Electronic Supplementary Information

A novel universal colorimetric sensor for simultaneous dual targets detection through DNA-directed self-assembly of graphene oxide and magnetic separation

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1. Experimental section

1.1. Reagents and materials

Graphene oxide (GO) was purchased from Nanjing XFNANO Materials Tech Inc. Phosphate, phenolphthalein (PP), thymolphthalein (TP), ferric chloride ($FeCl_3$), ferrous chloride ($FeSO_4 \cdot 7H_2O$) were purchased from Sinopharm Chemical Reagent Co. Ltd (China). OTA, aflatoxins B1 (AFB1) and fumonisin B1 (FB1) were obtained from Sigma-Aldrich**.** semi-complementary OTA aptamer (DNA_1 and DNA_2), OTA aptamer, Fluorescent dye labeled OTA aptamer (FOTA aptamer), semi-complementary AFB1 aptamer (DNA_4 and DNA_5) and AFB1 aptamer were purchased from Sangon Biotech Co., Ltd. (China) (Their sequences were shown in Table S1). Phosphate buffered saline (PBS, Na_2HPO_4-NaH_2PO_4, 0.1 M) was prepared in the laboratory. Doubly distilled water was used throughout this work. All other reagents were of analytical reagent grade.

1.2. Apparatus

Transmission electron microscopy (TEM) were conducted using a JEOL 100 instrument (JEOL, Japan) with an accelerating voltage of 200 kV. Fourier transform infrared (FTIR) spectrum was received on a Fourier transform spectrometer (Tensor 27, Bruker). Atomic force microscopy (AFM) measurements were carried out using Bruker Innova Microscope instrument. Fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectra-photometer (Tokyo, Japan). UV–vis absorption spectra were measured by UV-2450 spectrophotometer (Shimadzu, Japan). All the photographs were taken using a Canon digital camera.

1.3. Preparation Fe_3O_4/GO composite

The magnetic Fe_3O_4-graphene oxide nanoparticles hybrid (Fe_3O_4/GO) was synthesized by in situ chemical co-precipitation of Fe^{2+} and Fe^{3+} in an alkaline solution in the presence of GO.[1, 2] Firstly, 50 mL GO (0.8 mg mL^{-1}) aqueous was sonicated for 1 h to transform the carboxylic acid groups to carboxylate anions and was purged with N_2 for 30 min. Then, the aqueous solution (50 mL) of $FeCl_3$ (0.055 g) and $Fe_2SO_4 \cdot 7H_2O$ (0.048 g) was purged with N_2 for 30 min. This solution was added dropwise to the as-prepared GO suspension solution under magnetic stirring, and the mixture was stirred overnight under a nitrogen atmosphere for complete ion exchange. Then, the resulting mixture was heated to 90 °C before NaOH aqueous solution (6 M) was added dropwise to above mixture to precipitate Fe^{2+}/Fe^{3+} ions for synthesis of magnetite (Fe_3O_4) particles and adjust the pH to 10.0. The mixture was stirred at 90°C for 1.5 h and then cooled to room temperature. The as-prepared Fe_3O_4/GO solution

was washed with PBS buffer (pH=7.4) repeatedly until neutral and was stored at 4°C for further use.

1.4. Preparation of PP-DNA_1-GO and DNA_2-Fe_3O_4/GO

The pure GO aqueous solution (0.4mg mL^{-1},1mL) was severe ultrasonic treatment for 2 h to break down too large layers. DNA_1 (5μM) in a PBS buffer was added to the GO solution. The sample was shaken at room temperature for 24 h for sufficient immobilization. To remove free unbound aptamers, the mixture was centrifuged at 14000 rpm for 15 min, the supernatant was discarded and the precipitation was re-suspended with 900μL PBS buffer. Then, a stock solution of PP in ethanol (20 mM, 100μL) was added into the as-prepared DNA_1-GO solution with gently shaking to allow adsorption of PP onto the GO surfaces. The solution was shaken at room temperature for 3 h to make PP-DNA_1-GO conjugates were formed, and an excess amount of free PP was removed by centrifugation (14000 rpm, 15 min) for two times. The resulting precipitate was dispersed in 1 mL with PBS and the solution was stored at 4°C for further use.

DNA_2 (5μM) in a PBS buffer was added to the Fe_3O_4/GO solution (1mL), The sample was shaken at room temperature for 24 h for sufficient immobilization, The DNA_2-Fe_3O_4/GO solution was centrifuged at 14000 rpm for 15 min to remove free unbound DNA_2 and re-suspended with 1mL PBS buffer and the solution was stored at 4°C for further use.

The preparation of TP-DNA_4-GO, DNA_5-Fe_3O_4/GO for dual target detection was similar with PP-DNA_1-GO, DNA_2-Fe_3O_4/GO and the TP concentration was changed

to 1.5 mM.

1.5. Procedures of the biosensor

We carried out the OTA colorimetric method as follows: OTA aptamer (50 μL, 40 μM) was added into solution that 500 μL of the PP-DNA_1-GO was mixed with 500 μL DNA_2-Fe_3O_4/GO and The mixture was keeping shaken for 12 h at room temperature. After that, the solution was separated by a strong magnet and the supernatant was removed, the GO assembly was re-dispersed in 900 μL PBS buffer. Then, 100 μL of OTA containing solution with various concentration was added to the GO assembly solution. After incubation at 37°C for 1.5 h, measured supernatant liquid was then separated by a magnetic field. The color of the supernatant solution was visually observed. Finally, each well was added release reagents (BW, pH 12.0) and The absorbance were recorded by UV-visible spectrophotometer at 552 nm.

The simultaneous detection of the two targets (OTA and AFB1) is carried out as follows: 1mL of PP-DNA_1-GO+ OTA aptamer+DNA_2-Fe_3O_4/GO was mixed with 1mL of TP-DNA_4-GO+AFB1 aptamer+DNA_5-Fe_3O_4/GO together and the next steps are the same as for detecting OTA.

1.6. Preparation of the real testing samples

The penaut sample was purchased from local supermarket., 11 g of the non-contaminated peanut milled together with 1 g of sodium chloride. Aliquots (4 g) of the peanut powder were then spiked with OTA at different concentrations and mixed in a vortex mixer, respectively. After the addition of 10 mL of extraction solvent (methanol:water=6:4 (v/v)), the samples were mixed using an orbital shaker for 30

min. After centrifugation at 6000 rpm for 10 min, the extract was passed through a 0.45 mm syringe filter and then adjusted to pH 7.4, followed by dilution with PBS buffer to a finally spiked OTA concentration of 10, 50, 100 ng mL^{-1}, respectively.

Table S1. Oligonucleotides used in this paper

DNA name	DNA sequence (5' to 3')
DNA_1	GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTG TCCGATGCTCCCTTTA
DNA_2	CGCCACCCACACCCGATCGTGTGTGTGTGTGT GTGTGTGTGTGTGTGT
OTA aptamer	GATCGGGTGTGGGTGGCGTAAAGGGAGCATC GGACA
FOTA aptamer	FAM-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA
DNA_4	GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTG TGGGCCTAGCGAAGGGCACGAGA
DNA_5	CACAGAGAGACAACACGTGCCCAACGTGTGT GTGTGTGTGTGTGTGTGTGTGTGT
AFB1 aptamer	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTG CCCTTCGCTAGGCCCACA

2. Results and discussion

2.1. Characterization of the Fe_3O_4/GO

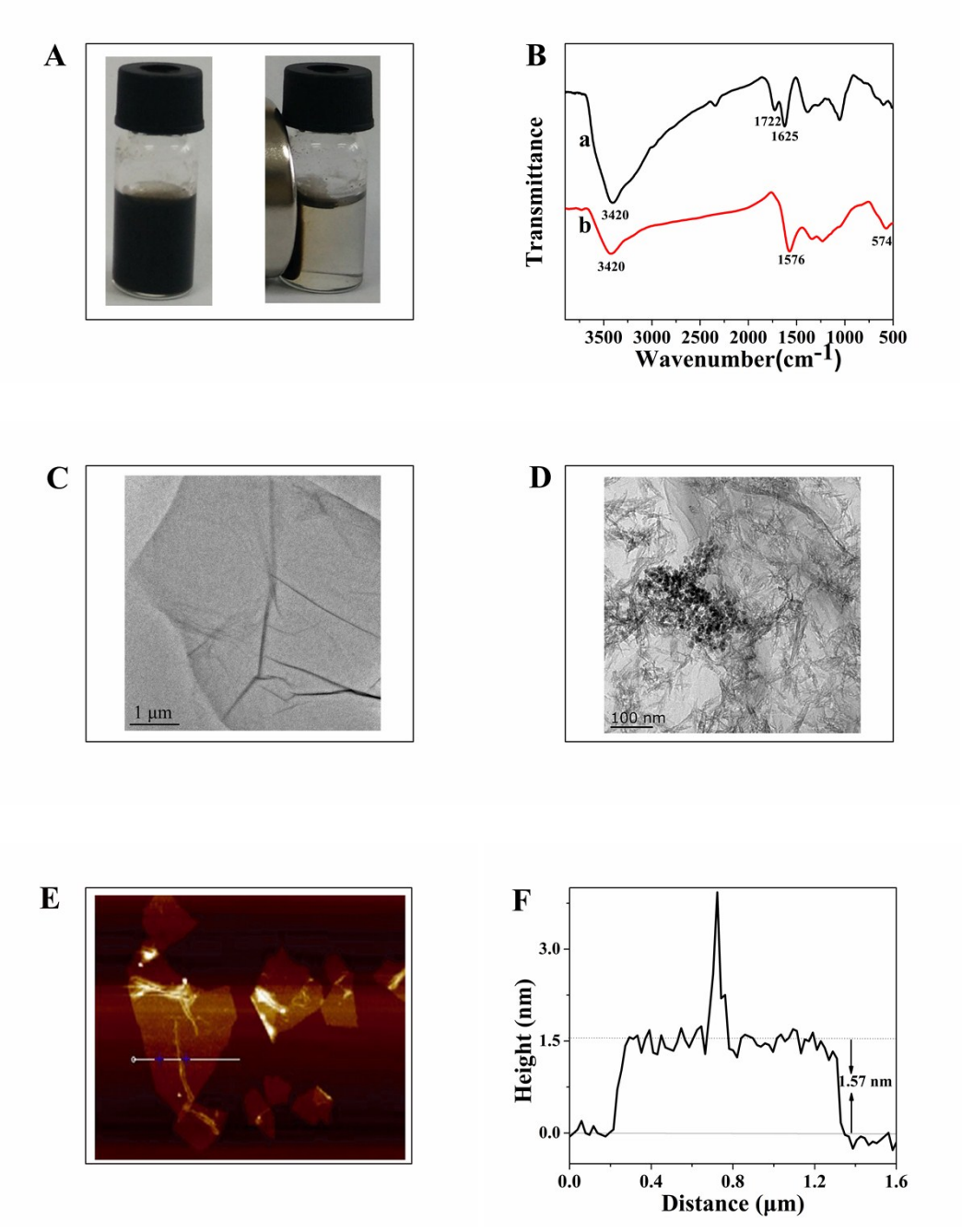

Fig. S1 The picture in (A) shows the Fe_3O_4/GO dispersed in the PBS solution (a) and magnetic separation (b), (B) FTIR spectra of GO (a) and Fe_3O_4/GO (b) composite. (C)TEM images of the as-prepared GO (C) and Fe_3O_4/GO (D). AFM images (E) and height profiles (F) of bare GO.

Fe_3O_4/GO was synthesized by a mixed solution method. As a result of the

magnetite content, the resulting Fe_3O_4/GO can quickly respond to external magnetic field (photograph b in Fig. S1A) in 2 min and be rapidly re-dispersed uniformly by hand-shaking. The excellent magnetic property enables Fe_3O_4/GO to be used for simple and efficient magnetic separation. Fig. S1B shows fourier transform infrared (FTIR) spectra of GO and Fe_3O_4/GO. In the spectra of GO, the peaks at 1722 cm^{-1} and 1625 cm^{-1} were respectively assigned to the C=O vibration of carboxylic groups and the skeletal vibration of the GO sheets. In the spectra of Fe_3O_4/GO, the peak at 1722 cm^{-1} corresponding to ν(C=O) of –COOH on the GO shifted to 1576 cm^{-1} due to the formation of $–COO^-$ after coating with Fe_3O_4 as previously reported.[3] Compared with that of GO, a new prominent absorption band appeared at about 574 cm^{-1} in the FTIR spectrum of the Fe_3O_4/GO composite, which corresponds to the stretching mode of Fe–O.[4, 5] The morphology and structure of GO and Fe_3O_4/GO nanocomposites was investigated by TEM, as shown in Fig. S1C. Fig. S1C demonstrates the representative view of free-standing GO nanosheets, revealing a stacked and rippled structure, which may be important for maintaining high surface area with a particular advantage of loading magnetic nanoparticles. Fig. S1D presents TEM images of as-prepared Fe_3O_4/GO nanocomposites. It can be seen clearly that two-dimensional GO nanosheets are homogeneously decorated by a number of Fe_3O_4 nanoparticles. Besides, we also confirm a monolayer state for the graphene sheets by the topographical profiles analyzed using atomic force microscopy, the height of bare GO sheets is approximately 1.57 nm (Fig S1 E, F).[6]

2.2. Optimization of pH Value of Phenolphthalein Solution

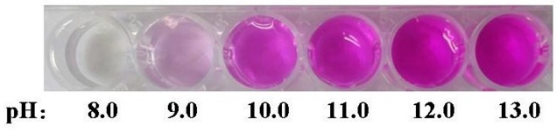

Fig. S2 Photographs of PP solution with pH intensities from 8.0 to 13.0 (left to right).

We compare the solubility of PP (50 μM) in water with different pH levels, and the results clearly show that when pH levels higher than 9.0, it gradually turned into soluble and the solution turned pink. When the pH value is 12.0, the pink color will reach the maximum intensity. Therefore, the pH value is 12.0 of BW was used to release the PP molecule from the GO surfaces (Fig. S2).

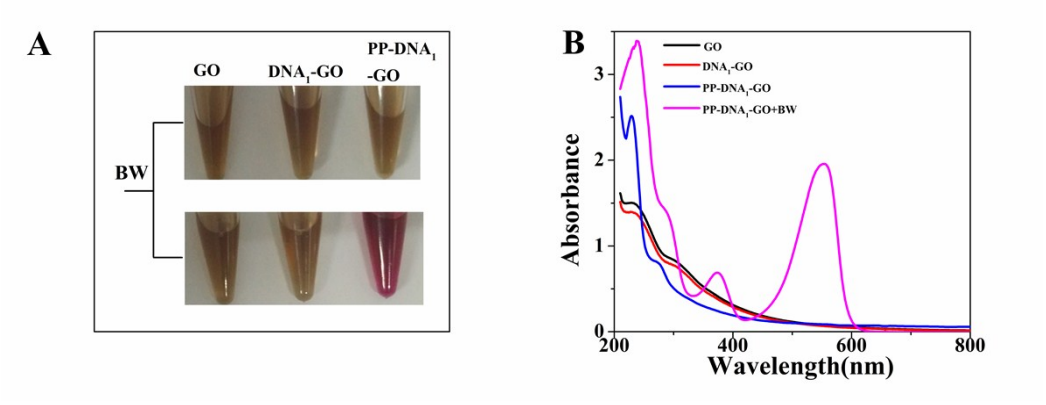

Fig. S3 (A) Bright-field images of solutions containing GO, DNA_1-GO, and PP-DNA_1-GO before and after treating with BW (pH 12.0). (B) Absorption spectra of solutions containing GO, DNA_1-GO, PP-DNA_1-GO, and PP-DNA_1-GO treated with BW (pH 12.0).

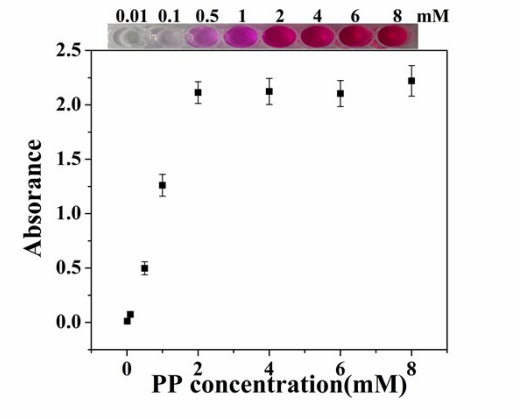

Fig. S4 Absorption intensities of PP-GO solution at 552 nm versus different concentrations of PP added into the GO (0.4 mg/mL) solution.

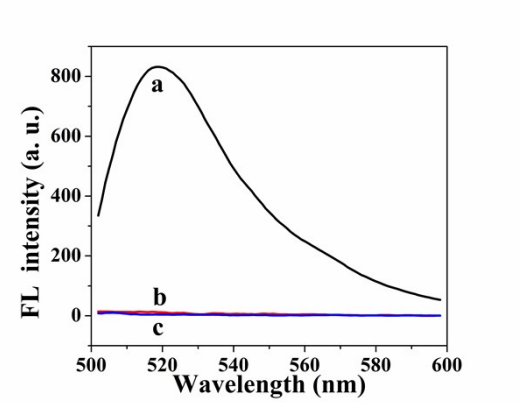

Fig. S5 Fluorescence intensity of FOTA aptamer (a), FOTA aptamer-GO (b), FOTA aptamer-Fe_3O_4/GO (c) in PBS buffer.

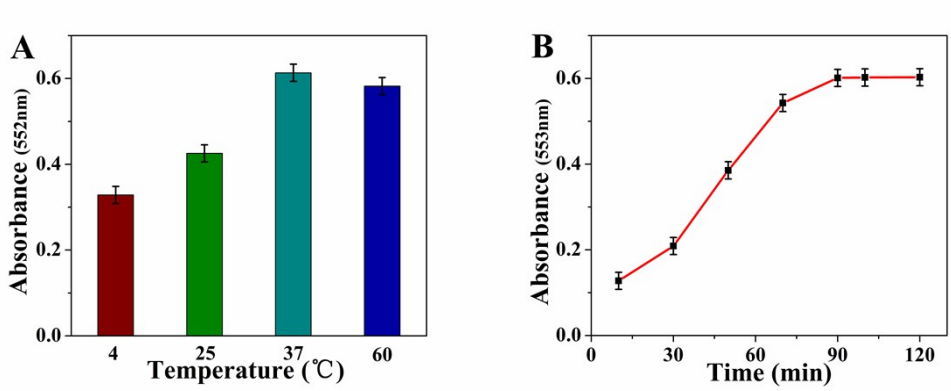

Fig. S6 The UV–vis absorption spectra responses of the supernatant (after treating with BW (pH=12.0) under the different reaction temperature (A) and reaction time (B) with OTA at the concentration of 100 ng mL^{-1}.

Table. S2 Comparison of the as-prepared methods for OTA with those reported in the literatures.

Detection method	Liner range (ng mL^{-1})	LOD (ng mL^{-1})	References
FL[a]	8-160	8	Wei et al (2015)[7]
	20-200	8.72	Sheng et al (2011)[8]
	10-80	9.64	Guo et al. (2011)[9]
	2-60	2	Lu et al (2015)[10]
EC[b]	12-60	12	Radi et al (2009)[11]
Colorimetry	8-250	8	Yang et al. (2011)[12]
	10-250	10	This work

[a] Fluorescence

[b] Electrochemistry

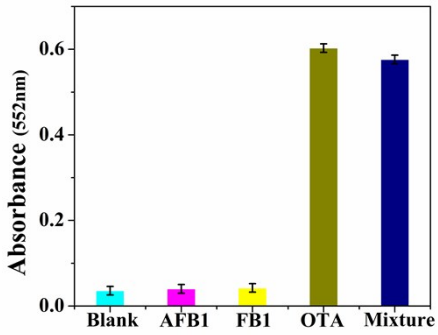

Fig. S7 The UV–vis absorption spectra intensity of the biosensor in the presence of different targets: blank (without OTA), AFB1(200 ng mL^{-1}), FB1 (200 ng mL^{-1}), OTA (100 ng mL^{-1}), and mixture: AFB1(200 ng mL^{-1}), FB1 (200 ng mL^{-1}), OTA (100 ng mL^{-1}).

Table S3 Results of OTA detection in peanuts (n=3).

Sample	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	RSD (%)
1	10	9.66	96.6	7.6
2	50	52.57	105.1	5.3
3	100	97.42	97.4	7.5

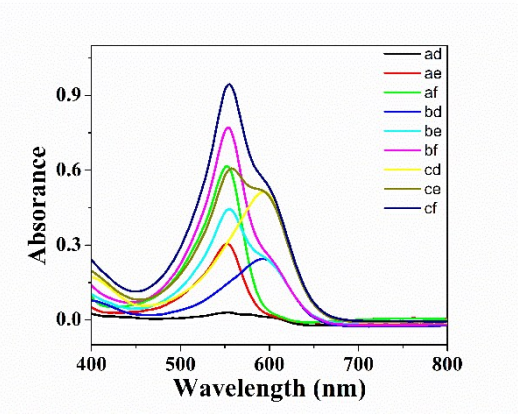

Fig. S8 The UV–vis absorption spectra curve in the presence of various concentrations of OTA and AFB1.

Table. S4: The concentration information of dual targets by analyzing the peak absorption at 552 nm and 594 nm.

	Added		**Found**	
	OTA (ng ml^{-1})	**AFB1 (ng ml^{-1})**	**OTA (ng ml^{-1})**	**AFB1 (ng ml^{-1})**
ad	0	0	0	0
ae	50	0	49.74	0
af	100	0	100.61	0
bd	0	50	0	48.73
be	50	50	48.08	45.52
bf	100	50	100.36	48.26
cd	0	100	0	102.32
ce	50	100	46.57	96.22
cf	100	100	100.15	101.37

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