Electronic Supplementary Information

Ultrasensitive biosensing platform based on luminescence quenching ability of fullerenols quantum Dots

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Experimental section

Reagents. All solvents and reagents were used as received without further purification. 5'-FAM labeled probe DNA (5'-FAM-AGTCAGTGTGGAAAATCTCTAGC-3'), target DNA (5'-GCTAGAGATTTTCCACACTGACT-3'), single-base mismatched target DNA (5'-GCTAGAGATTGTCCACACTGACT-3'), double-base mismatched target DNA (5'-GCTAGAGATTGTCCACGCTGACT-3'), 3'-TAMRA labeled OTA aptamer (5'-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-TAMRA-3') were supplied by Sangon Biotechnology Co., Ltd (Shanghai, China). Standard solutions of ochratoxin A (OTA), aflatoxin B1 (AFB1), fumonisin B1 (FB1), zearalenone (ZEN) and deoxynivalenol (DON) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other reagents were all from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The solvents and reagents were used as received without further purification. All aqueous solutions were prepared in ultrapure water obtained from a Milli-Q purification system (purified by Milli-Q biocel from Millipore China Ltd) (Millipore, Kankakee, IL, USA).

Instruments. The size and morphology of FOQDs were characterized by a HITACHI H-7000FA transmission electron microscope with an acceleration voltage of 100 kV. FT-IR spectrum of FOQDs was measured on a Nicolet iS10 FT-IR Spectrometer (Thermo Scientific, USA) with the KBr pellet technique. The UV-vis absorption measurements were conducted on a UV-2550 UV-vis spectrometer (Shimadzu, Japan). The fluorescence spectra were recorded on a RF5301 fluorescence spectrometer (Shimadzu, Japan).

Detection of target ssDNA. In order to select an appropriate concentration of FOQDs to do the following fluorescence recovery experiments, a fixed amount of 5'-FAM labeled probe ssDNA (20 nM) was incubated with increasing amounts of FOQDs in Tris-HCl buffer (10 mM, 5 mM $MgCl_2$, pH 7.4,) at room temperature (25 °C) for 1 h, and then fluorescence measurements were carried out. In a typical assay process, various concentrations of target ssDNA (0, 0.05 nM, 0.2 nM, 1 nM, 5 nM, 10 nM, 20 nM, 50 nM, 80 nM, 100 nM, 200 nM) were first mixed with 5'-FAM labeled probe ssDNA and the mixture was incubated at room temperature for 2 h. Afterwards a same amount of 0.050 mg/mL FOQDs was added individually into the above mixtures followed by incubation for another 1 h at room temperature. Finally, the fluorescence intensity the reaction mixture was recorded under the excitation of 480 nm. To examine the specificity of this DNA detection assay, single-base mismatched and double-base mismatched target DNA with a same concentration of 20 nM were added into the FAM labeled probe DNA-FOQDs system respectively in place of target DNA following the same experimental procedures.

Detection of OTA in Tris-HCl buffer. 40 nM TAMRA-labeled OTA aptamer was mixed with different amounts of FOQDs in Tris-HCl buffer (10 mM, 5 mM KCl, 5 mM $CaCl_2$, pH 8.5) and the mixtures were gently shaken at room temperature for 1 h, and then fluorescence measurements were carried out. In order to do the fluorescence recovery experiments, different concentrations of OTA (0, 0.01 ng/mL, 0.05 ng/mL, 0.1 ng/mL, 0.5 ng/mL, 0.8 ng/mL, 1ng/mL) were first added into 40 nM TAMRA-labeled OTA aptamer in 10 mM Tris-HCl buffer (pH 8.5, 5 mM KCl, 5 mM $CaCl_2$) and

incubated at room temperature for 2 h. Afterwards, 6.7 μg/mL of FOQDs was added individually into the above mixtures followed by incubation for another 1 h at room temperature. Finally, the fluorescence intensity of the reaction mixture was recorded under the excitation of 550 nm.

Detection of OTA in grape juice. In order to determine the applicability of this OTA biosensor in grape juice, grape juice sample 100-fold diluted with 10 mM Tris-HCl buffer (pH 8.5, 5 mM KCl, 5 mM $CaCl_2$) was used as the detection medium and the detection procedure was the same as that in the aqueous buffer solution. And standard addition method was also applied to detect the concentration of OTA in practical grape juice samples.

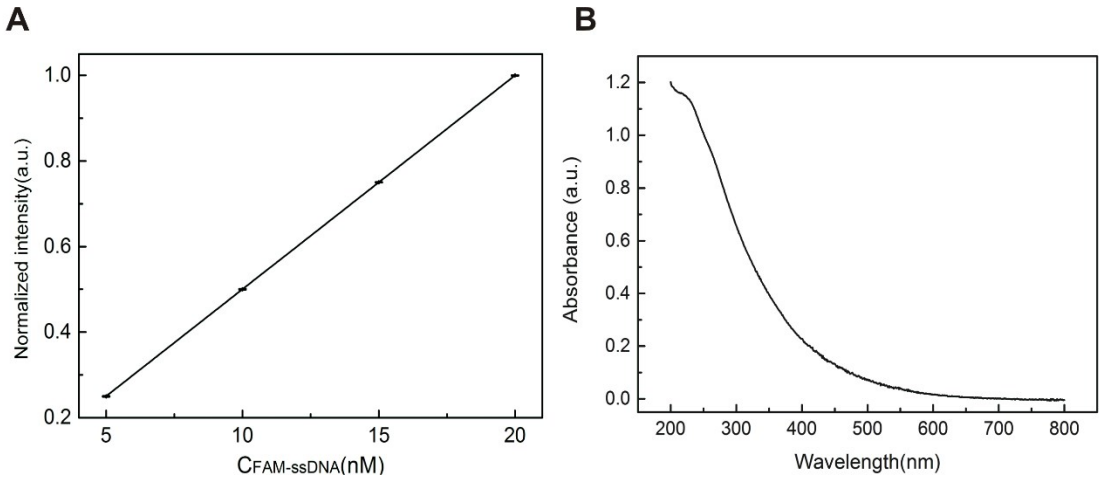

Fig. S1. (A) Concentration dependent fluorescence response of FAM-ssDNA. (B) The UV-vis absorption spectra of FOQDs.

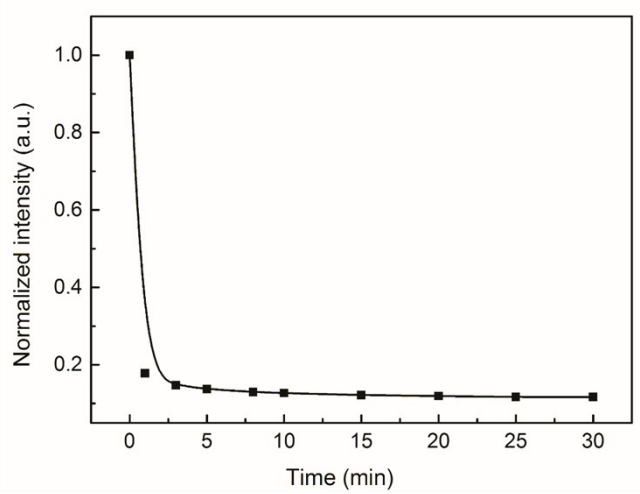

Fig. S2. Time dependence of the luminescence quenching efficiency in the presence of 20 nM 5'-FAM-labeled probe ssDNA and 0.050 mg/mL FOQDs. All experiments were performed in Tris-HCl buffer (10 mM, 5 mM $MgCl_2$, pH 7.4) under excitation at 480 nm.

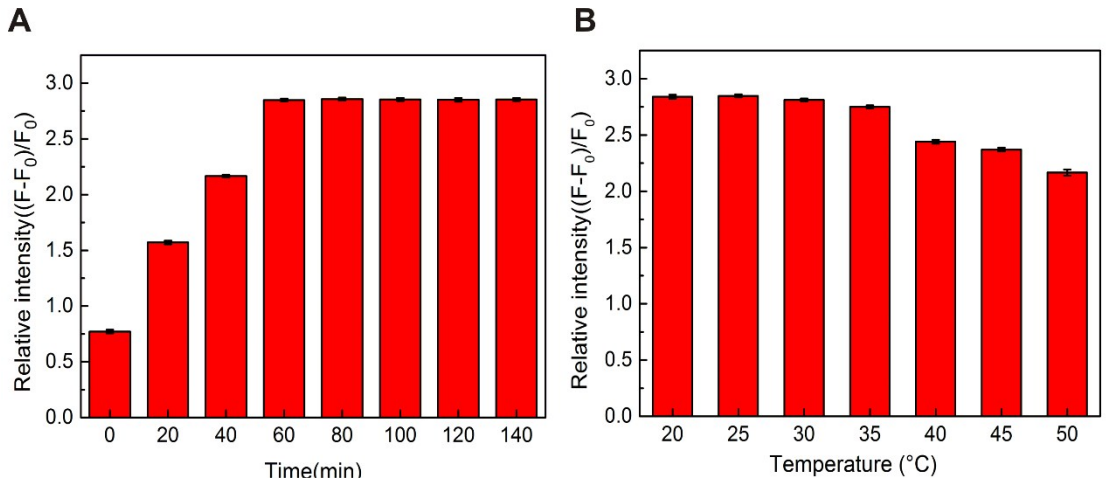

Fig. S3. (A) Effect of incubation time for FAM-ssDNA and target ssDNA on fluorescence recovery of FAM-ssDNA. Experiments were conducted in the presence of 20 nM FAM-ssDNA, 10 nM target ssDNA and 0.05 mg/mL FOQDs in Tris-HCl buffer (10 mM, 5 mM $MgCl_2$, pH 7.4) under excitation at 480 nm at room temperature. The time for fluorescence quenching was 1h. (B) Effect of incubation temperature on

fluorescence recovery of FAM-ssDNA. Experiments were conducted in the presence of 20 nM FAM-ssDNA, 10 nM target ssDNA and 0.05 mg/mL FOQDs in Tris-HCl buffer (10 mM, 5 mM $MgCl_2$, pH 7.4) under excitation at 480 nm. The incubation time for fluorescence recovery and quenching was 2h and 1h, respectively.

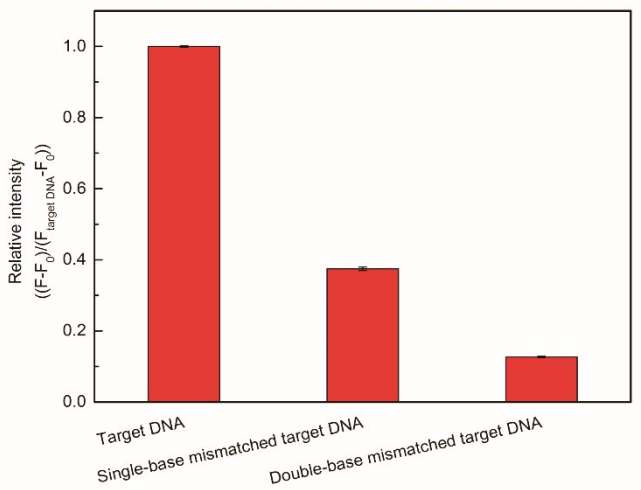

Fig. S4. Relative fluorescence intensity ($(F_{other\ target}-F_0)/(F_{target\ DNA}-F_0)$) of the biosensor in the presence of target DNA, single-base mismatched target DNA and double-base mismatched target DNA respectively, F_0 is the fluorescence intensity in the absence of target DNA, data were presented as average ±SD from three independent measurements. The concentration of all the three ssDNA sequences was 20 nM, Experiments were conducted in the presence of 20 nM 5'-FAM labeled probe DNA and 0.050 mg/mL FOQDs in Tris-HCl buffer (10 mM, 5 mM $MgCl_2$, pH 7.4) under excitation at 480 nm.

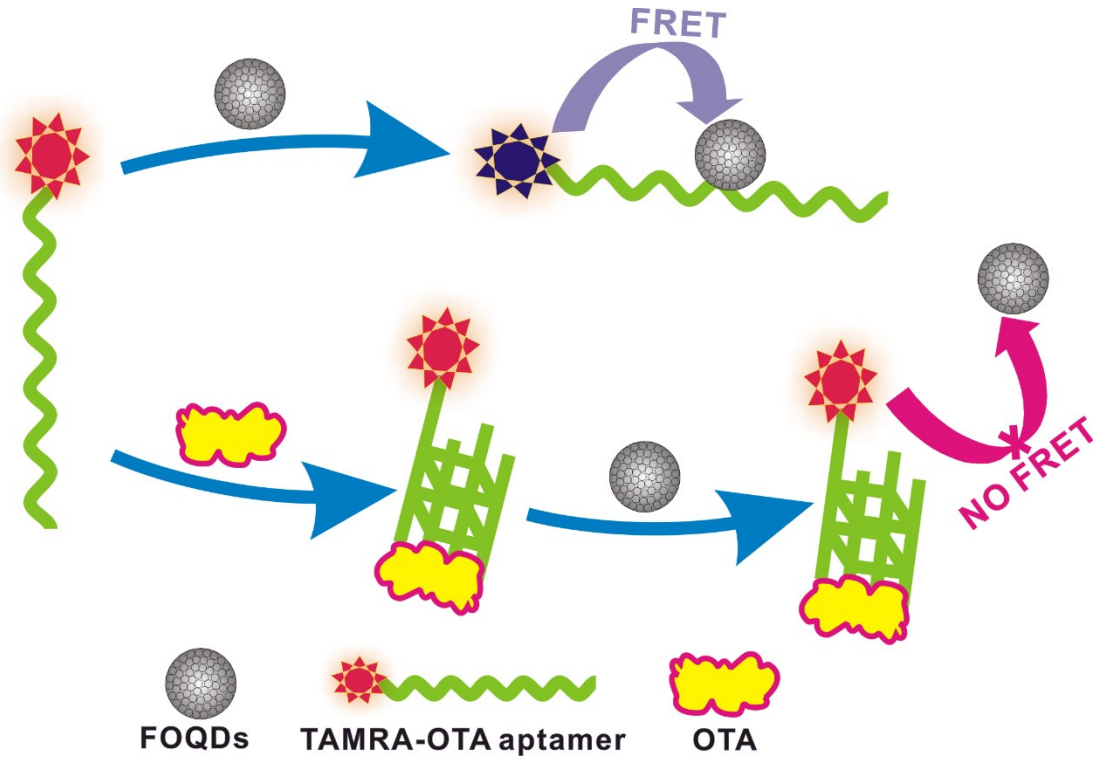

Fig. S5. Schematic illustration of the biosensing platform for OTA detection on the basis of PET from TAMRA-labeled OTA aptamer to FOQDs.

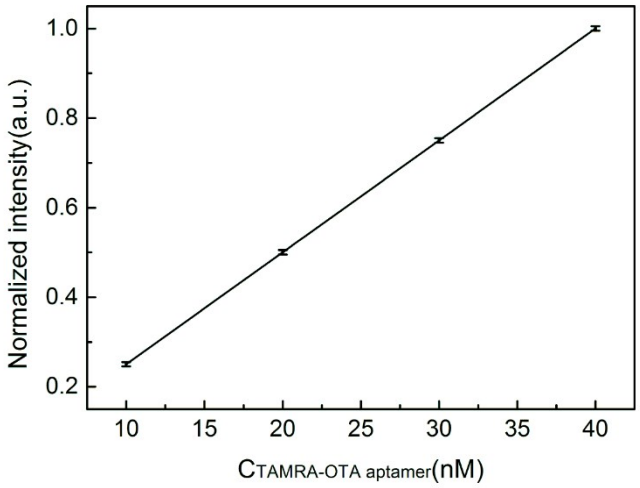

Fig. S6. Concentration dependent fluorescence response of TAMRA-OTA aptamer.

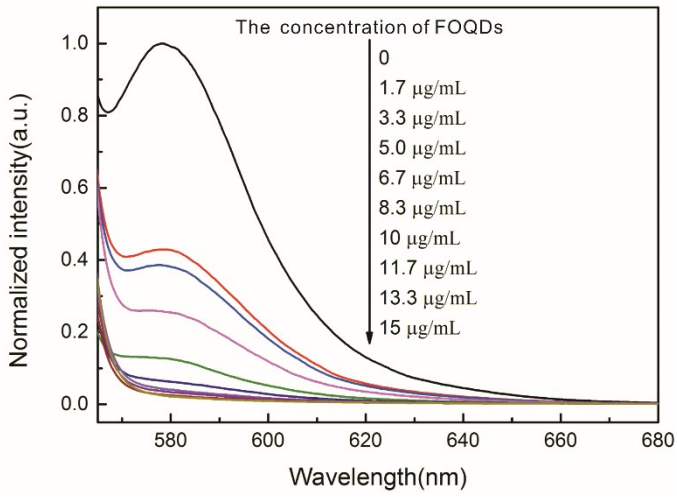

Fig. S7. Luminescence quenching of TAMRA labeled OTA aptamer (40 nM) in the presence of various concentrations of FOQDs (0, 1.7 μg/mL, 3.3 μg/mL, 5.0 μg/mL, 6.7 μg/mL, 8.3 μg/mL, 10 μg/mL, 11.7 μg/mL, 13.3 μg/mL, 15 μg/mL). The experiments were conducted in Tris-HCl buffer (10 mM, 5 mM KCl, 5 mM $CaCl_2$, pH 8.5) under excitation at 550 nm.

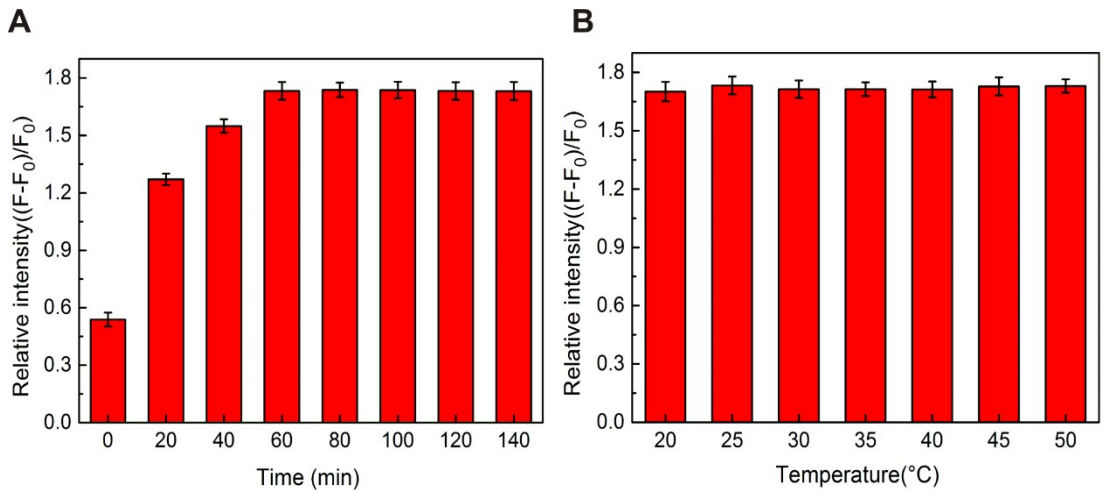

Fig. S8. (A) Effect of incubation time for TAMRA-OTA aptamer and OTA on fluorescence recovery of TAMRA-OTA aptamer. Experiments were conducted in the presence of 40 nM TAMRA-OTA aptamer, 0.6 ng/mL OTA and 6.7 μg/mL FOQDs in Tris-HCl buffer (10 mM, 5 mM KCl, 5 mM $CaCl_2$, pH 8.5) under excitation at 550 nm

at room temperature. The incubation time for fluorescence quenching was 1h. (B) Effect of incubation temperature on fluorescence recovery of TAMRA-OTA aptamer. Experiments were conducted in the presence of 40 nM TAMRA-OTA aptamer, 0.6 ng/mL OTA and 6.7 μg/mL FOQDs in Tris-HCl buffer (10 mM, 5 mM KCl, 5 mM $CaCl_2$, pH 8.5) under excitation at 550 nm. The incubation time for fluorescence recovery and quenching was 2h and 1h, respectively.

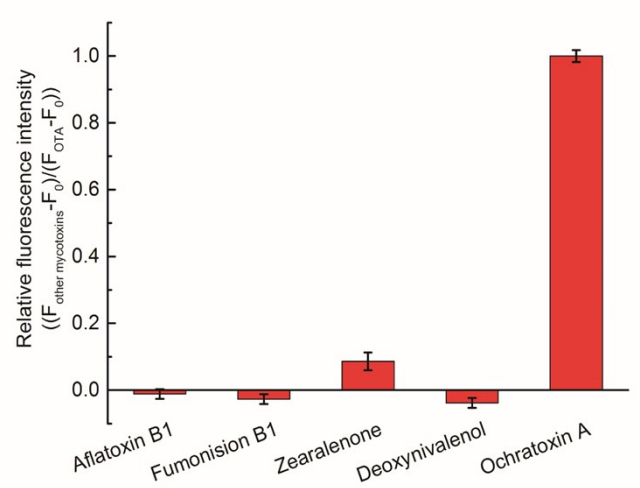

Fig. S9. Relative fluorescence intensity ($(F_{other\ target}-F_0)/(F_{target\ DNA}-F_0)$) of the biosensor in the presence of aflatoxin B1, fumonision B1, zearalenone, deoxynivalenol and ochratoxin A respectively, F_0 is the fluorescence intensity in the absence of mycotoxin, data were presented as average ±SD from three independent measurements. The concentration of all the three mycotoxins was 1 ng/mL, Experiments were conducted in the presence of 40 nM 3'-TAMRA labeled OTA aptamer and 6.7 μg/mL PdNPs in Tris-HCl buffer (10 mM, 5 mM KCl, 5 mM $CaCl_2$, pH 8.5) under excitation at 550 nm.

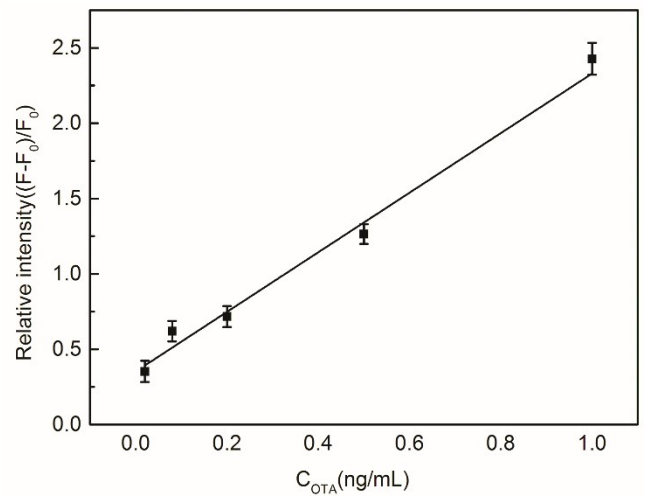

Fig. S10. The linear relationship between the fluorescence recovery (at 580 nm) and the concentration of OTA within the range from 0.02-1 ng/mL, data were presented as average ±SD from three independent measurements. Experiments were conducted in the presence of 40 nM TAMRA-OTA aptamer and 6.7 μg/mL FOQDs in grape juice 100-fold diluted with Tris-HCl buffer (10 mM, 5 mM KCl, 5 mM $CaCl_2$, pH 8.5) under excitation at 550 nm and emission at 580 nm.

Table S1. Determination of OTA in three real grape juice samples.

Sample no	Added (ng/mL)	Found (ng/mL)[a]	Recovery(%)	RSD(%,n=3)
1	0.05	0.048	96	4.2
2	0.1	0.11	110	2.7
3	0.5	0.47	94	1.3

[a]Mean value of three determinations by the biosensor.

Table S2. Reproducibility were evaluated for OTA biosensor

Sample no	Added (ng/mL)	Intra-day[a] (n=11)		Inter-day[b] (n=11)	
		Found±SD (ng/mL)	RSD (%)	Found±SD (ng/mL)	RSD (%)
1	0.03	0.029±0.0012	4.1	0.031±0.0011	3.5
2	0.05	0.050±0.0014	2.8	0.049±0.0022	4.5
3	0.08	0.079±0.0029	3.7	0.078±0.0023	2.9
4	0.2	0.21±0.0025	1.2	0.20±0.0064	3.2
5	0.6	0.58±0.013	2.2	0.59±0.0071	1.2

[a]The determination are carried out in eleven replicates in the same day;

[b]The determination are carried out in eleven different days.