



## Analytical Methods

## Development of a graphene oxide nanosheet and double-stranded DNA structure based fluorescent “signal off” aptasensor for ochratoxin A detection in malt

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## ABSTRACT

A “signal off” fluorescent aptasensor based on graphene oxide (GO) nanosheet and double-stranded DNA structure was fabricated for OTA detection. In the absence of OTA, the aptamer and its complementary DNA (cDNA) formed double-stranded conjugates that could coexist with GO, presenting fluorescence responses. Then, the presented OTA was captured by the aptamers, causing the release of cDNA-FAM probes. The free probes were adsorbed by GO, leading to an OTA concentration-dependent fluorescence quenching through fluorescence resonance energy transfer. Under optimum conditions, the fluorescent aptasensor exhibited outstanding sensitivity with a LOD of 11 pg/mL and a wide dynamic range of 0.04–30 ng/mL. The selectivity of the aptasensor was confirmed against other four mycotoxins, and the feasibility and reliability were verified by determining OTA in the spiked malt samples with satisfactory recovery of 95.50%–112.05%. This aptasensing platform could be adapted to measure other mycotoxins by replacing the aptamer sequences for food safety.

## Introduction

Mycotoxin contamination in a variety of foods, feed and other commodities largely consumed by humans and animals, as well as the consequent serious threat to health, safety, global economy and society development has been a thorny issue in the world (Cao et al., 2021; Leite et al., 2021; Liew & Mohd-Redzwan, 2018). Of 400 classified mycotoxins, ochratoxin A (OTA) is the most toxic member of ochratoxins produced by certain fungi species from the genera *Aspergillus* and *Penicillium* due to its confirmed hepatotoxicity, nephrotoxicity, teratogenicity, carcinogenicity, and genetic toxicity (Huang et al., 2019; Tao et al., 2018). It has been classified as a group 2B human carcinogen by the International Agency for Research on Cancer (IARC) (Schrenk et al., 2020). OTA is regarded as a cumulative mycotoxin with high incidence rate in many consumed matrices, and low-dose, chronic, and long-term exposure through food intake and environmental pollution has been a

global concern (Klingelhöfer et al., 2020). Of them, OTA contamination in the malt matrix has attracted more and more attention. As one of the important raw materials for popular foods and many medicinal products, malt is consumed hugely in the world. However, in the whole process from production, processing to storage, OTA contamination is always unavoidable. Thus, the maximum residue limits (MRLs) for OTA have been officially set in many countries and international organizations to guarantee food and relevant sample safety with MRL of 5 µg/kg regarding OTA in malt (European Commission, 2006). To satisfy these detection limits, it is of great urgency and importance to develop a sensitive, convenient and efficient method for accurate monitoring of OTA.

Until now, various analytical techniques, such as liquid/gas chromatography coupled to different detectors, have been developed for routine identification and detection of OTA due to their fascinating sensitivity, accuracy and specificity for standard analysis in well-

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equipped laboratory (Cina et al., 2021; Kaya et al., 2019; Wei et al., 2019). However, they are greatly restricted for extensive application by expensive instrument, high cost- and time-consumption, tedious pre-treatment, large organic solvent waste, and skilled technicians. Fortunately, fluorescent sensors based on biomolecules (e.g. antibody and aptamer) as the specific recognition elements have attracted much attention for rapid analysis of diverse targets. Of them, aptamer-based fluorescent sensors, abbreviated as fluorescent aptasensors, with wide applications in different fields (Fang et al., 2021; Zhang et al., 2021; Zhao et al., 2021) have provided a preferred choice for OTA detection thanks to their miniature size, portability, excellent sensitivity, rapid response, and low consumption of toxic organic solvents for on-site monitoring.

Aptamer, also called as “artificial antibody”, has become the predominant alternative in developing highly-selective inspection technologies. As a recognition element, it displays many outstanding advantages compared to antibody, such as easy *in vitro* synthesis and modification, strong specificity and affinity between aptamer and target, good stability and activity, low cost, convenient transportation, and long-term preservation (Alkhamis et al., 2019; Torabi et al., 2020). In addition, aptamer can adopt a reversible conformational change to specifically and tightly bind with the target. It has shown great potential in the robust and rapid analysis of small molecules like mycotoxins based on fluorescence transducers (Shkempi et al., 2021).

As regard to the fluorescent aptasensing of mycotoxins, some nanomaterials with fascinating fluorescence quenching ability are widely introduced in the combination with fluorescent substances to construct diverse analytical platforms. Of them, graphene oxide (GO), a two-dimensional (2-D) carbon nanomaterial with the unparalleled advantages of large surface area, excellent electrical conductivity, good biocompatibility and high dispersibility in the water, has been confirmed with superior fluorescence quenching capability (Alex et al., 2021; Ghosh et al., 2021; Morales-Narvaes & Merkoci, 2019). High proportion of 2-D nanosheet structure and large specific surface area of GO facilitate the adsorption of biomolecules, such as a single-stranded DNA (ssDNA) with aromatic rings of bases, on its surface through  $\pi$ - $\pi$  conjugation. In addition, as a superior nanoquencher, GO can effectively quench the fluorescence of various fluorescent materials like organic dye, quantum dots, and carbon dots through fluorescence resonance energy transfer (FRET), which is beneficial for developing the FRET-based biosensing platforms (Zhang et al., 2017). In most reports, the fluorescent aptasensors were often designed based on single-stranded aptamer and GO (Cheng et al., 2018; Wu et al., 2019; Yang et al., 2018). After the addition of target molecules, the adsorbed single-stranded aptamers with labels were desorbed from the GO surface, causing the restoration of fluorescence to achieve quantitative monitoring of the targets. However, in our preliminary experiments, other non-covalent effects between GO and combined conjugate of fluorescence probe (aptamer-carboxyfluorescein (FAM)) and target were observed. It indicated the incomplete recovery of the fluorescence probe, which might reduce the sensitivity of the aptasensor to a certain extent and affect the actual detection of OTA.

Therefore, in this study, a GO nanosheet and double-stranded DNA structure of aptamer-complementary DNA (cDNA) based fluorescent “signal off” aptasensor was developed for the detection of OTA using FAM labeled cDNA and GO nanosheets, aiming at largely improve the sensitivity. Considering the affection of the non-covalent effect between aptamer-OTA conjugate and GO, cDNA modified FAM was selected as the signal probe. The fluorescence of FAM-cDNA would be always quenched by GO nanosheets according to the amount released, which was directly related with OTA content to achieve accurate quantification. Through observing fluorescence emission spectra of FAM in the system, the feasibility of this strategy was confirmed. And the aptamer concentration, the incubation time of aptamer and cDNA-FAM probes, the GO nanosheets concentration and the incubation time of targets were systematically investigated. Considering that guanine (G) on DNA

would weaken the fluorescence intensity of FAM, it was important to select the 5'-end of cDNA modified fluorophore and ensure to form a saturated double-stranded structure. Thanks to its simple preparation, fast response, and high specificity, this work provided a simple, sensitive, and reliable analytical tool for practical detection of mycotoxins in the fields of food safety and environmental monitoring.

## Material and methods

### Materials and apparatus

The OTA aptamer and its cDNA sequences were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd (Shanghai, China, <https://www.sangon.bioon.com.cn>), which were listed as follows:

OTA aptamer: 5'-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3'.

cDNA: 5'-FAM-TGT CCG ATG CTC CCT TTA CGC CAC CCA 5' CAC CCG ATC-3'.

Graphene Oxide nanosheet solution was purchased from Nanjing XF Nano Material Tech Co., Ltd. (Nanjing, China). Ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEN), aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), T-2 toxin (T-2) were purchased from Qingdao Pribolab Biological Engineering Co. Ltd. (Qingdao, China). The used phosphate buffered saline (PBS, 0.01 M, pH7.2-7.4) solution was purchased from Beijing Solarbio Science & Technology Co., Ltd. And other reagents were of analytical grade without further purification. Malt samples were collected from the local medicinal material market.

### Instrumentation

All fluorescent measurement was performed on a FL970 fluorophotometer from Shanghai Techcomp Instrument Ltd. (Shanghai, China) equipped with FL Analyze 4.0 software. Slits for the excitation and emission measurements were set at 5 nm in width. The excitation wavelength was set at 495 nm. The fluorescence spectra were collected with an emission wavelength range of 495–580 nm, and the maximum fluorescence responses occurred at 520 nm were finally recorded.

### Establishment of OTA fluorescent detection

According to the experimental protocol, several important experimental conditions were optimized. Then, a standard curve was established to achieve quantitative detection of OTA according to the following procedure. First, 1  $\mu$ M of aptamer solution, 0.5  $\mu$ M of cDNA-FAM probe and PBS solution were sequentially placed in a brown centrifuge tube for incubation under slight shaking for 40 min at 37 °C. Then, 10  $\mu$ L of 1 mg/mL GO was added to the final volume 270  $\mu$ L for co-reaction for 15 min. The fluorescence intensity of the mixed solution at the maximum emission of 520 nm with a 495-nm irradiation was determined as  $F_1$ . Then, 30  $\mu$ L of the spiked PBS solution with different concentrations of OTA (0.04, 0.1, 1, 5, 10, and 30 ng/mL) was added into the tube containing the aptamer-cDNA-FAM/GO nanosheet quenching system, respectively, for incubation under slight shaking for 30 min at 37 °C. And the fluorescence intensity was measured as  $F_2$ . The difference value ( $\Delta F$ ) of  $F_1$  and  $F_2$  were calculated for quantitation of OTA in PBS solutions.

### Specificity evaluation

To evaluate the selectivity of the aptasensor for OTA over other mycotoxins, AFB<sub>1</sub>, ZEN, DON, and T-2 toxin at the same concentration (30 ng/mL) with OTA as reference substances, were added to the aptamer-cDNA-FAM/GO nanosheets system, respectively. They were measured under the same condition as OTA to compared the induced changes in fluorescence intensity ( $\Delta F$ ).

### Preparation and measurements of malt samples

To verify the reliability of the fluorescent aptasensor, the spiked malt samples were analyzed according to the standard addition method in advance. Briefly, the confirmed OTA-free malt sample was ground into powder by a high-speed disintegrator and passed through a 1-mm aperture sieve. One gram of malt powder spiked with different concentrations of OTA were weighed into a centrifuge tube with the addition of 2 mL of acetonitrile, followed by vortexing vigorously and ultrasonical extraction for 20 min. Then, the extraction was centrifuged at 8000 rpm for 5 min, and the supernatant was taken for filtrating through a 0.22- $\mu\text{m}$  membrane. Afterwards, the resultant solution was diluted 15 times with PBS solution and used for recovery evaluation according to the above procedure.

## Results and discussion

### Detection principle of the aptasensor

Here, a fluorescent “signal off” aptasensor was fabricated for detecting OTA based on a double-stranded DNA of aptamer-cDNA competitive mode and GO nanosheet quenching strategy. GO nanosheet can adsorb ssDNA on its surface through  $\pi$ - $\pi$  stacking interaction with the ring structure of nucleobases (Li et al., 2020). Due to the short distance between luminescent material and GO, the fluorescence was further quenched through FRET when the fluorescence emission spectra of luminescent material and absorption spectra of GO were overlapped (Zhao et al., 2020). However, in the preliminary experiment, after the introduction of target OTA, it was found that the aptamer-FAM as probe did not significantly recover fluorescence, but was affected by non-covalent effects and continued to be adsorbed on GO after binding to the target. Thus, the cDNA corresponding to the anti-OTA aptamer was designed and labeled by FAM, which has been used as the fluorescence probe of the system. In this aptasensing system, cDNA-FAM, aptamer and GO nanosheet functioned as fluorescence donor, OTA recognition element and fluorescence quencher, respectively.

As shown in Scheme 1, in the absence of target OTA, the aptamer naturally bound to cDNA to form aptamer-cDNA-FAM composite through the base complementary pairing. The double-stranded structure could coexist with GO nanosheets in solution system, exhibiting strong fluorescence responses. After OTA was introduced, it was recognized and captured by the aptamer preferentially to obtain the aptamer-OTA conjugate with a folded structure, triggering the dissociation of aptamer-cDNA-FAM composite and the release of cDNA-FAM. The released FAM-labeled single-stranded cDNA was adsorbed onto the GO nanosheets, and the fluorescence of which was quenched by GO nanosheets through FRET in a “signal off” mode. Then, the quenched

fluorescence value resulted from the introduced OTA was calculated for quantitation of OTA.

### Characterization of the GO-based fluorescent aptasensor

First of all, the fluorescent sensor based on the principle of FRET should meet the requirements of spectral overlap between the quencher and the fluorophore. As shown in Fig. 1, the emission spectrum of FAM overlapped with the UV-vis absorption spectrum of GO nanosheets, so that when GO shortened the distance from FAM by adsorbing ssDNA, FRET would be triggered.

In order to verify the feasibility of the aptasensor, each step for fabrication was characterized through recording the fluorescence emission spectra of the cDNA-FAM probes upon the introduction of GO nanosheets, aptamer, and target OTA, respectively. As shown in Fig. 2a, the fluorescence of pure cDNA-FAM probes was almost quenched after the addition of GO nanosheets, confirming that GO with excellent fluorescence quenching ability adsorbed the single-stranded cDNA to quench the fluorescence of the labeled FAM through FRET. When aptamer recognized and bound with cDNA to form the double-stranded aptamer-cDNA-FAM conjugate, the system emitted strong fluorescence (Fig. 2b), even if GO nanosheets were introduced, the fluorescence only caused slight reduction (Fig. 2c). These findings indicated that the aptamer-cDNA-FAM conjugates and GO nanosheets could co-exist in the mixed solution system without interaction. However, when the target OTA was added, it would compete with cDNA to bind with the aptamer

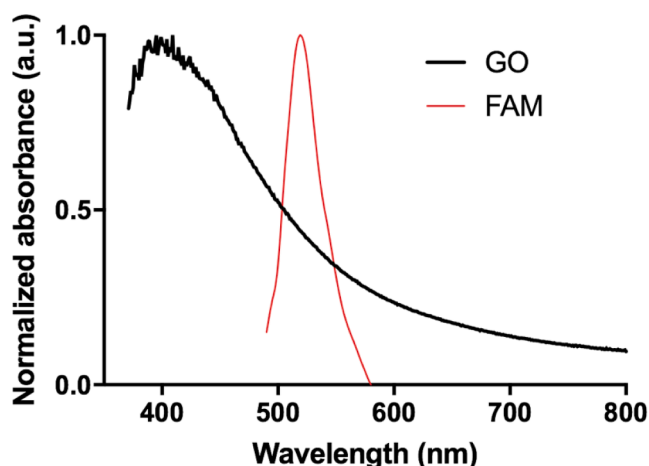
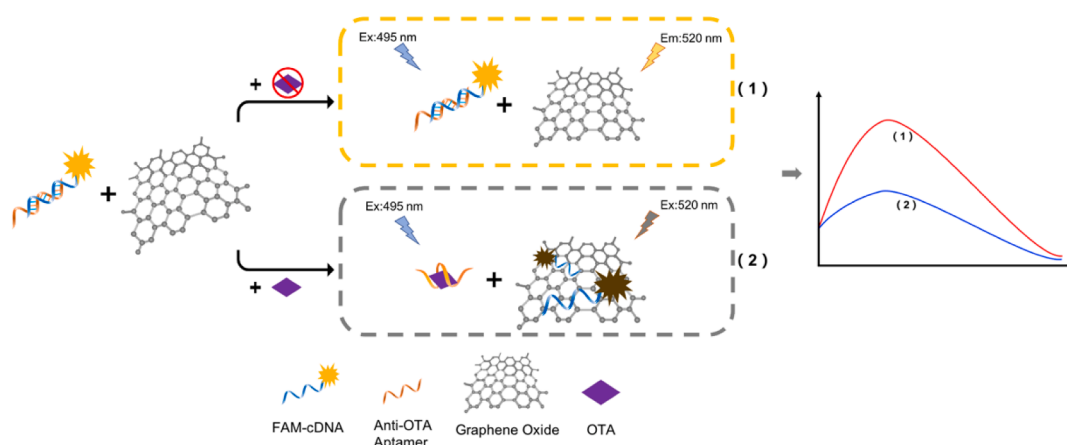


Fig. 1. Emission spectrum of FAM labelled aptamer probe and UV-vis absorption spectrum of GO nanosheets.



Scheme 1. Illustration of a fluorescent aptasensor based on the double-stranded DNA competition mode and GO for OTA detection.

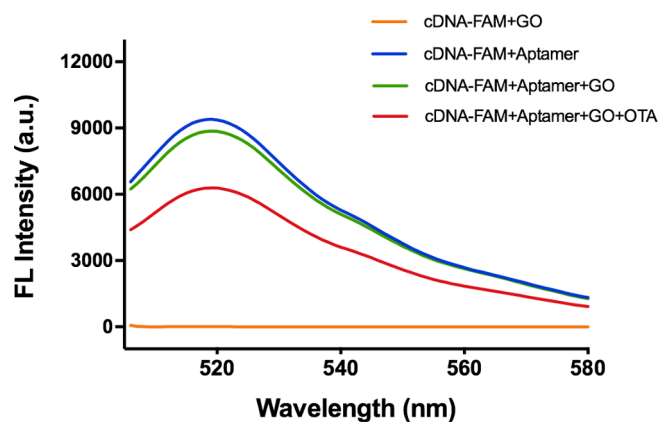


Fig. 2. Fluorescence spectra of (a) 0.5  $\mu\text{M}$  of cDNA-FAM and 1 mg/mL of GO nanosheets; (b) 0.5  $\mu\text{M}$  cDNA-FAM and 1  $\mu\text{M}$  aptamer; (c) 0.5  $\mu\text{M}$  cDNA-FAM, 1  $\mu\text{M}$  aptamer, and 1 mg/mL GO; (d) 0.5  $\mu\text{M}$  cDNA-FAM, 1  $\mu\text{M}$  aptamer, 1 mg/mL GO, and 100 ng/mL OTA after incubation at 37  $^{\circ}\text{C}$ .

due to the stronger affinity between aptamer and the target OTA, resulting in the dissociation of aptamer-cDNA-FAM double-stranded structure and the release of cDNA-FAM. Then, the released free cDNA-FAM probes were adsorbed onto the surface of GO nanosheets, causing the reduction of fluorescence intensity (Fig. 2d). The OTA concentration-dependent reduced fluorescence intensity was recorded for quantitation of OTA. The above results indicated the successful fabrication of the aptasensor for subsequent analysis of OTA.

#### Optimization of crucial experimental conditions

In order to achieve the optimal detection performance of the

aptasensor for OTA, some crucial parameters, such as the aptamer concentration, the incubation time of aptamer and cDNA-FAM probes, the GO nanosheets concentration and the incubation time of OTA were systematically optimized.

#### Aptamer concentration

The added amount of aptamer was firstly optimized since it could directly affect the sensitivity of subsequent experiments. In order to better choose the added amount of aptamer, 0.5  $\mu\text{M}$  of the cDNA-FAM fluorescence probes firstly interacted with the GO nanosheets to form fluorescence quenching conjugates in this optimization step. And then the added concentration of aptamer was selected according to the degree of recovery of the fluorescence intensity as the concentration increased. As shown in Fig. 3A, the fluorescence intensity gradually increased with increasing added concentration of the aptamer, which reached the highest value when 1  $\mu\text{M}$  aptamer was added, and then tended to stable regardless of the continue increase of aptamer solution. This indicated that when the cDNA-FAM fluorescence probe was at a fixed concentration, 1  $\mu\text{M}$  aptamer could bind to it with high efficiency, so that free fluorescence probes in the system as few as possible were quenched by GO to cause waste. On the other hand, it could form the largest amount of double-stranded structure to react with the target, and avoid excess aptamer and target OTA binding to increase sensitivity. Therefore, 1  $\mu\text{M}$  aptamer solution was introduced in constructing the aptasensor.

#### Incubation time of aptamer and cDNA-FAM probes

After the aptamer was introduced, the adequate binding with cDNA-FAM was necessary. Here, the fluorescence emission spectra of the double-stranded aptamer-cDNA-FAM conjugate after sufficient incubation were measured. Fig. 3B displayed that the fluorescence intensity

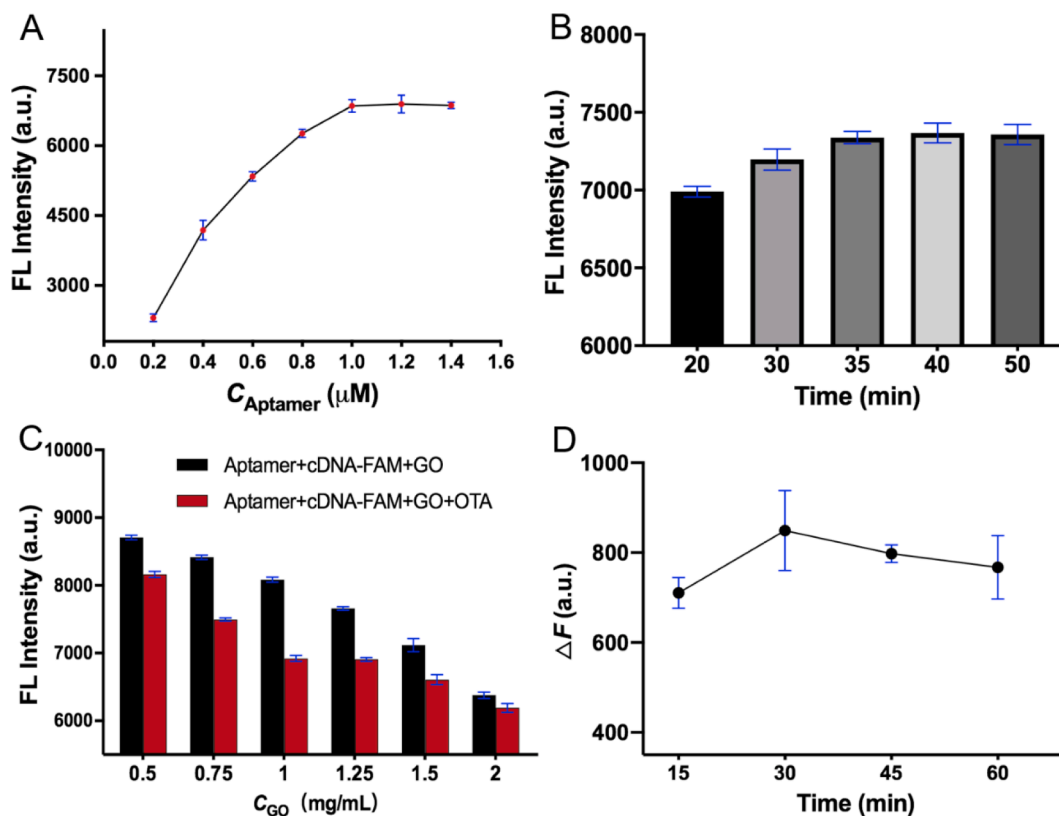


Fig. 3. Optimization of experimental conditions of (A) the OTA aptamer concentration ( $C_{\text{aptamer}} = 0.5 \mu\text{M}$ ), (B) the incubation time of aptamer (0.5  $\mu\text{M}$ ) and cDNA-FAM (0.5  $\mu\text{M}$ ), (C) the GO nanosheets concentration ( $C_{\text{aptamer}} = 0.5 \mu\text{M}$ ,  $C_{\text{cDNA-FAM}} = 0.5 \mu\text{M}$ ), and (D) the incubation time of OTA (100 ng/mL) ( $n = 3$ ).



increased with lengthening the incubation time. This phenomenon might be due to that the single-stranded cDNA-FAM fluorescence probes themselves would bend into the spatial structure, causing the fluorescence quenching effect of guanine (G) on the FAM fluorophores. The double-stranded rigid structure formed by base pairing between the aptamer and cDNA weakened the quenched fluorescence caused by G, giving an enhanced fluorescence responses of FAM. It was important that ensuring the formation of a saturated double-stranded structure because that could reduce the quantitative calculation error between the fluorescence reduction caused by G and the fluorescence quench caused by GO. After a 40-min incubation, the fluorescence intensity was stable, showing sufficient conjugation of aptamer and cDNA-FAM probes. Thus, 40 min was determined as the optimal incubation time for the aptamer and the cDNA-FAM fluorescence probes.

#### GO nanosheets concentration

The quenched fluorescence of cDNA-FAM probes by GO nanosheets was directly relevant with OTA content. Then, the added amount of GO nanosheets should also be controlled to achieve maximum detection efficiency. Here, the fluorescence intensity of the aptamer-cDNA-FAM/GO conjugates in the absence and presence of OTA was determined to calculate the difference value ( $\Delta F$ ) for optimizing the added amount of GO. As could be seen in Fig. 3C, the fluorescence intensities  $F_1$  of the double-stranded aptamer-cDNA-FAM conjugate, as well as  $F_2$  due to the added OTA were all reduced with increasing the added amount of GO nanosheets. When 1 mg/mL of GO nanosheets solution was applied, the highest  $\Delta F$  value was observed, which was selected as the best added concentration of GO nanosheets.

#### Incubation time of OTA

The incubation time of OTA in the aptamer-cDNA-FAM/GO nanosheets system was investigated. It could be found in Fig. 3D that the  $\Delta F$  values increased with prolonging the incubation time because of more combinations of aptamer and OTA. When the incubation time was controlled in 30 min,  $\Delta F$  reached the highest value. Subsequently, the  $\Delta F$  values decreased due to the detached probes from GO nanosheets caused by continuous heating and vibration. Therefore, an incubation time of 30 min was adequate for incubating OTA in the aptasensing system for its quantitation.

#### Quantitative measurement of OTA

Under the above optimum conditions, the developed GO nanosheets based fluorescent “signal off” aptasensor was utilized for the quantitative determination of OTA at an excitation wavelength of 495 nm and an emission wavelength of 520 nm. The fluorescence intensity  $F_2$  of the aptamer-cDNA-FAM/GO nanosheets system in the presence of OTA decreased with increasing OTA concentration while the difference value ( $\Delta F$ ) of fluorescence intensity increased with increasing the concentration of OTA. The calibration curve exhibited a good linear relationship between  $\Delta F$  versus OTA concentration was linear in a wide range of 0.04–30 ng/mL (Fig. 4). The regression equation could be expressed as:  $\Delta F = 17.061c + 490.13$  with  $R^2 = 0.9904$ , where  $\Delta F$  presents the difference value of fluorescence intensity in the aptamer-cDNA-FAM/GO nanosheets system before and after adding OTA. The limit of detection (LOD), which was calculated as the concentration of OTA by gradually diluting the standard with PBS solution, was measured about 11 pg/mL. As presented in Table 1, the newly-developed fluorescent “signal off” aptasensor based on GO nanosheets and double-stranded DNA competition mode of aptamer-cDNA conjugate offered obvious advantages in sensitivity compared with other nanomaterials and single-stranded DNA based aptasensors, especially in complicated matrices like malt. A sufficiently wide detection range and extremely low detection limit exhibited that it is an achievable, sensitive, and promising method for

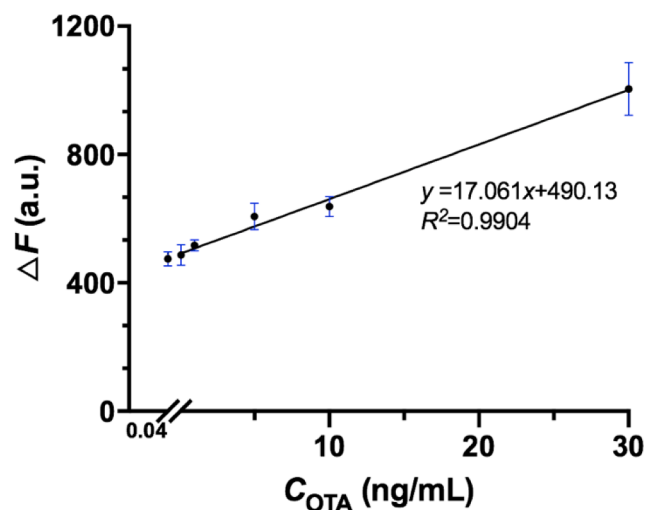


Fig. 4. The relationship between the changed fluorescence intensity ( $\Delta F$ ) and the added OTA concentration (0.04, 0.1, 1, 5, 10, and 30 ng/mL) ( $n = 3$ ).

Table 1

Comparison of the proposed fluorescence “turn off” aptasensor with other nanomaterials based aptasensors.

Detection system	LOD	Sample	Ref.
A fluorescence aptasensor based on semiconductor quantum dots and MoS <sub>2</sub> nanosheets	1 ng/mL	Red wine	(Lu et al., 2017)
A dual-channel fluorescence aptasensor based on Texas red-labelled P-OTA-36 and GO	15.17 ng/mL	White wine	(Wang et al., 2019)
Aptamer-based fluorescent sensor by quenching of gold nanoparticles	9.148 ng/mL	Cornmeal, beer, coffee	(Lv et al., 2017)
An aptasensor based on single-walled carbon nanohorn	6.932 ng/mL	Red wine	(Lv et al., 2016)
A fluorescence aptasensor based on a double-stranded DNA competition mode and GO nanosheets	0.011 ng/mL	Malt	<b>This work</b>

assaying OTA.

#### Specificity investigation

The proposed aptasensor should be selective enough for specific assay of trace OTA without interferences from other analogues or mycotoxin molecules. In order to evaluate the specificity of this developed aptasensor for OTA, other mycotoxins, such as AFB<sub>1</sub>, ZEN, DON, and T-2 toxin as interfering substances were measured. As shown in Fig. 5, only OTA induced a significantly higher  $\Delta F$  values than other tested mycotoxins. The  $\Delta F$  value was around 1200 a.u. for OTA, and was less than 400 a.u. for AFB<sub>1</sub>, ZEN, DON, and T-2 toxins, which would not affect the detection of OTA. All these findings powerfully proved the high selectivity and specificity of the proposed fluorescent aptasensor for OTA detection.

#### Real sample analysis

Here, malt sample was selected for OTA detection to investigate the feasibility and reliability of the developed fluorescent “signal off” aptasensor in real-world application. The OTA-free malt samples were spiked with known low (1 ng/mL), medium (10 ng/mL) and high (30 ng/mL) concentrations of OTA, followed by the above-describe procedure to prepare the tested sample solution for OTA analysis by using the developed fluorescence aptasensor. As shown in Table 2, the average recovery rate ranged from 95.50% to 112.05%. This illustrated that the

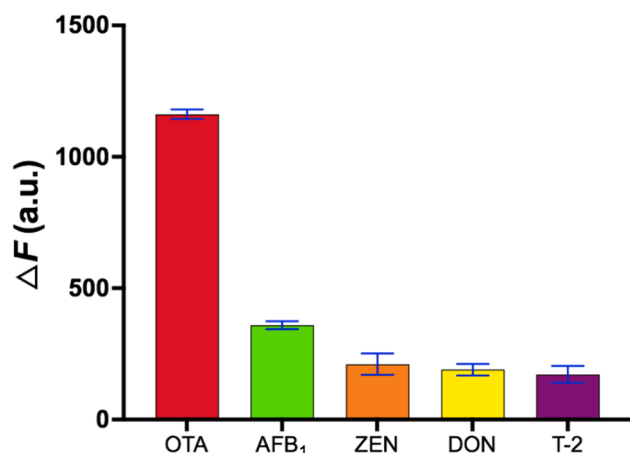


Fig. 5. Specificity of the fluorescence aptasensor toward OTA against other mycotoxins at the same concentration (30 ng/mL) ( $n = 3$ ).

Table 2

Recovery measured by the proposed fluorescence aptasensor in malt samples ( $n = 3$ ).

Sample	OTA added (ng/mL)	Measured (ng/mL)	Recovery (%)
1#	1	0.96 ± 0.09	96.67
2#	10	11.20 ± 0.70	112.05
3#	30	28.65 ± 0.96	95.50

quantitative analysis of OTA in malt samples by the newly-developed fluorescent aptasensor could be potentially applied to monitor OTA contamination levels in real malt samples.

## Conclusions

In this study, a novel aptasensor based on the fluorescence quenching from GO nanosheets on FAM fluorophores labeled on cDNA was developed for OTA detection. On the positive sides: 1) Compared with most single-stranded reactions, this work deliberately selected cDNA-FAM as signal probe and first pointed out that combining the double-stranded structure with GO nanosheets to construct the fluorescence system could avoid the sensitivity of system reducing caused by the non-covalent effect between the aptamer-target conjugate and the quencher; 2) Under the optimum conditions, the developed fluorescent aptasensing platform exhibited outstanding sensitivity with a LOD of 11 pg/mL and a wide dynamic range over 0.04 ng/mL to 30 ng/mL. The selectivity of this aptasensor for OTA was confirmed against other tested mycotoxins, and the feasibility and reliability of which was verified by determining OTA in the spiked malt samples; 3) In addition, in order to avoid the influence of guanine on the fluorophore, the important reminders were given to choose the end of DNA that far away from guanine labeling FAM and to form a saturated double-stranded structure during the reaction. Simultaneously, due to the commonality of fluorophores and quenchers, there are also many further improvement strategies that can be explored through: 1) Replacing FAM with nanoparticles exhibiting better properties, such as quantum dots or up-conversion nanoparticles; 2) Increasing GO functionality, such as using magnetic GO to separate and reduce interference; 3) Designing and adding a new reaction program to achieve signal amplification; and 4) Conforming the method certainty for its practical application in real samples through other standard approaches, such as HPLC-MS/MS.

In conclusion, the proposed method with low cost, high sensitivity, simple operation, strong specificity, and rapid response provided a reliable solution for OTA detection in the field of on-site food analysis and could further be a universal aptamer-based platform for the analysis

of more mycotoxins and other contaminants.

## CRedit authorship contribution statement

**Qing Zhang:** Formal analysis, Writing – original draft. **Linzi Kang:** Resources, Methodology. **Pengfei Yue:** Formal analysis, Writing – original draft. **Linchun Shi:** Data curation, Supervision. **Meng Wang:** Validation, Investigation. **Lidong Zhou:** Conceptualization, Supervision. **Haiping Zhao:** Supervision, Funding acquisition. **Weijun Kong:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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