-color DNA-templated silver nanoclusters for modular design of FRET aptasensors toward multiplexed detection

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Materials. $AgNO_3$ and other chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). We obtained AFB1 and OTA from Sigma-Aldrich (USA). The DNA oligonucleotides sequences names as DNA1 and DNA2 (Table 1) were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). The aptamers possessing sequences against AFB1 (Apt1), OTA (Apt2), prostate-specific antigen (Apt3), carcinoembryonic antigen (Apt 4) (Table S1) were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Aflatoxin B1 (AFB1), fumonisin B1 (FB1), zearalenone (ZEN), glucose, bovine serum albumin (BSA), vitamin B2, KCl, $CaCl_2$, and $MgSO_4$ were obtained from Aladdin reagent Co., Ltd. Ochratoxin A (OTA) and deoxynivalenol (DON) were purchased from Cato Research Chemicals Inc. Aflatoxin B2 (AFB2) was obtained from Sigma-Aldrich.10 mM PBS (pH 7.4) and Tris-HCl buffer (pH 7.4, containing 50 mM Tris-HCl and 1.0 mM EDTA) were used for aptasensor fabrication and detection procedure. We used deionized and ultrapure water to conduct all experiments.

Instruments. There are various kind of characterization tools available that were used as evidence for the completion of reactions and to check property of their AgNCs-DNA. UV-vis absorption spectra were studied using a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were recorded using a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan). Transmission electron microscopy (TEM, JEOL 2100, Japan) was used for morphology analysis of the specimens. X-ray photoelectron spectroscopy (XPS) was taken on a multi-technique surface analysis system (Thermo Electron Co., USA).

Preparation of MoS_2 nanosheets. A modified liquid exfoliation method with a little modification were used to prepare MoS_2 nanosheets.[1] Firstly, MoS_2 powder (300 mg) was added to a mixture of ethanol and water (45% v/v) in a flask (250 mL), which was sonicated for 24 h to produce a dark green suspension. We collected the supernatant after centrifuging at 6000 rpm for 20 min. The collected supernatant was further centrifugated at 12000 rpm for 30 min and the precipitation was baked at 70 °C in oven. Finally, the as-prepared MoS_2 nanosheets were dispersed in Tris-HCl buffer (10 mM, pH 7.4) for further use.

Design of DNA templates for AgNCs synthesis. Two DNA strands, designated as DNA1 and DNA2, were designed to incorporate a template region at the 5' terminus for AgNCs-DNA synthesis, along with a hybridization region tailored at the 3' terminus to facilitate aptamer capture (Table S1). This hybridization segment comprises five guanine sequences (-GGGGG, G5)

specifically designed to promote aptamer binding. In parallel, Apt1 or Apt2 were designed to integrate with the AgNCs-DNA signal tags through five cytosine sequences (-CCCCC, C5) positioned at their 3' ends (Table S1). This design ensured the efficient synthesis of AgNCs-DNA probes and their subsequent hybridization with the respective aptamers.

Synthesis of AgNCs-DNA1 and AgNCs-DNA2. Initially, a 100 μM DNA1 solution was centrifuged at 10,000 rpm for 5 min and then diluted to 75 μL using PBS buffer (10 mM, pH 7.4). This solution was heated in a water bath at 90 °C for 1 h and subsequently cooled to room temperature. Afterwards, 10 μL of 1.5 mM $AgNO_3$ and 30 μL of PBS buffer (10 mM, pH 7.4) were added to 100 μL of the pretreated DNA1 solution, followed by vigorously stirring at room temperature for 1 h. Thereafter, 35 μL of freshly prepared 1.5 mM $NaBH_4$ was added, followed by incubation in the dark at 4.0 °C for 16 h to yield AgNCs-DNA1. A similar protocol was adhered to for the synthesis of the AgNCs-DNA2 probe, with the sole exception of replacing DNA1 with DNA2.

Conjugation of AgNCs-DNA with aptamer. To conjugate AgNCs-DNA1 with Apt1, resulting in AgNCs-DNA1/Apt1, we combined 100 μL of Apt1 with 100 μL of the obtained AgNCs-DNA1 solution. Following this, 300 μL of TE buffer (consisting of 50 mM Tris-HCl and 1.0 mM EDTA at pH 7.4) was added, bringing the total volume to 500 μL. This mixture was then shaken in the dark at room temperature for 2 h to promote hybridization between the G5 and C5 sequences. The resulting AgNCs-DNA1/Apt1 probes were subsequently stored at 4 °C for later use. Analogously, AgNCs-DNA2/Apt2 probes were prepared using the same methodology, substituting AgNCs-DNA2 and Apt2 as the fluorescence indicator and aptamer, respectively.

Construction of the FRET aptasensor and detection procedure. Initially, 200 μL of AgNCs-DNA1/Apt1 and 200 μL of AgNCs-DNA2/Apt2 were combined and vigorously shaken for 5 min at room temperature. Subsequently, 300 μL of MoS_2 nanosheets solution at 1.5 mg mL^{-1} was introduced to quench the fluorescence of the AgNCs-DNA/Apt. After shaking for 7 min, the adsorption between the MoS_2 nanosheets and the AgNCs-DNA/Apt probes led to a significant reduction in the fluorescence intensity of the probes. The resulting MoS_2@AgNCs-DNA/Apt was then diluted with TE buffer (pH 7.4) to a total volume of 800 μL, forming the aptasensor. To detect the presence of AFB1 and OTA, 100 μL aliquots of mycotoxin solutions, varying in concentration, were introduced into 400 μL of the MoS_2@AgNCs-DNA/Apt suspension. This mixture was then

incubated at 37 °C for 50 min while being agitated. After the incubation period, the fluorescence spectrum of the solution was recorded using an excitation wavelength of 310 nm.

Repeatability, long-term stability, and practicality of the aptasensor. The aptasensor's repeatability was examined by conducting six consecutive fluorescent measurements by incubation it with AFB1 and OTA at a concentration of 1 ng mL^{-1}. These parallel assessments yielded a low relative standard deviation (RSD) of 4.5%, demonstrating the aptasensor possessed good repeatability. Moreover, its long-term stability was further investigated by monitoring its fluorescence response upon weekly incubation with AFB1 and OTA (1 ng mL^{-1} each). The experimental data revealed that, even after three weeks, the aptasensor retained 95.4% of its initial response for 1 ng mL^{-1} AFB1 and 92.5% for 1 ng mL^{-1} OTA, thereby exhibiting satisfactory long-term stability. To further investigate the potential practicality of the developed method, extensive testing was performed to evaluate the recovery percentages of AFB1 and OTA in spiked peanut samples. These samples were intentionally spiked with different concentrations of mycotoxins (0.5, 1, 5 ng mL^{-1}). The recovery rates for AFB1 in the peanut samples ranged from 97.8% to 100.6% (RSD < 9.8%), while the recovery rates for OTA in peanut samples ranged from 97.4% to 106.7% (RSD < 8.2%). A detailed overview of these results is available in Table S3. These findings strongly indicated the suitability of the developed assay for the quantitative determination of AFB1 and OTA.

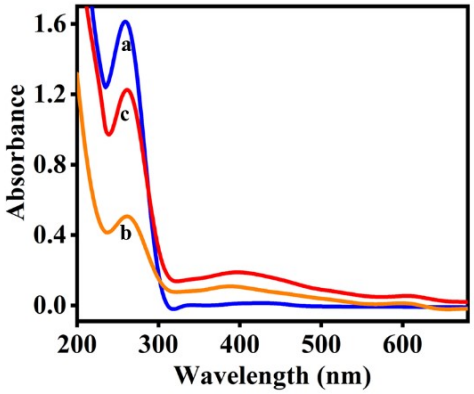

Fig. S1 UV-vis absorption spectra of (a) free DNA2, (b) AgNCs-DNA2, and (c) AgNCs-DNA2/Apt2.

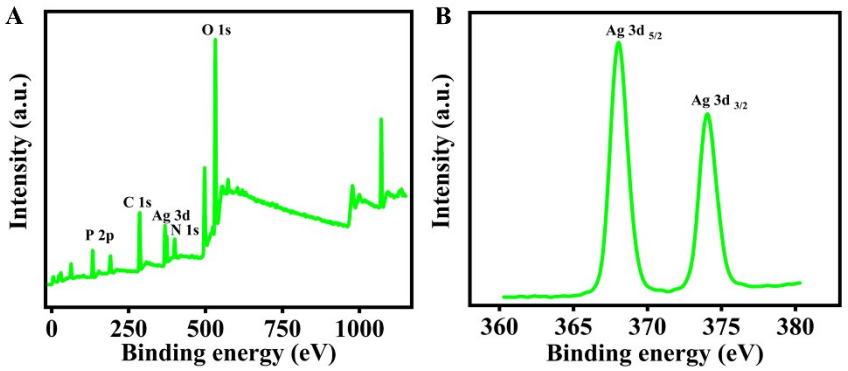

Fig. S2 (A) XPS wide scan survey spectrum of AgNCs-DNA2. (B) High-resolution XPS spectrum of Ag $3d_{5/2}$ and Ag $3d_{3/2}$ of AgNCs-DNA2.

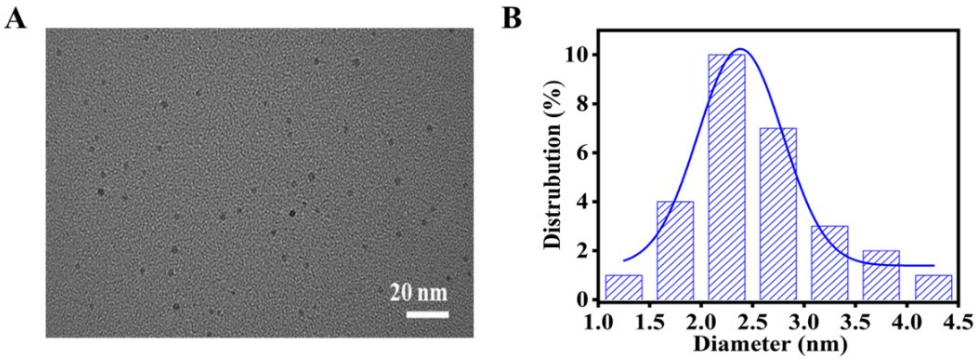

Fig. S3 (A) TEM image and (B) particle size distribution histogram of AgNCs-DNA2.

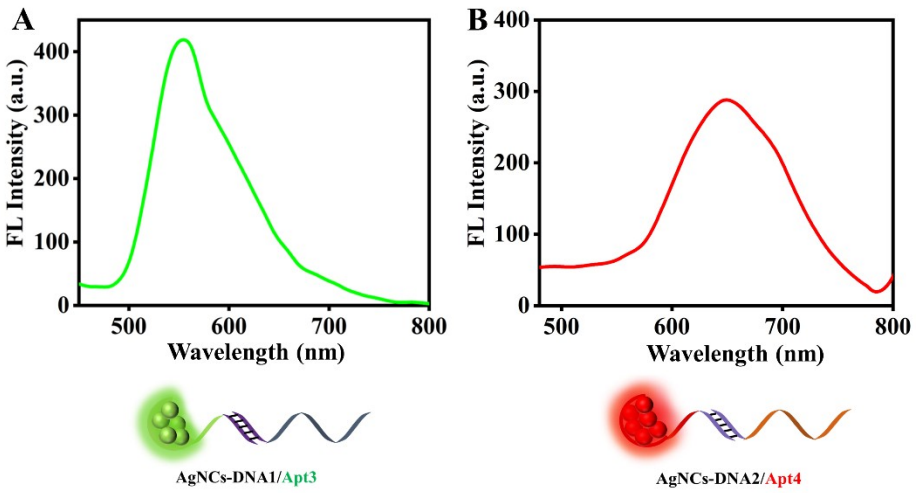

Fig. S4 The fluorescence spectra of (A) AgNCs-DNA1/Apt3 and (B) AgNCs-DNA1/Apt4.

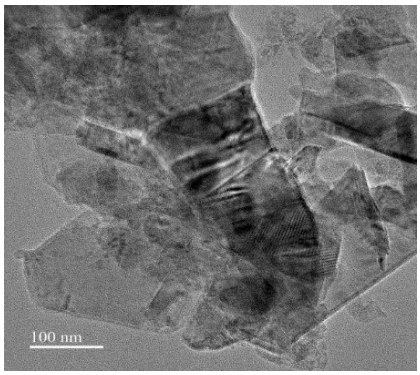

Fig. S5 TEM image of MoS_2 nanosheets.

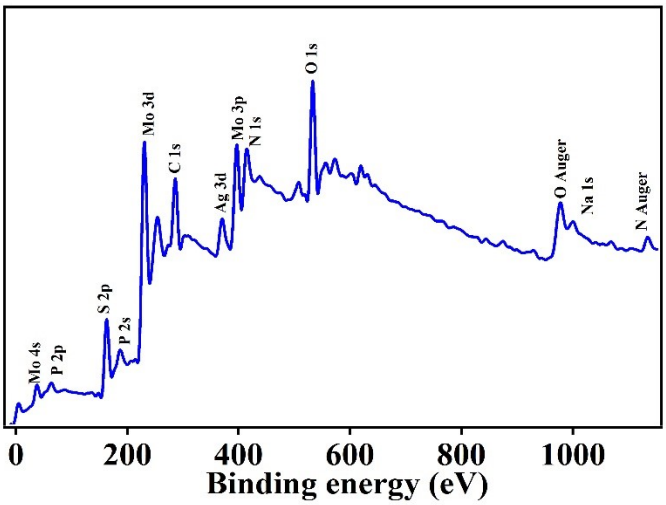

Fig. S6 The wide scan XPS of MoS_2@AgNCs-DNA/Apt bioconjugates.

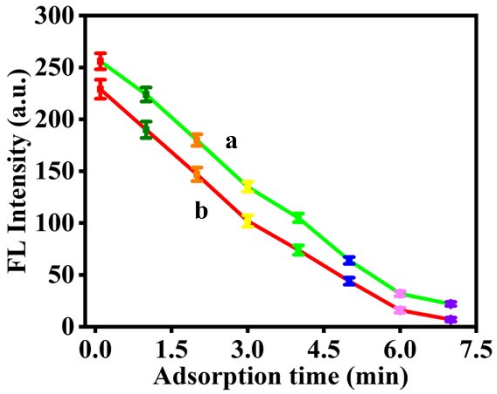

Fig. S7 Effect of the adsorption time between (a) AgNCs-DNA1/Apt1 or (b) AgNCs-DNA2/Apt2 and MoS_2 nanosheets.

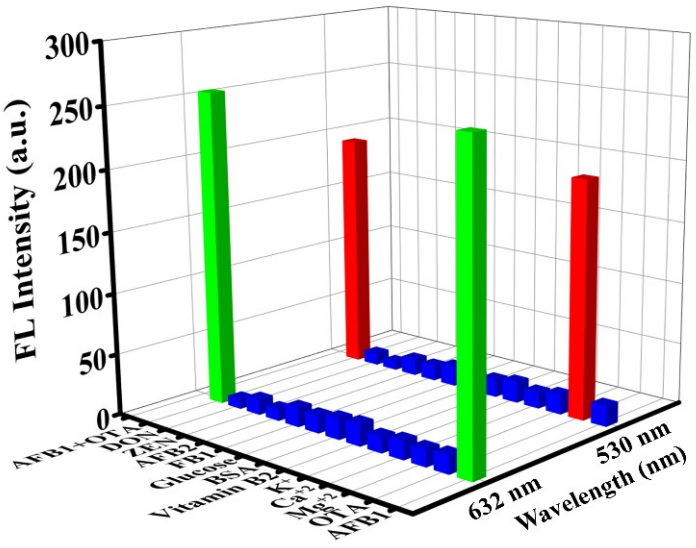

Fig. S8 Selectivity of the aptasensor toward the detection of AFB1 and OTA with other interfering substances at a fixed concentration of 100 ng mL^{-1}.

Table S1 The information of DNA sequences used in this work.

No.	Sequence Information (from 5' to 3')	References
DNA1	TAT ATG TCC CCC CCC CAT ATA TA *GGGGG*	2
DNA2	ATATAGGCTACCCCCCCCCCCCTAGCCTGTGC *GGGGG*	3
Apt1	GTT GGG CAC GTG TTGTCT CTC TGT GTC TCG TGC CCT TCG CTA GG CCC *CCCCC*	4
Apt2	GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA *CCCCC*	5
Apt3	ATT AAA GCT CGC CAT CAA ATA*CCCCC*	6
Apt4	ATACCAGCTTATTCAATT*CCCCC*	7

Table S2 Comparison between our method and other fluorescence aptasensors for AFB1/OTA detection.

Signal probe	Linear range (ng mL^{-1})		LOD (pg mL^{-1})		References
	AFB1	OTA	AFB1	OTA	
AgNCs-DNA	−	0.01−0.30	−	2	5
Au nanorods-DNA	−	0.01−10	−	5	8
AgNCs-DNA	0.001−0.05	0.001−0.05	0.3	0.2	9
AuNCs-DNA	0.005−100	−	3.4	−	4
Carbon dots/CdZnTe quantum dots	0.01–10	0.02–5.0	3.3	7.1	10
AgNCs-DNA	0.1−120	0.1-100	1.2	3.1	This work

Table S3 Simultaneous detection of AFB1 and OTA in spiked peanut samples (n = 3).

Samples	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	RSD (%)
AFB_1	0.50	0.503	100.6	5.2
	1.0	0.997	99.7	9.7
	5.0	4.890	97.8	9.8
OTA	0.5	0.487	97.4	8.2
	1.0	1.067	106.7	6.8
	5.0	4.934	98.68	7.8

Notes and references

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