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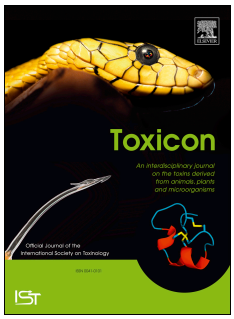


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# A Direct Determination of AFBs in vinegar by aptamer-based surface plasmon resonance biosensor

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**Abstract:** Aflatoxin (AFB) is one of the most toxic fungal metabolites produced by *Aspergillus flavus*, which may contaminate food and agricultural products. Herein, an aptamer-based surface plasmon resonance (SPR) biosensor was developed to detect AFBs. The chosen aptamer showed comparable interaction with the two AFBs, namely aflatoxin B1 (AFB1) and aflatoxin B2 (AFB2). Such phenomenon was rarely reported, and might lead to a simultaneous detection of both AFBs. In this study, AFB1 was used to systematically establish the detection method. In the SPR system, streptavidin proteins were immobilized on the surface of a CM5 sensor chip as a cross-linker and biotin-aptamers were captured through streptavidin-biotin interaction. After optimization, the assay showed a dynamic range between 0.09 and 200 ng·mL$^{-1}$ (linear range from 1.5 to 50 ng·mL$^{-1}$ and a LOD of 0.19 ng·mL$^{-1}$) of AFB1 in buffer. As expected, the aptasensor showed high specificity towards AFB1 and AFB2, but hardly bound to other toxins with similar structures, including ochratoxin A (OTA), ochratoxin B (OTB), Zeralenone (ZEA) and T-2 toxin (T-2). Determination of AFB1 in vinegar was further performed using the SPR biosensor. Recoveries of AFB1 from spiked samples ranged from 96.3 to 117.8%. The developed SPR assay is a simple, fast and sensitive approach for the detection of residual AFBs in agricultural products and foodstuffs like vinegar.

**Keywords:** Aflatoxin; SPR; Aptamer; Vinegar analysis

## 1. Introduction

Mycotoxins, secondary metabolites produced by fungi, are the most widespread potential contaminants which pose health risks for humans and animals(Kong et al., 2016). They are commonly regarded as pathogenic factors of liver cancer, and even trace amount could cause serious health problems. It is reported by the Food and Agriculture Organization (FAO) that 25% of the world's food crops are contaminated by mycotoxins(Smeesters et al., 2015). Aflatoxin (AFB), which is one of the most common contaminants in spoiled food and feed, is a secondary metabolite produced by *Aspergillus flavus*. So far, several aflatoxin analogs have been identified, among which aflatoxin B1 (AFB1) is the most carcinogenic and can cause acute toxic effect. The toxicity of AFBs was much higher than ochratoxin, and AFB1 has been listed as group I carcinogens by the International Agency for Research on Cancer (IARC). The AFBs have been detected in wines(Giovannoli et al., 2015; Wei et al., 2015), groundnuts(Chauhan et al., 2015), ground corns(Luo et al., 2014; Seok et al., 2015), wheat(Iqbal et al., 2014), walnuts,(Golge et al., 2016) cheeses(Pietri et al., 2016) and soybean sauces(Liu et al., 2015). Therefore, it is very important to establish highly sensitive and simple methods for the determination of AFBs in food and agricultural products.

Several analytical methods have been developed to detect AFBs in foodstuffs and agricultural products, such as high performance liquid chromatography (HPLC)(Afsah-Hejri et al., 2011), liquid chromatography–tandem mass spectrometry (LC-MS/MS)(Capriotti et al., 2014; Dzuman et al., 2015), immunochromatographic assay (ICA) strip(Kong et al., 2017a; Kong et al., 2017b; Kong et al., 2017c), surface plasmon resonance (SPR)(Zhu et al., 2015) and enzyme-linked immunosorbent assay (ELISA)(Lee et al., 2004; Liu et al., 2013a; Kong et al., 2017d). Chromatographic methods, including HPLC and LC-MS, are the most acceptable ones for the detection of mycotoxins. They have been widely used for mycotoxins detection in the past few years owing to their high sensitivity(Golge et al., 2016; Liu et al., 2013b). However, the pre- or post-column derivatization is relatively complicated in high performance

liquid chromatography with fluorescence detection (HPLC-FLD), while the tedious sample preparation and expensive instrumentation have also limited the application of LC-MS. As a specific and low-cost technique, ELISA is easy to perform and analyze numerous samples simultaneously. However, the conventional ELISA is limited by the properties of antibody, such as laborious, time-consuming and instability due to their denaturation and degradation(Li et al., 2015; Toh et al., 2015).

SPR is one of the dominant optical technologies which occurs at the interface between thin conducting films and media of different refractive indexes(Yola et al., 2014a). SPR technique was developed to study biomolecular interaction based on the differences in the refractive index of molecules on the surface(Li et al., 2012), and can be applied to monitor the molecular interactions in real-time(Canoa et al., 2015). Recently, SPR biosensor has been applied in biochemical inspection(Mazumdar et al., 2010; Wang et al., 2009a; Wang et al., 2009b), food safety analysis(Ashley and Li, 2013; Park et al., 2014), quantification of proteins and toxins, and determination of affinity-binding constants. Yola *et al*(Yola et al., 2014b) have described a highly sensitive SPR-based imprinted nanosensor for the determination of amoxicillin, and the method was also applied to determine citrinin in red yeast rice(Atar et al., 2015), patulintoxin(Pennacchio et al., 2014) and oxytocin in milk samples(Yola et al., 2015).

Aptamers are single-stranded oligonucleotides selected through an *in-vitro* process named Systematic Evolution of Ligands by Exponential Enrichment (SELEX). Aptamers have been considered as a good alternative to antibodies for many obvious advantages, such as low cost and easy modification with biotin, hydrosulphonyl and enzymes, which make them attractive in the field of mycotoxins detection. In this study, we developed a sensitive and rapid aptamer-based SPR method for the detection of AFB1, and it was successfully applied for the direct detection of AFB1 in vinegar. Such aptamer-based SPR method is promising for the rapid detection of various biotoxins.

## 2. Materials and methods

### 2.1 Chemicals and regents

Aflatoxin B1, Aflatoxin B2 (from *Aspergillus flavus*) and related mycotoxins (ochratoxin A, ochratoxin B, Zeralenone, T-2 toxin) were purchased from Pribolab (Singapore) and dissolved in DMSO at a concentration of 2 $mg \cdot mL^{-1}$. Standardized vinegar samples were obtained from a local supermarket (Beijing, China). N-hydroxysuccinimide, ethyl 1-Ethyl-3-(dimethylaminopropyl) carbodiimide, DMSO and Tris base were purchased from Sigma Aldrich (China). Streptavidin (SA) was also purchased from Sigma Aldrich (China) and dissolved in 1×PBS. The AFB1-aptamer is patented by Neoventures Biotechnology Inc. (Canada) (Patent: PCT/CA2010/001292). Biotinylated aptamer ligand of sequence biotin 5′-GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCGCTAGGCCCACA -3′ was obtained from Sangon Biotech (Shanghai, China) and reconstituted in 0.1M Tris-HCl (pH 7.4). Ethanolamine–HCl (pH 8.5), 10mM sodium acetate (pH 5.5, 5.0, 4.5, 4.0), CM5 sensor chips and PBS were purchased from GE Healthcare (China). The Isothermal Titration Calorimeter (MicroCal™ iTC200), SPR biosensor (BIAcore T200) were obtained from GE Healthcare Bio-Sciences AB (America). All solutions were prepared using ultrapure water obtained from a Milli-Q ultrapure water system (Millipore Corporation, Bedford, MA, US).

**2.2 Isothermal titration calorimetry (ITC)**

ITC measures the heat changes that occur when two substrates interacting. ITC was performed using a MicroCal iTC200 instrument (Malvern Instruments Ltd., UK). The thermal equilibration step at 25°C was followed by an initial 60 s delay step and a subsequently 0.4 μL injection. All samples were degassed and temperate before measurement. To ensure the buffers matching, AFBs and aptamer were dissolved in the same buffer (0.1 M Tris, pH 7.4) with 1% DMSO. The experiments were performed in the sample cell containing 5 μM aptamer and the syringe containing 64μM AFB1 or AFB2. The parameter was set as the preliminary experiments. In detail, the reference power was 10 $\mu cal \cdot s^{-1}$ and the syringe stirring speed was 800 rpm. The assay consisted of the blank and binding experiments. The heat change was taken by subtracting the blank from the binding result. The binding experiments consisted 17 injections, including 16 successive 2.0μL injections every 180 s and one 0.4μL

injection at first injection. Blank experiment was carried out under the same conditions as binding experiments by directly injecting AFB1 or AFB2 into the buffer. Data were fit to a one set of sites binding model using the calorimeter software.

**2.3 pH scouting**

Before immobilizing SA on the sensor chip, an appropriate immobilization pH and SA concentration were optimized. In particular, the optimal pH of immobilization should be higher than 3.5 and lower than the isoelectric point of the protein, so that the opposite net charges of the surface and protein could drive the protein to concentrate on the surface. The procedure to obtain the appropriate immobilization pH is called ‘‘pH scouting’’. Briefly, the SA was respectively diluted in 10 mM sodium acetates at various pH values (pH 5.5, 5.0, 4.5, 4.0) to a final concentration of 50μg·mL$^{-1}$. The flow rate was 10 μL·min$^{-1}$ and the contact time was 120s. After each injection, a washing solution (50mM NaOH) was injected to remove any remaining ligands.

**2.4 Surface preparation**

SPR measurement was performed using a BIAcore T200 instrument with four flow channels. SA was immobilized covalently on a CM5 sensor chip by amine coupling. The affinity of SA for biotin is extremely high, with an equilibrium dissociation constant of about $10^{-15}$ M. Hence, the biotinylated aptamer can be captured by the SA on the surface of the sensor chip. The surface of the CM5 sensor chip is covered with a matrix of carboxymethylated dextran, a flexible unbranched carbohydrate polymer forming a thin surface layer. For the immobilization of SA, 20 mM sodium acetate buffer (pH 4.5) was used as a dilution buffer, and PBS buffer was used as a running buffer at a flow rate of 10 μL·min$^{-1}$. The surface was activated by injecting the mixture containing equal volumes of 0.1 M N-hydroxysuccinimide (NHS) and 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to generate reactive succinimide esters. 50μg·mL$^{-1}$ SA was passed over the surface and the primary amine groups of SA reacted spontaneously with the succinimide esters on the surface of the sensor chip. The excess reactive groups were blocked by injecting 1 M ethanolamine-HCl (pH 8.5) at a flow rate of 10 μL·min$^{-1}$ for 7 min. The online

response was applied to characterize the sensor surface. Final immobilization levels were determined in resonance units (RU, where 1RU=1pg/mm$^2$). For the biotin tagged aptamer, the flow buffer was changed to Tris-HCl buffer. And the flow rate was set to 10 μL·min$^{-1}$. 20μM biotin tagged aptamer was injected and captured onto the surface of channel 2 only. The channel 1 without aptamer was prepared with the same immobilization procedure.

**2.5 SPR assay**

The instrument system was equilibrated with the Tris-HCl running buffer (pH=7.4) at 25±0.1°C. Different concentrations of standard AFBs (0.09–200 ng·mL$^{-1}$) were prepared by serial dilutions in Tris–HCl from stock solution of 2 mg·mL$^{-1}$ in DMSO. To reduce the bulk contribution, it was necessary to match the refractive index of samples and buffer. The AFB1 stock solution was diluted 1000 times to reduce the DMSO concentration to 0.1%. The running buffer was also added with DMSO to a concentration of 0.1% and was used to prepare the dilution samples. All buffers of the experiments were filtered (0.22 μm).

For the generation of the calibration curves and determination of AFB1, the standard AFB1 was diluted in Tris-HCl (pH 7.4) and injected for 120s onto the aptamer-modified sensor surface at a flow rate of 30 μL·min$^{-1}$. The binding between the captured aptamer and AFB1 was monitored in real-time. After each binding reaction, a dissociation time of 300 s was applied to allow the signal returning to the baseline. The binding responses were acquired 5 s after the end of the AFB1 injection and were measured relative to the baseline. At least 3 samples with the same concentration of AFBs were detected. The calibration curve for AFB1 was obtained from over eleven calibration points.

The vinegar sample was prepared by spiking with different concentrations of AFB1 (10 ng/ml, 50 ng/ml, 100ng/ml). In detail, 5g of spiked samples was diluted by Tris-HCl in the volumetric flask of 25mL, and then mixed. The liquids were filtered through a 0.22μm filter and diluted 2-fold with water to minimize the matrix. The diluted extract was passed through an Imunoaffinity chromatography Column (IAC, Pribolab, Singapore). The column was washed with 10 mL Tris-HCl, 20 mL (2×10

mL) water and then dried with air. The AFB1 were eluted by passing quartic 0.5 mL of methanol through the IAC column at a flow rate of 2~3 $mL \cdot min^{-1}$ and collected in the EP tube. The eluate was then evaporated to dryness at 65℃ in water bath and the residue was re-dissolved with 1 mL Tris-HCl (pH=7.4) including 0.1% DMSO. The data was analyzed by BIAcore T200 Evaluation Software Version 2.0 (GE Healthcare, US).

## 3. Results and discussion

### 3.1 Interaction between the aptamer and aflatoxins

ITC experiments were firstly performed to confirm the binding status and intensity of AFB1 and AFB2 to the biotin-modified aptamer. The data from ITC was shown in Fig.1 and all of the data were fitted by one-site model within the ITC analysis software. The results showed a strong binding event between AFB1 and the biotin-aptamer with an equilibrium constant $Ka=1.51\times10^{6}\pm1.68\times10^{5}\ M^{-1}$ (Fig. 1A). AFB2 also had a strong interaction with this aptamer, with an equilibrium constant $Ka=8.01\times10^{5}\pm2.03\times10^{5}\ M^{-1}$. The chemical structure of AFB2 is similar to AFB1, as it is a 8, 9-dihydro derivative of AFB1 lacking the critical unsaturated double bond. Although several researches indicated that AFB2 had lower toxicity than AFB1, it also exhibits significant toxic effects and is labeled as a Group I human carcinogen by IARC. AFB1 and AFB2 usually appear simultaneously in the contaminated agricultural products and cause serious toxic effects. This aptamer showed comparable binding abilities to AFB1 and AFB2, and thus provided a chance for simultaneous detection of both aflatoxins.

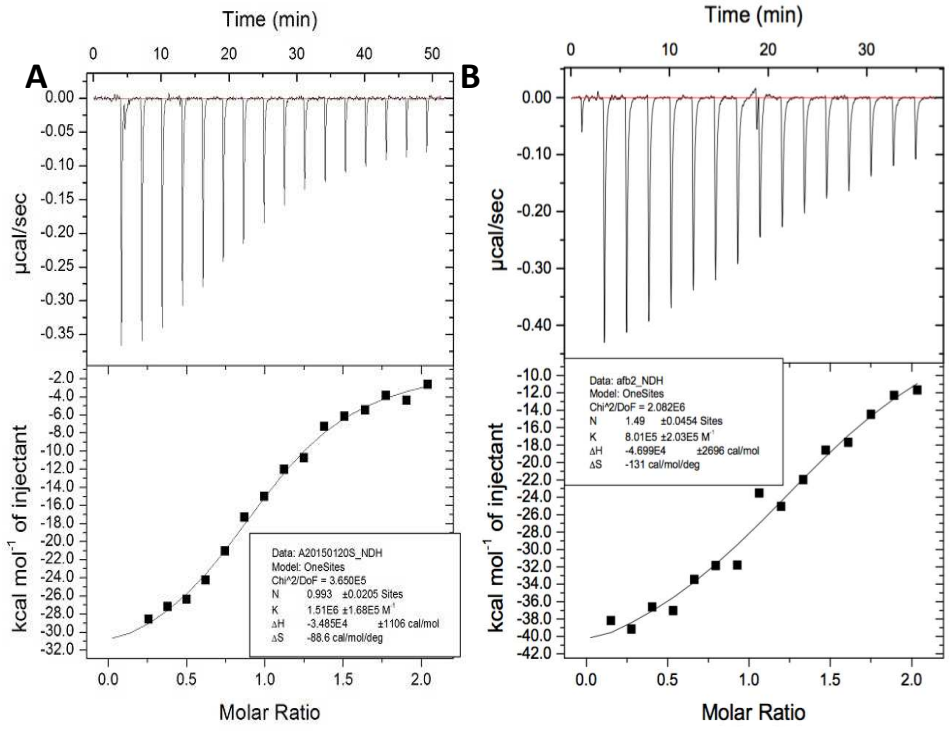


Fig.1 ITC data curves and binding isotherms for titration of AFB1 and AFB2 with aptamer. (A) The binding affinity between AFB1 and aptamer in 10 mM Tris, pH 7.4. (B) The binding affinity between AFB2 and aptamer in the same binding buffer.

### 3.2 Establishment of aptamer-based AFB1 detection by SPR

*Surface preparation of the sensor chip*

As AFB1 is the more toxic and dominant one in the contaminated product compared to AFB2, the aptamer-based SPR method was established and optimized using AFB1 standard. Firstly, the sensor chip surface was prepared. In the pH scouting program, high immobilization level in sodium acetate (pH 4.5) was found (Fig.2), therefore, sodium acetate (pH 4.5) was chosen as the dilution buffer for SA in the immobilization assay. The primary amine groups of SA reacted spontaneously with the reactive succinimide esters which were activated by the mixture of EDC and NHS. And then the ethanolamine was added to deactivate excess reactive groups (Fig.3A). The immobilization levels were 2914 and 3152 RU for channel 1 and 2, respectively.

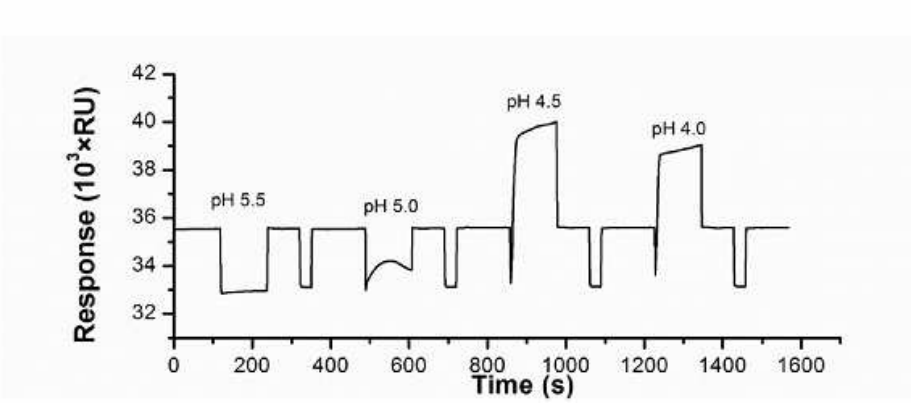


Fig.2 pH scouting of SA immobilization. Response of immobilization assay with different pH and sodium acetate (pH 4.5) was chosen as the dilution buffer.

SA as a cross-linker was successfully immobilized onto the CM5 chip surface by amine coupling which could be used to capture the biotinylated aptamer with high efficiency (the equilibrium dissociation constant was about $10^{-15}$ M). During BIAcore analysis, the dissociation of biotinylated aptamer from the surface of SA was generally negligible which ensures the stabilization of capture. The equipment was equilibrated with the running buffer until a stable baseline was achieved. The aptamer was loaded and captured onto the surface of Channel2 only (Fig.3B).

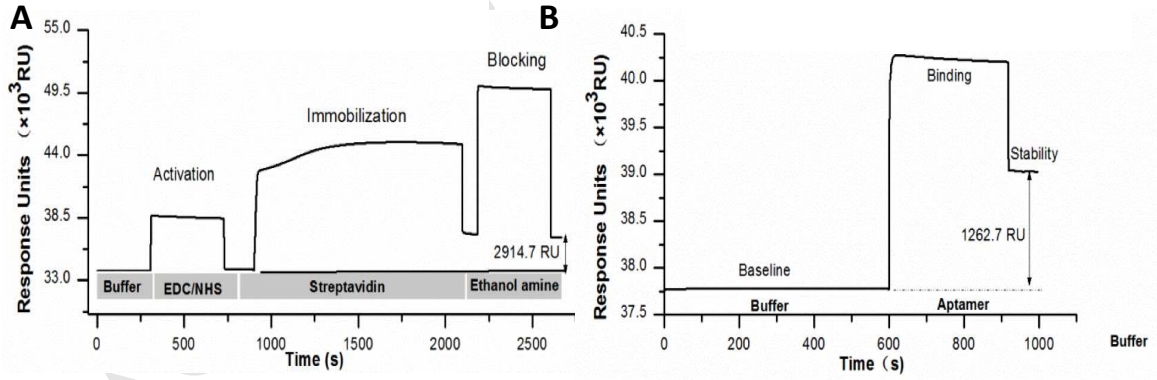


Fig.3 The sensorgram for the immobolization of streptavidin and the capture of the biotin tagged aptamer on a CM5 chip. (A) SA was passed over the surface and the esters reacted spontaneously with uncharged amino groups or other nucleophilic groups to link the SA covalently to the dextran. result, 2914 RU. (B) Streptavidin immobilized on the sensor chip surface was used to capture biotinylated aptamer with high efficiency. result, 1262 RU.

*Parameter optimization and AFB1 assessment*

Surface plasmon resonance (SPR) is an optical phenomenon which occurs at the interface between thin conducting films and media of different refractive indexes. For a certain incident wavelength, the variation of resonance angle is determined by the refractive index of the medium which is related to the concentration of the analyte attached on the surface of the thin conducting films. In the light of the literature(Shim et al., 2014b), Tris-HCl buffer showed the best performance on the binding of AFB1 and aptamer and was chosen as the running buffer instead of PBS buffer in the immobilization performance. As shown in Fig. S1, the relative response between AFB1 and aptamer returned to the base line rapidly, which indicated that AFB1 bound to the aptamer with a quick dissociation rate constant (Fig. S1). Accordingly, the injection and dissociation time were set to 60s and 120s, respectively.

Response curves were recorded by injecting different concentrations of AFB1 (0.09 to 200 ng·mL$^{-1}$) onto the sensor chip with immobilized SA and aptamer. Each sample was injected in triplicate and a calibration plot of the relative response at 5s was plotted against AFB1 concentration (Fig. 4).

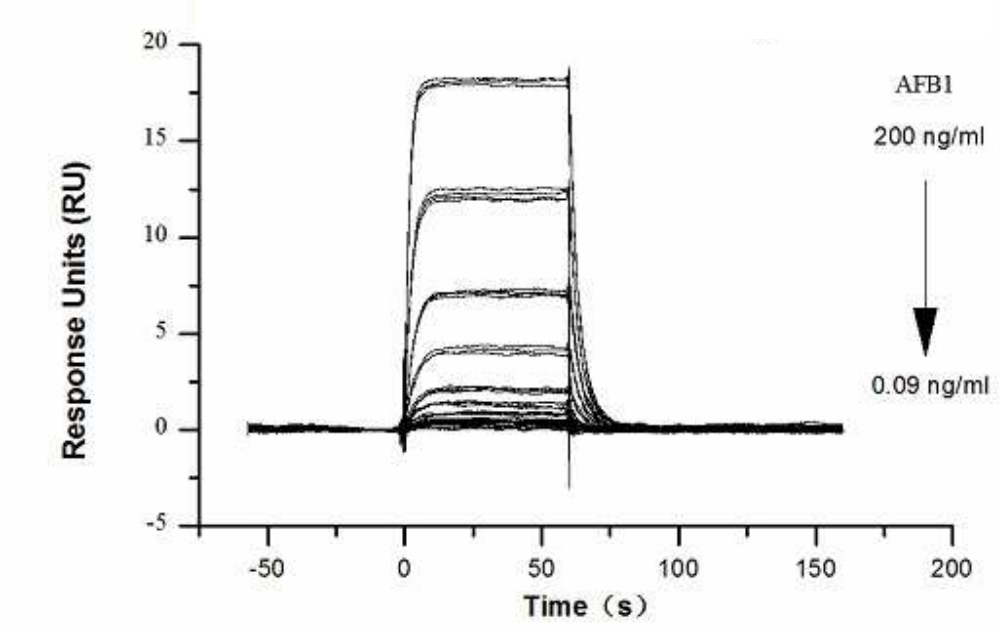


Fig.4 Response plot of AFB1 for the aptamer based SPR biosensor. Injection of AFB1 (0.094–200 ng/mL).

Evaluation of AFB1 concentration was achieved by a fully defined

four-parameter equation in BIAcore for fitting the curve to the calibration data points. The 4-parameter equation is $y = R_{hi} - \frac{R_{hi}-R_{lo}}{1+(\frac{x}{A_1})^{A_3}}$, where y and x are the plot coordinates. In addition, $R_{hi}$ and $R_{lo}$ are fitting parameters that corresponded to the maximum and minimum response levels, respectively. In this experiment, $R_{hi}$ was 2.307; $R_{lo}$ was 0.00323; $A_1$ was 93.33; $A_2$ was 1.094 and the chi-squared value was 0.0037 (Fig. 5A). Moreover, the biosensor exhibited a linear detection range from 1.5 to 50 ng·mL$^{-1}$ of AFB1 with good fitness ($R^2$=0.9821, Fig. 5B). The limit of detection was established as 0.19 ng·mL$^{-1}$ for AFB1, which were determined from the mean measurements of representative blank samples (n=8).

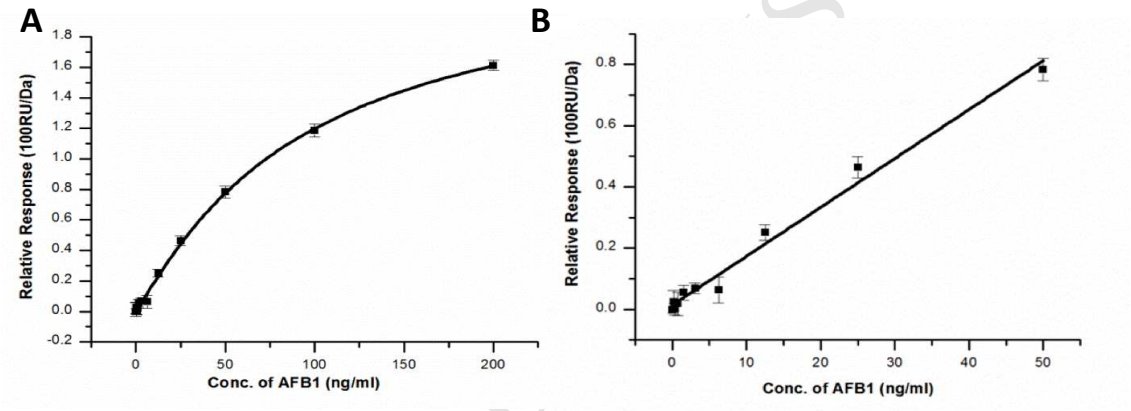


Fig.5 Response plot for the aptamer based SPR biosensor (A) and calibration plots of AFBs for the aptamer based SPR biosensor (B). Data points are the average plus±one standard deviation (n=3, LOD=0.19ng/mL, S/N=3).

*Selectivity of the SPR*

In order to determine the selectivity of this method, several relevant molecules, including AFB2, ochratoxin A (OTA), ochratoxin B (OTB), Zeralenone (ZEA) and T-2 toxin (T-2), were measured under the same concentration of 20 ng·mL$^{-1}$ as AFB1. As shown in Fig. 6, the responses of AFB1 and AFB2 were respectively 1.05RU and 1.28RU after molecular weight adjustment, which were obviously greater than OTA (0.17RU), OTB (0.20RU), T-2 (0.10RU) and ZEA (0.07RU). This result is expectable because the aptamer showed comparable affinity to both AFB1 and AFB2, and it further confirms that such aptamer-based SPR assay is available for the simultaneous detection of AFB1 and AFB2. Actually, previous studies had used this aptamer for the

detection of AFB1(Seok et al., 2015; Shim et al., 2014b) and AFB2(Luan et al., 2015), respectively. However, the comparison between these two AFBs using this aptamer was not clearly conveyed. Based on the results of this study, detection of total AFBs might be applicable. In addition, the biosensor showed very low binding rate to the other mycotoxins with similar structure to AFBs, indicating the aptamer-based SPR assay has a high specificity.

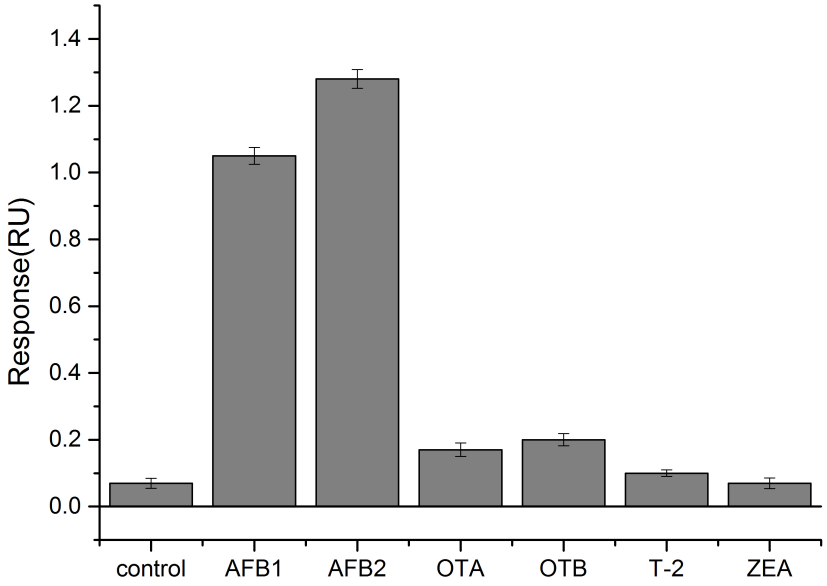


Fig.6 Specificity of the sensor chip surface toward different mycotoxin samples: AFB1, AFB2, OTA, OTB, T-2, ZEA, The concentration of tested mycotoxin was 20ng·mL$^{-1}$.

*Reusability*

When AFB1 injection stopped and the buffer passed through the surface of the chip, the response signal quickly returned to the basic, indicating that AFB1 binding to the aptamer in a fast-on and fast-off manner. That means AFBs can dissociate in the buffer without regeneration reagent and quickly enter the next cycle. In this way, the chip can be reused for a variety of times without effect on the sensitivity of the assay, and thus reduce the consumption of the ligands and decrease the cost.

In general, this aptamer-based SPR assay is a simple, sensitive, fast and economic method for the detection of AFBs. The sensitivity of this method is much higher than most of the antibody-based methods and showed a wide detection range. Additionally,

this approach did not have complicated preparation procedure and only requires 3 minutes for each cycle of detection (Table 1). More importantly, the aptamer ensures high specificity, but is not as expensive as those antibodies.

Table 1. Comparison of various analytical methods for AFB1 detection.

| Method | LOD | Linearity | Time | Reference |
|---|---|---|---|---|
| Immunochromatography assay | 2.5 ng·mL$^{-1}$ | 0.1-20 ng·mL$^{-1}$ | <2 h | (Sun et al., 2006) |
| Clean-up tandem immunoassay □column | 5 ng·mL$^{-1}$ | - | >20 min | (Goryacheva et al., 2007) |
| Enzyme immunoassay | 2 ng·mL$^{-1}$ | 10-50 ng·mL$^{-1}$ | >35 min | (Saha et al., 2007) |
| UHPLC-FLD | 2 ng·mL$^{-1}$ | - | 15 min | (Corcuera et al., 2011) |
| Real time quantitative PCR | 0.1 ng·mL$^{-1}$ | 0.1-10 ng·mL$^{-1}$ | >2 h | (Babu and Muriana, 2011) |
| Chemiluminescence competitive aptamer assay | 0.11 ng·mL$^{-1}$ | 0.1-10 ng·mL$^{-1}$ | >20 min | (Shim et al., 2014a) |
| SPR using neutrophil porcine elastase | 0.97 ng·mL$^{-1}$ | 1.67-17.8 ng·mL$^{-1}$ | 45 min | (Cuccioloni et al., 2008) |
| NRL array biosensor | 0.6-1.4 ng·mL$^{-1}$ | - | 30 min | (Sapsford et al., 2006) |
| RT-qPCR aptasensor | 25 fg·mL$^{-1}$ | 5×10$^{-5}$ ng·mL$^{-1}$ | >2 h | (Guo et al., 2014) |
| Immunochromatography | 0.5 ng·mL$^{-1}$ | - | 15 min | (Shim et al., 2007) |
| Surface-enhanced Raman scattering | 0.48pg·mL$^{-1}$ | 1-1000pg·mL$^{-1}$ | - | (Li et al., 2016) |
| Surface plasmon resonance □biosensors □ | 0.19 ng·mL$^{-1}$ | 1.5 to 50 | 3 min | This work |

| | | | | |
|---|---|---|---|---|
| | | ng·mL$^{-1}$ | | |

### 3.3 Analysis of vinegar samples by the SPR assay

The SPR biosensor was further applied to the detection of AFB1 in vinegar. The European Commission has established that the maximum level of AFB1 is 2 μg·kg$^{-1}$ for unprocessed cereals and all products derived from cereals. In this studies, vinegar samples were spiked with different concentrations of AFB1 (2,10,20 ng/ml) and subject to the dection using the SPR assay(Fig. 7). As shown in Table 2, the mean absolute recoveries for the detection assay of AFB1 in vinegar were 94.1–125.9%. This result indicates that the aptamer-based SPR assay is applicable in the detection of AFBs in complex samples.

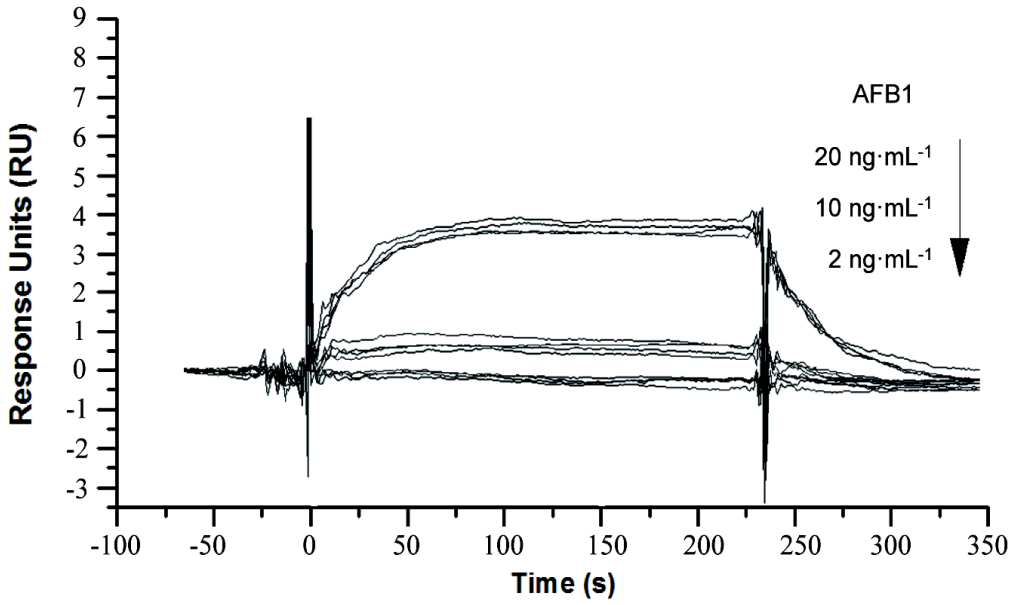


Fig.7 Response plot for the aptamer based AFB1 SPR biosensor in spiked vinegar samples and injection of AFB1 (2, 10, 20 ng/mL).

Table 2. Recovery studies of vinegar at three levels by SPR

| sample | spiked (ng·mL$^{-1}$) | actual concentration of AFB1 in buffer (ng·mL$^{-1}$) | Absolute recovery (%)(SPR) | CV(%) |
|---|---|---|---|---|
| vinegar | 2 | 2.3570 | 117.8 | 4.71 |
| | 10 | 10.6792 | 106.8 | 3.54 |
| | 20 | 19.2614 | 96.3 | 3.78 |

## 4. Conclusions

Herein, an aptamer-based SPR method was successfully developed for the rapid detection of AFBs. The linear range of the biosensor was from 1.5 to 50 $ng \cdot mL^{-1}$ (the limit of detection was 0.19$ng \cdot mL^{-1}$), which is sensitive enough for the rapid screening of AFB1 in accordance with the EU standard (2 $\mu g \cdot kg^{-1}$). The overall analysis time was only 3 minutes, which is superior to most of the other techniques. In addition, satisfactory recoveries were obtained in vinegar samples that were spiked with different concentrations of AFB1. Compared to the previously reported methods for AFBs detection, the present SPR aptasensor has the advantages of high sensitivity, high specificity and shortly detection time, so that it is a highly promising approach for the quick detection of AFBs in agricultural products and foods.

## Acknowledgements

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## Conflict of Interest

The authors have no conflict of interest.

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# Highlights

The Title: *A Direct Determination of AFBs in vinegar by aptamer-based surface plasmon resonance biosensor*

- We establish SPR aptasensor for the systematical detection of AFBs.
- The chosen aptamer showed comparable interaction with the two AFBs, namely AFB1 and AFB2. Such phenomenon was rarely reported.
- The biosensor was applied to detect AFB1 in a concentration range of 0.19-200 ng/mL.
- The approach was successfully applied to detect AFB1 in vinegar samples.
- comparing with other methods like HPLC, Vinegar AFB1 concentrations obtained were comparable with our SPR assay.