

# An aptamer-based biosensor for detection of aflatoxin M<sub>1</sub>

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Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>), one of the most toxic mycotoxins, imposes serious health hazards. AFM<sub>1</sub> had previously been classified as a group 2B carcinogen<sup>1</sup> and has been classified as a group 1 carcinogen by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO).<sup>2</sup> Determination of AFM<sub>1</sub> thus plays an important role for quality control of food safety. In this work, a sensitive and

reliable aptasensor was developed for the detection of AFM<sub>1</sub>. The immobilization of aptamer through a strong interaction with biotin–streptavidin was used as a molecular recognition element, and its complementary ssDNA was employed as the template for a real-time quantitative polymerase chain reaction (RT-qPCR) amplification. Under optimized assay conditions, a linear relationship (ranging from 1.0×10<sup>-4</sup> to 1.0 μg L<sup>-1</sup>) was achieved with a limit of detection (LOD) down to 0.03 ng L<sup>-1</sup>. In addition, the aptasensor developed here exhibits high selectivity for AFM<sub>1</sub> over other mycotoxins and small effects from cross-reaction with structural analogs. The method proposed here has been successfully applied to quantitative determination of AFM<sub>1</sub> in infant rice cereal and infant milk powder samples. Results demonstrated that the current approach is potentially useful for food safety analysis, and it could be extended to a large number of targets.

## Introduction

Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>), one of the most toxic contaminants in dairy products, is a metabolite produced by dairy cows as a result of being fed with feeds contaminated with Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>).<sup>3-5</sup> Once present in dairy products, AFM<sub>1</sub> poses a hazard to humans (especially infants) who consume them.<sup>6</sup> Taking this threat into consideration, many countries have set maximum limits for AFM<sub>1</sub> and established various regulations.<sup>7,8</sup> The European Union (EU) set a maximum tolerated level of AFM<sub>1</sub> to 0.050 μg/kg for adult consumption and subsequently more restrictively to

0.025 µg/kg for food for infants and children.<sup>9</sup> In China and the United States, the maximum limit for AFM<sub>1</sub> is 0.5 µg/kg in milk.<sup>6,10</sup> Hence, the development of simple, sensitive and selective methods to determine the presence and level of AFM<sub>1</sub> is much in demand for food safety organizations to implement regulatory requirements.

Approaches have been developed in recent years for quantitative determination of AFM<sub>1</sub> including high-performance liquid chromatography (HPLC) with fluorescence detection (FLD),<sup>6,11-13</sup> high-performance liquid chromatography (HPLC) combined with mass spectrometry (MS).<sup>8,14,15</sup> However, the quantitative approaches require complicated pretreatment, professional operators and expensive instruments. Some immunological methods like enzyme-linked immune sorbent assay (ELISA)<sup>16-18</sup> and immunosensors<sup>19-21</sup> have also been reported for AFM<sub>1</sub> detection. There are disadvantages in both preparation of the antibody and its stability and this limits its application in the field. Recently, application of aptamer-based biosensors for mycotoxins has shown a remarkable potential with the advantages of low cost, high stability, easy synthesis and ease of modification compared with antibodies. Since the report of an aptamer for ochratoxin A (OTA) in 2008,<sup>22</sup> many aptasensors for OTA and AFB<sub>1</sub> have been developed for feed and food safety.<sup>23-29</sup> In our previous study,<sup>27</sup> an aptasensor based on qPCR was successfully designed for AFB<sub>1</sub> determination with high sensitivity. Aptasensors were developed for the detection of AFM<sub>1</sub> using electrochemical methods and impedance spectroscopy techniques.<sup>30,31</sup> However, the

selectivity of the aptamer for AFM<sub>1</sub> was not clear since only irrelevant OTA was chosen as an interference to study the cross-reaction. Cross-activity tests between other toxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub> and AFG<sub>2</sub>) should be carried out to answer the question whether the aptasensor is suitable for quantifying the AFM<sub>1</sub> concentration in real samples.<sup>30</sup> Recently, an aptamer specific to AFM<sub>1</sub> with a dissociation constant (K<sub>d</sub>) value of 35 nM has been reported.<sup>32</sup> To the best of our knowledge, aptasensor based on this aptamer for AFM<sub>1</sub> detection have not been reported.

In this study, a new aptamer-based biosensor to sensitively and selectively detect AFM<sub>1</sub> was developed, combining the advantages of strong recognition ability of the aptamer to AFM<sub>1</sub> and excellent amplification efficiency of the RT-qPCR technique to improve sensitivity. Six complementary ssDNA fragments were designed to explore the binding sites between the specific aptamer and AFM<sub>1</sub>. The presence of AFM<sub>1</sub> induced the release of complementary ssDNA because of forming an aptamer/AFM<sub>1</sub> complex, leading to the reduction in the amount of PCR template and the increase in cycle numbers. Quantification of AFM<sub>1</sub> has been achieved according to the linear relationship between the change of the PCR amplification signal and AFM<sub>1</sub> levels.

## **Results and Discussion**

### **Optimization of the amplification of complementary ssDNA**

The complementary ssDNA is applied as the subsequent PCR template. The

melting curve in this step is a key factor which affects amplification efficiency and primer specificity. The concentration of the complementary ssDNA and specificity of the primer should thus be optimized. Amplification curves are shown in Fig. S1(A). As the concentration of complementary ssDNA decreases over the range of  $1 \times 10^{-3}$  to 10 nM the cycle number ( $C_t$ ) increased. Corresponding to the amplification curve the standard curve relating the cycle number threshold ( $C_t$ ) and the complementary ssDNA in the range of  $1 \times 10^{-3}$  to 10 nM is shown in Fig. S1(B), demonstrating the sensitive and quantitative detection of the complementary ssDNA with high amplification efficiency (103.1%) and a good linear relationship with a high correlation coefficient (0.995). The linear regression equation was described by  $C_t = -3.2495 \log C + 36.363$ , where  $C_t$  is cycle threshold number and  $C$  is the concentration of the complementary DNA. The optimal concentration of the complementary ssDNA was 10 nM with the lowest  $C_t$  values. The PCR melting curves are shown in Fig. S2, demonstrating the specificity of the PCR amplification without the appearance of primer dimers or other nonspecific DNA products since an obvious single peak was observed at 80°C.

### **Optimization of streptavidin and the biotinylated aptamer**

Other factors would affect the performance of this method including the concentrations of streptavidin, the biotinylated aptamer and the complementary ssDNA and these all should be optimized. Under a fixed concentration of complementary ssDNA at 10 nM, the concentrations of streptavidin and the

biotinylated aptamer were analyzed for the PCR amplification signal change (Fig. S3). The adsorptive power of streptavidin to PCR tubes and the binding ability of streptavidin-coated tubes to biotinylated aptamer was primarily detected using different concentrations of streptavidin (Fig. S3). This also shows that there was a clear difference of Ct values between the control group and streptavidin-coated tubes, which showed the intense adsorptive ability between biotinylated aptamer and streptavidin-coated tubes. In analysis of the Ct values at different concentrations of streptavidin, the Ct value reached the lowest level when the concentration of streptavidin was  $2.5 \text{ ng mL}^{-1}$ . This graph also shows that the Ct values decreased with the increase of the amount of aptamer when aptamer levels were below  $10.0 \text{ nM}$  and also that the Ct values increased with the amount of aptamer above  $10.0 \text{ nM}$  mainly because of steric hindrance. Thus,  $2.5 \text{ ng mL}^{-1}$  of streptavidin and  $10 \text{ nM}$  of aptamer were the optimal conditions for RT-qPCR amplification.

### **AFM<sub>1</sub> determination**

Under optimal conditions, the amplification curves and calibration curve of this aptasensor for different levels of AFM<sub>1</sub> with AFM<sub>1</sub> DNA1 were determined by RT-qPCR (Fig. 1). As is shown in Fig. 1(A), the cycle number increased with an increase in AFM<sub>1</sub>. More complementary ssDNA would be released when more AFM<sub>1</sub> is present in the reaction system, which leads to a decrease in the amount of the PCR template and an increase in cycle number. Meanwhile, a good linear relationship between Ct values and AFM<sub>1</sub> levels in the range of  $1 \times 10^{-4}$  to  $1 \text{ } \mu\text{g L}^{-1}$  was obtained as

indicated in Fig. 1(B) with the limit of detection  $0.03 \text{ ng L}^{-1}$  ( $S/N=3$ ). The linear regression equation was described by  $Ct=3.703 \log C + 20.736$  ( $R^2=0.998$ ), where  $Ct$  is cycle threshold number and  $C$  is  $AFM_1$  concentration. All the detection conditions of the other five  $AFM_1$  DNA were identical to those used in the  $AFM_1$  DNA1 procedure, allowing a comparison of the performance of amplification curves (Fig. 2). Results clearly show the determination of  $AFM_1$  DNA1 has the best results with excellent amplification efficiency, indicating the binding site between the specific aptamer and  $AFM_1$  mainly exists in the position close to 5'-terminal of the aptamer. In addition, the aptasensor in this study demonstrates a high sensitivity in  $AFM_1$  determination in comparison to other current approaches (Table 1).

### **Specificity analysis**

The specificity of the aptasensor plays an important role in the development and practicality of this method. In order to evaluate the specificity of the detection system, the change of PCR amplification produced by other five mycotoxins (including OTA, ZEN,  $FB_1$ ,  $AFB_1$  and  $AFB_2$ ) was determined. As shown in Fig.3, the detection of OTA, ZEN,  $FB_1$ , as well as the control, had no obvious  $Ct$  value changes at the concentration of  $1 \text{ ng mL}^{-1}$  among the five mycotoxins. However,  $AFB_1$  and  $AFB_2$ , the structural analogs of  $AFM_1$ , resulted in a slight increase of the  $Ct$  values but no significant effect ( $P>0.05$ ). In addition, a similar result was detected using a mix of these five mycotoxins without  $AFM_1$  (Mix1). The corresponding  $Ct$  value of  $AFM_1$  in a mix of these five mycotoxins (Mix2) was slightly lower than the  $Ct$  value of  $AFM_1$

alone with no significant difference between Mix2 and AFM<sub>1</sub>. This sensing system thus has high specificity for the detection of AFM<sub>1</sub> owing to the high recognition ability of the biotin-labeled aptamer to the target, and the inability of the aptamer to recognize other mycotoxins.

### **Repeatability analysis**

The repeatability of this method is an important issue for the development and practical implementation of AFM<sub>1</sub> detection, which was assessed by analyzing the Ct values of the same sample (1.0 ng mL<sup>-1</sup> AFM<sub>1</sub>) five times. As indicated in Fig. S4, results showed a good repeatability of the measurements with a relative standard deviation (RSD) of 5.0%.

### **Method validation**

In order to evaluate feasibility and reliability of this method, we applied it to the determination of different concentrations of AFM<sub>1</sub> in infant rice cereal samples and infant rice cereal samples. As shown in Table 2, The recoveries of the spiked infant rice cereal and infant rice cereal samples were in the range of 84–106% and 68–80.3%, correspondingly, indicating that the proposed aptasensor was suitable for quantitative determination of mycotoxins in food samples for quality control of food safety. However, the relatively low recovery in the infant rice cereal samples might be due to sample pretreatments, and the future work would focus on improving sample pretreatments to AFM<sub>1</sub> determination for food safety.



## Experimental

### Methods

The aim of this work was to develop an aptasensor for the detection of AFM<sub>1</sub>. The schematic of the sensing method is described in Fig. 4. The aptasensor is based on conformational change of the aptamer owing to the formation of AFM<sub>1</sub>/aptamer complex and release of complementary ssDNA and signal amplification by RT-qPCR. Firstly, a strong interaction of biotin–streptavidin (Fig. 4A) results in the immobilization of the aptamer on the surface of streptavidin-coated PCR tubes. The complementary ssDNA, as the amplification template of PCR, is partly hybridized with the single-strand aptamer to form dsDNA, which is stable in the absence of AFM<sub>1</sub>, resulting in no obvious changes of the amount of the complementary ssDNA as the template for RT-qPCR amplification. Upon the addition of AFM<sub>1</sub>, a binding event between the aptamer and AFM<sub>1</sub> (Fig. 4B) induces a conformational change in the aptamer that leads to the release of the complementary ssDNA, with the result that the amount of template was reduced (Fig. 4C). As a consequence, for the aptasensor, a strong signal change in PCR amplification was observed, which can be used for the quantification of the concentration of AFM<sub>1</sub>.

### Materials and reagents

Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) was purchased from Sigma-Aldrich (USA). Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) was obtained from the National Standard Reference Center (Beijing, China). Ochratoxin A (OTA), zearalenone (ZEN), aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), and fumonisin (FB<sub>1</sub>) were

purchased from Pribolab Co. Ltd (Singapore). Streptavidin was obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China). Other chemicals such as sodium bicarbonate ( $\text{NaHCO}_3$ ), anhydrous calcium chloride ( $\text{CaCl}_2$ ), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), ethylenediaminetetraacetic acid (EDTA), sodium chloride ( $\text{NaCl}$ ), potassium chloride ( $\text{KCl}$ ), 2-Amino-2-(hydroxymethyl)-1,3-propanediol (Tris), , and sodium citrate ( $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$ ) were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Water was purified with a Milli-Q purification system.

The SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> II (includes SYBR<sup>®</sup> Premix Ex Taq<sup>®</sup> (2×)(SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> II (Perfect Real Time)) & ROX Reference Dye II(50×)) were purchased from Takara Bio Co. Ltd. (Dalian, China). In order to explore the binding sites between the specific aptamer and AFM<sub>1</sub>, the complementary DNA fragment of the aptamer was designed with six alternative sequences. The aptamer with 3'-terminal biotin groups was chemically synthesized by Genecreate Biological Co. Ltd. (Wuhan, China), and the complementary DNA fragments were chemically synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China) and purified by HPLC. Their sequences are as follows:

AFM<sub>1</sub> Aptamer<sup>32</sup>:

5'-ATCCGTCACACCTGCTCTGACGCTGGGGTTCGACCCGGAGAAATGCATTCCCCTGTGGTGTT  
GGCTCCCGTAT-3'

Complementary DNA (AFM<sub>1</sub> DNA):

AFM<sub>1</sub> DNA1:

5'-GGTGTGACGGATAATCTGGTTTAGCTACGCCTTCCCCGTGGCGATGTTTCTTAGCGCCTTAC  
-3'

AFM<sub>1</sub> DNA2:

5'-AGCGTCAGAGCAAATCTGGTTTAGCTACGCCTTCCCCGTGGCGATGTTTCTTAGCGCCTTAC  
-3'

AFM<sub>1</sub> DNA3:

5'-CGGGTCGACCCCAATCTGGTTTAGCTACGCCTTCCCCGTGGCGATGTTTCTTAGCGCCTTAC  
-3'

AFM<sub>1</sub> DNA4:

5'-AATGCATTTCTCAATCTGGTTTAGCTACGCCTTCCCCGTGGCGATGTTTCTTAGCGCCTTAC-  
3'

AFM<sub>1</sub> DNA5:

5'-ACACCACAGGGGAATCTGGTTTAGCTACGCCTTCCCCGTGGCGATGTTTCTTAGCGCCTTA  
C-3'

AFM<sub>1</sub> DNA6:

5'-ATACGGGAGCCAAATCTGGTTTAGCTACGCCTTCCCCGTGGCGATGTTTCTTAGCGCCTTAC  
-3'

Upstream primer: 5'-AATCTGGTTTAGCTACGCCTTC-3'

Downstream primer: 5'-GTAAGGCGCTAAGAAACATCG-3'

### **Immobilization of the aptamer**

Based on our previous studies,<sup>27</sup> we carried out immobilization of the aptamer with some modifications. The details are as follows. PCR tubes were treated with 50  $\mu$ L 0.8% glutaraldehyde solution at 37°C for 5 h in order to improve their

adsorbability. After washing three times with ultrapure water, 50  $\mu\text{L}$  of streptavidin dissolved in 0.01 M carbonate buffer solution was added and incubated at 37°C for 2 h. Next, the tubes were washed twice with PBST (10 mM PBS, pH 7.2, 0.05% Tween-20). The aptamer and its complementary ssDNA were mixed sufficiently in a hybridization buffer (750 mM NaCl, 75 mM  $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7$ , pH 8.0) in the ratio 1:1 (v/v), and 50  $\mu\text{L}$  of the mixture was added to each tube and incubated at 37°C for 1 h. Then the tubes were subsequently washed three times with hybridization buffer to remove the uncombined DNA fragments.

#### **RT-qPCR measurements for AFM<sub>1</sub>**

50  $\mu\text{L}$  of AFM<sub>1</sub> standard solution was added and incubated with Tris buffer (10 mM Tris, 120 mM NaCl, 5 mM KCl, 20 mM  $\text{CaCl}_2$ , pH 7.0) at 45°C for 1 h. Then all PCR tubes were washed three times with the Tris buffer to remove the uncombined AFM<sub>1</sub> and the released complementary ssDNA.

In the next step, RT-qPCR was carried out using the ABI 7500 Real-Time PCR System (USA). The 50  $\mu\text{L}$  PCR mixture consisted of 2  $\mu\text{L}$  of 10  $\mu\text{M}$  upstream and downstream primers, respectively, 25  $\mu\text{L}$  SYBR<sup>®</sup> Premix Ex Taq<sup>®</sup> (2 $\times$ ), 1  $\mu\text{L}$  of ROX Reference Dye II(50 $\times$ ) and 20  $\mu\text{L}$  water. The reaction conditions of real-time PCR were as follows: an initial denaturation for 30 s at 95°C, followed by 40 cycles of denaturation for 5 s at 95°C, and annealing for 34 s at 60°C. The amplification efficiency (E) of RT-qPCR was determined using the formula,  $E=10^{(-1/\text{slope})}-1$ , where the slope is estimated from the standard curve.<sup>27,38</sup> Fluorescence measurements

were taken after each annealing step. A melting curve analysis was performed from 60°C to 95°C to detect potential nonspecific products with the following conditions: an initial denaturation for 15 s at 95°C, followed by 40 cycles of denaturation for 1 min at 60°C, and annealing for 15 s at 95°C.

### **Specificity analysis**

In order to assess the selectivity of this aptasensor and investigate whether presence of other mycotoxins could interfere with the detection of AFM<sub>1</sub>, the following mycotoxins, including OTA, ZEN, FB<sub>1</sub>, AFB<sub>1</sub> and AFB<sub>2</sub>, were applied to the aptasensor. These mycotoxins were used at the same concentration of 1 ng mL<sup>-1</sup>. All other experimental conditions were identical to that for AFM<sub>1</sub> determination, and the change in cycle number among these mycotoxins was compared.

### **Method validation**

Application of the method to infant rice cereal samples and infant milk powder was conducted to validate the AFM<sub>1</sub> determination. Infant rice cereal samples were spiked with AFM<sub>1</sub> at 5×10<sup>-4</sup>, 5×10<sup>-3</sup> and 0.05 ng mL<sup>-1</sup> (3 replicates per treatment), and infant milk powder samples were spiked with AFM<sub>1</sub> at 5×10<sup>-4</sup>, 5×10<sup>-2</sup> and 0.1 ng mL<sup>-1</sup> (3 replicates per treatment). Each sample was accurately weighed (0.5 g) after drying into 10 ml centrifuge tubes. Then, 2.5 mL of 70% methanol in water was added to extract AFM<sub>1</sub> from the sample. The entire mixture was vortexed for 5 min using Vortex-Genie 2 (Scientific Industries, USA) and subsequently centrifuged at 10,000 g for 10 min. The supernatant was collected and concentrated to 0.5 mL under a

nitrogen stream. Finally, each residue was re-dissolved in 2 ml of aqueous methanol solution (5%) and subjected to RT-qPCR.

### **Statistical analysis**

Each analysis (aflatoxin calibration curve standards and test samples) was performed in triplicate. Amplification curves for AFM<sub>1</sub> were plotted with Origin 8.0 software (OriginLab Corporation, Northampton, MA, USA). Simple linear regression analysis of the cycle threshold number (Ct) values on log concentrations of AFM<sub>1</sub> were carried out using Microsoft Excel. Standard deviations (SDs) and means for Ct values were obtained from three replicates.

### **Conclusions**

In this work, we describe a reliable and sensitive aptamer-based biosensor for determination of AFM<sub>1</sub>, which simultaneously combined the advantages of high recognition power of the aptamer to AFM<sub>1</sub> and excellent amplification efficiency of RT-qPCR technique to improve sensitivity. Under optimal conditions, a good linear relationship existed between Ct values and AFM<sub>1</sub> levels over the range from  $1 \times 10^{-4}$  to  $1 \mu\text{g L}^{-1}$  with high sensitivity ( LOD =  $0.03 \text{ ng L}^{-1}$  ). The detection of five other mycotoxins was limited, but there may be a small cross-reaction with AFB<sub>1</sub>. This shows its value in the determination of AFM<sub>1</sub> for food safety with an acceptable selectivity. Importantly, this method can be applied to the detection of AFM<sub>1</sub> in infant rice cereal and infant rice cereal samples with satisfactory recoveries.

Therefore, this aptasensor has a highly potential application for biologically small molecules.

## Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 21305158), the Special Fund for Agro-scientific Research in the Public Interest (201403071), Modern Agro-Industry Technology Research System of the PR China (nycytx-04-01), Project of risk assessment on raw milk (GJFP2015009), and The Agricultural Science and Technology Innovation Program (ASTIP-IAS12).

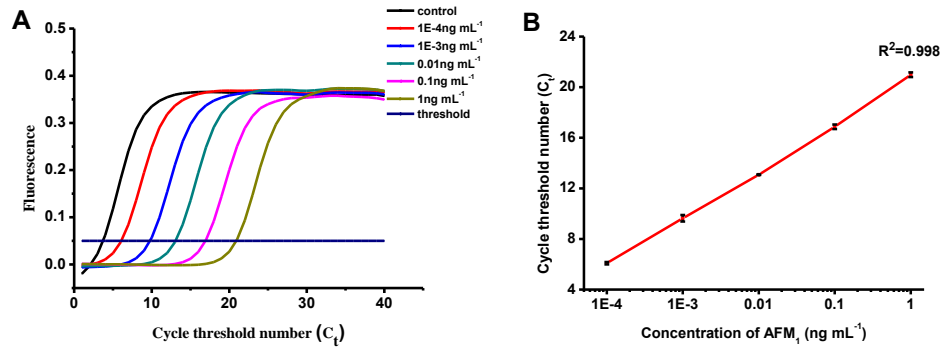
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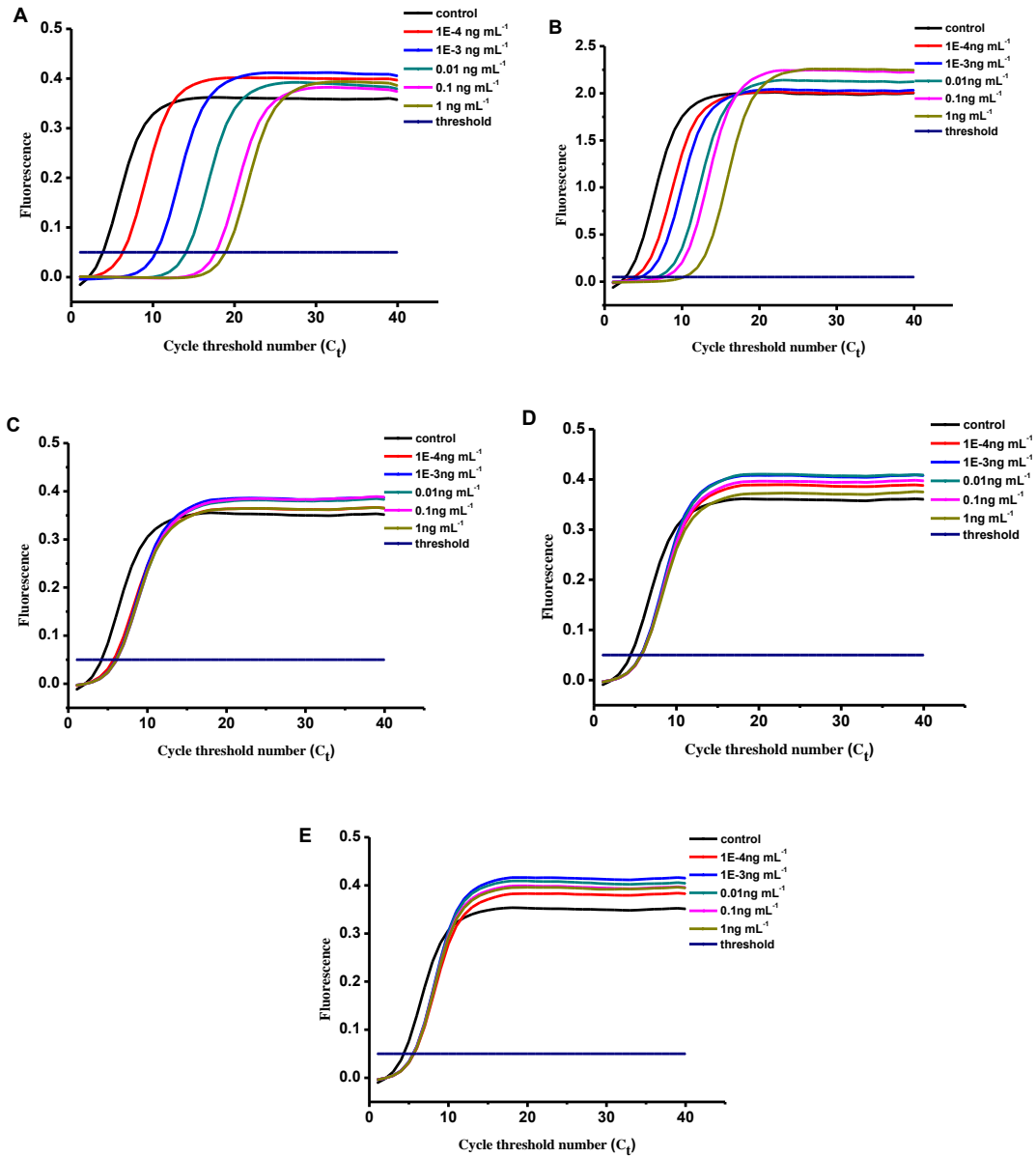
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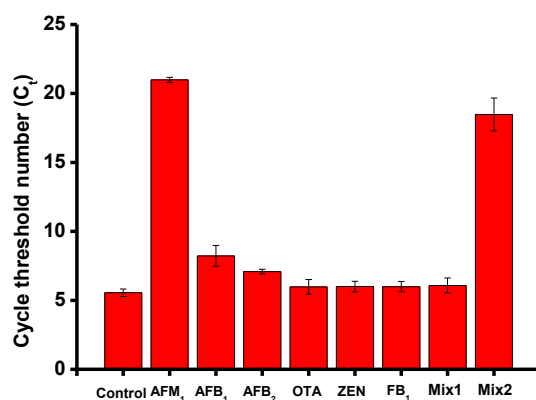
**Fig. 1.** (A) The amplification curves at different concentrations of AFM<sub>1</sub> in the range of  $1 \times 10^{-4}$  to  $1 \mu\text{g L}^{-1}$  for the determination of AFM<sub>1</sub> DNA1, including the negative control without AFM<sub>1</sub>. (B) The standard curves between the AFM<sub>1</sub> concentration and the Ct value in the range of  $1 \times 10^{-4}$  to  $1 \mu\text{g L}^{-1}$ .



**Fig. 2.** The amplification curves at different concentrations of AFM<sub>1</sub> in the range of  $1 \times 10^{-4}$  to  $1 \mu\text{g L}^{-1}$  for the determination of different AFM<sub>1</sub> DNA, including the negative control without AFM<sub>1</sub>. (A) AFM<sub>1</sub> DNA2; (B) AFM<sub>1</sub> DNA3; (C) AFM<sub>1</sub> DNA4; (D) AFM<sub>1</sub> DNA5; (E) AFM<sub>1</sub> DNA6.

**Table 1.** Comparison of the sensitivity of currently available methods for the detection of AFM<sub>1</sub>.

No.	Method	LOD	reference
1	LC/MS/MS	6 ng L <sup>-1</sup>	33
2	Fluorometric sensor	50 ng L <sup>-1</sup>	34
3	Electrochemical immunosensors	1 ng L <sup>-1</sup>	35
4	Indirect competitive ELISA	0.04 µg L <sup>-1</sup>	3
5	Impedimetric biosensor	1 µg L <sup>-1</sup>	36
6	HPLC	6 ng L <sup>-1</sup>	11
7	Cellular biosensor	5 ng L <sup>-1</sup>	37
8	Direct chemiluminescent ELISA	1 ng L <sup>-1</sup>	21
9	DART-MS	0.1 µg L <sup>-1</sup>	10
10	SPE-UPLC–MS/MS	0.25 ng L <sup>-1</sup>	15
11	RT-qPCR based aptasensor	0.03 ng L <sup>-1</sup>	This work

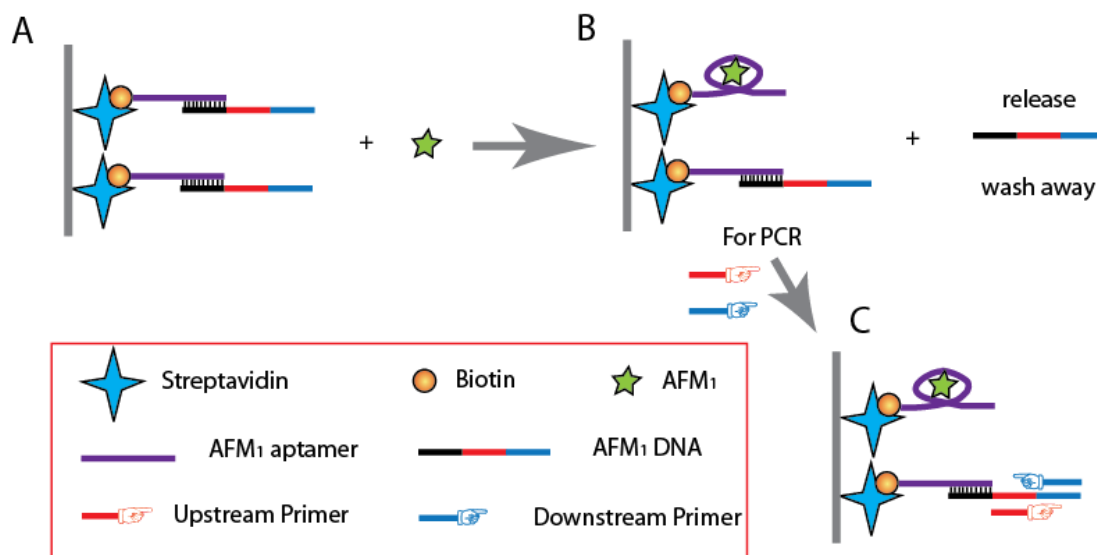


**Fig. 3.** The Ct values in the absence and presence of 1 ng mL<sup>-1</sup> mycotoxins including AFM<sub>1</sub>, OTA, ZEA, AFB<sub>1</sub>, AFB<sub>2</sub>, FB<sub>1</sub>, Mix1 (OTA, ZEA, AFB<sub>1</sub>, AFB<sub>2</sub>, FB<sub>1</sub>) and Mix2 (OTA, ZEA, AFB<sub>1</sub>, AFB<sub>2</sub>, FB<sub>1</sub>, and AFM<sub>1</sub>). The experiment conditions are as following: complementary ssDNA 10 nM, aptamer 10 nM, and streptavidin 2.5 ng mL<sup>-1</sup>. Means and standard deviations are shown with three replicates for each treatment.

**Table 2.** Determination of AFM<sub>1</sub> spiked into infant rice cereal samples.

Sample	Spiked concentration (pg/mL)	Detected concentrations Mean <sup>a</sup> ±SD <sup>b</sup> (pg/mL)	Recovery (%)
Infant rice cereal	50.0	45.0±5.7	90
	5.0	5.3±0.3	106
	0.50	0.42±0.02	84
Infant milk powder	100.0	80.3±6.4	80
	50.0	36.9±1.8	74
	0.50	0.34±0.02	68

a. The mean of three replicates; b. SD=standard deviation



**Fig. 4.** Schematic to illustrate the aptasensor for detection of Aflatoxin M<sub>1</sub>.